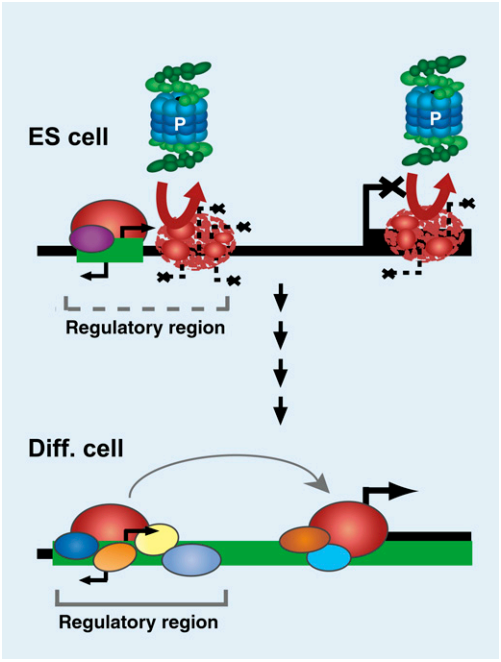
Research Roundup



DILLON/ELSEVIER

In ES cells (top), the proteasome (P) degrades transcription factors to prevent inappropriate transcription.

Proteasomes on ES cell patrol

ES cell chromatin is more active and open than that of differentiated cells, yet tissue-specific genes are somehow kept quiet. A report by Henrietta Szutorisz, Niall Dillon (MRC Clinical Sciences Centre, London, UK), and colleagues suggests that proteasomes police such tissue-specific loci, degrading incoming transcription factors before they can initiate transcription.

The tissue-specific genes VpreB1 and λ5 share the same locus and are both strongly activated in pre–B cells but repressed in ES cells. The group now finds that repression in ES cells requires proteasome activity. Proteasome inhibition in ES cells led to increased transcription and the recruitment of a number of general transcription factors to the locus.

This transcriptional up-regulation, however, occurred all over the locus rather than at the genes' normal transcription start sites. The novel transcription start sites were mainly located around a known intergenic regulatory region. The many transcription factor binding sequences in such a region would be likely to attract and assemble nonspecific transcription factor complexes in the open chromatin environment of ES cells. Thus it appears that the proteasome's job is to ensure that these complexes don't inappropriately activate transcription.

Proteasome levels are similar in all cell types, yet in pre–B cells the transcription complex necessary for VpreB1 and λ5 activation must be proteasome resistant. "There's some evidence that certain kinds of protein motifs get recognized by proteasomes," says Dillon. He suggests that these motifs might be available in ES cells but then get masked when the proteins form complexes that include tissue-specific transcription factors.

Although the exact mechanism is unknown, proteasome inhibition also up-regulated other tissue-specific loci in ES cells, suggesting that proteasome policing might be a general mechanism for keeping inappropriate transcription in check. JCB

Reference: Szutorisz, H., et al. 2006. *Cell.* 127:1375–1388.

Stabilizing the microtubule scaffold

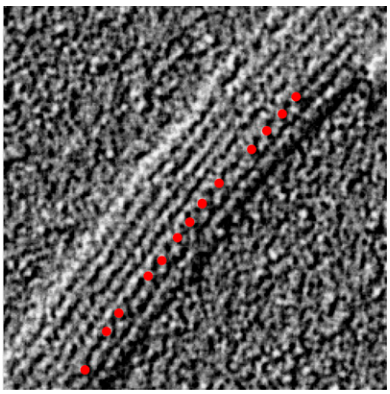
Combining stability with movement is a perpetual challenge for engineers. Linda Sandblad, Damian Brunner, Andreas Hoenger (EMBL, Heidelberg, Germany), and colleagues now report just such an engineering feat for a microtubule binding protein. Mal3p, they show, binds in such a way as to both stabilize microtubules and allow free-flowing transportation along their length.

Microtubules are not just the cell's scaffolding, they are also the highways for transporting factors and vesicles. To perform their various functions, microtubules interact with a number of motor proteins and other microtubule-associated proteins (MAPs), the most conserved of which are the end binding proteins, such as EB1.

Despite its name, the precise mode in which EB1 binds to microtubules was unknown. Light microscopy had revealed an accumulation of the yeast EB1 homologue, Mal3p, at the microtubule plus end, but also a faint signal along the microtubule length. Sandblad et al. used metal shadowing electron microscopy, in which a fine layer of metal is sprayed onto the sample, to look more closely at Mal3p binding. Like snow blown onto a tree, the metal builds up and brings the topography of the sample into sharp relief.

The team observed that Mal3p molecules aligned along the length of the microtubule but typically all in a single one of the many surface grooves. This, they showed, was the microtubule's seam.

The seam is formed by the closure of tubulin lattice sheets—like the edges of a sheet of paper rolled into the shape of a tube. The unique presence of Mal3p at these seams suggests Mal3p acts like sticking tape to hold the two edges of the sheet together. The binding of Mal3p thus provides stability and yet leaves the rest of the microtubule surface free for proteins to motor along to their cellular destinations. JCB



HOENGER/ELSEVIER

Mal3p (highlighted in red) stabilizes a microtubule by binding along its seam.

Reference: Sandblad, L., et al. 2006. *Cell.* 127:1415–1424.

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Transient silencing made permanent

All cancers have genes that are permanently silenced by DNA methylation. Yeshayahu Schlesinger, Howard Cedar (The Hebrew University, Israel), and colleagues, and Martin Widschwendter (UCL, London, UK), Peter Laird (USC, Los Angeles, CA), and colleagues inspect these irreversibly silenced genes. In normal tissues, they find, these genes carry transient repression signals, which are inappropriately made permanent in cancer.

Large parts of the genome get methylated and thus permanently silenced in the early embryo, but genes associated with CpG islands are spared. Some are instead transiently repressed by a complex called polycomb. This prevents inappropriate expression during development, but allows repression to be reversed when needed.

Irreversible silencing by DNA methylation does occur at many CpG island genes during cancer progression. Schlesinger et al. now show that >60% of genes that are methylated in colon cancer are marked by polycomb in normal tissues. In an accompanying paper, Widschwendter et al. calculated from previously published data that polycomb target genes are 12-fold more likely than nontargets to be methylated in cancer.

Different sets of CpG island genes get methylated in different cancers, but both groups found that, regardless of cancer type, the correlation between methylation and polycomb tagging is consistent.

No causal link between transient repression and permanent silencing marks, or between methylation of genes and cancer, has been established. Cedar speculates, however, that methylation of polycomb-tagged CpG genes could be an early, even causative, event in cancer. "Polycomb target genes are required for differentiation," he explains. "Therefore, cells have a mechanism for getting rid of polycomb. If, prior to that, these genes get abnormally methylated, the cell gets stuck in a state of proliferation, unable to differentiate."

The model suggests that cancer might originate from adult stem cells rather than from cell dedifferentiation. Perhaps an abnormally active DNA methyltransferase in certain stem cells incorrectly targets transiently repressed loci. JCB

References: Schlesinger, Y., et al. 2007. *Nat. Genet.* doi:10.1038/ng1950.
Widschwendter, M., et al. 2007. *Nat. Genet.* doi:10.1038/ng1941.

Pom1p prevents a spreading middle

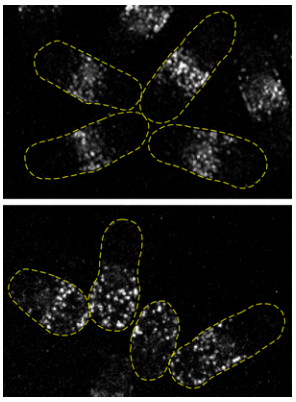
Cells often take on a perfect hourglass figure as they divide into two equal daughter cells. Precise positioning of the cytokinetic waistline in fission yeast requires inhibitory signals from the cell poles, Neal Padte, Fred Chang (Columbia University, New York, NY), and colleagues now report.

The waistline in *Schizosaccharomyces pombe* is positioned by a belt-like ring of mid1p at the cell's midpoint. Mid1p, located in the plasma membrane, then recruits myosin and other contractile ring proteins to separate the cell into two.

Padte and colleagues predicted by computer simulation that mid1p is in the middle because its diffusion to the poles is forbidden. They found that, in yeast cells lacking a polar kinase called pom1p, mid1p was no longer in a band around the middle, but was instead spread out. This in turn caused misplaced or multiple myosin contractile rings to form and the yeast to divide asymmetrically.

Spreading of mid1p in pom1p-deficient cells was only seen in one direction, however. Although pom1p is located at both poles, it is usually enriched at one. What prevents mid1p from creeping toward the other pole is currently under investigation. JCB

Reference: Padte, N., et al. 2006. *Curr. Biol.* 16:2480–2487.



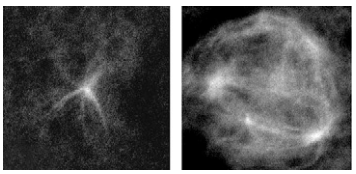
CHANG/ELSEVIER

Mid1p spreads from the yeast cell's middle toward one of the poles in the absence of Pom1p (bottom).

Clustering creates a spindle

Individual lobbyists might have a hard time promoting their cause, but get a large group of like-minded individuals together, and there's action. Similarly, the spindle assembly action of Aurora B starts only once this kinase comes together with other chromosomal passenger complex (CPC) members, according to a new study by Alexander Kelly, Hironori Funabiki, and colleagues (Rockefeller University, New York, NY).

Spindle assembly at the chromosomes is controlled by the Aurora B kinase, which deactivates microtubule-destructing proteins MCAK and Op18. The CPC member Dasra A is required for the rest of the CPC to assemble on chromosomes. Kelly et al. now show that this chromosomal loading serves to cluster the CPC components. It is this clustering that then kicks off the Aurora B pathway.



FUNABIKI/ELSEVIER

Clustering of CPC components (right) promotes spindle formation (white) in the absence of chromosomes.

The team induced clustering of the CPC proteins in cell extracts devoid of chromatin using antibodies to a CPC component. This clustering was sufficient to phosphorylate downstream targets of Aurora B and to generate spindles lacking chromosomes.

Clustering, the team showed, allowed the CPC components to effectively phosphorylate each other and ultimately activate Aurora B. They suggest that cytoplasmic phosphatases would dampen the activity of Aurora B that is not bound to chromosomes. Thus, clustering of CPC components by loading onto chromatin provides a "very simple mechanism to spatially control the kinase activity," says Funabiki. JCB

Reference: Kelly, A., et al. 2007. *Dev. Cell.* 12:31–43.