Table SI. Summary of mutational analyses of residues involved in the proposed molecular switch mechanism of eIF5B

Mutation/ Location	Phenotype (References)	Putative function of the mutated residue during the conformational switch.	Effect of the mutant in the context of the domain release mechanism.
E434A Switch 1; in the activated GTP-bound G domain at the end of helix α' (Fig. 2D).	Moderate slow growth phenotype. (Shin et al, 2002)	In structures of the inactive G domain Glu434 is not defined. In the GTP-bound state, Glu434 forms part of the secondary coordination shell for the Mg^{2+} ion by interacting with one of the two water molecules of the primary shell; its carboxylate group thereby occupies a position analogous to that of Asp50 in switch 1 of EF-Tu.	Mutation of Glu434 to Ala would cause the loss of an indirect interaction between switch 1 and the Mg^{2+} ion. This destabilizes the GTP-bound conformation of switch 1 and thereby indirectly the entire active site. Moreover, the loss of a stabilizing interaction for the primary coordination shell of the Mg^{2+} ion might cause a rearrangement within the active site that is unfavorable for GTP hydrolysis.
T439A G2 motif in switch 1 (Fig. 2).	Severe slow growth phenotype. Binds GTP but lacks detectable GTPase activity. Active in subunit joining (with reduced rates). Fails to be released from the formed 80S complexes and stabilizes the Met-$tRNA_i^{Met}$. (Shin et al, 2002)	Together with Gly479, one of the critical determinants for the recognition of the γ-phosphate. Moves ~20 Å upon GTP binding to interact with one of the γ-phosphate oxygens and to coordinate the Mg^{2+} ion and W^{cat} in the active site, thereby stabilizing switch 1 in its active GTP-conformation.	An Ala in lieu of Thr is unable to form the contact to the Mg^{2+} ion. Thus, switch 1 fails to be activated in response to GTP binding and remains in the inactive conformation; the GTPase center is not or only partially formed, preventing the positioning of His^{cat} and W^{cat} for GTP hydrolysis. Switch 2 can still interact with the γ-phosphate for partial activation, allowing release of domain III and interactions with the ribosomal complexes to promote subunit joining.
A444V Beginning of strand β3, following switch 2. Side chain points toward domain II (Fig. 2 and S7B/C).	No apparent growth phenotype. Specific suppressor of G479A: restores WT growth rates and general translation, ribosome-dependent GTPase activity, subunit joining and *GCN4* translational control. Allows GDP to activate eIF5B for ribosome binding. Reduces the ability of the GDP-bound form to dissociate from the 80S ribosome. Ribosome binding of eIF5B-G479A,A444V is governed by a nucleotide-no nucleotide switch. (Shin et al, 2007)	The side chain of Ala444 points directly toward β10 and thereby forms part of the tightly packed hydrophobic interface between G domain and domain II. Upon GTP binding, Ala444 moves nearly 2 Å closer toward the active site (passively) following the movement of Asp476 in the G3 motif of switch 2 which interacts with the GTP-bound Mg^{2+} ion.	The increased volume of Val in lieu of Ala444 would cause a repulsion between domain II and the N-terminus of strand β3, causing the latter to move toward Asp476. In order to avoid a steric clash, Asp476 has to move toward the P loop. Thus, a Val in position 444 would induce – now actively and constitutively – the same movement in Asp476 that is normally induced by the binding of GTP and Mg^{2+}, thereby reducing the energy barrier that has to be overcome by nucleotides to shift switch 2 toward the binding pocket, compensating the increased energy penalty due to G479A. Thereby, GDP is able to induce a GTP-like position of the G3 motif and the shift of switch 2 (not the GTP-conformation) that promotes the release of domain III. Accordingly, domain III is also unable to reassociate stably with the G domain after GTP hydrolysis, which prevents dissociation of eIF5B from the ribosome as long as GDP is bound.

G479A G3 motif in switch 2 (Fig. 2, S4A/B and S7B/C).	Severe slow growth phenotype. Reduces affinity to GDP/GTP and impairs ribosome dependent GTPase activity and subunit joining activity. (Shin et al, 2007)	Gly479 stabilizes switch 2 in the active form and positions His^{cat} and W^{cat}. Moves ~8 Å upon GTP binding to form a direct contact to the γ-phosphate. During the rearrangement, Gly479 performs a peptide flip of ~160° which (according to the eIF5B·GDP structures) occurs simultaneously to the movement of Asp476 toward the Mg^{2+} ion.	The 160° peptide flip is energetically not allowed for a residue other than Gly, including Ala. Thus, the G479A mutation would stabilize the inactive form of switch 2 by preventing the conformational transition to the active state even in the presence of GTP. Consequently, GTP-bound eIF5B remains in the inactive form with domain III attached to switch 2, unable to promote subunit joining.
H480E **H480I** Switch 2; C-terminal to the G3 motif (Fig. 2D and 4A/B).	Severe slow growth phenotype. Impairs ribosome dependent GTPase activity. Forms stable complexes with 80S ribosomes. Active in subunit joining, but fails to be released from 80S ribosomes; impairs the formation of the first peptide bond. (Shin et al, 2009; Shin et al, 2002)	His480 (His^{cat}) in eIF5B is homologous to His84 in EF-Tu and most likely directly involved in GTP hydrolysis. During the GTP-induced molecular switch, His480 follows the movement of Gly479 and is positioned close to W^{cat} and the γ-phosphate.	His480 has no direct contribution to the conformational switch of eIF5B upon GTP binding. In the GTP-bound form, His480 is positioned virtually identical to His84 in EF-Tu, suggesting the same critical role in GTP hydrolysis. Thus, mutation of His480 to Glu or Ile and the resulting GTPase deficiency would prevent the conformational switch back to the inactive form of switch 2 that is required to retract domain III to trigger the dissociation of eIF5B from the ribosome.
H505Y In the loop following strand β5; next to the P loop (Fig. 4D).	No growth phenotype. Lacks GTPase activity. Reduces the affinity to ribosome. Suppressor for T439A: restores growth; reduces rates of subunit joining, allows release from ribosome without GTP hydrolysis. (Acker et al, 2009; Shin et al, 2002)	In the model of eIF5B·GTP on the ribosome, the side chain of His505 points toward H95 of the 28S rRNA at a distance of 3.5 Å, suggesting a direct interaction with the phosphate backbone.	In the proposed mode of binding for eIF5B·GTP to H95 and the SRL, the bulky Tyr side chain in lieu of His would on the one hand impair the formation of a direct contact and on the other hand cause steric clashes with the rRNA. Thus, the Tyr mutation would interfere with the productive interactions of the G domain to the SRL, resulting in a reduced affinity of mutant eIF5B for the 60S subunit, causing a reduced GTPase activity.
D740R C-terminal third of helix α8; points toward the G domain (Fig. 3A/B and S7B/C).	No apparent growth phenotype. Specific suppressor of the G479A mutant: restores growth rates and general translation in yeast as well as *GCN4* translational control. Partially restores ribosome dependent GTPase activity. (Shin et al, 2007)	Asp740 lies at the backside of domain III at a distance of more than 30 Å from the P loop (regardless of the nucleotide state of the G domain). In inactive (apo and GDP-bound) eIF5B, Asp740 interacts directly with Arg489 of the inactive switch 2. Upon the GTP-induced rearrangement of switch 2, Asp740 looses its contact to Arg489 but remains otherwise unchanged. Thus, Asp740 has no *direct* influence on the active GTP-bound conformation of eIF5B but only an indirect effect by stabilizing the inactive form.	D740R results in the loss of one of the interactions that stabilize switch 2 in its inactive conformation and its contact to domain III. Moreover, the bulky, positively charged side chain of the Arg would result in steric and electrostatic repulsion of Arg489 in switch 2. Thus, D740R would destabilize the inactive conformation of switch 2 to facilitate the release and thereby activation of domain III in the G479A mutant for productive interactions with the ribosome. As D740R does not specifically favor the GTP-conformation of switch 2 but only disfavors the inactive conformation, D740R restores general translation but is only partially able to restore GTPase activity.