Abstract

A key task of translation initiation is to select a proper start codon. The start codon defines not only the position from which the protein translation is initiated but also the reading frame in which the protein sequence is decoded. The accuracy of start codon recognition is controlled by number of initiation factors (eIFs) and their mutual interactions. One of them, the largest, is called eukaryotic initiation factor 3 (eIF3). The exact mechanism by which eIF3 affects the accuracy of start codon recognition is not understood. Here, using CryoEM, formaldehyde gradient cross-linking and various biochemical methods, we elucidated contributions of individual human eIF3 subunits to this intricate process.

In particular, my work and the work of our collaborators revealed that eIF3d promotes loading of eIF3 to the ribosome at the onset of initiation and that the minimalistic eIF3 subcomplex (YLC – for Yeast Like Complex) still preserves basic eIF3 functions, like formation of the 43S pre-initiation complex (PIC) and mRNA recruitment. Another study then implicated human eIF3c is fine-tuning the fidelity of start codon recognition, as downregulation of eIF3c causes a codon-specific decrease in start codon recognition fidelity and an increase in the eIF5 abundance; eIF5 is together with eIFs 1, 1A and 2 one of the crucial players in this process. I found that eIF3c interacts with eIF5 through the tip of its unstructured N-terminal tail via three specific residues. Interestingly, I also discovered that this specific contact is lacking in the trypanosomatids, indicating that these organisms differ in their way of achieving start codon fidelity. In fact, this structural work described a number of other peculiar features of the 43S PIC specific to kinetoplastids and revealed previously unknown position-specific details of unstructured tails of several key eIFs, and most notably the previously enigmatic C-terminal domain of eIF5. Importantly, these findings have opened a possibility of a development of druggable target(s) to combat these parasites.

Last but not least, using the knock-in Crispr/Cas9 system, I successfully established two homozygous human HEK293T stable lines with two separate single point mutations in the eIF5-binding site of human eIF3c, and employing luciferase reporter assays, I demonstrated that the

disruption of the eIF3c-eIF5 contact reduced the fidelity of start codon selection and cell fitness. My ongoing work is now dedicated to fully characterize these first-ever generated AUG selection mutants in mammals and explore the importance of eIF3 in this fundamental process directly in a mouse model that will be generated.