International *Dictyostelium* Conference **2006**

Hotel La Fonda, Santa Fe, New Mexico, USA
Sept 17-22, 2006

Organizers: **Adam Kuspa**
Richard Gomer
Richard Sucgang
Meeting Coordinator: **Charlotte Cherry**

TIME	SUN, 9-17	MON, 9-18	TUES, 9-19	WED, 9-20	THURS, 9-21	FRI, 9-22
7:00-8:30		BREAKFAST	BREAKFAST	BREAKFAST	BREAKFAST	BREAKFAST
8:30-10:10		**Session I** **Cell Adhesion** *A. Kuspa*	**Session III** **Development I** *C. Thompson*	**Session VI** **Polarity & Chemotaxis II** *C. Chung*	**Session IX** **Development III** *L. Kim*	
		M.B. Duran 1 A.S. Kowal 2	D. Brazill 1 Y. Tang 2	R. Kay 1 A. Kortholt 2	N. Heise 1 D. Bakthavatsalam 2	
		Session II **Genome Analysis** *L. Eichinger*	V. McMains 3 M. Katoh 4	D. Wessels 3 D. Soll 4	J. Hadwiger 3 M. Cabral 4	
		D. Queller 1 R. Kay 2				
10:10-10:40		BREAK	BREAK	BREAK	BREAK	
10:40-12:10		**Session II, cont'd** **Genome Analysis** *L. Eichinger*	**Session III, cont'd** **Development I** *C. Thompson*	**Session VI cont'd** **Polarity & Chemotaxis II** *C. Chung*	**Session X** **Phagocytosis** *C. Damer*	
		W. Nellen 3 R. Sucgang 4 R. Chisholm 5	V. Sonakya, R. Dottin 5 A. Kuspa 6 G. Shaulsky 7	C. Beta 5 X. Xu 6 W. Rappel 7	M. Clarke 1 T. Jin 2 T. Khurana 3	
12:10		LUNCH	LUNCH	LUNCH	LUNCH/Boxed	
13:00		Free Time 13:00-19:00	Free Time 13:00-16:00	Free Time 13:00-16:30	**BANDELIER** 13:00-18:00	
16:00	**REGISTRATION**		**Session IV**			
16:30			**Polarity & Chemotaxis I** *T. Jin*	**Session VII** **Actin and Cytoskeleton** *C. Parent*		
			R. Teo 1 A. Harwood 2 L. Kim 3	A. M-Taubenberger 1 D. Robinson 2		
17:15-17:45			BREAK	BREAK		
17:15			**Session IV cont'd** **Polarity & Chemotaxis I** *T. Jin*	**Session VII cont'd** **Actin and Cytoskeleton** *C. Parent*		
			P. Kriebel 4 M. Lombardi 5 R. Firtel 6	P. Steimle 3 C. Saxe 4 C. Chung 5		
19:00-20:30	**RECEPTION**	DINNER 19:00-20:30	DINNER 19:00-20:30	DINNER	DINNER & Entertainment 19:00-22:00	
20:30-22:00	19:00-21:00	**POSTER SESSION**	**Session V** **Development II** *M. Katoh*	**20:00 Session VIII** **Oxygen, Stress & Death** *D. Brazill*		
		Note: Posters will be up all week	C. Thompson 1 J. Williams 2 P. Schaap 3 W. Loomis 4	C. West 1 Z. Wang 2 J. Na 3 21:15-21:30 BREAK		
				21:30-22:20 Session VIII cont'd **Oxygen, Stress & Death** *D. Brazill*		
				G. Souza 4 P. Golstein 5		

I Cell Adhesion 9/18/2006, from 8:30 to 9:20.

Chair: Adam Kuspa

1	M. Berenice Duran	Overexpression of the *Dictyostelium discoideum paxB* gene interferes with normal cell-cell cohesion, cell-substrate adhesion, and cell sorting
2	Anthony S. Kowal	Elucidating the role of *Dictyostelium discoideum* SadA protein in cell-substrate adhesion

II Genome Analysis 9/18/2006, from 9:20 to 12:10.

Chair: Ludwig Eichinger

1	David Queller	Microsatellite evolution; mutation rates and variability
2	Robert Kay	Copy number variation between *Dictyostelium discoideum* strains
3	Wolfgang Nellen	DNA Methylation and epigenetic gene silencing in *Dictyostelium*.
4	Richard Sucgang	Process engineering high throughput restriction enzyme insertional mutagenesis in *Dictyostelium*
5	Rex L. Chisholm	dictyBase Update

III Development I 9/19/2006, from 8:30 to 12:10.

Chair: Chris Thompson

1	Derrick Brazill	Quorum sensing in *Dictyostelium discoideum* is dependent on Phospholipase D activity.
2	Yitai Tang	CnrN, a putative PTEN-like phosphatase, is required for counting factor-mediated group size regulation in Dictyostelium
3	Vanessa McMains	The role of the Presenilin/γ-secretase complex in cell differentiation and nutrient capture.
4	Mariko Katoh	The commitment for development in *Dictyostelium*
5	Vikas Sonakya	Knockdown of the PP2A-Catalytic Subunit Gene by RNAi Arrests Development of *Dictyostelium*
6	Adam Kuspa	A new cell type that mediates innate immunity and detoxification during *Dictyostelium* development
7	Gad Shaulsky	The molecular basis of social behavior in *Dictyostelium*

IV Polarity and Chemotaxis I 9/19/2006, from 16:00 to 19:00.

Chair: Tian Jin

1	Regina Teo	A role for PLC in chemotaxis?
2	Adrian J. Harwood	Prolyl oligopeptidase and multiple inositol polyphosphate phosphatase (MIPP) modulate inositol signalling and chemotaxis via gene regulation
3	Leung Kim	Glycogen Synthase Kinase 3 and Srn1 inhibit PI3K membrane localization in *Dictyostelium* cells.
4	Paul Kriebel	Vesicle trafficking is essential for the proper cellular distribution of the adenylyl cyclase ACA and cAMP secretion during chemotaxis and streaming.
5	Maria Lombardi	Traction Force Microscopy in *Dictyostelium* Reveals Distinct Roles for Myosin II Motor and Actin Cross-linking Activity in Maintaining Cell Polarity
6	Rick Firtel	Rap and Ras as regulators of cell polarity and chemotaxis.

V Development II 9/19/2006, from 20:30 to 22:00.

Chair: Mariko Katoh

1	Chris Thompson	Unravelling the DIF signalling pathway using genetics and microarrays
2	Jeff Williams	DIF-inducible tyrosine phosphorylation of STATc is regulated by the protein tyrosine phosphatase PTP3 and the *Dictyostelium* homologue of the Cbl proto-oncogene
3	Pauline Schaap	cAMP production by adenylyl cyclase G induces prespore differentiation in *Dictyostelium* slugs
4	Bill Loomis	Regulation of Sporulation by Cytokinins

VI Polarity and Chemotaxis II 9/20/2006, from 8:30 to 12:10.

Chair: Chang Chung

1	Robert Kay	PI3kinases, the plasma membrane and cell movement
2	Arjan Kortholt	Chemotaxis in reverse gear by polarity inversion of PLC/PI3Kinase signaling
3	Deborah Wessels	PTEN Plays a Fundamental Role in Basic Cell Motility, Which is Also Manifested in pten-Cells as a Reduction in the Efficiency of Chemotaxis
4	David R. Soll	*Dictyostelium discoideum* Amoebae Read Both the Increasing and Decreasing Temporal Gradients of cAMP in the Front and Back of
5	Carsten Beta	Microfluidic tools for the study of cell dynamics
6	Xuehua Xu	A locally controlled inhibitory mechanism in GPCR-mediated chemoattractant sensing
7	Wouter-Jan Rappel	Directional sensing in eukaryotic chemotaxis: a balanced inactivation model

VII Actin and Cytoskeleton 9/20/2006, from 16:30 to 19:00.

Chair: Carole Parent

1	Annette Müller-Taubenberger	Regulation of Dictyostelium cytokinesis by a SIN-related pathway
2	Douglas Robinson	Dynamic Cortical Rearrangements, Mechanosensing, and Mechanical Feedback of Living Cells
3	Paul Steimle	Myosin heavy-chain kinase A from Dictyostelium possesses a novel actin-binding domain that cross-links actin filaments
4	Charles Saxe	The N-terminus of *Dictyostelium* Scar interacts with Abi and HSPC300 and is essential for proper regulation and function
5	Chang Y. Chung	Regulation of Polarized F-actin Organization in Chemotaxing Cells

VIII Oxygen, Stress and Death 9/20/2006, from 20:00 to 22:00.

Chair: Derrick Brazill

1	Chris West	Role of Skp1 prolyl hydroxylation and glycosylation in culmination in *Dictyostelium*
2	Zhuo A. Wang	Role of a cytoplasmic prolyl 4-hydroxylase in oxygen-dependent development in *Dictyostelium*
3	Jianbo Na	Microarray analysis reveals STATc as a key regulator of the Dictyostelium transcriptional response to hyperosmotic shock
4	Glaucia Mendes Souza	KeaA, a *Dictyostelium* kelch-domain protein that regulates the response to stress and development
5	Pierre Golstein	Necrotic cell death in *Dictyostelium*

IX Development III 9/21/2006, from 8:30 to 10:10.

Chair: L. Kim

1	Norton Heise	Functional analysis of a UDP-GlcNAc:Thr polypeptide alpha-N-acetyl-D-glucosaminyltransferase-like (pp-alpha-GlcNAcT) gene of *Trypanosoma cruzi* using *Dictyostelium discoideum*
2	Deenadayalan Bakthavatsalam	Cell density sensing is mediated by a G protein coupled receptor
3	Jeff Hadwiger	G Protein Specificity in Chemotaxis and Development
4	Matt Cabral	TagA and AcbA Interact to Regulate Cell Fate Specification and Spore Encapsulation.

X Phagocytosis 9/21/2006, from 10:40 to 12:10.

Chair: Cynthia Damer

1	Margaret Clarke	Phagocytosis of bacteria by *Dictyostelium* amoebae.
2	Tian Jin	A vesicle surface tyrosine kinase regulates phagosome maturation
3	Taruna Khurana	Rheb Positively Regulates TOR Complex 2 to Suppress Phagocytosis: A Potential Pathway to Coordinate Nutrient Particle Capture and Growth

Posters

101	*Eryong Huang*	**bZIP Transcription Factors in Dictyostelium discoideum**
102	*Dhamodharan Neelamegan*	**Use of Dictyostelium enzymes in the development of glycoconjugate vaccines against gram negative bacterial infection.**
103	*Bill Loomis*	**Global Proteomics of Differentiating Cells**
104	*Catherine Pears*	**Requirement for DNA-PK in restriction enzyme-mediated integration of plasmids into the genome of Dictyostelium.**
105	*Nilgun Isik*	**Elmo Proteins regulate Phagocytosis and Cell Migration in Dictyostelium discoideum**
106	*Brenda J. Blacklock*	**Fatty Acid Elongation in Dictyostelium discoideum.**
107	*Rebecca Fernandez*	**Dictyostelium discoideum RnoA is involved in development past the aggregate stage as well as aggregation at low cell densities**
108	*Harish Padh*	**Transfection efficiency in Dictyostelium is enhanced by inhibition of endocytosis**
109	*Harish Padh*	**Expression of human erythropoietin gene in Dictyostelium discoideum**
110	*D. A. Cotter*	**The Dispersal of Microbial Eukaryotes, including the Dictyosteliacea, through the Attraction of Drosophila melanogaster**
111	*Lorenzo Santorelli*	**Mechanisms of cheating behavior in the social amoeba Dictyostelium discoideum**
112	*Xin-Hua Liao*	**Purification of new factors associated with TOR complex 2 in Dictyostelium discoidium**
113	*Petra Fey, Karen E. Pilcher, Pascale Gaudet*	**New Annotation Features in dictyBase**
114	*W.Jonathan Ryves*	**Dynamics of Cell Penetrating Peptide use in living Dictyostelium.**
115	*Anupama Khare*	**Mechanisms of Cheating in Dictyostelium discoideum**

Posters

116	*Eric M. Just*	**dictyMart and dictyCyc: New Ways to Explore Data in dictyBase**
117	*Daniela C. Gonzalez*	**Identification of PP1 interacting proteins in Dictyostelium discoideum**
118	*Thomas Keller*	**Genetic selections for DIF insensitive and hypersensitive mutants**
119	*Hiroshi Ochiai*	**The PKB/AKT related kinase may be engaged in the transition from the first finger stage to the slug stage**
120	*Petros Batsios*	**Knockout of the Ste20-like kinase DstA affects phagocytosis and development**
121	*Christopher Dinh*	**A strategy to generate 23,000 individual barcoded D. discoideum insertion mutants for the parallel analysis of phenotypes**
122	*Wouter N. van Egmond*	**GbpC is the only cGMP-target in Dictyostelium**
123	*B. Ewa Snaar-Jagalska*	**Single-molecule analysis of cAR1-YFP during chemotaxis and internalization**
124	*Paul Steimle*	**Cellular Studies of Dictyostelium Myosin II Heavy Chain Kinase-C Subdomains**
125	*Andrew Heidel*	**Comparative Genomics in the Social Amoebae**
126	*Ran-Der Hwang*	**Regulation of actin cytoskeletal architecture by Fimbrin A.**
127	*Tomaz Curk*	**Discovering patterns of gene regulation in *Dictyostelium discoideum* using rule-based clustering**
128	*Xiuli Huang*	**Nutrient Sensing in Dictyostelium through the TOR pathway**
129	*Beth Ford and Jessica Sazama*	**Two Distinct Roles for a Novel Anti-Adhesive Protein During Dictyostelium Growth and Development**
130	*Daniel F. Lusche*	**Dictyostelium as a model for understanding the most common genetic disease efflicting humans, polycystic kidney disease.**

Posters

131	*Cynthia K. Damer*	**Copine A is required for normal cytokinesis, contractile vacuole function, and development in Dictyostelium**
132	*Daniela C. Gonzalez*	**The P450 oxidoreductase, RedA, controls development beyond the mound stage in Dictyostelium discoideum**
133	*Scott Gruver*	**The Role of 3'-phosphoinositides in Chemotaxis**
134	*Richard Kessin*	**The *Dictyostelium discoideum* genome and its polyketide synthase genes**
135	*Vikas Sonakya*	**Elucidation of the role of Phosphoprotein Phosphatase A in growth and development of *Dictyostelium discoideum***
136	*Chris West*	**Two Predicted Diglycosyltransferase Genes Contribute to Spore Coat Polysaccharide Biosynthesis, Cellulose Deposition, and Resistance of Spores to Stress in *Dictyostelium discoideum***
137	*Chris Janetopoulos*	**Temporal and Spatial Regulation of Phosphoinositide Signaling Regulates Cytokinesis**

Abstracts for Talks

Overexpression of the *Dictyostelium discoideum paxB* gene interferes with normal cell-cell cohesion, cell-substrate adhesion, and cell sorting

M. Berenice Duran, Derrick Brazill

Department of Biological Sciences, Center for the Study of Gene Structure and Function, Hunter College, City University of New York
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The development of the eukaryote *Dictyostelium discoideum* displays many of the features of animal embryogenesis such as regulated cell-cell adhesion and morphogenesis. As in animals, *Dictyostelium* cell adhesion molecules have a mechanical function. As a result, these molecules may interact with the signal-transduction processes governing development. One such mammalian protein is the focal adhesion molecule Paxillin. Paxillin functions as a docking site on the plasma membrane for signaling and structural proteins. To gain a better understanding of the regulation of *paxillin*, we are studying the *Dictyostelium discoideum* orthologue *paxB*.

Using the Tet-off system we created an inducible overexpressor line of *paxB*. PaxB overexpressing cells (PaxBOE) exhibit an increase in cell-cell clumping, both in non-nutrient buffer and in HL-5. This clustering behavior is indicative of a possible cell-cell cohesion activity. In contrast, PaxBOE cells are less adhesive to the substratum when starved in non-nutrient buffer, suggesting a possible role in cell-substrate adhesion.

PaxBOE cells aggregate to form mounds, but subsequent morphogenesis is blocked. However, addition of 20% wild type cells is able to rescue fruiting body formation. This suggests a non-cell autonomous role for *paxB*. In these chimeras, wild-type cells are predominantly localized to the anterior one-third of the slug, and the middle section of the spore mass. In addition, fewer PaxBOE cells were found in the spore mass. Taken together this suggests *paxB* plays a role in cell differentiation. We propose that the PaxB protein is required for proper cell-cell cohesion, cell-substrate adhesion, cell sorting and development passed the mound stage.

Cell Adhesion on 9/18/2006 from 8:30 to 9:20. Chaired by *Adam Kuspa*.

Elucidating the role of *Dictyostelium discoideum* SadA protein in cell-substrate adhesion

Anthony S. Kowal, Geraldine V.Amargo, Rex L. Chisholm

Northwestern University, Chicago, IL

How cells interact with substrates such as food particles or the surfaces along which they migrate, is a very important question in cell and cancer biology. The social amoeba *Dictyostelium discoideum* has proven to be a useful model in which to address these questions, due in part to the ease of genetic manipulation. One of the proteins found to be important for cell-substrate adhesion is SadA. SadA is a predicted 9-pass transmembrane protein, resides in the plasma membrane, and includes features present in other well characterized mammalian proteins known to have a role in substrate adhesion. SadA mutant cells are deficient in phagocytosis, cell-substrate adhesion, mitosis and exhibit disorganized actin cytoskeletons. To begin to understand how SadA mediates substrate adhesion, we focused in on SadA's C-terminal cytoplasmic tail. The 32 amino-acid long tail contains a number of serine residues which are highly predicted to be phosphorylated, suggesting that this may be a region for protein-protein interactions. We began by mutating S943, S944, and S950 to either alanine (to mimic a non-phosphorylated state) or glutamic acid (to mimic a phosphorylated state), and attempted to rescue SadA null cells. Both constructs were capable of rescuing the SadA null phenotype equally well, suggesting that these sites are not important for SadA function. We are now focusing our efforts on S924,S925, and S940,S941 to determine if these residues are important for SadA function. Our studies with *Dictyostelium* will help us to understand substrate adhesion in an evolutionary less complex eukaryote.

Microsatellite evolution; mutation rates and variability

David Queller, Clea Scala, Sara Middlemist, Ryan McMullen, Marcus Kronforst, Natasha Mehdiabadi, Katie Stephens, Prince Buzombo, Joan Strassmann

Rice University, Houston, TX

The genome paper showed *D. discoideum* to be very rich in microsatellite repeats, constituting over 10% of the genome. Most surprising was the large number of trinucleotide repeats inside genes that coded for long stretches of amino acids in proteins. For example, over 2000 genes had runs of 20 or more glutamines or asparagines, and several other amino acids, usually small polar ones, were commonly represented. Why does *D. discoideum* have so many coding repeats? Do they play some functional role, or are simply the result of the forces of mutation and drift that drive microsatellite evolution in non-coding regions? To begin to address these questions, we studied both their mutational rates, and their variability in natural populations. We first hypothesized that unusually high microsatellite mutation rates might drive microsatellite expansion. Mutations that add or delete repeats are thought to occur by slippage during replication. We estimated mutation rates for 52 loci via a mutation accumulation experiment with 90 lines taken through about 1000 cell generations and 70 single cell bottlenecks that fix mutations. The estimated slippage mutation rate was actually lower than for most organisms: 6.37×10^{-6}. For loci with fewer than 40 repeats, the rate was even lower: 8.49×10^{-7}. Therefore, high mutation rates do not drive the extraordinary abundance of microsatellites.

Variability in natural populations might provide insight into selection on these loci. We predicted that, if selection acts strongly on these repeats, alleles should be distributed rather tightly around an optimal number. We first examined natural variation in detail at three loci that each had 2 or more long amino acid repeats: *dimA*, *atg1*, and *yakA*. Repeat number quite variable in all repeat regions suggesting that selection does not act strongly on repeat number. To get a more powerful signal of selection, we compared 50 pairs of repeat loci, one member of each pair inside a coding region and the other nearby but in a non-coding region. Pairs were matched for repeat number and motif. We expected selection to make the repeats less variable in coding regions, but there was no significant difference between coding and non-coding regions, indicating that selection is not acting strongly on these coding sequences, or that it acting equally strongly in coding and non-coding regions.

The extreme AT-richness of the *D. discoideum* genome may have played an important role. The reduced sequence complexity in AT-rich (or CG-rich) genomes means that more small repeat sequences will be formed by random substitutions, which are then subject to slippage mutation and drift. It is possible that *D. discoideum* has evolved its low slippage mutation rates to help protect against an unusually high input of microsatellite repeats.

Copy number variation between *Dictyostelium discoideum* strains

Gareth Bloomfield, Jason Skelton, Yoshi Tanaka, Al Ivens, and Robert Kay

Sanger Centre and MRC Laboratory of Molecular Biology, Cambridge, UK

We have found that duplications typically of many kilobases in length are common amongst laboratory strains of *Dictyostelium*, and could underlie much of the phenotypic differences observed between them. The genome sequence of Ax4 (and Ax3, and the strains derived from them) famously contains a single large inverted duplication of approximately 750 kilobases on chromosome 2. Previous genetic studies had also found evidence for copy number variation (CNV) in other strains. We have used array comparative genomic hybridisation (array CGH) to survey the genomes of various laboratory strains and wild isolates. Large duplications are widespread among laboratory strains, at diverse, apparently random sites in the genome. Strains affected, apart from Ax4, include Ax2, DH1 and NC4 itself. In contrast, most recent wild isolates appear to lack these large rearrangements, but provide evidence of polymorphic or deleted loci. Smaller scale copy number polymorphisms are also found, and can be informative in tracking lines of descent in the history of laboratory strains. Strikingly, the increased gene-dosage of duplicated genes causes a roughly corresponding increase in RNA levels, potentially leading to functional, phenotypic alterations in affected strains. CNV may also make gene disruption problematic if two copies instead of one have to be targeted. We have identified lines that are free of detectable duplications; the use of such strains (as well as careful maintenance of stocks) should facilitate comparison of results obtained in different laboratories.

DNA Methylation and epigenetic gene silencing in *Dictyostelium*.

Branimira Borisova, Blaga Popova, Balint Foeldesi, Markus Kaller, Xiaoxiao Zhang, Manu Dubin, Christian Hammann and Wolfgang Nellen

Dept. Genetics, Kassel University, 34132 Kassel, Germany

With the detection of DNA methylation Dictyostelium has become an interesting model system to study chromatin remodelling. Similar to Drosophila, *dnmA* is the only DNA methyltransferase gene in the genome and responsible for a low but highly specific level of asymmetric C-methylation. The in vivo function of Dnmt2-like methyltransferases is controversially discussed [1, 2] but it now appears clear that they contribute to silencing of retroelements [3] and probably to the regulation of other protein coding genes. The question how specific asymmetric C residues are recognized and (de novo) methylated is still open. We provide evidence that DNA methylation is linked to the RNA interference pathway but that different mechanisms regulate silencing or downregulation of different genomic loci.

RNA interference is believed to be a self reinforcing gene silencing machinery that amplifies the initial dsRNA signal by RdRPs and eventually leads to degradation of specific mRNAs. We have shown that RNAi is stringently controlled and that silencing depends on a minimal threshold level of transcribed dsRNA. Surprisingly, primary siRNAs are rapidly lost and only secondary siRNAs (RdRP products) are detected by Northern blot [4]. The function of Argonaute proteins, the RNase EriA and the RNA helicase HelF on RNAi regulation will be discussed-

1. Goll, M.G., et al., *Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2*. Science, 2006. **311**(5759): p. 395-8.
2. Jeltsch, A., W. Nellen, and F. Lyko, *Two substrates are better than one: dual specificities for Dnmt2 methyltransferases*. Trends Biochem Sci, 2006.
3. Kuhlmann, M., et al., *Silencing of retrotransposons in Dictyostelium by DNA methylation and RNAi*. Nucleic Acids Res, 2005. **33**(19): p. 6405-17.
4. Popova, B., et al., *HelF, a putative RNA helicase acts as a nuclear suppressor of RNAi but not antisense mediated gene silencing*. Nucleic Acids Res, 2006. **34**(3): p. 773-84.

Process engineering high throughput restriction enzyme insertional mutagenesis in *Dictyostelium*

Jie Song, Chris Dinh, Adam Kuspa, and Richard Sucgang

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Large scale systematic mutagenesis is an essential tool for the functional characterization of any completely sequenced genome, and this is no different for *Dictyostelium discoideum*. We have been concentrating our efforts on high-throughput insertional mutagenesis through restriction enzyme mediated integration (REMI), and have constructed an efficient pipeline for producing and cataloging mutants. We have been distributing knockout plasmids to our colleagues from this collection through our website (`http://dictygenome.org`) for the last few years. We will describe the functional aspects of the pipeline design, which codifies the different outcomes of REMI, and abstracts away many sources of human error. Mining the data from the production workflow has unearthed evidence of mutational hotspots in REMI.

dictyBase Update

Rex L. Chisholm, Eric M. Just, Sohel N. Merchant, Karen E. Pilcher, Pascale Gaudet, Petra Fey, Warren A. Kibbe

dictyBase, Center for Genetic Medicine, Northwestern University, 676 N. St. Clair Street, Chicago, IL, 60611

Over the past year, the dictyBase team has made efforts to improve usability by implementing new methods of annotation and data retrieval.

First, in an attempt to fully integrate the Dicty Stock Center with dictyBase, we have linked strains with their corresponding genes, such that there are reciprocal links on the Strain Records and the Gene Pages. Furthermore, dictyBase curators have begun systematic literature-based strain curation in addition to capturing mutant phenotype information in the database. To improve consistency in phenotype annotation, we have created a controlled vocabulary for describing phenotypes.

To provide an open forum for any member of the community to contribute comments about any gene in the genome, we have introduced a "wiki"-based Community Annotation tool (http://wiki.dictybase.org). Wiki pages are linked to every Gene Page.

We have also implemented a method of viewing biochemical pathways in *Dictyostelium*. The dictyCyc tool, based on the Pathway Tools software, allows users to search and browse predicted biochemical pathways and the gene products responsible for those enzymatic reactions. All predicted pathways for a particular gene are listed on the Gene Page, providing a direct link to a pathway graphic in dictyCyc.

Finally, dictyMart is a powerful database query system which can generate fully customized datasets. Users can search based on chromosomal location, dictyBaseID, and Gene Ontology annotations, among other criteria. The result set can include various annotations such as chromosomal location, Gene Ontology annotations, dictyBaseIDs, gene product annotations, and sequences. The results can be viewed in tabular format in a browser or exported as Microsoft Excel files, and sequences can be output as FASTA files. The implementation of these new data and annotation features are intended to maximize access to the increasing body of information in the dictyBase database.

dictyBase is supported by grants from NIGMS and NHGRI.

Quorum sensing in *Dictyostelium discoideum* is dependent on Phospholipase D activity.

Yi Chen, Derrick Brazill

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Cell-density sensing, or quorum sensing, plays a vital role in eukaryotic processes ranging from organ formation and regeneration to disease progression in trypanosome infections. The social amoeba *Dictyostelium discoideum* is the simplest eukaryote to display quorum sensing. Starving *Dictyostelium* cells are able to calculate the concentration of starving cells by simultaneously secreting and sensing a glycoprotein called conditioned medium factor (CMF). When the number of starving cells is high, the level of CMF is also high. The high levels of CMF maintain Gα2 in its active form, thus allowing signaling through cAR1 to proceed, and aggregation to occur.
Phospholipase D (PLD) is a key player in CMF signaling. We have identified the gene *pldB*, which has homology to mammalian PLD1. Cells lacking *pldB* have decreased cellular PLD activity and cells overexpressing *pldB* have increased PLD activity, suggesting that PldB is a functioning phospholipase. In addition, CMF is unable to regulate the activity of Ga2 in cells lacking *pldB*, which is consistent with PldB being in the CMF pathway. PldB is a negative regulator of CMF signaling as cells lacking *pldB* aggregate at very low cell density while cells overexpressing *pldB* are unable to aggregate. Addition of phosphatidic acid, a product of PLD activity, mimics overexpression of *pldB*, arguing that the enzymatic activity of PldB is important in quorum sensing. The inability of *pldB* overexpressing cells to aggregate may be due to the fact that they are unable to chemotax towards cAMP. In agreement with this, F-actin staining shows that these cells do not produce filopodia, suggesting that the overexpression of *pldB* disrupts actin organization. Thus, PldB appears to be integral to CMF-mediated cell-density sensing.

CnrN, a putative PTEN-like phosphatase, is required for counting factor-mediated group size regulation in Dictyostelium

Yitai Tang, Richard Gomer

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Much remains to be understood about how the size of groups of cells or tissues is regulated. *Dictyostelium* cells aggregate to form multi-cellular fruiting bodies during development. As a part of negative feedback loop, Counting factor (CF), a secreted protein complex, decreases the number of cells in aggregates and fruiting bodies. We used a second-site suppressor screen to identify CF signal transduction components, and found that an insertion in *cnrN* affects group size. Knocking-out *cnrN* in wild-type cells resulted in the formation of abnormally small fruiting bodies. The *cnrN*$^-$ phenotype was rescued by the expression of CnrN. *cnrN*$^-$ cells are insensitive to either addition or depletion of CF, indicating that CnrN may be a downstream effector of CF. The predicted amino acid sequence of CnrN has similarity to dual specificity phosphatases and has 40% identity to the *Dictyostelium* 3'-phosphoinositide phosphatase PTEN. *cnrN*$^-$ cells have an increased random cell motility, which is consistent with computer simulations and previous observations indicating that high random cell motility causes the formation of small fruiting bodies. However, *cnrN*$^-$ cells have normal levels of cell-cell adhesion which is another group size regulator. cAMP-stimulated actin polymerization was abnormally high in *cnrN*$^-$ cells, indicating that CnrN regulates cell motility by regulating the cytoskeleton. PIP3-initiated Akt recruitment to the plasma membrane potentiates cell motility in *Dictyostelium*. Both cAMP-stimulated PIP_3 levels and Akt membrane translocation were abnormally high in *cnrN*$^-$ cells. Our results suggest that CnrN may increase group size in *Dictyostelium* in part by inhibiting the PIP3 signaling pathway and cell motility.

The role of the Presenilin/γ-secretase complex in cell differentiation and nutrient capture.

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The γ-secretase complex [Presenilin (PS), Nicastrin (Nct), Aph1 and Pen2)] is responsible for intramembrane proteolysis of type 1 single pass transmembrane proteins. Cleavage of such substrates is required for a variety of essential cellular and developmental functions. In some instances proteolysis induces the release of a membrane tethered transcription factor, while in others mechanism of signaling transduction is still not known. Principle substrates of γ-secretase include β-Amyloid Precursor Protein and Notch, and mutations in the γ-secretase complex have been strongly linked to Alzheimer's disease, defects in Notch signaling, and embryonic lethality. Since *Dictyostelium* does not have orthologs of these traditional substrates, it may present a unique system to reveal novel functions of γ-secretase and associated downstream pathway components. Indeed, I have demonstrated that in *Dictyostelium* the γ-secretase complex plays two important, yet very distinct, roles during times of growth and development.

Through the analyses of a series of single and double mutations of the *PS*, *Aph1*, and *Nct* genes, I have demonstrated that γ-secretase is necessary for cell fate specification during development, primarily in the differentiation of spore cells. I have also identified a role for the γ-secretase complex in nutrient capture; γ-secretase mutants have defects in phagocytosis. Studies using a γ-secretase-specific inhibitor suggest that proteolytic activity is required for the developmental processes, and studies are in progress to assess the effect on phagocytosis. I have identified several novel candidate substrates for γ-secretase in *Dictyostelium*, and am expressing fluorescent-tagged variants to examine if they are subject to processing during development in wild-type and γ-secretase mutant cells. I hope to use these potential substrates to quantify enzymatic activity within the various mutants, to identify each component's contribution to activity, and to identify novel functions downstream of γ-secretase cleavage. This assay would also allow verification of the efficacy of γ-secretase inhibitors.

The commitment for development in *Dictyostelium*

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Upon starvation, *Dictyostelium* cells halt cell proliferation, start a succession of morphogenesis, and finally form dormant spores. Spores can survive long periods of starvation, and when nutrients are abundant, they germinate and transit to the growth phase again. *Dictyostelium* cells can also dedifferentiate. Developing cells at almost any stage can be made to dedifferentiate and resume growth by mechanical dissociation and subsequent exposure to a fresh nutritional source[1]. Our previous studies have shown that dedifferentiation is regulated by a dedicated genetic program[2]. One of the most curious phenomena in this context is that *Dictyostelium* slugs can migrate across a lawn of bacteria and proceed to culminate despite the abundance of food. We describe this phenomenon as commitment and argue that it is one of the natural developmental processes. We found that starving cells can revert to the vegetative state when nutrients are supplied only within the first few hours of development. After some time, the cells commit and proceed into development regardless of the presence of food. We analyzed this phenomenon using wild type and several mutant strains and found that commitment requires intact cAMP signaling components such as AcaA, CarA and YakA. We found that starvation in suspension cannot induce commitment and that cAMP pulses were necessary. We also found that commitment is a physiological phenomenon that was not due to a physical barrier between the amoeba and the bacterial food source. Finally, we found that commitment is accompanied by loss of phagocytic ability that is dependent upon pulsatile cAMP signals.

* These authors contributed equally

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Knockdown of the PP2A-Catalytic Subunit Gene by RNAi Arrests Development of *Dictyostelium*

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Protein dephosphorylation by phospho-protein phosphatase PP2A is implicated in the regulation of several physiological processes such as transcription, RNA splicing, translation, and signal transduction. This heterotrimeric enzyme contains an A subunit which serves as a scaffolding molecule to coordinate the assembly of the holoenzyme, a B subunit which determines substrate selectivity and subcellular localization, and a C or catalytic subunit. RNA interference (RNAi) is a sequence-specific silencing of homologous genes in eukaryotic cells using double stranded RNA. To study the role of PP2A in development, we used RNAi to reduce the expression of the C subunit (PP2Ac) gene in *Dictyostelium discoideum*. In previous experiments, we have shown that double stranded RNA synthesized from long (1200 bases) inverted repeat DNA could reduce the mRNA expression of the development-specific Dictyostelium transcription factor coded by the mybB gene and block aggregation (Otsuka, Cogill, Kuan, Dottin and Gross unpublished). In the current experiments, we demonstrated the use of polymerase II promoter to direct the in vivo synthesis of shRNA (short hairpin RNA) from cloned synthetic oligonucleotides. Extrachromosomal plasmids with shRNA coding constructs were used to transform in AX3 cells. The transformed cells show a arrest in development after aggregation at the mound stage. Analysis of PP2Ac mRNA using Northern blots shows a substantial loss of PP2Ac mRNA in development. The results show that PP2A is essential for the progression of development beyond the mound stage. Further more, short synthetic oligonucliotides can now be used to inactivate important genes in Dictyostelium.

A new cell type that mediates innate immunity and detoxification during *Dictyostelium* development

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Last year, we described a previously unrecognized cell type that appears to be dedicated to detoxification and provides a form of innate immunity during development. We call these cells "Scavenger" cells, or "S" cells, because of their likely role in protecting the prespore cells from various insults that might otherwise compromise spore production or viability. S cells move throughout mounds and slugs, accumulate toxins within intracellular vesicles, engulf bacteria, and then aggregate into clumps of 3-10 cells that are left behind in the slime trail or at the base of the fruiting body. Thus, these cells provide a plausible mechanism for the removal of toxins and pathogens from multicellular structures.

We have been able to isolate S cells by fluorescence-activated cell sorting to study their properties. S cells appear to be highly active phagocytes as they rapidly accumulate latex beads, or bacteria. When disaggregated slugs cells are mixed with latex beads, or *Legionella* bacteria, S cells accumulate almost all of the beads, or bacteria, after 1 hour. When slugs are allowed to reform and migrate for 24 hours, almost all of the beads, or *Legionella*, are found within S cells that have been left behind in the slime trail. Thus, S cells have the potential to provide a form of innate immunity to *Dictyostelium* by removing pathogenic bacteria prior to fruiting body formation.

A transcriptional profile of S cells suggests that they are related to PstAB cells, but their scattered distribution throughout the slug indicates they cannot be true PstAB cells. We suggest that Scavenger cells be called PstS cells to conform to the generally accepted terminology for prestalk cells set out by Jeff Williams and colleagues. The transcriptional analysis also revealed S cell-enriched expression of several genes, including a 10- to 20-fold enrichment over slug cells in the expression of the tirA gene. The tirA gene contains a TIR domain signaling module that is common to innate immune pathways in other species. During development, tirA mutants produce S cells that are more sensitive to Legionella infection. Somewhat surprisingly, vegetative tirA mutants grow normally in defined media, but they arrest growth and lose cell viability when plated on bacterial lawns, suggesting an impaired tolerance for bacteria.

We have observed what appear to be S cells in four other species suggesting that innate immunity is a common feature of dictyostelids. Innate immunity involving specialized phagocytes has not been observed previously outside of the metazoa. Thus, *Dictyostelium* may shed light on the evolution and function of innate immune systems in plants and animals.

The molecular basis of social behavior in *Dictyostelium*

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Dictyostelium cells can form chimeric fruiting bodies in nature and under controlled laboratory conditions[1]. The potential for social conflict in chimeric fruiting bodies is manifested in the cell type choice: spores are viable and stalk cells are dead. Cheaters are defined as cells that make more than their fair share of spores in chimerae, and previous work by Ennis *et al*. has demonstrated that cheating can be explored with genetic tools[2]. Cheating would greatly hinder the evolution of social systems, so some of the open questions in the field deal with mechanisms that prevent or limit cheating. We have decided to apply a genetic approach to these questions and developed tools to identify mutations that cause cheating without overt morphological consequences. We carried a pool of several thousand mutants through twenty cycles of growth and development in chimerae and identified mutants that can cheat on the parental strain. We also carried pools of 250 mutants through ten cycles of selection and identified additional mutants. Finally, we used a strong cheater as a selective pressure and identified mutants that resist cheating. Altogether, we have identified about 100 mutations that are involved in cheating. The mutated genes are expected to participate in processes such as ubiquitin-mediated protein modification, signal transduction, polyketide synthesis, cytoskeletal processes, etc. These results suggest that social behavior is regulated by various pathways and that it can be explored using molecular genetic tools.

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2. Ennis, H. L., Dao, D. N., Pukatzki, S. U. & Kessin, R. H. Dictyostelium amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proc. Natl. Acad. Sci. USA* **97**, 3292-3297 (2000).

This work is supported by the FIBR program of the National Science Foundation.

A role for PLC in chemotaxis?

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Inositol phosphates are intimately associated with *Dictyostelium* chemotaxis. During chemotaxis, phospholipase C (PLC) is rapidly activated upon cAMP stimulation to produce $InsP_3$. However, no apparent chemotaxis phenotype was observed when the PLC gene, *pipA*, was disrupted. This suggests that $InsP_3$ is not required for chemotaxis. We have revisited this question using lithium to globally lower inositol within the cell. Lithium attenuates chemotaxis, but paradoxically we found that rather than making cells more sensitive to lithium, loss of PLC makes cells less sensitive. Consistent with these observations PLC over-expression leads to increased lithium sensitivity. These results again suggest that an $InsP_3$ is not an important molecule regulating chemotaxis.

How does PLC regulate chemotaxis? PIP2 is both the substrate for PLC and PI3-kinase. This latter enzyme generates PIP3 at the leading edge in response to cAMP2. PIP3 is an important molecule for chemotaxis as it drives actin polymerization. The effects of lithium on chemotaxis, notably on cell speed and polarity, match the effects of the PI3-kinase inhibitor LY294002 and a double *pi3k1/pi3k2* null mutant, which has reduced synthesis of PIP3 (Loovers et al., 2006). We propose PLC attenuates PIP3 by degrading PIP2. Loss of PLC increases PIP2 and reduces the effects of lithium.

Loovers H, Postma M, Keizer-Gunnink I, Huang Y, Devreotes, P& van Haastert, P. (2006). Mol Biol Cell *17* 1503-13

Prolyl oligopeptidase and multiple inositol polyphosphate phosphatase (MIPP) modulate inositol signalling and chemotaxis via gene regulation

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Conventionally, the cellular concentration of IP_3 is regulated through activation of phospholipase C (PLC). However, the enzyme Prolyl oligopeptidase (PO) has been shown to attenuate IP3 though an unknown mechanism. Here, we report that in *Dictyostelium* PO negatively regulates multiple inositol polyphosphate phosphatase (MIPP) activity to control the degradation of higher order inositol phosphates. By disruption of the MIPP gene, *mippA* , we show that PO acts directly through MIPP to reduce the effect of lithium on chemotaxis.

Over-expression of MippA elevates the cellular IP_3 concentration, however it leads to lithium hypersensitivity. This indicates that loss of PO does not confer lithium resistance through elevation of IP_3. Instead we find that altered PO and MIPP activity lead to elevated expression of the genes that control inositol synthesis and recycling. Consistent with this, we show that elevation of IMPase or inositol synthase leads to lithium resistance. These results define a novel signalling pathway where PO and MIPP controls inositol signalling through elevated gene expression.

Polarity and Chemotaxis I on 9/19/2006 from 16:00 to 19:00. Chaired by *Tian Jin*.

Glycogen Synthase Kinase 3 and Srn1 inhibit PI3K membrane localization in *Dictyostelium* cells.

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Glycogen Synthase Kinase 3 (GSK3) is a multifunctional kinase involved in diverse cellular activities such as cell fate decision, cell cycle, metabolism, and cell polarity control in various eukaryotes. Dictyostelium gsk3-null cells are not only defective in cell differentiation, but also in directional cell migration. Here, we report that *gsk3*-null cells suffer elevated level of phosphatidylinositol-3,4,5-triphosphate (PIP3) before and after stimulation by chemoattractants, and consequently are defective in gradient sensing. *Dictyostelium* PI3Ks translocalize from cytosol to the plasma membrane in response to chemoattractant stimulation. In *gsk3*$^{-}$ cells, however, PI3K significantly mis-localized to the plasma membrane in the absence of stimulation. A PI3K1 substitution mutant was generated, whose potential GSK3 phosphorylation sites were abolished. This PI3K1 mutant constitutively localized to the plasma membrane of unstimulated wild type cells, supporting that GSK3 normally inhibits PI3K membrane localization. We suggest that maintaining low level of PIP3 by GSK3 before and after the stimulation is essential for effective chemotaxis. Furthermore, we have identified a REMI mutant, Srn1, showing similar phenotype as *gsk3*-null cells. Srn1 is a GPI anchored Superoxide Dismutase, which is radically different from GSK3. We will discuss the mechanisms of PIP3 regulation by Srn1 and GSK3.

Polarity and Chemotaxis I on 9/19/2006 from 16:00 to 19:00. Chaired by *Tian Jin*.

Vesicle trafficking is essential for the proper cellular distribution of the adenylyl cyclase ACA and cAMP secretion during chemotaxis and streaming.

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We are interested in understanding the signaling mechanisms that control cell polarization and chemotaxis. These responses are important in many processes such as wound healing, neuronal growth and leukocyte cell migration. In *Dictyostelium*, the binding of cAMP to G protein-coupled cAMP receptors (cARs) activates a variety of effectors including the adenylyl cyclase ACA, which converts ATP to cAMP. cAMP acts both internally, to alter gene expression through PKA, and externally to relay the chemoattractant signal. We have shown that ACA is enriched at the back of chemotaxing cells and proposed that this provides a compartment from which cAMP is secreted allowing cells to orient themselves head to tail and form streams. Interestingly, we also found that ACA labels rapidly moving intracellular vesicles. We investigated whether these vesicles are involved in the trafficking of ACA using Fluorescence Recovery After Photobleaching (FRAP). As a control we followed the fate of cAR1. These studies show that in nonpolar cells, bleached cAR-1 recovers first in areas near unbleached membranes, consistent with recovery by diffusion. In contrast ACA recovers uniformly across the bleached area, consistent with vesicle delivery to the cell periphery. Moreover, in polarized chemotaxing cells specifically bleached at the tail, ACA not only recovers in a manner consistent with vesicle delivery but the recovery is earlier and more extensive. These results suggest that ACA is replenished via vesicle fusion to the cell periphery and implicate vesicle trafficking as a mechanism for ACA tail enrichment in polarized cells. These results also suggest that the machinery for the enrichment in polarized cells is developmentally regulated. We also find that when the actin cytoskeleton is depolymerized using latrunculin A, the trafficking of ACA vesicles is disrupted. In fact, the vesicles completely disappear. Under these conditions the fluorescent recovery of ACA changes to a pattern consistent with membrane diffusion, where the areas near unbleached membranes recover first. This result indicates that the actin cytoskeleton is required for the delivery of ACA vesicles to the cell periphery. We also observe that ACA vesicle movement is significantly reduced in nocodazole-treated cells, and that under these conditions, the fluorescent recovery of ACA is consistent with membrane diffusion. These data indicate that the microtubules are also required for the delivery of ACA vesicles to the periphery. In accordance with these findings, we also find that ACA vesicles co-localize with microtubules and most strikingly, that nocodazole-treated cells have strong streaming defects thereby suggesting that vesicle trafficking is required for cAMP secretion. Together, our findings establish a role for vesicle trafficking in ACA localization and cAMP secretion.

Polarity and Chemotaxis I on 9/19/2006 from 16:00 to 19:00. Chaired by *Tian Jin*.

Traction Force Microscopy in *Dictyostelium* Reveals Distinct Roles for Myosin II Motor and Actin Cross-linking Activity in Maintaining Cell Polarity

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Cell movement requires the generation of protrusive forces at the leading edge and contractile forces at the cell rear to facilitate retraction. In addition, the transmission of contractile force to the substratum, via cell adhesions, results in the production of traction forces that "pull" the cell forward. It is widely accepted that the interaction of myosin II with actin is the primary mechanism for producing contractile forces. This is supported by the finding that myosin II inhibition prevents cell movement due to failure of the rear to retract. However, *Dictyostelium* amoebae that lack myosin II are still capable of moving, although at a slower rate compared with wild-type cells. We have developed a gelatin traction force assay and used it to investigate the role of myosin II in traction force generated by randomly moving wild-type, myosin II essential light chain (*mlcE*$^-$) null, and myosin II (*mhcA*$^-$) null mutant *Dictyostelium discoideum* cells. Our data shows that while wild-type cells generate the largest forces, *mlcE*$^-$ and *mhcA*$^-$ null cells can generate small traction forces independently of myosin II motor function, approximately 63% and 90% less than wild-type, respectively. However, rapid movement can occur in all three cell types when an asymmetric distribution of traction stresses (high at the cell rear and low at the front) is present. Together these data suggests that the distribution of forces, not the magnitude is efficient for developing, maintaining, and regenerating cell polarity. We conclude that myosin II actin cross-linking activity is sufficient for stabilizing an asymmetric distribution of forces, while myosin II motor function is required for its development and regeneration.

Polarity and Chemotaxis I on 9/19/2006 from 16:00 to 19:00. Chaired by *Tian Jin*.

Rap and Ras as regulators of cell polarity and chemotaxis.

TJ Jeon, Stacey Lee, Susan Lee, Pascale Charest, Kosuke Takeda, Rick Firtel,

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A new model on the role of Rap1 and Ras in controlling cell polarity and chemotaxis will be discussed.

Unravelling the DIF signalling pathway using genetics and microarrays

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DIF-1 is a central regulator of cell fate choice and pattern formation. It is thought that a subset of cells respond to DIF and differentiate as pstO cells due to intrinsic biases. These cells are initially scattered amongst prespore cells but subsequently sort out. Little is known about the DIF signalling pathway or the DIF target genes that regulate differential prespore and pstO cell behaviour. We have therefore addressed these questions using a genetic selection strategy and genome wide microarray profiling.

(1) Previously we developed a selection strategy based on the 8-Br-cAMP monolayer assay to identify mutants (e.g. *dimA*$^-$) that remained as detergent resistant spores in the presence of DIF. However, the cloning of the *dimB* gene highlighted that many DIF signalling mutants would not be identified using this strategy. We therefore developed an alternative selection strategy based on the cAMP removal assay in which DIF-resistant mutants are enriched by failing to produce stalk cells. Using a novel mutagenic restriction enzyme (*Tsp*509I), a REMI library of 20,000 transformants was generated and subjected to two rounds of selection. The insertion site in several mutants has been identified. One such mutant (*dimC*$^-$) contained an insertion in the potential promoter region of a GATA family transcription factor. GATA expression peaked at 6 hours and was 30-fold reduced in the recapitulated *dimC*- mutant. The mutant is DIF-resistant and fails to make stalk cells in both the cAMP removal and 8-Br-cAMP assays. Interestingly, however, DIF-1 is still able to repress spore cell formation in the *dimC*- mutant, unlike in *dimA*$^-$ cells, suggesting it is only required to regulate a subset of DIF responses. This idea is supported by the subtle growth and plaque morphology of the mutant. Phenotypic and expression data for the *dimC*$^-$ mutant and a model for *dimC* function will be presented.

(2) We have used microarrays to identify DIF-1 target genes during normal development. RNA was extracted at 13 time points during development, labelled and hybridized to a *Dictyostelium* microarray representing over 5500 non-redundant genes. The microarray gene expression profile of wild type cells was compared to that of the *dmtA*$^-$ and *dimA*$^-$ DIF-1 signalling mutants. In order to compare differential gene expression between experimental conditions (reproducibility, treatment and time), analysis of variation (ANOVA) was used to identify the source of the variation. From this, we have defined a set of approximately 100 DIF-1 target genes. In order to validate this set of genes, the developmental expression profile of representative genes was tested by qPCR. From this, we have defined several genes that exhibit robust DIF dependent expression. For example, one DIF induced gene is predicted to encode a ubiquitin protein ligase. Expression of this gene peaks at 6hrs and is strongly enriched in prestalk cells. A knockout mutant has been generated and exhibits a strong slugger phenotype. Further characterisation of the mutant phenotype as well as studies of potential regulatory targets will be presented.

Development II on 9/19/2006 from 20:30 to 22:00. Chaired by *Mariko Katoh*.

DIF-inducible tyrosine phosphorylation of STATc is regulated by the protein tyrosine phosphatase PTP3 and the *Dictyostelium* homologue of the Cbl proto-oncogene

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STATc, a Signal Transducer and Activator of Transcription protein is nuclear localised in pstO cells of the slug. In cells at four hours of development STATc can be activated, i.e. tyrosine phosphorylated, by exposure to either DIF-1 or hyper-osmotic stress. Tyrosine phosphorylation induces dimerisation and nuclear accumulation of STATc. The Cbl proteins down-regulate metazoan signalling pathways by targeting receptor tyrosine kinases for ubiquitination. They contain a phosphotyrosine-binding region, comprised of a four-helix bundle, an EF hand and an SH2 domain, linked to a ring finger E3 ubiquitin-ligase domain. CblA is a Dictyostelium homologoue of the metazoan Cbl proteins. Because of the role of Cbl proteins in down-regulating tyrosine kinase signalling pathways, we analysed STAT activation in the cblA null strain. In the parental strain tyrosine phosphorylation of STATc is induced by DIF-1 but in the null strain this response is attenuated. Because this is the opposite result from that expected if CblA down-regulated the tyrosine kinase we sought a tyrosine phosphatase that might be the Cbl target.

Previous work by Rick Firtel and Tony Hunter showed that hyper-osmotic stress causes phosphorylation of the protein tyrosine phosphatase PTP3 and identified a 130kD protein as a likely PTP3 substrate. We show that p130 is STATc and that STATc is indeed a direct PTP3 substrate. We further show that CblA fulfils its function, as a positive regulator of STATc tyrosine phosphorylation, by binding to and down-regulating PTP3. Over the same time scale as the DIF-1 induced increase in tyrosine phosphorylation of STATc, PTP3 undergoes a DIF-induced pI shift that is consistent with an increase in its phosphorylation level. This suggests a STATc activation mechanism whereby DIF-1 induces serine-threonine phosphorylation of PTP3, decreasing PTP3 activity and so leading to an increase in STATc tyrosine phosphorylation. Consistent with this model, over-expression of a substrate trapping form of PTP3, a likely dominant PTP3 inhibitor, leads to semi-constitutive STATc tyrosine phosphorylation. If correct, this constitutes a novel STAT activation mechanism and it re-focusses the search for the STATc activation mechanism: away from the tyrosine kinase that modifies STATc and onto the serine-threonine kinase that modifies PTP3.

Development II on 9/19/2006 from 20:30 to 22:00. Chaired by *Mariko Katoh*.

cAMP production by adenylyl cyclase G induces prespore differentiation in *Dictyostelium* slugs

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Encystation and sporulation are crucial developmental transitions for solitary and social amoebas, respectively. While little is known of encystation, sporulation requires both extra- and intracellular cAMP. After aggregation of social amoebas, extracellular cAMP binding to surface receptors and intracellular cAMP binding to PKA act together to induce prespore differentiation. Later, a second episode of PKA activation triggers spore maturation. Adenylyl cyclase B (ACB) produces cAMP for maturation, but the cAMP source for prespore induction is unknown. We show that adenylyl cyclase G (ACG) is translationally upregulated in prespore tissue after aggregation. acg null mutants show reduced prespore differentiation, which becomes very severe when ACB is also deleted. Partial rescue of ACG function by ACB is due to upregulation of ACB in the prespore region of acg null structures. The role of ACG in spore formation is deeply conserved and can be traced back to induction of encystation in solitary amoebas.

Development II on 9/19/2006 from 20:30 to 22:00. Chaired by *Mariko Katoh*.

Regulation of Sporulation by Cytokinins

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Cytokinins are N6 substituted adenine derivatives that play various roles in the growth and development of plants by activating two-component phosphorelay pathways. The germination inhibitor, discadenine, is a cytokinin found in Dictyostelium which has been shown to affect plant root growth as well as spore germination possibly acting through the histidine kinase DhkB in *Dictyostelium*. We have previously found that double mutants lacking both the SDF-2 receptor, DhkA, and DhkB form fewer spores than either of the single mutants. These results suggest that the germination inhibitor might also play a role in the initiation of sporulation.

The cytokinins, discadenine, isopentenyl adenine and zeatin, not only block germination but also induce rapid sporulation in KP cells. dhkB- null cells do not sporulate in response to the cytokinins but still respond to SDF-1, SDF-2 and GABA by rapid sporulation. Cells lacking the late adenylyl cyclase ACR fail to respond to cytokinins although cells lacking either of the other adenylyl cyclases, ACA or ACG, respond to cytokinins normally. Since the N-terminal portion of ACR carries a response regulatory region, it is likely that phosphorelay from DhkB activates its adenylyl cyclase activity leading to an increase in internal cAMP and PKA activity. Inhibition of PKA with H89 blocks induction of sporulation by cytokinins.

We knocked out one of the genes encoding isopentenyl transferase (iptA) which catalyzes the first step in the biosynthesis of the cytokinins, isopentenyl adenine (I6 Ade) and discadenine. These cytokinins were extracted from wild type and mutant fruiting bodies and purified by HPLC before being quantified by UV absorbance and mass spectroscopy. Cytokinin levels were reduced ~10 fold in the iptA- strain. Since the mutant makes less than half as many viable spores as wild type strains, it appears that the cytokinins are actively involved in sporulation.

A mutant strain, DG1110, was isolated in a near-saturation REMI screen for morphological mutants and found to form long, thin stalks and unstabled spores similar to acrA- mutants. The gene mutated in DG1110 encodes a 62 kDa protein with two potentially transmembrane hydrophobic domains. The mutant strain responds to GABA, SDF-1 and SDF-2 normally but does not sporulate in response to cytokinins. It is possible that strain DG1110 is missing the receptor for isopentenyl adenine and discadenine.

The cytokinin signal transduction pathway is independent of the SDF-2 pathway in that mutants lacking ACA, ACG, GrlE, PI3K, PKB R1, DhkA, RegA, or AcbA still respond to isopentenyl adenine. It appears that there are multiple overlapping and partially redundant signal transduction pathways leading to sporulation in *Dictyostelium*.

Development II on 9/19/2006 from 20:30 to 22:00. Chaired by *Mariko Katoh*.

PI3kinases, the plasma membrane and cell movement

Oliver Hoeller, David Traynor, Paul Langridge, and Robert Kay

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We will report on two areas: a genetic test of the role of PI3kinases and PIP_3 in cell movement and on the 'surface area problem'.

The rapid recruitment of PI3kinases and PH domain proteins to the plasma membrane is the first detectable polarized event in gradient sensing, and this, together with the defective chemotaxis of the PI3K1/2 and PTEN null cells, suggests a central role for PI3kinases in gradient sensing. We have tested this idea by genetically disrupting all five type-I PI3kinase genes and also made a sextuple KO of these genes plus PTEN. Although the latter mutant lacks all known ways of generating asymmetric PIP3 in the plasma membrane it and the quintuple PI3kinase mutant can both chemotax to cyclic-AMP fairly efficiently.

We are interested in how moving cells deal with their surface area: it is likely to change, as they change in shape, yet the plasma membrane is expected to be barely extensible. The surface area of moving *Dictyostelium* cells can be estimated from 3D reconstructions of confocal stacks and in this way we detect changes of up to 30% in a few minutes, well beyond that allowed by membrane stretching. The likeliest explanation is that endocytosis and exocytosis of membrane is coupled to cell movement so as to adjust the surface area. Consistent with this idea, aggregation-competent cells endocytose their membrane at a rapid rate, although it is accompanied by very little fluid, as previously reported. It has been suggested that exocytosis occurs at the leading edge of a cell, thus supplying new membrane at the site of pseudopod expansion and creating a flow of membrane away from it. Experiments attempting to detect this flow will be reported.

Chemotaxis in reverse gear by polarity inversion of PLC/PI3Kinase signaling

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Many eukaryotic cells move in the direction of chemical compounds. Cell movement in embryonic development is orchestrated by a multitude of attractants and repellents. Cells become polarized due to actin filaments in the front that induce the formation of local pseudopodia, and acto-myosin filaments in the back of the cell that inhibits pseudopod formation. Chemoattractants, such as cAMP in *Dictyostelium* or fMLP in neutrophils, induce the activation of PLC and PI3-kinase at the front of the cell, leading to the local depletion of the phospholipid PI(4,5)P2 and formation of PI(3,4,5)P3. It has been shown that PI(3,4,5)P3 at the leading edge is a very strong inducer of pseudopod extensions. In this presentation we show that a cAMP analog inhibits PLC instead of activation, thereby reversing the polarity of PI(4,5)P2. This leads to the formation of PI(3,4,5)P3 in the back of the cells and chemotaxis away from the source. These results indicate that cell polarity may drive the direction of cell movement, such that cells can even chemotax backwards.

Polarity and Chemotaxis II on 9/20/2006 from 8:30 to 12:10. Chaired by *Chang Chung*.

PTEN Plays a Fundamental Role in Basic Cell Motility, Which is Also Manifested in pten-Cells as a Reduction in the Efficiency of Chemotaxis

Deborah Wessels, Daniel Lusche, Spencer Kuhl and David R. Soll

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Models have been developed in which the role of PTEN, a PIP3 phosphatase, is to generate intracellular PIP3 gradients that are basic to cell polarization and oriented movement in a spatial gradient of cAMP. In these models, PIP3 is released intracellularly in the anterior end of a cell, and PTEN, which is localized in the cortex in the back half of the cell, hydrolyzes PIP3, thus accentuating an intracellular PIP3 gradient increasing anteriorly. We present evidence that in fact, PTEN plays a fundamental role in the efficiency of persistent translocation in buffer (i.e., in the absence of attractant) as well as in the process of chemotaxis. pten- cells in buffer cannot suppress lateral pseudopod formation, especially in the posterior half of a cell, resulting in a decrease in persistent translocation. In a spatial gradient, the majority of pten- cells exhibit positive chemotactic indices, but the indices are on average lower than in wild-type cells, because pten- cells continue to form aberrant lateral pseudopods in the posterior of the cell, causing unwarranted turns that decrease chemotactic efficiency. This single defect also decreases the efficiency of the response of pten- to the different phases of simulated temporal and natural waves of cAMP. The dynamics and localization of PTEN are similar to those of myosin II, and, what is more, pten- cells and the myosin heavy chain phosphorylation mutant 3XASP, which expresses a MHC that constitutively mimics phosphorylated MHC, exhibit remarkably similar behavioral defects, suggesting either interactions or interdependencies of myosin II and PTEN in the suppression of lateral pseudopods during basic cell motility and chemotaxis. Our results are not consistent with a role for PTEN as a PIP3 phosphatase essential for sensing a spatial gradient of cAMP. Rather, they are more consistent with a structural role in the suppression of lateral pseudopod formation

Dictyostelium discoideum Amoebae Read Both the Increasing and Decreasing Temporal Gradients of cAMP in the Front and Back of Waves.

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In nature Dictyostelium amoebae respond to waves of cAMP in the process of chemotaxis. These waves possess both spatial and temporal information. In the front of a wave, cells experience a positive spatial gradient of cAMP and an increasing temporal gradient, and in the back a negative spatial gradient and a decreasing temporal gradient. Several years ago, it was demonstrated that many of the complex changes in cell behavior associated with the different phases of a natural wave were in response to the temporal information. We have, therefore, begun a detailed analysis of cell behavior in 2D and 3D in a series of four temporal waves that mimic the temporal, but not spatial, dynamics of the average natural wave. The four normal simulated temporal waves had a period of seven minutes and were each composed of an increasing temporal gradient of cAMP beginning at 10^{-8} M and ending at 10^{-6} M cAMP over 3.5 minutes, and a deceasing temporal gradient beginning at 10^{-6} M and ending at 10^{-8} M over 3.5 minutes. Cells exhibited little or no response to the first wave, then exhibited a repeated sequence of behaviors associated with the different phases of each of the last three waves, most notably increased velocity and decreased lateral pseudopod formation in the front, a decrease in locomotion at the peak and erratic behavior associated with increased lateral pseudopod formation in the back. By manipulating the shape of the wave, we have tested whether cells assess the decreasing temporal gradient in the back of the wave. If each of the four waves consisted of a normal increasing gradient in the front, but an immediate return to buffer at the peak and continued buffer for 3.5 minutes (i.e., each wave lacked the decreasing gradient in the back), cells did not exhibit the normal responses associated with the last three in a series of four normal temporal waves, most notably they did not exhibit a surge in persistent translocation in the front. Under these conditions, motility appeared inhibited rather than stimulated in the front of each wave. Experiments were also performed in which 1) the first two waves were normal, while the last two lacked the decreasing gradient (i.e., involved an immediate return to buffer at the peak), and 2) after an increasing gradient in the front, cAMP concentration was held at peak concentration (10^{-6} M) for 3.5 minutes. Our results demonstrate that a cell must pass through one normal temporal wave, which primes it for normal responses to subsequent waves. In a series of four waves, if the first two are normal, and the third lacks the decreasing temporal gradient in the back, it loses priming. Our results also indicate that cells do indeed "read" decreasing temporal gradients of cAMP in the back of natural waves and that the decreasing temporal gradient is necessary for priming. The results underscore the importance of temporal gradients of cAMP in natural chemotaxis. They suggest that it is limiting, and in many cases misleading, to assess only the response of cells to a standing spatial gradient of cAMP, especially when analyzing mutants. Examples will be given of mutants that undergo chemotaxis relatively normally in spatial gradients of cAMP, but respond abnormally to temporal gradients, which causes major behavioral defects during natural chemotaxis.

Polarity and Chemotaxis II on 9/20/2006 from 8:30 to 12:10. Chaired by *Chang Chung*.

Microfluidic tools for the study of cell dynamics

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Understanding of directional sensing has seen a rapid advance through the use of fluorescent fusion proteins. Further progress in this field will rely on experimental techniques that provide quantitative control of chemical stimuli on the length scales of individual cells, with a temporal resolution that matches the time scales of the intracellular signaling events. We combine microfluidic techniques with the photo-chemical release of caged signaling agents to expose single cells to well-defined stimuli with high spatial and temporal resolution. Gradients of well-defined shape can be generated on micrometer length scales and subsecond switching times between different concentration profiles can be readily achieved. We apply this approach to quantify intracellular translocation of fluorescently labeled proteins in chemotactic *Dictyostelium* cells responding to complex stimuli with cAMP.

Polarity and Chemotaxis II on 9/20/2006 from 8:30 to 12:10. Chaired by *Chang Chung*.

A locally controlled inhibitory mechanism in GPCR-mediated chemoattractant sensing

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The signaling network underlying cAMP chemoattractant sensing consists of cAR1 GPCR-mediated signals, differential activation/deactivation of G-proteins and membrane bound enzymes, protein translocation and lipid modification. It has been proposed that an increase in cAMP receptor occupancy activates two antagonistic signaling processes: a rapid ìexcitationî that triggers cell response and a slower ìinhibitionî that turns off the responses. Several molecular mechanisms of the excitatory process have been revealed. cAMP binds to cAR1 and induces dissociation of Galpha2/Gbeta-gamma heterotrimeric G- protein. Free Gbeta-gamma activates Ras leading to the activation PI3K, which converts PIP2 into PIP3 on the inner plasma membrane. The phostatase PTEN acts as a direct antagonist of PI3K, dephosphorylating PIP3 to PIP2. While the molecular mechanisms of the inhibition process remain unknown. Quantitative measurement and modeling of the spatiotemporal dynamics of these known components in different regions of the cell in response to stimulation offers a way to reveal the principles and the mechanisms underlining the inhibition process. In this study, we designed sequential stimulation protocols to experimentally detect temporal and spatial aspects of the inhibition process around the membrane of single living cells. We found that repeated transient cAMP stimuli, unlike a sustained cAMP stimulation, induced repetitive PIP3 transient responses without detectable refractory periods. This result supports the notion that receptor-mediated excitation and inhibition processes rise and fall by following a fast and a slow temporal mode, respectively. Furthermore, we experimentally detected the asymmetric distribution of the inhibition process in a cell resided in a cAMP gradient. Exposing a cell to a sustained cAMP gradient led to a stable PHCrac-GFP accumulation in the front of the cell. A sudden withdrawal of the cAMP gradient from this polarized cell led to a rapid return of G-protein dissociation, PTEN and PHCrac-GFP distribution to basal levels around basal levels around the cell membrane. Interestingly, we found that there was a short time period during which re-activation of receptor/G-protein by imposing a uniform stimulation or the same spatial gradient induced a clear PHCrac-GFP translocation to the back but not the front of the cell. This novel inverted PIP3 response indicates that a sustained cAMP gradient induces an asymmetrically distributed inhibition that acts on the signaling pathway between G-protein and PI3K, which is surprisingly stronger in the front of the cell.

Polarity and Chemotaxis II on 9/20/2006 from 8:30 to 12:10. Chaired by *Chang Chung*.

Directional sensing in eukaryotic chemotaxis: a balanced inactivation model

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Many eukaryotic cells, including *Dictyostelium discoideum* amoebae, fibroblasts and neutrophils, are able to respond to chemoattractant gradients with high sensitivity. Recent studies have demonstrated that following the introduction of a chemoattractant gradient, several chemotaxis pathway components exhibit a sub-cellular reorganization that cannot be described as a simple amplification of the external gradient. Instead, this re-organization has the characteristics of a switch, leading to a well-defined front and back. Here, we propose a new directional sensing mechanism in which two second messengers are produced at equal rates. The diffusion of one of them, coupled with an inactivation scheme, ensures a switch-like response to external gradients for a large range of gradient steepness and average concentration. Furthermore, our model is able to reverse the sub-cellular organization rapidly and its response to multiple simultaneous chemoattractant sources is in good agreement with recent experimental results. Finally we propose that the dynamics of a heterotrimeric G-protein might allow for a specific biochemical realization of our model.

Polarity and Chemotaxis II on 9/20/2006 from 8:30 to 12:10. Chaired by *Chang Chung*.

Regulation of Dictyostelium cytokinesis by a SIN-related pathway

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Cell division is a well-coordinated process that involves mitosis bringing about division of the genetic material, followed by cleavage of the cell body. Our work aims at a more detailed understanding of these processes in particular the role of the cytoskeleton and the regulatory components.
To identify proteins important for cytokinesis, a REMI screen was performed that enabled the isolation of a multinucleated mutant with a peculiar cytokinesis defect. The gene affected in the REMI mutant encoded a Ser/Thr-kinase that we named septase, because it is crucial for proper cleavage furrow formation. The septase is homologous to Cdc7p from *Saccharomyces pombe*. Cdc7p is a central regulator of the septation-initiation network (SIN) of *S. pombe*. SIN controls events at the end of mitosis, initiates contraction of the actin ring and synthesis of the division septum enabling cytokinesis in fission yeast. A partially parallel pathway in *Saccharomyces cerevisiae* is the mitotic-exit network (MEN). MEN is also required for cytokinesis, but its main role is regulating the inactivation of cyclin-dependent kinases at the end of mitosis required for mitotic exit. The proteins constituting the SIN and MEN signaling cascades are quite conserved between the two yeasts, and some orthologs have been identified in filamentous fungi, *Caenorhabditis*, *Drosophila* and higher eukaryotes.
SIN components present in *Dictyostelium* were identified by searching the genome database. Amongst potential upstream regulators of the septase are the polo-like kinase (PLK), a GAP (Bub2), and a GTPase (Spg1). Downstream effectors of the septase may involve at least one of the NDR kinases (NdrB) and Mob1 proteins.
Expressing SIN-homologous proteins of *Dictyostelium* with fluorescent protein tags showed that Bub2, Spg1, and MobB and MobC localize to centrosomes and allowed visualization of these proteins in comparison to other marker proteins during the cell cycle.
We have started a functional analysis of the SIN-homologous proteins in *Dictyostelium*. This approach involves the generation of knock-out mutants as well as the identification of specific interactions by employing the tandem affinity purification method and the expression of protein domains for *in vitro* assays.

Supported by the Deutsche Forschungsgemeinschaft (SFB 413).

Dynamic Cortical Rearrangements, Mechanosensing, and Mechanical Feedback of Living Cells

Janet C. Effler, Kristine D. Girard, Scot C. Kuo, Pablo A. Iglesias, and Douglas N. Robinson

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Cell cortices rearrange dynamically to complete cytokinesis, crawl in response to chemoattractant, build tissues and make neuronal connections in environments where they are subjected to mechanical disturbances such as from neighboring cells or tissues. Highly enriched in the cortex, actin, myosin-II, and actin crosslinkers facilitate cortical movements. On the molecular scale, myosin-II generates mechanical force and senses mechanical loads, which alters its mechanochemistry. However, in the context of a whole nonmuscle cell where myosin-II acts on a complex, randomly arrayed cytoskeletal network, it is not known how myosin-II drives cell shape change, while promoting mechanical resistance. First, we used high resolution laser-based particle tracking (LPT) to probe the nanoscale, nonequilibrium cortical properties of living cells. From LPT studies, myosin-II mechanochemistry and dynacortin-mediated actin crosslinking control cortex dynamics in *Dictyostelium*. Consistent with its low duty-ratio, myosin-II does not directly drive active motility on long time-scales. Instead, myosin-II and dynacortin antagonistically regulate other active processes in the living cortex. Second, we discovered a novel mechanosensory system that is dependent upon myosin-II and that ensures robust cytokinesis. Mitotic cells sense and respond to applied forces by redistributing contractile ring proteins, including myosin-II, to the site of the mechanical disturbance, rejecting the disturbance, and completing symmetric cytokinesis. Our observations indicate that the spatial and temporal changes of cell shape during cytokinesis are controlled by mechanical feedback that overrides normal spindle signals and that directs the contractile apparatus anywhere along the cortex. This feedback system may provide critical regulation that ensures successful cell division. Our combined effort is revealing the interface between myosin-II-driven active cortical rearrangements and passive cortical properties from actin crosslinkers that govern cell shape dynamics.

Actin and Cytoskeleton on 9/20/2006 from 16:30 to 19:00. Chaired by *Carole Parent*.

Myosin heavy-chain kinase A from *Dictyostelium* possesses a novel actin-binding domain that cross-links actin filaments

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Myosin II in *Dictyostelium* and nonmuscle cells exists in a dynamic equilibrium between a cytoplasmic pool of monomers and a cytoskeleton-associated assembly of bipolar filaments. Myosin II filament disassembly in Dictyostelium is driven by phosphorylation of the myosin II heavy chain (MHC) "tail" region via the activities of three structurally-related MHC kinases: MHCK-A, -B, or -C. All three of the MHCKs share homologous catalytic and WD-repeat domains. MHCK-A is the most extensively-studied of the MHC kinases. Recent studies from our lab have revealed a unique relationship between MHCK-A and F-actin, whereby F-actin is a potent activator of MHCK-A activity (50-fold); and MHCK-A, in turn, possesses the ability to organize actin filaments into bundles. In contrast to MHCK-A, there is essentially no information about the structure-function relationships defining the activities of the MHCK-B and -C enzymes. We have examined the in vivo function of the WD-repeat domain of MHCK-C by over-expressing either full-length MHCK-C (MHCK-C++) or a truncated version of MHCK-C lacking the WD-repeat domain (MHCK-C-ΔWD++) in Dictyostelium cells, and then assaying for myosin II-dependent activities such as cytokinesis and multicellular development. As has been reported previously (Betapudi et al., 2005, Mol Biol Cell, 16:2248), MHCK-C++ cells exhibited cytokinesis defects with reduced growth in suspension culture and increased multinuclearity. Interestingly, we found that MHCK-C-Δ-WD++ cells also do not proliferate in suspension culture; however, this growth defect is not accompanied by an increase in multinuclearity. In addition, the MHCK-C-Δ-WD++ cells, unlike their MHCK-C++ counterparts, retain the ability to undergo multicellular development. Together, these results suggest that the WD-repeat domain of MHCK-C may not serve the same myosin targeting function as the WD-repeat of MHCK-A. Moreover, our results indicate that under certain conditions, MHCK-C may exhibit activities outside of its known MHC kinase function in the cell.

The N-terminus of *Dictyostelium* Scar interacts with Abi and HSPC300 and is essential for proper regulation and function

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Scar/WAVE proteins, members of the conserved Wiskott-Aldrich Syndrome (WAS) family, promote actin polymerization by activating the Arp 2/3 complex. A number of proteins, including a complex containing Nap1, PIR121, Abi1/2 and HSPC300, interact with Scar/WAVE, though the role of this complex in regulating Scar function remains unclear. Here we identify a short N-terminal region of Dictyostelium Scar that is necessary and sufficient for interaction with HSPC300 and Abi in vitro. Cells expressing Scar lacking this N-terminal region show abnormalities in F-actin distribution, cell morphology, movement, and cytokinesis. This is true even in the presence of wild type Scar. The data suggest that the first 96 amino acids of Scar are necessary for participation in a large molecular weight protein complex, and that this Scar-containing complex is responsible for the proper localization and regulation of Scar. The presence of mis-regulated, or unregulated Scar has significant deleterious effects on cells and may explain the need to keep Scar activity tightly controlled *in vivo* either by assembly in a complex or by rapid degradation.

Regulation of Polarized F-actin Organization in Chemotaxing Cells

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The actin cytoskeleton is highly polarized in chemotaxing *Dictyostelium* cells, with F-actin assembled predominantly in the anterior leading edge and to a lesser degree in the cell's posterior. In a previous study, we demonstrated that WASP localizes on vesicles and these vesicles appear to be preferentially distributed at the leading edge and uropod of chemotaxing cells. We have examined the role of PCH family proteins, CLP1 and CLP2, in the regulation of polarized WASP localization and F-actin organization. The PCH family members share a similar domain organization, including an amino-terminal FCH domain followed by a coiled-coil domain, proline-glutamic acid-serine-threonine–rich (PEST) sequences, and a SH3 domain. CLP1 and CLP2 appear to be functionally redundant and deletion of both CLP1 and 2 cause a loss of polarity and severe defects in chemotaxis. WASP and CLP1 are colocalized on vesicles and interactions between SH3 domain of CLP1/2 and the proline-rich repeats of WASP are required for vesicular localization. Polarized distribution of vesicles is absent in cells lacking CLP1/2 or RacC, a major regulator of WASP in *Dictyostelium*. Onset of cellular polarity causes change of CLP1 localization from centrosome to vesicles and RacC is required for this change of localization. Our results suggest that polarized F-actin organization in chemotaxing cells is controlled by biased delivery of vesicles containing WASP , CLP1/2, and RacC to the leading edge and uropod presumably via interaction of FCH domain of CLP1/2 with microtubules.

Role of Skp1 prolyl hydroxylation and glycosylation in culmination in *Dictyostelium*

Christopher M. West[1], Zhuo A. Wang[1], Hanke van der Wel[1], and Ira J. Blader[2]

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A biochemical screen for glycosylation of nuclear and cytoplasmic proteins netted a novel pathway consisting of five cytoplasmic glycosyltransferases acting on Skp1 in the social amoebazoan *Dictyostelium*. Skp1 is best known as a subunit of the SCF-class of E3 ubiquitin ligases responsible for regulating the lifetime of cell cycle and developmental regulatory proteins. The glycan chain is assembled on 4-hydroxyproline, in contrast to the more common attachment of *O*-linked glycans to Thr or Ser. Skp1 proline is hydroxylated by an ortholog of a cytoplasmic/nuclear prolyl hydroxylase described in animal cells, where this oxygen-dependent enzyme has been hypothesized to be a physiological oxygen sensor. Genetic inactivation of the prolyl hydroxylase P4H1 blocks Skp1 glycosylation and inhibits culmination, as assessed morphologically and quantitated by counting spores. Inhibition ranges from 80-99.9%, but occasionally culmination is only delayed and cells must be developed at 15-18% to observe full inhibition. The reason for this variation is not known, but incubating cells in the presence of 40% O2 fully rescues culmination (but not Skp1 glycosylation), indicating that O2 influences cells in multiple ways. Genetic inactivation of the second glycosyltransferase in the pathway does not inhibit culmination, indicating that inhibition is not due to inability to form the full glycan chain. The slug-to-fruit switch is normally regulated by multiple signals operating through prestalk A tip cells via a protein kinase A (PKA)-dependent mechanism. PKA overexpression partially rescues culmination in P4H1-null cells, indicating that P4H1 functions upstream of PKA. Mutant cells fail to express an *ecmA*-driven marker in tip cells, suggesting an effect on prestalk tip cells. Small numbers of RFP-labeled wild-type cells migrate to the tip and rescue the switch, suggesting that P4H1 is involved in signaling culmination non cell autonomously. The requirement for P4H1 can be overridden by expression of P4H1 in prestalk cells, but not by overexpression of an enzyme-dead mutant of P4H1. The results suggest that prolyl 4-hydroxylation mediates a signaling pathway that contributes to regulation of the slug-to-fruit switch, a critical life cycle event that controls the location of sporulation in the soil microenvironment. Biochemical and bioinformatics studies suggest the occurrence of the Skp1 modification pathway in the intracellular pathogen *Toxoplasma gondii*, indicating that *Dictyostelium* will be a useful model for understanding developmental regulation in other difficult-to-study protists. Supported by NIH GM-03759.

Role of a cytoplasmic prolyl 4-hydroxylase in oxygen-dependent development in *Dictyostelium*

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Development is dependent on O_2 in *Dictyostelium*, suggesting that this organism may be a good model system for understanding O_2 regulation in cells. Concentrations as high as 10% (ambient = 21%) selectively inhibit culmination and inhibition is fully reversible when cultures are returned to normal atmosphere. In contrast, growth is normal at an O2 concentration as low as 2.5%. Overexpression of protein kinase A under control of its own promoter partially rescues culmination, suggesting that O_2 signals through known pathways that regulate the slug-to-fruit switch. P4H1, the *Dictyostelium* ortholog of cytoplasmic animal prolyl 4-hydroxylases, is an O_2-sensitive enzyme in vitro. Genetic inactivation of P4H1 inhibits culmination similar to mild hypoxia. Here we show that overexpression of P4H1 bypasses hypoxic inhibition, suggesting that the hypoxic signal is mediated by P4H1 oxygen-substrate starvation. Skp1, which is best known as a subunit of E3(SCF) ubiquitin ligases responsible for regulating the lifetime of cell regulatory proteins, is the only detectable acceptor target of P4H1, based on an indirect assay for measuring the level of P4H1 substrates which accumulate in a P4H1-null strain. Two almost identical Skp1 genes (Skp1A & Skp1B) are present in the Dictyostelium genome and are expressed. Development of a Skp1B-disrupted strain is normal. To obtain independent evidence that the function of P4H1 is mediated via Skp1A, we developed a method to mutate the codon for the P4H1-target proline in the Skp1A gene locus. Current evidence indicates that Skp1A does not tolerate a Pro143Ala substitution except in the presence of Skp1B. Other amino acid substitutions are being tested to identify a residue that cannot be hydroxylated by P4H1 but still supports basic Skp1 functions. These results suggest that P4H1 is a critical mediator of oxygen-dependent development in *Dictyostelium*, but further studies are required to establish the predicted role of Skp1 in oxygen signaling. Supported by NIH GM-37539.

Microarray analysis reveals STATc as a key regulator of the Dictyostelium transcriptional response to hyperosmotic shock

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Dictyostelium discoideum is frequently subjected to environmental changes in its natural habitat, the forest soil. In order to survive the organism had to develop effective mechanisms to sense and respond to such changes. When cells are faced with a hypertonic environment a complex response is triggered. It starts with signal sensing and transduction and leads to changes in cell shape, the cytoskeleton, transport processes, metabolism and gene expression. Certain aspects of the *Dictyostelium* osmotic stress response have been elucidated, however, so far no comprehensive picture is available.
To better understand the *D. discoideum* response to hyperosmotic conditions, we performed gene expression profiling using DNA-microarrays. The transcriptional profile of cells treated with 200 mM sorbitol for one hour and during a 2-hour time course was analysed. The results of the time course revealed a time-dependent induction or repression of 809 genes, more than 15% of the genes on the array, which peaked 45 to 60 minutes after the hyperosmotic shock. The differentially regulated genes were applied to cluster analysis and functional annotation using gene ontology (GO) terms. Two main responses appear to be the down-regulation of the metabolic machinery and the up-regulation of the stress response system, including STATc. Further analysis of STATc revealed that it is a key regulator of the *Dictyostelium* transcriptional response to hyperosmotic shock. Approximately 25% of the differentially regulated genes were dependent on the presence of STATc. We analysed the promoter region of one STATc-regulated gene, *rto*A, and located an essential region. However, we were unable to detect a direct interaction with STATc. Based on our results we conclude that at least two, possibly three, signal transduction pathways get activated in *Dictyostelium* cells subjected to hypertonicity.

Oxygen, Stress and Death on 9/20/2006 from 20:00 to 22:00. Chaired by *Derrick Brazill*.

KeaA, a *Dictyostelium* kelch-domain protein that regulates the response to stress and development

Luciana Mantzouranis, Raquel Bagattini, Fernando Toshio Ogata, Milton Yutaka Nishiyama-Junior, Ricardo Zorzetto Nicoliello VÍncio and Glaucia Mendes Souza

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YakA is a protein kinase required for the regulation of several stress responses in Dictyostelium. YakA acts by regulating the expression of the cAMP-dependent protein kinase pkaC in response to nutrient starvation, oxidative, nitrosoative and thermal stresses. PKA-C is necessary for the inhibition of the cell cycle that follows treatment with hydrogen peroxyde and components that generate nitric oxide. This protein kinase is also known for its role in the regulation of several developmental genes that will orchestrate aggregation and differentiation during Dictyostelium development. To identify components that might modulate the nitrosoative/oxidative stress responses, *yakA*$^-$ second site suppressors were isolated from a pool of insertional mutants treated with sodium nitroprusside (SNP). DNA from a confirmed SNP resistant clone was isolated, the mutated gene was cloned by plasmid rescue and its sequence determined. The gene DG1106, previously mutated by the Developmental Gene Program was identical to the mutated gene. Due to its similarity to kelch proteins, the mutated gene was named keaA. The gene keaA codes for a kelch domain protein with six kelch repeats at the C-terminus and a zf-C3HC4 domain at the N-terminus. An additional feature is a cysteine-rich sequence located in the mid-portion of the protein.

A mutation in keaA suppresses the hipersensitivity of yakA$^-$ cells to oxidative and nitrosoative stresses as well as death induced by SNP and H2O2. The SNP-treated double mutant yakA$^-$/keaA$^-$ shows a growth profile similar to treated wild-type cells and no extensive lysis is observed. Similarly, no lysis is observed when the double mutant was treated with H2O2. To determine if a mutation in keaA might suppress the faster cell cycle phenotype of yakA- cells growth curves were performed. The double mutant yakA$^-$/keaA$^-$ shows growth rates similar to yakA- cells, indicating that the faster cell cycle phenotype is not suppressed by a mutation in keaA. Moreover the growth profile of keaA- cells indicates that this gene is necessary for growth. This growth deficiency is even more pronounced when cells are diluted to very low density (under 10^5/ml).

SNP and H2O2 treatments induce cAMP synthesis in wild-type cells but the induction is not significant in yakA-, acaA- and keaA- cells. This indicates that these genes are necessary for cAMP production in response to these stresses. A morphological analysis of keaA- during multicellular development indicated that, although this gene is not absolutely required for aggregation, a delay in aggregation is observed depending on the starvation conditions. keaA- cells express very low levels of pkaC and carA during aggregation and culmination. This may be the reason why these cells are delayed in the completion of the developmental process.

With the aim of confirming a role for keaA in development the effect of over-expressing the cysteine-rich domain and Kelch domain was analysed. The results indicate a role for the cysteine-rich domain in the regulation of development. The mutant cells take as long as wild-type cells to reach the stage of tight mounds, however the mutant cells present smaller aggregates and therefore smaller culminants and fruiting bodies. keaA expression was analysed in wild type cells during exponential growth and the results indicate an induction of keaA similar to the observed for genes responsive to pre-starvation.

Finally, to investigate how treatment of cells with compounds that generate nitrosoative or oxidative stresses would impact on transcript levels cDNA microarrays are being used. Through this technique and qPCR analysis we could identify genes that are regulated by keaA as example dscA, csbB, cycB and pefA.

Oxygen, Stress and Death on 9/20/2006 from 20:00 to 22:00. Chaired by *Derrick Brazill*.

Necrotic cell death in *Dictyostelium*

P Golstein, C Laporte, A Kosta, G Klein, L Aubry, D Lam, E Tresse, and MF Luciani

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While necrotic cell death is attracting considerable interest in particular from a pathophysiological point of view, its molecular bases are still poorly understood. Investigations in simple biological models, taken for instance outside the animal kingdom, may benefit from less interference from other cell death mechanisms and from better experimental accessibility, while providing phylogenetic information. Can necrotic cell death occur outside the animal kingdom?
In the protist *Dictyostelium,* developmental stimuli lead in a monolayer technique to vacuolar "stalk" cell death. We previously showed that under the same conditions *atg1* autophagy mutant cells do not vacuolize, but still die. We now report that these cells undergo a stereotyped sequence of events characteristic of necrotic cell death. This sequence included swift mitochondrial uncoupling with mitochondrial DC-FDA fluorescence, ATP depletion and increased oxygen consumption. This was followed by perinuclear clustering of dilated mitochondria. Rapid plasma membrane rupture then occurred, which was evidenced by time-lapse videos and quantified by FACS. Of additional interest, developmental stimuli and classical mitochondrial uncouplers triggered a similar sequence of events, and exogenous glucose delayed plasma membrane rupture in a non-glycolytic manner.
The occurrence of necrotic cell death in the protist *Dictyostelium* (1) provides a very favorable model for further study of this type of cell death, and (2) strongly suggests that the mechanism underlying necrotic cell death was present in an ancestor common to the Amoebozoa protists and to animals and has been conserved in evolution.

Oxygen, Stress and Death on 9/20/2006 from 20:00 to 22:00. Chaired by *Derrick Brazill*.

Functional analysis of a UDP-GlcNAc:Thr polypeptide alpha-N-acetyl-D-glucosaminyltransferase-like (pp-alpha-GlcNAcT) gene of *Trypanosoma cruzi* using *Dictyostelium discoideum*

Norton Heise[1,2], Divyendu Singh[1], Hanke van der Wel[1], Altan Ercan[1], Luciana Loureiro Penha[2], Lucia Mendonca-Previato[2], Jose Osvaldo Previato[2], and Christopher M. West[1]

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Mucin-like glycoproteins are the major surface components in different life-cycle stages of the protozoan parasite *T. cruzi*, the causative agent of Chagas' disease in humans. Evidences suggests that these surface mucins likely participate in both host- and vector-parasite interactions. Although these heterogeneous and highly *O*-glycosylated glycoproteins are known to be encoded by multigene families of hundreds of genes, all of their *O*-glycans are attached at their reducing ends by an α-*O*-*N*-acetylglucosamine linked to Thr. The enzyme responsible for the addition of this first sugar residue, a pp-αGlcNAcT, has been characterized biochemically (Previato *et al*., 1998, JBC **273:** 14982) and shown to be a Golgi resident protein (Morgado-Díaz *et al*., 2001, Parasitology **123:** 33). Inhibition of mucin-type *O*-glycosylation biosynthesis at the point of GlcNAc addition to the protein seems to be a good drug target to be explored against the parasite.

The social amoeba *Dictyostelium discoideum* also assembles *O*-linked glycans on mucin-type domains in spore coat proteins and GPI-anchored cell surface proteins, and these, like those of *T. cruzi*, are linked via α-*O*-*N*-acetylglucosamine linked to Thr (but also Ser). Recently, the Golgi-associated enzyme that catalyzes formation of this linkage was cloned and demonstrated to be encoded by the *modB*-locus that previous studies had shown is required for a major class of *O*-linked glycosylation. In order to characterize the *T. cruzi* enzyme at the molecular level, the *modB* gene sequence was used to search the *T. cruzi* genome sequence database (El-Sayed *et al*., 2005, Science **309**: 409) using BLAST. This search yielded three type-2 membrane protein sequences showing similarity to the *Dictyostelium* Golgi pp-±GlcNAcT (Wang *et al*., 2003, JBC **278:** 51395). Two of these are predicted to be catalytically active whereas the third is not, and all three are conseerved and syntenic throughout the trypansomatid family where sequence information is available, including *T. brucei* and *Leishmania major*.

The predicted biochemical function of the *T. cruzi* pp-αGlcNAcT-like sequences was tested by expression in growth phase *modB*-mutant *Dictyostelium* cells, using an integrating expression vector for secretory proteins (pVS4). *modB*-mutants lack selected carbohydrate epitopes recognized by several monoclonal antibodies. One of the three catalytic domain constructs partially complemented the absence of endogenous pp-αGlcNAcT based on Western blotting using mAb 54.2 or the lectin wheat germ agglutinin. These results confirm the predicted glycosyltransferase activity of this gene. Current studies are examining the utility of the *Dictyostelium* expression system for investigating the biochemical mechanism of the enzyme, the function of the other two related genes, and the effect of inhibitors.

Supported by OCAST, CNPq, FAPERJ.

Development III on 9/21/2006 from 8:30 to 10:10. Chaired by *L. Kim*.

Cell density sensing is mediated by a G protein coupled receptor

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Much remains to be understood about how cells sense their local density. When *Dictyostelium* cells starve, they secrete a glycoprotein called conditioned medium factor (CMF). At a high density of starving cells, as indicated to cells by a high extracellular concentration of CMF, the cells aggregate. RpkA is a G protein coupled receptor (GPCR) in *Dictyostelium* which contains a seven transmembrane region and a C-terminal PIP5 kinase domain. Like cells lacking CMF, rpkAØ cells fail to aggregate, have altered expression of developmentally regulated genes, and do not generate cAMP pulses. Similar to wild type cells, rpkAØ cells secrete CMF upon starvation. Unlike wild type cells, rpkAØ cells do not form streams in the presence of recombinant CMF (rCMF) and do not induce the production of inositol trisphosphate in response to rCMF, indicating that RpkA regulates CMF signal transduction. Inactivation of G alpha1, a negative regulator of CMF signaling, rescues the developmental defect of the rpkAØ cells. The developmental defect of rpkAØ cells is also rescued with expression of RpkA, a kinase-dead RpkA, or the transmembrane fragment of RpkA. This indicates the transmembrane domain of RpkA is necessary and sufficient for CMF signaling. Together, our data suggest that RpkA is a G protein coupled receptor required for cell density sensing.

G Protein Specificity in Chemotaxis and Development

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Dictyostelium uses a variety of G proteins during chemotaxis and development to mediate cellular responses when stimulated by extracellular signals. The specificity of cellular responses appears to be provided primarily by the receptor and coupled Gα subunit because other downstream signaling components can often function in multiple pathways. Previous phenotypic analyses of knockout or over-expression mutants suggest Gα subunits contribute to pathway specificity but whether this specificity is due to Gα subunit-receptor interactions only or other Gα subunit interactions has not been established. Studies of Gα subunits in other organisms indicate the Gα subunit carboxyl terminus is important for the coupling to specific receptors and so to address the role of Gα subunit functional specificity in *Dictyostelium*, chimeric Gα subunits were created with replacements in the carboxyl terminus. A chimeric Gα2/4 subunit (Gα2 subunit with a Gα4-specific carboxyl terminus) but not a chimeric Gα5/4 subunit partially rescued folate-stimulated cGMP accumulation and chemotactic movement in a *gα4⁻* mutant. In wild-type cells, the Gα5/4 subunit severely inhibited chemotaxis to folate and decreased aggregate size in developing populations demonstrating this subunit shares many features with the wild-type Gα5 subunit. These results suggest Gα subunit structure outside of the carboxyl terminus provides important determinants for cellular responses independent of receptor specificity. Furthermore, a comparison of wild-type and chimeric Gα subunit phenotypes and structure reveals a potential role for Gα subunit-MAP kinase interactions in the regulation of cellular responses.

TagA and AcbA Interact to Regulate Cell Fate Specification and Spore Encapsulation.

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The tag gene family (tagA, B, C and D) are predicted ABC transporters containing a serine protease domain. Genetic evidence for tagA, B, and C indicates they are involved in cell signaling events during development. Previously we have provided evidence that TagA is required for proper cell fate determination, as well as, correct expression of some cell type specific markers (1 and in press). The tagA mutant produces an overabundance of PstB cells and expresses the cotB marker in mature stalk cells. AcbA is an acyl-CoA binding factor and a homolog to the precursor of DBI a peptide that modulates the activity of the GABAA receptor in neurons (2). It is known that AcbA is the precursor for SDF-2 signaling peptides, and compelling evidence suggests that TagC has a trypsin-like enzymatic activity that converts AcbA into SDF-2 peptides (2,3). SDF-2 peptides stimulate spore encapsulation late in development and we are testing the hypothesis that AcbA-derived peptides are produced by TagA and act early in development to specify cell fate (3,4,5). ;; Here we show evidence that the ABC transporter/serine protease TagA, and AcbA interact in a pathway or network to control cell fate specification and spore encapsulation. Mis-expression of the cell type specific marker cotB in stalk cells during development as well as an overabundance of pstB cells in the TagA and AcbA knockouts illustrate a similarity of cell fate specification defects. Furthermore, chimeric mixtures of the two mutants reveal a failure of either mutant to suppress the mis-expression of cotB in the other, or to prevent the other mutant from contributing to the stalk as wild-type cells can. The tagA mutant fails to release SDF-2 peptides during culmination and it overproduces SDF-1, phenocopying the acbA mutant. Overexpression of the protease domain from TagA produces multi-tipped aggregates, but fails to do so in the acbA mutant, suggesting that acbA acts downstream from tagA. Indirect immuno-fluorescence studies show that anti-TagA and anti-AcbA antibodies stain puncta, presumably vesicles, that are distinct from the endoplasmic reticulum and Golgi apparatus. TagA is predominantly found on endosomal vesicles, indicated by co-localization with GFP-N-Golvesin, while AcbA is found predominantly on endosomes, but also in other locations, consistent with the known role of AcbA orthologs in lipid metabolism. Thus, it is possible that AcbA is accessible for processing by TagA within the cell. In 22-hour cells, AcbA or AcbA peptides localize in what appear to be vesicles that are located just underneath the plasma membrane. Treatment of 22-hour cells with SDF-2 peptide appears to cause the release of AcbA from these vesicles since the staining pattern disappears. Such a mechanism would provide a means for the autocrine release of SDF-2 peptides during spore encapsulation. AcbA fails to properly localize to these vesicles during late development in the tagA mutant, further supporting the hypothesis that TagA processes AcbA. Minimally, TagA is required for the proper localization of AcbA or its processed products during the late stages of development. Finally, we show that the TagA serine protease has trypsin-like specificity in vitro. We plan to continue to define the biochemical relationship between TagA and AcbA and explore the possibility that DhkA acts as the receptor for AcbA cleavage products in cell fate specification.

Development III on 9/21/2006 from 8:30 to 10:10. Chaired by *L. Kim*.

Phagocytosis of bacteria by *Dictyostelium* amoebae.

Margaret Clarke and Lucinda Maddera

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Dictyostelium cells are professional phagocytes that avidly consume bacteria, their natural prey. Using fluorescent probes, we have monitored the initial steps in this process in living amoebae. The prey was live E. coli expressing cytoplasmic GFP or DsRed-Express. Using probes that bind differentially to newer and older actin filaments, we have visualized the assembly and disassembly of actin filaments responsible for extending the phagocytic cup to engulf a bacterium, and, after the phagosome has sealed, for propelling the phagosome away from the site of uptake. In bacteria expressing fluorescent proteins that are susceptible to proteolysis, we have monitored loss of that fluorescent signal and staining of the bacterial contents with Neutral Red, indicating permeabilization of the cell wall and acidification of the cytoplasm. This occurs during a period of microtubule-based transport that promotes fusion of the phagosome with microtubule-associated acidic endosomes; the phagosome merges with the highly dynamic endocytic mixing compartment. These events occur within the first six or seven minutes after formation of the phagosome. We have also tracked the phagosome membrane during this interval. PHcrac-GFP, which binds to membranes enriched in PI(3,4,5)P3, brightly labels the phagocytic cup and new phagosome, but fades shortly after internalization. At this time, 2FYVE-GFP, which binds to membranes enriched in PI3P, becomes bright. The 2FYVE-GFP label persists, revealing the changing morphologies and interconnections of the phagosome membrane during mixing/recycling. We are currently comparing these aspects of trafficking for phagosomes containing E. coli vs. virulent and avirulent strains of L. pneumophila.

A vesicle surface tyrosine kinase regulates phagosome maturation

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Phagocytosis is an evolutionarily conserved process that is crucial for host defense against microbial pathogens and for obtaining nutrients in *Dictyostelium discoideum*. Phagocytosed particles are delivered via a complex route from phagosomes to lysosomes for degradation, but the molecular mechanisms involved in the phagosome maturation process are not well understood. Here, we have identified a novel vesicle associated receptor tyrosine kinase-like protein, VSK3, in *D. discoideum* and demonstrated its novel role in phagosome maturation. VSK3 resides on the membrane of late endosomes/lysosomes with it C-terminal kinase domain facing the cytoplasm. Both inactivation of VSK3 by gene disruption as well as over-expression of VSK3 reduced the rate of phagocytosis, while over-expression of VSK3 lacking the kinase domain had no effect. Though the protein is not involved in the engulfment process, it is required for the fusion of phagosomes with late endosomes/lysosomes. These findings, along with the remarkable similarities between *D. discoideum* and metazoans in the known mechanisms that govern phagocytosis, suggest that regulated tyrosine kinase signaling on the surface of endosome/lysosomes may represent a general control mechanism for phagosome maturation in all phagocytes.

Rheb Positively Regulates TOR Complex 2 to Suppress Phagocytosis: A Potential Pathway to Coordinate Nutrient Particle Capture and Growth

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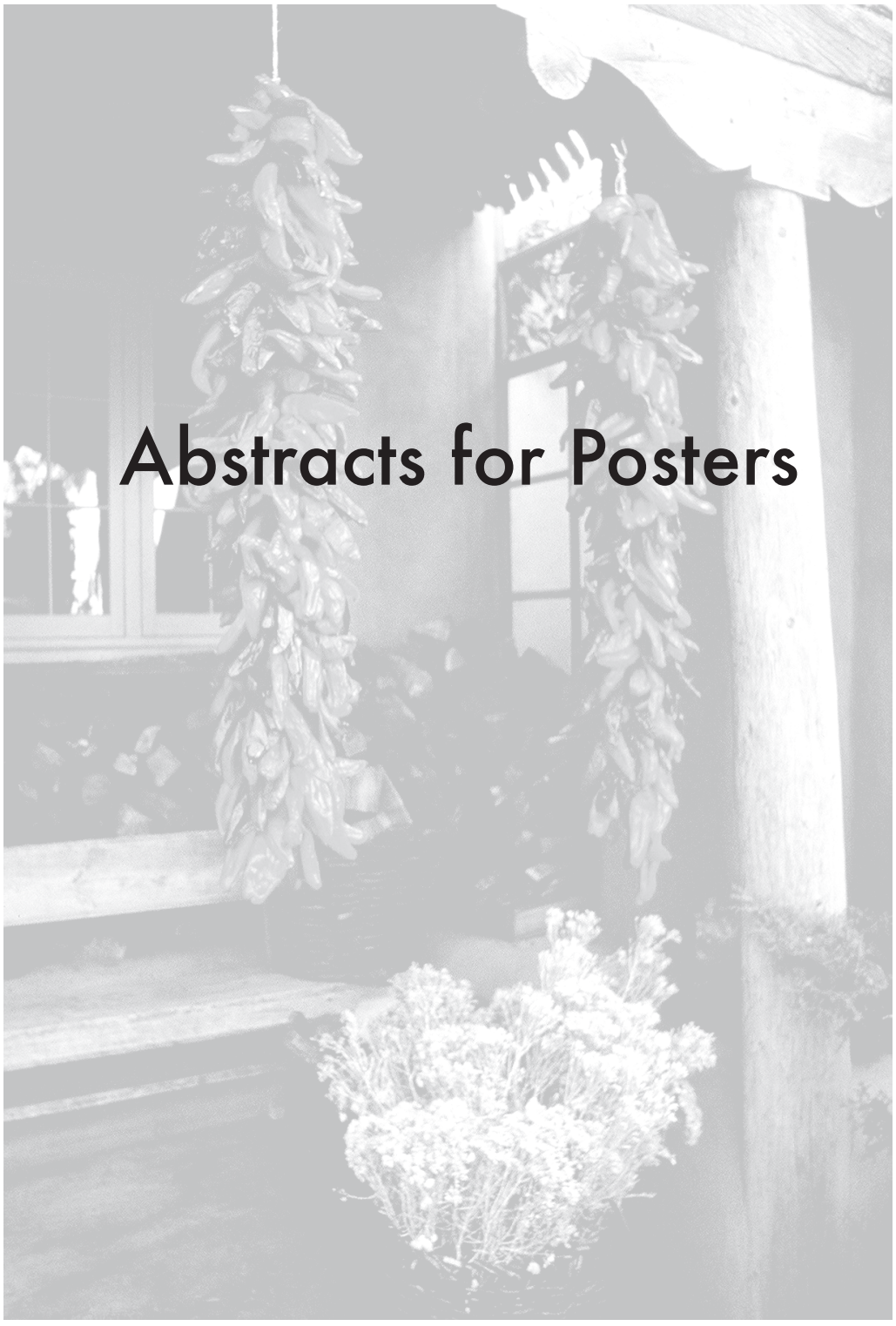
NIDDK/National Institutes of Health, Bethesda, MD 20892

TOR Complex 2 (TORC2) is required for actin cytoskeletal polarization, while TORC1 is required for growth. Since *Dictyostelium* engulf bacteria for nutrient uptake by an actin-dependent process, we focused on TORC2 as a potential co-regulator with TORC1 of cellular growth. Surprisingly, we show that loss of TORC2 components Pia, RIP3, and LST8 promotes nutrient particle uptake, via phagocytosis, while inactivation of TORC1, by depletion of TORC1-specific raptor, has no effect on phagocytosis. Furthermore, epistatic data show that activated Rheb suppresses phagocytosis in a TORC2-dependent manner, suggesting that TORC2 and TORC1 are subject to positive, co-regulation by the Rheb pathway. We also show that TORC2 may be indirectly inhibited by rapamycin by a mechanism that requires a productive TOR/raptor complex and suggest that TORC2 and TORC1 complexes may be in equilibrium. The integrated and balanced regulations of TORC2 and TORC1 may be critical to coordinate and optimize growth with energy needs.



Abstracts for Posters

bZIP Transcription Factors in Dictyostelium discoideum

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Transcription factors (TF) play a pivotal role in the regulation of gene expression. We are studying the function of a major family of TFs, the basic region leucine zipper (bZIP) TFs in *Dictyostelium discoideum*. bZIPs are known to be involved in a number of intracellular signaling processes that regulate differentiation. There are 19 bZIPs in *Dictyostelium*, and we have characterized 2 of them. DimA and DimB were found to be required for DIF-1 signaling, and they homo- and heterodimerize to regulate DIF-1 induced cellular processes (Thompson *et a.l* 2004, Huang *et al*. 2006). However, central questions remained such as the candidates of the cAMPresponse element binding protein (CREB) and other bZIPs that could form heterodimers with DimA and/or DimB. Compared to other species, bZIPs in *Dictyostelium* have relatively greater potential of heterodimerization based on our computational analysis of their leucine zipper regions. Therefore, they might be able to increase the variety of their regulatory capacity by building a complex interaction network. We implemented a bottom-up strategy to construct deletion mutants of each bZIP with the goal of finding their individual functions. Preliminary morphological data reveal several CREB like bZIPs where the mutant strains are aggregationless or have severe morphological defects in early development. Mutants that show similar phenotypes and have high predicted dimerization scores, calculated using the Base Optimized Weights algorithm (Fong *et al*. 2004), will be test in vitro for protein-protein interations. We also tested the transcription patterns of the bZIPs. Several bZIPs show similar developmental expression patterns to that of the *dimA* and *dimB*. Among those, at least two slightly favor prespore expression, similar to *dimA* and *dimB*. One of the TF genes, bZIP19, showed a strong preferential expression in prestalk cells and the knockout mutant formed small gnarled fruiting bodies. We are continuing to analyze these genes with the ultimate goal of understanding the function all the bZIPs in *Dictyostelium* and how they interact to regulate gene expressions in various biological processes.

Dhamodharan Neelamegan

102

Use of Dictyostelium enzymes in the development of glycoconjugate vaccines against gram negative bacterial infection.

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Lipopolysaccharide (LPS) of Gram negative bacteria are responsible for several pathophysiological effects during infection. Glycoconjugate vaccine strategies involve generation of LPS – protein conjugates to promote long lasting immune responses to the otherwise poorly immunogenic saccharide epitopes. The LPS must be deacylated prior to conjugation to preclude the endotoxicity of the LPS molecule. We make use of Dictyostelium enzymes in obtaining deacylated LPS molecules retaining key epitopes like phosphoethanolamine (PEtn) groups, which are sensitive to chemical deacylation methods.

Dictyostelium in their natural habitat use bacteria as a food source for growth, and they produce several cell lysing enzymes to break down the bacterial cell wall. We make use of two *Dictyostelium* enzymes, fatty acid amidase I (FAAI) and fatty acid amidase II (FAAII) to deacylate two N-linked fatty acid present on LPS molecule. The amidase secreted during axenic growth removes one fatty acid from LPS containing two N-linked fatty acids. Another amidase activity was found to be cellular and produced when the cells undergo starvation or when grown on bacteria. The cellular amidase uses the substrate with one N-linked fatty acid. Using the described enzymes, glycoconjugates were made from N meningitidis LPS and rabbits were immunized, the immune sera analyzed showed good responses and good cross reactivity to LPS from other meningococcal strains. The *Dictyostelium* enzymes are also capable of acting on purified LPS from other pathogenic bacteria and therefore provide a useful tool in manipulating LPS for use in vaccine development.

Bill Loomis

103

Global Proteomics of Differentiating Cells

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We have used liquid chromatography coupled to tandem mass spectroscopy (LC-MS/MS) to characterize proteins present in total extracts prepared from exponentially growing cells, prespore and prestalk cells separated after 18 hrs of development. 4194 unique proteins were recognized on the basis of one or more tryptic peptides. 1530 proteins were unequivocally characterized(>= 5 peptides) of which 1100 (72%) did not change appreciably or went down during development. These included the ribosomal proteins and many house-keeping proteins. Of the 430 proteins that increased >2x during development, 200 were preferentially found in prespore cells and 168 were preferentially found in prestalk cells although many of them were only marginally enriched in one or the other cell type.

28 prespore proteins and 28 prestalk proteins from the LC-MS/MS analyses had matching mRNA data on microarrays confirmed by whole mount in situ hybridization. There was excellent agreement between the protein and mRNA data concerning the degree of cell type enrichment. Prestalk proteins were less enriched than prespore proteins, possibly because many of the prestalk proteins were enriched in only a subset of prestalk cells. Microarray data was available for 334 of the 1530 well characterized proteins. The majority (227) of these mRNAs and proteins showed no cell type enrichment.

Proteins that accumulated during development correlated well with mRNAs that accumulated during development. On the other hand, the protein products of mRNAs that became less abundant during development did not decrease to a comparable extent. It seems that most vegetative proteins are fairly stable during development. ;; These preliminary results are being confirmed with samples that have been stable isotope labeled to increase reliability of quantification. Moreover, evidence for post-translational modifications of specific proteins is being extracted from the MS/MS data. Already the proteomics data have confirmed that a relatively small proportion of the genetic repertoire of *Dictyostelium* is deployed uniquely during development and that most of the changing physiology of the cell types results from changes in only a few hundred proteins.

Catherine Pears

104

Requirement for DNA-PK in restriction enzyme-mediated integration of plasmids into the genome of Dictyostelium.

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DNA double strand breaks (DSBs) can be repaired by either homologous recombination or non-homologous end-joining (NHEJ). In vertebrates, the first step in NHEJ is recruitment of the DNA-dependent protein kinase (DNA-PK) to DNA termini. DNA-PK consists of a catalytic subunit (DNA-PKcs) that is recruited to DNA ends by the Ku70/Ku80 heterodimer. Although Ku has been identified in a wide variety of organisms, DNA-PKcs was thought to be restricted to vertebrates. We previously reported the identification of DNA-PKcs in Dictyostelium, and that strains in which the genes encoding either DNA-PKcs or Ku80 have been deleted by homologous recombination are sensitive to DSBs when hatching from spores. Here we report a function for DNA-PK in vegetative cells during restriction enzyme mediated integration (REMI) of transfected plasmids. Strains which are lacking Ku show no increase in linearised plasmid integration into the genome in the presence of compatible restriction enzyme whereas strains lacking DNA-PKcs show a reduced efficiency. This data is consistent with a role for these proteins in NHEJ and with REMI being dependent on this pathway.

Nilgun Isik

105

Elmo Proteins regulate Phagocytosis and Cell Migration in Dictyostelium discoideum

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The ELMO/Ced-12 family of proteins is conserved throughout evolution in eukaryotic cells and has been shown to regulate cell shape, cell migration and phagocytosis by ultimately regulating the actin cytoskeleton in C. elegans and mammalian cells. ELMO/Ced-12 forms a complex with CrkII/Ced-2 and Dock180/Ced-5 to serve as a functional guanine nucleotide exchange factor for Rac/Ced-10, leading to the reorganization of the actin cytoskeleton during phagocytosis and cell movement. Two ELMO-like genes, ELMO A and B, are present in the *D. discoideum* genome. To determine if these genes encode functional homologues of metazoan ELMO proteins, we performed both gene disruption and over-expression experiments. We found that vegetative elmo A- and elmo B-null cells have increased rates of phagocytosis relative to wild-type cells, while cells over-expressing ELMO A have the opposite phenotype with decrease phagocytosis rates. In addition, both elmo A- and elmo B-null cells are developmentally delayed when starved on non-nutrient agar and display chemotaxis defects with increased psuedopod formation and excessive F-actin localization within pseudopods relative to wild-type cells. Using total internal reflection microscopy, we find that ELMO A is excluded in regions of intense actin polymerization near the plasma membrane of vegetative cells; together with the observation that more F-actin is observed in chemotaxing elmo A-null cells, these data suggest a negative regulatory relationship between ELMO and F-actin. Our preliminary data suggest that ELMO is a broadly conserved family of proteins that functions to regulate phagocytosis and cell shape changes during chemotaxis.

Brenda J. Blacklock

106

Fatty Acid Elongation in Dictyostelium discoideum.

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Elongation of *de novo* synthesized fatty acids to long chain and very long chain fatty acids may be accomplished by two alternative pathways; one, found primarily in plants, is centered on the 3-ketoacyl-CoA synthase (KCS) condensing enzymes and the other involves orthologs of the ELO proteins (for elongation) found in organisms as widely ranging as plants, microalgae, worm, fish, and mammals. These fatty acid elongation pathways provide long chain and very long chain fatty acids for a wide range of complex lipids including membrane sphingolipids and glycerolipids, triacylglycerols, and the epicuticular waxes found in plants. We are interested in the complementary and contrasting roles of the elongation pathways in long chain and very long chain fatty acid biosynthesis, and the possible role of complex lipids, in which elongation products are found, in signal transduction and development.
Dictyostelium discoideum demonstrates its early divergence from the branch of the tree of life leading to the Metazoa by the presence of one *KCS* and four *ELO* genes in its genome. *D. discoideum*, therefore, presents an opportunity to study the fatty acid elongation pathways, and physiological processes dependent on fatty acid elongation, present in plants and animals in a more tractable organism. Analysis of *D. discoideum* fatty acids revealed that at least one fatty acid elongation pathway is highly active and uses a mono-unsaturated C16 fatty acid substrate, and that polyunsaturated fatty acids can function as elongation substrates. The profile of fatty acids found throughout *D. discoideum* development suggests that fatty acid elongation is developmentally regulated. Further progress toward characterizing *Dictyostelium* fatty acid elongation pathways and functional characterization of the DdKCS and DdELOs will be presented.

Rebecca Fernandez

107

Dictyostelium discoideum RnoA is involved in development past the aggregate stage as well as aggregation at low cell densities

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ARNO, ARF nucleotide-binding site opener, is a guanine nucleotide exchange factor that regulates ADP-ribosylation factors. Phospholipase D (PLD), shown to be activated by ARNO, has been intimately linked to a multitude of cell signaling pathways including those leading to tumor formation and cancer metastasis. The PLD homolog in *Dictyostelium discoideum*, PldB, has been implicated in cell density sensing, morphogenesis, and cell differentiation. To elucidate the relationship between ARNO and PLD in cellular processes the *arno* homolog in *Dictyostelium discoideum*, *rnoA*, was characterized. Low cell density assays demonstrated that overexpression of *rnoA*, as well as the overexpression of *pldB*, compromise the ability of cells to aggregate at lower densities as compared to wild type cells, suggesting that RnoA and PldB are both involved in the same developmental pathway. In addition, the overexpression of *rnoA* led to a developmental arrest at the aggregate stage suggesting a developmental role for RnoA outside the regulation of PldB. Chimeric assays revealed that the addition of twenty percent wild type cells to *rnoA* overexpressing cells allows normal fruiting body formation, indicative of a non cell autonomous role for RnoA. In these chimeras, *rnoA* overexpressing cells were uniformly distributed throughout the fruiting body signifying that RnoA, while being important for development, is not involved in differentiation. Similar to GFP-PldB, green fluorescent protein tagged RnoA was found predominately in acidic vesicles, which is consistent with the two proteins interacting with each other. While these data illustrate strong parallels between RnoA and PldB functions, the differences observed in the results suggest that while RnoA may potentially regulate PldB, RnoA also functions independently of PldB.

Harish Padh

108

Transfection efficiency in Dictyostelium is enhanced by inhibition of endocytosis

Bhavesh Vats, Harish Padh

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Transfection efficiency of non-viral vectors in eukaryotes is very poor compared with viral derived vectors. Methods for application of non-viral vectors in eukaryotes are generally derived from the highly efficient ones used for prokaryotes while intracellular organelle structures and traffic are much more complex in eukaryotes perhaps resulting in poorer transfection efficiency based on unit DNA. Viral vectors used in gene therapy pose their own risk in humans whereas non-viral plasmid-mediated transfection, despite the limitation of efficiency, has the major advantage that it raises none of the safety concern of biological vectors for human therapy. Therefore, for better gene therapy protocols safer non-viral vectors would be preferred if their transfection efficiency could be improved and brought to the level that of viral vectors. Indeed, the advent of gene therapy has provided the impetus for improving, by appropriate chemical design, the efficiency of classical transfection agents.

The passage of foreign DNA from extra-cellular space to nucleus of a eukaryotic cell is poorly understood. The dogma is that DNA-chemical agent complex (for example, calcium phosphate salt) is taken up by the cell invariably by endocytosis. Endocytosis is a multi-step process involving binding, internalization, formation of endosomes, fusion with lysosomes and lysis. The extremely low pH and hydrolytic enzymes within the endosomes as well as lysosomes generally bring about degradation of entrapped DNA along with the associated complexes. Moreover, the DNA that survives and escapes endosomal-lysosomal degradation must dissociate from the condensing agent before or after entering the nucleus.

Dictyostelium provides an excellent system to decipher passage of DNA to nuclei and improve transfection efficiency by intervention. In *D. discoideum*, for the calcium-phosphate method, the transformation efficiency is 10^{-5} to 10^{-6} which can be increased two-fold by synchronizing cells. In order to increase the transformation efficiency in *Dictyostelium discoideum*, we modified commonly used calcium-phosphate transfection method to inhibit/ disrupt the endocytic machinery in the cells during the process. We used endocytic inhibitor 150 mM NaCl at low temperature and achieved higher number of transformants per μg DNA as compared to the conventional calcium-phosphate mediated transformation process (approx. 2.3 fold). Caffeine, an inhibitor of endocytosis in *D. discoideum*, also shows similar effect on transformation efficiency (approx. 2.6 fold) as does NaCl at low temperature during the calcium-phosphate mediated transformation. Similar results were obtained when two different genes were inserted individually in a plasmid with same backbone, implying that it is not a sequence specific improvement in transfection efficiency.

We postulate that inhibition of hydrolytic degradation in endo-lysosomal compartment results in efficient transfer of extra-cellular DNA to the nuclei. Further work is required to understand the mechanism behind the inhibition/ disruption of the endocytic machinery leading to increased number of transformant per microgram DNA. It will be useful to determine the average copy number of the integrated plasmid in the transformants obtained by the conventional method and the modified method.

Harish Padh

109

Expression of human erythropoietin gene in Dictyostelium discoideum

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Erythropoietin (EPO) is a glycoprotein hormone produced in the peri-tubular cells in the kidneys and stimulates the maturation of erythrocytes in the bone marrow, thereby increasing the hemoglobin level in circulation. It is the main drug used to cure many types of anemia, such as anemia in chronic renal diseases, chronic anemia during cancer treatment, anemia in preterm infants. Mature human EPO protein contains 166 amino acids, and has one O - linked and three N - linked glycosylation site at Ser126 and Asn24, Asn38, Asn83 respectively. Removal or modification of the glycan chains from hEPO results in altered in-vivo & in-vitro activity. In year 2005, the global market of recombinant erythropoietins, the leader in sales of biopharmaceutical products, was 12.4 billion USD with annual growth of 7 %. The Indian EPO market was estimated to be 20 million USD crore with growth rate of 20 %.

This protein when expressed in prokaryotic expression systems shows activity in vitro but not in vivo. This is due to lack of glycosylation machinery in prokaryotes. Thus, the protein has to be expressed in eukaryotic systems. Due to its clinical importance, to have a near human version of the functional protein, the recombinant version is produced in CHO cells which glycosylate the protein.

In the quest to develop *Dictyostelium discoideum* as an alternative heterologous protein expression system, we cloned a 2.43 kbp portion of the human epo gene from a mammalian vector in a modified version of the pA15GFP vector constructed by removal of the gfp gene and insertion of a MCS. The axenic *D. discoideum* strain Ax-2 was used in the study. Transformants were obtained by the calcium-phosphate transformation procedure and selected in HL-5 medium with 10 μg/ml of G418. The stability of the transgene in the vegetative cells across many generations and through development cycle was confirmed by sequence specific PCR using primers specific for epo gene. The presence of the transcript was ascertained from the total RNA by synthesis of the cDNA and its PCR amplification using sequence specific primers. The recombinant EPO was identified by immuno-blotting and compared with the recombinant protein produced in CHO cells. The protein expressed in *D. discoideum* was relatively smaller in molecular weight than the EPO expressed in CHO cells. Instead of a stack of proteins which are seen in case of CHO recombinant EPO, distinct bands were seen when the protein was expressed in *Dictyostelium discoideum*.

In spite of the promising result, the cDNA sequence is to be confirmed, pattern of glycosylation needs to be analyzed as well as activity of the protein is to be characterized. Further work in this direction is required for optimizing expression and making *Dictyostelium discoideum* as a viable expression system for the production of EPO.

D. A. Cotter

110

The Dispersal of Microbial Eukaryotes, including the Dictyosteliacea, through the Attraction of Drosophila melanogaster

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The fruiting body of *Dictyostelium discoideum* consists of a tapering column of dead stalk cells supporting a viscous droplet of spores called the sorus. The individual spores of the sorus are surrounded by a hygroscopic matrix of materials resulting from the sporulation process as well as from the apoptosis of stalk cells. We have shown previously that the matrix materials regulate the transcription of at least 1000 genes involved in sporulation, dormancy and germination. The matrix materials have at least five separate functions which are expressed temporally and are under environmental control. To investigate a sixth possible function of the matrix, i.e., spore dispersal, we chose to work with *Drosophila melanogaster*. The flies were attracted to open cultures of *Dictyostelium discoideum, mucoroides,* and *purpureum*, grown on streaks of *Esherichia coli* or *Klebsiella aerogenes* from compost piles containing fruit and vegetable material (including tomatoes). These 20 mL Petri dish cultures gave rise to F1 fly generations within 10-12 days, though not an F2 generation, possibly due to exhaustion of the medium. The release of the F1 flies in a room (6.7m x 3.3m by 3m) containing additional plates of these three species of social amoebae resulted in an F2 generation. This process of fly release to fresh plates was repeated until an F10 generation was obtained, clearly demonstrating that the flies could readily grow and reproduce on the two-membered cultures. Non-nutrient agar did not attract flies in the laboratory. Bacterial streaks attracted flies carrying propagules of the social amoebae and other microbial organisms. Commonly transferred prokaryotes included members of the Enterobacteraceae and Streptomyces spp.; commonly transferred eukaryotes included *Geotrichum candidum, Rhizopus spp., Mucor spp., Aspergillus niger, Colpoda spp*., and various soil amoebae. *G. candidum* was obtained universally and transmitted through all generations. Flies also transmitted nematodes and mites in a few isolated cases. In laboratory experiments larvae of *D. melanogaster* consumed soral materials of *D. purpureum* as evidenced by purple staining of their guts. Both larvae and adults flies readily transmitted propagules of the social amoebae within and between Petri dishes in the laboratory. Experiments are in progress to determine if the notoriously contaminated fruit flies are actually attracted to a volatile component of the *D. discoideum* soral matrix.

Lorenzo Santorelli

111

Mechanisms of cheating behavior in the social amoeba Dictyostelium discoideum

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Under starvation, *D. discoideum* cells undergo a process of differentiation into spores and dead stalk cells. In a mix between two or more genetically different clones, a conflict may arise in deciding which cells will become spores and survive and which will become stalk and die. The clone that differentiates more spores than its fair share is called “cheater” and the other “loser”. Our goal is to isolate and identify the genetic mechanism(s) that regulate and modulate this social behavior. To simulate evolutionary selection for cheaters, 10,000 knockout mutants generated using REMI were subjected to several rounds of development, spore germination and growth in a mixed population. Only the spores were taken to the next generation, enabling cheaters to become overrepresented in the evolving population. Southern Blot and quantitative PCR analyses confirmed that some of the analyzed knockout strains increased in relative abundance during the selection. Mutants exhibiting a normal morphological phenotype were picked and isolated after 10 and 20 generations and tested for their cheating ability in a pairwise mixture with the parental wild type. If proved to be cheaters, the mutations were identified and recapitulated in a fresh wild type strain. To identify additional mutants, we implemented a lower complexity screen where 16 pools of 250 REMI mutants each were subjected to ten cycles of selection. In this case, genomic DNA was isolated from the last round and cloned and sequenced en masse. This procedure yielded about 140 genes that are potentially responsible for cheating behavior. We found that few genetic pathways were overrepresented in the mutant list. They include ubiquitin-mediated protein modification, signal transduction and polyketide synthesis. Our findings suggest that more than social cheating involves more than one pathway.

Xin-Hua Liao

112

Purification of new factors associated with TOR complex 2 in Dictyostelium discoidium

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The TOR (Target of Rapamycin) pathway plays a central role in sensing and responding to nutrients, stress, and energy states. TOR complex 1 (TORC1), which is sensitive to rapamycin, regulates protein synthesis and cell growth, while TOR complex 2 (TORC2), which is considered insensitive to rapamycin, regulates the actin cytoskeletal function. Recently, however, our lab and other labs have found that long-term treatment with rapamycin can alter pathways that appear to function downstream of TORC2, indicating that TORC2 may be a target of rapamycin. To dissect further the functions of TORC2 and to study protein interactions within the complex, we have created "knock-in" *Dictyostelium* cell lines of TORC2 components *Pia*, *Rip3*, and *Lst8* that carry ectopic TAP tags. We are using these cells to purify the various TORC2 components as multi-protein complexes. Purified complexes have been identified by silver staining and we are scaling the purification scheme to obtain definitive peptide sequences. This work may help elucidate the mechanisms for TOCR2 regulation, the relationship between TORC1 and TORC2, intermediate factors upstream and downstream of TORC2, and novel TORC2-associated factors. These data may also explain the mechanistic effects of rapamycin on TORC2 and impact functional studies of rapamycin during clinic trials.

Petra Fey, Karen E. Pilcher, Pascale Gaudet

113

New Annotation Features in dictyBase

Petra Fey, Karen E. Pilcher, Pascale Gaudet, Sohel N. Merchant, and Rex L. Chisholm

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To improve integration of the Dicty Stock Center with dictyBase, curators have recently started literature-based annotation of Dictyostelium strains. Previously, strain curation was done exclusively by the Stock Center as strains were received. The goal of this transition was to seamlessly combine Stock Center strain information with dictyBase phenotype annotations. As a first step, curators linked all strains that existed in the Stock Center to their corresponding genes. This assures that all available mutant strains are accessible from the Gene Page. Strain curation is now an integral part of the literature curation process.

Strains and phenotypes are found on the Gene Page. Phenotypes are listed by mutant type such as 'null' or 'overexpressor.' Strains are listed by name. Currently both the strain section and the phenotype sections link to separate detail pages. To represent phenotypes more accurately, we are in the process of developing a new Strain and Phenotype tool that will link phenotype information directly to the strain, which in turn links to the gene mutated in the strain. For systematically capturing phenotype data, we created a phenotype ontology consisting of a controlled vocabulary. This is also a step towards the ability to search mutant phenotypes. We will show and explain the process of phenotype curation at dictyBase.

dictyBase now has a Community Annotation tool: wiki.dictybase.org. Each Gene Page has a link to a wiki page similar to Wikipedia (wikipedia.org). It allows users to enter information about any gene in dictyBase. We will demonstrate how to use the community annotation tool and show examples of what users have entered.

dictyBase is supported by grants from NIGMS and NHGRI.

W.Jonathan Ryves

114

Dynamics of Cell Penetrating Peptide use in living Dictyostelium.

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We have recently reported the use of the cell penetrating peptides (CPP) in living amoeba (1). These experiments used the 16 amino acid penetratin molecule fused to a peptide inhibitor of PKA (PKI) as a method for delivering a bioactive peptide cargo. Addition of the CPP-PKI to the media of wild-type Dictyostelium cultures caused a dose-dependent abrogation of the aggregation response to starvation, an effect consistent with the inhibition of the cAMP-dependent protein kinase (PKA). Furthermore in a strain of amoeba which has constitutively high PKA activity associated with precocious aggregation and spore / stalk pattern disruption, CPP-PKI was able to restore near-normal cell aggregation and pattern formation.
We now report on our further research into CPP association with living amoeba using a fluorescently labelled CPP (fCCP) fused to another cargo, this a PKA substrate peptide (PKAsub). Cell entry of fCCP-PKAsub was followed by cell fixation with protocols using para-formaldehyde and observed with fluorescence microscopy. Penetration was found to be rapid (within minutes) and pervasive throughout the cytosol and nucleus, while we observed F-Actin concentrated at the leading edges in the same cells. We conclude that the dynamics of fCCP entry and clearance from pre-loaded cells will be crucial in devising further in vivo experimental protocols.

(1). Use of a penetratin-linked Peptide in dictyostelium (2006) Ryves WJ and Harwood AJ., Mol. Biotechnol., 33(2), pp123-32.

Anupama Khare

115

Mechanisms of Cheating in Dictyostelium discoideum

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The long-term objective of this project is to better understand social behavior in *Dictyostelium*, through the identification of genes and biochemical pathways involved. Due to its unique developmental cycle – where multicellular structures are formed by aggregation of a large number of cells – *Dictyostelium* can form chimera. This property makes it possible for certain strains to form more than their fair share of spores in a chimeric fruiting body, thereby increasing their representation in the next generation. Such strains are called "cheaters". In order to identify genes involved in cheating, we have selected for several mutants that cheat on wild-type cells and shown that cheaters are found in nature as well. One of the questions raised by these findings is how come cheaters do not take over the entire population. We are testing the hypothesis that strains can evolve the ability to resist cheating. To identify mutants that can resist cheating, we carried out a genetic screen using the presence of a cheater in the population as a selective pressure. We generated a pool of mutants by REMI (Restriction-Enzyme Mediated Integration) mutagenesis, mixed it with the selector cheater, and allowed the mixture to form fruiting bodies. The cheater should cheat on the mutant pool leading to a significant enrichment of cheater-resistant mutants in the population. We observed an enrichment of certain mutants when we subjected the population to several rounds of such selection. These mutants are resistant to the selector cheater. Some, but not all of them, cheat on the wild type strain. Analysis of the mutant genes suggests that the cheating (and resistance) may involve cytoskeletal processes. We are currently studying these mutants to elucidate the actual mechanism of cheating involved.

dictyMart and dictyCyc: New Ways to Explore Data in dictyBase

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Since December, 2002, dictyBase has been hosting, annotating, and distributing the *Dictyostelium discoideum* genome sequence. The continued genome annotation has led to increased demand for an advanced query tool as well as primed the genome for further analysis for pathway information. Two new resources have been released by dictyBase: dictyMart facilitates advanced queries and data retrieval, while dictyCyc displays newly aggregated biochemical pathway information.

dictyMart is a database query system that allows a user to combine search criteria and generate custom data sets. Users can search genes based on chromosomal location, dictyBaseID, and Gene Ontology annotations, among
other criteria. The results can include various annotations such as location, Gene Ontology annotations, gene products, or sequences. Results can be viewed in a browser or downloaded as a Microsoft Excel file. An introduction to query construction and various filter and export options will be discussed. dictyMart is implemented with open-source software from the European Bioinformatics Institute (EBI) called BioMart.

dictyCyc provides visualization of predicted biochemical pathways in dictyBase. The Gene Page now shows the names of biochemical pathways in which a gene's product is involved. The link takes the user to a clickable graphical depiction of the pathway featuring all of the reactions, reactants, enzymes, and protein complexes involved in the pathway as well as the genes which produce each known protein subunit. A tour of the pathway browser and an overview of the pathway prediction process will be presented. Pathways are predicted and displayed by the Pathway Tools software from SRI.
dictyBase is supported by grants from NIGMS and NHGRI.

Daniela C. Gonzalez

117

Identification of PP1 interacting proteins in Dictyostelium discoideum

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Annotation of the recently sequenced genome of *D . discoideum* revealed at least 15 genes that encode putative members of the serine/threonine phosphatase family such as PP1, PP2A, PP2B, PP4, PP5 and PP6. Type 1 ser/thrh phosphatase consists of a well-conserved catalytic subunit (PP1c) and one or more variable regulatory subunits. In mammals, more than fifty polypeptides that bind PP1c have been identified, originating holoenzymes with distinct cell locations and specificities. Search for regulatory/interacting subunits using both bioinformatics and two hybrid screenings approaches revealed 33 potential candidates to interact to DdPP1c. Using these tools we have identified and characterized orthologs of PP1 inhibitors such as PP1-inhibitor-2 (DdI-2) and Ypi1 (DdI-3), a novel PP1 inhibitor described in budding yeast. DdI-2 and DdI-3 interact with *D. discoideum* PP1 catalytic subunit on yeast two-hybrid assays and the respective recombinant products obtained in bacteria is capable of inhibiting the phosphorylase phosphatase activity of PP1c (DdPP1c) in vitro. The domains of DdI-2 involved in the interaction with DdPP1c were mapped by two-hybrid interaction assays with DdI-2 deleted mutants. These are the first PP1 interacting proteins identified in *D. discoideum.*
Supported by FAPESP, CNPq and CAPES.

Thomas Keller

118

Genetic selections for DIF insensitive and hypersensitive mutants

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DIF-1 is a chlorinated alkyl phenone required to regulate the decision between the prespore and prestalkO cell fates during *Dictyostelium* development. However, little is known about the genes required to transduce the DIF-1 signal. To date only the transcription factors dimA, dimB, STATc and mybE have been shown to be part of the signal transduction pathway itself. In addition two further genes, rblA and culB appear to influence the sensitivity of cells to DIF. Therefore, to further understand the DIF-1 signal transduction pathway, we have developed genetic selection strategies to identify DIF-1 signaling mutants.

(1) DIF insensitive mutants: Previous selections were based on the 8-Br-cAMP monolayer assay, in which DIF-1 insensitive mutants differentiate as detergent resistant spores rather than dead stalk cells. The *dimA*- mutant was identified in this way from a REMI library mutagenized with *Dpn*II. However, this strategy would not identify DIF-resistant mutants that are unable to make viable spores (e.g. *dimB*-). Furthermore, some DIF signaling genes might not contain accessible *Dpn*II sites or may exhibit grow defects when disrupted (e.g. *mybE*-) and therefore prove impossible to isolate. To circumvent these problems we (i) developed and optimized an alternative selection based on the cAMP removal assay in which DIF-resistant mutants would be expected to be enriched due to a failure to produce dead stalk cells and instead remain as viable amoebae (ii) used a novel mutagenic restriction enzyme (*Tsp*509I) to generate a REMI library of 20,000 transformants. The recognition sequence for *Tsp*509I is AATT, rather than the GC rich (GATC) recognition sequence of the conventional REMI enzyme *Dpn*II. Consequently, *Tsp*509I cuts far more frequently than *Dpn*II, including within promoter regions. We identified insertion sites by inverse PCR and developed a simple novel PCR based strategy to generate recapitulation vectors. This methodology led to the isolation of a novel DIF resistant mutant (*dimC*-) and will be described in detail.

(2) DIF hypersensitive mutants: Previously several labs have reported mutants that both preferentially become prestalk cells in chimera with wild type cells and are DIF hypersensitive (e.g. rblA and culB). We have therefore devised a novel approach, in which mutants that preferentially become prestalk cells in chimera are enriched. Mutant cells were made sporey by growth in G+ medium and mixed with stalky G- wild type cells in a 1:9 ratio. At the slug stage of development the anterior 25% of several hundred slugs (>10x library coverage) was removed and disaggregated. Control experiments illustrated that prestalk sorting mutants would be enriched more that 20 fold from a single round of selection, once wild type cells have been killed by selection in blasticidin containing medium. Consistent with this idea, we found that the number of mutants exhibiting obvious morphological defects when developed clonally increased dramatically over 3 rounds of selection. As expected, these mutants sort to the anterior 25% of chimeric slugs. The site of insertion in several mutants has been determined and often lies in key signaling genes (e.g. rap-GAP and a serine/threonine kinase).

Hiroshi Ochiai

119

The PKB/AKT related kinase may be engaged in the transition from the first finger stage to the slug stage

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In *Dictyostelium*, the molecular signal that triggers entry of the first finger into slug are not known; the protein kinase B related kinase (PKBR kinase) may be involved in the transition. The *pkbR* null organisms have a strong tendency remain to be the first finger stage, although during the further development the organisms present a wide range of terminal phenotypes, including tight mounds, abnormal first fingers, normal-looking fruiting bodies. A PKBR kinase has a kinase domain and a C-terminal one related to those of protein kinase B (PKB/AKT), but not pleckstrin homology (PH) domain that is common among other PKB.
We previously reported that the pkbR null organisms express the prestalk-specific gene, ecmA, but not the ecmB whose expression usually appears at the core region of the slug (Dicty 2004). Here, we analyzed the expression patterns of acaA, dhkC of a histidine kinase, cudA essential for normal culmination, and cotC as a prespore marker in the *pkbR* null organisms by in situ hybridization. In short, acaA in the pkbR null organisms express acaA mRNA at the prestalk A region of the first fingers of 22 hr, although the expression of dhkC, amtC,and cudA mRNAs are almost nothing or decreased. At early tight aggregation stage the low level expression was seen on the top region of pkbR null organisms. It was reported that ectopic CudA expression in pkbR null strains restores culmination (Kirsten et al. 2005). We over-expressed CudA in the pkbR null cells, but it provided no rescue. The present results suggest that the pkbR gene is involved in a signal pathway from the first finger stage to the slug stage.

Knockout of the Ste20-like kinase DstA affects phagocytosis and development

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Fast rearrangements in the actin cytoskeleton are controlled by various actin-binding proteins, which are often regulated by phosphorylation. A Ste20-like kinase was previously shown to phosphorylate the actin binding protein severin in vitro (Eichinger *et al*., J. Biol. Chem. 273:12952-12959, 1998). The Ste20 group of kinases in *Dictyostelium discoideum* has 13 members in the germinal centre kinase (GCK) subfamily. To characterize the function of these GCK kinases several null mutants and GFP overexpressing strains were generated.
D. discoideum cells lacking DstA (Dictyostelium Ste20-like kinase A) show a defect in phagocytosis, whereas cells expressing GFP-DstA in the wildtype background engulf particles more efficiently. In addition, DstA null cells exhibit a delay in development with a tendency to form aberrant long streams on Klebsiella. GFP-DstA localizes to patches in the plasma membrane and to intracellular membrane compartments distinct from organells stained for F-actin, comitin or annexin VII. During chemotaxis in a cAMP gradient the mutant cells frequently form lateral pseudopods which indicates a chemotaxis or polarity defect.

Christopher Dinh

121

A strategy to generate 23,000 individual barcoded D. discoideum insertion mutants for the parallel analysis of phenotypes

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For the past few years we have provided researchers with mutant resources in the form of knockout plasmids cloned from REMI insertion pools (http://dictygenome.org). About 1,000 genes have been disrupted and their knockout plasmids are available to regenerate mutants for the study of specific genes. We have now constructed a set of barcoded REMI insertion mutants whose phenotypes can be analyzed semi-quantitatively and in parallel. We began by constructing over 800 distinct insertion plasmids, suitable for REMI mutagenesis, that each contain a unique barcode consisting of a random 60-mer DNA sequence (the tag) bounded on each side by universal 20-mer oligonucleotide priming sites that are suitable for PCR amplification of the tag within complex mixtures of DNA. We used 768 of these plasmids as integrating vectors using three different restriction enzymes in REMI transformation experiments, where each transformant was grown clonally, in order to generate a library of 23,000 individual tagged *D. discoideum* mutant strains. From this library, 30 pools of 768 uniquely barcoded strains were created. The unique barcode within each strain can be used as strain identifier, which makes it possible to follow the relative abundance of each barcode in the population of mutants and provides the opportunity to gauge the performance (e.g., growth or sporulation) of the strains in the pool during various phenotypic challenges. Each mutant can be identified by PCR amplification of barcode tags unique to the insertion site, followed by hybridization to microarrays containing sequences complementary to each tag. This system will be useful for performing genetic analyses where relative, semi-quantitative phenotypes are desirable, such as in drug resistance studies.

Wouter N. van Egmond

122

GbpC is the only cGMP-target in Dictyostelium

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GbpC is a high affinity target for the second messenger cGMP in *Dictyostelium*. Previously, it was shown that a cell line in which the gene coding for gbpC is disrupted has lost all high affinity binding sites for cGMP. In this study, we analyzed the remaining low affinity cGMP binding sites in gbpC null lysates. This was done using a biochemical and a bio-informatical approach. In the first approach, we used cGMP- coupled agarose beads to perform pull-down assays and subsequent mass spectrometry analysis, to identify novel unknown cGMP binding proteins. From replacement studies with cGMP, we show that Nucleoside diphosphate kinase (NDPK), the protein that catalyzes GTP-formation from GDP, is responsible for the remaining low affinity cGMP-binding in gbpC null cells. Moreover, in the bioinformatical approach we could identify a novel gene that encodes a protein with a putative cyclic Nucleotide Binding Domain (cNBD). This protein encodes a member of a heavy metal transporter family, Ancient Conserved Domain Protein, and was therefore named DdACDP. Following purification of this domain fused with GST, we show that the cNBD of this protein is able to bind cGMP. For NDPK and DdACDP, the affinities for cGMP are much lower as the maximal cellular concentration of this second messenger. Overall, we conclude that gbpC is the sole target of cGMP and therefore plays a key role in transducing the cGMP-signal in *Dictyostelium*.

B. Ewa Snaar-Jagalska

123

Single-molecule analysis of cAR1-YFP during chemotaxis and internalization

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Novel fluorescent methods have enabled the direct visualization of the individual cAMP receptors in living cells. Here we show by single-molecule microscopy an agonist-induced increase in mobility of cAR1-YFP at the leading edge of *Dictyostelium* cells. This mobility shift was concurrent to the uncoupling/activation of the G□-protein downstream to the receptor. We propose that the receptor mobility is directly involved in gradient-sensing and provides a new mechanistic basis for the primary amplification steps in current theoretical models describing polarity in chemotactic signalling.
The single-molecule experiments in real time also revealed that the fraction of cytosolic receptors increases after persistent agonist stimulation. The internalization of single GPCR molecules was abolished in the phosphorylation deficient mutant, cm1234-YFP or when cells were stimulated with an antagonist Rp-cAMPS, which does not induce receptor phosphorylation. In cells disaggregated from mounds a lower level of cAR1-YFP was only detected in cytosol while cm1234-YFP level was not down-regulated. In addition, cm1234-YFP over-expression in a wild-type background causes mound-stage arrest. Together these observations suggest that phosphorylation-dependent internalization reduces normal cAR1 activity and permits development beyond the mound stage.

Cellular Studies of Dictyostelium Myosin II Heavy Chain Kinase-C Subdomains

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Myosin heavy chain kinase A (MHCK-A) catalyzes the disassembly of myosin II filaments in *Dictyostelium discoideum* via phosphorylation of the "tail" region of the myosin heavy chain. Under various cellular conditions, MHCK-A is recruited to actin-rich cortical sites and is preferentially enriched at sites of pseudopod formation. MHCK-A possesses an amino-terminal "coiled-coil" domain (498 amino acids; and designated C1-498) that mediates the oligomerization, cellular localization, and actin binding activities of the kinase. We have shown previously that C1-498 interaction with F-actin leads to a 40-fold increase in the catalytic activity of MHCK-A, and thus may represent a potent mechanism for achieving highly-localized and robust disassembly of myosin II filaments in the cell. In the current study, we examined the actin-binding characteristics of the "coiled-coil" domain as a means of gaining insight into the mechanisms by which F-actin-mediated activation of MHCK-A, and thus myosin II filament disassembly, can be regulated in the cell. Co-sedimentation assays revealed that GST-tagged C1-498 protein binds cooperatively to F-actin with an apparent K_D of ~0.5 μM F-actin and a stoichiometry of ~5:1 (actin:C1-498). By contrast, assays of "coiled-coil" domain truncations lacking either amino-terminal (C121-498) or carboxy-terminal (C1-452) regions exhibited ~50% reductions in actin binding activity, and a combined truncation lacking both ends (C121-452) exhibits complete loss of actin binding activity. Studies examining the nature of the interaction with F-actin revealed that the coiled-coil domain of MHCK-A competes with tropomyosin for binding to F-actin, suggesting that these two coiled-coil-rich proteins share common mechanisms for interaction with F-actin. We also show via chemical cross-linking that the "coiled-coil" domain of MHCK-A (C1-498) indeed oligomerizes, forming assemblies containing four to five coiled-coil monomers. Perhaps most surprisingly, we found that the coiled-coil domain (C1-498) cross-links actin filaments into bundles, indicating that MHCK A can affect the cytoskeleton in two important ways: (1) by driving myosin II-filament disassembly via myosin II heavy-chain phosphorylation, and (2) by cross-linking/bundling actin filaments. Collectively, our results suggest that changes in the accessibility of actin-binding determinants in the "coiled-coil" domain may lead to localized cross-linking of actin filaments which, in turn, may activate MHCK-A-mediated disassembly of myosin II filaments at specific sites in the cell.

Andrew Heidel

125

Comparative Genomics in the Social Amoebae

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Dictyostelium discoideum has notable strengths as a model system for the study of cell migration, cell differentiation, development and generation of multicellularity. These well-studied aspects of *D. discoideum* biology plus the diversity in closely related species, permit a comparative genomics approach to address several functional and evolutionary questions. For this study we plan to sequence the genomes of the closely related species *Polysphondylium pallidum* and *D. fasciculatum* allowing a comparison between these species and the already sequenced *D. discoideum*. Ultimately, all efforts in this direction aim at the elucidation of genome and feature evolution in the entire social amoebae group. On a large scale, these genomic comparisons can determine important motifs in chromosome structures such as telomeres, centromeres and rDNA. Genome-wide comparisons of gene duplication and divergence can also illustrate how the genomes have evolved as a whole. We also plan to study gene regulation by identifying conserved elements in promoter elements and miRNAs through the technique of phylogenetic shadowing. On a smaller scale we plan to investigate families of genes associated with social amoebae life history. We present here our initial steps in a comparative analysis of social amoebae.

Ran-Der Hwang

126

Regulation of actin cytoskeletal architecture by Fimbrin A.

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The fimbrins, also known as plastins, are members of the family of actin-binding proteins. FimA has a calcium-binding domain (EF hands) and has been shown to have calcium regulated actin-binding activity *in vitro*[1], but the *in vivo* significance has not been characterized. This protein is unique among the F-actin-binding domain (ABD)- containing proteins in possessing a tandem repeat of the ABD within a single polypeptide chain. In order to understand what regulates fimbrin's binding to actin filaments in the cell, we generated fluorescent fusion proteins, encoding either the entire protein or its various domains, and examined the localization of these fluorescent proteins in living cells. Preliminary analysis indicates that fimbrin localized to newly formed pseudopods as well as macropinosome cups, but only weakly in the peripheral cortex. The localization of fimbrin truncated constructs encompassing both actin-binding domains, but devoid of EF hands (ΔEF), was indistinguishable from that of the full-length protein. However, the first actin-binding domain (ABD1) alone or the EF hands plus first actin-binding domain (EA1) both strongly localized to the cortex of the cell. Surprisingly, the expression of the second actin-binding domain (ABD2) alone leads to the formation of a large aggregate of actin filaments. These aggregates have highly organized arrays of filaments similar to the Hirano bodies formed in cells expressing a fragment of the 34kd actin binding protein[2]. ABD2 transfected cells also show a reduced rate of growth. The localization of GFP--actinin showed essentially the same pattern as full-length fimbrin. In contrast, GFP-ABP120 was highly enriched in the peripheral cortex, macropinosome cups, and new protrusions. These experiments show that while the fimbrin, -actinin, or ABP120 actin-binding domains have a high degree of sequence similarity, their actin-binding activities exhibit wide variations.

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Tomaz Curk

127

Discovering patterns of gene regulation in *Dictyostelium discoideum* using rule-based clustering

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Computational inference of relations between the structure of regulatory regions and gene expression can greatly assist in experiment planning and validation in an otherwise tedious discovery process of transcription regulatory mechanisms. For this task we have developed a rule inference-based clustering method that is able to identify clusters of highly co-expressed genes and similar structures of the regulatory region. Standard "cluster-first" approaches begin by gene expression-based clustering. Cluster-specific putative binding sites are then identified for each cluster. The success of the latter can greatly depend on the composition of the gene clusters. In contrast, the method we have developed starts with a set of known or putative binding sites and tries to infer rules describing clusters of co-expressed genes. The proposed rule-based clustering is able to efficiently derive complex relations between elements in the regulatory region. Rules are of the form "IF *structure* THEN *expression profile*", where *structure* is an assertion over the binding sites in the gene promoter sequence and *expression profile* an average profile of genes that match the *structure*. The descriptive language used to define the *structure* is rich; the conditions on the structure may include assertions on the presence of binding sites, conditions on the distance of putative binding sites from the transcription and translation start site, distance between putative binding sites, and their relative and absolute orientation relative to a reference element. We have performed genome-wide analyses of the regulatory regions of *Dictyostelium discoideum* using a compendium of gene expression data from Van Driessche et al. 2002, Van Driessche et al. 2005, and Booth, et al. 2005. The results are presented in the form of intuitive and easy-to-navigate web pages. Links to external supporting sources (*e.g.* TRANSFAC) on identified known binding sites are given. We also show how rule-based clustering can be used to identify the stretch of regulatory region which holds the most information about gene expression.

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Xiuli Huang

128

Nutrient Sensing in Dictyostelium through the TOR pathway

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TOR (Target Of Rapamycin) is a member of the PIKK related family of protein kinases and a well known regulator of cell growth, ribosomal biogenesis, and protein synthesis. In this study we used Dictyostelium cells as a model system to dissect the role of TOR with respect to nutrient requirements and response. As in most systems, Dictyostelium TOR is part of two distinct complexes: TORC1 contains TOR, LST8 and Raptor, whereas TORC2 is constituted of TOR, LST8, RIP3 and Pia. TOR kinase is regulated by a wide range of signals, including growth factors and nutrients. Dictyostelium cells grow exponentially if provided with nutrient rich medium. Here we examine cell response to changes in nutrient strength and glucose concentrations by analyzing cellular growth rates as well as the phosphorylation of 4E-BP, a major target of TORC1. We observed that cells grow slower in diluted media, which was paralleled by a reduction in TOR activity as defined by levels of p4E-BP. We are also defining limiting factors (amino acids, etc.) in media which may be essential for cells to grow at normal rates.

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

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

In growing cells, AmpA has a second and distinct function. AmpA protein accumulates as a function of cell density and is associated with the cell periphery. Gene inactivation results in an increase in cell-cell clumping and cell-substrate adhesion, while overexpression of AmpA reduces both clumping and substrate adhesion. AmpA also influences the size of plaques formed on bacterial lawns. The plaque size alteration is due to differences in the ability of cells to migrate out of the plaque into the bacterial lawn in search of food. Comparison of migration of wt and mutants cells to folic acid indicates that AmpA null cells are defective in migration on top of agarose but migrate normally under agarose, while AmpA overexpressing cells behave exactly the opposite, migrating efficiently on top of agarose but are unable to migrate under agarose. Additionally AmpA influences the basal level of actin polymerization in growing cells. All of the results are best explained by a role for AmpA in influencing cell substrate adhesion which in turn influences cell traction and actin polymerization. Direct measurements of cell traction using the properties of elastic polymers are currently in progress in collaboration with Drs. Jennie Leach and Jeffrey Jacot. A model for the role of AmpA as a secreted autocrine factor controlling cell adhesion, cell traction and actin polymerization in response to increasing cell density in growing cells is presented.

Current work focuses on identifying AmpA interacting partners. Large quantities of a AmpA-His fusion protein have been generated and are being used to identify interacting proteins and REMI mutagenesis is being used to identify second site suppressors of AmpA overexpressing and null mutants.

Daniel F. Lusche

130

Dictyostelium as a model for understanding the most common genetic disease efflicting humans, polycystic kidney disease.

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Calcium plays a role in a variety of chemical reactions and biological functions. We have begun to investigate its role in basic cell motility and chemotaxis by varying extracellular calcium concentrations. We have analysed wild type cells (Ax2), a null mutant for the IP3 receptor-like gene *iplA*$^-$, which is believed to encode an intracellular calcium channel, and a null mutant for the polycystic kidney disease PKD2-like gene, *pkd2l*$^-$, a putative second calcium channel. We have found the following:

1) Increasing calcium to 20mM increases both the speed and efficiency of persistent translocation in the absence of exogenous chemoattractant in aggregation competent wild type cells. Similarly, it enhances the motile behavior of aggregation competent *iplA*$^-$ and *pkd2l*$^-$ cells, but not to the same extent as in wildtype cells.These resuts indicate first that extracellular calcium is important for chemotaxis in a spatial gradiet of cAMP and that LPS is not optimal for chemotaxis presumably because of too low a calcium concentration.

2) The chemotactic index and and proportion of the population exhibiting a chemotactic response is reduced by half in *iplA*$^-$ cells, and is barely positive in *pkd2l*$^-$ cells in a spatial gradient of cAMP generated in the standard solution (LPS) or in tricine buffer. Increasing the extracellular concentration of calcium to 20mM increases to a small degree the efficiency of chemotaxis in wild type cells, but nearly normalizes chemotaxis in both mutants. These results indicate that both PKD2L and IPLA play roles in maintaining optimal intracellular calcium levels for chemotaxis.

Because polycystic kidney disease is the most common genetic disease in humans, we have now begun a collaboration with researchers at the Mayo Clinic to test whether the same defects measured in *Dictyostelium discoideum pkd2l*$^-$ cells are manifested by polymorphnuclear leucocytes of patients with this disease, and if increased calcium normalizes such abnormalities.

Cynthia K. Damer

131

Copine A is required for normal cytokinesis, contractile vacuole function, and development in Dictyostelium

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Copines make up a family of soluble, calcium-dependent membrane binding proteins found in a variety of eukaryotic organisms. In an earlier study, we identified six copine genes in the *Dictyostelium* genome and focused our studies on *cpnA*. Our previous localization studies of a GFP-tagged CpnA in *Dictyostelium* suggested that CpnA may have roles in contractile vacuole function, endolysosomal trafficking, and development. To test these hypotheses, we created a *cpnA*$^-$ knockout strain in *Dictyostelium*. The *cpnA*$^-$ cells exhibited normal growth rates and a slight cytokinesis defect. When placed in starvation conditions, *cpnA*$^-$ cells appeared to aggregate, form mounds, and fingers with normal timing; however, they were delayed or arrested in the finger stage. When placed in water, *cpnA*$^-$ cells formed unusually large contractile vacuoles indicating a defect in contractile vacuole function, while endocytosis and phagocytosis rates in the *cpnA*$^-$ cells were similar to wild type cells. Our studies indicate that CpnA has roles in cytokinesis and contractile vacuole function, and is required for normal development, specifically in the late stages of development prior to culmination. In addition, we used real time RT-PCR to determine the expression patterns of all six copine genes during development. The six copine genes were found to be expressed in vegetative cells with each gene exhibiting a distinct pattern of expression throughout development. All of the copine genes, except *cpnF*, show an up-regulation of expression at one or two developmental transitions, suggesting that copines may be important regulators of *Dictyostelium* development. We are currently using affinity chromatography and MALDI-TOF-mass spectrometry to isolate and identify proteins that bind to CpnA.

Daniela C. Gonzalez

132

The P450 oxidoreductase, RedA, controls development beyond the mound stage in *Dictyostelium discoideum*

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We isolated a mutant strain of *Dictyostelium discoideum* following restriction enzyme-mediated integration (REMI) mutagenesis, which develops only to the mound stage. Morphogenesis in this strain is arrested following aggregation and the mounds accumulate a bright yellow pigment not normally seen at this stage. We found that the insertion had disrupted a gene, *redA,* that encodes a member of the diflavin oxidoreductase family of enzymes. Recapitulating the mutation by homologous recombination produced a strain with the same phenotype. The *Dictyostelium* genome carries three genes of the diflavin oxidoreductase family. The products of two of these genes, *redA* and *redB*, are predicted to be NADPH-cytochrome-P450 oxidoreductases. *redA* transcripts are present during growth and early development but then decline, reaching undetectable levels after the mound stage. The mound-arrest phenotype is cell-autonomous suggesting that the defect occurs within the cells rather than in intercellular signaling. Supported by CNPq, CAPES and FAPESP.

Scott Gruver

The Role of 3'-phosphoinositides in Chemotaxis

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During chemotaxis cells must translate an asymmetric extracellular cue into directionally biased movement. Several studies in a variety of systems have, to a greater or lesser extent, implicated 3'-phosphoinositide (3'-PI) signaling as a key component in the gradient amplification pathway that regulates cell polarity and chemotaxis yet the precise role of 3'-PI's in these cellular processes remain an open question. We address this question using a combination of both brightfield and fluorescence time lapse imaging and statistical analysis of *wildtype, pi3k 1/2-,* and *pten-* cell lines. The chemotactic movements of all three cells lines are spatially and temporally complex. Analyzing several measurements of chemotactic ability in the standard pipette assay revealed that the chemotaxis of wildtype cells depends on their position and/or time in the chemoattractant gradient, such that cells chemotax more efficiently as they approached the gradient source. Interestingly, *pi3k 1/2- and pten-* also show a similar trend but with an overall lower chemotaxis efficiency suggesting that 3'-phosphoinositide signaling is not involved in this phenomenon of increasing efficiency with increased proximity to the gradient source but regulates other aspects of chemotaxis. Quantitative fluorescence imaging of the spatial-temporal dynamics of 3'-PIs as evidenced by membrane bound, GFP-fused PH domain with 3'-PI specificity provide quantitative data to explore the dynamic interrelationship between signaling, morphology, and chemotaxis variables. These measurements show the regulation of the 3'-phosphoinositide signaling pathway to be spatially and temporally complex. Analysis of this data set using multivariable time series analysis to examine the ability of combinations of variables across different time scales to predict the chemotactic movements of cells will be discussed.

Richard Kessin

134

The *Dictyostelium discoideum* genome and its polyketide synthase genes

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Polyketides are important chemical substances for both pharmaceutical and agro-industries. Today, polyketide antibiotics, antifungals, antiparasitics, animal growth promotants, natural insecticides, immunosuppressives, cytostatics and anticholesterolemics are in commercial use. Polyketides are synthesized by Type I modular and Type II iterative polyketide synthases (PKS's) in bacteria, Type I iterative PKS's in fungi and by chalcone synthases in plants (The nature of polyketide antibiotic synthesis will be explained at the poster with an animated cartoon).

As soil organisms that exist in a world of bacteria, fungi, nematodes, and other potential predators, the Dictyostelia should also have a rich chemical repertoire. The genome of *Dictyostelium discoideum* is exceptionally rich in polyketide synthase genes (PKS) encoded by more then 40 recognizable genes spread on all six chromosomes. At least one of the PKSs is dedicated to the synthesis of Differentiation Inducing Factor, as shown by Rob Kay and his collaborators. We know of no other organisms with as many PKS genes.

We are using bioinformatics and gene expression approaches to confirm existing and to detect novel PKS genes. This sequence and expression information was used to ask how the PKS genes of *Dictyostelium* evolved. Similarity searches revealed all forty three known putative PKS genes present in the dictyBase. These were analyzed further using an approach based on hidden Markov models implemented within the HMMER program. Additional analyses showed that 3 dictyBase pairs of PKS genes (6 genes) could be combined into 3 single genes. In addition, 3 novel genes have been identified. Therefore, our analysis also revealed 43 PKS genes in total. PKS proteins are highly modular. Detailed annotation of all genes showed that they encoded β-ketoacylsynthase (KS), acyltransferase (AT), dehydratase (DH), methyltransferase (MT), enoylreductase (ER), ketoreductase (KR) and acyl carrier protein (ACP) domains expected in a polyketide synthase. In about half of the genes, there is an additional domain translated downstream of the ACP. In two genes, these domains show homology to chalcone synthases, which creates a novel structure. Thirty-two of the PKS genes occur in thirteen potential gene-clusters (containing 2-5 genes each). At present we do not know whether any of the putative clustered PKS genes represent an individual biosynthetic pathway. Twelve single genes were found and they resemble Type I iterative genes.

We have constructed a phylogenetic tree by neighbor joining of the KS domain sequences. This showed that the *D. discoideum* sequences formed a separate but very diverse group with distances between extreme members nearly as great as the distance to bacterial and fungal sequences. Many of the retrieved PKS genes are transcribed. We found expressed sequence tags for many PKS genes in cDNA libraries and we have recovered information on transcription of others by examining gene array experiments. Real time PCR techniques using primers to the 3' ends of the genes were performed with 10 selected PKS genes to obtain their time course of expression. All ten genes tested showed different expression patterns during growth and development.

Vikas Sonakya

135

Elucidation of the role of Phosphoprotein Phosphatase A in growth and development of *Dictyostelium discoideum*

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Reversible protein phosphorylation is an essential regulatory mechanism in many cellular processes. The protein phoshatases (PPases) are highly regulated enzymes that mediate protein dephosphorylation and play an important control in signal transduction, growth and development. Phosphoprotein Phosphatase 2A (PP2A) is an abundant, highly conserved, heterotrimeric serine/threonine phosphatase in eukaryotic cells. To elucidate the role of PP2A in eukaryotic growth and development we focused on mutating or reducing the expression of the gene encoding the catalytic subunit PP2Ac of *Dictyostelium discoideum*, a eukaryotic microorganism and a model system for studying eukaryotic development. RNA interference (RNAi) was used to reduce the level of PP2Ac mRNA by using shRNA (short hairpin RNA) complementary to four positions in the PP2Ac gene cloned. The shRNA templates were transformed in *Dictyostelium discoideum* using extra-chromosomal plasmids (pMB12n and pDXA3H). The transformed cells showed an arrest in development at the mound stage and reduced mRNA levels.

In addition two site specific mutations were incorporated in the PP2Ac gene on an integrating plasmid pBORP. The mutations (G160A and G244A) were designed to alter amino acids Asp54Asn and Asp82Asn in the protein products of the transforming PP2Ac gene but not to affect the endogenous gene. The transformed cells, selected with G418, were observed for growth and development changes. The doubling time of mutagenic strains G160A and G244A was drastically increased to more than 60 hrs compared to 16 hrs for wild type cells. The cells were also defective in development and arrest at mount stage. Since the transformed cells retained the wild type PP2Ac gene the mutations in the transforming gene conferred a dominant negative phenotype on the cells. Therefore both RNAi and site specific mutation experiments show that PP2Ac is important in growth and development beyond the mound stage in *Dictyostelium discoideum.*

Chris West

136

Two Predicted Diglycosyltransferase Genes Contribute to Spore Coat Polysaccharide Biosynthesis, Cellulose Deposition, and Resistance of Spores to Stress in *Dictyostelium discoideum*

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The spore coat is a trilaminar cell wall that is formed *de novo* at the cell surface of differentiating spores of *Dictyostelium discoideum*. *Dictyostelium* is a soil amoeba with a multicellular developmental cycle and an NIH model organism for cellular regulation. The coat is assembled from 1) a vesicular pool of protein and a Gal/GalNAc-rich polysaccharide (GPS) derived from the Golgi and stored in the prespore vesicle, 2) a separate late-synthesized pool of distinct proteins including SP65 and CB55 (*tenB*), and 3) cellulose formed *de novo* at the cell surface. The GPS is recognized by the lectins RCA-I and soybean agglutinin and is localized to the inner and middle layers of the coat, but its significance for spore coat structure and function is not known. In a related species, *Dictyostelium mucoroides*, a similar spore polysaccharide consisting of the repeating trisaccharide Galbeta1,3(Galbeta1,6)GalNAcalpha1,3- has been described and a galactosyltransferase involved in its biosynthesis was an important prespore marker in the 1960's. A search of the genome sequence of *D. discoideum* for potential genes that encode a Golgi-localized glycosyltransferase with separate domains for forming inverting and retaining glycosidic linkages, which might direct formation of the repeating disaccharide backbone of the GPS, yielded two candidates (*pgtA* & *pgtB*). Disruption of either gene by homologous recombination greatly reduces labeling of spores with the RCA-I lectin. Mutant spores lack resistance to hypertonic stress and exhibit increased permeability to the DNA dye DAPI. Co-development of mutant and wild-type cells show that incorporation of GPS into coats and the spore defect are cell autonomous. Electron microscopy shows that the middle cellulosic layer of the spore coat is attenuated and variable. Based on the predicted topology of *pgtA* and *pgtB*, alternating sugars of the GPS polymers are proposed to be extended by separate domains of a single glycosyltransferase protein within the lumen of the Golgi apparatus. Genetic disruption of predicted biosynthetic enzymes for the spore coat GPS has revealed an important functional role for this cell wall polysaccharide in cellulose organization and the stress-resistance of spores. (Supported by NSF MCB-0240634, NIH R01-GM-37539, and the Presbyterian Health Foundation)

Chris Janetopoulos

137

Temporal and Spatial Regulation of Phosphoinositide Signaling Regulates Cytokinesis

Chris Janetopoulos, Morgan Sammons, Carrie Elzie, Jane Borleis, and Peter Devreotes

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Cells must undergo dramatic changes in cell polarity during motility and cell division. Polarized distributions of PI(3,4,5)P3, which are clearly important for regulating cell morphology during migration, also appear to play an important role during cytokinesis, the final event in cell division. In chemotaxis, polarity is established by local accumulation of PI(3,4,5)P3 at the cell's leading edge, achieved through temporal and spatial regulation of PI3 kinases and the tumor suppressor, PTEN. We find that as migrating *D. discoideum* cells round up to enter cytokinesis, PI(3,4,5)P3 signaling is uniformly suppressed. Then, as the spindle and cell elongate, PI3 kinases and PTEN move to and function at the poles and furrow, respectively. Cell lines lacking modulation of this enzymatic activity are defective in cytokinesis, and cannot divide in suspension. The cells continue to grow and duplicate their nuclei, generating large multinucleate cells. Furrows that fail to ingress between nuclei are unable to stably accumulate myosin filaments or suppress actin-filled ruffles. We propose that phosphoinositide-linked circuits, similar to those that bring about asymmetry during cell migration, also regulate polarity in cytokinesis.

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