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Nucleotide correlations that suggest tertiary interactions in the TV-replacement loop-containing mitochondrial tRNAs of the nematodes, *Caenorhabditis elegans* and *Ascaris suum*

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ABSTRACT

In the predicted secondary structures of 20 of the 22 tRNAs encoded in mitochondrial DNA (mtDNA) molecules of the nematodes, *Caenorhabditis elegans* and *Ascaris suum*, the TΨC arm and variable loop are replaced with a loop of 6 to 12 nucleotides: the TV-replacement loop. From considerations of patterns of nucleotide correlations in the central regions of these tRNAs, it seems highly likely that tertiary interactions occur within five sets of binary and ternary combinations of nucleotides that correspond in location to nucleotides known to be involved in tertiary interactions in yeast $tRNA^{Phe}$ and other standard tRNAs. These observations are consistent with the nematode TV-replacement loop-containing mt-tRNAs being folded into a similar L-shaped functional form to that demonstrated for standard tRNAs, and for the bovine DHU (dihydrouridine) arm replacement-loop-containing mt-$tRNA^{Ser(AGY)}$. However, the apparent occurrence in nematode mt-tRNAs of tertiary bonds common to standard tRNAs contrasts with the situation in bovine mt-$tRNA^{Ser(AGY)}$ where the functional form is dependent on an almost unique set of tertiary interactions. Because three of the proposed conserved tertiary interactions in the nematode mt-tRNAs involve nucleotides that occur in the variable loop in standard tRNAs, it seems more likely that in nematode mt-tRNAs it is the TΨC arm rather than the variable loop that has undergone the greatest proportional decrease in nucleotide number.

INTRODUCTION

The secondary structure potential of transfer RNA (tRNA) molecules has been conserved to a remarkable degree during the evolution of living organisms. All tRNAs encoded in prokaryotic DNAs, eukaryotic nuclear DNAs and organelle DNAs, except those of metazoan (multicellular animal) mitochondria, can be folded into a similar clover leaf form comprising the four arms and connecting variable loop and other nucleotides shown in Figure 1A. Among these tRNAs (designated standard tRNAs) are found a number of nucleotides that are highly conserved and referred to as invariant, and other nucleotides that are conserved as purines or pyrimidines and are referred to as semi-invariant (1–4).

Extensive X-ray diffraction studies of yeast nuclear-encoded $tRNA^{Phe}$ revealed a tertiary structure that has an overall L shape (5–10). One arm of the L is formed by the DHU (dihydrouridine) stem being arranged on top of, but at a slight angle to, the anticodon stem. The second arm of the L is formed by linear alignment of the TΨC and amino-acyl stems. The DHU and TΨC loops bulge in close proximity to each other outside the elbow of the L structure. These same studies of yeast $tRNA^{Phe}$ also revealed the occurrence of specific, mainly non-Watson–Crick type, tertiary hydrogen bonding between highly conserved nucleotides, that were clearly responsible for stability of the final L shape (11,12). Three-dimensional tertiary structures have also been worked out from X-ray diffraction data for initiator $tRNA^{Met}$ of *Escherichia coli* and yeast (13–15), yeast $tRNA^{Asp}$ (16,17), and for *E.coli* $tRNA^{Gln}$ and yeast $tRNA^{Asp}$ bound to their corresponding amino-acyl synthetases (18,19). Although some differences concerning the configurations of the ends of the amino-acyl stems and anticodon loops were noted among the different free tRNAs, and between free and bound tRNAs, all tRNAs examined had the same overall L shape, and available data support the notion that this structure is maintained in each case by the same set of tertiary interactions described for yeast $tRNA^{Phe}$.

Because all tRNAs of an organisms must fit into the same space in the ribosome to deliver their attached amino acid, it is reasonable to postulate that, as supported by presently available data, tRNAs have a common tertiary form. However, examination of tRNAs from different prokaryotes and eukaryotes (4) indicate that although nucleotide combinations involved in some of the tertiary interactions in yeast $tRNA^{Phe}$ are highly conserved among the tRNAs of single organisms, some are not. In the latter cases, this implies that either alternative hydrogen bonding can occur between similarly located but different nucleotides, or that not all of the tertiary interactions found in

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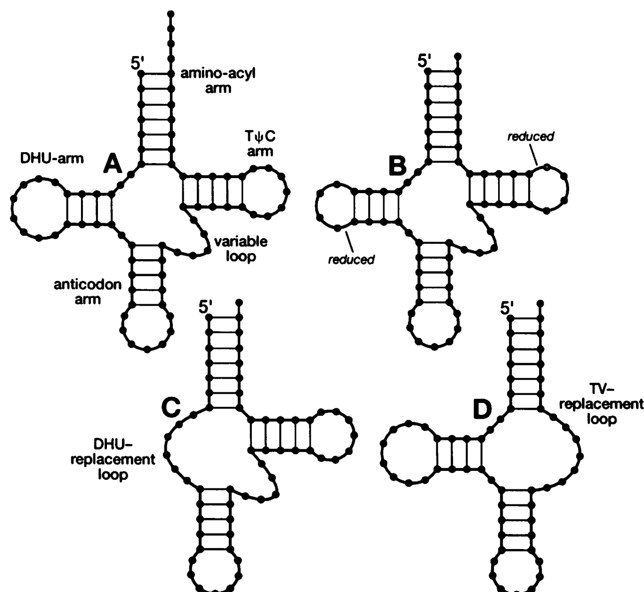

Figure 1. Secondary structures of standards tRNAs (A), the majority of metazoan mt-tRNAs (B), dihydrouridine (DHU) arm-replacement loop-containing metazoan mt-tRNAs (Ser (AGN), and Ser (UCN) in nematodes) (C), and nematode TΨC and variable loop (TV)-replacement loop-containing mt-tRNAs (D).

yeast tRNA^{Phe} occur in all tRNAs. Some alternative hydrogen bonding possibilities between similarly located, but diverse nucleotide combinations have been described (11).

Extra tertiary hydrogen bonds have been shown to occur in the folded structure of yeast initiator tRNA^{Met}, including three that bridge the DHU and TΨC loops, which involve a set of nucleotides that are characteristic of eukaryotic, nuclear-encoded, initiator tRNAs (15).

In contrast to the uniformity of secondary structure of standard tRNAs, a great deal of variation has been noted among tRNAs encoded in the mitochondrial DNA (mtDNA) molecules of metazoa. Each metazoan mtDNA encodes only 22 tRNAs, but because of an extended wobble mechanism of anticodon–codon recognition, this number of tRNAs is sufficient to decode all of the twelve or thirteen protein genes common to these mtDNAs (reviewed in references 20 and 21). The majority of metazoan mt-tRNAs have an overall secondary structure that resembles that of standard tRNAs (Fig. 1B). However, these mt-tRNAs show substantial variation in both size and sequence of their DHU and TΨC loops, and some nucleotides in these loops that are highly conserved among standard tRNAs are conserved to a lesser extent among metazoan mt-tRNAs (detailed in reference 20).

Encoded in most metazoan mtDNAs are one or two kinds of tRNA that, relative to standard tRNAs, have gross structural changes. In all known metazoan mt-tRNAs that specify serine and recognize AGY, AGY/A or AGN codons, the DHU arm is replaced with a simple loop of between 5 and 13 nucleotides (Fig. 1C; 22–25). The mt-tRNA$^{Ser(UCN)}$ of the nematodes *Caenorhabditis elegans* and *Ascaris suum*, but not of other metazoa examined to date, also contain a DHU arm-replacement loop (25,26). In all 20 of the remaining mt-tRNAs of *C.elegans* and *A.suum* the TΨC arm and variable loop are replaced with a loop of between 6 and 12 nucleotides (the TV-replacement loop; 23–25; Fig. 1D).

For the bovine DHU-replacement loop-containing mt-tRNA$^{Ser(AGY)}$, a tertiary structure model has been derived from chemical probing data (28). This structure is similar in overall shape to that of standard tRNAs, but smaller, and stabilized by a set of mainly unique tertiary hydrogen bonds. However, based on computer modeling studies, it has been proposed recently that DHU replacement loop-containing mt-tRNAs are of two tertiary structural kinds, the overall shapes of which are distinguished from that of standard tRNAs by a greater angle between the helical domain formed by the aminoacyl and TΨC stems, and that formed by the anticodon and DHU stems (29).

At this time, there is a dearth of experimental data regarding tertiary folding of the variously modified, four-armed metazoan mt-tRNAs, and of the TV-replacement loop-containing nematode mt-tRNAs. We have noted that in the *C.elegans* and *A.suum* TV-replacement loop-containing tRNAs, many of the invariant and semi-invariant nucleotides are conserved to a higher degree than is found among four-armed mt-tRNAs from a variety of metazoa (see reference 20), and that among these nematode mt-tRNAs, nucleotides at other positions, particularly in the 5′ end-proximal region of the TV-replacement loop, are highly conserved. Stimulated by these observations, we have examined the sets of *C.elegans* and *A.suum* mt-tRNAs for nucleotide correlations that might indicate the occurrence of tertiary interactions similar to those found in standard tRNAs, and thus provide clues as to how TV-replacement loop-containing tRNAs attain their final folded form.

MATERIALS AND METHODS

Data regarding nematode mt-tRNAs presented and discussed in this paper are based on the secondary (folded) structures of the 20 *C.elegans* TV (TΨC arm and variable loop)-replacement loop-containing mt-tRNA genes shown in Figure 2, and on the secondary structure of all but one of the corresponding 20 *A.suum* TV-replacement loop-containing mt-tRNA genes given in Figure 2 of reference 27. The exception in the latter case is the secondary structure of the *A.suum* mt-tRNA^{Arg} gene which is given in reference 26. All sequences are in the EMBL Data Library: accession numbers X54252; X54253. Frequencies of nucleotides at specific positions in prokaryotic and eukaryotic tRNA genes, mentioned in the text, were calculated from gene sequences listed in reference 4. Prokaryotic values were based on all tRNA genes listed for *Escherichia coli* and *Bacillus subtilis*, and eukaryotic values were based on all nuclear-encoded tRNA genes listed for *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and mouse (or human in cases where mouse gene sequences were not available).

RESULTS

The hydrogen bondings that maintain the tertiary structure in yeast tRNA^{Phe} occur between nucleotides that are conserved to varying degrees among standard tRNAs, and located in the central region of the tRNA secondary structure; DHU and TΨC arms, variable loop and nucleotides between the DHU arm and the amino-acyl and anticodon arms (see Fig. 3A). A consensus diagram of the corresponding central region of the *C.elegans* and *A.suum* TV-replacement loop-containing tRNAs, summarizing the degrees to which specific nucleotides are highly conserved in the two species is given in Figure 4. The sets of *C.elegans* and *A.suum* TV-replacement loop-containing mt-tRNAs were

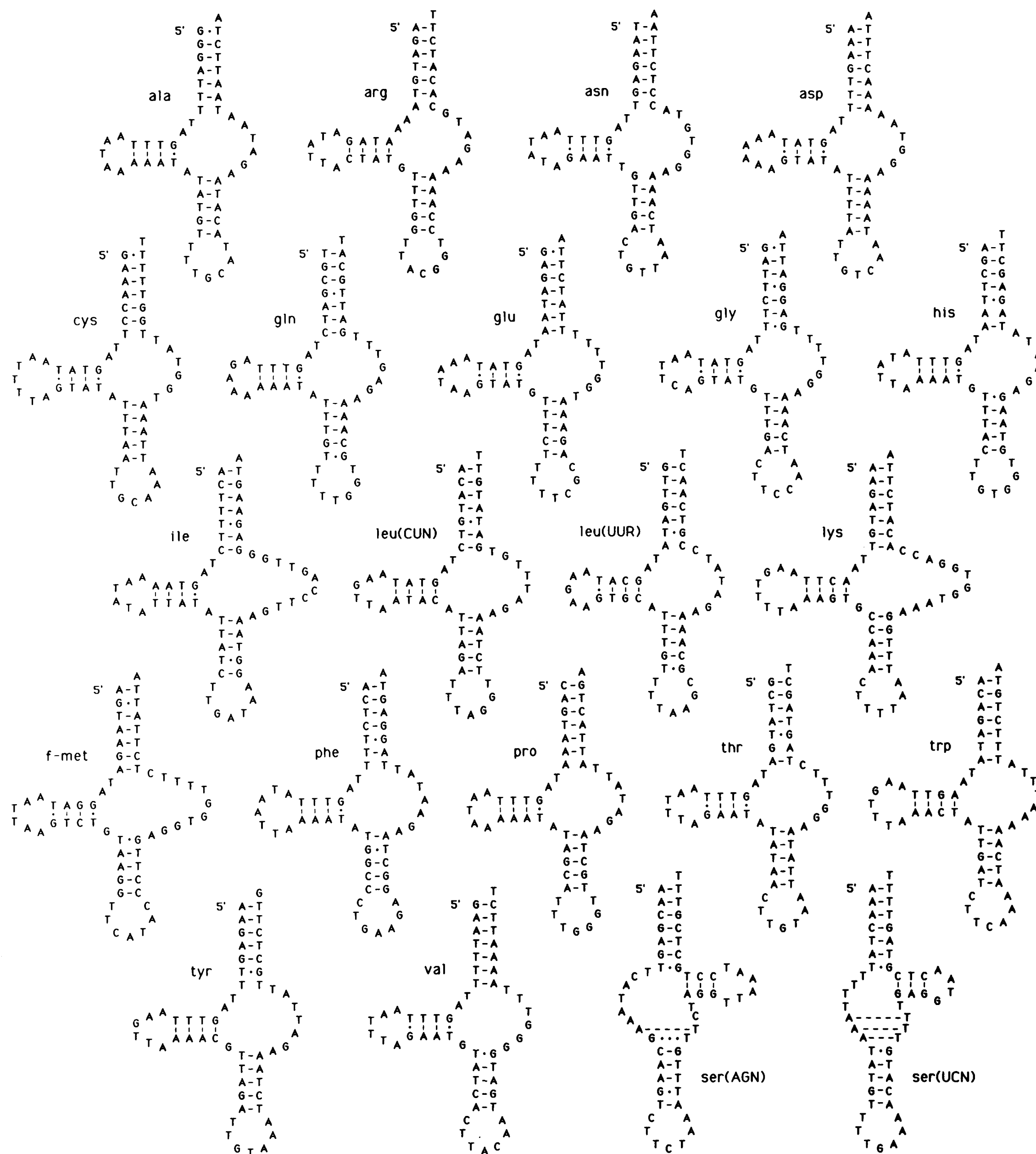

Figure 2. The 20 TV (TΨC arm and variable loop)-replacement loop-containing mt-tRNA genes and two DHU (dihydrouridine) arm-replacement loop-containing tRNA genes of *Caenorhabditis elegans* shown in the presumed secondary structural forms of the corresponding tRNAs.

examined for specific nucleotide correlations that would be consistent with the presence in these tRNAs of the tertiary interactions similar to those that occur in yeast $tRNA^{Phe}$ (Fig. 5).

In yeast $tRNA^{Phe}$, U8 is hydrogen bonded to A14 in the DHU loop, and to this nucleotide pair is hydrogen bonded A21, also in the DHU loop. This nucleotide combination is found in about 95% of the various tRNAs of prokaryotes and eukaryotes. Conservation of the U8·A14·A21 nucleotide interaction in nematode mt-tRNAs is supported by the very high conservation of all three of the nucleotides involved (U8 and A14, 95% and 100%, respectively, in both species; A21, 100% in *C.elegans* and 85% in *A.suum*).

The third nucleotide in the variable loop of the yeast $tRNA^{Phe}$, m^7G46 is hydrogen bonded to G22 (of the DHU stem nucleotide pair, C13–G22) to form the nucleotide triple m^7G46·G22–C13 (Fig. 3). This G46·G22–C13 combination is found in 60 and 40% of prokaryotic and eukaryotic tRNAs. In 6% of eukaryotic tRNAs, U replaces C in position 13 (G46·G22–U13) and A46·A22–U13 occurs in 8% of these tRNAs. Neither of the latter two combinations occur in prokaryotic tRNAs, but it has been proposed that both G46·G22–U13 and A46·A22–U13 can be maintained by hydrogen bonding in eukaryotic tRNAs (11). Among the remaining prokaryotic and eukaryotic tRNAs are 12 and 14 other

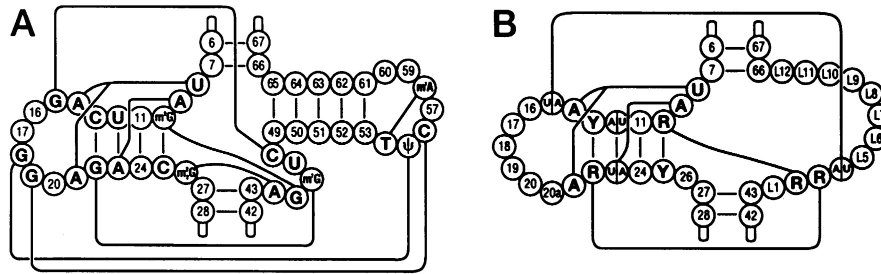

Figure 3. Diagram of the central regions of the secondary structure of yeast tRNA^{Phe} (A), and the consensus secondary structure of the TV-replacement loop-containing mt-tRNAs of *C.elegans* and *A.suum* (B). In the yeast diagram are shown all of the tertiary bondings indicated from X-ray diffraction data (5−10). In the *C.elegans*/*A.suum* diagram are shown tertiary bondings that correspond to some of those found in yeast tRNA^{Phe}, that are predicted from specific nucleotide correlations among the *C.elegans* and *A.suum* mt-tRNAs (see Fig. 5 and text). m^{2}G, 2-methyl guanine; m$^2_2$G dimethyl guanine; m^{7}G, 7-methyl guanine; T, ribothymine; Y, pseudouridine; m^{1}A, methyl adenine.

nucleotide 46·12−13 combinations indicating either that hydrogen bonding of nucleotides 46 and 22 is extremely flexible or that this interaction does not occur in many standard tRNAs. In eight *C.elegans* and seven *A.suum* mt-tRNAs the L3 (equivalent to nt 46)·22−13 combination is GL3·G22−U13, and in nine *C.elegans* and eight *A.suum* mt-tRNAs it is AL3·A22−U13. The remaining three *C.elegans* and five *A.suum* mt-tRNAs do not fall into this homopurine (L3·22)−U13 pattern. That the L3·22−13 interaction exists in at least 17 *C.elegans* and 15 *A.suum* mt-tRNAs is supported by the above mentioned demonstration of plausible hydrogen bondings for both the GL3·G22−U13 and AL3·A22−U13 triplets. Also, this interpretation is supported by the observation that AL3·A22−U13 in *C.elegans* tRNA^{Gln} is replaced by GL3·G22−U13 in *A.suum* tRNA^{Gln}.

The second nucleotide in the variable loop of yeast tRNA^{Phe}, G45, is hydrogen bonded (a single bond) to m^{2}G10 (of the first DHU stem nucleotide pair, m^{2}G10−C25) to form the nucleotide triple G45·m^{2}G10−C25 (Fig. 3). This triple occurs in 55% and 61% of eukaryotic and prokaryotic tRNAs. In a further 22% and 7%, G45·G10 also ocurrs, but as G45·G10−U25. Other 45·10−25 combinations in eukaryotic and prokaryotic tRNAs are A45·G10−C25 (11% and 14%), U45·G10−C25 (7% and 7%), and one and five other combinations of lesser frequencies. Among *C.elegans* and *A.suum* mt-tRNAs there are 17 and 15, respectively, that have a G in the second position (L2) of the TV-replacement loop and a G in position 10 (Fig. 5). In 14 (70%) each of the *C.elegans* and *A.suum* mt-tRNAs, there occur GL2·G10−U25, and in three *C.elegans* and one *A.suum* mt-tRNAs there occur GL2·G10−C25. Additionally, in the Arg, Lys and Trp mt-tRNAs of both *C.elegans* and *A.suum* there is AL2·A10−U25. Thus, the constancy of occurrence of the homopurine (L2·10)−pyrimidine 25 in the L2·10−25 combination, particularly of GL2·G10, in most nematode mt-tRNAs again suggests conservation of the (L2)45·10−25 tertiary interaction that occurs in standard tRNAs.

In yeast tRNA^{Phe}, C48, the variable loop nucleotide adjacent to the TΨC stem, is hydrogen bonded to the second nucleotide in the DHU loop, G15, (Fig. 3). In 98% and 90% of prokaryotic and eukaryotic tRNAs, this combination is either G15·C48 (79% and 74%) or A15·U48 (19% and 16%). Other 15·48 combinations occur with relative frequencies of G·U > A·C > G·A > C·G. In all but one *C.elegans* and three *A.suum* mt-

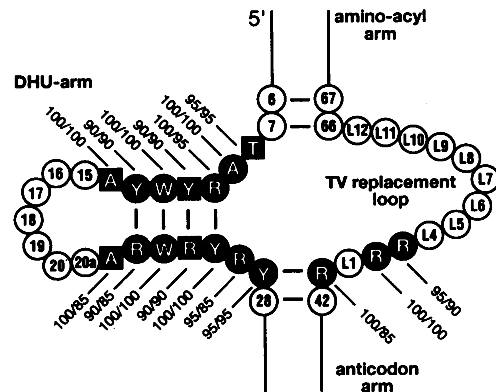

Figure 4. Diagram of the central region of the consensus secondary structure of the 20 *C.elegans* and 20 *A.suum* mt-tRNA genes that each contain a TV-replacement loop. The number of nucleotides shown in the DHU loop and the TV-replacement loop are the maximum numbers observed. Letters in solid squares identify nucleotides or nucleotide combinations that occur among *C.elegans*/*A.suum* mt-tRNA genes with frequencies (as percentages) shown by the accompanying numbers, and are also constant nucleotides in prokaryotic and eukaryotic nuclear-encoded tRNAs. Letters in solid circles identify other nucleotides or nucleotide combinations that occur in *C.elegans*/*A.suum* mt-tRNA genes with frequencies (as percentages) shown by the accompanying numbers. Eight of these nucleotides (nt 9, 10, 13, 22, 25, 26, 27, 43) are considered semi-invariant in prokaryotic and eukaryotic nuclear-encoded tRNAs (2,3). The numbers shown in the open circles reflect the conventional numbering system for yeast tRNA^{Phe} (nt 6−7, 28, 42, 66, 67; reference 4), and the numbering of TV-replacement loop nucleotides (L1−L12; 25) A, adenine; T, thymine; R, adenine or guanine; Y, cytosine or thymine; W, adenine or thymine. Modified from Figure 8 of reference 25.

tRNAs, there are the nucleotide combinations A15·UL4 (equivalent to nt 48) (16 in *C.elegans*; 10 in *A.suum*) or U15·AL4 (3 in *C.elegans*; 7 in *A.suum*) (Fig. 5), suggesting that the tertiary interaction between nucleotides 15 and 48 is conserved in nematode mt-tRNAs. Further support for this view is provided by the observation that there is a strictly conserved nucleotide change in this position between four corresponding mt-tRNAs of the two nematodes; the *C.elegans* Cys, Pro, Thr and Tyr mt-tRNAs each has the combination A15·UL4, while the four corresponding mt-tRNAs of *A.suum* each has U15·AL4 (Fig. 5). However, the high frequency G15·C48 and A15·U48 combinations in prokaryotic and eukaryotic tRNAs are not joined by Watson−Crick bonds: the two strands are parallel rather than

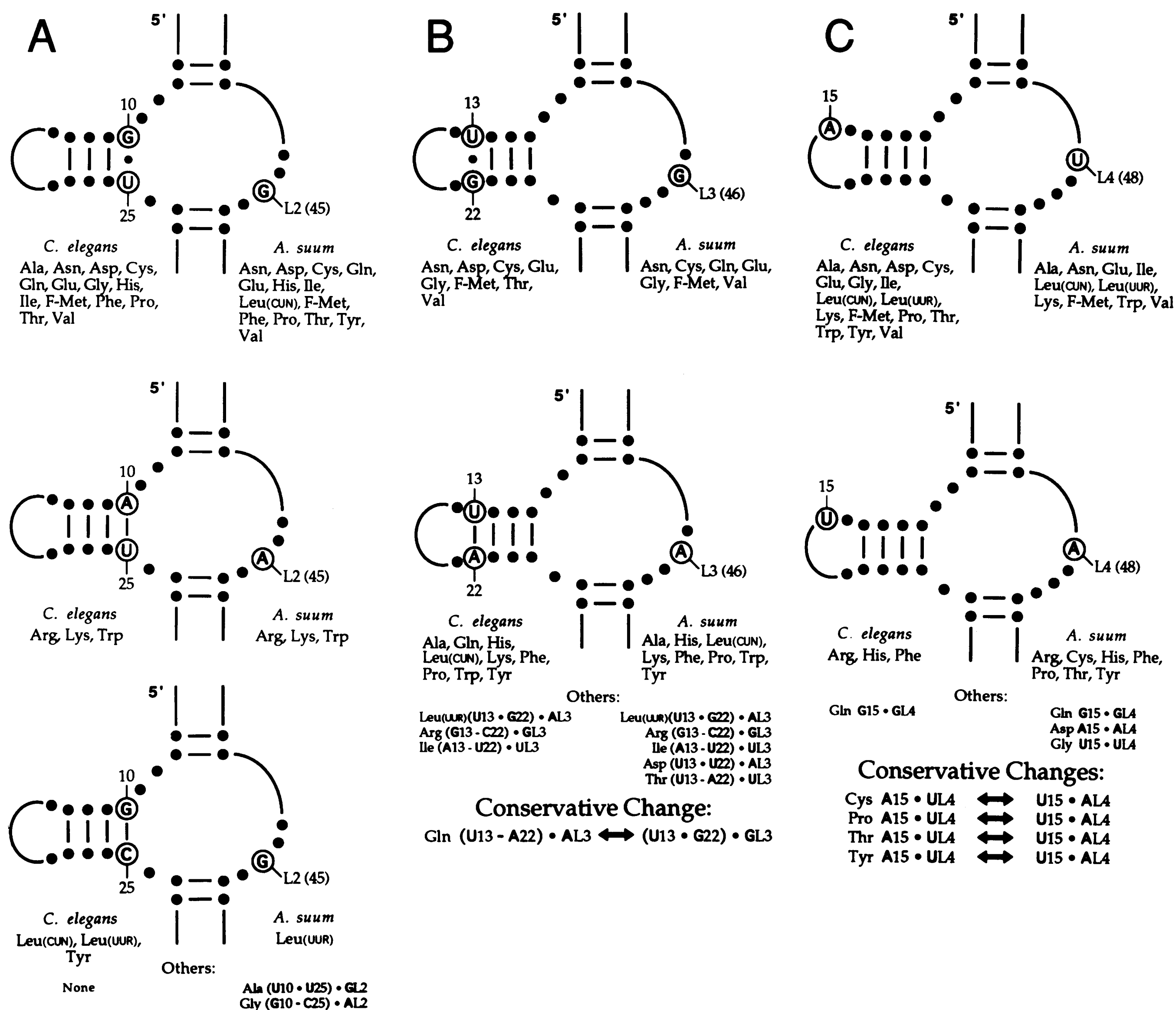

Figure 5. The distribution among the 20 TV-replacement loop-containing mt-tRNAs of *C.elegans* and *A.suum* of three different nucleotide combinations that in yeast RNA^{Phe} are involved in tertiary bonding. Column A, the nucleotide pair 10–25 in the DHU stem and nucleotide L2 (nt 45 in yeast tRNA^{Phe}) in the TV-replacement loop. Column B, the nucleotide pair 13–22 in the DHU stem and nucleotide L3 (nt 46) in the TV-replacement loop. Column C, nucleotide 15 in the DHU loop, and nucleotide L4 (nt 48) in the TV-replacement loop. Other nucleotide combinations that do not fit those diagramed are shown at the bottom of each column. Also shown at the bottoms of columns B and C are conservative changes in respective nucleotide combinations that occur between some corresponding mt-tRNAs of *C.elegans* and *A.suum*.

anti-parallel (11). Therefore, the joining of U15 and AL4 in nematode mt-tRNAs must involve bonds not found in prokaryotic and eukaryotic tRNAs.

The implied interpretation from the *C.elegans* and *A.suum* data that L4 in nematode mt-tRNAs corresponds to nucleotide 48 rather than nucleotide 47 (the fifth rather than the fourth nucleotide in the variable loop) of yeast tRNA^{Phe} is supported by the observation that in yeast tRNA^{Asp}, although there is a four nucleotide variable loop, all four tertiary interactions involving the variable loop nucleotides occur (16).

Nucleotide A9 in yeast tRNA^{Phe} is hydrogen bonded to A23 (of the DHU stem nucleotide pair U12–A23) to form the nucleotide triple A9·A23–U12. The tertiary interaction between correspondingly located nucleotides in *E.coli* initiator tRNA^{Met} involves bonding of a G in position 9 with a C in position 23 to yield the nucleotide triple G9·C23–G12 (12). However, these two combinations, A9·A23–U12 and G9·C23–G12, are found in only 20% and 21% of eukaryotic tRNAs. A third combination, G9·G23–C12, has the highest frequency (32%) in these tRNAs. Low frequencies of ten other 9·23–12 combinations occur in the remaining tRNAs. In prokaryotic tRNAs, A9·A23–U12 occurs in 53% of tRNAs and G9·C23–G12 occurs in 19%. The highest frequency 9·23–12 combination in eukaryotic tRNAs, G9·G23–C12, is present in only 5% of prokaryotic tRNAs. Again, there is a high number (nine) of other low frequency combinations. Among *C.elegans* and *A.suum* mt-tRNAs there is clearly a non-random occurrence of nucleotides in the 9·23–12 positions. Nucleotide 9 is always A. However, the 12–23 pair

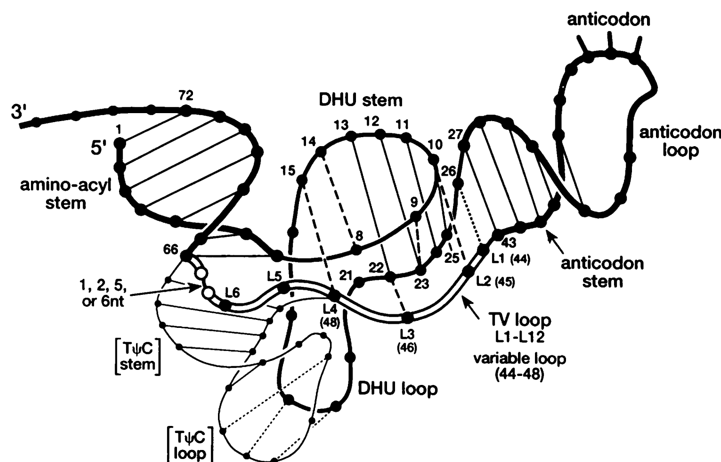

Figure 6. The tertiary structure of yeast tRNA^{Phe} as depicted in reference 8 on which is superimposed a possible partial consensus tertiary structure of the *C.elegans* and *A.suum* TV-replacement loop-containing mt-tRNAs. Solid dots represent nucleotides of both kinds of tRNA and follow the numbering system used for yeast tRNA^{Phe}, except that nucleotides 44, 45, 46 and 48 in the yeast tRNA^{Phe} variable loop also carry the labels of the first four nucleotides (L1, L2, L3, L4) of the nematode tRNA TV-replacement loops. The nematode TV-replacement loop (nt L1–L12) is shown as an open double line superimposed over the yeast variable loop (nt 44–48) and connecting with the first nucleotide (nt 66) of the common amino-acyl stem. The TΨC stem and loop that is unique to the yeast tRNA^{Phe} is shown as a thin line. Solid straight lines represent Watson–Crick pairings of nucleotides in stem regions. Dashed lines involving the nucleotide combinations 8·14, 9·23–12, L2 (nt 45)·10–25, L3 (nt 46)·22–13 and L4 (nt 48)·15, indicate tertiary bondings found in yeast tRNA^{Phe} that are also predicted to occur in the nematode mt-tRNAs. Dotted lines connecting the nucleotide combinations L1 (nt 44)–26, 18–55, 19–56 and 54–58 are tertiary interactions found in yeast tRNA^{Phe}, but not predicted in nematode mt-tRNAs.

is either A12–U23 (nine tRNAs in both *C.elegans* and *A.suum*) or U12–A23 (eleven tRNAs in both *C.elegans* and *A.suum*). These observations suggest that in about half of each set of nematode mt-tRNAs there occurs the A9·A23–U12 interaction found in yeast tRNA^{Phe}. The high frequency of the constant combination A9·U23–A12 in the remaining half of each set of *C.elegans* and *A.suum* mt-tRNAs suggests that a tertiary interaction is also provided by this nucleotide set. However, an A9·U23–A12 combination occurs only rarely in eukaryotic tRNAs and has not been recorded in prokaryotic tRNAs.

In yeast tRNA^{Phe}, m$^2_2$G26, the nucleotide between the DHU and anticodon stems, is hydrogen bonded to the first nucleotide of the variable loop, A44. But again, G26·A44 is not a highly conserved combination among eukaryotic and prokaryotic tRNAs: 42% and 20% respectively. In eukaryotic tRNAs there are ten other 26·44 combinations. Of these G26·U44, A26·A44, and A26·G44 are found in 14%, 10%, and 8% tRNAs, and the remaining seven combinations are all of lower frequency. Among prokaryotic tRNAs there are seven other 26·44 combinations, the most frequent of which are A26·G44 (23%), G26·U44 (16%), A26·A44 (16%), and A26·C44 (15%). Among the *C.elegans* and *A.suum* mt-tRNAs are found, respectively, 8 and 6 different 26·44 nucleotide combinations. However, in *C.elegans* 80% of mt-tRNAs have either G26·A44 (30%) or A26·A44 (50%). These combinations are less frequent in *A.suum* mt-tRNAs: G26·A44, 25%; A26·A44, 20%. Also, G26·U44 occurs in 20% of *A.suum* mt-tRNAs. Therefore, correlations of 26·46 position nucleotides among the *C.elegans* and *A.suum* mt-tRNAs in regard to possible conservation of tertiary bonding are less convincing than are the other nucleotide combinations considered.

Examination of the nematode mt-tRNAs did not reveal evidence for the occurrence within these tRNAs of the equivalent of three other tertiary interactions found in standard tRNAs. In standard tRNAs, hydrogen bonding between the invariant nucleotides, G18 and pseudouridine (Ψ)55, and G19 and C56 (a Watson–Crick base pair), hold together the DHU and TΨC loops at the outside elbow of the L-shaped tertiary structure (Figs. 3 and 6). A single G is found in only 14% and 30%, respectively, of the DHU loops of *C.elegans* and *A.suum* mt-tRNAs, the lowest occurrence of Gs in DHU loops among all sets of mt-tRNAs examined to date (20). Also, comparisons of nucleotides in the DHU and TV-replacement loops of the nematode mt-tRNAs failed to reveal any constancy of nucleotide combinations suggestive of one or more common interactions between these regions of nematode mt-tRNAs, other than those discussed above. Further, evidence for a nucleotide pair equivalent to the ribothymine 54 (T54)–m^{1}A58 in standard tRNAs (Fig. 3) was not obtained.

DISCUSSION

The data presented in this paper are interpreted as indicating that at least five of the nucleotide combinations involved in tertiary interactions in yeast tRNA^{Phe} (Figs. 3A and 6), and probably the majority of other standard tRNAs, are also involved in tertiary interactions in nematode TV-replacement loop-containing mt-tRNAs (Figs. 3B and 6). For the 20 *C.elegans* and 20 *A.suum* mt-tRNAs, respectively, 14 and 13, are predicted to have all 5 interactions, 4 and 4 mt-tRNAs would have a single exception, and 2 and 3 mt-tRNAs would have two exceptions (Fig. 5).

Among prokaryotic and eukaryotic tRNAs, the constancy of specific nucleotide combinations in the 8·21·14 and 15·48 positions indicates that the tertiary interactions found within these nucleotide sets in yeast tRNA^{Phe} are likely to occur in virtually all prokaryotic and eukaryotic tRNAs. However, consideration of the variety of combinations of nucleotides at positions 46·22–13, 45·10–25, 9·23–12, and 26·44 among prokaryotic and eukaryotic tRNAs, strongly suggests that tertiary interactions may not occur within all of these groups of nucleotides in all prokaryotic and eukaryotic tRNAs.

In the tertiary structure of yeast tRNA^{Phe}, the bases of the variable loop nucleotides 45, 46 and 48 that interact with

nucleotides in the DHU stem, lie in the major groove of the DHU stem helix (5; Fig. 6). These interactions, together with the bonding between nucleotides 26 and 44, complement the stacking of nucleotide pairs in the DHU and anticodon stems (Fig. 6). Conservation of the three (or possibly, in some cases, four) variable loop-DHU stem interactions in nematode mt-tRNAs, as suggested by our data, is therefore consistent with maintenance in these tRNAs of the same relative positions of the DHU and anticodon stems as occur in standard tRNAs. Interestingly, in yeast $tRNA^{Phe}$, nucleotide 47, which is absent in yeast $tRNA^{Asp}$ (16) and, as we propose, in nematode mt-tRNAs, does not take part in overall nucleotide stacking (5).

In yeast $tRNA^{Phe}$, the tertiary bondings of nucleotides 8 and 9 (located between the amino-acyl and DHU stems) with nucleotide 14 in the DHU loop, and nucleotide 23 in the DHU stem, respectively (Fig. 3A), stabilize the amino-acyl stem at an approximate right angle to the stacked DHU and anticodon stems (Fig. 6). Again, conservation of the equivalent interactions in nematode mt-tRNAs is consistent with the tRNAs having an overall L-shaped functional structure (Fig. 6). There may exist other tertiary interactions in nematode mt-tRNAs that substitute for the DHU loop-TΨC loop interactions of standard tRNAs (Fig. 6), that are not apparent from our nucleotide correlation considerations.

The proposal from the present data that the L-shaped tertiary structure of TV-replacement loop-containing mt-tRNAs is stabilized by the equivalent of at least five of the tertiary interactions that occur in standard tRNAs, contrasts with the situation in the bovine DHU-replacement loop-containing mt-$tRNA^{Ser(AGY)}$. In this case, as deduced from chemical probing data, stability of the L-shaped structure results from an almost unique set of tertiary interactions (28). Unusual tertiary interactions are also postulated to occur in the two computer-based DHU-replacement loop-containing mt-$tRNA^{Ser}$ models (29).

In most standard tRNAs, the TΨC arm and variable loop together comprise 22 nucleotides. In contrast, the sizes of the TV-replacement loops in 17 out of 20 of the *C.elegans* and *A.suum* mt-tRNAs are between 6 and 8 nucleotides. The remaining three mt-tRNAs of each species, f-Met, Ile and Lys, have TV-replacement loops of 11, 12 (or 11) and 12 nucleotides, respectively (Fig. 2; 27). Our interpretation of the nucleotide correlation data presented implies that nucleotides L1−L4 of the TV-replacement loop are homologous to 4 out of 5 nucleotides in the variable loop of standard tRNAs. Therefore, it is the TΨC arm that has undergone the greatest reduction in these nematode mt-tRNAs. From the remaining nucleotides (L5−L11, or L5−L12) of each of the TV-replacement loops of *C.elegans* mt-$tRNA^{f\text{-}Met}$, mt-$tRNA^{Ile}$ and mt-$tRNA^{Lys}$ (Fig. 2), a small TΨC arm comprising a 2 nucleotide pair stem and a 3 or 4 nucleotide loop could be formed. However, this structure would be conserved in only one of the corresponding *A.suum* mt-tRNAs (Lys). In the *C.elegans* mt-$tRNA^{Ile}$ TV-replacement loop (Fig. 2), nucleotides L5−L8 (5′ CCAG) are complementary to the following L9−L12 (5′ TTGG), consistent with the possibility that in this case the entire original TΨC loop has been eliminated.

Examination of the sets of 21 four-armed mt-tRNAs of human (30), chicken (31), a sea urchin (*Paracentrotus lividus*; 32) and *Drosophila yakuba* (33) revealed that among the mt-tRNAs of each species, all five nucleotide correlations noted for the nematode TV-replacement loop-containing mt-tRNAs also occur, but at lower frequencies (unpublished data). This may indicate that each of the five proposed tertiary interactions are present in some of the four-armed mt-tRNAs of each species examined, but a variety of other bondings are utilized to hold the different mt-tRNAs of a set into a common, presumably L-shaped, structure.

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