This loss of viability continued throughout life, and, by the age of 7 months, ~10-fold fewer double-mutant mice were alive compared with *Ku80* single-mutant mice.

Rad54$^{-/-}$ *Ku80*$^{-/-}$ mice were hypersensitive to low doses of ionizing radiation, whereas, at the same dose, *Ku80*$^{-/-}$ mice were moderately sensitive. In addition, double mutants showed a significantly increased level of DSBs, as more than 90% of embryonic fibroblasts derived from *Rad54*$^{-/-}$ *Ku80*$^{-/-}$ mice contained γ-H2ax foci, and most of these contained a large number of foci, unlike single-mutant cells.

So, the HR and NHEJ pathways are important for survival and for genomic integrity, and seem to function synergistically in repairing DSBs. It is now important to establish the origin of these DSBs to understand how HR and NHEJ collaborate to maintain a stable genome.

Arianne Heinrichs

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maturation pathway from early to late endosomes. They also showed that CI-MPR can be found both in these retromer-containing tubules (which are probably *en route* back to the TGN) and in the intralumenal vesicles of endosomes.

To establish a functional link between retromer and CI-MPR, Bonifacino and co-workers used siRNA to deplete HeLa cells of VPS26 or VPS35. They observed that this resulted in a marked decrease in CI-MPR levels, which was due to an increase in its delivery to, and degradation in, lysosomes. These studies therefore "…indicate that retromer prevents the delivery of CI-MPR to lysosomes, probably by sequestration into endosome-derived tubules from where the receptor returns to the TGN." And, together, these papers have highlighted a retrieval role for the mammalian retromer.

Rachel Smallridge

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WEB SITES

Matthew Seaman's laboratory: http://www.cimr.cam.ac.uk/people/profiles/seaman_matthew_profile.html

Juan Bonifacino's laboratory: http://dir2.nichd.nih.gov/nichd/cbmb/Juan_Bonifacino.html

RNA SPLICING

A localizing cut

Reporting in *Nature*, Olivier Hachet and Anne Ephrussi reveal a new role for messenger RNA splicing — regulating the cytoplasmic localization of mRNA.

Until now, it was thought that the cytoplasmic localization of *oskar* mRNA to the posterior pole of the *Drosophila melanogaster* oocyte was specified by the 3′ untranslated region (UTR) alone. However, deposition of the human exon–exon junction complex (EJC) on mRNA is splicing-dependent and RNA-sequence independent, and two of the human EJC components are homologues of the *D. melanogaster* EJC proteins that are needed to localize *oskar* mRNA. This prompted the authors to investigate whether splicing regulates *oskar* mRNA localization.

Hachet and Ephrussi found that deleting all three *oskar* introns did not affect the transportation of this so-called *oskΔi(1,2,3)* mRNA into the oocytes of transgenic flies, in which no endogenous *oskar* mRNA was produced. However, by mid-oogenesis the localization of *oskΔi(1,2,3)* mRNA was defective (diffuse), with only a small amount present at the posterior pole by late oogenesis. A series of intron deletions showed that only intron 1 was required for the localization of *oskar* mRNA. The *oskar*-mRNA-dependent localization of the EJC protein Y14 to the posterior pole was also shown to depend only on the presence of intron 1. But is it the splicing of intron 1 or sequence-specific information in intron 1 that determines the localization of *oskar* mRNA?

To answer this question, the authors replaced the intron-1 sequence with the intron-3 sequence. This *osk(i3 in i1)* mRNA was correctly localized to the posterior pole, as was Y14. So the intron-1 sequence itself isn't required for localization, but splicing of the first exon–exon junction is.

Although *oskar* transcripts with no 3′ UTR fail to localize correctly, these results indicate that the 3′ UTR alone is not sufficient for the localization of *oskar* mRNA. Indeed, Hachet and Ephrussi found that the *oskar* 3′ UTR can only drive localization in the presence of (appropriately spliced) endogenous *oskar* mRNA, and that it is independent of Oskar protein, which indicates that other proteins are also involved. So, it seems that the 3′ UTR promotes the splicing-dependent assembly of *oskar* mRNA into an *oskar* mRNP complex. And as EJC components Y14 and Mago nashi are needed to localize *oskar* mRNA, it is the splicing-dependent deposition of the EJC at the first exon–exon junction that couples splicing to the correct cytoplasmic localization of *oskar* mRNA at the posterior pole of the oocyte.

The authors suggest that where the EJC is deposited on an mRNA molecule determines the architecture of the localization complex that is formed (by mediating interactions between factors that are bound to different regions of the mRNA). And, more broadly, they suggest this could explain why the EJC is not always involved in cytoplasmic-mRNA localization.

Natalie Wilson

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WEB SITE

Anne Ephrussi's laboratory: http://www-db.embl-heidelberg.de/jss/emblGroups/g_33.html