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Le cycle cellulaire : vers une biologie intégrative de la division cellulaire

Cell cycle: bridging scales in cell division

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Rapport sur la Conférence

Conference Report

Report

With the generous financial and logistical support of the CNRS, and the very capable organization and support of Mrs. Nathalie Babic, the Jacques Monod conference on the Cell Cycle, held on October 11-15, 2014 in the picturesque village of Roscoff on the Brittany Coast (France) reached its 13th edition in the series. Attendants suffered one day of very stormy weather, but otherwise the climate was generally pleasant and enjoyable. The conference, titled "Bridging Scales in Cell Division", aimed at identifying problem in a continuum of biological scales, ranging from the detailed molecular events that subtend to the cell cycle, to their higher-level consequences at the cellular and organismal level. While the scientific Presidency (Andrea Musacchio) and vice-Presidency (Renata Basto) abided by the traditional format of the meeting, a new type of lecture, the "landmark lecture", was introduced to give adequate coverage to the historical development of the cell cycle field, to the special benefit of the younger audience. Prof. John Tyson, from Virginia Tech College in Blacksburg (USA) provided a very balanced account of the development of cell cycle studies and how mathematical formalism followed suite with fundamental predictions, for instance regarding the role of trigger waves. The oral presentations, regardless of whether delivered by invited speakers or by speakers selected from the posters, were recognized as being almost invariably of the highest quality, and were followed by lively rounds of stimulating discussion whose conclusion required almost invariably the intervention of the chairperson for the sake of timekeeping. There were two poster sessions, both of which were well attended and extremely lively. The interest of the topic, the scientific quality, and the fascinating and friendly atmosphere of Roscoff together contributed to the great success of the meeting and motivated many of its participant to propose its continuation. Indeed, many participants recognize that the meeting is unique and voiced their desire that the meeting be continued and a new edition held in the future. We have not received the results of the evaluation at the time of writing of this report, but we have every reason to believe that a high level of satisfaction was reached and that this will reflect in a very good evaluation.

The goal of cell division is to generate two daughter cells from a mother cell. Traditional work on the cell division cycle focused on the identification of the factors that drive the progression of the cycle and the correct ordering of cell cycle events, and their regulators that instate feedback control capable of arresting the cell cycle clock. This body of cell cycle research is of crucial importance because many fundamental aspects of the cell cycle remain unclear and require further elucidation. In addition to this fundamental cell cycle research, there is considerable interest in the mechanisms whereby the cell cycle oscillator regulates crucial events such as spindle assembly or the deposition of new CENP-A chromatin at the centromere. Finally, the question how the cell cycle, and in particular mitosis, shapes tissues in multicellular organisms attracts considerable attention. To provide each of these aspects with adequate coverage, the conference was organized in seven sessions, including:

Session I: Cell cycle transitions

Session II: Phosphatases

Session III: Spindle & Spindle checkpoint

Session IV: Chromosome instability

Session V: Asymmetry in cell division

Session VI: Chromatin and chromosome architecture

Session VII: Cytokinesis

The first evening, after a reception and dinner, was occupied by the Keynote lecture, given by **David Morgan** (UCSF, USA). His lecture provided an overview of the spectacular recent advancements in the study of a crucial machine in cell division, the anaphase promoting complex/cyclosome (APC/C), and in particular on the significance of recent structural studies. Morgan concluded his overview with important recent results from his laboratory showing that the so-called co-activators of the APC/C are more than simple substrate adapter, and rather act as catalytic activators of the APC/C by decreasing its Michaelis constant towards the E2 (Ub-conjugating) enzyme. The role of a new motif (the ABBA motif) found in the Bub1 and BubR1 proteins and required for an interaction with the Cdc20 co-activator was also discussed.

On the next day, the "Landmark Lecture" of **John Tyson** (Virginia Tech, Blacksburg VA, USA) focused on the questions that mathematical modelling can help solve in the area of cell cycle regulation. Tyson showed how the past 25 years had witnessed great advancements in the study of the cell cycle, and that such advancements had been paralleled by studies of mathematical modelling. The lecture was an excellent historical excursus on the topic and discussed the role of mathematical modelling to understand the basis of irreversible transitions in the cell cycle. The robustness of the cell cycle control network and the role of molecular fluctuations in yeast cells were also discussed.

The first session (Cell Cycle Transitions) analysed the events that cause the cell cycle to progress and their regulation. **Damien Coudreuse** from the Institute of Genetics and Development of Rennes (France) discussed the regulation of synthetic cell cycles in fission yeast engineered so that only one cyclin-dependent kinase and one cyclin are required for cell division. **Anna Castro** (Centre de Recherche de Biochimie Macromoléculaire, Montpellier, France) presented exciting unpublished work demonstrating that the kinase Greatwall, which is essential to promote mitotic entry and to maintain the mitotic state, is also a crucial role of cell proliferation downstream from the Akt/PKB kinase. The meiotic G2/M-phase transition in starfish oocyte was the topic of the presentation of **Takeo Kishimoto** (Ochanomizu University, Tokyo, Japan), who showed that the Cyclin B/Cdk1 complex phosphorylates Arpp19/Ensa to inhibit PP2A-B55 and that this requires an initial activation of cyclin B-Cdk1 by Akt/PKB. **Thomas Mayer** (University of Konstanz, Germany) reported that the APC/C is essential for entry into meiotic M-phase. In vertebrates, immature oocytes are arrested at prophase of meiosis I, an arrests lasting up to four decades in human. Mayer reported that depletion of Cdh1 from *Xenopus laevis* did not affect the prophase-I arrest of immature oocytes but rather their entry into MI upon progesterone (PG) stimulation. Additional studies indicated that the APC/CCdh1-mediated destruction of the phosphatase PP6c was essential to mediate the progesterone induced activation of Cdk1/cyclin-B and, consequently, for the entry of immature oocytes into the first meiotic M-phase. **James Ferrell** (Stanford University, USA) demonstrated the usefulness of trigger waves as a means to support Cdk1 activation in very large cells like eggs. Using cell free *Xenopus* egg extracts in thin Teflon tubes and a fluorescence microscopy assay for mitosis, it was possible to demonstrate the existence of such trigger waves. The control of cohesin cleavage in meiosis was the topic of the presentation of **Wolfgang Zachariae** (Max Planck Institute of Biochemistry, Martinsried, Germany), who uses budding yeast to investigate how centromeric cohesin is cleaved at the onset of anaphase II and how this event is coordinated with exit from meiosis II and spore formation, the yeast equivalent of gametogenesis. **Lionel Pintard** (Jacques Monod Institute, Paris, France) reported an investigation of the molecular mechanisms governing mitotic entry during animal development, showing that the mitotic kinase Cdk1 phosphorylates SPAT-1/Bora to regulate its interaction with Polo-like kinase 1 (PLK1) and to promote PLK1 activation and mitotic entry in *C. elegans*. **Rene Medema** (The Netherlands Cancer Institute, Amsterdam, The Netherlands) reported that a cell's decision to irreversibly withdraw from the cell cycle is made within a few hours following damage in G2 cells,

and that a permanent arrest is dependent on induction of p53 and p21, resulting in the nuclear translocation of Cyclin B1 and the activation of APC/C^{Cdh1} several hours later. Inhibition of APC/CCdh1 activity fails to prevent cell cycle withdrawal, whereas preventing nuclear retention of Cyclin B1 allow cells to remain in cycle. In an interesting talk related to that of Lionel Pintard, **Olivier Gavet** (Institut Gustave Roussy, Villejuif, France) reported that the activation of Plk1 takes place in late G2. It precedes Cyclin-B1-Cdk1 one by a few minutes and relies on upstream Aurora-A kinase, thus clarifying some upstream molecular steps leading to CyclinB1-Cdk1 initial activation and mitotic entry. Finally, **Arnaud Echard** (Institut Pasteur, Paris, France), reported how midbody remnant after cytokinesis abscission in mammalian cells become engulfed by an actin-dependent phagocytosis-like mechanism, possibly suggesting a mechanism by which the remnants of the midbody may signal over long distances between cells.

The second session (Phosphatases) started with an interesting talk by **Mathieu Bollen** (University of Leuven, Belgium), who congratulated the organizers for dedicating an entire session to phosphatases, important cell cycle regulators that have not been given adequate attention previously. Bollen discussed the intricate relationship between PP1 and PP2 phosphatases and how Repo-Man acts as a chromosome-based scaffold to regulate these proteins and their interaction with the Aurora B kinase. **Nelio Rodrigues** (University College London, United Kingdom) reported that kinetochore-localized PP1/Sds22 inactivate cortical Moesin and elicit relaxation of the actomyosin cortex at mid-anaphase. Delocalization of PP1/Sds22 from the kinetochores through KNL1 depletion prevents polar blebbing at anaphase and impairs cell elongation, thus showing direct coupling between anaphase chromosome movements and cell division. Extending the role of phosphatases at kinetochores, **Ulrike Gruneberg** (Sir William Dunn School of Pathology, Oxford, United Kingdom) reported that PP2A-B56 opposes Mps1 phosphorylation of Knl1 to promote spindle assembly checkpoint silencing. Similar results, but with an important twist, were presented by **Adrian Saurin** (University of Dundee, United Kingdom), who showed that SAC signalling recruits PP2A-B56 to kinetochores to antagonize Aurora B and promote PP1 recruitment. PP1 in turn silences the SAC and delocalizes PP2A-B56. Interestingly, there appears to be a PP1/PP2A phosphatase relay based on the physical interaction of these proteins in a single complex, as reported by **Iain Hagan** (University of Manchester, United Kingdom) in an interesting presentation.

The third section (Spindle and Spindle checkpoint) was extensive and started with an impressive presentation by **Gohta Goshima** (Nagoya University, Japan) who demonstrated the power of biochemical reconstitution in the study of microtubule plus-end dynamics. Regretfully, Jordan Raff was forced to cancel his participation to the meeting due to personal reasons and was replaced by **Derek McCusker** (European Institute of Chemistry and Biology, Bordeaux, France), who discussed how nano-scale clustering of the Rho GTPase module into cortical membrane domains contributes to establishing a polarity axis in budding yeast. **Jens Lüder**, from the Institute for Research in Biomedicine in Barcelona (Spain), discussed how γ-TuRCs associates with minus ends of non- centrosomal spindle microtubules and becomes then transported to minus ends by motors dynein such as HSET and Eg5. The exciting presentation of **Arshad Desai** (University of California, San Diego, USA) diverged from that in the abstract and focused on the remarkable mechanism of anaphase chromosome separation in meiotic *C. elegans* cells, and more specifically on the role of the nuclear envelope protein ELYS in this process. **Andrea Musacchio** (Max-Planck-Institute of Molecular Physiology, Dortmund, Germany) discussed the mechanism of kinetochore recruitment of the Bub1 and BubR1 components of the spindle assembly checkpoint, demonstrating how these paralogs diverged substantially to perform their function optimally. In as logical follow-up, **Ariane Abrieu** (CNRS, Montpellier, France) reported how in C. elegans Plk1 replaces Mps1 as the kinase responsible for the phosphorylation of the Knl1 kinetochore scaffold, which in turn promotes kinetochore recruitment of Bub1. The

next presentation, from **Andrea Ciliberto** (IFOM, Milan, Italy), focused on the systems level implications arising from the two functions that the Cdc20 protein performed in the cell cycle, as a promoter of cell cycle progression and APC/C activator, and on APC/C inhibition as a component of the spindle assembly checkpoint. **Susanne Lens** (University Medical Center Utrecht, Utrecht, The Netherlands) reported that in addition to BubR1, also Shugoshin-1 (Sgo1) contributes to the recruitment of PP2A-B56 to centromeres and kinetochores. The different pools of phosphatase activity may work in in concert to regulate Aurora B activity and kinetochore substrate phosphorylation to appropriately tune the stability of kinetochore-microtubule attachments. In a related talk, **Andreas Wallek** (Max Planck Institute of Biochemistry, Martinsried, Germany) analysed the role of Sgo1 in the centromeric recruitment of the regulatory subunit of the protein phosphatase 2A Rts1 during mitosis. Interestingly, Sgo1 was essential to maintain the enrichment of condensin complexes on centromeric chromatin, and was responsible for the turnover of kinetochore-microtubule attachments by stabilizing Ipl1/Aurora B. **Stefan Westermann** from the Research Institute of Molecular Pathology (IMP, Vienna, Austria) presented a "skateboard model" to clarify how asymmetric Kar3 motors in *S. cerevisiae* interact with microtubules, and suggested that the Kar3 complex may rectify a potentially diffusible enzyme with a non-catalytic binding mechanism mediated by the Kar3 binding partners Cik1 or Vik1.

Moving now into the fourth session (Chromosome instability), **Rong Li** (Stowers Institute for Medical Research, Kansas City, USA) reported the development of a single-cell quantitative assay for direct measurement of chromosome instability (CIN) in budding yeast. Genes that affect CIN in a dosage-sensitive manner were identified in a genome-wide assay. The screens brought to light that heterogeneous aneuploidy generated by CIN scales directly with the degree of overall growth inhibition under diverse stress conditions. A strategy of "evolutionary trap" (ET) to target the adaptabilty and fitness of aneuploid populations under stress was presented. In her impressive talk **Kerstin Knouse** (Massachusetts Institute of Technology, Cambridge, USA) presented her measurements of the incidence of somatic aneuploidy in mouse and human tissues, demonstrating that aneuploidy occurs much less frequently in the liver and brain than previously reported and is no more prevalent in these tissues than in skin, arguing against a positive role for aneuploidy in organ function. **Renata Basto** (Institut Curie, Paris, France) showed that aneuploidy causes brain size reduction in developing Drosophila brain due to decrease in the number of proliferative neural stem cells. Anueploid cells exited the cell cycle and underwent premature differentiation. In his talk, **Stephen S. Taylor** (University of Manchester, United Kingdom) discussed the result of a screen aiming to test the competing network model to explain a cell's decision to undergo death in mitosis or to undergo slippage. Remarkably, the transcription factor Myc was the only factor identified in the screen and in additional secondary and ternary screens whose presence is clearly required for death in mitosis.

In his exciting talk that started the fifth session (Asymmetry in cell division), **Michael Lampson** (University of Pennsylvania, Philadelphia, USA) described a mechanism leading to unequal chromosome segregation during female meiosis, in violation of Mendel's First Law. Such "meiotic drive" depends on asymmetric spindle microtubules and asymmetric centromere strength. Equally excitingly, **Patrick Meraldi** (University of Geneva, Switzerland) reported that old and new centrosomes behave differently in mitosis, even during symmetric cell division, and that this influences the ability of polar chromosomes to align on the metaphase plate, suggesting differences in the stability of kinetochore microtubules for old and new centrosomes. **Péter Lénárt** (European Molecular Biology Laboratory, Germany) reported on studies of centriole elimination in starfish oocytes in which a GFP marker was established to follow the entire process of centrosome elimination *in vivo*, revealing that the mother centriole is always extruded into the second polar body, whereas a single daughter centriole remains in the mature egg. **Wang Xuan** (ETH, Zürich,

Switzerland) reported on the behaviour of transfected exogenous DNA containing repetitive LacO sequences in mammalian cells. The results were consistent with the existence of a cellular response pathway recognizing both plasmid and telomeric extra-chromosomal DNAs, resulting in their clustering outside the nucleus. **Pierre Gönczy** (EPFL, Lausanne, Switzerland) reported that in human cells the ternary complex members LGN and $G\alpha_{i1-3}$, but not NuMA, are dispensable for cortical dynein enrichment during anaphase, in contrast to their requirement in metaphase. NuMA associates with PtdInsP (PIP) and PtdInsP$_2$ (PIP$_2$) phosphoinositides *in vitro*, and the depletion of such phosphoinositides prevents NuMA cortical localization *in vivo*, thus revealing a function of plasma membrane phospholipids in cortical dynein distribution during mitosis. **Fumio Matsuzaki** (RIKEN Center for Developmental Biology, Japan) reported on the role of FGF18 in the self-renewal potential of radial glia, i.e. neural stem cells.

Session VI was opened by a talk by **Sebastian Müller** (Institut Curie, Paris, France), who replaced Genevieve Almouzni who was forced to decline her participation. The talk reported on the role of CENP-C in the interaction with the specialized histone chaperone HJURP, which is responsible for the deposition of new CENP-A (CenH3) at the centromere during mitotic exit. On similar notes, **Ana Stankovic**, from the Instituto Gulbenkian de Ciência (Oeiras, Portugal) reported the identification of phosphorylation sites on Mis18BP1 (the largest member of the Mis18 complex) that counteract its kinetochore localization and activity. Furthermore, a cyclin interaction site within the CENP-A specific chaperone HJURP was identified that prevented HJURP phosphorylation. Co-expression of proteins carrying mutations at these CDK-responsive residues was sufficient to induce premature CENP-A assembly, uncoupling cell cycle progression from nascent CENP-A deposition. **Daniel Gerlich** (IMBA, Vienna, Austria) showed that adhesion between chromosomes is low during mitosis but increases at mitotic exit resulting in chromosomes clustering prior to nuclear envelope reformation. An imaging-based RNAi screen identified Ki-67 as a candidate chromosome surface adhesion regulator. In his talk, **Thomas Gligoris** (University of Oxford, United Kingdom) reported a biochemical and structural characterisation of the cohesin's Smc3/kleisin DNA-exit gate that was instrumental to unequivocally demonstrate entrapment of chromosomes within chemically formed cohesin rings *in vivo*. In a related talk, **Christian Haering** (EMBL, Heidelberg, Germany) reported on the organization of Condensin rings and on the role of a DNA sensor formed by the two HEAT-repeat subunits of condensin, which appears to activate the SMC ATPase-dependent transport of chromosome fibers into the condensin ring. **Sylvie Tournier** (Université de Toulouse, France) reported on a new role of Aurora B in telomere dispersion and disjunction during mitosis, suggesting that Aurora B targets distinct heterochromatin domains, centromeres and telomeres to control chromosome segregation. **Kota Nagasaka** (Japanese Foundation for Cancer Research, Tokyo, Japan) reported on results obtained with a newly developed live cell sensor to measure the timing of sister chromatid resolution during the cell cycle.

After the extraordinary banquet, the meeting punctually resumed the next morning with the final session (Session 7) on Cytokinesis. The first speaker in the session, **Phong Tran** (Institut Curie, Paris, France), discussed the role of the fission yeast kinesin-14 Pkl1p, which functions to focus MT minus ends at the poles, and whose depletion leads to unfocused and unstable spindle poles, with aberrant MT protrusions away from the main spindle mass, leading to spindle positioning asymmetry and subsequent chromosome missegregation during cytokinesis. In her interesting talk, **Olga Afonso** (Universidade do Porto, Portugal) discussed a conserved feedback control mechanism that delays chromosome decondensation and NER in response to incomplete chromosome separation during anaphase, and that is based on an Aurora B gradient associated with the spindle midzone. **Esben Lorentzen** (MPI of Biochemistry, Martinsried, Germany) reported the

reconstitution and structural analysis of active Rab11-GTP in complex with the C-terminal effector domain of Rabin8. The structural work has important implications for vesicular transport to the cleavage furrow and the base of the cilium. **Anne Paoletti** (Institut Curie, Paris, France) reported on her exciting work on the characterization of the molecular basis for the specification of the division plane in *S. pombe*, which is based on a feedback mechanism involving the positioning factor Mid1/Anillin and its regulation of a Pom1/DYRK kinase gradient emanating from cell tips. In the last presentation of the meeting, **Kristyna Kotynkova** (London Research Institute, Potters Bar, United Kingdom) reported on her interesting and surprising studies on the role of the conserved RhoGEF factor Ect2. A mutant protein unable to bind centralspindlin was largely able to support cytokinesis in cells depleted of endogenous Ect2, a finding inconsistent with current models of cleavage furrow formation and positioning