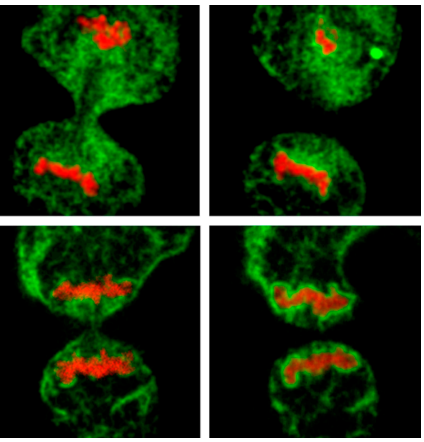
In This Issue



Knockdown of reticulon (bottom) speeds formation of the nuclear envelope, indicated here by a smooth membrane around chromatin (red).

Kick out the reticulons to close the envelope

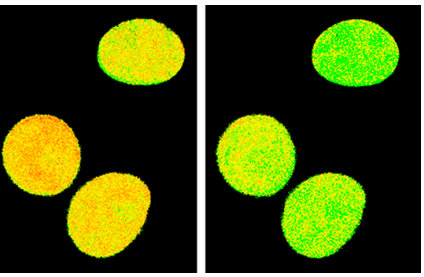
The nuclear envelope reforms when tube-forming reticulons are ejected from chromatin-associated ER tubules, say Anderson and Hetzer.

The nuclear envelope was once thought to disintegrate into vesicles during mitosis, but growing evidence suggests it is absorbed into the endoplasmic reticulum. Recent in vitro experiments by these authors have shown that, at the end of mitosis, ER tubules surround chromatin and gradually flatten out to form the nuclear envelope.

To test if the same process occurs in vivo, the authors tagged ER proteins, envelope proteins, and histones with fluorescent dyes. Chromatin remains free of ER membrane through metaphase, but by telophase, ER tubules started to attach to the chromatin, the authors showed. Once a few tubules were immobilized, more tubules slid into place alongside them, eventually coating the entire surface of the chromatin. As the nuclear envelope reformed, the ER tube-forming protein, reticulon, was cleared from the chromatin-associated membrane and collected in the surrounding ER tubules. The clearance of reticulon, which induces membrane curvature, coincided with flattening of the nuclear envelope.

From the onset of anaphase, complete closure of the NE took ~10 min. Ejection of reticulons from the tubules was rate-limiting because overexpression delayed closure, and knockdown hastened it. "Formation of the nuclear envelope from tubular endoplasmic reticulum requires massive reorganization," says PI Martin Hetzer, "so it's not too surprising that reticulons create a bottleneck as they are cleared." These results appear to clinch the case for the ER as the source of the nuclear envelope. RR

Anderson, D.J., and M.W. Hetzer. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805140.



When Ran binds to RCC1 (right), FRET (yellows and reds) decreases as chromatin binding increases.

Better binding with a flick of the tail

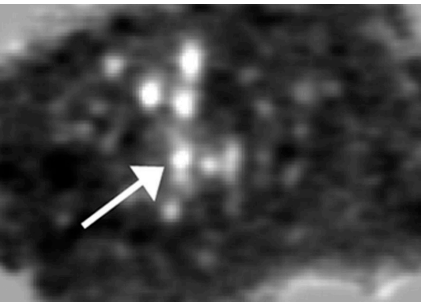
When RCC1's tail gets between it and chromatin, it gets a little help from its partner, Ran, to move the tail aside, according to Hao and Macara.

RCC1 is a chromatin-binding protein essential for chromosome condensation, mitosis, and nuclear envelope assembly, and is also the only known exchange factor for the all-important Ran GTPase. RCC1 is shaped like a doughnut with a tail. Both parts of the protein have been implicated in chromatin-binding—removal of either portion weakens, but does not prevent, chromatin binding—which suggests a synergy between doughnut and tail, but the mechanistic details have been lacking.

Binding of Ran to one side of the doughnut prompts the other side to bind chromatin, but because the doughnut is a rigid structure, Ran binding cannot simply induce an allosteric change in its shape. The authors thus looked more closely at the tail's potential function. Without the tail, RCC1 lost its ability to bind to DNA, but, surprisingly, bound more strongly to histones. This suggested that the tail might, at times, obstruct the protein's histone binding site.

The authors attached FRET fluorophores to both tail and doughnut, and found that when Ran was added, FRET efficiency dropped, in keeping with a movement of the tail away from the body of the protein. The authors propose that "Ran binding swings the tail outward, exposing the belly of the doughnut where histones bind, while moving the tail closer to the DNA." In keeping with this model, RCC1 bound to both DNA and histones more strongly in the presence of Ran. RR

Hao, Y., and I.G. Macara. 2008. *J. Cell Biol.* doi:10.1083/jcb.200803110.



Vesicles fusing with plasma membrane in adrenal chromaffin cells show up as bright spots with interference reflection microscopy.

Vesicles roar, and don't collapse

Hormone-containing vesicles open wide, then shut again without collapsing into the plasma membrane, say Llobet et al.

The phrase "kiss and run" is used to refer to the retrieval of a whole vesicle back into the cell after fusion with the plasma membrane. Although kiss and run occurs in fast fusion vesicles, where the pore opening is very small, slow fusion vesicles, which open wide, were thought to collapse into the plasma membrane. To get a closer look at vesicle dynamics, the authors turned to interference reflection microscopy (IRM), which allows direct visualization of membrane deflections of as little as ten nanometers. They attached adrenal chromaffin cells—a favorite model cell type for vesicle release studies—to a coverslip, and watched as individual vesicles fused with the plasma membrane. Each fusion event caused a deformation of the membrane, visible as a bright spot due to a change in the distance between membrane and coverslip.

The authors showed that each spot on the surface quickly brightened, in keeping with a dilation of the vesicle mouth after fusion, and then gradually dimmed. "We call it 'roaring,'" PI Leon Lagnado

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says, "since its behavior is reminiscent of the MGM lion." The team's movies of vesicle opening dynamics confirmed previous results that both the speed of opening and the ultimate size of the pore were calcium dependent, with lower concentrations causing faster and smaller openings.

When they treated vesicles with strontium to prevent them from closing, the bright spots remained, rather than disappearing as would be expected if the vesicle collapsed into the membrane. And when they inhibited dynamin, which promotes vesicle scission, vesicles could still close even though their departure from the membrane was blocked, indicating that vesicle closure is regulated independently from scission.

"I suspect this process is common to other hormone-secreting cells," Lagnado says, which in general use similar large vesicles, but it is unlikely to be seen in neurotransmitter-releasing vesicles, which are much smaller and which are known to collapse into the plasma membrane. The direct visualization allowed by IRM should be useful for answering a wide variety of other outstanding questions about vesicle fusion. **RR**

Llobet, A., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200807034.

A weak signaler gets in close

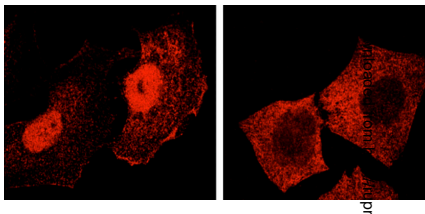
When c-Met can't shout, it moves closer to get its signal heard, according to Kermorgant and Parker.

c-Met is the plasma membrane receptor for hepatocyte growth factor (HGF), and signals by phosphorylating the transcription factor STAT3. But it's a weak activator of STAT3, and STAT3 faces a gauntlet of inactivating phosphatases that stand between the plasma membrane and the nucleus. This suggested to the authors that c-Met may not rely on long-distance diffusion of STAT3 to get its message across.

Looking at live cells, the team found that HGF stimulation caused c-Met and STAT3 to colocalize on endosomes at the periphery, where STAT3 became phosphorylated. But blocking microtubule trafficking of endosomes prevented active STAT3 from accumulating in the nucleus, confirming that diffusion was insufficient to get it there, and that its transport within endosomes, along with c-Met, was needed to deliver the signal. Not surprisingly, endosome trafficking was not required if the gauntlet of phosphatases was inactivated.

"Information flow from receptors is not simply a switch thrown at the cell surface," says PI Peter Parker. "The spatial distribution of signaling components is important, and a Western blot doesn't necessarily tell you much about what the pathway is doing in the cell." Although strong signal activators may be able to rely on peripheral signaling alone to get their message across, Parker suggests, for weaker ones, where they signal from may determine whether they are heard at all. **RR**

Kermorgant, S., and P.J. Parker. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806076.



Nuclear accumulation of STAT3 (left) is prevented by blocking endosome shuttling of c-Met (right).

SRC1, central to the nuclear periphery

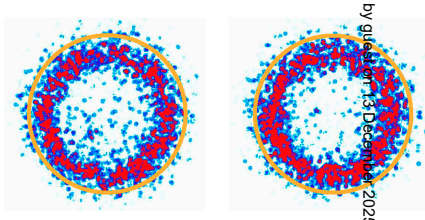
In yeast a handful of transcription-coupled export (TREX) factors, which package nascent mRNAs and eject them from the nucleus, have been identified. But Grund et al. were on the search for more. Now they've found SRC1, whose TREX credentials turn out to be just half of its story.

Grund et al. discovered SRC1's potential role in the TREX pathway by showing that it could compensate for the lack of certain TREX factors in yeast. Because little was known about SRC1, the next step was to remove it from yeast to see how it affected transcription. Only a small number of genes were misregulated, but interestingly these were skewed toward genes residing near telomeres. Members of the PHO family of genes, for example, which are located in subtelomeric regions, were up-regulated in the *Src1* mutant strain.

Chromatin immunoprecipitation showed that the SRC1 protein was enriched at telomeres and subtelomeres. Analysis of the structure and localization of SRC1 showed that it was an integral membrane protein that appeared to be specifically embedded in the inner nuclear membrane. It was possible that by interacting with both telomeres and inner nuclear membrane, SRC1 might bring genes, such as the PHO family, into the proximity of transcription repressors at the periphery—a region previously considered a site of silencing. Without SRC1, however, the active PHO genes remained at the nuclear periphery.

Exactly how SRC1 might repress PHO genes is thus so far unclear. And exactly why a potential TREX pathway factor would be working as a transcription repressor is also unclear. Although the periphery was historically considered as a site of silencing, recently it has been found that certain genes relocate there for activation, and it is thought that patches of inactive heterochromatin (such as telomeres) alternate with active chromatin near the nuclear pores (for easy mRNA export). The authors propose that SRC1 might somehow act at the interface between the two. **RW**

Grund, S.E., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200803098.



SRC1-regulated PHO genes (red and blue) locate near the nuclear membrane (orange circle) in the presence (left) or absence (right) of SRC1.