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Arc1p is required for cytoplasmic confinement of synthetases and tRNA

Marie-Pierre Golinelli-Cohen · Marc Mirande

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Abstract In yeast, Arc1p interacts with ScMetRS and ScGluRS and operates as a tRNA-Interacting Factor (tIF) in *trans* of these two synthetases. Its N-terminal domain (N-Arc1p) binds the two synthetases and its C-terminal domain is an EMAPII-like domain organized around an OB-fold-based tIF. *ARC1* is not an essential gene but its deletion (*arc1⁻* cells) is accompanied by a growth retardation phenotype. Here, we show that expression of N-Arc1p or of C-Arc1p alone palliates the growth defect of *arc1⁻* cells, and that bacterial Trbp111 or human p43, two proteins containing EMAPII-like domains, also improve the growth of an *arc1⁻* strain. The synthetic lethality of an *arc1⁻los1⁻* strain can be complemented with either *ARC1* or *LOS1*. Expression of N-Arc1p or C-Arc1p alone does not complement an *arc1⁻los1⁻* phenotype, but coexpression of the two domains does. Our data demonstrate that Trbp111 or p43 may replace C-Arc1p to complement an *arc1⁻los1⁻* strain. The two functional domains of Arc1p (N-Arc1p and C-Arc1p) are required to get rid of the synthetic lethal phenotype but do not need to be physically linked. To get some clues to the discrete functions of N-Arc1p and C-Arc1p, we targeted ScMetRS or tIF domains to the nuclear compartment and analyzed their cellular localization by using GFP fusions, and their ability to sustain growth. Our results are consistent with a model according to which Arc1p is a bifunctional protein

involved in the subcellular localization of ScMetRS and ScGluRS via its N-terminal domain and of tRNA via its C-terminal domain.

Keywords Methionyl-tRNA synthetase · Arc1p · p43 · tRNA-Interacting Factor · Subcellular organization

Introduction

Aminoacyl-tRNA synthetases catalyze the formation of an ester bond between a specific amino acid and the 3'-end of a cognate tRNA [1]. This family of 20 enzymes has to decode the genetic information into amino acids with high fidelity and protein-tRNA interactions play a key role in this process. Prokaryotic synthetases are built of two major functional domains constituting the catalytic core: the catalytic domain, which contains the active site and interacts with the acceptor arm of the tRNA molecule; the second domain generally interacts with the anticodon domain of the tRNA [2, 3]. Many eukaryotic enzymes possess additional domains appended to the N- or C-terminal end of the catalytic core [4]. Some of them are hydrophobic and are involved in protein-protein interactions within a unique multisynthetase complex found only in higher eukaryotes [5]; when others are cationic and interact non-specifically with tRNAs (tRNA-Interacting Factor, tIF). In some cases, tIF domains act as cofactors in *cis* to the catalytic domain of a synthetase. This is the case of the N-terminal domain of mammalian LysRS [6], the repeat domain of human MetRS [7], the EMAPII-like (endothelial monocyte-activating polypeptide II) domain of plant

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MetRS [8]. The EMAPII domain was first identified in human p43, a non-synthetase protein associated with the multisynthetase complex [9] and is also found in yeast Arc1p, which makes a complex with yeast MetRS (*ScMetRS*) and GluRS (*ScGluRS*) [10].

In the present study, we looked at three different proteins containing EMAPII-like domains (Arc1p, p43, or Trbp111) from three different types of organisms (lower and higher eukaryotes and prokaryotes). Arc1p from *Saccharomyces cerevisiae* can be divided into three distinct domains (Fig. 1). The N-terminal domain (N-Arc1p) interacts with the N-terminal domains of *ScMetRS* and of *ScGluRS* [11–13]. The C-terminal domain (C-Arc1p, EMAPII-like domain) binds non-specifically tRNAs (tIF domain). The presence of the M domain (M-Arc1p) improves the affinity of C-Arc1p for tRNAs [10, 11]. The association of Arc1p with *ScMetRS* and *ScGluRS* improves the catalytic efficiency of the two synthetases by lowering their K_m value for tRNAs [11, 13]. The EMAPII-like domain of Arc1p acts as a cofactor in *trans* for the two synthetases.

p43 is one of the three auxiliary proteins associated with nine aminoacyl-tRNA synthetases in a multi-enzyme complex ubiquitous to higher eukaryotes [14]. As Arc1p, p43 from *Homo sapiens* can be divided into three distinct domains (Fig. 1). The C-terminal domain (C-p43, EMAPII domain) is capable of binding tRNAs (tIF domain) [9]. The N-terminal domain (N-p43) has no similarity of sequence with N-Arc1p and is involved in the interaction of p43 with the multisynthetase complex. It is not yet proven that p43 can act as a cofactor in *trans* for a synthetase and stimulate its activity [15].

Finally, Trbp111 from *Aquifex aeolicus* presents high similarity of sequence with the N-terminal end of the EMAPII-type domain (Fig. 1) and binds tRNAs without specificity of sequence (tIF domain) [16].

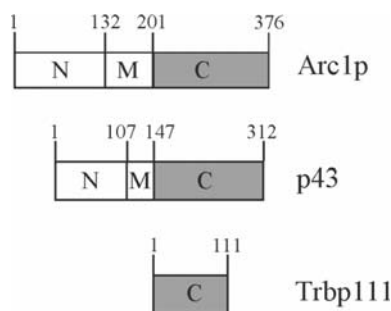


Fig. 1 Schematic representation of the sequences of *S. cerevisiae* Arc1p, of *H. sapiens* p43, and of *A. aeolicus* Trbp111. Grey bars represent the OB-fold domain of the EMAPII-like regions of the three proteins

Trbp111 binds the convex side of the tRNA and may form a ternary complex with the synthetase [17]. The common point between these three proteins is the presence of an EMAPII-type domain capable of binding tRNAs non-specifically.

Previously, we showed that fusion proteins between the catalytic core of a synthetase (*MetRS* from *Oryza sativa*) and a tIF domain (C-Arc1p, C-p43) could restore cell viability of a yeast strain carrying a deletion of the gene coding for *ScMetRS* (*mes1*⁻). Thus, tIF domains may act indifferently in *trans* (interaction between a synthetase and a protein carrying a tIF domain) or in *cis* (fusion of *OsMetRS* with C-Arc1p or C-p43) of the catalytic core of a synthetase. Unexpectedly, p43 or Arc1p could restore or improve growth even if they do not interact with *MetRS*. These results established that Arc1p/p43 is able to sustain growth of a yeast strain independently of its function of tIF for *MetRS* and *GluRS* and suggested that Arc1p might have another function in addition to its role of tIF in *trans* for the two synthetases.

Here, we looked at the effects of the expression of separate domains of Arc1p on the growth of *arc1*⁻ cells. Expression of N-Arc1p could palliate the growth defect of the *arc1*⁻ strain and co-expression of N-Arc1p with C-Arc1p or with another tIF domain restored viability of *arc1*⁻*los1*⁻ cells. The cytoplasmic sequestration of *ScMetRS* fused to a nuclear localization signal was increased by expression of N-Arc1p. We propose a model according to which Arc1p has a dual function: it would be involved in the subcellular localization of *ScMetRS* and *ScGluRS* via its N-terminal domain and of tRNAs via its C-terminal domain.

Materials and methods

DNA manipulations, yeast strains, and media

All recombinant DNA manipulations were carried out using standard protocols [18]. Restriction endonucleases and DNA modification enzymes were purchased from either Roche, or New England Biolabs. Nucleotide sequences were determined by automatic sequencing (MWG Biotech). The following previously described plasmids were used: pRS314 (*CEN/ARS, TRP1*), pRS315 (*CEN/ARS, LEU2*), pRS425 (2 μ , *LEU2*) [19], pJG4-5 (2 μ , *TRP1*) [20], pEGFP-N1 (Clontech; Kitts, PA), pRS314-pPGK-tPGK (*CEN/ARS, TRP1*), pRS315-pPGK-tPGK (*CEN/ARS, LEU2*), pJG4-5-pPGK-tPGK (2 μ , *TRP1*) [21], pRS316-URA3-*MetRS* (*CEN/ARS, URA3*) [12] and pUN100-MES1 (*CEN/ARS, LEU2*) [10] expressing

S. cerevisiae MetRS (*ScMetRS*), pUN100-Arc1 (*CEN/ARS, LEU2*) expressing *S. cerevisiae* Arc1p (Arc1p; GenBank GI 1620459) [10], and pRS314-MOs expressing *O. sativa* MetRS (*OsMetRS*; GenBank GI 4091007) [21]. The yeast strains used in this study are: *arc1⁻los1⁻* (**a**, *los1::HIS3, arc1::HIS3, ade2, leu2, ura3, trp1, his3, pHT4467-URA3-ADE3-LOS1*) [10], *arc1⁻mes1⁻* (**a**, *mes1::HIS5, arc1::HIS3, ade2, leu2, ura3, trp1, his3, pRS316-URA3-MetRS*) [12]. Yeast cells were transformed using the lithium chloride method [22] and were grown in rich YPG medium (0.5% (w/v) yeast extract, 0.5% (w/v) Bacto peptone, 3% (w/v) glucose) or in minimal YNB medium (0.7% (w/v) yeast nitrogen base without amino acid, 2% (w/v) glucose) containing the necessary amino acids, and bases except those used for selection. Yeast growth studies and complementation assays were performed as described previously [21]. Expression of GFP-containing proteins, C-p43-containing proteins and *ScMetRS* were checked by Western blotting using antibodies raised against GFP (Clontech), against the C-terminal domain of p43 [23], or against *ScMetRS* (gift from Franco Fasiolo).

Construction of yeast expression vectors

ScMetRS, *OsMetRS*, p43 and its isolated domains, and Trbp111 were expressed in yeast under the control of the PGK promoter. Arc1p and its isolated domains were expressed under the control of the natural Arc1p promoter except in pJG4-5-Arc1p and pJG4-5-N-Arc1p (pPGK promoter). The nuclear localization signal (NLS) sequence from the commercially available pJG4-5 plasmid with a CATA sequence before the starting ATG was inserted into the *Bgl*III site of pRS314-pPGK-tPGK by hybridization of two oligonucleotides (pRS314-NLS). The green fluorescent protein (GFP) gene was PCR-amplified using pEGFP-N1 as template, *Bgl*III and *Bam*HI sites were introduced at the 5'- and 3'-ends of the sequence, respectively. After *Bam*HI/*Bgl*III digestion, the cassette was introduced into the *Bgl*III site of pRS314-pPGK-tPGK (pRS314-GFP).

Cloning of Trbp111, *ScMetRS*, p43 domains, and Arc1p domains in yeast expression vectors

Gene coding for *A. aeolicus* Trbp111 (GenBank access number GI2983087) was amplified using ptrbp111 [16] (gift from Paul Schimmel) as template and *Bam*HI sites were introduced at both ends of the sequence. The PCR product was digested by *Bam*HI and introduced into the *Bgl*III site of pRS314-pPGK-tPGK and of

pJG4-5-pPGK-tPGK (pRS314-Trbp111, pJG4-5-Trbp111). *ScMetRS* gene (GenBank access number GI3923) was excised from pUN100-MetRS by *Bam*HI digestion and introduced into the *Bgl*III site of pJG4-5-pPGK-tPGK and of pRS314-pPGK-tPGK (pJG4-5-*ScMetRS*, pRS314-*ScMetRS*). pRS315-*ScMetRS* was constructed by in vivo recombination in yeast between pRS314-*ScMetRS* and pRS315-pPGK-tPGK. The *Bam*HI/*Xba*I fragments of pTRE2/p43^{Arf}Hs and of pTRE2/p43^{EMAPII}Hs (V. Shalak & M. Mirande, unpublished data) were inserted into the *Bgl*III site of pRS314-pPGK-tPGK and of pJG4-5-pPGK-tPGK (pRS314-(M+C)-p43, pJG4-5-(M+C)-p43, pRS314-C-p43, pJG4-5-C-p43). pRS314-N-p43 and pRS315-N-p43 expressing the N domain of p43 fused to an His-Tag at the N-terminal end were constructed by insertion of the *Nco*I/*Bsp*I fragment of pET28b/N-p43Hs (V. Shalak & M. Mirande, unpublished data) into the *Bgl*III sites of pRS314-pPGK-tPGK and of pRS315-pPGK-tPGK. pRS314-ARC1ΔC, pRS315-ARC1ΔC expressing (N+M)-Arc1p, pRS315-ARC1N, pRS314-ARC1N expressing N-Arc1p, pRS135-ARC1C, pRS425-ARC1C expressing C-Arc1p, pRS315-ARC1ΔN, pRS425-ARC1ΔN expressing (M+C)-Arc1p are generous gifts from Kyriaki Galani, George Simos and Ed Hurt. The sequences coding for Arc1p and N-Arc1p were PCR-amplified and *Bam*HI sites were introduced at both ends. After *Bam*HI digestion, cassettes were introduced into the *Bgl*III site of pJG4-5-pPGK-tPGK (pJG4-5-Arc1p, pJG4-5-N-Arc1p).

Cloning of NLS-X, X-GFP, and NLS-X-GFP genes in yeast expression vectors

The sequences coding for Trbp111, C-Arc1p, (M+C)-p43, and *ScMetRS* were PCR-amplified without the starting ATG and *Bam*HI (Trbp111, C-Arc1p) or *Bgl*III ((M+C)-p43, *ScMetRS*) sites were introduced at both ends of the sequences. After *Bam*HI or *Bgl*III digestion, cassettes were inserted into the *Bgl*III site of pRS314-NLS to construct pRS314-NLS-X plasmids. pEGP-N1-p43^{Arf} plasmid (V. Shalak & M. Mirande, unpublished data) expressing (M+C)-p43 protein fused to the GFP sequence was digested by *Eco*RI and *Eag*I and the cassette was inserted into the *Bgl*III site of pRS314-pPGK-tPGK (pRS314-(M+C)-p43-GFP). Sequences coding for Trbp111, C-Arc1p, *ScMetRS*, for the same proteins fused to the NLS peptide, and for NLS-(M+C)-p43 were PCR-amplified without Stop codon and with *Bam*HI sites at both ends. After digestion, cassettes were inserted into the *Bgl*III site of pRS314-GFP vector to construct pRS314-X-GFP and pRS314-NLS-X-GFP plasmids.

Expression and purification of HisTag-Arc1p and HisTag-N-Arc1p

The sequences expressing Arc1p and N-Arc1p were PCR-amplified using pUN100-Arc1p as template and *NcoI/XhoI* sites were introduced at the 5'- and the 3'-ends, respectively. After *NcoI/XhoI* digestion, cassettes were introduced into the *NcoI/XhoI* sites of pET28b vector to construct pET28b-Arc1p and pET28b-N-Arc1p plasmids. Proteins were expressed in *E. coli* BL21(DE3) and purified as described [23]. Proteins concentrations were determined by using calculated absorption coefficients of 0.58 and 1.07 A_{280} units $\text{mg}^{-1} \text{cm}^2$, respectively for Arc1p and N-Arc1p.

Purification of ScMetRS

ScMetRS was expressed in *arc1⁻mes1⁻* cells under the strong constitutive PGK promoter using the multicopy pJG4-5-ScMetRS (2 μ , *TRP1*) plasmid. Cells were grown at 28°C in 3 l of YPG supplemented with adenine (40 $\mu\text{g/l}$) to an $A_{600 \text{ nm}}$ of 8. Cells were washed, resuspended in 24 ml of extraction buffer (100 mM Tris-HCl pH 8.0, 100 mM KCl, 10 mM MgCl_2 , 1 mM EDTA, 10% glycerol, 10 mM β -mercaptoethanol), and lysed in an Eaton Press at 6,000 Psi. The lysate was diluted by addition of 50 ml of extraction buffer containing 1 mM DFP and centrifuged at 50,000g for 15 min at 4°C to remove cell debris. Following precipitation of nucleic acids with Polymin P at 0.2% and centrifugation at 50,000g for 15 min at 4°C, the clear supernatant was applied to a Q-Sepharose FF column (Amersham Pharmacia Biotech., 16 \times 224 mm) equilibrated in 20 mM Tris-HCl pH 7.0, 10 mM β -mercaptoethanol, 100 mM KCl, 10% glycerol, and eluted with a 900 ml linear gradient of KCl from 100 to 400 mM. Fractions containing ScMetRS (eluted at ~200 mM KCl) were dialyzed against 50 mM potassium phosphate pH 7.5, 10 mM β -mercaptoethanol, 10% glycerol, loaded on a Ceramic HA (Biorad, 16 \times 177 mm), and eluted with a 500 ml linear gradient of potassium phosphate from 50 to 180 mM. Fractions containing ScMetRS were dialyzed against 20 mM Tris-HCl pH 7.0, 10 mM β -mercaptoethanol, 10% glycerol, loaded on a Mono Q HR 5/5 (Amersham Pharmacia Biotech., 1 ml) equilibrated in 20 mM Tris-HCl pH 7.0, 100 mM KCl, 10 mM β -mercaptoethanol, 10% glycerol, and eluted with a 50 ml linear gradient of KCl from 100 to 400 mM. Fractions containing ScMetRS (eluted at ~150 mM KCl) were dialyzed against 25 mM potassium phosphate pH 7.5, 55% glycerol, 2 mM dithiothreitol, and stored at -20°C. Protein concentration was determined

by using a calculated absorption coefficient of 1.32 A_{280} units $\text{mg}^{-1} \text{cm}^2$.

Sedimentation equilibrium

Ultracentrifugation experiments were conducted as described previously [24] in a Beckman Optima XL-A analytical ultracentrifuge using an An 60 Ti rotor and a double-sector cell with a path length of 12 mm. Equilibrium was verified from the superimposition of duplicate scans. The experimental sedimentation equilibrium data were fitted to a model for a single homogeneous species following the equation:

$$c(r) = c(r_{\text{ref}}) \exp\{[M_r(1 - \bar{v}\rho)\omega^2/2RT](r^2 - r_{\text{ref}}^2)\}$$

where $c(r)$ is the protein concentration at radial position r , $c(r_{\text{ref}})$ is the concentration of the protein at an arbitrary reference radial distance r_{ref} , M_r is the molecular mass, \bar{v} is the partial specific volume of the solute (0.734 for Arc1p at 4°C), ρ is the density of the solvent, ω is the angular velocity of the rotor, and R and T are the molar gas constant and the absolute temperature, respectively.

Confocal microscopy

Cells were grown overnight to an optical density at 600 nm of 0.3–1 at 28°C in YNB medium containing all the necessary amino acids and bases necessary for growth. Cells were added into 8-well Lab-Tek II chambers (Nalge Nunc International) and immediately observed by confocal laser scanning microscopy using a Leica TCS SP2 confocal microscope.

Aminoacylation assays

Initial rates of tRNA aminoacylation were measured at 25°C in 0.1 ml of 20 mM imidazole-HCl pH 7.5, 150 mM KCl, 0.5 mM DTT, 5 mM MgCl_2 , 3 mM ATP, 52 μM ^{14}C -labeled methionine (NEN, 58 Ci/mol) and saturating amounts of tRNA (except otherwise stated), as previously described [8]. Brewer's yeast tRNA^{Met} partially purified on a BD cellulose resin with a NaCl gradient (0.45–1 M) in the presence of 10 mM MgSO_4 (methionine acceptance of 240 pmol/ A_{260}) was used as tRNA substrate. Preincubation of ScMetRS with Arc1p or N-Arc1p was performed at 25°C for 10 min in 20 mM Tris-HCl pH 7.0, 50 mM NaCl, 2 mM DTT, 10% glycerol. Proteins were diluted in 10 mM Tris-HCl pH 7.5, 10 mM β -mercaptoethanol, and 4 mg/ml BSA just before use.

Results

Expression of isolated domains of Arc1p stimulates growth of *arc1⁻* cells

Deletion of *ARC1*, the gene coding for Arc1p, is not lethal for yeast but introduces a growth defect which is larger at non-optimal temperatures (20°C or 37°C) than at physiological growth temperature (28°C) [10, 21]. Expression of Arc1p from a centromeric plasmid could palliate the growth defect phenotype of *arc1⁻* strain. Complementation assays suggested that the continuity of Arc1p is not a prerequisite for its activity [11]. In that connection, we first wanted to determine whether the isolated domains of Arc1p could restore normal growth of *arc1⁻* strain. Isolated domains of Arc1p (N-Arc1p, C-Arc1p, (N+M)-Arc1p, (M+C)-Arc1p, Fig. 1) were expressed in an *arc1⁻mes1⁻* strain transformed by a centromeric plasmid expressing *ScMetRS* (*arc1⁻* phenotype). For clarity, the *arc1⁻mes1⁻* strain transformed with a centromeric plasmid expressing ‘protein X’ will be noted *arc1⁻mes1⁻(protein X)*. Similar notation will be used for the *arc1⁻los1⁻* strain, carrying gene disruptions of *ARC1* and *LOS1*.

At 20°C, expression of C-Arc1p slightly improved the growth of *arc1⁻mes1⁻(ScMetRS)* cells when expression of N-Arc1p had much larger effects (Fig. 2A). Expression of Arc1p or of only N-Arc1p had very similar effects on the growth of the cells (Fig. 2A). Similar results were obtained at 28°C (Fig. 2B). At 37°C, expression of C-Arc1p had no effect on the growth of the cells when expression of N-Arc1p clearly stimulated it (Fig. 2C). These results show that

N-Arc1p and C-Arc1p are likely to be involved in different mechanisms, and suggest that the growth limiting factors are different at 20°C and 37°C. Similar results were obtained when (N+M)-Arc1p and (M+C)-Arc1p were expressed instead of N-Arc1p and C-Arc1p (Fig. 2A–C). Expression of C-Arc1p or of (M+C)-Arc1p from 2 μ plasmids gave similar results (data not shown).

Expression of tIF domains from different origins stimulated the growth of *arc1⁻* cells

N-Arc1p interacts with *ScMetRS* and *ScGluRS* and is responsible for the assembly of the ternary complex [11–13]. Thus, the effect of the expression of N-Arc1p in an *arc1⁻* background could be the consequence of its association with the two synthetases. Previously, we have shown that p43 from *H. sapiens* (Fig. 1) does not interact with *ScMetRS* and with *ScGluRS* [21]. As expected, expression of N-p43 did not improve the growth of *arc1⁻mes1⁻(ScMetRS)* strain at 37°C (Fig. 3A), 20°C, and 28°C (data not shown).

By contrast, C-Arc1p does not associate with *ScMetRS* and *ScGluRS* but could also palliate the absence of Arc1p at 20°C (Fig. 2A). C-Arc1p is able to interact non-specifically with tRNAs (tIF domain) [11]. The C-terminal domain of p43 and the protein Trbp111 from *A. aeolicus* possess high similarities of sequence with C-Arc1p and are also able to interact non-specifically with tRNAs [9, 16]. To test if the effects observed when C-Arc1p was expressed were essentially due to its ability to interact with tRNAs, we looked at the replacement of Arc1p by p43, C-p43, (M+C)-p43, or Trbp111. As observed with C-Arc1p (Fig. 2A),

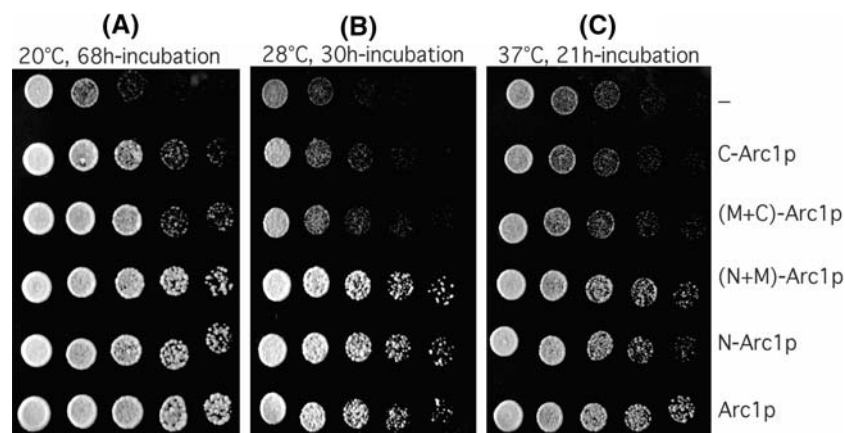


Fig. 2 Expression of N-Arc1p can palliate the growth defect of *arc1⁻mes1⁻(ScMetRS)* strain. When specified, cells were transformed by a centromeric plasmid expressing Arc1p or a domain of Arc1p. Cells were grown in minimal medium, serially diluted,

and aliquots were spotted onto YPG plates containing adenine and tryptophan. Plates were incubated at the temperature and for the time indicated. Plates are representative of at least two independent experiments

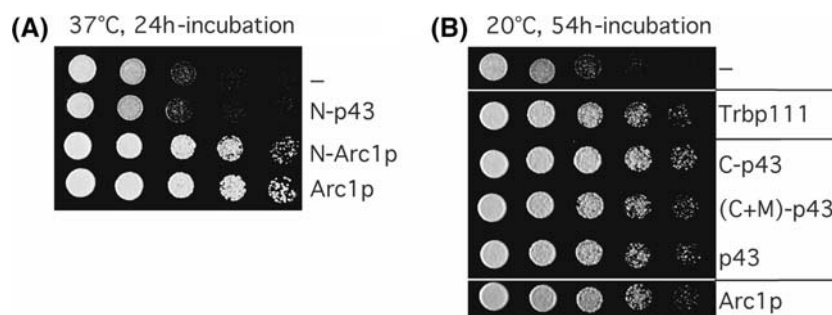


Fig. 3 Expression of tIF domains of different origins improves the growth of *arc1⁻mes1⁻(ScMetRS)* strain. When specified, cells were transformed by a centromeric plasmid expressing N-p43, N-Arc1p, or Arc1p (panel **A**), or expressing a tIF domain from different origins (Arc1p, p43, Trbp111) (panel **B**). Cells were

grown in minimal medium, serially diluted, and aliquots were spotted onto YPG plates containing adenine and tryptophan. Plates were incubated at the temperature and for the time indicated. Plates are representative of at least two independent experiments

expression of these tIFs was also able to improve the growth of *arc1⁻mes1⁻(ScMetRS)* at 20°C (Fig. 3B). Thus, expression of another tIF (p43, C-p43, (M+C)-p43, Trbp111) may functionally replace C-Arc1p in vivo. These tIFs cannot interact with *ScMetRS* and *ScGluRS*. The improvement of the growth can only be linked to their ability to interact with tRNAs but does not require their association with the synthetases.

Co-expression of (N+M)-Arc1p with a tIF domain can restore viability of *arc1⁻los1⁻* cells

Yeast *Los1p* is involved in tRNA export from the nucleus to the cytoplasm [25, 26]. Disruption of *LOS1* does not cause any apparent growth defect, suggesting that export of tRNAs still continues in the absence of *Los1p* at least to an extent that supports normal cell growth [25]. When the single deletion of chromosomal *ARC1* or *LOS1* is not lethal for yeast, the double deletion is lethal; expression of Arc1p from a centromeric plasmid restores viability of *arc1⁻los1⁻* cells.

Even if expression of C-Arc1p or of N-Arc1p improves the growth of *arc1⁻* cells (Fig. 2), expression of isolated domains of Arc1p (N-, C-, (N+M)-, (M+C)-Arc1p) could not restore viability of *arc1⁻los1⁻* cells, but co-expression of C-Arc1p (or of (M+C)-Arc1p) with N-Arc1p could rescue *arc1⁻los1⁻* cells [11] (Fig. 4A). Thus, when each isolated domain of Arc1p cannot restore the viability of *arc1⁻los1⁻* cells, co-expression of N-Arc1p with C-Arc1p was able to restore it. Co-expression of N-p43 with C-Arc1p could not restore the viability of *arc1⁻los1⁻* cells (Fig. 4A).

Expression of tIF-containing proteins could palliate the *arc1⁻* phenotype. Interestingly, co-expression of (N+M)-Arc1p with (M+C)-p43 (Fig. 4B), with p43 (data not shown) or with Trbp111 (Fig. 4C) could also rescue *arc1⁻los1⁻* cells. Similar results were obtained

when N-Arc1p was expressed instead of (N+M)-Arc1p but complementation was less straightforward (Fig. 4B, C). Addition of the M-domain may stabilize N-Arc1p in vivo. Complementation was improved when N-Arc1p was co-expressed with a tIF-containing protein expressed from a multicopy plasmid (data not shown). These results clearly establish that Arc1p is a bifunctional protein. The two functional domains of Arc1p (N-Arc1p and a tIF domain) do not need to be physically linked and can be provided as individual proteins.

N-Arc1p does not affect the catalytic activity of *ScMetRS*

Arc1p and N-Arc1p were expressed in *E. coli* as fusion proteins with an His-Tag at the C-terminal end and proteins were purified as described (Materials and methods). Arc1p was subjected to equilibrium sedimentation analysis in 50 mM potassium phosphate pH 7.5, 10% glycerol, and 1 mM DTT (Fig. 5). Experimental data could be fitted to a monodisperse solute with M_r of $43,445 \pm 1,000$, as compared to a theoretical mass of 42,084 for a monomer. These data contrast with an earlier study showing that Arc1p is eluted from a Superdex 200 gel filtration column as an entity of apparent molecular mass of 85 kDa, suggesting that it may form a dimer in solution [27]. The later approach does not directly address the mass of a protein but is related to its Stokes radius. Thus, we can conclude that Arc1p is a monomer with an elongated shape. Arc1p and *ScMetRS* [28] are monomers in solution, which is consistent with the formation of a 1:1 complex. In the case of N-Arc1p, it formed a polydisperse solute and we could not find adequate buffer conditions for analysis.

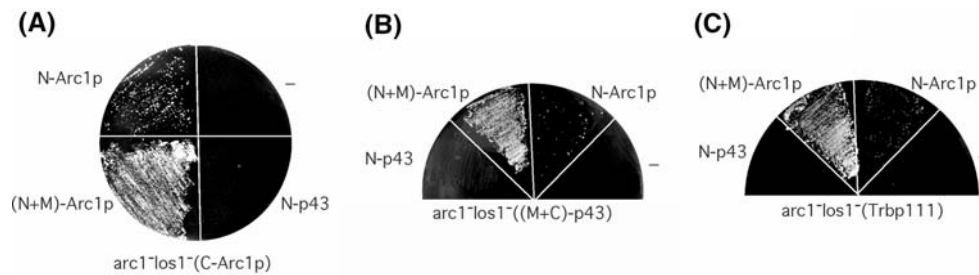


Fig. 4 Co-expression of N-Arc1p or of (N+M)-Arc1p with a tIF domain can restore the viability of *arc1⁻los1⁻* cells. *arc1⁻los1⁻* strain was co-transformed by a centromeric plasmid expressing C-Arc1p (panel **A**), (M+C)-p43 (panel **B**) or Trbp111 (panel **C**),

and by a second centromeric plasmid expressing N-Arc1p, (N+M)-Arc1p or N-p43 when specified. Tests of complementation were conducted. Plates were incubated for four days at 28°C and are representative of at least two independent experiments

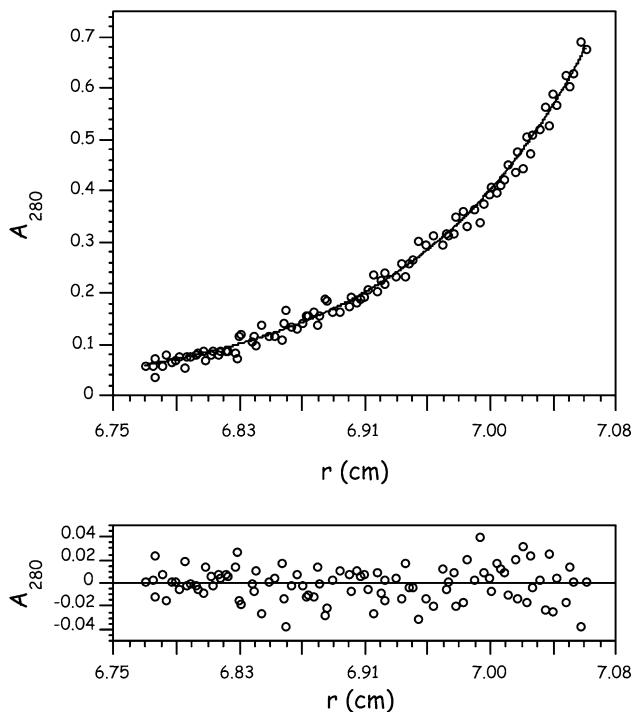


Fig. 5 Arc1p is a monomer in solution. Arc1p (initial concentration of 17 μ M) was analyzed by equilibrium sedimentation at 15,000 rpm in 50 mM potassium phosphate pH 7.5, 10% glycerol, and 1 mM DTT at 4°C. Experimental values (○) were fitted (—) to a monodisperse 43,445 Da solute. The residuals are indicated

Because the N-terminal domain of Arc1p is involved in the interaction of Arc1p with ScMetRS, association of N-Arc1p with ScMetRS might affect the catalytic efficiency of the synthetase by inducing a conformational change of the active site. However, it has been reported that Arc1p stimulates the activity of ScMetRS whereas N-Arc1p does not [11]. We also observed that Arc1p stimulates the aminoacylation activity of ScMetRS in vitro (with a 3- to 4-fold increase in k_{cat}/K_m), but addition of N-Arc1p had no effect on the catalytic activity of ScMetRS (Fig. 6).

N-Arc1p keeps ScMetRS in the cytoplasm

N-Arc1p does not stimulate the catalytic activity of ScMetRS in vitro. We investigated the possibility that N-Arc1p may have a role in the cellular localization of ScMetRS and thus may affect the viability of an *arc1⁻mes1⁻* strain. Indeed, formation of the ternary complex between Arc1p, ScMetRS, and ScGluRS keeps the three proteins into the cytoplasm [29].

The cytoplasm of eukaryotic cells is believed to be highly organized [30, 31]. We surmised that N-Arc1p could be involved in the cytoplasmic compartmentalization of MetRS at the sites of active protein synthesis. To analyze the effect of N-Arc1p on the cytoplasmic

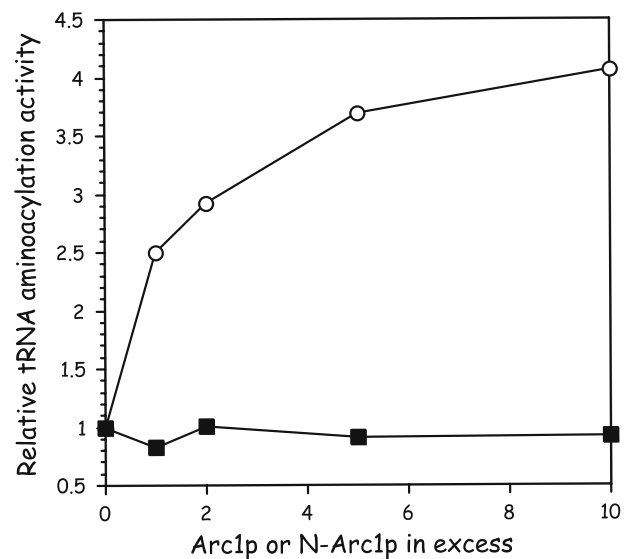


Fig. 6 Arc1p, but not N-Arc1p, stimulates ScMetRS activity. ScMetRS (2.5 μ M) was pre-incubated with Arc1p (○) or N-Arc1p (■) for 10 min at 25°C. Proteins were diluted and immediately subjected to the aminoacylation assay. Reactions were performed at 25°C for 10 min in the presence of 1.13 nM ScMetRS and of limiting amounts of tRNA (420 nM tRNA^{Met})

compartmentalization of *ScMetRS*, we chose to address *ScMetRS* to another cellular compartment. We introduced a nuclear localization signal (NLS) at the N-terminus of *ScMetRS*. When expression of *ScMetRS* restored viability to *arc1⁻mes1⁻* cells, expression of the synthetase fused to the NLS could not rescue the strain (Fig. 7A). Similarly, *OsMetRS* rescued *arc1⁻mes1⁻* cells but NLS-*OsMetRS* did not (Fig. 7B). When NLS-*ScMetRS* was co-expressed with Arc1p, the cell viability of *arc1⁻mes1⁻* was clearly restored (Fig. 7A). Interestingly, co-expression of NLS-*ScMetRS* with N-Arc1p only gave similar results even if complementation was less efficient (Fig. 7A). Co-expression of Arc1p or N-Arc1p with NLS-*OsMetRS*, two proteins that cannot form stable complexes with *OsMetRS* [21], could also not restore cell viability (Fig. 7B). These data suggest that interaction of the N-terminal domain of Arc1p with *ScMetRS* triggers its proper subcellular localization and is essential for complementation.

To ascertain that N-Arc1p restored viability of an *arc1⁻mes1⁻* strain by triggering cytoplasmic sequestration of *ScMetRS*, we analyzed the cellular localization of *ScMetRS* by confocal microscopy. We expressed the synthetase tagged with the GFP at its C-terminal end (*ScMetRS*-GFP). The expression and

the stability of the fusion protein were checked by Western blot. Clearly, the synthetase was only cytoplasmic (Fig. 7C). When an NLS was fused to the synthetase (NLS-*ScMetRS*-GFP), its localization was only nuclear (Fig. 7C) (note that wild type *ScMetRS*, without a GFP-Tag, is also present in these strains). When Arc1p was co-expressed with NLS-*ScMetRS*-GFP, the synthetase was mostly in the nucleus but a clear signal was also detected in the cytoplasm (Fig. 7C). Similarly, when N-Arc1p was co-expressed with NLS-*ScMetRS*-GFP, some of the synthetase was also observed in the cytoplasm (Fig. 7C). These results are consistent with the complementation experiments (Fig. 7A) showing that complementation is more effective in the presence of Arc1p, than when only N-Arc1p is added. Clearly, the formation of a complex between NLS-*ScMetRS* and N-Arc1p (or Arc1p via its N-terminal domain) allows to sequester in the cytoplasm some of the synthetase fused to a strong NLS and thus restores cell viability. NLS-*OsMetRS* cannot form a complex with N-Arc1p or Arc1p, which would keep the synthetase in the cytoplasm and, consequently, co-expression of the two proteins cannot restore the viability of *arc1⁻mes1⁻* cells (Fig. 7B).

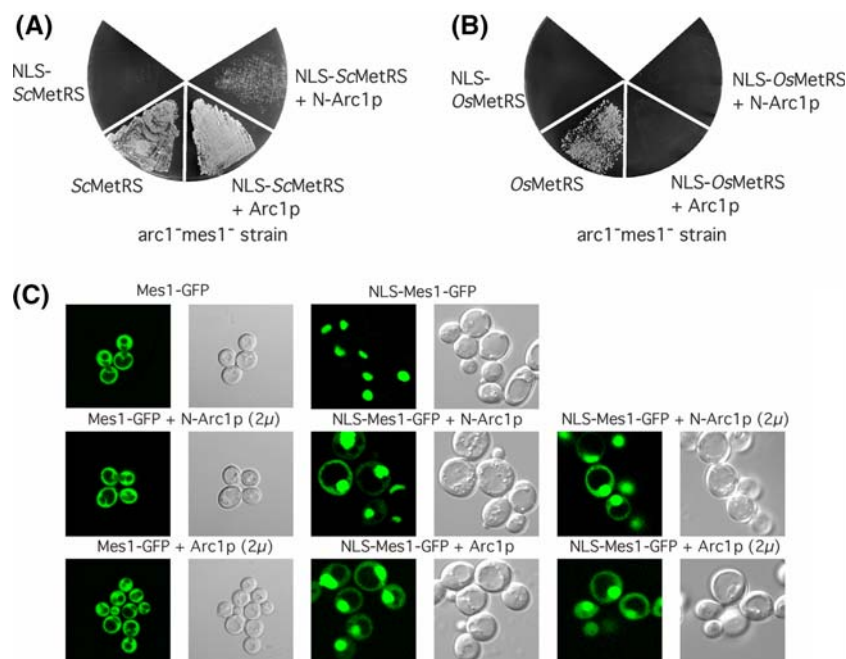


Fig. 7 N-Arc1p keeps *ScMetRS* in the cytoplasm and restores cell viability. (Panels **A**, **B**) *arc1⁻mes1⁻* strain was transformed by a plasmid expressing *ScMetRS* or NLS-*ScMetRS* (panel **A**), or by a plasmid expressing *OsMetRS*, or NLS-*OsMetRS* (panel **B**). When specified, cells were co-transformed by a second plasmid expressing Arc1p or N-Arc1p. Tests of complementation were

conducted. Plates were incubated for four days at 28°C and are representative of at least three independent experiments. (Panel **C**) Localization by confocal microscopy of *ScMetRS*-GFP (Mes1-GFP) and NLS-*ScMetRS*-GFP (NLS-Mes1-GFP) in *arc1⁻mes1⁻* cells in the absence and in the presence of N-Arc1p or of Arc1p

tIF domains have to be cytoplasmic for cell viability

Co-expression of a tIF domain with N-Arc1p is strictly necessary to rescue the *arc1⁻los1⁻* strain (Fig. 4). Full-length Arc1p was only observed in the cytoplasm of yeast cells but it is proposed that Arc1p shuttles between the nucleus and the cytoplasm [29]. However, we have seen that a human protein, C-p43, and a bacterial protein, Trbp111, are also able to rescue the *arc1⁻los1⁻* strain and to functionally replace C-Arc1p. Because it is unlikely that C-p43 and Trbp111 have the signal sequences required to efficiently shuttle between the nuclear and cytoplasmic compartments in yeast, we decided to look at the effect of the cellular localization of these tIF-containing proteins on their ability to rescue an *arc1⁻los1⁻* strain.

(M+C)-p43 and Trbp111 were expressed as fusion proteins with GFP in yeast using centromeric plasmid. Their expression and stability were checked by Western blot, and their cellular localization was analyzed by confocal microscopy. All the tIF-containing proteins displayed strictly cytoplasmic patterns (Fig. 8B) and their co-expression with N-Arc1p rescued *arc1⁻los1⁻* cells (Fig. 8A). Then, the tIF-containing proteins were fused to a NLS. NLS-tIF-GFP proteins were only nuclear (Fig. 8B). Interestingly, addition of a NLS to (M+C)-p43 or to Trbp111 impaired their ability to restore cell viability of *arc1⁻los1⁻(N-Arc1p)* (Fig. 8A). Conversely, a construct of NLS-Arc1p could complement the *arc1⁻los1⁻* strain, but it displayed a cytoplasmic localization [29]. Thus, tIF domains have to be cytoplasmic to be able to restore cell viability.

Discussion

Dual function of Arc1p

Arc1p forms a complex with yeast MetRS and GluRS and modulates their aminoacylation efficiency [10, 13]. Here, we show that Arc1p is a monomer in solution, which is consistent with the formation of a 1:1:1 complex between Arc1p, MetRS, and GluRS. The N-terminal domain of Arc1p binds the two synthetases; its C-terminal moiety is a tRNA-binding domain. In the present study, we showed that expression of N-Arc1p stimulates the growth of *arc1⁻* cells with an efficiency similar to that obtained with full-length Arc1p. However, N-Arc1p does not affect the *in vitro* aminoacylation reaction catalyzed by ScMetRS. We investigated the possibility that N-Arc1p may have a role on the cytoplasmic compartmentalization of ScMetRS. To test this possibility, we addressed ScMetRS to another

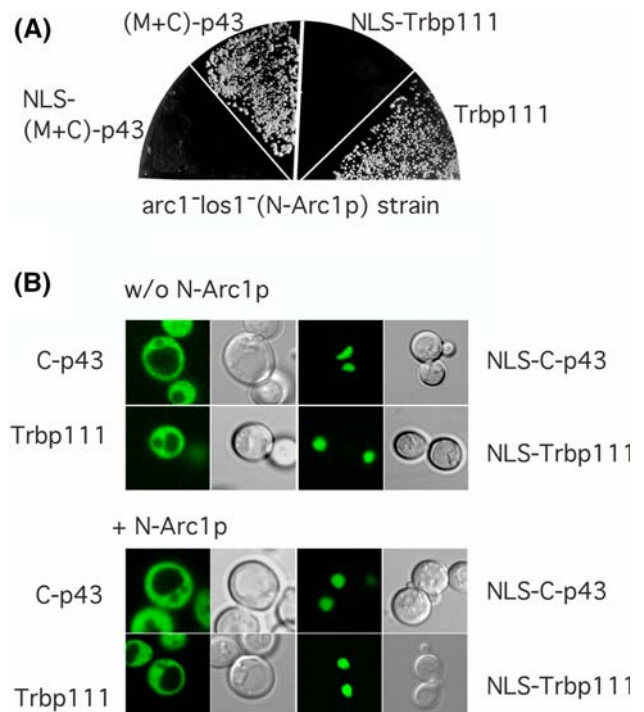


Fig. 8 tIF domains have to be cytoplasmic for cell viability. (panel **A**) *arc1⁻los1⁻* strain expressing N-Arc1p from a centromeric plasmid was co-transformed by a second plasmid expressing Trbp111, (M+C)-p43, or one of these proteins fused to a nuclear localization signal (NLS). Tests of complementation were conducted. Plates were incubated for four days at 28°C and are representative of at least three independent experiments. (panel **B**) Localization by confocal microscopy of tIF-GFP and NLS-tIF-GFP in *arc1⁻los1⁻* cells in the absence and in the presence of N-Arc1p

compartment. When addressed to the nucleus, ScMetRS was not able to rescue *arc1⁻mes1⁻* cells. But, when N-Arc1p (or Arc1p) was co-expressed with NLS-ScMetRS, cell viability was restored and the synthetase was partially recovered in the cytoplasm (5–10%). With the expression system we used, plasmid-born NLS-ScMetRS was expressed at a level 5–10 times higher than that of endogenous MetRS (as measured by MetRS activity in the corresponding yeast extracts). Thus, the cytoplasmic retention of 5–10% of NLS-ScMetRS corresponded to a wild-type level of cytoplasmic MetRS and was sufficient to sustain cell viability. Expression of N-Arc1p is sufficient to impair the NLS-directed translocation of ScMetRS into the nucleus. Because ScMetRS is localized in the cytoplasm, the function of N-Arc1p is probably not restricted to the prevention of translocation of ScMetRS into the nucleus but might be involved in the proper subcellular localization of the synthetase.

We showed previously that p43, the human homolog of Arc1p, may replace Arc1p in yeast even if p43

does not form a complex with *ScMetRS* and *ScGluRS* [21]. This result suggested that the tRNA binding capacity of these two proteins may have a cellular role not directly related to the formation of a complex with the two synthetases. Here, we showed that C-Arc1p, human p43 or C-p43, and *A. aeolicus* Trbp111, which share homologous EMAPII-like domains capable of non-specific interactions with tRNAs, have similar effects in vivo and stimulate the growth of *arc1⁻* cells. Thus, the function of C-Arc1p is directly linked to its ability to interact with tRNA. In an *arc1⁻los1⁻* background, N-Arc1p or C-Arc1p alone could not rescue the strain, but coexpression of N-Arc1p with C-Arc1p or with another tIF domain did. Thus, when nucleocytoplasmic transport of tRNAs is partially defective, the two functions provided by the N- and C-domain of Arc1p are required to sustain growth. The tIF domains are functional in vivo only when expressed in the cytoplasm.

Implications for cellular organization of the translation machinery

In yeast as well as in other eukaryotic cells, because protein synthesis occurs in the cytoplasm, tRNAs have to be efficiently transported from the nucleus, where they are transcribed, to the cytoplasm, where they are aminoacylated and used as substrates in ribosomal protein synthesis. Until very recently, one dogma of cellular biology stipulated that retrograde transport of tRNA from the cytoplasm to the nucleus does not occur. As a consequence of this belief it is generally accepted that tRNA aminoacylation may also occur in the nucleus. Indeed, the presence of aminoacylated tRNAs in the nucleus of yeast has been convincingly reported by several independent studies [32–34].

However, significant amounts of aminoacyl-tRNA synthetases have never been observed in the nucleus. In yeast, analyses of cellular localization of aminoacyl-tRNA synthetases [12] (this study) or of EF1A [33] fused to GFP suggest that components of the protein synthesis machinery are mainly clustered within the cytoplasm. In mammalian cells, the presence of aminoacyl-tRNA synthetases in the nucleus is also a controversial matter. By immunofluorescence microscopy, methionyl-tRNA synthetase has been described as a purely cytoplasmic protein [35, 36] or has been reported to be also localized to the nucleolus [37]. Analysis of the cellular distribution of GFP fusion proteins showed that synthetases [38, 39] (Kaminska and Mirande, unpublished results) and elongation factors [40] are efficiently excluded from the nucleus of human cells. Thus, the steady-state nuclear level of the

components of translation in eukaryotic cells is, at the very least, quite low. If aminoacyl-tRNA synthetases are present within the nuclear compartment, it seems very unlikely that they may sustain efficient aminoacylation of tRNAs.

Our previous results [21] suggested the possibility that the dogma of irreversible nucleocytoplasmic transport of tRNA during the cellular cycle of translation in eukaryotes has to be re-evaluated. We suggested for the first time that another explanation that could fit with all the observations published to date would be to consider the possibility that, after aminoacylation within the cytoplasm, tRNAs may follow a retrograde transport pathway to join the nuclear compartment if adequate conditions for efficient protein synthesis are not fulfilled within the cytoplasm (Fig. 9). Very recently, two independent studies showing that tRNAs do indeed cross the nuclear barrier from the cytoplasm to the nucleus [41, 42] lent credit to our alternative hypothesis.

According to this scheme, if tRNA aminoacylation is defective (ts mutants of aminoacyl-tRNA synthetases [32, 34], amino acid starvation, addition of synthetase inhibitors [33], or absence of the tIF associated in *cis* or in *trans*), if EF1A is limiting (after disruption of *TEF2*, one of the two genes that encode the eukaryotic elongation factor EF1A [33]) or if the ternary complex of aminoacyl-tRNA with GTP and EF1A is unstable (mutations in *TEF1* or *TEF2* genes) it is conceivable to observe nuclear accumulation of deacylated or acylated tRNAs that would not be efficiently sequestered within the cytoplasmic compartment. In addition, after dissociation from the elongating ribosome, tRNA has to be recycled in translation. The tIFs proteins associated in *cis* or in *trans* to eukaryotic synthetases, such as Arc1p or p43, might be involved in preventing the nuclear re-import of tRNAs. Similarly, it was reported that overexpression of EF1A restored growth of a *los1⁻arc1 Δ C* strain [33]. In the cytoplasm, these tRNA-binding proteins may also participate in the process of tRNA export by interacting with the newly exported tRNAs and thus by sequestering them into the cytoplasmic fraction. The localization of Arc1p in the vicinity of the nuclear periphery is consistent with its possible involvement at the cytoplasmic side of the nuclear pore complex [10]. In line with this model, in an *arc1⁻* strain, the two functions of Arc1p are disabled: (i) appropriate subcellular localization of *ScMetRS* and *ScGluRS* via the N-terminal domain of Arc1p and (ii) sequestration of tRNA into the translation process via the C-terminal domain. As the nuclear tRNA export or re-export system is working efficiently, restoration of a single of these functions

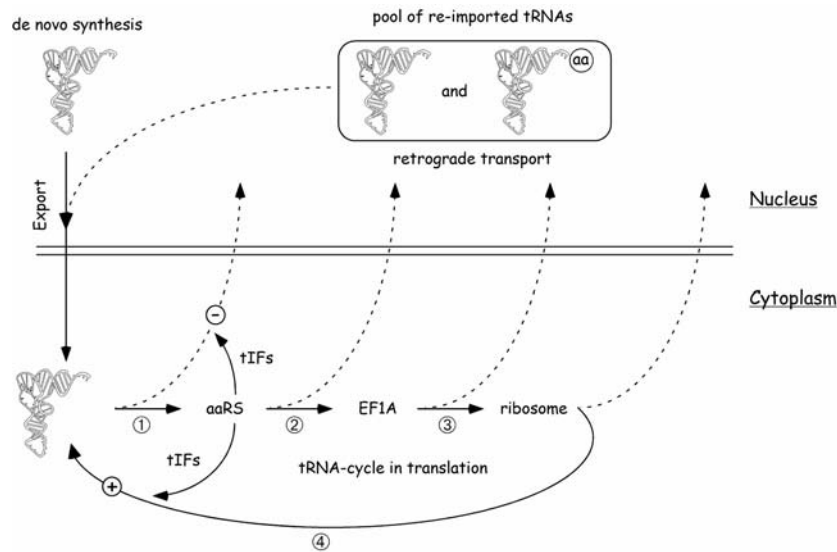


Fig. 9 A schematic view of tRNA shuffling between the nuclear and cytoplasmic compartments. In *S. cerevisiae*, newly synthesized tRNAs are exported from the nucleus via Los1p and other unknown pathways to enter the translation machinery. They are aminoacylated by aminoacyl-tRNA synthetases (①), form a ternary complex with EF1A and GTP (②), are the donor of amino acids for ribosomal protein synthesis (③), and are recycled in the translation process (④). Under some circumstances, e.g. when one of the components of the protein

synthesis machinery is defective, deacylated tRNAs, or aminoacylated tRNAs underwent retrograde transport, and might be subsequently re-exported to the cytoplasm. The tIFs proteins associated in *cis* or in *trans* to aminoacyl-tRNA synthetases (Arc1p in this study) may prevent leakage of tRNAs in the nuclear compartment (–) and stimulate binding of deacylated tRNAs to the synthetase (+) (see text for details)

could be sufficient to support cell growth. In an *arc1⁻los1⁻* background in which tRNA export is also limiting due to the *los1⁻* phenotype, addition of N-Arc1p and of a tRNA sequestering domain would be necessary.

In mammals, several pieces of evidence suggest that tRNA is effectively chaperoned in the translational process to ensure its vectorial handover from one component to the other (that is from aminoacyl-tRNA synthetase to elongation factor, from elongation factor to ribosome, and from ribosome to aminoacyl-tRNA synthetase) without dissociation in the cytoplasmic fluid [30, 31]. Functional interaction between aminoacyl-tRNA synthetases and elongation factor has been described [7, 43–45]. These studies suggest that the limiting step in tRNA aminoacylation could be the dissociation of aminoacylated tRNA from the synthetase and that EF1A may facilitate this step, thus contributing to the vectorial handover of tRNA. Interestingly, in the permeabilized cell system developed by Deutscher and coworkers, exogenously added aminoacyl-tRNAs were poor substrates in protein synthesis [30, 31]. According to the scheme shown Fig. 9, one interesting possibility would be that recycling of tRNA molecules that escape the cytoplasmic

tRNA-chaperoned pathway have to follow the retrograde transport pathway to reach the nucleus and to re-enter the tRNA cycle in translation via the export machinery. Retrograde transport of tRNA could be a salvage pathway by which cells subjected to deleterious growth conditions maintain an important pool of tRNA rapidly mobilizable to resume protein synthesis when favorable conditions for protein synthesis are recovered. The nucleus might be a reservoir for storage and maintenance of a tRNA pool rapidly assimilable into translation.

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References

1. Ibba M, Söll D (2000) Aminoacyl-tRNA synthesis. *Annu Rev Biochem* 69:617–650
2. Buechter DD, Schimmel P (1993) Aminoacylation of RNA minihelices – implications for tRNA synthetase structural design and evolution. *Crit Rev Biochem Mol Biol* 28:309–322
3. Schimmel P, Giegé R, Moras D, Yokoyama S (1993) An operational RNA code for amino acids and possible relationship to genetic code. *Proc Natl Acad Sci USA* 90:8763–8768
4. Mirande M (1991) Aminoacyl-tRNA synthetase family from prokaryotes and eukaryotes: structural domains and their implications. *Prog Nucleic Acid Res Mol Biol* 40:95–142
5. Mirande M, Lazard M, Martinez R, Latreille MT (1992) Engineering mammalian aspartyl-tRNA synthetase to probe structural features mediating its association with the multi-synthetase complex. *Eur J Biochem* 203:459–466
6. Francin M, Kaminska M, Kerjan P, Mirande M (2002) The N-terminal domain of mammalian Lysyl-tRNA synthetase is a functional tRNA-binding domain. *J Biol Chem* 277:1762–1769
7. Kaminska M, Shalak V, Mirande M (2001) The appended C-domain of human methionyl-tRNA synthetase has a tRNA-sequestering function. *Biochemistry* 40:14309–14316
8. Kaminska M, Deniziak M, Kerjan P, Barciszewski J, Mirande M (2000) A recurrent general RNA binding domain appended to plant methionyl-tRNA synthetase acts as a cis-acting cofactor for aminoacylation. *EMBO J* 19:6908–6917
9. Quevillon S, Agou F, Robinson JC, Mirande M (1997) The p43 component of the mammalian multi-synthetase complex is likely to be the precursor of the endothelial monocyte-activating polypeptide II cytokine. *J Biol Chem* 272:32573–32579
10. Simos G, Segref A, Fasiolo F, Hellmuth K, Shevchenko A, Mann M, Hurt EC (1996) The yeast protein Arc1p binds to tRNA and functions as a cofactor for the methionyl- and glutamyl-tRNA synthetases. *EMBO J* 15:5437–5448
11. Simos G, Sauer A, Fasiolo F, Hurt EC (1998) A conserved domain within Arc1p delivers tRNA to aminoacyl-tRNA synthetases. *Mol Cell* 1:235–242
12. Galani K, Grosshans H, Deinert K, Hurt EC, Simos G (2001) The intracellular location of two aminoacyl-tRNA synthetases depends on complex formation with Arc1p. *EMBO J* 20:6889–6898
13. Graindorge JS, Senger B, Tritsch D, Simos G, Fasiolo F (2005) Role of Arc1p in the modulation of yeast glutamyl-tRNA synthetase activity. *Biochemistry* 44:1344–1352
14. Kerjan P, Cerini C, Semeriva M, Mirande M (1994) The multienzyme complex containing nine aminoacyl-tRNA synthetases is ubiquitous from *Drosophila* to mammals. *Biochim Biophys Acta* 21:293–297
15. Guigou L, Shalak V, Mirande M (2004) The tRNA-interacting factor p43 associates with mammalian arginyl-tRNA synthetase but does not modify its tRNA aminoacylation properties. *Biochemistry* 43:4592–4600
16. Morales AJ, Swairjo MA, Schimmel P (1999) Structure-specific tRNA-binding protein from the extreme thermophile *Aquifex aeolicus*. *EMBO J* 18:3475–3483
17. Nomanbhoy T, Morales AJ, Abraham AT, Vortler CS, Giegé R, Schimmel P (2001) Simultaneous binding of two proteins to opposite sides of a single transfer RNA. *Nat Struct Biol* 8:344–348
18. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press
19. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19–27
20. Gyuris J, Golemis E, Chertkov H, Brent R (1993) Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell* 75:791–803
21. Golinelli-Cohen MP, Zakrzewska A, Mirande M (2004) Complementation of yeast Arc1p by the p43 component of the human multisynthetase complex does not require its association with yeast MetRS and GluRS. *J Mol Biol* 340:15–27
22. Ito H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkali cations. *J Bacteriol* 153:163–168
23. Shalak V, Kaminska M, Mitnacht-Kraus R, Vandenabeele P, Clauss M, Mirande M (2001) The EMAPII cytokine is released from the mammalian multisynthetase complex after cleavage of its p43/proEMAPII component. *J Biol Chem* 276:23769–23776
24. Agou F, Waller JP, Mirande M (1996) Expression of rat aspartyl-tRNA synthetase in *Saccharomyces cerevisiae*. Role of the NH₂-terminal polypeptide extension on enzyme activity and stability. *J Biol Chem* 271:29295–29303
25. Hurt DJ, Wang SS, Lin YH, Hopper AK (1987) Cloning and characterization of LOS1, a *Saccharomyces cerevisiae* gene that affects tRNA splicing. *Mol Cell Biol* 7:1208–1216
26. Hopper AK, Schultz LD, Shapiro RA (1980) Processing of intervening sequences: a new yeast mutant which fails to excise intervening sequences from precursor tRNAs. *Cell* 19:741–751
27. Deinert K, Fasiolo F, Hurt EC, Simos G (2001) Arc1p organizes the yeast aminoacyl-tRNA synthetase complex and stabilizes its interaction with the cognate tRNAs. *J Biol Chem* 276:6000–6008
28. Fasiolo F, Gibson BW, Walter P, Chatton B, Biemann K, Boulanger Y (1985) Cytoplasmic methionyl-tRNA synthetase from Bakers' yeast. A monomer with a post-translationally modified N terminus. *J Biol Chem* 260:15571–15576
29. Galani K, Hurt E, Simos G (2005) The tRNA aminoacylation co-factor Arc1p is excluded from the nucleus by an Xpo1p-dependent mechanism. *FEBS Lett* 579:969–975
30. Negrutskii BS, Deutscher MP (1991) Channeling of aminoacyl-tRNA for protein synthesis *in vivo*. *Proc Natl Acad Sci USA* 88:4991–4995
31. Stapulionis R, Deutscher MP (1995) A channeled tRNA cycle during mammalian protein synthesis. *Proc Natl Acad Sci USA* 92:7158–7161
32. Sarkar S, Azad AK, Hopper AK (1999) Nuclear tRNA aminoacylation and its role in nuclear export of endogenous tRNAs in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 96:14366–14371
33. Grosshans H, Hurt E, Simos G (2000) An aminoacylation-dependent nuclear tRNA export pathway in yeast. *Genes Dev* 14:830–840
34. Steiner-Mosonyi M, Mangroo D (2004) The nuclear tRNA aminoacylation-dependent pathway may be the principal route used to export tRNA from the nucleus in *Saccharomyces cerevisiae*. *Biochem J* 378:809–816
35. Dang CV, Yang DC, Pollard TD (1983) Association of methionyl-tRNA synthetase with detergent-insoluble components of the rough endoplasmic reticulum. *J Cell Biol* 96:1138–1147

36. Mirande M, Le Corre D, Louvard D, Reggio H, Pailliez JP, Waller JP (1985) Association of an aminoacyl-tRNA synthetase complex and of phenylalanyl-tRNA synthetase with the cytoskeletal framework fraction from mammalian cells. *Exp Cell Res* 156:91–102
37. Ko YG, Kang YS, Kim EK, Park SG, Kim S (2000) Nuclear localization of human methionyl-tRNA synthetase and its role in ribosomal RNA synthesis. *J Cell Biol* 149:567–574
38. Tolkunova E, Park H, Xia J, King MP, Davidson E (2000) The human lysyl-tRNA synthetase gene encodes both the cytoplasmic and mitochondrial enzymes by means of an unusual alternative splicing of the primary transcript. *J Biol Chem* 275:35063–35069
39. Ling C, Yao YN, Zheng YG, Wei H, Wang L, Wu XF, Wang ED (2005) The C-terminal appended domain of human cytosolic leucyl-tRNA synthetase is indispensable in its interaction with arginyl-tRNA synthetase in the multi-tRNA synthetase complex. *J Biol Chem* 280:34755–34763
40. Bohnsack MT, Regener K, Schwappach B, Saffrich R, Paraskeva E, Hartmann E, Görlich D (2002) Exp5 exports eEF1A via tRNA from nuclei and synergizes with other transport pathways to confine translation to the cytoplasm. *EMBO J* 21:6205–6215
41. Takano A, Endo T, Yoshihisa T (2005) tRNA actively shuttles between the nucleus and cytosol in yeast. *Science* 309:140–142
42. Shaheen HH, Hopper AK (2005) Retrograde movement of tRNAs from the cytoplasm to the nucleus in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 102:11290–11295
43. Reed VS, Wastney ME, Yang DCH (1994) Mechanisms of the transfer of aminoacyl-tRNA from aminoacyl-tRNA synthetase to the elongation factor 1 α . *J Biol Chem* 269:32932–32936
44. Negrutskii BS, Budkevich TV, Shalak VF, Turkovskaya GV, El'skaya AV (1996) Rabbit translation elongation factor 1 α stimulates the activity of homologous aminoacyl-tRNA synthetases. *FEBS Lett* 382:18–20
45. Negrutskii BS, Shalak VF, Kerjan P, El'skaya AV, Mirande M (1999) Functional interaction of mammalian valyl-tRNA synthetase with elongation factor EF-1 α in the complex with EF-1H. *J Biol Chem* 274:4545–4550