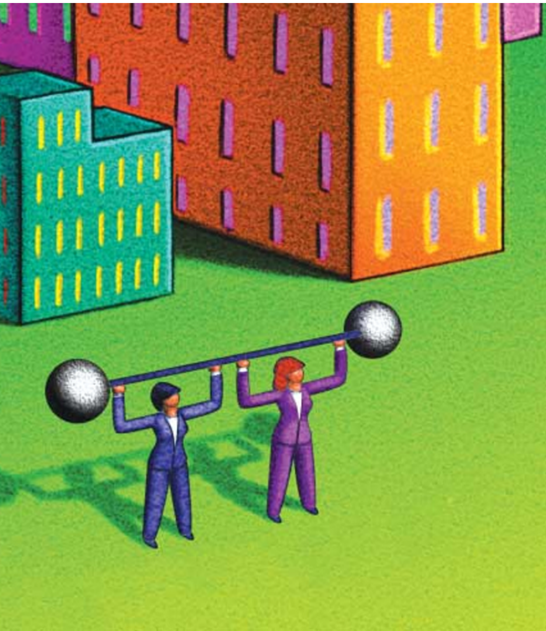
DNA REPAIR

It takes two



DNA double-strand break (DSB) repair occurs by at least two distinct pathways — homologous recombination (HR) and non-homologous end-joining (NHEJ). Both pathways are clearly important for maintaining genome stability, but little is known about how they are coordinated. Now, two papers in *Genes & Development* show that the pathways might collaborate in the repair of DSBs that arise after DNA replication.

Mice that lack the NHEJ-specific factor DNA ligase IV (Lig4) have a moderate mutant-repair phenotype, whereas deficiency for the HR factor Rad54 causes a more mild phenotype. Mills *et al.* created double-mutant mice that lacked both *Rad54* and *Lig4* to study the relationship between HR and NHEJ in DSB repair. They found that embryonic fibroblasts that were derived from these mice showed a severe proliferation defect compared with cells derived from either *Rad54* or *Lig4* single-mutant mice.

To examine whether this proliferation defect was due to a high level of DSBs, the authors carried out immunofluorescence experiments to detect the phosphorylated histone-H2A variant of H2ax (γ-H2ax), which is a marker for the presence of DSBs. Nearly 100% of double-mutant cells contained γ-H2ax foci, compared with 50% of *Lig4*$^{-/-}$ cells.

Because p53 deficiency can rescue the proliferation defect of double-mutant cells, Mills *et al.* used cells from triple-mutant mice that were also defective in p53 to study chromosomal abnormalities. The level of chromosome and chromatid instability in *Rad54*$^{-/-}$ *p53*$^{-/-}$ cells was low, compared with a significantly increased level of chromosome breakage in *Lig4*$^{-/-}$ *p53*$^{-/-}$ cells. Triple-mutant cells showed an even higher level of fragmented chromosomes, as well as a much larger proportion of broken sister chromatids. The high proportion of single-chromatid breaks in cells that are deficient for both Rad54 and Lig4, compared with either deficiency alone, implies that there is a cooperative role for both pathways in post-replication DSB repair.

Taking a similar approach, Couëdel *et al.* generated double-mutant mice that combine the HR mutant *Rad54* with the moderately affected NHEJ mutant *Ku80*. Significantly fewer double-mutant mice survived at birth compared with *Ku80* single-mutant mice.

MEMBRANE TRAFFICKING

Bring it back

Yeast have a protein complex known as 'retromer' that retrieves the receptor Vps10 (vacuolar protein sorting-10) from endosomes and returns it the Golgi. In mammalian cells, endosome-to-*trans*-Golgi-network (TGN) retrieval is poorly understood, although several proteins or protein complexes are thought to be involved in the endosome-to-TGN retrieval of mannose-6-phosphate receptors (MPRs) — the mammalian counterparts of Vps10. So, might the mammalian retromer be one of the protein complexes involved?

Two papers in *The Journal of Cell Biology* now answer this question with a resounding "yes". In the first paper, Matthew Seaman showed that VPS26, a mammalian retromer subunit, is localized to endosomal membranes. As this location fits with the suspected role for the mammalian retromer, he then studied endosome-to-TGN retrieval in a cell line that was derived from *Vps26*$^{-/-}$ mice and in HeLaM cells that had been treated with small interfering RNA (siRNA) against *VPS26*.

Seaman found that, in both cases, a loss of *VPS26* expression disrupted the trafficking of the cation-independent MPR (CI-MPR). In the mouse *Vps26*$^{-/-}$ cells, there was an increased degradation of CI-MPR in lysosomes, whereas in the siRNA-treated cells, there was an increased cycling of CI-MPR between endosomes and the cell surface. This difference is probably due to cell-type-specific trafficking pathways for CI-MPR.

In the final part of his study, Seaman used reporter constructs and antibody-uptake assays to show that a CI-MPR reporter construct moves from the cell surface to the Golgi through VPS26-positive endosomes and that, in the anti-*VPS26* siRNA-treated cells, this construct is retained in endosomes. Together, these data "…provide compelling evidence that [VPS26] (and therefore retromer) is required for efficient retrieval of the CI-MPR from endosomes to the Golgi."

In the second paper, Juan Bonifacino and colleagues used a yeast two-hybrid system and deletion mutants to show that two non-overlapping regions of the CI-MPR cytosolic domain can independently interact with VPS35 (another mammalian retromer subunit). This prompted them to look at the potential role of retromer in CI-MPR trafficking, and they used immunofluorescence microscopy to show that retromer mainly localizes to endosomes, where it partially colocalizes with CI-MPR.

Using immunogold cryo-electron microscopy for increased resolution, they showed that retromer is associated with tubular–vesicular profiles that emanate from early endosomes or from intermediates in the

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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

References and links

ORIGINAL RESEARCH PAPERS Mills, K. D. *et al*. Rad54 and DNA ligase IV cooperate to maintain mammalian chromatid stability. *Genes Dev.* **18**, 1283–1292 (2004) | Couëdel, C. *et al*. Collaboration of homologous recombination and non-homologous end-joining factors to the survival and integrity of mice and cells. *Genes Dev.* **18**, 1293–1304 (2004)

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

References and links

ORIGINAL RESEARCH PAPER Hachet, O. & Ephrussi, A. Splicing of *oskar* RNA in the nucleus is coupled to cytoplasmic localization. *Nature* **428**, 959–963 (2004)

WEB SITE

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

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