9 Summary

The three dimensional structure of the non-translating 80S ribosome was determined using cryo-electron microscopy and single particle reconstruction. A 150 Å long rod-shaped density at the 60S ribosomal subunit was identified as the main helix of extension segment 27 (ES27) and could be observed in two main positions: (i) close to the L1 arm (ii) or following a 90º turn, close to the exit site of the tunnel in the large ribosomal subunit. We propose that this rotating rRNA structure controls the access to the exit tunnel in the large ribosomal subunit for non-ribosomal factors, possibly interacting directly with the nascent chain or nascent chain interacting factors.

The structure of the translating 80S ribosome in association with the protein conducting channel (PCC) was determined using cryo-electron microscopy and single particle reconstruction. The structure revealed four connections between the PCC and the ribosome formed by distinct rRNA and protein elements across a gap of 10-20 Å. The observed closed conformation of the PCC was found to be independent of its functional state. Furthermore, it was estimated by transmembrane domain fitting that the oligomeric PCC is formed by three Sec61α,β,γ trimers. A model describing the cotranslational transport of proteins through the PCC and across the ER membrane and the integration of transmembrane proteins, proposes two main functional states of the ribosome-PCC complex.

Using a fluorescent nucleotide exchange assay, we identified the β-subunits of the two homologous trimeric PCCs in yeast to be the guanine nucleotide exchange factors (GEFs) for the β-subunit of the signal recognition particle receptor involved in cotranslational protein transport across the membrane of the endoplasmic reticulum. Both the cytosolic domain of Sec61β as well as the holo Sec61β, when part of the isolated trimeric Sec61p complex, function as the GEF for SRβ, whereas the same Sec61β, when part of the heptameric complex that facilitates posttranslational protein transport, is inactive as the GEF for SRβ.