receptor Ptch1. Interestingly, Sanders et al. (2013) find that tagged Boc and Cdon localize within filopodia of responding cells. Furthermore, sustained contact is observed between ZPA-extended and Cdo/Boc-expressing filopodia. However, there is no direct evidence of a filopodial engagement of Shh by filopodial localized Cdon or Boc. As noted above, Shh particles actively traffic within filopodia of ZPA cells. In contrast, Cdon and Boc are reported to occupy fixed positions in their filopodia. This raises the question of how these factors would then traffic Shh to Ptch1 in the responding cell.

The work of Sanders et al. (2013) highlights the importance of incorporating dynamic imaging into what is a largely static framework of amniote development. The data are thought provoking, but the system is challenging. To go beyond the correlative to the mechanistic will require highly specific ways of modifying Shh trafficking processes to exclude filopodia while leaving other possible routes of signal delivery intact. There is also a need to devise specific ways of modulating filopodial dynamics that leave all other aspects of cell function intact. Twenty years following the discovery of Shh, Sanders et al. (2013) uncover a new opportunity for fresh insights into the workings of a key, vertebrate morphogen.

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A Close Look at Wiggly Chromosomes

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In a recent issue of *Cell*, Fisher et al. (2013) use high-resolution time-lapse imaging to peer into bacterial genome (nucleoid) structure. The nucleoid, an elastic filament confined via an internal network, undergoes periodic fluctuations critical in relieving tension. Programmed tethers and their release highlight a primordial mechanical cycle for chromosome segregation.

The genome sequence revolution has slowly captured the imagination of the public, but the genome is much more than the ATCGs that made up the DNA polymer. Much remains mysterious about the behavior of the genome in living cells. First is the DNA packaging problem. For humans, ~2 m of DNA must be packed into a 20–30 μm nucleus, and for bacteria, ~2 mm DNA must be packed into a 1 μm cell. Second is the accessibility problem. How can the genome be dramatically compacted yet simultaneously provide huge protein conglomerates such as RNA and DNA polymerase with access to specific genes? The genome is far from being a static information warehouse. Rather, it is a mechanically active entity that is constantly altering its shape. A consequence of the constant shape shifting is that, on average, any given gene may be exposed within the population. To study how the genome is organized, it is therefore critical to keep cells alive. Long polymers such as DNA in a confined space require a very different solution than most biologists have been trained to study. Rather than worrying about salt concentration, pH, and osmolarity, we need to be concerned with concepts such as viscosity, confinement, tethering, and thermal noise. Unlike gases, if we mix two long chain polymers in a confined space, they will segregate simply based on the penalty incurred (entropic repulsion) when the two chains collide.

In a recent issue of *Cell*, Fisher et al. (2013) provide one of the first high-resolution live-imaging series of the bacterial genome (known as a nucleoid). The authors show that the nucleoid exhibits waves of density changes that propagate from end to end. The implications of these findings are startling and provide critical new ways to think about our genetic makeup as we move away from bucket chemistry to take into account physics and statistical mechanics. The bacterial genome is not simply stuffed in the cell. Far from being amorphous, there is internal organization to the nucleoid, as evidenced by its helical shape and clear separation from the cell wall. The evidence comes from imaging with a variety

of nucleoid labels, such as fluorescent repressor operator system (FROS) (Wiggins et al., 2010), Fis (Hadizadeh Yazdi et al., 2012), and the nucleoid-associated protein HU (Fisher et al., 2013). The work of Fisher et al. (2013) provides direct evidence for a structure that is longitudinally bundled and radially confined. By using rapid image acquisition, the authors find that these bundles are dynamic and that waves of density can be seen to flux through the long axis of the nucleoid. Polymer confinement is generated by an internal structure that ratchets down chromatin domains—not like stuffing your sleeping bag into its pouch. The internal structure observed here may be analogous to protein-based chromosome scaffolds observed in eukaryotic chromosomes (Earnshaw et al., 1985) or constraints from protein complexes such as condensin and cohesin (Stephens et al., 2011) together with DNA entanglements (Kawamura et al., 2010).

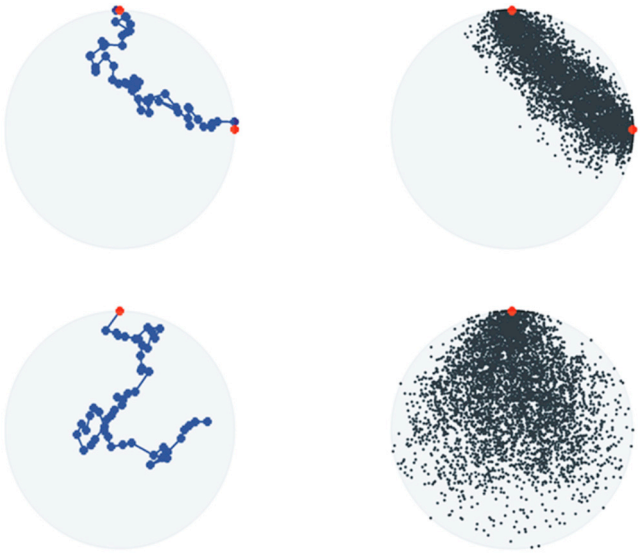

Figure 1. Brownian Fluctuation of Bead-Spring Polymer Chains
Singly tethered polymer chains explore more space relative to doubly tethered chains. Top: doubly tethered chain; bottom: singly tethered chain. Snapshots in time (left), ensemble behavior over time (right). Model by Paula A. Vasquez, Department of Mathematics, UNC-Chapel Hill.

A key insight from Fisher et al. (2013) is the consequence of tethering (Figure 1). The longitudinal pulses within the nucleoid correlate with the time and number of what are known as the T1–T4 transitions. T1–T4 transitions reflect discrete events toward the completion of replication and segregation. The transitions are abrupt and have been proposed to reflect release of tethered sites (Bates and Kleckner, 2005; Joshi et al., 2011). Direct visualization of a snapping event provides evidence for rapid elastic recoil of a specific locus (Fisher et al., 2013). Snapping events within confined chromatin polymers have also been observed in cohesin and condensin-rich pericentromeres in budding yeast (Stephens et al., 2013). Tethers emerge as a critical concept in considering the behavior of DNA polymers. If you take a slinky and let it randomly fluctuate, the ends and the middle exhibit comparable effective spring constants. However, if you simply tether one end of the slinky, a gradient of spring constants is generated (stiff at tether site, soft at untethered end). Thus, tethering provides a mechanism to generate heterogeneity along an otherwise homogeneous polymer chain and is likely to have profound biological implications. Evidence of snaps and tethers raises questions about the types of cellular tethers (are there programmed tethers like eukaryotic interphase telomere tethering and nonprogrammed or stochastic tethering interactions emerging from entanglements?) and how the cell dissolves these attachments at the appropriate time and place.

The DNA polymer is fundamentally a knotted mess. Replication constantly generates catenates, whereas positive and negative supercoils lead and follow transcription. The local environment of any given gene in a long-chain polymer would be a physicist's nightmare to compute and would take a biologist an eternity to reconstitute. The cell, of course, has this figured out: the system thrives on thermal noise. Take a ball of yarn and let your kitten or granddaughter (in my case) mess around for a few hours. If you try to untangle the yarn by pulling a strand, you will rapidly tighten a knot hidden in the interior. Alternatively, if you shake the whole mess—from the inside and out—the knots will loosen. Indeed, Fisher et al. (2013) propose that the longitudinal waves play a fundamental role in deconvolving an otherwise knotted mess of supercoils and entanglements that could prematurely stress programmed tethers. The periodic undulations act to massage the nucleoid, thereby relieving tension. This perspective offers new insight into bacterial chromosome segregation, as well as cellular mechanisms for relieving stress and strain.

Looking beyond the immediate dynamics of chromatin behavior, one of the more profound concepts to come from the Fisher et al. (2013) study is the connection of waves (or cycles) of mechanical undulations to early mechanisms of segregation and cell-cycle regulation. As biologists, we are taught to think of building complexity to execute a process. From the physics, however, the fidelity of a process depends on decreasing complexity and closer adherence to thermodynamics. While DNA-binding proteins that mediate chromatin-skeletal interactions have evolved from simple plasmid-based mechanisms in bacteria to complex kinetochore-based mechanisms in eukaryotes, there is likely an internal mechanical cycle conserved between these systems that predates these solutions for managing complex DNA polymers during cell division.

Is the motion observed by Fisher et al. (2013) completely thermal? We know that chromosomal motion is an energy-requiring process. The machines that drive chromosome motility—e.g., enzymes moving along the helix, enzymes breaking and sealing covalent bonds—do not generate coherent directed motion. Instead of putting heat into the system to increase molecular motion, randomly directed enzymatic activity increases the number of conformational states (ΔS, from the Gibbs free energy equation). This provides a source of energy for the extent and magnitude of chromosome fluctuations that are key to keeping the chromosome network fluid.

Finally, we need to determine the magnitude of forces driving these undulations and, for that matter, segregation of the polymer. Are these chromosomal systems tuned to just beat thermal noise or are they

significantly stronger? There have been several attempts to measure absolute forces on chromosomes in living cells, particularly during mitosis, when there is a large refractive index difference between the chromosome and the surrounding media. The theoretical calculations from Stokes' law show that you only need to hydrolyze 25 ATP molecules to move a huge grasshopper chromosome. Using finely calibrated microneedles, Nicklas (1988) measured a stall force of 700 pN per chromosome. These measurements have gone untested for over 30 years and have been the gold standard in the field. Very recently, a group using calibrated optical traps found that the force required to stall movement was much closer to the theoretical values (Ferraro-Gideon et al., 2013). While these are early days in force measurements and observing chromosome dynamics in live cells, the small cadre of biologists and physicists using optical methods and theory is growing, and they are showing us productive new ways to think about chromosomes.

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