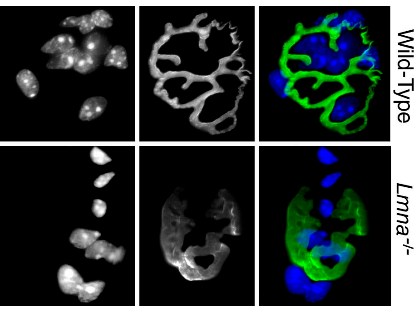
In This Issue



Lmna$^{-/-}$ **animals have disorganized neuromuscular synapses (green) without postsynaptic nuclei (blue).**

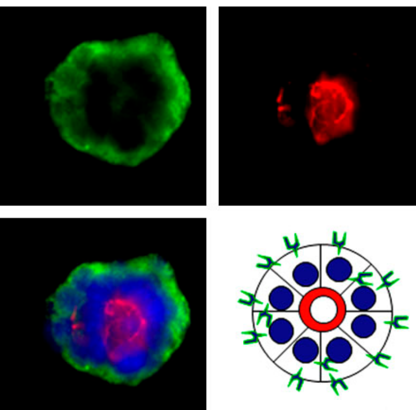
Lamin A/C deficiency is unnerving

Mutations in the nuclear intermediate filament lamin A/C (*LMNA*) gene are associated with Emery-Dreifuss muscular dystrophy, but cause the disease by unknown mechanisms. Méjat et al. show that one mechanism involves the disruption of neuromuscular junctions.

Muscle fiber cells contain hundreds of nuclei. In normal fibers, several nuclei cluster together under the cell membrane at sites of neuronal contact. These postsynaptic nuclei synthesize the components of the neuromuscular junction that specify the overlying membrane as the target site for innervation. The authors found that *LMNA*-deficient animals (including those with a point mutation in *LMNA* that in humans can cause Emery-Dreifuss disease) failed to position nuclei into these postsynaptic clusters. This prevented the proper organization of the neuromuscular junction and disrupted muscle fiber innervation, says author Alexandre Méjat.

The authors showed that either loss or mutation of *LMNA* disrupted nuclear positioning by causing the mislocalization of two other proteins: Nesprin-1, which spans the outer nuclear membrane and anchors nuclei to the actin cytoskeleton, and SUN2, which spans the inner nuclear membrane, linking Nesprin to lamin A/C. Although lamin A/C is ubiquitously expressed, *LMNA* defects specifically affected striated and skeletal muscle because Nesprin-1 and SUN2 are highly expressed in these tissues. Samples from Emery-Dreifuss muscular dystrophy patients exhibit similar hallmarks of skeletal muscle functional denervation, suggesting the authors are on the right track.

Méjat, A., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200811035.



Acini in 3D laminin-rich gels (red: apical surface; blue: nuclei; green: basolateral surface).

It takes two to party with chromatin

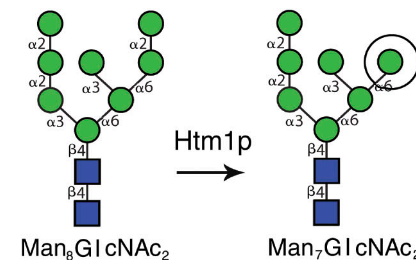
Xu et al. have discovered that two sustained signals combine to support milk production by mammary cells.

In vivo, mammary epithelial cells synthesize milk proteins when stimulated by the hormone prolactin. Yet, when these cells are cultured on flat, plastic dishes they do not make milk proteins even when prolactin is added to the cultures. The authors found that the cells on plastic became polarized with separate apical and basolateral surfaces. The prolactin receptor is present on the cells, but is localized on their basolateral side—attached to the dish—and thus cannot be accessed by prolactin applied to the apical surface.

Xu et al. showed that if prolactin was applied basolaterally to cells on plastic it could activate STAT5, the transcription factor that controls the expression of milk proteins—but this signal was transient and insufficient to support milk protein production. A second signal was required to achieve the sustained STAT5 activation and subsequent chromatin rearrangements that make milk genes accessible for transcription. This signal was supplied when the cells were grown in a laminin-111–rich gel: under these conditions, cells form into hollow spheroids called acini with their prolactin receptor exposed to the exterior.

Xu et al. showed that laminin-111 signaling (via a laminin receptor) synergizes with the prolactin signal to sustain STAT5 activation, though exactly how these two signals come together is so far unknown.

Xu, R., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200807021.



Htm1p produces $Man_7GlcNAc_2$, which Yos9p can recognize by its exposed α1,6-mannose ring (circled).

Htm1p: getting a "sugar-handle" on misfolded proteins

Clerc et al. show that the yeast protein Htm1p flags misfolded secretory proteins for degradation.

When first inserted into the endoplasmic reticulum (ER), proteins are decorated with an elaborate, branching chain of sugars. This improves the water solubility of the proteins and helps them fold in the ER lumen. But, as proteins repeatedly try to fold into their final shape, glucose and mannose sugar rings are sequentially lopped off the original chain to yield the $Man_8GlcNAc_2$ oligosaccharide. If a protein takes too long to fold it is degraded. It was thought that $Man_8GlcNAc_2$ flagged misfolded proteins to divert them for degradation. However, this model has recently been called into question.

It turns out that the subtly different $Man_7GlcNAc_2$ sugar is the degradation flag, says author Markus Aebi; he and his team have also worked out the protein players for producing this flag and for recognizing it. Htm1p, which was known to be required for degrading misfolded proteins, trims

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one further mannose ring from $Man_8GlcNAc_2$ to make $Man_7GlcNAc_2$. This was unexpected because no mannosidase activity was detected when Htm1p was first characterized (although it homologous to mannosidase enzymes).

$Man_7GlcNAc_2$ is then recognized by a protein called Yos9p. This protein specifically binds to the exposed mannose residue left after Htm1p's trimming. Yos9p was already thought to "proofread" glycans that signal protein misfolding and target them for degradation, but until now the specific signal sought by Yos9p wasn't clear. The work therefore provides important insights into how this arbiter of protein quality control operates in the ER.

Clerc, S., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200809198.

Lamin B locks up Oct-1

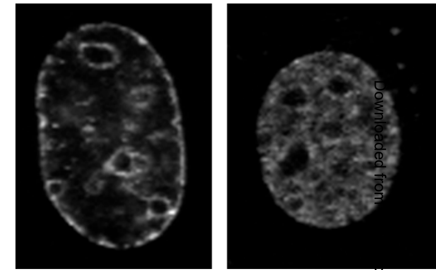
A large fraction of the transcription factor Oct-1 is associated with the inner nuclear envelope, but how and why it is retained there was unknown.

As for how, Malhas et al. show that Oct-1 binds to lamin B1, a prominent intermediate filament that lines the nuclear envelope, and in cells expressing a drastically truncated mutant of lamin B1, Oct-1 was disassociated from the nuclear envelope.

This left the question, why? The authors asked whether disrupting lamin B1–Oct-1 interactions could affect the expression of genes regulated by Oct-1. Indeed, in cells with truncated lamin B1, they found that expression of several Oct-1–regulated genes was altered because more Oct-1 could bind at these genes' promoters. Among the genes was a group involved in the oxidative stress response. As a result, these mutant cells accumulated higher levels of reactive oxygen species than wild-type cells.

It remains to be seen whether and how lamin B1–Oct-1 interactions are actively regulated in cells to help control gene expression. But, it is evident from these results that perturbation of lamin B1–Oct-1 interactions can make cells more vulnerable to oxidative stress. This could be particularly important in aging cells, where nuclear envelope integrity (and lamin B1 localization) is often perturbed, says author David Vaux. Lamins support the structure of the nucleus, and compromised nuclear structure has been a suspected cause of aging; another type of lamin, lamin A, is known to cause a premature aging disease when faulty. Increased production of reactive oxygen species—due to the perturbation of lamin B1 in mature cells—could be another way in which lamins contribute to the aging process.

Malhas, A.N., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200804155.



Oct-1 localizes to the nuclear envelope in wild-type (left) but not lamin B mutant cells (right).

CERT loss puts the brakes on growth

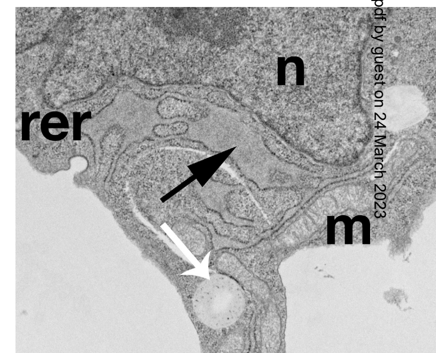
Wang et al. provide new insights into how ceramide transfer protein (CERT) affects cell growth and survival.

Many cancer therapeutic agents cause ceramide-dependent apoptosis, but the cell biology of this lipid is poorly understood. Recently, it was shown that CERT is required to transport ceramide from the endoplasmic reticulum (ER), where it is synthesized, to the Golgi, where it undergoes processing to create complex sphingolipids including sphingomyelin—a major component of plasma membranes. To learn more about CERT and ceramide in vivo, Wang et al. made CERT-deficient mice.

CERT-deficient embryos, they found, die around embryonic day 11.5. To explore whether increased ceramide levels and subsequent apoptosis could be to blame, the authors examined the embryos' cells. The ER of CERT-deficient cells was swollen, as ceramide was trapped in the organelle. This impaired ER function and also activated cellular stress pathways. Some of the trapped ceramide overflowed into mitochondria, causing these organelles to bloat too. How ceramide is transmitted from the ER to mitochondria remains unclear, but as with the ER, the ceramide accumulation impaired mitochondrial function.

Surprisingly, the stress and organelle malfunctions were not enough to kill the cells, as the cells up-regulated several adaptive responses. The cells did exhibit impaired growth rates however, as they adapted to these stressful conditions. In the growing embryos, this resulted in retarded organogenesis—the animals died when their hearts failed to develop properly. The implication for cancer therapy, on the other hand, is that targeting the CERT pathway might slow or stop a tumor's growth, but may not kill it.

Wang, X., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200807176.



CERT-deficient cells have swollen rough ER (black arrow) and distended or disintegrating mitochondria (white arrow).

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