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An Innovative Method to Produce Recombinant Human Telomerase Reverse Transcriptase (hTERT).

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Telomerase is a ribonucleoprotein complex that adds telomeric DNA repeats to the end of chromosomes, which are iteratively shortened as cells divide. Activation of the complex is critical to the immortalization of normal cells. Telomerase has become an important molecular marker for the diagnosis, prognosis, and treatment of cancer. The lack of purified hTERT, the catalytic subunit of complex, is a major problem. The primary difficulty in generating active recombinant hTERT appears to be the protein folding. We used a GFP-hTERT fusion to monitor folding in insect cells. Folding was not improved by the co-expression of the human heat shock proteins hsp70 and hsp90. We used the intrinsic cellular chaperones that reside in specific cytoplasmic compartments to assist the folding process. We directed the transport of the GFP-hTERT fusion to mitochondria and the endoplasmic reticulum (ER). The GFP fluorescence was enhanced when the GFP-hTERT fusion was directed to the ER, and the folding and solubility of the ER-transported protein was improved. This improvement makes it possible to purify and assay the recombinant hTERT protein.