The choreography of DNA replication has been a mysterious black box. How is replication targeted to different, reproducibly localized DNA domains at the early, middle, and late stages of S phase? Rather than invoking a homunculus, Anje Sporbert, Cristina Cardoso (Max Delbrück Center for Molecular Medicine, Berlin, Germany), and colleagues have come up with a domino model that runs itself once the replication program gets started.

Cardoso’s group focused on the dynamics of PCNA, a sliding clamp that aids in DNA polymerase processivity. Recovery of fluorescence after bleaching of PCNA was slow, suggesting that PCNA from within a given replication focus is recycled to load at each new Okazaki fragment. But the picture over the longer term is very different. Based on pulse labeling of DNA replication and PCNA, bulk PCNA is released from the sites that were replicated earlier and keeps up with the sites of ongoing replication. The recycling at this timescale is indirect—it is unbleached PCNA from the nucleoplasm that assembles at the new replication foci. Importantly, these foci are found preferentially right next to the recently bleached foci.

Earlier workers mapping DNA replication sites had noted, but had not dwelt upon, this close apposition of sequential replication foci. Cardoso believes that the pattern is crucial. “We think the major DNA replication program could be explained by this,” she says. Activity at early replication foci may transmit a signal down the DNA strand to activate neighboring foci, either by pulling physically on the DNA (like undoing a shoelace) or by an unknown chromatin-modifying mechanism. “The program would be fixed,” says Cardoso, “as long as the first sites to fire were fixed.”

Reference: Sporbert, A., et al. 2002. Mol. Cell. 10:1355–1365.

Hearts grow with the flow

A properly formed heart comes only after effort—the pumping of cells through a primitive heart to generate gene-activating shear forces. That is the conclusion of Jay Hove, Reinhard Köster, Scott Fraser, Morteza Gharib, and colleagues (California Institute of Technology, Pasadena, CA), who have come up with a method for watching heart development by culturing developing zebrafish on microscope stages.

The researchers tracked the movement of blood cells against the background of fluorescently stained blood serum. High-speed imaging (1,000 frames/s) and digital particle-tracking yielded movement vectors for the blood cells. The cells moved at speeds of up to 1.5 mm/s in a primitive, valveless heart and 0.5 cm/s in a more developed heart. The resultant churning forces should be more than enough to activate the many endothelial genes known to respond to shear stress.

The high speeds surprised the group. The small scale of the developing heart was thought to slow down blood cells because of frequent collisions of cells with vessel walls. But apparently the zebrafish has a mighty heart, even at the age of 37 h.

If flow was blocked with a bead, the juvenile hearts continued to beat but failed both to form one heart chamber and to loop correctly. Thus, says Köster, “it’s not just a genetic program that directs heart development.” He hopes to determine which developmental genes are responding to flow forces to shape the development of the heart.

Reference: Hove, J.R., et al. 2003. Nature. 421:172–177.

Replicating dominos



Posed to migrate

Epithelial cells are ready to run off at any moment, at least based on the results of Olga Speck, Richard Fehon (Duke University, Durham, NC), and colleagues. They have found that moesin, previously thought to be a stolid structural component, is actually a signaling protein that maintains epithelial cell identity by suppressing Rho activity.

Ezrin, radixin, and moesin (ERM)—represented only by Moesin in flies—are proteins that link actin to various transmembrane proteins at the apical surface of epithelial cells. The link was thought to help maintain epithelial structures such as microvilli, but Fehon’s group found that flies lacking Moesin had epithelial cells that lost polarity, dropped out of monolayers, and migrated invasively.

Moesin appears to act by suppressing Rho. Interfering with ERM proteins in mammalian epithelial cells boosted Rho activity, and fly Moesin mutants improved when Rho1 dose was halved. Rho1 overexpression in wild type, however, phenocopied Moesin loss.

Thus epithelial cells must be actively maintained in their polarized state, lest they slip off into the motile night. This instability may reflect the transitions from structured epithelium to motile founder cells that occur frequently during development—a transition that carcinoma cells apparently recapitulate when they become metastatic.

Reference: Speck, O., et al. 2003. Nature. 421:83–87.