Abstract

In eukaryotes, translation initiation is guided by up to twelve protein initiation factors (eIFs) and begins with the formation of the 43S pre-initiation complex (PIC) composed of the small ribosomal subunit (40S), eIF2.GTP/Met-tRNA$_i^{Met}$ ternary complex, and eIFs 1, 1A, 3 and 5. The 43S PIC subsequently interacts with the 5´end of an mRNA (an mRNA recruitment step) and thus formed 48S PIC travels in 5´ to 3´ direction along the mRNA leader sequence to locate the AUG start codon (this presumably linear movement is generally known as scanning). Start site selection results in the dissociation of the initiation factors and joining of the large (60S) ribosomal subunit to form the 80S initiation complex poised for elongation. Eukaryotic initiation factor 3 (eIF3) plays a critical role in most of these events; however, the molecular details of most of its contributions are still unknown to us. Previous *in vivo* studies generated numerous mutations in all eIF3 subunits with specific defects either in the PICs assembly or in the following steps such as scanning, AUG recognition, etc. To understand the exact role of eIF3 in this intriguing process at the molecular level, we have embarked on a study that aims to dissect the individual functions of each eIF3 subunit in translation initiation using the purified mutant eIF3 complexes in the *in vitro* reconstitution system featuring several functional assays. Based on our preliminary results with some eIF3a/TIF32 , b and c mutants here we show that at least two of these *in vitro* assays, namely the 43S formation and mRNA recruitment protocols, are able to reveal and "measure the extent" of particular mechanistic effects of distinct eIF3 mutants.