Regulatory elements in eIF1A control the fidelity of start codon selection by modulating tRNAMet binding to the ribosome

Adesh K. Saini,1,3 Jagpreet S. Nanda,2,3 Jon R. Lorsch,2,5 and Alan G. Hinnebusch1,4
1Laboratory of Gene Regulation and Development, Eunice K. Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA; 2Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

eIF1A is the eukaryotic ortholog of bacterial translation initiation factor IF1, but contains a helical domain and long unstructured N-terminal tail (NTT) and C-terminal tail (CTT) absent in IF1. Here, we identify elements in these accessory regions of eIF1A with dual functions in binding methionyl initiator tRNA (Met-tRNAiMet) to the ribosome and in selecting AUG codons. A pair of repeats in the eIF1A CTT, dubbed Scanning Enhancer 1 (SE1) and SE2, was found to stimulate recruitment of Met-tRNAiMet in the ternary complex (TC) with eIF2GTP and also to block initiation at UUG codons. In contrast, the NTT and segments of the helical domain are required for the elevated UUG initiation occurring in SE mutants, and both regions also impede TC recruitment. Remarkably, mutations in these latter elements, dubbed scanning inhibitors SI1 and SI2, reverse the defects in TC loading and UUG initiation conferred by SE substitutions, showing that the dual functions of SE elements in TC binding and UUG suppression are mechanistically linked. It appears that SE elements enhance TC binding in a conformation conducive to scanning but incompatible with initiation, whereas SI elements destabilize this conformation to enable full accommodation of Met-tRNAiMet in the P site for AUG selection.

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Identification of the translation initiation codon in eukaryotes typically occurs by a scanning mechanism in which the small (40S) ribosomal subunit recruits the methionyl initiator tRNA [Met-tRNAiMet] in a ternary complex (TC) with the GTP-bound form of eIF2 to form the 43S preinitiation complex (PIC). The 43S PIC then binds to the mRNA near the m7G-capped 5' end and scans the leader, using complementarity with the anticodon of the initiator as a key means of identifying the AUG start codon [Pestova et al. 2007]. The mechanisms involved in ribosomal scanning and in distinguishing AUG from non-AUG triplets by the scanning PIC are not fully understood.

The factors eIF1 and eIF1A have been shown to stimulate scanning and assembly of a stable 48S PIC at AUG, and eIF1 also blocks recognition of near-cognate triplets, in a reconstituted mammalian system [Pestova and Kolupaeva 2002]. eIF1 appears to act with eIF1A to promote an open, scanning-conducive conformation of the PIC [Lomakin et al. 2000; Fekete et al. 2007; Passmore et al. 2007], and it impedes GTP hydrolysis by the TC in the absence of perfect base-pairing between the P-site codon and anticodon of Met-tRNAiMet [Unbehaun et al. 2004]. Consistent with this, recognition of AUG elicits dissociation of eIF1 from the 40S subunit and accelerates Pi release from eIF2GDP-Pi in a reconstituted yeast system [Algire et al. 2005; Maag et al. 2005].

The mechanisms of scanning and AUG selection are being dissected with genetic tools in budding yeast. Substitutions in the three subunits of eIF2, eIF5, and eIF1 were described that increase the frequency of initiation at the UUG start codon of his4-301 mRNA, restoring the ability to synthesize histidine [His+ pheno-type] [Yoon and Donahue 1992; Donahue 2000]. A subset of a subset of Sui- Suppressors of initiation codon mutant) substitutions affecting eIF1 (encoded by SUI1) appear to act simply by accelerating eIF1 dissociation from the PIC [Cheung et al. 2007]. Conversely, overexpression of
wild-type eIF1 suppresses the Sui− phenotypes of the SU15 and SU13-2 substitutions in eIF5 and eIF2β, respectively (Valasek et al. 2004; Fekete et al. 2007), conferring the Ssu− (Suppression of Sui−) phenotype. These findings support the notion that eIF1 is a “gatekeeper” that impedes start codon selection and whose inhibitory functions are eliminated at AUG codons, at least partly, by its dissociation from the 40S subunit. Presumably, overexpression of eIF1 prevents its release from the 40S subunit, which would otherwise occur at a higher frequency at UUGs in Sui− mutants, and thereby allows scanning to continue downstream.

The eIF1A contains an oligonucleotide/oligosaccharide-binding (OB) fold domain related to that present in bacterial translation initiation factor IF1, but additionally contains a helical domain comprised of helix α2 and a 3₁₀ helix connected by a short linker region [Loa], plus structured N or C strands that pack against different surfaces of α2. eIF1A also contains long unstructured N-terminal tail [NTT] or C-terminal tail [CTT] (Fig. 1A; Battiste et al. 2000). The original genetic selections that yielded Sui− mutations affecting eIF1, eIF5, or subunits of eIF2 failed to identify Sui− mutations in TIF11 encoding yeast eIF1A (Donahue 2000). However, we found previously that removing the unstructured CTT and the C-terminal region of eIF1A appeared to have opposite effects on scanning and start codon recognition.

Interestingly, the ΔC truncation, or double-Ala substitution of eIF1A CTT residues Phe-131 and Phe-133, also impairs loading of the TC on 40S subunits in PIC assembly. A well-established manifestation of this defect is the perturbation of translational control of GCN4 mRNA by four short upstream ORFs [uORFs]. Ribosomes that have translated the 5′-most uORF in GCN4 mRNA [uORF1] and resumed scanning can bypass the remaining three uORFs [uORF2–uORF4] and reinitiate at the GCN4 AUG when the levels of TC are reduced by eIF2α phosphorylation by protein kinase Gcn2 in amino acid-starved cells. The reduced TC concentration enables a fraction of the reinitiating 40S subunits to rebind TC only after bypassing uORF2–uORF4, but before reaching the GCN4 start codon. A similar shift in reinitiation from uORF2–uORF4 to the GCN4 ORF also occurs in eIF mutants where the rate of TC loading on 40S subunits is impaired, constitutively derepressing GCN4 translation independently of Gcn2 [the Gcd− phenotype] (Hinnebusch

**Figure 1.** Mapping the SE elements in the CTT of eIF1A. (A) Schematic showing the domains in eIF1A, indicating the residue number that begins each domain [above], and locations of the SEs in the CTT sequence [below]. [Constructs a–p] Schematics indicating amino acids missing [red dashes] in different TIF11 alleles. [Right] Relative growth, on a scale of 0–10, of his4-301 tif11Δ strains harboring the indicated TIF11 alleles on LEU2 plasmids under the conditions described in B. [B–D] Slg− and His*/Sui− phenotypes of strains harboring selected TIF11 alleles, described in A, were determined by spotting serial 10-fold dilutions on synthetic complete medium lacking leucine [SC-L] supplemented with 0.3 mM His (+His) or 0.0003 mM His [−His] and incubating for 3 d (+His) or 6 d [−His] at 30°C.
The ΔC and the F131A,F133A mutations confer Gcd− phenotypes by this mechanism, and also reduce the rate of TC loading on 40S subunits in the reconstituted yeast system (Olsen et al. 2003; Fekete et al. 2005, 2007). Thus, the eIF1A CTT appears to have dual functions in promoting TC binding and suppressing UUG initiation, but it was unclear whether these two functions are related.

While the F131A,F133A substitution in eIF1A increases the UUG:AUG initiation ratio measured using HIS4-lacZ reporters differing in these start codons, it does not confer the His+/Sui− phenotype observed for the ΔC truncation, suggesting that other residues removed by ΔC besides F131,F133 act to enhance scanning and block initiation at UUG codons (Fekete et al. 2005). Hence, we set out to identify all residues in eIF1A that participate in this key initiation function. In the process, we discovered that two loosely conserved ~10-residue repeats in the CTT, dubbed scanning enhancers [SEs], are the critical elements that both inhibit UUG initiation and promote TC binding to the 40S subunit, and we provide genetic and biochemical evidence that these activities are mechanistically linked. We further demonstrate that segments of the helical domain of eIF1A function with the NTT to impede scanning and promote start codon recognition and also negatively regulate TC binding. Our results support a model in which SE elements stabilize a mode of TC binding that is conducive with scanning but incompatible with initiation, and thereby block selection of near-cognate codons, whereas scanning inhibitor [SI] elements antagonize this mode of TC binding to enable AUG recognition.

Results

Identification of partially redundant SE elements in the eIF1A CTT

The F131A,F133A mutation in the eIF1A CTT increases the UUG:AUG initiation ratio, but unlike the ΔC truncation, does not confer a His+/Sui− phenotype in his4-301 cells (Fekete et al. 2005; 2007). To identify the other CTT residues responsible for the strong Sui− phenotype of Δ108–153, we first constructed the set of nested deletions shown in Figure 1A [constructs b–f] with a common end point at the predicted N-terminal residue of the unstructured CTT [Asp-119] (Battiste et al. 2000). The TIF11 alleles on a LEU2 plasmid were introduced into a his4-301 tif11Δ ura3 strain harboring wild-type TIF11 on a URA3 plasmid, and the latter TIF11− plasmid was evicted by counterselection on medium containing 5-fluoroorotic acid (5-FOA) (Boeke et al. 1987). Western analysis of whole-cell extracts [WCEs] of these and other mutants described below showed that none of their phenotypes could be attributed to reduced eIF1A expression [Supplemental Fig. S1]. The deletion mutants were tested for His+/Sui− phenotypes by examining cell growth on (-His) medium containing only 0.1% of the amount of histidine added to +His medium to fully supplement His− auxotrophs. Only the largest deletion [Δ119–133], which removed F131 and F133, produced a His− phenotype while also conferring a strong slow-growth [Sui−] phenotype on +His medium [Fig. 1A [constructs a–f], B [lanes a,e,f]]]. Given that substituting F131 and F133 alone did not have a His− phenotype (Fekete et al. 2007), we surmised that residues between 119 and 130 are functionally redundant with F131 and F133. We provisionally dubbed this hypothetical element Scanning Enhancer 1 (SE1) and that containing F131,F133 SE2, and hypothesized that a strong Sui− phenotype results only when SE1 and SE2 are impaired simultaneously.

To support the idea that SE1 is functionally redundant with SE2, we constructed another set of deletions with a common end point at F133, and thus all lacking F131,F133 of SE2 [Fig. 1A, constructs g–i]. Examination of these mutants revealed that removing residues from F133 to S125 was required to evoke His+ and Sgl− phenotypes [Fig. 1B [lanes i vs. h], summary in 1A [constructs g–i]]. Comparing constructs i and h in Figure 1A suggested that the C-terminal boundary of SE1 lies between S125 and E127. To map the N-terminal boundary of SE1, we deleted one residue at a time beginning with D119 from construct Δ128–133, which lacks F131, F133 of SE2 [Fig. 1A, constructs j–l]. Moderate His+ and Sgl− phenotypes appeared only with removal of F121 (Fig. 1C, lanes 1 vs. k). Together, these results suggested that SE1 maps between F121 and E127 [Fig. 1A, schematic].

The aforementioned results for constructs e and f in Figure 1A suggested that F131,F133 occupy the N terminus of SE2. To map the C terminus of SE2, we deleted residues from the extreme C terminus of eIF1A in a construct where SE1 is removed by Δ119–130 [Fig. 1A, constructs m–p]. Moderate His+ and Sgl− phenotypes appeared only with the deletion of residues 134–136 in construct p [Fig. 1D, lanes p vs. o], thus suggesting that SE2 extends from F131 to A136 [Fig. 1A, schematic].

To test directly the conclusion that SE1 and SE2 have overlapping functions in blocking UUG initiation, we constructed complete, multiple-Ala substitutions of SE1 and SE2. The SE1* mutation conferred only a slight His+ phenotype [Fig. 2B, lanes a,b] and a correspondingly small increase in the UUG:AUG initiation ratio measured using HIS4-lacZ reporters [Fig. 2C, bars a,b]. The SE2* mutation, in contrast, conferred Sgl−, a stronger His− phenotype [Fig. 2B, lanes b,c], and a much larger increase in the UUG:AUG initiation ratio [Fig. 2C, bars b,c]. Strikingly, the SE1*,SE2* double mutation is lethal, preventing eviction of the TIF11* plasmid [data not shown]. However, combining SE1* with an incomplete substitution of SE2 in which F131 is retained [mutation SE2* + F131] yields a viable mutant with strong Sgl− and His− phenotypes [Fig. 2B, lane d] and an even larger UUG:AUG initiation ratio than does SE2* alone [Fig. 2C, bars c vs. e]. By assaying a pair of luciferase [LUC] reporters with UUG or AUG start codons, we confirmed that SE1*,SE2* + F131 provokes a larger increase in the UUG:AUG initiation ratio than does SE2* or SE1* [Fig. 2E, bars a–d]. These findings support the idea that SE1 and SE2 are partially redundant elements that block initiation at UUG, and suggest that SE2 is more crucial than SE1 for this function.
Noting that SE₁ and SE₂ comprise a loosely conserved repeat of nine to 10 residues, each containing a pair of Phe residues [Fig. 2A], we sought to demonstrate that the Phe pairs are the critical residues in SE₁ and SE₂ blocking UUG recognition. First, we generated constructs combining a complete substitution of one SE element with an incomplete substitution of the other SE that retains both Phe residues and compared them with construct SE₁*,SE₂* containing only one Phe residue. Both constructs retaining two Phe residues [Fig. 2B, lanes c,f] produce weaker Slg⁻ and His⁺ phenotypes [Fig. 2B] and lower UUG:AUG ratios [Fig. 2C] compared with that with only a single Phe [Fig. 2C, bar d]. Furthermore, a construct [Fig. 2C, bar g] in which all of the residues in the two SEs are substituted except for the four Phe residues produces no Slg⁻ or His⁺ phenotype [Fig. 2B, lanes g vs. a] and only a slight increase in the UUG:AUG ratio compared with wild type [Fig. 2C, bars g vs. a]. These results suggest that the number of Phe residues is the critical determinant of the Slg⁻ and Sui⁻ phenotypes of SE mutations.

Finally, we compared substitutions of only the two Phe residues in SE₁ versus those in SE₂. F₁₂₁,F₁₂₃⁺,F₁₃₁,F₁₃₃‣ A provokes a stronger His⁺ phenotype [Figs. 2B, lanes h,i] and a larger increase in UUG:AUG ratio [Fig. 2D] compared with F₁₂₁,F₁₂₃,A. Although neither double-Phe substitution confers a Slg⁻ phenotype, the quadruple-mutant F₁₂₁,F₁₂₃,F₁₃₁,F₁₃₃‣ A is lethal (data not shown). Thus, F₁₂₁,F₁₂₃ are less critical than F₁₃₁,F₁₃₃, but become essential for blocking UUG initiation in the absence of F₁₃₁,F₁₃₃. [The fact that the F₁₃₁,F₁₃₃‣ A mutant is His⁺ and Slg⁻ does not contradict our previous findings, as we had analyzed a Flag-tagged version of this allele. The Flag tag reduces the His⁺/Sui⁻ phenotypes and exacerbates the growth defects of various TIF11 mutations [Fekete et al. 2007].] Together, the results in Figure 2 indicate that the phenylalanines are the critical residues in SE₁ and SE₂, which act redundantly to suppress UUG initiation and support cell growth.

Evidence that elimination of both SEs is lethal owing to an intolerable defect in scanning or non-AUG initiation

It is possible that precisely substituting both SEs is lethal because of an intolerable increase in non-AUG initiation. To test this idea, we asked whether the lethality could be overcome by the Ssu mutation 17–21 in the NTT of eIF1A or by overexpressing eIF1, both of which reduce UUG initiation in other Sui⁻ mutants. Indeed, 17–21 suppresses the lethality of SE₁*,SE₂*, although the resulting mutant retains Slg⁻ and His⁺/Sui⁻ phenotypes (Fig. 3A, †His and –His, segments c vs. a) and a high UUG:AUG ratio of 0.5 ± 0.05. Importantly, additionally overexpressing eIF1 from a high-copy (hc) SUI1 plasmid improved the growth rate and nearly eliminated the His⁺/Sui⁻ phenotype.
of the \( SE_1^*, SE_2^*, 17-21 \) strain (Fig. 3A, +His and −His, segments d vs. c). Similarly, introducing either 17–21, hc \( SUI1 \), or both into the viable mutant \( SE_1^*, SE_2^* + F_{131} \) suppressed its His\(^+\)/Sui\(^−\) phenotype (Fig. 3A, −His, segments f–h vs. e) and (for 17–21) diminished the elevated UUG:AUG ratio in this viable Sui\(^−\) mutant (Fig. 2E, bars e vs. d).

Consistent with the above findings, \( \Delta 117–153 \), which removes both \( SE_1 \) and \( SE_2 \), is also lethal, and this lethality is suppressed by 17–21 (Fig. 3B, 5-FOA, lanes c vs. d). Interestingly, \( \Delta 117–153 \) confers a His\(^+\)/Sui\(^−\) phenotype in viable cells also harboring \( TIF11^* \), and this dominant His\(^+\)/Sui\(^−\) phenotype is suppressed by 17–21 or hc \( SUI1 \) (Fig. 3B, −His, lanes c,d and e,f). As shown above for \( SE_1^*, SE_2^*, 17-21 \), introducing hc \( SUI1 \) into the viable \( \Delta 117–153, 17-21 \) mutant eliminates its residual His\(^+\)/Sui\(^−\) phenotype (Fig. 3C, lanes c vs. d).

The aforementioned results indicate that the lethality of eliminating both SEs involves an extreme defect in non-AUG initiation that is lessened by an Ssu\(^−\) mutation in eIF1A or overexpressing eIF1. The dominant Sui\(^−\) phenotype of \( \Delta 117–153 \) further indicates that this defective protein can compete with wild-type eIF1A for incorporation into PICs, but then fails to block UUG initiation effectively. Given the proposed function of eIF1 in promoting the open, scanning conformation of the PIC, and the ability of eIF1 overexpression or the 17–21 mutation to suppress the lethality and Sui\(^−\) phenotypes caused by eliminating both SE elements, we infer that the SE elements promote the open, scanning conformation of the PIC (hence, their designation as SEs). In contrast, the eIF1A NTT inhibits this open conformation and is regarded as an SI element, which we dubbed SI\(^\circ\).

Identification of a second SI element in the eIF1A helical domain

Our previous finding that deleting residues 108–153 from eIF1A \( \Delta 117–153 \) eliminates its residual His\(^+\)/Sui\(^−\) phenotype (Fekete et al. 2005) is consistent with the elimination of both SE elements, but...
seems at odds with the lethality observed here for Δ117–153 and SE1*,SE2*, which likewise eliminate both SEs. Because the lethality of the latter mutations is suppressed by the 17–21 mutation in SI1, we reasoned that Δ108–153 is viable because it deletes a second SI element [ provisionally dubbed SI3] that is located just upstream of the SEs and would be left intact by the smaller truncation Δ117–153. Supporting this interpretation, extending the deletion in Δ117–153 by only the single codon for Asn-116 [producing Δ116–153] suppressed the lethality of Δ117–153 [data not shown]. Furthermore, Δ116–153 confers a smaller increase in the UUG:AUG initiation ratio than does Δ117–153 when these alleles are compared in situations where Δ117–153 is not lethal, either in the presence of TIF11* [Fig. 3D] or hc SUI1* [Fig. 3E]. Δ116–153 also confers weaker Slg− and His*/Sui− phenotypes than does Δ117–153 in the presence of hc SUI1* (Supplemental Fig. S2A, lanes d vs. e). Thus, it appears that Asn-116 belongs to the putative SI2 and its elimination by Δ116–153 suppresses the lethal, hyper-Sui− phenotype of removing both SEs in Δ117–153.

To determine whether elimination of N116 fully inactivated SI2, we examined the Sui− phenotypes of constructs that extend the deletion of C-terminal domain (CTD) residues further upstream to also remove P110, G107, or Q106 [Fig. 3F, constructs c–f]. Compared with Δ116–153, these larger deletions improved the growth rate on complete medium and progressively reduced the Sui− phenotype [Fig. 3G, +His and −His, lanes d–f vs. c] and the UUG:AUG initiation ratio [Fig. 3H, bars c–e vs. b]. [The weaker His* phenotype observed for Δ116–153 vs. Δ110–153 likely reflects the relatively poor growth of the Δ116–153 mutant evident on +His medium.] Thus, extending the deletion of CTD residues from N116 to Q106, removing the entire C strand, 310 helix, and linker Loα [Fig. 3F], constitutes a step-wise reduction in SI2 function.

To confirm this last conclusion and also map the N-terminal boundary of SI2, we made nested Ala substitutions from residue 106 to 109 [the last residue of α2 and Loα linker] in the lethal Δ117–153 truncation [Fig. 4A, “A” substitutions in constructs c–f]. We reasoned that substitutions encroaching into the N terminus of SI2 should confer an Ssu− phenotype and suppress the lethal, hyper-Sui− phenotype of Δ117–153. Indeed, substituting both P106 and G107, but not Q106 alone, suppressed the lethality of Δ117–153, and the more extensive substitutions that include residues 108 and 109 conferred even higher growth rates [Supplemental Fig. S2B, 5-FOA, lanes c–f vs. b; results summarized in Fig. 4A, +His]. Importantly, the 106–109 substitution [106–109Ala] also suppressed the lethality of the hyper-Sui− mutation SE1*,SE2*,106–109Ala with alanines (As) in the indicated residues L26Y28K29 and their predicted contacts with the N strand (Fig. 4B). The coordinates of human eIF1A (PDB:1D7Q) were used to generate structural models using PyMOL software, showing selected side chains in stick representation.
phenotypes of the viable mutant \( SE_1^*, SE_2^* + F_{131} \) \( F_{131} \) [Fig. 4B, sections h vs. g]. Together, the results suggest that SI2 spans residues 107–117 and encompasses Laa310 helix, and most of the structured C strand in the eIF1A CTD.

Because all of the components of SI2 belong to the helical domain [Fig. 4C], we asked whether altering residues in the Laa310 helix, or C strand that interacts directly with \( \alpha_2 \) [Battiste et al. 2000] would also confer an Ssu\(^-\) phenotype by disrupting SI2 function. We first examined the effects of Ala substitutions in five such C strand residues: Glu108, Pro110, Glu111, Ile115, and Asn116 (Fig. 4C). This mutation [EPEIN-Ala] suppressed the lethality of the hyper-Ssu\(^-\)/C0 allele \( D_{117–153} \) (Supplemental Fig. S2B, 5-FOA, lanes b vs. g) as described above for Ssu\(^-\)/C0 mutation \( 106–109\)Ala. Because the structured N strand of the N-terminal domain (NTD) (residues 26–33) also packs against \( \alpha_2 \), we then asked whether substituting N strand residues Leu26, Tyr28, and Lys29 that are predicted to contact residues in \( \alpha_2 \) or Laa (Fig. 4D) would likewise confer Ssu\(^-\) phenotypes. Indeed, this triple-Ala substitution [LYKAla] suppressed the lethality of \( D_{117–153} \) (Supplemental Fig. S2B, 5-FOA, lanes b vs. h) and diminished the elevated UUG:AUG initiation ratio conferred by \( D_{116–153} \), closely resembling the Ssu\(^-\)/C0 mutation \( 17–21 \) (Fig. 3H, bars f–g vs. b). Consistent with these findings, the N and C strand mutations LYKAla and 106–116Ala decreased the Sgl\(^-\) and His\(^+\)/Sui\(^-\) phenotypes of the viable mutant \( SE_1^*, SE_2^* + F_{131} \) [Fig. 5A, sections a–c vs. d], and, along with 106–116Ala, also reduced the UUG:AUG ratio in \( SE_1^*, SE_2^* + F_{131} \) cells [Fig. 5B, bars f–h vs. e]. These results indicate that proper packing of both N and C strands against \( \alpha_2 \) is required for the function of SI2 in supporting UUG initiation in Sui\(^-\) mutants of eIF1A.

We asked next whether mutations affecting SI2 can suppress the Sui\(^-\) phenotype of SUI3-2, encoding the S264Y substitution in eIF2b. Remarkably, 106–116Ala and LYKAla both diminish the Sui\(^-\)/His\(^+\) phenotype of SUI3-2 cells (Fig. 5C, data not shown) and reduce the elevated UUG:AUG initiation ratio conferred by SUI3-2 [Fig. 5D], mimicking the Ssu\(^-\)/C0 mutation \( 17–21 \) in SI1 in both respects. Interestingly, the DEAR-AASA mutation described previously [Fekete et al. 2007], which substitutes Asp98, Glu99, and Arg101 of \( \alpha_2 \) itself and eliminates contacts with C or N strand residues [Fig. 5E], also confers an Ssu\(^-\) phenotype in SUI3-2 cells [Fig. 5C,D]. Thus, the integrity of the entire helical domain is required.

Figure 5. SI substitutions suppress the Sui\(^-\) phenotype of SUI3-2. (A) Sgl\(^-\) and His\(^+\)/Sui\(^-\) phenotypes of his4-301 strains containing the indicated TIF11 alleles were determined as in Figure 4B. (B) his4-301 strains with the indicated TIF11 alleles plus the dual luciferase reporter pRaugFFuug were assayed as described in Figure 2E. (C) Sgl\(^-\) and His\(^+\)/Sui\(^-\) phenotypes of his4-301 strains with the indicated TIF11 alleles and containing either plasmid pRSSU13-S264Y-W harboring SUI3-2 (lanes 2–7) or empty vector (lane 1) were determined as in Figure 1B, except that the cells were spotted on SC-LW medium. (D) Strains described in C harboring the AUG or UUG HIS4-lacZ reporters were analyzed as in Figure 2C. (E) Predicted interaction of eIF1A residues D98E99A100R101 with C and N strand residues is depicted as in Figure 4C.
to support the elevated UUG initiation rate in SUI3-2 cells. Finally, eliminating the entire NTT by the ΔN mutation confers a stronger Ssu− phenotype compared with 17–21 [Fig. 5D], indicating that 17–21 only partially impairs S1.

**Gcd−** phenotypes of Sui− mutations in SE1SE2 are suppressed by Ssu− mutations in S1

We showed previously that the ΔC and F131,133A mutations impair TC binding to 40S subunits. When introduced into Flag-tagged eIF1A, these substitutions derepress translation of GCN4 mRNA independently of eIF2α phosphorylation by GCN2. This Gcd− phenotype was attributed to a reduced rate of TC loading on 40S subunits that have translated uORF1 and resumed scanning, allowing them to bypass uORF2–uORF4 and reinitiate at GCN4 without any decrease in TC formation [Olsen et al. 2003; Fekete et al. 2005, 2007]. Consistent with these findings, the SE2* mutation (which eliminates F131,F133) confers a pronounced Gcd− phenotype, derepressing by ~10-fold the GCN4-lacZ reporter containing all four uORFs in nonstarved GCN2 cells (Fig. 6A, Nonstarvation, bars c vs. a). The SE1* mutation confers a smaller derepression of GCN4-lacZ, and adding to it the Ala substitutions of all SE2 residues except F131 [mutant SE1*,SE2*+F131] evokes a stronger derepression than does SE1* alone [Fig. 6A, Nonstarvation, bars d vs. b]. None of these mutations increases expression of a GCN4-lacZ construct lacking all four uORFs in nonstarvation conditions (Supplemental Fig. S3A), confirming that they diminish the translational repression imposed by the uORFs. Importantly, co-overexpressing all three eIF2 subunits and tRNA\[^{\text{Met}}\], the components of TC, from an hc plasmid (hc TC) reduces the derepression evoked by the SE1*, SE2*, and SE1*,SE2* + F131 mutations [Fig. 6C]. This supports the idea that their Gcd− phenotypes arise from slower TC loading on reinitiating 40S subunits scanning downstream from uORF1, which is overcome by mass action at higher concentrations of TC.

![Figure 6](image.png)

In vivo and in vitro TC loading defects of SE substitutions are suppressed by S1 substitutions. (A) GCN2 strains containing the indicated TIF11 alleles and GCN4-lacZ reporter plasmid p180, depicted at the top, were cultured in repressing (Nonstarvation) or derepressing (Starvation) medium (SC-LU) or derepressing (Starvation) medium (SC-LU lacking isoleucine and valine and supplemented with 0.5 μg/mL sulfometuron), and β-galactosidase activities were assayed in WCEs. (B) GCN2 strains containing the indicated TIF11 alleles were analyzed as in A under nonstarvation conditions. (C) GCN2 strains harboring the indicated TIF11 alleles, GCN4-lacZ reporter plasmid p180, and either empty vector, hc SUI1, or hc TC plasmid p4385 were assayed under repressing conditions as in C, except that cells were grown in SC-LUW. (D) GCN2 strains harboring the indicated TIF11 alleles plus the AUG or UUG HIS4-lacZ reporters and either empty vector, hc SUI1, or hc TC were analyzed as in Figure 3D. (E) GCN2 strains harboring the indicated TIF11 alleles, GCN4-lacZ reporter plasmid p180, and either empty vector or SUI3-2 were assayed under derepressing conditions as in C. (F) Binding of TC to 40S subunits as a function of the concentration of 40S subunits [K\text{d}], or time [k\text{eq}], measured as the fraction of [\[^{35}\text{S}\]Met]-tRNA\[^{\text{Met}}\] associated with 40S subunits in a native gel assay containing saturating eIF1 (1 μM), model mRNA(AUG), and wild-type or mutant eIF1A proteins at 1 μM. Errors are mean deviations of at least three measurements. (G) Effects of eIF1A substitutions on the rate of ribosomal subunit joining. Kinetics of subunit joining measured using purified 40S and 60S subunits at 100 nM and 200 nM, respectively, and wild-type or mutant eIF1A proteins at 1 μM by monitoring the change in intensity of light scattering over time. Kinetics were biphasic for wild type, 106-116Ala, and 17–21, but monophasic for the other three eIF1A mutants. [k1 and k2] Rate constants for the first and second phases, respectively; [α1/α2] ratio of amplitudes of the first to second phases; na not applicable owing to monophasic reactions. Errors are mean deviations of at least three measurements.
Substituting SE1*,SE2* impairs TC binding in vitro in a manner suppressed by Ssu− substitutions in SI elements

To test our prediction that SE and SI substitutions have opposite effects on TC loading, we measured their effects on TC binding to 40S subunits in the yeast reconstituted system [Algire et al. 2002, Acker et al. 2007]. We first examined the effects of SE and SI substitutions on the equilibrium binding constant of eIF1A for 40S subunits in the presence of saturating concentrations of wild-type eIF1, using a previously established assay [Maag and Lorsch 2003]. The results in Supplemental Fig. S4A indicated that only the two mutants harboring the LYKAa substitution displayed an appreciable reduction in eIF1A affinity for 40S subunits. To compensate for these defects in subsequent TC-binding assays, we used eIF1 and eIF1A at concentrations of 1 μM, >80-fold above the Kd values measured for all mutant eIF1A proteins.

To assay TC loading, preformed TC containing [35S]-labeled Met-tRNA\text{\textsubscript{Met}} was incubated with purified 40S subunits, a model mRNA with an AUG start codon, eIF1, and either wild-type or mutant eIF1A, and TC binding to the 40S subunit was monitored by an electrophoretic mobility shift [Kolitz et al. 2009]. Importantly, the SE1*,SE2* compound substitution markedly decreased the affinity [greater than eightfold increase in Kd] and rate (>35-fold decrease in k\text{obs}) of TC binding to the PIC [Figs. 6F; Supplemental Fig. S4B]. The individual SE1* and SE2* substitutions also reduced the k\text{obs} for TC loading, but by smaller amounts—less than twofold and twofold, respectively [Fig. 6F]. These results fit with the fact that SE1* and SE2* produce moderate and strong Gcd− phenotypes, respectively [Fig. 6A], whereas SE1*,SE2* is lethal in vivo.

In contrast, the Ssu− substitutions LYKAa, 106–109Ala, and 106–116Ala all increased the rate of TC loading in vitro compared with that seen for wild-type eIF1A [Fig. 6F]. Remarkably, all three Ssu− substitutions also greatly reduced the deleterious effects of SE1*,SE2* on the affinity and rate of TC loading, restoring the Kd values to <0.5 nM and increasing k\text{obs} by factors of 7–11 compared with the the SE1*,SE2* mutant alone [Fig. 6F]. The SI1 substitution 17–21 also strongly suppressed the defective rate of TC binding conferred by SE1*,SE2*, although it did not suppress the reduction in TC-binding affinity [Fig. 6F]. These results suggest that both SI and SI2 negatively regulate the rate of TC loading, and that substitutions in these regions compensate for the impaired TC binding conferred by SE substitutions. The fact that SI mutations co-suppress the TC-binding defects in vitro and the Gcd− and Sui− phenotypes in vivo of SE mutations strongly suggests that all of these defects are linked mechanistically to the rate and stability of TC binding.

Substituting the SE elements stabilizes a closed conformation of the 40S subunit competent for subunit joining

Cryo-electron microscopy (cryo-EM) reconstructions of the 40S subunit in the presence or absence of eIF1 and eIF1A demonstrated that the two factors synergistically stabilize the open conformation of the subunit, and biochemical experiments strongly suggested that TC initially binds to this open state [Passmore et al. 2007]. Accordingly, we hypothesized that the eIF1A SEs might stimulate the rate of TC loading, at least in part, by maintaining the open conformation of the 40S subunit. To test this possibility, we exploited the observation that eIF1 and eIF1A synergistically impede joining of the 60S with the 40S subunit, consistent with the idea that the open conformation is not receptive to subunit joining and is instead optimized for scanning, whereas subunit joining occurs in the closed conformation following AUG recognition and eIF1 release [Acker et al. 2006]. Hence, if mutating the SE elements shifts the equilibrium from the open to closed conformation of the 40S subunit, this should stimulate the rate of 60S joining with the 40S subunit in vitro.

Spontaneous joining of the 40S and 60S subunits follows biphasic kinetics, with the fast and slow phases having roughly equal amplitudes. These two phases likely correspond to different conformational states of the subunit; the fast phase may be 60S joining to the closed state of the 40S subunit and the slow phase may be the conversion of the open to closed state of the 40S subunit [Acker et al. 2006]. In the presence of the SE1*,SE2* mutant, the kinetics of subunit joining were completely monophasic, with a rate constant (0.05 s\textsuperscript{−1}) similar to that of the fast phase of joining observed for wild-type eIF1A [Fig. 6G; Supplemental Fig. S4C]. This is consistent with the idea that substituting the SE elements shifts the conformational equilibrium of the 40S subunit in favor of the closed state, which is competent for subunit joining but not for the initial stage of TC loading. In contrast, the SI substitutions 17–21 and 106–116Ala have the opposite effect of increasing the amplitude of the slow phase of the subunit joining reaction.
ing both Phe pairs in the eIF1A SEs increases UUG

Our results indicate that both F131 and F133 contribute to the function of SE2 (Fig. 2), but F121 might suffice for SE1 function.

The fact that Δ117–153 is lethal but the more extensive truncation Δ108–153 (ΔC) is viable was the critical clue that the latter disrupts an element whose elimination compensates for the lethality of deleting both SEs. By systematically testing additional mutations for suppression of the lethality of Δ117–153, we determined that this new element includes the Lαα, 310 helix, and residues in the structured N and C strands that contact α2 in the helical domain. Similar to the 17–21 mutation in the NTT, disrupting the integrity of the helical domain suppresses Sui− mutations in the SE elements and in elf2β [SUI3-2], conferring the Ssu− phenotype. Hence, we conclude that α2 and its associated N and C strands comprise a functional unit, dubbed SI2, that acts in conjunction with SI1 to arrest scanning and promote start codon selection.

Interestingly, mutations affecting SE1 and SE2 also confer strong Gcd− phenotypes that can be suppressed by overexpressing TC, indicating reduced rates of TC loading during reinitiation on GCN4 mRNA. Just as in blocking UUG initiation, SE2 is more critical than SE1 in promoting TC loading and repressing GCN4 translation. Our biochemical analysis in the reconstituted system supports this conclusion by revealing reduced rates of TC binding that are more severe for SE2 versus SE1 substitutions, and of greatest severity when both elements are lacking. This last result indicates that SE1 and SE2 have overlapping functions in TC loading in addition to blocking UUG initiation. The strong correlation between the effects of different SE mutations on the degree of Sui− phenotypes in vivo and the severity of Gcd− phenotypes and TC binding defects in vitro provides evidence that the increased UUG initiation in SE mutants is linked mechanistically to their defective TC binding.

Remarkably, Ssu− mutations in either SI element diminish the Gcd− phenotypes of both SE mutations and the elf2β Sui− mutation SUI3-2, thus suggesting that the Ssu− mutations compensate for defects in TC binding. Our biochemical data support this conclusion, as defects in TC binding provoked by the SE1*,SE2* substitution are partially suppressed by multiple Ssu− substitutions in SI1 or SI2, all of which elicit an increased rate of TC binding when present in otherwise wild-type elf1A. Together, these results provide compelling evidence that the ability of SI mutations to suppress UUG initiation in Sui− mutants involves their ability to compensate for TC loading defects. This leads us to the surprising deduction that the wild-type SI elements act to oppose continued scanning and promote start codon recognition by negatively regulating TC binding.

How might the defect in TC binding provoked by SE mutations lead to increased UUG initiation? We begin by proposing that the SEs stabilize TC binding to the PIC in a conformation that is compatible with scanning but incompatible with initiation. For example, the SEs could
bind Met-tRNA\textsubscript{Met} in a way that prevents it from being fully engaged with the P site—a mode of binding we dub the “P\textsubscript{out}” state, which would be associated with the open, scanning conformation of the PIC (Fig. 7A). The SEs could promote the P\textsubscript{out} mode of TC binding directly (e.g., by interacting with the anticodon stem–loop [ASL] of the initiator), or indirectly by stabilizing the open conformation of the 40S subunit, to which TC initially binds (Passmore et al. 2007). In either case, entry of AUG into the P site and its perfect complementarity with the anticodon of the initiator would be required to overcome the SEs and enable Met-tRNA\textsubscript{Met} to be fully accommodated in the P site and trigger downstream steps in the initiation pathway. We dub this second conformation the “P\textsubscript{in}” state, and envision that it is characteristic of the closed, scanning-incompatible conformation of the PIC (Fig. 7A). These two states could be identical to the two states that bind TC weakly or stably, respectively, that we detected previously in kinetic studies [Kolitz et al. 2009].

As SE substitutions would impair the ability of eIF1A to stabilize the P\textsubscript{out} conformation in which TC initially binds, this would account for their deleterious effect on TC binding to the 40S subunit in vitro and their Gcd\textsuperscript{−} phenotypes in vivo. By destabilizing P\textsubscript{out}, SE mutations would also facilitate the P\textsubscript{out}-to-P\textsubscript{in} transition at UUG codons, which occurs without a perfect codon–anticodon match, and this would help explain their Sui\textsuperscript{−} phenotypes (Fig. 7B). Thus, although SE substitutions reduce the rate of TC loading, once TC is bound to the 40S subunit, the transition from P\textsubscript{out} to P\textsubscript{in} would occur more frequently at near cognate codons. We found that TC overexpression suppresses the Gcd\textsuperscript{−} phenotypes of SE mutants [Fig. 6C] but does not reduce the elevated UUG:AUG ratio in SE\textsubscript{1*},SE\textsubscript{2*} + F\textsubscript{131} cells (Fig. 6D). These findings are consistent with our model, as increasing TC levels should boost the rate of TC binding to the P\textsubscript{out} state, reducing the Gcd\textsuperscript{−} phenotype, but should not mitigate the increased probability of P\textsubscript{out}-to-P\textsubscript{in} transitions at UUG codons that, in our model, contribute to the Sui\textsuperscript{−} phenotypes of SE mutants.

Our proposal that SE elements stabilize TC binding in a conformation that facilitates scanning but is incompatible with initiation predicts that Met-tRNA\textsubscript{Met} binds in the P site of the scanning PIC [the P\textsubscript{out} state] in a manner distinct from that seen in crystal structures of bacterial 70S-mRNA-tRNA complexes [Berk et al. 2006; Korostelev et al. 2006; Selmer et al. 2006]. In fact, this prediction is strongly supported by results of directed hydroxyl radical mapping of the mammalian eIF1A in reconstituted 43S PICs [Yu et al. 2009]. This work revealed that the CTT extends into the P site, threading under the Met-tRNA\textsubscript{Met} in a configuration that would obstruct Met-tRNA\textsubscript{Met} binding to the P site in the manner observed in bacterial 70S complexes. Hence, it was concluded that AUG recognition and formation of the closed complex would likely require removal of the eIF1A CTT from the P site. Indeed, we argued previously that the eIF1A CTT would be ejected from the P site on AUG recognition [Fekete et al. 2007], based on its physical displacement from eIF1 in the PIC [Maag et al. 2005] and its AUG-dependent functional interaction with eIF5 [Maag et al. 2006]. It seems likely, therefore, that the SEs sterically block the P\textsubscript{in} mode of TC binding in addition to stabilizing the P\textsubscript{out} conformation [Fig. 7A]. This idea is very attractive because SE mutations would then facilitate the P\textsubscript{out}-to-P\textsubscript{in} transition at UUGs [conferring Sui\textsuperscript{−} phenotypes] in two ways: by destabilizing P\textsubscript{out} and also removing a steric impediment to P\textsubscript{in} (Fig. 7B).

Figure 7. (A) Model describing the positive and negative effects of the SE and SI elements of eIF1A, respectively, on TC binding in the P\textsubscript{out} conformation, which is conducive to scanning, and the second function of the SEs in blocking TC binding in the P\textsubscript{in} conformation, which is incompatible with scanning and permissive for initiation. (B) SE inactivation destabilizes P\textsubscript{out} reducing TC loading and conferring the Gcd\textsuperscript{−} phenotype and also enhancing the P\textsubscript{out}-to-P\textsubscript{in} transition at UUGs to confer the Sui\textsuperscript{−} phenotype. The P\textsubscript{out}-to-P\textsubscript{in} transition and UUG initiation is further stimulated by loss of the inhibitory effect of the SEs on P\textsubscript{in}. (C) SI inactivation stabilizes P\textsubscript{out} promoting TC loading and replacing Gcd\textsuperscript{−} phenotypes with Gcn\textsuperscript{−} phenotypes, and suppressing the P\textsubscript{out}-to-P\textsubscript{in} transition at UUGs to confer the Ssu\textsuperscript{−} phenotype. (D,E) Hypothetical model depicting the different conformations of initiator tRNA in the P\textsubscript{out} (D) and P\textsubscript{in} (E) states and the proposed roles of the eIF1A SE elements in stabilizing initiator binding in P\textsubscript{out}, where the initiator is not fully accommodated in the P site, and impeding initiator binding in P\textsubscript{in}, where the initiator is more fully engaged with the P site. On AUG recognition, the SEs are ejected from the P site to allow greater accommodation of the initiator in the P\textsubscript{in} state.

A

B

C

D

E
Combining the results of Yu et al. (2009) with our finding that SE elements promote TC binding to the scanning-conducive conformation of the PIC, we propose that, in the P\textsubscript{out} state, the SEs occupy the P site in a manner that blocks accommodation of the initiator ASL without preventing the codon–anticodon interactions required during scanning (Fig. 7D). The SEs might interact directly with the anticodon or ASL of the initiator to stabilize TC bound in this scanning conformation of the PIC. Pairing with the AUG start codon would lead to ejection of the SEs from the P site and allow more extensive P-site engagement of the initiator ASL in the P\textsubscript{in} conformation, as depicted in Figure 7E. The P\textsubscript{in} conformation might resemble the “30S P/I” state visualized in a cryo-EM model of the bacterial 30S initiation complex (Simonetti et al. 2008), and further conformational changes would presumably occur on subunit joining before reaching the classical P-site binding of tRNA seen in bacterial 70S structures (Berk et al. 2006; Korostelev et al. 2006; Selmer et al. 2006). Our model in Figure 7, D and E, fits with the notion that accommodating initiator tRNA in different intermediate states is a key feature of the small ribosomal subunit (Simonetti et al. 2008).

We can readily incorporate the functions of eIF1A SI elements into our model simply by proposing that they oppose the SEs and destabilize the P\textsubscript{out} state to which TC initially binds (Fig. 7A). This can explain our finding that SI mutations partially suppress the defective TC binding conferred by the SE\textsubscript{1}/C\textsubscript{0} mutations in vitro and, consistently, suppress the Gcd\textsuperscript{−} phenotypes of SE mutations in vivo (Fig. 7C). At the same time, SI mutations would shift the equilibrium back from P\textsubscript{in} to P\textsubscript{out} promoting the scanning conformation of the PIC and suppressing UUG initiation in Sui\textsuperscript{−} mutants, i.e., their Ssu\textsuperscript{−} phenotypes (Fig. 7C). The function of the wild-type SI elements in destabilizing the P\textsubscript{out} state can be viewed as a driving force that facilitates the P\textsubscript{out}-to-P\textsubscript{in} transition required for AUG selection. Because the NTT also appears to interact directly with the P site (Yu et al. 2009), it might play a direct role in antagonizing initiator binding to the P\textsubscript{out} state (Fig. 7D).

Results of our previous studies suggested that TC binds initially to the open conformation of the PIC, stabilized synergistically by eIF1 and eIF1A (Passmore et al. 2007). Hence, it is plausible that the SEs could stimulate TC loading by stabilizing the open conformation of the 40S rather than interacting directly with the initiator. Indeed, we found that inactivation of the SEs accelerates the rate of 60S subunit joining, which is thought to proceed only from the closed conformation of the 40S. Remarkably, SI substitutions had the opposite effect and partially suppressed the more rapid 60S joining conferred by the SE substitutions, as would be expected if the wild-type SIs antagonize the open conformation as the means of promoting start codon recognition. Thus, it appears likely that the SEs and SIs regulate the rate of TC loading, at least partly, by their opposing effects on the open-to-closed conformational transition of the 40S subunit. However, it is noteworthy in this connection that eIF1 overexpression did not suppress the Gcd\textsuperscript{−} phenotype of an SE mutation even though it suppressed the Sui\textsuperscript{−} phenotype (Fig. 6C,D). As eIF1 promotes the open conformation, one might expect that overexpressing eIF1 would rescue TC binding in SE mutants if this defect arises only from reduced occupancy of the open state. Hence, the SEs likely stimulate TC loading by a second, possibly direct, mechanism in addition to promoting the open conformation of the 40S subunit.

In its simplest formulation, our model posits that the SEs in eIF1A help to recruit TC in the open, scanning conformation and block initiator accommodation at non-AUGs, whereas SIs drive the transition to the closed state in which the initiator is fully engaged in the P site at AUG codons (Fig. 7). IF1, the bacterial ortholog of eIF1A, lacks the CTT, NTT, and helical domain (Supplemental Fig. S6; Carter et al. 2001), whereas the archaeal ortholog (aIF1A) lacks only the C strand and unstructured CTT (Supplemental Figs. S7, S8). Similar to bacteria, it appears that many mRNAs in archaea use the Shine-Delgarno (SD) sequence upstream of the start codon to recruit the 30S subunit directly to the initiation region (Dennis 1997; Londei 2005), obviating the scanning mechanism. Thus, in bacteria and archaea, the presence of SD-facilitated AUG selection is correlated with the absence of SEs, consistent with the role of these eukaryotic elements in promoting a scanning-competent intermediate in the initiation pathway (Fig. 7D). Considering that archaea resemble eukaryotes in employing a TC with aIF2-GTP for initiator recruitment (Pedulla et al. 2005), the helical domains in aIF1/aIF1A might also play a role in promoting TC binding in the P\textsubscript{in} state. Recent in vitro findings suggest that bacterial IF1 functions in stabilizing a conformation of the 30S initiation complex that is incompatible with subunit joining, which can be overcome by a favorable SD sequence (Milon et al. 2008). Thus, although IF1 lacks the SE elements, it carries out one of the functions we ascribe to the SE, of stabilizing a small subunit conformation incompatible with subunit joining. This raises the possibility that a region of the OB fold—the main structural element shared between eIF1A and IF1—could augment this aspect of SE function.

Materials and methods

Plasmids and yeast strains

Plasmids and yeast strains used in this study are listed in Supplemental Tables S1 and S2, respectively. Descriptions of plasmid constructions and site-directed mutagenesis are given in the Supplemental Material. The his4-301 yeast strain H3582 [MATa ura3-52 trp1Δ63 leu2-3, leu2-112 his4-301 (ACG) tif11Δ p3392 <TIF11, URA3>] was transformed with single-copy or hc LEU2 plasmids harboring various TIF11 alleles on SC-L medium, and the resident TIF11+ URA3 plasmid p3392 was evicted by selection on 5-FOA medium to obtain the mutant strains listed in Supplemental Table S2.

Biochemical assays with yeast extracts

Assays of β-galactosidase activity in WCEs were performed as described previously [Moehle and Hinnebusch 1991]. Measurements of luminescence in WCEs were conducted essentially as described [Dyer et al. 2000]. For Western analysis, WCE extracts
were prepared by trichloroacetic acid extraction as described previously [Reid and Schatz 1982], and immunoblot analysis was conducted as described [Olsen et al. 2003].

Biochemical assays in the reconstituted yeast system

Reagent preparation is described in the Supplemental Material. Fluorescence anisotropy measurements to determine Kd values for 40S binding of eIF1A were performed as described previously using wild-type eIF1A Fluorescein-labeled at the C terminus and competing its 40S binding with unlabeled eIF1A mutants [Maag and Lorsch 2003, Maag et al. 2006]. For all experiments, buffer conditions were 30 mM HEPES (pH 7.4), 100 mM potassium acetate (pH 7.4), 3 mM MgCl2, and 2 mM dithiothreitol. TC binding was measured by gel mobility shift assays as described previously [Algire et al. 2002]. Kinetics of ribosomal subunit joining in the presence of wild-type or mutant eIF1A was measured by light scattering on an SX.180MV-R stopped-flow fluorometer (Applied Photophysics) [Acker et al. 2009].

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Erratum

Genes & Development 24: 97–110 (2010)

Regulatory elements in eIF1A control the fidelity of start codon selection by modulating tRNAi\textsuperscript{Met} binding to the ribosome
Adesh K. Saini, Jagpreet S. Nanda, Jon R. Lorsch, and Alan G. Hinnebusch

In the above-mentioned article, the label for the third row of data in Figure 6G, “$a_1/a_2$,” is incorrect. Instead, it should read “$a_2/a_1$,” the ratio of amplitudes of the second [slow] phase to the first [fast] phase of the subunit joining reaction. The descriptions and interpretations of these data in the Results and Discussion sections require no corrections.
Regulatory elements in eIF1A control the fidelity of start codon selection by modulating tRNA\textsubscript{i\text{Met}} binding to the ribosome

Adesh K. Saini, Jagpreet S. Nanda, Jon R. Lorsch, et al.

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