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Reverse transcriptase mediated binding of primer tRNA to the viral genome

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SUMMARY.

A complex between $tRNA^{Trp}$ (beef) and 35 S RNA from avian myeloblastosis virus is obtained when the mixture is preincubated in the presence of reverse transcriptase at 35° C. The tRNA-RNA complex is active in initiating DNA synthesis catalyzed by reverse transcriptase. The interaction of tRNA with reverse transcriptase involves the partial unwinding of the acceptor stem of tRNA, as evidenced by nuclease digestion with RNAase T1 and micrococcal nuclease. When $tRNA_2^{Glu}$ (coli), having a high degree of similarity with primer tRNA at the level of the acceptor stem, was used as primer for DNA synthesis, a low but significant level of incorporation was obtained, if the reaction was performed at 35° C, while a high incorporation, similar to the one obtained with $tRNA^{Trp}$ was obtained when the annealing between $tRNA_2^{Glu}$ and 35 S RNA was performed at 80° C. Our evidences point out to an important role of the viral DNA polymerase in positioning the primer on the RNA genome.

INTRODUCTION.

All DNA polymerases need a short primer for initiating DNA synthesis. RNA-dependent DNA polymerases (reverse transcriptase) are no exception to this rule, as it has been shown that they use tRNA partially base paired to the viral genome as primer in the synthesis of DNA (1-4). Reverse transcriptase from avian myeloblastosis virus (AMV) is a dimeric enzyme with an α subunit of 60,000 molecular weight and a β subunit of 90,000 molecular weight (4). The α subunit alone possesses the ability to catalyze DNA synthesis (DNA and RNA dependent) as well as to degrade exonucleolytically the RNA strand of a DNA-RNA hybrid (RNAase H activity) (4). The β subunit contains the complete sequence of the α subunit and seems to be involved in the interaction of the primer template complex with the enzyme (5, 6). The primer in the case of AMV is chicken $tRNA^{Trp}$ (7). The specific interaction of $tRNA^{Trp}$ with avian reverse transcriptase has been shown with different techniques (8-11). It has also been shown that $tRNA^{Trp}$ from beef liver

has a sequence almost identical to $tRNA^{Trp}$ (chicken) (12). Beef liver $tRNA^{Trp}$ can serve as *in vitro* primer of DNA synthesis directed by the AMV 35 S RNA as efficiently as the homologous primer (13).

Primer tRNA is found base paired near the 5' end of AMV 35 S RNA. A region of about 16 nucleotides from the 3' end of tRNA seems to be involved in this base pairing (14, 15).

How is $tRNA^{Trp}$ positioned on the viral genome ? Since tRNA is a highly structured macromolecule it is hard to believe that base pairing with the viral genome will not require the presence of factors able to destabilize both tRNA and 35 S AMV RNA.

In this communication we show that reverse transcriptase catalyses the binding of primer tRNA to the 35 S RNA. This binding is probably preceded by the unwinding of the acceptor stem of tRNA by AMV DNA polymerase. Evidences are presented that the enzyme-tRNA interaction is involved in the positioning of the primer, by using tRNAs other than the primer $tRNA^{Trp}$, like $tRNA_2^{Glu}$ (coli) and $tRNA^{Val}$ (beef), for the initiation of RNA-directed DNA synthesis by AMV reverse transcriptase. A preliminary account of this work has been presented (16).

MATERIALS.

AMV pellets and purified AMV reverse transcriptase were obtained from Life Sciences Inc. St Petersburg, Florida, U.S.A. *E. coli* tRNA nucleotidyl transferase was a kind gift of Dr D.S. Carré and was purified as described before (17).

Radiochemicals were purchased from New England Nuclear Co. $|^{32}P$ gamma$|$ -ATP and T4 polynucleotide kinase were kind gifts of Dr G. Keith, Strasbourg. RNAase T1 and micrococcal nuclease were obtained from Sigma Chem. Co, St Louis, Missouri, U.S.A. or P.L. Biochemicals, Wisconsin, U.S.A. The purification of $tRNA^{Trp}$ and $tRNA^{Val}$ from beef liver have been published elsewhere (18). $tRNA^{Trp}$ (yeast) was a kind gift of Dr G. Keith and $tRNA_2^{Glu}$ (coli) was purchased form Boehringer Mannheim.

Poly rA and oligo dT_{12} were obtained from P.L. Biochemicals and annealed as described previously (19).

METHODS.

1. *Purification of AMV reverse transcriptase.*

The method of Kacian *et al.* was used to purify the enzyme from

pelleted virions (20). Homogeneity was ascertained by polyacrylamide electrophoresis in the presence of SDS (21).

2. Preparation of 35 S AMV RNA.

Viral 70 S RNA was purified from the frozen viral pellet by the phenol method (22). In order to eliminate the endogeneous primer, 70 S RNA was heated 1 min at 80° C and centrifuged in a 5 to 20 % sucrose gradient for 3 hours at 4° C in the 6 x 5 rotor of the MSE ultracentrifuge at 200,000 x g. High molecular weight RNA was pooled and precipitated with ethanol. Occasionally when some endogenous primer tRNA was still present, 70 S RNA was treated with dimethylsulfoxide prior to the sucrose gradient centrifugation (23).

3. Reverse transcriptase assays.

a) With poly rA-dT_{12} template : In a final volume of 100 µl the incubation mixture contained : 50 mM Tris-HCl pH 8.3 ; 40 mM KCl ; 6 mM $MgCl_2$; 10 µg of bovine serum albumin ; 0.5 mM $|^3H|$dTTP (70 cpm/pmole) ; 2 O.D. 260/ml poly rA-dT_{12} and different amounts of enzyme.

b) With 35 S RNA-tRNA template : The incubation mixture (100 µl) contained Tris-HCl, KCl, $MgCl_2$ and bovine serum albumin as above. Other components were 0.5 mM of dATP, dCTP, dGTP and 5 µM $|^{32}P$-alpha| dGTP (2 000 cpm/pmole ; 0.15-0.20 O.D. 260 35 S AMV RNA and different amounts of primer and enzyme. Incubation was carried out at 37° C for different lenghs of time. The incubation was stopped by addition of 0.1 ml of 20 % cold trichloracetic acid plus 0.1 M pyrophosphate carrier. The radioactive material was collected on nitrocellulose membranes (Schleicher et Schüll 0.45 µm), washed with 2 % cold trichloracetic acid and counted in a PPO-POPOP-toluene scintillation mixture.

4. 5' end labeling of tRNA.

The dephosphorylation step was carried out in 100 µl with 100 mM Tris-HCl pH 8 ; 0.5 O.D. 260 of tRNA and 0.1 units of bacterial alkaline phosphatase (Sigma type III-R). Incubation was carried out at 55° C for 60 min. Phenol extraction was performed and the aqueous layer was precipitated overnight with ethanol at -20° C. tRNA was disolved in 0.07 ml of 10 mM Tris-HCl pH 7.5 ; 1 mM spermidine and 0.1 mM EDTA. To 0.07 ml of this solution were added 10 µl of a buffer containing 500 mM Tris-HCl pH 9.5 ; 100 mM $MgCl_2$; 50 mM DTE ; 20 µl of $|^{32}P$-gamma| ATP (1 000-3 000 Ci/mmole) and 2 units of polynucleotide kinase. Incubation was carried out at 37° C for 30 min. Reaction was stopped by adding 0.1 ml of 4 M ammonium acetate plus 0.1 mg of the unlabeled homologous tRNA and precipitated with 0.6 ml of

ethanol at -20° C. The specific activity of the resulting tRNA was between 10,000 and 30,000 cpm/pmole.

5. 3' end labeling of tRNA.

Terminal adenosine was eliminated by periodate oxidation and β-elimination. Labeling with $|^3H|$ ATP or $|^3H|$ CTP was performed with the E. coli tRNA nucleotidyltransferase as described previously (17). The specific activities of the tritium labeled tRNAs were 500 cpm/pmole for the cytidine labeled and 700 cpm/pmole for the adenosine labeled tRNA.

6. Hybridation of tRNA 35 S AMV RNA.

Viral 35 S RNA and tRNA were heated at 80° C in 10 mM Tris-HCl pH 7.5 and 200 nM NaCl. The mixture was cooled slowly.

7. Sepharose 4B chromatography.

The column of Sepharose 4B (Pharmacia) (0.90 x 40 cm) was equilibrated in a buffer containing 300 mM NaCl, 30 mM sodium acetate pH 4.5 and 0.5 % SDS. Samples were equilibrated in the same buffer before gel filtration. Counting was performed in a dioxane-PPO-POPOP scintillation mixture (24).

8. Analysis of cDNA initiated by $tRNA^{Trp}$ (beef) and $tRNA_2^{Glu}$ (coli).

The synthesis of ddTMP terminated DNA fragments was made essentially as described by Bina-Stein et al. (25). AMV 35 S RNA was annealed to $tRNA^{Trp}$ (beef) or $tRNA_2^{Glu}$ (coli) as described above. The hybrids were precipitated with ethanol, recovered by centrifugation and resuspended in 0.04 ml of 10 mM Tris-HCl pH 7.5 containing 0.15 mCi of $|^{32}P|$ alpha dATP (625 Ci/mmole). The solution was made 50 mM Tris-HCl pH 8.3, 10 mM $MgCl_2$, 100 mM KCl and 10 mM β-mercaptoethanol in a final volume of 0.10 ml. AMV reverse transciptase was added (70 units) and the reaction mixture incubated 1 min at 40° C. Subsequently 0.05 ml aliquots were removed and added to tubes containing the four dNTPs to obtain a final concentration of 57 µM each and 14.2 µM ddTTP. Incubation was carried out for 15 min at 40° C, then 0.02 ml of a solution containing the four dNTPs was added and the reaction continued for 10 min.

The reaction were stopped by addition of 0.01 ml of 1 M NaOH, heated for 5 min at 100°C, brought to pH 4.5 with sodium acetate and precipitated with 2.5 volumes of ethanol overnight. The pellets were recovered by centrifugation, lyophilized and resuspended in 0.01 ml of buffer containing 5 mM Tris borate pH 8.3, 1 mM EDTA, 7 M urea, 0.02 % xylene cyanol FF and 0.02 % bromophenol blue, heated at 100° C for 20 seconds and loaded in a

20 cm x 40 cm 20% polyacrylamide gel slab. Electrophoresis was carried out at 30 V/cm in a 50 mM Tris borate pH 8.3, 7 M urea, EDTA system until the bromophenol marker reached the bottom of the gel. The gel was then covered with a polyethylene film and autoradiographed with a Kodak X-Omat-R film in the presence of a Cronex Lighting-Plus intensifying screen (DuPont).

RESULTS.

1.- Binding of $tRNA^{Trp}$(beef) to AMV 35 S RNA.

AMV RNA and primer $tRNA^{Trp}$ are able to form a stable complex if the mixture is heated to over 70°C and reannenling is performed under mild conditions. As it can be seen in Figure 1 if the annealing of 5' [^{32}P] tRNA and 35 S AMV RNA is done at 35°C and the mixture submitted to Sepharose 4B

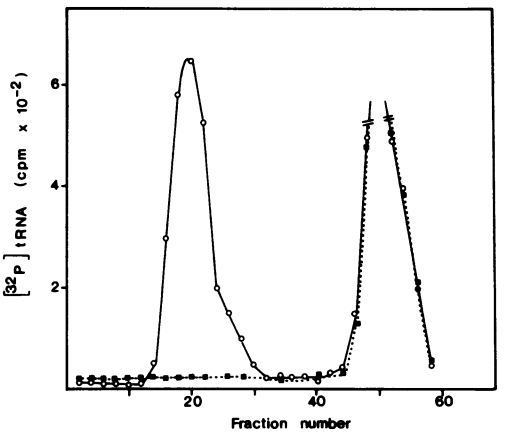

Figure 1 .- Sepharose 4B chromatography of the complex between $tRNA^{Trp}$(beef) and AMV RNA annealed at 35°C in the presence (○) or absence (■) of reverse transcriptase.
Annealing mixture in a final volume of 0.1 ml: 50 mM Tris pH 8.3 6 mM $MgCl_2$, 6.7 pmoles of 5' labeled tRNA, 3.5 µg of 35 S RNA, and 17 units of reverse transcriptase. After 20 min at 35°C the reaction was stopped with 0.05 ml of 15% SDS,3M NaCl. Gel filtration was performed as indicated in the text.

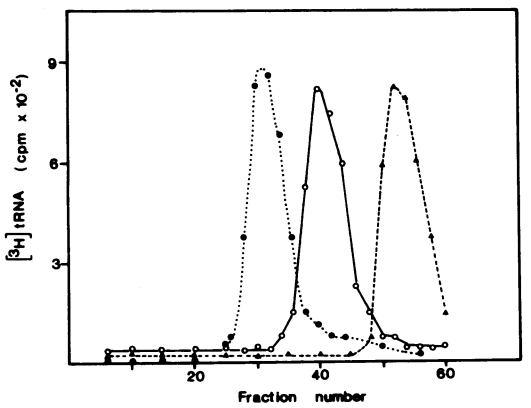

Figure 2 .- Sepharose 4B chromatography of $tRNA^{Trp}$(beef)tritium labeled . (△) tRNA alone, (○) tRNA annealed to AMV RNA as in Figure 1, (●) tRNA-AMV RNA complex having served for initiation of DNA. Conditions as in the previous Figure except that 36 pmoles of tritiated tRNA and 1.4 µg of AMV RNA were used. DNA synthesis was performed as indicated in Methods in the presence of unlabeled dNTPs for one hour.

gel chromatography, no radioactivity is found associated with viral RNA in the position of the void volume. If the annealing is done at the same temperature but in the presence of reverse transcriptase a fraction of radioactive tRNA is now found associated with 35 S RNA. Gel chromatography is performed in the presence of SDS to eliminate RNA-protein interactions. Figure 2 shows also a chromatographic profile performed this time with a partially degraded RNA. For this experiments fractions of AMV RNA between 15 and 20 S were pooled from the sucrose gradient described in Methods and precipitated with ethanol. This RNA will not be excluded in the Sepharose column although it will be clearly eluted before tRNA. As seen in Figure 2 if the complex $tRNA^{Trp}$-RNA is used for DNA synthesis in the presence of reverse transcriptase and the four dNTPs, and the product submitted to gel filtration, the radioactivity of $[^3H]$tRNA is found associated with a material heavier than the primer-template complex. The AMV RNA used in these experiments had no detectable endogenous primer tRNA activity.

2.- Unwinding of the acceptor stem of tRNA by reverse transcriptase.

Figure 3 describes the effect of reverse transcriptase on the digestion of 5' labeled tRNA by RNAase T_1. No digestion of the 5' end of tRNA is observed when labeled $tRNA^{Trp}$(beef) or $tRNA^{Val}$(beef) are incubated with the nuclease. In the presence of reverse transcriptase the 5' end becomes clearly susceptible to nuclease attack. This effect seem to be unspecific, under the conditions used, since $tRNA^{Val}$(beef) was digested to a level similar to that of $tRNA^{Trp}$. Digestion of both tRNAs in the absence of reverse

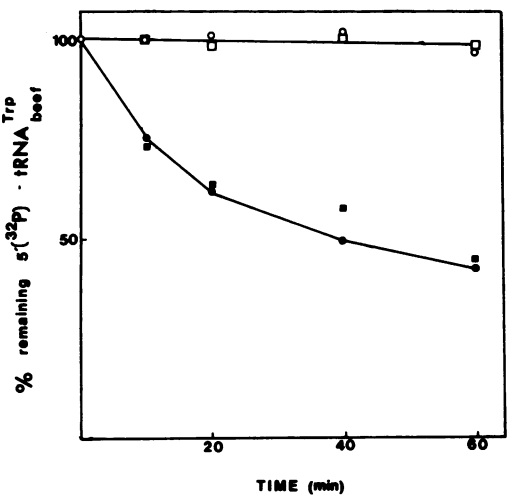

Figure 3 .- RNAase T_1 digestion of 5' labeled tRNA.

In a final volume of 0.1 ml the digestion mixture contained: 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 45 pmoles of tRNA, labeled as described in the text, 0.6 units of RNAase T_1 and 60 units of reverse transcriptase. Incubation was done at 37°C and the acid precipitable material determined as in Methods.
(□) $tRNA^{Trp}$ plus RNAase T_1
(○) $tRNA^{Trp}$ plus reverse transcriptase
(■) $tRNA^{Trp}$ plus RNAase T_1 plus reverse transcriptase
(●) $tRNA^{Val}$ plus RNAase T_1 plus reverse transcriptase

transcriptase is also observed if they are denatured by heating in the presence of EDTA (not shown).

In Figure 4 is described the digestion of 3' labeled tRNA by micrococcal nuclease. Primer tRNA was labeled at the 3' terminal adenosine with $|^3H|$ATP (Figure 4A) and at the first cytidine of the CCA end with $|^3H|$CTP using E.coli tRNA nucleotidyltransferase as described in Methods. As seen in Figure 4A the 3' is digested by nuclease in the presence or absence of reverse transcriptase. A clear protection against nuclease digestion is observed in the presence of 35 S viral RNA due probably to base pairing between tRNA and the viral genome.In the presence of 35 S RNA and in the absence of reverse transcriptase no protection was observed (not shown). Similar results were obtained with cytidine labeled tRNA as seen in Figure 4B.

3.- Use of $tRNA_2^{Glu}$ as primer of reverse transcriptase.

In order to study the importance of tRNA structure in serving as primer in reverse transcription we looked whether tRNAs other than $tRNA^{Trp}$ were able to initiate RNA directed DNA synthesis. We have chosen $tRNA_2^{Glu}$(coli) and $tRNA^{Val}$(beef) since they have different degrees of similarity to tRNA tryptophane. Table I shows the sequence of the last 16 nucleotides of $tRNA^{Trp}$

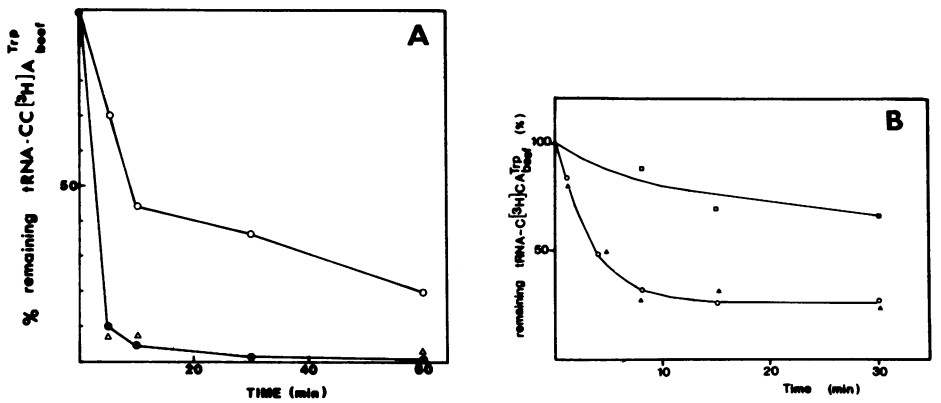

FIGURE 4 .- Micrococcal nuclease digestion of 3' labeled $tRNA^{Trp}$(beef) In a final volume of 0.1 ml the incubation mixture contained 50 mM Tris ph 8.3, 6 mM $MgCl_2$, 150 pmoles of tRNA, 0.014 O.D.260 of 35 S RNA, 60 units of reverse transcriptase and 0.125 units of nuclease (Sigma). Incubation was at 20°C and radioactivity determined as in the text.(A) (●) Adenosine labeled tRNA plus nuclease, (△) plus nuclease plus reverse transcriptase, (○) plus both enzymes plus 35 S RNA.(B) (▲) Cytidine labeled tRNA plus nuclease, (○) plus nuclease plus reverse transcriptase, (□) plus both enzyme plus 35 S RNA.

TABLE I . - Sequences of $tRNA^{Trp}$(beef), $tRNA_2^{Glu}$(coli) and $tRNA^{Val}$(beef) near the 3' end.

	TψC Stem	Acceptor Stem	3' End	Reference
$tRNA^{Trp}$(beef)	C A C G U	C G G G G U C	A C C A	12
$tRNA_2^{Glu}$(coli)	C C C C U x x x	A G G G G A C x x x x x	G C C A x x x	26
$tRNA^{Val}$(beef)	C C G G G	C G G A A A C	A C C A	27

(beef), $tRNA_2^{Glu}$ (coli) and $tRNA^{Val}$(beef).The number of residues identical to $tRNA^{Trp}$(beef) are only slightly different in both tRNAs (10 for $tRNA^{Val}$ and 11 for $tRNA_2^{Glu}$) but and additional similarity can be found in the case of the bacterial $tRNA_2^{Glu}$ that is the presence of four G in a row. As seen in Table II under the usual conditions of incubation at 35°C, $tRNA^{Trp}$(beef) is recognized very efficiently, while $tRNA_2^{Glu}$ (coli) initiates DNA synthesis at a low but significant rate. In the case of $tRNA^{Val}$(beef) no primer activity was observed (11). If the annealing between tRNA and viral RNA is done at 80°C and the complex used for DNA synthesis, $tRNA_2^{Glu}$ (coli) becomes a very efficient primer while $tRNA^{Val}$(beef) is still not able to initiate DNA synthesis.

Having shown that a bacterial tRNA was able to serve as primer of AMV reverse trasncriptase it seemed important to show if $tRNA_2^{Glu}$ (coli) was able to initiate at the same site than $tRNA^{Trp}$. For this purpose we used the DNA chain terminator ddTTP (25). The radioactive transcription products initiated by both primers were isolated and submitted to polyacrylamide electrophoresis and subsequent autoradiography. The results of such experiment are shown in Figure 5.

TABLE II .- Primer activities of bovine and bacterial tRNAs annealed to AMV RNA at different temperatures. The assay is described under Methods. Incubation was carried out at 35°C for 60 min.

	cpm dGMP incorporated into DNA			
	Temperature of Annealing			
	35°C		80°C	
	Amount of tRNA used (pmoles)			
	18	36	18	36
$tRNA^{Trp}$(beef)	15250	21300	16080	22083
$tRNA_2^{Glu}$(coli)	1720	3025	11830	14085
$tRNA^{Val}$(beef)	<500	<500	<500	<500

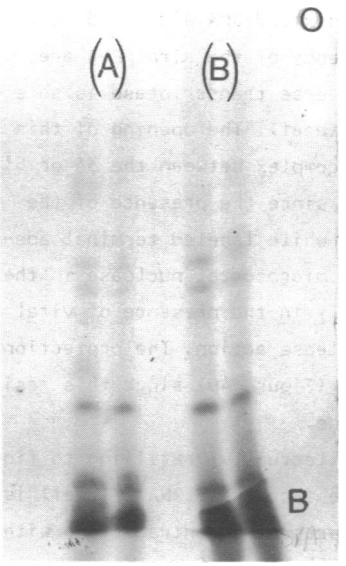

FIGURE 5 .- Polyacryalamide electrophoresis of the ddTMP fragments of cDNA primed by:

$tRNA^{Trp}$(beef) (A)

$tRNA_2^{Glu}$(coli) (B)

O = Towards origin

B = Towards bottom

The experimental details are given in the text.

DISCUSSION.-

The aim of our project is to study the role played by the enzyme reverse transcriptase in the binding of primer tRNA to the viral genome.

Primer tRNA is found partially base paired to a region near the 5' end of the viral genome. By using nuclease digestion it has been shown that about 16 nucleotides at the 3' end are involved in base pairing with the 35 S RNA (14,15). The annealing of tRNA to the viral RNA implies the opening of two highly structured macromolecules. In our hands no complex is obtained if the annealing is done at 35°C. As shown in Figure 1 if the reaction at 35°C is done in the presence of reverse transcriptase, the formation of a stable complex tRNA-AMV RNA eluting in the position of the void volume of the column was obtained. The presence of SDS eliminates the possibility of interactions between the viral DNA polymerase and tRNA or 35 S RNA.The experiment shown in Figure 2 suggests strongly that the complex tRNA-35 S RNA is active in initiating DNA synthesis in the presence of reverse transcriptase.

What is the mechanism used by reverse transcriptase to facilitate the base pairing between primer and template?. Recently it has been reported that reverse transcriptase unwinds a double stranded hybrid DNA-RNA while it will not dissociate the complex between tRNA and AMV RNA(28). Together the RNAase H and unwinding activities of the enzyme give support to the model of

reverse transcription based on the degradation of the 5' region of viral RNA, to allow the base pairing between the newly synthesized DNA and the 3' end of AMV RNA since the sequences at the 5' and 3' ends of the viral RNA are identical (29,30). As seen in Figures 3 and 4 reverse transcriptase is able to partially unwind the acceptor stem of $tRNA^{Trp}$(beef). The opening of this region does not imply the formation of a strong complex between the 3' or 5' final residues of tRNA and reverse transcriptase since the presence of the enzyme makes the 5' end susceptible to RNAase T_1 while labeled terminal adenosine or cytydine at the 3' end are digested by micrococcal nuclease in the presence or absence of reverse transcriptase; only in the presence of viral RNA does the 3' end become protected against nuclease action. The protection is stronger in the case of cytidine labeled tRNA (Figure 4B) since this residue is more stabilized than the terminal adenosine.

Although it would have been intellectually gratifying to find that the unwinding effect is primer specific, the fact that $tRNA^{Val}$(beef) is also opened by reverse transcriptase does not represent a contradiction with the in vivo situation because of the different affinities of reverse transcriptase for different tRNAs. In the presence of limiting amounts of tRNA the enzyme will form a specific complex with primer $tRNA^{Trp}$ (8), while at a high ratio tRNA/enzyme a complex $tRNA^{Val}$(beef) with AMV reverse transcriptase has been observed (11). In the infected cell the situation should thus be favourable for the selection of $tRNA^{Trp}$.

Is the appropriate 3' end sequence of tRNA the only requisite for a tRNA to serve as primer of reverse transcriptase? We have tried to answer this question by initiating DNA synthesis with tRNAs having different degrees of similarity with $tRNA^{Trp}$(Table I). The results shown in Table II indicate that the structural analogies between $tRNA_2^{Glu}$(coli) and primer $tRNA^{Trp}$ allow it to serve as primer in the RNA-directed DNA synthesis catalyzed by reverse transcriptase, but only if the annealing is performed at high temperature. At 35°C under conditions where $tRNA^{Trp}$ is efficiently possitioned on the viral genome, $tRNA_2^{Glu}$ is a poor initiator of DNA synthesis. These results support the idea that reverse transcriptase recognizes very specific structural features of primer tRNA. The experimental evidences shown in Figure 5 indicate that $tRNA_2^{Glu}$ seem to initiate at the same site as $tRNA^{Trp}$.The difference at the level of the high molecular weight fragments may reflect the different efficiencies of both primers as seen in Table II. The sequence analogy between both tRNAs would favour the idea that the site of initiation on the viral genome is the same. However a question remains to be answered concer-

ning the fact that $tRNA_2^{Glu}$ (coli) which is unwound at the 3' end (as the tRNAs shown in Figure 3) by reverse transcriptase is not annealed by the enzyme to the viral genome. Other regions of tRNA primer seem to be important in the binding to the viral genome. By ultraviolet irradiation of the complex between $tRNA^{Trp}$(beef) and AMV reverse transcriptase we have found a strong interaction between the dihydrouridine and anticodon loops of tRNA and the enzyme (our unpublished results). Our working hypothesis implies that before the binding of primer tRNA to the viral template, the formation of a highly specific complex tRNA-reverse transcriptase is compulsory. Although other tRNAs can form a complex with the enzyme,those complexes are not active in the transfer of tRNA to the initiation site.

While this work was under progress several lines of evidences have indicated, indirectly, that a viral protein, probably reverse transcriptase, may be involved in the selection of host tRNAs (31-33). The experimental evidences presented in this article correlates well with these results and indicate that the interactions between $tRNA^{Trp}$ and reverse transcriptase may lead to the positioning of primer tRNA on the viral genome, as well as to direct the enzyme to the correct initiation site in the template RNA.

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REFERENCES.

1 - Taylor,J.M. (1977) Biochim.Biophys.Acta Reviews on Cancer.CR4,57- 71.

2 - Waters,L.C. and Mullin,B.C. (1977) Progress in Nucleic Acid Research and Molecular Biology. 20, 131-160.

3 - Bishop,J.M. (1978) Ann.Rev.Biochemistry. 47, 35-88.

4 - Verma,I.M. (1977) Biochim.Biophys.Acta Reviews on Cancer. CR4, 1-38.

5 - Gibson,W.and Verma,I.M. (1974) Proc.Natl.Acad.Sci.U.S. 71, 4991-4994.

6 - Araya,A.,Labouesse,J.,and Litvak,S. (1979) Biochem.Biophys.Res.Comm. 88, 9-15.

7 - Harada,F.,Sawyer,R.C.,and Dahlberg,J.E. (1975) J.Biol.Chem.250, 3487- 3497.

8 - Haseltine,W.M.,Panet,A., Smoler,D.,Baltimore,D., Peters,G.,Harada,F., and Dahlberg,J.E. (1977) Biochemistry 16, 3625-3632.

9 - Hizi,A.,Leis,J.P., and Joklik,W.K. (1977) J.Biol.Chem. 252, 6878-6884.

10 - Panet,A., and Berliner,H. (1978) Virology 26, 214-220.

11 - Araya,A. and Litvak,S. (1979) Cold Spring Harbour Symposia on DNA Replication. 43, in the press.

12 - Fournier,M.,Labouesse,J.,Dirheimer,G.,Fix,C.,and Keith,G. (1979) Biochim.Biophys.Acta. 521, 198-208.

13 - Baroudy,B.M.,Fournier,M.,Labouesse,J.,Papas,T.S. and Chirijkian,J.G. (1977) Proc.Natl.Acad.Sci.U.S. 74, 1889-1893.

14 - Cordell,B.,Starnezer,E.,Friedrich.R.,Bishop,J.,and Goodman,H. (1976) J.Virol. 19, 548-558.

15 - Taylor,J.M.,and Illmensee,R. (1976)J.Virol. 16, 553-558.

16 - Araya,A.,Labouesse,J., and Litvak,S. (1978) Cold Spring Harbor Meeting on tRNA. Abstracts,page 95.

17 - Carré,D.S.,Litvak,S.,and Chapeville,F. (1970) Biochim.Biophys.Acta. 224, 371-380.

18 - Fournier,M.,Dorizzi,M.,Sarger,C.,and Labouesse,J. (1976) Biochimie 58, 1159-1167.

19 - Tarrago-Litvak,L.,Viratelle,O.,Darriet,D.,Dalibart,R.,Graves,P.V. and Litvak,S. (1978) Nucleic Acid Res. 5, 2197-2210.

20 - Kacian,D.L.,Watson,K.F.,Burny,A.,and Spiegelman,S.(1971) Biochim.Biophys.Acta. 246, 365-383.

21 - Laemmli,U.K. (1970) Nature 220, 680-685.

22 - Coffin,J.M. and Billeter,M.A. (1976) J.Mol.Biol. 100, 293-318.

23 - Faras,A.J., Garapin,A.C.,Levinson,W.E.,Bishop,J.M.,and Goodman,H. (1973) J.Virol. 12, 334-342.

24 - Bray,G.A. (1960) Analyt.Biochem. 1, 279-285.

25 - Bina-Stein,M.,Thoren,M.,Salzman,N., and Thompson,J.A. (1979) Proc. Natl. Acad. Sci. U.S. 76, 731-735.

26 - Ohashi,Z., Harada,F.,and Nishimura,S. (1972) FEBS Letters 20, 239-241.

27 - Piper,P.W. (1975) Eur.J.Biochemistry 51, 295-304.

28 - Collet,M.S.,Leis,J.P.,Smith,M.S.,and Faras,A.J. (1978) J.Virol. 26, 498-509.

29 - Haseltine,W.M.,Maxam,A.L.,and Gilbert,W. (1977) Proc.Natl.Acad.Sci.U.S. 74, 989-993.

30 - Schwartz,D.E.,Zamecnik,P.C.,and Weith,H.C. (1977) Proc.Natl.Acad.Sci. U.S. 74, 994-998.

31 - Panet,A., Weil,G.,and Friis,R.R. (1978) J.Virol. 28, 434-443.

32 - Levin,J.G.,and Seidman,J.G. (1979) J.Virol. 29, 328-335.

33 - Sawyer,R.C.,and Hanafusa,H. (1979) J. Virol. 29, 863-871.