Heterochromatin gets dynamic

The sleeping giant of the cell, heterochromatin, is really a bustling complex whose components turn over rapidly, say two groups led by Richard Festenstein (Imperial College, London) and Thierry Cheutin and Tom Misteli (National Cancer Institute [NCI], Bethesda, MD). Both groups used fluorescence recovery after photobleaching (FRAP) to track the behavior of heterochromatin protein 1 (HP1). HP1 was not static but moving around, as Misteli puts it, in the “same sort of range” as transcription factors. Although both groups found that HP1 was dynamic, Festenstein’s mobility values were lower than those found by Misteli. Part of the difference may lie in varying bleaching and microscopy methods, but both researchers suggest the importance of the different cell types used. Festenstein looked in primary T cells—a resting cell type—and when he stimulated the T cell receptor to activate these cells, his new values for HP1 mobility increased almost to the level seen by Misteli in his transformed cells. Thus, an increase in heterochromatin protein dynamics may help kick-start a T cell as it takes on invading microbes. The details of how cell activation increases mobility remain to be determined, but the overall high level of mobility is already causing a rethinking of standard models of heterochromatin. The important point, says Misteli, is not to confuse stable with static. “We think most stable structures are dynamic,” he says. “You can generate stable structures from dynamic components.” The dynamic behavior may, however, indicate that dimers or small oligomers of HP1 may be the building blocks of heterochromatin, rather than the large oligomeric networks portrayed in many textbooks.

Also up for grabs is the accessibility of both heterochromatin DNA and the ever-important histone tails (the target of HP1 binding). With the dynamic behavior of HP1, says Festenstein, “now you get an opportunity for access.” Thus, heterochromatin is not dormant but subject to constant competition for binding between HP1 and the activators and remodeling factors that seek to bring DNA into the open.


Life on a bed of needles

Cells pull on their environment during adhesion, contraction, or movement, but mapping those forces is no easy task. Past methods have relied on the wrinkling or deformation of a planar substrate—akin to the scrunching of a sheet when someone sits down on a bed. Now, John Tan, Christopher Chen, and colleagues (Johns Hopkins University, Baltimore, MD) have put forward an alternative method using arrays of microfabricated, bendy posts.

The wrinkling methods map the location of the forces indirectly and rely on deconvolving one of several possible solutions. But, says Chen, “because the posts move independently, if a post moves you know it’s because the cell is pulling on it—there’s really no other explanation. For the other methods you need to know where the adhesions are, or make other assumptions.” For example, the new method would spot the influence of an adhesion even if it lacked all known cell adhesion molecules. Second, the posts can be varied in height. The tips of the posts all lie in one plane, but the bases of some can be raised to make shorter, stiffer posts in any pattern desired. This will allow the group to measure how the cell responds to differing resistance, without having to alter the hardness of the substrate’s material, which might itself alter adhesion properties.

As an initial experiment, Tan and Chen have compared adhesion size with the magnitude of pulling force—an experiment that has yielded divergent conclusions in two recent studies. Using the posts, the size of adhesions correlates with magnitude of force for adhesions larger than 1 μm², but some smaller adhesions generated much larger forces. “We see both types of reported relationships in the same cell,” says Tan. “That suggests both groups are right.”

The tops of the posts are coated with fibronectin using microprinting, thus keeping the cells restricted to the tops. This may be more physiological than it sounds. “The way cells seek out and find these posts may be similar to the way cells seek out collagen fibers in a loose network,” says Chen. Limited microprinting restricts the spreading of the cells. Such cells could not contract in response to serum, yet still responded to activated RhoA.

Polarity feeds back

Polarizing cells must first pick a direction. The external cues that guide this choice are obvious: bud scars or mating partners for budding yeast, and chemoattractant gradients for Dictyostelium and neutrophils. But polarization still occurs, albeit in a random direction, when these cues are either removed or made uniform.

Now, Roland Wedlich-Soldner, Rong Li (Harvard Medical School, Boston, MA), and a group of mathematical modelers have come up with an explanation for this intrinsic polarization in budding yeast. In wild-type situations, the intrinsic mechanism may be used to solidify the direction originally dictated by the external cue.

Normally, the bud scar acts as a site for activating Cdc42. Somehow, expression of activated Cdc42 is by itself sufficient to polarize cells. This polarization is now shown to involve the formation of a cap of Cdc42 on the plasma membrane. The cap’s location is independent of any obvious cue, including bud scars, microtubule arrays, and lipid localization. The polarization does depend on transport apparatus—actin cables, a type V myosin motor, and vesicle exocytosis—and Cdc42 cofractionates with a secretory vesicle marker.

The researchers suggest that an initial stochastic grouping of Cdc42 on the plasma membrane stimulates the formation of actin cables and thus the vesicle-based delivery of more Cdc42. A similar positive feedback is seen in neutrophil chemotaxis, where lipids stimulate Rho GTPases to produce more lipids.

Thus, says Li, there are “intrinsic mechanisms [that] are sufficient to break symmetry without contributions from external cues.” Yeast cells lacking this feedback can still respond to a normal external cue, but their inefficient polarization results in secretion in both mother and daughter cells making the cells fat.


Linking spindle to furrow

Once it gets going, cytokinesis is a simple squeeze. The complicated machinery is devoted instead to localizing the cytokinesis furrow. Now, Gregory Somers and Robert Saint (Australian National University, Canberra, Australia) have provided a link from mitotic spindle to contractile furrow that may explain how one positions the other.

The group started with the furrow-localized protein Pebble (PBL), a guanine nucleotide exchange factor (GEF) and thus activator for Rho1 and actin remodeling. Two-hybrid and coimmunoprecipitation experiments with PBL turned up RacGAP50C. The worm homologue of RacGAP50C, CYK-4, is essential for cytokinesis completion and binds a kinesin-like protein that bundles microtubules in the central spindle.

Consistent with these interactions, RacGAP50C was found in an inner ring near central spindle microtubules, abutting an outer ring of PBL. How these rings affect each other is unclear. PBL and RacGAP50C, despite being an activating GEF and an inhibitory GTPase-activating protein (GAP), interact synergistically rather than antagonistically. Somers and Saint find that the GAP activity is not directed at Rho1 but is required for cytokinesis. Perhaps the logic behind the activity will be tied up in the need to regulate the timing of PBL activation.


Exocytosis in action

Ger Kreitzer, Enrique Rodriguez-Boulan (Cornell University, New York, NY), and colleagues have provided the first visualization of targeted exocytosis in polarized epithelial cells.

Exocytic events can be seen by specialized microscopy techniques that selectively illuminate the bottom of cells. But to see exocytosis on the lateral side of a polarized cell, the researchers had to comb through many confocal images looking for events in which fluorescence intensity diminished due to emptying rather than movement of a vesicle or tubule. One clue was the spread of fluorescence visible only after release of an exocytic cargo.

“Technologically this is not so difficult,” says Kreitzer. “But the analysis was very labor intensive.” The reward was a direct readout of fusion events. Basolateral cargoes were located in the most apical two thirds of the cytoplasm and fused with the corresponding region of basolateral membrane. Apical cargoes were concentrated in approximately the top 4 μm of cytoplasm. Their fusion was not observed in polarized cells, and thus was presumed to be restricted to the apical membrane. Neither cargo fused with the basal membrane.

Microtubule depolymerization is known to result in mislocalization of apical membrane proteins, and Kreitzer and colleagues saw fusion of apical cargoes with basolateral membranes of nocodazole-treated cells. This correlated with syntaxin 3—a fusion machinery protein normally restricted to apical membranes—mislocalizing to basolateral membranes. Thus, syntaxin localization may direct delivery of apical and basolateral cargoes. But it is also possible that syntaxin 3 is itself an apical cargo, and that the real regulation is at the level of loading cargoes onto the correct motors or arrays of microtubules.