PROTEIN SYNTHESIS

A moment's hesitation

Nature has developed quality-control mechanisms to ensure accurate protein synthesis. In a report in *Molecular Cell*, Hayes and Sauer now show that when a bacterial ribosome pauses during polypeptide synthesis, the messenger RNA that is being translated is cleaved at the codon that occupies the ribosomal A site. They propose that this quality-control mechanism — which they refer to as A-site mRNA cleavage — together with another translational quality-control system (the tmRNA system), provides a way of reducing translational errors and therefore the production of harmful polypeptides.

Ribosomal pausing can occur at rare codons or stop codons because the cognate transfer RNA or protein-release factor, which is needed for translation termination, is scarce. The authors used a version of the *Escherichia coli ybeL* gene, which encodes a protein that ends with two proline residues (YbeL-PP), to study its effects — the carboxy-terminal proline residue of YbeL-PP causes inefficient translation termination, which results in ribosome pausing. Northern-blot analysis of cellular RNA showed a truncated *ybeL-PP* mRNA that was close in size to a control *ybeL* transcript, which ends after the second base of the stop codon. More precise mapping confirmed that *ybeL-PP* mRNA was cleaved at, or near, the stop codon.

Interestingly, Hayes and Sauer found that tmRNA (a specialized RNA that ensures the tagging of polypeptides for degradation) is not essential for A-site mRNA cleavage. Yet, when tmRNA is present, it ensures that the polypeptide at the pausing ribosome is tagged for degradation — thereby linking A-site mRNA cleavage to the tmRNA quality-control system.

By introducing an amber stop codon in the *ybeL-PP* gene, which prevents efficient translation of the *ybeL-PP* transcript, the level of truncated mRNA was reduced significantly. So, translation is clearly important for A-site mRNA cleavage.

By growing cells in the presence of a proline analogue instead of proline, both ribosomal pausing and polypeptide tagging were reduced, which correlated with a reduction in the level of truncated transcript. The same effect on A-site mRNA cleavage was shown when protein release factor 1 (RF1), which is known to reduce ribosomal pausing, was overproduced. This led the authors to conclude that both translation and ribosome pausing are required for A-site mRNA cleavage. Indeed, when Hayes and Sauer did the reverse experiment, using conditions that increased ribosomal pausing, the level of truncated mRNA increased.

Next, the authors tested the *E. coli* RelE toxin, which is known to be a mediator of A-site mRNA cleavage, and a range of other bacterial toxins for their possible involvement in *ybeL-PP* mRNA cleavage *in vivo*. None of the toxin-deleted strains had any effect on A-site mRNA cleavage, as shown by northern blots and by assaying for polypeptide tagging. The ribosomal alarmone (p)ppGpp (a guanine nucleotide that is synthesized when ribosomes stall) also does not seem to have a role in either the cleavage or tagging process.

As is often the case in science, new findings give rise to further questions — the nuclease that is responsible for A-site mRNA cleavage is still elusive, and the authors speculate that the ribosome itself might mediate this cleavage reaction. In addition, whether a similar mechanism exists in other bacteria, archaebacteria or eukaryotes is, for the moment, anyone's guess.

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References and links

ORIGINAL RESEARCH PAPER Hayes, C. S. & Sauer, R. T. Cleavage of the A site mRNA codon during ribosome pausing provides a mechanism for translational quality control. *Mol. Cell* **12**, 903–911 (2003)

IN BRIEF

APOPTOSIS

Cytochrome *c* binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis.

Boehning, D. *et al. Nature Cell Biol.* 9 Nov 2003 (DOI: 10.1038/ncb1063)

That small concentrations of Ca^{2+} can inhibit inositol-1,4,5-trisphosphate receptor (Ins(1,4,5)P_3R) function is a well-known feedback mechanism to prevent excess Ca^{2+} release. Boehning and colleagues now show that physiological quantities of cytochrome *c* can block this Ca^{2+}-mediated inhibition of Ins(1,4,5)P_3R function. Early in apoptosis, cytochrome *c* released from mitochondria translocates to the endoplasmic reticulum and binds Ins(1,4,5)P_3R. This sensitizes Ins(1,4,5)P_3R to increased Ca^{2+} release, causing a large cytochrome *c* release that amplifies the apoptotic signal.

TELOMERES

DNA damage foci at dysfunctional telomeres.

Takai, H. *et al. Curr. Biol.* **13**, 1549–1556 (2003)

A DNA damage checkpoint response in telomere-initiated senescence.

d'Adda di Fagagna, F. et al. *Nature* 5 Nov 2003 (DOI: 10.1038/nature02118)

The groups of de Lange and Jackson now show that dysfunctional telomeres activate a DNA-damage-checkpoint response. de Lange and colleagues uncapped telomeres by inhibiting the duplex TTAGGG repeat binding factor (TRF2), which is essential for telomere protection. Known DNA-damage-response factors, including 53BP1, γ-H2AX, Rad17, ATM and Mre11, became associated with the uncapped dysfunctional telomeres at 'telomere dysfunction-induced foci' (TIFs). And inhibiting the upstream protein kinase activators of the DNA-damage checkpoint reduced the accumulation of 53BP1. Using human diploid fibroblast (HDF) cell lines that undergo senescence as a result of telomere shortening, Jackson and colleagues detected a 'senescence-associated DNA damage focus' (SDF) of γ-H2AX colocalized with 53BP1, MDC1, NBS1 and SMC1 pS966. This telomere-initiated senescence triggers a complete DNA-damage response — dysfunctional telomeres contribute directly to this response. Accumulation of the DNA-damage-response factors was also seen when TRF2 was inhibited in immortalized human fibroblasts.

PROTEIN ASSEMBLY

An essential role of Sam50 in the protein sorting and assembly machinery of the mitochondrial outer membrane.

Kozjak, V. *et al. J. Biol. Chem.* 15 Oct 2003 [epub ahead of print]

Kozjak and colleagues report the identification of Sam50, which associates with Mas37 to form the *Saccharomyces cerevisiae* mitochondrial outer membrane sorting and assembly machinery (SAM complex). Using an *in organello* assembly assay, Sam50 was shown to be crucial for the assembly of β-barrel proteins of the outer membrane, such as Tom40 and porin. In addition, Sam50 is only the second mitochondrial outer membrane protein that is known to be essential for cell viability.

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