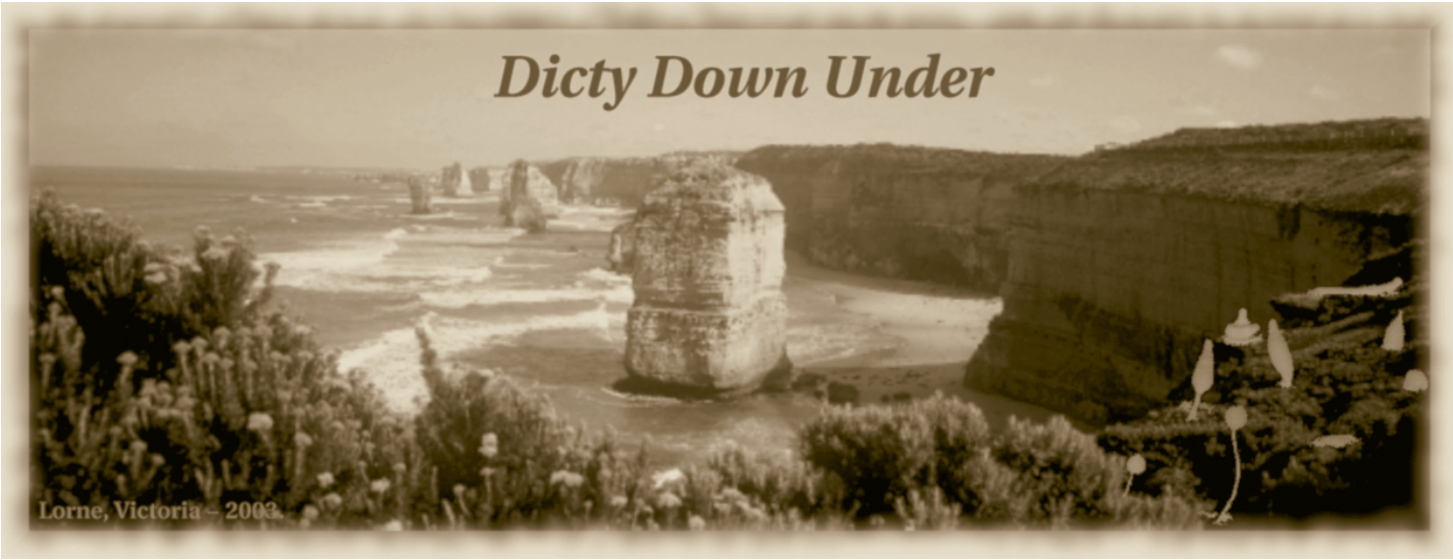

International *Dictyostelium* Conference

Erskine House, Lorne, Victoria, Australia.

June 29 - July 4, 2003

Schedule of Oral Presentations

Monday June 30

09:10 Welcome, Paul Fisher
Announcements, Peter Beech

09:30 – 10:50 ***Session 1.*** **Chair: J. Franke**

The wild side of a social amoeba

1. *Dictyostelium discoideum* in its natural context
J.E. Strassmann, D. Castillo, A. Fortunato, K. Foster, M.Landi, T.Platt, L. Santorelli, M. Smith, D. Queller

2. Genetic Heterogeneity and Social Behaviour in Cellular Slime Molds
S. Kaushik, V. Nanjundiah

Sleeping and waking: sporulation and germination

3. Tyrosine phosphorylation of actin, actin rod formation and spore coat stability during *Dictyostelium* sporulation depend on the MADS-box transcription factor srfA
R. Escalante, Y. Yamada, D. Cotter, L. Sastre, M. Sameshima

4. Anatomy of an adenylyl cyclase osmosensor
S. Saran, E. Alvarez-Curto, P. Schaap

10:50 – 11:20 Morning Tea

11:20 – 12:40 ***Session 2.*** **Chair: C. Weijer.**

A closer look: amoebae under the microscope

5. Quantitative microscopy-visualizing signaling events in single living cells
X. Jiao, J. Chim, X. Xu, T. Jin

6. Spectral Confocal FRET Imaging Reveals the Spatial-temporal Activation of the Heterotrimeric G-proteins in Single Chemotactic Cells
X. Xu, X. Jiao, T. Jin

7. The early cAMP wave propagation revisited: phenomenology and genetic dissection
S. Sawai, E.C. Cox

8. Mechanism of cell movement within closely-packed cell aggregates
K. Inouye

Monday

13:00 – 14:30 Lunch

15:30 – 16:00 Afternoon Tea

19:00 Opening Dinner

Music Theatre Australia is thrilled to present feature entertainment by internationally acclaimed Australian songsters Pot-Pourri performing favourite hits from opera to Broadway with a touch of comedy and magic.

Tuesday July 1

09:10 – 10:50 ***Session 3*** **Chair: P. Schaap**

The beginning of the end: from growth to early development

9. *Dictyostelium* as a model study for Nramp1 function in phagocytosis and resistance to pathogens
B. Peracino, C. Skriwan, A. Balest, A. Balbo, B. Pergolizzi, A. Noegel, M. Steinert, S. Bozzaro

10. Integrity of the actin cortex ensures reliable chromosome segregation in mitosis
G. Gerisch, J. Faix, J. Koehler, A. Mueller-Taubenberger

11. Precise function and subcellular localization of *dia2* expressed specifically during the growth/differentiation transition (GDT) of *Dictyostelium* cells
S. Hirose, A. Amagai, Y. Maeda

12. Spontaneous oscillations of ERK2 activation during development of *Dictyostelium*
Y. Miyazaki, D. Ikeno, M. Kuwayama, M. Maeda

13. Dd-STATb, a *Dictyostelium* STAT protein with a highly unusual SH2 domain, is a regulator of cell growth and early development
N.V. Zhukovskaya, M. Fukuzawa, M. Tsujioka, K.A. Jermyn, T. Kawata, J.G. Williams

10:50 – 11:20 Morning Tea

11:20 – 12:40 ***Session 4*** **Chair: A. Mueller-Taubenberger**

Pointing the bones: cytoskeleton and cell polarity

14. GskA, the cytoskeleton and cell polarity
E. Dalton, H. Williams, D. Wessels, D. Soll, Adrian J. Harwood

15. A cyclase associated protein in the regulation of the actin cytoskeleton and cell polarity in *Dictyostelium*
H. Sultana, A.A. Noegel

16. Regulation of Actin Cytoskeleton and Cellular Polarity by WASP during Chemotaxis
C.Y. Chung, S. Meyers, R.A. Firtel, Y. Lee

17. Pseudopod extension requires membrane trafficking events
D. Traynor, S. Reichelt, H. Yamaguchi, R.R. Kay

13:00 – 14:30 Lunch

Tuesday

14:30 – 15:40 ***Session 5*** **Chair: W. Loomis**

A social attraction: signalling and chemotaxis

18. Finding Their Way - How Cells Sense Direction
R. Meili, S. Lee, S. Merlot, H. Szemenzei, F. Du, A. Sasaki, Rick Firtel

19. A cGMP mediated myosin II signalling pathway that plays an important role in chemotaxis
L. Bosgraaf, D. Veltman, J.M. Goldberg, J.L. Smith, D. Wessels, D. Soll, P.J.M. van Haastert

20. A Gα-dependent Network that Antagonizes Global Chemotactic Responses
J. Brzostowski, C. Parent, A.R. Kimmel

Preserving the common wealth: the Stock Centre

21. The *Dictyostelium* stock centre.
J. Franke

15:40 – 16:10 Afternoon Tea

16:10 Workshop I
The Dicty *Stock Centre*
J. Franke

19:00 – 20:30 Dinner

20:30 Poster Session I

Wednesday July 2

Excursion to Sovereign Hill Gold-Mining Village

Buses depart at 8.30 am. Return for dinner at 8.00 pm, lunch provided.

Thursday July 3

09:10 – 10:50 ***Session 6*** **Chair: S. Bozzaro**

Making maps: The genome

22. A high resolution HAPPY map of the *Dictyostelium discoideum* genome
J.A. Pachebat, A.T. Bankier, B.A. Konfortov, P.H. Dear, G. Glöckner, K. Szafranski, R. Sucgang, M.-A. Rajandream, R Davies, G. Bloomfield, A.A. Noegel, The *Dictyostelium* Genome Sequencing Consortium

23. Sequence and analysis of chromosomes 1, 2 and 3
L. Eichinger

24. The *Dictyostelium* Genome Sequencing Project: Progress on Sequencing and Assembling Chromosomes 4, 5 and 6
R. Sucgang, J. Song, The Baylor Seq. Team, R. Gibbs, A. Kuspa, P. Davis, D. Saunders, Sanger Inst. Seq. Team, M.A. Rajandream, R. Davies, M. Quail, N. Hamlin, B. Barrell, J. Pachebat, A. Bankier, B. Konfortov, P. Dear, R.R. Kay, J.G. Williams, W.F. Loomis

25. PKA, GBF and LagC control of gene expression as seen on genome-wide microarrays
N. Iranfar, Danny Fuller, W.F. Loomis

26. DictyBase – a new *Dictyostelium* genome database
P. Fey, E.M. Just, P. Gaudet, P.A. Dyck, W.A. Kibbe, R.L. Chisholm

10:50 – 11:20 ***Morning Tea***

11:20 – 12:40 ***Session 7*** **Chair: A. Harwood**

Understanding DIFferences: DIF signalling and developmental gene regulation

27. Polyketide signalling in development
I. Sarafimidis, T.Saito, T. Suarez, D. Stetsenko, J-C Yang, G. Taylor, D. Neuhaus, R.R. Kay

28. DIF-dependent and independent expression of the cell type specific genes in *Dictyostelium* as revealed by *in situ* hybridization and microarray analyses
M. Maeda, T. Maruo, H. Sakamoto, M. Tomisako, T. Morio, H. Urushihara, Y. Tanaka, N. Iranfar, D. Fuller, W.F. Loomis

29. A bZIP/bRLZ transcription factor required for DIF signaling in *Dictyostelium*
C.R.L. Thompson, Q. Fu, C. Buhay and G. Shaulsky

30. Co-operative promoter interactions between a myb site and a C-rich element mediate DIF-regulated gene activation
M. Fukuzawa, M. Tsujioka, J.G. Williams

13:00 – 14:30 Lunch

Thursday

14:30 – 15:50 ***Session 8*** **Chair: D. Cotter**

Choosing a vocation: cell type determination

31. TagA, a putative serine protease/ABC transporter of *Dictyostelium* that is required for cell fate determination at the onset of development
J.R. Good, M. Cabral, S. Sharma, J. Yang, N. Van Driessche, C.A. Shaw, G. Shaulsky, A. Kuspa

32. A putative ariadne-like ubiquitin ligase is required for *Dictyostelium* development
N. Whitney, L. McGill, R. Lunsford, D. Lindsey

33. Genetic interactions of the E3 ubiquitin ligase component FbxA with cAMP metabolism and a histidine kinase signaling pathway during *Dictyostelium discoideum* development
T. Tekinay, H.L. Ennis, M.Y. Wu, M.K. Nelson, R.H. Kessin, and D.I. Ratner

34. Analysis of regulatory elements within the promoter sequence of the developmentally expressed gene encoding 5' nucleotidase and subsequent identification of a potentially novel transcription factor in the model system *Dictyostelium discoideum*
C.M. Eristi, M. Ubeidat, N.S. Wiles, B.R. Joyce, C.L.Rutherford

15:50 – 16:20 Afternoon Tea

16:20 Workshops – The Genome and DictyBase

Workshop III will run straight on from Workshop II and may therefore begin earlier than scheduled.

16:20 – 17:00 Workshop II
Genome Workshop
L. Eichinger, T. Morio, J. Pachebat, A. Kuspa

17:00 – 18:30 Workshop III
Learn to navigate the new Dictyostelium database: DictyBase
E. Just, P. Gaudet, P. Fey, R. Chisholm

19:00 – 20:30 Dinner

20:30 Poster Session II

Friday July 4

09:30 – 10:50 ***Session 9*** **Chair: R. Kay**

Group dynamics: From cell type determination to morphogenesis

35. *trishanku*, a novel gene that is involved in regulating cell type proportions and the stability of the differentiated state in *Dictyostelium discoideum*
J.K. Jaiswal, N. Mujumdar, H.K.MacWilliams, V. Nanjundiah

36. Spatial expression and functional characterization of ammonia transporter genes in *Dictyostelium*
S. Follstaedt, J. Kirsten, C. Singleton

37. Prespore-specific antigen: a cell adhesion molecule at the migratory slug stage of *Dictyostelium discoideum* development
V.M. Bowers-Morrow, S.O. Ali, K.L. Williams

38. Defining the molecular targets of valproic acid
K. Gregory, C. Shaw, S. Sharma, G. Shaulsky, R.S.B. Williams

10:50 – 11:20 Morning Tea

11:20 – 12:40 ***Session 10*** **Chair: J. Williams**

Shaping society: Signalling and morphogenesis

39. Identification of Erf2 homologs in *Dictyostelium*: Potential protein palmitoyltransferases
B. Wells, R.E. Gundersen

40. Prolyl oligopeptidase and regulation of inositol phosphates
M. Keim, R. Williams, A.J. Harwood

41. Aardvark and morphogenesis
J. Reynolds, W.J. Ryves, A.J. Harwood

42. The role of cARs in the control of cell polarization and movement
D. Dormann, G. Weijer, C.J. Weijer

13:00 – 14:30 Lunch

Friday

14:30 – 15:30 ***Session 11.*** **Chair: Y. Maeda**

The power to act: Mitochondria

43. Mitochondrial transcription in *Dictyostelium*
P. Le, B. Jayawardena, U. Greferath, P.R. Fisher, C. Barth

44. Mitochondrial FtsZs in *Dictyostelium* amoebae
P.R. Gilson, X.-C. Yu, D. Hereld, C. Barth, A. Savage, B. Kiefel, S.T. Lay, P.R. Fisher, W. Margolin, P.L. Beech

45. Mitochondrial disease and phototaxis in *Dictyostelium* slugs
P.R. Fisher, M. Kotsifas, S.T. Lay, Z. Wilczynska, L. Said, A. Ahmed, C. Barth

15:30 – 16:00 Afternoon Tea

19:00 Conference Banquet
Music Theatre Australia is thrilled to present entertainment by jazz group, the Fly Right Trio.

Poster Sessions

All posters can be displayed for the duration of the meeting.
Please mount posters on boards according to your poster number.

The presentation of the posters will be in two sessions:
Session I: Tuesday 20:30 – 22:00, Posters 1-12
Session II: Thursday 20:30 – 22:00, Posters 13-22

1. A DNA microarray resource for the *Dictyostelium* community
G. Bloomfield, J. Skelton, N. Nikolaidou-Katsaridou, B. Barrell, R. Kay , A. Ivens

2. Organisation of the cytoskeleton by actin-binding proteins and their regulators
R. Arasada, H. Son, L. Eichinger, A. Gloss, R. Rost, A. Vogel, J. Faix, M. Schleicher

3. Secretion of cysteine proteases during multicellular development of *Dictyostelium discoideum*
K.Z. Kirmani, L.E. Beneteau, D.A. Brock, K.E. Gale, R.H. Gomer, M. Sameshima, D.A. Cotter

4. The *cis*-acting domain for bi-directional transcription on the *fkbp2*/*dia1* promoter(s) that switch transcriptional directions during the growth/differentiation transition (GDT) of *Dictyostelium* Cells
S. Hirose, C. Pears, A. Amagai, Y. Madea

5. Contributions of mitochondrial DNA to cell differentiation and pattern formation in *Dictyostelium* development
J. Chida, H. Yamaguchi, A. Amagai, M. Tanaka, Y. Maeda

6. Mitochondrial gene disruption and phototaxis in *Dictyostelium discoideum*
L.M. Said, Z. Wilczynska, M. Hayes, P.R. Fisher.

7. Chaperonin 60 import into *Dictyostelium* mitochondria occurs cotranslationally
A. Ahmed, P.R. Gilson, P.L. Beech, P.R. Fisher

8. A search for STATa target genes and analysis of a STATa activation domain by use of *in situ* hybridisation
N. Shimada, Z. Katagiri, M. Fukuzawa, T. Maruo, J.G. Williams, M. Maeda, T. Kawata

9. The first intron is necessary for the STATc dependent stress response of *rtoA*
M. Tsujioka, T. Araki, T. Abe, M. Fukuzawa, M. Meima, P. Schaap, T. Morio, H. Urushihara, M. Katoh, M. Maeda, Y. Tanaka, I. Takeuchi, J.G. Williams

10. Functional analysis of transcription factor genes in *Dictyostelium*: expression analysis and generation of disruptants
H. Kuwayama, S. Obara, K. Yuasa, K. Uchida, T. Morio, H. Urushihara, S. Kuhara, M. Maeda, Y. Tanaka

11. Regulation and expression of alkaline phosphatase during *Dictyostelium* development
B. Joyce, M. Ubeidat, C. Eristi, N. Wiles, C.L. Rutherford

12. Regulation of development by Ddp24 genes, a family of genes involved in COPI/II-coated vesicle trafficking
T. Morio, Y. Kuwabara, M. Sawayama, H. Kuwayama, Y. Kawabe, Y. Tanaka

13. A novel pathway of prestarvation response: involvement of a *Dictyostelium* homologue of TRAP1
T. Morita, A. Amagai, Y. Maeda

14. *Dictyostelium* stress responses and the YakA pathway
R. Bagattini, L. Mantzouranis, R. Mascarenhas, W.B. Ribeiro, G.M. Souza

15. FbiA, a potential target of ubiquitin-mediated degradation, regulates cell-type proportioning in *Dictyostelium discoideum*
M.S. Foresther, K.A. McFeaters, N.A. Spardy, M.J. Niederst, E.A. Wilson, D.S. McGill, C. Moré, J.A. Christman, T. Abe, M.K. Nelson

16. A novel growth factor (psi factor) regulates cell-type specific gene expressions in *Dictyostelium discoideum*
H. Minamisawa, N. Shimada, T. Kawata, A.A. Oohata

17. Is the expression of the tRNA gene-targeted retrotransposon TRE-A regulated by RNAi?
O. Siol, T. Dingermann, T. Winckler

18. Fluorescent protein markers used in live cell imaging of dynamic processes in *Dictyostelium*
A. Mueller-Taubenberger, T. Bretschneider, J. Koehler, K.I. Anderson, S. Diez, G. Gerisch

19. Hypertonic signal promotes stability of *Dictyostelium* spores
Y. Yamada, M. Sameshima

20. The *lodA* gene of *Dictyostelium* is required for adenylyl cyclase (ACA) stimulation during aggregation
A. Carver, M. Rai, C. Singleton

21. Characterisation of the flavoprotein subunit of succinate dehydrogenase (SdhA) in *Dictyostelium discoideum*
S.T. Lay, Z. Wilczynska, P.R. Fisher

22. Identification and characterization of a mitochondrial DNA polymerase in *Dictyostelium*
M. Mokbel, C. Barth

Oral presentations

Dictyostelium discoideum in its natural context

J. Strassmann, D. Castillo, A. Fortunato, K. Foster, M. Landi, T. Platt, L. Santorelli, M. Smith, D. Queller

Rice University, USA

D. discoideum has been studied extensively in the laboratory on agar at 20^0 C. But much less is known about how it lives in the wild, something that is true for many model organisms. We have explored aspects of the social and physical environment of *D. discoideum*. Populations of *D. discoideum* sampled in Massachusetts, Virginia, North Carolina, Tennessee, Texas and Japan show significant genetic differentiation, but at low levels. This indicates that spores are probably transported quite widely. Another factor that leads to genetic variation among isolates is sexual reproduction. Greater levels of linkage disequilibrium between linked loci than unlinked loci indicate that sexual reproduction occurs in nature. Within a site, and even within a 0.2 gm sample, there is great genetic variation within isolates indicating that genetically distinct clones often coaggregate, setting the stage for between-clone competition. We have shown that genetically distinct clones mix in the laboratory environment and that some clones exploit other clones by preferentially getting into the sorus. Since coaggregation and exploitation probably also occur in nature, many genes may function in competition. For example, Bozzaro's group has shown that *csA* is influenced by both the social and the physical environment. The csA knockout seems to develop normally on agar. On soil it does not aggregate as well as wildtype, making fewer and smaller fruiting bodies. If *csA-* is mixed equally with wildtype cells, on the smooth surface of agar knockouts succeed in aggregating and become spore, not stalk. But on the rougher surface of soil, the knockout fails to aggregate as well, causing it to be underrepresented in spore cells compared to wildtype. This combination of effects make *csA* the first single-gene greenbeard gene discovered in any organism.

The social and physical environments could also interact. Slugs formed from chimeras migrate less far than slugs formed from equal numbers of cells of pure clones. This pattern is robust to substrate type, true on both soil and agar. Fruiting bodies in the wild are much smaller than in the laboratory, probably because of the paucity of food. This may mean aggregating with non-clonemates is necessary to achieve larger slug sizes for migration through the soil. We further examined the impact of physical environment on competition between genetically distinct clones. We competed 8 pairs of clones in 8 environments, varying moisture, temperature and pH. The outcome of competition changed direction with environment in two of the 8 pairs. Overall, both the physical environment and the social environment have an impact on developmental processes in *D. discoideum.*

Genetic Heterogeneity and Social Behaviour in Cellular Slime Molds

S. Kaushik, V. Nanjundiah

Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

We have carried out observations on genetic chimaeras made up of wild isolates of *Dictyostelium giganteum* and *D. purpureum*. The aim was to see whether the probability of spore formation by an amoeba was influenced by whether it is in a clonal aggregate or not. In chimaeric slugs and sori clones tend to be found in spatially contiguous clusters. They differentiate into spores in numbers that reflect initial mixing ratios and genetic relatedness. When clones are mixed, probabilities of spore formation cannot be arrayed in a simple linear hierarchy. Other experiments on mixtures of laboratory strains of *D. discoideum* indicate that the production of, and sensitivity to, DIF-like extracellular signals influences the probability that an amoeba differentiates into a spore or a stalk cell. Overall, it appears that complex epigenetic interactions underlie social behaviour in Dictyostelid amoebae.

Tyrosine phosphorylation of actin, actin rod formation and spore coat stability during *Dictyostelium* sporulation depend on the MADS-box transcription factor srfA

R. Escalante[1], Y. Yamada[2], D. A. Cotter[3], L. Sastre[1] and M. Sameshima[2]

[1]Instituto de Investigaciones Biomédicas CSIC/UAM. Spain; [2]The Tokyo Metropolitan Institute of Medical Science. Japan; [3]University of Windsor, Canada.

The initial process of spore maturation in *Dictyostelium* is encapsulation of prespore cells with a tri-lamellar case at the mid-culmination stage. The spore maturation process also involves dramatic changes in the cytoskeleton; actin rods are formed both in the nucleus and cytoplasm. These rods first appeared as bundles of actin tubules hexagonally cross-linked (1). As the spores mature modules of rods are associated into thicker rods. Besides, 50% of actin molecules are phosphorylated in tyrosine after encapusulation (2). Actin phosphorylation is coincident with rod maturation and might contribute to the formation and stability of elongated actin rods in the spores. The MADS-box transcription factor srfA is involved in spore differentiation in *Dictyostelium*. The expression of this gene is activated by the cAMP-dependent protein kinase during mid-culmination (3). SrfA-dependent gene expression is essential for spore maturation since strains lacking srfA display abnormal spore morphology and loss of viability (4). Extremely reduced expression of spiA in the mutant suggests that srfA might be involved only in late events of spore differentiation. Here a detailed structural analysis of mutant spores has been performed to gain insight into the specific aspects of spore differentiation in which srfA is involved.

Two main structural defects have been observed. One is the formation of actin rods. SrfA mutant spores showed the initial stages of rod formation but no mature rods were found in older spores either in the nucleus or the cytoplasm. Moreover, phosphorylation of actin is strongly reduced in the mutant. The other defect observed was the formation of the spore coat. Young srfA$^-$ spores show basically normal trilaminar coat structures suggesting that release of prespore vesicles and basic assembly of the coat takes place in the absence of srfA. However, the outer layer gets wavier as the spore ages and suffers a progressive degradation suggesting a late defect in the stability of the spore coat. Taken together, these results suggest that SrfA is involved in late events of spore maturation necessary for spore stability.

1. Sameshima et al., 2001. J Struct. Biol. *136*, 7-19; 2. Kishiet al., 1998. J Cell Sci. *111*, 2923-2932; 3. Escalante et al., 2002. Mech. Develop. *117*, 201-208; 4. Escalante et al., 1998. Development *125*, 3801-3808.

Anatomy of an adenylyl cyclase osmosensor

S. Saran, E. Alvarez-Curto, P. Schaap

University of Dundee

Adenylyl cyclase G (ACG) is a structural homolog of the large class of adenylyl cyclases from Euglenazoan parasites. The enzyme is activated by high osmolality and mediates inhibition of spore germination by this stress factor. The catalytic domains of all eukaryote nucleotidyl cyclases are active as dimers and dimerization often mediates activation. To investigate the role of dimerization in ACG activation, we co-expressed ACG under a constitutive promoter with an ACG construct that lacked the catalytic domain (ACG)cat) and was driven by a UV-inducible promoter. After UV-induction of ACG)cat, cAMP production by ACG was strongly inhibited, but the fold-stimulation by high osmolality was not reduced. Size-fractionation of native ACG showed that dimers were formed between ACG molecules and between ACG and ACG)cat. However, high osmolality did not alter the dimer/monomer ratio. This indicates that ACG activity requires dimerization via a region outside the catalytic domain, but that dimer formation does not mediate activation. To establish whether ACG required auxiliary sensors for osmostimulation, we expressed ACG cDNA in a yeast adenylyl cyclase null mutant. In yeast, cAMP production by ACG was similarly activated by high osmolality as in *Dictyostelium*. This indicates that the ACG osmosensor is intramolecular, which defines ACG as the first characterized primary osmosensor in eukaryotes.

Quantitative microscopy-visualizing signaling events in single living cells

X. Jiao, J. Chim, X. Xu, T. Jin

NIAID/NIH, USA

The recent advances in fluorescence microscopy and developments of new fluorescent probes make imaging a powerful technique for studying signal transduction inside single living cells with fine spatial and temporal resolution. Using a new generation of Laser Scanning Confocal Microscope (LSM 510 META), we are applying FRET microscopic imaging method and data analysis to monitor dynamic interactions between two proteins at the subcellular level. In addition, we are developing methods to visualize chemical gradients and changes of intracellular Ca^{2+} and cAMP levels. Combining imaging, spectroscopy and quantitative analyses, biochemical reactions in signal transduction pathways could be studied in single living cells.

Spectral Confocal FRET Imaging Reveals the Spatial-temporal Activation of the Heterotrimeric G-proteins in Single Chemotactic Cells

X. Xu, X. Jiao, T. Jin

National Institutes of Health, U.S.A.

Both the spatial and temporal aspects of heterotrimeric G-protein activation in single *Dictyostelium discoideum* cells were visualized as changes in fluorescence resonance energy transfer (FRET) between $G\alpha_2$ and $G\beta$ subunits tagged with cyan and yellow fluorescent proteins. G-protein coupled receptors detect chemoattractant gradients and direct cell movements by activating heterotrimeric G-proteins. To elucidate molecular mechanism of directional sensing, it is essential to determine spatial activities of the G protein in single living cells. Toward that end, we have applied living cell imaging of fluorescence resonance energy transfer (FRET) to monitor dissociations of $G\alpha_2$ and $G\beta\gamma$ subunits upon cAMP stimulations. Until recently, it had been difficult to obtain reliable FRET images in living cells with conventional system based on filter stets and bandpass acquisition systems because of overlapping emission spectra of FRET donor and acceptor pairs. A new Laser Scanning Confocal Microscope (LSM510 META) allows us to simultaneously acquire spectrally-resolved images of multiple fluorescent signals from a single cell and quantitatively analyze the contributions of various fluorophores with high spatial resolution to each pixel of an image. Applying this technology, we quantitatively analyzed spatiotemporal dynamics of heterotrimeric G-protein activities and downstream biochemical changes in single cells exposed to various chemoattractant stimulations. A global increase in chemoattractant concentration elicited uniform activation of G-proteins associated with the membrane of non-polarized cells. In contrast, a brief directional stimulation resulted in asymmetric and persistent G-protein dissociation, accompanied by delayed and transient PH-domain reporter redistribution to the cell membrane. In a steady chemoattractant gradient, the extent of G-protein activation reflected the local concentration of the ligand, whereas changes in membrane lipid composition were much more asymmetric across the cell body. These latter findings provide insight into how signals arising from shallow chemoattractant gradients are amplified to provide the intracellular compass guiding highly directional cellular migratory responses.

The early cAMP wave propagation revisited: phenomenology and genetic dissection

S. Sawai, E. C. Cox

Princeton University, USA

We have characterized the early wave phenomena observed by means of dark-field optics for wild-type and mutant strains under various conditions. Spatial features such as wave geometry and temporal features such as signal onset time and frequency were analysed. By testing different initial conditions, we see how heterogeneity of cells along the developmental path correlates with appearance of spiral waves. We will also introduce wave phenotypes of regA, rdeC and other mutant strains having defects in the feedback module of the oscillatory circuit. These cell lines each have a distinct and characteristic behavior such as propagating plateau, labyrinthine pattern and loss of oscillation. Our findings provide a basis for understanding the design principle behind the oscillatory circuit as well as proof of principle for the mutant screening based on dark-field wave phenotypes that is currently underway in our group.

Mechanism of cell movement within closely-packed cell aggregates

K. Inouye

Kyoto University, Japan

Cell rearrangement is an important component of embryonic development, and it plays a central role in pattern formation in Dictyostelium. However it is not quite clear how cells change positions within 3-dimensional tissues. Studies on the mechanism of cell movement have been performed with cells attached to flat surfaces, and it has not been shown whether the mechanism is the same for cell movement in 3-dimensional tissues.

In general, the situation of motile cells in a 3-dimensional tissue is quite different from that of cells in isolation in two respects. Firstly, the substratum of movement is their neighbouring cells, which may also be moving in the same or different direction, and secondly, there are very strong constraints on the direction of pseudopod extension. These characteristics of cell movements in a tissue provide the following problems, which are interesting from both theoretical and experimental points of view. (1) How does a cell located deep in a closely-packed tissue get traction for its movement? (2) Could it not be that coherent motion of cells, such as rotational cell movement, is an intrinsic property of a tissue composed of motile cells without requiring an additional mechanism to organise cell movements?

Here I will describe some characteristics of cell movements in isolation and within aggregates, and show that there are two types of cell movement, the "crawling" movement and "gliding" movement. The "crawling" movement is commonly seen with cells in isolation, and appears to be primarily driven by cortical expansion at the leading edge of elongating pseudopodia, whereas contraction of the cell cortex provides the propulsive force of the "gliding" movement, which is uncommon in solitary cells but prevalent in cells in multicellular stages. Analysis of F-actin dynamics in gliding cells indicates the importance of the attachment/detachment of the actin cytoskeleton to the cell membrane in this type of cell movement. Based on these and other observations, I will propose a possible mechanism for cell movement within 3-dimensional tissues. Possible mechanisms of cell sorting and generation of coherent cell motion will also be discussed.

Dictyostelium as a model study for Nramp1 function in phagocytosis and resistance to pathogens

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Dictyostelium amoebae are professional phagocytes, which ingest bacteria as the principal source of food. We have cloned the *Dictyostelium* homologue of human Nramp1, an endo-lysosomal membrane protein that confers on macrophages resistance to infection by a variety of intracellular bacteria or protozoa. The *Dictyostelium Nramp1* gene encodes a protein of 53 kDa with 11 putative transmembrane domains. The *Nramp1* gene is transcribed during the growth-phase and down regulated to barely detectable levels upon starvation. To gain insights into its function, Nramp1 or the vatB subunit of the V-H^+ATPase were fused with GFP and expressed in cells. As expected, GFP-vatB was inserted in membranes of acidic compartments, in addition to the contractile vacuole system. In contrast, GFP-Nramp1 decorates only a subset of acidic vesicles, the Golgi and non-acidic vesicles, possibly post-lysosomes. Late, but not early, endosomes and phagosomes were decorated with GFP-Nramp1, whereas GFP-vatB labelled both early and late endo- and phagosomal vesicles.

Cells expressing an *Nramp1* antisense gene construct displayed reduced *E. coli*, *L. pneumophila* and *M. avium* phagocytosis, and were more permissive hosts than wild-type cells for intracellular growth of *L. pneumophyla* and *M. avium*. *Dictyostelium* is thus the lowest professional phagocyte, in which Nramp1 appears to play a role in phagocytosis and resistance to pathogens.

A null mutant (AB3) has been generated by homologous recombination and is now being used to confirm the results obtained with the antisense cells and to study the mechanism of action of Nramp1.

Integrity of the actin cortex ensures reliable chromosome segregation in mitosis

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Cleavage of the cell body is brought about by the actin network in the cell cortex, and segregation of chromosomes is mediated by microtubule arrays that form the mitotic apparatus organized by centrosomes.

Studying mitosis in *Dictyostelium* mutants that are deficient in actin-binding proteins associated with the cell cortex, we found that some of these proteins are required for precise segregation of the chromosomes. In mutants lacking either the actin-interacting protein 1 (Aip1) or the cortexillin I/II dimer, an actin-bundling protein, atypical mitotic complexes and enlarged nuclei with varying DNA content were observed. These alterations are linked to the dissociation of centrosomes into the cytoplasm and their irregular re-association with the nuclei. Live imaging showed how coalescing mitotic complexes give rise to a multipolar spindle, and how excess centrosomes are eliminated by mitotic cleavage between anucleate and nucleated portions of a cell.

Centrosomal aberrations were also observed in mutants lacking both Aip1 and coronin, another conserved regulator of actin dynamics. Electron microscopical investigation of these mutants revealed an altered morphology of the actin cortex and a higher F-actin content. It is tempting to speculate that the inability of these mutants to rapidly rebuild their filamentous actin is responsible for the observed severe phenotype.

Our data indicate that proteins of the actin system contribute to the normal function of centrosomes, suggesting that appropriate organization of the actin cortex is a prerequisite for the reliable segregation of chromosomes in mitosis. We conclude that reversible separation of centrosomes from nuclei, caused by deficiencies in actin-binding proteins, give rise to perpetuated genomic instability. Many of the proteins constituting the actin system in *Dictyostelium* are related to mammalian proteins. Therefore, similar deficiencies in mammalian actin-binding proteins might contribute to the aneuploidy observed in malignant tumour cells.

Precise function and subcellular localization of *dia2* expressed specifically during the growth/differentiation transition (GDT) of *Dictyostelium* cells

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In *Dictyostelium discoideum* Ax-2 cells, a checkpoint (PS-point) of the growth/differentiation transition (GDT) has been precisely specified. Accordingly, extensive studies on the PS-point should lead us to understand the mechanism of GDT. We have already identified several genes (*dia1*, *dia2* and *dia3*) specifically expressed during the GDT. It has been shown that the expression of the *dia2* gene is augmented in response to starvation around the PS-point, and that its inactivation by antisense-RNA impaired the progress of differentiation and morphogenesis of *Dictyostelium* cells (Chae et al., 1998). The present work has demonstrated that destruction of the *dia2* gene gives a severe effect on cellular differentiation; the DIA2-null cells never differentiated, thus resulting in no aggregation. Western blot analysis using the DIA2-specific antiserum have demonstrated that the amount of the DIA2 protein reach its maximum 6 hr after starvation, as the case of the *dia2* transcription profile. Interestingly, the DIA2 protein appeared even during the growth phase in a cell-density dependent manner, particularly from the exponential growth phase ($>5x10^6$ cells/ml). This suggests that the DIA2 expression might be under the control of prestarvation factor (PSF). We also made a GFP-tagged form of the DIA2 protein. The DIA2-GFP fusion protein seemed to be located in cytosolic vesicles and endoplasmic reticulum (ER).

Chae et al., (1998) Biochem. Biophys. Res. Commun., 252, 278-283.

Spontaneous oscillations of ERK2 activation during development of *Dictyostelium*

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The MAP kinase ERK2 is activated when extracellular cAMP binds to the G-protein coupled cAMP receptor CAR1 and is essential for the subsequent transient rise in cAMP level. In the absence of ERK2 the cAMP signal is not relayed and chemotactic aggregation fails to occur. Conflicting reports have appeared concerning the role of G-beta in the signal transduction pathway leading to ERK2 activation (Knetsch et al., 1996; Maeda et al., 1996). Using highly specific antibodies to ERK2 together with antibodies that recognize the phosphorylated forms of ERK1 and ERK2, we reinvestigated the role of heterotrimeric G protein in ERK2 activation. We find that addition of cAMP leads to the rapid activation of ERK2 in both wild type and G-beta null cells but that the proportion of ERK2 that is phosphorylated is reduced in the mutant cells. It appears that both G-protein independent and G-protein dependent pathways function to activate ERK2.

Computer simulation of a circuit that can produce spontaneous oscillations in developing *Dictyostelium* cells has indicated that ERK2 is activated for about 3 minutes during the front of a wave and then returns to basal levels over the next 4 minutes (Laub and Loomis, 1998). We found that dissociated cells in suspension show spontaneous oscillations of ERK2 activation with a 7 minute periodicity. The amplitude of the changes in ERK2 activation in dissociated cells in suspension increases during the aggregation stage to reach a peak at 10 hours. Spontaneous oscillations were not observed in *carA*$^-$ null cells lacking the surface receptor CAR1. However, spontaneous changes in ERK2 activation could be seen in *gpbA*$^-$ and *acaA*$^-$ cells, lacking G-beta and the adenylyl cyclase ACA respectively, although the periods were prolonged and the amplitudes deminished. These observations support our conclusion that activation of ERK2 is partially G-protein independent.

The Laub-Loomis model suggests that ERK2 activation is attentuated when PKA is activated following the accumulation of internal cAMP. Since spontaneous oscillation can still be observed in *acaA*$^-$ cells, it appears that the adenylyl cyclase ACR generates sufficient cAMP to periodically activate PKA as well as CAR1.

Dd-STATb, a Dictyostelium STAT protein with a highly unusual SH2 domain, is a regulator of cell growth and early development

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STAT (Signal Transducer and Activator of Transcription) proteins are commonly the effectors in cytokine signalling pathways and are also important developmental regulators in both mammals and flies. STATs are activated by tyrosine phosphorylation, usually by a member of the JAK kinase family, and then dimerise with another STAT molecule before moving to the nucleus. The four *Dictyostelium* STAT proteins, Dd-STATa-d, all contain a DNA binding domain, an SH2 domain and a site of tyrosine phosphorylation. Dd-STATa and Dd-STATc are respectively activated by cAMP and DIF and they regulate different aspects of multicellular development. Dd-STATb is unique, among SH2 domain containing proteins, in that the universally conserved and essential SH2 domain arginine residue, that is primarily responsible for phosphotyrosine binding, is replaced by a leucine. There is also a 21 amino acid insertion within the Dd-STATb SH2 domain. Despite these two major abnormalities, Dd-STATb is biologically functional; Dd-STATb null cells have a growth defect, revealed when they are co-cultured over several growth cycles with parental cells. Also, micro-array assay revealed several genes that are either under-expressed or over-expressed in the null strain and one of these, discoidin 1, is also over-expressed during early development. As would be predicted from its structure, the Dd-STATb SH2 domain does not bind *in vitro* to tyrosine phosphorylated peptides. Tyrosine phosphorylation and dimerisation of metazoan STAT proteins requires sequential SH2 domain:phosphotyrosine interactions and again, as expected, we are unable to detect tyrosine phosphorylation of Dd-STATb. Remarkably, despite these deficiencies, the Dd-STATb protein sediments at the size expected for a homo-dimer. Furthermore, after mutation of the predicted tyrosine phosphorylation site to phenylalanine the mutant protein still sediments as a dimer and it is nuclear enriched. These observations suggest a novel mode of dimerisation, that is independent of a canonical SH2 domain:phosphotyrosine interaction but that generates a biologically active transcription factor.

GskA, the cytoskeleton and cell polarity

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The protein kinase GSK-3 is a well-characterized component of a number of important signaling pathways, including Insulin and Wnt signaling in animals. Observations in a number of cell biological systems suggest that GSK-3 may also play important roles in regulating the cytoskeleton and cell polarity[1]. Interaction with β-catenin, the downstream target of GSK-3 in the Wnt signaling pathway, can explain some but not all of these GSK-3 regulatory roles. Homologues of both GSK-3 (GskA) and β-catenin (Aardvark; Aar) are present in *Dictyostelium*, and offer a chance to study their role in a highly motile cell.

Previous observations suggest that GskA may interact with the cytoskeleton. Expression of a mutant GskA protein (VE267,268GR) leads to loss of cell polarity and reduced motility[2]. A similar phenotype is seen when Aar is over expressed, and the effect of the GskA-(VE267,268GR) mutation is lost in cells that lack *aar*. To investigate these phenotypes further we have carried out a detailed analysis of *aar* mutant chemotaxis. These results indeed suggest a defect within the cytoskeleton. In parallel studies, we have expressed new GskA mutant proteins and these also appear to lead to cytoskeletal defects.

1. A.J. Harwood & Braga, V.M.M. (2003) cdc42 & GSK-3: signals at the crossroads. Nature Cell Biology **5**, 275-277.

2. Fraser E., et al (2002) Identification of the Axin and FRAT/GBP binding region of glycogen synthase kinase-3. J. Biol. Chem. **277**, 2176-2185

A cyclase associated protein in the regulation of the actin cytoskeleton and cell polarity in *Dictyostelium*

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The link between signal transduction and subsequent alterations in the cytoskeleton is a central theme in cytoskeleton research. A protein that may provide this link is the cyclase associated protein, CAP, first found in *Saccharomyces cerevisiae*. The CAP homologue of *Dictyostelium discoideum* is a PIP_2 regulated G-actin sequestering protein which is present in the cytosol and shows enrichment at the plasma membrane. For the better understanding of CAP in the regulation of the actin cytoskeleton and of cell polarity in *Dictyostelium* we have made use of *Dictyostelium* mutants affected in signalling pathways like mutants deficient for ACA, G-protein α2 and βγ subunits, the ACA-regulator PIA, PI3-Kinase and protein kinase A (PKA) to see if these signalling molecules are important for targeting of CAP to the cell cortex, and also in the relocalisation of CAP upon a stimulus (phagocytosis, pinocytosis and chemotaxis). Our studies revealed that ACA, Gα2, Gβγ, PIA and PI3-Kinase are not required for correct functioning of CAP and for targeting of CAP to the cell cortex. However, we found that expression of CAP has a positive effect on phagocytosis of the Gβγ mutant and completely rescued the severe impairment in pinocytosis of the PI3-Kinase mutant.

We also observed that aggregation deficient aca cells expressing GFP-CAP were able to form EDTA sensitive cell contacts and develop into mounds and thus, CAP is a multifunctional protein with many activities at the cellular level. Taken together, all our analysis and future studies are aiming at unraveling a link of CAP with the cytoskeleton and signalling pathways in the cell.

Regulation of Actin Cytoskeleton and Cellular Polarity by WASP during Chemotaxis

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Wiskott-Aldrich Syndrome protein (WASP) is a key downstream component connecting multiple signaling pathways to F-actin polymerization, a process essential for the formation of lamellipodia and filopodia during chemotaxis. We took many approaches to elucidate roles of WASP during chemotaxis. 1) We created a WASP gene replacement strain ($WASP^{tet}$ cells), in which WASP expression is under the control of the tet promoter, so we can tightly control the expression of WASP with tetracycline. $WASP^{tet}$ cells show significant defects in polymerizing F-actin in response to cAMP stimulation and in establishing axial polarity in the chemoattractant gradient. Their motility is also severely impaired. 2) Understanding of the domain(s) responsible for the subcellular localization of WASP would help us to dissect signaling pathways controlling WASP function. In migrating cells, WASP localizes at the leading edge and uropod where F-actin is actively assembled. We identified Basic (B) domain of WASP as a major localization motif. B domain appears to bind to phosphoinositides and site-directed mutagenesis of lysine residue(s) in this domain abolished the binding. WH1 domain of WASP appears to stimulate F-actin assembly, independent from VCA domain activating Arp2/3 complex. Overexpression of the WH1 domain leads to a higher degree of F-actin polymerization as well as a mislocalization of F-actin accumulation within the cytosol. Mutations of highly conserved, aromatic amino acid residues in WH1 domain abolished this stimulatory effect. The ability of the WH1 domain to stimulate actin polymerization, however, may be independent of endogenous WASP expression. Mutations within the SH3 binding domain of WASP (poly-proline region) result in a severe WAS phenotype. Our results also show that cells expressing a DdWASP lacking the whole polyproline domain plus V domain (DdWASPΔPro) or the last two poly-proline repeat and the V domain (DdWASPΔV) have aberrant F-actin organization. Cells overexpressing DdWASPΔPro are not polarized and cannot extend pseudopodia effectively, probably because they lack dynamic regulation of the F-actin cytoskeleton. Similarly, cells overexpressing DdWASPΔV do not polarize, but they manage to extend pseudopodia and move very slowly. These results suggest that these mutants of WASP exert different effects on the machinery of F-actin assembly and that WASP activity plays a very important part in the regulation of the actin cytoskeleton during *Dictyostelium* chemotaxis.

Pseudopod extension requires membrane trafficing events

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The pseudopod of an amoeboid cell is thought to be extended by the force of actin polymerization at its leading edge. Pseudopod extension must also require a local increase in membrane area, but it is not known whether this is provided simply by deformation of the plasma membrane or by the local insertion of membrane vesicles to supply fresh membrane. The N-ethyl-maleimide Sensitive Factor (NSF) is required for most membrane trafficking events. Thompson and Bretscher (Development **129**, 4185, 2002) found that cells of a *Dictyostelium* mutant in which NSF is temperature sensitive, round up and become essentially immotile after a few minutes at the restrictive temperature and suggested that this might due to a loss of polarity.

We have investigated the response of these cells to gradients of the chemoattractant cyclic-AMP, supplied from a micropipette. We find that they are readily polarized in these conditions, as marked by the attraction of PI3kinase and a PH-domain protein to the membrane facing the needle. They are also capable of efficient actin polymerization at this site, as detected with ABP120-GFP. This often results in a bulge, or small extension, towards the needle, but rarely in the extension of a pseudopod. The process is repeated at different sites when the needle is moved around the cell. Thus by these criteria, the 'actin motor' of the pseudopod is fully functional in these cells, yet is unable to drive more than a slight extension of the pseudopod. Since exocytosis of pre-loaded FITC dextran by mutant cells ceases after 10 minutes at the restrictive temperature, our results suggest that membrane insertion is also required for pseudopod extension.

Finding Their Way - How Cells Sense Direction

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Chemotaxis, or directional movement up a soluble chemical gradient, is a highly conserved property key of many eukaryotic cells types that regulates cellular processes that range from the migration of polymorphonuclear leukocytes and macrophage to a site of inflammatory, directed movement of fibroblasts during wound healing, metastasis of cancer cells, and the aggregation of *Dictyostelium* cells to form a multicellular organism. We have identified several pathways that regulate the formation of the leading edge and control cell polarity in chemotaxing cells, including those controlled by PI3K and a MAP kinase cascade. We have shown that PI3K localizes to the leading edge, where it is activated downstream from Ras to form the lipid products PI(3,4,5)P_3/PI(3,4)P_2. In turn, localized activation of PI3K leads to the spatially-restricted localization of PH domain-containing proteins, which bind to these lipid product and then regulate different aspects of cell polarity, F-actin polymerization and leading edge protrusion, and myosin II assembly and trailing edge retraction. We have also demonstrated that the tumor suppressor PTEN, a 3' inositol-PI(3,4,5)P_3/PI(3,4)P_2-specific phosphatase, negatively regulates this pathway and is required for restricting the formation of PI(3,4,5)P_3/PI(3,4)P_2-containing lipid domains, and thus PH domain localization, to the leading edge. We have identified a potentially new pathway that genetically is required for proper chemotaxis. Three genes, two of which were independently discovered in *Dictyostelium*, are part of the TOR2 complex in yeast associated with polarized actin formation. Knockouts of these genes results in chemotaxis defects and affect the PI3K pathway. One of these, *Dictyostelium* RIP3, is a Ras-binding protein. We have further demonstrated that MEK1 and ERK1 localize to the leading edge and are required for proper chemotaxis. The control of MEK1's subcellular localization is regulated, in part, via chemotractant-mediated MEK1 SUMOylation. I will highlight some of our recent findings on new components of these pathways. I will also present results suggesting that the PI3K pathway also functions to regulate the MEK1/ERK1 MAP kinase cascade.

A cGMP mediated myosin II signalling pathway that plays an important role in chemotaxis

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Thanks to the genome sequencing project, the genes that produce, degrade and bind cGMP have recently been cloned. We have identified two guanylyl cyclases, two putative cGMP targets and three phosphodiesterases in *Dictyostelium*. Cell lines in which the two guanlylyl cyclases are disrupted have no detectable cGMP anymore, indicating there are only two guanlylyl cyclases in *Dictyotelium*; the transmembrane GCA and the soluble sGC. Disruption of two phosphodiesterases, which belong to a novel class II subtype, results in very high cGMP levels. The putative cGMP target protein GbpC displays a complex domain structure and contains Ras, kinase, RasGEF, Leucine rich repeat, cyclic nucleotide binding and GRAM domains. The other putative cGMP target, GbpD, is homologous to the C-terminal half and contains RasGEF, GRAM and nucleotide binding domains.

Disruption of the GbpC gene results in the loss of all high affinity cGMP binding capacity in lysates and a dramatic drop of cAMP induced myosin II phosphorylation. Remarkably, myosin II no longer translocates to the cell cortex after stimulation with cAMP. Furthermore, these mutants are unable to chemotax properly in a spatial gradient chamber and fail to polarize. Disruption of the gbpD gene results in hyper-polarized cells that chemotax more efficiently towards cAMP. Furhermore, myosin II phosphorylation is increased in GbpD minus cells. Thus, GbpD seems to act as an antagonist of GbpC. Preliminary results with GFP-fusion proteins indicate that sGC and GbpC are translocated to the membrane after cAMP stimulation. We will present a model that explains the role of cGMP on myosin II regulation.

A Gα-dependent Network that Antagonizes Global Chemotactic Responses

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Chemotaxing cells (e.g. *Dictyostelium*, neutrophils, etc.) are able to sense and move directionally in chemoattractant gradients that differ by <2% across the cell body. This shallow extracellular gradient is amplified into a very steep intracellular gradient, with selected signaling components localized specifically to the leading edge or rear of moving cells. This is despite the fact that receptors are uniformly distributed, and essentially uniformly stimulated (+/- 2%). Thus, it is hypothesized that activating, as well as, inhibiting pathways function downstream of receptor signaling to create the steep intracellular gradient. We have uncovered a novel inhibitory signaling pathway involving the G alpha subunit Gα9 during chemotaxis of *Dictyostelium* and suggest that this is part of a regulated inhibitory loop. The model is defined both genetically and biochemically using "loss-of-function" and "gain-of-function" mutations of Gα9. Gα9 regulates receptor sensitization/de-sensitization for chemotactic response. Mechanistically, Gα9 exerts specific effects within 10 sec. of receptor stimulation to negatively regulate multiple downstream pathways. Most dramatically, cells lacking the Gα9 subunit are hyperpolarized and hyperchemotactic, whereas cells expressing constitutively activated Gα9 exhibit a reciprocal phenotype. Finally, *gα9*-null mutations are able to repair polarity, chemotaxis, and aggregation defects in cells that only express non-phosphorylatable receptors which do not undergo normal adaptive/de-adaptive receptor cycling. Thus, loss of the Gα-mediated inhibitory network can compensate for a compromised receptor activation pathway.

The Dicty stock center

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The *Dictyostelium* stock center will be the central repository for strains and mutants of *Dictyostelium discoideum* and other cellular slime molds. In addition we envision storing useful vectors and the plasmid constructs that were used to label cells or to generate knockout mutants. We also hope to become a source of reliable information regarding culture methods and strain and plasmid information. Published strains and plasmids will be requested, although we encourage all to send us materials. The stock center can be billed for material sent in. Anyone can ask for material, which will be sent upon request following receipt of a Fedex or credit card account number. It is hoped that we will have a web presence before the Dicty2003 conference, and eventually ordering of strains and plasmids will be available on line. The first several months have been spent developing the database, getting the facility constructed, ordering equipment and materials, etc. We now have three large liquid nitrogen tanks ready (one off premises), and we are ready to receive materials. Currently more than 60 strains have been stored, and an additional couple of major shipments have been received. One of the initial aims is to safeguard some of the older collections, including collections of chemical mutants that have been used in earlier asexual genetics studies.

A high resolution HAPPY map of the *Dictyostelium discoideum* genome

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HAPPY mapping is an in-vitro PCR based technique that examines the co-segregation of sequence-tagged site (STS) markers in a panel of randomly broken genomic DNA. This technique is accurate and avoids many of the artefacts associated with physical maps.

Sequencing of the 34 Mb *D. discoideum* genome by the sequencing consortium is close to completion. The genome was sequenced using a combined chromosome specific- and whole genome- shotgun library approach. However the genome is extremely AT rich and contains numerous complex repeats, resulting in cloning and sequencing artefacts. In addition chromosome specific libraries suffer from cross-contamination, and chromosomes 4 and 5 co-migrate as a blob requiring their analysis as a single entity. These factors have created significant difficulties in the assignation and ordering sequence contigs along chromosomes.

To overcome this problem, we have made an accurate high-resolution HAPPY map of the entire *D. discoideum* genome, using STS markers selected from previously mapped genes, and sequence contigs from the chromosome specific projects. As the project progressed, interim HAPPY maps have been assembled on a chromosome-by-chromosome basis. Markers are assembled into linkage groups, which are then placed onto chromosomes and ordered relative to each other by integrating linkage data with sequence scaffold and previously mapped genetic loci information. The HAPPY maps have proved useful in defining the order of sequence contigs along the chromosomes, and aided in gap closure, enabling the chromosome 2 sequence to be published (Nature 418, 79-85, 2002).

Here we report on the completion of the high-resolution HAPPY map of the *D. discoideum* AX4 genome, and describe its integration with sequence contig scaffolds to create supercontigs spanning each of the chromosomes.

Sequence and analysis of chromosomes 1, 2 and 3

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Currently, efforts are underway that aim at determining the complete genome sequence of *Dictyostelium discoideum*. As strategy a whole chromosome shotgun (WCS) approach has been chosen and each of the six *Dictyostelium* chromosomes has been assigned to project partners. The project is well advanced, chromosome 2 has already been published, and it is expected that the sequences of chromosomes 1 and 6 and a gene catalogue for the complete genome will be available end of this year. The genome sequence will constitute the basis for genome-wide functional analyses and will undoubtedly further accelerate *Dictyostelium* research into a number of fundamental biological processes.

Chromosomes 1 (C1), 2 (C2) and 3 (C3) are part of the DFG funded project and comprise approximately 5, 8 and 6 megabases (Mb) of sequence, respectively, representing about 55 % of the genome. Due to cloning and sequencing biases caused by the extremely high average A+T content of 78% and long complex repetitive elements with only few polymorphisms sequencing and assembly of the chromosomes is a challenge. Nevertheless, it was possible to assemble the C1 and C2 sequences to a high standard by a seed- and BLAST-based cyclic assembly strategy, which, after finishing, resulted in three C1 and 51 C2 contigs. For C3 shotgun sequencing is finished and the assembly has been started.

The gene prediction program geneID predicted 1,760 protein coding genes on C1 and 2,799 on C2. This results in a surprisingly high gene density of about 1 gene per 2.8 kb, which is surpassed only by *Saccharomyces cerevisiae* (one per 2 kb) and is similar to that of *Schizosaccharomyces pombe* (one per 2.5 kb). If we assume that the other chromosomes have a similar gene density, we expect around 12,000 genes in the *D. discoideum* genome, approximately twice as many as in yeast and close to the 13,600 in *Drosophila*. Analysis of the C2 encoded genes showed that a significant number of the genes have higher similarities to genes of vertebrates than to those of other fully sequenced eukaryotes. This analysis strengthened the view that the evolutionary position of *D. discoideum* is located prior to the branching of metazoa and fungi but after the divergence of the plant kingdom, placing it close to the base of metazoan evolution.

The *Dictyostelium* genome project is a joint effort of groups in the USA, the UK, Germany and Japan. We thank all members of the consortium for their contributions.
Genome Project websites:
http://www.uni-koeln.de/dictyostelium/
http://genome.imb-jena.de/dictyostelium/
http://dictygenome.bcm.tmc.edu/
http://www.sanger.ac.uk/Projects/D_discoideum/
http://db.dictybase.org/
http://www.csm.biol.tsukuba.ac.jp/cDNAproject.html
http://www.mrc-lmb.cam.ac.uk:80/happy/happy-home-page.html

The *Dictyostelium* Genome Sequencing Project: Progress on Sequencing and Assembling Chromosomes 4, 5 and 6

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The Sanger and Baylor teams of the *Dictyostelium* Genome Sequencing Consortium have focused on assembling and finishing the sequences of chromosomes 4, 5 and 6 - roughly 45% of the genome. Chromosome 6, the smallest of the chromosomes at 3.9 megabases, was assembled using the high-density HAPPY map of chromosome 6 to confirm the order and orientation of the scaffolds. The sequence confirms the order of genes established by long range restriction mapping and REMI-RFLP methods. Chromosome 6 encodes 1400-1600 predicted proteins giving an average of 1 gene per 2.5 kb, which is about the same density predicted on chromosome 2. Additional structural features of chromosome 6 will be presented.

The shotgun sequence data for chromosomes 4 and 5 was processed as a single assembly, since the two chromosomes cannot be separated on pulsed field gels. We used the whole genome HAPPY map to assign the resulting contigs to their respective chromosomes. To date, we have produced over 9 Mb of contigs anchored to markers in the ~11 Mb making up chromosomes 4 and 5. Analyses and progress on these chromosomes will be presented, as well as tools to help the research community explore and utilize the genome sequence data.

PKA, GBF and LagC control of gene expression as seen on genome-wide microarrays

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Genome-wide expression analyses have shown that many developmentally regulated genes are robustly expressed in Dictyostelium cells developed in shaken suspension if they are treated with pulses of 30 nM cAMP at 6 minute intervals between 2 and 6 hrs of development and then given 300 μM cAMP. Under these conditions only small clumps of a dozen or so cells are formed after 6 hrs of development. Using stringent criteria we find that only three genes, *carA*, *gbaB* and *pdsA*, are expressed in the absence of cAMP treatment and are independent of adenylyl cyclase activity in the cells. Pulsing wild type or *acaA*$^-$ cells results in higher levels of expression of these genes. Another 15 genes are expressed in pulsed cells starting at 2 hrs of development as long as the cells have one or the other of the developmental adenylyl cyclases, ACA or ACR, but not if both are missing. Later genes appear to be strictly ACA-dependent since they are not expressed in pulsed *acaA*$^-$ cells unless the cells have constitutive PKA activity. Increasing PKA activity appears to initiate expression of sets of genes as development proceeds.

Strains lacking the DNA binding protein GBF express the pulse-independent genes and most of the ACA-independent genes except for *lagC* and *lagC2*. Pulsed *gbfA*$^-$ null cells also express 13 ACA-dependent genes which start up between 2 and 6 hrs of development. Later genes are not expressed in pulsed *gbfA*$^-$ cells. Pulsed *lagC*$^-$ mutant cells show a very similar expression profile, expressing the early genes but not the post-aggregation genes. Only two of the GBF-dependent genes are expressed in *lagC*$^-$ null cells suggesting that most of the consequences of the lack of GBF result from the failure to express *lagC*. Three genes appear to be subject to complex control since they are expressed in *gbfA*$^-$ cells but not in *lagC*$^-$ cells.

Pulsed *lagC*$^-$ cells that have constitutive PKA activity resulting from *pkaC* driven by its own promoter only express 5 of the 22 post-aggregation genes indicating that the LagC signal transduction pathway acts in concert with PKA to couple cell-cell adhesion to the temporal sequence of differential gene expression.

Strain TL130 lacks both developmental adenylyl cyclases but carries *pkaC* driven by the strong actin 15 promoter. While unable to aggregate chemotactically, cells of this strain can form mounds by accretion. However, morphogenesis is arrested shortly thereafter. The expression profile of TL130 cells developed in suspension without treatment with cAMP is very similar to those in *gbfA*$^-$ and *lagC*$^-$ cells. However, expression of *lagC* is greatly delayed and the GBF-/LagC-dependent genes are not expressed. This can account for the morphological arrest at the tight mound/finger stage.

When TL130 cells in suspension are given cAMP pulses followed by high levels of exogenous cAMP, they express *lagC* on time and express most late genes, including the classical prespore specific genes. Since ACA is mutated in these cells, they must be responding to exogenous cAMP in some way other than stimulation of adenylyl cyclase activity.

DictyBase – a new *Dictyostelium* genome database

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The new dictyBase, accessible from http://dictybase.org, provides the scientific community with a database that aims to integrate all currently available *Dictyostelium* information. Each gene has a locus page from which all relevant information is linked, such as a chromosomal map, gene names and aliases, gene sequences, a BLAST server, protein info, Gene Ontology (GO) annotations, literature, and researchers.

The chromosomal map consists of the 'generic genome browser' (Gbrowse; http://www.gmod.org/ggb/index.shtml). Gbrowse allows choosing any chromosomal range, zooming and centering, and to retrieve an annotation by position, name, or key word search. You find alignments of genes or gene models with ESTs and contigs. This is currently especially useful for the fully sequenced chromosome 2 (*Glöckner, et al. (2002) Nature 418:79-85*). Eventually, all gene names in dictyBase will follow the proposed Demerec nomenclature (http://dictybase.org/Nomenclature%20proposal.htm), and researchers are able to reserve names for genes that are work in progress. All known sequence information (genomic -, coding -, translated sequences) is linked to the locus page and can be subjected directly to a BLAST search. External links include all Entrez Nucleotide and Entrez Protein records. The Gene Ontology (GO), (http://www.geneontology.org/) is an independent database linked to dictyBase. The objective of GO is to provide a controlled vocabulary for the description of molecular function, biological process, and cellular component of gene products. The terms are to be used across different databases, providing a uniform platform for different model organism databases.

The information in our database (gene names and products, GO annotations, literature) is constantly being updated and improved by manual curation. PubMed references are linked automatically to a locus page; subsequently curators insure that the papers are appropriately linked to genes, and sort the papers into different subject categories. Manual curation will make dictyBase a trustable scientific resource. In the near future the database will also display information about strains available from the *Dictyostelium* stock center at Columbia. Requests will be produced directly from the pages describing the strain or mutation.

We believe that dictyBase, especially as more genomic data becomes available, will provide invaluable tools for the scientific community. We strongly encourage researchers to help in this effort by contributing their expert advice.

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Polyketide signalling in development

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DIF-1, and the closely related DIF-2 and DIF-3, are polyketide signals regulating stalk cell differentiation during development. Their production is essentially abolished in the *dmtA* null mutant, lacking the terminal methyl transferase of the DIF-1 biosynthetic pathway. Yet this mutant is still able to make stalk cells. We have therefore initiated a systematic search for additional differentiation factors released by these cells, which might be responsible for inducing stalk cell differentiation.

Polyketide synthesis is blocked by the generic inhbitor cerulenin, which inhibits the expression of both prestalk and prespore gene expression in cells cultured at high density, where endogenous factors might be expected to accumulate in the medium. Differentiation is restored to these cells by conditioned medium, obtained from cells developing without the inhibitor. Differentiation can even be stimulated strongly by Cd^{2+} ions, which are know to stimulate polyketide synthesis in fungi. These results suggest the presence of additional, unknown, polyketide signals in *Dictyostelium* development.

We have analysed the differentiation inducing factors released into the medium of the *dmtA* mutant during normal development, using HPLC to resolve them, and a number of different assays for cell differentiation to detect them. Four major activities are resolved, of which two appear to be intermediates in DIF-1 biosynthesis, which would be expected to accumulate in this strain. One activity is largely uncharacterized and the final one, provisionally named 'Terminator', is able to stimulate both stalk and spore cell differentiation. Although highly purified and analysed by both mass-spec and NMR, its chemical nature is currently unknown.

DIF-1 is able to stimulate prestalk gene expression rapidly by acting through defined regions of the relevant promoters. However the DIF receptor and signal transduction pathway are largely unknown. We have approached this problem genetically by screening for REMI mutants in which an ecmB-lacZ reporter is not induced by DIF-1. The design and outcome of this screen will be described.

DIF-dependent and independent expression of the cell type specific genes in D*ictyostelium* as revealed by *in situ* hybridization and microarray analyses

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Addition of DIF-1, a chlorinated alkylphenone, to *Dictyostelium* cells developing in dispersed monolayers leads to terminal differentiation of stalk cells. Moreover, DIF-1 induces the prestalk genes *ecmA* and *ecmB* in cells dissociated from aggregates. However, expression of initial prestalk genes *tagB* and *carB* is not affected by DIF-1. A mutant that is unable to synthesize DIF-1 due to the lack of DMT1, the methyltransferase that catalyzes the last step in DIF-1 biosynthesis, was found to develop well but failed to express a reporter gene controlled by the PstO specific regulatory region of *ecmA*. Expresssion of ecmO-gal could be induced in *dmtA*$^-$ cells by co-developing them with wild type cells or adding DIF-1. It was proposed that DIF-1 is essential for differentiation of PST-O cells. We analyzed 30 genes that are preferentially expressed in PstO cells by *in situ* hybridization of *dmtA*$^-$ slugs. Expression of 18 of these genes was significantly reduced in the DIF-less strain but expression of the other 12 PstO genes was not affected by the lack of DIF-1. We also examined PstA or PstAB genes and found that they were expressed in the expected cell types at the same levels in mutant and wild type slugs. Among 12 PstAO genes that we examined, 5 genes were expressed in both PstA and PstO cells in the *dmtA*$^-$ strain as in wild type strains while others were only expressed in PstA cells in slugs of the *dmtA*$^-$ strain. It appears that DIF-1 is essential for expression of some PstO genes but that other PstO genes are expressed in a DIF-independent manner. Recently, we identified 62 prespore genes by microarray analyses and analyzed expression pattern of 22 prespore genes in the *dmtA*- strain.. Since DIF-1 is known to repress several prespore genes in cells dissociated from aggregates, we expected to see increased expression of prespore genes in *dmtA*$^-$ slugs. However, most were expressed at wild type or reduced levels in mutant slugs. Although these genes are expressed uniformly in the prespore zone of wild type slugs, two-thirds of them were expressed in a graded manner in mutant slugs with the level decreasing towards the back. Our results show that the *dmtA*$^-$ slugs express prestalk genes normally except for a subset in PstO cells. Moreover, they clearly generate a region where DIF-independent PstO genes are expressed. Surprisingly, DIF-1 was found to affect expression of some prespore genes in the posterior region of slugs. While DIF-1 does not appear to be a morphogen affecting initial cell type divergence or terminal differentiation in fruiting bodies, it seems to modulate gene expression in both prespore and prestalk cells.

A bZIP/bRLZ transcription factor required for DIF signaling in *Dictyostelium*

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The developing *Dictyostelium* slug exhibits clear anterior-posterior pattern with the prestalk and prespore cell types arranged into tissues along this axis. The question of how this pattern arises is fundamental to our understanding of the control of *Dictyostelium* development. However, the patterning mechanism itself has also been the subject of much debate, with both morphogen gradients and a process more akin to lateral inhibition proposed. Nevertheless, the stalk cell inducing small diffusible molecule DIF-1 has remained central to both ideas. Furthermore, these studies have been aided considerably by the generation of a mutant specifically defective in DIF biosynthesis (dmtA), which has allowed the study of DIF-1 function during normal development.

In order to further understand this patterning process and its control in *Dictyostelium*, it is important to gain insights into the molecular nature of the DIF-1 response pathway and ultimately how each component may be influenced by other signals. However, to date few components of the DIF-1 signaling pathway have been identified. We took a forward genetic approach to identify mutants in these genes. We reasoned that since DIF-1 diverts cells from the spore cell fate to the stalk cell fate in 8-Br-cAMP monolayer assays, a mutant defective in the DIF-1 response would be more likely to produce viable spores in the presence of DIF-1 than wild type cells. We therefore used this as the basis of a screen to enrich for DIF insensitive mutants (dims) or DIF undersensitive mutants (dums). From a library of ~30000 mutants, we isolated one dim (dimA) and two dum mutants. dimA, shows no response to DIF-1 in all conditions tested. Furthermore dimA is clearly required for DIF-1 signalling in vivo since the mutant exhibits indistinguishable morphogical and cell type specific defects to those of the dmtA mutant. However, key differences lie in the cell autonomous nature of the phenotype and the finding that dimA produces normal levels of DIF-1. Since the disrupted gene in dimA encodes a transcription factor of the bZIP or bRLZ classes, we propose that dimA encodes the key transcriptional regulator required to integrate DIF-1 signaling.

Secondly, we have been using a microarray based approach to identify DIF target genes in vivo. Samples were initially taken from the dmtA mutant, dmtA+DIF (rescued) and Ax2+DIF at various developmental time points and the expression profiles of 5000 genes compared to wild type. In this way we could detect potential DIF induced genes (low in dmtA) and DIF repressed genes (high in DIF treated samples). Furthermore, we have now strengthened this data by intersecting these lists with those genes misexpresed in the DIF non-responsive mutant dimA. These data provide valuable insights into the mechanism and timing of DIF action.

Co-operative promoter interactions between a myb site and a C-rich element mediate DIF-regulated gene activation

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Analysis of the promoter of the *ecmA* gene led to the discovery of the pstA and pstO prestalk cell sub-types. Mapping of the 121 nt region that directs pstO-specific gene expression then suggested an important role for a direct repeat of the sequence TTGA, located at the cap-site distal end of the 121-mer. This acts as a binding site for Dd-STATa and, when a 53 nt sub-fragment containing the TTGA repeat site is multimerised and placed adjacent to a G box (a binding site for GBF), it directs strong expression throughout the prestalk region. However, despite the fact that the 53-mer derives from within a region that directs pstO expression (i.e. the 121-mer), it helps direct stronger expression in the pstA cells than the pstO cells. Furthermore, Dd-STATa is activated by cAMP and is required for tip differentiation, not for pstO differentiation. Therefore we have re-analysed the 121-mer and find that point mutations in the TTGA repeats do not impair pstO expression. Instead, in the cap-site proximal one-third of the 121-mer, there is a C–rich sequence and a closely situated 7 nucleotide dyad, AACAGTT. Mutations in either element eliminate pstO-specific expression. Moreover, when a 30-mer, that contains both elements, is tetramerised it directs pstO-selective gene expression and the lacZ reporter is DIF inducible. The dyad site fits the consensus sequence for a myb binding site and band-shift competition assays, using a sea urchin myb site (AACCGTT), support this. There is a previous report of a myb site that is essential for prestalk-specific expression of the GPI gene (1) and we show that a 28 nt region of the PDE late promoter, that is necessary for DIF inducibility (2), contains a perfect match to the ecmO myb site. There are at least 7 *Dictyostelium* genes that contain one or more myb repeat elements but only three of these have been studied previously. We will report progress in identifying the myb gene that we believe helps mediate DIF-inducible gene expression.

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TagA, a putative serine protease/ABC transporter of *Dictyostelium* that is required for cell fate determination at the onset of development.

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The *tag* genes of *Dictyostelium* are predicted to encode multi-domain proteins consisting of serine protease and ATP-binding cassette transporter domains. We have identified a novel *tag* gene, *tagA*, which is involved in cell type differentiation. The *tagA* mRNA accumulates during the first four hours of development, whereas TagA protein accumulates between two and ten hours of development and decreases thereafter. Wild type cells express *tagA* in prespore cells and mature spores, defining *tagA* expression as prespore-specific. However, *tagA* mutant cells that activate the *tagA* promoter do not sporulate, but instead form part of the outer basal disc and lower cup of the fruiting body. TagA mutant aggregates elaborate multiple prestalk cell regions during development and produce spores asynchronously and with low viability. TagA mutants produce about twice as many prestalk cells as the wild type as judged by a prestalk cell reporter construct. When mixed with wild-type cells *tagA*$^-$ cells become overrepresented in the prestalk cell population, suggesting that this phenotype is cell-autonomous. These results suggest that TagA is required for the specification of an initial population of prespore cells in which *tagA* is expressed. Expression profiling uncovered a delay in the transcriptional program between 2 and 6 hours, coincident with TagA expression, revealing an early function for TagA. TagA also appears to play a general role in cell fate determination since *tagA* mutants express a spore coat protein gene (*cotB*) within vacuolated cells that form part of the stalk and they express a prestalk/stalk-specific gene (*ecmB*) within cells that become spores. The expression of TagA at two hours of development, the observed coincident delay in the transcriptional program and the subsequent mis-expression of cell-type specific genes provide evidence for cell fate determination beginning in some cells much earlier than previously believed.

A putative ariadne-like ubiquitin ligase is required for *Dictyostelium* development

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The formation of a fruiting body by *Dictyostelium* cells provides a simple model system to investigate how developing cells form functional structures. Using REMI mutagenesis, we have isolated a mutant, designated *rbrA*, which forms slugs that are unable to phototax or develop into fruiting bodies. The deduced amino acid sequence of *rbrA* is highly similar to ariadne-like ubiquitin ligases, which are members of the RBR family. RBR genes encode proteins with two RING finger domains separated by an IBR (in-between-RINGS) domain. They are widespread in eukaryotes and include *parkin*, a gene implicated in Parkinson's disease. Many RBR family proteins appear to function in ubiquitination. *rbrA* mRNA is present in growing cells and throughout development. *rbrA*-null cells are much smaller than wild type cells and initially develop more quickly than wild type cells. Prespore-specific genes are expressed temporally in developing *rbrA*-null cells as in wild-type cells, but at much higher than wild type levels. Immunofluorescence using antibodies against cell-type-specific markers to stain cells from disassociated slugs show that prestalk cell numbers are greatly reduced in *rbrA*-null slugs. GFP expression and *in situ* hybridization studies indicate that cells expressing prestalk markers do not localize to the tip of slugs formed from *rbrA*-null cells - the prestalk region does not form. The *rbrA* phenotype can be rescued by mixing a small amount of wild-type cells with developing mutant cells. Mixtures containing 10% wild-type cells could phototax and form fruiting bodies. Increasing the per cent wild-type resulted in increasing stalk height. In the chimeric slugs, *rbrA*-null cells preferentially locate to the prespore region, while wild type cells preferentially locate to the prestalk region. These results suggest that a putative ariadne-like ubiquitin ligase is required for cell-type proportioning and pattern formation.

Genetic interactions of the E3 ubiquitin ligase component FbxA with cAMP metabolism and a histidine kinase signaling pathway during *Dictyostelium discoideum* development

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Dictyostelium amoebae altered in a gene called *fbxA*, which is thought to encode a component of an SCF E3 ubiquitin ligase, have defective regulation of cell-type proportionality. In chimeras with wild-type cells, the mutant amoebae form mainly spores, leaving the construction of stalks to wild-type cells. To examine the role of FbxA and regulated proteolysis we have recovered the promoter of *fbxA* and shown that it is expressed in a pattern resembling that of a prestalk-specific gene until late in development when it is also expressed in developing spore cells. Because *fbxA* mutant cells are developmentally deficient in pure culture, we were able to select suppressor mutations that promote sporulation of the original mutant. One suppressor mutation resides within the gene *regA*, which encodes a cAMP phosphodiesterase linked to an activating "response regulator" domain. In another suppressor, there has been a disruption of *dhkA*, a gene encoding a "two-component" histidine kinase known to influence *Dictyostelium* development. RegA appears precociously and in greater amounts in the *fbx*A mutant but not in an *fbx*A/*dhk*A double mutant, where RegA is restored to wild-type levels. Because the basis of *regA* suppression might involve alterations in cAMP levels during development, the concentration of cAMP was determined in all strains. The level of cAMP is relatively constant during multi-cellular development in all strains except the *dhkA* mutant, in which it is reduced at least 6-fold. The level of cAMP in the double mutant *dhkA/fbxA* is relatively normal. The level of cAMP in the various mutants does not correlate with spore formation, as would be expected on the basis of our present understanding of the signaling pathway leading to the induction of spores. Altered amounts of RegA and cAMP early in the development of the mutants suggest that both *fbxA* and *dhkA* genes act earlier than previously thought.

Analysis of regulatory elements within the promoter sequence of the developmentally expressed gene encoding 5'nucleotidase and subsequent identification of a potentially novel transcription factor in the model system *Dictyostelium discoideum*

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Progression through the life cycle of *Dictyostelium discoideum* reveals an increase in phosphatase activity that appears to coincide with the onset of cell differentiation in this organism. It was previously shown that the enzyme, which appeared to be localized to the boundary between the prestalk and prespore cells in the culmination stage, had a high pH optimum and a high affinity for the 5'AMP substrate. Activity assays using artificial substrates has resulted in reference of the enzyme as a classical alkaline phosphatase (ALP), while activity due to the high substrate specificity of the enzyme with 5'AMP has resulted in reference of the enzyme as a 5'-nucleotidase (5NT). The high substrate affinity of 5NT for 5'AMP has implicated the enzyme for a role in the regulation of cAMP. cyclic AMP is degraded to 5'AMP by cAMP phosphodiesterase. The roles of cAMP as a morphogen, chemoattractant, and effector of gene regulation during the life cycle of *Dictyostelium*, has led to interest and further investigation of the developmental regulation of 5NT. In order to definitively attribute the phosphatase activity localized at the boundary between the prestalk and prespore cells as being ALP or 5NT, analysis of mutants with the disrupted gene encoding 5NT or ALP was performed. Results from these studies provided evidence that the activity localized to this interface region was ALP. Although 5NT activity was not localized to this region, its developmental regulation throughout the life cycle and involvement in regulating cAMP has led to us to further analyze the mechanisms regulating expression at the level of transcription. In order to identify *cis*-acting regulatory elements in the sequence upstream from the 5NT coding region, a series of internal and 5' promoter deletions were produced and fused to a luciferase reporter gene. Luciferase assays of the deletion constructs led to the identification of three potential regulatory elements within the promoter that were subsequently analyzed further by electromobility gel shift assays. Specific binding of a protein to the sequence –307 and –226bp from the transcription start site prompted the isolation of the binding factor through a series of chromatography techniques. The highest sequence similarity found was with folic acid synthase. Whether the association of this protein with the upstream promoter affects expression of 5NT will be the subject of future investigations.

trishanku, a novel gene that is involved in regulating cell type proportions and the stability of the differentiated state in _Dictyostelium discoideum_

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The establishment and maintenance of proper cell type proportions is a characteristic feature of *Dictyostelium* development. We have identified a novel gene, *trishanku* (*tri*), whose disruption leads to the formation of fruiting bodies with a thickened stalk and subterminal spore mass. The absence of *tri* has no detectable effects on growth, the growth-to-development transition or early aggregation. Early in development, however, cell-cell adhesion weakens and aggregation streams break up, leading to a decrease in aggregate size. The cell type proportions in the slug appear normal, but experiments with stable and labile reporters reveal an increased transdifferentiation between prestalk and prespore cells. Because of this, many prespore cells have a history of prestalk marker expression. During culmination such cells move to the upper cup and ultimately differentiate into stalk. The bond between the upper cup and spore mass appears to loosen midway through culmination and the spore mass stops ascending while the stalk continues to elongate. *Tri* is expressed during all stages of growth and development. Transcript accumulation is low in vegetative cells and occurs mainly during the late G2 phase of the cell cycle. In development the transcript peaks during the first finger stage and subsequently localizes to the prespore zone. The predicted Tri protein contains a putative nuclear localization signal and a strongly conserved BTB protein-protein interaction domain; this domain is found in other proteins associated with a variety of functions including the regulation of transcription, chromatin structure and actin condensation.

Spatial expression and functional characterization of ammonia transporter genes in *Dictyostelium*

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Ammonia is an important signaling molecule involved in the regulation of development in *Dictyostelium*. During aggregation, ammonia gradients are established, and the ammonia concentration in the immediate environment or within a particular cell throughout development may vary. This is due to the rate of cellular ammonia production, to its rate of loss by evaporation to the atmosphere or by diffusion into the substratum, and to cellular transport by ammonia transporters (AMTs). Recent efforts in genome sequencing have identified three ammonia transporters in Dictyostelium. In addition to physically altering the levels of ammonia within cells, the AMTs also may play a role in ammonia signaling. The three transporter genes, amtA, amtB, and amtC are expressed in growing cells and throughout development. They exhibit a complex and dynamic spatial pattern of expression as development progresses. AmtB and amtC are primarily prespore specific in their expression. AmtC, however, is also expressed in a small group of cells at the very anterior tip of first fingers and slugs. In contrast, amtA is primarily expressed in various prestalk and ALC cells, but in a very dynamic way with changes in its expression among the prestalk subtypes as development progresses. Two of the amt genes have been disrupted, and the null strains are being characterized. AmtB null cells show only subtle phenotypic aberrations during development with the severity dependent upon the substrate/solution upon which the cells are developing. AmtC null cells possess a strong slugger mutant phenotype. We assume the slugging behavior of *amtC*$^-$ is due to altered ammonia levels or altered ammonia signaling within the anterior most tips of the first fingers/slugs.

Prespore-specific Antigen: a cell adhesion molecule at the migratory slug stage of *Dictyostelium discoideum* development

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Prespore-specific Antigen (PsA) is selectively expressed on the surface of prespore cells at the multicellular migratory slug stage of *Dictyostelium discoideum* development. It is a developmentally regulated glycoprotein which is anchored to the cell membrane through a glycosyl phosphatidylinositol (GPI) anchor. We present the results of an *in vitro* immunological investigation of the hypothesis that PsA functions as a cell adhesion molecule, and of a ligand binding assay indicating that PsA has cell membrane binding partner(s). This is the first evidence to implicate a direct role for a putative cell adhesion molecule in cell-cell adhesion during the multicellular migratory slug stage of *D. discoideum* development. Cell-cell adhesion assays were carried out in the presence or absence of the monoclonal antibody MUD1 which has a single antigenic determinant: a peptide epitope on PsA. These assays showed specific inhibition of cell-cell adhesion by MUD1. Further, it was found that a purified recombinant form of PsA (rPsA), can neutralize the inhibitory effect of MUD1; the inhibitory effect on cell-cell adhesion is primarily due to the blocking of PsA by the monoclonal antibody. The resistance of aggregates to dissociation in the presence of 10mM EDTA (ethylenediaminetetraacetic acid) indicates that PsA mediates EDTA-stable cell-cell contacts, and that PsA-mediated cell adhesion is likely to be independent of divalent cations such as Ca^{2+} or Mg^{2+}.

Current work is aimed at identifying PsA's receptor(s). We present:

(i) The results of ligand binding assays performed with PsA, following separation of HU2421 membrane proteins via 1D SDS-PAGE electrophoresis, and transfer to nitrocellulose membrane. These results confirm that PsA does not bind homophilically, and indicate that it binds to a number of membrane proteins with apparent molecular weights in the range 20-100 kDa.

(ii) The results of ligand binding assays with PsA, following separation of HU2421 membrane proteins via 2D-PAGE electrophoresis, and transfer to nitrocellulose membrane. These results indicate PsA binds to a number of protein spots on a 2D gel, with apparent molecular weights in the range 20-120 kDa.

(iii) the results of applying mass spectrometric techniques to identify PsA's receptor(s).

Defining the molecular targets of valproic acid

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Valproic acid, a short chain fatty acid, is the most widely prescribed anti-epileptic drug worldwide. It is now increasingly being used in the treatment of manic depression due to its low toxicity and wide spectrum activity. It has the serious disadvantage, however, of being teratogenic, causing neural tube defects in 2% of cases if taken during the first trimester of pregnancy. Although a molecular mechanism has been proposed for its anti-epileptic and teratogenic effects, the mechanism by which it and lithium – the most commonly used anti-manic drug - functions in the treatment of manic depression remains unknown.

The effect of valproic acid on *Dictyostelium* is to severely retard development at the mound stage, causing only a small number of fruiting bodies to be formed with short stalks and round spore heads. This fruiting body morphology is distinct to that caused by lithium.

To identify genes involved in the effects of valproic acid, we have employed both a growth and a development screen to identified 25 genes conferring resistance to this drug. These mutants have been further characterized by their resistance to both lithium and non-teratogenic analogues of valproic acid, which has provided an indication of the possible role of each gene in the anti-manic, anti-epileptic or teratogenic function of valproic acid. Most of these mutants can be divided into three categories:

1. RNA transcription/modification
2. Putative MAP Kinase signaling
3. Lipids/small GTPase signaling

I will present the initial results of this screen and briefly describe the other approaches being used to identify the mechanism of action of valproic acid.

Identification of Erf2 homologs in *Dictyostelium*: Potential protein palmitoyltransferases.

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The heterotrimeric guanine nucleotide binding proteins (G proteins) are essential components of a wide variety of eukaryotic cellular signalling pathways. Heterotrimeric G proteins consist of a 40 kDa α-subunit, a 36 kDa β-subunit and a small 8-10 kDa γ- subunit. Acting as molecular switches, G proteins relay molecular information from membrane bound receptors to downstream intracellular effectors. It has been shown that most G-protein α-subunits require lipid modification by myristic acid and/or palmitic acid for proper localization and function. Protein palmitoylation is a post-translational, reversible thioester linkage of palmitic acid (C16:0) to a cysteine residue of a substrate protein. Palmitoylation of G-proteins occurs specifically in the N-terminal region of the α-subunit, however the mechanism by which palmitoylation occurs in eukaryotic cells has proven to be elusive. In the 'social amobae', *Dictyostelium discoideum*, the transition from vegetative growth to multicellular development has been shown to be dependent upon the G-protein G2. The G2 α-subunit is myristoylated and palmitoylated, *in vivo* and both modifications are required for proper function. Gα2 in *Dictyostelium* offers an excellent system from which to examine protein palmitoylation. Recently, Erf 2p, a 41 kDa membrane bound protein, was identified as a Ras palmitoyltransferase in *Saccharomyces cerevisiae*. To determine whether Erf 2 homologs exist in *Dictyostelium*, a BLAST search against the sequenced genome was performed. The search yielded a family of twelve Erf 2p homologs within the *Dictyostelium* genome. We are in the process of elucidating the expression patterns of these twelve putative palmitoyltransferases during the *Dictyostelium* life cycle using RT-PCR and Real Time-PCR. In addition, *in vitro* transcription and translation of each of the putative *Dictyostelium* palmitoyltransferases is to be attempted in order to test palmitoylatransferase activity towards myristoylated Gα2 and other potential substrates.

Prolyl oligopeptidase and regulation of inositol phosphates

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Activation of phospholipase C (PLC) in response to cell stimulation and formation of Ins(1,4,5)P_3 is common to plants, animals and yeasts. In *Dictyostelium*, PLC is activated by cAMP in aggregating cells. Ins(1,4,5)P_3 however is only one of many inositol phosphate (InsP) species, the roles of these other species is less clear.

The mood stabilizing drugs lithium and VPA lower the intracellular concentration of Ins(1,4,5)P_3 and inhibit *Dictyostelium* aggregation. By screening for *Dictyostelium* mutants that can aggregate in the presence of 10 mM lithium, we have identified a number of genes required for the regulation of Ins(1,4,5)P_3. The mutant *lisA* lacks the enzyme prolyl oligopeptidase (PO), and we show that changing PO activity in an number of ways alters the concentration of Ins(1,4,5)P_3.

To investigate the mechanism of the PO interaction, we have examined the effect of manipulating its activity on the complete spectrum of InsP species. We find that PO activity regulates both the total mass of InsP in the cell and the ratio of $InsP_6$ to $InsP_5$ and $InsP_4$. The change in $InsP_6$:$InsP_{4/5}$ ratio can be explained by regulation of the activity of Mulitple Inositol Polyphosphate Phosphatase (MinsPP), but how can the total InsP mass change be explained?

Inositol synthase converts glucose-6-phosophate to inositol monophosphate and is essential for the *de novo* synthesis of inositol. In yeast it has been proposed that expression of the yeast inositol synthase gene (*ino1*) is regulated by the ratio of $InsP_6$:$InsP_{4/5}$. We propose that through modulation of MInsPP activity PO regulates expression of the *Dictyostelium ino-1* gene. We present expression data and genetic evidence to support this hypothesis.

Aardvark and Morphogenesis

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β-catenin and the protein kinase GSK-3 are central components of the Wnt signalling pathway in animals. This signal transduction pathway is important for cell fate determination and oncogenesis, through its regulation of gene expression. Although widely studied, the molecular mechanisms of the pathway remain to be elucidated. *Dictyostelium* contains homologues of GSK-3, gskA, and β-catenin, Aardvark (Aar). The interactions of these genes shows both similarities and differences to the animal Wnt-stimulated pathways. By further investigating the function of these proteins in *Dictyostelium*, we hope to draw common conclusions concerning basic properties of Wnt-mediated signal transduction.

Mutants lacking *gskA* or *aar* genes have overlapping, but distinct phenotypes. We have identified a number of *Dictyostelium* targets of GSK-3, the sum of which could account for a complex phenotype. We have also shown that Aar plays two separate cellular roles. As seen in animals, it is required for cell adhesion through its structural role in the Adherens Junction. These junctions form late during *Dictyostelium* development and are required for the integrity of the nascent stalk tube. Consequently loss of *aar* results in formation of ectopic stalks during culmination and fruiting bodies that collapse due to weak stalks. There are however some phenotypes in common to *gskA* and *aar* mutants; namely, effects on cell motility during early development and reduced expression of the prespore gene *psA*. We are investigating how GskA and Aar interact to regulate these processes.

Sequence analysis indicates 10 Arm repeats in Aar, preceded by a cluster of potential phosphorylation sites in the N-terminus. There is also a potential binding site for the Adherens Junction-associated protein α-catenin, within the first Arm repeat. Deletion analysis demonstrates that the Arm repeats and both of these sites are required for Aar function. The equivalent N-terminal phosphorylation sites of β-catenin are substrates for GSK-3, however the biochemical analysis of Aar shows that these are phosphorylated by an alternative kinase. We have discovered, however, an alternative GskA phosphorylation site within the Arm region. Phosphorylation of this site may explain the observed interactions between GskA and Aar.

The role of cARs in the control of cell polarization and movement

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We are investigating the role of the various cAMP receptors in later development to better understand their individual role in the reception and transduction of cAMP signals. We have made double knockouts of cAR2 and cAR4 in a variety of genetic backgrounds. cAR2 knockouts can develop into slugs, with normal levels of prespore cells, but are strongly impaired in migration. These slugs go on to form somewhat small but normal proportioned fruiting bodies. Also cAR2/cAR4 double knockout strains continue to form slugs and fruiting bodies. cAR4 null mutants form slugs that migrate and show a photo-tactic response towards light, but their orientation is much less precise compared to that of their parent strains.

The finding that the cAR2/cAR4 null strains can form slugs implies that expression of cAR1 in prestalk cells is sufficient to drive prestalk cell sorting and slug formation, since cAR3 is only expressed in prespore cells. Investigation of CRAC localization and the organization of the actin cytoskeleton reveals that prestalk cells expressing only cAR1 are normally polarized. Inhibition of cAMP signaling through cAR1 by the use of the cAR1 specific antagonist IPA, results in an immediate loss of polarity of prestalk cells, as manifested by the random extension of pseudopodia. This shows that cAMP signaling is required for polarization of protrusive activity, and slug migration.

During our investigation of the organization of the actin cytoskeleton in slug cells we observed prestalk cell specific actin rich protrusive lamelopodia at the front of these cells. These structures are first observed in slugs and are not found in aggregation streams and mounds. They may be a manifestation of the increased chemotactic movement force that allows prestalk cells to sort out from prespore cells and take up their position in the front of the slug. The organization of these lamelopodia is dependent on cAMP signaling through cAR1 and we are now investigating the signaling pathways involved.

Mitochondrial transcription in *Dictyostelium discoideum*

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In previous work, we found the circular mitochondrial genome of *Dictyostelium discoideum* to be transcribed into eight major, polycistronic transcripts encoding polypeptides, ribosomal RNAs and interspersed transfer RNAs. Most of these polycistronic transcripts are subsequently processed into smaller mono-, di- or tricistronic RNAs. The 5' ends of these polycistronic transcripts have been determined by primer extension analysis and by aligning the potential transcription start sites, a consensus sequence representing a potential promoter region was identified. The identified promoter region did not reveal any significant sequence homologies to known promoter regions from other organisms (Barth *et al.*, 2001, *Curr Genet* **39**, 355 – 364). To determine if these sequences represent genuine transcription initiation sites or whether the 5' ends have been generated by post-transcriptional processing, Reverse Transcriptase - PCR was performed. The results obtained suggest that the eight polycistronic transcripts do not represent primary transcripts, but are processed from a single primary transcript. The promoter region for the single primary transcript is proposed to be located in a non-coding region between the *atp1* and *rnl* genes.

To verify and characterize the putative promoter region in DNaseI footprinting experiments, the nuclear gene of the *Dictyostelium* mitochondrial RNA polymerase has been cloned for expression of the gene in *Escherichia coli* cells and subsequent purification of the gene product. The *Dictyostelium* protein exhibits a high sequence homology to the RNA polymerases of T3/T7 bacteriophages and to mitochondrial RNA polymerases of *Chenopodium album, Arabidopsis thaliana* and of various yeasts.

In addition, the nuclear-encoded genes of two potential *Dictyostelium* mitochondrial transcription factors have been cloned for expression in *E. coli* cells. The gene products display high sequence homologies to known DNA binding proteins, such as high mobility group proteins (HMG), myb-related proteins and other proteins involved in gene expression. Mitochondrial targeting prediction software such as Helicalwheel plot, Predator, MitoProt and TargetP suggest the proteins to be localized in the mitochondria. The identified promoter region, the purified mitochondrial RNA polymerase and the mitochondrial transcription factors will be used to establish an *in vitro* transcription system to further characterize transcription of the *Dictyostelium* mitochondrial genome.

Mitochondrial FtsZs in *Dictyostelium* amoebae

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FtsZ is the principal component of the bacterial cell division apparatus, where it forms a ring at the mid-point of dividing cells. Though the protein is absent from fungi, animals and the mitochondria of higher-plants, the mitochondria of many protists, including *Dictyostelium*, have retained FtsZ. We have investigated the role that FtsZs might play in mitochondrial division in *Dictyostelium discoideum. Dictyostelium* amoebae have two nuclear-encoded FtsZs, FszA and FszB, targeted to the inside of mitochondria. Null mutations of *fszA* and/or *fszB* result in *Dictyostelium* mitochondria becoming greatly elongated — indicating that a decrease in mitochondrial division has occurred. In support of this, anti-FszA and FszA-GFP localisations show belts and/or puncta in mitochondria that may be recent or future sites of division. FszB-GFP, in contrast, locates to a sub-mitochondrial body that is usually located at one end of the organelle. We propose that the two FtsZs of *Dictyostelium* act in mitochondrial division, though a decreased level of division can still take place in their absence, probably due to the action of other mitochondrial division proteins. This is the first genetic evidence for FtsZ in mitochondrial division, and the first demonstration of two differentially localised FtsZs within the one organelle.

Mitochondrial disease and phototaxis in *Dictyostelium* slugs

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We previously reported that phototactic signal transduction in *Dictyostelium* is impaired by disruption of the mitochondrial large subunit rRNA gene in a subpopulation of the mitochondria (Biochem. Biophys. Res. Comm. 234, 39-43). In a subset of the disruptants growth was also impaired. In more recent work we cloned and characterized the single, nuclear chaperonin 60 gene (*hspA*) of *Dictyostelium* and found it to be expressed in vegetative cells, but down-regulated during early development. The protein however remains at the same levels throughout development. Unusually for a member of the Hsp60 gene family, *hspA* is not stress-inducible. The mitochondrial protein it encodes is required for proper folding of an unknown subset of imported and mitochondrially-encoded mitochondrial proteins. By expressing an *hspA* antisense RNA from different numbers of copies of an integrating plasmid construct, we were able to obtain gene dose-dependent antisense inhibition of *hspA* mRNA expression. This resulted in defects in phototaxis, thermotaxis, growth and morphogenesis and allowed, for the first time, *in vivo* genetic dose-response curves to be constructed relating the severity of an underlying genetic defect in the mitochondria to the phenotypes it produces. The pattern of phenotypes was similar to that observed previously with disruption of the mitochondrial *rnlA* gene. Phototaxis (and thermotaxis) were more sensitively impaired by *hspA* antisense inhibition than growth or morphogenesis. For example, phototaxis was dramatically impaired in all antisense transformants while growth in shaken culture in HL5 medium became exponentially slower only when plasmid copy numbers exceeded 60. Transformants carrying an equivalent plasmid construct with the same *hspA* fragment in the sense orientation were wild type with respect to all tested phenotypes even at high copy numbers. The results suggest either a specific role for the mitochondria in phototactic signal transduction (eg. in Ca^{2+} signalling) or that signal transduction is more sensitive than other cellular activities to mitochondrial insufficiency in energy provision to the cell. To clarify these issues we are now seeking to determine whether disruption of *any* mitochondrial gene causes similar patterns of phenotypes and whether cytosolic and/or mitochondrial Ca^{2+} signalling is impaired in mitochondrially diseased strains.

Poster presentations

Poster Abstract 1

A DNA microarray resource for the *Dictyostelium* community

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With the aim of ultimately producing a DNA microarray covering all of the genes in the *Dictyostelium* genome we have so far made arrays covering ~ 400 mostly previously-characterised genes, and subsequently ~ 3500 predicted genes from genome sequence data. Our method is to amplify a 200-400 bp segment of each gene by PCR, and covalently attach one strand to modified glass slides. A custom piece of software uses BLAST alignments to select primers that will amplify sequences relatively specific to each gene, in order to reduce cross-hybridisation of similar sequences. Test experiments using these arrays demonstrate their usefulness in generating biologically-relevant data. We are now working through the remaining genes in the genome assembly designing primers for an array covering >90% of genes, that will be ready towards the end of this year. This array will be made freely available to the *Dictyostelium* community, with priority given to UK-based researchers. Please visit our website (http://www.sanger.ac.uk/PostGenomics/PathogenArrays/Dicty/) for fuller, updated information.

Poster Abstract 2

Organisation of the cytoskeleton by actin-binding proteins and their regulators.

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I. To investigate the regulatory events upstream of profilin, we previously reported the identification of LmpA, a suppressor of the profilin minus phenotype. Here we report the discovery and initial characterisation of two new homologs of LmpA, both of them sharing several features with LmpA. However, they show intriguing differences in their developmental regulation, and exhibit different sorting signals of either the di-leucine or tyrosine type. Recently we generated a null mutant of *lmpB* which is currently being analysed.

II. Profilin III, a third homolog of profilin was identifed in the *Dictyostelium* genome databank. The profilin III transcript is not developmentally regulated. The recombinant protein cosediments with poly-L-proline, inhibits the actin polymerisation, and PIP_2 competes with G-actin binding. The low expression of profilin III mRNA suggests a distinct role of profilin III because a low protein concentration argues against an actin sequestering function.

III. We also cloned and characterised two members of the *Dictyostelium discoideum* STE20-like kinase family and named them DST-1 and DST-2 (***D****ictyostelium* **ST**E-20-like kinase). Based on homology and domain structure both belong to the GCK subfamily of STE20–like kinases. Both DST-1 and DST-2 phosphorylate the actin-binding protein severin, a gelsolin like F-actin fragmenting molecule. Phosphorylation experiments in the presence or absence of the catalytic subunit of cAMP-dependent protein kinase (PKA) from bovine heart showed that DST-2 was phosphorylated and activated by PKA *in vitro*.

IV. Rac GTPases are important regulators of the actin cytoskeleton and consequently influence the shape and movement of the cells. We identified a novel RacGEF that harbours armadillo repeats in the N-terminal region, a coiled-coil domain in the central part and a RacGEF domain at the C-terminus. The RacGEF null mutants show severe cytokinesis defects when grown in suspension and a highly dynamic and substantial disorganised actin cytoskeleton that forms membrane ruffles and numerous cell protrusions. Measurements of cell motility showed that the loss of RacGEF correlates with an approximately two fold increase in cell speed. Complementation of the mutant with the entire GFP-tagged RacGEF rescued the aforementioned phenotypes, demonstrating the specificity of this RacGEF in these processes.

Poster Abstract 3

Secretion of cysteine proteases during multicellular development of *Dictyostelium discoideum*

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The cysteine proteases of *Dictyostelium discoideum* represent an interesting area of study in cellular biology. At first glance the cysteine proteases appear to be enzymes that are simply involved in the recycling of endogenous proteins and the degradation of externally acquired proteins. While this might be true, in 1997 Cotter *et al* stressed that the enzymes might also have direct roles in development. In 1993 Bonner *et al* hypothesized that the cysteine proteases are creatively involved in slug migration. Through the use of pure gelatin media and infused gelatin agar, our lab has been able to show that *D. discoideum* secretes cysteine proteases during multicellular development. In 1995 Freeze *et al* emphasized that proteolytic enzyme activity decreased as *D. discoideum* proceeds through development. Through the use of zymography, our lab has been able to verify this hypothesis. In 2002 Brock *et al* reported that the *cprF* gene product is part of the counting factor complex, a secreted multi-meric complex that serves as a negative regulator during the aggregation process. Through the use of zymography, our lab has confirmed that *D. discoideum* counting factor mutant strains do not exhibit any defects in processing of the cysteine proteases, as the enzymatic bands migrate no differently than the bands of the wild-type strain *AX4*.

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Poster Abstract 4

The *cis*-acting domain for bi-directional transcription on the *fkbp2*/*dia1* promoter(s) that switch transcriptional directions during the growth/differentiation transition (GDT) of *Dictyostelium* cells

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The precise mechanism of growth/differentiation transition (GDT) is a crucial issue to be solved in cellular and developmental biology. We have already demonstrated that *dia1*, one of genes specifically expressed in response to differentiation of starving *Dictyostelium discoideum* cells from the PS-point (a growth/differentiation checkpoint in the cell cycle), negatively regulates the GDT by suppressing cAMP signaling cascades (Hirose et al., 2000). The promoter region of *dia1* has an intriguing feature with another gene (*fkbp2*) at the opposite end of *dia1* ORF. The fkbp2 gene is specifically expressed at the vegetative stage, and rapidly eliminated in response to deprivation of nutrients. Thus, the expressions of *dia1* and *fkbp2* were inversely regulated during the GDT. It indicates that the promoter region of *dia1* and *fkbp2* has a sort of control mechanisms to switch transcriptional directions. Analysis of the promoter region must offer us significant information about how a set of genes involved in the GDT are up-regulated or down-regulated. We applied DNA EMSA to study about *cis*-elements on the *fkbp2*/*dia1* promoter(s). The EMSA assays using the full promoter region have demonstrated the presence of different DNA-protein complexes between vegetatively growing cells and differentiating cells. Here, it is of interest to note that the *cis*-elements were a highly conserved repeat (AAACTGATTA GCTCGATCCCCT). We are now assaying β - galactosidase activity driven under a series of deleted promoters to specify essential parts of the *fkbp2*/*dia1* promoter(s).

Hirose et al. (2000) Development, 127:3263-270

Poster Abstract 5

Contributions of mitochondrial DNA to cell differentiation and pattern formation in *Dictyostelium* development

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Beyond our imagination, increasing evidence indicates that mitochondria have novel and crucial functions as the regulatory machinery of growth/differentiation transition, cell-type determination, cellular movement and pattern formation, Here we tried to prepare ρ^0-like cells with a reduced amount of mitochondrial DNA (mtDNA) from *Dictyostelium discoideum* Ax-2 cells, by means of exposure to about 30 μM ethidium bromide (EtBr) in axenic medium. As a result, we have succeeded in getting ρ^0-like cells with about one-fourth of mtDNA compared to non-EtBr-treated cells. The vegetatively growing ρ^0-like cells were found electron-microscopically to have enlarged nucleoli and greatly transformed mitochondria. Importantly, when they were starved, they exhibited a marked delay of differentiation and abnormal morphogenesis: somewhat irregular-shaped slugs were formed after a prolonged time (ca. 48 hrs) of incubation at 22°C, but failed to develop to fruiting bodies. Analyses using *ecmAO*-gal cells (prestalk cells; pstAO) and *D19*-gal cells (prespore cells; psp) have demonstrated that the clear anterior prestalk /posterior prespore pattern as observed in normal slug is considerably disordered in ρ^0-like cells, presumably due to incomplete sorting between the prestalk and prespore cells, and that the ratio (about 0.25 in normal slugs) of pstAO/psp cells is increased and reaches about 1.0. This indicates that prespore differentiation is fairly inhibited in the ρ^0-like cells, while that differentiation to prestalk cells is rather enhanced. Preferential inhibition of prespore differentiation in ρ^0-like cells was also confirmed by immunostaining of prespore-specific vacuole (PSV) using FITC-conjugated anti-spore IgG. Moreover, slug derived from ρ^0-like cells exhibited impaired phototaxis. Taken together these results strongly suggest the great importance of mtDNA in the regulatory mechanisms of cell differentiation and pattern formation.

Poster Abstract 6

Mitochondrial gene disruption and phototaxis in *Dictyostelium discoideum*

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It was previously reported that disruption of the mitochondrial large subunit rRNA gene (*rnl*) caused phototaxis deficiencies (Wilczynska *et al.*, 1997). *rnl* is required for efficient mitochondrial protein synthesis and thus expression of genes. This led to the hypothesis that disruption of genes essential for mitochondrial ATP synthesis (oxidative phosphorylation) leads to impairment of phototaxis in *Dictyostelium discoideum*.

To understand how mitochondrial dysfunction affects phototactic signal transduction, various mitochondrial genes in *Dictyostelium discoideum* have been targeted for disruption and the effects on phototaxis examined. The genes selected for disruption involved those that encode subunits of oxidative phosphorylation complexes including *atp1* (atp synthase subunit 1*)* (Hayes, 1999), *cob* (cytochrome b of cytochrome reductase*), nd5* (NADH-Q reductase subunit 1*), cox3* (cytochrome c oxidase*), atp6* (atp synthase subunit 6) and *nd2* (NADH-Q reductase subunit 2). Open reading frames termed ORF796 (Hayes, 1999) and ORF1740 were also targeted for disruption. The products of these genes have unknown functions (not thought to be linked to oxidative phosphorylaiton).

To target these genes for disruption, portions of the target genes were amplified by PCR and cloned into the *Dictyostelium* shuttle vector pDNeo2. The resulting constructs were transformed into strain AX2G and the resulting transformants tested for phototaxis defects. Control transformations with pDNeo2 lacking any insert were also performed.

It was found that disruption experiments targeted at *all* the genes resulted in significantly higher levels of phototaxis mutants than the frequency obtained from transformation of *Dictyostelium discoideum* with only pDNeo2. To verify that the targeted disruption was effective the putative mitochondrial disruptants need to be further tested for plasmid insertions into the mitochondrial genome using Southern hybridisation analysis with vector (pDNeo2) and mitochondrial DNA probes.

Plasmid insertions into mitochondrial DNA in the mutants could result in phototaxis deficiencies either because of ATP depletion or impairment of some other mitochondrial function. If ATP depletion is the indirect cause of phototaxis deficiencies it might be expected that phototaxis mutants would arise at high frequency only when essential mitochondrial genes are targeted. This however was not the case. Phototaxis mutants were found at elevated frequency in these experiments regardless of which mitochondrial gene was targeted, those required for ATP production (eg. *cob*) and those presumed not to be (eg. ORF796). This suggests that the phototaxis defects in mitochondrial mutants may derive from a direct involvement of mitochondria in signal transduction rather than from the indirect effects of ATP depletion.

Poster Abstract 7

Chaperonin 60 import into *Dictyostelium* mitochondria occurs cotranslationally

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In other organisms there is evidence that mitochondrial protein import occurs cotranslationally. We constructed plasmids encoding apoaequorin (a Ca^{2+}-sensitive luminescent protein) and GFP fused to variable N-terminal regions of chaperonin 60 (the first 23, 40, 80, 97 and 150 amino acids), as well as an N-terminal region (the first 82 amino acids) of the mitochondrial DNA topoisomerase II. The constructs were used to transform *D. discoideum* AX2 cells and transformants were tested using epifluorescence microscopy for colocalization of the GFP with the fluorescent mitochondrial stain MitoTracker Red. All except the first 23 amino acids of chaperonin 60 were found to successfully target GFP to the mitochondria. These results confirmed that *Dictyostelium* chaperonin 60 is a mitochondrial protein and showed that the first 23 amino acids were insufficient to constitute a competent mitochondrial targeting signal, even though sequence analysis using 'MitoProt II 1.0a4' predicted a mitochondrial peptidase cleavage site at amino acid 18.

Expression levels for our constructs were assessed by fluorescence microscopy in the case of GFP and by bioluminescence in the case of aequorin. In the latter case, transformants were lysed in a buffer containing excess Ca^{2+} in order to discharge total aequorin after its *in vivo* reconstitution with the cofactor coelenterazine *h.* The total emitted light was used as a measure of aequorin activity and compared with that of strain HPF275, an AX2 derivative expressing cytoplasmic aequorin (Nebl & Fisher, 1997). The expression of the mitochondrially targeted GFP and aequorin fusion proteins was much lower than the non-targeted (cytoplasmic) forms with or without the leading 23 amino acids from chaperonin 60. This suggests that translation is retarded by the process of mitochondrial protein import and provides the first evidence in *Dictyostelium* that mitochondrial import is cotranslational. Cells expressing mitochondrially targeted aequorin will help us determine the possible role(s) of mitochondrial calcium signalling in the physiology of *D. discoideum.*

Reference: Nebl & Fisher (1997), J. Cell Sci. 110, 2845-2853

Poster Abstract 8

A search for STATa target genes and analysis of a STATa activation domain by use of *in situ* hybridisation

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STATs (signal transducers and activators of transcription) are transcription factors, which lie at the end of cytokine and growth signal transduction pathways. STATs also play crucial roles in embryogenesis, including those pattern formation processes that require organised cell movement.

There are four STAT genes, *Dd-STATa~d*, in *Dictyostelium*. STATa is activated by cAMP and translocates into the nuclei of tip cells, a sub-set of the prestalk A cells. The STATa null mutant loses the tip cells, supposed to function as an "organiser" in the slug, and it fails to culminate. Therefore, genes regulated by STATa presumably have important functions for culmination. As STAT signalling networks are known to be evolutionally conserved, hunting such genes is of central importance. However, *cudA* and *ecmB* are the only target genes of STATa thus far known.

In this study, we searched for STATa target genes by of *in situ* hybridisation. cDNA clones from the Japanese cDNA project, whose gene expressions patterns are known to be prestalk-specific (Maeda et al., 2003), were chosen as probes and we compared their expression patterns in Ax2 and STATa-null strains. Among 54 cDNA clones analysed, we picked up 13 candidate STATa target genes. These showed reduced or no gene expression in the STATa null strain. STATa could serve as a direct activator of these genes, or STATa may activate their expression indirectly. Most of these genes were expressed in the cone shaped mass of prestalk AB cells in the wild-type strain. This cone is missing in the STATa-null strains. However, it is of course possible that the prestalk AB cells are simply absent from the STATa-null strain, rather than there is direct activation by STATa. We also found several PstA and PstAO-expressed genes whose expression was down-regulated in the null mutant. This runs contrary to the fact that the STATa-null strain expresses both the *ecmAO::lacZ* and *ecmA::lacZ* markers. To confirm the relationship between STATa and these new PstA and PstAO genes, promoter analyses and construction of knockout strains is underway.

We further investigated how STATa activates some of the potential 'target genes. Expression of mutated STATa proteins in a STATa null background revealed that a very small region, composed of 18 amino acids containing a part of a potential leucine zipper, is necessary for activation of prestalk AB-type genes. The region perfectly overlaps with sequences necessary for cAMP-induced tyrosine phosphorylation and nuclear translocation. This indicates the leucine zipper region has a very important role(s) for STATa functioning.

Poster Abstract 9

The first intron is necessary for the STATc dependent stress response of *rtoA*

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Hyper-osmotic stress activates Dd-STATc as well as DIF (differentiation inducing factor for stalk cells). It is known to elevate intracellular cGMP and cAMP levels and the membrane permeant analogue 8-bromo-cGMP rapidly activates Dd-STATc, while 8-bromo- cAMP is a much less effective inducer. Micro-array analysis identified two genes, *gapA* and *rtoA*, that are induced by hyper-osmotic stress. The induction of *gapA* and *rtoA* is entirely dependent upon Dd-STATc and both genes are rapidly inducible with 8-bromo-cGMP. Again, 8-bromo-cAMP is a much less potent inducer than 8-bromo-cGMP. From these data, we have recently proposed that Dd-STATc functions as a transcriptional activator in a novel cGMP-mediated stress response pathway. The analysis of the regulatory element of *rtoA* demonstrates that two parts are necessary for the stress response. One is within 208 bps upstream of the transcription start site and the other is a certain element in the first intron, which is the first example of a regulatory intron in *Dictyostelium*.

Poster Abstract 10

Functional analysis of transcription factor genes in *Dictyostelium*: expression analysis and generation of disruptants

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Expression profiling during *Dictyostelium* development by the microarray technology showed that hundreds of genes dynamically change their expression levels at developmentally remarkable stages such as aggregation, cell differentiation and fruiting body formation. Moreover, clustering analysis of the expression profile revealed existence of several large groups of co-expressed genes. These findings imply that regulatory mechanisms each of which controls the expression of co-expressed genes are integrated to a huge regulatory network in development. Therefore, to elucidate transcriptional regulatory mechanism of each co-expressed gene group should be important for understanding the overall regulatory network. For this purpose, we are investigating transcription factors found in *Dictyostelium* genome and cDNA database.

First, we identified candidates for transcription factor genes from the database by the following two ways. In one way, we selected protein sequences categorized as transcriptional control or transcription factor from *Saccharomyces cerevisiae* and *Arabidopsis thaliana* ORF database and TRANSFAC transcription factor database. Then the selected sequences were subjected to BLAST similarity search against *Dictyostelium* cDNA contig database and, thus, contigs being significantly similar ($e < 0.01$) to functional domains of the transcription factors were chosen. In another way, we obtained amino acid sequences of conserved domains among transcription factors by searching with the key words, transcription factor, DNA bind and DNA-bind, against the CDD (Conserved Domain Database and Search Service). The amino acid sequences were compared to *Dictyostelium* ORF sequences predicted by Dr. Loomis (http://dicty.sdsc.edu/annotationdicty.html) and again ORF sequences with significant similarity were selected. Finally, we identified 55 independent ORFs potentially encoding transcription factors.

Second, we investigated temporal and spatial expression pattern of the ORFs by real-time quantitative PCR analysis and in situ hybridization. Based on the expression pattern, the ORFs were classified into several groups.

Third, to investigate the function, we are now making disruption mutants of all the selected 55 genes and subsequently characterizing their phenotypes. The details of all those data will be presented.

Poster Abstract 11

Regulation and expression of Alkaline Phosphatase during *Dictyostelium* development

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It has been speculated that Alkaline Phosphatase (ALP) may play a vital role in the development of *Dictyostelium* because of its striking localization pattern. During the time-course in development ALP activity becomes localized to a narrow band at the interface of the prestalk and prespores zones. Previous experiments also suggested that ALP activity was developmentally regulated. Determination of the mechanisms that result in the cell-specific localization of this enzyme may provide invaluable information in understanding cell differentiation in *Dictyostelium*. We amplified 880 base pairs of the *alp* 5' flanking region from genomic DNA and fused it to a *LacZ* reporter gene. *In situ β-galactosidase* assays of *Dictyostelium* transformants revealed a localization pattern that correlated well with authentic ALP activity that utilized a BCIP/NBT reaction mixture. *ß-galactosidase* assays of transformants containing the full-length *alp* promoter fused with the lacZ gene showed a steady increase in activity from early in development until mid culmination stage. *Alp* expression appeared to increase 3 to 4 fold between vegetative and mid culmination stages but then decreased slightly in late culminants and mature fruiting body structures. Transcription start site mapping was accomplished by primer extension assays using labeled *alp* promoter-specific oligonucleotides. The resulting primer extension band indicated a single transcription start site that is 144 nucleotides upstream of the start codon. A potential TATA box domain was present beginning at -29 and ending at -24. 5' Promoter deletion analysis of the *alp* 5' flanking region indicated the presence of a novel *cis*-acting element between -787 and -517. Preliminary internal deletion analysis of this region suggested a transcription factor binding domain between -642 and -574. After deduction of the exact *cis*-acting regulatory sequences present in the 5' flanking region we will attempt to identify and purify the corresponding tr*ans*-acting factors.

Poster Abstract 12

Regulation of development by Ddp24 genes, a family of genes involved in COPI/II-coated vesicle trafficking

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The membrane-spanning protein p24 is a component of COPI- and COPII-coated vesicles involved in the vesicular transport system between the endoplasmic reticulum and the Golgi apparatus. p24 is considered to play a role for coat assembly. In addition, there are multiple p24 proteins in a organism and it has been suggested that each of them plays a role for cargo sorting. It implies that p24 proteins might commit higher biological process such as development by regulating the transport of certain cargo molecule. However, quite a few studies in this context have been done because many studies have used yeast and mammalian cultured cells which are difficult to investigate multicellular organization.

To investigate the role p24 proteins for development, we identified four p24 genes (*Ddp24A*, *B*, *C*, and *D*) in *Dictyostelium discoideum*. Their deduced amino acid sequences have significant structural similarity to p24 proteins in other organisms including *Polysphondylium*, another cellular slime mold. *Ddp24A*, *B* and *C* are developmentally regulated and are maximally expressed between aggregation and mound stages. On the other hand, the expression of *Ddp24D* was hardly detected during development.

We have successfully generated mutants lacking *Ddp24A* or *Ddp24C*. *Ddp24A* null cells showed a delay of development after forming mounds, although they aggregated in the same way as Ax2 cells. Under the submerged condition, *Ddp24A* mutant cells showed delayed aggregation. In addition, the mutant stalks were easy to bend so that the fruiting bodies fell easily. Microscopic observation of the mutant stalks revealed unusual arrangement of cells in stalk tube, which caused locally thin and thick regions in the stalks.

On the other hand, Ddp24C mutant cells showed slower growth both in HL-5 medium and on bacterial lawn. During development, the mutant cells formed small aggregates. Interestingly, Ddp24C mutant stalks gave the same phenotype as Ddp24A mutant with abnormal arrangement of stalk cells and stalks easy to bend and fall down.

As well as the study we reported previously (Kawabe *et al.*, *Gene* **239**, 75-79, 1999), this is one of the first reports showing that p24 proteins are involved in regulation of development.

Poster Abstract 13

A novel pathway of prestarvation response: involvement of a *Dictyostelium* homologue of TRAP1

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Dd-TRAP1 is a *Dictyostelium* homologue of TRAP-1, a human protein that binds to the type1 tumor necrosis factor (TNF) receptor. TRAP-1 has a putative mitochondrial localization sequence and shows significant homology to members of HSP90 family. We previously reported that Dd-TRAP1 was predominantly located in the cell membrane/cortex, co-localizing with F-actin during growth, and translocated to mitochondria coupling with differentiation (Morita et al., 2002).

Recently, the translocation of Dd-TRAP1 to mitochondria was found to occur at the mid-late exponential growth phase (>2-3×10^6 cells/ml). Such a movement of Dd-TRAP1 from the cell cortex to mitochondria was also induced by conditioned medium in which *Dictyostelium discoideum* Ax-2 cells had been grown up to the late exponential growth phase (8×10^6 cells/ml). Thus the conditioned medium has to contain a prestarvation factor-1 (PSF-1) with the activity of Dd-TRAP1 translocation. Preliminary experiments have demonstrated that the PSF-1 activity was heat-instable and resistant to pronase-E treatment. Prestarvation genes such as *discoidin I* and *car1* are known to be expressed during the growth phase, coupling with an increased number of cells in growth medium. The expressions of differentiation-associated genes like *dia1* and *dia3* were augmented in Dd-TRAP1-overexpressing cells (Dd-TRAP1^{OE} cells) as compared with parental Ax-2 cells, but the expression kinetics of *discoidin I* and *car1* in Dd-TRAP1^{OE} cells during the growth phase was almost the same as that in Ax-2 cells. This strongly suggests the existence of another novel pathway of prestarvation response in addition to a given pathway as realized by Discoidin I and CAR1 expressions. Therefore, it is of importance to identify PSF capable of making the Dd-TRAP1 protein moved to mitochondria for cellular differentiation. Chemical identification of molecule(s) interacting with Dd-TRAP1 is also under investigation.

Morita, T., Amagai, A. and Maeda, Y. (2002) Unique behavior of a *Dictyostelium* homologue of TRAP-1, coupling with differentiation of *D. discoideum* cells. Exp. Cell Res., 280, 45-54.

Poster Abstract 14

Dictyostelium stress responses and the YakA pathway

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Under nutrient depletion *Dictyostelium* cells arrest growth and undergo changes in gene expression, decreasing vegetative mRNAs and inducing the expression of *pkaC*. YakA is an effector of these changes, regulating the decrease of vegetative mRNA expression and the increase of PKA activity that will ultimately regulate expression of adenylyl cyclase, cAMP synthesis and the induction of development. During growth YakA controls the intervals between cell divisions. *yakA* null cells are hyper sensitive to nitrosoative/oxidative stress and a second-site mutation in *pkaC* suppresses this sensitivity. Likewise, a second-site mutation in *keaA*, supresses the death induced by SNP. High copy suppressors of the *yakA* related phenotypes revealed new targets of the YakA regulation. A role for *papA*, *gefA*, *mvpB* and *lapA* has been identified. RasC and a 14-3-3 protein have also been found to be targets of YakA and PKA-C in the regulation of *Dictyostelium* stress responses. The over-expression of a truncated gef construct and the PDGF-associated protein *papA* accelerates development but not in the *pkaC* null background, indicating that they act upstream of this kinase. All high-copy suppressors inhibit growth, even in the absence of *pkaC* indicating a role for these elements in the control of the cell cycle. Finally, treatment of cells with compounds that generate nitrosoative or oxidative stresses lead to alterations in transcript levels for all the above mentioned genes, in a YakA and PKA-C-dependent manner. The data seems to point YakA and PKA-C as key regulators of nutrient, nitrosoative and oxidative stresses.

Poster Abstract 15

FbiA, a potential target of ubiquitin-mediated degradation, regulates cell-type proportioning in *Dictyostelium discoideum*

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The FbiA protein of *Dictyostelium discoideum* was identified via yeast two-hybrid analysis in a search for proteins capable of interacting with the WD-40 repeat region of the F-box/WD-40 repeat-containing protein FbxA. Based on analogy to FbxA homologues in other systems, this FbiA-FbxA interaction suggests that FbiA is likely to be targeted for FbxA-dependent, ubiquitin-mediated degradation. The *fbiA*$^-$ null mutant develops more slowly than wild-type cells, but is able to undergo culmination. The resulting fruiting bodies, however, are unusually tall, with longer stalks and smaller sporeheads than wild-type culminants. Furthermore, transformation with cell-type specific *lacZ* reporter constructs reveals that the *fbiA*$^-$ null mutant produces structures with an unusually high prestalk:prespore cell ratio. Hence it appears that FbiA normally plays some role in either inhibiting the prestalk fate or promoting the prespore fate. This finding is consistent with a model in which the unusually low prestalk:prespore ratio of *fbxA*$^-$ mutants results from the accumulation of abnormally high levels of FbiA in the absence of FbxA-mediated ubiquitination and degradation. The original cDNA clone of *fbiA* lacks the gene's 5' end. Nonetheless, we have found that a myc-tagged version of this truncated FbiA protein (myc-FbiA*) shows a dose-dependent ability to complement both the slow developmental rate and increased stalk:spore ratio of the *fbiA*$^-$ null mutant. Interestingly, overexpression of myc-FbiA* in a wild-type strain background leads to a phenotype reminiscent of the *fbiA*$^-$ null mutant (increased stalk:spore ratio). Hence, it appears possible that myc-FbiA* has a dominant negative effect on FbiA function when native FbiA is present in cells, perhaps due to the formation of a non-functional mixed multimer. The C-terminal region of FbiA is homologous to proteins in humans, mice, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. pombe*, *S. cerevisiae*, *N. crassa*, and *P. falciparum*. The function of these FbiA homologues is, however, unknown. Hence, further characterization of FbiA's role in *Dictyostelium* development may shed light on the function of this evolutionarily conserved protein family.

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Poster Abstract 16

A novel growth factor (psi factor) regulates cell-type specific gene expressions in *Dictyostelium discoideum*

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Previously, we found a secreted factor which induces prespore cell differentiation and cell division of prespore cells in *Dictyostelium discoideum* (Oohata et al., 1997). The factor was termed ψ factor (prespore-inducing factor) and was elucidated to be a glycoprotein. The gene encoding ψ factor, *psiA*, was isolated based on the partial amino acid sequence of the purified factor (Kawata et al., submitted). *psiA* encodes a novel protein composed of 557 amino acids. Both *psiA* null and overexpression strains developed normally, however, conditioned media from these strains showed substantial differences in ψ activity compared with that of parental strain (Ax2).

In this study, we investigated the effects of ψ factor on expression of some cell-type specific genes by use of Northern hybridisation, β-galactosidase staining and *in situ* hybridisation in both *psiA* null and overexpression strains. Among them, expression of prespore-specific *rnrB* gene, encoding a small subunit of ribonucleotide reductase, was reduced dramatically in *psiA* overexpressing strain. In contrast, a remarkable difference in spatial pattern of gene expression was observed in *psiA* null strain: the *rnrB* gene expressed in prestalk (pstA) cells unlike in those of Ax2. Furthermore, although no spatial difference was observed as to prestalk specific genes such as *ecmB* and an *ecmB* homologue, timing of their expression shifted earlier in *psiA* null strain than that observed in Ax2 development and delayed in *psiA* overexpression strain. These findings suggest that ψ factor acts inhibitory on these genes in prestalk cells, particularly on *rnrB* gene in pstA cells. Tsang and co-workers analysed *rnrB* promoter and showed the GC-rich element (box A and B) may be involved in the inhibitory mechanism in *rnrB* gene expression in prestalk cells (Bonfils et al., 1999). It is interesting to understand how ψ factor acts through such an element of *rnrB* promoter.

On the other hand, we examined effects of ψ factor on stalk cell differentiation in the presence of DIF-1 under submerged culture. *psiA* null cells differentiated into stalk cells efficiently as well as Ax2 cells, however, stalk cell differentiation in *psiA* overexpression cells was inhibited markedly.

Taken together, we propose that ψ factor antagonises DIF-1 for prestalk/stalk cell differentiation in addition to inducing prespore cells.

Poster Abstract 17

Is the expression of the tRNA gene-targeted retrotransposon TRE-A regulated by RNAi?

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The tRNA gene-targeted retroelements (TREs) are a family of seven retrotransposons that integrate in close vicinity to tRNA genes. It has been known for some time that TRE5-A produces both plus strand ("sense") and minus strand ("antisense") transcripts. Plus strand transcripts encode proteins essential for retrotransposition of TRE5-A. Expression of TRE5-A from both strands is a naturally selected, endogenous system that may allow the study of RNAi-mediated effects in *D. discoideum*. An argument that steady-state levels of TRE5-A transcripts may be maintained at an intermediate level by RNAi-controlled pathways comes from the observation that cultivation of cells with antimycin A increases both plus strand and minus strand TRE5-A RNAs. Antimycin A is known to block the respiratory chain and may therefore deplete the cellular ATP pool (RNAi has been shown to depend on ATP). Work from W. Nellen's laboratory has demonstrated that in *D. discoideum* RNAi requires RNA-dependent RNA polymerases (Rrp's). RrpA null cells, which have no RNAi, overexpress both sense and antisense TRE5-A transcripts.

Maintainance of TRE5-A transcripts depends on a cellular protein, CbfA (C-module-binding factor; CMBF). Depletion of CbfA in mutant cells drastically reduces both plus and minus strand transcripts of TRE5-A. To investigate whether CbfA maintains TRE5-A transcripts by acting as a general inhibitor of RNAi, we developed an artificial RNAi test system that lacked any TRE5-A genomic sequences. We expressed the neomycin phosphotransferase gene (*neo*) under the control of a constitutive sense promoter (*act15*) and an inducible antisense promoter (*rnrB*). This artificial system exactly mimicked the natural expression profile of TRE5-A in that the leakiness of the *rnrB* promoter produced low levels of stable antisense RNA. Induction of the *rnrB* promoter with methansulfonic acid methyl ester (MSM) did not increase antisense *neo* RNA levels but instead resulted in simultaneous loss of both sense and antisense *neo* transcripts. Thus, low plus strand transcript (mRNA) levels can be maintained in *D. discoideum* cells even in the presence of antisense transcripts, but elevation of antisense RNAs above a critical threshold level seems to induce a strong RNAi response that rapidly eliminates all homologous transcripts. Importantly, MSM-induced destruction of *neo* transcripts was unchanged in a mutant underexpressing CbfA. This argues against a role of CbfA as a general regulator of antisense-mediated gene regulation (i.e. RNAi) and points to specific functions of CbfA in the regulation of TRE5-A expression. Since CbfA binds to the antisense promoter of TRE5-A *in vitro*, our current model is that CbfA may function as a repressor of the TRE5-A antisense promoter such that steady-state levels can be maintained below the threshold levels detected by the RNAi machinery. Preliminary studies show that the 25 kDa carboxy-terminal part of the 115 kDa CbfA protein is sufficient to re-establish wildtype TRE5-A transcription levels in CbfA-depleted mutants.

Poster Abstract 18

Fluorescent protein markers used in live cell imaging of dynamic processes in *Dictyostelium*

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The introduction of fluorescent proteins as molecular tags has opened a wide field of applications in order to monitor the locations, redistribution and movement of proteins in living cells [1]. GFP and its variants can be fused to many proteins without altering their intrinsic functions.

In order to study actin-myosin dynamics at the substrate-attached surface of migrating cells, we have combined TIRF microscopy with the expression of GFP or YFP fusion proteins as probes in live *Dictyostelium* cells. Both actin and myosin II form networks of motile filaments immediately beneath the cell surface. Two patterns of actin assembly can be distinguished: highly dynamic, loose meshworks and dense filament assemblies. The dense assemblies are sites of Arp2/3 complex recruitment, prominent at leading edges, but also transiently found at distal foci and propagating actin waves. Actin meshworks are found allover the substrate-attached surface and are characterized by a very high turnover rate.

We will provide examples showing fusions of GFP, YFP and the red variants HcRed and mRFP1either individually or in combination with each other and will also discuss some of the drawbacks using this technology.

[1] G. Gerisch and A. Mueller-Taubenberger. GFP-fusions as fluorescent reporters to study organelle and cytoskeleton dynamics in chemotaxis and phagocytosis. *Meth. Enzymol.* **361**, 320-337 (2003).

Poster Abstract 19

Hypertonic signal promotes stability of *Dictyostelium* spores

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Dictyostelium spores are highly specialized dormant cells. Spore maturation is initiated with rapid encapsulation during culmination, followed by further maturation processes that involve changes in the cytoskeleton (1, 2). Spore maturation is controlled by cAMP dependent protein kinase (PKA). Activation of PKA in *rdeC* (HTY506) cells, a putative null allele of *pkaR*, or K-P cells (3) which over expresses the catalytic subunit *pkaC*, leads to precocious differentiation of spores. We report here that hypertonic condition during spore maturation promotes stability of *rdeC* or K-P spores.

Under either high or low osmotic conditions, about 50% of *rdeC* cells differentiated into viable spores. However, spores formed under low osmotic condition became aberrant and a spore fraction decreased in a short period. In contrast, spores formed under high osmotic conditions remained viable at considerably high rate. Requirement for high osmolarity for spore stability was also observed in spore differentiation of *rdeC* and K-P cells under submerged conditions. Hypertonic signal is known to stabilize spores by inhibiting germination. However increasing osmolarity after spore encapsulation did not prevent the decrease of spore fraction, indicating that the hypertonic condition affected before encapsulation to form stable spores. These results suggested that high osmotic condition, in addition to PKA activation is required for formation of stable spores, although PKA activation is sufficient for induction of encapsulated spores that are capable to germinate.

1. Kishi et al. (1998) J Cell Sci. 111, 2923-2932.
2. Sameshima et al. (2001) J Struct Biol 136, 7-19.
3. kind gift from C. Anjard

Poster Abstract 20

The *loda* gene of *Dictyostelium* is required for adenylyl cyclase (ACA) stimulation during aggregation

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The *lodA* gene (loss of development) was identified as having amino acid sequence identity with the relA and spoT genes of bacteria. These genes produce proteins involved in (p)ppGpp production and activation of the stringent response in response to amino acid starvation. We were interested if a similar detection system for amino acid starvation functioned during the initiation of development for *Dictyostelium* cells. Indeed, *lodA* null cells are unable to develop. However, we have been unable to establish any (p)ppGpp synthetase or hydrolase activity for the LODA protein. There are technical reasons why these negative findings may not be informative, and whether LODA in involved in (p)ppGpp metabolism remains unknown. The null strain has been characterized and several deficiencies were found. When plated for development, the cells do not induce the pulse-induced genes, such as *carA*, *aca*, and others. Growth specific genes are not shut off as is normally the case. While *pkaC* also is not induced, *pufA* and *gdt1* seem to be regulated normally. Development of the *lodA* null strain is not rescued by comixing with wild type cells nor by ectopic expression of CAR1, ACA, or PKAC. However, mixing with wild type and ectopic expression of PKAC does rescue development. Taken together, the findings suggest that the LODA protein is required for stimulation of ACA during aggregation.

Poster Abstract 21

Characterisation of the flavoprotein subunit of succinate dehydrogenase (SdhA) in *Dictyostelium discoideum*

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Here we describe the cloning, sequencing and functional characterization of the *Dictyostelium discoideum* genes for the flavoprotein subunit (SdhA) of succinate dehydrogenase. Succinate dehydrogenase is an essential enzyme of the tricarboxylic acid (TCA) cycle that is responsible for the oxidation of succinate to fumarate. It is a heterotetrameric enzyme composed of four nuclear-encoded subunits, a flavoprotein subunit containing the active site of the enzyme, an iron sulfur protein and two hydrophobic membrane anchoring subunits. Together they form Complex II of the electron transport chain which is located in the inner mitochondrial membrane of eukaryotes.

Sequence comparison of the *Dictyostelium sdhA* gene with homologs from other organisms reveal that the gene sequence has been highly conserved throughout evolution. The *sdhA* gene contains no introns and encodes a protein of about 64 kDa that is targeted to the mitochondria. This localization was supported by the expression and observation of SdhA-GFP fusion protein in *Dictyostelium* cells. Transcription of the gene is down regulated during early *Dictyostelium* development in response to starvation, while the level of the flavoprotein subunit remained constant throughout the life cycle. In an attempt to understand how mitochondrial dysfunction affects phototactic signal transduction, targeted disruption, gene knockout, antisense inhibition and interference RNA (RNAi) experiments were performed using cloned gene sequences.

Consistent with the essential role of succinate dehydrogenase in cellular energetics, we were unable to isolate mutants in which the *sdhA* gene had been disrupted. In addition, the RNAi construct was lethal in cells at high copy numbers. To obtain transformants containing low copy numbers (<10) of the RNAi construct it was necessary to use it in cotransformations with the vector (pDNeo2) lacking the insert. Orientation in phototaxis as well as growth and morphogenesis was impaired in *sdhA* transformants containing low copy numbers of the RNAi-expressing plasmid in the genome. In contrast, transformants containing an antisense RNA-expressing plasmid did not show decreased levels of *sdhA* mRNA or protein, nor was any apparent effect on phototactic accuracy or growth observed. To investigate further the possible impact of decreased SdhA activity in *Dictyostelium* cells, an *sdhA* construct carrying a point mutation (His77Ser) at a conserved histidine residue was created and introduced into cells. This residue has been found in other organisms to be necessary for the covalent binding of FAD and for enzyme activity of Complex II. To assess the effect of the substitution in *Dictyostelium*, Northern and Western blot analysis and measurement of the energy status of resultant transformants will be conducted.

Poster Abstract 22

Identification and characterisation of a mitochondrial DNA polymerase in *Dictyostelium*

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Mitochondrial DNA polymerase gamma (mtDNA pol γ) is the sole enzyme found so far in a variety of organisms to be devoted to mitochondrial DNA (mtDNA) replication. Database searches of the *Dictyostelium discoideum* genome database have suggested that a mtDNA pol γ-like enzyme is not involved in *D. discoideum* mtDNA replication. The results obtained from the searches indicated that there are no homologous mtDNA pol γ sequences present in the *D. discoideum* genome. This also seems to be the case in plants, where a mtDNA pol γ-like enzyme has not been discovered yet. To date, the enzyme that mediates the replication of the *D. discoideum* mtDNA has not been identified.

In order to identify the gene of a *D. discoideum* mitochondrial DNA polymerase (mtDNA pol), searches of the *D. discoideum* genome database were performed based on sequence homology to known *Escherichia coli* DNA polymerase (DNA pol) sequences. One potential *D. discoideum* mtDNA pol gene sequence was edited to obtain an open reading frame and translated into a protein sequence that was used for BLAST searches and sequence alignments. The alignments demonstrated that the potential *D. discoideum* mtDNA pol has very low similarity to known mtDNA pol γ sequences from different organisms, but it shows a high identity to DNA polymerase A sequences. Using the software Predator, MitoProt and HelicalWheel, it was demonstrated that the potential *D. discoideum* mtDNA pol encodes a protein that is highly likely to be translocated into the mitochondria. Various molecular techniques were used to isolate, characterise and clone the gene for the potential *D. discoideum* mtDNA pol. These included Southern and Northern hybridisation analysis to demonstrate the presence of the gene in the *D. discoideum* genome and to assess its transcription. Reverse Transcription Polymerase Chain Reaction has been performed to obtain a cDNA copy of the gene, demonstrating the presence of an intron in the 5' portion of the gene. To confirm the subcellular localisation of the potential *D. discoideum* mtDNA pol, a 5' portion of the gene has been cloned into the *D. discoideum* expression vector pA15GFP to create a fusion protein that allows targeting of the green fluorescent protein (GFP) to the mitochondria. With the identification and characterisation of *D. discoideum* mitochondrial DNA polymerases, more can be learnt about the molecular processes involved in *D. discoideum* mtDNA replication and repair.

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