HIGHLIGHTS

PCNA

<http://ca.expasy.org/cgi-bin/niceprot.pl?P18248>

Polymerase α

<http://ca.expasy.org/cgi-bin/niceprot.pl?Q9DE46>

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...".

Arianne Heinrichs

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)