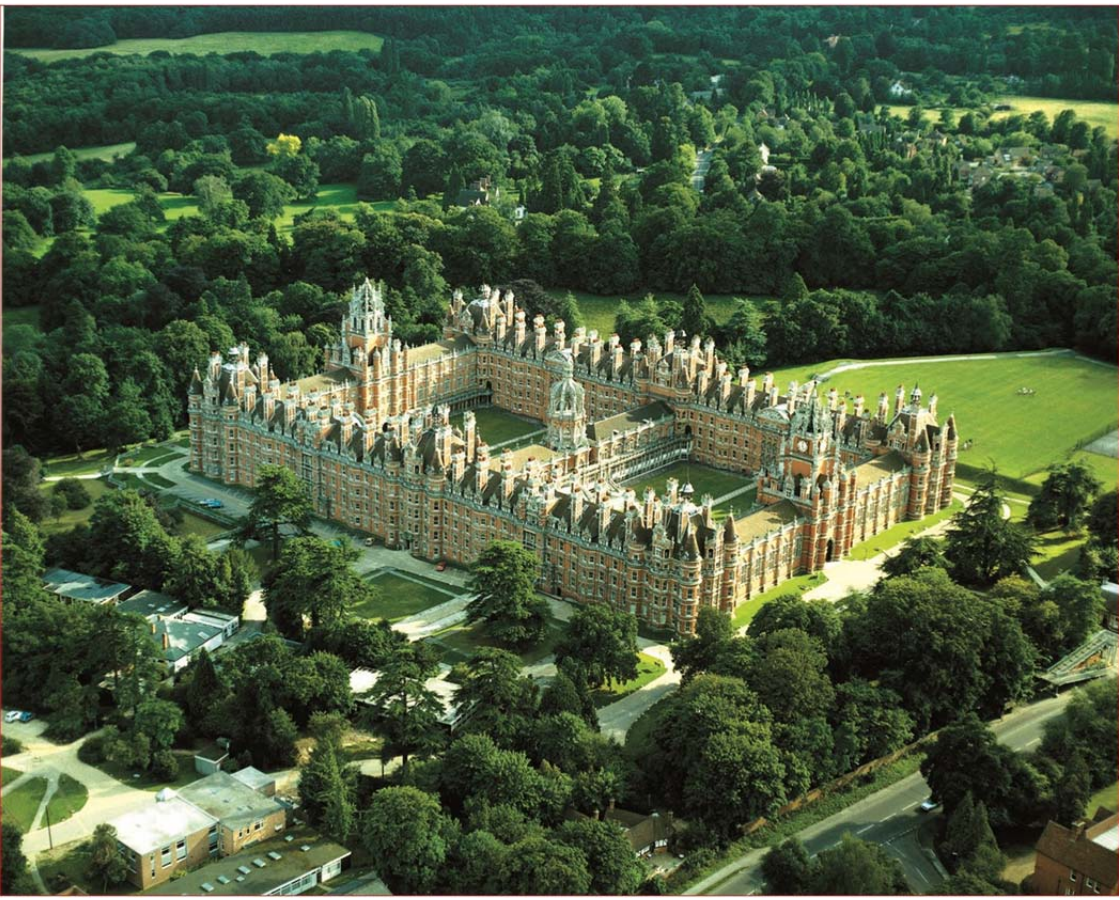
Dicty 2015



Royal Holloway University of London

August 9th–13th

London, United Kingdom

Annual Dictyostelium Conference

2015

August 9-13

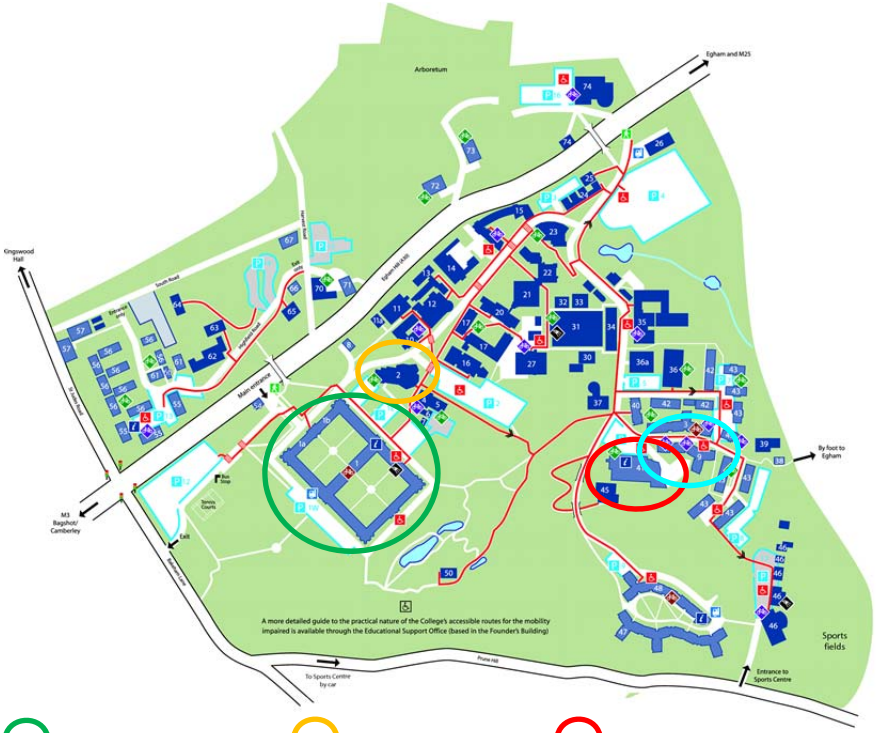
Royal Holloway University of London

Organisers
Robin Williams
Arjan Kortholt
Douwe Veltman

Dicty2015 is sponsored by

And special thanks to Johannes Zanker
for providing the images for the conference bag.

Map and information



Founders Building
- *Dining room*
- *Crosslands Suite*
- *Picture Gallery*

Windsor Building

The HUB Reception

Tuke Hall Accomodation

All talks are held in the Windsor Auditorium (Windsor Building).

Accommodation check-in:	Sunday, The Hub Reception
Registration:	Sunday, Windsor Building
Breakfast:	Mon-Thur 7.30-9am, Founders Dining Room
Lunch:	Windsor Building
Dinner:	Sun 18.00-19.30, Founders Dining Room Mon-Tue 18.30-20.00, Founders Dining room
Bar Service:	Sun 19.00-21.00, Crosslands Suite Mon-Tue 19.30-23.00 Crosslands Suite
Poster Sessions:	Mon-Tue 20.00-21.30, Windsor Building
Conference Banquet:	Wed, Reception 18.30-19.00 Picture Gallery Banquet 19.15-21.00 Founders Dining Room English Village Dance 21.00-23.00
Contact:	CMS@rhul.ac.uk
Organisers mobile: (emergency only)	+44(0)7946548516

Conference programme

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Chemotaxis and Development I</td><td>Wednesday 12th

Cytokinesis and Nuclear Organization</td><td>Thursday 13th

Chemotaxis and Development II</td></tr>
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Pick up lunch pack

Excursion
Windsor Castle or
Fuller's Griffin Brewery</td><td>12.30-13.45
lunch</td></tr>
<tr><td>14.00-15.10
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Poster Session I
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even numbers</td></tr>
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Sunday, August 9

12.00		Registration desk open until 18.00
17.00	*1*	Dicty Jails: a new tool for long-term single-cell imaging. **Ana Teresa López Jiménez**
17.30	*2*	Src1 a protein of the inner nuclear membrane is interacting with the lamin-like protein NE81 in Dictyostelium discoideum. **Petros Batsios**
18.00		Dinner

Monday, August 10 BIOMEDICAL & HOST PATHOGEN INTERACTIONS

Morning session chair: Adrian Harwood

09.30 *3* Dictyostelium as a biomedical model: Understanding the MCT ketogenic diet. **Robin Williams**

10.00 *4* Roco4: A model to investigate LRRK2 inhibitor binding. **Bernd Gilsbach**

10.20 *5* Impedance spectroscopy of Dictyostelium discoideum development: from collective oscillations to single cell adhesion dynamics. **Marco Tarantola**

10.40-11.10 coffee break

11.10 *6* Cell death in Dictyostelium: investigations on causality. **Pierre Golstein**

11.30 *7* How Ligand Breakdown Shapes and Potentiates Chemotactic Gradients. **Robert Insall**

11.50 *8* Towards the Application of CRISPR for Whole Gene and Nucleotide-Specific Mutagenesis. **Alan Kimmel**

12.10 *9* Amoeba Tastes Bitter: A Novel Non Animal Model for Bitterness Research. **Marco Cocorocchio**

12.30-14.00 lunch

Afternoon session chair: Annette Müller-Taubenberger

14.00 *10* Bacteria capture and killing by NOX-dependent DNA extracellular traps predates the evolution of animals. **Thierry Soldati**

14.30 *11* Identification of macrame - a Novel regulator of microtubule dynamics and lysosomal trafficking. **Jason King**

14.50 *12* Nutritional immunity and iron homeostasis in Dictyostelium. **Salvatore Bozzaro**

15.10-15.40 coffee break

15.40 *13* Interactions between amoebae and non-pathogenic bacteria: a genetic approach. **Pierre Cosson**

16.00 *14* Is the TOR pathway controlled by Mycobacterium marinum? **Elena Cardenal-Muñoz**

16.20 *15* The Crop Sows the Farmer: Burkholderia bacteria infectiously induce the proto-farming symbiosis of Dictyostelium amoebae and food bacteria. **Susanne DiSalvo**

16.40 Flash talks

17.00-18.30 Break

18.30-20.00 Dinner

20.00-21.30 Poster Session I - odd numbers

Tuesday, August 11 **CHEMOTAXIS AND DEVELOPMENT I**

Morning session chair: Peter van Haastert

09.00 *16* Systems biology of cellular rhythms: from microorganisms to mammalian cells. **Albert Goldbeter**
09.30 *17* Identification of a novel chemoattractant GPCR that regulates both chemotaxis and phagocytosis. **Tian Jin**
10.00 *18* A pseudopod-centred model for cell migration also replicates phagocytosis (plus a bit of amplification). **Matthew Neilson**
10.20 *19* Understanding cell movement in the back of traveling waves of chemoattractant. **Monica Skoge**

10.40-11.10 coffee break

11.10 *20* Investigating the effects of inositol depletion in Dictyostelium. **Anna Frej**
11.30 *21* Function of Rho GTPase in Gradient Sensing. **Hiroshi Senoo**
11.50 *22* Regulation of Population Density in Dictyostelium by Extracellular Polyphosphate. **Patrick Suess**
12.10 *23* Why does Dictyostelium discoideum have so many identical actin genes? **Edward Tunnacliffe**

12.30-14.00 lunch

Afternoon session chair: Richard Gomer

14.00 *24* Noise dampening is required for robust cell fate oscillator dynamics. **Chris Thompson**
14.30 *25* Gene regulation by c-di-GMP in Dictyostelid social amoebas. **Zhi-hui Chen**
14.50 *26* A novel ubiquitin like domain containing protein is required for early development and cAMP propagation in D.discoideum. **Satoshi Kuwana**

15.10-15.40 coffee break

15.40 *27* The new face of dictyBase. **David Jimenez-Morales**
16.00 *28* Characterization of Dictyostelium genes by parallel phenotyping of barcoded mutants. **Adam Kuspa**
16.20 Flash talks
16.40 *29* Introducing a new dictyExpress:from raw reads to gene expression browsing. **Miha Stajdohar** and **Blaz Zupan**

17.00-18.30 Break

18.30-20.00 Dinner
20.00-21.30 Poster Session II - even numbers

Wednesday, August 12 CYTOKINESIS AND NUCLEAR ORGANIZATION

Morning session chair: Jonathan Chubb

09.00 *30* Cell shape through the cell cycle. **Buzz Baum**

09.30 *31* The STE group kinase SepA controls cell migration and cleavage furrow formation. **Annette Müller-Taubenberger**

10.00 *32* ADP-ribosylation of histone H2B in repair of DNA double strand breaks. **Catherine Pears**

10.20 *33* Measurement and modelling of stochastic transcription dynamics. **Adam Corrigan**

10.40-11.10 coffee break

11.10 *34* Spacing Remodelers in Dictyostelium Control Transcription Through Distinct Effects on Nucleosome Size, Positioning and Occupancy. **Mark Robinson**

11.30 *35* Proteolytic regulation in Dictoystelium mitochondria. **Elinor Thompson**

11.50 *36* C-module-binding factor supports retrotransposition of TRE5-A by suppressing an RNAi pathway. **Thomas Winckler**

12.10 *37* REMI-seq – generation of a genome-wide mutant resource for Dictyostelium functional genomics. **Amy Baldwin**

12.30 Pick up lunch pack

13.00 Departure for Windsor Castle and Fuller's Griffin Brewery

18.30 Reception, Picture Gallery,
Banquet, Founders Dining Room
English Village Dance

Thursday, August 10 **CHEMOTAXIS AND DEVELOPMENT II**

Morning session chair: Elinor Thompson

09.30 *38* Formin A function during migration in confined environments. **Jan Faix**

10.00 *39* Polychromatic 'greenbeard' genes determine patterns of aggregation in a social amoeba. **Nicole Gruenheit**

10.20 *40* Locally Regulated Ras Signaling Reveals Inhibitory Process in GPCR-mediated Chemotaxis. **Xuehua Xu**

10.40-11.10 coffee break

11.10 *41* A Cud-type transcription factor regulates Dictyostelium spore differentiation. **Yoko Yamada**

11.30 *42* Ras activation during cell movement in buffer and chemotaxis. **Peter van Haastert**

11.50 *43* The Protein Kinase C Orthologue PkcA Regulates the Actin Cytoskeleton. **Derrick Brazill**

12.10 *44* Macropinosomes and pseudopods are associated with distinct spatial patterns of PIP_3 and SCAR/WAVE. **Douwe Veltman**

12.30-13.45 lunch

Departure

Talks

Talk 1

Dicty Jails: a new tool for long-term single-cell imaging

Ana Teresa López Jiménez[1], Matthieu Délincé[2], Monica Hagedorn[3] John D. McKinney[2], Thierry Soldati[1]

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[2]School of Life Sciences, École Polytechnique Fédéral de Lausanne (EPFL). 1015 Lausanne, Switzerland.

[3]Section Parasitology, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Straße 74
D-20359 Hamburg, Germany

Abstract

Recent progress in single-cell techniques has evidenced that cell populations are intrinsically heterogeneous, and that the average cell behaviour can be irrelevant to the outcome of biological processes. This is especially relevant to the monitoring of intracellular infections, which result from the interaction of populations of host and pathogens, and give rise to a variety of complex interdependent responses.

We use long-term single-cell imaging to study the infection of *Dictyostelium* and the intracellular pathogen *Mycobacterium marinum*, the causative agent of fish tuberculosis . Similarly to the human pathogen *Mycobacterium tuberculosis, M. marinum* hampers the maturation and acidification of the phagosomal compartment in which it resides. Afterwards, *M. marinum* breaks its vacuole to gain access to the cytosol and eventually escapes by cell lysis or by a non-lytic mechanism called ejection.

Like many other professional phagocytes, *Dictyostelium* is highly motile making it unsuitable for single-cell long-term imaging. Therefore, a microfluidic device to trap infected cells was designed. The *Dictyostelium* cell cycle and motility are not impaired in this device and can be recorded for several days.

We have started dissecting the infection process using different cellular markers such as VacuolinA-GFP, which accumulates at the mycobacterium-containing compartment. Exciting results showed that the induction of the differentiation cycle triggers the curing of the *M. marinum* infection. We thus engaged in deciphering how important pathways such as autophagy, exocytosis or ejection specifically impact on the fate of the infection, using mutants such as *atg1-*, *wash-* or *racH-*, respectively.

Talk 2

Src1 a protein of the inner nuclear membrane is interacting with the lamin-like protein NE81 in Dictyostelium discoideum

Petros Batsios[1], Xiang Ren[2], Irene Meyer[1], Otto Baumann[1], Denis Larochelle[2] and Ralph Gräf[1]

[1]*Institut für Biochemie und Biology, Universität Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Deutschland*

[2]*Department of Biochemistry and Molecular Biology, Clark University, 15 Maywood St., Worcester, MA 01610-1477, USA*

Abstract

The nuclear envelope (NE) consists of the outer and inner nuclear membrane (INM), whereby the latter is bound to the nuclear lamina. We identified Src1 as a *Dictyostelium* homologue of group II LEM-domain proteins, such as the human lamin and chromatin-binding INM protein MAN1. Yet, in contrast to MAN1 Src1 lacks a LEM-domain. Both endogenous Src1 and GFP-Src1 localize to the NE during the entire cell cycle. During interphase Src1 is concentrated at sites associated with nucleoli as shown by antibody staining indicating that Src1 may be required to bind nuclei at the NE in order to facilitate transport of ribosome subunits into the cytosol. As proven by immunogold-EM stainings, GFP-Src1 is exclusively located at the INM. Cells with high GFP-Src1 overexpression formed filamentous nuclear protrusions similar in morphology and dynamics to those formed in mutants overexpressing a C-terminal fragment of the Dictyostelium lamin NE81 fused to GFP which led to the idea that Src1 and NE81 could interact. This was confirmed when soluble, truncated mRFP-Src1 co-localized at cytosolic clusters consisting of an intentionally mis-localized mutant of GFP-NE81. Furthermore, Src1 interacted in a BioID biotinylation assay, independently proving an interaction between both proteins. Our results show that lower eukaryotes contain conserved proteins of the inner NE that are interacting with a lamin-based nuclear lamina as in higher cells. This is interesting not only from an evolutionary point of view, but also makes these amoebae an interesting experimental platform to study the role of lamina-chromatin interactions in chromatin dynamics and cell differentiation.

Talk 3

Dictyostelium as a biomedical model: Understanding the MCT ketogenic diet

Pishan Chang[1], Katrin Augustin[1], Kim Tan[4], Karin Boddum[2], Sophie Williams[2], Min Sun[2], John Terschak[3], Jorge D. Hardege[3], Karin Borges [4*], Philip E. Chen[1], Matthew C. Walker[2], Robin S.B. Williams[1]

[1]Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham, TW20 0EX, UK. [2] Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, WC1N 3BG, UK. [3] School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK.[4] Department of Pharmacology, School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia.

Abstract

Our research has focused on employing *Dictyostelium* as a simple model system for biomedical research. The model provides a range of advantages including rapid growth, single and multiple cell stages, well characterized development, rapid genetic ablation or protein tagging and the use of isogenic cultures. This research approach is also consistent with changing funding priorities in the UK, with an increased focus on biomedical or health-related areas. Here we will outline a long-standing research area in our laboratory into epilepsy treatments that was initiated in *Dictyostelium* and translated to mammalian systems.

We employed *Dictyostelium* in our initial studies to better understand the molecular mechanisms of the commonly used epilepsy treatment valproic acid. Using *Dictyostelium,* we showed that the drug blocked turnover of phosphoinositides, and we were able to demonstrate a similar effect in the mammalian brain during seizure progression. Using *Dictyostelium*, we then identified a range of compounds with enhanced activity in this mechanism. One of these compounds, decanoic acid, is a major constituent of a diet used to treat drug resistant epilepsy, the medium chain triglyceride (MCT) ketogenic diet. We then showed that decanoic acid, which is elevated in the plasma of patients on the diet, acts to directly control seizure activity in multiple animal seizure models. Notably, this effect could not be reproduced for ketones, suggesting that the diet may not act via ketogenesis. We further identified a primary target for decanoic acid in this therapeutic role.

This project highlights the use of *Dictyostelium* as a simple and versatile model system for biomedical research, and successful translation to mammalian pre-clinical research models.

Talk 4

Roco4: A model to investigate LRRK2 inhibitor binding

Bernd K. Gilsbach[1], Genta Ito[2], Dario R. Alessi[2], Peter J.M van Haastert[1], Alfred Wittinghofer[3] Arjan Kortholt[1]

[1]*Department of Cell Biochemistry, University of Groningen, 9747 AG, Groningen, The Netherlands;*

[2] *MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, United Kingdom*

[3]*Structural Biology Group, Max Planck Institut für Molekulare Physiologie, 44227 Dortmund, Germany*

Abstract

LRRK2 is a multi-domain protein, including a GTPase and kinase domain, which is found to be mutated in Parkinson´s disease (PD) patients. So far there is no established therapy for PD. Given its prevalence in cases of PD, and the role of the kinase and GTPase, LRRK2 represents a clear molecular target for therapeutic development. However, purification of suitable amounts and quality of LRRK2 to undertake structural and biochemical characterization is not feasible. We previously have use *Dictyostelium discoideum* Roco4 as model to study the structural and biochemical characteristics of the LRRK2 kinase domain (REF). Furthermore, the biochemical tractability of Roco4 allows *in vitro* screening of inhibitor libraries, whereas the unique phenotype of *roco4*-null mutants and its rescue by the Roco4-LRRK2-kinase chimera can be used for *in vivo* testing and screening.

The Roco4 kinase domain can be purified in high amounts and crystallized under various conditions. Nevertheless the wild-type protein binds only with low affinity to LRRK2 specific inhibitors therefore we introduced two mutations F1106L and F 1161L which increased the inhibitor binding up to 175 fold. Furthermore the structural analysis of the two co-crystallized inhibitors LRRK2-Inh1 and Compound 19 showed the parts of the inhibitors do not contribute to binding and give space for optimization of these compounds.

Together our data show that Roco4 is a suitable model system to obtain insight in the binding mechanism, optimization of the current and identification of new LRRK2 inhibitors.

Talk 5

Impedance spectroscopy of Dictyostelium discoideum development: from collective oscillations to single cell adhesion dynamics

Leonhardt, H., Gerhardt, M., Krüger, K., Schäfer, E., Aue, D., Polo, E., Westendorf, C., Oikawa, N., Beta, C., Geil, B., Janshoff, A., Bodenschatz, E. and Tarantola, M.

Abstract

D. d. synchronizes itself during early development with waves of cAMP. We have quantified these collective phenomena on the intercellular level by applying electric impedance measurements. We showed that D. d. seeded on micrometer-sized electrodes provoke impedance oscillations, which can be attributed to cAMP dependent oscillations [1]. We recorded time dependent correlation of Electrical Cell-substrate Impedance Sensing (ECIS) signals and changes in height detected by TIRF-microscopy by comparing light intensity of subtracted BF images from both assays. A high impedance signal IZI was found to correlate with a high fluorescence intensity and thus with a small cell-surface-distance. The arrangement of D. d. analyzed on bright field images revealed synchronous formation of cell aggregates contributing to impedance oscillations, while a decrease of circularity <C> occurs out-of phase. Modeling calculations show that cells in united structures generate higher impedance values than the same number of isolated cells [2]. In addition, we analyze starving cells in microfluidic devices in combination with an impedimetric assay [3]. The results provide a quantitative understanding of the collective cell morphology during early starvation.

In a recent work, we present electrical impedance measurements of single amoeboid cells on microelectrodes. Wild type cells and mutant strains are studied that differ in their cell-substrate adhesion strength. We recorded the projected cell area by time-lapse microscopy and observed quasi-periodic oscillations of the cell shape. These were correlated with long-term variations in the impedance signal and did not show any systematic dependence on the adhesion strength. In contrast, short-term impedance fluctuations were clearly correlated with the adhesion strength. Interference reflection microscopy recordings showed that altered cell-substrate adhesion corresponds to changes in the area of close contact between the ventral surface of the cell and the substrate. We thus conclude that long-term trends in the cellular impedance signal of single cells are caused by oscillatory changes in the projected cell area, while short-term fluctuations can be attributed to local changes in the cell-substrate distance that are related to the formation of adhesion sites. We propose a method to separate both contributions to the impedance signal based on band-pass filtering.

[1] Schäfer, E., Westendorf, C., Bodenschatz, E., Beta, C., Geil, B. and Janshoff, A., "Shape Oscillations of Dictyostelium discoideum Cells on Ultramicroelectrodes Monitored by Impedance Analysis", Small, March 2011, Vol. 7(6), 723 pp.

[2] Schäfer, E., Tarantola, M., Polo, E., Westendorf, C., Oikawa, N., Bodenschatz, E., Geil, B. and Janshoff, A., "Chemotaxis of Dictyostelium discoideum: Collective Oscillation of Cellular Contacts", PLOS One, January 2013, Vol. 8, e54172 pp.

[3] Schäfer, E., Aue, D., Tarantola, M., Polo, E., Westendorf, C., Oikawa, N., Bodenschatz, E., Geil, B. and Janshoff, A., "Collective behavior of Dictyostelium discoideum monitored by impedance analysis", Commun. Integr. Biol., May 2013, Vol. 6(3), e23894 pp.

[4] Leonhardt, H., Gerhardt, M., Krüger, K., Tarantola, M. and Beta, C., "Cell-substrate impedance fluctuations of single amoeboid cells encode cell-shape and cell-substrate adhesion dynamics", Phys. Rev. E, 2015, submitted

Talk 6

Cell death in Dictyostelium: investigations on causality

Pierre Golstein

Centre d'Immunologie de Marseille-Luminy, INSERM-CNRS-Université de la Méditerranée, Case 906, 13288 Marseille Cedex 9, France

Abstract

Dictyostelium discoideum, a eukaryote, a protist, a slime mold, is unicellular in favorable conditions. It shows upon starvation multicellular development leading to fruiting bodies. Each of these fruiting bodies is made of a mass of spores on top of a stalk. This stalk is made of dying or dead cells.

This cell death, developmental by definition, can be mimicked in vitro in cell monolayers allowing morphological study and genetic manipulations. Dictyostelium offers two main advantages for the study of this cell death. First, its genome, now sequenced, is small, compact, and haploid, favoring insertional mutagenesis. Second, from a cell death point of view this genome does not encode caspases or bcl-2 family members, thus in this model organism there is no apoptosis machinery that could interfere with the non-apoptotic cell death mechanisms at play.

The results to be reported include a description of successive steps of this developmental cell death as mimicked in vitro (including paddle cell formation, rounding, vacuolization and cellulose shell formation), their governing exogenous two main signals, an insertional mutagenesis study partially identifying the corresponding signaling pathways, and considerations of where death proper is located in these phenomenological and molecular pathways.

Talk 7

How Ligand Breakdown Shapes and Potentiates Chemotactic Gradients

Luke Tweedy[1], David Knecht[2], Robert Insall[1]

[1]*Beatson Institute for Cancer Research, Glasgow G61 1BD, UK*

[2]*University of Connecticut, 91 N. Eagleville Rd., Storrs, CT 06269, USA*

Abstract

Breakdown of chemoattractants such as cAMP is essential for their biological functions. Disruption of cAMP phosphodiesterase, for example, completely blocks Dictyostelium development as cAMP levels grow too constitutively high to allow pulsatile signalling.

We have recently found in another system that uses chemotaxis - melanoma cells migrating towards LPA - that attractant breakdown can cause a more fundamental behaviour shift. Melanoma cells break down LPA that is globally present in saturating levels. By doing this they create chemoattractant gradients that are low where cell density is highest. These gradients have a number of unexpected effects - they can operate over arbitrarily large distances, unlike fixed gradients, whose range is limited to short distances. Although the population responds as a whole, only a variable and complex proportion of the individual cells move at any one time; we provide computational models and direct measurements to show how this works.

Consideration of the effects of attractant breakdown shows that even in established assays, such as Dunn chamber and micropipette assays, the enzymes that break down attractants fundamentally change the shape of the gradients and the responses of the cells.

Talk 8

Towards the Application of CRISPR for Whole Gene and Nucleotide-Specific Mutagenesis

Wenli Bai, Alan R. Kimmel

Laboratory of Cellular and Developmental Biology

National Institute of Diabetes and Digestive and Kidney Diseases

National Institutes of Health, Bethesda, MD 20892, US

Abstract

Although the efficiency of targeted gene disruption by homologous recombination in Dictyostelium is high compared to more complex genomes, site-specific mutagenesis and epitope tagging are still problematic. To overcome these issues, we have been developing methods for Dictyostelium-specific application of the CRISPR/Cas9 genome editing system. We will discuss vector details, experimental guidelines, but also current limitations.

Talk 9

Amoeba Tastes Bitter: A Novel non Animal Model for Bitterness Research

Marco Cocorocchio[1], Paul L. R. Andrews[2], Robin S. B. Williams[1]

[1] School of Biological Sciences, Centre of Biomedical Sciences, Royal Holloway University of London, Egham, United Kingdom,

[2] Division of Biomedical Science St George's University of London, London, United Kingdom

Abstract

A large number of therapeutically active drugs have a bitter taste. This often causes an aversive reaction in both children and adults, leading to decreased patient compliance and in some cases to severe reactions such as nausea and vomiting. Research into bitter taste perception often employs animal models that are expensive, time consuming and causes long lasting distress. Thus, there is an urgent need to develop new strategies for the identification of bitter substances in the early phase of drug screening. Since we have shown that, like humans, the social amoeba *Dictyostelium discoideum* is able to respond to bitter molecules, we set out to investigate if the model was able to predict the bitterness of specific molecules. Here, twelve bitter substances (ibuprofen, azelastine hydrochloride, caffeine, chlorhexidine digluconate, potassium nitrate, paracetamol, quinine and five blinded compounds) were employed to investigate the changes in cell behaviour (inhibition of protrusion formation) as a read-out for bitterness response. We show that *Dictyostelium* cell behaviour was inhibited by all compounds in a dose dependent manner, with calculated IC_{50} values enabling comparison to that obtained using the rat *in vivo* Brief Access Taste Aversion (BATA) test and human sensory panel models test. Using both known and blinded compounds, *Dictyostelium* cell behaviour shows a significant correlation to both rat and human bitterness response. Therefore, *Dictyostelium* shows promise as replacement pre-screening platform for the investigation of bitter tastants.

Talk 10

Bacteria capture and killing by NOX-dependent DNA extracellular traps predates the evolution of animals

Xuezhi Zhang[1], Olga Zhuchenko[2], Adam Kuspa[2], and Thierry Soldati[1].

[1] *Department of Biochemistry, Sciences II, University of Geneva, 30 Quai Ernest-Ansermet, CH-1211 Geneva-4, Switzerland.*

[2] *Molecular and Human Genetics, Baylor College of Medicine, Houston TX 770301, USA*

Abstract

The first line of defence against bacteria are phagocytic cells of the innate immune system. These cells kill bacteria via oxygen-dependent (e.g. ROS) and oxygen-independent (e.g. chemicals, enzymes and microbicidal peptides) mechanisms. While multicellular organisms use phagocytosis to kill microbes and initiate a sustained immune response, phagocytic amoebae internalise bacteria as nutrients, via mechanisms of recognition, signalling and killing that are surprisingly conserved. In particular, at the transition to multicellularity, eukaryotic organisms acquired NOX enzymes to generate ROS.

Dictyostelium is a social amoeba that feeds by phagocytosis and has a rudimentary but highly conserved cell-intrinsic immune system. It is genetically and biochemically tractable and has emerged as a powerful and experimentally versatile model organism. Solitary Dictyostelium amoebae feed on bacteria in the soil until depletion of the food induces a developmental program in which ~100,000 amoebae aggregate, form a migrating slug and eventually undergo terminal differentiation into the spores and stalk cells that comprise the fruiting body. In addition, the slug also contains a population of Sentinel cells that are the only slug cells able to phagocytose bacteria to be left behind during slug migration. We propose that Sentinel cells represent a form of amoebal innate immunity analogous to mammalian neutrophils.

Extracellular traps (ETs) produced by neutrophils are reticulated nets of DNA decorated with antimicrobial granules that contribute to defense against bacteria. Sentinel cells are able to elaborate DNA-based ETs morphologically and functionally similar to those of neutrophils. The production of ETs is stimulated by LPS and by Gram-negative bacteria. Most interestingly, ETs production requires the generation of ROS by Sentinel cells, via NOX enzymes. These results emphasise that, like in neutrophils from CGD patients, NOX-dependent ROS production is essential to ET formation, and that the origin of DNA-based ETs as a mechanism for bacterial defense predates the appearance of animals.

Talk 11

Identification of *macrame* – a novel regulator of microtubule dynamics and lysosomal trafficking

Jason S. King

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Abstract

Autophagy is required by cells to capture and remove damaged cellular components as well as survive periods of starvation. A genetic screen to identify new components required for autophagic degradation identified a new gene required for cells to degrade their cytosol, survive amino acid starvation and complete development. Null mutants however also have poor bacterial and axenic growth, indicating a general lysosomal defect. This gene (which we have named macrame*)* encodes a highly conserved, but previously unstudied protein. Mechanistically, we show that macrame is a fundamental regulator of microtubule dynamics, and thus essential for proper lysosomal trafficking and degradation.

Talk 12

Nutritional immunity and iron homeostasis in Dictyostelium

Barbara Peracino[1], Simona Buracco[1], Raffaella Cinquetti[2], Alessandra Vollero[2], Elena Signoretto[3], Michela Castagna[3], Elena Bossi[2] and Salvatore Bozzaro[1]

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Abstract

Iron is an essential element for all cells, being involved in many cellular processes as enzyme cofactor or component of Fe-S or heme prosthetic groups. Bacteria acquire iron from the environment by secreting siderophores, and many intracellular pathogens, such as legionella or mycobacteria, scavenge iron from the phagosomal milieu. Professional phagocytes, such as macrophages or *Dictyostelium*, have developed mechanisms to manipulate metal ion concentration to control microbial growth, a process known as "nutritional immunity". The phagosomal iron transporter Nramp1 plays a paradigmatic role in this process. In *Dictyostelium*, Nramp1 displays both nutritive and protective function, favouring cytosolic accumulation of iron upon bacteria digestion and simultaneously starving ingested pathogens from iron. Such a dual role is similar to that of the macrophage ortholog, which recycles iron from ingested erythrocytes while starving engulfed pathogens. *Dictyostelium* Nramp1 shares with the mammalian ortholog several features: their protein sequences are highly conserved, in both cases transport is regulated by a proton gradient, is electrogenic and specific for Fe^{2+} and Mn^{2+}, not Fe^{3+} or Cu^{2+}. Dictyostelium also harbours Nramp2, which is located in the contractile vacuole and synergistically with Nramp1 regulates iron homeostasis. Nramp2 differs for a more divergent protein sequence and for mediating non-electrogenic transport of Fe^{2+} only. In vivo iron efflux from phagosomes via Nramp1 requires reduction of Fe^{3+} to Fe^{2+}. Of three putative ferric reductases, at least one is expressed in plasma membrane and phagosomes. Other major regulators of mammalian iron homeostasis, such as mitoferrin, frataxin and Aco-1, are conserved in Dictyostelium.

Talk 13

Interactions between amoebae and non-pathogenic bacteria : a genetic approach

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Abstract

Dictyostelium amoebae can feed upon a variety of non-pathogenic bacteria. For this, they must ingest, kill and digest the bacteria to which they are confronted. We have previously isolated and characterized Dictyostelium mutants that lost the ability to feed upon bacteria. For some mutants, this was due to a defect in their ability to kill bacteria (e.g. kil1, kil2 mutant cells). For others (e.g. fspA mutant), this was linked to an inability to sense the presence of bacteria.

We have recently isolated new Dictyostelium mutants unable to feed upon non-pathogenic bacteria, and are currently analyzing their phenotypes. Our current results reveal the existence of distinct mechanisms for sensing and killing of different types of bacteria.

Talk 14

Is the TOR pathway controlled by *Mycobacterium marinum*?

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Abstract

The professional phagocyte *Dictyostelium* has become a good model to investigate how eukaryotic cells respond to bacterial infection. We use *Dictyostelium* as a host model for tuberculosis studies. Like in macrophages, *Mycobacterium marinum* (bacterium causing a tuberculosis-like disease in frogs and fish) modifies the phagosome of *Dictyostelium* to establish a replicative niche, which might shield against intracellular immune responses but limits the bacterial access to nutrients. Maybe as a consequence, early after the infection *M. marinum* breaks its niche, leading to the recruitment of host autophagy markers such as p62, Atg8/LC3 and ubiquitin. Autophagy is part of the innate immune response against intracellular pathogens, even though some bacteria benefit from autophagy-derived nutrients.

Amoebae lacking Atg1/ULK1, the major kinase responsible for autophagy induction, show increased numbers of lipid droplets (LDs, the main nutrient source for mycobacteria), while proliferation of *M. marinum* is enhanced. Since TOR kinase regulates both autophagy and LDs homeostasis in eukaryotes, we wonder whether *M. marinum* actively controls TOR in order to obtain lipids from the host and thus support its proliferation. We observe changes in the localization of some TOR pathway components (e.g. Rheb, Lst8, Raptor) during infection, which mimic the changes observed under starvation. By applying several biochemical and microscopy approaches, we are trying to decipher the signaling cascades responsible for the different localization of TOR pathway proteins, as well as the correlation between these changes in localization and TOR activity.

Talk 15

The Crop Sows the Farmer: *Burkholderia* bacteria infectiously induce the proto-farming symbiosis of *Dictyostelium* amoebae and food bacteria.

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Abstract

The initiation of a new symbiotic association can allow for an organism to rapidly acquire novel traits by gaining access to the genetic repertoire of their partner. However, symbionts may begin as pathogens that only subsequently become beneficial. In the *Dictyostelium discoideum* farming symbiosis, certain amoebas (farmers) persistently associate with bacterial partners. These bacterial partners can incur a reproductive cost but also provide their host with novel beneficial capabilities, such as carriage of bacterial food (proto-farming) and defense against competitors. Here, we explore the role of bacterial associates in the initiation, maintenance, and phenotypic effects in new farming symbioses. We demonstrate that two clades of farmer-associated *Burkholderia* isolates colonize *D. discoideum* non-farmers and infectiously endow them with farmer-like characteristics, suggesting that *Burkholderia* symbionts are a major driver of the farming phenomenon. Under food rich conditions, *Burkholderia* colonized amoebas produce fewer spores than their uncolonized counterparts, but the severity of this effect differs between *Burkholderia* genotypes and in some cases, between new and old amoeba hosts, suggesting some role for co-evolution within the association. However, *Burkholderia* colonization also leads to secondary food carriage by amoeba hosts, which may be conditionally adaptive because it can confer an advantage to the amoeba host when grown in food limiting conditions. *Burkholderia* can be found inside amoeba spores demonstrating that it can invade and survive within host cells. These results change our understanding of the *Dictyostelium* farming symbiosis by establishing that the bacterial partner, *Burkholderia*, is an important causative agent of the farming phenomenon.

Talk 16

Keynote speaker

Systems biology of cellular rhythms: from microorganisms to mammalian cells

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Abstract

Rhythmic phenomena occur at all levels of biological organization, with periods ranging from milliseconds to years. Cellular rhythms originate from the regulatory feedback loops that control the dynamics of biochemical processes and represent a phenomenon of temporal self-organization. They illustrate how an emergent property, autonomous oscillatory behaviour, arises from molecular interactions in regulatory networks. In my talk I will address the dynamical bases of cellular rhythms in both microorganisms and animals. In microorganisms, examples range from metabolic oscillations in yeast to cyclic AMP oscillations in *Dictyostelium* cells, *Min* protein oscillations in *E. coli*, and circadian oscillations in cyanobacteria. In plant and animal cells, circadian oscillations originate from transcription-translation regulatory loops. The network of cyclin-dependent kinases that controls progression through the mammalian cell cycle is regulated in a way that makes it prone to operate in a periodic manner. Focusing on the latter system I will address the balance between cell cycle arrest and cell proliferation. This balance is controlled by the combined effect of growth factors, the levels of activators (oncogenes) and inhibitors (tumour suppressors) of cell cycle progression, the extracellular matrix, and contact inhibition. Supra-threshold changes in the level of any of these factors can trigger a switch in the dynamical behaviour of the Cdk network corresponding to a bifurcation between a stable steady state, associated with cell cycle arrest, and sustained oscillations of the various cyclin-dependent kinases, corresponding to cell proliferation.

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Talk 17

Identification of a novel chemoattractant GPCR that regulates both chemotaxis and phagocytosis

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Abstract

Eukaryotic phagocytes search and destroy invading microorganisms via chemotaxis and phagocytosis. Different receptors mediate signaling pathways to control the remodeling of actin cytoskeleton that generates either chemotaxing leading fronts or phagocytic cups. Here, using a quantitative phosphoproteomic approach, we uncovered an orphan GPCR for folic acid, a chemoattractant for Dictyostelium discoideum. D. diccoideum cells are professional phagocytes that chase bacteria via chemotaxis and eat them via phagocytosis. We showed that this new GPCR is required for both chemotaxis toward folic acid and phagocytosis of bacteria for food. Thus, our study suggests that a chemoattractant GPCR-mediated signaling pathways that control not only directional cell migration but also phagocytosis.

Talk 18

A pseudopod-centred model for cell migration also replicates phagocytosis (plus a bit of amplification)

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Abstract

Phagocytosis (the process by which a cell consumes a solid particle) is involved in a variety of processes, such as nutrient-acquisition, immune-response and the removal of cell debris. Upon encountering a foreign particle, the actin cytoskeleton of a phagocytic cell undergoes local reorganisation, which causes the cell to stretch itself around the particle until it has been engulfed. We demonstrate that, with a few simple modifications, our existing computational model for pseudopod-centred cell migration and chemotaxis can be used to simulate phagocytosis. Using this modified model, we investigate the effects of phagocytic signalling and cell-particle adhesion on a cell's ability to perform phagocytosis.

Dictyostelium cells are capable of performing effective chemotaxis (that is, directed migration in response to an external stimulus) over a surprisingly broad range of chemical concentrations. One way in which a cell could achieve this large dynamic range is by incorporating a variable amount of amplification into its signalling system, such that the chemotactic sensitivity is maximised relative to the ambient concentration. We show that, with the inclusion of a dynamic signal-amplification term, our existing cell migration model can produce chemotaxis over a wider range of concentrations. This produces a form of adaptation that is slower, and works differently, from the short-term adaptation seen in LEGI models. Our model is then used to investigate the effects of variable amplification on a cell's ability to chemotax in shallow gradients.

Talk 19

Understanding cell movement in the back of traveling waves of chemoattractant

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Abstract

During aggregation Dictyostelium cells migrate chemotactically towards the source of traveling waves of cAMP. To do so, they must follow the spatial gradient in the front of the wave, which leads toward the wave source, and ignore the opposing spatial gradient in the back of the wave, which points away from the source – the "back-of-the-wave" problem. Our recent work has shown that 5-hour-developed, but not 3-hour-developed, cells continue migrating toward the wave source in the back of the wave - thus remembering the gradient direction seen in the front of the wave - for over 2 minutes in traveling waves with a "natural" period of 6 minutes. Here we explore the molecular basis for the movement of cells in the back of the wave using mutants, inhibitors, and fluorescent reporters of the chemotaxis signaling network. We found that caffeine strongly inhibits persistence and increases reversal in the back of the wave, and traced this effect to impairment of the TORC2-PKB pathway. In contrast, we found that persistence is decreased without increasing reversal in mutants lacking myosin II and cGMP production.

Talk 20

Investigating the effects of inositol depletion in *Dictyostelium.*

Anna Frej[1], Jonathan Clark[2], Caroline Le Roy[3], Peter Thomason[4], Andrew Davidson[4], Sergio Lilla[4], Grant Churchill[5], Sandrine P Claus[3], Robert Insall[4], Phillip Hawkins[2], Len Stephens[2] and Robin SB Williams[1]

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Abstract

Regulating inositol signalling is a proposed mechanism of action for bipolar disorder treatments, including valproic acid (VPA) and lithium. VPA and lithium reduce inositol (1,4,5)-trisphosphate (IP_3) levels in *Dictyostelium* and mammalian neurons. VPA was suggested to inhibit inositol-3-phosphate synthase (INO1) in inositol biosynthesis although the specific mechanism remains unknown.

We investigate consequences of inositol depletion and a role for INO1 in cell function and as a potential target for VPA. We show that a cell line with ablated *ino1* is unable to grow or develop unless supplemented with inositol. Inositol depletion enhances autophagy, loss of substrate adhesion and reduced cytokinesis. Overexpression of *ino1* rescues these effects, confirming an essential requirement for INO1 in growth and development.

We also examined the cellular and physiological effects of INO1 loss. The absence of inositol and INO1 protein caused a change in *Dictyostelium* metabolism. Inositol depletion led to a decrease in the levels of amino acids and phospholipids, with the exception of phosphatidylinositol 4,5-bisphosphate (PIP2). To further investigate the role of INO1, we have identified potential binding partners to help dissect its role in basic cell function.

Our data provides a new insight into the cellular and physiological roles of INO1, including cell survival, metabolism and phospholipid signalling.

Talk 21

Function of Rho GTPase in Gradient Sensing

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Abstract

A fundamental question in chemotaxis is how cells translate unstable extracellular cues into robust intracellular signaling? In Dictyostelium chemotaxis, gradients of the chemoattractant cAMP are converted to the local activation of Ras GTPase and the production of phosphatidylinositol 3,4,5 triphosphate (PIP3) at the leading edge of migrating cells. The conversion of extracellular chemical gradients to intracellular polarized signaling is called directional sensing and serves as an internal compass to determine the direction of cell movements.

Using biosensors for Ras activation and PIP3 production, I found that cells lacking a Rho GTPase, RacE, fail to orient Ras and PIP3 signaling events in cAMP gradients. As a result, racE-null cells failed to maintain the proper position of Ras and PIP3 signaling and therefore showed impaired chemotaxis. Importantly, an active form of RacE was located at the rear region facing away from cAMP gradients, suggesting that RacE spatially restricts Ras and PIP3 signaling to the front of cells from the back.

Identification and characterization of RacE binding partners will likely help reveal how RacE spatially restricts Ras activation and PIP3 production. I was specifically interested in finding proteins that bind to both RacE and Ras. Using combination of immunoprecipitation and mass spectrometry, we identified a novel protein, GflB, that binds to both RacE and Ras, as a candidate component that links RacE to Ras signaling. I generated gflB-null cells and found that gflB-null cells generate excess lateral pseudopods and are impaired chemotaxis, similar to racE-null RacE cells. These results suggest defects in directional sensing in gflB-null cells. At the meeting, I will discuss how GflB controls directional sensing and chemotaxis toward cAMP.

Talk 22

Regulation of Population Density in Dictyostelium by Extracellular Polyphosphate

Patrick Suess and Richard Gomer

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Abstract

Much remains to be understood about how a population of cells senses its density or the number of cells, and stops proliferation when the density or total number reaches a preset value. In the 1970's, Yarger and Soll found that *Dictyostelium* cells secrete a factor that induces stationary phase in shaking culture. We found that the factor appears to be inorganic polyphosphate. In shaking culture, extracellular polyphosphate concentrations increase as the cell density increases, and if the concentration of polyphosphate found at stationary phase is added to cells at mid-log, proliferation is inhibited. Adding an exopolyphosphatase to cell cultures decreases extracellular polyphosphate and causes cells to proliferate to abnormally high cell densities. Biotinylated polyphosphate shows saturatable binding at concentrations similar to those present at stationary phase, and this binding is competed by unlabeled polyphosphate as well as stationary phase conditioned media. Despite the presence of polyphosphate in all eukaryotes, its synthesis and regulation is poorly understood. We found that cells lacking InsP6K, which adds an extra phosphate to IP6, or cells lacking the secreted proteins AprA and CfaD, reach abnormally high cell densitites, do not enter a stationary phase, and show decreased extracellular polyphosphate levels. Conversely, cells lacking the phospholipase D PldB have increased extracellular polyphosphate and reach stationary phase at abnormally low cell densities. These findings may provide insight into polyphosphate synthesis and the nature of cell density and cell number regulation in higher eukaryotes.

Talk 23

Why does *Dictyostelium discoideum* have so many identical actin genes?

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Abstract

Gene duplication is important for the generation of novel genes and functions and evolutionary theory predicts entirely redundant genes should diverge over time. *Dictyostelium discoideum* has a large actin gene family which consists of 32 genes, 17 of which encode a single protein isoform. Other eukaryotes such as yeasts (1 gene), *Drosophila* (6) and humans (6) have far fewer genes, with each isoform encoded by a separate gene. Other Dictyostelids have a similar expanded actin gene family, implying functional value. To investigate why this organism has maintained such a large complement of genes encoding identical proteins, we are using 1. RNAseq data to reevaluate early expression data based on hybridisations, 2. single cell transcriptomics to characterise the cell variability in actin expression during development, 3. live transcription imaging to monitor the acute transcriptional dynamics of individual family members, 4. knockouts to investigate gene crosstalk and 5. localisation and stability assays to address the importance of 3' UTR elements.

Talk 24

Noise dampening is required for robust cell fate oscillator dynamics

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Abstract

Developmental cell fate choice and proportioning is characterised by its robustness and reproducibility. Consequently, heterogeneity (also termed noise and stochasticity) in cell behaviours, cell signalling and responses are often thought to be a hindrance. However, recent observations of 'salt-and-pepper' differentiation, which is seen in examples raging from competence in B. subtilis, lineage specification in the mouse blastocyst, to stalk and spore differentiation in Dictyostelium have challenged this view. In fact, in these cases such heterogeneity has been proposed to be required for normal cell fate choice and symmetry breaking. Our research addresses the extent to which such heterogeneity is necessary, how something that is inherently variable can be harnessed to result in a reproducible outcome, and what is the source of this heterogeneity.

Our most recent studies (Chattwood et al, eLife 2014) revealed that the interplay between dynamic heterogeneity in Ras-GTPase activity and heterogeneity in nutritional status is required for normal lineage priming and salt and pepper differentiation. This is because such heterogeneity sets the intrinsic response threshold to lineage specific differentiation signals. Indeed new RNA seq data reveals that both conditions bias cells towards the same cell fate (ie the outcome of the bias at the slug stage is identical). Despite this, we find that a surprising inverse relationship between genes under Ras and nutritional control during growth when lineage priming takes place (ie opposite gene expression profiles lead to the same fate outcome). We have used modelling and experimentation to explain this observation, which reveals the existence of a novel system required to buffer against extrinsic variation. We will describe evidence that this system facilitates the robust running of an ultradian cell fate oscillator.

Talk 25

Gene regulation by c-di-GMP in Dictyostelid social amoebas

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Abstract

Dictyostelid social amoebas aggregate to form fruiting bodies upon starvation, in which the cells differentiate into spores and stalk cells. We detected a diguanylate cyclase gene in this eukaryote, which proved to be highly similar to prokaryote diguanylate cyclases. The *Dictyostelium* diguanylate cyclase, *DgcA*, was expressed in prestalk cells and produced c-di-GMP that was secreted to induce stalk cell differentiation (1, 2). We compared transcriptional profiles of *dgca-* and wild-type cells to identify genes that are controlled by c-di-GMP signalling. We prepared fusion constructs of the promoters of the upregulated genes with *LacZ* and examined the expression patterns of the genes in developing fruiting bodies. Apart from genes expressed in the stalk, we detected several genes that were expressed very late in cells that remained amoeboid at the upper- and lower boundaries of the spore head. This set defines a novel class of developmentally regulated genes for this organism. *In vitro* studies showed that the upper- and lower cup genes were strongly upregulated by stimulation with c-di-GMP. We examined expression of c-di-GMP regulated genes in several mutants that show a similar developmental defect as the *dgca-* mutant and identified a number of potential downstream targets of c-di-GMP.

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Talk 26

A novel ubiquitin like domain containing protein is required for early development and cAMP propagation in *D.discoideum.*

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Abstract

Prestalk A (pstA) cells comprise the tip region of the slug, and are labeled with the ecmA marker (a subfragment of the *ecmA* gene). We had previously shown that a transcription factor MrfA is involved in pstA differentiation and suggested a possible function of pstA cells for tip dominance. However, further study using *omt12* gene marker suggests that there is another pstA population intermingled in the tip region. Unlike ecmA-positive pstA cells, the novel pstA cells (pstA1) appeared in the vegetative phase and then sorted into tip region. When growing cells were separated into the pstA1 and the remaining cells, the pstA1-enriched population develops several hours faster than the other cell populations, including unsorted cells.

To investigate pstA1 differentiation, we used REMI mutagenesis and FACS enrichment to isolate a mutant *DG1037*- that exhibited a significant reduction of the *omt12*p marker expression. Since the *DG1037* encodes a ubiquitin-like domain containing protein (UBL), we named the gene *ubdA.* UBL is also present in mammals, but its biological function is largely unknown. While starved *ubdA*- cells show normal chemotaxis to a cAMP source, they never create aggregative stream by themselves. cAMP pulsing does not rescue *ubdA*- phenotypes including not only csA and cAR1 expressions but also cAMP production. Interestingly, in chimera with 10% of the pstA1 cells, the mutant cells can aggregate.

These results suggest that *ubdA* is involved in early development via pstA1 differentiation and we propose that the pstA1 acts as an early organizer towards tip formation and cAMP wave propagation.

Talk 27

The new face of dictyBase

Siddhartha Basu, David Jimenez-Morales, Petra Fey, Robert J. Dodson, and Rex L. Chisholm

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Abstract

dictyBase is the most comprehensive, current, and highly curated database for Dictyostelid genomics available online. Our primary mission is to provide the biomedical research community with high quality data and tools that enable original research. However, outdated infrastructure has significantly constrained our ability to implement the latest technological advances and new datasets. To overcome these limitations, a major overhaul is currently being completed [1]. In this process, the user interface is being redesigned and modernized with the goal of improving navigation and facilitating the integration of new tools. We are using modern frameworks (AngularJS and ReactJS) for developing dynamic web applications that will increase the adaptation to screen sizes of dictyBase on most web enabled devices. We will present the principal changes affecting the front page and most popular sections of dictyBase. A beta version of the new dictyBase will be released to the Dicty community at the meeting. Hands-on sessions will be available during the poster session.

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Talk 28

Characterization of *Dictyostelium* genes by parallel phenotyping of barcoded mutants.

Christopher Dinh[1], Zhiyi Liu[1], Anup Parikh[2], Jo Ann Ong[1], Jamie Martinez[1], Nhat Mai[1], Theresa Luong[1], Vy-Thao Dinh[1], June Hu[1], Jie Song[1], Elizabeth Villegas[2], Bin Liu[1], Richard Sucgang[1], Blaz Zupan[2], Gad Shaulsky[2], and Adam Kuspa[1,2,3]

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Abstract

The ability to phenotype mutants in parallel is useful for providing insights into gene function at the whole-genome level. We have produced a set of individually barcoded *D. discoideum* insertion mutants and have used them in parallel analyses of phenotypes that provide accurate inferences of gene function. In order to make them we constructed 768 barcoded Restriction Enzyme-Mediated Integration (REMI) plasmids, each with unique 60-mer oligonucleotide tags flanked by 20-mer primer sites (barcodes) that can be amplified by PCR. The 768 plasmids were linearized with *BamH*I, *EcoR*I, or *Sph*I restriction enzymes and used to mutagenize AX4 in >1,000 REMI transformations, using *Dpn*II, *Apo*II, or *Nla*III, respectively. Transformed cells were immediately cloned into 96-well plates in order to obtain a broad sampling of genomic insertions sites, and 30 strains from each of the 768 barcoded plasmids were stored individually, producing an indexed library of 23,040 mutants. To conduct parallel phenotyping experiments we consolidated the mutants into 30 pools of 768 strains such that each strain in a pool carried a unique barcode. We demonstrated that we could identify each of the barcodes by carrying out PCR amplification on DNA prepared from the pooled strains, followed either by hybridization to DNA microarrays containing the 768 60-mer tags from each barcode (plus control 60-mers), or by direct sequencing of the population of amplicons. In various genetic enrichment experiments, we challenged triplicate pools of mutants through cycles of a particular condition in order to reveal mutants that showed a higher fitness under those conditions compared to other mutants in the pool. We inferred higher fitness based on the increased abundance of a mutant, as estimated from the change in abundance of its DNA barcode. We tested growth in the presence of sub-lethal concentrations of 24 different drugs that inhibit the growth of *Dictyostelium*, in order to find mutants that display relative resistance, identifying potential drug targets. We also tested for increases in sporulation efficiency by putting the mutants through rounds of development in order to identify new cheater mutants, and we enriched for resistance to infection by *Legionella pneumophila*. We retested 3-10 strains from each pool that were enriched in each of the three replicates of the experiment and we could confirm their phenotypes without exception. For example, we identified dozens of mutants that were enriched after multiple rounds of infection by *L. pneumophila* and identified the mutated genes. Many of the genes encode proteins involved in autophagy, signaling, and membrane trafficking. Some of them have human orthologs that have not been reported to be associated with *L. pneumophila* pathogenesis. We tested one of these, the C2-domain containing protein KIAA0528, in an RNAi knockdown experiment in human macrophages derived from HL-60 cells, using shRNAs targeting KIAA0528. We observed 2.5- to 5-fold reduction in KIAA0528 mRNA in these cells, depending on the shRNA used, and observed concomitant resistance to killing by *L. pneumophila* infection.

Talk 29

Workshop

Introducing a new dictyExpress: from raw reads to gene expression browsing

Miha Stajdohar[1, 2, 3] and Blaz Zupan[2, 3]

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Abstract

Most likely, you have already been using dictyExpress [1], an interactive, exploratory gene expression data analytics web app. It sits on the dictyBase web page, and is engaged every time you click on a gen expression link in any of gene home pages. dictyExpress has been serving gene expression profiles from over 1000 microarray Dicty experiments from Baylor College of Medicine. In the past month, we have replaced the old dictyExpress with a new app. Instead of expressions from microarrays, dictyExpress now offers only data from NGS reads. The app has been entirely reimplemented, and yes, you can now view the expression of your favorite gene on the iPhone. dictyExpress still supports all the familiar operations, including expression profile viewer, GO browser, differential expression visualization, experiment comparison, hierarchical clustering. Under the hood, the most essential change is the data management. One can now upload NGS reads, process them with a click of a button and publish them online. In this workshop, we will introduce the new dictyExpress, show how easy it is to add new NGS experiments, discuss its computational pipeline and demo its programming interface to data mining toolbox Orange [2].

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Talk 30

Keynote speaker

Cell shape through the cell cycle

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Abstract

We are interested in the dynamics of cell shape control in animals. More specifically, we are interested in the mechanisms by which the actin cytoskeleton controls mitotic cell shape changes during normal and cancer divisions in cell culture and in a tissue context, where they are subject to external forces, and where division contributes to patterning and morphogenesis. As model systems we use the fly, human cells in culture and patient samples. In addition, more recently we have extended this work to study cell shape through the cycle in Sulfolobus, since it turns out that much of the machinery controlling eukaryotic cell architecture has homologues in Archaea. Our studies use a combination of live cell imaging, genetics, microfabrication and mechanical perturbations to study the molecular, cellular and physical processes involved. My talk will focus on recent work in which we have studied how eukaryotic cells actively remodel their actin and microtubule cytoskeletons as they enter and exit mitosis. I will discuss the ways in which these two filament systems work together, via either co-regulation or crosstalk, to ensure that genetic material, mitochondria and cell mass are fairly and accurately segregated at division.

Talk 31

The STE group kinase SepA controls cell migration and cleavage furrow formation

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Abstract

Septase (SepA) is a STE20 group kinase that had been identified in a screen for cytokinesis-defective mutants in *Dictyostelium discoideum*. Functional studies revealed that SepA is crucial for proper cleavage furrow formation, and plays a role in development. SepA just as its upstream regulator, the GTPase Spg1, is associated with centrosomes. Live-cell imaging studies showed that SepA-null cells have unimpaired nuclear divisions and proceed normally through mitosis. However, often cleavage furrows were either not formed at all or were atypical and extremely asymmetric. Outside of the furrow, the cortical actin showed a strong ruffling activity. These results led to the conclusion that SepA is involved in the spatial and temporal control system organizing cortical activities in mitotic and post-mitotic cells.

More recently, SepA has been identified as a downstream target of ErkB (Nichols and Kay, in preparation). ErkB regulates chemotaxis towards folate and cAMP in *Dictyostelium*, and SepA belongs to the list of proteins that are phosphorylated in response to these chemoattractants. We therefore aimed at a more detailed analysis of the migratory behaviour of SepA-null cells. In under-agar folate assays, the speed of the SepA-null cells is strongly reduced. In contrast to wild-type cells, the shape of SepA-null cells is unusually elongated under these conditions. Expression of SepA in the null-background rescues the defects of the mutants almost completely. In summary, our data suggest that SepA controls cortical activities both in migration and during cell division. Currently we aim at a deeper understanding of the signalling network regulating SepA.

Talk 32

ADP-ribosylation of histone H2B in repair of DNA double strand breaks

Alina Rakhimova, Seiji Ura, Duen-Wei Hsu, Hong Yu Wang, Nicholas Lakin, Catherine Pears

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Abstract

DNA is susceptible to many forms of damage that, if unrepaired, can lead to the accumulation of mutations and diseases such as cancer. Double strand breaks (DSB) are one of the most toxic lesions and the cell has evolved multiple ways to repair this to prevent such damage. The two major repair pathways are homologous recombination, in which the damage is repaired by copying information from a sister chromatid or homologous chromosome, and non-homologous end-joining in which the ends are repaired and re-ligated. One of the earliest responses to DNA damage, including DSBs, is activation of ADP-ribosyl transferases that ligate ADP moieties onto target proteins. Inhibitors of these enzymes have potential for chemotherapy as they sensitize tumour cells to DNA damaging agents such as ionizing radiation. ADP-ribosyl transferases are conserved in *Dictyostelium* and we have previously shown Adprt1a to be the major form involved in DSB repair, influencing the choice between alternative repair pathways. Although histones are known to be ADP-ribosylated following DNA damage, the sites of modification and importance of this in repair of damage, including DSBs, is not known. Here we report that *Dictyostelium* H2B is a target for modification by Adprt1a *in vitro* and map sites of modification. H2B is also ADP-ribosylated in response to induction of DSBs in cells and we use gene replacement to investigate the importance of this post-translational modification in repair of DSBs.

Talk 33

Measurement and modelling of stochastic transcription dynamics

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Abstract

What does it mean when we say that a gene is "on"? Standard approaches to measure transcription evaluate population averages of steady state RNA level. These methods, although highly useful, give a homogenous assessment of transcriptional output and mask the underlying dynamic events. Recent single cell studies have forced a re-evaluation of this view, revealing that transcription can occur in a series of irregular bursts, or pulses, with strong periods of activity interspersed by long periods of inactivity, and very little transcription occurring in most cells at any one time. Transcriptional bursts reflect the process of transcription itself, and provide access to deeper understanding of transcriptional mechanism. Here we investigate the processes underlying transcriptional bursts by quantitative imaging of single gene transcriptional events in individual living cells, in combination with molecular genetics and mathematical modelling. Early models based upon fixed cells suggest either a single permissive state or alternating inactive and permissive states. In contrast, our live cell data indicate a continuum of transcriptional states, with a slowly fluctuating initiation rate converting the gene from low to high levels of activity, with the properties of the fluctuations defined by the core transcription machinery. This behaviour enables a wide dynamic range of transcriptional responses, faithfully transmitting developmental or environmental information in the highly stochastic cell environment.

Talk 34

Spacing Remodelers in Dictyostelium Control Transcription Through Distinct Effects on Nucleosome Size, Positioning and Occupancy.

Mark Robinson[1], James Platt[1,2], Nick Kent[1], Alan Kimmel[2] and Adrian Harwood[1]

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Abstract

Nucleosome positioning and occupancy are important determinants of the chromatin landscape, actively regulating transcription. Dynamic remodelling of this landscape is essential for cellular differentiation, replication and DNA repair. The CHD and ISWI families of ATP-dependent chromatin remodelers possess the ability to sample DNA linker length and space nucleosomes evenly *in vitro*. Studies in yeast have shown aberrant organisation of the chromatin landscape and transcriptional disruption upon loss of these proteins, yet precisely how the former results in the later remains unclear. Knockout mutants were generated for the major remodelers and their phenotypes examined. MNase-seq and RNA-seq was used to investigate the relationship between chromatin structure and gene transcription of the four spacing remodelers: ChdA, ChdB, ChdC and Isw. All four remodeler mutants displayed distinct, non-redundant phenotypes and variant RNA profiles. Nucleosome mapping further reveals distinct regions and mechanisms of activity for all four remodelers. Quantitative analysis of nucleosome profiles allowed detection of mutant-specific relationships between the size, positioning and occupancy of nucleosomes across genes and their transcriptional regulation.

Talk 35

Proteolytic regulation in *Dictoystelium* mitochondria

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Abstract

Proteolysis is increasingly recognised as a key regulatory mechanism in cell biology, proteases comprising 2-5% of organism genomes across evolution. They govern protein activation, localisation, exposure of cryptic binding sites and release of neoproteins: indeed, altered protease expression and substrate-proteolysis are important in pathogenesis, including Parkinson's and other neurodegenerative diseases. Proteolysis takes place within cell membranes via families of evolutionarily well-conserved intermembrane proteases. Amongst these are the 'rhomboid' serine proteases, enzymes known to influence development, signalling and infection in a range of eukaryotes and prokaryotes. Of four predicted enzymatically-active rhomboids in *D. discoideum*, we have found three to be important in the mitochondrion. The conserved eukaryotic, mitochondrial 'PARL'-type protease (RhmD) cannot be inactivated but the *rhmA*-rhomboid knockout responds poorly to chemoattractant, shows decreased motility and displays aberrant mitochondrial ultrastructure and altered respiration. A further rhomboid knockout, *rhmB*$^{-}$, has reduced growth rates and folate chemotaxis and, interestingly, a double *A/B*-mutant is unable to phagocytose prey bacteria. Whereas RhmA-GFP is visualised at the contractile vacuole, RhmB-GFP colocalises with MitoTracker to the mitochondrion, but transcription of A and B both peak during multicellular growth and, in qPCR, rhmB transcription is differentially regulated in *rhmA*$^{-}$ cells. Since transcription levels are also altered of our predicted *Dictyostelium* orthologues of *Saccharomyces cerevisiae* mitochondrial rhomboid-substrates, we are examining (co-)localisation of orthologue-RFP in GFP-expressing and *rhmA*$^{-}$ and *B*$^{-}$ cells, while conducting reporter studies with the remaining rhomboids C and D.

Talk 36

C-module-binding factor supports retrotransposition of TRE5-A by suppressing an RNAi pathway

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Abstract

Transposable elements are found in almost all organisms and play a central role in shaping their host's genomes. Because integration into genomic DNA is immanent to transposition, mobile elements represent a constant threat to the viability of their hosts. The genome of *Dictyostelium discoideum* is haploid and densely packed with genes, and, therefore, control of transposon activity is of particular importance for maintaining genome integrity in this organism. Previous RNA sequencing experiments revealed that the expression of several transposable elements in the *D. discoideum* genome is deregulated in a mutant lacking the C-module-binding factor (CbfA). At the same time, the CbfA mutant shows a ~200-fold overexpression of the gene coding for the Argonaute-like protein AgnC, suggesting that CbfA may indirectly support the amplification of transposable elements by limiting the availability of AgnC in a distinct RNA interference pathway. We used the retrotransposon TRE5-A, which maintains an active population in *D. discoideum* cells and for which we have an in vivo retrotransposition assay, as a model to evaluate this assumption. We show that TRE5-A transcripts accumulate in an *agnC* knockout strain to higher level than in wild-type cells. In contrast, TRE5-A expression vanishes in cells that overexpress *agnC* in a CbfA wild-type background, and this is followed by a more than 90% reduction of the retrotransposition activity of the TRE5-A population. It thus appears that the endogene cbfA was “hijacked” by the retrotransposon machinery to eliminate a defense mechanism against transposition, which certainly had a profound effect on genome evolution.

Talk 37

REMI-seq – generation of a genome-wide mutant resource for Dictyostelium functional genomics

Amy Baldwin[1], Nicole Gruenheit[2], Sarah Jaques[1], Thomas Keller[2], Rex Chisholm[3], Christopher Thompson[2] and Adrian Harwood[1]

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Abstract

We are combining Restriction Enzyme Mediated Integration (REMI) mutagenesis with NGS technology to create a genome-wide set of gene knockout mutants. Using this process, we aim to create a near total collection of *Dictyostelium* mutants as a new resource for the *Dictyostelium* community. The resource will comprise individual, sets and large pools of mutants, with the position of REMI mutations searchable via dictyBase.

To date, we have generated more than 11,000 insertion mutants by REMI and stored them in a gridded format for later access. The REMI insertion sites for ~1,000 of these mutants have been identified and include 280 mutants with 'in-gene' insertions that are not currently catalogued in the Dicty stock centre. Of the ~1,000 mutants processed to date, 63% have in-gene insertions (37% have inter-genic insertions) and 4% contain insertions attributed to hotspots. 84% of the stocks contain a single mutant with a single insertion.

This new resource will produce a step change in *Dictyostelium* genetics. The principle benefits will be the on-line availability of independent and multi-allelic mutants for nearly all *Dictyostelium* genes and the capacity to conduct complex phenotyping of protein families. Another key benefit will be the ease at which whole genome phenotypic screens can be conducted; parallel sequencing and population fitness studies on bar-coded pools of random mutants will probe both lethal and sub-lethal sensitivity changes, for example to developmental signals, toxins or drugs. Proof of principle experiments have demonstrated a linear response and determined a broad dynamic range of the system.

Talk 38

Formin A function during migration in confined environments

Nagendran Ramalingam[1,2], Christof Franke[3], Evelin Jaschinski[4,], Moritz Winterhoff[3], Yao Lu[5], Stefan Brühmann[3], Alexander Junemann[3], Helena Meier[3], Angelika A. Noegel[6], Igor Weber[7], Hongxia Zhao[5], Rudolf Merkel[4], Michael Schleicher[1], and Jan Faix[3]

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Abstract

Eukaryotic cells can move by distinct modes of action. Fast amoeboid cell migration, as utilized by leukocytes or Dictyostelium amoebae, is characterized by weak adhesion, formation of actin-rich pseudopods or hydrostatic pressure-driven blebs in their fronts and myosin II-driven contractility in the rear. However, it remained elusive how the contractile machinery in the trailing edge is regulated and how this system is localized. Here we identify Diaphanous-related formin A (ForA) from Dictyostelium discoideum as a critical regulatory component of this machinery also comprising the actin crosslinker cortexillin and IQGAP-related proteins. ForA exerts canonical formin activity in vitro, rapidly relocalises to new prospective ends in repolarizing cells and is required for cortical integrity. Intriguingly, the speed of randomly migrating forA-null cells is markedly increased in unconfined environments, whereas compression under agar strongly impairs motility due to extensive, unproductive blebbing in their rear. Our findings therefore strongly suggest ForA to generate a specialized subset of filaments as the basis of a protective cortical actin sheath in the cell rear to withstand the increased contractile forces imposed by myosin II in response to mechanical stress that are required for efficient cell migration in 2D-confined environments. Finally, we show that the localization of ForA to the rear of polarized cells is mediated by its PI(4,5)P2-specific C2 domain.

Talk 39

Polychromatic 'greenbeard' genes determine patterns of aggregation in a social amoeba

Nicole Gruenheit[1], Katie Parkinson[1], Jennifer A. Howie[1], Jason B. Wolf[2] and Christopher R.L. Thompson[1]

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[2] *Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK*

Abstract

Multicellularity requires a great degree of cooperation among cells to ultimately benefit the multicellular individual. This can be achieved using kin recognition mechanisms, which follow genome wide relatedness, or a phenotypic marker, where a gene encodes both a recognizable characteristic, such as a green beard, and altruistic behaviour towards others bearing the same mark. In this case individuals need only exhibit a genetic relationship at this 'greenbeard' locus. However, a mark like this will inevitably sweep to fixation, simultaneously loosing its discriminatory nature unless multiple variations of the same mark, e.g. multiple beard colours, are used.

In *D. discoideum* aggregations can consist of multiple different genotypes. This raises the question of what prevents the emergence of disruptive cheaters that contribute a smaller proportion of cells to the stalk. Also, natural isolates vary in their ability to co-aggregate, with some strains partially excluding one another. It is currently unknown whether this is driven by a kin recognition or a greenbeard mechanism.

Here we tested whether tgrB1 and tgrC1 encode a muliticoloured greenbeard using co-occurring strains known to contain cheaters and losers. These strains exhibit complex partner specific non-transitive patterns of segregation, with segregation most prevalent in cases where facultative occur in chimeric development. Overall genetic distance is insufficient to explain these segregation patterns, which is inconsistent with a kin recognition mechanism. Instead, extensive patterns of sequence divergence, which affect the strength of *in vitro* TgrB1 and TgrC1 binding, can explain the observed segregation patterns suggesting a mechanism for cheater avoidance by providing a phenotypic mark akin to a multicoloured greenbeard.

Talk 40

Locally Regulated Ras Signaling Reveals Inhibitory Process in GPCR-mediated Chemotaxis

Xuehua Xu and Tian Jin

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Abstract

Optimal polarization and chemotaxis in a wide range of concentration gradient require inhibition processes. No molecular mechanism of inhibition has ever been identified. Here, we reveal a locally controlled inhibitory process at the step of Ras activation by its negative regulator, a C_2 domain containing Ras GAP, C_2RasGAP1. C_2RasGAP1 localizes at leading edge of chemotaxing cells and plays essential roles in persistent adaptation of Ras activation. The alteration of Ras activation results in impaired directional sensing and an ultimately excessive polymerization of F-actin in the cells, leading to impaired chemotaxis. Remarkably, the impaired ability of directional sensing and chemotaxis in *c2rasgap* knockout cells was chemoattractant concentration dependent. Taken together, our results uncover a mechanism by which cells achieve optimal polarization and chemotaxis in a concentration-independent manner, the essence of an inhibitory process required for directional sensing and chemotaxis.

Talk 41

A Cud-type transcription factor regulates Dictyostelium spore differentiation

Yoko Yamada and Pauline Schaap

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Abstract

Spore differentiation of social Dictyostelid amoebas likely evolved from encystation of solitary amoebas by adopting the encapsulation mechanism into a multicellular developmental process. At the time of spore maturation, signals exchanged between prestalk and prespore cells ultimately converge on the activation of PKA in prespore cells, which subsequently induces expression of spore-specific genes, exocytosis of prespore specific vescicles that contain preassembled spore coat proteins and cellulose synthesis at plasma membrane to form fully encapsulated spores. Many components of the pathways controlling spore formation are still unknown and most notably the downstream targets of PKA. To identify genes in the spore pathway, we isolated a mutant with a spore defect after REMI mutagenesis of D. discoideum, and identified a Cud-type transcription factor, spdA (sporulation defective A) as the defective gene. Cud family genes are found in Amoebozoa and plants and are conserved between Dictyostelid taxon groups. spdA- cells differentiate into prespore cells but form virtually no viable spores. We show that spdA acts cell-autonomously in spore formation and is essential for induction of some spore-specific genes by PKA.

Talk 42

Ras activation during cell movement in buffer and chemotaxis

Peter van Haastert, Ineke Keizer-Gunnink and Arjan Kortholt

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Abstract

Activation of Ras plays an important role during chemotaxis. In a shallow gradient of cAMP activation of receptor and heterotrimeric G-proteins are approximately proportional to the local concentration of cAMP, whereas the activation of Ras is much stronger at the leading than at the back of the cell. Previously we have used a very sensitive assay to detect Ras activation during chemotaxis (Kortholt et al, 2013, J Cell Sci, 126, 4502-13). This assay is based on the co-expression of RBD-Raf-GFP and cytosolic RFP. RBD-Raf-GFP only binds to active Ras-GTP and thus translocates to the membrane upon Ras activation. Cytosolic RFP is used to determine the cytosolic volume of these boundary pixels; the GFP-RFP difference yields quantitative and very sensitive information on Ras activation. We have now used this assay to investigated in detail how dynamic Ras patches of cells in buffer are regulated by the cytoskeleton, and how gradients of chemoattractant influence Ras patches. A picture emerges of excitability, symmetry braking and phase separation of Ras/cytoskeleton interactions that is coupled to basal cell movement. In this picture, chemotaxis is a relatively simple positional bias of Ras activation by the gradient of chemoattractant to mediate directional movement.

Talk 43

The Protein Kinase C Orthologue PkcA Regulates the Actin Cytoskeleton

Singh, S., Garcia, R., Khan, M., Shah, S., Brazill, D.

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Abstract

Quorum sensing, the ability of to monitor the local density of cells and modify behavior accordingly, is imperative for regulated tissue development and upkeep. While cell density-sensing (quorum-sensing) pathways have been well-characterized in bacteria, these pathways are poorly understood in eukaryotes. In *Dictyostelium discoideum*, the CMF quorum-sensing pathway ensures proper development by preventing the initiation of development until enough starving cells are present. This is accomplished, in part, through regulating the actin cytoskeleton. Here we describe a putative protein kinase C orthologue, PkcA, which regulates quorum sensing and actin in *D. discoideum*. We found that cells overproducing PkcA are unable to aggregate even at high cell density, as if they are blind to CMF. Conversely, cells lacking PkcA aggregate at low cell density, as if in the presence of CMF. However, this aggregation occurs in the absence of coherent streaming. The aberrant aggregation in both mutants appears to be caused by defects in cAMP chemotaxis and adhesion. Cells overexpressing PkcA have defective cAMP chemotaxis and enhanced cell-substrate adhesion. Cells lacking PkcA have decreased cell-cell and cell-substrate adhesion. The adhesion defects in the PkcA mutant cells appear to be caused by F-actin mislocalization. In keeping with a role in actin regulation, cells lacking PkcA also have a cytokinesis defect. Taken together, the results suggest that PkcA may regulate actin during vegetative growth, as well as during starvation for CMF quorum sensing.

Talk 44

Macropinosomes and pseudopods are associated with distinct spatial patterns of PIP_3 and SCAR/WAVE.

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Abstract

Actin pseudopods and macropinosomes contain similar components, but are architecturally and functionally different. We show they are differentiated by PIP_3 and the key actin regulator, SCAR/WAVE. PIP_3 patches, historically associated with chemotactic signal processing, invariably exclude SCAR from their centres, leading to a ring of SCAR and actin around the edge of the patch. This causes protrusion of circular ruffles and macropinosomes, but not pseudopods. PIP_3 patches therefore cause macropinocytosis and oppose migration by blocking pseudopod progression. PIP_3 patches always lead to rings of SCAR, whether they are basal, at cell-cell contacts, or caused by uniform chemottractant stimulation. In contrast, motile pseudopods show continuous recruitment of SCAR, accompanied by much lower levels of PIP_3, in shallow gradients and only modestly increased by chemoattractants. PIP_3 patches are common in chemotactic cells of axenic strains, but are absent from wild type cells. We propose that circular SCAR recruitment at the edges of PIP_3 patches provides a conserved template for cupped actin structures.

Posters

Poster 1

Antibodies for everybody

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Abstract

Antibodies are essential reagents for all aspects of biological research. Monoclonal antibodies have proven valuable to study a wide variety of processes in Dictyostelium discoideum. However the number of antibodies available has remained relatively limited, and previously characterized hybridomas are sometimes lost.

We are developing a new service at the University of Geneva, open to all academic laboratories : our service generates recombinant antibodies against new targets, stores indefinitely the plasmids encoding the antibodies, produces the antibodies, and distributes them to the scientific community. If adopted widely by our scientific community, this could represent a valuable new tool for the Dictyostelium community.

Poster 2

Understanding the role of vacuolins/flotillins in the biogenesis of the *Mycobacterium marinum* niche

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Abstract

We use *Dictyostelium* as a host to study mycobacterial infections. *Dictyostelium* encodes three vacuolins (A, B and C), found on the compartment containing *Mycobacterium marinum*, a close cousin of *Mycobacterium tuberculosis*. In addition, vacuolin B knockout (KO) cells appeared more resistant to mycobacterial infections. Therefore, we study whether vacuolins play a role in the establishment of a permissive niche.

Vacuolins are homologous to the metazoan flotillins. Recently, flotillins were shown to interact with the recycling machinery and be involved in receptor recycling. Consequently, we also want to understand whether vacuolins are involved in recycling in *Dictyostelium*.

We demonstrate that vacuolin C is mainly localized to lysosomes, whereas vacuolin A and B are found in postlysosomes. In addition, biochemical fractionation experiments show that vacuolins, like flotillins, are strongly associated with membranes, are palmitoylated and can be found in a Triton X-100 insoluble fraction.

Furthermore, our GTP-Trap pulldown experiments suggest that vacuolins can interact with several subunits of the v-ATPase and different Rab proteins. We are further characterizing these interactions and investigating if vacuolins can interact with the recycling machinery.

After showing that the vacuolin B KO cells previously described were in fact a double vacuolin B and C mutant, we decided to generate new single and multiple KO mutants. The new single vacuolin B KOs only show a mild resistance to infection, conforming that we need to further explore the role of vacuolins in infection using multiple KO strains.

Poster 3

Delineating the Immunity Functions of Reactive Oxygen Species Using *Dictyostelium discoideum* as a Model Phagocyte

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Abstract

Reactive oxygen species (ROS) are key components of the immune response to intracellular pathogens. Deleterious mutations in the ROS-generating phagocyte NADPH oxidase (NOX) underlie chronic granulomatous disease, marked by severe, recurring bacterial and fungal infections. NOX comprises a heterodimer of transmembrane proteins: Nox2, the catalytic subunit, and $p22^{phox}$, which recruits cytosolic proteins required for activation. Vegetative *Dictyostelium* expresses NoxA, CybA, and NcfA, homologs of Nox2, of $p22^{phox}$, and of the NOX regulator $p67^{phox}$, respectively. Expression of two additional Nox homologs, NoxB and C, appears to be limited to developmental stages.

Using fluorescence-based assays, we have observed that *Dictyostelium* produces ROS when exposed to bacterial products such as lipopolysaccharide (LPS) and that the rate of ROS production varies based on the type of LPS. ROS production is decreased in *noxabc* triple null or *cyba* null mutants. We expressed fluorescent protein fusions of CybA and of NcfA in wild-type and mutant amoebae. CybA localizes to the plasma membrane, macropinosomes, and bead-containing phagosomes independently of NoxA. NcfA is cytosolic and is enriched at the leading edge, forming macropinosomes, and phagocytic cups but disappears rapidly after closure. Enrichment of NcfA is independent of CybA and NoxA and requires a predicted Rac-binding domain in its N-terminus. These results suggest that Rac activity recruits NcfA to phagocytic/macropinocytic sites to facilitate interaction with membrane-localized CybA and NoxA and that, in the absence of activating signals, NcfA is not retained. We are currently imaging infections to determine whether NcfA is recruited to microbe-containing phagosomes.

Poster 4

Packaging of non pathogenic bacteria in secreted multilamellar bodies by *Dictyostelium discoideum*

Valérie E. Paquet[1,2], Marius Dybwad[3], Janet M. Blatny[3] and Steve J. Charette[1,2]

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Abstract

In the environment, amoebae chase bacteria as food source. Some bacteria are able to avoid the enzymatic degradation by the phagocytic pathway through various mechanisms. Bacteria that can survive amoeba predation are called amoeba resistant bacteria (ARB). It is already known that some ARB find themselves packaged in multilamellar bodies (MLB), which are composed of multiple concentric membrane layers produced and secreted by amoebae. Packaged bacteria are protected from harsh conditions and the packaging process is suspected to promote the persistence of bacteria in the environment. Until now, only pathogenic bacteria were studied regarding their ARB capacity and the packaging process. We propose that a link exists between the capacity of bacteria to resist to amoeba grazing and their capacity to be included in MLB regardless their pathogenic status. Using *Dictyostelium discoideum* amoeba model, the grazing resistance have been assessed for about one hundred environmental bacterial isolates. Bacteria displaying an ARB phenotype have been then tested for their capacity to be packaged and confirmed by two microscopic methods. Ten bacteria from *Cupriavidus*, *Micrococcus* and *Staphylococcus* genera were packaged in MLB secreted by *D. discoideum*. These results confirm that bacteria packaging by amoeba is not restricted to pathogenic bacteria and suggest a more complex role for this process in microbial ecology.

Poster 5

Identification of proteins associated with multilamellar bodies produced by *Dictyostelium discoideum*

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Abstract

Dictyostelium discoideum amoebae produce multilamellar bodies (MLBs) when fed digestible bacteria and secrete them in the milieu. The lipid composition of MLBs is mainly amoebal in origin, suggesting that MLB formation is a protozoa-driven process, which could play significant roles in amoeba's physiology. Therefore, the main objective of this study was to identify major proteins present on MLBs in order to get a better understanding of the molecular mechanisms governing MLB formation. MLBs were purified from a co-culture of amoebae and digestible bacteria using a sodium bromide density gradient. Using SDS-PAGE and mass spectrometry, four major proteins were identified, including the GP17 protein, a protein with an unknown function. The presence of GP17 on MLBs was confirmed by immunofluorescence using a specific anti-GP17 antibody. The localization of GP17 was assessed in axenic condition and in co-culture with digestible bacteria. Another major protein found on MLB is one with an unknown function and is recognized by the H36 antibody. This antibody was used as a MLB marker in a previous study. Finally, identification of proteins associated with MLBs and their functions may help to attribute a potential physiological role to these structures. The elucidation of the biochemical composition of MLBs and the subsequent development of molecular tools such as MLB markers may allow further analyses to better understand the molecular mechanisms of MLB formation.

Poster 6

Analyses of DrkA kinase in STATa activation

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Abstract

Dictyostelium STATa is a structural and functional homologue of metazoan signal transducers and activators of transcription (STATs) and controls stalk cell differentiation. STATa is activated by phosphorylation on Tyr702 when cells are exposed to extracellular cAMP. Although two tyrosine kinase-like (TKL) proteins, Pyk2 and Pyk3, have been definitively identified as STATc kinases no kinase is known for STATa. From the screening of a kinase overexpressor library, we identified DrkA, a member of the TKL family and the *Dictyostelium* receptor-like kinase (DRK) subfamily, as a candidate STATa kinase. The *drkA* gene is selectively expressed in prestalk A (pstA) cells, where STATa is activated. In a null mutant for *drkA*, cAMP induced STATa activation is almost completely eliminated. Recombinant DrkA protein is auto-phosphorylated on tyrosine, and an in vitro kinase assay shows that DrkA can phosphorylate STATa on Tyr702 in a STATa-SH2 (phophotyrosine binding) domain dependent manner. These observations strongly suggest that DrkA is one of the key regulators of STATa tyrosine phosphorylation and are consistent with it being the direct activating STATa kinase.

Poster 7

Metabolism of the signaling molecule glorin during multicellular development of *Polysphondylium* species

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Abstract

Signaling with the unconventional dipeptide glorin (*N*-Propionyl-gamma-L-glutamyl-L-ornithin-delta-lactam-ethylester) may be the most ancient form of intercellular communication in the evolution of multicellularity in the Dictyostelid social amoebae. We previously observed that several taxa are responsive to glorin in a droplet chemotaxis assay under conditions which required the generation of a glorin gradient by secreted glorin-degrading enzymes known as glorinases. We used *Polysphondylium violaceum* and *P. pallidum* as model species and HPLC-MS technology to determine glorinase activities produced by the cells during the first hours of starvation. Bioactivity-guided fractionation of buffer supernatants and proteomics methods are being applied to identify glorinases from *P. pallidum* cells.

Poster 8

The Future of Curation at dictyBase

Petra Fey, Siddhartha Basu, Robert J. Dodson, David Jimenez-Morales, and Rex L. Chisholm

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Abstract

The complete dictyBase overhaul and introduction of state of the art software infrastructure will allow curators to begin annotating new biological features and use existing annotations to represent and connect data in novel ways. Curated protein interactions via the Gene Ontology (GO) will be used to represent protein-protein interactions. Curators already privately annotate spatial expression with the *Dictyostelium* anatomy ontology and we recently started annotating *Dictyostelium* disease orthologues with their respective disease ontology (DO) terms. The updated database will also allow representing GO annotations with 'GO extensions', which add deeper context to those annotations. In the near future HTML5 technology will revolutionize the way curators add annotations to the database, allowing the direct editing of gene pages. Furthermore, it will open the door for direct community annotations on the gene page for interested users.

Basu S, Fey P, Jimenez-Morales D, Dodson RJ, Chisholm RL. dictyBase 2015: Expanding data and annotations in a new software environment. Genesis. 2015 Jun 19. doi: 10.1002/dvg.22867

Poster 9

The regulation of macropinosome maturation by PIKfyve

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Abstract

The macropinocytic pathway is poorly understood, yet plays pivotal roles in nutrient acquisition, immune defence and is subverted in many diseases such as cancer, atherosclerosis and neurodegenerative diseases. Macropinocytosis is a critical component of the innate immune system; dendritic cells and macrophages act as sentinels, surveying their environment for threats through macropinocytic sampling. Although important in defence against infection, many pathogens hijack the macropinocytic pathway, using it as an entry mechanism and preventing maturation to allow establishment of intracellular niches.

Despite its importance, the molecular mechansisms of maturation and protein recycling remain elusive. We have so far identified two proteins that are important in the maturation pathway. PIKfyve is a phosphatidylinositol 5 kinase that is essential for the production of $PI(3,5)P_2$. Inhibition of PIKfyve manifests in a swollen vesicle phenotype and severe defects in vesicle trafficking. A possible PIKfyve effector protein, SNXA, localises to macropinosomes in a PIKfyve dependent manner. Furthermore SNXA knockout cells appear to have defects in macropinosome maturation. I aim to explore the role between PIKfyve and SNXA and to decipher their role in controlling macropinosome maturation.

Poster 10

The mitochondrial TOB/SAM complex in different stages of *Dictyostelium discoideum* life cycle.

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Abstract

The slime mold *Dictyostelium discoideum* life cycle gives an interesting insight into complexity of changes when unicellular organism is transformed to multicellular one.

In our research we focused on the TOB/SAM (Topogenesis of the Mitochondrial Outer Membrane β-barrel Proteins/Sorting and Assembly Machinery) complex. It is located in the outer mitochondrial membrane and is involved in insertion of mitochondrial β-barrel proteins as well as assembly of the TOM complex (Translocase of the Outer Membrane) regarded as a general gate for proteins imported into mitochondria. Interestingly, available data indicate that the TOB/SAM complex exists in various forms that may differ in subunit identity, size and number and the number of the TOB/SAM complex forms increases in mitochondria of multicellular organisms. Consequently one could speculate about the functional relationship between the TOB/SAM complex and multicellularity.

Therefore we studied the TOB/SAM complex in the following four stages of *D. discoideum* life cycle: unicellular, early starvation, aggregation and culmination. The obtained results indicate that the complex consists of two subunits, namely Tob55/Sam50 (~50 kDa) and Metaxin (~50 kDa). The latter (DDB_G0276899) is a homologue of human Metaxin. It was detected by antibody against human Metaxin1 (Mtx1) and displays distinct similarity to the protein. Molecular weight of the TOB/SAM complex is about 250-300 kDa in mitochondria isolated from cells from all studied stages except from the aggregation stage where the complex seem to have an additional form with molecular weight of about 450 kDa.

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Poster 11

Pks26 null mutant showed loser phenotype

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Abstract

Altruism is a cooperative behavior that increases the fitness of other individuals. It is important and ubiquitous system in nature, but its genetic basis is difficult to examine. Social amoebae *Dictyostelium discoideum* shows altruistic behavior, and is amenable to genetic manipulation.

In 2008, more than 100 genes have been reported to be involved in altruistic behavior in *Dictyostelium* by the use of REMI mutants. One of them, *pks26* REMI mutant was suggested to be a loser, which differentiate less spores than its proportional share when mixed with its parental strain.

We focused on this gene to examine the involvement of polyketide in the mechanisms of Altruism. We created knockout mutant of *pke26* by homologous recombination and examined its phenotype. The growth of *pks26* null mutant was normally, but its development was slightly different from its parental strain Ax2. The *pks26* null mutant showed rapid development and its fruiting body had longer stalk than that of Ax2. We mixed *pks26* null cells with Ax2 cells and found the null cells decreased in its frequency through successive generations. This result suggests that *pks26* null mutant is a loser mutant. The detailed developmental features of *pks26* null mutant will be discussed.

Poster 12

Autophagy and ESCRT machinery: a plasma membrane repair mechanism during mycobacterial egress?

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Abstract

Nearly two million people are dying from tuberculosis every year (WHO) and the global burden of the infection with *Mycobacteria tuberculosis* remains enormous. Despite intense research no effective vaccines or treatment targets have been developed in the last decades. In contrast to the difficult and time-consuming work with the strictly human pathogen *M. tuberculosis*, its close relative *M. marinum* in combination with the amoeba *Dictyostelium discoideum* was established as a tractable model to study the mechanisms of mycobacterial pathogenicity and cellular host defense.

While host cell entry and the establishment of a replication compartment are well understood, little is known about the egress of mycobacteria from their host cells and how the infection spreads between cells. In *Dictyostelium,* a F-actin based structure, the ejectosome, has been shown to allow non-lytic egress of mycobacteria (Hagedorn *et al*., 2009). It remains unclear how the host cell maintains its integrity during the process of bacterial egress in which the host plasma membrane ruptures locally. Applying microscopy techniques we were able to show that the autophagic machinery is specifically recruited to the distal pole of ejecting bacteria. If autophagy is impaired, cell-to-cell transmission is reduced, the plasma membrane becomes compromised and the host cells eventually die (Gerstenmaier *et al.,* 2015). Furthermore, we could show that factors of the ESCRT machinery localize to ejecting bacteria in a similar pattern as the autophagy machinery does. We propose that an interplay of the autophagic and ESCRT machinery seals the "large" wound that is generated by egressing mycobacteria.

Poster 13

Gip1 regulates the dynamic ranges of chemotaxis through shuttling of heterotrimeirc G proteins.

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Abstract

Chemotaxis is the cellular behavior that combines motility with the perception of chemical gradient. *Dictyostelium discoideum* display a typical chemotactic response to cAMP at the early stage of multicellular development. In this case, cAMP is recognized by a sensor unit which sends signals via several signaling modules to cellular motility unit. Trimeric G protein, Gα2 and Gβγ, is essential component of the sensor unit. We are interested in how this unit process chemical information and found a novel regulator of heterotrimeric G proteins, Gip1 (trimeric G prtotein interacting protein 1). Although a cell lacking this novel factor can complete the development process to make fruiting bodies, early on abnormal aggregation pattern was observed. Closer examination has shown that chemotaxis was abrogated especially at higher concentrations of cAMP. This data suggests that Gip1 extends the dynamic ranges of chemotaxis to higher cAMP concentrations. We also noticed that Gip1 controls trimeric G proteins shuttling between the plasma membrane and the cytosol and mediates the cAMP triggered translocation of trimeric G proteins. These behaviors appear to explain the defects of Gip1 perturbation in gradient sensing.

Poster 14

Structure activity correlation of 4-methyl-5-pentylbenzene-1, 3-diol (MPBD), a differentiation-inducing factor of *Dictyostelium discoideum.*

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Abstract

4-methyl-5-pentylbenzene-1,3-diol(MPBD) is a polyketide produced by SteelyA polyketide synthase. The functional analysis of MPBD revealed that it regulated chemotaxis in aggregation stage and spore maturation in the last step of the development. To understand the structure-activity correlation of MPBD, we created 4 derivatives of MPBD and investigated their chemotaxis regulation in *Dictyostelium* cells.

One of them, 4-ethyl-5-pentylbenzene-1, 3-diol (EtPBD), recovered chemotaxis activity in *stlA* null mutant to the same level with MPBD *in vitro*. Two of the derivatives, 1,5-dimethoxy-2-methyl-3-pentyl-benzene (Me protected MPBD), and 4-methyl-3-pentyl-phenol (Dehydroxy-MPBD) recovered moderate level of chemotaxis in *stlA* null mutant. These results suggest that chain length at 4 position of these molecules is important for chemotaxis. The spore maturation activity of these derivatives will also be discussed.

Poster 15

Gradient response of giant *Dictyostelium discoideum* cells

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Abstract

Many current models of eukaryotic gradient sensing rely on the interplay of a localized activator with a global, fast diffusing inhibitor. Based on this mechanism, we expect that gradient sensing is cell size dependent. To probe this dependence, giant cells of the social amoeba *Dictyostelium discoideum* offer a promising perspective. They can be generated in different sizes by electric pulse-induced fusion from a suspension of native, normal-sized amoeba. We have previously used this system to study the dynamics of combined actin and PIP3 waves in large unbounded domains. Here, we show first results of giant cells that are exposed to gradients of cAMP. Our experiments indicate that, in contrast to normal-sized cells, the gradient response of giant cells initially does not involve the entire cell body. Instead, individual protrusions are formed that are comparable in size to individual native cells. They migrate towards the chemical source, while the main body of the cells remains static or even moves in another direction. Only after 1 to 2 minutes also the movement of the main body is affected and follows the protrusion in gradient direction, suggesting that initial gradient sensing is confined to small subsets of the giant cell that eventually bias the movement of the main cell body.

Poster 16

Axenic cultivation of 'non-axenic' *Dictyostelium* cells

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Abstract

Methods and media for the short term cultivation of wildtype ('non-axenic') *Dictyostelium discoideum* cells are reported.

Poster 17

Prespore CudA requires CudD for nuclear accumulation

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Abstract

CudA (culmination-defective A) is a transcription factor involved in stalk tube initiation and spore maturation at the final step of *D. discoideum* morphogenesis. The expression of *cudA* and its protein coincides in the tip and in the prespore region of a slug. When *cudA* is complemented in the prestalk region of *cudA* null, culmination is restored but no spores are formed (glassy spore head). During analysis of other *cud* family members, we have isolated a *cudD* mutant showing glassy spore heads. This led us examine the correlation between CudA and CudD.

CudA is abolished from nuclei in prespore cells of the *cudD* mutant while the tip CudA maintains nuclear localization. CudA in the *cudD* mutant becomes nuclear localized when cells are treated with leptomycin B, an inhibitor for exportin, suggesting that the nuclear accumulation of CudA is regulated via nuclear export. A moderate level of interaction between CudA and CudD is detected in yeast two-hybrid analysis, suggesting that CudD may act as an export inhibitor for CudA.

In the *cudD* mutant, the expression of *cudA*-dependent *cotC* is reduced to 20% of that in wild-type, while in a *cudD* overexpressor that increases to 260%. The expression of *cudA*-independent *pspA* is almost unaffected. Interestingly, similar results are obtained in *cudA* expression, suggesting that CudA itself regulates *cudA* expression.

CudD localizes in nuclei of disaggregated *cudA*- slug cells. Taken together, our results indicate that CudD does not require CudA for nuclear accumulation, but is necessary for inhibiting export of CudA from nucleus.

Poster 18

Exploring a Potential Role for FbxA in DIF Signaling

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Abstract

The original characterization of mutants lacking FbxA, an F-box WD-40 protein that is part of an SCF E3 ubiquitin ligase complex, focused on the resulting cell-autonomous sorting defect ("cheater" phenotype), decreased prestalk:prespore ratio, and tendency to arrest as slugs. However, several aspects of the *fbxA*$^-$ phenotype (fragile slugs; reduced pstO population; culminants with slipped spore heads) are similar to those subsequently reported for mutants with defects in DIF synthesis or DIF-responsive transcription factors. Development on DIF-containing agar did not rescue the fragile slugs or aberrant culminants characteristic of the *fbxA*$^-$ null mutant, as would have been predicted for a mutant with a defect in DIF synthesis. Hence, we hypothesized that FbxA instead plays a role in response to DIF. We used the *ecmB-lacZ* reporter to confirm that *fbxA*$^-$ mutants display the altered lower cup expression and reduced basal disc characteristic of DIF response mutants. However, a global effect on DIF responses seems unlikely as neither *fbxA*$^-$ mutants nor cells overexpressing FbxA showed any striking alteration in DIF-induced nuclear localization of DimB or StatC in cells starved in shaking suspension for either 4 or 8 hours. Whether any difference in relocalization occurs later in development (when *fbxA* is more highly expressed), or if the kinetics of DIF-induced phosphorylation vary, is currently unknown. Similarly, we have yet to directly measure the effect of altered FbxA levels on DIF's regulation of *pspA*, *ecmA*, and *ecmB* transcription. Our analyses may eventually reveal a previously unappreciated role for FbxA in this complex signaling network.

Poster 19

Functional conservation of adenylate cyclase A (ACA) throughout Dictyostelia

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Abstract

In *Dictyostelium discoideum*, ACA produces extracellular cAMP which acts as chemoattractant in the coordination of aggregation and culmination. All Dictyostelids species are subdivided into four major groups. Like *D. discoideum*, group 4 species use cAMP as chemoattractant. Other group species do not use cAMP as chemoattractant. However, cAMP receptor null mutant in group 2 species *Polysphondylium pallidum* revealed that extracellular cAMP is also required for fruiting body formation.

To understand evolution of cAMP signaling in Dictyostelia, we analyzed ACA function in other group species. Recent genome projects of six Dictyostelia species revealed that ACA orthologous genes are conserved throughout the Dictyostelid phylogeny. Most Dictyostelia have a single ACA gene, except for the group 2 species, which have three ACA genes. Expression of *D. lacteum* ACA in *D. discoideum* ACA null mutant partially restored aggregation and fruiting body formation, showing that these orthologues produce cAMP. Single or double ACA knockout mutant in *P. pallidum* still formed fruiting bodies and normal spores and stalk cells, although one of the double ACA mutants formed abnormal fruiting bodies. This indicates that the *P.pallidum* ACAs have overlapping functions. The specific ACA inhibitor SQ22536 completely blocked aggregation and also inhibited post-aggregative development in *P.pallidum*, indicating that ACA has functions both in aggregation and post-aggregative development in *P. pallidum*. SQ22536 also inhibited aggregation of group 1 and 3 species, suggesting that the role of ACA in the aggregation stage is conserved throughout the Dictyostelid phylogeny, even though group 1-3 species do not use cAMP as chemoattractant.

Poster 20

Effects of temperature on growth and development of dictyostelids - Their diversity and ecological implications

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Abstract

The distribution of dictyostelids is worldwide, inhabiting various climatic zones from the tundra to the tropics. To see whether the global distributions of dictyostelids are correlated with their temperature tolerance, we examined the effects of temperature over the range of 0 ~ 37˚C on growth and development of 30 species isolated from the temperate, tropic or subarctic zones. We found that some "thermophilic" species grew optimally and formed fruiting bodies at 28˚C or above, while some "cold resistant" species grew and developed normally at 4˚C. Surprisingly, some of the latter grew and formed normal fruiting bodies even at 0˚C. All species isolated from the subarctic zone showed cold resistance and did not survive at temperatures above 28˚C. Species in group 3, clades 2A and 2B tended to be thermophilic and many cold resistant species belonged to group 4. This is consistent with the fact that many species isolated from the tropic regions are group 3 species and that the many of those isolated in the cold regions belong to group 4. Additionally, comparison between the highly cold-resistant species *D. rosarium* and a modestly cold-resistant *D. discoideum* revealed that there are differences in cell shape and F-actin localization at 0˚C.

Poster 21

Mechanical basis of cell sorting phenomena

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Abstract

"Cell sorting", changes in the relative position of cells within a tissue, is an important component of development, and its role in the establishment of the prestalk-prespore pattern in *Dictyostelium discoideum* has been well documented. It will also be involved in segregation between genetically distinct cells of the same species and between cells of closely related species that share a common chemoattractant for aggregation.

In past studies of cell sorting, cell adhesion has been the main subject to be focused. However, cell sorting is an active process, and in *Dictyostelium* development in particular, it takes place while the cells are exhibiting massive movement. Then, two questions arise concerning its mechanism; (1) how a cell changes position when embedded in a tissue of motile cells, and (2) how the difference between cell types affect their movement. To answer them, we performed various types of mixing experiments, including the one demonstrating suppression of interspecific segregation by heterologous expression of a *tgr* gene, and also some theoretical analysis. On the basis of these and old results, we put forward a hypothetical mechanism for cell sorting.

Poster 22

The botanical curcumin negatively regulates transcription of antioxidant genes and increases reactive oxygen species in *Dictyostelium discoideum*

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Abstract

Herbal dietary supplements represent a $20 billion per year industry. However, little is known about their safety and efficacy. An example of this is the phytochemical curcumin, which is extensively used worldwide with claims that it has curative effects against microbial, viral, and inflammatory diseases and preventative effects against cancer and Alzheimer's disease. The therapeutic effects of curcumin are often attributed to antioxidant properties, although other mechanisms have also been suggested. *Dictyostelium discoideum* has been proven to be an excellent lead genetic system for interrogating the mechanism of action of a number of drugs used in human medicine. Therefore, we have used this model system to investigate the mechanism of action of the botanical curcumin. Our studies show that: 1) Curcumin has a dose-dependent inhibitory effect on cell proliferation; 2) The transcription of the antioxidant catalase (*cat*) A gene, and the superoxide dismutase (*sod*) *A*, *B* and *2* genes are reduced in the presence of curcumin; 3) Catalase A specific enzyme activity is lowered in response to curcumin; 4) The level of superoxide increases in the presence of curcumin; 5) The anti-oxidant N-acetylcysteine does not have an effect on cell proliferation or catalase A specific activity; and 6) Curcumin has no effect on cell proliferation in *Dictyostelium purpureum*. These results indicate that *Dictyostelium discoideum* can be used as a non-mammalian model to assess the molecular mechanisms underlying the pharmacological effects of botanicals and also to better understand the roles of oxidants on amoebal physiology.

Poster 23

Amoeboid cells as a transport system for micro objects

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Abstract

The transport and positioning of micron-sized objects in complex geometries is often accomplished by fluid flow. However, under geometric constrains, like dead end structures, this is difficult to achieve. An alternative approach would be the use of magnetic or optical tweezers. Yet these techniques require a lot of time to rearrange many objects, since it has to be done one by one. Here, we propose a novel approach exploiting the chemotactic behavior of single cells. We use cells of the social amoeba *Dictyostelium discoideum* to transport particles of different sizes. The cells are guided by a gradient of the chemoattractant cAMP, which can be established with the help of a gradient chamber or through photo-uncaging to direct the cells in a more specific fashion. To analyze this transport in more detail, the force applied to a micron-sized bead by a *Dictyostelium* cell is measured with the help of an optical trap.

Poster 24

Developing a non-animal model system to investigate bitter tastants as new treatments for asthma

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Abstract

Bitter tasting compounds have recently been heralded as potential new treatments for asthma, as they relax airways and inhibit inflammatory cell function. A major obstacle in the development of bitter tastants as new medications for asthma is a lack of knowledge about the molecular mechanism of their action in airway muscle cells. Although bitter compounds are agonists for the G protein-coupled bitter taste receptors (TAS2Rs), our preliminary findings in human bronchi suggest that the bitter tastants may act via distinct signalling pathways. We are employing *Dictyostelium discoideum* as a non-animal model to identify these distinct mechanisms, since *Dictyostelium* does not express TAS2Rs. We will screen a *Dictyostelium* REMI library for mutants resistant to the growth-inhibitory effects of candidate bitter tastants, which will provide insight into the molecular identity of the signalling pathways that mediate these effects. We will also investigate the conservation of signalling pathways by expressing human TAS2Rs in *Dictyostelium* and observing whether they confer sensitivity to their cognate bitter tastant agonists. Ultimately, we wish to identify novel treatments and their molecular targets to aid in the treatment of asthma and other airway-obstructive diseases, without the use of animals.

Poster 25

Finding the target of valproic acid

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Abstract

Valproic acid (VPA) is a broad spectrum anti-epileptic drug that is also used to treat bipolar disorder and migraine. Although VPA is very effective in seizure control, it has a number of adverse side effects highlighting a need to develop improved therapeutic agents. Research in this area has been primarily focused on using animal models, however, *Dictyostelium* has recently shown promise as an alternative model to study the molecular mechanism of VPA. Using *D. discoideum*, VPA has been shown to block phosphoinositide signalling, and this effect has been verified in animal seizure models, where VPA blocks the rapid reduction in phosphoinositide turnover during epileptic seizures. In *D. discoideum,* this effect of VPA is independent of phospholipid kinase activity and inositol synthesis and recycling, suggesting the molecular target may be a component of the phosphoinositide salvage pathway. The aim of this project is to use *D. discoideum* to investigate three proteins involved in the phosphoinositide salvage pathway as potential VPA targets. These proteins are cytidine-diphosphate diacylglycerol synthase (CDS), diacylglycerol kinase-α (DGKA) and phosphatidylinositol synthase (CDIPT). Knockout mutants for each encoding gene will therefore be tested for resistance to the effect of VPA in development and overexpression of each gene will rescue the wild type phenotype. Preliminary data, using a single knockout for DGKA, shows gene ablation gives rise to an aberrant development morphology, but the mutant does not show altered resistance to VPA during developmental. Continued analysis of the inositol salvage pathway as a target of VPA will examine the remaining proteins to identify the target of VPA in the treatment of epilepsy.

Poster 26

Analysing the γ-secretase Complex Using the Simple Model Organism *Dictyostelium*

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Abstract

Alzheimer's disease (AD) is a neurodegenerative condition resulting in cognitive decline, dementia, and decreased quality of life. AD affects 35 million people in the over-60 population worldwide, with a predicted 115 million people affected by 2050. The causes of AD are multifactorial and mutations in the γ-secretase complex contribute to the pathogenesis and progression of the condition. The γ-secretase complex is composed of four subunits: one of two catalytic Presenilin proteins (Psen1/2); Nicastrin (Ncstn); Anterior pharynx defective 1 (Aph-1) and Presenilin enhancer 2 (Pen-2). The complex acts via a proteolytic mechanism leading to generation of amyloid-β, and via a non-proteolytic (scaffolding) mechanism. Whilst Presenilin proteins have been extensively researched, fewer studies have investigated the roles of the remaining three proteins. In mammalian models it is difficult to study this complex as deletion of subunits results in embryonic lethality. *Dictyostelium* shares all γ-secretase components with mammals and is the simplest model organism with two presenilin proteins. This project aims to determine the function of Ncstn, Aph-1 and Pen-2 in the *Dictyostelium* γ-secretase complex by genetic ablation of individual components. The resultant mutants will be characterised, and rescued by overexpression of *Dictyostelium* and human proteins. The non-proteolytic role of the complex will also be analysed by mutating key motifs and domains to better understand the structural role of the complex and its components. Finally, formation of the complex in *Dictyostelium* will be analysed through the use of fluorescently tagged complex components. This work will enhance understanding of proteolytic and non-proteolytic functions of the γ-secretase complex, and its role in AD.

Poster 27

Roco4 diffusion kinetics *in vivo*: dimerization, complex formation and/or organelle binding?

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Abstract

LRRK2 is a large multidomain protein, consisting of several protein binding domains, a dimerization domain and two catalytic domains (kinase and GTPase). Mutations in various domains of LRRK2 may lead to Parkinson's Disease and have been found to increase the kinase activity of LRRK2. All purified or co-immunoprecipitated LRRK2 fragments are dimeric, and LRRK2 kinase activity seems to be dependent on dimerization. Recently it was shown that in live CHO-K1 cells LRRK2 is monomeric and inactive in the cytosol, but attains pre-dominantly as an active dimer at the membrane. These results suggest that LRRK2 cycles between a low active monomeric state and high active dimeric state. However, the regulation of dimerization and translocation are not well understood . I am using Fluorescence Correlation Spectroscopy (FCS) in *Dictyostelium discoideum* to measure diffusion times of Roco4,. Here I will discuss the implication of these data in the context of the molecular function and activation mechanism of Roco4.

Poster 28

Structural characterization of phenylalanine hydroxylase from *Dictyostelium discoideum*

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Abstract

Phenylalanine hydroxylase (PAH) catalyzes the hydroxylation of the amino acid L-phenylalanine to L-tyrosine in the presence of the specific cofactor tetrahydrobiopterin (BH_4) and O_2. The PAH from *Dictyostelium discoideum* (*Dic*PAH) has a different cofactor specificity and regulatory functions though it high sequence similarities to mammalian PAHs. It has been reported that *Dic*PAH is able to use both L-*erythro*-tetrahydrobiopterin (BH_4) and its stereoisomer D-*threo*-BH4 (DH_4) for its function *in vitro*. Moreover, *Dic*PAH is devoid of the characteristic regulatory mechanisms of mammalian PAH such as positive cooperativity for Phe and activation by pre-incubation with the substrate.

To understand the dual cofactor specificities and different allosteric regulation of *Dic*PAH, we have determined three-dimensional structures of an apo-*Dic*PAH_RC$_{1-415}$ and its complexes with the BH_2 and both BH_2 and L-norleucine (NLE) at 2.07, 2.07 and 2.39 Å, respectively. The overall structures of the catalytic domain show significant similarities to other mammalian PAHs and bacteria PAHs with well-conserved residues around the BH_2 and Fe(III) binding sites. It is found that the NLE binding of *Dic*PAH_RC$_{1-4151}$ caused local conformational changes in residues 262-269 and similar large global conformational changes like in the human PAH structure. Comparing *Dic*PAH_RC$_{1-415}$ with the rat PAH_RC structure, the N-terminal regulatory domain of *Dic*PAH has a shorter N-terminal loop and rotated about 9° away relatively to the catalytic domain, producing more exposed active site than that in the rat PAH_RC structure. These structural differences at the regulatory domain seem to be associated with the different regulation of *Dic*PAH.

Poster 29

GflB, a Gα2 stimulated Rap1-specific GEF is important for *Dictyostelium* chemotaxis and development

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Abstract

Binding of chemoattractant to G protein-coupled receptors (GPCRs) leads to the activation and dissociation of the heterotrimeric G-protein complex. The dissociated Gα-GTP and Gβγ dimer interact with their respective effector proteins to induce complex rearrangements of the actin/myosin network which subsequently drives cell movement up the chemoattractant gradient. It is however still unclear how heterotrimeric G proteins transduce the intracellular signal to the actin/myosin II-based moving apparatus. With a pull-down proteomic screen we identified a novel RasGEF and RhoGAP containing protein, GEF-Like Protein B (GflB), as Gα2 binding partner. GflB localizes to the leading edge and functions as a Gα stimulated, Rap1-specific GEF that is important for the balance between Ras and Rap signaling during chemotaxis. The kinetics of GflB translocation are fine-tuned by GSK-3 mediated phosphorylation. Cells lacking *gflB* display impaired actin and myosin dynamics, resulting in defective chemotaxis. Our observations demonstrate that GflB is a central, upstream regulator of chemoattractant-mediated cell polarity and cytoskeletal reorganizations by directly linking Gα activation to monomeric G-protein signaling.

Poster 30

Dictyostelium Roco4 as model for LRRK2-mediated Parkinson's Disease

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Abstract

Dictyostelium Roco4 belongs to the conserved family of Roco proteins. The most prominent family member, human LRRK2 (Leucine Rich Repeat Kinase 2), has been associated with both familial and idiopathic Parkinson's Disease (PD). Although several potential LRRK2 mediated pathways and interaction partners have been identified, yet much about the cellular functions remain unknown, including the underlying molecular mechanisms of enhanced LRRK2 catalytic activity of several PD associated mutations. Important understanding of LRRK2 has come from our work with *Dictyostelium* Roco family proteins whereby especially Roco4 provides a genetic and structural homology. Here I show that disruption of *roco4* results in impaired development, phototaxis, stalk formation, and ATP metabolism, phenotypes which are known to be affected by dysregulated mitochondrial homoeostasis. The LRR (Leucine Rich Repeat) containing N-terminus of both LRRK2 and Roco4 is essential for their function *in vivo*, suggesting intramolecular regulation of protein activity. The *roco4*-null cells also provided an excellent tool to investigate this complex activation mechanism in more detail. Studies with chimera proteins of Roco4 and LRRK2 *in vivo* have shown that the LRR determines the specificity of the chimera and that the kinase part is freely exchangeable; thus the kinase of human LRRK2 in the context of Roco4 restores the *roco4*- phenotype. Importantly, the N-terminus of Roco4 directly binds and inhibits kinase activity *in vitro*. Together this strongly suggests that the N-terminus of LRRK2/Roco4 is essential for the intramolecular regulation of kinase activity and most likely determines the input and/or output specificity of the proteins by binding to upstream and/or downstream regulators.

Poster 31

ErkB is a master protein kinase that regulates a core set of chemotaxis proteins

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Abstract

We have taken a global phosphoproteomic approach to expand the inventory of proteins involved in chemotaxis. By using SILAC and phosphopeptide enrichment, we have quantified changes in protein phosphorylation in response to chemoattractants at specific sites across several thousand proteins. Among these, we have identified several hundred proteins whose phosphorylation is changed at least 3-fold following cAMP stimulation. These phosphorylation changes can be grouped into specific kinetic classes, with both increases and decreases observed. However, responses to cAMP stimulation are complex and not limited to chemotaxis; other processes, including signal relay and developmental gene expression, also reside downstream.

By comparing cAMP data with responses to the vegetative chemoattractant folate, we found a small set of proteins whose phosphorylation is increased in both cases. We propose that this set represents a group of core chemotactic proteins. The majority of these proteins are phosphorylated at a single novel peptide consensus sequence, suggesting that a single master kinase is responsible. A mutant screen, confirmed by further phosphoproteomic experiments, demonstrated that the protein kinase ErkB regulates these phosphorylation sites. We have revisited the chemotaxis defects of the erkB$^-$ null, and investigated the chemotaxis functions of candidate proteins from the 'core' chemotactic set.

Poster 32

Epigallocatechin gallate alters growth and PIP_3 signaling in *Dictyostelium discoideum*

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Abstract

Epigallocatechin gallate (EGCG), a flavonoid found at high levels in green tea, has received significant recent attention due to its potential health-promoting activities. EGCG appears to exert its effects via a variety of mechanisms, including regulating the activities of cell surface and intracellular signaling proteins. We have previously shown that EGCG blocks the *Dictyostelium* life cycle at aggregation, a defect that likely arises from reduced cell motility and resembles defects seen with a loss of PI3K activity. Here, we show that treatment with EGCG affects growth and PIP_3 signaling in *Dictyostelium*. Axenic growth is inhibited by as little as 100μM EGCG, a concentration that exhibits low cytotoxicity. EGCG also blocks membrane association of CRAC-GFP in cells treated with cAMP, suggesting that PIP_3 does not accumulate in the presence of the catechin. EGCG has no dramatic effects on membrane association of PTEN-GFP or on its redistribution in response to cAMP. Given the important role of PIP_3 signaling in cancer and inflammation, these results suggest a possible mechanism by which EGCG may impact human health.

Poster 33

Dictyostelium as a non-animal model to identify pathways that drive resistance of homologous recombination defective tumours to PARP inhibitors

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Abstract

Tumour cells are often defective in DNA repair and are consequently sensitive to DNA damaging agents used in chemo- and radio-therapy. The inherent repair defects in these cells can be exploited in synthetic lethal strategies to target tumour cells. For example, families with high risk of breast and ovarian cancer lack BRCA2, a protein essential for DNA double-strand break (DSB) repair by homologous recombination (HR). Survival of these tumour cells is dependent on a pathway involving Poly (ADP-Ribosyl) Transferases (PARPs) and treatment with PARP inhibitors (PARPi) results in cell death. However, after an initial clinical response, some tumours develop resistance to PARPi. Identifying pathways driving the desensitization of HR-defective cells to PARPi will provide novel therapeutic targets that, when inhibited, will overcome resistance. Previously, we reported that DNA repair pathways, including PARPs, are conserved in *Dictyostelium*. Here we extend these findings to establish *Dictyostelium* as a model to identify pathways that drive resistance of tumours to PARPi. We screened several clinical relevant PARPi for effectiveness in *Dictyostelium* and show that synthetic lethality between PARPi and HR-deficiency is conserved in *Dictyostelium*, illustrating this system is a tractable model for these studies. Finally, disruption of the non-homologous end-joining (NHEJ) DSB repair pathway suppresses sensitivity of HR-defective strains to PARPi. These data indicate *Dictyostelium* is an effective model to identify pathways that drive sensitivity of HR-defective cells to PARP inhibitors and disrupting the NHEJ pathway can counteract this effect.

Poster 34

REMI-seq – generation of a genome-wide mutant resource for *Dictyostelium* functional genomics

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Abstract

REMI-seq combines Restriction Enzyme Mediated Integration mutagenesis with NGS technology to rapidly identify gene knockout mutants on a large scale. We aim to use this method to generate a near total collection of *Dictyostelium* mutants. This will create a community resource that comprises individual, sets and large pools of mutants, with the loci of REMI mutations searchable on dictyBase.

Insertion sites are identified by sequencing the insert/genomic DNA junctions. The junctions are captured using MmeI, a type IIC restriction enzyme, indexed with sequencing adapters and amplified by PCR. Following size selection, the target DNA is sequenced using an Illumina MiSeq. A bioinformatic pipeline has been designed to deconvolve the sequencing data and catalogue the mutants. To date, we have generated and stored more than 11,000 insertion mutants and stored them in an individual and accessible format. So far, we have sequenced ~1,000 mutant insertion sites and identified 280 mutants with 'in-gene' insertions that are not currently catalogued in the dictyBase stock centre.

We believe that this new resource will produce a step change in *Dictyostelium* genetics, offering on-line availability of independent and multi-allelic mutants for nearly all *Dictyostelium* genes. Providing a capacity to conduct complex phenotyping of protein families and whole genome phenotypic screens specific to the user's need.

Poster 35

Evidence that chromatin modifications regulate fluctuating gene expression and control 'salt-and-pepper' differentiation

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Abstract

How cells are able to make robust cell fate decisions that lead to the correct numbers of different cell types is a fundamental question in developmental biology. In many cases cell communication and positional signaling provides an explanation. However, the mechanism underpinning 'salt-and-pepper' differentiation, which is seen in examples raging from competence in B. subtilis, lineage specification in the mouse blastocyst to stalk and spore differentiation in Dictyostelium is still poorly understood. One possibility is that fate decisions made in this way reflect fluctuating gene expression patterns within a population, ensuring that only some are competent or primed to adopt a particular fate at any one moment.

Modifications to chromatin are known to influence dynamic gene expression. We therefore hypothesized that lineage choice could be affected through removal of important chromatin marks. To test this prediction we created mutants in several chromatin modification enzymes in *D. discoideum.* Consistent with this idea, found that knockout of the histone methyl-transferase set1, affects this cell fate choice and strongly biases cells towards a pre-stalk fate. Based on RNA-seq expression we have discovered that set1 is required to repress a specific set of developmental genes during growth, when lineage priming takes pace. Moreover, we find that the pattern of misexpression suggests a key role in a cross repression feedback loop required for buffering against fluctuations in cell extrinsic noise, a feedback loop we have discovered to be essential to ensure the correct pre-stalk proportioning based on different positive pre-stalk inputs.

Poster 36

Understanding cell type sorting out by differential migration and adhesion

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Abstract

Salt and pepper differentiation followed by sorting out represents a fundamental mechanism of pattern formation during development. Cells initially differentiate scattered due to heterogeneity in their response thresholds to a uniform concentration of differentiation inducing signals. These cells then sort out from one another, a process thought to involve differential cell migration/and or cell adhesion. A good model to study this process is Dictyostelium, which only contains two main cell types, prespore and prestalk, which differentiate intermingled before sorting into discrete tissues. It has been proposed that that cell type differentiation endows cells with different gene expression profiles, which in turn causes cells to acquire a different cell properties such as cell shape, rigidity, motility and adhesion which can be used to drive the differential behaviour of the cell types. To test this idea, we first determined the cell type specific gene expression profile of prestalk and prespore cells. RNA sequencing was performed on FACS sorted prespore (psA-GFP) and prestalk (ecmAO-RFP) cells. A cell type index of pst/(pst+psA) was used to identify prespore and prestalk specific genes. By classifying cell type specific genes, a group of myosin II structural and regulatory genes were first identified as pre-stalk specific and we decided to study their functions in sorting and morphogenesis. The developmental phenotype of these myosin II structural and regulatory gene mutant cells showed different levels of defects in morphogenesis. The chimeric development of mutant cells vs actin::15 labelled Ax4 cells revealed that cells lacking myosin II structural and regulatory genes failed to sort to their given region. Finally, we have developed novel assays to directly measure the cell motility of wild type and mutant cells during cell sorting and cAMP mediated chemotaxis in a 3D environment.

Poster 37

The role of the Dictyostelium Akt homologs in macropinocytosis

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Abstract

Macropinocytosis is an actin driven process whereby cells can engulf relatively large volumes of media, for example in immune sampling, growth of some Ras-driven cancers and entry of certain pathogens such as Salmonella into host cells.

In Dictyostelium a patch of active Ras is produced at the membrane, stimulating the class I PI3K's to make a PIP3 patch. The SCAR/WAVE complex is recruited to the edge of the PIP3 patch, driving actin polymerisation and forming a macropinocytic cup which closes, delivering medium into the cell. Increased macropinocytosis has been established as a way in which axenic cells can drastically increase their fluid uptake compared to wild strains, allowing propagation in nutrient broth. This has been achieved by the inactivation of NF1, which acts to limit the size of patches of active Ras. In its absence, the active Ras and PIP3 patches can grow larger giving more voluminous macropinosomes.

The Dictyostelium homologs of Akt, PkbA and PkbR1 have long been known as being important for chemotaxis, as well there being a notable axenic growth defect in the double mutant. Here we explored this growth defect further, finding a reduction in the volume of medium taken up by the mutant and determined that this was due to the volume of the macropinosomes being smaller than in the parental strain. I propose that PkbA/PkbR1 act to increase the size of the macropinosomes, most likely by affecting the size of the active Ras or PIP3 patches.

Poster 38

Analysis of Intracellular Calcium Channels in *Dictyostelium* Development.

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Abstract

Calcium is a ubiquitous intracellular signal responsible for controlling numerous cellular responses including development, differentiation and proliferation. Calcium is stored in both neutral and acidic stores and its release though gated channels has been implicated in regulating development and cell fate choice in *Dictyostelium*. This research aims to understand the roles of the calcium channel proteins found on acidic (two-pore channel proteins (TPCs), mucolipin (TRP-ML)), and neutral (IplA) stores in calcium signaling during *Dictyostelium* development.

A series of strains that are disrupted in the one or more of genes encoding calcium channels have been generated. All disrupted mutants (including one lacking all three of these channels) are able to form fruiting bodies. However, strains lacking the TPC channel show a delay in early development. *tpc*-null cells also show preference for a stalk cell fate, consistent with the TPC channel playing a role in development. *In vitro* vesicles derived from intracellular organelles can take up calcium and release it upon stimulation with arachidonic acid. In vesicles from *tpc*-null cells, the rate of uptake of Ca^{2+} is increased, suggesting Ca^{2+} homeostasis might be altered in the absence of the channel. *In vivo* analysis of intracellular Ca^{2+} responses is being carried out in strains expressing the sensitive Ca^{2+} indicator YC-Nano.

These strains deficient in one or more of the calcium channels controlling release from both neutral and acidic Ca^{2+} stores, in conjunction with real-time Ca^{2+} detection, will facilitate analysis of the roles of the individual channels and stores in *Dictyostelium* development.

Poster 39

Identifying the molecular targets of cannabis derivatives using a simple model system

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Abstract

Cannabis has been used for the treatment of medical conditions such as rheumatism, inflammation, seizures and pain management for many centuries. Cannabinoids, compounds derived from cannabis are now also licensed as pharmaceuticals in the treatment of multiple sclerosis and cannabidiol (CBD) the most abundant non-psychotropic cannabinoid is undergoing phase III clinical trials as a treatment for refractory epilepsy. Exactly how cannabinoids exert their therapeutic affects remains unknown. Identifying the molecular targets of cannabinoids would allow the design of pharmaceuticals with greater efficacy and with more tolerable side effect profiles. This study aims to identify the molecular targets of CBD, cannabidivarin (CBDV) and cannabidiolic acid (CBDA) using *D.discoideum* as a model system. We have shown that *D.discoideum* growth is blocked by all three cannabinoids in a dose dependant manner. Screening a restriction enzyme mediated integration (REMI) library with each of these cannabinoids has identified 25 mutants showing resistance to the effect of these cannabinoids on growth, with some mutants showing resistance to multiple compounds. From these mutants four genes have been independently identified that are likely to confer resistance to cannabinoids. One of these genes is involved in mitochondrial electron transport while another is potentially linked to myelin sheath maintenance and neurotransmitter regulation. Thus, this project will identify new mechanisms of cannabinoids that are likely to relate to the treatment of a range of human disorders.

Poster 40

Calcium signalling is essential for arrestin recruitment to the plasma membrane

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Arrestins in mammalian cells form an important part of G-protein coupled receptor signalling: they bind to the phosphorylated tails of GPCRs and can both cause endocytic down-regulation of the receptor and act as signalling platforms for downstream effectors.

Cao et al (MBC 25, 3210, 2014) described a pair of related Dictyostelium proteins with arrestin domains, which appear to control the frequency of cAMP oscillations in aggregation, as their double mutant produces cAMP waves at twice the frequency of wild-type. They also showed that a reporter for one of these proteins, AdcC-GFP, is transiently recruited to the plasma membrane in response to cAMP stimulation, and that (surprisingly) this depends on the presence of extracellular calcium.

We have followed up the calcium dependence of AdcC translocation. We find that translocation is abrogated by mutation of the presumed IP3 receptor (IplA), which abolishes calcium signalling in response to cAMP. Like cAMP, ATP causes a rapid calcium influx into cells and transient recruitment of AdcC-GFP to the membrane, but in this case both responses are independent of IplA. We have separately identified the channel essential for the calcium response to ATP, and find that mutation of this channel blocks AdcC translocation. Treatment of cells with a calcium ionophore, to elevate intra-cellular calcium independent of receptor activation, also causes AdcC translocation.

Our results show that coincident calcium signalling is required for AdcC recruitment to the plasma membrane after ligand stimulation of cells, and that this calcium signal may be sufficient.

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