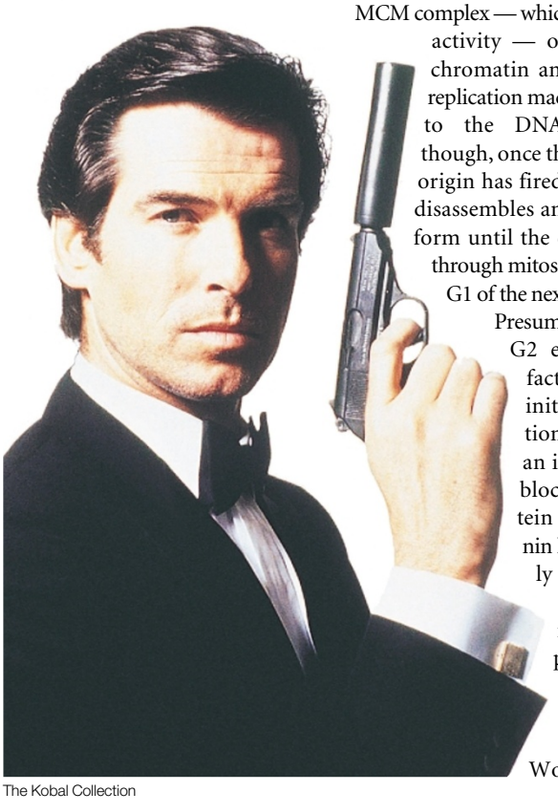
# HIGHLIGHTS



DNA REPLICATION

## Licence to kill

Cells are faced with a problem each time they divide — how can they ensure that their chromosomal DNA is duplicated only once per cell cycle? The first clues came five years ago, when components of the complex that 'licenses' the DNA for a single round of replication were identified. But this still left the question of how licensing is regulated. Reports in *Science* and *Nature Cell Biology* now fill in this piece of the puzzle, connecting the activities of two components with positive and negative effects on the replication of DNA.



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Replication follows a stepwise pattern of protein assembly. Binding of the 'origin-recognition complex' at initiator elements in the DNA is followed by attachment of two further proteins, Cdc6 and Cdt1. Next, during G1, these proteins recruit the so-called MCM complex to form the 'pre-replication complex' (pre-RC). At this stage the cell is licensed for replication, probably because the MCM complex — which has helicase activity — opens up the chromatin and allows the replication machinery access to the DNA. Crucially, though, once the replication origin has fired, the pre-RC disassembles and cannot reform until the cell has gone through mitosis and entered G1 of the next cell cycle.

Presumably nuclei in G2 either lack a factor needed to initiate replication, or contain an inhibitor that blocks it. A protein called geminin had previously been shown to block DNA replication by preventing loading of the MCM complex. So Wohlschlegel and colleagues have asked what its target might be. They used immunoprecipitation to show that geminin interacts with the MCM-loader Cdt1 in human cells. They then used an *in vitro* system of DNA replication to test whether geminin inhibits replication by targeting Cdt1. They found that geminin-dependent inhibition of pre-RC formation can be counteracted by excess Cdt1, supporting the idea that geminin targets Cdt1 to inhibit re-replication.

Tada and co-workers approached the problem using fractionated egg extracts from *Xenopus laevis*. They showed that geminin blocks the licensing ability of one fraction, termed RLF-B, and purification of RLF-B revealed the active ingredient to be Cdt1. The authors confirmed that geminin and RLF-B interact and antagonize one another. Finally they found that the geminin present during metaphase is enough to completely block origin assembly.

Geminin, then, has a licence to kill DNA replication — at least temporarily. Whether it acts only through Cdt1, and whether it is redundant with other regulatory mechanisms, are just some of the next questions to be tackled.

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### References and links

**ORIGINAL RESEARCH PAPERS** Wohlschlegel, J. A. *et al.* Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. *Science* **290**, 2309–2312 (2000) | Tada, S. *et al.* Repression of origin assembly in metaphase depends on inhibition of RLF-B/Cdt1 by geminin. *Nature Cell Biol.* **3**, 107–113 (2001)
**WEB SITE** Xenbase