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Genetics

The *sup-7(st5)* X gene of *Caenorhabditis elegans* encodes a $tRNA^{Trp}_{UAG}$ amber suppressor

(amber nonsense suppressors)

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ABSTRACT **In earlier studies, we identified in *Caenorhabditis elegans* two informational suppressors *sup-5 III* and *sup-7 X* and recently showed that these suppressors acted via an altered tRNA to suppress translational termination at amber (UAG) stop codons. We now show that the *sup-7(st5)* suppressor is a $tRNA^{Trp}_{UAG}$ amber suppressor. These studies utilized a radiolabeled purified tRNA fraction to identify hybridizing genomic sequences in a phage genomic library. DNA sequence analysis of the hybridizing segment of one clone showed that the probe recognized a $tRNA^{Trp}_{UGG}$ sequence. The *sup-7* gene was shown to be one of an 11 or 12 member $tRNA^{Trp}$ family by Southern blot analysis, taking advantage of an *Xba* I restriction site induced in the anticodon sequence by the mutational event to suppressor. Sequence analysis of a recombinant λ clone containing *sup-7* gene proved that *sup-7(st5)* is a $tRNA^{Trp}_{UAG}$. This conclusive proof of the nature of *sup7(st5)* will permit unambiguous interpretation in genetic applications, and the availability of the cloned sequences may allow the *sup-7* gene to be used to select for the reintroduction of DNA into *C. elegans*.**

Nonsense mutations introduce in-phase translation stop codons (UAG, UAA, or UGA) into the polypeptide-coding sequence of genes. Translation of the mRNA is terminated prematurely, producing a concomitantly truncated polypeptide product (1). The effects of such mutations can be alleviated by nonsense suppressors, which are most commonly tRNA genes altered so that the tRNA product can decode the premature termination signal, producing a full-length polypeptide (2). Usually, but not always, the tRNA is altered at the anticodon to permit it to pair with one of the termination codons. Nonsense suppressors have been described in bacteria (3) and yeast (4) and more recently through genetic engineering in mammalian cell lines (5). These suppressors have proven to be powerful genetic tools. In particular, they allow nonsense mutations, including those in essential genes, to be studied as conditional mutations, they provide a convenient means of deducing the nucleotide sequence of suppressible mutations and in some instances can be used to create known amino acid substitutions at specific sites (6), and they have been important in probing tRNA function and biosynthesis.

The potential utility of nonsense suppressors in genetic studies of eukaryotic development led us to attempt to identify nonsense suppressors in the small nematode *Caenorhabditis elegans*. *C. elegans* is the subject of detailed genetic investigations into various aspects of development, including cell determination, regulation of gene expression, and morphogenesis. In a series of experiments reported previously (7–9) we identified several suppressors by using classical genetic approaches and have characterized these suppressors with genetic and biochemical techniques.

In initial studies we used reversion analysis of *unc-15 I* and *unc-13 I* mutants (10, 11) to recover suppressors that defined the loci *sup-5 III* and *sup-7 X*. Both *unc-15* and *unc-13* mutants are severely paralyzed and, after mutagenesis, revertants can be recognized by their improved movement among thousands of paralyzed animals. Both *sup-5* and *sup-7* suppressors have properties expected of nonsense suppressors. They are dose-dependent, dominant suppressors that act only on specific alleles of many genes. When tested against mutant alleles of the genes *unc-54 I* and *unc-15 I*, which encode the major myosin heavy chain (12) and paramyosin (11), respectively, the suppressors act only on a proportion of null alleles to partially restore full-length gene products. Deletions extending through *sup-7* lack suppressor activity, so that suppression requires an altered *sup-7* product not its absence (8).

More recently, we demonstrated that *sup-5* and *sup-7* act on amber mutations via an altered tRNA (9). On the one hand, the suppressible allele *unc-54(e1300)* was shown by DNA sequence analysis to contain an amber codon at position 1906 of the 1966-residue coding sequence. On the other hand, *in vitro* translation experiments showed that tRNAs from *sup-5* and *sup-7* strains, but not from wild-type, promoted read-through only of amber terminators with several different mRNAs.

These experiments do not establish what alteration of the tRNA has occurred nor do they reveal which if any of the tRNA species is mutated. To answer these questions we have used recombinant DNA techniques to identify and isolate the *sup-7* gene. Sequence analysis shows it to be a $tRNA^{Trp}_{UAG}$ gene.

MATERIALS AND METHODS

Growth and Maintenance of Nematode Strains. All nematode strains used in this study were derived from the N2 strain of *C. elegans* var. Bristol (10). Maintenance and growth were as described (10, 13). Strains used in addition to N2 were RW2070[*dpy-18(e364) III;sup-7(st5) X*] and RW3291[*dpy-18(e364); sup-7(st5)/stDf1*] (8).

Bacterial Strains, Cloning Vehicles, Media, and General Methods. *Escherichia coli* strains C600 and Q364 were used for propagation of bacteriophage λ1059 (14). The pBR322 and recombinant plasmids were grown in *E. coli* strain RR1. *E. coli* was grown in either L broth or CY broth (14). General methods for growth of phage and plasmids, recovery of DNA, gel electrophoresis, and Southern blotting were as described (9, 14, 15). DNA sequencing was done by the method of Maxam and Gilbert (16) with minor modifications.

Preparation of a tRNA Suppressor Probe. A tRNA fraction containing suppressor activity was obtained by RPC-5 and Sepharose 4B chromatography of a total tRNA fraction from

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Abbreviations: bp, base pair(s); kb, kilobase(s).

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sup-7(st5) animals (3). This fraction was radiolabeled with cytidine 3',5'-[5'-^{32}P]bis(phosphate) (Amersham) using T4 RNA ligase (P-L Biochemicals) (17). In some experiments this fraction was subjected to further purification by electrophoresis of the sample through a 40-cm 20% acrylamide gel. The band containing the suppressor activity, identified previously by Norma Wills (personal communication), was cut out of the gel and the tRNA was recovered by electrophoretic elution.

Preparation of DNA Probes. To obtain a DNA probe for the tRNA^{Trp} gene family, a 185-base-pair (bp) *Tha* I–*Hae* III fragment was recovered from the pRW42 clone by preparative acrylamide gel electrophoresis. The fragment was subcloned into the pBR322 *Bam*HI site using *Bam*HI linkers (Collaborative Research, Waltham, MA). Plasmid DNA (pRW55) was prepared and digested with *Bam*HI; the 185-bp fragment was gel purified and self-ligated before nick-translation (18). A *sup-7* specific probe was derived by gel purification of the 1.5-kilobase (kb) *Sal* I/*Eco*RI insert from pRW83, followed by nick-translation. DNA probes had specific activities of 0.5–2 × 10^8 dpm/μg.

Screening Genomic Libraries. Genomic libraries were prepared from N2 and RW2070 DNA by ligating *Mbo* I partial-digest fragments into *Bam*HI-digested λ1059 DNA (9, 14). For experiments using the gel-purified tRNA probe, ≈10^4 insert-containing phage from the RW2070 library were screened with 5 × 10^6 cpm of probe by using the method of Benton and Davis (19). Hybridization was carried out in 50% formamide/1% Sarkosyl/0.5 M NaCl/0.1 M sodium phosphate, pH 7.0/5 mM ethylenediamine tetraacetate at 42°C overnight. Library screening with DNA probes was carried out as described (9). In one case, 3.2 × 10^4 insert-containing phage from the RW2070 library were screened with 1 × 10^6 cpm of the 185-bp probe. In the second, 1 × 10^4 insert-containing phage were screened with 1 × 10^6 cpm of the nick-translated 1.3-kb insert from pRW91.

RESULTS

Recovery of Genomic Sequences Hybridizing to a Purified tRNA Probe. Of several possible approaches to isolating the *sup-7* gene, we elected to take advantage of the ability of a tRNA molecule to hybridize to its gene. A fraction enriched for suppressor tRNA was obtained after passage of total tRNA over an RPC-5 column and elution from a Sepharose 4B column (9). This fraction still contained four RNA species detectable after two-dimensional acrylamide gel electrophoresis, but suppressor activity was localized to one of these, as assayed in a wheat germ translation system programed with brome mosaic virus coat protein (Norma Wills, personal communication).

To use this Sepharose 4B fraction as a probe to recover genomic sequences, the RNAs were radiolabeled at the 3'-OH end with cytidine [^{32}P]bis(phosphate) (17) and hybridized to a λ phage library of *sup-7(st5)* genomic DNA. Seven phage clones were recovered for initial analysis. By restriction enzyme analysis, 2 clones contained DNA encoding rRNA (rDNA) (20) and another 4 contained a repeated sequence of about 950 bp, which proved to contain the nematode 5S genes (B. Honda, personal communication). Only 1 clone, λ2070-RW7, was a candidate for containing a tRNA gene. We removed the contaminating 5S and rRNA sequences from the Sepharose 4B fraction by electrophoresis of the end-labeled RNAs through a 20% acrylamide gel and elution of labeled bands. The band identified previously as having suppressor activity hybridized specifically to λ2070-RW7 DNA, whereas other bands hybridized with 5S- and rRNA-encoding clones (data not shown).

The genomic library of *sup-7* DNA was rescreened with the gel-purified tRNA probe and an additional 14 hybridizing clones were recovered. Only 1 contained 5S genes, whereas the other 13 clones identified five distinct genomic segments, as determined by restriction enzyme digestion, agarose gel electrophoresis, and Southern blotting (Fig. 1). One of these segments corresponded to that already found in λ2070-RW7. Each genomic segment contained a single hybridizing fragment. The similar intensity of signal from each of the hybridizing fragments from the five different segments suggests that our probe recognized only one tRNA gene family, with the different clones containing different members of the same family rather than different tRNA species.

The Recombinant Phage λ2070-RW7 Contains a tRNA$^{Trp}_{UGG}$ Sequence. To determine which tRNA family had been recognized by the probe, we arbitrarily selected λ2070-RW7 for further analysis. The hybridizing 2.15-kb *Hin*dIII fragment was subcloned into pBR322, and the hybridizing region was further localized by restriction mapping and Southern blotting (Fig. 2). This region was sequenced in both directions from a *Tha* I site and contains a tRNA$^{Trp}_{UGG}$ gene based on its homology with other sequenced eukaryotic tRNA$^{Trp}_{UGG}$ genes, including the CCA anticodon (21) (Fig. 2). The A and B promoter blocks are similar to the blocks identified in studies of the tRNA$^{Pro}_{CCA}$ gene of *C. elegans* (22) and with tRNA genes from other organisms (23, 24).

The sequencing of the tRNA gene in λ2070-RW7 led us to postulate that our tRNA probe was detecting a family of tRNA^{Trp} genes, one member of which corresponded to the *sup-7* gene. Furthermore, examination of the sequence around the anticodon shows that if *sup-7(st5)* represents a change in the anticodon encoding sequence from CCA to CTA, as expected for an amber suppressor, this would create a new *Xba* I site (T*CTAGA*) not present in the wild type. This change could be recognized by Southern blot analysis and would identify the fragment containing the *sup-7* gene.

Southern Blot Hybridization of Wild-Type and Suppressor DNAs Shows *sup-7* Is Likely a tRNA$^{Trp}_{UAG}$. To test the predic-

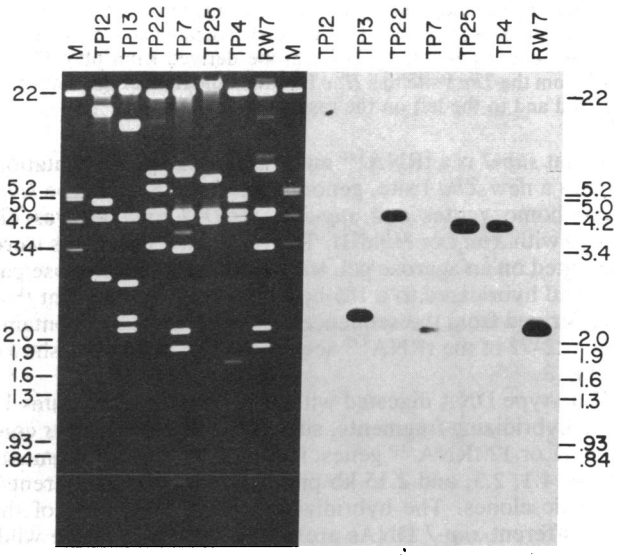

FIG. 1. Phage clones recovered from a *sup-7* genomic library by using a ^{32}P end-labeled tRNA probe. DNA from representative clones was digested with *Hin*dIII, and fragments were separated by agarose gel electrophoresis and either (*i*) stained with ethidium bromide or (*ii*) blotted onto nitrocellulose and hybridized with a purified tRNA fraction with suppressor activity, end-labeled with cytidine [^{32}P]bis(phosphate). The positions of the marker fragments in kb are indicated. Of the 7 clones shown, λ2070-RW7 and λ2070-TP7 share restriction fragments as do λ2070-TP4 and λ2070-TP25. The hybridizing sequence of λ2070-TP12 remains joined to the left arm of the phage vector after *Hin*dIII digestion and has transferred incompletely. Other experiments show it contains a genomic sequence distinct from the other 4 represented.

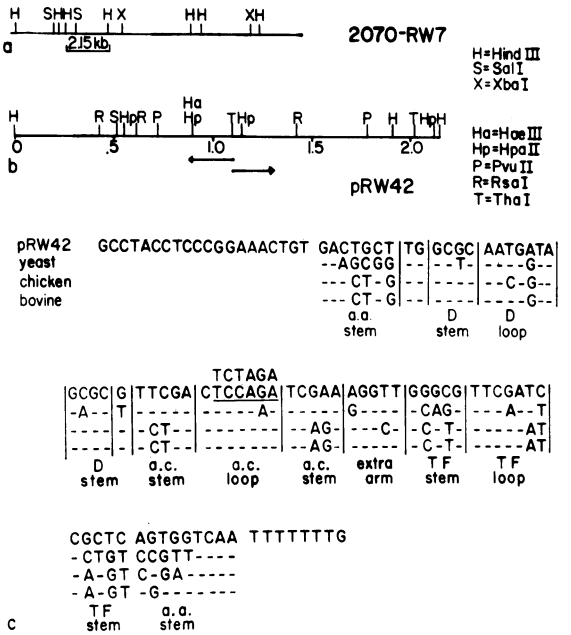

FIG. 2. Sequence of the hybridizing region of λ2070-RW7. (*a*) Restriction map of λ2070-RW7 insert, with the position of the hybridizing 2.15-kb *Hin*dIII fragment indicated. This fragment was subcloned into the *Hin*dIII site of pBR322. (*b*) Restriction map of the 2.15-kb *Hin*dIII fragment in plasmid pRW42. Hybridization to Southern blots of restriction digests localized the hybridizing sequence to the central 250-bp *Hpa* II fragment. The regions sequenced are indicated by arrows under the map. (*c*) Partial sequence of pRW42 in both directions from the *Tha* I site compared to yeast, chicken, and bovine $tRNA^{Trp}$ sequences. Dashes indicate sequence identity with pRW42, and bases that differ are given. Vertical lines divide the sequence into the likely aminoacyl (a.a.) stem, dihydrouracil (D) stem and loop, anticodon (a.c.) stem and loop, extra arm, and T-pseudouracil (TF) stem and loop. The *Tha* I site used for sequencing is in the distal D stem of the tRNA cloverleaf structure. The CCA of the anticodon sequence, if changed to CTA, is a subset of the *Xba* I recognition site T*CTAGA*, as indicated above the nematode sequence. The 185-bp DNA probe derived from pRW42 extends from the *Tha* I site to a *Hae* III site to the right of the sequence depicted and to the left on the restriction map above.

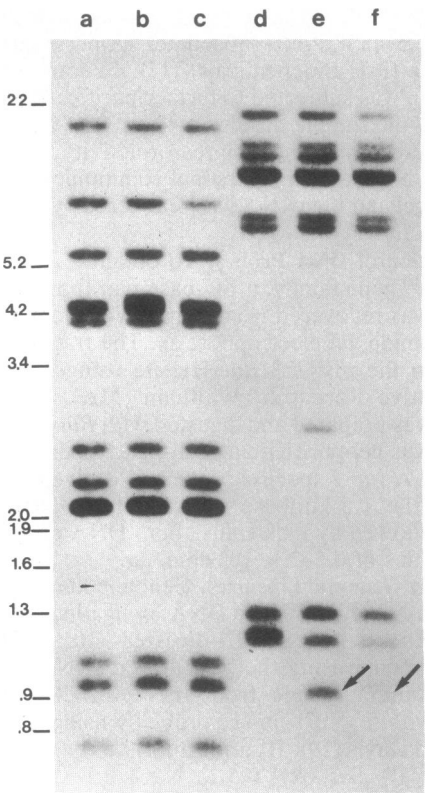

FIG. 3. Identification of *sup-7(st5)* within the $tRNA^{Trp}$ gene family. DNA from wild-type (N2) (lanes a and d), *sup-7(st5)* (strain RW2070) (lanes b and e), and *sup-7(st5)/stDf1* (strain RW3291) (lanes c and f) was digested with *Hin*dIII (lanes a–c) or *Xba* I (lanes d–f). After digestion and electrophoresis through a 0.7% agarose gel, the DNA was transferred to nitrocellulose and hybridized with the 185-bp probe derived from pRW42, which contains nucleotides 22–72 of the $tRNA^{Trp}$ sequence. (See Fig. 2.) Eleven distinct fragments can be distinguished in the wild-type *Hin*dIII-digested DNA (lane a). The fragment at 2.15 kb gives a more intense hybridization signal because it has complete homology with the probe. Other bands give weaker signals and presumably are recognized by the tRNA portion of the probe. The greater intensity of the band at 4.3 kb could reflect a greater extent of homology with the probe or could result from comigration of 2 or more hybridizing fragments. Fragments of the same mobility also hybridize in DNA from the two suppressor strains (lanes b and c), but the signal from the 7.5-kb *Hin*dIII fragment of the *sup-7(st5)stDf1* strain, which is haploid for the *sup-7* gene, is weaker than in either wild type or *sup-7(st5)*. The *Xba* I digests of the three strains (lanes d–f) are similar with the exception of a doublet present at 1.2 kb in wild type, which is reduced to a singlet in the *sup-7(st5)* strains. These strains have a new fragment of 0.95 kb (arrows) not present in wild type. The signal from this fragment is weaker again in the *sup-7(st5)/stDf1* strain in the original autoradiograph and is barely visible in this photograph. The minor band at 3 kb in lane e was not present in other digests and is assumed to result from partial digestion of this sample.

tion that *sup-7* is a $tRNA^{Trp}$ and that the *sup-7(st5)* mutation creates a new *Xba* I site, genomic DNA from wild-type *sup-7(st5)* homozygotes and *sup-7(st5)/stDf1* animals was digested with *Xba* I or *Hin*dIII. The restriction fragments were separated on an agarose gel, transferred to nitrocellulose paper, and hybridized to a 185-bp *Tha* I–*Hae* III fragment that was derived from the sequenced $tRNA^{Trp}$ gene and contains bases 22–72 of the $tRNA^{Trp}$ sequence. The results are shown in Fig. 3.

Wild-type DNA digested with *Hin*dIII (lane a) contains 11 or 12 hybridizing fragments, suggesting that *C. elegans* contains 11 or 12 $tRNA^{Trp}$ genes, including 4 *Hin*dIII fragments of 4.4, 4.1, 2.3, and 2.15 kb previously found in different λ genomic clones. The hybridizing *Hin*dIII fragments of the two different *sup-7* DNAs are of the same size as the wild-type fragments, showing that there is not extensive rearrangement around any of the genomic $tRNA^{Trp}$ genes in these strains (lanes b and c).

The band intensities of wild-type and the *sup-7(st5)/stDf1* DNAs digested with *Hin*dIII are similar with the exception of the band at 7.5 kb, which is less intense in the *sup-7(st5)/stDf1* DNA. This strain with the *sup-7(st5)* allele on one homologue and a deficiency removing *sup-7* in the other is effectively haploid for a small region containing *sup-7*. The underrepresentation of the 7.5-kb *Hin*dIII fragment in this hybridization, which was reproduced in other experiments, suggests that this fragment is contained in the region removed by the deficency, a result consistent with the hypothesis that *sup-7* is a $tRNA^{Trp}$ gene contained on the 7.5-kb *Hin*dIII fragment.

In contrast to results of the *Hin*dIII digests, *Xba* I digests of wild-type and *sup-7* DNAs resulted in a fragment of differing mobility. In general, the hybridizing fragments are the same in wild-type and suppressor DNAs. However, a doublet is present in wild type at 1.2 kb (lane d), and only a single band is seen in the *sup-7* DNA (lanes e and f). It is replaced by a fragment of just less than 1 kb in the *sup-7* strains, which is absent in wild type. As expected, this fragment is underrepresented in the *sup 7(st5)/stDf1* strain. The *sup-7* gene thus resides in a 1.2-kb *Xba* I fragment in wild type and the mutation *sup-7(st5)* introduces a new *Xba* I site,

likely to be in the anticodon loop sequence of a $tRNA^{Trp}$ gene.

The *sup-7(st5)* Gene Is a $tRNA^{Trp}_{UAG}$. *Hin*dIII and *Xba* I restriction digests and Southern blotting of the available set of five putative $tRNA^{Trp}$ sequences showed that each of them contained sequences hybridizing to the 185-bp probe from λ2070-RW7, but none of these corresponded to the fragment sizes predicted for the *sup-7* gene. To recover additional sequences, the λ2070 library was rescreened, in this case with the 185-bp DNA probe derived from λ2070-RW7. Of an additional 28 positive clones recovered, 5 contain the expected 1.0-kb *Xba* I fragment and 3 of these 5 contain the complete 7.5-kb *Hin*dIII fragment. Restriction enzyme digests indicate all 3 are derived from the same genomic segment and are partially overlapping (Fig. 4).

The *sup-7* gene was localized to a 1.3-kb *Eco*RI–*Sal* I fragment near the *Sal* I site. DNA sequencing from the *Sal* I site was performed, and, by homology with the λ2070-RW7 sequence, a $tRNA^{Trp}$ gene was identified (Fig. 4). This gene contains the CTA anticodon, expected of an amber suppressor. The sequence was confirmed by sequencing of the opposite strand from the *Dde* I site.

To establish precisely the nature of the *sup-7* mutation, the wild-type *sup-7* allele was recovered from a wild-type genomic library, by using a unique sequence from the *sup-7* clone as probe. The sequence leftward from the *Sal* I site shows that the only difference between the *sup-7* suppressor and wild type is the substitution of a T for a C at position 35, changing the anticodon sequence to CTA from CCA (Fig. 4).

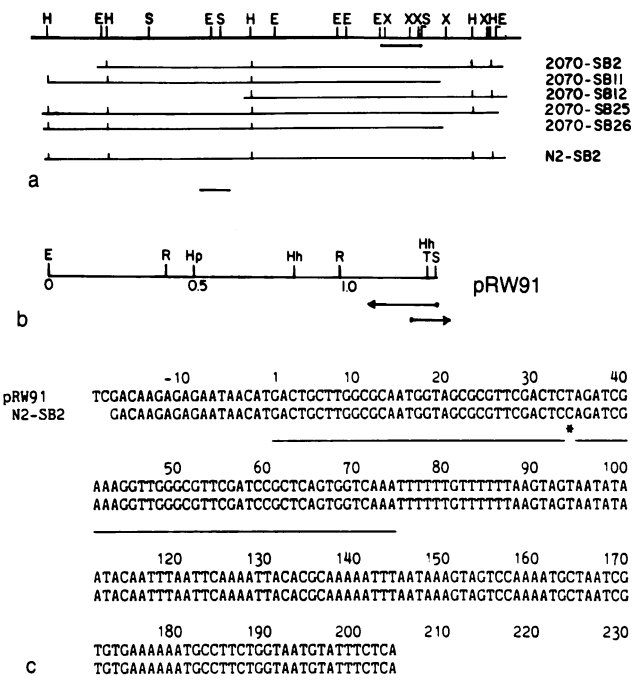

FIG. 4. Sequence of *sup-7(st5)* in λ2070-SB2. (*a*) Restriction map of clone λ2070-SB2 containing a hybridizing 7.5-kb *Hin*dIII fragment and 0.95-kb *Xba* I fragment. The presence of both of these fragments identified this clone unambigiously as containing the *sup-7* gene. The hybridizing sequence was localized to a 1.3-kb *Eco*RI–*Sal* I fragment, which was subcloned into pBR322 to give plasmid pRW91. The bar represents 1 kb. Restriction enzyme abbreviations are the same as in Fig. 2. (*b*) Restriction map of the pRW91 insert. The regions sequenced from the *Sal* I site and *Dde* I site are indicated. Restriction enzyme abbreviations are as in Fig. 2, and Hh is *Hha* I. (*c*) The sequence of pRW91 from the *Sal* I site. A $tRNA^{Trp}$ gene with a CTA anticodon sequence is present beginning at position 1 as indicated. The opposite strand was sequenced from the *Dde* I site to confirm the sequence. The wild-type sequence, shown below, was determined from the *Sal* I site of λN2-SB2. This phage was recovered from a wild-type (N2) library in λ1059 by using the *Eco*RI–*Sal* I fragment from pRW91. The underlined portion indicates the extent of the tRNA sequence. The only substitution in the region sequenced was the C → T transition in the anticodon sequence (marked by the asterisk).

DISCUSSION

Our results demonstrate that the amber (UAG) nonsense suppressor *sup-7(st5)* of *C. elegans* is a member of the $tRNA^{Trp}$ gene family and contains a single base-pair substitution changing the anticodon from CCA to CTA. We used an end-labeled purified RNA fraction with suppressor activity to recover hybridizing genomic sequences from a library of the mutant DNA. DNA sequence analysis of one clone showed that $tRNA^{Trp}$ genes were recognized by the probe. Further analysis of the sequence predicted the creation of an *Xba* I restriction enzyme site in the $tRNA^{Trp}$ gene if the CCA anticodon sequence mutates to CTA. Genomic Southern blots comparing wild-type and *sup-7* DNA digests hybridized with a DNA probe from a $tRNA^{Trp}$ gene demonstrated an extra *Xba* I site in *sup7(st5)* gene among the 11 or 12 members of this family. Sequencing of the *sup-7(st5)* gene confirmed the presence of the CTA anticodon and comparison with the sequence of the wild-type *sup-7* allele shows that the only substitution in the region sequenced is the C → T mutation in the anticodon.

The *sup-7(st5)* suppressor is analogous in almost every respect to nonsense suppressors found in yeast and in bacteria and their phages and to those introduced into mammalian cells (3–5). It was isolated based on its ability to suppress other mutations (7, 8), it acts on specific alleles of many genes by partially restoring gene product (7, 8), and it results from a base change of the tRNA anticodon. In addition to these commonly recognized properties, it also shares the characteristic of having adverse effects on growth (8, 25). Although tryptophan tRNAs have not yet been identified as suppressors in yeast, they are known in *E. coli* (26). The $tRNA^{Trp}_{UAG}$ suppressor in *E. coli* is unusual in that the anticodon change results in mischarging of the tRNA with glutamine (27). We do not know yet if the *sup-7* $tRNA_{UAG}$ will behave in a similar manner.

The *sup-7* gene has extensive homology with other known eukaryotic $tRNA^{Trp}$ sequences (21) and shares general features with all eukaryotic tRNAs (23, 24). It has 55 of 72 nucleotides identical to bovine and chicken $tRNA^{Trps}$ and 13 of the 17 differences are located in the stem regions of the tRNA secondary structure. It is less homologous with the yeast $tRNA^{Trp}$ sequences, sharing only 48 nucleotides. Unlike the *Dictyostelium* gene sequence, from which it differs by 30 nucleotides, *sup-7* does not contain an intervening sequence (28). The *sup-7* sequence forms a secondary structure, which is in agreement with rules for tRNAs in general. The gene also contains sequences that match well the consensus sequences for A and B RNA polymerase III promoter blocks, as defined both for nematode tRNAs in a heterologous system (22) and for polymerase III genes of other organisms (23, 24). It is followed by a run of Ts that can serve as a transcription termination signal (29). Whether these similarities to other organisms' tRNA are sufficient to allow the *sup-7* gene to be expressed and the transcript to be processed correctly in heterologous systems remains to be tested. We do know the *sup-7* tRNA is charged by a yeast S-100 in the *in vitro* translation system (9).

The wild-type *sup-7* sequence differs from the λ2070-RW7 $tRNA^{Trp}$ sequence at position 18, where *sup-7* has a G and the λ2070-RW7 sequence has an A. In yeast, such differences in the mature wild-type tRNA encoding sequences have not been found between different members of tRNA gene families (30, 31). This difference in the two *C. elegans* $tRNA^{Trp}$ sequences is likely to be important as it involves an invariant nucleotide of the A block of the RNA polymerase III pro-

moter (23, 24). The G at position 18 in *sup-7* corresponds to the consensus nucleotide, as would be expected from its known *in vivo* suppressor activity. The A at this position in the λ2070-RW7 sequence is the exception. In yeast, an A substitution at the corresponding position of the *SUP-4* $tRNA^{Tyr}$ gene results in reduced transcription and incomplete processing (24). If the *C. elegans* RNA polymerase III has similar specificity, the λ2070-RW7 sequence would represent a dysfunctional gene. On the other hand, the A in the λ2070-RW7 sequence could have other consequences, such as tissue-specific expression.

In addition to *sup-7(st5)*, various other suppressors have been identified in *C. elegans*. In particular, 30 additional suppressor isolates have been recovered in reversion experiments using *unc-15(e1214)* and *unc-13(e450 or e1091)* (7, 8). Genetic mapping places these isolates in only two chromosomal regions, one corresponding to the position of *sup-7* and the other on LG III containing *sup-5 III*. The *sup-5(e1464)* suppressor has a spectrum of suppression similar to *sup-7*, suggesting that it recognizes the same terminator as *sup-7* and may insert the same amino acid. In preliminary experiments, a fragment recognized by the λ2070-RW7-derived probe is of lower molecular weight in *Xba* I digests of *sup-5* DNA, suggesting that indeed *sup-5* is another member of the tryptophan tRNA family. We do not know if the other 28 isolates mapping to these regions are in fact in the *sup-7* and *sup-5* genes. The dominance of the suppressors prevents complementation testing, and linkage data alone are insufficient to assign these other isolates to *sup-7* and *sup-5*. Possible clustering of tRNA genes, as seen in other organisms, could place several tRNA genes in a relatively small genetic unit (32). *Xba* I digests of DNA from these isolates should determine which $tRNA^{Trp}$ gene, if any, is mutated.

Suppressors derived from other tRNA species or recognizing other nonsense codons would be useful, but as yet none is known in *C. elegans*. The possible limitation of the present set of 30 isolates to the $tRNA^{Trp}$ family could result from the requirements for a particular amino acid at the sites mutated in the *unc-13* and *unc-15* alleles used. Mutagen specificity might also limit the variety obtained. All 30 isolates described were induced with ethylmethane sulfonate, which induces principally G·C to A·T transitions in prokaryotes (33). In the small sample of point mutations sequenced so far, it seems to have a similar specificity in *C. elegans* (six of seven are G·C to A·T transitions; unpublished data). Other mutagens and other alleles must be used in attempts to expand suppressor genetics in *C. elegans*. The determination of specific point mutations in genes such as *unc-54* will help direct these efforts.

We anticipate that *sup-7* and other nonsense suppressors will be applied widely in genetic studies of *C. elegans*. As in microorganisms, nonsense suppressors provide a genetic means of inferring the molecular consequences of a mutation. They also can provide a means of recovering nonsense mutations in a homozygous form for essential genes. This can be particularly effective in a self-fertilizing diploid organism like *C. elegans*, in which clonal reproduction of an animal heterozygous for the suppressor produces progeny lacking the suppressor as well as those containing the suppressor.

The suppressor may also have applications unique to metazoans. In particular, it may be possible to obtain animals that are mosaic for the suppressor in somatic cells. Mosaic animals have been identified by Herman *et al.*, using fragments of the X chromosome (34). If the suppressor could be recovered on a mitotically unstable chromosomal or episomal fragment, it in combination with a suppressible allele of a gene of interest could result in a mosaic animal. With the use of appropriate controls, this could allow one to deduce the tissue of expression of the gene of interest. Finally, *sup-7* as a small, constitutively expressed dominant gene, for which strong selections exist, is an ideal candidate for use in DNA transfection experiments with *C. elegans*. Such a method would be extremely valuable in the analysis of genes in *C. elegans*.

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