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Sciences du Vivant - Environnement et Développement durable

CONFÉRENCES JACQUES-MONOD

Roscoff (France), 26-30 avril 2008

Cycle de division cellulaire et stabilité du génome

The Cell Cycle and Genomic Stability

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Conférence Jacques Monod
„Cycle de division cellulaire et stabilité du génome“

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La série de conférences Jacques Monod dédiée au cycle cellulaire débuta par une première conférence organisée par Eric Karsenti et Tim Hunt à Roscoff en septembre 1988. C'est lors de cette conférence inaugurale que les participants réalisèrent pour la première fois qu'ils travaillaient tous sur la même kinase et que le rôle et l'importance des complexes cycline/kinase-dépendantes des cyclines (Cdk) furent conceptualisés largement tels que nous les connaissons aujourd'hui. Entre temps, trois des participants à la première conférence ont obtenu le prix Nobel de médecine pour ces découvertes. Depuis 1988, la série de conférences Jacques Monod consacrée au cycle cellulaire a toujours joui d'une très grande réputation dans le domaine et toutes les éditions précédentes étaient d'une très haute tenue et marquées par une très bonne atmosphère d'échanges et de discussions. La conférence de cette année, la neuvième de la série, célébrait donc le 20ème anniversaire de ces conférences. Elle fut organisée par Jonathon Pines et Yves Barral et dédiée à la stabilité du génome, et fut l'occasion de combiner un regard sur le chemin accompli, notamment au travers de la conférence inaugurale de Tim Hunt, le découvreur des cyclines et l'un des lauréats du prix Nobel, et de s'interroger sur les prochaines étapes à franchir.

Le cycle cellulaire est le processus au cours duquel la cellule se prépare et finalement accomplit sa division en deux cellules filles au potentiel génétique équivalent. Ce processus qui inclut la réplication de l'ADN et des centrosomes, la formation du fuseau mitotique, la ségrégation des chromosomes et finalement le clivage de la cellule (cytocinèse), est régulé de façon très précise de façon à prévenir des divisions indues et à assurer que toutes les étapes du processus soient accomplies dans le bon ordre et de façon satisfaisante. Au cours des 20 dernières années il est devenu clair que des dysfonctionnements de ces régulations sont impliqués dans le développement de cancers, soit parce qu'ils permettent à la cellule de se diviser indépendamment des restrictions

imposées par le tissu où elle se trouve, soit parce qu'ils permettent la déstabilisation du génome et ainsi l'accumulation des mutations nécessaires à la tumorigénèse. La conférence 2008 s'est penchée prioritairement sur les mécanismes assurant la stabilité du génome. Sept thèmes furent abordés dans ce contexte :

1- Mécanismes assurant l'établissement de la cohésion des chromatides sœur au cours de la réplication du génome.
2- Mécanismes de régulation de l'entrée en mitose.
3- Mécanismes de réplication des centrosomes et d'assemblage du fuseau mitotique.
4- Attachement des chromosomes au fuseau et mécanismes du checkpoint d'assemblage du fuseau.
5- Régulation de l'anaphase par le complexe APC/cyclosome.
6- Régulation de la cytocinèse.
7- Le rôle de l'aneuploïdie dans la tumorigénèse.

La conférence, qui sût retrouver la convivialité et le niveau de discussion des précédentes éditions fût très appréciée par tous les participants. Il y fût décidé de continuer cette série, si le CNRS le permet, et le vice président Yves Barral y fût invité à organiser la prochaine conférence avec l'aide de Ariane Abrieux.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled *The Cell Cycle and Genomic Stability* Roscoff, 26-30 April 2008

The recent Jacques Monod conference on the Cell Cycle: Genomic Stability continued the high standards set by previous meetings. The quality of the talks was excellent and both poster sessions were very lively and overflowing with participants. 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 50 % oversubscribed and the overwhelming support from the participants for organising another meeting in 2 years time (62/62 respondents). We are very grateful to the CNRS for supporting the meeting.

Six themes were addressed at the conference: DNA replication and chromatid cohesion; the decision to enter M phase; Centrosomes and spindle assembly; Chromosome attachment and the spindle assembly checkpoint; The anaphase promoting complex; Cytokinesis and cell division.

Tim Hunt (LRI, Clare Hall) gave the plenary lecture in which he reviewed the progress made in the field over the last 20 years, and presented his own recent research on how exit from mitosis is regulated, which has revealed an important role for the PP2A and calcineurin, a calcium-dependent phosphatase.

1) DNA replication and chromatid cohesion

For daughter cells to inherit identical copies of the genome it is important for newly replicated DNA to remain attached to its sister chromatid until mitosis. This is achieved by the cohesions and Kim Nasmyth (Oxford) presented his elegant work showing that cohesin's form a ring to bind the replicated DNA strands together and Jean-Paul Javerzat (Bordeaux) gave insights into how cohesin is loaded onto DNA and stabilised during

DNA replication. Marcel Mechali (Montpellier) showed how DNA replication origins alter their strength when cells differentiate, and can be remodelled by mitotic Xenopus extracts. Paola Vagnerelli (Edinburgh) gave a short talk (selected from the abstracts) on her work on the chromatin structure of the centromere and how this was altered in the absence of the condensin complex. Mitsuhiro Yanagida showed further how condensins and cohesions are recruited to centromeres in the fission yeast, and how this interacts with the DNA damage pathway. Finally Aude Dupré (short talk) showed that the mos-MAP kinase pathway represses DNA replication in meiosis by acting on the Cdc6 protein.

2) The decision to enter M phase

Sally Kornbluth (Duke) linked DNA damage and and mitotic entry through the Aven protein, a newly identified activator of ATM, and Anne Royou (UC Santa Cruz, short talk) showed that the *Drosophila* DNA damage response kinase, Grps, blocks entry to mitosis by preventing cyclin B-Cdk activation and independently blocking its nuclear import. Pat O'Farrell (UCSF, short talk) showed that cyclin B in *Drosophila* has a rate-limiting role in mitotic entry in early embryos and that its activity is controlled by both proteolysis and phosphorylation on the 'T-loop' of the Cdk that until now had been thought to be constitutive. Catherine Jessus (Paris) showed that cyclin B-Cdk1 is also the main trigger of mitosis in Xenopus oocytes and that the mos-MAPK pathway and Polo-like kinase (Plk) are not, as previously proposed upstream, but act as part of a positive feedback loop downstream of cyclin B-Cdk1. David Glover (Cambridge, short talk) showed that the Greatwall or Scant kinase is part of this downstream control in *Drosophila* acting on Plk. Takeo Kishimoto (Tokyo, short talk) also showed that Plk1 was downstream of Cdk1 in starfish oocytes and that together Cdk1 and Plk1 were required for MPF activity. Iain Hagan (Manchester) showed that the recruitment of Plk to the spindle pole body (equivalent to the animal cell centrosome) in fission yeast, which is an important trigger of mitosis, is controlled by the TOR stress pathway and cyclin B-Cdk1 in G2 phase.

3) Centrosomes and spindle assembly

Centrosomes were dealt with in more detail in the third session, in particular their role in nucleating the mitotic spindle. Prasad Jallepalli (New York, short talk) used somatic cell mutation to demonstrate that the separase protein is required for centrioles to separate in human cells. Erich Nigg showed that the Plk4 kinase (and later Monica Bettancourt-Dias, Lisbon, short talk) has a crucial role in regulating centriole biogenesis, and Erich Nigg also showed that the Bora protein is an important regulator of centrosome maturation and subsequent spindle assembly through binding the Aurora A kinase and competing for Aurora A with the TPX2 protein. Isabelle Vernos (Barcelona) showed that the first 43 amino acids of TPX2 are required to localise it and Aurora A to the centrosomes and microtubules and thus for spindle assembly, and that the TACC3 protein is a crucial substrate. Hiro Funabiki (New York) demonstrated that the other Aurora family member, Aurora B, was also required for spindle assembly in *Xenopus* egg extracts and that it was activated by both microtubules through the INCENP protein, and by chromosomes acting to concentrate Aurora B. In addition to the centrosome-dependent spindle assembly pathway there is a parallel pathway dependent on the Ran GTPase and Eric Karsenti (EMBL) showed that the Cdk11 kinase is the key chromosome-associated player in this. Monica Gotta (Geneva) identified the G-proteins that are subsequently responsible for positioning the spindle within a *C. elegans* embryo in response to polarity cues, and found that these act through a dynein-dependent mechanism. Valerie Doye (Paris) addressed the intriguing question of what some of the components of the nuclear pore do when they reloacalise to the kinetochores in mitosis. She found that depleting the Nup107-160 complex causes subsequent problems in chromosome attachment and the loss of RanGAP, RanBP2 and Crm1 from kinetochores.

4) Chromosome attachment and the spindle assembly checkpoint

Mary Dasso (NIH) continued the theme of how Ran acts on the spindle. She showed that the Nup107-160 recruits the gamma tubulin ring complex to the kinetochore and that this is essential for Ran-dependent nucleation and stabilisation of microtubules. Patrick Meraldi (Zurich, short talk) showed that kinetochore microtubules are particularly important to form a bi-polar spindle if centrosomes don’t properly separate before nuclear

envelope breakdown. Andrea Musacchio (Milan) presented the crystal structure of the Nuf2/Ndc80 complex that binds microtubules at the kinetochore. Andrew Murray (Harvard) had begun to determine the minimum requirement for a functional kinetochore. Using a lac operator array on a yeast chromosome he targeted individual kinetochore proteins to the array with a lac I fusion and assayed chromosome segregation. Bill Earnshaw (Edinburgh) had built a minimal centromere in human cells. He used an array of alpha satellite DNA with alternating repeats of a CENP-B binding motif and a tet operator on a circular plasmid. He then altered chromatin structure by targeting transcription activators or repressors with the tet repressor and assayed the effect on chromosome segregation. The proper segregation of chromosomes depends crucially on their attachment to the mitotic spindle in a bi-polar fashion; therefore, cells have a Spindle Assembly Checkpoint (SAC) that prevents anaphase when chromosomes are incorrectly attached. Kevin Hardwick (Edinburgh, short talk) characterised some of the SAC complexes in fission yeast, and Roger Karess (Paris) showed that one of the most conserved components, Mad2, was not required for viability in *Drosophila,* whereas another, BubR1, was essential because of an additional role in chromosome attachment. Stephen Taylor (Manchester, short talk) showed that the Bub1 protein was essential for the checkpoint in the mouse.

5) The anaphase promoting complex

The Spindle Assembly Checkpoint prevents anaphase by blocking the proteolysis of securin and cyclin B directed by the Anaphase Promoting Complex/Cyclosome (APC/C), a ubiquitin ligase. David Morgan (UCSF) showed that securin proteolysis in yeast is coupled to rapid chromosome segregation by a positive feedback with the Cdc14 phosphatase. Jonathon Pines (Cambridge) showed that the SAC acts on the APC/C by converting the APC/C activator, Cdc20, into a substrate of the APC/C. Hiro Yamano (Oxted, UK, short talk) found that it is the amino terminus of Cdc20 that activates the APC/C against particular substrates.

6) Cytokinesis and cell division

The last event in cell division is the formation of the 2 daughter cells – cytokinesis. Bruno Goud (Paris) reviewed the role of the Rab proteins in membrane traffic in mitosis and three short talks from Hemmo Meyer (Zurich), Manuel Mendoza (Zurich) and Daniel Gerlich (Zurich) revealed the role of the Aurora B protein in regulating nuclear envelope reassembly through p97/Cdc48 in *Xenopus* and in preventing abscission in budding yeast and mammalian cells. Anne Paoletti (Paris) showed how the Mid1 protein and the Polo-like kinase select the site for cytokinesis in fission yeast. Yves Barral (Zurich) showed that there is a barrier at the bud neck in budding yeast that prevents the passage of rDNA circles between mother and daughter cells and is crucial to determining the proliferative life span of the two cells.

7) Aneuploidy and tumorigenesis

The final session of the conference was largely devoted to the connection between genomic stability and tumourigenesis. Tony Hunter (Salk Institute) showed that the Chk1 kinase that responds to DNA damage is regulated by ubiquitination catalysed by Fbx6/SCF complex, and that Chk1 itself is important in regulating a block to homologous replication when additional DNA damage lesions are generated after DNA damage repair has begun. Willy Krek (Zurich) provided evidence that the VHL tumour suppressor stabilises microtubules in the primary cilium, and elucidated a pathway connecting VHL and the PTEN tumour suppressor that may be particularly important in preventing tumours in kidney cells. Lastly, Renata Basto (Cambridge, short talk) and Don Cleveland (La Jolla) both addressed the question of whether genomic instability could be the cause of cancer. Renata Basto showed that multiple centrosomes in *Drosophila* brain cells perturbed asymmetric division and this could generate tumours, whereas Don Cleveland showed that mice lacking the CENP-E protein mis-segregated chromosomes at a higher rate than normal and in some tissues this caused tumours, but not in others.

Suggestions for Improvements

The advent of the new Gulf Stream Hotel requires that more time be set aside for lunch and dinner breaks to allow for the walk to the hotel. In light of this the conference guidelines may need to be revised to reduce the required number of speakers.

A larger room for the poster sessions would be an improvement. A larger venue for the coffee breaks would also be welcome. The train schedule required that the Wednesday morning session be quite short. Again the guidelines for the conference could be altered to reflect this. Wireless access could be improved – it was difficult to establish an account for many participants.

The requirement to raise 20% of the funds for the conference proved a challenge because of the rules restricting advertising sponsorship in the meeting program and on the web site, and the fixed fees. Many of the companies approached declined to sponsor the meeting because they felt that they would receive insufficient publicity for the fee requested. The CNRS may wish to review its policy on advertising if future conferences will also be dependent on raising external funds.

Lastly, although some participants commented that the meeting was too focussed, others declared that it covered a broad range of exciting topics. We think this probably reflects the emphasis given to the participant's particular specialty since the cell cycle field has expanded so much that no one meeting can be expected to cover all aspects in a satisfactory manner.