Telomerase activity reconstituted in vitro with purified human telomerase reverse transcriptase and human telomerase RNA component.

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Telomerase is a specialized reverse transcriptase that catalyzes elongation of the telomeric tandem repeat, TTAGGG, by addition of this sequence to the ends of existing telomeres. Human telomerase reverse transcriptase (hTERT) has been identified as a catalytic enzyme involved in telomere elongation that requires telomerase RNA, human telomerase RNA component (hTR), as an RNA template. We established a new method to express and purify soluble insect-expressed recombinant hTERT. The partially purified FLAG-hTERT retained the catalytic activity of telomerase in a complementation assay in vitro to exhibit telomerase activity in telomerase-negative TIG3 cell extract and in a reconstitution assay with FLAG-hTERT and purified hTR in vitro. FLAG-hTERT (D712A) with a mutation in the VDV motif exhibited no telomerase activity, confirming the authentic catalytic activity of FLAG-hTERT. The reconstituted complex of FLAG-hTERT and hTR in vitro was detected by electrophoretic mobility shift assay, and its activity was stimulated by more than 30-fold by TIG3 cell extract. This suggested that some cellular component(s) in the extract facilitated the reconstituted telomerase activity in vitro. Geldanamycin had no effect on the reconstituted activity but partially reduced the stimulated activity of the reconstituted telomerase by the TIG3 cell extract, suggesting that Hsp90 might contribute to the stimulatory effect of the cellular components.

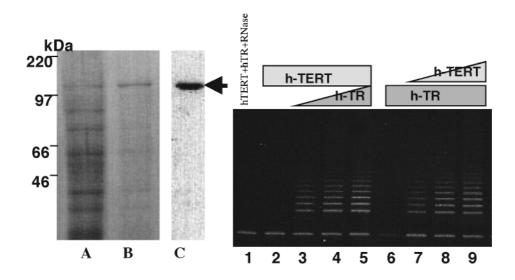


Figure. In vitro reconstitution of telomerase activity with two components.

Solubilized fraction of insect-expressed FLAG-hTERT (A) and partially purified (B) detected by CBB staining and western blotting with anti-FLAG antibody (C). Telomerase activity of FLAG-hTERT with varying amount of template hTR (lanes 2-5) and hTR and varying amount of FLAG-hTERT (lanes 6-9). Equimolar amount of hTR and FLAG-hTERT was pretreated with RNaseA, then subjected to TRAP assay.