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ORIGINAL ARTICLE

eIF5A has a function in the cotranslational translocation of proteins into the ER

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Abstract The putative eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved and essential protein present in all organisms except bacteria. To be activated, eIF5A requires the conversion of a specific residue of lysine into hypusine. This hypusine modification occurs posttranslationally in two enzymatic steps, and the polyamine spermidine is the substrate. Despite having an essential function in translation elongation, the critical role played by eIF5A remains unclear. In addition to demonstrating genetic interactions with translation factors, eIF5A mutants genetically interact with mutations in *YPT1*, which encodes an essential protein involved in endoplasmic reticulum (ER)-to-Golgi vesicle transport. In this study, we investigated the correlation between the function of eIF5A in translation and secretion in yeast. The results of in vivo translocation assays and genetic interaction analyses suggest a specific role for eIF5A in the cotranslational translocation of proteins into the ER, but not in the posttranslational pathway. Additionally, we observed that a block in eIF5A activation up-regulates stress-induced chaperones, which also occurs when SRP function is lost. Finally, loss of eIF5A function affects binding of the ribosome-nascent chain complex to SRP. These results link eIF5A function in translation with a role of SRP in the cell and may help explain the dual effects of eIF5A in differential and general translation.

Keywords Translation elongation · Hypusine · eIF5A · Cotranslational translocation · Endoplasmic reticulum

Abbreviations

eIF5A	Eukaryotic translation initiation factor 5A
TIF51A	Gene encoding eIF5A in yeast
eEF2	Eukaryotic translation elongation factor 2
EFT2	Gene encoding eEF2 in yeast
eEF1A	Eukaryotic translation elongation factor 1A
EF-P	Elongation factor P
SRP	Signal recognition particle
ER	Endoplasmic reticulum
RNC	Ribosome-nascent chain
CPY	Carboxypeptidase Y
DPAP B	Dipeptidyl aminopeptidase B

Introduction

The putative eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved and essential protein that is present in archaea and eukaryotes but not in bacteria (Schnier et al. 1991; Chen and Liu 1997). It was initially purified from the ribosomes of reticulocyte lysates, and eIF5A was also shown to stimulate the synthesis of methionyl-puromycin in vitro (Benne and Hershey 1978). However, a controversy has existed for years about whether eIF5A plays a direct role in translation. Meanwhile, several other functions have been attributed to eIF5A,

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including roles in cell cycle progression, nucleocytoplasmic transport, mRNA degradation and apoptosis (Zanelli and Valentini 2007; Park et al. 2010; Marra et al. 2007). Nevertheless, it was recently demonstrated that eIF5A physically interacts with the 80S ribosome, as well as with translation elongation factors eEF1A and eEF2. In addition, eIF5A co-fractionates with monosomes and polysomes in a translation-dependent manner (Jao and Chen 2006; Zanelli et al. 2006). Mutants of eIF5A show an accumulation of polysomes instead of polysome run-off, and they cause an increase in the average time required for ribosomes to transit along mRNAs (Gregio et al. 2009; Saini et al. 2009). Moreover, eIF5A interacts functionally with elongation factor eEF2 (Dias et al. 2012). These results not only support the notion that eIF5A plays a direct role in translation but also suggest that its role is specifically in translation elongation rather than translation initiation.

eIF5A is activated by a unique posttranslational modification, in which a specific lysine residue (K51 in yeast) is converted into hypusine over two steps: first, the enzyme deoxyhypusine synthase (Dys1 in yeast) transfers an aminobutyl moiety from the polyamine spermidine to the amino group of the specific lysine residue, forming deoxyhypusine; then, addition of a hydroxyl group is catalyzed by the deoxyhypusine hydroxylase (Lia1 in yeast) (Park et al. 2010).

Although bacteria do not have an eIF5A orthologue, they have instead the elongation factor P (EF-P), which shares important structural features with eIF5A (Park et al. 2010; Dias et al. 2013). EF-P does not contain a hypusine residue; however, in a subset of bacterial species, EF-P does undergo a posttranslational modification which involves the addition of a β-lysine to a specific lysine residue, corresponding to the hypusine modification site in eIF5A (Navarre et al. 2010; Yanagisawa et al. 2010; Bailly and de Crécy-Lagard 2010; Roy et al. 2011; Park et al. 2012). This EF-P modification, called β-lysylation, is analogous to the hypusine modification of eIF5A and is important for the function of EF-P in the translation and cell growth of *E. coli* (Yanagisawa et al. 2010; Park et al. 2012).

The budding yeast *Saccharomyces cerevisiae* has been used as a model system to study eIF5A, and several genetic interactions have already been identified (Valentini et al. 2002; Zanelli and Valentini 2005; Frigieri et al. 2007, 2008; Dias et al. 2012). One interesting genetic interaction is synthetic lethality caused by mutation of both eIF5A and Ypt1 (Frigieri et al. 2008), a protein essential for the fusion of endoplasmic reticulum (ER) vesicles to the Golgi (Segev 2001). Although we demonstrated that eIF5A does not work directly in the vesicle trafficking, this factor is associated to ribosomes bound to membranes, implying a function for eIF5A in translation at the ER (Frigieri et al. 2008).

To better understand the link between eIF5A and the secretory pathway, in this study, we analyzed the involvement of eIF5A in the translocation of proteins into the ER. We demonstrate that eIF5A is important for the cotranslational translocation of proteins into the ER, but not for the posttranslational translocation pathway. Additionally, genetic analyses revealed that interactions only occur between eIF5A and factors essential for the cotranslational translocation pathway. Interestingly, when hypusine formation and eIF5A activation are blocked, we observed up-regulation of the expression of stress-induced chaperones, a phenotype also seen in mutants with loss of function of the cotranslational translocation pathway and the consequent accumulation of unfolded proteins in the cytosol. Finally, we show that eIF5A function is important for the ribosome-nascent chain (RNC) complex to bind the signal recognition particle (SRP). Collectively, these

Table 1 The yeast strains used in this study

Strain	Genotype	Source
SVL14	*MATa ade2 his3 leu2 trp1 ura3 can1 tif51A-1* (P83S)	Lab collection (Valentini et al. 2002)
SVL82	*MATa ade2 his3 leu2 trp1 ura3 can1*	Lab collection
SVL613	*MATa leu2 trp1 ura3 his3 dys1::HIS3 [DYS1/TRP1/CEN]* (pSV520)	Lab collection
SVL614	*MATa leu2 trp1 ura3 his3 dys1::HIS3* [*dys1-1* (W75R, T118A, A147T)/*TRP1*/*CEN*] (pSV730)	Lab collection (Galvão et al. 2013)
VZL1021	*MATα ura3 leu2 his3 trp1 ade2 sec61-101*	Davis Ng
VZL1246	*MATα ura3 leu2 his3 trp1 ade2 can1 rpl25::HIS3* [*RPL25*-GFP/*LEU2*/*CEN*]	Martin Pool
VZL1248	*MATa ura3 ade2 trp1 leu2 his3 sec65-1*	Martin Pool
VZL1257	*MATα his3 leu2 ura3 tif51A-1::natMX4 can1 lyp1*	Lab collection
VZL1292	*MATa ade2 his3 leu2 trp1 ura3 can1* [$eft2^{H699K}$/2μ/*TRP1*] (pVZ1370)	Lab collection
Y07842[a]	*MATa his3 leu2 ura3 sec61-2::kanMX4*	Brenda Andrews
Y07900[a]	*MATa his3 leu2 ura3 sec62-ts::kanMX4*	Brenda Andrews
Y11000[a]	*MATa his3 leu2 ura3 sec65-1::kanMX4*	Brenda Andrews
1623[a]	*MATa his3 leu2 ura3*	Brenda Andrews

[a] Li et al. (2011)

Table 2 The plasmids used in this study

Plasmid	Description	Source
pSV59	*CEN*, *LEU2*	Lab collection
pSV60	*CEN*, *URA3*	Lab collection
pSV65	*2μ*, *URA3*	Lab collection
pSV107	*TIF51A*, *2μ*, *URA3*	Lab collection
pSV138	*TIF51A*, *CEN*, *URA3*	Lab collection
pSV146	*TIF51A*, *CEN*, *LEU2*	Lab collection
pSV364	*2μ*, *URA3*	Lab collection
pSV374	*2μ*, *LEU2*	Lab collection
pVZ1064	*SSCPY-URA3*, *HIS3*, *CEN*	Davis Ng
pVZ1110	*GAL1-DAP2-6xHis*, *2μ*, *URA3*	Open biosystems/Lab collection
pVZ1359	*SEC65*, *SRP21*, *SRP72*, *2μ*, *URA3*	Colin J. Stirling
pVZ1360	*SCR1*, *SRP14*, *SRP54*, *SRP68*, *2μ*, *LEU2*	Colin J. Stirling
pVZ1370	$eft2^{H699K}$, *2μ*, *TRP1*	Lab collection

results reveal a close connection and specific interaction between eIF5A and the translocation of proteins into the ER via the cotranslational pathway.

Materials and methods

Yeast strains, plasmids and standard procedures

The yeast strains and plasmids used in this study are listed in Tables 1 and 2, respectively. The procedures for cell growth and genetic manipulations were performed according to standard protocols (Guthrie and Fink 1991).

Western blot analysis

Yeast strains were grown to mid-log phase and were subsequently lysed in protein extraction buffer (20 mM Tris–HCl, at pH 7.5, 2 mM dithiothreitol, 2 mM EDTA and 5 μg/mL each of pepstatin, leupeptin, aprotinin and chymostatin). Total protein extracts were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. The proteins of interest were detected through immunoblotting, using specific antibodies and the chemiluminescence detection system.

Growth analysis and temperature sensitivity assay

Yeast strains were grown under permissive conditions, and equal amounts of the cultures were subsequently harvested. Tenfold serial dilutions were plated onto specific media and grown at the indicated temperatures for 3 days.

Synthetic genetic interactions

Strains carrying double knockout alleles or mutations were generated by standard crossing and sporulation methods. Spores of interest were grown in the presence of nourseothricin (*tif51A-1::natMX4*) and geneticin to select for other mutant genes (*kanMX4* marker). The strains were then transformed with a plasmid encoding *TIF51A* or an empty vector. Briefly, the plasmid shuffle assay (Lorsch 2007) was used to select the strains that, due to natural plasmid loss, did not harbor the *TIF51A/URA3* plasmid after being grown in media containing uracil. This selection allows for an analysis of the gene interactions both with and without a copy of the wild-type gene in an isogenic background.

Subcellular fractionation

The subcellular fractionation of the lysed cells was based on previously described protocols (Frey et al. 2001; Dalley et al. 2008). The pellet of cells was prepared according to the western blot protocol, including a treatment with 0.1 mg/mL cycloheximide for 5 min just before lysis. The lysates were cleared of cellular debris by centrifugation at $1{,}200\times g$ for 10 min, and the membranes were pelleted by centrifugation at $18{,}000\times g$ for 20 min. The pellet was solubilized by the addition of 1.5 % (weight/volume) CHAPS in the presence of 500 mM KOAc. Insoluble materials were removed by high-speed centrifugation. The resulting supernatant was centrifuged for 1 h at $256{,}000\times g$ to generate a ribosome-enriched pellet and a postribosomal supernatant. The total protein extract (T), postribosomal supernatant (S) and ribosomal-enriched pellet (P) were each analyzed by western blot. The western blot signals of the samples were quantified using ImageScanner III and IQ Tools (GE Healthcare Life Sciences). The values of the pellet fractions, normalized by their respective total fractions, were plotted in percentages relative to the wild type (assuming wild type = 1.0) using Microsoft Excel software (Microsoft, Redmond, Washington, USA).

Results and discussion

A mutant of eIF5A shows a cotranslational translocation defect, which is not due to impaired general translation elongation

Considering the involvement of eIF5A in elongation and its functional connection to the secretory pathway (Frigieri et al. 2008; Gregio et al. 2009), we hypothesized that eIF5A might act during ER-associated protein synthesis.

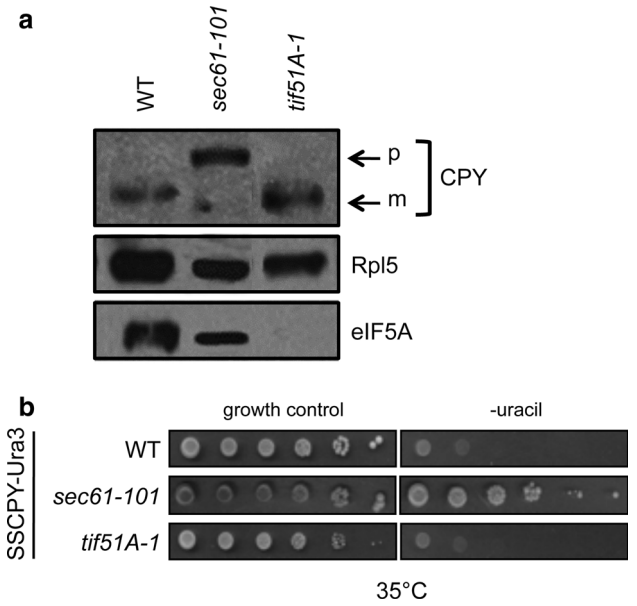


Fig. 1 eIF5A does not play a role in the posttranslational translocation of proteins into the ER. **a** The in vivo translocation of the endogenous CPY protein. The wild-type (SVL82), *sec61-101* (VZL1021) and *tif51A-1* (SVL14) strains were grown to mid-log phase at 25 °C and then shifted to the restrictive temperature (37 °C) during 3 h. Ten micrograms of total protein extract from each strain were immunoblotted with anti-CPY to detect the precursor (pCPY = 69 kDa) and the mature (mCPY = 61 kDa) forms of the protein. Anti-Rpl5 was used as a loading control. Anti-eIF5A was used to detect the protein mutant depletion under restrictive growth conditions. **b** Tenfold serial dilutions of the same strains harboring the reporter SSCPY-Ura3 (pVZ1064) were plated onto SC-histidine (growth control) and SC-uracil to test the ability to grow due to the cytoplasmic accumulation of SSCPY-Ura3. Both plates were incubated at semi-permissive temperature (35 °C) for 3 days

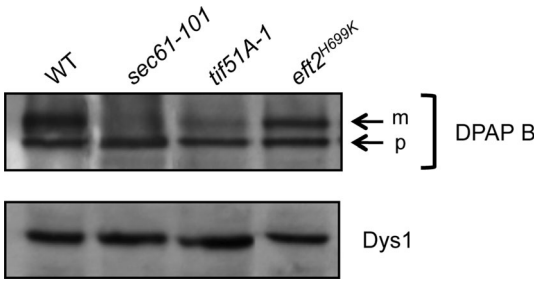


Fig. 2 eIF5A, but not eEF2, plays a role in the cotranslational translocation of proteins into the ER. The wild-type (SVL82), *sec61-101* (VZL1021), *tif51A-1* (SVL14) and $eft2^{H699K}$ (SVL1292) strains, all carrying the reporter DPAP B plasmid (pVZ1110), were grown to mid-log phase at the permissive temperature (25 °C). The expression of the reporter DPAP B was induced by the addition of 2 % galactose for 3 h. For SVL14, SVL82 and VZL1021, DPAP B induction time was coincident to the shift time at the restrictive temperature, 3 h at 37 °C. The dominant-negative $eft2^{H699K}$ was grown at 25 °C as translation elongation defect already occurs at this condition (Ortiz and Kinzy 2005). The total protein extract was immunoblotted using anti-6×His (DPAP B) to detect both the precursor (pDPAP B = 93 kDa) and the mature (mDPAP B = 120 kDa) forms of DPAP B. Dys1 was used as a loading control

To test this possibility, we used a *tif51A-1* mutant strain, which has depleted eIF5A (Valentini et al. 2002), and reporters of protein translocation into the ER (Ng et al. 1996). Because the mechanisms governing the cotranslational and posttranslational pathways of protein translocation into the ER differ significantly, in particular the factors associated with the translational machinery, we tested reporters specific to both pathways (Ng et al. 1996).

We first investigated the posttranslational pathway using the vacuolar carboxypeptidase Y (CPY) as a reporter. The targeting of CPY to the ER occurs in a SRP-independent manner (Ng et al. 1996). CPY maturation occurs normally in wild-type cells but is abnormal in cells that exhibit a general translocation defect, such as *sec61* mutants (Stirling et al. 1992; Ng et al. 1996). As shown in Fig. 1a, the wild-type strain showed correct sorting of CPY, which undergoes proteolysis to yield its vacuolar mature form (61 kDa), consequently decreasing its molecular weight. As in the wild type, the *tif51A-1* mutant also exhibits only the mature form of CPY. On the other hand, the *sec61-101* mutant exhibits an accumulation of the precursor form of CPY (69 kDa). These results agree with previous evidence, which indicated that different eIF5A mutants did not display defects in CPY maturation (Frigieri et al. 2008).

To extend our analysis of the potential link between eIF5A and ER-associated protein synthesis, we used a second reporter. The signal sequence of CPY was fused to the N-terminus of the entire *URA3* ORF (SSCPY-Ura3), leading to the production of the fusion protein SSCPY-Ura3, which is artificially targeted to the ER exclusively via the posttranslational pathway (Ng et al. 1996). In a wild-type strain, SSCPY-Ura3 is efficiently targeted to the ER and this fusion protein cannot complement an *ura3* strain because it lacks access to its natural cytosolic substrate. In contrast, cells with a defect in the posttranslational pathway accumulate SSCPY-Ura3 in the cytoplasm and can grow in medium without uracil. The results generated using this reporter are shown in Fig. 1b. In contrast to the *sec61-101* mutant, the eIF5A mutant *tif51A-1* expressing an SSCPY-Ura3 reporter cannot grow in the absence of uracil, indicating that the posttranslational translocation pathway is intact in these cells. These results demonstrate that eIF5A does not play a role in the posttranslational translocation of proteins into the ER.

Next, we asked whether eIF5A is necessary for the cotranslational translocation pathway. We used the vacuolar dipeptidyl aminopeptidase B (DPAP B) as a reporter because this protein is exclusively translocated via the cotranslational SRP-dependent pathway (Ng et al. 1996). In the wild-type strain, DPAP B is targeted to the ER and undergoes several N-glycosylations, which increase its molecular weight from 93 to 120 kDa (Fig. 2). In contrast,

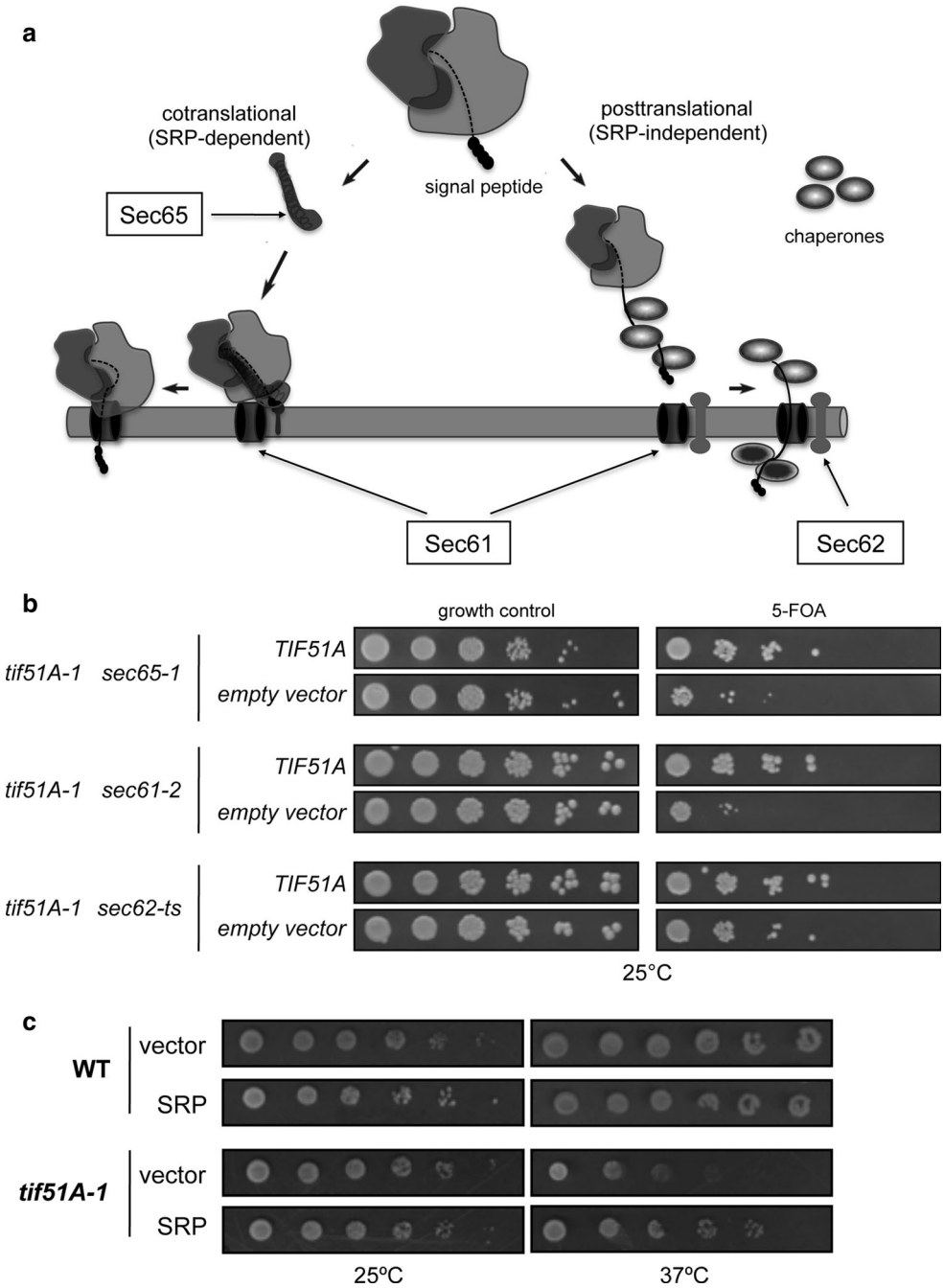


Fig. 3 Genetic interactions occur specifically between eIF5A and cotranslational translocation factors. **a** A simplified scheme for protein translocation into the ER via the cotranslational (SRP-dependent) and the posttranslational (SRP-independent) pathways. The proteins in the black boxes, Sec65, Sec61 and Sec62, were assayed. **b** The genetic interactions between *tif51A-1* and either *sec65-1*, *sec61-2* or *sec62-ts*. The indicated double mutant strains harboring *TIF51A* (pSV138 or pSV146) or the empty vector (pSV59 or pSV60) were plated onto SC-leu,-ura (growth control) and SC+5-FOA media and grown at the permissive temperature (25 °C) for 3 days. **c** Rescue of the temperature-sensitive phenotype of the *tif51A-1* by overexpression of the SRP complex. Tenfold serial dilutions of the wild-type (SVL82) and *tif51A-1* (SVL14) strains, either harboring all of the SRP complex components (pVZ1359+pVZ1360) or harboring empty vectors (pSV364+pSV374), were plated onto SC-leu,-ura and grown at both the permissive (25 °C) and the restrictive temperatures (37 °C) for 3 days

in the *sec61-101* mutant, the targeting of DPAP B to the ER is inefficient, and no mature form of the protein is observed (Fig. 2). The eIF5A mutant *tif51A-1* showed a defect in maturation of DPAP B, comparable to that seen in the *sec61-101* mutant (Fig. 2). Considering that the CPY targeting is correct in the *tif51A-1* mutant, these results suggest that eIF5A function is necessary specifically for the cotranslational translocation of proteins into the ER.

Since cotranslational translocation is coordinated with the elongation step of protein synthesis (Mason et al. 2000; Brodsky 1998) and because eIF5A mutants show a general defect in translation elongation, we next tested whether another translation elongation mutant might also display a defect in the cotranslational translocation pathway. We used DPAP B to assess the translocation abilities of a strain harboring the elongation factor 2 (eEF2) dominant-negative mutant $eft2^{H699K}$. The protein eEF2 is a canonical translation elongation factor, and the $eft2^{H699K}$ mutant strain exhibits impaired elongation of polypeptides during protein synthesis, a phenotype shared with eIF5A mutants (Dias et al. 2008, 2012; Gregio et al. 2009). As observed in the right-most lane of Fig. 2, similarly to wild-type cell,

DPAP B is correctly targeted to the ER in the *eft2*^{H699K} mutant. This result strongly suggests that the involvement of eIF5A in the cotranslational translocation pathway is specific and not merely a consequence of a general block in the elongation step of protein synthesis.

eIF5A interacts genetically only with factors in the cotranslational translocation pathway

To confirm the functional relevance of eIF5A for the cellular secretory pathway, we searched for genetic interactions between eIF5A and translocation factors. As depicted in Fig. 3a, we chose the genes of factors that are essential for the cotranslational translocation pathway (*SEC65*) or for the posttranslational pathway (*SEC62*) and the primary translocon component (*SEC61*), which is essential for both pathways.

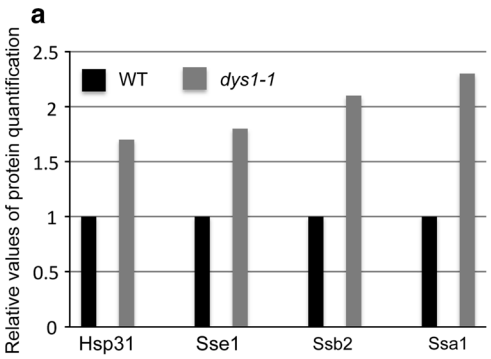
We first tested the effect of combining mutants of the translocation factors described above and the eIF5A mutant *tif51A-1* in a single haploid strain. Interestingly, we observed a growth defect at the permissive temperature (25 °C) in the double mutant strains carrying *tif51A-1* and either *sec65-1* or *sec61-2* (empty vector), but no defect was noticed in the double mutant *tif51A-1 sec62-ts* (empty vector), when compared to the wild type (*TIF51A*). This genetic assay shows a synthetic sick genetic interaction only between *tif51A-1* and the mutants affecting the cotranslational pathway, *sec65-1* and *sec61-2* (Fig. 3b).

In cotranslational targeting, binding of SRP to the signal peptide is a limiting step. Because of this fact, and because of the greater quantity of ribosomes in a cell than SRPs (Ogg and Walter 1995), we next tested whether an overexpression of genes coding for all SRP components would rescue the growth defect of the *tif51A-1* mutant at the restrictive temperature. As shown in Fig. 3c, the overexpression of SRP does cause a partial rescue of the growth defect.

Together, these results further support the idea that the function of eIF5A is important for the cotranslational targeting of proteins to the ER.

Blocking eIF5A activation increases the levels of stress-induced chaperones

The functional correlations between eIF5A and the cotranslational pathway described herein are very similar to those reported for Rpl25-GFP. The ribosomal protein L25 (Rpl25) is an important mediator of the ribosome interaction with SRP (Halic et al. 2004, 2006), and a strain expressing an Rpl25-GFP fusion protein has defects in the cotranslational translocation but not in the posttranslational pathway (Dalley et al. 2008). In addition, some stress-induced chaperones are up-regulated in the Rpl25-GFP



b

Gene	Hsf1-binding motif	Hsf1 regulation
HSP31	CTTCATGAAA	potential
SSE1	TAAGATCTTCTAAGG	confirmed
SSB2	TTTCAAGAAA	potential
SSA1	AGAACATTCTAGAAA	confirmed

[1] Hsf1-binding motif and regulation were obtained using "Search for Associations" tool from YEASTRACT Database (http://www.yeastract.com).

Fig. 4 Loss of eIF5A function causes the up-regulation of stress-induced chaperones. **a** Differentially expressed chaperones identified by mass spectrometry in 2D-DIGE proteomic maps of *dys1-1* mutant (SVL614) compared to its isogenic wild-type strain (SVL613) after growth at 25 °C. Each bar represents relative staining intensities of chaperones Hsp31, Sse1, Ssb2 and Ssa1 from 2D-gels of biological triplicates (data not shown). **b** The genes encoding the same up-regulated chaperones are transcriptionally regulated by the transcription factor Hsf1, according to the confirmed or potential motifs indicated (YEASTRACT Database)

strain (Dalley et al. 2008), a phenotype reminiscent of SRP dysfunction in the cell (Arnold and Wittrup 1994; Mutka and Walter 2001). To verify if the loss of function of eIF5A also triggers an up-regulation of stress-induced chaperones, we looked for these proteins in 2D gel-based proteomic maps of eIF5A-defective mutants (data not shown). However, the eIF5A mutants described so far need a temperature shift to exhibit the defect in eIF5A function, which also induces heat-shock responses and alters the expression of chaperones, compromising this analysis. Instead, we used the deoxyhypusine synthase mutant *dys1-1*, which shows a 60 % reduction in hypusine-containing eIF5A at permissive temperature. Besides, even under permissive growth condition (25 °C), the *dys1-1* mutant shows defects of translation (total protein synthesis and polysome profile) that are identical to the eIF5A mutants at their restrictive temperature (Galvão et al. 2013).

Among the five chaperones identified in our 2D-DIGE experiments, we found that the stress-induced chaperones Hsp31, Sse1, Ssb2 and Ssa1 were up-regulated in the *dys1-1* mutant when compared to the wild type (Fig. 4a). Stress-induced chaperones become up-regulated in response to the accumulation of unfolded proteins in the cytosol, and the Hsf1 transcription factor is determinant for their induction (Sorger 1991; Albanèse et al. 2006). Figure 4b shows the information on Hsf1 regulation as well as the Hsf1-binding

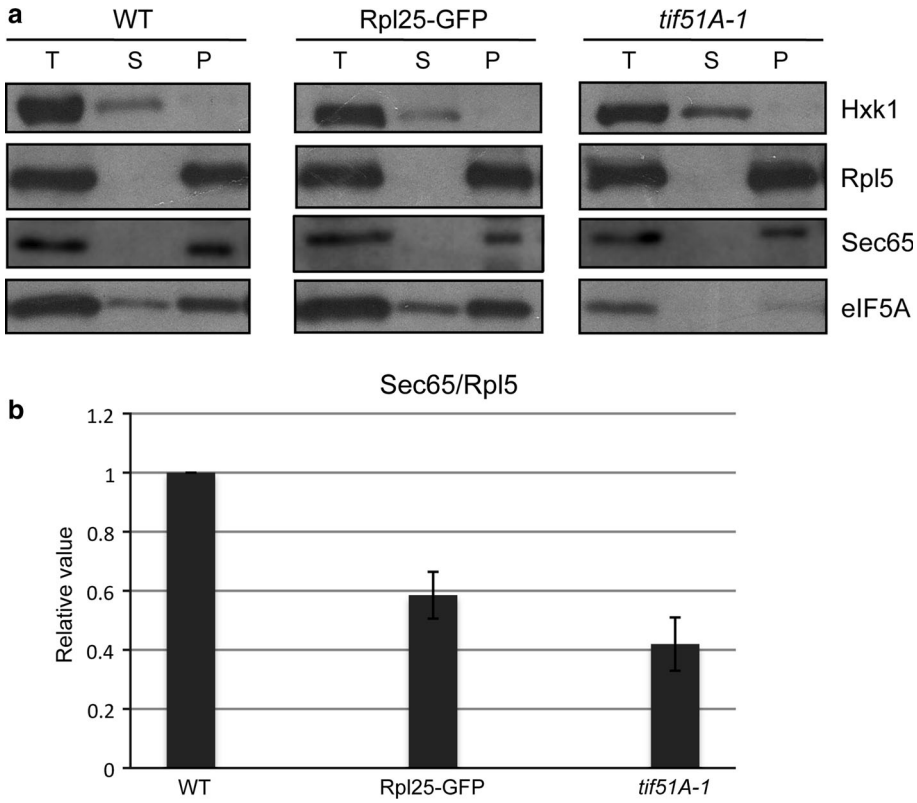


Fig. 5 eIF5A is important to the binding of the ribosome-nascent chain complex to SRP. **a** The wild-type (SVL82), Rpl25-GFP (VZL1246) and *tif51A-1* (SVL14) strains were grown to mid-log phase at the permissive temperature (25 °C) and then shifted to 37 °C for 3 h. The total extract (T), supernatant (S) and pellet (P) from a 256,000×*g* centrifugation were immunoblotted against Hxk1, Rpl5, Sec65 and eIF5A using specific polyclonal antibodies. **b** The quantification of the Sec65 present in the ribosome-enriched fraction (P) normalized by the ribosomal protein Rpl5. The graph represents the relative values of Sec65/Rpl5 obtained from independent replicates, assuming the wild-type strain to have a value of 1

motifs that are present in the promoters of the genes coding for Hsp31, Sse1, Ssb2 and Ssa1. Therefore, our results examining stress-induced chaperones in the eIF5A mutant are also very similar to those observed for the Rpl25-GFP strain (Dalley et al. 2008) and SRP mutants (Arnold and Wittrup 1994; Mutka and Walter 2001), and they again support the concept that eIF5A functions during the cotranslational targeting of proteins to the ER.

eIF5A is important for the binding of SRP to the ribosome-nascent chain complex

Because eIF5A binds to ribosomes and has an essential function during translation elongation (Zanelli et al. 2006; Jao and Chen 2006; Gregio et al. 2009; Saini et al. 2009), we also tested whether eIF5A depletion interferes with the binding of ribosome to SRP. We used a strategy of ultra-centrifugation to purify the ribosomes, which allows for the co-purification of bound SRP (Frey et al. 2001; Dalley et al. 2008). For this experiment, we also included Rpl25-GFP, which has decreased association of ribosomes with SRPs (Dalley et al. 2008). Therefore, the total cell extracts (T) were separated into postribosomal supernatants (S) and ribosomal pellet fractions (P) by ultracentrifugation. The quantity of purified complexes was detected by blotting the ribosomal protein L5 (Rpl5) and the SRP subunit Sec65. Samples were also blotted for the cytosolic hexokinase Hxk1 and eIF5A. As shown in Fig. 5a, all pellet fractions (P) contained ribosomes (Rpl5) and SRP (Sec65) complexes, which were absent in the supernatant fractions (S). On the other hand, Hxk1 was present only in the S fractions, as expected. The depletion of eIF5A in the *tif51A-1* mutant was also seen in all fractions, compared to the wild-type strain (Fig. 5a). Because the presence of Sec65 in the P fractions is dependent on ribosome precipitation, we quantified and normalized the amount of Sec65 relative to Rpl5 (Fig. 5b). In comparison to the wild-type strain, we observed the known defect in the Rpl25-GFP strain in the binding of RNCs to SRPs (Dalley et al. 2008). Similarly, the eIF5A mutant *tif51A-1* demonstrated a decreased association between ribosomes and SRPs.

These results are consistent with the defects observed in the cotranslational translocation pathway of eIF5A mutants and with the genetic interactions between eIF5A and cotranslational translocation factors. Furthermore, our data suggest that the primary function of eIF5A in the translocation of proteins during translation is the efficiency of binding of SRP to elongating ribosomes.

To our knowledge, this is the first study showing that a translation factor has been directly linked with the function of SRP in targeting the RNC to the ER. Additionally, this discovered link between the function of eIF5A in the ribosome and SRP binding to the RNC supports the hypothesis that eIF5A acts differentially in the translation

of a specific subset of mRNAs, although the loss of eIF5A function ultimately induces general defects in translation elongation (Zanelli and Valentini 2007; Park et al. 2010).

It is important to note that the eIF5A structural homolog EF-P has recently been shown to function preferentially in the translation of proteins containing poly-proline tracts and other amino acid motifs, such as YIRYIR (Ude et al. 2013; Doerfel et al. 2013; Hersch et al. 2013). However, not all of the proteins containing these motifs are decreased in a *Salmonella* strain deficient in EF-P, suggesting that the role EF-P plays in the translation of specific proteins is more complex than was initially determined by an analysis of poly-proline tracts (Hersch et al. 2013). Interestingly, several proteins identified as more dependent on EF-P for their translation are related to membrane integrity and motility (Bullwinkle et al. 2012; Hersch et al. 2013), which require a number of proteins that are translocated in an SRP-dependent manner (Zhang et al. 2012). It remains to be determined whether eIF5A also affects translation in an amino acid tract-dependent manner similarly to EF-P and whether this is correlated with SRP-dependent translation.

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Conflict of interest We declare that we have no conflict of interest.

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