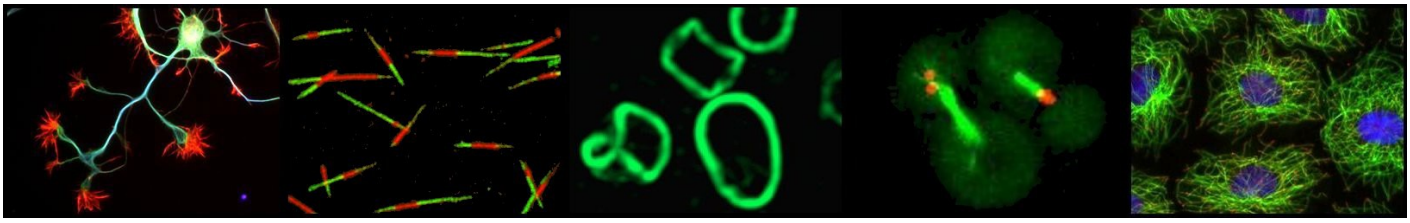

Les 2èmes Journées du Réseau France Microtubules
(French Microtubule Network)
6 et 7 juillet 2015, Université Grenoble Alpes

POSTER SESSIONS

AMPHITHEATRE BOUCHERLE – UNIVERSITE GRENOBLE ALPES/CHU

LUNDI 06 JUILLET 2015 -
13h45 – 15h30 **POSTER SESSION**

MARDI 07 JUILLET 2015 -
13h15 – 14h30 **POSTER SESSION**

Poster N° 1

Title : Evidence for new C-terminally truncated variants of α- and β-tubulins.

Authors : Aillaud Chrystelle[abc], Bosc Christophe[abc], Saoudi Yasmina[abc], Sago Laila[d], Taulet Nicolas[e], Cieren Adeline[abc], Peris Leticia[abc], Denarier Eric[abc], Tort Oliviaf[ghi], Janke Carsten[ghi], Redeker Virginie[dj], Andrieux Annie[abc], Moutin Marie-Jo[abc]*.

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Abstract :

Cellular α-tubulin can bear various carboxy-terminal sequences: full length tubulin arising from gene neosynthesis is tyrosinated and two truncated variants, corresponding to detyrosinated and Δ2 α-tubulin, result from the sequential cleavage of one or two C-terminal residues, respectively. Here, by using a novel antibody named 3EG, highly specific to the –EEEG C-terminal sequence, we demonstrate the occurrence in neuronal tissues of a new αΔ3-tubulin variant corresponding to α1A/B-tubulin deleted of its three last residues (EEY). αΔ3-tubulin presents a specific distribution pattern: its quantity in brain is similar to that of αΔ2-tubulin around birth, but much lower in adult tissue. This truncated α1A/B-tubulin variant can be generated from αΔ2-tubulin by the deglutamylases CCP1, CCP4, CCP5, CCP6, but not by CCP2 and CCP3. Moreover, using 3EG antibody, we identify a C-terminally truncated α-tubulin form with the same –EEEG C-terminal sequence. Using mass spectrometry, we demonstrate that β2A/B-tubulin is modified by truncation of the four C-terminal residues (EDEA). We show that this newly identified βΔ4-tubulin is ubiquitously present in cells and tissues and that its level is constant throughout the cell cycle. These new C-terminally truncated α- and β-tubulin variants, both ending with –EEEG sequence, are expected to regulate microtubule physiology.

Poster N° 2

Microtubules and progression of glioblastoma: role of the protein Tau.

Alessandra Pagano, Gilles Breuzard, Alexis Douence, Soazig Douillard, Sonia Bastonero, José Luis, Vincent Peyrot, Pascale Barbier, Hervé Kovacic.

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Microtubule (MT) associated protein Tau is a MT stabilizing protein known to be implicated in neurite outgrowth and cell polarity. Besides, its expression seems to be correlated with resistance to MT-targeting therapies and metastatic potential in many cancer such as breast cancer. We aimed to study the implication of Tau in glioblastoma cancer invasion. We stably down regulated Tau expression in the glioblastoma cell line U87 by a shRNA approach and we studied cell migration and invasion. Random motility on fibronectin matrix (cell speed and total distance) was reduced by 40% in cells down regulating tau (shTau cells) compared to vector control transfected cells (shctrl cells). Cell transmigration (Boyden chambers) was also significantly reduced by 80%. As a complementary approach, we studied cell evasion from spheroids on fibronectin matrix. Cell evasion was significantly reduced in shTau cells compared to shctrl cells at two days of evasion. More interestingly, we noticed that after one day shTau cells presented a complete disassembly of the spheroid compared to shctrl cells showing cell evasion from a still intact spheroid. A similar behaviour was confirmed in experiments of 3D invasion through collagen matrix, suggesting strongly an involvement of Tau in cell adhesion and cell-cell interaction. To explain the migratory defects of shTau cells we are also currently studying the MT dynamics and the cross-talk of actin cytoskeleton and MT network in shTau cells compared to shctrl cells. Our preliminary results indicate a role for Tau in glioblastoma cell invasion and deserve further investigation in vitro and in vivo.

Poster N° 3

Title : Modeling of dynamic instability of MTs with aging and impact of drugs

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Abstract :

Microtubules (MTs) are long tube polymers of tubulin, found throughout the cytoplasm. They are characterized by dynamic instabilities involved in a number of cellular processes, including cell division and migration. Microtubule-targeted drugs induce perturbation in their instabilities making them attractive for anti-cancer therapies.

Recent studies as [1] show that MTs age might play a crucial role in the effects of microtubule targeted drugs on MT instabilities. The aim of this work is to improve modeling of MT instability by introducing phenomenon of aging of MTs.

We propose a new deterministic mathematical model inspired by the work of P. Hinow et al. [2] to simulate the behavior of a MT population with presence of stabilizing and destabilizing drugs. The model couples transport equations with ordinary differential equations (ODE) with nonlocal terms endowed with suitable boundary conditions for both catastrophe and rescue. The mathematical model takes into account results of biological observations provided by the pharmacologist of our interdisciplinary research group [3].

New model allows us to demonstrate the pharmacological action of some anti-microtubule drugs on MT population through their influence on MT "aging" and, thus, on MT instabilities. Numerical results are in a good agreement with biological observations.

The program is funded thanks to the support of the A*MIDEX project (n° ANR-11-IDEX-0001-02) funded by the «Investissements d'Avenir» French Government program, managed by the French National Research Agency (ANR) and the support of Plan Cancer 2014, INSERM.

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2. P. Hinow, V. Rezania, J.A. Tuzszynski. *Continuous model for microtubule dynamics with catastrophe, rescue, and nucleation processes*. Phys Rev E Stat Nonlin Soft Matter Phys. 80 (3 Pt 1): 031904, 2009.

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Poster N° 4

α- and β-tubulin repertoire and post-translational modifications in platelets and during their biogenesis.

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Blood platelets, which are essential to arrest bleeding, are produced by giant cells in the bone marrow, the megakaryocytes (MKs). In the final stages of their biogenesis, microtubules (MTs) assemble into circular sub-membranous structures (the marginal band) thus determining their typical flat discoid shape. The biological importance of the marginal band is illustrated by the presence of abnormal spheroid platelets in patients with mutations in platelet tubulin. This outstanding feature suggests that platelet tubulin has specific biochemical and structural properties. Incorporation of specific tubulin isotypes and the generation of specific tubulin post-translational modifications (PTMs), as well as the interaction of platelet MTs with a distinct set of MAPs and motors are expected to coordinate the assembly of the marginal band during platelet formation.

Our aim was to perform an extensive biochemical characterization and quantitative analysis of the components of the MT cytoskeleton during platelet biogenesis to understand how they contribute to the assembly of the marginal band. Using quantitative RT-PCR and mass spectrometric analysis, we established the late appearance of the β1-, α4A-, and α8-tubulin isotypes during megakaryocyte maturation. Quantitative MS is underway to establish their relative content in MTs purified from circulating platelets. Functional importance of the β1- and α4A-isotypes was assessed in β1-tubulin knockout and α4A-tubulin mutant mice. These exhibited a 60% and 25% drop in platelet count, respectively; their platelets did not display the characteristic discoid shape, and the number of MT coils was dramatically decreased to 2-4 coils, vs. 10-12 coils in wild type mice. To analyse the involvement of tubulin PTMs in the assembly of the marginal band, we have studied expression of tubulin-modifying enzymes (from TTLL and CCP families) by quantitative RT-PCR during megakaryopoiesis. We found an upregulation of enzymes involved in polyglutamylation and polyglycylation at late stages of MK maturation. The level of these specific PTMs is currently evaluated in the tubulin pool from cultured megakaryocytes and native platelets. Altogether, the available results suggest that a timely combination of selected isotype assembly and PTMs during the final stages of platelet biogenesis contributes to the formation of the MT marginal band.

Poster N° 5

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[*]

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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.

Poster N° 6

Title : Normal morphogenesis in plants lacking PPB : new light on PPB function

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

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Abstract :

The TTP complex (TON1, TRM, PP2A) controls the organization of cortical microtubule arrays in plant cells, both during interphase and at the G2/M transition for preprophase band (PPB) setup. 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.

Mutant Arabidopsis plants specifically devoid of PPB display suprinsingly mild defects in term of morphology and fertility. Planes division in embryo, shoot apical meristem, asymetrics divisions (meristemoïds, root initials) and young sister cells volumes were analysed.

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. However, the PPB is necessary to reduce variations in division site positioning, and as such should be viewed more as part of a noise-reducing or reinforcement process rather than as a "decision-making" one.

Poster N° 7

Title : Dynamic microtubules catalyse formation of Navigator-Trio complexes to regulate neurite extension

Authors : Jeffrey van Haren[1*], Jérôme Boudeau[2*], Susanne Schmidt[2], Sreya Basu[1], Zhe Liu[1], Dave Lammers[1], Jeroen Demmers[3], Jabran Benhari[2], Franck Grosveld[1], Anne Debant[2], and Niels Galjart[1]

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Abstract :
Neurite extension is regulated by multiple signalling cascades that ultimately converge to the actin and microtubule (MT) networks. Rho GTPases play a pivotal role in controlling the actin cytoskeleton remodelling in the growth cone, while the dynamic behaviour of MTs is largely regulated by microtubule plus-end tracking proteins (+TIPs), which associate with the growing ends of MTs.
Here we show that the +TIP Navigator 1 (NAV1), which is expressed in the developing nervous system of mammals, is important for neurite outgrowth and interacts with Trio, a Rho-GEF that plays key roles in axon outgrowth and guidance in the developing nervous system by activating the Rho GTPases Rac1 and RhoG. We find that NAV1 and Trio co-localise at the plus-end of MTs and in *foci* that accumulate near the cytoplasmic membrane of growth cones. We then demonstrate that Trio is recruited to the MT plus-ends by NAV1, and also interacts with the 'core' +TIP EB1 *via* an SRIP motif, which makes Trio a novel SxIP motif-containing +TIP. We demonstrate that binding of Trio to NAV1 at MT plus-ends enhances the affinity of Trio for its target GTPases RhoG and Rac1, and is required for Trio to induce Rac1 activation and neurite outgrowth. Strikingly, we find that EB1-mediated recruitment of Trio to MT plus-ends is also required for Trio to induce proper neurite outgrowth. Finally, we show that stabilisation of the MT network by paclitaxel impairs both the NAV1-Trio interaction and the accumulation of these proteins in neurite extensions, which further indicates that dynamic MTs are required for the targeting of Trio and NAV1 to the cell periphery.
Altogether, our data indicate that EB1-labelled ends of dynamic MTs promote the formation and targeting to the growth cone plasma membrane of functional NAV1-Trio complexes, which in turn regulate neurite outgrowth by selectively activating Rac1 and RhoG. Collectively, our data reveal a novel functional link between dynamic MTs, actin remodelling and neurite extension.

Poster N° 8

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.

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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.

Poster N° 9

Title :

Aurora A mutations prevent rapid cyclin B degradation following Spindle Assembly Checkpoint satisfaction in *Drosophila* neuroblasts.

Authors :
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Abstract :
In Drosophila neuroblasts, the proper formation and alignment along the polarity axis of a microtubule-based structure, the mitotic spindle, is crucial for stem cell renewal, differentiation and accurate chromosome segregation to ensure tissue homeostasis. Drosophila mutants for sas-4 and aurA genes display brain tumors with extra neuroblasts, triggered by loss of cell identity due to spindle alignment defect. These mutants also show defective mitotic spindle assembly and consequent mitotic delay caused by the activation of the Spindle Assembly Checkpoint (SAC). In an attempt to determine if generating aneuploidy by compromising genetically spindle formation would compromise neuroblast proliferation, we inactivated the SAC by removing the SAC protein Mad2 from aurA and sas-4 mutants. Removal of the SAC in the sas-4 mutant impaired NB proliferation and chromosome segregation and is lethal. By contrast, in the aurA mutant removing the SAC did not prevent brain overgrowth, tumor formation, and chromosome segregation was normal. Monitoring Mad2 and cyclin B dynamics in live aurA neuroblasts revealed that chromosome alignment and SAC satisfaction were not coupled to cyclin B degradation. As a consequence, aurA mutants NBs are delayed in mitosis, allowing enough time to form a proper spindle and perform accurate chromosome segregation in a SAC-independent manner. We therefore show for the first time the existence of an Aurora A-dependent mechanism that promotes timed and efficient cyclin B degradation.

Poster N° 10

Eribulin suppresses ch-TOG microtubule plus-end localization and cancer cell chemotaxis

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Microtubule targeting agents (MTAs) are widely used as antineoplastic drugs, in particular for the treatment of breast cancer. MTAs target the mitotic spindle, block cell division and lead to apoptosis. Recent studies have challenged the fact that the antimitotic activity of MTAs is the only mechanism responsible for their antitumoral potency. This is supported in part by the fact that non-cytotoxic concentrations of MTAs disrupt dynamics of interphase microtubules and affect cell migration which could impair tumor angiogenesis and metastasis. The underlying molecular mechanisms however are still poorly defined. Eribulin mesylate is a new MTA recently approved for advanced breast cancer treatment, after failure of taxane-containing regimens, and have shown a significant survival improvement. We have investigated how non-cytotoxic concentrations of eribulin, that interacts with a new class of binding site on b-tubulin, affect microtubule dynamics and directed migration of breast cancer cells. We had previously established that directed cell migration is strongly linked to stabilization of microtubules at the cell leading edge, which is controlled by microtubule +end interacting proteins (+TIPs). We observed that four hours of exposure to subnanomolar concentrations of eribulin was sufficient to prevent cortical microtubule capture and inhibit breast cancer cell chemotaxis. These effects being reminiscent of those observed when +TIP expression was altered, we investigated the impact of eribulin on +TIPs. Eribulin induced a dose-dependent depletion of prototypical +TIPs, EB1 and CLIP-170 from microtubule +ends when treated with concentration above 0.1nM. Interestingly, at 0.1 nM, eribulin had no significant effect on EB1 and CLIP-170 comets but delocalized ch-TOG, a tubulin polymerase binding at the tip of microtubule +ends. This dose of eribulin was also sufficient to impact microtubule dynamics and directed migration. In addition, down regulation of ch-TOG led to a similar inhibition of microtubule growth speed, capture and of chemotaxis. Collectively, our data suggest that MTA binding to microtubule +end induced the delocalization of ch-TOG, an initiating step leading to alterations in microtubule dynamics and cancer cell migration.

Title :

How stathmin modulates anti-cancer drugs activity

Authors :

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Abstract :

Microtubules dynamic is tightly regulated by microtubule associated proteins (MAPs). Stabilizing MAPs such as Tau favors microtubules polymerization while destabilizing MAPs such as Stathmin induce depolymerization upon binding to tubulin. Because of its importance, microtubules dynamic is also the target of a whole class of anti-cancer drugs called microtubule-targeting agents (MTAs), which similarly to MAPs can be either stabilizers such as Taxanes or destabilizers such as *Vinca* alkaloids. Several cellular studies have shown that stathmin level of expression would impact anti-cancer drugs activity. Thus understanding the interplay between MTAs and MAPs becomes clinically relevant in tumors which overexpress stathmin. We investigated the molecular mechanism by which stathmin affects the function of two families of MTAs commonly used in the Clinics: *Vinca* alkaloids and Taxanes. Using Isothermal Titration Calorimetry, as well as other classical biochemical methods applied to tubulin we addressed this question *in vitro*. We showed that stathmin potentiates vinblastine or vinflunine binding and depolymerizing activity on microtubules. Interestingly we have also found that on the contrary paclitaxel loses its microtubule stabilizing activity in the presence of stathmin. Our results give molecular grounds to how stathmin can be a modulator of MTA activity and binding to tubulin. This double mechanism is of great biological significance in clinics to target tumors that overexpress active stathmin compared to the normal surrounding tissues, and could potentially be used in personal medicine in order to orient anticancer treatment.

Poster N° 12

Title : Molecular mechanisms and regulation of microtubule bundling by tau: differential roles of tau projection domain and repeat motifs.

Authors : Auréliane Elie, Eléa Prezel, Virginie Stoppin-Mellet, Julie Delaroche, Christophe Bosc, Ninon Zala, Laurence Serre, Anne Fourest-Lieuvin, Marylin Vantard and Isabelle Arnal

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Abstract : Microtubules (MTs) are key components of the eukaryotic cytoskeleton and are involved in major cellular events including cell division, motility and morphogenesis. MTs are organized into stable bundles within axons and dendrites to maintain the polarized shape of neurons and to insure cargo transport. Tau is one of the neuronal MAPs (Microtubule-Associated Proteins) that promote MT assembly and bundling. Although the MT-stabilizing properties of tau have been widely studied, the mechanisms by which this protein spatially organizes MTs remain elusive. By reconstituting *in vitro* MT bundling, we could identify molecular features involved in MT organization by tau. We used various tau isoforms and truncated fragments either in tau's N-terminal projection domain or C-terminal MT-binding repeats to decipher the role of each subdomain in MT bundling. We found that the projection domain of tau has an inhibitory effect on MT bundling whereas the two hexapeptides responsible for the formation of Alzheimer paired helical filaments (PHFs) are fundamental in this process. We also showed that tau phosphorylations on specific sites, with some of them being abnormally phosphorylated in Alzheimer's disease, differentially regulates MT bundling and stabilization. Overall, our study reveals that tau modulates MT bundling and dynamics *via* distinct domains, and that these two activities can be dissociated by tau phosphorylation.

Title: Functional implications of posttranslational glycylation in mammalian cilia

Authors: Sudarshan Gadadhar, Annemarie Wehenkel, Cecilia Rocha, Ngoc-Lan Julie Nguyen, Carsten Janke

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Abstract: Cilia, organelles that have a key role in mammalian cell functioning, are of two types: Motile cilia, involved in cell motility and other mechanical tasks, and primary cilia – for cell-cell communication and organ development. Both types of cilia are composed of a core structure, the axoneme, a highly structured microtubule assembly. Tubulin, the major building block of microtubules, and thus axonemes, is modified with a variety of posttranslational modifications, notably glutamylation and glycylation, in axonemes. These modifications are thought to fine-tune the biophysical properties, as well as the interactions of axonemes with microtubule-associated proteins. Glycylation, a modification confined to cilia, is catalyzed by enzymes TTLL3 and TTLL8, which belong to tubulin tyrosine ligase like (TTLL) family.

With the aim of understanding the mechanism of microtubule glycylation and its role in mammalian ciliogenesis and ciliary functioning, we focus on combining mouse physiology and histology with cell biology and biochemical approaches, like lentivirus-based knockdown approaches, *in vitro* glycylation assays, immunoprecipitation studies and immunofluorescence microscopy. For this, knockout mouse models are used to study the role of TTLL3 and TTLL8 in ciliogenesis and organ development. Previous work depicted a conserved role for glycylation in stabilizing axonemes. Preliminary findings further indicate that cells with differences in length of cilia differ in extent of glycylation, suggesting that the extent of tubulin glycylation is somehow related to ciliary length. We also observe that other proteins are glycylated by these enzymes in cilia, and their modification might play key roles in ciliary function. Mice lacking the glycylation enzymes have been observed to show organ-specific phenotypes and a higher propensity to develop tumors, highlighting the importance of glycylation in organ development. Thus, posttranslational glycylation appears to be essential for stability and functioning of both, primary and motile cilia, and besides tubulin, other glycylation substrates might be involved in this function.

Poster N° 14

Title : Biochemical and Structural Insights into Microtubule Perturbation by CopN from *Chlamydia pneumoniae*

Authors : Agata Nawrotek[1], Beatriz G. Guimarães[2], Christophe Velours[1], Agathe Subtil[3], Marcel Knossow[1] and Benoît Gigant[1]

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Abstract :

Although the actin network is commonly hijacked by pathogens, there are few reports of parasites targeting microtubules. The proposed member of the LcrE protein family from some *Chlamydia* species (e.g. pCopN from *C. pneumoniae*) binds tubulin and inhibits microtubule assembly in vitro. From the pCopN structure and its similarity with that of MxiC from *Shigella*, we definitively confirm CopN as the *Chlamydia* homolog of the LcrE family of bacterial proteins involved in the regulation of type III secretion. We have also investigated the molecular basis for the pCopN effect on microtubules. We show that pCopN delays microtubule nucleation and acts as a pure tubulin-sequestering protein at steady state. It targets the β subunit interface involved in the tubulin longitudinal self-association in a way that inhibits nucleotide exchange. pCopN contains three repetitions of a helical motif flanked by disordered N- and C-terminal extensions. We have identified the pCopN minimal tubulin-binding region within the second and third repeats. Together with the intriguing observation that *C. trachomatis* CopN does not bind tubulin, our data support the notion that, in addition to the shared function of type III secretion regulation, these proteins have evolved different functions in the host cytosol. Our results provide a mechanistic framework for understanding the *C. pneumoniae* CopN-specific inhibition of microtubule assembly.

Ref: Nawrotek et al. (2014) *J Biol Chem* 289, 25199–210.

Poster N° 15

Title : Regulation of microtubule dynamics by the stress kinase JNK in epithelial cells

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Abstract :
The laboratory has previously shown that the stress kinase JNK (c-Jun N-terminal Kinase) regulates microtubule dynamics, by increasing polymerization and depolymerization speeds as well as rescue frequencies in epithelial cells. While several neuronal MAPs are known JNK substrates, data are missing for epithelial MAPs. We are studying two new putative substrates of JNK: β-tubulin and the +TIP CLIP-170. We found respectively one and two JNK dependent phosphorylation -sites in β-tubulin and CLIP-170. We are currently characterizing these phosphorylations in *in vitro* kinase assays and *in cellulo*. The effects on microtubule dynamics, and physiological cellular functions will be addressed. Since JNK is upregulated in several pathologies, especially cancers, available phosphoproteoms could be screened for β-tubulin and CLIP-170 JNK-dependent deregulation.

Title : Modeling the dynamical Tau protein - Microtubule interaction

Authors : Jordan Hervy, Dominique J. Bicout

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Abstract :

Tauopathies such as Alzheimer's disease or Parkinson syndromes are examples of disorders associated with a dysfunction of tau proteins to properly regulate the network of microtubules (MTs) in axons. Understanding how and in what way Tau protein affect the microtubule dynamics represent therefore a major challenge in understanding Tauopathies. In this work, we were interested in the dynamical interaction between Tau protein and microtubule in the absence of dynamic. Based on the experimental observations we constructed a model describing the interaction Tau - MT as a dynamical process "on - off" in which the free Tau ("off") may be confined ("on") on the MT which it moves along a one-dimensional diffusion process and then followed by either an attachment on the MT or a detachment in the "off" state. This model is characterized by 5 parameters that can be extracted from experiments : k_on the confinement rate on the MT for a free Tau, D the diffusion coefficient of the one-dimensional diffusion for a Tau confined on the MT, k_b the attachment rate for a Tau confined along the MT, k_c the detachment rate of Tau binded on the MT, and k_f the escape rate of a Tau protein. We have derived the analytical expressions of the main features of the Tau interaction - MT, namely the stationary distribution and the means displacements of Tau along the MT, probability and dwell time of Tau on the microtubule.

Title : Biological effects of repeated electroconvulsive seizures in MAP6 null mice, an animal model of psychiatric disorders

Authors : Julie Jonckheere (1), Gaëlle Dall' Igna (1,2), Jean-Christophe Deloulme (1), Nicolas Chauliac (1,2), Christophe Porcher (4), Annie Andrieux (1), Jérôme Holtzmann (1,2), Marie-Françoise Suaud-Chagny (3), Sylvie Gory-Fauré (1)

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Abstract :

Drug-resistant depression and life-threatening situations necessitated the revival of a non-drug therapy: the electroconvulsive therapy (ECT). Despite its high efficacy, the mechanisms underlying the beneficial results of ECT are not understood yet. The current hypotheses come from results obtained with electroconvulsive stimulation (ECS), the animal counter-part of ECT. However, results are often inconclusive, mainly because data are frequently based on unchallenged animals that do not exhibit behavioral nor biological defects found in depression. As a consequence, the biological effects driven by ECS are rarely time-correlated with behavioral improvements. Thus we aim to develop a translational approach using MAP6-KO mice to decipher the biological effects of ECT. This mice exhibit some behavioral and biological features, and pharmacological response relevant to some aspect of the major depressive disorder. The objective of this project is to analyze the biological effects of repeated ECS, on an animal model of psychiatric disorders: the MAP6-KO mice.

Poster N° 18

Title : Evolution of proteins from the MAP65 family within nematodes

Authors : Caroline Launay1, Isabelle Arnal2, Marie Delattre1

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Abstract :
The mitotic spindle is a microtubule (MT) network, whose position must be tightly controlled during cell division. The role of external forces on spindle positioning has been extensively studied in many systems. In contrast, the importance of the mechanical properties of the central spindle- the region that connects each spindle pole- to convey these forces remains to be explored. We have shown that in different species of nematodes, the mitotic spindle is asymmetrically positioned through different combinations of spindle movements compared to those described in the reference species *C. elegans,* which reflect changes in the regulation of the mechanical forces acting on the spindle. We performed tubulin immuno-staining in a subset of species and found very different widths of MT overlap for some of them. We hypothesised that changes in MT cross-linking in the central spindle could modify the material properties of the spindle. These changes would influence the coupling between centrosomes thereby affecting spindle motion during its orientation.

It has previously been suggested that the major cross-linker of MTs, the MAP65 protein, might have adapted to the different MT architecture in yeast, humans and plants. Based on the sequence divergence between MAP65 proteins from different nematode species, we hypothesize that MAP65 may have different ability to bundle and organize MTs depending on the species observed. Consequently, evolution of MAP65 proteins may be responsible for different material properties of the central spindle across worm species. We are testing these hypotheses by a bottom-up approach, combining *in vivo* and *in vitro* experiments. We have already shown that two different recombinant MAP65 proteins from worms behave as functional MAPs *in vitro* and can be used for further analysis on purified MTs. In parallel, we are creating transgenic *C. elegans* embryos, in which the endogenous *MAP65* gene is replaced by the *MAP65* sequence of another nematode species. In these replacement lines, we will analyse the localisation of the MAP65 protein, in particular the size of the overlap midzone, analyse the shape of the spindle, its elongation, its deformation and its positioning during anaphase. With this project, we aim to provide detailed characterization of proteins of the MAP65 family- beyond the well-characterized yeast, humans and plants orthologs- in order to study the evolvability of these essential proteins.

Title : Role of centrosomal proteins in skin cell differentiation

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Abstract: The centrosome is the major microtubule organizer in somatic animal cells; it nucleates and organizes microtubules in a radial array emanating from the PCM. In many differentiated cell type such as neurons, epithelial cells or myoblasts, the centrosome disassembles or loses its activity, PCM proteins are reorganized and set up a specific microtubule network. During differentiation of epidermal cells, microtubules have been shown to relocate to the cortex. They are involved in the formation and maintenance of intercellular cell junctions enabling the formation of a proper skin barrier.

We investigated the role of centrosomal proteins in this process and observed that a subset of PCM proteins such as ninein or PCM1 are relocated to the cortex. Because ninein is known to be involved in microtubule anchoring, we generated ninein-KO mice. These mice develop a stratified epidermis with slightly altered expression and localization of several cell junction proteins. In parallel, siRNA-treatment of primary keratinocyte cultures interferes with the formation of a cortically anchored microtubule network.

Poster N° 20

Investigation of the EB1 interactome reveals novels players in microtubule capture and tumor cell motility

Authors : Aurélie Mangon, Habib Bouguenina, Danièle Salaun, Emilie Baudelet, Luc Camoin, Stéphane Audebert, Pascal Verdier-Pinard and Ali Badache

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Abstract : Overexpression of the ErbB2 receptor tyrosine kinase in breast cancer is associated with poor prognosis. Our group investigates the mechanisms by which ErbB2 contributes to tumor cell motility, invasion and metastasis. Cell migration involves reorganization of numerous subcellular structures like the actin cytoskeleton, adhesion sites and microtubules (MTs). We have previously identified a pathway by which ErbB2 controls MT capture and their stabilization at the leading edge of migrating cells. This pathway involves the recruitment to the cell leading edge of a MT capture complex that is supposed to interact with MT + ends. EB1 is a +end-tracking protein (+TIP), that contributes to MT dynamics and interaction with cortical structures. EB1 act as a hub recruiting other +TIPs, including proteins harboring an SxIP motif that allows interaction with its EBH domain. In order to identify novel actors involved in MT cortical stabilization and cell motility, we carried out the characterization of the EB1 interactome in breast cancer cells, via affinity pulldown on the one hand and proximity labeling on the other hand. EB1-EGFP pulldown identified many known EB1 partners, but also novel EB1-binding proteins. Surprisingly, proximity labeling, which reveals proteins in the vicinity of EB1 in vivo, uncovered a very limited number of candidates. Among the few proteins identified in both assays, we found NAV1, MMG and iASPP, a protein known to interact with p53 and inhibit its pro-apoptotic function. Several studies showed that iASPP is overexpressed in various cancers, including breast cancer. Our preliminary experiments show that iASPP knockdown inhibits ErbB2-driven cell motility and disturbs MT capture. Moreover, mutation of a potential SxIP motif in the N-terminal region of iASPP prevented its interaction with EB1 and induced the same defect in MT capture, warranting further investigations. On-going work aims at defining how the newly identified EB1-partners contribute to regulate MT dynamics and tumor cell motility.

Poster N° 21

Title : Molecular mechanisms of gamma-tubulin ring assembly, and implications for human development

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

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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. Finally, we analyzed the consequences of point mutations in the GCP4 gene in cultured cells from patients. We focused on a role of GCP4 in cell division, microtubule organization, and ciliogenesis.

Poster N° 22

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.

Poster N° 23

Title : Microtubule dynamics and mechanical forces in the one-cell *C. elegans* embryo

Authors : Wallis Nahaboo[1], Malgorzata Borowiak[1,2], Dirk Trauner[2], Oliver Thorn-Seshold[1,2] , Marie Delattre[1]

Address : [1] *Laboratory of the Molecular Biology of the Cell, ENS Lyon, Lyon France,* [2] *Department of Chemistry and Pharmacy, 5-13 Butenandtstrasse Ludwig-Maximilians-Universität, München, Munich 81377, Germany*

Abstract : During mitotis, chromosomes are connected to a microtubule-based spindle. The displacement of the spindle poles and/or the activity of kinetochore microtubules generate mechanical forces that segregate the chromatids. Using laser destruction of the centrosomes during *C. elegans* mitosis, we showed that neither of these mechanisms is necessary to achieve proper chromatid segregation. We demonstrated that an outward force generated by the spindle midzone is sufficient for anaphase in mitotic cells. We next identified candidate molecules involved in this outward force. We found that SPD-1/MAP-65 and BMK-1/kinesin-5 act as a brake opposing the force generated by the spindle midzone. Conversely, we revealed that two microtubule-growth and nucleation agents, Ran and CLASP, are necessary for the establishment of the outward force. Besides their established role during spindle formation, we uncovered a new function for Ran and CLASP during anaphase (1.).

We will also present a new tool to optically control microtubule dynamics with high spatiotemporal precision and full reversibility. Our Photostatins PST-1 molecule promotes microtubule depolymerization when irradiated with 390-430 nm light, but quickly revert to an inactive form in the dark or by irradiation with 500-530 nm light. Using *C. elegan*s embryos, we show that PST-1 controls mitosis with single-cell resolution and with a temporal response on the scale of seconds. This unique tool opens the way to the study of MTs dynamics in the *C. elegans* spindle and beyond this model organism. More generally PSTs hold promise as a new class of precision chemotherapeutics (2.).

1. Nahaboo & al, MBoC, 2015
2. Borowiak, Nahaboo & al, Cell, accepted

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 to decrease EB1 accumulation at growing MT ends. FRAP analyses indicate that ATIP3 silencing accelerates EB1 turnover on its recognition site at growing MT ends, supporting a novel mechanism for negative regulation of EB1 function and MT dynamics at the plus ends.

Given the potent tumor suppressor effects of ATIP3, these findings may have clinical relevance in the field of cancer. The identification of ATIP3-EB1 complexes as molecular markers and/or therapeutic targets in breast cancer is under investigation.

Visualization of microtubule dynamic instability in glioblastoma by intra-vital two-photon microscopy: effect of microtubule targeting agents.

Sarah Oddoux [1], Clément Ricard [1,2,3,4], Raphaël Bergès [1], Marie Petit [1,2], Geneviève Rougon [3,4], Diane Braguer [1], Franck Debarbieux [3,4], Stéphane Honoré [1,2].

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Glioblastoma multiforme is the most common and malignant form of gliomas, characterized by highly aggressive growth and invasive behavior that accounts for the poor overall survival of patients. We have previously shown that the over-expression of EB1, a microtubule-binding protein, is both a marker of bad prognostic and of sensitivity to vincristine, a microtubule targeting agent (MTA). Microtubules are primary targets of chemotherapeutic treatments as they are essential for cell proliferation and migration. Microtubules functions require them to be dynamic. Therefore MTAs are key drugs in chemotherapy as they alter microtubule dynamics, which in turn prevents cell proliferation and migration. Mathematic models of microtubule dynamics in response to MTAs are currently being developed in collaboration with Marseille Institute of Mathematics to help discriminate anti-mitotic from anti-migratory effects of MTAs. However these models are based on data obtained on glioblastoma cells in culture.

In order to validate these models *in vivo* we have designed a protocol allowing for the first time the follow-up and the quantification of microtubules dynamics in glioblastoma cells implanted in the mouse brain. We have developed GL261 glioblastoma cells expressing microtubule markers tagged with the green fluorescent protein. These cells were cultured to form spheroids before their implantation in the cortex of mice. The bone above the implantation area was replaced by a glass window allowing to follow the tumor progression and the microtubule dynamics over time on the same animal using intravital two-photon microscopy. To gain information about the tumor micro-environment we used transgenic mice expressing fluorescent proteins in various cell types of interest (neurons, astrocytes and immune cells). Vascular remodeling was also highlighted by intravenous injection of a fluorescent marker.

Mice will be treated with vincristine following two protocols and the results will be put in perspective to those obtained on cultured cells. This will allow us to compare results obtained both *in vitro* and *in vivo* and implement our mathematical models for the evaluation of molecules of interest for glioblastoma treatment. This project represents a major technical breakthrough to broaden the panel of preclinical tests to evaluate the potential of MTAs in glioblastoma treatment.

Poster N° 26

Title :

Triadin and CLIMP-63 form a link between triads and microtubules in muscle cells

Authors :

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Abstract :

Muscle contraction is achieved when an efficient excitation signal at the plasma membrane triggers intracellular calcium release. This process called “excitation-contraction (E-C) coupling” relies on a macromolecular protein complex, spanning the plasma membrane and the sarcoplasmic reticulum (SR), containing the calcium channel of the SR, the ryanodine receptor (RyR). This calcium release complex is present exclusively in highly organized membrane structures called triads. A triad is composed of two SR terminal cisternae surrounding a plasma membrane transverse-tubule. This architecture is essential to sustain the activity of the calcium channel RyR1, which is located in the membrane of SR terminal cisternae. However, little is known about the molecular mechanisms allowing the formation and maintenance of SR terminal cisternae. Triadin is a member of this complex, present in the SR membrane and interacting with RyR1. Deletion of the triadin gene leads to partial disorganisation of SR membranes in skeletal muscles, with abnormal orientation of part of the triads. We have shown in a non muscle cell model that triadin expression leads to important modification of the endoplasmic reticulum (ER) morphology, already observed with the expression of proteins regulating ER morphology, and known as “rope-like structures”. These modifications of ER morphology are correlated to alteration of the microtubule network. It thus suggests that in skeletal muscle, triadin could play a role in the structure of sarcoplasmic reticulum to allow efficient E-C coupling. For the present work, using mass spectrometry analysis of the proteins co-immunoprecipitated with triadin, we have identified a putative triadin partner which could interact with triadin and with the microtubules, and therefore anchor the sarcoplasmic reticulum to the microtubule network. Using different deletion mutants of both proteins we identified the domains of each protein important for this interaction.

Title: Microtubule microdomains with a GTP-bound conformation: mechanisms of formation and control of microtubule rescues

Authors:
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Abstract:
We have evidence previously that small inner microtubule regions that retain a GTP-bound tubulin conformation (termed GTP islands) strongly correlate with the location of future microtubule rescues (Dimitrov et al., Science, 2008, 322:1353-6).
Further analysis of the mechanisms that underlie the occurrence of GTP islands reveal that microtubule crossings play an important role in this process, both *in vivo* and *in vitro*. By making growing microtubules climb over or jump from nano-pillars of various heights, we provide evidence that the edges of the nano-pillars can be as effective as microtubule crossings to generate the islands. This data suggests that the local curvature of the microtubule wall at the pillar's edges is responsible for the occurrence of a tubulin conformation or of lattice defects that are recognized by the anti-GTP-tubulin antibody.
Also, as GTP islands are recorded in the microtubules before the occurrence of rescues, we tested whether the overall organization of the microtubule network could influence its own dynamics. Computer simulations in which the network displayed a meshed vs radial microtubule arrangement and mixed both random and crossing-dependent GTP islands suggested that catastrophe and rescue frequency should be significantly higher in meshed networks. This could be due to higher GTP island density at the cell periphery. Accordingly, cell recovery from nocodazole treatment with or without the inhibition of microtubule nucleation by Golgi elements, which resulted in more or less radial microtubule arrays, resulted in the modulation of the density of GTP islands at the cell periphery.
Altogether, the way microtubules interact with each other within a network provides new clues into how they may influence their own dynamics and into how the network could adapt its dynamic behaviour according to changes in cell organization or morphology.

Poster N° 28

Title : Kinase activities which regulate the tubulin polymerization status in non fertilized marine invertebrate eggs

Authors : Gérard Prulière, Janet Chenevert, Céline Hébras, Lydia Besnardeau and Alex McDougall

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Abstract : Ascidian oocytes are blocked in metaphase of the first meiotic division with a small asterless meiotic spindle as well as a cortical network of short and disorganized microtubules. Most of the tubulin remains unpolymerized, a fact which raises the question of how the equilibrium between monomeric and polymeric tubulin is controlled in these quite large cells (140µm). We find that treatment of the oocytes with specific inhibitors of Polo-like kinase or of the MAPK pathway leads to a tremendous polymerization of tubulin into multiple cytaster structures similar to those obtained after addition of purified centrosomes to Xenopus egg extracts (Piel and Bornens 2005). Using in vivo tracking of fluorescent proteins and immuno-labelling approaches, we show that these cytoplasmic asters concentrate many of the factors involved in the formation of the spindle during normal mitoses (γ-tubulin, RAN, TPX2, NDEL, NUP153, Numa, phospho-aPKC). Moreover, these asters form a multi-spindle connected network which was observed to contract. Inhibition of dynein using ciliobrevin or of EG5 using monastrol leads to destruction of this network, indicating that its formation also involves motor activities. The meiotic spindle, which is normally deprived of real poles, accumulates large foci of gamma-tubulin and forms many long astral microtubules under Polo-like and MAPK kinase pathway inhibitor treatment. After a temperature controlled depolymerization/polymerization cycle, we observe that γ-tubulin abnormally aggregates at the level of meiotic chromosomes and at the poles of the cytoplasmic asters prior the re-initiation of microtubule polymerization. Similar results were obtained for oocytes from other ascidian species as well as Amphioxus and an important tubulin polymerization after kinase inhibition was also observed in several sea urchins species. Our results demonstrate that both Polo Kinase and the MAPK pathway are essential to prevent polymerization of the high tubulin monomer concentration stored in marine oocytes to ensure rapid cleavages during development of the embryos.

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.

Poster N° 30

Title : Dynamic regulation of BDNF axonal transport by neuronal activity

Authors : Rodier, J.-A. (Grenoble)[1,2], Charlot, B. (Montpellier)[3,4], Genoux, A. (Grenoble)[1,2], Saudou, F. (Grenoble)[1,2,5], Cazorla, M. (Grenoble)[1,2]

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Abstract :

Dynamic remodeling of axonal connections is a sine qua none condition to adapt proper responseto environmental stimuli. BDNF, the most abundant neurotrophin in the adult brain, is a key regulator of axonal remodeling, dendritic sprouting and synaptic plasticity, and its release at the synapse depends on neuronal activity. But how does neuronal activity direct axonal vesicles containing BDNF toward activated synapses to promote branching? Here we developed microfluidics systems compatible with high-resolution videomicroscopy connected to multielectrode arrays (MEA) (see poster by Cazorla et al.) to isolate corticostriatal connections and to monitor BDNF axonal transport within active axonal networks. Using this multicomplex system with segregated cortical and striatal primary cultures, we analyzed the evolution of BDNF trafficking during the maturation of the corticostriatal pathway. The formation and maturation of the network was assessed using a variety of fluorescent markers and sensors: MAP-2 (dendritic marker) and Tau-1 (axonal marker) to analyze the growth of dendritic and axonal branches; synaptophysin (presynaptic marker) and PSD-95 (postsynaptic marker) to analyze the formation of synaptic contacts; iGluSnFr (glutamate sensor) to visualize active glutamatergic corticostriatal synapses; GCamp5 (Calcium sensor) to analyze global and individual neuronal activity. We found that corticostriatal formation and maturation correlate with changes in BDNF trafficking properties. We are now studying the molecular and cellular events responsible for this regulation and its effect on the connection probability of the corticostriatal pathway.

Poster N° 31

Title :

Tau-tubulin interaction by isothermal titration calorimetry

Authors :

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Abstract :

Microtubule associated proteins tau are intrinsically disordered proteins that can bind to tubulin and promote its polymerization into microtubules. While tau aggregates are a hallmark of Alzheimer disease, tau is also found to be overexpressed in various types of cancer and associated with resistance to microtubule targeting drugs therapy. Despite intensive investigation of tau-tubulin binding, the precise mechanism of this process is still controversial. Recently we started tackling this issue using isothermal titration calorimetry and demonstrated complex curves in favor of two distinct tau binding modes to tubulin. To decipher the exact mode / mechanism of tau binding to tubulin we are currently performing isothermal titration calorimetry using constructs of tau and stathmin, in combination with analytical ultracentrifugation, turbidimetry, cosedimentation assay and transmission electronic microscopy, in order to distinguish and characterize separately these two binding modes.

Poster N° 32

Title : HTT phosphorylation and the neurotrophin retrograde axonal signaling

Authors : Scaramuzzino C[1,2], Pla P[4], Dard R[4], Saudou F[1,2,3]

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Abstract : Mutation of huntingtin (HTT) leads to Huntington disease (HD), a devastating neurodegenerative disorder characterized by the degeneration of striatal and cortical neurons. The degeneration of striatal neurons is partly due to a reduced capacity of cortical neurons to synthetize and transport BDNF to the striatum coupled with an impairment of the TrkB signaling in striatal dendrites. However, the mechanism leading to the degeneration of cortical neurons remain to be elucidated. Normally, HTT regulates the vesicular transport in axons and dendrites. The directionality of this transport is regulated by HTT phosphorylation status at serine 421 (S421). In particular, S421 dephosphorylation of HTT by calcineurin detaches kinesin from vesicles and promotes retrograde transport. However, the mechanism triggering the selective retrograde transport of the TrkB-BDNF signaling endosomes in response to trophic factors has not been elucidated. To investigate this mechanism, we used state-of-the-art microfluidic chambers and videomicroscopy approaches to analyze TrkB trafficking in distal part of cortical axons, speculating that HTT and possibly its dephosphorylation is a key step controlling retrograde transport of signaling endosomes. We found that in control situation TrkB-containing vesicles are in a steady state with a preferential anterograde transport. Moreover, after 5 minutes of BDNF infusion in the synaptic compartment of the microfluidic chambers, we identify that there is a massive but transient inversion to retrograde flow of TrkB containing vesicles. Treatment of neurons with calcineurin inhibitor (such as FK506) reduced significantly the retrograde flow, indicating a role of calcineurin in the BDNF-induced retrograde flow of TrkB receptors. We are now investigating the requirement of HTT dephosphorylation in this process using neurons derived from mice that carry point mutations at S421.

In vitro study of MAP6 interactions with microtubules

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Microtubules are highly dynamic structures regulated *in vivo* by several factors including MAPs (microtubule-associated proteins). Among them are the family of MAP6 isoforms (also called STOP proteins), originally identified in the laboratory due to their capacity to protect microtubules from depolymerisation induced by hypothermic exposure. MAP6 knockout mice present behavioural disorders underpinned by neuronal abnormalities involving, at least partly, microtubules defects. However, the mode of action of MAP6 on microtubule dynamics is still largely unknown. We have recently established purification procedures for recombinant MAP6 isoforms expressed in insect cells. We have shown that MAP6-N binds directly to tubulin and enhances microtubule nucleation. We have characterized the interaction of MAP6-N with microtubules and shown using TIRF microscopy that it displays a strong effect on the microtubule dynamics. The protein stimulates microtubules plus-end elongation by regulating their growth rate, catastrophes and rescues. Furthermore, we have observed that the protein is able to regulate the microtubule minus-end dynamics. Preliminary data on the structure-function of MAP6 are also presented.

Title: APC enhances EB binding at microtubule ends in vitro

Authors : Serre, L., Ramirez-Rios, S., Delaroche, J., Arnal, I.

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Abstract: For nearly two decades, the stories of End-Binding (EB) and Adenomatous Polyposis Coli (APC) proteins have been overlapping. EB1 and EB3 were both identified through their binding to APC and its homolog APC-Like (APC-L) (APC-L) (Su et al., 1995; Nagakawa et al. 2000). Cellular depletion of EB or APC led to the mis-segregation of chromosomes, suggesting that both proteins were participating to the same biological pathway (Draviam et al., 2006).

We intend to decipher in reconstituted cell-free systems the interaction between APC, EBs and microtubules. In particular, we showed that the c-terminal region of APC enhanced the localization of EB3 or EB1 to the ends of microtubules. In addition to the SXIP motif already defined as a prerequisite for the APC/EB1 interaction (Honappa et al., 2005), we suggest that this process is more complex and would involve other regions conserved between the different APCs. The presence of several potential EB-binding sites on APC may be related to the large panel of cellular functions requiring the APC/EB crosstalk and a multi-level regulation.

Title : Insight the molecular function of the TRM proteins in microtubule array organization

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Abstract :

Plant cortical microtubule arrays are highly organized structures that play key functions in the acquisition of the plant architecture. Our group has identified a key regulatory complex named the TTP complex (for TON1, TRM, PP2A), involved in plant cortical microtubule arrays formation. Despite the acentrosomal nature of plant cells, all members of this complex share similarity with animal centrosomal proteins involved in ciliary and centriolar/centrosomal functions. The Arabidopsis TRM component of the TTP complex is encoded by 34 genes that probably specify different developmental functions of the complex during cell elongation and division.

Mutations in the TRM subgroup 1 (*trm1-2-3-4*) lead to mild phenotypes such as smaller organs. Cell length is also reduced confirming involvement of this TRM subgroup in cell elongation. Cortical microtubule array anisotropy in actively elongating cells is affected in comparison to that of similar wild type cells. Since microtubule dynamics and nucleation events are widely accepted as the main factors involved in plant microtubule arrays shaping and reorganization and since the geometry of microtubule nucleation is reversed from branched to parallel in some *pp2a* mutant, dynamic parameters of individual microtubule and nucleation events are under scrutiny in these *trm* mutants.

Poster N° 36

GSK3-mediated MAP1B phosphorylation regulates neurite branching and microtubules dynamics

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Abstract :
The microtubule-associated protein MAP1B, a protein essential to neuron regeneration, is known to play an important role in neurite and axon extension: neurites of *map1b-/-* mice are more highly branched than their wildtype counterparts. Here, we investigated the role of GSK3-mediated MAP1B phosphorylation in fine-tuning of neurite branching and the underlying microtubule (MT) dynamics. On wildtype adult dorsal root ganglion neurons, MAP1B phosphorylation is locally reduced at branching points, and inhibition of GSK3-mediated MAP1B phosphorylation further increases branching from growth cones and distal neurite shafts.

While *map1b-/- n*eurites are not affected by GSK3 inhibition, transfection of *map1b-/-* neurons with full-length *map1b*-cDNA restores a normal branching phenotype. Our analysis of *map1b*-cDNA-transfected COS-7 cells, and of mutant mice lacking tyrosinated MTs, indicates a preferential association of phospho-MAP1B with labile MTs. Interestingly, inhibition of GSK3-mediated MAP1B phosphorylation protects both tyrosinated labile, and acetylated stable MTs against depolymerization, while detyrosinated MT are less abundant in presence of MAP1B.

We conclude that phosphorylation of MAP1B by GSK3 is a key process for the control of the local balance between acetylated/detyrosinated-stable and tyrosinated-labile MTs by MAP1B.

Poster N° 37

Title : Tau inhibits the tracking of EBs at microtubule ends in cells.

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Abstract : End-binding proteins (EBs, mainly EB1 and EB3) are plus-end tracking proteins (+TIPs) that preferentially associate with the growing end of microtubules. Structural microtubule-associated proteins (MAPs) have recently emerged as potential regulators of EB functions. We investigate here the functional interplay between EBs and tau, one of the major neuronal structural MAP. Using *in vitro* reconstitution assays, we show that tau inhibits EBs tracking at microtubule growing ends. Tau and EBs form a complex *via* the C-terminal region of EBs and the microtubule-binding sites of tau. To test whether tau could also inhibit the EB tracking in cells, we labelled endogenous EB1 in mouse embryonic fibroblasts (MEFs) transfected with tau. Both the density and length of EB1 comets were decreased in the presence of Tau, reflecting *in vitro* results. Microtubule dynamics were tested in live cells with EB3-GFP as a marker. No differences were found in dynamic parameters when control cells were compared with cells expressing tau, but the length of EB3-GFP comets was reduced as for endogenous EB1 comets. Hence, the inhibition of EB tracking does not reflect a reduction of microtubule growth rate. Taken together these results show a crosstalk between Tau and EBs and could point out a role of tau in the regulation of EBs properties.

Title : Mathematical modelling of MT dynamics in the presence of EB1 and cancer chemotherapy drugs

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Abstract :
Understanding how microtubule (MT) targeting agents (MTAs) alter MT dynamics has been studied both experimentally and theoretically. It has been well established that MTAs exert their cytotoxic affect on MTs by suppressing MT dynamic instability. However, at low non-cytotoxic levels, more interesting dynamics have been observed, such as an increase in MT dynamic instability. Also, it has recently been discovered that the EB family of tip tracking proteins sensitize the affect of MTAs on MT dynamics *in vitro* [1] and in *vivo* [1,2].

Here, we develop a mathematical model, based on the work of Hinow et al. [3], to describe the action of EBs on MT dynamics. To our knowledge, no such model has been developed. Also, we further extend this model to account for the action of MTAs, at both low non-cytotoxic and high cytotoxic levels, on MT dynamics. Our goal is to not only describe the action of MTAs on MTs, but to also understand how the EB proteins work to sensitize the MTA affect on MT dynamic instability.

[1] Berges et al. *EB1 protein overexpression correlates with glioblastoma progression and sensitizes to vinca-alkaloids in vitro and in vivo*. Oncotarget, 2014.

[2] Mohan et al. *EB proteins sensitize to microtubules to the action of microtubule-targeting agents*. PNAS, 2013.

[3] Hinow et al. *Continuous model for microtubule dynamics with catastrophe, rescue, and nucleation processes.* Physical Review E. 2009.