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Determining the Functions of eIF3 in Yeast Canonical Translation Initiation

Canonical translation initiation in yeast requires the coordination of multiple eukaryotic initiation factors (eIFs) with 40S and 60S ribosomes. Eukaryotic initiation factor 3 (eIF3) is essential for the assembly of the 43S PIC, the binding of mRNA to the eIF4F complex, and scanning and AUG recognition (Pestova, 2002). However, the roles of each subunit of yeIF3 (a/b/c/i/g) during yeast canonical translation initiation in the 43S PIC are unknown, as there is a lack of structural information about its location and interactions within the 43S PIC.

A novel technique allowed us to purify each subunit separately to determine how yeIF3a/c/b/i/g binds and interacts in the 43S PIC. We have analyzed a modular multicomponent system by collecting and analyzing datasets for elF3entry (aCTD/b/i/g), elF3full (a/c/b/i/g), eIF3a/c/b, and eIF3b/i/g. Gel-based assays were performed to assess eIF3's role in PIC formation and mRNA recruitment. Cryo-EM single particle analysis methods were used for structural analyses. Finally, cryoDRGN heterogeneity analysis was conducted to determine how individual eIF3 subunits influence 40S head and body dynamics.

We have determined how yeIF3a/c/b/i/g binds and interacts during yeast canonical translation initiation. In the eIF3full dataset, eIF3b/i/g binds to both sides of the 40S subunit. In the eIF3entry dataset, eIF3b/i/g is present on both sides of the 40S subunit and can bind to the 40S ribosome without eIF3a/c. The eIF3b/i/g dataset has neither eIF3a/c nor eIF3aCTD, so eIF3b/i/g poorly binds to the 40S subunit. In the eIF3a/c/b dataset, eIFb binds to the 40S subunit without eIF3i/g. Additionally, eIF3a/c stabilizes the head of the 40S. Finally, eIF3full is proficient in both PIC binding and mRNA recruitment; eIFentry can do both PIC binding and mRNA recruitment, albeit very slowly; and eIF3a/c/b is proficient in PIC binding but inactive in mRNA recruitment.

Our novel findings inform of previously unknown functions regarding yeIF3 in the 43S PIC. They show how the coordinated interaction of eIF3 subunits with the 40S ribosome supports efficient initiation. Our findings provide a robust framework for understanding how each module contributes to the overall translation mechanism. This modular mechanism model, supported by structural and biochemical data, comprehensively explains how eIF3's independent yet cooperative subunit interactions guide 43S PIC assembly and mRNA recruitment. As the most complex translation initiation factor, understanding the structure and functions of eIF3 is essential to unlocking new knowledge regarding ribosome function and protein synthesis, which is crucial for all cellular functions and life itself.