Deciphering the dynamics of divergent co-translational assembly pathways

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Protein-protein interactions are at the heart of all biological processes in the cell, with the ribosome emerging as a platform, orchestrating the nascent-chain interplay dynamics. Here we study the assembly pathways of two homologous complexes from the N-terminal acetyltransferases (NATs) family. Comparison of NatA and NatB by selective ribosome profiling revealed opposite assembly pathways, where highly homologous subunits serve diverging functions. In each complex, we identified a dedicated subunit binding its partner as it emerges from the ribosome. Molecular dynamics simulations revealed that co-translationally engaged subunits show binding energy clustering, where only a few “hotspot” residues mediate the entire interface formation. α-helices harboring hotspots were highly thermolabile, folding and unfolding during simulations, depending on their partner subunit to avoid misfolding. In vivo hotspot mutations caused aggregation, leading to growth defects. Conservation analysis revealed that missense NATs variants, causing neurodevelopmental and neurodegenerative diseases, are disrupting putative hotspots clusters. We propose a model based on the distribution of interface energy as a strong predictor of cotranslational assembly interactions and thermostability, providing basis for future synthetic interface design.