passive process, in which nuclear size and cell size scale independently of each other but proportionately with nuclear activity. It also remains to be determined whether the size of the nucleus affects gene expression (Figure 1B, green arrow). The identification of the local, regional, and global factors that determine nuclear size will help establish the precise mechanism that determines the nuclear:cell volume ratio and its relationship to nuclear function.

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Relax, Kinetochores Are Exquisitely Sensitive to Tension

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By estimating the absolute levels of tension at kinetochores in dividing yeast cells and relating these measurements to kinetochore detachment probability, Mukherjee et al. (2019) quantify in this issue of *Developmental Cell* the force sensitivity of the mitotic error correction system.

During mitosis, chromosomes must be segregated with perfect fidelity in order to avoid aneuploidy, a condition where cells have too many or too few chromosomes. Chromosome segregation is orchestrated by kinetochores, large multi-protein complexes that attach chromosomal DNA to dynamic spindle microtubules. Kinetochore-microtubule attachments are initially made at random, with a high chance for error. To avoid mis-segregation, cells have evolved a robust regulatory system to sense and selectively release erroneous attachments. Correctly "bi-oriented" sister kinetochore pairs are attached to microtubules emanating from opposite sides of the cell, so they come under tension from opposing spindle forces. Incorrectly attached kinetochores are more relaxed, lacking tension. For over half a century, it has been recognized that these different levels of tension are probably crucial mechanical cues: tension is somehow responsible for stabilizing the correct attachments. Conversely, the lack of tension on incorrect attachments allows them to release, giving another chance for proper attachments to form (Nicklas and Koch, 1969). This tension-dependent stabilization is widely believed to be the fundamental basis for accuracy in mitosis.

The concept that tension-dependent stabilization explains mitotic accuracy was established by the pioneering work of Bruce Nicklas, who showed that incorrectly attached chromosomes in meiotic grasshopper spermatocytes will spontaneously reorient and that tugging on an incorrectly attached chromosome with a glass microneedle can prevent this corrective reorientation (Nicklas and Koch, 1969). As Nicklas himself explained, the hypothesis was first proposed even earlier, in nascent form, by Roland Dietz (Dietz, 1958). Since then, a great deal more support has come from additional studies in a variety of cell types. Because direct, mechanical manipulation of individual chromosomes is prohibitively difficult in most cells, a variety of clever methods have been devised to perturb tension less directly. A common strategy has been to treat cells with microtubule-altering drugs, such as nocodazole or taxol. However, these drugs affect many intracellular processes, and, thus, the interpretation of their effects on kinetochore-microtubule attachment stability has been controversial (Magidson et al., 2016). More finely targeted approaches have included the engineering (in budding yeast) of un-replicated mini-chromosomes carrying one or two active kinetochores (Dewar et al., 2004),

the inhibition (in human cells) of midzone motors that stabilize spindle architecture (Lampson et al., 2004), and the depletion (from *Drosophila* S2 cells) of "chromokinesins" that push on chromosome arms (Cane et al., 2013). Such studies have all been generally consistent with the hypothesis that tension stabilizes kinetochore-microtubule attachments *in vivo*, and they have provided important clues about the underlying mechanism(s). However, because the absolute magnitude of tension was not measured, the sensitivity of kinetochores to tension *in vivo* remained unclear. Moreover, a crucial question was left unanswered: just how much force is needed to stabilize a kinetochore-microtubule attachment in a mitotic cell? A new study in this issue of *Developmental Cell* by Mukherjee and co-workers (2019) now provides an answer.

Building on previous work from the same lab (Chacón et al., 2014), Mukherjee et al. (2019) use a combination of genetic approaches and live-cell imaging to manipulate and quantify kinetochore tension in budding yeast. The authors find that kinetochores are highly responsive to tension. A drop of only ~1 pN, which represents only ~20% of the normal tension per kinetochore, was apparently sufficient to increase kinetochore-microtubule detachment dramatically, by ~5-fold. This relaxation-induced detachment required the activity of Aurora B kinase, an essential regulator of error correction (Tanaka et al., 2002; Biggins and Murray, 2001), and it led to chromosome mis-segregation when cells were allowed to proceed to anaphase. These findings add to the growing body of evidence that tension suppresses Aurora-triggered kinetochore detachment. They also quantitatively relate detachment probabilities to absolute tension levels for the first time, revealing extraordinary tension sensitivity in the mitotic error correction system.

To estimate absolute levels of tension *in vivo*, the authors first measured fluctuations of fluorescent, centromere-associated lacO arrays during metaphase (Chacón et al., 2014). If certain assumptions hold, these fluctuations in the distance between sister lacO spots will quantitatively reflect the stiffness, κ, of the structures through which they are connected. A key assumption is that the fluctuations are driven solely by thermal energy and not by active (energy-transducing) external processes, such as microtubule or molecular motor dynamics. To avoid the latter possibilities, Mukherjee et al. (2019) measured fluctuations over very short (~1 s) time intervals during which active motility made an apparently negligible contribution (Chacón et al., 2014; Mukherjee et al., 2019). With the stiffness κ in hand, the authors then measured the distance between sister lacO spots, Δx, and computed inter-kinetochore force using Hooke's law, $F = \kappa \cdot \Delta x$. Essentially the same method is used routinely to measure forces with laser traps and microneedles, although these devices are calibrated in isolation, where no external attachments or non-thermal forces can cause errors in the estimation of κ. But as applied by Mukherjee and co-workers (2019), the calculation rests on an implicit assumption that κ is a property of the pericentromeric "inter-kinetochore spring," uninfluenced by the other spindle structures through which the lacO spots were also connected during their calibration measurements. Nevertheless, their method provides a straightforward way to estimate the absolute forces at kinetochores *in vivo*, which has otherwise proven very challenging. In wild-type budding yeast, the estimated pericentromere tension was ~5 pN, enough to provide a useful mechanical signal (Chacón et al., 2014).

Mukherjee et al. (2019) also use kinesin-5 deficient yeast strains to perturb tension across the pericentromere. Kinesin-5 motors normally help establish and maintain spindle bipolarity by crosslinking and sliding interpolar microtubules, thereby generating outward forces that are transmitted to chromosomes via kinetochore-attached microtubules (Lampson et al., 2004). Deleting the yeast kinesin-5 motors, Kip1 or Cin8, or deleting the microtubule bundling protein, Ase1, decreased the average pericentromere tension from 4.6 pN in wild-type cells down to 4.0, 2.8, or 3.3 pN in the mutants, respectively. The mutant cell types also displayed increased propensity for kinetochore detachment, as measured by the collapse of two distinct fluorescent centromere-lacO signals into a single diffraction-limited spot. The probability of detachment increased sharply and monotonically as the inter-kinetochore tension fell, indicating that the error correction machinery responds very sensitively to decreased tension at kinetochores.

To prevent premature separation of sister chromatids that are not yet bi-oriented, mitotic cells rely on another surveillance system known as the spindle assembly checkpoint. This fail-safe system delays mitotic progression by inhibiting the metaphase-to-anaphase transition (Biggins and Murray, 2001). As further validation, Mukherjee et al. (2019) examined whether or not the mutant cells with reduced tension tended to mis-segregate their chromosomes when permitted to enter anaphase. Cells from which both Mad2 and Cin8 were deleted (thereby disabling the spindle checkpoint response) displayed high levels of anaphase chromosome mis-segregation, consistent with the low-tension mutants having high kinetochore-microtubule detachment rates. This interpretation was further supported by the observation that low-tension mutants with normal *MAD2* spent extra time in metaphase, presumably because relaxation-induced detachments triggered the spindle assembly checkpoint.

It is widely believed that selective phosphorylation of relaxed kinetochores by Aurora B kinase (Ipl1 in budding yeast) underlies mitotic error correction (Biggins and Murray, 2001; Tanaka et al., 2002). Mukherjee et al. (2019) lastly sought to determine whether Aurora B activity was necessary for the high detachment rates observed in low-tension mutants. They used a temperature-sensitive allele, *ipl1-321*, that can be inactivated when cells are shifted to 37°C. They observed that tension dependence of the detachment rate was lost when Ipl1 was deactivated. Simulations with a steeply tension-dependent kinetochore phosphorylation rate constant fit the detachment gradient well. Furthermore, assessment by mass spectrometry of the phosphorylation status of a key target of Ipl1, Dam1, showed that phosphorylation scaled with decreasing tension.

The measurement of absolute force levels in living cells has been a major challenge for those seeking to understand intracellular mechanical signaling. By estimating absolute levels of tension at kinetochores in dividing cells and relating their measurements to kinetochore detachment probabilities, Mukherjee et al. (2019) reveal the exquisite tension sensitivity of the mitotic error correction system.

The challenge now is to explain how such sensitivity arises.

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The Bacterivore's Solution: Fight and Flight to Promote Survival

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Bacterial avoidance and innate immune response are two ways by which *C. elegans* respond to pathogenic bacteria. In this issue of *Developmental Cell*, Kumar et al. (2019) and Singh and Aballay (2019) demonstrate that bacterial colonization is essential to induce both responses, which may be associated with somatic and reproductive longevity.

While germophobia may be a psychological issue for humans, avoiding pathogenic bacteria is a matter of life and death for *Caenorhabditis elegans*. Upon exposure to certain pathogenic bacteria, worms can die within only hours or days, in contrast to their normal lifespan of 3 weeks (Tan et al., 1999). To avoid this fate, worms may run away, and this protective avoidance behavior can be elicited within minutes (rapid) or hours (late), dependent upon the type of pathogen. In this issue of *Developmental Cell*, findings from Kumar et al. (2019) and Singh and Aballay (2019) reveal that bacterial colonization and consequent bloating of the intestine are responsible for the late avoidance behavior in *C. elegans*.

Singh and Aballay (2019) study avoidance behaviors upon exposure to pathogenic bacteria. They discover that with pathogenic *Pseudomonas aeruginosa*, wild-type worms elicit a late avoidance behavior, which is positively correlated with bacterial colonization in the intestine. They further confirm that the inhibition of bacterial colonization abrogates the avoidance response, while the elevation of bacterial colonization caused by defects in either pharyngeal pumping or defecation motor program (DMP) is sufficient to elicit the avoidance response. Interestingly, like the long-lived pharyngeal pumping mutants, the DMP mutants also show increased lifespan when grown on *E. coli* that are mildly pathogenic. Kumar et al. (2019) reach a similar conclusion through characterization of a mutant from a forward genetic screen. In a previous genetic screen, the authors identified a loss-of-function mutation in *phm-2*, which extended reproductive span in *C. elegans* (Hughes et al., 2011). They now show that a mutation in *phm-2* causes abnormalities in pharyngeal grinder function and that this mutation extends lifespan. Interestingly, this mutation also leads to increased avoidance behavior and bacterial colonization in the intestine, which are associated with its pro-longevity effects. Together, these two studies suggest that bacterial colonization induces an avoidance behavioral response and consequently increases organismal longevity under normal growth conditions (Figure 1).