Research Roundup

Mother puts organelles in their place

Like a strict parent, the mother centriole keeps order in the cell by telling other organelles where to sit, according to new work by Jessica Feldman, Wallace Marshall (University of California, San Francisco, CA), and Stefan Geimer (Universität Bayreuth, Bayreuth, Germany).

Marshall's team is interested in what controls the intracellular geometry of organelle positioning. To address this topic, they focus on one organelle that is well-known for its specific positioning: the centriole.

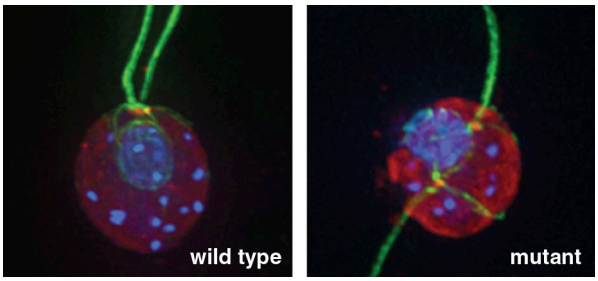
The tethered pair of mother and daughter centrioles is the major component of the centrosome complex and also promotes the assembly of cilia. Thus cilia can act as a cell surface indicator of centriole positioning. The team used the unicellular alga *Chlamydomonas reinhardtii,* which normally has two cilia at its apex, to scan for mutants in which cilia were misplaced.

In certain misplaced cilia mutants, the fibers that normally tether mother and daughter centrioles were absent. The team found that whereas the mother centriole in these mutants continued to locate at the cell's apex, the daughter's position was random. Thus it seems that the mother's tight rein on the daughter is what keeps the young one in position.

Centrioles are normally closely linked to the nucleus, but in mutants that lacked the fibers connecting these two, it was again the nucleus that displayed a randomized positioning, not the centriole. The authors found evidence that the centrioles position two other organelles—the contractile vacuole and microtubule rootlets. From these combined data, they suggest that the mother centriole might in fact coordinate the positions of a whole subset of organelles.

The nucleus in mammalian cells is also found associated with centrosomes, indicating that the guiding influence of mother might well be conserved. JCB

Reference: Feldman, J.L., et al. 2007. *PLoS Biol.* 5:e149.



MARSHALL/PLOS

Centrioles correctly position cilia (green), and also intracellular organelles.

Imaging individual transcription factors

Structural analysis, biochemistry, and theoretical models have built a picture of how individual transcription factors find, bind, and regulate their target genes. Now, for the first time, Johan Elf, Gene-Wei Li, and Sunney Xie (Harvard University, Cambridge, MA) provide moving pictures of this process in living cells.

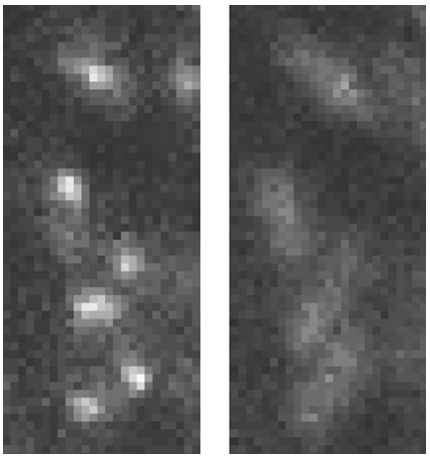
The *lac* operon of *Escherichia coli* has been one of the most well-studied model systems of transcriptional regulation. When a lac repressor protein binds its operator sequence upstream of the operon, transcription is repressed. Upon binding of lactose metabolites (or analogues thereof, such as IPTG), the repressor dissociates from the operator, which enables transcription. Previous studies measured the kinetics of lac repressor binding and dissociation indirectly by analyzing the accumulation of gene products.

To follow directly the kinetics of individual lac repressors, the team expressed a fluorescent lac repressor fusion protein. Despite lac autorepression, wild-type *E. coli* cells still have ~20 copies of the repressor monomer per cell—too many to pick out individual factors from the fluorescent blur. So Elf et al. decreased this number to just a few monomers by modifying the autorepression sequence.

Using a sensitive camera in combination with a high-intensity stroboscopic laser that allowed millisecond exposure times, the team as able to capture individual repressor molecules as they moved around the cell.

In the absence of IPTG, the repressor molecules are detected as single localized spots, indicating the repressor's specific binding to the lac operator. When IPTG was added, the repressors dissociated within seconds and diffused rapidly through the cell. Upon removal of IPTG, the repressors found their binding sites in ~1 min.

During this search phase, repressors nonspecifically bound and scanned DNA for up to 5 ms at a time. Between scans, the repressors hopped to other DNA segments in less than 0.5 ms. These are the first quantitative measurements of how DNA binding proteins search for their target sequences in a living cell.



XIE/ELSEVIER

Millisecond exposure (left) captures single-molecule movement that otherwise appears as a blur (right).

To follow individual molecules in larger, more complex cells, additional difficulties must be overcome, such as 3D tracking in large volumes. Nevertheless, the team plans to adapt the technology to see to what extent transcription factor kinetics can also be probed in eukaryotes. JCB

Reference: Elf, J., et al. 2007. *Science.* 316:1191–1194.