International Dictyostelium Conference

September 04 -09, 2007

Rotenburg an der Fulda, Germany

Organizers: Wolfgang Nellen and Markus Maniak

Venue:

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Timetable

Time	Tue, 04	Wed, 05	Thu, 06	Fri, 07	Sat, 08	Sun, 09
07:30		BREAKFAST				
08:30	Opt. visit to Fulda	Growth Phase and Pathogens	Cell Motility	Myosins and Cytokinesis	Disease Proteins and Trafficking	Departure
12:30	LUNCH					
14:30	Arrival and Registration	Differentiation and Patterning	Chemotactic Signalling	Leisure Event	Features of the Genome	Departure
18:30	DINNER				BANQUET	
20:30	Get together	Posters	Posters	Free Time	Disco	
22:30						

Session 1: Growth, Phagocytosis and Pathogens (Chair: Gomer, R./Soldati, T.)

8:30 – 10:10

Kimmel, A.R.: **The Actions of TOR Complexes 1 and 2 in Control of Growth and Development**

Bakthavatsalam, D.: **An autocrine negative feedback loop that regulates cell proliferation requires G-protein-associated components**

Clarke, M.: **Illuminating the Phagocytic Pathway of Dictyostelium through High Resolution Light Microscopy of Living Cells.**

Leippe, M.: **"What kills the prey": Molecular characterization of antimicrobial polypeptides of Dictyostelium discoideum**

COFFEE BREAK

10:50 - 12:30

Bozzaro, S.: **The Nramp (Natural Resistance Associated Membrane Protein) family in Dictyostelium**

Hagedorn, M.: **Identification of host-factors that affect cellular immunity during mycobacteria infection**

Pukatzki, S.: **The bacterial type VI secretion system targets the host cytoskeleton**

Maniak, M.: **Competition of Targeting Signals in vivo**

LUNCH BREAK

Session 2: Differentiation and Patterning (Chair: Kay, R./Mutzel, R.)

14:30 - 16:10

Gross, J.: **Cell patterning in D. discoideum.**

Keller, T.: **Identification of a DIF Regulated GATA Transcription Factor Reveals Novel Roles For DIF Signaling in Dictyostelium**

Suarez, T.: **PadA, an NmrA-like protein involved in *Dictyostelium* development and cell differentiation.**

Blagg, S.: **Breaking down the DIF pathway – a novel Ubiquitin Ligase involved in Dictyostelium development.**

COFFEE BREAK

16:50 – 18:30

Yamada, Y.:**CudA defines a new family of eukaryotic transcription factors and is important for prespore and tip gene expression**

Sastre, L.: **The transcription factor SRFB is required for regulation of several actin-cytoskeleton related functions and multi-cellular development**

Parkinson, K.: **Cell type specific regulation of Rap activity is required for morphogenesis and patterning in Dictyostelium**

Marwan, W.: **The Sporulation Control Network of *Physarum polycephalum* Probed by Plasmodial Fusion of Mutants**

DINNER

20:30 – 22:30

POSTERS in the Panoramastudio

Session 3: Cell Motility (Chair: Steimle, P./Schleicher, M.)

8:30 – 10:10

Cox, E.: **A search strategy for eukaryotic cells**

Wessels, D.: **How A Cell Crawls: Pseudopod Extension and Cellular Translocation Are Not Driven by Myosin II-Dependent Posterior Cell Contraction**

Gerisch,G.: **Dynamic actin patterns**

Beta, C.: **Actin dynamics in SCAR-deficient cells**

COFFEE BREAK

10:50 - 12:30

Heinrich, D.: **Actin–microtubule crosstalk in the viscoplastic intracellular space and sub-second actin dynamics during cell spreading**

Schulz, I.: .**Identification and characterization of CP39, a novel centrosomal core protein identified by proteomic screening of the Dictyostelium centrosome**

Hickinson, D.N.: **Alcohol leaves Dicty in a spin: PLD and butan-1-ol in motility and endocytosis.**

Ryves, J.: **Use of penetratin-based peptides to study cell motility and chemotaxis.**

LUNCH BREAK

Session 4: Chemotactic Signalling (Chair: Weeks, G./Williams, J.)

14:30 - 16:10

Bodenschatz, E.: **PH-domain translocation in well controlled chematractant gradients.**

Rappel, W.J.: **Noise and directional sensing: a numerical modeling study**

Sawai, S.: **Intrinsic oscillations of cAMP in Dictyostelium cells**

Kriebel, P.: **Vesicular trafficking is required for chemoattractant delivery at the trailing edge of chemotaxing Dictyostelium cells.**

COFFEE BREAK

16:50 – 18:30

de Keijzer, S.: **Single-molecule imaging of sub-steps in the early events of the chemotaxis signaling cascade.**

Kortholt, A.: **PLC regulation of PI(3,4,5)P3-mediated chemotaxis**

Firtel, R.: **Regulation of cell polarization and chemotaxis by Ras and Rap1**

Williams, R.S.B.: **Understanding phospholipase A2 signalling in Dictyostelium as a target for fatty acids**

DINNER

20:30 – 22:30

POSTERS in the Panoramastudio

Fri, Sept. 7th

Session 5: Myosins and Cytokinesis (Chair: Chisholm, R./Noegel, A.)

8:30 – 10:10

Crawley, S.: **Characterization of the Light Chain Composition of the Dictyostelium Myosin I Family**

Steimle, P.: **The Cellular Activities of Myosin Heavy Chain Kinases B and C Are Specified by Homologous WD-Repeat Domains**

Coté, G.P.: **Structural Characterization of the Atypical Kinase Catalytic Domain of the Dictyostelium Myosin II Heavy Chain Kinase**

Mondal, S.: **Linking Ras to myosin function: RasGEF Q, a Dictyostelium exchange factor for RasB, affects myosin II functions**

COFFEE BREAK

10:50 - 12:30

Insall, R.H.: **Abi (abelson interactor) Mutants Reveal Specific Roles for the SCAR/WAVE Complex in Cytokinesis**

Müller-Taubenberger, A.: **Exploring the role of NDR kinases in cytokinesis of Dictyostelium discoideum**

Rohlfs, M.: **Ste20-like kinases in Dictyostelium discoideum - Implications for cytokinesis, phagocytosis and development**

Franke, J.: **Announcement: The Dicty Stock Center**

Maniak, M.: **Announcement: The Leisure Activities**

LUNCH BAGS

Leisure Activity: Documenta-Visit or Canoe Trip

DINNER

Session 6: Disease-Related Proteins and Trafficking (Chair: Rivero, F./Brazill, D.)

8:30 – 10:10

Schaap, P.: **Roots of cAMP signalling in the Dictyostelids**

Escalante, R.: **Functional genomics in Dictyostelium: a homologue of the accute pancreatitis-related gene VMP1 is involved in multiple aspects of Dictyostelium cell biology**

Thompson, C.R.L.: **An intracellular P2X receptor required for osmoregulation in Dictyostelium**

Siu, C.-H.: **Involvement of ATP, Calmodulin and V-H(+)-ATPase in the Transport of the Cell Adhesion Molecule DdCAD-1 by Contractile Vacuoles**

COFFEE BREAK

10:50 - 12:30

Sriskanthadevan, S.: **The C-terminal Domain of the Soluble Cell Adhesion Molecule DdCAD-1 is Involved in the Non-classical Transport Pathway via Contractile Vacuoles**

Fisher, P.R.: **AMPK-mediated mitochondrial disease in Dictyostelium causes increased susceptibility to Legionella infection.**

Hodges, A.: **Exploring how Dictyostelium can tolerate a large number of endogenous proteins containing long uninterrupted polyQ stretches that are in the disease-causing range in humans**

Golstein, P.: **Autophagic and necrotic cell death in Dictyostelium**

LUNCH BREAK

Session 7: Features of the Genome (Chair: Winckler, T./Eichinger, L.)

14:30 - 16:10

Urushihara, H.: **Comparative genomic analysis of Acytostelium subglobosum, a group 2 species without stalk-cell differentiation**

Heidel, A.: **Mitochondrial Genome Evolution in the Social Amoebae**

Pears C.J.: **Repair of DNA Double Strand Breaks by Non Homologous End Joining in Dictyostelium**

Dittmann, R.: **Telomere structure in social amoebas**

COFFEE BREAK

16:50 – 18:30

Söderbom, F.: **The small RNA repertoire of Dictyostelium: microRNA candidates, small antisense RNAs and multiple classes of repeat-associated RNAs**

Nellen, W.: **Epigenetics**

Muramoto, T.: **Visualization of nuclear functions in living cells**

Announcements: **Dicty 2008 and Dicty 2009**

BANQUET

Abstracts for Lecture Sessions

Session 1: Growth, Phagocytosis and Pathogens

Session 2: Differentiation and Patterning

Session 3: Cell Motility

Session 4: Chemotactic Signalling

Session 5: Myosins and Cytokinesis

Session 6: Disease-Related Proteins and Trafficking

Session 7: Features of the Genome

The Actions of TOR Complexes 1 and 2 in Control of Growth and Development

Liao, X.-H., Huang, X., Khurana, T., Rosel, D., and Kimmel, A.R.

LCDB/NIDDK, National Institutes of Health, Bethesda, MD 20892, USA

The TOR (Target of Rapamycin) pathway plays a central role in sensing and responding to nutrients, stress, and energy states. TOR complex 1 (TORC1), comprised of TOR, Raptor and Lst8, is sensitive to Rapamycin and regulates protein synthesis and cell growth; TOR complex 2 (TORC2), comprised of TOR, Pia, RIP3, and Lst8, is considered insensitive to the direct actions of Rapamycin and regulates the actin cytoskeleton. TORC1 is subject to positive regulation by activation of the small G protein Rheb, and negative regulation via TSC2, a Rheb GAP. However, a connection of the TSC2/Rheb pathway with TORC2 has not been clear. Based upon several growth- and developmental-specific read-outs that are dependent upon either TORC1 or TORC2, we suggest that both TORC2 and TORC1 are subject to positive, co-regulation by the Rheb pathway. Further, while Rapamycin is suggested to be a specific inhibitor of TORC1, our data indicate that Rapamycin is also able to inhibit TORC2-dependent events, albeit indirectly, and by a mechanism that requires a productive TOR/Raptor complex 1. TORC2 and TORC1 activities may be in functional equilibrium and the integrated and balanced regulations of TORC2 and TORC1 may be critical in Dictyostelium to coordinate and optimize growth with energy needs and to organize development. To dissect further the functions of TORC1 and TORC2 and their mechanistic relationships, we have created "knock-in" Dictyostelium cell lines that carry ectopic TAP tags of TORC1 components TOR, Raptor, and Lst8, of TORC2 components TOR, Pia, RIP3, and Lst8, and of the upstream regulators TSC2 and Rheb.. We are using these cells to study the interaction dynamics of TOR complexes 1 and 2 under varying culture conditions by tandem affinity purification coupled to mass spectrometric analyses.

An autocrine negative feedback loop that regulates cell proliferation requires G-protein-associated components

D. Bakthavatsalam, J.M. Choe, and R.H. Gomer

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Despite evidence for their existence, little is known about secreted factors and their associated signal transduction pathways that act in negative feedback loops to repress the proliferation of the secreting cells. We previously found that growing Dictyostelium cells secrete a protein called AprA that slows the proliferation of cells (Brock and Gomer, 2005). To elucidate the function of AprA, we expressed and purified recombinant AprA (rAprA). When added to growing cells, rAprA slows proliferation with an EC50 of ~0.1µg/ml. At concentrations above 1µg/ml, rAprA slows proliferation by 20-25%. Even at very high concentrations, rAprA does not completely inhibit proliferation, suggesting that AprA functions to limit but not completely inhibit proliferation. Using rAprA as a standard, we found that growing cells accumulate ~0.025 µg/ml AprA at low log phase, with the concentration increasing to ~0.6 µg/ml at stationary phase, indicating that growing cells accumulate a physiologically effective concentration of AprA. To elucidate the AprA signal transduction pathway, we examined the effect of rAprA on a variety of Dictyostelium mutants. CrlA has similarity to 7-transmembrane receptors, and crlA^{-} cells proliferate faster than wild-type (Raisley et al., 2004). Cells lacking CrlA, Gβ, Gα8 or Gα9 were essentially insensitive to rAprA, indicating that these proteins are necessary for AprA signal transduction. Cells lacking Gα1, Gα3, Gα4, Gα5 or Gα7 were sensitive to rAprA. Together, the data suggest that AprA requires some G protein-associated components to function in an autocrine negative feedback loop to repress proliferation.

Brock, D.A., and Gomer, R.H. (2005). A secreted factor represses cell proliferation in Dictyostelium. Development 132, 4553-4562. Raisley, B., Zhang, M., Hereld, D., and Hadwiger, J.A. (2004). A cAMP receptor-like G protein-coupled receptor with roles in growth regulation and development. Developmental biology 265, 433-445.

Illuminating the Phagocytic Pathway of Dictyostelium through High Resolution Light Microscopy of Living Cells.

Margaret Clarke*, Ulrike Engel+, and Günther Gerisch$

*Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; +University of Heidelberg, Nikon Imaging Center, Heidelberg, Germany; $Max-Planck-Institut, Martinsried, Germany

New insights into phagocytic behavior and the relationship of events in phagosome maturation have been gained through observation of living cells expressing fluorescently-tagged proteins. Probes included mRFP-LimE, which labels actin filaments, VatM-GFP (a subunit of the V-ATPase), mRFP-a-tubulin, and GFP-MyoB. The prey was a yeast mutant defective in cytokinesis, so many of the yeast had buds. Although budded yeast can be ingested by Dictyostelium cells, we frequently observed abortive attempts at phagocytosis of such yeast. When phagocytosis was initiated at one pole of a budded yeast, the forming phagocytic cup closely followed the contour of the yeast and attempted to seal at the narrow neck that linked the mother to the bud. Such attempts were characterized by high levels of actin accumulation. One example occurred in a cell expressing mRFP-LimE and GFP-MyoB; both proteins grew extremely bright at the mother-bud neck. The bright signals persisted for more than three minutes, whereupon the mRFP-LimE and GFP-MyoB disappeared, and the cell abandoned the yeast. It was not uncommon for Dictyostelium cells to succeed in severing the mother-bud link and sealing the phagosome. An example was recorded for a cell expressing mRFP-a-tubulin and VatM-GFP; microtubules and VatM-GFP-positive vesicles interacted closely with the nascent phagosome during the extended period of uptake. A yeast recently exocytosed (or abandoned) was often phagocytosed moments later by another cell or even the same cell. A new phagosome became surrounded promptly by VatM-GFP-positive vesicles, which delivered VatM-GFP to the phagosome membrane. Prior to exocytosis, a period of very active vesiculation occurred, which removed VatM-GFP from the phagosome membrane. Not long afterwards, flickers of mRFP-LimE appeared intermittently about the phagosome; often exocytosis followed. In cells expressing GFP-MyoB and mRFP-LimE, both proteins were enriched about the phagosome during exocytosis.

"What kills the prey": Molecular characterization of antimicrobial polypeptides of Dictyostelium discoideum

Herbst, R.§ and Leippe, M.+

§Ruhr University of Bochum, Zoology/Parasitology, Universitätsstr. 150, 44780 Bochum +University of Kiel, Zoophysiology, Zoological Institute, Olshausenstr. 40, 24098 Kiel

Amoeboid protozoa are widespread in nature and highly diverse, they are often free-living in soil and water and use other microorganisms as a food source. Its important role as a general model in cell biology aside, Dictyostelium discoideum is a free-living amoeba and as such an interesting model for amoebae that harbour human-pathogenic bacteria and/or are human pathogens by themselves. Although D. discoideum feeds on bacteria, the knowledge about the antibacterial armamentarium that may kill and degrade the engulfed prey is scarce. Recently, we isolated and characterized the major Dictyostelium lysozyme (ALY), the founding member of a novel lysozyme class (1). Here, we report on a multifarious gene family that potentially codes for homologues of amoebapores and naegleriapores, the pore-forming peptides of Entamoeba histolytica and Naegleria fowleri, we characterized previously in detail (2, 3). The dictyostelian amoebapore-like (DAL) peptides appear to be often contained in larger prepromultipeptide precursors, which simultaneously may give rise to a variety of mature peptides upon proteolytic processing. Selected DAL peptides were recombinantly expressed and found to be potently active against bacteria. We assume that the function of these peptides is the rapid killing of bacteria inside the phagolysosome before degradation by lysozymes takes place.

1) Müller, I., Subert, N., Otto, H., Herbst, R., Rühling, H., Maniak, M. and Leippe, M. (2005) A Dictyostelium mutant with reduced lysozyme levels compensates by increased phagocytic activity. J. Biol. Chem. 280, 10435-10443. 2) Herbst, R., Ott, C., Jacobs, T., Marti, T., Marciano-Cabral, F. and Leippe, M. (2002) Pore-forming polypeptides of the pathogenic protozoon Naegleria fowleri. J. Biol. Chem. 277, 22353-22360. 3) Leippe, M., Bruhn, H., Hecht, O. and Grötzinger, J. (2005) Ancient weapons: the three-dimensional structure of amoebapore A. Trends Parasitol. 21, 5-7.

The Nramp (Natural Resistance Associated Membrane Protein) family in Dictyostelium

Barbara Peracino, Alessandra Balest and Salvatore Bozzaro

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Nramp's are membrane transporters for divalent metals (Fe, Mn, Zn, Cd). They are widely distributed in pro- and eukaryotes. Two genes, Nramp1 and Nramp2, exist in mammals. The Nramp1 protein is expressed exclusively in endo-lysosomal vesicles of phagocytes, whereas Nramp2 is expressed on the surface of several tissues, in particular brain, intestinal brush border, kidney and reticulocites. Nramp2 is the major iron transporter, with the transferrin receptor, and mutations have been correlated with congenital microcytic anemia. Nramp1 mutations have, instead, been correlated with increased susceptibility to tuberculosis, leprosy, legionnaire' disease, salmonellosis and leishmaniosis. Dictyostelium cells harbour a single copy of both genes. Nramp1 is expressed during growth and down-regulated during development, whereas Nramp2 is expressed continuously. We have generated single mutants for Nramp1 or Nramp2 and a double mutant is being generated. Nramp1 gene disruption results in increased susceptibility to infection by Mycobacteria or Legionella, whereas its overexpression protects against Legionella infection. By studying iron transport in purified phagosomes, we have shown that Nramp1 acts by depleting iron from the phagosomes, if an ATP source is provided (Peracino et al., Traffic, 7,22-38, 2006). The phenotype of the Nramp2 knockout mutant is currently under investigation. The cellular localization of GFP-fused Nramp1 or Nramp2 is strikingly different. Nramp1 decorates endo-lysosomal vesicles, and is recruited to macropinosomes and phagosomes shortly after their formation. By contrast, Nramp2 is enriched in the membrane of the contractile vacuole network and in the plasma membrane. Nramp2 is not recruited in, or is rapidly recycled from, the macropinosomal membrane, and does not decorate phagosomes. We hypothesize that a network of Nramp1 and Nramp2 regulates iron and other divalent cation homeostasis in the cell.

Identification of host-factors that affect cellular immunity during mycobacteria infection

Hagedorn, M., Gopaldass, N., Sattler, N. and Soldati, T.

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Tuberculosis, caused by the pathogen M. tuberculosis is a leading cause of death in the world. The pathogen Mycobacterium marinum, a close cousin to M. tuberculosis, provides a useful model to study the pathogenic mechanisms of tuberculosis in genetically tractable model organisms, such as Drosophila and zebrafish. Using the amoeba Dictyostelium discoideum as a host, we identified and characterized mycobacterial and host factors, which influence the balance of complex and dynamic host-pathogen interactions. Dictyostelium is easily infected with M. marinum resulting in a persistent infection. We find that two host proteins – the flotillin homologue vacuolin, which appears to be raft-associated also in Dictyostelium, and p80, a predicted copper transporter – accumulate at the vacuole during pathogen replication until it finally ruptures and the bacteria are released into the cytosol. Flotillin-1 accumulation at the replication niche of M. marinum and its rupture were also observed in human peripheral blood monocytes and microglial cells. Three vacuolin isoforms are encoded in the Dictyostelium genome, two of which (VacA and VacB) are expressed in vegetative cells. By infecting various Dictyostelium mutants, we show that the absence of vacuolin B renders the host more immune to M. marinum. The small GTPase RacH has been shown to regulate vacuolin distribution in the endocytic pathway and its absence markedly decreased acidification of early phagosomes. Lack of RacH renders the host more susceptible to M. marinum proliferation but inhibits its cell-to-cell spreading. Overall, using Dictyostelium as a host model system opens the door to a vast variety of microscopical, genetic and biochemical experimental approaches in order to further elucidate host-pathogen interactions, and specifically the mechanisms of mycobacteria pathogenesis.

The bacterial type VI secretion system targets the host cytoskeleton

Ma, Amy T.*, Mekalanos, John J.*, and Pukatzki, Stefan$

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Microbial pathogens have developed sophisticated strategies to infect host cells. Some interactions between host and pathogen have evolved in environmental reservoirs where bacteria must contend with eukaryotic predators such as amoebae and fungi. When in contact with humans, pathogens apply some of the strategies that they acquired in the environment to exploit the human host. With this concept in mind, we have pioneered the use of the environmental amoeba Dictyostelium discoideum as a genetically accessible host model to identify such basic virulence mechanisms. This approach has led to the discovery of the type VI protein secretion pathway in Vibrio cholerae, which confers cytotoxicity toward amoebae and mammalian macrophages. In attenuated V. cholerae mutants, the majority of transposon insertions lay within a 14.2-kb region, containing 18 highly conserved genes. Mutations in these genes abolish the ability of V. cholerae to interfere with the actin cytoskeleton of infected host cells. One of the type VI genes, VgrG-1, contains a putative actin-crosslinking domain and is responsible for the observed rounding of infected host cells. It turns out that VgrG-1 not only forms a complex with other VgrG's to minimally secrete hemolysin co-regulated protein (Hcp), but also crosslinks actin monomers to injure infected host cells – a process that requires direct contact. We propose that VgrG1 is a modulator of the type VI system by adding unique features to the system, namely actin crosslinking. As a large number of Gram-negative bacterial pathogens carry type VI genes to confer virulence in animals and cultured macrophages, we propose that the type VI secretion system serves an important conserved function in microbial pathogenesis. The components of the type VI system, therefore, represent exciting new targets for antimicrobial drug-based therapies.

Competition of Targeting Signals in vivo

Christian Schmauch and Markus Maniak

Zellbiologie, Universität Kassel, Germany

After their translation and folding in the cytoplasm, proteins end up in various cellular destinations. They may be imported into the nucleus or the peroxisome, associate with a membrane, or rather become part of large, highly localized cytoplasmic structures such as the cytoskeleton. The protein's localisation is thought to be primarily governed by the binding-strength to its immediate target, such as an import receptor for an organelle or the major component of the cytoskeleton e.g. actin. Many of such binary interactions have been quantified in vitro.
To qualitatively assess the localisation properties of proteins in vivo, we have experimentally provided a set of actin-binding proteins with competing targeting information and expressed them at various concentrations to analyse the strength of the signal that governs their subcellular localisation. Our microscopic observations indicate that the targeting preference of most cytoskeletal proteins is dominated by organellar sorting signals. Among these, the nuclear localisation signal of SV40 is strongest, followed by the oligomerized PHB domain that targets vacuolin, a flotillin/reggie-related protein to the endosomal surface, and finally the tripeptide SKL mediating transport into the lumen of the peroxisome. Although all of these signals override the preference for actin-binding of most tested cytoskeletal proteins, one actin-associated protein, coronin, can only be misled by the nuclear localisation signal.
Because the targeting behaviour of this model set of hybrid proteins in living Dictyostelium amoebae correlates surprisingly well with the affinities of their constituent signals derived from in vitro experiments conducted in various other organisms, we consider this approach as a valid in vivo support to complement past and future biochemical studies.

Cell patterning in D. discoideum.

Gross,J.

Biochemistry, Oxford.

Aggregates of starved Dictyostelium amoebae are divided transversely into an anterior zone of cells containing highly acidic intracellular compartments and a posterior zone of cells with less acidic compartments. I will outline a model according to which the initial differential cell sorting and later patterning of aggregates is influenced by the activity of vesicular Ca2+/H+ exchangers that convert the difference in acidity between the acidic compartments of anterior and posterior cells into a difference in the amount of sequestered Ca2+ and subsequently in the height of the Ca2+ transients generated in response to successive cAMP waves. The difference in the amplitudes of the Ca2+ transients in turn controls the amplitude of the relayed cAMP signals, and a combination of intracellular Ca2+ and cAMP transients affects the choice of pathway of differentiation. The predicted Ca2+/H+ exchangers have been demonstrated experimentally. The difference in vesicle acidity and hence in Ca2+/H+ exchanger activity is initially controlled by the combinations of pumps and fluxes associated with different stages of the cell cycle, and the “correct” proportion of anterior to posterior cells in aggregates is established subsequently by a reaction diffusion system based upon a slowly diffusing weak acid and a fast diffusing weak base (ammonia) both highest in the anterior zones of aggregates. Further aspects of this model, as well as supporting evidence, will be discussed.

Identification of a DIF Regulated GATA Transcription Factor Reveals Novel Roles For DIF Signaling in Dictyostelium

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DIF-1 is a key signaling molecule that regulates pstO cell differentiation. To date only the transcription factors dimA, dimB, STATc and mybE are known regulators of the DIF signal transduction pathway. To isolate a wider range of DIF-1 insensitive mutants (dims), two modifications were made to a previous REMI mutant screen. Firstly, the cAMP removal assay was used to enrich for DIF-resistant mutants that fail to differentiate as dead stalk cells and remain as viable amoebae. Secondly, Tsp509I was chosen as the mutagenic enzyme. One mutant identified in this way (dimC-), is a hypomorphic allele of a GATA family transcription factor (gataC). Consistent with the idea that GataC regulates DIF responsive gene expression, gataC expression is induced by DIF and GataC rapidly translocates to the nucleus in response to DIF. GataC- null cells fail to make stalk cells in both the cAMP removal and 8-Br-cAMP assays. The behaviour in the 8-Br-cAMP assay was however distinct from other dims, suggesting gataC is only required to regulate a subset of DIF responses. This idea is supported by its behaviour when prestalk and prespore markers were used to measure DIF responses in monolayer and dissociated cell assays. Developing gataC- cells show hallmark DIF signaling morphological defects. Despite this, expression of ecmO, ecmA and ecmAO reporter genes was normal. However, pstB cell differentiation was highly aberrant. PstB cells were found towards the rear of mutant slugs instead of at the prestalk/prespore boundary. In addition basal disc differentiation was severely affected. Importantly, both defects are phenocopied in the dmtA-, dimA- and dimB- DIF signaling mutants. We have therefore identified a novel factor required for DIF signal transduction and a new role for DIF signaling in the differentiation of the pstB population of anterior like cells and basal disc.

PadA, an NmrA-like protein involved in *Dictyostelium* development and cell differentiation.

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We have isolated a REMI mutant impaired in *in vitro* DIF-1-induced responses. The recapitulated *padA*$^-$ allele is thermo sensitive. At the permissive T, *padA*$^-$shows slower growth than the wild type strain and completes development after 36 h, producing small and sparse fruiting bodies with a low number of mature spores. At the restrictive T, 27ºC, *padA*$^-$ can not grow on axenic medium. When *padA*$^-$ cells grown at 22ºC are set to develop at 27ºC, they are unable to form proper fruiting bodies. Development is severely affected at this T and is blocked before culmination. The mutant is also defective in both stalk and spore formation. Development of the *padA*$^-$ mutant is hypersensitive to the inhibitory effect of ammonia and all phenotypic defects can be complemented when the *padA* gene is expressed under a constitutive promoter. Further analysis showed that the mutant is impaired in prestalk specific genes expression in slugs at the restrictive T. When *padA*$^-$ cells were developed in chimeras together with a small proportion of wild-type cells, the *padA*$^-$ mutant cells avoided the tip of the slug at the restrictive T.

Structural predictions of PadA using several threading methods show that this protein belongs to the short-chain dehydrogenases/reductases (SDR) superfamily and is a distant homologue of NmrA, a negative transcription-regulating protein that binds to GATA factors in response to the presence of ammonium. NmrA proteins have been described in fungi.

The mRNA levels of the putative ammonium transporters *amtA* and *amtC*, involved in the culmination/slug choice, are deregulated during development in the mutant strain at the restrictive temperature.

We have identified a pleiotropic gene involved in *Dictyostelium* vegetative growth, cell differentiation and development.

Breaking down the DIF pathway – a novel Ubiquitin Ligase involved in Dictyostelium development.

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During development, cells which are destined to form the stalk and related supporting structures of the fruiting body (Prestalk cells) differentiate in the mound, and then sort to the front of the motile slug. DIF-1 is a morphogen involved specifically in the differentiation of Prestalk 'O' (PstO) cells during Dictyostelium development. DIF-1 induced targets should therefore regulate PstO behaviour. We have identified a DIF-1-induced Ubiquitin Ligase (UblA) that is crucial for normal development, and is expressed specifically in the PstO region. The Ubql protein contains an N-terminal Filamin domain (indicating possible interactions with Filamin or related proteins) and a C-terminal HECT domain (indicating involvement in targeting proteins for degradation by the proteasome). A ublA null mutant shows markedly increased slugging on plaques, and severely delayed culmination. Interestingly, PstO cells are mislocalised in the ublA null mutant slugs; they are found at the back as opposed to the PstO collar region at the front of the slug. As UblA contains both a Filamin and a HECT domain, part of its function may be to regulate Filamin levels, so we tested this genetically. Interestingly, it was found that disrupting the Filamin gene in the ublA null background was able to rescue the PstO cell mislocalisation and slugging phenotypes. This suggests that UblA may regulate Filamin activity by degradation via the proteasome, and that this process is necessary for normal development and patterning of Dictyostelium Prestalk cells.

CudA defines a new family of eukaryotic transcription factors and is important for prespore and tip gene expression

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CudA is required for efficient prespore-specific gene expression and we show, by ChIP analysis, that CudA is bound in vivo at the promoters of three co-regulated prespore genes: cotA, cotB and cotC. ECudA, an Entamoeba CudA homologue that retains activity when expressed in E. coli, binds to a specific region of the cotC promoter in vitro. The ECudA binding site is a dyad and, consistent with a symmetrical binding site, CudA forms a homodimer in a yeast two-hybrid system. Mutation of ECudA half-sites reduces cotC expression in prespore cells. The conserved, presumptive DNA binding domain of CudA is similar in sequence to domains in two Arabidopsis proteins and one Oryza protein. Significantly, these are the only proteins in the two plant species that contain an SH2 domain. Such a structure, with a DNA binding domain located upstream of an SH2 domain, suggests that the plant proteins are orthologous to metazoan STATs. Consistent with this notion the DNA sequence of the ECudA half binding site, GAA, is identical to metazoan STAT half-sites; although the relative positions of the two halves of the dyad are reversed. These results define a hitherto unrecognised class of transcriptional regulator and suggest a model for the evolution of STATs and their DNA binding sites. CudA is also expressed in the extreme slug tip. Previous genetic analyses suggested that the cudA-expressing cells endow the tip with its biological properties (Fukuzawa et al., 1997). In order to identify potential transcriptional targets for CudA, we sought genes with a CudA-like expression pattern in the slug tip. One previously characterised gene (Maeda et al., 2003), that encodes an expansin-like protein, shows just such a pattern and is not expressed in a cudA null strain. The espl7promoter contains two separate regions that bind to EcudA. Mapping studies suggest that transcription of espl7 occurs by the synergistic action of CudA and other proteins.

Fukuzawa, M., Hopper, N. and Williams, J. (1997) cudA: A Dictyostelium gene with pleiotropic effects on cellular differentiation and slug behaviour. Development, 124, 2719-2728. Maeda, M., Sakamoto, H., Iranfar, N., Fuller, D., Maruo, T., Ogihara, S., Morio, T., Urushihara, H., Tanaka, Y. and Loomis, W.F. (2003) Changing patterns of gene expression in Dictyostelium prestalk cell subtypes recognized by in situ hybridization with genes from microarray analyses. Euk. Cell, 2, 627-637.

The transcription factor SRFB is required for regulation of several actin-cytoskeleton related functions and multi-cellular development

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The Serum Response Factor (SRF) is an important regulator of cell proliferation and differentiation. D. discoideum contains two genes coding for SRF-homologous proteins, srfA and srfB. SrfA is expressed during development and is required for slug migration, morphogenesis and terminal spore differentiation. In contrast, srfB is expressed in vegetative cells and during development, with maximal expression between 6 and 12 hours. This gene is expressed under the control of three alternative promoters with different cell-type specific patterns of expression, although the mRNAs generated code for the same protein. Promoter 1 drives expression in vegetative cells and in scattered cells in mound and finger structures. Promoters 2 and 3 specifically direct transcription in PstAB prestalk cells, in the first cells that migrate to form the stalk and in stalk and lower-cup cells. Analysis of an srfB-interrupted strain (srfBKO) has shown that this gene is required for regulation of several actin-cytoskeleton-related functions, such as cytokinesis, phagocytosis and macropinocytosis. Starved srfBKO cells showed impaired chemotaxis to cAMP and defective lateral pseudopodia inhibition. SrfBKO cells presented increased spontaneous motility but low directionality towards cAMP and increased cell-cell and cell-substrate adhesion. When developing under shaking, the appearance of EDTA-stable contacts was delayed. In contrast to WT, they failed to form streams under water, but aggregated on Nitrocellulose filters or agar two hours earlier than WT cells, mainly by accretion, and timely formed fruiting bodies. srfBKO cells showed increased slug formation under conditions that favor direct culmination in WT cells. The phenotypic alterations observed for this mutant make us suggest that srfB is involved in the regulation of the actin cytoskeleton, as described for the vertebrate SRF gene. In adition, srfB could participate in the regulation of developmental processes such as slug culmination.

Cell type specific regulation of Rap activity is required for morphogenesis and patterning in Dictyostelium

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The regulation of cell adhesion and cell motility plays an important role in pattern formation during Dictyostelium development. Consequently great efforts have been made to identify genes that control these processes. One such gene is Rap1 as it has been implicated in adhesion and motility in vegetative Dictyostelium cells. However, a potential role of Rap1 in the regulation of multicellular development has not been determined. In order to study the role of Rap1 in cell fate choice and pattern formation, we have generated a mutant in a potential Rap1 GTPase activating protein (RapGAPA). RapGAPA- cells have increased levels of active Rap1 compared to wild type cells when stimulated with cAMP, indicating that RapGAPA regulates Rap1 activity. Furthermore, RapGAPA- cells exhibit hallmark phenotypes of other known mutants with increased levels of active Rap1, including increased substrate adhesion and abnormal F-actin distribution. However, unlike these other mutants, RapGAPA- cells did not exhibit impaired motility or chemotaxis, indicating that RapGAPA may only regulate specific roles of Rap1. We show that RapGAPA is required for normal development. Firstly, streams of aggregating cells break up as a result of decreased cell-cell adhesion. Secondly, the RapGAPA- cells do not form a migratory slug. Instead development is blocked at the tipped mound stage, although culmination does eventually occur. RapGAPA- cells exhibit a cell autonomous defect in prestalk cell differentiation. Using cell type specific markers, we demonstrate that this is because RapGAPA is required for the correct sorting behaviour of a specific subtype of prestalk cells, the prestalk A cells.

The Sporulation Control Network of *Physarum polycephalum* Probed by Plasmodial Fusion of Mutants

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Somatic complementation by fusion of two mutant plasmodia and mixing of their cytoplasms occurs when the genetic defect of one fusion partner is cured by the functional gene product provided by the other. We have found that complementation of mutational defects in the network mediating stimulus-induced commitment to sporulation of *Physarum polycephalum* may reflect time-dependent changes in the signaling states of its molecular building blocks. The analysis of somatic complementation effects that occur in a time-dependent manner after sporulation has been triggered, can be used to systematically probe network structure and dynamics. A predictive dynamic model of the sporulation control network is built from experimental data employing Petri nets as a modeling framework. We are currently setting up a platform for the systematic isolation of mutants and subsequent map-based cloning of genes tagged by chemically induced point mutation.

A search strategy for eukaryotic cells

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Eukaryotic cells are large enough to detect signals and then orient to them by averaging signal strength over the length and breadth of the cell. Amoebae, fibroblasts, neutrophils and growth cones all behave in this way. Little is known however about searching behavior in the absence of a signal. Do individual cells have a search strategy when they are beyond the range of the signal, as appears to be the case for animals in search of food? Here we ask if single, isolated, *Dictyostelium* and *Polysphondylium* amoebae bias their motion in the absence of external cues. We find that their behavior is well described by a biased walk, improving their chances of success. Unlike strategies used by foraging animals, amoebae restrict their turning angle, remember their last turn, and turn away from it on the next turn. We have modeled our data and compared it to the Brownian (or random) expectation, and to other classes of biased walks that are believed to characterize searching strategies in large animals. We suggest that other eukaryotic cells may employ a similar strategy when seeking conditions or signal sources not yet within range of their detection system.

How A Cell Crawls: Pseudopod Extension and Cellular Translocation Are Not Driven by Myosin II-Dependent Posterior Cell Contraction

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Lacking a quantitative 4D description of how a cell migrates on a flat surface, we have relied on suspect perceptions to model locomotion, interpret the behavioral defects of cytoskeletal and regulatory mutants, and deduce the function of molecules in locomotion and chemotaxis. This has led to inaccurate models and in some cases, inaccurate or blatantly wrong interpretations of mutant defects. In turn, this has led to misinterpretations of the role of the mutated genes in cell migration and chemotaxis. To obtain a legitimate 4D description, we employed 3D-DIAS software to reconstruct and obtain morphometric data on the behavior of D. discoideum cells. The resulting detailed description proved incompatible with popular posterior contraction-anterior expansion models for cell migration. Additionally, by similarly reconstructing and motion-analyzing the myosin II phosphorylation mutant 3XASP, we provide evidence that normal cortical localization of myosin II, while necessary for the suppression of lateral pseudopod formation, does not play a role in either anterior pseudopod extension or uropod retraction during cell migration. Our 4D description has revealed a number of morphometric events, including coordinate anterior migration of the anterior boundary between pseudopod and cell body with the posterior tip of the uropod, constancy of the summed volumes of pseudopods regardless of translocation dynamics, posterior-anterior translocation of major cell body mass, with a substantial 3D component, following transient anterior pseudopod extension, the fixed relationship between landmarks at the cell periphery and the substrate, and a number of other characteristics that can now be incorporated into a new and far more accurate and complex model for cell migration. This more accurate 4D description can now be used to interpret the true behavioral defects of new cytoskeletal and regulatory mutants and, hence, the function of mutated genes, and to reinterpret old mutants.

Dynamic actin patterns

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The actin network in the cell cortex is continuously generating distinct structures that either mediate cell motility, endocytosis, or cell division. To study the capacity of the actin system to self-organize into dynamic three-dimensional patterns, we focused on free-running actin waves. The spatio-temporal pattern of myoB, the Arp2/3 complex, and coronin in the waves has been elucidated. By photobleaching the dynamics of these proteins is examined with regard to the mechanism of wave propagation. The relevance of this dynamics to the protrusion of a leading edge is discussed.

Actin dynamics in SCAR-deficient cells

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The dynamical properties of the actin cytoskeleton provide the basis for motility, phagocytosis, and division of eukaryotic cells. Directed polymerization of actin in the cell cortex has been identified as the underlying source of force generation. A key player in the formation of a dense cortical actin network is the seven-subunit Arp2/3 complex that initiates the nucleation of branches on existing filaments. Its activity is controlled by SCAR/WAVE proteins of the WASp (Wiscott-Aldrich Syndrome protein) family that are downstream effectors of receptor-mediated signalling pathways. Here we analyze the temporal patterns of actin polymerization in the cortex of mutant cells lacking members of the pentameric SCAR complex. The results highlight the actin machinery as a self-organizing system that can be described by the concepts of non-equilibrium dynamics. We furthermore report evidence that the cortical dynamics is linked to the chemosensory pathway, so that receptor signals are transmitted to the actin system, even if SCAR is missing.

Actin–microtubule crosstalk in the viscoplastic intracellular space and sub-second actin dynamics during cell spreading

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This work is motivated by the questions how actin is involved in force generation in the intracellular space, and how actin effects the cell's interaction with its environment (substrate). To gain insight into the nature of the dynamic mechanical properties of the cytoplasmic space, we analyzed the motion of magnetic colloidal force probes (R = 0.7 μm) inside the cell in the absence and presence of external forces (F = 20 -700 pN). The roles of the actin cortex, the microtubule (MT) aster and their crosstalk were explored by comparing the behavior of wild type (WT) cells, mutants and drug treated cells. The correlated motions of the MTs and the centrosome were evaluated by micro-fluorescence of GFP-labelled MTs. The velocity distributions of the colloids under varying conditions can be presented by log-normal distributions with long tails. The colloid responses are strongly non-linear and are mostly directed perpendicular to the applied force, showing that the cytoplasm behaves as an active viscoplastic body with time and force dependent drag coefficients. Nano-Newton loads excerted on the soft MTs are balanced by traction forces arising at the MT ends coupled to the actin cortex and the centrosome. The actin cortex responds to forces on a sub-second time scale, made possible by continuous actin re-polymerization everywhere in the cell cortex. Further, we investigated the actin re-organization dynamics on a sub-second time scale during the initial cell spreading process on surfaces with varying adhesion strength. The cells were labeled with fluorescent proteins to visualize the actin network and the proteins associated with its function and regulation. We complemented high speed scanning confocal microscopy, on the whole cell body, with TIRF microscopy in the substrate plane, in order to monitor the non-monotonic cell spreading behavior accompanied by continuous actin-filament turnover. I will present the repertoire of cellular activities.

Identification and characterization of CP39, a novel centrosomal core protein identified by proteomic screening of the Dictyostelium centrosome

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The centrosome is a nucleus-associated organelle required for cell cycle progression and all microtubule-dependent processes. Analysis of its function is complicated by the fact that the majority of its up to 100 different proteins show only more or less dynamic binding activities but no catalytic activity. Thus further information is expected from comparative biology and proteomic analysis of the protein inventory of different centrosome types. We used mass spectrometry to analyze the proteome of isolated Dictyostelium centrosomes. The initial centrosome isolation protocol was optimized to achieve higher enrichment of centrosomal components including a new density gradient isolation step for nucleus/centrosome complexes and a DNase treatment. To distinguish between centrosomal and contaminating proteins within the preparation, the protein composition of the centrosome-enriched fraction was compared to that of a centrosome-depleted fraction. Therefore we used 2D-PAGE, 16-BAC/SDS-PAGE and differential ITRAQ-labeling followed by cation exchange. In addition to 12 known components associated with Dictyostelium centrosomes, 33 new candidates were identified. Of these, 6 could already be localized to isolated centrosomes either by GFP-fusions or immunofluorescence microscopy. This includes a potential homolog of the yeast SPB component Bbp1p, which is currently investigated in our lab. Bbp1p tethers the yeast SPB to the half bridge via binding to Mps2p and the SUN-1 orthologue Mps3p. In addition we identified CP39, a novel centrosomal protein encoded by geneDDB0233886. CP39 localizes to the centrosome only during interphase, but not during mitosis. By confocal deconvolution immunofluorescence microscopy and electron microscopy we could show, that CP39 is part of the centrosomal core complex. The only other known component of the layered core of the Dictyostelium centrosome, the kinase DdNek1, is a potential regulator for the cell cycle dependent behavior of CP39.

Alcohol leaves Dicty in a spin: PLD and butan-1-ol in motility and endocytosis.

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D. discoideum contains three Phospholipase D (PLD) genes. We have generated single knockouts of each and a cell line devoid of all PLD activity to investigate the roles of PLD in fluid uptake and cell migration. pldA null cells show no obvious phenotype during growth or development. pldB is expressed later in development and pldB null cells show defects in phototaxis during the slug stage. pldC null cells show misregulation during aggregation resulting in grossly enlarged territory sizes. Butan-1-ol is a primary alcohol that has classically been used as a tool to investigate PLD activity. Butan-1-ol blocks PLD signalling by directing the product of PLD mediated lipid hydrolysis from phosphatidic acid towards production of a phosphatidyl-butanol species. D. discoideum treated with butan-1-ol show a striking response exemplified by a cessation of fluid uptake activities and cell rounding. The cells remain rounded throughout treatment but recover after removal of the butan-1-ol. Surprisingly cells deficient in PLD did not resemble those treated with butan-1-ol. To investigate the butan-1-ol induced rounding response we looked at the cytoskeleton using GFP ? tubulin and RFP limE makers. We have additionally shown the butan-1-ol response is due to dramatic and pronounced alteration of the microtubule structure within a cell. This is observed as a collapse of the radial arrangement of microtubules and spiralling of the centrosome. Overall our data show that PLD is involved in subtle signalling events during development and the cell rounding response to butan-1-ol is not mediated via a PLD pathway but butan-1-ol has additional, currently unknown, signalling targets within the cell.

Use of penetratin-based peptides to study cell motility and chemotaxis.

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Gene disruption provides a powerful method to remove and analyse specific gene function in Dictyostelium. There are however limitations to this approach. First, genes may be pleotropic, multi-functional or work in dependent series. This means for example that loss of a gene may have an effect in early development, which has a knock-on effect at later stages. Second, cells often appear to make compensatory changes to suppress long-term loss of gene product. The possibility of compensatory changes is a current concern in analysis chemotaxis. These problems can be overcome by acute inhibition of protein function just prior to analysis through specific low molecular weight inhibitors (drugs) or inducible RNAi. We have been developing a third approach based on cell penetrating peptides (CPP). We have established that CPPs based on the 16 amino acid penetratin sequence can rapidly enter living amoeba (1). Using this approach we designed a specific inhibitor of the cAMP-dependent protein kinase (PKA) based on the PKI sequence. This provides a particular useful test as PKA has multiple functions throughout development, making it difficult to specifically investigate its effect on chemotaxis. To make this technology cost-effective, we have established a method to produce CPP-based peptides and small proteins in bacteria. The generation should enable targeting of protein:protein interactions, in addition to enzyme action.

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

PH-domain translocation in well controlled chematractant gradients.

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We use the photo-chemical release of caged cAMP in microfluidic devices to expose chemotactic cells to spatio-temporally well controlled chemoattractant stimuli (switching time approx. 0.5 sec and arbitrarily shaped gradients) We apply this technique to the study of intracellular translocation of fluorescently labeled PH-domain proteins. Single chemotactic cells were exposed to localized, well defined gradients and their translocation response was quantified as a function of the external gradient steepness. We find as a function of gradient steepness that the translocation signal sets in with a finite response and at steep gradients. At shallow gradients no translocation signal could be measured, although the cells showed a chemotactic response.

Noise and directional sensing: a numerical modeling study

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Eukaryotic cells can detect and respond to very shallow gradients. In these gradients, stochastic effects due to Brownian motion of the ligand molecules and binding uncertainties will start playing a role. We have quantified these stochastic effects via direct numerical simulations and have coupled the resulting external noisy gradient signal to two directional sensing models. Furthermore, we briefly discuss preliminary results for a highly simplified one-dimensional geometry in which some of the calculations can be done analytically.

Intrinsic oscillations of cAMP in Dictyostelium cells

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It is well documented that Dictyostelium cells respond to extracellular cAMP stimuli by synthesizing intracellular cAMP. To date it remains unclear how the signaling is initiated and by what molecular mechanism the oscillations are sustained. We have revisited the cAMP dynamics of (pre-)aggregating cells by live-cell imaging using FRET-based cAMP sensor protein. Our approach allows one to measure the time course of the cAMP response directly at the single-cell level. Upon transient stimulation (30sec to 2 min) of extra-cellular cAMP in the range of 10nM-10uM, intracellular cAMP begin to rise within 5 sec and peaks at 30 sec followed by decrease to the baseline level in the next few minutes. The observed dynamics are consistent with what is known from the population-level biochemical assay. The response is inhibited when cells are treated with LY294002 or SQ22536 and therefore it is PI3kinase and ACA dependent. Using this single-cell based assay, we discovered that under prolonged cAMP stimulation (6-20min), cAMP responses do not perfectly adapt but rather display persistent oscillations of ~3 min period as long as extra-cellular cAMP is present. The intrinsic oscillations are shut off upon removal of extra-cellular cAMP. The degree of damping of the intra-cellular cAMP oscillations varies from cell to cell and when statistically averaged, it is consistent with the known population-level cAMP profile. The oscillations are observed in a pkaR-null strain, therefore they are independent of cAMP-mediated regulation of PKA. Our observation suggests that in addition to de-adaptation of ACA mediated by clearing of extracellular cAMP, there is an independent mechanism that can sustain oscillations within a single cell. The observed intrinsic oscillations may be responsible for the appearance of pace-maker cells.

Vesicular trafficking is required for chemoattractant delivery at the trailing edge of chemotaxing Dictyostelium cells.

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We have shown that the adenylyl cyclase ACA is enriched at the back of chemotaxing Dictyostelium cells, allowing them to align head-to-tail and form streams. Interestingly, we also determined that ACA labels highly dynamic intracellular vesicles. Here we investigate the role of these vesicles in controlling the asymmetric distribution of ACA. Using fluorescence recovery after photobleaching (FRAP) we show that the asymmetric distribution of ACA at the back of cells requires vesicular trafficking. This trafficking results in a local accumulation of intracellular vesicles at the back of cells and involves intact actin and microtubule networks. Intriguingly, we also observe that migrating cells leave behind trails containing ACA. Since migrating cells maintain a polarized distribution of ACA, we reasoned that ACA synthesis is be required to preserve ACA asymmetry and streaming. Using a combination of cell biological and biochemical approaches, we show that ACA is indeed rapidly synthesized. Furthermore, we find that cycloheximide treatment abolishes ACA enrichment at the back of migrating cells and induces serious streaming defects. Finally, using immuno-EM analyses we show intracellular ACA labeling on highly tubular, membranous structures and on multi-vesicular bodies that appear to be fusing with the plasma membrane, which is also labeled with ACA. Together these experiments define a mechanism for streaming where de novo protein synthesis and endocytosis are required for the polarized distribution of ACA vesicles at the back of migrating cells. These vesicles fuse with the plasma membrane at the back and are released proximally as well as in trails, potentially attracting nearby cells to form streams. We propose that similar compartmentalization and shedding mechanisms exist in mammalian cells during embryogenesis, wound healing, neuron growth, and metastasis.

Single-molecule imaging of sub-steps in the early events of the chemotaxis signaling cascade.

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During chemotaxis, the dynamic properties of the cAR1 receptors are anticipated to be influenced by the formation of protein-protein and protein-lipid networks and their possible linkage with the cytoskeleton. This could control their accessibility to cAMP and to downstream signaling proteins, hence forming an important regulatory mechanism for cellular signaling. The signal transduction cascade is ultimately based on specific reactions between one molecule and a second. And as such interactions are usually not completely synchronized in time; bulk studies will only yield information on the average properties of the interactions. In order to obtain more detailed information about the existence of subpopulations, domains, and sub-steps in the interaction processes, it is necessary to follow the events taking place at a single-molecule level in real-time. To this date, only the spectral and photophysical characteristics of the yellow-fluorescent protein mutant, eYFP, were sufficient for single-molecule microscopy. The bleaching rate is very fast, though, and to observe the events for longer periods of time we used a new labeling technique. The HaloTag technology (Promega) is based on the formation of a covalent bond between the HaloTag™ protein, fused to the protein of interest, and a synthetic ligand that carries a fluorescent label of varying colors. These fluorescent labels are much more photostable and photobleaching is no longer necessary since the amount of fluorescence is controllable by adjusting the concentration of the Halo ligand. In this TIRFM study, single cAR1-Halo molecules were followed at the plasma membrane under different physiological conditions, i.e. cAMP stimulation, disruption of actin and tubulin cytoskeleton and in different genetic background. In conjunction with this, other Halo-fused proteins were also followed at the membrane. These data will be discussed in relevance of understanding the process of gradient sensing.

PLC regulation of PI(3,4,5)P3-mediated chemotaxis

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Generation of a PI(3,4,5)P3 gradient within the plasma membrane is important for cell polarization and chemotaxis in many eukaryotic cells. The gradient is produced by the combined activity of PI3K to increase PI(3,4,5)P3 on the membrane nearest the polarizing signal and PI(3,4,5)P3 dephosphorylation by PTEN elsewhere. Common to both of these enzymes is the lipid PI(4,5)P2, which is not only the substrate of PI3K and product of PTEN, but is also important for membrane binding of PTEN. Consequently, regulation of PLC activity, which hydrolyses PI(4,5)P2, could have important consequences for PI(3,4,5)P3 localization. We investigate the role of PLC in PI(3,4,5)P3 mediated chemotaxis in Dictyostelium. plc-null cells are insensitive to the PI3K inhibitor LY294002 and produce little PI(3,4,5)P3 after cAMP stimulation, as monitored by the PI(3,4,5)P3-specific PH-domain of CRAC (PHCRACGFP). In contrast, PLC overexpression elevates PI(3,4,5)P3 and impairs chemotaxis in a similar way to loss of pten. PI3K localisation at the leading edge of plc-null cells is unaltered, but dissociation of PTEN from the membrane is strongly reduced in both gradient and uniform stimulation with cAMP. These results indicate that local activation of PLC can control PTEN localization and suggest a novel mechanism to regulate the internal PI(3,4,5)P3 gradient.

Regulation of cell polarization and chemotaxis by Ras and Rap1

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Members of the Ras family of small GTPases play critical roles in controlling chemotaxis. Both Ras and Rap1 are activated at the leading edge of chemotaxing cells and regulate distinct downstream effector pathways. Ras mediates cell polarity, chemotactic speed, and directional sensing through downstream effectors that include PI3K and TORC2, which act together to regulate Akt/PKB and a related kinase PKBR1, and F-actin polymerization through members of the PIP3-responsive Dock180 family of RacGEFs. In contrast, Rap1 controls cell attachment at the leading edge and contractility through the regulation of MyoII assembly and disassembly and via MyoII-independent pathways. We have examined the regulation of Ras through the identification and functional analysis of a RasGEF complex and through the analysis of a RasGAP that helps control the timing of the Ras response. The RasGEF complex contains two RasGEFs, a scaffolding protein, PP2A, and several other components. The majority of the components have been disrupted, and biochemical and phenotypic analysis of single and multiple KO mutants has provided an understanding of the role of individual components and the complex as a whole in regulating chemotaxis. Through deletional analysis, we have obtained a “crude” understanding of the organization of the complex, while phospho-proteomic analyses has suggested that the complex is regulated by differential phosphorylation. By characterizing the ability of cells to spatially regulate leading edge functions in different RasGEF and RasGAP null mutant backgrounds, we have further insight into the mechanisms by which cells spatially regulate leading edge functions how cells polarize and orient in chemoattractant gradients.

Understanding phospholipase A2 signalling in Dictyostelium as a target for fatty acids

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Phospholipase A2 (PLA2) enzymes catalyse the hydrolysis of membrane phospholipids at the sn-2 position to release free fatty acids including the essential fatty acid arachidonic acid (AA). The activity of this enzyme has been associated with seizure progression, since PLA2-catalysed overproduction of AA has been shown in the brain following seizures (Bazan et al., 2002), and in psychiatric disorders such as bipolar disorder (manic depression)(Ross et al., 2006). The role of the PLA2 signalling pathway in these disorders remains unclear. The epilepsy and bipolar disorder treatment, valproic acid (VPA), a branched chain fatty acid, reduces PLA2–catalysed AA release by 28-33% in the mammalian brain, by an unknown mechanism (Chang et al., 2001). Two other bipolar disorder treatments, lithium and carbamazepine also reduce AA turnover, thus implicating PLA2 inhibition as a target for bipolar disorder treatments. VPA blocks development in Dictyostelium at 1 mM – close to therapeutic concentrations given to patients during treatment. To investigate the effects of VPA we have screened a REMI library for mutants resistant to VPA, and identified a gene encoding a homologue to the human calcium insensitive phospholipase A2 (iPLA2alpha). The human homologue is a relatively uncharacterised protein, and no previous association has been made with the activity of this enzyme and the effects of VPA. Ablation of this protein in Dictyostelium partially overcomes the VPA-induced block in aggregation. To analyse the biochemistry of this process, we have developed an in vivo assay for PLA2, and shown VPA acutely blocks PLA2 activity. We have been able to employ this assay to determine the chemical characteristics of PLA2 inhibitory compounds, based around the core structure of VPA. These studies offer a chance to better understand the therapeutic effects of fatty acids, including VPA, on PLA2 signalling.

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Characterization of the Light Chain Composition of the Dictyostelium Myosin I Family

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Dictyostelium discoideum possess a complement of seven class I myosins which includes three long-tailed isoforms (MyoB, C and D), three shorted-tailed isoforms (MyoA, E and F) and a single myosin I, MyoK, which lacks both a neck region and a tail. Studies from a wide range of organisms has demonstrated that myosin I activity can be potentially regulated through calcium chelation by the myosin light chains associated with the neck region of the molecule. To further understand the regulatory mechanisms controlling myosin I function in Dictyostelium we have identified and characterized the light chain composition of MyoA, B, C, D and E. The three long-tailed myosins associate with unique myosin light chains. MyoD co-purified with two copies of a 16 kDa light chain, termed myosin light chain D (MlcD), which lacks high-affinity calcium binding sites. MyoB associates with single copy of a novel 8 kDa light chain (MlcB). MlcB is composed of only 2 EF-hands and is therefore comparable to a single lobe of a typical myosin light chain. MlcB bound Ca2+ with a Kd value of 0.2 μM and underwent a Ca2+-induced change in conformation that increased alpha-helical content and surface hydrophobicity. A MyoB head-neck construct bound MlcB and displayed an actin-activated Mg2+ATPase activity that was insensitive to Ca2+. MyoC associates with two copies of another unique 8 kDa light chain (MlcC). MlcC does not bind calcium rendering MyoC activity insensitive to in vivo calcium level changes. In contrast to the long-tailed isoforms, the short-tailed myosins MyoA and MyoE each associate with two copies of calmodulin. However, despite possessing calmodulin as a light chain, the actin-activated Mg2+ATPase activity of a MyoA head-neck construct was insensitive to changes in Ca2+ levels within a physiologically relevant range.

The Cellular Activities of Myosin Heavy Chain Kinases B and C Are Specified by Homologous WD-Repeat Domains

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Myosin II filament disassembly in Dictyostelium is driven by phosphorylation of the myosin II heavy chain (MHC) “tail” region via the activities of three structurally-related MHC kinases: MHCK-A, -B, or -C. All three MHCKs share homologous catalytic and WD-repeat domains. While MHCK-A has been studied extensively, comparatively little is known about the MHCK B and MHCK C enzymes. In the studies presented here we examined the in vivo function of the WD-repeat domains of MHCK-B and C by over-expressing in Dictyostelium cells either the full-length enzymes (MHCK-B++ or C++) or truncated versions lacking their WD-repeat domains (MHCK-B-Δ-WD++ or C-Δ-WD++). These cell lines were then analyzed for myosin II-dependent activities such as cytokinesis and multicellular development. As has been reported by others, we found that the MHCK-B++ and MHCK-C++ cells grew poorly and exhibited markedly increased multinuclearity when cultured in suspension. Interestingly, we found that cells over-expressing Δ-WD truncations of MHCK B or C do not grow well in suspension culture; however, this growth defect is not accompanied by increased multinuclearity. We also found that the MHCK-C-Δ-WD++ cells, unlike their MHCK-C++ counterparts, retain the ability to undergo multicellular development. Together, these results indicate that the WD-repeat domains of MHCK-B and C direct these kinases to phosphorylate myosin II heavy chain. Our localization studies of GFP-MHCK-C have revealed that only the full-length kinase (not the truncations tested) exhibits cAMP-stimulated translocation to the cell cortex, and that this activity is lost when cells are treated with latrunculin A. Further studies are in progress examining the relationships between the structural domains of the MHCK-B and C enzymes and their biochemical activities, ultimately with the goal of understanding how the cell coordinates the activities of the MHC kinases in the dynamic context of a nonmuscle cell.

Structural Characterization of the Atypical Kinase Catalytic Domain of the Dictyostelium Myosin II Heavy Chain Kinase

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The alpha kinases are a small but widespread family of eukaryotic protein kinases that exhibit no detectable sequence homology to the superfamily of "typical" serine, threonine and tyrosine protein kinases. The first member of the alpha kinase family to be identified was Dictyostelium myosin II heavy chain kinase A (MHCK A). MHCK A consists of a N-terminal coiled-coiled domain that binds actin filaments, a central kinase catalytic domain and a C-terminal WD repeat domain that interacts with myosin II. MHCK A promotes the disassembly of myosin II filaments by phosphorylating three threonine residues located within kinases (MHCK B-D) areαthe myosin II tail. Three other Dictyostelium closely related to MHCK A and function in a cooperative manner to regulate myosin II assembly and activity. A minimal MHCK A kinase catalytic domain (A-CAT; residues 552-841) that retains a high level of activity has been characterized. Assays with a variety of substrates show that A-CAT strongly prefers to phosphorylate threonine rather than serine residues. A hydrophobic residue in the P-2 position and basic residues in the P+1 and P+2 positions were found to be critical for peptide substrate recognition by A-CAT. To date, structural information is available for only a single alpha kinase: the kinase domain of the mouse TRPM7 cation channel, which shares about 30% sequence identity with A-CAT. The high resolution X-ray crystal structure of A-CAT in the presence of Mg2+ and nucleotides has now been solved. The overall structure of A-CAT is very similar to that of the TRPM7 kinase domain; however, significant differences are observed in the conformation of a flexible glycine-rich loop that is one of the distinguishing features of the alpha kinases. In addition, the X-ray structure provides new insights into the active site of the alpha kinases and suggests a catalytic mechanism quite different from that employed by typical protein kinases

Linking Ras to myosin function: RasGEF Q, a Dictyostelium exchange factor for RasB, affects myosin II functions

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RasGEF Q, a Ras guanine nucleotide exchange factor (RasGEF) from Dictyostelium, is a 143 kDa protein containing RasGEF domains separated by a DEP domain. We show that RasGEF Q is the predominant exchange factor for RasB and that RasB is activated by cAMP, and that the DEP domain acts as an autoregulatory domain in regulating activation of RasGEF Q. Overexpression of the GEF domain of RasGEFQ constitutively activates RasB and leads to a cytokinesis defect in suspension resembling the phenotype of cells expressing a constitutively active RasB and of myosin null mutants. Furthermore, RasGEF Q- mutants show myosin II overassembly as a result of higher levels of unphosphorylated myosin and are defective in suppression of lateral pseudopods during chemotactic migration. RasGEF Q can bind to F-actin and has the potential to form complexes with MHCK A that contain active RasB. Together our results suggest that starvation signals through RasGEF Q to activate RasB, which then regulates processes requiring myosin II by regulating MHCK A. In addition to cell polarity defects RasGEF Q- mutants also have defects in cell sorting, developmental patterning and slug motility.

Abi (abelson interactor) Mutants Reveal Specific Roles for the SCAR/WAVE Complex in Cytokinesis

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The SCAR/WAVE complex is an essential regulator of actin function, but the mechanisms controlling its activity remain unclear. One subunit, Abi1/2, is particularly well studied because of its connection with tyrosine kinase signalling. Here we analyze the roles of the unique Dictyostelium Abi. We find that abiA mutants show less severe defects than scar- cells, indicating – unexpectedly - that SCAR retains partial activity in the absence of Abi. Furthermore, abiA mutants have a serious defect in cytokinesis, which is not seen in other SCAR complex mutants. Detailed examination of cytokinesis reveals that SCAR normally promotes directed migration away from the plane of cleavage; SCAR protein localises to polar ruffles, and scar mutant daughter cells migrate away from one another much less accurately than wild type. In abiA mutants, however, inappropriate SCAR-dependent movement actively interferes with cell division, causing a far more severe cytokinesis defect. We conclude that normal cytokinesis requires SCAR activity, regulated through multiple pathways.

Exploring the role of NDR kinases in cytokinesis of Dictyostelium discoideum

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Proper cytokinesis requires the concerted action of a huge number of proteins. We have identified a septation initiation network (SIN)-related pathway in Dictyostelium discoideum. In Schizosaccharomyces pombe this signaling cascade controls events at the end of mitosis, and initiates contraction of the actin ring system and synthesis of the division septum. SIN is highly analogous to the mitotic exit network (MEN) of budding yeast. Some of the components of these two pathways have also been found in higher eukaryotes. In Dictyostelium an almost complete set of SIN-homologous proteins is present. Central to the predicted pathway in Dictyostelium is the Cdc7p-homologous kinase septase (sepA) that was identified during a screen for cytokinesis-defective mutants. Septase is crucial for proper cleavage furrow formation. Potential regulators upstream of septase are the polo-like kinase (Plk1), a GTPase activating protein (Bub2) and a GTPase (Spg1). Plk1 localises to centrosomes and spindles only during mitosis, whereas Bub2 and Spg1 are found permanently at centrosomes. Among potential effectors of septase are four NDR kinases (NdrA-D) and three Mob1 proteins (MobA-C). NDR (nuclear Dbf2-related) kinases were shown to regulate essential cellular processes from yeast to humans, and we are analysing the involvement the Dictyostelium NDR kinases in respect to cytokinesis. By tandem affinity purification NdrB and MobB were found to interact. We are also investigating the subcellular distribution of NdrA and NdrB by expression of GFP-tagged fusion proteins and the use of specific antibodies. In order to dissect specific interactions and functions, full length and truncated constructs of septase and NDR kinases are purified and tested in in-vitro assays. Mutants of the NdrA kinase were generated and are currently characterised.

Ste20-like kinases in Dictyostelium discoideum - Implications for cytokinesis, phagocytosis and development

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Ste20-like kinases are Ser/Thr kinases that are involved in regulation of MAP kinase cascades, apoptosis, ion transport, stress response and the cytoskeleton. D. discoideum contains several Ste20-like genes representing the two subfamilies PAK and GCK. We are currently investigating the function of the 13 D. discoideum GCKs which show in their N-terminal kinase domains up to 74% sequence identity as compared to mammalian homologs. As shown for Krs1 the C-terminal half can inhibit the kinase domain (Arasada et al., 2006). According to gene disruptions and expression of GFP fusion proteins we found that GCKs are involved in the regulation of chemotaxis, development, phagocytosis and cytokinesis. Disruption of the svkA gene which codes for the severin kinase (Eichinger et al., 1998) caused a defect in cytokinesis with up to 30 nuclei per cell. This phenotype arises from the frequent disability of svkA-minus cells to sever the last cytosolic connection during cell division. The important role of SvkA during late stages of cytokinesis is further supported by GFP-SvkA constructs that localize to the midzone of dividing cells. This localization is mediated by the C-terminal domain. A kinase dead SvkA-K134A mutant could not rescue the phenotype, suggesting that both localization and kinase activity are fundamental for the function of SvkA during cytokinesis. The kinase DstA (DDB0216377) is the closest relative to SvkA. The most striking phenotype of DstA-minus cells is their ability to form streams in full medium. Phagocytosis is reduced and colonies on bacteria show very long streams. In diluted nutrient medium (less than 10% full medium) AX2 cells and mutant are indistinguishable in their expression of CsA. However, in difference to AX2 DstA-minus mutants form elaborate streams and express the prespore marker pspA/Mud1. The data suggest that the lack of DstA leads to a defect in uptake or utilization of nutrients, and induces development already in full medium.

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Roots of cAMP signalling in the Dictyostelids

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Both intracellular and secreted cAMP have multitudinous role in Dictyostelium development. Secreted cAMP mediates the aggregation of starving amoebas and induces the differentiation of prespore cells. Intracellular cAMP acting on cAMP dependent protein kinase (PKA) triggers initiation of development and the maturation of spores and stalk cells. cAMP activation of PKA also prevents spore germination under the prevalent conditions of high osmolality in the spore head. The osmosensing adenylyl cyclase, ACG, produces cAMP for prespore differentiation and inhibition of spore germination. Using a comparative approach, we show that ACG originally acted as a drought sensor that regulated encystation of single amoebas and that its function was elaborated during Dictyostelid evolution to control cell differentiation and pattern formation during multicellular development. ACG genes, osmolyte-activated ACG activity and osmo-regulation of spore germination were detected in species that span the dictyostelid phylogeny. Unlike the evolutionary young species D.discoideum, older dictyostelids have retained the ancestral mechanism of encystation from solitary amoebas. In these species and other solitary amoebas, encystation is independently triggered by starvation or by high osmolality. Osmolyte-induced encystation was accompanied by an increase in cAMP and prevented by inhibition of PKA, indicating that ACG and PKA activation mediate this response. We propose that high osmolality signals approaching drought in soil amoebas and that this cAMP-mediated stress response is at the root of all developmental cAMP signalling in the Dictyostelids.

Functional genomics in Dictyostelium: a homologue of the accute pancreatitis-related gene VMP1 is involved in multiple aspects of Dictyostelium cell biology

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We are interested in the study a subset of genes of unknown function conserved between human and the social amoeba Dictyostelium discoideum. The selected genes are absent in simpler unicellular yeasts lacking the complexity of the multicellular developmental stages displayed by the social amoeba Dictyostelium. We have generated a collection of mutants in those genes and some of them show interesting phenotypes that might be connected to human diseases. A summary of the project and an update of the mutant collection will be presented. One of those genes is VMP1, a human protein of unknown function that is strongly and rapidly induced in pancreas during acute pancreatitis and has been proposed to play a role in the molecular basis of the disease. Acute pancreatitis is an auto-digestive disease occurring when hydrolytic enzymes, that should only be active when secreted to the digestive system, are abnormally activated leading to cell damage and inflammation. The pathogeny of the disease is not completely understood but membrane traffic and the secretory pathway seem to be involved. Dictyostelium VMP1 homologue was among our collection of genes and was selected for further study. The gene was disrupted by homologous recombination and later complemented by the expression of VMP1 fused to GFP. The complemented strain was used to determine the localization of the protein in the endoplasmic reticulum. Analysis of the phenotype showed a dramatic defect in osmoregulation and other aspects of cell biology that will be described in detail. We have also disrupted the gene in strains that express markers of endoplasmic reticulum, golgi and contractile vacuole to investigate the effects of the mutation in the morphology of these organella. Our phenotypic study suggests a possible role of the protein in membrane traffic and protein secretion. Our current efforts to determine the molecular function of the protein will be presented and discussed in the context of the disease.

An intracellular P2X receptor required for osmoregulation in Dictyostelium

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Soon after the discovery of the importance of intracellular ATP in the 1930s, ATP was also shown to be released from cells. However, knowledge of the extracellular roles of nucleotides has progressed slowly. Receptors for ATP of the ion channel (P2X) and G protein-coupled (P2Y) types were identified in the early 1990s. These are now known to be represented throughout vertebrate genomes, but absent from Drosophila and C.elegans. In mammals, P2X receptors regulate taste, bladder emptying, oxygen sensing, inflammation and pain. Consequently, there is great interest in understanding P2X receptor structure/function as they provide novel therapeutic targets. We identified a weakly related gene in Dictyostelium (~10% identity), and discovered, with the use of heterologous expression in human embryonic kidney cells, that it encodes a membrane ion channel activated by ATP. Site-directed mutagenesis revealed essential structure–function conservation with P2X receptors of higher organisms. Therefore, despite such low homology, essential properties of a P2X receptor (ATP binding, channel gating and permeation) are conserved between amoeba and man. Consequently, attention can now be drawn to those few parts of the molecule critical to these functions. These studies therefore represent significant advances for the study of P2X receptor structure and function. Surprisingly, in Dictyostelium, the receptor was found in on intracellular membranes, especially the contractile vacuole. Gene disruption resulted in cells that were unable to regulate cell volume in hypotonic conditions. Cell swelling in the mutant was accompanied by a marked inhibition of contractile vacuole emptying. These findings therefore demonstrate a new functional role for P2X receptors on intracellular organelles. Given the ubiquitous presence of their ligand ATP, this raises the possibility that P2X receptors might also function as intracellular ion channels in other cell types, including higher organisms.

Involvement of ATP, Calmodulin and V-H(+)-ATPase in the Transport of the Cell Adhesion Molecule DdCAD-1 by Contractile Vacuoles

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In the classical protein transport pathway, proteins are processed through the rER-Golgi pathway. However, an increasing number of soluble proteins are now known to utilize non-classical pathways for secretion. The Dictyostelium Ca2+-dependent cell adhesion molecule DdCAD-1 (encoded by the cadA gene) is synthesized at the onset of development as a soluble protein, which lacks both signal peptide and transmembrane domains. Previous studies have shown that it is transported to the plasma membrane by contractile vacuoles. To elucidate the molecular mechanisms involved in its transport, an in vitro import assay was established utilizing contractile vacuoles isolated from cadA-null cells and the cytosol of wild-type cells. Initial characterization shows that the import of DdCAD-1 into contractile vacuoles requires exogenous ATP and Ca2+. However, mutating the DdCAD-1 Ca2+-binding sites has little effect on its import, suggesting the involvement of a Ca2+-dependent factor in the cytosol. Co-IP studies coupled with mass spectrometry led to the identification of Ca2+-calmodulin as a carrier protein that docks DdCAD-1 onto the contractile vacuole. Calmodulin binds to the proteolipid subunit vatP of V-H(+)-ATPase on the contractile vacuoles and unloads its cargo due to its higher affinity for vatP. DdCAD-1 then binds to the bafilomycin A1-binding site of vatP and is imported into the lumen of contractile vacuoles. Calmodulin interacts with vatP and DdCAD-1 through the same site, while DdCAD-1 utilizes different sites in its interaction with calmodulin and vatP. DdCAD-1 bound on the lumenal surface is targeted for surface presentation, while unbound DdCAD-1 is secreted. Interestingly, discoidin-I and several mammalian proteins containing a calmodulin-binding motif can also be imported into contractile vacuoles, suggesting that a variation of this protein transport mechanism is utilized in vertebrate cells. (Supported by the Canadian Institutes of Health Research.)

The C-terminal Domain of the Soluble Cell Adhesion Molecule DdCAD-1 is Involved in the Non-classical Transport Pathway via Contractile Vacuoles

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The cell adhesion molecule DdCAD-1 is encoded by the cadA gene. It is synthesized as a soluble protein at the onset of Dictyostelium development. The NMR solution structure of DdCAD-1 reveals two domains with β-sandwich architecture. The N-terminal domain resembles the structure of protein S of Myxococcus xanthus and mediates cell-cell adhesion via homophilic binding, while the function of the C-terminal domain remains to be elucidated. We have previously shown that DdCAD-1 is transported to the plasma membrane for either surface expression or secretion through contractile vacuoles. To elucidate the mechanism that underlies this novel protein transport pathway, recombinant proteins containing different domains of DdCAD-1 were used in pull-down experiments. Results show that the C-terminal domain of DdCAD-1 binds to Ca2+-bound calmodulin. The calmodulin-DdCAD-1 complex is responsible for the docking of DdCAD-1 onto the cytoplasmic surface of contractile vacuoles. In addition, the C-terminal domain of DdCAD-1 alone is sufficient for import into contractile vacuoles in the presence Ca2+-bound calmodulin and ATP. Chemical crosslinking studies using sulfo-SBED led to the identification of a calmodulin-binding site in DdCAD-1. Furthermore, microsphere-to-cell binding assays using cadA-null cells show that the C-terminal domain of DdCAD-1 is capable of tethering DdCAD-1 to a transmembrane anchoring protein on the cell surface. Co-capping experiments show that the calmodulin-DdCAD-1 complex can bind to cadA-null cells, indicating that the C-terminal domain of DdCAD-1 has distinct binding sites for calmodulin and the putative anchoring protein. Therefore, DdCAD-1 contains at least three distinct protein binding sites; one is present in the N-terminal domain and involved in its adhesive function during the initial stage of development, while two are associated with the C-terminal domain and involved in its import into contractile vacuoles. (Supported by the CIHR.)

AMPK-mediated mitochondrial disease in Dictyostelium causes increased susceptibility to Legionella infection.

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The cytopathology of mitochondrial disease has traditionally been believed to result from insufficient ATP to support normal cellular activities. Mitochondrial dysfunction in Dictyostelium leads to a consistent pattern of aberrant phenotypes regardless of whether it is caused by heteroplasmic disruption of rnl, the mitochondrial large rRNA gene or by antisense inhibition of expression of chaperonin 60, an essential nuclear-encoded mitochondrial protein (Wilczynska et al., 1997; Kotsifas et al., 2002). These phenotypes are impaired phototaxis and thermotaxis, slow growth and deranged multicellular development with a misdirection of cells into the stalk pathway. At higher levels of severity of the underlying genetic disorder (eg. more severe antisense inhibition) aggregation is also impaired. We reported recently (Bokko et al., 2007) that all of these phenotypes are caused by the activity of an energy-sensing cellular alarm protein AMPK. Ectopic overexpression of a truncated, active AMPK catalytic subunit (AMPKαT) mimics mitochondrial dysfunction in otherwise healthy cells, while antisense inhibition of AMPK expression in mitochondrially diseased cells suppressed all aberrant phenotypes. We have created mitochondrial disease by heteroplasmic disruption of a number of other mitochondrial genes and find that regardless of which gene is targeted for disruption, the pattern of phenotypes is the same. While phagocytosis and pinocytosis rates are normal in mitochondrially diseased cells, we have found that the amoebae are more susceptible to Legionella infection. This phenotype is also mediated by AMPK as shown by the fact that it is mimicked by AMPKαT overexpression and suppressed by AMPKα antisense inhibition in mitochondrially diseased cells. These results suggest that the downstream targets of AMPK signalling include one or more of the proteins regulating the ability of Legionella to replicate in a host cell.

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Exploring how Dictyostelium can tolerate a large number of endogenous proteins containing long uninterrupted polyQ stretches that are in the disease-causing range in humans

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There are 9 dominantly inherited polyQ diseases in humans arising from expansion of a CAG repeat at the level of DNA in the associated gene. A repeat length > 34-44 CAGs (depending on the gene) results in protein aggregation of the mutant protein in specific neurons and cell death. There are 68 putative genes in D. discoideum containing an uninterrupted stretch of largely CAA repeats1 that would give rise to ≥40Q at the protein level, suggesting there may be mechanism(s) to stabilise toxic polyQ structures and deal with polyQ degradation. Apart from spc97 (51Q)2, none of these proteins have previously been studied. A selection of 23 genes were analysed by RT-PCR to determine their developmental expression. PolyQ-containing proteins were assessed by immunoblot using the 1C2 antibody, that detects proteins containing >39Q as a result of multimeric binding to a polyQ epitope. Of these, 19 were detectable by RT-PCR. The majority (15) were expressed throughout development, while 4 had a more restricted pattern of expression: e.g. Q48c; DDB0188933 was only expressed at the loose aggregate stage. Cloning of the cDNA for a selection of these genes is underway to enable functional studies to be carried out in Dictyostelium, mammalian cells and by in vitro biophysical characterisation. N-terminal mutant (82Q) and normal (23Q) human huntingtin (htt) was over-expressed in Dictyostelium. Whilst a diffuse pattern of staining in the cytoplasm was seen in many cells, a significant number had 1-3 large, strongly stained foci within the cytoplasm, regardless of polyQ length. Expression of full-length mutant or normal htt (~10kb) was unsuccessful probably due to its size and high GC content. Further co immunostaining and co-immunoprecipitation experiments are underway to determine the subcellular localisation of these strongly staining spots and characterise other proteins that may be associated with these foci.

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Autophagic and necrotic cell death in Dictyostelium

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Investigations in simple biological models, taken for instance outside the animal kingdom, may benefit from less interference from other cell death mechanisms and from better experimental accessibility, while providing phylogenetic information. We previously showed that the protist Dictyostelium discoideum is a genetically tractable model for developmental autophagic vacuolar cell death. Further studies showed that: 1. The procedure that induced autophagy, vacuolization and death in wild-type cells led in atg1 autophagy gene mutant cells to impaired autophagy and to no vacuolization, demonstrating that atg1 is required for vacuolization. Unexpectedly, however, cell death still took place, with a non-vacuolar and centrally condensed morphology. We further investigated this developmentally-induced cell death occurring in an autophagy mutant, and found that it included a stereotyped sequence of events characteristic of necrotic cell death. Of additional interest, developmental stimuli and classical mitochondrial uncouplers triggered a similar sequence of events. Thus, Dictyostelium can show starvation-induced autophagic or necrotic cell death. 2. The Dictyostelium genome does not encode the main protein families at play in apoptotic cell death, namely the caspase (except an irrelevant paracaspase) and the bcl-2 families. Thus, the autophagic and necrotic cell death in Dictyostelium can take place with no interference from the apoptosis machinery, making this an even better model to study non-apoptotic cell deaths. 3. A genetic analysis of these cell death pathways may lead to some clarification of how the cell shifts from starvation-induced autophagy to autophagic cell death, or from energy-depletion lesions to necrotic cell death. A study of a relevant mutant obtained by random mutagenesis will be presented.

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Comparative genomic analysis of Acytostelium subglobosum, a group 2 species without stalk-cell differentiation

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The process of fruiting body formation in the cellular slime molds is one of the simplest examples of cell differentiation, and serves as a good model system for studies of developmental mechanisms. However, in the fruiting bodies of Acytostelium, stalks are made of empty cellulose tubes and all the cells survive starvation as spores. Since the acellular stalks do not represent a Utopia of equal right for survival but does a primitive stage of multicellularity unaccompanied by division of labor, we may ask about the genetic information necessary for cell differentiation by comparing Acytostelium with the well-developed Dictyostelium discoideum. Thus, we started the genome analysis of Acytostelium, where genetic control of development was totally unknown. Among the six Acytostelium species available, Acytostelium subglobosum, strain LB1, was chosen for analysis because of its relative easiness for our handling growth and development. Rough examination of genomic nature was attempted first by constructing and analyzing a SalI fragment library, which revealed a high G+C content (56%) of the genome and a moderately high gene density as D. discoideum. Currently, the whole-genome shotgun sequencing is being carried out. By a first survey of the results, even at the approximate coverage of 1/2 x, the existence of orthologous genes was noticed for most of those involved in spore and stalk cell differentiation in D. discoideum, suggesting the fundamental role of gene expression control for establishment of cell-type differentiation.

Mitochondrial Genome Evolution in the Social Amoebae

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A whole genome sample sequencing project in the social amoebae group provided us with a set of complete mitochondrial genomes from Dictyostelium citrinum, Polysphondylium pallidum and D. fasciculatum. These genomes plus the previously sequenced mitochondrial genomes of the model species D. discoideum and the related solitary amoeba, Acanthamoeba castellanii, within this deeply branching phylogenic group allow comparative genomic studies of the mitochondrial genomes. Mitochondrial genomes differ greatly in size and gene content across eukaryotes, however the mitochondrial genomes of social amoebae and A. castellanii are largely similar, permitting the differences to be clearly traced to a point in the phylogeny and to determine the frequency of genomic changes. Differences include a segmental rearrangement, tRNA content and location and group-I introns in cox1/2. We also calculated the evolutionary rate of change for every protein-coding gene, to identify fast-evolving and slow-evolving genes. Finally the genetic code in the social amoebae is the universal code while nearby relatives such as A. castellanii and farther relatives including all fungi and animals have alternative codes, so we investigate if social amoebae have the potential to change codes.

Repair of DNA Double Strand Breaks by Non Homologous End Joining in Dictyostelium

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DNA double strand breaks (DSBs), can be repaired by either homologous recombination (HR) or non-homologous end-joining (NHEJ). The first step in mammalian NHEJ is recognition of DNA termini by Ku. Ku serves as a platform to recruit different proteins to DNA DSBs including the DNA dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs). DNA-PKcs kinase activity is activated upon Ku-dependent DNA end-binding and phosphorylation of the nuclease Artemis by DNA-PKcs regulating its nuclease activity, allowing it to cleave ssDNA overhangs in order to make them compatible for ligation. Although Ku has been identified in a wide variety of organisms, until recently DNA-PKcs and Artemis were thought to be restricted to vertebrates. However, we identified a functional orthologue of DNA-PKcs in Dictyostelium (1). In order to assess the genetic requirements for NHEJ in Dictyostelium, we have analysed restriction enzyme mediated integration (REMI) of linearised plasmid DNA into the genome. DNA-PKcs and Ku80 are required for efficient REMI of compatible DNA termini into the genome of Dictyostelium. In addition, Dictyostelium are capable of processing DNA termini in order to facilitate REMI of incompatible DNA termini into the genome. The genetic requirements for these processing events will be discussed.

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Telomere structure in social amoebas

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Telomeres are important DNA-repeats at the end of chromosomes present in all known eukaryotes except e.g. Drosophila. They prevent the chromosomes from DNA-loss during S-phase or protect it from end-to-end fusion (recombination) with other chromosomes. During DNA synthesis telomeres are shortened, which finally can lead to cell senescence if the original length is not restored. Several conserved and species-specific proteins are involved in processes related to telomere length. One of them, the cellular ribonucleoprotein telomerase, can attach new repeats to the telomere and so counteracts the shortening. From the genome analysis of D. discoideum we learned that this species lacks the common eukaryote telomere repeats. Instead, parts of the rDNA palindrome sequences seem to be attached to all chromosome ends. The mechanism by which this is achieved and how the chromosome ends are maintained is currently unknown. So we set out to investigate the structure of the telomeres in D. discoideum in more detail. In a first step we analysed the telomere differences between the wild type NC4, and the two derived lab strains AX2 and AX4. This analysis showed that there is turnover of rDNA palindrome sequences over longer time periods. Currently, different Dictysteliida genomes from all 4 different phylogenetic groups are being sequenced. Our group is engaged in the analysis of D. fasciculatum, a group 1 member, and P. pallidum, a group 2 member. We found, that both D. fasciculatum, and P. pallidum (PN500) actually have a canonical eukaryote telomeric repeat. Thus, the unique mechanism by which chromosome ends are maintained evolved within the social amoebae. This evolutionary change has to be accompanied by a functional change in at least some of the gene products involved in normal eukaryote telomere maintenance. Therefore, we compared the respective protein sequences of D. fasciculatum and D. discoideum to each other. Results of this comparison will be presented.

The small RNA repertoire of Dictyostelium: microRNA candidates, small antisense RNAs and multiple classes of repeat-associated RNAs

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Small RNAs play crucial roles in regulation of gene expression in many eukaryotes. We have cloned and characterized 18-26 nt RNAs in Dictyostelium. This survey uncovered developmentally regulated microRNA candidates whose biogenesis, at least in one case, is dependent on a Dicer homolog, DrnB. Furthermore, we identified a large number of 21 nt RNAs originating from the DIRS-1 retrotransposon. Small RNAs from another retrotransposon, Skipper, were significantly up-regulated in strains depleted of the second Dicer-like protein, DrnA, and a putative RNA-dependent RNA polymerase, RrpC, respectively, whereas the expression of DIRS-1 small RNAs was not altered in any of the analyzed strains. This suggests the presence of multiple RNAi pathways in Dictyostelium. In addition, we isolated several small RNAs with antisense complementarity to mRNAs. At least three of these small RNAs may be derived from longer antisense transcripts.

The siRNA/miRNA machinery in Dictyostelium

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We have previously identified the RNA helicase HelF as a negative regulator of RNAi. We present biochemical data on the affinity of the HelF and Dicer dsRBDs to miRNAs and dsRNA that are consistent with a competition of the two proteins for substrates. We have started to analyze proteins and RNA that associate with HelF and with the EriA protein, a homolog of C. elegans Eri-1 that serves as a negative regulator of RNAi. Preliminary data on the identification of interaction partners will be presented. The recent description of miRNA candidates in Dictyostelium prompted us to investigate the effect of heterologous miRNA expression. Surprisingly, a strong developmental deffect was observed when mammalian prelet-7 was overexpressed in Dictyostelium cells. We currently investigate if this is due to specific targets or to a general overload of the miRNA machinery.

Visualization of nuclear functions in living cells

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Conventional methods for studying transcription such as Northern blot and microarray measure averaged mRNA levels of cell populations at fixed time points. However, these methods ignore the dynamic nature of transcription and cell-cell variations. We have previously developed a method to directly visualize the nascent RNA of defined genes in living cells and demonstrated that transcription of a gene can be discontinuous. To address how transcriptional behavior varies between different genes, we selected 4 “housekeeping” genes, abpE, act5, cinD and rpl15, and 4 developmentally induced genes, cofC, csaA, hspF and zfaA, and have monitored their transcription in living cells using fluorescent microscopy. We are also interested in the relationship between transcription and the cell cycle. We have developed a fluorescent marker, for use in living cells which allows us to assess whether a cell is in S-phase. We have made precise measurements of cell cycle phase timing and its variations under different conditions. In addition, we have been exploring the relationship between the cell cycle and differentiation.

Abstracts for Poster Sessions

#1-10	Dicty, Friends and Family
#11-21	Chemotaxis and Motility
#22-31	Actin and Associates
#32-42	Factors Regulating Development
#43-49	Organelles and Microtubules
#50-56	Features of the Genome

A modular set of Dictyostelium vectors with inducible expression

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We designed a set of vectors that allows some basic experiments on expressed genes, such as immuno blotting (FLAG), protein purification (GST), localization (GFP and mRFP) and protein-protein interaction (TAP). The set is optimized for ease of use and has the following convenient properties: MODULAR: Vectors are build from separate modules. The basic expression vector consists of four modules, (1) "E. Coli replication", (2), "Resistance marker", (3) "Dd replication" and (4) "Expression cassette". Modules can easily be exchanged, allowing for example the change of resistance marker from G418 to hygromycin. SMALL: All modules were trimmed down to their smallest possible size, while retaining their functional properties. Small vector sizes increase the ease of cloning and allow for the expression of relatively large inserts. An extrachromosomal expression vector can be constructed from the modules that is only 6.8 kb small. EXTRACHROMOSOMAL: All vectors were constructed to replicate extrachromosomally, as transfectants are most easily obtained using extrachromosomal vectors Fusion tags such as GFP or mRFP are also considered modules and these can be inserted into the "Expression cassette". The open reading frame of all constructed tags have the same distance to the muliple cloning site. Thus, an inserted gene that fuses in frame to one tag, will fuse in frame to all tags. Furthermore, the small size of the vector allowed the combination of the 2-plasmid tetracyclin-inducible expression system that is available for Dictyostelium into a single plasmid. Modules were created that encode a transactivator that is either activated (tet-on) or repressed (tet-off) by increasing amounts of doxycylin. By inserting such a transactivator module into the vector and replacing the constitutively active promoter by an inducible promoter, a vector was constructed in which the expression of a luciferase reporter gene could be regulated over a 10,000-fold range.

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Growth of Myxamoebae of the Cellular Slime Mold Dictyostelium discoideum in Suspension and Immobilized form on Living Bacteria

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Dictyostelium discoideum is of considerable interest as an expression system for the production of recombinant glycoproteins owing to its ability to perform a whole spectrum of post-translational modifications. However, the cultivation of this social amobae is not as easy as that of other common microbial expression systems. In the present study, D. discoideum AX2-R2 was grown on living E. coli B/r bacteria in shake-flasks and in a standard stirred tank bioreactors. The maximum cell densities of D. discoideum detected in the shake-flasks and in bioreactor were almost identical with 1.3x107 ml-1 and 1.4x107 ml-1, respectively. More than 99% of the bacterial biomass was consumed in both cases. Since immobilization technique often is applied in order to achieve high cell concentrations, D. discoideum was cultivated in the presence of different porous supports with the aim of studying its growth in an immobilized state using living bacteria as substrate. For small scale, a shake-flask cultivation system with external loop was applied, while on a larger scale a bioreactor was coupled with an external loop of a glass column containing a fixed bed of the porous support. Under these conditions, the maximum cell density of immobilized D. discoideum cells was 5.8x107 ml-1 on porous sintered glass (SIKUG 041) and 2.4x106 ml-1 on silicone foam (ImmobaSil R), respectively. The success of the immobilization process was documented by scanning electron microscopy.

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The N-glycosylation potential of Dictyostelium discoideum

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N-linked glycosylation is a major post-translational modification of proteins, but details about structures and enzymes responsible for their biosynthesis are lacking for many organisms. In the case of Dictyostelium discoideum, there were a number of relevant studies in the period 1985-1995; however, in the last few years, more modern analytical techniques have not been used to study N-glycans from this organism. Thus, considering also the sequencing of the genome, we believe the time is ripe to reappraise and extend the old data on this topic. Our initial data was based on Western blotting using an antiserum raised against a plant glycoprotein (horseradish peroxidase), which recognises core alpha1,3-fucose; these data clearly indicate that core fucose is expressed by AX3. Different proteins get fucosylated at early and later stages of development. Indeed, in the wild-type, the glycans expressed undergo a developmental shift as soon as the pseudoplasmodial stage is reached, from larger species such as Hex8HexNAc4Fuc1 and Hex8HexNAc3 to smaller types like Hex4HexNAc2Fuc1. Linkage analysis of Hex8HexNAc4Fuc1 revealed the finding of a novel structure with both inter- and bisecting GlcNAc as well as core fucose. Such fucosylation is missing from the HL250 mutant strain, even though both strains possess a core alpha 1,3-fucosyltransferase activity, as shown by HPLC and linkage analysis. Thus, the origin of the fucosylation defect in HL250 is verified to be due to a mutation in the GDP-D-Mannose-4,6-Dehydratase (GMD) gene. GMD is one of the two enzymes necessary for enzymatic transformation of GDP-mannose into GDP-fucose. Expression of recombinant forms of the two relevant Dictyostelium enzymes showed that the AX3 GMD is active, whereas the HL250-encoded form is not. In summary, modern methods verify that Dictyostelium has a novel N-glycomic potential and that it possess core fucosylation of the type found in plants and invertebrates.

Acquisition of novel pathways for catabolism of L-methionine in Escherichia coli during long-term chemostat co-culture with Dictyostelium discoideum

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We report on experiments aimed a selecting an artificial symbiosis between Dictyostelium discoideum Ax-2 and Escherichia coli MG1655 by long-term continuous co-cultivation in suspension. Co-culturing the organisms in a mineral medium supporting growth of E. coli invariably leads to the emergence of predation-resistant bacteria after a few dozen generations, entailing wash-out of the amoebae. A regimen of forced syntrophy was therefore developed, where growth of the bacteria was dependent on the capacity of D. discoideum to transform L-methionine into a source of sulfur for E. coli. In two independent experiments under these conditions, both organisms persisted for hundreds of generations in chemostat co-cultures. Eventually, however, the amoebae disappeared from the culture, with the E. coli population growing at high biomass yield, suggesting emancipation of the bacteria from supply of sulfur by D. discoideum. Inspection of the growth characteristics of these E. coli cells revealed that they had evolved the capacity to grow on L-methionine as the sole source of sulfur, albeit at the expense of reduced growth yield in the presence of limiting amounts of nitrogen. A model for the evolved pathway allowing utilization of L-methionine will be presented. To block emergence of this pathway, in a third experiment a co-culture was run with L-methionine as the sole source of sulfur and nitrogen. Again, predation-resistant E. coli were selected which are able to utilize L-methionine as their sole source of sulfur and nitrogen. Analysis of the evolved metabolic pathway is under way. We plan to identify and delete the E. coli genes that were recruited for evolution of the novel pathways to design new adaptive scenarios for experimental evolution of a symbiotic association.

Protists bearing gifts: Amoebae as natural hosts of emerging pathogens

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The number and widespread nature of some free-living amoebae such as the Acanthamoebae makes them major predators of a variety of microbial communities. The capacity of Acanthamoeba spp. to predate single cells or graze microbial biofilms coupled with the ability of some ingested microbes to survive and multiply within amoebae, positions Acanthamoeba as a significant reservoir of environmental microbes. Pathogens such as Legionella spp., Mycobacteria spp., and Francisella spp. exploit Acanthamoeba as both a replicative niche and a 'safe haven'. In addition members of the Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, the Bacteroidetes, and the Chlamydiales have been found as stable endosymbionts of various Acanthamoeba isolates. Recent findings have revealed an even more intricate trilateral associations - Acanthamoeba containing two phylogenetically distinct bacterial species, suggesting that free-living amoeba might be hot spots for lateral gene transfer between intracellular bacteria. Host-prey associations and the predation-survival struggles that result have allowed for the evolution of varied virulence factors. The ongoing genome sequencing project of Acanthamoeba castellanii offers new perspectives to study virulence strategies of pathogens regarding their persistence and multiplying in natural hosts. By investigating the transcriptional and translational profile of Amoebae infected with pathogens we wish to gain a genome scale perspective on the evolution of virulence in a subset of these pathogens. Comparison with data from the surrogate host Dictyostelium discoideum should help in identifying accords and differences in the pathogenicity of intracellular microbes. The extensive studies of mechanisms involved in phagocytosis in the social amoeba give us a platform by which we can analyse the interactions and compare it with other systems such as Caenorhabditis elegans, Drosophila melanogaster and mammals.

Dictyostelium transcriptional response upon exposure to Pseudomonas aeruginosa: common and specific effects from PAO1 and PA14 strains.

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Pseudomonas aeruginosa is an opportunistic human pathogen, infecting immunocompromised patients and causing persistent respiratory infections in people affected from cystic fibrosis. It has been reported differences in the virulence of different Pseudomonas strains and isolates. PA14 shows higher virulence than PAO1 in a wide range of hosts including insects, nematodes and plants and the precise cause of this difference is not known. It is likely that the interaction of Pseudomonas aeruginosa with these environmental hosts, including amoebas is important for the maintenance and evolution of pathogenic mechanisms. In this context the social amoeba Dictyostelium discoideum has been described as a suitable host to study virulence of Pseudomonas and other opportunistic pathogens. We have now compared the virulence of PAO1 and PA14 using Dictyostelium and studied the transcriptional response of the social amoeba upon infection. Our results showed a higher virulence for PA14 over PAO1 in Dictyostelium using different platting assays. Dictyostelium cells were exposed to PAO1 or PA14 (mixed with an excess of the non-pathogenic bacterium Klebsiella aerogenes for food supply) and after 4 hours, cellular RNA extracted. A three way comparison was made using microarrays between RNA samples from cells treated with the two different strains and control cells exposed only to Klebsiella. There was a broad overlap in the transcriptional response: 359 genes showed evidence of similar differential regulation in both conditions compared with the control. Effects on metabolism, signaling, stress response and cell cycle can be inferred from the genes affected. Interestingly, there are 170 genes differentially regulated between PAO1 and PA14. Our results suggest the induction of a common transcriptional response and also a specific response for each of the strains that might contribute to their different virulence.

Exploitation and co-existence in strains of Dictyostelium giganteum

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We have been working with Dictyostelids isolated from undisturbed forest soil in the Mudumalai reserve, southern India. A molecular phylogeny based on small subunit ribosomal RNA (SSU rDNA) partial gene sequences from 17 wild isolates belonging to 5 species and four isolates from three laboratory strains indicates their pattern of relatedness. The experiments reported below were carried out with genetically distinguishable strains of Dictyostelium giganteum. The strains intermingle freely in the laboratory. In other words, they can form chimaeric aggregates and fruiting bodies. When they do so, one strain often exploits the other during sporulation - that is, it forms a disproportionate number of spores relative to the other. Despite this, the strains co-exist in close proximity in the wild. We have begun to address questions that are raised by this observation. 1) What might account for the co-existence of different strains of same species even when, one is more efficient at sporulating than the other in a chimaera? 2) How do group-level traits such as aggregation territory size, overall spore forming efficiency and strain productivity (the spore forming efficiency of a given strain when part of a mixture) compare between clonal and chimaeric groups? On the basis of the cases studied so far, our preliminary findings are as follows: (a) There is a trade-off between different life cycle components of fitness, and this may help two strains to co-exist; (b) With respect to territory size and spore forming efficiency, but not strain productivity, clonal groups and chimaeras are comparable. We discuss these results in the light of evolutionary models for cooperative behaviour in the cellular slime moulds.

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Genetic mechanisms underlying cheating and its control in D discoideum development

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Development of multicellular organisms is built on a cooperative framework. The complexity of social behaviour seen between cells during development is manifest in the temporal and spatial expression of genes. This must be a highly coordinated process for a properly patterned organism to form. However, within this cooperative framework, competition between cells is still permissible and must be tightly controlled. If competitive processes are not controlled then cheaters can emerge that exploit the cooperative system. I am interested in the genetic mechanisms of competitive processes during development and their control. Dictyostelium is an excellent model to study cooperation and competition because development is a social behaviour. During development, cells differentiate into two cell types; viable spores and dead stalk. By preferentially sorting to the spores in a mixing experiment, a mutant strain could 'cheat death'. One genetic mutant, ChtA, has been shown to exhibit cheating behaviour in mixing experiments but is controlled by its inability to develop in isolation (1). We have designed a modified version of the screen used to isolate ChtA to find more cell-fate choice mutants that preferentially become spores. Importantly, the modifications of this screen allow for analysis of a particularly interesting class of mutants: those that cheat and have a normal phenotype. Explaining 'subtle' phenotypes like these might benefit from a genetic approach. After two rounds of selection morphological mutants, including ChtA, were enriched. In addition, two techniques have been developed that will be used for high-throughput analysis of behaviour and mutant genotypes in future rounds.

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Comparative Genomics in Social Amoebas

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After completion of the D. discoideum genome in 2005 (1,2) it was obvious that a comparative genomics approach has to follow this endeavour to address the following questions: i) How different are the members of this evolutionary branch in terms of general genomic properties ii) Which genes are species-specific and which genes are genus specific iii) Can we define evolutionary events which led to the formation of this group of organisms iv) Can we find synteny between distantly related species? Sequencing of two species within the social amoebas is currently under way. The targeted species D. fasciculatum belongs to group 1 and P. pallidum to group 2 according to (3). P. pallidum is a cosmopolitan species, whereas D. fasciculatum is restricted to middle Europe. So far we have accumulated enough data to make rough estimates for both species on genome structures, gene similarities, and synteny. One striking difference between the model organism D. discoideum and the related species is the different nucleotide bias in their genomes. Furthermore, D. discoideum seems to occupy a unique and extreme position among social amoebas in other genomic properites like repeat content and palindrome length. Based on our data we will present first insights in the genome evolution of social amoebas.

1 Glöckner, G. et al. (2002): Sequence and analysis of chromosome 2 of Dictyostelium discoideum. Nature 418:79-85
2 Eichinger, L. et al. (2005): The genome of the social amoeba Dictyostelium discoideum. Nature 435:43-57
3 Schaap, P. et al. (2006): Molecular phylogeny and evolution of morphology in the social amoebas. Science 314:661-3

Dictyostelids from the Mediterranean region and systematics

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Dictyostelids are found living in a variety of soils types and vegetation. Previously, almost nothing was known about dictyostelids from southern Europe and especially from the Mediterranean region. For this reason we started a project with these objectives: make an inventory of the dictyostelids in this part of the world, develop a biosystematic study of these species, analyse the taxonomic value of morphological and molecular features, establish distribution and ecology, make this information publicly available by online system. Sampling was done in Mediterranean woodlands and largely evergreen oak forests. 22 species of dictyostelids were isolated, belonging to the 3 traditional genera (Romeralo & Lado, 2006). The first taxonomic study of the Iberian dictyostelids was presented (Romeralo PhD thesis 2007). The results increased the number of reported species at the Iberian Peninsula by 300%. Since individual isolates were often difficult to differentiate, sequences were determined for the internally transcribed spacer (ITS) region of the ribosomal RNA operon. This was the first study of its kind for species of dictyostelids. These data proved to be very useful for phylogeny at different taxonomic levels (Romeralo et al, 2007a). Morphological and molecular data were also used to address problematic species for example, revision of the taxonomic concept for “Dictyostelium sphaerocephalum” (Romeralo et al, 2007b). The first molecular phylogeny of the Dictyostelia (Schaap et al, 2006) showed four major groups. This phylogeny was based on a very slowly evolving gene (SSU rDNA), and therefore did not resolve many of the species level relationships. In order to resolve these, we sequenced the ITS region for all species. By combining ITS and SSU rDNA sequences, we are now able to resolve most of the fine level relationships as well (Romeralo, Spiegel & Baldauf, in prep). We are now working on a formal revision of Dictyostelia (Romeralo, Baldauf & Schaap, in prep).

The evolution of cAMP receptor diversity and function in the Dictyostelids

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In *Dictyostelium discoideum*, cAMP signaling plays an important role for aggregation and cell differentiation. *Dictyostelium discoideum* has four cAMP receptors (cAR1-cAR4), which show different expression patterns and functions during development. The molecular phylogeny of the Dictyostelids shows subdivision of all known species into 4 major groups. *D. discoideum* lies within the most derived Group 4.

We investigated cAMP receptor diversity throughout the Dictyostelid phylogeny. All tested group 4 species have 4 receptor genes which are similar to cAR1-cAR4. The group 2 species *Polysphondylium pallidum* has two cAR genes, *tasA* and *tasB.* They are most similar to each other and then to cAR1. Both are expressed during post-aggregative development and *tasA* was shown earlier to function in branch formation. All tested group 2 species except the Acytostelids also have 2 cAR genes which are most similar to *tasA* and *tasB*. From species in Group1 and Group 3, we isolated only a single cAR gene. All cARs in Group 1, 2 and 3 are more similar to cAR1 than to any of the other *D. discoideum* cARs.

To analyze functional evolution of cAMP receptor, we made *tasB* single or *tasA tasB* double knockout mutants in *Polysphondylium pallidum*. The *tasB* knockout mutant is more sensitive to inhibition of fruiting body formation when culminating directly on the bacterial growth plates. When developed on non-nutrient agar, the *tasB* knockout mutant formed fruiting bodies like wild type cells. On the other hand, the phenotype of a double *tasB tasA* knockout mutant is quite severe. *tasB*$^{-}$*tasA*$^{-}$ cells aggregate but never form normal fruiting bodies. Instead they form stunted club-like structures, which contain random arrays of stalk cells and round microcyst-like cells instead of spores. These results suggest that extracellular cAMP signaling is essential for fruiting body morphogenesis and spore differentiation in *P.pallidum.* Though essential for proper formation of the stalk, extracellular cAMP signalling is not required for differentiation of the stalk cells.

Evolution of extracellular cAMP phoshosdiesterases

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The Dictyostelid amoebae are a diverse group of organisms that show a simple form of multicellularity with a range of phenotypes. In the model system Dictyostelium discoideum, extracellular cAMP pulses coordinate the aggregation of starving amoebas and are also implicated in the subsequent formation of migrating slugs and culminating fruiting structures. cAMP is produced by an adenylyl cyclase A, and degraded by an extracellular phosphodiesterase, PdsA. Together with the cAMP receptors, cARs, these enzymes are essential for oscillatory cAMP signalling. We are interested in the evolutionary history of extracellular cAMP signalling and searched for PdsA genes in the four major subdivisions of the Dictyostelids. The PdsA gene is deeply conserved in the dictyostelids with gene duplications in some branches. There are striking differences in developmental regulation of PdsA across the Dictyostelid phylogeny. Additionally the younger PdsA proteins appear to have a higher affinity for cAMP.

Single-molecule analysis of cAR1-eYFP during chemotaxis

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Until now, the dynamics of the chemoattractant-receptor has been neglected in models describing directional sensing mechanisms. Using Single Molecule Microscopy the dynamic behavior of cAR1-eYFP molecules was studied. In resting cells two receptor populations where found, a mobile and an immobile population. Treatment with latrunculin reduced the immobile fraction 2 coupling, via cytoskeleton anchoring, controlsαsize suggesting that G the mobility of cAR1. When studying receptor behavior in chemotaxing cells, the fraction of mobile receptors was found to be larger at the anterior while the posterior receptor populations remained distributed like in resting cells. Furthermore, we found this mobility shift to be 2-protein. An in silicoαlinked to the uncoupling/activation of the G model confirmed that a single activated/mobile receptor can activate multiple G-proteins thereby providing a mechanistic basis for a primary amplification step at the level of the G-proteins in current theoretical models describing directional sensing. Currently we are conducting diffusion analysis of cAR1-eYFP to get insight in membrane organisation and possible membrane domains in both resting and chemotaxing cells.

Quantitative analysis of cell movement and fluorescence intensities

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The use of fluorescent proteins has enabled scientists to visualize protein behaviour in living cells. GFP- and RFP-fusion proteins have proven very useful, especially in studies of dynamic cellular processes such as chemotaxis. The increasing use of fluorescent tags calls for more sophisticated ways of analysis. The program Quimp was developed to simultaneously quantify cell movement and membrane associated fluorescence intensities using active contours (Dormann D., Libotte T., Weijer C.J., Bretschneider T. Cell Motil Cytoskeleton 2002 Aug;52(4):221-30). We have greatly improved the cell movement analysis by Quimp. By keeping track of the position of the nodes that define the cell outline, individual parts of the cell membrane (or -cortex) can be followed in time. We show that the program accurately measures extension and retraction of the cell, as well as fluorescent intensities of (multiple) fusion proteins. The use of 2D color plots enables easy interpretation of protein localization within a moving cell. Furthermore, the local movement of the cell can now be quantitatively correlated with fluorescent intensities.

Dictyostelium Chemotaxis is Regulated and Sensitized by Extracellular Signal Degradation

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Starvation of the social amoebae, Dictyostelium discoideum, induces a developmental program where individual cells sense and migrate towards an attractant molecule in a process known as chemotaxis. This behavior is guided by cyclic adenosine monophosphate (cAMP), which is detected by a family of G protein-coupled receptors. Soon after the onset of starvation Dictyostelium cells release cAMP. This stimulates cAMP receptors and initiates the expression of a set of developmentally regulated genes. This signaling is propagated by the highly regulated synthesis and degradation of extracellular cAMP. The degradation is controlled by the phosphodiesterase PdsA. Cells lacking PdsA (pdsA-) do not express cAMP receptors or other components required for signal reception and relay. These cells remain unpolarized and are unable to sense or move toward a cAMP source. The exogenous addition of partially purified PdsA to pdsA- cells during development rescues this defect and allows the pdsA- cells to express the components required for signal recognition and polarize. The cellular distribution of signaling molecules in treated pdsA- cells is identical to wild type cells and the cells are able to move towards a point source of cAMP. However, the differentiated pdsA- cells fail to align in a head-to-tail fashion during chemotaxis and respond to a narrower range of cAMP concentrations. When DTT is used to inhibit PdsA during chemotaxis, wild type cells exhibit similar behavior to differentiated pdsA- cells. Furthermore, treating chemotaxing wild type cells with exogenous PdsA decreases the range and the speed of cells responding to a point source of cAMP. This work shows that signal degradation is required for gene expression as well as for proper gradient dissemination. We propose that PdsA plays a key role in shaping cAMP gradients, thereby allowing cells to sense a wide range of attractant concentrations and properly relay signals to neighboring cells.

Direct measurements of intracellular cAMP concentration in Dictyostelium cells

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Intercellular signaling of starved Dictyostelium cells is known to be mediated by intracellularly synthesized cAMP that the cells release and respond to extracellularly. The molecular mechanisms that govern the initiation and maintenance of the signaling processes as well as how cell aggregation centers are determined remain largely unknown. Here we present a new approach that allows us to measure intracellular cAMP concentration at the single and multiple cell level, using a FRET-based cAMP sensor protein combined with live-cell imaging. Using this technique, we are able to recover the well-documented large-scale cell aggregation wave pattern, and we are able to show that these pattern result from oscillations of cytosolic cAMP concentration at the single cell level. Moreover, at low cell density we observe responsiveness to sub-nM extracellular cAMP concentration suggesting that very little cAMP is necessary to start the communication pattern.

3D Reconstruction and Motion Analysis of Living Cells

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How cells crawl, or translocate, is fundamental to a wide array of biological phenomena ranging from Dictyostelium discoideum aggregation, to white blood cell function and embryogenesis. Furthermore, animal cell locomotion involves a large number of regulatory and cytoskeletal components that give rise to unexpectedly complex behaviors that can, in turn, be manipulated to induce directional movement or chemotaxis. In an effort to develop a realistic model of how a cell crawls and reads the spatial and temporal information in gradients of chemoattractant, we developed the Dynamic Image Analysis System (DIAS), an integrated imaging and software system for high-resolution 2D and 3D motion analysis and dynamic reconstruction of live cells. DIAS provides quantitative data of centroid based parameters such as velocity and direction of movement in two and three dimensions as well as analyses of dynamic cell shape changes, extension and retraction of pseudopodia and filopodia, organelle movements, and cytoplasmic flow. We have applied these tools to comparisons of mutant and wild type Dictyostelium amoebae, analysis of human neutrophil behavior in genetically based diseases that may affect immune cell behavior (Shwachman-Diamond Syndrome, Polycystic Kidney Disease) and to embryogenesis in several model organisms, including some with known mutations. We will demonstrate how the application of these technologies has led to accurate behavioral phenotypes in Dictyostelium, human neutrophils and embryogenesis in C. elegans and zebrafish. We will also present unique 3D-DIAS capabilities that are under-utilized but may be of value for some studies through collaboration.

Identification of costars as a novel gene involved in Dictyostelium chemotaxis

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Chemotaxis, a process in which cells move towards the source of diffusible chemoattractants, is crucial in many physiological and pathological processes. Through analyzing mutants generated by REMI mutagenesis, we have identified a novel chemotaxis-related gene, which we named costars (cosA). The predicted 82-aa Costars protein shares significant homology to the C-terminal part of STARS, a mammalian striated muscle activator of Rho signaling. RT-PCR analysis found that expression of cosA peaked at early stages of development. Examination of cosA- mutant found aberrant developmental morphology both on bacterial lawns and on non-nutrient plates, severely defective chemotaxis responses to cAMP in the small-drop assay, and abnormal actin distribution patterns in TRITC-phalloidin staining; all these defects were corrected by expressing a full-length cosA cDNA in the mutant. In vivo actin polymerization assay showed significantly increased levels of actin assembly in cosA- mutant. Consistent with the reported actin-binding activity of the C-terminal part of STARS, results of in vitro actin sedimentation assay suggest that Costars binds to actin. Interestingly, this gene appears to be highly conserved among diverse species; the Dictyostelium and mammalian Costars (mCostars) proteins share 69% identities and 83% similarities in their amino acid sequences. Expression of the human mCostars in cosA- Dictyostelium cells rescued defects in development, chemotaxis to cAMP, and actin distributions, indicating that mCostars is an ortholog of the Dictyostelium counterpart. In vitro actin sedimentation assay showed binding of GST-mCostars to actin. HA-mCostars colocalized with actin, but not F-actin, in immunofluoresence cell staining. Overexpression of mCostars in breast cancer cells resulted in increased cell migration in the transwell assay. Taken together, our results suggest that Costars proteins participate in regulation of actin cytoskeleton and cell migration.

The Role of Calcium in Basic Cell Motility, Lateral Pseudopod Formation and Chemotaxis

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The capacity to suppress lateral pseudopod formation is carefully orchestrated during basic motile behavior in the absence of chemoattractant, and during chemotaxis (Wessels et al., 1984 Cell Motil. Cytoskeleton, 27; Wessels et al. 1998, Cell Motil.Cytoskeleton 41; Soll et al., 2002, J. Musc. Res. Cell Motil. 23). By mutational analysis, several genes have been demonstrated to play a role in this process, including myosin II, myosin IB, myosin IF, myosin IA, clathrin, sphingosine-1-phosphate lyase, and, most recently, the tumor suppressor pten (Wessels et al, 1991, Cell Motil. Cytoskeleton 20; Titus et al., 1993, Mol. Biol. Cell 4 ; Wessels et al. 1996, Cell Motil.Cytoskeleton 33; Stites et al. 1998, Cell Motil.Cytoskeleton 39; Wessels et al. 2000, J. Cell Sci. 113; Zhang et al, 2002, J. Cell Sci. 115; Falk et al., 2003, J. Cell Sci. 116; Kumar et al. 2004, Cell Motil. Cytoskeleton 59; Heid et al., 2004, J. Cell Sci 117; Heid et al., 2005, J. Cell Sci.118; Wessels et al. 2007, J. Cell Sci. 121). Because of the role calcium homeostasis plays in a variety of cellular functions including myosin function, we have examined its role in cell motility and chemotaxis. We demonstrate that the concentration of extracellular calcium has a dramatic influence on cell behavior and the suppression of lateral pseudopod formation. At suboptimal concentrations, cells are slow. Less persistent and exhibit a decrease in chemotactic efficiency. Cells abnormally form multiple lateral pseudopods just as they do in the mutants 3XASP, S-1-P lyase-, pten-, clathrin-, myoA-, myoB- and myoF-. Increasing extracellular calcium to the optimum concentration rescues some but not all of these mutants, leading to interesting models for the regulation of lateral pseudopod formation during basic cell motility and chemotaxis.

Domain analysis of cGMP-binding protein C

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GbpC is the high affinity target for the second messenger cGMP in Dictyostelium. GbpC is a 300 kDa-protein and has a domain topology that is conserved among proteins that belong to the ROCO-family. These proteins have a central domain core, consisting of a Ras GTPase called ROC, a COR and a MAPKinase-kinase-kinase domain. Furthermore, GbpC has Leucin-Rich Repeats, a RasGEF, DEP, GRAM and two cGMP-Binding domains. This domain structure suggests an intramolecular signalling cascade, in which cGMP-binding causes the RasGEF domain to activate ROC, thereby leading to full activation of the kinase domain, which serves as the output for this protein. In order to investigate this putative activation mechanism, several mutations and truncations of the GbpC gene were created and expressed in GbpC null cells. In a small population assay, GbpC null cells show no chemotaxis anymore under specific circumstances. The ability of all constructs to rescue this phenotype was investigated, which gives indications about the importance of the different domains for full activity of GbpC. Using this assay, we show that the Leucin Rich Repeats, ROC- and Kinase domain of GbpC are all essential for activity. On the contrary, cGMP-binding only partly contributes to, but is not essential for, full activity. Further analysis of the C-terminal part of GbpC shows that GbpC's RasGEF domain is indeed capable of catalyzing GDP/GTP exchange on its own Ras-domain in vitro, and that most likely, the GRAM-domain is responsible for cGMP-dependent membrane localization of the protein. These findings lead to a model for the activation mechanism of GbpC during chemotaxis.

A genome wide REMI screen for new regulators of chemotaxis and cytokinesis

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Chemotaxis, or cell migration in response to chemical cues, is regulated by a number of redundant signaling pathways. cAMP binding to the G protein-coupled receptor, cAR1, initiates this signaling, which ultimately results in actin polymerization and migration up the chemoattractant gradient. Despite the significant progress that has been made in this field, there are still unknowns. To address this, we are using a collection of Restriction Enzyme Mediated Integration (REMI) –induced mutant strains (a kind gift from the Kay Laboratory), to screen for new regulators of chemotaxis. First, we have isolated clonal populations of over 700 morphological strains that display defects during fruiting body formation on bacterial lawns. Second, we are screening these strains for their ability to chemotax using both the Small Population Assay and the Agar Cutting Assay. Using these well-established techniques, we are able to determine each strain's ability to respond to multiple doses of cAMP and folate as compared to wild-type cells. We also monitor cAR1 expression, induction of cell polarity and aggregate morphology under a variety of conditions. To date, we have found that many of the morphologically defective strains respond either weakly or not at all to chemoattractants. These are being further characterized with respect to actin polymerization, cGMP production and PIP3 levels after stimulation. Since some genes that are involved in aggregation also play a crucial role in cytokinesis, we are further screening the morphological mutants for cytokinesis ability in collaboration with the Robinson Laboratory. Strains are grown in suspension culture to determine growth rate and nuclei distribution per cell. We have observed some overlap in mutants that do not respond to chemoattractants and those that display growth defects and multinucleate cells. Genes will be identified and all information displayed in a database which we will make accessible to the community.

Relevence of association between Filamin and an IQ-GAP related protein, GAPA in cell division

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Filamin is an actin cross-linking protein allowing actin filaments to form orthogonal networks. Apart from its role in actin cytoskeleton organization, Filamin is also required for slug phototaxis. We identified the IQ-GAP related protein GAPA as an interactor for Dictyostelium Filamin. GAPA has been shown to be required for cytokinesis. We found that the interaction is through the actin binding domain (ABD) of Filamin and the GAP domain in GAPA. GAPA is enriched in the cleavage furrow in dividing cells, and expression of GFP-tagged GAPA completely rescued the strong cytokinesis defect in GAPA null cells. Enrichment of GAPA in the cleavage furrow was unaltered in myosin II null cells, indicating that the role of GAPA in cytokinesis is independent of myosin II. Filamin null cells do not show defects in cytokinesis, but show the presence of nuclei free particles, or particles with an extremely small nucleus. We have also confirmed by GST pulldown and immunoprecipitations interaction between GAPA and cortexillin, another actin binding protein required for cytokinesis. Our future work focuses on the relevance of the GAPA - filamin interaction in regulating proper cell division.

Myosin Heavy Chain Kinase-A Can Inhibit Myosin II Activity by Competing Directly with Myosin II for Binding to Actin Filaments

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Myosin heavy chain kinase A (MHCK-A) catalyzes the disassembly of myosin II filaments in *Dictyostelium discoideum* via phosphorylation of the "tail" region of the myosin heavy chain. MHCK-A possesses an amino-terminal "coiled-coil" domain (amino acids 1-498) that mediates the oligomerization, cellular localization to the actin-rich cell cortex, and actin binding activities of the kinase. Recent studies from our lab have revealed a unique relationship between MHCK-A and F-actin, whereby F-actin is a potent activator of MHCK-A activity (50-fold); and MHCK-A, in turn, possesses the ability to organize actin filaments into bundles. In the current study, we examined the effects of "coiled-coil" domain binding to F-actin on the ability of myosin II (S1 fragment) to interact with F-actin. Our results demonstrate that S1-myosin II binding to F-actin is inhibited in a dose-dependent manner by the "coiled-coil" domain of MHCK-A, and that this is not due to "coiled-coil" domain binding to the myosin II. Over-expression of the "coiled-coil" domain in *Dictyostelium* cells leads to decreased growth rates and increased multinuclearity when these cells are cultured in suspension, suggesting that "coiled-coil" domain can inhibit myosin II binding to F-actin in vivo and thus may play a physiologically significant role in regulating myosin II-mediated contraction in the cell. By contrast, cells over-expressing the carboxy-terminal WD-repeat domain of MHCK-A do not exhibit such defects in cytokinesis. Nevertheless, preliminary studies indicate that the WD-repeat domain may be involved in regulating "coiled-coil" domain binding to F-actin since pull-down assays with GST-tagged WD-repeat domain and untagged "coiled-coil" domain have revealed a direct interaction between these domains. We speculate that the disruption of this intramolecular interaction is a key regulatory (activating) event that may be triggered by autophosphorylation of the kinase.

The D. discoideum actinome

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The Dictyostelium discoideum genome contains a relatively large number of genes that code for actin and actin-related proteins. We have analyzed the D. discoideum actinome and compared it with data from 10 genomes of selected model organisms. The D. discoideum actinome comprises a total of 42 actins and actin-related proteins. Among them are 17 conventional actins (Act15-group) that share identical amino acid sequences but are encoded by 17 distinct genes. eleven conventional actins have distinct changes in their amino acid sequences, eight proteins with a characteristic actin sequence profile are actin-related proteins (Arps), six genes are potential pseudogenes. The Act15-group forms two gene clusters with eleven genes on chr2 and four on chr5 which suggests waves of duplication events especially on chr2. A homology search of the human genome using a protein sequence from the Act15-group as a query finds -isoform as best hit (bits/score = 724; E-value: 0.0). This suggests that the Act15-group reached already in D. discoideum a nearly perfect protein sequence which changed only marginally upwards to higher eukaryotes. Despite the high number of actin genes, in a protein preparation more than 95% of total actin in the amoeba consists of only one sequence variant (Vandekerckhove and Weber, 1980), which now turned out to be an actin from the Act15-group. This suggests that the other actin variants do not play a major role in the overall microfilament system but might be very specific regulators of cytoskeletal dynamics in space and time.

Vandekerckhove J, Weber K (1980) Vegetative Dictyostelium cells containing 17 actin genes express a single major actin. Nature 284:475-477

Actin-related proteins in *Dictyostelium discoideum*

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Actin-related proteins (Arps) constitute an evolutionarily conserved protein family. Functional studies showed important activities for the Arp2/3 complex in actin filament dynamics and for Arp1 in dynein-mediated movement of vesicles along microtubules. More recently, Arps and conventional actin have also been reported as constituents of chromatin remodeling complexes. The knowledge on the nuclear functions of Arps was achieved mostly from work with budding yeast and not much is known on the role of Arps in other organisms.
All Arp subfamilies specified in mammals are also present in *Dictyostelium discoideum*. We have started to explore the expression and functions of the Arps in *Dictyostelium* by creating null mutants (Arp5, Arp8). Mutant cells lacking Arp8 have a pleiotropic phenotype with defects during development. GFP-Arp8 localizes to the nucleus consistent with the notion that Arp8 is a component of the INO80 complex. INO80 is one of the ATP-dependent chromatin remodeling complexes studied in yeast and contains Arp4, Arp5, Arp8 and actin. Database searches showed that genes for most of the INO80 constituents are also present in *Dictyostelium*.
In order to study the subcellular localization we used fluorescently labeled and Flag-tagged fusion proteins (Arp1, Arp4, Arp6, Arp8, and Arp11). *Dictyostelium* Arp1 is located at centrosomes corresponding to the distribution described for Arp1 (centractin) in other organisms, whereas Arp4 and Arp6 are also in the nucleus indicating an involvement in chromatin reorganization.

Possible components of the signalling cascade from endosomes to the cytoskeletal machinery in Dictyostelium discoideum.

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A novel class of lysozymes was recently identified in D. discoideum. The knock-out of its main member, AlyA, leads to increased phagocytic rates without affecting macropinocytosis (Müller et al, 2005). The esterase Gp70 was found to be up-regulated in the AlyA-null cells, and over-expression of Gp70 in wild type cells (AX2) leads to the same phenotype as AlyA knock-out. These results raised the idea of a signalling pathway between reduced lysozyme levels, elevated Gp70 content, and increased phagocytosis. A cDNA-microarray analysis on both mutants revealed a series of genes up-regulated in AlyA-null cells, but not in Gp70 over-expressing cells, as well as eight equally down-regulated genes in both strains. These genes seem to be possible candidates for the signalling cascade from phagosomes to the cytoskeletal machinery. Our current data shows the involvement of SSE346, SSJ758 and SLB350 in the signalling cascade between Gp70 and increased particle internalization. Interestingly, the knock-out of these proteins led to divergent phenotypes. The SSE346-KO strains are characterized by increased phagocytic rates as well as enlarged plaques on bacterial lawns, when compared to the wild type. SSJ758-KO leads to enlarged plaques but exhibit normal phagocytosis. On the contrary, SLB350-KO show increased phagocytosis but no variation in plaque size. We are currently analysing other candidates in order to understand the succession of events linking the lysosomal defect with the cytoskeletal adaptation.

Müller, I., Subert, N., Otto, H., Herbst, R., Rühling, H., Maniak, M. and Leippe, M. (2005) A Dictyostelium Mutant with Reduced Lysozyme Levels Compensates by Increased Phagocytic Activity. J. Biol. Chem. 280, 10435-10443, 2005

Characterization of Coronin7 in Dictyostelium discoideum

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WD-repeat proteins play a role in a variety of cellular events, including signal transduction, transcriptional regulation, cytoskeleton remodelling and regulation of vesicular trafficking. One family of WD-repeat containing proteins are the coronins. All coronins have a conserved N-terminal core domain that includes seven WD-repeats folding into a three dimensional propeller like structure. Our work focuses on the coronin7 homolog in Dictyostelium. In contrast to other coronins, coronin7 possesses two WD-repeat motifs that form a core region in the N-terminal and another one at the C-terminal region. The homologous C. elegans POD-1 and Dictyostelium coro are known to regulate the actin cytoskeleton, but also govern vesicular trafficking as indicated by mutant phenotypes. Furthermore, mutants have severe developmental defects. Analysis of Dictyostelium coronin7 GFP fusions and immunofluorescence studies with specific monoclonal antibodies show localization at actin-rich structures in the cells. Dynamic accumulation at phagocytic and pinocytic cups leads to the assumption that coronin7 may participate in the remodelling of the cortical actin cytoskeleton. The 105 kDa protein is expressed throughout Dictyostelium development and is especially prominent during early aggregation state. Furthermore we present data from a coronin7 knockout cell line where first experiments show premature development in respect to wild type.

Functional characterization of a VacuolinA-Myc-VASP hybrid

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In a targeting experiment to manipulate the actin coat of endosomes in Dictyostelium discoideum VASP was fused to Vacuolin A linked by a myc-tag. VASP (vasodilator-stimulated-phosphoprotein) is an actin-bundling protein which localizes to areas where actin is highly dynamic such as lamellopodia and filopodia tips. The vacuolin isoforms A and B are specific protein markers for late neutral endosomes. In wild type Dictyostelium-cells the late neutral phase in the endocytic pathway is characterized by the appearance of 8-10 vacuolin- and actin-positive endosomes. A strong expression of the VAM-VASP (VacuolinA-Myc-VASP) construct in Dictyostelium discoideum leads to the formation of only a single but huge vacuolin-marked vesicle instead which appears loaded with F-actin and for that reason called actin-ball. Besides the accumulation of endogenous Vacuolin the actin balls also feature a remarkable amount of endogenous VASP and other actin-interacting proteins like Aip1, ABP34 and Coronin. The actin balls are not surrounded or filled with membranes and do not take part at the endocytic pathway. Furthermore these balls affect the cell by decreasing the rate of pino-, phago- and transcytosis, by slowing down the cell division and by reducing the formation of filopodia, because proteins that usually participate in these processes but are now accumulated in the actin balls. Single deletions of different domains in the VAM-VASP construct lead to the conclusion that the formation of the actin balls mostly depends on the F-actin binding domain and the tetramerization domain which are located in the VASP protein. The localization of VASP to late neutral endosomes by the fusion of the protein to vacuolin is also a condition that supports the formation of actin balls, probably by increasing the local density of the hybrid.

Characterization of a complex formed by PakB, DAbp1 and myosin I

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Dictyostelium PakB has been identified as the protein kinase that activates myosin I by phosphorylating the TEDS site in the motor domain. Yeast two-hybrid screen using PakB-1-178 as bait identified the Dictyostelium homologue of actin binding protein 1 (DAbp1) as a binding partner. The binding interaction is mediated by the C-terminal SH3 domain of DAbp1 and PxxP motifs located within PakB. Pull down assays were performed using N-terminal fragments of PakB expressed in Dictyostelium as GFP fusion proteins and the SH3 domain of DAbp1 expressed in bacteria as a GST fusion protein. The binding site for the SH3 domain of DAbp1 was localized to a PXXP motif located between residues 58 to 79 of PakB. A PakB fragment containing residues 1-79 was recruited, like PakB, to the leading edge of cells during chemotaxis and to macropinocytic cups, but a fragment containing PakB residues 1-58 remained diffuse in the cytoplasm. This result suggests that the DAbp1 binding site may be involved in recruiting PakB to the cell cortex. However, GFP-PakB-1-178 still localized to the leading edge of DAbp1 null cells or when the DAbp1 binding site was eliminated by mutation. These result indicate that recruitment of PakB involves other binding partners in addition to DAbp1. An interaction was identified between the MyoB SH3 domain and a motif located between residues 97 and 120 of PakB, suggesting that localization of PakB may also involve myosin I. Studies have also been performed on the interactions mediated by DAbp1. A proline-rich region located in the middle portion of DAbp1 was shown to bind the C-terminal DAbp1 SH3 domain. The internal SH3 domain binding site was narrowed down to fragment encompassing residues 237-313, which include three typical PXXP binding motifs. This region of DAbp1 also binds the SH3 domains of amphiphysin and myosin I. DAbp1 thus provide a means to form a complex linking PakB and myosin I to membranes.

Actin dynamics in SCAR-deficient cells

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The dynamical properties of the actin cytoskeleton provide the basis for motility, phagocytosis, and division of eukaryotic cells. Directed polymerization of actin in the cell cortex has been identified as the underlying source of force generation. A key player in the formation of a dense cortical actin network is the seven-subunit Arp2/3 complex that initiates the nucleation of branches on existing filaments. Its activity is controlled by SCAR/WAVE proteins of the WASp (Wiscott-Aldrich Syndrome protein) family that are downstream effectors of receptor-mediated signalling pathways. Here we analyze the temporal patterns of actin polymerization in the cortex of mutant cells lacking members of the pentameric SCAR complex. The results highlight the actin machinery as a self-organizing system that can be described by the concepts of non-equilibrium dynamics. We furthermore report evidence that the cortical dynamics is linked to the chemosensory pathway, so that receptor signals are transmitted to the actin system, even if SCAR is missing.

Filamin repeat segments required for photosensory signalling in *Dictyostelium discoideum*

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Filamin is an actin binding protein responsible for crosslinking F-actin filaments and stabilising the cortical actin network. It is ubiquitous in eukaryotes and its basic structure is well conserved, consisting of an N-terminal actin binding domain followed by a series of repeated segments which vary in number in different organisms. *D. discoideum* is a well established model organism for the study of signalling pathways and the actin cytoskeleton and as such makes an excellent organism in which to study a cytoskeletal protein like filamin. Ddfilamin plays a putative role as a scaffolding protein in a photosensory signalling pathway and this role is thought to be mediated by the unusual repeat segments in the rod domain.

In order to study the role of filamin in phototaxis, a filamin null mutant, HG1264, was transformed with constructs each of which expressed wild type filamin or a mutant filamin with a deletion of one of the repeat segments (referred to as filamin#1 through filamin#6). Transformants expressing the full length filamin to wild type levels completely rescued the phototaxis defect in HG1264, however if filamin was expressed at lower than wild type levels the phototaxis defect was not restored. The transformants lacking any one of the repeat segments 2-6 retained defective phototaxis and thermotaxis phenotypes, whereas transformants expressing filamin#1 exhibited phenotypes ranging from severely deranged as seen in the filamin null mutant through to complementation of the phenotype to wild type levels. Immunofluorescence microscopy showed that filamin lacking any of the repeat segments still localised to the same actin rich areas as wild type filamin. Ddfilamin has been shown to interact with RasD and IP experiments demonstrated that this interaction did not rely upon any single repeat segment as RasD still immunoprecipitated with filamin regardless of which repeat was absent.

This paper demonstrates that wild type levels of filamin expression are essential for the formation of functional photosensory signalling complexes and that each of the repeat segments 2-6 are absolutely required for filamin's role in phototaxis. By contrast, repeat segment 1 is not essential provided the mutated filamin lacking repeat segment 1 is expressed at a high enough level. The defects in photo/thermosensory signal transduction caused by the absence of the repeats are due neither to mislocalisation of filamin nor to the loss of RasD recruitment to the previously described photosensory signalling complex, as all mutated forms of filamin lacking one of the repeat segments still localise to the same areas as wild type filamin and still interact with RasD. Presumably repeat segments 2 to 6 are required for the recruitment of signalling proteins other than RasD.

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Characterisation of Kame, a novel JmjC domain-containing protein in *Dictyostelium discoideum*.

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Since their discovery, JmjC domains have been found in organisms from bacteria to humans. Recent phylogenetic analysis and studies of the domain architecture that there are seven distinct groups (Klose et al., 2006). Of these groups three have been studied because of their ability to demethylate histones through interactions with α-ketoglutarate and Fe (II). Binding of these cofactors is required for enzymatic function and occurs at conserved residues within the JmjC domain. Many of the uncharacterised JmjCcontaining proteins also contain these conserved residues (Klose et al., 2006). Easily manipulated model organisms such as *Dictyostelium discoideum* have proven invaluable when determining the functions of uncharacterised proteins. A novel JmjC domain-containing protein, Kame, was discovered in *D. discoideum* and its functions were analysed in antisense inhibition and overexpression studies.

Antisense inhibition of Kame expression increases the rates of phagocytosis, pinocytosis, growth on bacterial lawns and growth in liquid while overexpression of the full length protein causes a slight impairment of all of these phenotypes. Transformants.overexpressing the full length protein are also defective in the switch from cellular.growth to development. Overexpression of the N-terminal portion of the protein mimics antisense inhibition in that it also leads to increased rates of growth on bacteria. Conversely overexpression of the C-terminal portion (JmjC domain) appears to mimic overexpression of the full length protein and appears to be lethal at high levels.

Phenotypes such as phototaxis and thermotaxis appear to be unaffected. The results suggest functions for this novel protein in the regulation of endocytosis, growth and development.

Klose, R.J., Kallin, E.M. & Zhang, Y. (2006). JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet* **7**, 715-727.

Two-hybrid screening for binding partners of C-module-binding factor CbfA

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CbfA was initially identified as a DNA-binding activity specific for the C-module, a promoter activity within the Dictyostelium retrotransposon TRE5-A. Later we found that CbfA plays a crucial role in early development by binding to and regulating the promoter of the aggregation-specific adenylyl cyclase ACA. CbfA is a multidomain protein that is thought to be involved in chromatin remodeling. The factor contains a "jumonji domain" (JmjC). Such domains were shown in other proteins to facilitate the oxidative demethylation of lysine side chains in histone H3 proteins. On the other hand CbfA contains a "carboxy-terminal domain" (CTD) that may facilitate gene regulatory functions of CbfA independently of the remainder of the protein. In order to identify putative interacting partners of CbfA, we constructed high quality cDNA libraries from growing D. discoideum cells in a yeast two-hybrid system. We report on the results of initial screenings of these cDNA libraries using either the JmjC domain or the CTD of CbfA as bait.

Differential regulation of the antiproliferative gene dpc3 during growth and differentiation of D. discoideum

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We have isolated and functionally characterized dpc3, the D. discoideum homolog of the antiproliferative gene PC3/Tis/Tob, which has roles in the control of cell cycle and differentiation during vertebrate neurogenesis. This gene is tipically expressed in cells during the last cell cycle before the mitosis that precedes proliferation arrest and commitment to terminal differentiation. Dpc3 ORF is 1272 nt long and encodes a peptide of 423 aa with 49% homology with the human Tob1. During vegetative growth Dpc3 gene is expressed in a cell cycle-dependent way, with a peak in mid-late G2, and it is also expressed in development when it is already detectable during aggregation. At postaggregative stages dpc3 is expressed in cells with the morphological characteristics of ALC, and in pstA cell, while at culmination ALC-specific expression is switched off, resulting in a stalk-specific pattern in mature culminants. Vegetative and ALC-specific expression are dependent on expression of rblA, the D. discoideum ortholog of Rb (retinoblastoma). On the contrary, stalk-specific expression is independent of rblA, suggesting that dpc3 is controlled by different mechanisms in vegetative growth and development.

Identifying molecules that regulate DIF-responsive bZIP transcription factors.

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Differentiation-inducing factor-1 (DIF-1) is a diffusible signalling molecule required for the differentiation of a subset of prestalk cells. Several factors are known to influence a cell's response to DIF-1, however, very little is known about the signalling pathway itself. DimA and DimB are bZIP transcription factors that have been identified as part of the DIF-1 signalling pathway. bZIP transciption factors act by homo- and heterodimerisation with other bZIP transcription factors. Under DIF-1 induction, DimA and DimB translocate to the nucleus where they regulate the expression of prespore and prestalk markers. In order to identify molecules that regulate bZIP activity two approaches have been taken. (1) Yeast 2-hybrid screen. Deletion studies of both DimA and DimB have been carried out to identify regions required for nuclear import. Two domains have been identified in each of the proteins. These are the DNA-binding/dimerisation domain and second domain downstream. A deletion construct of DimA, based on these two domains was generated and used in a 2-hybrid screen. Hits have been found in several proteins including a third bZIP transcription factor (bzpP), a FAD-binding oxidoreductase and a glutathione synthase (gshB). Progress in studying the potential function of these proteins in DIF signalling will be presented. (2) Tandem affinity purification (TAP) Tag. We have replaced the endogenous dimB gene with a TAP-tagged version. The expression of dimB and its translocation to the nucleus in response to DIF-1 is unaffected by the addition of a C-terminal TAP Tag. Therefore, we are using this strain to isolate dimB protein complexes by a two-step affinity purification method and identifying individual proteins by mass spectrometry. We will present data showing purification of DimB-TAP in response to DIF stimulation.

Suppression of partially active Dictyostelium STATa mutation by overexpressing a segment of SUN domain containing protein

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The transcription factor Dd-STATa, a functional homologue of metazoan STAT (Signal Transducers and Activators of Transcription) proteins, is necessary to complete development. We have isolated at least 18 putative multicopy suppressors of Dd-STATa by use of genetic screening. One of them encodes a segment of the sunB gene, whose product harbors the SUN (Sad1 and UNC) domain, a C-terminal region common to the C. elegans protein UNC-84 and the S. pombe Sad1 protein. Suppressor a1403 encodes the first 261 amino acids of SunB protein (SunB-ori); overexpression of a1403 cDNA suppressed the Dd-statA mutation, as the strain efficiently formed fruiting bodies, though with short stalk. In contrast, overexpression of the SunB-ori::GFP fusion protein as well as of full-length SunB protein poorly suppressed the parental mutation, although the GFP-tagged full-length SunB (SunB::GFP) fusion protein did moderately suppress the phenotype. Thus, it is likely that SunB-ori is acting in a dominant-negative fashion. The sunB gene is fairly uniformly expressed during all developmental stages and throughout whole multicellular structures in both wild type and Dd-STATa null strains. In spite of such uniform expression of the sunB gene, SunB::GFP protein gradually accumulated in prestalk cells as development proceeded. So far, screening of sunB null mutant has been unsuccessful. Instead, we obtained a putative sunB knockdown mutant that showed reduced growth rate and an aberrant developmental morphology. The sunB gene appears to function in both growth and development. Overexpression of the a1403 cDNA restored the expression of a limited set of putative Dd-STATa target genes slightly but did not change the amount of the phosphorylated form of truncated Dd-STATa, GFP::STATa(core). Sun B's suppression mechanism may not be through Dd-STATa directly and remains unknown. However, morphological reversion implies some sort of functional relationship between Dd-STATa and sunB.

1. Shimada, N. and Kawata, T. (2007) Evidence that non-coding RNA dutA is a multicopy suppressor of Dictyostelium STAT protein, Dd-STATa. Eukaryotic Cell, 6, 1030-1040.

Isolation and characterization of putative Dictyostelium PIAS homologue, a protein inhibitor of activated STAT

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In mammals, there are protein families that inhibit STAT (Signal Transducers and Activators of Transcription) activity. One of them is the PIAS (protein inhibitor of activated STAT) family that was initially identified by a two-hybrid screen for the ability to interact with STAT proteins. We isolated a putative Dictyostelium homologue of PIAS, Dd-PIAS, by homology searching. The Dd-PIAS gene encodes a protein consisting of 843 amino acids with a predicted molecular weight of 94.5 kDa. Dd-PIAS protein possesses representative domains or motifs found in mammalian PIAS including SAP (SAF-A/B, ACINUS, PIAS), LXXL-like, PINIT, RLD (RING-finger-like zinc-binding) and carboxyl-terminal highly acidic (AD) domains. The Dd-PIAS promoter fused to a lacZ reporter construct revealed the expression of the Dd-PIAS gene to be specific to prespore and pstA cells at the slug and culmination stages. There was no pstO expression during late developmental stages. The Dd-PIAS null mutant displayed a wild type phenotype but when the Dd-PIAS::GFP fusion protein was overexpressed by its own promoter, transformants showed no phototaxis and formed coiled slugs when developed on water agar. When growing on a bacterial lawn, the overexpressor formed a plaque with a rough edge, slightly similar to that found in a Dd-STATc-null mutant. Interestingly, the expression of the Dd-PIAS gene was reduced in the Dd-STATa-null mutant. Genetic interactions between Dd-PIAS and Dictyostelium STAT proteins are under investigation.

The Dictyostelium response to hyperosmotic shock

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Dictyostelium discoideum is frequently subjected to environmental changes in its natural habitat, the forest soil. In order to survive the organism had to develop effective mechanisms to sense and respond to such changes. When cells are faced with a hypertonic environment a complex response is triggered. It starts with signal sensing and transduction and leads to changes in cell shape, the cytoskeleton, transport processes, metabolism and gene expression. To better understand the D. discoideum response to hyperosmotic conditions, we performed cell biological, biochemical and gene expression profiling experiments. The transcriptional profile of cells treated with 200 mM sorbitol during a 2-hour time course was analysed. The results revealed a time-dependent induction or repression of 809 genes, more than 15% of the genes on the array, which peaked 45 to 60 minutes after the hyperosmotic shock. The differentially regulated genes were applied to cluster analysis and functional annotation using gene ontology (GO) terms. The most prominent response was the down-regulation of the metabolic machinery. Most genes encoding enzymes of the major metabolic pathways were down-regulated in response to hypertonicity. However, some genes were up-regulated which provided valuable clues for the synthesis of osmolytes. Biochemical analysis of putative osmolytes showed that Dictyostelium uses a mixture of osmolytes to counteract external osmolarity. Another important response was the up-regulation of the stress response system, including STATc. Comparison of wild-type and STATc knock-out cells showed that STATc is a key regulator of the Dictyostelium transcriptional response to hyperosmotic shock. Approximately 20% of the differentially regulated genes were dependent on the presence of STATc. Based on our results we conclude that at least two, possibly three, signal transduction pathways get activated in Dictyostelium cells subjected to hypertonicity.

Serine phosphorylation in Dictyostelium Paxillin is dependent on cell-substrate adhesion

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The adhesion of cells to other cells and to the surrounding extracellular matrix is a key element for survival, proliferation, differentiation and migration. Such adhesion is critical in the development of Dictyostelium discoideum. Cell-cell adhesion is an important component of D. discoideum morphogenesis and is required for multicellular development past the aggregation stage. One adhesion regulator protein, PaxB, is an orthologue of mammalian Paxillin. Paxillin is a focal adhesion molecule that provides a docking site on the plasma membrane for signaling and structural proteins. We have previously shown the overexpression of PaxB increases cell-cell cohesion. In the current study, we demonstrate PaxB mediated cell-cell cohesion is decreased in the presence of EDTA. This suggests adhesion mediated by PaxB is calcium dependent. We also show PaxB undergoes serine phosphorylation. In addition, the serine phosphorylation of PaxB is greater in cells adhered to a substrate than cells in suspension. In contrast, PaxB serine phosphorylation in the presence of EDTA is less in adhered cells than cells in suspension. Taken together the data suggest PaxB serine phosphorylation is stimulated by cell-substrate adhesion, and the PaxB mediated adhesion is EDTA sensitive.

Dictyostelium discoideum RnoA, PldB, and PaxB form a potential complex at the actin cytoskeleton during adhesion.

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Proper organization of the actin cytoskeleton is essential for cell motility and adhesion. ARNO, ARF nucleotide-binding site opener, is a guanine nucleotide exchange factor in mammalian cells that has been linked to the physiological processes of cell motility and rearrangement of the actin cytoskeleton. Phospholipase D (PLD), the enzyme that catalyzes the creation of the second messenger phosphatidic acid, has been implicated in cell migration and has also been functionally linked to the actin cytoskeleton. The adaptor protein paxillin mediates interactions between the extracellular matrix and the cytoskeleton by serving as a docking site for an assortment of signaling molecules, including those involved in the organization of the actin cytoskeleton. Given their similarity of function, we decided to investigate potential interactions between the Dictyostelium discoideum ARNO, PLD, and paxillin homologs RnoA, PldB, and PaxB, respectively. Previous work has shown RnoA, PldB, and PaxB to be involved in cellular processes such as adhesion, motility, and chemotaxis which lead to differentiation and development in Dictyostelium discoideum. RnoA, PldB, and PaxB all co-localize with actin at the cell-substrate interface, as well as the cell cortex. These findings suggest RnoA, PldB, and PaxB may play a role in the organization of actin, particularly during adhesion. In addition, immunoprecipitates of RnoA were found to contain PldB. PldB was likewise able to immunoprecipitate RnoA. Similarly, immunoprecipitates of PaxB contain both RnoA and PldB. In cells lacking pldB, RnoA did not co-immunoprecipitate with PaxB, indicative of the possible involvement of PldB for an interaction between PaxB and RnoA to occur. These results highlight a potential complex between RnoA, PldB, and PaxB, as well as between their mammalian homologs ARNO, PLD, and paxillin.

Tyrosine phosphorylation in Dictyostelium discoideum PaxB is impaired during adhesion

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Adhesion is required for cell differentiation, proliferation, motility, migration and hence, survival. Paxillin, an adapter protein in higher organisms, is present in focal adhesions and is involved in cell-substrate interactions and cell movement. Vertebrate paxillin is highly phosphorylated on tyrosine residues Y31 and Y118, although a role of phosphorylation in paxillin regulation has not been well established. To address this, we are examining the role of tyrosine phosphorylation in PaxB, a Dictyostelium discoideum paxillin homologue. PaxB contains thirteen potential tyrosine phosphorylation sites in its amino acid sequence. To gain a better understanding of the role of tyrosine phosphorylation of PaxB, the tyrosine phosphorylation status of PaxB was determined during cell-substrate adhesion, cell-cell cohesion and development. Tyrosine-phosphorylated PaxB was detected in vegetative cells as well as during development, with the highest levels of phosphorylation reached at 12 hours of development. PaxB tyrosine phosphorylation levels were undetectable after 18 hours of development. We find that suspended vegetative Dictyostelium discoideum cells displayed a higher level of tyrosine- phosphorylated PaxB than cells adhered to a surface. When calcium dependent adhesion was inhibited by addition of 10 mM EDTA, tyrosine phosphorylation of PaxB in adhered cells decreased, suggesting a potential role for tyrosine-phosphorylated PaxB in calcium-dependent adhesion. In addition, PaxB tyrosine phosphorylation decreased during cell clumping, indicating a possible role of tyrosine phosphorylation in cell-cell cohesion activity. These data illustrate a potential role for tyrosine-phosphorylated PaxB in cell-substrate adhesion, cell-cell cohesion and development.

Two Roles for an Anti-Adhesive Protein During Dictyostelium Growth And Development.

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The ampA gene encodes a novel protein that modulates cell adhesion and developmental patterning. The AmpA protein is necessary in a non-cell autonomous manner to prevent premature differentiation of prespore cells. In ampA null cells a prespore marker is expressed in cells at the mound periphery that will normally differentiate into prestalk cells. A supernatant source and synthetic peptides from the AmpA protein can prevent this misexpression. Expression in growing cells reveals a second function for AmpA. AmpA loss results in an increase in cell adhesion, and a reduction in F actin with a concomitant increase in G actin. Overexpression of AmpA reduces adhesion and increases F actin. As a result of these changes in the cytoskeleton and in adhesion, AmpA also influences cell migration. In comparison to wt cells, AmpA null cells are defective in migration on top of agarose but migrate normally under agarose. AmpA overexpressing cells show the opposite behavior, migrating well on top of agarose but unable to migrate under agarose. Cell traction measurements using elastic polymers, undertaken in collaboration with Drs. Leach and Jacot indicate that both AmpA null and overexpressing cells are reduced in their levels of cell traction suggesting a critical level of AmpA is necessary for optimal levels of actin polymerization and substrate adhesion to enable efficient migration. AmpA-tap tag constructs appear to function like wt AmpA and are being used with AmpA-His fusion proteins to identify interacting proteins. REMI mutagenesis is being used to identify second site suppressors of AmpA overexpression. Candidates have been identified which increase adhesion and alter the levels of polymerized actin but none suppress the developmental effects of AmpA overexpression. The fact that suppressors of some AmpA functions but not others are identified is consistent with the observation that some AmpA functions are cell autonomous and others non-cell autonomous.

Role of the exocyst complex and its regulators in Dictyostelium discoideum

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The exocyst is an evolutionary conserved complex originally identified in S. cerevisiae. It is composed of eight subunits: sec3, sec5, sec6, sec8, sec10, sec15, exo70 and exo84. The complex is required for post-Golgi transport and tethering of vesicles to the plasma membrane. All subunits have helical bundles, which are packed against each other in an elongated rod-like structure. Homologs of each subunit have been identified in diverse organisms including insects and mammals. A BLAST search in DictyBase revealed a single homolog for each protein. One goal is to investigate the function of the exocyst by generating knock out mutants and cells expressing fluorescently-tagged Sec8 and Sec15. We follow two strategies to generate Sec15 and Sec3 deletions. A “classical” knock out in haploid and diploid cells, and a tetracycline-regulatable knock down. Live cell imaging revealed a localization of the exocyst on the bladders of the contractile vacuole before and during expulsion, similar to what has been shown for LvsA and drainin. This was confirmed by immunofluorescent staining for calmodulin. Additionally, we could show a colocalization with vacuolin, but not ER or Golgi markers. We speculate that the complex tethers both types of vacuoles before exocytosis or expulsion to the plasma membrane. Furthermore, we are interested in the regulation of the complex. In other systems it has been shown that Rab11 and Sec4, a homolog of Dictyostelium Rab8, interact with Sec15. In total the Dictyostelium genome encodes 52 Rab GTPases. Concentrating on predicted endosomal Rab GTPases, we use fluorescent tagging to study their localization. Rab11a, Rab11c and Rab8a localize to the contractile vacuole, but Rab11b was found on vacuolin positive post lysosomes and in a juxtanuclear region. This distribution partly overlaps with the exocyst. We will explore this potential functional link by monitoring precisely the spatial and temporal colocalisation of the exocyst and Rab GTPases.

The Lipid Droplet Proteome of Dictyostelium discoideum.

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Lipid droplets - (LDs) also called lipid bodies - are omnipresent organelles in yeast, plants, and animals as well as some prokaryotes. The droplets have the function to store energy in form of triacylglycerols and also to save sterols and precursors of membrane phospholipids. It is assumed that LDs arise from the membrane of the ER and are surrounded by a phospholipid monolayer. Their interior consists entirely of neutral lipids; however a number of proteins are present on the droplet surface. Despite the importance of LDs in lipid metabolism and disease, their protein content and the function of droplet-associated proteins is not clear. The aim of this project is the identification of the proteins sitting on the outside of the LDs of the model organism D. discoideum. Their formation of LDs was induced by the addition of palmitic acid to the growth medium. Subsequently, LDs were purified through sucrose gradient centrifugation and analysed by mass spectroscopy. Using this method and by fluorescence microscopy Perilipin, a member of the evolutionarily related family of PAT proteins (Perilipin, Adipophilin TIP47) known to be associated with lipid droplets, could be detected. Proteins, which have a role in lipid and fatty acid metabolism were found as well. The next step is to analyse unknown proteins of the lipid droplet proteome regarding their function as derived from the phenotype induced in D. discoideum if the respective gene is disrupted or overexpressed.

Characterization of LC-FACS proteins in Dictyostelium discoideum

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Long-Chain-Fatty Acyl-CoA Synthetases (LC-FACS) catalyze the activation of fatty acids (FAs) consisting of 16 to 22 C-atoms by adding a Coenzyme A to the C1 atom of the FA. This activation is required for storage of FAs as Triacylgycerol or their degradation in the β-Oxidation to provide energy for the cell. In Dictyostelium we have identified two LC-FACS proteins, LC-FACS 1 and LC-FACS 2 encoded by the genes fcsA and fcsB respectively. LC-FACS 1 is associated with the cytosolic side of the endosomal membrane for the first few minutes of endosome formation and remains bound to it until the endosomes are neutralised. Cells deficient in LC-FACS 1 that were constructed by homologous recombination and carry the disrupted fcsA-gene show a decreased uptake of FAs from endosomes but no other conspicuous defect. LC-FACS 2 is a transmembrane protein which contains an N-terminal ER-targeting sequence. GFP fused to the C-terminus of LC-FACS 2 localizes in the ER whereas the LC-FACS 2 bearing an internal myc-epitope is transported to peroxisomes. Mutants lacking LC-FACS 2 show wildtype-like uptake of FAs from endosomes but show reduced phagocytosis instead. To verify these results and to better understand the function of LC-FACS proteins in Dictyostelium further research will be done on the double mutant (fcsA-/fcsB-) and a fcsA-overexpressor strain in a fcsB--background.

von Löhneysen, K., Pawolleck, N., Rühling, H. and Maniak, M. (2003) A Dictyostelium long chain fatty acyl coenzyme A-synthetase mediates fatty acid retrieval from endosomes. Eur J Cell Biol, 82, 505-514.

Characterization of the mitochondrial Ras-related GTPase Miro of Dictyostelium discoideum

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Recently a new subfamily of GTPases, Miro (mitochondrial Rho GTPases) has been described. Miro consists of a Ras-like GTPase, which bears similarity to other Rho proteins, two EF-hand domains that serve as calcium binding regions and an additional GTPase domain at the C-terminus followed by a short transmembrane region, which targets the protein to the outer mitochondrial membrane. S. cerevisiae cells lacking the Miro homologue Gem1p showed collapsed globular or grape-like mitochondria. In D. melanogaster strong overexpression of Miro led to disruptive mitochondrial distribution. Furthermore loss of dMiro impaired larval locomotion and disrupted subcellular distribution of mitochondria in neurons and muscles. In vertebrates two isoforms of Miro are present that play roles in mitochondrial trafficking. An interaction of Miro with kinesin-binding proteins GRIF-1 and OIP106 has been reported, suggesting that Miro GTPases form a link between the mitochondria and the microtubule trafficking apparatus. One Miro gene (gemA) has been identified in the model organism D. discoideum. This gene encodes a protein of 658 amino acids with the characteristic domain architecture of other Miro proteins. We have used homologous recombination to generate a D. discoideum knockout strain lacking gemA. The recombination event was verified using PCR, Southern blot analysis and RT-PCR. A monoclonal antibody that specifically recognizes the first 530 residues of the protein was used to confirm absence of Miro in the knockout strain. Furthermore we could show mitochondrial anchoring of Miro by immunfluorescence analysis. We will report on the effects of gemA deletion in processes like growth, phagocytosis, fluid phase endocytosis and exocytosis, development and chemotaxis. Characterization of D. discoideum Miro applying methods that specifically address the mitochondrial function will follow, as well as the identification of potential regulators and effectors of Miro.

The role of TM9 proteins in the regulation of cell physiology.

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TM9 proteins constitute a well-defined family of proteins characterized by the presence of nine transmembrane domain and a high degree of homology, and previous studies have suggested that they might play a role in the endocytic pathway. There are three TM9 proteins in yeast *Saccharomyces cerevisiae* and in *Dictyostelium discoideum* amoebae. To study their role, the corresponding genes were deleted in budding yeast and in *Dictyostelium*, and the phenotype of the mutants was analyzed. Together, our results suggest that TM9 proteins participate to an intracellular signaling pathway that ultimately regulates several aspects of cellular physiology, in particular intracellular transport.

The centrosome on a leash: characterization of proteins involved in the attachment of the centrosome to the nucleus

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Centrosome/nucleus attachment is crucial for proper mitosis and nuclear positioning. It requires dynein/dynactin and a protein complex (connector) involving the nuclear envelope (NE) protein SUN1. There is no common scheme for the partners of SUN1 participating in centrosome/nucleus attachment in different organisms. We show that Dictyostelium SUN1 is a useful marker protein for the connector. Cells expressing full-length GFP-SUN1 at low levels show no aberrant phenotype. In interphase and mitosis, GFP-SUN1 localized to the NE with some bias towards its centrosomal side. The centrosome is connected to the NE by a thin GFP-labeled line with a bright dot directly adjacent to the centrosome. Live cell imaging reveals that the connection is very elastic, since its length varies constantly. The dot at the centrosome was also labeled at isolated centrosomes using SUN1 specific antibodies (by A. Noegel; Cologne). Upon GFP-SUN1 overexpression we observed cells with unattached, supernumerary centrosomes, cytoplasts and nuclei of different size as a further indication for a role of SUN1 in centrosome/nucleus attachment. Our proteomic analysis of the centrosome has revealed a Dictyostelium orthologue of Bbp1p, a spindle pole body component associated with to SUN1 in budding yeast. Analysis of GFP-DdBbp1 interphase cells reveals that DdBbp1 is localized to the nuclear side of the centrosome/nucleus connector. In mitosis, it behaves like a kinetochore protein with up to six detectable spots associated with the chromosomes. While a role of DdBbp1 in centrosome/nucleus attachment is not yet clear, we previously proved this for the dynein regulator DdLIS1. Hypomorphic DdLIS1 mutants showed an increased distance of centrosomes and nuclei, while the thin GFP-SUN1 connection inbetween was still intact, but increased in length. This indicates that dynein and its regulators are not required for the attachment itself, but for keeping the short distance between centrosomes and nuclei.

Functional analyses of Dictyostelium TACC a centrosomal DdCP224 interactor identified by Tandem Affinity Purification.

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Tandem affinity purification (TAP) is a method originally established in yeast to isolate highly purified protein complexes in a very gentle and efficient way. We have modified TAP for Dictyostelium applications and have proved it as a useful method to isolate microtubule (MT)-associated protein complexes containing EB1 and the XMAP215 homologue DdCP224. Both proteins are centrosomal components that also bind to microtubule tips. DdCP224 is involved in centrosome duplication and cytokinesis, whereas EB1 assists in spindle formation. At the MT tip these two proteins are thought to be part of a complex that links MTs to the cell cortex in a dynein/dynactin-dependent manner. Besides confirmation of the known interaction between EB1 and DdCP224, TAP followed by mass spectrometry revealed a novel interaction between DdCP224 and a TACC- protein (**t**ransforming **a**cidic **c**oiled **c**oil). This interaction was also approved by co-immunoprecipitation. Furthermore both proteins were co-localized at the centrosome and microtubule tips using specific antibodies in immunofluorescence microscopy. During mitosis, both proteins co-localized at the spindle poles and at the microtubule overlap zone of the central spindle. GFP-TACC was found at the same locations as the endogenous protein and, thus, is the first live cell marker for microtubule tips in Dictyostelium. The C-terminal TACC domain alone turned out to be sufficient for both centrosomal and microtubule tip-localization of the protein and interaction with DdCP224. In Drosophila, TACC has been shown to be required for centrosomal localization of the DdCP224 orthologue msps. However, in Dictyostelium TACC depletion by RNAi to an extent of more than 95% had no detectable effect on DdCP224 localization and resulted in no obvious mutant phenotype regarding growth, development or the microtubule cytoskeleton. Further investigations to elucidate the function of TACC by identification of other binding partners through TAP are under the way.

Nuclear Organisation of Transposons and transgenes in Dictyostelium discodium

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We are interested in the interplay between nuclear organisation, chromatin modifications and RNAi pathways and how they regulate gene expression and chromosome organization. Antibodies against different histone modifications and fluorescence in-situ hybridisation (FISH) were applied to interphase nuclei. We confirmed the existence of a distinct euchromatic compartment and a single chromocenter containing almost all the constitutive heterochromatin. The heterochromatic compartment contains all the repetitive DIRS1 retrotransposons. During mitosis DIRS1 sequences are at the leading edge of the chromosomes (directed towards the poles), supporting the idea that DIRS1 belong to centromeric regions in Dictyostelium. The Skipper retrotransposon is also contained within the chromocenter, but additional copies were found in 10-15 distinct foci within the euchromatic compartment. DIRS1 and Skipper were analysed using chromatin immunoprecipertation (ChIP) and both are decorated with histone H3 dimethylated at lysine position 9 (H3K9me2). myc-HcpB, a homolog of HP1, binds directly to the DIRS1-containing chromatin. Preliminary results on integrated multicopy trangenes suggest that most are localised in the heterochromatin compartment. We are currently investigating the relationship between chromatin location, copy number and expression level of transgenes. The effect of RNAi pathway mutants on chromatin modifications and on nuclear structure was studied in order to investigate a possible link between RNA processing and chromatin. Preliminary results from the mutants suggest that centromeric heterochromatin may be expressed at considerable levels, this would be consistent with recent data from yeast that centromeres must be transcribed to maintain silencing.

Mapping of histone modifications in Dictyostelium

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Posttranslational modifications of histones and other chromatin components play a major role in epigenetic gene regulation. While there is a constantly growing map of these modifications in other model organisms, only a few of them including H3K9- and H3K4 methylation have been studied in Dictyostelium so far. Allthough histones are usually highly conserved, some diffenrences in the amino acid sequence could provide different targets for the modification machinery. Using mass spectroscopy to investigate protein modifications, we found that several histone modifications known from other model organisms like H3K79 methylation are also present in Dictyostelium. While these modifications are highly conserved across species, we found a previously unknown H2B modification. As the modification site is not conserved in other species it provides an interesting target to investigate its function and the connected pathways, leading to further insight on how different epigentic states evolved or changed during evolution. It has previously been shown that chromatin modifications change during the developmental cycle of Dictyostelium (Chubb et al, 2006). We have initiated experiments to elucidate global changes in the histone code during development and will present first experiments.

Chubb, J.R., Bloomfield, G., Xu, Q., Kaller, M., Ivens, A., Skelton, J., Turner, B.M., Nellen, W., Shaulsky, G., Kay, R.R., Bickmore, W.A., Singer, R.H. (2006). Developmental timing in Dictyostelium is regulated by the Set1 histone methyltransferase. Dev Biol. 2006 April 15; 292(2): 519–532.

Chromatin remodelling and inositol phosphate signalling

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Inhibition of the enzyme prolyl oligopeptidase (PO) in Dictyostelium leads to resistance to the effects of lithium via a mechanism that requires the enzyme multiple inositol polyphosphate phosphatase (MIPP). Our analysis demonstrates that this is due to an indirect mechanism via increased expression of a number of genes that regulate the synthesis and metabolism of inositol phosphates, including myo-inositol synthase (ino1) and inositol monophosphatase (IMPase). In yeast, the chromatin remodelling factors, Ino80 and Swi/SNF2 are required for expression of ino1, and it has been proposed that they are regulated by a higher order inositol phosphate, InsP5. As MIPP regulates the cellular concentration of InsP5, it may act to mediate the effects of PO inhibition through alteration of the InsP5 concentration. Consistent with this we have isolated a lithium resistant mutant, lisG, which encodes a chromatin remodelling factor of the chromo-helical domain (CHD) protein family. In addition to lisG, the Dictyostelium genome contains representatives of all four families of ATP dependent chromatin remodelling factors: CHD, Swi/SNF, Isw1 and Ino80. We are currently examining the role of Ino80. This forms a multimeric complex that includes actin- related protein 8 (Arp8). We have established that over-expression of Arp8 elevates expression of ino1, indicating that as in yeast the Ino80 complex controls ino1 gene expression in Dictyostelium and that Arp8 protein may be limiting for gene expression.

A Comparative Analysis of Spliceosomal Genes in Dictyostelium discoideum

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Little is known about pre-mRNA splicing in *Dictyostelium discoideum*, which seems to play an important role in this organism as two thirds of its genes contain at least one intron. Based on 267 known human genes involved in splicing we identified 165 non-redundant putative spliceosomal proteins and related factors in *D. discoideum*. A core set of spliceosomal small nucleoproteins (snRNPs), PRP19 complex proteins, and late-acting proteins are highly conserved in *D. discoideum*, *S. cerevisiae*, *D. melanogaster*, and *H. sapiens*. In non-snRNPs and H complex families, *D. discoideum* homologs are closer to those in fly and human than to those in yeast. Several splicing regulators, including SR proteins and CUG-binding proteins, together with five putative exon junction complex (EJC) proteins have been identified in *D. discoideum* but not in yeast. This comprehensive catalog of spliceosomal proteins provides useful information for future studies of splicing in *D. discoideum*.

As an ongoing effort at dictyBase we are examining gene models and genomic sequences in comparison with ESTs and cDNA sequences. To date, this led to the identification of 24 genes that have clear evidence of alternative splicing. This is contrary to the previous belief that no regulated alternative splicing exists in unicellular organisms. Together with our identification of a number of alternative splicing regulators, these results strongly suggest that alternative splicing regulation plays an important role in the biology of *D. discoideum*. The efficient genetic and biochemical manipulation of this organism will certainly further our understanding of pre-mRNA splicing in general.

Successful searches in dictyBase

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dictyBase has a powerful search tool that allows users to search various fields in the database: Gene Name & Synonym, Gene Products, Descriptions, Gene Ontology Terms, Colleagues, Authors, Phenotypes, ESTs, Strains, Plasmids, dictyBase IDs, GenBank Accessions and Web Pages, including the NewsLetters archive, the ListServ and the Techniques. The wildcard (*) can be used anywhere in a search string to widen search results. This is also a quick Search for Gene Names Only option, comparable to the Google "I Feel Lucky" button, that provides direct access to gene pages when searching by Gene Names or Synonyms. The Stock Center has a separate search tool where, in addition to strain names and strain IDs, various characteristics can be searched, including the depositor of a strain to the Stock Center, the parent strain or the mutagenesis method. The Genome Browser can also be searched for gene names, dictyBase IDs or EST names, in addition to genomic coordinates. dictyMart provides an excellent way to do complex queries by limiting chromosomal location, GO annotation, or dictyBaseID, for example. Note that dictyMart uses the % character as a wildcard. Facilitating searching using controlled vocabularies: In addition to the Gene Ontology that we have used for gene annotation since the early days of dictyBase, we now use a Phenotype Ontology for annotation of mutant strains. Thus, to look for defects in aggregation it is possible to search on 'aggregation', which returns phenotypes involving aberrant aggregation and their associated genes. We have introduced a new, more systematic way to describe strains called the Strain Descriptor. The strain descriptor does not replace the published strain name or the Systematic Name (of the HG101 format), but provides a quick overview so that a Gene Page with a long list of strains is more intelligible. Because the Strain Descriptor always contains the primary gene name it allows to find strains by searching for gene names.

dictyBase Strain and Phenotype Curation

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We recently changed the way phenotypes are stored in the database. Phenotypes that used to be linked to genes are now linked to strains, which in turn link to the gene(s) mutated. This represents phenotypes more accurately, as those are characteristics of strains rather than genes. Since a Strain Record is needed to curate phenotypes, dictyBase curators now annotate strains from the published literature, which appear in the Dicty Stock center as "unavailable". Curators read papers to find the genetic modification, the parent strain, the transformation vector, the selection marker and a description of the strain. This information is added to the database in order to produce a Strain Record. Once the strain is created, curators add phenotype annotations to the strain. For example the chcA- strain is associated with the phenotypes 'abolished cytokinesis', 'abolished sporulation' and 'aberrant fruiting body morphology'. Researchers are encouraged to submit their strains to the Stock Center. To provide more consistent annotations, we are using a phenotype ontology. An ontology is a set of defined terms with hierarchical relationships to one another. Terms from the Phenotype Ontology are composed of two parts: the entity modified in the mutant, and a value qualifying that modification. For example, a ”short stalk” phenotype qualifies the stalk as having “decreased height”. The hierarchical relationships link different phenotypes through common parent terms, for example, 'decreased stalk height’ and 'increased sorus size' are types (therefore children) of 'aberrant fruiting body morphology'. Phenotype terms often also contain synonyms for wider searchability. The use of a consistent vocabulary facilitates searching as well as allows grouping of related phenotypes based on specific criteria: one can find all phenotypes related to aggregation (abolished aggregation, delayed aggregation, etc.), or all delayed phenotypes (delayed aggregation, delayed culmination, etc.).

Dictyostelium Annotation in UniProtKB/Swiss-Prot

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Sequencing of the Dictyostelium discoideum genome, together with the Dictyostelium discoideum cDNA Project, is generating a large amount of nucleotide sequence data. Studying the encoded protein sequences is a key step in interpreting the D. discoideum genomes, to aid Dictyostelium research and also to allow broader insights into cellular communication, movement and development in eukaryotes. UniProt (Universal Protein Resource) provides a central resource on protein sequences and functional annotation. UniProt Knowledgebase (UniProtKB) is the central database of protein sequences, and contains the manually annotated UniProtKB/Swiss-Prot section and the automatically annotated UniProtKB/TrEMBL section. UniProt databases are freely available and can be accessed online at http://www.uniprot.org. D. discoideum curation in UniProtKB/Swiss-Prot focuses on proteins whose function or expression pattern has been determined experimentally, with priority given to proteins with mammalian homologs and those for which knockout data is available. UniProtKB/Swiss-Prot release 53.2 (26-June 2007) contains 346 D. discoideum. entries. In addition, more than 16,000 D. discoideum entries are present in UniProtKB/TrEMBL. For each protein, sequences and experimental data are collated and summarized in a UniProtKB/Swiss-Prot entry using controlled vocabulary and free text. All D. discoideum entries in UniProtKB/Swiss-Prot cross-reference to dictyBase and we work to ensure that the information we provide complements that in dictyBase. D. discoideum proteins are also annotated with biological process, molecular function and cellular component gene ontology (GO) terms; the GO database and dictyBase are just two of more than 60 databases cross-referenced in UniProtKB. With ongoing and increased curation of D. discoideum protein sequences UniProt aims to support Dictyostelium research.

Wu CH, Apweiler R, Bairoch A, Natale DA, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Mazumder R, O'Donovan C, Redaschi N and Suzek B (2006) The Universal Protein Resource (UniProt): an expanding universe of protein information. Nucleic Acids Res. 34(Database issue):D187-91.

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