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Another Cut for Lysine tRNA: Application of the Hyperprocessing Reaction Reveals Another Stabilization Strategy in Metazoan Lysine tRNAs[1]

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Recently, we revealed that the cloverleaf structure of some eukaryotic tRNAs is not always stable *in vitro*, and the denatured structures of these tRNAs are sometimes detected in bacterial RNase P reactions. We have designated the unusual internal cleavage reaction of these tRNAs as hyperprocessing. We have developed this hyperprocessing strategy as a useful tool for examining the stability of the tRNA cloverleaf structure. There are some common features in such unstable, hyperprocessible tRNAs, and the criteria for the hyperprocessing reaction of tRNA are extracted. Metazoan initiator methionine tRNAs and lysine tRNAs commonly fit the criteria, and are predicted to be hyperprocessible. The RNase P reactions of two metazoan lysine tRNAs from *Homo sapiens* and *Caenorhabditis elegans*, which fit the criteria, resulted in resistance to the internal cleavage reaction, while one bacterial lysine tRNA from *Acholeplasma laidlawii*, which also fits the criteria, was internally cleaved by the RNase P. The results showed that the metazoan lysine tRNAs examined are very stable without base modifications even under *in vitro* conditions. We also examined the 3′-half short construct of the human lysine tRNA, and the results showed that this RNA was internally cleaved by the enzyme. The results indicated that the human lysine tRNA has the ability to be hyperprocessed but is structurally stabilized in spite of lacking base modifications. A comparative study suggested, moreover, that the acceptor-stem bases should take part in the stabilization of metazoan lysine tRNAs. Our data strongly suggest that the cloverleaf shape of other metazoan lysine tRNAs should also be stabilized by means of similar strategies to in the case of human tRNA(Lys3).

Key words: hyperprocessing, lysine tRNA, metazoa, RNase P.

Transfer RNA (tRNA) molecules are basic, ubiquitous molecules, and play important roles in the protein synthesis system. In most cases, the tRNA molecule has a common cloverleaf structure, consisting of four major stems, three major loops, and one extra variable loop. For a long time, this molecule was thought to be stable and rigid. Recently, however, some reports appeared showing that the cloverleaf structure of the tRNA molecule is not always stable without base modifications, or under some *in vitro* conditions (*1–11*). Some tRNAs show a change in conformation from the cloverleaf form to another one. In the case of *Drosophila* initiator methionine tRNA, the acceptor stem and anticodon stem melted under *in vitro* conditions, the D- and T-stems/loops being retained as core hairpins, to newly yield double-hairpin folding (*4*). The conformational changes of the tRNA molecule were experimentally confirmed using the *Escherichia coli* ribonuclease P (RNase P) RNA ribozyme reaction. The internal cleavage reaction on tRNA was designated as hyperprocessing. The phenomenon was not specific to this tRNA: conformational changes of *Drosophila* alanine tRNA, histidine tRNA, and human tyrosine tRNA were also detected (*2, 12*). These four tRNAs commonly exhibit complementarities between the acceptor-stem region and anticodon-stem with an extra loop region that promote disruption of the canonical cloverleaf folding and yield unexpected double hairpin folding. The presence of unexpected complementarities in the tRNA molecule is, of course, disadvantageous for the stability of tRNA. The hyperprocessing reaction of tRNAs is only observed *in vitro*, however, the hyperprocessing reaction strategy can be applied to evaluation of the stability of the cloverleaf folding of each tRNA molecule. Study on *in vitro* transcribed tRNA without base modifications itself might not be important. But the information obtained through such studies will be useful for consideration of the structural properties of RNA and for the preparation of tRNA by transcription, and moreover such information will explain the biological roles of the base modifications in tRNA molecules. One of the merits of our hyperprocessing strategy is the high sensitivity of the reaction: the content of double-hairpin shaped tRNA, which becomes the substrate for the RNase P reac-

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tion, is considered to be low but can be easily detected through accumulation of the cleaved products of the RNase P reaction.

In this paper, we determined the criteria, the common features, for the hyperprocessing reaction of tRNA molecules. Also, we applied the hyperprocessing strategy to evaluation of the stability of the cloverleaf structure of metazoan lysine tRNAs. Our hyperprocessing strategy can be a useful tool for evaluating the stability of the cloverleaf folding of the targeted tRNA molecules.

MATERIALS AND METHODS

Preparation of RNAs and Other Chemicals—E. coli and *Bacillus subtilis* ribonuclease P RNAs were prepared by *in vitro* transcription from the pGEM-3Z–derived plasmid DNAs with T7 RNA polymerase according to the method described previously (*2, 9*).

The human tyrosine tRNA precursor, fly initiator methionine tRNA precursor, fly alanine tRNA precursor, and fly histidine tRNA precursor were prepared by *in vitro* transcription from the pGEM-3Z–derived plasmid DNAs as described previously (*2, 3, 12*). Synthetic DNAs were used for the cloning of human, worm, and bacterial lysine tRNAs: 5′-AATTC ACCCG ATCTA GCCCG GATAG CTCAG TCGGT AGAGC ATCAG ACTTT TAATC TGAGG GTCCA GGGTT CAAGT CCCTG TTCGG GCGCC AGGA-3′, 5′-AGCTT CCTGG CGCCC GAACA GGGAC TTGAA CCCTG GACCC TCAGA TTAAA AGTCT GATGC TCTAC CGACT GAGCT ATCCG GGCTA GATCG GGTG-3′ (for human

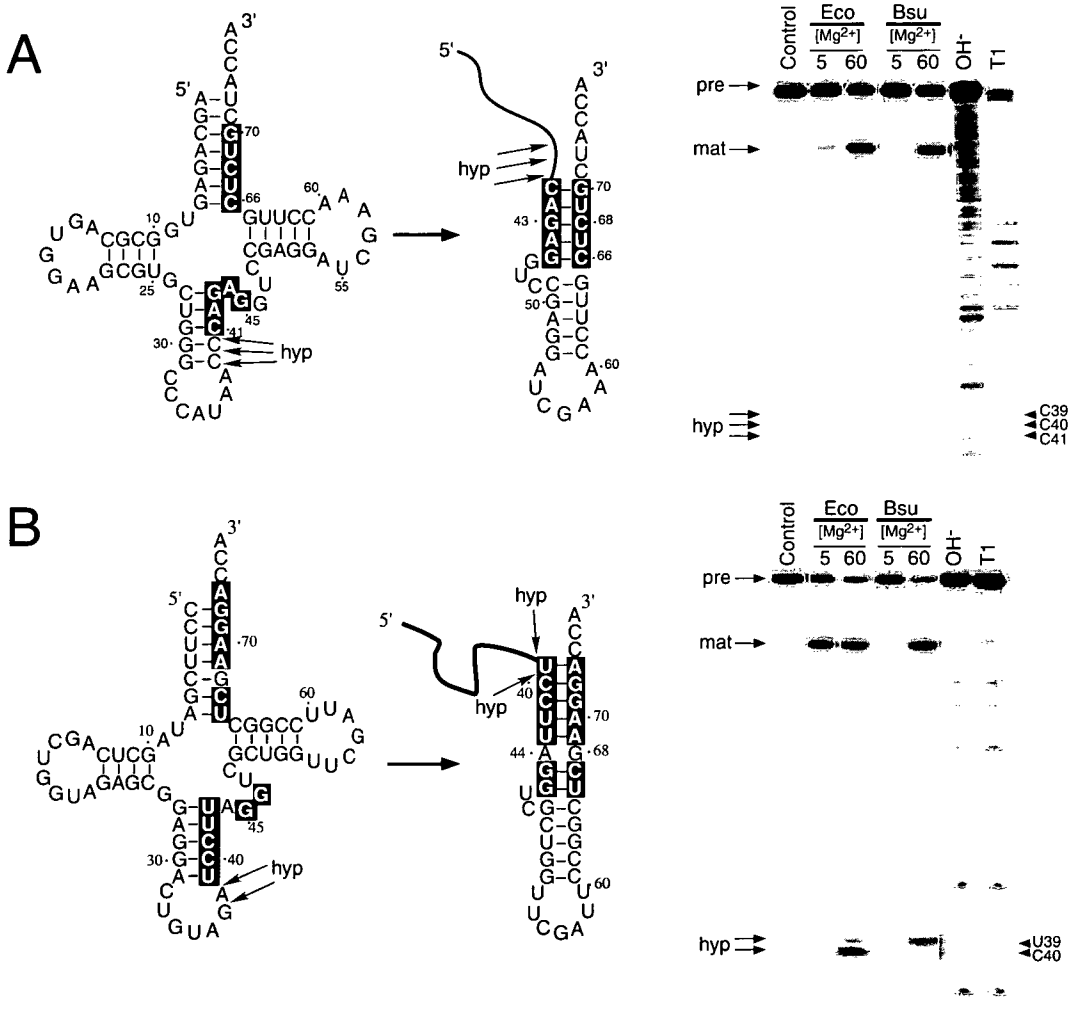

Fig. 1. **Examples of metazoan floppy tRNAs.** The cloverleaf structure of these tRNAs is disrupted, through melting of the acceptor- and anticodon-stems, to yield an alternative large 3′-hairpin, the extended T-hairpin, under *in vitro* conditions. The 3′-extended T-hairpin of these tRNAs is recognized as a substrate by bacterial ribonudease P, and is internally cleaved at the indicated sites (*2, 4, 12*). (A) Fly initiator methionine tRNA. Complementary regions C^{41}–G^{45} and C^{66}–G^{70} contribute to the formation of a new extended T-hairpin (*4*). 12 nM pre-tRNA was used as the substrate. 0.36 μM bacterial RNase P RNA was used. (B) Human tyrosine tRNA. Complementary regions U^{39}–G^{46} and U^{66}–A^{73} contribute to the formation of a new extended T-hairpin (*12*). 10 nM pre-tRNA was used as the substrate. 0.36 μM bacterial RNase P RNA was used. "Eco" and "Bsu" represent the reactions in the presence of *E. coli* and *B. subtilis* RNase P RNA, respectively. "Control" represents for the reactions in the absence of an enzyme. "OH^-," "T1," and "U2" represent the size markers, respectively. "Pre," "mat," and "hyp," on the left side of the photos, represent for the tRNA precursor, mature tRNA, and internally cleaved (hyperprocessed) product(s), respectively. The base position numbering is according to Sprinzl *et al.* (*21*).

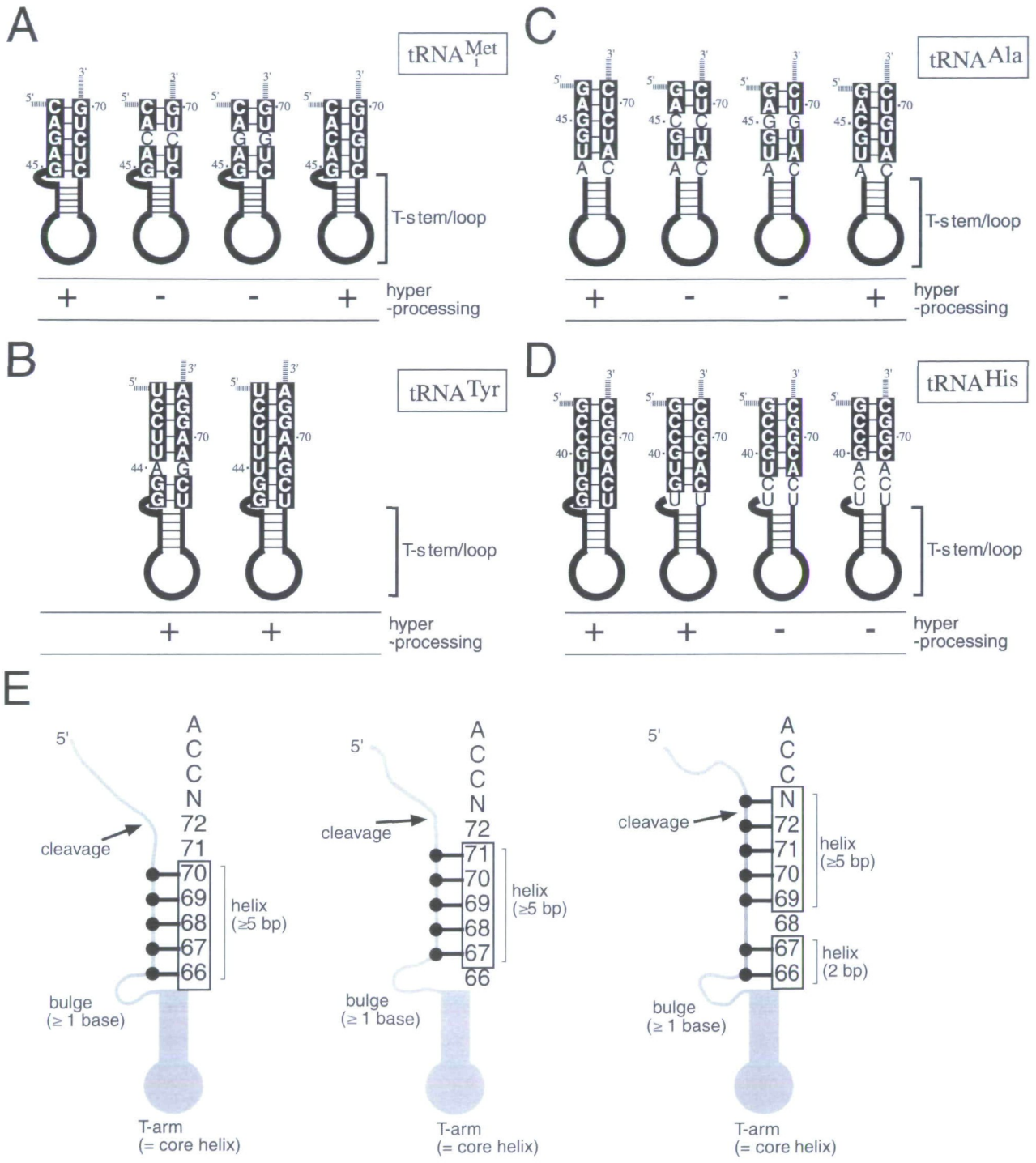

Fig. 2. **Criteria for the hyperprocessing reaction of "floppy" tRNAs by RNase P.** Some tRNAs have internally complementary sequences that contribute to the formation of a 3′-extended T-hairpin, the intact T-stem and TΨC-loop being retained, under *in vitro* conditions (*2, 4, 12, 22*). The results of mutant analyses of tRNAs are summarized: (A) in the case of fly initiator methionine tRNA (*4*), (B) in the case of human tyrosine tRNA (*12*), (C) in the case of fly alanine tRNA (*2*), and (D) in the case of fly histidine tRNA (*2*). (E) The T-hairpin comprising the 3′-half molecule of these floppy tRNA is recognized as a substrate by bacterial RNase P. Until now, the requirements for the internal cleavage reaction of RNase P, the hyperprocessing reaction, are categorized into three types: (i) the N^{66}–N^{70} region is included in the helix formation of more than five base-pairs in length in the extended T-hairpin (left); (ii) the N^{67}–N^{71} region is included in the helix formation (middle); (iii) and the discontinuous N^{66}–N^{67} and N^{69}–N^{73} regions are included in the helix formation (right; Ref. *12*). Base N^{68} in the third type can be base-paired, as shown in the first type (*12*). The base position numbering is according to Sprinzl *et al.* (*21*).

tRNA^{Lys}), 5′-AATTC ACCCG ATGTA GCCCG GTTAG CTCAG TCGGT AGAGC ACCAG ACTCT TAATC TGGTT GTCGC GGGTT CGAGC CCCGC ATTGG GCTCC AGGA-3′, 5′-AGCTT CCTGG AGCCC AATGC GGGGC TCGAA

CCCGC GACAA CCAGA TTAAG AGTCT GGTGC TCTAC CGACT GAGCT AACCG GGCTA CATCG GGTG-3′ (for *C. elegans* $tRNA^{Lys}$), 5′-AATTC CTTTT AATCT GAGGT CCAGG GTTCA AGTCC CTGTT CGGGC GCCAG GA-3′, 5′-AGCTT CCTGG CGCCC GAACA GGGAC TTGAA CCCTG GACCT CAGAT TAAAA GG-3′ (for the 3′-half of human $tRNA^{Lys}$), 5′-AATTC CTTTT AATCT GAGGT CCAGG GTTCA AGTCC CTGTT CGGGC ACCAG GA-3′, 5′-AGCTT CCTGG TGCCC GAACA GGGAC TTGAA CCCTG GACCT CAGAT TAAAA GG-3′ (for the 3′-half of the human $tRNA^{Lys}$ G73A mutant), 5′-AATTC ACCCG ATGTA GCATC CATAG CTCAG TTGGT AGAGC AACAG ACTCT TAATC TGTGG GTCCA CGGTT CGAGC CCGTG TGGGT GTACC AGGA-3′, and 5′-AGCTT CCTGG TACAC CCACA CGGGC TCGAA CCGTG GACCC ACAGA TTAAG AGTCT GTTGC TCTAC CAACT GAGCT ATGGA TGCTA CATCG GGTG-3′ (for *Acholeplasma laidlawii* $tRNA^{Lys}$). These DNAs were cloned onto pGEM-3Z (Promega) using *Eco*RI and *Hin*dIII restriction sites. The cloned sequences were confirmed by DNA sequencing.

Cleavage Assaying of RNAs—E. coli and *B. subtilis* RNase P RNAs, the tRNA precursors, and artificial hairpin substrates were prepared by *in vitro* transcription with T7 RNA polymerase ("T7 RNA polymerase," TOYOBO) using cleaved DNA templates. tRNA precursors were labeled at the 3′-end with [5′-^{32}P]pCp and T4 RNA ligase according to the methods described previously. The hyperprocessing reaction was performed at 37°C, for 120 min on a 10 μl

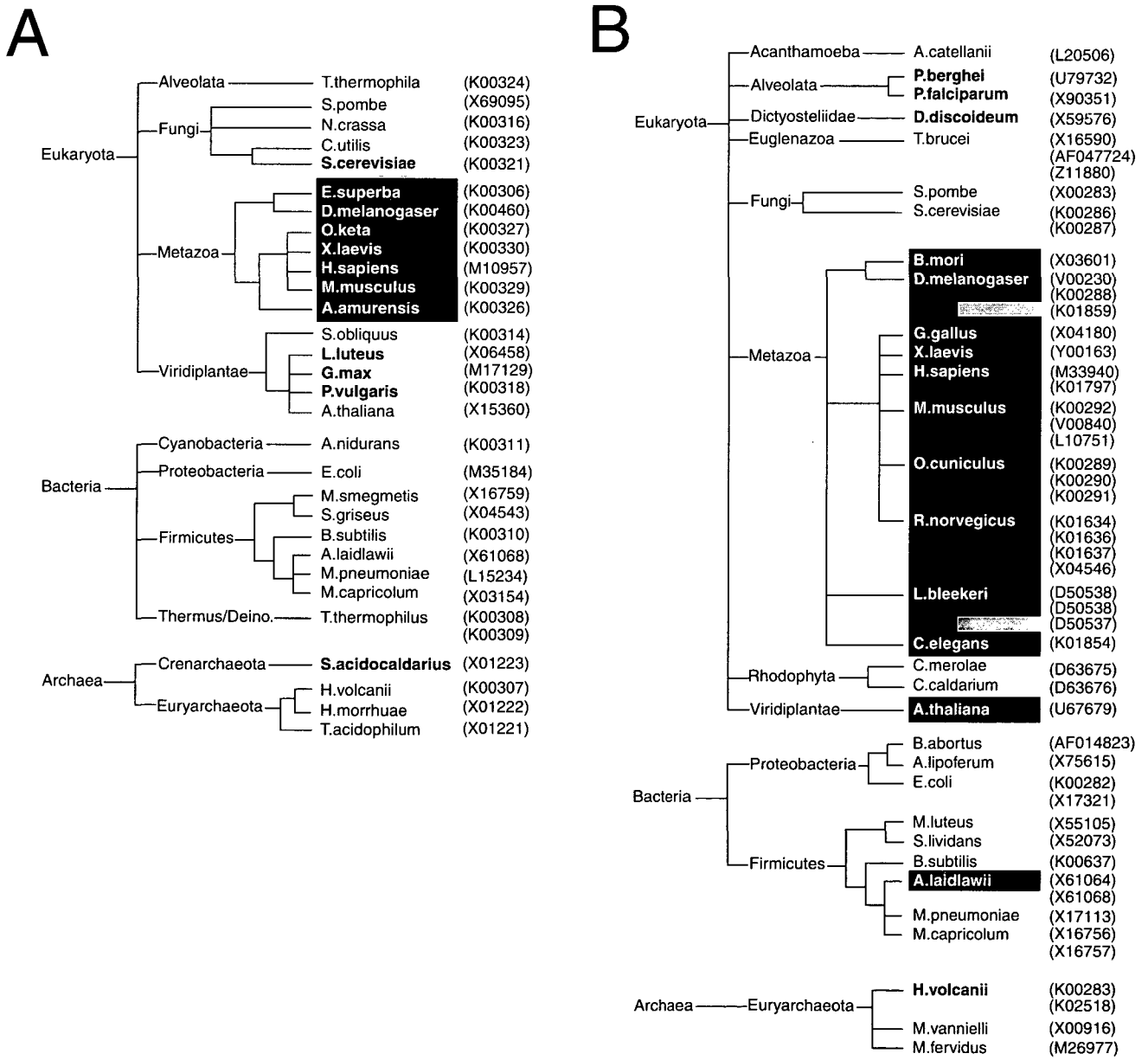

Fig. 3. **Prediction of floppy tRNAs.** The hyperprocessible tRNAs are phylogenetically analyzed according to the criteria given in Fig. 2. The tRNAs that fit the criteria are shown in closed boxes with white digits. The tRNAs that fit the criteria with a one-base replacement are shown in shaded boxes. The GenBank accession numbers are shown on the right. (A) Initiator methionine tRNA. (B) Lysine tRNA. (C) Complementary regions in lysine tRNAs.

C

Origin	Complementary regions	
anticodon: 'UUU'	39 44	66 71
human Lys3	UCUGAG	UUCGGG
mouse Lys5	UCUGAG	UUCGGG
mouse Lys	UCUGAG	UUCAGG
rat Lys5	UCUGAG	UUCGGG
rabbit Lys3	UCUGAG	UUCGGG
frog Lys	UCUGAG	UUCGGG
fly Lys5	UCCAAG	UUCGGG
squid Lys	UCUGAG	UGUGGG
squid Lys	CUCGG	UUGGG
anticodon: 'CUU'	40 44	67 71
human Lys1	CUCAG	UUGGG
chicken Lys1	CUCAG	UUGGG
mouse Lys4	CUCAG	UUGGG
rat Lys1	CUCAG	UUGGG
rat Lys2	CUCAG	UUGGG
rat Lys3	CUCAG	UUGGG
rabbit Lys1	CCCAG	UUGGG
rabbit Lys2	CUCAG	UUGGG
fly Lys2	CUCAG	UUGGG
fly Lys4	CUCAGG	CUUGGG
worm (C.elegans)	UCUGGU	AUUGGG
worm (B.mori)	CUCAG	UUGGG
squid Lys	CUCGG	UUGGG
plant Lys1	CUUGU	GUGGG
plant Lys2	CUUGU	GUGGG
plant Lys3	CUUGU	GUGGG
	38 43	66 70
A.laidlawii Lys	AUCUG	UGGGU

Fig. 3C

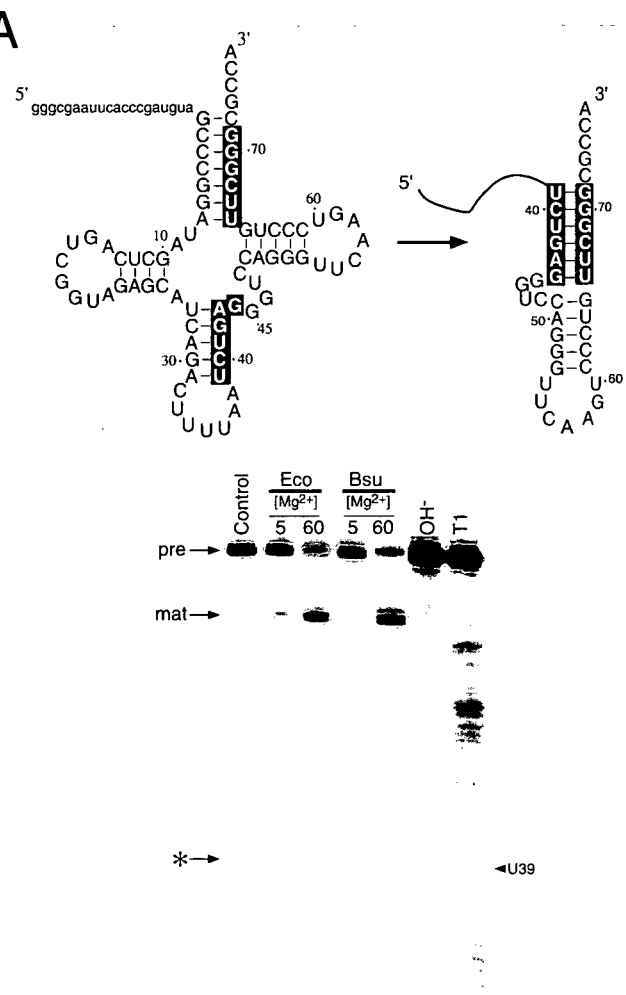

Fig. 4A

reaction scale, under the standard conditions with 5 or 60 mM Mg^{2+} (0.36 μM *E. coli* or *B. subtilis* RNase P RNA, 10–12 nM RNA substrates, 100 mM NH_4Cl, 5 or 60 mM $MgCl_2$, 5% [w/v] polyethylene glycol, 50 mM Tris-HCl; pH 7.6), and the reaction products were subjected to 20% PAGE with 8 M urea and then analyzed as described previously (*4*).

RESULTS AND DISCUSSION

Hyperprocessing Reaction as a Detection Tool for Floppy tRNAs—The cloverleaf shape of some tRNA molecules is not always stable, and is sometimes disrupted to yield an alternative "hyperprocessible" structure. The denatured tRNAs with the hyperprocessible structure are further recognized as substrates by bacterial RNase P enzymes and are then internally cleaved by the enzymes. At present, four hyperprocessible eukaryotic tRNAs are known: *Drosophila* initiator methionine tRNA (*4*), alanine tRNA, histidine tRNA (*2*), and human tyrosine tRNA (*12*). The hyperprocessing reaction of these tRNAs was examined at first using the *E. coli* enzyme, and then the hyperprocessing reaction of *Drosophila* alanine and histidine tRNAs by the *B. subtilis* enzyme was reported (*13*). These data strongly suggest that the hyperprocessing reaction of such a floppy tRNA is a common feature of bacterial RNase P enzymes.

Figure 1 shows the RNase P reactions on these four eukaryotic tRNAs with *E. coli* and *B. subtilis* RNase P ribozymes *in vitro*. The results indicated that these tRNAs are commonly hyperprocessible, floppy tRNAs, and that the *B. subtilis* enzyme as well as the *E. coli* one catalyzed the hyperprocessing of these tRNAs. So, we decided to use both the *E. coli* and *B. subtilis* enzymes as tools to examine the cloverleaf shape of the tRNA molecule in the following experiments.

Criteria Extraction and Prediction of Floppy tRNAs—The above results showed the examples of hyperprocessible, floppy tRNAs. Considering that the bacterial RNase P enzymes recognize hairpin RNA with a CCA-3′ tag sequence as well as the cloverleaf tRNA precursor, the hairpin formation of the 3′-half of the denatured tRNA is required for the hyperprocessing reaction. The results of mutant analyses of the RNase P reactions on these tRNAs are summarized in Fig. 2: the results for *Drosophila* initiator methionine tRNA (A), human tyrosine tRNA (B), *Drosophila* alanine tRNA (C), and histidine tRNA (D) are shown. The extracted criteria for the hyperprocessing reaction of tRNA are also summarized in Fig. 2E: (i) complementarity is required between the acceptor-stem region (around N^{66}–N^{73}) and the anticodon-stem region (around N^{32}–N^{48}) to form a new helix, (ii) the length of the newly formed helix is more than five bases or more, (iii) the T-stem/loop is retained and is used as a core hairpin, (iv) the newly formed

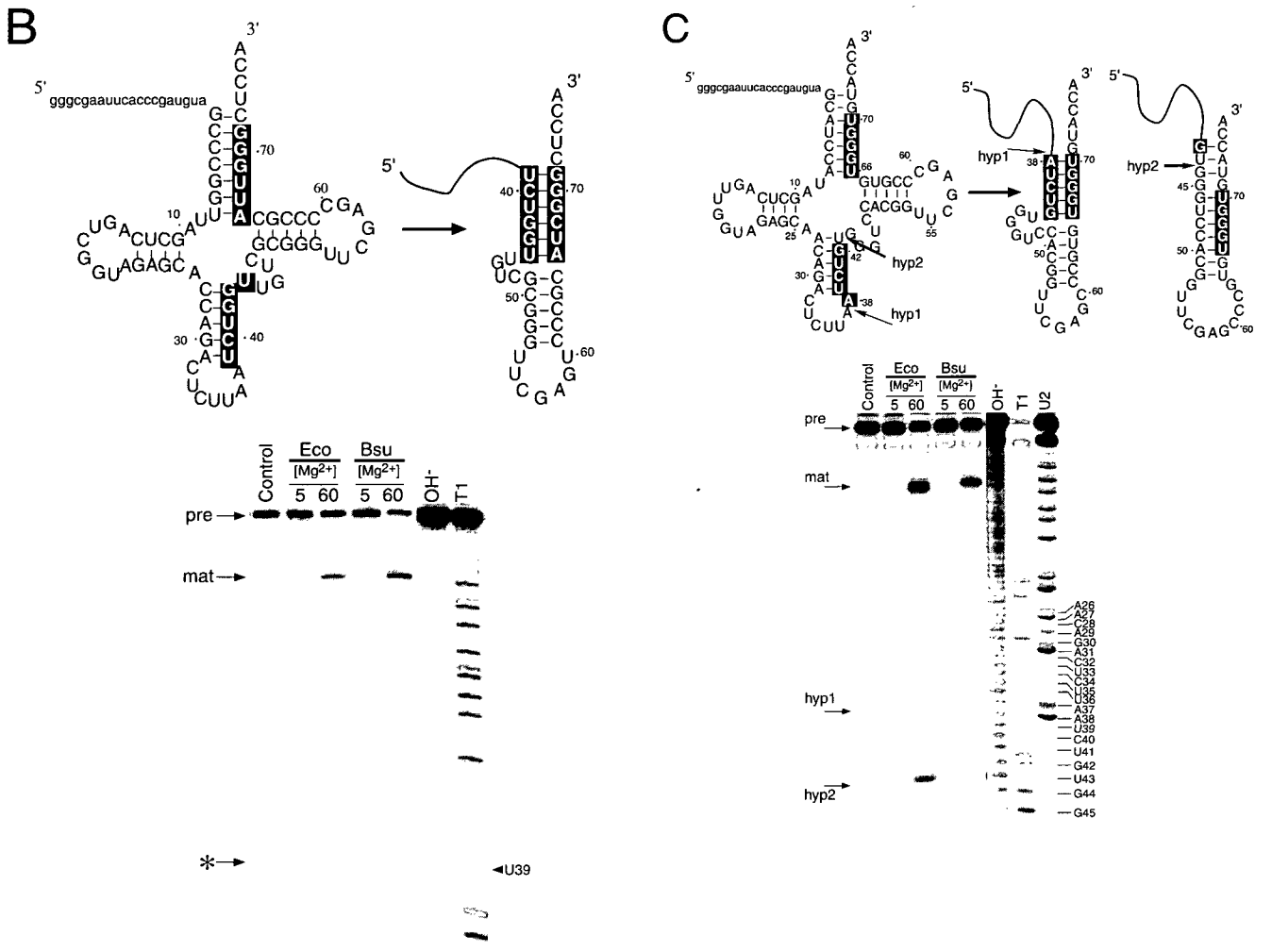

Fig. 4. **RNase P reactions on lysine tRNAs.** The RNase P reactions on two metazoan lysine tRNAs and one bacterial lysine tRNA were examined. (A) Human lysine tRNA precursor. (B) *C. elegans* lysine tRNA preursor. (C) Bacterial (*Acholeplasma laidlawii*) lysine tRNA precursor. In each reaction, 10 nM pre-tRNA and 0.36 μM RNase P RNA were used. Putative 3′-hairpin structures are shown with the cloverleaf structures. "pre," "mat," and "hyp," on the left of the photos represent the tRNA precursor, mature tRNA, and internally cleaved (hyperprocessed) product(s), respectively. The asterisks with arrows in A and B indicate the putative cleavage positions for the RNase P RNAs. For details, see Fig. 1.

helix should be located on or adjacent to the remaining T-stem/loop, (v) and the reactions are performed by bacterial RNase P.

With these criteria, we searched many tRNA sequences and found that many eukaryotic tRNAs fit the criteria. Interestingly, metazoan initiator methionine tRNAs and metazoan lysine tRNAs commonly fit the criteria (the two lysine tRNAs from fly and squid are exceptions, but they can fit the criteria with only one-base replacement; see Fig. 3, A and B). In the case of metazoan initiator methionine tRNAs, the base sequences are highly conserved, and the tRNAs commonly fit the hyperprocessing criteria as in the case of *Drosophila* tRNA. On the other hand, the metazoan lysine tRNAs are divided to two groups according to the anticodon bases, "CUU" and "UUU" (see Fig. 3C). Among "CUU" lysine tRNAs, metazoan and plant tRNAs commonly fit the criteria: the N^{40}–N^{44} and N^{67}–N^{71} regions are complementary (in the cases of fly Lys4 and *C. elegans* Lys, the N^{39}–N^{44} and N^{66}–N^{71} regions are complementary). Among "UUU" lysine tRNAs, seven metazoan tRNAs commonly fit the criteria: the N^{39}–N^{44} and N^{66}–N^{71} regions are complementary, or the N^{40}–N^{44} and N^{67}–N^{71} regions are complementary. Moreover, one bacterial "CUU" lysine tRNA fits the criteria.

Based on the above results, we chose two metazoan lysine tRNAs, human $tRNA_3^{Lys}$ and *Caenorhabditis elegans* $tRNA^{Lys}$, and one bacterial (*A. laidlawii*) lysine tRNA to examine the RNase P reactions to evaluate the stability of the cloverleaf shape of the tRNAs.

RNase P Reactions on Lysine tRNAs—In human $tRNA_3^{Lys}$, the $U^{39}C^{40}U^{41}G^{42}A^{43}G^{44}$ and $U^{66}U^{67}C^{68}G^{69}G^{70}G^{71}$ regions are complementary and these sequences are expected to contribute to the formation of a new helix on the T-stem/loop *in vitro*: the putative cleavage site is the A^{37}–A^{38} or A^{38}–U^{39} bond (Fig. 4A; top). The results for the RNase P reactions on this tRNAs are shown in Fig. 4A (bottom). The reactions were performed with both *E. coli* and *B. subtilis* ribozymes. The normal processing reaction was efficiently catalyzed by both enzymes, while the hyperprocessing reaction on this tRNA was not detected. These data

indicate that the human $tRNA_3^{Lys}$ is resistant to the hyperprocessing reaction, which suggests that the cloverleaf structure of this tRNA is stable in spite of the presence of the risky complementary sequences.

The same results were obtained for the *C. elegans* $tRNA^{Lys}$ (see Fig. 4B). In this worm tRNA, the $U^{39}C^{40}U^{41}G^{42}G^{43}U^{44}$ and $A^{66}U^{67}U^{68}G^{69}G^{70}G^{71}$ regions are complementary and these sequences are expected to contribute to the formation a new helix on the T-stem/loop *in vitro*: the putative cleavage site is the A^{37}–A^{38} or A^{38}–U^{39} bond (Fig. 4B; top). The normal processing reactions were efficiently catalyzed, while no hyperprocessing reaction was observed (Fig. 4B; bottom).

On the other hand, the results for the bacterial $tRNA^{Lys}$ were different. In *A. laidlawii* $tRNA^{Lys}$, the $A^{38}U^{39}C^{40}U^{41}G^{42}$ and $U^{66}G^{67}G^{68}G^{69}U^{70}$ regions are complementary and these sequences are expected to contribute to the formation of a new helix on the T-stem/loop *in vitro*: the putative cleavage site is the U^{35}–U^{36} or A^{37}–A^{38} bond (Fig. 4C; top). The normal processing reaction on this tRNA was, of course, ob-

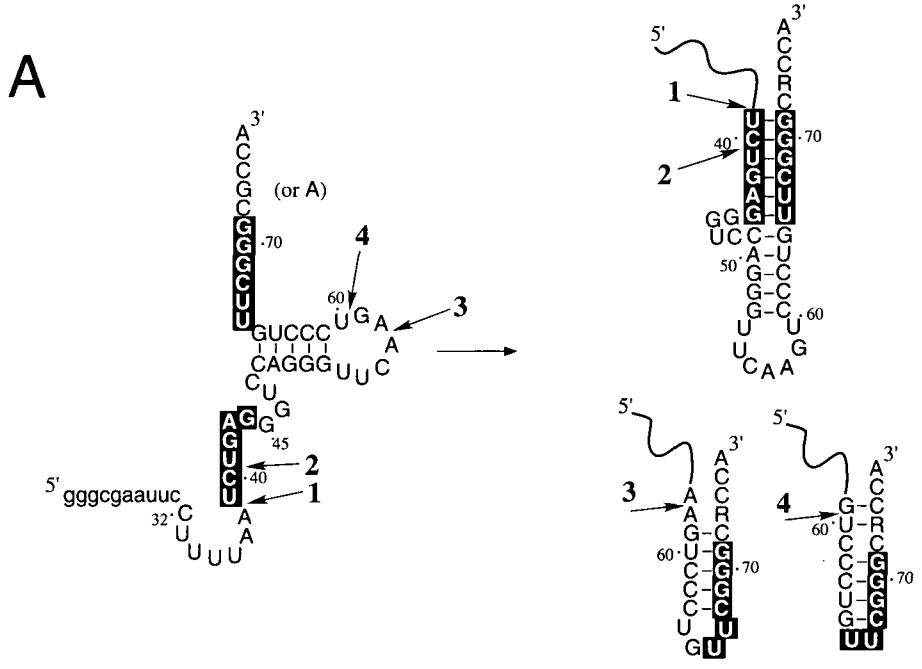

Fig. 5. **RNase P reactions on model substrates derived from $tRNA^{Lys}$.** (A) Schematic representation of the secondary structure of the hairpin substrate derived from human $tRNA_3^{Lys}$. Two substrates, containing "G" and "A" at position 73, respectively, were examined. The C^{32}–A^{76} region (containing the anticodon-loop, the strand of the anticodon-stem, the extra loop, the T-stem, the TΨC-loop, the strand of the acceptor-stem, and the RCCA regions) of the substrate is derived from human $tRNA_3^{Lys}$; other bases in the 5′-leader region, including the *Eco*RI site, are derived from the pGEM-3Z plasmid DNA. Complementary regions U^{39}–G^{44} and U^{66}–G^{71} are marked. The cleaved sites in the substrate are indicated by arrows. (B) RNase P reaction on the substrate RNAs. 12 nM RNA and 0.36 μM RNase P RNA were used for each reaction.

served, and moreover, the hyperprocessing reaction on this tRNA was also observed (Fig. 4C; bottom).

The above results showed that the two metazoan lysine tRNAs were resistant to the hyperprocessing reaction, which indicated that the cloverleaf structure of these tRNAs is highly stabilized even under the harsh *in vitro* conditions. Considering that the base sequences of the metazoan lysine tRNAs are highly conserved (see Figs. 3C and 6C), the other metazoan tRNAs, which were not examined in this study, are also expected to be stabilized and to be resistant to the hyperprocessing reaction.

RNase P Reactions on a Model Hairpin Substrate—The results in Fig. 4 showed that the metazoan lysine tRNAs were resistant to the hyperprocessing reaction. We then prepared hairpin RNAs derived from the human $tRNA_3^{Lys}$ to examine whether or not the $U^{39}C^{40}U^{41}G^{42}A^{43}G^{44}$ and $U^{66}U^{67}C^{68}G^{69}G^{70}G^{71}$ complementary regions have the ability to form a new helix, as shown in Fig. 4A. The putative secondary structures of the RNAs are shown in Fig. 5A. In the model RNA, the C^{32}–C^{72} and $G^{73}CCA$–3′ regions are derived from the wild type sequence. The results of the RNase P reactions are shown in Fig. 5B. The results showed that the hairpin RNA containing the 3′-half sequence of the human lysine tRNA was internally cleaved by the RNase P at several sites, which indicates that the U^{39}–G^{44} and U^{66}–G^{71} strands formed, at least partially, the expected helix (see the "product-1" and "product-2" bands). We also examined another RNA which contains A^{73} instead of G. The results of the RNase P reaction on this mutant were the same as for the G^{73} hairpin RNA.

These data indicate that the 3′-half fragment of human $tRNA_3^{Lys}$, corresponding to the C^{32}–$C^{72}G^{73}CCA$ region, has the ability to form the alternative hairpin, which could be recognized as a substrate by the bacterial RNase P and should be cleaved internally at the expected sites. Compared with the results shown in Fig. 4A, the human lysine tRNA has the ability to be hyperprocessed, as we predicted in Fig. 2, however, the cloverleaf structure of this tRNA should be stabilized through some strategies at the molecular level.

How the Metazoan Lysine tRNA Is Stabilized?—Two metazoan lysine tRNAs were resistant to the hyperprocessing reaction, while the bacterial lysine tRNA was sensitive to the reaction. What is the difference between them? Sequence comparison of them, shown in Fig. 6, indicated that many bases are conserved in the three tRNAs. Structural studies on the human lysine tRNA showed that the anticodon stem and loop region (C^{28}–G^{42}) of this tRNA are highly stabilized through base stacking (*14–18*), and other studies on various tRNAs showed that the interactions between the D-loop and the TΨC-loop stabilize the tertiary L-shaped structure of the tRNA molecule (*19, 20*). Our results for the metazoan tRNAs were consistent with these results even though the tRNAs used in our study lack the base modifications, but these results can not explain the sensitivity of the bacterial lysine tRNA to the hyperprocessing reaction: the bases in the D-stem/loop, anticodon-stem/loop, and T-stem/loop are also highly conserved in the three tRNAs (see Fig. 6). The differences are mainly found in the bases located in the acceptor-stem: the bases at N^3, N^{70}, N^5, N^{68}, N^6, N^{67}, and N^{73} of the bacterial tRNA differ from the metazoan bases. Considering the results in Fig. 5, *i.e.* that base G^{73} or A^{73} in the 3′-half short construct of human lysine tRNA did not affect the hyperprocessing reaction of RNAs, the other bases in the acceptor-stem should also take part in the stabilization of the cloverleaf structure of the metazoan tRNAs.

Conclusions—As shown by the above results, the bacterial RNase P is a molecular scissor which can be used as a sensitive detection tool for hairpin-folded denatured tRNA. Although the "criteria" we presented in this report are not complete, they would be useful for predicting "hyperprocessible" tRNAs: according to our experience, the tRNAs which did not fit the "criteria" have never been hyperprocessed.

Our hyperprocessing strategy revealed that the cloverleaf structure of metazoan lysine tRNAs was efficiently retained by the base sequence alone, without the base modifications.

As shown by the above results, the hyperprocessing strategy is a useful tool for evaluating the tRNA stability, and for revealing the hidden molecular strategy for the stabilization of tRNA molecules.

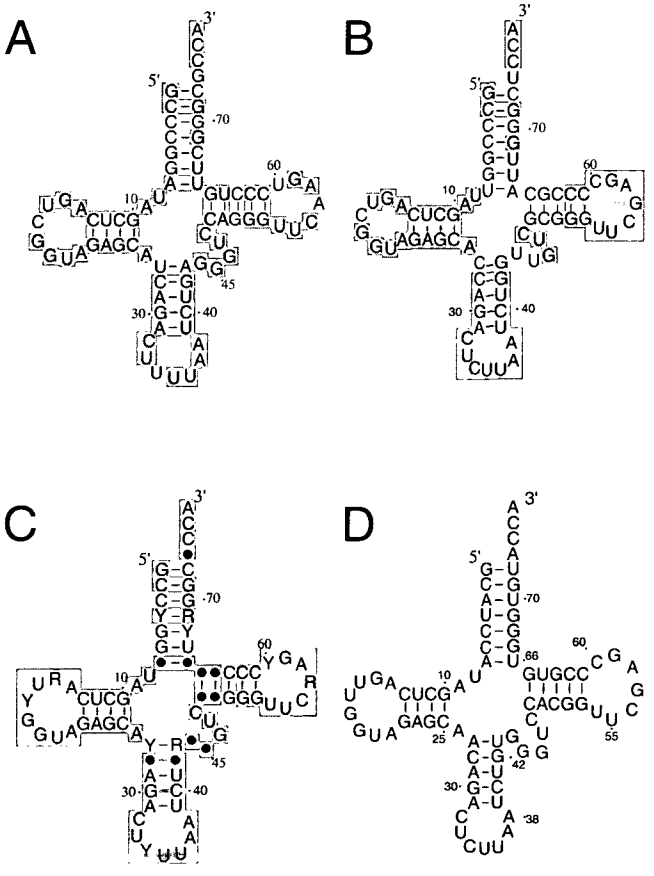

Fig. 6. **Comparison of lysine tRNAs.** (A) Human $tRNA_3^{Lys}$. The conserved bases as to the bacterial (*A. laidlawii*) $tRNA^{Lys}$ are indicated by a grey back ground. (B) *C. elegans* $tRNA^{Lys}$. (C) The conserved bases in metazoan lysine tRNAs. (D) *A. laidlawii* $tRNA^{Lys}$.

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