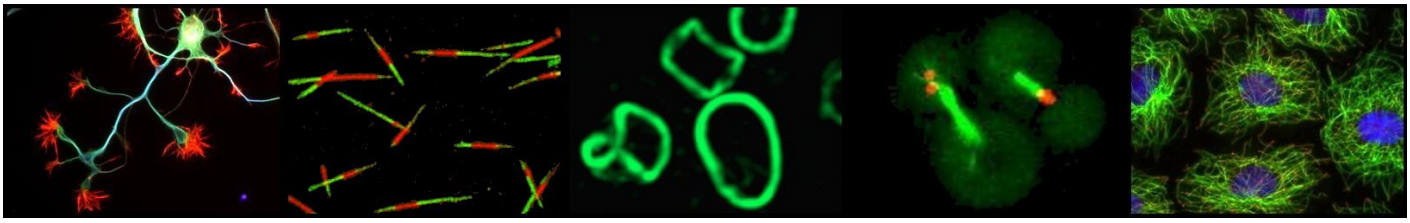

Les 2èmes Journées du Réseau France Microtubules
(French Microtubule Network)
6 et 7 juillet 2015, Université Grenoble Alpes

LUNDI 06 JUILLET 2015 - AMPHITHEATRE BOUCHERLE – UNIVERSITE GRENOBLE ALPES/CHU

9h00 Welcome & Registration

9h30-10h30	**Seminar**	**Invited Speaker Niels GALJART (Rotterdam, The Netherlands)**

10h30 - 11h	**SESSION 1**	**MICROTUBULE ASSEMBLY AND REGULATIONS (I)**

- **EB1-gold reveals the GTP-cap architecture of growing microtubule ends.** Frank BAZILE
- **Direct evidence for the interaction of stathmin with microtubules in cells: an investigation made by real-time confocal imaging coupled to FRET and FRAP spectroscopies**. Gilles BREUZARD

COFFEE BREAK

11h30 – 12h45	**SESSION 2**	**MICROTUBULE ASSEMBLY AND REGULATIONS (II)**

- **EB1 links microtubule network organization and touch response in *Arabidopsis thaliana*.** Frédéric COQUELLE
- **Unravelling the mechanoperception and transduction pathway controlling the microtubule response to mechanical stress.** Stéphane VERGER
- **Investigating the position of GCPs 4, 5, 6 in the Gamma-tubulin ring complex.** Dorian FARACHE,
- **Microtubule and actin organization by the neuronal MAP tau.** Isabelle ARNAL
- **Role of tubulin polyglutamylation in neurodegeneration.** Maria M. MAGIERA

LUNCH

13h45 – 15h30 **POSTER SESSION**

COFFEE BREAK

15h30-16h30 **SESSION 3 MICROTUBULES AND THEIR REGULATORS: NEW THERAPEUTIC TARGETS? (I)**

- **Microtubule-bound septins together with tyrosinated and polyglutamylated tubulin confer cell adaptation to Taxol® and impact on cell motility.** Anita BAILLET
- **The role of primary cilia in colon homeostasis and tumor development.** Ruizhi TANG
- **End-binding 1 protein overexpression correlates with glioblastoma progression and sensitizes to Vinca-alkaloids in vitro and in vivo.** Raphael BERGES
- **Microtubule-associated tumor suppressor ATIP3 interacts with EB1: clinical relevance in breast cancer.** Clara NAHMIAS

COFFEE BREAK

17h15-18h00 **SESSION 4 MICROTUBULES AND THEIR REGULATORS: NEW THERAPEUTIC TARGETS? (II)**

- **IPP51, a chalcone acting as a microtubule inhibitor with in vivo antitumor activity against bladder carcinoma.** Véronique MARTEL-FRACHET
- **A new LIMK inhibitor stabilizes microtubules and has anticancer activity.** Chloé PRUNIER
- **Photoswitchable microtubule inhibitors for localised optical control of MT dynamics, mitosis, and cell death.** Oliver THORN-SESHOLD

20H00 DINER PRISE DE LA BASTILLE

TIPs and TAPs to control microtubule behaviour.

Niels Galjart, Department of Cell Biology, Erasmus MC, The Netherlands.

The microtubule (MT) cytoskeleton is essential for many processes, including mitosis, cell polarity and shape, and ciliogenesis. The dynamic behaviour of MTs is regulated by a multitude of MT-associated proteins (MAPs). MT plus-end tracking proteins, or +TIPs, are MAPs which specifically associate with the ends of growing MTs, thereby regulating both MT behaviour and the interactions of MTs with other cellular structures. Mammalian CLASP1 and -2 are interesting +TIPs as these proteins are capable of selectively stabilizing MTs at the edge of cells in a signal-dependent manner. In addition, CLASPs promote MT growth at the Golgi.

We recently designed a method to efficiently express tubulin dimers, the building blocks of MTs, in mammalian cells. This allowed us to isolate functional tagged tubulin dimers using a rapid two-step protocol. An optimized mass spectrometry-based approach was subsequently employed to identify tubulin-associated proteins (TAPs) in cultured HEK293 cells. Interestingly, CLASPs and a few other +TIPs are present in this "tubulome". In addition, we find selected centrosomal components and motor proteins, and numerous other factors. Mutant tubulins, containing mutations that are found in patients with brain malformations, bind CLASPs and other TAPs less efficiently and expression of these mutants in cells impairs intracellular processes involving these factors. We propose that a core network of tubulin-binding +TIPs, including the CLASPs, promotes MT growth at plus ends and other intracellular sites. Together our results provide a resource for investigating tubulin interactions and functions, widen the spectrum of tubulin-related disease mechanisms, and shed light on how TIPs and TAPs control microtubule behaviour.

Title : EB1-gold reveals the GTP-cap architecture of growing microtubule ends

Authors : Audrey Guesdon[*], Franck Bazile[*], Rubén M. Buey Renu Mohan, Solange Monier, Morgane Angevin, Claire Heichette, Ralph Wieneke, Robert Tampé, Laurence Duchesne, Anna Akhmanova, Michel O. Steinmetz, Denis Chrétien

These authors contributed equally to this work[*]

Address : Institute of Genetics and Development of Rennes, UMR6290 CNRS, University of Rennes 1, Campus universitaire de Beaulieu, 35042 Rennes Cédex, France

Abstract : The structure of microtubule ends and the nucleotide state of tubulin are fundamental to microtubule dynamics. The end-binding protein EB1 tracks growing microtubule ends by recognizing GTP-tubulin dimers forming the terminal GTP-cap of microtubules. Here, we used EB1 conjugated to gold nanoparticles in combination with cryo-electron tomography to investigate the structure of the EB1-binding region of growing microtubules. We found that the EB1-binding sites are located on the outer surface of both open-curved and straight regions of tubulin sheets, and extend into closed regions of the microtubule lattice. Microtubules assembled in the presence of either GTP, GTP analogues or in cell extracts display similar, outwardly curved tubulin sheets, suggesting that microtubule extremities are curved independently of the nucleotide state of tubulin. Together, our results visualize the architecture of the GTP-cap at unprecedented resolution, and suggest a model that relates tubulin conformational changes to GTP-hydrolysis during microtubule assembly.

Title: Direct evidence for the interaction of stathmin with microtubules in cells: an investigation made by real-time confocal imaging coupled to FRET and FRAP spectroscopies.

Authors: Gilles Breuzard, Roqiya Nouar, Sonia Bastonero, Svetlana Gorokhova, Pascale Barbier, François Devred, Hervé Kovacic and Vincent Peyrot.

Address: Aix-Marseille Université, Inserm, CRO2 UMR_S 911, Faculté de Pharmacie, 13385, Marseille, France

Abstract:

Background. Stathmin is a prominent destabilizer of microtubule (MT), acting either by sequestering tubulin or by binding to the MT plus-ends, as shown by multiple *in vitro* studies. However, the question about the molecular mechanisms of stathmin binding to tubulin/MTs remains unexplored in cell. In the current study, we developed quantitative FRET (Förster Resonance Energy Transfer) and FRAP (Fluorescence Recovery After Photobleaching) imaging to investigate stathmin-tubulin interactions in the cell and to explore the impact of stathmin phosphorylation on this interaction.

Observations. FRET imaging shows a discrete distribution of the interaction of stathmin with tubulin in the cytosol, consistently with its sequestering activity. Remarkably, we have also observed a significant fraction of stathmin bound at the plus-end and along the length of MTs. This pool of stathmin corresponds to the binding of stathmin phosphorylated on serine 38 and/or serine 25. In order to further characterize this novel interaction, we expressed truncated stathmin in cells and found that the C-terminal domain of stahmin was the major contributor to this binding. By FRAP, we determined that the binding exchange of stathmin with soluble tubulin dimers is significantly modified by the presence of MTs providing compelling evidence that stathmin can bind to MTs in cells.

Conclusions. We were able to localize and quantify the stathmin-tubulin interactions in the complex environment of cell cytoplasms. We propose a new model of interaction between stathmin and tubulin/MT in cell, where (i) un-phosphorylated stathmin sequesters tubulin in the cytosol, (ii) fully phosphorylated stathmin detaches from tubulin/MTs, and (iii) partially phosphorylated stathmin (on serine 38- and/or serine 25) binds to the MT wall. In conclusion, stathmin bound along the MT would be readily available to participate in the protofilament dissociation when the moving plus-end of a depolymerising MT reaches that stathmin molecules.

Title : EB1 links microtubule network organization and touch response in *Arabidopsis thaliana*.

Authors : Arthur T. Molines[1], Jessica Marion[1], Salem Chabout[2], Laetitia Besse[3], Valérie Nicolas[4], Jim P. Dompierre[3], Grégory Mouille[2], Béatrice Satiat-Jeunemaître[1,3], and Frédéric M. Coquelle[1]

Address : [1]*Group "Dynamics of Cell Compartmentation in plant cells" , Department of Cell Biology, Institute for Integrative Biology of the Cell (I2BC), UMR 9198 CNRS – CEA - Université Paris-Sud, Saclay Plant Sciences, 91198, Gif-sur-Yvette Cedex, France.*
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[4]*Microscopy Facility, Institut Paris-Sud d'Innovation Thérapeutique (IPSIT), Université Paris-Sud, Chatenay-Malabry, France*

Abstract :
In plants cells, the fine-tuning of the microtubule (MT) network organization is crucial for multiple processes such as division and growth. However, the precise molecular mechanisms sustaining the overall MT array deployment are not well understood. We question here the involvement of AtEB1a and AtEB1b, two of the three A. thaliana orthologs of the +TIPs (plus-End-Tracking Proteins) EB1 (End-Binding 1), in the cortical MT network architecture. Unlike animals, precise functions and regulations of EB1 plant counterparts are still to be elucidated. We tackled the study of EB1 functions in elongating epidermal cells using chimeric lines of plants expressing GFP-fused tubulin in wild type or in double mutant background. Using confocal and TIRF microscopy, combined with anisotropy calculation of fibers distribution, we observed a significant disorganization of the MT network in the double mutant. Super-resolution microcopy, combined with an original image analysis process, revealed a marked decrease of MT bundling in EB1-defective seedlings. Moreover, double mutated plants display significant defects in root thigmotropism, underlying the functional relationships between MTs and development. Altogether, our data suggest that EB1a and b contribute both to the bundling and to the 3D organization of plant MTs, the two events being possibly linked. We are currently investigating both cell wall architecture and cell mechanical properties in order to correlate the plant growth defect to the sub-cellular phenotype.

Title : **Unravelling the mechanoperception and transduction pathway controlling the microtubule response to mechanical stress.**

Authors : Stéphane VERGER, Olivier Hamant

Address : RDP/LJC - ENS Lyon 46, Allee d'Italie 69364 Lyon cedex 07 France

Abstract : Because they guide the ordered deposition of cellulose microfibrils in the cell wall, cortical microtubules (CMTs) play a central role in regulating plant morphogenesis. Many factors such as hormones, biotic and abiotic cues have been shown to influence the dynamics and orientation of CMTs in plants. Among these signals, mechanical stress has emerged as one of the major signals controlling microtubule dynamics and orientation: microtubule align along maximal tension so as to mechanically reinforce the cell wall in that direction (Hamant et al., 2008, Uyttewaal et al., 2012, Sampathkumar et al., 2014). Because mechanical stress also depends on tissue shape, this provides a feedback loop in which shape and stress control each other via the CMTs. Yet, the identity of the molecular players implicated in sensing and transducing these mechanical signals to the CMTs remains unknown. In order to identify such factors, we have set up a genetic screen that is based on the response of plant growth to mechanical perturbations, on a secondary visual screen based on the CMT response to ablations and on a suppressor screen of katanin mutants (which display a delayed response to mechanical stress). In parallel, a candidate gene approach is conducted on known regulators of microtubule dynamics. Altogether this approach should allow us to identify early molecular players of this mechanotransduction pathway as well as important regulators of the microtubules behavior in response to mechanical stress. I will present the overall approach and my first results.

Title : Investigating the position of GCPs 4, 5, 6 in the Gamma-tubulin ring complex

Authors : Dorian Farache, Alain Jauneau, Marie-Hélène Remy, Laurent Emorine, Laurence Haren, Andreas Merdes

Address : Centre de Biologie du Développement, Université Toulouse III, 118 route de Narbonne, 31062 Toulouse

Abstract :

Gamma-tubulin ring complexes (gamma-TuRCs) are large multiprotein complexes that act as templates for the nucleation of microtubules. Their main component is gamma-tubulin, laterally associated into a helical structure. Gamma-tubulin molecules are themselves anchored to a helical core of GCP proteins. These GCPs belong to a family of proteins with similar core structure. Among these, GCPs 2 and 3 are the most abundant ones. Additional proteins are present at a low stoichiometric ratio, including GCPs 4, 5, 6, and several smaller subunits. GCPs 4, 5, and 6 are necessary in humans to stabilize the gamma-TuRC. We performed a series of biophysical and biochemical experiments to determine the nearest neighbours of GCPs 4, 5, 6 within the complex, and to conclude on their role in gamma-TuRC assembly. Further, we constructed chimaeric proteins to learn which domains define the function of specific GCPs.

Title: Microtubule and actin organization by the neuronal MAP tau

Authors : A Elie, E Prezel, C Guérin, E Denarier, S Ramirez-Rios, L Serre, A Andrieux, A Fourest-Lieuvin, L Blanchoin and I Arnal

Address: Grenoble Institut des Neurosciences, Grenoble

Abstract :

The crosstalk between microtubules and actin is essential for many cellular processes. However, the molecular mechanisms underlying microtubule/actin organization by cross-linkers remain poorly understood. To challenge this question, we focus on tau, a neuronal microtubule-associated protein (MAP) that promotes microtubule (MT) stabilization in axons and has also been proposed to interact with actin. Using reconstituted cell-free system and TIRF microscopy, we developed an original assay to visualize the concomitant assembly of microtubules and actin. We show that tau co-organizes the two networks by inducing the guided polymerization of actin filaments along microtubules and the growth of microtubules along actin filament bundles. Our study further reveals that tau repeated motifs, initially characterized as tubulin-binding sites, are required to bridge microtubules and actin. We also confirm a direct interaction between endogenous actin and tau by FRET microscopy in primary neuronal cultures, and show that tau can co-localize with microtubules and actin in growth cones of developing neurons. Overall we identify tau as a molecular linker between microtubule and actin networks, leading to a co-alignment of the two cytoskeletons, which might be essential for various neuronal functions.

Title : Role of tubulin polyglutamylation in neurodegeneration

Authors : Maria M. Magiera, Patricia Marques de Souza, Tiziana Giordano, Julie Nguyen, Méghane Sittewelle and Carsten Janke

Address : Institut Curie, CNRS-UMR3348, Orsay

Abstract :

Microtubules (MTs) are components of the cytoskeleton, which, by associating with various microtubule associated proteins (MAPs) or molecular motors, are involved in neuronal architecture, transport, survival and regeneration. The various roles of MTs are controlled by posttranslational modifications of tubulin, the MT building block, such as acetylation, detyrosination, delta-2 tubulin and polyglutamylation. We focus on polyglutamylation, a modification that adds secondary glutamate chains on tubulin molecules. Based on the finding that neurodegeneration in a Purkinje cell degeneration (pcd) mouse model is due to the excess of tubulin polyglutamylation in the cerebellum, we now want to understand the universal role of tubulin polyglutamylation in neurodegeneration. To this aim we have created animals with hyperglutamylation of tubulin in all brain regions. These mice show a massive and more global level of neuronal defects. If, in contrast, hyperglutamylation is constrained to specific neuronal, degeneration is restricted to those neurons. We are now exploring the mechanisms behind observed neurodegeneration, which we believe can be linked to the defects in axonal transport, a mechanism known to be deregulated in many human neurodegenerative diseases.

Microtubule-bound septins together with tyrosinated and polyglutamylated tubulin confer cell adaptation to Taxol® and impact on cell motility

Benjamin Targa[1], Laurence Froidevaux-Klipfel[2], Isabelle Cantaloube[1], Mahasen Saati[1], Christian Poüs[1,3] and Anita Baillet[1]

[1]*Laboratoire de Biochimie et Biologie Cellulaire, INSERM UMR-S 1193, Université Paris Saclay, Faculté de Pharmacie, Châtenay-Malabry, France*
[2]*Laboratoire d'Immunologie, INSERM UMR-S 996, Clamart, France*
[3]*Laboratoire de Biochimie-Hormonologie, Hôpital Antoine Béclère, AP-HP, Clamart, France*

Acquired resistance to the microtubule (MT)-stabilizing agent Taxol® is a major obstacle for successful chemotherapy and limits its use as an anticancer drug. We evidenced a new mechanism of Taxol® resistance acquired by MDA-MB 231 breast cancer cells which involves i) MT enrichment in retyrosinated and long-chain polyglutamylated tubulin and ii) overexpression and relocalization from the actin cytoskeleton to the MT network of several septins, a family of filamentous GTPases implicated in cytokinesis, membrane compartmentalization and cell migration. Altogether, these modulations enhance CLIP-170 and MCAK recruitment to MTs, which would in turn compensate Taxol®-mediated inhibition of MT dynamics.

Here, we show that the overexpression of TTL, TTLL5 and 11 together with that of a panel of septins that comprised the SEPT9_i1 isoform were necessary and sufficient to make septin filaments localize to MTs, where they facilitated the recruitment of tubulin polyglutamylation enzymes. These changes, which also enhanced CLIP-170 and MCAK recruitment to MTs, allowed a variety of sensitive cells (not only MDA-MB 231 but also Hela, CHO or RPE-1) to partially resist Taxol®.

In addition, wound healing experiments revealed that migration is partially restored in the resistant MDA-MB 231 cells compared to their Taxol®-treated sensitive counterpart. Nevertheless, the directionally persistent migration and the subcellular localization of paxillin in focal adhesions is still perturbed. Also, while the overall level of tubulin acetylation does not differ between Taxol®-sensitive and -resistant MDA-MB 231 cells, resistant cells that migrate in response to wounding failed to polarize tubulin acetylation on the MTs oriented toward their leading edge.

Altogether, our data shed light on the importance of the interplay between septin filaments and the MT cytoskeleton in Taxol® resistance. They also provide new insights into how post-translational tubulin modifications and septins may be involved in metastasis, which may represent potential new therapeutic targets to address in the future.

Title : The role of primary cilia in colon homeostasis and tumor development

Authors : Ruizhi Tang[1,2], Laura Papon[1,2], Cecilia Rocha[1,2,3,4,5,6], Carsten Janke[3,4,5,6], Michael Hahne[1,2,7]

Address : [1]IGMM CNRS UMR5535, Montpellier, France, [2]Université Montpellier Sud de France, Montpellier, France, [3]Institut Curie, Orsay, France, [4]PSL Research University, Paris, France, [5]CNRS UMR3306, Orsay, France, [6]INSERM U1005, Orsay, Franc, [7]Academic Medical Center, Amsterdam, The Netherlands

Abstract : The primary cilium is a sensory organelle expressed on nearly all eukaryotic cells. Primary cilia are assembled on the basis of a microtubule component, the axoneme. The functions of primary cilia are modulation of signaling pathways including WNT and Hedgehog (HH), which are essential in the regulation of intestinal homeostasis. We have recently described that glycylation, a posttranslational modification of microtubules, is crucial in the maintenance of primary cilia. We observed a decreased number of primary cilia and increased cell proliferation in the colon in mice deficient for the glycylase TTLL3, which is the only glycyclase expressed in the colon. Despite this overproliferation, TTLL3-deficient mice display a normal tissue architecture of the colon. However, loss of glycylase activity promotes induced colon carcinogenesis. In my PhD project we will focus on the consequences of a complete loss of primary cilia in intestinal epithelial cells and intestinal myofibroblasts respectively. For this, we will use villin-cre-mediated recombination to generate mice in which components of the ciliary transport machinery (Kif3A or Ift88) are knocked out specifically in the intestine. Mice will be analyzed for altered colon homeostasis and altered susceptibility for induced colon carcinogenesis. In addition, we will investigate altered WNT and HH signaling by the analysis of respective target genes in colon and the use of WNT and HH reporter constructs in isolated colonic epithelial cells or intestinal organoid cell cultures. My work should provide direct proof that the primary cilia regulate proliferation of colon epithelium.

End-binding 1 protein overexpression correlates with glioblastoma progression and sensitizes to *Vinca*-alkaloids *in vitro* and *in vivo*

Raphael Berges[(1)], Nathalie Baeza-Kallee[(1)], Emeline Tabouret[(1,2)], Olivier Chinot[(1,2)], Marie Petit[(1,2)], Anna Kruczynski[(3)], Dominique Figarella-Branger[(1,2)], Stephane Honore[(1,2)] and Diane Braguer[(1,2)].

(1) : Aix-Marseille Université, INSERM, CRO2 UMR_S 911, Marseille, France.
(2) : APHM, CHU Timone, Marseille, France.
(3) : Centre de Recherche d'Oncologie Expérimentale, Institut de Recherche Pierre Fabre, Toulouse, France.

End-binding 1 protein (EB1) is a key player in the regulation of microtubule (MT) dynamics. In the present study we investigated the role of EB1 in glioblastoma (GBM) tumor progression and its potential predictive role for response to *Vinca*-alkaloid chemotherapy. Immunohistological analysis of the 109 human GBM cases revealed that EB1 overexpression correlated with poor outcome including progression-free survival and overall survival. In order to decipher the implication of EB1 in GBM disease, its expression was knocked down or up-regulated in several human U87 GBM clones. Downregulation of EB1 by shRNA inhibited cell migration and proliferation *in vitro*. Conversely, EB1 overexpression promoted them and accelerated tumor growth in orthotopically-transplanted nude mice. Furthermore, EB1 expression was evaluated in stem-like GBM6 and GBM9 cells isolated from two GBM patients. GBM6, that display *in vivo* a higher tumorigenicity with a more infiltrative pattern of migration than GBM9, largely overexpressed EB1 as compared to GBM9. GBM6 showed strong and EB1-dependent migratory potential. The predictive role of EB1 in the response of GBM cells to *Vinca*-alkaloid treatment was investigated. Vinflunine (VFL) and vincristine (VCR) increased survival of EB1-overexpressing U87 bearing mice as compared to control. Besides, VFL and VCR were more effective to inhibit cell migration and proliferation in EB1-overexpressing clones than in controls. MT dynamics was increased in EB1 overexpressing U87 cells. The cellular sensitization to *Vinca*-alkaloids was linked to a stronger alteration of MT dynamic instability in these cells, thus inhibiting the increase induced by EB1 overexpression. Altogether, our results show that EB1 expression level has a prognostic value in GBM, and that *Vinca*-alkaloid chemotherapy could improve the treatment of GBM patients with EB1-overexpressing tumor.

Title : Microtubule-associated tumor suppressor ATIP3 interacts with EB1 : clinical relevance in breast cancer

Authors : Anne Nehlig, Angie Molina, Lauriane Velot, Sylvie Rodrigues-Ferreira, Benjamin Bouchet, Diane Braguer, Stephane Honoré, Clara Nahmias

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Abstract :

Microtubule-associated protein ATIP3, the major product of tumor suppressor MTUS1 gene, is markedly down-regulated in aggressive breast tumors of the triple-negative subtype and is a prognostic marker of poor survival in breast cancer patients. Previous studies from our lab have shown that ATIP3 is a potent microtubule stabilizer and that its re-expression in breast cancer cell lines reduces cell proliferation, migration and polarity, as a consequence of decreased microtubule dynamic instability.

Our recent studies indicate that ATIP3 directly interacts with end-binding protein EB1, a master regulator of MT dynamics. Surprisingly, ATIP3 does not accumulate with EB1 at MT growing ends but displays back-tracking properties. In situ ATIP3-EB1 molecular complexes are detected in the cytosol and along the MT lattice by Proximity Ligation Assay. The minimal EB1-interacting domain is both necessary and sufficient to reduce EB1 accumulation at growing MT ends, highlighting the importance of ATIP3-EB1 interaction on ATIP3 function. FRAP analyses reveal that ATIP3 silencing accelerates EB1 turnover at growing MT ends, supporting a novel mechanism for negative regulation of EB1 association with its recognition site at MT plus ends. Clinical relevance of ATIP3-EB1 interaction in breast cancer will be presented.

Title : IPP51, a chalcone acting as a microtubule inhibitor with *in vivo* antitumor activity against bladder carcinoma.

Authors : V. Martel-Frachet[1], M. Keramidas[1], A. Nurisso[2], S. DeBonis[3], C. Rome[4], A. Boumendjel[5], X. Ronot[1], D.A. Skoufias[3] and JL Coll[1]

Address : [1]Unité INSERM/UJF U823, Equipe 5, Institut Albert Bonniot, Grenoble.
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Abstract :
Flavonoids are naturally plant polyphenols which are classified into different subclasses, including chalcones, which are the flavonoids precursors. Various molecules derived from flavonoids have been studied *in vitro* and *in vivo* for their potential anticancer activity. We have identified 1-(2,4-dimethoxyphenyl)-3-(1-methylindolyl) propenone (IPP51), a new methoxylated chalcone derivative, which selectively inhibits proliferation of tumor-derived cells versus normal non-tumor cells. IPP51 treatment induces a cell cycle arrest at the prometaphase stage, which leads to apoptosis. IPP51 inhibits tubulin polymerization in an *in vitro* assay with purified tubulin. Molecular docking showed that the indol group of IPP51 can be accommodated in the colchicine binding site of tubulin, which was confirmed by an *in vitro* competition assay, demonstrating that IPP51 can compete for colchicine binding to soluble tubulin. In cells, IPP51 induces an increase in soluble tubulin, interferes with spindle formation, mitotic chromosome alignment and induces activation of spindle-assembly checkpoint. Furthermore, IPP51 inhibits *in vitro* capillary-like tube formation by endothelial cells, indicating that it has anti-angiogenic activity. Finally, in a human bladder xenograft mouse model, IPP51 inhibited tumor growth without signs of toxicity. Altogether, these findings suggest that IPP51 is an attractive new microtubule-targeting agent with potential chemotherapeutic value.
Martel-Frachet et al., In press, Oncotarget

Title : A new LIMK inhibitor stabilizes microtubules and has anticancer activity

Authors : *[1]Chloé Prunier, [2]Véronique Josserand, [3]Evelyne Beerling, [3]Anoek Zoemer, [1]Renaud Prudent, [2]Amandine Hurbin, [4]Leanne de Koning, [5]Pascale Cohen, [2]Jean-Luc Coll, [3]Jacco van Rheenen, [1]Marc Billaud, and [1]Laurence Lafanechère*

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Abstract :

LIM Kinases (LIMKs) function as central network hubs that coordinate actin dynamics through cofilin phosphorylation and microtubule dynamics, through a still unknown mechanism. When LIMKs are inhibited, microtubules are stabilized and microfilaments are severed and disorganized. LIMKs are also enzymes whose activity is more important in cancers than in normal tissue. Consequently, their inhibition selectively targets tumors and could offer a large therapeutic window. LIMKs are thus considered as emerging targets for cancer therapy and an increasing number of inhibitors are reported in the literature. Moreover, due to their indirect stabilizing effect on microtubules, LIMKs inhibitors could represent an attractive alternative strategy in Taxol ® resistant cancers.

Our team has identified a highly selective cell permeable LIMKs inhibitor, called "Pyr1" (Prudent et *al.*, 2012). We have used this inhibitor 1) to investigate the effect of LIMKs inhibition on breast cancer development and 2) to test the hypothesis that LIMKs inhibition could show some efficacy in paclitaxel resistant cancers. We have shown that Pyr1 is toxic on breast cancer cell lines resistant to paclitaxel. On different models of mice tumor xenografts, Pyr1 shows potent antitumor activity on primary and secondary tumors, with no detectable undesirable side effects. The antitumor effect is often more potent than that of paclitaxel. Moreover Pyr1 is active on paclitaxel resistant xenografts. Finally, we have evaluated the antimetastatic activity of Pyr1 *in vitro* and using intravital microscopy through a mammary window (coll. with J. van Rheenen, Hubrecht Institute). *In vitro*, Pyr1 shows a strong effect on cell invasion, resulting mainly from its effect on motility. Inside the tumors, Pyr1 induces a striking change of cell morphology. Many of the cells show a rounded phenotype, while the others remain elongated. Careful analysis of the motility indicated that Pyr1 reduces the motility of elongated cells while it induces an amoeboid-like motility in rounded cells adopted. In this model, Pyr1 did not prevent the metastasis spreading, but metastasis were found much smaller. These results indicate that LIMKs inhibitors such as Pyr1 could represent a pharmacological alternative to overcome the resistances often observed when tumors are treated with taxanes. Moreover, they could be potent agents to reduce the size of metastasis.

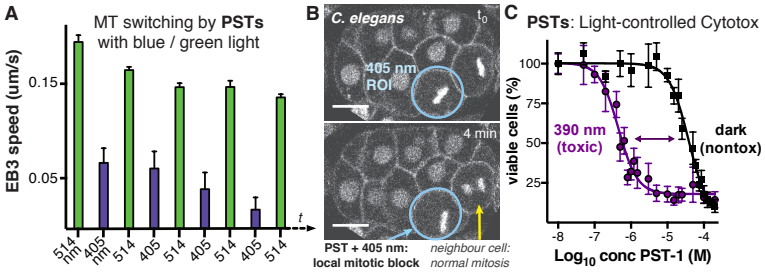
Photoswitchable microtubule inhibitors for localised optical control of MT dynamics, mitosis, and cell death

Authors : M. Borowiak[1], W. Nahaboo[2], M. Reynders[1], M. DeLattre[2], D. Trauner*[1], O. Thorn-Seshold*[1]

Address : [1]Ludwig-Maximilians-Universität, Munich, Germany; [2]École Normale Supérieure de Lyon, France

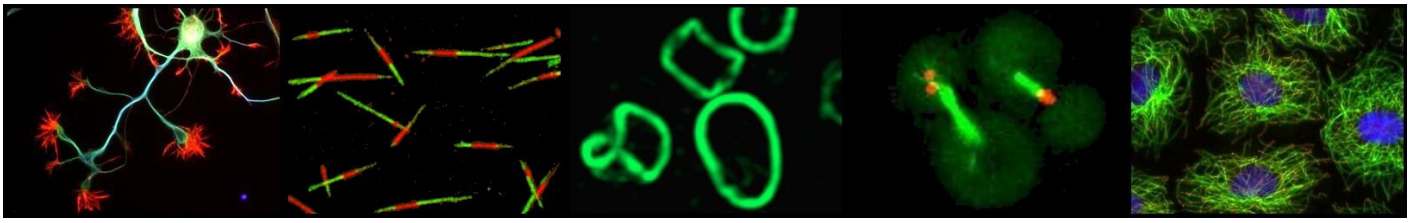
Abstract : Current inhibitors of microtubule dynamics (nocodazole, colchicine, Taxol, *Vinca*) cannot be targeted to specific cells or tissues, or timed to act in response to specific cell events. Thus they cannot spatiotemporally address MT-dependent processes in research, and they cause dose-limiting (spatially off-target) side-effects in cancer chemotherapy.[1]

Photostatins (**PSTs**) are the first spatiotemporally specific MT inhibitors. **PSTs** are colchicine derivatives that can be switched on and off by the researcher, with full reversibility, by applying blue or green light. **PSTs** turn MT dynamics on and off *in cellulo* with a temporal response under one second (A). **PSTs** pause and restart mitosis in single selected cells within living embryos, using standard microscopy for local illumination (B). **PSTs** can tissue-specifically destroy MTs in live mice, and are >250 times more toxic under blue light than in the dark (C), which is highly promising for tumour-targeted chemotherapy.[2]



Outlook: We are developing **PSTs** for subcellular MT inhibition and 2-photon control. We want to examine the antitumour/VDA potential of **PSTs** *in vivo* through collaborative experiments. We are interested to design new **PSTs** for biology experimenters with specific needs (model systems, set wavelengths, dual targeting) and we seek partners for such collaborative research.

[1] Mitchison, *Chem. Biol.* **2002**, 1275: Cytoskeleton Inhibitors.
[2] Borowiak, Nahaboo, DeLattre, *et al.*, Trauner & Thorn-Seshold, *Cell* **2015** (accepted): Photoswitchable microtubule inhibitors.



MARDI 07 JUILLET 2015 - AMPHITHEATRE BOUCHERLE – UNIVERSITE GRENOBLE ALPES/CHU

9h00-10h30 SESSION 5 MICROTUBULES AND CELL DIVISION (SPINDLE ORGANIZATION)

- **Proper timing of spindle assembly is critical for correct chromosome alignment in mouse oocytes.** Isma BENNABI

- **The microtubule depolymerase Kif2 (kinesin 13) localizes to a cortical structure which positions the spindle asymmetrically during unequal cleavages.** Janet CHENEVERT

- **Yeast GSK-3 kinase regulates astral microtubule function via phosphorylation of the microtubule-stabilizing kinesin Kip2.** Dimitris LIAKOPOULOS

- **Ensconsin/MAP7 is required for various aspects of cell division.** Régis GIET

- **Huntingtin is required for mitotic spindle orientation.** Sandrine HUMBERT

- **Different ways to build a mitotic spindle in the mouse brain: from normal to pathological conditions.** Véronique MARTHIENS

COFFEE BREAK

11h00-12h15 SESSION 6 MICROTUBULES, CELL MORPHOGENESIS AND DIFFERENTIATION

- **Microtubule-Associated Protein 6 (MAP6) mediates neuronal connectivity through Semaphorin 3E-dependent signaling for axonal growth.** Jean-Christophe DELOULME

- **A role for GSK3β in controlling the Navigator-Trio-mediated crosstalk between microtubule dynamics and actin remodelling during neuronal morphogenesis.** Jabran BENHARI

- **The characterization of a G2/M TRM isoform sheds new light on the preprophase band function in plant cell division plane positioning.** Martine PASTUGLIA

- **Motor-driven marginal band coiling promotes cell shape change during platelet activation.** Karin SADOUL

- **Cell anisotropy and microtubules.** Vincent MIRABET

LUNCH

13h15 – 14h30 **POSTER SESSION**

14h30-15h45 **SESSION 6** **MICROTUBULES, ORGANELLE TRANSPORT AND CELL MIGRATION**

- **Regulation of cortical microtubule capture and cell migration by a microtubule minus end-associated protein complex.** Habib BOUGUENINA
- **Kinesin-14 transports large DNA molecules through the cytoplasm of eukaryotic cells** Giovanni CAPPELLO
- **Microtubule dependent nuclear domain organization during skeletal muscle fibers development**. Vincent GACHE
- **Huntington's disease: huntingtin and the control of intracellular dynamics**. Frédéric SAUDOU
- **The structure of apo-kinesin bound to tubulin links the nucleotide cycle to movement.** LuYan CAO

COFFEE BREAK

16h15-16h45 **CLOSING SESSION** **ORGANIZATION OF THE NEXT FRENCH MT NETWORK MEETING, 2017**

Title : Proper timing of spindle assembly is critical for correct chromosome alignment in mouse oocytes

Authors : Isma Bennabi, Isabelle Quéguiner, Marie-Hélène Verlhac and Marie-Emilie Terret

Address : CIRB, Collège de France, UMR7241/U1050, 11 place Marcelin Berthelot, 75005 Paris

Abstract :

Most cells possess centrosomes acting as major microtubule organizing centers (MTOCs). In mitosis, the two duplicated centrosomes rapidly promote bipolar spindle formation, organizing the poles of the spindle. Oocytes are an exception to this rule, lacking centrioles and thus canonical centrosomes, imposing alternative modes of spindle formation. In mouse oocytes, microtubules are nucleated from multiple acentriolar MTOCs (aMTOCs) that have to be sorted and clustered to form a bipolar spindle. Interestingly, whereas the entire process of mitosis lasts approximately one hour, spindle formation in mouse oocytes is a very slow process (approximately eight hours). **Our goal is to elucidate the mechanisms regulating acentriolar spindle formation.** In particular, we want to understand why it is such a slow process, which is strongly error prone. We used the microtubule motor HSET (kinesin-14) as a tool to answer this question. We show that modifying HSET levels in mouse oocytes perturbs the timing of spindle assembly and alters spindle shape. Indeed, spindle bipolarisation is precocious in oocytes overexpressing HSET and in contrast delayed in oocytes treated with an HSET inhibitor. In particular, the timing of aMTOCs sorting and clustering is affected. Interestingly, this has major consequences on chromosome behavior since oocytes overexpressing HSET have a high rate of misaligned chromosomes. Altogether our results suggest that even though the process of meiosis I spindle assembly in mouse oocytes is slow, a precise timing of events, is essential to avoid further increase in chromosome segregation errors.

Title : The microtubule depolymerase Kif2 (kinesin 13) localizes to a cortical structure which positions the spindle asymmetrically during unequal cleavages

Authors : Janet Chenevert, Vlad Costache, Lydia Besnardeau, Gerard Pruliere, Alex McDougall

Address : Laboratoire de Biologie de Développement de Villefranche-sur-mer, CNRS/UPMC - UMR7009, Observatoire Océanologique 06230

Abstract :

Asymmetric positioning of the spindle can be caused by localized cortical complexes which capture or shorten microtubules. Embryos of the ascidian, a marine invertebrate chordate, possess a striking example of a cortical structure which can control spindle positioning: the Centrosome Attracting Body (CAB). The CAB contains determinant mRNAs and germ plasm, and it is partitioned into the small daughter cells (micromeres) during a series of asymmetric cell divisions. The CAB is necessary and sufficient to promote unequal cleavage, however how it attracts a centrosome is not understood. We are investigating the relationship between the CAB, microtubules, and spindle position. Detailed analysis using fixed samples and tracking of spindle poles in live cells shows that one centrosome migrates toward the cortex during mitosis and its aster shrinks as it approaches the CAB region. We find that that the microtubule depolymerase Kif2 (kinesin 13) is highly enriched in the cortical CAB. In embryos injected with a Kif2 dominant negative construct, the posterior centrosome fails to migrate toward the cortex and both asters appear the same size. Kif2 cycles on and off the CAB with each division. We also noted that spindles do not align precisely toward the CAB but rather towards the adjacent midline cortex. The dynein partners Pins (LGN) and NuMA (Mud) localize to this midline site during mitosis. The CAB is generally poor in microtubules, however paclitaxel treatment reveals a transient bundle of stable microtubules connecting the attracted centrosome to a point midline to the CAB. We propose a mechanism with long-range and short-range components to explain unequal cleavages in the ascidian embryo: the CAB and kinesin 13 cause aster asymmetry and orient the spindle posteriorly, then Pins-Numa-dynein pulls the centrosome toward the midline cortex.

Title : **Yeast GSK-3 kinase regulates astral microtubule function via phosphorylation of the microtubule-stabilizing kinesin Kip2**

Authors : Hauke Drechsler[1,2], Ann Na Tan[1] and Dimitris Liakopoulos[1,3]

Address : [1]Biochemistry Centre Heidelberg (BZH), INF 328, 69120 Heidelberg, Germany

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[3]Centre de Recherche de Biochimie Macromoléculaire (CRBM), CNRS UMR 5237, 1919, route de Mende, 34293 Montpellier Cedex 05, France

Abstract :

The *S. cerevisiae* kinesin Kip2 stabilises astral microtubules and facilitates spindle positioning through transport of microtubule-associated proteins, such as the yeast CLIP-170 homologue Bik1, dynein and the Adenomatous Polyposis Coli-related protein Kar9 to the plus ends of astral microtubules. Here, we show that Kip2 associates physically with its processivity factor Bim1, the yeast homologue of the EB1 plus end-tracking protein. This interaction requires an EB1-binding motif in the N-terminal extension of Kip2 and is negatively regulated by phosphorylation through Mck1, the yeast Glycogen Synthase Kinase 3. In addition, Mck1-dependent phosphorylation decreases the intrinsic microtubule affinity of Kip2. Reduction in Kip2 phosphorylation leads to stabilisation of astral microtubules and accumulation of Kip2, dynein and Kar9 at microtubule plus ends, while loss of Mck1 function leads to defects in spindle positioning. Furthermore, we provide evidence that a subpopulation of Mck1 at the bud-cortex phosphorylates Kip2. We propose that yeast GSK-3 spatially controls astral microtubule dynamics and the load of dynein and Kar9 on astral microtubule plus ends by regulating Kip2 interactions with Bim1 and microtubules.

Ensconsin/MAP7 is required for various aspects of cell division.

Régis GIET
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Abstract :
Ensconsin/MAP7 mutant neuroblasts (neural Stem cells) display shorter metaphase spindles, a defect caused by a reduced microtubule polymerization rate and enhanced by centrosome ablation. In agreement with a direct effect in regulating spindle length, Ensconsin over-expression triggered an increase in spindle length in S2 cells, whereas purified Ensconsin stimulated microtubule polymerization in vitro. Interestingly, ensc null mutant flies also display defective centrosome separation and positioning during interphase, a phenotype also detected in kinesin-1 mutants. Our results suggest that Ensconsin cooperates with its binding partner Kinesin-1 during interphase to trigger centrosome separation. However, Ensconsin promotes microtubule polymerization during mitosis to control spindle length, independently of Kinesin-1. Interestingly, spindle size and mitotic timing are normal in Khc-depleted and mutant Nbs, indicating a dependence of the short spindle phenotype on Ensconsin but not Kinesin-1. These data demonstrate that early centrosome separation after cytokinesis is a prerequisite for correct centrosome segregation, a process in which Ensconsin and Kinesin-1 appear to be key players. It also reveals that centrosome –associated proteins are not the only molecules required for the regulation of centrosome separation in stem cells.
In addition, Ensconsin also seems to play a key role during spermatocyte division. By contrast to *ensc* fly brain NBs, cytokinesis frequently fails during meiosis 1 and 2 suggesting that Ensconsin contribution to cell division varies between different cell types. In agreement with this hypothesis Ensconsin association with spindle MTs is down regulated during early (but not late) meiosis. We propose that this specific regulation is correlated with the presence of high tubulin levels during spermatid elongation.

Title : Huntingtin is required for mitotic spindle orientation

Authors : S. Humbert

Address : GIN – U836 Inserm – Université Joseph Fourier

Abstract :
The bulk of interest in the huntingtin protein has centered on the fact that, when mutated, huntingtin causes Huntington's disease (HD), a devastating neurodegenerative disorder. Given the adult onset and dysfunction and death of adult neurons characterizing HD, most studies have focused on the toxic effects elicited by mutant huntingtin in post-mitotic neurons. In contrast, the roles of the wild-type protein have been overlooked. I will describe how huntingtin regulates mitotic spindle orientation in different types of progenitors. I will also discuss how this function of huntingtin has potential implications for progenitor self-renewal and differentiation properties.

Title : Different ways to build a mitotic spindle in the mouse brain: from normal to pathological conditions

Authors : Véronique Marthiens, Diana Vargas-Hurtado, Benoit Coddens, Carole Pennetier, Renata Basto
Address : Institut Curie/CNRS UMR144, 12 rue Lhomond, 75005 Paris

Abstract :
Centrosomes function as the main microtubule-organizing centers in animal cells. During mitosis, they participate in the building of a bipolar spindle ensuring a proper transmission of the duplicated genome to each daughter cell. The presence of more than two centrosomes in a cell, or centrosome amplification, has been described in cells/tissues of patients affected by developmental disorders and cancer. Our lab is interested in analysing the consequences of having too many centrosomes on tissue development and homeostasis at the scale of an organism. To that aim, we have recently developed a mouse model where embryonic neural stem cells carry extra centrosomes. We observed a drastic reduction of brain size at birth, a phenotype reminiscent of a neurodevelopmental disorder called human primary microcephaly (MCPH). We demonstrated that the presence of extra centrosomes triggers multipolar divisions at early stages of development and produces daughter cells with high levels of aneuploidy (loss or gain of whole chromosomes). These cells are rapidly removed from the cycling population by p53-dependent apoptosis. At late stages of development, supernumerary centrosomes gather together at the two spindle poles enabling bipolar divisions that generate a viable progeny with low levels of aneuploidy. More, we now show that adult neural stem cells form a bipolar spindle in the presence of extra centrosomes in a way that prevents the generation of mitotic errors. To explain these differences, we have explored the level of expression and sub-cellular localization of candidate proteins described as centrosome clustering factors in genetic screens. A systematic comparison of these factors in the mouse brain at early and late stages of development has unravelled that neural stem cells use different strategies to build a mitotic spindle over time. We therefore provide *in vivo* evidences that the same cells display different capacities to cluster extra centrosomes and generate aneuploidy over time that may rely on differences between stages in the pathways triggering the formation of a bipolar spindle.

Title : Microtubule-Associated Protein 6 (MAP6) mediates neuronal connectivity through Semaphorin 3E-dependent signaling for axonal growth

Authors : JC Deloulme[1], S Gory-Fauré[1], A Andrieux's team[1], E Barbier's team[1], H Lahrech's team[2], P Robinson's team[3], F Mann's team[4]

Address : 1/Univ. Grenoble Alpes, Grenoble Institut Neurosciences, INSERM, U836, CEA, iRTSV 2/CEA, LETI, Clinatec 3/Univ Sydney, Australia 4/Aix-Marseille Univ, CNRS, IBDM UMR 7288

Abstract :

Structural microtubule associated proteins (MAPs) stabilize microtubules, a property that was thought to be essential for development, maintenance and function of neuronal circuits. However, deletion of the structural MAPs in mice does not lead to major neurodevelopment defects. We now demonstrate a role for MAP6 in brain wiring that is independent of microtubule binding. We find that MAP6 deletion disrupts brain connectivity and is associated with a lack of post-commissural fornix fibers. MAP6 contributes to fornix development by regulating axonal elongation induced by Semaphorin 3E. We show that MAP6 acts downstream of receptor activation through a mechanism that requires a proline-rich domain distinct from its MT stabilizing domains. We also show that MAP6 directly binds to SH3 domain proteins known to be involved in neurite extension and semaphorin function. Thus MAP6 is critical to interface guidance molecules with intracellular signaling effectors during the development of cerebral axon tracts.

Title : A role for GSK3β in controlling the Navigator-Trio-mediated crosstalk between microtubule dynamics and actin remodelling during neuronal morphogenesis

Authors : Jabran Benhari[1], Serge Urbach[2], Susanne Schmidt[1], Niels Galjart[3], Anne Debant[1], and Jérôme Boudeau[1]

Address :

1- Centre de Recherche en Biochimie Macromoléculaire, CNRS UMR 5237, University of Montpellier, 1919 route de Mende, 34293 Montpellier, France
2- Plate-forme de Protéomique Fonctionnelle, Institut de Génomique Fonctionnelle, CNRS UMR 5203/Inserm U661, Montpellier, France
3- Department of Cell Biology and Genetics, Erasmus MC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Abstract :

During neuronal morphogenesis, axon outgrowth and guidance rely on well-coordinated dynamics of the actin and microtubule (MT) networks. However, the molecular events behind this crosstalk remain poorly understood. We have recently demonstrated that the RhoGEF Trio, which plays key roles in axon outgrowth and guidance in the developing nervous system, operates at the interface between these two cytoskeletal systems. Trio is recruited at the growing ends of dynamic MTs by MT plus-end tracking proteins (+TIPs) Navigator 1 (NAV1) and EB1, and is targeted to the growth cone plasma membrane where it locally activates its target GTPases Rac1 and RhoG to promote actin remodelling and neurite extension (Van Haren *et al.*, Curr. Biol., 2014). These data have revealed a novel functional link between dynamic MTs, actin remodelling and neurite extension. However, the signalling pathways regulating the activities of the Trio-NAV1-EB1 complex as a cytoskeletal coordinator remain to be elucidated.

By using a proteomic approach, we have identified the serine-threonine kinase GSK3β as a Trio interactor. GSK3β is a key regulator of several aspects of neuronal morphogenesis, and is known to control MT dynamics and to regulate +TIP binding to EB1. Nevertheless, treatment of cells by the GSK3β inhibitors SB216763 or LiCl had no effect on Trio or NAV1 binding to EB1. We then found that GSK3β phosphorylates NAV1 *in vitro* and in cells, and that GSK3β inhibition in cells impairs the interaction between Trio and NAV1, which suggests that phosphorylation of NAV1 by GSK3β may modulate the assembly of Trio-NAV1 complexes at MT ends. Since the binding of Trio to NAV1 is required for Trio to induce Rac1 activation, we tested whether the abrogation of this interaction caused by GSK3β inactivation could alter the function of Trio. Indeed, we found that Trio-mediated Rac1 activation is impaired by treatment of cells with SB216763 and LiCl. All together, these data suggest that GSK3β plays a role in the morphogenic function of the Trio-NAV1 complex by controlling the association of these proteins at MT plus-ends.

In order to further characterise the role of NAV1 phosphorylation, we have mapped by mass spectrometry the residues phosphorylated by GSK3β on NAV1 in SB216763-treated *versus* control cells. Three phosphorylated residues were identified, two of them being located within the microtubule-binding domain (MTB) of NAV1, suggesting a role of these phosphorylation events in the MT tracking properties of NAV1. We are now making use of phospho-null mutants of NAV1 to test whether the direct phosphorylation of NAV1 by GSK3β is sufficient to inflect the Trio-NAV1 association at MT plus-ends and whether it alters their functions during neuronal development.

Finally, we are investigating the upstream signals that could control the effect of GSK3β in the formation of the Trio-NAV1 complex at MT pus-ends. Both Trio and NAV1 have been assigned a role in mediating the effects of netrin-1/DCC attractive signalling in neurons, and interestingly, it has been reported that netrin-1/DCC-mediated signalling regulates GSK3β activity in neurons. By using cultured mouse embryonic cortical neurons, we are therefore testing the attractive hypothesis whereby GSK3β could modulate the functions of the Trio-NAV1 complex in neuronal morphogenesis in response to netrin-1.

Title : The characterization of a G2/M TRM isoform sheds new light on the preprophase band function in plant cell division plane positioning.

Authors : Martine Pastuglia, Estelle Schaefer, Katia Belcram, Magalie Uyttewaal, David Legland, Magali Goussot, Yann Duroc and David Bouchez

Address : IJPB – INRA, Route de Saint Cyr, 78026 Versailles Cedex

Abstract :

In land plants, the division plane is set up pre-mitotically, during the G2 to M phase transition and is faithfully predicted by the position of the preprophase band (PPB), a transient, premitotic, cortical microtubule array. The molecular pathways leading to preprophase band formation are still largely unknown. Our team has identified a regulatory complex, the TTP complex (TON1/TRM/PP2A) involved in cortical microtubules organization during both interphase and PPB formation.

Recent characterization of G2/M specific TRMs contributing to a mitotic isoform of the TTP complex allowed us to produce triple mutant Arabidopsis plants specifically devoid of PPB, with no cumulative defects carried over from misfunction of the interphase cortical array of microtubules (ICMT) prior to G2/M. These mutant plants display surprisingly mild defects in term of morphology and fertility. Analysis of the geometry of cell division in the root meristem of such PPB-less plants revealed that the mean orientation of division planes was not altered in the mutant compared to the wild type.

We thus conclude that the PPB, contrarily to a widely accepted view, is not a primary determinant of division plane positioning, since plants specifically lacking PPBs are able to position their division planes almost properly.

Title : Motor-driven marginal band coiling promotes cell shape change during platelet activation

Authors : ***Boubou Diagouraga, Alexei Grichine, Arnold Fertin, Jin Wang, Saadi Khochbin and Karin Sadoul***

Address : Institut Albert Bonniot La Tronche, BP170 F-38042 Grenoble Cedex 9

Abstract :

Circulating quiescent platelets have a flat, discoid shape maintained by a peripheral ring of microtubules, called the marginal band. Platelets are activated after vessel injury and undergo a major shape change known as disc-to-sphere transition. It has been suggested that actomyosin contracts the marginal band to a smaller ring promoting the spherical shape. Instead microtubule motors of the dynein family slide microtubules apart leading to marginal band extension. The limited available space forces the marginal band to coil, which induces the spherical shape of the activating platelet. Actomyosin contraction will then compress the coiled marginal band and newly polymerising microtubules within the coiled ring will short-cut their original path and form the smaller microtubule ring observed in activated platelets.

Title : Cell anisotropy and microtubules

Authors : Vincent Mirabet, Arezki Boudaoud, Olivier Hamant, Henrik Jonsson, Elliot Meyerowitz

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Abstract :

Morphogenesis in plants is mainly due to anisotropic growth of tissues, indeed plant cells are tightly bound together and very rarely migrate. Plant cells regulate their growth through controlled expansion of their rigid wall. Inside this rigid wall, cellulose microfibrills' orientation is anisotropic, and their direction guides the wall expansion. Inside the cell, the microtubule network is a major actor of cellulose orientation. As these proteic fibrills undergo constant reshuffling, it is crucial to understand how their configuration is maintained and regulated to understand cell wall anisotropy. For some years now, it has been shown that stress and cell deformations are factors modulating microtubule orientations. Nevertheless, little is known about the influence of shape on the microtubule orientation. It this study, I use a 3D model of cells and simulate virtual microtubules following standart interactions rules as defined in the literature. I show that cell shape can be of strong influence on the spontaneous properties of the microtubule network. Knowing those spontaneous configurations is crucial to be able to separate observations between normal configurations and altered ones.

Title : Regulation of cortical microtubule capture and cell migration by a microtubule minus end-associated protein complex.

Authors : Habib Bouguenina, Danièle Salaun, Stéphane Audebert, Pascal Verdier-Pinard, Ali Badache

Address : Centre de recherche en Cancérologie de Marseille, Aix-Marseille Université ; Inserm ; Institut Paoli-Calmettes ; CNRS, Marseille, France.

Abstract :

Microtubules (MT) are polarized structures with a minus-end tethered at the MT organizing centers and a plus-end exploring the cytoplasm, until meeting stabilizing structures. MT plus- end capture at the cell cortex is involved in various cellular functions including polarized cell migration and mitotic spindle positioning. Cortical MT capture is governed by different mechanisms and work in the field, including ours, has allowed identifying MT plus-end interacting cortical structures; however, recent data also suggest plus/minus-end interplay which remains poorly understood. Using targeted proteomics and high resolution imaging we showed that EB1, a master plus-end tracking protein, interacted with a set of proteins localized at MT nucleating centers, which form a large complex including the scaffold AKAP9, protein kinase A, a non-muscular isoform of myomegalin and the centrosomal protein, CDK5RAP2. Unexpectedly, individual knockdown of these proteins affected cortical MT capture and breast cancer cell migration. Biochemical and functional characterization of the complex revealed an order of organization with a pivotal role for myomegalin. Prevention of myomegalin-EB1 interaction was sufficient to disturb microtubules cortical stabilization, suggesting that EB1's ability to associate with the minus-end complex is required for microtubule plus-end capture. Our current efforts are aimed at revealing the EB1-associated minus-end complex exact architecture and at defining the molecular mechanism whereby it controls MT plus-ends dynamics and impacts directed cell migration.

Title : Kinesin-14 transports large DNA molecules through the cytoplasm of eukaryotic cells

Authors : F Farina, C Delevoye, D Klopfenstein, C Schmidt, JL Viovy, M Quanz, M Dutreix, and G Cappello

Address : Laboratoire Interdisciplinaire de Physique, Centre National de la Recherche Scientifique, Université Joseph Fourier, Unité Mixte de Recherche 5588, 38402 St. Martin d'Hères, France

Abstract :

We investigate the transport of short exogenous DNA molecules in the cytoplasm of eukaryotic cells.
Various types of exogenous DNA molecules are commonly used in gene and molecular therapy. To be effective, the DNA molecules must reach the nuclear membrane and enter the nucleus.

Using an in-cell single-molecule approach, we determine that the kinesin-14 actively transports the DNA fragments toward the minus-end of the microtubules and promote the nuclear internalization. With an in-vitro reconstituted minimal system, we prove that the tail of kinesin-14 has a specific affinity for double stranded oligonucleotides with more than 32 base-pairs. Thus, this molecular motor is sufficient alone to bind and transport DNA molecules and to put them in contact with nucleoporins NUP50 and NUP153.

Title : Microtubule dependent nuclear domain organization during skeletal muscle fibers development

Authors : Gache Vincent[1]

Address : ENS-Gerland/LBMC/MNCA 46 allée d'italie 69007 Lyon

Abstract :

Myonuclei actively position themselves throughout muscular development. Growing evidences support a direct connection between regulation of nuclear positioning, myonuclear domains establishment, microtubule architecture maintenance and normal function of muscles.

Incorrect positioning of nuclei in the center of myofibers is a hallmark of a class of muscular diseases called centronuclear myopathies, which includes myotubular myopathy.

Here we report nuclei behavior analysis using temporal interference of microtubule related proteins and highlight crucial role of few molecular motors and MAPs on myonuclear domains establishment.

Title : Huntington's disease: huntingtin and the control of intracellular dynamics

Authors : Frédéric Saudou[1,2]

Address : [1]Grenoble Institute of Neurosciences, Univ. Grenoble Alpes, [2]INSERM U836, Grenoble, France

Abstract :

Huntington's disease is caused by the abnormal polyglutamine expansion in the N-ter part of huntingtin (HTT), a large protein of 350kDa. Over the past years, we proposed that HTT acts a scaffold for the molecular motors and through this function, regulates the efficiency and directionality of vesicular transport along microtubules in neurons. This function is conserved in Drosophila. In particular, HTT controls the microtubule-based fast axonal transport (FAT) of neurotrophic factors such as BDNF. PolyQ expansion in HTT alters this function, leading to a decrease in neurotrophic support and death of striatal neurons. Interestingly, the defect in transport might not be restricted to axons but could also involve defects in the retrograde transport of TrkB in striatal dendrites.

In addition to the role of HTT in scaffolding the molecular motors both in cortical and striatal neurons, we found that HTT scaffolds GAPDH on vesicles and that vesicular GAPDH is necessary to propel vesicles in GAPDH deficient neurons. Here we will extend these findings and discuss how HTT by specifically localizing the glycolytic machinery on vesicles may supply constant energy for the transport of vesicles over long distances in axons. We will also discuss how this machinery is altered in disease situation. Finally, we will extend the function of HTT as a scaffold for dynamin1 for regulating intracellular dynamics in health and disease.

Keywords: huntingtin, fast axonal transport, BDNF, microtubules, glycolysis, energy, dynamin, endoplasmic reticulum

The structure of apo-kinesin bound to tubulin links the nucleotide cycle to movement

LuYan Cao, Weiyi Wang, Qiyang Jiang, Chunguang Wang, Marcel Knossow, Benoît Gigant

I2BC, Centre de Recherche de Gif, Centre National de la Recherche Scientifique, Gif sur Yvette, France.

Kinesin-1 is a dimeric motor protein that moves processively towards the (+)-end of microtubules as it hydrolyses ATP. In the kinesin nucleotide cycle, the binding of the kinesin–ADP motor domain to microtubules accelerates ADP release by more than three orders of magnitude and results in nucleotide-free microtubule-bound kinesin. Subsequent ATP binding initiates a mechanical step. But the connection between the kinesin nucleotide cycle and movement has remained elusive, mostly because the structure of apo-kinesin was not known. Here, we determine the 2.2 Å resolution structure of nucleotide-free kinesin bound to tubulin and, from the comparison with the structures of detached kinesin–ADP and tubulin-bound kinesin–ATP, we identify three motor subdomains that are invariant in all nucleotide states, though each subdomain moves en bloc. When kinesin–ADP binds to tubulin, the changes of the relative orientations of the subdomains lead to remodeling of the nucleotide-binding site and ADP release. When ATP is bound, it bridges the three subdomains, changing their orientations to allow docking of the neck linker, a 15 amino acids peptide C-terminal to the motor domain core, onto the motor domain. This gives rise to movement. In combination with mutational studies, the structures show that neck linker docking also gates the ATPase activity because the neck linker first residue latches the motor domain in a hydrolysis-efficient conformation. By revealing how ATP controls the power stroke, our results define a structural framework for understanding the transformation of chemical energy into mechanical work by (+)-end directed kinesins.

Organisateurs

Commanditaires