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Commentary

Telomerase catalysis: A phylogenetically conserved reverse transcriptase

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Replication of telomeres, the ends of eukaryotic chromosomes, is the responsibility of the enzyme telomerase. Since its discovery 13 years ago, research on this unusual DNA polymerase has revealed a series of surprises. The first of these was the realization that information within the enzyme itself determines the sequence of its product: a portion of a telomerase RNA subunit is the template that dictates the nucleotides added onto the telomere (1, 2). The interest in telomere replication has increased further during the past several years because of observations indicating that maintenance of telomere length by telomerase could provide the molecular basis for determining the lifespan of cells in culture (3).

The most recent insight has been the discovery that telomerase is a reverse transcriptase (4, 5), with catalysis provided by a protein subunit with striking similarities to conventional reverse transcriptases. The genes encoding the TERT (for telomerase reverse transcriptase; reviewed in ref. 6) proteins have been recovered from a diversity of species. Each of these proteins exhibits sequence features previously observed in reverse transcriptases, as well as a telomerase-specific T motif (ref. 7 and references therein). Mutation of key residues predicted to be critical for catalysis (by comparison to the reverse transcriptase active site) abolishes telomerase activity in yeast and humans (4, 5, 8), and expression of the human RNA and TERT subunits in an *in vitro* translation system is sufficient to reconstitute activity (8, 9). Although reliance on a templating RNA component had already suggested parallels between this enzyme and other RNA-dependent DNA polymerases, the demonstration that the telomerase catalytic subunit exhibited structural and enzymatic similarities to conventional reverse transcriptases provided direct mechanistic support for these comparisons. This result also has striking implications for reverse transcriptase evolution (10, 11), by demonstrating that such enzymes are not employed solely for replication of parasitic genetic elements but are also necessary for normal cellular proliferation. Furthermore, the cloning of the human TERT (hTERT) subunit permitted a direct test of the hypothesized role of telomere replication in the lifespan of normal human cells in culture: ectopic expression of hTERT in telomerase-negative human diploid fibroblasts restored enzyme activity and conferred an ability to proliferate well beyond the normal senescence point (12).

In this issue of the *Proceedings*, one aspect of telomerase research has come full circle, with the cloning of the TERT protein from the ciliate *Tetrahymena* (7, 13), the source of the first discovered telomerase activity. The ciliated protozoa have contributed greatly to our understanding of telomere biology because of an unusual feature of ciliate development. During the formation of a new macronucleus after mating, *de novo* addition of telomeres occurs on the ends of hundreds of thousands of newly formed minichromosomes (14). Thus, ciliates such as *Tetrahymena*, *Euplotes,* and *Oxytricha* have proven to be rich sources of the factors required for telomere replication and maintenance. As a consequence, the first two telomerase-associated proteins, p80 and p95, were identified after purification of the *Tetrahymena* telomerase complex (15). On the basis of limited sequence similarities with other polymerases, p95 was proposed to contain the catalytic active site of this enzyme (15). However, p95 showed no homology to the emerging family of TERT proteins. This presented a potential puzzle, invoking the possibility of an alternative class of telomerase enzymes that utilized a different catalytic mechanism.

This possibility has now been laid to rest by two reports in this issue, from the Cech and Collins laboratories, showing that the *Tetrahymena* telomerase relies on a reverse transcriptase subunit for catalysis (7, 13). The gene encoding the *Tetrahymena* TERT protein was cloned by using a molecular approach, and the predicted protein displayed both the expected seven reverse transcriptase motifs and the T motif. Expression of this TERT protein and the RNA subunit in reticulocyte extracts was sufficient to reconstitute polymerization activity (although the high processivity observed with native enzyme was not achieved with this reconstituted core complex), and catalysis by the reconstituted enzyme was abolished by mutations similar to those previously tested for yeast and human telomerases (13). The *Tetrahymena* catalytic subunit protein also could be coimmunoprecipitated with p95, p80, and the RNA subunit, arguing that all four components are present in a single complex (13). Expression of the TERT mRNA was observed to increase dramatically after mating (7), consistent with the greatly increased requirements for telomere addition during macronuclear development.

Thus, it is now clear that the well characterized *Tetrahymena* enzyme has a catalytic subunit that shows both structural and evolutionary conservation with other telomerases. This result argues for the conceptually satisfying view that telomerase, regardless of its source, has a phylogenetically conserved core that minimally consists of the RNA component and a TERT protein. However, this conservation does not hold up as well when potential holoenzyme-associated proteins, isolated in several different systems, are compared. The telomerase reverse transcriptase subunit was discovered and characterized as a result of parallel biochemical and genetic endeavors in *Euplotes* and *Saccharomyces cerevisiae*; these efforts also led to the identification of telomerase-associated proteins in both species. Extensive purification of an active enzyme from *Euplotes aediculatus* resulted in a complex consisting of the RNA and catalytic protein subunits, as well as a copurifying 43-kDa protein; however, p80- and p95-like proteins were not detected in this complex (16). An alternative strategy for the identification of yeast telomerase components relied on a screen for mutants of yeast that exhibited an *in vivo* telomere replication defect (17, 18). This uncovered *EST2*, the yeast homolog of the telomerase reverse transcriptase gene, as well

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as additional *EST* genes that, when mutated, exhibited a mutant phenotype identical to that displayed by mutations in the core enzyme complex. Est1, Est2, and Est3 proteins each coimmunoprecipitate with yeast telomerase activity (ref. 19; T. R. Hughes, R. G. Weilbaecher, and V.L., unpublished observations), suggesting that Est1 and Est3 could be components of the yeast holoenzyme. However, the yeast enzyme has not been purified sufficiently to be able to assess whether all three Est proteins are present in a single complex with the telomerase RNA. Strikingly, the telomerase-associated Est proteins show no similarity to any of the ciliate proteins. Furthermore, a search of the completely sequenced yeast genome has not revealed any homologs of the *Tetrahymena* p80 and p95 proteins.

So what is the explanation for the lack of convergence between these different sets of telomerase-associated proteins? Several hypotheses come to mind, which are not necessarily exclusive. The first possibility is that telomerase may be a large holoenzyme with a number of associated proteins, and efforts in these three organisms have succeeded in identifying only a partial subset. In support of this possibility, human, rat, and murine homologs of p80 have been isolated (20, 21), and the human p80 homolog has been shown to be in a complex with the hTERT subunit of telomerase (22), consistent with a similar demonstration for the equivalent *Tetrahymena* proteins (13). This finding excludes the possibility that p80 is a ciliate-specific telomerase protein and also raises the expectation that a similar mammalian p95 homolog may follow. This cross-species conservation does make the lack of a recognizable yeast version even more puzzling, but perhaps yeast homologs may not be readily identified on the basis of primary sequence. In fact, one proposed set of orthologs may be p95 and the yeast Est1 protein, as these two telomerase-associated proteins have a similar set of *in vitro* biochemical properties. Both proteins exhibit low-affinity, but sequence-specific, binding to single-strand telomeric DNA substrates (23, 24). In addition, both proteins interact, albeit nonspecifically, with RNA *in vitro* (23, 24). Such properties argue for roles in recognition of the telomeric DNA substrate and interaction with the telomerase RNA.

Alternatively, the diversity of telomerase-associated proteins may be a reflection of the differing requirements faced by telomerase in various biological situations, such that although the enzyme core may be conserved, at least a subset of the proteins that associate with the holoenzyme will be species-specific. One obvious species difference that may be mediated by components of the telomerase holoenzyme is the substantial variation in telomere length, ranging from 50 bp for some ciliates to $\geq$50 kb in one species of mouse. In addition, an enzyme that is responsible for *de novo* telomere addition as a part of chromosome healing might be expected to have differing cofactor requirements than an enzyme complex that is responsible for telomere length maintenance during vegetative growth. Even within a single species (*Euplotes crasses*), biochemical differences have been noted between telomerase isolated from vegetatively growing cells versus enzyme from mated cells (25).

Thus, although the basis for the differences in holoenzyme composition between different species is not yet understood, the results of the last year have shown that a phylogenetically conserved TERT protein is common to all telomerases. This has provided specific insight into the mechanism of telomerase catalysis, as well as establishing a sound foundation for a future detailed understanding of the composition of the telomerase holoenzyme. With four components of the most thoroughly studied telomerase now identified and available, *Tetrahymena* once again establishes itself as a system that will contribute important information about the biochemical activities of the components of this unusual DNA polymerase.

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