Mitochondrial Form of a tRNA Synthetase Can Be Made Bifunctional by Manipulating Its Leader Peptide†

Chien-Chia Wang,*,‡ Kuang-Jung Chang,‡ Huei-Lin Tang,‡ Chia-Jung Hsieh,‡ and Paul Schimmel*,§

Department of Life Science, National Central University, Chung-Li, Taiwan 32054, and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, Mail Drop BCC-379, La Jolla, California 92037

Received April 15, 2002; Revised Manuscript Received October 3, 2002

ABSTRACT: Previous studies showed that yeast *VAS1* encodes both the cytoplasmic and mitochondrial forms of valyl-tRNA synthetase (ValRS), using alternative transcription and translation. The ValRS isoforms have identical polypeptide sequences, except for a 46-amino acid leader peptide that functions as a mitochondrial targeting signal. Although the two forms of the enzyme exhibit indistinguishable tRNA specificities in vitro, they cannot substitute for each other in vivo because of their different localizations. Here we show that the 46-residue leader sequence can be divided into two nonoverlapping peptides, each of which retains the ability to target the enzyme into mitochondria. The engineered proteins (with truncated leader sequences) are dual-targeted, rescuing both the cytoplasmic and mitochondrial defects of a *vas1* knockout strain. Thus, in addition to alternative splicing and alternative translation initiation as mechanisms by which a single gene can encode cytoplasmic and mitochondrial activities, the inherent characteristics of a single polypeptide may enable it to be distributed simultaneously between two cellular compartments. This mechanism may explain how certain other single genes in *Saccharomyces cerevisiae* provide dual functions.

In prokaryotes, there are typically 20 aminoacyl-tRNA synthetases (aaRSs),[1] one for each amino acid (*1*−*4*). In eukaryotes, protein synthesis occurs not only in cytoplasm but also in organelles such as mitochondria (and chloroplasts in plants) (*5*). Except in some algae, all aaRSs are encoded by nuclear genes and imported post-translationally into their respective compartments. The compartmentalization of the protein synthesis machinery within the cytoplasm and organelles of eukaryotes leads to isoaccepting tRNA species that are distinguished by their nucleotide sequences, subcellular locations, and enzyme specificities. Thus, eukaryotes such as yeast commonly have two genes encoding distinct proteins for the same aminoacylation activity, with one protein localized to the cytoplasm and the other to the mitochondria. Each enzyme aminoacylates the isoaccepting tRNAs within its respective cell compartment but is sequestered from the isoacceptors localized in other compartments.

Precursor forms of nuclear-encoded mitochondrial proteins harbor targeting and sorting signals that guide them to various locations within the mitochondrion. These transit signals are present as cleavable amino-terminal signal sequences or as parts of mature proteins. The most common and best studied are the N-terminal signal sequences. These transit peptides are highly variable in length (around 20−60 amino acid residues) and sequence (*6*). So far, no conserved sequences or structural motifs have been identified for these N-terminal presequences. Common features include being rich in hydroxylated and basic residues, being deficient in acidic residues, and the propensity to form an amphipathic α-helix in membrane-like environments (*7*). The amphipathic nature of these signals is believed to play an important role in specific recognition by the mitochondrial receptors.

In contrast to most tRNA synthetases, two *Saccharomyces cerevisiae* genes, *HIS1* (*8*) and *VAS1* (*9*), encode both the mitochondrial and cytosolic forms (valyl- and histidyl-tRNA synthetases, respectively). Distinct mRNAs are produced from each of these genes. The mRNAs of these genes encode two alternative in-frame initiation codons. The mitochondrial form of the enzyme is translated from the first initiation AUG codon on the "long" message, while the cytosolic form is translated from the second in-frame AUG codon on the "short" message (Figure 1). As a consequence, the mitochondrial enzymes have the same polypeptide sequences as their cytosolic counterparts, except for a short amino-terminal mitochondrial targeting sequence. The transit peptide is subsequently cleaved off upon import into the matrix of mitochondria. In vitro studies confirmed that two ValRS isoforms exhibit similar chromatographic properties and identical tRNA specificities (*10*). However, because the two isozymes are targeted to distinct compartments, they cannot substitute for each other in vivo (*9*). A similar observation has been made for genes encoding *Arabidopsis thaliana* alanyl-tRNA synthetase, threonyl-tRNA synthetase, and

† This work was supported in part by Grant NSC 90-2311-B-008-004 (to C.-C.W.) from the National Science Council (Taiwan, ROC), by Grant GM23562 (to P.S.) from the National Institutes of Health, and by a fellowship from the National Foundation for Cancer Research.

* To whom correspondence should be addressed. C.-C.W.: phone, 886-3-426-0840; fax, 886-3-422-8482; e-mail, dukewang@cc.ncu.edu.tw. P.S.: phone, (858) 784-8970; fax, (858) 784-8990; e-mail, schimmel@scripps.edu.

‡ National Central University.

§ The Scripps Research Institute.

[1] Abbreviations: ADH, alcohol dehydrogenase; aaRS, aminoacyl-tRNA synthetase; 5-FOA, 5-fluoroorotic acid; PCR, polymerase chain reaction; ValRS, valyl-tRNA synthetase.

10.1021/bi025964c CCC: $25.00
Published on Web 01/21/2003

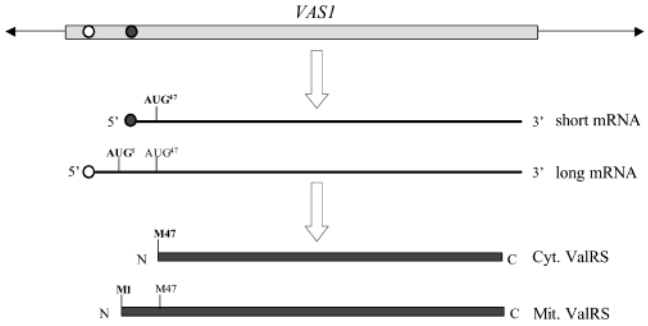

FIGURE 1: Alternative transcription and translation of *VAS1*. Two major transcripts are derived from *VAS1*, the only gene in *S. cerevisiae* encoding ValRS. The longer message uses the first AUG codon (AUG^1) to initiate the translation of the mitochondrial form of ValRS, while the shorter message uses the second AUG codon (AUG^{47}) to initiate the translation of the cytoplasmic form. Note that the longer message contains both AUG codons (AUG^1 and AUG^{47}), while the shorter message contains only the second AUG codon (AUG^{47}). The 138 bp DNA segment between the two initiator AUG codons encodes a 46-residue leader peptide that functions as a mitochondrial targeting signal.

valyl-tRNA synthetase (*11*), as well as for *Neurosporra crassa* valyl-tRNA synthetase (*12*). In these instances, one nuclear gene encodes two distinct proteins, one of which targets the cytoplasm and the other mitochondria. The mechanism by which these single nuclear genes can target proteins to two cellular compartments has not been established.

Here we investigated the possibility that one gene could encode both mitochondrial and cytoplasmic enzymes by a mechanism not previously described. In particular, we wondered whether a single tRNA synthetase, translated from a single AUG initiation codon, could divide between the cytosol and mitochondria. This possibility could occur if the "signal sequence" was inefficient and did not completely target the protein to the mitochondria. Our interest in this possibility came from the desire to understand more completely the versatility of single proteins and the diversity of mechanisms for sorting proteins among cell compartments. In addition, we think that new mechanisms are at least a formal possibility to be considered with systems such as the single genes encoding both forms of the above-mentioned *A. thaliana* and other tRNA synthetases (such as *S. cerevisiae* glycyl-tRNA synthetase) (*13*) where the mechanism for targeting and sorting has not been established. For these purposes, we chose *S. cerevisiae* ValRS and attempted to create enzyme constructs that enabled both the cytoplasmic and mitochondrial activities to arise from a protein translated from a single (rather than two) AUG initiation codon.

EXPERIMENTAL PROCEDURES

Construction of Various VAS1 Constructs. Cloning of *VAS1* from the yeast *S. cerevisiae* followed standard protocols. For cloning *VAS1* into a low-copy number yeast shuttle vector pRS315 (*14*), a pair of oligonucleotides with an *Eag*I site and an *Xho*I site, respectively, were employed to amplify the *VAS1* gene (using yeast genomic DNA as a template) via PCR. The "forward" primer with an *Eag*I site was located 300 bp upstream of the *VAS1* open reading frame, while the "reverse" primer with an *Xho*I site was located immediately upstream of the stop codon. The 3.6 kb PCR fragments were subsequently digested with *Eag*I and *Xho*I prior to cloning into pRS315 for expression and complementation assays. For cloning *VAS1* into a high-copy number yeast shuttle vector (pADH) with a constitutive *ADH* promoter, a forward primer with an *Eag*I site (located 6 bp upstream of the *VAS1* open reading frame) was paired with the aforementioned reverse primer (with an *Xho*I site) to amplify the open reading frame of *VAS1*. The PCR fragments were subsequently digested with *Eag*I and *Xho*I and then cloned into pADH for expression and complementation assays. Note that pADH is a derivative of pQB169 (Cubist Pharmaceuticals, Inc., Lexington, MA), with the addition of an *Eag*I site and an *Xho*I site into the preexisting multiple cloning site and of a short sequence encoding a six-His tag immediately following the *Xho*I site. Various point mutations, such as M1A (ATG^1 to GCG^1) and M47A (ATG^{47} to GCG^{47}), were subsequently introduced into appropriate sequences following standard protocols.

For cloning mitochondrial ValRS derivatives with partial N-terminal deletions into pRS315, an *Nde*I site was inserted at the first initiation codon (ATG^1) of the wild-type gene, yielding $VAS1^{NdeI}$. Various DNA fragments containing base pairs 31−550, 61−550, and 100−550 (encoding peptides containing amino acid residues 11−183, 21−183, and 34−183, respectively) were independently amplified as *Nde*I−*Bsm*I fragments by PCR using $VAS1^{M47A}$ as a template (*Bsm*I is a native restriction site in *VAS1*). The PCR fragments were first digested with *Nde*I and *Bsm*I and then used to replace the corresponding fragment in $VAS1^{NdeI}$, yielding $VAS1^{\square 1-10/M11/M47A}$, $VAS1^{\square 1-20/M21/M47A}$, and $VAS1^{\square 1-33/M34/M47A}$. For construction of $VAS1^{M1/\square 21-46/M47A}$, a DNA sequence containing base pairs −300 to 60 (relative to the ATG^1 initiation codon) of *VAS1* was amplified by PCR as an *Eag*I−*Nde*I fragment and then used to replace the corresponding fragment in $VAS1^{\square 1-46/M47}$ (see below). The expression of these constructs was analyzed by a Western blot method (data not shown). For construction of $VAS1^{\square 1-46/M47}$ in pRS315, a DNA fragment containing base pairs 139−550 (encoding amino acid residues 47−183) was amplified by PCR as an *Nde*I−*Bsm*I fragment using wild-type *VAS1* as a template and then used to replace the corresponding fragment in $VAS1^{NdeI}$.

Complementation Assays for the Cytoplasmic Function of VAS1. A diploid yeast strain that has a *VAS1* deletion in one of the two sets of chromosomes was purchased from Research Genetics (Huntsville, AL). A haploid *vas1* knockout strain, designated CW1, was derived from this heterozygous diploid strain following standard protocols. Briefly, a low-copy number yeast shuttle vector pRS316 (with a *URA3* marker) containing wild-type *VAS1* was transformed into the *VAS1*-mutated diploid strain (a/α, *his3*-□*1*/*his3*-□*1*, *leu2*-□*0*/*leu2*-□*0*, □*vas1*/*VAS1*, *met15*-□*0*/*MET15*, *lys2*-□*0*/*LYS2*, *ura3*-□*0*/*ura3*-□*0*) prior to sporulation and tetrad dissection to maintain the growth ability of the resulting □*vas1* haploid strain. Complementation assays for the cytoplasmic function of plasmid-borne *VAS1* and its derivatives with the *LEU2* marker were tested by introducing into CW1 the second plasmid and determining the ability of the transformants to grow in the presence of 5-fluoroorotic acid (5-FOA). The cultures were incubated at 30 °C for 3−5 days or until colonies appeared. The transformants evicted the maintenance plasmid with the *URA3* marker in the presence of 5-FOA. Thus, only an enzyme with the cytoplasmic ValRS activity encoded by the second plasmid (with the *LEU2* marker) could rescue the growth defect.

Complementation Assays for the Mitochondrial Function of VAS1. Because *VAS1* is bifunctional, assay of mitochondrial ValRS activity for constructs that already expressed a functional cytoplasmic ValRS could be done by testing the transformants on YPG (yeast extract/peptone/glycerol) plates following 5-FOA selections. However, assay of mitochondrial ValRS activity for constructs that did not encode a functional cytoplasmic ValRS required an additional plasmid to provide the essential, cytoplasmic ValRS activity (after eviction of the original maintenance plasmid on 5-FOA plates). To be consistent, assays for mitochondrial ValRS were carried out for all the constructs, regardless of whether these constructs expressed a functional cytoplasmic ValRS, by cotransforming CW1 with a second maintenance plasmid that expressed a functional cytoplasmic ValRS but was defective in mitochondrial ValRS activity. (In this report, *VAS1*^{M1A} or *VAS1*$^{\square 1-46/M47}$ cloned into pRS313 was used as the second, cytoplasm-only, maintenance construct.)

Briefly, CW1 was cotransformed with the test plasmid (with a *LEU2* marker) and the second maintenance plasmid (with a *HIS3* marker). The first maintenance plasmid with the *URA3* marker was evicted from the cotransformants, while the second maintenance plasmid with the *HIS3* marker was stably retained in the presence of 5-FOA. As a result, all cotransformants survived 5-FOA selections, due to the presence of the cytoplasmic ValRS derived from the second maintenance plasmid. The cotransformants were further tested on YPG plates for their mitochondrial phenotypes. A yeast cell cannot survive on glycerol without functional mitochondria. Because the second maintenance plasmid encoded only the cytoplasmic form of ValRS, the cotransformants could not grow on YPG plates unless a functional mitochondrial ValRS was encoded by the test plasmid.

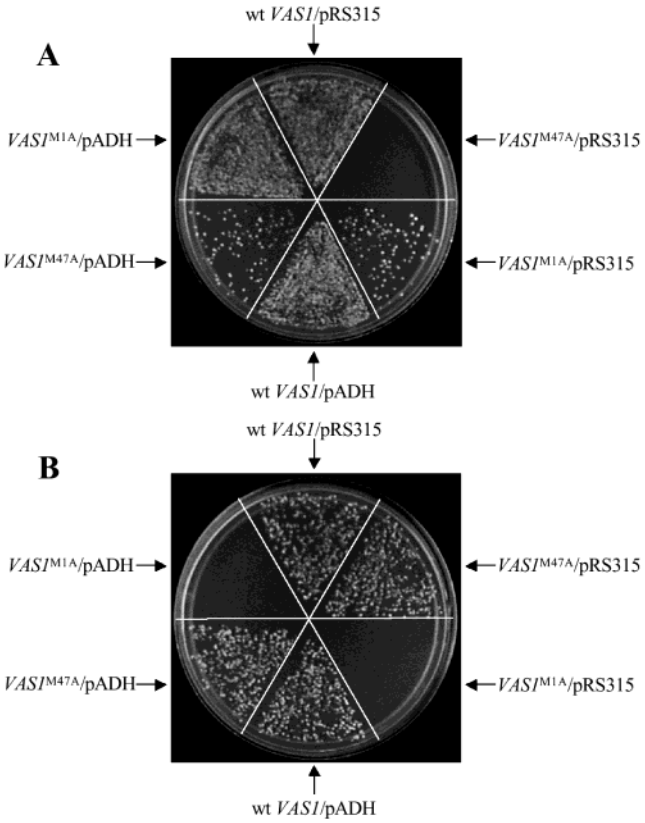

FIGURE 2: Complementation of the *vas1* knockout strain CW1 by the two forms of ValRS. Complementation of the cytoplasmic and mitochondrial phenotypes of the *vas1* knockout strain was shown by the ability to lose the maintenance plasmid and grow on 5-FOA and YPG plates, respectively. (A) Complementation assays on a 5-FOA plate. (B) Complementation assays on a YPG plate.

RESULTS

Strategy for Studying Differential Localization of the VAS1 Gene Products. Although the two forms of the yeast valine enzyme share most of the same polypeptide sequences (Figure 1), the mitochondrial enzyme cannot substitute for its cytoplasmic counterpart in vivo, and vice versa (*9*). These observations suggest that the compartmentalization of the ValRS isozymes is efficient. Both enzyme forms are addressed to their respective compartments, i.e., the cytoplasm and mitochondria. Cytoplasmic aaRSs are essential for the survival of the cell under any condition, while the mitochondrial enzymes are essential only when the mitochondrial function is required, such as growth on a medium containing glycerol (a nonfermenting carbohydrate) as the sole carbon source. These properties were used to test a particular aaRS for its cytoplasmic and mitochondrial functions.

Engineering a Dual Targeted ValRS Protein. As shown in Figure 2, the cytoplasmic form of ValRS (encoded by *VAS1*^{M1A}), when expressed from a low-copy number plasmid vector pRS315, could rescue the growth defect of a *vas1* knockout strain on a 5-FOA plate (Figure 2A). In contrast, it could not restore the growth phenotype of the knockout strain on a YPG plate (Figure 2B). Thus, the cytoplasmic ValRS, when expressed at low levels, could not substitute for its mitochondrial counterpart. This result is consistent with previous observations, in which the ValRS isozymes were expressed from the yeast chromosomes rather than plasmids (*9*). We were motivated to ask whether a dual targeted ValRS could be designed without changing its expression level. To accomplish this objective, the efficiency of mitochondrial import was manipulated such that only a fraction of the preproteins was imported into the mitochondria with the rest remaining in the cytoplasm. To this end, various truncations were independently introduced into the N-terminal transit peptide of the mitochondrial ValRS precursor to impair its import efficiency. Many of these truncations removed the first initiation codon (AUG). The resulting constructs were tested for their ability to rescue the *vas1*-deleted allele. To preclude the possible interference caused by the cytoplasmic form of ValRS, a missense mutation that changed the ATG^{47} codon to a GCG^{47} codon (Met-47 to Ala-47) was introduced. As a consequence, none of the native ATG initiators, ATG^{1} and ATG^{47}, were available. To initiate the translation of these deletion mutants, an ATG codon was independently introduced at the beginning of each new open reading frame (Figure 3A).

As shown in panels B and C of Figure 3, deletion of the 10 N-terminal amino acid residues had no discernible effect on its complementation activity. The truncated protein (*VAS1*$^{\square 1-10/M11/M47A}$) behaved as a mitochondrial enzyme (Figure 3C). Deletion of the 20 N-terminal amino acid residues yielded an enzyme (*VAS1*$^{\square 1-20/M21/M47A}$) that could complement the cytoplasmic as well as the mitochondrial defects of a *vas1* knockout strain. *VAS1*$^{\square 1-20/M21/M47A}$ rescued the growth phenotypes of the knockout on both 5-FOA and

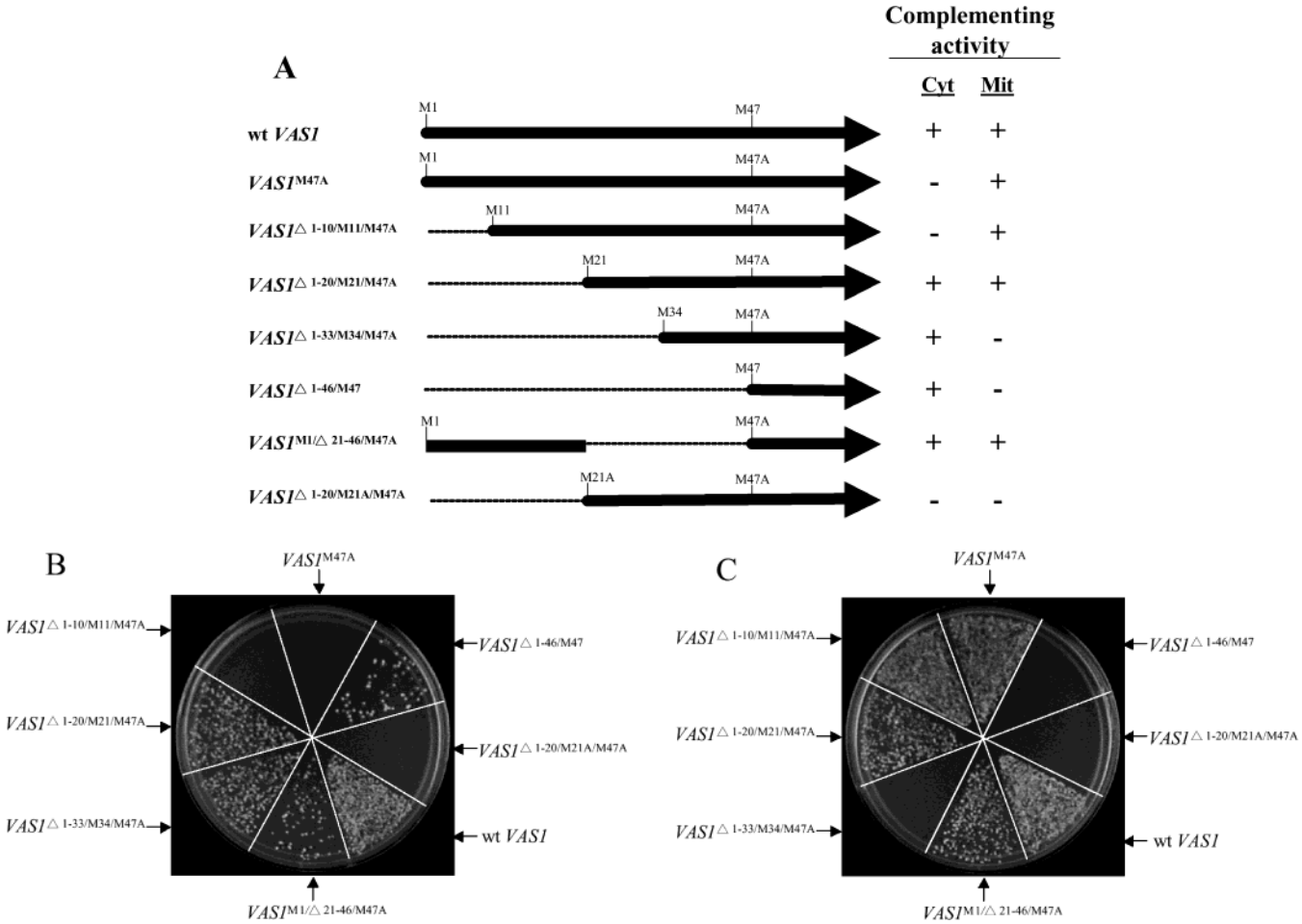

FIGURE 3: Complementation of the *vas1* knockout strain CW1 by *VAS1* mutants with a truncated mitochondrial targeting signal. CW1 was transformed with the various mutants of *VAS1* and then tested for its growth phenotypes. (A) A schematic summary of the *VAS1* constructs and their complementation activities. (B) Complementation assays on a 5-FOA plate. (C) Complementation assays on a YPG plate. Construction of the *VAS1* mutants with a truncated mitochondrial targeting signal is as described in Experimental Procedures. For clarification, the initiation methionines (M1 and M47) of the cytoplasmic and mitochondrial forms of ValRS as well as their corresponding mutations (M1A and M47A, respectively) are indicated. Dashed lines denote deleted portions of the segment encoding the leader peptide of the mitochondrial form of ValRS. All the *VAS1* constructs in the assay used a native *VAS1* promoter for transcription. M11, M21, and M34 denote ATG codons artificially introduced into $VAS1^{\square 1-10/M11/M47A}$, $VAS1^{\square 1-20/M21/M47A}$, and $VAS1^{\square 1-33/M34/M47A}$, respectively.

YPG plates, and the transformants appeared after growth for 3 days at 30 °C (Figure 3B,C). Further deletion to residues 33 and 46 yielded truncated enzymes ($VAS1^{\square 1-33/M34/M47A}$ and $VAS1^{\square 1-46/M47}$, respectively) that could no longer act as mitochondrial enzymes and rescued only the cytoplasmic defect of the knockout allele. It should be pointed out that a mitochondrial ValRS enzyme without the leader peptide ($VAS1^{\square 1-46/M47}$) is identical to its cytoplasmic counterpart.

The bifunctional nature of *VAS1* can be attributed to alternative transcription and translation, in which two initiators, ATG^1 and ATG^{47}, serve as the translation start sites for the mitochondrial and cytoplasmic enzymes, respectively. To ensure that the primary translation product of $VAS1^{\square 1-20/M21/M47A}$ is dual targeted and responsible for both the cytoplasmic and mitochondrial ValRS activities, we tested whether other potential translation initiators were present in addition to the artificially introduced initiator ATG^{21} in $VAS1^{\square 1-20/M21/M47A}$. Mutation of the ATG^{21} codon (Met-21) to a GCG^{21} codon (Ala-21) completely abolished the complementing activity of $VAS1^{\square 1-20/M21A/M47A}$ in both compartments (Figure 3B,C), suggesting that the ATG^{21} codon is the sole translation initiation codon for $VAS1^{\square 1-20/M21/M47A}$. Thus, only primary translation products, initiated at the ATG^{21} codon, are responsible for both the cytoplasmic and mitochondrial ValRS activities.

These results showed that a truncated signal peptide is sufficient for directing a significant fraction of the preproteins into mitochondria. Whether the part of the signal peptide that was removed, containing N-terminal residues 1−20, also had mitochondrial targeting activity was next investigated. Pursuant to this objective, an internal deletion construct ($VAS1^{M1/\square 21-46/M47A}$) was made by removing part of the leader peptide (residues 21−46) from the native mitochondrial precursor. $VAS1^{M1/\square 21-46/M47A}$ rescued both the cytoplasmic and mitochondrial defects of the *vas1* knockout (Figure 3B,C). Thus, the peptide containing N-terminal residues 1−20 retains at least part of the mitochondrial targeting activity that is sufficient for directing the preproteins into the mitochondria.

Expression-Based Dual Targeting of the Wild-Type Enzyme. To further test our conclusions, the biological role of ValRS as a cytoplasmic or mitochondrial enzyme was manipulated by altering the expression level of the isozymes. To this end, the wild-type *VAS1* gene (wt *VAS1*), $VAS1^{M1A}$, and $VAS1^{M47A}$ were subsequently cloned into a high-copy number plasmid (pADH) under the control of a constitutive

Table 1: A Schematic Summary of the *VAS1* Constructs and Their Complementation Activities[a]

construct	vector/promoter	complementing activity	
		Cyt	Mit
wt *VAS1*	pRS315/*VAS1* promoter	+	+
VAS1^{M1A}		+	−
VAS1^{M47A}		−	+
wt *VAS1*	pADH/ADH promoter	+	+
VAS1^{M1A}		+	+
VAS1^{M47A}		+	+

[a] Plasmid pRS315 is a low-copy number yeast shuttle vector, while pADH is a high-copy number yeast shuttle vector with a constitutive ADH promoter.

ADH (alcohol dehydrogenase) promoter. Each construct was then tested for complementing activity. Both the cytoplasmic (*VAS1*^{M1A}/pADH) and mitochondrial (*VAS1*^{M47A}/pADH) forms of ValRS, when overexpressed, rescued the cytoplasmic defect of the knockout strain. The transformants appeared after growth for 2−3 days on a 5-FOA plate (Figure 2A). However, only the mitochondrial enzyme, but not the cytoplasmic enzyme, could rescue the mitochondrial defect of the *vas1*-deleted allele. These transformants appeared after growth for 2−3 days on a YPG plate (Figure 2B).

The most likely explanation for these results is that the mitochondrial transport machinery is somehow overloaded by the highly expressed preproteins, leading to retardation of at least a fraction of the precursor molecules in the cytoplasm. Because ValRS cannot discriminate the tRNA^{Val} isoacceptors in the cytoplasm from those in mitochondria (*10*), the retarded precursor molecules can efficiently aminoacylate the cytosolic tRNA^{Val} species and sustain the growth of the knockout strain on a FOA plate. In contrast, the cytoplasmic enzyme, even when highly expressed, could not move across the mitochondrial membranes and thus could not rescue the growth phenotype of the knockout on a YPG plate (Figure 2B).

The results are summarized in Table 1. Consistent with these observations, when the mitochondrial enzyme was expressed from a high-copy number plasmid (pRS425) under the control of a native *VAS1* rather than a constitutive *ADH* promoter, complementation for the cytoplasmic defect of the knockout allele was weak. Transformants only appeared after growth for 4−5 days on FOA plates (data not shown).

DISCUSSION

Through artificial manipulations, a single gene product was distributed between two cell compartments in amounts sufficient to allow cell growth on distinct media. As shown in Figures 2 and 3, truncation of part of the leader peptide (e.g., *VAS1*$^{\square 1-20/M21/M47A}$ and *VAS1*$^{M1/\square 21-46/M47A}$) enabled the otherwise "monofunctional" mitochondrial precursors to act in both compartments. Most likely, *VAS1*$^{\square 1-20/M21/M47A}$ and *VAS1*$^{M1/\square 21-46/M47A}$ have a reduced affinity for mitochondrial receptors of the import machinery, resulting in colocalization of the molecules in both compartments.

In contrast, the overexpressed preprotein (*VAS1*^{M47A}/pADH) probably exceeded the capacity of the import machinery, thus leading to retardation of the precursor molecules in the cytoplasm. However, overexpression per se was not sufficient to "force" a cytosolic protein into the mitochondria. Thus, overexpression of cytoplasmic ValRS or a leaderless form of mitochondrial ValRS did not permit import (Figure 2B). In contrast, although deletion of the leader peptide of COXVa (encoding the precursor form of mitochondrial cytochrome *c* oxidase subunit Va) severely impairs import of the protein, overexpression of a leaderless form of the enzyme overcomes the requirement for a signal peptide in mitochondrial import (*15*). This observation suggests that COXVa contains a second, cryptic, internal targeting signal that normally does not play a significant role in import but can become active in the absence of the primary signal. However, there seems to be no alternative signal embedded in the internal sequence of ValRS.

The yeast *FUM1* gene appears to take a route different from the ones described here, but with the same net result. *FUM1* encodes only one primary translation product that is responsible for both the cytoplasmic and mitochondrial fumarase activities in vivo (*16*). The preproteins are apparently first targeted to the mitochondrial matrix, and then a significant fraction of the processed proteins arrives back in the cytoplasm. As a result, the mature forms of the cytoplasmic and mitochondrial fumarases have the identical molecular size.

HIS1 (the gene encoding histidyl-tRNA synthetase) (*8*) and *VAS1* (the gene encoding valyl-tRNA synthetase) (*9*) encode both the cytoplasmic and mitochondrial enzymes by alternative use of two in-frame translation initiators. This strategy is also used in yeast for other single genes that encode both cytoplasmic and mitochondrial forms. Examples include at least three yeast tRNA-processing enzymes, encoded by *TRM1*, *MOD5*, and *CCA1*. Each of these genes contains more than one in-phase ATG codon and encodes two or more protein products destined for distinct compartments (*17*). However, a single isoform can coexist in distinct organelles (*18*, *19*). For example, the majority of Cca1p-I, translated from the first in-frame ATG codon, is in mitochondria, but a small fraction of this isoform is also found in the cytosol. Thus, Cca1p-I may be the closest natural example to the strategy described here. Still, the main contribution to cytosolic enzyme activity comes from Cca1p-II and Cca1p-III, which are initiated from ATG2 and ATG3.

The leader peptide of ValRS does not form the typical amphiphilic α-helix associated with some mitochondrial import sequences (*9*). Either of two nonoverlapping pieces of the 46-amino acid peptide promoted import to a level sufficient to allow growth on glycerol. At the same time, some protein was also distributed to the cytoplasm. Although both nonoverlapping peptides are required to target the protein exclusively to mitochondria, each peptide independently contained enough information to achieve at least some import. Thus, these peptide sequences can direct the appended protein simultaneously to two compartments.

ACKNOWLEDGMENT

We are grateful to Jim D. Karam of Tulane University (New Orleans, LA) and Brian Nordin of The Scripps Research Institute for critical reading of the manuscript.

REFERENCES

1. Carter, C. W., Jr. (1993) *Annu. Rev. Biochem. 62*, 715−748.
2. Martinis, S. A., and Schimmel, P. (1996) in *Escherichia coli and Salmonella Cellular and Molecular Biology* (Neidhardt, F. C., Ed.)

2nd ed., pp 887−901, American Society for Microbiology, Washington, DC.
3. Giegé, R., Sissler, M., and Florentz, C. (1998) *Nucleic Acids Res. 26*, 5017−5035.
4. Pelchat, M., and Lapointe, J. (1999) *Biochem. Cell Biol. 77*, 343−347.
5. Maréchal-Drouard, L., Weil, J. H., and Dietrich, A. (1993) *Annu. Rev. Cell Biol. 8*, 115−131.
6. Neupert, W. (1997) *Annu. Rev. Biochem. 66*, 863−917.
7. Lemire, B. D., Fankhauser, C., Baker, A., and Schatz, G. (1989) *J. Biol. Chem. 264*, 20206−20215.
8. Natsoulis, G., Hilger, F., and Fink, G. R. (1986) *Cell 46*, 235−243.
9. Chatton, B., Walter, P., Ebel, J.-P., Lacroute, F., and Fasiolo, F. (1988) *J. Biol. Chem. 263*, 52−57.
10. Felter, S., Diatewa, M., Schneider, C., and Stahl, A. J. (1981) *Biochem. Biophys. Res. Commun. 98*, 727−734.
11. Souciet, G., Menand, B., Ovesna, J., Cosset, A., Dietrich, A., and Wintz, H. (1999) *Eur. J. Biochem. 266*, 848−854.
12. Kubelik, A. R., Turcq, B., and Lambowitz, A. M. (1991) *Mol. Cell. Biol. 11*, 4022−4035.
13. Turner, R. J., Lovato, M., and Schimmel, P. (2000) *J. Biol. Chem. 275*, 27681−27688.
14. Sikorski, R. S., and Hieter, P. (1989) *Genetics 122*, 19−27.
15. Dircks, L. K., and Poyton, R. O. (1990) *Mol. Cell. Biol. 10*, 4984−4995.
16. Wu, M., and Tzagoloff, A. (1987) *J. Biol. Chem. 262*, 12275−12282.
17. Martin, N. C., and Hopper, A. K. (1994) *Biochimie 76*, 1161−1167.
18. Boguta, M., Hunter, L. A., Shen, W. C., Gillman, E. C., Martin, N. C., and Hopper, A. K. (1994) *Mol. Cell. Biol. 14*, 2298−2306.
19. Wolfe, C. L., Hopper, A. K., and Martin, N. C. (1996) *J. Biol. Chem. 271*, 4679−4686.

BI025964C