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Chromosome biology: Too big to fail

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Spindles are microtubule-based machines that segregate chromosomes during cell division. Spindle morphology and dynamics are malleable based on forces within the spindle, and a new study reveals the extreme plasticity of the *Saccharomyces cerevisiae* spindle to adapt and segregate engineered mega-chromosomes.

Cells must faithfully segregate their chromosomes during cell division. Failure to properly separate chromosomes can lead to negative outcomes, including aneuploidy[1] and cellular death[2], which are associated with human health conditions such as cancer progression[3] and congenital birth defects[4]. In mitosis, replicated chromosomes consist of two identical sister chromatids that are pulled apart by the spindle, a dynamic molecular machine. The spindle is a bipolar structure, composed of microtubules that attach to sister chromatids and generate sufficient force to retract them towards the poles during anaphase[5] (Figure 1). Microtubules in the spindles fall into three categories based on their role: first, kinetochore microtubules attach and pull chromosomes apart via the kinetochore, a protein structure that assembles on centromeric DNA; second, interpolar or core microtubules provide structural integrity for the spindle; and third, astral microtubules reach away from the main spindle to position it within the cell[6] (Figure 1A). While the spindle is a critical and highly conserved cellular machine, its morphology and dynamic capabilities are highly variable across species, and even within species. Previous studies have shown that various spindle features, including the length of the spindle and the number of microtubules, can vary based on the number of chromosomes[7,8] and attachments to the spindle[9]. However, we have lacked a clear understanding of how chromosome demands on the spindle ultimately shape its structure and function, and how dynamic the spindle can be in response to their chromosome loads. In a study published in this issue of *Current Biology*, Kunchala *et al.*[10] utilize the genetically engineered 'mega-chromosomes' in *Saccharomyces cerevisiae* to probe the plasticity of the spindle. Findings from this study suggest that the spindle is highly responsive, altering its morphology and segregation dynamics in response to the mega-chromosome karyotype. Ultimately, the spindle is able to adaptively accommodate to the load, revealing that, for cells, chromosome segregation is too big to fail (Figure 1B).

Spindles are tasked with the critical role of pulling chromosomes apart, but this function is highly complex. This singular task requires spindles to generate force,

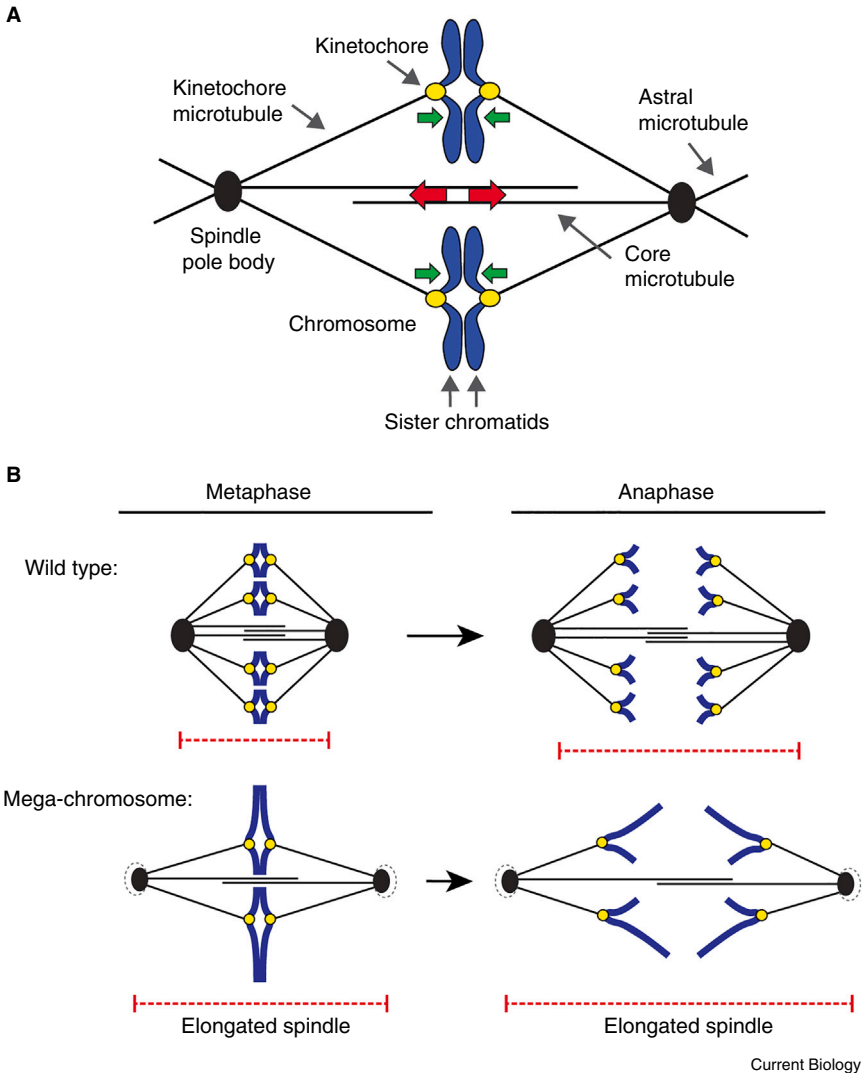

Figure 1. Chromosome segregation by the spindle.
(A) Chromosomes (blue) consist of two sister chromatids that are segregated by the spindle. The spindle consists of kinetochore, core and astral microtubules emanating from spindle pole bodies. Chromosomes attach to the spindle via proteinaceous kinetochores (yellow), and these attachments provide inward force (green arrows) that counter outward forces (red arrows) from the core microtubules, ultimately resulting in spindle length. (B) Wild-type *S. cerevisiae* have 16 chromosomes that attach and segregate on the spindle. Kunchala *et al.* found that fusing the yeast genome into two mega-chromosomes impacted spindle morphology and dynamics, resulting in smaller spindle pole bodies, fewer kinetochore and core microtubules, elongated metaphase and anaphase spindles, and slower anaphase dynamics to separate the mega-chromosomes.

sense tension, understand directionality, measure distance, and alter these parameters based on the chromosomal load they are tasked with segregating. In their new study, Kunchala *et al.*[10] utilized an innovative approach to investigate the relationship between spindles and chromosomes. In 2018, both the Qin[11] and the Boeke[12,13] groups used CRISRP to genetically engineer the genome of *S. cerevisiae*, fusing its native 16 chromosomes together into a few mega-chromosomes. Kunchala *et al.* made use of these strains, specifically those containing two mega-chromosomes. The group investigated the resulting spindle impacts of increased chromosome size yet fewer attachments. This approach differed from previous studies where ploidy[7,8] or number of attachments[9] were altered but not chromosome size. Kunchala *et al.* found that mega-chromosomes introduced additional burden on *S. cerevisiae*, making them intolerant to over-expression or deletion of genes related to spindle assembly, stability and elongation. Specifically, screens using the Harvard Institute of Proteomics library[14] showed that the over-expression of genes associated with nuclear division were the least tolerated by mega-chromosome strains, including genes such as ASE1 and BIM1 (microtubule-associated proteins), KAR3 and KIP3 (microtubule motors), and CBF2 and SLK19 (kinetochore proteins). Similarly, deletion of these typically non-essential genes resulted in lethality or negative growth phenotypes in the mega-chromosome strains.

Using high-resolution electron tomography and live confocal microscopy, Kunchala *et al.*[10] characterized the impact of the mega-chromosomes on spindle morphology and dynamics. Spindles in mega-chromosome strains had on average fewer kinetochore microtubules (characterized as 'non-core' microtubules in the new study) and fewer core microtubules compared with spindles with wild-type chromosomes (Figure 1B). This reduction in core microtubules likely results in greater instability for the spindle under the strain of mega-chromosomes, as the study found that spindles are more bent with a higher degree of curvature compared to with wild type. The authors also discovered in live confocal microscopy that spindles frequently collapse in anaphase, also likely due to the increased burden. Previous studies had shown that metaphase spindle length is the result of opposing forces (force balance model[9]), with chromosome attachments to the spindle generating inward pulling forces and poleward directed kinesins on the core microtubules generating outward pushing forces (Figure 1A). The new findings from Kunchala *et al.* aligned with this model, showing that reduced number of chromosomal attachments (two attachments from the two mega-chromosomes) resulted in longer metaphase spindles, and this elongation could be partially reversed with the addition of more centromeres attaching to the spindle[10]. The study also confirmed previous findings[7–9,15] that the size of the spindle pole bodies, the site of microtubule nucleation in *S. cerevisiae*, is correlated with microtubule number, with

smaller spindle pole bodies producing fewer microtubules. However, they also found that this scaling is non-linear, with a predicted minimum size for the structure (Figure 1B).

The Kunchala *et al.* study discovered a novel and significant relationship between chromosomes and the behavior of anaphase spindles. No previous study has quantified an effect on anaphase spindle morphology and dynamics as a result of chromosome load. Kunchala *et al.* found that in their mega-chromosome strains, the final length of the anaphase spindle and its rate of elongation are significantly increased[10]. This adaptive elongation of the anaphase spindle could be a response to the reduced inward force created by fewer chromosome attachments. Kunchala *et al.* also discovered that the elongated arms of the mega-chromosomes pose a segregation challenge. The theoretical maximum length of a chromosome arm is half the length of the spindle — for example, if a spindle is 10 μm long at the end of anaphase, the maximum length of a chromosome arm must be 5 μm, otherwise arms would not fully separate. Kunchala *et al.* found that one of their mega-chromosome strains contained a chromosome arm longer than the theoretical maximum, and suggested the elongated anaphase spindle length could be a response to fully separate the chromosomes. To support this claim, Kunchala *et al.* demonstrated that cytokinesis is prevented through the NoCut pathway, which monitors the clearance of chromosome arms into the daughter cell[16]. Deletion of NoCut gene *BOI1* resulted in deleterious impacts on the mega-chromosome strains.

Further characterization of live spindle dynamics revealed that mega-chromosome strains grow slowly due to metaphase and anaphase challenges. In addition to slower anaphase times due to reduced spindle elongation velocity, cell growth is inhibited by activation of the spindle checkpoint. The spindle checkpoint is a surveillance system that prevents the transition to anaphase until all chromosomes are correctly oriented on the spindle[17]. The spindle checkpoint is typically dispensable in *S. cerevisiae*, as chromosomes usually align correctly on the metaphase spindle. However, Kunchala *et al.* found that mega-chromosome strains are highly dependent on the checkpoint to guarantee accurate chromosome segregation. Deletion of the *MAD1* spindle checkpoint gene results in lethality for some strains and faster division for others, showing their reliance on the checkpoint to slow growth and ensure proper separation. Additionally, growth is slowed due to observed anaphase spindle collapse as cells try to separate the mega-chromosomes.

The study by Kunchala *et al.* represents a novel approach to probe the relationship between chromosome load and the resulting morphological and dynamic responses from the spindles. This study has revealed a novel mechanism in which spindles adaptively respond to the size of their chromosome burdens, elongating their metaphase and anaphase spindles, altering microtubule numbers, and changing their segregation dynamics to account for the increased burden (Figure 1B). Genes associated with spindle assembly, stability and elongation that are redundant or non-essential in wild-type yeast become indispensable in the presence of mega-chromosomes. Similarly, surveillance mechanisms such as the spindle assembly checkpoint and the NoCut pathway become essential for cells to survive the burden posed by mega-chromosomes. The Kunchala *et al.* study contributes significantly to our understanding of the spindle, its plasticity and adaptability to achieve the ultimate goal of chromosome segregation in the face of large chromosomal loads. The work is critical for our basic understanding of spindles and chromosome segregation, but also has important implications for synthetic biology. Many efforts are underway to engineer synthetic genomes in single-cell organisms[18] or synthetic chromosomes as platforms to deliver extra-genomic content in plants[19] and animals[20], but the response of the spindle to additional chromosomal burdens is critical for this work. Perhaps most importantly, the study by Kunchala *et al.* shows us the importance of chromosome segregation and the role of the spindle — no matter how big and burdensome, it is simply too important to fail.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Paleontology: Ediacaran ecology drove ocean ventilation

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Fluid dynamics modeling of an Ediacaran ecosystem illustrates an important positive feedback loop between early multicellular organisms and environmental water flow. Early communities thus helped to chemically shape new environments where oxygen-dependent organisms could thrive.

Proficiencies in geology and biology are expected, and upholding the rigors of the scientific method is essential, but the most underappreciated attribute required for the study of paleontology is creativity. Not only are paleontologists often limited by the quantity and quality of fossil material, but we can also be hindered by a lack of appropriate modern analogs. But these challenges encourage us to be clever, they provide a little more freedom, or maybe fewer rules, for us to develop hypotheses — they inspire us to be creative. Perhaps nowhere in geological time is this more apparent than at the dawn of animals during the Ediacaran Period (635–539 million years ago). Around the middle of this period, some 575–565 million years ago[1,2], eukaryotic cells accomplished a remarkably strange new life mode compared to the nearly four billion years prior. These novel organisms, often called the 'Ediacara biota'[3,4] or 'vendobionts'[5], were the proto-eumetazoan pioneers of complex multicellularity, many of which remain biologically, physiologically and ecologically enigmatic today — and that's why many of us love them! Given their wide range of morphological diversity and notably sessile life modes (at least in the earliest iterations of vendobiont communities), one of the most inviting uses of paleontological creativity has been to craft conjectures on the structure and function of their ecosystems. For example, without evident orifices or other dedicated feeding structures, how did these organisms feed? How did they respire? And how did they interact with each other, if at all, or their environments?

Now, a new study in a recent issue of *Current Biology* by Susana Gutarra, Imran Rahman and colleagues[6] seeks to explore a specific part of the structure and function of these early multicellular ecosystems: how did these 'vendobionts' interact with their surroundings? More precisely, how did the sessile epifauna of the earliest Ediacara biota communities influence the hydrodynamics of their immediate environment? This new work builds upon several previous efforts[7–9] that have employed a simulation technique known as 'computational fluid dynamics' to provide quantitative predictions of fluid-flow phenomena around objects, in their cases most often virtual models of fossil organisms. While computational fluid dynamics have been used for decades in engineering fields, for example in hydrodynamic and aerodynamic modeling, few researchers have applied it to paleontological or paleoecological studies; benthic communities of the Ediacaran provide a prime opportunity for creative application of this technique.

Gutarra and colleagues[6] chose to model three distinct fossil-bearing bedding surfaces (the 'D', 'E', and Lower Mistaken Point surfaces; Figure 1) from the Mistaken Point Ecological Reserve in Newfoundland, Canada. This is a geologically and paleontologically rich region of the world. Mistaken Point is a UNESCO World Heritage Site renowned for its extensive deep-water Ediacaran communities and contains the official lower boundary, or 'golden spike', for the Cambrian Period[10]. The fossiliferous surfaces themselves are truly spectacular; its well-exposed bedding planes capture snapshots of in-place benthic paleocommunities inhabiting the seafloor of moderately deep paleoenvironments[3]. Just a decade ago,

