

PARP retains Ku at double-strand breaks

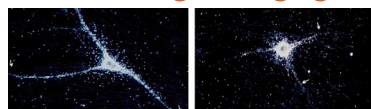
Couto et al. describe how a poly ADP-ribose polymerase (PARP) helps repair DNA double-strand breaks (DSBs) by promoting the accumulation of a key protein required for nonhomologous end joining (NHEJ).

PARPs are activated by DNA damage, catalyzing the poly ADP-ribosylation (PARylation) of proteins at both single-strand breaks and DSBs. In vertebrate cells, PARP1 and PARP2 regulate the repair of single-strand lesions and promote DSB repair by homologous recombination. But how PARylation promotes DSB repair by the NHEJ pathway—and which PARP is responsible—is unclear. Couto et al. investigated the functions of PARPs in *Dictyostelium*, a genetically tractable organism that expresses many DNA-repair proteins found in vertebrates but not lower organisms such as yeast.

Couto et al. discovered that two *Dictyostelium* PARPs, Adprt1b and Adprt2, were required to repair single-strand DNA lesions. PARylation at DSBs, however, was principally carried out by a third PARP, Adprt1a. The NHEJ pathway was less active in *Dictyostelium* lacking Adprt1a because the Ku complex, which recognizes DNA ends and recruits the NHEJ repair machinery, no longer accumulated on chromatin after DNA damage.

The Ku70 subunit contains a motif that binds to poly ADP-ribose. A version of Ku70 lacking this domain failed to accumulate on DNA after DSB induction and was unable to rescue the NHEJ defects of cells lacking wild-type Ku70. Senior author Nicholas Lakin now wants to identify which proteins are PARylated by Adprt1a in order to recruit or retain Ku70 at DSBs. Couto, C.A.-M., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201012132.

RNA targeting gets competitive



Compared to control neurons (left), BC1 RNA (white) isn't delivered to dendrites in cells lacking hnRNP A2 (right).

Muslimov et al. reveal how noncanonical structural motifs target RNAs to neuronal dendrites and describe how this might go awry in a neurodegenerative disease.

Many neuronal RNAs are delivered to specific locations within the nerve cell. This targeting is governed by RNA-binding proteins that often recognize the three-dimensional structure of the RNA rather than its specific nucleotide sequence. RNA structures are complex, in part because ribonucleotides aren't restricted to forming the standard "Watson-Crick" base pairs found in DNA. Noncanonical pairing between the purine bases guanine and adenine, for example, helps to form a stem-loop structure in BC1 that targets this regulatory RNA to neuronal dendrites.

Muslimov et al. found that these noncanonical base pairs

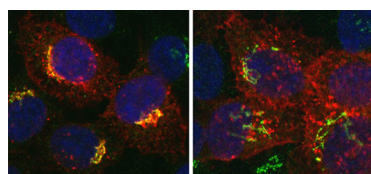
were essential for the interaction of BC1 with the RNA-targeting protein hnRNP A2 and that this interaction was required for BC1's delivery to dendrites. mRNA encoding the protein kinase PKM ζ relied on a similar interaction for its dendritic localization.

hnRNP A2 has also been proposed to bind stem-loop structures formed by noncanonical base pairing within the CGG repeats found in FMR1 mRNA. These CGG repeats are abnormally expanded in patients with the neurodegenerative disorder fragile X-associated tremor/ataxia syndrome (FXTAS). Muslimov et al. found that RNA containing a high number of CGG repeats inhibited BC1's interaction with hnRNP A2 and disrupted its delivery to dendrites, an effect reversed by increased amounts of hnRNP A2.

The repeat expansion found in FXTAS patients may thus perturb RNA targeting by competing for transport factors such as hnRNP A2. Senior author Henri Tiedge now wants to identify other RNAs that target to dendrites using noncanonical base pairing and to uncover additional proteins that interact with these motifs.

Muslimov, I.A., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201010027.

A COG in the retrograde transport machinery



Compared to control cells (left), TGN38 (red) isn't recycled back to the TGN (green) in Cog6-depleted cells (right).

Laufman et al. reveal how a membrane-tethering complex promotes the transport of vesicles from endosomes to the trans-Golgi network (TGN).

The conserved oligomeric Golgi (COG) complex helps to recycle

proteins back through the Golgi apparatus by tethering retrograde transport vesicles to their target membranes. Whether the complex has a similar function in docking endosome-derived vesicles to the TGN has remained unclear, however.

Laufman et al. found that endosome-to-TGN trafficking was impaired in cells lacking the COG subunit Cog6. Many of the SNARE proteins that promote the fusion of endosome-derived vesicles with the TGN were mislocalized in Cog6's absence.

In particular, the SNARE Syntaxin 6 no longer localized to the Golgi, and its expression level was reduced by proteasomal degradation. Cog6 bound directly to Syntaxin 6, suggesting that the tethering complex stabilizes the SNARE, perhaps by promoting its association with other Golgi-localized SNARE proteins.

Endosome-to-TGN transport was restored in Cog6-depleted cells by boosting levels of the vesicle SNARE VAMP4. Because vesicle SNARE overexpression often compensates for the loss of upstream tethering factors, this suggests that the COG complex does in fact tether endosome-derived vesicles to the TGN. The complex therefore appears to control transport to the TGN in two ways—by tethering vesicles and by stabilizing Syntaxin 6—as well as controlling retrograde trafficking to earlier Golgi compartments. Senior author Sima Lev says this probably allows the two transport steps to be coordinated. She now wants to investigate whether COG subunits interact with additional components of the TGN fusion machinery.

Laufman, O., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201102045.