International Dictyostelium Conference

2009

Estes Park, Colorado, USA
Sept 23-28, 2009

Organizers: **Alan Kimmel**
Tian Jin

Meeting Coordinator: **Marilyn Parham**

	SUN	MON	TUES	WED	THUR	FRI	
Brkfst	none	7-9	7-9	7-8:30	7-9 Check-out	7-9 Depart	
Morn/Talks	none 10:20-10:40	9-12 Coffee 10:20-10:40	9-12 Coffee 9:30-9:50	8:30-11:00 Coffee 10:20-10:40	9-12 Coffee	none	7
talks	7 talks	6 talks	8 talks				
Lunch	none	12-1:30	12-1:30	NOON/BUSES	12-1:30	none	
Aft/Talks	none Arrive Check-in	2:30-5:30 Coffee 3:50-4:10 7 talks	2:30-5:30 Coffee 3:50-4:10 8 talks	excursions	2:30-5:00 Coffee 3:30-3:50 6 talks	none	
Dinner	Reception	6-7:30 6-8	6-7:30	on your own	Banquet none 6:30-8:30		
Evening	none	7:30-10:30 22 posters	7:30-10:30 22 posters	none	9-stars	none	

Schedule of Oral Presentations

Monday 9:00-12:00 AM (7 Talks)
Session 1: Genomics and Whole-Genome Based Analyses

Chair: Catherine Pears

1. SEXUAL GENETICS AND GENOME SEQUENCING. Gareth Bloomfield, Justin Pachebat and Rob Kay

2. CONSERVATION AND CHANGE IN CYCLIC AMP SIGNALLING GENES DURING SOCIAL AMOEBA EVOLUTION. Yoshinori Kawabe, Hajara Lawal, Christina Schilde, Andrew Heidel, Gernot Gloeckner and Pauline Schaap

3. GENE REPERTOIRE OF ACYTOSTELIUM SUBGLOBOSUM, A GROUP 2 SPECIES WITHOUT STALK-CELL DIFFERENTIATION. Hideko Urushihara, Hidekazu Kuwayama, Hiroshi Kagoshima, Tadasu Shin-I, Takehiko Itoh, Tateaki Tanigichi, Kyoko Uchi, Yoko Kuroki, Yuji Kohara, Asao Fujiyama

4. WHOLE GENOME SEQUENCING OF NATURAL ISOLATES OF DICTYOSTELIUM DISCOIDEUM. Elizabeth Ostrowski, Yufeng Shen, Mariko Katoh, Debra Brock, Christopher Dinh, Richard Sucgang, Gad Shaulsky, Stephen Richards, Richard Gibbs, Adam Kuspa, Joan Strassmann, David Queller

Coffee Break

5. TRANSCRIPTOME CONSERVATION OF THE D. DISCOIDEUM AND D. PURPUREUM DEVELOPMENTAL PROGRAMS REVEALED THROUGH RNA-SEQUENCING. Anup Parikh, Edward Miranda, Mariko Katoh, Danny Fuller, Gregor Rot, Lan Zagar, Tomaz Curk, Rui Chen, Blaz Zupan, William F. Loomis, Adam Kuspa and Gad Shaulsky

6. THE CARBOXY-TERMINAL DOMAIN OF DICTYOSTELIUM C-MODULE-BINDING FACTOR (CBFA) IS AN INDEPENDENT GENE REGULATORY ENTITY Jörg Lucas, Annika Bilzer, Lorna Moll, Markus Bohnert, Hideko Urushihara, Ludwig Eichinger, Gernot Glöckner, Oliver Siol, Thomas Winckler

7. DICTYBASE 2009: FACELIFT AND IMPROVED DATA ACCESSIBILITY. Yulia Bushmanova, Petra Fey, Siddhartha Basu, Pascale Gaudet, Warren A. Kibbe, and Rex L Chisholm

Monday 2:30-5:30 PM (7 Talks)
Session 2: Actin-Related Processes

Chair: Tom Egelhoff

1. LOCALIZATION AND FUNCTION OF WASP RELATED PROTEIN A (WRPA) IN DICTYOSTELIUM CELLS IN RELATION TO LOCALIZED F-ACTIN ASSEMBLY DURING CHEMOTAXIS. Alexander Feoktistov, Yunxiang Zhao, Janani Balasundaram, Chang Y. Chung

2. AN ELMO PROTEIN MAY LINK G-PROTEIN-COUPLED RECEPTOR (GPCR) SIGNALING TO ACTIN REARRANGEMENT IN DICTYOSTELIUM DISCOIDEUM. Jianshe Yan, Vassil Mihaylov, Xuehua Xu, Joseph Brzostowski, Carole Parent, and Tian Jin

3. PAXB AND PLDB INTERACT TO REGULATE ACTIN-BASED PROCESSES. Jelena Pribic, May Kong, Megan Kelley, Max Colten, Derrick Brazill

4. INVESTIGATING THE ROLE OF ARP2 PHOSPHORYLATION IN LIVING CELLS. Peter Thomason, Mehreen Zaki, Robert Insall

Coffee Break

5. DICTYOSTELIUM DISCOIDEUM RNOA INTERPRETS CAMP MEDIATED CHEMOTACTIC SIGNALS TO INFLUENCE ACTIN ORGANIZATION. Rebecca Fernandez, Antonella Jimenez, Derrick Brazill

6. JOINT CONTROL OF ACTIN DYNAMICS BY CORONIN AND AIP1 IS CELL MOTILITY. Hellen C. Ishikawa-Ankerhold, Günther Gerisch, Annette Müller-Taubenberger

7. THE ACTIN RELATED PROTEIN ARP8 REGULATES CELL CHEMOTAXIS. Ben Rogers

Tuesday 9:00-12:00 AM (7 Talks)
Session 3: Signaling Pathways and cAMP Responses

Chair: Joe Brzostowski

1. A DICTYOSTELIUM CHALONE USES G PROTEINS TO REGULATE PROLIFERATION. Deenadayalan Bakthavatsalam, Jonathan M. Choe, Nana E. Hanson, and Richard H. Gomer

2. G PROTEIN AND MAP KINASE SIGNALING. Hoai-Nghia Nguyen, Brent Raisley, and Jeff Hadwiger

3. DIFFERENTIAL REGULATION AND ACTION OF AKT AND PKBR1 DURING GROWTH AND DEVELOPMENT OF *DICTYOSTELIUM*. Xin-Hua Liao, Jonathan Buggey, and Alan R. Kimmel

4. THE ROLE OF A C2 DOMAIN CONTAINING RASGAP IN G PROTEIN COUPLED RECEPTOR MEDIATED CHEMOTAXIS. Xuehua Xu

Coffee Break

5. REGULATION OF DIRECTIONAL SENSING THROUGH THE SPATIOTEMPORAL CONTROL OF RAS. Pascale G. Charest, Zhousin Shen, Ashley Lukoduc, Atsuo Sasaki, Steve Briggs, and Rick Firtel

6. SIGNALING EVENTS IN CHEMOTAXIS. Peter Devreotes, Yoichiro Kamimura, Meghdad rahdar, Jane Borleis, Yu Long, JONATHAN Franca Koh, Kristen Swaney, Michelle Tang, and Stacey S. Willard
7. FOLIC ACID CHEMOTAXIS USING VEGETATIVE DICTYOSTELIUM DISCOIDEUM. Alayna Roberts, Kamalakkannan Srinivasan, Carrie Elzie, Liwei Jiang, and Chris Janetopoulos

Tuesday 2:30-5:50 PM (8 Talks)
Session 4: Modeling Cell Motility

Chair: Satoshi Sawai

1. SINGLE CELLS, FORAGING STRATEGIES AND BIASED RANDOM WALKS: HOW DO CELLS FIND HIDDEN TARGETS? Edward C. Cox, Liang Li, Rob Cooper, and Simon Tolic-Norrelykke Cox

2. A GENERAL SCALING LAW GOVERNING DIRECTED AND NON-DIRECTED CELL MOTILITY. Scott Gruver, Alka Potdar, Peter Cummings, Chang Chung

3. THREE- AND FOUR-DIMENSIONAL OF VISUALIZATION OF DICTYOSTELIUM CHEMOTAXIS CELL MIGRATION USING OPTICAL COHERENCE TOMOGRAPHY (OCT). Sara Rey, Wolfgang Drexler and Adrian Harwood

4. EXTERNAL AND INTERNAL NOISE LIMITS TO EUKARYOTIC CHEMOTAXIS. Fuller,D., Chen,W., Adler, M.,Groisman, A., Levine, H., Rappel, W-J. and Loomis, W. F.

Coffee Break

5. HIGH CALCIUM PARTIALLY OR COMPLETELY NORMALIZES THE DEFECTS IN MOTILITY AND CHEMOTAXIS OF MUTANTS *SGLA*$^{-}$, *CHCA*$^{-}$ AND *PTENA*$^{-}$, BUT NOT *MHCA*$^{-}$ OR 3XASP. Daniel F. Lusche, Deborah Wessels, David R. Soll

6. TWO MODELS FOR CELL MOTILITY. Wouter-Jan Rappel, Inbal Hecht, Mathias Buenemann, Leonard Sander and Herbert Levine

7. PSEUDOPOD FORMATION DURING MOVEMENT AND CHEMOTAXIS. Peter J.M. van Haastert and Leonard Bosgraaf

8. A SECOND CHEMOTAXIS SYSTEM OVERLAPPING THE cAMP SYSTEM DURING AGGREGATION? Deborah Wessels, Spencer Kuhl, Amanda Scherer, Brent Raisley and David R. Soll

Wednesday. 9:00-12:00 AM (6 Talks)
Session 5: Bacterial Recognition and Endocytic Functions

Chair: Brenda Blacklock

1. LOSS OF AUTOPHAGY 9 RESULTS IN DEFECTS IN DICTYOSTELIUM DEVVELOPMENT, IN PHAGOCYTOSIS AND IN THE CLEARANCE AND REPLICATION OF LEGIONELLA PNEUMOPHILA. Alexandra Ley, Can Ünal, Sze Man Tung, Budi Tunggal, Michael Steinert and Ludwig Eichinger

2. PHOSPHOINOSITIDE SIGNALING AND NRAMP PROTEINS IN DICTYOSTELIUM RESISTANCE TO LEGIONELLA INFECTION. Alessandra Balest, Barbara Peracino and Salvatore Bozzaro

3. GENETIC AND TRANSCRIPTIONAL EVIDENCE FOR BACTERIAL DISCRIMINATION IN D. DISCOIDEUM. Waleed Nasser, Anup Parikh, Roshan Miranda, Chris Dinh, Rui Chen, Gad Shaulsky and Adam Kuspa

Coffee Break

4. FARMERS COME IN MANY SHAPES AND SIZES: A NEW MICROBE SYMBIOSIS. Debbie Brock, David Queller, and Joan Strassmann

5. ADDRESSING THE FUNCTIONS OF ARRESTIN-DOMAIN CONTAINING PROTEINS IN *DICTYOSTELIUM.* Dorian Guetta, Karine Langou, Didier Grunwald, Gérard Klein and Laurence Aubry

6. EFFECT OF STARVATION ON THE ENDOCYTIC PATHWAY OF *DICTYOSTELIUM* CELLS. Ewan W. Smith, Wanessa C. Lima, Steve J. Charette, Pierre Cosson

Thursday 9:00-12:00 AM (8 Talks)

Session 6: Modeling of Human Disease-Related Pathways/Developmental Recognition

Chair: Margaret Nelson

1. PRESENILIN-SIGNALING REGULATES GROWTH AND CELL FATE PATTERNING: *DICTYOSTELIUM* AS A NOVEL SYSTEM FOR FUNCTIONAL STUDIES OF THE PS/γ-SECRETASE COMPLEX. Vanessa C. McMains, Michael Myre, and Alan R. Kimmel

2. PLA_2 INHIBITION AND LIPID SIGNALLING IN *DICTYOSTELIUM*: CHARACTERISING THERAPEUTIC TARGETS IN VPA TREATMENT. Nadine Pawolleck, Frauke Hänel, Markus Maniak, Robin SB Williams

3. UNDERSTANDING SHORT CHAIN FATTY ACID UPTAKE MECHANISMS IN *DICTYOSTELIUM.* Nicole Terbach, Dmitri Gordienko, Nigel A. Brown[2], Robin SB Williams

4. A HUNTINGTIN ORTHOLOG IN *DICTYOSTELIUM* AND ITS ROLE IN MULTICELLULAR DEVELOPMENT. Michael A. Myre, Amanda Lumsden, Marcy E. Macdonald and James F. Gusella

Coffee Break

5. THE UNCONVENTIONAL MECHANISMS OF SECRETION OF ACBA AND PROCESSING INTO SDF-2 SIGNALING PEPTIDE ARE CONSERVED IN MICE, YEASTS AND DICTYOSTELIUM. Christophe Anjard, Ravi Manjithaya, Suresh Subramani, Juan Duran, Vivek Malhotra, Megan E. Williams, Margarita Berhens and William F. Loomis

6. A CYTOPLASMIC PROLYL HYDROXYLATION AND GLYCOSYLATION PATHWAY MODIFIES SKP1 TO REGULATE O_2-DEPENDENT DEVELOPMENT IN *DICTYOSTELIUM*. Christopher M. West, Zhuo A. Wang, and Hanke van der Wel

7. CHEATER RESISTANCE IS NOT FUTILE: MECHANISMS OF CHEATING AND COUNTER-CHEATING IN D. DISCOIDEUM. Anupama Khare, Lorenzo Santorelli, Joan Strassmann, David Queller, Adam Kuspa and Gad Shaulsky

8. TWO NOVEL SH2 DOMAIN PROTEINS INTERACT TO REGULATE DICTYOSTELIUM GENE EXPRESSION DURING GROWTH AND EARLY DEVELOPMENT. Chris Sugden, Gareth Bloomfield, Alan Ivens, Annette Muller Taubenberger and Jeffrey G. Williams

Thursday. 2:30-5:30 PM (6 Talks)

Session 7: Vegetative Processes and Cellular Interactions

Chair: Derrick Brazill

1. IDENTIFICATION AND CELL CYCLE-DEPENDENT LOCALIZATION OF NOVEL CENTROSOMAL CORE COMPONENTS IN DICTYOSTELIUM. Ralph Gräf, Oliver Kuhnert, Irene Schulz

2. THE CELL SHAPE CHANGES OF CYTOKINESIS. Douglas Robinson

3. REGULATION OF CELL-CYCLE GENES BY RETINOBLASTOMA IN *DICTOSTELIUM*. Kimchi Doquang, Gareth Bloomfield, Asa MacWilliams, Adrian Tsang, and Harry MacWilliams

Coffee Break

4. THE POLYMORPHIC E-SET REPEAT PROTEINS TIGER-B1 (TGRB1) AND TIGER-C1 (TGRC1) ARE SELF/NON-SELF RECOGNITION MOLECULES IN *D. DISCOIDEUM*. Shigenori Hirose, Rocio Benabentos, Gad Shaulsky and Adam Kuspa

5. AN ALPHA-CATENIN HOMOLOGUE IN D. DISCOIDEUM AND ITS ROLE IN CELL-CELL ADHESION. Daniel J. Dickinson, William I. Weis & W. James Nelson

6. THE ROLE OF COPINE A (CPNA) IN DICTYOSTELIUM DEVELOPMENT. Cynthia K. Damer, Kerry A. Lepley, Jaimie M. Pineda, Alex C. Donaghy, and Tasha S. Smith

Poster Sessions:

#1 - Mon. Eve. 7:30-10:30 PM

22 posters

Asghar
Baskar
Benabentos
Brazill
Cooper
da Silva
Egelhoff
Ford
Hirose
Huber
Keizer-Gunnik
Kelsey
Kortholt
Kuhl
Lindsey
Lusche
Nguyen
Raisley
Silliker
Taniguchi
West
Yim

#2 - Tues. Eve. 7:30-10:30 PM

22 posters

Basu
Blacklock
Dinh
Gaudet
Hong
Jimenez
Kay
Le Coadic
Lepley
Li
McQuade
Macro
Mao
Nelson
Nair
Pineda
Poloz
Schaap
Smith
Sonakya
Vincelli
Yamada

Oral Presentations

SEXUAL GENETICS AND GENOME SEQUENCING

Gareth Bloomfield[1], Justin Pachebat[2] and Rob Kay[1]

1. MRC Laboratory of Molecular Biology, Cambridge, UK
2. Dept of Plant Sciences, University of Cambridge, UK

We have identified the mating type locus of D. discoideum and sequenced it from a number of isolates of different mating behaviours. Deletion of the locus from Ax2 results in cells unable to produce macrocysts when paired with cells of complementary mating type. Introduction of the locus from a complementary-type strain into the null strain renders it able to mate with Ax2. This brings the possibility of using sexual genetics as a tool in Dictyostelium research a step closer.

The projects to sequence the Dictyostelium discoideum genome and ESTs were enormous achievements. Advances in sequencing technology now enable sequencing of much higher throughput at dramatically lower cost. We have resequenced Ax4, as well as Ax2 and DdB (the immediate parent of both axenic strains), using Solexa/Illumina sequencing to generate 10-20 million short reads that could then be mapped onto the existing genome sequence. This has identified thousands of positions in the current database sequence that should be corrected, as well as points of divergence between the different strains. We are using this technique in an attempt to identify the axenic loci and will discuss the difficulties inherent in this approach.

Abstract 2

CONSERVATION AND CHANGE IN CYCLIC AMP SIGNALLING GENES DURING SOCIAL AMOEBA EVOLUTION.

Yoshinori Kawabe[1], Hajara Lawal[1], Christina Schilde[1], Andrew Heidel[2], Gernot Gloeckner[2] and Pauline Schaap[1]

[1]College of Life Sciences, University of Dundee, UK, [2]Leibniz Institute for Age Research, University of Jena, Germany

cAMP plays a ubiquitous role in controlling almost all aspects of *D.discoideum* development. As a secreted signal it regulates expression of different classes of genes and coordinates cell movement during aggregation and morphogenesis. As an intracellular messenger it controls entry into development, spore and stalk cell maturation and spore germination. About 13 proteins are directly responsible for synthesis, detection and degradation of cAMP, while a much larger number is involved in upstream regulation of these proteins or downstream processing of the cAMP signal.

In the past 10 years we have used a combination of gene amplification and functional gene analysis across species to retrace patterns of conservation and change in some cAMP signalling genes across all Dictyostelids. This work will be summarized and used to formulate a scenario how the diverse cAMP signalling mechanisms in modern dictyostelids evolved from a stress response in their unicellular ancestors.

Very recently sequencing and assembly of the genomes of the early diverging Dictyostelids *P.pallidum* and *D.fasciculatum* were completed. This provides tremendous opportunities for understanding how cell- and developmental processes in the Dictyostelids evolved. Conservation and change in broad set of genes associated with cyclic nucleotide signalling will be discussed.

Abstract 3

GENE REPERTOIRE OF ACYTOSTELIUM SUBGLOBOSUM, A GROUP 2 SPECIES WITHOUT STALK-CELL DIFFERENTIATION

Hideko Urushihara[1], Hidekazu Kuwayama[1], Hiroshi Kagoshima[2], Tadasu Shin-I[2], Takehiko Itoh[3], Tateaki Tanigichi[2], Kyoko Uchi[1], Yoko Kuroki[2], Yuji Kohara[2], Asao Fujiyama[2,4]

[1]University of Tsukuba, Tsukuba, Japan; [2]National Institute of Genetics, Mishima, Japan; [3]Mitsubishi Research Institute, Tokyo, Japan;[4]National Institute of Informatics, Tokyo, Japan

We have been analyzing the genome structure of *Acytostelium subglobosum*, which forms tiny fruiting bodies with acellular stalks. By the end-sequencing of whole genome shotgun libraries and a fosmid library, we have generated the genome assembly of 20 Mbp in total length, the longest scaffold extending to 1.47 Mbp. In addition, we constructed cDNA libraries from vegetative and developmental cells, and sequenced randomly chosen 15,000 clones from both ends. Those EST sequences were clustered into 3,300 UniGenes. The *A. subglobosum* gene models have been constructed by mapping the EST assembly to the genome, followed by the mapping of *Dictyostelium discoideum* protein sequences. The updated *Acytostelium* gene database contains 9,269 gene models. Although most developmental genes of *D. discoideum* seem to have *A. subglobosum* counterparts, closer examination revealed that some of the family members were missing in *A. subglobosum*. This could affect the developmental trait of this species, because the individual family members tend to play different roles during growth and development in *D. discoideum*.

Abstract 4

WHOLE GENOME SEQUENCING OF NATURAL ISOLATES OF DICTYOSTELIUM DISCOIDEUM

Elizabeth Ostrowski[1], Yufeng Shen[2], Mariko Katoh[3], Debra Brock[1], Christopher Dinh[4], Richard Sucgang[4], Gad Shaulsky[3], Stephen Richards[2], Richard Gibbs[2], Adam Kuspa[4], Joan Strassmann[1], David Queller[1]

[1]Department of Ecology and Evolutionary Biology, Rice University, Houston TX 77005
[2]Human Genome Sequencing Center, Baylor College of Medicine, Houston TX 77030
[3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030
[4]Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston TX 77030

Comparison of the genome sequences of different species and isolates within a species can elucidate their evolutionary history and reveal the selective forces that are operating on the genome. We are combining whole-genome sequencing with a molecular evolution approach to identify rapidly evolving genes in *Dictyostelium discoideum* and to test the hypothesis that selective conflict drives rapid evolution in the genes that underlie social interactions.

To that end, we have recently completed sequencing of more than 12 isolates of *D. discoideum* and several other closely related species. The *D. discoideum* sequences are being compared to one another, to the laboratory strain AX4, and to several related species to uncover evidence of rapid adaptive evolution. Comparison of the *D. discoideum* strains shows that they differ at approximately 0.1% of sites in the genome. The distribution of polymorphism is highly skewed: most genes show little or no genetic change, whereas relatively few show extremely high levels of polymorphism and/or a high rate of nonsynonymous to synonymous substitution (dN/dS), suggesting that they are evolving under strong balancing or positive selection.

Abstract 5

TRANSCRIPTOME CONSERVATION OF THE D. DISCOIDEUM AND D. PURPUREUM DEVELOPMENTAL PROGRAMS REVEALED THROUGH RNA-SEQUENCING

Anup Parikh[1], Edward Miranda[1], Mariko Katoh[1], Danny Fuller[2], Gregor Rot[3], Lan Zagar[3], Tomaz Curk[3], Rui Chen[1], Blaz Zupan[1,3], William F. Loomis[2], Adam Kuspa[1] and Gad Shaulsky[1]

1 Baylor College of Medicine, Houston, TX, USA
2 University of California San Diego, La Jolla, CA, USA
3 University of Ljubljana, Ljubljana, Slovenia

Cross species comparison of genomes and transcriptomes is a powerful tool for understanding underlying biological processes, accurate functional classification of genes, and discovery of novel elements. Using Illumina sequencing of mRNAs, we have captured the global transcriptional profiles of the developmental programs of D. discoideum and D. purpureum. The morphology and transcriptional profiles in these two species have been largely conserved even after greater than 300 million years of divergence. We also captured the transcriptional profiles of prespore and prestalk cells from both species. The RNA-seq results correlate well with previously known markers and reveal many new transcripts with unique developmental regulation and cell type enrichment.

Our analysis provides the first complete picture of the transcriptional landscape in both D. discoideum and D. purpureum. The complete dataset will be available through a genome browser and exploration of over 13,900 genome elements will be possible through an interactive web interface at http://www.ailab.si/dictyexpress, which currently displays all of our published microarray data.

Abstract 6

THE CARBOXY-TERMINAL DOMAIN OF DICTYOSTELIUM C-MODULE-BINDING FACTOR (CBFA) IS AN INDEPENDENT GENE REGULATORY ENTITY

Jörg Lucas(1), Annika Bilzer(1), Lorna Moll(2), Markus Bohnert(1), Hideko Urushihara(3), Ludwig Eichinger(2), Gernot Glöckner(4), Oliver Siol(1), Thomas Winckler(1)

1 School of Biology and Pharmacy, Institute of Pharmacy, Department of Pharmaceutical Biology, University of Jena (Germany) 2 Institute for Biochemistry I, Medical Faculty, University of Cologne, Joseph-Stelzmann-Strasse Cologne (Germany), 3 Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki (Japan), 4 Fritz-Lipmann-Institute for Age Research, Jena (Germany)

CbfA is a multidomain protein that belongs to the family of jumonji-type (JmjC) transcription regulators. CbfA and a related D. discoideum CbfA-like protein, CbfB, share a paralogous domain arrangement that includes the JmjC domain – presumably a chromatin-remodeling activity – and two zinc finger-like (ZF) motifs. The CbfA and CbfB proteins have completely different carboxy-terminal domains, suggesting that the plasticity of such domains may have contributed to the adaptation of the CbfA-like transcription factors to the rapid genome evolution in the dictyostelid clade. In transcriptome analyses we found that CbfA regulates at least 250-500 genes during the vegetative growth of D. discoideum cells. Functional annotation of these genes revealed that CbfA predominantly controls the expression of gene products involved in several housekeeping functions. Interestingly, about one half the of CbfA-dependent genes does not require the entire CbfA protein. Instead, the carboxy-terminal domain of CbfA mediates this transcription regulation.

Abstract 7

DICTYBASE 2009: FACELIFT AND IMPROVED DATA ACCESSIBILITY

Yulia Bushmanova, Petra Fey, Siddhartha Basu, Pascale Gaudet, Warren A. Kibbe, and Rex L Chisholm

dictyBase, Northwestern University, Chicago, IL, USA

To improve data representation and accessibility in dictyBase, we reengineered our gene page. All gene-related information is now being displayed on a single page in separate tabs that include Protein information, GO annotations, Phenotypes, References, and a direct access to BLAST. Separation of protein information into a tab allowed us to display additional data, obtained from UniProt and InterPro. Associated sequences open in a new tab and alternative transcripts are accessible via nested tabs. Another tab provides a shortcut to a BLAST server where gene-specific sequences are displayed and upon selection pre-fill the sequence field.

We have also redesigned and reorganized the front page and other static pages. The links are grouped under newly organized top bars. Dynamic sidebars appear on the left with context-dependent menus. Those changes were achieved using Web2.0 technologies. This allowed the development of content-enriched and interactive web interfaces, while at the same time improving the organization and accessibility of dictyBase information and data.

dictyBase is supported by grants from the NIH (GM64426 and HG00022)

Abstract 8

LOCALIZATION AND FUNCTION OF WASP RELATED PROTEIN A (WRPA) IN DICTYOSTELIUM CELLS IN RELATION TO LOCALIZED F-ACTIN ASSEMBLY DURING CHEMOTAXIS

Alexander Feoktistov, Yunxiang Zhao, Janani Balasundaram, Chang Y. Chung

Department of Biological Sciences and Pharmacology, Vanderbilt University, Nashville, TN

The WASP family of proteins, including WASP, N-WASP, and SCAR or WAVE, has emerged as important adaptor molecules that connect multiple signaling pathways to regulate the actin cytoskeleton in response to a chemoattractant. Dictyostelium cells express WASP, as well as WRPs (WASP related protein A and B). WRPa contains many of the domains present in WASP, specifically the actin/Arp2/3 binding VCA region, the RacC-binding GBD domain, and the SH3 binding poly proline domain. Analysis of wild type, wrpA null, and YFP-WRPa expresseing wrpA null Dictyostelium cells revealed that WRPa is required for proper chemotaxis in response to a cAMP gradient, and YFP-WRP expressed in a null background rescued chemotactic defects. wrpA null cells extend more pseudopods and their average life span is longer than that of wild type cells, causing more turns and inefficient chemotaxis. Upon cAMP stimulation, YFP-WRPa translocates into detergent-insoluble cytoskeleton fraction at 30 sec after stimulation. In addition, YFP-WRPa appears to be localized at the membrane cortex of a retracting psuedopod. These results suggest that WRPa might be required for the reorganization of F-actin that ius important for the regulation of the dynamics of psuedopod extension and retraction.

Abstract 9

AN ELMO PROTEIN MAY LINK G-PROTEIN-COUPLED RECEPTOR (GPCR) SIGNALING TO ACTIN REARRANGEMENT IN DICTYOSTELIUM DISCOIDEUM

Jianshe Yan[1], Vassil Mihaylov[2], Xuehua Xu[3], Joseph Brzostowski[4], Carole Parent[2], and Tian Jin[1]

[1]Chemotaxis Signal Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD; [2]Laboratory of Cellular and Molecular Biology, CCR, NCI, NIH, Bethesda, MD; [3]Georgetown University, School of Medicine, Department of Oncology; [4]NIH/NIAID/LIG Imaging Facility Rockville, MD

One of the basic questions in cell migration is how a GPCR chemosensing network regulates assembly of the actin network required for directional force generation during chemotaxis. ELMO (EnguLfment and cell Motility) protein family has been implicated in this process. ELMO was first discovered as an essential component involved in engulfment of dead cells in C. elegans. DOCK180 and ELMO form a complex that functions as a bipartite GEF to optimally activate Rac and promote actin cytoskeleton rearrangement. DOCK-ELMO complexes also receive signals from GPCRs, integrins and tyrosine kinases to activate Rac, leading to cell migration. However, how GPCRs and other receptors activate DOCK-ELMO has not been determined in any system. We have been using D. discoideum, as a model system to study the functions of ELMO proteins in GPCR-mediated signaling network leading to chemotaxis. We found one member of ELMO family may be the missing connection between the GPCR/G-protein, the chemoattractant sensing machinery, and the actin cytoskeleton, the machinery of cell movement.

Abstract 10

PAXB AND PLDB INTERACT TO REGULATE ACTIN-BASED PROCESSES

Jelena Pribic, May Kong, Megan Kelley, Max Colten, Derrick Brazill

Hunter College, The Graduate Center of CUNY

The actin cytoskeleton plays a fundamental role in various processes including differentiation, migration, endocytosis and exocytosis. An adapter protein Paxillin as well as an enzyme Phospholipase D (PLD), has been associated with processes based on the actin cytoskeleton regulation. Such regulation is critical for the development of *Dictyostelium discoideum*. To gain better insight into the roles of Paxillin and PLD and to investigate their potential interactions, we study the Paxillin and PLD homologs, PaxB and PldB, respectively. It has been reported that cells lacking *paxB* arrest at the mound stage due to a defect in cell sorting. Here we show that overexpression of *pldB* in these cells rescues the cell sorting defect and allows development to proceed, suggesting an interaction between PaxB and PldB. Additionally, PaxB and PldB exhibit interactions in several actin-required processes including cAMP chemotaxis, exocytosis and actin polymerization. However, phagocytosis, another actin-based process, does not depend on this interaction. Altogether, our data indicate that PaxB and PldB act in the same pathways to control actin reorganization required processes

Abstract 11

INVESTIGATING THE ROLE OF ARP2 PHOSPHORYLATION IN LIVING CELLS

Peter Thomason, Mehreen Zaki, Robert Insall
Beatson Institute for Cancer Research, Glasgow, UK

The Arp2/3 complex is considered to be the most important regulator of actin polymerization in migratory cells. Several mechanisms are directed towards the control of Arp2/3 complex activity, such as its regulation by nucleation-promoting factors (NPFs) of the WASP and Scar/WAVE families. The majority of current efforts are focused on identifying and describing the mechanisms of regulation of these NPFs, rather than of the Arp2/3 complex itself. Recently in vitro studies have shown that phosphorylation of the Arp2/3 complex on Ser/Thr and Tyrosine residues is required for its ability to stimulate actin polymerization. Proteomic analysis has identified three putative phosphorylation sites on Arp2, at residues Thr 235, Thr 236 and Tyr 200, the phosphorylation status of which seem to be regulated by extracellular ligands. We are investigating the importance of phosphorylation of these Arp2 residues by using parasexual replacement of the Dictyostelium wild-type Arp2 gene with site-specific mutants. We will present data on the viability of these strains together with (where appropriate) an analysis of their developmental and chemotaxis phenotypes.

Abstract 12

DICTYOSTELIUM DISCOIDEUM RNOA INTERPRETS CAMP MEDIATED CHEMOTACTIC SIGNALS TO INFLUENCE ACTIN ORGANIZATION

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Management of actin cytoskeletal dynamics is essential for cell function. ARNO, an Arf guanine nucleotide exchange factor, has been implicated in actin regulation but its exact role remains unknown. To explore ARNO's role in this regulation and in actin mediated processes, the *Dictyostelium discoideum* homolog RnoA was examined. Encoded by *secG*, RnoA expression peaks during aggregation and mound formation. Antisense reduction of RnoA prolongs aggregation, delaying development. RnoA overexpression causes mound stage arrest, which is rescued by addition of wildtype cells. RnoA does not inhibit cell sorting as RnoA overexpressing (RnoAOE) cells and wildtype cells are evenly distributed throughout the fruiting body in chimeric mixtures. RnoA antisense and RnoAOE cells fail to stream during aggregation or to chemotax toward cAMP. This chemotactic defect is specific to cAMP chemotaxis, as both RnoA mutants chemotax to folate. F-actin staining of the RnoA mutants demonstrates alterations in RnoA expression can modify actin organization. Together, the results suggest proper RnoA levels are important for response to cAMP signaling in order to coordinate F-actin organization during development.

Abstract 13

JOINT CONTROL OF ACTIN DYNAMICS BY CORONIN AND AIP1 IS REQUIRED FOR PROPER CYTOKINESIS, ENDOCYTIC TRAFFIC, AND CELL MOTILITY

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Coronin and actin-interacting protein 1 (Aip1) are actin-binding proteins that either inhibit actin polymerization or enhance the disassembly of actin filaments. Cells of *Dictyostelium discoideum* lacking both proteins are delayed in growth and early development, and often fail to proceed to fruiting body formation. Coronin/Aip1-null cells are characterized by an increase in filamentous actin that causes a thickening of the cell cortex as compared to wild-type and single-mutant cells. This imbalance has severe consequences for cell motility, cytokinesis and endocytosis. Coronin/Aip1-null cells show numerous surface protrusions enriched in filamentous actin and cofilin. Although motility is slowed down, the double-mutant cells are still capable of orientating in a gradient of chemoattractant. Not only is the formation of a cleavage furrow strongly affected in the double-mutant cells but also the function of centrosomes in the formation of bipolar spindles. Endocytic trafficking from the acidic to the neutral compartment is impaired in the double-mutant cells causing delayed exocytosis. We discuss how these deficiencies might be linked to the aberrant regulation of actin polymerization in cells lacking both coronin and Aip1.

Abstract 14

THE ACTIN RELATED PROTEIN ARP8 REGULATES CELL CHEMOTAXIS

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The actin related protein Arp8 is a subunit of the Ino80 complex required for ATP-dependent chromatin re-modelling activity and gene regulation. Arp8-null mutants are delayed in aggregation and fail to express carA, acaA and pdsA, which are required for cAMP signalling. Expression of these genes is restored by addition of exogenous cAMP pulses, suggesting that the cells are able to respond to cAMP pulses, but not initiate them. However, arp8 null cells rescued by cAMP pulses, still have an additional chemotactic phenotype, as they can move along low, but not high concentration cAMP gradients. We find that arp8 null mutants have elevated expression of the inositol biosynthetic genes impA1 and ino1. We have previously shown that increased expression of impa1 leads to elevated levels of PIP3 signalling, and indeed observe elevated PIP3 signalling in arp8 mutants. PIP3 signalling is attenuated by lithium treatment, and we find that chemotaxis of arp8 null cells is rescued by lithium. Based on these results, we suggest that the Ino80 complex is involved in the modulation of PIP3 signalling.

Abstract 15

A DICTYOSTELIUM CHALONE USES G PROTEINS TO REGULATE PROLIFERATION

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In mammals, the sizes of tumor metastases and some tissues may be regulated by negative feedback loops in which autocrine secreted factors called chalones inhibit proliferation. Little is known about chalones, and how cells sense them. A secreted protein, AprA, is a *Dictyostelium* chalone. Cells lacking AprA proliferate faster than wild type cells, and adding recombinant AprA (rAprA) to cells slows proliferation. We find that cells lacking the G proteins Gα8 and Gβ proliferate faster than wild-type cells despite secreting normal or high levels of AprA. Compared to wild-type cells, the proliferation of *gα8*$^-$ and *gβ*$^-$ cells is weakly inhibited by rAprA. Like other ligands that activate G proteins, rAprA induces the binding of [^{3}H]GTP to membranes, and GTPγS inhibits the binding of rAprA to membranes. Both rAprA-induced [^{3}H]GTP binding and the GTPγS inhibition of rAprA binding require Gα8 and Gβ. Our study shows that Gα8 and Gβ are part of the signal transduction pathway used by AprA to inhibit proliferation in *Dictyostelium*, thus showing that a chalone signal transduction pathway uses G proteins.

Abstract 16

G PROTEIN AND MAP KINASE SIGNALING

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G protein-coupled receptors regulate many different developmental responses in Dictyostelium but the mechanisms for allowing pathway specificity downstream of the receptors remain largely undetermined. A comparison of Gα subunit structure has lead to the identification of putative MAPK docking sites (D-motifs) in some subunits suggesting that interactions of Gα subunits with MAP kinases might be an important step in providing signal transduction pathway specificity. MAP kinase activation occurs in response to cAMP and folate stimulation but only the responses to folate require the G protein function. Alteration of the Gα4 D-motif reduces the association of this subunit with the MAPK ERK2 and impacts late developmental morphogenesis. A similar alteration of the amino terminal D-motif of the Gα5 subunit eliminates the ability of the Gα5 subunit to promote precocious development. Alterations of the Gα4 or Gα5 D-motifs have little impact on MAPK activation but these alterations might affect the localization of MAPK activity during signal transduction.

Abstract 17

Differential Regulation and Action of AKT and PKBR1 during Growth and Development of *Dictyostelium*

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AKT and PKBR1 are related AGC kinases that play pivotal roles in *Dictyostelium* chemotaxis during growth and development. AKT has a PH domain and is transiently recruited to the membrane by interaction with PIP3, whereas PKBR1 is myristoylated and is persistently at the membrane. The disparate locations of AKT and PKBR1 indicate the potential for different activation mechanisms and function. Nonetheless, AKT and PKBR1 both require phosphorylation within their kinase (PDK1) domains and within their HM motifs. Chemoattractant stimulation of AKT and PKBR1 was studied during growth, by folate, and during development, by cAMP. Under both situations, AKT activation requires PI3K, while activation of PKBR1 is PIP3-independent. TORC2 is another key module for chemotaxis and regulation for AKT and PKBR1. Phosphorylation of PKBR1 at both the PDK1 and HM sites is completely eliminated in *Dictyostelium* that lack TORC2 components Pia, RIP3, or lst8, but, following folate stimulation, AKT phosphorylations persist at both sites. We also show that PDK1 phosphorylation of AKT and PKBR1 requires HM phosphorylation, but, in contrast to what has been previously assumed, phosphorylation by TORC2 is insufficient to activate either AKT or PKBR1. We also investigated the proteins transiently phosphorylated by AKT and PKBR1 following chemoattractant stimulation. Interestingly, the phosphorylation profiles of substrates were different in *akt*- or *pkbr1*- null strains, depending upon stimulation with either folate or cAMP. We suggest that AKT and PKBR1 preferentially phosphorylate optimal substrates. We have investigated the pathways of activation of AKT and PKBR1 by different chemoattractants, and discovered that these two related kinases are distinctively regulated by upstream factors and function differently through phosphorylating specific substrates. Collectively, these data provide functional proof for differences in the regulation of AKT and PKBR1 and context for complexity of PDK1 and TORC2 regulation of multiple AGC protein kinases in other systems.

Abstract 18

THE ROLE OF A C2 DOMAIN CONTAINING RASGAP IN G PROTEIN COUPLED RECEPTOR MEDIATED CHEMOTAXIS

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The role of a C2 domain containing RasGAP in G protein coupled receptor mediated chemotaxis. Ras signaling plays critical roles in G protein coupled receptor mediated chemotaxis during the development of Dictyostelium discoideum. The central question of chemotaxis is how a chemotaxing cell adapts to uniform stimuli and amplifies a chemoattractant gradient into a polarized biochemical response leading to directional cell movement. Ras signaling mediated by GEFs and GAPs is supposed to be a key step of signaling amplification during chemosensing. We identified and analysis the function of a C2 domain containing RasGAP (C2RasGAP1) in chemotaxis.

Abstract 19

Regulation of directional sensing through the spatiotemporal control of Ras

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A cell's ability to detect and orient themselves in chemoattractant gradients has been the subject of numerous studies, but the underlying molecular mechanisms remain largely unknown. Ras activation is the earliest polarized response to chemoattractant gradients downstream from heterotrimeric G proteins in Dictyostelium and inhibition of Ras signaling induces directional migration defects. Activated Ras is enriched at the leading edge, promoting the localized activation of key chemotactic effectors, such as PI3K and TORC2. In Dictyostelium, Ras controls cell motility, chemotaxis and signal relay, acting, in part, through the regulation of PI3K and TORC2, downstream from GPCR and heterotrimeric G proteins. To understand this process, we have investigated the mechanism by which Ras is spatially and temporally controls and the pathways that are regulated by Ras and by which Ras is regulated.

Abstract 20

SIGNALING EVENTS IN CHEMOTAXIS

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The mechanisms of sensing shallow gradients of extracellular signals is remarkably similar in *Dictyostelium* amoebae and mammalian leukocytes. An extensive series of studies have indicated that the upstream components and reactions in the signaling pathway are quite uniform while downstream responses such as PIP_3 accumulation and actin polymerization are sharply localized towards the high side of the gradient. Uniform stimuli transiently recruit and activate PI3Ks and cause PTEN to be released from the membrane while gradients of chemoattractant cause PI3Ks and PTEN to bind to the membrane at the front and the back of the cell, respectively. This reciprocal regulation provides robust control of PIP_3 and leads to its sharp accumulation at the anterior. A similar PIP_3-based "polarity circuit" plays a key role in cytokinesis where PI3Ks and PTEN move to and function at the poles and furrow, respectively, of the dividing cell. Disruption of PTEN broadens PI localization and actin polymerization in parallel, leading to vigorous extension of lateral pseudopodia. However, lowered levels of PIP_3 do not greatly interfere with either chemotaxis or cytokinesis, suggesting that additional PIP_3-independent pathways act in parallel.

We have recently shown that activation of TorC2 and PKB occurs at the leading edge of chemotaxing cells and plays a critical role in directed cell migration. Within seconds of stimulation of chemotactically sensitive cells, two PKB homologs, PKBA and PKBR1, transiently phosphorylate at least 10 proteins. The enzymes are activated by phosphorylation of their hydrophobic motifs (HMs) through TorC2 and subsequent phosphorylation of their activation loops (ALs). Cells deficient in both PKBs or TorC2 lack most of the phosphorylated substrates and are specifically impaired in directional sensing. Evidence suggests that RasC activates TorC2. Activation of PKBA requires PIP_3 while activation of PKBR1, a myristoylated form persistently bound to the membrane, is independent of PIP_3. This redundant circuit explains previous observations on the role of PIP_3 in chemotaxis. In cells lacking PTEN, high levels of PIP_3 overactivate PKBA and impair chemotaxis by triggering many extraneous pseudopodia. In the absence of PIP_3, chemotaxis can occur almost normally because PKBR1 is activated by TorC2 independently of PIP_3. In all cases, optimal PKB substrate phosphorylation is strongly correlated with robust chemotaxis.

FOLIC ACID CHEMOTAXIS USING VEGETATIVE DICTYOSTELIUM DISCOIDEUM

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Chemotaxis plays an essential role in the life cycle of the social amoeba Dictyostelium discoideum. Cells migrate towards food during vegetative growth and also chemotax during the stages that lead to the formation of fruiting bodies. Many of the genes that regulate chemotaxis have been isolated by analysis of mutant lines that were chemotactically defective when aggregating after starvation on bacterial lawns. Use of such screens has been largely successful, but sometimes results in the misidentification of chemotaxis mutants that are in fact defective in other processes, such as the proper regulation of developmental genes that are required for cAMP-mediated chemotaxis. Cells that are competent for folic acid chemotaxis, on the other hand, need only be grown in the presence of bacteria and do not require a complex developmental program for proper expression of signaling proteins. Presumably, the same directional sensing module guides a cell during cAMP- and folic acid-mediated chemotaxis. We have used folic acid micropipette assays to demonstrate that many of the same signaling molecules are used for both chemotactic pathways. We are now using folic acid chemotaxis to further characterize a number of mutants that have been previously documented as being defective in cAMP-mediated chemotaxis.

Abstract 22

Single cells, foraging strategies and biased random walks: how do cells find hidden targets?

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We know how organisms and cells find targets they can see or smell. We do it all the time. But what should we do if the targets are so remote or hidden that we can neither see nor smell them? We can imagine two radically different strategies. One, random searching in a Brownian sense, and two, the lawnmower search. Both guarantee that the target will be found, but the first could well take forever, and the second implies very sophisticated algorithms. It also takes a long time.

I will introduce the subject with a review of some of the new experimental work on foraging strategy, then turn to our own work with single foraging *Dictyostelium* amoebae, where we have shown that starving amoebae have a strategy that lies somewhere between the lawn mower and Brownian search. To accomplish this task they have use a new kind of biased random walk that encodes memory of the past. This memory is used to improve performance in the future. I will then ask if this strategy has been selected over a wide range of length scales.

Li, L., Nørrelykke, S.F., and Cox, E,C, (2008). Persistent cell motion in the absence of external signals: a search strategy for eukaryotic cells. *PLoS ONE* **3,** e2093.

Abstract 23

A GENERAL SCALING LAW GOVERNING DIRECTED AND NON-DIRECTED CELL MOTILITY

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Cell motility is a fundamental process with relevance to embryonic development, immune response, and metastasis. Cells move either spontaneously, in a non-directed fashion, or in response to chemotactic signals, in a directed fashion. Even though they are often studied separately, both forms of motility share many complex processes at the molecular and subcellular scale, e.g., orchestrated cytoskeletal rearrangements and polarization. In addition, at the cellular level both types of motility include persistent runs interspersed with reorientation pauses. Because there is a great range of variability in motility among different cell types, a key challenge in the field is to integrate these multi-scale processes into a coherent framework. We analyzed the motility of individual Dictyostelium cells with bimodal analysis, a method that compares time spent in persistent versus reorientation mode. Unexpectedly, we found that reorientation time is coupled with persistent time in an inverse correlation, i.e., if one increases, the other must decrease. Surprisingly, the inverse correlation holds for both non-directed and chemotactic motility, so that the full range of Dictyostelium motility can be described by a single scaling relationship. Additionally, we found an identical scaling relationship for three human cell lines, indicating that the coupling of reorientation and persistence holds across species and making it possible to describe the complexity of cell motility in a surprisingly general and simple manner. Armed with this new perspective, we analyzed the motility of Dictyostelium mutants, and found four in which the coupling between reorientation and persistent modes was altered. Our results point to a fundamental underlying principle, described by a simple scaling law, unifying mechanisms of eukaryotic cell motility at several scales.

Abstract 24

Three- and Four-dimensional of visualization of Dictyostelium chemotaxis cell migration using Optical Coherence Tomography (OCT)

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Due to limitations in optical microscopy, cell chemotaxis is usually studied on two dimensional (2D), optically transparent surfaces, most often these are glass cover slips. These are conditions far from those experienced by cells in vivo or in their natural environment. In Dictyostelium, it is difficult to follow cells moving on optical opaque surfaces, such as nitrocellulose membranes, and when they move in three dimensional (3D) environments.

Optical Coherence Tomography (OCT) is a non-invasive label-free imaging technique, which offers the potential to visualize moving cells on both opaque surfaces and in 3D matrices with a depth (z-axial) depth of > 400 m. Here, we use OCT time lapse video-microscopy to investigate Dictyostelium cells undergoing chemotaxis in 3D (2D+time) and 4D (3D+time) environments. We will compare cell chemotaxis in these environments to those previously recorded for cells moving on conventional glass surfaces. Our results demonstrate the utility of OCT for cell tracking and offer a powerful method to analyse cell chemotaxis in complex environments.

Rey et al (2009) J Biophotonics (in press) 10.1002/jbio.200910027

Abstract 25

EXTERNAL AND INTERNAL NOISE LIMITS TO EUKARYOTIC CHEMOTAXIS

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The chemotactic response of cells can be considered as the outcome from two distinct steps: establishment of spatial differences in the distribution of receptors with bound chemoattractant on the cell's surface; and the response to these differences by the signal transduction pathways leading to directed motility. We have used microfluidic devices that generate stable exponential cAMP gradients such that the proportional difference across a cell (ie. gradient steepness) is constant across the full width of the chamber in the different devices. We have found that cells respond to a 1.25% difference across a 10 mm cell with maximal chemotactic accuracy at 14 nM. The cells respond to steeper gradients at lower mid-point concentrations.

Data from these experiments were analyzed for mutual information in the receptor response to cAMP concentrations and directionality of motion. The external and internal noise limits to chemotaxis could be calculated for the various conditions. These analyses set the foundations for further experiments using strains in which pertinent components are compromised by genetic lesions.

Abstract 26

High calcium partially or completely normalizes the defects in motility and chemotaxis of mutants *sglA*$^-$, *chcA*$^-$ and *ptenA*$^-$, but not *mhcA*$^-$ or 3XASP

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High extracellular calcium (> 5 mM) and high extracellular potassium (40 mM) support efficient cell motility and chemotaxis. Several mutants of *Dictyostelium discoideum*, including *sglA*$^-$, *chcA*$^-$, *ptenA*$^-$, *mhcA*$^-$ and 3XASP, have been shown to have similar defects in polarity, the suppression of the lateral pseudopod formation, basic cell motility, chemotaxis and myosin II localization in the cell cortex, when analyzed in the buffered saline salts solution BSS, which contains potassium as its major cation. The behavioral defects exhibited in BSS are strikingly similar to those exhibited in tricine buffer containing a low concentration of extracellular calcium (< 5 mM). We show that high extracellular calcium, but not high extracellular potassium, can partially or completely normalize these defects in the mutants *sglA*$^-$, *chcA*$^-$ and *ptenA*$^-$, but not in the mutants *mhcA*$^-$ and 3XASP, leading to an interesting regulatory model.

Abstract 27

TWO MODELS FOR CELL MOTILITY

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Dictyostelium cells move up a gradient using a crawling motion. This crawling is characterized by pseudopodal extensions at the front of the cell, followed by retraction of the rear of the cell. Here we report preliminary results for two separate models that describe these processes.

The first model simulates pseudopodal extensions through the formation of membrane "patches" that are generated by a biochemical model. The second model describes the internal forces and the forces between the cell and the substrate and investigates the necessary conditions on these forces during motion.

Abstract 28

PSEUDOPOD FORMATION DURING MOVEMENT AND CHEMOTAXIS

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Dictyostelium cells extend pseudopodia for movement. To investigate how cells move and navigate we developed a computer algorithm to characterize the direction, size and timing of the extension of ~6000 pseudopodia by Dictyostelium cells in buffer and shallow cAMP gradients. Two types of pseudopodia may be formed: splitting of an existing pseudopod, or extending a de novo pseudopod at regions devoid of recent pseudopod activity. Split-pseudopodia are extended at ~60 degrees relative to the previous pseudopod, mostly as alternating Right/Left/Right steps leading to relatively straight zigzag runs. De novo pseudopodia are extended in nearly random directions thereby interrupting the zigzag runs. A shallow gradient of cAMP induces a small bias in the direction of pseudopod extension towards the attractant, which is amplified ~5-fold by the persistence of the subsequent pseudopodia to move in the same direction. Persistent movement is controlled by PLA2 and cGMP signaling to the cytoskeleton. Two other signaling pathways, PI3-kinase and a soluble guanylyl cyclase, determine the orientation of the emerging pseudopodia towards the cAMP gradient.

Abstract 29

A SECOND CHEMOTAXIS SYSTEM OVERLAPPING THE cAMP SYSTEM DURING AGGREGATION?

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We have discovered that aggregation-competent *Dictyostelium* amoebae possess a second chemotactic system that is separate and distinct from the classical cAMP chemotaxis system. Furthermore, through the application of a custom designed microfluidic chamber, we have been able to separate the chemotactic response of individual *Dictyostelium* cells to cAMP and to the newly discovered chemoattractant. The second chemoattractant, may be relayed with cAMP. We tentatively hypothesize that the two chemoattractants function simultaneously to orchestrate natural aggregation.

Abstract 30

LOSS OF AUTOPHAGY 9 RESULTS IN DEFECTS IN *DICTYOSTELIUM* DEVVELOPMENT, IN PHAGOCYTOSIS AND IN THE CLEARANCE AND REPLICATION OF *LEGIONELLA PNEUMOPHILA*.

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*equal contribution

The professional phagocyte *Dictyostelium discoideum* is a useful model for the study of the medically relevant infection of host cells by pathogenic microorganisms. The microarray-based analysis of differential gene expression of *D. discoideum* after infection with *Legionella pneumophila* resulted in the identification of 240 genes specific for the pathogenic response (Farbrother *et al*., *Cell. Microbiol.* 3:438-56, 2006). Of these more than 50 genes have clear orthologues in higher eukaryotes including man. They were placed into different functional categories and one category with three differentially regulated genes was macroautophagy (hereafter autophagy).

Autophagy contributes to many physiological and pathological processes, including starvation, cell differentiation, morphogenesis, programmed cell death, cancer and neurodegenerative disorders. In contrast, its possible involvement in the host response to pathogens is still a matter of debate. Therefore, we selected the up-regulated *atg9* gene for further studies. The ATG9 protein is highly conserved from yeast to man and yeast ATG9 is important in the formation of the pre-autophagosome structure (PAS), the site of autophagosome assembly. *Atg9* knock-out cells had growth defects in medium as well as on bacterial lawns and displayed severe developmental defects, consistent with the well-known role of autophagy in *Dictyostelium* development (Otto *et al.*, *J. Biol. Chem.* 279:15621-29, 2004). Surprisingly, the ATG9 mutant also had a strong phagocytosis defect that was particularly apparent when infecting the cells with *L. pneumophila*. However, those *Legionellae* that entered the host could multiply better in the mutant in comparison to wild-type cells. This appeared to be due to a less efficient clearance of *L. pneumophila* in the early and a more efficient replication in the late phase of infection in the ATG9$^-$ cells. Our results are consistent with a protective role of ATG9 and hence autophagy during pathogen infection.

Abstract 31

PHOSPHOINOSITIDE SIGNALING AND NRAMP PROTEINS IN DICTYOSTELIUM RESISTANCE TO LEGIONELLA INFECTION

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The Dictyostelium genome harbours two genes homologous to mammalian Nramp's (Natural Resistance Associated Membrane Proteins). Nramp's proteins are divalent metal cation transporters, with a preference for iron and manganese. Dictyostelium Nramp1 is located on endo- and phago-lysosomal membranes, while Nramp2 is exclusively found on the membrane of the contractile vacuole. Like the mammalian homolog, Dictyostelium Nramp1 is a resistance marker again invasive bacteria, such as mycobacteria, legionellae and salmonellae. Nramp1 disruption favours, while constitutive expression inhibits intracellular growth of legionella. Genetic or pharmacological inactivation of PI3K, but not PLC, results in enhanced legionella intracellular growth and reversion of the protective effect of Nramp1 constitutive expression. To be effective, PI3K inhibitors should be added at the onset of infection, delimiting a window of two-hours post-infection for their activity. The effect of the PI3K inhibitors is not mediated by increased bacterial uptake or alterations of the actin cytoskeleton, rather the Legionella appears to subvert a PI3K-dependent intracellular vesicle fusion pathway.

Abstract 32

GENETIC AND TRANSCRIPTIONAL EVIDENCE FOR BACTERIAL DISCRIMINATION IN D. DISCOIDEUM

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D. discoideum feeds within bacteria-rich soils so it is likely to have evolved mechanisms that enable it to discriminate among different bacteria. Such mechanisms would allow the amoeba to respond appropriately to different bacteria for optimal feeding and to potentially avoid exploitation by pathogens. tirA mutant amoeba grow poorly on the gram-negative bacterium Klebsiella aerogenes (Chen et al., Science, 317:678), but they grow normally on the gram-positive bacterium Bacillus subtilis. The previously defined bsg (B. subtilis growth defective) mutants exhibit the inverse phenotype (Newell et al., J. Gen. Microbiol. 100:207). We will describe the initial genes identified in a genetic screen based on these observations, as well as the changes in the transcriptome of amoebae growing on gram-negative versus gram-positive bacteria. Dictyostelium amoebae appear to have an intricately regulated response to different bacteria. Characterization of the molecular basis of this regulation should shed light on the antibacterial defense mechanisms of amoebae and may shed light on innate immune responses in other organisms.

Abstract 33

FARMERS COME IN MANY SHAPES AND SIZES: A NEW MICROBE SYMBIOSIS

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We have identified a novel facultative farming symbiosis between a eukaryotic soil amoeba, *Dictyostelium discoideum*, and one or more bacteria species. *D. discoideum* has long been known for its predation on bacteria which it engulfs by phagocytosis. We now report a continuing interaction with bacteria as *D. discoideum* enters its social stage, which turns a predator/prey relationship into a symbiosis. We show that about a third of all wild clones are able to carry bacteria through the sporulation process. We call these clones farmers because they then use the carried bacteria for food once the spores hatch. Those clones that farm differ from the non-farmers in a number of important ways including entering the social stage before exhausting all food sources, little slug migration, context-dependent differences in spore production, and the ability of farmers to immediately re-associate with their bacterial partners after the association has been disrupted using antibiotics. In addition to food bacteria, farmer clones consistently carry other bacteria species that are not eaten suggesting they may have other functions, possibly including defense. Microbes that can successfully interact long term with bacteria could offer insights into areas such as antibiotic resistance and this new, simple system has great potential.

Abstract 34

ADDRESSING THE FUNCTIONS OF ARRESTIN-DOMAIN CONTAINING PROTEINS IN *DICTYOSTELIUM*

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Besides canonical arrestins known to regulate GPCR-dependent signalling, a large family of arrestin-domain containing proteins (Adc) exists that harbors extra domains in addition to the arrestin core. Dictyostelium genome encodes 6 Adc proteins (AdcA-F) with extensions carrying lipid-binding and/or protein-protein interaction domains. AdcA contains a FYVE domain immediately downstream of the arrestin core and a histidine rich tri-repeat in the N-terminal part. We showed that AdcA is located on the macropinocytic and phagosomal pathways and restricted to the early compartments. A functional FYVE domain is necessary for endosomal recruitment of AdcA. The histidine-rich domain self-oligomerizes upon Zn2+ or Ni2+ binding and may therefore be responsible for the oligomeric state of the protein in vivo. We also showed that AdcA interacts with ArfA in a nucleotide-dependent manner. Because adcA disruption alters recycling from macropinosomes, our model proposes that AdcA functions to retrieve membrane proteins from the macropinosomes to allow their return to the plasma membrane via recycling endosomes.

Abstract 35

EFFECT OF STARVATION ON THE ENDOCYTIC PATHWAY OF *DICTYOSTELIUM* CELLS

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The behavior of Dictyostelium discoideum upon starvation is abundantly documented. However little is known about the effect of starvation on the endocytic pathway. Here we show that the general structure of the endocytic pathway is maintained in starved cells, but that its dynamics are significantly slowed down. The behavior of the endocytic pathway of apm3 and lvsB mutant cells during starvation was also evaluated. Some of the defects previously seen in these mutant cells in unstarved conditions persist when cells are starved, notably the slower transfer of endocytosed material between endocytic compartments. Other parameters, such as the rates of endocytosis or the fusion of post-lysosomes to the cell surface are affected differentially in starved and non-starved mutant cells. The results obtained in starved cells provide a different perspective and help to identify the primary defects resulting from a specific genetic inactivation event. Consequently, our results confirm the quantitative role of lvsB in the transit between lysosomes and post-lysosomes as well as the involvement of apm3 in the qualitative composition of endocytic compartments.

Abstract 36

Presenilin-Signaling Regulates Growth and Cell Fate Patterning: *Dictyostelium* as a Novel System for Functional Studies of the PS/γ-Secretase Complex

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Presenilin (PS) is the catalytic moiety of the γ-secretase complex. PS/γ-secretase components are well-conserved among metazoa, but presence/function in more distant species is not resolved. To elucidate molecular mechanisms intrinsic to presenilin function or dysfunction, we have examined PS/γ-secretase function in *Dictyostelium*. *Dictyostelium* have highly diverged orthologs for each PS/γ-secretase component, but lack endogenous APP, Notch, and other characterized PS/γ-secretase substrates. We created single and double mutants for the γ-secretase component genes. WT *Dictyostelium* is capable of amyloidogenic processing of ectopically expressed human APP to generate $A\beta_{40}$ and $A\beta_{42}$ peptides; strains deficient in γ-secretase components cannot produce Aβ peptides but accumulate "ectodomain shedding" intermediates of APP that are identical to α- and β-CTFs of mammalian cells. We further demonstrate that *Dictyostelium* require PS/γ-secretase for phagocytic growth and prespore/spore fate specification in a cell-autonomous manner, demonstrating that PS-signaling is an ancient process that arose prior to metazoan radiation.

Abstract 37

PLA_2 INHIBITION AND LIPID SIGNALLING IN *DICTYOSTELIUM*: CHARACTERISING THERAPEUTIC TARGETS IN VPA TREATMENT

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Phospholipase A_2 (PLA_2) proteins function to generate lysophospholipids and fatty acids contributing to phospholipid metabolism and cell signalling. PLA_2 activity is of potential therapeutic interest since enzymatic activity is increased in a range of medical conditions including seizures. The seizure-control drug Valproic Acid (VPA) is known to inhibit this signalling pathway.

Here, we employ *Dictyostelium* to analyse the mechanism of VPA-induced attenuation of PLA_2 activity, using the radio-labelled fatty acid arachidonic acid (AA) to visualise and quantify pathway activity. We show that VPA and related compounds inhibits PLA_2 signalling, giving rise to an increased accumulation (and reduced excretion) of lipid compounds. These lipid changes are distinct to that caused by specific PLA_2 inhibitors, suggesting a novel mechanism of action. We show that this VPA-catalysed effect is unlikely to be through reduced AA incorporation into lipids, and is unlikely to be related to other cellular effects of VPA (inositol attenuation and teratogenicity) as shown by structure/function studies.

This study therefore provides insight into the molecular mechanisms of PLA_2 inhibition by VPA.

Abstract 38

UNDERSTANDING SHORT CHAIN FATTY ACID UPTAKE MECHANISMS IN *DICTYOSTELIUM*

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Valproic acid (VPA), a branched short chain fatty acid, is the most commonly prescribed anticonvulsant, and despite over 40 years of use, the mechanism of its cellular uptake is currently unknown. Our central research interest concerns the molecular effects of VPA, using the simple model *Dictyostelium discoideum*.
Screening a *Dictyostelium* mutant library for isolates resistant to a VPA block in development identified one mutant lacking a human bicarbonate transporter family homologue, Slc4. Proteins related to this family have previously been linked to fatty acid transport, and the *slc4* null mutant show partial resistance to VPA during development and a reduced uptake of ^{3}H-VPA, thus indicating a role of the transporter in VPA uptake. ^{3}H-VPA transport was further characterized using anion transporter and proton gradient inhibitors, temperature and pH dependence, and various structural analogues of VPA.

These results implicate bicarbonate transporters as a novel and essential mechanism for VPA uptake.

Abstract 39

A HUNTINGTIN ORTHOLOG IN *DICTYOSTELIUM* AND ITS ROLE IN MULTICELLULAR DEVELOPMENT

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Huntington's Disease (HD) is caused by the expansion of a polyglutamine tract (polyQ) in huntingtin, a large HEAT repeat protein whose normal function is largely unknown. The absence of huntingtin in knock-out mice results in embryonic lethality. In contrast, null mutants in *Drosophila* are viable with no overt developmental defects. In an attempt to create a model system to elucidate huntingtin normal function, we generated a null mutant of the *Dictyostelium* huntingtin ortholog (DdHtt). *Dictyostelium* cells lacking endogenous huntingtin are viable. However, DdHtt-null cells stream poorly and appear to form aggregates largely by accretion. When assayed under phosphate buffer, DdHtt-null cells become round and aggregation is completely abolished. DdHtt-null cells exhibit delayed, asynchronous development and construct fruiting bodies with a glassy sorus containing round, defective spores. When developed as chimeras with wild type cells, DdHtt-null cells fail to populate the pre-spore region of the slug in a cell-autonomous manner. These phenotypes offer potential insight to defining normal functions of huntingtin that may be dysregulated in humans in HD and sets the stage for examining the fundamental role of this unique protein during multicellular development.

Abstract 40

THE UNCONVENTIONAL MECHANISMS OF SECRETION OF ACBA AND PROCESSING INTO SDF-2 SIGNALING PEPTIDE ARE CONSERVED IN MICE, YEASTS AND DICTYOSTELIUM.

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We have found that the yeasts Saccharomyces cerevisiae and Pichia pastoris secrete ACBP by an unconventional mechanism and process it into SDF-2 like peptide upon nitrogen starvation. As in Dictyostelium, GRASP is required for ACBP secretion in both yeasts. Mutants unable to produce SDF-2 activity in Dictyostelium and yeasts were identified and correspond to proteins involved in various membrane trafficking pathways.

In mice, as well as humans, ACBP has been shown to be the precursor of neuropeptides called endozepines or DBI. In the brain, these peptides compete for Diazepam (Valium) binding sites on GABAA receptors. We have used Dictyostelium encapsulation as a bioassay to study the production of endozepines by animal cells. We have found that astrocytes can be triggered to rapidly produce endozepines by addition of steroids. ACBP secretion is insensitive to BrefeldinA, an inhibitor of the classical secretion pathway. Astrocytes secrete ACBP by an unconventional pathway and process it extracellularly. They can also process recombinant Dictyostelium AcbA into SDF-2. The signaling pathway is being further investigated pharmacologically.

Abstract 41

A CYTOPLASMIC PROLYL HYDROXYLATION AND GLYCOSYLATION PATHWAY MODIFIES SKP1 TO REGULATE O_2-DEPENDENT DEVELOPMENT IN *DICTYOSTELIUM*

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ABSTRACT

Dictyostelium is an obligate aerobe that monitors O_2 for informational purposes. Whereas only 2.5% O_2 is required for proliferation, 12% is required for culmination, and O_2 influences slug polarity, slug migration, and cell-type proportioning. *Dictyostelium* expresses a cytoplasmic prolyl hydroxylase that mediates O_2-sensing in animals, but lacks the HIFalpha transcription factor subunit target. The major and possibly only substrate of *Dictyostelium* P4H1 is Skp1, a subunit of the SCF-class of E3-ubiquitin ligases related to the VHL-class that mediates hydroxylation-dependent HIFalpha-degradation in animals. The O_2-requirement for *Dictyostelium* culmination is increased by P4H1-gene disruption and reduced by overexpression. Reduction of Skp1 gene number reduces the O_2-requirement for culmination, whereas an increase raises the O_2-requirement and selectively disrupts spore differentiation in a hydroxylation-dependent fashion. In an O_2-dependent submerged development model that bypasses culmination, P4H1 is required for spore but not stalk cell differentiation. Skp1 hydroxyproline is modified by successive glycosylation reactions forming a pentasaccharide cap. Though disruption of the second glycosyltransferase gene does not affect the O_2-requirement, blocking addition of the fourth sugar is similar to blocking prolyl hydroxylation. The results suggest that Skp1 contributes to the breakdown of an unknown activator of culmination/sporulation by a mechanism that is inhibited by hydroxylation/glycosylation, and exerts modification-dependent dominant negative effects when overexpressed. It is proposed that, in *Dictyostelium* and other protists including *Toxoplasma gondii*, O_2-dependent prolyl hydroxylation and glycosylation act in hierarchical fashion to mediate environmental control of development, via protein degradation rather than indirectly via transcription as found in animals.

Abstract 42

CHEATER RESISTANCE IS NOT FUTILE: MECHANISMS OF CHEATING AND COUNTER-CHEATING IN D. DISCOIDEUM.

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Cheaters threaten sociality by exploiting the advantages of social behavior while avoiding the costs. In Dictyostelium, cheaters make more than their fair share of spores in chimerae. Many genes confer cheater phenotypes when mutated (Santorelli et al. 2008. Nature 451:1107-10) and chtC is one of the strongest cheaters. Here we show that chtC cheats by forcing its victims to become prestalk. That conclusion relies on the observation that tagA-null cells, that do not form stalks in chimerae with the wild type, are not exploited by chtC. We hypothesized that sociality could be protected from cheaters through the evolution of cheater-resistance. To test that possibility, we mixed populations of REMI mutants with chtC and collected spores after development. After 4-6 rounds of spore selection in chimerae, we found many mutants that resisted cheating. Intriguingly, most of the strains were not cheaters when tested against the wild type. We propose that the evolution of such noble cheater-resistant mutants is a mechanism that may protect social cooperation.

Abstract 43

TWO NOVEL SH2 DOMAIN PROTEINS INTERACT TO REGULATE DICTYOSTELIUM GENE EXPRESSION DURING GROWTH AND EARLY DEVELOPMENT

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Of the 13 Dictyostelium SH2 domain proteins only three are functionally unassigned. LrrB, one of the three, is comprised of two protein interaction domains: an SH2 domain and a leucine-rich repeat domain. Analysis of a null mutant indicates that LrrB is required for correct slug anatomy, phototaxis and culmination. Growth and early development appear normal in the mutant but expression-profiling reveals that three genes expressed in growth and early development are greatly under-expressed in the LrrB null strain. Orthodox TAP tagging indicates that LrrB binds to a 14-3-3 protein and that this binding changes during early development. Comparative TAP tagging shows that LrrB also interacts, via its SH2 domain and in a tyrosine phosphorylation dependent manner, with two structurally related proteins: CceA and CceB. Interaction of LrrB with CceA is of particular significance because CceA itself is one of the three functionally unassigned SH2 domain proteins. Thus, just as in metazoa but on a greatly reduced numerical scale, an interacting network of SH2 domain proteins regulates specific Dictyostelium gene expression.

Abstract 44

IDENTIFICATION AND CELL CYCLE-DEPENDENT LOCALIZATION OF NOVEL CENTROSOMAL CORE COMPONENTS IN DICTYOSTELIUM

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We have identified 34 new centrosomal candidate proteins through mass spectrometrical proteome analysis of isolated Dictyostelium centrosomes. We used GFP fusion proteins to localize the candidate proteins in whole cells and on isolated centrosomes. So far we have identified nine new genuine centrosomal proteins including four components of the heretofore almost uncharacterized centrosomal core structure, two corona proteins and two proteins localized at a zone between that of corona marker and core proteins. Two novel core proteins, CP39 and CP55, are involved in centrosome biogenesis. GFP-CP39 dissociates from the centrosome in prophase, presumably after centrosome duplication, and reappears at the spindle poles in telophase. Its overexpression elicits supernumerary centrosomes. Thus, it may serve as a seed for the aggregation of centrosomal material culminating in the formation of a cytosolic MTOC. In contrast, GFP-CP55 is localized to the centrosome during the entire cell cycle. The null mutant is characterized by bloated centrosomes and supenumerary, cytosolic MTOCs that were generated during mitosis. We hypothesize a role of CP55 in maintenance of structural coherence of the centrosome.

Abstract 45

THE CELL SHAPE CHANGES OF CYTOKINESIS

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Cytokinesis is a fascinating shape change in which a cell divides into two in only 5 min! Fundamentally, cytokinesis is a mechanical process, and initially, we focused on how myosin-II motors and actin-associated proteins interact to control these mechanics. We have also discovered a mechanosensory checkpoint controller that monitors cytokinesis shape change, and the enzymatic properties of myosin-II are precisely tuned for this control system though many of these same properties are expendable for cytokinesis itself. Finally, we have identified a pathway that includes the tumor suppressor 14-3-3, microtubules, and the RacE small GTPase, which modulates cortical mechanics and cytokinesis dynamics. Interestingly, 14-3-3 proteins are implicated in several human cancers and here we specifically link 14-3-3 to cytokinesis and cortical mechanics, two cellular processes where defects are linked to tumor progression and metastasis. In sum, we are beginning to connect the regulatory pathways to the mechanical networks that control cytokinesis shape change dynamics.

Abstract 46

REGULATION OF CELL-CYCLE GENES BY RETINOBLASTOMA IN *DICTOSTELIUM*

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The proliferative cycle of most eukaryotic cells is divided into G1, S, G2 and M phases. At the "major control point", just before the G1/S transition, genes required for the S-phase are activated by the transcription factor E2F. E2F is sequestered in early G1 by the retinoblastoma protein Rb. In late G1, there is an autocatalytic amplification of G1 cyclin transcription, triggered by nutrients or growth factors acting on the cyclin promoters. The G1 cyclins drive cyclin-dependent kinases to inactivate Rb, releasing E2F. Components of this pathway are found in such far-flung eukaryotic groups as animals and plants, diatoms and ciliates.

In *Dictyostelium*, a prominent G1 phase is lacking. At least one gene whose product is required in S-phase, *rnrB*, is expressed in growing cells in late G2. Three genes normally required at the G1/S transition, *cyclinD*, *cdh1*, and *emi1*, are expressed in Dicty primarily in developing cells. Dicty does have, however, an ortholog of the canonical G1 regulator Rb, *rblA*, which is expressed in growth and development. To clarify the role of this protein in the Dicty cell cycle, we have made transcriptional profiles of an *rblA* hypomorph using both microarray technology and massively parallel RNA sequencing. We find that the vast majority of presumed DNA replication genes are upregulated in this strain. These include genes coding for enzymes of deoxyribonucleotide metabolism, DNA polymerases, ligases, and topoisomerase, histones, chromatin assembly factors and the chromosome cohesion complex – over 70 genes in all. The regulation of these genes by Rb is thus conserved in Dicty, though the time point of their expression probably differs from other eukaryotes.

Specific proteins are also required during the mitotic phase of the eukaryotic cell cycle. The factors that induce mitotic genes, however, are obscure; mitosis is thought to be initiated not by transcriptional events, but by protein phosphorylation. In *Dictyostelium*, mitotic genes appear to be controlled by *rblA*. Over 80 such genes are overexpressed in the *rblA* hypomorph; these include genes for mitotic effectors such as condensins, kinesins, centrosomal, spindle and kinetochore components, and dynamins, as well as genes for regulatory proteins (aurora kinase, polo kinase, CDK1).

In *Dictyostelium*, lacking G1, it "makes sense" to have DNA replication and mitotic genes regulated by a common signal. Among eukaryotes, such a cell cycle could in principle be ancestral or derived. An ancestral, G1-less cell cycle is unfortunately hard to reconcile with modern concepts of eukaryote evolution. G1 regulation is clearly homologous in animals and

plants, so one would have to place Amebozoans near the root of the eukaryotic tree, and assume that G1 originated in a later, common ancestor of higher cells. This would require vastly different rates of evolution in different groups of eukaryotes. In addition, studies of gene fusions argue that "no extant eukaryote branched from the tree before the divergence of animals and plants" (*Current Biology* **13**, R665).

An alternative hypothesis is that *Dictyostelium* has rearranged a more complex cycle, which had different inducers for DNA-replication and mitotic genes. Such a cycle is known, for example, in budding yeast. Arguing against this idea is the breadth and uniformity of the transcriptional response to Rb in Dicty. ORC, MCM, RFC, GINS, and SMC are all multiprotein complexes required in chromosomal replication. In animals, plants, and fungi, less than half of the corresponding genes are cell-cycle regulated, while in Dicty, all are regulated by *rblA*. Among mitotic genes, 8 of 10 anaphase-promoting complex subunits are *rblA*-regulated in Dicty, while only one is modulated in the human cell cycle, and none/two are regulated in *S. cerevesiae/S. pombe*. One does not have the impression that Dicty cell cycle-related transcription was recently, disruptively reorganized.

A final interpretation is that the ancestral eukaryotic cell cycle had distinct G1/S and G2/M transitions, but that DNA-synthesis and mitotic genes were both regulated by Rb. The Dicty cell cycle would be only a modest simplification of such a scheme. There are in fact several hints that Rb regulates mitotic genes as well as S-phase genes in animal cells. A handful of mitotic genes have been identified as Rb-repressed in microarray studies in both mammals and insects. Rb family members are required for repression of mitotic genes in response to DNA damage. Most strikingly, proteins of "LINC" complex, which has been implicated in G2/M transcription in human cells, interact with Rb-family members in *Caenorhabditis* and *Drosophila*.

At least three LINC subunits have Dicty orthologs, and all are strongly overexpressed in the *rblA* hypomorph. All in all, G2/M transcriptional regulation by Rb may be very similar in Dicty and higher cells; *Dictyostelium* is thus a promising system for studying this new aspect of cell cycle biology.

Abstract 47

The polymorphic E-set repeat proteins Tiger-B1 (TgrB1) and Tiger-C1 (tgrC1) are self/non-self recognition molecules in *D. discoideum*.

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In nature, genetically distinct *D. discoideum* cells can co-develop, a condition that is susceptible to exploitation by cheaters. Kin recognition has been proposed as one mechanism to stabilize cell cooperation and confound cheating. We hypothesize that *tgrB1* (*lagB1*) and *tgrC1* (*lagC1*), genes that we have shown to be highly polymorphic in wild isolates of *D. discoideum* (Benebentos et al., Curr Biol. 19:567), function in kin recognition. The Tiger-C1 protein (TgrC1) is a transmembrane adhesion protein with multiple immunoglobulin-like domains, and *tgrB1* encodes a similar protein. *tgrB1* and *tgrC1* knockout mutants each segregate from the parental strain (AX4) during aggregation (and not from each other) whereas *tgrD1*, *csaA*, and *cadA* mutants do not, suggesting that differential adhesion alone does not account for the segregation. We have replaced the *tgrB1* and *tgrC1* genes in AX4 with divergent genes from wild strains and found that single gene replacements behave like *tgrB1* and *tgrC1* null mutants whereas double-replacement strains develop normally. The *tgrB1*/*C1* double replacement strains segregate from AX4 during development suggesting that these two Tiger proteins function as recognition proteins.

Abstract 48

"AN ALPHA-CATENIN HOMOLOGUE IN D. DISCOIDEUM AND ITS ROLE IN CELL-CELL ADHESION"

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In metazoans, Adherens Junction (AJ)-mediated cell-cell adhesion is essential for early embryonic development and is dysfunctional in cancer. AJs are composed of transmembrane cadherins and their cytoplasmic partner proteins alpha- and beta-catenin. We sought to understand to the evolution of AJs using Dictyostelium as a model system. We identified an alpha-catenin homologue in D. discoideum, which we named alpha(Dd)-catenin. A beta-catenin homologue called Aardvark has been previously described (Grimson et al. 2000). During development, alpha(Dd)-catenin is recruited to cell-cell contacts in an Aardvark-dependent manner. Disruption of the alpha(Dd)-catenin gene by homologous recombination results in abnormal fruiting body morphology, similar to loss of Aardvark. Although alpha(Dd)-catenin also has homology to the alpha-catenin related protein Vinculin, biochemical studies showed that alpha(Dd)-catenin is functionally more similar to metazoan alpha-catenin than vinculin. Our work provides insight into the evolution of multicellularity and paves the way for studies examining the role of Dictyostelium AJs in multicellular development and morphogenesis.

Abstract 49

THE ROLE OF COPINE A (CPNA) IN DICTYOSTELIUM DEVELOPMENT

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We are studying the role of CpnA in *Dictyostelium* development. Time-lapse imaging revealed that *cpnA*- cells exhibited delayed aggregation and made large mounds that did not break up into several fingers, as did the parental wildtype mounds. Each mound of *cpnA*- cells formed one large slug that was much less motile than the smaller slugs of the wildtype cells. The large slugs of the *cpnA*- cells displayed no phototaxis, negative thermotaxis, and did not culminate into fruiting bodies, although prespore and prestalk cell patterning appeared to be normal. When *cpnA*- cells were mixed with a small percentage of wildtype cells, the phototaxis, thermotaxis, slug size, and culmination defects were rescued. However, conditioned media from wildtype cells was not able to rescue the culmination defect suggesting that wildtype cells must be in close proximity for rescue. In EGTA, *cpnA*- cells formed fruiting bodies with short stalks. Preliminary studies suggest that *cpnA*- cells are more adhesive than wildtype cells suggesting CpnA's role in development may in part be to regulate calcium-dependent adhesion.

Poster Session I

Abstract P1

Glorin-mediated gene expression in *Polysphondylium pallidum*

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The recently established dictyostelid phylogeny (Science 314:661-663, 2006) suggests that intercellular communication with peptide acrasins such as N-propionyl-γ-L-glutamyl-L-ornithine-δ-lactam-ethylester (glorin) is an ancestral property, while communication with the "simpler" metabolites such as cAMP or pterins is a derived trait. Recent advances in whole-genome sequencing of *Dictyostelium discoideum* and *Polysphondylium pallidum* now provide the basis for a genome-wide survey of genes involved in glorin communication in ancient dictyostelids and evaluation of their fates in species that switched from peptide acrasins to other signaling systems. We will be reporting on first results of experiments aimed at investigating glorin-mediated gene expression in developing *P. pallidum* amoebae.

Abstract P2

CONSERVED ACTION OF ADENOSINE AND ITS ANTAGONIST CAFFEINE ON AGGREGATION AND PATTERN FORMATION IN CELLULAR SLIME MOLDS.

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Concentration gradients of morphogens during embryonic development determine the cell-fate of individual cells. However, it is not clear if morphogens identified in one group of organisms have a conserved and redundant action across all others in the same group. Using cellular slime mold *Polysphondylium* as a model, we examine the effect of adenosine, one of the morphogens identified in *Dictyostelium* promoting large aggregate formation and favouring stalk-cell differentiation. When adenosine is tested on a different genus *Polysphondylium,* there is indeed large aggregate and slug formation and there is a bias towards stalk cell differentiation pathway. This suggests there is an evolutionarily conserved aggregation pathway common to slime molds though they may use different signalling molecules as *Dictyostelium*, makes use of cAMP as a first messenger while *Polysphondylium* synthesises a modified dipeptide glorin in response to starvation. In the presence of adenosine, there is accelerated cell division, increased cell movement, an increase in intracellular glucose levels and enhanced glorin secretion all of which contributing towards large aggregate formation. Besides, adenosine and its antagonist caffeine overrides the effect of *Dictyostelium* mutants impaired in aggregate size and restore their parental aggregate size indicating that adenosine regulated pathway acts downstream of the mutant genes. Like in *Dictyostelium*, caffeine favours multiple tip formation in *Polysphondylium* indicating pathways that are common to slime molds even though their temporal patterns of stalk cell differentiation in these two organisms are differentially regulated.

Abstract P3

THE TIGER GENE FAMILY ENCODES TRANSMEMBRANE, IMMUNOGLOBULIN/E-SET REPEAT PROTEINS THAT HAVE DIVERSE ROLES IN CELL-CELL ADHESION, DEVELOPMENT AND KIN-RECOGNITION

Rocio Benabentos, [1] Shigenori Hirose,[2] W. Justin Cordill,[2] Yue Wang,[1] Anup Parikh,[1] Roshan Miranda,[1] Elizabeth Ostrowski,[3] Joan Strassmann,[3] David Queller,[3] Richard Sucgang,[2] Adam Kuspa,[1,2,3] and Gad Shaulsky[1,3]

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D. discoideum is susceptible to chimerism and to cheating but it has cellular recognition mechanisms that may allow it to avoid exploitation by discriminating and preferentially aggregating with closely related cells. The tiger (tgr, Transmembrane, IPT, IG, E-set, Repeat protein) gene family encodes single pass transmembrane proteins with repeated immunoglobulin-like domains. Members of this family are good candidates for having cell adhesion, communication and recognition functions because of their predicted localization at the membrane and their developmentally regulated expression. Also, some of the tiger genes show high polymorphism in natural populations and signals of positive selection indicating rapid evolution and possible roles in dynamic functions such as kin recognition. One example is the proteins TgrB1 and TgrC1 (formerly LagB1 and LagC1) which are putative co-receptors that mediate kin discrimination through allele specific interactions. We have performed experiments where we replaced one or both wild type alleles with the alleles from natural isolates to test this hypothesis. Our results support a role in kin recognition.

Abstract P4

CMF REQUIRES PLDB TO REGULATE G PROTEIN DISSOCIATION AS MEASURED BY FRET

Sibnath Ray, Yi Chen and Derrick Brazill

Hunter College

Quorum sensing in *Dictyostelium* is required for efficient entry into the development phase of its life cycle. Starving cells monitor the density of other starving cells by secreting and sensing Conditioned Medium Factor, CMF. When the density of starving cells is high, as measured by high levels of CMF, aggregation is allowed to proceed. CMF controls aggregation by regulating signaling through the chemoattractant cAMP receptor and its associated G protein, Gα2βγ. Here we use fluorescence resonance energy transfer (FRET) to monitor the dissociation of Gα2 from Gβγ. We find that along with cAMP, CMF increases the dissociation of the G protein. In fact, CMF can augment the dissociation induced by cAMP. The ability of CMF to affect dissociation is dependent on PldB, a phospholipase D orthologue. PldB is also required for CMF to decrease the cAMP-stimulated GTPase activity associated with Gα2. In addition, cells lacking PldB exhibit altered membrane localization of Gβγ. These results suggest the CMF alters the kinetics of cAMP-induced G protein signaling using phospholipase D activity

Abstract P5

HOW TO GO STRAIGHT

Robert Cooper, Ted Cox

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Chemotaxis in Dictyostelium modulates an underlying process for random motion. Even without external signals, this random motility already exhibits some structure. Wandering amoebas bias their random walk so that left turns are preferentially followed by right turns and vice-versa, thus creating a zig-zag path. This biased random walk results in a straighter path and a larger mean squared displacement than would a pure random walk. We investigate how Dictyostelium amoebas can create this zig-zag pattern of motion to extend their persistence length. In particular, a vector from the centrosome to the nucleus remains correlated with the previous direction of motion for several minutes into the future. Since the positioning of the nucleus toward the leading edge helps to stabilize pseudopodia, this persistence of this vector could help cells to maintain their directional persistence.

Abstract P6

Serine/threonine phosphatases in *Dictyostelium discoideum*: expression profile during development and heat, osmotic and oxidative stresses.

Layla Farage and Aline M. da Silva

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Analyses of *Dictyostelium* genome and yeast two hybrid screenings revealed at least 15 genes encoding catalytic subunits from ser/thr phosphatase (PSTP) family such as PP1, PP2A, PP2B, PP4, PP5 and PP6 and several genes encoding potential PP1 and PP4 partners. To survey the function of some *Dictyostelium* PSTP-related genes we examined their expression level under different conditions during growth and development. Using RT-qPCR, we found that PP1, PP4, PP5 and PP6 catalytic subunit genes are expressed throughout *Dictyostelium* life cycle while transcript levels of PP4 and PP6 increased upon exposure of growing cells to heat shock. Interestingly, we found an increase in transcript levels of three PP1 interactors – DdI-2, DdI-3 (Inhibitor-2 and Inhibitor-3 orthologs, respectively) and DDB_G0292194 (hypothetical ORF) – upon exposure of growing cells to heat shock. Hyper osmotic shock up-regulates the expression of both DdI-2 and DDB_G0292194 while only the latter was up-regulated by oxidative stress. In addition, both DdI-2 and DDB_G0292194 are up-regulated during development. These results suggest a role for these genes in stress responses and development. The potential novel PP1c interactor protein encoded by DDB_G0292194 bears a forkhead (FHA) domain near its N-terminus between two Asparagine-rich sites. A potential RVXF motif is found after the FHA domain. Using yeast two-hybrid assays, we found that interaction of DDB_G0292194 with PP1 catalytic subunit might be independent of FHA domain. Supported by FAPESP and CNPq.

Abstract P7

STUDIES ON DICTYOSTELIUM MYOSIN II KINASES AND PHOSPHATASES

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Dictyostelium discoideum myosin II heavy chain kinase A (MHCK A) plays key role in regulation of myosin II assembly and localization through phosphorylation of three residues located near the C-terminus of the myosin heavy chain. Phosphorylation drives myosin II from a filamentous to a monomeric state and plays a central role in inhibiting the cellular activity of myosin II. MHCK A has three distinct domains: a 50-kDa N-terminal coiled-coil region, 35-kDa central catalytic domain, and a C-terminal WD-repeat domain. Earlier studies have shown autophosphorylation as a critical activator for MHCK A in vitro. To understand the biochemical mechanism by which autophosphorylation activates MHCK A, and the in vivo roles of this autophosphorylation, we have initiated mass spectroscopy studies to map the autophosphorylation sites, and we have created a corresponding array of alanine and aspartic acid substitution mutant kinase constructs. Biochemical analysis and in vivo studies are in progress to assess the behavior of these mutant kinase constructs.

Biochemical studies indicate PP2A as the probably phosphatase responsible for MHC dephophosphorylation to allow myosin II assembly. We have created gene disruption lines for the PP2A "B55" targeting subunit. Recent studies on these cell lines will also be presented.

Abstract P8

TWO ROLES FOR AN ANTI-ADHESIVE PROTEIN DURING DICTYOSTELIUM GROWTH AND DEVELOPMENT

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The ampA gene encodes a novel protein that modulates cell adhesion 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. A supernatant source and synthetic peptides from the AmpA protein can prevent this misexpression. A model for AmpA acting via a lateral inhibition mechanism to prevent cells from assuming a prespore fate is proposed.

Expression in growing cells reveals a second function for AmpA. AmpA loss results in an increase in cell adhesion, and a reduction in F actin with a concomitant increase in G actin. Overexpression of AmpA reduces adhesion and increases F actin. As a result of these changes in the cytoskeleton and in adhesion, AmpA also influences cell migration. In comparison to wild type cells, AmpA null cells are defective in migration on top of agarose but migrate normally under agarose. AmpA overexpressing cells show the opposite behavior, migrating well on top of agarose but unable to migrate under agarose.

In order to determine how AmpA affects actin polymerization, wild type and AmpA over-expressing strains have been created containing an Actin binding domain fused to GFP. This has allowed us to visualize actin dynamics in living cells. Preliminary results suggest that a subset of AmpA over expressing cells polymerize significantly more actin than wild type and the excess actin appears to concentrate at the rear of the migrating cells.

Abstract P9

THREE GDT PROTEIN KINASES PLAY DIVERSE ROLES IN THE GROWTH-DEVELOPMENT TRANSITION IN *DICTYOSTELIUM DISCOIDEUM*.

Shigenori Hirose, Guokai Chen and Adam Kuspa
Baylor College of Medicine

Dictyostelium initiates a developmental program in response low food availability and multiple steps occur during the growth to development transition (GDT). The prestarvation response appears to initiates the GDT, and it prepares cells for subsequent nutrient shortage (Clarke et al., Dev Genet 9, 315-326). Within the GDT, a number of vegetative genes are down-regulated and developmental gene expression is induced. The end of the GDT process appears to be ensured by a process of commitment, which prevents cells from returning back to the growth phase even when presented with nutrients (Katoh et al., Eukaryot Cell 6, 2038-045).
The GDT tyrosine kinase-like protein kinase subfamily members have been reported to have phenotypes that implicate them in GDT regulation. We will describe data that provide new insights into the roles of GDT1, GDT2, and GDT4 in the growth to development transition.

Abstract P10

Identification of the mechanisms that control EGF-like peptide enhanced cell movement in *Dictyostelium discoideum*

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Cell movement is a fundamental cellular process. Although much is known about the signals and mechanisms that regulate this event, there are still many aspects that remain to be discovered. The Epidermal Growth Factor (EGF) stimulates cell movement in normal and cancerous cells by binding to the EGF receptor (EGFR). In mammalian cells, EGF-like (EGFL) peptides have also been shown to enhance cell movement by binding to the EGFR. We have recently shown that a synthetic EGFL peptide (DdEGFL1) based on the amino acid sequence of the extracellular, calmodulin-binding protein cyrA, enhances both random cell motility and cAMP-mediated chemotaxis in *Dictyostelium discoideum*. We are currently performing research to identify the receptor as well as downstream intracellular targets that are affected by DdEGFL1 treatment. Our research may ultimately identify an EGFL peptide dependent signaling pathway in *Dictyostelium* that may provide a model system for understanding how these peptides function in higher organisms.

Abstract P11

DIRECTIONAL MOVEMENT IN *DICTYOSTELIUM DISCOIDEUM* BY THE REPELLENT 8CPT-CAMP

Ineke Keizer-Gunnink

Rijks University, Grouingen Netherlands

In aggregation-competent Dictyostelium cells a gradient of cAMP induces directional upgradient movement. Directionality is determined by various signalling pathways, the major one resulting in upgradient PI(3,4,5)P3 patches, F-actin polymerisation and pseudopod formation. The cAMP-analogue 8CPT-cAMP is not a chemoattractant but instead a repellent. Previously we have presented a model where the downgradient movement by 8CPT-cAMP is induced by Gα1-mediated inhibition of PLC (versus Gα2-mediated stimulation of PLC by cAMP), leading to upgradient PI(4,5)P2 and association of PTEN and downgradient accumulation of PI(3,4,5)P3 (Keizer-Gunnink et al, 2007; J Cell Biol 177(4):579-85). Chemotaxis towards cAMP is mediated by multiple parallel signaling pathways, including RasC/G, PLC, PI3K, TORC2, PLA2, and sGC; inhibition of one pathway lead to a small reduction of chemotaxis, because other pathways are sufficient for chemotaxis. In this study we investigated the requirement of these signalling pathways for repulsion of cells away from 8CPT-cAMP. We identify multiple auto-activating loops that are triggered by 8CPT-cAMP with inverse spatial polarity.

Abstract P12

UNCOVERING COMPONENTS OF THE AMPA PATHWAY USING REMI MUTAGENESIS

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The ampA gene encodes a protein that modulates cell-cell and cell-substrate adhesions, and developmental patterning in Dictyostelium. Differences in phenotypes between AmpA wildtype, overexpressor, and knockout cells suggest a role for AmpA in growing cells as a secreted autocrine factor controlling cell adhesion and actin polymerization.

Current work focuses on identifying genes involved in AmpA mediated pathways. REMI mutagenesis was used to isolate second site suppressors of an AmpA overexpressing primary mutation. Screens comparing plaque size and substrate adhesion were used to identify suppressors. AmpA overexpressing cells produce large plaques on bacterial lawns and show decreased substrate adhesion, so the production of small plaques and increased adhesion would signify suppression.

Several candidate suppressor mutants were isolated; three of those candidate genes have been sequenced and shown to encode 1) a 9 spanning transmembrane protein, 2) a Sap DNA binding domain containing protein that also has a PTEN domain and 3) a protein containing a membrane bending Barr domain. The identification and characterization of the three genes is presented.

Abstract P13

A RAP/PI3K REGULATORY CIRCUIT CONTROLS PSEUDOPOD FORMATION

Arjan Kortholt[*§], Parvin Bolourani[†], Ineke Keizer-Gunnink[*],Gerald Weeks[‡], Alfred Wittinghofer[§], and Peter J.M. Van Haastert[*‖]

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Description:

During chemotaxis, Ras proteins are key intermediates in directional sensing, cellular motility and cell polarity. Rap proteins belong to the Ras superfamily of small GTPases. GbpD, a *Dictyostelium discoideum* guanine exchange factor specific for Rap1, has been implicated in adhesion, cell polarity and chemotaxis. Cells overexpressing GbpD are flat, exhibit strongly increased cell-substrate attachment, and extend many bifurcated and lateral pseudopodia. Phg2, a serine/threonine-specific kinase, mediates Rap1-regulated cell-substrate adhesion, but not cell polarity or chemotaxis (Kortholt et al., 2006). Using the strong GbpDOE phenotype the role of GbpD/Rap1 in cell polarity and chemotaxis was characterized in more detail. Together our data indicate that a GbpD/Rap/PI3K pathway controls pseudopod formation and cell polarity.

Kortholt, A., Rehmann, H., Kae, H., Bosgraaf, L., Keizer-Gunnink, I., Weeks, G., Wittinghofer, A. and van Haastert, P. J. (2006). Characterization of the GbpD-activated Rap1 pathway regulating adhesion and cell polarity in Dictyostelium discoideum. J. Biol. Chem. 281, 23367-23376.

Abstract P14

THE DEVELOPMENT AND APPLICATION OF A MICRO-MIXER AND DIFFUSION HYBRID MICROFLUIDICS DEVICE TO STUDY THE INDIVIDUAL EFFECTS OF MULTIPLE AND COMBINED CHEMOATTRACTANTS

Spencer Kuhl

University of Iowa

Several iterations of custom, one-of-a-kind microfluidics devices have been designed and constructed in collaboration with Translume Inc., of Ann Arbor, Michigan. Using syringe pumps to individually control flow rates through the device, we can induce dynamic or stable gradients and rapid changes in shear force, all within a very large range of flow rates due to the hybrid microfluidics design, which uses chevron-shaped micro-mixers and diffusion rather than pure diffusion to obtain highly manipulatable gradients. We will discuss the merits of using fused silica in the construction of the microfluidics device versus one time use PDMS (Polydimethylsiloxane). Factors taken into consideration include optical clarity, devise reusability, the capacity to use the device at much more extreme temperatures, and the ability to maintain structural integrity given high pressure within the chamber. Using this chamber, we discovered there may be two chemoattractant systems functioning during natural Dictyostelium aggregation.

Abstract P15

IDENTIFICATION OF UBIQUITIN CONJUGATES AT THE GROWTH-TO-DEVELOPMENT TRANSITION

Trudi Johnson, David Sky, and David F. Lindsey
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The ubiquitin processing protease, UbpA, is required for the growth-to-development transition. *ubpA*$^{-}$ cells accumulate ubiquitin-containing species that likely include ubiquitin conjugates of proteins that must be degraded for the growth-to-development transition to occur. To identify these proteins, we purified ubiquitin-containing species by S5a-affinity chromatography from both wild-type and *ubpA*$^{-}$ cells. MuDPIT mass spec analysis identified 102 proteins by more than one peptide and with higher peptide number in mutant than wild-type. In addition, 26 proteins containing GlyGly-modified lysine residues were identified. These included ubiquitin (Lys-6, 48, 53), components of phagosomes, and proteasome regulatory subunits repressed at the onset of development, such as PsmD8. In addition, we report that UbpA was required for growth-stage expression of *lmcA* and *lmcB*. Preliminary results suggest that LmcA and LmcB form a complex and may interact with a cytidine deaminase and ubiquitinated proteins, but not with ubiquitin. These results will lead to a better understanding of the mechanisms cells use to sense starvation stress and respond by making the transition from growth to development.

Abstract P16

The sodium-hydrogen exchanger Nhe1 acts as the putative "receptor" for the potassium effects on motility and chemotaxis.

Daniel F. Lusche, Daniel E. Ryerson, Deborah Wessels and David R. Soll

The W.M. Keck Dynamic Image Analysis Facility, Department of Biology, The University of Iowa, Iowa City, IA 52242

Extracellular calcium and potassium are major regulators of cell behavior (Lusche et al., 2009). They function in part by regulating myosin II localization in the posterior cell cortex, which in turn is essential for normal cell polarity, the suppression of lateral pseudopod formation, efficient cell motility and efficient chemotaxis. Calcium and potassium differ in their capacity to facilitate motility and the suppression of lateral pseudopod formation, leading to the hypothesis that they function through a different channel, exchanger or receptor at the membrane. Here, we provide evidence that the Na-H exchanger, Nhe1 (Patel and Barber, 2005), appears to be the functional "receptor" for the potassium effects. A strain lacking the exchanger is defective in basic motile behavior, chemotaxis, lateral pseudopod suppression and myosin II localization. These behavioral defects are completely rescued by incubating the cells in high extracellular calcium. High extracellular potassium, however, does not normalize these defects. We conclude that the exchanger is necessary for the effects on behavior of extracellular potassium, but not calcium.

Abstract P17

FUNCTIONAL ANALYSIS OF MAP KINASES ERK1 AND ERK2 DURING GROWTH AND DEVELOPMENT.

Nghia Nguyen, Brent Raisley, and Jeff Hadwiger.

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MAP kinases are often associated with the regulation of cell proliferation, cell differentiation, cell development and responses to stress. Only two MAP kinases, ERK1 and ERK2, exist in Dictyostelium with 57% identity in amino acid sequences. Whether ERK1 and ERK2 have overlapping functions or they separately function during growth and development still remain to be determined. We have compared the phenotypes of cells lacking either ERK1 or ERK2 and cells lacking both ERKs and found that ERK1 function has little effect on the developmental life cycle compared to ERK2. However, the slow plaque growth rate of erk1- cells on bacterial lawns compared to wild-type cells implies a possible role for ERK1 in cell proliferation. The analysis of chemotaxis and chemoattractant-stimulated phosphorylation of MAPKs suggests ERK1 is not necessary for response to cAMP or folate. The cytoplasmic/nuclear ratio of GFP-ERK1 or GFP-ERK2 does not appear to change when cells are stimulated by cAMP or folate.

Abstract P18

A STRATEGY FOR ANALYZING THE ROLE OF THE VARIOUS DOMAINS OF cAR1 IN THE CELLULAR ASSESSMENT OF SPATIAL GRADIENTS, TEMPORAL GRADIENTS AND NATURAL WAVES OF cAMP, AND THE RAPID ADDITION OF HIGH CONCENTRATIONS OF cAMP

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Mutants of cAR1 have been created in which various domains have been deleted. Expression of these GFP-tagged mutant receptors under the control of the wild type promoter provides a unique scenario for investigating the role of receptor domains in receptor localization, down regulation and the discrimination of natural cAMP wave information that includes positive and negative spatial gradients, positive and negative temporal gradients and high peak concentrations of cAMP. DIAS analysis of these receptor mutants using Zigmond-type and microfluidic chambers to separately generate the three types of information will provide new insights into cAR1 function.

Abstract P19

A SEARCH FOR GENES INVOLVED IN A QUORUM SENSING MECHANISM THAT REGULATES MATING COMPETENCY IN *DIDYMIUM IRIDIS*

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Didymium iridis is a sexually reproducing plasmodial slime mold with free-living haploid and diploid stages. A compatible mate is necessary for mating to occur, but cells must also be triggered to mate by high cell densities. We propose that a quorum sensing mechanism is responsible for this cell counting ability and that it regulates the transition to mating competency. In order to find genes involved in mating competency we used suppression subtractive hybridization. cDNA from mating competent cells was subtracted with cDNA from pre-competent cells; a total of 163 clones comprising 34 contigs were analyzed. Sixteen contigs were found to contain putative conserved domains; twelve contigs contained significant alignments to *Dictyostelium discoideum* (*E*-values were from 8.00 *E*-04 to 7.00 *E*-52). Eighteen sequences did not contain putative conserved domains, or have similarity to NCBI database sequences, with one exception that showed a significant alignment (to *D. discoideum E*-value, 2.00 *E*-23). Many of the conserved domains had functions consistent with quorum sensing, such as: transporter, secretion, transmembrane, and cell signaling.

Abstract P20

PHASE RESPONSE ANALYSIS OF CHEMOTACTIC MOVEMENT

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(2): ERATO Complex Systems Biology Project, JST

The directional movement of fast moving amoeboid cells is mediated by actin-filled protrusions of the cell surface pseudopodia. In the absence of external stimuli, pseudopodia are extended spontaneously in more or less random directions. We characterized the chemotactic response of Dictyostelium cells undergoing morphological changes to a spatially uniform cAMP stimulus. A circular map constructed around the centroid of a cell allows decomposition of the dynamic responses in cell shape into a small set of Fourier modes. By simultaneous visualization of F-actin reorganization using a fluorescent protein, a statistical relationship between F-actin localization at the cell cortex and the amplitude of the Fourier modes was derived. We demonstrate that the response time of cell shape change is determined by the phase of the second and third Fourier modes. Based on these results, we discuss how single cells adapt to a specific level of stimulus through a history-dependent process with respect to cellular shape change.

Abstract P21

GLYCOSYLATION OF RECOMBINANT *DICTYOSTELIUM DISCOIDEUM* CELL SURFACE GP130 IMPLICATED IN CELL ADHESION

Christa L. Feasley[a], Jennifer M. Johnson[a], Christopher M. West[a], and Catherine P. Chia[b]

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Abstract

The cell surface glycoprotein gp130 from the social amoeba *Dictyostelium discoideum* has been implicated in modulation of cell-cell and cell-substratum interactions. gp130 consists of an 85 kD polypeptide with 18 candidate N-glycosylation sequons and is probably GPI-anchored, and information about the N-glycans may help identify molecular determinants of the cellular activities. PNGase-F-digestion of a highly-purified, non-anchored version of s-gp130 secreted from *Dictyostelium* released 85% of all sugars, and the 29 kDa MW shift suggested that the majority of sequons are utilized. MALDI-ToF-ToF-MS and MS/MS analysis of native and permethylated glycans, sugar composition analysis, and exoglycosidase digestions indicated that the major N-glycan is a $Man_8GlcNAc_4$ species with bisecting and intersecting GlcNAc's, a glycan previously characterized in the 1980's before mass spectrometry. Low levels of $Man_8GlcNAc_3$, $Man_8GlcNAc_2$ and other glycans were also detected. PNGase-A digestion of s-gp130 released additional N-glycans consistent with core alpha3-fucosylation, which was confirmed by reactivity with a specific antibody. HPLC enriched 2-aminobenzamide-labeled N-glycans revealed lower abundance sulfated and methyl-phosphorylated high mannose N-glycans, structures also described previously. s-gp130 N-glycans were much less heterogeneous than total GPI-anchored or secretory protein glycans. N-glycan site mapping, based on incorporation of $H_2{}^{18}O$ or conversion of Asn to Asp during PNGase-F digestion, or retention of GlcNAc after Endo-H digestion, confirmed modification of 12 sequons, some variably, with glycans identified by enzymatic release. The majority of these were confirmed directly by MS analysis of proteolytic glycopeptides, based on mass matching, detection of glycan oxonium ions, and MS/MS sequencing. These findings will inform new strategies to probe the functions of N-glycans in gp130.

Abstract P22

THE EFFECT OF METHYLGLYOXAL ON CELL CYCLE IN *DICTYOSTELIUM DISCOIDEUM*

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Reduced glutathione (GSH) serves as a primary redox buffer and its depletion causes growth inhibition or apoptosis in many organisms. We reported that the null mutant ($gcsA^{-}$) of *gcsA* encoding gamma-glutamylcysteine synthetase shows G1 phase arrest and developmental defect upon GSH depletion. And the effect of GSH deprivation on growth and cell cycle was augmented by knockdown ($alrA^{as}$) of alrA (aldose reductase). AlrA was prominently induced in $gcsA^{-}$ and can metabolize methylglyoxal (MG). The concentration of intracellular methylglyoxal in $gcsA^{-}/alrA^{as}$ was higher than that of $gcsA^{-}$. The treatment of MG on wild type of *D. discoideum* showed similar effects on cells suggesting MG accumulated by GSH depletion is a primary factor inducing cell cycle arrest of $gcsA^{-}$ cells. To see how MG affects cell cycle, we investigate the effect of MG on well-known cell cycle regulation factors such as cyclin and cyclin-dependent kinase (cdk). We monitored cyclin mRNA expression pattern using Northern blot analysis and cdk activity. Northern blot analysis and activity measurement revealed that transcriptional level of cyclin B and cdk1 activity was changed by GSH depletion and MG treatment.
This work was supported by BK 21 and the Korea Research Foundation (KRF) grant funded by The Korea Government (MEST) (2009-0075708).

Poster Session II

Abstract P23

A PRIMER FOR BULK DATA EXCHANGE AT DICTYBASE

Siddhartha Basu, Yulia Bushmanova, Eric Just, Pascale Gaudet, Petra Fey, Warren A. Kibbe, and Rex L Chisholm

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dictyBase provides whole-genome data to facilitate synchronization between dictyBase and public repositories as well as supporting whole-genome analysis by our users. Genomic information and annotations are synchronized with public gateways such as GenBank, UniProtKB and the GO consortium. Data is exchanged bidirectionally, starting with an export of dictyBase annotations to GenBank. This data is automatically integrated into UniProtKB via TrEMBL, after which it is fed into the InterPro domain analysis pipeline. The resulting domain information is imported back into dictyBase.

The Downloads repository (http://dictybase.org/Downloads/) contains various types of curated data. DNA sequences and annotations for chromosomes, transcripts, and polypeptides can be downloaded in FASTA and GFF3 formats. Functional data provided are protein domains, phenotypes and gene ontology annotations, as well as phenotype and anatomy ontologies and publications. Mappings between dictyBase and UniProtKB identifiers along with gene names are also available.

The batch 'ID converter' tool allows translating various database identifiers using a customized input list. DictyMart, another bulk extraction tool, enables user-customized exports that group different identifiers with their genomic and functional datasets.

dictyBase is supported by grants from the NIH (GM64426 and HG00022)

Abstract P24

CLONING AND FUNCTIONAL CHARACTERIZATION OF *DICTYOSTELIUM DISCOIDEUM* FATTY ACID ELONGASE GENES

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Elongation of fatty acids is accomplished in the ER by the iterative addition of two carbon units to *de novo* synthesized fatty acids and provides long and very long-chain fatty acids for a wide range of complex lipids. *Dictyostelium discoideum*, possesses examples of the two distinct condensing enzyme families that catalyze the first reaction in fatty acid elongation; one, found primarily in plants, the 3-ketoacyl-CoA synthases (KCSs), and the other, the ELOs, which are found in mammals, microalgae, worm, fish, and plants. *Dictyostelium*, thus, represents a useful model for understanding fatty acid elongation in both animals and plants. Analysis of *D. discoideum* lipids revealed that fatty acid elongation is highly active and that both saturated and unsaturated fatty acids are substrates for elongation in *D. discoideum*. We have cloned the genes and cDNAs for each of the KCS and ELOs found in the *D. discoideum* genome. Functional characterization of DdKCS (CutA) demonstrated a substrate specificity for very long-chain saturated fatty acids, while EloA is highly active in the elongation of $16{:}1^{\Delta 9}$ to $18{:}1^{\Delta 11}$, the most abundant *D. discoideum* fatty acid. Progress toward identification of essential residues for the ELO-catalyzed condensation reaction will also be presented.

Abstract P25

GENETIC SCREEN WITH BARCODED REMI VECTORS IDENTIFIES D. DISCOIDEUM MUTANTS ALTERED IN RESPONSE TO CISPLATIN

Christopher Dinh**,** Bin Liu, Vy-Thao Dinh, June Hu, Thuy-Ngan Nguyen, Anup Parikh, Richard Sucgang, Gad Shaulsky and Adam Kuspa

Cisplatin is widely used to treat a variety of cancers. Despite its usefulness, cisplatin is toxic and often results in drug-resistant tumors. To improve our understanding of the molecular response of eukaryotic cells to cisplatin, we have carried out a genetic screen using barcoded REMI insertion vectors and parallel analyses of cisplatin resistance/sensitivity. We describe the development of 23,000 barcoded D. discoideum insertion mutants and our use of the relative abundance of barcodes as a surrogate for mutant fitness in mixed populations. We show that the change in the relative abundance of a barcode during the drug challenge is predictive of the phenotype of the mutant harboring the barcode vector. In our initial screens, we have identified over 100 mutants that are less sensitive than the parental strain to cisplatin. Since the deconvolution of barcode frequencies does not require recovery of viable cells, we have also identified to cisplatin sensitive mutants.

Abstract P26

THE GENE ONTOLOGY'S REFERENCE GENOME PROJECT: A UNIFIED FRAMEWORK FOR FUNCTIONAL ANNOTATION ACROSS SPECIES

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Complete functional annotation of genomes is a powerful tool for researchers; however, such annotation is a time-consuming task limited by the availability of experimental data. The function of genes for which there is no experimental data can often be predicted via comparison to related, annotated sequences of known function. We describe here the Reference Genome project, an effort from the Gene Ontology (GO) Consortium to fully annotate twelve genomes to rigorous standards: human, plus eleven organisms that are important models in biomedical research. We use phylogenetic relationships to infer the function(s) of ancestral proteins and propagate these annotations to their descendants. This endeavor faces many difficult challenges: the determination of reference protein sets; the identification of gene families for curation; the application of best practices for annotation; the development of methodologies for evaluating progress; and the development of software tools to support this effort.

This work is supported by NHGRI grant #HG002273 and NIGMS #GM081084-01A1.

Abstract P27

A VESICLE SURFACE TYROSINE KINASE REGULATES PHAGOSOME MATURATION

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Phagocytosis is crucial for host defense against microbial pathogens and for obtaining nutrients in Dictyostelium discoideum. Phagocytosed particles are delivered from phagosomes to lysosomes for degradation, but the molecular mechanism regulating phagosome maturation remains unclear. Using D. discoideum as a model system, we plan to reveal important components involved in phagosome maturation. We have identified 3 novel vesicle-associated receptor tyrosine kinases, VSK1-3, in D. discoideum. Our previous study suggests that localized VSK3 tyrosine kinase signaling on the surface of endosome/lysosomes represents a new control mechanism for phagosome maturation. My research focuses on identification of the targets of VSK kinases. This study will provide a foundation for understanding the molecular mechanism of VSK signaling that regulate phagosome maturation.

Abstract P28

DICTYAP, THE *D.DISCOIDEUM* ANNOTATION PROGRAM AT THE UNIPROTKB/SWISS-PROT DATABASE.

Jiménez S,[1] and the UniProt Consortium[1,2,3].

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The *Dictyostelium discoideum* annotation program, dictyAP, was established in order to promote the close collaboration between UniProtKB and the model organism database, dictyBase. These two databases are doing complementary work, and while dictyBase annotates all the genome relevant information, UniProtKB/Swiss-Prot is specialized in the comprehensive annotation of proteins.

Gene products that are well conserved across species can be used to create templates (which we call UniRules), which define annotations that can be propagated to confirmed matches. These gene products are used to build multiple alignments to produce eukaryotic-wide automated annotation. We have currently 1'300 *D.discoideum* candidates for the generation of such UniRules.

In Release 15.3 there are 3'748 *D.discoideum* entries in UniProtKB/Swiss-Prot. Of these 2'700 have orthologs in other species, 1'705 are associated with functional information, and 436 have evidence at protein level (protein sequence, X-ray crystallography information, post-translational modification etc..).

Our goal for 2009 is to provide the scientific community with entries corresponding to the *Dictyostelium discoideum* proteins that have been experimentally characterized.

Abstract P29

THE SURFACE AREA PROBLEM IN CELL MOVEMENT

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Moving cells measurably change in surface area as they change shape: for instance producing a pseudopod from a rounded cell can require an increase of around 20% in surface area over a couple of minutes. Since membranes are barely stretchable, we have proposed that cells can adjust the extent of their plasma membrane by regulating their rates of endocytosis and exocytosis. To test the role of exocytosis in cell movement, we examined a fast-acting temperature sensitive mutant in an exocytic gene – secA – isolated by Mark Bretscher. At the restrictive temperature, cells of this mutant rapidly cease movement, though they can still be polarized by a cAMP gradient; and though actin is polymerized under the membrane, it cannot drive extension of a pseudopod. We further speculate that cells might adjust the balance of their endocytic cycle through sensing membrane tension using stretch operated channels. Three such channels predicted from the genome of been knocked out and the phenotypes of the mutants will be reported.

Abstract P30

SPECIFIC ROLES OF MAGNESIUM AND A PHAGOSOMAL P-ATPASE IN INTRACELLULAR BACTERIAL KILLING.

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Presenter : Marion Le Coadic

University Medical Center, Dpt of cell physiology and metabolism

Bacterial ingestion and killing by phagocytic cells is essential to protect the human body from infectious microorganisms. Production of reactive oxygen species, lysosomal enzymes and the specific ionic composition of phagosomes may all contribute to intracellular bacterial killing. Here we used *Dictyostelium discoideum* amoebae to study intracellular killing mechanisms. In a random genetic screen, we identified Kil2, a new gene product specifically involved in killing of gram-negative *Klebsiella pneumoniae* bacteria. Kil2 is a type V P-ATPase present in the phagosomal membrane. Knockout kil2 cells did not kill efficiently *Klebsiella*, but this defect was rescued by the addition of magnesium ions, suggesting that Kil2 may function as a magnesium pump in the phagosomal membrane. Interestingly, kil2 knockout cells still killed efficiently gram-negative *Pseudomonas aeruginosa* as well as gram-positive *Bacillus subtilis*, and did not show enhanced susceptibility to *Mycobacterium marinum* intracellular replication. Systematic analysis of killing-defective mutants will hopefully lead to a better understanding of the complex relationships between phagocytic cells and pathogenic bacteria.

Abstract P31

The role of Copine A in phototaxis and thermotaxis in developing slugs of Dictyostelium discoideum

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Copines are a group of membrane binding proteins of unknown function found in many eukaryotic organisms. *Dictyostelium* have six copine genes and we are focusing on copine A (*cpnA*). When under starvation conditions, *Dictyostelium* cells aggregate into mounds that become migrating slugs, which exhibit phototaxis and thermotaxis. To study the role of *cpnA* in development, we compared the slug behavior of cells lacking the *cpnA* gene (*cpnA*- cells) to the slug behavior of wildtype cells. We found that the *cpnA*- slugs were much larger than wildtype slugs. In addition, we found that *cpnA*- slugs exhibited no phototaxis and negative thermotaxis, while wildtype slugs exhibited positive phototaxis and thermotaxis. When *cpnA*- cells were mixed with a small percentage of wildtype cells (5-10%), the phototaxis, thermotaxis, and slug size defects were rescued. Our results indicate that *cpnA* plays a role in intercellular signaling important in regulating slug size, phototaxis, and thermotaxis.

Abstract P32

UNDERSTANDING THE EFFECT OF POPULATION STRUCTURE ON SOCIAL INTERACTION IN *D. DISCOIDEUM*

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Confronting starvation, solitary *Dictyostelium discoideum* can co-aggregate with cells of different genetic background to form multicellular fruiting bodies. The fact that only the spores are viable while the stalk eventually dies arises questions with respect to social interaction and altruistic cooperation. We ask how population structure affects social behavior in *D. discoideum*. A molecular population genetic analysis of 24 natural *D. discoideum* strains show that they fall into three distinct population groups based on genetic ancestry and relatedness. We test if a higher degree of kin discrimination is observed in chimeras composed of within-group vs. between-group strain pairs. The relative amount of strains in chimeric fruiting bodies formed from equal amounts of two *D. discoideum* individuals were quantified by analysis of SNP levels via pyrosequencing. Greater variance in relative levels of strains in different chimera comparisons would indicate a higher level of discrimination and poorer cooperation among non-identical individuals. Statistical analysis revealed that both the degree of chimerism and dominance are mainly strain-dependent and chimeric-specific, although there does appear to be a significant difference in within- vs. between-group chimeras.

Abstract P33

ISOPRENYLCYSTEINE METHYLATION IS REQUIRED FOR NORMAL GROWTH AND ENDOCYTOSIS IN *DICTYOSTELIUM*

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Many members of the Ras superfamily of small monomeric GTPases require methylation of isoprenylated carboxy-terminal cysteine residues for proper localization and activity. Because Ras GTPases are often overactive in malignancies, inhibitors of isoprenylcysteine methyltransferase are being tested as potential antitumor drugs. The cellular effects of these inhibitors are poorly understood, however. We are characterizing the effects of loss of isoprenylcysteine methyltransferase activity on *Dictyostelium* amoebae, with an emphasis on characterizing defects of growth and vesicle trafficking. Amoebae lacking methyltransferase activity grow slowly on bacterial lawns and fail to grow in shaking or adherent axenic culture. Methyltransferase-deficient cells also migrate slowly towards folate and have reduced rates of endocytosis. The altered signaling pathways that lead to these defects are being further investigated.

Abstract P34

UNDERSTANDING CLATHRIN-MEDIATED ENDOCYTOSIS IN *DICTYOSTELIUM*

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In eukaryotes, clathrin-mediated endocytosis (CME) is an essential process required for the internalization of membrane receptors. In addition to clathrin, a vast network of accessory and regulatory proteins are required for CME. Many components of this CME machinery exist in *Dictyostelium* providing an opportunity to study the physiological role of CME during single and multicellular stages. However, while the role of clathrin in osmoregulation and cytokinesis in *Dictyostelium* has been documented, demonstration of a role for clathrin in endocytosis has been lacking due to the absence of a bona fide *Dictyostelium* CME receptor. We are using biochemical approaches to identify membrane proteins that are internalized in *Dictyostelium* by CME. Additionally real-time imaging of fluorescently tagged components of the CME machinery allows us to study the dynamics of these proteins. In combination these studies will provide information about the role of CME in *Dictyostelium*, as well as making *Dictyostelium* more amenable as a model system for studying CME.

Abstract P35

Identification of the target proteins of CpnA in Dictyostelium

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Copines are calcium-dependent membrane-binding proteins found in diverse eukaryotic organisms. Copines are characterized by having two C2 domains at the N-terminus followed by a VWA domain at the C-terminus. Copines are thought to act in signaling pathways bringing target proteins to membranes in response to an increase in calcium concentration. Experiments using affinity chromatography and mass spectrometry indicate that discoidin, S-adenosylhomocysteine hydrolase (SAHH), and actin are candidate target proteins of CpnA. To corroborate these findings, we are performing immunoprecipitations using an antibody to GFP with *Dictyostelium* cells expressing GFP, GFP-CpnA, or GFP fused to the VWA domain of CpnA (Ado). The GFP antibody pulls down GFP and the GFP-fusion proteins and VatD co-precipitated with GFP-CpnA. Western blots using antibodies against these candidate target proteins indicated that discoidin and SAHH were not pulled down specifically with CpnA or the Ado. Yeast-two hybrid with Ado as the bait will be performed to identify and verify the interaction between candidate proteins and CpnA in *Dictyostelium*.

Abstract P36

EXPLORING THE TRANSCRIPTIONAL REGULATION OF FBIA, AN EVOLUTIONARILY-CONSERVED PROTEIN INVOLVED IN CELL-TYPE PROPORTIONING IN *DICTYOSTELIUM DISCOIDEUM*

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FbiA is an evolutionarily-conserved protein identified via its interaction with the WD-40 repeat region of FbxA, a component of an SCF E3 ubiquitin ligase. This interaction, as well as the phenotypes of null mutants, suggests that FbxA-mediated ubiquitination of FbiA plays a role in cell-type proportioning. Here, however, we focus on transcriptional regulation of *fbiA*. Prior analysis via RT-PCR and *in situ* hybridization revealed a complex, developmentally-regulated expression pattern. Of particular interest was the gradient visible in the prespore region of preculminants at the start and end of peak expression. We have used *in situ* analysis of mutant strains to investigate a potential role for SDF-2 in this regulation. We have also used reporter constructs to identify an 800 bp region upstream of *fbiA* sufficient to confer accurate temporal and spatial regulation. Staining from a reporter expressing a labile version of β-galactosidase confirms the prespore enrichment previously observed, whereas data from a stable β-galactosidase reporter suggest that *fbiA* is expressed early in development in a subset of cells that go on to form prestalk cells.

Abstract P37

UNDERSTANDING FUNCTIONAL ROLE OF POLYKETIDE SYNTHASES AND ASSOCIATED PROTEINS IN *DICTYOSTELIUM DISCOIDEUM*

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Dictyostelium genome analysis has revealed the presence of an unprecedented number of Polyketide Synthases (PKSs). PKSs are multi-functional enzymes, capable of synthesizing diverse metabolites with varied biological functions. Our lab is interested in dissecting out how these forty functional PKSs (DiPKSs) could play a role during the morphogenesis of this organism. Recently, we showed that one of the developmental regulating factors, MPBD, is a biosynthetic product of DiPKS1. By combining genetic and biochemical studies, we are presently investigating the relevance of DiPKS2. Transcriptional profile reported earlier suggests that this gene shows differential temporal expression (4-10 h and 22 h of development). Our studies further indicate cell-type specific expression of dipks2. We are also attempting to identify metabolites produced by this PKS. However, in order to obtain functional form of PKSs, a key step involves their post-translational modification by phosphopantetheinyl transferases (PPTases). We have identified 2 classes of PPTases from *Dictyostelium* and shown their distinct functions and relevance in its biology.

Abstract P38

COPINE A EXPRESSION IN WILDTYPE CELLS AND PRESTALK AND PRESPORE PATTERNING IN CPNA- CELLS WITHIN DEVELOPING DICTYOSTELIUM

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Copines comprise a family of calcium-dependent, membrane-binding proteins found in various eukaryotes. Cells lacking the cpnA gene are arrested in the finger/slug stage of development. To determine if the prespore and prestalk cell localization and patterning is disrupted in cpnA- cells, plasmids containing prespore (cotB, pspA) and prestalk (ecmAO, ecmB) marker gene promoter sequences upstream of the lacZ gene were transformed into wildtype and cpnA- cells, allowing for the localization of prespore and prestalk cells to be mapped within developing structures when stained with x-gal. In addition, the cpnA promoter sequence was cloned into a plasmid upstream of the lacZ gene and the plasmid transformed into wildtype cells to determine the cpnA expression pattern in developing Dictyostelium. Our results indicate that prespore and prestalk patterning is normal in cpnA- cells until development arrest at the finger/slug stage. Preliminary studies suggest that cpnA is preferentially expressed in the prestalk region during the slug stage, but is expressed in prespore cells during culmination and spores within the fruiting body.

Abstract P39

COLCHICINE AFFECTS PATTERN AND POLARITY IN *DICTYOSTELIUM DISCOIDEUM* VIA A MECHANISM NOT INVOLVING MICROTUBULES.

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The microtubule disrupting drug, colchicine, has been shown to have an effect on pattern and polarity in many biological systems. In *Dictyostelium*, colchicine has been shown to induce multiple axis formation and stalk cell differentiation. At 0.01M, colchicine prevented pseudoplasmodia from culminating while terminal stalk cell differentiation occurred at the posterior end. At 0.05M, colchicine led to the reorganization of single pseudoplasmodia to form several mounds. Each mound then accumulated mature stalk cells at its posterior end. Here we show that colchicine's effect on the establishment of pattern and polarity in *Dictyostelium* is independent of its microtubule-disrupting activity. Pseudoplasmodia treated with nocodazole and vinblastine, other microtubule-disrupting drugs, at concentrations that disrupted microtubular networks, as verified by immunostaining, resulted in the formation of normal fruiting bodies. Interestingly, colchicine also increased motility of *Dictyostelium* amoeba, up to 580% of control, an effect not seen with nocodazole or vinblastine. Overall, colchicine is affecting pattern and polarity via some non-microtubule mediated mechanism that may involve alterations in cell motility.

Abstract P40

CONSERVATION AND CHANGE IN CYCLIC AMP SIGNALLING GENES DURING SOCIAL AMOEBA EVOLUTION.

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cAMP plays a ubiquitous role in controlling almost all aspects of *D.discoideum* development. As a secreted signal it regulates expression of different classes of genes and coordinates cell movement during aggregation and morphogenesis. As an intracellular messenger it controls entry into development, spore and stalk cell maturation and spore germination. About 13 proteins are directly responsible for synthesis, detection and degradation of cAMP, while a much larger number is involved in upstream regulation of these proteins or downstream processing of the cAMP signal.

In the past 10 years we have used a combination of gene amplification and functional gene analysis across species to retrace patterns of conservation and change in some cAMP signalling genes across all Dictyostelids. This work will be summarized and used to formulate a scenario how the diverse cAMP signalling mechanisms in modern dictyostelids evolved from a stress response in their unicellular ancestors.

Very recently sequencing and assembly of the genomes of the early diverging Dictyostelids *P.pallidum* and *D.fasciculatum* were completed. This provides tremendous opportunities for understanding how cell- and developmental processes in the Dictyostelids evolved. Conservation and change in broad set of genes associated with cyclic nucleotide signalling will be discussed.

Abstract P41

dictyExpress: an interactive web interface to *D. discoideum* gene expression data

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In the past we have deposited *D. discoideum* gene expression profiles as static pictures on dictyBase, or in supplemental data files of relevant publications. We are replacing this mode of data access with an interactive application, which renders several useful visualizations, supports queries, and allows the user to perform data analytics. dictyExpress is an interactive web-based application that incorporates the Baylor College of Medicine expression database and an analytical server. dictyExpress supports data retrieval, gene selection, display of expression time courses, gene ontology enrichment analysis, hierarchical clustering, retrieval of genes correlated with a given target profile and linking to dictyBase. The application is available at www.ailab.si/dictyexpress and can be linked directly from specific dictyBase gene pages. Future versions will include RNA-sequencing data. We will provide short tutorials at the poster session for people who bring their laptop computers

Abstract P42

COPINE A HAS A ROLE IN CALCIUM-REGULATED INTERCELLULAR SIGNALING DURING DICTYOSTELIUM DEVELOPMENT

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Copines are calcium-dependent membrane-binding proteins found in numerous eukaryotic organisms including Dictyostelium. Cells lacking CpnA are arrested in the finger/slug stage of development. Time-lapse imaging revealed that cpnA- cells formed larger mounds and less motile slugs than wildtype cells. Mixing cpnA- cells with a small percentage of wildtype cells rescued the developmental defect. The percentage of wildtype spores in these chimeric fruiting bodies was the same as in the original mixture of cells. Wildtype conditioned media was unsuccessful in rescuing the developmental defect in cpnA- cells, which suggests that cell proximity is important for rescue. In the reverse mixing experiment, a small percentage of cpnA- cells did not preferentially differentiate into stalk or spore cells when mixed with wildtype cells. When cpnA- cells were developed in EGTA, they were able to form fruiting bodies, but with short stalks. Preliminary studies indicate that cpnA- cells are more adherent than wildtype cells. Our results suggest that CpnA has a role in calcium-regulated intercellular signaling during Dictyostelium development.

Abstract P43

KNOCKDOWN OF *DICTYOSTELIUM* GENES BY RNAI USING A TETRACYCLINE INDUCIBLE POLYMERASE III PROMOTER

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RNA interference (RNAi) is a now standard technique for analyzing gene function in some eukaryotes. Our lab had previously used long inverted repeat DNA sequences (~1200 bases) to mediate RNAi-induced silencing of adenylyl cyclase A, phosphoprotein phosphatase (PP2A) and transcription factor MybB to illustrate their involvement in growth and development of *Dictyostelium discoideum* (Otsuka *et al*, unpublished work). Here, we report the use of a tetracycline (tet) inducible RNA polymerase III (Pol IIII) promoter-based system to regulate the expression of short oligonucleotides (~20-21bp) sequences targeting the catalytic subunit of phosphoprotein phosphatase PP2Ac or *Dictyostelium* homologs of kelch repeat proteins (KRH1 and KRH2). In the presence of tetracycline PP2A-RNAi transformed cells were arrested at the mound stage, whereas development was delayed in KRH-RNAi cells. These results indicate that PP2A and KRH proteins are essential for normal development. Therefore, the tet inducible system may be highly efficient in synthesis of small RNAs and can be used to characterize genes involved in development of *Dictyostelium*.

Supported by NIH G12 RR03037

Abstract P44

THE DICTY STOCK CENTER - 2009 UPDATE

Marc Vincelli, Petra Fey, Jakob Franke, Rex L. Chisholm, and Pascale Gaudet,

DictyStocks at Northwestern University

The Dicty Stock Center is a central repository for Dictyostelium discoideum strains, isolates of other cellular slime mold species, plasmids, and commonly used bacteria. These rapidly growing collections currently contain 1440 different strains and 508 plasmids. The catalogs are continuously updated. The strain collection includes: 300 natural isolates, 100 different species, over 350 null mutants, and over 150 marked strains. Dictyostelium strains are curated during the process of literature based curation at dictyBase. Strains and their phenotypes are linked to genes in dictyBase. During the past year all of the Stock Center materials were transferred from Columbia University to Northwestern University. The Dicty Stock Center at Northwestern re-opened in April 2009 when the new grant for the stock center began. Between April and mid-June, we have received 88 orders (close to 10 orders a week). In total, 153 strains and 186 plasmids were requested, with an average of 4 items per order, with orders coming from 12 different countries. The Stock Center encourages all community members to deposit strains as they are published.

The Dicty Stock Center is funded by NIH grant 1R01GM087371-01.

Abstract P45

GENE REGULATION BY DIF AND IDENTIFICATION OF A NEW PRESTALK CELL SUB TYPE

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The DimB and MybE transcription factors bind to sites in the ecmA promoter and are required for DIF induction of ecmA in monolayer assay. Genome-wide micro-array analyses on parental, mybE- and dimB- cells identified additional genes that depend on MybE and DimB for their DIF inducibility. Surprisingly, it also identified many other genes that are induced by DIF but under negative control of MybE and DimB. Therefore there presumably is an alternate DIF activation pathway for these genes. Further characterization of rtaA, one of the MybE and DimB repressed genes, identified a new sub-set of the anterior like cells that become the upper cup, which we term pstU. Two other prestalk sub-types, pstO and pstB cells, are also induced by DIF and this raises an issue of inductive specificity. Differences between precursor cells in the activity of DimB and MybE may explain one signaling molecule DIF can induce more than one cell type.

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