Research Roundup

Does the Kai clock rotate?

Jinmin Wang (Yale University, New Haven, CT) suggests that an ancient circadian clock rotates much like a hand on an analogue clock. His analysis of cyanobacterial clock protein structures reveals a similarity with the F$_1$-ATPase rotary motor.

The cyanobacterial clock is controlled by the KaiA, KaiB, and KaiC proteins, which form a complex at night that falls apart in the day. Mutations causing more stable complexes correspond to a longer periodicity. But little is known about how the timing of complex formation is controlled. Wang analyzed the recently solved structures of the Kai proteins to suggest a mechanism.

When KaiC is ATP-bound, the Kai complex is stable. But when ATP is bumped off by autophosphorylation near the ATP-binding site, the complex falls apart. Autophosphorylation is stimulated in vitro by KaiA.

Wang realized that the ATP-binding domains of KaiC hexamers resemble the F$_1$-ATPase ring. He proposes that KaiA fits inside the ring much like the $\gamma$ subunits fit inside the F$_1$-ATPase ring. Based on previous structures, KaiA dimers are too large to fit inside the ring. But Wang proposes that KaiA dimers must first be activated by the extension of helical domains. This proposed extension makes KaiA dimers resemble $\gamma$ subunits. The need for KaiA activation would also explain the two-hour delay between the rise in KaiA and KaiC levels at dusk and complex formation. The stimulus for such KaiA activation is unclear.

In Wang’s model, KaiA is expected to contact at most two KaiC monomers at a time and stimulate their autokinase activity. Autophosphorylation would both displace ATP and provide energy for the rotation of KaiA to new KaiC subunits. This cycle would repeat until the KaiC hexamer lacks ATP completely and the complex falls apart.

The rotation of KaiA might be hindered by KaiB, which Wang predicts bridges KaiA and KaiC below the ring. This suggestion fits with previous in vitro data showing that KaiB slows KaiC autophosphorylation. In fact, says Wang, “the autophosphorylation rate of KaiC is very slow, about three to four hours per [dimer].” Thus, one round of KaiA rotation should be approximately equivalent to the span of a night, or one rotation of an average wall clock.

Reference: Wang, J. 2005. JCB. 169:5.H9253/H9254

Stiffening under pressure

Natural networks, such as collagen gels or cytoskeletal webs, have the ability to increase their stiffness with increasing strain. This unique feature is an advantage over most synthetic fibers. If blood vessels were made of rubber tubing, for example, the pressure from a heart beat would vastly increase vessel diameter. But collagen’s nonlinear elasticity prevents such a drastic endothelial deformation.

This ability is usually explained by the heterogeneous nature of biological gels—perhaps tauter filaments take over at increasing strains. But in vitro measurements by Storm et al. now show that uniform biopolymer gels also exhibit nonlinear elasticity. The authors then produced a mathematical model that explains this behavior based on the characteristics of the individual polymer filaments within a cross-linked network.

“We show that strain stiffening comes about automatically because of the semiflexible nature of the chains in the biomaterials,” says Janmey. “They’re not exactly straight, but they’re not randomly highly coiled either.” That flexibility allows for short range movement under mild strain, but under greater strain the filaments reach the end of their leash and stop extending. Slightly stiffer polymers (actin, collagen), with shorter leashes, stop extending at lower strains. Softer gels, such as intermediate filaments, take larger deformations before they stiffen.

The model makes certain assumptions that might not hold true in vivo. For one, networks were assumed to form randomly. But Arp2/3 complexes, for instance, bias actin filament branching at specific angles. Additionally, cross-links were assumed to stay put under strain, although they are probably labile in cells. Determining the effects of these variations on strain stiffening will require further modeling.

Beyond biological interests, the findings might also prove useful to engineers. “If you wanted to make a material that stiffens as you deform it,” says Janmey, “filaments as stiff as intermediate filaments would create a new type of [synthetic] polymer.”

Reference: Storm, C., et al. 2005. Nature. 435:191–194.

A hypothetical extension (left to right) of KaiA makes it slim enough to fit inside the KaiC ring.
Myosin reins in neurites

Myosin II pulls growth cones in the right direction, as shown by Stephen Turney and Paul Bridgman (Washington University, St. Louis, MO). Growing neurons in the developing embryo are directed by guidance cues such as laminin-1 (LN1), which steer the extension of neurite growth cones. Bridgman had previously noticed that neuronal growth cones contain high levels of myosin II. As this motor generates force on the cytoskeleton, he figured it might be involved in turning neurites in response to guidance cues.

Such was the case for LN1, as shown by the growth of neurites at borders between LN1 and polyornithine substrates. Normally, growing neurites rapidly retreat from polyornithine and turn back into the laminin surface. But when myosin II activity was inhibited, the neurites ignored the change in substrate and grew over polyornithine.

Turning depended on the activation of integrins—the LN1 receptors. The subsequent activation of focal adhesion kinases might activate or recruit myosin II. On polyornithine, both myosin II and focal complexes are randomly distributed. On LN1, however, myosin IIB concentrated in the transitional domain of the growth cone—intermingled with or just behind the new front of focal complexes. Myosin placement in relation to adhesion sites might pull neurites toward more LN1 and away from unwanted substrates.

Reference: Turney, S.G., and P.C. Bridgman. 2005. Nat. Neurosci. doi:10.1038/nn1466.

Inequality made equal

Forces generating asymmetry give rise to left–right (LR) differences in internal organs such as the lungs and liver. Now, Yasuhiko Kawakami, Juan Carlos Izpisua Belmonte, and colleagues (Salk Institute, La Jolla, CA), and Julien Vermot and Olivier Pourquié (Stowers Institute for Medical Research, Kansas City, MO) show that these forces are buffered by the action of retinoic acid (RA) to ensure symmetry in vertebrae and muscle formation.

Vertebræ and muscle are derived from the somites, which form as symmetric segmentations along the anterior–posterior axis. These segments form near the node, a mass of cells that provides positional information to organize the body plan. In mice and chicks, the node contains ciliated cells that generate fluid movement to produce LR asymmetry.

The Salk group shows that this ciliated system is conserved in zebrafish. They also find that loss of the system causes asymmetric somite formation. As RA gradients help to time somitogenesis, the authors investigated whether it coordinates the LR system with somite formation. Indeed, blocking RA production led to more somites on the left side, and this asymmetry depended on the LR information flow.

A similar RA buffer also operates in chicks and mice, according to the Missouri group. The details regarding how RA influences LR patterning are not clear. RA down-regulates FGF activity, and this antagonism is known to help time somite formation via oscillations in gene expression. In the absence of RA, these oscillations were faster on the left side than they were on the right.

The loss of Zip1 is known not to impair homologue pairing. But Roeder hypothesizes that it may become more important when other mechanisms that contribute to pairing are impaired.

References: Kawakami, Y., et al. 2005. Nature. 435:165–171. Vermot, J., and O. Pourquié. 2005. Nature. 435:215–220.

Centromeric links in meiosis

Results from Tomomi Tsubouchi and Shirlleen Roeder (Yale University, New Haven, CT) suggest that centromeres might bring meiotic chromosomes together for a round of speed dating to find their ultimate partner.

Meiotic homologue pairing involves an interplay between genetic recombination and the formation of the synaptonemal complex (SC), which bridges homologous chromosomes. The new results show that one SC protein, Zip1, also bridges nonhomologues at early stages of prophase I in budding yeast.

Zip1 was seen at centromeres in foci, with the number of foci matching the number of chromosome pairs in the cell. The pairs did not need to be homologues—even in haploid cells, which lack homologues, chromosomes paired at their centromeres.

The number of centromeric Zip1 foci remained constant throughout prophase even as the number of homologous pairs increased, suggesting that partners are exchanged until the right pairing is achieved. “Zip1 may hold centromeres together long enough for chromosomal homology to be assessed,” suggests Roeder. The onset of recombination might then signal Zip1 extension, which was seen proceeding from centromeric regions in later stages of prophase.

The loss of Zip1 is known not to impair homologue pairing. But Roeder hypothesizes that it may become more important when other mechanisms that contribute to pairing are impaired.

Reference: Tsubouchi, T., and G.S. Roeder. 2005. Science. 308:870–873.