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Roscoff (France), 5-9 septembre 2012

Division cellulaire : de la mécanique des molécules uniques aux organismes multicellulaires

Cell division: from single molecule mechanics to multicellular organisms

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée :

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La XII^{ème} édition des Conférences Jacques Monod dédiée au Cycle Cellulaire (Septembre 2012) a été plébiscitée. Le nombre de postulants a largement dépassé le nombre de places disponibles. Le but de cette conférence était de rassembler des scientifiques fascinés par la division cellulaire, mais qui approchent la question à partir de différentes perspectives, de par leurs différences de formation et de savoir-faire. Nous avions donc invité un panel de physiciens qui développent de nouvelles techniques pour disséquer la mécanique des enzymes, de biochimistes et de biologistes structuraux qui sont en train de reconstituer des machines multimoléculaires du cycle cellulaire, de chimistes qui développent de plus en plus d'inhibiteurs spécifiques d'enzymes clés comme les moteurs moléculaires et les kinases, de physiologistes qui commencent à étudier comment la perturbation des gènes du cycle cellulaire affecte certains tissus, et de médecins qui explorent comment la perturbation du cycle cellulaire favorise l'apparition de cancers.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Cell division: from single molecule mechanics to multicellular organisms

Roscoff, September 5-9, 2012

GENERAL ASPECTS

The recent Jacques Monod conference on the Cell Cycle: "**from single molecule mechanics to whole organisms**" was the 12th edition of this conference series and developed along the aims established by previous editions, in term of opening the field to the new generations of scientists, carrying new questions, yet preserving the high standards set by the previous meetings. The quality of the talks was outstanding, and the discussion after each talk was extensive and led to very stimulating new ideas. Both poster sessions were very lively and extremely well attended. Once again the unique venue at Roscoff generated a remarkable atmosphere at the conference, which was marked by constructive, friendly and productive discussions. The success of the conference can be judged by it being largely oversubscribed.

As always, Mrs Lidoreau mastered the organization of the administrative issues before, during and after the conference, and Alain provided strong support to solve all technical issues during the various sessions.

Administrative issues

There were 28 invited speakers (10 from France, 11 from the rest of Europe and 7 from outside Europe: USA and Japan). Unfortunately, Tony Hyman, Roger Karess and Stephen Taylor could not attend the conference due to unforeseen circumstances. The invited speakers gave 20 minutes talks followed by 5 minutes for discussion.

Twenty outstanding short presentations were selected from abstracts to give a 10-minute talk followed by 5 minutes for discussion.

65 posters were presented in either of two sessions.

Two editors, representing the EMBO journal (Hartmut Vodermaier) and PLOS Biology (Ines Alvarez-Garcia), also attended the conference and provided awards for two poster prizes that were granted to Cristina Ghenoiu (Rockefeller, New York) and Franz Meitinger (DKFZ Heidelberg, Germany).

When classified according to professional status, the 112 participants were grouped as editors (2), Ph.D students (21), Post-docs (25), junior group leader (31) and senior (33) group leaders.

The participants are currently working in the following 14 countries: Austria (5), Canada (1), Denmark (2), England (21), France (25), Germany (14), Italy (4), Japan (5), The Netherland (5), Norway (2), Portugal (3), Spain (6), Switzerland (9), and USA (10).

SUMMARY of the TALKS

During cell division a cell duplicates its genome and segregates it into the two future daughter cells. This requires tight control of several crucial mitotic steps. Based on the work that the guest speakers chose to present and on the abstracts selected for oral presentation, we organized the program into the following eight sub-sessions: (i) Meiosis, (ii) Poles & Polo, (iii) Cohesion, (iv) Centromere & kinetochore, (v) CDK inhibitors, (vi) Spindle & checkpoint, (vii) Cytokinesis and (viii) Integrated views.

To introduce the conference, **Jonathon Pines** (The Gurdon Institute, University of Cambridge, UK) gave a plenary lecture, reminding how the first conferences he attended in Roscoff more than 20 years ago, gave birth to the "Cell Cycle" field. Nowadays, this field expanded so much that it cannot fit in a single "Jacques Monod" conference. Indeed, most research groups are now super specialized, restricting their research to chromosome duplication, mitosis, or the switch between quiescence, proliferation and apoptosis. The lab of Jonathon Pines is using gene targeting to generate cell lines in which one allele of the gene for an APC/C substrate or SAC protein is tagged with a fluorescent protein. Using this quantitative assay and chemical inhibitors, Pines demonstrated that the spindle assembly checkpoint is extremely sensitive to MPS1 activity levels. This finding opens new questions regarding homeostatic control of the mitotic checkpoint.

1- Meiosis

Jan Ellenberg (EMBL-Heidelberg, Germany) opened and illuminated the session. He presented the latest results from his team providing strong evidence that the meiosis I spindle divides only after reaching the cell cortex. His team developed efficient RNAi-mediated gene knock-down in oocytes that overcomes the maternal load. He also introduced the light sheet imaging technique that they will use in the near future for long term imaging of the oocyte from meiosis I to early embryonic division. **Marie-Hélène Verlhac** (Collège de France, France) studies the regulation of spindle positioning by actin dynamics in mouse oocytes. Her team compared cytoplasmic Arp2/3-regulated, versus cortical formin-regulated actin. They measured the mechanical properties of the cortex, in presence or absence of the MAPK signaling pathway that regulates cortical stiffness. This allowed them to show that a decrease in cortical tension is required for accurate spindle positioning at the cell cortex, a prerequisite to achieve the first asymmetric meiotic division. **Thierry Lorca** (Université de Montpellier, CRBM, France) used Xenopus egg extracts to study regulatory phosphorylations of Arpp19, an inhibitor of the PP2A-B55 phosphatase. He showed that RSK rather than PKA is responsible to generate the PP2A binding epitope onto Arpp19, at a position that they identified. **Takeo Kishimoto** (Tokyo Institute of Technology, Japan) revisited the notion of "MPF" (Maturation Promoting Factor) defined in 1971, and characterized as being CDK1-Cyclin B in the late 1980's. Reminding that MPF is one order of magnitude more active than purified CDK1-Cyclin B, he showed that a combination of purified CDK1-Cyclin and Greatwall kinase reproduces full MPF activity. **Thomas Mayer** (University of Konstanz, Germany) demonstrated that beyond its role in maintaining the Meiosis II arrest of Xenopus egg extracts, XErp1/Emi2 is also the APC/C inhibitor responsible to ensure timely metaphase-to-anaphase transitions during early embryonic cell cycles. He also showed that while Cdk1-Cyclin B antagonizes XErp1 function, PP2A and PKA are stimulating it.

2- Poles & Polo

Building on her previous results obtained in Drosophila, **Renata Basto** (Institut Curie, France) developed new mice overexpressing PLK4 in the brain. She showed that 33% of neural stem cells had extra centrosomes. The main observed defect is the production of tripolar spindles, and their maintenance until anaphase. This raises the percentage of anaphase cells displaying lagging chromosomes, increases the mitotic index and promotes cell death. These defects could be responsible for the human neurodevelopmental disorder microcephaly. **David Pellman** (Dana-Farber Cancer Institute, USA) used a 3D culture system for human mammary epithelial cells. He showed that producing extra centrosomes by either cytokinesis failure or transient PLK4 overexpression, promotes the formation of invasive structures in 3D. They found that cells with extra centrosomes have increased microtubule nucleation and Rac1 activation and that Rac1 inhibition suppresses the invasive phenotype. **Claude Prigent** (IGDR, France) presented results showing that Nucleophosmin/B23 activates Aurora A at the centrosome. **Vladimir Joukov** (Dana-Farber Cancer Institute, USA) presented experiments performed in Xenopus egg extracts showing that beads coated with Cep192 function as a scaffolding and activating factor for Aurora A and Plx1, which recruits pericentriolar material capable to perform MTOC functions. **Fumiko Toyoshima** (Kyoto University, Kyoto) discovered a previously unrecognized role for pregnenolone, a cholesterol metabolite, in centriole cohesion. Their results suggest that pregnenolone plays an essential role in recruiting Sgo1 to the centrosome to protect centriole cohesion from the Plk1-induced centriole disengagement in early mitosis. **Cayetano Gonzalez** (IRB-Barcelona, Spain) is studying the asymmetric division of neuroblasts in Drosophila. He showed that the daughter centrosome retains more pericentriolar material, and remains at the apical cortex, contrary to the motile mother centriole that will be delivered to the differenciating daughter cell. He further showed that this is regulated via PLK1-dependent phosphorylation of Centrobin. **Marcos Malumbres** (CNIO, Spain) described the newly generated conditional PLK1-/- mice. He showed that germline ablation of Plk1 results in embryonic lethality due to mitotic abnormalities at the morula stage. Furthermore, PLK1 heterozygosity also promotes several abnormalities. Finally, **Iain Hagan** (University of Manchester, UK) used an analogue-sensitive constitutively active Plo1 mutant to show that the centrosome/SPB acts as a major signaling hub to control diverse cell cycle decisions at remote locations.

3- Replication & Cohesion

Katja Wassmann (University of Paris VI, France) described a novel mechanism of deprotection of centromeric cohesin in mouse oocyte meiosis II. Using localization data obtained by super-resolution microscopy, she proposes a regulatory mechanism of PP2A inhibition that depends upon shugoshin. **Jean-Paul Javerzat** (IBGC, Bordeaux, France) studies the establishment and maintenance of sister-chromatid cohesion in *S. cerevisiae*. He showed that cells devoid of the *pds5* gene are proficient for sister-chromatid cohesion establishment but cohesion is eroded during the G2 phase and is eventually lost when the G2 phase is extended. **Ana Losada** (CNIO, Spain) documented the specific roles of cohesion factors Pds5A and Pds5B in mouse cells. After generating distinct knock-out alleles of Pds5A and Pds5B, her team characterized mouse embryonic fibroblasts completely lacking one or the other protein. They thus found that Pds5B is essential for centromeric cohesion. **Eric Weiss** (Northwestern University, USA) reported the first structure of the *S. pombe* Mob2-Cbk1 complex at 3 angstroms resolution. They identified a novel kinase-docking motif that recruits the Cbk1/Mob2 complex to its direct *in vivo* substrates.

4- Centromere & Kinetochore

Andrew McAinsh (Warwick Medical School, UK) described the development of a next generation automatic kinetochore/spindle pole-tracking assay in which they have been able to achieve a temporal resolution of 2 seconds per 3D image stack. This is providing new insights into sister kinetochore and spindle behavior in mitosis and driving the development of new mathematical models of chromosome motion. **Toru Hirota** (JFCR, Tokyo, Japan) developed a fluorescence-based sensor for the protease separase that mediates cohesin cleavage. They found that separase undergoes an abrupt activation shortly before anaphase onset in the vicinity of chromosomes. Subsequent to its proteolytic activation, separase then binds to and inhibits a subset of cyclin B1-cdk1, which antagonizes cdk1-mediated phosphorylation on chromosomes and facilitates poleward movement of sisters in anaphase. **Iain Cheeseman** (Nine Cambridge Center, USA) showed that the Ska1 complex diffuses on the microtubule lattice, tracks with depolymerizing microtubules, and stabilizes the formation of curved protofilaments. He further showed by electron-microscopy that the Ska1 complex binds onto stabilized peeled protofilaments, while Ndc80 does not. However, the Ndc80 and Ska1 complexes bind to microtubules synergistically. **Geert Kops** (University Medical Center Utrecht, The Netherlands) showed that vertebrate BubR1 is an unusual pseudokinase. Indeed, putative catalysis by human BubR1 is dispensable for error-free chromosome segregation. However, BubR1 cooperates with PLK1 to stabilise kinetochore-microtubule interactions by regulating PP2A-B56 mediated de-phosphorylation of Aurora B substrates at the kinetochore-microtubule interface. **Andrea Musacchio** (MPI, Dortmund, Germany) detailed the structure of the intrakinetochore platform KNL1 protein, highlighting how its various domains participate to the recruitment of Bub1 and BubR1. **Todd Stukenberg** (University of Virginia, USA) found that microtubules originating at kinetochores after the release from a nocodazole arrest act as conduits to take Aurora B to kinetochores, where Aurora B phosphorylates its substrates. He also showed that the kinetochore proteins Ndc80 and the CENP H/I/K complex inhibit dynein removal of Mad1, when kinetochore-nucleated microtubules are present. This ensures that the signal persists until kinetochores are placed under bipolar pulling forces by attachments to the spindle. **Stefan Westermann** (IMP, Vienna) used bioinformatic, biochemical and structural approaches to elucidate the function of centromere proteins in budding yeast. He identified the histone-fold protein $Cnn1^{CENP\text{-}T}$ as a direct centromere receptor of the microtubule binding Ndc80 complex. Another essential link in the kinetochore framework is formed by the yeast homologs of the human centromere proteins CENP-U and CENP-Q. They demonstrated that the respective yeast proteins Ame1 and Okp1 form a DNA-binding heterodimer that directly interacts with the Mtw1 complex. **Tatsuya Nishino** (NIG, Shizuoka, Japan) described structural and biochemical analyses of CENP-T-W and CENP-S-X and found that they are dimeric histone fold proteins that are able to engage in a tetrameric assembly, which is required for functional kinetochore formation *in vivo*.

5- CDK inhibitors

Laurent Meijer (ManRos Therapeutics, France) gave an overview of his work focused on pharmacological inhibitors of cyclin-dependent kinases (CDKs), and their applications against cancers, neurodegenerative diseases, renal diseases and inflammation. They have optimized roscovitine derived CDK inhibitors to nanomolar active drugs, one of which they aim to bring to clinical evaluation. He also offered the opportunity to his student **Vincent Guen**, to talk for five minutes about his recent findings showing that the complex CDK10-Cyclin M forms a novel cyclin-dependent kinase that regulates ETS2 degradation.

6- Spindle & Checkpoint

David Barford (ICR London, UK) used a combination of X-ray crystallography and single particle electron microscopy to solve the structure of the APC/C. He also presented structural and mechanistic studies of the *S. pombe* MCC that explains how the closed conformation of Mad2 (C-Mad2) promotes MCC assembly and how the MCC inhibits the APC/C towards D box and KEN box-dependent substrates. **Don Cleveland** (LICR San Diego, USA) used protease-cleavable forms of Mad2 and BubR1 to demonstrate that once bound onto the APC/C, only BubR1 (but not Mad2) is required to maintain APC/C inhibition. This was further demonstrated *in vivo*. He also provided evidence that Mad2 is required for Cdc20 to bind to BubR1 within the MCC. **Patrick Meraldi** (ETH Zurich, Switzerland) developed a high-resolution kinetochore and centriole tracking-assay in HeLa cells, to show that the spindle checkpoint can also detect spindles with unequal half-spindle lengths. To generate such asymmetric spindles, they blocked centriole duplication and monitored the resulting mixture of cells with different centriole numbers. Their functional data imply that the unequal half-spindle lengths are caused by different microtubule dynamics at each spindle pole, with faster microtubule depolymerization at the spindle pole containing only one centriole. **Rocio Sotillo** (EMBL-Monterotondo, Italy) used state-of-the-art mouse genetic strategies to demonstrate that overexpression of the mitotic checkpoint protein Mad2, commonly found up-regulated in human tumors, develop CIN and (subsequently) aneuploid tumors in a variety of epithelial tissues, including breast. **Ariane Abrieu** (Université de Montpellier, CRBM, France) used Xenopus egg extracts to demonstrate that CDK-dependent potentiation of MPS1 kinase activity is essential to the mitotic checkpoint. **Prasad Jallepalli** (MSKCC, New York, USA) coupled quantitative proteomics and chemical genetics to identify Mps1-dependent phosphorylations *in vivo*. Quantitation of over 20,000 phosphorylation sites revealed Mps1-regulated modifications on numerous kinetochore proteins including Mps1 itself, Histone H2A, the mitotic checkpoint complex protein BubR1, and the outer kinetochore proteins KNL1 and Ska3. Mutation of Mps1 N-terminal autophosphorylation sites elevated kinase activity and interfered with efficient chromosome bi-orientation, suggesting a tyrosine kinase-like autoregulatory mechanism. **Jonathan Millar** (University of Warwick, UK) presented his latest findings showing that association of PP1 to the Spc7/Spc105/KNL1 family of kinetochore proteins is necessary to stabilize microtubule-kinetochore attachments and silence the SAC.

7- Cytokinesis

Yves Barral (ETH Zurich, Switzerland) studies the mechanisms leading to the asymmetric segregation of DNA circles, which are formed upon excision of circular DNA molecules from the genome. He showed how DNA circles attach to the nuclear envelope, while chromosomal DNA does not. This provides an explanation to their previous finding that DNA circles are retained in the yeast mother cell and not in the bud. **Simonetta Piatti** (Université de Montpellier, CRBM, France) performed a genetic screen for novel regulators of septin dynamics. They found that the Rho1 GTPase (the yeast counterpart of metazoan RhoA) and its target protein kinase C (Pkc1) are involved in the deposition and stabilization of the septin ring during the cell cycle. **Masanori Mishima** (University of Warwick, UK) studies the formation and maintenance of microtubule structures during cytokinesis. He discovered that a direct interaction between centralspindlin and PRC1 is critical for maintenance of the integrity of the central spindle. **Jonas F. Dorn** (Université de Montréal, Canada) is measuring and modeling the mechanics of cytokinesis. Ring closure in the *C. elegans* zygote is non-concentric (asymmetric), providing an additional readout for testing models of cytokinesis.

Importantly, asymmetric cytokinesis is universal among metazoan cell types examined, yet is mechanically impossible according to the classic sliding filament hypothesis, which has been the prevalent model of cytokinesis for 40 years. They find that a minimal mechanical feedback loop among membrane curvature, cytoskeletal alignment and contractility can reproduce the kinetics and geometry, i.e. asymmetry, of cytokinesis in control cells. **Mark Petronczki** (LRI, Cancer Research UK) identified an atypical C1 domain in the MgcRacGAP subunit of centralspindlin, a conserved component of the midzone and midbody, as a membrane tether that binds to phosphoinositide lipids. These results may illustrate the molecular basis for a long-postulated function of the midbody, the anchoring of the cleavage furrow. **Michael Glotzer** (University of Chicago, USA) recently identified a novel protein, NOP–1, that functions in parallel to CYK–4 to promote RhoA activation. In addition, they have discovered an inhibitory mechanism that suppresses centralspindlin-mediated contractility. **Helen Mattews** (University College London, UK) showed that Ect2 is also required in a RhoA-dependent manner for cell rounding at mitotic onset. Ect2 RNAi cells have profound defects in the mitotic actin cortex and are mechanically softer, leading to defects in spindle formation. **Anna Caballe** (King's College London, UK) used live cell imaging of dividing cells to reveal acceleration in midbody abscission upon CHMP4C depletion. She found that CHMP4C interacts with the chromosomal passenger complex (CPC) through Borealin, and that the kinase Aurora B regulate CHMP4C by phosphorylation, a requirement to the abscission checkpoint. **Arnaud Echard** (Institut Pasteur, France) addressed the role of ERM (Ezrin, Radixin, Moesin) activation in spindle orientation in mammalian cells both in culture and *in vivo*. Using fibronectin-coated micropatterns, he showed that ERM proteins and their asymmetric activation by the SLK kinase are required for the proper orientation of the mitotic spindle. Furthermore, he confirmed these results *in vivo* in apical progenitors of the mouse embryonic neuroepithelium.

8- Integrated views

Thomas Surrey (Cancer Research UK) established a novel cell-free system from single *Drosophila* embryos that supports successive mitotic nuclear division cycles with native characteristics. Using this new assay in combination with laser microsurgery he showed that in addition to anaphase spindle extension, migration of microtubule asters is crucial for the efficient distribution of nuclei throughout cytoplasmic space. Encapsulating nuclei in micro-fabricated chambers revealed that the early *Drosophila* embryo cytoplasm encodes a developmental program that ensures a distinct inter-nuclei distance that is independent of available space. **Matthieu Piel** (Institut Curie, France) showed that the timing of abscission might be dependent upon the forces that daughter cells exert on each other. Unexpectedly, he showed that such forces delay abscission. They propose that force dependent abscission delay could insure cohesion of the two daughter cells during the re-adhesion process, which could be important for tissue cohesion *in vivo*, and could also allow control of relative positioning of the two daughter cells.

CONCLUSIONS

A strong request to organize another meeting in the next 2 years (in 2014) was clearly and strongly voiced by many participants. Andrea Musacchio (Dortmund, Germany) accepted to become the next President, and Renata Basto (Paris, France) was elected to become the next vice-president.