Abstract

In eukaryotes, the eIF2.GTP.tRNA$_i^{Met}$ ternary complex (TC) plays an important role in AUG start codon recognition. The heterotrimeric eIF2 complex consists of a core eIF2**γ** subunit to which binds eIF2**α** and eIF2**β** subunits. Cryo-EM structure suggests that the eIF2**β** interacts with the eIF2**γ** subunit via **α**1-helix (residues 127-143). The eIF2**β** C-terminal zinc-binding domain (ZBD) is subdivided into the S-loop, R-loop, and the T-loop. However, the role of the eIF2**β** ZBD in the TC formation is not clearly understood. Previously, the eIF2**β**^{S264Y} mutation (S-loop) was reported to show an intrinsic GTPase activity, defect in the TC formation leading to the de-repression of GCN4 expression (gcd$^-$ phenotype), and relaxed stringency of the AUG codon recognition (Sui$^-$ phenotype). However, we showed that the eIF2**β**^{S264Y} mutation has an eIF2**β**-**γ** interaction defect, raising the possibility that the eIF2**γ** has another binding site for the eIF2**β** subunit in addition to the eIF2**β**-**γ** interaction via **α**1-helix. To gain insights into the mechanism of TC formation, we screened for the eIF2**β**^{S264Y} intragenic suppressor mutation. We identified the eIF2**β**^{T238A} mutation in the ZBD that suppressed the slow growth (Slg$^-$), Gcd$^-$, and Sui$^-$ phenotype of the eIF2**β**^{S264Y} mutation. Biochemical analysis of the eIF2**β**^{S264Y} mutation reveals the Met-tRNA$_i^{Met}$ and eIF2**β** interaction defects with the eIF2**γ** subunit. The intragenic suppressor mutation eIF2**β**^{T238A} in the T-loop restores the eIF2**β**^{S264Y} binding with the eIF2**γ** subunit but not the Met-tRNA$_i^{Met}$ binding defect in the free TC. However, the Met-tRNA$_i^{Met}$ binding to the eIF2**β**$^{S264Y/T238A}$ double mutation is stable when associated with the 40S ribosomes, explaining the partial rescue of the Gcd$^-$ phenotype. The eIF2**β** R-loop appears to interact with the acceptor arm of the Met-tRNA$_i^{Met}$. Consistently, the eIF2**β**^{N252D} mutation showed Slg$^-$, Gcd$^-$, and Sui$^-$ phenotype, which can be partially suppressed by the T-loop eIF2**β**^{T238A} mutation. This suggests that the eIF2**β** R-loop and the T-loop cooperate for the stable binding of the Met-

$tRNA_i^{Met}$ to the eIF2 complex. Interestingly, the eIF2**β**^{T238A} mutation also partially suppressed the Slg$^-$, Gcd$^-$, and Sui$^-$ phenotype of the core GTPase eIF2**γ**^{N135D} switch-I mutation, suggesting that the eIF2**β** ZBD modulates the Met-$tRNA_i^{Met}$ binding by stabilizing the eIF2**γ** switch-I region. However, even though the eIF2**β**^{T238A} mutation rescued the eIF2**β**^{S264Y} mutant's binding defect, it did not rescue the growth defect associated with the eIF2**γ**^{V281K} mutation (an equivalent MEHMO syndrome mutation in yeast that shows an eIF2**β** binding defect). Our data suggest that in addition to the previously known interaction of eIF2**β** with the eIF2**γ** subunit via its **α**1-helix, the eIF2**β**-ZBD also interacts with the eIF2**γ** subunit via guanine nucleotide-binding interface; thus, the eIF2**β**-**γ** interacts via two distinct binding sites.