STRUCTURE WATCH

A radial way to anneal DNA

Eukaryotes have many tools to repair damaged DNA. One such tool — used to repair DNA double-strand breaks — is *RAD52*, which was discovered in yeast as a gene that helps protect against DNA damage caused by X-ray radiation, but that also repairs double-strand breaks during meiotic recombination. *In vitro*, the Rad52 protein binds to the Rad51 recombinase enzyme and stimulates its catalytic activity; Rad52 also interacts with single-stranded DNA (ssDNA) and induces annealing of complementary strands to form a duplex. But how does it promote DNA-duplex formation?

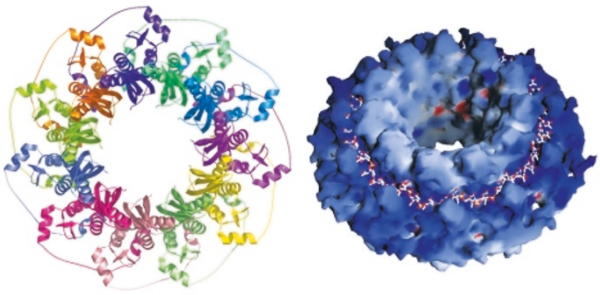
Wigley and colleagues now present the crystal structure of the ssDNA-binding amino-terminal domain (NTD) of Rad52. The NTD binds ssDNA in an unusual way, exposing every fourth nucleotide to attack by hydroxyl radicals in footprinting experiments. The structure reveals the basis for this repetitive pattern of DNA binding, and shows how Rad52 might promote the formation of double-stranded DNA (dsDNA).

The Rad52 NTD molecules are arranged as an undecameric ring, or wheel (see left-hand figure). Each Rad52 subunit has a core mixed α/β-fold domain that is connected to the carboxy-terminal helix by a flexible linker. The latter helix might be important in mediating Rad52's oligomeric state. The most striking feature of the Rad52 wheel, though, is a deep groove running along its entire outer rim (see right-hand figure). Amino acids at the bottom of this groove are positively charged (dark blue), whereas the residues that form the walls are hydrophobic. The groove is sufficiently wide to accommodate ssDNA, but appears to be too narrow for dsDNA (a ball-and-stick representation of ssDNA is modelled in the groove of the right-hand figure). The arrangement of amino acids indicates that the negatively charged DNA backbone might associate with the positively charged surface of the protein, leaving the DNA bases protruding out of the groove. Such an orientation might position the bases in such a way that allows the efficient formation of dsDNA. Further, the oligomeric nature of Rad52 might account for the four-base periodicity observed in footprinting experiments — the distance between each subunit in the Rad52 wheel is ~22 Å, which could accommodate precisely four bases of extended ssDNA. Together, the structural characteristics of the Rad52 wheel indicate that two Rad52 oligomers might come together like interlocking cogs and, in doing so, bring together complementary strands of DNA and promote energetically favourable duplex formation.

It is interesting to note that the name *RAD* was coined as a geneticist's shorthand for radiation sensitivity. How appropriate then that it also means 'wheel' in German.

Andreas Ladurner, Assistant Editor, Nature Structural Biology

REFERENCE Singleton, M. R. *et al.* Structure of the single-strand annealing domain of human RAD52 protein. *Proc. Natl Acad. Sci. USA* **21**, 13492–13497 (2002)



DEVELOPMENT

Please release me

Concentration-dependent cellular responses are often induced by the localized production of secreted proteins. One such protein — Hedgehog (Hh) — stimulates responses in distant cells, yet it is expected to remain in the membrane by virtue of its cholesterol and palmitoyl modifications. An insight into how this is possible comes from work published in *Cell*, in which DispA is identified as an essential factor for releasing Hh.

The authors first identified and characterized two murine homologues of *Drosophila disp*, *mDispA* and *mDispB*, which they propose encode 12-membrane-spanning proteins. On the basis of the early embryonic expression (E7.5–E9.5) of *mDispA*, and its ability to rescue a *disp* mutation, the authors studied the role of mDispA in early mammalian Hh signalling by deleting the exon encoding 11 of the 12 transmembrane domains. Homozygous embryos died at around E9.5, showing several patterning defects.

So Ma *et al.* further studied mDispA's function using fibroblasts from *mDispA*$^{-/-}$ embryos. A reporter construct containing binding sites for Gli — a transcription factor that is a downstream target of the Hh pathway — showed that *mDispA*$^{-/-}$ cells responded normally to exogenous Sonic Hh (Shh). This indicated that the *mDispA*$^{-/-}$ phenotype could be due to the failure either to modify Shh or to present or release it to neighbouring cells.

To test the former possibility, Ma *et al.* transfected constructs encoding full-length Shh (to which both palmitoyl and cholesterol can be added) or ShhN (which cannot be modified by cholesterol) into *mDispA*$^{-/-}$ cells and compared the electrophoretic mobility of the resultant proteins. Shh was efficiently cleaved and showed a higher mobility than ShhN, so the lack of Shh signalling in *mDispA*$^{-/-}$ embryos can't be due to defective Shh processing.

To confirm that the signalling defect arises from the failure to release or present Shh, ShhN-expressing *mDispA*$^{-/-}$ cells were transfected with *mDispA* or *mDispB* expression constructs and mixed with cells containing a Gli reporter. mDispA, but not mDispB, increased the amount of ShhN available for signalling to the reporter cells. Further experiments measuring the release of Shh showed that DispA functions to set Hh proteins free from cell membranes.

The authors propose that Disp proteins have a relatively simple function in initiating communication by catalysing signal release. On the basis of sequence similarity and subsequent mutational analysis, they have shown that Disp proteins are members of the bacterial RND family of transporters, which now has a new function — in membrane release of Hh.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Ma, Y. *et al.* Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of Dispatched. *Cell* **111**, 63–75 (2002)

FURTHER READING Ingham, P. W. & McMahon, A. P. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* **15**, 3059–3087 (2001)