DNA REPAIR

Sensing breakdown

The ATR (ATM- and Rad3-related)-dependent checkpoint pathway signals both replication stress and various DNA-damage events. Reporting in *Science*, Zou and Elledge have now identified a 'common intermediate' for DNA damage and replication problems, that can be 'sensed' by the ATR-dependent checkpoint. Zou and Elledge, as well as Cimprich and colleagues, in *Current Biology*, also provide new insight into the mechanism underlying the intranuclear translocation of checkpoint proteins following DNA damage.

Single-stranded (ss)DNA had previously been proposed as the common intermediate required for checkpoint activation. However, ssDNA is coated with replication protein A (RPA), which led Zou and Elledge to study the function of RPA. They treated cells with ionizing radiation, and noticed that ATR — which exists as a complex with ATRIP (ATR-interacting protein) — formed nuclear foci, together with RPA. RPA colocalized completely with the ATR–ATRIP complex, the recruitment of which was RPA dependent.

Chk1 protein kinase is an ATR substrate that is phosphorylated in response to DNA damage or replication blocks. When expression of RPA70 (the largest subunit of RPA) was inhibited with small interfering RNA, phosphorylation of Chk1 was reduced compared with control cells. The authors found that RPA regulated ATR-mediated phosphorylation of Chk1, in response to both replication blocks and DNA damage.

Zou and Elledge hypothesized that RPA might function in damage recognition, by recruiting ATR–ATRIP directly to damage sites. This turned out to be correct, as RPA stimulated the binding of purified ATRIP to ssDNA in an *in vitro* binding assay, and ATRIP association recruited ATR to RPA–ssDNA complexes. Moreover, the recruitment of ATR–ATRIP to ssDNA was necessary for the phosphorylation of Rad17, an ATR substrate associated with ssDNA. So, the recruitment of ATR–ATRIP to ssDNA enables the activation of its DNA-bound substrates.

Adding to its significance, RPA function is conserved — in yeast, RPA was required for the recruitment of Ddc2 (the yeast homologue of ATRIP) to DNA damage *in vivo*. And the checkpoint-defective RPA mutant strain *rfa1-t11* was unable to recruit Ddc2 to ssDNA.

In agreement with Zou and Elledge's data, Cimprich and colleagues found that RPA and ATR co-localized to sites of DNA damage, and that intranuclear translocation occurring in response to DNA damage is a regulated process. Whereas Zou and Elledge identified a regulatory role for RPA in this process, the Cimprich group noted that an ATR mutant lacking kinase activity failed to relocalize, so they assigned an additional regulatory function to ATR.

So, although some of the details of the ATR checkpoint activation mechanism need to be clarified, Zou and Elledge concluded that "…an apparent activation may be achieved by the simultaneous enrichment of ATR–ATRIP and its substrates at the sites of DNA damage". The versatility of the ATR-dependent checkpoint, which can respond to various types of DNA damage and stalled replication forks, is achieved by recognizing the common intermediate, RPA–ssDNA. This simple model for DNA-damage signalling is highly conserved, as prokaryotes sense RecA–ssDNA and eukaryotes, RPA–ssDNA.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Zou, L. & Elledge, S. J. Sensing DNA damage through ATRIP recognition of RPA–ssDNA complexes. *Science* **300**, 1542–1548 (2003) | Barr, S. M. *et al.* ATR kinase activity regulates the intranuclear translocation of ATR and RPA following ionizing radation. *Curr. Biol.* **13**, 1047–1051 (2003)

IN BRIEF

CYTOKINESIS

Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation.

Casenghi, M. *et al*. *Dev. Cell* **5**, 113–125 (2003)

A role for Plk1 phosphorylation of NudC in cytokinesis.

Zhou, T. *et al*. *Dev. Cell* **5**, 127–138 (2003)

Both groups identified new substrates for Polo-like kinase 1 (Plk1), which has important functions in mitosis. Casenghi and colleagues characterized a centrosomal Plk1 substrate, named Nlp (nein-like protein). They found that Nlp associates with γ-tubulin and that it stimulates microtubule nucleation. This process is required for spindle formation and occurs when centrosomes undergo a structural reorganization, known as centrosome maturation, in preparation for mitosis. Phosphorylated Nlp dissociates from centrosomes and no longer binds γ-tubulin, and expression of a Nlp mutant lacking Plk phosphorylation sites causes aberrant mitotic spindles. So, displacement of Nlp from the centrosome might be necessary for centrosome maturation and spindle assembly. Zou *et al.* identified NudC (nuclear distribution gene C) as a Plk1 substrate *in vitro* and *in vivo*. Depletion of NudC by RNA interference induces multiple defects during mitosis, including multinucleation and cell arrest at the midbody stage, which indicates that NudC has a role in cytokinesis.

UBIQUITYLATION

Requirement for ubiquitin in Tat-mediated transactivation of the HIV-1 promoter.

Brès, V. *et al. Nature Cell Biol*. **5**, 754–761 (2003)

Ubiquitylation has emerged as a means to regulate transcription, so Brès *et al.* investigated whether Tat, the HIV-1 transactivator, is ubiquitylated and what effect this might have. They found that the E3 RING-finger protein Hdm2 ubiquitylates Tat on lysine 71 and that, rather than targeting Tat for degradation, ubiquitylation increases Tat transactivation. This process is required for Tat-mediated HIV-1 replication, although the exact mechanisms remain unclear.

SIGNALLING

QSulf1 remodels the 6-*O* sulfation states of cell surface heparin sulfate proteoglycans to promote Wnt signaling.

Ai, X. *et al*. *J. Cell Biol*. 2003 July 21 (DOI:10.1083/jcb.200212083)

The heparin sulphate (HS) chains of HS proteoglycans (HSPGs) bind ligands and affect signalling, but the regulation of sulphation is unclear. Here, the sulphatase QSulf1 is characterized as a 6-*O* endosulphatase — it removes internal sulphates from disaccharides. QSulf1, either at the cell surface or the Golgi, desulphates HSPGs to promote Wnt signalling. Ai *et al.* suggest that, in the absence of QSulf1, HSPGs bind Wnt ligands with high affinity, but that QSulf1-mediated desulphation of HSPGs lowers Wnt binding affinity, thereby allowing Wnts to be 'presented' to their receptors.