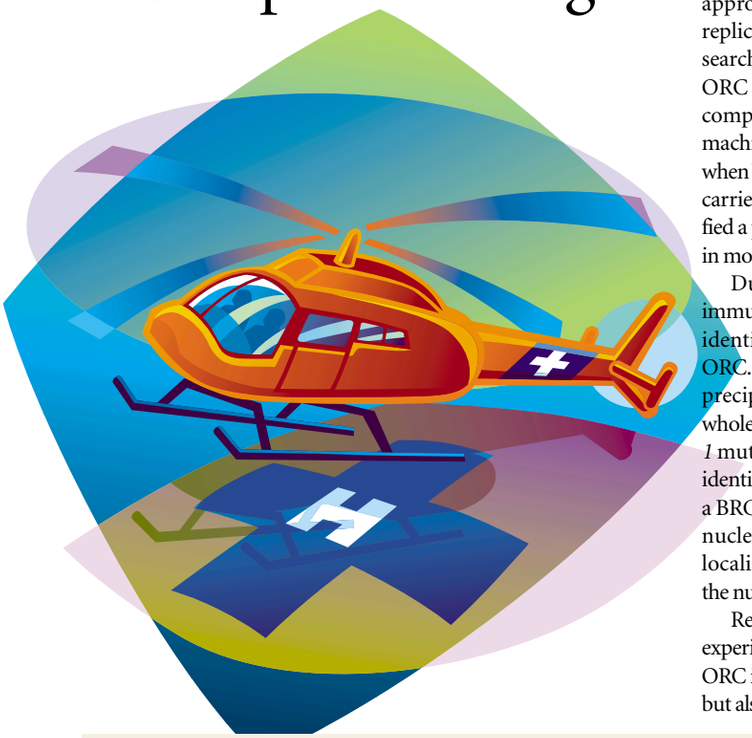
DNA REPLICATION

A complex landing



The origin-recognition complex (ORC) is a molecular landing pad. At various times during the cell division cycle, ORC nucleates the assembly of appropriate protein complexes at replication origins. For this reason, a search for proteins that interact with ORC is often used to uncover new components of the DNA-replication machinery. But, as they report in *Cell*, when Yi-Chieh Du and Bruce Stillman carried out such a search, they identified a protein that seems to be involved in more than just DNA replication.

Du and Stillman started with immunoprecipitation experiments to identify proteins that interact with ORC. Seven proteins were specifically precipitated from wild-type yeast whole-cell extracts, but not from *orc2-1* mutant extracts, one of which was identical to yeast Yph1. Yph1 contains a BRCT domain, as well as a putative nuclear-localization signal, and is localized mainly in — or near to — the nucleolus.

Reciprocal immunoprecipitation experiments confirmed that Yph1 and ORC interact both *in vitro* and *in vivo*, but also showed that several other proteins interact with Yph1. To analyse these complexes, the authors separated them by glycerol-gradient sedimentation, followed by western blotting. Two main complexes were detected, and mass-spectrometry analysis showed that the smaller one contained Yph1, Erb1 and Ytm1. The larger complex contained these proteins, but also trapped factors that are involved in cell-cycle regulation, checkpoint control, ribosome biosynthesis and chromatin remodelling.

Both Erb1 and Ytm1 are involved in biosynthesis of the 60S ribosomal subunit, and Ytm1 is also essential for the G1–S transition. So, Du and Stillman next investigated the ribosome profile of a temperature-sensitive *Yph1* mutant strain (*yph1-td*). They could not detect free 60S ribosomal subunits in the *yph1-td* cells, which indicates that Yph1 is needed for the synthesis or stability of this subunit.

How might this role for Yph1 in ribosome biogenesis link to its possible function in DNA replication? The authors noticed that the levels of Yph1 varied in cells that were grown

DNA REPLICATION

Converging pathways

As the Yph1 story shows, evidence for a link between DNA replication and ribosome biogenesis is growing. This link is strengthened by a second report in *Cell* from Chun Liang and colleagues, who describe Noc3 ('nucleolar complex-associated protein') as another new binding partner for ORC. Noc3 is the first basic helix–loop–helix protein that has been shown to be involved in replication initiation, and the surprise is that, like Yph1 and its associated proteins, Noc3 is required for the processing of pre-ribosomal RNAs.

Liang's group used a genetic screen to identify previously unknown initiation proteins for DNA replication in budding yeast. Specifically, they looked for proteins that interact with Mcm5 — one of the so-called 'minichromosome maintenance' (MCM) proteins. Along with ORC and various other proteins (including Cdc6 and Cdt1), the MCMs bind origins of replication.

The authors identified the *NOC3* gene as a suppressor of an *mcm5* mutant, and then used reciprocal co-immunoprecipitations to show that the Noc3 and Mcm5 proteins interact directly *in vivo*. They also detected the interaction of Noc3 with Mcm2 and Orc1 (a subunit of ORC).

To test whether Noc3 is essential for the initiation of DNA replication, Liang and co-workers created a temperature-sensitive *noc3-td* mutant strain. A plasmid-loss assay showed that Noc3 is indeed required for replication initiation, and FACS analysis of wild-type and mutant cells throughout the cell cycle confirmed that Noc3 is needed for entry into — but not progression through — S phase.

Initiation proteins such as ORC, Cdc6 and the MCMs can all bind to chromatin, and a chromatin-binding assay showed that Noc3 also shares this property. What's more, it remained bound at all stages of the cell cycle, which indicates that, like ORC, Noc3 is constitutively bound.

During the M–G1 transition, the 'pre-replicative complex' (pre-RC) — which consists of at least ORC, Cdc6, Cdt1 and the MCM proteins — is assembled at replication origins. The MCMs are then maintained at the pre-RC to allow other replication proteins to be loaded. So, could Noc3 be needed for the formation or maintenance of the pre-RC? To test this, the authors asked what would happen to loading of the various components in the *noc3-td* cells. They found that little or no Cdc6 or Mcm2 was loaded onto chromatin in these cells, which indicates a role for Noc3 in this process. Conversely, when a temperature shift was used to remove Noc3 from chromatin, Mcm2 was also released. Finally, ORC was shown to be necessary for the stable association of Noc3 with chromatin.

Conventional wisdom has it that ORC loads Cdc6 and the MCMs onto chromatin to form the pre-RC. So the discovery of Noc3 as an intermediary in this process is an important find. How this function sits with the role of Noc3 in ribosome biogenesis is a question for further study, but it raises the tantalizing possibility that, like Yph1, Noc3 might also be involved in coordinating several cellular pathways.

Alison Mitchell

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WEB SITE
Chun Liang's laboratory:
http://www.ust.hk/~webbich/profiles/cliang.html

under different conditions, and showed that there is a correlation between the levels of Yph1 and the rate of proliferation that occurs in response to the energy source that is used. So, levels of Yph1 are high when glucose is plentiful and proliferation is high, but low (or absent) when slow proliferation occurs due to poor energy sources.

When these findings are combined, a picture emerges in which Yph1 might help to link processes that require high levels of energy — for example, DNA replication and ribosome biogenesis — to a regulatory mechanism that senses the amount of an available energy source. Elegant though this explanation is, however, the authors point out that Yph1 could turn out to be involved in these processes independently.

Alison Mitchell

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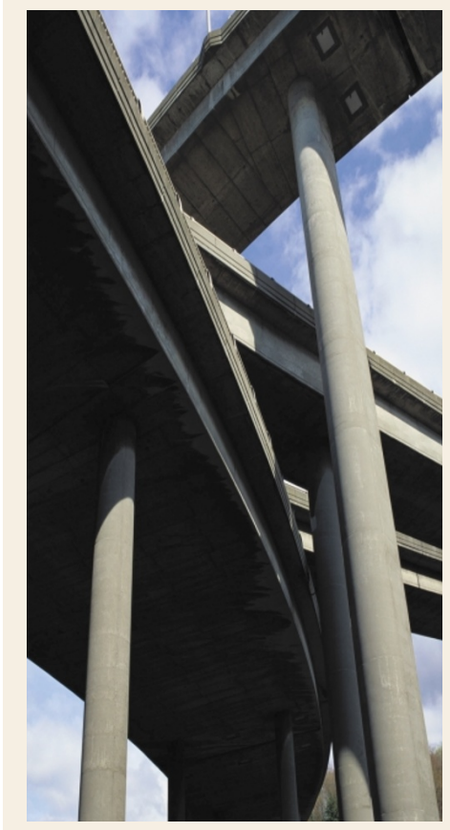

PROTEOLYSIS

A new family

It has generally been thought that the presenilin component of the γ-secretase complex catalyses the intramembrane proteolysis of transmembrane (TM) substrates such as Notch and β-amyloid precursor protein. However, recent observations have indicated that, in fact, another component of this complex might be responsible for this catalytic activity. But now, in *Science*, Martoglio and co-workers reaffirm the catalytic role of presenilin by reporting the identification of a new presenilin-like aspartic protease — human signal peptide peptidase (SPP).

Many integral membrane proteins need a signal sequence for correct membrane insertion, and signal peptidase releases the signal-sequence peptide into the membrane after insertion. So, where does SPP come in? It is thought that SPP catalyses the intramembrane proteolysis of signal peptides, and releases functional signal-peptide fragments — for example, cell-surface epitopes — from the endoplasmic reticulum (ER) membrane.

Martoglio and colleagues therefore set about identifying human SPP. They modified a known SPP inhibitor to contain a photoreactive group (to allow irreversible covalent binding of SPP on inhibitor activation by ultraviolet (UV) light) and a biotin moiety (to enable detection of tagged SPP). The authors then mixed this inhibitor (called TBL_4K) with detergent-solubilized ER membranes, and exposed the mixture to UV light. This method allowed them to isolate two differentially glycosylated forms (42 and 40 kDa) of SPP.

Using mass-spectrometric analysis of the 42-kDa form and database searches, the authors identified more than 15 proteins from many species that are homologous to human SPP. Unfortunately, these proteins have unknown functions, although the authors noted that the most highly conserved regions contain YD and LGLGD motifs in two putative TM segments. These motifs are characteristic of presenilin-like aspartic proteases.

The authors verified that the TBL_4K-targeted protein is an SPP by expressing it in *Saccharomyces cerevisiae*. They observed TBL_4K-sensitive SPP activity, and also showed that mutating the conserved aspartate residue in the LGLGD motif to alanine abolishes SPP catalytic activity without affecting TBL_4K labelling.

Analysis of the amino-acid sequence and potential glycosylation sites of human SPP allowed the authors to propose that it has a seven-TM topology with its amino terminus in the ER lumen, its carboxyl terminus (which contains an ER-retrieval signal) in the cytosol, and the YD and LGLGD aspartic-protease motifs in the middle of adjacent TM helices. The latter feature is found in presenilins, although the orientation of these TM helices is reversed in presenilins. This fits with the opposite orientation of presenilin and SPP substrates — presenilin substrates are type I TM proteins, whereas SPP substrates have a type II orientation.

The identification of SPP as a presenilin-like aspartic protease by Martoglio and co-workers supports the view that presenilins are proteases. Among the components of the γ-secretase complex, only the presenilins now resemble a known protease. This work has potentially identified a new family of polytopic membrane aspartic proteases, and future studies of SPP might help to determine the mysterious mechanism of intramembrane proteolysis.

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WEB SITES

Bruno Martoglio's laboratory: http://www.bc.biol.ethz.ch/groups/martoglio/martoglio.html

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