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The first architecture of telomerase holoenzyme

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Telomerase is a reverse transcriptase that maintains genome integrity by adding DNA repeats to chromosome ends called telomeres. Ever since the discovery of telomerase in *Tetrahymena* by Carol W. Greider and Elizabeth Blackburn in 1984, the telomerase holoenzyme drew great attention due to its central roles in regulating stem cell renewal and the immortality of many cancer cell types. Despite expanded studies of telomerase function in many organisms, little was known about the molecular architecture of telomerase holoenzyme. Because of its low abundance and complexity, the purification and reconstitution of homogeneous telomerase holoenzyme has consistently presented a challenge for structural studies.

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For the first time, Jiang and colleagues successfully reconstituted a 500 kDa *Tetrahymena* telomerase holoenzyme *in vitro* and used electron microscopy (EM) to elucidate unprecedented details on its physical and functional architecture. Six out of seven *Tetrahymena* holoenzyme proteins (telomerase, p65, p75, p50, p19 and Teb1) and telomerase RNA were co-localized, assembled and purified. A predominant conformation and preferred orientation of telomerase holoenzyme was identified by comparing both negative staining EM and cryo-EM images. The three-dimensional (3D) reconstructions of telomerase holoenzyme were carried out using 2,220 particles from EM images with a resolution of 25 Å using an automated random conical tilt (RCT) method. Antigen-binding fragment (Fab) against the protein tag or subunit variants were used to pinpoint the exact positions and arrangements of holoenzyme subunits within the telomerase holoenzyme. Homology models and the known crystal and NMR structures were used to fit the electron microscopy density map. The enzymatic activity of the telomerase

holoenzyme or subcomplex was validated by primer extension assays. The subunit interaction network was identified, and the structure and activity contributions of subcomplex to that of holoenzyme were compared. Positional dynamics of the subcomplex were proposed based on telomerase EM class averages. The study identified the unexpected role of previously uncharacterized p50, which serves as a central hub between the catalytic core and the accessory proteins, thus promoting telomerase activity.

In summary, Jiang and colleagues have clearly defined the telomerase subunit interaction network, as well as the arrangement and molecular interactions between *Tetrahymena* telomerase protein and RNA components. Their work provides exquisite detail on the structure of the *Tetrahymena* telomerase core complex. Towards the ultimate goal of obtaining high resolution structures of telomerase holoenzyme, Jiang and colleagues revolutionize our understanding of the molecule's assembly and shed significant light on the structural features of human telomerase holoenzymes.

Reference:

Jiang, J., Miracco, E.J., Hong, K., Eckert, B., Chan, H., Cash, D.D., Min, B., Zhou, Z.H., Collins, K., and Feigon, J. (2013). The architecture of Tetrahymena telomerase holoenzyme. Nature *496*, 187-192.