Tension at EMBO's Aneuploidy Workshop

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The EMBO 2010 Workshop on Chromosome Segregation and Aneuploidy, held between 19 and 23 June at Edinburgh University's Royal College of Surgeons, highlighted basic mitotic mechanisms, chromosome-based defects linked to human diseases and the processes that connect them.

During cell division, protein assemblies called kinetochores segregate the replicated chromosomes (sister chromatids) so that each daughter cell receives a complete copy of the genome (Fig 1). When chromosome segregation goes awry, it can generate aneuploid cells containing abnormal numbers of chromosomes. These cells are usually inviable, but those that do survive can contribute to genetic diseases, including cancer.

The 2010 EMBO Workshop on Chromosome Segregation and Aneuploidy, organized by Bill Earnshaw, Kevin Hardwick, and Margarete Heck (all at U. Edinburgh, UK) brought together researchers from the basic and clinical fields to discuss chromosome segregation and aneuploidy, and how the latter might contribute to tumour transformation and genetic disease.

Preventing aneuploidy

To prevent aneuploidy, a cell must detect and resolve errors that could lead to chromosome segregation defects. A major area of research highlighted at this meeting was the mechanism by which the spindle assembly checkpoint (SAC) detects errors and relays this information to delay cell-cycle progression. There was an exciting debate about whether the sensed errors include the absence of kinetochore–spindle attachments or defects in the forces produced at spindle-bound kinetochores. Thus, while the meeting was highly collegial, it was not without ‘tension’.

Two mitotic kinases—Aurora B and Mps1—were subjects of intense focus. Aurora B—part of the chromosome passenger complex (CPC; Ruchaud *et al*,

2007)—corrects improper chromosome–spindle attachments, promoting bi-orientation of all sister chromatids. It is thought that Aurora B might indirectly contribute to the SAC through its kinetochore–microtubule destabilizing activity. However, others have proposed that Aurora B directly controls SAC function. The lab of Susanne Lens (U. Medical Center Utrecht, the Netherlands) has investigated this by generating a mutant in the CPC subunit INCENP that is SAC defective. They found that the mutant can still correct errors in chromosome segregation, suggesting that the SAC control and error correction roles of the CPC are separable. Similarly, by partly depleting the kinetochore component Nuf2—which reduces kinetochore–microtubule binding, but leaves the SAC intact—and then inhibiting Aurora B activity, Andrea Musacchio's lab (European Institute of Oncology, Italy) observed an override of the checkpoint arrest, suggesting that Aurora B plays a direct role in SAC function.

When interkinetochore tension is generated, the SAC is silenced (Fig 1), and must remain silent throughout the remainder of mitosis. At anaphase onset, cohesion between sister chromatids, and thereby tension, is lost from all chromosomes. The laboratories of Mark Petronczki (Clare Hall Laboratories, UK)—working with human cells—and Frank Uhlmann (Cancer Research UK, London)—working with budding yeast—have addressed why the checkpoint is not re-engaged at anaphase. The reason seems to be the relocation of the CPC from kinetochores to the spindle midzone. Uhlmann and colleagues

showed that relocation of the CPC from kinetochores on dephosphorylation of the CPC subunit INCENP/Sli15 inactivates the checkpoint (Mirchenko & Uhlmann, 2010). Petronczki's lab anchored Aurora B to centromeres, thereby preventing its relocation at anaphase onset. They found that this recruits a subset of SAC proteins to anaphase kinetochores (Vázquez-Novelle & Petronczki, 2010). Taken together, these results suggest that CPC relocation removes the tension sensor from kinetochores to inactivate the checkpoint, thereby preventing its re-engagement when tension is lost in anaphase.

New studies also highlighted the role of the Mps1 kinase in SAC signalling, showing that Mps1 is required for the localization of SAC proteins to tension-less kinetochores. However, which checkpoint proteins require Mps1 activity for their recruitment to kinetochores remains unclear. These inconsistencies reflect the degree of Mps1 inhibition, the time when Mps1 is inhibited—before or after entry into mitosis—and the cell type that is studied.

The lab of Geert Kops (U. Medical Center Utrecht, the Netherlands) studies how Mps1 contributes to error correction and SAC activation. Mps1 has a short half-life at kinetochores and Kops's lab showed that Mps1 itself controls this turnover. To analyse this turnover, they stably targeted Mps1 to kinetochores as a Mis12–Mps1 fusion protein, and observed an extended metaphase delay due to the recruitment of multiple SAC proteins to fully attached, bi-oriented kinetochores. This finding suggests that Mps1 can directly promote SAC signalling

Fig 1 | Mechanisms contributing to aneuploidy. Left: Cell division process in wild-type cells producing identical diploid daughter cells. The cell cycle stages, ploidy and essential activities are shown. Middle: Aneuploidy caused by the presence of a sister chromatid pair that is merotelically attached to the mitotic spindle. Merotelic attachments occur during wild type mitosis but are quickly corrected. However, when a merotelically bound chromosome pair is not corrected, this results in a lagging chromosome during anaphase and an aneuploid daughter cell. Right: Aneuploidy caused by the presence of multiple centrosomes leading to a multipolar spindle. Multipolar mitosis leads to the death of most daughter cells unless centrosomes cluster. Although the progeny shown here have lost chromosomes, in a multipolar mitosis daughter cells can also gain chromosomes. Adapted from Weaver and Cleveland (2006).

and that removal of Mps1 from bi-oriented kinetochores is a prerequisite for the transition to anaphase. Stephen Taylor (U. Manchester, UK) discussed his lab's work with a new Mps1 inhibitor (AZ3146) and an antibody that recognizes specifically Mad2 that is recruited to unattached kinetochores. They showed that Mps1 inhibition after cells enter mitosis prevents Mad2 recruitment, and hence SAC activation (Hewitt *et al*, 2010). When the Musacchio lab inhibited Mps1 activity with the drug Reversine, they observed that Mps1 activity is required for accurate chromosome segregation. In particular, the correction of syntelically attached sister chromatids—in which both sister kinetochores are bound to the same centrosome—was inhibited in the absence of Mps1 activity (Santaguida *et al*, 2010). The Taylor and Musacchio labs further showed that Aurora B, known to be required for the correction of syntelic and merotelic—in which one kinetochore is bound to one centrosome, the other to both centrosomes (Fig 1)—is required for Mps1 localization to kinetochores, suggesting that Aurora B acts upstream to Mps1 (Hewitt *et al*, 2010; Santaguida *et al*, 2010). It remains unclear whether Mps1 also acts separately in the correction of merotelically bound sister chromatids from Aurora B.

While numerous studies have explored the downstream role of the SAC in inhibiting cell-cycle progression in the presence of chromosome segregation defects, it remains unclear how the latter contribute to SAC signalling. Todd Stukenburg (U. Virginia, USA) described his work in collaboration with Dan Burke, showing that certain kinetochore proteins thought to have exclusively structural roles are also required for SAC activity. Traditionally defined SAC components—such as Mad2—respond to a lack of microtubule occupancy and interkinetochore tension, caused for example by microtubule depolymerization. By contrast, Aurora B/Ipl1 and BubR1/Mad3 are required for the cellular response to a lack of interkinetochore tension. By using a budding yeast *mad3* phosphomutant, which mimics the lack of phosphorylation by Ipl1, the tension-responsive branch of the SAC was eliminated. By using this strain, Burke's lab screened the yeast gene deletion collection for mutants that cannot grow in the presence of a spindle-depolymerizing drug. They identified the conserved kinetochore proteins Chl4 (CENP-N), Ctf19 (CENP-P) and Ctf3 (CENP-I), suggesting that these

might function specifically in the occupancy branch of the SAC. They further demonstrated that CENP-I is required to arrest HeLa cells in the presence of spindle poison nocodazole, when the SAC was comprised by reducing Aurora B activity.

Generating aneuploidy

The presence of one (trisomy) or two (tetrasomy) extra copies of a chromosome characterizes many childhood cancers. By applying multi-colour labelling and holographic real-time microscopy to Wilm's tumour cells (kidney cancer in children), as well as statistically analysing chromosome transmission patterns, David Gisselson's team (Lund U., Sweden) found that tripolar spindles and incomplete cytokinesis are the most likely cause of asymmetrical chromosome segregation in childhood cancers. Cells with supernumerary centrosomes—which are likely to form multipolar spindles—have problems satisfying the SAC. However, if the chromosome pairs bound to the tripolar spindle have all their kinetochores under tension (Fig 1), tripolar mitosis can satisfy the SAC. This explains why erroneously dividing Wilm's tumour cells are not always halted by the mitotic checkpoint.

Duane Compton (Dartmouth U., USA) emphasized the difference between aneuploidy—which refers to a cell state in which the number of chromosomes differs from the standard ploidy—and chromosomal instability (CIN)—which refers to the frequency of chromosome mis-segregation. One significant cause of CIN is the persistence of merotelic attachments of chromosomes to spindle microtubules; merotelic attachments occur during normal mitosis, but become quickly resolved (Fig 1). Compton's lab showed that many cancer cell lines with CIN have hyper-stable kinetochore-microtubule attachments. These might reduce the ability to correct wrongly oriented attachments, and thus contribute to chromosome mis-segregation and cancer transformation. They confirmed this hypothesis by destabilizing kinetochore microtubules through the overexpression of microtubule-depolymerizing proteins (MCAK, Kif2b). This treatment restored correct chromosome segregation in tumours xenografted on mice, resulting in enhanced mouse survival. These observations have important clinical implications, as reversing CIN might be a promising anti-cancer strategy.

Agents that disrupt spindle microtubules are a key tool in the clinic. However, many

questions about tumour response to these compounds remain unanswered. Kip Sluder's lab (U. Massachusetts, USA) found that even a brief treatment of cells with anti-microtubule drugs can result in cell death. Surprisingly, this does not occur by arresting the cells in mitosis. Instead, the cells proceed through normal cell division when the drug is removed, but arrest terminally in interphase. This does not seem to be a consequence of DNA damage and is an exciting alternative way of using these compounds.

Increases in chromosome mis-segregation can occur naturally. As women get older, the frequency of miscarriage and trisomic pregnancies significantly increases. The majority of trisomic pregnancies result from errors during the first meiotic division when pairs of recombinant homologous chromosomes disjoin. Research in Mary Herbert's lab (Newcastle U., UK) indicates that female reproductive ageing in mice is characterized by a dramatic decline in the ability of oocytes to synchronously segregate their homologous chromosomes during anaphase of meiosis I. This problem seems to be caused by a reduction in cohesin complexes that stabilize the linkages between the replicated chromosomes. The observed diminution in cohesin is linked to reduced levels of the shugoshin protein, which protects centromere cohesin from precocious removal. These observations might explain a long-standing puzzle and connect age-related birth defects to basic cell division processes.

Environmental causes of aneuploidy

Few errors occur during normal mitosis, but external perturbations—such as carcinogens—can influence the generation of aneuploidy. Industrial contaminants such as zinc or lead chromate are known to cause lung tumours. The lab of John Wise (U. Southern Maine, USA) has found that chromate anions can cause chromosomal aberrations and aneuploidy. Chromate also triggers a decrease in Mad2 levels, resulting in a bypass of SAC activity. Furthermore, depending on the particular chromate exposure conditions, the number of centrosomes increases (Fig 1). Together, these anomalies might significantly contribute to lung cancer transformation. Similarly, silicon dioxide, from which silicones are produced, is known to cause lung cancer. Preliminary results from the lab of Micheline Kirsch-Volders (U. Brussels, Belgium) showed that uptake of amorphous

silica nanoparticles stimulates microtubule nucleation and causes an increase in centrosome numbers, consequences that obstruct faithful chromosome transmission during mitosis (Fig 1).

In addition to environmental hazards, drugs developed to treat human disease might cause aneuploidy as a side effect. These include AZT, 3TC and nevirapine, which have been used since the 1990s to treat HIV/AIDS. The lab of Ofelia Olivero (National Cancer Institute, USA) has studied their effect on chromosome segregation *in utero* by exposing pregnant *Erythrocebus patas* monkeys to combinations of these drugs. When the fetal bone marrow mesenchymal cells were analysed, a 5–7-fold increase in chromosome mis-segregation was observed and centrosome amplification was increased fivefold. This strongly indicates that children of HIV-infected women who are exposed *in utero* to nucleoside analogue drugs, might sustain significant carcinogenic insults, and should therefore be subjected to long-term surveillance.

At the end of this exciting workshop, Geert Kops and Susanne Lens volunteered to organize the next EMBO Aneuploidy Workshop in 2013 in Utrecht. As the audience unanimously voted to pass the torch to them, we look forward to discussing progress in chromosome segregation and aneuploidy, while enjoying a slice of aged Gouda, an ice cold Grolsch, and a brined herring sandwich.

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