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Reverse Transcriptase as the Major Determinant for Selective Packaging of tRNA's into Avian Sarcoma Virus Particles

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Mutants of avian sarcoma virus which lack a functional DNA polymerase were found to be nonselective in the incorporation of host cell tRNA's into virus particles. In contrast, mutants which possess a functional DNA polymerase but lack the viral genome RNA contained a specific subset of the host cell tRNA population, indistinguishable from that of the wild-type virus. Thus the reverse transcriptase, and not the viral RNA, is probably the major factor determining which tRNA's are incorporated into avian sarcoma virus particles. Supporting evidence was obtained in an in vitro binding assay between purified reverse transcriptase and unfractionated cellular tRNA's. However, the subset of tRNA's which associated with the genome in the 70S complex was determined primarily by the viral RNA. In the absence of DNA polymerase, the 70S RNA complex in mature virus particles contained the normal complement of associated tRNA's with the exception of $tRNA^{Trp}$, the primer for RNA-directed DNA synthesis.

Up to 30% of the RNA in retrovirus particles is tRNA, presumably derived from the host cell during virus budding (8, 12, 15, 32, 36, 42). The majority of these tRNA's are released when virus particles are extracted with protein denaturants and are referred to as free tRNA's to distinguish them from the subset which is associated with the viral genome RNA in a 60 to 70S complex (8, 12, 32, 36, 42). Heat denaturation of this complex yields two subunits of 35 to 40S genome RNA and the associated tRNA's (7, 13, 15, 32, 34, 36, 39, 42). When the 70S RNA complex is used to direct DNA synthesis catalyzed by the viral reverse transcriptase enzyme, one of these associated tRNA's serves as a primer for DNA synthesis (4, 7, 14, 15, 30-32, 39, 42, 43). In the avian sarcoma virus (ASV) system, the primer has been characterized as a cellular tRNA for tryptophan ($tRNA^{Trp}$) (14, 18, 38, 42, 43).

Although the role of the other tRNA's in retroviruses is unclear, some selection must operate during their incorporation into virions, since only a subset of the total host cell tRNA population is packaged, and in reproducible amounts (11, 12, 30-33, 36, 41, 42). In avian cells, $tRNA^{Trp}$ represents only a small fraction (2%) of the total cellular tRNA's, whereas it makes up about 30 to 50% of the tRNA's in ASV particles (14, 15, 36, 38). Moreover, different viruses, even those growing in the same type of host cell, package different subsets of tRNA (30, 36, 37). Since selection is probably mediated by a structural component of the virus, we have examined the tRNA content of mutants of ASV deficient in one or more of these components in an attempt to identify which is the major determinant for tRNA packaging.

The structural components of ASV virions are encoded by three distinct genetic regions: *gag* (internal structural proteins), *pol* (RNA-dependent DNA polymerase) and *env* (envelope glycoproteins) (5). Nonconditional mutants of ASV have been described which are defective in either *pol* or *env* or both (17). In addition, Linial et al. have recently described a variant of ASV which contains the viral structural proteins but is deficient in genomic RNA (25). We have analyzed the free and genome-associated tRNA populations of several of these mutant viruses and find that although the genome RNA clearly influences which tRNA's are included in the 70S RNA complex, the virion reverse transcriptase is the major determinant in selective encapsidation of cellular tRNA's into virus particles.

Previous studies have indicated a specific binding between avian myeloblastosis virus (AMV) DNA polymerase and the $tRNA^{Trp}$ primer (2, 16, 20, 21, 29). We have extended these studies to show that under appropriate conditions, in vitro binding of purified DNA polymerase to unfractionated cellular tRNA's can mimic the selective packaging of tRNA's by ASV virions.

MATERIALS AND METHODS

Cells and viruses. All of the cells used in this study were clones of transformed turkey or quail cells

chronically releasing particles of ASV defective in one or more functions (Table 1). Three strains of turkey embryo fibroblast cells transformed by a DNA polymerase-deficient variant of the Bryan strain of ASV (α BH-RSV) have been described by Murphy (26). Two of these, designated α40T and α48T, released viral particles which lacked detectable RNA-dependent DNA polymerase activity (*pol*$^-$). The particles released by α40T cells were additionally defective in the envelope glycoprotein (*env*$^-$), whereas those from α48T behaved phenotypically as *env*$^+$. The third clone, 16T, released particles of the classical BH-RSV(−) type which were *pol*$^+$ *env*$^-$ (26). Additional variants, released by lines of transformed quail cells and unrelated to the BH-RSV-derived mutants, were generously provided by M. Linial. These included the SE52d cell line, which produces *pol*$^-$ particles apparently due to deletion of about 30% of the sequences in the polymerase gene (24), and the SE21Q1b cell line, which releases particles containing the normal complement of viral proteins but no viral RNA (designated *rna*$^-$ in this study) (25).

All of the cells were propagated in Dulbecco-modified Eagle medium supplemented with 10% tryptose phosphate, 4% fetal calf serum, 1% chick serum, and 1% dimethyl sulfoxide.

Assay of RNA-dependent DNA polymerase activity. The viral particles released from each cell type were assayed for reverse transcriptase activity. After an initial clarification at 12,000 × *g* for 10 min, virus was harvested from the culture medium by centrifugation at 100,000 × *g* for 60 min. The virus pellets were suspended and equal portions were assayed in reactions containing 50 mM Tris-hydrochloride (pH 8.3), 50 mM NaCl, 10 mM magnesium acetate, 5 mM dithiothreitol, 0.02% Nonidet P-40 (NP-40), 2.5 μg of oligodeoxyguanosine-polyribocytidine (1:10, wt/wt), and 10 μCi of [^{3}H]dGTP (Radiochemical Centre, Amersham, U.K.; 6.2 Ci/mmol) in a total volume of 0.125 ml. The assay measured incorporation of [^{3}H]-dGMP into acid-precipitable material in a 1-h incubation at 37°C. After removal of the culture medium to perform this assay, the cells were dissociated with trypsin and counted. The DNA polymerase results were then expressed as counts per minute of [^{3}H]-dGMP incorporated per minute by particles released from 10^6 cells in a 24-h period.

Analysis of viral RNAs. Subconfluent cultures of the various cell types were labeled with ^{32}P in phosphate-free Eagle medium containing 5% dialyzed fetal calf serum, 1% dimethyl sulfoxide, and 0.1 to 0.2 mCi of $^{32}PO_4^{3-}$ per ml. The medium was changed at 24-h intervals, and virus was harvested from the pooled culture fluids by centrifugation (36). The virion RNA was isolated by pronase-sodium dodecyl sulfate treatment and phenol extraction, and the 70S RNA was separated from the free small RNAs by sucrose gradient centrifugation as previously described (32, 36). In the case of 21Q1b particles in which no 70S RNA complex was detected, the RNA prepared from the viral particles was separated into polyadenylate-containing and polyadenylate-deficient fractions by chromatography on polyuridylate [poly(U)]-Sepharose (9).

The free and 70S-associated tRNA's were further analyzed by electrophoresis in two dimensions in polyacrylamide slab gels (22, 36). The 70S RNA recovered from the sucrose gradients or, in the case of 21Q1b particles, the polyadenylated RNA fraction was heated to 90°C for 2 min before loading onto the gel. The ^{32}P-labeled tRNA species were detected by autoradiography of the gel, using Kodak No-Screen X-ray film. In some experiments, RNAs detected as spots on the autoradiograph were excised and eluted from the gel in order to quantitate them relative to the genome RNA trapped at the gel origin, or to characterize them by RNase T1 digestion and fingerprinting as described previously (32, 35, 36).

TABLE 1. *Phenotype and reverse transcriptase activity of ASV variants*

Cell type	Phenotype of released particle	RNA-dependent DNA polymerase[a]	Source and reference
16T	*pol*$^+$ *env*$^-$	73,000	Murphy (26)
α40T	*pol*$^-$ *env*$^-$	175	Murphy (26)
α48T	*pol*$^-$ *env*$^+$	110	Murphy (26)
SE52d	*pol*$^-$ *env*$^+$	370	Linial (24)
SE21Q1b	*pol*$^+$ *env*$^+$ *rna*$^-$	33,970	Linial (25)

[a] Expressed as counts per minute of [^{3}H]dGMP incorporated per minute by particles produced by 10^6 cells in a 24-h period.

In vitro binding of DNA polymerase to tRNA. ^{32}P-labeled 4S RNA was prepared from chicken embryo fibroblast cells essentially as described previously (20). The 4S RNA fraction recovered after sucrose gradient centrifugation was subjected to two cycles of gel filtration on columns of Sephadex G-100 (40 by 1 cm) in order to remove free $^{32}PO_4^{3-}$ and contaminating RNAs greater than 4S in size. From 10^5 to 10^6 cpm of this labeled RNA, equivalent to approximately 0.1 to 0.5 μg of total tRNA, was incubated in the presence and absence of 30 U of AMV reverse transcriptase (a gift from J. W. Beard, Life Sciences Inc., St. Petersburg, Fla.) in a buffer containing 50 mM Tris-hydrochloride (pH 8.3), 60 mM NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1% NP-40, and 5% glycerol. Incubation was performed at various temperatures for 10 min, and the samples were then analyzed by gel filtration in a temperature-controlled column of Sephadex G-100 (35 by 0.5 cm). The column was eluted with 100 mM potassium phosphate buffer (pH 7.5) containing 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.1% NP-40, and 5% glycerol. Each column fraction was assayed for [^{32}P]RNA by direct counting of Cerenkov radiation, and alternate fractions were assayed for reverse transcriptase activity essentially as described above for the virus particles, but using oligodeoxythymidylate-polyriboadenylate [oligo (dT)·poly(rA)] as a template. The results were expressed as total counts per minute of [^{3}H]dTMP incorporated per assay.

Column fractions containing the peak polymerase activity were pooled and precipitated by the addition of carrier RNA (100 μg/ml) and cetyltriethylammo-

nium bromide at 0°C (3). The precipitates were recovered by centrifugation at 16,000 × *g* for 10 min, resuspended in 1 M NaCl, and precipitated again with 2 volumes of ethanol at −20°C. The ^{32}P-labeled RNAs associated with the polymerase were analyzed directly by two-dimensional (2-D) gel electrophoresis (22, 36).

RESULTS

Free tRNA's in defective ASV particles. Labeled RNA was prepared from each of the different ASV variants described in Table 1. The low-molecular-weight RNAs recovered after sucrose gradient centrifugation of the virion RNAs were fractionated by 2-D gel electrophoresis. Virus particles of the *pol*$^+$ *env*$^-$ phenotype released by the 16T cells contained seven major fractions of tRNA (Fig. 1a). This pattern was indistinguishable from that of wild-type ASV, and the numbering system conforms to that previously used by Sawyer and Dahlberg (36). Elution and RNase T1 oligonucleotide fingerprinting of the various fractions (data not shown) confirmed these assignments and in some cases identified the tRNA species (35). For example, spot 1 had the fingerprint of tRNA^{Trp}, the major primer for RNA-directed DNA synthesis (18). The position of tRNA^{Trp} in other panels of Fig. 1 is indicated by the arrow. The pattern of viral tRNA's obtained from the 16T particles was less complex than that of the total tRNA population of the 16T cells (Fig. 1f). However, the *pol*$^-$ particles released by α40T, α48T, and SE52d cells contained a free 4S RNA population similar to that of the cell (Fig. 1b–d). In contrast to the variants defective in polymerase, the 21Q1b particles, with normal levels of structural proteins but no viral genome RNA, contained a specific subset of the host cell tRNA's similar to that of wild-type ASV (Fig. 1e).

tRNA's associated with the viral genome RNA. All of the *pol*$^-$ variants of ASV described in Table 1, as well as the *pol*$^+$ *env*$^-$ virus from 16T cells, were found to contain normal proportions of 70S and 4S RNAs (data not shown). In the case of particles produced by 21Q1b cells, the RNA ranged from 4S up to approximately 30S in size, as described originally by Linial et al. (25). To distinguish clearly between the free and associated small RNA populations in this virus, the viral RNA was subjected to chromatography on poly(U)-Sepharose, and the polyadenylated RNA was taken to represent pseudogenomic RNA. The high-molecular-weight RNA complexes prepared from the various viruses were heated to 90°C for 2 min and applied to 2-D gels under conditions which trapped RNA greater than 8S in size at the gel origin (32, 36). The 4 to 5S regions of these gels are depicted in Fig. 2. Figure 2a shows the pattern of associated tRNA's observed with 16T virions and is identical to that of wild-type ASV (36). The predominant species (spot 1) was tRNA^{Trp}, and low amounts of spots 3, 5, and 7 were also discernible. The *pol*$^-$ variants α48T and SE52d contained markedly reduced amounts of tRNA^{Trp} (Fig. 2b, c), whereas spots 5 and 7 and 5S RNA were still detected. The 21Q1b virions contained very small amounts of tRNA associated with the high-molecular-weight RNA and showed no obvious selectivity compared with total cellular tRNA (Fig. 2d).

In vitro binding of DNA polymerase to tRNA. The studies described above support the hypothesis that the viral reverse transcriptase may be the sole determinant for selective packaging of tRNA's into ASV virions. We therefore decided to examine the in vitro binding of tRNA to purified reverse transcriptase in an attempt to find conditions which would reproduce the in vitro selectivity.

Panet et al. previously reported a gel filtration assay which could demonstrate specific binding between AMV reverse transcriptase and tRNA^{Trp} (29). Under their conditions, the purified enzyme also bound low levels of tRNA$_4^{Met}$ (spots 5 and 6) but showed no affinity for other virion tRNA's. By carrying out the assay at elevated temperatures and in the absence of magnesium, the specificity of the binding reaction can be modified to include the other viral tRNA's. Figure 3 shows a gel filtration assay performed in 1 mM EDTA at 25°C. In the absence of reverse transcriptase, chicken cell 4S RNA eluted in the included volume of a Sephadex G-100 column as a symmetrical peak (Fig. 3a). Prior incubation of the RNA with AMV reverse transcriptase under these conditions caused a small fraction of the RNA to coelute with the enzyme in the excluded volume of the gel filtration column (Fig. 3b). The RNAs in fractions containing the peak of DNA polymerase activity, and in the corresponding fractions of the control column (Fig. 3a), were analyzed by 2-D gel electrophoresis (Fig. 4). In the absence of added enzyme, only a trace of RNA was detected in the excluded volume; this corresponded to 5S RNA which had not been removed during the original purification of the cell 4S RNAs (Fig. 4a). However, in the presence of reverse transcriptase, a subset of the tRNA's, corresponding in identity and relative abundance (data not shown) to the free 4S RNAs of ASV, coeluted with the peak of polymerase activity (Fig. 4b).

The binding assay was repeated under a variety of other conditions. Using the same ratio of enzyme to RNA but at low temperature (4°C) and in the presence of magnesium, the specificity

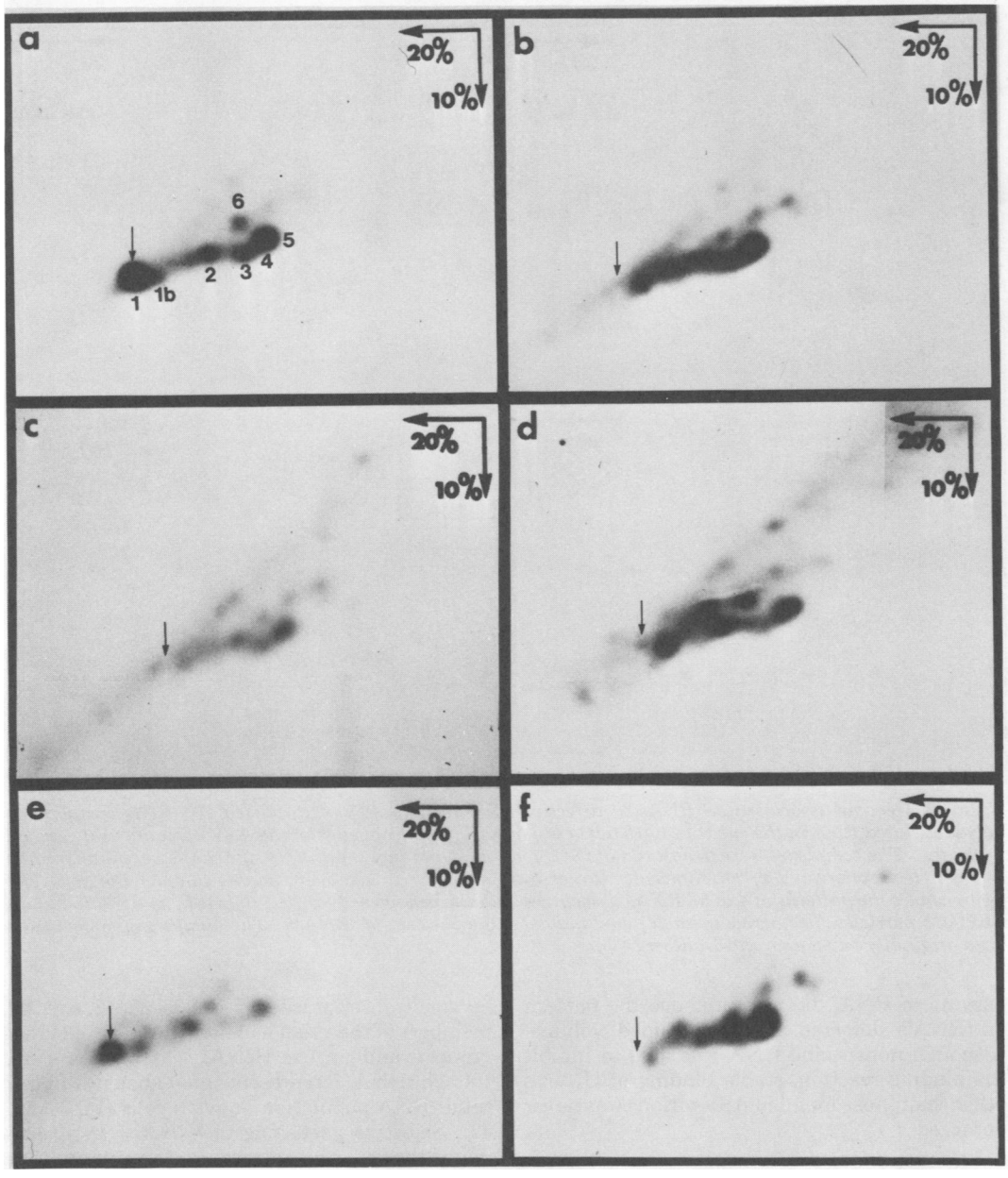

FIG. 1. *Free 4S RNAs in defective ASV particles. The free, virion 4S RNAs were prepared from ^{32}P-labeled cultures of (a) 16T, (b) α48T, (c) α40T, (d) SE52d, and (e) SE21Q1b cells according to standard procedures (32, 36). The 4S RNAs were further fractionated by electrophoresis in two dimensions in polyacrylamide slab gels (22, 36). The first dimension (top to bottom) was in 10% acrylamide; the second dimension (right to left) was in 20% acrylamide. The ^{32}P-labeled RNAs were detected by autoradiography and numbered according to Sawyer and Dahlberg (36). The downward arrow in each panel indicates the position of spot 1, i.e., tRNA^{Trp}. For comparison, the pattern of total tRNA's from turkey embryo fibroblasts, analyzed in the same way, is shown in panel f.*

of the binding reaction was essentially as reported by Panet et al. (29; data not shown). Thus, two parameters, temperature and divalent cation, appeared to be critical in governing the specificity of the reaction since altering other components in the system, such as the ratio of

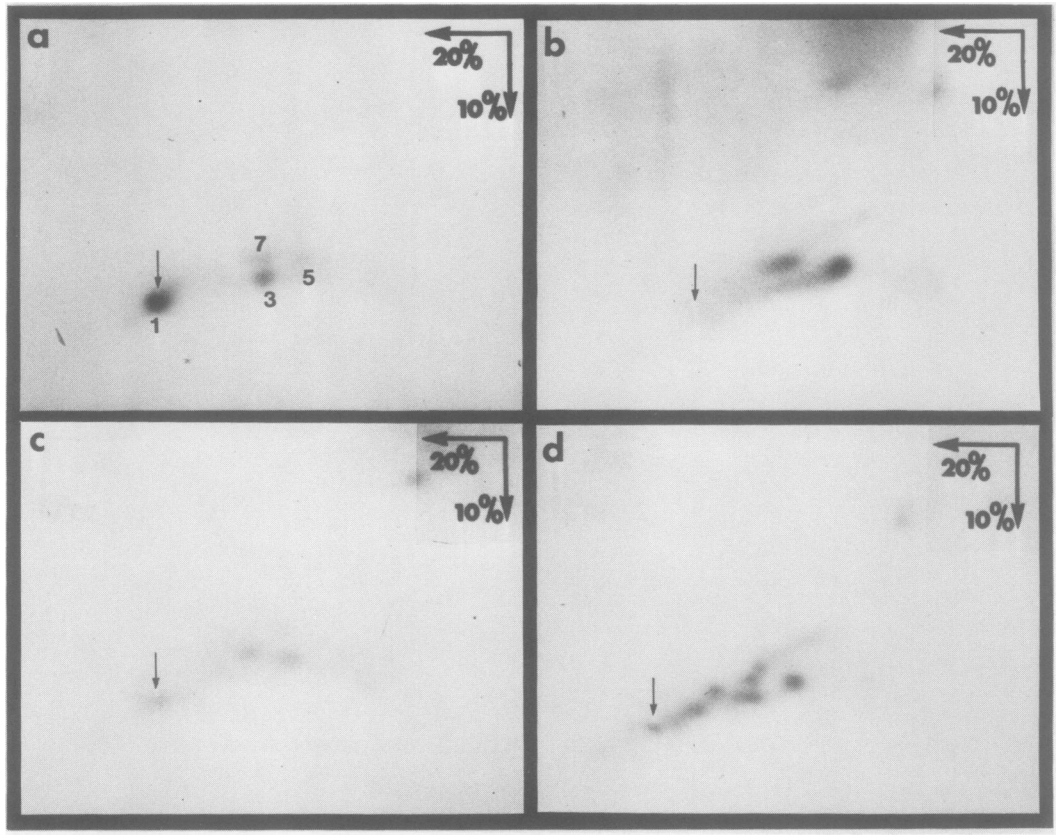

FIG. 2. *Genome-associated 4S RNAs in defective ASV particles. The ^{32}P-labeled 70S RNA complex (or polyadenylated RNA in the case of 21Q1b particles) was isolated from each of the ASV variants as described in the text. The complexes were denatured at 90°C for 2 min, and any small RNAs released were analyzed by 2-D gel electrophoresis (32, 36). Molecules larger than about 8S in size were unable to enter the gels. The figure shows the patterns of 4 to 5S RNAs associated with the genomes of (a) 16T, (b) α48T, (c) SE52d, and (d) SE21Q1b particles. The arrow in each panel indicates the position of tRNA^{Trp}. The numbering system is as used originally by Sawyer and Dahlberg (36).*

enzyme to RNA, did not influence the pattern of tRNA's detected in the excluded volume. Although nonspecific RNA was able to inhibit the binding reaction, stable binding of tRNA's other than those found in ASV virions was never observed.

DISCUSSION

The encapsidation of host cell tRNA's into retrovirus particles is not a fortuitous consequence of budding from the cell membrane, but rather a specific, controlled mechanism. Only a subset of the tRNA population is selected for inclusion in the virion, and in amounts not representative of their relative abundances within the host cell (11, 30–33, 36, 42). Of this subset, one tRNA in particular has been the focus of attention, namely, the tRNA serving as primer for RNA-directed DNA synthesis by the viral reverse transcriptase (4, 7, 14, 30–33, 42, 43). All members of the avian leukosis and sarcoma virus group examined use tRNA^{Trp} as primer, even though tRNA^{Trp} represents only about 2% of the total tRNA population in avian cells (14, 38, 39, 42). Selective packaging of tRNA^{Trp} in virions might therefore be necessary to ensure that adequate amounts of primer are present for efficient DNA synthesis when the virus infects another cell. The reason for encapsidation of the other virion tRNA's remains obscure since no function has yet been ascribed to them (39, 42). It is possible that some may be involved in linking the genome RNA subunits together in the 70S complex, or in interactions with RNA binding proteins within the virus core (42). In this context, it is worth noting that in another retrovirus system, mouse mammary tumor virus, only the primer and one other tRNA are found in the virus (31).

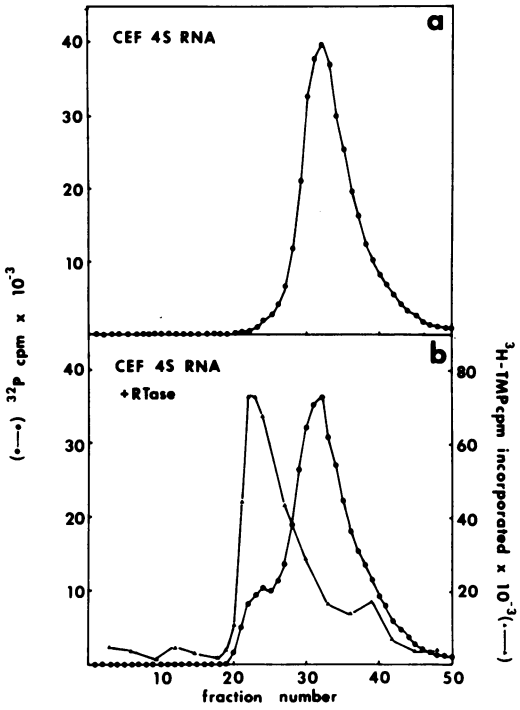

FIG. 3. *Binding of tRNA's to AMV reverse transcriptase. ^{32}P-labeled 4S RNA from chicken embryo fibroblasts was incubated (a) alone or (b) in the presence of 30 U of AMV reverse transcriptase as described in the text. Incubation was performed at 25°C. The samples were then subjected to gel filtration in a temperature-controlled (25°C) column of Sephadex G-100. Each fraction was assayed for [^{32}P]RNA by direct counting of Cerenkov radiation, and equal portions of alternate fractions were taken to assess reverse transcriptase activity using poly(rA)·oligo(dT) as the template-primer.*

In its role as primer, $tRNA^{Trp}$ must interact with the viral reverse transcriptase. Since the initial observations by Panet et al. (29) that purified AMV reverse transcriptase could specifically bind to $tRNA^{Trp}$ in vitro, this interaction has been regarded as the most likely mechanism for enriching for primer tRNA in virus particles. For this reason, we decided to examine a series of defective ASV variants which lack detectable reverse transcriptase activity. Two of these, α40T and α48T, were derived by Murphy from an original stock of αBH-RSV (17, 26). These, together with the classical BH-RSV(−) virions released by 16T cells, permitted examination of particles with three distinct phenotypes: *pol*$^+$ *env*$^-$, *pol*$^-$ *env*$^-$, and *pol*$^-$ *env*$^+$ (see Table 1). The results presented in Fig. 1 showed that in the absence of functional polymerase, no selection appeared to operate over which tRNA's were packaged into virus particles, confirming the findings of Sawyer and Hanafusa (37). In addition, they confirmed that the presence or absence of the envelope glycoproteins did not influence the packaging of tRNA's. To exclude the possibility that the results obtained were peculiar to the particular defect in αBH-RSV, an independently generated variant, SE52d, was also examined. Whereas α mutants derived from BH-RSV appear to be devoid of immunoprecipitable reverse transcriptase protein, SE52d encodes a truncated polymerase protein as a result of deletion of about 30% of the sequences in the polymerase gene (24, 27). As shown in Fig. 1d, SE52d resembled the other α mutants in showing no selectivity over the free 4S RNAs encapsidated.

Since the tRNA primer also interacts directly

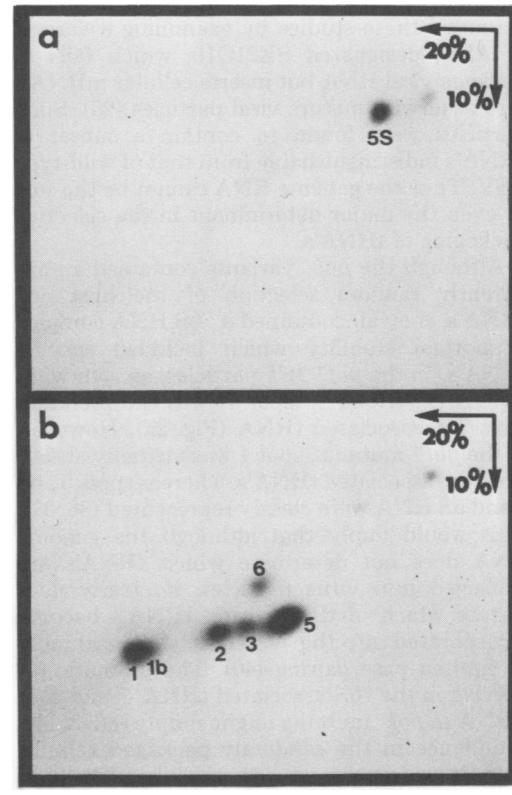

FIG. 4. *Characterization of tRNA's binding to AMV reverse transcriptase. Fractions 21 to 25 from each of the elution profiles shown in Fig. 3 were pooled, and the RNAs were precipitated by addition of cetyltriethylammonium bromide (3). The recovered RNAs were then analyzed directly by 2-D gel electrophoresis (22, 36). (a) RNAs eluting in the void volume in the absence of reverse transcriptase; (b) RNAs coeluting with AMV reverse transcriptase. The identity of each of the numbered spots was confirmed by RNase T1 digestion and oligonucleotide fingerprinting (35; data not shown).*

with the viral genome RNA via a short region of base complementarity (6, 10), it is conceivable that this interaction could account for high levels of primer within virus particles. The presence of other tRNA's would in this case reflect random, less stable binding to other regions of the genome RNA (40, 41, 42). However, such a mechanism cannot account for the observation that the majority of the viral tRNA's, including most of the primer, occur free in virions; i.e., they are not associated with the genome RNA as normally extracted with protein denaturants. Furthermore, experiments conducted on murine leukemia virus grown in the presence of actinomycin D implied that even though such virions contained almost no genomic RNA, they nevertheless contained the normal levels and relative amounts of tRNA (23). We have confirmed and extended these studies by examining a variant of ASV, designated SE21Q1b, which fails to package viral RNA but inserts cellular mRNA's into otherwise mature viral particles (25). Such particles were found to contain a subset of tRNA's indistinguishable from that of wild-type ASV. Thus the genome RNA cannot be the sole or even the major determinant in the selective packaging of tRNA's.

Although the *pol*$^-$ variants contained an apparently random selection of the host cell tRNA's, they all contained a 70S RNA complex of normal stability which included specific tRNA's. In the *pol*$^+$ 16T particles, as with wild-type ASV, $tRNA^{Trp}$ (spot 1) was the predominant 70S-associated tRNA (Fig. 2a). However, in the *pol*$^-$ mutants, spot 1 was virtually absent from the associated tRNA's, whereas spots 5 and 7 and 5S RNA were clearly represented (36, 37). This would imply that although the genome RNA does not determine which tRNA's are packaged into virus particles, it clearly does dictate which of these virion tRNA's become incorporated into the 70S complex, presumably by limited base pairing (40). The dramatic reduction in the 70S-associated $tRNA^{Trp}$ and spot 3 RNA in *pol*$^-$ mutants might simply reflect low abundance in the randomly packaged cellular tRNA's as compared with the normally high concentration in wild-type virus. The lack of $tRNA^{Trp}$ was not a consequence of loss or alteration of the primer-binding site as determined by reassociation experiments with ^{32}P-labeled $tRNA^{Trp}$ and SE52d genomic RNA (data not shown). However, these data do not rule out the possibility that reverse transcriptase plays a role in association of the primer with the genome RNA (1, 6, 39). They do exclude the suggestion that the primer tRNA is responsible for linking the two genome RNA subunits in the ASV 70S complex (19).

The absence of tRNA selection in *pol*$^-$ mutants, and the lack of involvement of *env*, imply that the reverse transcriptase may be the sole determinant for this selection. Alternatively, one or more of the internal structural proteins could act in conjunction with the polymerase to control packaging of tRNA's (23). If the former premise were to hold, it should be possible to mimic the selection in vitro in a binding reaction between purified reverse transcriptase and tRNA's. Previous studies on this interaction were performed at low temperature in the presence of magnesium ions and relied on the fact that binding to reverse transcriptase would cause a tRNA to be excluded from Sephadex G-100 in gel filtration (2, 16, 20, 21, 29). Specific binding of $tRNA^{Trp}$ (spot 1) and small amounts of $tRNA_4^{Met}$ (spots 5 and 6) were reported (29). Subsequent studies using different binding assays suggested that the purified enzyme might have an affinity for all tRNA's, but with a wide variation in binding constant (21, 28). By carrying out the gel filtration assay at elevated temperature in the absence of magnesium, we have determined conditions in which purified AMV DNA polymerase will select, in vitro, a specific subset of chick cell tRNA's which is indistinguishable in content and relative abundances from the free tRNA population of ASV. Therefore, we conclude that RNA-directed DNA polymerase is the major determinant in the selective packaging of tRNA's into ASV.

Curiously, work on mammalian retrovirus systems has failed to demonstrate equivalent interactions with the appropriate primer tRNA (20, 28, 42). This may be partly due to the fact that the mammalian reverse transcriptase is isolated as a single polypeptide, possibly equivalent to only the α subunit of the AMV enzyme (16, 20, 21, 28). The α subunit has been shown to be inactive or at least several orders of magnitude less efficient in tRNA binding (16, 20, 21). If, as seems likely in view of general similarities between retroviruses, an analogous mechanism were to operate in mammalian systems, then the mouse mammary tumor virus reverse transcriptase would be expected to be highly selective since only two isoacceptor tRNA's for lysine occur in appreciable amounts in the virion (31).

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