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# Complementation of Yeast Arc1p by the p43 Component of the Human Multisynthetase Complex does not Require its Association with Yeast MetRS and GluRS

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Yeast Arc1p, human p43 and plant methionyl-tRNA synthetase (MetRS) possess an EMAPII-like domain capable of non-specific interactions with tRNA. Arc1p interacts with MetRS (*MES1*) and GluRS and operates as a tRNA-interacting factor (tIF) in *trans* of these two synthetases. In plant MetRS, the EMAPII-like domain is fused to the catalytic core of the synthetase and acts as a *cis*-acting tIF for aminoacylation. We observed that the catalytic core of plant MetRS expressed from a centromeric plasmid cannot complement a yeast *arc1<sup>-</sup> mes1<sup>-</sup>* strain. Overexpression of the mutant enzyme from a high-copy number plasmid restored cell growth, suggesting that deletion of its C-terminal tIF domain was responsible for the poor aminoacylation efficiency of that enzyme *in vivo*. Accordingly, expression of full-size plant MetRS from a centromeric plasmid, but also of fusion proteins between its catalytic core and the EMAPII-like domains of yeast Arc1p or of human p43 restored cell viability. These data showed that homologous tIF domains from different origins are interchangeable and may act indifferently in *trans* or in *cis* of the catalytic domain of a synthetase. Unexpectedly, co-expression of Arc1p with the catalytic core of plant MetRS restored cell viability as well, even though Arc1p did not associate with plant MetRS. Because Arc1p also interacts with yeast GluRS, restoration of cell growth could be due at least in part to its role of cofactor for that enzyme. However, co-expression of human p43, a tIF that did not associate with plant MetRS or with yeast GluRS and MetRS, also restored cell viability of a yeast strain that expressed the catalytic core of plant MetRS. These results show that p43 and Arc1p are able to facilitate tRNA aminoacylation *in vivo* even if they do not interact physically with the synthetases. We propose that p43/Arc1p may be involved in sequestering tRNAs in the cytoplasm of eukaryotic cells, thereby increasing their availability for protein synthesis.

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**Keywords:** Arc1p; EMAPII; tRNA-interacting factor; methionyl-tRNA synthetase

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Abbreviations used: MetRS, methionyl-tRNA synthetase; tIF, tRNA-interacting factor; EMAPII, endothelial monocyte-activating polypeptide II; *Os*, *Oriza sativa*; *Ec*, *Escherichia coli*; *Hs*, *Homo sapiens*; aa, aminoacyl.

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## Introduction

Aminoacyl-tRNA synthetases catalyze the formation of an ester bond between an amino acid and the 3' end of a tRNA in a two-step reaction. First, the amino acid is activated and an enzyme-bound adenylate intermediate is formed. Then, the amino acid is transferred to the 3' end of the tRNA molecule.<sup>1</sup> Aminoacyl-tRNA synthetases have to decode the genetic information into amino acids with high fidelity and protein-tRNA

interactions play a key role in this process. Binding of a tRNA molecule to a synthetase is thought to proceed in two steps: an initial broad-specificity interaction between the tRNA and the synthetase is followed by a more precise recognition that involves conformational changes of both the synthetase and the cognate tRNA<sup>2</sup> and contributes to tRNA specificity as non-specific tRNAs are normally less efficiently aminoacylated.<sup>3</sup>

Prokaryotic synthetases, which represent the elementary synthetase throughout evolution, are built of two major functional domains constituting the catalytic core: the catalytic domain contains the active site and interacts with the acceptor arm of tRNA; the second domain generally interacts with the anticodon arm.<sup>4,5</sup> Eukaryotic enzymes present a catalytic core closely related to the respective prokaryotic enzyme, but possess additional domains appended to the N or C-terminal end.<sup>6</sup> In higher eukaryotes, polypeptide extensions are hydrophobic and participate in the formation of the multi-synthetase complex,<sup>7</sup> or are cationic and interact non-specifically with tRNA to improve the catalytic efficiency of the enzyme. According to their primary structures, tRNA-interacting factors (tIF) can be divided into three major families. The first one is found in eukaryotic LysRS, AspRS and AsnRS.<sup>8–11</sup> A repeated domain is found in human MetRS, HisRS, TrpRS, GlyRS and in the linker region of glutamyl-prolyl-tRNA synthetase.<sup>12–15</sup> The last type of tIF (EMAPII-like domain) is appended to plant MetRS<sup>16</sup> or human TyrRS<sup>17</sup> and acts in *cis* of the catalytic domain of the synthetase. The EMAPII domain was first identified in human p43, a non-synthetase protein associated with the multi-synthetase complex,<sup>18</sup> and is identical with a tumor-derived factor (endothelial monocyte-activating polypeptide II) that alters endothelial functions.<sup>19</sup> The yeast protein Arc1p (aminoacyl-tRNA synthetase cofactor I), which makes a ternary complex with GluRS and MetRS, possesses a C-terminal domain homologous to EMAPII and acts as a tIF in *trans* of these two synthetases.<sup>20,21</sup>

Methionyl-tRNA synthetases display an especially variable structural organization throughout evolution. The enzyme can be limited to the catalytic core as in *Aquifex aeolicus*, or may have an appended C-terminal domain involved in dimer formation as in *Escherichia coli*.<sup>22,23</sup> Plant and human MetRS possess C-terminal extensions with unrelated sequences. In both cases, this domain interacts non-specifically with tRNA, and acts in *cis* to the catalytic core to improve the catalytic efficiency of the enzyme in the aminoacylation reaction.<sup>15,16</sup> In yeast, Arc1p possesses a tRNA-binding domain at its C-terminal end. The N-terminal moiety of Arc1p interacts with the eukaryotic-specific N-terminal domains of GluRS and MetRS. Association with Arc1p provides a tIF in *trans* to these two enzymes which improves their catalytic efficiency.<sup>20,24,25</sup> Arc1p is required for optimal growth and is essential for viability of

yeast in the absence of the nuclear pore-associated protein Los1p involved in nuclear tRNA export.<sup>26</sup>

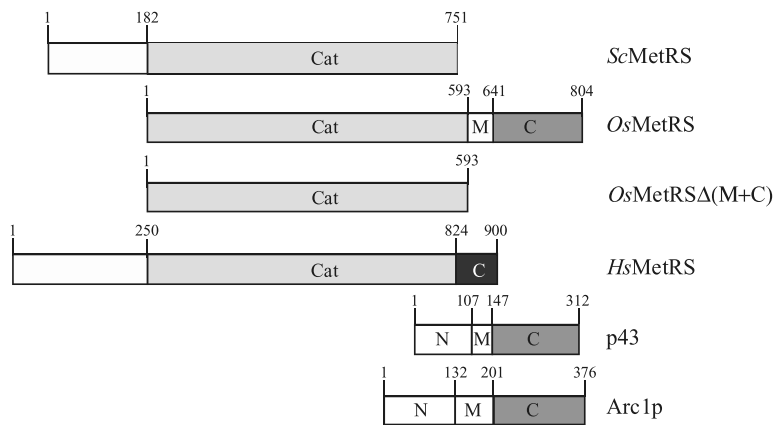
Because EMAPII-like domains are found appended to various proteins and are widely distributed among living organisms, we investigated the possibility of rescuing Arc1p-deficient yeast cells with fusion proteins containing EMAPII-like domains from different origins. Using MetRS from the rice *Oriza sativa* (OsMetRS), we showed that in the absence of its C-terminal tIF, the catalytic core of plant MetRS could not complement an *arc1<sup>-</sup>mes1<sup>-</sup>* yeast strain. We constructed a series of fusion proteins that tested the capacity of various EMAPII-like domains to act as functional *cis*-acting tRNA-binding domains *in vivo*. To further address the function of these EMAPII-like domains, we tested for the absolute requirement of their association in *cis* or in *trans* of the synthetases to achieve complementation. Our results establish that human p43 or yeast Arc1p are also able to sustain growth of yeast strains independently of their function of tIF for MetRS and GluRS. These data suggest that in addition to its role of tIF for two synthetases, Arc1p may be directly involved in sequestering tRNA in the cytoplasm of yeast.

## Results

### OsMetRS can replace ScMetRS *in vivo*

Deletion of *MES1*, the gene coding for ScMetRS, is lethal for yeast,<sup>25</sup> but deletion of *ARC1* only introduces a growth defect.<sup>20</sup> Expression of Arc1p from a centromeric plasmid (pUN100-Arc1) palliates the growth defect of an *arc1<sup>-</sup>* strain. Arc1p forms a complex with ScMetRS and ScGluRS;<sup>20</sup> the N-terminal domain of Arc1p (Figure 1) interacts with the N-terminal domains of the two synthetases.<sup>24,25</sup> The C-terminal domain of Arc1p (EMAPII-like domain) binds tRNA and improves the catalytic efficiency of ScMetRS by lowering its  $K_m$  value for tRNA<sup>Met</sup> in the aminoacylation reaction.<sup>20,24</sup> These findings suggested that, in yeast, Arc1p operates as a *trans*-acting tRNA interacting factor (tIF) for two aminoacyl-tRNA synthetases (GluRS and MetRS).

MetRS from the rice *Oriza sativa* (OsMetRS) can be divided into three distinct domains (Figure 1): the catalytic core at the N-terminal end with high sequence similarity with prokaryotic MetRS (50% identities); an EMAPII-like domain at the C-terminal end (53% identities with the C-terminal domain of Arc1p); and a median (M) domain with no sequence similarity with any known protein.<sup>27</sup> *In vitro*, the (M + C) domain of OsMetRS clearly provides tRNA-binding properties to the native enzyme and acts as a *cis*-acting tIF for aminoacylation.<sup>16</sup> We tested whether OsMetRS or its isolated catalytic domain obtained after deletion of the (M + C) domain could rescue an *arc1<sup>-</sup>mes1<sup>-</sup>* yeast strain. For the clarity of this paper, we will name *arc1<sup>-</sup>mes1<sup>-</sup>* (protein A, protein B, etc.)



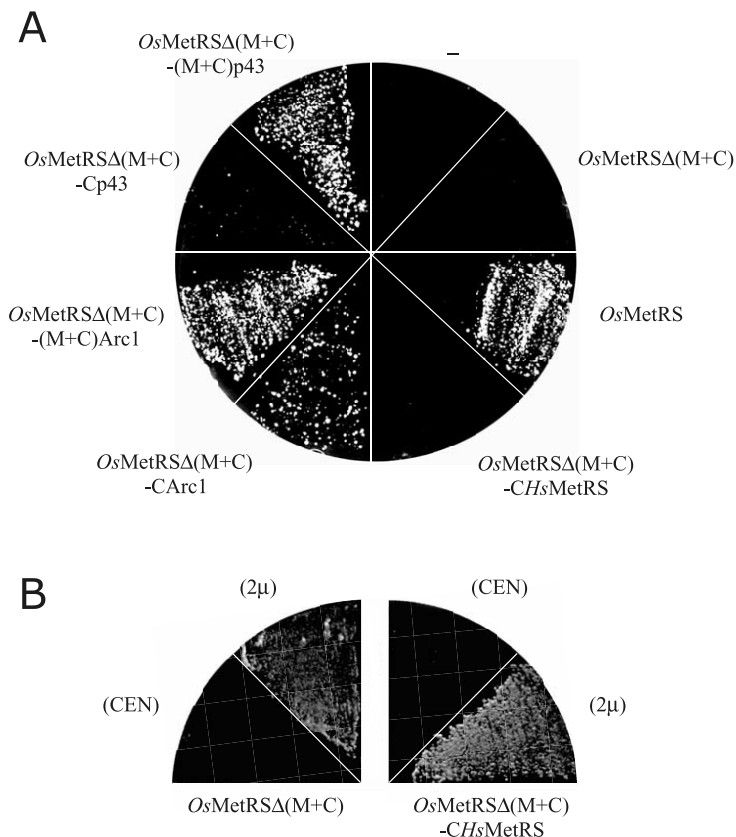
**Figure 1.** Schematic representation of the sequences of MetRSs from *S. cerevisiae* (ScMetRS), *O. sativa* (OsMetRS) or *H. sapiens* (HsMetRS), of *H. sapiens* p43 (p43) and of *S. cerevisiae* Arc1p (Arc1p). A light grey bar represents the catalytic core of the different MetRSs. A black bar represents the C-terminal extension of HsMetRS. Grey bars represent EMAPII-like domains at the C-terminal end of OsMetRS, Arc1p and p43.

the yeast strain with gene disruptions of *ARC1* and *MES1* transformed with the plasmids expressing protein A, protein B, etc. Thus, the *arc1<sup>-</sup> mes1<sup>-</sup>* strain transformed with the centromeric plasmid expressing ScMetRS will be noted *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS). If a 2 $\mu$  plasmid is used, then the notation will be *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS/2 $\mu$ ).

A *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) strain expressing ScMetRS from a centromeric plasmid containing *URA3* was transformed with a second centromeric plasmid containing *TRP1* and expressing OsMetRS. After overnight culture in the presence of uracil, cells were plated on a growth medium containing 5'-fluoroorotic acid (FOA), which achieves the counter selection of cells containing

*URA3* plasmids. This complementation assay showed that expression of OsMetRS restored cell viability (Figure 2A).

Previous *in vitro* studies have shown that deletion of the M and C-terminal domains of OsMetRS produces a stable monomeric enzyme with a tenfold decrease in  $K_m$  for tRNA in the aminoacylation reaction.<sup>16</sup> When this MetRS species, OsMetRS $\Delta$ (M + C), was expressed from a centromeric plasmid, cell viability was not restored (Figure 2A). Thus, deletion of the (M + C) domain of OsMetRS also impairs the catalytic efficiency of the enzyme *in vivo*. However, when OsMetRS $\Delta$ (M + C) was expressed from a multicopy plasmid, cell viability was restored



**Figure 2.** OsMetRS and fusion proteins derived from OsMetRS can replace ScMetRS in yeast. A, *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) strain was transformed by centromeric plasmids expressing OsMetRS or a fusion protein derived from OsMetRS and complementation experiments were performed as described in Materials and Methods. Plates were incubated for four days at 28 °C. B, *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) strain was transformed by centromeric (CEN) or multicopy (2 $\mu$ ) plasmids expressing OsMetRS $\Delta$ (M + C) and OsMetRS $\Delta$ (M + C)-CHsMetRS and tests of complementation were conducted. Plates shown in A and B are representative of at least three independent experiments.



(Figure 2B). This result suggests that increasing the expression level of the catalytic core of *OsMetRS* could palliate its poor aminoacylation efficiency.

### The C-terminal domain of *OsMetRS* can be replaced by other tRNA-binding domains

p43 is one of the three auxiliary proteins associated with nine aminoacyl-tRNA synthetases in a multi-enzyme complex ubiquitous to higher eukaryotes.<sup>28</sup> As Arc1p, p43 can be divided into three distinct domains (Figure 1). The C-terminal domains of both yeast Arc1p and human p43 are capable of binding tRNA. In both cases, the affinity of the C-terminal domain of the protein for tRNA is improved in the presence of the M domain.<sup>18,20,24</sup> Like *OsMetRS*, human MetRS (*HsMetRS*) possesses a C-terminal domain capable of binding tRNA (Figure 1).<sup>15</sup> However, no sequence similarity exists between the (M + C) domain of *OsMetRS* and the C-terminal domain of *HsMetRS*; the EMAPII-like domain of *OsMetRS* has an OB-fold conformation whereas the C-terminal domain of *HsMetRS* corresponds to a helix-turn-helix motif.<sup>12,29</sup> We expressed chimeric proteins made of the catalytic core of *OsMetRS* (*OsMetRS* $\Delta$ (M + C)) and of C-terminal fragments of yeast Arc1p, human p43 or *HsMetRS* capable of binding tRNA, and we tested the activity of the fusion proteins in an *in vivo* complementation assay.

Five different fusion proteins were expressed. *OsMetRS* $\Delta$ (M + C)-CArc1 and *OsMetRS* $\Delta$ (M + C)-Cp43 containing the C-terminal domain of yeast Arc1p and human p43, respectively, could clearly complement an *arc1*<sup>-</sup> *mes1*<sup>-</sup> strain (Figure 2A). The efficiency of complementation was correlated with the tRNA-binding potency of the domain

fused to *OsMetRS* $\Delta$ (M + C). Indeed, complementation was clearly improved when the M domain of Arc1p or of p43 was also present (*OsMetRS* $\Delta$ (M + C)-(M + C)Arc1 and *OsMetRS* $\Delta$ (M + C)-(M + C)p43, respectively) (Figure 2A). Thus, addition of a tRNA-binding domain to the catalytic core of *OsMetRS* restored cell viability. However, when the C-terminal domain of *HsMetRS* was fused to the catalytic core of rice MetRS (*OsMetRS* $\Delta$ (M + C)-*CHsMetRS* (Figure 2A)), only few cells grew in the complementation assay. This could be due to the tRNA-sequestering function of this domain<sup>15</sup> that might impair the rate of Met-tRNA<sup>Met</sup> release *in vivo*. As for *OsMetRS* $\Delta$ (M + C), expression of *OsMetRS* $\Delta$ (M + C)-*CHsMetRS* using a multicopy plasmid improved the ability of the fusion protein to complement the *arc1*<sup>-</sup> *mes1*<sup>-</sup> strain (Figure 2B).

These results are consistent with the interpretation according to which EMAPII-like tRNA-binding domains are functional *cis*-acting tIFs for the catalytic core of *OsMetRS*, improve the efficiency of tRNA aminoacylation *in vivo* and, consequently, restore cell viability. These results also showed that the (M + C) domain of human p43, the human homolog of yeast Arc1p, can fulfill the role of tIF in *cis* to the catalytic core of a synthetase.

Yeast strains *arc1*<sup>-</sup> *mes1*<sup>-</sup> rescued by *ScMetRS*, *OsMetRS*, *OsMetRS* $\Delta$ (M + C)-(M + C)Arc1, and *OsMetRS* $\Delta$ (M + C)-(M + C)p43 grew at similar rates on rich medium (Table 1 and Figure 3). A growth rate identical with that of the *arc1*<sup>-</sup> *mes1*<sup>-</sup> (*ScMetRS*, Arc1p) strain was never reached, indicating that the presence of a tIF domain acting in *cis* of MetRS cannot completely palliate the growth defect of the *arc1*<sup>-</sup> *mes1*<sup>-</sup> strain. This result is consistent with the finding that Arc1p acts in *trans* of MetRS but also of GluRS.<sup>20</sup> Therefore, rescuing the

**Table 1.** Doubling-times ( $\tau$ ) at 20, 28, and 37 °C of *arc1*<sup>-</sup> *mes1*<sup>-</sup> strains expressing yeast MetRS (*ScMetRS*), rice MetRS (*OsMetRS*) or a fusion protein derived from rice MetRS, with or without co-expression of an auxiliary protein

MetRS <sup>c</sup>	Auxiliary protein <sup>a</sup>		$\tau$ (min) <sup>b</sup>		
	Protein	Plasmid <sup>d</sup>	20 °C	28 °C	37 °C
<i>ScMetRS</i>	-	-	252 ± 9	141 ± 13	188 ± 17
<i>ScMetRS</i>	-	CEN <sup>e</sup>	266 ± 5	-	-
<i>ScMetRS</i>	Arc1p	CEN	182 ± 9	109 ± 7	136 ± 2
<i>ScMetRS</i>	p43	CEN	241 ± 6	-	191 ± 11
<i>ScMetRS</i>	p43	2 $\mu$	229 ± 6	-	-
<i>ScMetRS</i>	EF1A	CEN	245 ± 2	-	-
<i>OsMetRS</i>	-	-	234 ± 4	142 ± 3	225 ± 11
<i>OsMetRS</i>	Arc1p	CEN	186 ± 6	123 ± 4	143 ± 10
<i>OsMetRS</i>	EF1A	CEN	234 ± 5	-	-
<i>OsMetRS</i> $\Delta$ (M + C)-(M + C)Arc1	-	-	258 ± 2	-	223 ± 27
<i>OsMetRS</i> $\Delta$ (M + C)-(M + C)p43	-	-	234 ± 3	-	224 ± 29
<i>OsMetRS</i> $\Delta$ (M + C)	Arc1p	CEN	197 ± 3	-	162 ± 13

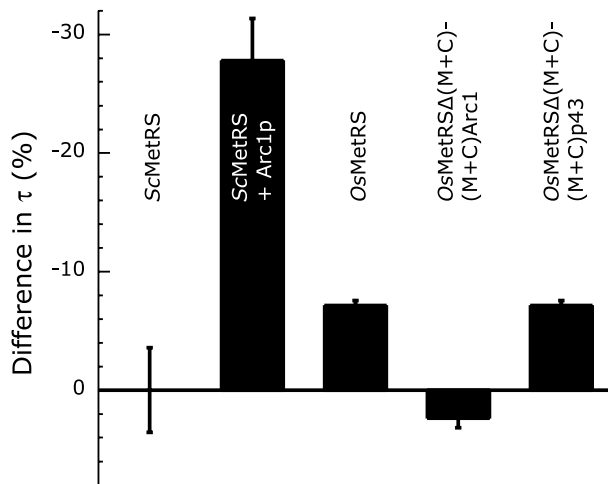
<sup>a</sup> Protein co-expressed with MetRS. A dash (-) indicates that no protein was co-expressed.

<sup>b</sup> Doubling-time values ( $\tau$ ) are the average of at least three independent experiments. Experiments were performed as described in Materials and Methods.

<sup>c</sup> MetRS were expressed from a centromeric plasmid (pRS316 for *ScMetRS* and pRS314 for *OsMetRS* and for fusion proteins derived from *OsMetRS*).

<sup>d</sup> CEN, centromeric plasmid (pUN100-Arc1 for the expression of Arc1p, pRS315-pPGK-tPGK for the expression of p43 and EF1A); 2 $\mu$  for multicopy plasmid (pJG4-5-pPGK-tPGK).

<sup>e</sup> pRS314-pPGK-tPGK.



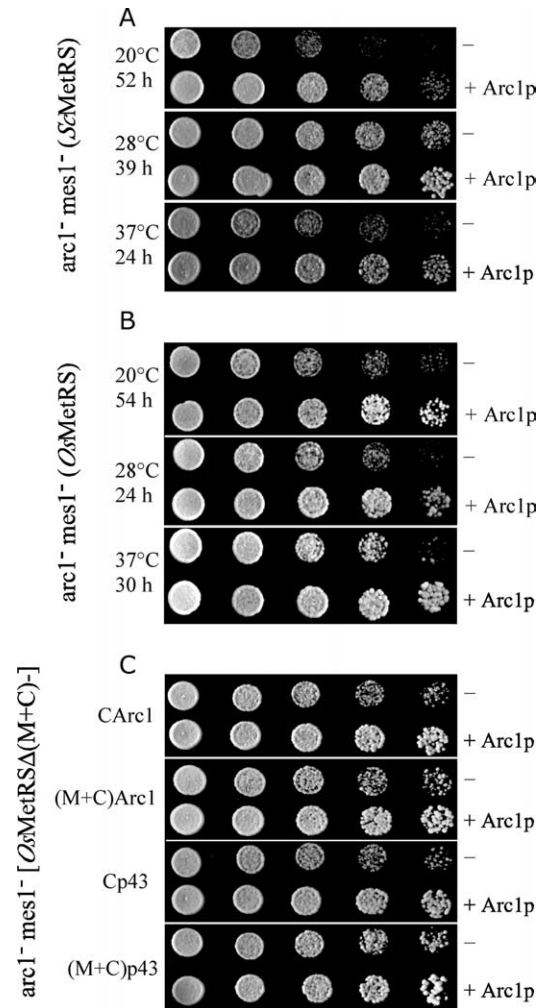
**Figure 3.** Comparison of the growth rates of *arc1<sup>-</sup> mes1<sup>-</sup>* strains rescued by different MetRS species. Differences between growth rates at 20 °C are expressed by the difference between the doubling-time ( $\tau$ ) of the strain and the  $\tau$  value of *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) strain ( $\tau = 252(\pm 9)$  minutes) and are the average of at least three independent experiments. The different MetRSs (as indicated) and Arc1p are expressed from centromeric plasmids.

activity of MetRS alone is not sufficient to restore a wild-type phenotype.

### Arc1p stimulates growth of *arc1<sup>-</sup> mes1<sup>-</sup>* cells expressing *OsMetRS* or a fusion protein thereof

As shown,<sup>20</sup> the growth defect phenotype of *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) can be palliated by co-expression of Arc1p from a centromeric plasmid (strain *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS, Arc1p)). Expression of Arc1p in *trans* of ScMetRS significantly improved the growth rate of the strain in solid (Figure 4A) and liquid media (Table 1). Effects were larger at non-optimal temperatures (at 20 °C or 37 °C, the doubling-time of the strain,  $\tau$ , was improved by 30%) than at physiological growth temperature (a 20% improvement of  $\tau$  at 28 °C). When cells were transformed with a control plasmid (pRS314-pPGK-tPGK) or with a plasmid expressing an unrelated human protein (pRS315-EF1A), the growth rate was not significantly affected (Table 1).

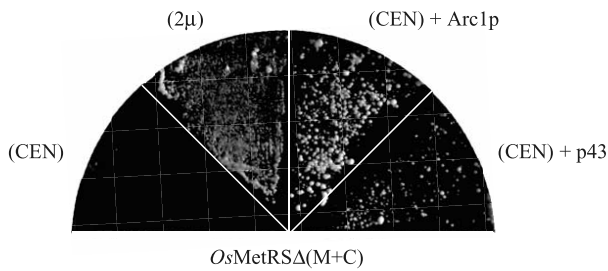
Similarly, co-expression of Arc1p with *OsMetRS* (Figure 4B and Table 1) or with fusion proteins containing the catalytic core of *OsMetRS* fused to EMAPII-like tIFs (Figure 4C) also improved cell growth. Because Arc1p cannot interact with *OsMetRS* (see below), these data are consistent with the function of tIF acting in *trans* of yeast GluRS attributed to Arc1p. Thus, restoration of a wild-type growth phenotype is likely to require that both MetRS and GluRS are functionally linked to tIFs that may be associated in *cis* or in *trans* of their catalytic core.



**Figure 4.** Arc1p can palliate the growth defect of *arc1<sup>-</sup> mes1<sup>-</sup>* strains expressing ScMetRS, *OsMetRS* or a fusion protein derived from *OsMetRS*. Cells transformed by centromeric plasmids were grown in minimal medium, serially diluted, and aliquots were spotted onto YPG plates containing adenine and tryptophan, when necessary. Where indicated, yeast cells also expressed Arc1p from pUN100-Arc1. A, *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) strain. B, *arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRS*) strain. Plates were incubated at the temperatures and for the times indicated. C, *arc1<sup>-</sup> mes1<sup>-</sup>* strains expressing fusion proteins derived from *OsMetRS* $\Delta$ (M + C). Cells were incubated at 37 °C for 35 hours. Plates are representative of at least two independent experiments.

### Arc1p can restore cell viability of a yeast strain with a defective MetRS

The weak *in vivo* aminoacylation efficiency of the catalytic core of rice MetRS can be palliated by its overexpression (Figure 2B) or by fusion of a tIF domain (Figure 2A). Surprisingly, co-expression of Arc1p with *OsMetRS* $\Delta$ (M + C) also restored viability of *arc1<sup>-</sup> mes1<sup>-</sup>* cells (Figure 5). This result was unexpected, since Arc1p was not supposed to interact with the catalytic core of rice MetRS. Indeed, Arc1p operates as a *trans*-acting tIF for

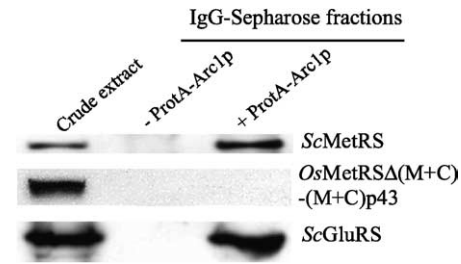


**Figure 5.** Ability of Arc1p and p43 to rescue *arc1<sup>-</sup> mes1<sup>-</sup>* cells expressing *OsMetRSΔ(M + C)*. A *arc1<sup>-</sup> mes1<sup>-</sup>* (*ScMetRS*) strain was transformed with a centromeric (CEN) or multicopy ( $2\mu$ ) plasmid expressing *OsMetRSΔ(M + C)* and, where indicated, with a centromeric plasmid expressing Arc1p or p43. Tests of complementation were performed at 28 °C for four days. This plate is representative of at least three independent experiments.

*ScMetRS* by interacting with tRNA *via* its C-terminal domain and with *ScMetRS* *via* its N-terminal domain.<sup>20,24,25</sup> Outside the catalytic core, *OsMetRS* has no similarity of sequence with *ScMetRS*; especially *OsMetRS* does not have a polypeptide extension similar to that found at the N-terminal end of *ScMetRS* and responsible for its interaction with Arc1p.<sup>24,25</sup> Thus, we did not expect that *OsMetRS* could interact with Arc1p.

We first sought to verify that Arc1p does not form a complex with *OsMetRS*. To find out whether Arc1p associates physically with *OsMetRS*, Arc1p was affinity-purified as an epitope-tagged fusion protein containing the IgG-binding domain derived from *Staphylococcus aureus* protein A (ProtA-Arc1p). ProtA-Arc1p was functional as well as Arc1p.<sup>20</sup> As a control, IgG-Sepharose was incubated with a soluble extract of *arc1<sup>-</sup> mes1<sup>-</sup>* (*ScMetRS*, ProtA-Arc1p) strain. As expected, analysis by Western blotting using antibodies directed to *ScMetRS* or *ScGluRS* showed that both synthetases co-purified with ProtA-Arc1p (Figure 6). A similar experiment was performed with *arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRSΔ(M + C)*-(M + C)p43, ProtA-Arc1p) extract. Using antibodies raised against the C-terminal domain of p43, we found that *OsMetRSΔ(M + C)*-(M + C)p43 was not co-purified with ProtA-Arc1p (Figure 6). Thus, Arc1p does not interact with the catalytic core of *OsMetRS* as it does with the N-terminal domain of *ScMetRS*. Therefore, in the absence of protein-protein interaction, Arc1p cannot act as a tIF *in trans* of *OsMetRSΔ(M + C)*.

The restoration of viability of *arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRSΔ(M + C)*) following co-expression of Arc1p suggested that MetRS activity is not the limiting factor for growth of *arc1<sup>-</sup>* strains. Moreover, the growth rate of the resulting strain *arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRSΔ(M + C)*, Arc1p) was very similar to that of *arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRS*, Arc1p), or even to that of *arc1<sup>-</sup> mes1<sup>-</sup>* (*ScMetRS*, Arc1p) corresponding to wild-type (Figure 7 and Table 1).



**Figure 6.** Arc1p does not interact with the catalytic domain of *OsMetRS*. ProtA-Arc1p from *arc1<sup>-</sup> mes1<sup>-</sup>* cells co-transformed with plasmids that expressed *ScMetRS* or *OsMetRSΔ(M + C)*-(M + C)p43 was affinity-purified on IgG-Sepharose. Proteins absorbed on IgG-Sepharose were analyzed by Western blotting with antibodies directed to *ScMetRS*, p43 or *ScGluRS*. The corresponding crude extracts were analyzed in parallel. Negative controls were performed with crude extracts of cells that did not express ProtA-Arc1p.

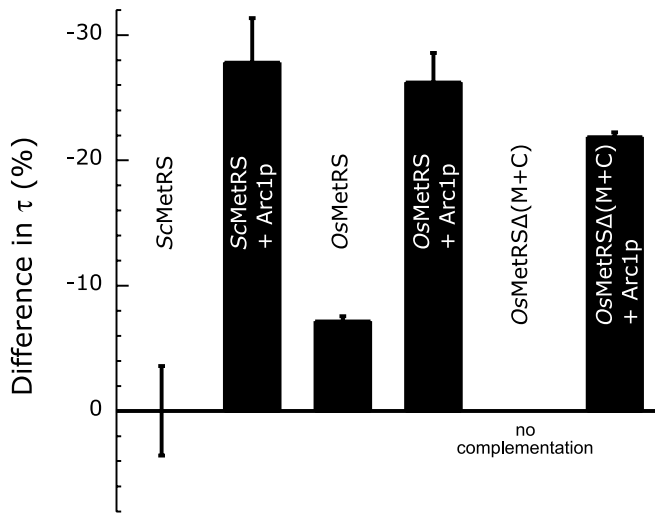
Thus, because Arc1p does not associate with rice MetRS, these data suggested that the main consequence of the lack of Arc1p would be to lower the catalytic efficiency of GluRS. Alternatively, another not yet defined function of Arc1p could be the primary defect of strains with a null-allele of *ARC1*.

#### Human p43 can restore Arc1p

Because p43, the human homolog of yeast Arc1p, was not supposed to form a complex with either yeast MetRS and GluRS or with rice MetRS, we anticipated that it might be a good tool to test for the absolute requirement of a tIF acting in *cis* or in *trans* of MetRS or GluRS to rescue *arc1<sup>-</sup>* strains. As shown above with Arc1p, co-expression of human p43 with the catalytic core of *OsMetRS* also restored viability of *arc1<sup>-</sup> mes1<sup>-</sup>* cells (Figure 5). The growth rate of this strain ( $\tau$  at 20 °C of 249( $\pm$ 4) minutes) was similar to that of *arc1<sup>-</sup> mes1<sup>-</sup>* (*ScMetRS*) ( $\tau$  at 20 °C of 252( $\pm$ 9) minutes) (Table 1).

As Arc1p, p43 possesses an EMAPII domain capable of non-specific interaction with tRNA.<sup>18</sup> Its N-terminal end is involved in dimerization of the protein<sup>30</sup> and interacts with ArgRS and p38, two other components of the mammalian multi-synthetase complex.<sup>31</sup> Except for the C-terminal domain, p43 has no significant similarity of sequence with Arc1p and we hypothesized that human p43 does not interact with *ScMetRS*, *ScGluRS*, or *OsMetRS*. To look for a possible interaction between human p43 and *ScMetRS*, *ScGluRS* or *OsMetRS*, His-tagged p43 protein was incubated with cell extracts from yeast containing *ScMetRS* and *ScGluRS* (*arc1<sup>-</sup> mes1<sup>-</sup>* (*ScMetRS*)) or *OsMetRSΔ(M + C)*-(M + C)p43 (*arc1<sup>-</sup> mes1<sup>-</sup>* (*OsMetRSΔ(M + C)*-(M + C)p43)). Using affinity-purified antibodies directed to the His-tag, we observed that *ScMetRS*, *ScGluRS* and *OsMetRSΔ(M + C)*-(M + C)p43 were not co-immunoprecipitated with His-tagged p43





**Figure 7.** Expression of Arc1p stimulates growth rates of *arc1<sup>-</sup>mes1<sup>-</sup>* strains rescued by different MetRS species. Differences between growth rates at 20 °C are expressed as for Figure 3 and are the average of at least three independent experiments. The different MetRSs (as indicated) and Arc1p are expressed from centromeric plasmids.

(Figure 8A). In a control experiment, mammalian ArgRS that interacts with p43<sup>31</sup> was recovered within the immunoprecipitate (Figure 8A). By immunoprecipitation and Western blot, we were able to show the interaction between ArgRS and p43, two proteins that associate with an apparent dissociation constant  $K_d$  value of 93 nM<sup>31</sup> but not between p43 and ScMetRS, ScGluRS or OsMetRS. Due to the sensitivity of this assay, we might expect to demonstrate the formation of a complex with a  $K_d$  value up to 5–10  $\mu$ M. Thus, p43 cannot associate with ScMetRS, ScGluRS or OsMetRS at protein concentrations prevailing *in vivo* and, consequently, p43 cannot act as a tIF *in trans* of these enzymes.

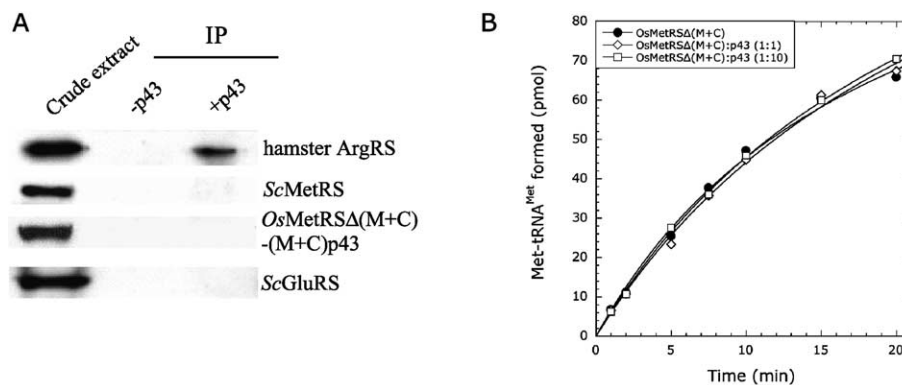
As an additional control experiment, we also examined the possibility that p43 only transiently associates with OsMetRS $\Delta$ (M + C) but may still stimulate its activity. The time course of tRNA<sup>Met</sup> aminoacylation catalyzed by OsMetRS $\Delta$ (M + C) was followed in the absence or in the presence of

one or ten molar excess of p43 in the assay, under conditions where tRNA was not saturating (Figure 8B). The tRNA<sup>Met</sup> aminoacylation capacity of the enzyme was not affected by addition of p43 in the assay. These results show that human p43 interacts neither physically nor functionally with the catalytic core of rice MetRS.

#### Expression of human p43 improves growth of *arc1<sup>-</sup>* cells

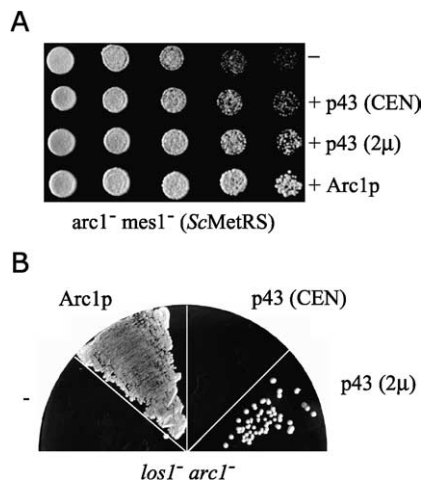
If p43 is able to replace Arc1p in a MetRS and GluRS-independent pathway, it should be able to rescue the growth defect phenotype of the *arc1<sup>-</sup>mes1<sup>-</sup>* (ScMetRS) strain. Expression of human p43 from a centromeric plasmid did only slightly affect the growth rate of the strain (Figure 9A). Interestingly, expression of p43 from a multicopy plasmid significantly improved the growth of *arc1<sup>-</sup>mes1<sup>-</sup>* (ScMetRS) (Figure 9A and Table 1).

Similarly, p43 may also rescue a *los1<sup>-</sup>arc1<sup>-</sup>*



**Figure 8.** p43 does not interact with ScMetRS, OsMetRS or ScGluRS and does not interfere with OsMetRS activity. A, Cell extracts of strain W303-1B expressing hamster ArgRS, of strain *arc1<sup>-</sup>mes1<sup>-</sup>* expressing ScMetRS and ScGluRS, or of strain *arc1<sup>-</sup>mes1<sup>-</sup>* expressing OsMetRS $\Delta$ (M + C)-(M + C)p43 were incubated with His-tagged human p43 or not (negative control). Immunoprecipitates (IP) obtained with anti-(His-tag) antibodies were analyzed by Western blotting with antibodies directed to hamster ArgRS, ScMetRS, p43 or ScGluRS. The corresponding crude extracts were analyzed in parallel. B, The activity of OsMetRS $\Delta$ (M + C) was determined in the tRNA<sup>Met</sup> aminoacylation reaction in the presence or in the absence of p43, as described in Materials and Methods.





**Figure 9.** p43 palliates the growth defect of an *arc1<sup>-</sup> mes1<sup>-</sup> (ScMetRS)* strain and rescues a *los1<sup>-</sup> arc1<sup>-</sup>* strain. A, *arc1<sup>-</sup> mes1<sup>-</sup> (ScMetRS)* cells co-transformed with a centromeric (CEN) or multicopy (2μ) plasmid expressing human p43 or with a centromeric plasmid expressing Arc1p were grown in minimal medium, serially diluted, and spotted onto YPG plates. The plate was incubated 68 hours at 20 °C. B, *los1<sup>-</sup> arc1<sup>-</sup>* strain was transformed with plasmids expressing human p43 (centromeric (CEN) or multicopy (2μ)) or yeast Arc1p (centromeric) and tests of complementation were conducted. The plate shown in B was incubated for six days at 28 °C and is representative of at least three independent experiments.

strain. Yeast Los1p is involved in tRNA export from the nucleus to the cytoplasm.<sup>32,33</sup> Disruption of *LOS1* does not cause any apparent growth defect, suggesting that export of tRNA still continues in the absence of Los1p at least to an extent that supports normal cell growth.<sup>32</sup> When the single deletion of chromosomal *ARC1* or *LOS1* is not lethal for yeast, the double deletion is lethal. This synthetic lethality suggested the involvement of Arc1p in tRNA export or, more generally, in the cytoplasmic localization of tRNA.<sup>26</sup> As shown, expression of Arc1p restored cell viability<sup>26</sup> (Figure 9B). Expression of human p43 from a centromeric plasmid could not rescue a *los1<sup>-</sup> arc1<sup>-</sup>* strain, but its expression from a 2μ plasmid achieved the complementation (Figure 9B).

Thus, even if p43 cannot interact with yeast MetRS and GluRS, its expression improves the growth rate of cells lacking Arc1p. All these findings suggest that Arc1p is involved in a cellular pathway independent of its ability to associate with MetRS and GluRS, and that p43 may also fulfill this function *in vivo*.

## Discussion

Arc1p forms a ternary complex with ScMetRS and ScGluRS and acts as a tIF in *trans* of both synthetases to improve their catalytic efficiencies.<sup>20,24</sup> The human homolog of Arc1p, the

p43 protein, is associated within a multi-enzyme complex containing nine aminoacyl-tRNA synthetases and two other non-synthetase proteins.<sup>18</sup> Yeast Arc1p, human p43 as well as rice MetRS (OsMetRS) possess EMAPII-like C-terminal domains.<sup>16,18,20</sup> *In vitro*, the EMAPII-like domain of OsMetRS interacts non-specifically with tRNA, decreases the  $K_m$  value of the enzyme for tRNA<sup>Met</sup>, improves the catalytic efficiency of MetRS at the non-saturating tRNA concentrations that prevail *in vivo* but does not significantly affect its  $k_{cat}$  value.<sup>16</sup> The EMAPII-like domain of OsMetRS acts as a tIF in *cis* of the catalytic core of the enzyme (OsMetRSΔ(M + C), with our notation). Whereas the tRNA-binding capacity of the EMAPII domain of human p43 has been clearly shown *in vitro*,<sup>18,30</sup> its possible involvement *in vivo* as a tIF in *trans* of an aminoacyl-tRNA synthetase was not demonstrated. The results reported here reveal that the EMAPII-like domain of OsMetRS and the EMAPII domain of human p43 have the potential to act as functional tIFs *in vivo* and put new perspectives on the role of Arc1p in yeast.

### EMAPII-like domains are functional tIFs for aminoacyl-tRNA synthetases

Our results clearly showed that the EMAPII-like domains of yeast Arc1p and of human p43 can act as tIFs in *cis* when fused to the catalytic core of a synthetase. Fusion proteins containing the C or the (M + C) domain from Arc1p or from p43 fused to the C-terminal end of the catalytic core of OsMetRS expressed from a low copy number plasmid restored viability of *arc1<sup>-</sup> mes1<sup>-</sup>* cells. Similarly, addition of the N-terminal tRNA-binding domain of yeast glutamyl-tRNA synthetase or of the C or the (M + C) domain of Arc1p to the C terminus of *E. coli* AlaRS or to the N terminus of *E. coli* GlnRS converted them into functional yeast enzymes *in vivo* and *in vitro*.<sup>34–36</sup> In these studies, expression of *E. coli* GlnRS from a high copy number plasmid did not complement the yeast knockout.<sup>37</sup> Here, we show that the catalytic core of OsMetRS can complement the *arc1<sup>-</sup> mes1<sup>-</sup>* strain when expressed from a high copy number plasmid. These results indicate that the catalytic core of OsMetRS alone is able to form a productive complex with yeast tRNA<sup>Met</sup>, but its low affinity for tRNA has to be compensated for by an increase of its *in vivo* concentration to restore cell viability. Thus, an efficient synthetase-tRNA complex can be restored *in vivo* either by increasing the cellular concentration of the enzyme or by enhancing its affinity for yeast tRNA (addition of a *cis*-acting tIF to the catalytic core).

The polybasic M-domain of Arc1p or of p43 improves the affinity of the EMAPII-like domain for tRNA *in vitro*<sup>20,24</sup> (V. Shalak & M.M., unpublished results). In our experiments, the efficiency of complementation of *arc1<sup>-</sup> mes1<sup>-</sup>* cells was clearly correlated with the affinity for tRNA of the domain fused to the catalytic core of OsMetRS: the

(M + C) domains of Arc1p and p43 proved to be more effective than the corresponding C domains. These results are consistent with the interpretation according to which the major effect of the fusion of a non-specific RNA binding domain to the catalytic core of an eukaryotic synthetase is to lower the  $K_d$  value of the enzyme for tRNA.

Remarkably, the same tIF may act in *trans* (Arc1p with ScMetRS) or in *cis* (C-Arc1p with the catalytic domains of OsMetRS, EcAlaRS or EcGlnRS) of a synthetase and, in the latter case, may be appended at the N (C-Arc1p with the catalytic domain of EcGlnRS) or at the C (C-Arc1p with the catalytic domains of OsMetRS or EcAlaRS) terminus of the catalytic core of a synthetase. All these data suggest that the peptide segment that links the catalytic domain of the synthetase and the tIF is flexible enough to contribute a productive interaction with the tRNA molecule, irrespective of the polarity of the fusion and to accommodate the different tRNA-binding modes of class-I (MetRS and GlnRS) and class-II (AlaRS) aminoacyl-tRNA synthetases.

### Dual function of Arc1p/p43

Strikingly, co-expression of human p43 also rescued the *arc1<sup>-</sup> mes1<sup>-</sup>* strain that expressed the catalytic core of rice MetRS from a low copy number plasmid. Because p43 does not form a complex with OsMetRS or with yeast GluRS, p43 could not act as a tIF in *trans* of either of these two enzymes. Moreover, expression of p43 in *arc1<sup>-</sup> mes1<sup>-</sup>* (ScMetRS) also improved the growth rate of the cells even though p43 does not interact with ScMetRS and ScGluRS. Similarly, Arc1p also rescued the *arc1<sup>-</sup> mes1<sup>-</sup>* (OsMetRS $\Delta$ (M + C)) strain. The N-terminal domain of Arc1p interacts with the N-terminal domain of ScMetRS but also with the N-terminal domain of ScGluRS.<sup>25</sup> OsMetRS does not possess a domain similar to the N-terminal extension of ScMetRS and has no similarity of sequence with ScMetRS outside the catalytic core. The complex between ScMetRS and Arc1p can be easily isolated from cell extracts of yeast but formation of a similar complex between Arc1p and OsMetRS was not observed. However, because Arc1p also associates with yeast GluRS and provides GluRS with enhanced tRNA binding capacities, complementation of *arc1<sup>-</sup> mes1<sup>-</sup>* (OsMetRS $\Delta$ (M + C)) with Arc1p might be attributed to its *trans*-acting effect on GluRS. Thus, in the yeast system, the involvement of Arc1p in a MetRS and GluRS-independent pathway might have been observed only in a double mutant strain carrying deletions of the N-terminal domains of both MetRS and GluRS.

Human p43 also rescued a *los1<sup>-</sup> arc1<sup>-</sup>* strain. Yeast Los1p is a nuclear export factor for tRNA.<sup>38,39</sup> However, *LOS1* is a non-essential gene which provides strong evidence for the existence of parallel tRNA nuclear export pathways in yeast. *ARC1* is synthetically lethal upon the disrup-

tion of *LOS1*, which led to the suggestion that Arc1p might also be directly involved in tRNA export.<sup>40</sup> However, all the available data strongly suggest that Arc1p is efficiently sequestered in the cytoplasm of yeast. In an exhaustive study, Galani *et al.*<sup>25</sup> showed that Arc1p, MetRS and GluRS are excluded from the nucleus unless the Arc1p–MetRS–GluRS complex is disrupted. Therefore, the emergence of this ternary complex can be seen as a means to prevent leakage of these enzymes into the nuclear compartment, and thus to confine components of the translational apparatus to the cytoplasm, where translation occurs.

All models of nuclear tRNA export in yeast already published are based on the idea that tRNA aminoacylation can occur in the nucleus, and that a Los1p-independent pathway is directly linked to nuclear tRNA aminoacylation.<sup>41</sup> In support of these hypotheses, temperature-sensitive (ts) mutations of three different aminoacyl-tRNA synthetases (*mes1-1*, *ils1-1* and *tys1-1* encoding thermosensitive methionyl-, isoleucyl- and tyrosyl-tRNA synthetases, respectively) were accompanied by a nuclear accumulation of tRNAs.<sup>42</sup> Likewise, at non-permissive temperature, tRNA accumulation inside the nucleus is observed in a *cca1-1* ts mutant that encodes a thermosensitive tRNA nucleotidyl-transferase, an essential enzyme involved in CCA addition at the 3' end of tRNA molecules.<sup>41,42</sup> Moreover, accumulation of non-aminoacylated tRNAs by amino acid starvation or by addition of specific inhibitors, or of free aminoacylated tRNAs in cells with reduced amounts of EF1A or with mutant alleles of *TEF2* resulted in nuclear accumulation of tRNA. Therefore, strains that are defective for tRNA aminoacylation or for formation of the ternary complex EF1A-GTP-aatRNA show accumulation of tRNA inside the nucleus. Taken nuclear aminoacylation for granted, these results have been interpreted as the requirement for aminoacylation and EF1A for nuclear tRNA export.<sup>41</sup> However, nuclear EF1A has never been observed and the presence of aminoacyl-tRNA synthetases in the nuclear compartment remains a controversial question. It should also be noticed that inhibition of protein synthesis by means that would not affect the capacity of tRNA to associate with major cytoplasmic proteins has no effect on tRNA compartmentalization within the cell. For instance, mutations in the genes encoding eIF2 $\gamma$ , the  $\gamma$  subunit of the eukaryotic initiation factor 2 that binds tRNA<sup>Met</sup>, or eIF2B $\gamma$ , the  $\gamma$  subunit of the guanine nucleotide exchange factor for eIF2, are synthetically lethal with a *LOS1* deletion.<sup>38</sup> However, nuclear accumulation of tRNA could not be detected in the *sui2-1* ts mutant of eIF2 $\alpha$ , in strains with *gcd11* mutant alleles of eIF2 $\gamma$  or in the *gcd1-501* ts mutant of eIF2B $\gamma$ .<sup>41</sup> Similarly, treatment of cells with cycloheximide that blocks ribosomal protein synthesis and causes accumulation of the ternary complex EF1A-GTP-aatRNA did not result in nuclear accumulation of tRNA.<sup>41</sup> Thus, when translation is blocked at the initiation step

or at the ribosomal elongation stage, two processes that do not affect the level of tRNA aminoacylation *in vivo* and that do not impair formation of the ternary complex EF1A-GTP-aatRNA, tRNA is not preferentially recruited in the nuclear compartment.

As discussed by Görlich and co-workers,<sup>43</sup> nuclear transport pathways seem to synergize to confine protein components of the translation machinery to the cytoplasm of eukaryotic cells. One alternative explanation to the data recapitulated above is to consider that aminoacyl-tRNA is confined to the cytoplasm *via* protein-tRNA interactions and that tRNAs in excess that are not trapped in the cytoplasm by components of the translation machinery are able to diffuse (or to be transported) to the nuclear compartment. Accordingly, overexpression of ScMetRS suppressed the nuclear accumulation of tRNA<sup>Met</sup> observed in a strain with a *los1* null mutation<sup>44</sup> and overproduction of eEF1A also restored growth of the synthetically lethal *los1<sup>-</sup> arc1ΔC* strain.<sup>41</sup> Similarly, because p43 and Arc1p are potent tRNA binding proteins *per se*, they might confine tRNA to the cytoplasm thereby increasing the concentration of tRNA available for protein synthesis. The possible substitution of Arc1p by its human homolog p43 will allow us to address the involvement of p43/Arc1p in the sequestration of tRNA in the cytoplasm of yeast independently of their role of tIF for two aminoacyl-tRNA synthetases.

## Materials and Methods

### DNA manipulations, yeast strains, and media

All recombinant DNA manipulations were carried out using standard protocols.<sup>45</sup> 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*),<sup>46</sup> pYeDP10 (*2μ, URA3*),<sup>47</sup> pJG4-5 (*2μ, TRP1*),<sup>48</sup> pRS316-URA3-MetRS (*CEN/ARS, URA3*) expressing *S. cerevisiae* MetRS (ScMetRS),<sup>25</sup> pUN100-Arc1 (*CEN/ARS, LEU2*) expressing *S. cerevisiae* Arc1p,<sup>20</sup> pRS425-ProtA-Arc1 (*2μ, LEU2*) expressing *S. cerevisiae* Arc1p fused to protein A at its N-terminal end (ProtA-Arc1p) (K. Galani & G. Simos, unpublished results), and pYeDP60-ArgRS (*2μ, URA3, ADE2*) expressing hamster ArgRS.<sup>49</sup> The yeast strains used in this study were: *los1<sup>-</sup> arc1<sup>-</sup>* (**a**, *los1::HIS3, arc1::HIS3, ade2, leu2, ura3, trp1, his3*, pHT4467-URA3-ADE3-LOS1),<sup>20</sup> *mes1<sup>-</sup> arc1<sup>-</sup>* (**a**, *mes1::HIS5, arc1::HIS3, ade2, leu2, ura3, trp1, his3*, pRS316-URA3-MetRS),<sup>25</sup> and W303-1B (**α**, *leu2, his3, trp1, ura3, ade2*). Yeast cells were transformed using the lithium chloride method<sup>50</sup> 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 acids, 2% glucose) containing the necessary amino acids and bases except those used for selection. For protein expression, cultures were performed in YPG or YNB medium; for ArgRS expression, cultures were performed in YPGal

medium (0.5% yeast extract, 0.5% Bacto peptone, 2% (w/v) galactose) containing tryptophan.

### Construction of yeast expression vectors

Proteins were expressed in yeast under the control of the PGK promoter except Arc1p and ProtA-Arc1p (ADH1 promoter), ScMetRS (*MES1* natural promoter), and hamster ArgRS (GAL10-CYC1 promoter). The pPGK-tPGK cassette was excised from pYeDP10 by BamHI/EcoRI digestion and inserted into the BamHI/EcoRI sites of pRS314 (pRS314-pPGK-tPGK), and the BamHI/HindIII sites of pRS315 (pRS315-pPGK-tPGK). The SacI/EcoRI cassette containing pPGK and tPGK of pRS314-pPGK-tPGK was inserted into the SacI/SphI sites of pJG4-5 (pJG4-5-pPGK-tPGK).

### Cloning of the different MetRSs in yeast expression vectors

Using pYeDP10 plasmid as template, the BglIII/SnaBI fragment of tPGK was amplified by PCR and EcoRI/Sall sites were introduced at the 3' end of the sequence. After BglIII/Sall digestion, tPGK was introduced into the XhoI site following the stop codon of pET-28b-MOsΔC plasmid (pET-28b-MOsΔC-tPGK\*) expressing the catalytic core of the plant *O. sativa* MetRS (*OsMetRS*; GenBank GI 4091007) deleted of the seven proline residues located at positions 4–10 and truncated of its M and C-terminal domains (stop codon introduced just after the Glu codon at position 593), *OsMetRSΔ(M + C)*<sup>16,27</sup> (Figure 1). The HindIII/XhoI fragment was PCR-amplified and a XhoI site followed by a stop codon and a Sall site were introduced just after the Glu593 codon. After HindIII/Sall digestion, the PCR product was inserted into the HindIII/XhoI sites of pET-28b-MOsΔC-tPGK\* (pET-28b-MOsΔC\*-tPGK\*).

The (M + C)-terminal domain of *O. sativa* MetRS was PCR-amplified from the M13-MOs43-2 plasmid<sup>27</sup> (Figure 1) and XhoI sites were introduced at the 5' and 3' ends of the sequence. The C-terminal domain of *Homo sapiens* MetRS (*HsMetRS*; GenBank GI 14043021; Figure 1) was excised from pET-28b-MHs plasmid<sup>15</sup> by Sall/XhoI digestion. The C domain (residues 201–376) and the (M + C) domain (residues 132–376) of *S. cerevisiae* Arc1p (GenBank GI 1620459; Figure 1) were PCR-amplified from pUN100-Arc1 plasmid and Sall and XhoI sites were introduced at the 5' and 3' ends, respectively. The C domain (residues 147–312) and (M + C) domain (residues 107–312) of *H. sapiens* p43 (GenBank GI 4758265; Figure 1) were PCR-amplified from pET-28b-p43 plasmid<sup>30</sup> and XhoI sites were introduced at the 5' and 3' ends of the sequence. After XhoI or Sall/XhoI digestion of the PCR products, C or (M + C) domain cassettes were inserted into the XhoI site of pET-28b-MOsΔC\*-tPGK\* to construct the different pET-28b-MOsΔC\*-Xdomain-tPGK\* plasmids expressing *OsMetRSΔ(M + C)*-X domain proteins. The different X domains fused to the C-terminal end of *OsMetRSΔ(M + C)* are: the C and (M + C) domains of yeast Arc1p noted CArc1 and (M + C)Arc1, respectively; the C and (M + C) domains of human p43 noted Cp43 and (M + C)p43, respectively; and the C domain of *HsMetRS* noted CHsMetRS. Native plant MetRS, corresponding to the natural fusion of *OsMetRSΔ(M + C)* with its (M + C) domain (Figure 1) is noted *OsMetRS*.

The NcoI/NdeI fragment of pET-28b-MOs was introduced into the BglIII site of pRS314-pPGK-tPGK vector



(pRS314-5'MOs-tPGK). pRS314-MOsΔC\*-X domain plasmids were produced by BglIII digestion of pRS314-5'MOs-tPGK, EagI/EcoRI digestion of the pET-28b-MOsΔC\*-Xdomain-tPGK\* plasmids or of pET-28b-MOsΔC-tPGK\* plasmid, and recombination in yeast. pRS315-MOsΔC (and pRS315-MOsΔC\*-CHsMetRS) and pJG4-5-MOsΔC (and pJG4-5-MOsΔC\*-CHsMetRS) plasmids were produced by XbaI/EcoRI digestion of pRS314-MOsΔC (and pRS314-MOsΔC\*-CHsMetRS), BglIII digestion of pRS315-pPGK-tPGK and pJG4-5-pPGK-tPGK, and recombination in yeast.

### Expression of human p43 and EF1A in yeast

pRS315-p43 expressing human p43 was constructed by insertion of the BamHI/XbaI fragment of pTRE2-p43 plasmid (V. Shalak & M.M., unpublished results) into the BglIII site of pRS315-pPGK-tPGK vector. The pJG4-5-p43 plasmid was constructed by BamHI/EcoRI digestion of pRS315-p43, BglIII digestion of pJG4-5-pPGK-tPGK and recombination in yeast. pRS315-EF1A expressing human EF1A was constructed by insertion of the NdeI/BamHI fragment of pET-16b-EF1A plasmid (gift from Jan Dijk, Leiden, The Netherlands) into the BglIII site of pRS315-pPGK-tPGK plasmid.

### Complementation assays

*los1<sup>-</sup> arc1<sup>-</sup>* and *mes1<sup>-</sup> arc1<sup>-</sup>* yeast strains transformed with the desired plasmid(s) were grown overnight to 40 hours in YNB medium containing all the necessary amino acids and bases including uracil. For counter selection for cells containing *URA3* plasmids, the cultures were typically diluted to an optical density at 600 nm of 1 and 50 μl were plated on a YNB agar plate containing uracil, 5'-fluoroorotic acid (FOA; Euromedex) at 1 mg/ml and the necessary amino acids and bases except those used for selection. Plates were incubated for four to six days at 28 °C.

### Yeast growth studies in liquid medium

Overnight pre-culture of the desired yeast strain was performed at 28 °C in YNB medium containing all the necessary amino acids and bases. Cultures were performed at the desired temperature in YPG containing adenine and tryptophan (if necessary) and were inoculated with YNB pre-culture. After 12–15 hours, aliquots were removed and absorbances at 600 nm were measured. For each culture, the absorbance at 600 nm was plotted as a function of time and data were fitted with a single-exponential equation using KaleidaGraph Software. The doubling-time ( $\tau$ ) was calculated from the parameter of the exponential fit. To be able to compare results of experiments performed at different days, they were normalized using one strain as reference.

### Yeast growth studies on agar plate

Overnight culture of the desired yeast strain was grown at 28 °C in YNB medium containing all the necessary amino acids and bases. Absorbances at 600 nm were measured and cultures were diluted to an absorbance of 0.3. Serial dilutions were made with a dilution factor of 4. For each culture and dilution, a 6 μl drop was deposited on a YPG plate containing adenine and tryptophan. Plates were incubated at the temperature and for the time specified in the text.

### p43 immunoprecipitation

Cells were grown in 150 ml of YNB (or YPGal for ArgRS expression) to an optical density at 600 nm of 1. Cultures were centrifuged, and cells were washed with extraction buffer (100 mM Tris-HCl (pH 8.0), 10 mM MgCl<sub>2</sub>, 1 mM DTT) and resuspended in 0.6 ml of extraction buffer. Cells were broken mechanically with glass beads, centrifuged, and supernatants were collected and kept on ice. A 2 μM solution of His-tagged human p43<sup>30</sup> in A50T buffer (8 mM Na<sub>2</sub>HPO<sub>4</sub>, 15 mM KH<sub>2</sub>PO<sub>4</sub>, 2.7 mM KCl, 50 mM NaCl, 0.1% (v/v) Triton X-100) containing 0.2% (w/v) of bovine serum albumin was prepared. A total of 100 μl of yeast extract was incubated for 30 minutes at 4 °C with 50 μl of His-tagged p43 solution or with 50 μl of A50T-BSA buffer (control). A pre-adsorption step was performed: 10 μl of a 6.8 mg/ml solution of goat IgG was added to each sample. After 15 minutes at 4 °C, 20 μl of a 1 : 1 slurry of protein A-Sepharose (Amersham Biosciences) in A50T buffer was added. After 30 minutes at 4 °C, samples were pelleted by centrifugation and supernatants were recovered. The specific immunoprecipitation step was conducted by the addition of 10 μl of a 100 μg/ml of penta-His antibody (Qiagen). After one hour at 4 °C, 25 μl of a 1 : 1 slurry of protein A-Sepharose in A50T buffer were added. After 30 minutes at 4 °C, samples were pelleted by centrifugation and supernatants removed. Pellets were washed five times with 1 ml of A100T buffer (A50T containing 100 mM NaCl) and once with 1 ml of 10 mM Tris-HCl (pH 7.5). Samples were analyzed by SDS-PAGE on a 10% (w/v) polyacrylamide gel.<sup>51</sup> ScMetRS, ScGluRS, hamster ArgRS and OsMetRSΔ(M + C)-(M + C)p43 proteins were detected by Western blotting using antibodies raised against ScMetRS or ScGluRS (gifts from Franco Fasiolo, Strasbourg, France), against the catalytic domain of hamster ArgRS,<sup>49</sup> or against the C-terminal domain of p43.<sup>30</sup>

### Interaction of ProtA-Arc1p with MetRS and GluRS

*arc1<sup>-</sup> mes1<sup>-</sup>* cells co-expressing ProtA-Arc1p and ScMetRS (interaction of Arc1p with ScMetRS and ScGluRS) or OsMetRSΔ(M + C)-(M + C)p43 were grown overnight in 150 ml of YNB. Negative controls were performed with cells expressing one of the two MetRS (ScMetRS or OsMetRSΔ(M + C)-(M + C)p43) only (no co-expression of ProtA-Arc1p). Yeast extracts were prepared as described above; 100 μl of extract were incubated for one hour with 50 μl of IgG-Sepharose (Amersham Biosciences). After one hour at 4 °C, samples were centrifuged and supernatants removed. Pellets were washed five times with 1 ml of A100T buffer and once with 1 ml of 10 mM Tris-HCl (pH 7.5). Samples were analyzed by SDS-PAGE on a 10% polyacrylamide gel. Proteins were detected by Western blotting.

### Aminoacylation assay

OsMetRSΔ(M + C) and p43 were purified as described.<sup>16,30</sup> OsMetRSΔ(M + C) (0.1 μM) was pre-incubated at 4 °C for 20 minutes with p43 (0, 0.1 or 1.0 μM) in 10 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, and bovine serum albumin at 4 mg/ml. Time course of aminoacylation of tRNA<sub>i</sub><sup>Met</sup> was conducted at 25 °C. tRNA aminoacylation was performed in 20 mM imidazole-HCl buffer (pH 7.5), 100 mM KCl, 0.5 mM DTT, 7 mM MgCl<sub>2</sub>, 2 mM ATP, 60 μM <sup>14</sup>C-labeled



methionine (NEN; 50 Ci/mol) and non-saturating amounts of tRNA (1  $\mu$ M) as described.<sup>16</sup> Homogeneous yeast tRNA<sub>i</sub><sup>Met</sup> obtained by *in vitro* transcription<sup>16</sup> (methionine acceptance of 1150 pmol/*A*<sub>260</sub>) was used as tRNA substrate. The reaction mixture contained catalytic amounts of OsMetRSΔ(M + C) (10 nM) and increasing amounts of human p43 (0, 10 or 100 nM). At different time intervals, aliquots were withdrawn, quenched in ice-cold 5% (w/v) trichloroacetic acid, filtered on GF/C filters (Whatman) and processed for liquid scintillation counting.

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