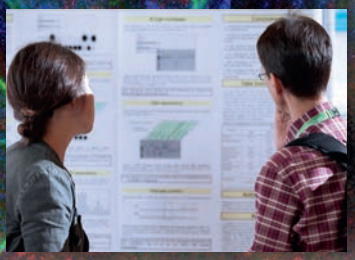
Biochemical Society
Advancing Molecular Bioscience

BSCB BRITISH SOCIETY FOR CELL BIOLOGY

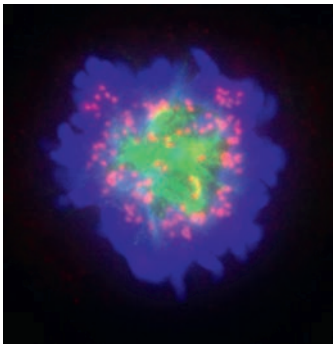
Programme & Abstracts

A joint conference of the British Society for Cell Biology and the Biochemical Society

The Dynamic Cell 2014



4–7 September 2014
Robinson College, Cambridge, UK



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British Society for Cell Biology
British Society for Developmental Biology

Joint Spring Meeting

celebrating the 50th Anniversary of the BSCB

12th - 15th April 2015

University of Warwick

Kyo Agata
Enrique Amaya
Hilary Ashe
Julian Blow
Ineke Braakman
Andrea Brand
Siobhan Braybrook
Frank Constantini
Anna Cuervo
Caroline Dean
Liam Dolan
Kristian Franze
Clare Futter
Susan Gasser
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Anne Grapin-Botton
Edith Heard
Carl-Philipp Heisenberg
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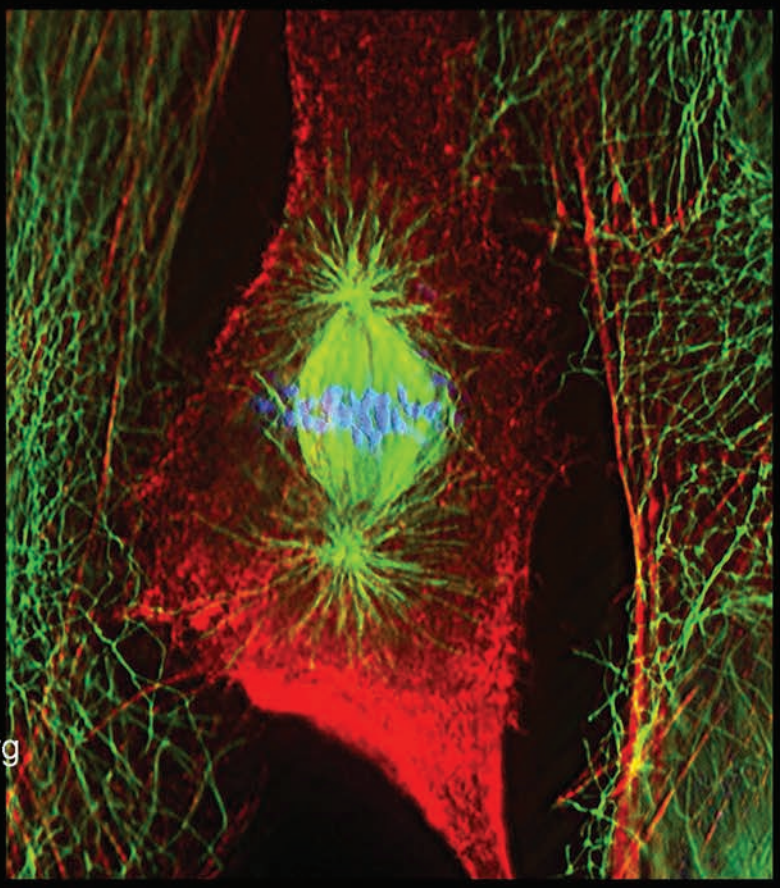
Wieland Huttner



Andrew Johnson
Rob Klose
Peter Landsdorp
Roberto Mayor
Irene Miguel-Aliaga
Elke Ober
Terry Orr-Weaver
Norbert Perrimon
Mathieu Piel
Chris Ponting
Peter Reddian
Tristan Rodriguez
Christiana Ruhrberg
Erik Sahai
Luca Scorrano
Mike Sheetz
Jose Silva
Anne Straube
Clare Waterman
Fiona Watt
Tamotsu Yoshimori
Phil Zegerman

Plenary lectures by:

Brigid Hogan and Jennifer Lippincott-Schwartz

Topics include:
Cellular Responses, DNA Replication, Epigenetics, Metabolism, Morphogenetic Movements/Cell Migration, Organogenesis, Physical Biology/Mechanical Forces, Protein Homeostasis and Regeneration and/or Reprogramming

Scientific Organisers: Jo Begbie, Jenny Nichols, Kate Nobes and Grant Wheeler

www.bscb-bsdb-meetings.co.uk

Image by Dr Mette Mogensen

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Contents

Welcome

The Biochemical Society and British Society for Cell Biology are delighted to welcome you to Robinson College for the Focused Meeting entitled 'The Dynamic Cell'. Stephen Royle, Jeremy Carlton, Ulrike Gruneberg and James Wakefield have worked hard to prepare the scientific programme for this conference and the Societies would like to offer sincere thanks to them.

Please take some time to visit the trade exhibitors at this conference for detailed product and technical information. The Biochemical Society and British Society for Cell Biology are grateful for their funding and support, which together with that of the sponsors, ensures that the Focused Meetings can continue to provide an exciting, high-quality programme with an emphasis on emerging life science topics.

Finally, our thanks to Amy Brown and Nick Milne at the Robinson College for their support in the organization of this conference. I wish you an enjoyable and rewarding conference.

Sheila Graham
Honorary Meetings Secretary for the Biochemical Society

Stephen J. Royle
Meetings Secretary for the British Society for Cell Biology

Introduction

Dear Participant,

Welcome to Dynamic Cell 2014!

This meeting is jointly organized by the British Society for Cell Biology (BSCB) and the Biochemical Society. In creating Dynamic Cell, we wanted to assemble a stellar line up of speakers that showcased the world's best cell biology, from a range of organisms and across a collection of distinct, but interrelated, research specialisms. We hope you agree that this aim has been fully realized and look forward to an inspiring and enjoyable focused meeting. We are also delighted to host a prestige BSCB plenary lecture from Prof Jim Spudich and to present the Biochemical Society's 2014 Novartis Medal to Prof Jeff Errington, the 2014 GlaxoSmithKline award to Prof Juan Martin-Serrano and the 2014 Early Career Research Award in Cell Biology to Dr Melina Schuh.

We also wished to strengthen links between the BSCB and the Biochemical Society and hope that this meeting highlights the importance of these societies to UK bioscience.

The University of Cambridge was founded in 1209 and is the world's third-oldest surviving university. Its mission is to contribute to society through the pursuit of education, learning and research at the highest international levels and is consistently ranked among the foremost universities in the world.

Cambridge is a collegiate university, made up of 31 self-governing colleges that accommodate the academics, undergraduate and postgraduate student bodies. Separate to this are the Schools, Faculties and Departments that house the research activities and organize the teaching syllabi. In addition to the university's research and teaching departments, a number of world-leading research institutes are affiliated with the university, adding to the research environment in this city.

Robinson College is one of the newer colleges, founded in 1977 with a gift from the philanthropist Sir David Robinson and is unique in that it is the only Cambridge college designed from its inception to be for graduate and undergraduate students of either sex. The college is set in landscaped gardens and is distinctive for its architecture and use of red brick; indeed, Robinson was recently named in the Daily Telegraph's '50 most inspiring buildings in Britain'.

Our conference dinner will be held in King's College. The college was founded in 1441 by King Henry VI and its chapel with its gothic architecture is one of the most famous Cambridge landmarks. Famous scientists associated with King's College include Fred Sanger, Sydney Brenner and Alan Turing.

We would like to thank the Biochemical Society's meeting's office, particularly Charlotte Dooley and Frances van Klaveren, for organizational support. We are also grateful for the support of the BSCB, the Biochemical Society and our sponsors for helping his meeting happen.

We hope you enjoy and take inspiration from this meeting,

Jeremy Carlton, Ulrike Gruneberg, Stephen Royle and James Wakefield

Overview

Thursday 4 September 2014

11:00–14:20	Registration
13:00–14:20	Lunch
14:20–14:30	Welcome and Introduction
14:30–15:50	**Molecular Control of Chromosome Segregation**
15:50–16:20	Coffee/Tea Break
16:20–18:00	**Molecular Control of Chromosome Segregation** (continued)
18:00–18:45	**The Biochemical Society's 2014 Early Career Research Awards**
19:00–20:30	Dinner

Friday 5 September 2014

09:00–10:40	**Cargo Sorting in the Endocytic Pathway**
10:40–11:10	Coffee/Tea Break
11:10–12:30	**Cargo Sorting in the Endocytic Pathway** (continued)
12:30–13:30	Lunch and Poster Viewing 1
13:30–14:50	***In-Vitro* Analysis of Molecular Motors**
14:50–15:20	Coffee/Tea Break
15:20–16:10	***In-Vitro* Analysis of Molecular Motors** (continued)
16:10–17:10	**The BSCB Plenary Lecture**
17:10–18:30	Poster Session 1
18:30–19:30	**The Novartis Award Medal and Prize**
19:30–21:00	Dinner

Saturday 6 September 2014

09:00–10:20	**Membrane Dynamics during Cytokinesis**
10:20–10:50	Coffee/Tea Break
10:50–12:10	**Membrane Dynamics during Cytokinesis** (continued)
12:10–13:10	Lunch and Poster Viewing 2
13:10–14:50	**Cell Migration and the Cytoskeleton**
14:50–15:20	Coffee/Tea Break
15:20–16:40	**Cell Migration and the Cytoskeleton** (continued)
16:40–18:00	Poster Session 2
18:00–19:00	**GlaxoSmithKline Award Lecture**
19:30–22:00	Conference Dinner at King's College

Sunday 7 September 2014

09:00–10:40	**Cargo Sorting in the Secretory Pathway**
10:40–11:10	Coffee/Tea Break
11:10–12:30	**Cargo Sorting in the Secretory Pathway** (continued)
12:30–13:00	Closing remarks and Meeting Close
13:00–14:00	Lunch

All lectures will take place in the Auditorium.

The registration fee includes lunch and refreshments, which will be served in the Dining Hall. Along with a Conference Dinner on 6 September 2014, which will be held at King's College, Cambridge.

The residential – registration fee also includes three nights' accommodation (4, 5 and 6 September) in a single en suite room, along with breakfast and dinner at the Garden Restaurant. Breakfast will be served during 07:30–09:00 and dinner will be served during 19:00–20:30.

Sponsors and Exhibition

The Organizers would like to express their thanks and gratitude to the conference sponsors:

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The Trade Exhibition is situated in the Dining Hall. Please visit the trade stands to benefit from technical and product advice.

Organizers

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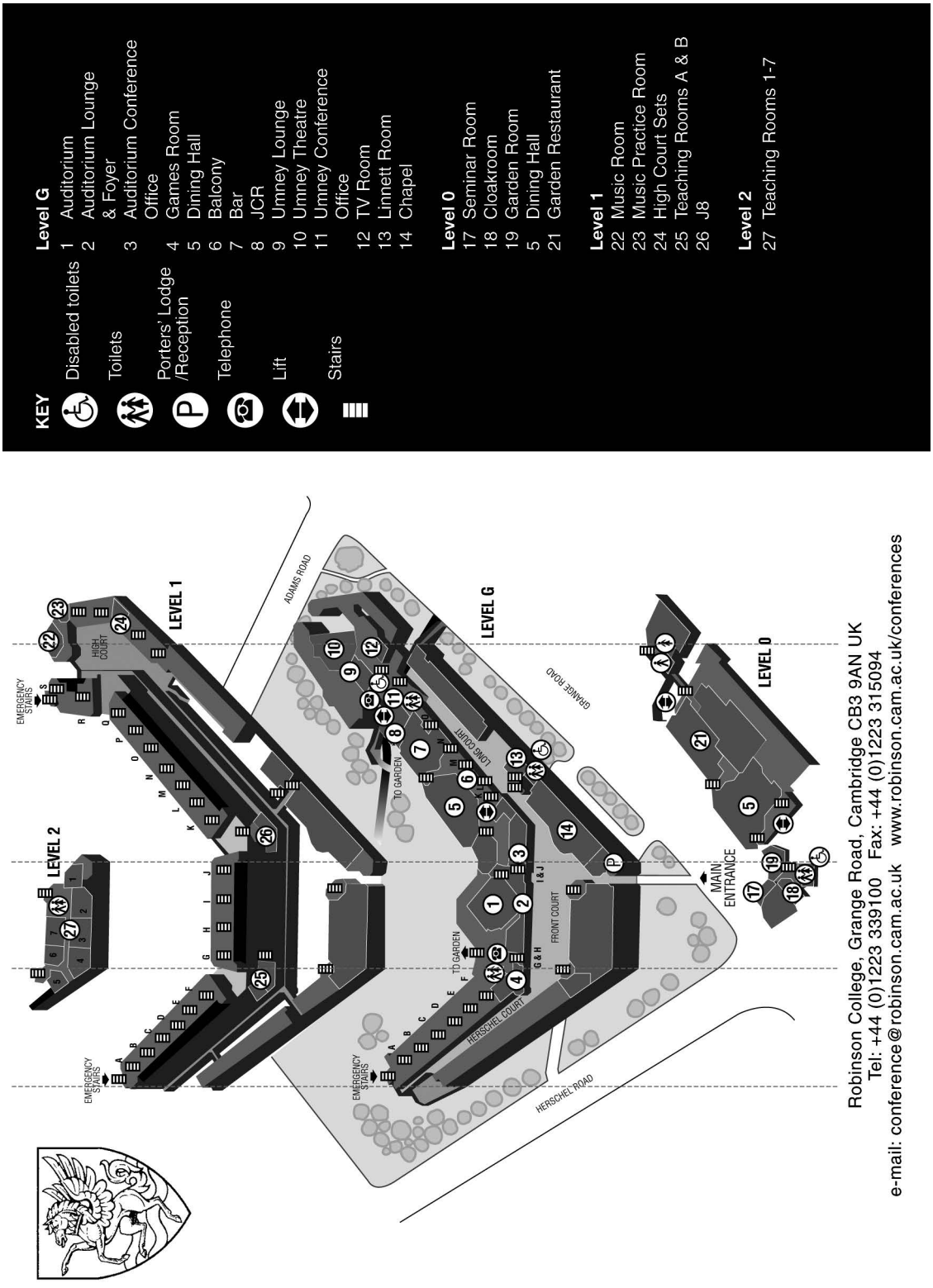
King's College London, UK

James Wakefield (J.G.Wakefield@exeter.ac.uk)
University of Exeter, UK

Venue Map

Registration will take place at: Robinson College, in the Dining Hall.



Participant Information

Membership of the Biochemical Society

Membership is open to anyone with an interest in biochemistry and the wider biosciences. The Society has a network of over 6,900 members worldwide, with membership categories to cover scientists at any stage in their career - from Undergraduate Member right up to Full Member. Benefits of membership are numerous and include:

- Subscription to *The Biochemist* magazine (pick up a sample copy at the registration desk)
- Grants and bursaries to attend international conferences
- Reduced registration fees for Biochemical Society conferences
- Access to FEBS Fellowships
- Personal subscription to the *Biochemical Journal* and *Biochemical Society Transactions* and much much more.

Sign up to pay by direct debit and receive a 5% discount on your membership fees annually (note this is only available with a UK bank account).

If you are interested in joining or learning more about the Society, please speak to Charlotte Dooley during the conference.

Membership of the British Society for Cell Biology

The goal of the BSCB is simple – to promote all aspects of cell biology in the UK. We are a not-for-profit organisation with ~1,400 members. We have no paid employees: all our officers and committee members work for free. If you're reading this, the chances are that the BSCB exists for you. Please join us and get involved in supporting your cell biology community. BSCB Members benefits include:

- Eligibility to apply for the popular Honor Fell/CoB Travel Bursaries (Full registration and accommodation costs for the Annual BSCB Spring Meeting; up to £300 for other UK meetings; up to £400 for EU meetings and up to £500 for meetings in the rest of the world)
- Access to BSCB Summer Studentships
- Access to support for Small Meetings
- Special discounts on Journal subscriptions and textbooks
- The chance to enter our Image and Science Writing Competitions
- Free annual glossy hard-copy Newsletter and periodic e-Newsletters

If you are interested in joining or learning more about the Society, please speak to co-organiser James Wakefield during the conference.

Poster Sessions

The Poster Session will take place in the Dining Hall.

Each poster has been assigned a number, which appears alongside its abstract in this booklet. Presenting authors are requested to stand alongside their posters during their poster session, as indicated above.

Posters that are Odd numbered should be mounted by 09:00 and removed by 19:30 on Friday 5 September. Even numbered posters should be mounted by 09:00 and removed by 19:30 on Saturday 6 September. Velcro will be provided at the registration desk.

The Societies cannot be held responsible for lost or damaged posters.

All accepted poster abstracts are available to view online at www.biochemistry.org/conferences.

Poster Prizes

Biochemical Society and British Society for Cell Biology Poster Prize

Biochemical Society
Advancing Molecular Bioscience

BSCB BRITISH SOCIETY FOR CELL BIOLOGY

A Biochemical Society and British Society for Cell Biology Poster Prize of £250 will be awarded to the best poster presented at this meeting by a researcher in the early stages of their career (post-graduate or first post-doctoral position).

***Biochemical Journal* Poster Prize**

BJ BIOCHEMICAL JOURNAL

The Biochemical Journal Poster Prize of £100 will be awarded to the second best poster prize at this meeting.

Entrants for both poster prizes must be PhD students or post-doctoral scientists within five years of receiving their PhD. To enter, please collect a sticker from the registration desk before the start of your poster session.

Delegates will be asked to vote on the best poster – please ensure you collecting a voting slip from the registration desk.

Oral Communications

Oral communications selected from poster abstracts will be given in the lecture theatre. Please refer to the Scientific Programme for timings.

Continuing Professional Development

The Dynamic Cell has been approved for the purposes of Continuing Professional Development (CPD) by the Society of Biology. Participants can claim 75 credits for attending. These points are valid if attendees are registered on the Society of Biology CPD scheme. Approval signifies that the Society of Biology recognizes the Dynamic Cell event is of merit to the development needs of participants. If you require a CPD certificate for this conference please request one during the post event feedback questionnaire which will be sent to you by email shortly after the event.

Security

Badges must be worn for the duration of the conference, both for security purposes and for entry to the lectures and social events.

Facilities Information

Accommodation

Residential registration includes three nights' accommodation for 4, 5 and 6 September in Robinson College. Keys can be collected from the Porters Lodge upon arrival. The Porters Lodge is open 24 hours a day. Check in time is after 13:00 and check out is by 09:30 on the day of departure. A luggage room will be available before check-in and after check-out, please ask at the registration desk for more information. Please note there will be a charge of £15 for lost keys.

Address:

Robinson College
Grange Road
Cambridge
CB3 9AN
UK

Parking

Car Parking is available at the Wilberforce Sports Ground for those who have previously bought and received car parking permits from the Biochemical Society. Please make sure that your permit is clearly displayed, entry will be refused to those without a permit.

The entrance to the car park is on the corner of Adams Road and Wilberforce Road. Once inside the Sports Ground please follow the signs to the parking area. Please note that the car park is only 500 yards from the Porters' Lodge of Robinson College.

There is unrestricted street parking around the College in Adams Road, Herschel Road and Sylvester Road which is readily available at weekends and in the evenings. Wilberforce Road, at the top of Adams Road, is usually empty and within 5 minutes walk of the College.

Medical Services

Please contact the Biochemical Society registration desk in the case of a minor incident. If it is an emergency, please call 999 directly and inform the Biochemical Society member of staff at the registration desk.

Internet Access

WiFi is available throughout the college. Please ask at the registration desk for the password.

Further Information

Biochemical Society Transactions

All Speakers have been invited to prepare a manuscript for publication in *Biochemical Society Transactions*. The proceedings will be published in Volume 43 part (1) due to be published in February 2015. Single issues of *Biochemical Society Transactions* are available for purchase (£30). If you did not order the Conference issue during the registration process, you may do so at the conference registration desk or after the meeting by emailing conferences@biochemistry.org

Certificates of Attendance

Attendees requiring a Certificate of Attendance for the meeting should contact the Biochemical Society and BSCB registration desk.

Attendees at the Conference

A delegate list will be sent by email to all attendees after the conference. Please note that this list is intended for use only to promote networking between scientists. You do not have permission to use this list for any other purpose, and any other use may infringe the Data Protection Act 1998. The list contains the names and affiliations of all attendees. Contact details are included only for attendees who gave their permission during the registration process.

Liability

The Biochemical Society and BSCB will assume no responsibility whatsoever for damage or injury to persons or property during the meeting. Participants are advised to arrange their own personal travel and health insurance.

Tweeting and Blogging

The Biochemical Society and BSCB encourages the discussion of its conferences via Twitter, Facebook and similar social networks. In order to promote discussion and the exchange of information, delegates who wish to Tweet are asked to use the Biochemical Society hashtag:

#Cell_dynamic

Whilst we encourage respectful tweeting, we expect delegates to refrain disseminating unpublished work on the internet.
Speakers will be made aware of this policy, and have the right to ask delegates not to disseminate their research via the Internet. If a Speaker makes this request, delegates are asked not to discuss the relevant work in this way.

Delegates are respectfully asked to refrain from communicating using mobile devices whilst lectures are in progress.

Photography

Please note that photographs taken at this event may be used for promotional purposes by the Biochemical Society and BSCB, e.g. by inclusion on the Biochemical Society and BSCB websites and/or marketing materials. If you have any concerns or queries regarding this, please visit the registration desk or contact the Conference Office on +44(0)20 7685 2450 or by email at conferences@biochemistry.org.

We ask that you refrain from taking photos of posters and slides

Useful Contacts

Venue

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Porters Lodge	Tel +44 (0)1223 339 100

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British Society for Cell Biology

James Wakefield (membership enquires)	Tel: +44 (0)1392 724670 email: j.g.wakefield@exeter.ac.uk
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Scientific Programme

Thursday 4 September 2014

11:00–14:20 Registration

13:00–14:20 Lunch

14:20–14:30 Welcome and Introduction

Molecular Control of Chromosome Segregation

Chair: Ulrike Gruneberg (University of Oxford, UK)

Abstract	Time	Title and Author
S001	14:30–15:00	*The Ran-GTP gradient spatially regulates the activity of XCTK2 within the spindle* **Claire Walczak** (Indiana University, USA)
S002	15:00–15:30	*Protein complexes responsible for centrosome segregation in mitosis* **Fanni Gergely** (University of Cambridge, UK)
P005	15:30–15:50	*Temporal control of cell division: switches, refractory periods and feedback control* **Selected Oral Communication – Silvia Santos** (Imperial College London, UK)

15:50–16:20 Coffee/Tea Break

S003	16:20–16:50	*Sharpening the anaphase switch* **Jonathan Millar** (University of Warwick, UK)
S004	16:50–17:20	*Regulation of the chromosomal passenger complex in cancer* **Susanne Lens** (Utrecht University, The Netherlands)
P021	17:20–17:40	*The spindle checkpoint regulates Cdc20 activity and turnover to control mitotic progression* **Selected Oral Communication – Kevin Hardwick** (University of Edinburgh, UK)
P075	17:40–18:00	*A mitotic exit ubiquitome from human cells* **Selected Oral Communication – Catherine Lindon** (University of Cambridge, UK)
S005	18:00–18:45	**The Biochemical Society's 2014 Early Career Research Awards** *New insights into aneuploidy in mammalian eggs* **Melina Schuh** (MRC Laboratory of Molecular Biology, UK)

19:00–20:30 Dinner (Residential registrants)

Friday 5 September 2014

Cargo Sorting in the Endocytic Pathway		
Chair: Stephen Royle (University of Warwick, UK)		
S006	09:00–09:30	*Endocytic cargo selection and clathrin coat assembly* **Linton Traub** (University of Pittsburgh, USA)
S007	09:30–10:00	*The how and why early endosomes move: lessons from a fungal model system* **Gero Steinberg** (University of Exeter, UK)
P020	10:00–10:20	*CLIP-170 spatially modulates receptor tyrosine kinase localization to coordinate cell migration* **Selected Oral Communication – Kossay Zaoui** (McGill University, Canada)
P088	10:20–10:40	*A membrane-driven conformational switch in AP2 activates clathrin recruitment* **Selected Oral Communication –Bernard Kelly** (University of Cambridge, UK)
10:40–11:10 Coffee/Tea Break		
S008	11:10–11:40	*Adaptor protein complexes* **Margaret Robinson** (University of Cambridge, UK)
S009	11:40–12:10	*Endosomal sorting orchestrated by retromer* **Peter Cullen** (University of Bristol, UK)
P094	12:10–12:30	*Ysc84 is a novel, PI(4,5)P_2 regulated, actin-capping protein functioning in early stages of yeast endocytosis* **Selected Oral Communication – Agnieszka Urbanek** (University of Sheffield, UK)
12:30–13:30 Lunch and Poster Viewing 1 (Odd numbered posters)		
***In-Vitro* Analysis of Molecular Motors**		
Chair: James Wakefield (University of Exeter, UK)		
S010	13:30–14:00	*The structure and mechanisms of dynein* **Andrew Carter** (MRC-LBM, UK)
S011	14:00–14:30	*Reconstitution of a hierarchical +TIP interaction network controlling microtubule end tracking of human dynein* **Thomas Surrey** (CRUK-LRI, UK)
P012	14:30–14:50	*Myosin Va and dynamic actin oppose microtubules to drive long-range organelle transport* **Selected Oral Communication – Alistair Hume** (University of Nottingham, UK)
14:50–15:20 Coffee/Tea Break		

S012	15:20–15:50	*Molecular mechanisms of myosin function* **Michelle Peckham** University of Leeds, UK)
P102	15:50–16:10	*Cut7-driven microtubule sliding reverses direction depending on motor density* **Selected Oral Communication – Robert Cross** (Warwick Medical School, UK)
S013	16:10–17:10	**The BSCB Plenary Lecture** *The myosin family of molecular motors: nature's exquisite nanomachines* **James Spudich** (Stanford University School of Medicine, USA)

17:10–18:30 Poster Session 1 (Odd numbered posters)

S014	18:30–19:30	**The Novartis Medal and prize** *Cell cycle dynamics in* Bacillus subtilis **Jeff Errington** (Newcastle University, UK)

19:30–21:00 Dinner (Residential residents)

Saturday 6 September 2014

Membrane Dynamics during Cytokinesis

Chair: Jeremy Carlton (King's College London, UK)

S015	09:00–09:30	*Precision timing mechanisms for anaphase onset and cytokinesis in human cells* **Francis Barr** (University of Oxford, UK)
S016	09:30–10:00	*Plant cytokinesis – a tale of membrane traffic and fusion* **Gerd Juergens** (Tubingen University, Germany)
P054	10:00–10:20	*Essential role of ESCRT-III-associated kinase in the regulation of abscission timing* **Selected Oral Communication – Anna Caballe** (King's College London, UK)

10:20–10:50 Coffee/Tea Break

S017	10:50–11:20	*Dividing cells regulate their lipid composition and localisation* **Ulrike Eggert** (King's College London, UK)
S018	11:20–11:50	*Regulation of midbody formation and function by mitotic kinases* **Pier Paolo D'Avino** (University of Cambridge, UK)
P055	11:50–12:10	*The roles of the oncoprotein GOLPH3 in contractile ring assembly and membrane trafficking during cytokinesis* **Selected Oral Communication – Maria Grazia Giansanti** (Sapienza University of Rome, Italy)

12:10–13:10 Lunch and Poster Viewing 2 (Even numbered posters)

Cell Migration and the Cytoskeleton		
Chair: Patrick Caswell (University of Manchester, UK)		
S019	13:10–13:40	*Control of directional cell migration by the microtubule cytoskeleton* **Anne Straube** (University of Warwick, UK)
S020	13:40–14:10	*Role of the actin cytoskeleton in 3D invasive migration* **Laura Machesky** (CRUK Beatson Institute for Cancer Research, UK)
P004	14:10–14:30	*Oncogene-like induction of cellular invasion from centrosome amplification* **Selected Oral Communication – Susana Godinho** (Barts Cancer Institute, UK)
P084	14:30–14:50	*Syndecan-4 controls integrin recycling to regulate cell migration and the extracellular microenvironment* **Selected Oral Communication – Mark Morgan** (University of Liverpool, UK)

14:50–15:20 Coffee/Tea Break

S021	15:20–15:50	*Pushing with actin: from cells to pathogens* **Vic Small** (Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna)
P087	15:50–16:10	*Dynamic anisotropies in cytoskeletal organization induced by ROS and Rho signalling underlie multicellular sensing and spatial patterning in a* Drosophila *epithelium* **Selected Oral Communication – Maithreyi Narasimha** (Tata Institute of Fundamental Research, India)
P073	16:10–16:30	*Drink or drive: competition between macropinocytosis and cell migration* **Selected Oral Communication – Douwe Veltman** (Medical Research Council, Cambridge, UK)

16:40–18:00 Poster Session 2 (Even numbered posters)

S022	18:00–19:00	**GlaxoSmithKline Award** *A helping hand from enveloped viruses to uncover the final events of cell division* **Juan Martin-Serrano** (King's College London School of Medicine, UK)

19:30–22:00 Conference Dinner at King's College

Sunday 7 September 2014

Cargo Sorting in the Secretory Pathway		
Chair: Isabelle Jourdain (University of Exeter, UK)		
S023	09:00–09:30	*Parallels between ER-Golgi traffic and autophagy* **Susan Ferro-Novick** (University of California, San Diego, USA)
S024	09:30–10:00	*Morpho-functional changes in the organisation of the secretory pathway in* D. melanogaster *upon starvation* **Catherine Rabouille** (Hubrecht Institute for Stem Cell Research and Developmental Biology, The Netherlands)
P026	10:00–10:20	*Protein sorting and glycan biosynthesis* **Selected Oral Communication – Daniel Ungar** (University of York, UK)
P095	10:20–10:40	*Sterol traffic in yeast is mediated by a newly discovered family of StART proteins* **Selected Oral Communication – Louise Wong** (University College London, UK)
10:40–11:10 Coffee/Tea Break		
S025	11:10–11:40	*Mechanism of sorting and export of bulky procollagen VII from the endosplasmic reticulum* **Vivek Malhotra** (CRG, Spain)
P057	11:40–12:00	*Regulation of exocytosis by the exocyst complex* **Selected Oral Communication – Mary Munson** (University of Massachusetts Medical School, USA)
S026	12:00–12:30	*The small GTPase Arf1 modulates mitochondrial morphology and function* **Anne Spang (Biozentrum Basel, Switzerland)**
12:30–13:00 Closing remarks and meeting close		
13:00–14:00 Lunch		

Posters

P001	**Investigation of proteoglycan traffic in the late secretory pathway** D.P. Buser and M. Spiess
P002	**Poster withdrawn**
P003	**GPVI clustering in platelets imaged by super resolution microscopy** N.S. Poulter, D.M. Owen, S.M. Jung, M. Moroi, E. Gardiner, R. Andrews, R.W. Farndale, S.G. Thomas and S.P. Watson
P004	**Oncogene-like induction of cellular invasion from centrosome amplification** S. Godinho, R. Picone, M. Burute, M. Thery and D. Pellman
P005	**Temporal control of cell division: switches, refractory periods and feedback control** S. Santos and J.E. Ferrell Jr.
P006	**Poster withdrawn**
P007	**GIMAP5 affects calcium homeostasis by regulating the movement of organelles** S. Ramanathan, D. Serrano, S. Ilangumaran and C. Lavoie
P008	**Human MOB2 acts as a positive regulator of cell migration and invasion in human fibrosarcoma cells** S.-S. Fan, Y.-c. Liu, L.-m. Choo, M.-h. Dong and W.-t. Chao
P009	**Robust mitotic spindle formation is achieved by the co-ordinated action of multiple microtubule generating pathways** D. Hayward, J. Metz, C. Pellacani and J.G. Wakefield
P010	**Functional characterization of the Nesprin-2 – SMC interaction** C. Mroß, M. Munck, S. Neumann and A.A. Noegel
P011	**Poster withdrawn**
P012	**Myosin Va and dynamic actin oppose microtubules to drive long-range organelle transport** R. Evans, C. Robinson, D. Briggs, D.J. Tooth, J.S. Ramalho, M. Cantero, L. Montoliu, S. Patel, E. Sviderskaya and A. Hume
P013	**On the mechanism of durotaxis in motile cells** M. Riaz, M. Versaevel and S. Gabriele
P014	**A novel actin filament population that regulates ER-to-Golgi trafficking** A.J. Kee, L. Yang, E. Polishchuk, R. Polishchuk, R. Weigert, P. Gunning and E.C. Hardeman
P015	**Role of myosins 10 and 18a in migration of prostate cancer cells** K.A. Makowska, K.J. White, S.S. Rosenfeld, C.M. Wells and M. Peckham
P016	**WAFL knockdown impairs cell migration and invasion** B.J. Tyrrell, X. Yu and L.M. Machesky
P017	**Linking ESCRT function to genomic instability and DNA damage** J.E. Willan, A. Cleasby, F. Stefani, P.G. Woodman, H. Bryant and B. Ciani
P018	**The role of MELK in cell division** A.P. Sagona and M. Mishima

P019	**Analysis of Weibel Palade Body exocytosis and subsequent compensatory endocytosis using correlative electron microscopy** T.D. Nightingale, N.L. Stevenson, I.J. White and D.F. Cutler
P020	**CLIP-170 spatially modulates receptor tyrosine kinase localization to coordinate cell migration** K. Zaoui, C.A. Parachoniak and M. Park
P021	**The spindle checkpoint regulates Cdc20 activity and turnover to control mitotic progression** K.G. Hardwick, K. May, K. Paraskevopoulos, O. Sen, I. Yuan and J. Zich
P022	**Development of tools to study polarised secretion using super resolution microscopy** H. Zenner, S. Munro and D. St Johnston
P023	***P. aeruginosa* lectin LecB triggers bacterial invasion via induction of PI3K, the small GTPase Rac and subsequent actin cytoskeleton rearrangement** K. Kühn and W. Römer
P024	**Poster withdrawn**
P025	**Investigating the role of mitotic MAPs in the early *Drosophila* embryo** S.J. Scott, P. Jones, S. Campbell, K.J. Heesom and J. Wakefield
P026	**Protein sorting and glycan biosynthesis** K.M. Wilson, N.P. Cottam and D. Ungar
P027	**RNA polymerase III promotes cell migration and ECM remodelling through increased expression of its product, $tRNA_i^{Met}$** C.J. Clarke, J. Birch, A.D. Campbell, D. Sumpton and J.C. Norman
P028	**Application of adhesive protein micropatterns to study dynamics of actomyosin filaments during epithelial to mesenchymal transition** B. Richards, J. Walker, K. Straatman, C. Binns, A. Hudson and M. Kriajevska
P029	**Poster withdrawn**
P030	**The role of the coxsackie and adenovirus receptor in lung cancer cell proliferation** R. Pike, G. Santis and M. Parsons
P031	**Hormetic effects of the flavonoids quercetin, galangin and chrysin on 3T3 pre-adipocyte growth and development** T. Diya, D. Cadagan, S. Merry, P. Gowland and C. Towlson
P032	**Endocytosis and S6-kinase: is there a link?** A. Malik, A. Piechnik, A. Lew, E. Liszewska, K. Kotulska and J. Jaworski
P033	**Cellular characterization of bleb-like structures induced by pore-forming toxins** C. Brito, F. Mesquita, D. Cabanes and S. Sousa
P034	**Snaps of SNARES and Muncs - IF localisation of endogenous Syntaxin11 and Munc18-2 in human cytotoxic T-lymphocytes** N. Dieckmann, Y. Hackmann and G.M. Griffiths

P035	**Cross-linking (3D) proteomics of the augmin complex suggests a mechanism for gamma-tubulin dependent microtubule-templated microtubule nucleation** J.W.i-C. Chen, A. Chen, K. Rogala, J. Rappsilber, C.M. Deane and J. Wakefield
P036	**IQGAP3 is a novel player of cytokinesis** M. Leone and F.B. Engel
P037	**The epsilon subunit of the CCT molecular chaperone links actin folding to MRTF-A/SRF-dependent gene expression** K. Elliott, M. Spiess, A. Svanström, R. Karlsson and J. Grantham
P038	**Anillin's role in asymmetric ingression of epidermal cells** A. Mariotti and A. Piekny
P039	**Rab7b and the actomyosin cytoskeleton: novel roles in intracellular trafficking and cell migration** C. Progida, M. Borg and O. Bakke
P040	**How does clathrin bind AP-2?** L.A. Wood and S.J. Royle
P041	**Functional roles of key apical complex molecules in collective cell migration** H. Wang, J. Luo, Z.-h. Xu and J. Chen
P042	**Signals from the outside? : the role of GPCRs in cytokinesis** A.E.G. Booth and U. Eggert
P043	**TMEM115: a new rhomboid-like protein potentially involved in Golgi retrograde traffic and lipid metabolism** A. Moncada Pazos and M. Freeman
P044	**Probing cytokinesis: identifying new proteins involved in cell division** A.L. Wilson, S.J. Terry and U. Eggert
P045	**Switching on cytoplasmic dynein: processive movement induced by BICD2N and dynactin** M.A. Schlager, H.T. Hoang, L. Urnavicius, S.L. Bullock and A. Carter
P046	**Characterisation of Golgi-localised GORAB protein** T.M. Witkos, M. Joensuu, E. Jokitalo and M. Lowe
P047	**The actin filament bundling protein drebrin plays a role in prostate cancer cell motility** A.E. Dart, D.C. Worth and P.R. Gordon-Weeks
P048	**Role of phosphatases in chromosome segregation** D. Conti
P049	**PP2A-B56 opposes Mps1 phosphorylation of Knl1 and thereby promotes spindle assembly checkpoint silencing** A. Espert, P. Uluocak, R. Nunes. Bastos, D. Mangat and U. Gruneberg
P050	**Distinct levels in Pom1 gradients limit Cdr2 activity and localization to time and position division** P. Bhatia, O. Hachet, M. Hersch, S.A. Rincón, M. Berthelot-Grosjean, S. Dalessi, L. Basterra, S. Bergmann, A. Paoletti and S. Martin

P051	**Cytoskeleton remodelling and cell motility of normal and glaucomatous trabecular meshwork cells are substrate compliance dependent** B. Liu, B. Lukasz, J. Kilpatrick, D. Wallace, C. O'Brien and S. Jarvis
P052	**From nucleosome to midbody: extrachromosomal activity of histone H2B in cytokinesis** L. Monteonofrio, C. Rinaldo and S. Soddu
P053	**Poster withdrawn**
P054	**Essential role of ESCRT-III-associated kinase in the regulation of abscission timing** A. Caballe, D. Wenzel, M. Agromayor, J. McCullough, L. Labrador, S.L. Alam, J. Carlton, J.J. Skalicky, M. Kloc, W.I. Sundquist and J. Martin-Serrano
P055	**The roles of the oncoprotein GOLPH3 in contractile ring assembly and membrane trafficking during cytokinesis** S. Sechi, G. Colotti, G. Belloni, A. Frappaolo and M.G. Giansanti
P056	**Molecular insights into the meiotic microtubule organising centre that directs vigorous nuclear movement during fission yeast sexual differentiation** K. Straatman, C. Funaya, Y. Connolly, A. Grallert, D.L. Smith, C. Antony, K. Sawin and K. Tanaka
P057	**Regulation of exocytosis by the exocyst complex** M. Munson
P058	**Dynamic flux of microvesicles modulate parasite-host cell interaction of *Trypanosoma cruzi* in eukaryotic cells** M.I. Ramirez Sr., I. Evans, A. Mojoli and I. de Almeida
P059	**Kif1C is required for podosome formation in vascular smooth muscle cells** A. Bachmann, N. Efimova, A. Feoktostov, U. Theisen and I. Kaverina
P060	**Regulation of spindle orientation in mitosis by p37/p47 adaptor proteins of CDC48/p97** B.H. Lee, P. Meraldi Sr. and M. Gotta Sr.
P061	**The apical complex provides a regulated gateway for secretion of invasion factors in apicomplexan parasites** N.J. Katris, G.G. Van Dooren and R.F. Waller
P062	**Poster withdrawn**
P063	**The requirement of barbed end capping of actin filaments for faithful cytokinesis** S.J. Terry and U. Eggert
P064	**Network-guided connectivity mapping predicts new microtubule stabilizing agents and drug sensitivity in cancer cell lines** R.L. Shrestha
P065	**The end-binding protein EB2 maintains a dynamic microtubule population essential for reorganisation of the microtubule array during polarisation** J.R. Gadsby, D.A. Goldspink, J. Perkins, P.P. Powell, P. Thomas, E.K. Lund, J. Gavrilovic and M.M. Mogensen
P066	**Comparative proteomics define membrane architecture and host-parasite interface** C. Gadelha, W. Zhang, B. Wickstead, B.T. Chait and M.C. Field

P067	**Molecular dissection of the Dam1 complex** S.K. Talapatra, J. Zou, J. Rappsilber and J. Welburn
P068	**N-WASP/WIP mediated "actin-hotspots" drive 3D cell migration through direct force coupling to the nucleus** T. Zech, O. Chatzidoukaki and L.M. Machesky
P069	**Defective axonal retrograde transport of signalling endosomes and MAP kinase signaling in a mouse model of autosomal dominant spinal muscular atrophy** C. Garrett, M. Barri, A. Kuta, V. Soura, W. Deng, E. Fisher, G. Schiavo and M. Hafezparast
P070	**Poster withdrawn**
P071	**The RhoGAP Myosin 9b and its role in the migration of myeloid cells** S.A. Hemkemeyer, B. Lohmann, U. Honnert, S. Viehmann, P.J. Hanley and M. Bähler
P072	**Targeting of intracellular farnesyl protein transferase modulates actin dynamics and epithelial cells morphology** A. Markiv, M.G. Zariwala and D. Renshaw
P073	**Drink or drive: competition between macropinocytosis and cell migration** D.M. Veltman, D.A. Knecht, R.H. Insall and R.R. Kay
P074	**Integrin trafficking reprograms the actin cytoskeleton for migration in 3D-matrix** N. Paul, D.M. Green and P.T. Caswell
P075	**A mitotic exit ubiquitome from human cells** M. Min, U. Mayor, G. Dittmar and C. Lindon
P076	**Spatial organisation of early secretory pathway in mammalian cells is required for collagen secretion** V.J. Miller and D.J. Stephens
P077	**The microfilament severing and capping protein gelsolin binds directly to the molecular chaperone CCT in a calcium-induced active state** A. Svanström and J. Grantham
P078	**Identification of vaccinia virus F12 and E2 as kinesin light chain interacting proteins preferentially binding the KLC2 C-terminal tail** D.C.J. Carpentier, W.N.D. Gao, H. Ewles and G.L. Smith
P079	**CDK1 phosphorylation of Tiam1 and PAK activation regulate centrosome separation in mitosis** H.J. Whalley, G. White, E. Castaneda-Saucedo and A. Malliri
P080	**Regulation of the ESCRTIII component CHMP4C by the chromosomal passenger complex during cytokinesis** L. Capalbo, R. Nunes. Bastos, F.A. Barr and P. D'Avino
P081	**Functional role of the basic insertion within loop2 in the head domain of myosin IX** S.J. Oeding, U. Pieper, U. Honnert and M. Bähler
P082	**Endophilin marks and controls a clathrin-independent endocytic pathway** A. Ferreira

P083	**Structural insights into the organization of the cavin membrane coat** O. Kovtun, V. Tillu, R.G. Parton and B.M. Collins
P084	**Syndecan-4 controls integrin recycling to regulate cell migration and the extracellular microenvironment** M.R. Morgan, J. Batson and M.J. Humphries
P085	**PTEN regulates PAR3 positioning and mitotic spindle orientation during colorectal gland morphogenesis** R.K. Deevi, J. McClements, D. Tkoz, A. Fatehullah, M. Nagavardhini and F.C. Campbell
P086	**Insights into the trafficking and polarized secretion of major proteinase inhibitor cystatin C in retinal pigment epithelium: consequences for development of age-related macular degeneration** L. Paraoan
P087	**Dynamic anisotropies in cytoskeletal organisation induced by ROS and Rho signalling underlie multicellular sensing and spatial patterning in a *Drosophila* epithelium** S. Saravanan, S. Muliyil and M. Narasimha
P088	**A membrane-driven conformational switch in AP2 activates clathrin recruitment** B.T. Kelly, S. Graham, N. Liska, P. Dannhauser, S. Höning, E. Ungewickell and D. Owen
P089	**Structural basis and functional implications of Vps33A recruitment to the human HOPS complex by Vps16** S.C. Graham, L. Wartosch, S.R. Gray, E.J. Scourfield, J.E. Deane, J.P. Luzio and D.J. Owen
P090	**Hepatitis D virus recruits clathrin by mimicking host cell motifs** J. Muenzner, B.T. Kelly and S.C. Graham
P091	**Arpin-mediated idling and reductions in cell speed are associated with turns** R. Gorelik and A. Gautreau
P092	**A functional interplay between the leucine rich repeat kinase 2 and p21 activated kinase 6 in neuronal cytoskeleton** L. Civiero, M. Cirnaru, A. Beylina, U. Rodella, I. Russo, E. Belluzzi, E. Lobbestael, L. Reyniers, P. Lewis, C. Van Den Haute, V. Baekelandt, L. Bubacco, G. Piccoli, M.R. Cookson, J.-M. Taymans and E. Greggio
P093	**Modelling crowding behaviour of kinesin 1** N. Jenkins, R.A. Cross and S. Grosskinsky
P094	**Ysc84 is a novel, PI(4,5)P_2 regulated, actin-capping protein functioning in early stages of yeast endocytosis** A. Urbanek, E. Allwood, F. Gardiner, A.P. Smith, W.I. Booth and K.R. Ayscough
P095	**Sterol traffic in yeast is mediated by a newly discovered family of StART proteins** L. Wong, A. Gatta, Y.Y. Sere, D.M. Calderón, A. Zhuravleva, S. Cockcroft, A.K. Menon and T. P. Levine
P096	**Fission yeast Sec3 bridges the exocyst complex to the actin cytoskeleton** I. Jourdain, H.C. Dooley and T. Toda
P097	**Dissecting the roles of the IP_3 receptors in migration of fibroblasts** C. Martin-Granados, R. Butler and C.W. Taylor

P098	**Small molecule inhibitors of Rho signalling: characterization and target identification of the Rhodblocks** O. Lancaster and U. Eggert
P099	**E-cadherin apical sorting by a p200/AP-1/clathrin/RAB-11 pathway during morphogenesis** G. Gillard, M. Shafaq-Zadah, O. Nicolle and G. Michaux
P100	**The formin FMNL3 in angiogenesis** M. Richards and H. Mellor
P101	**Beta 1-integrin - c-Met crosstalk: an endosomal inside-in signalling** R. Barrow-Mcgee, N. Kishi, C. Joffre, L. Menard and S. Kermorgant
P102	**Cut7-driven microtubule sliding reverses direction depending on motor density** M. Britto, A. Goulet, K. Zehra, C. Adams, R. MacKay, C. Moores and R.A. Cross

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S001 The Ran-GTP gradient spatially regulates the activity of XCTK2 within the spindle

Lesley N. Weaver, Stephanie C. Ems-McClung, Sidney L. Shaw and Claire E. Walczak
Indiana University, Bloomington, USA

The ability of cells to nucleate microtubules (MTs) in the vicinity of the chromosomes and to organize them into a bipolar MT array is a fundamental mechanism of spindle assembly. Cues from chromatin, such as the Ran-GTP gradient, initiate a signaling cascade that promotes the release of spindle assembly factors to form the spindle. We previously showed that the minus-end directed Kinesin-14 XCTK2 is regulated by Ran-GTP. Binding of importin α/β to the tail of XCTK2 inhibits MT binding, which is reversed in the presence of Ran-GTP, suggesting that the Ran gradient around chromatin may modulate XCKT2 crosslinking. Here we show how XCTK2 is regulated in different regions of the *Xenopus* spindle. Using FRAP of GFP-XCTK2 we found that the turnover of XCTK2 is spatially regulated within the spindle, and this spatial regulation is influenced by the Ran-GTP gradient. Flattening the Ran-GTP gradient by the addition of a mixture of RanQ69L and RanT24N blocked the ability of excess XCTK2 to stimulate spindle assembly and caused bundling of free MTs within the extract. The stimulation of spindle assembly and the MT bundling activities of XCTK2 are dependent on the presence of both the XCTK2 motor and tail domains, suggesting that motor-dependent MT cross-linking is critical to XCTK2 function. Together our results suggest that XCTK2 is a key Ran-regulated factor whose activity is temporally and spatially controlled within the spindle.

Notes:

S002 Protein complexes responsible for centrosome segregation in mitosis

Fanni Gergely and Pavithra L. Chavali
University of Cambridge, Cambridge, UK

Centrosomes are normally located at the mitotic spindle poles in somatic animal cells. Maintaining a connection between the centrosomes and spindle poles is crucial not only for spindle formation, but also for correct segregation of centrosomes. Inheriting a single functional centrosome by each daughter cell is a prerequisite for subsequent centrosome duplication cycles in proliferating cells and cilia formation in quiescent or differentiating cells. We have previously described a vital function for the highly conserved centrosomal and microtubule-binding protein, CDK5RAP2, in maintaining centrosome-spindle pole attachment in the presence of spindle forces. Using a combination of affinity purification, biochemical fractionation and mass spectrometry approaches here we demonstrate that CDK5RAP2 acts in a dynamic multiprotein complex at the centrosome-spindle pole interface. Its key interactors include the minus-end directed microtubule motors, HSET and dynein, which play non-redundant roles in centrosome-spindle pole attachment. Remarkably, stability of CDK5RAP2 depends on intact centrosomes and centrosomal localization, indicating that the protein complex must have evolved to specifically function at centrosomes. We propose that via interactions with dynein and HSET the centrosomal platform formed by CDK5RAP2 serves to capture and subsequently cross-link kinetochore microtubules with centrosomal microtubules. Such a mechanism would ensure a stable position for centrosomes within spindle poles, consequently bringing about the correct transmission of centrioles into daughter cells.

Notes:

S003 Sharpening the anaphase switch

Jonathan Millar
University of Warwick, Warwick, UK

The spindle assembly checkpoint (SAC) is thought to be the primary control switch that dictates the timing of anaphase onset. Components of the checkpoint include Mad1, Mad2, Mad3(BubR1), Bub3 and the Bub1, Mph1(Mps1), and Aurora B kinases. The checkpoint is activated in early mitosis when individual kinetochores are not bound to spindle microtubules. This causes Mad2 to undergo a conformational change which triggers its association with Mad3 and Cdc20 to form the mitotic checkpoint complex (MCC), a potent inhibitor of the APC/C. When all sister kinetochores are bi-oriented the SAC is switched off. This allows APC/C to target Cyclin B and Securin for destruction. Inactivation of Cyclin B/Cdk1 triggers re-localisation of Aurora B kinase, and other components of the chromosome passenger complex (CPC), to MKLP2 (kinesin-6) at the spindle midzone. Similarly, we find that in fission yeast re-localisation of CPC depends on phospho-dependent interaction of CPC with the C-terminus of Klp9 (kinesin-6), a homologue of mammalian MKLP2. Surprisingly, when this interaction is disrupted, bi-oriented kinetochores congress to the metaphase plate but APC/C activation is delayed by a mechanism that requires Sgo2 and some (Mad3, Bub1 and Mph1), but not all (Mad1 and Mad2), components of the SAC. These data indicate that phospho-dependent interaction of CPC with Klp9 sharpens the anaphase switch by terminating a novel Mad3-dependent, but Mad1 and Mad2-independent, APC/C-inhibitory pathway that is distinct from the SAC.

Notes:

S004 Regulation of the chromosomal passenger complex in cancer

Amanda Meppelink[1], Lilian Kabeche[2], Sanne Hindriksen[1], Martijn Vromans[1], Jan Koster[3], Duane Compton[2] and Susanne Lens[1]
[1]University Medical Center Utrecht, Utrecht, Netherlands
[2]Geisel School of Medicine at Dartmouth, Hanover, USA
[3]Academic Medical Center, Amsterdam, Netherlands

Chromosomal instability (CIN) is a hallmark of cancer and is frequently caused by persistent defects in kinetochore-microtubule (k-MT) attachments. However, the molecular basis for this frequent mitotic defect in cancer cells remains unknown. The chromosomal passenger complex (CPC), consisting of Aurora B kinase, INCENP, survivin and borealin is essential to obtain proper k-MT attachments and we hypothesize that the function of this complex might be perturbed in some cancers. We are analyzing public cancer genome databases as well as microarray datasets for deviations in CPC members or its regulators. A recent analyses of microarray data of colon cancer identified Shugoshin-1 (Sgo1) as a gene with increased expression in CIN+ versus CIN- tumors. Sgo1 overexpression led to increased Sgo1 levels at centromeres, hyperstabilized k-MTs, and increased the frequency of chromosome segregation errors in anaphase. Elevated centromeric Sgo1 recruited excess PP2A-B56 to centromeres that counteracted Aurora B kinase activity and undermined efficient correction of k-MT attachment errors. Sgo1-depeleted cells displayed reduced centromeric localization of Aurora B, but the concomitant loss of centromeric PP2A-B56 maintained stable Aurora B activity. Thus, the activity of Aurora B kinase at centromeres of unattached chromosomes is balanced by the Sgo-1-dependent recruitment of PP2A-B56 to appropriately tune the stability of k-MT attachments. We propose that Sgo1 overexpression causes CIN by disturbing this balance by recruiting excess PP2A-B56 to centromeres without the concomitant extra recruitment of Aurora B kinase.

Notes:

S005 New insights into aneuploidy in mammalian eggs

Melina Schuh
MRC Laboratory of Molecular Biology, Cambridge, UK

All animal life starts with the fertilization of an egg. An egg and a sperm fuse and together they form a new embryo. But surprisingly, eggs frequently contain an incorrect number of chromosomes. Depending on the age of the woman, 10-50% of eggs are chromosomally abnormal. This makes defects in the egg the most common cause of pregnancy losses and human aneuploidy such as Down's syndrome. In this presentation, I will talk about exciting new insights into the causes of aneuploidy in mammalian eggs.

Notes:

S006 Endocytic cargo selection and clathrin coat assembly

Linton M. Traub
University of Pittsburgh, Pittsburgh, USA

In eukaryotic cells, the efficient conveyance of select transmembrane components and lumenal macromolecules between membrane-bounded organelles typically depends on small, short-lived carrier vesicles or tubules. To allow for cargo selectivity at different organelle sorting stations, the formation of transport vesicles is governed by various, biochemically distinct, polymeric proteinaceous coats, which assemble at defined membrane surfaces. Possibly the best studied of these is the endocytic clathrin coat, responsible for the swift internalization of many, but not all, cell surface constituents. During its lifetime, a clathrin-coated assemblage coordinates three temporally linked processes: cargo selection, local membrane remodeling, and regulated rupture from the plasma membrane. These integrated activities are orchestrated by a large suite of discrete endocytic factors that display characteristic temporal behaviors at the assembly zone relative to the terminal membrane scission event. The most variable step is the initiation of the coat at nascent assembly sites with an apparent random distribution over the cell surface. Several mechanistic models to explain the nucleation of clathrin coats at the cell surface have been proposed. The role of the recently identified muniscin family of early-arriving pioneer proteins in the establishment of clathrin bud sites will be examined.

Notes:

S007 The how and why early endosomes move: lessons from a fungal model system

Gero Steinberg
University of Exeter, Exeter, UK

Long-range motility of early endosomes (EEs) is a common feature of neurons and filamentous fungi. This process is driven by the molecular motors dynein and kinesin-3, but the ways by which these molecular motors cooperate is poorly understood. Furthermore, the biological reason for the constant motility of EEs is unknown. Recently, a combination of quantitative light microscopy, genetic screening and mathematical modeling in the fungal model system *Ustilago maydis* provided novel insight into the mechanism and cellular role of motor-mediate bi-directional membrane trafficking. These studies identified hook proteins as coordinators of dynein and kinesin-3 and revealed an unexpected linkage between membrane trafficking and protein translation. This talk will summarise these results, but will also present unreported roles of EE motility in fungal pathogenicity.

Notes:

S008 Adaptor protein complexes

Margaret S Robinson
University of Cambridge, Cambridge, UK

Adaptor protein complexes (APs) facilitate cargo selection and vesicle formation in a number of different trafficking pathways. Five APs have been identified, as well as the more distantly related COPI complex. APs 1 and 2, both of which are associated with clathrin-coated vesicles (CCVs), are the best characterised of the APs; however, there are still a number of unanswered questions about AP-1 function. Previously we have investigated AP-1 using knockdowns and knocksideways; more recently we have added two additional approaches, gene trapping and CRISPR/Cas genome editing, to determine the role of AP-1 in decoding acidic cluster sorting signals. We have also been exploring the links between AP-4, AP-5, and hereditary spastic paraplegia. Finally, using a newly designed bioinformatics tool, we have discovered a missing link between APs and COPI. This complex, which we have named TSET, provides new insights not only into the evolution of the AP family, but also into the evolution of the earliest eukaryotes.

Notes:

S009 Endosomal sorting orchestrated by retromer

Matthew Gallon[1], Kirsty J. McMillan[1], Ian J. McGough[2], Florian Steinberg[3] and Peter J. Cullen[1]
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[2]MRC National Institute for Medical Research, London, UK
[3]University of Freiburg, Freiburg, Germany

Retromer is an ancient, highly conserved heterotrimeric protein complex composed of VPS26, VPS29 and VPS35. Retromer is localised to various compartments of the endosomal network. From here retromer functions as a molecular machine controlling the endosomal sorting of numerous transmembrane spanning proteins (termed cargos) to the plasma membrane, trans-Golgi network (TGN) or specialised organelles (e.g. lysosome and lysosome-related organelles).
Retromer is present in every eukaryotic cell and knock-outs in model organisms are unviable as retromer regulates sorting of cargos involved in establishment of epithelial polarity and the secretion of various morphogens. Retromer is also associated with disease, being hijacked by pathogens and necessary for non-amyloidogenic processing of amyloid precursor proteins. More recently, a retromer mutation, VPS35(p.D620N), and mutations in retromer-associated proteins, have been linked with familial late onset Parkinson disease (PD). These patients are asymptomatic prior to clinical diagnosis, implying that retromer pathway mutations pre-dispose dopaminergic neurons to age-related neurodegeneration. This likely arises from an altered ability of retromer to control sorting of neuroprotective cargos. Evidence of reduced retromer expression in brains of sporadic PD patients, further supports this hypothesis.
Here we will discuss how, through studying retromer, we are gaining an increased understand of the etiology and pathology of these complex neurodegenerative diseases, especially for our limited understanding of the cell biology of sporadic and familial PD.

Notes:

S010 The structure and mechanisms of dynein

Andrew Carter, Simon L. Bullock, Max A. Schlager and Ha Thi Hoang
MRC Laboratory of Molecular Biology, Cambridge, UK

Cytoplasmic dynein is an ~1.4 MDa multi-protein complex that transports many cellular cargoes towards the minus ends of microtubules. Several *in vitro* studies of mammalian dynein have suggested that individual motors are not robustly processive, raising questions about how dynein-associated cargos can move over long distances in cells. Here, we report the production of a fully recombinant human dynein complex from a single baculovirus in insect cells. Individual complexes very rarely show directional movement *in vitro*. However, addition of dynactin together with the N-terminal region of the cargo adaptor BICD2 (BICD2N) gives rise to unidirectional dynein movement over remarkably long distances. Single molecule fluorescence microscopy provides evidence that BICD2N and dynactin stimulate processivity by regulating individual dynein complexes, rather than by promoting oligomerisation of the motor complex. Negative stain electron microscopy reveals the dynein-dynactin-BICD2N complex to be well ordered, with dynactin positioned approximately along the length of the dynein tail. Collectively, our results provide insight into a novel mechanism for co-ordinating cargo binding with long distance motor movement.

Notes:

S011 Reconstitution of a hierarchical +TIP interaction network controlling microtubule end tracking of human dynein

Christian Duellberg[1], Martina Trokter[2], Rupam Jha[1], Indrani Sen[3], Michel O. Steinmetz[3] and Thomas Surrey[1]

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Several proteins, collectively termed +TIPs, bind to growing microtubule end regions. +TIPs form dynamic interaction networks. Potentially competitive and hierarchical interaction modes play an important role in the behaviour of this network. The reasons that determine which +TIPs are recruited to the limited number of binding sites in the microtubule end regions remain poorly understood. Here we investigated how human dynein, the major minus end directed motor and an important +TIP, is targeted to growing microtubule ends in the presence of different +TIP competitors. We use TIRF microscopy and a biochemical *in vitro* reconstitution assay. We found that a hierarchical recruitment mode is used to target the dynein complex to growing microtubule ends in the presence of competitors. Our results highlight how the connectivity and hierarchy within dynamic +TIP networks are orchestrated. We will discuss similarities and differences between the mechanisms of plus end loading of human and yeast dynein and possibilities of how processive motility could be regulated after loading.

Notes:

S012 Molecular mechanisms of myosin function

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Over 30 different classes of myosins; molecular motors that walk along actin filament tracks, have been identified through phylogenetic analyses (Sebé-Pedrós et al., Genome Biol. Evol. 6: 290-305 (2014)). The human genome contains 39 myosin genes, divided up into 12 different classes (Peckham, Biochem Soc Trans. 39:1142–1148 (2011)). The structure, cellular function, and biochemical properties of many of these isoforms remains poorly characterized. Moreover, controversy remains as to whether some myosins are monomers or dimers. Myosins 6, 7a and 10 contain a stable single alpha helical (SAH) domain, situated just after the canonical lever. SAH domains are found in many proteins, and may function as a stiff spacer. In myosins, the SAH domain can extend the lever allowing myosins to take longer steps (Baboolal et al., PNAS, 106: 4189-4194 (2009). As these myosin isoforms work in a crowded environment, we questioned if the SAH domain might also act as a spring-like element between the motor and the tail, preventing the motor becoming detached from its track, when the cargo becomes trapped in the dense actin meshwork. Atomic Force Microscopy (AFM) and other experiments showed that SAH domains unfold at relatively low forces and have a high propensity to refold. These results suggest that in myosins with SAH domains, this domain could unfold and refold under relatively low forces in the cell, enabling these motors to carry cargoes in a crowded environment.

Notes:

S013 The myosin family of molecular motors: nature's exquisite nanomachines

James Spudich
Stanford University School of Medicine, Stanford, USA

The molecular basis of how myosin motors work has been significantly advanced by single molecule studies of myosins II, V and VI. Myosin V moves processively by stepping arm-over-arm, walking along the 36-nm pseudo-repeat of an actin filament by swinging its long lever arms through an angle of ~70°. Myosin VI, a considerably different myosin family member, was the biggest challenge to the lever arm hypothesis of myosin movement. It has a very short light-chain-binding domain (the conventional lever arm). Nevertheless, the molecule steps processively 36 nm along an actin filament. We now understand how this molecular motor achieves this feat, and the motor that might have disproved the swinging lever arm hypothesis ended up corroborating it.
After 40 years of developing and utilizing assays to understand the molecular basis of energy transduction by the myosin family of molecular motors, all members of my laboratory are now focused on understanding the underlying biochemical and biophysical bases of human hypertrophic (HCM) and dilated (DCM) cardiomyopathies. HCM and DCM result from single missense mutations in one of several sarcomeric proteins. Associated with HCM and DCM worldwide are heart failure, arrhythmias, and sudden cardiac death at any age. We are using *in vitro* molecular studies of biochemically reconstituted human sarcomeric protein complexes to lay the foundation for understanding the effects of HCM- and DCM-causing mutations on power generation by the contractile apparatus of the sarcomere.

Notes:

S014 Cell cycle dynamics in *Bacillus subtilis*

Romain Mercier, Yoshikazu Kawai and Jeff Errington
Newcastle University, Newcastle upon Tyne, UK

The peptidoglycan wall is a hallmark of bacterial cells and was probably present in their last common ancestor, at around the dawn of cellular life on earth. The last common ancestor also probably possessed a sophisticated division machine based around the widely conserved FtsZ protein. This tubulin-like protein assembles into a ring structure at the site of impending division and then recruits 10 or so well conserved proteins that help bring about cytokinesis in most bacteria. How cell wall synthesis is regulated during growth and division remains an important, largely unsolved problem in bacteria. We have recently been studying strange cell wall deficient, or “L-form” bacteria, as a way to gain insights into the control of wall synthesis and cell division. Using *B. subtilis* as a model we discovered that switching from the walled to the L-form state is surprisingly simple, requiring only one or two genetic changes. However, the L-forms are remarkably altered in growth and proliferation. The FtsZ-based division machine becomes completely dispensible, and the cells divide, instead, by a membrane blebbing mechanism. We can now generate L-forms in various other bacteria, including the Gram negative *E. coli*, and find that their properties are largely similar to those of *B. subtilis*, including ability to dispense with the proteinaceous division machine. These studies of L-forms have wide implications, from mechanisms of antibiotic resistance, to the origins of life.

Notes:

S015 Precision timing mechanisms for anaphase onset and cytokinesis in human cells

Ricardo Nunes Bastos, Michael Cundell and Francis A. Barr
University of Oxford, Oxford, UK

Cytokinesis is triggered following separase activation and chromosome segregation in a series of highly coordinated events. In mammalian cells, the central spindle forms in anaphase A as the chromosomes segregate. This is triggered when PP2A-B55 removes inhibitory phosphorylations from the microtubule associated protein PRC1 (protein regulator of cytokinesis). PRC1 together with a series of kinesin motor protein complexes formed by MKlp1, MKlp2, and KIF4A results in the formation of an array of antiparallel overlapping microtubules that act as a molecular ruler determining the cell division site in anaphase B. Recruitment of Polo like kinase 1 to this structure triggers activation of the ECT2 RhoGEF, and hence RhoA activation and actomyosin mediated cell cleavage during telophase. Finally, recruitment of ESCRT complex to the compressed remnants of the central spindle, a structure often termed the midbody, results in membrane remodelling and abscission to generate two separated daughter cells. Both the spatial control and temporal ordering of these events requires to action of protein kinases of the Aurora and Polo families, which are opposed by PPP family phosphatases.

Notes:

S016 Plant cytokinesis – a tale of membrane traffic and fusion

Gerd Juergens
Tubingen University, Tubingen, Germany

Cytokinesis separates the forming daughter cells. Higher plants have lost the ability to constrict the plasma membrane in the division plane. Instead, TGN-derived membrane vesicles are targeted to the centre of the division plane and generate, by homotypic fusion, the partitioning membrane named cell plate. The cell plate expands in a centrifugal fashion until its margin fuses with the plasma membrane at the cortical division site. Mutant screens in *Arabidopsis* have identified a cytokinesis-specific syntaxin named KNOLLE and an interacting Sec1/Munc18 (SM) protein named KEULE both of which are required for vesicle fusion during cytokinesis. KNOLLE is only made during M-phase, targeted to the division plane and degraded in the vacuole at the end of cytokinesis. Our studies address mechanisms of KNOLLE trafficking and interaction of KNOLLE with different SNARE partners and with SM-protein KEULE, ensuring membrane fusion in cytokinesis.

Notes:

S017 Dividing cells regulate their lipid composition and localisation

Ulrike Eggert
King's College London, London, UK

How cells regulate and execute cytokinesis, the final step in cell division, remain major unsolved questions in basic biology. I will focus on our work to understand how the plasma membrane and membrane trafficking participate in cytokinesis. Although it is known that cell membranes undergo dramatic structural rearrangements during cytokinesis, and it is obvious that membrane rearrangements are needed to seal daughter cells after severing, very little is known about whether (and how) specific lipids are involved in cytokinesis. Using small molecule and RNAi perturbations, we have identified which lipid families participate in cytokinesis. Using LC-MS-based lipid profiling, we showed that the lipidome changes with the cell cycle and that 11 lipids with very specific chemical structures accumulate in dividing cells. To begin to form hypotheses about the lipids' possible functions, we evaluated the mechanical properties of membranes in live dividing cells and their isolated lipids by AFM. We conclude that cells actively regulate and modulate their lipid composition and localization during division, with both signaling and structural roles likely. This work has broader implications for the active and sustained participation of lipids in basic biology

Notes:

S018 Regulation of midbody formation and function by mitotic kinases

Pier Paolo D'Avino, Zuni Bassi, Luisa Capalbo and Callum McKenzie
University of Cambridge, Cambridge, UK

The final separation of the two daughter cells that occur at the end of cell division – cytokinesis - requires the coordinated action of many proteins that control sequential events. First, cells determine cleavage plane position through signals generated by the spindle microtubules (MTs), which during anaphase re-organise into an array of antiparallel and interdigitating MTs known as the central spindle. Central spindle and astral MTs promote ingression of the cleavage furrow that bisects the dividing cell. Furrow ingression is driven by assembly and contraction of an actomyosin contractile ring. Finally, new membrane, in the form of vesicles transported along the central spindle MTs, is inserted at the cleavage site. At this late stage the central spindle forms a compact structure known as the midbody (MB), which contains at its centre an electron-dense structure, the MB ring or Flemming body, important for cell abscission. All these sequential events are regulated by post-translational modifications, such as phosphorylation/dephosphorylation and protein degradation. Two kinases have been recently implicated in the formation and regulation of the MB, Citron kinase and Aurora B. I will present data indicating that a cross-regulation between these two kinases is essential for proper MB formation and function.

Notes:

S019 Control of directional cell migration by the microtubule cytoskeleton

Anne Straube
University of Warwick, Coventry, UK

Directional cell migration requires the establishment and maintenance of long-term differences in structure and function between the front and rear of a cell. Many cell types require the microtubule cytoskeleton to support cell polarity and guide directional migration. This requires the asymmetric organisation of the microtubule array, tight control of microtubule dynamics and directed microtubule-based transport processes.
I will show that in human epithelial cells, cell protrusion depends on the density of subcortical microtubule assembly. We find that changes to microtubule assembly caused by perturbation of individual microtubule regulators result in a range of different cell migration phenotypes through the differential regulation of cell protrusions.
Furthermore, microtubule-dependent transport of integrins contributes to directionally persistent cell migration through stabilization of an extended cell rear. The balance of rear adhesion and contractility determines tail lifetime and tail retraction correlates to and precedes changes in migration direction. I propose a rear-drag mechanism whereby the counterforce originating from a well-anchored tail serves to maintain directionality of the force-generating leading edge of the cell.

Notes:

S020 Role of the actin cytoskeleton in 3D invasive migration

Laura M. Machesky
The Beatson Institute for Cancer Research, Glasgow, UK

Signaling to actin dynamics via the major actin nucleation promoting proteins such as WASP-family proteins and Arp2/3 complex are coordinated by small GTPases to facilitate migration. When cells move across a 2D substratum, they produce prominent lamellipodia and filopodia, which in 3D appear differently organised into elongated protrusions, actin puncta and spikes. We have been studying the role of Arp2/3 complex based actin networks in migration in 3D and *in vivo* in the spread of cancer. The actin bundling protein fascin also makes a very important contribution to tumour cell migration and invasion. When cells migrate in 3D matrix, they assemble punctate actin structures resembling invadopodia that concentrate Arp2/3 complex and N-WASP but do not depend on Scar/WAVE. Fascin mediates the formation of spikes that facilitate invasion into 3D matrix. However, proteins implicated in invadopodia assembly *in vitro*, do not necessarily show a role in tumour invasion *in vivo*, but seem to be important for metastatic dissemination. I will discuss the different requirements for dynamic actin structures *in vitro* in 2D, 3D and *in vivo*, focussing on our recent work on N-WASP and fascin.

Notes:

S021 Pushing with actin: from cells to pathogens

Vic Small
University of Vienna, Vienna, Austria

By employing a combination of live cell imaging and electron tomography (ET) we have determined the structure of lamellipodia in states of protrusion, treadmilling and initiation (1). We have shown that lamellipodia are formed via the formation of subsets of actin filaments joined by branch junctions. Image averaging produced a model of branch junctions in situ at 2.9nm resolution and revealed a close fit to the electron density map of the Arp2/3-actin complex *in vitro*. A subclass of bacteria, including Listeria, Rickettsia and viruses, such as Vaccinia and baculovirus exploit the actin machinery of host cells to generate propulsive actin comet tails to disseminate their infection. Using electron tomography, we have shown (2) that baculovirus generates at its rear a fish-bone like array of subsets of branched actin filaments, with an average of only four filaments engaged in pushing at any one time. In both these studies, the combination of negative staining-ET for higher filament resolution and cryo-ET for preserving overall 3D morphology was crucial for obtaining a complete structure-function analysis of actin driven propulsion.
1. Vinzenz, M. et al., 2012. Actin branching in the initiation and maintenance of lamellipodia. J. Cell Sci. 125:2775-85. 2. Mueller, J. et al., 2014. Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion.. PLoS Biol. 2014 Jan;12(1):e1001765. doi: 10.1371/journal.pbio.1001765. Epub 2014 Jan 14.

Notes:

S022 A helping hand from enveloped viruses to uncover the final events of cell division

Juan Martin-Serrano
King's College London School of Medicine, London, UK

We investigate the Endosomal Sorting Required for Transport (ESCRT) machinery and how this eukaryotic pathway promotes retroviral assembly and related aspects of cell biology such as endosomal sorting. One of our ongoing goals is the identification and functional characterization of novel components of the ESCRT machinery as a way to uncover host factors for enveloped viruses and new cellular processes facilitated by this pathway. Accordingly, we have a longstanding interest in the mechanisms employed by retroviruses to recruit the membrane-remodeling ESCRT complex. This work with viruses has been essential to uncover the unexpected role of the ESCRT machinery in cytokinetic abscission, the last step in cell division. My talk will cover some of our contributions to this field, with emphasis on the intersection between virology and cell biology and how we can exploit these disciplines to illuminate each other

Notes:

S023 Parallels between ER-Golgi traffic and autophagy

Susan Ferro-Novick, Saralin Davis, Juan Wang, Jingzhen Ding and Jinzhong Zhang
University of California, San Diego, La Jolla, USA

ER-derived COPII coated vesicles are usually targeted to the Golgi. However, during cell stress these vesicles are diverted to the canonical macroautophagy pathway where they become a membrane source for the autophagosome, a unique compartment that targets unwanted proteins for degradation. We have shown that when macroautophagy is induced the TRAPPIII complex, a conserved autophagy-specific guanine nucleotide exchange factor for the Rab GTPase Ypt1 (Rab1 in mammals), is recruited to the preautophagosomal structure (PAS) by Atg17. In parallel with this event, Atg9 vesicles move from the Golgi to the PAS to initiate autophagosome formation. We have proposed that activated Ypt1 recruits the putative membrane curvature sensor Atg1 to the PAS to tether Atg9 vesicles to COPII vesicles. Consistent with this proposal, COPII vesicles and the ER-Golgi fusion machinery are needed for macroautophagy. Current studies, which show that Ypt1/Rab1 regulates ER-Golgi traffic and macroautophagy by a common mechanism, will be discussed.

Notes:

S024 Morpho-functional changes in the organisation of the secretory pathway in D. melanogaster upon starvation

Catherine Rabouille
Hubrecht Institute-KNAW, Utrecht, The Netherlands

Stress, including amino-acid starvation, leads to the protein translation inhibition and formation of large cytoplasmic ribonucleoprotein particles that stored or degraded untranslated mRNAs, the Stress Granules and Processing Bodies, respectively. Here, we show that amino-acid starvation also leads to the inhibition of protein transport through the secretory pathway and formation of a novel non-membrane bound stress assembly, the Sec Body (SB) that incorporate key ER exit site components. Sec Bodies are not lipid droplets or autophagosomes. Although their formation does not require membrane traffic through the early secretory pathway, it requires Sec16 and Sec23/24AB. Furthermore, we show that they are not terminal aggregates and act as a reservoir for the re-building of functional ERES. Second, we investigate the relationship between Sec Bodies and stress granules/P-bodies and show that the formation of these stress assemblies provide a pro-survival mechanism for cell during starvation.

Notes:

S025 Mechanism of sorting and export of bulky procollagen VII from the endosplasmic reticulum

Vivek Malhotra
CRG, Barcelona, Spain

TANGO1 is localized to the ER exit site where its SH3 like domain binds procollagen VII in the lumen of the ER. The coiled coil domains of TANGO1 on the cytoplasmic side of the ER bind cTAGE5. Both TANGO1 and cTAGE 5 contain a proline rich domain (PRD) that binds Sec23 and Sec24 of the COPII coats. These interactions promote the sorting and recruitment of procollagen VII to the site of COPII coat assembly. Knockdown by siRNA of TANGO1 or cTAGE 5 inhibits procollagen VII export from the ER. The export of equally bulky procollagen I, however, is independent of TANGO1 and cTAGE5. TANGO1 also interacts with SLY1 on the cytoplasmic side and our new data reveals that SLY1 and its ER specific interacting partner syntaxin 18 are required for procollagen VII but not procollagen I export. Based on our new findings I suggest that TANGO1 and cTAGE5 sort procollagen VII and recruit it to the ER exit site. This site then grows by SLY1 and syntaxin 18 mediated fusion of membranes most likely from the ERGIC. This patch of ER is segregated from other export domains and employed for the release of procollagen VII. Thus bulky collagens are sorted from each other in the ER and exported by different routes for their release from cells.

Notes:

S026 The small GTPase Arf1 modulates mitochondrial morphology and function

Anne Spang[1], Karin Bernadette Ackema[1], Stefan Böckler[2], Shyi Chyi Wang[3], Ursula Sauder[1], Heidi Mergentaler[1], Benedikt Westermann[2], Frederic Bard[3], Stephan Frank[4] and Jürgen Hench[4]
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[2]University of Bayreuth, Bayreuth, Germany
[3]IMCB, Singapore, Singapore
[4]Institute of Pathology, University Hospital Basel, BASEL, Switzerland

The small GTPase Arf1 is indispensable for the generation of different types of transport vesicles along the secretory pathway by interaction with cargo and by recruiting coat components to membranes. In addition, Arf1 is involved in autophagy and modulate the activity of lipid modifying enzymes. We uncovered a novel, conserved role for Arf1 and its guanine nucleotoide exchange factor GBF1 in the mitochondrial morphology and function. This role is independent of Arf1's fucntion in the secretory pathway or autophagy. Arf1 does not directly regulate the dynamin-like protein Drp1/Dmn1 or the mitofusin Fzo1. However, in an *arf1* mutant, Fzo1 levels are reduced and Fzo1 appears to cluster on mitochondrial membranes. This clustering can be prevented by overexpression of the AAA-ATPase Cdc48/VCP/p97, indicating that Fzo1 might become misfolded in the *arf1* mutant. Arf1 is enriched in membrane fractions that contain mitochondria and ER-mitochondria contacts. However, in the absence of Arf1 function, ER-mitochondrial contact sites are still present. Yet, their function might be altered and lipid exchange between the organelles is potentially perturbed. We propose a regulatory role of Arf1 at ER-mitochondrial contact sites, which is essential for mitochondrial functionality.

Notes:

P001 Investigation of proteoglycan traffic in the late secretory pathway

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The information for the intracellular sorting of proteins along the secretory pathway resides either directly in the amino acid sequence of a polypeptide or in posttranslational modifications. N- and O-glycans have been previously shown to contribute to apical protein sorting in polarized cells. The third major class of glycans, the glycosaminoglycans (GAGs), has also been implicated to operate as apical and basolateral sorting signal. More recently, the effect of glycosaminoglycan chains on protein transport and sorting has been studied in non-polarized cells in general. Using short sequence tags encoding known GAG attachment sites, soluble secretory and transmembrane cargo proteins could be converted into proteoglycans. Modification of polypeptides with GAG chains altered the transport characteristics and accelerated TGN-to-cell surface transport in comparison to their GAG-free derivatives. The changes in exocytic transport from the TGN suggested that GAG chains direct proteoglycans into distinct carrier populations and pathways en route to the plasma membrane. However, the underlying sorting machinery and mechanisms that drive rapid TGN exit of proteoglycans remain elusive. With the aim to identify the components that sort proteoglycans in the TGN and mediate accelerated transport to the cell surface, we sought to isolate TGN-derived proteoglycan-ferrying carriers to analyze their molecular content by mass spectrometry. The proteomic inventory of such carriers might reveal cargo receptors specific for proteoglycans or allow us to link them to known transport vehicles in the late secretory pathway.

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P002 Poster withdrawn

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P003 GPVI clustering in platelets imaged by super resolution microscopy

Natalie S. Poulter[1], Dylan M. Owen[2], Stephanie M. Jung[3], Masaaki Moroi[3], Elizabeth Gardiner[4], Robert Andrews[4], Richard W. Farndale[3], Steven G. Thomas[1] and Steve P. Watson[1]

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GPVI is the main signalling receptor for collagen on platelets and is detected as a monomer and dimer. Biochemical techniques have shown that once bound to collagen dimeric GPVI signals through the ITAM of the associated FcRγ, leading to the formation of the LAT-based signalosome and platelet activation. However, localization of the signalling components has not been visualised by light microscopy due to the platelets size and the constraints on resolution imposed by the wavelength of light. Direct stochastic optical reconstruction microscopy (dSTORM), a super resolution microscopy technique, permits visualization of single molecules at resolutions of ~20nm and allows quantitative comparisons between experimental treatments. We have combined dSTORM and cluster analysis with high affinity anti-GPVI pan and dimer-specific antibodies to quantitatively compare GPVI clustering in platelets spread on different substrates. On fibrous collagen, GPVI clusters are highly organized along the fibre and contain dimeric GPVI. Platelets spread on collagen-related peptide (CRP) have small GPVI clusters distributed over the surface. There are twice as many receptors in clusters formed on collagen than those formed on CRP and the receptors are closer together (nearest neighbour distance in clusters: ~7nm and 11nm respectively). Our results demonstrate that dSTORM is a powerful technique for visualizing platelet proteins and allows quantitative comparisons. We will extend this work to 2-colour imaging to build up a picture of the receptors and signalling components at the platelet surface.

Notes:

P004 Oncogene-like induction of cellular invasion from centrosome amplification

Susana Godinho[1], Remigio Picone[2], Mithila Burute[3], Manuel Thery[3] and David Pellman[2]

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Centrosome amplification has long been recognized as a feature of human tumors, however its role in tumorigenesis remains unclear. Centrosome amplification is poorly tolerated by non-transformed cells, and, in the absence of selection, extra centrosomes are spontaneously lost. Thus, the high frequency of centrosome amplification, particularly in more aggressive tumors, raises the possibility that extra centrosomes could, in some contexts, confer advantageous characteristics that promote tumor progression. Using a three-dimensional model system and other approaches to culture human mammary epithelial cells, we find that centrosome amplification triggers cell invasion. This invasive behavior is similar to that induced by overexpression of the breast cancer oncogene ErbB2 and indeed enhances invasiveness triggered by ErbB2. We show that, through increased centrosomal microtubule nucleation, centrosome amplification increases Rac1 activity, which disrupts normal cell-cell adhesion and promotes invasion. These findings demonstrate that centrosome amplification, a structural alteration of the cytoskeleton, can promote features of malignant transformation.

Notes:

P005 Temporal control of cell division: switches, refractory periods and feedback control

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Mitosis is triggered by the activation of Cdk1-cyclin B1 and its translocation from the cytoplasm to the nucleus. Positive feedback loops regulate the activation of Cdk1-cyclin B1 and help make the onset of mitosis irreversible and all-or-none in character. We recently showed that an analogous process, spatial positive feedback, regulates Cdk1-cyclin B1 redistribution at the onset of mitosis (*Santos, SDM et al Cell 2012*). Triggering spatial positive feedback promoted both the rapid translocation of Cdk1-cyclin B1 to the nucleus and induced premature mitosis in the majority of free cycling cells. Surprisingly, we observed that even G1-cells underwent mitosis. In other words, even cells in which DNA was not fully replicated were able to undergo cell division. These observations raised the question on *when do cells become competent to undergo a new division cycle* and *how is this mechanism controlled*. By combining live cell imaging of cell cycle biosensors with computational approaches we found that the presence of a negative feedback in the Cdk1-cyclin B1 core network imposes a refractory period in which cells are unable to respond to a mitotic trigger. If negative feedback is weakened, the refractory period is shortened and cells can re-enter a new division cycle soon after dividing. Our results suggest that negative feedback Cdk1-cyclin B1 may be the basis for a long and complete interphase and is essential for maintenance of chromosome number and for reliable segregation of genetic information to daughter cells.

Notes:

P006 Poster Withdrawn

Notes:

P007 GIMAP5 affects calcium homeostasis by regulating the movement of organelles

Sheela Ramanathan, Daniel Serrano, Subburaj Ilangumaran and Christine Lavoie
Université de Sherbrooke, Sherbrooke, Canada

Introduction: Mature T cells that lack a functional GIMAP5 protein (GTPase of the immune associated nucleotide binding protein 5) are unable to survive in the secondary lymphoid organs. The precise mechanisms by which GIMAP5 mediates the survival of T cells are not known. We have previously shown that GIMAP5 deficiency impairs TCR-induced calcium influx. We have also shown that this reduced calcium influx is associated with the inability of mitochondria to sequester calcium in a microtubule dependent manner. The regulation of calcium response was also observed in 293T cells overexpressing GIMAP5 protein. In this study we identified the domains of GIMAP5 required for the calcium flux and interaction with microtubules. **Methodology**: Interaction of GIMAP5 or the different deletion mutants with subcellular structures was assessed by confocal microscopy. Intracellular calcium flux was determined by videomicroscopy. **Results:** The transmembrane domain located at the C-terminal of the GIMAP5 protein is involved in anchoring the protein on lysosomes and other vesicles in the cell. N-terminal region of GIMAP5 is required for the interaction with the microtubules. However, interaction with the microbules, and the membrane anchor regulate the calcium homeostasis in the cell. **Conclusions**: Our results suggest that GIMAP5 may be part of the complex that mediates the movement of organelles on microtubules. Thus GIMAP5 may have a role in regulating the crosstalk between organelles and cytoskeleton in the maintenance of the intracellular calcium homeostasis in T lymphocytes.

Notes:

P008 Human MOB2 acts as a positive regulator of cell migration and invasion in human fibrosarcoma cells

Seng-Sheen Fan, Yi-ching Liu, Lai-mun Choo, Meng-han Dong and Wei-ting Chao
Tunghai University, Taichung, Taiwan

Mps-One-Binder proteins (MOB) are important components to control cellular processes, such as cell proliferation, cell migration, morphogenesis, and apoptosis. MOB family proteins can be found from yeast, *Drosophila* and mammals. In *Drosophila*, Mob1 protein acted as tumor suppressor to control the Hippo pathway and photoreceptor morphogenesis. Human MOB2 (hMOB2) is known to bind with NDR1/2 and participates in regulating cell apoptosis and centrosome duplication. In mouse neuron cell, MOB2 regulates the organization of actin filaments and participates in neurite formation. It has been shown that the actin dynamics is highly regulated during cell migration. We are interested to investigate whether MOB2 plays a role in cell migration. Using immunocytochemistry, we found hMOB2 was localized at the cytoplasm, nucleus, and cell edge. In migrating cells, we found that hMOB2 was mainly localized in the leading edge. In the wound healing assay and transwell migration assay, we found that depletion of hMOB2 expression leaded to reduce the rate of cell migration invasion. We also found that expression of full-length hMOB2 could rescue the cell migration activity in hMOB2 depleted cells. Furthermore, the expression and the localization of focal adhesion complex were significantly increased in MOB2 depleted cells. Together, the results suggest that hMOB2 acts as a positive regulator of cell motility and invasion in human fibrosacroma cells.

Notes:

P009 Robust mitotic spindle formation is achieved by the co-ordinated action of multiple microtubule generating pathways

Daniel Hayward[1], Jeremy Metz[1], Claudia Pellacani[2] and James G. Wakefield[1]
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[2]Universita di Roma "La Sapienza", Rome, Italy

The mitotic spindle is defined by its bipolar symmetry and organised mass of microtubules, which drive chromosome alignment and segregation. Although different cells have been shown to employ different molecular pathways to generate the microtubules required for spindle formation, how these pathways are co-ordinated within a single cell is poorly understood. We have tested the limits within which the *Drosophila* embryonic spindle forms, disrupting the inherent temporal control that overlays mitotic microtubule generation, interfering with the molecular mechanism that generates new microtubules from pre-existing ones, and disrupting the spatial relationship between microtubule nucleation and centrosome function. Our work uncovers previously undescribed routes to spindle formation in embryos and establishes the central role of Augmin in all microtubule-generating pathways. It also demonstrates that the contributions of each pathway to spindle formation are integrated, highlighting the remarkable flexibility with which cells can respond to perturbations that limit their capacity to generate microtubules.

Notes:

P010 Functional characterization of the Nesprin-2 – SMC interaction

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Centre for Biochemistry, Institute of Biochemistry I, medical Faculty, Cologne, Germany

Nesprins (Nuclear Envelope Spectrin Repeat Proteins) are nuclear envelope proteins that are part of the LINC (Linkers of the nucleoskeleton to the cytoskeleton) complex. Mutations of proteins in the LINC complex cause diseases which are termed laminopathies, for example dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy. Recently a new domain was found in the central rod domain of Nesprin-2, a structural maintenance of chromosomes (SMC) domain. Notably SMC-proteins have roles in a wide range of cellular processes including mitosis and DNA damage repair, in which Nesprins have currently not been described. SMC proteins form heterodimers among each other, which are supported by non-SMC proteins. One of these complexes is the heterodimer of SMC2/4, which is also known as condensin and plays a central role in chromosome condensation.
Interestingly our group showed that a loss of the Nesprin-2 isoforms which contain the SMC-domain leads to swollen chromosomes and increased chromosomal areas. Similar phenotypes have been described after a loss of SMC2 or SMC4 which form the condensin complex. Thus the increased chromosomal area could be a result of condensation defects upon Nesprin-2 deficiency. In order to understand this newly discovered function of Nesprin-2 in chromosome condensation we are characterizing the interaction between Nesprin-2 and SMC proteins. Regarding these aims, the interaction between Nesprin-2 and SMC2/SMC4 has been shown in pull down and immunoprecipitation with lysates from a human keratinocyte cell line. Additionally, an overlay assay revealed that the Nesprin-2 and SMC2 interaction is a direct interaction. Strikingly, we found that the interaction is strongest in S-Phase, but is also present in mitosis. Knock down of Nesprin-2 isoforms which contain the SMC-domain did not lead to an overall change in localization of SMC2, SMC3 and SMC4 at least during mitosis. The Nesprin SMC protein interaction has not been described so far and therefore this project offers the possibility to substantially expand the repertoire of Nesprin-2 functions.

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P011 Poster withdrawn

Notes:

P012 Myosin Va and dynamic actin oppose microtubules to drive long-range organelle transport.

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In animal cells microtubule and actin tracks, and their associated kinesin/dynein and myosin motors, are thought to perform complementary roles regulating long- and short-range transport, respectively. For several cargo (including melanosomes) microtubule transport is bi-directional, and thus it is assumed that centrifugal and centripetal transport, to and from the plasma membrane, mirror one another in their requirement for microtubules and actin. The 'co-operative capture' model exemplifies this view, and suggests that melanosome distribution in melanocyte dendrites is maintained by long-range transport on microtubules followed by actin/myosin-Va-dependent tethering. In this study we used cell normalisation technology to quantitatively examine the contribution of microtubules and actin/myosin-Va to organelle distribution in melanocytes. Surprisingly, our results indicate that microtubules are essential for centripetal but not centrifugal transport. Instead we find that microtubules retard a centrifugal transport process that is dependent upon myosin-Va and a population of dynamic F-actin. Functional analysis of mutant proteins indicates that myosin-Va works as a transporter dispersing melanosomes along actin tracks whose +/barbed ends are oriented towards the plasma membrane. Overall our data highlight the role of myosin-Va and actin in transport, and not tethering, and suggest a new model in which organelle distribution is determined by the balance between microtubule-dependent centripetal and myosin-Va/actin-dependent centrifugal transport.

Notes:

P013 On the mechanism of durotaxis in motile cells

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Many dynamic processes, including cytoskeletal polymerization and cell-substrate interactions, determine morphological and directional parameters of migrating cells. A large number of studies have characterized the mechanical behavior of stationary cells in response to matrix stiffness changes, however there have been no comprehensive efforts to elucidate the mechanisms by which matrix stiffness determines motile cell behavior. To this end, we have studied the mechanosensitive response of fish keratocytes, which are among the fastest moving animal cells. Based on a large population of cells, our results demonstrate that keratocyte morphological parameters are significantly influenced by the matrix stiffness over a wide range of rigidities (from kPa to GPa). These observations suggest that matrix stiffness can be a valuable tool for controlling the natural phenotypic variability of keratocytes. Furthermore, migrating parameters were also observed to be controlled by matrix stiffness, suggesting that the substrate rigidity dictates the level of polarization and directionality of motile cells. We further demonstrate that the physical linkage between the cytoskeleton and the substrate is significantly affected by the matrix stiffness and is required for cell polarization. On the whole, our results are consistent with a quantitative physical model in which the overall keratocyte behavior (shape, polarization and directionality) emerges from the impact of the matrix stiffness on the formation of focal adhesions that creates a frictional slippage, which, in turn, balances myosin-mediated contractile forces.

Notes:

P014 A novel actin filament population that regulates ER-to-Golgi trafficking

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Although the actin cytoskeleton is critical for Golgi morphology and vesicle trafficking, the actin filaments associated with the ER and Golgi have not been clearly defined. We have shown that the actin-associated protein, tropomyosin (Tm), defines in an isoform specific manner the function of actin filament populations. In this study, we have identified a specific Tm isoform, Tm4 at the Golgi colocalised with β-actin. Tm4 and β-actin localisation was sensitive to the Golgi disrupting drug, Belfeldin A (BFA), and with BFA removal, Tm4 and β-actin re-association with the Golgi was observed at the early stages of Golgi reformation. In mouse embryonic fibroblasts (MEFs) that lack the full-length Tm4 protein, the reestablishment of Golgi morphology after BFA treatment was altered (Golgi more dispersed) compared to wild-type cells. Using the temperature sensitive VSV-G(ts045)-KDEL construct, ER-to-Golgi but not Golgi-to-ER trafficking was shown to be altered in the Tm4 mutant cells. This anterograde trafficking defect was specific to Tm4 as exogenous Tm4 was able to rescue ER-to-Golgi trafficking in the Tm4 mutant cells. The Tm4-dependant anterograde trafficking was dependant on Myosin II activity. Consistent with impaired export from the ER, EM analysis of the Tm4 mutant MEFs revealed a number of ER and Golgi dysmorphologies including the proliferation of swollen ER structures. In conclusion, we have identified a novel Tm4/β-actin filament population that has an important role in ER-to-Golgi vesicle trafficking.

Notes:

P015 Role of myosins 10 and 18a in migration of prostate cancer cells

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Myosins are molecular motors involved in cell migration and motility, and it is likely that their activity could contribute to metastatic spread of cancers. To test this idea, we used quantitative PCR and immunoblotting to analyse myosin expression pattern in prostate cancer cell lines with high (PC3) and low (LNCaP) metastatic potential. We found that 5 myosins, including Myo10 and Myo18a, significantly increased their expression in cells with higher metastatic potential (PC3). We next used siRNA to knockdown myosin expression in prostate cancer cells, and we found distinct changes in cell morphology, adhesion, and cell migration of prostate cancer cell lines for Myo10 and Myo18a. Knockdown of Myo10 in PC3 cells significantly increased spread area, and reduced numbers of filopodia and speed of migration. Knockdown of Myo18a also significantly increased spread area, did not affect filopodia or migration speed, but increased numbers of non-muscle myosin 2a filaments. We also observed a drastic effect of Myo10 knockdown on morphology of a glioblastoma cell line, suggesting that Myo10 might also be important in other types of cancer. This is the first demonstration of the role of these myosins in migration of prostate cancer cells, and suggests that relative expression levels of different myosins are important for determining cellular phenotype.

Notes:

P016 WAFL knockdown impairs cell migration and invasion

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In 2011 Hanahan and Weinberg defined 10 hallmarks of cancer that included "Activating invasion and metastasis". This process involves primary tumor cells invading into their local microenvironment and migrating away from the primary tumor followed by metastasis to different organs, which dramatically reduces patient survival. In an effort to further understand this process we have performed an invasion screen based on the circular invasion assay (Kam et al, 2008 BMC Cancer). In this screen we analysed 433 genes and obtained just over 100 positive hits.
WAFL (Wiskott-Aldrich syndrome protein and FKBP-like), also known as FKBP15, was one of the positive hits. WAFL is a 133kDa protein that contains four identifiable domains of which two, the WH1 (WASP Homology 1) domain and acidic C-terminus, are found in the WASP (Wiskott-Aldrich syndrome protein) family of actin regulators. WAFL was first identified as a protein overexpressed in Ulcerative Colitis (Viklund et al, 2008, Int J Colorectal Dis.) and later linked to endocytic transport (Viklund et al, 2009, Exp Cell Res.). Interestingly, WAFL also interacts with the WASH complex.
At present the role of WAFL in cells is not clear, here we validate that WAFL knockdown impairs cell invasion in the circular invasion assay and investigate further the consequences of WAFL knockdown on cell migration and invasion.

Notes:

P017 Linking ESCRT function to genomic instability and DNA damage

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ESCRT (endosomal sorting complex required for transport) is a multiprotein complex better known for its functions in viral budding and multivesicular body biogenesis. However, ESCRT complexes have recently been shown to have crucial roles in cytokinesis and mitosis. Four ESCRT complexes exist, ESCRT-0, I, II and III. In humans, ESCRT-III is composed of the CHMP protein family, which includes CHMP1 through 7, together with the AAA ATPases VPS4A and B, which promote disassembly of the ESCRT complexes. CHMP is an acronym for either CHromatin Modifying Proteins or CHarged Multivesicular Proteins to reflect the major biological roles for this family. Depletion of ESCRT-III subunits generates defects in mitosis and cytokinesis, preventing accurate chromosomal segregation, which in turn generates DNA damage and genomic instability. In this context, some CHMP proteins are found associated with micronuclei and lagging chromatin, but the significance of this occurrence is largely unexplored. We are investigating the chromatin-associated roles of ESCRT-III by dissecting the mechanisms that regulate their nuclear localisation and examining the influence of ESCRT-III subunits depletion on the cellular sensitivity to DNA damaging agents. Our overarching aim is the dissection of the mechanisms that link ESCRT function to the maintenance of genomic stability.

Notes:

P018 The role of MELK in cell division

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Maternal embryonic leucine kinase (MELK) is a member of the PAR-1/Kin1/SAD-1 protein kinase superfamily and is involved in various cellular processes, including cell division. It contains a KA1 (kinase-associated-1) domain, which is reported to bind to acidic phospholipids and is proposed to target the kinases to membranes. Using HeLa cells, we confirmed previous observations in *Xenopus* embryos that endogenous MELK localizes to the cell cortex, cleavage furrow and early midbody. Depletion of MELK results in multinucleate/giant-nucleate cells, indicating a role of MELK in cell division. By live microscopy we observed that the mutation in KA1 domain of GFP-tagged MELK affects its cortical localization. As MELK has been reported to act in a conserved PAR-4/LKB1 pathway, possible partners of MELK in cell division were searched in this pathway. Among them, MO25 (CAB39) colocalized with MELK at the midbody and also presented localization at spindle poles. Depletion of CAB39 resulted in defects in the spindle and in a multinucleate phenotype. We are currently trying to elucidate further the roles of MELK and its partners in cell division.

Notes:

P019 Analysis of Weibel Palade Body exocytosis and subsequent compensatory endocytosis using correlative electron microscopy

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Weibel Palade bodies (WPB) are the intracellular storage organelle of endothelial cells. These massive secretory organelles (up to 5 μm in length) contain a whole host of pro-inflammatory and pro-haemostatic proteins that are essential for an appropriate physiological response to tissue injury. The organelle contents include integral membrane proteins such as P-selectin and secreted proteins, the most abundant of which is Von Willebrands factor (VWF). Following exocytosis some of the membrane and integral proteins necessarily need to be retrieved by compensatory endocytosis. The extent of compensatory endocytosis using biochemical assays was determined, however, we also required analysis of these events at higher resolution. A spinning disk microscopy assay was therefore developed for time resolution of the point of exocytosis, and for the first time using correlative light and electron microscopy we were able to monitor compensatory endocytosis relative to the point of fusion. Furthermore, we were able to monitor intermediates of exocytosis using electron microscopy by changing the external pH, and demonstrate that WPB can fuse at previous sites of exocytosis. Overall this gives a high resolution analysis of the events surrounding WPB exocytosis.

Notes:

P020 CLIP-170 spatially modulates receptor tyrosine kinase localization to coordinate cell migration

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Endocytic sorting of activated receptor tyrosine kinases (RTKs) between recycling and degradative processes controls signal duration, location and surface complement of RTKs. Microtubule plus-end tracking proteins play essential roles in various cellular activities including translocation of intracellular cargo. Mechanisms through which RTKs recycle back to the plasma membrane following internalisation in response to ligand remain poorly understood. We find that net outward-directed movement of endocytic vesicles containing the hepatocyte growth factor (HGF) RTK, requires recruitment of the microtubule-binding protein, CLIP-170 as well as the association of CLIP-170 to microtubule plus-ends. In response to HGF, entry of Met into Rab4 positive endosomes results in Golgi-localized γ-ear-containing Arf-binding protein 3 (GGA3) and CLIP-170 recruitment to an activated Met RTK complex . We conclude that CLIP-170 acts to co-ordinate the recycling and the transport of Met-positive endocytic vesicles to plus-ends of microtubules towards the leading edge thereby promoting cell migration.

Notes:

P021 The spindle checkpoint regulates Cdc20 activity and turnover to control mitotic progression

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The spindle checkpoint is a surveillance system that delays anaphase onset until all chromosomes are properly attached to the mitotic spindle. Several protein kinases have critical roles to play in spindle checkpoint signalling, but the mechanisms by which they inhibit mitotic progression remain unclear. In fission yeast the mitotic checkpoint complex (MCC) generated at unattached kinetochores is Mad2-Mad3-Cdc20. In mitosis we detect a stable interaction between the MCC and the anaphase-promoting complex (APC/C). Assembly and maintenance of MCC-APC/C requires Mps1, Bub1 and Aurora (Ark1) kinase activities and the small APC/C sub-unit Apc15. Efficient checkpoint release requires Protein Phosphatase 1 (PP1) activity and Apc14. We have identified ~20 phosphorylation sites in the three MCC proteins (Cdc20, Mad2 and Mad3). Mutational analyses show that certain modifications of Mad2 and Mad3 stabilise their interactions within MCC-APC/C and that these are important for maintenance of spindle checkpoint arrest. Mutation of phosphorylation sites in Cdc20 and its C-terminal –IR motif affect APC/C binding and mitotic timing. Cdc20 gets poly-ubiquitinated by the APC/C and is continually degraded during mitosis and we find that the ability of the MCC to bind to APC/C has a profound effect on the stability (half-life) of Cdc20. We will present recent data and structural models as to how the checkpoint acts to regulate Cdc20-APC/C interactions and activity, and how these in turn regulate Cdc20 homeostasis and mitotic progression.

Notes:

P022 Development of tools to study polarised secretion using super resolution microscopy

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Cell polarity and membrane trafficking are intertwined processes that ensure that tissue homeostasis is maintained. Whilst much of the machinery related to both processes has been well described, how they are linked and the specific tools required for polarised trafficking are less clear. We are using super resolution microscopy, combined with molecular biology tools in an attempt to functionally describe sorting for discrete plasma membrane destinations, as well as destinations themselves. The model we are using is the follicle cell of the Drosophila egg chamber and thus it has been necessary to develop a range of tools that will enable us to complete this study. This has included optimising the use of dyes, compatible with super resolution microscopes, in the egg chambers, as well as synchronisation techniques to allow us to study the biosynthetic pathway.

Notes:

P023 *P. aeruginosa* lectin LecB triggers bacterial invasion via induction of PI3K, the small GTPase Rac and subsequent actin cytoskeleton rearrangement

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P. aeruginosa is a major opportunistic and nosocomial pathogen causing severe infections of the pulmonary tract with a particular risk for cystic fibrosis patients. Currently, antibiotic resistance becomes an increasing problem; hence, it is important to identify the proteins in the dynamic cellular processes that are crucial for *P. aeruginosa* infection and might represent potential drug targets for therapy.
In many cases, pathogens hijack the host cellular apparatus in order to promote and/ or spread the invasion. However, for *P. aeruginosa*, the inducing bacterial factors and the specific signaling pathway are only partly described.
Potent virulence factors of *P. aeruginosa* are lectins that contribute to the specific recognition and the initial adhesion to the host cell. In our study we analyzed if and how the *P. aeruginosa* lectin LecB may additionally induce kinase signaling and subsequent cytoskeleton rearrangement in order to promote bacterial invasion.
We found that LecB is capable to induce the activity of PI3K and of the small GTPase Rac as well as actin cytoskeleton rearrangement. Regarding bacteria, the *P. aeruginosa* Δ*lecB* mutant strain showed reduced invasiveness. Taken together, these results show that the extracellular bacterial factor LecB facilitates the invasion of *P. aeruginosa* by the induction of host cell signaling.

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P024 Poster withdrawn

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P025 Investigating the role of mitotic MAPs in the early *Drosophila* embryo

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Cell division is a fundamental biological process. It is driven by the formation of a microtubule (MT)-based mitotic spindle that ensures faithful chromosome segregation and, subsequently, a MT-based central spindle that facilitates cytokinesis. Defects in these structures can lead to chromosomal instability and/or cell polarisation defects. Therefore, understanding how MTs are formed and how their behaviour is regulated is important for understanding diseases such as cancer, microcephaly, and neuronal disorders. The nucleation, length and the dynamics of MTs are determined by MT associated proteins (MAPs). Following on from previous work in our lab (Hughes et al., PLoS Biology, 2008) we have undertaken quantitative, comparative MAP proteomics, comparing the complement of MAPs from *Drosophila* early embryos either stochastically transiting through the cell cycle, or thoseat the metaphase-anaphase transition.
We demonstrate that, as expected, many known MAPs important for cell division increase their association with MTs during mitosis. In addition, our approach identifies classes of proteins, not previously characterised as MAPs, which bind MTs during mitosis. Through generation of transgenic flies expressing GFP-fusions to these proteins, we are investigating both their cell-cycle dependent localisation and identifying their interacting partners. We are also generating antibodies against some of these proteins in order to undertake interfering antibody injections in embryos. We will present our initial investigations into some of these classes of MAPs, verifying our approach with the previously characterised proteins Asp and TD60.

Notes:

P026 Protein sorting and glycan biosynthesis

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Secreted and membrane proteins undergo different posttranslational modifications, most importantly glycosylation, while they moving through the Golgi. Modifying enzymes are non-uniformly distributed amongst the Golgi cisternae, a distribution maintained by vesicular sorting. At the centre of the protein interaction networks responsible for the targeting of enzyme-carrying vesicles to the various cisternae, is the conserved oligomeric Golgi (COG) complex. This hetero-octameric complex can be divided into two lobes, lobe A containing subunits 1-4, while lobe B subunits 5-8. COG's defects cause glycosylation abnormalities in all studied model organisms, and congenital glycosylation disorders in humans. Using recently developed cell free vesicle transport and glycan analysis assays the role of different COG mutations in the various transport steps at the Golgi was assessed. While glycan profiling suggests that lobe B defects cause a disruption of late-Golgi enzyme trafficking and lobe A defects disrupt early to medial enzyme sorting, a cell-free assay shows a more nuanced picture. Cell-free reconstitution of galactosyltransferase trafficking indicates that lobe A may inhibit lobe B's function during late-Golgi vesicle transport. These data imply that in patients with congenital glycosylation disorders, who are suffering from COG deficiencies, a primary cause for the cellular defect could be an imbalance of the different COG subunits.

Notes:

P027 RNA polymerase III promotes cell migration and ECM remodelling through increased expression of its product, $tRNA_i^{Met}$

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RNA Polymerase III (Pol III) is responsible for transcription of small non-coding RNAs. Bioinformatic data indicate that Pol III transcripts are expressed at higher levels in melanoma metastases by comparison to the primary tumour. We demonstrate here that raised Pol III activity enhances the invasive and metastatic capacity of tumour cell lines through both cell autonomous and non-autonomous mechanisms.
Increased expression of a single Pol III product, $tRNA_i^{Met}$, increases migratory velocity of immortalised mouse embryonic fibroblasts (MEF3T3) by approximately 1.5-fold. This was opposed by pre-incubation with blocking antibodies which target either $\alpha_5\beta_1$ integrin or fibronectin, suggesting an alteration in integrin dependent cell migration. Furthermore, WM266.4 melanoma cells overexpressing $tRNA_i^{Met}$ have an increased capacity to colonise the lungs when injected into the tail vein of nude mice.
To investigate this further, we analysed the extracellular matrix (ECM) produced by MEF3T3 $tRNA_i^{Met}$ cells. Compared to control, the ECM derived from $tRNA_i^{Met}$-overexpressing cells was able to support increased migration velocity of fibroblasts. Moreover, control MEF3T3 cells treated with conditioned media from $tRNA_i^{Met}$ overexpressing cells also produced ECM that supported increased cell migration.
To determine what factor(s) were driving formation of a pro-migratory ECM, mass spectrometry was conducted on proteins secreted from MEF3T3 $tRNA_i^{Met}$ cells. This data indicates that $tRNA_i^{Met}$ overexpression can drive secretion of a more collagen rich ECM.
Taken together, these data suggest that Pol III can act via $tRNA_i^{Met}$ to alter integrin dynamics and ECM deposition which, in turn, is capable of promoting cell migration and metastases.

Notes:

P028 Application of adhesive protein micropatterns to study dynamics of actomyosin filaments during Epithelial to Mesenchymal Transition

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Epithelial-Mesenchymal transition (EMT) is a biological process during which adherent epithelial cells with defined apical-basal polarity reversibly shift to a mesenchymal phenotype due in part to loss of cell-cell contacts. Non-muscle myosin IIA (NMIIA), an actin-based molecular motor, is involved in the distribution of the actin cytoskeleton which undergoes modification during EMT. It is particularly important in the formation of actin stress fibres, which are vital for maintaining functions including cell polarity, adhesion and migration. Adhesive protein micropatterns are custom designed micrometre-scale constructs composed of extracellular matrix proteins (ECM). They have become a popular and flexible method for the investigation of cytoskeletal modifications. They are capable of influencing the shape and cytoskeletal arrangement of cells through the formation of focal adhesions between the patterned ECM and the surface of cells. In this study, a selection of micropatterns were designed and produced to study the dynamics of NMIIA filament formation. Photolithography and focused ion beam etching were investigated for the production of silicon moulds to produce elastomeric stamps for micro-contact printing. Designs were drawn using software, and film and chrome photomasks were produced to generate stamps. Results from the different masks were compared and assessed. A novel micro-contact printing procedure was developed, and fluorescently labelled fibronectin micropatterns were successfully produced. These were used to investigate distribution of NMIIA in wild type and mutant cells with inducible EMT and GFP-tagged NMIIA.

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P029 Poster withdrawn

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P030 The role of the coxsackie and adenovirus receptor in lung cancer cell proliferation

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The coxsackie and adenovirus receptor (CAR) is a transmembrane adenovirus receptor and an important molecule in controlling cell-cell adhesion through homodimer formation between adjacent epithelial cells. Previous work has shown that CAR may play a role in proliferation. Our own analysis has shown that CAR expression alters epidermal growth factor (EGF)-dependent proliferation and migration in A549 lung cancer cells. Furthermore, CAR and the EGF receptor (EGFR) are able to form a biochemical complex and colocalise in these cells. Moreover, we performed a recent mass spectrometry screen to identify novel intracellular binding partners for CAR. One of the interaction partners identified using this approach was the kinesin-like protein, Kif22. Kif22 is a microtubule motor that is important for chromosome separation. Therefore, we hypothesised that CAR may play a role in cell proliferation in lung cancer through co-operation with Kif22 downstream of EGFR signalling. Current experiments are utilising biochemical and microscopy approaches to further define the interaction between CAR and Kif22 and whether this complex can mediate EGF-dependent signalling changes leading to enhanced cell growth. To understand the relationship between CAR, Kif22 and EGFR in a disease context, the proteins will be studied in tissue samples, as well as in an *in vivo* model. This work will provide an understanding of how these proteins coordinate to drive lung cancer development and progression.

Notes:

P031 Hormetic effects of the flavonoids Quercetin, Galangin and Chrysin on 3T3 pre-adipocyte growth and development

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Natural dietary plant flavonoids are known to impact on the growth and development of mammalian cells. Flavones and flavonols are two widely studied flavonoid classes but little is known concerning their effects on pre-adipocytes. Proliferation and differentiation, hallmarks for cell growth and development, have been suggested to be mutually exclusive. An increase or decrease in proliferation would impact on the available cells for differentiation. Pre-adipocyte proliferation after treatment with flavonoids was investigated using the model cell line 3T3-L1. Proliferation was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 5 days of incubation. At supra-physiological concentrations, Quercetin, Galangin and Chrysin significantly reduced proliferation ($p<0.05$, t-test) whereas proliferation was encouraged (up to 20%) at concentrations below 22.2 μM. Combining Quercetin, a flavonol, and Chrysin, a flavone, showed similar results. This study suggests that flavonoids at physiological concentrations may delay the differentiation of pre-adipocytes. In the delayed phase, non-committed pre-adipocytes could potentially become progenitors of other cell types and this would reduce the risk of obesity by pre-adipocyte diversion. It can be concluded that flavonoids have a hormetic effect on adipocyte growth and development dependent on flavonoid concentration. Investigation of a wider range of flavonoid combinations that may be more effective modifiers of both the proliferation and differentiation of pre-adipocytes is warranted.

Notes:

P032 Endocytosis and S6-kinase: is there a link?

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Ribosomal protein S6-kinase (S6K) plays a crucial role in maintaining cell homeostasis and increased S6K activity is linked to pathologies, such as tuberous sclerosis (TSC). This genetic disease is characterized by tuber formation in different tissues, including skin and brain. S6K is mainly known for its role in regulating translation, but recent studies revealed that its activity is also important for other aspects of cell metabolism, e.g. transcription and lipid biosynthesis. Here we present data that point to clathrin-mediated endocytosis (CME) as another S6K-dependent process. In particular, we identified μ-adaptin, a protein involved in CME, as a possible S6K-interacting protein. We further confirmed role of S6K in regulation of CME, showing that pharmacological inhibition of S6K activity alters transferrin uptake. In contrast, in tuberous sclerosis patients' fibroblasts, which show S6K overactivation, CME is remarkably increased. Importantly, endocytosis and proper protein sorting are currently considered key elements of cellular signaling. Therefore, disturbed internalization of membrane receptors could account for deregulation of cell proliferation, growth and migration, which are all hallmarks of TSC. *This work has been financed by FNP grant POMOST/2013-7/10.*

Notes:

P033 Cellular characterization of bleb-like structures induced by pore-forming toxins

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During infection many bacteria secrete pore-forming toxins (PFTs) that disrupt the plasma membrane (PM) integrity, causing Ca^{2+} influx from the extracellular environment. This stimulates repair mechanisms such as PM blebbing, which allow isolation and recovery of membrane lesions. Blebbing is regulated by the actomyosin cytoskeleton, however the specific regulators of this process remain unknown. We found that the challenge of host cells with different bacterial PFTs (Listeriolysin O (LLO) or Aerolysin O) induces Ca^{2+}-dependent blebbing followed by the formation of bleb retraction structures at the cell surface. Characterization of these structures showed that they display a unique cytoskeletal organization and recruit different cytoskeletal components such as actin, non-muscle myosin-II (NMII) isoforms, Filamin and others. We showed that depletion of NMIIA inhibits the formation of these structures and renders cells susceptible to intoxication. In contrast, NMIIB is not required for this process. In addition, the analysis of other cellular organelles showed that PFT intoxication causes endoplasmic reticulum (ER) vacuolation and the recruitment of ER proteins to the bleb retraction structures. Interestingly, pre-treatment of cells with Brefeldin A, an inhibitor of the ER-to-Golgi anterograde transport, reduces the number of bleb-like structures following PFT intoxication, suggesting the importance of ER in the regulation of toxin-induced blebbing responses. Here we will characterize these cytoskeletal structures and discuss the role of cytoskeleton and ER components during membrane blebbing and cell survival responses to PFTs.

Notes:

P034 Snaps of SNARES and Muncs - IF localisation of endogenous Syntaxin11 and Munc18-2 in human cytotoxic T-lymphocytes

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Cytotoxic T-lymphocytes (CTLs) are key players of the mammalian immune system capable of eliminating infected and tumourigenic cells through targeted delivery of cytotoxic substances.
Pro-apoptotic factors are released from secretory lysosomal related organelles (LROs) at a highly organised CTL-target interface that is known as the immune-synapse. Precise delivery of LRO-cargo is a highly dynamic process that requires complex machinery. The study of the immunodeficiency condition Familial Haemophagocytic Lymphohistiocytosis (FHL) identified several proteins that are essential for CTL killing. Mutations in the Sec/Munc protein Munc18-2 and its binding partner the SNARE protein Syntaxin11 (Stx11), have been shown to cause FHL type 5 and 4 respectively and it has been suggested that Munc18-2 delivers the SNARE protein to its site of action. A key question that remains to be answered is how Munc18-2 and Stx11 function in secretion.
CTLs represent an excellent model system for the study of Munc18-2 and Stx11 involvement in targeted secretion. We have generated new antibodies for detection of endogenous Munc18-2 and Stx11 by immuno-fluorescence. Munc18-2 localised to the cytoplasm, secretory granule membranes and the plasma membrane. Stx11 was exclusively detected on the plasma membrane where it appeared to accumulate at the leading edge of migrating cells. These findings support the model of Munc18-2 chaperoning Stx11 from lysosomal compartments to the plasma membrane where it seems likely that it takes part in the final fusion events involved in secretion.

Notes:

P035 Cross-linking (3D) Proteomics of the Augmin complex suggests a mechanism for gamma-tubulin dependent microtubule-templated microtubule nucleation

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Since its discovery in Drosophila, the conserved hetero-octomeric Augmin complex has radically changed our understanding of microtubule (MT) generation during mitosis. Augmin amplifies MT number during mitosis and without it the density of MTs within the mitotic spindle is dramatically reduced, such that chromosome alignment and mitotic progression are perturbed. Human Augmin subunits associate with the MT nucleating complex γ-TuRC and removal of Augmin, through RNAi, mutation or immuno-depletion, removes the fraction of γ-TuRC normally present within the spindle, without affecting centrosomal levels. The current model is therefore that Augmin acts as a molecular linker between an existing MT and a γ-TuRC, allowing the nucleation of new MTs from the walls of pre-existing ones. However, due to its multi-subunit complexity and its poor sequence conservation, little is known of the molecular and structural basis of Augmin function in any organism. Even in the absence of structural data, here we have used cross-linking/mass spectrometry (CLMS) and endogenously purified Augmin to determine the orientation of subunits with one another and their molecular relationship to both MTs and the γ-TuRC. Not only does this provide an advance in our understanding of MT-templated MT nucleation, but it also demonstrates that CLMS can guide structural investigations into the biology of multi-protein complexes.

Notes:

P036 IQGAP3 is a novel player of cytokinesis

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The mammalian heart is a terminally differentiated organ. During development cardiomyocytes reduce their proliferation rate until they stop dividing after birth. The loss of proliferation is associated to cytokinesis failure resulting in the presence of binucleated cardiomyocytes. To date this phenomenon is poorly understood. Here, we correlated microarray gene expression data from embryonic day 11 to postnatal day 10 with the developmental dynamics of cardiomyocyte proliferation to i) identify novel cytokinesis proteins and ii) understand the biological mechanisms underlying cardiomyocyte cytokinesis failure. We hypothesize that all cytokinesis proteins exhibit a similar gene expression profile during heart development. Our analysis suggests IQGAP3 as a potential cytokinesis protein. IQGAP3 belongs to the family of IQ motif containing GTPase Activating Proteins. Little is known about this protein, except for its positive role in both neurite outgrowth and epithelial cell proliferation. Our data show that IQGAP3 expression correlates with cardiomyocyte proliferation during development. In fact IQGAP3 expression is high during early developmental stages and low after birth. In contrast, the gene expression profiles of the other IQGAP family members did not correlate with the rate of cardiomyocyte proliferation. Mitosis stages-specific immunofluorescence analyses revealed that IQGAP3 is first detectable in anaphase at the cleavage furrow. After furrow ingression and midzone constriction, it localizes around the midbody. This localization pattern is present not only in cardiomyocytes but also in different cell lines (HL-1, NIH3T3 and HeLa) suggesting a conserved role of IQGAP3 during mitosis. Collectively, our data suggest that IQGAP3 is a novel cytokinesis protein.

Notes:

P037 The epsilon subunit of the CCT molecular chaperone links actin folding to MRTF-A/SRF-dependent gene expression.

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The eukaryotic molecular chaperone CCT is a multi-subunit oligomer essential for the correct folding of newly synthesized actin molecules. It consists of eight distinct subunits, each encoded by an individual gene, all of which are essential in yeast. Actin filaments, which are highly dynamic with regard to their assembly/disassembly are controlled by a vast array of actin-binding proteins, many of which are modulated by receptor activation at the cell surface. The actin assembly state is registered by the myocardin-related transcription factor-A (MRTF-A)/Serum Response Factor (SRF) pathway, which in turn regulates the transcription of a large number of genes, including actin itself and numerous actin binding proteins. Here, using cultured mammalian cells as a model system, we show that the epsilon subunit of CCT, when monomeric, is a component of the MRTF-A/SRF pathway. This reveals a complex level of interplay between the dependence of actin for folding upon the CCT oligomer and mediation of actin transcription via the MRTF-A/SRF pathway and monomeric CCTepsilon. This work provides further evidence that the role of CCT extends beyond protein folding, which is mediated by the assembled CCT oligomer, to include activities of the free, monomeric CCT subunits.

Notes:

P038 Anillin's role in asymmetric ingression of epidermal cells

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Cytokinesis occurs at the end of mitosis to divide the cell into two daughter cells. An actomyosin contractile ring forms at the equatorial cortex and ingresses to pinch in the cytosol and membrane. Anillin is a highly conserved protein that binds to components of the ring, mitotic spindle and membrane, and is a key regulator of cytokinesis. The division plane occurs perpendicular to the axis of asymmetry in epithelial cells to ensure that each daughter cell inherits apicobasal polarity. However, these cells undergo dramatic shape changes that involve reorganization of the cytoskeleton during mitosis, and it is not clear how the daughter cells re-establish polarity. Interestingly, ingression of the contractile ring is shifted off-centre starting from the junction-free side of the cell, and ending near the apical, junction-rich side of the cell. Previous studies revealed that asymmetric ingression occurs in other organisms, particularly in the one-cell *C. elegans* embryo, where it is anillin-dependent (Maddox et al., 2007). We hypothesize that anillin is part of an intrinsic mechanism that mediates asymmetric ingression in mammalian epithelial cells, which contributes to polarity by marking junction-free zones. In dividing MDCK cells, anillin is broadly enriched along the junction-free cortex, and is uncoupled from myosin, which remains slightly apically enriched. The mechanisms that promote junction-free enrichment of anillin likely involve the mitotic spindle and DNA, which are apically positioned. We are continuing to characterize and explore the intrinsic machinery that regulates the localization of contractile proteins in epithelial cells.

Notes:

P039 Rab7b and the actomyosin cytoskeleton: novel roles in intracellular trafficking and cell migration

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Rab proteins are small GTPases that specifically regulate the transport between the different compartments of the endomembrane system in eukaryotic cells. One member of this protein family, Rab7b, was originally described as an isoform of Rab7a, which regulates endosomal maturation. However, we found that this small GTPase is a completely new Rab. Indeed, Rab7b has a different role in the intracellular traffic compared to Rab7a, regulating the retrograde transport from late endosomes to the TGN. By a yeast two-hybrid screening, we have recently identified a novel interactor of Rab7b, which revealed to us a new role for Rab proteins outside their canonical role in intracellular traffic. Here, we illustrate the functional significance of this interaction by showing that the direct binding to this effector, a cytoskeleton component, is important not only for the regulation of the intracellular transport, but also for the correct cell polarization required in processes such as cell adhesion and migration.

Notes:

P040 How does clathrin bind AP-2?

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Clathrin-mediated endocytosis (CME) is the major route of internalisation at the plasma membrane. A key step in CME is the engagement of the adaptor protein, which in turn binds the cargo and membrane that is to be internalised. The major adaptor protein at the plasma membrane is the AP-2 complex.
Despite intense study, it is unclear how clathrin binds AP-2. It was originally thought that there was one binding site on AP-2, the clathrin box motif on the hinge of β2-adaptin, and that this was recognised by a single groove on the N-terminal domain (NTD) of clathrin. However, subsequent work found evidence for four interaction sites on the NTD in addition to a binding site on the clathrin heavy chain 'ankle'. Finally, why clathrin only recognises AP-2 at the plasma membrane and not in the cytoplasm is also not understood.
In order to study clathrin-AP-2 interactions in isolation from other components of CME, we created a synthetic protein where the hinge and appendage region of β2-adaptin was fused to the alpha chain of CD8. This protein, but not a cytosolic version, is recognised by clathrin in cells, suggesting that membrane localisation may be important for clathrin-AP-2 recognition. After clathrin binding, the synthetic construct is efficiently internalised. This internalisation is independent of endogenous AP-2 and suggests that we have reconstituted a minimal form of CME in living cells. We will describe the functional testing of pairwise combinations of β2-adaptin and clathrin mutants to delineate the molecular details of the clathrin-AP-2 interaction.

Notes:

P041 Functional roles of key apical complex molecules in collective cell migration

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Cells not only migrate individually but can also migrate collectively under developmental, physiological and diseased conditions. During collective migration, cells often maintain cell-cell junctions and apical polarity, which are essential characteristics of epithelia. But, how the cell junction and apical polarity molecules contribute to collective migration is largely unknown. Here, we utilize *Drosophila* border cells in the ovary as a collective migration model system to study functional roles of key apical complexes, including Crumbs/Stardust/Patj complex and Par3/Par6/aPKC complex. During *Drosophila* oogenesis, a coherent cluster of about 8 border cells undergo invasive and directional migration between and over the nurse cells. Within the border cell cluster, members of Crumbs or Par complex were localized in the cell junction between border cells at high level, and they were also present at low level in the outward cortical region of border cells (facing the nurse cells), which have the potential to form actin-rich lamellipodial protrusions. We found that loss of function in any member of the Crumbs complex caused ectopic and irregularly-shaped protrusions to form in the outward cortical region, while aPKC and Par6 but not Par3 were enriched in these ectopic protrusions along with F-actin, p-myosin, Arp2 and ENA/VASP. Further genetic, histochemical, and live imaging analyses revealed the existence of two functionally distinct populations of apical complex molecules, one population acting at apical junction region to likely maintain the adherens junction and the other acting at the outward cortical region of border cells to promote actin-rich protrusion, partly through the Rac pathway.

Notes:

P042 Signals from the outside? : the role of GPCRs in cytokinesis

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The process of cytokinesis is not yet fully elucidated and remains a major unsolved question in biology. In our lab, we use both biological and chemical manipulation of cells in order to investigate cytokinesis at a cellular and molecular level.
In cultured cells it is unclear how/if the extracellular environment may contribute towards regulating cell division and whether extracellular signals are communicated to the cytokinetic machinery. Obvious candidates for signal transduction are the large family of G-protein Coupled Receptors (GPCRs). We have previously published data showing that depletion of a number of GPCRs result in cytokinetic defects in cell culture models. Subsequent work has been focused on characterising these defects.
Depletion of the somatostatin receptor 5 (SSTR5) in HeLa cells results in 11% binucleates. The binucleates primarily result from failure in the final stages of cytokinesis (abscission), suggesting SSTR5 may be important for the correct function of the abscission machinery. The depleted cells are characterised by a stark perturbation in their actin cytoskeleton resulting in increased stress fibres and blebs. These phenotypic changes can be reverted using inhibitory drugs targeting the Rho/ROCK signalling pathway, suggesting that SSTR5 depletion may result in activation of this pathway.
We are in the process of exploring further how SSTR5 is involved in regulating cytokinesis. A key question is whether the involvement is through canonical GPCR signalling pathway, i.e. signalling through the trimeric G-proteins.

Notes:

P043 TMEM115: a new rhomboid-like protein potentially involved in Golgi retrograde traffic and lipid metabolism

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TMEM115 is a recently identified non-proteolytic member of the Rhomboid-like superfamily of proteins. It is a Golgi polytopic membrane protein, conserved from plants to humans and expressed in most adult and embryonic tissues. To elucidate the biological role of TMEM115, we have recently generated flies and mice lacking this gene; both cause a significant reduction of normal lifespan. In the case of mice, we have observed a striking phenotype with KO animals unable to gain weight and never surviving beyond weaning. In parallel, we have employed high-throughput screens in yeast to identify interacting partners of TMEM115 and pathways in which it is involved. These approaches have identified links between TMEM115 and COG4, as well as suggested involvement with lipid metabolism. Consistent with these observations, our preliminary experiments in mammalian cells suggest an implication of TMEM115 in Golgi retrograde trafficking and its necessity for normal lipid droplet formation. In summary, our work demonstrates the biological importance of TMEM115 and reinforces the need to understand its molecular mechanism of action in the cell.

Notes:

P044 Probing cytokinesis: Identifying new proteins involved in cell division

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Cytokinesis is the evolutionarily conserved process by which a cell compartmentalises its cytoplasm and two daughter nuclei in to two individual cells. This process involves highly spatially and temporally co-ordinated actions of cell cycle regulation, cytoskeleton rearrangements and membrane reorganisation to ensure each of the two daughter cells created are endowed with a complete set of intact chromosomes and cytoplasmic organelles.
Although in recent years our knowledge of the cytokinetic process has increased, many of the mechanisms which underlie this crucial event remain a mystery. Work in our lab utilises RNAi techniques to dissect these mechanisms to try and form a more complete picture of cytokinesis and the signalling events which underlie it.
During an RNAi screen to identify novel proteins involved cytokinesis, several surprising hits were discovered; these included CDC42EP1. CDC42EP1 is a member of a family of CDC42 effector proteins, whose activity is believed to be negatively regulated by the small GTPase CDC42. We have shown the CDC42EP1 plays a role in the maintenance of the integrity of the final stage in cytokinesis. This role may be mediated by its interaction with septins.

Notes:

P045 Switching on cytoplasmic dynein: processive movement induced by BICD2N and dynactin

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Cytoplasmic dynein is a 1.4 MDa protein complex that transports cargos towards the minus ends of microtubules. It functions together with the multiprotein complex dynactin and other cofactors. The role for these components is only partially understood. Here we recombinantly express full human cytoplasmic dynein from a single baculovirus in insect cells. This allows us to purify large quantities of fluorescently labeled dynein complexes that are well behaved and complete over gel filtration. *In vitro* motility assays show that the complexes do not move processively along microtubules, consistent with a previous study of recombinant human dynein by Trokter et al. (2012). Addition of pig brain purified dynactin, a known processivity factor for dynein, also fails to stimulate processive movement. Previous work showed that the N terminus of the BICD2 adaptor molecule (BICD2N) stabilizes the dynein/dynactin complex. We find that the addition of both dynactin and BICD2N to recombinant dynein complexes induces long processive runs along the microtubules. We propose that adaptor proteins such as BICD2 can serve as an "ignition key" for the dynein/dynactin motor complex, thus coordinating cargo binding with processive movement.

Notes:

P046 Characterisation of Golgi-localised GORAB protein

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GORAB is a protein that localises to the *trans*-Golgi network (TGN) and is known to interact with Rab6. The loss of GORAB leads to geroderma osteodysplastica (GO), an autosomal recessive disorder which results in lax skin, precocious skin aging, osteoporosis, susceptibility to fractures and joint hyperelasticity. Although GO first had been described in 1950, the molecular cause of the disease was found only recently. However, both the mechanism of pathogenesis in GO patients and the functions of GORAB are poorly defined.
In this study, we investigated the cellular functions of GORAB. We found that GORAB is not evenly distributed in the TGN but rather localises to distinctive TGN domains. We established a network of interactions with Golgi-localised proteins, including several small GTPases, that are known to be involved in protein transport. Moreover, we observed morphological changes of the Golgi apparatus in primary cells derived from GO patients in comparison to cells obtained from healthy individuals. Together, this data suggests a role of GORAB in protein trafficking in the Golgi apparatus.

Notes:

P047 The actin filament bundling protein drebrin plays a role in prostate cancer cell motility.

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We show here that the actin-filament-bundling protein drebrin plays an important role in prostate cancer cell motility and invasion. Drebrin is particularly abundant in developing neurons where it co-ordinates microtubule/actin filament interactions that underlie neuritogenesis and neuronal migration. In developing neurons, the binding of drebrin to actin filaments is regulated by Cdk5 phosphorylation at S142 and phosphorylation mutants of S142 inhibit neuronal migration. We found that drebrin, and drebrin phosphorylated at S142, are present in the human prostate cancer cell lines PC-3 and DU-145, where they co-localise with actin filaments in filopodia. To test if drebrin has a role in PC-3 cell motility and invasiveness we conducted *in vitro* assays in the presence of the small, organic molecule BTP2, which inhibits drebrin binding to actin filaments. BTP2 inhibited the motility of PC-3 cells in 2D (random motility and wound closure) and 3D (Matrigel invasion) assays in a concentration-dependent manner. Live cell imaging showed that BTP2 inhibition was associated with changes in filopodia dynamics and response to chemotactic gradients. Our findings identify a potential role for drebrin in prostate cancer cell motility and we aim to establish if drebrin is a suitable therapeutic target or biomarker for the disease. To address these aims we are focussing our current experiments on the development of an *in vivo* metastatic model and are assessing the levels of drebrin expression in human prostate cancer samples.

Notes:

P048 Role of phosphatases in chromosome segregation

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Chromosomal instability (CIN) is a hallmark of cancers. How chromosomes attach to microtubules is not fully understood and we are studying proteins that are essential for the early event of chromosome-microtubule capture. Our studies confirm that the phosphatase PP2A plays an important role in congression. We will present data to show how the phosphatase controls chromosome-microtubule attachment.

Notes:

P049 PP2A-B56 opposes Mps1 phosphorylation of Knl1 and thereby promotes spindle assembly checkpoint silencing

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The spindle assembly checkpoint (SAC) monitors correct attachment of chromosomes to microtubules, an important safeguard mechanism ensuring faithful chromosome segregation in eukaryotic cells. A key unresolved question is how the SAC signal is turned off once all the chromosomes have successfully attached to the spindle. Mps1 phosphorylation of Knl1 results in recruitment of the SAC proteins Bub1, Bub3 and BubR1 to the kinetochore and production of the wait-anaphase signal. SAC silencing is therefore expected to involve a phosphatase opposing Mps1. Here we demonstrate *in vivo* and *in vitro* that BubR1-associated PP2A-B56 is a key phosphatase for the removal of the Mps1 mediated Knl1 phosphorylations necessary for Bub1/BubR1 recruitment in mammalian cells. SAC silencing is thus promoted by a negative feedback loop involving the Mps1 dependent recruitment of a phosphatase opposing Mps1. Our findings extend the previously reported role for BubR1-associated PP2A-B56 in opposing Aurora B and suggest that BubR1-bound PP2A-B56 integrates kinetochore surveillance and silencing of the spindle assembly checkpoint.

Notes:

P050 Distinct levels in Pom1 gradients limit Cdr2 activity and localization to time and position division

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Where and when cells divide are fundamental questions. In rod-shaped fission yeast cells, the DYRK-family kinase Pom1 is organized in concentration gradients from cell poles and controls cell division timing and positioning. Pom1 gradients restrict to mid-cell the SAD-like kinase Cdr2, which recruits Mid1/Anillin for medial division. Pom1 also delays mitotic commitment through Cdr2, which inhibits Wee1. Here, we describe quantitatively the distributions of cortical Pom1 and Cdr2. These reveal low profile overlap contrasting with previous whole-cell measurements and Cdr2 levels increase with cell elongation, raising the possibility that Pom1 regulates mitotic commitment by controlling Cdr2 medial levels. However, we show that distinct thresholds of Pom1 activity define the timing and positioning of division. Three conditions—a separation-of-function Pom1 allele, partial downregulation of Pom1 activity, and haploinsufficiency in diploid cells—yield cells that divide early, similar to pom1 deletion, but medially, like wild-type cells. In these cells, Cdr2 is localized correctly at mid-cell. Further, Cdr2 overexpression promotes precocious mitosis only in absence of Pom1.Thus, Pom1 inhibits Cdr2 for mitotic commitment independently of regulating its localization or cortical levels. Indeed,we show Pom1 restricts Cdr2 activity through phosphorylation of a C-terminal self-inhibitory tail. In summary, our results demonstrate that distinct levels in Pom1 gradients delineate a medial Cdr2 domain, for cell division placement, and control its activity, for mitotic commitment.

Notes:

P051 Cytoskeleton remodelling and cell motility of normal and glaucomatous trabecular meshwork cells are substrate compliance dependent

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Marked extracellular matrix (ECM) remodelling occurs in human optic nerve head in primary open angle glaucoma (POAG) patients. This alternation of the physical properties of the tissue during glaucoma progression dramatically impacts upon cell behaviour. This study investigates the effects of substrate rigidity, using analogues for the ECM, as a possible pathophysiologic factor in glaucoma progression. Human trabecular meshwork (HTM) cells sense their mechanical environment, leading to changes in morphology, proliferation, cytoskeleton remodelling, motility and cell stiffness. Rhodemin-phalloidin labeling and confocal microscopy demonstrate cells seeded on soft surface are devoid of stress fibers and F-actin patches are formed in peripheral extension at the cell edge. This cytoskeleton remodelling, contributes significantly to the change of the cell stiffness. Moreover, the localisation of Rac and Cdc42, members of the Rho GTPases family, forms the link between the substrate compliance modulation and F-actin reorganization. In additions, we have demonstrated that cells have a significant higher motility rate on soft surfaces, but this phenomenon is cell disease state dependent. These results provide insight into modulation of substrate stiffness may be a mechanism to direct cell migration and disease progression.

Notes:

P052 From nucleosome to midbody: extrachromosomal activity of histone H2B in cytokinesis

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Histones are the constitutive components of chromosomal nucleosomes but a few extrachromosomal activities of specific histones have been described. Recently, we have found that histone H2B localizes at the midbody where it is phosphorylated at S14 by HIPK2, a cell fate decision kinase in response to DNA damage and in development, whose activity is required for abscission and prevention of tetraploidization. Histones bind nucleic acids but we observed that H2B and HIPK2 localize at the midbody independently from the presence of DNA. Thus, we speculated that RNA might be responsible for the H2B cytokinetic localization. This hypothesis was also stimulated by the observation that DDR focus formation requires site-specific DICER and DROSHA RNA products. Here, we show that in mitosis-enriched HeLa cells, lack of RNA induced by RNase A treatment do not impair cytokinetic-specific localization of H2B and cell division. In addition, no defects in H2B localization are observed in DICER-defective HCT116 cells, indicating that H2B localizes at the midbody independently of RNA. To further investigate the role of H2B at the midbody, we studied the effects induced by depletion of H2B variants with specific short interfering RNA (siRNA). In HeLa cells, H2B depletion results in prevention of cell cleavage, accumulation of long intercellular bridges and abscission defects. Additional experiments with single variant-specific siRNAs resulted in the appearance of comparable defects only with some siRNAs (*i.e.,* H2B.B, H2B.M, H2B.K, or H2B.E). These results support a role for H2B in cytokinesis and indicate that some H2B variants are preferentially involved in this function.

Notes:

P053 Poster withdrawn

Notes:

P054 Essential role of ESCRT-III-associated kinase in the regulation of abscission timing

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In late stages of cytokinesis, the Endosomal Sorting Complex Required for Transport (ESCRT) machinery mediates abscission of the intercellular bridge connecting daughter cells. ESCRT-III proteins are known to form filaments that drive cortical constriction on either side of the midbody and mediate membrane fission of opposing membranes. In response to unsegregated chromosomes, the NoCut checkpoint relies on Aurora B to trigger a genome protection mechanism that induces abscission delays via the ESCRT-III subunit CHMP4C. Nevertheless, the molecular mechanisms underlying abscission regulation by NoCut remain poorly understood. Here we characterize the key role of ESCRT-III-associated kinase (EAK) in the modulation of abscission timing, which requires both its kinase activity and interactions with ESCRT-III. We provide evidence of a functional connection between EAK and CHMP4C, and identify this kinase as an essential component of the NoCut pathway. Finally, we show that EAK phosphorylates ESCRT-III proteins. We identify EAK phosphorylation sites on ESCRT-III and expose their importance for NoCut activation and ESCRT-III polymer assembly. Thus, we have revealed a new component of the timer controlling abscission in different physiological situations via phosphorylation of ESCRT-III proteins.

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Notes:

P055 The roles of the oncoprotein GOLPH3 in contractile ring assembly and membrane trafficking during cytokinesis

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The highly conserved Golgi phosphoprotein 3 (GOLPH3) protein has been described as a Phosphatidylinositol 4-phosphate [PI(4)P] effector at the Golgi. GOLPH3 has been recognized as a potent oncogene, involved in the development of several human tumors. However the precise roles played by GOLPH3 in tumorigenesis are not yet understood. We have characterized the *Drosophila* homologue of human GOLPH3 during cell division. GOLPH3 accumulates at the cleavage furrow and is required for successful cytokinesis in *Drosophila* spermatocytes and larval neuroblasts. In premeiotic spermatocytes GOLPH3 protein is required for maintaining the organization of Golgi stacks. In dividing spermatocytes wild type function of GOLPH3 enables maintenance of centralspindlin and Rho1 at cell equator and stabilization of Myosin II and Septin rings. The molecular mechanism underlying GOLPH3 function in cytokinesis is strictly dependent on the ability of this protein to interact with PI(4) P. Mutations that abolish PI(4)P binding, impair recruitment of GOLPH3 to both the Golgi and the cleavage furrow. Moreover telophase cells from mutants with defective GOLPH3-PI(4) P interaction, fail to accumulate PI(4)P-and Rab11-associated secretory organelles at the cleavage site. Finally GOLPH3 protein interacts with components of both cytokinesis and membrane trafficking machineries in *Drosophila* cells. Based on these results we propose that GOLPH3 acts as a key molecule to coordinate phosphoinositide signaling with actomyosin dynamics and vesicle trafficking during cytokinesis.

Notes:

P056 Molecular insights into the meiotic microtubule organising centre that directs vigorous nuclear movement during fission yeast sexual differentiation

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During fission yeast meiotic prophase, a specialised microtubule organising centre transiently appears next to the yeast centrosome, the spindle pole body (SPB) (Funaya et al., *Curr Biol*., **22**, 562-574 (2012)). We named the structure the radial microtubule organising centre (rMTOC) as it organises radial microtubules (rMTs) which, with help of dynein-dynactin complex, drive vigorous oscillatory nuclear movement. We identified a protein called Hrs1 (also known as Mcp6) as an essential structural component of the rMTOC and a large-scale Hrs1 immuno-affinity purification found Mto1, a Cep215/CDK5RAP2 homologue, to be a major constituent of the Hrs1 immuno-complex. *In vitro* translated Hrs1 and Mto1 also directly interact with each other. Furthermore, localisation of Hrs1 and Mto1 to the SPB is interdependent. Collectively, Hrs1 and Mto1 are likely to form a stable structural scaffold of the rMTOC. In the absence of Hrs1, the rMTOC does not develop, and the nuclear movement is substantially abrogated. Nonetheless, in average, about 10 MTs are still running in the vicinity of the SPB, and dynein-dynactin complex is found on the MTs. This observation is surprising because as few as two opposing MTs at the SPB are expected to produce an oscillatory nuclear movement (Vogel et al., *PLoS Biol*., **7**, (2009)). We hypothesise that these remaining MTs have lost a firm anchoring at the SPB in the absence of rMTOC and are only loosely associated with the SPB. We examine this hypothesis and explore the role of Hrs1-Mto1 complex in the MT anchoring function.

Notes:

P057 Regulation of exocytosis by the exocyst complex

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Eukaryotic cells are crowded with membrane-bound vesicles that transport cargo between subcellular organelles, and to the plasma membrane for secretion. SNARE proteins are core components of the membrane fusion machinery, and their regulation is crucial for the precise specificity and timing of vesicle fusion. SNAREs at the plasma membrane are controlled in part by the exocyst complex, which is essential for cellular growth, secretion and endocytosis. It is a hetero-octameric protein complex localized to sites of secretion on the plasma membrane, and is thought to function in quality control through specific tethering of secretory vesicles. Our studies indicate functional cooperation between the yeast exocyst complex and the SNARE regulatory protein Sec1 to regulate the specificity and timing of SNAREs and membrane fusion. We identified conserved residues on the exocyst subunit Sec6 that are required for proper complex localization. Studies of the exocyst architecture and interactions with binding partners had previously been hindered by the challenges of obtaining purified complexes; we recently developed a successful purification strategy for obtaining stable exocyst complexes. We are investigating their structure and stoichiometry using biochemical, genetic and proteolytic disruptions of the complex, mass spectrometry and binding studies. By altering solution conditions, we can isolate novel subcomplexes that are likely to be important for assembly and function. Moreover, negative stain EM reveals our first view of the overall structure of the intact yeast exocyst complex.

Notes:

P058 Dynamic flux of microvesicles modulate parasite-host cell interaction of *Trypanosoma cruzi* in eukariotic cells

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Extracellular vesicles (Exosomes,Microvesicles and apoptotic bodies) from eukaryotic cells are involved in multiples functions altering host cells. We have seen the involvement of microvesicles (MVs) (0.2 -1 μm size) during parasite-host cell contact, using *Trypanosoma cruzi* and THP1 cells. During the interaction an increase of intracellular calcium activate scramblase modifying phospolipids polarity of the membrane with budding of microvesicles with phosphotidilserin exposure. We are interested to know how is the contribution of different stages of *T cruzi* (metacyclic, blood trypomastigotes and epimastigotes) at the MVs formation. We stained differentially THP-1 and parasites membranes searching for stained MVs on the supernatant. We observed MVs from THP-1 (green) and parasite (red) in a $Ca^{2}+$ dependent process. All stages can release membranes contribuiting for MVs formation .Moreover using NBD fluorocrome (FRET methodology) we detected a decrease in fluorescence energy transfer, showing that MVs released from stained THP-1 membranes can fuse with MVs released from parasite. The fusion process was inhibited at 4ºC.Interestingly, MVs from trypomastigotes present a higher fusogenic capacity than other stages.The greatest fusogenic potential observed for MVs from trypomastigotes coincide with a higher parasite proteins of MVs from trypomastigotes as seen by proteomics . The microvesicles are processed by eukariotic cell in a IP3 , calcium and actin process.The understanding of intracellular traficking and effect of microvesicles at the host cell are under investigation and could be important to define new chemoterapeutic targets .

Notes:

P059 Kif1C is required for podosome formation in vascular smooth muscle cells

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During atherosclerosis and in response to vascular injury, vascular smooth muscle cells (VSMCs) de-differentiate and form podosomes, adhesion structures that mediate extracellular matrix remodelling. Matrix remodelling enables VSMCs to migrate, invade and contribute to the formation of vascular lesions. We show that microtubules and the microtubule motor Kif1C are essential for *de novo* podosome formation in VSMCs. Kif1C is a plus end directed organelle transporter involved in integrin recycling. However, how Kif1C selects and loads cargo and which cargo is essential for podosome formation remains to be understood. Using an RNAi rescue system and truncated constructs of Kif1C we show that the PTPD1 binding site and the FHA domain are essential for Kif1C's podosome inducing function. However the FHA domain plays a merely structural role as the FHA domain from CHK2 can replace it. We will present data dissecting the contribution of known Kif1C cargos to podosome formation in VSMCs.

Notes:

P060 Regulation of spindle orientation in mitosis by p37/p47 adaptor proteins of CDC48/p97

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Spindle orientation and positioning are two important aspects of mitosis, which determines the cell division plane. This fundamental aspect plays a significant role in development and has shown links to neurological diseases and cancer. Mitotic spindle orientation and positioning in metaphase are regulated by cortical proteins such as dynein/dynactin (the 'pullers' of the spindle), which are recruited by NuMa/LGN/Gαi to the cortex.
The conserved and multifunctional CDC48/p97 protein is a type II AAA ATPase that has a central role in the ubiquitin system and has been implicated in several cellular processes. CDC48/p97 main function is carried out by extracting proteins from complexes and binding to different adaptors defines its specificity. The adaptor proteins p37/p47 are required for *Endoplasmic Reticulum*/Golgi apparatus maintenance during interphase. Interestingly, p37/47 co-depletion results in mitotic spindle orientation defects, delay in centrosome separation, and Aurora A accumulation at the centrosome.
The aim of my project is to understand how p37/47 adaptor proteins are involved in the regulation of spindle orientation and positioning in HeLa cells. Current experiments show that p37 depleted cells possess altered spindle dynamics in metaphase. Furthermore, the levels of cortical proteins regulating spindle positioning and orientation are affected.
I will present our progress in the characterization of the role of these conserved cofactors of CDC48/p97 in cell division and spindle orientation.

Notes:

P061 The apical complex provides a regulated gateway for secretion of invasion factors in apicomplexan parasites

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The apical complex is the definitive cell structure of phylum Apicomplexa, a huge group of single-celled obligate intracellular parasites that includes the malaria parasite Plasmodium, and Toxoplasma. The apical complex is the focal point of the parasite's cytoskeleton and coordinates cell motility, invasion of host animal cells, and formation of daughter cells within the mother cell. It is an ultrastructurally elaborate structure consisting of mobile rings and a battery of diverse secretory organelles that are delivered in a stage-specific manner to the host via this cellular nozzle. Despite the importance of this structure, its molecular composition is relatively poorly known and few studies have experimentally tested its functions. We are characterising novel components of the *Toxoplasma gondii* apical complex and using them to explore the mechanics and function of this structure. These studies show that during cell division, nascent apical complexes first assembled on the newly duplicated centrosomes, and then detach to initiate new cell cytoskeletons that form the basis of internally generated daughter cells. During invasion events, super-resolution microscopy reveals remarkably dynamic molecular mechanics, including a proteinaceous tube that lines the the apical complex turning inside out. Inducible knockdowns of apical complex components reveal that not only is this the site of delivery of invasion factors to the host cell, but it also regulates the timing of their release. These studies highlight the sophistication of this structure that has doubtlessly been instrumental in the success of this parasite group.

Notes:

P062 Poster withdrawn

Notes:

P063 The requirement of barbed end capping of actin filaments for faithful cytokinesis.

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Cytokinesis is the final stage of cell division and is tightly regulated to ensure the correct inheritance of DNA and organelles to each daughter cell. This requires the co-ordinate action of the cytoskeleton and membrane trafficking systems. Although understanding cytokinesis has greatly increased over the years, many aspects of its regulation remain unknown. A candidate we have validated from using image based RNAi screening is CAPZβ, a subunit of the heteromeric actin barbed end capping complex CAPZ. We found that CAPZβ depletion leads to increases in the total F-actin content of cells, resulting in dramatic alterations in actin dynamics. Despite these changes cells are still able to cycle normally through mitosis and are able to initiate a cytokinetic furrow. Furrow kinetics are altered with cells taking a longer time to furrow. Although activated myosin and contractile ring proteins are recruited normally, cytokinesis failure seems to occur by cells not being able to stabilize their furrow. Inhibiting actin polymerization with an Arp2/3 inhibitor does not rescue cytokinesis, indicating that polymerization of branched actin structures are not contributing to the cytokinesis defect. In contrast, treatment with low doses of the pan-formin inhibitor SMIFH2 can rescue cytokinesis, suggesting the mechanism for the cytokinesis defect is linked to the overgrowth of formin based linear actin filaments. Thus our results indicate that cytokinesis requires a fine balance between actin filament capping and formin based actin polymerization.

Notes:

P064 Network-guided connectivity mapping predicts new microtubule stabilizing agents and drug sensitivity in cancer cell lines

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Paclitaxel, the most widely used drugs against aggressive cancers. By combining a network-guided transcriptional signature matching strategy and high-resolution cell biology assays, we identified and validated refined gene signatures of response to paclitaxel, which are predictive of treatment outcome and can be used to identify novel microtubule stabilising drugs. We generated a signature summarising the consensual transcriptional response to paclitaxel from the Connectivity Map (a large database of transcriptional responses to drug treatment). Then we refined this signature by systematic reduction of its overlap with the transcriptional responses induced by proteasome inhibitors and microtubule destabilisers, identified as neighbours of paclitaxel in a drug similarity network based on gene expression data. By further querying the Connectivity Map for drugs eliciting a transcriptional response similar to the resulting refined signatures, we predicted that glipizide and splitomicin stabilise microtubules in human cells. In agreement, we find that glipizide and splitomicin treatment reduces interphase microtubule growth rates and transiently increases the percentage of mitotic cells – all consistent with glipizide and splitomicin being microtubule stabilisers. Finally, by mining a large drug screening dataset, we show that human cancer cell lines whose basal transcriptional profile is anti-correlated to our refined signatures of microtubule stabilisation are significantly more sensitive to paclitaxel and docetaxel treatment. Thus we provide a proof of concept for a reproducible computational strategy to identify refined signatures that characterise drug's mode of action and are predictive of drug response.

Notes:

P065 The end-binding protein EB2 maintains a dynamic microtubule population essential for reorganisation of the microtubule array during polarisation

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The ability of cells to polarise is critical for their function, whether they are part of an apico-basal epithelial sheet, or are migratory, with a defined front and rear. Rearrangement of microtubules is essential in establishing and maintaining specific polarised morphologies. For example, differentiated columnar epithelial cells form non-centrosomal arrays of microtubules, with minus ends anchored at apical sites and plus ends extending into basal regions, whilst migrating cells have centrosomally anchored microtubules extending into the leading edge, where the balance between dynamic and stable microtubules is critical for efficient motility. Such microtubule rearrangements are driven by their inherent dynamics, and influenced by +TIPs such as the EB family. EB1/3 promote microtubule growth, and will induce bundle formation when overexpressed. By contrast, EB2 has a reduced affinity for the plus end and its overexpression does not induce bundle formation.
We have recently established EB2 as an essential regulator of microtubule reorganisation during apico-basal epithelial polarisation; its expression is required initially, and is later reduced. Examination of microtubule dynamics reveals that EB2 loss causes an increase in microtubule pausing and a reduction in shrinking events. Additional data will be presented regarding how the influence of EB2 on microtubule dynamics affects cell polarity. Overall, we suggest that the expression of EB2 is required to maintain a dynamic microtubule population, whilst its down-regulation results in microtubule stabilisation and bundle formation.

Notes:

P066 Comparative proteomics define membrane architecture and host-parasite interface

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Surface membrane organization and composition is key to cellular function, and membrane proteins serve many essential roles in endocytosis, secretion and cell recognition. The surface of parasitic organisms, however, is a double-edged sword; this is the primary interface between parasites and their hosts, and those crucial cellular processes must be carried out while avoiding elimination by the host immune defenses. For extracellular African trypanosome parasites, little is known about most of the proteins that reside at this interface. This severely limits functional and evolutionary studies, and hampers the development of treatments. Here we describe a combined biochemical, proteomic and bioinformatic approach to describe a high-confidence surface proteome for the human pathogen *Trypanosoma brucei*.
The 'surfeome' contains proteins previously localized to the surface and many novel components with membrane characteristics. Extensive validation by cellular localization suggests that the majority of surfeome constituents are bona fide surface-associated proteins, including several ones of unknown function. Interestingly, receptor-like molecules are almost exclusively species-specific, whereas transporter-like ones are conserved to model organisms. We also present a first examination of sorting signals and show that proteins with different biochemical characteristics access combinations of specialized membrane regions, suggesting the existence of distinct surface domains defined by boundaries which restrict diffusion.
The approach developed here is generally applicable to the study of surface membranes, and reveals a paradigm that has important implications for the function of the trypanosome cell surface.

Notes:

P067 Molecular dissection of the Dam1 complex

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All eukaryotic cells segregate their chromosomes equally between two daughter cells at each division. This process needs to be robust, as errors in this process leads to catastrophic effect on the cell and on the organism as a whole. Different microtubule associated proteins function together to control spindle assembly and chromosome segregation. The spatial and temporal control of their activity is key to perform accurate chromosome segregation leading to successful cell division. Dam1 protein complex in budding yeast is one such example. It forms a ring complex around microtubule enabling robust microtubule kinetochore attachment.
Our aim is to understand how the Dam1 subunits are organised in the ring complex and how it is interacting with the microtubules. No high-resolution information is available to date. Using *in vitro* crosslinking combined with mass spectrometry, we have identified intra and intermolecular subunit interactions within the Dam1 complex. This enables us to generate a high-resolution map of the molecular arrangement of the Dam1 complex. In addition, we are currently performing analysis of the microtuble-Dam1 complex interactions. Together these observations will facilitate understanding the role of Dam1 complex both at the molecular level and further in the cellular context.

Notes:

P068 N-WASP/WIP mediated "actin-hotspots" drive 3D cell migration through direct force coupling to the nucleus

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The actin nucleation promotion factor N-WASP is up-regulated in breast cancer and is coupling pseudopod extension and matrix degradation to facilitate invasive cancer cell migration.
Cells migrating through 3D matrices form hybrid adhesion structures termed “actin-hotspots”, which contain N-WASP and display hallmarks of both focal adhesions and invadopodia.
We have identified a novel interaction between the N-WASP Interacting Protein, WIP, and the guanine nucleotide exchange factor ARHGEF. ARHGEF7 localises to actin-hotspots in 3D matrices. Loss of ARHGEF7 abolished cancer cell invasion, but increased matrix degradation and pseudopod extension in collagen matrices.
These seemingly contradictory results can be explained by our finding that ARHGEF7 is part of a N-WASP/WIP↔ARHGEF7↔PAK2 signalling cascade that is required for adhesion site turnover and force coupling. Loss of ARHGEF7 leads to less tension being applied by actin on adhesion sites as well as the nuclear envelope resulting in loss of motility.
The nucleus can act as limiting factor in 3D cell migration. Migrating cells need to actively squeeze the nucleus through matrix pores. Knockdown of nuclear envelope actin binding proteins called Nesprin-1 and -2 severely affect 3D cell migration. We have found a reciprocal regulation of Nesprin-2 and ARHGEF7 function.
We here show that direct force coupling from actin-hotspots to the nuclear membrane is required for 3D cell migration and we propose that this could be the mechanism to establish polarity during 3D cell migration.

Notes:

P069 Defective axonal retrograde transport of signalling endosomes and MAP kinase signaling in a mouse model of autosomal dominant spinal muscular atrophy

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Fast axonal retrograde transport is mediated by cytoplasmic dynein, a multi-subunit motor protein composed of two homodimerised heavy chains (DYNC1H1) and multiple intermediate, light intermediate and light chains. Autosomal dominant mutations in *DYNC1H1* cause spinal muscular atrophy with lower extremity predominance (SMA-LED), Charcot-Marie-Tooth disease (CMT), and intellectual disability. A phenylalanine to tyrosine substitution mutation (F580Y) in the tail domain of DYNC1H1 leads to autosomal dominant motor function deficit and loss of spinal cord motor and proprioceptive neurons in the Legs at odd angles (*Loa*) mouse model for SMA-LED. Our live-cell imaging and biochemical analysis of cultured primary motor neurons and embryonic fibroblasts, isolated from homozygous and heterozygous *Loa* embryos, have revealed that the velocity of microtubule minus-end movement of brain derived neurotrophic factor (BDNF) and epidermal-growth-factor (EGF) induced signalling endosomes is significantly reduced in *Loa* motor neurons and embryonic fibroblasts. This in turn leads to altered activation of the extracellular signal-regulated kinases (ERK) 1/2 and c-Fos expression in both mutant cell types. The motor neurons, however, exhibit a strikingly abnormal ERK1/2 and c-Fos response to serum-starvation induced stress, providing a possible explanation for how mutations in the ubiquitously expressed DYNC1H1 lead to neuron-specific disease.

Notes:

P070 Poster withdrawn

Notes:

P071 The RhoGAP Myosin 9b and its role in the migration of myeloid cells

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Mammals express diverse myosins that can be grouped into 11 different classes. Among them class IX encompasses myosin 9a and 9b (Myo9b). The tail-domain of class IX myosins comprises a GAP-domain that negatively regulates RhoA/-B/-C in a spatially restricted manner. Myo9b knockout ($Myo9b^{-/-}$) mice exhibit altered immune responses: Macrophages and dendritic cells have decreased motility and reduced chemotactic efficiency. They fail to polarize because of increased Rho signaling leading to increased cell contractility. The aim of this project is to elucidate the regulation of Myo9b to understand how RhoA is regulated in a spatially restricted manner and acts cell autonomously in $Myo9b^{-/-}$ immune cells. Primary murine neutrophils were isolated from bone-marrow and compared to human myeloid HL-60 leukemia cells differentiated into neutrophils. Cells were analyzed for morphological phenotype by confocal microscopy; motility was investigated by performing chemotaxis assays, measuring cell velocity and chemotactic efficiency. Myo9b-deficient neutrophils show a reduced velocity and chemotactic efficiency in a 2D-chemotaxis assay compared to wildtype cells. Human HL-60 neutrophils migrate with a much slower velocity than primary murine cells but show a similar chemotactic capacity.
CONCLUSION Primary Myo9b-deficient neutrophils display the same phenotype as previously described for DCs and macrophages. HL60-neutrophils represent a good cell line model to further study the regulation of Myo9b in cellular migration. Experiments designed to explore the regulation of Myo9b are currently ongoing.

Notes:

P072 Targeting of intracellular farnesyl protein transferase modulates actin dynamics and epithelial cells morphology

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Farnesyl transferase inhibitors (FTIs) are being actively investigated to target signal-transduction pathways responsible for the proliferation and survival of cancer cells. These molecules are targeting intracellular farnesyl protein transferase, an enzyme that catalyses the transfer of a farnesyl moiety to the C-terminal cysteine of the substrate protein with the CAAX terminal motif. Rho GTPases are small signalling proteins that regulate many aspects of intracellylar actin dynamics, cell polarisation and cell migration contain the CAAX terminal motif. Rho GTPases require farnesylation for their cell membrane attachment and are potential targets for FTIs. Our study has demonstrated that tipifarnib, an orally active nonpeptidomimetic farnesyl transferase inhibitor, administered in therapeutic doses leads to changes in Caco2 epithelial cell morphology, actin polimerisation and microvillus formation. Our observations provide evidence for the complexity of FTIs function and their potential as anticancer drugs and warrant further investigation.

Notes:

P073 Drink or drive: competition between macropinocytosis and cell migration.

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The cytoskeleton is utilised for a variety of cellular processes, including migration, adhesion and endocytosis. The required molecular components are often shared between different processes, but it is not well understood how cells balance their use. We find that macropinocytosis and cell migration are negatively correlated. Frequent drinkers migrate only poorly and vice versa. Both processes are balanced by the lipid PIP3. Enhanced PIP3 signaling causes a shift towards macropinocytosis and inhibits motility by redirecting the major actin filament nucleator in pseudopods, the SCAR/WAVE complex. High-resolution microscopy shows that patches with high levels of PIP3 recruit SCAR/WAVE on their periphery, resulting in circular ruffles and engulfment. Results shed a new light on the role of PIP3, which is traditionally thought to stimulate cell motility.

Notes:

P074 Integrin trafficking reprograms the actin cytoskeleton for migration in 3D-matrix

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Endocytic trafficking of integrins is critical in the regulation of cellular processes including invasion and metastasis of cancer cells, and Rab-coupling protein (RCP)-dependent recycling of alpha-5 beta-1 integrin promotes invasion into fibronectin (FN)-rich 3D-extracellular matrix (ECM).
The localised trafficking of alpha-5 beta-1 at the tips of protrusions inactivates the RhoGTPase Rac, and activates RhoA at the front of invading cells via the RacGAP1-IQGAP1 complex. This local activation of RhoA drives extension of long pseudopodial processes tipped with actin spikes to promote invasion. Bursts of actin polymerisation are accompanied by an accumulation of Rab11 containing vesicles, suggesting that trafficking integrins carry the signal to stabilise protrusions and promote actin polymerisation. This is a novel and surprising finding because in the current dogma, Rac activity is thought to drive Arp2/3-mediated actin polymerisation at the leading edge.
Inhibition of Arp2/3 with CK-666 had little influence on the speed or persistence of migration in 3D-matrices. Furthermore, Arp2/3 was not required for alpha-5 beta-1-induced bursts of actin spikes. However, inhibition of formin-dependent actin polymerisation abrogated 3D migration, actin-spike formation and invasion into FN-rich matrix. Preliminary evidence indicates that FHOD3 (a Diaphanous-related formin) is specifically and non-redundantly required for integrin-driven invasion. This suggests that alpha-5 beta-1 and RhoA reprogram the actin cytoskeleton via an alternate formin-dependent pathway to promote invasion of cancer cells in 3D-matrix.

Notes:

P075 A mitotic exit ubiquitome from human cells

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Mitotic cell division is a highly dynamic process involving profound morphological and biochemical changes to the cell. Many of these changes are mediated through targeted ubiquitination of cellular components. One critical player is the ubiquitin ligase known as Anaphase Promoting Complex/Cyclosome (APC/C), which drives mitotic exit via the ubiquitin-mediated destruction of mitotic cyclins. During mitotic exit, the APC/C is thought to target a large number of protein substrates in a temporally and spatially controlled manner. However, an absence of rigorous cell-based assays to investigate ubiquitination *in vivo* has limited knowledge of the identity, roles and regulation of key ubiquitin-mediated steps. Here we report an *in vivo* ubiquitin tagging system that allows efficient purification of ubiquitin conjugates from synchronised cell populations. Coupling this system with mass spectrometry, we have identified a series of mitotic regulators and cellular components specifically targeted for polyubiquitination during mitotic exit. We find that many are new substrates of the APC/C. We functionally validate KIFC1 and RacGAP1/Cyk4 as two such targets involved respectively in timely mitotic spindle disassembly and cell spreading after mitosis.
Many of the targets identified by our proteomic study generate functional clusters that indicate other processes regulated by polyubiquitination during the re-establishment of interphase in daughter cells.

Notes:

P076 Spatial organisation of early secretory pathway in mammalian cells is required for collagen secretion.

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Regulated trafficking of newly synthesised proteins is vital for cellular function, with one third of human proteins estimated to enter the trafficking pathway. Proteins leave the endoplasmic reticulum (ER) in COPII-coated vesicles. These COPII vesicles form at specific subdomains of the ER, termed transitional ER (tER). The integrity of these cup-like areas requires the structural proteins TFG and Sec16. Why COPII vesicle formation is spatially organised by being restricted to these defined sites is not known. RNAi depletion of TFG reduces the amount of COPII coat proteins present on the ER and in the organization of tER. TFG depletion leads to only mild alterations in Golgi morphology and no significant defect in the trafficking of small cargoes. We have used stable isotope labelling of amino acids in cell culture combined with mass spectrometry to define the role TFG, and more widely of tER and Golgi organization, in secretion. Our data show that the higher-level organisation of COPII vesicle formation is required for efficient transport of collagens.

Notes:

P077 The microfilament severing and capping protein gelsolin binds directly to the molecular chaperone CCT in a calcium-induced active state

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Cancer is based on DNA damage, uncontrolled mitosis, repressed apoptosis and the development of metastasis. The latter process relies on the cells ability to move, which is in turn controlled by actin filaments and regulatory proteins that influence actin dynamics. Additionally, actin requires the molecular chaperone CCT to be correctly folded. We aim to investigate an extended role for CCT in actin dynamics by studying the previously described interaction between CCT and the actin filament severing and capping protein gelsolin *in vitro*. Gelsolin exists in either a closed (inactive) or a calcium-induced open (active) conformation. We purified recombinant HIS-tagged gelsolin and initially confirmed that it could adopt an open or closed conformation upon adding or chelating calcium. Furthermore, the severing of *in vitro* polymerized actin filaments by gelsolin occurred only in the presence of calcium. Using an immunoprecipitation assay, we demonstrate that only the calcium-induced open state of gelsolin binds to CCT. We therefore postulate that the binding of gelsolin to CCT at high calcium concentrations may provide the cell with a tool to regulate filament severing/capping during calcium signaling.

Notes:

P078 Identification of vaccinia virus F12 and E2 as kinesin light chain interacting proteins preferentially binding the KLC2 C-terminal tail

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Vaccinia virus utilises microtubule mediated trafficking at three distinct stages of its replication cycle; entry, morphogenesis and egress. Egress of the fully formed intracellular enveloped virion (IEV) to the cell surface is currently the best characterised of these processes, however, both the cellular and viral proteins involved and the mechanism for selection and modulation of the correct trafficking complexes remain poorly characterised. The A36 protein directly links IEVs to kinesin-1 through an interaction with the tetratricopeptide repeat (TPR) region of kinesin light chain (KLC). Deletion of A36, however, does not completely abrogate IEV egress, suggesting other proteins can mediate virus/kinesin-1 interaction. Deletion of the viral F12 or E2 proteins results in loss of virus egress. We present the first evidence that the F12/E2 complex interacts with KLC, displaying a strong preference for associating with the KLC2 isoform. E2 is necessary and on its own is sufficient for binding to KLC. The F12/E2 interaction with KLC was mapped outside the KLC TPR region, to the variable C-terminal tail specific to KLC2. Our observations support a role for F12/E2 in kinesin-1 mediated IEV egress but their exact contributions remain elusive. These two proteins display different expression kinetics during infection (F12 is early and E2 is late). We postulate that this may modulate the correct timing and targeting of IEV egress by binding a specific subset of kinesin-1 complexes.

Notes:

P079 CDK1 phosphorylation of Tiam1 and PAK activation regulate centrosome separation in mitosis

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Centrosome separation is critical for bipolar spindle formation and the subsequent accurate segregation of chromosomes during mitosis. Kinesin-5 (Eg5) is a microtubule motor essential for pushing centrosomes apart during bipolar spindle assembly. We previously found that Tiam1 and its substrate, the Rac GTPase, both localise to centrosomes and counteract Eg5 in early mitosis. As a consequence, Tiam1 depleted cells can more easily escape the monopolar arrest normally induced by Eg5 inhibition. More recently we have identified S1466 as a novel CDK consensus site on Tiam1 which is highly phosphorylated in mitosis. We have confirmed using a phospho-specific antibody that S1466 is phosphorylated in early mitosis in a CDK1-dependant manner, and that the phosphorylated pool of Tiam1 is localised on the centrosomes. Interestingly, we have found that S1466 of Tiam1 is required for antagonism of centrosome separation in mitosis; as introduing a S1466A mutation abolishes rescue of the mitotic phenotypes seen on Tiam1 depletion. In addition, we have found that phosphorylation of S1466 of Tiam1 is required for activation of group I p21-activated kinases (PAKs) on the centrosomes in prophase. We show that depletion of PAK1 or PAK2 also leads to increased intercentrosomal distance, and allows cells to more easily bipolarise under Eg5 inhibition. These results demonstrate a novel function for PAK downstream of Tiam1 phosphorylation in regulating bipolar spindle assembly, and may also have important implications for cancer therapy.

Notes:

P080 Regulation of the ESCRTIII component CHMP4C by the Chromosomal Passenger Complex during cytokinesis

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Cytokinesis is the final stage of mitosis and requires many proteins that control sequential events to physically separate the two daughter cells. The Chromosomal Passenger Complex (CPC) and in particular its enzymatic component Aurora B, has been proposed to monitor the final separation of the two daughter cells at the end of cytokinesis in order to prevent cell abscission in the presence of DNA at the cleavage site. Recent studies indicate that abscission is mediated by the assembly of filaments comprising components of the Endosomal Sorting Complex Required for Transport-III (ESCRT-III) and we published that one of its component CHMP4C is regulated by the CPC using two concomitant events: the binding of the CPC subunit Borealin to CHMP4C's N-terminal half and Aurora B phosphorylation of three serine residues on CHMP4C's C-terminal tail. We have found that Aurora B phosphorylates CHMP4C *in vivo* and that the entire CPC is necessary for proper CHMP4C phosphorylation and localisation during cytokinesis. I will discuss these results and their implications on how the CPC controls abscission timing through regulation of ESCRTIII.

Notes:

P081 Functional role of the basic insertion within loop2 in the head domain of myosin IX

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Myosins are molecular motors with the ability to convert chemical energy into directed mechanical force along actin filaments. Class IX myosins show several unique motor properties compared to the members of other myosin classes. Remarkably, they are able to take multiple successive steps along actin filaments without dissociating, although they are single-headed motors. The mechanism of the single-headed processive movement of myosin IX still remains to be determined.
It was shown that a basic insertion of approximately 120 aa within loop 2 in the head domain tethers myosin to the actin filament and ensures a persistent binding to F-actin during the whole ATPase cycle. Interestingly, a calmodulin-binding site (CaM-BS) could be identified within the basic insertion. Calmodulin (CaM) is sensitive to alterations in the Ca^{2+} concentration and reacts with a conformational change. Therefore Ca^{2+}/CaM may regulate the myosin IX motor properties.
To elucidate the contribution of CaM to the binding behavior of the insertion, actin-binding assays were performed and show that the insertion binds and bundles actin filaments in the absence, but not in the presence of apoCaM or Ca^{2+}/CaM. To investigate further the role of the CaM-BS, synthetically produced peptides containing the putative sequence of the CaM-BS were tested for CaM binding by fluorescence spectroscopy. It could be shown that the peptide bound to CaM. However, the binding of CaM was not significantly modified by varying Ca^{2+}-concentrations.

Notes:

P082 Endophilin marks and controls a clathrin-independent endocytic pathway

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Endocytosis is the process by which cells internalise macromolecules and regulate the surface levels of membrane receptors. Proteins containing a BAR domain can sense, stabilise or induce curvature of cellular membranes supporting endocytosis amongst other cellular processes. Endophilin is a member of the BAR domain protein superfamily that functions during endocytosis recruiting dynamin which promotes scission of the pinched plasma membrane to form clathrin coated vesicles. The recruitment of dynamin and other partners is mediated by the SH3 domain of endophilin, located at the C-terminus.
Recent data from our lab shows that endophilin marks and controls a previously undescribed pathway of endocytosis, independent from the well-established clathrin-mediated endocytosis. Upon stimulation of specific membrane receptors by their ligands, endophilin-positive membrane tubes and vesicles are formed and rapidly move towards early endossomes.
My recent work consists in defining the binding motifs of endophilin in some of these membrane receptors. Furthermore, was assessed the effect of endophilin in regulation of the downstream signalling of these receptors. Because some of the identified receptors are also involved in cell migration, the role of this novel endocytic pathway in cell migration is being evaluated. The data collected so far reveals that this new endocytic route is essential in regulating the number of surface receptors, cell signalling and migration.

Notes:

P083 Structural insights into the organization of the cavin membrane coat

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Caveola membrane invaginations are a striking feature of many vertebrate cell types, and are critical for cell signaling, endocytosis and mechanotransduction. Their formation depends on the caveolins and the cavin peripheral membrane proteins (cavin1, cavin2, cavin3 and cavin4), although there is currently no atomic level information addressing the mechanisms that underpin caveola assembly. Here we show that a minimal N-terminal domain of the cavin proteins (the HR1 fragment) is required and sufficient for their homo and hetero-oligomerisation. The crystal structures of mouse cavin1 and zebrafish cavin4 HR1 domains reveal highly conserved trimeric coiled-coil architectures, with unique intra-subunit interactions that determine the specificity of coiled-coil formation. A conspicuous feature of the HR1 domain is a basic surface patch, conserved among all cavins and across all species, which we show can mediate interaction with negatively-charged membrane lipids including phosphoinositides. Mutations in this domain prevent membrane association and perturb caveolae formation *in vivo*. Interestingly the cavin proteins possess intrinsic membrane remodeling properties *in vitro*, that we propose is important for the formation of caveolae. Finally, we show that full-length cavin proteins possess characteristic rod-shape structures that reflect the coiled-coil architecture of the HR1 assembly domain and have dimensions corresponding closely to the striations observed on the surface of caveolae *in vivo*. We therefore propose the striations forming the common coat of caveola are composed of polymerised cavin trimers.

Notes:

P084 Syndecan-4 controls integrin recycling to regulate cell migration and the extracellular microenvironment

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Microenvironment remodelling and cell migration are essential for tissue morphogenesis, homeostasis and repair, and for the pathogenesis of inflammatory and neoplastic disease. Adhesion contacts are integrin-mediated complexes that integrate the extracellular microenvironment with the contractile machinery of the cell. Consequently, the spatial and temporal co-ordination of adhesion receptor dynamics is essential for cell migration and microenvironmental remodelling. We have identified the membrane-intercalated adhesion receptor syndecan-4, as a regulatory control point that coordinates integrin recycling in order to regulate cell migration, fibronectin fibrillogenesis and the biomechanical characteristics of the matrix. We have shown that the non-receptor tyrosine kinase Src phosphorylates syndecan-4 to suppress activation of the small GTPase Arf6. Syndecan-4-mediated Arf6 activity differentially regulates recycling of the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrin heterodimers to the membrane, to control adhesion complex dynamics. Thus, syndecan-4 phosphorylation functions as a molecular switch targeting specific integrin heterodimers to the membrane to modulate cell migration and fibronectin fibrillogenesis. Indeed, by co-ordinating engagement of either $\alpha_5\beta_1$ or $\alpha_v\beta_3$ integrins at the cell surface, syndecan-4 phosphorylation controls 1) fibronectin secretion, 2) fibronectin remodelling and 3) the application of mechanical force; the three critical functions required to establish a viable and stable fibronectin-rich extracellular matrix.
We propose that during wound healing *in vivo*, precise spatiotemporal regulation of syndecan-4 phosphorylation is required to control integrin trafficking and dynamically co-ordinate cell migration and the integrity and biomechanical properties of the extracellular microenvironment.

Notes:

P085 PTEN regulates PAR3 positioning and mitotic spindle orientation during colorectal gland morphogenesis

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Background: Three-dimensional (3D) gland morphology is maintained by PTEN, through coupling the GTPase cdc42 to atypical protein kinase C (aPKC) at the apical membrane (AM). PTEN knockdown generates morphology defects including AM mispositioning and a multilumen glandular phenotype. AM alignment is linked to apical junction (AJ) positioning and mitotic spindle orientation (MSO) but PTEN regulation of these phenomena remains unclear. The aim of this study was to investigate PTEN regulation of AJ positioning and MSO during 3D gland morphogenesis.
Methods: We used PTEN-expressing wild type (wt) Caco-2 and PTEN stably-deficient Caco-2 ShPTEN cells to generate 3D glands in culture. In developing glands, PAR3, NHERF-1 and tubulin were used as AJ, AM and microtubule markers respectively. MSO was assessed by microtubule alignment against cells' long axis. To probe the cdc42/aPKC pathway, glands were transfected with dominant negative (DN) or constitutively active (CA) cdc42 or treated by an aPKC pseudosubstrate inhibitor (aPKCI) or an aPKC activator (aPKCA).
Results: PTEN deficiency induced misorientation of the mitotic spindle, displacement of PAR3 and AM misalignment. These abnormalities were phenocopied by DN cdc42 transfection or aPKCI treatment. Transfection of CA cdc42 or aPKCA treatment restored AM alignment and overall gland morphology, in PTEN-deficient glands.
Conclusions This study shows for the first time that PTEN regulates PAR3 positioning and MSO through the cdc/aPKC pathway to control AM alignment and overall 3D gland morphology.

Notes:

P086 Insights into the trafficking and polarized secretion of major proteinase inhibitor cystatin C in retinal pigment epithelium: consequences for development of age-related macular degeneration

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The cysteine proteinase inhibitor cystatin C is among the top 2% most abundantly expressed proteins in the retinal pigment epithelium (RPE), which has an essential role in supporting the function of the neuroretina. Development of age-related macular degeneration (AMD), the leading cause of legal blindness in the Western world, is associated with multiple malfunctions of the RPE. A variant of cystatin C was correlated with increased risk of developing exudative AMD. The targeting and processing through the secretory pathway of RPE cells of cystatin C, synthesized as a precursor, are essentially dependent on an N-terminal 26-amino acid hydrophobic signal sequence.
In striking contrast to the wild-type precursor cystatin C conspicuously targeted to the Golgi apparatus, the AMD-associated variant B (A25T) precursor cystatin C associates also with mitochondria. Secretion of variant B is also reduced by approximately 50% compared with that of the wild-type cystatin C. In addition, the temporal and spatial expression of cystatin C *in situ* RPE in relation to age shows a significant reduction of cystatin C level in the posterior RPE. The preferential basolateral secretion together with age-related reduction in expression and targeting point towards a function of cystatin C in regulation of proteolytic activity and maintaining the structure and function of the Bruch□s membrane/choroid.
Impairment of secretion, alongside possible misfolding or aggregation of proteins in the RPE cells emerge as critical molecular events underlying macular degeneration.

Notes:

P087 Dynamic anisotropies in cytoskeletal organisation induced by ROS and Rho signalling underlie multicellular sensing and spatial patterning in a *Drosophila* epithelium.

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The morphogenesis and resilience of epithelial tissues relies on dynamic, heterogeneous and coordinated cell behaviors. We have used the amnioserosa, an active participant during Drosophila dorsal closure, a model for both epithelial fusion and wound healing, to investigate the origin and nature of cues that influence individual cell behaviors, drive transitions between behaviors and enable their coordination. Using single cell genetic and nanoscale laser perturbations (to influence chemistry and mechanics respectively), 4D confocal microscopy and quantitative morphometry, we show that transitions in cell behavior (pulsed apical constriction to cell delamination-an epithelial to mesenchymal transition) result from dynamic asymmetries in the spatial organization and dynamics of the actomyosin cytoskeleton. These asymmetries, specifically the restriction of actomyosin dependent contractility to either medial or circumapical pools or to specific cellular interfaces, are in turn influenced by stochasticities and asymmetries in physical cues (geometry and tension) and chemical signals (mitochondrial remodeling and oxidative stress signaling) that act both cell-autonomously and non-autonomously. We demonstrate that mitochondrial ROS influences the spatial organization of the actomyosin contractility through its dose-dependent effect on the distribution of the Rho-kinase ROCK to circumapical (high ROS) or medial (low ROS) pools. Collectively, our findings invoke the interplay between chemical and mechanical signals for multi-cellular sensing and the spatial patterning of epithelia during morphogenesis and maintenance. They also provide an explanation for compromised tissue integrity and resilience in metabolic and oncological pathologies.

Notes:

P088 A membrane-driven conformational switch in AP2 activates clathrin recruitment

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Clathrin-mediated endocytosis (CME) is a vital cellular process governing the internalization of cell-surface proteins. During CME, membrane-binding clathrin adaptors recruit and polymerize clathrin to form a clathrin-coated “pit” (CCP) into which cargo proteins are sorted. AP2 is the central adaptor in CME, mediating recruitment of cargo and accessory proteins. It exists in at least two conformations: a cytosolic, “inactive” conformation, and a plasma membrane-bound, “active” conformation. Binding of AP2 to the plasma membrane phosphoinositide PtdIns(4,5)P_2 drives AP2 from the “inactive” to the “active” conformation, allowing the complex to recognize and bind transmembrane protein cargo. We determined a new structure of AP2 that includes the clathrin-binding β2-hinge, revealing that the canonical clathrin-binding motif in the β2-hinge is sequestered in the “inactive” conformation of AP2. This autoinhibition is relieved when AP2 binds to the plasma membrane, because the sequestration of the β2-hinge is incompatible with the “active” conformation of AP2. By developing an AP2-dependent budding assay, we show that membrane recruitment by PtdIns(4,5)P_2 and cargo triggers clathrin binding and clathrin-coated bud formation. This switching mechanism restricts clathrin recruitment and polymerization to the plasma membrane, thereby linking AP2’s membrane recruitment to its key functions of clathrin and cargo binding.

Notes:

P089 Structural basis and functional implications of Vps33A recruitment to the human HOPS complex by Vps16

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The homotypic fusion and vacuole protein sorting (HOPS) multi-subunit complex is required for late endosome-lysosome and autophagosome-lysosome fusion in mammals. We have determined the crystal structure of the human HOPS subunit Vps33A, confirming its identity as member of the Sec1/Munc18 (SM) family of proteins that regulate membrane fusion by binding directly to SNARE proteins. Previous studies have shown that a small hydrophobic pocket in domain 1 of SM proteins is important for SNARE regulation as it binds a peptide at the N terminus of syntaxin-family SNAREs. Vps33A lacks this hydrophobic pocket, consistent with the hypothesis that the remainder of the HOPS complex acts to recruit Vps33A, and thus SM protein function, to the site of membrane fusion. We identified that Vps33A binds residues 642-736 of HOPS subunit Vps16 and we solved the structure of this complex to 2.6 Å resolution. Mutations at the binding interface disrupt the Vps33A–Vps16 interaction both *in vitro* and in cells, preventing recruitment of Vps33A to the HOPS complex. Functional assays show that disruption of the Vps33A–Vps16 interaction prevents autophagosome-lysosome fusion and inhibits delivery of endocytic cargo to lysosomes. The Vps33A–Vps16 complex provides a structural framework for studying the association between SM proteins and tethering complexes and our functional studies confirm that recruitment of Vps33A via Vps16 is required for HOPS activity.

Notes:

P090 Hepatitis D virus recruits clathrin by mimicking host cell motifs

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Hepatitis D virus (HDV) is a small, enveloped RNA virus which can sustain infection only in cells simultaneously infected with Hepatitis B virus (HBV). Superinfection of chronic HBV carriers with HDV can cause fulminant acute hepatitis or severely aggravate hepatic diseases like liver cirrhosis and hepatocellular carcinoma. Hepatitis D virus encodes only one protein, the Hepatitis D antigen, which occurs in a small (HDAg-S) and large (HDAg-L) isoform. Previous studies demonstrated that clathrin, a cellular protein involved in endocytosis and intracellular trafficking, binds to HDAg-L and that this interaction is required for the release of enveloped virus-like particles from host cells. We sought to identify the molecular basis of the clathrin:HDAg-L interaction and solved crystal structures of HDAg-L peptides from two HDV genotypes bound to the N-terminal domain of clathrin. Our structures reveal that both HDAg-L peptides bind multiple sites on clathrin, including one site which was previously structurally uncharacterized. We are currently investigating the functional relevance of the observed binding sites. Our studies aim not only to enhance our understanding of the HDV egress pathway but also to identify molecular targets for novel therapeutics to disrupt the virus life cycle.

Notes:

P091 Arpin-mediated idling and reductions in cell speed are associated with turns

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We recently reported a novel protein, Arpin, which inhibits the Arp2/3 complex, a molecular machine that nucleates actin filaments at the cellular front in the lamellipodium. There, new actin filaments polymerize and exert force to protrude the edge forward. Arp2/3-based positive feedback signaling is the basis for lamellipodial persistence, which drives effective directional migration. Indeed, we observed that Arpin is associated with decreases in two major parameters of cell migration: speed and directional persistence. To understand how Arpin controls these parameters, in this work we re-examined migration trajectories of three different cell types with Arpin perturbations. In all three systems, we found that speed and turn angle are negatively correlated, suggesting a universal linkage between these parameters. Arpin-mediated decreases in instantaneous speed were associated with increases in turn angle. Importantly, in all three systems Arpin induced episodes of idling, defined as moving below a speed threshold. This threshold is a percentage of the average speed, calculated using the first derivative of log mean squared displacements. Observed idling episodes interrupted and effectively shortened duration of the active phase of migration, consistent with a model in which Arpin counteracts a positive-feedback circuit that sustains the lamellipodium over time. Finally, Arpin-associated idling episodes were followed by turns, implicating idling as a means for enacting a turn.

Notes:

P092 A functional interplay between the Leucine rich repeat kinase 2 and p21 activated kinase 6 in neuronal cytoskeleton

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Leucine rich repeat kinase 2 (LRRK2) is a large multidomain protein with GTPase and kinase domains with a still unknown function. Mutations in *LRRK2* are the most common genetic cause of Parkinson disease (PD), the second most common neurodegenerative disease. Interestingly, the assembly of abnormal cytoskeleton elements is thought to be one major characteristic of neurodegenerative diseases. Emerging evidence suggests that LRRK2 influences i) cell migration, ii) vesicular trafficking, neurite outgrowth and synaptogenesis and iii) inflammation, all pathways that require the presence of a functional cytoskeleton. Accordingly, LRRK2 interacts with the major components of cell cytoskeleton, actin and tubulins. In this context, our recent findings describe a novel LRRK2-mediated pathway in neurons that involves the activation of the p21 activated kinases (PAKs), a class of enzymes that act downstream of small GTPases in different morphogenetic processes through remodeling of the actin cytoskeleton. Our data show that i) LRRK2 forms a functional complex with PAK6 *in vitro* and *in vivo* at the level of actin filaments, ii) LRRK2 is required for PAK6-dependent neurite growth in cortical primary neurons, iii) PAK6 is not activated in LRRK2 knock-out mice and iv) PAK6 is hyper-phosphorylated in brains from LRRK2-G2019S carries and idiopathic PD patients. Concluding, our findings point to PAK6 as a novel LRRK2-downstream effector and further support the involvement of LRRK2 in the regulation of neuronal cytoskeleton architecture in health and disease.

Notes:

P093 Modelling crowding behaviour of Kinesin 1

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Kinesins are a family of motor proteins that are primarily involved in cargo transport along the microtubule cytoskeleton and cross-linking bundles of microtubules. Kinesins can be both processive and non-processive and while most kinesins are minus-end directed, there are some which step in the opposite direction. The motor often operates in situations where the local occupancy of Kinesins on the microtubule is high. Even in these high density conditions the transport properties of the system remain very efficient. We present a theoretical model for studying this crowding behaviour with a particular focus on Kinesin 1. The model used is similar to those used in analysis of traffic dynamics on road networks. The approach involves using both analytical methods and simulations in order to choose rate parameters which give rise to the observed *in vitro* behaviour. A primary feature of the model is that it captures the behaviour of observing a peak motor current at densities greater than 50%. This is achieved by considering internal states within the framework of a simple exclusion process model.

Notes:

P094 Ysc84 is a novel, PI(4,5)P2 regulated, actin - capping protein functioning in early stages of yeast endocytosis

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Regulation of endocytosis via actin cytoskeleton has been extensively studied in yeast. Polymerization of actin is proposed to provide the driving force for invagination against the turgor pressure that creates tension at the plasma membrane. Most actin regulatory proteins arrive during slow-movement phase, marked by rapid burst of actin polymerization followed by fast-movement phase and scission. However, live cell-imaging shows that Ysc84 localizes to endocytic sites after Las17 but before other known actin binding proteins, suggesting it may function at an early stage of invagination. Ysc84 was demonstrated to bind actin filaments via its conserved N-terminal domain. What is interesting, is that full length Ysc84 could only bind actin when its SH3 domain interacted with Las17, homologue of WASP. While Ysc84 has homologous in other organisms including human Sh3yl-1, little is know of its nature of interaction with actin and how this interaction affects actin filament assembly. The aim of the study is to find out whether the mechanism of interaction with actin is conserved between Ysc84 homologues and whether it could be use to model disease pathways associated with actin cytoskeleton like: cell migration, invasiveness. Here we identify key residues involved in Ysc84 actin and lipid binding and reveal previously unknown actin capping activity, which is negatively regulated by PI(4,5)P2. Ysc84 mutants defective in lipid or actin-binding interaction were characterized *in vivo* and show distinc phenotypes indicating the importance of these interaction for regulating early stages of endocytosis.

Notes:

P095 Sterol traffic in yeast is mediated by a newly discovered family of StART proteins

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Sterol traffic from the ER to the plasma membrane is a fundamental cellular process that is poorly understood. One thing is clear: SNARE-mediated vesicular traffic is not involved. Instead, sterol traffic is thought to be mediated by cytoplasmic lipid transfer proteins (LTPs), each containing a hydrophobic cavity that binds specific lipids one at a time. But the precise LTPs have not been identified. StART proteins make up a large family of conserved cytoplasmic LTPs some of which bind sterol. Studies in humans have been slow to make progress on the role of StARTs in bulk traffic of sterols, so we have studied this process in budding yeast. Although this organism was previously described as lacking START proteins, we discovered a new sub-family of StART proteins that has 6 members in yeast, as well as members in all other eukaryotes. We have carried out *in vitro* studies of the individual domains from new StART proteins for lipid specificity and 3D structure. In addition, we have determined *in vivo* localisations and functions of the full length StART proteins that indicate that they transfer sterol across ER-plasma membrane contact sites.

Notes:

P096 Fission yeast Sec3 bridges the exocyst complex to the actin cytoskeleton

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The exocyst complex tethers post-Golgi secretory vesicles to the plasma membrane prior to docking and fusion. In this study we identify Sec3, the missing component of the *Schizosaccharomyces pombe* exocyst complex (*Sp*Sec3). *Sp*Sec3 shares many properties with its orthologs, and its mutants are rescued by human Sec3/EXOC1. Although involved in exocytosis, *Sp*Sec3 does not appear to mark the site of exocyst complex assembly at the plasma membrane. It does however mark the sites of actin cytoskeleton recruitment and controls the organization of all three yeast actin structures:the actin cables, endocytic actin patches and actomyosin ring. Specifically, *Sp*Sec3 physically interacts with For3 and *sec3* mutants have no actin cables as a result of a failure to polarize this nucleating formin. *Sp*Sec3 also interacts with actin patch components and *sec3* mutants havedepolarized actin patches of reduced endocytic capacity. Finally, the constriction and disassembly of the cytokinetic actomyosin ring is compromised by the absence of Sec3. We propose that a role of *Sp*Sec3 is to spatially couple actin machineries and their independently polarized regulators. As a consequence of its dual role in secretion and actin organization Sec3 appears as a major coordinator of cell morphology in fission yeast.

Notes:

P097 Dissecting the roles of the IP_3 receptors in migration of fibroblasts

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Establishment of a front-rear polarity axis is required for directional migration of cells. Interestingly, gradients of cytosolic Ca^{2+} increasing from front to rear have been observed in several cell types. Directed migration also requires local Ca^{2+} flickers near the leading edge of the cell which activate myosin and modulate focal adhesions. Evidence derived from studies in fibroblasts and endothelial cells supports the current model in which spatially polarised components of the Ca^{2+} signalling machinery act in a coordinated manner to promote cell migration. In fact, receptor tyrosine kinase signalling, phospholipase C, PtdIns(3,4,5)P_3, diacylglycerol, active protein kinase C, Ca^{2+} pumps and stromal interaction molecule 1 are polarised in migrating endothelial monolayers. Despite the well-established role that the IP_3 receptors play in eliciting Ca^{2+} signals the individual contribution of the three IP_3 receptor subtypes to polarised cell migration needs to be further defined. Hence, we have studied the role of the IP_3 receptors in migration of fibroblasts using a high throughput 2D wound healing assay.

Notes:

P098 Small molecule inhibitors of Rho signalling: characterization and target identification of the Rhodblocks

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Bioactive small molecules are an important component of the biologist's toolbox to study basic cell biology. Small molecules provide distinct advantages to conventional genetic approaches to studying protein function; they act quickly and often reversibly. Prominent examples include a variety of small molecules directly targeting the actin and microtubule cytoskeletons in cells. However, the availability of small molecules with known protein targets that can be used in cell biology assays is relatively limited. The Rho GTPase pathway signals between the microtubule-based mitotic spindle and actin cytoskeleton during cytokinesis to control cell division. It also controls various other cellular processes including cell adhesion, migration and morphogenesis. Recently our research group developed a novel strategy to identify small molecules targeting the Rho pathway during cytokinesis, by screening for compounds that enhance a partial Rho RNAi phenotype in *Drosophila* tissue culture cells (Castoreno et al., Nat. Chem. Biol. **6**, 457-463, 2010). Twelve new small molecules, the Rhodblocks, were identified in this way. Rhodblock 6 was shown to be a Rho-kinase inhibitor, verifying this new screening approach. Molecular targets for the remaining eleven Rhodblocks have yet to be identified. Here we present data further characterizing two Rhodblocks with different cytoskeletal phenotypes that are beginning to provide clues to their function and suggest a diversity of cellular targets amongst the Rhodblocks; Rhodblock 4 induces cell rounding and blebbing in adherent cells; Rhodblock 12 disrupts microtubule organization in cells. We therefore also discuss work towards a systematic genetic approach to Rhodblock target identification.

Notes:

P099 E-cadherin apical sorting by a p200/AP-1/clathrin/RAB-11 pathway during morphogenesis

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Membrane traffic can be divided in two essential steps: sorting between cargos and transport to various destinations. In epithelial cells, a specific sorting event is required to differentially traffic apical and basolateral proteins; while basolateral sorting has been extensively studied, apical sorting mechanisms are still elusive. To characterise apical sorting *in vivo* we choose to examine the apical delivery of E-cadherin (E-cad) in *C. elegans* epidermal cells.
A systematic RNAi screen designed to find genes required for the strict E-cad apical localisation identified clathrin, the clathrin adaptor AP-1 and p200/SOAP-1, a physical AP-1 interactor; their loss induced a basolateral localisation of E-cad without affecting the apico-basal diffusion barrier. Surprisingly glycosphingolipids were not required in that process. We further found that SOAP-1 controls AP-1 localisation which is itself required for Rab11 subapical localisation. Furthermore Rab11 depletion induced a strong loss of the apical E-cad pool. We concluded that SOAP-1/AP-1 control E-cad sorting upstream of its apical delivery via Rab11 endosomes in the biosynthetic and/or recycling pathways.
This function of AP-1 in E-cad apical delivery is essential during embryonic morphogenesis, a process controlled by junction rearrangement and driven by acto-myosin contractions: we found that the E-cad basolateral accumulation was correlated in time and space with a strong loss of cell-cell adhesion and a dramatic loss of actin organisation.
We therefore propose that a molecular pathway including SOAP-1, AP-1 and clathrin acts upstream of Rab11 to apically deliver E-cadherin in *C. elegans* epidermal cells and control cell-cell adhesion rearrangement during morphogenesis.

Notes:

P100 The formin FMNL3 in angiogenesis

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Angiogenesis is a multi-stage process describing blood vessel formation from pre-existing vasculature and involves cell polarisation, elongation, migration and lumenogenesis. Previous work identified the formin and cytoskeletal regulator FMNL3 as being required for angiogenesis during Zebrafish development. Formin function is regulated by Rho GTPases and interestingly, we found FMNL3 to bind to and act as an effector of Cdc42, with FMNL3 depletion in endothelial cells (ECs) resulting in aberrant cell polarisation and directional migration. Others have observed defects in lumenogenesis during angiogenesis in Zebrafish development following FMNL3 depletion. Lumenogenesis involves the definition of apical and basolateral membranes, and the correct localisation of proteins to these regions. During spreading, ECs form distinct apical regions which are concentrated for proteins known to localise apically and to be required for lumenogenesis including Podocalyxin-Like 1 (PDXL1), Phospho-Moesin, VE-Cadherin and F-actin. Using this system to study apical polarisation and trafficking, FMNL3 was identified as a protein required for the correct localisation of PDXL1 to the apical membrane. In an *in vitro* angiogenesis assay, the localisation of VE-Cadherin to the cell-cell junctions bordering the luminal membrane, a process which coincides with PDXL1 localisation to the luminal membrane, was found to be perturbed. Together, this shows that FMNL3 functions in the regulation of cell polarisation, where it is responsible for the trafficking of PDXL1 to the apical membrane to allow lumen formation.

Notes:

P101 Beta 1-integrin - c-Met crosstalk: an endosomal inside-in signalling

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Receptor Tyrosine Kinases (RTK) and integrins cooperate to stimulate cell migration, survival and tumour metastasis. However, how these two major classes of transmembrane receptor molecules cooperate is not yet fully understood. Recently endocytosis has been shown to play a major role in RTK signaling and in integrin function. We provide evidence that the internalisation of an integrin can influence the endosomal signalling of an RTK. We report that b1-integrin plays a non-adhesive role in c-Met signalling through controlling: 1) c-Met endocytosis; 2) c-Met sustained signalling on autophagosomes; Mechanistically, b1-integrin plays the role of an adaptor through linking c-Met to $p52^{ShcA}$ through its NXXY domains in the cytoplasmic tail. This input of b1-integrin to autophagosomal c-Met signalling is ligand-adhesion independent such that it occurs in cells growing in suspension. This sustained c-Met signalling on the autophagosomes leads to protection against anoikis, anchorage-independent growth *in vitro*,tumorigenesis and lung metastasis *in vivo*. While RTK-integrin cooperation has been assumed to occur at the plasma membrane and has mostly been described to necessitate integrin "inside-out" or "outside-in" signalling, our results suggest the existence of a novel mode of integrin signalling, in cooperation with RTK, which we term "an endosomal inside-in signalling", occurring on autophagosomes. This non-adhesive role of b1-integrin may be necessary in the early steps of metastasis, through enhancing c-Met signalling and consequent cell survival.

Notes:

P102 Cut7-driven microtubule sliding reverses direction depending on motor density

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[3]University of Warwick, Coventry, UK

Cut7, the only kinesin-5 in *S. pombe*, is essential for mitosis. We have found using *in vitro* reconstitution that lawns of full length Cut7 tetramers slide microtubules (MTs) bidirectionally, with MT sliding velocity and direction dependent on motor crowding and ionic strength. Dense lawns of full length Cut7 drive minus end directed MT sliding at high ionic strength. As ionic strength is lowered, sliding velocity progressively reduces and ultimately MTs slide slowly (~10-20 nm s^{-1}) in the opposite direction. Sparse surfaces of full length Cut7 drive fast minus end directed sliding under all buffer conditions assayed. Our data are broadly in line with the previously-reported directional reversal behaviour of *S.cerevisae* Cin8, Kip1, and truncated *S. pombe* Cut7. However in contrast to earlier reports we find that for Cut7, MT sliding velocity does not depend on MT length. We also find that lawns of truncated Cut7 monomers drive only plus end directed MT sliding, consistent with our cryoEM reconstruction of the monomer. This shows that plus end directed strokes are the basal activity of individual Cut7 motor heads and that directional reversal is an emergent property of interacting head-pairs. We now propose a possible mechanism for directional reversal, in which processive minus end directed steps are inhibited by motor crowding, causing basal, nonprocessive, plus end directed strokes to dominate. Simulations establish that such a model can in principle account for the observed behaviour.

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Monday, 20th April 2015

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Online registration

1st British Microtubule Meeting

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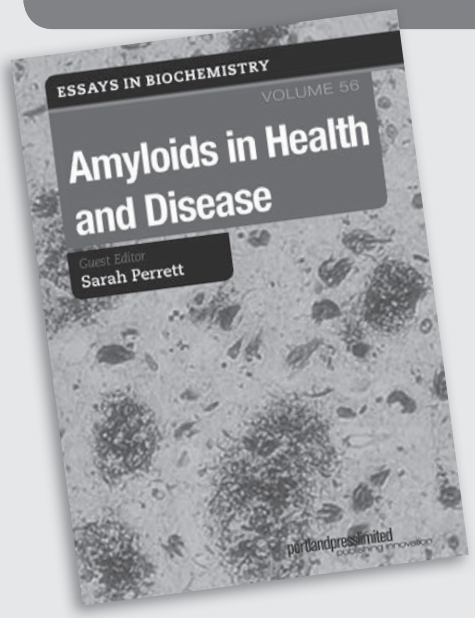

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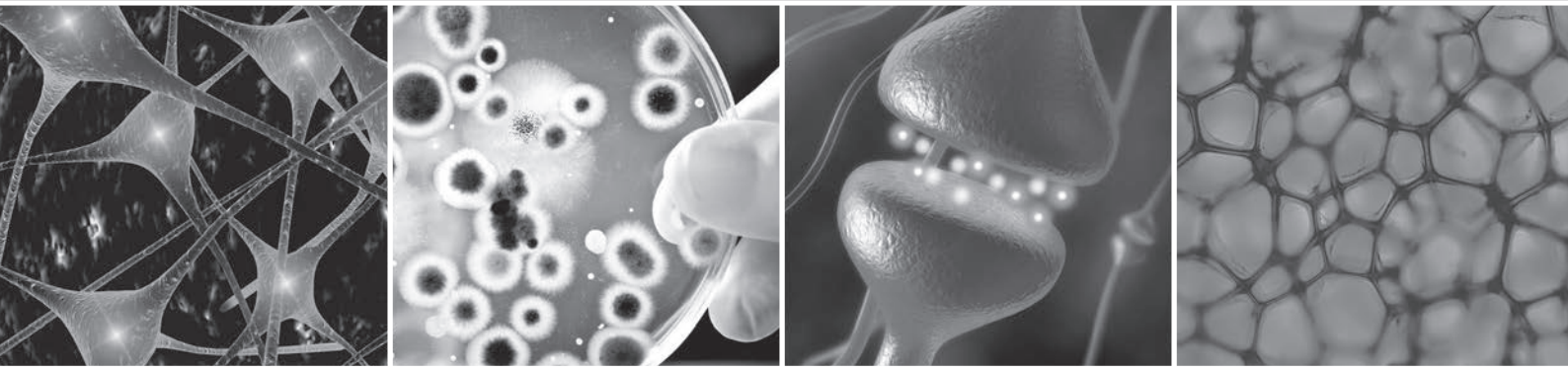

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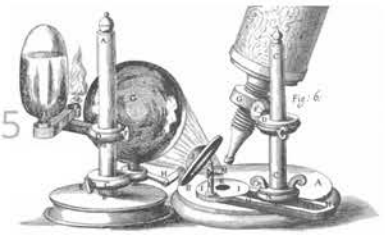
BSCB

British Society for Cell Biology

The BSCB was founded 1965 in order to promote the advancement of research in all branches of cell biology and to encourage the interchange of information. The Society organises two annual conferences and supports a limited number of other meetings across the UK relevant to cell biology. It helps promote the career development of young cell biologists and provides both travel funds for young researchers and summer research bursaries to undergraduates to work in BSCB member's labs. The Society awards the Hooke Medal each year to a UK-based cell biologist who has made outstanding contributions to their field. The BSCB runs annual competitions for cell biology imagery and science writing and also provides a range of highly popular informational resources for school pupils that are used across the globe. We also play an increasing role in raising awareness of science policy and funding issues in the UK.

The Society is supported by our membership and by a generous grant from The Company of Biologists, who publish Journal of Cell Science, Development, Journal of Experimental Biology and Disease Models and Mechanisms.

BSCB Hooke Medal 2015



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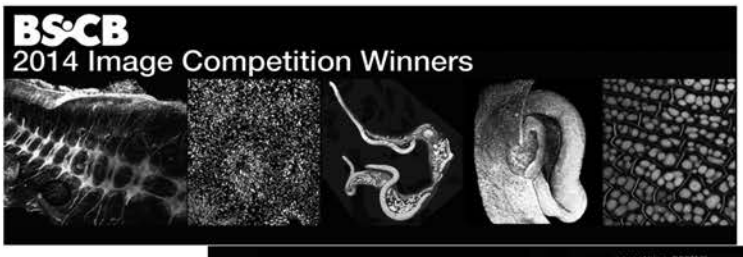

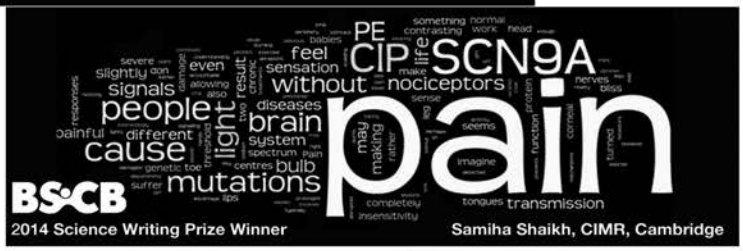

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