Steady-state levels of imported tRNAs in *Chlamydomonas* mitochondria are correlated with both cytosolic and mitochondrial codon usages

Elizaveta Vinogradova[1,2], Thalia Salinas[1,3], Valérie Cognat[1], Claire Remacle[3,*] and Laurence Maréchal-Drouard[1,*]

[1]Institut de Biologie Moléculaire des Plantes, Unité Propre de Recherche 2357, associated with Université Louis Pasteur, Centre National de la Recherche Scientifique, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France, [2]Faculty of Biology, Lomonosov Moscow State University, Leninskie Gory, Moscow 199991, Russia and [3]Génétique des microorganismes, Institut de Botanique B22, Université de Liège, B-4000 Liège, Belgium

ABSTRACT

The mitochondrial genome of *Chlamydomonas reinhardtii* only encodes three expressed tRNA genes, thus most mitochondrial tRNAs are likely imported. The sharing of tRNAs between chloroplasts and mitochondria has been speculated in this organism. We first demonstrate that no plastidial tRNA is present in mitochondria and that the mitochondrial translation mainly relies on the import of nucleus-encoded tRNA species. Then, using northern analysis, we show that the extent of mitochondrial localization for the 49 tRNA isoacceptor families encoded by the *C. reinhardtii* nuclear genome is highly variable. Until now the reasons for such variability were unknown. By comparing cytosolic and mitochondrial codon usage with the sub-cellular distribution of tRNAs, we provide unprecedented evidence that the steady-state level of a mitochondrial tRNA is linked not only to the frequency of the cognate codon in mitochondria but also to its frequency in the cytosol, then allowing optimal mitochondrial translation.

INTRODUCTION

To compensate the evolutionary loss of mitochondrial tRNA genes, import of nucleus-encoded tRNAs into mitochondria occurs in all eukaryotic kingdoms (1). An intriguing aspect of tRNA import concerns the proportion of nucleus-encoded tRNA found in mitochondria. It has long been believed that only a few percent of a cytosolic tRNA is present in mitochondria (2). However, some evidence supports the idea that a differential distribution of nucleus-encoded tRNAs between the cytosol and mitochondria does exist. Indeed, in *Leishmania tarentolae*, some tRNAs can be classified as mainly cytosolic and others as mainly mitochondrial (3,4). In wheat mitochondria tRNA^{Leu}UAA was shown to be in higher amount in mitochondria than in the cytosol (5). So far, such a difference in the cytosolic and mitochondrial localization of tRNAs has not been carefully studied, as well as the basis for that.

The mitochondrial genome of *Chlamydomonas reinhardtii* only encodes three tRNAs (6). The idea that, in this organism, plastids can provide tRNAs to mitochondria has been put forward (7,8). Here, we first show that no plastidial tRNA is imported into *Chlamydomonas* mitochondria and that most of the mitochondrial tRNAs are nucleus-encoded and imported from the cytosol. As both cytosolic and mitochondrial codon usages are highly and differentially biased (6,9) (Figure 1), we thought that this alga was the appropriate model to answer the following question: is the efficiency of the nucleus-encoded tRNA import into mitochondria adapted to the need of the mitochondrial translation machinery? Here the sub-localization of the tRNAs representing the 49 isoacceptor families encoded by the *C. reinhardtii* nuclear genome (9) and their extent of import into mitochondria were analyzed. We propose that the mitochondrial localization of tRNA isoacceptors is tightly linked to both the nuclear and the mitochondrial codon usages.

*To whom correspondence should be addressed. Tel: +32 4366 3812; Fax: +32 4366 2960; Email: c.remacle@ulg.ac.be
Correspondence may also be addressed to Laurence Maréchal-Drouard. Tel: +33 388 417 244; Fax: +33 388 614 442;
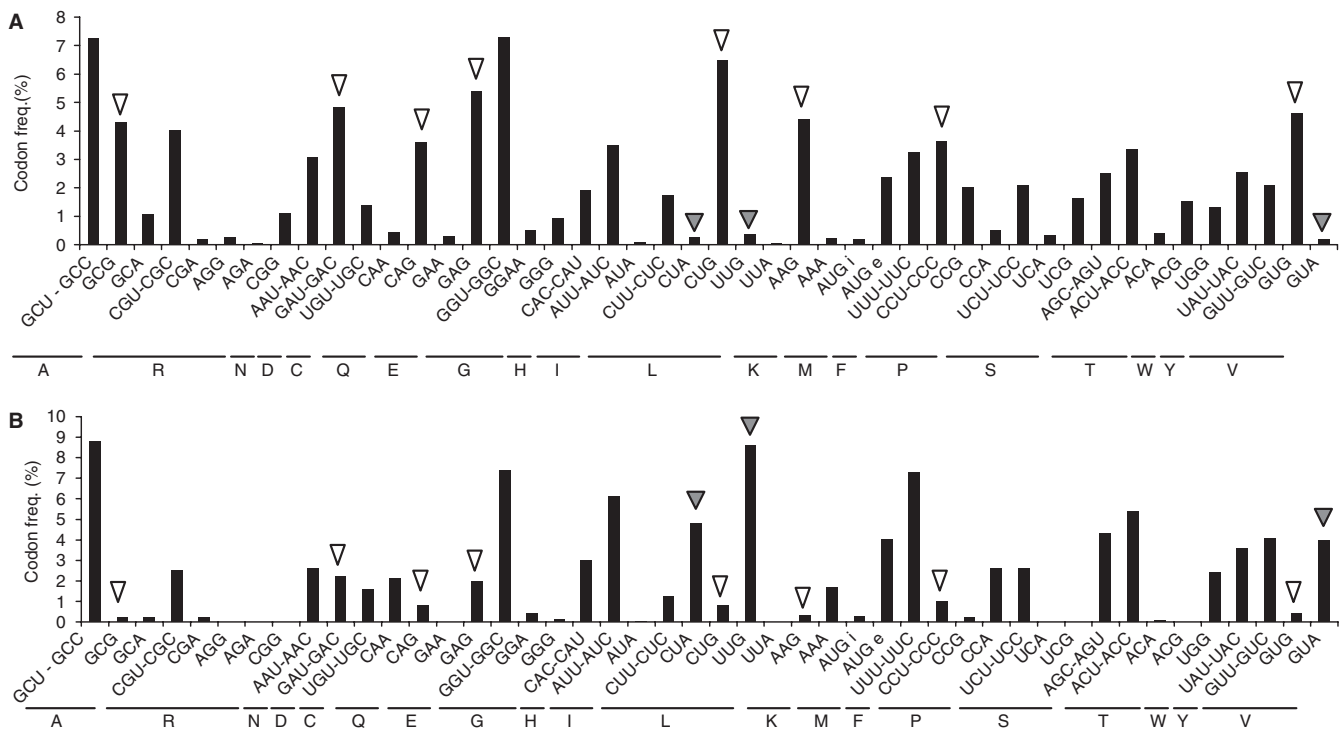Email: laurence.drouard@ibmp-ulp.u-strasbg.fr

Figure 1. Histograms for codon frequency in *C. reinhardtii*. (**A**) Cytosolic codon usage. (**B**) Mitochondrial codon usage. For (A) and (B) codons have been grouped according to classical wobble rules and aminoacids are represented with the one-letter code. White (high frequency in the cytosol but weak in the mitochondria) and grey (low frequency in the cytosol but high in the mitochondria) arrow heads indicate some codons differentially used in the cytosol and mitochondria.

MATERIALS AND METHODS

Purification of mitochondria and chloroplasts

Crude mitochondrial fraction was isolated from the cell-wall-less mutant *cw15 mt*[+] by digitonin treatment according to (10). The mitochondrial fraction was then loaded on a discontinuous Percoll gradient (13%/21%/45%). Purified mitochondria were recovered at the 45/21 interface and washed two times in MET buffer (Mannitol 280 mM, Tris–HCl pH 7 10 mM, EDTA 0.5 mM, 0.1% BSA) by 10 min centrifugation at 11 000 *g*. Chloroplast fraction was recovered according to ref. (11).

Total, mitochondrial and plastidial RNA isolation

Total tRNA extract was prepared from whole cells as described in ref. (12). For mitochondrial and plastidial RNA preparation, the same method was used, but because of the low complexity of the RNA population, the LiCl precipitation step was omitted.

Northern analysis and quantitation of tRNA import

Hybridization of oligonucleotides (Supplementary data, Table S1) to northern blots of total, mitochondrial or plastidial RNAs from *C. reinhardtii* were performed as in ref. (9). Signals obtained on a phosphorimager were quantified using the MacBas software. For quantitation, RNA fractions were analyzed by northern blots such that hybridization signal obtained with each specific probe was linear with respect to the amount of RNA loaded on the gel. To determine the percentage of a given tRNA localized in mitochondria, we first determined the fraction of the three mitochondria-encoded tRNAs present in total (i.e. whole cell) tRNA fraction. Hybridization of 3 µg of total or mitochondrial tRNA fractions with probes directed against the three mitochondria-encoded tRNAs (Figure 2B) have shown that 4% of the whole-cell fraction is mitochondrial. In other words, 3 µg of mitochondrial tRNAs are extracted from 25 times more cells than 3 µg of total tRNAs. This value allowed us to normalize the signal for a given tRNA in total and mitochondrial tRNA fractions with respect to the same number of cells. Then, the extent of mitochondrial localization was estimated by calculating the percentage of a given tRNA in the mitochondrial fraction relative to the abundance of that RNA in the whole cell fraction as follow: 100× (Phosphorimager units in mitochondrial fraction)/(Phosphorimager units in total fraction × 25) = 4× (Phosphorimager units in mitochondrial fraction)/(Phosphorimager units in total fraction). For tRNA[Ala] isoacceptors hybridizing to the same oligonucleotides (Table 1), RT–PCR followed by sequence analysis of clones allowed us to calculate the percentage of each isoacceptor among the whole-cell and mitochondrial tRNA populations.

Cloning and sequencing of RT–PCR products

Transfer RNAs purified on 15% denaturing polyacrylamide gel (12) were used as substrate for RT–PCR amplification and cloned into PCR-2-TOPO (Invitrogen).
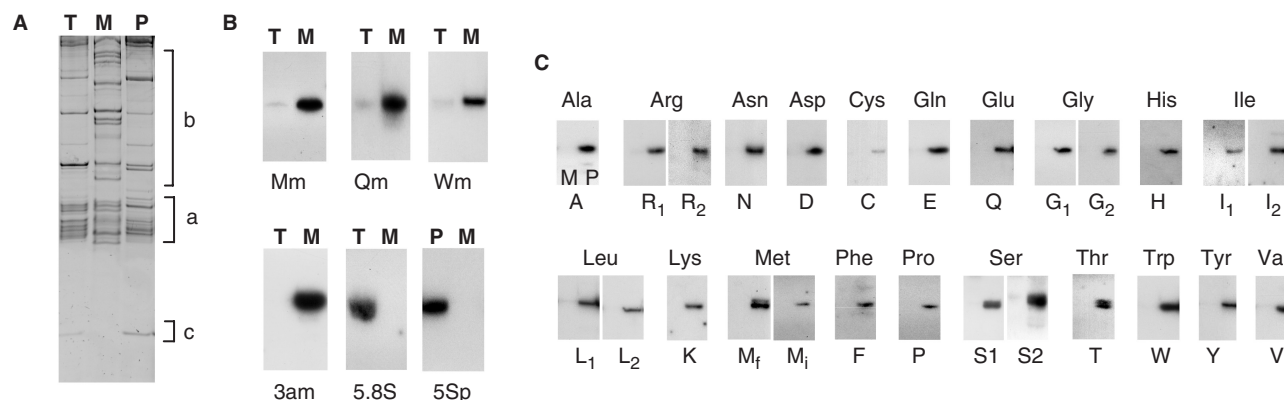
Figure 2. Mitochondria of *C. reinhardtii* do not import plastidial tRNAs. (**A**) Whole-cell (T), mitochondrial (M) and plastidial (P) RNA fractions separated on polyacrylamide gel and stained with ethidium bromide. Regions corresponding to tRNAs (a), rRNAs (b) and plastidial 3S rRNA (c) are indicated. (**B**) Hybridization of probes specific to mitochondria-encoded tRNA^{Met} (Mm), tRNA^{Gln} (Qm) and tRNA^{Trp} (Wm) and to mitochondrial 3a rRNA (3am), cytosolic 5.8S rRNA (5.8S), plastidial 5S rRNA (5Sp) to northern blots of whole-cell (T), mitochondrial (M) or plastidial (P) RNAs. (**C**) Hybridization of probes specific to plastidial tRNA genes to northern blots of mitochondrial (M) and plastidial (P) RNAs. Name of probes is given below each northern experiment (see Supplementary data Table S1 online).

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All available sequence data for *C. reinhardtii* used in this paper were previously described (9,13,14). This information was used to design oligonucleotide probes specific for the corresponding rRNAs and tRNAs (Supplementary data, Table S1).

RESULTS

The three tRNA genes encoded by the mitochondrial genome are expressed

As a first step towards the analysis of the *C. reinhardtii* mitochondrial tRNA set, mitochondrial RNA was extracted. This RNA fraction (Figure 2A) mainly contains a dozen of RNA fragments corresponding to ribosomal RNA (rRNA) pieces (13) and tRNAs. The mitochondrial tRNA profile was different to the profile observed with whole cell or plastidial tRNA fractions. Northern blots (Figure 2B) indicate that the three mitochondrial tRNA genes are expressed. Whereas a probe directed against the mitochondrial L3a rRNA [one of the 10 rRNA fragments constituting the large rRNA; (13)] gave a strong signal, almost no cytosolic 5.8S rRNA or plastidial 5S rRNA (<0.15%) was detected in the mitochondrial fraction. Thus this fraction is not significantly contaminated either by cytosolic or by plastidial RNAs and can be considered as a true mitochondrial fraction.

Plastidial tRNAs are not present in *C. reinhardtii* mitochondria

With only three mitochondria-encoded tRNAs, tRNA import is a prerequisite for the *Chlamydomonas* mitochondrial protein synthesis. While the import of nucleus-encoded tRNAs into mitochondria is a wide spread phenomenon (1), previous genetic studies suggest that, in this alga, chloroplasts could provide mitochondria with tRNAs (7). To address the question of the nuclear and/or plastidial origin of the imported tRNAs, we first performed northern experiments with probes specific to each plastidial tRNA (Supplementary data, Table S1). For each probe, a signal was detected in the plastidial RNA fraction whereas no signal was obtained in the mitochondrial one (Figure 2C), thus demonstrating that the mitochondrial translation machinery does not rely on the import of plastidial tRNAs.

The steady-state levels of imported tRNAs found in mitochondria are highly variable

The above data suggest that the mitochondrial tRNA population mainly consists of nucleus-encoded tRNA species. Northern blots containing known amounts of the whole-cell and mitochondrial tRNA fractions hybridized with oligonucleotide probes specific for each cytosolic tRNA (Supplementary data, Table S1) allowed us to determine their sub-cellular distribution (Figure 3A). For each tRNA, we calculated the ratio between its steady-state level in mitochondria and in the whole-cell fraction (Figure 3B and Table 1). The most striking aspect of this analysis is the high variation of the partitioning among the tRNAs (from 0% to 98%). The following conclusions can be drawn.

(i) If we assume that a percentage less than or equal to 0.15% (the maximal level of 5.8S rRNA cytosolic marker estimated in the mitochondrial fraction) is most likely the result of cytosolic contamination, then 15 tRNAs can be considered as cytosol-specific.

(ii) For 31 tRNAs, the extent of mitochondrial localization ranges from 0.2% to 26%. This reflects the important fluctuation of the level of nucleus-encoded tRNAs present in mitochondria.

(iii) For three tRNAs, the percentage is equal or above 80%. Up to now, in all organisms in which tRNA import has been studied, no nucleus-encoded tRNA has been localized exclusively in

Table 1. Steady-state levels of imported tRNAs in mitochondria relative to their abundance in the cytosol in *Chlamydomonas reinhardtii*

Amino acids	Anticodons	Codons	% cyto	% mito[a]	mito/cyto[b]	Probes	%[c]
Ala	AGC	GCU-GCC	7.24	8.84	1.22	A1	8.8
	CGC	GCG	4.29	0.19	0.44	A1-A2	0.2
	UGC	GCA	1.06	0.23	0.22	A1-A2	
Arg	CCG	CGG	1.1	0	0.00	R1	0.10
	ACG	CGU-CGC	4.04	2.49	0.62	R2	0.15
	CCU	AGG	0.27	0	0.00	R3	0.1
	UCG	CGA	0.19	0.1	0.53	R4	0.65
	UCU	AGA	0.07	0	0.00	R5	0
Asn	GGU	AAU-AAC	3.09	2.59	0.84	N	3.5
Asp	GUC	GAU-GAC	4.83	2.24	0.46	D	1.6
Cys	GCA	UGU-UGC	1.39	1.55	1.11	C	3.5
Gln	UUG	CAA	0.43	2.07	4.76	Q1	0.1
	CUG	CAG	3.6	0.84	0.23	Q2	2.8
Glu	UUC	GAA	0.28	0	0.00	E1	0.5
	CUC	GAG	5.41	2.04	0.38	E2	3.5
Gly	GCC	GGU-GGC	7.27	7.32	1.01	G1	3.6
	UCC	GGA	0.5	0.42	0.84	G2	4.2
	CCC	GGG	0.94	0.1	0.11	G3	0.15
His	GUG	CAC-CAU	1.9	2.94	1.55	H	6.5
Ile	AAU	AUU-AUC	3.49	6.09	1.74	I1	4.7
	UAU	AUA	0.1	0.03	0.3	I2	0.1
Leu	CAG	CUG	6.48	0.87	0.13	L1	0.3
	CAA	UUG	0.39	8.52	21.8	L2	85
	UAA	UUA	0.06	0	0.00	L3	0.6
	UAG	CUA	0.26	4.73	18.19	L4	19
	AAG	CUU-CUC	1.73	1.33	0.77	L5	0.2
Lys	CUU	AAG	4.42	0.32	0.07	K1	2.1
	UUU	AAA	0.24	1.68	7.00	K2	98
Met	CAU	AUG i	0.21	0.27	1.3	Mi	0.1
	CAU	AUG e	2.38	4.07	1.23	Me	1.9
Phe	GAA	UUU-UUC	3.24	6.99	2.17	F	4.4
SeC	UCA	UGA	nd	0	0.00	SeC	0
Pro	AGG	CCU-CCC	3.65	1	0.27	P	0.2
	CGG	CCG	2.02	0.16	0.08	P	
	UGG	CCA	0.52	2.62	5.04	P	
Ser	AGA	UCU-UCC	2.09	2.66	1.26	S1	0.1
	UGA	UCA	0.32	0	0.00	S2	0.1
	CGA	UCG	1.63	0	0.00	S3	0.15
	GCU	AGC-AGU	2.52	4.31	1.72	S4	0.4
Thr	AGU	ACU-ACC	3.35	5.37	1.61	T1	26
	CGU	ACG	1.55	0	0.00	T2	0.1
	UGU	ACA	0.41	0.06	0.15	T3	0.7
Trp	CCA	UGG	1.31	2.4	1.81	W	0
Tyr	GAC	UAU-UAC	2.56	3.56	1.39	Y	10.6
Val	AAC	GUU-GUC	2.1	4.3	2.04	V1	4.5
	CAC	GUG	4.61	0.39	0.08	V2	0.15
	UAC	GUA	0.21	4.02	19.14	V3	80

Nuclear tRNA gene content (identified by anticodons), minimal potential codon recognition pattern (codons), cytosolic codon usage (% cyto) and name of probes are given as in (9), (see also supplementary data, Table S1 online).
[a]Mitochondrial codon usage (% mito) is according to (6).
[b]Ratio codon frequency (mito/cyto).
[c]The steady-state levels of imported tRNAs relative to their abundance in the cytosol are given in percentage. Mean values are given (see legend of Figure 3 for detailed explanations).

mitochondria. With an import level of 98%, tRNA[Lys]UUU can be considered as the first example of such a tRNA.

(iv) Comparing tRNA isoacceptors, the extent of mitochondrial localization is highly variable. For example, tRNA[Leu]CAG and tRNA[Val]CAC are mainly cytosol-specific whereas tRNA[Leu]CAA and tRNA[Val]UAC are mainly localized in mitochondria.

No correlation between the amount of a given tRNA in the cytosol and the extent of its mitochondrial localization was found.

A substantial variation of the distribution of cytosolic tRNAs in mitochondria was described in wheat (5) or *Leishmania* (3,4), but such a detailed analysis on the important variability of the mitochondrial localization of
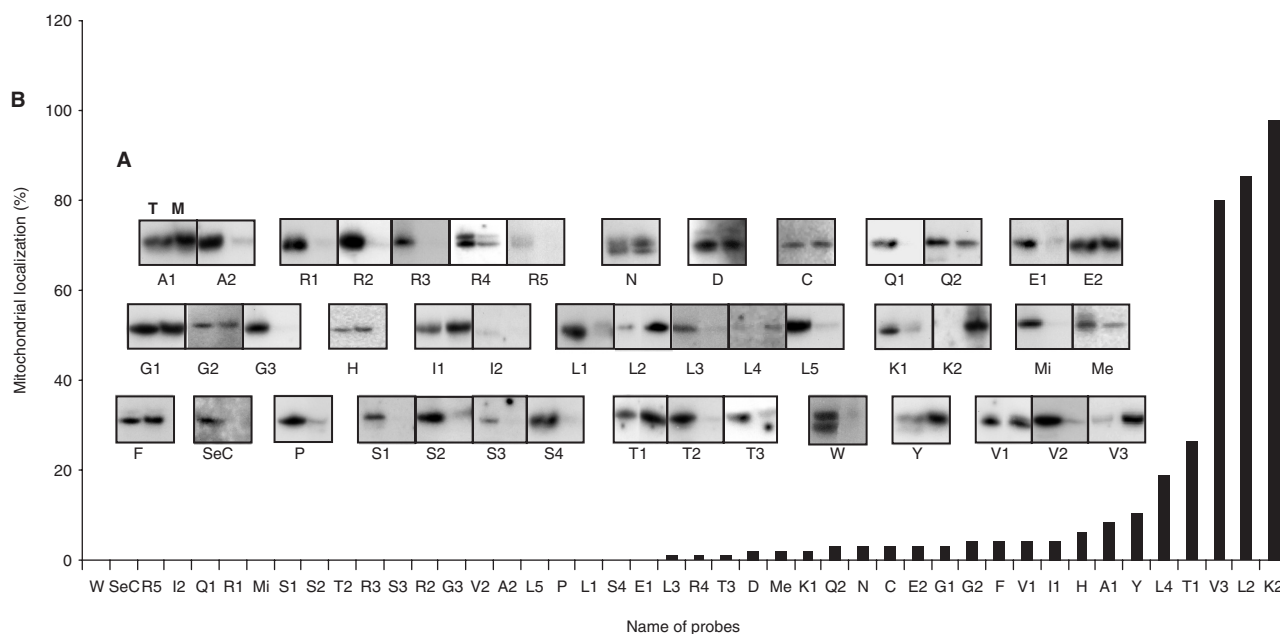
Figure 3. Expression and sub-localization of the nucleus-encoded tRNAs of *C. reinhardtii*. (**A**) Whole-cell RNA fraction (T) and mitochondrial RNA fraction (M) separated on polyacrylamide gel and hybridized against probes specific to cytosolic tRNA genes (Table 1 and Supplementary data, Table S1). Name of probes is given below each northern experiment. (**B**) Extent of mitochondrial import for each cytosolic tRNA in percentage. For each tRNA, two to four independent northern blot experiments were performed. Three and four independent total and mitochondrial RNA preparations were used for the whole set of northern experiments. For each probe, a typical experiment is presented in (A) but the percentages of extent of mitochondrial import given in (B) are the mean values obtained with two to four independent northern blot experiments. Error bars are not presented on the histogram because, at this scale, they will not be visible for most tRNAs. The standard deviations observed are usually less than 5%. Only for two probes, Y and Me, a higher deviation of around 10% was observed.

nucleus-encoded tRNAs was never reported. We then addressed the question of the reasons for such a differential import.

Biological significance for the differential import of cytosolic tRNAs into mitochondria

The three mitochondria-encoded tRNA^{Trp}, tRNA^{Gln}UUG and tRNA^{Met} genes are expressed in *Chlamydomonas* mitochondria. As expected, cytosolic tRNA^{Trp} and tRNA^{Gln}UUG are not in the organelle. In contrast, the second cytosolic tRNA^{Gln} isoacceptor, tRNA^{Gln}CUG, is imported into mitochondria. The mitochondria-encoded tRNA^{Met} gene encodes an elongator tRNA^{Met-e}. An initiator tRNA^{Met-i} and an elongator tRNA^{Met-e} are expressed from *C. reinhardtii* nuclear DNA (9). Surprisingly, cytosolic tRNA^{Met-i} is not brought into mitochondria whereas cytosolic tRNA^{Met-e} is.

In *Chlamydomonas* mitochondria, the codon distribution is highly biased: seven codons occur very infrequently and nine codons are not used (Figure 1) (6). In the cytosol of *Chlamydomonas*, selenocysteine is inserted into selenoproteins by a tRNA^{Sec} that recognizes the UGA stop codon (15). In *Chlamydomonas* mitochondria, there is no UGA stop codon and no selenoproteins, and, as expected, tRNA^{Sec} remains in the cytosol. The other unused codons are recognized by tRNA^{Leu}UAA, tRNA^{Ser}UGA, tRNA^{Ser}CGA, tRNA^{Thr}CGU, tRNA^{Glu}UUC, tRNA^{Arg}CCG, tRNA^{Arg}CCU and tRNA^{Arg}UCU. These tRNAs, *a priori* not required by the mitochondrial

translation machinery, are either cytosol-specific or very inefficiently addressed to the organelles.

In the mitochondrial genome, 77 Arg codons out of 80 are CGU and CGC. These codons must be read, according to wobble rules, by the cytosolic tRNA^{Arg}ACG, assuming that the A is post-transcriptionaly modified in inosine. Surprisingly, the tRNA^{Arg}ACG is mostly exclusively in the cytosol. In mammalian mitochondria, tRNA^{Arg}UCG is sufficient for reading all four CGN codons (16). Whether the tRNA^{Arg}UCG present in *Chlamydomonas* mitochondria is sufficient for reading all arginine codons will need to be addressed. Similarly, two tRNA isoacceptor families (tRNA^{Pro} and tRNA^{Ser}) gave unexpected data: the amount of imported tRNA was very low whatever the isoacceptor tRNA considered. In order to assess this result, probes covering other regions of tRNA^{Pro} and tRNA^{Ser} isoacceptors were used but gave similar results (data not shown).

Among the other tRNA isoacceptor families, the extent of mitochondrial localization is highly variable and there is no correlation between the abundance of a given tRNA in the cytosol and the extent of its mitochondrial localization. For some of them, it was just the opposite: although a tRNA was present in large amount in the cytosol (e.g. tRNA^{Leu}CAG or tRNA^{Val}CAC), the level of imported tRNA in mitochondria was very low whereas tRNAs present at low concentration in the cytosol (e.g. tRNA^{Leu}CAA or tRNA^{Val}UAC) could be highly found in mitochondria. For some tRNA isoacceptors, the preferential mitochondrial localization is clearly linked to the high frequency of
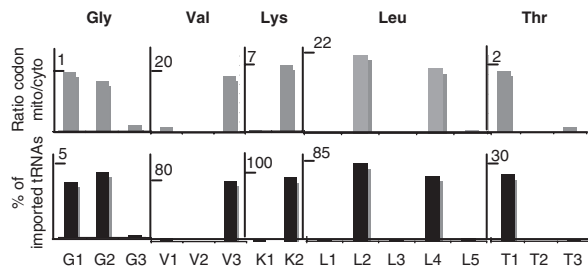
Figure 4. Link between codon usages and the steady-state levels of imported tRNAs. Histograms (in grey) showing the ratios mitochondrial codon frequency/cytosolic codon frequency (mito/cyto) for five aminoacids (Gly, Val, Lys, Leu, Thr) and histograms (in black) showing the percentages of the steady-state levels of imported tRNA isoacceptors into mitochondria relative to their abundance in the whole-cell fraction. Name of tRNAs is as in Figure 3.

the corresponding codon in the organelle (e.g. tRNA^{Leu}CAA). However, the level of different imported tRNAs relative to their abundance in the whole-cell fraction can be within the same range of magnitude although the frequency of the mitochondrial codons they can recognize greatly differs (e.g. tRNA^{Gly}GCC versus tRNA^{Gly}UCC). Looking for other reasons to explain that, the ratio mitochondrial codon frequency/cytosolic codon frequency was calculated for codons read by tRNA^{Gly}, tRNA^{Leu}, tRNA^{Lys}, tRNA^{Thr} and tRNA^{Val} isoacceptors (Figure 4A). A strong link between the extent of their mitochondrial localization and the ratio was observed. When this ratio is high, meaning that the corresponding codons are frequently used in mitochondria as compared to the cytosol, then the percentage of imported tRNAs is high. In contrast, this percentage is low when the corresponding codons are rare in mitochondria but often used in the cytosol. Thus, the level of imported tRNAs is linked not only to the mitochondrial codon usage, but also to the cytosolic one.

DISCUSSION

The *Chlamydomonas* mitochondrial translation machinery does not rely on the import of plastidial tRNAs and depends on the import of nucleus-encoded tRNAs. Out of 49, 31 cytosolic tRNA isoacceptors (representing all amino acids but one, tryptophan) were found in mitochondria. This process is quite specific and a few nucleus-encoded tRNAs not required by the mitochondrial translation machinery are not or not significantly detected in *Chlamydomonas* mitochondria. These data are in agreement with what was observed so far: only essential nucleus-encoded tRNAs are present within mitochondria (1).

However, a few interesting discrepancies are worth to be discussed. In higher plant mitochondria, a single tRNA^{Gln}, with a UUG anticodon, is sufficient to decode the two CAA and CAG glutamine codons (16). The presence of two isoaccepting tRNA^{Gln} (with the CUG and UUG anticodons) in *Chlamydomonas* mitochondria thus raises the question of their apparent redundancy in term

of codon recognition. The most likely explanation is that the mitochondria-encoded tRNA^{Gln}UUG contains a modified U in the 'wobble' position so as to prohibit the recognition of the CAG codon.

Concerning tRNA^{Met}, there is, on one hand, an apparent redundancy of two potential tRNA^{Met-e} and, on the other hand, an apparent lack of a tRNA^{Met-i} in *Chlamydomonas* mitochondria. Bacterial and organellar tRNA^{Met-i} are only functional when carrying a formylated methionine. The formyl group is added to the methionine of the charged tRNA^{Met-i} by a methionyl–tRNA^{Met} formyltransferase. Such an enzyme is likely present in *Chlamydomonas* mitochondria (Supplementary data, Figure S1) suggesting that one out of the two tRNA^{Met-e} is formylated within the organelle. A similar situation was found in *Trypanosoma brucei* where no mitochondrial tRNA^{Met} genes exist. In this organism, whereas the eukaryotic tRNA^{Met-i} remains exclusively in the cytosol, a fraction of the cytosolic tRNA^{Met-e} becomes formylated after import into mitochondria and is then used as initiator (17).

According to the mitochondrial codon usage, the CCA proline codon and the UCY and AGY serine codons are frequently used but the corresponding nucleus-encoded tRNAs were not found in the organelle. Obviously, *Chlamydomonas* mitochondrial translation requires a minimal set of one tRNA^{Pro} and two tRNA^{Ser} but these tRNAs remain to be identified. Four possibilities may be hypothesized to explain these results. First, we cannot exclude that our northern experiments failed to detect these tRNAs. However, the use of several independent probes for the same tRNA isoacceptor makes this hypothesis unlikely to occur. Second, we could hypothesize that the same set of tRNAs is not always present in the organelle because *Chlamydomonas* mitochondrial genes would not be expressed at the same time or would not use the same codon usage. This hypothesis is not correct because, with the exception of the reverse-transcriptase like gene, the seven other mitochondrial genes encode proteins of respiratory complexes which must be expressed at the same time. Furthermore, the codon usage pattern of the eight mitochondrial genes is mostly identical (6). Third, a single copy gene can be sufficient to produce a tRNA mostly localized in mitochondria [e.g. tRNA^{Val} UAC, tRNA^{Lys}AAA; (9)]. Thus, we cannot exclude that some nuclear tRNA genes, giving raise to tRNAs mostly present in mitochondria, may have escaped detection. In this context, it is worth to note that very recent findings such as the existence of permuted tRNA genes, split tRNA genes or tRNA genes with multiple introns demonstrate that tRNA genes may easily escape detection (18). Finally, as shown in *Leishmania* (19) editing events modifying the anticodon of imported tRNAs also represent a possibility to explain this discrepancy, although editing has not yet been demonstrated in *Chlamydomonas* mitochondria. To clarify the situation, the precise characterization of the tRNA^{Ser} and tRNA^{Pro} species used during mitochondrial translation in *Chlamydomonas* will need to be assessed. In this respect, fractionation on 2D-gel of a large amount of *Chlamydomonas* mitochondrial tRNA

extract will provide material to identify by aminoacylation tests the tRNAs of interest.

Up to now, only tRNAs of eukaryotic origin were shown to be imported into mitochondria. The presence of nucleus-encoded tRNAs mainly localized in mitochondria raises the question of their origin. Do they correspond to prokaryotic tRNA genes that were once inserted into the nuclear genome and are now expressed and addressed back to the organelle? Phylogenetic trees show that these tRNAs are not related to prokaryotic tRNAs but to eukaryotic ones (9). Thus, in course of evolution, a very few nuclear tRNA genes of eukaryotic origin have been recruited exclusively to provide mitochondria with essential tRNAs.

In *Chlamydomonas*, the cytosolic tRNA abundance is adjusted to the codon usage (9). Here, in most cases, we found a good correlation between the set of nucleus-encoded tRNAs present in mitochondria and the mitochondrial codon usage. This reveals an unprecedented degree of adjustment of the tRNA populations not only within the cytosol but also within the mitochondria of this green alga. In the trypanosomatid *L. tarentolae* preferential import of some tRNAs has been observed (3,4) but whether the abundance of the imported tRNAs is correlated to the frequency of the cognate codons in this organism has not been studied yet. In another trypanosomatid, *T. brucei*, the extent of mitochondrial localization varies from 1 to 7.5% for the 15 nucleus-encoded tRNAs studied but, contrary to what we observed in *Chlamydomonas*, no correlation between the differential import efficiencies and the mitochondrial codon usage was found (2). Another piece of evidence suggesting that the tRNA mitochondrial import pathway is regulated comes from studies on the *S. cerevisiae* mitochondrial tRNA^{Lys}. Import of this tRNA is essential under stress conditions (20). It is probably too early to draw conclusions but we can wonder whether the level of regulation of tRNA mitochondrial import depends on the mechanism involved and on the organism considered. In *Chlamydomonas*, with regards to the mitochondrial localization of tRNA, the system seems to be able to adapt relatively easily not only by acquiring the possibility to bring new cytosolic tRNA species into mitochondria depending on the set of mitochondrial tRNA genes, but also by regulating the abundance of each tRNA so as to optimize mitochondrial translation. The steady-state level of a nucleus-encoded tRNA in mitochondria is mainly determined by two parameters: its import efficiency and its rate of degradation in the mitochondria. The question of mitochondrial tRNA stability has not often been addressed. Whereas cytosolic tRNA^{Gly} isoacceptors are differentially localized *in vivo* in dicotyledonous plant mitochondria, no difference in their stability has been seen *in vitro* in potato mitochondria (21). In *T. brucei*, no significant differences in the kinetics of degradation of several tRNAs were observed (2). Thus, it seems that the abundance of a nucleus-encoded tRNA in mitochondria depends on its import efficiency rather than on its degradation rate once inside the mitochondrial matrix. A strong correlation between the abundance of the nucleus-encoded tRNAs and codon usage is observed in

Chlamydomonas mitochondria, and we now need to understand how such a correlation can be established. Two major hypotheses, which are not exclusive, can be proposed. First, this correlation can be a result of co-evolution between the mitochondrial genome and tRNA import processes. As previously noted, in *Chlamydomonas*, during evolution, the number of tRNA genes has been adapted so as to adjust the cytosolic tRNA abundance to the codon usage (9). It is also worth to mention that, in higher plants, the set of nucleus-encoded tRNA species found in mitochondria is complementary to the set of the mitochondria-encoded tRNA species and both the mitochondria-encoded and nucleus-encoded tRNA populations vary in parallel in different species and maintain the complementarity between the two tRNA sets (22). These variations are likely to be the result of selective pressure during evolution. In the same line, we can speculate that the steady-state levels of imported tRNAs in *Chlamydomonas* mitochondria result from evolution. A second equally attractive explanation which also should be explored, is that the mitochondrial genome codes for factors that regulate tRNA import. In the latter case, fine-tuning of tRNA import would be necessary to rapidly and efficiently adapt the tRNA population to the needs of *Chlamydomonas* mitochondria. Such regulation requires feed back control from the mitochondria to the cytosol by unknown factors and has not been observed so far. Whether tRNAs themselves or other factors play an important role, and if so how, is a crucial question to be solved. The availability of efficient mitochondrial transformation in *Chlamydomonas* (23) will provide an invaluable tool for manipulating the mitochondrial tRNA gene content or the codon usage in this organelle. Looking for the modification of the balance of nucleus-encoded tRNAs between the cytosol and the mitochondria will then allow us to determine if the information residing in the mitochondrial genome can regulate tRNA import efficiency and/or if it is the result of a co-evolution between the mitochondrial genome and tRNA import process.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

FUNDING

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