because a direct assessment of courage is not possible in non-human species. Intriguingly, however, the internal state elicited by vMT activation seems to be perceived as positive, because the mice prefer to stay in a chamber in which they can receive the vMT stimulation, given a choice. Future studies that examine the correlation between vMT activity and courage in humans will help us to further understand the emotional state encoded by the vMT.

It is also still unclear whether tail rattling in mice can effectively deter a predator in the wild. But this behaviour did successfully stop my daughter from bothering her pets. ■

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STRUCTURAL BIOLOGY

A closer look at human telomerase

The telomerase enzyme performs a crucial role by maintaining protective caps of repetitive DNA sequences at the ends of chromosomes. A structure of human telomerase casts fresh light on its architecture. SEE ARTICLE P.190

MICHAEL D. STONE

The chromosomes of eukaryotic organisms, which include plants, animals and fungi, are capped by protective structures called telomeres[1]. In certain types of proliferative cell, these structures are maintained by the telomerase enzyme. Inherited genetic mutations that compromise telomerase function cause disorders characterized by deterioration of proliferative tissues[2], whereas increased expression of telomerase supports unbridled cell growth in most human cancers[3]. Efforts to develop telomerase-based therapeutics have been hampered by an incomplete understanding of the structure and organization of the telomerase complex. On page 190, Nguyen *et al.*[4] report a structure of human telomerase bound to DNA, providing an unprecedented view of how the enzyme complex is organized and establishing a framework for drug discovery.

Telomerase is a ribonucleoprotein (RNP) enzyme — part RNA, part protein. To retain normal function, the enzyme must contain at least a telomerase reverse transcriptase (TERT) protein subunit and a long, non-coding telomerase RNA (TR). Telomerase complexes also usually include several species-specific proteins that are important for regulating RNP assembly[5]. The enzyme maintains telomeres by catalysing the addition of simple DNA-sequence repeats onto the ends of linear chromosomes. The catalytic TERT subunit uses a small portion of TR as a template for these repeats.

Vertebrate telomerases also belong to the family of box H/ACA RNP complexes, which generally modify essential cellular RNAs such as transfer RNAs, ribosomal RNA and spliceosomal RNA[6]. Curiously, despite possessing the RNA and protein components of H/ACA RNPs, telomerase does not exhibit the RNA-modifying activity typically associated with this class of complex. Nevertheless, mutations in those components impair telomere maintenance and cause diseases related to telomerase deficiency[2].

Human telomerase has low abundance in cells, and attempts to reconstitute it from its purified components have been thwarted by protein insolubility and the low efficiency of RNP assembly. This challenge can be overcome by expressing TERT and TR in cultured human cells that naturally express large quantities of the other telomerase proteins needed for RNP assembly[7]. This approach was used to reconstitute human telomerase and produce the first low-resolution structure[8] of the enzyme using electron microscopy (EM). However, telomerase complexes made in this way can contain different subunits and have varying structures, both of which limit the resolution of EM structures.

Nguyen *et al.* report an improved purification procedure that yields substantially more-active and more homogeneously assembled telomerase. Armed with this highly purified enzyme, the authors used cryo-electron microscopy (cryoEM) to reconstruct the structure of human telomerase at subnanometre resolution. This resolution is three times better than that of the previously reported structure, and allowed the authors to reliably work out how the various telomerase components

correlate with the regions of electron density mapped out by cryoEM.

The authors' structure has a bilobal shape (Fig. 1) consistent with the earlier low-resolution structure. This shape was previously interpreted[8] as a telomerase dimer — that is, each lobe was thought to contain a copy of the TERT and TR subunits. Nguyen *et al.* now propose a markedly different interpretation. They point out that the structure of one of the lobes is consistent with high-resolution models[9] of TERT and TR, which comprise the 'catalytic core' of the telomerase complex. They also compare their data with the crystal structure[10] of an H/ACA RNP bound to a single 'hairpin' RNA, and postulate that the second lobe contains the H/ACA RNP. Moreover, they propose that this H/ACA lobe includes a single copy of the TCAB1 protein, which regulates telomerase trafficking in the nucleus[11]. The bilobal architecture results from a remarkably extended RNA scaffold, consistent with the general model that TRs provide a flexible scaffold for telomerase-associated proteins[12]. The two distinct lobes interact through two bridging RNA helices.

The discrepancies between Nguyen and colleagues' structure and previous reports of the composition and organization of human telomerase are probably rooted in the different methods used to purify the enzyme, which might yield distinct telomerase subcomplexes and, in some cases, promote formation of a telomerase dimer. Nevertheless, the present structure supports the hypothesis that telomerase from a range of organisms can function as a monomer[13,14].

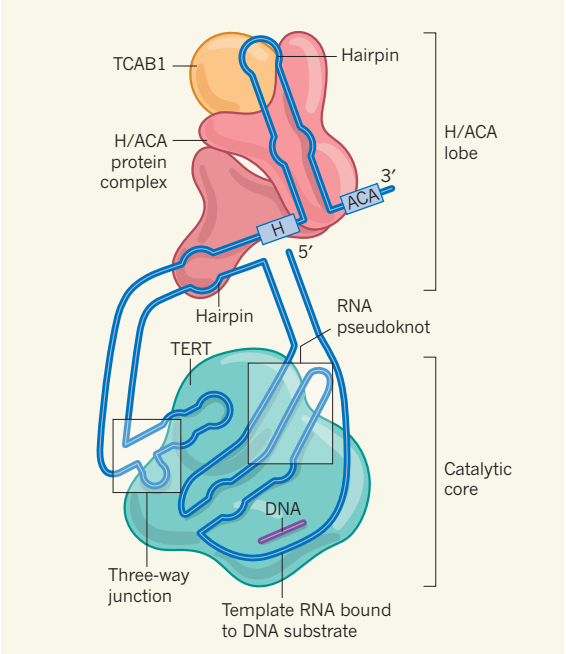

Figure 1 | Cartoon of the human telomerase enzyme. Telomerase enzymes catalyse the addition of short DNA-sequence repeats to protective caps (telomeres) at the ends of linear chromosomes. Nguyen *et al.*[4] report a structure of human telomerase obtained using cryo-electron microscopy. The overall structure is bilobal, and consists of an extended RNA (blue) that acts as a scaffold for various proteins. The authors suggest that the H/ACA lobe — named after the H and ACA motifs within the RNA — contains two copies of the tetrameric H/ACA protein complex bound to two 'hairpin' regions of the RNA scaffold, as well as a TCAB1 protein. This lobe facilitates the assembly of the telomerase enzyme from its components, and controls trafficking of the enzyme in the nucleus. The other lobe is proposed to contain the enzyme's catalytic core, which includes the TERT protein, together with the pseudoknot and three-way junction of the RNA. The RNA sequence that is used as a template for the DNA repeats is also in the catalytic core, bound to a DNA substrate.

In Nguyen and co-workers' reconstruction of the catalytic core of human telomerase, the active site of the ring-shaped TERT subunit contains the template RNA bound to a DNA substrate. The two regions of TR required for catalytic function *in vitro* are known as the template/pseudoknot domain (t/PK) and the conserved regions 4/5 (CR4/5) domain. In the authors' structure, the evolutionarily conserved pseudoknot fold in the t/PK domain resides on the 'back' side of the TERT ring, far from the active site. This is highly consistent with the pseudoknot's location in the previously reported structure[13] of telomerase from the protozoan *Tetrahymena thermophila*, and suggests that this essential TR element indirectly promotes telomerase function, perhaps by guiding proper RNP assembly or by influencing the conformation of TERT subdomains during catalysis.

The authors' model of the H/ACA lobe is the first structure of a eukaryotic H/ACA RNP, and includes two complete sets of the four H/ACA proteins (dyskerin, NOP10, NHP2 and GAR1) bound to RNA hairpins. The structure reveals interesting differences in the way in which each set of H/ACA proteins interacts with its respective hairpin. The foundation of the H/ACA lobe structure is made by dyskerin subunits, which bind to the base of each RNA hairpin and also make extensive contacts with each other. The remaining three H/ACA proteins seem to make numerous contacts with one of the RNA hairpins, but substantially fewer with the other. Lastly, the TCAB1 protein binds to a structure within TR known as the CAB box, found at the apex of the H/ACA lobe. These results provide a structural explanation for a wealth of previously reported biochemical data[15,16] that defined the subunit stoichiometry, as well as the RNA-hairpin sequence and structure requirements for stable assembly, of the TR H/ACA RNP.

The structural segregation of the catalytic core and H/ACA lobe raises the question of why the telomerase complex has retained the large H/ACA RNP throughout evolution. Part of the answer is likely to be that TCAB1 is needed to mediate trafficking of telomerase components during RNP assembly. However, another striking feature of the H/ACA lobe is that its proteins seem to promote a specific conformation of TR that brings the CR4/5 domain close to the catalytic core, where it is needed to support catalysis. This bears a remarkable resemblance to the *T. thermophila* telomerase RNP, in which an RNA-binding protein called p65 is required to remodel TR and promote contacts with the catalytic TERT subunit[13,17]. This resemblance suggests that telomerases from different organisms have co-opted different types of RNA-binding protein to promote RNP assembly. The idea that the H/ACA proteins direct RNA folding can be tested experimentally, using the new structural model as a framework with which to design and interpret incisive experiments.

Nguyen and colleagues' work sets a new bar for structural models of human telomerase, but there is still work to be done. A complete view of telomerase structure and function will require atomic-resolution models of the enzyme in its various functional states. Advances in cryoEM techniques, together with other structural and biophysical tools, will no doubt continue to enhance our appreciation of the intricate workings of this fascinating enzyme. ■

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