Dispatches

Cell Size: Chromosomes Get Slapped by a Midzone Ruler

Spatial and temporal coordination of mitotic events has been generally attributed to the coincidental outcome of increasing cyclin-dependent kinase activity. A recent study reports that mitotic events and structures previously considered to be independently controlled are capable of trans-regulation to ensure genomic integrity.

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During metazoan development, cell size reduces dramatically whereas the genome size is unchanged. To account for this incongruity, mitotic structures become proportionally smaller as cell size decreases. Although a smaller spindle could be constructed by decreasing microtubule length, a particular mystery is how chromosome condensation mechanisms coordinate the degree of compaction with cell size. In a recent study published in *Science* that set out to determine regulators of chromosome compaction, Neurohr *et al.* [1] turned to an organism that normally does not face this scaling challenge — budding yeast.

Neurohr *et al.* [1] showed that yeast cells could faithfully segregate a 45% longer chromosome by adapting anaphase condensation. In order to introduce a longer chromosome without creating aneuploidy, the authors fused the two longest chromosomes (IV and XII) using *in vivo* homologous recombination. The resulting chromosome (*LC(XII:IV)*) contained full genetic activity and, after artificial deactivation of one of the two centromeres, resulted in no overt growth defects compared with wild-type yeast. This tolerance of an increase in the length of a single chromosome, which was predicted to cause segregation errors, indicated the existence of a cellular compensatory mechanism.

To cope with a longer chromosome, one possible adaptation could be to elongate the spindle in anaphase with a different rate or to a different extent. However, the elongation kinetics of the distance between the two spindle pole bodies did not change in cells carrying the *LC(XII:IV)* chromosome compared with the wild-type cells, and the spindle reached the same final length in anaphase. The finding that spindle function was unaltered in cells harbouring an abnormally long chromosome implied that the adaptive mechanism involves modification of the chromosome itself. In support of this hypothesis, cells mutant for a subunit of the condensin complex (*smc2-8*) showed segregation defects of the *LC(XII:IV)* chromosome.

Contrary to metazoans, individual yeast mitotic chromosomes cannot be observed using light microscopy, making the study of DNA condensation and segregation particularly challenging in this system. To circumvent this problem, Neurohr *et al.* [1] used integrated *tet* and *lac* repressor arrays at the *TRP1* and *LYS4* loci, respectively, on chromosome IV and fluorescently tagged TetR and LacI repressor proteins to assess chromosome condensation and segregation [2]. Unlike bulk chromosome segregation, the *TRP1* and *LYS4* loci on *LC(XII:IV)* sister chromatids segregated after anaphase spindle elongation, suggesting that this segregation might depend on an anaphase-specific change in DNA compaction. To assess chromatin compaction, the authors measured the distance between the *TRP1* and *LYS4* loci, which decreased during mitosis in a condensin-dependent manner [2]. In late anaphase, the distance between *TRP1* and *LYS4* in *LC(XII:IV)* decreased compared with the distance between these loci on the non-fused chromosome IV. This suggests that the presence of a longer chromosome arm activates a higher order of compaction. Interestingly, 'adaptive hyper-condensation' (as Neurohr *et al.* [1] termed this process) occurs only on the long chromosome, indicating that a 'chromosome ruler' activity modifies the state of compaction depending on chromosome length (Figure 1A).

The fundamental role of mitotic chromosome condensation is to provide compaction of the genome, allowing complete and accurate segregation by the microtubule-based spindle during anaphase. The spindle machinery pulls chromosomes towards opposite poles during anaphase via two distinct mechanisms termed anaphase A and B. In budding yeast, anaphase B is the major mechanism that physically segregates the chromosomes [3]. In anaphase B, antiparallel sliding of non-kinetochore interpolar microtubules (nkMTs) elongates the entire spindle, ensuring spatial segregation of the duplicated genome before cytokinesis. During anaphase B, the spindle midzone, comprising overlapping nkMTs and an assemblage of microtubule-associated proteins, ensures spindle integrity. In budding yeast, the chromosomal passenger complex (CPC) is a three-protein complex containing the conserved Aurora B kinase. The CPC localizes to the inner-centromere region of chromosomes during metaphase, then transits to the spindle midzone in anaphase. CPC activity at the spindle midzone has been shown to regulate cytokinesis and overall spindle integrity during anaphase, at least in part by Aurora B kinase activity (reviewed in [4]). Additionally, Aurora B regulates chromosome condensation [2,5–7] via direct phosphorylation of serine 10 of histone H3 [8].

Given the localized activity of the CPC, Neurohr *et al.* [1] concluded that it would be an excellent candidate to act as a ruler in adaptive hyper-condensation. To test this hypothesis, the authors used a temperature-sensitive mutant of Aurora B (*ipl1-321*): *ipl1-321* mutant cells with normal chromosomes exhibited a minor defect in compaction of *TRP1* and *LYS4* loci, a defect enhanced in cells carrying *LC(XII:IV)*. The same phenotype was observed in a non-phosphorylatable histone H3 S10A mutant, indicating that histone H3 modification by Aurora B regulates

chromatin structure during anaphase to ensure complete segregation [9]. In support of a local versus global effect of Aurora B activity, the segregation accuracy of *LC(XII:IV)* was reduced in cells in which the CPC was unable to localize to the spindle midzone. This defect was rescued by a mutation in Sli15/INCENP (a second CPC protein) that resulted in constitutive targeting of the CPC to the midzone.

Taken together, Neurohr *et al.* [1] have shown that long chromosome arms spanning the spindle midzone in late anaphase trigger an adaptive hyper-condensation response that involves Aurora B phosphorylation of histone H3 S10. In metazoans, this chromosome arrangement occurs in the form of merotelic kinetochore attachment; a low-frequency event in normal tissues [10]. In this situation, a single kinetochore is attached to both spindle poles, resulting in a lagging chromosome in the middle of the anaphase spindle, juxtaposed to the spindle midzone. Testing whether adaptive hyper-condensation occurs in metazoans will be challenging due to the infrequency of merotely and due to chromosome size differences: however, this could be a model to test whether the observations in yeast reflect mechanisms conserved in human cells.

Regardless of whether or not there is direct conservation of yeast adaptive hyper-condensation, ruler-like mechanisms must exist in metazoans. During embryogenesis, multiple rounds of cell division without an increase in embryo size diminish the size of cells and of mitotic structures, including chromosomes and spindles (Figure 1B). The results presented by Neurohr *et al.* [1] suggest that the spindle midzone can trans-regulate chromosome compaction. An additional example of trans-regulation, described by Greenan *et al.* [11], is the observation that embryonic mitotic spindle length is dictated by centrosome size through a gradient of Aurora A kinase. In sum, these studies raise important paradigms of subcellular scaling, but they do not provide a complete answer to the quandary of size regulation of mitotic structures.

One hypothesis is that spindle length is determined by the physical limit of the cell boundary. Wuhr *et al.* [12] correlated metaphase spindle length with cell size changes during *Xenopus laevis* development. The 1.2 mm fertilized egg develops into a larva containing 12 μm cells. In the smaller cells, metaphase spindle length scales linearly with respect to cell size, in support of the idea that the cell boundary restricts spindle elongation. Interestingly, this observation was not true for larger cells; the metaphase spindle approached a maximum length of approximately 60 μm. This suggests that the spindle length is limited, independently of cell size, possibly by a trans-regulation mechanism, such as that described by Neurohr *et al.* [1], intrinsic to mitotic structures [13].

Overall, the study by Neurohr *et al.* [1] helps us to understand the mechanisms responsible for compaction and segregation of the genome in response to spatial constraints. However, many questions

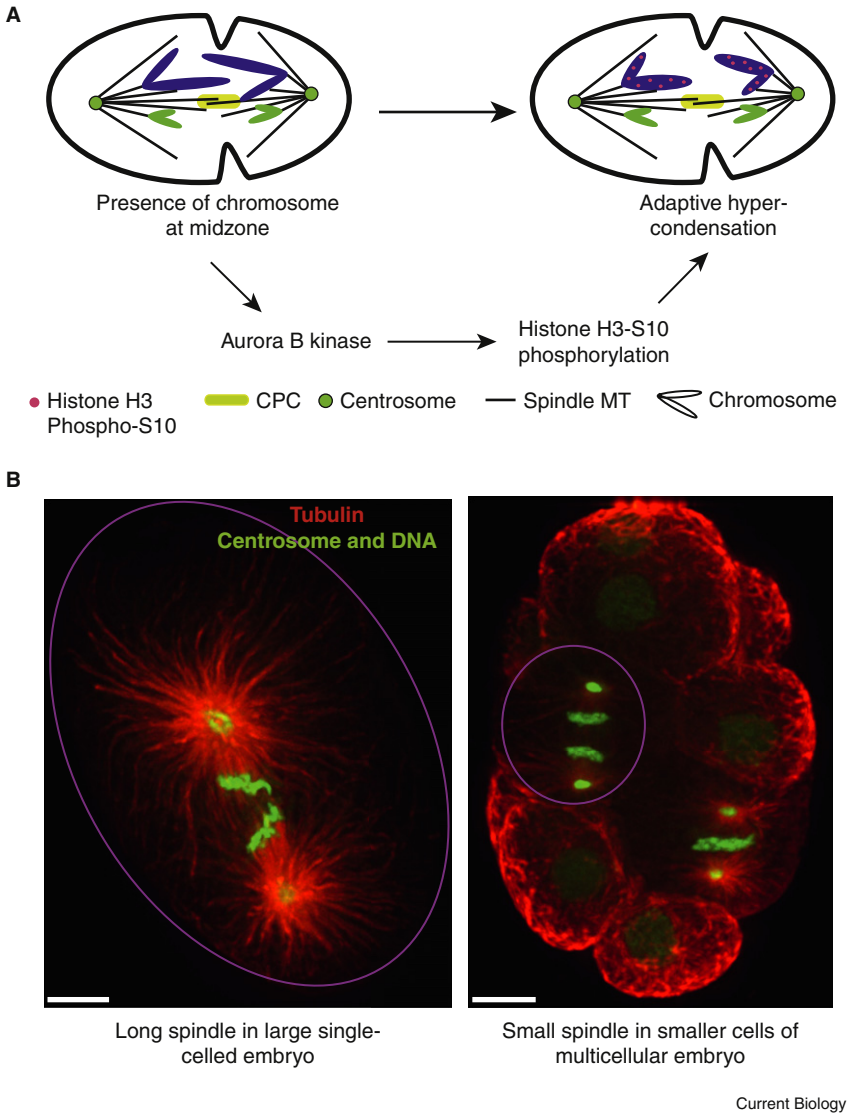

Figure 1. Adaptive chromosome hyper-condensation.

(A) A midzone ruler ensures complete chromosome segregation. In late anaphase, chromosome arms in close proximity to the spindle midzone undergo hyper-condensation, moving the arms out of the path of the cytokinetic furrow. Adaptive hyper-condensation is an example of trans-regulation involving histone H3 phosphorylation on S10 by the midzone-localized kinase Aurora B. (B) During *Caenorhabditis elegans* embryonic development, bigger cells (one-cell stage embryo) have longer spindles, larger centrosomes, and bigger chromosomes compared with smaller cells (multicellular stage), even though the genome is exactly the same in both cases. *C. elegans* embryos are shown co-expressing GFP–histone H2B (green) and GFP–γ-tubulin (green) and stained with anti-tubulin antibody (red). Scale bars = 5 μm.

regarding size regulation remain unanswered. For instance, the majority of DNA compaction occurs during prophase well before the chromatin is released into the cytoplasm, and before assembly of the spindle or spindle midzone. Therefore, midzone-based trans-regulation cannot be the entire answer to measuring chromosome compaction in mitosis. It will be of great interest to follow how this new model will influence the next studies in this field.

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Perceptual Learning: Visual Function Improved by LTP/LTD-like Stimulation

A new behavioral training approach has been found significantly to improve visual function; the results further attest to the high degree of plasticity in sensory systems.

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Our sensory systems, once fully developed, do not remain static. Instead, these systems are plastic and can be modified as a result of repeated exposure to stimuli. Research on this issue has included both behavioral and neurophysiological studies. Behavioral research has used a variety of techniques that result in perceptual learning (improved performance with practice) [1–3], including techniques in which stimuli are repeatedly presented at near threshold levels resulting in dramatic changes in detection and discrimination performance. Neurophysiological research has used techniques such as long-term potentiation (LTP) or long-term depression (LTD) training, in which cells are repeatedly stimulated (at relative high rates for LTP or low rates for LTD) resulting in changes in synaptic connections [4–6]. Although it is generally assumed that behavioral and neurophysiological studies are examining related if not the same mechanisms, the methodologies are quite different and, as a result, there has been no research showing a direct link between the results of these two different phenomena.

This issue was examined recently in an interesting and surprising study reported in this issue of *Current Biology* by Beste *et al.* [7], in which LTP/LTD-like visual stimulation was presented to human observers. Subjects viewed a fixation cross with bars presented on either side of fixation; LTP/LTD-like stimulation occurred by varying the luminance of the bars at different rates. For LTP-like stimulation [1–3] the luminance was repeatedly varied at 20 Hz for five seconds, followed by a five second period with no stimulation, over a 40 minute period. For LTD-like stimulation [8], the luminance was varied at 1 Hz for a 40 minute period. This type of stimulation is analogous to direct electrical stimulation of cells (at 20 Hz or 1 Hz, respectively) in neurophysiological studies of LTP and LTD.

Irrelevant distractor information was presented by varying the orientation of the bars (vertical or horizontal) and by varying the salience of the distractor information (by changing the length to width ratio of the bars). During the stimulation period, subjects passively viewed the bars and responded to a fixation task. Five experimental groups, in which stimulation was unilateral or bilateral and orientation might be varied, and two control groups, in which ubjects were presented with either the background screen with fixation or no display during the 40 minutes, were run. The experimental groups included a unilateral LTP stimulation (the 20 Hz presentation occurred on just one side of fixation), a bilateral LTP stimulation (the 20 Hz presentation occurred on both sides of fixation), a unilateral LTP stimulation group where orientation of the bar (rather than luminance) was varied, a unilateral LTD stimulation group with only luminance varied, and a unilateral LTD group where orientation instead of luminance was varied. Before and after passive stimulation, the subjects performed a change-detection task in which they