Protein synthesis

DNA-repair enzyme turns to translation

Alan J. Warren

A key DNA-repair enzyme has a surprising role during the early steps in the assembly of ribosomes—the molecular machines that translate the genetic code into protein. See p.291

Every minute, each human cell constructs up to 7,500 ribosomes—essential intracellular factories that decode instructions from genes to make all the proteins in the body. Ribosomes are assembled from four distinct ribosomal RNA (rRNA) molecules and 80 different proteins, which form small and large subunits, in a complex process involving more than 200 assembly factors. A better understanding of the underlying mechanisms might help to explain the devastating consequences of genetic mutations known as ribosomopathies that affect this assembly pathway. On page 291, Shao et al. identify an unexpected role for the enzyme DNA-dependent protein kinase (DNA-PK) — a core component of the machinery for repairing DNA double-strand breaks (DSBs) — in the early steps of ribosome assembly.

Cells must repair DSBs promptly, because they threaten genomic stability and can lead to cell death or cancer. Non-homologous end joining (NHEJ) is a main pathway for DSB repair. A dimeric protein complex called KU initiates this process by binding to the broken DNA ends, then recruiting the DNA-PK catalytic subunit (DNA-PKcs) to form the active DNA-PK enzyme (Fig. 1a). DNA-PK, through its kinase activity, adds phosphate groups to the side chains of serine and threonine amino acids in other proteins, and heavily regulates itself by phosphorylating a cluster near its threonine 2609 (T2609) residue. This activity leads to the recruitment of other enzymes, such as Artemis, that process and join the broken DNA strands.

In a comprehensive series of genetic experiments, Shao and colleagues established that both the kinase activity of DNA-PKcs and phosphorylation at its T2609 cluster are crucial for blood development (haematopoiesis) in mice. Mice that entirely lacked DNA-PKcs and the tumour-suppressor protein p53 developed a type of blood cancer and died. By contrast, animals that did not have p53 and carried a mutant form of DNA-PKcs lacking kinase activity survived. However, they developed a disease of the bone marrow reminiscent of a blood cancer called myelodysplastic syndrome. Moreover, mice in which amino-acid residues in the T2609 cluster were replaced by alanine residues (which could not be phosphorylated) died at four weeks old and had severe p53-dependent anaemia associated with reduced protein synthesis. This condition was reminiscent of the ribosomopathy Diamond–Blackfan anaemia (DBA), which is caused by mutations in any one of 18 different ribosomal proteins.

Shao et al. showed that deletion of the KU protein completely restored haematopoiesis in mice that had mutations in the T2609 cluster, ruling out defective DNA repair alone as the explanation for the blood disorders. What, then, might DNA-PK be doing in this context?

The first precursor of the small ribosomal subunit, known as the small-subunit processome, is assembled around an RNA called U3 (ref. 3; Fig. 1b) in a subcellular compartment called the nucleolus. Shao and colleagues confirmed previous reports4,5 that a proportion of KU and DNA-PKcs resides in the nucleolus. These observations suggested a link between KU, DNA-PKcs and ribosome assembly. The authors provided evidence that supports this link by using U3 as ‘bait’ to identify components of the small-subunit processome, which included DNA-PKcs and KU, but not other NHEJ factors.

The small ribosomal subunit is partly comprised of an RNA called 18S. The researchers found that unprocessed precursors of 18S rRNA accumulated in cells that lacked DNA-PKcs kinase activity, but did not accumulate when KU was deleted, too. Moreover, mice and cell lines lacking DNA-PKcs kinase activity showed reduced global protein synthesis. The authors used a technique called infrared crosslinking immunoprecipitation (irCLIP) to track down DNA-PKcs and KU to a specific location of the processome, near U3. Finally, they found that a structured fragment of U3...
drives the assembly of DNA-PK and stimulates its catalytic activity in vitro, although does so much less efficiently than can DNA.

Taken together, these observations suggest a model in which KU recruits DNA-PKcs to the small-subunit processome. In the case of kinase-defective DNA-PK, the mutant enzyme’s inability to regulate its own activity gives the protein a new function, blocking the processing of precursor rRNA into mature 18S rRNA in the small-subunit processome. The resulting defect in global protein synthesis drives a p53-dependent loss of red-blood-cell precursors—a cell type that has an especially high physiological demand for protein synthesis. The parallels with NHEJ are intriguing: in that pathway, the complete deletion of DNA-PKcs results in only a minor reduction in repair fidelity, and the joining of broken DNA ends is retained. By contrast, the kinase-inactive DNA-PKcs mutant is wholly unable to carry out end joining.

The specific role of DNA-PK in precursor rRNA processing, and how it recognizes precursor rRNA in vivo, remains unclear. However, structural analysis of the yeast small-subunit processome8 has revealed that U3 acts as a molecular guide that docks the processome onto the precursor rRNA by forming four evolutionarily conserved duplexes (hinges) between the two components: two hinges in a highly branched region of the precursor rRNA, and two in a region that will become the mature 18S rRNA. These hinges are a prerequisite for three cleavage events, mediated by an RNA-cleaving nuclease enzyme, that release the 18S rRNA ready to make the small subunit.

Shao et al. show that DNA-PK and KU primarily interact with U3 at this hinge region. Thus, much as DNA-PKcs recruits the DNA-cleaving enzyme Artemis during the NHEJ processing of DNA ends7, with U3, DNA-PKcs might also help to recruit specific RNA-cleaving nucleases (such as UTP24) to the small-subunit processome to cleave the precursor rRNA for ribosome construction.

Structural studies suggest that the binding of DNA-PKcs to KU and DNA could regulate the activation of DNA-PKcs kinase activity allosterically, that is, by changing the conformation of the enzyme. In the future, it will be interesting to compare RNA- and DNA-dependent conformational changes in DNA-PKcs. The physiological relevance of the broad array of RNA partners identified by Shao et al. in their irCLIP analysis also remains to be dissected.

Shao and colleagues’ study has identified an interesting player in ribosome assembly that might efficiently couple DNA DSB repair with processing of precursor rRNA, which is highly transcribed from the naturally unstable ribosomal DNA template. Broadly, the findings encourage us to critically evaluate how dynamic redistribution of DNA-PK might allow the cell to couple DSB repair with the regulation of protein synthesis. And, although further studies are required, we might have taken a step closer to deciphering the mysterious ribosomopathies.

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### Palaeontology

**Tiny fossil sheds light on miniaturization of birds**

Roger B. J. Benson

A tiny skull trapped in 99-million-year-old amber suggests that some of the earliest birds evolved to become miniature. The fossil illustrates how ancient amber can act as a window into the distant past. See p.245

Dinosaurs were big, whereas birds—which evolved from dinosaurs—are small. This variation is of great importance, because body size affects lifespan, food requirements, sensory capabilities and many other fundamental aspects of biology. The smallest dinosaurs' weighed hundreds of grams, but the smallest living bird, the bee hummingbird (*Mellisuga helenae*), weighs only 2 grams. How did this difference come about, and why? On page 245, Xing et al. describe the tiny, fossilized, bird-like skull of a previously unknown species, which they name *Oculudentavis khaungraae*.

The discovery suggests that miniature body sizes in birds evolved earlier than previously recognized, and might provide insights into the evolutionary process of miniaturization.

Fossilization of bones in sediments such as clay, silt and sand can crush and destroy the remains of small animals, and can flatten and decay soft parts such as skin, scales and feathers. By contrast, preservation of small animals in Burmese amber (which formed from the resin flows of coniferous trees about 99 million years ago) helps to protect their soft parts. A wide range of invertebrates and small

**Figure 1** | Computed tomography scan of the skull of *Oculudentavis khaungraae*. Xing et al. have characterized this 99-million-year-old fossil bird.