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A Synthetic Intron in a Naturally Intronless Yeast Pre-tRNA Is Spliced Efficiently In Vivo†

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***Saccharomyces cerevisiae* glutamine $tRNA_{CAG}$ is encoded by an intronless, single-copy gene, *SUP60*. We have imposed a requirement for splicing in the biosynthesis of this tRNA by inserting a synthetic intron in the *SUP60* gene. Genetic analysis demonstrated that the interrupted gene produces a functional, mature tRNA product in vivo.**

About 10% of the tRNA genes in *Saccharomyces cerevisiae* contain introns (8). All tRNA introns are removed from pre-tRNA by a single nuclear enzyme system composed of a tRNA-splicing endonuclease and ligase (2, 10). In tRNA splicing there are no conserved primary sequence elements in the introns or at the splice site junctions, whereas these types of conserved sequence elements play an important role in both rRNA and mRNA splicing (9). In tRNA splicing, the most important element of recognition appears to be the three-dimensional conformation of the pre-tRNA. All precleavage intermediates in *S. cerevisiae* can be folded into a consensus secondary structure that has the following unique features (6, 13). (i) The introns always interrupt the anticodon at the same position. (ii) The 5′ and 3′ splice junctions reside in single-stranded regions. (iii) Introns participate in secondary structure, the most common feature being the anticodon helix formed by base pairing between the intron and the anticodon. (iv) Finally, the exon domains of pre-tRNA are folded into a configuration resembling that of mature tRNA.

Given the similarity between pre-tRNA and mature tRNA structures and the lack of a role for conserved primary sequence elements in the introns or at the splice sites, we expected the configuration of the exons to play an important if not predominant role in substrate recognition by the splicing enzymes. We have examined this problem by inserting a synthetic intron into an *S. cerevisiae* tRNA gene that lacks an intron. We then determined whether the intron is removed and a functional tRNA is produced in vivo. To our knowledge, this is the first experiment of its kind to be attempted in any organism.

For these studies we chose the single-copy essential gene *SUP60*, which encodes glutamine $tRNA_{CAG}$ (14; I. Edelman, Ph.D. thesis, University of Wisconsin, Madison, 1987). An allele of *SUP60* conferring amber (UAG) nonsense suppression has recently been characterized (Edelman, Ph.D. thesis). The *SUP60* gene is well suited for these studies, because simple genetic tests are available to assess the in vivo expression of both the wild-type (*SUP60*$^+$) and amber suppressor (*SUP60-a*) alleles (Edelman, Ph.D. thesis).

The synthetic intron was designed to have the typical features of a tRNA intron listed above (Fig. 1). It is not, however, related in primary sequence to any tRNA intron found in nature. The *SUP60* gene was mutagenized in vitro (5) with an oligonucleotide [5′-CTGGGGTTGTTCGGATGC CTAATTTAT(T/C)AGAACCGAAAGTG-3′] to construct both the wild-type and amber suppressor alleles of *SUP60*, each containing an intron 12 bases in length. Correct construction of the synthetic genes was confirmed by DNA sequence analysis (12).

The intron-containing genes *SUP60*$^+$*::IVS* and *SUP60-a::IVS*, encoding wild-type and amber suppressor tRNA, respectively, were subcloned (7) from M13 phages to a centromeric plasmid vector (YCp50) (11). The plasmids bearing the intron-containing tRNA genes were transformed (4) into a haploid *S. cerevisiae* strain containing the *trp1-1* amber suppressible mutation (W169-31a [*MATa trp1-1 leu2-3,112 ura3-52 pep4-3*]). Transformants were tested for suppression of *trp1-1* by assaying growth in media lacking tryptophan. The strain containing the YCp*SUP60-a::IVS* plasmid was able to grow in the absence of tryptophan, whereas the strain containing the YCp*SUP60*$^+$*::IVS* plasmid was auxotrophic for tryptophan. This result shows that *SUP60-a::IVS* acts as an amber suppressor. We presume, therefore, that the synthetic intron was spliced from glutamine pre-tRNA.

The single-copy *SUP60* gene is required for normal vegetative growth at 30°C (14; Edelman, Ph.D. thesis). Since *SUP60* deletions cause inviability, we also used restoration of viability as another test for splicing of the synthetic intron from glutamine pre-$tRNA_{CAG}$. A diploid strain heterozygous for *SUP60*$^+$ and a deletion, *SUP60-Δ2::LEU2* (WD-1c [*MATa/MATα sup60*$^+$*/sup60-Δ2::LEU2 leu2-3/leu2-3,112 trp1-1/trp1-1 ura3-52/ura3-52*]), was transformed with the plasmid YCp*SUP60*$^+$*::IVS*. The *SUP60-Δ2::LEU2* allele was marked by insertion of the *LEU2* gene adjacent to the deleted tRNA gene. After sporulation of the diploid, viable progeny were recovered that contained the recessive lethal *SUP60* deletion marked by *LEU2*. Progeny of this type always carried the YCp*SUP60*$^+$*::IVS* plasmid, suggesting that viability was restored to an otherwise lethal genotype by plasmid-encoded glutamine $tRNA_{CAG}$.

We assayed for plasmid stability during nonselective mitotic growth and found that the plasmid could not be lost from strains carrying *SUP60-Δ2::LEU2*. However, when these strains were mated with a wild-type strain, the plasmid was no longer required and was readily lost. Thus, a *SUP60*

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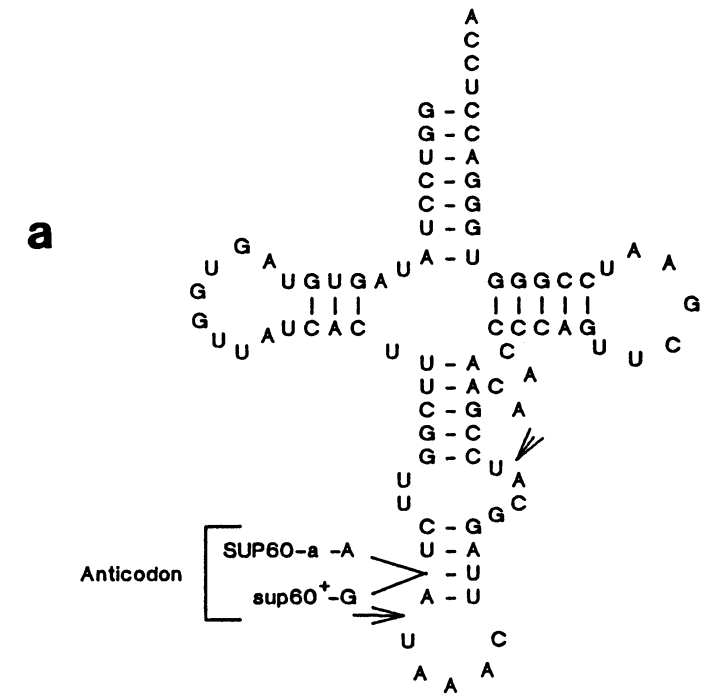

b

tRNA Gene	Suppression of *trp1-1*	Restore viability to *sup60Δ 2 :: LEU2*
SUP60-a :: IVS	YES	NO
sup60^{+} :: IVS	NO	YES

FIG. 1. (a) Proposed secondary structure for the intron-containing glutamine pre-tRNA$_{CAG}$. The arrows indicate the expected sites of intron excision. (b) Summary of the phenotypic analysis of *SUP60-a::IVS* and *SUP60*$^{+}$*::IVS*.

gene containing an intron can provide the missing function whose absence causes lethality in strains deleted for *SUP60*.

We also compared strains whose growth rates depend on a glutamine tRNA gene that either contains or lacks an intron by examining colony size at various time points after plating. This method can distinguish growth rate differences of 10% or greater (1). We found that the growth rates were indistinguishable. Based on this criterion, we suggest that splicing of the intron occurs efficiently enough to support a wild-type rate of growth.

In summary, these experiments show that the *S. cerevisiae* glutamine tRNA$_{CAG}$ gene expresses a functional product in vivo when interrupted by a synthetic intron. The results of the phenotypic assays described above support the view that the intron is removed from glutamine pre-tRNA and the exons are religated efficiently by the tRNA-splicing enzymes. Since glutamine tRNA is intronless in nature, one plausible explanation for our results is that the tRNA splicing enzymes recognize the general features of tRNA three-dimensional shape as a central feature of enzyme-substrate interaction.

This view is further supported by a substantial body of published data suggesting a negligible role for tRNA introns in their own removal (3). Despite some conservation in the secondary structure of introns, these features of introns may not be critical to splicing. Most notably, the consensus secondary structure rules for introns in *S. cerevisiae* lack universality and do not apply, for example, to tRNA introns in *Schizosaccharomyces pombe*. In this species, tRNA introns generally lack an anticodon helix and other structures typical of *S. cerevisiae* introns. Furthermore, *Schizosaccharomyces pombe* intron-containing pre-tRNAs are cleaved efficiently in vitro by *S. cerevisiae* tRNA-splicing endonuclease (3). Thus, secondary structures within the intron are not obligate features of the substrate needed for recognition by the splicing enzymes.

Our results have some bearing on the relative paucity of introns among yeast tRNA genes. We have shown that for at least one of the predominant intronless tRNA genes, the introduction of an intron represents a tolerable disruption of the tRNA coding sequence. We cannot be certain whether these tRNA genes have been intronless throughout their evolution or whether introns originally present in the genes were lost. It does, however, appear that present-day intronless tRNA genes have retained the capacity to acquire new introns and are capable of functioning in their presence.

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