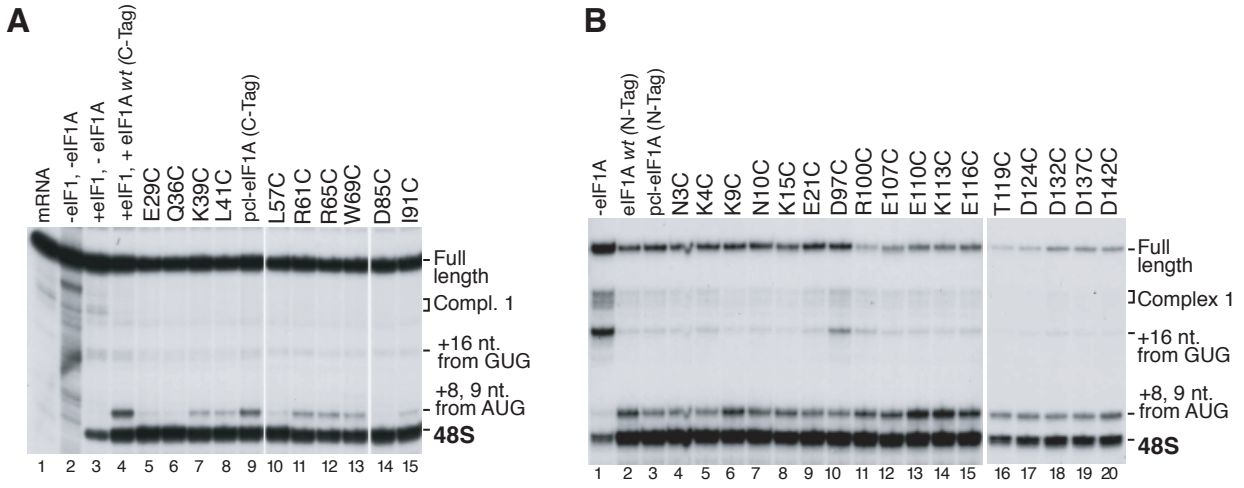
Activity of eIF1A mutants

Wild type (*wt*) eIF1A contains cysteines at positions 50 and 58, neither of which is surface-exposed. Whereas substitution of C50 by serine in eIF1A tagged with His_6 at either N- or C-terminus (**p**artially **c**ysteine-**l**ess eIF1A mutants, pcl-eIF1A) did not influence eIF1A's activity in 43S complex formation (data not shown) or in assembly of 48S complexes on native β-globin mRNA (Fig. S1A, compare lanes 4 and 9; Fig. S1B, compare lanes 2 and 3), substitution of C58 reduced eIF1A's activity in both assays (data not shown). Fully active pcl-eIF1A mutants with a C50S substitution and the non-exposed C58 were therefore used to construct 27 eIF1A mutants containing additional single surface-exposed cysteines. The activity of eIF1A cysteine mutants was verified by their ability to promote 48S complex formation on native β-globin mRNA in an *in vitro* reconstituted translation system assayed by toe-printing (Figs. S1A, B). In the absence of eIF1A, 48S complexes do not form efficiently on the initiation codon of native β-globin mRNA, and a large proportion of ribosomal complexes instead assembles aberrantly near the 5'-end of the mRNA (complex I) (Pestova et al., 1998) and at the GUG triplet in the β-globin 5'-UTR (Pestova and Kolupaeva, 2002) (e.g. Fig. S1B, lane 1). All eIF1A cysteine mutants were active in promoting efficient formation of 48S complexes and in suppressing assembly of aberrant ribosomal complexes on β-globin mRNA (Figs. S1A, B). Additional toe-prints +8-9 nt downstream of the AUG codon most likely represent initiation complexes assembled on the AUG codon, but in which the 3'-portion of mRNA is not properly fixed in the 40S subunit's mRNA-binding cleft (Battiste et al., 2000; Pisareva et al., 2008).

Figure S1. eIF1A cysteine mutants and their activities in 48S complex formation

Toe-printing analysis of initiation complexes assembled on β-globin mRNA in the presence of 40S subunits, Met-$tRNA_i^{Met}$, eIFs 2/3/4A/4B/4F/1 and eIF1A mutants as indicated. Positions of assembled ribosomal complexes are marked on each panel.



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Supplementary Figure 1