REPLICATION

Three of a kind

Two mechanisms for damage-induced replication arrest have been described so far; one that involves activation of the DNA-damage checkpoint and a checkpoint-independent one that causes slowing of replication-fork progression. Now, reporting in *The Journal of Cell Biology*, Matthew Stokes and Matthew Michael have uncovered a third damage-induced replication-arrest pathway.

The authors had previously shown that when damaged sperm chromatin from *Xenopus* (treated with the alkylating agent methyl methanesulfonate (MMS)) was incubated with undamaged sperm chromatin, replication of the undamaged DNA was delayed. This result was confirmed when they co-incubated MMS-treated DNA plasmids with undamaged sperm chromatin in a DNA replication assay, and found that replication of the sperm chromatin was inhibited. They also showed that the period of co-incubation was important — replication of the sperm chromatin was affected only during the first 10 min of co-incubation. So, the block occurs early on in the replication process.

Next, Stokes and Michael used damaged plasmid DNA that had been immobilized on magnetic beads. After incubation of the plasmid with *Xenopus* egg cytosol, the plasmid beads were collected and the supernatant recovered. Incubating this extract (which had been exposed only transiently to damaged DNA) with sperm chromatin in a replication assay blocked the replication of sperm chromatin. This led the authors to believe that the extract contains an inhibitor of replication.

But which replication step is blocked by this inhibitor? To answer this question, Stokes and Michael co-incubated plasmid and sperm chromatin as in the standard DNA-replication assay, and then separated the plasmid and the rest of the extract from the chromatin, which they tested for chromatin-associated proteins by immunoblotting. They found that co-incubating damaged plasmid, but not undamaged plasmid, resulted in a reduction in the amount of chromatin-associated proliferating cell nuclear antigen (PCNA), which is an essential replication factor. However, the recruitment of DNA polymerase (pol) α to chromatin was unaffected. This implies that the block occurs after pol α binds to chromatin and before PCNA is loaded onto the replication fork.

Using an inhibitor of checkpoint kinases to block the DNA-damage checkpoint pathway, the authors showed that the ability of MMS-treated plasmid to block replication of sperm chromatin was unaffected, which means that MMS-induced replication arrest is checkpoint independent. Interestingly, they also showed that stimulation of replication arrest of undamaged sperm chromatin activates the checkpoint response.

So, although the diffusible inhibitor is still elusive, the data indicate that "…the coupling of replication arrest and checkpoint activation pathways suggest an integrated response to MMS-induced damage…".

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References and links

ORIGINAL RESEARCH PAPER Stokes, M. P. & Michael, W. M. DNA damage-induced replication arrest in *Xenopus* egg extract. *J. Cell Biol.* **163**, 245-255 (2003)

STRUCTURE WATCH

Fix in place

Aurora A — an oncogenic serine/threonine protein kinase — is important in cell-cycle progression, and early in mitosis it's required for mitotic spindle assembly. It's activated by the microtubule-associated protein TPX2, which also localizes Aurora A to spindle microtubules, and by phosphorylation. Despite its importance in cell division and cancer, the mechanism of Aurora-A activation has remained unclear. Now, though, in *Molecular Cell*, Conti and colleagues provide new insights by describing the crystal structures of phosphorylated human Aurora A alone and in complex with the minimal activating domain of TPX2.

Comparing the structure of phosphorylated TPX2-bound Aurora A with other kinases showed that it closely matches the active conformation of kinases. In this structure, the 'activation segment' of Aurora A, which contains the crucial phosphothreonine, is in a conformation that is competent for substrate binding. However, in the absence of TPX2, although the overall structure of phosphorylated Aurora A remains very similar, the activation segment adopts an inactive conformation, in which the crucial phosphothreonine is accessible to deactivating phosphatases. So, although TPX2 binding triggers no global conformational change in Aurora A, it pulls on the activation segment using a lever-arm-like mechanism, which moves the phosphothreonine into a buried position and fixes the active conformation in place. And, as the intermolecular interaction between Aurora A and TPX2 resembles the intramolecular interaction of the catalytic core of cAMP-dependent protein kinase with its flanking extensions, this mechanism could be a common theme in kinase regulation.

REFERENCE Bayliss, R. *et al.* Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol. Cell* **12**, 851–862 (2003)

Getting a grip on GRIP

Four golgins — large coiled-coil proteins that have functions in Golgi structure and vesicle traffic — are targeted to the *trans*-Golgi membrane by their GRIP domain. This targeting is mediated by GRIP binding to the Arf-like (Arl) GTPase Arl1, which is also Golgi localized. But, what is the molecular basis of this targeting? Munro and colleagues now provide clues in *Molecular Cell*, by describing the 1.7-Å-resolution crystal structure of human Arl1-GTP in complex with the GRIP domain of the human golgin-245.

In the structure, the GRIP domains form dimers and each monomer contains three anti-parallel α-helices arranged in an S shape. One face of the monomer is involved in dimer formation, while the opposite face binds Arl1. So, each GRIP homodimer binds two Arl-GTPs, and it does this using two α-helices from each monomer. A comparison of this structure with other GTPase–α-helical-effector complexes indicates that, despite the lack of sequence and topology conservation, this recognition of a pair of α-helices might be a common structural basis for effector binding. The bivalent interaction is, however, unique to the Arl1-GTP–GRIP complex, and this interaction might be a way to increase the residence time of this complex on the Golgi membrane. The structure also indicates how this complex might interact with Golgi membranes — through the amino-terminal myristoyl group of each Arl1 and the carboxy-terminal tail of each GRIP.

REFERENCE Panic, B. *et al.* Structural basis for Arl1-dependent targeting of homodimeric GRIP domains to the Golgi apparatus. *Mol. Cell* **12**, 863–874 (2003)