In This Issue

Text by Ben Short
bshort@rockefeller.edu

Mismatch repair provides a security patch for translesion synthesis

Tsaalbi-Shtylik et al. reveal that DNA mismatch repair (MMR) proteins suppress UV-induced mutagenesis by removing nucleotides introduced by error-prone DNA polymerases.

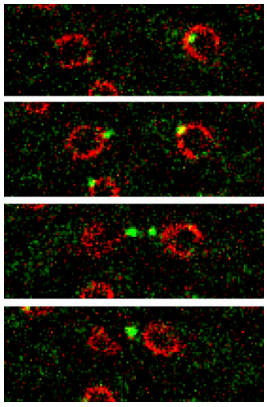
The MMR pathway is best known for its role in correcting the rare mistakes of replicative DNA polymerases. However, the MMR proteins Msh2 and Msh6 have also been implicated in preventing the introduction of DNA mutations following exposure to low, physiological doses of UV light. UV-damaged nucleotides can't undergo base pairing and are therefore not replicated by normal DNA polymerases. This results in patches of single-stranded DNA that can be filled in by specialized DNA polymerases in a process called translesion synthesis (TLS). These polymerases are error prone, however, so they frequently incorporate incorrect nucleotides opposite the photo-damaged nucleotide.

Tsaalbi-Shtylik et al. found that Msh2 and Msh6 recognize the incorrect nucleotides introduced by TLS opposite photolesions and trigger their removal, regenerating patches of single-stranded DNA around photo-damaged nucleotides. These patches induced the Chk1 DNA damage signaling pathway that arrests cells in S phase. If the single-stranded patches weren't filled in before the subsequent S phase, they were converted to double-strand breaks that initiated apoptosis.

In the absence of Msh2/Msh6, nucleotides incorrectly incorporated by TLS remained in place, reducing checkpoint activation and cell death but introducing an increased number of DNA mutations. Patients with Lynch syndrome, who inherit mutations in MMR genes, may therefore suffer a high incidence of colorectal cancers because their intestinal cells are susceptible to genotoxic insults that cause similar damage to UV light. Senior author Niels de Wind now wants to investigate whether other MMR proteins are also involved in this "post-TLS" repair pathway.

Tsaalbi-Shtylik, A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201408017.

For fission yeast nuclei, it takes two to tango



In this time-lapse series, dynein (green) accumulates on the spindle pole bodies as two haploid nuclei (delineated by their nuclear envelopes, red) approach each other.

Two minus end–directed microtubule motors work in parallel to bring haploid nuclei together during fission yeast mating, Scheffler et al. reveal.

Mating yeast cells must move their nuclei toward each other so that they can fuse and undergo meiosis. In budding yeast, nuclear congression is driven by Kar3, a member of the kinesin-14 family of minus end–directed motor proteins. To investigate whether the kinesin-14 Klp2 performs a similar function in fission yeast, Scheffler et al. trapped cells in microfluidic chambers so they could follow the mating process by live imaging.

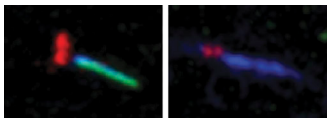
Nuclear congression was delayed in the absence of Klp2 but, unlike in budding yeast lacking Kar3, haploid nuclei eventually converged and fused together. The process was also delayed in fission yeast lacking the minus end–directed motor dynein, the researchers found, and almost completely blocked in cells lacking both motor proteins.

Klp2 localized to microtubules emanating from the spindle pole bodies associated with each haploid nucleus, and could potentially bring the nuclei together by sliding antiparallel microtubules past each other. Dynein, in contrast, localized to the spindle pole bodies themselves, where it might pull on microtubules emanating from the other nucleus once Klp2 has brought them into close proximity. Dynein's localization and its ability to promote nuclear congression depended on its light intermediate chain, the researchers discovered.

In higher eukaryotes, nuclear congression is driven by dynein but not kinesin-14. Fission yeast are thus unique in their reliance on two minus end–directed motors operating in parallel, a mechanism that may improve the efficiency of nuclear congression if the rod-shaped cells mate and fuse at odd angles.

Scheffler, S., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201409087.

Tmem231 keeps cilia in the zone



Compared with a wild-type cilium (left), Arl13b (green) is absent from the cilium (blue) of a cell lacking Tmem231 (right). The ciliary basal body is labeled red.

Roberson et al. demonstrate that a protein linked to human disease regulates ciliary membrane composition by organizing the organelle's transition zone.

The Meckel syndrome (MKS) complex assembles at the transition zone between the ciliary basal body and axoneme, where it controls the localization of ciliary membrane proteins. Mutations in the genes encoding MKS complex proteins disrupt ciliary membrane composition and cause MKS, a human disease characterized by cystic kidneys, polydactyly, and defects in bile duct formation. A transmembrane protein called Tmem231 binds to the MKS complex protein B9d1, but its contribution to the assembly and function of the complex is unclear.

Mutations in human *TMEM231* have also been linked to MKS, but *Tmem231* knockout mice die too early in embryogenesis to display any hallmarks of the disease. Roberson et al. knocked out *Tmem231* in a mixed genetic background, which allowed the animals to survive long enough to develop MKS-like symptoms. MKS complex components, including B9d1, failed to assemble at the ciliary transition zone in these mice, resulting in the loss of key signaling proteins, such as Arl13b and Inpp5e, from the ciliary membrane.

Roberson et al. identified several additional *TMEM231* mutations, not only in MKS patients but also in two siblings with orofaciodigital syndrome type 3. All of these mutations disrupted the MKS complex's localization and function in cultured fibroblasts. Senior author Jeremy Reiter now wants to understand how the complex regulates ciliary composition and to investigate why the symptoms of MKS are more severe than those of orofaciodigital syndrome type 3.

Roberson, E.C., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201411087.