GENEVA, SWITZERLAND

August 20 to 24

# Dicty 2017

# Meeting Booklet

UNIVERSITÉ DE GENÈVE

LS² Life Sciences Switzerland

# Map and information


All talks are held in the room Cosmos

| | |
|---|---|
| Breakfast: | Mon-Thu 6:30-10:00, Restaurant des Arts and Terrasse |
| Lunch: | Mon, Tues, Thu, Terrasse or Observatoire |
| Dinner: | Sun-Wed, Terrasse or Observatoire |
| Coffee breaks: | Mon-Thu, Foyer Pause |
| Poster sessions: | Mon-Tues from 20:00, room Odyssée |
| Workshops: | Mon-Tues from 20:00, rooms Pluton, Jupiter and Uranus (I and II) |

Contact: conference@hotel-chavannes.ch / reception@hotel-chavannes.ch
0041 22 9608181

dicty2017@gmail.com

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Poster prizes

Poster presenter 1 :

Poster presenter 2 :

Poster presenter 3 :

Poster presenter 4 :

Poster presenter 5 :

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Flash talk prize

Flash talk presenter :

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Talk prizes

Talk presenter on Monday 21st :

Talk presenter on Tuesday 22nd :

Talk presenter Wednesday 23rd or Thursday 24th :

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Prizes will be awarded
before lunch on Thursday 24th!

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# Annual Dictyostelium Conference 2017

## August 20-24

## Best Western Hotel Chavannes-de-Bogis
## Geneva, Switzerland

**Organisers**
**Thierry Soldati**
**Pierre Cosson**
**and their groups**

# Socials and excursion

**(more information on site)**

## Ice Breakers

Sunday August 20th, from arrival to the Keynote lecture, various opportunities for networking: quiz, photo booth, archery contest etc… accompanied by snacks and drinks.

## Meet the speaker

Every day, you will be able to sign-up for "meet the speakers" events, at lunch or dinner

## Antibodies contest

UNIVERSITÉ DE GENÈVE RECOMBINANT ANTIBODIES

During the conference, the Geneva Antibody Facility will hold a contest to generate recombinant antibodies against 3 selected candidate *Dictyostelium* proteins. Ideally, these candidates should:

1. Be useful to as many laboratories as possible. Think "actin", "gel loading controls", "organelle marker", "developmental marker".
2. Recognize a small, abundant, non-glycosylated protein (to increase chances of recognizing the endogenous protein).

We (Wanessa Lima and Pierre Cosson) welcome your suggestions during the meeting!

## Excursion to Geneva (Wednesday, August 23rd)

FREE WALK GENEVA

**Sunday August 20th and Monday August 21st**

Do not forget to sign up for the excursion at the entrance of the conference room!

**Wednesday, August 23rd**

| | |
|---|---|
| 12:30 | Pick up lunch pack |
| 12:45 | Coaches depart conference center (entrance) for the excursion |
| 13:15 | Boat departs from Coppet, cruise to Geneva |
| 14:00 | Guided tour of Geneva in small groups (http://www.freewalk.ch/geneva) |
| 17:00 – 17:30 | Coaches depart Geneva train station (Rue de Malatrex), back to the hotel |

## Dinner party (Wednesday August 23rd)

GENEVA SWING

| | |
|---|---|
| 18:30 | Barbecue at the poolside |
| 19:30 | Animations |
| | Swing dancing exhibition and initiation course (dancing teachers Jerôme Cadoret and Ana Barros, http://genevaswing.ch) |
| | Lost in Swing music band (http://www.lostinswing.com) |

# Conference programme

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**Sunday, August 20th**

| | |
|---|---|
| 12:00 | Arrival and registration open until 18:00<br>Ice breaker, photo booth<br>Tea, coffee, drinks and cookies |
| 18:00 – 18:05 | Welcome and foreword : Thierry Soldati and Pierre Cosson |
| 18:05 – 19:00 | **Keynote Lecture : Michel MILINKOVITCH**<br>"A living cellular automaton : when Charles Darwin meets John von Neumann & Alan Turing" |
| 19:30 | Dinner |

**Monday, August 21st**

## CELL MIGRATION & CHEMOTAXIS

### Chairpersons : Cristina Bosmani & Caroline Barisch

| | |
|---|---|
| 8:30 – 9:10 | **Keynote : Michael WAY**<br>"New levels of complexity in Arp2/3-driven actin polymerization" |
| 9:10 – 9:30 | **Peter VAN HAASTERT**<br>"New signaling networks for symmetry breaking and directed cell movement" |
| 9:30 – 9:50 | **Igor WEBER**<br>"Rac1 dynamics in *Dictyostelium* cells" |
| 9:50 – 10:10 | **Wouter-Jan RAPPEL**<br>"Movement of *Dictyostelium* cells on patterned substrates" |
| 10:10 – 10:45 | Coffee Break |
| 10:45 – 11:10 | **Dicty keynote : Peter DEVREOTES**<br>"Biasing of a Signal Transduction Excitable Network (STEN) directs cell migration" |
| 11:10 – 11:30 | **David KNECHT**<br>"Self-generated gradient formation and the role of chemoattractant degradation in gradient sensing" |
| 11:30 – 11:50 | **Kees WEIJER**<br>"Investigations of the modes of oscillatory signalling in Dictyostelids" |
| 11:50 – 11:55 | **Flash talk : Zully SANTIAGO**<br>"Choice of cell motility structures is regulated by TalA, a cortex-to-membrane crosslinking protein" |
| 11:55 – 12:00 | **Flash talk : Masahiro UEDA**<br>"Trimeric G-protein shuttling for chemotaxis ranges" |
| 12:00 – 12:05 | **Flash talk : Ted COX**<br>"Telling right from wrong: receptor occupancy and kinetic proof-reading" |
| 12:05 – 13:30 | Lunch Break |

**Monday, August 21st**

## HOST-PATHOGEN INTERACTIONS

### Chairpersons : Louise Lefrançois & Wanessa Du Fresne Von Hohenesche

13:30 – 14:10 **Keynote : Bruno LEMAITRE**

"The foreign within: *Drosophila-Spiroplasma* interaction as a model of insect endosymbiosis"

14:10 – 14:30 **Hubert HILBI**

"*Dictyostelium* as a model for *Legionella*-phagocyte interactions"

14:30 – 14:50 **Yann HÉCHARD**

"*Legionella pneumophila* prevents proliferation and DNA replication of *Acanthamoeba castellanii*"

14:50 – 15:10 **Caroline BARISCH**

"Take it with a pinch of salt! Zinc intoxication to control mycobacteria infection!"

15:10 – 15:45 Coffee Break

15:45 – 16:10 **Dicty keynote : Adam KUSPA**

"Distinct lectins moderate interactions with bacteria during growth and development of *Dictyostelium discoideum*"

16:10 – 16:30 **Debra BROCK**

"The love/hate relationships between *Dictyostelium* and *Burkholderia* bacteria"

16:30 – 16:50 **Dawn TAYLOR-MULNEIX**

"*Bordetella bronchiseptica* exploits the complex life cycle of *Dictyostelium* as an amplifying transmission vector"

16:50 – 17:10 **Christopher WEST**

"Molecular mechanisms of oxygen sensing in *Dictyostelium* and other protists"

17:10 – 17:15 **Flash talk : Ana Teresa LÓPEZ-JIMÉNEZ**

"The "DictyJail": a new microfluidic tool for long-term single-cell imaging of mycobacteria infection"

17:15 – 17:20 **Flash talk : Camila VALENZUELA MONTENEGRO**

"*Salmonella Typhimurium* requires SopB and SifA to survive intracellularly in a SCV-like compartment in *Dictyostelium discoideum*"

17:20 – 18:30 Break

18:30 – 20:00 Dinner

**20:00 – 23:00 Poster and Workshop Session I, drinks and snacks**

20:00 – 20:05 **Flash talk : Christophe ANJARD**

"Short and long distance signals regulate vegetative cell motility"

20:05 – 20:10 **Flash talk : Nasna NASSIR**

"Non-canonical functions of telomerase reverse transcriptase in *D. discoideum*"

20:10 – 20:15 **Flash talk : Vlatka ANTOLOVIC**

"Generation of single cell transcript variability by repression"

**Tuesday, August 22nd**

## DISEASE MODELING & TOOLS

### Chairpersons : Ana Teresa López-Jiménez & Nabil Hanna

8:30 – 9:10 **Keynotes : Amy BALDWIN**
"GWDI-bank – Introducing the genome wide *Dictyostelium* insertion resource for functional genomics"
**and Nicole GRUENHEIT**
"High content parallel phenotyping in *Dictyostelium*"

9:10 – 9:30 **Katelyn MROCZEK**
"A *Dictyostelium discoideum* model for Alzheimer's disease and other Tauopathies"

9:30 – 9:50 **Peter THOMASON**
"Gaining insights into the rare inherited disease HPS using *Dictyostelium*"

9:50 – 10:10 **Devdutt SHARMA**
"Identifying new roles for the $\gamma$ -secretase complex in *Dictyostelium discoideum*"

10:10 – 10:45 Coffee Break

10:45 – 11:10 **Dicty keynote : Robin WILLIAMS**
"Investigating the active functional groups of curcumin controlling biological activity"

11:10 – 11:30 **Annette MÜLLER-TAUBENBERGER**
"Intranuclear and cytoplasmic actin rod assembly in *Dictyostelium discoideum*"

11:30 – 11:50 **Pierre STALLFORTH**
"Natural Products from Amoeba-Bacteria Interactions"

11:50 – 11:55 **Flash talk : Lavanya MUTHUKUMAR**
"Determining the cellular pathways involved in *D. discoideum* polyketide synthase metabolism**"**

11:50 – 11:55 **Flash talk : Isabella GUIDO**
"Sensing mechanism of cells under the influence of electric fields"

12:00 – 12:05 **Flash talk : Wanessa DU FRESNE VON HOHENESCHE**
"The Geneva Antibody Facility"

12:05 – 13:30 Lunch Break

**Tuesday, August 22nd**

## MULTICELLULAR DEVELOPMENT & EVOLUTION

**Chairpersons : Joe Dan Dunn & Jade Leiba**

13:30 – 14:10 **Keynote : Marcos GONZÁLEZ-GAITÁN**
"The basis of the asymmetric targeting of endosomes during asymmetric division"

14:10 – 14:30 **Gad SHAULSKY**
"The role of allorecognition signaling in tissue integration"

14:30 – 14:50 **Azam GHOLAMI**
"Control of pattern formation in *Dictyostelium discoideum*"

14:50 – 15:10 **John NICHOLS**
"Cell and molecular transitions of dedifferentiation"

15:10 – 15:45 Coffee Break

15:45 – 16:10 **Dicty keynote : Joan STRASSMANN**
"Learning from mistakes"

16:10 – 16:30 **Falk HILLMANN**
"Functional genomics of a protosteloid amoeba suggests alternative routes to fruiting body formation"

16:30 – 16:50 **Wolfgang HEIDEN**
"A simulation model for dynamic thermotaxis of *Dictyostelium* slugs"

16:50 – 17:10 **Yoko YAMADA**
"The transcription factor sporulation deficient A is a master regulator of *Dictyostelium* sporulation"

17:10 – 17:15 **Flash talk : Rosa HERBST**
"Polyketide synthase null mutant resembles prestalk A cells"

17:15 – 18:30 Break

18:30 – 20:00 Dinner

**20:00 – 23:00 Poster and Workshop Session II, drinks and snacks**

20:00 – 20:05 **Flash talk : Stephanie SANTARRIAGA**
"Polyphosphate induces a phase-separated chaperone complex during *Dictyostelium discoideum* development"

20:05 – 20:10 **Flash talk : Eunice DOMÍNGUEZ-MARTÍN**
"IreA and autophagy cooperate for the survival response to ER-stress in *Dictyostelium discoideum*"

20:10 – 20:15 **Flash talk : Ikumi HAYAKAWA**
"Genes responsible for species recognition in social amoebae"

**Wednesday, August 23rd**

## GENE EXPRESSION

### Morning session chairpersons : Elena Cardenal-Muñoz & Tania Jauslin

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|---|---|
| 8:30 – 9:10 | **Keynote : Françoise STUTZ**<br>"Non-coding transcription, gene regulation and replication in the yeast *S. cerevisiae*" |
| 9:10 – 9:30 | **Fredrik SÖDERBOM**<br>"Abundant non-coding RNA involved in early *Dictyostelium discoideum* development is conserved in Dictyostelid social amoebae" |
| 9:30 – 9:50 | **Christian HAMMANN**<br>"The Elongator complex of *Dictyostelium discoideum*" |
| 9:50 – 10:10 | **Adrian HARWOOD**<br>"Nucleosome positioning and gene regulation" |
| 10:10 – 10:45 | Coffee Break |
| 10:45 – 11:10 | **Dicty keynote : Jonathan CHUBB**<br>"Causes and consequences of unpredictable gene expression" |
| 11:10 – 11:30 | **Christopher THOMPSON**<br>"A buffered noisy cell fate oscillator in *Dictyostelium*" |
| 11:30 – 11:50 | **Huaqing CAI**<br>"Nucleocytoplasmic shuttling of a GATA transcription factor functions as a development timer" |
| 11:50 – 12:10 | **William SALVIDGE**<br>"The role of the histone methyl-transferase COMPASS complex in controlling variable gene expression and cell type proportioning" |
| 12:15 | Pick up lunch pack |
| 12:45 | Coaches depart conference center for the excursion |
| 13:15 | Boat departs from Coppet, cruise to Geneva |
| 14:00 | Guided tour of Geneva in small groups |
| 17:00 – 17:30 | Coaches depart train Geneva station, back to the hotel |
| 18:30 | Dinner party, BBQ at the poolside |
| 19:30 | Animations, music and dance floor |

**Thursday, August 24th**

## CELLULAR ORGANIZATION, TRANSPORT & SIGNALLING

**Chairpersons : Romain Bodinier & Otmane Lamrabet**

| | |
|---|---|
| 8:30 – 9:10 | **Keynote : Florence NIEDERGANG**<br>"How to build a phagosome: macrophages solutions to remodel membranes and cytoskeleton" |
| 9:10 – 9:30 | **Catherine BUCKLEY**<br>"Coordination of Rho and Ras small GTPases in macropinocytic and phagocytic cup formation" |
| 9:30 – 9:50 | **Peggy PASCHKE**<br>"How are macropinosomes formed?" |
| 9:50 – 10:10 | **Jade LEIBA**<br>"Role of DrkD in cell spreading and phagocytosis in *Dictyostelium*" |
| 10:10 – 10:45 | Coffee Break |
| 10:45 – 11:10 | **Dicty keynote : Alan KIMMEL**<br>"Quantifying live bacterial sensing for novel regulators of chemotaxis" |
| 11:10 – 11:30 | **Tian JIN**<br>"Chemoattractant GPCRs mediate both chemotaxis and phagocytosis for catching and ingesting bacteria" |
| 11:30 – 11:50 | **Catherine PEARS**<br>"Two-pore channel protein TPC2 in early *Dictyostelium* development" |
| 11:50 – 12:10 | **Irene MEYER**<br>"Centrosomes and nuclear pore complexes in semi-closed mitosis" |
| 12:10 – 12:30 | **Patrick SUESS**<br>"Extracellular polyphosphate regulates proliferation and development" |
| 12:30 – 13:00 | Awards ceremony<br>Closing and farewell address |
| 13:00 | Lunch |
| 14:00 | Departure |

# Workshops information

Monday August 21st and Tuesday August 22nd

Starting at 20h00 (simultaneously with Poster sessions)

Rooms Pluton, Jupiter and Uranus (I and II)

**Amy BALDWIN and Nicole GRUENHEIT**
(abstracts in Meeting booklet)

"GWDI-bank – Introducing the genome wide *Dictyostelium* insertion resource for functional genomics" and "High content parallel phenotyping in *Dictyostelium*"

**Petra FEY**
(abstract in Posters booklet)

"dictyBase 2017: new tools and interfaces"

**Allyson E. SGRO**
(abstract in Posters booklet)

"Working toward proof-by-control of the signaling rules governing collective decision making in *Dictyostelium*"

**Daniel IRIMIA**
(abstract in Posters booklet)

"The Next Dicty World Race"

# Talks

# A living cellular automation: when Charles Darwin meets John von Neumann & Alan Turing

**Michel Milinkovitch**

*University of Geneva, Switzerland*

# New levels of complexity in Arp2/3 driven actin polymerization

**Michael Way**

*The Francis Crick Insitute, UK*

# New signaling networks for symmetry breaking and directed cell movement.

Youtao Liu, Marjon Kamp, Ineke Keizer-Gunnink, Arjan Kortholt and **Peter J.M. van Haastert**

*University Groningen, Netherlands*

Central to chemotaxis is the molecular mechanism by which a shallow spatial gradient of chemoattractant induces symmetry breaking of activated signaling molecules. First, we studied the movement and shape of amoeboid cells in buffer, which constantly deform by making new extensions. Are cell shape changes random, or are they the consequence of an underlying order? And if there is order, what is the mechanism and function? Careful investigations reveal that amoeboid cells in the absence of external cues do not move randomly, but exhibit persistence: cells have a tendency to extend new alternating right/left protrusions in a similar direction as previous protrusions1. We have used a sensitive assay for local Ras activation (Ras*) at the membrane *Dictyostelium* cells2. Patches of Ras* are responsible for protrusions and shape changes. We manipulated *Dictyostelium* cells to increasingly inhibit the F-actin-based cytoskeleton (F-actin, IQGAP, myosin). Starting from the full inhibition of F-actin-based cytoskeleton we show that Ras* is low and nearly uniform at the entire plasma membrane. F-actin leads to high uniform Ras*. The F-actin-associated cytoskeleton induces a series of symmetry breakings: first, local inhibition of Ras* by IQGAP/cortexillin converts uniform Ras* into multiple Ras* patches with rotational symmetry; second, inhibition of Ras* by myosin in the rear induces a polarized cell with Ras* in the front with mirror symmetry; finally, SCAR induces left-right asymmetry of Ras*, leading to gliding mirror symmetry that is characteristic for the persistent left-right gait of amoeboid cells in the absence of external cues. Computer simulations and mutant analysis reveal that these symmetry breaking prepare the cell for very efficient chemotaxis. Subsequently, we have used an extensive and efficient proteomic approach to identify further components of this basal signaling pathway that collectively form the dynamic signaling network for chemotaxis. We have used these proteomic data to get more insight in the mechanism by which: (1) the heterotrimeric G protein cycle is regulated3 (2) heterotrimeric G proteins induce symmetry breaking in small G protein signaling4, and (3) symmetry breaking in G protein signaling induces cytoskeleton rearrangements and subsequently cell migration.

1. Bosgraaf, L., and Van Haastert, P. J. M. (2009). The ordered extension of pseudopodia by amoeboid cells in the absence of external cues. PLoS One 4, e5253.
2. Kortholt, A. et al. (2013). Ras activation and symmetry breaking during *Dictyostelium* chemotaxis. J. Cell Sci. 126, 4502–4513
3. Kataria, R. et al. (2013) *Dictyostelium* Ric8 is a nonreceptor guanine exchange factor for heterotrimeric G proteins and is important for development and chemotaxis. Proc. Natl. Acad. Sci. U. S. A. 110, 6424–9.
4. Liu, Y. et al. (2016). A Gα-Stimulated RapGEF Is a Receptor-Proximal Regulator of *Dictyostelium* Chemotaxis. Dev. Cell 37, 458–72.

# Rac1 dynamics in *Dictyostelium* cells

Vedrana Filić, Maja Marinović, Marko Šoštar, Vlatka Antolović, Jan Faix*, and **Igor Weber**

*Division of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia; *Institute for Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany*

*Dictyostelium* cells are capable of completely reversing their polarity within 20 seconds, and thus undergo the fastest spontaneous re-polarization among eukaryotic cells. Signalling by small Rho GTPases plays an essential role in this process. Biosensors are therefore needed that are able to match these fast dynamics and enable protracted imaging at high recording rates. However, standard tools used to investigate Rho GTPase activity in living cells such as probes based on fluorescence resonance energy transfer (FRET), bimolecular fluorescence complementation (BiFC), and photoactivation, are of limited usefulness in quickly migrating cells because short time available for image acquisition often results in an unacceptably low signal-to-noise ratio. Attempts to remedy this effect by increasing the intensity of illumination are restricted by photobleaching of probes and the cell photosensitivity. We describe characterization of a new fluorescent probe that selectively binds to active forms of Dictyostelium discoideum Rac1 GTPases, and demonstrate its excellent properties for live cell imaging. The probe is based on the GTPase-binding domain (GBD) from DPAKa kinase, and was selected on the basis of yeast two-hybrid screen, GST pull-down assay, and FRET measurements by fluorescence lifetime imaging microscopy (FLIM). The probe binds specifically to active Rac1 and has a low cytoplasmic background, thus enabling quantitative determination of the Rac1 activity in the cell membrane over an order of magnitude. The main advantage of DPAKa(GBD) in comparison to similar probes is its finely graded intensity distribution along the entire plasma membrane, which enables measurements of the Rac1 activity in different parts of the membrane. Overexpression of DPAKa(GBD)-DYFP probe induces no adverse effects on cell motility, cytokinesis and growth, thus enabling long-term imaging with negligible photobleaching and phototoxicity. Rac1 GTPases play a dual role in the regulation of actin assembly, which is a key determinant of cell polarity. At the cell front, Rac1 activates the Scar/WAVE complex and thereby stimulates the Arp2/3-mediated actin polymerization. At the cell back, Rac1 regulates stability of the cell cortex by initiating formation of a complex containing IQGAP-related protein DGAP1 and a heterodimer of actin-bundling proteins cortexillins. We will discuss the possible consequences of the Rac1 interaction with multiple partners on its dynamics in living motile cells. Additionally, we will propose that DGAP1 unifies the roles of an effector and a sequestrator in relation to Rac1. Unlike classical effectors, which bind their activators transiently leading to short-lived signalling complexes, interaction between DGAP1 and Rac1-GTP could be stabilized by the formation of a complex with cortexillins. Since the oppositely localized Rac1 effector, Scar/WAVE, promotes actin polymerization at the cell front, competition between DGAP1 and Scar/WAVE for the common activator Rac1-GTP might provide the basis for the oscillatory re-polarization typically seen in randomly migrating *Dictyostelium* cells. This hypothesis is further being investigated by combining imaging and modelling approaches.

# Movement of *Dictyostelium* cells on patterned substrates

Richa Karmakar, Elisabeth Ghabache, Edgar Gutierrez, Alex Groisman, Brian Camley, and **Wouter-Jan Rappel**

*University of California, San Diego, USA*

We use a micropatterned surface to investigate the movement of *Dictyostelium* cells restricted to 1D stripes. The cells are exposed to periodic waves of chemoattracant that are generated by a microfluidic device. We track individual cells as well as clusters of cells and determine their chemotactic index. Consistent with our earlier work, we find that individual cells exhibit cellular memory and that they maintain direction towards the wave source in the back of the wave. Interestingly, preliminary data reveals that clusters of cells exhibit a larger memory than single cells. We also present a model that can reproduce the observed data.

# Biasing of a Signal Transduction Excitable Network (STEN) directs cell migration

**Peter N. Devreotes**, Sayak Bhattacharya, Marc Edwards, Pablo A. Iglesias, Thomas Lampert, Xiaoguang Li, Yuchuan Miao, David Zhang

*Departments of Cell Biology and Electrical Engineering and Computer Science*
*Johns Hopkins University, School of Medicine, Baltimore, MD 21205*

Recent studies in *Dictyostelium* suggest that cell migration is mediated by waves of signal transduction activity that drive cytoskeletal events. To further investigate this hypothesis, we used chemically induced heterdimerization to acutely activate specific nodes in the STEN. We found that migratory behavior are switched from amoeboid to keratocyte-like and oscillatory modes within minutes. This can be achieved by synthetically decreasing PIP2 levels, increasing Ras/Rap-related activities, and many other perturbations. The perturbations at these key nodes of an excitable signal transduction network initiate a causal chain of events: The threshold for network activation is lowered, the speed and range of propagating waves of signal transduction activity increases, actin driven cellular protrusions expand and, consequently, the cell migratory mode transitions ensue. Conversely, innately keratocyte-like and oscillatory cells are promptly converted to amoeboid by inhibition of Ras effectors with restoration of directed migration. We use computational analysis to explain how thresholds control cell migration and discuss the architecture of the signal transduction network that gives rise to excitability. We show that the STEN can be biased by rapid recruitment of G-proteins βγ-subunits suggesting a mechanism for chemotaxis.

# Self-generated gradient formation and the role of chemoattractant degradation in gradient sensing

**David A. Knecht**, Luke Tweedy, and Robert H. Insall

*Univeristy of Connecticut, USA*

It is well known that cells are able to migrate directionally in response to diffusional chemotactic gradients. However, the role of chemoattractant degradation by cells in gradient sensing has received much less attention. We have found that cells are able to locally degrade a uniform concentration of chemoattractant to form a "self-generated gradient" of chemoattractant. As long as the cells are initially inhomogeneous, they will move away from their starting point for long distances as they continuously generate a local gradient. Using the folate chemotaxis assay, we have modeled the self-generated gradient formation and measured the gradient to show its formation. Cells have both cell surface and secreted folate deaminase activities which degrade folate to a form that does not activate the folate receptor. Cells increase the amount of folate deaminase secreted in response to the presence of folate allowing them to modulate folate degradation. We have found no evidence that the cellular responses to folate are the result of the production of a chemorepellent by cells. We are also examining the extent to which degradation plays a role in modifying diffusional gradients. Methotrexate (a structural analog of folic acid which stimulates the folate receptor but is not degraded by folate deaminase) will allow us to test whether cells respond to diffusional gradients that are not modified by degradation. Preliminary evidence indicates a greater role for degradation than previously suspected. We have purified the secreted folate deaminase and are in the process of sequencing the protein in order to generate folate deaminase mutants to further test this hypothesis.

# Investigations of the modes of oscillatory signalling in Dictyostelids

Gail Singer[1], Tsuyoshi Araki[2], Hajara Lawal[1], Pauline Schaap[1], **Kees Weijer**[1]

*[1]School of Life Sciences, University of Dundee, Dundee UK; [2]Sophia University Tokyo, Japan*

It has been well established that *Dictyostelium* cell communicate by propagating waves of cAMP. These cAMP waves direct locally coordinated periodic movement of cells to result in aggregation and later development. We have previously visualised these coordinated movement waves as lightscattering waves during all stages of development. We now report the direct visualisation and characterisation of cAMP waves during the aggregation mound and slug stages of development using a cAMP specific Fret sensor. Furthermore although periodic cAMP signalling appears to be limited to group 4 species, we found strong evidence for the existence of oscillatory signalling, measured as light scattering waves, coordinating morphogenesis among representative species of all four major groups of Dictyostelids. We are currently trying to elucidate the molecular basis for the observed oscillatory cell-cell signalling in other species.

# The foreign within: *Drosophila-Spiroplasma* interaction as a model of insect endosymbiosis

**Bruno Lemaitre**

*EPFL, Lausanne, Switzerland*

# *Dictyostelium* as a model for *Legionella*-phagocyte interactions

**Hubert Hilbi**

*University of Zurich, Switzerland*

*Dictyostelium discoideum* is a versatile model to analyze pathogen-phagocyte interactions. The causative agent of Legionnaires' pneumonia, *Legionella pneumophila*, is a facultative intracellular bacterium that grows in free-living amoeba and macrophages within a specific membrane-bound compartment, the *Legionella*-containing vacuole (LCV). LCV formation is a complex process, during which the pathogen vacuole interacts with the endosomal, secretory and retrograde vesicle trafficking pathways, and tightly associates with the endoplasmic reticulum. The *L. pneumophila* Icm/Dot type IV secretion system (T4SS) is essential for LCV formation and translocates the astonishing number of about 300 different "effector proteins" into host cells, where they subvert cytoskeleton dynamics, vesicle trafficking and signal transduction. To analyze *L. pneumophila*-phagocyte interactions, intact LCVs are purified from infected *D. discoideum* amoeba and characterized by proteomics. Moreover, *D. discoideum* deletion mutants and strains producing fluorescently tagged proteins allow assessing pathogen vacuole formation, intracellular bacterial replication and modulation of cell migration by wild-type and mutant *L. pneumophila*. Recent studies in our lab employ tandem fluorescently labelled *D. discoideum* strains to elucidate by live cell fluorescence microscopy and imaging flow cytometry the role of phosphoinositide lipids, small and large GTPases, and other conserved host components for *L. pneumophila*-phagocyte interactions.

# *Legionella pneumophila* prevents proliferation and DNA replication of *Acanthamoeba castellanii*

Luce Mengue[1], Matthieu Régnacq[2], Willy Aucher[1], Emilie Portier[1], **Yann Héchard**[1] and Ascel Samba-Louaka[1].

[1]*Laboratoire Ecologie et Biologie des Interactions, Microbiologie de l'Eau, Université de Poitiers, UMR CNRS 7267;* [2]*Signalisation et Transports Ioniques Membranaires, Université de Poitiers, CNRS ERL 7368, Poitiers, France.*

*Acanthamoeba castellanii* is an amoeba considered as a "trojan horse" of the microbial world as it allows multiplication and protects several bacteria. *Legionella pneumophila*, the causative agent for legionellosis, is one of the most studied *A. castellanii* resisting bacteria. Many host cellular pathways are modulated by *L. pneumophila* such as phagocytosis and autophagy. Our objective was to test whether *L. pneumophila* would modulate the proliferation of *A. castellanii*. We found that *L. pneumophila* is able to impair proliferation of infected *A. castellanii*. This effect seemed controlled by an effector secreted by the type IV secretion system (T4SS) of *L. pneumophila* since a T4SS mutant (dot A) did not impair proliferation. Time lapse microscopy showed that, in addition to impair cell division, *L. pneumophila* induced modifications in shape, motility of *A. castellanii*. Use of Edu, an analogue of thymidine, demonstrated that infection inhibited DNA replication within *A. castellanii*. Thus, we searched for cyclin dependent kinase (CDK) genes in the *A. castellanii* genome and found one gene, CDC2b, which is similar to the main cell cycle regulator gene in human CDK1 ) and in *Saccharomyces cerevisiae* (CDC28). By expressing amoebal CDC2b gene in a CDC28 mutant of *Saccharomyces cerevisiae*, we establish that the CDC2b could be a cyclin-dependent kinase. To our knowledge, *L. pneumophila* could be the first bacterium regulating the eukaryotic cell cycle through down-regulation of the host CDK expression. In conclusion, *L. pneumophila* impairs *Acanthamoeba castellanii* cell cycle by a mechanism which remains to be elucidated.

# Take it with a pinch of salt! Zinc intoxication to control mycobacteria infection!

**Barisch, C.,** Kalinina, V., Lefrançois, L., Appiah, J., Koliwer-Brandl, H., Hilbi, H., & Soldati, T.

*University of Geneva, Switzerland*

Nutritional immunity describes the battle raging at the interface between a host and its intracellular pathogen for access to (micro)nutrients. In the case of transition metals, recent studies revealed a "double edge sword" problem. Indeed, phagocytes can restrict intravacuolar bacterial growth either by depleting essential ions, such as iron, or accumulate them, for example copper and zinc, to intoxicating concentrations. Despite recent efforts, the roles of NRAMP transporters in iron depletion, and ZnT transporters in zinc accumulation during Mycobacterium tuberculosis infection of macrophages are still unclear. We use the *Dicytostelium/Mycobacterium marinum* system to study the role of iron, zinc and their transporters during mycobacterial infection. We visualised and quantitated the appearance of free zinc in bead- or *M. marinum*-containing vacuoles (MCVs). In regular phagosomes, free zinc is delivered immediatley after particle uptake, mainly by fusion with "zincosomes" of endosomal origin. We localised the four *Dictyostelium* ZnT transporters in endosomes, the contractile vacuole and at the MCV membrane and study the impact of knockouts (KOs) on the homeostasis of zinc. We show that an *M. marinum* KO mutant in CtpC, a cation efflux transporter, is attenuated in intracellular growth. We also demonstrate that the virulence of this mutant is further reduced in a *Dictyostelium* zntA KO. Strikingly, in a *Dictyostelium* zntB KO that is depleted in endosomal zinc *M. marinum* growth is also attenuated. Finally, we bring evidence that *nramp* KOs of Dictyostelium are more susceptible to infection, illustrating the opposite strategies of bacterial control exerted by the host via the homeostasis of different transition metals.

# Distinct lectins moderate interactions with bacteria during growth and development of *Dictyostelium discoideum*

Christopher Dinh, Timothy Farinholt, Olga Zhuchenko, and **Adam Kuspa**

*Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030 USA*

The predation of bacteria by amoebae versus the colonization of amoebae by bacteria is an ancient interspecies competition. Extant social amoebae such as *Dictyostelium discoideum* have been shaped by this evolutionary history and have intricate mechanisms to optimize their survival in complex soil environments (see e.g., 1). During development the amoebae form a mobile multicellular slug comprised of several distinct cell-types, including Sentinel cells that function as an innate immune system capable of clearing the slug of potentially harmful bacteria (2). Sentinel cells also elaborate DNA-based Extracellular Traps (ETs) that trap and kill bacteria that are similar to the ETs produced by neutrophils (3). Bacterial carriage during development in some wild strains of *D. discoideum* that results in bacterial endosymbiosis (4) would appear to be incompatible with the co-occurrence of Sentinel cell innate immunity. Therefore, we have been investigating bacterial carriage to better understand the regulation of the "kill versus cooperate" options that bacteria and amoebae face during growth and development, and to examine the interplay between *D. discoideum's* innate immune system and its microbiome. We have found that wild strains that carry bacteria during development secrete carbohydrate-binding proteins (lectins) during the growth to development transition that influence amoebal survival and induce bacterial endosymbiosis. We will provide evidence that the lectins Discoidin A (DscA) and Discoidin C (DscC) bind and protect bacteria from killing by antibacterial proteins secreted by D. discoideum. DscA and DscC also induce bacterial endosymbiosis, resulting in live bacteria within amoebae and spores, through a process we call Lectin-Induced Modified Bacterial Internalization, or LIMBI. LIMBI also leads to high-efficiency transformation when amoebae are presented with DscA-coated *E. coli* carrying *Dictyostelium* shuttle plasmids demonstrating that LIMBI is distinct from the phagocytosis pathway used for bacterial feeding. CadA is also secreted by carrier strains at higher levels compared to non-carrier strains. We have found that CadA is a also lectin that binds bacteria and is required for colony formation on lawns of Gram(-) bacteria. CadA mediates bacterial clumping in vitro and appears to be required in vivo for the formation of a transient physical barrier between growing amoebae and bacteria. These functions may account for the impaired colony formation of *cadA*- mutants on bacterial lawns. We will also briefly describe two additional classes of secreted lectins that appear to be involved amoebae-bacteria interactions. Our work suggests that *D. discoideum* amoebae control interactions with bacteria using at least four distinct classes of secreted lectins, which modulate bacterial feeding and innate immune responses, presumably to regulate their microbiome and optimize survival.

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# The love/hate relationships between *Dictyostelium* amoebae and *Burkholderia* bacteria

**Debra A. Brock,** Longfei Shu, Susanne DiSalvo, David C. Queller, and Joan E. Strassmann

*Washington University in St. Louis, USA*

Symbiotic relationships are common and can range from beneficial (love) to virulent (hate). Bacteria are frequent partners in these associations. How do bacteria go from free-living to evolve into obligatory endosymbionts? A first step in the trajectory could be facultative symbiosis within host cells. We have previously described a facultative symbiosis between the social amoeba Dictyostelium discoideum and certain *Burkholderia* bacteria. Some lineages of wild amoebae known as farmers have stable interactions with different bacterial partners that are both food and weapons. We have also reported two major clades of *Burkholderia* as the most prominent bacterial partners of *D. discoideum* farmers. Here, we explore the fitness impacts of pairing various *Dictyostelium* hosts with the same or unfamiliar farmer-associated *Burkholderia*. We further characterized the morphological impacts of this symbiosis on *D. discoideum* by measuring their terminal fruiting body form and by taking detailed photographs of amoebae and spores using scanning electron and fluorescent confocal microscopy. First, we found viable spore production for naïve *D. discoideum* hosts is significantly reduced in the presence of *Burkholderia*, indicating the association between naïve *D. discoideum* and *Burkholderia* is more virulent. However for the beneficial trajectory, we found evidence of partner adaptation because the natural hosts of clade 2 *Burkholderia* are less harmed by their own bacteria than are naïve hosts. We further used fluorescently-labelled *Burkholderia* and found that *D. discoideum* spores are significantly more infected by Clade 2 *Burkholderia* than by Clade 1 *Burkholderia*. These results suggest that the degree of fitness detriment imposed by mixing and matching *Burkholderia* with various partners may correlate with infection frequency. We also observed Clade 1 and Clade 2 *Burkholderia* had different effects on fruiting body morphology. *D. discoideum* colonized by Clade 1 *Burkholderia* produced tall, thin stalks that were unable to hold the spore mass of the fruiting body aloft. We found the opposite phenotype for the Clade 2 *Burkholderia*. Here the phenotype is short with a sturdy stalk. Besides the morphological impact of the two clades of *Burkholderia*, we investigated the origins and trajectory of the symbiosis. We previously established that *Burkholderia* can be taken up from the environment through horizontal transmission and stably partner with *D. discoideum*. Now we show that *Burkholderia* hide in vacuoles to avoid being killed by the host. These vacuoles are vertically transmitted to the next generation inside *D. discoideum* spores. Importantly, carriage of *Burkholderia* induces carriage of other bacteria that could not become carried on their own. We found that both food bacteria and *Burkholderia* symbionts were ingested through phagocytosis and were surrounded by multi-lamellar bodies. In the presence of *Burkholderia* symbionts, these bacteria were resistant to phagocytic digestion. The carriage of food bacteria is an important benefit for *D. discoideum* and may help maintain the symbiotic relationship. This eukaryote-bacterium symbiosis spans the range of mutualism (love) to virulence (hate) in interactions between the social amoeba *D. discoideum* and farmer-associated *Burkholderia*, and presents a valuable opportunity to gain insight and to explore the process of host-symbiont lifestyle trajectories.

# *Bordetella bronchiseptica* exploits the complex life cycle of *Dictyostelium discoideum* as an amplifying transmission vector

**Dawn L. Taylor-Mulneix**, Liron Bendor, Bodo Linz, Israel Rivera, Valerie E. Ryman, Kalyan K. Dewan, Shannon M. Wagner, Emily F. Wilson, Lindsay J. Hilburger, Laura E. Cuff, Christopher M. West, Eric T. Harvill

*University of Georgia, Athens, GA, USA*

Multiple lines of evidence suggest that *Bordetella* species have a significant life stage outside of the mammalian respiratory tract that is yet to be defined. The BvgAS two component system, a paradigm for a global virulence regulon, controls expression of many "virulence factors" expressed in the Bvg+ phase that are necessary for respiratory tract infection. A similarly large set of highly conserved genes are expressed under Bvg- phase growth conditions that are not required for respiratory infection, but are hypothesized to be important in an undefined extra-host reservoir or niche. Here we show that Bvg- phase genes are involved in the novel ability of *Bordetella bronchiseptica* to grow and disseminate via the complex life cycle of the amoeba *Dictyostelium discoideum*. Unlike bacteria that serve as an amoeba food source, *B. bronchiseptica* evades amoeba predation, survives within the amoeba for extended periods of time, incorporates into the amoeba sori, and disseminates along with the amoeba to novel locations. Remarkably, *B. bronchiseptica* continues to be transferred with the amoeba over months, through multiple life cycles of amoeba growth on lawns of other bacteria, thus demonstrating that *B. bronchiseptica* can expand and disperse geographically via the complex *D. discoideum* lifecycle. *B. bronchiseptica* within the sori can efficiently infect mice, indicating that amoeba may represent an environmental reservoir and a vector within which pathogenic *bordetellae* can expand and disseminate to encounter and infect new mammalian hosts. These data identify amoeba as potential environmental reservoirs as well as amplifying and disseminating vectors for the *B. bronchiseptica*, and reveal an important role for the Bvg- phase in these interactions.

# Molecular Mechanisms of Oxygen Sensing in *Dictyostelium* and other Protists

**Christopher M. West**[1,4], Xiangzhong Xu[1], M. Osman Sheikh[1,5], David Thieker[2,5], Gordon Chalmers[3,5], Andrew Boland[1], Alexander Eletski[5], Robert J. Woods[1,5], John N. Glushka[5], James H. Prestegard[1,5], and Brad Bendiak[6]

*[1]Depts. of Biochemistry & Molecular Biology, [2]Chemistry, and [3]Computer Science, [4]Center for Tropical and Emerging Global Diseases, [5]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602 USA; [6]Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045 USA.*

Oxygen sensing is key for the success of all aerobic cells, and many organisms have evolved non-heme dioxygenases such as prolyl hydroxylases to measure dissolved O2. *Dictyostelium* is a unicellular social amoeba that lives in the soil where it feeds on bacteria. When starved, the amoebae aggregate into a multicellular slug that migrates to the soil surface to form a fruiting body, and they integrate multiple environmental cues (e.g., oxygen, ammonia, light) to guide these transitions. *Dictyostelium*, like animals, utilizes a nucleo-cytoplasmic prolyl hydroxylase (PhyA) to sense O2. In animals, hypoxia signals a new condition, which triggers new transcription via HIFα, whereas in amoebae it is high O2 that signals a new condition, which we propose controls protein turnover via the SCF class of E3-polyubiquitin ligases and the proteasome. The *Dictyostelium* mechanism involves the prolyl hydroxylation and subsequent glycosylation of Skp1, a subunit of SCF complexes, by six enzymatic steps that appear to be dedicated to Skp1. Disruption of phyA increases the threshold for O2 signaling by a mechanism that can be suppressed by proteasome inhibition. To understand how the glycan functions, we used NMR to investigate the effects of the glycan on Skp1 conformation, to determine the linkages of the sugars, and to characterize its relaxation dynamics. We then used molecular dynamics, guided by the constraints of the NMR data, to investigate the effects of the glycan on Skp1 conformation. These studies show an altered ensemble of Skp1 conformations that can explain the increased interactions with three F-box proteins – putative substrate receptors of the SCF complex – that were observed in proteomics based Skp1 interactome studies. Further analysis of two genomically tagged F-box proteins reveal that their steady state levels are decreased in a developmentally regulated fashion when Skp1 is glycosylated. One of these is JcdI, a predicted lysyl hydroxylase. These studies support the model that environmental signals influence *Dictyostelium* development through modulation of the turnover of proteins whose disposal enables progression to subsequent stages en route to terminal fruiting body development and sporulation. A similar mechanism is thought to operate in other protists including the agent for human toxoplasmosis, *Toxoplasma gondii*.

# Workshop

## GWDI-bank – Introducing the genome wide *Dictyostelium* insertion resource for functional genomics

**Amy Baldwin**[1], Nicole Gruenheit[2], Sarah Jaques[1], Balint Stewart[2], Hassan Ahmed[2], Thomas Keller[2], Petra Fey[3], Rex Chisholm[3], Christopher Thompson[2] and Adrian Harwood[1]

*[1]Cardiff School of Biosciences, Hadyn Ellis Building, Maindy Road, Cardiff University, Cardiff, UK, CF24 4HQ; [2]Faculty of Life Sciences, Michael Smith Building, Oxford Road, The University of Manchester, Manchester, UK, M13 9PT; [3]Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA, 60611*

In an age of –omic technologies it is essential to be able to quickly acquire and study targets. We have developed a pipeline for the large-scale generation of gene knockout mutants by combining restriction enzyme-mediated integration (REMI) mutagenesis with NGS technology, namely REMI-seq. Using this method; we have created a genome-wide collection of *Dictyostelium* mutants as a new resource for the international research community. The resource comprises both individual and large pools of mutants. The position of REMI mutations will be searchable via dictyBase, and the Dicty Stock Centre will distribute the resource. The resource, referred to as the Genome Wide *Dictyostelium* Insertion bank, or GWDI-bank for short, comprises approx. 23,000 individually banked mutants with known insertion sites. This includes ~14,000 different genomic loci, of which 69% are intragenic. Approx. 5,500 different genes have at least one insertion; there are multiple alleles available for the majority of these genes. A further ~1,000 genes have an insertion within 500 bp upstream of their start codon. Validation of the resource has established that the REMI-seq pipeline is robust; inverse PCR demonstrated that mutants contain an insertion at the expected loci and a screen for developmental phenotypes revealed the expected phenotypes when the insertion occurred in a previously characterised gene. 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 many *Dictyostelium* genes, the capacity to conduct complex phenotyping of protein families, and the ease at which whole genome phenotypic screens can be conducted.

# Workshop

## High content parallel phenotyping in *Dictyostelium*

**Nicole Gruenheit**, Amy Baldwin, Balint Stewart, Sarah Jaques, Rex Chisholm, Adrian Harwood, Christopher Thompson

*University of Manchester, UK*

Genetically tractable, simple eukaryotic cells like *Dictyostelium* are immensely valuable tools for discovery genetics and biomedical research. One key element is their potential for high content genetic screening.However, despite the plethora of existing bio-resources, including complete genome sequence and transcriptional profiles, full exploitation of these data is hampered by our inability to efficiently link genotype, transcriptome or proteome level information to phenotype. To date, it has only been possible to generate pools of mutants by REMI and to isolate mutants with a desired phenotype. However, this approach is hampered by the fact that (a) the complexity of the initial pool of mutants was unknown (b) the causative mutation in each mutant of interest had to be identified one by one (this laborious process necessitated that only a handful of mutants could be studied) (c) only positive selections could be carried out that enriched for mutants that increased in frequency (e.g. drug resistant), whilst hypersensitive mutants that decreased in frequency were lost. To remove these key bottlenecks, we have developed a novel technique (REMI-seq), which combines REMI mutagenesis with NGS technology to create a genome-wide set of 'barcoded' single gene mutants with defined insertion sites. After insertion at DpnII or NlaIII sites, 20 bp fragments can be extracted using a type III endonuclease, MmeI (which cuts 18/20 bp downstream of its recognition sequence) and an I-SceI meganuclease site (there are no other I-SceI sites in the Dictyostelium genome). Addition of Illumina sequencing adaptors after digestion allows these fragments to be efficiently isolated from contaminating gDNA. This novel REMI-seq methodology allows us to identify insertion sites from both single mutants and pools of mutants en masse.Most importantly, this allows researchers to screen for mutants that exhibit changes in fitness when challenged (e.g. in developmental signalling or drug sensitivity). This is because within a mixed population of multiple mutants, the number of reads of each unique sequence tag (or "barcode") provides a quantitative measure of the relative abundance of that mutant. When these populations are subjected to selection conditions, mutants that increase or decrease their frequency can be identified by changes in barcode read counts. We have carried out proof of principle experiments to determine the dynamic range of this method, which illustrates linearity over a 10,000x fold range. Furthermore, trial selections of a pool of 30,000 mutants grown in HL5 or on different bacterial species illustrates the biological value of the data. We will also highlight the utility of the resource to describe essential genes for other aspects of the life cycle such as differentiation and in biomedical screening for drug sensitivity. These data sets will not only provide proof of principle studies, but also highly useful data for the research community. Finally, because the pools of mutants will be made available to the research community, we will present a step-by-step analysis pipeline of the data and highlight best practice for use of this resource.

# A *Dictyostelium discoideum* model for Alzheimer's disease and other Tauopathies.

**Katelyn H. Mroczek**, Sarah J. Annesley and Paul R. Fisher

*Discipline of Microbiology, Department of Physiology, Anatomy and Microbiology, La Trobe University, Vic 3086, Australia*

Alzheimer's disease is the most common form of dementia and is becoming a more prevalent neurodegenerative disorder in an aging population. One of the hallmarks of the disease is the abnormal accumulation of the Tau protein into aggregates in neurons in affected regions of the brain. This characteristic feature is also found in other neurodegenerative diseases which are collectively known as tauopathies. In normal conditions, Tau binds dynamically to microtubules aiding in their assembly and stability, dependent on the phosphorylation state of the protein. In disease-affected neurons, hyperphosphorylation leads to the accumulation of the Tau protein into aggregates, mainly neurofibrillary tangles (NFT) which have been seen to colocalise with other protein aggregates in neurodegeneration. One such protein is α-synuclein, the main constituent of Lewy bodies (LB), the hallmark of Parkinson's disease. In many neurodegenerative diseases, the colocalisation of NFT and LB has been observed. We have expressed full length human Tau in *Dictyostelium discoideum* alone, and in combination with α-synuclein, to study the toxicity and interactions of these proteins. Previous results of *D. discoideum* strains expressing wild type α-synuclein showed reduced growth on bacterial lawns, reduced phagocytosis rates, and a slight phototaxis defect. The expression of Tau has no effect on growth rates, but causes a moderate phototaxis defect. When Tau is coexpressed with α-synuclein it rescues the α-synuclein-mediated growth defect and produces a more severe defect in phototaxis than either protein alone. This suggests an interaction between the two proteins. Western blot analysis indicates that Tau is able to be phosphorylated by *D. discoideum* kinases, which is the first step in the eventual accumulation of the Tau protein in the human brain. DuolinkTM immunofluorescence experiments suggest that Tau interacts both with tubulin on the microtubules of *D. discoideum*, and with the α-synuclein protein at the cortex of the cell. Together these results suggest that *D. discoideum* could be a viable simple model system for the study of tauopathies and interactions of human proteins involved in neurodegeneration.

# Gaining insights into the rare inherited disease HPS using *Dictyostelium*

**Peter Thomason** and Robert Insall

*CRUK Beatson Institute, UK*

Hermanksy-Pudlak Syndrome (HPS) is a rare inherited human disease caused by mutation in any of at least 9 HPS genes. Symptoms can be from mild to severe, and include defects to vision, hemostasis, and lung function. All of the HPS genes encode proteins involved in the trafficking of secreted proteins through the cell in specialised compartments termed lysosome-related organelles (LRO). Three separate multi-protein complexes are necessary for LRO biogenesis, termed BLOCs 1, 2 and 3, which act at different stages in the sorting/secretory pathway. We discovered the genes for BLOC2 in *Dictyostelium* by virtue of a screen for mutants having disrupted exocytosis. The functions of the BLOCs have been very elusive, but are now beginning to be solved. BLOC1 is a sorting complex that acts at sorting/recycling endosomes to direct cargoes to nascent LRO, and BLOC3 is an activator of Rab32/38 later in this trafficking pathway. No molecular function has yet been proposed for BLOC2, but we will present evidence for this function, as well as discuss the challenges that remain in working out the mechanism of its action.

# Identifying new roles for the γ-secretase complex in *Dictyostelium discoideum*

**Devdutt Sharma**[1], Philip Beesley[1], Jason King[2], Robin SB Williams[1]

[1]*School of Biological Sciences, Royal Holloway, University of London, TW20 0EX, UK;*
[2]*Department of Biomedical Sciences, University of Sheffield, Sheffield, S10 2TN, UK*

The γ-secretase complex consists of four proteins (presenilin 1/2, nicastrin, anterior pharynx defective 1 and presenilin enhancer 2) and has been widely investigated as a key player in early-onset Alzheimer's disease. The complex is required for development in mammalian models, where ablation of any component results in embryonic lethality, and has distinct proteolytic and non-proteolytic structural roles. The vast majority of research in this area has focused on the catalytic role of the complex, with few studies exploring its non-catalytic role. In *Dictyostelium*, recent studies have examined orthologues of each component of the complex to show that presenilin proteins are required for multicellular development, through a non-catalytic structural role. Interestingly the human presenilin protein, lacking catalytic activity, also rescues this developmental defect, suggesting a conserved structural function for this protein across evolution. Here we show that loss of the other components of the γ-secretase complex also blocks *Dictyostelium* development, suggesting that the complex (not just presenilin proteins) are required for this structural role. Since lysosomal trafficking has been demonstrated to be necessary for *Dictyostelium* development, and aberrant lysosomal trafficking has been implicated in Alzheimer's disease pathology, we continued to investigate this function. We show that cells lacking a complete γ-secretase complex exhibit defects in macropinocytosis, and share defects with cells exhibiting aberrant autophagy and protein ubiquitination. Furthermore we show that this change in lysosomal trafficking is also reliant on the structural role of the complex, since both the non-catalytic *Dictyostelium* presenilin B, and Human presenilin 1 protein are able to rescue these autophagic defects. Our research therefore shows that, in *Dictyostelium*, the γ-secretase complex plays a key role in lysosomal trafficking and autophagy through a non-catalytic structural role.

# Investigating the active functional groups of curcumin controlling biological activity

Marco Cocorocchio[1], Balint Stewart[2], Amy Baldwin[3], Adrian Harwood[4], Chris Thompson[3], Paul L. R. Andrews[2], **Robin S. B. Williams**[1]

*[1]Centre of Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham, TW20 0EX, UK; [2] Faculty of Life Sciences, Manchester University, Manchester, M13 9PL, UK; [3] Neuroscience & Mental Health Research Institute, Cardiff University, Cardiff, CF24 4HQ, UK; [4] Division of Biomedical Science St George's University of London, London, SW17 0RE, UK*

Natural compounds often have complex molecular structures, various cellular effects and multiple molecular targets. These characteristics make them difficult to analyse using a classical pharmacological approach. Curcumin, derived from turmeric, is a complex molecule possessing wide-ranging biological activities and cellular mechanisms, and has been investigated as a potential therapeutic treatment for Alzheimer's disease and cancer. Here, we developed an innovative approach to examine the physiological effects and molecular targets of curcumin using *Dictyostelium discoideum*. Curcumin reduced *D. discoideum* growth with an IC50 of 44μM, slowed multicellular development, and caused acute effects on cell behaviour with an IC50 of 2.3μM. Analysis of a range of structurally related compounds provided distinct potencies on growth, development and cell behaviour, highlighting the active functional groups of curcumin that confer specific effects. Molecular mechanisms underlying these distinct effects were then investigated by the analysis of mutants resistant to some of these compounds. Two mutants were analysed in detail: (1) the PsenB null mutant, lacking the presenilin 1 orthologue; and (2) and the PsrA null mutant, lacking the protein phosphatase 2A regulatory subunit. This study therefore provides an innovative approach to analyse complex natural products to identify the chemical basis biological activity and molecular mechanisms of natural products, with implication on the development of novel therapeutics for the treatment of disease.

# Intranuclear and cytoplasmic actin rod assembly in *Dictyostelium discoideum*

Hellen Ishikawa-Ankerhold and Annette **Müller-Taubenberger**

*LMU Munich, Germany*

Intranuclear and cytoplasmic rods are aggregates consisting of actin and cofilin that are formed in consequence of chemical or mechanical stress conditions. The formation of actin rods is associated with a variety of pathological conditions in humans, such as certain myopathies and some neurological disorders. It is still not well understood what exactly triggers the formation of rods, whether other proteins are involved, and what the underlying mechanisms of rod assembly or disassembly are. We have used *Dictyostelium discoideum* to study appearance, stages of assembly, composition, stability, and dismantling of intranuclear rods. Our data show that intranuclear rods, in addition to actin and cofilin, are composed of a distinct set of other proteins comprising actin-interacting protein 1 (Aip1), coronin (CorA), filactin (Fia), and the 34 kDa actin-bundling protein B (AbpB). A finely tuned spatio-temporal pattern of protein recruitment was found during formation of rods. Aip1 is important for the final state of rod compaction indicating that Aip1 plays a major role in shaping the intranuclear rods. In the absence of both Aip1 and CorA, rods are not formed in the nucleus, suggesting that a sufficient supply of monomeric actin is a prerequisite for rod formation. More recently, we have explored the conditions that lead to the induction of cytoplasmic actin rods that show a greater variability in appearance and shape.

Reference: Ishikawa-Ankerhold HC, Daszkiewicz W, Schleicher M, Müller-Taubenberger A. 2017. Actin-interacting protein 1 contributes to intranuclear rod assembly in *Dictyostelium discoideum*. Sci. Rep. 7: 40310.

# Natural products from amoeba-bacteria interactions

**P. Stallforth**, M. Klapper, R. Herbst, S. Götze, R. Barnett, J. Arp

*Hans Knöll Institute Jena, Germany*

Interacting microbial communities are a very rich source of structurally diverse natural products. Soil-dwelling bacteria, for instance, are constantly exposed to a multitude of threats: bacteriophages can infect and kill bacteria; bacterial competitors fight for the same resources; amoebae and nematodes are voracious predators. These evolutionary pressures have shaped intricate bacterial defense strategies. Secondary metabolites are selective and efficient mechanisms that can repel and kill both competitors and predators. Microbial predator-prey interactions thus offer an almost inexhaustible source of novel natural products with diverse functionality. Since protozoan grazing is the major source of bacterial mortality, and hence a significant selection pressure in soil eco-systems, we investigate bacterial secondary metabolites involved in defense against amoebal predation. Dictyostelids, in particular, are ubiquitous, highly bacterivorous, and commonly encountered soil inhabitants. We identified novel bacterial secondary metabolites that protect the producing bacteria against amoebal predation. We describe the isolation, structure elucidation, and synthesis of these structures, as well as proposed biosynthetic pathways.

# The basis of the asymmetric targeting of endosomes during asymmetric division

**Marcos González-Gaitán**

*University of Geneva, Switzerland*

# The role of allorecognition signaling in tissue integration

Shigenori Hirose, Gong Chen, Peter Kundert, Adam Kuspa and **Gad Shaulsky.**

*Baylor College of Medicine, Houston, TX 77030, USA.*

TgrB1 and TgrC1 are polymorphic trans-membrane proteins that mediate allorecognition and post-aggregative development in *D. discoideum*. We will present several lines of evidence TgrC1 acts as a ligand and TgrB1 acts as a receptor during the interaction between adjacent compatible cells in the process of post-aggregative tissue formation. We also found that signal transduction downstream of TgrB1 involves several small GTPases and their regulators. Using chimerae of compatible and incompatible cells at different proportions we are beginning to evaluate how individual cells begin to cooperate as they become incorporated into a tissue. The most intriguing observations suggest that soluble signals like cAMP, which are critical during aggregation when cells behave as individuals, are replaced with short-range contact-mediated signaling as the cells form functional tissues within the loose aggregate.

# Control of pattern formation in *Dictyostelium discoideum*

T. Eckstein, E. Vidal, A. Bae, V. Zykov, E. Bodenschatz, **A. Gholami**

*MPI for Dynamics and Self-Organization, Germany*

A classic example of self-generated patterns in nature is found in the social amobae *Dictyostelium discoideum*. When starved, millions of individual cells signal each other with the signaling molecule cyclic adenosine monophosphate (cAMP). cAMP waves in the form of spiral or target patterns propagate in cell populations and direct aggregation of individual cells to form centimeter-scale Voronoi domains and eventually multicellular fruiting bodies. In this study, we control the shape of Voronoi domains by introducing periodic geometrical obstacles with different size and periodicity in the system. We observe that the obstacles act as aggregation centers and the periodic arrangement of the obstacles is reflected directly in the corresponding Voronoi domains.

# Cell and molecular transitions of dedifferentiation

**John Nichols**, Jonathan Chubb

*MRC Laboratory for Molecular Cell Biology, University College London, Gower St, London, WC1E 6BT, United Kingdom*

During dedifferentiation, partially or terminally differentiated cells revert to a previous developmental state with increased developmental potential. This phenomenon plays an important role in tissue repair and wound healing, and can be artificially forced to produce induced pluripotent stem cells. However, the mechanisms underlying dedifferentiation are not understood in any experimental system. When disaggregated and given growth media, differentiating *Dictyostelium* cells readily dedifferentiate. To determine the essential features of the dedifferentiation transition, we have used several high temporal density transcriptomics time series to characterise the gene expression changes occurring during dedifferentiation. Our data reveal the major gene expression phases of the process, illuminate the relationships between dedifferentiation and forward development, and suggest the triggers of the transcriptional responses underlying dedifferentiation. The interdependence of the various gene expression responses is being investigated using live imaging of single cell gene expression.

# Learning from mistakes

**Joan E Strassmann**, David C. Queller, Debbie Brock, Suegene Noh, Tamara Haselkorn, Susanne DiSalvo

*Washington University in St. Louis, USA*

In the nearly 20 years since we began to work on social amoebae we have learned a great deal from our mistakes. In this talk I tell the story of some of those mistakes and the fascinating discoveries they led to. Here are the six things we thought were true but were not. 1. Different clones of *Dictyostelium* will not mix in the social stage. 2. Fruiting bodies are sterile; they contain no bacteria. 3. Carried bacteria are all food for *Dictyostelium*. 4. Farming is a genetic trait. 5. *Dictyostelium* cannot be cured of farming with antibiotics. 6. Positive spot tests mean all spores carry *Burkholderia*.

# Functional genomics of a protosteloid amoeba suggests alternative routes to fruiting body formation

**Falk Hillmann**, Silvia Novohradská, Gilian Forbes, Iuliia Viediernikova, Thomas Spaller, Thomas Winckler, Pauline Schaap, Gernot Glöckner

*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Germany*

Establishment of multicellularity represents a major transition in eukaryote evolution. Dictyostelids have evolved a relatively simple multicellular stage resulting in a fruiting body supported by a stalk. In contrast *Protostelia* produce only one single stalked spore. Thus, the major difference in the developmental cycle of *Protostelia* and social amoebae seems to be the establishment of multicellularity. To separate spore development from multicellular interactions we analyzed the genome and transcriptome of *Protostelium fungivorum*, a solitary amoeba isolated at a forest site near Jena and revealed high rates of fruiting body formation under laboratory conditions. Its live-style is exclusively mycophagous, but the food spectrum is broad within the fungal clade including several ascomycetous and basidiomycetous yeasts. Trophozoites and fruiting bodies were morphologically similar to the type strain of *Protostelium mycophagum* whose genome was also sequenced. The genomes of the two protosteloid species were slightly larger than for Dicytostelids and during fruiting body formation nearly 4000 genes were upregulated, corresponding to specific pathways required for the differentiation process. Highly enriched functional categories included genes of the cell cycle, DNA replication and cytoskeleton reorganization. An overall comparison with genes involved in the development of Dictyostelids revealed a conservation of more than 500 genes, but these are also mainly present in the non-fruiting body forming species *Acanthamoeba castellanii*. Moreover, expression regulation of those genes differs between *P. fungivorum* and its multicellular relative *D. discoideum*. Although within Amoebozoa differentiation to sporocarps (fruiting bodies) is common, our current genome analysis suggests that each group could have exploited different routes to achieve this. Most remarkable is the large repertoire and diversity between species in genes that mediate environmental sensing and signal processing. This likely reflects an immense adaptability of the single cell stage to varying environmental conditions. We surmise that this signaling repertoire could have provided sufficient building blocks to accommodate the demands for cell-cell communication in the early multicellular forms.

# A simulation model for dynamic thermotaxis of *Dictyostelium* slugs

**Wolfgang Heiden** and Jana Esser

*Bonn-Rhein-Sieg University of Applied Sciences, Germany*

Introduction: *Dictyostelium* slugs are known to show positive or negative thermotaxis, depending on the temperature gradient orientation related to the temperature during slug formation, and can even adapt to a dynamically changing gradient. For better understanding the parameters that trigger this behavior, we have developed a simulation model for slug thermotaxis by modifying a model by Pilat, which itself was based on an earlier model by Marée et al..

Methods: While the original model covers only positive thermotaxis with a slug following a single pacemaker cell, we introduced a dynamically changing temperature gradient and a realistic numerical relation of pacemaker to non-pacemaker cells. In our model (following the concept introduced by Marée and continued by Pilat), temperature exerts its influence on slug migration via the delay between two pulses of cAMP emission as related to the temperature at (and temperature gradient around) the cell position. (This delay can be understood as a simplified consequence of Ammonia production proportional to the environment temperature). Interaction of slug cells is modeled as inelastic impulses from non-pacemaker to pacemaker cells, together with a spin component. Collision among non-pacemaker cells, however, had to be modeled as elastic impulses.

Results: It is shown that a slug in the modified model always moves in the direction of change to a temperature gradient, even in case of an inverting gradient during the migration phase. The model is also (more or less) independent of the start temperature, although the time before a continuous migration differs depending on the initial situation, i.e. whether starting with positive or negative thermotaxis.

Conclusions: This model seems to be sufficient to direct *Dictyostelium* slugs along a changing temperature gradient towards the origin of gradient changes (which is usually the boundary surface between soil and air), even if the pacemaker cells are located rectangular or opposite to this direction directly after slug formation. In case of the latter, pacemaker cells are pushed through the slug until they end up at the opposing pole, then leading slug migration along the temperature gradient.

# The transcription factor sporulation deficient A is a master regulator of *Dictyostelium* sporulation

**Yoko Yamada** and Pauline Schaap

*School of Life Sciences, University of Dundee, UK*

Sporulation in *Dictyostelium* fruiting bodies is derived from amoebozoan encystation with both being induced by cAMP acting on PKA. Using tagged mutagenesis to find other pathway components, we identified a nuclear protein, spdA, without which no viable spores are formed. Expression of prespore genes, encoding spore coat proteins, is strongly reduced in spda- cells, while expression of many spore genes is absent. Chromatin immunoprecipitation (ChIP) of a spdA-YFP gene fusion showed that these genes directly bind to spdA, which identifies spdA as a transcriptional regulator. Sequencing of spdA-YFP ChIPed DNA fragments revealed that spdA binds 117-166 (pre)spore promoters, including those of other transcription factors that activate spore gene expression. These factors do not activate spdA expression and their null mutants have less severe sporulation phenotypes than spdA, identifiying spdA as the major sporulation inducer.

# Non-coding transcription, gene regulation and replication in the yeast *S. cerevisiae*

**Françoise Stutz**

*University of Geneva, Switzerland*

# Abundant non-coding RNA involved in early *Dictyostelium discoideum* development is conserved in Dictyostelid social amoebae.

Lotta Avesson, Johan Reimegård, Jonas Kjellin, Zhen Liao, Gernot Glöckner, Pauline Schaap, and **Fredrik Söderbom**

*Uppsala University, Sweden*

In recent years, the discovery of numerous non-coding (nc)RNAs has dramatically changed the view of how organisms control their biological processes. Small ncRNAs such as micro (mi)RNAs and small interfering (si)RNAs have been explored mainly in multicellular organisms, i.e. plants and animals, where they are crucial to ensure proper gene expression. Other classes of ncRNAs have also been demonstrated to play important roles in different biological processes. We previously identified an abundant and highly expressed class of ncRNA (Class I RNAs), 42 – 65 nt long, involved in multicellular development in the social amoeba *Dictyostelium discoideum*. In order to understand if Class I RNAs are unique to *D. discoideum* or also present in other organisms, we searched for Class I RNA genes in genomes from species representing each major group of dictyostelid social amoeba. Numerous Class I RNA genes were identified in all of these species, which separated from their last common ancestor some 600 million years ago. In contrast, genomes from strictly unicellular Amoebozoa showed no evidence of this class of RNA. Analysis of Class I RNAs from the different social amoeba species revealed several conserved features. They harbor a short stem-structure, connecting the 5´ and 3´ ends, and a conserved sequence element. In addition, the genes are preceded by a putative promoter sequence. Based on apparent lack of synteny and analysis of primary sequence, it appears as if the expansion of Class I RNA genes took place largely after the divergence of Dictyostelia. Hence, our results show that Class I RNA is an ancient abundant class of ncRNA, likely specific to dictyostelid social amoeba, and could play a role in their evolution and unique multicellular life style.

# The Elongator complex of *Dictyostelium discoideum*

Manfred A. Schäck and **Christian Hammann***

*Ribogenetics and Biochemistry Laboratory, Department of Life Sciences and Chemistry, Jacobs University Bremen gGmbH, Campus Ring 1, 28759 Bremen, Germany*

Transfer RNAs (tRNA) are among the most posttranscriptionally modified RNA molecules in nature. The RNA modifications contribute to the stability of the secondary and tertiary structure of the tRNA. Anticodon modifications at wobble position U34 are particularly important, since they help to modulate the codon - anticodon interaction and wobble recognition during translation. The eukaryotic Elongator complex catalyzes the first step in the Elongator dependent U34 modification pathway. The complex consists of 6 proteins (Elp1-Elp6) that can be divided into two subcomplexes (Elp123 and Elp456). Both, Elp123 and Elp456 form heterodimers, resulting in an overall stoichiometry of the holocomplex with two proteins of every subunit. Elongator dysfunction has severe effects on different cellular processes and Elongator mutants in yeast and *A. thaliana* display specific stress phenotypes. Elongator dysfunction is lethal in most higher metazoa. In humans, brain specific splicing defects in Elongator mRNAs are associated with the formation of neuropathies including amyotrophic lateral sclerosis, familial dysautonomia and rolandic epilepsy. Here, we present the first investigation on Elongator dependent anticodon U34 modification in *Dictyostelium discoideum*. Pulldown studies with ectopically overexpressed Elongator subunits reveal that also the *D. discoideum* Elongator complex consists of 6 proteins. The two subcomplexes Elp123 and Elp456 are mainly found independently. Native PAGE and gel filtration experiments on the Elp456 subcomplex and recombinant Elp456 suggest, however, that the subcomplex might be larger in the amoeba than observed in other organisms. Using the viral 2A peptide skipping sequence, we show that Elp3, the enzymatic protein of the Elongator, is instable. This peptide sequence allows to discriminate between translational efficiency and protein stability of different proteins. We postulate that Elp3 is stabilized by formation of the complex. Normally, glutamine codon usage in *Dictyostelium* is biased with 96% CAA to 4% CAG. CAA is read by the Elongator-dependently modified tQ(UUG), and CAG by tQ(CUG), which is not modified by the Elongator complex. Notably, the genome of Dictyostelium features a corresponding tRNA gene ratio of 13:1 for tQ(UUG):tQ(CUG).

We report the first unconditional t(QCUG) null mutant in an eukaryotic model organism. In the corresponding strain, and a series of mutant strains, in which the Elongator pathway in *D. discoideum* is disrupted at all major steps, we observe increased doubling times, a phenotype that is even more pronounced under stress conditions. To address the cellular function of Elongator dependent tRNA modifications, we investigated the decoding capability of glutamine tRNAs on artificial gene constructs in these strains. Specifically, Poly-glutamine leaders with different CAA/CAG codon ratios fused to GFP were generated and expression differences measured via flow cytometry and Western blotting. In elp3 deletion strains, translation of reporter genes with CAA codons was significantly reduced, while overexpression of unmodified tQ(UUG) partially rescued the expression level of the reporter genes. Our data indicate further that tQ(UUG) can wobble over the CAG codon in *D. discoideum*.

# Nucleosome positioning and gene regulation

Mark Robinson, James Platt, Nick Kent, Alan Kimmel* & **Adrian J Harwood**

**NIDDK, National Institutes of Health, Bethesda, MD, USA, Cardiff University, UK*

Nucleosome positioning and occupancy are important determinants of the chromatin landscape and actively regulate gene transcription. Dynamic remodelling of this landscape is essential for cellular differentiation, replication and DNA repair. Within the SNF2 ATPase superfamily of chromatin remodelers, the CHD and ISWI proteins 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 disruption of transcription when chd1 and isw1/2 are ablated. However, the situation in other organisms is more complicated with the presence of multiple CHD proteins, including two subfamilies not present in yeasts. Aberrant function of these CHD proteins is associated with human diseases, including neuroblastoma, epileptic encephalopathy, learning disabilities and Autism Spectrum Disorders. To examine this more complex situation, we have investigated nucleosome patterning and gene transcription in *Dictyostelium*. *Dictyostelium* possesses three CHD genes and a single ISW gene. ChdA is homologous to CHD1, whereas ChdB and ChdC belonging to the type III subfamily found in animals. Each of Chd gene is expressed in growing cells, but show different patterns of expression during development and cell differentiation. Knockout mutants generated for each gene show distinct transcriptional profiles, indicating that they control different gene subsets. Nucleosome mapping of ChdC mutants revealed a very specific deficit, causing increased nucleosome spacing, but only affecting a small subset of genes. In contrast, ChdA, ChdB and Isw mutants had little effect on nucleosome spacing, but distinct and differing affects on nucleosome position, occupancy and DNA contact size. Not only did each remodeler have different functional effects, they targeted different gene sets, consistent with their different effects on transcription. This indicates individual remodelers act independently, in a non-redundant manner, targeting different chromatin regions. Although each remodeler can alter transcription of specific genes, the majority of their target genes show altered nucleosome pattern without an associated change in gene expression. This suggests a complex, but developmentally regulated, pattern of chromatin regulation, where each chromatin remodeler has distinct chromatin targets, activities and specific effects on gene expression.

# Causes and consequences of unpredictable gene expression

Ed Tunnacliffe, Agnes Miermont, Vlatka Antolovic and **Jonathan R. Chubb**

*Universyty College London, UK*

Transcriptional output is inherently noisy. Whilst some systems have developed features to reduce the impact of this noise on biological regulation, in many systems, noise may be actively harnessed to make cells different from each other. However, the origins of noisy transcription are largely unclear, and the degree to which the noise in a specific gene can accurately predict a cell decision has been difficult to accurately estimate. We have used single cell transcriptomics, live cell imaging of transcription and molecular genetics to investigate the origins of noisy transcription and its impact upon cell regulation. Our data suggest that noisy transcriptional signatures can be completely specified by a gene's promoter, and are independent of its genomic context. We also show that the expression level of a single noisy gene at the onset of development can provide a partially accurate prediction of the final fate of the cell.

# A buffered noisy cell fate oscillator in *Dictyostelium*

Nicole Gruenheit[1], Edward J. Johnson[1], Katie Parkinson[1], Koki Nagayama[1], Jack Llewellyn[1,2], Balint Stewart[1], Thomas Keller[1], Wouter van Zon[1], Simon L. Cotter[2] and **Christopher R.L. Thompson[1]***

*[1]Division of Developmental Biology and Medicine, Faculty of Biology, Medicine and Health, The University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK; [2]School of Mathematics, Faculty of Science and Engineering, The University of Manchester, Alan Turing Building, Manchester, M13 9PL, UK*

During embryonic development, cell differentiation must be tightly controlled in space and time to ensure robust and reproducible tissue patterning and proportioning. One idea is that differentiation is aided by cell heterogeneity because it increases the spectrum of differentiation capabilities of cells in a uniform environment. Most notably, this leads to random 'salt-and-pepper' differentiation where reproducible proportions of different cell types still arise. Examples range from competence in *B. subtilis,* to lineage specification in the mouse blastocyst. In these cases, cells are thought to be transiently and dynamically 'primed' to adopt a particular lineage, with priming thought to affect the likelihood that a cell will respond to signals that trigger differentiation. Despite this emerging framework, few lineage priming genes have been identified, and two questions remain. (1) How are lineage priming dynamics determined to ensure the correct the number of lineage-primed cells are present in a population? (2) Do differentiating cells all follow the same path towards the differentiated state? Understanding these dynamics will be crucial as they lie at the underlie developmental patterning by this mechanism. We will report on how our use of single cell RNA-seq, live cell imaging, cell fate mapping and mathematical modeling in *Dictyostelium* has revealed a novel system in which a buffered noisy cell fate oscillator based on the cell cycle can be used to achieve robust cell type proportioning in vivo.

# Nucleocytoplasmic shuttling of a GATA transcription factor functions as a development timer

**Cai H**, Katoh-Kurasawa M, Muramoto T, Santhanam B, Long Y, Li L, Ueda M, Iglesias PA, Shaulsky G, Devreotes PN.

*Institute of Biophysics, Chinese Academy of Sciences, China*

Self-organized propagating waves of cAMP observed in *Dictyostelium* represent one of the first known examples of biological oscillations. Periodic cAMP signals serve as chemoattractants and, by an unknown mechanism, time the acquisition of chemotactic competence during multicellular development. We discovered that a GATA family transcription factor, GtaC, decodes the information in cAMP signaling. GtaC exhibits rapid and synchronized nucleocytoplasmic shuttling in response to cAMP waves. This behavior requires coordinated action of an intrinsic nuclear localization signal and receptor-mediated phosphorylation. Disrupting GtaC shuttling by adding an exogenous NLS or mutating the residues involved in phospho-cycling leads to precocious development. While cAMP is required for chemotactic gene expression, it also drives GtaC out of the nucleus. As a result, each cAMP oscillation generates a transient burst of transcription, and the decline of cAMP allows GtaC to return to the nucleus and resensitizes the system. We demonstrate that this design enables cells to modulate developmental gene expression by counting cAMP waves.

# The role of the histone methyl-transferase COMPASS complex in controlling variable gene expression and cell type proportioning

**W. Salvidge**, N. Gruenheit, C. Thompson

*University of Manchester, UK*

During multicellular development, cells must make fate decisions that reproducibly generate the correct cell type proportions. It is remarkable that in certain developmental scenarios, seemingly identical cells in a homogenous environment can achieve this. It is thought that this is possible because cell populations exhibit reproducible cell-cell variation in gene expression. How these differences are generated has been intensely studied over the past decade, with transcriptional bursting emerging as an important factor for driving variability between cells. Furthermore, it is thought that chromatin structure around gene promoters is a key regulator of transcriptional bursting. However, key questions remain. What factors regulate chromatin structure at the molecular level? Is the activity of chromatin regulators governed by random processes or entrained by external or hidden factors such as cell cycle positioning, cell volume, metabolism etc? Are the proportions of cells exhibiting different bursting patterns regulated to ensure normal cell fate choice and proportioning? To address these questions, we investigated whether different regulators of chromatin structure affect the pre-stalk/pre-spore fate decision in *D. discoideum*. Members of the COMPASS complex, which are responsible for generating methylation on histone 3 at position lysine 4 (H3K4me), were found to play a key role in controlling the balance of cell types in multicellular development. Single cell RNA-sequencing revealed genes normally regulated by this modification to represent a specific class of hyper variable genes. These genes are transcriptionally repressed in most cells. However some cells have a higher tendency to express these transcripts at low levels. Following differentiation, this repression is removed and high levels of transcription are observed in specific cell types. Our data suggest that COMPASS activity is controlled by extrinsic factors, so only a subpopulation of cells express these transcripts and are primed to adopt the stalk cell fate. Mutations that decrease H3K4me levels result in premature homogeneous expression of these genes, and a severe cell type imbalance. Our data thus reveal a novel mechanism linking specific regulation of transcriptional bursting to cell fate propensity.

# How to build a phagosome: macrophages solutions to remodel membranes and cytoskeleton

**Florence Niedergang**

*Institut Cochin, Paris, France*

# Coordination of Rho and Ras small GTPases in macropinocytic and phagocytic cup formation

**Catherine Buckley** and Jason King

*University of Sheffield, UK*

How cells regulate formation of macropinocytic and phagocytic cups is not well understood yet is critical during the immune response. Dendritic cells rely on macropinocytosis to sample their external environment for threats, whereas professional phagocytes, such as macrophages, capture invading pathogens by phagocytosis to prevent infection. *Dictyostelium* hunt and feed on bacteria in the wild and undergo constitutive macropinocytosis, making them an ideal model to study cup formation. Formation of macropinosomes and phagosomes requires the generation of complex cup shaped protrusions. Actin polymerisation is critical to generate the force required to extend the membrane and form the rims of the cup. This polymerisation is tightly regulated by members of the Rho and Ras family of small GTPases. Despite its importance, how Rho and Ras activity is coordinated to control local membrane extension and form these complex cup shapes is unknown. We have identified a novel protein, RGBarG, which contains an RCC1 domain, small GTPase regulatory domains (RhoGEF and RasGAP) and a BAR domain. We show that RGBarG localises to macropinocytic and phagocytic cups and is essential for normal formation of both structures. Loss of RGBarG affects the ability of cells to phagocytose particles and bacteria of different sizes and geometries and cells are unable to form stable macropinosomes. This demonstrates the importance of coordinated small GTPase activity in cup formation.

# How are macropinsomes formed?

**Peggy I Paschke**, René Martin, Hans-Joachim Knoelker, Robert R Kay

*MRC-LMB Cambridge, UK*

Macropinocytosis is an efficient way for cells to take up large volumes of medium into intracellular vesicles, from which they can extract nutrients and other useful molecules. It's important for a wide spectrum of human biology, including antigen sampling by immune cells, uptake of drugs and has been hijacked by pathogens as a major route of entry. Recent data suggest that macropinocytosis is a widely used method for feeding by cancer cells and may be implicated in the spread of neurodegenerative disease within the brain.

Considering it's importance, macropinocytosis is still poorly understood. Driven by the actin cytoskeleton it can be studied advantageously in axenic *Dictyostelium* cells, which use it for feeding. It has been shown recently, that the first step for the formation of a macropinsome is the establishment of an active Ras patch. Following this step PI3-kinases are binding to the activated Ras and produce the signal molecule PIP3. The resulting PIP3 patch is a crucial platform for the recruitment of PIP3 binding proteins like for example protein kinases or actin binding or regulating proteins. Many of those have been shown to be necessary for the formation of a macropinosome. But surprisingly still just little is known how those signalling patches are formed at the plasma membrane. Using high through put flowcytometry, microscopy and molecular genetics we try figure out which cellular components are important for the patch formation. By now we have the first hints that point out an important role of different actin interacting proteins for this process.

Currently we are investigating which proteins are of particular importance for patch formation. Our major focus is to understand how the different components of the actin cytoskeleton help to establish patch formation and this way enable a cell to form macropinosome.

# Role of DrkD in cell spreading and phagocytosis in *Dictyostelium*

**J. Leiba**, A. Sabra, R. Bodinier, A. Marchetti and P. Cosson

*University of Geneva, Switzerland*

*Dictyostelium discoideum* is a widely used model to study molecular mechanisms controlling cell adhesion, cell spreading, and phagocytosis. In this study, we isolated and characterized a DrkD KO *Dictyostelium* mutant. The predicted DrkD protein contains leucine rich repeats and a serine/threonine kinase domain. DrkD KO cells have an increased motility and spread more efficiently than WT cells on a glass surface. They also show increased phagocytosis. These results demonstrate the involvement of DrkD in the control of cell spreading and phagocytosis. The underlying molecular mechanisms, as well as the exact link between DrkD and phagocytosis, are currently under investigation.

# Quantifying live bacterial sensing for novel regulators of chemotaxis

Netra Pal Meena and **Alan R, Kimmel**

*National Institute of Health*, Bethesda, MD USA

Aspects of innate immunity, including chemotaxis, derive from characteristics inherent to ancient phagocytes. Although ligand chemotaxis has been biochemically investigated using mammalian and model systems that share many mechanistic pathways for chemotaxis, precision of chemotaxis towards ligands being actively secreted by live bacteria is not well studied. We have quantified *Dictyostelium* chemotaxis towards live gram positive and gram negative bacteria. We demonstrate high sensitivity to multiple bacterially-secreted chemoattractants. Additive/competitive assays indicate that intracellular signaling-networks for multiple ligands utilise independent upstream adaptive mechanisms, but common downstream targets, thus amplifying detection at low signal propagation, but strengthening discrimination of multiple inputs. These analyses allow us to dissect potential for new chemoattractants and effects of additional factors.

# Chemoattractant GPCRs mediate both chemotaxis and phagocytosis for catching and ingesting bacteria

Miao Pan, Xi Wen and Xuehua Xu, **Tian Jin**

*NIAID, NIH, USA*

The current dogma is that human phagocytes, such as neutrophils, use chemoattractant GPCRs to mediate chemotaxis for catching bacteria, and then switch to phagocytic receptors, such as those for complements or IgGs that opsonize bacteria, for phagocytosis. However, several lines of evidence indicate that this model is incomplete. 1. Upon a bacterial infection, neutrophils arrive the infection site in several minutes to immediately fight bacteria without the full engagement of complements and IgGs. 2. Phagocytes in lower organisms, such as *D. discoideum* and *Drosophila*, do not have the capacity to generate complements or IgGs, but they can engulf bacteria efficiently. Therefore, phagocytes must have another mechanism to internalize bacteria. Seventy years ago, it was reported that neutrophils can effectively ingest surface-associated bacteria in the absence of opsonins during the recovery in *Pneumicoccal Pneuminia*. Recent studies also showed that neutrophils are able to efficiently engulf unopsonised microbes when they are placed on a surface. The molecular mechanism underlying the "surface-dependent and opsonnin-independent phagocytosis" is not known. Our recent studies have discovered that chemoattractant GPCR-mediated signaling network controls not only chemotaxis to chase bacteria but also phagocytosis to engulf unopsonzied bacteria in both *D. discoideum* (Pan et al., Dev Cell, 2016) and neutrophils. The social amoeba *D. discoideum* is a professional phagocyte that catches bacteria via chemotaxis and engulf them as food via phagocytosis. Although folic acid released by bacteria was shown to be the chemoattractant for *D. discoideum* to seek bacteria more than 40 years ago, a folic acid receptor had not been identified. We recently developed a quantitative phosphoproteomic technique to discover signaling components. Using this approach, we identified the long sought after folic acid receptor, fAR1. We found that fAR1 GPCR mediates signaling events to control not only chemotaxis to folic acid but also engulfment of folic acid-coated particles. We propose that the chemoattractant GPCR-mediated-migration as well as engulfment is an evolutionarily conserved mechanism, and human neutrophils may also use this mechanism to chase and engulf bacteria to fight infections. Once bacterial infection occurs in a human body, neutrophils leave the bloodstream, migrate to infection sites, catch bacterial pathogens, and then ingest them. Neutrophils catch bacterial pathogens by detecting formulate-peptides generated by bacteria and migrating toward them via chemotaxis. Then, they bind and engulf bacteria by recognizing specific signals on bacterial surface via phagocytosis. Recently, we discovered that formyl-peptide receptors (fPR GPCR) coupled with heterotrimeric Gi proteins mediate chemotaxis as well as phagocytosis of fMLP-coated particles and *E. coli* (Wen and Jin, in preparation). Our studies revealed an evolutionarily conserved mechanism that directs professional phagocytes migrating toward bacteria via chemotaxis and promotes them to engulf bacterial via "surface phagocytosis" as an essential part of innate immunity.

# Two-pore channel protein TPC2 in early *Dictyostelium* development

Fusheng Chang, Julian Gross, Antony Galione, **Catherine Pears**

*University of Oxford, UK*

Calcium (Ca2+) is a ubiquitous signaling molecule in eukaryotic cells. Cytosolic levels are kept low by pumping Ca2+ out of the cell or by sequestering in both neutral and acidic intracellular stores. Increases in cytosolic Ca2+ are triggered by its release through gated channels on these stores or on the plasma membrane and have been implicated in regulating numerous cellular responses including development. The release of Ca2+ from neutral stores is relatively well characterized compared to that from acidic stores. Two-pore channel proteins (TPCs) are one of a number of Ca2+ channels found on acidic stores. The *Dictyostelium* genome has a single gene predicted to encode a TPC2 orthologue and disruption of this gene causes a delay in early development. Expression of a genetically encoded Ca2+ sensor, YC- Nano, can be used to monitor transient increases in cytosolic Ca2+ in living cells and reveals that a reduced and delayed cAMP-induced increase in cytosolic Ca2+ is apparent in *tpc2-* cells, compared to parental Ax2 cells. The pH of acidic vesicles in *tpc2-* cells is decreased and, consistent with this, tpc2- cells are more sensitive than parental Ax2 cells to inhibition of aggregation by weak bases. Analysis of gene expression in *tpc2-* cells, reveals altered expression of early developmental genes and a reduction in cytosolic Ca2+ levels already apparent in vegetatively growing cells. These results suggest that loss of TPC2 causes alterations in both Ca2+ and H+ ion balance in Dictyostelium and impacts on early development, revealing a previously unknown role for acidic compartments in this process.

# Centrosomes and nuclear pore complexes in semi-closed mitosis

**Irene Meyer**, Petros Batsios, Tatjana Peter, Kristina Mitic, and Ralph Gräf

*University of Potsdam, Germany*

Centrosomes root back to the last eukaryotic common ancestor (LECA). Organisms possessing cilia have centrosomes with centrioles, which are also capable of functioning as basal bodies in cilia formation. Organisms lacking cilia for locomotion such as amoebozoans contain centrosomes without centrioles. We investigate the centrosome in *Dictyostelium* to study those centrosomal functions that are independent of centrioles. The spheroid *Dictyostelium* centrosome consists of a three-layered core structure, embedded in a matrix containing microtubule-nucleation complexes, the corona. We have characterized most if not all structural centrosomal components on a molecular level and have allocated most individual components successfully either to the core or the corona structure. To elucidate their mutual protein interactions we employed proximity-dependent biotin identification (BioID) to map protein-protein interactions on the biochemical level. In the course of these experiments we also found nuclear pore complex (NPC) as subunits as integral components of the centrosome. The functional link between centrosome and NPC proteins could lie in a shared role in the dynamic re-organization of the nuclear envelope (NE) during mitosis, where tubulin dimers, spindle-assembly factors and other proteins need to get access to chromosomes. In different eukaryotes this is achieved by nuclear envelope breakdown (open mitosis), by specific transport through the intact NE (closed mitosis), or by permeabilization of the NE through partial disassembly of NPCs (semi-closed mitosis). There appears no common scheme within the individual eukaryotic supergroups and, thus, the situation in the LECA is unknown. Among all model organisms *Dictyostelium* is unique, as it possesses a nuclear lamina based on an ancestral lamin, and a semi-closed mitosis with an intact NE. Three scenarios how tubulin dimers and other spindle assembly factors enter the nuclear matrix are conceivable: Either the mitotic NE becomes permeabilized through modification of nuclear pore complexes (NPCs), or through the fenestrae that are formed during insertion of the duplicating centrosome into the NE, or by a combination of both mechanisms. An involvement of the centrosomal fenestrae would be an unprecedented novel mechanism. Its existence is supported by observations in a strain depleted of the centrosomal central layer protein CP75, which appeared to be defective in NE permeabilization and subsequent spindle formation. CP75 dissociates from mitotic centrosomes after NE permeabilization, and it binds to the NPC component Nup53, which in turn dissociates from NPCs during mitosis. This argues that duplication of the centriole-free *Dictyostelium* centrosome is tightly coupled to NE modification including partial disassembly of NPCs. Indeed, Nup53 is known to be involved in membrane shaping and it could therefore be involved in centrosome insertion. Moreover, we could show that central FG-repeat Nups dissociate from mitotic NPCs while transmembrane and inner ring Nups remain. We hypothesize that NE permeabilization occurs in two steps: (1) entry of the mitotic centrosomal core structure into the NE along with its permeabilization and (2) further permeabilization by partial disassembly of NPCs. The understanding how the NE is permeabilized during mitosis in Dictyostelium to allow spindle assembly will bring us closer to an understanding of the situation in the LECA

# Extracellular polyphosphate regulates proliferation and development

**Patrick M. Suess** and Richard H. Gomer

*Texas A&M University, USA*

Linear chains of five to hundreds of phosphates called polyphosphate are found in organisms ranging from bacteria to humans, but their function is poorly understood. In *Dictyostelium*, polyphosphate is used as an extracellular signal that inhibits cytokinesis of vegetative cells in an autocrine negative feedback loop. To elucidate how cells respond to this unusual signal, we examined the effect of polyphosphate on mutants lacking possible signal transduction components. In diluted growth media, cells lacking the putative G-protein coupled receptor GrlD or RasC showed no polyphosphate-induced proliferation inhibition. In undiluted media, these same mutants exhibited polyphosphate-induced proliferation inhibition, suggesting that in the presence of high nutrients, polyphosphate uses a different mechanism to inhibit proliferation. To further elucidate how cells respond to polyphosphate, we did proteomic analysis of cells treated with physiological levels of polyphosphate. Polyphosphate caused proteasome protein levels to decrease. In high and low nutrients, polyphosphate decreased proteasome activity, induced phosphorylation of Akt and Erk1, and induced expression of the early onset developmental molecule CsA. All of these effects required GrlD and RasC. In low but not high nutrients, polyphosphate also induced aggregation, and this effect required GrlD, RasC, Akt, and Erk1. In human leukemia cells, polyphosphate also decreased proteasome activity and cell proliferation, suggesting the existence of a conserved polyphosphate response mechanism.

# Flash talks

The numbering of the poster boards corresponds to the page number of the respective abstract

# Choice of cell motility structures is regulated by TalA, a cortex-to-membrane crosslinking protein.

**Zully Santiago** and Derrick Brazill

*Department of Biological Sciences, The Graduate Center, Hunter College, The City University of New York, New York, USA*

Cells effectively modify their motility in response to environmental conditions. A cell moving freely experiences much less hydrostatic pressure from compaction than one moving through other cells, tissue, or debris. Yet much of what is understood about cell motility has been determined from studies using cells on slides, an environment free from such stressors. Under these conditions, motility is dominated by actin-based structures: pseudopodia, filopodia, and lamellipodia. However, under conditions mimicking the compression of cells in biological environments, motility is dominated by blebbing, where the cell membrane detaches from the cortex. *Dictyostelium discoideum* utilizes all four of these motility structures, making it an ideal model organism to investigate cell motility in response to the environment and the molecular players involved. In order to better understand cell choice in utilizing these structures in response to pressure, we employed an under agarose assay where we altered the hydrostatic pressure by varying the agarose concentration. We found that wild-type cells under low-pressure agarose (0.4 percent) used filopodia and blebs to an equal extent. However, under high-pressure agarose (0.7 percent), this shifts predominately to blebs, suggesting that high pressure favors bleb-based motility. This ability to modify the use of motility structures is due in part to TalA, a protein that links the membrane to the cortex. Cells lacking TalA make fewer blebs than wild-type cells under both pressures. In addition, when shifted to high pressure, cells lacking TalA do not increase their use of blebs. Taken together, this suggests that TalA is not only required for efficient blebbing as is seen in wild-type cells, but is also needed to modulate the use of blebs in response to pressure changes in the environment.

# Trimeric G-protein shuttling for chemotaxis ranges

Takero Miyagawa[1], Yoichiro Kamimura[2], Hiroyasu Koteishi[2], Kohei Takeshita[3], Atsushi Nakagawa[3], and **Masahiro Ueda**[1,2]

*[1]Grad. Sch. Front. Biosci., Osaka Univ.; [2]QBiC, RIKEN; [3]IPR, Osaka Univ. Japan*

Chemotactic cells can sense chemical gradients over a wide range of concentrations; however, the underlying wide-range sensing mechanisms are only partially understood. Recently, we found that a novel regulator of G proteins, G protein-interacting protein1 (Gip1), is essential for extending the chemotactic range of *Dictyostelium discoideum* cells. Gip1 was found to bind and sequester heterotrimeric G proteins in cytosolic pools. Receptor activation induced G protein translocation to the plasma membrane from the cytosol in a Gip1-dependent manner, causing a biased redistribution of G protein on the membrane along a chemoattractant gradient. To reveal the structural basis of G protein shuttling, we determined the crystal structure of C-terminal region of Gip1 that is essential for binding to the Gβγ subunit. The structural analysis revealed that the C-terminal region of Gip1 had a hydrophobic cavity composed of six α-helices. Biochemical analyses revealed that the hydrophobic cavity was important for the complex formation with the lipid modification of the Gβγ subunit. This mechanism can offer an explanation for the sequestration of Gαβγ in the cytosol. We will discuss the possible mechanisms of Gip1-dependent trimeric G-protein shuttling for wide range chemotaxis.

# Telling right from wrong: receptor occupancy and kinetic proof-reading

**Edward C Cox**

*Princeton UNiversity, 119 Jadwin Hall, Princeton, NJ 08544*

If you ask a colleague how the *Dictyostelium* cAMP receptor distinguishes between cAMP and, say, cGMP they will say "it's the binding constant" (some will add "stupid"). There is some truth to this answer, but it cannot be the only way the two are told apart because they differ from each other by the equivalent of just one or two H-bonds. This amounts to a discrimination energy difference of just a few kcal/mol, and thus an error rate of a few percent at steady state. This problem appears in many different systems—DNA replication, amino acid activation, and tubulin polymerization, to name three—and it has been solved, at least for DNA replication, by kinetic proofreading, where a slowing of a forward rate allows a back-reaction to remove the errant nucleotide. There is a canonical signature for all proof-reading systems that can be found for the cAMP receptor, and I will suggest that much of its complexity is a reflection of a proofreading step following binding.

# The "Dicty Jail": a new microfluidic tool for long-term single-cell imaging of mycobacteria infection.

**Ana T López-Jiménez**[1], Matthieu Delincé[2], Monica Hagedorn[3], John D McKinney[2], Thierry Soldati[1].

*[1]University of Geneva, Biochemistry, Geneva, Switzerland; [2]École Polytechnique Fédéral de Lausanne, School of Life Sciences, Lausanne, Switzerland; [3]Jacobs University, Life Sciences and Chemistry, Bremen, Germany.*

Tuberculosis is a highly infectious disease caused by *Mycobacterium tuberculosis*. In order to study the mechanisms of bacteria pathogenicity and host defenses, we use the model system composed by *Mycobacterium marinum* (the causative agent of tuberculosis in fish) and Dictyostelium discoideum (a free-living amoeba that feeds on bacteria by phagocytosis). After phagocytic uptake by *D. discoideum*, *M. marinum* does not follow the normal bactericidal pathway that leads to lysosomal degradation. Instead, *M. marinum* is able to hamper phagosomal acidification and maturation, thus, tailoring a permissive niche for its replication. At later stages, the compartment ruptures, enabling *M. marinum* to access the cytosol. Finally, the bacteria escape the cell by lysing the host or by a non-lytic mechanism called ejection. Niche breakage and ejection are dependent on the secretion of the mycobacterium membranolytic peptide ESAT-6. These stages of the infection have been investigated at the bulk population level, which is intrinsically phenotypically heterogeneous. To gain a more comprehensive understanding of how cell-cell variations impact the infection outcome, we have committed to dissecting the infection at the single cell level. For this, we use a microfluidic device for visualization of different combinations of mutants and fluorescent reporters at a spinning disc confocal microscope for several days. Inspection of single infected cells revealed a great diversity of complex fates and timings that we have quantified. Surprisingly, host death was shown to contribute importantly to *M. marinum* release and propagation of the infection by efferocytosis. We have confirmed previous observations that viability of the host is improved when the bacteria are incapacitated in ESAT-6 secretion. We also observed different dynamics in the intracellular trafficking of the bacteria, using markers such as VacuolinA-GFP or LifeAct-GFP. Importantly, we also monitored how pathways such as autophagy, exocytosis or ejection determine the release of the bacteria to the extracellular medium, using mutants such as *atg1-*, *wshA-* or *racH-*, respectively. All these pathways not only seriously impact bacterial fate in standard medium conditions, but also contribute to the spontaneous bacteria release that is induced when infected *D. discoideum* cells are subjected to starvation, which leads to curing of the multicellular stage form by a combination of cell-autonomous and cell-exclusion mechanisms.

# *Salmonella typhimurium* requires SopB and SifA to survive intracellularly in a SCV-like compartment in *Dictyostelium discoideum*

**Camila Valenzuela**, Andrea Sabag, Ítalo M. Urrutia, Magdalena Gil, Macarena Varas, Jost Enninga and Carlos A. Santiviago

*Institut Pasteur, France*

*Salmonella* survival within eukaryotic cells is explained, in part, by its ability to establish an intracellular compartment known as the *Salmonella* containing vacuole (SCV). To this end, *Salmonella* exploits several effector proteins secreted by type-three secretion systems T3SS−1 and T3SS−2. We previously reported a role for both T3SS in the survival of *Salmonella typhimurium* in the social amoeba *Dictyostelium discoideum*. In this work, we evaluated the role played by two effector proteins in this process. We chose SopB (secreted by T3SS-1) and SifA (secreted by the T3SS-2) because these proteins play major roles in the formation and maturation of the SCV in other cell types. We performed infection assays using axenic cultures of *D. discoideum* AX2 infected with *S. typhimurium* wild-type strain 14028s, derived mutants ΔsopB and ΔsifA and complemented strains. Intracellular bacteria were recovered at different times from *D. discoideum* and titrated. Our results showed that ΔsopB and ΔsifA mutants were recovered at ~5-fold lower levels than the wild-type strain at 6 h post-infection, indicating that these mutants are defective in intracellular survival in the amoeba. This phenotype was complemented using plasmids carrying wild-type copies of the deleted genes and these strains showed wild-type levels of intracellular survival. Next, we assessed whether *S. typhimurium* was confined in a vacuolar compartment containing a SCV marker found in other cellular models. For this, we performed infections of *D. discoideum* cells expressing a GFP fusion of the vacuolar ATPase (VatM-GFP). According to our results, viable wild-type bacteria were detected laying within VatM-GFP+ vacuoles up to 4 h of infection. In the case of ΔsopB and ΔsifA mutants, only a few bacteria were detected within VatM-GFP+ vacuoles at 3 h of infection, and most *D. discoideum* cells contained remnants of killed bacteria. Altogether, our results suggest that *S. typhimurium* resides in a vacuolar compartment in *D. discoideum* and requires effectors SopB and SifA to avoid degradation by this amoeba. We are currently characterizing the proteome of this SCV-like compartment in *D. discoideum*.

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# Short and long distance signals regulate vegetative cell motility

Joseph d'Alessandro, Alexandre Solon, Yoshinori Hayakawa, François Detcheverry, Jean-Paul Rieu, Charlotte Rivière, **Christophe Anjard**

*Claude Bernard University Lyon 1, France*

*Dictyostelium* is considered to be a solitary amoeba while food is available and turning into a social cell upon starvation. We are showing here that, even when food in plentiful, *Dictyostelium* cells interact with each other through multiple mechanism both by contact and through secreted factors to regulate their motility and spreading.
We created small colonies using micro-stencils and tracked the cell trajectories as they spread out in order to better investigate the onset of these collective behaviors (1). From data analysis and simulation of an elementary model, we demonstrate that contact interactions act to speed up the early population spreading by promoting individual cells to a state of higher persistence, which constitutes an as-yet unreported contact enhancement of locomotion. a phenomenon we called CEL (Contact enhancement of Locomotion). Our findings also suggest that the current modelling paradigm of memoryless active particles may need to be extended to account for the history-dependent internal state of motile cells. At longer time, the average cell speed is reduced by secreted Quorum Sensing factor (QSF) that accumulates in the media and acts at long range to prevent cells to drift too far apart from the others (2). The cAMP-independent response to this unidentifed, high-molecular-weight molecule includes a reduction of the cell movements, in particular through the down-regulation of a mode of motility with high persistence time. Using indirect estimation of the QSF concentration and mathematical analysis of the cells' response, we demonstrate that the QSF production is under negative feedback: the secretion rate decreases linearly as a function of the concentration, which gives the cells a way to detect when they exceed a density threshold. The combination of CEL and QSF effects on cell motility might result into an optimization of space utilization.

1- Contact enhancement of locomotion in spreading cell colonies. (2017 J. d'Alessandro, A. Solon, Y. Hayakawa, C Anjard, F. Detcheverry , Nature physics
2- Collective regulation of cell motility using an accurate density sensing system. J. d'Alessandro, J.P Rieu, C. Rivièrey and C. Anjard. Submitted to interface

# Non-canonical functions of telomerase reverse transcriptase (tert) in *Dictyostelium discoideum*.

**Nasna Nassir** and Ramamurthy Baskar

*Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology, Madras. Chennai-600036. India.*

The catalytic subunit of telomerase enzyme is the telomerase reverse transcriptase (TERT) which adds specific repetitive sequences to the chromosomal ends. Though TERT plays an important role in telomere maintenance, the non-canonical roles of TERT are poorly understood. As growth and development are clearly separated in its life cycle, we chose Dictyostelium discoideum to study the non-canonical functions of TERT. Although expression of TERT mRNA is seen throughout the life cycle, beyond growth, the expression is elevated suggesting significant role of TERT in multicellular development. By homologous recombination, we generated tert knockout clones exhibiting both growth and developmental defects. The mutant cells were arrested in G0 phase of the cell cycle and growth in axenic media was severely affected due to defective pinocytosis. A prominent phenotype of the mutant is the breaking of aggregation streams and delayed development. Some of the defects in tert knockout could be attributed to impaired cAMP relay and faulty cell adhesion. Though the speed of the mutant cells during random movement is comparable to that of wild type, they are defective in chemotaxis towards cAMP as well as folic acid. Further, our results suggest that defective directional sensing and altered F actin polymerization could possibly manifest in these phenotypic defects. Both growth defect and the pathway of stream breaking in tert mutants are currently being studied. Besides telomere maintenance, TERT thus has pleiotropic roles in *Dictyostelium discoideum*.

# Generation of single cell transcript variability by repression

**Vlatka Antolović**, Agnes Miermont, Adam M. Corrigan and Jonathan R. Chubb

*University College London, UK*

Gene expression levels vary greatly within similar cells, even within clonal cell populations. These spontaneous expression differences underlie cell fate diversity in both differentiation and disease. The mechanisms responsible for generating expression variability are poorly understood. Using single cell transcriptomics, we show that transcript variability emerging during *Dictyostelium* differentiation is driven predominantly by repression rather than activation. The increased variability of repressed genes was observed over a broad range of expression levels, indicating variability is actively imposed, and not a passive statistical effect of the reduced numbers of molecules accompanying repression. These findings can be explained by a simple model of transcript production, with expression controlled by the frequency, rather than the magnitude, of transcriptional firing events. Our study reveals that the generation of differences between cells can be a direct consequence of the basic mechanisms of transcriptional regulation.

# Determining the cellular pathways involved in *D. discoideum* polyketide synthase metabolism.

**Lavanya Muthukumar**[1], Shweta Saran[3], Rajesh S Gokhale[2], Kiragandur Manjunath[1].

*[1]Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bengaluru, Karnataka, 560056, India. [2]National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110020, India. [3]School of Life Sciences, Jawaharlal Nehru University, New Mehrauli Road, New Delhi, 110067, India*
**Corresponding author: lavanyamkumar@gmail.com*

Polyketide Synthases (PKS) are enzymes that make a diverse class of secondary metabolites called Polyketides, many of which are pharmacologically active compounds. The invivo roles of these Polyketides have not been defined clearly. Polyketides are found in several classes of organisms such as Plants, Fungi and Bacteria. Genome sequencing has revealed that the soil dwelling social amoeba *Dictyostelium discoideum* has the largest number of putative Polyketide Synthases of which 35 are type I PKSs. In this study, functional characterization by microarray analysis of the slug stage of the a few PKS gene knockout strains indicates that Polyketides might play a role in cell adhesion, vesicle trafficking and mitochondrial function pathways in *D. discoideum*.

# Sensing mechanism of cells under the influence of electric fields

**Isabella Guido**, Eberhard Bodenschatz

*Max-Planck Institute for Dynamics and Self-Organization, Germany*

Cells have the ability to detect continuous current electric fields and respond to them with a directed migratory movement. Dictyostelium discoideum cells, a key model organism for the study of eukaryotic chemotaxis, orient and migrate toward the cathode under the influence of an electric field. Whereas genes and proteins that might play a role in electric migration as well as that define the migration direction have been previously investigated, the underlying sensing mechanism remains unknown. In this study we investigated the response to electric field of vegetative cells and cells starved in buffer for short time. Short starved cells exhibit electrotactic behavior for about one hour and then they migrate randomly. Vegetative cells cannot sense the electric stimulus at all and migrate randomly in an electric field. By analyzing these different effects of electric fields on the two kind of cells we observed that conditioned medium factor ,a protein secreted by the cells when they begin to starve, is essential for the cells to sense and respond to the electric field. These results show for the first time which can be a reliable cellular pathway triggering the electric sensing in biological cells.

# The Geneva Antibody Facility

**Wanessa du Fresne Von Hohenesche**

*University of Geneva, Switzerland*

The Geneva Antibody Facility has been created in 2015 with the main objective of increasing the access and the use of recombinant antibodies by the academic research community. For that, the key point was the production of non-commercially available antibodies at an affordable price, and with an open-access and collaborative spirit. Our facility is currently divided in two main parts: Discovery and Production. The Discovery section uses phage display technology to specifically select antibodies against any desired target of any desired species. The Production section is responsible for the generation of antibody-containing vectors (that produce a mini-antibody with the scFv antigen-binding portion fused to a Fc portion) not only from antibodies generated by us, but also from any antibody to which the aminoacid sequence is available. To complement this task, our newest project is the building of an open-access recombinant antibody database, with the ambitious task of retrieving all antibodies with a known target and known sequence (right now, this information is scattered or hidden in databases and in the scientific literature). For the *Dictyostelium* laboratories, our facility might be an invaluable tool: (i) as a depository of already existent antibodies (including new antibodies developed by us, against *Dictyostelium* proteins such as Far1, NoxABC, VacABC, DrkAD, Phg1a, SibA, among others); and (ii) as it allows any laboratory to develop new antibodies (that will be available and distributed to the whole community). This facility should be collectively used by the *Dictyostelium* community as a common strategy to increase the power of the *Dictyostelium* model system.

# Polyketide synthase null mutant resembles prestalk A cells

**Rosa Herbst**, Markus Günther, Tom Burks, and Pierre Stallforth

*Chemistry of Microbial Communication, Leibniz Institute for Natural Product Research and Infection Biology, 00745 Jena, Germany*

Polyketides are a major class of natural products produced by microorganisms, plants, and invertebrates. They play pivotal roles as toxins and antibiotics, but also during differentiation or inter- and intracellular communication processes. These secondary metabolites are synthesized by polyketide synthases (PKS), which are large multienzyme protein complexes. The genome of *Dictyostelium discoideum* exhibits more than 40 pks genes, distributed over the six chromosomes, with some of them located in cluster of 2- 5 genes (1). All PKS in D. discoideum belong to the class of type I synthases, i.e., proteins that contain all necessary domains for the stepwise biosynthesis of their respective natural product. There are two exceptions, namely steely A and B, which are hybrid proteins with an additional type III domain fused to the type I PKS. Their respective metabolites, 4-methyl-5-pentylbenzene-1,3-diol (MPBD) and differentiation inducing factor-1 (DIF-1), are the only isolated and biochemically characterized polyketides of D. discoideum so far. Both are secreted molecules with important functions during the differentiation process towards spore and stalk cells(2,3) . The products and the corresponding function of the remaining 38 PKS still remain unclear. Our approach is to generate pks-null mutants and analyze the resulting phenotypes with respect to behaviour of the cells during development and predation. The mutant presented here exerts striking morphological alterations during its developmental cycle, including a missing slug stage and multiple tip formation. RT-qPCR analyses of the expression profile of several developmentally regulated genes revealed that the vegetative amoebae already display features of prestalk A cells.

(1) Zucko, J, et al., Bioinformatics (2007), 23 (19):2543-2549
(2) Anjard, C. et al., Eukaryot. Cell (2011), 10 (7):956-963
(3) Saito, T., et al., Dev. Biol. (2008), 317 (2):444-453

# Polyphosphate induces a phase-separated chaperone complex during *Dictyostelium discoideum* development

**Stephanie Santarriaga**, Alicia Fikejs, K. Matthew Scaglione

*Medical College of Wisconsin, USA*

*Dictyostelium's* proteome is comprised of a vast array of single sequence repeats and as such represents a unique model organism to investigate proteostasis. Upon conditions of cellular stress, the social amoeba *Dictyostelium discoideum* undergoes a developmental process, transitioning from a unicellular amoeba to a multicellular fruiting body. The mechanisms *Dictyostelium* employ to maintain proteostasis during its developmental process are unknown. Here, we show that a novel alpha-crystallin domain containing protein, Hsp48, is essential for *Dictyostelium* development. Loss of Hsp48 leads to the presence of ubiquitin-positive inclusions in cells that fail to complete development. We further show that Hsp48 forms a biomolecular condensate that is stabilized by a highly-charged, intrinsically disordered C-terminus. Formation of this biomolecular condensate is driven by polyphosphate, and removal of polyphosphate kinase prevents its formation. Together, these data demonstrate an essential role for a polyphosphate-Hsp48 biomolecular condensate in maintaining proteostasis during *Dictyostelium* development.

# IreA and autophagy cooperate for the survival response to ER-stress in *Dictyostelium discoideum*

**Eunice Domínguez-Martín**[1,2], Laura Kawasaki[2], Laura Ongay-Larios[2], Roberto Coria[2]* and Ricardo Escalante[1]*

*[1]Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM). Arturo Duperier 4. 28029 Madrid. Spain; [2]Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México D.F., México.*

The Unfolded Protein Response (UPR) is an adaptive pathway that restores cellular homeostasis when endoplasmic reticulum (ER) stress is caused by any alteration of the ER protein folding capacity. Ire1 ER-resident kinase/ribonuclease, the only UPR sensor that has been conserved during evolution, transmits ER information to the nucleus trough the non-conventional splicing of Hac1 (yeast)/Xbp1 (metazoans) mRNA. Autophagy contributes also to the recovery of cell homeostasis after ER-stress; still, many questions regarding the interplay between UPR and autophagy remain. We have addressed these questions in the model system *Dictyostelium discoideum*; first, we described the ER-stress response and identify IreA as the single bonafide Ire1 orthologue in this system. Both, IreA kinase and ribonuclease activities, resulted indispensable for the tunicamycin(TN)-induced ER-stress response; which induced an ireA-dependent specific gene expression program, though an Xbp1 orthologue was not identified. Autophagy was activated in *Dictyostelium* under TN treatment, and mutants lacking autophagy presented an increased sensitivity to TN. Interestingly, IreA resulted dispensable for autophagy activation, since accumulation of the early autophagic marker GFP-Atg18 occurred in the absence of IreA under TN treatment. However, these autophagic structures were abnormal and trapped at the ER, which appeared to be collapsed. Therefore, the degradative capacity of IreA-lacking cells was compromised, leading to ubiquitin-positive protein aggregates accumulation and a reduced capacity to degrade cytosolic proteins. Our data suggests that both IreA and autophagy contribute to survival under ER stress. As autophagosomes emerges from specialized regions of the ER in *Dictyostelium* we hypothesize that unresolved ER-stress in cells lacking IreA may cause alterations on the ER structure that lead to a late blockade in autophagosome formation.

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*Co-senior authors.

# Genes responsible for species recognition in social amoebae

**Ikumi Shibano-Hayakawa**[1,2] and Kei Inouye[1]

*[1]Department of Botany, [2]Department of Physics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan*

Self/non-self recognition is important in the life history of social amoebae. In particular, mixing between different species has to be avoided during their development. However, while the mechanism for kin discrimination in *Dictyostelium discoideum* (Dd) has been studied in detail, little is known about the molecular basis of interspecific recognition. In this study, we focused on the interaction between *D. purpureum* (Dp) and Dd, and sought to find Dp genes that, when heterologously expressed in Dd, induce chimeric development. When mixed and starved, Dd and Dp cells coaggregate towards a common centre by cAMP chemotaxis, but segregate from each other within the mound to form separate fruiting bodies. Under conditions where stream formation of the two species was synchronised, an early sign of segregation was seen already within aggregating streams. Namely, cells of each species tended to align lengthwise to form files of either species within a stream, suggesting the involvement of adhesion molecules responsible for end-to-end cell contact. An obvious candidate was Contact site A (csaA), which was originally identified as an end-to-end adhesion factor of aggregating Dd cells. We also considered Tiger C1 (tgrC1), which is precociously expressed to complement the function of Contact site A in csaA-null mutant cells. The wild-type Dp strain used in the study has one gene homologous to Dd csaA and 3 pairs of genes structurally similar to the Dd tgrB1/C1 pair. We introduced these Dp genes individually into Dd Ax2 cells, and examined their effects on cell sorting. When mixed with Dp cells in equal proportions, Dd cells expressing one of the Dp tiger family genes, which will be referred to here as Dp-tgr2752C, were not excluded from Dp cells, and formed chimeric slugs and fruiting bodies. No other transformants formed chimeras with Dp at comparable levels. The Dp-tgr2752C gene is developmentally regulated and maximally expressed at the mound stage. Like Dd tgrC1, it is highly polymorphic in wild isolates of the same mating group. These results suggest that Dp-tgr2752C has similar functions as tgrC1 in Dd and that it is also a component of the species recognition system. Possible evolutionary history of self/non-self recognition systems in social amoebae will also be discussed.

# Posters

The numbering of the poster boards corresponds to the page number of the respective abstract

# Squalene synthase has a novel peroxisomal location in the slime mould *Dictyostelium discoideum*

**Murtakab Y. Al-hejjaj**, Mayyadah A. Alkuwayti, Donald J. Watts and Ewald H. Hettema

*University of Sheffield, UK*

# *Dictyostelium discoideum* as a model for the evaluation of teratogenic compounds

**Robert Baines**

*University of Manchester, UK*

# Design of a chemical probe of a eukaryotic signal involved in multicellular development

**R. Barnett**, Jena/DE, P. Stallforth, Jena/DE

*Robert Barnett, Leibniz Institute for Natural Product Research and Infection Biology e.V. –Hans-Knöll-Institute (HKI), Beutenbergstrasse 11a, 07745 Jena, Germany*

In this work we carry out a structure-activity-relationship (SAR) of a eurkaryotic signaling molecule, create a chemical probe, and carry out photoaffinity labeling studies to identify the cognate receptor. Glorin is a small signaling molecule which regulates multicellular aggregation in several species of social amoeba [1] (e.g., *Dictyostelium caveatum* and *Polysphondylium violaceum*). The receptors for the signaling molecule and the enzymes responsible for its inactivation are currently unknown. A detailed overview of this glorin-based signaling process is important to gain insight into the origin of multicellularity in eukaryotes. We plan to identify the glorin receptor via a proteomics approach. Therefore it was necessary to create a glorin-based chemical probe, which can bind to the receptor and be subsequently crosslinked to the protein. Then the protein-bound probe can be fished out of the cell lysate via affinity chromatography. An SAR study was carried out utilizing our published synthesis of glorin [2] to synthesise derivatives and determine a potential site of modification, such that the chemical probe still binds the receptor. A chemical probe was then synthesized containing both a diazirine and an alkyne moiety. Photoaffinity labeling studies were then carried out to attempt to identify the glorin receptor and other glorin-associated proteins.

Literature:
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# Revisiting the role of vacuolins in phagocytosis, membrane trafficking and in the biogenesis of the *Mycobacterium marinum* niche

**Cristina Bosmani**, Monica Hagedorn, Thierry Soldati

*University of Geneva, Switzerland*

*Mycobacterium tuberculosis* is the causative agent of tuberculosis in humans and is able to manipulate the phagosome maturation pathway to establish a permissive compartment where it can proliferate. We use *Mycobacterium marinum*, a close cousin, and *Dictyostelium* as a surrogate macrophage to study host-pathogen interactions during mycobacterial infections. *Dictyostelium* has three vacuolins (A, B and C) that localize at Mycobacteria-containing compartments and are upregulated upon infection. *Dictyostelium* vacuolins are homologs of the metazoan flotillins. Flotillins can oligomerize, form microdomains at the plasma membrane and serve as signaling platforms. In addition, flotillins are involved in Transferrin-Receptor and E-cadherin recycling to the plasma membrane via interactions with recycling machinery components. We want to understand whether vacuolins are involved in the establishment of a permissive *M. marinum* niche by altering endosomal recycling. We found that vacuolin C, a previously uncharacterized vacuolin, localizes to lysosomes, whereas vacuolins A and B are found on postlysosomes, as previously observed. In addition, we demonstrate that vacuolins, like flotillins, are strongly associated with membranes, are palmitoylated, and can be found in detergent-resistant membranes. To determine the role of the three vacuolins, we generated single and multiple knock-outs by homologous recombination. We found that deletion of vacuolin genes dramatically impairs the phagocytic uptake of several types of particles with different sizes, and results in motility defects. We suggest that vacuolins are involved in recycling of plasma membrane proteins involved in adhesion and particle recognition. In addition, we find that vacuolin triple knock-outs are impaired in lysosomal enzyme trafficking. Finally, we show that the knock-out of vacuolins confers partial resistance to infection with *Mycobacterium marinum*, suggesting that vacuolins are important host factors that are manipulated by the pathogen to establish its permissive compartment.

# Ether-Linked Lipid Biosynthesis is Important for Growth, Chemotaxis, Growth and Development in *Dictyostelium discoideum*

Eric Lau, Noura Maziak, Iurii Gurkov, Rachel Zufferey and **Derrick Brazill**

*Hunter College, CUNY, USA*

Inositol phospholipids play key regulatory roles in numerous cellular functions including growth, vesical trafficking, cytoskeletal organization and signal transduction. In general, differentially phosphorylated inositol head groups bind to specific protein domains, recruiting the associated protein to the membrane, and regulating its activity. While much is known about the function of the phosphorylated inositol head group, the role of the diacylglycerol backbone is less well understood. Interestingly, while the inositol phosphorylation is highly conserved across different taxa, the lipid backbones vary. Specifically, in *Dictyostelium discoideum*, inositol phospholipids contain ether-linked lipids. To better understand the synthesis and the function of these ether lipid-containing inositol phospholipids, we are studying the role of an enzyme potentially involved in ether lipid biosynthesis, fatty acyl-CoA reductase/acyl transferase (FARAT). Sequence and biochemical analyses suggest that FARAT is a bifunctional enzyme that provides the two substrates required for ether lipid biosynthesis, a fatty alcohol and 1-acyl dihydroxyacetonephosphate. We find that GFP-FARAT partially localizes to sites of lipid synthesis, consistent with its proposed function. Cells lacking FARAT have altered growth kinetics compared to wild-type AX2 cells, with an extended lag phase and increased doubling time, implying a role in growth. While able to proceed through development, cells lacking FARAT produce fruiting bodies with weakened stalks, suggesting a role in stalk cell differentiation or maturation. Most interestingly, cells lacking FARAT are defective in folate chemotaxis, implying that ether lipid biosynthesis may be important in the production of phospholipid signaling molecules. Taken together, this argues that ether-linked inositol phospholipids play a variety of important roles in *Dictyostelium discoideum*.

# Differential effects of transition metals on *Dictyostelium* cell growth and resistance to *Legionella pneumophila* infection.

**Simona Buracco***, Barbara Peracino* and Salvatore Bozzaro*

**Department of Clinical and Biological Sciences, University of Turin, Italy*

Transition metals play fundamental roles in all organisms. Iron is the most abundant transition metal in cells, being involved in processes such as respiration, energy production and gene regulation. Similarly to iron, copper participates in redox reactions, regulating many enzymes, including cytochrome c oxidase and Cu-Zn superoxide dismutase. Zinc is the second most abundant transition metal, is redox inert, but has structural and catalytic roles, stabilising negative charges of substrates or organizing protein subdomains in zinc motifs. Excess iron, zinc or copper are toxic, thus cells have developed complex mechanisms to regulate their homeostasis. Tight regulation of transition metal bioavailability also regulates host-pathogen interactions. For intracellular pathogens, the source of metals is the cytoplasm of the host, which in turn can manipulate intracellular metal traffic following pathogen recognition. It is established that iron is withheld from the pathogen-containing vacuole, whereas for copper and zinc the evidence is unclear. Indeed most infection studies in mammals have concentrated on the effects of metal deficiency or overloading at organism level. Thus, zinc deficiency and supplementation have been shown to correlate with, respectively, high risk of respiratory tract infections, and recovery from severe infection. While it is clear that iron, zinc or copper deficiency or overloading affect lymphocyte proliferation/maturation, thus adaptive immune response, an understanding of the processes at macrophage level is very limited, except for iron. The early identification in a mouse mutant susceptible to mycobacterial infection of the iron Nramp1 transporter allowed dissecting Nramp1 role in professional phagocytes, from *Dictyostelium* to macrophages. Nramp1 regulates iron efflux from the phagosome, thus starving pathogenic bacteria for iron. Similar studies for zinc or copper are missing, also due to the large number of copper and zinc transporters. In *Dictyostelium*, zinc and copper transporters include 12 and 4 members, respectively. To assess the role of zinc or copper in *Dictyostelium*, we have used an indirect approach, namely growing cells under conditions of metal depletion or overloading, then testing cell development, phagocytosis and resistance to *Legionella pneumophila* infection. Iron deficiency or overload inhibits *Dictyostelium* cell growth within two generations, suggesting that this metal is essential for growth and toxic at high concentration. Surprisingly, zinc or copper deficiency failed to affect growth, with cells duplicating normally for more than 20 generations. Zinc or copper overloading inhibited cell growth, respectively at 50- or 500-fold the physiological concentration, suggesting that cells are very efficient in controlling homeostasis of these metals. Zinc, but not copper, deficiency during growth blocked development at mound stage. A 20-fold excess of zinc during growth failed to alter development, whereas a 200-fold excess of copper inhibited aggregation at loose mounds. *Legionella* infection was inhibited in both wild type or Nramp1/NrampB KO mutant cells grown under iron deficiency, whereas its proliferation was enhanced in cells grown under iron overload, with Fe(III) accelerating bacterial proliferation and cell lysis faster than Fe(II). In contrast to iron, zinc and copper deficiency or excess did not affect *Legionella* infection, compared to control cells. Thus iron appears to play a major role in controlling resistance to pathogens, confirming previous results with the Nramp1 KO mutant, whereas zinc or copper appear to be dispensable, at least with wild type *Legionella pneumophila*.

# Exploring *Mycobacterium bovis* host-pathogen interactions using *Dictyostelium discoideum*.

**Rachel Butler**[1], Louise Lefrancois[2], Tom Mendum[1], Huihai Wu[1], Jana Baron[1], James Barber[1], Martin Vordermeier[3], Mick Coad[3], Thierry Soldati[2], Graham Stewart[1].

[1]*Microbial Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK;* [2]*Department of Biochemistry, University of Geneva, Switzerland;* [3]*TB Immunology and Vaccinology, Department of Bacteriology, Addlestone, Surrey, UK*

*Mycobacterium bovis*, a member of the *M. tuberculosis* complex, causes bovine tuberculosis, one of the most important veterinary health problems in the UK. In the absence of improved control, the projected economic burden to the UK over the next decade is predicted to be £1 billion. Control is likely to require an integrated approach with vaccination of cattle representing a key component. Presently, the *M. bovis* BCG vaccine is the most encouraging vaccination option, yet studies suggest that it has a protective efficacy of only ~70% underlining the need for strategic development of new vaccines. In this NC3Rs funded project, our vision is to develop non-sentient infection models for mycobacterial infection that are genetically tractable for both host and pathogen. This will allow the comprehensive description of the host and pathogen genes important to the intracellular host-pathogen interaction, informing rational development of novel vaccines that stimulate enhanced immunity against *Mycobacterium bovis*. The aim of the project is to examine how *Mycobacterium bovis* manipulates the fundamental processes of innate immunity. To achieve this we have established and characterised mycobacterial infection models involving the amoeba *Dictyostelium discoideum* as the host phagocyte. We have selected libraries of mutant bacteria in both *Dictyostelium* and primary bovine macrophages, to identify which pathogen genes are necessary for intracellular survival and which host genes are important for resistance and susceptibility to infection.

# CnrD and CueA, two potential new selective autophagy receptors in *Dictyostelium*

**Elena Cardenal-Muñoz**, Imen Ayadi, Jason S King and Thierry Soldati

*University of Geneva, Switzerland*

The functional conservation of the autophagy pathway between the professional phagocyte *Dictyostelium* and mammalian macrophages, in addition to the tractability of this amoeba, make *Dictyostelium* an excellent model system to uncover the molecular mechanisms of autophagy. We recently published various tailored methods to reveal the precise stage in which the autophagy pathway is regulated in *Dictyostelium*. Thus, we have demonstrated that in this amoeba, as occurs in macrophages infected with *Mycobacterium tuberculosis*, *Mycobacterium marinum* antagonistically induces autophagosome formation while repressing the autophagic flux. Upon uptake by the host cell, *M. marinum* damages the membrane of its containing vacuole (MCV), what reduces the activity of TORC1, the main inhibitor of autophagy, increases the transcription of autophagy genes, induces the formation of autophagosomes and recruits the autophagy machinery to the bacterial niche. However, these damages are likewise essential to devoid the MCV of proteolytic activity. The membrane disruptions caused by the pathogen likely alters the content or properties of the MCV, impeding the killing of the bacteria within autolysosomes. We are now focusing on the study of selective autophagy in *Dictyostelium*. Apart from a p62-homolog, *Dictyostelium* has two CUE domain-containing proteins, CnrD and CueA, which are potential receptor candidates. They aggregate in autophagy-null cells, are degraded upon autophagy induction, and colocalise with Atg8 and mycobacteria at MCVs. One of these proteins is of special interest, since it might be the ancestor of a human protein, CUEDC1, about which very little is known.

# The fate of multilamellar bodies produced and secreted by *Dictyostelium discoideum* amoebae

**Steve J. Charette***, Alix M. Denoncourt, Alicia F. Durocher, and Valérie E. Paquet

*1. Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, Quebec City, QC, Canada; 2. Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Hôpital Laval, Quebec City, QC, Canada; 3. Département de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Quebec City, QC, Canada*

The amoeba *Dictyostelium discoideum* produces and secretes multilamellar bodies (MLBs), mainly made of amoebal membranes, upon digestion of bacteria. The fate of MLBs after their secretion is not clear, and it was proposed that they may serve as a food source. The aim of this study was to determine if protozoa (*D. discoideum* itself and the ciliate *Tetrahymena*) can internalize and digest secreted *D. discoideum* MLBs as a potential nutrient source. Our results showed that MLBs were ingested by naive *D. discoideum* amoebae (i. e. cells not exposed to bacteria and consequently not producing MLBs). Only a small fraction of the ingested MLBs were found in cells' post-lysosomes compared to undigestible beads suggesting that they are digested by naive amoebae. *D. discoideum* MLBs were also ingested by the ciliate *Tetrahymena pyriformis*. MLBs internalized by the ciliate were compacted into pellets and expelled in the extracellular medium without obvious signs of digestion. The results of this study provide new insights on the biological function of MLBs and, considering that MLBs are also involved in bacteria packaging, suggest additional layers of complexity in microbial interactions.

# Investigating the active functional groups of complex chemicals controlling biological activity

**Marco Cocorocchio**[1], Balint Stewart[2], Amy Baldwin[3], Adrian Harwood[4], Chris Thompson[3], 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]Faculty of Life Sciences, Manchester University, Manchester, M13 9PL; [3]Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, CF24 4HQ; [4]Division of Biomedical Science St George's University of London, London, United Kingdom*

Natural compounds often have complex molecular structures and unknown molecular targets. These characteristics make them difficult to analyse using a classic pharmacological approach. Curcumin, the main curcuminoid of turmeric, is a complex molecule possessing wide-ranging biological activities and unknown cellular mechanisms. The aim of this project is to provide an innovative approach to investigate the physiological effects and molecular targets of curcumin and structurally related compounds using the model system *Dictyostelium discoideum*. This approach will facilitate the identification of the active functional groups of these molecules that confer specific cellular and organismal effects. Curcumin showed chronic effects in reducing growth with an IC50 of 44μM and slowing multicellular development, and acute effects on cell behaviour with an IC50 of 2.3μM. Analysis of a range of structurally related compounds (demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, FLLL31, EF-24, UBS-109 and curcumin-pyrazole) provided distinct effects on growth, development and cell behaviour providing a ranking order of potency for each compound and effect. From this analysis, the presence of methoxy groups provided the key functional groups for the inhibition of acute cell behaviour and development, whereas loss of these group enhanced growth inhibition. Molecular mechanisms underlying these effects have been investigated by identifying mutants resistant to each compounds and validated in resistance to growth and cell behaviour. We therefore provide a model where the distinct effects of curcumin and derivatives on growth, development and acute cell behaviour are modulated by specific active groups through distinct molecular mechanisms. These findings demonstrate that *Dictyostelium* may be used as a model for the prediction of bioactive functional groups of structurally related compounds.

# *Drosophila melanogaster* infection with *Mycobacterium marinum* as an alternative of the macrophage model for testing new anti-tuberculosis compounds

**Jonathan A. G. Cox[1]***, Cathy Slack[1], Michael Stich[2]

*[1]School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK; [2]School of Engineering and Applied Science, Aston University, Aston Triangle, Birmingham, UK; *j.a.g.cox@aston.ac.uk*

Tuberculosis (TB) kills 1.5 million people per year and 1 in 3 people globally are estimated to carry a latent TB infection. *Drosophila melanogaster* is a well-established model system for the study of host-pathogen interactions, but remains extensively underexplored for TB research. Adult flies can be easily injected with measured doses of bacteria allowing screening methodologies that would be prohibitively expensive or laborious in vertebrate hosts. Injection of *D. melanogaster* with *Mycobacterium marinum* causes a lethal infection, killing flies with a 50% lethal dose (LD50) of 5 CFU.ml-1. Importantly, the initial stages of the infection closely resemble the early stages of *M. marinum* infection in vertebrates. Like vertebrates, *D. melanogaster* has bactericidal phagocytic macrophages, known as hemocytes, and during the early stages of infection the bacteria localise to and proliferate within these hemocytes. Thus, *M. marinum* would appear to successfully subvert macrophages in a similar manner in flies and vertebrates. A *D. melanogaster*-*M. marinum* infection model therefore offers a convenient, cost-effective, and entirely novel screening step for new candidate anti-tuberculosis agents. This model uses fluorescence microscopy and classical microbiological techniques to track the infection of *D. melanogaster* hemocytes in real time with *M. marinum*-GFP. The varying outcomes of treatment with and without positive and negative control compounds in a fixed dose regimen (with respect to the compound's minimal inhibitory concentration) will provide a platform for modelling the population dynamics of *M. marinum*-GFP infection in *D. melanogaster* hemocytes. This mathematical model will subsequently be employed to assess the outcomes of experimental compound testing in this system. Fundamentally, this project addresses an important issue in in the TB drug discovery pipeline by a novel and interdisciplinary strategy for advanced-hit compound assessment that meets the principles of replacement, reduction and refinement (3Rs).

# *Dictyostelium discoideum* as a model to study phosphate homeostasis

**Yann Desfougères** and Adolfo Saiardi

*UCL MRC-LMCB, UK*

Phosphate is an essential component of life. However, how phosphate homeostasis is regulated in eukaryotes remains poorly understood. Most of our current knowledge comes from studies in the yeast *Saccharomyces cerevisiae*. The yeast has allowed us to uncover a link between phosphate homeostasis and inositol pyrophosphates. However, many of the genes identified in yeast defining the "PHOsphate pathway" are not conserved in other eukaryotes. We have established the social amoeba *Dictyostelium discoideum* as an alternative model to study phosphate homeostasis in eukaryotes and to investigate the intricate relationship between inorganic phosphate (Pi), inositol polyphosphates (InsPs), and inorganic polyphosphate (polyP). In our quest to define the complex enzymology of amoeba inositol phosphate synthesis, we discovered a new activity for the highly conserved inositol-tetrakisphosphate 1-kinase (ITPK1). This opens a new perspective to understand the function of the highly phosphorylated inositol phosphates that appears to be more complex than previously thought. *D. discoideum* readily incorporates radioactive phosphate [Pi] into IP6 suggesting a high turnover. Surprisingly however, the pool of labelled IP6 has a longer half-life than ATP. We are also investigating how inorganic polyphosphate (polyP) is produced, where it is stored and why it is important during amoeba development. Our recent results show that polyP is secreted during starvation, that this process requires the membrane fusion machinery, and that polyP is degraded after secretion.

# Transmembrane transcription factors implicated in *Dictyostelium* ER-stress signaling

**Eunice Domínguez-Martín**[1,2], Alvaro de Pedroso[1], Olivier Vincent[1], Roberto Coria[2]* and Ricardo Escalante[1]*

[1]*Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM). Madrid. Spain;*
[2]*Instituto de Fisiología Celular, UNAM, D.F, México.*

Conditions that alter the protein folding capacity of the endoplasmic reticulum (ER) may lead to ER stress; to avoid detrimental consequences, ER-status is monitored by transmembrane protein sensors that, by different mechanisms, fire the unfolded protein response (UPR). In plants and animals, the ER-status is quickly communicated to the nucleus by Atf6, an ER membrane-bound basic leucine zipper (bZIP) transcription factor that is trapped at the ER. Under ER-stress, Atf6 is transported to the Golgi where it is processed by regulated intramembrane proteolysis. Free Atf6 bZIP is then transported to the nucleus where it participates in a specific transcriptional response. A BLAST analysis of *Dictyostelium* genome could not detect any Atf6 homologues; though we have identified the presence of a putative transmembrane domain in the protein sequence of four bZIPs: bzpC, bzpD, bzpJ and bzpM. By fusing the amino-terminus of this bZIPs with a GFP tag, we observed that all of them were processed after ER stress, independently of the IreA kinase/ribonuclease mediated UPR pathway. GFP-bzpD and GFP-bzpJ were localized at the ER and were transported to the nucleus in cells under ER-stress. Though, bzpD was accumulated earlier than bzpJ at the nucleus. In addition, the steady state localization of bzpD and bzpJ differed; while GFP-bzpD presented a more homogeneous ER localization, GFP-bzpJ localized at discrete regions over the ER. Further, only GFP-bzpD presented a clear transient accumulation at the Golgi just before it was transported to the nucleus. GFP-bzpM and GFP-bzpC expression could only be detected by western blot. Our data suggests that bzpD may be the *Dictyostelium* Atf6 functional orthologue; while, bzpJ, bzpC and bzpM may regulate other ER-specialized responses. Recently, other ER membrane-bound Atf6-like transcription factors have been identified in human cells; this, together with our results, suggests that ER transmembrane bZIPs may sense and regulate specific ER requirements, integrating a complete homeostatic response, that appeared early during evolution.

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*Co-senior authors.

# The pathways regulating spore and cyst differentiation in the two branches of Dictyostelia show a small conserved core with many branch-specific innovations

**Qingyou Du**, Zhi-hui Chen, Yoko Yamada, Christina Schilde and Pauline Schaap

*University of Dundee, UK*

Unicellular Amoebozoa and many other protists transform into dormant cysts in response to environmental stress. Multicellular Dictyostelia form spores in fruiting bodies when starved, but some species have retained encystation as an alternative survival strategy. Increased cAMP levels acting on PKA trigger spore formation and we previously showed with that this is also the case for amoebozoan encystation. In a search for additional genes that regulate the medically relevant process of encystation, we deleted the genes alxA, amdA, bzpF, dmpA, dokA, splA, srfA, stkA and YakA, which are essential for sporulation or PKA activity in the genetic model *Dictyostelium discoideum*, in the encysting Dictyostelid *Polysphondylium pallidum*. Deletion of all genes but one had no effects on development to spores or cysts in *P. pallidum*. However, deletion of amdA, encoding AMP deaminase, prevented encystation in response to stress and blocked development to fruiting bodies at the tipped mound stage. The cause of the developmental defect was not clear and was also much more severe than in the *D. discoideum* amda- mutant, which still forms fruiting bodies. The encystation defect resulted from accumulation of 5'AMP. Both 5'AMP and its dephoshorylated product adenosine proved to be potent inhibitors of encystation in wild-type cells. This Dictyostelia are subdivided into two main branches with *D. discoideum* and *P. pallidum* belonging to branch II and I, respectively. While each branch inherited some functionally conserved cAMP signalling genes for sporulation and encystation from the amoebozoan ancestor, it appears that many conserved genes acquired different biological roles in the branch I and II lineages.

# Reactive oxygen species contribute to the immune response of *Dictyostelium discoideum* to mycobacterial infection

**Joe Dan Dunn**, Xuezhi Zhang, and Thierry Soldati

*University of Geneva, Switzerland*

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. We are using *Dictyostelium discoideum* as a model phagocyte to delineate the events leading to ROS production and to identify ROS contributions to the immune response to *Mycobacterium* tuberculosis. Phagocyte NOX comprises Nox2, the catalytic subunit, and p22phox, which recruits cytosolic proteins required for activation. *D. discoideum* expresses NoxA, CybA, and NcfA, homologs of Nox2, of p22 phox, and of the NOX regulator p67phox, respectively. We have observed that *D. discoideum* produces ROS when exposed to microbial products and that ROS production is decreased in mutants lacking NoxA or CybA. Phagocyte NOX localizes to the plasma membrane and phagosome. During bead uptake by *D. discoideum*, CybA from the plasma membrane is present on nascent phagosomes and removed prior to acidification. Concomitantly with neutralization, phagosomal CybA is replenished from acidic compartments. During *Mycobacterium* uptake, CybA is rapidly lost from the phagosome and redelivery is impaired. During uptake of beads and *Mycobacterium*, NcfA from the cytosol is enriched at phagocytic cups and transiently associates with nascent phagosomes. NcfA is recruited to the membrane via its Rac-binding domain and potentially couples cytoskeletal remodeling events such as migration and phagocytosis with NOX activation. In intracellular growth assays, *Mycobacterium* mutants lacking ROS-detoxifying enzymes exhibited decreased growth in *D. discoideum*. *Mycobacterium* strains expressing redox-sensitive GFP will be used to assess the oxidative stress experienced by bacteria during infection; this will enable us to determine whether ROS function as signaling molecules or anti-microbial effectors.

# Workshop

## dictyBase 2017: new tools and interfaces

**Petra Fey**, Siddhartha Basu, and Rex L. Chisholm

*Northwestern University, USA*

We are working on a completely new infrastructure for dictyBase. This new cloud based system represents a complete overhaul of both the database and the software that supports it. Here we report the initial release of an advanced query tool named dictyMine and a new ordering system for the Dicty Stock Center (DSC). dictyMine is based on the biological data warehouse system InterMine (http://intermine.org). In this release, all genomes, their annotations, and Gene Ontology (GO) will be available for querying. dictyMine allows you to login and save your queries for later use. Alternatively, users may take advantage of a collection of 'canned' queries to start, e.g. "Protein sequences of curated genes with their UniProt mapping" or "Sequencing Center Ids mapped to Gene Ids". Queries are easily adapted to change the search. The Dicty Stock Center is a widely-used resource for teachers and researchers in diverse institutions worldwide. A couple of years ago, the NIH required that we implement charges, which accelerated the need for an improved and more efficient ordering process. Here we present the new streamlined DSC ordering, which saves the user's time when logged in, and provides the DSC staff with complete information to process the order. The checkout now includes payment information, and provides a more robust and stable interface. While these are the first 'beta' versions of new user interfaces and functions, our work 'under the hood' continues and new items will be released periodically. Slowly the current database will be replaced by the new, producing an entirely new experience for dictyBase users.

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# Functional analysis of the core autophagy protein ATG12

**Sarah Fischer**, Ramesh Rijal, Roman Konertz, Susanne Meßling and Ludwig Eichinger

*Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany*

Macroautophagy is an intracellular degradative pathway that is highly conserved in all eukaryotic cells. The process is crucial for cellular homeostasis and serves as a response to different stresses such as e.g. starvation or the appearance of protein aggregates. During macroautophagy cytosolic material becomes enclosed in newly generated double-membrane vesicles, the so-called autophagosomes. Upon maturation, the autophagosome fuses with the lysosome for degradation of the cargo. Atg12 is one of the core autophagy genes (Atg) and has been shown to be involved in the expansion of the double-membrane of the growing autophagosome. For autophagosome formation, two ubiquitin-like conjugation reactions are indispensable. The ubiquitin-like protein ATG12 plays an essential role in the first conjugation reaction, which results in the formation of a tetrameric complex composed of an ATG16 homodimer and two ATG12-ATG5 heterodimers. This complex then acts as an E3-like enzyme in the covalent linkage of the ubiquitin-like ATG8(LC3) to the phospholipid phosphatidyl-ethanolamine (PE). To decipher the cellular function of ATG12, gene replacement mutants of atg12 in *Dictyostelium discoideum* AX2 wild-type and ATG16$^-$ cells were generated. The generated ATG12$^-$, ATG16$^-$ and ATG12$^-$/16$^-$ cells had complex phenotypes and showed significant similar defects in cellular viability, autolysosome maturation and fruiting body formation, which implies that both proteins together with ATG5 act, as expected, as a functional unit in autophagy. Furthermore, we found that ablation of either one or both proteins also resulted in similar defects in axenic growth, macropinocytosis and protein homeostasis, as evidenced by an increase in ubiquitylated proteins, the appearance of ubiquitin-positive protein aggregates and a decrease in proteasomal activity. In contrast, phagocytosis and spore viability were much more affected in ATG12/16 double knock-out cells, suggesting that both proteins fulfill autophagy-independent functions in these cellular processes where they appear to act in parallel. RNAseq and RT-PCR analyses of AX2 and mutant strains revealed major transcriptional changes in a large number of genes. Among other changes we observed an up-regulation of several core autophagy genes, as e.g. atg5, atg9 and the two atg8 paralogues, indicating a positive feed-back loop for the expression of autophagy genes in the mutant strains. Future studies will focus on: (1) the identification of novel ATG12 interacting proteins, (2) a possible role of ATG12 in the cross-talk between autophagy and proteasomal degradation and (3) the role of ATG12 in autophagy-independent cellular processes.

# Elucidating cytoskeletal dynamics in the social amoeba *Dictyostelium discoideum* using spatiotemporal manipulation of membrane signaling

**Sven Flemming**, Kirsten Sachse and Carsten Beta

*University of Potsdam, Germany*

Motile cells like makrophages, stem cells or cancer cells show complex spatiotemporal pattern formation in the actin cytoskeleton. These patterns can be influenced by external cues like chemoattractants which leads to directed movement but can also occur without an external stimulus, for example as self-sustained actin oscillations or waves. We use the social amoeba *Dictyostelium discoideum* as a model organism to elucidate how different components of the signaling pathways contribute to these actin dynamics. In giant *D. discoideum* cells, which we create by electrofusion, occurrence and interaction of actin waves can be observed in a less restricted environment. Currently we are developing systems that will allow us to manipulate downstream targets of the cAMP signaling pathway. This includes approaches using inducible dimerization systems such as the optogenetic TULIP system that can be utilized to recruit proteins to certain cellular compartments. In another approach, photo uncaging of cAMP is used to induce signaling in selected regions of the cell to investigate how this influences the actin dynamics at the cell cortex. Based on these approaches we hope to provide the basis for detailed spatiotemporal perturbation experiments to probe the dynamics of actin oscillations and waves at the cortex of motile amoeboid cells.

# Oxygen and PhyA drive dramatic metabolic and transcriptional changes during *Dictyostelium* development

**Elisabet Gas-Pascual**[1,4], Honghe Jin[2], Scott Harding[3], Lydgia Jackson[5], David Dyer[5], Muatasem Ubeidat[6], Xiaoxiao Sun[2], Ping Ma[2], CJ Tsai[3], and Christopher M. West[1,4]

*[1]Depts. of Biochemistry & Molecular Biology, [2]Statistics, and [3]Genetics, [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602 USA; [5]Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 71304 USA; [6]Department of Biological Sciences, Southwestern Oklahoma State University, Weatherford, OK 73096 USA*

Sensing and responding to temporal and physical gradients of O2 is key for the survival of all aerobic cells. In response to starvation, *Dictyostelium* amoebae aggregate into a multicellular slug that migrates to the soil surface to become, via an O2-triggered mechanism, a fruiting body. We have identified a novel paradigm in the social amoeba *Dictyostelium*, in which O2 levels regulate the proteome via activation of the SCF family of E3 polyubiquitin ligases. Key to the mechanism are a series of posttranslational modifications, including prolyl hydroxylation by PhyA and glycosylation, of the adaptor subunit Skp1 of SCF complexes. Our previous data indicate that O2 is rate-limiting for prolyl hydroxylation but other factors including intermediates in central carbon metabolism and sugar nucleotide precursors for the glycosyltransferases are likely to also be important. When *Dictyostelium* is starved, ATP generation is fueled by massive proteolysis, which also supports gluconeogenesis to generate precursors for synthesis of cell wall glycoproteins and polysaccharides, and glycogen and trehalose for energy storage. Using GC-MS, we assessed the steady state metabolomic profile of *Dictyostelium* cells at low (2.5%) and high (ambient or 21%) O2. The study identified a large number of compounds. Of the confident assignments, the comparison revealed substantial decreases of amino acids and trehalose and other sugars at low O2, while maintaining critical intermediates in gluconeogenesis and pyrimidine/purine metabolism.

Previous studies revealed that massive shifts in the transcriptome are closely related to changes in morphogenesis, and can be regarded as a molecular phenotype. We therefore enlisted time-course RNA-seq studies to begin to assess how slugs respond to O2 levels and to search for novel regulatory mechanisms that control O2-dependent development. A statistical method was employed to analyze data from five sequential time points (from 10-18 hr) without replicates. Incubation at 5% O2, which blocks the slug-to-fruit switch, led to massive changes in the transcriptome compared to 21% O2. While many of the time course differences could be attributed to an interruption of the developmental program, other changes suggested the occurrence of compensatory reactions whose significance remains to be explored. Disruption of phyA results in an increased O2-requirement for slugs to fruit, from 5-10% for wild-type cells to 21-40%. A parallel RNA-seq analysis was performed on phyA– cells at 5% O2, which did not fruit, and at 21% O2, the threshold at which delayed fruiting was just allowed. This comparison was designed to separate proximal effects of PhyA action from more indirect effects on the developmental program per se, and to possibly identify genes associated with the phyA– override mechanism that allows development at high O2. A surprisingly large number of genes are proximately affected by phyA irrespective of consequences on morphogenesis, suggesting that PhyA and Skp1 may comprise a regulatory hub for a host of homeostatic routines that ensure adaptation to changing environments.

# Nuclear envelope proteins in the amoebozoan *Dictyostelium discoideum*

Petros Batsios, Otto Baumann, Martin W. Goldberg, Marianne Grafe, Denis A. Larochelle, Daria Lisin, Irene Meyer, Kristina Mitic, Xiang Ren, Reimer Stick and **Ralph Gräf**

*University of Potsdam, Germany*

The nuclear envelope (NE) consists of the outer and inner nuclear membrane (INM), whereby the latter is bound to the nuclear lamina. With NE81 we have identified a nuclear lamina protein in the amoebozoan *Dictyostelium* clearly evolutionarily related to metazoan lamins. Microscopic data indicate that tagged versions of full length NE81 as well as NE81 lacking the nuclear localization signal and/or the lipid modification CaaX-signal (GFP/Flag/His-myc-NE81deltaNLSdeltaCLIM) are capable of cell cycle-dependent assembly of higher order structures. Field emission scanning EM of NE81 expressed in *Xenopus* reveals formation of filamentous structures at *Xenopus* nuclei that are very reminiscent of *Xenopus* lamin B2 expressed in the same system. In parallel His-Myc-tagged NE81deltaNLSdeltaCLIM was purified from *Dictyostelium* extracts. The purified protein was soluble at high-salt conditions, but assembled to higher order structures at low-salt conditions as shown by negative staining EM and high resolution light microscopy. Though proximity-dependent biotin identification (BioID) we could identify two protein interactors of NE81 at the INM, Sun1 and Src1. Src1 is a *Dictyostelium* homologue of the helix-extension-helix family of proteins, which also includes the human lamin-binding protein MAN1. Both endogenous Src1 and GFP-Src1 localized to the NE during the entire cell cycle and was enriched at regions of nucleolar attachment to the NE. Electron microscopy and light microscopy after differential detergent treatment indicated that Src1 resides in the INM. FRAP experiments with GFP-Src1 cells suggested that the protein exists in two fractions, a mobile and and immobile one, the latter of which could be stably engaged in forming the nuclear lamina together with the lamin NE81. Src1 interaction with NE81 could also be confirmed in a mis-localization assay where soluble, truncated mRFP-Src1 localized to artificial clusters consisting of an intentionally mis-localized mutant of GFP-NE81. Expression of GFP-Src1_1-646, a fragment C-terminally truncated after the first transmembrane domain, disrupted interaction of nuclear membranes with the nuclear lamina, as cells formed protrusions of the NE that were dependent on cytoskeletal pulling forces. Protrusions were dependent on intact microtubules but not actin filaments. We present evidence that Src1 naturally occurs in two isoforms, one representing full-length Src1 and one corresponding to the shorter Src1_1-646 fragment with only one transmembrane domain. While full-length Src1 is concentrated at sites of nucleolar attachment to the NE, the short isoform may be distributed more evenly along the NE.
Our results indicate that Src1 is required for integrity of the NE and highlight *Dictyostelium* as a promising model for the evolution of nuclear architecture.

# Accumulation of autophagic and ESCRT-machinery: A case of frustrated autophagy?

Lilli Gerstenmaier[1] and **Monica Hagedorn**[1,2]

*[1]past: Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; [2]Research Group Hammann, Jacobs-University, Bremen, Germany*

Disease-causing mycobacteria, such as *Mycobacterium tuberculosis*, establish their initial infection within macrophages by causing phagosomal maturation arrest. While entry into the host cell by phagocytosis is well studied, very little is known how mycobacteria egress to spread the infection at later stages.
In our laboratory we used *M. marinum*, a close relative of *M. tuberculosis*, combined with *Dictyostelium discoideum* to better understand the underlying molecular mechanisms that lead to non-lytic egress of pathogenic mycobacteria from their host cell via the ejectosome structure. By combining, light and high-resolution single cell microscopy we were able to show that the autophagic, as well as the ESCRT machinery are specifically recruited to the distal pole of ejecting bacteria. In mutants of either machinery, ejectosomes are still present, however, cell-to-cell transmission is reduced. Furthermore, microscopy studies reveal that the ejectosome is leaky if autophagy is blocked. We propose that the recruited membrane at the pole might function as a patch to seal the egress site. Furthermore, we hypothesize, that the membrane accumulation at the distal pole represents a usually transient stage during autophagosome closure.

# Mycobacteria-*Dictyostelium* RNAseq reveals transcriptional pathways essential during the infection course

**Nabil Hanna**[1], Astrid Melotti[2], Frédéric Burdet[3], Marco Pagni[3], Pierre Cosson[2] and Thierry Soldati[1]

*[1]University of Geneva, Department of Biochemistry, 30 Quai Ernest Ansermet, Sciences II, Geneva, Switzerland; [2]CMU, Department of Cell Physiology and Metabolism, Geneva, Switzerland; [3]Swiss Institute of Bioinformatics,Vital-IT Unil,CIG, Lausanne, Switzerland*

Tuberculosis remains the most pervasive infectious disease and the recent emergence of multiple or even fully drug-resistant strains increases the risk and emphasizes the need for more efficient and better drug treatments. We use an integrated approach to dissect and model the relationship between mycobacteria and their host. The experimentally versatile *Dictyostelium discoideum – M. marinum* infection model provides a powerful and ethically un-concerning system to study mycobacteria pathogenicity. We have already brought evidence that this is an efficient strategy to identify new anti-infective chemicals. Now, we are using the technological developments in high throughput RNA-sequencing to determine transcriptional signatures triggered by mycobacteria. A full analysis of *Dictyostelium -M. marinum* 'contact' experiment (mixing both organisms for 4 hours at MOI 400) provided a fingerprint of the transcriptome of the host cell in the presence of mycobacteria, and helped us identifying specific markers of the presence of intracellular mycobacteria (GO term: chemotaxis, actin binding, phagolysosome,…). On the other hand, a time-resolved transcriptomic analysis of *Dictyostelium* during infection, covering the critical stages of entry, establishment of a permissive niche, and proliferation was performed to decipher the different host pathways impacted during the mycobacterial infection course.

# *Dictyostelium* versus *Klebsiella pneumoniae*: chemical compounds can tip the balance

**Estelle Ifrid**

*University of Geneva, Switzerland*

On one side of the ring, *Klebsiella pneumoniae* is a gram-negative bacterium responsible for severe nosocomial infections. Worldwide, multi-drug resistant *K. pneumoniae* are a great public health concern.
On the other side, the amoeba *Dictyostelium discoideum* is capable of ingesting and killing *K. pneumoniae* bacteria. It shares conserved molecular mechanisms with macrophages. Our general aim is to understand the molecular mechanisms that determine whether the phagocytic *Dictyostelium* or the pathogenic *K. pneumoniae* survive when they encounter. The specific aim of this study was to identify compounds that affect the outcome of this host-pathogen interaction. *Dictyostelium* phg1a mutant cells kill inefficiently *K. pneumoniae* bacteria and fail to feed upon them. We used this observation to develop an assay adequate to screen a library of compounds. Among 8'000 compounds tested, we identified 14 compounds that restore growth of phg1a *Dictyostelium* on *K. pneumoniae* bacteria. Further characterization revealed that one of these compounds facilitates intracellular killing of *K. pneumoniae* by phg1a *Dictyostelium*. We are currently studying how this compound modifies host-pathogen interactions.

# Workshop

## The Next Dicty World Race

**Daniel Irimia**

*Massachusetts General Hospital, Harvard Medical School, USA*

Building on the success of the past races, the next Dicty World Race will feature a number of technological innovations, logistic changes, and enhanced outcomes. The race will start with a series of "local" competitions, in the lab of the participants. For this, we will employ new network-enabled microscopes and newly designed microfluidic devices shared with the participants by the generosity of our sponsor CytoSmart. The images of dicty cells moving through the microfluidic channels will be automatically uploaded, stored, and analyzed in a cloud. The top 12 fastest and smartest cells will compete in the Final of the Race, organized in Boston, for the Winner Title, which will come with a $3,000 line of credit for purchases from our sponsor BioTechne. A database of motility phenotypes for all the cells tested will be made available to the participants, and will continue to grow through subsequent races or independent efforts, providing a new standard for cell motility for Dicty cells and mutants.

# Autophagy induction reduces polyQ aggregates toxicity in *D. discoideum* as model system

**Mukul Jain**[1, 2], Bano Saidullah[2], and Shweta Saran[1]

[1]*School of Life Sciences, Jawaharlal Nehru University, New Delhi -110067, India;*
[2]*School of Sciences, IGNOU, New Delhi-110068*

A hallmark of CAG repeat disorder is the production of polyQ repeat that forms aggregates in the nucleus and cytoplasm of affected neurons. Proteins with longer polyQ repeats aggregate more rapidly and cause disease at a former age. The cellular mechanism causing toxicity due to polyQ repeats in *D. discoideum* is not yet known. Literature shows that expansion of 103Q in *D. discoideum* forms soluble aggregates, in this study we show that a range of polyQ (43Q-80Q) form aggregates where as in high polyQ (120Q, 216Q) show diffused aggregates pattern. We engineered *D. discoideum* cells to express varied polyQ repeat lengths and show their toxicity levels, growth and development. Aggregates forming strains show multitipped phenotype which is similar to autophagic mutant's phenotype and display a drastic decrease in percent cell viability during growth. Ubiquitin colocalise with polyQ aggregates, showed accumulation of ubiquitin positive aggregates and as well inhibitor of ubiquitin/proteasome pathway had effect on aggregation polyQ in *D. discoideum*, suggesting that the ubiquitination of polyQ aggregates previously noted in mammalian cells may inherently be required for polyQ aggregates degradation. Autophagy, which is a degradation pathway being utilized by the cell to get rid of aggregates was analyzed by using different regulatory drugs and under autophagy mutants. These observations establish *D. discoideum* as a system for studying the polyQ aggregates degradation.

Keywords: Autophagy, Ubiquitin proteosomal system, polyQ, *D. discoideum*

# RapC is required for cytokinesis and cell migration in *Dictyostelium*

Byeonggyu Park and **Taeck J. Jeon**

*Chosun University, Korea*

Ras proteins are small, monomeric GTPases that act as crucial regulators of a number of cellular signaling pathways, including proliferation, cell migration, differentiation and apoptosis. There are 19 Ras subfamily proteins in *Dictyostelium* genome database. The functions of most of these Ras proteins in development and cell migration have not been studied yet. Here we investigated the roles of RapC in cell migration and development. RapC has the highest homology (50.9 % amino acid identity) with RapA, which is a key regulator in cell adhesion and cell migration. rapC null cells displayed flattened and spread morphology and strong cell-substrate adhesion compared to wild-type cells, which are opposite phenotypes to RapA and RapB. In addition, cells lacking RapC had a cytokinesis defect and showed slower migration speed and lower migration indexes than wild-type cells in chemotaxis. These phenotypes of rapC null cells were rescued by expressing GFP-RapC. GFP-RapC was localized to the plasma membrane. These results suggest that RapC plays important roles in cell morphogenesis, cytokinesis, and migration.

# National BioResource Project (NBRP) of cellular slime molds in Japan

**Yoichiro Kamimura**[1,2], Hidekazu Kuwayama[3], Masahiro Ueda[1,2]

*[1]Laboratory for Cell Signaling Dynamics, Quantitative Biology Center, RIKEN, Japan; [2]Laboratory of Single Molecule Biology, Osaka Univ., Japan; [3]Faculty of Life and Environmental Sciences, Univ. of Tsukuba, Japan*

National BioResource Project (NBRP) of cellular slime molds in Japan was started by Dr. Hideko Urushihara in 2007 in order to accelerate *Dictyostelium* studies and increase new users. Our missions include (1) the organization of stable storage and back-up, (2) strategic collection of research-accelerating resources, (3) intensive quality control, (4) infeunicormation and technical support for new users. We have already collected and stored around 1,000 strains and around 400 plasmids which are mainly resources originating in Japan. The stocked strains include field-isolates which are currently contributing to discovery of pharmacological lead compounds for drug development. These resources are available to any person through the website (http://nenkin.nbrp.jp). Please visit the website of NBRP of cellular slime molds and to find materials which may be useful for your research. Furthermore, we accept your deposits of bioresources for *Dictyostelium* studies from around the world. If you have submissions, please contact to us (nbrpnenkin@shigen.info). We would be grateful for your contribution to and comments on this project.

# Mechanics and dynamics of *Dictyostelium discoideum* adhesion

**N.Kamprad**, C.Westendorf, L.Mamoyan, A.Bae, M.Tarantola

*Max Planck Institute for Dynamics and Self-Organization*
*Am Faßberg 17, 37077 Göttingen, Germany*

Motile cells exert traction on the substratum in order to extend anterior pseudopodia and retract the rear. While the cytoskeleton generates the protrusive and contractile forces, interaction of the ventral cell surface with the underlying support is necessary for the transmission of these forces. We focus on *Dictyostelium discoideum* (D.d), analysing cell-substrate interactions. The advantage of this amoeba is that it adheres non-specifically to substrates without using integrins, which are well-studied major adhesion protein in other eukaryotes. Actin-rich areas, called actin foci, well known to be involved in D.d. adhesion processes. We perform co-localization studies of the actin label LimE with other proteins, which are well known to be involved in the process of adhesion. Measurements are carried out using total internal reflection fluorescence microscopy (TIRF) of single cells in the early developmental stage. We follow the dynamics of proteins like the transmembrane adhesion protein SadA or the protein complex Arp2/3, which is involved in the crosslinking of gel-like actin networks. Furthermore the actin anchoring proteins TalinA and TalinB are analysed. In addition, the SCAR-complex, which nucleates the process of actin polymerization, was studied. Moreover, we assessed the distribution of actin foci relevant to adhesion against the foci involved in the process of coating endocytotic vesicles. In a collaborative project, we analysed negative regulators of adhesion and motility by employing knock out strains for the corresponding adhesion proteins and quantified morphology, contact area and adhesion forces by confocal microscopy, reflection interference contrast microscopy (RICM) and single cell force spectroscopy (SCFS).

# ATG16 mediates the autophagic degradation of the 19S proteasomal subunits PSMD1 and PSMD2

Qiuhong Xiong, **Malte Karow**, Sarah Fischer, Susanne Meßling and Ludwig Eichinger

*Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany*

Autophagy and the ubiquitin proteasome system are the two major cellular processes for protein and organelle recycling and clearance in eukaryotic cells. Evidence is accumulating that these two pathways are interrelated through adaptor proteins. We found that PSMD1 and PSMD2, both components of the 19S regulatory particle of the proteasome, directly interact with *Dictyostelium discoideum* autophagy 16 (ATG16), a core autophagosomal protein. ATG16 is composed of an N-terminal domain which is responsible for homo-dimerization and binding to ATG5 and a C-terminal half with seven WD40 repeats. Deletion analysis of ATG16 showed that the N-terminal half of ATG16 interacted directly only with PSMD1, while the C-terminal half interacted with both, PSMD1 and PSMD2. In AX2 wild-type cells RFP-PSMD1 as well as RFP-PSMD2 was enriched in large puncta, reminiscent of autophagosomes. These puncta were absent in ATG16$^-$ and ATG9$^-$/16$^-$ cells and weaker and less frequent in ATG9$^-$ cells, showing that ATG16 was crucial and the autophagic process per se important for their formation. Co-expression of ATG16-GFP or GFP-ATG8a (LC3) with RFP-PSMD1 or RFP-PSMD2, respectively, in ATG16$^-$ or AX2 cells revealed many instances of co-localization, suggesting that RFP-PSMD1 or RFP-PSMD2 positive puncta constitute autophagosomes. Furthermore, we confirmed by LysoTracker labeling and by a proteolytic cleavage assay that PSMD1 and PSMD2 were present in lysosomes. We provide evidence that ATG16 directly interacts with the 19S proteasome regulatory particle components PSMD1 and PSMD2. In vivo, ATG16 is required for their enrichment in ATG8a (LC3) positive puncta which mature into autolysosomes. Thus, ATG16 links autophagy and the ubiquitin protease system.

# Glycogen synthase kinase 3 determines life cycle choice in the encysting Dictyostelid *Polysphondylium pallidum*.

**Yoshinori Kawabe**[1,2], Takahiro Morio[2], Yoshimasa Tanaka[2], and Pauline Schaap[1]

*[1]School of Life Sciences, University of Dundee, Dundee, DD15EH, UK; [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, 305-8572, Japan*

Glycogen synthase kinase 3 (GSK3) regulates many cell fate decisions in animal development. In multicellular structures of the group 4 dictyostelid *Dictyostelium discoideum*, GSK3 promotes spore over stalk-like differentiation. We investigated whether, similar to other sporulation inducing genes, such as cAMP dependent protein kinase (PKA), this role of GSK3 is derived from an ancestral role in encystation of unicellular amoebas. We deleted GSK3 in *Polysphondylium pallidum*, a group 2 dictyostelid which has retained encystation as an alternative survival strategy. Loss of GSK3 reduced aggregate size and inhibited cytokinesis of cells in suspension as also occurs in *D. discoideum*, but did not affect spore or stalk differentiation in *P. pallidum*. However, gsk3- amoebas entered into encystation under conditions that in wild-type favour multicellular sporulation. The gsk3- cells were hypersensitive to osmolytes, which are known to promote encystation, and cyst inducing factors that are secreted during starvation. GSK3 was not itself regulated by these factors, but inhibited their effects. Thus, while PKA is essential for entry into either encystation or sporulation, GSK3 selectively directs dictyostelids into multicellular development.

# Transcriptome analysis of host-pathogen interaction reveals massive increase of specific tRNA sequences during *Mycobacterium* infection

**Kjellin J**., Pränting M., Bach F., Höppner M., Vaid R., Grabherr M., Hagedorn M. & Söderbom F.

*Uppsala University, Sweden*

*Dictyostelium discoideum* is an established model for host-pathogen interactions that have increased our knowledge of the infection process of several intracellular bacterial pathogens. In this study, we performed a global characterization and comparison of the early transcriptional responses of *D. discoideum* upon infection with *Mycobacterium marinum* and *Legionella pneumophila* by high throughput sequencing of both small RNAs and mRNAs. We detect distinct transcriptional responses on mRNA level following the two infections. *M. marinum* infection induce e.g. the ESCRT machinery and multiple autophagy related genes, while genes connected to processes such as phagolysosomes and phagocytosis are repressed. During *L. pneumophila* infection, only one of the autophagy-related genes, atg9, showed increased expression and no induction of the ESCRT machinery was detected. Two RNAi related genes, agnB and eriA, are up-regulated in response to both infections. Comparison with previously published data showed that this increase is not seen in response to Escherichia coli B/r, utilized as food by *D. discoideum*, suggesting a role of RNAi in the response to pathogenic bacteria. *M. marinum* infection have a major impact on the small RNA population. Specific 5' tRNA halves, mainly from tRNA-Asp, constitute almost 40 % of the small RNA sequencing libraries from cells infected by *M. marinum*. In contrast, no increase of tRNA halves was observed in response to *L. pneumophila* infection. Through analysis of publically available small RNA data, we found that 5' tRNA halves are generated also in mammalian cell in response to *M. marinum* and M. smegmatis but not *L. pneumophila* infection, suggesting that this is a conserved response that is either triggered by Mycobacteria or repressed by *L. pneumophila*.

# Bacterial alkaloids prevent amoebal predation

**M. Klapper**, S. Götze, R. Barnett, K. Willing, P. Stallforth

*Leibniz-Institute for Natural Product Chemistry and Infection Biology - Hans-Knöll-Institute Jena, Germany*

Bacteria are constantly exposed to a multitude of threats: bacteriophages can infect and kill bacteria; amoebae, nematodes, and insects can prey on prokaryotes, and competitor strains fight for the same resources. In order to survive in this battlefield, bacteria have evolved highly effective defense mechanisms. [1,2] Because killing and deterring the antagonists are powerful ways to thrive in this environment, bacteria display a great diversity of toxins and antibiotics that selectively act on their enemies. Amoebae are voracious and ubiquitous predators to bacteria that cause constant depletion of huge bacterial reservoirs. This puts both organisms under strong evolutionary selection pressure: the bacteria have evolved mechanisms to prevent grazing and the amoebae must counteract or surmount these mechanisms in order to survive. [3] We focus on the interactions between the eukaryotic soil amoeba *Dictyostelium discoideum* and various soil bacteria. We isolated one particular *Pseudomonas fluorescens* strain from forest soil, which displays great toxicity against *D. discoideum*. Bioassay-guided fractionation allowed the identification of amoebicidal natural products. The discovered pyreudiones bear a tetramic acid moiety and belong to the class of bacterial pyrrolizidine alkaloids. Their structures were elucidated by spectroscopic methods and chemical synthesis. Whole genome sequencing of the producer strain allowed identifying their biosynthetic genes. Generation of gene deletion mutants unable to produce the pyreudiones showed that these toxins are sufficient and necessary to prevent amoebal predation. [4]

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# Actin crosslinking proteins localize based on regionally different affinities for F-actin

Michael Lemieux and **David A. Knecht**

*Univeristy of Connecticut, USA*

Actin cross-linking proteins (ACLP's) represent a subgroup of actin binding proteins (ABP's) that are responsible for the cross-linking of individual actin filaments to form a globally-connected actin cytoskeleton. We have examined potential regulators of the localization of two of the most abundant of these ACLP's, α-actinin (αA) and filamin (FLN). We show that αA and FLN localize distinctly in polarized cells and newly-forming macropinosomes and phagosomes. This distinct localization is not explained by competition between the two proteins for F-actin binding sites. We have used photo-activatable fluorescent protein tagged versions of the proteins (mEOS3.2) to investigate the dynamics of each protein at different F-actin rich structures. The data demonstrates that the dissociation rates of αA and FLN are different depending on the structure to which they are bound. Newly formed F-actin structures at the tips of macropinosomes, phagosomes and protrusions have high affinity for αA and low affinity for FLN. More distal sites, which are presumed to be older F-actin filaments, have a higher affinity for FLN than αA. Further, modification of the F-actin binding affinity of the ACLP's dramatically altered the localization of each of these proteins. Taken together, this data suggests that a major determinant in the distinct localization of different ACLP's is a unique affinity for different F-actin structures within the cell. We hypothesize that the ACLP's are recognizing the difference in structure of ATP-F-actin vs. ADP-F-actin.

# Genome Wide Mutagenesis strategies in *Dictyostelium discoideum* and *Mycobacterium marinum* to decipher the conserved genetic basis of mycobacteria intracellular infections

**Louise H Lefrançois**[1], Tom Mendum[2], Rachel Shrimpton[2], Frédéric Burdet[3], Marco Pagni[3], Graham Stewart[2] & Thierry Soldati[1]

*[1]University of Geneva, Faculty of Sciences, Geneva, Switzerland; [2]FHMS, University of Surrey, Guildford, United Kingdom; [3]Swiss Institute of Bioinformatics, Lausanne, Switzerland*

This study aims at understanding how mycobacteria manipulate the fundamental processes of cell autonomous innate immunity, in particular the phagosomal environment. Because genetic analyses of host factors are difficult in diploid mammalian cells, we propose to use *Dictyostelium discoideum* as a model host phagocyte. Our plan is to develop the host-pathogen system *D. discoideum-M. marinum* as a powerful genetically tractable model. To that purpose, we will apply genome-wide mutagenesis and high throughput sequencing in *M. marinum* and *M. bovis* (Transposon, Tn-Seq) and a similar approach in *D. discoideum* (Restriction enzyme mediated insertion, REMI-Seq). The precise identification and relative abundance of insertions before and after selection will allow us to quantitatively compare the compositions of pools with a high dynamic range. As a proof of principle, we validated the approach by applying the Tn-Seq method for the first time to *M. marinum*. We identified ± 10 % of genes are strictly essentials for growth of *M. marinum* in medium. Then, we tested whether *M. marinum* can use similar carbon sources as *Mycobacterium tuberculosis* (fatty acids and sterols), by performing selections of pools in defined media. In addition, we infected *D. discoideum* and bovine macrophages with M. marium or M. *bovis* Tn pools to identify genes required for survival and replication in these two phagocytes. Our preliminary data highlight that the major virulence mechanisms to infect macrophages are also necessary in *Dictyostelium*, such as the well known virulence factors PDIM or the ESX-1 secretion system. Finally, infection of *D. discoideum* REMI pools with *M. marinum* will reveal host genes implicated in resistance and susceptibility to infection.These promising and innovative approaches will allow a comprehensive definition of the conservation of host and pathogen genes and strategies important for the intracellular interactions during infection of macrophages and *D. discoideum*.

# Tuberculosis boosts HIV-1 infection of macrophages by triggering IL-10/STAT3-dependent tunneling nanotube formation

Shanti Souriant, Luciana Balboa, Karine Pingris, Denise Kviatcovsky, Céline Cougoule, Claire Lastrucci, Aicha Bah, Romain Gasser, Renaud Poincloux, Brigitte Raynaud-Messina, Talal Al Saati, Sandra Inwentarz, Susana Poggi, Eduardo Jose Moraña, Pablo González-Montaner, Marcelo Corti, Bernard Lagane, Isabelle Vergne, Marcelo Kuroda, Maria del Carmen Sasiain, Olivier Neyrolles, Isabelle Maridonneau-Parini, **Geanncarlo Lugo-Villarino** and Christel Vérollet

*Institute de Pharmacologie et de Biologie Structurale (IPBS), Toulouse, France*

Introduction: The tuberculosis (TB) bacillus, *Mycobacterium tuberculosis* (Mtb), and the human immunodeficiency virus (HIV-1) are known to act synergistically and impact the progression of one another in co-infected patients. Yet, the mechanisms involved in amplification of HIV-1 pathogenesis by Mtb remain poorly understood. Recently, we showed that TB skews human monocyte differentiation towards an M(IL-10) anti-inflammatory macrophage program, whose abundance in vivo correlates with disease severity.

Methods: We used two independent in vitro models that mimic a TB-associated microenvironment to differentiate human monocytes into M(IL-10) macrophages. The first model is based on the use of conditioned medium from Mtb-infected macrophages (CmMTB), and the second one applies pleural effusion (PE) fluid from TB patients (PE-TB). M(IL-10) macrophages are then infected with HIV-1, and we measure the level of p24 in the culture supernatants and in HIV-1 infected cells by the appearance of Gag-positive cells by immunofluorescence. A functional profile of these cells is assessed to better understand the effect imparted by TB on HIV-1-infected macrophages. We correlate our in vitro findings in co-infected patients and non-human primates (NHPs).

Results: We reveal that HIV-1 production is exacerbated in M(IL-10) macrophages through the formation of tunneling nanotubes (TNT), which favor viral cell-to-cell transmission. Strikingly, pharmacological inhibition of IL-10/STAT3 signaling or TNT formation fully abolished HIV-1 enhanced production in M(IL-10) cells. Those M(IL-10) macrophages accumulate in blood of co-infected patients and in lungs of experimentally co-infected NHPs, which correlates with disease severity. Finally, TNT-like structures among M(IL-10) cells were found in the lung of co-infected NHPs.

Conclusions: Our study shows that M(IL-10) macrophages are key players in exacerbation of HIV-1 infection in a TB co-infection setting, and reveal TNT formation as an unsuspected target to develop novel therapeutic approaches against HIV-1/Mtb co-morbidity.

# Morphogenesis of social amoeba *Dictyostelium discoideum* as an inspiration for swarm robotics: aggregation phase

**Mohammad Parhizkar**, Giovanna Di Marzo Serugendo

*University of Geneva, Switzerland*

As computer scientists, we are particularly interested in understanding the different phases of *D. discoideum* behaviour, and providing agent-based models we can later integrate into artificial engineered systems. In the last decade, bio-inspired multi-agent systems have become increasingly useful for the design of artificial systems. Social insects such as termite, honeybee and ant colonies provide examples of swarm intelligence. Besides them, *Dictyostelium discoideum* provides another well-known example thanks to its ability of self-aggregation; dynamic self-assembly and self-disassembly. This plays a significant role in their development process for building complex multicellular organisms. Our aim is to study and understand the different life cycle phases of *D. discoideum*, developing a multi-agent model to ultimately use this model to engineer swarm of robots. In our model, the environment communicates through reaction-diffusion mechanisms and the self-organizing agents move following excitation fronts. We show that despite its simplicity the model exhibits interesting self-organizing properties. Our model is a discrete dynamical system in time, space and state. This paper provides a description of our agent-based model for a part of the aggregation phase of *D. discoideum* life cycle, which goes from starvation, to streaming and aggregation territories. The model is composed of two parts: the cells' behaviour during the pre-aggregation phase (starvation and synchronizing for starting the aggregation); and the cells' behaviour during the aggregation phase (regular cell vs. center). The key character of our model is the cells' self-assessment and self-generated gradients arising from six chemical factors: PSF, CMF, Adenosine, cAMP, PDE and CF released by each individual amoeba. In pre-aggregation, cells use quorum sensing to investigate the food ratio to amoebae population size by PSF. If the concentration of PSF is above the threshold that means the starvation has happened and the cells need to inform the others by releasing CMF. Whenever the concentration of CMF is above the threshold, cells start the aggregation phase. During the aggregation phase, selection of centers happens first. Becoming an autonomous center depends on cell's age, concentration of CMF and concentration of adenosine. Cells use two levels of quorum sensing, one to choose the proper aggregation cluster to join, and all other for formation of new (late) autonomous centers. When concentrations of cAMP and CF are both high enough, cells discover that the population needs a new center. Finally, we also modeled "Chemotaxis", where cells decide to move based on the highest gradient of cAMP. In this work, Matlab simulations show interesting multicellular behaviors, such as stream formation, homogeneous size territories, late centers and centers inhibition. The obtained results show that a simple and effective model of individual cells is able to exhibit emergent properties resulting from the interaction among individual cells. Future work will consider models for the remaining phases of D. discoideum behaviour, and translation of this model into artificial systems, such as swarms or micro-robots.

# CBP7 plays a negative role in cell migration and development in *Dictyostelium*

**Byeonggyu Park** and Taeck J. Jeon*

*Chosun University, Korea*

Calcium ions are involved in a variety of cellular signaling. In *Dictysotelium*, fourteen genes encoding calcium binding proteins (CBP) have been found. The functions of most of these CBP proteins in development have not been studied yet. CBP7, one of 14 CBPs, is composed of 169 amino acids and contains four EF-hand domains. Here, we investigated roles of CBP7 in *Dictyostelium* development and found that CBP7 appears to play some important roles in the cell aggregation step during development. Surprisingly, cells expressing CBP7 showed severe development defects, complete loss of development, while wild-type cells formed aggregates within 6-8hr development and finally fruiting bodies. CBP7-overexpressing cells did not even aggregate and just stayed at the single-cell growing stages in the development-inducing condition. Cell sorting experiments using mixed cells of wild-type cells and CBP7 overexpressing cells confirmed that CBP7-overexpressing cells had much lower migration and aggregation speed in the aggregation stage of development compared to wild-type cells. The four EF-hand domains in CBP7 appear to be essential for functions of CBP7 in development. Cells expressing CBP7 with mutations in the EF domain showed normal development. Our results suggest that CBP7 plays an important role in development through the four EF-hand domains, possibly calcium binding.

# Rapid and efficient genetic engineering of *Dictyostelium discoideum* amoeba without the need for axenisation

**Peggy I Paschke**, David A Knecht, David Traynor, Thomas D Williams, Robert H Insall, Jonathan R Chubb, Robert R Kay, Douwe M Veltman

*MRC-LMB Cambridge, UK*

*Dictyostelium* has a mature technology for molecular-genetic manipulation, based around transformation using several different selectable markers, marker re-cycling, homologous recombination and insertional mutagenesis, all supported by a well-annotated genome. However this technology is optimized for mutant axenic cells growing in liquid medium and is difficult to apply to wild-type cells, which grow only on bacteria. Moreover, the mutations that are present in axenic lab strains disturb Ras signalling. This results in execessive macropinocytosis and impedes cell migration, potentially confusing the interpretation of signal transduction and chemotaxis experiments what makes non-axenic wild-type cells a much better model system for those approaches. We have made a series of incremental and more radical improvements to *Dictyostelium* molecular genetics, basing our methods around selection on bacteria, and including improved transformation protocols and vectors that are at the same time workable under axenic conditions. These methods allow wild-type cells to be manipulated as easily as axenic ones. Additionally genes can be knocked out that are conditionally essential for macropinocytotic growth. An extra advantage is the speed up of transformations for all strains due to the quicker cell growth on bacteria. A workable number of cells can be obtained in a few days rather than weeks.

# Iron homeostasis in *Dictyostelium*: potential role of putative ferric reductases.

**Barbara Peracino** & Salvatore Bozzaro

*University of Torino, Italy*

Iron is the most abundant transition metal in all living organisms, and is essential for several cellular activities, including respiration, oxygen transport, energy production and regulation of gene expression. Iron starvation is used by professional phagocytes, from *Dictyostelium* to macrophages, as a form of defence mechanisms against intracellular pathogens. We have shown that *Dictyostelium* cells express the proton-driven iron transporter Nramp1 and the homolog NrampB (Nramp2) in the membrane of phagosomes and the contractile vacuole, respectively. Their disruption affects *Dictyostelium* susceptibility to infection by *Legionella pneumophila* and *Mycobacterium* avium. Nramp1 and NrampB transport ferrous but not ferric ions. The *Dictyostelium* genome does not contain ferric reductases of the STEAP family, which in macrophages are responsible for ferric ion reduction in phagosomes/endosomes. It harbours, however, three genes encoding putative Fe(III) chelate reductases of the cytochrome b561 family. To assess their potential role in *Dictyostelium* iron homeostasis and resistance to pathogenic bacteria, we have cloned the genes and generated KO mutants as well as GFP-fused proteins. One of the three gene products is expressed in the plasma membrane and transiently in phagosomes/macropinosomes, whereas the remaining two are expressed in the membrane of the endoplasmic reticulum. Single and double mutants for two of the three genes have been generated, whereas disruption of the third gene (DDB_G0279437) has been unsuccessful, despite repeated attempts, suggesting that its disruption is probably lethal. The single and double KO mutants generated so far display reduced growth rate on E. coli B/2 and K. aerogenes. Development is normal and susceptibility to *L. pneumophila* infection is unaltered. Attempts are under way to generate a conditional triple mutant, to assess potential compensatory effects of the DD_BG0279437 gene product.

# Untangling the complexity of torc2 regulation by using pia/rictor mutants

**Barbara Pergolizzi***, Cristina Panuzzo*, Salvatore Bozzaro* and Enrico Bracco°

**Department of Clinical and Biological Sciences and °Department of Oncology, University of Turin, Italy*

The activation of the RasC-TORC2-AKT/PKB module, upon cAMP binding to Car1, regulates cell polarization during *Dictyostelium* chemotaxis. TORC2 also mediates GPCR-dependent stimulation of adenylyl cyclase A (ACA), enhancing cAMP relay and developmental gene expression. Thus, mutants defective in the TORC2 Pia/Rictor subunit, such as the temperature-sensitive mutant HSB1, encoding a point mutation at codon 917 (G917D), are impaired in chemotaxis and development. Near-saturation mutagenesis of HSB1 mutant led to isolation of a suppressor mutant, named HSB1HECTPH1- (Pergolizzi et al. 2017), in which spontaneous chemotaxis and development were restored. TORC2-dependent PKBs phosphorylation and chemotactic cell polarization were rescued, whereas PIA-dependent ACA stimulation was not restored but bypassed. The hectphA gene responsible for the phenotype reversion encodes for a HECT ubiquitin ligase, homologous to mammalian HERC1, but contain a PH domain. How HECTPH1 inactivation contributes to the phenotype reversion remains to be ascertained. Whether the Pia G917D mutation affects TORC2 integrity/assembly or interaction with substrates is unknown. In C. elegans a single point mutation of the Rictor gene at codon 1120 (G1120E) resembles the rictor deficiency phenotype (Jones et al., 2009). By mimicking this mutation in the human Rictor ortholog (G934E), severe impairment in the Rictor-Sin1 interaction, and thus TORC2 assembly, has been detected (Aimbetov et al 2012). Since the aminoacid residue G917 is conserved throughout evolution, corresponding to the human Rictor codon 884, we started a functional study to assess whether the Pia G917D mutation affects TORC2 integrity in *Dictyostelium* and mammalian cells. We show that both, the Pia G917D mutation and Rictor mutation G934E, corresponding to G963E in PIA, when overexpressed in AX2 cells cause developmental delay in *Dictyostelium* cells and affect TORC2 assembly/integrity. Experiments are in progress to assess if the corresponding Pia G917D mutation in mammals altersTORC2 assembly/integrity, similar to the Rictor G934E.

# Altruism, cheating, farming: are they adaptive in the wild:

**David Queller**, Suegene Noh, Katherine Geist, Tyler Larsen, Xiangjun Tian, Joan Strassmann

*Washington University in St. Louis, USA*

*D. discoideum* has become a model system for the evolution of social interactions in the lab, both for altruism and cheating within the species and for a symbiotic farming interaction with *Burkholderia* bacteria. The problem with this and other microbial model systems is that we cannot watch the interactions in the wild habitat, making it hard to assess their natural adaptive significance. We therefore developed several novel indirect strategies for assessing the importance of cheating, altruism, and farming in the wild. For cheating, we tested the prediction that cheating genes involved in continual evolutionary conflict would show more adaptive evolution than other genes. We identified potential cheating genes using RNAseq at the tight aggregate stage to pull out genes that changed expression in chimeras relative to clonal aggregates. Consistent with the cheating conflict prediction, those genes showed more adaptive evolution than other genes. We then tested whether prestalk cells are selected by altruistic kin selection or by direct selection to reproduce. Again using RNAseq data, this time to identify genes upregulated in prestalk versus prepore cells, we tested theoretical predictions about the relative amounts of selection and variation in those genes. Kin selection should weaken selection in prestalk cells in proportion to their relatedness to spore cells, which because relatedness is quite high, should result in little weakening. Direct selection on prestalk cells should weaken selection on them, compared to prespore cells about four-fold because prestalk cells are about four times rarer than prespore cells. Using snp variation as an index of the strength of selection (more selection -> less variation), the results prove consistent with kin selection and strongly inconsistent with direct selection. For the symbiosis with *Burkholderia*, we wanted to understand whether this trait was on balance beneficial or harmful in the wild. We allowed both *Burkholderia* and *D. discoideum* to evolve separately in the lab reasoning that, in the absence of the partner, each would tend to lose adaptation associated with partner interactions. If adaptation was naturally mutualistic, loss of those traits would make the evolved lines more harmful. If adaptation was antagonistic, the losses would make them more beneficial. Early results of this experiment are mixed, but with a trend towards supporting the mutualism hypothesis.

# *Dictyostelium discoideum* cell-intrinsic immunity

**Lyudmil Raykov**, Thierry Soldati

*University of Geneva, Switzerland*

The social amoeba *Dictyostelium discoideum* is a recognized model phagocyte to study processes of cell-intrincic defences, which are conserved in professional phagocytes of the animal immune system, such as macrophages or neutrophils. We use *D. discoideum* as a host cell for the pathogenic bacterium *Mycobacterium marinum*, a close relative of *Mycobacterium* tuberculosis, to study the molecular mechanisms of the host-pathogen interactions during infection. Following uptake by the host cell, *M. marinum* inhibits host defense mechanisms such as autophagy and lysosomal-dependent degradation, to establish a permissive niche with endosomal features, where it replicates. Studies in animals showed a group of interrelated host factors involved in stress signaling transduction and pathogen detection. These proteins are the E3-type ubiquitin ligases TNF-associated factors (TRAFs), the guanylate binding proteins (GBPs, a family of cytokine-induced large GTPases), the tripartite motif (TRIM) proteins known to play a dual role as receptors and regulators of autophagy, and finally the signal transducers and activators of transcription (STATs). However, little is known about the upstream sensors, recognizing the pathogen and promoting activation and recruitment of these factors, and the downstream actors, relaying the stress signal and leading to differential expression of defense genes. Therefore, to identify relevant relationships and potential partners in these immune pathways, we are exploiting and characterizing the, TRAF, GBP, TRIM and STAT orthologs in *D. discoideum*. We are monitoring by live microscopy the intracellular localization of all these proteins, previously tagged with fluorescent proteins, in normal and stress conditions. In addition, we are analysing mycobacterial survival within *D. discoideum* knockout cells lacking these factors. Finally, we aim at identifying the targets of the infection-relevant STAT transcription factors and at screening for relevant interaction partners by applying the ChIP (chromatin immunoprecipitation) and APEX2 (proximity labelling with ascorbate peroxidase) techniques, respectively.

# Functional characterization of *Dictyostelium discoideum* UBXD9

**Jana Riehl**, Ramesh Rijal, Christoph Clemen and Ludwig Eichinger

*Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany*

The homo-hexameric triple A ATPase p97, also known as VCP, is involved in a variety of cellular processes as e.g. autophagy, chromatin-associated degradation and ER associated degradation (ERAD). To fulfil its distinct functions, p97 interacts with a large number of different cofactors. The family of Ubiquitin Regulatory X (UBX) domain containing (UBXD) proteins constitutes the largest subgroup of p97 binding proteins. The UBX domain is an evolutionarily conserved 80 amino acids region that shares structural similarity with ubiquitin and is usually located in the C-terminal region of UBXD proteins. In addition, UBXD proteins can contain further ubiquitin related protein motifs in the N-terminal part. Co-immunoprecipitation experiments using total cell lysates from *Dictyostelium discoideum* cells ectopically expressing p97WT-RFP resulted in the mass spectrometry-based identification of an uncharacterized UBXD protein. Sequence analyses showed that this protein with an apparent molecular mass of 95 kDa is one of 11 UBXD proteins in *D. discoideum*. Similarity searches further revealed that the *Dictyostelium* protein is the orthologue of the human TUG (Tether containing UBX domain for GLUT4) protein, also known as ASPL or UBXD9, which is one out of 13 members of the human UBXD protein family. UBXD9 proteins contain the conserved TUG-UBL1 (TUG-Ubiquitin Like 1) domain of approximately 60 amino acids at the N-terminus and an UBX domain in the C-terminal part. Furthermore, our sequence analyses revealed that all UBXD9 members harbor in addition a coiled coil domain preceding the UBX domain. *D. discoideum* UBXD9 is localised in the cytoplasm and in the nucleus. Previous studies found a corresponding localisation of the human TUG/ASPL/UBXD9 orthologue. Similar to human UBXD9, *D. discoideum* UBXD9 interacts directly with p97 and it is thought, that UBXD9 trimers cause the disassembly of p97 hexamers to monomers. To identify and characterize the p97 interacting domains of UBXD9 further, we created GST-tagged UBXD9 truncation constructs and performed pull down assays. The results showed that all truncation products that contain the UBX domain strongly interact with p97. In addition, the N-terminal TUG-UBL1 possibly mediates weak binding to p97. We will next examine which UBXD9 domains can trigger the disassembly of p97 hexamer, to elucidate the mechanism of UBXD9-mediated p97 monomerisation. We hypothesize that p97 monomerisation constitutes an important regulatory mechanism of p97.

# *Bordetella bronchiseptica* modulates *Dictyostelium discoideum* phagocytic pathway and avoids predation

**Israel Rivera**, Valerie E. Rayman, Kalyan K. Dewan, Shannon M. Wagner, Eric T. Harvill

*University of Georgia, USA*

*Bordetella bronchiseptica* is a gram-negative cocobacili that infects the respiratory tracts of mammals, including immuno-compromised humans. Recent work has shown that *B. bronchiseptica* is able to exploit the social amoeba *Dictyostelium discoideum* as an environmental reservoir. Upon internalization *B. bronchiseptica*'s hijacks *D. discoideum* intracellular environment and acquires protection against environmental hazards (e.g., antibiotic treatment). To understand the mechanism that enables the bacteria to persist intracellularly, we characterized *D. discoideum* transcriptional changes during *B. bronchiseptica* internalization. We exposed *B. bronchiseptica* to *D. discoideum* in a 100:1 ratio for one hour, followed by one hour of Gentamicin treatment to kill extracellular bacteria. RNA was then isolated and sequenced (RNA-seq) for downstream analysis. In our results we observed a downregulation of *D. discoideum* genes that are involved in early membrane trafficking. These include cell signaling manA and nagA as well as a large number of actin polymerization genes. In contrast, we observed the upregulation of genes involved in transmembrane movement of indigestible substances including: abgG2 and abgG21. Our results suggest that upon internalization, *B. bronchiseptica* disrupts *D. discoideum* ability to digest bacteria by modulating cytoskeletal movement and vesicle transport, thus leading to bacterial persistence inside amoeba. Ultimately, understanding the mechanism by which *B. bronchiseptica* is capable of exploiting amoeba as an environmental niche will expand our knowledge on the bacteria virulence spectrum and serve as a model to understand key genes involved in intracellular persistence.

# Identification of a new chaperone that suppresses polyglutamine aggregation in *Dictyostelium*

Stephanie Santarriaga, Adam Kanack, Alicia Fikejs, Holly Haver, **Matt Scaglione**

*Medical College of Wisconsin, USA*

The polyglutamine diseases are a group of nine dominantly inherited neurodegenerative diseases caused by the expansion of polyglutamine tracts within specific genes. Expansion of these polyglutamine tracts results in aggregation of the proteins encoded by these genes, ultimately resulting in neuronal death. Unlike other model organisms, *Dictyostelium* naturally contains numerous proteins with long polyglutamine repeats, and we have shown that *Dictyostelium* has an unusual ability to resist protein aggregation. Here, we have performed an unbiased forward genetic screen and identified a single *Dictyostelium* specific gene responsible for suppressing polyglutamine aggregation. We show that this gene suppresses polyglutamine aggregation in *Dictyostelium*, and deletion of this gene results in similar amounts of polyglutamine aggregates in *Dictyostelium* as observed in human cells. We further show that this protein suppresses polyglutamine aggregation in human cells, selectively targeting aggregation prone proteins for degradation by the proteasome. Interestingly, this *Dictyostelium* protein selectively targets aggregation prone polyglutamine proteins for degradation and has no effect on the levels of soluble polyglutamine expanded protein. Together our data identify a novel route to suppress polyglutamine aggregation in *Dictyostelium*, and show that this route may be harnessed to prevent polyglutamine aggregation in human cells.

# Cell-substrate adhesion and contact guidance of *Dictyostelium discoideum* on surfaces of varying geometries

C. Blum[1], **M. Schröder**[1,2], M. Tarantola[1], E. Bodenschatz[1,2]

*[1]Max Planck Institute for Dynamics and Self-Organization, Am Fassberg 17, 37077 Göttingen; [2]Institute for Nonlinear Dynamics, Faculty of Physics, Georg-August University, Friedrich-Hund-Platz 1, 37077 Göttingen*

In embryogenesis as well as in immunology, cell-substrate adhesion is essential for directed migration of cells. Furthermore, less complex organisms such as the social amoeba *Dictyostelium discoideum* (D. d.) hunt for bacteria in the soil requiring directed migration, too. Since D. d. cells lack stress fibers and integrins, they are highly suitable to investigate the physics of cell adhesion and motility. To understand the role of cortical actin during cell-substrate adhesion, we inhibited the Arp2/3 complex with the inhibitor CK666 and characterized initial adhesion employing single cell force spectroscopy. Furthermore, we used electric cell-substrate impedance sensing to relate the inhibition of the Arp2/3 complex of D. d. cells to their shape oscillations and the initial adhesion. Until now, many studies on the D. d. are performed on planar surfaces whereas in its natural environment, the soil, migrating D. d. cells are exposed to a porous, anisotropic and often fluid-filled environment. For this reason, we assess D. d. cells crawling on optical fibers as well as on designed sinusoidal surfaces. The surfaces vary in period as well as in amplitude and have both planar and circular wave shapes. Motion is characterized by the curvature anisotropy parameter. We show that D. d. cells statistically prefer the direction of the radius of maximal curvature. Furthermore, by fluorescence labeling and 3D imaging via spinning disc confocal laser scanning microscopy of contributors to adhesion, we try to understand this symmetry breaking. Further research comprises the analysis of curvotaxis with the help of finite element simulations reconstructing the behavior on curved surfaces.

# Workshop

## Working toward proof-by-control of the signaling rules governing collective decision making in *Dictyostelium*

**Allyson E. Sgro**[1,2] and Thomas Gregor[1]

*[1]Joseph Henry Laboratories of Physics and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA; [2]Current Address: Department of Biomedical Engineering, Boston University, Boston, MA, USA*

*Dictyostelium discoideum* is a classic model system for studying cellular decision making in many different contexts, ranging from chemotaxis, to quorum sensing, to developmental fate. While several of these decisions are regulated at some level by the dynamics of intra- and intercellular cyclic AMP (cAMP) signaling within and between single cells, only recently has quantitative visualization of cAMP signaling at the single-cell level become possible; quantitative control of a single cell, though, has remained elusive. To allow for direct quantitative control of cAMP production to interrogate how decision making is driven at the level of single cells, we have implemented a light-activated adenylyl cyclase with low dark activity and highly-tunable cAMP production over the concentration ranges critical during collective signaling and aggregation in *Dictyostelium*. Using this tool, we were able to test the potential spatiotemporal signaling rules that *Dictyostelium* follows during the aggregation process. We have found that while the aggregation process is robust to many different signaling frequencies, quorum sensing appears to override cAMP signaling. Going forward we are working to elucidate the rules governing aggregation center formation and probing the limits of our control over decision making in a collective multicellular system.

# PkcA regulates chemotaxis in *Dictyostelium discoideum*

**Sean Singh**, Isabella Jansen, Derrick Brazill

*Hunter College, USA*

*Dictyostelium discoideum* is reliant on proper chemotaxis for growth and development. During vegetative growth, amoebae hunt bacteria by chemotaxing towards bacterial metabolic byproducts, such as folic acid. During early development, amoebae aggregate by migrating up cAMP gradients generated by starving cells. In later development, cAMP chemotaxis is used for cell sorting and morphogenesis. In all cases, it is critical that the cells be capable of moving efficiently towards a chemoattractant source. While cAMP chemotaxis has been extensively studied in *Dictyostelium*, many of the signaling molecules involved in linking chemoattractant reception to motility are still not well characterized. One such molecule is PkcA, a *Dictyostelium discoideum* Protein Kinase C-orthologue. We have previously shown that aggregation in starving cells is dependent on PkcA and that PkcA plays a role in organizing the actin cytoskeleton in response to cAMP, suggesting a role for PkcA in regulating cAMP chemotaxis. To examine the function of PkcA during chemotaxis, we utilize the well-established under-agarose chemotaxis assay. We find that under a cAMP gradient, starved cells lacking PkcA have decreased speed, directionality, and chemotactic index, when compared to wild-type cells. This suggests that PkcA plays a role in regulating multiple parts of cAMP chemotaxis. Additionally, cells lacking PkcA have a decreased chemotactic index towards folic acid when compared to wild-type cells, suggesting that PkcA may play a role in folic acid signaling in vegetative cells. Taken together, our results suggest that PkcA may be involved in linking chemoattractant reception to cytoskeletal organization, in both vegetative, and starving cells.

# Functional analysis of wild-type and N471D strumpellin in *D. discoideum*

**Lin Song**[1], Ramesh Rijal[1], Laura Park[2], Robert Insall[2], Rolf Schröder[1], Christoph S. Clemen[1] and Ludwig Eichinger[1]

*[1]Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany;*
*[2]Beatson Institute for Cancer Research, Switchback Road, Glasgow G61 1BD, UK*

Hereditary Spastic Paraplegias (HSPs) are clinically characterized by lower limb weakness and spasticity. Several point mutations of human strumpellin (Str), with N471D being the most frequent one, have been shown to cause HSP (SPG8). To investigate the molecular function of wild-type and StrN471D, we generated *Dictyostelium discoideum* $Str^-$ cells as well as cells that ectopically express StrWT-GFP or StrN471D-GFP in AX2 wild-type and $Str^-$ cells and analyzed the resulting strains. Here, we describe their phenotypes in comparison to AX2 in cell division, cell growth, macropinocytosis, exocytosis, lysosomal properties and secretion of lysosomal enzymes. We found that knock-out of strumpellin resulted in significant defects in all of these cellular processes. Expression of StrWT-GFP in $Str^-$ cells rescued all of the observed defects while expression of StrN471D-GFP could only rescue some of the defects, indicating the importance of the StrN471D residue for full functionality of the protein. The results indicate that strumpellin plays a key role in the endo-lysosomal system. The N471D mutation apparently interferes with some of the essential functions of strumpellin in this system. In summary, our data provide a basis for a better understanding of the molecular mechanism of SPG8.

# Subcellular positioning of nuclei in multinuclear *Dictyostelium* cells

**Maike Stange**[1],Marius Hintsche[1],Kirsten Sachse[1], Angelo Valleriani[2], Alexander Anielski[1], Matthias Gerhardt[1], Carsten Beta[1]

[1]*Institute of Physics and Astronomy, University of Potsdam, Potsdam, Germany;* [2]*Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany*

A fundamental question of cell biology is how cells establish and maintain a well-defined size. Here we investigated to what extent the microtubule cytoskeleton can set a predefined cell size, independent of an enclosing cell membrane. We used a new self-established aggregation method and subsequent electropulse-induced cell fusion to form giant multinuclear cells of *Dictyostelium discoideum*. Based on confocal imaging of cells that expressed mRFP-histone2B and GFP-α-tubulin as fluorescent markers, we determined the subcellular distributions of nuclei and centrosomes in the giant cells using a computer based analysis. The two- and three-dimensional imaging results showed that the positions of nuclei in giant cells do not fall onto a regular lattice. However, a comparison with model predictions for random positioning revealed that the subcellular arrangement of nuclei in giant cells maintains a low but still detectable degree of ordering. This can be explained by the steric requirements of the microtubule cytoskeleton, as confirmed by the effect of thiabendazole.

# *Dictyostelium discoideum* is providing insight to intracellular infection stages of *Aspergillus fumigatus*

**Iuliia Viediernikova** [1,2], Joe Dan Dunn [3], Thierry Soldati [3] and Falk Hillmann [1]

*[1] Junior Research Group Evolution of Microbial Interaction, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena, Germany; [2] Friedrich Schiller University Jena, Germany; [3] Department of Biochemistry, University of Geneva, Switzerland*

In the past years *Dictyostelium discoideum* has become a compelling and conclusive phagocyte model to study the defence strategies of pathogens against innate immunity. Fates of bacterial infections are increasingly deciphered with the help of *D. discoideum*, but this model has thus far a minimal application in the field of fungal biology. The human pathogenic fungus *Aspergillus fumigatus* causes fatal infections in immunocompromised individuals. According to the current knowledge, highly specific virulence factors are absent in this fungus. This suggests that some general fungal virulence traits have emerged before the appearance of innate immune systems of vertebrates. Furthermore, filamentous fungi like *A. fumigatus* and soil amoeba like *D. discoideum* or even fungivorous species are sharing similar natural environments for millions of years. Investigating this antagonistic interaction could help us to identify factors that drive the infection process. One crucial trait of *A. fumigatus* is the ability of its small, airborne spores to withstand alveolar macrophages through an arrest of phagolysosome maturation. In previous studies, we have shown that major virulence determinants, like certain mycotoxins as well as the green pigment dihydroxynaphtalene (DHN)-melanin protect *A. fumigatus* against the soil amoeba *D. discoideum*. Spores lacking a melanin layer were internalized at higher rates and phagosomes matured through fusion of lysosomes. These phagolysosomes sequentially acquired different proteins (Rab GTPases, V-ATPases) during maturation, ultimately leading to progressive acidification. Interestingly, amoeba cells infected with wild-type conidia were acidified notably less than melanin deficient mutants, leading to maturation arrest of their phagolysosomes. Using single cell pH assays, we could show that acidification with DHN-melanin deficient conidia was transient and was followed by neutralization of the phagolysosome. Using fluorescent reporters, we found that in *D. discoideum* the non-catalytic subunit VatB was recruited to phagosomes within the first 40 min independent if conidia of the wild-type or of the melanin deficient mutant were ingested by the amoeba. Nevertheless, the pH with melanized conidia remained constant suggesting that the amoeba ATPase was either defective or its assembly was inhibited. CybA, the orthologue of the small NADP oxidase (NOX) subunit of innate immune cells was recruited to phagolysomes at later stages, but occurred independent of any acidification event. At later stages of infection, potential damage was detected in infected cells through the recruitment of the repair escort machinery and autophagy markers. We are currently aiming to elucidate how *A. fumigatus* targets the host cell. This knowledge could provide a vital key to better antifungal drug design.

# Insights into macropinocytosis from high throughput flow cytometry

**Thomas D. Williams**, Robert R. Kay

*MRC-Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, UK, CB2 0QH*

Axenic *Dictyostelium discoideum* strains are able to grow in liquid media due to a large increase in fluid uptake by macropinocytosis (MP) allowing them to obtain more nutrients than their parental wild isolates. MP has also been implicated in host invasion by certain pathogens, antigen sampling by immune cells, prion spreading and proliferation of Ras-driven cancers. Despite the biological and clinical importance of MP, relatively little is known about it. Recent work by our lab has revealed some of the genetic and molecular underpinnings of MP in *D. discoideum*. The main causative mutation that allows axenic growth is deletion of the RasGAP NF1. In the absence of NF1 patches of active Ras at the plasma membrane, which activate PI3Ks to make PIP3, are larger. Around the edge of the active Ras/PIP3 patch a circular ring of the SCAR complex is formed and directs actin polymerization to form a cup-shaped structure. Macropinosome formation is completed when this closes, internalising a droplet of fluid. We have developed a high throughput flow cytometry assay in order to measure the rate of MP with single cell resolution simultaneously in many strains or conditions. Compared to previous ways of measuring MP the experimental scale made possible by this technique allows far more variables to be assessed and thus facilitates more detailed investigation. We have used this technique to identify nutrients that cause MP to be upregulated and have investigated the physiological regulation of MP. A set of knockout mutants has also been screened for defects in MP and investigated further using confocal microscopy in order to identify more key proteins and improve our knowledge of macropinocytic cup formation.

# Metabolism of the signaling molecule glorin during early multicellular development of *Polysphondylium pallidum*

Daniel Raszkowski[1], Robert Barnett[2], Luke Tweedy[3], Robert Insall[3], Dirk Hoffmeister[4], Pierre Stallforth[2], **Thomas Winckler[1]**

*[1]Pharmaceutical Biology, Institute of Pharmacy, Friedrich Schiller University Jena, Germany; [2]Junior Research Group 'Chemistry of Microbial Communication', Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany; [3]Cancer Research UK Beatson Institute, Glasgow, UK; [4]Pharmaceutical Microbiology, Institute of Pharmacy, Friedrich Schiller University Jena, Germany*

Glorin was first described by John Bonner and colleagues as the acrasin that mediates aggregation of *Polysphondylium violaceum*. It seems that polysphondylids, which are represented in group 2 of the phylogenetic tree and are distantly related to *P. violaceum*, and also many group 1 species including *D. fasciculatum* have the capacity to break down glorin and respond chemotactically to the resulting glorin gradient. This suggests that glorin-based communication is widespread among social amoebae and may be ancestral to other acrasin systems. Here, we used *P. pallidum* as a model organism to investigate the metabolism of glorin. We describe a major metabolite produced by a "glorinase" enzymatic activity that is both membrane-bound and secreted in soluble form by starving *P. pallidum* cells. Formation of the metabolite is the first step in the inactivation of the glorin signal, because the isolated metabolite neither mimics glorin's activity of inducing gene expression in starving *P. pallidum* cells nor does it serve as a chemoattractant. Further, we present a chemically synthesized glorin derivative (glorinamide) that is resistant to glorinase activity and acts as a stable glorin mimetic. Ongoing work is aimed at the purification and proteomics-based identification of glorinase in cell-free supernatants of aggregating *P. pallidum* cells.

# Concentration-dependent gradient-sensing dynamics and chemotaxis, and their regulation in *Dictyostelium discoideum*

**Xuehua Xu**, Wen Xi, and Tian Jin

*National Institute of Health (NIH), USA*

Eukaryotic cells chemotax through a large range of chemoattractant concentration through adaptation, while the components and fundamental functions of adaptation are not fully understood. We have previously shown that a temporal-spatially regulated, yet concentration-dependent gradient sensing dynamics are essential for G protein coupled receptor-mediated chemotaxis in *Dictyostelium discoideum*. Recently, we identified C2GAP1 as a key regulator of Ras adaptation and long-range chemotaxis. Here, we reveal the role of C2GAP1 in regulating the concentration dependent tempo-spatial dynamics of gradient sensing and chemotaxis in *D. discoideum*.

# Notes

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