



International *Dictyostelium* Conference 2008

Tsukuba International Congress Center





International *Dictyostelium* Conference 2008

September 15th to September 20th, 2008

Tsukuba International Congress Center

Supported by Japanese Society of Developmental Biologists

Local Organizing Committee

*Tomoaki Abe, Hiroyuki Adachi, Yuzuru Kubohara, Kei Inouye, Seido Nagano,
Masazumi Semeshina, Hideko Urushihara, Taro Uyeda, Shigehiko Yumura*

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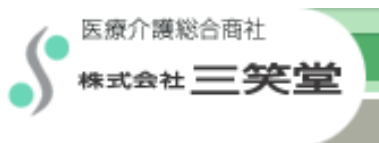
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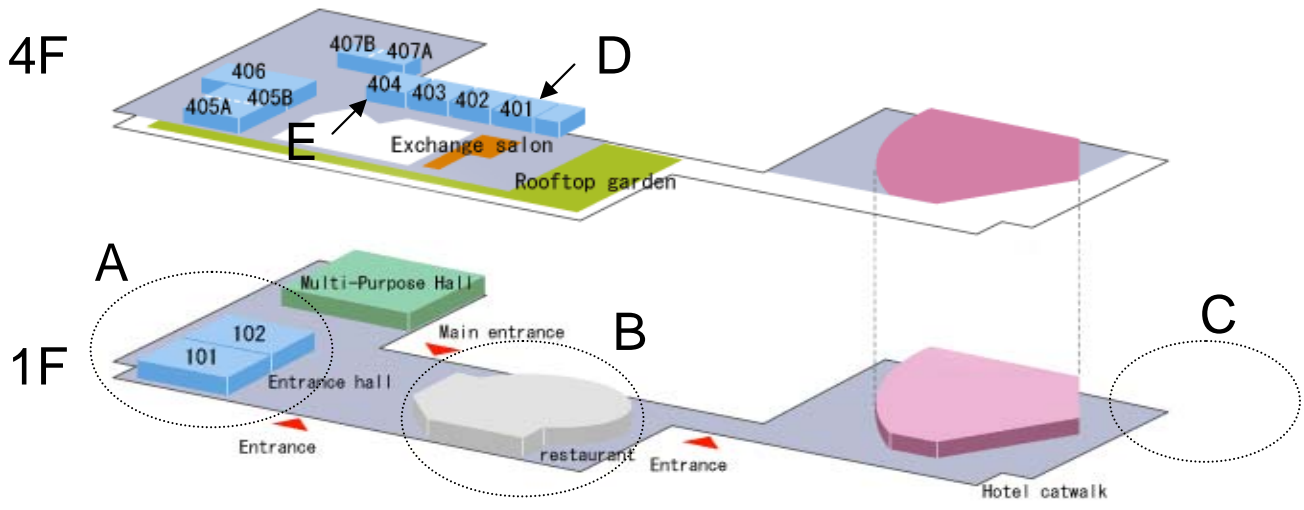
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Program at a glance

	Monday 15th	Tuesday 16th	Wednesday 17th	Thursday 18th	Friday 19th	Saturday 20th
9:00 – 10:20	/	Session 1	Session 5	Session 9	Session 11	Session 15
		Coffee break	Coffee break	Coffee break	Coffee break	Coffee break
10:50 – 12:10		Session 2	Session 6	Session 10	Session 12	Workshop
		Lunch	Lunch	Excursion	Lunch	/
14:00 – 15:20		Session 3	Session 7		Session 13	
		Coffee break	Coffee break		Coffee break	
16:00 – 17:20		Session 4	Session 8	Session 14		
	Dinner	Dinner	Break			
19:00 – 21:00	Mixer	Poster session	Poster session	Open	Banquet	

Floor guide (Tsukuba International Congress Center)



- # Talks and posters: room 101 and 102 (connected) – A
- # Mixer, lunch and dinner: Restaurant Espoir – B
- # Lunch, dinner and group discussion: Restaurant CASA in Epochal – C
- # DictyBase advisory board meeting (Tuesday lunch time): room 401 – D
- # Comparative genomics satellite meeting (Wednesday lunch time): room 404 – E

Schedule of oral presentations

Tuesday, 9:00-10:20

Session 1 : Genome and Evolution I

Chair: Ludwig Eichinger

1. Segregation of wild isolates during development in *D. discoideum*
Elizabeth Ostrowski, Mariko Katoh, Gad Shaulsky, David Queller, Joan Strassmann
2. Heterogeneity and co-existence in cellular slime moulds
Santosh Sathe, Bandhana Katoch, Sonia Kaushik, Albert Lalremruata, Ramesh K. Aggarwal, Vidyanand Nanjundiah
3. The evolutionary importance of chimerism and cheating
David C. Queller, Sara Middlemist, David Castillo, Joan E. Strassmann
4. Activated cAMP receptors turn cysts into spores
Yoshinori Kawabe, Takahiro Morio, Yoshimasa Tanaka, Pauline Schaap

Tuesday, 10:50-12:10

Session 2 : Differentiation and Morphogenesis I

Chair: Chris Thompson

1. Functional analysis of Steely Polyketide Synthase
Tamao Saito, Robert R. Kay
2. Identification and initial characterization of DIFase coding gene
Francisco Velazquez, Paul Flatman, Simon Gilbert, Rob Kay
3. Activation of STATc by Stress and DIF
Tsuyoshi Araki, Judith Langenick, Wouter N. van Egmond, Peter J. M. van Haastert, Shuai Chen, Carol MacKintosh, Annette Müller-Taubenberger, Jeffrey G. Williams
4. DimB and MybE: the odd couple?
Yoko Yamada, Tsuyoshi Araki, Natasha Zhukovskaya, Susan Ross, Rob Kay, Gareth Bloomfield, Al Ivens, Jeff Williams

Tuesday, 14:00-15:20

Session 3 : Chemotaxis and Cell Signalling

Chair: Tian Jin

1. 3'-phosphoinositides regulate coordination between speed and directional accuracy during chemotaxis
Scott Gruver, John Wikswo, Chang Chung
2. PIP3-independent activation of TorC2-PKB at the cell's leading edge mediated chemotaxis.
Yoichiro Kamimura, Peter N Devreotes
3. Chromatin re-modelling and the regulation of chemotaxis
Ben Rogers, Annette Mueller-Taubenberger, Adrian Harwood
4. The role of inositol synthesis in chemotaxis
Regina Teo, Kimberley Lewis, W Jonathan Ryves, Adrian J Harwood

Tuesday, 16:00-17:20

Session 4 : Cytoskeleton and Cell motility I

Chair: Shigehiko Yumura

1. Correlated waves of actin filaments and PIP₃ in *Dictyostelium* cells.
Yukako Asano, Akira Nagasaki, Taro Q.P. Uyeda
2. Does arginylation matter for actin-dependent activities in *Dictyostelium*?
Annette Müller-Taubenberger, Petros Batsios, Michael Schleicher
3. Modulation of actin structure and function by phosphorylation of Tyr-53 and profilin binding
Kyuwon Baek, Xiong Liu, François Ferron, Shi Shu, Edward D. Korn, Roberto Dominguez
4. PaxB and PldB work to regulate actin-based processes
M. Berenice Duran, Asif Rahman, Max Colten, Derrick Brazill

Wednesday, 9:00-10:20

Session 5 : Differentiation and Morphogenesis II

Chair: Rob Kay

1. Regulation of Differential Cell Adhesion by Rap1 is Required for Cell Type Patterning and Morphogenesis in *Dictyostelium*
Katie Parkinson, Parvin Bolourani, David Traynor, Nicola L. Aldren, Robert R. Kay, Gerald Weeks, Christopher R. L. Thompson
2. Analysis of dedifferentiation pathway in *Dictyostelium discoideum*
Mariko Katoh, Gad Shaulsky
3. A steroid signal regulates sensitivity to GABA during culmination
Christophe Anjard, William F. Loomis
4. Vacuole formation during stalk cell differentiation
Toru Uchikawa, Kei Inouye

Wednesday, 10:50-12:10

Session 6 : Recognition and Sexual Cycle

Chair: Tomoaki Abe

1. Developmental changes in N-glycan fucosylation in *Dictyostelium*
Birgit Schiller, Josef Voglmeir, Alba Hykollari, Gerald Pörtl, Karin Hummel, Rudolf Geyer, Iain B.H. Wilson
2. Rapid evolution in the lag gene family reveals a role in self-recognition
Rocio Benabentos, Shigenori Hirose, Richard Sucgang, Tomaz Curk, W. Justin Cordill, Mariko Katoh, Yue Wang, Elizabeth Ostrowski, Joan Strassmann, David Queller, Blaz Zupan, Gad Shaulsky, Adam Kuspa
3. The *Dictyostelium* mating type locus
Gareth Bloomfield, Jason Skelton, Alasdair Ivens, Yoshimasa Tanaka, Robert R. Kay
4. Involvement of the PKC activity in translocation of the ZYG1 protein into the cell cortex during *Dictyostelium* development
Aiko Amagai, Shinya Urano, Harry MacWilliams, Kazuo Yamamoto, Yasuo Maeda

Wednesday, 12:10-12:20

Announcements (Dicty Stock Center — Jacob Franke)

Wednesday, 14:00-15:20

Session 7 : Cell adhesion and Cytoskeleton

Chair: Taro Uyeda

1. Elucidating a role for the *Dictyostelium discoideum* SadA protein in cell-substrate adhesion: A Role for the Tail
Anthony S. Kowal, Rex L. Chisholm
2. Myosin Heavy Chain Kinase C Mediates Cell Adhesion via a Myosin II-Independent Mechanism
Alyssa Chowdhury, Atiya Franklin, Linzi Hyatt, Paul Steimle
3. Functional analysis of NDR kinases in *Dictyostelium discoideum*
Peter Kastner, Michael Schleicher, Annette Müller-Taubenberger
4. Microtubule-based motor protein functions in *Dictyostelium*
Michael Koonce, Irina Tikhonenko, Dilip Nag, Douglas Robinson

Wednesday, 16:00-17:20

Session 8 : Host-pathogen interaction

Chair: Annette Müller-Taubenberger

1. Phagocytosis and host-pathogen interactions in *Dictyostelium*. Re-examining some open questions on PLC, PI3K, Nramp1 and actin in phagocytosis and infection.
Salvatore Bozzaro, Alessandra Balest, Barbara Peracino, Barbara Pergolizzi, Alessio Sillo
2. Genetic and genomic evidence for bacterial recognition by dictyostelids
Waleed Nasser, Richard Sucgang, Adam Kuspa
3. The *Dictyostelium* response to infection with *Legionella*: From microarray analysis to functional characterisation — a top-down approach.
Rui Tian, Can Ünal, Sachin Kumar, Michael Steinert, Ludwig Eichinger
4. The *Dictyostelium* Pod-1 homolog Crn7 is a coronin-like protein involved in actin-driven processes and infection with *Legionella pneumophila*
M. C. Shina, C. Ünal, A. Müller-Taubenberger, M. Steinert, A. A. Noegel

Thursday, 9:00-10:20

Session 9 : Cytoskeleton and Cell motility II

Chair: Dave Knecht

1. Understanding phosphatidylinositol turnover as a therapeutic target using *Dictyostelium*
Ben Orabi, Nadine Pawolleck, Robin S.B. Williams
2. A RasGEF/Sca1/PP2A Signaling Complex Controls the Ras-TORC2-AKT/PKB Pathway
Pascale G. Charest, Atsuo T. Sasaki, Zhouxin Shen, Steve Briggs, Richard A. Firtel
3. *Dictyostelium* talin homologue, talin A, is involved in tail retraction in directed cell migration
Masatsune Tsujioka, Shigenobu Yonemura
4. Quantitative analysis of amoeboid cell motility
Ruedi Meili, Juan C. del Alamo, Baldomero Alonso-Latorre, Juan C. Lasheras, Rick Firtel

Thursday, 10:50-12:10

Session 10 : Gene regulation

Chair: Takefumi Kawata

1. Visualisation of transcriptional firing in individual living cells
Tetsuya Muramoto, Jonathan Chubb
2. Interactions between modifications of histone H3.
Duen-Wei Hsu, Jonathan Chubb, Po-Hsien Liu, Louis C. Mahadevan, Catherine Pears
3. Mitochondrial Processing Peptidase (MPP) in *Dictyostelium discoideum*: MPP activity is controlled via processing of α -MPP by a novel protease during development
Koki Nagayama, Tetsuo Ohmachi
4. Characterization of the mitochondrial Ras-related GTPase Miro of *Dictyostelium discoideum*
Georgia Vlahou, Jürgen-Christoph von Kleist-Retzow, Rudolf J. Wiesner, Francisco Rivero

Friday, 9:00-10:20

Session 11 : Chemotaxis and Oscillation

Chair: Paul Fisher

1. Dynamic interactions between receptors and G-proteins prior to and after receptor activation in *Dictyostelium discoideum*
Carrie Elzie, Jennifer Colby, Morgan Sammons, Chris Janetopoulos
2. Folic acid is a potent chemoattractant of amoebae of a protist, *Vahlkampfia sp.*, as the case for vegetative amoebae of the cellular slime molds
Yasuo Maeda, Aiko Amagai
3. A phase transition to collective behavior in *Dictyostelium* cell populations
Thomas Gregor, Koichi Fujimoto, Satoshi Sawai
4. Study of Excitability and Synchronization in *Dictyostelium* by the Newly Developed Micro-laboratory
Shunsuke Sakurai, Satoshi Konishi, Seido Nagano

Friday, 10:50-12:10

Session 12 : Differentiation and Morphogenesis III

Chair: Adrian Harwood

1. Functional characterisation of adenylyl cyclase B in *Dictyostelium*
Zhihui Chen, Pauline Schaap
2. Regulation of filamin complex activity by a novel class of HECT ubiquitin ligase is required for cell sorting and morphogenesis in *Dictyostelium*
Simone Blagg, Thomas Keller, Sarah J. Annesley, Qikai Xu, Paul R. Fisher, Christopher R. L. Thompson
3. Calcium Signalling in *Dictyostelium* and its Implications in Polycystic Kidney Disease and Mucopolysaccharidosis Type IV
Claire Y Allan, Paul R Fisher
4. Specificity of the Presenilin/ γ -secretase Complex in Control of Development
Vanessa McMains, Marielle Young, Alan R. Kimmel

Friday, 12:10

Announcements (dicty2009 — Alan Kimmel)

Friday, 14:00-15:20

Session 13 : Cytoskeleton and Cell Motility III

Chair: Rex Chisholm

1. Cytoskeletal assembly and maturation: How do different actin binding proteins cooperate and compete in assembling actin filament arrays
David A. Knecht
2. WASP Family proteins controlling actin dynamics in *Dictyostelium discoideum*
Michael Carnell, Robert Insall
3. Functional roles of VASP phosphorylation in the regulation of chemotaxis and osmotic stress response.
Wan-Hsin Lin, J. Scott Gruver, Sharon E. Nelson, Chang Y. Chung
4. An ELMO-like protein associated with actinomyosin restricts spurious F-actin events to coordinate phagocytosis and chemotaxis
Nilgun Isik, Joseph Brzostowski, Tian Jin

Friday, 16:00-17:20

Session 14 : Genome and Evolution II

Chair: Pauline Schaap

1. Cryptic species, phylogenetics, and sex in four *Dictyostelium* species
Joan E. Strassmann, Natasha Mehdiabadi, Chandra Jack, Sara Kalla, Marcus Kronforst, David C. Queller
2. *Dictyostelium* anatomy and phenotypes
Pascale Gaudet, Jeffery G. Williams, Petra Fey, Rex L. Chisholm
3. Comparative Genomics in Social Amoebas
Gernot Glöckner, Andrew Heidel, Ludwig Eichinger, Angelika A. Noegel, Pauline Schaap, Matthias Platzer
4. The evolutionary genomics of *Dictyostelium discoideum*
Jonathan Flowers, Angela Stathos, Elizabeth Ostrowski, David Queller, Joan Strassmann, Michael Purugganan

Saturday, 9:00-10:00

Session 15 :

Chair: Hiroyuki Adachi

1. An RnoA-PldB-PaxB complex potentially regulates actin cytoskeleton and adhesion in *Dictyostelium discoideum*
Rebecca Fernandez, Derrick Brazill
2. Structural Dynamics of Cytokinesis
Alexandra Surcel, Elizabeth Reichl, Janet Effler, Yixin Ren, Douglas Robinson
3. Dynamic interactions between chemoattractant GPCR and heterotrimeric G-proteins in membrane of living cells revealed by FRAP and Single-molecule imaging
Xuehua Xu, Joseph A. Brzostowski, Pavel Tolar, Tian Jin

Saturday, 10:30-11:30

Workshop : Community Resources

Chair: Jeffery Williams

1. New tools at dictyBase
Petra Fey
2. An update on the Dicty Stock Center and its future
Jacob Franke
3. Two resource sites from Japan
Hideko Urushihara

Poster sessions

(Grouped by keyword provided by the authors)

Genome

1. Developmental genes in *Acytostelium subglobosum*, a group 2 species without cell-type differentiation
Hideko Urushihara, Hidekazu Kuwayama, Kyoko Uchi, Yuji Kohara, Hiroshi Kagoshima, Tadasu Shin-i, Kazuko Ohishi, Takehiko Itoh, Tadatsugu Taniguchi, Asao Fujiyama
2. dictyBase: Update 2008
Petra Fey, Pascale Gaudet, Siddhartha Basu, Eric Just, Daniel Schneider, Yulia Bushmanova, Warren A. Kibbe, Rex L. Chisholm

Differentiation

3. *Dictyostelium* PKB/AKT related kinase may function as an activator for a putative switch of stalk formation
Hiroshi Ochiai, Koki Nagayama, Kosuke Takeda, Masashi Fukuzawa, Shigeharu Takiya, Tetsuo Ohmachi
4. A new mode of prespore gene regulation: DdMybF functions as a repressor for prespore differentiation
Kei Tomita, Masashi Fukuzawa
5. Dla1, La-like RNA-binding domain-containing protein, is important for cell growth and cell-type proportioning of *Dictyostelium discoideum*.
Toshinori Usui, Kiyotaka Shibata, Tomoaki Abe
6. Some cations affect on development of the cellular slime mold, *Dictyostelium discoideum*.
Yuka Satou, Yuushin Kamada, Saburo Uchiyama
7. Developmental inhibitor of *Acytostelium subglobosum* that forms fruiting bodies without cell differentiation
Kyoko Uchi, Hidekazu Kuwayama, Ryuji Yoshino, Hideko Urushihara
8. Growth kinetics and differentiation in *Dictyostelium*
Noritaka Masaki, Satoshi Sawai
9. Biological activities of DIF-1 derivatives
Yuzuru Kubohara, Haruhisa Kikuchi, Yoshiteru Oshima
10. A novel differentiation inducing factor with low molecular weight in *Dictyostelium discoideum*: its purification and characterization
Yoshiaki Takaya, Masashi Fukuzawa, Rie Hotta, Manabu Nakagawa, Akiko A. Oohata

Morphogenesis

11. Cotransporters and the uptake of the epilepsy treatment, Valproic acid, using the biomedical model *Dictyostelium discoideum*
Nicole Terbach, Dmitri Gordienko, Nigel A. Brown, Robin S. B. Williams
12. SunB, a novel type of SUN-domain-containing protein, is implicated in cytokinesis and development in *Dictyostelium discoideum*
Nao Shimada, Hiroyuki Adachi, Koji Yoda, Takefumi Kawata

Gene regulation

13. New components of the *Dictyostelium* PKA pathway revealed by Bayesian analysis of transcriptional profiling data
Anup Parikh, Eryong Huang, Chris Dinh, Blaz Zupan, Adam Kuspa, Devika Subramanian, Gad Shaulsky
14. shRNA- Directed Knockdown of Gene and Analysis of *Dictyostelium* Development
Vikas Sonakya, Hideshi Otsuka, Marie McAnuff, Subrata Chowdhury, Albana Thomaraj, Julian Gross, Thomas Winkler, Robert Dottin

Sexual cycle

15. Differential gene expression between sexually mature and immature cells of *Dictyostelium discoideum*
Saeki, K., Satoh, T., Muramoto, T., Skeleton, J., Kay, R., Kuwayama, H., Urushihara, H.
16. Functional analysis of *rasX* group genes
Yoko Furuya, Mariko Kunitani, Tetsuya Muramoto, Hidekazu Kuwayama, Hideko Urushihara

Cell adhesion

17. Analyses of integrin-beta-like proteins in *Dictyostelium*
Zenjiro Sampei, Keisuke Yokota, Masakazu Saito, Naoko Masuyama, Kunito Yoshida, Tatsuaki Kudo, Koji Yoda, Kei Inouye, Hiroyuki Adachi
18. PaxB regulates actin dependent processes
Jelena Pribic, May Kong, Derrick Brazill

Cell motility

19. SecA is required for cell motility
Roberto Zanchi, Mark S. Bretscher, Robert R. Kay
20. Heterogeneity in cell motility after induction of starvation correlates with the developmental fate of amoebae
S Pavana Gowry, G Rajani Kanth, V. Nanjundiah, Gopal Pande
21. Screening of genes involved in cell migration in *Dictyostelium*.
Akira Nagasaki, Taro Q.P. Uyeda

22. Directional migration in *Dictyostelium* cells induced by repeated stretch of the substratum
Yoshiaki Iwadate, Yuki Ishiyama, Shigehiko Yumura
23. Switching direction in electric signal-induced cell migration by cGMP and phosphatidylinositol signaling
Masayuki J. Sato, Wouter N. van Egmond, Hiroaki Takagi, Peter J. M. van Haastert, Toshio Yanagida, Masahiro Ueda
24. A cell number counting factor regulates both speed and direction of a cell during development to regulate group size in *Dictyostelium discoideum*
Jonghyun Roh, Yeonjeong Yu, Hyeri Seo, Soomin Son, Gyeongyun Go, Deborah Wessels, David R Soll, Richard H Gomer, Wonhee Jang
25. A novel expression system for dominant negative mutant actin in *Dictyostelium*
Taro Q.P. Noguchi, Noriko Kanzaki, Hironori Ueno, Keiko Hirose, Taro Q.P. Uyeda

Cytoskeleton

26. Comparison of the properties of actin aggregates induced by fragments of different actin binding proteins
Andrew Maselli, Jeannie Ramos, Denise Patrick, Ran-der Hwang, David Knecht
27. Role of PTEN for localization of myosinII in *Dictyostelium* cells.
Md. Kamruzzaman Pramanik, Miho Iijima, Yoshiaki Iwadate, Shigehiko Yumura
28. Analyses on a novel protein involved in cytokinesis in *Dictyostelium*
Hironori Inaba, Yuya Takaki, Takahiro Sasaki, Yuji Iwasa, Takehiro Hara, Takashi Yamamoto, Jun Sawaguri, Koji Yoda, Hiroyuki Adachi
29. Complex constructs including G-actin aggregates in *Dictyostelium discoideum* spores.
Masazumi Sameshima
30. Effects of Tyr-53 mutations on polymerization of *Dictyostelium* actin and cell phenotype
Xiong Liu, Shi Shu, Bin Yu, Edward D. Korn
31. Regulation of actin cytoskeletal architecture by Fimbrin A
Ran-der Hwang, Chin-chi Chen, David A. Knecht
32. Visualizing myosin-actin interaction with a genetically encoded fluorescent strain sensor.
Sosuke Iwai, Taro Q. P. Uyeda
33. Single-molecule imaging analysis of heterotrimeric G protein dynamics in early signaling events of chemotactic responses.
Yukihiro Miyanaga, Tatsuo Shibata, Masahiro Ueda

34. Identification of PKBA substrates that link PIP3 to the cytoskeleton
Michelle Tang, Yoichiro Kamimura, Peter Devreotes, Miho Iijima

Chemotaxis

35. Towards a quantitative input-output relationship in the *Dictyostelium* cAMP relay response
Thomas Gregor, Koichi Fujimoto, Satoshi Sawai
36. Adaptive responses regulated by the chemoattractant seven- transmembrane receptor CAR1
Joseph Brzostowski, Satoshi Sawai, Carole A. Parent, Dale Hereld, Alan R. Kimmel
37. Self-organization in the phosphatidylinositol lipids signaling pathway
Yoshiyuki Arai, Tatsuo Shibata, Satomi Matsuoka, Toshio Yanagida, Masahiro Ueda
38. Modeling adaptation and intrinsic cytosolic cAMP oscillations in isolated *Dictyostelium* cells.
Koichi Fujimoto, Thomas Gregor, Satoshi Sawai
39. Understanding clathrin-mediated endocytosis in *Dictyostelium*
Laura Macro, Jyoti K. Jaiswal, Sanford M. Simon
40. Antisense RNA inhibition of *Dictyostelium* β -MPP induces expression of nuclear-encoded mitochondrial proteins in retrograde regulation manner
Koki Nagayama, Shiori Itono, Hiroshi Ochiai, Tetsuo Ohmachi

Endo- and exocytosis

41. Dynamics of *Escherichia coli* gene expression in symbiotic relationship building with *Dictyostelium discoideum*
Kumiko KIHARA, Kotaro MORI, Naoaki ONO, Shingo SUZUKI, Akiko KASHIWAGI, Chikara FURUSAWA, Tetsuya YOMO

Stress response

42. Expression and biochemical characterization of *Dictyostelium discoideum* fatty acid amide hydrolases
Dhamodharan Neelamegan, Frank St. Michael, Suzanne Lacelle, James C. Richards, Andrew D. Cox

Host-pathogen interaction

43. A DNA oligonucleotide-assisted genetic manipulation that increases transformation and homologous recombination efficiencies
Hidekazu Kuwayama, Toshio Yanagida, Masahiro Ueda
44. The biosynthesis of GDP-Fucose in *Dictyostelium*
Alba Hykollari, Birgit Schiller, Josef Voglmeir, Stefan Karl, Iain B. H. Wilson

Others

45. Determining the role of abc transporters in *Dictyostelium discoideum* development
Edward Miranda, Olga Zhuchenko, Adam Kuspa, Gad Shaulsky
46. Biologically active small molecules produced by cellular slime molds
Haruhisa Kikuchi, Shinya Ishiko, Aiko Amagai, Yasuo Maeda, Kohei Hosaka,
Yuzuru Kubohara, Yoshiteru Oshima
47. Startup of NBRP-nenkin, the Japanese Stock Center
Hideko Urushihara, Taro Q. P. Uyeda, Hidekazu Kuwayama, Akira Nagasaki,
Shin-ichi Kawakami, Yu Hachikubo, Keiichiro Ui, Reiko Nishijima
48. A taxonomic revision of the genus *Polysphondylium*
Shin-ichi Kawakami, Hiromitsu Hagiwara, Tetsuo Hashimoto
49. The TOR signalling pathway controls Batten Disease phenotypes in *Dictyostelium*
Paige K Smith, Paul R Fisher

Oral Presentations

Segregation of wild isolates during development in *D. discoideum*

Elizabeth Ostrowski¹, Mariko Katoh², Gad Shaulsky², David Queller¹, Joan Strassmann¹

¹Department of Ecology and Evolutionary Biology, Rice University, Houston TX, USA,

²Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

In *D. discoideum*, multicellular development results in the death of approximately 20% of the cells, which form a cellular stalk that lifts up and supports the spores. Because aggregation can occur between genetically different cells, the process is susceptible to invasion by cheaters—individuals who derive a fitness benefit from the formation of the stalk, but fail to contribute their fair share to its production. One mechanism that might limit or prevent cheating is kin discrimination—if individuals can recognize and restrict fruiting body formation to relatives, then the fitness benefit provided by the dead stalk cells will accrue disproportionately to related individuals, who indirectly pass on their genes. Consistent with this possibility, recent work has shown that, in mixes of genetically distinct isolates of *D. purpureum*, cells can segregate from non-identical cells, forming fruiting bodies that are comprised primarily of one clone or the other.

We sought to determine whether genetically distinct wild isolates of *D. discoideum* are capable of distinguishing and then segregating from one another during multicellular development. We mixed cells of the lab strain AX4 (labeled with GFP) with cells of each of 16 different wild isolates and allowed them to undergo multicellular development. Following development, we sampled fruiting bodies from the mixes, estimated the proportion of labeled spores in each one, and used the variance in this measure as an estimate of the degree of segregation. In a control mix of genetically identical cells, among fruiting body variance in the proportion of labeled cells was low, indicating that the cells did not segregate. By contrast, mixes of genetically distinct strains showed higher variance, indicating greater segregation, and there was a significant correlation between the genetic distance between mixed strains based on microsatellite loci and the degree to which they segregate when mixed. Using time lapse microscopy, we monitored the development of the mixes. We found that genetically distinct isolates initiate development at the same time and form common aggregation streams, but as development progresses into the tight aggregate stage, they increasingly segregate into different aggregates, although they rarely exclude one another completely. Taken together, our results demonstrate the existence of self-nonsel recognition in *D. discoideum*, an ability that may act to exclude potential cheaters.

Heterogeneity and co-existence in cellular slime moulds

Santosh Sathe¹, Bandhana Katoch¹, Sonia Kaushik¹, Albert Lalremruata², Ramesh K. Aggarwal², Vidyanand Nanjundiah^{1,3}

¹Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India, ²Centre for Cellular and Molecular Biology, Hyderabad 500007, India, ³Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560012, India.

Following starvation, cellular slime mould (CSM) amoebae form social groups with division of labour. Eventually some cells die (and form a stalk) whereas others survive (and form spores). This constitutes an extreme example of so-called ‘altruistic’ and ‘selfish’ behaviour within a social group. In the course of our attempt to understand the origin and maintenance of social behaviour in the CSMs, we have measured fitness-related traits in single cells, clonal groups and genetic mosaics of *Dictyostelium giganteum* and *D. purpureum* strains isolated from undisturbed forest soil.

Evidence will be presented in support of the following assertions: (i) Single fruiting bodies that develop from tiny soil particles or animal dung show a significant extent of genetic heterogeneity; (ii) Clonal groups and chimaeras, among the latter being those formed by mixing siblings (macrocyt progeny), are very similar with regard to many traits including aggregation territory sizes and overall spore forming efficiencies; (iii) When pairs of strains are made to aggregate and form genetically mosaic groups, one strain tends to be more efficient at sporulation than the other (though in general, mosaics exhibit a higher variability than clones); (iv) Relative sporulation efficiencies can be arranged in a transitive hierarchy; (v) A strain which is low in the hierarchy does significantly better than expected when the group consists of three strains instead of two; (vi) In the cases studied by us, genetic similarity within groups does not appear to be an important mediator of social behaviour; and (vii) In addition to other factors, trade-offs between different life cycle components of fitness and phenotypic complementation may help distinct strains to co-exist in the wild.

The evolutionary importance of chimerism and cheating

David C. Queller, Sara Middlemist, David Castillo, Joan E. Strassmann
Department of Ecology and Evolutionary Biology, Rice University

Social amoebas can form chimeric fruiting bodies, allowing one clone to cheat another with respect to who forms the sterile stalk. Numerous genes affect cheating, but we want to understand whether chimerism and cheating have been important in the evolutionary history of *Dictyostelium*. As with most model systems, it can be difficult to understand behavior in the field, but we have used several approaches. First, different clones are found in close proximity to each other in soil, suggesting they could join in the same fruiting body. However, fruiting bodies collected from dung were usually, though not always, clonal. Part of this results from recognition and segregation of clones that initially aggregate together. Though recognition currently reduces chimerism, it also suggests that chimerism has been historically important; recognition only makes sense if it is important to exclude other clones or to treat them differently. Several indirect tests give mixed results. First, the tendency of chimeric slugs to travel less far is consistent with them having evolved to avoid the front pre-stalk region, but other explanations are possible. We also investigated the importance of cell health for getting into spores, reasoning that if competition against other clones is important, cells should use every advantage they have. The results were ambiguous. As predicted, unhealthy cells with either too little glucose or too much acid, were worse at competing to be spores in chimeras. But they were no worse than predicted by their spore production when alone. Finally, we conducted a mutation accumulation experiment, putting 90 cell lines through about 1000 generations and 70 single-cell bottlenecks. The effect of the bottlenecks is to reduce the effect of selection and allow mutations to accumulate. Traits that have been selected and maintained by selection should show a decline when random mutations accumulate. We found this to be true for the ability to cheat, consistent with it being a trait maintained by selection.

Activated cAMP receptors turn cysts into spores

Yoshinori Kawabe¹, Takahiro Morio², Yoshimasa Tanaka², Pauline Schaap¹

¹College of Life Sciences, University of Dundee, Dundee, UK., ²Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

Spore forming Dictyostelid social amoebas evolved from cyst forming solitary amoebas by inventing a unique form of multicellularity where amoebas assemble into fruiting structures. In the derived species *Dictyostelium discoideum*, secreted cAMP coordinates aggregation and fruiting body morphogenesis, and triggers expression of aggregation and sporulation genes. To investigate how and when these roles of cAMP evolved, we studied the heterogeneity and function of cAMP receptor (cAR) genes throughout the social amoeba phylogeny. Our data show that the gene duplications that gave rise to the four *D. discoideum* cARs occurred late in dictyostelid evolution. Most Dictyostelia have only a single cAR that has duplicated independently in the Polysphondylids and Acytostelids. When the two cAR genes of the basal species *Polysphondylium pallidum* are disrupted, aggregation remains normal, but subsequent fruiting body formation becomes aberrant. The stunted structures consist of normal but disorganized stalk cells that support a mass of spherical cysts instead of elliptical spores. Encystation is an ancestral survival strategy in *P. pallidum* that occurs when aggregation is prevented. Similar to normal cysts, but unlike spores, the cAR null cysts cannot withstand freeze-drying, but like cysts and spores they are detergent resistant. We show that cAMP induces spore gene expression in *P. pallidum* wild type cells, but not in the cAR null mutants, which is the most likely cause of their sporulation defect. Our data indicate that spores are evolutionary derived from cysts, which is supported by recent evidence that the formation of both requires increased intracellular cAMP levels. Spores differ from cysts in also requiring extracellular cAMP. This suggests a mechanism whereby accumulation of secreted cAMP inside aggregates instructs the starving cells to form spores and not cysts.

Functional analysis of Steely Polyketide Synthase

Tamao Saito¹, Robert R. Kay²

¹Faculty of Science, Hokkaido University, Japan, ²MRC Laboratory of Molecular Biology, Cambridge, UK

Polyketides are a ubiquitous class of natural products produced by bacteria, plants and fungi. Like most natural products, they are generally produced to bring an ecological advantage to the producer species by affecting other organisms in the same environment. Polyketides are made by polyketide synthases (PKSs), which are currently classified into three groups: type I, type II, and type III-the chalcone synthase-PKS. *Dictyostelium* has accomplished an important piece of PKS engineering in which a type III PKS is fused to a multi-domain type I PKS, to give a large protein of around 3,000 amino acids. We found two such hybrid “Steely” enzymes encoded in *Dictyostelium* genome and one of them, SteelyB makes the (pre)stalk cell inducer DIF-1.

We focus on SteelyB PKS and try to address two questions concerning its function. The first question is whether or not SteelyB makes any other polyketides in addition to the DIF-1 precursor. The second question is how the two components of the fusion cooperate with each other: for instance is the growing polyketide transferred directly from one to the other, or is a shuttle protein involved?

In the slug stage, SteelyB makes DIF-1 in the prespore region, but in the fruiting body, it no longer makes DIF-1 but makes the precursor of the late chlorinated compounds that accumulate in the stalk. In accordance with this, we detect the *stlB* expression in the stalk region of the fruiting body. This expression profile of *stlB* is distinct from that of *dmtA*. To address the second question, we made a TAP-tag knock-in mutant into the *stlB* gene locus. This knock-in mutant made the predicted products by *in vivo* labeling. With the pull-down fraction of SteelyB protein, we found the full synthesis of DIF-1 polyketide, THPH, from simple acetate and malonate substrates in a single reaction vessel, suggesting that ancillary proteins are not required for SteelyB activity.

Identification and initial characterization of DIFase coding gene

Francisco Velazquez, Paul Flatman, Simon Gilbert, Rob Kay
Laboratory of Molecular Biology-Medical Research Council. Cambridge. United Kingdom

The study of DIF molecules and their role in *Dictyostelium discoideum* development has followed a tortuous path. Identified as prestalk-inducing factors, DIF's signalling machinery has been hunted for decades. Although the work of many laboratories has improved our understanding of the role of DIF, the intricate puzzle of DIF signalling is still far from solved. Here we report the identification of the gene encoding DIF-1 3(5)- dechlorinase, otherwise named DIFase, and the initial characterization of null and overexpressing strains. DIFase was first identified as an activity and the enzymatic features determined, but DIFase gene remained unknown until now. With a joint approach based on in silico searches and protein purification we have succeeded in identifying the DIFase gene in *D. discoideum*. It carries the same protein domain organization (i.e. GST_N/GST_C domains) as glutathione-S-transferases. *D. discoideum* has another 14 proteins with a similar domain organization, although DIFase is responsible for at least 90% of DIF-3 production. DIFase expression is developmentally regulated and it shows a prestalk specific expression pattern at tipped mound and slug stages. The development of null and over expressing strains has been compared with the parental Ax2 strain. We also present the expression pattern of different subpopulation markers (e.g. *ecmA:lacZ*, *ecmB:lacZ*...) in the null background.

Activation of STATc by Stress and DIF

Tsuyoshi Araki¹, Judith Langenick¹, Wouter N. van Egmond², Peter J. M. van Haastert², Shuai Chen³, Carol MacKintosh³, Annette Müller-Taubenberger⁴, Jeffrey G. Williams¹

¹Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, ²Department of Molecular Cell Biology, University of Groningen, 9751 NN Haren, The Netherlands, ³The MRC Protein Phosphorylation Unit, The Sir James Black Centre, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, ⁴Institute for Cell Biology (ABI), Ludwig Maximilians University, Schillerstr. 42, Munich 80336, Germany

When *Dictyostelium* cells are exposed to hyper-osmotic stress or to DIF-1 (DIF) STATc is rapidly tyrosine phosphorylated. Previous observations suggest a non-paradigmatic mode of STAT activation, whereby DIF or stress-induced serine-threonine phosphorylation of the PTP3 protein tyrosine phosphatase inhibits its activity towards STATc (Araki *et al.*, 2008). Cbl proteins down-regulate metazoan signalling pathways by ubiquitylating receptor tyrosine kinases, thereby targeting them for degradation. In a *cblA*- strain early development occurs normally but migrating *cblA*- slugs frequently fragment and the basal disc of the culminants that are formed are absent or much reduced. These are characteristic features of mutants in signalling by DIF. Tyrosine phosphorylation of STATc is induced by DIF but in the *cblA*- strain this response is attenuated relative to parental cells. Interestingly, stress activation is normal in the *cblA*- strain; so the two pathways can be genetically uncoupled. CblA fulfils this function, as a positive regulator of DIF induced STATc tyrosine phosphorylation, by constitutively down-regulating PTP3: the protein tyrosine phosphatase responsible for de-phosphorylating STATc. We identify two serine residues in PTP3, S448 and S747, that rapidly increase in phosphorylation level after DIF and stress treatments and we use phospho-specific antibodies against them to characterise the stress response. We show that 14-3-3 protein binds to PTP3 and that binding increases after stress treatment. Inactivation of the S448 site greatly decreases binding while mutation of S747 causes constitutive 14-3-3 binding. We do not yet know the significance of the binding but it seems not to relate to the known stress induced translocation of PTP3 into endosomal vesicles (Gamper *et al.*, 1999). cGMP is a candidate second messenger for *Dictyostelium* hyper-osmotic stress responses and 8-bromo cGMP, a membrane permeant form of cGMP, is a known activator of STATc. Genetic ablation of GbpC, the sole *Dictyostelium* cGMP binding protein and a founding member of the ROCO protein family, prevents 8-bromo cGMP-induced STATc activation. However, osmotic stress-induced activation of STATc is normal in the *gbpC*- strain. Moreover, in the parental strain, 8-bromo cGMP does not stimulate phosphorylation of S448 and S747 of PTP3. These facts suggest parallel stress activation pathways. We present evidence that intracellular calcium may be the parallel second messenger by showing that agents such as BHQ (2,5-Di-*t*-butyl-1,4-benzohydroquinone) that elevate intracellular calcium levels are STATc activators and that BHQ stimulates phosphorylation of S448 and S747 of PTP3. We therefore propose that cGMP may exert its stimulatory effect on STATc by activating the STATc tyrosine kinase while, in parallel, calcium signalling represses STATc dephosphorylation via its inhibitory effect on PTP3.

- Araki *et al.* (2008) *Development* 135: 1347-53.
- Gamper *et al.* (1999) *J. Biol. Chem.* 274: 12129-38.

DimB and MybE: the odd couple?

Yoko Yamada¹, Tsuyoshi Araki¹, Natasha Zhukovskaya¹, Susan Ross¹, Rob Kay², Gareth Bloomfield², Al Ivens³, Jeff Williams¹

¹College of Life Sciences, University of Dundee, ²MRC laboratory of Molecular Biology, Cambridge,

³Wellcome Trust Sanger Institute

DimB is a bZIP transcription factor that accumulates in nuclei rapidly after cells are exposed to DIF. We present pharmacological evidence to show that this occurs via a signalling pathway distinct from that which activates STATc (see abstract by Araki et al.). In order to understand how DIF regulates DimB we studied its phosphorylation, initially using 2D gels and then by mass spectrometry. Thus far we have identified a site near the C terminus that shows an increase in phosphorylation after cells are treated with DIF. We raised an antibody that is specific for this phosphorylated site and used the antibody to confirm that the site is inducibly modified. Mutation of the site does not reduce DIF induced nuclear accumulation, perhaps indicating a redundancy of function with other regulated sites, but the antibody is a valuable tool for tracing the DIF signalling pathway. Previously described CHIP analyses showed that, after DIF induction, DimB binds to the promoter of the *ecmA* gene. In vitro mapping experiments identified two DimB binding sites within the *ecmA* promoter and also two binding sites for MybE: a single Myb domain transcription factor. In monolayer assay both the DimB null and the MybE null are insensitive to DIF induction. At the slug stage the *mybE* null shows no expression of *ecmA*AO:*lacZ* (i. e. *lacZ* under the control of the entire *ecmA* promoter) in *pstO* and ALC. However, the situation for DimB becomes more complex when we consider normal development; the genetic evidence suggests that in Ax2 cells DimB is a repressor of *ecmA* transcription rather than an activator. We have not yet resolved this apparent paradox for *ecmA* but, rather than draw conclusions based on just one promoter, we performed genome wide micro-array analysis on parental, *mybE*- and *dimB*- cells that were either DIF induced or left untreated. This identified 46 genes that depend on both MybE and DimB for their inducibility but 77 genes were only detectably DIF induced in *mybE*- cells, 5 genes were only induced in *dimB*- cells and 6 genes were inducible in both the *dimB*- and *mybE*- cells. This indicates that, even in the monolayer assay where both MybE and DimB function as apparent activators for *ecmA*, many genes fall under the negative control of the two transcription factors. We are currently characterising some of these genes further, to broaden our picture of DIF regulated gene expression.

3'-phosphoinositides regulate coordination between speed and directional accuracy during chemotaxis

Scott Gruver¹, John Wikswa², Chang Chung¹

¹Department of Pharmacology, Vanderbilt University, ²Department of Physics and Biomedical Engineering, Vanderbilt University

The PI3K/PTEN pathway, as the regulator of 3'-phosphoinositide (3'-PI) dynamics, has emerged as a key regulator of chemoattractant gradient sensing during chemotaxis in *Dictyostelium* and other cell types. Previous results have shown 3'-PIs to be important for regulating basal cell motility and sensing the direction and strength of the chemoattractant gradient. We examined the chemotaxis of wild-type cells and cells lacking PTEN or PI3K1 and 2 using analytical methods that allow us to quantitatively discern differences between the genotypes ability to sense and efficiently respond to changes in gradient steepness during chemotaxis. We find that cells are capable of increasing their chemotactic accuracy and speed as they approach a micropipette in a manner that is dependent on the increasing strength of the concentration gradient and 3'-PI signaling. Further, our data shows that 3'-PI signaling affects a cell's ability to coordinate speed and direction in order to increase chemotactic efficiency. Using a new measurement of chemotactic efficiency that reveals the degree of coordination between speed and accuracy, we find that cells also have the capacity to increase their chemotactic efficiency as they approach the micropipette. Like directional accuracy and speed, the increase in chemotactic efficiency of cells with increased gradient strength is sensitive to 3'-PI dysregulation. Our evidence suggests that receptor-driven 3'-PI signaling regulates the ability of the cell to capitalize on stronger directional inputs as well as minimize the effects of inaccurate turns in order to increase chemotactic efficiency.

PIP3-independent activation of TorC2-PKB at the cell's leading edge mediated chemotaxis.

Yoichiro Kamimura, Peter N Devreotes

Johns Hopkins University, School of Medicine, Department of Cell Biology

Chemoattractants trigger multiple signal transduction pathways that lead to cytoskeletal rearrangements and directional motility. Although PIP3 is well characterized as a second messenger, chemotaxis can still occur in the absence of PIP3 and the identities of the PIP3 independent pathways remain unknown. Here we show that Tor complex 2 (TorC2)-PKBR1 controls PIP3 independent chemotaxis. In *Dictyostelium* cells two PKB (Protein Kinase B) homologs, PKBA and PKBR1, are present. Membrane localization of PKBA is regulated via PIP3 dependent PH domain, while PKBR1 is anchored through post translational myristoylation. Within seconds of stimulating cells, the two PKB homologs mediate transient phosphorylation of at least eight proteins, including Talin B, PI4P 5-kinase, two RasGefs, and a RhoGap. PKBR1 is the major PKB activity and PKBA provides a minor redundant activity in vivo. Surprisingly, all of the substrates are phosphorylated with normal kinetics in cells lacking PI 3-kinase activity. Cells deficient in TorC2 or PKBR1 activity show reduced phosphorylation of the endogenous substrates and are impaired in chemotaxis. PKBR1 is activated through phosphorylation of the hydrophobic motif (T470) via TorC2 and subsequent phosphorylation of the activation loop (T309). The immunofluorescent staining of chemotactic cells by phospho-specific antibodies against the hydrophobic motif and the activation loop show that these chemoattractant-inducible phosphorylation events are restricted to the cell's leading edge. Activation of TorC2 depends on heterotrimeric G-protein function and intermediate G-proteins, including Ras GTPases. The data lead to a model where cytosolic TorC2, encountering locally activated small G-protein(s) at the leading of the cell, becomes activated and phosphorylates PKBs. These in turn phosphorylate a series of signaling and cytoskeletal proteins, thereby regulating directed migration.

Chromatin re-modelling and the regulation of chemotaxis

Ben Rogers¹, Annette Mueller-Taubenberger², Adrian Harwood¹

¹School of Biosciences, Cardiff University, UK, ²Institute for Cell Biology / ABI, Ludwig Maximilians University Munich, Germany

Homologues of the four classes of ATP-dependent chromatin remodelling factors are present in *Dictyostelium*. Here we describe the role of two distinct factors during chemotaxis.

LisG was isolated as a lithium resistant mutant and encodes a homologue of the human CHD protein CHD8. In *Dictyostelium*, LisG is a component of a novel signal pathway, which requires the prolyl oligopeptidase gene, *dpoA* and is altered by changes in IP₅. Mutants of *lisG* cause up-regulation of the inositol synthase (*ino1*) gene, which then leads to lithium resistance. We have generated two alleles of *lisG*, one lacking the first 90 amino acids of the N-terminus, the other is a complete null mutation. These alleles confer different chemotactic phenotypes. Cells possessing the N-terminal deletion of LisG (deltaNlisG) move faster than wild type cells. This confers lithium resistance because although lithium suppresses cell speed, it remains higher than wild type cells under equivalent conditions. In contrast, *lisG* null mutant cells exhibit chemotaxis defects, but remarkably these are reversed by lithium. These observations suggest that LisG is a component of a gene regulatory network mediating changes in cell chemotaxis.

Arp8 is an actin-related protein, which is required for function of the Ino80 protein complex. Named after the ATP-dependent helicase Ino80 of yeast, this complex is required for expression of the *ino1* gene. We show that *ino1* gene expression in *Dictyostelium* is dependent on Arp8. Furthermore, we have found that *arp8* null mutants have slow aggregation and exhibit a delay to chemotaxis when plated in a cAMP gradient. This delay is overcome by lithium treatment and is persistent in lithium resistant mutants.

We will discuss how changes in gene expression via altered chromatin remodelling may alter cAMP-mediated chemotaxis in *Dictyostelium*.

The role of inositol synthesis in chemotaxis

Regina Teo, Kimberley Lewis, W Jonathan Ryves, Adrian J Harwood
Cardiff School of Biosciences

It seems a remarkable fact that the three families of lithium sensitive enzymes all have the potential to regulate myo-inositol within the cell. This raises the question of whether the cellular effects of lithium converge on myo-inositol synthesis.

The first lithium targets to be discovered belonged to a family of monophosphatases, which include Inositol MonoPhosphatase (IMPase) and Inositol PolyPhosphatase (IPP). These enzymes are key in recycling and synthesis of myo-inositol, and lithium treatment causes inositol depletion. Here we show that by inhibiting IMPase, lithium treatment reduces levels of PIP3 leading to chemotaxis phenotypes that match those seen for a complete PI-3-kinase null mutant (*pi3k1-5*) and in wild type cells treated with the PI3k inhibitor, LY294002. Consistent with this action, over-expression of IMPase reverses the effects of lithium and elevates PIP3 signalling.

The second lithium target is the multifunctional serine/threonine protein kinase Glycogen Synthase Kinase 3 (GSK-3). As suggested by its name, among its many targets is glycogen synthase, which controls the rate of glucose conversion to glycogen. Inhibition or loss of GSK-3 would be expected to reduce the concentration of glucose-6-phosphate, the substrate of inositol synthase. We therefore predict that loss of GSK-3 would have an indirect effect on the levels of inositol available to the cell. Indeed we find that loss of the *Dictyostelium* homologue of GSK-3, *gskA*, causes a severe chemotactic defect. Consistent with our hypothesis over-expression of IMPase can reverse this aspect of the *gskA* null phenotype.

The third lithium target is PhosphoGlucoMutase (*pgm*), which catalyses both the forward and reverse conversion of glucose-1-phosphate to glucose-6-phosphate. Again it has the potential to regulate the cellular concentration of inositol. Over-expression of *pgm* in *gskA* null cells rescues its chemotactic effects.

Our current results indicate that inositol depletion leads to defective chemotaxis, and many of the effects of lithium treatment can be reversed by over-expression of the relevant genes that are involved in the phosphoinositol synthetic pathway.

Correlated waves of actin filaments and PIP₃ in *Dictyostelium* cells.

Yukako Asano, Akira Nagasaki, Taro Q.P. Uyeda
National Institute of Advanced Industrial Science and Technology (AIST)

Chemotaxis-deficient *amiB*-null mutant *Dictyostelium* cells show two distinct movements: 1) they extend protrusions randomly without net displacements; 2) they migrate persistently and unidirectionally in a keratocyte-like manner. Here, we monitored the intracellular distribution of phosphatidylinositol (3,4,5)-trisphosphate (PIP₃) to gain insight into roles PIP₃ plays in those spontaneous motilities. In keratocyte-like cells, PIP₃ showed convex distribution over the basal membrane, with no anterior enrichment. In stalled cells, as well as in wild type cells, PIP₃ repeated wave-like changes, including emergence, expansion and disappearance, on the basal membrane. The waves induced lamellipodia when they approached the cell edge, and the advancing speed of the waves was comparable to the migration speed of the keratocyte-like cells. LY294002 abolished PIP₃ waves in stalled cells and stopped keratocyte-like cells. These results together suggested that keratocyte-like cells are “surfing” on the PIP₃ waves by coupling steady lamellipodial protrusions to the PIP₃ waves. Simultaneous live observation of actin filaments and PIP₃ in wild type or stalled *amiB*-null cells indicated that the PIP₃ waves were correlated with wave-like distributions of actin filaments. In latrunculin-treated, rounded *amiB*-null cells, PIP₃-rich semicircles and actin-rich peripheral segments rotated synchronously. In those cases, PIP₃ waves often followed actin waves, suggesting that PIP₃ induces local depolymerization of actin filaments. Consistent with this idea, cortical accumulation of PIP₃ was often correlated with local retraction of the periphery. We propose that the waves of PIP₃ and actin filaments are loosely coupled with each other and play important roles in generating spontaneous cell polarity.

Does arginylation matter for actin-dependent activities in *Dictyostelium*?

Annette Müller-Taubenberger, Petros Batsios, Michael Schleicher
Institute for Cell Biology, Ludwig Maximilians University Munich, Schillerstr. 42, 80336 München, Germany

Arginylation is a posttranslational modification that is only poorly understood at its molecular level. Recently, arginylation of β -actin was described to regulate actin filament properties, β -actin localization, and lamella formation in motile cells (Karakozova et al., 2006, Science 313, 192-196). The highly conserved enzyme Arg-transfer RNA protein transferase (Ate1) mediates arginylation. In order to test whether arginylation plays a role for actin-dependent processes in *Dictyostelium discoideum* we knocked out the gene encoding Ate1 and investigated the phenotype of *ate1*-null cells. Similar to mouse fibroblasts deficient for *ate1* the *Dictyostelium ate1*-null cells are smaller in size compared to wild type. Random motility and chemotaxis were not significantly altered in *ate1*-null cells. However, visualization of actin cytoskeleton dynamics by live cell microscopy using GFP-Lim(delta), a marker for filamentous actin, indicated significant changes in comparison to wild-type cells. *Ate1*-null cells lack almost completely the actin patches observed at the substrate-attached surface of wild-type cells and are only weakly adhesive. We currently test whether actin itself is arginylated in *Dictyostelium* and whether the differences observed in the organization of filamentous actin structures are directly or indirectly linked to arginylation.

Modulation of actin structure and function by phosphorylation of Tyr-53 and profilin binding

Kyuwon Baek¹, Xiong Liu², François Ferron¹, Shi Shu², Edward D. Korn², Roberto Dominguez¹

¹Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA, ²Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA

KB and XL contributed equally to this work

On starvation, *Dictyostelium* cells aggregate to form multicellular fruiting bodies containing spores that germinate when transferred to nutrient-rich medium. This developmental cycle correlates with the extent of actin phosphorylation at Tyr-53 (pY53-actin), which is low in vegetative cells but high in viable mature spores. We established a procedure to isolate pY53 actin from *Dictyostelium* and characterized its properties (Liu et al. (2006) PNAS, 103: 13694-13699). To extend this study, we obtained high-resolution crystal structures of both pY53-actin and unphosphorylated actin complexed with gelsolin segment 1 and unphosphorylated actin complexed with profilin. In the structure of pY53-actin, the phosphate group on Tyr-53 makes hydrogen-bonding interactions with residues of the DNase I-binding loop (D-loop) of actin, resulting in a more stable conformation of the D-loop than in the unphosphorylated structures. A more rigidly folded D-loop may explain some of the previously described properties of pY53-actin, including its increased critical concentration for polymerization, reduced rates of nucleation and pointed end elongation, and weak affinity for DNase I. We show now that phosphorylation of Tyr-53 inhibits subtilisin cleavage of the D-loop and reduces the rate of nucleotide exchange on actin. The structure of profilin-*Dictyostelium*-actin is strikingly similar to previously determined structures of profilin- β -actin and profilin- α -actin. By comparing this representative set of profilin-actin structures with other structures of actin we highlight the effects of profilin on the actin conformation. In the profilin-actin complexes, subdomains 1 and 3 of actin close around profilin, producing a 4.7° rotation of the two major domains of actin relative to each other. As a result, the nucleotide cleft becomes moderately more open in the profilin-actin complex, probably explaining the stimulation of nucleotide exchange on actin by profilin.

PaxB and PldB work to regulate actin-based processes

M. Berenice Duran, Asif Rahman, Max Colten, Derrick Brazil
Hunter College, Department of Biological Sciences, Center for the Study of Gene Structure and Function

The proper integration of the actin cytoskeleton with adhesion is essential to numerous cellular processes including proliferation, differentiation and migration. Such regulation is critical for the development of *Dictyostelium discoideum*, which depends upon cell-cell adhesion, cell motility and cellular differentiation. To better understand this process, we have been studying the role of two proteins, PaxB and PldB in the actin-dependent processes of motility, chemotaxis, pinocytosis and exocytosis.

It has been shown that PaxB, like its mammalian ortholog paxillin, is integral in controlling cell-cell and cell substrate adhesion. This regulation may be through controlling the actin cytoskeleton, as cells overproducing PaxB display misregulated actin. These cells also display defects in random motility, chemotaxis towards folate and chemotaxis towards cAMP. PaxB most likely has an active role in these activities, as PaxB is localized to the pseudopods during chemotaxis. Interestingly, overproduction of PaxB also inhibits pinocytosis and exocytosis, although a direct link to these processes has not been shown. PldB, the ortholog to phospholipase D, has also been shown to regulate the actin cytoskeleton and random motility, much like PaxB. We find that, like PaxB, overproduction of PldB disrupts chemotaxis. As was seen with PaxB, PldB appears to have an active role in this regulation as PldB is localized to pseudopods during chemotaxis.

Taken together, our data suggest that PaxB and PldB are integrally involved in controlling motility and chemotaxis. This is most likely through the regulation of the actin cytoskeleton. The defects we observed in pinocytosis and exocytosis are most likely indirect effects caused by misregulation of the actin cytoskeleton.

Regulation of Differential Cell Adhesion by Rap1 is Required for Cell Type Patterning and Morphogenesis in *Dictyostelium*

Katie Parkinson¹, Parvin Bolourani², David Traynor³, Nicola L. Aldren¹, Robert R. Kay³, Gerald Weeks², Christopher R. L. Thompson¹

¹Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, ²Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, ³MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH

Regulated cell adhesion and motility play important roles in pattern formation during development. Consequently great efforts have been made to identify genes that control these processes. One candidate is Rap1, as it has been implicated in the regulation of adhesion and motility in cell culture. In order to further study the role of Rap1 during multicellular development, we have generated a mutant in a potential Rap1 GTPase activating protein (RapGAPB) in *Dictyostelium*. rapGAPB- cells have increased levels of active Rap1 compared to wild type cells when stimulated with cAMP, indicating that RapGAPB regulates Rap1 activity. Furthermore, rapGAPB- cells exhibit hallmark phenotypes of other known mutants with hyperactivated Rap1, including increased substrate adhesion and abnormal F-actin distribution. We also found that RapGAPB is expressed at highest levels in prestalk cells and is required for normal development. Firstly, streams of aggregating rapGAPB- cells break up as a result of decreased cell-cell adhesion. Secondly, rapGAPB- cells exhibit cell autonomous defects in prestalk cell patterning. Finally, using cell type specific markers, we demonstrate that this is because RapGAPB is required for the correct sorting behaviour and adhesive properties of the different cell types. To further understand Rap1 regulated adhesion, we performed a suppressor screen in order to identify genes that interact with RapGAPB. Disruption of a number of novel genes with a highly similar structure to that of classic adhesion molecules rescued the rapGAPB- phenotype. Studying these genes will enable further understanding of Rap1 regulated differential cell adhesion.

Analysis of dedifferentiation pathway in *Dictyostelium discoideum*

Mariko Katoh, Gad Shaulsky

Department of Molecular and Human Genetics, Baylor College of Medicine

In the soil amoeba *Dictyostelium discoideum*, dedifferentiation can be induced by mechanical disaggregation of developing multicellular structures and subsequent exposure to a fresh nutritional source. Developing cells at almost any stage have the potential to dedifferentiate and resume growth. Our previous studies have shown that dedifferentiation is regulated by a dedicated genetic program. We therefore decided to screen for mutants that have defects in the dedifferentiation process. Disaggregated slug cells, in the absence of nutrients, can re-aggregate rapidly and progress to the culmination stage within a short period while 'skipping' the first 10 hrs of development. The property of rapid re-aggregation is lost after 12-16 hours of incubation in the presence of nutrients (erasure). We rationalized that the timing of erasure would be altered if mutations occurred in key regulatory dedifferentiation genes and screened for mutations that attenuate ("persistent") or accelerate ("forgetful") dedifferentiation. Persistent mutants should retain the capacity for rapid re-development even after 12-hr incubation for erasure so they should form spores rapidly for short re-development time. Forgetful mutants, after short erasure incubation, should form spores later than wild type cells in re-developmental progression. We enriched for these mutants by successive rounds of development and dedifferentiation until defective dedifferentiation mutants took over the population. Interestingly, we isolated two different alleles of the ABC transporter gene *tagA*, one in the persistent and the other in the forgetful mutant screens. We will describe the characterization of several *tagA* alleles. We are also testing whether the PKA pathway is directly involved in regulating dedifferentiation.

A steroid signal regulates sensitivity to GABA during culmination

Christophe Anjard, William F. Loomis

Cell and Developmental Biology, UCSD, La Jolla CA 92093, USA

cAMP levels increase sharply during culmination resulting in high levels of PKA activity that trigger rapid encapsulation. Activation of the adenylyl cyclase AcrA by cytokinins acting through DhkB as well as inhibition of the phosphodiesterase RegA by SDF-2 acting through DhkA works as a coincidence detector to finely control PKA activity. SDF-2 is a 34 amino acid peptide that is cleaved from its precursor acyl CoA binding protein (AcbA) by the surface protease TagC. For AcbA to be released from prespore cells it has to follow an unconventional pathway into vesicles that are secreted in response to GABA acting through the GPCR GrIE. Extracellular glutamate is a competitive inhibitor of GABA induction of AcbA secretion. Since it has long been known that extracellular glutamate accumulates to high levels ($> 1\text{mM}$) throughout late development, it was unclear how GABA might be effective.

We have found that a small, heat stable, protease resistant, hydrophobic signal, SDF-3, is released during culmination and acts through the GPCR, GrlA, coupled to a hetero-trimeric G protein containing Ga4, to overcome glutamate inhibition and induce sporulation by facilitating GABA signaling. Drugs targeting steroid biosynthesis block production of SDF-3. Mutant strains lacking either GrlA or Ga4 fail to accumulate SDF-2 in culminants and sporulate poorly. Cells of these mutant strains do not respond to SDF-3 or hydrocortisone. While grlA- null cells cannot synergize with cells lacking the GABA biosynthetic enzyme GadA, they synergize well with cells lacking GrIE which can produce GABA but cannot respond to it. Synergy was determined by accumulation of SDF-2.

Cells treated with purified SDF-3 or 10 nM hydrocortisone respond to 10 nM GABA by rapid sporulation even in the presence of 10 mM glutamate. It appears that extracellular GABA increases and glutamate inhibition decreases in response to SDF-3. We have found that while glutamate acts through GrIE when it is coupled to trimeric G protein containing Ga9, GABA acts through GrIE when it is coupled to trimeric G protein containing Ga7. Perhaps activation of the GrlA pathway by steroid binding shifts the equilibrium toward Ga7 such that glutamate is no longer inhibitory and GrIE can fully respond to GABA.

Vacuole formation during stalk cell differentiation

Toru Uchikawa, Kei Inouye

Department of Botany, Graduate School of Science, Kyoto University

Vacuoles in fungi and plants are known to play various roles in the cellular system such as storing materials, degrading waste matters, controlling the osmotic pressure, and maintaining the turgor pressure. In the cellular slime mold, stalk-cell vacuoles are formed in a short time during the fruiting body formation, but their origin and functions are not well understood. We are studying the mechanism of stalk-cell vacuole formation in monolayer culture of prestalk cells dissociated from slugs in the presence of 8-Br-cAMP and in normal development using time-lapse video microscopy.

Basic dyes such as neutral red are well-known markers of the “autophagic vacuoles” (autophagolysosomes), which is thought to be a precursor of the stalk-cell vacuole. In monolayer culture of prestalk cells stained with neutral red or LysoTracker, we confirmed that vital-dye-positive vesicles fuse together to become large vacuoles. To investigate the dynamic behavior of vacuole membranes, we searched for marker molecules localized specifically on vacuole membranes, and Ammonium transporter A (AmtA) attracted our attention. AmtA had been shown to be expressed primarily in prestalk cells at developmental stages and has a function to regulate the differentiation of stalk cells and spores by activating the DhkC phosphorelay which in turn inhibits PKA activity (1,2). We found that in stalk cells the AmtA-GFP fusion protein was localized predominantly on the membrane of stalk-cell vacuoles. At the vegetative and early developmental stages, AmtA-GFP was localized mainly on lysosomal membranes and also detected on several kinds of membranes such as endoplasmic reticulum, endosomes, nuclear envelope and mitochondrial membranes, but not on the cell membrane and contractile vacuoles. Time-lapse microscopic analysis revealed predominant localization of AmtA-GFP on autophagolysosomes and repeated fusion of these structures to form vacuoles in prestalk cells. On the other hand, contractile vacuoles, which are rich in H⁺-ATPase as in plant vacuoles, remained separate from autophagolysosomes and stalk-cell vacuoles. We concluded that stalk-cell vacuoles mainly originate from autophagolysosomes.

We also developed a method to observe the behavior of individual living cells within intact fruiting bodies. With this technique, we investigated where stalk-cell vacuoles are formed in the tissue and how they develop. Based on these observations, possible mechanisms for the formation of stalk-cell vacuoles and their functions will be discussed.

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2. Singleton et al. (2006). *Eukaryotic Cell*, 991-996.

Developmental changes in N-glycan fucosylation in *Dictyostelium*

Birgit Schiller¹, Josef Voglmeir¹, Alba Hykollari¹, Gerald Pörtl¹, Karin Hummel², Rudolf Geyer³,
Iain B.H. Wilson¹

¹Departement für Chemie, Universität für Bodenkultur, A-1190 Wien, Austria, ²Vetomics,
Veterinärmedizinische Universität, A-1220 Wien, Austria, ³Institut für Biochemie,
Justus-Liebig-Universität, D35292 Giessen, Germany

Although correlations between the properties of its protein-bound N-glycans and developmental differentiation in *Dictyostelium discoideum* have been reported some twenty or more years ago, the exact structures of slime mould N-glycans and the nature of the attached fucose residues have not been determined. It has been suggested that core α 1,3 fucose epitopes decrease during development, while addition of “peripheral fucose” is supposed to increase. Furthermore genome analysis revealed the presence of an α 1,6-fucosyltransferase gene, whilst products of this glycosyltransferase have not been detected until now. N-glycan preparations as well as crude lysates were therefore prepared of axenically grown AX3 as well as early tipped cell mounds, late mounds and whole fruiting bodies. These samples were subjected to Western blotting using an antibody specific for α 1,3-fucose (anti-HRP). Results indicated the presence of this epitope throughout development, suggesting an increase in this type of glycosylation. This data though was contradicted by RP-HPLC profiles of the fluorescently labeled glycans and their subsequent mass-spectrometry analysis. We thus postulate that the amount of core α 1,3-fucosylated glycans does not increase during development, and believe the lower binding of antibody to lysates from midlog-phase cells to be due to the highly unusual structure of the major glycan assembled during growth. This novel glycan contains both inter- and bisecting N-acetylglucosamine residues as well as the anti-HRP reactive core α 1,3-fucose. Data from glycan digestions and from GC-MS analysis of a fucosyltransferase product further confirm the nature of the α 1,3-linkage. The amount of this large oligomannosidic glycan decreases as the cells differentiate while smaller fucosylated glycans appear, some of them also carrying inter- and bisecting N-acetylglucosamines. We further detect by reversed-phase HPLC a relative increase in a late-eluting peak (11 glucose units), indicative for α 1,6-fucose. Treatment of the purified peak with α 1,6-fucosidase results in appearance of the non-fucosylated form, showing that this novel structure is not merely a degradation product of the large glycan assembled during growth. Developmental and cohesion defects in *D. discoideum* have been associated with alterations in the glycosylation pattern. It is also known that especially fucosylated structures are associated with numerous human diseases. Studying the alteration of glycans carrying fucose epitopes during the development of single *D. discoideum* cells to multicellular fruiting bodies with distinct cell types will help to shed light on mechanisms such as cell-recognition and adhesion.

Rapid evolution in the lag gene family reveals a role in self-recognition

Rocio Benabentos¹, Shigenori Hirose¹, Richard Suggang¹, Tomaz Curk², W. Justin Cordill¹, Mariko Katoh¹, Yue Wang¹, Elizabeth Ostrowski³, Joan Strassmann³, David Queller³, Blaz Zupan^{1,2}, Gad Shaulsky^{1,3}, Adam Kuspa^{1,3}

¹Baylor College of Medicine, Houston, TX, ²University of Ljubljana, Ljubljana, Slovenia, ³Rice University, Houston, TX

The *D. discoideum* gene lagC encodes the cell-cell adhesion glycoprotein gp150, which is essential for development. Exhaustive searching of the genome revealed 55 paralogous genes that appear to have arisen from a series of gene duplications and subsequent sequence divergence. Some of the lag-genes are arranged in clusters, in which sequence divergence correlates with map distance, consistent with evidence of recent duplication events and the potential for common function and transcriptional regulation. Genes like lagC, which encode plasma membrane proteins with multiple extracellular immunoglobulin repeats, participate in self-recognition in other organisms and their sequences are highly polymorphic within natural populations. We compared data from massively parallel sequencing of *D. discoideum* two wild isolates and from the published reference sequence and found substantial sequence polymorphism in several members of the lag-gene family, including lagC (which we have renamed lagC1). We also found that the highly polymorphic lag-genes are arranged in pairs and their transcription is coordinately regulated. We sequenced these polymorphic genes in several wild isolates of *D. discoideum* and found evidence for positive selection, further implicating the genes as components of a self/nonself-recognition mechanism. Mutation analysis and gene replacement experiments support the idea that lagC1, and its tandem gene lagB1, are indeed involved in self-recognition. In addition, computational analysis shows a high correlation between the lagB1 and lagC1 sequences and the ability of wild strains to segregate from one another.

The *Dictyostelium* mating type locus

Gareth Bloomfield^{1,2}, Jason Skelton², Alasdair Ivens², Yoshimasa Tanaka¹, Robert R. Kay¹
¹MRC Laboratory of Molecular Biology, Cambridge, UK, ²Wellcome Trust Sanger Institute, Hinxton, UK

Wild isolates of *Dictyostelium discoideum* typically fall into two complementary mating types. Under certain conditions, when cells of each mating type meet they initiate the sexual cycle of the social amoebae, which leads to the formation of macrocysts. Germination of at least some macrocysts appears to be preceded by meiosis, during which recombination between the parental chromosome occurs at high frequency. The haploid progeny, when cloned, are of one or other mating type, implying that this phenotype is determined by a single locus. We have identified a locus that distinguishes between the two types and is required for macrocyst production, thus possessing the properties expected of the mating type locus. Using microarray-based comparative genomic hybridisation to identify sequences present in strains of mating type I (such as NC4) but not in mating type II (exemplified by V12), we found a single gene present in all type I but in none of the type II strains examined. Deletion of this gene, *matA*, renders the mutant cells unable to form macrocysts. Moreover, its deletion removes a block to growth of parasexual diploids formed in crosses with strains of the opposite mating type. The same locus in type II strains contains 3 genes, of which one, *matC*, is homologous to *matA*. The other genes, *matB* and *matD*, have little similarity to sequences in the public databases; notably though *matD* has a predicted signal peptide and GPI-anchor. Most interestingly, in two other strains the locus contains homologues of *matB* and *matD*, but neither *matA* nor *matC*: these are the self-fertile isolate AC4 and WS2162 which mates with both type I and type II strains but not with itself. These findings hint at how mating behaviour has evolved as well as how it might be regulated at the molecular level.

Involvement of the PKC activity in translocation of the ZYG1 protein into the cell cortex during *Dictyostelium* development

Aiko Amagai¹, Shinya Urano², Harry MacWilliams³, Kazuo Yamamoto¹, Yasuo Maeda²

¹Department of Biomolecular Science, Graduate School of Life Sciences, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan, ²Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan, ³Zoologisches Institute Munchen, Ludwig-Maximilians-Universitat Munchen, Luisenstr., 14, 80333 Munchen, Germany

We have previously demonstrated that a potent plant hormone, ethylene induces sexual development including zygote formation through enforced expression of a novel gene, *zyg1*, in *Dictyostelium* cells (Amagai *et al.*, 2007). After the BLAST search, ZYG1, the product of *zyg1* gene, is predicted to have several phosphorylation sites by PKC. The number of zygotes formed was found to increase by a PKC activator (TPA: phorbol ester) and markedly inhibited by a PKC inhibitor (staurosporin). This raised the possibility that phosphorylation of ZYG1 by PKC might be involved in zygote formation of *D. mucoroides-7* (Dm7) cells. *D. discoideum* Ax2 cells also form multinucleate cells by their fusion and engulf surrounding cells by phagocytosis under certain conditions. As these events are markedly influenced by TPA and staurosporine, they might be caused by the same mechanism operating on zygote formation of Dm7 cells. The activated PKC is generally known to be located in the cell membrane. Therefore, it is quite possible that ZYG1 also may be localized in the cell membrane. To test this possibility, we introduced two types of fusion genes (*gfp-zyg1* and *zyg1-gfp*) into Ax2 cells and analyzed behaviors of the fusion proteins (GFP-ZYG1 and ZYG1-GFP), using an UV microscope and a laser scanning confocal microscope. As a result, GFP-ZYG1 and ZYG1-GFP were found to be translocated to the cell cortex of multinucleate cells. They also translocated close to the regions of cell-to-cell contacts and phagocytotic cups. Importantly, such translocation of ZYG1 protein into the cell cortex was greatly affected by the PKC activity: Coupled with inhibition of the cell fusion and cell engulfment by the staurosporine, the translocation of ZYG1 was almost completely blocked. Taken together these results suggest that the phagocytotic process as well as the cell fusion for zygote formation may be caused through the signaling pathway in which the phosphorylation of ZYG1 by PKC is involved.

Elucidating a role for the *Dictyostelium discoideum* SadA protein in cell-substrate adhesion:
A Role for the Tail

Anthony S. Kowal, Rex L. Chisholm

Center for Genetic Medicine and Department of Cell and Molecular Biology, Northwestern
University, Chicago, IL USA

In a genetic screen for proteins involved in cell-substrate adhesion, our lab identified the SadA (Substrate Adhesion Deficient A) gene. SadA is a putative 9-pass transmembrane protein containing three EGF-like repeats in its first extracellular domain that is localized to the plasma membrane in SadA-GFP expressing cells. sadA- cells are unable to adhere to a surface, exhibit a disrupted actin cytoskeleton and a cytokinesis defect. The mutant cells are also unable to phagocytose latex beads. We hypothesized that the carboxy terminal tail of SadA plays an important role in its function. Consistent with this hypothesis, overexpression of the SadA-Tail in wild-type cells results in decreased adhesion, and a SadA tail deletion construct is unable to rescue the adhesion defect. Mutation analysis of the seven serine residues in the SadA tail suggests that two sites S924 and S925 are important for SadA function. Whereas SadA S924AS925A is capable of wild-type levels of adhesion, SadA S924ES925E shows decreased adhesion. Intriguingly, GST pull-down assays showed different profiles of interaction partners with wild-type tail, SadA S924AS925A and S924ES925E fusion proteins. We are in the process of verifying two candidate proteins, ABP50 and Cortexillin A, found in the pull-down assays, as putative binding partners of the SadA tail. Our data suggest that these two serine residues might be potential sites for regulating protein:protein interactions important for cell adhesion.

Myosin Heavy Chain Kinase C Mediates Cell Adhesion via a Myosin II-Independent Mechanism

Alyssa Chowdhury, Atiya Franklin, Linzi Hyatt, Paul Steimle

University of North Carolina at Greensboro Department of Biology Greensboro, NC 27402

Numerous studies in *Dictyostelium* have demonstrated that myosin II plays a central role in cell adhesion, whereby myosin II recruitment and activation at the cell cortex facilitates localized detachment of the cell from substrate and myosin II inactivation stabilizes adhesion to substrate. It has been reported recently that cell adhesion is controlled, in part, by cellular signals mediated by Rap1 and its effector, Phg2. Activation of this pathway leads to increased cell adhesion by inducing myosin II heavy chain (MHC) phosphorylation and disassembly of myosin II filaments (Jeon et al. 2007 *Journal of Cell Biology* 176:1021-1033). Myosin II filament disassembly in *Dictyostelium* is driven by phosphorylation of the MHC “tail” region via the activities of three structurally-related MHC kinases: MHCK-A, -B, or -C. All three MHCKs share homologous catalytic and WD-repeat domains.

In the studies presented here we examined the roles that the individual MHCKs play in regulating myosin II function in the context of cell adhesion. Assays in which knockout cell lines lacking each of the MHCKs were subjected to increasing rotational speeds (up to 200 rpm) revealed that cells lacking MHCK-C exhibit significantly reduced adhesion to substrate when compared with cells lacking either MHCK-A or -B. Complementary assays demonstrated that cells over-expressing MHCK-C exhibit extremely stable adhesion to substrate, with nearly 90% of these cells remaining attached at 200 rpm. By comparison, far fewer cells over-expressing MHCK-A (55%) or B (85%) remained attached at 200 rpm. Unexpectedly, we found that the elevated level of adhesion exhibited by cells over-expressing MHCK-C is myosin II independent since co-expression of a myosin II with a nonphosphorylatable heavy chain (3xALA/MHCK-C cells) does not restore normal levels of adhesion, nor does it lead to destabilization of adhesion in response to sodium azide treatment (25 μ M), even though these cells indeed exhibit the myosin II-dependent “cringe” response when exposed azide.

We further show biochemically that a truncation of MHCK-C lacking the N-terminal WD-repeat domain (MHCK-C- Δ -WD) is unable to phosphorylate MHC, and is unable to drive myosin II filament disassembly in vivo. We found that cells over-expressing MHCK-C- Δ -WD display elevated adhesion levels comparable to those observed for cells over-expressing full-length MHCK-C. In addition, MHCK-C- Δ -WD cells still exhibit the myosin II-dependent “cringe” response upon treatment with sodium azide, but do not detach from the substrate, as is observed with AX2 or 3xALA (no MHCK-C) background cell lines. Thus, even when MHCK-C is expressed as a truncation (MHCK-C- Δ -WD) that cannot control myosin II activity, it can still drive cells to form highly stable attachments to substrate.

Taken together, these studies support a model in which MHCK-C, which co-localizes with myosin II at the cell rear, can facilitate cell adhesion by catalyzing the disassembly of myosin II bipolar filaments (via MHC phosphorylation) and by driving the formation of myosin II-independent attachments to an external surface. In the context of cell migration, these activities of MHCK-C may play a role controlling the rate and direction of cell locomotion during chemotaxis or multicellular development.

Functional analysis of NDR kinases in *Dictyostelium discoideum*

Peter Kastner, Michael Schleicher, Annette Müller-Taubenberger
Ludwig Maximilians University Munich, Institute for Cell Biology, Schillerstrasse 42, 80336
Muenchen, Germany

NDR (nuclear Dbf2-related) kinases are a subgroup of the AGC (protein kinase A (PKA)/PKG/PKC-like) class of protein kinases. The genome of *Dictyostelium discoideum* encodes four NDR group kinases, NdrA, B, C and D. Sequence comparisons indicate that NdrA and B correspond to the human kinases NDR1 and 2 that were shown to play a role in centrosome duplication. NdrC and D are related to the large tumor suppressors LATS1 and 2. In addition to the different NDR kinases, in *D. discoideum* three potential NDR activators of the Mob1 group, MobA, B and C are expressed. Our current working hypothesis is that these different Mob1 proteins may specifically activate one distinct NDR kinases of the pool of four. In *Drosophila melanogaster*, homologs of NDR kinases play a role in development, and in *Schizosaccharomyces pombe* a NDR kinase (Sid2p) is part of the septation initiation network (SIN), which is important for the final stages of cytokinesis. We recently disclosed a SIN-homologous pathway involved in the regulation of cytokinesis in *D. discoideum*. Upstream components of this pathway include a polo-like kinase (PLK), a GAP (Bub2), a GTPase (Spg1), and a central Ser/Thr-kinase (SepA, a homolog to the SIN-kinase Cdc7p). The effectors downstream of SepA are not specified yet, but may include members of the Ste20-like and NDR group of kinases. Therefore, we started to explore the *D. discoideum* NDR kinases and test their potential involvement in cytokinesis. NdrA was shown to localize to the cytoplasm. Deletion of the *ndrA* gene results in a phenotype with reduced growth. NdrA mutant cells have defects in phagocytosis, but cytokinesis is unaffected. NdrB kinase localizes to centrosomes. By immunoprecipitation studies and tandem affinity purification (TAP) MobB was found to interact with NdrB. Moreover, pull-down experiments of the mainly cytoplasmic MobB revealed a potential interaction with a PI3-kinase related protein kinase indicating a regulation by phosphatidylinositol. The data suggest that NdrB together with MobB may be a component downstream of SepA in the SIN-related pathway of *D. discoideum*.

Microtubule-based motor protein functions in *Dictyostelium*

Michael Koonce¹, Irina Tikhonenko¹, Dilip Nag¹, Douglas Robinson²

¹Division of Molecular Medicine, Wadsworth Center, Albany NY, 12201-0509, USA, ²Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA

Kinesin and dynein are two families of motor proteins that drive organelle and microtubule movements in eukaryotic cells. These movements are essential for organelle trafficking, endomembrane distribution, cell shape, and cell division. Most eukaryotes contain multiple family members, with each isoform thought to participate in a subset of functions. *Dictyostelium discoideum* contains a single dynein motor, and 13 kinesin isoforms grouped into 10 families. While dynein appears essential for viability, a complete functional survey of kinesins has not yet been performed in this organism. Published works from other groups have addressed the individual activities of 6 kinesins (kif1, kif2, kif3, kif 5, kif7, kif12). Using gene disruption strategies, we have addressed the individual function(s) of the seven remaining kinesin isoforms in *Dictyostelium*. Only two of the kinesin genes (kif3, Rohlk et al, 2008, and kif6) appear absolutely required for viability. Interestingly, kinesin isoforms that are considered essential for viability in metazoans (kif2, kif4, kif8, kif13) can be deleted in *Dictyostelium*. Phenotypes range from none detectable to significant growth defects; some demonstrate mitotic chaos when coupled with other genetic perturbations. We will present here details of these phenotypes as well as a functional survey of microtubule-based motors in *Dictyostelium*. The goal of our ongoing project is to establish a minimal microtubule-based motility proteome for this basal eukaryote, enabling us to contrast motor functions here with the often far more elaborate motor families in the metazoans.

Phagocytosis and host-pathogen interactions in *Dictyostelium*. Re-examining some open questions on PLC, PI3K, Nramp1 and actin in phagocytosis and infection.

Salvatore Bozzaro, Alessandra Balest, Barbara Peracino, Barbara Pergolizzi, Alessio Sillo
Department of Clinical and Biological Sciences University of Turin, Italy

Dictyostelium is a professional phagocyte, which takes up both pathogenic and non-pathogenic bacteria, albeit with different kinetics.

Uptake assay under shaking helps discriminating between macropinocytosis and phagocytosis, the latter process requiring tight bacterial binding for ingestion. We present some evidence that *Legionella pneumophila*, in contrast to other non-pathogenic or pathogenic bacteria, such as *E. coli*, *K. aerogenes*, *N. meningitidis* and *M. avium*, is taken up very inefficiently, possibly by macropinocytosis. *L. pneumophila* is, nevertheless, a more virulent pathogen for *Dictyostelium* cells than *N. meningitidis* and *M. avium*.

We had previously shown that phagocytosis of *E. coli* is sensitive to PLC, but not PI3K inhibitors. In view of contradictory results in the literature, we have re-examined the role of PLC and PI3K in uptake of *E. coli* or *L. pneumophila*, and their potential role in infectiveness by *L. pneumophila*. We confirm that *E. coli* phagocytosis is inhibited by PLC inhibitors, but is insensitive to pharmacological or genetic PI3K inactivation. In contrast, *Legionella* uptake is partially sensitive to PI3K, in addition to PLC.

Legionella infection is totally blocked by drugs affecting PLC activity or actin polymerization. We show that the inhibitory effect of actin-depolymerizing drugs on infection can be completely explained with a block of bacterial uptake, while intracellular bacterial growth is insensitive to the drugs.

Despite the reduced uptake, and in contrast to PLC inhibitors, *Legionella* infection is strongly enhanced by inhibiting PI3K, as previously reported by H. Hilbi and confirmed by us. In an attempt to unravel the mechanism of action of PI3K in *Legionella* intracellular growth, we have studied the dynamics of LCV (*Legionella* Containing Vacuole) maturation by using cells expressing GFP-fused proteins or antibodies against endosomal or post-lysosomal markers. It appears that the LCV avoids fusion with endosomal markers but acquires quite early post-lysosomal markers.

A marker that is recruited to both early and late phagosomes is the iron transporter Nramp1. We have shown that constitutive expression of GFP-Nramp1 protects the cells from *Legionella* intracellular growth. Remarkably, PI3K inhibitors relieve the protective effect of GFP-Nramp1, suggesting that PI3K may act upstream of Nramp1. PI3K inhibitors also display a slight inhibitory effect on GFP-Nramp1 recruitment to LCV. Whether this limited interference of PI3K inhibitors on LCV intracellular traffic and fusion with Nramp1 vesicles is sufficient to explain the stimulatory effect of the drug on intracellular growth remains open.

Genetic and genomic evidence for bacterial recognition by dictyostelids

Waleed Nasser, Richard Suggang, Adam Kuspa

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston Texas, 77030 USA

Our recent discovery of Sentinel (S) cells in *D. discoideum* suggests that the dictyostelids possess a form of innate immunity (1). TirA, a protein containing a Toll/Interleukin-1 receptor (TIR) domain, functions in S cells and is also required for survival of *D. discoideum* on live bacteria. This suggests that components of innate immune signaling have been retained in the amoebozoa lineage from their common ancestor with plants and animals. We are exploring the possibility that bacterial recognition systems are used by *D. discoideum* to allow vegetative amoeba to respond to changes in the microbial flora in the soil and thereby avoid exploitation by pathogens. We will describe genetic and genomic screens for potential components of bacterial recognition. TIR and leucine rich repeat (LRR) domains are found in Toll-like receptors in animals and resistance proteins in plants that are central mediators of pathogen recognition. We will describe a survey of the *D. discoideum* genome that identified 35 proteins with different combination of TIR, LRR, or protein kinase domains, some of which may represent bacterial recognition receptors. We will also describe the initial results of a genetic screen that is based on our finding that *tirA* mutant amoebae die on lawns of Gram-negative bacteria, but grow normally on lawns of *Bacillus subtilis*, a Gram-positive bacteria. From a screen of several thousand REMI insertion mutants we have identified a number of mutants that display differential growth on Gram-negative and Gram-positive bacteria, suggesting that specific recognition of bacteria is possible in *D. discoideum*. Characterizing pathways involved in the recognition of bacteria by *D. discoideum* may shed light on the evolution of innate immune systems in general and may help to illuminate the defense systems that amoebae use against soil bacteria.

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The *Dictyostelium* response to infection with *Legionella*: From microarray analysis to functional characterisation — a top-down approach.

Rui Tian¹, Can Ünal², Sachin Kumar¹, Michael Steinert², Ludwig Eichinger¹

¹Center for Biochemistry, Medical Faculty, University of Cologne, 50931 Köln, Germany, ²Institute for Microbiology, TU Braunschweig, Germany

The professional phagocyte *Dictyostelium discoideum* is a useful model for the study of the medically relevant infection of host cells by pathogenic microorganisms. The analysis of the time course of *D. discoideum* infection revealed that *L. pneumophila* not only triggers a stress response and interferes with intracellular vesicle fusion and destination but also profoundly influences and exploits the metabolism of its host.

Comparative studies with pathogenic and non-pathogenic *L. pneumophila* 24 hours post-infection identified 240 differentially expressed genes that appear to be involved in the pathogenic response (Farbrother et al., Cell. Microbiol. 3:438-56, 2006). More than 50 of these genes have clear orthologues in higher eukaryotes including human and through manual annotation we placed them in different functional categories. One category with three differentially regulated genes was macroautophagy (hereafter autophagy). Autophagy contributes to many physiological and pathological processes, including starvation, cell differentiation, morphogenesis, programmed cell death, cancer and neurodegenerative disorders. However, its role in infection processes is so far not clear. To gain insight into a possible function of autophagy during bacterial infection we selected the up-regulated autophagy 9 (ATG9) gene for further studies. The ATG9 protein is highly conserved from yeast to man and yeast ATG9 plays an important role in the formation of the pre-autophagosome structure (PAS), the site of autophagosome assembly. ATG9 knock-out cells displayed severe developmental defects, consistent with the well-known role of autophagy in *Dictyostelium* development (Otto et al., J. Biol. Chem. 279:15621-29, 2004). In addition, the ATG9 mutant had a strong phagocytosis defect and appeared to be less efficient in the clearance of *Legionella*. This is consistent with a protective role of ATG9 and hence autophagy in the early phase of infection when the pathogen establishes its replicative niche. Further studies are aimed to better understand the function of ATG9 during phagocytosis and infection.

The *Dictyostelium* Pod-1 homolog Crn7 is a coronin-like protein involved in actin-driven processes and infection with *Legionella pneumophila*

M. C. Shina¹, C. Ünal², A. Müller-Taubenberger³, M. Steinert², A. A. Noegel¹

¹Institute for Biochemistry I, Center for Molecular Medicine Cologne (CMMC) and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Medical Faculty, University of Cologne,, ²Institute for Molecular Infection-Biology, University of Würzburg, Germany and Technical University Braunschweig, Institute for Microbiology, Germany, ³Institute for Cell Biology and Center for Integrated Protein Science (CIPSM), Ludwig Maximilians University Munich

Coronins are multifunctional proteins containing WD40 repeat domains that form a β -propeller structure predestined for protein-protein interactions. Here we describe *corB*, one of two genes in *Dictyostelium discoideum* that code for coronin proteins. *corB* encodes a 962 amino acid protein, Crn7, that in contrast to the *corA* product coronin, contains two complete WD repeat domains thus resembling Crn7, the long coronin in man, and Pod-1 of *Drosophila melanogaster* and *Caenorhabditis elegans*. *Dictyostelium discoideum* Crn7 shares highest homology with its human counterpart and harbors also a proline-serine-threonine (PST) rich domain that is unique for *Dictyostelium discoideum* and human Crn7 and separates the two WD repeat domains. *Dictyostelium discoideum* Crn7 is present in the cytosol as well as in cytoskeletal and membrane fractions. During phago- and macropinocytosis Crn7 accumulates at phago- and pinocytic cups. Strains lacking Crn7 have severe defects in actin driven processes like phagocytosis, and development. Further, Crn7 affects the infection process by the pathogen *Legionella pneumophila* where it functions different from coronin. Our results suggest that Crn7 has specific roles in F-actin-mediated cytoskeletal rearrangements and that it is an important factor in protection against bacterial infection.

Understanding phosphatidylinositol turnover as a therapeutic target using *Dictyostelium*Ben Orabi, Nadine Pawolleck, [Robin S.B. Williams](#)

Biological Sciences, Bourne Laboratory, Royal Holloway University of London, Egham, Surrey, UK

Epilepsy costs between 0.1 and 1.12% of national healthcare budgets globally, with an estimated 3.4 million people diagnosed with active epilepsy in Europe. Valproic acid (VPA, 2 propyl pentanoic acid) is the most widely prescribed treatment for epilepsy worldwide, although unusually, how it works in this role is unclear. We have recently discovered that VPA acts by acutely attenuating phosphatidylinositol (PI) kinase signaling in *Dictyostelium*. This is highly significant, since this effect occurs within the time frame of VPA's control of seizures, and PI signaling is likely to be associated with epilepsy progression. Preliminary data also suggests the effect occurs in mammalian systems.

To measure PI kinase signaling in *Dictyostelium*, we have adapted a mammalian-based protocol for introducing radio-labeled ATP into cells, which allows incorporation of the label into lipids, and we can quantify this incorporation by thin layer chromatography (TLC) and phosphorimaging analysis. Using this approach VPA (0.5 mM) decreases PIP and PIP₂ production in living cells within a 6 minute period (by $74\pm 7\%$ and $79\pm 6\%$ respectively), suggesting an effect through inhibition of PI kinase activity. The effect is also seen by inhibition of phosphatidylinositol 3 kinase (PI3K) using specific pharmacological inhibitors (e.g. LY294002, 50 μ M, $83\pm 7\%$ and $80\pm 4\%$ respectively). We have employed a range of VPA-related compounds to show that this effect is not related to VPA's acidity or lipophilicity, although the inhibitory effect is partially dependent upon the acid group, since amide derivatives show reduced inhibition. Compounds with backbones and side chains of increasing length result in higher PI kinase inhibition, whereas shorter backbone lengths show reduced efficacy.

The trend in structural characteristics observed for VPA-like compounds giving increasing PI kinase inhibition is shared with the anti-epileptic efficacy of the compounds in a variety of animal models. For example, using pentylenetetrazol (PTZ)-induced seizure or audiogenic seizure models in mice, VPA-related compounds with increasing backbone and side chain length show better seizure control than VPA. These studies did not test the longer compounds that we have identified as stronger inhibitors of PI signaling. Furthermore, shorter backbone lengths gave reduced seizure control in these experiments - in agreement with reduced PI kinase inhibition. Other studies have identified VPA analogues showing increased anti-epileptic activity for multiple branched structures such as isopentyl pentanoic acid (PIA), and PIA also has a strong inhibitory effect on PI signaling. Thus, our results suggest that VPA's anti-epileptic effect is related to structural characteristics required for the inhibition of PI signaling in *Dictyostelium*. We are yet to determine if these drugs show similar effects in mammalian systems.

Although we are currently determining if these effects occur through direct or indirect mechanisms, our data suggests a potentially therapeutic effect of a mainstay treatment for epilepsy (VPA) via regulation of phosphatidylinositol signaling.

A RasGEF/Sca1/PP2A Signaling Complex Controls the Ras-TORC2-AKT/PKB Pathway

Pascale G. Charest¹, Atsuo T. Sasaki^{1,2}, Zhouxin Shen¹, Steve Briggs¹, Richard A. Firtel¹

¹Section of Cell and Developmental Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0380, ²Present address: Department of Systems Biology, HMS, Division of Signal Transduction, BIDMC, 77 Avenue Louis Pasteur, Boston, MA 02115

Although our knowledge of Ras function is already extensive, increasing evidences suggest that Ras signaling is much more complex than originally anticipated. In addition to couple receptor tyrosine kinases to mitogenic signaling pathways, increasing evidences suggest that Ras proteins also participate in the transduction of signals through G protein-coupled receptors (GPCR). Genetically tractable model organisms, such as *Dictyostelium discoideum*, have been proven to be useful tools to decipher the Ras signaling networks. In *Dictyostelium*, Ras controls cell motility, chemotaxis and signal relay, acting, in part, through the regulation of PI3K and TORC2, downstream from GPCR and heterotrimeric G proteins. The Aimless RasGEF (*gefA*), an orthologue of mammalian Sos, was suggested to be a regulator of these Ras functions. To get insight into the mechanisms regulating Ras signaling, we undertook a sequential affinity purification and mass spectrometry approach to identify proteins interacting with Aimless. We found that Aimless is part of a stable protein complex that includes another RasGEF, RasGEFH, PP2A, and a previously uncharacterized Armadillo-like repeat-containing protein of 175 kDa. Co-immunoprecipitation studies indicate that the latter acts as a scaffold, which we named Sca1, bringing together an Aimless-RasGEFH heterodimer and PP2A. Whereas disruption of *gefH* alone produced minor effects, cells that lack only Aimless, both RasGEFs, or Sca1 display reduced Ras and PKB activity as well as F-actin polymerization, and exhibit cell motility as well as chemotaxis defects. We identified RasC, an orthologue of mammalian H-Ras, as the major Ras protein regulated by the RasGEF/Sca1/ PP2A complex. In addition, preliminary data suggest that the Sca1-scaffolded signaling complex regulates PKB activity by controlling the Ras-dependent activation of TORC2, but not that of PI3K, which are both Ras effectors regulating PKB. Furthermore, phospho-proteomics coupled to biochemical studies revealed that Sca1 undergoes dynamic phosphorylation, which includes transient and chemoattractant-induced PKB-promoted phosphorylation, as part of a desensitization mechanism. Together, our data suggest that the RasGEF/Sca1/PP2A signaling complex selectively regulates the activation of a Ras-TORC2-PKB pathway, and undergoes PKB-promoted negative feedback regulation. Studies aiming at understanding the role of PP2A in the regulation of this Ras signaling pathway are underway.

Dictyostelium talin homologue, talin A, is involved in tail retraction in directed cell migration

Masatsune Tsujioka, Shigenobu Yonemura
RIKEN, CDB

Talin regulates adhesion molecules and links them to the actin cytoskeleton at cell-substrate adhesion sites. Two talin homologues have been discovered in various organisms including human and mouse, however, very little is known about their distinct functions. We compared the sub-cellular localizations of two talins (talin A and talin B) fused with GFP in the cellular slime mould *Dictyostelium discoideum*, which is an established model system to investigate cell motility. Talin A-GFP and GFP-talin B localized at the trailing and leading edges, respectively, in aggregating cells. However, talin A-GFP was found to be dispersed throughout the plasma membrane in mutant cells lacking myosin II, which also accumulates at the tail region to retract the trailing edge by contraction of actomyosin filaments. Advancing talin A-null cells showed features similar to myosin II-null cells in that they trailed long, thin tails. Therefore, we speculate that talin A, unlike talin B, binds adhesion molecules to actomyosin bundles so that constriction of these bundles facilitates the detachment of the trailing edge from the substrate. We also examined which regions determined the specific distributions of talin A and talin B. The C-terminal F-actin-binding regions of talin A and talin B fused with GFP showed the same distribution pattern as each full-length talin. As F-actin forms networks at the leading edges while forming bundles with myosin II at the trailing edge, we speculate that actin-binding regions of talin A and talin B bind distinct F-actin structures and that these differences lead to the distinct localization of the two talins. Biochemical assays revealed that the N-terminal membrane-binding domain of talin A associated with phospholipids, PIP2 and PIP3, whereas the equivalent domain of talin B bound exclusively to PIP3. Since PIP3 assembles at the leading-edge membrane and PIP2 has a tendency to accumulate at the trailing end of the plasma membrane in migrating cells, the membrane-binding domains of each talin may also be implicated in their specific localizations.

Quantitative analysis of amoeboid cell motility

Ruedi Meili¹, Juan C. del Alamo², Baldomero Alonso-Latorre², Juan C. Lasheras², Rick Firtel¹

¹Section of Cell and Developmental Biology, UCSD, La Jolla, CA, ²Department of Mechanical and Aerospace Engineering, UCSD, La Jolla, CA

Ameoboid motility consists of the repetition of protrusion, contraction and retraction. This cyclical mechanical process requires the spatiotemporal coordination of the biochemical pathways regulating the main force generators of F-actin polymerization and actomyosin contraction. To better understand this biomechanical integration, we assembled the necessary tools to study how individual components affect the mechanical output quantitatively. We developed an improved force cytometry method that uses an explicit calculation of the force field resulting in improved accuracy and resolution. We found that the time evolution of the strain energy exerted by migrating cells on their substrate is quasi periodic and can be used as a simple indicator of the stages of the cell motility cycle. The mean velocity of migration (V) and the period of the strain energy (T) are related through a hyperbolic law $V=L/T$, where L is a constant step length that remains unchanged in mutants with deficiencies in myosin II function, like *mhcA*- and *mlcE*-cells. Assigning a quantitative function to specific molecules is complicated by the inherent variability of the behavior of individual cells. We were able to address this difficulty with a novel statistical analysis of the spatial and temporal organization of the traction forces that the cells exert on the substrate. We took advantage of the cyclical nature of amoeboid motility and adapted phase averaging statistics originally developed for the analysis of oscillatory features of physical systems like turbulent flow. The results of our analysis are sequential normalized traction force maps, which can be identified with experimentally observable phases of motility such as protrusion, adhesion, contraction and relaxation. Comparison of wild-type maps with maps of cells with mutated myosin function helps define quantitatively the role of the affected molecules for mechanical function and organization on the cellular level.

Visualisation of transcriptional firing in individual living cells

Tetsuya Muramoto, Jonathan Chubb
College of Life Sciences, University of Dundee, UK

It is unclear how cells encode stability of transcriptional states during cell differentiation. To what level of robustness are transcriptional states maintained during cell decision making, and what factors generate robustness? The two cells from a mitosis share cytoplasmic and nuclear components, potentially including similar chromatin states. They also share, at least temporarily, their extracellular environment. To gain insight into how these forces dovetail to generate (or limit) transcriptional stability, we use a system which allows us to visualise nascent RNA in individual living cells using fluorescence microscopy [1]. We are now able to combine this technique with imaging protocols allowing capture of complete *Dictyostelium* cell cycles [2]. Our approaches are giving us insight into the magnitude of transcriptional stability during the cell cycle, the potential influence of this upon differentiation and molecular, sub-cellular and extra-cellular contributions to the minimisation of transcriptional noise.

[1] *Current Biology* (2006) 16: 1018-1025

[2] *Development* (2008) 135: 1647-1657

Interactions between modifications of histone H3.

Duen-Wei Hsu¹, Jonathan Chubb², Po-Hsien Liu¹, Louis C. Mahadevan¹, Catherine Pears¹

¹Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom, ²Division of Cell and Developmental Biology, College of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom

Histone modifications play a vital role in regulation of gene expression. Combinations of modifications on individual histone molecules generate binding interfaces for proteins that regulate DNA function including the transcription machinery. The complexity of histone modification increases in multicellular organisms and modifications likely arose to cope with the necessity to regulate the genes required for differentiation into different cell types. We have focussed on interactions between modifications at the N-terminus of histone H3 finding, for example, that trimethylation of lysine 4 targets proteins for rapid acetylation. Use of strains in which genes encoding H3 proteins, or their modifying enzymes, are altered has been used to identify the proteins responsible for these modifications and their importance.

Mitochondrial Processing Peptidase (MPP) in *Dictyostelium discoideum*: MPP activity is controlled via processing of α -MPP by a novel protease during development

Koki Nagayama, Tetsuo Ohmachi

Department of Biochemistry and Biotechnology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, 036-8561, Japan

The majority of mitochondrial proteins encoded by nuclear genes are synthesized in the cytosol as larger precursors with a mitochondrial targeting signal (MTS) sequence. During or after import of the precursor proteins into mitochondria, the presequences are removed by the mitochondrial processing peptidase (MPP) to form mature proteins. MPP consists of two subunits, α -MPP and β -MPP. A cDNA clone encoding the α subunit of *Dictyostelium* mitochondrial processing peptidase (Dd α -MPP) was cloned, sequenced and characterized. Dd α -MPP contains two well-conserved motif domains found in other α -MPPs, a distal basic amino acid recognition site and a glycine-rich domain, both of which play important roles in recognition of precursor proteins. Expression of Dd α -MPP mRNA is highest in vegetatively growing cells and in cells at early development, and it is strongly down-regulated after 10 hr development. The Dd α -MPP protein is expressed as two forms, designated as α -MPP_H and α -MPP_L, throughout the *Dictyostelium* life cycle. α -MPP_H, the larger protein is cleaved into the functional α -MPP_L. We were not able to isolate mutants, in which the α -*mpp* gene has been disrupted, suggesting that MPP plays an essential role in mitochondrial biogenesis. An antisense transformant (α A2) expressing α -MPP at lower level than the wild-type AX-3 strain was isolated to examine the function of the α -MPP protein. Development of the α A2 strain was normal until the slug formation stage (12 h development); but, there was a prolonged slug stage (to \sim 24 h), in which only α -MPP_H was present, and no α -MPP_L or MPP activity was detected. After then, α -MPP_L and MPP activity appeared again and eventually, normal fruiting bodies were formed with an approximately 8 hr delay compared with normal development. These results suggest that MPP activity is controlled via the processing of α -MPP by a novel protease, designated as MPP- α processing peptidase (MAPP), which cleaves the high molecular α -MPP_H to form the functional mature α -MPP_L.

Characterization of the mitochondrial Ras-related GTPase Miro of *Dictyostelium discoideum*Georgia Vlahou¹, Jürgen-Christoph von Kleist-Retzow^{2,3}, Rudolf J. Wiesner², Francisco Rivero^{1,4}¹Center for Biochemistry, Medical Faculty, University of Cologne, Germany, ²Institute of Vegetative Physiology and Center for Molecular Medicine Cologne, University of Cologne, Germany,³Department of Pediatrics, University of Cologne, Germany, ⁴The Hull York Medical School and Department of Biological Sciences, University of Hull, UK

Recently a new subfamily of evolutionarily conserved GTPases, Miro (mitochondrial Rho GTPases) has been described. Miro consists of a Ras-like GTPase, which bears similarity to other Rho proteins, followed by two EF-hand domains that serve as calcium binding regions, an additional GTPase domain and a short transmembrane region, which targets the protein to the outer mitochondrial membrane. In *A. thaliana* three orthologs of the Miro GTPases are known. The Miro1 mutant exhibits abnormally enlarged or tube-like mitochondrial morphology, leading to the disruption of continuous streaming of mitochondria in the growing pollen tube. *S. cerevisiae* cells lacking the Miro homologue Gem1p show collapsed globular or grape-like mitochondria. In *D. melanogaster* strong overexpression of Miro leads to disruptive mitochondrial distribution. Furthermore loss of dMiro impairs larval locomotion and disrupts the subcellular distribution of mitochondria in neurons and muscles. In vertebrates two isoforms of Miro are present that play roles in mitochondrial trafficking. An interaction of Miro with kinesin-binding proteins GRIF-1 and OIP106 has been reported, suggesting that Miro GTPases form a link between the mitochondria and the microtubule trafficking apparatus. One Miro gene (*gemA*) has been identified in the model organism *D. discoideum*. This gene encodes a protein of 658 amino acids with the characteristic domain architecture of other known Miro proteins. By immunofluorescence analysis we show mitochondrial anchoring of Miro. Using homologous recombination we have generated a *D. discoideum* knockout strain lacking *gemA*. The recombination event was verified using PCR, Southern blot analysis and RT-PCR. A monoclonal antibody that specifically recognizes the first 530 residues of the protein was generated and used to confirm the absence of Miro in the knockout strain. We examined the effects of *gemA* deletion in processes like growth, phagocytosis, fluid phase endocytosis and exocytosis, development, phototaxis and chemotaxis. To address specifically the mitochondrial function we examined concentrations of lactate and glucose, O₂ consumption, which was found to be increased in cells lacking *gemA* as well as mitochondrial size, number and morphology, which remained unchanged. Currently we are concentrating in the analysis of the association of mitochondria to microtubules. Identification of potential regulators and effectors of Miro will follow.

Dynamic interactions between receptors and G-proteins prior to and after receptor activation in *Dictyostelium discoideum*

Carrie Elzie, Jennifer Colby, Morgan Sammons, Chris Janetopoulos
Department of Biological Sciences, Vanderbilt University

Extracellular stimuli exert their effects on eukaryotic cells through serpentine G-protein-coupled receptors (GPCRs) and mediate a vast number of physiological responses. Excited receptors activate heterotrimeric G-proteins, consisting of three subunits, α , β and γ . In *Dictyostelium discoideum*, cAMP binds to the cAMP receptor cAR1, which is coupled to the heterotrimer containing the $G\alpha 2$ subunit. Until recently, there has been very little in vivo evidence describing how receptors influence the localization of the G-protein complex prior to and after ligand binding. It has been previously shown that the state of the heterotrimer could be monitored by changes in Forster Resonance Energy Transfer (FRET) between the $\alpha 2$ and β -subunits of *Dictyostelium discoideum*. We now report the kinetics of G-protein activation as a loss of FRET prior to and after cAMP addition using total internal reflection fluorescence microscopy (TIRFM). We also performed photobleaching experiments to measure membrane G-protein recovery times. Lastly, $G\alpha 2$ -subunit localization was analyzed in wild-type and mutant cell lines lacking various signaling components. Our data show that inactive and active G-proteins cycle between the cytosol and plasma membrane. These data suggest that cAR1 activation slows the $\alpha 2$ -subunit's membrane off-rate, while simultaneously promoting $\beta\gamma$ -subunit disassociation.

Folic acid is a potent chemoattractant of amoebae of a protist, *Vahlkampfia* sp., as the case for vegetative amoebae of the cellular slime molds

Yasuo Maeda¹, Aiko Amagai²

¹Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan, ²Department of Biomolecular Science, Graduate School of Life Sciences, Tohoku University, Katahira 2-1-1, Aoba-Ku, Sendai 980-8577, Japan

Folic acid (folate; vitamin Bc) is well recognized to be essential for the proper metabolism of the essential amino acid methionine as well as for the synthesis of adenine and thymine, two of the four bases that make up our genomic DNA. A folate deficiency has been implicated in a wide variety of disorder from Alzheimer's disease to depression, atherosclerosis, heart attack, stroke, cervical and colon cancer, cleft up and palate, dementia and hearing loss, and of course, neural tube defects. In the cellular slime molds including *Dictyostelium*, vegetative growth-phase cells are known to be chmotactically moved toward folate that is synthesized and secreted by bacterial food sources such as *Escherichia coli*. Intracellular folate signal transduction including G proteins, Ca²⁺ channels, and IP3 pathway has been reported in *D. discoideum*. To our surprise, the chemoattractant(s) of free-living protozoan amoebae remained to be determined thus far, possibly because of a total lack of the pertinent method for assaying chemotaxis. We recently isolated a primitive free-living amoeba from the soil of Costa Rica, and identified it as a new species of the genus *Vahlkampfia* belonging to a subclass Gymnamoebia in which *Entamoeba*, *Acanthamoeba* and *Naegleria* are included. Here, we have demonstrated using a quite simple but finely designed chemotaxis assay that the *Vahlkampfia* amoebae exhibit the polarized movement toward higher folate concentrations. Thus, folate may serve as an attractant during food seeking of amoeboid cells which naturally prey on folate-releasing bacteria in the soil. This raised the possibility that the function of folate might be evolutionary conserved, working well as a potent chemoattractant of amoeboid cells in a wide range of organisms as well as in the Protista and cellular slime molds.

A phase transition to collective behavior in *Dictyostelium* cell populationsThomas Gregor¹, Koichi Fujimoto², Satoshi Sawai^{1,2}¹Graduate School of Arts and Sciences, University of Tokyo, ²ERATO Complex Systems Biology Project, JST

Collective dynamics are widely observed during development of multicellular bodies and emerge as a result of communication among individual cells via signaling molecules. However, little is known experimentally of the fundamental features that describe how the highly nonlinear spatio-temporal dynamics at the single-cell level can give rise to coherent dynamics at the population level. The prototypical example is cell-to-cell signaling among social amoebae *Dictyostelium*, where a few hundred thousand cells aggregate to form a fruiting body. cAMP is synthesized and secreted periodically and serves as a cue that directs chemotaxis of individual cells. Here we use a FRET-based sensor protein, combined with live-imaging, to monitor cytosolic cAMP levels in developing *Dictyostelium* cells. Single cell resolution timelapse recordings of cell populations during the first 10 hours of development reveal the very onset of periodic, spike-like signaling, as well as sequential changes in the signaling frequency. The input-output relation for wild-type and mutant strains obtained from single cells in isolation indicates that the intracellular cAMP dynamics is governed by a PI3kinase-TORC2-actin dependent feedback loop. Collective cAMP oscillations in populations of cells under perfusion reveal a sharp phase transition between a decoupled state and collective behavior for a range of cell densities and dilution rates. These observations together with a mathematical model that we have constructed, suggest that the intact population is able to drive itself to this transition spontaneously during development.

Study of Excitability and Synchronization in *Dictyostelium* by the Newly Developed Micro-laboratoryShunsuke Sakurai¹, Satoshi Konishi², Seido Nagano¹¹Department of Bioinformatics, Ritsumeikan University, ²Department of Micro System Technology, Ritsumeikan University

On starvation *Dictyostelium* amoebae communicate each other by cAMP pulses, and the differentiation from the single cell-state to the multicellular-state is controlled by the different types of cAMP pulses and their corresponding cAMP receptors. To utilize such characters for the quantitative study of cellular communication, we have developed a new micro-laboratory system, where *Dictyostelium* amoebae are trapped within micro-wells (20-100 micro meters in diameter) such that steady production of cAMP from micro-wells can be guaranteed. In Dictyostelium 2000, held at University of Dundee, we have reported the computer simulation results of the cAMP wave packet propagation that is quite similar to the propagation of signals in neural networks when many *Dictyostelium* amoebae are put in a given plane by restricting the motion of cells. Now, we have successfully reproduced similar phenomena in the newly developed micro-laboratory system. By changing number of cells and types of cells in micro-wells and the spatial distribution of micro-wells, we can proceed further and expect external control of the development of cells trapped in the target well, too.

Functional characterisation of adenylyl cyclase B in *Dictyostelium*

Zhihui Chen, Pauline Schaap

School of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom

Adenylyl cyclase B (ACB) is one of the three adenylyl cyclases producing cAMP during development of *Dictyostelium*. It is expressed at low levels during growth and higher levels in the prestalk cells after aggregation [1-3]. ACB plays an essential role in stalk- and spore maturation [4], but nothing is known about the regulation of its activity. ACB has a primary structure composed of an N-terminal transmembrane domain, histidine kinase domain, three response regulator domains, the cyclase catalytic domain and a low complexity region at the C-terminus [4]. In order to understand the role of the different domains in regulation of cyclase activity, we expressed mutated forms of ACB that lack one or more domains, or carry point-mutations in amino-acids with suspected essential roles in enzyme regulation in an *acb*- null mutant and analysed their activity, subcellular localization and complementation of the *acb*- phenotype. Loss of the C-terminal low complexity region increased AC activity, indicating negative regulation by this domain. Truncation of the N-terminal transmembrane domain strongly decreased ACB activity. A novel histidine autophosphorylation motif was identified and loss of this motif in conjunction with the transmembrane domain again increased AC activity. Site-directed mutagenesis of the acceptor histidine (H565Q) in this motif also increased activity of the complete ACB protein. These results suggest that the histidine kinase motif negatively regulates ACB. Mutation of phosphoryl acceptor (D1010A) of response regulator 1, that is closest to the histidine kinase, decreased AC activity, while similar mutations in response regulators 2 and 3 gave rise to higher cyclase activity. This suggests that ACB activity may be regulated by input from two different phosphoryl transfer pathways. The phenotype of defective spore maturation in the *acb*- null mutant is restored by expression of full length ACB-YFP and by all mutant forms that retained 30% or more of full length ACB activity. ACB-YFP was localised intracellularly on vesicular membranes. After deletion of the transmembrane domains the protein became localized in the cytosol.

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Regulation of filamin complex activity by a novel class of HECT ubiquitin ligase is required for cell sorting and morphogenesis in *Dictyostelium*

Simone Blagg¹, Thomas Keller¹, Sarah J. Annesley², Qikai Xu³, Paul R. Fisher²,
Christopher R. L. Thompson¹

¹Faculty of Life Sciences Michael Smith Building University of Manchester Oxford Road
Manchester M13 9PT UK, ²Department of Microbiology La Trobe University VIC 3086
AUSTRALIA, ³Department of Molecular and Human Genetics Baylor College of Medicine One
Baylor Plaza Houston TX 77030 USA

Regulated protein destruction by HECT ubiquitin ligases is important for normal cellular function, whereas misregulation can lead to disease. However, few targets are known. We have identified a novel group of evolutionarily conserved HECT ligases with an N-terminal Filamin domain (HFNs). Filamin is a F-actin binding adapter protein found in large protein complexes, and is involved in diverse cellular processes including cell adhesion, motility and morphogenesis. The developmental cycle of the social amoeba *D. discoideum* provides a model system to study these processes because differential adhesion and motility are thought to drive cell sorting and cell type patterning, although the molecular basis is poorly defined.

We have found that *Dictyostelium hfnA* is specifically expressed in a subset of prestalk cells (pstO) and is dependent on the diffusible signaling molecule DIF-1. In a *hfnA*- mutant, pstO cells do differentiate, but fail to sort out. Disruption of filamin function in a *hfnA*- background effectively rescued pstO cell localization, whereas over-expression of the C-terminus of FIP (Filamin Interacting Protein) in a wild type background phenocopied the *hfnA*- pstO sorting defect. Furthermore, *hfnA*- cells exhibited slugger and phototaxis defects that are consistent with filamin complex hyperactivity. Both defects were also rescued by filamin disruption.

We propose that HfnA regulates filamin complex activity in a cell type specific fashion, through the breakdown of filamin complexes, and that this is important for correct movement and/or adhesion of the different cell types. We will present recent data testing this idea using real time 3D imaging of cell movement during morphogenesis, as well as direct measurements of cell type specific chemotaxis and adhesion.

This study provides a novel mechanism for filamin regulation, and reveals new roles for filamin and HECT ligases in development and cell patterning.

Calcium Signalling in *Dictyostelium* and its Implications in Polycystic Kidney Disease and Mucopolipidosis Type IV

Claire Y Allan, Paul R Fisher

La Trobe University, Bundoora, Victoria, Australia

Dictyostelium discoideum regulates intracellular calcium ($[Ca^{2+}]_i$) through Ca^{2+} regulated Ca^{2+} pumps and channels. During its lifecycle, amoebae are chemotactically responsive to folate and cAMP which stimulate a rise in $[Ca^{2+}]_i$. This study investigates the involvement of four proteins, two Ca^{2+} - ATPases and two Ca^{2+} permeable membrane channels, polycystin-2 and mucolipin-1, in $[Ca^{2+}]_i$ regulation in *Dictyostelium*. The human homologues of these Ca^{2+} channels are implicated in Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Mucopolipidosis Type IV. Therefore we are using *Dictyostelium* as a model to study these diseases.

The genes encoding the Ca^{2+} -ATPases, polycystin-2 and mucolipin-1 were cloned, and strains expressing either under or overexpressing each protein were produced. To investigate the role of each in Ca^{2+} signalling during chemotaxis, the strains were assayed for Ca^{2+} responses to chemoattractants. The amplitude of Ca^{2+} responses in the polycystin-2 and mucolipin-1 strains differed from wild type. Overexpression and underexpression of both proteins resulted in larger and reduced Ca^{2+} responses, respectively. The changes in amplitude suggest that these proteins contribute to Ca^{2+} influx into the cytosol as a result of chemoattractant stimulation. Ca^{2+} responses in the strains expressing decreased levels of the individual Ca^{2+} -ATPases, showed no significant deviation from wild type. This suggests a compensatory ability of other cellular Ca^{2+} -ATPases to regulate Ca^{2+} homeostasis. Varying extracellular $[Ca^{2+}]$ was found to alter the expression levels of these Ca^{2+} -ATPases and polycystin-2. This variation in expression is consistent with a role for these proteins in Ca^{2+} homeostasis.

The lysosomal storage disease, Mucopolipidosis Type IV, results from alterations in the amount of functional mucolipin-1. Overexpression, and mutational loss of function of mucolipin-1, results in abnormal accumulation of acidic vesicles. We also observed this phenotype in *Dictyostelium* strains with altered mucolipin-1 levels, which manifested as an increase in the amount of autofluorescent vesicles. Our results suggest that in *Dictyostelium* accumulation of these vesicles may result from altered cytosolic Ca^{2+} signals, including those required for lysosomal trafficking and maturation. These strains have also been found to exhibit phenotypes consistent with strains modelling the lysosomal disorder, Batten disease. These phenotypes include increases rates of phagocytosis and pinocytosis, and small yet normally proportioned fruiting bodies.

Polycystin-2 is encoded by one of two autosomal genes implicated in ADPKD, and is the most common monogenic cause of kidney failure in humans. In mammals polycystin-2 has been localized to the apical primary cilia of renal tubule and epithelial cells and ADPKD is characterized by a combination of renal and liver cyst formation and renal enlargement. We have observed that in *Dictyostelium* strains, decreasing the levels of polycystin-2 results in decreased nutrient uptake by pinocytosis and phagocytosis, conversely increasing expression enhances nutrient uptake. This is likely due to the respective decrease and increase intracellular Ca^{2+} , as Ca^{2+} is involved in phagocytic cup formation and induction of pinocytosis.

Specificity of the Presenilin/ γ -secretase Complex in Control of Development

Vanessa McMains, Marielle Young, Alan R. Kimmel
LCDB/NIDDK, National Institutes of Health, Bethesda, MD USA

The gamma-secretase complex [Presenilin (PS), Nicastrin (Nct), Aph1 and Pen2] cleaves single-pass transmembrane proteins to release intracellular domain moieties that regulate a variety of signaling pathways. Proteolytic activity appears to reside within PS. In metazoa, gamma-secretase is responsible for processing of Notch, resulting in Notch-dependent gene expression and subsequent cell fate specification; loss-of-function mutations in PS leads to Notch-like embryonic lethality. In humans, mutations in PS alter specificity in cleavage of Amyloid Precursor Protein (APP) and ultimately cause Alzheimer's disease. *Dictyostelium* has 2 genes that encode PS-like proteins and one each for Nct, Aph1, and Pen2. Loss of the two PS genes of *Dictyostelium* does not cause lethality, making it ideal to study gamma-secretase signaling. We have screened for novel functions of PS in *Dictyostelium* and for distinct roles of the different complex components. Although the genes in *Dictyostelium* display significant similarity to their mammalian counterparts, they are nonetheless highly diverged. To assess a definitive functional association with γ -secretase activity, we expressed the mammalian substrate APP in WT and mutant variants of *Dictyostelium*. gamma-secretase processing of APP was observed in WT cells, but not in strains lacking PS, Nct, and Aph1, or in multiply mutated stains. Analysis of single and double mutations of PS, Nct and Aph1 genes, indicates that all the components are required for proper prespore/spore differentiation by a cell-autonomous pathway. We have tentatively identified several endogenous proteins in *Dictyostelium* that are candidate substrates for gamma-secretase processing in vivo and are testing if these proteins are indeed subject to PS cleavage and if their processing is essential to regulate *Dictyostelium* development.

Cytoskeletal assembly and maturation: How do different actin binding proteins cooperate and compete in assembling actin filament arrays

David A. Knecht

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

Actin binding proteins organize actin filaments into orthogonal, parallel and anti-parallel arrays in order for the cell to carry out specific mechanical functions. Since all of these ABP's are present in cells, it is unclear how different forms of actin can be created if all ABP's bind to new actin filaments. We have begun to examine investigate this problem using three representative proteins: filamin, alpha-actinin and fimbrin. In vitro, filamin cross-links actin filaments into orthogonal arrays, alpha-actinin forms loose anti-parallel bundles and fimbrin forms tight parallel bundles. Alpha-actinin and fimbrin both have EF hands, while filamin does not. All three proteins associate with new protrusions formed in non-polarized cells. However, in polarized cells fimbrin and alpha-actinin are found at the front of cells while filamin is found in the lateral cortex and at the rear of the cell. In cells expressing both filamin and alpha-actinin, the two proteins show nearly complementary patterns of localization. Thus in the cell, there are actin filaments that bind one or the other, but not both ABP's. Surprisingly, the actin binding domains of these proteins show the same relative distribution indicating that the actin binding domain alone is sufficient to drive localization to specific sets of actin filaments. This localization of the ABD's is dependent upon the multimerization status of the probes. Filamin and alpha-actinin are normally found as homo-dimers in the cell. If the filamin ABD is expressed as a monomer, it localizes to the front of the cell. As a dimer, it is found in the lateral cortex and rear. As a tetramer, it forms an aggregate at the rear of the cell. Alpha-actinin remains at the front even when expressed as a dimer. We hypothesize that actin filaments are made at the front of polarized cells and mature and move rearward as the cell moves forward. The high affinity of filamin for actin leads to an asymmetric distribution of the protein, such that there is little free filamin at the front of the cell and more free alpha-actinin and fimbrin. Thus newly polymerized filaments associate primarily with those two ABP's. Over time, the filaments become associated with filamin and lose their affinity for alpha-actinin and fimbrin. We are developing a TIRF based in vitro assay to directly test this hypothesis.

WASP Family proteins controlling actin dynamics in *Dictyostelium discoideum*

Michael Carnell, Robert Insall

The Beatson Institute for Cancer Research

The actin cytoskeleton consists of a variety of dynamic filamentous structures within the cell that are fundamental for a vast array of cellular processes. A deeper understanding of its regulation and cellular function are necessary if mechanisms such as cellular motility, cytokinesis, and vesicle trafficking are to be fully comprehended. The arp2/3 complex is one of the best-characterized nucleators to date, and when activated a branched actin structure results.

A key question is how is this actin nucleator regulated, and how this can account for the variety of actin structures observed. WASP family proteins play an important role. This group of proteins is characterized by the presence of a C-terminal VCA domain, which is responsible for the binding and activation of the arp2/3 complex. The variability in the N-terminal regions between WASP family members allows varying signaling pathways to converge on the arp2/3 complex, each of which may result in different actin structures performing varying roles.

Our aim is to compare all WASP family proteins in the *Dictyostelium* genome, and further study the role of each in actin dynamics. So far we have identified an important and unexpected role for WASP family proteins in vesicle trafficking.

Functional roles of VASP phosphorylation in the regulation of chemotaxis and osmotic stress response.

Wan-Hsin Lin¹, J. Scott Gruver², Sharon E. Nelson², Chang Y. Chung^{1,2}

¹Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA, ²Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Vasodilator-stimulated phosphoprotein (VASP) has been identified as a major substrate of PKA and PKG and plays crucial roles in controlling F-actin-driven processes. Growing evidence indicates that VASP function is modulated by phosphorylation at multiple sites. In this study, we took advantage of *Dictyostelium* which possesses only one member of the Ena/VASP family to investigate the functional roles of VASP phosphorylation in response to osmotic stress or cAMP stimulus. Our results demonstrated that hyperosmotic stress with sorbitol and extracellular cAMP stimulation cause VASP phosphorylation at Ser141. VASP phosphorylation appears to be important in controlling VASP localization at the membrane cortex and the interaction with WASP and WIPa. VASP phosphorylation plays a negative role for the early steps of filopodia/microspikes formation, suggesting that unphosphorylated VASP might be required for the initiation of filopodia/microspikes formation. Analysis of chemotaxis of cells expressing VASP mutants showed that VASP phosphorylation is required for the establishment of cell polarity under cAMP gradient. Taken together, we conclude that unphosphorylated VASP is important for the regulation of membrane protrusion, whereas phosphorylated VASP for the regulation of cell polarity during directional movement.

An ELMO-like protein associated with actinomyosin restricts spurious F-actin events to coordinate phagocytosis and chemotaxis

Nilgun Isik¹, Joseph Brzostowski², Tian Jin¹

¹Chemotaxis Signal Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, USA, ²Laboratory of Immunogenetics Light Imaging Facility, National Institute of Allergy and Infectious Diseases, NIH, USA

Elmo proteins are known to positively regulate actin polymerization during cell migration and phagocytosis through activation of the small G-protein Rac. Here, we have identified a unique Elmo-like protein, ElmoA, in *Dictyostelium discoideum* that unexpectedly functions as a negative regulator of actin polymerization. Cells lacking ElmoA display an elevated rate of phagocytosis, increased pseudopod formation and excessive F-actin localization within pseudopods. ElmoA associates with cortex actin and the myosin II heavy chain. TIRF microscopic observations of functional ElmoA-GFP reveal that a fraction of ElmoA localizes near the presumptive actin/myosin II cortex and the levels of ElmoA and myosin II negatively correlate with that of polymerizing F-actin. F-actin-regulated dynamic dispersions of ElmoA and myosin II are interdependent and this regulation involves a putative actin-binding domain at the C-terminus of ElmoA. Taken together, our data suggest that ElmoA modulates actin/myosin II at the cortex to prevent excessive F-actin polymerization around the cell periphery, thereby maintaining proper cell shape during phagocytosis and chemotaxis.

Cryptic species, phylogenetics, and sex in four *Dictyostelium* species

Joan E. Strassmann¹, Natasha Mehdiabadi¹, Chandra Jack¹, Sara Kalla¹, Marcus Kronforst²,
David C. Queller¹

¹Rice University, ²Harvard University

Microbial individuals are difficult to assign to the correct species because they often lack distinguishing morphological traits. Nevertheless it is crucial to know if interactants are from the same or different species. For example, within species, genes for recognition of relatives and cooperation within families, can be selected. An allele conferring a competitive advantage can replace a less competitive allele within species but not between species because sexual recombination occurs only within species. Between species interactions often involve parasitism, predation, or competition, though mutualisms are also possible.

In the absence of morphological characters, species can be identified with DNA sequence information. Variable regions of nuclear ribosomal DNA and mitochondrial DNA flanked by conserved regions are ideal for delineating species. Since individual clones can be stored as spores and used repeatedly in interaction experiments, the identification of a set of clones to species will be very useful. Sequence information can also indicate whether or not a morphological species corresponds to an actual species.

Dictyostelium and *Polysphondelium* are increasingly being used as models for social evolution, which makes it particularly important to understand whether interactants actually come from the same species. It is also useful to understand population structure and the degree to which genetic variation tracks geographic distance within species. In the present study we sequenced informative regions of nuclear rDNA, and in some cases, also mtDNA, from at least 20 clones each of *D. discoideum*, *D. giganteum*, *D. purpureum*, and *P. violaceum* from a variety of sites. We found significant structure in the latter two species, and less in the first two. We found evidence for cryptic species that was supported by patterns of macrocyst formation in *D. purpureum* and *P. violaceum*, but not in *D. discoideum* or *D. giganteum*.

Dictyostelium anatomy and phenotypes

Pascale Gaudet¹, Jeffery G. Williams², Petra Fey¹, Rex L. Chisholm¹

¹dictyBase, Northwestern University, Biomedical Informatics Center, 750 N. Lake Shore Drive, Chicago, IL, 60611, USA., ²School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, United Kingdom

Dictyostelium genes are annotated at dictyBase in a variety of ways, such as the inclusion of Gene Ontology terms and other functional annotations. Ontologies can be defined as “collections of formal, machine-processable and human-interpretable representations of entities, and the relations between those entities”. The most well-developed and extensively used ontology for genomics is the Gene Ontology (GO), which contains over 20,000 terms describing molecular functions, biological processes and cellular components. Other ontologies exist that describe genomic sequences, cells, anatomy, and others entities. Scientific curators review the literature and associate ontology terms to genes and gene products as appropriate.

We have constructed an ontological description of the *Dictyostelium* anatomy that defines the structural makeup of *Dictyostelium* and its composing parts, including its different cell types¹. The anatomy is organized following the *Dictyostelium* life cycle. This ontology allows the annotation of species-specific events, such as phenotype and gene expression, that facilitates data retrieval by researchers using tools available at dictyBase. This promotes accurate annotation, which in turn facilitates searching and makes use of the ontology’s power for data mining and analysis.

We have implemented a new schema for phenotype annotation in dictyBase whereby phenotypes are associated with the strains studied, which in turn link to the gene(s) mutated in that strain. For each description of a genetically manipulated strain in the literature a strain record is created that describes the characteristics of the strain including the genetic modification, the parent strain, the transformation vector, the selection marker and a short description. Once the strain record is created, curators add phenotype annotations to the strain’s database entry. For example the *aarA*- strain is associated with the phenotypes ‘decreased sporulation’ and ‘aberrant fruiting body morphology’. Phenotypes are described using a pre-composed phenotype ontology based on the EQ pheno-syntax developed by the National Center for Biomedical Ontology² where the entity can come from other ontologies such as the Dicty anatomy¹, GO³, or ChEBI⁴ and the quality consists of simple terms such as ‘decreased’, ‘abolished’, and ‘precocious’. Phenotype terms often also contain synonyms to maximize searchability. The use of a consistent vocabulary facilitates searching and allows grouping of related phenotypes based on specific criteria.

dictyBase is supported by NIH grants GM64426 and HG00022.

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Comparative Genomics in Social Amoebas

Gernot Glöckner¹, Andrew Heidel¹, Ludwig Eichinger², Angelika A. Noegel², Pauline Schaap³, Matthias Platzer¹

¹Leibniz Institute for Age Research, Fritz-Lipmann Institute Beutenbergstr. 11 D-07743 Jena,

²University of Cologne Medical Faculty, Institute of Biochemistry I, Joseph-Stelzmann-Str. 52,

D-50931 Cologne, Germany, ³School of Life Sciences. University of Dundee, Dundee DD1 5EH, UK

After completion of the *D. discoideum* genome in 2005 (1,2) it was obvious that a comparative genomics approach has to follow this endeavour to address the following questions: i) How different are the members of this evolutionary branch in terms of general genomic properties ii) Which genes are species-specific and which genes are genus specific iii) Can we define evolutionary events which led to the formation of this group of organisms iv) Can we find synteny between distantly related species? To address these questions we sequenced two species within the social amoebas group to a depth of ~18 using Sanger sequencing and next generation sequencing technology. The targeted species *D. fasciculatum* belongs to group 1 and *P. pallidum* to group 2 according to (3). The genome size and coding density of both species is comparable to that of *D. discoideum*. We put all sequence and annotation data in a database dedicated to genome comparisons (4). An initial analysis revealed that synteny is restricted to only a handful of all genes indicating a frequent reshuffling of the genomes. This analysis also enables us to define the core gene set of social amoebas, which we currently estimate to comprise around 7500 genes.

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The evolutionary genomics of *Dictyostelium discoideum*

Jonathan Flowers¹, Angela Stathos¹, Elizabeth Ostrowski², David Queller², Joan Strassmann²,
Michael Purugganan¹

¹Department of Biology, Center for Genomics and Systems Biology, 100 Washington Square East,
New York University, New York, NY 10003 USA, ²Department of Ecology and Evolutionary
Biology, Rice University, 6100 Main St. Houston TX 77005 USA

Dictyostelium discoideum is a single-celled amoeba that, upon starvation or other types of environmental stress, can form multicellular entities that migrate and form fruiting bodies. Its status as a developmental model system and ability to form cooperative units has made this species a useful organism for studying the dynamics of social interactions. Here, we assess levels and patterns of SNP variation by assaying genome-wide molecular variation in *D. discoideum*, using samples from a Virginia population as well as several strains collected throughout the US. We focus mainly on genes in chromosome 4, but include markers throughout the genome. Our analysis of sequence variation in 24 clones indicates very low levels of single nucleotide polymorphism (SNP) in this species. Moreover, linkage disequilibrium (LD) in this species decays within less than 10 kb and there is evidence of evolutionary recombination consistent with widespread sexual reproduction in nature. The results from these studies will allow us to use genomic approaches to map genes underlying natural social variation and potential regions of selective sweeps in *D. discoideum*.

An RnoA-PldB-PaxB complex potentially regulates actin cytoskeleton and adhesion in *Dictyostelium discoideum*

Rebecca Fernandez, Derrick Brazill
Hunter College, CUNY

Regulation and organization of the actin cytoskeleton are fundamental processes for cell shape, motility, and adhesion. A number of proteins have been shown to be important in this regulation. ARNO, a mammalian guanine nucleotide exchange factor for ARFs, has been linked to physiological functions mediated by the actin cytoskeleton. Phospholipase D (PLD), the enzyme responsible for the production of the second messenger phosphatidic acid, has also been associated with cell migration and actin cytoskeleton rearrangements. Paxillin mediates interactions between the extracellular matrix and the actin cytoskeleton by serving as a docking site for both signaling molecules and actin associated proteins. Given their communal association with the actin cytoskeleton, we explored the interactions between the *Dictyostelium discoideum* ARNO, PLD, and paxillin homologs RnoA, PldB, and PaxB, respectively. Cells lacking PaxB are known to have significantly diminished cell-substrate adhesion. However, the overexpression of PldB can rescue the adhesion defect of paxB null cells, implying a genetic interaction between paxB and pldB. Furthermore, RnoA, PldB, and PaxB all co-localize with actin at the cell-substrate interface and at the cell cortex. Co-immunoprecipitation studies suggest RnoA, PldB, and PaxB interact during vegetative and starved conditions. This interaction does not appear to depend on a single protein as RnoA and PaxB are able to associate in the absence of PldB and RnoA and PldB are able to associate in the absence of PaxB. The interaction is also not dependent on F-actin as disruption of the actin cytoskeleton by Latrunculin A treatment did not disrupt RnoA, PldB, and PaxB interactions. These data suggest a possible role for RnoA, PldB, and PaxB interactions during adhesion.

Structural Dynamics of Cytokinesis

Alexandra Surcel, Elizabeth Reichl, Janet Effler, Yixin Ren, Douglas Robinson
Johns Hopkins School of Medicine, Department of Cell Biology

Contractile networks are integral contributors to cytokinesis and cell motility. These networks depend on the mechanochemistry of myosin-II to generate sliding force on the actin polymers. However, to be contractile, the networks must also be crosslinked by proteins and to change the shape of the cell, the network must be linked to the plasma membrane. Our work on cytokinesis dynamics has revealed the interplay between myosin-II, the actin network and crosslinker proteins in promoting cleavage furrow ingression. We demonstrate that myosin-II affects local dynamics by increasing furrow stiffness and by slowing furrow ingression. Conversely, regulators such as dynacortin, fimbrin, and RacE act globally in the cell cortex to antagonistically affect ingression while also influencing cortical tension. Our measurements reveal that the kinetics of actin crosslinkers are slower at the cleavage furrow cortex than at the polar cortex, correlating with the function of each crosslinker in cortical mechanics. We have also demonstrated that the cleavage furrow cortex of adherent *Dictyostelium* cells is assembled as a meshwork of actin filaments and that myosin is heterogeneously distributed rather than organized into a smooth uniform concentric belt. This morphology is more similar to the contractile meshwork found in adherent fibroblasts rather than the contractile ring found in *S. pombe* or HeLa cells. These studies indicate that it is the interactions between myosin-II and actin crosslinkers that increase local mechanical stress in the furrow region, coupled with a fluid pressure differential that squeezes cytoplasm from the midzone, driving furrow ingression. This hypothesis has been further supported by our discovery of a mechanosensory pathway that senses and controls cell shape change in *Dictyostelium*. We have identified a critical relationship between the actin crosslinker cortexillin-I and myosin-II, which forms the basis of this mechanosensory module. Both myosin-II and cortexillin-I require each other in order to be recruited to cortical sites of increased mechanical strain. Our results suggest a model in which myosin-II and cortexillin-I communicate through the actin filament to direct their co-localization in response to mechanical strain. These two sets of data taken together suggest that cytoskeleton dynamics and myosin-II localization are controlled by the combination of biochemical regulators and mechanical strain in the actin network.

Dynamic interactions between chemoattractant GPCR and heterotrimeric G-proteins in membrane of living cells revealed by FRAP and Single-molecule imaging

Xuehua Xu, Joseph A. Brzostowski, Pavel Tolar, Tian Jin

Chemotaxis Signal Section Laboratory of Immunogenetics National Institute of Allergy and Infectious Diseases, NIH

Ligand binding to GPCR triggers signaling events through heterotrimeric G-proteins. But it is not clear whether receptors and G-proteins form receptor/G-protein complexes in the membrane prior to receptor activation. Here, we have used FRAP and single-molecule imaging to characterize the behavior of YFP-tagged cAR1 GPCR and heterotrimeric G proteins in *Dictyostelium discoideum*. Our work suggests that ligand binding to cAR1 receptors promotes dynamic interactions between the activated receptors and heterotrimers G-proteins and thus facilitates activation of heterotrimeric G-proteins in living cells.

Workshop: Community Resources

1. New tools at dictyBase (Petra Fey)

- The first two topics on the dictyBase poster (P2), domains and textpresso, will be focused.

2. An update on the Dicty Stock Center and its future (Jacob Franke)

3. Two resource sites from Japan (Hideko Urushihara)

- NBRP-nenkin, Japanese stock center, (P47) and *Acytostelium* gene database (P1) will be detailed.

Posters

Developmental genes in *Acytostelium subglobosum*, a group 2 species without cell-type differentiation

Hideko Urushihara¹, Hidekazu Kuwayama¹, Kyoko Uchi¹, Yuji Kohara², Hiroshi Kagoshima², Tadasu Shin-i², Kazuko Ohishi², Takehiko Itoh³, Tadatsugu Taniguchi³, Asao Fujiyama⁴

¹University of Tsukuba, ²National Institute of Genetics, ³Mitsubishi Research Institute, ⁴National Institute of Informatics

Asexual development of *Dictyostelium discoideum* is known for its simplicity with cell differentiation into only two major cell types, spores and stalk cells. On the other hand, species in *Acytostelium* form fruiting bodies with acellular stalks. Apparently no cell differentiation takes place in the development of *Acytostelium*. We are interested in this multicellularity without cell differentiation, and have established an experimental system in *Acytostelium subglobosum*. Expecting that the genomic information associated to the ability of cell differentiation could be extracted by genome comparison between the species with and without cell differentiation, we performed the whole genome shotgun analysis of *A. subglobosum*. So far we obtained the nucleotide sequence of about 237 Mb from 345,000 reads, which were assembled into 6,800 contigs providing total extension of 33.2 Mb genomic sequence. The tblastx searches revealed that some contigs match with very high homology to stalk-cell specific genes in *D. discoideum* such as *ecmA* and *ecmB*. This suggests that evolutionary invention of stalk cells had been achieved by utilizing pre-existing genes rather than generating new genes. However, for more precise comparison, it is necessary to construct better gene models in *A. subglobosum* as well as to perform molecular biological studies. For the former, we are making efforts to add as many cDNA data as possible to the *Acytostelium* gene database, which is based on *D. discoideum* gene models. The database is now open for general access and users' feedback would be appreciated.

dictyBase: Update 2008

Petra Fey, Pascale Gaudet, Siddhartha Basu, Eric Just, Daniel Schneider, Yulia Bushmanova, Warren A. Kibbe, Rex L. Chisholm
dictyBase, Northwestern University, Chicago, IL, USA

At dictyBase we continually strive to provide new data and tools for our users. In the past year this included new linux-based servers that greatly improved performance and allowed us to more rapidly develop new features and incorporate new data sets. Exciting new features we have added in the past year include:

- A graphical display of InterPro **Protein Domains** has been incorporated into each gene page. The domain information is fetched on the fly from the Uniprot DAS (Distributed Annotation System) resource assuring current and accurate domain visualization for *Dictyostelium* proteins.
- In collaboration with WormBase, **Textpresso for Dicty**, an information extraction tool for *Dictyostelium* literature, was implemented [1]. This enables users to search full text Dicty papers and allows the download of references in multiple formats including EndNote.
- We started a collaboration with **UniProt/Swiss-Prot** to increase annotations of *Dictyostelium* proteins in this important protein database. Reciprocal links on the gene records facilitate users' access to complementary information in both databases.
- New **Gene Predictions** were generated with geneid using a customized training set based on *Dictyostelium* curated models, which identified 71 new genes.
- A new **Gene Identifier** has been added to every gene. This Gene ID has the format DDB_G1234567 and is stable regardless of the curation status of the gene. dictyBase IDs (DDB1234567) will continue to correspond to sequences associated with genes.
- The **Dicty Life analysis** of growth and development phenotypes of more than 2000 REMI mutants [2] was integrated into dictyBase. The phenotypes from this project are now annotations in dictyBase and the videos and other analyses generated by the study are linked to the relevant strain and gene pages in dictyBase.
- A **Curation Status** note has been added to gene pages to inform users about the annotation status and the date annotations were last reviewed.

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Dictyostelium PKB/AKT related kinase may function as an activator for a putative switch of stalk formation

Hiroshi Ochiai^{1,2,3}, Koki Nagayama², Kosuke Takeda^{3,4}, Masashi Fukuzawa², Shigeharu Takiya¹, Tetsuo Ohmachi²

¹Division of Genomedynamics, Creative Research Initiative" SOUSEI", Hokkaido University, Sapporo 060-0810, Japan, ²Department of Biochemistry and Biotechnology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan, ³Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, ⁴Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093, USA

In *Dictyostelium*, the molecular signal that triggers entry from first finger into slug is not known; the protein kinase B related kinase (PKBR kinase) may be involved in the transition. The *pkbR* null organisms have a strong tendency remain at the first finger stage, although during the further development the organisms present a wide range of terminal phenotypes, including tight mounds, abnormal first fingers, normal-looking fruiting bodies. A PKBR kinase has a kinase domain and a C-terminal one related to those of protein kinase B (PKB/AKT), but not pleckstrin homology (PH) domain that is common among other PKBs. Using promoter::*lacZ* constructs, we previously reported that the *pkbR* null mutant expresses the prestalk-specific genes, *ecmA/O*, *ecmA* and *ecmO*, but not the *ecmB*. which usually appears at the core region of a migrating slug. Using an *in situ* hybridization technique, we here compared the *pkbR* null mutant and parental Ax2 cells for expression of 12 genes necessary for culmination, and *cotC* gene necessary for prespore differentiation. Actually, The four two-component signal gene products that are involved in the regulation of cAMP signaling decreased distinctively during the delayed development of the *pkbR* null mutant. However, during the development a spore coat gene (*cotC*) product and three products of transcription factors that are localized in prestalk cells decreased, but **not** so much. Products of adenylate cyclase *acrA* gene also decreased similarly during development, although *acaA* product for aggregation is **not** so decreased. Importantly, *pka-C* gene product decreased clearly, although *pka-R* product **rather** continued to express in both prestalk and prespore cells. By contrast with the expression seen in the *pkbR* null mutant, the expression of almost all these genes of parental Ax2 cells increased greatly during development. A definite trend toward decrease of several gene products in *pkbR* null cells may reflect the presence of a hierarchic gene(s) that switches expression of these stalk related genes. The switch gene may be activated by the PKBR protein that is a serine/threonine kinase.

A new mode of prespore gene regulation: DdMybF functions as a repressor for prespore differentiation

Kei Tomita, Masashi Fukuzawa

Faculty of Agriculture and Life Science, Hirosaki University

Dictyostelium possesses a class of MYB transcription factors, the “SHAQKY” family MYBs, of a type contains a single MYB DNA binding domain found only in plant. DdMybE belongs to this family and is known to regulate the DIF signal transduction pathway towards pstO (and possibly a part of pstB) cell differentiation. We have been characterizing another single domain MYB, DdMybF, and will present evidences suggesting its possible function as a repressor for DIF-inducible prespore differentiation.

The mybF transcripts were detectable at vegetative cells and accumulated in two-peaks fashion at 8h and 24h of development. The mybF promoter-lacZ showed uniform scattered expression in slugs with slightly higher staining in the prestalk region. Mature spores showed a variety of staining intensity.

MybF null mutant develops in normal time course until slugs but struggles to culminate for a few days, resulting in very low numbers of mature fruiting bodies. Although the patterns of prestalk/stalk markers were normal, the prespore pattern was heavily affected. The pspA-lacZ was uniformly expressed in a slug, and the stalk of a fruit was completely blue. The cotC-lacZ showed the similar pattern. These suggest that MybF functions as a repressor for prespore pathway in the prestalk region.

The “presporely” phenotype of MybF null was also true in monolayer assay with pspA-lacZ. Cerulenin (50 μ M) and DIF are known to repress pspA-lacZ in wild-type cells. MybF null exhibits very high activity of pspA-lacZ even in the presence of these additives. Very interestingly, DIF rather induces pspA-lacZ expression in MybF null. We also found that, when cerulenin concentration was raised to 500 μ M at which no expression of pspA-lacZ was observed in Ax2, the DIF induction in the null was disappeared although there was still high expression of the gene.

These results suggest that DIF may have an intrinsic ability to induce prespore cells efficiently in the presence of other differentiation-inducing factors such as PSI-2 or MPBD, and DdMybF can be a major transcription factor to repress this pathway.

Dla1, La-like RNA-binding domain-containing protein, is important for cell growth and cell-type proportioning of *Dictyostelium discoideum*.

Toshinori Usui, Kiyotaka Shibata, Tomoaki Abe

Department of Biological Engineering, Faculty of Science and Engineering, Ishinomaki Senshu University, Ishinomaki, Miyagi 986-8580, Japan.

Dla1, a protein containing a highly homologous RNA-binding domain to metazoan La and Lhp1 in *S. cerevisiae*, was identified from a REMI mutant strain defective both in growth and development. La is known as the autoantigen of the anti-SSB autoantibody found in patients of Sjogren's syndrome, systemic lupus erythematosus, and neonatal lupus. La has been characterised as the transcription initiation factor for RNA polymerase III-transcribed genes, however, its complex multi-functionality has been suggested in many scientific reports. Dla1 is composed of 621aa and predicted MW is 68KDa. With real time PCR analysis, we found that its expression is constitutive throughout vegetative and developmental stages, although the mRNA-level is relatively high at the vegetative stage and at the early culmination stage. We produced Dla1⁻ strains with homologous recombination and analysed their phenotypes. When Dla1⁻ cells are fed on HL-5 medium, their growth rate is similar to wild-type Ax-2's, however, they grow significantly slower than Ax-2 when they are fed on *K. aerogenes* grown on SM-plates. Dla1⁻ cells can form fruiting bodies, however, they are unstable and many of them can not remain standing. Their basal discs are not properly formed, however, thick layers of basal disc cells are accumulated at the bottoms. We tested the lacZ staining patterns of cell-type specific markers, and found that EcmA and EcmB markers are highly over-expressed in Dla1⁻. The region stained with PspA marker is reduced on the contrary, particularly the unstained region occupied by rear-guard cells is increased. It was also confirmed with prespore-antigen staining. These results suggest that Dla1 shows multi-functionality during growth and development.

Some cations affect on development of the cellular slime mold, *Dictyostelium discoideum*.

Yuka Satou, Yuushin Kamada, Saburo Uchiyama
Department of Science Education, Faculty of Education, Iwate University

We are interested in some cations affecting the developmental fate of the cells of *Dictyostelium discoideum*. Kubohara (1) reported that zinc ions promote the conversion of pre-stalk or pre-spore cells to stalk cells under submerged conditions. In this study, we demonstrated an effect of some cations on the developmental fate of the cells in *D. discoideum* NC-4.

Cells were harvested at the log phase, and then cells were incubated with or without some cations for 2 hours. The cells incubated with cation were stained by fluorescent dye (CFSE) at the same time. Then they were mixed and allowed to aggregate and culminate on the cellophane sheet on the plane agar. Slugs on the cellophane sheet were put on a slide glass and observed under fluorescent microscope.

Cells treated with zinc ion (50mM) or sodium ion (100mM) were localized in the pre-spore and spore region of the slug. The results of the effect of the zinc ions were not coincide with the results reported by Kubohara (1). On the other hand, cells treated with copper ion (5mM) were localized in the pre-stalk region of the slug. Cells treated with calcium ion (100mM) were not affected on the distribution in the slug. Cells treated with calcium ion (100mM) and calcium ionophore (A23187) were excluded from the aggregates. To elucidate the effect of osmotic pressure, cells were treated with sucrose (200mM). The results show no effect on the distribution of treated cells in the slug. The possible role of these ions will be discussed.

1. Kubohara, Y. 1995. Zinc ions promote prestalk-to-stalk and prespore-to-stalk conversions in *Dictyostelium discoideum*. FEMS Microbiol. Lett. 134: 15-18.

Developmental inhibitor of *Acytostelium subglobosum* that forms fruiting bodies without cell differentiation

Kyoko Uchi, Hidekazu Kuwayama, Ryuji Yoshino, Hideko Urushihara
Graduate School of life and Environmental Sciences, University of Tsukuba

Asexual development or fruiting body formation in *Dictyostelium discoideum* is known for its simplicity with cell differentiation only into two cell types, spores and stalk cells. Cells of *Acytostelium*, classified as the most primitive genus of the cellular slime molds, however, form fruiting bodies with acellular stalks. Apparently no cell differentiation takes place in *Acytostelium*. We are interested in this multicellularity without cell differentiation, and taking a comparative genomic approach using *Dictyostelium discoideum* and *Acytostelium subglobosum* to reveal the genomic information associated with the ability of cell differentiation. As a parallel effort, we attempted to establish an experimental system in *A. subglobosum* for molecular biological and reverse genetic studies. When we isolated its clonal lines to eliminate an unknown yellow bacterium (UNY) co-existed in the original stock culture, their growth rates were much higher but multicellular development was much less extensive. Fruiting body formation was possible only at lower densities than 2.5×10^5 cells/cm². Interestingly, the development at higher cell densities were dramatically enhanced by the presence of live UNY, which could be substituted by activated carbon. These results suggest that UNY contributes to the multicellular development of *A. subglobosum* by inactivating the developmental inhibitor(s) secreted by the amoebae themselves. Since the inhibitory substance(s) was volatile, we suspect it to be either ammonia or ethylene, both having been reported to affect the multicellular development of *Dictyostelium* species.

Growth kinetics and differentiation in *Dictyostelium*Noritaka Masaki¹, Satoshi Sawai^{1,2}¹ERATO Complex Systems Biology Project, JST, ²Graduate School of Arts and Sciences, University of Tokyo

Feeding behavior of *Dictyostelium discoideum* cells ceases as cells enter multicellular development upon starvation. To investigate the kinetics of the transition, we studied the time course of the changes in the phagocytic activities. From flow-cytometric analyses of starved cells incubated together with fluorescently labeled bacteria, we discovered that the *Dictyostelium* cells consist of two distinct populations during the first 7 hr of development; one that remains in the feeding state and the other that switches to a non-feeding state. Expression level of a gene encoding cAMP receptor (*carA*) in the non-feeding population increases rapidly and peaks at 3hr. In contrast, *carA* expression of the cells that remain in the feeding state is low even after 4hr into starvation. In agreement with earlier studies, we found that strains deficient in cAMP signaling such as knockout mutants of adenylate cyclase (*aca-*) and *carA* do not lose phagocytic ability even after 7hr into starvation. Artificial application of cAMP pulses does not rescue the mutant phenotype. These results clearly demonstrate that differentiation of *Dictyostelium* cells from a feeding state to a non-feeding state is dependent on cell-cell cAMP signaling and that asynchrony of this transition is related to the heterogeneity in the expression level of the cAMP receptor. In a batch co-culture, we show that the relative growth rate of *Dictyostelium* cells is determined by the ratio between the number of bacteria and *Dictyostelium* and that there exists a critical ratio below which genes required for the cAMP signaling such as PDE are expressed. Based on these data and a dynamical model, we discuss how an asynchronous transition from a single-cell to a multicellular life-cycle may be viewed as an optimization problem in a fluctuating nutrient environment.

Biological activities of DIF-1 derivatives

Yuzuru Kubohara¹, Haruhisa Kikuchi², Yoshiteru Oshima²

¹Gunma University, Institute for Molecular and Cellular Regulation, ²Tohoku University, Graduate School of Pharmaceutical Sciences

The differentiation-inducing factor-1 (DIF-1) is a lipophilic signal molecule (chlorinated alkylphenone) that induces stalk cell differentiation in the cellular slime mold *Dictyostelium discoideum*. In addition, DIF-1 and its derivatives have been shown to possess anti-leukemic activity and glucose consumption-promoting activity in vitro in mammalian cells, which leads to the expectation that DIF derivatives may have therapeutic potential for the treatment of leukemia, obesity, and/or diabetes. In the present study, in order to assess the chemical structure-effect relationship of DIF-1 and to develop useful DIF derivatives, we synthesized 8 derivatives of DIF-1 and investigated their stalk cell-inducing activity in *Dictyostelium* cells and pharmacological activities in mammalian cells. Of the derivatives, two amide derivatives of DIF-1, whose hydrophobic indexes are close to that of DIF-1, induced stalk cell differentiation as strongly as DIF-1 in *Dictyostelium* cells, indicating that appropriate hydrophobicity should be important for their stalk cell-inducing activity. These results also suggest that the two agonists should be excellent tools suitable for the analysis of the mechanism of stalk cell differentiation in *D. discoideum*. Although some DIF derivatives were active in suppressing cell growth in human K562 leukemia cells and in promoting glucose consumption in mouse 3T3-L1 cells, they were almost inactive in suppressing cell growth in 3T3-L1 cells (non-transformed cells). These results suggest that DIF derivatives may be utilized for the medical treatment of leukemia, obesity, and/or diabetes.

A novel differentiation inducing factor with low molecular weight in *Dictyostelium discoideum*: its purification and characterization

Yoshiaki Takaya¹, Masashi Fukuzawa², Rie Hotta¹, Manabu Nakagawa³, Akiko A. Oohata⁴

¹Faculty of Pharmacy, Meijo University, ²Department of Biology, Faculty of Agriculture and Life Science, Hirosaki University, ³Chemical laboratory, Kansai Medical University, ⁴Biological laboratory, Kansai Medical University

Previously, we reported two prespore-cell inducing factors exist in conditioned medium; one is a glycoprotein named prespore-cell inducing factor (ψ factor, or PSI-1), and the other is a heat stable, dialyzable factor(s). Under submerged incubation of cells, co-existence of both factors induces efficiently prespore cell differentiation at very low cell densities in the presence of cAMP. In this study, we purified and characterized the latter dialyzable factor based on the ability of inducing prespore cell differentiation *in vitro*. Interestingly, the purified factor induced not only prespore but also prestalk specific genes. The factor began to be secreted after the starvation and appeared to cease secretion at aggregation, preceding expression of ψ factor gene. Based on NMR and mass spectra of the purified factor, it would possess quinone moiety with mol wt 208, and was putatively designated as a quinone derivative factor (QF). Twenty-six nM of the factor was required for a half maximal prespore cell differentiation under our *in vitro* conditions. We propose that QF plays a key role in the process of acquisition of differentiation commitment, before ψ factor specifically induces prespore cell differentiation.

Cotransporters and the uptake of the epilepsy treatment, Valproic acid, using the biomedical model *Dictyostelium discoideum*

Nicole Terbach¹, Dmitri Gordienko², Nigel A. Brown², Robin S. B. Williams¹

¹School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, United Kingdom, ²Division of Basic Medical Sciences, St. Georges University of London, London, United Kingdom

Valproic acid (VPA), a branched short chain fatty acid, has been used to treat epilepsy since 1967. It is, in fact, the most highly prescribed treatment for epilepsy. More recently, it has been found to be an effective treatment of bipolar disorder and for migraine prophylaxis. Effects on cancer, Alzheimer's disease and latent HIV are also being investigated. Despite the drug being used for over 40 years, the mechanism of its uptake into cells is currently unknown. We investigated the uptake of VPA into the biomedical model system *Dictyostelium discoideum* with a view of transferring the results to mammalian cortical cells in the future.

Since VPA blocks *Dictyostelium* growth and development, we screened a *D. discoideum* REMI mutant library for VPA resistant mutants, and identified one mutant resistant to VPA in both growth and developmental screens. The protein encoded by the ablated gene shows strong homology to members of the human bicarbonate transporter family SLC4 and contains a bicarbonate transporter domain and transporter signature. No other gene in the *Dictyostelium discoideum* genome is homologous to this family, so it is likely to represent the only member of its class in the model organism.

Transcription analysis of the *Dictyostelium* SLC4 gene showed expression throughout the 24 hour developmental cycle, but predominantly in growing cells. We overexpressed the gene with a GFP-tag and created knockouts using the Cre-loxP system. SLC4 ablation or treatment with anion transport inhibitors reduced the effect of VPA on *Dictyostelium* development, consistent with a role of this transporter in VPA uptake. ³H-VPA uptake was also analyzed in growing wild type, knockout and inhibitor treated cells and cells pulsed with cAMP for 5 hours. Competition studies of ³H-VPA with straight chain mono-, di- and tricarboxylic acids of different length and VPA derivatives like valpromide were conducted to determine the structural specificity of the VPA transport mechanism. Results from this project suggest that the SLC4 family of proteins are likely to play an important role in the uptake of this widely used therapeutic drug.

SunB, a novel type of SUN-domain-containing protein, is implicated in cytokinesis and development in *Dictyostelium discoideum*

Nao Shimada^{1,2,3}, Hiroyuki Adachi², Koji Yoda², Takefumi Kawata¹

¹Department of Biology, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan, ²Department of Biotechnology, University of Tokyo, Yayoi, Bunkyo-Ku, Tokyo 113-8657, Japan, ³Research Fellow of the Japan Society for the Promotion of Science

We isolated and analyzed the *sunB* gene, whose product harbors a novel type of Sad1 and UNC (SUN) domain. In accordance with the uniform expression of *sunB*, endogenous SunB protein was localized in all cells until culmination began. During culmination, the SunB protein seemed to be degraded in the stalk tube, prestalk AB (pstAB) cells. The SunB protein was localized in organelle membranes although this type of organelle has not yet been identified. We also obtained a *sunB* gene disruptant that showed a defect in cytokinesis. During development, the mutant strain formed a mound with multiple tips and failed to culminate. The mutation was cell autonomous and the mutant showed reduced expression of the prespore marker gene *pspA*, and elevated expression of marker genes for prestalk AB cells. Thus, the *sunB* gene appears to function in both cytokinesis and normal morphogenesis. Interestingly, pstAB cell-specific degradation of SunB may require Dd-STATA, a *Dictyostelium* homologue of the metazoan STAT (Signal Transducers and Activators of Transcription) proteins. To support this hypothesis, overexpression of a dominant negative SunB-GFP fusion protein in a hypomorphic Dd-STATA mutant strain suppressed the culmination defect. These data imply a negative regulation by Dd-STATA, directly or indirectly, on SunB function that might control entry into culmination.

New components of the *Dictyostelium* PKA pathway revealed by Bayesian analysis of transcriptional profiling data

Anup Parikh^{1,2}, Eryong Huang², Chris Dinh³, Blaz Zupan^{2,4}, Adam Kuspa^{1,3}, Devika Subramanian⁵, Gad Shaulsky^{1,2}

¹Graduate program in Structural Computational Biology and Molecular Biophysics, ²Department of Molecular and Human Genetics, ³Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, ⁴Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia, ⁵Department of Computer Science, Rice University, Houston, TX

Cellular function is a consequence of the concert activity of thousands of gene products. The expression and activity of these genes are regulated by complex networks and better understanding of these networks is essential to elucidating cell functions and pathologic phenotypes. The cAMP-dependent protein kinase (PKA) controls a wide range of cellular functions, and is implicated in many human diseases. PKA has been studied in numerous organisms for its role in development, memory and learning and cancer. The biochemical role of PKA in development and differentiation has been extensively studied in *Dictyostelium discoideum* but the underlying transcriptional regulatory network for the pathway is poorly understood. The wealth of knowledge about PKA signaling in *Dictyostelium*, as well as the large amounts of time series expression data available makes it an excellent system for computational modeling of the transcriptional network.

High throughput techniques in molecular biology, such as microarrays, provide a means to study cellular processes, but the inherent complexity, high variability and small sample size are major obstacles. Bayesian network models have a great potential for representing and uncovering pathways because of their ability to model complex multi-layered stochastic processes, as well as to integrate various sources of information. Using a systems biology framework, the accuracy of current modeling techniques can be improved by utilizing time series expression data, as well as biological knowledge from the literature. Furthermore, directed perturbation experiments, in which candidate genes are mutated, are crucial for validation of computational predictions, as well as refining the models to reflect causality relationships.

We have developed an iterative process that integrates modeling and experimental biology for discovery of new components of the PKA pathway. We have made computational predictions using single gene expansions of Bayesian networks centered on the core PKA pathway. The method ranked new genes as possible members of the known PKA pathway by calculating the addition score of the gene into the core Bayesian network. To test the predictive power of the method, we focus on the top 5% of the ranked genes. We first searched the literature and public databases for mutations in these genes. We postulated that mutations in genes involved in the PKA pathway should exhibit clear developmental phenotypes. Of the 209 top ranked genes 12 have previously been characterized in the literature and all 12 are important for proper development. To further validate our approach, we tested 5 mutations that have never been tested before for developmental defects. The proportion of developmental mutants in this group of genes is statistically significantly high (hypergeometric test; P-value < .05), supporting the hypothesis that new components in the pathway can be discovered by our approach. One of the genes we found encodes a putative bZIP transcription factor, which resembles CREB. The sequence similarity is supported by the ability of the protein to bind a CRE-containing DNA fragment in vitro.

shRNA- Directed Knockdown of Gene and Analysis of *Dictyostelium* Development

Vikas Sonakya, Hideshi Otsuka, Marie McAnuff, Subrata Chowdhury, Albana Thomaraj, Julian Gross, Thomas Winkler^a, Robert Dottin

Department of Biological Sciences, Hunter College, City University of New York, New York, NY10065, USA, ^aInstitut for Pharmazeutische Biologie, Universitat Frankfurt, Germany

In present research we provide evidence of an inducible down-regulation of specific genes using RNA interference in *Dictyostelium discoideum*. This system consists of an autonomous replicating *Dictyostelium* plasmid capable of transcribing cloned oligonucleotides into short hairpin RNA (shRNA). It contains a high efficiency tetracycline inducible pol III promoter from a *Dictyostelium* tRNA gene. Although the U6 and H1 mammalian promoters transcribe with high efficiency, they have not been identified in *Dictyostelium* or other eukaryotic microorganisms. Instead, typically, pol II promoters are used to transcribe long inverted repeat DNA to mediate RNAi. Nonetheless, using these long inverted repeat DNA sequences (1200 bases), we have previously shown that the knockdown of specific genes such as adenylyl cyclase A or transcription factor myb B, involved in early aggregation of *Dictyostelium*, blocks aggregation and arrests development. We report here the use of a Pol III tRNA promoter to mediate the knockdown of several *Dictyostelium* genes involved in development. While some oligonucleotides specifically knock down the catalytic subunit of phosphoprotein phosphatase PP2Ac, others target mRNA encoding homologs of proteins with kelch repeats (KRH1 and KRH2) that are thought to alter cAMP-dependent protein kinase A (PKA) activity. Therefore inducible, highly efficient synthesis of small RNAs can now be used to characterize genes and small RNAs involved in development of *Dictyostelium* and to identify their mammalian counterparts.

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Differential gene expression between sexually mature and immature cells of *Dictyostelium discoideum*

Saeki, K.¹, Satoh, T.¹, Muramoto, T.¹, Skelton, J.², Kay, R.³, Kuwayama, H.¹, Urushihara, H.¹
¹University of Tsukuba, ²Sanger Center, ³MRC Laboratory of Molecular Biology

The sexual process in *Dictyostelium discoideum* is initiated by maturation of amoebae to become fusion-competent cells or gametes under dark and submerged conditions. Those cells then undergo cell fusion with opposite mating-type cells to form zygotes. Since this process easily manipulated and cell fusion occurs synchronously *in vitro*, it is a superb experimental system for studying the cell fusion mechanisms. Moreover, it is interesting to know how *D. discoideum* amoebae differentially or commonly use the genes for alternative developmental pathways under different circumstances. We have focused our analysis on the genes induced or enhanced during sexual maturation. Construction of a gamete-specific subtraction library in KAX3 and its analysis have revealed the involvement of *gmsA*, which is homologous to a *Chlamydomonas* gamete-specific gene *a2*, in cell fusion, and *tmcB* and *tmcC*, which encode possible RNA-interacting proteins, in post-fusion processes. In the present study, we extended our analysis to a more genome-wide search by using DNA microarray carrying 9.2K genes in *D. discoideum*. In addition to KAX3, gene expression levels were compared between sexually mature and immature cells in V12, an opposite mating-type strain. Large scale analysis of down-regulated genes was performed for the first time. We found a total of 40 up-regulated (>2x) and 135 down-regulated (<1/2) genes. The microarray data were mostly confirmed by real-time RT-PCR. The knockout mutants of 2 up-regulated genes, *warA*, *egeA* (obtained from Dicty Stock Center) were normal in the sexual development. Through over-expression experiments of the down-regulated genes, we found that suppression of *ponA* was necessary for effective cell fusion. This gene encodes a membrane glycoprotein named Ponticullin that controls actin polymerization. Expression of its paralogue, *ponB*, was suppressed whenever *ponA* was enhanced, suggesting the possibility that *ponB* is required for cell fusion.

Functional analysis of *rasX* group genes

Yoko Furuya¹, Mariko Kunitani¹, Tetsuya Muramoto², Hidekazu Kuwayama¹, Hideko Urushihara¹
¹Graduate School of Life and Environmental Sciences, University of Tsukuba, ²Division of Cell and Developmental Biology, University of Dundee

We have been studying the sexual process of *Dictyostelium discoideum* as a model system for fertilization. To comprehend the genetic system controlling the sexual maturation and cell fusion, we constructed a gamete-specific subtraction library (FC-IC library) to enrich the sexuality-related genes, and analyzed the cDNA clones therein. According to the determination of expression specificities by real-time PCR, *rasZ* was the most enhanced gene during sexual maturation (400-fold increase). Among the 11 *ras* genes in *D. discoideum*, *rasZ* shares high similarities with *rasU*, *rasV*, *rasW*, *rasX*, and *rasY*, thus composing the *rasX* group. When we examined the expression patterns of all *rasX* group genes during sexual maturation and sexual and asexual development by real-time PCR, *rasV* showed a 40-fold increase in gamete and *rasW* showed continuous increase during sexual development. The similar analyses were performed on *rasG* group genes, and it was found that expression of *rasD* increased during sexual development. These results indicate that which *ras* to be used is precisely selective and that regulation of *ras* genes have both specific and common aspects in sexual and asexual development. We suspected that *rasX* group genes were mainly relevant for the sexual cycle and attempted to disrupt them. However, disruption of *rasZ* gene in KAX3 did not affect the sexual process. Neither did the knockout of 4 clustered *ras* genes (*rasV*, *rasW*, *rasX*, and *rasY*). Those genes could be involved in the stress response of differential culture conditions.

Analyses of integrin-beta-like proteins in *Dictyostelium*

Zenjiro Sampei¹, Keisuke Yokota¹, Masakazu Saito¹, Naoko Masuyama¹, Kunito Yoshida²,
Tatsuaki Kudo¹, Koji Yoda¹, Kei Inouye², Hiroyuki Adachi¹

¹Department of Biotechnology, The University of Tokyo, ²Department of Botany, Kyoto University

We found a family of eleven genes (dibA-dibK) encoding integrin-beta-like proteins, in the *Dictyostelium* cDNA and genome databases. Deduced amino acid sequences of the dib genes indicate the presence of an amino-terminal signal sequence, long cysteine-rich repeats, a transmembrane region, and a short carboxy-terminal tail carrying the NPXY motifs, known to be involved in talin binding of integrin beta chains. This overall structure and the size of the Dib polypeptides resemble those of metazoan integrin beta chains and are different from those of Sibs, reported previously as *Dictyostelium* adhesion molecules sharing some of the features of integrin beta chains. On the other hand, Dibs contain a PA14 domain in place of the vWF-A domain. Possible relationships between Dibs and integrin beta chains were found. Firstly, GFP-fusion proteins of most Dibs were present in the plasma and macropinosomal membranes, although strong signals were also observed on ER, Golgi and other vesicle membranes. Secondly, by immunoblotting of endoH-treated total cell extracts, Dibs were shown to be N-glycosylated. Thirdly, yeast two hybrid analyses revealed an interaction between the carboxy-terminal tail of some Dibs and talin A, talin B and human talin. Lastly, when DibA or DibE were overproduced in AX2 cells, vegetative cells were less adherent to the plastic Petri plates than the control cells, suggesting the involvement of Dibs in adhesion. On the other hand, knockout strains of DibB, DibC, DibD or DibE showed no clear phenotypes, suggesting functional redundancy among Dibs. We are currently examining the involvement of Dibs in cytokinesis, chemotaxis, and other aspects of cellular motility.

PaxB regulates actin dependent processes

Jelena Pribic, May Kong, Derrick Brazill
Department of Biological Sciences, Hunter College, CUNY

The actin cytoskeleton forms a membrane-associated network, whose proper regulation is essential for cell motility, chemotaxis, endocytosis and exocytosis. Paxillin, a focal adhesion molecule, is intimately involved in actin cytoskeleton reorganization. To gain a better understanding of the role of paxillin in these processes, we tested motility, chemotaxis, endocytosis and exocytosis in cells lacking and overexpressing *paxB*, the paxillin ortholog in *Dictyostelium discoideum*. We found that the speed of *paxB*⁻ cells is comparable to wild-type cells suggesting that PaxB is not involved in regulation of cell motility. When exposed to a folic acid gradient, *paxB*⁻ and wild-type cells exhibit similar speed and directionality. However, under the same conditions *paxB* overexpressing cells had normal speed but impaired directionality. These findings imply that PaxB does not play a direct role in chemotaxis to folic acid, but overexpression of *paxB* disrupts normal chemotaxis. When placed in cAMP gradient, starved *paxB*⁻ cells chemotaxed with higher speed but similar directionality as starved wild-type cells indicating that PaxB may play a negative role in chemotaxis to cAMP. In addition, *paxB*⁻ cells displayed normal pinocytosis, impaired exocytosis and enhanced phagocytosis, suggesting a role of PaxB in regulation of exocytosis and phagocytosis. Taken together, our data suggest that PaxB regulates chemotaxis, exocytosis and phagocytosis, processes mediated by actin cytoskeleton reorganization.

SecA is required for cell motility

Roberto Zanchi, Mark S. Bretscher, Robert R. Kay

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

Many motile cells, including *Dictyostelium discoideum* cells, continuously change their shape, generating protrusions, as they translocate. Since the plasma membrane is inelastic it cannot accommodate the cell changing morphology without continuously adjusting its surface area. We hypothesize that surface area is adjusted by regulating the endocytic cycle and therefore that membrane traffic to the plasma membrane is required for cell locomotion. To test this hypothesis, we studied the morphology and motility of a strain in which traffic to the plasmalemma is disrupted. We identified a temperature sensitive (*ts*) mutant of the SecA gene product, the homologue of yeast Sec1p, which is an essential component of the exocytic machinery. The SecA *ts* mutant has a severe growth defect at 27.5 °C. At this temperature, cells become rounded, showing large vacuole-like structures. Plasma membrane cycling is disrupted, as indicated by the lack of FM1-43 uptake above background levels. At the restrictive temperature random motility is abolished, while cells remain viable during the entire length of the experiments and they are able to recover to normal morphology if the temperature is lowered. The average speed is very low ($< 1.5 \mu\text{m}/\text{min}$) and maximum cell displacement rarely exceeds one cell diameter. Aggregation competent cells are readily capable of chasing a needle filled with chemo-attractant, but they fail to do so at the restrictive temperature. Finally, mutant cells at the restrictive temperature are still able to transduce the cAMP stimuli in actin polymerization as shown by the recruitment of ABD-120 GFP to the plasma membrane. We concluded that SecA is required for cell movement but not for polarization, suggesting that regulation of cell surface area is necessary to sustain cell motility.

Heterogeneity in cell motility after induction of starvation correlates with the developmental fate of amoebae

S Pavana Gowry¹, G Rajani Kanth¹, V. Nanjundiah², Gopal Pande¹

¹Centre for Cellular and Molecular Biology, Hyderabad, India, ²Indian Institute of Science, Bangalore, India

The velocity of axenically grown and motile *Dictyostelium discoideum* (AX2) cells was measured in three different conditions (a) in nutrient medium, (b) during early stage of starvation and (c) in replaced nutrient medium, after starvation. AX2 amoebae moved significantly faster in the starved condition in comparison to cells in nutrient medium before or after starvation. Based on the statistical analysis of the speed of individual cells, starved AX2 cells could be classified into 2 categories: slow and fast cells. This difference in cell velocity was not observed in nutrient medium. Similar analysis with starved amoebae of a mutant strain (Trishanku) of AX2, that is deficient in stalk formation, and with freshly starved wild type amoebae of *Polysphondylium pallidum* did not show the fast and slow categories of cells in any condition. However when AX2 cells were sorted by flow cytometry using the calcium sensitive dye FLUO-3, for low and high calcium content- a parameter known to be associated with prespore and pre stalk cell fate respectively, we found that the low calcium containing cells moved more slowly than high calcium containing cells. Fast moving cells showed a higher number of pseudopods per cell when compared to slow cells. Fluorescence microscopy of these cells showed that cell motility related molecules namely Actin, PTEN, PI3 kinase were differently organized in slow and fast cells. Based on the above analysis we propose that heterogeneity of cell motility during early starvation indicates the developmental fate of the starved amoebae wherein the fast moving cells belong to prestalk category and the slow moving cells belong to prespore category.

Screening of genes involved in cell migration in *Dictyostelium*.

Akira Nagasaki, Taro Q.P. Uyeda

National Institute of Advanced Industrial Science and Technology (AIST)

A single cell of wild-type *Dictyostelium discoideum* forms a visible colony on a plastic dish in several days, but due to enhanced cell migration, *amiB*-null mutant cells scatter over a large area and do not form noticeable colonies. Here, with an aim to identify genes involved in cell migration, we isolated suppresser mutants of *amiB*-null mutants that restore the ability to form colonies. From REMI (restriction enzyme-mediated integration)-mutagenized pool of double-mutants, we recovered 18 clones that restored the ability to form visible colonies, and identified the responsible genes from them. These genes can be categorized into several biological processes. One cell line, Sab16 (Suppressor of *amiB*) was chosen for further analysis, which had a disrupted phospholipase D *pldB* gene. To confirm the role of *pldB* gene in cell migration, we knocked out the *pldB* gene and over-expressed *gfp-pldB* in wild-type cells. GFP-PLDB localized to plasma membrane and on vesicles, and in migrating cells, at the protruding regions of pseudopodia. Migration speed of vegetative *pldB*-null cells was reduced to 73% of that of the wild-type. We then extended these studies to mammalian cells, which contain two PLD isoforms. We transfected rat bladder carcinoma cells with vectors expressing shRNA to deplete each PLD isoform. Depletion of both PLD1 and 2 by RNA interference reduced the velocity of the migration, but depletion of PLD2 inhibited motility more severely than that of PLD1. Furthermore, GFP-PLD2 was localized at protruding regions along the anterior edge in those cells. These results suggest that phospholipase D plays an important role in migration in *Dictyostelium* and mammalian cells and that our screening system is useful for the identification of genes involved in cell migration in general.

Directional migration in *Dictyostelium* cells induced by repeated stretch of the substratum

Yoshiaki Iwadate, Yuki Ishiyama, Shigehiko Yumura

Department of Functional Molecular Biology, Graduate School of Medicine, Yamaguchi University, Yamaguchi 753-8512, Japan

Cells show persistent migration even in the absence of chemoattractant. In order to migrate persistently, the cells must form their polarity for directional migration. How they form such a primal polarity is very interesting issue.

The migrating cells apply propulsive forces to substrata. At the same time, they receive the counter forces from the substrata. The amplitude of the counter forces changes dependently on the adherence of the cells to the substrata and the stiffness of the substrata. In order to form cell polarity for directional migration, do the cells evaluate the counter forces from the substrata? To investigate this possibility, application of stretching stimuli to migrating cells by stretching of elastic substrata seems to be useful, because this application mimics the counter forces from the substrata. However, fast migrating cells are easily peeled off from the substrata by the vibrating noise from the power source, such as a motor, of the commercial stretching devices because of their weak adhesiveness. Here, we developed a new stretching device using a shape-memory alloy instead of motors as the power source. This device allowed us to apply stretching stimuli to migrating *Dictyostelium* cells without vibrating noise.

Interestingly, in response to the stretching stimuli, wild-type *Dictyostelium* cells migrated perpendicular to the stretching direction. On the other hand, surprisingly, myosin II heavy chain-null cells migrated randomly. This observations strongly suggest that migrating *Dictyostelium* cells can sense mechanical signals from the substrata and form their polarity via myosin II-dependent process. Signaling cascade from the mechanical signals to myosin II accumulation will be discussed.

Switching direction in electric signal-induced cell migration by cGMP and phosphatidylinositol signaling

Masayuki J. Sato^{1,2}, Wouter N. van Egmond³, Hiroaki Takagi^{1,2}, Peter J. M. van Haastert³,
Toshio Yanagida¹, Masahiro Ueda^{1,2}

¹Laboratories for Nanobiology, Graduate School of Frontier Biosciences, Osaka University, ²JST, CREST, ³Department of Molecular Cell Biology, University of Groningen

Switching direction of cell movement in response to extracellular guidance cues has been found in various cell types and is an important cellular function for translocation during cellular and developmental processes. In direct current electric field (dcEF), many type of cells exhibit directional migration towards cathode or anode, called electrotaxis, where migration direction is depend on cell type. *Dictyostelium* cells migrate towards cathode in dcEF. We found that the preferential direction of migration during electrotaxis in *Dictyostelium* cells can be reversed through the genetic modulation of both soluble guanylyl cyclases (sGC) and the cGMP-binding protein GbpC, in combination with inhibition of phosphatidylinositol-3-OH kinases (PI3K). We further examined whether the N-terminus or catalytic domain of sGC controls migration direction in electrotaxis. We found that the catalytic domain is more essential than the N-terminus, a result different from chemotaxis studies. Therefore, it was concluded that cGMP is responsible for cathodal migration, while the N-terminus of sGC may enhance electrotactic efficiency by stabilizing the leading edge of migrating cell. Simultaneous suppression of cGMP and PI3K dependent signaling pathways caused reversal migration from cathode to anode through an unidentified signaling pathway. The observations provide the first identification of the genes required for directional switching in electrotaxis. Possible model for determining migration direction in electrotaxis will be discussed.

A cell number counting factor regulates both speed and direction of a cell during development to regulate group size in *Dictyostelium discoideum*

Jonghyun Roh¹, Yeonjeong Yu¹, Hyeri Seo¹, Soomin Son¹, Gyeongyun Go¹, Deborah Wessels², David R Soll², Richard H Gomer³, Wonhee Jang¹

¹Department of Life Science, Dongguk University, 3-26 Pil-Dong, Chung-gu, Seoul 100-715, Korea, ²W. M. Keck Dynamic Image Analysis Facility, Department of Biological Sciences, The University of Iowa, Iowa City, IA 52242, USA., ³Department of Biochemistry and Cell Biology, MS-140, Rice University, Houston, TX 77005-1894, USA.

Developing *Dictyostelium discoideum* cells form evenly sized groups of approximately 20,000 cells. A secreted 450 kDa protein complex called counting factor (CF) regulates the number of cells per group. We have used a combination of modeling and experiments to elucidate the underlying mechanism of CF-mediated group size regulation. Computer simulations predicted and experiments later confirmed that CF modulates group size by decreasing cell-cell adhesion and increasing random motility. Modeling also suggested a new possible mechanism for stream breakup involving the frequency of cell reorientation. In order to find out whether CF can regulate stream breakup in such a way, we analyzed the motility patterns of cells exposed to conditioned media collected from transformants lacking one of the four currently identified components of CF and to recombinant protein components of CF using 2-dimensional image analysis system. We show that a prediction made by computer simulations do actually occur in real life, and that different components of CF play different roles in random cell motility in developing *Dictyostelium* cells.

A novel expression system for dominant negative mutant actin in *Dictyostelium*

Taro Q.P. Noguchi^{1,2}, Noriko Kanzaki¹, Hironori Ueno¹, Keiko Hirose¹, Taro Q.P. Uyeda^{1,2}
¹RICE, AIST, ²Life and Environmental Sciences, Univ. of Tsukuba

Actin and myosin play critical roles in a wide range of fundamental eukaryotic processes, such as cell motility, vesicle transport, and muscle construction. It is generally accepted that myosin generates force by tilting the neck domain relative to the globular catalytic domain. However, several studies suggested that myosin binding induces cooperative conformational changes within actin filaments, and that these actin's structural changes play important roles in myosin movement. A number of studies reported dominant negative (DN) mutant actins that are toxic to cells even in the presence wild-type actin. For example, An and Mogami (1996) identified DN mutant actin alleles that impair flight of *D. melanogaster* even in the presence of three-fold excess normal actin genes. These phenotypes are consistent with the idea that the DN actins prevent myosin-induced cooperative conformational changes in copolymers made of DN and wild type actin subunits. Thus, DN mutant actins are very useful for understanding unknown actin functions related to the cooperative conformational changes. However, detailed biochemical analysis has not been achieved because the DN mutant actins are difficult to purify in sufficient quantities from fly muscle tissues and are expected to be toxic to the host cells of expression. We have therefore developed a novel actin expression system using *Dictyostelium*. In this system, the C terminus of actin molecule is fused with thymosin β , an actin monomer binding and sequestering protein, via a flexible linker. The thymosin β -fused actin moiety did not co-polymerize with endogenous actin in *Dictyostelium* cells, so that the recombinant actin would not exert toxic effects in the host cells, even if the mutant is DN in nature. The thymosin β -fused actin moiety was purified using His tag attached to thymosin and then cleaved by chymotrypsin immediately after the native final residue of actin to yield intact actin. Wild-type recombinant actin obtained this way polymerized normally as examined by the sedimentation assay, pyrene fluorescence assay, and electron microscopy, and moved at nearly normal speeds on surfaces coated with skeletal HMM. We thus concluded that the recombinant actin obtained by the novel system is normal, both functionally and structurally. Next, we introduced DN actin mutations (G63D, R95C, G156D, G156S, E226K, G268D, G301D, G302D, Q353@, W356@ ; stop codon indicated as @) identified by An and Mogami into the thymosin β -fused actin gene, and the resultant plasmids were used to transfect *Dictyostelium* cells. G301D, G302D, Q353@, and W356@ actins could not be purified due to very low levels of expression. We speculate that they were rapidly degraded in the cells since mRNA levels were normal. Four other DN mutant actins (G63D, G156D, G156S and G268D) affected polymerization ability. The remaining two DN mutant actins (R95C and E226K) impaired calcium regulation via the tropomyosin-troponin system. Thus, we were unable to identify, among An and Mogami's *Drosophila* DN mutant alleles, DN mutant alleles that exert toxicity by perturbing cooperative conformational changes of actin filaments. We are currently generating new mutant actins to identify mutant alleles that perturb myosin-induced cooperative conformational changes of actin filaments, by replacing glycine residues within actin to valine, alanine or other residues in a systematic manner.

Comparison of the properties of actin aggregates induced by fragments of different actin binding proteins

Andrew Maselli¹, Jeannie Ramos¹, Denise Patrick¹, Ran-der Hwang², David Knecht²

¹Chicago State University, Chicago, IL, ²University of Connecticut, Storrs, CT

Actin aggregations are a manifestation of the pathology associated with neurodegenerative disease. The prevalence of these structures in disease makes characterizing their formation and stability important. We report actin aggregate formation initiated by expression of fragments of two actin binding proteins (and compared their properties to aggregates induced by the actin stabilizing drug, jasplakinolide). Expression of the carboxy terminal half of ABP34 (aa 129-295) or a fragment of fimbrin which contains the second actin binding domain (aa 367-610) both lead to actin aggregate formation. Cells expressing either fragment show slowed growth in suspension and GFP-labeled aggregates are visible in the cytoplasmic bridge in cells during Cytokinesis B. The key difference between the aggregates is that the fim-abd2 aggregates appear to be less stable and disaggregate when cells are grown to high density. We see a 45% drop in the number of cells containing visible aggregates in cells expressing the fim-abd2 when cells are at low density (1.5×10^6) are compared with cell at high density (4.5×10^6). The ABP34-fragment expressing cells showed less than a 10% drop under the same conditions. When cells from the high density condition are diluted and allowed to grow, fim-abd2 aggregates rapidly reform. This effect appears to be independent of growth condition since cells grown on plates and in suspension show a similar effect. The configuration and degree of crosslinking of the aggregate is likely to play an important role in stability of the aggregates. The ultrastructure of the inclusions produced by these two ABP fragments show a range of configurations from amorphous regions with little order to regions of highly ordered filaments. Studies are underway to correlate the observations made at the light and EM levels. The markedly different behaviors of these two ABP-fragments when cells are a high density adds to our ability to use ABP-fragments in *Dictyostelium* as a model to study the formation and stability of cytoplasmic actin aggregates in disease states.

Role of PTEN for localization of myosinII in *Dictyostelium* cells.

Md. Kamruzzaman Pramanik¹, Miho Iijima², Yoshiaki Iwadate¹, Shigehiko Yumura¹

¹Applied Molecular Bioscience, Graduate School of Medicine, Yamaguchi University, Japan., ²Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

Tumour suppressor protein, PTEN dephosphorylates PIP (3,4,5)P₃ into PIP (4,5)P₂ and down regulates PI3kinase signaling. Thus this protein has been implicated in modulating phosphoinositide signaling responsible for many cellular processes such as polarity, migration, chemotaxis and cytokinesis. In chemotaxing neutrophils and *Dictyostelium* cells, PTEN is localized at the posterior site. Moreover, in dividing *Dictyostelium* cell, PTEN is localized at the furrow region. Both of these localization patterns of PTEN are same as myosin-II. Therefore, we hypothesized that PTEN may play an important role for myosin II localization. To examine this hypothesis, we studied co-localization of PTEN and myosin-II with time course adopting various approaches. PTEN-GFP and myosin-RFP were simultaneously expressed in the *Dictyostelium* cell and observed under confocal microscope. PTEN and myosin II co-localized at the posterior region of migrating cell and furrow region of the dividing cell (as previously reported). In addition, we found sequential co-localization of PTEN and myosin at the retracting pseudopod. Next, we observed myosin II localization in PTEN knock out cells. Less amount of myosin II was localized at the retracting pseudopod and retraction speed was slower than wild type cell. As PTEN knockout cell become multinucleated especially in shaking culture, we mimic suspension condition by embedding cells into soft agarose and observed myosin localization in dividing cells. Under this condition, myosin localization took place almost uniformly with a very few at the furrow region. From these studies, PTEN contributes to myosin II localization in different cellular processes including cell division, migration, and pseudopod retraction through its protein or lipid phosphatase activity.

Analyses on a novel protein involved in cytokinesis of *Dictyostelium*

Hironori Inaba, Yuya Takaki, Takahiro Sasaki, Yuji Iwasa, Takehiro Hara, Takashi Yamamoto, Jun Sawaguri, Koji Yoda, Hiroyuki Adachi

Department of Biotechnology, The University of Tokyo

To identify genes involved in cytokinesis of *Dictyostelium*, we have isolated cytokinesis mutants using REMI. After we reported the first gene *gapA* that encodes one of *Dictyostelium* IQGAP-related proteins, we screened more than 12,000 independent REMI transformants for cytokinesis mutants, and identified at least five genes of which disruption result in the defects of cytokinesis of this organism.

The gene identified from one of such mutants D411-2, encodes a novel protein with a putative coiled-coil domain. The regenerated knockout strain for the gene, KO6, showed cytokinesis defects both in the plate and suspension cultures. In the plate culture, the KO6 cells were very flat, and showed reversion of cytokinesis during furrow ingression and abscission. The KO6 cells also showed the reduced rates of phagocytosis and macropinocytosis.

The expression of GFP-D411-2p rescued the cytokinesis defects of the KO6 cells. As expected from the phenotypes of the KO6 cells, the fusion protein localized to crown, macropinosomal membrane and phagocytic cups in interphase. However, the fusion protein diffused to cytoplasm at the onset of cytokinesis, and localized fluorescence was not seen in the cell during cytokinesis. After the cells were lysed, GFP-D411-2p was partially associated with actin-rich cortical fraction, and was co-sedimented with the actin filaments polymerized from the depolymerized cortical fraction.

At this stage, although the precise role of D411-2p in cytokinesis is unclear, it is suggested that D411-2p participates in cytokinesis through the modulation of actin filaments that work commonly in cytokinesis, macropinocytosis and phagocytosis. We are currently examining the relationship between D411-2p and microtubules, another cytoskeleton that also plays crucial role in cytokinesis.

Complex constructs including G-actin aggregates in *Dictyostelium discoideum* spores.

Masazumi Sameshima

Department of Biology, Faculty of Agriculture and Life Science, Hirosaki University, Aomori
036-8561, Japan

Upon depletion of food sources, *D. discoideum* develops into a fruiting body carrying a mass of dormant spores. The dormancy of spores depends on both the high level of tyrosine phosphorylation of actin and the formation of F-actin bundles that include S-adenosyl-L-homocysteine hydrolase (1, 2, 3). Actin phosphorylation also regulates bending in plant, *Mimosa* (4). Here another new type of actin construct that is composed of G-actin molecules was found.

A cluster of lipid droplets embedded in an electron-dense matrix, had been observed in the spore cytoplasm using transmission electron microscopy (TEM). This matrix can be stained with anti-actin and anti-phosphotyrosine antibody by immunoelectron microscopy. However no fibrous structures were found on the TEM images, indicating the matrix is an aggregate of G-actin a part of which seems to be tyrosine-phosphorylated.

Electron-dense structures first appeared in prespores at the early culmination stage. Later lipid clusters embedded in an electron-dense matrix were observed in immature spores of mid culminants. During the germination process, complex constructs still existed in swollen spores, and then disappeared in emerged amoebae from spore capsules. Thus, a complex construct composed of a lipid cluster and a G-actin aggregate may functionally relate to sporulation and spore dormancy.

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Effects of Tyr-53 mutations on polymerization of *Dictyostelium* actin and cell phenotype

Xiong Liu, Shi Shu, Bin Yu, Edward D. Korn

Laboratory of Cell Biology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892, USA

We recently published (Liu et al. (2006), PNAS,103: 13694-13699) that phosphorylation of Tyr-53 of *Dictyostelium* actin (which occurs during normal development of *Dictyostelium* and under stress) inhibits filament nucleation and elongation in vitro, and destabilizes filaments. We are now characterizing the effects of substituting Phe, Ala, Asp and Glu for Tyr-53. Mutant and wild-type constructs, with an N-terminal Flag-tag, accounted for about 25% of the total actin when expressed in wild-type *Dictyostelium* AX3 cells, and the proteins were readily purified to electrophoretic homogeneity by affinity chromatography. The polymerization of N-FLAG-WT actin was very similar to polymerization of endogenous WT actin, and the Tyr53Phe mutation had little effect. However, polymerization of the Ala, Asp and Glu mutants was greatly impaired, similar to the effect of Tyr-53 phosphorylation. Arp2/3 VCA actin seeds, greatly accelerated polymerization of mutant actins, similar to their effect on Tyr-53 phosphorylated actin. Negative staining electron microscopy showed that, like Tyr-53 phosphorylated actin, the mutant actins formed only small aggregates under polymerization conditions. Cells expressing the four mutant actins grew as well as wild type cells in suspension culture, and FLAG-tagged WT and mutant actins co-localized with endogenous actin. Interestingly, the AX3 cells expressing Tyr53Ala, Tyr53Asp and Tyr53Glu mutants showed defects in development and streaming. Also, while the cells expressing the Tyr53Ala mutant chemotaxed in response to a cAMP gradient, they did not form streams like wild-type cells.

Regulation of actin cytoskeletal architecture by Fimbrin A

Ran-der Hwang, Chin-chi Chen, David A. Knecht
University of Connecticut, Storrs, CT

The fimbrins, also known as plastins, are members of the Calponin Homology superfamily of actin-binding proteins. Fimbrin has two calcium-binding domains (EF hands) and has been shown to have calcium regulated actin-binding activity in vitro in some organisms. This protein is unique among the CH domain containing proteins in possessing a tandem repeat of the actin binding domain (ABD) within a single polypeptide chain. In order to understand what regulates fimbrin's binding to actin filaments in the cell, we generated fluorescent fusion proteins, encoding either the entire protein or its various domains, and examined the localization of these fluorescent proteins in living cells. Fimbrin localizes to newly formed pseudopods as well as macropinosome cups, but only weakly in the peripheral cortex. Deletion of the EF hands (Δ EF) had no discernable effect on protein localization. However, the first actin-binding domain (ABD1) alone or the EF hands plus the first actin-binding domain (EA1) both localized more strongly to the cortex of the cell than the whole protein. Surprisingly, expression of the second actin-binding domain (ABD2) alone leads to the formation of a large aggregate of actin filaments. The actin aggregates induced by ABD2 were further investigated using the tetracysteine tagging system. We found that ABD2-4xCys was still able to generate actin aggregates, indicating that protein association through the GFP domain did not lead to filament aggregation. We have also used bacterially expressed fimbrin and its various domains to further investigate the function of the two actin-binding domains of fimbrin in cell free systems. The two actin-binding domains of fimbrin have different affinities for pure F-actin and induce different architectures of F-actin networks and have different effects on filament stability. These results suggest that the two ABDs are functionally different and cooperate in defining the function of the whole protein.

Visualizing myosin-actin interaction with a genetically encoded fluorescent strain sensor.

Sosuke Iwai, Taro Q. P. Uyeda

Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST)

Many proteins have been shown to undergo conformational changes in response to externally applied force *in vitro*. They include structural proteins such as muscle and cytoskeletal proteins, which are responsible for maintaining the structural integrity of cells, and mechanosensory proteins, which are involved in the transduction of mechanical signals to cells. In either case, protein conformational changes are believed to play important roles in the processes, although whether they occur *in vivo* remains unclear. To reveal the force-induced conformational changes, or strains, within proteins in living cells, a polypeptide-based “strain sensor” would be necessary. Toward this end, here we have developed a genetically encoded fluorescent strain sensor, by combining the proximity imaging (PRIM) technique (De Angelis *et al.*, 1998) and *Dictyostelium* myosin II and actin as a model system. PRIM depends on direct contact between two GFP molecules, which can lead to structural perturbations and concomitant spectral changes. Unlike FRET, PRIM is assumed to involve only two types of fluorescent excitation spectra corresponding to a monomer and a dimer GFP, so that an estimated excitation ratio will simply reflect a mixing ratio of the monomer and the dimer, in principle. Thus, PRIM would be useful for detecting the protein conformational changes *in vitro* and *in vivo*.

The developed PRIM-based strain sensor module (PriSSM) consists of the tandem fusion of a normal and circularly permuted GFP joined by a flexible linker. To apply strain to PriSSM, it was inserted between two motor domains of *Dictyostelium* myosin II. In the absence of strain, the two GFP moieties in PriSSM are in contact, whereas when the motor domains are bound to F-actin, PriSSM has a strained conformation, leading to the loss of contact and a concomitant spectral change. The spectral change was reversed when ATP was added, suggesting that the conformational and spectral changes of PriSSM were reversible. Using the sensor system, we examined whether the position of the lever arm in the rigor state was affected by the mutations within the motor domain. The mutational studies suggest that both the lever arm rigidity and the strong binding to F-actin are important for maintaining the poststroke position of the lever arm and the strained state of the sensor, consistent with the model that the optimal force generation requires the rigidity of the lever arm and the strong binding to F-actin. Moreover, the sensor was used to visualize the interaction between myosin II and F-actin in *Dictyostelium* cells. In normal cells, a large portion of myosin II proteins is detached from F-actin, presumably because of the low duty ratio of the motor protein. On the other hand, cellular ATP depletion or hyperosmotic stress increased the fraction of myosin II that binds to F-actin, suggesting that myosin II in the rigor state contribute to maintaining cortical tension in such stressed cells. Our PRIM-based strain sensor may provide a general approach for studying force-induced protein conformational changes *in vivo* as well as *in vitro*.

Single-molecule imaging analysis of heterotrimeric G protein dynamics in early signaling events of chemotactic responses.

Yukihiro Miyanaga¹, Tatsuo Shibata², Masahiro Ueda¹

¹Graduate School of Frontier Biosciences, Osaka University, ²Department of Mathematical and Life Science, Hiroshima University

Chemotactic signaling of *Dictyostelium* cells is mediated by heterotrimeric G proteins and their coupled receptors. According to the prevailing scheme, agonist stimulation of G protein-coupled receptors promotes the ternary complex formation with their coupled G protein, which leads to the dissociation into the activated $G\alpha$ and $G\beta\gamma$ subunits for signal transduction. However, the relevance of these early activation events of G proteins to the intracellular dynamics on plasma membrane has not been revealed. To reveal the intracellular dynamics of G proteins, we used single-molecule imaging techniques in living *Dictyostelium* cells, which provided direct measure of mobility in the plasma membrane and revealed a dynamic shuttling of G proteins between the membrane and cytoplasm. Before stimulation, G protein subunits, $G\alpha_2$ and $G\gamma$, associated with the membrane transiently and moved more rapidly than receptor, indicating an absence of precoupling. Agonist occupancy of receptors decreased $G\alpha_2$ lateral mobility to that of the receptor and increased its dwell time at the membrane. While $G\gamma$ interacted transiently with the receptors and dissociated rapidly from membrane even in the agonist-stimulated cells. These differences in membrane-binding properties between the G protein subunits suggest that the activated $G\alpha_2$ and $G\gamma$ subunits can transduce different types of signals, which would have the information about spatial gradient and average concentration of agonist, respectively.

Identification of PKBA substrates that link PIP3 to the cytoskeleton

Michelle Tang, Yoichiro Kamimura, Peter Devreotes, Miho Iijima
Department of Cell Biology, The Johns Hopkins Medical Institute

Tumor suppressor PTEN plays a key role in sensing of chemoattractant gradients. Disruption of PTEN in *Dictyostelium discoideum* causes a strong defect in sites of initiation and orientation of pseudopodia and causes cells to follow a circuitous route toward the attractant. This phenotype is caused by dramatically prolonged and broadened PIP3 accumulation and actin polymerization. However, the signaling events linking excess PIP3 to actin polymerization are unknown. There is evidence showing that one of the PKB homologs, PKBA, is activated after being recruited to the membrane through its PIP3-specific PH domain. Disruption of PKBA in *pten*- cells substantially reverses the chemotaxis defects. We compared the pattern of phosphorylated PKB substrates between *pkba*-/*pten*- and *pten*- cells. We identified three PKB substrates that might be critical in reversing *pten*- phenotype. To identify these substrates, a large scale purification method is developed, followed by immunoprecipitation and mass spectrometry. It is expected that disruption of these substrates in *pten*- cells will also reverse the phenotypic defects in the *pten*- cells, and provide an insight to the mechanisms by which PIP3 regulates the cytoskeleton.

Towards a quantitative input-output relationship in the *Dictyostelium* cAMP relay response

Thomas Gregor¹, Koichi Fujimoto², Satoshi Sawai^{1,2}

¹Graduate School of Arts and Sciences, University of Tokyo, ²ERATO Complex Systems Biology Project, JST

We revisit the input-output relationship between extracellular cAMP stimulus and intracellular response by live-cell imaging. Single cells in isolation were stimulated via perfusion over extended periods of time (tens of minutes) with cAMP concentrations that range over 7 orders of magnitude. Three typical response regimes are observed: 1) In the 10pM to 1nM range cells respond stochastically with a single cytosolic cAMP pulse that adapts perfectly on a fast ~ 3 minute time scale. 2) In the 1nM to 1 μ M range cells also respond with a fast pulse but cytosolic cAMP continues to oscillate afterwards upon tonic stimulus. These internal oscillations have a damped behavior that is stimulus dependent, but the cells do not fully adapt. 3) In the 1 μ M-10 μ M range cytosolic cAMP concentration oscillates continuously but damping is no longer observed. Fourier power spectra for the internal cAMP oscillations reveal periods ranging from 2 to 6 minutes for applied concentrations of 10 μ M to 1nM, respectively. Damping ranges from minute- (100pM) to hour- (100nM) timescales. The input-output relationship, quantified by computing the concentration integral over the stimulated time interval, shows a sharp transition around 1nM. Furthermore, using a purified version of the sensor protein we estimate both the absolute cytosolic cAMP concentration as well as the number of molecules released by a single cell during a cAMP pulsation.

Adaptive responses regulated by the chemoattractant seven- transmembrane receptor CAR1

Joseph Brzostowski¹, Satoshi Sawai², Carole A. Parent³, Dale Hereld⁴, Alan R. Kimmel⁵

¹Laboratory of Immunogenetics Light Imaging Facility, National Institute of Allergy and Infectious Diseases, NIH, USA, ²ERATO Complex Systems Biology Project, JST, Tokyo 153-8902, Japan,

³Laboratory of Cellular and Molecular Biology, CCR, National Cancer Institute, NIH, USA,

⁴National Institute on Alcohol Abuse and Alcoholism, NIH, USA, ⁵Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, USA

Chemotaxis is coordinated by both positively and negatively acting (adapting) intracellular pathways. For numerous eukaryotic cell-types, chemoattractant gradients are perceived by seven-transmembrane receptors (7-TMRs) coupled to heterotrimeric G proteins to activate downstream signaling networks. 7-TMRs activate a variety of signaling networks in addition to chemoattractant pathways, and in these other systems, receptor phosphorylation is required to turn off downstream signaling. The amoeba, *Dictyostelium discoideum*, uses the 7-TMR CAR1 to sense secreted cAMP to coordinate movement into aggregates in response to starvation. Essential to aggregate formation is the ability for most CAR1-mediated pathways to quickly adapt to a cAMP stimulus and for cells to rapidly destroy the cAMP ligand by secreted phosphodiesterases. CAR1 activates multiple networks, including the cAMP-synthesizing-pathway adenylyl cyclase A (ACA), and also initiates its own phosphorylation. We now demonstrate that receptor phosphorylation is required for adaptation of the ACA pathway: ACA does not adapt in cells expressing non-phosphorylatable CAR1 mutants in the presence of persist signal. Consistent with these results, we find that propagation of the cAMP signal is significantly compromised when mutant cells are allowed to aggregate on a solid substrate, but evidence of cAMP oscillations during aggregation is still observed, suggesting that the inability to shut down cAMP synthesis in biochemical assays can be overcome in situ.

Self-organization in the phosphatidylinositol lipids signaling pathway

Yoshiyuki Arai^{1,2}, Tatsuo Shibata^{2,3,4}, Satomi Matsuoka^{1,2}, Toshio Yanagida¹, Masahiro Ueda^{1,2}¹Graduate School of Frontier Biosciences, Osaka University, ²Japan Science and Technology Agency (JST), CREST, ³Department of Mathematical and Life Sciences, Hiroshima University, ⁴PRESTO, Japan Science and Technology Agency (JST)

Cell polarity and migration is regulated by the self-organized dynamics in cytoskeletal proteins, their regulators and molecular motors. It is known that the phosphatidylinositol (PtdIns) lipids signaling system plays an important role for such a process. At the leading edge of the motile cell, phosphatidylinositide 3-kinase (PI3K) catalyzes phosphorylation of PtdIns 4,5-bisphosphate (PtdIns(4,5)P₂) to PtdIns 3,4,5-triphosphate (PtdIns(3,4,5)P₃), which initiates the localization of cytoskeletal regulators. In contrast, at the lateral and tail region of the cell, tumor suppressor phosphatase tensin homolog (PTEN) degrades PtdIns(3,4,5)P₃. Such reciprocal enzymatic activities are essential for pseudopod formation and cell migration. Here, we report that the PtdIns lipids system exhibits self-organized, oscillatory traveling waves on the cell membrane without extracellular stimulation in *Dictyostelium discoideum* cells. We observed the translocation of the fluorescently labeled pleckstrin-homology domain of Akt/PKB and PTEN simultaneously as an indicator of PtdIns(3,4,5)P₃ and PtdIns(4,5)P₂ respectively. The localizations of the proteins were highly reciprocal, dynamically changing location along the membrane rotationally. Based on our experimental results, we proposed a mathematical model that can successfully reproduce several patterns observed experimentally. In our model, a novel, negative form of PTEN regulation membrane binding by PtdIns(3,4,5)P₃ is predicted.

Modeling adaptation and intrinsic cytosolic cAMP oscillations in isolated *Dictyostelium* cells.

Koichi Fujimoto¹, Thomas Gregor², Satoshi Sawai^{1,2}

¹ERATO Complex Systems Biology Project, JST, ²Graduate School of Arts and Sciences, University of Tokyo

FRET-based measurements of cytosolic cAMP at the single-cell level have enabled us to re-examine the underlying biochemical pathways that give rise to the oscillations and waves of extracellular cAMP. Based on our recent live-cell imaging data from wildtype and mutant strains under various stimulus conditions, we propose a reaction-kinetics model that describes how ligand-binding to the receptor regulates ACA through multiple regulatory modules consisted of Ras, PI3K, TORC2 and F-actin. Our numerical and analytical studies show that a putative negative feedback regulation of F-actin/ACA is necessary to account for the sustained intracellular cAMP oscillations in cells under persistent stimulation. The key signature of the cAMP response that we have experimentally uncovered is the existence of two adaptation kinetics of distinct time-scales. Based on the model simulations, we suggest that the fast activation and adaptation of a cAMP response results from the regulation of PIP3 whereas the slow time scale adaptation derives from actin-dependent inhibition of ACA. By extending the single-cell studies to a multicellular context, we demonstrate that the putative negative feedback and the resulting intrinsic intracellular cAMP oscillations are essential for the onset of the extracellular cAMP oscillations at the population level.

Understanding clathrin-mediated endocytosis in *Dictyostelium*

Laura Macro, Jyoti K. Jaiswal, Sanford M. Simon

Laboratory of Cellular Biophysics, Rockefeller University, 1230 York Ave, New York, New York 10021, USA

In eukaryotes, clathrin-mediated endocytosis (CME) is a major route of internalization of cell surface receptors (cargoes). The ability of the AP2 adaptor complex to simultaneously bind to cargo, clathrin and the plasma membrane makes it a key component of CME. In mammalian systems cargo molecules containing the consensus internalization motif YXX Φ (where Φ is a bulky hydrophobic residue) bind to the μ 2 subunit of AP2 and are used as markers of CME. Real-time imaging of cell surface receptor internalization by CME has allowed insights into the mechanism and machinery of CME. Many components of the CME machinery exist in *Dictyostelium* providing an opportunity to study the physiological role of CME during single and multicellular stages. However, while the role of clathrin in osmoregulation and cytokinesis in *Dictyostelium* has been documented, demonstration of a role for clathrin in endocytosis has been lacking due to the absence of a bona fide *Dictyostelium* CME receptor (cargo). For real-time imaging as well as biochemical analysis of CME in *Dictyostelium* we are identifying cargoes that are internalized in *Dictyostelium* by CME. Aside from providing insights into the role of CME in *Dictyostelium*, our study will also make *Dictyostelium* more amenable as a model system for studying CME.

Antisense RNA inhibition of *Dictyostelium* β -MPP induces expression of nuclear-encoded mitochondrial proteins in retrograde regulation manner

Koki Nagayama¹, Shiori Itono¹, Hiroshi Ochiai^{1,2}, Tetsuo Ohmachi¹

¹Department of Biochemistry and Biotechnology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, 036-8561, Japan, ²Creative Research Initiative SOUSEI (CRIS), Hokkaido University, Sapporo, 001-0021, Japan

Most mitochondrial proteins encoded by nuclear genes are synthesized with an N-terminal mitochondrial targeting signal (MTS) sequence, which is removed by mitochondrial processing peptidase (MPP). MPP consists of two subunits, α -MPP and β -MPP. A cDNA encoding the *Dictyostelium* β -MPP (Dd β -MPP) was cloned and sequenced. The cDNA clone encodes a protein with a typical MTS sequence at the N-terminus. Dd β -MPP contains an inverted metal-binding motif (HXXEHX₇₆E) which is essential to the catalytic activity of MPP and a -2 Arg recognition site which is a binding site required for cleaving precursor proteins. Western blot analysis of the mitochondrial subfractions revealed that Dd β -MPP is located in the mitochondrial matrix and membrane, whereas Dd α -MPP is located only in the matrix. The expression level of β -MPP mRNA was highest in vegetative cells and at the early development stage, and strikingly decreased at 5-10 h of development, indicating that transcription of the β -MPP gene is strongly down-regulated at this time. The Dd β -MPP protein is constantly expressed throughout the *Dictyostelium* life cycle.

In a transformant expressing the antisense RNA of the β -MPP gene, unexpectedly, the β -MPP protein and its mRNA increased about 1.8-fold and 4.5-fold relative to the wild type, respectively. Expression of other mitochondrial proteins, α -MPP and Cox IV, was also induced, suggesting that antisense RNA inhibition of the β -MPP gene induces gene expression of mitochondrial proteins. Also, when the wild-type AX-3 strain was treated with metformin or 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), which is an activator of AMP-activated protein kinase (AMPK), nuclear-encoded mitochondrial proteins, such as α -MPP, β -MPP and Cox IV, were induced as in the case of the expression of these proteins in the β -MPP antisense transformant. These results suggest that *Dictyostelium* has a mitochondrial retrograde regulation via AMPK signaling, which is the pathway of the transfer of information from the mitochondria to the nucleus.

Dynamics of *Escherichia coli* gene expression in symbiotic relationship building with *Dictyostelium discoideum*

Kumiko Kihara¹, Kotaro Mori¹, Naoaki Ono², Shingo Suzuki², Akiko Kashiwagi⁴, Chikara Furusawa^{2,3}, Tetsuya Yomo^{1,2,3}

¹Graduate School of Frontier Biosciences, Osaka University 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan, ²Graduate School of Information Science and Technology, Osaka University 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan, ³Kaneko Complex Systems Biology Project, ERATO, JST, ⁴Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

Todoriki et al. (2002) established an experimental symbiotic system composed of *Escherichia coli* and *Dictyostelium discoideum* to examine how symbiosis occurs. Even though *E. coli* and *D. discoideum* have a prey-predator relationship, these two species achieved a state of stable coexistence within mucoidal colonies after several weeks of cocultivation (which we named “symbiotic colony”)[Todoriki et al., 2002]. Although it takes three months for *E. coli* stress gene expression levels to decrease when coexisting with their symbiotic counterpart *D. discoideum* [Matsuyama et al., 2004], physiological changes in *E. coli* have been evident much earlier [Yamada et al., 2008]. Thus, much earlier processes before the three month period are likely important in the evolution of this new symbiotic system. We report here the global gene expression of *E. coli* within one month of the co-culture. We found that the transcriptional profile of symbiotic *E. coli* stress gene expression significantly decreased within 6 days of coculture.

Expression and biochemical characterization of *Dictyostelium discoideum* fatty acid amide hydrolases

Dhamodharan Neelamegan, Frank St. Michael, Suzanne Lacelle, James C. Richards, Andrew D. Cox

Institute for Biological Sciences, National Research Council, Ottawa, K1A 0R6, CANADA.

Dictyostelium discoideum cells elaborate several bacterial cell lysing enzymes when they grow using bacteria or liquid medium as food sources. In our laboratory we have made use of the extracellular amidase activity produced by *Dictyostelium* cells to modify bacterial lipopolysaccharide (LPS) enabling LPS structure function studies. The extracellular amidase can use different bacterial O-de-acylated LPS (LPS-OH) as its substrate and can preferentially hydrolyze one or both of the N-linked fatty acids of different LPS-OH. Production of recombinant *Dictyostelium* amidases was undertaken to enhance the use of the enzymes in *in vitro* hydrolyses of N-linked fatty acids from LPS-OH. Two potential fatty acid amide hydrolase genes, *ddAI* and *ddAII* were identified from the available *Dictyostelium* genome database. DdAI and DdAII proteins were over-expressed and purified from both *E.coli* and *Dictyostelium* systems. Both the over-expressed proteins DdAI and DdAII exhibit fatty acid amide hydrolase activity. *Dictyostelium* cell fractionation studies show that both DdAI and DdAII are not readily accessible as soluble proteins. DdAI and DdAII were solubilised using 1% Triton X-100 which enhanced the downstream purification of the proteins and their use in *in vitro* assay.

A DNA oligonucleotide-assisted genetic manipulation that increases transformation and homologous recombination efficiencies

Hidekazu Kuwayama¹, Toshio Yanagida², Masahiro Ueda²

¹Graduate School of Life and Environmental Sciences, University of Tsukuba, ²Graduate School of Frontier Biosciences, Osaka University

Artificial gene alteration by homologous recombination in living cells, termed gene targeting, presents fundamental and considerable knowledge of in vivo gene function. In principle, application of this method is possible to any types of genes and transformable cells. However, its success is limited due to low frequency of homologous recombination between endogenous targeted gene and exogenous transgene. Here, we describe a general gene-targeting method that the co-transformation of DNA oligonucleotides (oligomers) that were simply designed to be identical to both ends of the homologous flanking regions of the targeting construct; this feature significantly increased the homologous recombination frequency and transformation efficiency in all 4 examined cases. Using this strategy, both targeted alleles of diploid cells were simultaneously replaced in a single transformation procedure. Thus, the simplicity and versatility of this method applicable to any sort of cell may increase the application of gene targeting engineering.

The biosynthesis of GDP-Fucose in *Dictyostelium*

Alba Hykollari, Birgit Schiller, Josef Voglmeir, Stefan Karl, Iain B. H. Wilson
Departement fuer Chemie ,Universitaet fuer Bodenkultur , A -1190 Wien, Austria

Fucose is a modification of the N- and O-glycans of many organisms, including those of animals, plants and invertebrates and is responsible for a multitude of highly specific processes. All fucosyltransferases use a nucleotide activated version of the sugar, GDP-fucose. This substrate can be synthesised by two pathways, a de novo and a salvage pathway. Previous Western blotting analyses confirmed that core fucose is expressed on the N-glycans of the *Dictyostelium discoideum* AX3 “wild-type” strain. At early and later stages of development different proteins become fucosylated; in contrast, the mutant strain HL250 possesses a fucosylation defect, even though its core α 1,3-fucosyltransferase is active. The defect is verified to be a mutation in the GDP-D-Mannose-4,6-Dehydratase (GMD) gene. GMD is one of the two enzymes (the second being an epimerase/reductase named GER) necessary for enzymatic transformation of GDP-mannose into GDP-fucose. The cDNAs encoding both enzymes GMD and GER were amplified from the cDNA of AX3 and HL250 and subsequently expressed. Western blotting analysis confirmed that recombinant forms of GMD and GER from both the AX3 and HL250 strains can be expressed in *E. coli*. However, whereas GDP-mannose was converted to GDP-fucose by the AX3 GMD/GER “pair”, the recombinant HL250 form of the GMD protein was not active, in contrast to HL250 GER. The sequencing of two independent GMD cDNA clones derived from the HL250 strain could confirm the presence of a point mutation. The observation of the mutant strain HL250, with non-fucosylated glycans, revealed a reduced rate of cell proliferation, altered aggregate formation and (as shown by others) distorted spore coat structure. This highlights the importance of fucosylated glycans in *Dictyostelium discoideum*.

Determining the role of abc transporters in *Dictyostelium discoideum* development

Edward Miranda, Olga Zhuchenko, Adam Kuspa, Gad Shaulsky
Program in developmental Biology, Baylor College of Medicine

ATP-binding cassette (ABC) transporters can translocate a broad spectrum of molecules across the cell membrane, including lipids, peptides, amino acids, carbohydrates, ions, and xenobiotics. ABC genes are also known for the role they play in resistance towards anticancer agents in chemotherapy of cancer patients. The genome sequence of the soil amoeba *Dictyostelium discoideum* revealed 68 members of the ATP-binding cassette (ABC) superfamily of genes. This number is almost double the number of ABC transporters encoded in the human genome. The function of most of these transporters is not known, but considering that *Dictyostelium* is a soil organism, ABC transporters might provide resistance to xenobiotics they might encounter. In addition, this abundance of ABC transporters might indicate multiple functions. We hypothesize that the ABC transporters have functional similarities that may correlate with their structural similarities. The functional similarity is reflected in their effect on survival and on developmental cell fate in *Dictyostelium discoideum*. We are testing these hypotheses through a systematic study of mutations in these transporters.

We are currently categorizing ABC transporters based on the transcriptional profile of the respective mutants because transcriptional profiling using microarrays yields high-resolution phenotypes for mutant analysis. Transcriptional profiling is being done on 68 strains that carry different ABC gene-knockouts to functionally classify the genes and to obtain a genetic network that may connect them. Transcriptional phenotyping will be supported by morphological characterization of ABC mutant strains. We hypothesize that the functional similarities obtained for a cluster of ABC transporters through transcriptional phenotyping will be manifested through their cellular and molecular phenotypes. Through these analyses we hope to characterize the physiological functions of these evolutionarily conserved ABC genes.

Biologically active small molecules produced by cellular slime molds

Haruhisa Kikuchi¹, Shinya Ishiko¹, Aiko Amagai², Yasuo Maeda², Kohei Hosaka³, Yuzuru Kubohara⁴, Yoshiteru Oshima¹

¹Graduate School of Pharmaceutical Sciences, Tohoku University, ²Graduate School of Life Sciences, Tohoku University, ³Gunma University School of Health Sciences, ⁴Institute for Molecular and Cellular Regulation, Gunma University

Natural product chemistry deals with the isolation, structure elucidation, and study of chemical characteristics of substances, especially small organic compounds, produced by living organisms. Biologically or pharmacologically active natural products are used in the development of new drugs and molecular probes. To discover more novel natural compounds, it is necessary to utilize more living organisms. The cellular slime molds are frequently used as model organisms in various fields of basic science such as cell biology, developmental biology, and biophysics. However, they have not been yet used in natural product chemistry. Recently, we have focused on the utility of cellular slime molds as a resource for novel drug development. Many novel compounds were isolated from the fruiting bodies of various species of cellular slime molds. Total syntheses and biological evaluation of these compounds were also carried out. It was shown that dictyopyrones and dictyomedins may regulate *Dictyostelium* development. Amino sugar derivatives such as furanodictines and dictyoglucosamines induced neuronal differentiation of rat PC-12 cells. In addition, brefelamide inhibited the cellular proliferation of 1321N1 human astrocytoma cells. These results show that cellular slime molds are promising sources in natural product chemistry.

Startup of NBRP-nenkin, the Japanese Stock Center

Hideko Urushihara¹, Taro Q. P. Uyeda², Hidekazu Kuwayama¹, Akira Nagasaki², Shin-ichi Kawakami¹, Yu Hachikubo², Keiichiro Ui¹, Reiko Nishijima¹

¹Graduate School of Life and Environmental Sciences, University of Tsukuba, ²Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology

NBRP (the National Bio Resource Project) is a Japanese national project by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The purpose of this project is to collect, preserve, and provide bioresources, basic materials for life science research, and also to upgrade them responding to the demands of the science today through developing technologies, genome analysis, and others. The cellular slime molds, “nenkin” in Japanese, have been added to the list of those bioresources since August 2007, and University of Tsukuba and National Institute of Advanced Industrial Science and Technology (AIST) are collaborating for this. Since we already appreciate the great contribution of Dicty Stock Center, NBRP-nenkin collaborates with it and in some aspects complements it. The biggest and unique mission of NBRP-nenkin is to rearrange and distribute cDNA resources collected by Japanese cDNA Project. We eliminate redundancy and select the best clones for each gene from nearly 100,000 *E. coli* clones and rearrange them in 96-well plates. We have now 33 plates with full-length clones. We are also making efforts to collect the strains generated and/or maintained in Japan. Since shipping genetically modified organisms to and from overseas is laborious and sometimes very difficult in Japan, NBRP-nenkin takes care and collaborate with Dicty Stock Center. Please visit our site below and check our clones and strains. We will welcome your comments, suggestions, and requests. http://nenkin.lab.nig.ac.jp/en_top

A taxonomic revision of the genus *Polysphondylium*Shin-ichi Kawakami¹, Hiromitsu Hagiwara², Tetsuo Hashimoto¹¹Graduate School of Life and Environmental Sciences, University of Tsukuba, ²Department of Botany, National Museum of Nature and Science

Dictyostelid cellular slime molds (CSMs) are a small taxonomic group consisting of approximately 110 species. However, CSMs have some species complexes that are difficult to classify. On the other hand, Schaap *et al.* (2006) analyzed CSMs phylogenetically, but any taxonomic rearrangements have not been done until now. We here pick up and revise one species complex, *Polysphondylium pallidum*—*P. album* complex that forms a single phylogenetic clade but is one of most confused taxonomically. A representative species, *P. pallidum* Olive and its morphologically similar species, *P. album* Olive have already been emended (Kawakami and Hagiwara 2008). In addition, eight new taxa from Japan, Oman, and Sierra Leone have been found so far. In this study, a morphological comparison among all the species and new taxa made their differences and common features more clear. Phylogenetic relationships of 14 species and 8 new taxa based on the partial sequences of LSU rRNA gene D1/D2 region showed the monophyly of them. Moreover, phylogenetic analysis of SSU rRNA gene sequences of 14 species and 5 new taxa demonstrated that two morphological characters, aggregation pattern and spore size, were important for taxonomic criteria in this complex. Finally, we established a new genus and transferred this species complex to it because this complex was phylogenetically and morphologically distinct from the type species of *Polysphondylium*, *P. violaceum*.

- Kawakami and Hagiwara, *Mycologia* 100: 111–121 (2008).
- Schaap *et al.*, *Science* 314: 661–663 (2006).

The TOR signalling pathway controls Batten Disease phenotypes in *Dictyostelium*

Paige K Smith, Paul R Fisher

Department of Microbiology, La Trobe University, Bundoora, VIC

Batten Disease is the common name given to a group of lysosomal storage disorders known as the Neuronal Ceroid Lipofuscinoses (NCLs). Eight forms of the disease have been identified and are characterized by their age of onset. Whilst each is genetically different, symptoms are similar in the different forms of the disease. Lysosomal disease signaling has been poorly characterized in mammals due to a lack of model systems and the complexity of the pathways in higher eukaryotes. This study involved the characterization of the type two variant (NCL2) in *Dictyostelium discoideum* and subsequent investigation into the proteins involved in disease symptoms.

NCL2 is caused by a mutation in the enzyme Tripeptidyl-peptidase-1 (TPP-1), a serine peptidase involved in the cleavage of large proteins following their internalization to the lysosomal vesicle. Mutants with decreased TPP-1 expression were created and phenotypic analysis was conducted. Whilst growth rates are severely diminished in the mutants, the uptake of nutrients via phagocytosis and pinocytosis is significantly increased. We also present evidence that the TOR signaling pathway mediates the phenotypic effects of TPP-1 deficiency, thereby shedding light on Batten Disease symptoms in this model organism.

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List of Participants

Tomoaki Abe

Faculty of Science and Engineering,
Ishinomaki Senshu University,
Ishinomaki, Miyagi 986-8580, Japan
Email: tabe@isenshu-u.ac.jp

Hiroyuki Adachi

The University of Tokyo
Graduate School of Agricultural and Life Sciences
1-1-1 Yayoi, Bunkyo-ku
Tokyo 113-8657, Japan
Email: adachih@mail.ecc.u-tokyo.ac.jp

Claire Y Allan

La Trobe University
Department of Microbiology
Kingsbury drive, Bundoora, Victoria 3086, Australia
Email: c_y_a_001@hotmail.com

Aiko Amagai

Tohoku University, Graduate School of Life
Sciences
Katahira 2-1-1, Aoba-Ku
Sendai 980-8577, Japan
Email: aiamagai@mail.tains.tohoku.ac.jp

Tsuyoshi Araki

University of Dundee
School of Life Sciences
WTB/MSI Complex, University of Dundee
Dow Street., Dundee DD1 5EH, UK
Email: t.araki@dundee.ac.uk

Gareth Bloomfield

MRC Laboratory of Molecular Biology
Hills Road, Cambridge
CB2 0QH, UK
Email: garethb@mrc-lmb.cam.ac.uk

Salvatore Bozzaro

University of Turin
Dept. Clinical and Biological Sciences
Ospedale S. Luigi, Orbassano (Torino) 10043, Italy
Email: salvatore.bozzaro@unito.it

Derrick T Brazill

Hunter College
Department of Biological Sciences
695 Park Avenue, New York
NY 10065, USA
Email: brazill@genectr.hunter.cuny.edu

Joseph A Brzostowski

National Institutes of Health
12441 Parklawn Drive, Rockville, MD 20852, USA
Email: jb363a@nih.gov

Michael John Carnell

The Beatson Institute, The Beatson Institute
Garscube Estate, Bearsden, Glasgow
G61 1BD, UK
Email: m.carnell@beatson.gla.ac.uk

Pascale G. Charest

University of California, San Diego
Department of Cell and Developmental Biology
9500 Gilman Drive, La Jolla
CA 92093-0380, USA
Email: pcharest@ucsd.edu

Rex L Chisholm

Northwestern University
303 E Superior St, Chicago, IL 60611, USA
Email: r-chisholm@northwestern.edu

Ludwig Eichinger

University of Cologne
Institute for Biochemistry
Joseph-Stelzmann-str. 52, Koln, 50931, Germany
Email: ludwig.eichinger@uni-koeln.de

Petra Fey

Northwestern University
Biomedical Informatics Center
750 N. Lake Shore Drive, Chicago IL 60611, USA
Email: pfey@northwestern.edu

Paul R. Fisher

La Trobe University
Department of Microbiology
VIC 3086, Australia
Email: P.Fisher@latrobe.edu.au

Koichi Fujimoto

JST, ERATO Complex Systems Biology Project
c/o Kaneko
Dept. Pure & Appl. Sci. Univ. of Tokyo
3-8-1 Komaba Meguro Tokyo 153-8902, Japan
Email: fujimoto@complex.c.u-tokyo.ac.jp

Zhihui Chen

University of Dundee
Department of cell and developmental biology,
Dow street, Dundee, DD1 5EH, UK
Email: z.y.chen@dundee.ac.uk

Chang Yong Chung

Vanderbilt University
1215 21st Ave. South, Nashville
TN 37232-6600, U.S.A
Email: chang.chung@vanderbilt.edu

Rebecca S Fernandez

Hunter College, CUNY, Biological Sciences
695 Park Ave, New York
NY 10065, USA
Email: fernandez@genectr.hunter.cuny.edu

Richard Alan Firtel

UCSD, Cell and Developmental Biology
NSB rm. 6316,
9500 Gilman Drive, La Jolla, CA 92093-0380, USA
Email: rafirtel@ucsd.edu

Jakob Franke

Columbia University
Dept. Pathology
3959 Broadway, BHS T-19, New York
NY 10032, USA
Email: jf31@columbia.edu

Masashi Fukuzawa

Hirosaki University
Faculty of Agriculture and Life Science
3 Bunkyo-cho, Hirosaki
Aomori 036-8561, Japan
Email: fukuzawa@cc.hirosaki-u.ac.jp

Pascale Gaudet

Northwestern University
Biomedical Informatics Center
750 N. Lake Shore Dr , 11-160 60611, USA
Email: pgaudet@northwestern.edu

Gernot Gloeckner

Leibniz Institute for Age Research
Genome Analysis
Beutenbergstr 11 Jena, D-07745, Germany
Email: gernot@fli-leibniz.de

Thomas Gregor

University of Tokyo
University of Tokyo
3-38-8-102 Uehara, Shibuya-Ku 151-0064, Japan
Email: thomas.gregor@gmail.com

Scott Gruver

Vanderbilt University
Department of Pharmacology
Room 468 RRB; 23rd Ave. S at Pierce
Nashville, TN 37209, USA
Email: scott.gruver@vanderbilt.edu

Adrian J Harwood

Cardiff University
School of Biosciences
Museum Ave, Cardiff CF10 3US, UK
Email: harwoodaj@cf.ac.uk

Alba Hykollari

University of Natural Resources and Applied Life
Sciences, Vienna
Department of Chemistry
Muthgasse 18, Vienna 1190, Austria
Email: alba.hykollari@boku.ac.at

Hironori Inaba

The University of Tokyo, Graduate school of
Agricultural and Life Sciences
Department of Biotechnology
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Email: aa086087@mail.ecc.u-tokyo.ac.jp

Kei Inouye

Kyoto University
Department of Botany
Kitashirakawa Oiwake-cho, Sakyo-ku
Kyoto 606-8502, Japan
Email: inoue@cosmos.bot.kyoto-u.ac.jp

Yoshiaki Iwadate

Department of Functional Molecular Biology
Graduate School of Medicine
Yamaguchi University, Yoshida 1677-1
Yamaguchi 753-8512, Japan
Email: iwadate@yamaguchi-u.ac.jp

Sosuke Iwai

Research Institute for Cell Engineering
National Institute of Advanced Industrial Science
and Technology (AIST)
1-1-1 Higashi, Tsukuba 305-8562, Japan
Email: iwai-sosuke@aist.go.jp

Chris Janetopoulos

Vanderbilt University
Department of Biological Sciences
465 21st Ave South
Nashville, TN 37232, USA
Email: c.janetopoulos@vanderbilt.edu

Wonhee Jang

Dongguk University
Department of Life Sciences
3-26 Pil-dong Choonggu
Seoul 100715 Republic of Korea
Email: wany@dongguk.edu

Tian Jin

NIH
12441 Parklawn Dr.
Twinbrook II, 20852, USA
Email: tjjin@niaid.nih.gov

Peter M. Kastner

Institute for Cell Biology
Ludwig Maximilians University
Schillerstrasse 42, Muenchen 80336, Germany
Email: Peter.Kastner@lrz.uni-muenchen.de

Yoshinori Kawabe

Dundee University
School of Life Sciences
Msi/wtb Complex
Dow Street DUNDEE DD15PR, UK
Email: Y.Kawabe@dundee.ac.uk

Takefumi Kawata

Toho University
Department of Biology
2-2-1 Miyama, Funabashi 271-8410, Japan
Email: tkawata@bio.sci.toho-u.ac.jp

Kumiko Kihara

Osaka University
Graduate School of Frontier Biosciences
2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
Email: Kihara_Kumiko@bio.eng.osaka-u.ac.jp

Alan R. Kimmel

NIH, 50/3351
Bethesda, MD 20892, USA
Email: ark1@helix.nih.gov

Yoichiro Kamimura

Johns Hopkins University, School of Medicine
Department of Cell Biology
725 N Wolfe St Rm116 WBSB
Baltimore, MD 21205, USA
Email: ykamimu1@jhmi.edu

Mariko Katoh

Baylar College of Medicine
Department of Molecular and Human Genetics
One Baylar Plaza, Rm S930, Houston, Tx 77030,
USA
Email: mkatoh@bcm.tmc.edu

Shin-ichi Kawakami

University of Tsukuba,
Graduate School of Life and Environmental
Sciences
Tennodai 1-1-1, Tsukuba 305-8572, Japan
Email: shkawkaw@yahoo.co.jp

Robert R Kay

MRC Laboratory of Molecular Biology
Hills Rd, Cambridge CB2 0QH, UK
Email: rrk@mrc-lmb.cam.ac.uk

Haruhisa Kikuchi

Tohoku University
Graduate School of Pharmaceutical Sciences
Aoba-yama, Aoba-ku, Sendai 980-8578, Japan
Email: hal@mail.pharm.tohoku.ac.jp

David Knecht

University of Connecticut
91 N. Eagleville Rd., Storrs 6269, USA
Email: david.knecht@uconn.edu

Michael P Koonce

Wadsworth Center, Empire State Plaza
Albany, NY 12201-0509, USA
Email: koonce@wadsworth.org

Yuzuru Kubohara

Gunma University
Institute for Molecular and Cellular Regulation
3-39-15 Showa-machi, Maebashi 371-8512, Japan
Email: kubohara@showa.gunma-u.ac.jp

Adam Kuspa

Baylor College of Medicine
Department of Biochemistry
One Baylor Plaza, Houston 77030, USA
Email: akuspa@bcm.tmc.edu

Hikdekazu Kuwayama

University of Tsukuba
Graduate School of Life and Environmental
Sciences
Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan
Email: hidekuwayama@biol.tsukuba.ac.jp

William F. Loomis

UCSD
9500 Gilman drive, La Jolla
CA 92093-0368, USA
Email: wloomis@ucsd.edu

Laura Macro

Rockefeller University
1230 York Ave, Box 346, New York
NY 10021, USA
Email: macro.laura@gmail.com

Anthony S Kowal

Northwestern University
Center for Genetic Medicine
303 East Superior, Lurie 7-250
Chicago, IL 60611, USA
Email: askowal@northwestern.edu

Mariko Kunitani

University of Tsukuba
Graduate School of Life and Environmental
Sciences
Tennodai, Tsukuba, Ibaraki 305-8577, Japan
Email: kunitani@nenkin.gene.tsukuba.ac.jp

Satoshi Kuwana

Faculty of Agriculture and Life Science
Hirosaki University, 1 bunkyo-cho, Hirosaki
Aomori 036-8560, Japan
Email: satoshi.kuwana@hotmail.co.jp

Xiong Liu

Lab of Cell Biology, NHLBI, NIH
9000 Rockville Pike, Bethesda
MD 20892, USA
Email: liux@nhlbi.nih.gov

Annette Muller-Taubenbe

Ludwig Maximilians University Munich
Institute for Cell Biology,
Schillerstr. 42, Munchen 80336, Germany
Email: amueller@lrz.uni-muenchen.de

Yasuo Maeda

Tohoku University
Graduate School of Life Sciences
Tsunogorou 2-10-11-103, Aoba-ku
Sendai 980-0874, Japan
Email: ymaeda@mail.tains.tohoku.ac.jp

Noritaka Masaki

ERATO Complex Systems Biology Project, JST
The University of Tokyo
Komaba 3-8-1, Meguro-ku 153-8902, Japan
Email: nmasaki@complex.c.u-tokyo.ac.jp

Edward Roshan Miranda

Baylor College of Medicine
Program in Developmental Biology
8450, Cambridge st, apt 2159, Houston
Texas 77054, USA
Email: miranda@bcm.edu

Tetsuya Muramoto

University of Dundee
Collage of Life Science
Dow Street, Dundee
DD1 5EH, UK
Email: t.muramoto@dundee.ac.uk

Akira Nagasaki

AIST
Higashi 1-1-1, Tsukuba
Ibaraki 305-8562, Japan
Email: a-nagasaki@aist.go.jp

Dhamodharan Neelamegan

National Research Council
Institute for Biological Sciences
100 Sussex Drive, Ottawa
Ontario K1A0R6, Canada
Email: dhamodharan.neelamegan@nrc.gc.ca

Hiroshi Ochiai

Hokkaido University
Creative Research Initiative SOUSEI
North 10 jo, West 8 chome, Kita-ku
Sappor 060-0810, Japan
Email: hochiai@sci.hokudai.ac.jp

Marie McAnuff

Hunter College
Center for the Study of Gene Function and
Structure
695 Park Ave, 932 HN, New York
New York 10065, USA
Email: mcanuff@genectr.hunter.cuny.edu

Yukihiro Miyanaga

Osaka University
Graduate School of Frontier Biosciences
1-3 Yamadaoka, Suita, Osaka 565-0871, Japan
Email: yukihiro@phys1.med.osaka-u.ac.jp

Seido Nagano

Ritsumeikan University
1-1-1 Nojihigashi, , Kusatsu
Shiga 525-8577, Japan
Email: nagano@sk.ritsumei.ac.jp

Koki Nagayama

Hirosaki University
Department of Biochemistry and Molecular Biology
3 Bunkyo-cho, Hirosaki 036-8651, Japan
Email: i205015@stu.hirosaki-u.ac.jp

Taro Q.P. Noguchi

Research Institute for Cell Engineering (RICE),
AIST
Tsukuba Central 4, 1-1-1 Higashi, Tsukuba
Ibaraki 305-8562, Japan
Email: taro-noguchi@aist.go.jp

Tetsuo Ohmachi

Hirosaki University
Department of Biochemistry and Biotechnology
3 Bunkyo-cho, Hirosaki 036-8651, Japan
Email: tohmachi@cc.hirosaki-u.ac.jp

Akiko A. Oohata

Kansai Medical University
Department of Liberal Arts
6-1012, 5-ban, Kuzuhahanazono-cho
Hirakata 573-1121, Japan
Email: oohata@makino.kmu.ac.jp

Elizabeth Ostrowski

Rice University
Department of Ecology and Evolutionary Biology
EEB Department, MS 170, 6100 Main St.
Houston, TX 77005-1892, USA
Email: ostrowski@rice.edu

Gopal Pande

Centre for Cellular and Molecular Biology
Uppal Road, Hyderabad 500007, India
Email: gpande@ccmb.res.in

Anup Parikh

Baylor College of Medicine
One Baylor Plaza, S930 77030, USA
Email: anup.parikh@gmail.com

Katie Parkinson

University of Manchester
Michael Smith Building, Oxford Road,
Manchester M13 9PT, UK
Email: katie.parkinson@manchester.ac.uk

Catherine Jane Pears

Oxford University
Biochemistry Department
South Parks Road,, Oxford OX1 3QU, UK
Email: catherine.pears@bioch.ox.ac.uk

Md. Kamruzzaman Pramanik

Yamaguchi University
Applied Molecular Bioscience
Graduate School of Medicine
Yoshida1677-1, Yamaguchi 753-8512, Japan
Email: kpramanik2003@yahoo.com

Jelena Pribic

City University of New York
Hunter College, Biology Department
695 Park Avenue, New York City
10065, USA
Email: jelenapribicbg@yahoo.com

Michael Purugganan

New York University
Department of Biology
100 Washington Square East, New York, NY
10003, USA
Email: mp132@nyu.edu

David C Queller

Rice University
6100 Main St, MS-170,
130 Anderson Biology 77005, USA
Email: queller@rice.edu

Nirmala Rai

Everest Institute of Environmental Health Science
Kupondole, Lalitpur,
Nepal 977
Email: janata@enet.com.np

Kenneth B. Raper

University of Wisconsin
Madison, Wisconsin 53706, USA
Email: account@host.domain

Kentaro Saeki

Graduate School of Life and Environmental Science
University of Tsukuba
Tennodai, Tsukuba, Ibaraki 305-8577, Japan
Email: kentaro@nenkin.gene.tsukuba.ac.jp

Tamao Saito

Hokkaido University
Faculty of Science
kita10 nishi8 Sapporo 060-0810, Japan
Email: tasaito@sci.hokudai.ac.jp

Masazumi Sameshima

Hirosaki University
Department of Biology
3 Bunkyou-cho, Hirosaki
Aomori 036-8561, Japan
Email: msameshi@cc.hirosaki-u.ac.jp

Norimitsu Sasaki

Faculty of Science
Toho University
2-2-1 Miyama, Funabashi
Chiba 274-8510, Japan
Email: 6208006s@nc.toho-u.ac.jp

Santosh Lahu Sathe

Indian Institute of Science
Centre for Ecological Sciences
Boy's hostel (1T3), IISC, Yeshwantpur road
Bangalore 560012, India
Email: santosh_sathe@ces.iisc.ernet.in

Masayuki J. Sato

Osaka University
Laboratories for Nanobiology
Graduate School of Frontier Biosciences
1-3 Yamadaoka, Suita, Osaka 565-0871, Japan
Email: satom@phys1.med.osaka-u.ac.jp

Satoshi Sawai

University of Tokyo
Graduate School of Arts and Sciences
3-8-1 Komaba, Meguro-ku 153-8902, Japan
Email: cssawai@mail.ecc.u-tokyo.ac.jp

Pauline Schaap

Dundee University
MSI/WTB/JBC complex
Dow Street, Dundee DD1 5EH, UK
Email: p.schaap@dundee.ac.uk

Birgit Schiller

University of Natural Resources and Applied Life
Sciences, Vienna
Department of Chemistry
Muthgasse 18, Vienna 1190, Austria
Email: birgit.schiller@boku.ac.at

Hiroshi Senoo

Faculty of Agriculture and Life Science
Hirosaki University
1 bunkyo-cho, Hirosaki
Aomori 036-8560, Japan
Email: hakusanhakusan@gmail.com

Gad Shaulsky

Baylor College of Medicine
One Baylor Plaza, BCM225; S930, Houston
TX 77030, USA
Email: gadi@bcm.edu

Nao Shimada

The University of Tokyo
Graduate school of Agricultural and Life Sciences
1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Email: ashimada@mail.ecc.u-tokyo.ac.jp

Maria Christina Shina

University of Cologne
Medical Faculty, Centre for Biochemistry
Joseph-Stelzmann Strasse 52, Cologne 50931,
Germany
Email: mshina0@uni-koeln.de

Paige Katherine Smith

La Trobe University
Department of Microbiology
Kingsbury Drive Bundoora, Victoria 3086,
Australia
Email: unlucky_wally@yahoo.com.au

Paul A. Steimle

University of North Carolina at Greensboro
Department of Biology
312 Eberhart, Greensboro 27402, USA
Email: p_steiml@uncg.edu

Alexandra Surcel

Johns Hopkins School of Medicine
Cell Biology Department
725 N Wolfe Street, Baltimore
MD 21205-2105, USA
Email: asurcel1@jhmi.edu

Akira Tanaka

Hokkaido University
Faculty of Science
Kita10, Nishi8
Sapporo 060-0810 Japan
Email: attatta@ec.hokudai.ac.jp

Regina Teo

Cardiff School of Biosciences
Museum Avenue, Cardiff
CF10 3US, UK
Email: teor@cf.ac.uk

Pavana Gowry Sistla

Centre for Cellular & Molecular Biology
Uppal Road, Hyderabad 500007
India
Email: pavanags@ccmb.res.in

Vikas Sonakya

Hunter College
932HN, 695 Park Ave., New York
NY 10021, USA
Email: sonakya@genectr.hunter.cuny.edu

Joan E Strassmann

Rice University
Department of Ecology & Evolutionary Biology
6100 Main St, MS-170, 130 Anderson Biology
77005, USA
Email: strassm@rice.edu

Yoshiaki Takaya

Meijo University
Faculty of Pharmacy
150 Yagotoyama, Tempaku
Nagoya 468-8503, Japan
Email: ytakaya@ccmfs.meijo-u.ac.jp

Michelle Tang

The Johns Hopkins University
Cell Biology Department
WBSB 116, 725 N. Wolfe St Bltimore
MD 21205, USA
Email: mtang6@jhmi.edu

Nicole Terbach

Royal Holloway University of London
School of Biological Sciences
Egham, Surrey
TW20 0EX, UK
Email: n.j.terbach@rhul.ac.uk

Chris Thompson

University of Manchester
Faculty of Sciences
Michael Smith Building, Oxford Road, Manchester
M13 9PT, UK
Email: christopher.thompson@manchester.ac.uk

Junya Tsuchida

Agriculture and Life Science
Hirosaki University
1 bunkyo-cho, Hirosaki
Aomori 036-8560, Japan
Email: junya.tsuchidaman@gmail.com

Kyoko Uchi

University of Tsukuba
Graduate School of Life and Environmental
Sciences
1-1-1Tennodai, Tsukuba, Ibaraki 305-8577, Japan
Email: kyokouchi@nenkin.gene.tsukuba.ac.jp

Saburo Uchiyama

Iwate University
Department of Science Education
3-18-33 Ueda, Morioka city 020-8550, Japan
Email: uchiyama@iwate-u.ac.jp

Hideko Urushihara

Graduate School of Life and Environmental
Sciences
University of Tsukuba
1-1-1 Tennoudai, Tsukuba
Ibaraki 305-8572, Japan
Email: hideko@biol.tsukuba.ac.jp

Taro Q.P. Uyeda

Biomedical Information Research Center
National Institute of Advanced Industrial Science
and Technology
2-42 Aomi, Koto 135-0064, Japan
Email: t-uyeda@aist.go.jp

Kei Tomita

Faculty of Agriculture and Life Science
Hirosaki University
1 bunkyo-cho, Hirosaki
Aomori 036-8560, Japan
Email: rknhb605@ybb.ne.jp

Masatsune Tsujioka

RIKEN, CDB
2-2-3 Minatojima-minamimachi, Chuo-ku
Kobe 650-0047, Japan
Email: mas-tsujioka@cdb.riken.jp

Toru Uchikawa

Department of Botany, Graduate School of Science
Kyoto University
Sakyo-ku, Kyoto city 606-8502, Japan
Email: uchikawa@cosmos.bot.kyoto-u.ac.jp

Seiji Ura

The Beatson Institute for Cancer Research
Garscube Estate, Switchback Road
Bearsden, Glasgow
G61 1BD, UK
Email: s.ura@beatson.gla.ac.uk

Toshinori Usui

Department of Biological Engineering
Ishinomaki Senshu University
1 Shinmito, Minamisakai, Ishinomaki,
Miyag 986-8580, Japan
Email: dl200001@isenshu-u.ac.jp

Francisco Velazquez

Laboratory of Molecular Biology-MRC
Hills Road, Cambridge
CB2 0QH, UK
Email: fv@mrc-lmb.cam.ac.uk

Georgia Vlahou

University of Cologne
Medical Faculty, Center for Biochemistry
Joseph-Stelzmann-Str.52
Cologne 50931, Germany
Email: gvlahou@uni-koeln.de

Yuko Wakamatsu

Faculty of Agriculture and Life Science
Hirosaki University
1 bunkyo-cho, Hirosaki
Aomori 036-8560, Japan
Email: sasa_sa600@yahoo.co.jp

Jeff Williams

University of Dundee
School of Life Sciences
Dow St DD1 5EH, UK
Email: j.g.williams@dundee.ac.uk

Robin S.B. Williams

Royal Holloway, University of London
Department of Biological Sciences
Egham Hill, Egham, Surrey
TW20 0EX, UK
Email: robin.williams@rhul.ac.uk

Yoko Yamada

University of Dundee
College of Life Sciences
Dow Street, Dundee
DD1 5EH, UK
Email: y.yamada@dundee.ac.uk

Shigeiko Yumura

Yamaguchi University
Department of Biology
Yoshida, 1677-1
Yamaguchi 753-8512, Japan
Email: yumura@yamaguchi-u.ac.jp

Roberto Zanchi

MRC Laboratory of Molecular Biology
Hills Road, Cambridge
CB2 0QH, UK
Email: rz@mrc-lmb.cam.ac.uk

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