STRUCTURE WATCH

A complex interaction

The cyclin-dependent kinase inhibitor Sic1 must be destroyed to allow cells to progress through the cell cycle, and this destruction occurs when Sic1 is phosphorylated on at least six of its nine Cdc4-phosphodegron (CPD) sites. This phosphorylated Sic1 is bound by the WD40 domain of the F-box protein Cdc4, which takes Sic1 to the SCF^{Cdc4} (Skp1/Cullin/F-box protein) ubiquitin-ligase complex — a multisubunit E3 enzyme. Here, Sic1 is ubiquitylated, which targets it for proteolytic destruction. To clarify the basis of phospho-epitope recognition by Cdc4, and to further understand how E3 enzymes orientate their substrates, Sicheri and colleagues now describe in *Cell* the 2.7-Å X-ray crystal structure of a Skp1–Cdc4 complex bound to a CPD phosphopeptide.

The structure showed that a core CPD motif — Leu-Leu-pThr-Pro — binds to an eight-bladed β-propeller WD40 domain in Cdc4, and it also clarified how F-box proteins present substrates for ubiquitin transfer. Furthermore, the authors found that the low-affinity binding of CPD motifs in Sic1 to Cdc4 reflects a structural incompatibility with the CPD-binding site in Cdc4. When they re-engineered Cdc4 to optimize Sic1 binding, lower phosphorylated forms of Sic1 were ubiquitylated. These data explain the phosphorylation threshold for Sic1 binding, and indicate "…an equilibrium binding mode between a single receptor site in Cdc4 and multiple low-affinity CPD sites in Sic1".

REFERENCE Orlicky, S. *et al.* Structural basis for phosphodependent substrate selection and orientation by the SCF^{Cdc4} ubiquitin ligase. *Cell* **112**, 243–256 (2003)

An active Holliday

During genetic recombination, two homologous DNA molecules exchange strands to form a four-way DNA (Holliday) junction, and the subsequent action of junction-resolving enzymes determines the final genetic outcome. In the absence of Mg^{2+}, the centre of this junction is unstacked and open, and the four helical arms point towards the corners of a square. However, in the presence of Mg^{2+}, the junction folds to form one of two possible stacked X-structures, in which two DNA strands run straight through a pair of stacked helices and the other two are swapped between helical pairs. It is thought that transitions occur between these alternative stacking conformers, but no direct evidence has been presented to support this idea. Now, though, in *Nature Structural Biology*, Ha and colleagues describe the use of single-molecule methodology to detect these transitions in real time.

The authors found that the processes of conformer transition and branch migration both have the unstacked, open structure as the common intermediate, but that conformer transitions occur much faster than branch migration steps. Correlations have been observed between the dominant stacking conformation and the resolution of a Holliday junction, which indicates that DNA sequences can affect the outcome of genetic recombination by biasing the stacking conformation. The results of this study therefore indicate that "…sequence-dependent conformer bias can be fully manifested even in a fully branch-migratable junction and can determine the extent of genetic information exchange upon junction resolution".

REFERENCE McKinney, S. A. *et al.* Structural dynamics of individual Holliday junctions. *Nature Struct. Biol.* **10**, 93–97 (2003)

P53

Parc keeper

It's a recurring theme in cell biology — in the complex and crowded environment of the cell, where you are is just as important as what you are. The tumour suppressor p53 is needed in the nucleus to prevent the growth of abnormal or damaged cells by activating genes involved in cell-cycle arrest and apoptosis. But p53 is not required during normal cell growth when there is no DNA damage and, consequently, large amounts of p53 can be found in the cytoplasm of unstressed cells. This, and the finding that many tumour cells have abnormally high levels of cytoplasmic p53, indicates that sequestration of p53 in the cytoplasm could represent a non-mutational control mechanism.

Now, in *Cell*, Gu and colleagues report the purification and characterization of a crucial factor in this process called Parc (p53-associated, parkin-like cytoplasmic protein). Parc is present as part of a large complex (~1 MDa) in the cytoplasm, and associates tightly with p53, with its amino terminus appearing to interact directly with the carboxyl terminus of p53. Sequence analysis showed that Parc contains several domains, including Ring–IBR–Ring and CCH (C-terminal Cullen homology domain) signature motifs, which are found in proteins associated with ubiquitin-ligase activity. But despite having an intrinsic ubiquitin-ligase activity, the authors found no direct evidence to suggest that Parc regulates p53 stability through ubiquitylation.

Gu and co-workers found that overexpression of Parc promoted the cytoplasmic sequestration of ectopic p53. Neuroblastoma cells were also found to express abnormally high cytoplasmic levels of Parc, and RNA-interference-mediated downregulation of Parc in these cells induced nuclear localization of p53 and p53-mediated apoptosis.

Taken together, these results suggest that Parc is a crucial regulator of the subcellular localization of p53. Whether Parc interacts with a protein called Mdm2, another ubiquitin ligase recently shown to be involved in the nuclear export of p53, will shed light on what seems to be yet another level of control of this remarkable protein.

Simon Frantz, Associate Editor,
Nature Reviews Drug Discovery

References and links

ORIGINAL RESEARCH PAPER Nikolaev, A. Y. *et al.* Parc: a cytoplasmic anchor for p53. *Cell* **112**, 29–40 (2003)
FURTHER READING Kastan, M. B. & Zambetti, G. P. Parc-ing p53 in the cytoplasm. *Cell* **112**, 1–2 (2003)