Text by Ben Short
bshort@rockefeller.edu

Adding complexity to the nuclear pore complex

Two studies describe how nucleoporins affect muscle differentiation and budding yeast lifespan.

Nuclear pore complexes (NPCs) span the inner and outer membranes of the nuclear envelope and mediate transport between the nucleus and cytoplasm. In recent years, however, NPCs and the proteins that form them, known as nucleoporins, have also been linked to nuclear events including chromatin organization and gene expression, and to physiological processes such as differentiation and aging. Two papers now define the function of individual nucleoporins in mouse muscle differentiation (1) and budding yeast lifespan (2).

NPCs are composed of ∼30 different nucleoporins. In 2012, Martin Hetzer and colleagues at the Salk Institute in La Jolla, California, discovered that one particular nucleoporin, gp210/Nup210, is required for both neuronal and muscle differentiation. Myoblasts lacking Nup210, for example, undergo apoptosis instead of fusing together to form multinucleate myotubes (3).

To investigate how gp210/Nup210 promotes muscle differentiation, Hetzer and his graduate student Sebastian Gomez-Cavazos first determined which parts of the protein were required for myoblast fusion (1). Nup210 is a large transmembrane protein with a small C-terminal region facing the NPC, and a large, well-conserved N-terminal domain that protrudes into the perinuclear space between the inner and outer nuclear membranes. Nup210 mutants lacking the luminal domain failed to promote myoblast differentiation, whereas a version of the protein lacking the C-terminal domain supported myotube formation, even though it no longer localized efficiently to NPCs. "We wondered then whether Nup210 was acting away from nuclear pores," Hetzer says. Indeed, Nup210 mutants completely unable to localize to NPCs still supported muscle differentiation as long as the protein's N-terminal luminal domain was retained in the perinuclear space, which is continuous with the ER lumen.

But what does Nup210's luminal domain do to promote myotube formation? Muscle differentiation normally activates a mild stress response in the ER that helps to maintain calcium homeostasis and protein folding. Too much stress, however, can induce an ER-specific caspase cascade that triggers cell death. Gomez-Cavazos and Hetzer found that the ER stress response was highly activated in gp210/Nup210-deficient myoblasts undergoing differentiation. Inhibiting the ER stress-specific caspase cascade blocked cell death and restored the cells' ability to form differentiated myotubes. Nup210's luminal domain therefore appears to help muscle cells tolerate stressful conditions within the ER lumen/perinuclear space throughout differentiation. "It might be involved in calcium homeostasis, or it could act as a protein chaperone," Hetzer speculates.

> "The fact that they have opposite effects on lifespan was really surprising."

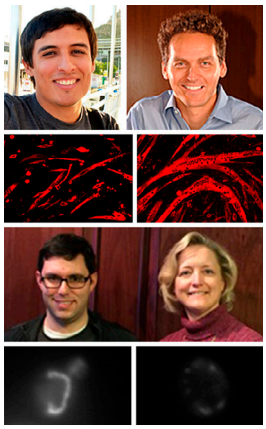
Meanwhile, Susan Wente, from Vanderbilt University in Nashville, Tennessee, was interested in following up on a previous observation from Hetzer's group that nucleoporins are down-regulated over time, increasing the leakiness of NPCs in aged rat neurons (4). Other groups have also linked changes in NPC composition to the aging of various organisms, but whether these changes are a cause or consequence of cellular aging is unclear. Wente and colleagues, led by postdoc Christopher Lord, decided to test whether nucleoporins directly affect the replicative lifespan of budding yeast, i.e. the number of daughters that a mother cell can produce before dying or becoming senescent (2).

Lord et al. examined the lifespan of yeast mutants whose nucleoporins lacked the FG repeats that fill the nuclear pore and assist the transport of karyopherin proteins and their cargoes. Removing the FG repeats from Nup116 decreased yeast replicative lifespan, whereas deleting the repeats of Nup100 increased yeast longevity. "These two nucleoporins are paralogues and are thought to be functionally redundant," Wente says. "So, the fact that they have opposite effects on lifespan was really surprising."

Both mutations made NPCs more leaky, however, indicating that aging isn't caused by changes in NPC permeability. Instead, the researchers found, removing the FG repeats of Nup116 decreased lifespan by disrupting the transport of a karyopherin called Kap121. This, in turn, reduced mitochondrial respiration, presumably because Kap121 helps export factors that promote mitochondrial function. Overexpressing Gsp1, a Ran GTPase that stimulates karyopherin-mediated nuclear transport, improved mitochondrial function and rescued the lifespan of *nup116ΔFG* yeast. Wild-type yeast lost full-length Nup116 as they aged, and overexpressing Gsp1 extended the lifespan of these cells too. Wente and colleagues now want to identify the critical cargoes of Kap121, and to investigate how Nup100 regulates replicative lifespan.

1. Gomez-Cavazos, J.S., and M.W. Hetzer. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201410047.
2. Lord, C.L., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201412024.
3. D'Angelo, M.A., et al. 2012. *Dev. Cell*. 22:446–458.
4. D'Angelo, M.A., et al. 2009. *Cell*. 136:284–295.

Focal Point



Two papers describe how different nuclear pore complex proteins affect cell differentiation and aging. (Top row, left to right) Sebastian Gomez-Cavazos and Martin Hetzer reveal that the nucleoporin Nup210 promotes muscle differentiation by maintaining nuclear envelope/ER homeostasis via its luminal domain. (Second row) Instead of fusing into multinucleate myotubes, differentiating myoblasts lacking Nup210 exhibit increased ER stress and apoptosis (left). Suppressing the ER stress-specific caspase cascade blocks apoptosis and restores myotube formation (right). (Third row, left to right) Christopher Lord, Susan Wente, and colleagues (not pictured) demonstrate that nucleoporins directly affect yeast cell aging. Removing the FG repeats from Nup116, for example, decreases replicative lifespan, apparently by inhibiting the karyopherin-mediated transport of factors required to maintain mitochondrial function. (Bottom row) The potentiometric dye Mitotracker shows that, compared with a wild-type cell (left), mitochondria are fragmented and have a lower membrane potential in yeast lacking the karyopherin Kap121 (right).

PHOTOS COURTESY OF THE AUTHORS; HETZER PHOTO COURTESY OF JOE BELCOVSON, SALK INSTITUTE FOR BIOLOGICAL STUDIES