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Mechanism of Signal Sequence Handover From NAC To SRP on Ribosomes During ER-protein Targeting

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Localization of nascent proteins to the appropriate organelle is essential for cell function and homeostasis. Almost 30% of these nascent proteins consist of membrane proteins that would need to be targeted to the endoplasmic reticulum (ER) membrane by the signal recognition particle (SRP), while being synthesized on ribosomes. To prevent protein aggregation, the cell has to maintain high fidelity in the protein targeting process. The nascent polypeptide-associated complex (NAC) interacts with newly synthesized proteins at the ribosomal tunnel exit and competes with SRP to prevent mistargeting of cytosolic and mitochondrial polypeptides to ER. How NAC antagonizes SRP and how this is overcome by ER targeting signals is unknown. Here, we reconstituted in vitro a reaction with signal-containing RNC (RNC_{SS}) mixed with both NAC and SRP and analyzed the complexes formed by cryo-electron microscopy (cryo-EM). We resolved two complexes within the particles, a pre-cargo handover RNCss•NAC complex, and a ternary post-cargo handover RNCss•NAC•SRP complex that revealed the molecular interactions between NAC and SRP on the ribosome. In addition, we investigated the functional implications of the cryo-EM structures by biochemical, in-vivo and single molecule experiments. Together, we found that NAC uses two domains with opposing effects to control SRP access. The core globular domain prevented SRP from binding to signal-less ribosomes, whereas a flexibly attached domain transiently captured SRP to permit scanning of nascent chains. The emergence of an ER targeting signal destabilized NAC's globular domain and facilitated SRP access to the nascent chain. These findings elucidate how NAC hands over the signal sequence to SRP and imparts specificity of protein localization. This study further proposes that the role for NAC as a sorting factor extends beyond the recruitment of SRP to orchestrate a multitude of nascent chain processing events [1].

References:

[1] Jomaa, A et al., Cell Reports 36(2) (2021), p. 109350. doi: 10.1016/j.celrep.2021.109350.