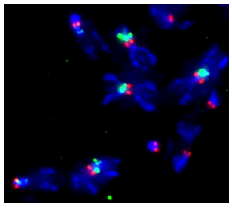
In This Issue

Text by Ben Short
bshort@rockefeller.edu

Satellite RNA guides kinetochore assembly



SAT III RNA (green) localizes near CENP-C (red) at the centromeres of mitotic chromosomes (blue).

Rošić et al. reveal that a noncoding RNA transcribed from repetitive DNA sequences promotes kinetochore assembly and chromosome segregation during mitosis.

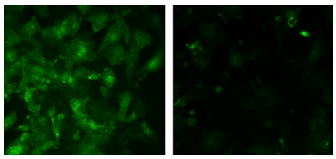
Centromeres—the chromosome regions where kinetochore proteins assemble and attach chromosomes to the mitotic spindle—are defined by epigenetic factors such as the histone variant CENP-A rather than by their specific DNA sequence. Centromeric chromatin is often characterized, however, by the presence of repetitive DNA sequences called satellite repeats, and some evidence suggests that RNAs transcribed from these regions might help cells identify the location of their centromeres.

Rošić et al. found that noncoding RNA transcribed from the satellite III (SAT III) repeats on the *Drosophila* X chromosome localized to the centromeres of most mitotic chromosomes. Depleting these SAT III transcripts caused defects in the segregation of all these chromosomes, not just the X chromosome. The researchers frequently saw chromosomes that failed to move to the spindle poles during anaphase. These lagging chromosomes showed reduced levels of centromere and kinetochore proteins including CENP-A, CENP-C, and Spc105, indicating that they are unable to attach to the mitotic spindle correctly.

SAT III transcripts interacted with CENP-C, suggesting that the RNA helps to recruit or stabilize this protein at centromeres, thereby promoting CENP-A incorporation and kinetochore assembly. Senior author Sylvia Erhardt thinks that noncoding RNAs transcribed from other satellite repeats may similarly act as epigenetic markers of centromeres. She now wants to investigate how SAT III transcription is regulated and to determine how the RNA interacts with CENP-C.

Rošić, S., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201404097.

mTORC2 helps brown adipose tissue fuel up



GLUT1 (green) is transported to the surface of brown adipocytes treated with a β_3-adrenoceptor agonist (left). This translocation is blocked in the presence of an mTOR inhibitor (right).

The kinase complex mTORC2 is the key regulator for adrenoceptor-stimulated glucose uptake in brown adipocytes, Olsen et al. reveal.

Brown adipose tissue can take up large amounts of glucose from the bloodstream to use as a fuel source to generate body heat. The sympathetic nervous system stimulates glucose uptake by activating the β_3-adrenoceptor, but the signaling pathway leading to GLUT1-dependent glucose import is largely unknown.

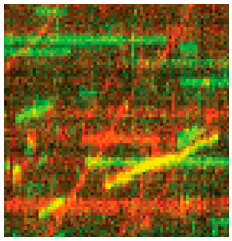
Insulin induces glucose uptake by activating the kinases PI3K and Akt, but Olsen et al. found that inhibiting these kinases had no effect on β_3-adrenoceptor–stimulated glucose import. In contrast, inhibiting the mTOR kinase—a key regulator of cell metabolism—blocked glucose uptake in both mouse and human adipocytes in response to β_3-adrenoceptor activation. mTOR inhibitors had no effect on cAMP-dependent GLUT1 expression but impaired the transporter's subsequent delivery to the plasma membrane.

mTOR operates in two complexes, known as mTORC1 and mTORC2. Olsen et al. found that the latter complex was required for glucose uptake by brown adipocytes, and they think that it promotes GLUT1 translocation by regulating the actin cytoskeleton.

Because brown adipose tissue can take up so much glucose from the bloodstream, stimulating this pathway could have both acute and long-term effects on glucose homeostasis in rodents and, perhaps, humans. It could therefore be an effective treatment for both type II diabetes and obesity. Senior author Tore Bengtsson now wants to investigate this possibility in mice.

Olsen, J.M., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201403080.

Building modular motor complexes



A kymograph shows the velocity of kinesin-1/kinesin-3 complexes (yellow) compared with individual kinesin-1 (green) and kinesin-3 (red) motors in vitro.

Norris et al. devise a method to control protein complex assembly in cells and use this approach to investigate how kinesin motor proteins coordinate their activities.

Little is known about how multiple motor proteins cooperate to transport cellular cargos, mainly because it is difficult to control the number and arrangement of motors in a given transport complex. Norris et al. designed a plasmid-based system that enables well-defined motor complexes to be assembled in vivo.

The complexes consist of helical scaffolds that can be linked at different positions to specific proteins of interest. When Norris et al. coupled kinesin-1 motors to either end of a 20-nm scaffold, the complex moved similarly to individual kinesin-1 molecules in vitro, indicating that each motor operated largely independently of the other. The motors showed even less cooperativity when they were separated by a longer, more flexible scaffold.

The researchers then paired kinesin-1 molecules with much faster kinesin-3 motors. Instead of moving at fast or intermediate speeds, most of the kinesin-1/kinesin-3 complexes moved at a slow, kinesin-1–like pace in vitro, again suggesting minimal cooperativity. The complexes also moved slowly on stable microtubules in vivo, but they travelled at faster, kinesin-3–like speeds on dynamic microtubules. Cargos might therefore recruit multiple motors in order to optimize their movement along different microtubule populations in different parts of the cell. Another possibility is that multiple motors may be required to generate enough force for cargo transport.

Norris et al.'s protein assembly system should help researchers to address these questions, as well as many other cell biological problems.

Norris, S.R., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201407086.