P53 FUNCTION

Do or die in the fly?

The p53 protein takes a two-pronged approach to restraining uncontrolled cell growth — it arrests the cell cycle to block cell proliferation, and activates apoptotic pathways to promote cell death. Or so the story goes. A paper by John Abrams and colleagues in *Proceedings of the National Academy of Sciences* now adds weight to the argument that this isn't always the case.

The authors were examining the functions of p53 in *Drosophila melanogaster* (Dmp53) through loss-of-function genetic analysis. They produced a targeted mutation at the *Dmp53* locus (*Dmp53*$^{-ns}$), and showed that flies with this disruption were viable and fertile, with no observable defects.

To activate programmed cell death in *Drosophila*, *reaper* (*rpr*) is one of three genes that need to be switched on. In response to γ-irradiation, Dmp53 is thought to do this by binding to a radiation-responsive enhancer upstream of *rpr*. The authors used a *rpr–lacZ* reporter transgene to show that this activation did not occur in *Dmp53*$^{-ns}$ mutant embryos, confirming that *rpr* is a transcriptional target of Dmp53. They also showed that another gene involved in cell death — *sickle* — was not induced after irradiation in *Dmp53*$^{-ns}$ mutants, indicating that this might be a Dmp53 target too.

Abrams and colleagues next checked the other side of the response — cell-cycle arrest. They irradiated wing discs, then looked for the presence of cells in mitosis. But they did not observe any cells in mitosis in either wild-type or the *Dmp53*$^{-ns}$ mutant wing discs after irradiation, suggesting that normal checkpoint functions were unaffected in the *Dmp53*$^{-ns}$ mutants.

Cells that lack *Dmp53* would be expected to show high rates of genomic instability, and the authors confirmed that, in response to moderate doses of ionizing radiation, *Dmp53*$^{-ns}$ embryos showed

NUCLEAR ASSEMBLY

Organize a get-together

In higher eukaryotes, the nuclear envelope (NE) breaks down during mitosis and nuclear pore complexes (NPCs) disassemble. So, what happens after mitosis? How do nucleoporins (Nups) reassemble to form NPCs that are incorporated into the new NE? This process has remained unclear, but now, in *Cell*, Mattaj, Doye and colleagues report that the conserved Nup107–160 complex has a crucial role in organizing this get-together.

The authors first used RNA interference in HeLa cells to deplete the levels of Nup107 and Nup133 — two components of the Nup107–160 complex. They found that this depletion reduced the levels of numerous Nups and decreased the density of NPCs in the NE — data that indicate a role for Nup107 and Nup133, and therefore the Nup107–160 complex, in NPC assembly.

To obtain more direct evidence for this role of the Nup107–160 complex, Mattaj, Doye and co-workers used an *in vitro* system based on *Xenopus* egg extract. First, they studied when the Nup107–160 complex functions in NE reassembly using sperm chromatin as a template for reassembly, and showed that Nup107 and Nup133 both associate with chromatin early, compared with a group of FG-repeat-containing Nups.

Next, the authors co-depleted Nup107 and Nup133 in this *in vitro* system and showed that, as in the mock-depleted controls, closed NEs formed on chromatin templates. However, in contrast to the controls, they saw essentially no NPCs on assembled nuclei in the Nup107/133-depleted extracts.

To verify that the co-depletion effects observed were specific to the removal of the Nup107–160 complex, Mattaj, Doye and colleagues tried to complement the defect. They purified the Nup107–160 complex from *Xenopus* egg extract and showed that NPC assembly and function could be restored only when the complex was added to the depleted extracts before closed NE formation. The Nup107–160 complex is therefore needed "…prior to NE closure for postmitotic NPC assembly into the NE".

In the final part of their study, the authors used the *in vitro* system to elucidate the step at which the Nup107–160 complex functions in NPC assembly. They showed that, in contrast to mock-depleted extracts, a group of FG-repeat-containing Nups could not associate stably with chromatin in extracts co-depleted of Nup107 and Nup133. This indicates that the Nup107–160 complex functions to recruit these FG-repeat-containing Nups to chromatin before they are assembled into NPCs and inserted into the NE.

The work of Mattaj, Doye and co-workers has therefore revealed a crucial role for the Nup107–160 complex in postmitotic NPC assembly. This has enabled them to propose a stepwise model in which this assembly is initiated on chromatin by early recruitment of the Nup107–160 complex. A question for the future is whether this complex also has a similar function during NPC assembly into NPC-containing NEs.

Rachel Smallridge

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WEB SITES

Valérie Doye's laboratory:
http://www.curie.fr/recherche/themes/detail_equipe.cfm/lang/_gb/id_equipe/28.htm

Iain W. Mattaj's laboratory:
http://www-db.embl-heidelberg.de/jss/emblGroups/g_45.html

considerably more mutagenesis than wild-type embryos. This indicates that Dmp53 can normally preserve genome stability by promoting apoptosis alone, and that failures in cell-cycle arrest are not required to account for genomic instability.

Another implication of these results is an evolutionary one. Similar observations have been made in studies with *Caenorhabditis elegans* p53, so Abrams and colleagues suggest that "…ancestral functions of p53 were intimately coupled to the regulation of cell death in the face of genotoxic challenge" and that "…an obvious corollary here is that checkpoint arrest by p53 may reflect a more recently invented function, specific perhaps to the vertebrate or mammalian lineage".

Alison Mitchell

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SYMBIOSIS

A cycle made for two

For human relationships to survive, there has to be some give and take — a rule that is also true for the symbiotic relationship between leguminous plants and nitrogen (N_2)-fixing bacteria (rhizobia). In root nodules, rhizobia are present as bacteroids that convert N_2 to ammonium (NH_4^+), and they had been thought simply to give plants NH_4^+ in exchange for the dicarboxylic acids they need for N_2 fixation. However, in *Nature*, Poole and colleagues now show that this symbiotic relationship is more complex than was previously thought and involves an amino-acid cycle.

The fact that bacteroids shut down NH_4^+ assimilation when they form a legume–rhizobia symbiotic relationship is known to be important for this symbiosis, but why? Could it be because plants give bacteroids the amino acids that the bacteria previously synthesized themselves? Isolated peribacteroid units from pea plants have been shown to secrete aspartate and alanine when incubated with dicarboxylic acids and glutamate, so amino-acid cycling is possible. But, is this cycling important for nodule metabolism?

To answer this question, Poole and co-workers first studied the effect of disrupting amino-acid transport in rhizobia. Free-living rhizobia that were mutated in both *aap* and *bra* — two ABC-type broad-specificity amino-acid transporters — were almost completely unable to take up a broad range of amino acids, although they could synthesize them. Peas nodulated with this mutant (known as RU1357) displayed features typical of plants unable to fix N_2, but the authors showed that these bacteroids did fix N_2. The problem was that the plants could not use the NH_4^+ made — an observation that contradicts the present models of bacterial and plant-nodule metabolism.

The authors next monitored the fate of $^{15}N_2$ fixed in nodules. Despite comparable levels of NH_4^+ production, the concentration of xylem amides was lower in RU1357-nodulated plants than in wild-type (A34) plants. In addition, although xylem-sap asparagine was ^{15}N enriched in RU1357-nodulated plants, the enrichment occurred at lower levels than in A34-nodulated plants and to a much lesser extent than for glutamine. The results indicate that, although the plants can still make amides, in the absence of bacteroid amino-acid transport, they cannot use the NH_4^+ released from bacteroids efficiently.

One explanation for this is that bacteroids give plants NH_4^+ and an amino acid such as aspartate for asparagine synthesis in the plant cytosol, and Poole and colleagues have proposed a model in which glutamate (or a precursor), in addition to dicarboxylic acids, is transported into bacteroids. In this model, glutamate enters through Aap/Bra and is used, together with dicarboxylic acids, to make aspartate or amino acids such as alanine. These amino acids are then secreted (possibly through Aap/Bra) and used by the plant to make asparagine.

In support of this model, the authors showed that bacteroid aspartate aminotransferase activity, which converts glutamate to aspartate, is essential for N_2 fixation, and that blocking amino-acid transport causes bacteroids to become carbon saturated (dicarboxylic acids can no longer be removed by being converted to amino acids and exported). So, in their relationship, plants give bacteroids amino acids, so that bacteroids can shut down NH_4^+ assimilation and, in return, "…bacteroids act like plant organelles to cycle amino acids back to the plant for asparagine synthesis". This cycle makes the plants and bacteria mutually dependent with an equal partnership, and therefore promotes the evolution of mutualism.

Rachel Smallridge

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WEB SITE

Philip Poole's laboratory:
http://www.ams.rdg.ac.uk/microbiology/rhizobium.htm