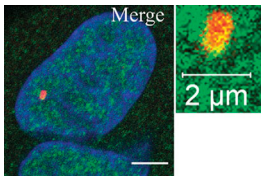
In This Issue

Text by Ben Short
bshort@rockefeller.edu

Histone H1 gets Pin'd onto chromatin



Pin1 (green) is enriched at a transcriptionally active region of the genome (red).

The proline isomerase Pin1 limits chromosome decondensation by stabilizing a histone's association with chromatin, Raghuram et al. reveal.

Histone H1 incorporates into nucleosomes to enhance chromatin folding and condensation. Phosphorylation by the cyclin-dependent kinase Cdk2 promotes H1's dissociation from chromatin, perhaps by altering the conformation of the histone's C-terminal domain. Raghuram et al. were therefore interested in the observation that, by phosphorylating serine–proline motifs, Cdk2 generates potential binding sites for the enzyme Pin1, which can alter protein conformations by changing the configuration of proline residues.

Raghuram et al. found that Pin1 bound to phosphorylated H1 and promoted the histone's dephosphorylation in vivo, probably by converting phosphoserine–proline bonds to the trans-configuration preferentially targeted by the phosphatase PP2A. In vitro experiments revealed that phosphorylation altered the conformation of nucleosome-associated H1 and that this was reversed by Pin1 binding. H1 was thus more stably associated with chromatin in cells expressing Pin1 compared with cells lacking the proline isomerase.

H1 is phosphorylated at transcriptionally active regions of the genome so that the surrounding chromatin relaxes enough to permit the transcriptional machinery access. Pin1 was also recruited to these regions, and, in the enzyme's absence, they became more decondensed than usual as H1 phosphorylation levels increased and the histone's association with chromatin was reduced.

Proline isomerization therefore counteracts H1 phosphorylation to prevent excessive decondensation during gene transcription. Senior author Michael Hendzel now wants to investigate Pin1's effect on chromatin structure and gene expression in more detail.

Raghuram, N., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201305159.

MAD2L2 helps mitotic cells take it slow

Listovsky and Sale describe how a protein slows cells' passage through mitosis by delaying activation of the anaphase-promoting complex/cyclosome (APC/C).

As a subunit of DNA polymerase ζ, MAD2L2 helps cells replicate damaged DNA and resolve chromosomes undergoing homologous recombination. The protein has also been shown to inhibit the cell cycle–regulated ubiquitin ligase APC/C in vitro, though how it does so and what effect this has in vivo is unknown.

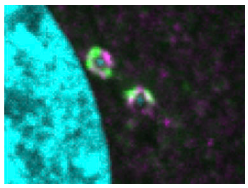
Once chromosomes are properly attached to the mitotic spindle, the APC/C targets proteins for degradation so that cells can segregate their chromosomes and exit mitosis. Listovsky and Sale found that cells lacking MAD2L2 degraded APC/C substrates prematurely and passed through mitosis quicker than wild-type cells.

To ensure that its substrates are degraded in the correct order, the APC/C is sequentially activated by two different proteins. CDC20 stimulates the ubiquitin ligase initially, but CDH1 then takes over to broaden the range of targets. Listovsky and Sale found that MAD2L2 bound to CDH1 and prevented it from associating with the APC/C early in mitosis. MAD2L2 itself was targeted for destruction by the APC/C and CDC20 at anaphase onset, freeing CDH1 to take over as the APC/C's activator. In cells lacking MAD2L2, CDH1 activated the APC/C prematurely, thereby accelerating mitotic exit.

MAD2L2-deficient cells often segregated their chromosomes incorrectly, perhaps because the loss of MAD2L2 is a double whammy that affects DNA polymerase ζ as well as mitotic progression. Defects in replication or recombination can result in problematic replication intermediates that lead to inaccurate chromosome segregation. Due to the premature activation of the APC/C, MAD2L2-deficient cells would also have less time to fix these errors before they divide.

Listovsky, T., and J.E. Sale. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201302060.

Ubiquitin isolates bacterial invaders



Two *Salmonella*-containing endosomes are decorated with ubiquitin (magenta) and Atg16L1 (green). DNA is stained blue.

Fujita et al. describe how the ubiquitination of host endosomal proteins helps capture invading bacteria inside autophagosomes.

In starving cells, double-membraned structures called autophagosomes engulf cytoplasmic content and deliver it to the lysosome for degradation and recycling. But autophagosomes can sometimes be more selective. In a process called xenophagy, for example, autophagosomes specifically form around invading bacteria. The engulfed pathogens are coated with ubiquitin, but which proteins are ubiquitinated, and how this aids bacterial capture, is unknown. One possibility is that proteins on the bacterial surface are ubiquitinated and then recognized by specific adaptor proteins, like p62, that link them to the autophagosomal protein LC3.

Using *Salmonella* or bacteria-mimicking beads, Fujita et al. found that, in fact, host endosomal proteins are ubiquitinated when xenophagy targets enter the cell and rupture the endosomal membrane. This ubiquitination was required to recruit several key autophagy proteins to bacteria-containing endosomes, including the ULK1 complex, which initiates autophagosome assembly, and the Atg16L1 complex, which helps conjugate LC3 to membrane lipids. These autophagy proteins were all recruited to damaged endosomes before LC3.

Atg16L1 bound directly to ubiquitin through its C-terminal WD domain. But Atg16L1 mutants lacking this domain were recruited to *Salmonella*-containing endosomes through two different backup mechanisms, including a direct interaction with the ULK1 subunit FIP200. Abolishing Atg16L1's localization was sufficient to prevent LC3's recruitment to ubiquitinated endosomes.

The researchers now want to investigate how cells recognize ruptured endosomal membranes and which host proteins are then ubiquitinated. Because the putative ubiquitin ligase targets host proteins, it can likely respond to a range of pathogens besides *Salmonella*.

Fujita, N., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201304188.