International Dictyostelium Conference 2005

August 13-18, 2005

Autrans, France

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PROGRAM

SATURDAY, AUGUST 13th

14:00-20:00 ***Registration of Delegates***

19:00-22:00 ***Welcome Party***

SUNDAY, AUGUST 14th

8:30-10:10 *PHAGOCYTOSIS-ENDOCYTOSIS*

Chair : Michael Schleicher

1. **CAP is essential for the functioning of the endo-lysosomal system and provides a link to the actin cytoskeleton**
   Hameeda Sultana, Francisco Rivero, Rosemarie Blau-Wasser, Stephan Schwager, Alessandra Balbo, Salvatore Bozzaro, Michael Schleicher and Angelika A. Noegel

2. **The *Dictyostelium* ESCRT machinery**
   Sara Mattei, Gérard Klein and Laurence Aubry

3. **Membrane sorting during phagocytosis**
   Valentina Mercanti, Steve J. Charette and Pierre Cosson

4. **TOR, the central controller of cell growth negatively regulates phagocytosis**
   Daniel Rosel, A. Majithia, T. Khurana and A.R. Kimmel

5. **Function and mechanism of action of *Dictyostelium* Nramp1 in bacterial infection**
   Salvatore Bozzaro, Barbara Peracino, Carina Wagner, Alessandra Balest, Alessandra Balbo, Barbara Pergolizzi, Angelika A. Noegel and Michael Steinert

10:10-10:40 ***Coffee break***

10:40-12:20 *CHEMOTAXIS I*

Chair : Rick Firtel

6. **Inositol phosphates and chemotaxis: PLC revisited**
   Jason King, Emma Dalton, Robin Williams and Adrian J. Harwood

7. **Prolyl oligopeptidase and the regulation of chemotaxis via inositol phosphates**
   Karina McQuillan, Jason King, Melanie Keim and Adrian J. Harwood

8. **A cAMP gradient induced a temporally inverted sensitivity-a novel phenomenon reveals that a local inhibition of PI3K is involved in chemoattractant gradient sensing**
Xuehua Xu, Martin Meier-Schellersheim and Tian Jin

9. **A PH domain-containing protein complex is formed at the plasma membrane in response to chemoattractant stimulation**
Vassil A Mihaylov, Frank I Comer, Li-Rong Yu, Timothy D Veenstra and Carole A Parent

10. **About the role of PI3-kinase mediated PI(3,4,5)P_3 signalling in chemotaxis**
Peter J.M. Van Haastert, Harriet Loovers, Marten Postma, Ineke Keizer, Leonard Bosgraaf and Douwe Veltman

12:30-14:00 Lunch

15:40-16:40 CHEMOTAXIS II
Chair : Günther Gerisch

11. **Leading the way - how cells sense and respond to chemoattractant gradients.**
Atsuo Sasaki, Susan Lee, Kosuke Takeda, Ruedi Meili and Richard A. Firtel

12. **Directional sensing and lipid signaling in chemotaxing *Dictyostelium***
Stacey Sedore Willard, Miho Iijima, Chris Janetopoulos, Francisca Vazquez, Lingfeng Chen and Peter Devreotes

13. **Polarized dynamics of single molecule cAR1-YFP during chemotaxis.**
Sandra de Keijzer, Arnauld Sergé, Piet H.M. Lommerse, Freek van Hemert, Herman P. Spaink, Thomas Schmidt and B. Ewa Snaar-Jagalska

16:40-17:10 Coffee break

17:10-18:30 CHEMOTAXIS III
Chair : Gerry Weeks

14. **ERK2 Activity is Regulated by Adaptive and Non-adaptive Pathways in Chemotaxing *Dictyostelium***
Joseph A. Brzostowski and Alan R. Kimmel

15. **A novel *Dictyostelium* RasGEF required for chemotaxis and development**
Maddalena Arigoni, Salvatore Bozzaro, Helmut Kae, Gerald Weeks and Enrico Bracco

16. **Signalling pathways of the RasGEF-containing proteins GbpC and GbpD leading to cell polarity and chemotaxis**
Arjan Kortholt, Leonard Bosgraaf, Helmut Kae, Gerald Weeks, Holger Rehman, Fred Wittinghofer and Peter J.M. Van Haastert

17. **Activated Rheb regulates growth and cell migration**
Amit R. Majithia, Taruna Khurana, Daniel Rosel and Alan R. Kimmel

19:00-20:30 Dinner

20:30-22:30 POSTER SESSION I

MONDAY, AUGUST 15th

8:30-10:10 GENE REGULATION/EXPRESSION I
Chair : Bill Loomis

18. **Distribution of transposable elements in social amoebae**
Gernot Glöckner, Elisha Westbrook and Thomas Winckler

19. **Identification of Regulatory Binding Sites and Corresponding Transcription Fractors Involved in the Developmental Control of 5'-nucleotidase and alkaline phosphatase Expression in *Dictyostelium discoideum***
Bradley R. Joyce, Natasha S. Wiles, Can M. Eristi and Charles L. Rutherford

20. **Evidence for DNA methylation in the *D. discoideum* genome**
Mariko Katoh, T. Curk, Q. Xu, B. Zupan, A. Kuspa and G. Shaulsky

21. **DNA double strand break repair pathways**
J. Hudson, D-W. Hsu, N. Zhukovskaya, K. Guo, J. G. Williams, Cathy Pears and N. Lakin

22. **C-module-DNA-binding factor regulates the aggregation-specific adenylyl cyclase**
Thomas Winckler, Sebastian Steube, Oliver Siol, Ilse Zündorf and Theodor Dingermann

10:10-10:40 Coffee break

10:40-11:40 GENE REGULATION/EXPRESSION II
Chair : Rob Kay

23. **A feed-forward loop regulates post-aggregation gene expression**
William F. Loomis, Negin Iranfar and Danny Fuller

24. **Visualising transcriptional pulses of a eukaryotic gene**
Jonathan R. Chubb and Robert H. Singer

25. **Modes of developmental gene expression and their relation to protein function**
Ezgi O. Booth, Nancy Van Driessche, Adam Kuspa and Gad Shaulsky

11:40-12:20 DIF SIGNALLING I
Chair : Pauline Schaap

26. **Identification of a novel polyketide synthase producing DIF-1**
Tamao Saito, Stephen Haydock, Atsushi Kato and Robert Kay

27. **The genome-wide regulation of gene expression by DIF**
Gareth Bloomfield, J. Skelton, T. Saito, R. Kay and A. Ivens

12:30-14:00 Lunch

15:40-16:40 DIF SIGNALLING II
Chair : Pauline Schaap

28. **Control of DIF Responses by bZIP Transcription Factor Interactions**
Eryong Huang, Simone Blagg, Thomas Keller, Gad Shaulsky and Chris Thompson

29. **Regulation of *Dictyostelium* prestalk-specific gene expression by a novel bZIP transcription factor**
Natasha Zhukovskaya, Masashi Fukuzawa, Tsuyoshi Araki, Yoko Yamada and Jeffrey Williams

30. **Non-vacuolar cell death in *Dictyostelium***
Pierre Golstein, Hannane Boukarabila, Artemis Kosta, Catherine Laporte, David Lam, Marie-Françoise Luciani and Emilie Tresse

16:40-17:10 Coffee break

17:10-18:30 EVOLUTION/ECOLOGY I
Chair : David Queller

31. **How *D. caveatum* inhibits the development of other Dictyostelids and then eats them**
Clement Nizak, Marin Roje, Frank J. Shin, Young P. Jang, Yasuhiro Itagaki, Koji Nakanishi, Stanislas Leibler and Richard H. Kessin

32. **The evolution of morphological complexity in the Dictyostelids**
Elisa Alvarez-Curto, Thomas Winckler, Michaela Nelson, James Cavender, Hiromitsu Hagiwara, Rupert Mutzel, Sandra Baldauf and Pauline Schaap

33. Social behaviour in mixtures of related and unrelated strains of *Dictyostelium giganteum*
Bandhana Katoch, Channabasavangowda, A. Sankarganesh, Sonia Kaushik and Vidyanand Nanjundiah

34. Kin recognition in *Dictyostelium*
Joan Strassmann, Natasha Mehdiabadi, Tiffany Talley-Farnham, Chandra Jack, Thomas Platt, Gad Shaulsky and David Queller

19:00-20:30 Dinner

20:30-22:30 WORKSHOP I
Chair : Rex Chisholm

35. Progress Report of the Dicty Stock Center (DSC)
Jakob Franke, Stephanie A. Marsh and Richard H. Kessin

36. dictyBase Workshop 2005
Karen E. Pilcher, Pascale Gaudet, Petra Fey, E. M. Just, S. N. Merchant, W. A. Kibbe and R. L Chisholm.

TUESDAY, AUGUST 16th

8:30-10:10 CYTOSKELETON I
Chair : Angelika Noegel

37. Dynamics of actin assembly in cell motility, chemotaxis, and phagocytosis
Günther Gerisch, Till Bretschneider, Annette Müller-Taubenberger, Stefan Diez and Kurt Anderson

38. Mechanical Induction of Actin-Mediated Phagosome Rocketing
Margaret Clarke, Annette Müller-Taubenberger, Kurt Anderson and Günther Gerisch

39. An interaction network links a myosin I to the membrane trafficking and actin nucleation machineries during phagocytosis
Yosuke von Heyden, Régis Dieckmann and Thierry Soldati

40. Investigation of the function of Copine A in *Dictyostelium*
Cynthia K. Damer, Marina Bayeva, Adam E. Goldman-Yassen, Emily S. Hahn, Lilian K. Ho, Lauren C. Naliboff, Javier Rivera and Catherine I. Socec

41. Ste20-like kinases in *Dictyostelium discoideum* are involved in phagocytosis, chemotaxis, development and cytokinesis
Meino Rohlfs, Rajesh Arasada, Petros Batsios and Michael Schleicher

10:10-10:40 Coffee break

10:40-11:40 CYTOSKELETON II

Chair : Margaret Clarke

42. A SIN-related pathway regulates cytokinesis in *Dictyostelium*
Annette Müller-Taubenberger, E. Burghardt, M. Schleicher and G. Gerisch

43. Cell mechanosensitivity and polarization under shear flow. How do cells reorient and how do proteins from the cytoskeleton relocalize under flow reversals ?
Jérémie Dalous, Till Bretschneider, Günther Gerisch and Franz Bruckert

44. Two mechanically distinct modes of protrusion formation in amoeboid movement
Kunito Yoshida and Thierry Soldati

11:40-12:40 EVOLUTION/ECOLOGY II

Chair : Yasuo Maeda

45. Control of cheating by high relatedness in a natural population
David Queller, Owen Gilbert, Kevin Foster, Natasha Mehdiabadi and Joan Strassmann

46. Comparing the *Dictyostelium* and *Entamoeba* genomes reveals an ancient split in the Conosa lineage
J. Song, Q. Xu, R. Olsen, W. F. Loomis, G. Shaulsky, A. Kuspa and Richard Sucgang

47. *Acanthamoeba polyphaga* mimivirus : Genome analysis of the largest known virus to date suggests possible links to *Dictyostelium*
Karsten Suhre

12:50-14:20 Lunch

14:30-20:00 Tour to the Caves of Choranche
or
Hiking in the Vercors

20:30 Banquet

WEDNESDAY, AUGUST 17th

8:30-10:10 STRESS & DISEASES I

Chair : Adrian Harwood

48. A system biology approach reveals Hg induced stress responses in the social amoeba *Dictyostelium discoideum*
Francesco Dondero, Jonsson H., Marsano F., Ranzato E., Pesce G., Boatti L., Bloomfield G., Skelton J., Magnelli V., Cavaletto M.,. Kay R., Griffin J. , Ivens A. and Viarengo A.

49. PI3K activity and small GTPase signalling are targeted by VPA: Implications for epilepsy, bipolar disorder, migraine and cancer treatment.
Xuehua Xu, Helmut Kae, Annette Müller-Taubenberger, Kathryn Adley, Nadine Pawolleck, Gerry Weeks, Jan Faix, Markus Maniak, Tian Jin and Robin SB Williams

50. Sphingolipid control of anticancer drug resistance in *Dictyostelium discoideum* and human cells
Junxia Min, Priya Sridevi, Arvan Chan, Bandhana Katoch, Hannah Alexander and Stephen Alexander

51. Functional characterization of DdLIS1 and DdDCX, two microtubule-associated proteins involved in lissencephaly
Irene Schulz, Jan Faix, Thi-Hieu Ho, Markus Rehberg and Ralph Gräf

52. The Shwachman-Diamond Syndrome Gene Encodes an RNA-Binding Protein That Localizes to the Pseudopod of *Dictyostelium* Amoebae During Chemotaxis
Deborah Wessels, Thyagarajan Srikantha, Spencer Kuhl, L. Aravimal and David R. Soll

10:10-10:40 Coffee break

10:40-11:40 STRESS & DISEASES II

Chair : Salvo Bozzaro

53. A new, resistant cell type in *Dictyostelium*: the aspidocyte
Yanni Serafimidis and Rob Kay

54. The role of AMP-activated protein kinase (AMPK) in phototaxis and mitochondrial diseases in *Dictyostelium discoideum*
Paul Bokko and Paul R. Fisher

55. Human versus *Dictyostelium*: comparative genomics to study the function of highly conserved genes of unknown function
Patricia Torija, Juan J. Vicente, Tiago B. Rodrigues, Alicia Robles, Sebastián Cerdán, Leandro Sastre, Rosa M. Calvo and Ricardo Escalante

11:40-12:20 CELL GROWTH & DIFFERENTIATION I

Chair : Alan Kimmel

56. Analysis of Phosphoprotein Phosphatase PP2A in Growth and Development of *Dictyostelium discoideum*
Chirag Mandavia, Srividhya Venkatesan, Avalon Garcia, Sara Anderson, Nicola James, Robert Salzler, Jinha Jung, Hideshi Otsuka, Julian Gross and Robert P. Dottin

57. Cnr1, a putative frizzled/smoothened-like protein participates in group size regulation and cell proliferation in *Dictyostelium discoideum*
Tong Gao, Debbie Brock, R. Diane Hatton, Yitai Tang, and Richard H. Gomer

12:30-14:00 Lunch

14:30-16:40 WORKSHOP II

Chair : Ed Cox & Adam Kuspa

58. Kenneth Raper, Elisha Mitchell and *Dictyostelium*
Eugene R. Katz

59. The *Dictyostelium* Kinome - analysis of the protein kinases from a simple model organism
Jonathan M. Goldberg, Gerard Manning, Allen Liu, Petra Fey, Karen E. Pilcher, and Janet L. Smith

60. Phenotyping and screening of *Dictyostelium* mutants based on dynamical features
Satoshi Sawai, Xiao-Juan Guan, Christopher Dinh, Jennifer Watt, Adam Kuspa and Edward C. Cox

61. A Deletion Set for *Dictyostelium*
Edward C. Cox and Adam Kuspa

16:40-17:10 Coffee break

17:10-18:30 CELL GROWTH & DIFFERENTIATION II

Chair : Jeff Williams

62. Innate immunity and detoxification mediated by specialized cells during *Dictyostelium* development
Guokai Chen, Olga Zhuchenko and Adam Kuspa

63. Aberrant stalk development and breakdown of tip dominance in *Dictyostelium* cell lines with RNAi-silenced expression of calcineurin B
Katrina Boeckeler, Rupert Mutzel and Barbara Weissenmayer

64. **Priming and processing of the spore differentiation factor SDF-2**
Christophe Anjard, Matthew Cabral, Adam Kuspa and Willliam F. Loomis

65. **Establishment of a system for elucidating precisely the function of individual mitochondrial genes**
Junji Chida, Masashi Tanaka, Aiko Amagai and Yasuo Maeda

19:00-20:30 Dinner

20:30-22:30 POSTER SESSION II

THURSDAY, AUGUST 18th

8:30-9:10 SEXUAL CYCLE
Chair : Richard Kessin

66. **Ethylene induces zygote formation through an enhanced expression of a novel zyg1 gene in *Dictyostelium mucoroides***
Aiko Amagai

67. **Gene sharing between sexual and asexual development in *Dictyostelium discoideum***
Hideko Urushihara, Sufen Li, Kinuyo Goto, Jin Hiraga, and Tetsuya Muramoto

9:10-9:30 Coffee break

9:30-10:50 BIOPHYSICAL APPROACHES OF CELL MOTION
Chair : Thierry Soldati

68. **The behaviour of *D. discoideum* from the point of view of the dynamical theory of nonlinear systems**
Lenka Sebestikova, Petra Vankova, Tomas Godula and Hana Sevcikova

69. **Threshold for Chemotactic Motion in *Dictyostelium discoideum***
Loling Song, Sharvari M. Nadkarni, Hendrik U. Boedeker, Carsten Beta, Albert Bae, Carl Franck , Wouter-Jan Rappel, William F. Loomis and Eberhard Bodenschatz

70. **Ramdom motion of *Dictyostelium discoideum*: quantitative analysis using model-free methods**
Hendrik Ulrich Boedeker, Carsten Beta and Eberhard Bodenschatz

71. **Measurements of the mechanical forces exerted by migrating *Dictyostelium* slugs using the flexible substrata method**
Jean-Paul Rieu, Catherine Barentin, Yasuo Maeda and Yasuji Sawada

12:00-14:00 Lunch

14:00 Departure

TALK ABSTRACTS

CAP is essential for the functioning of the endo-lysosomal system and provides a link to the actin cytoskeleton

Hameeda Sultana[1], Francisco Rivero[1], Rosemarie Blau-Wasser[1], Stephan Schwager[2], Alessandra Balbo[3], Salvatore Bozzaro[3], Michael Schleicher[2], Angelika A. Noegel[1]

[1] *Center for Biochemistry and Center for Molecular Medicine Cologne, Medical Faculty, University of Cologne, 50931 Köln, Germany*

[2] *Institute of Cell Biology, Ludwig-Maximilians-Universität, 80336 München, Germany*

[3] *Dipartimento di Scienze Cliniche e Biologiche, Ospedale S. Luigi, 10043 Orbassano, Italy*

Data from mutant analysis in yeast and *Dictyostelium* indicate a role for the cyclase-associated protein (CAP) in endocytosis and vesicle transport. We have used genetic and biochemical approaches to identify novel interacting partners of *Dictyostelium* CAP to help explain its molecular interactions in these processes. CAP associates and interacts with subunits of the highly conserved vacuolar H^+-ATPase (V-ATPase) and colocalizes to some extent with the V-ATPase. Furthermore, CAP is essential for maintaining the structural organization, integrity and functioning of the endo-lysosomal system as distribution and morphology of V-ATPase and Nramp1 decorated membranes were disturbed in a CAP mutant (CAP bsr) accompanied by an increased endosomal pH. Moreover, concanamycin A (CMA), a specific inhibitor of the V-ATPase, had a more severe effect on CAP bsr than on wild type cells and the mutant did not show adaptation to the drug. Also, the distribution of GFP-CAP was affected upon CMA treatment in the wild type and recovered after adaptation. Distribution of the V-ATPase in CAP bsr was drastically altered upon hypo-osmotic shock and growth was slower and reached lower saturation densities in the mutant under hyper-osmotic conditions. Taken together, our data unravel a link of CAP with the actin cytoskeleton and endocytosis and suggest that CAP is an essential component of the endo-lysosomal system in *Dictyostelium*.

The Dictyostelium ESCRT machinery

Sara Mattei, Gérard Klein and Laurence Aubry

Laboratoire de Biochimie et Biophysique des Systèmes Intégrés, DRDC, Cea-Grenoble, 17, rue des Martyrs, 38054 Grenoble cedex 9, France

The multivesicular body (MVB) is a platform where membrane proteins (from endocytic and biosynthetic pathways) targeted to the lysosome for degradation or maturation are sorted into intralumenal vesicles prior fusion with the lysosome. Biogenesis of the MVB and sorting of the membrane proteins involve multiprotein complexes, the ESCRT I, II and III subcomplexes as well as accessory proteins such as the adaptor Alix and the AAA-ATPase VPS4. Interestingly, the same machinary is hijacked by viruses such as HIV for their budding. Despite considerable recent progress in the understanding of the MVB sorting machinery and its biogenesis, many fundamental questions remain to be answered regarding the precise role of each individual component of the ESCRT machinery, the mechanisms of formation of intralumenal vesicles (forces underlying membrane curvature and fission) or how retroviruses divert this machinery for their budding.

We took advantage of *Dictyostelium* to dissect the specific function of each component. All ESCRT components and the associated proteins Alix and VPS4 are present in *Dictyostelium* genome. Analysis of the subcellular localisation of specific components suggests a phylogenetic conservation of the process. Similarly, overexpression of the dominant-negative form of VPS4, VPS4E/Q stabilizes Alix-positive endocytic subcompartments. A knock-out strategy allowed us to evidence a differential implication of the proteins in the process: the *alx* null mutant harbors a developmental defect that is not reproduced by *vps23* (*tsg101*) disruption, suggesting a more complex function for this actor.

Membrane sorting during phagocytosis

Valentina Mercanti, Steve J. Charette and Pierre Cosson

Dept of Cell Physiology and Metabolism, University of Geneva, School of Medicine, C.M.U, Switzerland.

Phagocytosis is the process by which cells internalize big particles such as bacteria. At the beginning of this process the particle binds to the cell surface, is engulfed in the phagocytic cup and internalized in a phagosome. The subsequent maturation of the phagosome, which involves delivery of endosomal and lysosomal proteins has been the subject of many studies. In comparison we know very little about membrane sorting during the first steps of phagocytosis and in particular in the phagocytic cup and the newly formed phagosome.
In this study we analyzed changes in the membrane composition during phagocytosis in *Dictyostelium discoideum*. Our results indicate that intense membrane sorting is initiated at very early stages of the phagocytic process. A quantitative description of these complex sorting events allowed us to compare membrane sorting during phagocytosis and macropinocytosis, and to evaluate alterations of membrane sorting in various mutant strains.

TOR, the central controller of cell growth negatively regulates phagocytosis

D. Rosel[1], A. Majithia[2], T. Khurana[2], A.R. Kimmel[2]

[1]*Charles University in Prague, Czech Republic*

[2]*National Institutes of Health, Bethesda, USA*

TOR, Target of Rapamycin (an immunosuppressant and anti-cancer drug), is a phosphatidylinositol kinase-related protein kinase, which regulates cell growth and the actin cytoskeleton. Recent studies have demonstrated that TOR functions as a part of two distinct protein complexes. TORC1 is a Rapamycin-sensitive complex that contains TOR, Raptor, and LST8 and controls cell growth in response to nutrients and growth factors. A second TOR complex, TORC2, is suggested to be insensitive to rapamycin, contains TOR, LST8, Pia (AVO1) and RIP3 (AVO2), and regulates actin polarization.
A signaling pathway that regulates TOR includes activation of PI3-K and its downstream effector Akt. Akt phophorylates and inactivates the Tuberous sclerosis complex (TSC) which acts as GAP toward the small G-protein Rheb. Rheb in turn directly activates TOR. We have utilized available null cell lines for genes involved in this pathway, and additionally constructed knockouts for Rheb, TSC2, Lst8, and the Rapamycin-sensor FKBP, for analysis of growth and growth-related processes. Our data confirm a TSC/Rheb pathway for TOR regulation in *Dictyostelium*. We show that TORC2 but not TORC1 negatively regulates phagocytosis, but that pinocytosis is not affected by either TOR. We propose new a model for TOR signaling. TORC1 positively regulates growth, whereas TORC2 negatively regulates phagocytosis. Since short-term treatment (45 minutes) with Rapamycin has no effect on phagocytosis, but long-term treatment (5 hours) activates phagocytosis, we propose that there may be an equilibrium between TORC1 and TORC2 complexes. Rapamycin therefore not only directly inhibits the TORC1, but it may also indirectly negatively modulate TORC2 activation.

Function and mechanism of action of *Dictyostelium* Nramp1 in bacterial infection

Salvatore Bozzaro, Barbara Peracino[1], Carina Wagner*, Alessandra Balest, Alessandra Balbo, Barbara Pergolizzi, Angelika A. Noegel[§], Michael Steinert*

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Nramp1 (Slc11a1) is an endo-lysosomal membrane protein that confers on macrophages resistance to infection by a variety of intracellular bacteria and protozoa. Nramp1 appears to act as membrane transporter of divalent cations, especially iron, manganese and zinc, but it is highly debated whether Nramp1 acts by depleting the phago-lysosome of metal cations, or rather importing them in the phagosomal lumen. It is also not clear how pathogens escape Nramp1 activity.

We found some years ago that *Dictyostelium* cells express an Nramp1 homolog during their growth phase. Similarly to macrophages, which are mutated in Nramp1, Dictyostelium knockout mutants are more susceptible than wild type cells to infection by Mycobacteria and Legionella. Overexpressing Nramp1 under the control of a constitutive promoter effectively protects the cells against *L. pneumophila* infection, but offers no additional advantage against *M. avium,* suggesting that these pathogens may use different mechanisms to neutralize Nramp1.

We have now studied the transport mechanism of Nramp1 by using purified phagosomes from control, Nramp1 knockout and overexpressing cells. We found that Nramp1 is essential for active iron transport and that Nramp1 eventually depletes iron from the phagosomes if an ATP source is provided. Since Nramp1 is not an ATPase, it is likely that ATP is required for activating the V-H^+ATPase pump in phagosomes, thus leading to H^+ accumulation in the phagosomal lumen and to Fe^{2+} export *via* Nramp1 powered by the H+ gradient in a symport mechanism. This model explains the apparently contradictory results obtained so far with macrophages, and shed some light on how pathogens can neutralize or even exploit Nramp1 to their advantage.

It is crucial in this model that Nramp1 and V-ATPase be located in the same compartment. By using GFP fusion proteins with Nramp1 and the vatB subunit of the V-ATPase, as well as anti-VatA antibodies, we show that in resting cells vatB and Nramp1 are present in partially separate compartments, and that Nramp1 is notably absent from the contractile vacuole, where most of vatB is located. Both vatB and Nramp1 are, however, rapidly recruited following macropinocytosis or phagocytosis of yeast and beads, and co-localize in endo- and phago-lysosomes. Experiments are in progress to study whether a similar recruitment occurs during *M. avium* or *L. pneumophila*.

Inositol phosphates and chemotaxis: PLC revisited

Jason King[1], Emma Dalton[1], Robin Williams[2], Adrian J. Harwood[1]

[1] *School of Bioscience, Cardiff University, Museun Ave, CF10 3US, UK*

[2] *Dept of Biology, University College London, Gower St, WC1E 6BT, UK*

Inositol phosphates are intimately associated with *Dictyostelium* chemotaxis. Regulated synthesis of the lipid inositol phosphate, phosphatidyl (3,4,5) trisphosphate (PIP3) by PI 3 kinase amplifies the response to cAMP and directs pseudopod formation. The role of soluble inositol phosphates (InsP) is less clear. Inositol (1,4,5) trisphosphate (InsP3) is rapidly generated by phospholipase C (PLC) mediated hydrolysis of phosphatidyl (4,5) bisphosphate (PIP2) upon cAMP stimulation. However, disruption of the single Dictyostelium PLC (pipA) gene appears to have no effect on chemotaxis, and furthermore loss of iplA, a putative InsP3 receptor, also has no effect. In contrast, lithium and valproic acid (VPA) treatment both reduce InsP3 and severely attenuate aggregation and the chemotactic response. Loss of prolyl oligopeptidase (DpoA) increases the basal level of InsP3 and restores the chemotactic response in the presence of lithium and VPA. To investigate this apparent contradiction, we have re-examined the pipA mutant in combination of lithium treatment and other mutants of InsP metabolism.

Despite complete loss of PLC activity, the pipA mutant still generates InsP3. This is due to up-regulation of the enzyme Multiple Inositol polyphosphate phosphatase (MIPP), which forms InP3 by dephosphorylation of higher order InsPs. The same enzyme is up-regulated in dpoA mutants. Consistent with these two observations we find that the pipA mutant is lithium resistant. We have identified and disrupted the *Dictyostelium* MIPP gene (mippA) that is expressed during chemotaxis. As predicted the mippA mutant is hypersensitive to lithium treatment. However when pipA and mippA mutations are combined, the double mutant is lithium resistant not hypersensitive. This places pipA genetically downstream of mippA. This could be explained if the major role of PLC during chemotaxis is to attenuate PIP signaling rather than to generate InP3.

Prolyl oligopeptidase and the regulation of chemotaxis via inositol phosphates

Karina McQuillan[1], Jason King[1], Melanie Keim[2], Adrian J. Harwood[1]

[1] *School of Bioscience, Cardiff University, Museun Ave, CF10 3US, UK*

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We have previously shown that loss of the *Dictyostelium* prolyl oligopeptidase gene (dpoA) elevates Inositol (1,4,5) trisphosphate (InsP3) and confers resistance to the effects of lithium and valproic acid; these observations suggest that DpoA regulates inositol phosphate (InsP) metabolism. We observe a peak of DpoA activity at the end of aggregation indicating that changes in DpoA activity may regulate aspects of development.

The molecular mechanism by which DpoA regulates inositol phosphates is unknown, but involves changes in activity of Multiple Inositol polyphosphate phosphatase (MippA). There are two general mechanisms by which DpoA could alter InsP metabolism. First, it could directly alter the flux of InsPs in the cell, and act to regulate the amount of free inositol for the production of phosphatidyl phosphates (PIPs). Second, in yeast higher order InsPs can regulate gene expression, and therefore PO could act indirectly through altered gene expression.

We find that a set of genes encoding enzymes required for the recycling and synthesis of inositol are regulated by altered DpoA activity. In contrast, genes encoding enzymes that regulate higher order InsP, including mippA and dpoA, are unaltered. Altered gene expression requires the mippA gene indicating that DpoA acts via altered MIPP activity. Furthermore, we find that the lithium resistant gene lisG encodes a chromatin re-modelling factor and could regulate altered gene expression. These results indicate that DpoA and MippA can regulate gene expression during *Dictyostelium* development.

A cAMP gradient induced a temporally inverted sensitivity-a novel phenomenon reveals that a local inhibition of PI3K is involved in chemoattractant gradient sensing

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A central question in chemotaxis is how a shallow external chemoattractant gradient is translated into highly polarized intracellular signaling events, which ultimately lead to cell polarization and directional movement. Many of the key molecules and biochemical signaling cascades that regulate chemotactic responses have been identified. A current challenge is to understand how these components work together to produce directional movement. We have determined the spatiotemporal dynamics of G-protein activation by FRET imaging and PIP3 levels in single living cells when they are suddenly exposed to a stable gradient (Xu et al, 2005). Our experiments revealed an unexpected dynamics of PIP3. Therefore, we have developed a new model that simulates the dynamic processes of a signaling network in a 'digital cell'. The signaling network in the digital cell allows it to respond to cAMP stimuli in computer simulations. The simulations quantitatively describe how cAMP binding to receptors triggers activation and membrane-recruitment of components that control the availability and/or activity of Gbetagamma, Ras, PI3K and PTEN in different regions of a cell in response to various cAMP fields (Meier-Schellersheim et al., unpublished). Our model proposes that the activation/inhibition of PI3K activity and the amount of membrane-bound PTEN are all regulated by the local receptor occupancy in various regions of the inner membrane of a cell. One counter intuitive conclusion generated from our interplay between experiments and modeling is that PI3K activity must be strongly inhibited at the front of the cell in a cAMP gradient after PHCrac-GFP distribution becomes highly polarized. To test this idea, we hypothesized that if the gradient is suddenly removed, the signaling components including the hypothetical inhibitory components would rapidly return to pre-stimulated states. If the inhibitory components withdraw more slowly from the cell membrane and the PI3K activity would be more strongly suppressed in the front, and then the back of the cell would be temporally more sensitive to cAMP stimulation. At this moment, a uniformly applied cAMP stimulation would induce a PHCrac-GFP translocation only to the back of the cells instead of a uniform response displayed by the naive cells. Our experiments demonstrated this temporally inverted sensitivity. One possible explanation is that if receptors remain desensitized or G-proteins are not completely re-associated when the second stimulation is applied, G-proteins would not be fully activated to induce PI3K activation in the front. To rule out this possibility, we determined the kinetics of G-protein re-association and re-activation in living cells using FRET analysis in real time. We found that the uniform cAMP stimulation triggered G-protein activation in both the front and back of a cell, but failed to induce PHCrac-GFP translocation in the front, indicating signaling components other than receptors and G-proteins were responsible for this inverted sensitivity. Therefore, this new phenomenon reveals a local inhibitory mechanism of PI3K involved in chemoattractnat gradient sensing.

A PH domain-containing protein complex is formed at the plasma membrane in response to chemoattractant stimulation

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A cell's ability to sense and respond to external directional cues is fundamental to diverse biological functions such as nerve growth, embryogenesis and innate immunity. In neutrophils and *Dictyostelium discoideum*, a model system for chemotaxis studies, PI3K signaling plays a pivotal role. PH-domain containing proteins that bind to the PI3K products $PI(3,4)P_2$ and $PI(3,4,5)P_3$, such as CRAC (Cytosolic Regulator of Adenylyl Cyclase) and Akt/PKB, are cytosolic under basal conditions, but undergo a dramatic redistribution when the cells are exposed to chemoattractants. In a gradient of chemoattractant, these proteins are rapidly and persistently recruited to the leading edge of the cell where they are proposed to spatially control the nucleation of a subset of signaling events. However, when a uniform saturating stimulus is applied, this recruitment to the plasma membrane is very transient due to an adaptation process that is not fully understood. This transient membrane recruitment of CRAC in *D. discoideum* is essential to its function as a regulator of chemotaxis and activator of adenylyl cyclase. Yet the mechanisms underlying these crucial functions of CRAC remain undetermined. Investigating these rapid and transient signaling events presents a significant challenge. We developed a rapid *in vivo* chemical crosslinking protocol that allows the capture of protein-protein interactions into stable complexes. Using this approach we show that, upon stimulation with chemoattractant, CRAC becomes part of high molecular weight protein complexes. These complexes are highly enriched at the plasma membrane and their formation and dissociation follow kinetics that mimic those of un-crosslinked CRAC. Interestingly, a short in-frame CRAC deletion mutant that fails to activate adenylyl cyclase but still translocates to the plasma membrane, shows a remarkable difference in the pattern of the formed complexes as well as in their distribution, with a prominent presence in the cytosol. These results suggest that CRAC determinants dynamically regulate protein-protein interactions at the plasma membrane. The crosslinked complexes have been purified using immobilized metal ion affinity chromatography and mass spectrometric analysis is currently under way to identify the components. These studies provide valuable insight into the signals that regulate chemotaxis, particularly in the context of deciphering the spatial cues involved in amplifying external gradients.

About the role of PI3-kinase mediated PI(3,4,5)P_3 signalling in chemotaxis

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It has been well documented by many laboratories that PH_{CRAC}-GFP localizes to the leading edge in chemotaxing cells, leading to actin polymerization and pseudopod formation at the position of these PH_{CRAC}-GFP patches. However, in buffer many pseudopodia are formed in the absence of PH_{CRAC}-GFP patches, and the PI3-kinase inhibitor LY29004 strongly inhibits PH_{CRAC}-GFP localization but has little effect on the direction of movement in a chemotactic gradient. Therefore we have set up a quantitative analysis of PH_{CRAC}-GFP localization, and the direction and speed of movement of the same cell in cAMP gradients that was formed in Zigmund-type chamber or delivered from a micropipette. We made the following observations:

When PH_{CRAC}-GFP patches are present, pseudopodia are always extended from these patches. Thus PH_{CRAC}-GFP (PI(3,4,5)P_3) patches are very strong inducers of pseudopod extension. There are many situation where PH_{CRAC}-GFP patches are not present, but pseudopodia are extended very efficiently in the direction of the cAMP gradient, for instance at increasing steepness of the gradients upto 500 pM/um the chemotaxis index increase to 0.8 but PH_{CRAC}-GFP patches are not yet detectable.

The input signal for chemotaxis is the absolute gradient dC/dx, while the input signal for PH_{CRAC}-GFP patches is the relative gradient dC/dx/C, suggesting that PH_{CRAC}-GFP patches do not mediate directional sensing.

We noticed that cells with a PH_{CRAC}-GFP patch make larger pseudopod extensions and have a larger speed than cells without PH_{CRAC}-GFP patches. Furthermore, upon withdrawal of the gradient cells with a PH_{CRAC}-GFP patch show a longer persistance of locomotion than cells without a PH_{CRAC}-GFP patch. These data suggest that the PH_{CRAC}-GFP patch at the leading edge of a cell is not a compass providing directional information, but more likely functions as a turbo, increasing the speed and persistance of locomotion. This turbo becomes active in steep gradients when the chemotaxis index is already high.

Leading the way - how cells sense and respond to chemoattractant gradients.

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During chemotaxis, eukaryotic cells recognize a shallow gradient of chemoattractant, which they amplify intracellularly to produce a highly polarized cell that moves up the gradient. Receptors and heterotrimeric G-protein subunits are distributed and activated almost uniformly along the plasma membrane, but PI(3,4,5)P_3, the product of PI3K, accumulates locally at the leading edge. We have identified pathways that help define the initial signals mediating the formation of an asymmetrical response at the leading edge and the feedback loops that amplify the signal and stabilize the newly forming pseudopod. We have expanded our analysis of the positive and negative feedback loops that control chemotaxis to the analysis of random cell movement. Our studies demonstrate that common regulatory circuits control both cellular responses. Pathways regulated by Ras are interconnected with those activating Rac and F-actin polymerization and together form a signaling network of intertwined feedback loops that amplify the signal at the leading edge while other pathways restrict the localization of these responses. Parallel studies in other labs on neutrophils suggest that these pathways have been highly conserved in evolution.

Directional sensing and lipid signaling in chemotaxing *Dictyostelium*

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Dictyostelium amoebae are able to dynamically respond to a shallow gradient of chemoattractant. Our data indicate that although upstream components of the signaling network governing chemotaxis are uniformly localized, downstream components are tightly localized to the portion of the cell that is exposed to the most chemoattractant. Upon receptor activation, PTEN is released from the plasma membrane and PI3K is recruited. During persistent uniform stimulation, downstream PIP_3 production and actin polymerization are transient, indicating that a repression mechanism is also activated by receptor stimulation. We propose that PIP_3 production and subsequent actin polymerization are regulated by a local excitation, global inhibition model in which PTEN and PI3K are critical reciprocal regulators of PIP_3 levels. PTEN and PI3K-regulated PIP_3 signaling results in directional sensing and pseudopod formation at the leading edge of the cell. Consistent with this model, disruption of PTEN function results in broadened PIP_3 accumulation and PI3K perturbation alters chemotactic efficiency. According to recent studies, this signaling mechanism may play a role in cytokinesis, since PTEN and PI3Ks are localized to the cleavage furrow and poles, respectively, in dividing cells. Disruption of these regulators impairs cytokinesis and results in large, multinucleate cells.

Polarized dynamics of single molecule cAR1-YFP during chemotaxis.

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Based on confocal imaging, the signalling components of the chemotaxis pathway (like PI3K, PTEN, AC) have a polarized distribution upon cAMP stimulation, except for the cAMP receptor, cAR1, and the coupled G-protein. Since distribution is not the only dynamic property of a receptor, the behaviour of single cAR1 on the apical membrane was followed in real time. The effect of cAMP stimulation on the mobility of cAR1 was investigated with Single Molecule Microscopy, where normal wide-field microscopy with laser excitation is combined with ultra-sensitive CCD camera detection. This gives us the possibility of detecting signals with a positional accuracy of 40 nm. Imaging cAR1-YFP expressed in car1 null cells showed a change in the mobility of the receptor. In both unpolarized and polarized cells two populations of receptors were found, a fast and an immobile fraction. Polarization of the cells was accomplished by chemotaxis needle assay. At the anterior of a polarized cell, the fraction of the fast receptor was larger (54% vs. 31%) compared with the posterior. The posterior was comparable to the unpolarized cell. There was a mobility shift between the unpolarized cell and the anterior of polarized cell. The fluidity of the membrane itself was investigated with a membrane marker (Concanavalin A) and was not different for anterior vs. posterior (excluding involvement of the fluidity in observed mobility shift). To further unravel the cause of the mobility shift we determined the dynamic properties of cAR1-YFP in different genetic backgrounds, a phosphorylation-deficient mutant of cAR1 and in Galpha2 null cells. Conformational change of the receptor due to phosphorylation had no effect on the monitored properties, whereas in absence of Galpha2 there was no mobility shift between unpolarized and polarized cells nor was there a difference between the anterior and posterior of polarized cells. However the number of fast receptors in Galpha2 null cells was comparable to the anterior of polarized car1 null cells, suggesting that the fast population represents Galpha2 uncoupled receptors. We suggest that the mobility shift of the receptors at the leading edge resembles the uncoupling/activation of G-protein and acceleration of intracellular signaling and possibly plays a role in establishing polarization during chemotaxis.

ERK2 Activity is Regulated by Adaptive and Non-adaptive Pathways in Chemotaxing *Dictyostelium*

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The ERK/MAP kinases have been implicated in the regulation of chemotactic signaling in mammalian cells and *Dictyostelium*. Chemotaxing *Dictyostelium* periodically synthesize and secrete cAMP, which in addition to its intracellular role, acts as a chemoattractant. The extracellular oscillatory cAMP signal is perceived by the seven-transmembrane receptor CAR1, which activates downstream networks by both G protein-dependent and -independent mechanisms. These downstream pathways respond transiently, adapting (de-sensitizing) to a non-fluctuating signal. It has been suggested that the activation/de-activation of ERK2 in Dictyostelium is required to regulate oscillatory cAMP production, and that deactivation of ERK2 is mediated by a PKA-dependent intracellular feedback mechanism. We have now tested this model by separately analyzing the activation and de-activation of ERK2 under controlled conditions that manipulate the level of the extracellular cAMP stimulus. We now show that the process of ERK2 activation is completely adaptive, but that ERK2 de-activation is non-adaptive and inhibited by the continuous presence of the cAMP ligand. In addition, we found that both activation and de-activation occur independently of G proteins and of ligand-induced production of intracellular cAMP. These data suggest that ERK2 activity is not controlled by a simple intracellular feedback mechanism through PKA and underscore the complexity of ERK2 regulation in control of chemotactic signaling and chemotaxis.

A novel *Dictyostelium* RasGEF required for chemotaxis and development

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Dictyostelium genome wide analysis revealed a surprisingly high number of Ras Guanine Nucleotide Exchange Factors (RasGEFs) despite the relatively small number of RasGAPs. Up to date only four RasGEFs have been characterized in *Dictyostelium*, which are all non-redundant either for growth or development. Here we report the identification and characterization of a fifth non-redundant *Dictyostelium* RasGEF named D.d.RasGEF M. Two mRNAs are differentially expressed during growth and development, and the developmental one is controlled by the heterotrimeric G-protein. Inactivation of the gene results in disappearance of both mRNAs and in cells that form small, flat aggregates and fail to develop further. The expression of genes required for aggregation is delayed in the mutant. Endogenous cAMP accumulates during early development to a much lower extent than in wild type cells. Adenylyl cyclase activation in response to cAMP pulses is also strongly reduced, by contrast guanylyl cyclase is stimulated to higher levels than in the wild type. Chemotaxis towards cAMP is impaired in the mutant, due to inability to inhibit lateral pseudopods. The actin polymerization response to cAMP is also altered. Cyclic AMP pulsing for several hours partially rescues the mutant. In vitro experiments suggest that RasGEF M acts downstream of the cAMP receptor but upstream of the G protein and acts as guanine exchange factor for RasG but not for RasC. These evidences together, indicate that RasGEF M plays a crucial role for the establishment of cAMP relay and for cell motility. We propose that RasGEF M is a component of a Ras regulated pathway, which integrate signals acting as positive or negative regulators for adenylyl cyclase or guanylyl cyclase, respectively. In addition, RasGEF M/RasG could regulate actin polymerization. Altered guanylyl cyclase, combined with the defective regulation of actin polymerization, could explain the chemotactic defect of the mutant.

Signalling pathways of the RasGEF-containing proteins GbpC and GbpD leading to cell polarity and chemotaxis

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The regulation of cell polarity plays an important role in chemotaxis. Previously, two proteins termed GbpC and GbpD were identified in Dictyostelium, which contain RasGEF and cyclic nucleotide binding domains. We show that gbpC-null cells display strongly reduced chemotaxis, because they are unable to polarise effectively in a chemotactic gradient. However, gbpD-null mutants exhibit the opposite phenotype: cells display improved chemotaxis and appear hyperpolar, because cells make very few lateral pseudopodia, whereas the leading edge is continuously remodelled. Overexpression of GbpD protein results in severely reduced chemotaxis. Cells extend many bifurcated and lateral pseudopodia, resulting in the absence of a leading edge. Furthermore, cells are flat and adhesive owing to an increased number of substrate-attached pseudopodia. This GbpD phenotype is not dependent on intracellular cGMP or cAMP. The GbpD overexpression phenotype is similar to that of cells overexpressing Rap1. In vitro experiments showed that GbpD specifically activates Rap and not any of the other *Dictyostelium* Ras proteins, suggesting a role of GbpD as RapGEF. The mammalian homolog of GbpD, PDZ-GEF, is also a cyclic-nucleotide-independent Rap-GEF mediating cell polarity and adhesion. In summary, GbpC is a high-affinity cGMP-binding protein that acts via myosin II, mediating the chemoattractant-induced establishment of cell polarity. GbpD induces the formation of substrate-attached pseudopodia, resulting in increased attachment and suppression of polarity.

Activated Rheb regulates growth and cell migration

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TOR (target of rapamycin) kinase complexes monitor signals from hormones, nutrients and energy levels to effect growth, proliferation, and cytoskeletal rearrangements. Rheb is a ras-like GTPase that, when GTP-bound, activates TOR to increase protein synthesis. Rheb over-expression causes oncogenic transformation while inactivation results in cell-cycle arrest. Rheb is in turn negatively regulated by the GTPase activating protein (GAP) complex TSC1/TSC2. Disruption of TSC1 or TSC2 causes the multi-organ tumor syndrome Tuberous Sclerosis marked by excess cell proliferation and aberrant migration. This presumably occurs through unregulated activation of Rheb and enhanced TOR signaling. However, the molecular events connecting Rheb to TOR remain unresolved, as do relationships to cytoskeletal regulation and cell migration.

Our current work expands the role of Rheb in cell growth, identifies a novel Rheb effector, and links Rheb to cytoskeletal regulation. We constructed and expressed constitutively active (RhebCA) and inactive (RhebDN) forms of Rheb in *Dictyostelium*. *Dictyostelium* exhibits distinct cellular growth and migratory phases making it ideal to dissect growth parameters from cytoskeletal control. Paradoxically, RhebCA expression, which activates TOR, causes severe growth suppression that is ameliorated by the TOR inhibitor rapamycin. These data indicate that frank activation of TOR is as deleterious to cell survival as its inactivation. We further show that RhebCA inhibits multiple actin-mediated processes including phagocytosis and cell migration. Utilizing a yeast two-hybrid system, we identify a novel interaction between Rheb and DGAP1, a homolog of mammalian IQGAPs that are involved in cell migration, adhesion and cytokinesis. DGAP1, like all IQGAPs, contains GAP-homology domains, but lacks essential catalytic residues. We localize the site of interaction with Rheb to a conserved C-terminal domain. Loss of DGAP1 rescues Rheb-mediated cytoskeletal defects. Taken together, our data suggest that DGAP1/IQGAP signals downstream of activated Rheb and may be universally important in cytoskeletal regulation and cell migration.

Distribution of transposable elements in social amoebae

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Transposable elements (TEs) fulfil various tasks within a cell. They contribute to genomic plasticity and therefore are a driving force of evolution. Moreover, in some organisms they form essential parts of chromosome structures such as the telomeres in *Drosophila*. The detailed analysis of TE distribution in the *D. discoideum* genome has revealed a TE dependent restriction to defined parts of the chromosomes. As a major result of this analysis we confirmed that DIRS elements seem to form functional centromeres in this species. We have now started to investigate the roles of TEs in shaping the genomes of other social amoebae. A first step for this goal is the analysis of the occurrence of TEs in the whole taxonomic group of Dictyosteliida. For this purpose we generated genomic libraries from four different species. From these libraries we obtained more than 1 Mb of raw sequence each. We will present a detailed analysis of repetitive elements in these species in the light of genome evolution.

Identification of Regulatory Binding Sites and Corresponding Transcription Fractors Involved in the Developmental Control of 5'-nucleotidase and alkaline phosphatase Expression in *Dictyostelium discoideum*

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Gene regulation is a critical aspect of normal development, energy conservation, metabolic control, and responses to environmental cues, diseases and pathogens in eukaryotic organisms. In order to appropriately respond to environmental changes and advance through the life cycle, an organism must manage the expression levels of a large number of genes by utilizing available gene regulation mechanisms. The developmental control of 5í-nucleotidase (5nt) and alkaline phosphatase (alp) expression in the model system *Dictyostelium discoideum* has provided a focal point for studies of gene regulation at the level of transcription. In order to identify temporally-regulated control elements within 5nt and alp promoter, 5í and internal promoter deletions were designed and fused to the luciferase and lacZ reporter genes, and reporter enzyme activity was measured in cells from the slug stage of development. Alternatively, cAMP induction experiments were performed on amoebae transformed with the mutagenized promoter constructs to identify control elements within the promoter influenced by the presence of cAMP. Results from these experiments enabled the identification of a 250 bp region within the 5nt promoter, which was used as a template for subsequent site-directed mutagenesis experiments. These experiments involved altering 6-12 bp regions of the promoter by substitution to precisely locate cis-acting temporally-regulated control elements. These experiments resulted in the discovery of two control regions between -530 and -560 bp and -440 and -460 from the ATG translation start site. In order to evaluate the functions of the cis-acting promoter control elements, electromobility gel shift assays were performed to identify specific DNA-protein interactions on the 5nt promoter. These assays enabled the detection of two sequence-specific binding proteins with a 0.13 Rf and 0.33 Rf. Both proteins were purified using a series of column chromatography techniques followed mass spectroscopic analysis. The purified proteins were identified as formyltetrahydrofolate synthase and hydroxymethylpterin pyrophosphokinase. These enzymes function in the biosynthetic pathway of tetrahydrofolate and the production folate coenzymes. The specific interactions of these enzymes with the 5nt promoter suggest these proteins may also function in regulation 5nt expression. A similar approach was used to identify cis-acting elements within the alp promoter. Mutagenized promoter-lacZ fusions resulted in the identification of a regulatory region within a -659 to -635 sequence. Gel shift analysis lead to the discovery of several specific DNA-protein interactions. One of these DNA-binding proteins has been purified and sequenced by mass spectroscopy. The purified protein was identified as TFII, a DNA-binding protein that interacts with the glycogen phosphorylase-2 (gp2) promoter. Sequence analysis of the two promoter targets suggests a possible binding site, ACAATG.

Evidence for DNA methylation in the *D. discoideum* genome

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Methylation of DNA at cytosine residues plays an important role in regulation of gene expression and genomic imprinting and is essential for mammalian development. In the past 15 years it has been accepted that there is no 5-methylcytosine in the *Dictyostelium* genome[1]. However, sequencing the *Dictyostelium* genome revealed a candidate DNA methyltransferase gene (dnmA). The genome sequence also revealed an unusual distribution of potential methylation sites, CpG islands, throughout the genome, suggesting a functional significance for DNA methylation[2]. DnmA belongs to the Dnmt2 subfamily and contains all the catalytic motifs necessary for C5 cytosine methyltransferases. Dnmt2 activity is typically weak in *Drosophila*, mouse and human cells.
We investigated the methylation status of *Dictyostelium* genomic DNA by Southwestern blotting with antibodies raised against 5-methylcytosine and detected low levels of the modified nucleotide. We searched the genome for potential methylation sites and found them in retrotransposable elements and in several genes. We then used Southern blot analysis with methylation sensitive enzymes and identified methylated sites in the guaB gene (Guanosine monophosphate synthesis) and in the DIRS repetitive element. We are currently analyzing the phenotype of the *dnmA* mutant.

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DNA double strand break repair pathways

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The genome is under constant assault from exogenous and endogenous sources that cause DNA damage, including UV, ionising radiation and errors in replication. One particularly potent form of DNA damage is DNA double strand breaks (DSBs), which, if not correctly repaired, can be highly mutagenic. Hence, to prevent genome instability, mechanisms exist to detect, signal and repair such damage.

Two mechanistically and genetically distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), contribute to the repair of DNA DSBs. HR involves information surrounding the break being copied from a homologous template to facilitate repair, whereas NHEJ is a direct ligation of DNA termini. In mammalian cells NHEJ requires the DNA dependent protein kinase (DNA-PK) complex, comprising the heterodimer Ku70/Ku80 and the DNA-PK catalytic subunit (DNA-PKcs). Orthologues of both Ku70 and Ku80 have been identified and extensively studied in genetically tractable model organisms such as *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. However, to date, no DNA-PKcs orthologue has been identified in these organisms, severely hindering a dissection of the role of DNA-PKcs in vivo. Here we report the identification of putative DNA-PKcs and Ku70/80 orthologues in *Dictyostelium* and describe experiments used to characterise the roles of these proteins in DNA DSB repair.

C-module-DNA-binding factor regulates the aggregation-specific adenylyl cyclase

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Aggregation of *Dictyostelium discoideum* cells is coordinated by cAMP. Mutants defective in the expression of ACA, Myb2, and CbfA (the C-module-binding factor) show similar phenotypes. They do not aggregate on their own, yet they are able to enter and complete development when stimulated with extracellular cAMP pulses provided artificially or by interspersed wild type cells. Mutant cells depleted in CbfA (*cbfA*^{am}) fail to express any of the cAMP-induced developmental genes; most striking is the complete lack of detectable *acaA* message. Loss of aggregation competence in *cbfA*^{am} cells is a direct consequence of missing ACA, since *cbfA*^{am} cells are rescued by ectopic *acaA* expression and complete development normally. This observation prompted us to investigate whether CbfA directly regulates the *acaA* promoter. Gel shift assays using fragments of the *acaA* promoter identified a DNA-binding activity that is missing in *cbfA*^{am} cells. This activity mimics CbfA's DNA-binding properties in that (i) the binding site in the *acaA* promoter is a highly A+T-rich sequence virtually consisting of long homothymidine runs, (ii) disrupting the homopolymeric thymidine runs with blocks of 10 bp oligo(dA) drastically reduced binding by the factor, (iii) deleting this binding site in the *acaA* promoter abolished binding by the factor, and (iv) replacing this binding site by of the well characterized CbfA binding site from the C-module of retrotransposon TRE5-A into the *acaA* promoter restored binding of the factor to crippled the *acaA* promoter. Finally, CbfA could be precipitated with biotinylated *acaA* promoter fragment. Taken together, these results suggest that CbfA binds to the *acaA* promoter and regulates the expression of ACA during aggregation.

A feed-forward loop regulates post-aggregation gene expression

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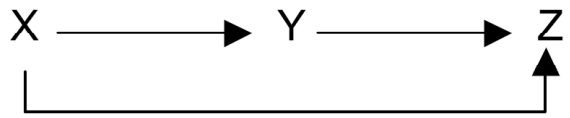
Feed-forward loops are prevalent among network motifs of *Bacillus subtilis* and *Saccharomyces cerevisiae*. In these loops transcription factor X regulates transcription of gene Y as well as later genes that depend on Y for expression:



With appropriate parameters such an arrangement requires a persistant input signal and results in rapid deactivation when the input goes off. A coherent feed-forward loop acts as a rapid response filter of noisy inputs, such as can be expected during multicellular development. In *Dictyostelium* the DNA-binding protein GBF and the signal transduction pathway instigated by LagC appear to form a feed-forward loop that regulates transcription of post-aggregation genes including those that encode the spore coat proteins.

Microarray analyses of cells developing in suspension with added cAMP pulses showed that expression of 3 pulse-independent, 11 pulse-dependent and 13 ACA dependent genes occurred normally in both *gbfA*$^-$ and *lagC*$^-$ null mutants except that *lagC* was not expressed ; in *gbfA*$^-$ mutant cells *lagC* is not expressed because its transcription is dependent on GBF, while in *lagC*$^-$ cells the gene is disrupted.

A set of 15 marker genes that normally start to be expressed after 8 hours of development are not expressed in either *gbfA*$^-$ or *lagC*$^-$ null mutants. To determine whether there is a linear dependent sequence from GBF to LagC to post-aggregation gene expression we transformed a *gbfA*$^-$ strain with an actin15::lagC construct. We found that constitutive expression of *lagC* did not result in expression of the post-aggregation genes. Likewise, constitutive expression of *gbfA* driven by the actin15 regulatory region did not overcome the block. It appears that both GBF and the signal transduction pathway that depends on the surface protein LagC are required for post-aggregation gene expression. Since expression of *lagC* is dependent on GBF, and GBF has been shown to directly bind to the regulatory regions of several post-aggregation genes, these post aggregation genes appear to be regulated by a feed-forward loop. LagC mediates cell-cell adhesion and appears to act as a check-point for multicellularity.

Visualising transcriptional pulses of a eukaryotic gene

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Gene transcription is hypothesized to be pulsatile, with bursts of polymerase activity occurring in response to cues intrinsic and extrinsic to the cell. We have visualized, for the first time, transcriptional pulses of a single defined developmental gene, in living cells. Using a method for the in vivo detection of specific RNAs at the site of their transcription, we observe pulses of transcriptional activity. Transcription can proceed as sequential pulses each lasting several minutes. I will present data defining the frequency, amplitude and duration of these pulses, and how these properties are regulated during development.

Modes of developmental gene expression and their relation to protein function

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When tested at the genome level, the correlation between gene expression and gene function is rather poor, but exceptions to this rule may be highly informative. For example, tightly co-regulated genes may be involved in processes where large protein assemblies must function, such as in ribosomal protein synthesis. In those cases, the function of un-annotated members of the group may be inferred from the annotation of the known members. We have used microarrays to analyze the expression of genes during the development of wild type and of 13 mutant *Dictyostelium* strains. Using a semi-supervised clustering method we found 17 groups of genes that were co-regulated during development of wild type cells. The groups were further subdivided into 57 groups when data from the mutant strains were considered. We then used the Gene Ontology (GO) annotations of the genes to find potential groups with common functions. Among the co-regulated genes, we found a group of early-expressed genes that encode cytoskeletal elements, a group of genes encoding calcium-binding proteins that are expressed in mid-development and a group of co-regulated cell-type specific genes. The largest and least expected group was of genes expressed in mid-late development, which contains many chemotaxis genes. Further analysis of the group revealed members that were not previously annotated as chemotaxis genes but nevertheless had chemotaxis phenotypes when mutated. We propose that chemotaxis genes may be co-regulated because the process involves many protein complexes and that other genes in the group may have yet unrecognized roles in chemotaxis.

Identification of a novel polyketide synthase producing DIF-1

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Polyketides, which include many drugs and antibiotics, are one of the most abundant classes of natural products. In *Dictyostelium*, they can act as signal compounds in development, such as DIF-1, and may also be key players in the interaction between Dictyostelium and other organisms in its environment. The genome contains approximately 40 polyketide synthase (PKS) genes, each encoding a large modular protein of over 2000 amino acids. As the first step in the analysis the PKS genes of *Dictyostelium*, we have identified the one encoding the PKS producing DIF-1 (referred to as disA). The identification was initially from its domain structure, in which there is a novel fusion of a chalcone synthase domain to the C-terminus of a Type I PKS, and confirmed by making a knock-out mutant. In the disA knock out strain, neither DIF-1 nor its precursors could be detected by chlorine labelling. However, DIF-1 production (and normal development) could be restored by supplying cells with the synthetic polyketide, or by mixed development with a dmtA mutant in which the final step in DIF-1 biosynthesis is blocked. As well as supporting identification of the disA gene, these results confirm the proposed DIF-1 biosynthetic pathway. The developmental phenotype of disA mutant is almost the same as that of the dmtA mutant. Though disA mutant has no detectable DIF-1 or its precursors, it can still make pre-stalk A cells and stalk cells. We also recognized some new aspects of the ëDIF-lessí phenotype in the disA mutant: it lacks a lower cup and the major part of the basal disc of the fruiting body. These developmental defects could be rescued by the presence of DIF-1 in the agar or by mixing with Ax2 or dmtA mutant cells. The phenotype of the disA mutant suggests that DIF-1 is the inducer of pre-stalk O and also of a class of anterior-like cells which produce the lower cup and basal disc.

The genome-wide regulation of gene expression by DIF

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The chlorinated polyketide DIF-1 is required for the induction of pstO cells in the developing slug, but previous work has only identified a handful of genes whose expression it controls. We have used microarrays, based on amplified ORF segments, to more comprehensively define the sphere of influence of DIF upon gene expression; to ask whether the three major DIFs produced during development have different effects; and to relate these DIF-regulated genes to prestalk and prespore differentiation. A three hour timecourse was carried out, beginning after nine hours of development in monolayers, comparing DIF-treated cells with controls. The expression of ~1200 genes is significantly altered (~16% of the total; controlling the false discovery rate at 5%), with evidence of two distinct phases. The genes affected cover a wide spectrum of predicted functions, including candidates for components of the DIF-degradative pathway and the early signalling response. The profiles for DIF-2 and DIF-3 are generally qualitatively similar to DIF-1, although quantitatively different. However, several genes appear to be more sensitive to DIF-2 or DIF-3, and are the subject of ongoing studies. This large scale study of the genes regulated by DIF lays a foundation for defining DIF-responsive promoter elements and for understanding how DIF contributes to prestalk and prespore cell differentiation.

Control of DIF Responses by bZIP Transcription Factor Interactions

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The small molecule DIF-1 plays an important role in the regulation of cell fate choice and proportioning in *Dictyostelium*. Previously, we described a key transcription factor, DimA, required for normal DIF-1 signal transduction. *dimA*$^-$ mutant cells fail to induce prestalk markers or repress prespore markers when treated with DIF in cell culture. Furthermore the *dimA*$^-$ mutant exhibits identical morphological and cell type differentiation defects to those of a mutant unable to synthesise DIF-1. One question that arises is how a single transcription factor can coordinate such diverse effects, including both the activation and repression of DIF-1 responsive genes? A possible solution comes from the finding that *dimA* encodes a bZIP transcription factor. bZIPs bind DNA as obligate dimers. Furthermore, heterodimerisation with other bZIPs can affect target gene specificity and activity. We therefore investigated whether heterodimerisation could play a role in the regulation of DimA activity and DIF-1 signal transduction.

Searches of the *Dictyostelium* genome reveal it to encode a further 18 DimA related bZIPs. We predicted that if any play a role in DIF-1 signaling they should exhibit similar phenotypes to *dimA*$^-$ when mutated. Using a knockout strategy, we found one mutant (*dimB*$^-$) failed to produce stalk cells in response to DIF-1 in monolayer assays. Furthermore, when developed, characteristic DIF-1 signaling defects in cell type differentiation and gross morphology were observed, suggesting that DimB could work together with DimA to regulate DIF-1 responses. Consistent with this idea we find that DimA and DimB directly interact in vitro. Interestingly, it is unlikely that DimA and DimB solely function as a heterodimer as the phenotypes of each mutant are similar but not identical. For example, unlike *dimA*$^-$, *dimB*$^-$ mutant cells do not make spore cells in the presence of DIF in Br-cAMP monolayer assays but die in a manner reminiscent of non-autophagic cell death. Epistasis analysis using a dimAB$^-$ double mutant suggests that dimB is normally required to repress this genetically uncharacterized response to DIF-1.

Finally we will present data which suggest DimA and DimB are direct targets of the DIF response pathway rather than permissive factors. Fusions of DimA and DimB to GFP reveal both to rapidly accumulate in the nucleus in response to DIF-1. Furthermore, we find that DimA accumulation is dependent on DimB, providing a possible molecular explanation for the epistatic relationship. These studies shed light on the complex interplay between transcription factors in the coordination of DIF-1 responses. We will discuss the implications of these findings for our understanding of regulation of DIF-1 signal transduction.

Regulation of Dictyostelium prestalk-specific gene expression by a bZIP and a MYB transcription factor

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The ecmA gene is specifically expressed in prestalk cells and its transcription is induced by the chlorinated hexaphenone DIF-1. We map two binding sites for a novel bZIP transcription factor within the ecmA promoter. Point mutagenesis of the cap-site proximal binding site greatly decreases reporter gene expression in pstO cells and anterior-like cells (ALC) but leaves pstA-specific expression unaffected. In a monolayer assay system that measures the induction of ecmA gene expression by exogenous DIF-1, null strains for the bZIP protein are DIF1 non-responsive. Hence we term the gene dimB (DIF-insensitive mutant B; see also preceding abstract by Thompson et al.). The DimB protein rapidly accumulates in the nucleus when cells are treated with DIF-1 and preliminary CHIP analyses suggest that it is associated with the ecmA promoter in vivo. In marked contrast to its essential role in monolayer induction, DimB is not required for ecmA gene expression during normal, multicellular development; suggesting that DimB may be redundant with another bZIP protein that is only active in the whole organism. We previously described a 22nucleotide region from within the distal part of the ecmA promoter that is both necessary and sufficient for prestalk-specific, DIF-inducible gene expression. The 22-mer was used to purify DdMybE, a novel MYB transcription factor. DdMybE contains a single DNA binding domain of a type previously found only in one large group of higher plant proteins: the SHAQKY proteins. We have now generated a complete mybE null strain and find that pstA-specific gene expression is unaffected but pstO and ALC-specific gene expression are drastically reduced. Thus we propose that DdMybE is the DIF-1 regulated activator of pstO and ALC specific gene expression and we will present a model for its interaction with DimB.

Non-vacuolar cell death in *Dictyostelium*

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To study caspase-independent cell death mechanisms, we are using as a model system *Dictyostelium discoideum*, which is haploid and genetically tractable, and shows programmed, developmental, apparently autophagic vacuolar cell death. In this eukaryote protist, development upon starvation leads to a fruiting body with a mass of spores at the tip of a stalk. This stalk is made of cells undergoing cell death. Methods exist to trigger differentiation in vitro without morphogenesis : these cells dying as a monolayer are easier to study than cells in a stalk. This developmentally regulated cell death was shown to be independent of conventional caspases, and, recently, of paracaspase. Thus, cell death in Dictyostelium can be used as a model for caspase-independent autophagic vacuolar cell death.

Using a combination of cell biology and molecular biology approaches, we showed the succession, in the pathway leading to *Dictyostelium* cell death, of cell polarization, immobilization and rounding, cellulose encasing, then massive vacuolization followed with membrane rupture. In this cascade of events, we could show by gene inactivation in particular of the atg1 autophagy gene, that cellulose encasing and, remarkably, vacuolization are not required for cell death. These studies thus showed that autophagy is not required in this autophagic vacuolar cell death model, and that there is an underlying non-apoptotic, non-autophagic cell death mechanism at play, which we are beginning to analyze. Also, a random insertional mutagenesis approach aims at identifying other molecules involved in this pathway.

How *D. caveatum* inhibits the development of other Dictyostelids and then eats them

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Dictyostelium caveatum is a very efficient predator of many, if not all Dictyostelid species. In mixtures with them, even in very small initial proportion, *D. caveatum* eventually emerges as the only survivor.

Two mechanisms are responsible for this behavior. First, we have confirmed and detailed by *in vivo* microscopy the phagocytic behavior predicted by David Waddell: *D. caveatum* cells rapidly ingest cells of other species but ignore each other. They can grow on these prey cells as they do on bacteria. A single large *D. caveatum* amoeba in an aggregate of *D. discoideum* can have many phagosomes that contain the remains of amoebae of *D. discoideum*.

Second, as is particularly clear in the case of small initial proportions of *D. caveatum* in mixtures, we have found that *D. caveatum* inhibits the development of amoebae of other species after the aggregation stage but before tip formation. Our available data suggest a defect in prestalk cell differentiation.

The inhibition of development can be observed when *D caveatum* cells and prey cells are isolated by a membrane if the pore size exceeds 500 Da. We have isolated compounds secreted by *D. caveatum* in starving conditions, and after several purification steps we recovered fractions in sufficient amount for structural analysis. A report of our progress with these interesting natural products will be presented.

The evolution of morphological complexity in the Dictyostelids

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One of the major goals of biology is to understand how species diversity and complexity was generated through adaptive evolution. Although this is, at least by the scientific community, acknowledged to be caused by natural selection acting on genetic variation, it has proven to be exceedingly difficult to identify specific mutations that caused phenotypic innovation in multicellular organisms. Although this is explainable by considering that mutations with large effects will be deleterious in well-integrated systems and that evolution has to work through accumulation of multiple minor mutations, it leaves us short of molecular evidence for macroevolution.

The Dictyostelids are a group of soil amoebae, which are as genetically diverse as the metazoans. They display conditional multicellularity where starving cells aggregate to form a motile slug-shaped pseudoplasmodium. This pseudoplasmodium eventually transforms into a fruiting structure consisting ball of spores, supported by a stalk. Depending on the species, the amoebae differentiate into two to five different cell types, with only the spore cells as the ultimate progenitors. Although each species forms characteristic and reproducible multicellular structures, both the size and shape of these structures show considerable plasticity, indicating that they are not as rigorously controlled as animal form. This property and their excellent genetic tractability makes the Dictyostelids a promising model system to identify the gene modifications that are utilized by adaptive evolution to generate phenotypic diversity.

By studying how morphological and genetic complexity evolved in the Dictyostelids we can firstly identify the ancestral core mechanisms of development and secondly the genetic changes and consequently the altered signalling mechanisms, that accompanied phenotypic innovations in more derived species. In this way the study of evolution helps us to understand development. Conversely, the identification of the specific genetic modification that caused phenotypic innovation will demonstrate at what level adaptive evolution acts to generate morphological complexity. Therefore, development can help us to understand evolution.

As a first step to understand the evolution of morphological complexity in the Dictyostelids we have constructed a molecular phylogeny of all known Dictyostelid species and we have mapped all described morphological traits to the phylogeny. This exercise gives us an initial impression of the history of character change in the Dictyostelids. With respect to fruiting body formation, Dictyostelid evolution appears to have progressed from subdivision of aggregates into small, clustered and branched fruiting bodies in the ancestral species to large, solitary unbranched fruiting bodies in the more derived species.

Social behaviour in mixtures of related and unrelated strains of *Dictyostelium giganteum*

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Starved amoebae belonging to genetically distinct strains of *Dictyostelium giganteum* were mixed in equal proportions and plated for development at a density of 10^5 cells/cm^2. A mixture consisted of two strains, and in some cases, three. Experiments were carried out with independent isolates, with the progeny of a cross, or with a parent-offspring combination. The outcome was monitored with respect to the numbers of spore and stalk cells in the resulting fruiting bodies that belonged to each component of the mix.

Our findings are as follows. (i) Strains exhibit a tendency to segregate themselves from other strains. One strain can inhibit the development of another, or separate 'pure' fruiting bodies can appear from the same aggregate, or distinct clumps of cells belonging to different strains form within the same slug or spore mass. (ii) Strains isolated from nearby locations are more likely to form chimaeric fruiting bodies than strains that come from more distant locations. (iii) In general, in any binary mixture the relative contribution of the two strains to the spore population is significantly different from 1:1; however, the spore forming efficiency of the population as a whole is constant across strains and combinations. (iv) The outcomes of all pair wise mixes make it possible to arrange strains in a transitive hierarchy of relative spore forming abilities; but the hierarchy no longer holds good when three strains are mixed. (v) Within a given fruiting body, when a strain contributes more to the spore population, it contributes less to the stalk population, but the two contributions need not be complementary. (vi) The relative contribution to the spore population is independent of whether the strains are genetically related. We infer that while evolution has favoured the formation of clonal social groups, once an aggregate has formed, cell fate is influenced more by complex non-linear interactions based on phenotypic differences between starved amoebae than by kinship per se.

Kin recognition in *Dictyostelium*

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The highly successful, speciose multicellular lineages share a single cell life cycle stage. This single cell bottleneck means that all cells in the multicellular organism are genetically identical. This trait reduces evolutionary conflict within the multicellular organism because all cells will share their genes. The social amoeba *Dictyostelium* forms a multicellular organism by aggregation of independent amoebae on the forest floor. We have previously shown in *D. discoideum* that these aggregations can be made up of genetically distinct amoebae. After aggregation there is conflict within the multicellular individual in a key arena: who becomes dead stalk and who becomes living spore. Some wild clones are better at avoiding contributing to the stalk, forcing the others in the multicellular organism to do so. We have also shown that chimeric individuals are hurt by this conflict because they are able to migrate less far towards light than are clonal individuals made up of the same cell number.

Dictystelium purpureum exhibits a different strategy. When starving amoebae are dense, they recognize those of different genotypes and do not aggregate with them, instead selecting individuals of their own clone. Thus fruiting bodies from dense mixtures are individually comprised of mostly only one clone. This requires both recognition of genetically different amoebae and discrimination against them. But when starving amoebae are not dense, they will aggregate with genetically distinct clones, forming mixed fruiting bodies. In some cases they will even aggregate with members of other species such as *D. discoideum* and *D. giganteum.* This conditional strategy allows starving cells of *D. purpureum* to avoid intra-organism conflict when they are dense and can select members of their own clone. It also allows them to achieve slugs of sufficient size for optimal migration when scarce by aggregating with genetically different clones. The social amoebae exhibit a rich variety of social systems partly because they have multicellular life stage that does not go through a single cell bottleneck. Comparative genomic approaches should tell us much about the organization and evolution of this diversity.

Progress Report of the Dicty Stock Center (DSC)

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The strain catalogue of the Dicty Stock Center contains nearly 700 strains and is growing rapidly. In the past year the collection of Bill Loomis was added, as well as a number of smaller collections. The older literature has been scanned to assemble requests for other large collections, including those of Steve Alexander (who has portions of the collections of David Francis, Reg Deering and Keith Williams), Rick Firtel, Guenther Gerisch, Dennis Welker, and Jeff Williams. Every week the literature is examined for new references, and if new strains and plasmids are published they are requested. If you have important collections, please discuss their preservation with Jakob Franke. So far, the response and cooperation have been good, but could be improved. The stock center will not only preserve your important material; it serves as a protection against disaster in your own lab. The stock center now maintains a plasmid collection which has been available since March 2005 and contains more than 120 plasmids. Multiple requests are received each week for plasmids and strains, and both can be ordered by means of a shopping cart mechanism from the dictyBase website. In November 2004 the new version of the Franke literature database was released (Dicty15); it contains 8132 references specific to the cellular slime molds. The references now contain PubMed ID numbers for rapid access. Another update will be available this summer. A grant renewal will be submitted before July 1, and we urge everyone to cooperate with the submission of materials, and to request strains and plasmids from the stock center. Funding depends on use.

dictyBase Workshop 2005

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**authors making equal contributions.*

dictyBase (www.dictybase.org) is the comprehensive model organism database for *Dictyostelium discoideum* that hosts the entire *Dictyostelium* genome sequence and related information. The power of using a relational database for storing biological information lies in the ability to make multiple connections between data such as genes, proteins, and publications. In this workshop, we will describe the ways to access this extensive data using the search function, the Genome Browser, and the BLAST Server. We will explain how get to the Gene Page, the central source of information for each gene.

Search Function: Every page in dictyBase has a Search box (http://dictybase.org/cgi-bin/search_news.cgi) in the upper right-hand corner. Searchable fields are Gene Names, Gene Products, Gene Ontology terms, Colleagues, and Authors.

Genome Browser: The Genome Browser (http://dictybase.org/db/cgi-bin/ggb/gbrowse/dictyBase) provides a graphical display of the genome and all associated sequences. This tool can be used not only to browse the genome but also to jump directly to a particular sequence such as a GenBank record or an EST. The Genome Browser is part of the GMOD (www.gmod.org) suite of open-source biological software.

BLAST Server: The dictyBase BLAST Server (http://dictybase.org/db/cgi-bin/blast.pl) allows you to search for all *Dictyostelium* sequences, including the full genome, the *Dictyostelium* GenBank records and ESTs. Several sequence datasets and adjustable parameters help to optimize your BLAST search.

Gene Page: This page displays all information relevant to a gene. Gene Products, Associated Sequences, Gene Ontology Annotations, Phenotypes, Expression data, Insertional Mutants, and published References are all available on a single Gene Page, linking to more detailed information pages.

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Dynamics of actin assembly in cell motility, chemotaxis, and phagocytosis

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In the fast moving cells of *Dictyostelium*, the cortical network of actin filaments is rapidly reorganized with polymerization rates as high as 3 to 5 μm per second. Superimposed on the actin network are dense actin assemblies that are associated with the Arp2/3 complex. Arp2/3-driven actin polymerization is induced by external stimuli such as chemoattractant or the adhesion of particles. However, intracellular patterns of Arp2/3 and actin assembly are also generated in the absence of stimuli. Remote from a leading edge, actin can polymerize in the form of patches or propagating waves. The spatio-temporal pattern of motor proteins and coronin that are recruited to the waves is subject of current studies.

Mechanical Induction of Actin-Mediated Phagosome Rocketing

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We have found that mechanical pressure can trigger actin-mediated rocketing of phagosomes. *Dictyostelium* amoebae that had been fed *S. cerevisae* were overlaid with agar. The agar was allowed gradually to dry, exerting increasing pressure on the cells. Presently, phagosomes containing yeast particles began to undergo rapid movement, sometimes back and forth, sometimes in long arcs, and sometimes in circular trajectories. This movement was mechanically triggered, because it occurred only if the agar was dried, it appeared in multiple adjacent cells at the same time, and it ceased if the agar was moistened again. Rocketing was examined in cells expressing GFP-tagged forms of Arp3, coronin, myosin-Ib, and alpha-tubulin, singly or in combination with mRFPmars-LimE, a red fluorescent marker for actin filaments. The results suggested that rocketing is triggered at the plasma membrane and is powered principally by actin. GFP-Arp3 displayed a small fluorescent ring or "print" where the phagosome pressed against the plasma membrane; this print was left behind or trailed as the particle moved away, but reappeared when the particle paused. Through-focus confocal microscopy revealed enrichment of GFP-Arp3 at both the upper and lower plasma membranes, but not in mid-cell focal planes. In contrast, actin filaments were associated with the rear of moving phagosomes in every focal plane. Prominent enrichment of myosin-Ib was observed at the area of contact between the phagosome and the plasma membrane. In cells expressing both GFP-MyoB and mRFPmars-ArpD, the MyoB ring at the plasma membrane was interior to the ArpD ring. We speculate that myosin-Ib, which binds both to the plasma membrane and to the Arp2/3 complex (through CARMIL), may play a central role in activating actin-mediated rocketing. Although rocketing occurred in nocodazole-treated cells, microtubules appeared to constrain and guide phagosome movement in normal cells. Rocketing phagosomes sometimes collided with and displaced the cell nucleus, indicating that rocketing particles exert considerable force. Protein assemblies resembling those associated with rocketing phagosomes were also generated when an exterior yeast particle was pressed against the plasma membrane, raising the possibility that plasma membrane deformation may help to initiate phagocytosis as well as rocketing.

An interaction network links a myosin I to the membrane trafficking and actin nucleation machineries during phagocytosis

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Dictyostelium is an ideal model system to study the actomyosin cytoskeleton, and its involvement in organelle transport, phagocytosis, exo- and endocytosis, and cell locomotion. MyoK is a very unconventional myosin that virtually lacks the usual tail/neck domain and instead is C-terminally farnesylated. It prominently features a 150 amino-acid insertion in loop 1 of the head domain, termed ìGPR loopì (Gly-Pro-Arg-rich) that shares 40-50% identity with the proline-rich domain of some WASp. This domain is elementary to the function of MyoK, as it contains multiple poly-Pro stretches that bind profilin-actin (whereas the Pro-rich-domain of WASp does not), as well as an SH3-binding motif. Pull-down assays, blot overlays and BIAcore revealed that Abp1 binds to GPR loop. Abp1 is expected to play an important role in the activation of the Arp2/3 mediated actin-nucleation machinery. Both MyoK and Abp1 null cells show defects in phagocytosis reminiscent of defects seen in other myosin and profilin mutants. Furthermore, immunofluorescence microscopy and phagosome purification showed that MyoK was present at very early stages of phagocytosis along with Abp1, profilin and Arp2/3. We recently observed that Dynamin, which also possesses an SH3 domain, is able to bind the GPR loop in blot overlays; Dynamin also belongs to the proteins recruited early to the phagosomes. We further observed direct binding of Dynamin to profilin, further unravelling the network governing the actin-turnover at sites of endocytosis.
We are dissecting further the role of MyoK and Abp1 in phagosome formation and maturation by applying a comparative proteomic strategy and hierarchical clustering algorithms to group proteins with similar time-profiles of abundance during phagosome maturation and thus derive a functional map of protein involvement.
In conclusion, we suggest that, as was proposed for the yeast class I myosins, MyoK represents an integrator of signal transduction cascades to actin polymerisation and membrane trafficking, enabling cells to generate the membrane protrusions necessary to locomote and take up particles.

Investigation of the function of Copine A in *Dictyostelium*

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Copines are soluble, calcium-dependent membrane binding proteins found in a variety of eukaryotic organisms. This family of proteins is characterized by two C2 domains at the N-terminus and a region similar to the A domain found in integrins at the C-terminus. The *Dictyostelium discoideum* genome contains five copine genes, *cpnA*-*cpnE*, and a possible sixth copine gene, *cpnF*. Our studies are focused on one of the copine genes, *cpnA*. CpnA is expressed throughout development and is capable of binding to membranes in a calcium-dependent manner *in vitro.* In fixed cells, a GFP-tagged CpnA is found on the plasma membrane and intracellular vacuoles. These GFP-CpnA-labeled vacuoles include contractile vacuoles, organelles of the endolysosomal pathway, and phagosomes. Cells overexpressing a GFP-tagged dominant negative version of CpnA (GFP-CpnAA^{DN}) have a growth defect with a doubling time 2-3 times longer than cells expressing GFP or GFP-CpnA. In fixed cells, GFP-CpnAA^{DN} is found throughout the cytoplasm and as small discrete patches within the cytoplasm. A *cpnA*$^{-}$ null mutant strain exhibits defects in cytokinesis, contractile vacuole function, development, and possibly phagocytosis. We are currently investigating CpnA's role in responses to different environmental stresses.

Ste20-like kinases in *Dictyostelium discoideum* are involved in phagocytosis, chemotaxis, development and cytokinesis

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The family of Ste20-like kinases in humans has 33 members including the 6 p21-activated kinases (PAK). The large subfamily of germinal center kinases (GCK) has 27 members with functions in apoptosis, cell proliferation, stress response, Golgi organisation, cell adhesion and organisation of the cytoskeleton. *Dictyostelium* has the rather high number of 17 GCKs, which is more than in worms (9) or flies (9). To characterise the function of 13 D. discoideum GCKs with significant homology to human Ste20-like kinases we generate and study as a first approach knock-out strains.
The knock-out of DST1 which was described as severin kinase (Eichinger et al., 1998, J. Biol. Chem. 273:12952-12959) has a strong cytokinesis defect. The mutant can accumulate up to 30 nuclei per cell even when grown on solid surface. In addition, DST1 minus-cells are defective in phagocytosis, sensitive to osmotic stress, have often a peculiar morphology during cell division, and are delayed in development by about 6 h. They show a phototaxis defect and form small, stumpy fruiting bodies. These findings imply a role of DST1 in the regulation of cytoskeletal dynamics that are involved in cytokinesis, motility and phagocytosis.
- Loss of DST2 on the other hand, leads to cells that move faster in random motility assays as compared to wild type cells. They also show biforked leading edges during chemotaxis implicating a role for DST2 in cell polarity.
- *Dictyostelium* cells lacking the Ste20-like kinase DST10 show a strong phagocytosis defect, form long streams on bacterial lawns, are delayed in development and phototaxis.
To further characterise the signalling pathways that lead to the observed phenotypes we will generate double knock-outs and try to identify binding partners or substrates of the kinases using tandem affinity purification (TAP).

A SIN-related pathway regulates cytokinesis in *Dictyostelium*

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The mitotic-exit network (MEN) and the septation-initiation network (SIN) control events at the end of mitosis in *S. cerevisiae* and *S. pombe*, respectively. SIN initiates contraction of the actin ring and synthesis of the division septum enabling cytokinesis. MEN is also required for cytokinesis, but its main role is regulating the inactivation of cyclin-dependent kinases at the end of mitosis required for mitotic exit. The components of these signaling cascades are quite conserved between the two yeasts and some orthologues have been identified in filamentous fungi, *Caenorhabditis*, *Drosophila* and higher eukaryotes. However, in most cases their functions are only insufficiently explored.
In an REMI approach to identify genes involved in cytokinesis we have isolated a multinucleated mutant with a peculiar cytokinesis defect. The corresponding gene encodes a kinase that is homologous to cdc7 from *S. pombe*. This kinase is a central regulator of SIN and its identification prompted us to search for additional components homologous to SIN pathway proteins. By sequence comparisons to the *Dictyostelium* genome database we were able to identify and clone two upstream components, a GTPase, Dd-Spg1, and a GAP, Dd-Bub2. Both proteins localize to centrosomes.
Current work concentrates on the characterization of the individual knock out phenotypes, the localization of the identified regulators in interphase cells and during mitosis, and the identification of further components of this regulatory pathway.

Cell mechanosensitivity and polarization under shear flow. How do cells reorient and how do proteins from the cytoskeleton relocalize under flow reversals?

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Dictyostelium cells have been recently shown to be mechanosensitive. Using GFP and mRFP fusion proteins, we showed that *Dictyostelium* cells under hydrodynamic shear stresses polarized with their leading edge facing the flow.
The actin cytoskeleton is a rigid protein network which is part of the mechanosensor that makes cells able to sense mechanical deformations.
However, the mechanosensory function of the cytoskeleton is not yet clear and we do not know which proteins may play a key role in this process. Moreover the mechanisms of cell reorientation under flow forces have not yet been studied.
Using a chamber enabling to change the direction of the flow, we studied the process of cell reorientation after a flow reversal. Do they perform U-turn or simply reverse their polarization? Is the myosin-II-dependent tail retraction or the leading edge protrusion the first event in the reorientation process? Is myosin-II necessary to sense forces and for actin relocalization?
In particular, the relocalization of proteins from the cytoskeleton, myosin-II and LIM (which is a marker for actin polymerization), has been quantified during the reorientation process by using active contours detection. We provided evidence that actin polymerization is leading the way and then, myosin-II-dependent efficient retraction follows.
Finally, we studied wild-type and myo-II-null mutant cells expressing LIM-GFP under flow reversals. Despite the bigger rigidity confered by myosin-II to the cytoskeleton, we showed that myosin-II is neither necessary for cells to sense mechanical forces nor to quickly reorient after a flow reversal.
Other structures like the microtubule network may play a role in the mechanosensing process. This is currently under investigation in our group.

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Two mechanically distinct modes of protrusion formation in amoeboid movement

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Cell motility is considered to be mediated by the coordination of contractile activity of myosin II at the posterior end and action of F-actin at the anterior end. Despite intense study, two contradictory roles of F-actin at the leading edge have been proposed. It is widely believed that the force produced by actin polymerisation directly pushes against the anterior membrane and leads to filopodia and lamellipodia protrusion. Alternatively, cortical F-actin can counteract the cytoplasmic pressure acting on the membrane. The contribution of native, controlled blebs to physiological cell motility has not been well appreciated. Interestingly it has been shown that the actin cortex of a polarized chemotactic *Dictyostelium* cell is more weakly linked to the plasma membrane at the anterior than the posterior, suggesting a bleb-like mechanism in normal cell motility. Using high resolution live microscopy, we found that *Dictyostelium* cell movement was often initiated by the sudden and successive formation of bleb-like structures along with elongating filopodia. Use of GFP-ABD as an F-actin probe revealed that blebs were first devoid of cortical F-actin, which appeared within our imaging interval (500 ms), resulting in the successive formation of F-actin arcs. Membrane extension and retraction, as well as centroid movement occur in pulsatile fashion. In wild-type cells, the velocities of membrane extension and retraction, as well as centroid velocity, decreased as the osmolarity of the external buffer increased. In myosin II-null cells, these velocities were constant over the tested osmolarity range. Spectral analysis showed that there are consistent peaks at frequencies of 0.2 Hz and its harmonics, irrespective of the tested conditions. These results suggest that *Dictyostelium* cells use two morphologically and mechanically distinct modes of protrusion formation, i.e., bleb-mode and filopodium-mode, both of which are tightly coupled to an unidentified oscillator.

Control of cheating by high relatedness in a natural population

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Cooperative groups are vulnerable to exploitation by 'cheaters', who selfishly use group resources while contributing little or nothing. The control of cheating genes was essential in major evolutionary transitions to cells, multicellular organisms, and societies, but it is poorly understood. Genetic relatedness is predicted to be important in control of cheating mutants in natural populations because high relatedness means cheaters will tend to be with each other, and possibly to cheat each other, rather than cheating others. However, the organisms where cheater mutants are known are generally microorganisms that do not have known relatedness structures. We document the power of relatedness in controlling the fbxA- cheater mutant in the social amoeba, *Dictyostelium discoideum*. We show that the cheater should be favored only at very low relatedness. At relatedness greater than 0.25, the cheating advantage is counteracted by cheaters dragging down their own reproduction. We also estimate relatedness in a natural population of fruiting bodies collected from white-tailed deer feces. Relatedness was much higher than the 0.25 threshold, with the majority of fruiting bodies being clonal. We therefore predicted that a fbxA- mutant could not spread in nature. In agreement with that prediction, we found no fbxA- phenotypes in 1200 clonal isolates from over 50 fruiting bodies collected from the field. We conclude that selection does not favor fbxA- and other cheaters with defective phenotypes when developed alone. Because relatedness is high, effective cheaters will have to be able to cheat wildtype without harming themselves.

Comparing the *Dictyostelium* and *Entamoeba* genomes reveals an ancient split in the Conosa lineage

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The *Dictyostelium discoideum* and *Entamoeba histolytica* genomes are the first Amoebozoa genomes to be sequenced. We compared these two genomes to each other and to other eukaryotic genomes to identify Amoebazoa specific genomic elements. Most of the 1500 orthologous gene families shared between the two amoebae are also shared with plant, animal, and fungal genomes. We found that only 42 gene families are distinct to the amoeba lineage, among these are a large number of proteins the encode repeats of the FNIP domain, and a transcription factor essential for proper cell type differentiation in *D. discoideum*. The small number of lineage-specific genes indicates an ancient split in the Conosa lineage leading to the archamoeba and the mycetozoa. An expanded phylogenetic tree, including the complete predicted proteomes of 23 eukaryotes, estimates that the divergence of these species is more than the divergence between the budding and fission yeasts and probably happened shortly after the Amebozoa split from the opisthokont lineage. Previous studies suggested that the acquisition of crucial metabolic enzymes resulted from recent horizontal gene transfer (HGT). We found that 44 of the 96 proposed HGT candidates from *E. histolytica* have orthologs in *D. discoideum*, arguing against recent HGT for these genes. The Amoebazoa specific genes may be useful in the design of novel diagnostics and therapies for amoebal pathologies.

Acanthamoeba polyphaga mimivirus : Genome analysis of the largest known virus to date suggests possible links to _Dictyostelium_.

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The objective of my presentation is to introduce the recently discovered [1] and sequenced [2] large DNA virus, *Acanthamoeba Polyphaga Mimivirus,* to the *Dictyostelium* community. Mimivirus mimics a microbial prey to its amoebaean "predator" to enter its host by phagocytosis. The virus takes Gram staining and may be detected under a light microscope (capsid size 400nm). Ghedin and Claverie [3] detected the presence of Mimivirus relatives in Sargasso Sea, indicating that the Mimiviridae may be quite ubiquitous in the environment. Although Mimivirus is only known (until now) to infect its "natural" host *Acanthamoeba Polyphaga,* a number of elements suggest that there may exist related large DNA viruses that can infect *Dictyostelium*. For instance, Mimivirus genome analysis revealed a very conserved promoter-like motif [4] that is found before *Dictyostelium* ribosomal proteins and also before proteins that are expressed in aggregation phase. Moreover, Mimivirus contains a family of paralogous proteins that is found in multiple copies in *Dictyostelium discoideum*, but not in *Entamoeba histolytica* nor in *Acanthamoeba castellanii*, nor in any other sequence database [O'Day & Suhre, in preparation]. Other Mimivirus paralogous gene families suggest that Mimivirus has evolved a large set of proteins to interfere with the host cell processes. I would like to invite the Dictyostelium community to actively search for a large DNA virus that may infect Dictyostelium, based on what we know about Mimivirus. Such a virus would be of inestimable value, as with its genome we would have access for the first time to a fully sequenced virus together with its eurakyotic host system.

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A system biology approach reveals Hg induced stress responses in the social amoeba *Dictyostelium discoideum*

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In this study, *Dictyostelium* amoebae were exposed to a hazardous contaminant, Hg, at sub-lethal concentrations. Our data demonstrate that nano/micromolar concentrations of Hg (0.5-2 μM) significantly affect important physiological parameters such as intracellular free calcium, lysosomal membrane stability and endocytosis rate, without altering cell replication rate or cell survival. To better understand the molecular mechanisms behind Hg-response, extracts of control and Hg-treated cells were analysed on 2-dimensional electrophoresis (2DE) gels. A high resolution 2DE map (pI-range 3-10) containing some 900 protein spots was obtained. Identification of excised spots was performed using MALDI TOF peptide mass fingerprinting, and the identities of some 150 proteins were revealed. Protein identities were grouped as follows: 17% structural proteins, 18% signal-transduction proteins, 24% metabolic enzymes, 12% stress-related proteins, 5% calcium-binding proteins, and 24% miscellaneous proteins. The results further demonstrated that the expression of 15 of the identified proteins was affected by Hg, e.g. glutathione-S-transferase, catalase, thioredoxin 3, GMP synthase, G protein α-subunit-like protein, acidic protein P2, and hypothetical protein gi28828580, all of which show a dramatic increase up to 4-fold in Hg-treated cells. Preliminary transcriptomic analysis, by means of DNA microarrays revealed the activation of several genes involved in toxicity resistance and detoxification (e.g. GSH-tranferases, ABC transporters, heavy metal carrier proteins, etc), metabolic and other redox processes (e.g. sod, GSH-reductase, thioredoxin, aldo/keto-reductase, etc), together with a number of genes not yet characterized. Finally, metabolomic profiles will be integrated in order to obtain a global overview of the molecular responses to Hg-exposure.

PI3K activity and small GTPase signalling are targeted by VPA: Implications for epilepsy, bipolar disorder, migraine and cancer treatment.

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Valproic acid (VPA) was identified in 1963 to acutely inhibit epileptic seizures. Since then, it has also been found to work in bipolar disorder, migraine and cancer treatment. The means by which VPA functions in these disorders remains obscure.

To understand how VPA works therapeutically, and as our previous work has shown VPA blocks development, we examined its effect on chemotaxis. We found VPA acutely blocks cell movement, a process that is at least partially controlled by PI3K activity. We therefore measured the effect of VPA on PI3K activity and showed high VPA concentrations blocked PI3K activity and low concentrations both slowed the rate and altered the size of the signal-induced PIP3 peak. Since Rac activity has been implicated to function downstream of PI3K in both *Dictyostelium* and in mammalian cells, we tested VPA and a PI3K inhibitor (LY29002) on cells containing a modified Rac1A enzyme, blocked in cycling in its active form. The phenotype of these cells - heavily reduced filopodia formation - was reversed by both drugs. Ras activity has also been shown to be modified by PI3K activity, and we found both VPA and LY29002 acutely reduced Ras G and C activation. These results thus suggest that Ras and Rac activity are partially reliant on VPA-sensitive PI3K activity. We further show that VPA strongly reduced phagocytosis and vesicle release, phenotypes also associated with PI3K inhibition.

We therefore propose a model for VPA action in the treatment of epilepsy, bipolar disorder, migraine and cancer through the inhibition of PI3K – a model which logically join the etiology of these disorders with a mechanism of drug action. This has widespread implications for the understanding of these medical conditions.

Sphingolipid control of anticancer drug resistance in *Dictyostelium discoideum* and human cells

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The focus of our laboratory is to understand the mechanisms for resistance to drugs used in cancer therapy, and to develop strategies for making tumor cells more sensitive to the drugs. We have used *Dictyostelium discoideum* as the primary genetic system to identify mutants with resistance to the widely used anticancer drug cisplatin, and then translated our findings to human cells. One of the identified genes encodes sphingosine-1-phosphate (S-1-P) lyase, the enzyme that functions in the degradation of the lipid S-1-P. We have pursued this observation in three directions.

1) Overexpressing or deleting the sphingosine kinases or S-1-P lyases alters drug resistance in predictable ways. The literature indicated that the bioactive lipids S-1-P and ceramide work as a rheostat, where a high S-1-P to ceramide ratio results in cell survival while a lower ratio would result in cell death. Therefore, we reasoned that the lack of S-1-P lyase activity altered the S-1-P/ceramide balance in favor of cell survival in the presence of cisplatin. To validate this hypothesis, we generated mutant strains that overexpressed either the S-1-P lyase or the sphingosine kinase, as well as sphingosine kinase null strains and studied the relationship between the levels of these enzymes and cisplatin resistance. As predicted, both sphingosine kinase null and S-1-P lyase overexpressing cells are more sensitive to cisplatin, while spingosine kinase overexpressing and S-1-P lyase null cells are more resistant to cisplatin. Significantly, the changes in sensitivity were only seen for cisplatin and the related platinum drug carboplatin, but not for other chemotherapeutic drugs. We have translated the findings with S-1-P lyase to human HEK293 and A549 cells. In agreement with the *D. discoideum* studies, overexpression of the human S-1-P lyase sensitizes both cell types primarily to platinum based drugs. The increase in sensitivity is mediated through the activation of p38 MAPK.

2) Overexpressing or deleting the ceramide synthases alters drug resistance. We wanted to determine the effect of modulating the ceramide part of the rheostat. Thus, we are studying the ceramide synthase family of enzymes in both *D. discoideum* and human cells. We have shown that altering the level of expression of some of these genes affects cell growth and sensitivity to cisplatin.

3) Selection of mutants resistant to the sphingosine kinase inhibitor DMS. The goal of this work is to identify proteins that interact with the ceramide/S-1-P pathway. Using a REMI mutant screen we have identified several mutants that confer resistance to DMS. We are currently studying the underlying mechanism of one of these genes.

Overall, the studies have identified the sphingolipid metabolizing enzymes as important new chemotherapeutic targets, and continue to validate *D. discoideum* as a powerful primary genetic system for anticancer drug discovery.

Functional characterization of DdLIS1 and DdDCX, two microtubule-associated proteins involved in lissencephaly

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Lissencephaly results from impaired cell migration of neuronal precursors during development of the neocortex. Mutations in the genes encoding LIS1 and doublecortin (DCX) are responsible for approximately 80% of all cases of lissencephaly. Since Dictyostelium is an outstanding model for studies on cell migration and development, we analyzed the homologues of LIS1 and doublecortin.
Dictyostelium LIS1 (DdLIS1) is a microtubule (MT) and centrosome-associated protein. Co-precipitation experiments revealed that DdLIS1 interacts with dynein, DdCP224 and the small GTPase Rac1A. Replacement of the DdLIS1 gene by the hypomorphic D327H allele or overexpression of an MBP-DdLIS1 fusion had no strong effects on development but disrupted various dynein-associated functions and altered actin dynamics. Our results show that DdLIS1 is required for maintenance of the MT cytoskeleton, Golgi apparatus and nucleus/centrosome association, and they suggest that LIS1-dependent alterations of actin dynamics could also contribute to defects in neuronal migration in lissencephaly patients.
DCX is characterized by two conserved tandem repeats of the DC-domain that are involved in microtubule (MT) binding. Although DCX has only been described in vertebrates where its expression was restricted to migrating, developing neurons, we have found a homologue in Dictyostelium. Its sequence similarity is mainly restricted to the DC-domains. GFP-DdDCX was localized along MTs, the centrosome and the cell cortex. Endogenous DdDCX was distributed similarly but it was only detectable after 8 h of development. DdDCX null mutants exhibited no phenotype. However, DdDCX null mutants additionally carrying the hypomorphic DdLIS1 allele are defective in formation of streams and aggregates during development, although they show normal chemotaxis along external cAMP signals. Stream formation turned out to be independent of MTs. Taken together, this suggests that DdDCX and DdLIS1 cooperate in cAMP signaling and that both proteins may also be involved in cytoskeleton-independent functions in brain development.

The Shwachman-Diamond Syndrome Gene Encodes an RNA-Binding Protein That Localizes to the Pseudopod of *Dictyostelium* Amoebae During Chemotaxis

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The Shwachman-Diamond Syndrome (SDS) is an autosomal disorder with multisystem defects. The SBDS gene, which contains mutations in a majority of SDS patients, encodes a protein with a deduced function in ribosome metabolism. Recently it was demonstrated by computer-assisted analysis of single cell behavior that the single defect of a polymorphonuclear leukocyte (PMN) of SDS patients is the incapacity to orient correctly in a spatial gradient of chemoattractant. Although the deduced function of the SBDS gene product is in ribosome metabolism, the behavioral defect exhibited by PMNs of SBDS patients suggested that it played a role in chemotactic orientation. Given that several proteins involved in chemotactic orientation localize to the pseudopod of cells orienting in a spatial gradient of attractant, we tested whether the SBDS gene product did the same. We identified and tagged the homolog of SBDS in the social amoeba Dictyostelium discoideum, a model for PMN chemotaxis, with green fluorescent protein. The SBDS protein remained dispersed equally throughout the cytoplasm of polarized cells migrating in buffer, but localized to the anterior pseudopod of cells undergoing chemotaxis in a spatial gradient of chemoattractant, suggesting that the SBDS protein played a direct role in chemotactic orientation.

A new, resistant cell type in *Dictyostelium*: the aspidocyte

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During a hunt for DIF-resistant mutants, we serendipitiously discovered a new cell type, which we call the aspidocyte (aspida; Greek for shield). Aspidocytes seem to be a resistant form of *Dictyostelium*, increasing survival against environmental toxins.

Aspidocytes form in isolation and are non-motile, irregular-shaped, refractile cells. They are resistant to detergent lysis where un-induced amoebae are completely lysed. However, they do not have a cell wall. Their resistance to detergent lysis is active, since pre-formed aspidocytes rapidly lyse when energy-poisoned with azide, and is accompanied by a cessation of endocytosis (monitored with fluorescent FM dyes) and sometimes by an unusual bi-laminar arrangement of F-actin beneath the plasma membrane (visualized using the ABD-GFP reporter for F-actin). Unlike spores, aspidocytes are not temperature resistant.

Aspidocytes are induced in a sensitized mutant background by Cd^{2+} alone, but a wide range of toxins can synergize. These include inhibitors of protein synthesis, RNA synthesis and membrane active agents. Aspidocytes are best induced with starving cells, but can also form in axenic medium. Wild-type cells can be induced by a combination of Cd^{2+} and a second inducer. Pre-formed aspidocytes are resistant to killing by Amphotericin B (membrane-disrupting) but not by Bleocin (damages DNA), suggesting that the aspidocyte state preferentially confers resistance to membrane active agents.

Several hours are required for fully-resistant aspidocytes to form, suggesting that they may rely on a specific program of gene expression. This possibility is being investigated in micro-array experiments. They lose detergent resistance fairly rapidly, once the inducing toxins are washed away and then resume amoeboid movement. We suggest that the aspidocyte is a survival form, of lesser resistance than spores, which may be an adaptation to resist sudden fluxes of environmental toxins.

The role of AMP-activated protein kinase (AMPK) in phototaxis and mitochondrial diseases in *Dictyostelium discoideum*

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The AMP-activated protein kinase (AMPK) is a metabolic-stress-sensing protein kinase that regulates metabolism in response to energy demand and supply by directly phosphorylating rate-limiting enzymes in metabolic pathways as well as controlling gene expression. Thus a decrease in the energy charge would activate the enzyme. Activated AMPK switches off anabolic pathways (fatty acid, triglyceride, cholesterol and protein synthesis and gene transcription) and switches on catabolic pathways such as glycolysis and fatty acid oxidation. We describe the cloning, sequencing and functional characterisation of the AMPK α subunit of *D. discoideum*. Sequence comparison of the *D. discoideum* AMPK α subunit with homologues from other eukaryotes reveals that the gene sequence is highly conserved. The *D. discoideum* AMPK α subunit encodes a protein about 39kDa, contains 3 introns and APE & DFG motifs. Preliminary pharmacological study showed that treatment of AX2 cells with graded concentrations of AICAR (a specific AMPK activator) caused dose-dependent phototaxis defects. To understand the role of AMPK in mitochondrial dysfunction and photosensory signal transduction we studied the effects of pharmacological activation, ectopic overexpression and antisense inhibition of AMPK as well as AMPK/*hspA* double antisense cotransformation of wild type cells. Northern blot and RT-PCR show that the AMPK α subunit gene is expressed during growth and throughout development. Plasmid construct copy numbers in all transformants were determined. Mutants overexpressing the AMPK α (catalytic) subunit gene showed phototaxis defects without obvious effects on development. Phototaxis defects became severe in transformants carrying higher copy numbers of the ectopic overexpression construct. The mRNA levels were high but only slight increases in the protein levels were observed. In contrast, antisense inhibition led to strong reductions of mRNA levels. These reductions resulted in moderately decreased levels of protein. Transformants containing an antisense RNA-expressing plasmid showed impaired aggregation on bacterial lawns but exhibited improved phototaxis on charcoal agar plates. The improvement of phototaxis and the impairment in development were more pronounced in transformants carrying higher plasmid construct copy numbers. Slugs of AMPK α subunit antisense transformants also migrate shorter distances, make fewer and smaller fruiting bodies. At very high copy numbers the mutant cells do not aggregate even on charcoal agar. The result suggests that AMPK functional attenuation (antisense-RNA) impairs the progress of aggregation and thus slowed or prevented development. This indicates that AMPK α subunit plays a role at least in initiation of development in *D. discoideum*. Although AMPK is widely studied in relation to diabetes, exercise physiology and energy homeostasis, its possible role in mitochondrial diseases appears not to have been considered previously. To investigate the possible role of AMPK in mitochondrial disease, we cotransformed AX2 cells with antisense plasmid constructs of hspA and AMPK α subunit. Antisense inhibition of chaperonin 60 expression *(hspA)* was shown previously to impair phototaxis and (at high copy numbers) development. Phototaxis and development were wild type in cotransformants. The result suggests that there is genetic interaction between AMPK and mitochondrial function *(hspA)* in phototaxis and development. The interaction suggests that ATP depletion caused by mitochondrial dysfunction activates AMPK whose downstream targets include proteins belonging to other signal transduction pathways, in this case phototaxis. The resulting perturbation of these pathways would result in impaired responses to corresponding signals, leading in this case to deranged phototaxis. This may explain why mitochondrial diseases present mostly as central nervous system and muscle disorders as some of the pathological consequences of ATP depletion in mitochondrial diseases could be due to chronic activation of AMPK.

Human versus *Dictyostelium*: comparative genomics to study the function of highly conserved genes of unknown function

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We have compared *Dictyostelium* and human proteomes and selected 66 genes of unknown function that were present in both species but absent from the complete proteomes of *S.cerevisiae* and *S.pombe*. Search of the predicted coding regions of these genes showed no characterized protein domains. We have initiated a systematic disruption by homologous recombination and so far, KO vectors for 31 of these genes have been generated and transformed in *Dictyostelium*. Among the 19 genes successfully interrupted, three of them showed obvious phenotypes. One of them, ng130 (DDB0204869), will be described in more detail. This gene codes for a highly conserved protein with homologs in plants, metazoa, fungi (except *S. cerevisiae* and *S. pombe*) and alpha-proteobacteria. No function has been assigned for this protein in any model system. Knock-out of this gene in *Dictyostelium* leads to pleiotropic defects both at the vegetative stage and development, including reduced cell mass, cell growth, phagocytosis and macropinocytosis. Developmental defects include an increased tendency to remain at the slug stage that can be attributed to abnormal levels of ammonia and also a reduced spore viability. Fusion of the protein with GFP has shown mitochondrial localization. No differences were found in mitochondrial mass or membrane potential. However, ATP levels in this strain were reduced to 70 % of those measured in wild type. Other metabolic defects have also been observed and will be described. Taken together our results suggest that ng130 plays an important role in cell bioenergetic and point out the importance of mitochondria in several aspects of development. Our current efforts to discover the biochemical function of this protein will be discussed

Analysis of Phosphoprotein Phosphatase PP2A in Growth and Development of *Dictyostelium discoideum.*

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Dephosphorylation by protein serine-threonine phosphatases plays essential roles in signal transduction. The heterotrimeric protein serine-threonine phosphatase PP2A is ubiquitously present in eukaryotic cells, and is the major phosphoprotein phosphatase in *Dictyostelium discoideum*. To elucidate the role of PP2A in protein phosphorylation, signal transduction, growth and development, we have created site-specific mutations in PP2Ac cDNA and transfected wild-type *Dictyostelium* cells. Mutations in the metal-binding residues of PP2Ac severely impair growth and development, and are presumed to be dominant-negative. A mutation altering one amino acid residue (P240H), in the PP2Ac subunit of the yeast Pph22p results in temperature-sensitive mitotic defects and impairment of catalytic activity *in vitro* (Evans and Hemmings 2000, *Genetics* **156**, 21-29.) The homologous mutation (P169H) on the *Dictyostelium* PP2Ac DNA also confers temperature-sensitive growth, some developmental defects and reduction in catalytic activity.

In previous experiments, we have transfected large inverted-repeat DNA fragments to induce RNA interference that severely reduced the mRNA of a development-specific *Dictyostelium* transcription factor gene, *mybB* (Otsuka, Cogill, Kuan, Dottin and Gross, unpublished) and blocked aggregation. We now express small shRNAs targeted against specific portions of mybB mRNA. Inhibition appears to depend on the particular shRNA sequence used. To extend this technology, we are using shRNA to knock down, but not eliminate the functioning of the essential gene, PP2Ac. These results show that PP2Ac is essential for growth and at multiple stages in *Dictyostelium* development. These approaches should delineate the mechanisms by which PP2A mediates signal transduction for eukaryotic cell proliferation and development.

Cnr1, a putative frizzled/smoothened-like protein participates in group size regulation and cell proliferation in *Dictyostelium discoideum*

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Much remains to be understood about the signals, receptors and pathways regulating *Dictyostelium discoideum* growth and development. In the course of a REMI mutagenesis we identified a clone with an insertion in a gene encoding a putative frizzled/smoothened-like protein. This gene was designated cell number regulator, cnr1. Northern blots show that cnr1 is expressed mainly during vegetative growth and early development. Disruption of cnr1 resulted in a mutant with larger aggregates and fruiting bodies, lower motility, higher adhesion, and an increased proliferation rate when compared to Ax2 parental cells. We previously found that counting factor (CF) decreases group size by increasing cell motility and decreasing cell-cell adhesion, and that CF is oversecreted by smlA- cells. When cnr1- cells were mixed with 10% smlA- cells, aggregate numbers increased and group size decreased. cnr1- cells were also sensitive to recombinant countin, CF45, CF50, and CF60B, which are components of CF. This indicates Cnr1 is not in the CF pathway. In addition, Cnr1 also behaves as a negative regulator of proliferation in *Dictyostelium*. Log phase cnr1- cells doubled every 9 hours while log phase Ax2 cells doubled every 16 hours. With DAPI stain, we found the percentage of multinuclear cells in cnr1- cells is about 3 times higher than that of Ax2 cells, which correlates with cnr1- cells proliferating faster than Ax2 cells. Western blots show that cnr1- cells have abnormally low level of AprA, a 60KDa protein secreted by in *Dictyostelium* cells that inhibits cell proliferation. This indicates that Cnr1 may inhibit cell proliferation by potentiating the expression of AprA. These results suggest that Cnr1 acts as dual functional protein regulating group size during development and inhibiting cell proliferation during growth.

Kenneth Raper, Elisha Mitchell and *Dictyostelium*

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The most important paper in the history of *Dictyostelium* is Kenneth Raper's 1940 paper "Pseudoplasmodium formation and organization in *Dictyostelium discoideum*". This epic manuscript was published in one of the most obscure journals in the world, the Journal of the Elisha Mitchell Scientific Society. I have been curious as to why Raper would have chosen this journal. To answer the question one must ask who Eisha Mitchell was, what was the Elisha Mitchell Scientifc Society, and what were their connections to Kenneth Raper and *Dictyostelium*. I will try to provide answers to all the above.

The *Dictyostelium* Kinome - analysis of the protein kinases from a simple model organism

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Dictyostelium discoideum is a widely studied model organism with both unicellular and multicellular forms in its developmental cycle. The *Dictyostelium* genome sequence has enabled the determination of its complement of kinase genes (the kinome), offering insights into kinase evolution and providing a focus for signaling analysis in this system. The *Dictyostelium* genome encodes 283 predicted kinases, similar to the count of the much more advanced *Drosophila*. It contains members of most kinase types shared by fungi and metazoans, as well as many kinases which appear to have been secondarily lost from the fungal lineage. This includes the entire TKL (tyrosine kinase-like) group, which likely mediates tyrosine kinase activity in the absence of any tyrosine kinase group members, and includes several novel receptor kinases. 39% of kinases are in *Dictyostelium*-specific families, indicating that protein kinases have played key roles in the adaptation of *Dictyostelium* within its niche.

Phenotyping and screening of *Dictyostelium* mutants based on dynamical features

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We have developed an automated imaging system to capture dynamical features such as darkfield wave propagation, cell streaming and slug migration during *Dictyostelium* development. Making use of the robotics, we have successfully obtained life-cycle movies of approximately 2000 REMI clones in addition to 200 or so morphological mutants isolated earlier by others. All clones were characterized semi-quantitatively by wavelet analysis and manual observation and screened for the presence of developmental defects as well as other features such as growth and cell morphology defects that were picked up during the parallel cell culture. A mutant-enriched subset of these clones of about 400 was further subjected to repeat runs and genotyping. Vector insertion sites in these clones were identified using combination of plasmid rescue and inverse PCR.

Previously, we have demonstrated that turbulent wave behavior is a signature of perturbation in the PKA pathway. Two clones with remarkably similar wave dynamics were also found in the present REMI mutant collection. When genotyped, vector insertion was found in the *rdeA* and *regA* genes, providing further evidence for the role of this signaling cascade in the early cAMP signaling. We will also discuss other newly uncovered mutations related to early and late developmental phenotype.

A Deletion Set for *Dictyostelium*

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With the completion of the *Dictyostelium* genome it is natural to ask "What next?" We propose a thorough analysis of this 35Mb genome by creating a deletion set of the 12, 500 known open reading frames. This is without question a large and ambitious goal, but if realized, an invaluable asset for the community, a starting point for most functional analyses, and a window into the function of, especially, those genes without obvious counterparts in other organisms. It would be very efficient, especially when measured against the cost of constructing deletions one at a time in individual laboratories.

How complicated would such a project be? Some of the difficulties are technical—deletion sets in large closely related families will pose problems (*actin*, for example), the abundance of long runs of amino acids that are under selection in the genome will often force less than ideal sequence choices, the efficiency with which individual deletions can be constructed is hard to predict, and the expected but largely unknown numbers of genes which are haplo lethal, will all pose challenges. There are also challenges of scale and cost.

We believe the tools now in hand are up to the task, and that most experimental problems can be foreseen and overcome. In rough outline, the way forward might involve the following steps: first, informatics to inform gene sequence choice and primer design for the construction of the appropriate knock-out sequences; second, PCR-based *in vitro* recombination and proofing; third, transformation and selection for the deletion; four, verification with PCR primers; five, phenotyping the strains; and finally, linking genotype and phenotype to dictyBase, and curating the deletion set in stock centers. Many of these steps can be automated robotically and in parallel.

These are our own views about how such a project might proceed. The main purpose of this discussion is however not to advocate one path over another, but to gather the views of others, and to see if there is support for such a project, one that will require commitment and cooperation from the entire community.

Innate immunity and detoxification mediated by specialized cells during *Dictyostelium* development

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We describe a previously unrecognized cell type that appears to be dedicated to the removal of toxins and which appear to provide a form of innate immunity. We have named these cells "Sentinel", or "S" cells because of their likely role in protecting the prespore cells from various insults that might otherwise compromise spore production or viability.

S cells move throughout mounds and the pstO and prespore regions of the slug, accumulate toxins within intracellular vesicles, aggregate into clumps of 3-10 cells and are then left behind in the slime trail, or at the base of the fruiting body. Thus, these cells provide a plausible mechanism for toxin removal from multicellular structures. S cells are the only cells that accumulate ethidium bromide (EtBr) during slug migration on EtBr-agar and we have used this property to isolate them by FACS to study their properties. S cells are as viable as prespore cells when plated on bacterial lawns, but are more sensitive to UV irradiation after they have accumulated EtBr. Several other toxins tested also accumulate in S cells' vesicles. Similar vesicles have been described in animal cells that contain several different ABC transporters and that appear to sequester toxins away from target organelles (1). Extracellular vesicles elaborated by vegetative *Dicyostelium* cells have also been proposed as a detoxification mechanism (2). We have evidence that developing cells also release toxin-filled vesicles and we have been able to purify them on sucrose gradients. In vitro, purified S cells rapidly accumulate EtBr into intracellular vesicles when they are mixed with these purified EtBr-vesicles. EtBr-laden cells also appear in naïve populations of disaggregated slug cells exposed to the EtBr-vesicles, or to free EtBr. These cells look like S cells and arise at the same proportion (~0.5%) as S cells, independent of the time of exposure, or the concentration of vesicles or EtBr used.

S cells appear to be highly active phagocytes as they rapidly accumulate latex beads, or *Legionella* bacteria. When disaggregated slugs cells are mixed with latex beads, or *Legionella*, S cells contain most of the beads, or bacteria, after 1 hour. When slugs are allowed to reform and migrate for 12 hours, almost all of the beads, or *Legionella*, are found within S cells. Thus, S cells have the potential to provide a form of innate immunity to *Dictyostelium*.

We have also observed a high frequency of bi-nucleated S cells in slugs migrating on EtBr-agar. In such cells, one of the nuclei is invariably stained with EtBr. Since dead cell nuclei stain with EtBr, but the nuclei of live cells' are never stained, this observation suggests that S cells also engulf dead cells in the slug. Thin-section electron microscopy, or other methods, will be needed to confirm this.

We have isolated mutants that have a propensity to form S cells in chimerae as well as mutants that produce higher and lower than the usual number of S cells. We have also observed what appear to be Sentinel cells in four other dictyostelid species suggesting a common mechanism for detoxification and innate immunity.

1. Rajagopal, A. and Simon, S. M. (2003). *Mol Biol Cell* **14**: 3389-99.
2. Tatischeff, I., et. al (1998). *Cell. Mol. Life Sci.* **54**: 476-487.

Aberrant stalk development and breakdown of tip dominance in *Dictyostelium* cell lines with RNAi-silenced expression of calcineurin B

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The cnbA gene of *Dictyostelium discoideum* which encodes the regulatory B subunit (CNB) of the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin was silenced by RNAi. We found a variety of silencing levels of CNB in different recombinant cell lines. Reduction of CNB expression in a given cell line was correlated with developmental aberrations. Cell lines with strongly reduced protein levels developed slower than wild type cells and formed short stalks and spore heads with additional tips. Whereas expression of the stalk-specific gene ecmB in mutant cells was repressed, there were no significant differences in the expression of the stalk-specific gene ecmA and the spore-specific gene pspA compared to wild type. Aberrant stalk development is a cell autonomous defect, while the breakdown of tip dominance can be prevented by the presence of as low as 10% wild type cells in chimeras. We propose that calcineurin mediates the effects of a differentiation-inducing factor (DIF)-elicited increase in free intracellular calcium in prestalk cells via the dephosphorylation of a cytosolic transcription complex component which activates the expression of prestalk genes.

Priming and processing of the spore differentiation factor SDF-2

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During culmination, the 34 amino acid peptide SDF-2 is released and binds to its receptor DhkA on both prespore and prestalk cells where it induces rapid encapsulation and the generation of further SDF-2. The precursor of SDF-2 is the acyl-CoA binding protein AcbA which is found only in prespore cells during culmination. It is concentrated in vesicles just under the surface and released in response to priming with SDF-2. Processing of AcbA requires the serine protease TagC which is restricted to prestalk cells. Thus the signal goes back and forth between the cell types to coordinate their terminal differentiation.
The material in the vesicles recognized by antibodies raised to recombinant AcbA does not appear to have been processed into the active peptide SDF-2, since no activity can be recovered from un-primed cells following lysis with Triton X100. Nor can prestalk cells process AcbA until they are primed. Primed cells can convert recombinant AcbA to SDF-2 within a few minutes and this process can be blocked by simultaneously adding the serine protease inhibitor TPCK. Mutants lacking TagC or DhkA do not process AcbA to SDF-2 even after priming. These observations suggest that priming of prestalk cells results in exposure of the TagC protease to the extracellular environment.
Nanomolar concentrations of gamma-aminobutyrate (GABA) trigger SDF-2 production by competent cells, apparently by binding to the putative G-coupled seven transmembrane receptor GrlE since mutants lacking GrlE show no response to GABA. GrlE is similar to animal GABA-B type of receptor and might be the first non-metazoan GABA receptor. GABA is generated by glutamate decarboxylase which is encoded by *gadA* in *Dictyostelium*. Expression of *gadA* occurs during the slug stage but only in prespore cells. It looks as if another intercellular loop is mediated by GABA to regulate the initial release and processing of AcbA to generate SDF-2.

Establishment of a system for elucidating precisely the function of individual mitochondrial genes

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We have previously demonstrated that ρ^{Δ} cells with a reduced amount (about 25%) of mitochondrial DNA (mtDNA), which were created by exposure of *Dictyostelium discoideum* Ax-2 cells to ethidium bromide (EtBr), exhibit delayed differentiation after starvation and stop their development at the slug stage. In addition, cell-type proportioning and patterning were quite abnormal in slugs derived from ρ^{Δ} cells: prestalk differentiation is enhanced, while prespore differentiation is markedly inhibited, thus suggesting the importance of mtDNA in a variety of cellular events. By means of the EtBr exposure method, however, we failed in creating ρ^{0} cells without any mtDNA. Thereupon, we have adopted a promising method of molecular genetics method to produce ρ^{0} cells. Ax-2 cells were transformed by the *Eco*RI gene fused to a mitochondrial targeting sequence (MTS), whose expression is under control of the tetracycline-regulated gene expression system. As was expected, mtDNA was found to be completely eliminated after removal of tetracycline from growth medium. Electron microscopic observations revealed that ρ^{0} cells contained only the presence of abnormal mitochondria, in which cristae were significantly disorganized. Staining of mitochondria with MitoTracker dye also showed that the mitochondrial transmembrane potential was completely vanished in ρ^{0} cells. Possibly because of these mitochondrial defects, ρ^{0} cells never proliferated in growth medium, and starved ρ^{0} cells failed to aggregate. Our final goal is to establish a system by which we can disclose precisely the function of individual mitochondrial genes in cellular development. For this, we are now attempting to disrupt specifically a certain mitochondrial gene such as *rps4* by a combination of a modification of the above method and homologous recombination.

Ethylene induces zygote formation through an enhanced expression of a novel zyg1 gene in *Dictyostelium mucoroides*

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Dictyostelium mucoroides-7 (Dm7) exhibits clear dimorphism in development depending upon environmental conditions such as light and water: macrocyst formation occurs as the sexual process and sorocarp formation as the asexual process. Ethylene known as a potent plant hormone induces sexual development (macrocyst formation) by inducing zygotes which are formed by cell fusion and subsequent nuclear fusion, while cAMP inhibits zygote formation and induces asexual sorocarp formation. Ethylene is biosynthesized from methionine through S-adenosyl-L-methionine (SAM) and 1- aminocyclopropane-1-carboxylic acid (ACC) in *Dictyostelium* cells as well as in higher plants. As the case for ethylene action, a gene, zyg1 was found to be tightly involved in zygote formation: Cells overexpressing zyg1 induce macrocyst formation by inducing zygote formation. Transformants which over- or under-produce ethylene were prepared to know if zygote formation actually occurs depending on the levels of ethylene production. As was expected, transformants overexpressing ACC oxidase which catalyzes the conversion from ACC to ethylene were found to overproduce ethylene and exhibit enhanced macrocyst formation. In contrast, transformants in which the expression of ACC oxidase was suppressed by means of RNAi exhibited a less level of ethylene synthesis, thus resulting in the inhibition of zygote formation. Importantly, the expression of zyg1 was also found to be significantly affected by the ethylene level synthesized. Taken together these results indicate that ethylene is surely involved in zygote formation through the zyg1 expression.

Gene sharing between sexual and asexual development in *Dictyostelium discoideum*

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Genes in AX4 is estimated to be about 12,500 by the genome analysis. The cDNA sequences obtained by the Japanese cDNA Project have been clustered into 6,700 genes, slightly above 50% of the estimation. Although this fact indicates that the number of cDNA clones sequenced from each library was not large enough, it also suggests that the significant fraction of gene repertoire is relevant to other cellular states than the “growth and (asexual) development”. In order to understand the overall genetic potential of *Dictyostelium*, studies of other processes such as macrocyst formation may now be important. We have been analyzing the genes involved in the process of macrocyst formation. When we constructed conventional and gamete-specific subtraction libraries and sequenced their clones, 179 out of 822 genes were not found in the cDNA libraries from other stages. On the other hand, the remaining genes are shared by vegetative and/or asexually developing cells. Although the specific genes are expected to play important roles in macrocyst formation, our previous studies indicate that common genes are important as well. In the present study, therefore, we focused on the latter shared genes, and attempted to pick up sexually important genes from the asexual developmental genes. First, we analyzed the function of four cAMP receptors during macrocyst formation, since it is known to use the cAMP-mediated cell aggregation system as in the asexual cycle. Next, we started to systematically analyze the sexual phenotypes of currently available knockout mutants of aggregation-related genes. Furthermore, we selected the aggregation-stage specific genes in the EST database, and analyzed their involvement in the sexual process. Analyses of those shared genes may reveal novel systems of gene expression in response to the environmental changes.

The behaviour of *D. discoideum* from the point of view of the dynamical theory of nonlinear systems

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It is well known, that *D. discoideum* is widely used for studies of self-organization processes in living organisms. The fundamental understanding of these processes is unthinkable without vast and detailed experimental investigation and elucidation of a tremendous variety of biochemical pathways that take place in the organism. Dynamical theory of nonlinear systems offers an additional level of understanding based on formulation of mathematical models of studied systems and their functional subsystems. The models build on the experimental knowledge gained with the aim to understand the self-organization processes on the basis of basic dynamical principles. These involve both nonlinearities of biochemical pathways and spatially oriented processes such as diffusion of chemical components, transport through the membrane, cell random motion and chemotaxis, etc.

In the presented paper, we will discuss the nonlinear phenomena occuring during vegetative and aggregation stages of the life cycle of *D. discoideum.* The discussion will be based on dynamical analysis of the mathematical models of both cells multiplication and synthesis of 3',5'-cyclic adenosine monophosphate in cells. We will illustrate the application of dynamical theories on several experimentally known phenomena, that include:

spreading of plaques on the bacterial lawn

formation of centers eliciting cAMP waves during early stages of aggregation

propagation of cAMP waves and formation of spiral and circular wave patterns

emergence of new aggregation centers during the propagation of cAMP waves

break up of large aggregates into a number of mounds.

Threshold for Chemotactic Motion in *Dictyostelium Discoideum*

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The chemotactic response of *Dictyostelium discoideum* cells to stationary, linear gradients of cyclic adenosine 3',5'-monophosphate (cAMP) is studied using microfluidic devices. For gradients equal to or higher than 10^{-12} M/μm, chemotactic motion was found. Below the threshold, the cells did not respond to cAMP and exhibited a constant basal motility. Above the threshold, the chemotactic response was observed to be determined by the steepness of the gradient. The chemotactic speed and the motility increased with increasing steepness of the gradient until 10^{-10} M/μm, at which concentration cells did not show any further increase in their chemotactic response. For concentrations above 10^{-9} M/μm the cells lost directionality and the motility returned to the sub-threshold level. Our data demonstrated that (1) dictyostelium chemotaxis is initiated and modulated by the change in the absolute chemo-attractant concentration gradient with a threshold of 10^{-12} M/μm; (2) further increase in gradient beyond 10^{-10} M/μm leads to the return of basal behavior; (3) microfluidic devices provide the means to establish, control, manipulate and remove a chemical gradient in a precise, reproducible and quantitative manner. Current work focuses on delineating the effects of an absolute concentration gradient and a relative concentration difference sensed by a cell body.

Ramdom motion of *Dictyostelium discoideum*: quantitative analysis using model-free methods

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Many types of cells are known to exhibit both directed and random types of motion. In particular, directed cell motion is usually overlaid by fluctuations causing frequent changes in the direction of propagation and therefore reducing the efficiency of the directional process. In order to understand the nature of the random processes in cell motion, we study the dynamics of Dictyostelium discoideum cells in microfluidic devices on the basis of cell trajectories. While previous works usually heuristically assume a model for random motion which is fitted to the experimental data via the corresponding distribution functions, we use a new model-free analysis method to quantitatively separate deterministic and stochastic parts of the cell dynamics. In this way, we obtain stochastic model equations (i.e. Langevin equations) using only very weak assumptions. We find that *Dictyostelium* cells in the absence of signalling undergo a random walk with multiplicative noise, which is different from the classical Ornstein-Uhlenbeck model for random walks. In addition, we show how the deterministic and stochastic components of the cell dynamics depend on the concentration of 3',5'-monophosphate (cAMP) and how the cell dynamics adapts to the presence of cAMP in the course of time. The experimental findings are discussed from different perspectives, and several mechanisms are proposed to explain the results.

Measurements of the mechanical forces exerted by migrating *Dictyostelium* slugs using the flexible substrata method

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Over the last decade, a large amount of information has been obtained on the distribution of cell movements along slug axis both in normal three-dimensional (3D) slugs (1) and in 2D slugs (2). But, the distribution of mechanical forces exerted by the migrating slug was never directly measured. Hence the mechanisms by which motive force is transmitted to the substrate and their location have been subject to numerous speculations and hypotheses. The only indirect measurement of motive force was made by Inouye et al. (3). Assuming a viscous drag in their analysis, the total motive force was found proportional to slug volume suggesting that the sum of the crawling movements of the whole cells is propelling the slug forward. Many models of slug migration have since simply postulated that the motive force is volumetric. However, the mechanisms by which interior cells can transfer forces to the substrate remain unclear. The role of extracellular matrix surrounding the slug (slime sheath) was also never investigated.

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POSTER ABSTRACTS

A ribosomal gene that affects morphogenesis and differentiation in *Dictyostelium discoideum*

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In the life cycle of *Dictyostelium discoideum* heterogeneities amongst vegetatively growing amoebae with respect to nutritional status, cell cycle position and levels of intracellular calcium are correlated with post- aggregation cell fate. In the course of an ongoing study of the cell cycle-calcium link we have isolated a *D discoideum* cDNA that can complement the yeast *cdc24-4* mutation. At 37^0C, *cdc24-4* mutants arrest post-Start in the G1 phase of the cell cycle and display a 7-8-fold increase in calcium influx at the restrictive temperature. The cDNA encodes the ribosomal protein S4.

s4 antisense cells show abnormal development. Most of the aggregates form undifferentiated multiple-tipped structures by 24hrs; however, one can see some spore and stalk cells too. The spores are viable and are spherical in shape in contrast to wild type spores which are ellipsoidal.*s4* antisense cells form normal-looking fruiting bodies after being pulsed for 6 hr with cAMP, or by artificially raising cellular calcium if cells are plated at high density. The presence of wild type cells can also rescue the phenotype; cell-cell contact is not needed. In both the cases, the spore morphology remains spherical. Antisense RNA-expressing cells also have larger aggregation territories than the wild type. When antisense-expressing cells are mixed with mutant cells known to be aberrant in group size regulation (*cf50,* which forms large aggregates and fruiting bodies and *smlA,* which forms small aggregates and fruiting bodies), fruiting bodies are formed in combination with *smlA* but not *cf50*. Again, the spore morphology remains spherical.

To explore the possible roles of *s4* in *D discoideum* growth and development, the gene was expressed in sense and antisense orientations under an actin 15 promoter. The transformants grow poorly under axenic conditions even at low selection pressures, suggesting that the stoichiometry of S4 relative to other cellular components is important. The effect of over- and under- expression of *s4* under its own putative promoter is being analysed.

These findings suggest a novel role for S4 in *D discoideum* development.

Shielding of cells by fluid flow

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The directed motion of cells along chemoattractant gradients is a fundamental aspect of many biological and medical phenomena. In a natural environment, chemotaxis often occurs under the influence of external fluid flow, e.g. in blood vessels. Shear forces are known to affect cellular dynamics and their influence on cell motility has been studied in detail. However, we show that another effect can strongly influence chemotactic motion under flow conditions: the interplay of geometry, flow speed, and diffusion can lead to a significant change of the concentration distribution in the vicinity of a cell. Recent developments in microfluidic techniques allow a precise experimental investigation of this scenario using *Dictyostelium discoideum* cells.

A novel PI(4,5)P_2 binding domain targeting the Phg2 kinase to the membrane in *Dictyostelium* cells.

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Phg2 is a ser/thr kinase involved in adhesion, motility, actin cytoskeleton dynamics and phagocytosis in *Dictyostelium* cells. In a search for Phg2 domains required for its localization to the plasma membrane, we identified a new domain interacting with PI(4,5)P_2 and PI(4)P membrane phosphoinositides. Deletion of this domain prevented membrane recruitment of Phg2 and proper function of the protein in the phagocytic process. Moreover, the overexpression of this PI(4,5)P_2 binding domain specifically had a dominant negative effect by inhibiting phagocytosis. Therefore plasma membrane recruitment of Phg2 is essential for its function. The PI(4,5)P_2 binding domain fused to GFP (GFP-Nt-Phg2) was also used to monitor the dynamics of PI(4,5)P_2 during macropinocytosis and phagocytosis. GFP-Nt-Phg2 disappeared from macropinosomes immediately after their closure. During phagocytosis, PI(4,5)P_2 disappeared even before the sealing of phagosomes as it was already observed in mammalian cells. Together these results demonstrate that PI(4,5)P_2 metabolism regulates the dynamics and the function of Phg2.

Two distinct roles for a novel anti-adhesive protein during *Dictyostelium* growth and development.

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The *ampA* gene encodes a novel protein that modulates cell-cell and cell-substrate adhesions and developmental patterning. The AmpA protein is necessary in a non-cell autonomous manner to prevent premature differentiation of prespore cells. In *ampA* null cells a prespore marker is expressed in cells at the mound periphery that will normally differentiate into prestalk cells. We demonstrate that a supernatant source of AmpA protein added extracellularly can prevent this premature mis-expression of the prespore marker. Synthetic oligopeptides are used to identify the domain of the AmpA protein and a 9 amino acid "active site" sequence that are important for preventing cells from premature prespore gene expression. A model for AmpA acting through a lateral inhibition mechanism to prevent cells at the mound periphery from assuming a prespore fate is discussed.

We also demonstrate that AmpA effects on cell agglutination are due to a second and distinct, cell associated role of AmpA. In growing cells, where the AmpA protein is associated with the cell periphery, gene inactivation results in an increase in cell-cell clumping. Overexpression of the *ampA* gene reduces clumping. Furthermore AmpA influences the size of plaques formed on bacterial lawns; overexpressing cells form large plaques while *ampA* null cells form tiny plaques. The plaque size alteration is due to differences in cell adhesion which influence whether cells can migrate out of the plaque into the bacterial lawn in search of food. Comparison of migration of AmpA overexpressing cells under different conditions suggests that cell associated AmpA influences cell traction and the basal level and organization of actin polymerization.

Phospholipase D regulates quorum sensing and differentiation in *Dictyostelium discoideum*.

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Quorum sensing, or the ability of a cell to sense the density of neighboring cells, plays a vital role in eukaryotic processes ranging from organ formation and regeneration to disease progression in trypanosome infections. The social amoeba *Dictyostelium discoideum* is the simplest eukaryote to display quorum sensing, and links its development to quorum sensing by using one G protein mediated signaling pathway to regulate a second G protein pathway. Starving *Dictyostelium* cells are able to calculate the concentration of starving cells by simultaneously secreting and sensing a glycoprotein called conditioned medium factor (CMF). When the number of starving cells is high, the level of CMF is also high. This allows signaling through cAR1 to proceed, and aggregation ensues.

Phospholipase D (PLD) is a key player in CMF signaling. We have identified the gene *pldB*, which has homology to mammalian PLD1. PldB is a negative regulator of CMF signaling as cells lacking *pldB* aggregate at very low cell density. In addition, these cells aggregate earlier than normal, possibly due to their early expression of cAR1. Localization studies with GFP-PldB place the protein in vesicles that may be endosomal/lysomal in origin. Finally, *pldB* appears also to be involved in later development. Experiments with chimeric organisms show that the *pldB* disruptants preferably localize to the tips of mounds and the stalk and cups of the fruiting body. This suggests that these cells preferentially develop along the prestalk pathway, implying that pldB may be involved in prespore development.

A secreted factor represses cell proliferation in *Dictyostelium*

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The concept of chalones or proliferation inhibiting factors has been popular for years but until the recent discovery of the factor myostatin no one had been able to purify a chalone. We have found that growing *Dictyostelium* cells secrete a 60 kDa protein called AprA for autocrine proliferation repressor (previously referred to as Ncf60) which might conform to the main properties of a chalone: an extracellular autocrine signal that negatively regulates proliferation without affecting cell viability. Growth medium conditioned by WT cells was size fractionated using a sieve column and fractions corresponding to ~150 kDa were able to inhibit the proliferation of WT and *aprA*$^-$ cells; this activity was not seen in growth medium conditioned by *aprA*$^-$ cells. AprA may thus form a complex with itself or other proteins. AprA purified by immunoprecipitation also slowed the proliferation of WT and *aprA*$^-$ cells. Video microscopy of growing cells indicated that in the presence or absence of AprA, cells appear healthy and motile, suggesting that AprA does not affect cell viability. Staining for Annexin V positive cells (a marker for dying cells) showed no significant difference comparing WT, *aprA*$^-$, and AprA overexpressing cells (AprA^{OE}) also suggesting that AprA does not affect cell survival/cell death. When compared with WT cells, *aprA*$^-$ cells have roughly twice as many cells with 2 or more nuclei as seen by DAPI staining. This correlates with the increased proliferation rate of *aprA*$^-$ as compared to WT cells. Conversely, AprA^{OE} cells, which proliferate slower than WT cells, have fewer cells with 2 or more nuclei than WT cells. Treatment of *aprA*$^-$ cells with immunoprecipitated AprA protein returns the number of two or more nuclei to WT levels. In addition to having an increased percentage of multinucleated cells during growth, *aprA*$^-$ cells form fewer spores during development. In summary, we have isolated an autocrine secreted factor that slows proliferation without affecting viability. AprA thus appears to have the properties of a *Dictyostelium* chalone.

Calcium mobilization stimulates *Dictyostelium discoideum* shear-flow induced cell motility

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Application of hydrodynamic mild shear stress to adherent *Dictyostelium discoideum* vegetative cells triggers active actin cytoskeleton remodeling resulting in net cell movement along the flow (Décavé et al. 2003 *J. Cell Sci.* **116** : 4331-43). The average cell speed is strongly stimulated by external calcium (Ca^{2+}, $K_{50\%}$ = 22 μM), but the directionality of the movement is almost unaffected. This calcium concentration is ten times higher than the one promoting cell adhesion to glass surface ($K_{50\%}$ = 2.5 μM). Addition of the calcium chelator EGTA or the Ca^{2+}-channel blocker gadolinium (Gd^{3+}) transiently stops cell movement. Monitoring the evolution of cell-surface contact area with time reveals that calcium stimulates cell speed by increasing the amplitude of both protrusion and retraction events at the cell edge, but not the frequency. As a consequence, with saturating external calcium concentrations, cells are sensitive to very low shear forces (20 pN; σ = 0.1 Pa). Moreover, a null-mutant lacking the unique Gβ subunit does not respond to external Ca^{2+} changes ($K_{50\%}$ > 1000 μM), although the directionality of the movement is comparable to that of wild-type cells. Furthermore, cells lacking the *Dictyostelium* homolog of the inositol 1,4,5-trisphosphate receptor (IP3-receptor) exhibit a markedly reduced Ca^{2+} sensitivity. Thus, calcium release from internal stores and calcium entry through the plasma membrane modulate cell speed in response to shear stress.

A method for isolation of rare knockout mutants arising from single or double homologous recombination in *Dictyostelium discoideum*

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Generation and characterization of knockout clones is a widely used approach to evaluate the specific function of a gene product in *Dictyostelium discoideum*. The mutant clones are generally obtained by insertion of a selection cassette by homologous recombination in the targeted gene. A frequent limitation to obtaining mutants is the very low frequency of homologous recombination. Here we present an easy method to identify those rare mutants, based on PCR analysis of pools of clones. This method not only allows the isolation of mutants created by double homologous recombination, but also of functional knockout mutants arising from a single recombination event. Our results reveal that single recombination events can be much more frequent and be an alternative way to obtain functional knockout mutants.

Phg2 and Arm play a role in the nutrient response in *Dictyostelium*

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The physiology of *Dictyostelium* amoebae is largely modulated by its response to nutrients. Nutrient starvation induces in particular the transition from growth to multicellular development. The molecular mechanisms by which nutrients regulate this transition are still poorly characterized to date.
Here we show that the growth-to-development transition in response to nutrients is altered in the previously characterized phg2 mutant. Phg2, which appears structurally related to Rho kinases, was previously characterized for its role in cellular adhesion and the control of the actin cytoskeleton. Our results indicate that it also participates to the cellular response to nutrients.
A protein interacting with the putative ras-binding domain of Phg2 was identified, and corresponded to the *Dictyostelium* homolog of the previously characterized mammalian Adhesion-Related molecule (Arm). Analysis of arm knock-out cells indicated that in *Dictyostelium* Arm is involved in the cellular response to nutrients, but not in the control of cellular adhesion.
These results suggest a partial overlap between the genes involved in the control of cell adhesion and phagocytosis, and genes involved in the response to nutrients.

RNA polymerase III transcription factor complex TFIIIB determines chromosomal integration sites of *Dictyostelium* retrotransposon TRE5-A

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Retrotransposon TRE5-A is a highly active mobile element that constantly shapes the *Dictyostelium discoideum* genome. Inspection of the *D. discoideum* genome has suggested a strong preference of TRE5-A for gene-less integration sites upstream of tRNA genes. Hence it has been argued that TRE5-A is not mutagenic, but there is still no formal proof for that assumption. We report on progress in the evaluation of two fundamental questions of TRE5-A biology: (i) how does TRE5-A recognize tRNA genes as potential integration sites, and (ii) how specific is integration site selection, i.e. what is the true mutagenic potential of TRE5-A?
We performed a systematic search for protein-protein interactions of TRE5-A-encoded proteins (ORF1p and ORF2p) with subunits of DdTFIIIB, a trimeric factor that initiates tRNA transcription. We found that TRE5-A ORF1p, but not ORF2p, interacts strongly with DdTFIIIB subunit DdTBP and to a lesser extent with the other two other DdTFIIIB subunits, DdBrf and DdBdp1. This result suggests that ORF1 protein is a component of the TRE5-A preintegration complex consisting at least of ORF1p and TRE5-A RNA , and that ORF1p is directly involved in the selection of integration sites. It remains unclear whether ORF2p, whose enzymatic activity is required for reverse transcription and integration, joins the TRE5-A preintegration complex in the cytoplasm or enters the nucleus separately.
To estimate the true specificity of TRE5-A integration it is required to follow integrations of artificial reporter elements introduced into AX2 cells. Since a complete TRE5-A cannot be maintained on plasmid vectors, we have started to explore what *cis*-acting sequences of a TRE5-A are required to act as a miniature fully active retroelement. Preliminary results show that the 3' end of TRE5-A (i.e. the C-module) is necessary and sufficient to support a full Retrotransposition cycle of the element.

A proteomics approach to decipher phagosome maturation and the role of the myosin I MyoK

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Myosins of class I have already been shown to be involved in cell motility and a variety of endocytic processes in *D. discoideum*. For example, the mutants of myosin I MyoK show a reduced phagocytic rate and cell motility, as well as a loss of cortical tension. During the ingestion of TRITC-labeled yeast, a fraction of MyoK localises at the phagocytic cup. The GPR loop of MyoK has also been shown to bind profilin, dynamin and Abp1. As a network of binding partners is building around MyoK, we want now to discover the direct and indirect effects of MyoK mutation at a molecular level especially during the process of phagocytosis. Therefore, we used 2D Fluorescence Differential Electrophoresis (DIGE) as a comparative tool. It allows us to analyse in a quantitative manner up to 12 different samples in a single run. We analysed total cell lysates of MyoK null cells versus their wild-type counterparts in the AX-2 and DH1-10 backgrounds. We also analysed the AX-2 cells in the suspension and adherent culture conditions.
On the other hand, we deciphered phagosome maturation in wild-type cells. Phagosomes were purified at six different time-points using a pulse/chase feeding protocol and analysed with conventional silver stained 2D gels. The results show striking differences in the protein profiles as phagosome matures. Clustering of the time-dependent protein profiles with a hierarchical algorithm should then allow the grouping of proteins into functional classes over the whole maturation period.
The two projects listed above should then be merged to compare phagosome maturation in wt and MyoK null cells using DIGE and subsequent clustering.

A tandem affinity purification (TAP) tag for use in *Dictyostelium discoideum*

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Tandem affinity purification (TAP) is an approach to protein complex isolation. The method involves fusing a tag to the C or N terminus of the target protein, then high affinity purification under native conditions, and subsequent protein identification using mass spectrometry/database search algorithms. The TAP tag consists of three modules including a calmodulin binding peptide, a TEV protease recognition site followed by two IgG binding domains of *Staphylococcus aureus* protein A [Puig et al. (2001) Methods 24: 218].

Using the computer program DNAworks [http://molbio.info.nih.gov/dnaworks], 24 oligonucleotides were designed for assembly using thermodynamically-based inside-out PCR [Gao et al. (2003) Nucleic Acids Research 31; e143] into a C-terminal TAP tag. We found that the assembly worked best using two primer pairs at a time, and accordingly constructed the tag in a total of 6 PCR reactions. After each reaction, the synthetic gene fragment was gel-purified prior to serving as template for the subsequent extension reaction. The 583 bp full-length TAP tag, optimized for *D. discoideum* codon usage, has been cloned between the Spe1 and Xho1 sites of pBluescript. This tag may be fused to target proteins of interest using the cloning sites, or alternatively the tag could be fused to the target gene by overlapping-ends PCR.

The TAP tag will be used to purify and characterize the Cdk1/cyclin cell cycle complex in D. discoideum. Cdk1 (formerly cdcB) is of key interest because of its many potential binding partners. Cdk1 is the only cyclin-dependent kinase in Dictyostelium of the Cdk1/2 class; Dictyostelium contains at least three cyclins of the cell-cycle regulating clade (cyclinA, cyclinB, and cyclinD) for which Cdk1 is the only plausible partner. *Dictyostelium* has no cyclin-dependent kinase inhibitors (CKIs) which are recognizable by homology, but this group of proteins is in general poorly conserved. Plant CKIs, now a well-established group, were only found fortuitously. In vertebrates, Cdk2 is also known to form complexes containing the retinoblastoma-susceptibility protein, which appears to have a role in regulating differentiation. We anticipate that studies of Cdk1-containing complexes will contribute to the understanding of the cell cycle and development in *Dictyostelium*.

Analysis of Phosphoprotein Phosphatase PP2A in Growth and Development of *Dictyostelium discoideum.*

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Dephosphorylation by protein serine-threonine phosphatases plays essential roles in signal transduction. The heterotrimeric protein serine-threonine phosphatase PP2A is ubiquitously present in eukaryotic cells, and is the major phosphoprotein phosphatase in *Dictyostelium discoideum*. To elucidate the role of PP2A in protein phosphorylation, signal transduction, growth and development, we have created site-specific mutations in PP2Ac cDNA and transfected wild-type *Dictyostelium* cells. Mutations in the metal-binding residues of PP2Ac severely impair growth and development, and are presumed to be dominant-negative. A mutation altering one amino acid residue (P240H), in the PP2Ac subunit of the yeast Pph22p results in temperature-sensitive mitotic defects and impairment of catalytic activity *in vitro* (Evans and Hemmings 2000, *Genetics* **156**, 21-29.) The homologous mutation (P169H) on the *Dictyostelium* PP2Ac DNA also confers temperature-sensitive growth, some developmental defects and reduction in catalytic activity.

In previous experiments, we have transfected large inverted-repeat DNA fragments to induce RNA interference that severely reduced the mRNA of a development-specific *Dictyostelium* transcription factor gene, *mybB* (Otsuka, Cogill, Kuan, Dottin and Gross, unpublished) and blocked aggregation. We now express small shRNAs targeted against specific portions of mybB mRNA. Inhibition appears to depend on the particular shRNA sequence used. To extend this technology, we are using shRNA to knock down, but not eliminate the functioning of the essential gene, PP2Ac. These results show that PP2Ac is essential for growth and at multiple stages in *Dictyostelium* development. These approaches should delineate the mechanisms by which PP2A mediates signal transduction for eukaryotic cell proliferation and development.

Dictyostelium transcriptional host cell response upon infection with *Legionella*

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Freshwater amoebae are the natural host of *Legionella pneumophila*, the causative agent of Legionnaires' disease. The professional phagocyte *Dictyostelium discoideum* lives in its natural habitat, the forest soil, on bacteria that are taken up by phagocytosis. It was shown that the medically relevant infection of host cells by pathogenic microorganisms can be investigated with *D. discoideum* as host and the facultative intracellular parasite *L. pneumophila*. Differential gene expression of the host after infection with *L. pneumophila* was investigated using DNA-microarrays, that carry more than 5,000 non-redundant EST clones, 450 probes derived from selected genes as well as appropriate positive and negative controls.

Investigation of a 48-hour time course of infection revealed several clusters of co-regulated genes, an enrichment of preferentially up- or down-regulated genes in distinct functional categories and also showed that most of the transcriptional changes occurred 24 hours after infection. A detailed analysis of the 24-hour time point post-infection was performed in comparison to three controls, uninfected cells and co-incubation with *L. hackeliae* and *L. pneumophila* Δ*dotA*. 131 differentially expressed *D. discoideum* genes were identified as common to all three experiments and are thought to be involved in the pathogenic response. Functional annotation of the differentially regulated genes revealed that apart from triggering a stress response *Legionella* apparently not only interferes with intracellular vesicle fusion and destination but also profoundly influences and exploits the metabolism of its host. For some of the identified genes e.g. RtoA involvement in the host response has been demonstrated in a recent study, for others such a role appears plausible. The results provide the basis for a better understanding of the complex host-pathogen interactions and for further studies on the *Dictyostelium* response to *Legionella* infection.

Characterisation and regulation of the exocyst complex in *Dictyostelium discoideum*

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The exocyst complex is an evolutionary conserved complex originally identified in *Saccharomyces cerevisiae* by TerBush et al.,1996. In yeast the complex is required for post-Golgi transport of vesicles and their tethering to the plasma membrane of the growing bud. It is composed of 8 subunits, and homologs of each of these proteins have been identified in diverse organisms, including plants, insects and mammals. Furthermore, a link between the exocyst complex and the Rab protein family has been reported. BLAST searches in DictyBase with the coding sequences of the human and yeast genes revealed only a single obvious homolog for each of the subunits in *Dictyostelium discoideum*. The goal of this project is to investigate the role of the exocyst complex and its potential regulators, the Rab proteins in D. discoideum, because it is an ideal model to study their function in focal exocytosis, during motility and phagocytosis. Focal exocytosis is likely providing membrane to the phagocytic cup during uptake, or recycling membrane back to the surface during endosomal transit. To investigate these hypotheses we choosed different approaches. (1) Localisation studies of the exocyst complex during cell motility, phagocytosis and on isolated phagosomes. (2) Co-localisation studies of the exocyst with known cellular compartment, such as late endosomes. (3) Impact of exocyst knock out mutations on cell motility, focal exocytosis, cell morphology and the different steps of phagocytosis like uptake, transit and exocytosis. (4) Localisation studies of the Rab proteins in exocyst mutants and co-localisation studies with the exocyst in wild type cells. Here I will report on our progress on these strategies that are borne to significantly improve our understanding of endosomal recycling, exocytosis and their regulation in *D. discoideum*.

Localization of Cell Types in the Slug of *Dictyostelium discoideum* based on Differential Sensitivity to cAMP and Ammonia

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Pre-stalk anterior-like cells and prespore cells are differentially localized in the migrating slug of *Dictyostelium discoideum*.

Our model for localization of these cell types is based on the differential chemotactic response of the cell types to cAMP and the differential sensitivity of the cell types to suppression of such chemotaxis by ammonia.

Our data is based on a drop assay that we have reported previously (Feit, Medynski & Rothrock (2001) J Biosci 26:157-66). Based on this assay, prestalk cells show much higher chemotactic sensitivity to cAMP than either anterior-like cells or prespore cells. Prestalk cells also show much less sensitivity to suppression of such chemotaxis by ammonia.

Anterior-like cells and prespore cells seem very similar in both cAMP and ammonia sensitivity. We are refining our measurements to see whether they differ at all.

We suggest that as anterior-like cells mature into prestalk cells, they dramatically increase in sensitivity to cAMP and decrease in sensitivity to ammonia, and that this accounts for their chemotactic recruitmen into the slug tip.

Two Distinct Roles for a Novel Anti-Adhesive Protein During *Dictyostelium* Growth And Development

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The ampA gene encodes a novel protein that modulates cell-cell and cell-substrate adhesions and developmental patterning. The AmpA protein is necessary in a non-cell autonomous manner to prevent premature differentiation of prespore cells. In ampA null cells a prespore marker is expressed in cells at the mound periphery that will normally differentiate into prestalk cells. We demonstrate that a supernatant source of AmpA protein added extracellularly can prevent this premature mis-expression of the prespore marker. Synthetic oligopeptides are used to identify the domain of the AmpA protein and a 9 amino acid ìactive siteî sequence that are important for preventing cells from premature prespore gene expression. A model for AmpA acting through a lateral inhibition mechanism to prevent cells at the mound periphery from assuming a prespore fate is discussed.

We also demonstrate that AmpA effects on cell agglutination are due to a second and distinct, cell associated role of AmpA. In growing cells, where the AmpA protein is associated with the cell periphery, gene inactivation results in an increase in cell-cell clumping. Overexpression of the ampA gene reduces clumping. Furthermore AmpA influences the size of plaques formed on bacterial lawns; overexpressing cells form large plaques while ampA null cells form tiny plaques. The plaque size alteration is due to differences in cell adhesion which influence whether cells can migrate out of the plaque into the bacterial lawn in search of food. Comparison of migration of AmpA overexpressing cells under different conditions suggests that cell associated AmpA influences cell traction and the basal level of actin polymerization. Current work focuses on identifying AmpA interacting partners. One approach is to create an AmpA-His fusion protein. This protein can be used to identify interacting partners of AmpA via affinity chromatography or immunoprecipitation. This fusion protein can also be used in rescue experiments to determine the biochemical and physiological properties of AmpA. A second approach to this problem is genetic. We are attempting REMI mutagenesis to create a second site suppressor of the AmpA mutant phenotype. This experiment would aid in the discovery of interacting proteins or pathways

An Overview of the Architecture of dictyBase

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Since December, 2002, dictyBase has been hosting and distributing the *Dictyostelium discoideum* genome sequence. The software and database behind dictyBase started out as a slightly modified version of *Saccharomyces* Genome Database (SGD). We have significantly extended the software and database schema to help us to better serve the Dictyostelium community. Here, we present an overview of the present structure of dictyBase, focusing on our adoption and extension of widely available bioinformatics tools.

Database schema: Web-based database applications have a database where data resides on disk and can be retrieved and inserted on demand. In dictyBase this is implemented in Oracle, a commercial database management system. The 'schema', or organization of the information in the database, is optimized for performance and flexibility. Recently, we have adopted the Generic Model Organism Database (GMOD) schema, Chado, for storing our sequence data and related information. The Chado schema allows great flexibility in storing different kinds of features, such as mRNA, tRNA, protein sequences, and untranslated regions as well as various relations among those features and groups of features. It also provides a common framework for dictyBase and other model organism databases (MODs).

Application server: In addition to the database there is application code which reads data from the database and organizes it into a more human comprehensible form. This 'application layer' takes data from the database and presents the data to the user as a web page. In dictyBase this is achieved using PERL. By combining the power of the popular PERL code library 'BioPerl' with our database, we have designed the dictyBase PERL application to logically group together data from the database to semantically imitate 'real world' concepts such as genes and transcripts. dictyBase also provides complete sets of genome data, organized in several different packages, available for download. Genome annotations are now available via GFF3 format which is a standard format set forth by the bioinformatics community. The GFF3 format has the full chromosomal sequence in addition to the locations of all the features and many annotations (GenBank Accession Numbers, GO terms). dictyBase is supported by grants from NIGMS and NHGRI.

Curation at dictyBase

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**authors making equal contributions.*

dictyBase is the comprehensive model organism database for *Dictyostelium discoideum* that hosts the entire *Dictyostelium* genome sequence and related information. Several aspects of gene structure and function are annotated, including gene model, gene product, phenotype, and Gene Ontology classification.

Before the curation process begins, data is integrated into the database automatically. Weekly imports include GenBank records, PubMed references, and Gene Ontology annotations. Other data are imported as they become available: updates to genome sequence, microarray data, and links to data from other projects, such as Baylor Insertional mutants and in situ hybridization images. dictyBase curators complement, correct and add information as appropriate.

Gene Model Curation: Curators examine the automated Gene Predictions and compare all available sequences, such as genomic sequence, independent GenBank records, ESTs, and sequence similarity with known proteins to make a Curated Model, the best available gene model for a gene. About 85% of Gene Predictions are accurate; the remaining are corrected manually.

Literature Curation: Curators read papers and assign Literature Topics, which are general categories aimed at providing a fast overview of the focus of publications. Original publications are also used to assign gene products, gene names, synonyms, brief descriptions, and name descriptions.

Phenotype Annotation: Curators extract information about mutant phenotypes from the literature. More information can be found on a separate page, where specific phenotypes are listed with their references and serve as a link to other *Dictyostelium* genes with the same phenotype. All phenotype annotations are entered manually.

Gene Ontology Annotation (GO): GO terms are imported electronically based on BLAST hits and InterPro domains. Curators upgrade, replace, and add to these annotations based on published data, as well as additional sequence analyses.

Accuracy, usability and service are our highest priorities. We work in close cooperation with researchers to ensure that these goals are met.

dictyBase is supported by grants from NIGMS and NHGRI.

Two different components regulate glucose-6-phosphatase in *Dictyostelium*

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Glucose-6-phosphatase, a key enzyme involved in gluconeogenesis and glycogenolysis, plays a major role in glucose homeostasis. In humans, the activity of this enzyme is elevated in diabetes type II, and mutations in this enzyme lead to glycogen storage diseases. Glucose-6-phosphatase is a multicomponent enzyme, located on the lumen side of the endoplasmic reticulum. How glucose-6-phosphatase is regulated is unknown, and identifying ways to inhibit this enzyme could decrease serum glucose levels in diabetics. We have found that a secreted factor that regulates group size in *Dictyostelium* regulates glucose-6-phosphatase activity. During development, *Dictyostelium* cells form groups of ~2 x 10^4 cells. The group size is regulated in part by a negative feedback pathway mediated by a secreted multi-polypeptide complex called counting factor (CF). The CF signal transduction pathway involves CF repressing internal glucose levels by regulating the activity of glucose-6-phosphatase. To understand how CF regulates glucose-6-phosphatase, we measured the activity of the enzyme in various conditions. Microsomal fractions were separated by ultracentrifugation from cell lines with different CF accumulation. The activity of glucose-6-phosphatase in the semi-purified microsomes had a negative correlation with the amount of CF present in these cell lines. Crude cytosol from wild-type cells and a cell line that lacks bioactive CF (countinØ cells) had almost no glucose-6-phosphatase activity. These cytosols were further fractionated into a fraction containing molecules greater than 10KDa (S>10K) and less than 10 KDa (S<10K). The fractions by themselves did not have a significant amount of glucose-6-phosphatase activity. However, the fraction that contained greater than 10 KDa from wild-type and countinØ cells significantly increased the activity of glucose-6-phosphatase, suggesting that this fraction contains one or more factors that potentiates the activity of glucose-6-phosphatase. In addition, the fraction that contained less than 10KDa from wild-type and countinØ cells significantly decreased the activity of glucose-6-phosphatase. We conclude that there exist one or more factors that increase the activity of glucose-6-phosphate in S>10K, and one or more factors that decreases the activity of glucose-6-phosphatase in S<10K, and we are currently investigating whether these factors are regulated by CF.

Regulation of the Batten Disease associated tripeptidyl peptidase I by AprA and AprB in *Dictyostelium discoideum*.

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Neuronal Ceroid Lipofuscinosis (NCL) or Batten Disease is an autosomal recessive neurodegenerative disease and is the most common neurodegenerative disease in children. NCL occurs at a rate of 1 to 5 per 100,000 births. Mutations in any of 6 identified genes will lead to the development NCL. Loss of function mutations in *CLN2* can lead to the onset of the late-infantile variant of NCL (LINCL). *CLN2* encodes the lysosomal protease tripeptidyl peptidase I (TPP I), and loss of TPP I activity is observed in fibroblasts derived from children with LINCL. Little is known about the regulation of TPP I, and no natural substrate for this peptidase has been identified. AprA and AprB are components of a secreted protein complex that modulates proliferation and development in *Dictyostelium discoideum*. *aprA*$^{-}$ and *aprB*$^{-}$ cells have increased proliferation and form large irregular fruiting bodies compared to wild-type cells. In contrast, AprA over-expressing cells (*aprA*^{OE}) proliferate slowly and form small fruiting bodies. *Ddcln2* was identified in a screen for second-site suppressors of the *aprA*^{OE} phenotype. Cells with mutant *Ddcln2 (aprA*^{OE}*/Ddcln2*^{m}*)* had increased proliferation compared to parental *aprA*^{OE} cells and formed large irregular fruiting bodies. *Ddcln2* encodes a predicted protein (DdTPP I) with sequence similarity to human TPP I. TPP I activity in protein lysates from *aprA*^{OE}*/Ddcln2*^{m} cells was significantly decreased compared to *aprA*^{OE} cells. Additionally, TPP I activity was significantly decreased in protein lysates from *aprA*$^{-}$ and *aprB*$^{-}$ cells compared to wild-type cells. Taken together, these data suggest that DdTPP I is regulated by AprA and AprB. Mutations of *Ddgnt4* and *Dddspp* were also identified as second-site suppressors of the *aprA*^{OE} phenotype. *Ddgnt4* encodes a predicted protein with sequence similarity to human Gnt I, an N-acetylglucosaminyltranferase that initiates the formation of complex N-glyans. The TPP I activity in protein lysates from *Ddgnt4* mutant cells (*aprA*^{OE}*/Ddgnt4*^{m}) was not significantly altered compared to *aprA*^{OE} cells, suggesting that DdGNT4 does not participate in the regulation of TPP I and may act downstream of DdTPP I in this pathway, or function independently of this pathway downstream of AprA. *Dddspp* encodes a predicted protein with similarity to human dentin sialophosphoprotein (DSPP), which is a member of the Small Integrin Binding LIgand N-linked Glycoprotein (SIBLING) family of proteins. Lysates from *Dddspp* mutant cells (*aprA*^{OE}*/Dddspp*^{m}) had decreased TPP I activity compared to *aprA*^{OE} cells, suggesting that DdDSPP is also involved in the regulation of TPP I. These data provide genetic evidence for a pathway that regulates TPP I downstream of AprA, AprB, and DdDSPP.

A probable serine/threonine protein kinase gene, *krsA*, is involved in cell aggregation during both sexual and asexual development of *Dictyostelium discoideum*.

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The two developmental modes in *Dictyostelium discoideum*, asexual and sexual, share molecular mechanisms to some extent, despite of the apparently very dissimilar outcome. For example, cell aggregation is commonly mediated by the chemotaxis to cAMP. To what extent the gene repertoire is shared and how differently the genes are regulated are interesting questions to understand the mechanism of developmental choice in *D. discoideum*. To answer these questions, we are analyzing the involvement of asexual developmental genes in the sexual process, and *vice versa*. In the present study, we focused on the aggregation-stage specific genes in the EST database, and examined if they function in the sexual process. We first selected the cDNA contigs containing the aggregation-stage clones at higher percentages (>= 60%) as well as at least one clone derived from the sexual-phase libraries and expressed at a high level. One of those genes, a probable serin/threonine protein kinase gene, *krsA* (kinase responsive to stress)-A, was examined in detail. After confirmation of the high expression levels at the aggregation stages of sexual and asexual development, we generated knockout mutants in KAX3 and V12, mutually complementary in mating type. The *krsA*-KO mutants formed large streams, which later break up, but developed to normal fruiting bodies on the agar surface. However, they failed to aggregate under submerged conditions. When the *krsA*-KO mutants of KAX3 and V12 were mated, the zygote development was severely disturbed. Namely, the cell aggregation around the zygotes was much less extensive and unable to form spherical aggregates. Furthermore, the outside wall of macrocyst was faint and irregular with frequent jumping out of the inner masses. These results suggest that *krsA* plays important roles in the regulation of cell interactions during sexual and asexual development in *D. discoideum*.

A proteomics approach to identify CbfA-regulated *Dictyostelium* proteins

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The DNA-binding protein CbfA, the C-module-binding factor, is essential for the progression from growth phase to development of *D. discoideum* cells. The most pronounced phenotype of CbfA-depleted mutant cells is a defect in phagocytosis of food bacteria, while starved cells show a strong aggregation defect that can be overcome by mixing mutant cells with wild type cells or application of artificial extracellular cAMP pulses. Expression profiling of starving CbfA mutants with DNA microarrays and Northern blots has suggested that CbfA-deficient cells fail to express the aggregation-specific adenylyl cyclase ACA and most other genes required for early and later development.
We applied 2D gel electrophoresis coupled with MALDI-TOF MS to search for genes that depend on CbfA for proper expression during growth and/or development. Total protein was prepared from AX2 and CbfA-depleted mutant cells at growth phase and after starvation. Differentially expressed proteins were reproducibly observed in conventional silver-stained 2D gels and after labeling of proteins with fluorescent dyes and analysis of 2D gels with the Amersham Ettan DIGE system. From a total of about 2.000 proteins analyzed on the individual gels, we found 27 proteins being differentially expressed in growing CbfA-depleted cells compared to wild type, and 21 proteins with altered expression in CbfA mutant cells starved for two hours. Identification of the differentially expressed proteins by MALDI-TOF MS is underway and results will be discussed in the context of known gene regulatory networks that control multicellular development of *D. discoideum* cells.

Functional evolution of cAMP receptor

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In *Dictyostelium discoideum*, cAMP signaling plays an important role for aggregation and cell differentiation. *Dictyostelium discoideum* has four cAMP receptors (cAR1-cAR4), which show different expression patterns and functions during development. *Polysphondylium pallidum* has two genes, tasA and tasB that are homologous to cARs. They are most similar to each other and then to cAR1. Both are expressed during post-aggregative development and tasA functions in branch formation. This suggests that function of cAMP receptors is evolutionally diversified at Dictyostelids. The molecular phylogeny of the Dictyostelids shows subdivision of all known species into 4 major groups. D. discoideum lies within the most derived Group4 and P. pallidum lies within the Group2. To analyze functional evolution of cAMP receptor, firstly, we isolated cAMP receptor genes by degenerate PCR from at least 2 species each group. From all of selected species, cAMP receptor gene was isolated. *D. mucoroides* and *D. rosarium*, which lie within Group 4 have 4 receptor genes which are similar to cAR1-cAR4. However we isolated only one receptor from *P. violaceum* which is at the base of Group 4. From *D. gleosporum* and *P. pseudocandidum* which lie within group 2, we isolated 2 receptor genes which are tasA and tasB homologs. Another Group 2 species *Acytostelium subglobosum* has 3 highly divergent genes. From two species each in Group1 and Group 3, we isolated only a single receptor gene. All cARs in Group 1, 2 and 3 are most similar to cAR1 than to any of the other D. discoideum cARs. Our data show that the gene duplications that gave rise to cAR2, cAR3 and cAR4 occurred late during Dictyostelid evolutions, and that other duplications of the most ancestral cAMP receptor cAR1 have occurred at least two more times. Now we are trying to disrupt the cAMP receptor of Group 1 species, which would demonstrate the basal most ancestral function of cAMP receptor.

A Rab21/LIM-only/CH-LIM Complex Regulates Phagocytosis *via* both Activating and Inhibitory Mechanisms

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We have identified two LIM domain proteins, LimF and ChLim, from *Dictyostelium* that interact with each other and with the small, Rab5-related, Rab21 GTPase to collectively regulate phagocytosis. To investigate *in vivo* functions, we generated cell lines that lack or overexpress LimF and ChLim and strains that express activating or inhibiting variants of Rab21. Overexpression of *LimF*, loss of *ChLim*, or expression of constitutively active Rab21 increases the rate of phagocytosis above that of wild-type. Conversely, loss of *LimF*, overexpression of *ChLim*, or expression of a dominant-negative Rab21 inhibits phagocytosis. Our studies using cells carrying multiple mutations in these genes further indicate that ChLim antagonizes the activating function of Rab21-GTP during phagocytosis; in turn, LimF is required for Rab21-GTP function. Finally, we demonstrate that ChLim and LimF localize to the phagocytic cup and phago-lysosomal vesicles. We suggest that LimF, ChLim, and activated Rab21-GTP participate as a novel signaling complex that regulates phagocytic activity.

Studying the non-vacuolar cell death in *Dictyostelium*

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We use *Dictyostelium*, and more precisely its HMX44A strain (Kay, 1987), to study programmed cell death. Through genetic approaches we wish to investigate the molecular mechanisms at play. While wild type cells showed DIF-induced vacuolar cell death (Cornillon et al., 1994, Levraud et al., 2003) inactivation of the *atg1* autophagy gene suppressed vacuolization but not cell death proper (Kosta et al., 2004). These results are consistent with a non-vacuolar type of cell death, present in both *atg1-* cells and in wild type cells, to which vacuolization (or a vacuolar cell death mechanism) is added in the latter. This non-vacuolar cell death might reflect an ancestor mechanism, perhaps related to extant necrosis (Golstein and Kroemer, 2005).
Atg1- Dictyostelium cells not only show this "necrotic" cell death mechanism, but also have additional experimental advantages (such as, upon induction of cell death, absence of aggregation and of vacuolization) compared to the initial HMX44A cells. To proceed we study cell death in parallel in both wild type and *atg1* mutant cells.
Taking *atg1-* cells as a starting material for further genetic manipulations raises a difficulty linked to the limited availability of selection markers in *Dictyostelium*. Thus, it has been difficult to create multiple mutations within an individual cell. This was recently achieved in *Dictyostelium* (Faix et al., 2004) using the CreLox system. The construction of such *agt1-* CreLox cells is in progress and it will be invaluable for both targeted mutagenesis and random mutagenesis experiments.
Furthermore, to shed light on this project from a different angle, we are using various electron microscopy techniques to characterize the ultrastructural morphology of *atg1-* and other mutants. We are also developing flow cytometry applications on *atg1-* cells. The different morphological characteristics of live and dead cells enable us to set up a simple and precise way to quantify cell death that is particularly useful to complement microscopic observations of the cells. The analysis of different probes in cytometry may provide some key markers to understand the cascade of events associated to non-vacuolar cell death.

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Identification by random insertional mutagenesis of genes involved in caspase-independent cell death mechanisms in *Dictyostelium*

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Apoptotic caspase-dependent cell death, the first mechanism of cell death described in detail, is still intensively studied. However, also caspase-independent cell death has been observed in many species including mammals. Nevertheless, the corresponding molecular mechanisms are largely unknown. Our present work makes use of a model organism, the protist *Dictyostelium discoideum*, which shows caspase-independent cell death during development. In starvation conditions, *Dictyostelium* cells aggregate, differentiate and morphogenize into a multicellular structure, called sorocarp, containing a mass of spores supported by a stalk. Cells in the stalk are considered dead on the basis of non-regrowth in rich medium and are vacuolized. This programmed cell death is therefore developmental and vacuolar, and in addition, caspase-independent since the *Dictyostelium* genome does not contain *caspase* genes.

To determine molecular mechanisms involved in this cell death, we are using a genetic approach based on random mutagenesis and developmental screening. More precisely, we generate mutants using Restriction Enzyme Mediated Integration. Mutants are first selected on developmental criteria. This screening is based on the hypothesis that cell death mutants shouldn't be able to give normal stalks. Moreover, we exclude mutants that are not able to undergo even early development, thus eliminating early signalization mutants. Subsequently, cells from mutants with abnormal development of stalks are induced to die in a monolayer test, in order to determine which of these mutants are resistant to death. This genetic approach has allowed us to isolate several cell death mutants, the study of which is in progress and should contribute to a better understanding of the molecular mechanisms leading to caspase-independent cell death in other systems.

A *Dictyostelium* homologue of the cbl oncogene encodes a regulator of STAT signalling

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The Cbl proteins are molecular adaptors that target proteins for ubiquitination and degradation. They contain a phosphotyrosine-binding motif, comprised of an EF hand and a variant SH2 domain, linked to a RING finger E3 ubiquitin-ligase domain. Cbl proteins have thus far been described only in animals but we show that *Dictyostelium* encodes a protein, Dd-CBLA, that contains the conserved sequence features of metazoan Cbl proteins. In a cblA null strain early development is accelerated by several hours, the slugs tend to bifurcate and the fruiting bodies often collapse. Because of the role of Cbl family members in regulating metazoan tyrosine kinase signalling pathways, we analysed the activation of two STAT proteins in the cblA null strain. Activation of Dd-STATa, in response to extracellular cAMP, occurs normally in the mutant but DIF-1 induced activation of Dd-STATc is three-fold decreased. These results suggest a role for Dd-CBLA in a DIF-1 regulated phosphotyrosine signalling pathway.

Acidic clusters are required for targeting a transmembrane protein to the contractile vacuole in *Dictyostelium* cells

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The mechanisms responsible for the targeting of transmembrane integral proteins to the contractile vacuole (CV) network are unknown. Here we show that the transfer of the cytoplasmic domain of a CV resident protein (Rh50) to a reporter transmembrane protein (CsA) was sufficient to address the chimera (CsA-Rh50) to the CV. We identified two clusters of acidic residues responsible for this targeting, and these motifs interacted with the γ-adaptin AP-1 subunit in a yeast protein-protein interaction assay. In order to identify the intracellular site where sorting to the CV took place, we followed the fate of the small fraction of CsA-Rh50 that reached the cell surface. Surface CsA-Rh50 was constitutively transported to the CV, but this transport occurred slowly, and via at least one intracellular endosomal compartment. Our results suggest strongly that the AP-1 clathrin adaptor complex plays a crucial role in sorting of membrane proteins to the contractile vacuole in *Dictyostelium discoideum*.

Hunting and Gathering behavior in *Dictyostelium discoideum*

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This study mainly focuses on modeling single cell motility in Dictyostelium amoeba on an agar surface in the absence of chemoattractant. Cells are observed retracing their paths from time to time. Statistical analysis shows that a power-law relationship between mean-squared amoeboid displacement and time characterizes their behavior. Further analysis indicates that cell trajectories consist of <Runs> and <Turns>. Unlike a Levy walk*, the lengths and times of runs are chosen at random from exponential distributions. More strikingly, amoebae are able to memorize their behavior from the last turn, and have a strong turning preference away from the last turn. This tendency is much stronger for short runs than for long runs. Calculation of autocorrelation functions for sequences of turns also indicates that memory does not persist beyond the nth+1 turn. Furthermore, longer runs have a tendency to be followed by relatively smaller turns. Velocity distributions, velocity autocorrelation functions and dependence of acceleration on velocity are investigated and shown to reject most characteristic properties of the Ornstein-Uhlenbeck process**.

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Transcriptional regulation of *dia1* and *impA* during the growth/differentiation transition

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impA and *dia1* are adjacent genes on chromosome 4 that are transcribed in opposite directions. They are separated by 654 bp of intergenic DNA. In contrast to many divergently transcribed genes that are co-expressed, *impA* is expressed only in growing cells while *dia1* is expressed only after the initiation of differentiation. Constructs were prepared with the intergenic region flanked by GFP on the *impA* side and DsRed on *dia1* side. Deletion of 112 bp proximal to the transcriptional start site of *impA* resulted in complete lack of expression of both reporter genes during growth or differentiation. Constructs carrying only the 112bp region flanked by the TATA boxes for the reporter genes resulted in expression in both directions. There appear to be *cis*-acting sites in this region that are necessary and sufficient for both leftward and rightward transcription. Two sequences [GAATTTTTTGAATTTT and its inverse] related to similar sequences in *carA*, and *pdsA* occur in the 112 bp region. These genes are co-regulated at the growth-to-differentiation transition.

At the other end of the *impA/dia1* intergenic region there are two copies of a motif that is also found in the *carA* regulatory region. Removing one copy of this repeat reduced expression in the *impA* direction 2-fold. Removing the second copy had no further consequences.

Removing the central portion of the intergenic region resulted in high levels of expression of *dia1* in growing cells indicating that this region contains a sequence involved in repression during the vegetative stage. Gel-shift experiments showed that a 80 kDa nuclear protein present in growing cells recognizes the sequence GAAGTTCTAATTGATTGAAG found in this region. This DNA binding activity is lost within 4 hours of the initiation of differentiation. Different nuclear proteins were found to recognize the repeated sequence proximal to *dia1*. One of these became prevalent after 4 hours of development. Together these regulatory components at least partially account for this aspect of the growth-to-differentiation transition.

Differential targeting affinities of cytoskeletal proteins

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Proteins that are tanslated on soluble ribosomes and become correctly folded in the cytoplasm may be transported into the nucleus, peroxisomes or remain in the cytoplasm. The cytoplasm itself is differentiated into many distinct targeting areas such as the inner leaflets of the plasma membrane, of endosomes or other organelles. The filaments of the actin cytoskeleton and microtubules provide additional targets. Fusions of cytoskeletal proteins to the green fluorescent protein or a myc-tag generally sort to their correct destination within the cytoplasm. If, however, an inert tag is provided with two different sorting signals, the distribution of the protein is governed by the relative affinities of the single components to their respective targets. We have observed previously, that a hybrid protein consisting of vacuolin (a protein associating with the membrane of the late endosome), a myc-tag (for specific detection), and cofilin (an actin depolymerising protein that is part of the cell cortex) sorts to the endosome until the capacity of binding sites is exceeded and then integrates into the cortical cytoskeleton (Drengk et al., 2003). Using the same strategy, we have presently analysed the distributions of five other cytoskeletal proteins, namely abp30, aip1, coactosin, coronin, and severin, to identify their relative affinities to their (unknown) binding sites in the cortex. Using the appropriate constructs, we are able to build a hierarchy of their affinities rooted on the peroxisomal sorting signal, which is the weakest of all tested.

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The gamete enriched *racF2* gene affects growth, sexual cell fusion, and asexual development in *Dictyostelium discoideum.*

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Dictyostelium discoideum undergoes three different reproductive strategies depending on environmental conditions; solitary growth by cell division, spore formation triggered by starvation, and sexual process of macrocyst formation under dark and submerged conditions. How the cells can beautifully respond to the environmental changes using the limited genetic resources is an intriguing question, and the genome-wide functional analysis of the genes is a hope to answer it. We have been analyzing the genetic system controlling the sexual process, and recently constructed a gamete-enriched subtraction library, FC-IC, to identify the genes responsible for the gamete maturation and fusion. Among the genes in the FC-IC library, 24 were confirmed to be expressed at higher (>5) levels in the fusion-competent cells or gametes compared to the fusion-incompetent cells. In the present study, we analyzed the *racF2* gene which is the second highest one of these 24 enriched genes. The Rac proteins are known to be involved in most of the actin-regulated processes such as membrane trafficking, motility and adhesion. They are also involved in the regulation of cellular processes as diverse as cytokinesis, gene expression, cell cycle progression and apoptosis. In *D. discoideum*, there are 15 members of Rac proteins, among which only *racF2*, *racF1* and *rac1b* are found in the FC-IC library. We successfully generated the *racF2* knockout mutants in V12 and overexpressors in KAX3 and V12, and analyzed their phenotypes. The *racF2* knockout mutants produced larger cell fusion products compared to the wild type cells. The overexpressors formed multinucleate cells and poorly grew in the suspension culture. They also showed a delay of asexual development. These results suggest that RacF2 is relevant for normal growth and sexual and asexual development in common. Thus, the reason for the higher level of *racF2* expression in the gamete remains elusive, and this gene may have yet unknown functions.

Characterization of *Dictyostelium discoideum* RacA, a member of the RhoBTB subfamily of RhoGTPases

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RacA belongs to the recently identified subfamily of Rho BTB proteins. Members of this subfamily are present in mammals, *Drosophila* and *Dictyostelium*. Rho BTB proteins are characterized by a modular organization, consisting of a GTPase domain, a proline rich region, a tandem of two BTB domains and a C-terminal region of unknown function. Recent publications postulate that BTB domain-containing proteins play a role in the ubiquitinylation pathway. Through binding to Cullin 3, a component of the ubiquitin E3 ligase, they confer substrate specificity to this complex.
To gain insight into the function of RhoBTB proteins we are characterizing the *Dictyostelium* representative RacA. To this end we have generated a knock out strain by inserting a blasticidin resistance cassette into the racA gene. More than 700 clones were examined in a negative PCR-screening. Potential knock out clones have been further analysed by Southern blotting using three different probes. Those clones still synthezised a racA mRNA-transcript as we could show by RT-PCR. Finally the knock out has been confirmed at the protein level using an affinity-purified polyclonal antiserum, which has been generated against a recombinant protein encompassing the C-terminal region of RacA. The knock out strain has been used for studying its behaviour in growth, development, chemotaxis, endo- and exocytosis and cytoskeletal structure. Additionally a « rescue » strain has been generated and examined accordingly.
In order to identify RacA binding partners affinity purification with a GFP-RacA fusion protein by means of a dual purification system, the Tandem Affinity Purification, is underway. As binding of RacA to several PAK proteins as well as WASP has been revealed by yeast-two-hybrid studies, this will next be confirmed by co-immunoprecipitation studies.

FbiA is a developmentally-regulated protein that regulates cell-type proportioning in *Dictyostelium discoideum*

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fbiA encodes a protein with a predicted molecular weight of 102 kDa and homology to proteins in humans, mice, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. pombe*, *S. cerevisiae*, *N. crassa*, and *P. falciparum*. The function of these FbiA homologues is unknown, although all include a region proposed as a consensus sequence for SAM-dependent methyltransferases. *fbiA* shows a complex, developmentally-regulated expression pattern: it is expressed at moderate levels in vegetative cells, drops during early development, rises again as mounds form, and then shows a dramatic but transient increase at the Mexican Hat/early pre-culminant stage. *in situ* analysis reveals that expression at the 15-16 hr peak is prespore-specific; expression at earlier stages is too weak to reliably detect any cell-type specificity. An expression gradient visible in structures at the start and end of the peak period suggests that the signal(s) that initiate and terminate this late expression peak may originate in the prestalk zone. Culminants derived from *fbiA*$^-$ null mutants are unusually tall, with longer stalks and smaller sporeheads than wild-type culminants. Furthermore, transformation with cell-type specific *lacZ* reporter constructs reveals that the *fbiA*$^-$ null mutant produces structures with an unusually high prestalk:prespore ratio. As judged by induction of *ecmA* expression in monolayer assays, the increased prestalk:prespore ratio of *fbiA*$^-$ null mutants is not due to increased sensitivity to DIF. FbiA may, however, influence DIF production, which would be consistent with its expression in prespore cells. FbiA was identified via yeast two-hybrid analysis in a search for proteins capable of interacting with the WD-40 repeat region of the F-box/WD-40 repeat-containing protein FbxA. Based on analogy to FbxA homologues in other systems, this FbiA-FbxA interaction suggests that FbiA may be targeted for FbxA-dependent, ubiquitin-mediated degradation.

A new essential gene involved in prestalk A differentiation: *padA*.

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We have isolated a REMI mutant showing delayed development and sparse and small fruiting bodies. Identification of the disrupted gene revealed a nucleotide sequence coding for a protein of unknown function and with no obvious homologues. Further studies showed that the gene (now named *padA*) is essential for growth and that the recapitulated *padA* mutant is thermo-sensitive for development. At 27ºC, it takes 24 hrs for the mutant to reach the mound stage and another 24hrs to make slugs. These then attempt to rise but appear short, thin and rough, and are unable to differentiate any further. Expression of the prestalk marker *ecmA* is abolished at 27ºC and significantly diminished at 22ºC. Other prestalk (*ecmO*, *ecmB*) and prespore (*pspA*) markers tested remained unchanged at both temperatures. When *padA* cells were co-developed with AX2 cells at the restrictive temperature, they were excluded from the tip of the slugs (pstA region) and were replaced by wild-type cells. Interestingly, this sorting disappeared at later stages of development. A similar “cheating” behavior has been described for mutant *dimA*, also involved in prestalk differentiation (Foster *et al*., 2004).
Mature stalk cells are a heterogeneous population in the *Dictyostelium* fruiting body, performing different functions (Sternfeld, 1998) and consequently there is a great diversity of prestalk cell types (Maeda *et al.,* 2003) probably arising through specific regulatory pathways. We believe we have identified a new gene involved in prestalk A cell differentiation that can be classified as a cheater in chimeras with wild-type cells. Having essential genes also performing crucial functions in prestalk differentiation could be a very powerful mechanism for the control of cheating in nature. The consequences of this suggestion are discussed.

Role of metabotropic GABAB receptor like proteins in *Dictyostelium discoideum*

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GPCRs are a superfamily of diverse integral membrane proteins that allow detection and transduction of a large variety of extracellular signals. In *Dictyostelium* only seven members of one family, the crl (cAMP receptor like) family, had been identified and studied in detail. The analysis of the genome sequence uncovered 48 additional putative GPCRs of which 45 can be grouped into the secretin (family 2), metabotropic glutamate/GABA B (family 3) and the frizzled / smoothened (family 5) families of receptors. The presence of family 2, 3 and 5 receptors in *Dictyostelium* was surprising because they had been thought to be animal-specific. GABA (gamma amino butyric acid), the principal inhibitory neurotransmitter in mammalian brain, signals through ionotropic (GABA(A)/GABA(C)) and metabotropic GABA(B) receptor systems. The functional GABAB receptor is a heterodimer of receptor 1 and receptor 2 subtypes. The *Dictyostelium* genome harbours 17 different genes encoding GABAB receptor like proteins. Of these, 15 were slightly more closely related to the subtype 2 and two appear to be closer to the subtype1. The analysis of one of each type of receptors named as GrlA and GrlJ is undertaken during this study. Both the receptors are expressed throughout the development of *Dictyostelium*. To gain more insight into the in vivo function of these proteins, we generated knockout mutants by homologous recombination, the analysis of which will be presented.

A Diaphanous-related formin is required for slug motility and normal development

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Formins are large, multi-domain proteins involved in the nucleation of linear actin filaments. These proteins were shown to be important for cytokinesis, cell polarity, microtubule dynamics and recently also for filopodia formation (1). Here we present evidence for the role of a Diaphanous-related formin (DRF) in cell motility during the development of *D. discoideum*. Biochemical analysis of a recombinant FH1FH2 fragment showed spontaneous nucleation and assembly of actin filaments in a dose-dependent manner in vitro. Furthermore, yeast-two-hybrid analysis indicated that the proline-rich FH1 domain interacts specifically with the profilin I isoform. The characterization of the null mutant revealed multiple defects in early and late development. We observed broken streams during early aggregation and severe inhibition of slug motility during phototaxis. The significant delay in development also led to the formation of small fruiting bodies. These defects could be rescued upon ectopic expression of the formin under the control of an actin-15 promoter. Taken together our findings indicate that this DRF is specifically involved in the regulation of the actin cytoskeleton during early and late development.

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Development of a cyclic nucleotide phosphodiesterase assay specific for the *Dictyostelium* developmental pacemaker RegA.

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The cyclic AMP phosphodiesterase RegA is a critical developmental pacemaker in *Dictyostelium*. RegA is a substrate for ubiquitination and proteolysis mediated by an SCF E3 ubiquitin ligase containing the specificity determinant FbxA. Mutation of the *regA* gene suppresses the severe sporulation deficiency of an *fbxA* null mutant [1], while levels of RegA polypeptide are greatly elevated in the *fbxA* single mutant [1, 2]. RegA has also been shown to form a multi-protein complex with FbxA [2]. Nevertheless, bulk cyclic AMP levels are not altered in *regA* or *fbxA* mutants as one might expect, but are altered by mutation of the histidine kinase gene *dhkA*, the other locus known to suppress *fbxA* mutation [1]. To elucidate the interaction between the ubiquitination, PKA and histidine kinase pathways, we intend to measure not just RegA polypeptide levels but also its enzymatic activity in a variety of genetic backgrounds.

Measurement of RegA activity is complicated by the existence in amoebae of five other cyclic nucleotide phosphodiesterases [3]. We have altered the customary assay conditions for RegA [4] by lowering the concentration of cAMP while adding cGMP as competitor. Use of the class I phosphodiesterase inhibitor IBMX and of a RegA specific antiserum allowed us to validate the specificity of our assay. Measurements of RegA activity in various developmental mutants are in progress.

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Evolutionary conservation of mechanisms controlling encystment and sporulation in solitary and social amoeba.

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D. discoideum sporulation and spore dormancy are controlled by activation of PKA. During sporulation the adenylyl cyclase, ACB, and the cAMP phosphodiesterase, RegA, play crucial roles in controlling cAMP levels. Maintenance of spore dormancy is controlled by the adenylyl cyclase, ACG, and RegA. ACG operates as an osmosensor and directly produces cAMP in response to osmotic pressure, keeping spores dormant in the spore head. High osmolarity also inhibits RegA by activating the osmosensing histadine phosphate, DokA. We aim to investigate whether these mechanisms are conserved throughout the social and solitary amoeba. We have found orthologues of ACG and RegA throughout the Dictyostelid phylogeny. High osmolarity was found to induce microcyst formation and inhibit germination in the social amoeba, *P. pallidum* and had the same effect on encystation and excystation of the solitary amoeba, *Acanthamoeba castellanii*. cAMP production was found during encystation of *A. castellanii* and biochemical evidence of the enzymes involved in cAMP production and degradation have been investigated.

Dictyostelium RacH is involved in endocytosis

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The small GTPases of the Rho family act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. In its active state Rho GTPases interact with a multitude of effectors that relay upstream signals to downstream targets. Although initially described as major regulators of cytoskeletal remodeling, Rho GTPases have been shown to be also involved in the regulation of cellular processes as diverse as endocytosis, vesicle trafficking, morphogenesis, cytokinesis, transcriptional activation and cell cycle progression. *Dictyostelium discoideum* is an attractive model to investigate structural and regulatory aspects of the actin cytoskeleton. Completion of the genome sequencing of *Dictyostelium* has revealed that in addition to 18 Rho GTPases, other components of Rho-regulated signalling pathways are also present in this organism, like RhoGDI, the Arp2/3 complex, PI3 kinases, PAK, Scar, WASP, formins, ELMO/DOCK complexes and numerous RhoGEFs and RhoGAPs.

We present our last data on the role of RacH, which is constitutively expressed throughout the complete developmental cycle of *Dictyostelium*. Initial studies performed with strains that overexpress the wild-type, constitutively active or dominant negative protein suggested that RacH is primarily involved in regulation of endocytosis, and has no impact on cell motility or chemotaxis. In support of this, RacH was found to be targeted to intracellular membranes of the nuclear envelope, ER and Golgi apparatus. We generated a RacH knockout strain using homologous recombination. Cells deficient in RacH have a decreased rate of fluid phase endocytosis and exocytosis, but no other apparent defects, confirming our previous observations. We have further addressed the requirements for subcellular localization of RacH by means of chimeric constructs, alanin exchange mutants and mutants that lack the insert region characteristic of Rho GTPases. Our results indicate that both the insert region and a region immediately upstream of the variable C-terminal region of RacH are required for localization at ER and Golgi membranes.

Looking for social genes in the social mold *Dictyostelium discoideum*

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During the unique life cycle of the social amoeba, *Dictyostelium discoideum*, cells propagate as free unicellular organisms, but under starving condition they undergo a process of aggregation which leads to the formation of a multicellular organism. The cells differentiate into spores that will give rise to the next generation, and into stalk cells, which are dead. The stalk cells are sacrificed to help the spores disperse and survive, much like somatic cells help to spread the germline of a metazoan multicellular organism. When the cells that form a *Dictyostelium* aggregate are not uniclonal, a conflict may arise in deciding which cells will become spores and survive and which will become stalk and die. In the case of a mix between two or more genetically different clones, the one that differentiates more spores is called “cheater” and the other “victim”. We hypothesize that some genetic mechanism(s) can regulate and modulate this social behavior. To test this hypothesis, we generated a large pool of mutants by REMI and selected for cheaters. To simulate evolutionary selection the mutant population was subjected to several rounds of development in a mixed population: after each round spores were collected, germinated and grown again. The process was repeated 20 times. At the end of 10 and 20 cycles, we were able to isolate several strong cheaters that were not morphologically different from the wild type. Through Real Time PCR analysis we have assessed that these cheaters became over-represented in the evolving population. In all cases, the mutants make more than their fare share of spores in chimera with wild type cells but appear to develop normally in pure populations. We are cloning the mutated genes in order to decipher the mechanisms that regulate cheating. Our analysis suggests that it is likely that cells can cheat by more than one mechanism.

Functions of a novel gene, *srsA*, in the starvation response and later development of *Dictyostelium* cells

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Growth and differentiation are fundamental characteristic of the cell and generally exclusive each other. In *Dictyostelium discoideum* Ax-2 cells, a specific checkpoint for the growth/differentiation transition (referred to as a growth/differentiation transition point; GDT-point) has been precisely identified in the mid-late G2 phase of the cell cycle. We have ever proposed that integration of two signaling pathways, starvation-specific and GDT point-specific one, is required for initiation of the differentiation program. Here we report functions of a novel gene, *srsA* (starvation-responsive small gene A), which is rapidly and transiently expressed in response to starvation. The *srsA* gene encodes a very small protein (SRSA) with a predicted molecular mass of 6.4 kDa. Starving *srsA*-null cells exhibited considerably delayed differentiation, coupling with delayed expressions of the developmentally regulated genes such as adenylyl cyclase (*acaA*) and cAMP receptor 1 (*carA*). The overexpression of *srsA* mRNA also gave an inhibitory effect on early differentiation, indicating that a proper level of *srsA* expression is required for normal differentiation. Interestingly, *srsA*-overexpressing cells exhibited precocious stalk differentiation during slug migration, just like *D. mucoroides* that migrates forming stalk cells at the slug tip, thus resulting in formation of abnormal fruiting bodies with a very long stalk and only a few number of mature spores. Since cAMP-dependent protein kinase (PKA) is known to be a key factor involved in starvation response and also in late differentiation into stalk or spore cells, the functional relationship between PKA activity and *srsA* expression is now under investigation.

Isolations of Dd-STATa multicopy suppressors via genetic screening

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Dd-STATa, a *Dictyostelium* homologue of the metazoan STAT (signal transducers and activators of transcription) proteins, is necessary in the slug for correct entry into culmination. Expression of a GFP fusion protein containing an N-terminal truncated form of Dd-STATa (aa 237-707) in the *Dd-STATa*-null mutant, a strain we call *GFP::STATa(core)*, partially rescues both the morphological defects of the *Dd-STATa*-null mutant and the expression of various potential Dd-STATa target genes that we previously identified (1). Using this "partially active" Dd-STATa strain as a parent strain, we have screened approximately 400,000 clones from a slug-stage cDNA library for their ability to partially rescue the culmination defect when overexpressed. Of the 14 putative suppressors so far identified in this manner, five have been recapitulated and confirmed to be multicopy suppressors of Dd-STATa. This is the first example of direct isolation of STAT suppressors. We will present our initial analysis of suppressor clones.

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Matrix Components Have Multiple Regulatory Roles in *Dictyostelium discoideum* Spore Dormancy

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We have begun to examine the characteristics of the viscous *Dictyostelium discoideum* sorus to evaluate its possible functions. It is recognised that the sorocarp of *D. discoideum* is constructed to aid the dispersal of spores by animals, rain, or dew formation (Kessin, 2001). The sorus is viscous and can stick to grazing animals. In addition, animals such as nematodes may ingest the sorus, and the spores themselves can pass through the digestive tract with full viability (Kessin, 2001).

We, and others, have found that components of the sorus may have additional roles:

1. The inter-spore material (the matrix) contains encapsulation factors that initiate sporulation.

2. The high osmotic pressure exerted by ammonium phosphate and other matrix molecules initially maintains dormancy by activating Adenylyl Cyclase G (ACG) and inhibiting the phosphodiesterase (Reg A) (see Cotter et al., 2000).

3. The same molecules, which initially exert an osmotic pressure on dormant spores, are also hygroscopic. Over several days, this characteristic results in an expansion of the sorus when the relative humidity is high at the soil or agar surface. The ammonium phosphate is largely responsible for the hygroscopic activity of the matrix, and the resulting expansion of the sorus. This was determined by separating matrix samples into fractions of greater than 5000 Daltons molecular weight and less than 5000 Daltons molecular weight, via Amicon Ultra-4 centrifugal filters. Then, a greater resolution was achieved by separating matrix samples chromatographically with 8 mL columns of Biorad P2 polyacrylamide beads (with a maximum pore size of 1600 Daltons); also, with columns of Sephadex G15. In all cases, the low-molecular weight material accounted for some 95% of the hygroscopicity of the matrix.

4. The matrix material, when diluted in distilled water, promotes growth of bacteria such as *Escherichia coli*, and fungi such as *Penicillium* spp. and *Saccharomyces cerevisiae*. This was shown with both liquid and solid nutrient-free media.

5. Diluted matrix serves as an excellent germination buffer at or about soral pH (i.e.: pH 6.2).

6. Furthermore, do the volatile aromatic components of the sorus attract agents of dispersal ?

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Characterisation of ATP Mediated Ca^{2+} Uptake in *Dictyostelium* Amoebae

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Extracellular ATP mediates numerous cellular functions via Ca^{2+} entry through cell surface ionotropic P2X receptors. In *Dictyostelium* it accelerates cellular aggregation and can alter aggregation territory size. The genome of this organism appears to harbour five P2X receptor homologues that we have named pxlA-E. We have characterised the ATP stimulated Ca^{2+} uptake in amoebae which is rapid (half maximal by 15 seconds) and requires only micromolar ATP concentrations for activation (half maximal is 1µM). The uptake is developmentally regulated and is both independent of, and additive with the Ca^{2+} entry pathway mediated by the *iplA* gene product. UTP and GTP both failed to stimulate Ca^{2+} uptake whereas adenosine invoked only a relatively small uptake that was additive with that mediated by ATP. The inhibitors PPADS and suramin did not block the ATP dependent Ca^{2+} uptake. Disruption of the pxlA gene had no apparent effect on growth, development or ATP mediated Ca^{2+} uptake.

Common and distinct functions of two talins in *Dictyostelium discoideum*

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Talin is a large cytoskeletal protein which is important not only for assembling and stabilizing focal adhesions but also for regulating integrin and other signaling molecules. The cellular slime mold *Dictyostelium discoideum* has two talin homologues, talA and talB. TalA null cells show weaker adhesion to the substrate and high proportion of multinucleated cells in the vegetative stage (Niewohner et al., 1997). In contrast, weaker exertion of motive force against substrate in talB null cells leads to the developmental arrest at the tight mound stage (Tsujioka et al., 1999; 2004). In this presentation, we created and analyzed a double mutant disrupting both talA and talB genes to investigate their functional redundancy and distinction. The talA/talB double mutant cells did not attach to the substrate at all in the vegetative stage. They also exhibited much severer motile defect during the development than the talB single mutant and the earlier developmental arrest at the loose mound stage, indicating that the two talins have common functions and compensate for the loss of each other in these respects in the single mutants. Meanwhile, no further alterations were demonstrated in the talA/talB double mutant in cyotokinesis compared with the talA null mutant. Moreover, overexpression of talB gene in talA null cells failed to rescue the cytokinetic defect, suggesting that talin A has specific roles in cyotokinesis. Detailed observation of cytokinesis revealed that talA null cells failed to cleave the thin bridge connecting the daughter cells after equatorial furrowing is complete, which implies that talin A is important for scission, the very final step of cytokinesis.

Genome wide analysis of the antitumor drug cisplatin resistance mechanisms

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Chemotherapy is frequently used to treat cancer. However, the efficacy of the treatment is often limited because some tumors are intrinsically resistant or may become resistant to drugs during the course of therapy. One example is cisplatin, a chemotherapeutic drug widely used to treat several malignancies, including lung cancer, head and neck cancer, and ovarian cancer. To fully exploit the potential of this drug in cancer therapy, it is important to understand why some cancer cells are resistant or become resistant to cisplatin. We plan to identify relevant regulatory networks using genomic approaches in *Dictyostelium discoideum*. We are in the process of comparing changes in global gene expression levels in wild type cells before and after treatment with cisplatin. Using statistical analysis of variation (ANOVA) we identified groups of genes that are reproducibly altered after drug treatment. Next, we will examine the annotation of the altered groups of genes, pick corresponding mutant strains from the Baylor College of Medicine knock-out collection and treat the mutants with cisplatin. We predict that mutations in these genes would alter the cells' sensitivity to cisplatin. In conclusion, our analyses are aimed at identifying networks involved in cisplatin resistance and will provide potential targets for modulating the response of cells to the anticancer drug cisplatin.

Development of *D. discoideum* after artificial stimulation with pulses of 3´,5´-cyclic adenosine monophosphate

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Stimulation of shaken suspensions of starving *Dictyostelium* cells by periodic addition of cAMP solution is a common technique used for preparation of aggregation competent cells utilized to study chemotactic response of the cells to extracellular cAMP gradients. The stimulation mimics effects of natural periodic passage of cAMP waves on transduction pathways and cell behaviour. Our work investigates the course of aggregation and other developmental stages of cells treated with this technique. The stimulation was carried out for 4 hours during which the drops of cAMP solution (10 μl of 100 nM cAMP) were periodically added every 6 min.

We found that the course of the aggregation stage in cells treated with pulses of cAMP is different when compared to the course of the aggregation stage in natural cells. We have found that the treated cells did not support propagation of cAMP waves and did not form streams though they did not lose the ability to chemotax. Individual cells can gather in tight aggregates, form mounds, and complete the development creating fruiting bodies. However, the completion of developmental process takes at least twice as much time as the development of natural cells.

A substantial portion of the delay was found to occur in the mound stage. The first mounds are formed already 10.5 hours after the beginning of starvation, i.e. even earlier than in natural cells, however, *D. discoideum* stays locked in the mound stage for another 14 hours. A significant amount of mounds is still present in the population 48 hours after the beginning of starvation.

Our observations suggest that the artificial stimulation of cells by cAMP pulses has a modifying effect on the course of transduction pathways and expression of genes governing the progress of the developmental cycle. The effects will be further studied by changing systematically parameters of the pulsation procedure.

Soluble guanylyl cyclase mediates both global inhibition and local stimulation of pseudopod extension during chemotaxis

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In a chemotactic gradient soluble guanylyl cyclase is localized at the leading edge of *Dictyostelium* cells where it is activated. It is known that the produced cGMP mediates myosin filament formation in the back of the cell.Two mutants were made: Mutant A has a deletion of the N-terminal segment and does not localize to the leading edge, but is still activated by chemoattractants. The mutant shows normal myosin filament formation in the back of the cell and suppression of lateral pseudopodia, but has strongly reduced chemotactic orientation. Mutant B has a point mutation in the catalytic domain; the mutant protein translocates properly to the leading edge. Although the mutant protein does not produce cGMP, it contributes significantly to chemotaxis compared to a guanylyl cyclase-null cell. These results demonstrate that localization of soluble guanylyl cyclase at the leading edge has two functions during chemotaxis: First, formation of cGMP that rapidly diffuses and leads to myosin filament formation in the back of the cell where it suppresses the formation of lateral pseudopodia. Secondly, local stimulation of pseudopodia in the direction of the gradient, independent of enzyme activity. We suggest that the C-terminal AAA-ATPase may mediate this second effect by interaction with other proteins.

Study of genes regulated by the transcription factor SrfA in *Dictyostelium discoideum*

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The transcription factor SRF (Serum Response Factor) plays a central role in proliferation and differentiation in a wide range of organisms (1). *Dictyostelium discoideum* SrfA, homologous to mammal SRF, is essential for slug migration and spore formation (2) (3). We have isolated genes whose expression is dependent on SrfA at slug and culmination stages using Microarrays and differential hybridation (4) (5). sig1 (SrfA Induced Gene 1) showed reduced mRNA expression levels in srfA- at the slug stage of development. This gene codes for a small protein of 89 aa. There are 12 very similar genes divided in two groups of 6 genes each in chromosome 2. For every group, the proteins have an identity above 85%. The similarity between the proteins of the two groups is about 60%. The expression of these genes has been studied by RT-PCR using specific primers and both groups showed an induction between 10 and 12 hours of development. Deletion of the six genes of every group didnít show any obvious phenotype. Double KO of the two groups or over-expression of one of the genes didnít show any phenotype either. A homologous to the human Synaptobrevin had been previously shown to be induced at culmination in a SrfA-dependent way (5). Strains that over-express Synaptobrevin (sybA) showed reduced growth rate in HL-5 (generation time about 14-16 hours) and lower phagocytosis. Strains expressing the GFP-SybA fusion protein showed localization of the protein in vesicles at the periphery of the cell. Specific markers of lysosomes, Golgi or macropinocytic vesicles didn't co-localized with GFP-Syb at any stage of the vesicular traffic from macropinocytosis to exocytosis. KO and RNAi strains are being generated to determinate the role of SybA in vesicular traffic or exocytosis, during vegetative growth and at culmination.

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cGMP controls developmental timing in *Dictyostelium*

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The cGMP-stimulated cGMP phosphodiesterase PdeD hydrolyses cGMP, which is implicated in the chemotactic response. We show here that pdeD loss-of-function mutants precociously expressed aggregative genes, which resulted in early onset of chemotactic signalling and development. This phenotype was accompanies by reduced growth of pdeD- colonies on bacterial lawns, but not in shaken suspension. Conversely, PdeD gain-of-function mutants grew faster on bacterial lawns, but showed a delay in aggregative gene expression. These data suggest that cGMP may play a role in the regulation of gene expression at the growth to development transition.

RNAi-mediated silencing of the putative transcription factor TacA affects growth and fruiting body formation in *Dictyostelium discoideum*

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Searching for *Dictyostelium* homologs of the calcineurin regulated NF-AT transcription factor family identified a putative transcription factor (tacA) containing a zinc finger DNA binding motif.
Constitutive overexpression of TacA caused no detectable phenotypic aberrations. In contrast silencing of expression of TacA by using an RNA interference construct under control of the Tet-Off promotor system strongly inhibited growth of the amoebae leading to a doubling time of around 30 hours compared to 16 hours of not induced cell lines. After 10 days of growth the cell density of tacA RNAi induced cell lines reached only half of not induced transformants.
Populations of RNAi tacA mutants formed only few, very small and crippled fruiting bodies after 3-4 days on HABP filters. Future work is aimed at establishing whether TacA is the target of calcineurin in *Dictyostelium*.

Analysis of the D. discoideum UNC-84

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A nuclear membrane protein termed UNC-84 (uncoordinated protein), first found in *Caenorhabditis elegans*, is involved in both nuclear anchoring and nuclear migration during early embryonic development. UNC-84 homologs from different species consist of at least one transmembrane domain and a SUN domain located at the C-terminus. Across the species, UNC-84 proteins show great variability in their protein sequence, except the highly conserved C-terminal SUN domain.

The *Dictyostelium* UNC-84 is a 105 kDa protein, composed of one transmembrane sequence followed by two coiled-coil domains and the highly conserved SUN domain located at the C-terminus. Dd-UNC-84, present throughout the development, is associated with the nuclear envelope. We have shown that it forms dimers and trimers via the two coiled-coil domains.
In the mammalian model, SUN-1 interacts directly with Nesprin-2, which is a giant member of the α-actinin superfamily. The *Dictyostelium* Nesprin-2 homolog, interaptin, represents a putative binding partner of the Dd-UNC-84. The distribution of Dd-UNC-84 in AX2 wild-type, interaptin over-expressing and interaptin-deficient AX2 mutants suggests a competitive localization of Dd-UNC-84 and interaptin at the nuclear envelope. Accordingly, a direct binding of interaptin to the Dd-UNC-84 could not be demonstrated by co-immunoprecipitation and GST-pull down experiments.

Metabolic enzymes and their influence on phagocytosis

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Two groups of enzymes, lysozymes and long-chain fatty-acyl-CoA-synthetases (LC-FACSs) are involved in phagocytosis in unexpected ways:

LC-FACS1 is a protein that associates with the cytosolic surface of acidic phagosomes, where it mediates fatty acid uptake from the diet, but does not contribute to phagocytosis itself (von Löhneysen et al., 2003). A closely related isoform, LC-FACS2, is a protein of the inner peroxisomal membrane. The corresponding knockout mutant shows strongly reduced phagocytosis. In contrast, the most divergent member of this protein family, LC-FACS3, originally isolated as a valproate-resistant REMI-mutant, exhibits a strongly increased rate of phagocytosis. We suspect that alterations in triglyceride levels signal to the cytoskeleton.

A cell line lacking the major vegetative lysozyme protein, alyA, compensates this defect by increased phagocytosis (Müller et al., 2005). Because this phenotype is correlated with increased expression of the gp70 esterase, (Yuan and Chia, 2000), we sought to identify the signalling components on the pathway from lysosome to esterosome to the cytoskeleton. Both, an alyA knockout stain and a gp 70 overexpressing cell-line were subjected to gene expression analysis by microarray technology, revealing a set of genes which are affected in both mutants in the same way. All of the genes tested thus far affect phagocytosis in a positive or negative way.

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LIST OF CONTRIBUTIONS

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NOTES

International Dictyostelium Conference 2005 - Planning

	SUNDAY 14	MONDAY 15	TUESDAY 16	WEDNESDAY 17	THURSDAY 18
8:30-8:50	Phagocytosis Endocytosis	Gene regulation & expression I	Cytoskeleton I	Stress & diseases I	Sexual cycle
8:50-9:10					
9:10-9:30					Coffee Break
9:30-9:50					Biophysical approaches to cell motion
9:50-10:10					
	Coffee Break				
10:40-11:00	Chemotaxis I	Gene regulation & expression II	Cytoskeleton II	Stress & diseases II	
11:00-11:20					
11:20-11:40					
11:40-12:00		DIF signalling I	Evolution-Ecology II	Cell growth & diff. I	
12:00-12:20					12:00-14:00 Lunch
12:20-12:40	12:30-14:00 Lunch			12:30-14:00 Lunch	
			12:50-14:20 Lunch		
14:30-15:40			*Tour to the Choranche Caves* *or* *Hiking in the Vercors*	WORKSHOP II	*Departure*
15:40-16:00	Chemotaxis II	DIF signalling II			
16:00-16:20					
16:20-16:40					
	Coffee Break			Coffee Break	
17:10-17:30	Chemotaxis III	Evolution-Ecology I		Cell growth & diff. II	
17:30-17:50					
17:50-18:10					
18:10-18:30					
	19:00-20:30 Dinner			19:00-20:30 Dinner	
20:30-22:30	*Posters I*	WORKSHOP I	20:30 Banquet	*Posters II*	