

Cleavage of tRNA within the mature tRNA sequence by the catalytic RNA of RNase P: Implication for the formation of the primer tRNA fragment for reverse transcription in *copia* retrovirus-like particles

(RNA enzyme/retrotransposon/"hyperprocessing"/tRNA secondary structure/*Drosophila*)

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ABSTRACT The retrovirus-like particles of *Drosophila* are intermediates of retrotransposition of the transposable element *copia*. In these particles, a 39-nucleotide-long fragment from the 5' region of *Drosophila* initiator methionine tRNA (tRNA_i^{Met}) is used as the primer for *copia* minus-strand reverse transcription. To function as primer for this reverse transcription, the *Drosophila* tRNA_i^{Met} must be cleaved *in vivo* at the site between nucleotides 39 and 40. When a synthetic *Drosophila* tRNA_i^{Met} precursor was incubated with M1RNA, the catalytic RNA of *Escherichia coli* RNase P, other cleavages within the mature tRNA sequence were detected in addition to the efficient removal of the 5' leader sequence of this tRNA precursor. One of these cleavage sites is between nucleotides 39 and 40 of *Drosophila* tRNA_i^{Met}. Based on this result, we propose a model for formation of the primer tRNA fragment for reverse transcription in *copia* retrovirus-like particles.

Drosophila cells contain virus-like particles that have about 5 kilobases (kb) of RNA homologous to the transposon *copia* (1). In addition to this 5-kb RNA, these particles also contain small cellular RNAs including tRNAs. Reverse transcriptase activity is also detected in the particles. These particles are therefore thought to be intermediates of transposition of *copia* and are called retrovirus-like particles (RVLPS). The *copia* RVLPS has been shown to replicate by a mechanism similar to that of vertebrate retroviral replication. We have however reported that the RVLPS has another type of priming mechanism, different from that of vertebrate retroviruses in the initiation of reverse transcription (2). The replication of all retroviruses so far examined is initiated by covalent addition of a deoxynucleotide to the 3' end of a cellular tRNA molecule that is hydrogen bonded to the primer binding site of the viral genome RNA (3). Generally, the 3'-terminal 18 nucleotides of the primer tRNA are complementary to the primer binding site of the viral genome RNA. Although this has been thought to be a universal mode of initiation of retroviral replication, it has been found that a different mode of tRNA priming is operating in *copia* RVLPS of *Drosophila*, where the first DNA extension does not start from the 3' end but from an internal site of the *Drosophila* initiator methionine tRNA (tRNA_i^{Met}) (2). In this case, an almost central 15-nucleotide sequence (around the anticodon loop, nucleotides 25–39 of the tRNA) but not the 3'-terminal region of the tRNA is complementary to the primer binding site of the RVLPS genome. After removal of the noncomplementary 3'-terminal region (nucleotides 40–75) of the tRNA, DNA synthesis starts from the newly formed 3' end (nucleotide 39)

of the 5' fragment of the tRNA, which is hydrogen bonded to the primer binding site.

Since there is no gene encoding this primer tRNA fragment in *Drosophila* cells (4) and since this fragment has the same modified bases as are found in the mature tRNA_i^{Met} of *Drosophila* (2), this primer tRNA fragment may be formed by further processing of mature tRNA_i^{Met} in *Drosophila* cells. We propose to call this further processing hyperprocessing. Hyperprocessing is distinguished from normal degradation of mature RNA in that the hyperprocessed product should have a biological function. We propose that cellular RNase P catalyzes the hyperprocessing of an altered conformational state of mature tRNA_i^{Met}. RNase P, which cleaves tRNA precursors to make mature 5' ends of tRNAs, is a ribonucleoprotein (for review, see ref. 5), and the RNA component of several prokaryotic variants of RNase P has catalytic activity (6).

In this paper we report that RNA component of *Escherichia coli* RNase P (M1RNA) cleaves a synthetic *Drosophila* tRNA_i^{Met} precursor at several sites within the mature tRNA sequence *in vitro*. Most of these cleavages can be explained by the known properties of M1RNA (7) and conformational changes of the substrate tRNA. One of the sites cleaved is between nucleotides 39 and 40 of this tRNA. We propose that hyperprocessing of *Drosophila* tRNA_i^{Met} at this site produces the primer tRNA fragment needed for *copia* retrotransposition.

MATERIALS AND METHODS

Enzymes and Chemicals. T7 RNA polymerase was obtained from Pharmacia. The restriction endonucleases *Apa* I, *Hind*III, *Pst* I, and *Xba* I, as well as DNA polymerase I Klenow fragment were from Toyobo (Osaka). RNase-free DNase was purchased from Promega. *Taq* DNA polymerase (from *Thermus aquaticus* YT1) was from Biotech International (Perth, Australia). T4 DNA ligase and reverse transcriptase from Rous-associated virus 2 were from Takara Shuzo (Kyoto). T4 polynucleotide kinase (3'-phosphatase-free) was from Boehringer Mannheim. T4 RNA ligase was prepared as described (8) or was from Takara Shuzo. [γ -³²P]ATP was from Amersham. [γ -³²P]pCp was synthesized by phosphorylation of 3'-CMP with [γ -³²P]ATP and 3'-phosphatase-free T4 polynucleotide kinase as described (9). Other chemicals were purchased from commercial sources.

Preparation of the Catalytic RNA Subunit of RNase P. The catalytic RNA subunit of RNase P of *E. coli* (M1RNA) was prepared by *in vitro* transcription with T7 RNA polymerase of plasmid pYAY (10). The *in vitro* transcript from pYAY

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Abbreviations: RVLPS, retrovirus-like particle; tRNA_i^{Met}, initiator methionine tRNA.

restricted by *Xba* I has a size of 428 bases and contains the M1RNA sequence (377 bases), vector and genomic sequences of 36 bases at the 5' end, and 15 bases at the 3' end of M1RNA. This transcript has previously been shown to have ribozyme activity (10). The reaction mixture for this transcription contained 40 mM Tris-HCl (pH 7.6), 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 200 units of RNasin, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM UTP, and 0.5 mM GTP, 1 μg of *Xba* I-linearized pYAY as template DNA, and 140 units of T7 RNA polymerase in a total volume of 100 μl. The mixture was incubated for 40 min at 37°C, and then 1 μl (1 unit) of RNase-free DNase was added and incubation was continued for 10 min at 37°C. After phenol extraction and ethanol precipitation, RNA was collected by centrifugation, dried, and dissolved in water to a concentration of 1 μg/μl.

Preparation of the Substrate RNA Containing the *Drosophila* tRNA^{Met} sequence. The substrate RNA was transcribed *in vitro* with T7 RNA polymerase from plasmid pDtY, which consists of a synthetic *Drosophila* tRNA^{Met} gene and the pGEM-2 vector (Promega). Two single-stranded DNAs, UtH51 (5'-TACAAGCTTGCAAGCAGAGTGGCGCAGTGGAAGCTGTGGGCCATAACC-3') and DtP60 (5'-GAACGTGAGTGTAGCAGAGCAAGGTTTCGATCCTCGGACCTCTGGGTTATGGGCCAGC-3') were synthesized using a solid-phase system on an Applied Biosystems DNA synthesizer. Since the 3'-terminal 15 nucleotides of these two DNAs were hybridizable to each other, the two DNAs were annealed and the long recessed 3' ends were filled in with dNTPs and *Taq* DNA polymerase. For this, the reaction mixture contained 0.5 μg of UtH51, 0.5 μg of DtP60, 50 mM KCl, 10 mM Tris-HCl (pH 8.5), 2.5 mM MgCl₂, gelatin at 0.2 mg/ml, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dTTP, and 0.2 mM dGTP in a total volume of 20 μl. The samples were layered with ≈20 μl of mineral oil to minimize evaporation. The mixture was heated at 95°C for 2 min and annealed at 37°C for 10 min, and then 0.5 unit of *Taq* DNA polymerase was added and this mixture was incubated at 70°C for 30 min. The reaction mixture was electrophoresed through an agarose gel and a double-stranded DNA of 96 base pairs (bp) was isolated from the gel. This DNA, containing *Hind*III and *Pst* I recognition sites at the 5' and 3' terminal regions, respectively, was digested with the two enzymes and then cloned into the *Hind*III and *Pst* I sites of the pGEM-2 vector to give pDtY. Transcription with T7 RNA polymerase from *Pst* I-linearized pDtY produced the 94-nucleotide-long RNA molecule shown in Fig. 2B. This RNA contained the 14-nucleotide long sequence (-18 to -5) from the pGEM-2 vector, 76 nucleotides (-4 to +72) from the *Drosophila* tRNA^{Met} gene, and a 4-nucleotide sequence, 5'-CCAC-3', at the 3' end. The sequence of this RNA was confirmed by reverse transcriptase sequencing essentially as described in the instructions to the Riboprobe system (Promega). The sequence of the terminally labeled RNA was also confirmed by enzymatic sequencing using RNase T1, RNase U2, *Staphylococcus aureus* nuclease, and alkaline or acid digestion as described (9).

Preparation of the Substrate RNA Having a Deletion of 5' Sequences of the tRNA. To obtain a 5'-deleted RNA substrate, plasmid pDtYS was constructed as follows. pDtY, containing single *Hind*III and *Apa* I sites (-9 to -4 and 28-33 in Fig. 2B, respectively), was treated with *Hind*III and *Apa* I and the resulting linear DNA was incubated with Klenow fragment of DNA polymerase I and four dNTPs to generate blunt ends. This linear DNA was circularized using T4 DNA ligase and transformed into *E. coli* HB101 to give pDtYS. Transcription with T7 RNA polymerase from *Pst* I-linearized pDtYS produced a 58-nucleotide-long RNA, which was an RNA with a deletion of 5' sequences from -4 to +32 (Fig. 4B) of the *Drosophila* tRNA^{Met}. This RNA substrate is expected to form the secondary structure shown in Fig. 1 Right and Fig. 4B.

Labeling of Substrate RNAs. The transcription mixtures for the RNA substrates were as described above for preparation of the catalytic RNA except that *Pst* I-linearized pDtY or pDtYS was used as the DNA template instead of pYAY. The transcripts were precipitated with ethanol, dried, dissolved in urea/dye loading mixture, and electrophoresed through 15% polyacrylamide/8 M urea gels. The RNAs were located by UV shadowing, eluted from the gels, precipitated with ethanol, dried, and labeled at the 3' end with [5'-³²P]pCp and T4 RNA ligase or at 5' end with [γ-³²P]ATP and T4 polynucleotide kinase as described (9). The labeled products were gel-purified and used as substrates for M1RNA.

Cleavage Reactions with the Catalytic RNA. Cleavage reactions were performed essentially as described by Guerrier-Takada *et al.* (6). The complete reaction mixture contained 50 mM Tris-HCl (pH 7.6), 100 mM NH₄Cl, 60 mM MgCl₂, 5% (wt/vol) polyethylene glycol, 1 μg of M1RNA (transcript of pYAY), and 2.5 fmol of 3'- or 5'-end-labeled substrate RNA (transcript of pDtY or pDtYS) in a total volume of 10 μl. Mixtures were incubated at 37°C for 1 hr and reactions were stopped by addition of 2 μl of 0.5 M EDTA. The products were separated by electrophoresis through 20% or 15% polyacrylamide/8 M urea gels and were detected by autoradiography. The cleavage sites were determined by comparison of the mobility of the products with those of a ladder of fragments prepared by partial alkaline or acid digestion of the substrates.

Cloning, Nucleic Acid Manipulations, and Analytical Methods. DNA manipulations and cloning techniques were performed essentially as described by Maniatis *et al.* (11). Labeling, controlled alkaline or acid hydrolysis, gel electrophoresis of RNA, and other analytical methods were as described by Krupp and Gross (9).

RESULTS

To function as the primer in the reverse transcription of *cop*ia, *Drosophila* tRNA^{Met} should be cleaved specifically between nucleotides 39 and 40. This site is located in the stable G+C-rich anticodon stem in the cloverleaf form of the tRNA (Fig. 1 Left) and appears to have no characteristic structural features. We noticed, however, that this tRNA has a 5-nucleotide sequence, 5'-CAGAG-3' (nucleotides 40-44), immediately 3' of the expected cleavage site. This sequence is hybridizable to the sequence 5'-CUCUG-3' (nucleotides 65-69). If a conformational change of this tRNA involving these two sequences occurs as shown in Fig. 1, nucleotide 39

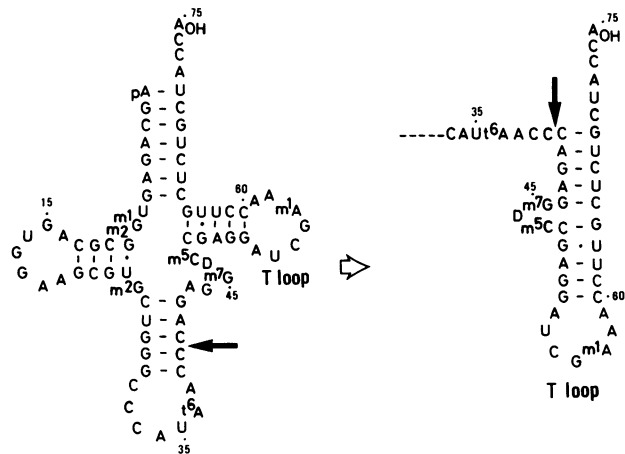


FIG. 1. A possible conformational change of *Drosophila* tRNA^{Met}. Boldface arrows indicate the site that should be cleaved to make the primer fragment for reverse transcription of the *cop*ia retrotransposon.

is now located 5' to a stem structure. This site (between nucleotides 39 and 40) is expected to be cleavable by RNase P, since the smallest substrate for this enzyme has been reported to retain only the domain of the aminoacyl stem and the TΨC stem and loop (7). We tested this possibility using M1RNA and an artificial *Drosophila* tRNA^{Met} precursor. The 3'-end-labeled tRNA precursor was incubated with M1RNA and the products were analyzed by polyacrylamide gel electrophoresis. As expected, this precursor was efficiently cleaved by M1RNA between nucleotides -1 and +1 (Fig. 2). In addition to this major product of the mature-sized tRNA, we detected several minor fragments representing products cleaved within the mature tRNA sequence. These fragments (arrows 1-7) are thought to be cleavage products generated by M1RNA, because their appearance depended on the presence of both M1RNA and Mg²⁺ in the reaction mixture. Furthermore, the mobilities of these fragments did not correspond to those of any fragments produced by partial alkaline hydrolysis, which would contain 5'-hydroxyl ends. This indicates that the fragments produced in the presence of M1RNA had 5'-phosphoryl ends, as expected for cleavage catalyzed by M1RNA. Three fragments (arrows 5, 6, and 7) indicate cleavage between nucleotides 37 and 38, 38 and 39, and 39 and 40, respectively. To further confirm the cleavage sites and also to detect the 5' fragment containing the actual *copia* primer sequence, the 5'-end-labeled tRNA precursor

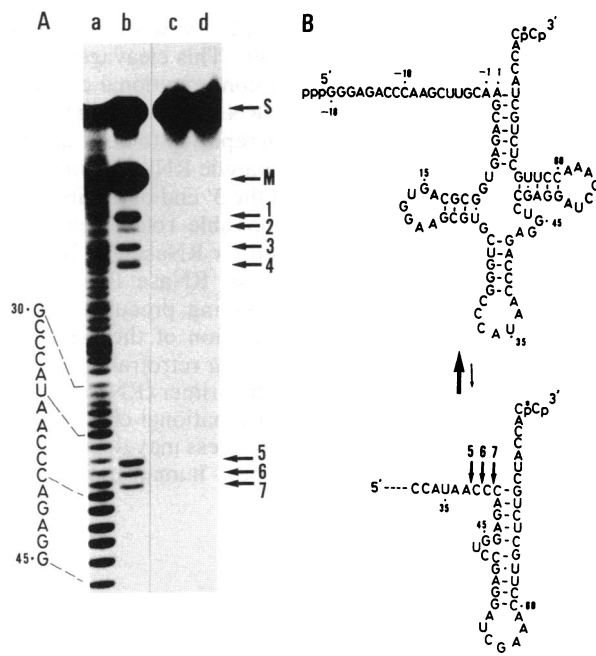


FIG. 2. Cleavage of the 3'-end-labeled tRNA^{Met} precursor of *Drosophila* by M1RNA. (A) Electrophoretic analysis of reactions using the 3'-³²P-labeled transcript of pDtY as substrate. Mixtures were analyzed in a 20% polyacrylamide/8 M urea gel. An autoradiogram of the gel is shown. Lanes: a, partial alkaline digest of the substrate; b, the complete reaction mixture; c, no added M1RNA; d, a stop solution (EDTA) was added to the complete reaction mixture before incubation. The nucleotide sequence of residues 30 to 45 is shown on the left. The nucleotide sequence was determined by comparison of the degradation patterns of this RNA (lane a) with those of partial alkaline or acidic digests in the enzymatic sequencing gel (data not shown). These bands can easily be recognized by the characteristic spacing they produce in the ladders; e.g., removal of a G results in a more pronounced increase of electrophoretic mobility as compared to removal of A, U, or C (9). Arrows 1-7, fragments found in lane b. S, substrate; M, mature-sized tRNA. (B) Schematic representation of the substrate and its possible conformational change. Arrows 5-7, cleavage sites corresponding to fragments 5-7 in A. Asterisk, the ³²P-labeled phosphate group.

was incubated with M1RNA and the products were analyzed (Fig. 3). Arrows 5, 6, and 7 in Fig. 3 again indicate cleavage between nucleotides 37 and 38, 38 and 39, and 39 and 40, respectively. This indicates that RNase P can catalyze cleavage between nucleotides 39 and 40, which is required to produce the primer tRNA fragment.

From the substrate specificity of M1RNA reported by McClain *et al.* (7), it seems reasonable to postulate that the conformational change of the tRNA shown in Figs. 1 and 2B may be necessary for cleavage between nucleotides 39 and 40. To test this prediction, we used a tRNA from which the sequence -4 to +32 had been removed (Fig. 4B) as substrate for the cleavage reaction. Because of the deletion of almost half of the 5' region of the tRNA, this substrate RNA is expected to take exclusively the secondary structure shown in Fig. 4B. As shown in Fig. 4A, using this substrate we detected much more efficient cleavage between nucleotides 37 and 38, 38 and 39, and 39 and 40 than when using the full-sized substrate tRNA (Fig. 2). These cleavage sites were also confirmed by the reaction using the 5'-end labeled pDtY transcript as substrate (data not shown). Fig. 4A also shows that this cleavage requires a Mg²⁺ concentration of >30 mM. These results indicate that the catalytic subunit of RNase P can catalyze cleavage of the tRNA^{Met} at the phosphodiester bond between nucleotides 39 and 40. These results suggest the possibility that the mature tRNA can exist in aqueous solution at least in part in the altered conformation as shown in Fig. 2B.

The other minor fragments produced from mature tRNA (arrows 1-4, Fig. 2A) suggested cleavage at the sites shown in Fig. 5. These cleavages also can be explained by conformational changes of the tRNA. The formation of "pseudo-aminoacyl stems" involving base pairing between the se-

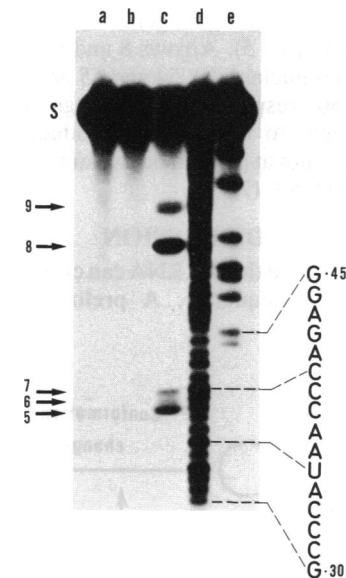


FIG. 3. Cleavage of the 5'-end-labeled tRNA^{Met} precursor of *Drosophila* by M1RNA: Electrophoretic analysis of reactions using the 5'-³²P-labeled transcript of pDtY as substrate. Mixtures were analyzed in a 15% polyacrylamide/8 M urea gel. An autoradiogram of the gel is shown. Lanes: a, no added M1RNA; b, a stop solution (EDTA) was added to the complete reaction mixture before incubation; c, the complete reaction mixture; d, partial acidic hydrolysis of the substrate; e, partial digest of the substrate by RNase T1. Although the bands for G-30 and G-42 are not visible in lane e, repeated analyses (data not shown) showed their presence. The nucleotide sequence of residues 30-45 is shown on the right. Assignment of the nucleotide sequence was done by G mapping from lane e and also as described in the legend to Fig. 2. Arrows 5-9, fragments found in lane c. Arrows 5, 6, and 7 correspond to the sites shown in Fig. 2. S, substrate.

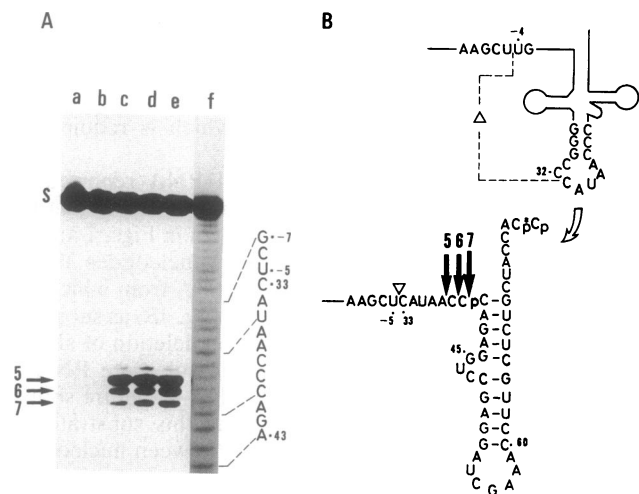


FIG. 4. Cleavage of the 5'-deleted substrate tRNA by M1RNA. (A) Electrophoretic analysis of reactions using the 3'-³²P-labeled transcript of pDtYS as substrate. The analysis was done as in Fig. 2A. Lanes: a-e, reaction mixtures containing Mg²⁺ at 0, 10, 30, 60, and 80 mM, respectively; f, partial alkaline digest of the substrate. The nucleotide sequence of residue -7 to +43 is shown on the right. The nucleotide sequence was determined as described in the legend to Fig. 2. Arrows 5, 6, and 7, major fragments produced, corresponding to the sites shown in B and also to fragments 5, 6, and 7 in Fig. 2. S, substrate. (B) Schematic representation of the substrate. (Upper) —△—, Region of the deletion in the *Drosophila* tRNA^{Met} precursor. (Lower) The deleted substrate and its possible secondary structure. Arrows 5, 6, and 7, cleavage sites. Asterisk, the ³²P-labeled phosphate group. ▽, The deletion site.

quence around the D loop and nucleotides 65–71 may be possible and this change could generate additional cleavage sites for M1RNA (Fig. 5). Arrows 8 and 9 in Fig. 3 indicate cleavage between nucleotides 54 and 55 and between nucleotides 59 and 60, respectively. Although these fragments have been thought to be cleavage products generated by M1RNA, they cannot at present be explained from the known properties of M1RNA (7).

DISCUSSION

We have demonstrated that M1RNA can cleave a tRNA within the mature tRNA sequence. A preliminary experiment

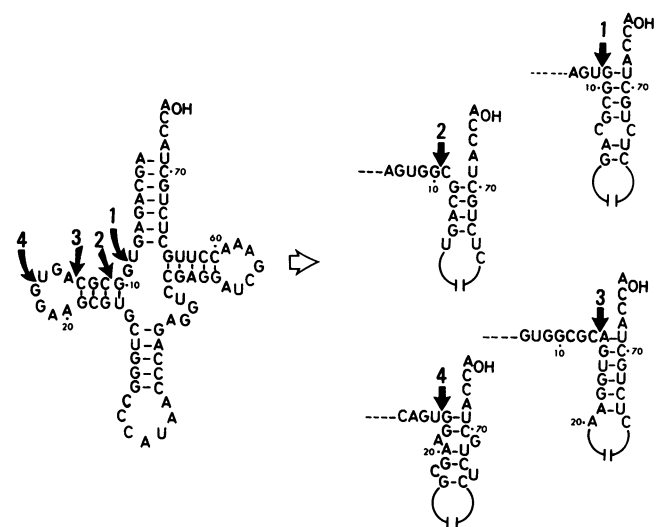


FIG. 5. Four types of possible conformational change of the *Drosophila* tRNA^{Met} sequence. Pseudo-aminoacyl stems can be formed by base pairing involving the sequences around the D loop. Arrows 1–4, sites of cleavage shown by arrows 1–4 in Fig. 2. These cleavages may be explained by these conformational changes and the specificity of M1RNA described by McClain *et al.* (7).

showed that natural tRNA^{Met} prepared from *Drosophila* cells is also cleaved by M1RNA at three sites (between nucleotides 37 and 38, 38 and 39, and 39 and 40). This cleavage appears dependent on the occurrence of a conformational change of the tRNA. A molecule other than a tRNA precursor or the 4.5S RNA of *E. coli* has previously been reported to be a substrate for RNase P. The enzyme or its catalytic RNA subunit alone cleaves the tRNA-like structure of the 3' end of turnip yellow mosaic virus RNA (12, 13). A possible role against viral infection *in vivo* has been discussed for RNase P (12).

Here we propose another role for RNase P *in vivo* in addition to its major role in processing precursor tRNA. RNase P may function in the formation of the primer for reverse transcription of the *Drosophila* retrotransposon *copia*. A model for the formation of the primer tRNA fragment is presented in Fig. 6. First, a conformational change of the *Drosophila* tRNA^{Met} occurs. This process may depend on an additional factor or factors. In type 1 human immunodeficiency

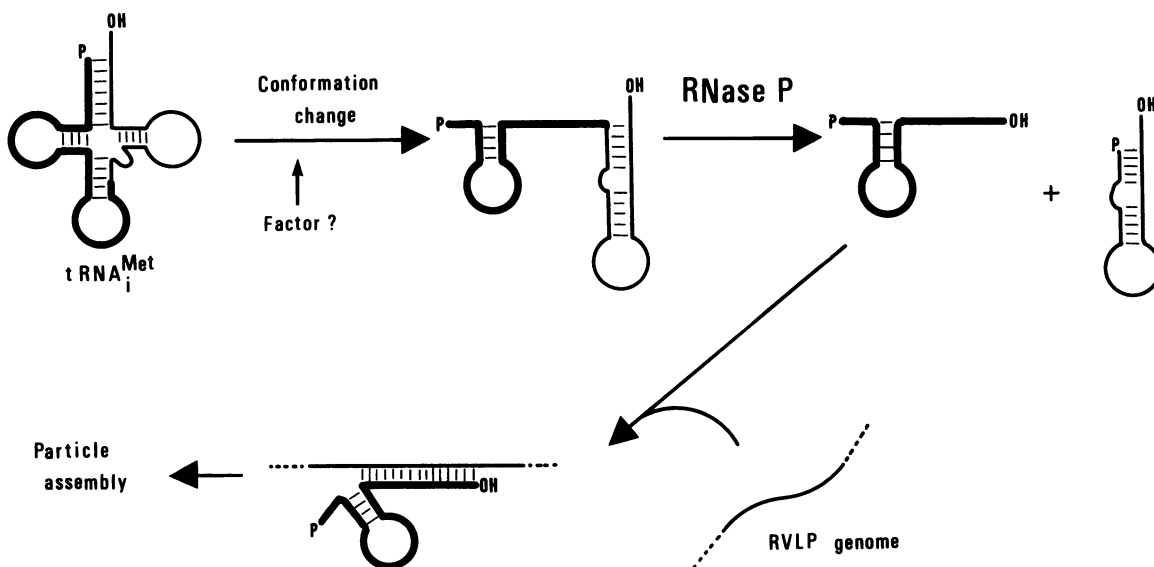


FIG. 6. A possible model for formation of the primer tRNA fragment for reverse transcription in the *Drosophila* retrotransposon *copia*. The primer segment in the *Drosophila* tRNA^{Met} is represented by a thick line.

ciency virus, a retrovirus, it has been reported that the reverse transcriptase specifically interacts with its cognate primer tRNA (14). The *copia* reverse transcriptase itself may be required for this conformational change. Conformational changes of RNA molecules have been demonstrated in the priming of ColE1 plasmid replication (15) and in translational control by attenuation (16). In vertebrate retrovirus replication, the higher order structure of the primer tRNA must change extensively. The aminoacyl stem and the TΨC stem of the tRNA must be unfolded for the molecule to function as a primer for reverse transcription (3). We therefore suggest that a similar conformational change can occur *in vivo*. In the next step in our model, the conformationally altered tRNA would be recognized and cleaved by *Drosophila* RNase P (Fig. 6). Since cleavage by RNase P generates 5'-phosphate and 3'-hydroxyl groups in the products (5), the cleaved product can be used directly for reverse transcription, which requires a 3'-hydroxyl end for priming. Concomitantly, this 5' fragment may be bound to the primer binding site of the genome and assembled into the RVLP.

We have used M1RNA of *E. coli* as an enzyme to catalyze the cleavage of *Drosophila* tRNA_i^{Met}. Although, so far as we know, an RNase P from *Drosophila* has yet not been described, it is likely that *Drosophila* cells contain an RNase P and that this enzyme has similar substrate specificities to those of prokaryotic RNase P. RNase P from several organisms is readily able to recognize substrates from different species (17) and this enzyme is represented in all cell types so far examined (ref. 18, ref. 19, and references cited therein). Carrara *et al.* (20) have reported that the RNase P from *Xenopus laevis* recognizes the aminoacyl stem of 7 bp of tRNA. The elimination of 1 bp from this stem causes a 50% reduction in the initial rate of cleavage by this enzyme. This observation is relevant to our model (Fig. 6). We assume that at least one of the cleaved sites may be located at the 5' side of this stem helical structure and that the substrate with only 5 bp in this stem, as in our model, may be cleaved to some extent, although the rate may be greatly reduced.

The cleavage of *Drosophila* tRNA_i^{Met} by M1RNA *in vitro* is not completely specific, as shown in Figs. 2 and 3. Although three fragments hybridizable to the primer binding site of the RVLP genome are produced by this *in vitro* reaction (5' fragments produced by cleavages 5, 6, and 7 in Figs. 2 and 3), only the fragment produced by cleavage between nucleotides 39 and 40 is utilized as primer for the reverse transcription of *copia* *in vivo* (21, 22). A plausible explanation for this is that the RVLP genome selects the longest primer, making the most stable hybrid with the primer binding site. Similar mechanisms of primer selection have been reported in plus-strand priming in retrovirus replication (23). Plus-strand synthesis is initiated from a plus-strand primer that consists of successive purine bases. This plus-strand primer is produced from the so-called polypurine tract of the viral RNA genome by RNase H action. Why is this polypurine tract the only oligoribonucleotide selected as a plus-strand primer, even though many other oligoribonucleotides are also produced by RNase H? Taylor and Sharmeen (23) suggest that this polypurine tract oligoribonucleotide makes the most stable interaction with the minus-strand DNA and that this binding stability contributes to the selection of the primer. This could also occur for selection of the primer for minus-strand synthesis in RVLP. The 5' molecule produced by cleavage between nucleotides 39 and 40 of the tRNA can make a 15-bp hybrid (including a G-U pair) with the primer binding site of the RVLP genome (2). This is the longest and hence the most stable base pairing for the three fragments and the site. This stability may be a determinant for selection of the primer molecule. Alternatively, it is possible

that the *Drosophila* RNase P may specifically cleave only between nucleotides 39 and 40 of the tRNA_i^{Met} *in vivo* with some factor(s) ensuring this specificity.

Priming of *copia* reverse transcription is the only known instance where a tRNA fragment has biological function. Specific cleavage of mature tRNAs has also been reported in the system of bacteriophage T4 (24, 25), although the biological function of the tRNA fragment itself is not known. It is possible, however, that hyperprocessed RNA fragments may be involved in the formation of pseudogenes and repetitive sequences in chromosomes by a mechanism involving self or bimolecular priming from these fragments (26).

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