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Characterization of Rous Sarcoma Virus *src* Gene Products Synthesized In Vitro

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The cell-free synthesis of three major proteins from virion RNA of nondefective Rous sarcoma virus (RSV), but not from RNA of transformation-defective deletion mutants, has been observed. The apparent molecular weights of these transformation-specific proteins are approximately 60,000 (60K), 25K, and 17K. Tryptic maps of methionine-containing peptides revealed the 17K, 25K, and 60K proteins to be overlapping in sequence. However, only partial homology was observed between the 17K, 25K, and 60K proteins synthesized from Schmidt-Ruppin strain, subgroup D, RSV RNA and those synthesized from Prague strain, subgroup B, RSV RNA. About half of the methionine peptides in the Schmidt-Ruppin strain, subgroup D, 60K protein were shared with the Prague strain, subgroup D, 60K protein, and the rest were distinct to each. The virion RNAs coding for the 60K, 25K, and 17K proteins were found to be polyadenylated and to sediment with maximal mRNA activity at about 23, 19 to 20, and 18S, respectively. In addition, transformation-specific proteins with molecular weights of 39K and 33K were observed by in vitro synthesis. These proteins are also related to the 60K, 25K, and 17K proteins and were synthesized from polyadenylated RSV RNA of approximately 21 to 22S. RNase T_1-resistant oligonucleotides were analyzed in parallel, and the *src*-specific oligonucleotides were found to be first present in equimolar amounts in those gradient fractions sedimenting at 21 to 22S. Our data suggest that synthesis of the 60K protein is initiated near the 5′ terminus of the *src* gene, whereas the 39K, 33K, 25K, and 17K proteins are initiated internally in the *src* gene. All of these proteins appear to be initiated independently, but they may have a common termination site.

The *src* gene of avian retroviruses is required for both the initiation and the maintenance of transformation in cultured fibroblasts and for the induction of sarcomas in vivo. Transformation-defective (td) deletion mutants of Rous sarcoma virus (RSV), which arise spontaneously upon passage of nondefective (nd) RSV in tissue culture cells, replicate normally but are unable to transform cells or induce sarcomas. These deletion mutants have been used to determine the boundaries and the location of the *src* gene within the viral genome. The largest deletions in td mutants represent ~20% of the 10,000-nucleotide genome and have been mapped between approximately 800 and 3,000 nucleotides from the 3′ terminus of the genomic RNA (10).

Despite the strong indications that the *src* gene codes for a protein responsible for transformation, until recently no such protein had been identified. During the past year, however, there have been several reports of identification of products of the *src* gene. We reported the cell-free synthesis of two sets of doublets having apparent molecular weights of 25,000 (25K) and 17K from virion RNA of the Prague strain, subgroup B (PR-B) of RSV that were not synthesized from virion RNA of td PR-B RSV (3). Size fractionation of the virion RNA revealed that the RNAs coding for the 25K and 17K proteins were polyadenylated RNAs of about 20 and 18S, respectively. We showed that the 25K and 17K proteins shared amino acid sequences, and both were distinct from the major translation product of full-length genomic RNA, the 76K polyprotein precursor to the viral internal structural proteins (27). Similar findings have been reported by Kamine et al., who observed synthesis of proteins with molecular weights of 60K, 25K, and 18K upon translation of virion RNA from PR-B RSV in vitro, but not from td PR-B RSV RNA (12). Purchio et al. likewise obtained synthesis of a 60K protein from 20 to 24S polyadenylated RNA isolated from PR-C RSV virions (24). As we will discuss below, this 60K protein is probably identical to the 55K protein that we observed to be synthesized from 25S polyadenylated RNA from PR-B RSV virions (3).

The cell-free synthesis of putative *src* gene products with molecular weights of 60K is particularly interesting in light of the recent identification by Brugge and Erikson (4) of a 60K tumor-specific antigen in RSV-transformed cells. They obtained serum from rabbits bearing Schmidt-Ruppin, subgroup D (SR-D) RSV-induced tumors. This serum specifically immunoprecipitated a 60K protein from both chicken and hamster cells transformed by RSV, even when the serum had been absorbed with disrupted virions to prevent immunoprecipitation of virion proteins.

In this report, we extend our previous characterization of cell-free translation products of RSV virion RNA (3). Two-dimensional analysis of tryptic peptides of the 17K, 25K, and 60K proteins has clearly demonstrated that these proteins have overlapping amino acid sequences. Comparison of these products synthesized from two different strains of RSV (PR-B and SR-D) has shown strain-specific differences in the 17K, 25K, and 60K proteins, manifested both by their electrophoretic mobility in acrylamide gels and by their tryptic peptides. The sizes of the polyadenylated RNAs coding for the 60K, 25K, and 17K proteins are consistent with the idea that these proteins are all coded for by the RSV *src* gene.

MATERIALS AND METHODS

Cells and virus. Primary cultures of chicken embryo fibroblasts were made from eggs obtained from SPAFAS. Cells were infected with recloned stocks of RSV. The PR-B, SR-D, and td SR-D clones were originally obtained from P. K. Vogt. After infection was established, virus was harvested at 12- to 24-h intervals. Cells were grown in medium 199 (Grand Island Biological Co.) supplemented with 2% tryptose phosphate broth (Difco), 1% calf serum (Microbiological Associates), 1% chick serum (Grand Island Biological Co.), 1% dimethyl sulfoxide (Mallinckrodt), and 10 U of Mycostatin (Squibb) per ml. Virus was pelleted from clarified supernatants by centrifugation at 17,000 rpm for 2 h in a Beckman 19 rotor. It was then resuspended and sedimented to equilibrium in a 20 to 55% sucrose gradient containing 0.1 M NaCl, 0.01 M Tris-hydrochloride (pH 7.4), and 0.001 M EDTA for 3 h at 25,000 rpm in a Beckman SW 27 rotor.

RNA preparation. RNA was extracted from purified virions with phenol-chloroform-sodium dodecyl sulfate (SDS), and the 70S RNA was isolated as described (6). The 70S RNA was denatured by heating at 100°C for 1 min, followed by quenching in ice water. Before sedimentation through sucrose gradients, the denatured RNA was bound to oligodeoxythymidylic acid [oligo(dT)]-cellulose to isolate polyadenylic acid [poly(A)]-containing molecules. The bound RNA was eluted with water, boiled, and bound to oligo(dT)-cellulose a second time. The denatured poly(A)-containing RNA was sedimented through a 10 to 25% sucrose gradient containing 10 mM sodium acetate (pH 5.2), 1 mM EDTA, and 0.1% SDS in a Beckman SW 50.1 rotor at 49,000 rpm for 3.5 h at 20°C.

Cell-free translation. The messenger-dependent rabbit reticulocyte lysate (22) was prepared with micrococcal nuclease (P. L. Biochemicals) at a concentration of 10 μg/ml and EGTA at 2.5 mM. Calf liver tRNA (Boehringer-Mannheim) was added at a concentration of 50 μg/ml. Translations were performed at 30°C for 1 h, usually in a volume of 10 μl containing [^{35}S]methionine (250 μCi/ml; specific activity, 600 Ci/mmol; Amersham). Translation products were subsequently incubated with 50 μg of RNase A (Worthington) per ml and 10 mM EDTA at 30°C for 10 min to digest tRNA's charged with [^{35}S]methionine. The inhibitor 7-methyl-GTP (m^{7}GTP) (P. L. Biochemicals) was used at 200 μM.

Gel electrophoresis. Polyacrylamide slab gels contained 14% acrylamide, 0.09% bis-acrylamide (Bio-Rad), 0.1% SDS, and the buffers described by Laemmli (14). Gels were run at 15 mA for approximately 4 h, and the radioactive proteins were subsequently detected by fluorography (16).

Tryptic peptide mapping. Cell-free translations were performed in the presence of 200 μM m^{7}GTP. Up to 200 μl of in vitro reaction were resolved by SDS-polyacrylamide electrophoresis on a single 2-mm-thick slab gel. After electrophoresis, the gel was shaken for 1 h in water with 5 g of mixed bed resin (Amberlite MB-3), to remove unincorporated [^{35}S]methionine, before being dried over a boiling-water bath. The dried gel was exposed to Kodak NS5T X-ray film for 1 to 5 days. Bands were excised from the gel by using suitable alignment markers. The backing paper was scraped from the dried gel bands, which were then cut up into small pieces and allowed to swell in a small volume of 0.05 M NH_4HCO_3–0.1% SDS. The swollen pieces were homogenized in a Tenbrock glass homogenizer, more buffer being added when necessary. Including washings, the final volume of buffer used was approximately 2 ml/100 mm^2 of gel. The homogenate was made 5% in 2-mercaptoethanol, boiled for 5 min, and then shaken overnight at 37°C in a rotary shaking water bath to elute the labeled protein. The gel fragments were pelleted for 10 min at 10,000 rpm in a Sorvall HB4 rotor at room temperature. The supernatant was carefully aspirated, and the gel fragments were washed for 2 h at 37°C with a volume of buffer half that used originally. The gel fragments were pelleted again, and the second supernatant was pooled with the first. A 75-μg amount of bovine gamma globulin was added as carrier to the pooled supernatants and mixed thoroughly. The protein was then precipitated by making the solution 20% in trichloroacetic acid and leaving at 0°C for 4 h. The precipitated protein was recovered by centrifugation for 15 min at 10,000 rpm in a Sorvall HB4 rotor at 4°C. The tube was drained thoroughly by inversion, and the pellet was washed successively with ethanol at −10°C and ethanol:ether (1:1) at −10°C with the same centrifugation conditions. The dried pellet was dissolved in 150 μl of chilled performic acid (30% H_2O_2 and 98% formic acid [1:9] incubated for 1 h at room temperature) and incubated for 2 h at 0°C. The performic acid solution was diluted with 3 ml of water, frozen, and lyophilized. The oxidized

protein was digested with 30 μg of L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone-treated trypsin (Worthington) in 0.5 ml of 0.05 M NH_4HCO_3 for 18 h at room temperature. A further 20 μg of trypsin was then added, and digestion continued for 4 h. The digest was diluted with 2 ml of water, frozen, and lyophilized. This procedure was repeated twice more until all the NH_4HCO_3 had been removed. The digest was finally dissolved in 0.5 ml of pH 4.7 electrophoresis buffer (7) and centrifuged for 2 min in a microcentrifuge at room temperature to remove any undigested core. The supernatant was carefully aspirated and lyophilized. The digest was finally dissolved in 10 to 20 μl of electrophoresis buffer and resolved in two dimensions on cellulose thin-layer plates as described by Gibson (7). Up to 50% of the digest could be used on 100-μm thin-layer plates without loss of resolution.

Oligonucleotide fingerprinting. ^{32}P-labeled 70S RSV RNA was digested with RNase T_1, and the resulting oligonucleotides were resolved by electrophoresis on Cellogel (Kalex) and homochromatography on DEAE-cellulose thin-layer plates (Analtech) as previously described (2). Autoradiograms were exposed at −70°C with intensifying screens (Dupont Cronex) and X-ray film (Kodak XR5) which had been preexposed to enhance detection (17).

RESULTS

Translation of RSV virion RNA. Previously we have shown that 25K and 17K proteins were synthesized in vitro from nd PR-B virion RNA but not td PR-B virion RNA and that the synthesis of these proteins was unaffected by the cap analog, m^7GTP (3). The m^7G-containing cap analogs inhibit protein synthesis from some capped mRNA's in the rabbit reticulocyte system (18). We have shown that synthesis of the RSV 76K *gag* gene product was inhibited to an extent of about 70% by m^7GTP (3). To extend our earlier analysis, we have carried out, in parallel, translation in the mRNA-dependent reticulocyte lysate of heat-denatured virion RNAs of PR-B, SR-D, and td SR-D RSV (Fig. 1). Comparison of the products from nd SR-D RNA and td SR-D RNA (tracks B and C) clearly reveals, in addition to the 25K and 17K proteins, an approximately 60K protein specific to the product of nd SR-D RNA.

In our earlier analysis of translation products of PR-B and td PR-B RNA, we observed, in addition to the 25K and 17K proteins specific to nd PR-B, a 60K product of both nd and td PR-B RNAs (3). Analysis of products synthesized in the presence of m^7GTP, which we have used to distinguish between products derived from the *gag* gene intiation site and other initiation sites (3), showed that there was a portion of the total 60K product made from nd PR-B RNA whose synthesis was resistant to m^7GTP. There was no m^7GTP-resistant protein synthesized from td PR-B RNA (3). Therefore, it appears that PR-B

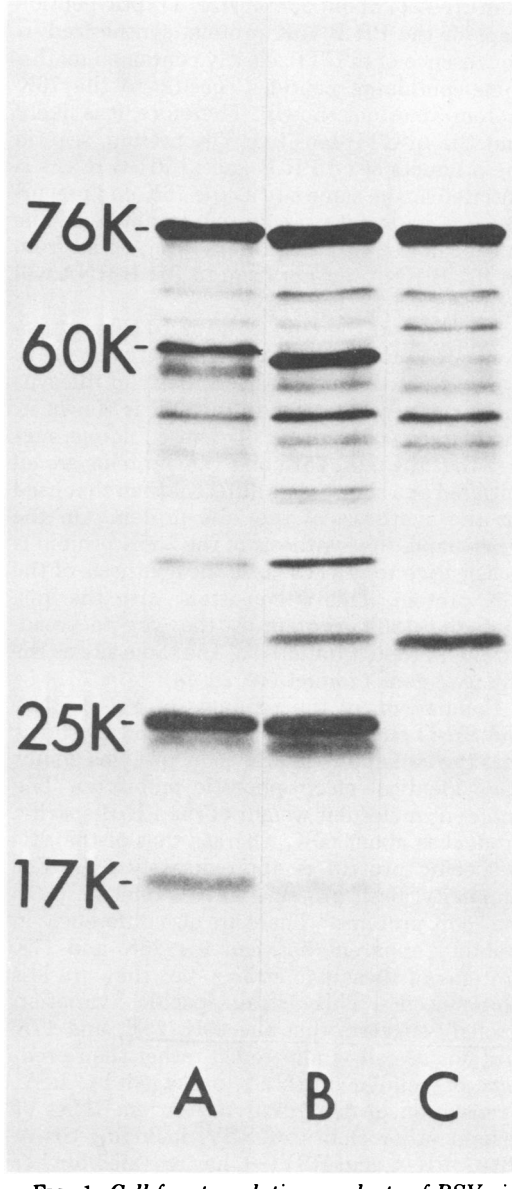


FIG. 1. *Cell-free translation products of RSV virion RNAs. Denatured 70S virion RNAs derived from (A) PR-B, (B) SR-D, and (C) td SR-D were translated in the mRNA-dependent reticulocyte lysate system in the presence of [^{35}S]methionine. The products were resolved on a 14% SDS-polyacrylamide slab gel which was subsequently fluorographed.*

virion RNA codes for two 60K proteins, having distinct initiation sites; synthesis of one of these proteins is sensitive to inhibition by m^7GTP, whereas synthesis of the other is resistant. Translation of size-fractionated polyadenylated PR-B RNA (Fig. 4) showed that 60K proteins were synthesized both from full-length RNA and

from RNA of about 23S in size. Tryptic peptide maps of the PR-B 60K protein, synthesized in the absence of m^7GTP, clearly contained methionine-containing peptides specific to the 76K protein (data not shown). Therefore, it is likely that the m^7GTP-sensitive 60K protein, seen in the products of nd PR-B and td PR-B RNAs is initiated at the same site as the 76K *gag* precursor protein, but is prematurely terminated. The m^7GTP-resistant 60K protein synthesized from nd PR-B RNA but not from td PR-B RNA will be discussed further below.

Synthesis of the 60K, 25K, and 17K proteins from SR-D RSV RNA is unaffected by m^7GTP. A demonstration of the insensitivity of the synthesis of these proteins to m^7GTP is shown in the marker tracks of Fig. 4. These findings suggest that the 60K, 25K, and 17K proteins are all initiated at a site or sites different than that used for the synthesis of the 76K protein. On the other hand, the synthesis of the 180K protein is as sensitive to m^7GTP as is the synthesis of the 76K protein. This is consistent with the idea that the 180K protein is the *gag-pol* readthrough product initiated at the same site as the 76K *gag* gene product (19, 20, 24).

Comparison of the products of PR-B RNA and SR-D RNA (Fig. 1, tracks A and B) shows that the two approximately 60K proteins do not have identical electrophoretic mobilities. The apparent molecular weight of the PR-B-specific product is about 60K, whereas that of the SR-D-specific protein is approximately 58K. For simplicity, both proteins will be referred to as the "60K proteins." There are also differences in mobility apparent between the 25K and 17K proteins of these two strains, but they are less pronounced. This strain-specific variation strongly suggests that the 60K, 25K, and 17K proteins are all virally coded rather than products of cellular mRNA's packaged by RSV. Translation of denatured 70S virion RNAs of several other clones of RSV, including SR-A, PR-C, PR-A, and RSV(−), has revealed further strain-specific differences in electrophoretic mobility of the 60K, 25K, and 17K proteins as well as of the 76K *gag* gene product (K. Beemon and T. Hunter, *In* J. Stevens, G. J. Todaro, and C. F. Fox [ed.], Proc. ICN-UCLA Symp. Persistent Viruses, in press). We have not observed synthesis of the 60K, 25K, or 17K proteins from any of the other td deletion mutants examined, including td PR-C, td SR-A, and partial deletion mutants of td SR-D, characterized by Lai et al. (15).

By tryptic peptide mapping we have shown a lack of homology between the 76K protein and the 25K and 17K proteins derived from PR-B virion RNA (3). We have extended these studies to include the PR-B m^7GTP-resistant 60K protein and the 60K, 25K, and 17K proteins of SR-D. In no case was there any homology to the 76K protein. The occurrence of methionine-containing tryptic peptides in common with p19 in our original peptide maps (3) has been largely abolished by the use of m^7GTP during the preparative synthesis of the 25K and 17K proteins. Clearly the p19 peptides were due to contamination of our preparations of 25K and 17K proteins with incomplete chains related to the 76K protein. We have also examined the 180K *gag-pol* read-through product and the virion glycoproteins by peptide mapping and found no apparent relationship between these proteins and the 60K, 25K, and 17K products. Similarly, we have immunoprecipitated translation products containing the 60K, 25K, and 17K proteins with antisera raised against the internal structural protein, p27, and against the major envelope glycoprotein, gp85, and have observed no cross-reaction. We, therefore, conclude that the 60K, 25K, and 17K proteins do not share sequences with any of the virion internal structural proteins, the viral polymerase, or the viral glycoproteins.

Relationship between the 60K, 25K, and 17K proteins. The lack of relatedness of the 60K, 25K, and 17K proteins to other viral gene products and the insensitivity of their synthesis to m^7GTP suggested that they might all be related to one another. We examined this possibility by comparative tryptic peptide mapping of [^{35}S]methionine-labeled proteins synthesized in the presence of m^7GTP. Comparisons of the methionine-containing tryptic peptides of the 60K, 25K, and 17K proteins of SR-D RSV and PR-B RSV are shown in Fig. 2. For each strain of RSV, the 60K, 25K, and 17K proteins appear to contain overlapping sequences. This was confirmed by analyzing mixtures of the 60K and 25K proteins together and of the 25K and 17K proteins together (data not shown). By using the knowledge that the proteins from each virus are overlapping, the peptides of the SR-D proteins have been numbered, with the lowest numbers being assigned to the peptides in the 17K protein, and so on. The peptides of the PR-B series have been given numbers where they are in common with SR-D peptides (see below) and otherwise have been assigned letters. From Fig. 2, it is obvious that all six peptides of the SR-D 17K protein are present in the 25K protein and that the 13 peptides in the 25K protein are present in the 60K protein. A similar pattern emerges for the PR-B 60K, 25K, and 17K proteins.

We also compared the peptide patterns of the

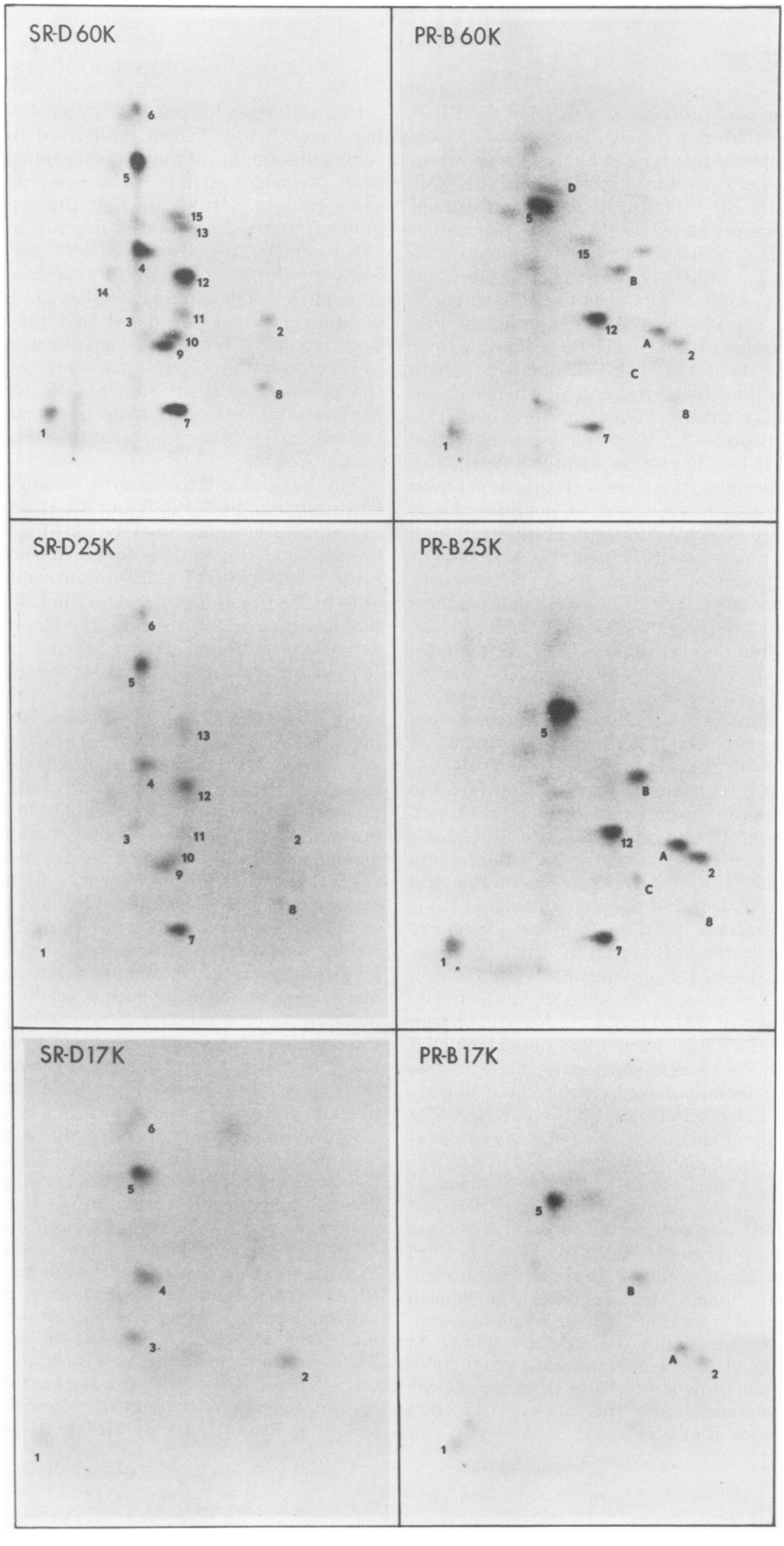
SR-D series of proteins with those of the PR-B series by analyzing suitable mixtures of digests. An example of this type of analysis is shown in Fig. 3, where peptide maps of the PR-B 25K protein, the SR-D 60K protein, and a mixture of the two are presented. On this basis, peptides in the PR-B proteins which are in common with peptides in the SR-D proteins have been assigned the same numbers as their SR-D counterparts. Peptides which are unique to the PR-B proteins have been given letters. It is apparent that the 17K proteins of SR-D and PR-B share three peptides (peptides 1, 2, 5), whereas there are three peptides unique to the SR-D 17K protein (peptides 3, 4, 6) and two peptides unique to the PR-B 17K protein (peptides A, B). For the 25K proteins, there are six common peptides (peptides 1, 2, 5, 7, 8, 12), seven peptides unique to SR-D (peptides 3, 4, 6, 9, 10, 11, 13), and three peptides unique to PR-B (peptides A, B, C). For the 60K proteins, there are seven common peptides (peptides 1, 2, 5, 7, 8, 12, 15), eight peptides unique to SR-D (peptides 3, 4, 6, 9, 10, 11, 13, 14), and four peptides unique to PR-B (peptides A, B, C, D). We conclude that for each virus the 60K, 25K, and 17K proteins form an overlapping set and that there is only partial homology between the two sets of proteins. The presence of strain-specific peptides in all three proteins suggests that the regions of homology between the two sets of proteins are not obviously clustered.

Sizing of RNA coding for the 60K, 25K, and 17K proteins. To localize the coding region for the 60K, 25K, and 17K proteins on the viral genetic map, we sized the polyadenylated RNA coding for these proteins. We had previously shown that the 25K and 17K PR-B proteins were synthesized from polyadenylated virion RNA sedimenting at 20 and 18S, respectively. We had also observed synthesis of a 55K protein (herein called 60K) from a 25S polyadenylated RNA (3). We have now extended these studies to a comparison of translation products of fractionated PR-B, SR-D, and td SR-D RNAs. For these experiments, 70S virion RNA was denatured by heat, and polyadenylated RNA was selected by two cycles of oligo(dT)-cellulose chromatography. Virion RNA from PR-B and from SR-D was sedimented in parallel sucrose gradients, with mouse rRNA markers in a third gradient. Each gradient fraction was precipitated with ethanol, and an equal portion of each fraction was translated. In Fig. 4 slab gels containing the translation products are shown. At both sides of each gel, translation products of unfractionated denatured 70S RNA are shown, as well as translation of this RNA performed in the presence of m^7GTP.

Size estimates for polyadenylated RNA coding for each protein can be derived from the sedimentation rate of the fraction containing the peak of mRNA activity. There is apparently some trailing of RNA towards the top of the gradient, particularly that coding for the major 76K product. This probably reflects the lack of ideal resolution of RNA by sedimentation through a nondenaturing sucrose gradient. In addition, it should be noted that the protein synthesis assay for mRNA activity is sensitive to very low levels of RNA, thus overemphasizing the presence of small amounts of RNA. For these reasons, we have used the fraction in which the maximal synthesis is found to estimate molecular weights.

The peak of mRNA activity coding for the 60K protein of both PR-B and SR-D was found to sediment at approximately 23S (Fig. 4, fractions 17 and 18). Both genomic RNAs show a peak of activity for the 25K protein sedimenting at 19 to 20S (fraction 20), and for the 17K protein sedimenting at 18S (fraction 21). The peak of activity for both the 76K *gag* precursor and the 180K *pol* precursor sedimented at 38S (fractions 10 to 11), suggesting that these proteins are synthesized from full-length virion RNA subunits. There are also a number of other products synthesized from RNA of approximately 38S; these are likely to be premature termination products representing the N terminus of the 76K protein. All of these size estimates are in good agreement with those we previously reported for the PR-B virion mRNA activity (3). In other experiments, polyadenylated RNA was selected from each gradient fraction of virion RNA after sedimentation rather than being selected before gradient fractionation. Similar RNA size estimates were obtained by both procedures (data not shown), thus ruling out the possibility that the smaller RNA species observed to code for the 25K and 17K proteins were generated by cleavage from the 5′ end of the RNA coding for the 60K protein.

In addition to these major products, proteins with molecular weights 39K and 33K were synthesized from both PR-B and SR-D virion RNAs of approximately 21 to 22S (Fig. 4). Tryptic peptide mapping showed that these proteins are related to the 60K protein (data not shown). Proteins of about 64K and 47K were made from SR-D RNA of about 28 to 29S, whereas proteins with molecular weights of 65K, and 44K, were made from PR-B RNA of about the same size. These proteins comigrate with translation products of 70S RNA immunoprecipitated with antiserum against gp85 (data not shown); therefore, it is likely that they are translated from


SR-D 60K
6
5
15
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3
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7
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PR-B 60K
D
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C
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SR-D 25K
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sequences within the *env* gene. Tryptic peptide mapping showed that the 64K and 47K proteins are related to one another but not to the 60K protein (data not shown). Pawson et al. (21) have similarly reported synthesis of a 70K, nonglycosylated *env* precursor by translation of 20 to 28S RNA from RSV-infected cells.

Poly(A)-containing virion RNA from td SR-D was also fractionated on a sucrose gradient, and individual fractions were translated, resulting in the products shown in Fig. 5. The left track contains translation products of SR-D 70S RNA. It is apparent from this experiment that none of the gradient fractions of td SR-D RNA codes for the synthesis of the 60K, 25K, or 17K protein, confirming our earlier observation obtained by translation of unfractionated 70S td SR-D RNA (Fig. 1). It is interesting to note that the 64K and 47K proteins seen above are present here but are synthesized from RNA sedimenting at 20 to 24S. Such a shift in sedimentation rate of polyadenylated RNA would be expected if these proteins are coded for by the *env* gene, because the td deletion results in transfer of the *env* gene to a site approximately 2,000 nucleotides nearer the 3′ terminus of the genome. It is evident that any td RSV RNA present in our preparations of nd RSV RNA would decrease the resolution of the mRNA activities for the 76K, 64K, and 47K proteins. The presence of td virions, however, would not compromise estimates of molecular weight for the virion RNA coding for *src*-related proteins.

A protein of apparent molecular weight of 34K was synthesized from td SR-D RNA of approximately 16 to 18S. A protein of similar electrophoretic mobility was also synthesized from SR-D and PR-B polyadenylated RNA of about 18S. We do not know the origin of the RNA coding for the 34K protein. It is possible that it is coded for by the 3′-terminal "common" region present in both nd and td viral RNA. Alternatively, it may be coded for by packaged cellular mRNA. Tryptic peptide maps show that the 34K protein is distinct from the virion structural proteins and from the 60K, 25K, and 17K proteins (data not shown). By comparison with samples kindly provided by Ray Erikson, we believe the 34K protein to be the same as the protein of apparent molecular weight 29K observed by Purchio et al. (24). It may also be identical to the 37K protein observed by Kamine et al. (12).

Oligonucleotides in RNA cosedimenting with mRNA activity for the 60K, 25K, and 17K proteins. For this highly structured RNA, size estimates based on sedimentation rates in nondenaturing gradients are not very precise. Therefore, we sought another method of identifying the portion of the viral genome coding for the 60K, 25K, and 17K proteins. To do this, we used the fact that the genomic RNA of RSV has been subjected to thorough physical characterization. In particular, the distance of large RNase T_1-resistant oligonucleotides from the 3′ poly(A) terminus of the RNA has been established (5, 9, 28, 29). The presence of specific T_1 oligonucleotides has also been correlated with specific biological functions, and three T_1 oligonucleotides have been assigned to the *src* gene which maps near the 3′ end of the genome (5, 28). In parallel gradients we sedimented poly(A)-containing PR-B RNA to assay for mRNA activities and ^{32}P-labeled, polyadenylated PR-B RNA. We used the ordered appearance of specific T_1 oligonucleotides in polyadenylated molecules of increasing size as a calibration for the size of the RNAs coding for particular proteins. We did not attempt to assay the mRNA activities of the ^{32}P-labeled RNA directly, but assumed that both labeled and unlabeled RNA molecules of a particular length would sediment to an equivalent position in parallel gradients.

Uniformly ^{32}P-labeled 70S PR-B virion RNA was denatured, and poly(A)-containing RNA was selected as described above. The RNA was somewhat degraded, so additional fragmentation was not employed. This RNA was sedimented in a sucrose gradient in parallel with the two gradients whose translation products are shown in Fig. 4. Individual gradient fractions were precipitated with ethanol. The RNA in each was subsequently digested exhaustively with RNase T_1 and fingerprinted by Cellogel electrophoresis and homochromatography. Autoradiograms of the resulting fingerprints of selected fractions are shown in Fig. 6. Spots 9 and 12 are the *src*-specific oligonucleotides (28); spot 12 consists of two unresolved oligonucleotides (2). Total 70S RNA was also fingerprinted to determine the molar ratio of oligonucleotides 9 and 12 in 70S RNA. If the ^{32}P-labeled virus population contained significant amounts of td virus, spots 9 and 12 would be present in less than equimolar amounts. Fig. 6A shows the fin-

FIG. 2. *Relationship between the 60K, 25K, and 17K proteins. SR-D and PR-B 60K, 25K, and 17K proteins, synthesized in vitro, in the presence of m^7GTP, were compared by two-dimensional mapping of methionine-containing tryptic peptides. Numbers are used to denote those PR-B peptides which are also found in SR-D proteins, whereas those that are unique to PR-B are represented by letters. Both common and unique peptides of SR-D are numbered. The SR-D and PR-B sets of peptide maps were run at different times.*

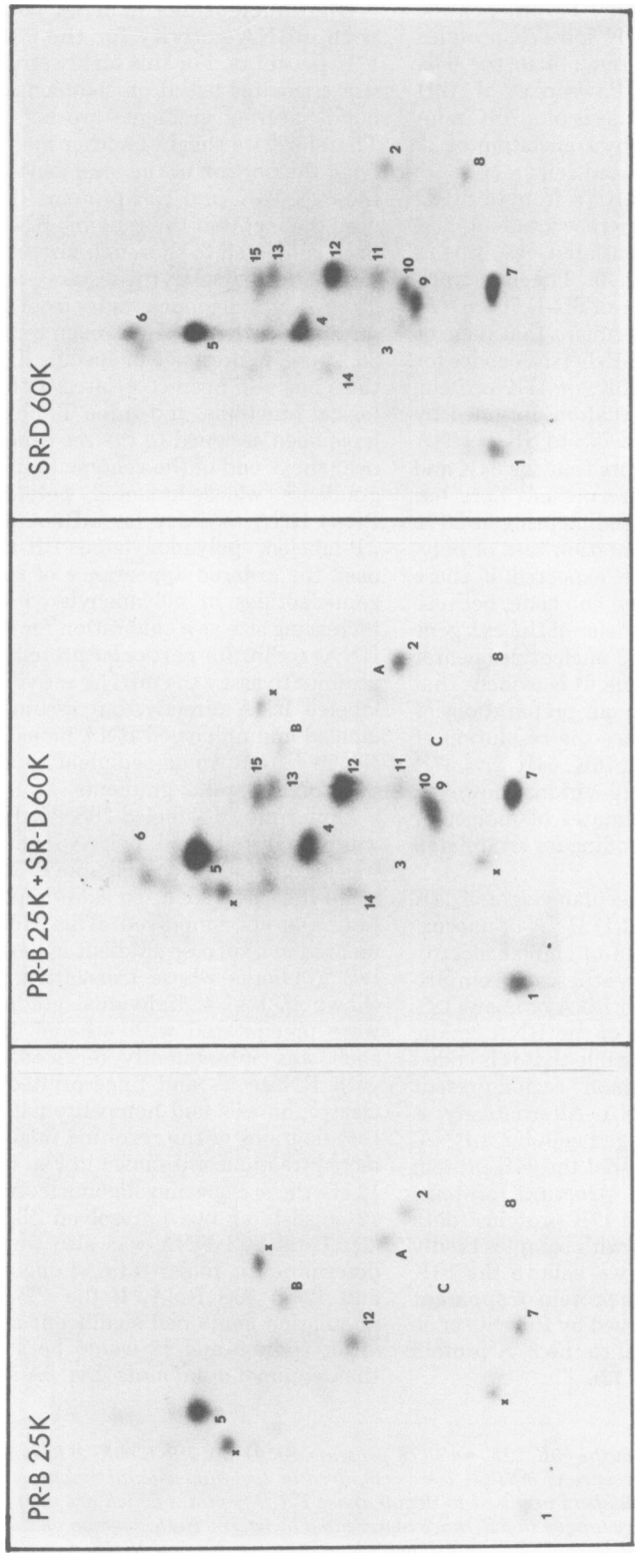

FIG. 3. *Comparison of the PR-B 25K protein with the SR-D 60K protein. A mixture of tryptic digests containing methionine-labeled peptides derived from these two proteins was mapped. Peptides are numbered as in Fig. 2. The PR-B 25K protein map shown here contains three peptides not apparent in the map of this protein shown in Fig. 2. These new peptides (marked X) are also found in the virion core protein, p19 (data not shown). Because this protein was translated without m^{7}GTP, it was contaminated with premature termination products of the 76K gag protein (3).*

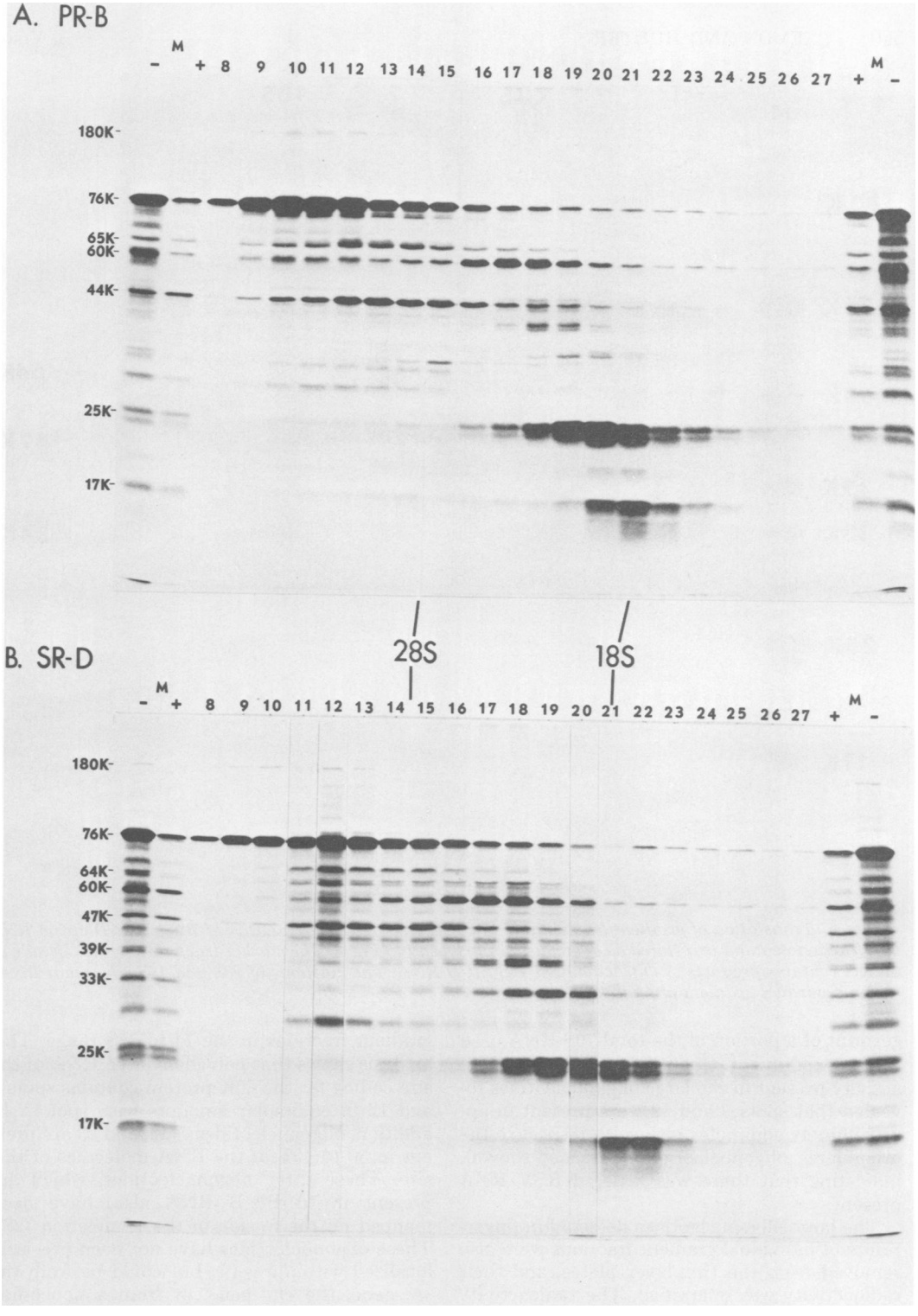

FIG. 4. *Sizing of RNA coding for the 60K, 25K, and 17K proteins. Fifty micrograms each of (A) PR-B and (B) SR-D 70S virion RNA were denatured by heat, and poly(A)-containing RNA species were selected. These were sedimented through sucrose gradients run in parallel. One-tenth of each gradient fraction was translated, and the [^{35}S]methionine products were subjected to electrophoresis through an SDS-polyacrylamide gel. The two marker tracks (M) on each end of the gel contain the products of translation of unfractionated 70S RNA, with (+) and without (−) m^{7}GTP. The positions of 28S and 18S ribosomal RNAs run in a parallel gradient are indicated.*

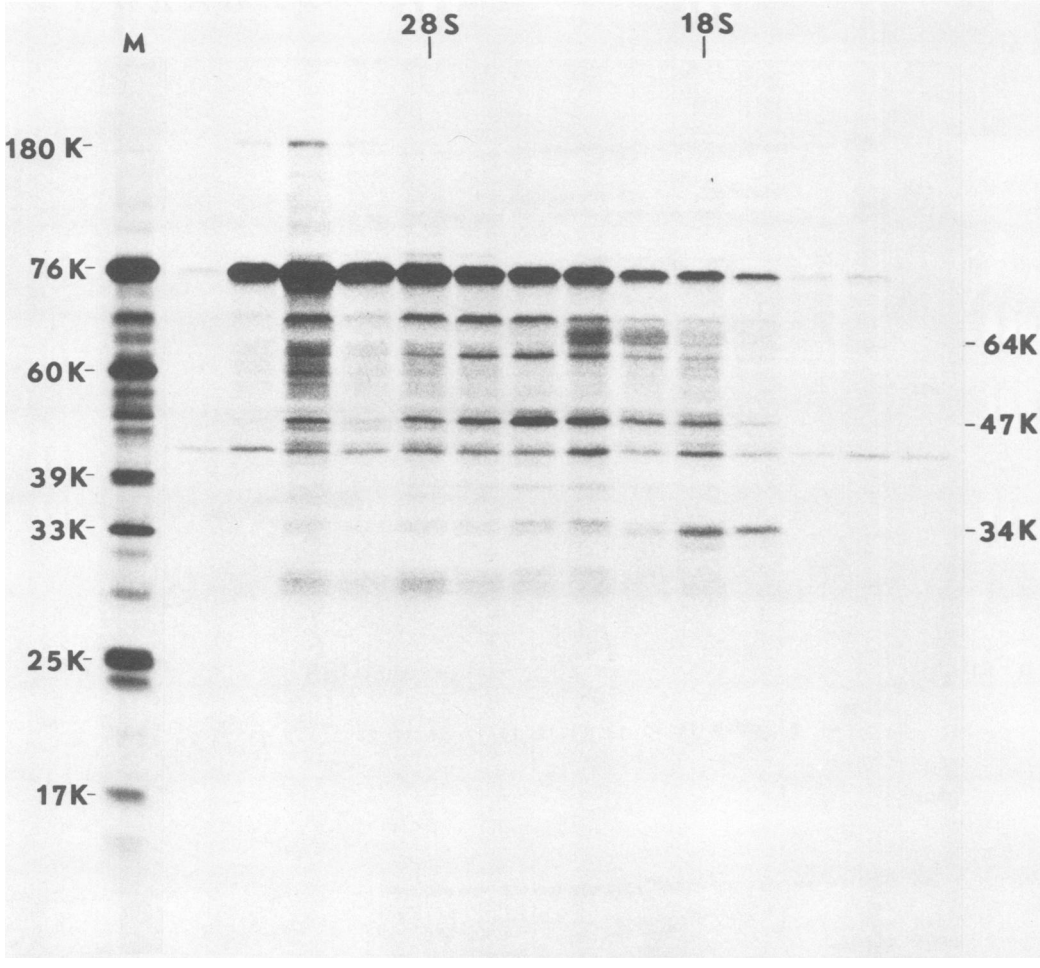

FIG. 5. *Translation of gradient fractionated td SR-D RNA. A 10-μg quantity of 70S td SR-D virion RNA was fractionated and translated as described in the legend to Fig. 4. The marker track (M) on the left side of the gel contains products of unfractionated 70S SR-D RNA. The positions of 28S and 18S ribosomal RNAs run in a parallel gradient are indicated.*

gerprint of a portion of the total 70S RNA used in this experiment. Quantification of the radioactivity present in the large oligonucleotides revealed that spots 9 and 12 were present in approximately equimolar ratio with respect to the other large oligonucleotides (data not shown), indicating that there was little td RSV RNA present.

The large oligonucleotides detected in fingerprints of individual gradient fractions were also removed from the thin-layer plates, and their radioactivity was quantified. The radioactivity per nucleotide in each oligonucleotide was standardized with respect to spot C, which is a conserved sequence present very near the 3′ terminus of RSV RNAs (28). In Fig. 7, the molar ratio of the large oligonucleotides is plotted for gradient fractions in the 14 to 25S range. This analysis shows that polyadenylated RNA of the size coding for the 60K protein contains spots 9 and 12 in equimolar amounts with spot C. In addition, oligonucleotides 7, 14, and 23 are present in 30 to 40% of the RNA molecules of this size. These three oligonucleotides, which are present in td PR-B RNA also, have been mapped on the 5′ side of the td deletion (28). These oligonucleotides have not been precisely localized within a gene, but could be from the *src* gene, the *env* gene, or from a noncoding region beteween these two genes. No significant amount of *env*-specific oligonucleotides (13, 19, 3; reference 29) was present in these fractions. This result suggests that the virion RNA coding for the 60K protein contains sequences from the

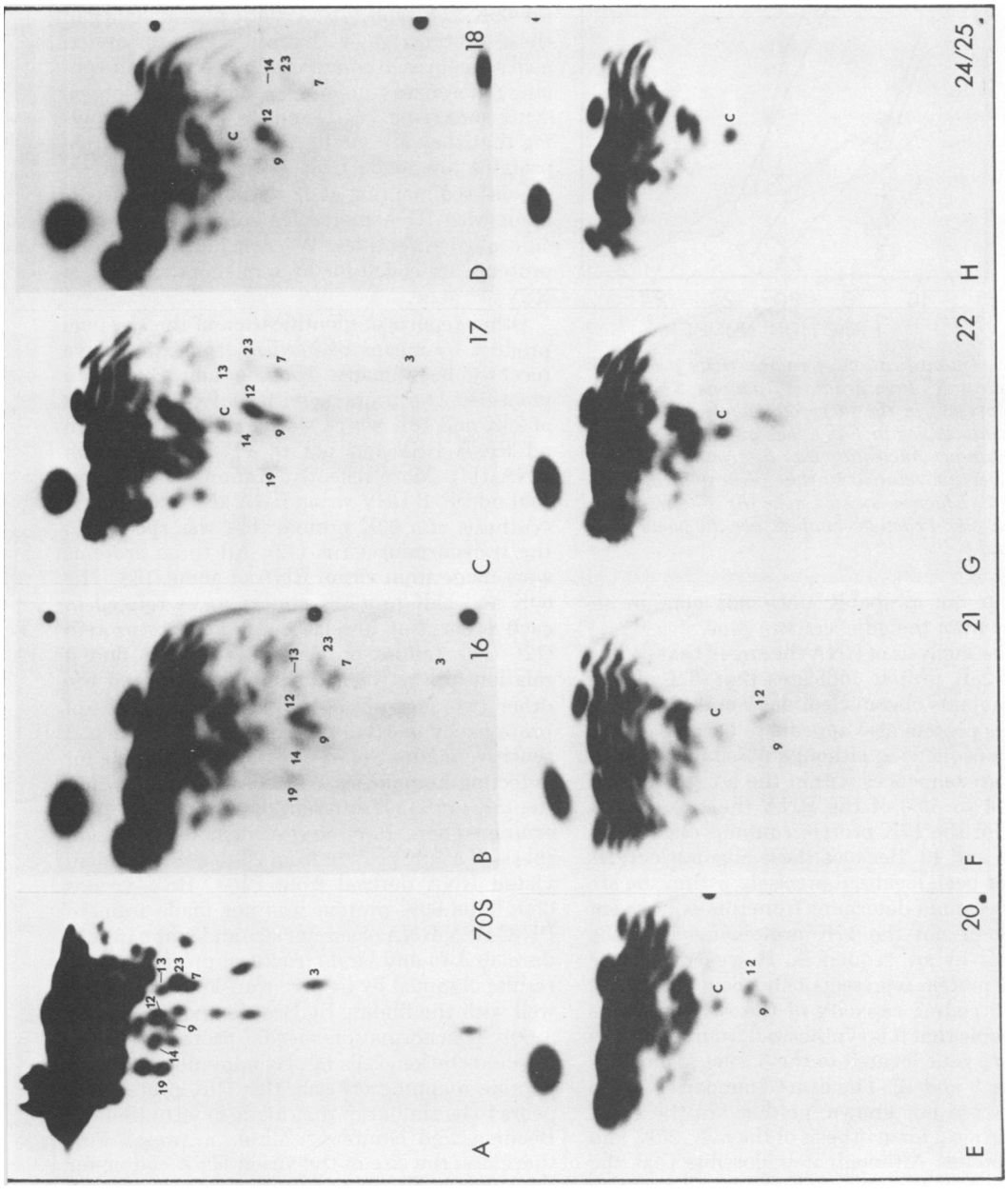

FIG. 6. *Analysis of oligonucleotides present in RNA cosedimenting with mRNA activity for the 60K, 25K, and 17K proteins.* ^{32}*P-labeled 70S PR-B virion RNA was denatured; poly(A)-containing species were selected by oligo(dT)-cellulose chromatography; and this RNA was sedimented in a sucrose gradient run in parallel with those of unlabeled PR-B and SR-D RNAs described in the legend to Fig. 4. Each gradient fraction was digested with RNase* T_1*, and the resulting RNase* T_1*-resistant oligonucleotides were resolved by two-dimensional fingerprinting and visualized by autoradiography. (A) contains the fingerprint of unfractionated 70S RNA, whereas the remaining fingerprints were made from individual gradient fractions, corresponding to those in Fig. 4. Oligonucleotides no. 9 and 12 are the src-specific oligonucleotides, whereas C is from the 3′ common region (28).*

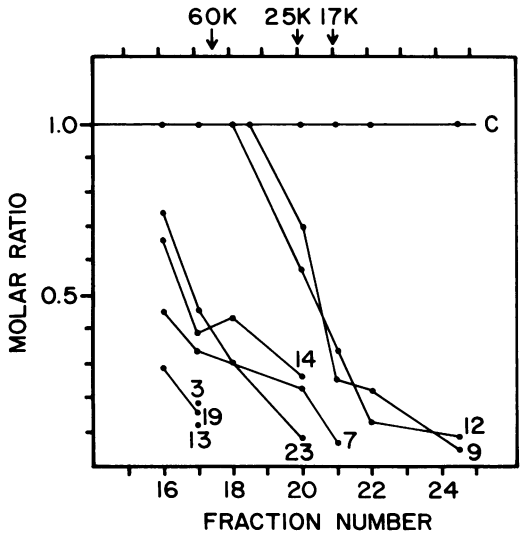

FIG. 7. *Quantification of radioactivity present in oligonucleotides from gradient fractions. The radioactivity present in the large oligonucleotides in the fingerprints shown in Fig. 6 was quantified. Counts per minute per nucleotide was determined for each oligonucleotide relative to that present in oligonucleotide C, which is located near the 3' terminus of the RNA (28). Fraction numbers are the same as in Fig. 4 and 6.*

src gene but probably does not contain sequences from the adjacent *env* gene.

Similar analysis of RNA the size of that coding for the 25K protein indicates that 70% of this RNA contains oligonucleotides 9 and 12. Therefore, this protein also appears to be synthesized from *src* sequences, although possibly it is initiated from sequences within the *src* gene. Only about 20 to 30% of the RNA the size of that coding for the 17K protein contains oligonucleotides 9 and 12. Because these oligonucleotides have not been localized precisely within the *src* gene, we cannot determine from this experiment whether or not the 17K protein may also be coded for by *src* sequences. However, because the 17K protein represents only about 25% of the assumed coding capacity of the *src* gene, it is conceivable that it is synthesized from sequences in the *src* gene located to the 3' side of oligonucleotides 9 and 12. The exact boundaries of the *src* gene are not known; neither are the exact initiation sites for synthesis of the 60K, 25K, and 17K proteins. Although it is possible that the translation of the 60K, 25K, and 17K proteins involves readthrough into the common region, it appears unlikely that synthesis of any of these proteins is initiated in the common region due to our finding that td RSV RNA, which contains the common region, does not code for the synthesis of the 60K, 25K, or 17K protein.

DISCUSSION

In vitro translation of virion RNA from transforming strains of RSV reveals several proteins not made from td RSV virion RNA. The apparent molecular weights of the most abundantly synthesized products unique to nd RSV RNA are 60K, 25K, and 17K. Tryptic peptide maps of these proteins show that they contain amino acid sequences in common. However, strain-specific differences were observed in these proteins synthesized from SR-D and PR-B RNAs, implying that they are virally coded products. These proteins are made from polyadenylated RNA species sedimenting at 18 to 23S, which cosediment with RNA molecules containing *src*-specific oligonucleotides. We conclude that these proteins are coded for by sequences within the RSV *src* gene.

Other reports of identification of the *src* gene product by means of in vitro translation have recently been made. Kamine and Buchanan identified two proteins with molecular weights of 25K and 18K which were made in vitro from nd PR-B RSV but not td PR-B RSV virion RNA (11). More recently, Kamine et al. found that nd PR-B RSV virion RNA also directed the synthesis of a 60K protein that was specific to the transforming virus (12). All three proteins were made from virion RNA of about 18S. The 60K and 25K proteins appear to be related to each other, but unrelated to the 18K protein (12). The failure of Kamine et al. to find a relationship between the 18K protein and the other two suggests either that the analysis of proteins by partial proteolysis is not such a sensitive method as tryptic peptide mapping for detecting homologies between proteins or that the 18K protein is different than the 17K protein examined here. Purchio et al. demonstrated synthesis of a 60K protein from 20 to 24S polyadenylated RNA derived from PR-C RSV virions (24). This 60K protein was not made from td PR-C RSV RNA of similar size and is apparently unrelated to any viral structural protein. These results obtained by in vitro translation correlate well with the finding by Brugge and Erikson of a 60K transformation-specific protein in RSV-infected chicken cells (4). By immunological and peptide mapping criteria, this 60K protein appears to be similar to that made in vitro (Sefton, Beemon, and Hunter, J. Virol., in press). Furthermore, the size of the virion RNA coding for the 60K protein (20 to 24S) is similar to the size of the *src*-specific RNA found in the cytoplasm of RSV-infected cells (about 21S) (8, 30). Although formal proof is lacking that the intracellular *src*-specific RNA is the mRNA for the *src* gene product, this seems likely to be the case. The intracellular *src*-specific RNA appears to

contain some sequences derived from the 5′ end of the genomic RNA (30) and thus may differ in structure from the virion RNA coding for the 60K protein (see below).

Relationship between the *src* gene products synthesized in vitro. We have shown that the 60K, 25K, and 17K proteins share amino acid sequences, such that the 17K protein is totally overlapped by the 25K protein, which is in turn totally overlapped by the 60K protein. The 60K protein appears to be the largest protein made in vitro containing these shared sequences. How is such a family of proteins generated and from precisely which part of the viral genome do they originate? The RNAs coding for the 60K, 25K, and 17K proteins are polyadenylated molecules of about 23S, 19 to 20S, and 18S in size, respectively. The differences in RNA size suggest that each protein has an unique mRNA activity and rules out the possibility that the 25K and 17K proteins are generated from the 60K protein by proteolytic processing. This conclusion is supported by the finding that all three proteins label with [^{35}S]formyl-methionine when synthesized in the presence of [^{35}S]f-met-tRNA$_f^{met}$ (data not shown). The simplest model to account for our data is shown in Fig. 8. We propose that the 60K, 25K, and 17K proteins have different N termini and share a common C-terminal region. Thus, the 25K and 17K proteins would be initiated at sites internal in the coding sequence for the 60K protein. Each protein would be coded for by a discrete RNA which would have a single active initiation site located at or near the 5′ end of the RNA. The prediction that the N-terminal sequences of the 60K, 25K, and 17K proteins are all different is being tested by examining the [^{35}S]formyl-methionine-labeled tryptic peptides of these proteins.

Because we do not know the exact positions of the initiation sites on these RNAs, we cannot determine exactly how far their coding regions extend toward the 3′ ends. In other eucaryotic mRNA's, however, the initiation site is not very far from the 5′ end. A further uncertainty in locating the coding region on these RNAs is the difficulty in determining their absolute size. It is possible that the coding sequences for the 60K, 25K, and 17K proteins extend into the 3′ "common" region shared by nd and td RSV RNAs (23, 28), bearing in mind that the limits of the *src* gene defined by the td deletion mutants do not necessarily define the actual *src* gene boundaries. It seems unlikely that the initiation site for any of these proteins is actually within the common region because we do not observe their synthesis from td RSV virion RNA. If the RNAs coding for the 60K, 25K, and 17K proteins are

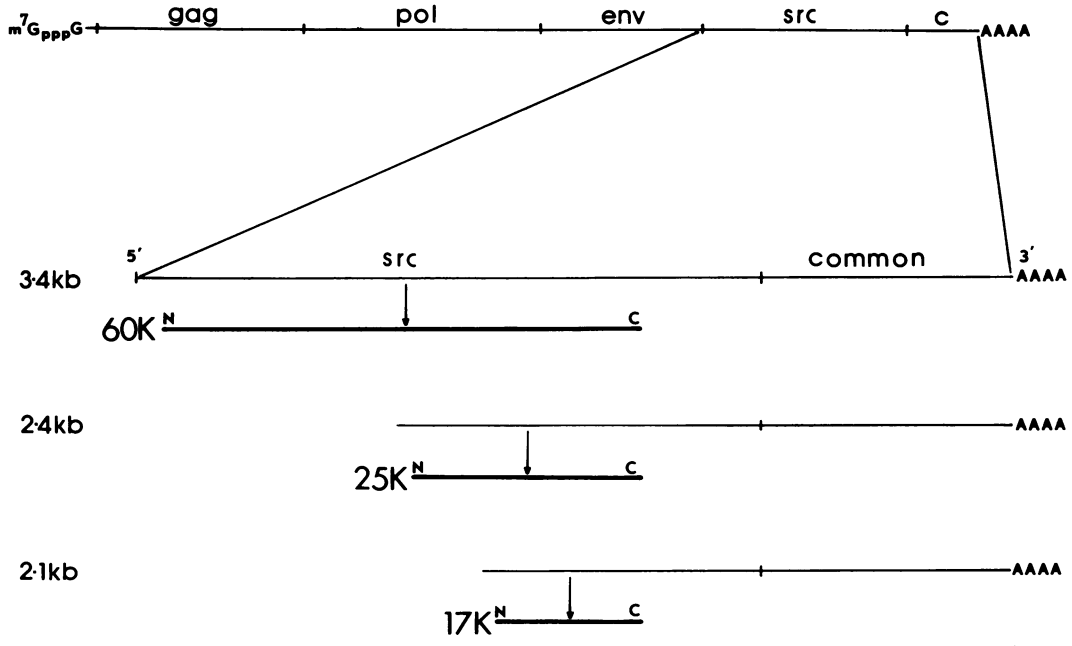

FIG. 8. *Proposed relationship between the major src gene products synthesized in vitro. The approximate sizes of the RNA species coding for the 60K, 25K, and 17K proteins are given on the left side of the figure in kilobases (kb). The 39K and 33K proteins are synthesized from RNAs intermediate in size to that coding for the 60K and that coding for the 25K proteins. The RSV genomic map shown at the top is from references 9 and 29.*

simply fragments, or at least essentially colinear representations of the 3′ end of the genomic RNA, then our sizing experiments indicate that the RNAs coding for the 60K and 25K proteins do indeed contain sequences from the *src* gene as defined by the td deletion mutants. By keeping in mind all the uncertainties and assuming that the initiation site of the 60K protein is near the end of the 23S RNA and that the 23S RNA is about 3,400 bases in length, then the 5′ end of the *src* gene corresponding to the N terminus of the 60K proteins lies about 3,300 bases from the 3′ end of the genome. The 3′ end of the *src* gene, corresponding to the C terminus of the 60K protein, would lie about 1,600 bases from the 3′ end of the genome. Making similar assumptions for the mRNA activities for the 25K and 17K proteins, both proteins would have C termini located at about 1,600 bases from the 3′ end of the genome. Thus, it is possible that all three *src*-related proteins share a common C terminus. If this genomic organization is correct, then there are about 1,600 bases beyond the end of the *src* gene with no assigned function. This region could potentially code for a fifth viral protein as large as 45K. A candidate for such a protein would be the 34K protein made from polyadenylated virion RNA of about 16S. Both the 39K and 33K proteins belong to the family of *src*-related proteins and have mRNA sizes intermediate between those of the 60K and 25K proteins. According to the model, the 39K and 33K proteins would share a common C terminus with the other *src*-related proteins. Because the 25K and 17K proteins were found to be relatively rich in methionine, compared with the 60K protein, the organization proposed in Fig. 8 indicates that most of the methionine residues in the 60K protein are in its C-terminal half.

An alternative hypothesis consistent with our data proposes that the presence of virion RNA molecules bearing partial deletions of varying lengths in the *src* gene may result in the synthesis of a family of *src*-related polypeptides from RNAs of different size. Such partial *src* deletion mutants have recently been described (13, 15). However, the nd virus used in our studies had been recently cloned, and there was no evidence for extensive deletions in the *src* gene apparent in the T_1 oligonucleotide experiment. Therefore, this hypothesis appears to be less likely than that proposed in Fig. 8.

The exact nature of the mRNA activities for the *src*-related proteins (60K, 39K, 33K, 25K, and 17K) and the *env*-related proteins (64K and 47K) originating from RSV virions remains unclear. Because we have ruled out the possibility that these proteins are coded for by cellular mRNA's, the most likely alternatives are either that these RNAs are fragments of the genomic RNA or that they are packaged virus-specific intracellular mRNA's. Generation of mRNA activity for the 60K, 25K, and 17K proteins does not appear to require growth of the virus in a transformed cell. Study of temperature-sensitive transformation mutants of RSV indicates that virions grown at either the permissive or the nonpermissive temperature contained RNA that was equally capable of coding for the 60K, 25K, and 17K proteins (Beemon and Hunter, in press). This suggests that RNAs coding for these proteins are not specifically generated by an enzyme from transformed cells. We have been unable to obtain unequivocal evidence that incubation of virus at 37°C yields more mRNA activity for the *src*-related proteins, even though the viral RNA becomes degraded by this treatment. It is clear, however, that all preparations of viral 70S RNA contain some RNA molecules shorter than full length. We have observed variability in the amounts of the various *src*-related proteins synthesized from different preparations of viral RNA, probably reflecting variations in the state of the RNA.

Our inability to find the 33K and 25K proteins in RSV-transformed cells (see below) implies that the mRNA's for these proteins do not exist in the cell. The 39K protein was detected in cells infected with one stock of RSV but not in other RSV-transformed cells. Therefore, it seems probable that there are multiple potential internal initiation sites within the *src* gene that are efficiently used in the mRNA-dependent reticulocyte lysate when genomic RNA is suitably fragmented. The ability of td SR-D RNA to code for the 64K and 47K proteins, which are probably *env*-related proteins, suggests that we should have observed the synthesis of *src*-related proteins if td RSV RNA contained the coding sequences for these proteins. Even if fragments of genomic RNA are active as mRNA's, this does not rule out the possibility that there are also packaged viral intracellular mRNA's which may be translated in vitro.

Strain specificity of the *src* proteins. Strain-specific differences in the 60K, 25K, and 17K proteins synthesized in vitro have been observed in electrophoretic mobility, tryptic peptide maps and in antigenicity (see below). Our two-dimensional tryptic peptide maps show that approximately half of the methionine-containing peptides in the PR-B and SR-D 60K proteins are common to both and about half are unique to each. It is quite unlikely that the large number of peptide differences observed between PR-B and SR-D are simply due to differential

modifications. Immunoprecipitation of in vitro products with antiserum from rabbits bearing SR-D RSV-induced tumors prepared by the method of Brugge and Erikson (4) brought down the 60K, 39K, 33K, and 25K nonvirion products of SR-D RNA. It did not, however, precipitate such products from PR-B RSV RNA. Similar strain-specific antigenicity has been observed for the 60K protein found in RSV-transformed cells (Sefton et al., J. Virol., in press).

These results are unexpected in light of the high degree of nucleic acid homology in the *src* gene observed by hybridization of different strains of RSV RNA to a specific $cDNA_{src}$ probe (25). Because the mobility of a tryptic peptide can be altered by a single amino acid change and because we are not examining the whole spectrum of tryptic peptides, it is difficult to estimate the extent of nonhomology between the *src* proteins that we have compared. It does appear, however, that the regions of nonhomology may be distributed throughout the *src* gene rather than being clustered. Perhaps the strain-specific differences in the *src* gene product are reflected in the strain-specific biological differences observed in transformed cells, including focus morphology (26) and water accumulation (1).

***src* protein in transformed cells.** Brugge and Erikson observed a tumor-specific antigen in transformed cells with an apparent molecular weight of 60K (4). These experiments have been confirmed, and tryptic peptide maps have shown that the 60K protein made in vitro is essentially identical to the 60K protein immunoprecipitated from transformed cells (Sefton et al., J. Virol., in press). We have not been able to detect tumor-specific antigens smaller than the 60K protein in SR-D RSV-transformed cells, although a 39K protein has been observed by immunoprecipitation from cells transformed by SR-A RSV. Because the antiserum from tumor-bearing animals is capable of recognizing the 60K, 39K, 33K, and 25K proteins synthesized in vitro from SR-D RNA, this suggests that if the 39K, 33K, and 25K proteins are present at all in SR-D-transformed chicken cells, they must be there in much lower amounts than the 60K protein. This could be due either to less efficient synthesis or to rapid turnover. The 17K protein is not recognized by this antiserum, and, therefore, we cannot determine whether the 17K protein is present in vivo. If multiple *src* gene products are detected in vivo, this could help explain the pleiotropic effects observed upon transformation (26). Regardless of whether or not the smaller *src*-related proteins are synthesized in transformed cells, they are likely to be very useful tools for structural and possible functional characterization of the *src* protein.

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LITERATURE CITED

1. **Bader, J. P., D. A. Ray, and N. R. Brown.** 1974. Accumulation of water during transformation of cells by an avian sarcoma virus. Cell **3**:307-313.
2. **Beemon, K., P. H. Duesberg, and P. K. Vogt.** 1974. Evidence for crossing-over between avian tumor viruses based on analysis of viral RNAs. Proc. Natl. Acad. Sci. U.S.A. **71**:4254-4258.
3. **Beemon, K., and T. Hunter.** 1977. *In vitro* translation yields a possible Rous sarcoma virus *src* gene product. Proc. Natl. Acad. Sci. U.S.A. **74**:3302-3306.
4. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346-348.
5. **Coffin, J. M., and M. A. Billeter.** 1976. A physical map of the Rous sarcoma virus genome. J. Mol. Biol. **100**:293-318.
6. **Duesberg, P. H., and P. K. Vogt.** 1973. RNA species obtained from clonal lines of avian sarcoma and avian leukosis virus. Virology **54**:207-219.
7. **Gibson, W.** 1974. Polyoma virus proteins: a description of the structural proteins of the virion based on polyacrylamide gel electrophoresis and peptide analysis. Virology **62**:319-336.
8. **Hayward, W. S.** 1977. Size and genetic content of viral RNAs in avian oncovirus-infected cells. J. Virol. **24**:47-63.
9. **Joho, R. H., E. Stoll, R. R. Friis, M. A. Billeter, and C. Weissmann.** 1976. A partial genetic map of Rous sarcoma virus RNA: location of polymerase, envelope and transformation markers, p. 127-145. *In* D. Baltimore, A. S. Huang, and C. F. Fox (ed.), Animal virology. Academic Press Inc., New York.
10. **Junghans, R. P., S. Hu, C. A. Knight, and N. Davidson.** 1977. Heteroduplex analysis of avian RNA tumor viruses. Proc. Natl. Acad. Sci. U.S.A. **74**:477-481.
11. **Kamine, J., and J. M. Buchanan.** 1977. Cell-free synthesis of two proteins unique to RNA of transforming virions of Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **74**:2011-2015.
12. **Kamine, J., J. G. Burr, and J. M. Buchanan.** 1978. Multiple forms of *sarc* gene proteins from Rous sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **75**:366-370.
13. **Kawai, S., P. H. Duesberg, and H. Hanafusa.** 1977. Transformation-defective mutants of Rous sarcoma virus with *src* gene deletions of varying length. J. Virol. **24**:910-914.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
15. **Lai, M. M. C., S. S. F. Hu, and P. K. Vogt.** 1977. Occurrence of partial deletion and substitution of the *src* gene in the RNA genome of avian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **74**:4781-4785.
16. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335-341.
17. **Laskey, R. A., and A. D. Mills.** 1977. Enhanced autoradiographic detection of ^{32}P and ^{125}I using intensifying screens and hypersensitive film. FEBS Lett. **82**:314-316.
18. **Lodish, H. F., and J. K. Rose.** 1977. Relative importance of 7-methyl-guanosine in ribosome binding and translation of vesicular stomatitis virus mRNA in wheat germ and reticulocyte cell-free systems. J. Biol. Chem.

252:1181-1188.

19. **Oppermann, H., J. M. Bishop, H. E. Varmus, and L. Levintow.** 1977. A joint product of the genes *gag* and *pol* of avian sarcoma virus: a possible precursor of reverse transcriptase. Cell **12**:993-1005.
20. **Paterson, B. M., D. J. Marciani, and T. S. Papas.** 1977. Cell-free synthesis of the precursor polypeptide for avian myeloblastosis virus DNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **74**:4951-4954.
21. **Pawson, T., R. Harvey, and A. E. Smith.** 1977. The size of Rous sarcoma virus mRNAs active in cell-free translation. Nature (London) **286**:416-420.
22. **Pelham, H. R. B., and R. J. Jackson.** 1976. An efficient mRNA-dependent translation system from reticulocyte lysates. Eur. J. Biochem. **67**:247-256.
23. **Pérez-Bercoff, R., and M. A. Billeter.** 1976. Characterization of the 3'-terminal region of the large molecular weight RNA subunits from normal and transformation-defective Rous sarcoma virus. Biochim. Biophys. Acta **454**:383-388.
24. **Purchio, A. F., E. Erikson, and R. L. Erikson.** 1977. Translation of 35S and of subgenomic regions of avian sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:4661-4665.
25. **Stehelin D., R. V. Guntaka, H. E. Varmus, and J. M. Bishop.** 1976. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. J. Mol. Biol. **101**:349-365.
26. **Vogt, P. K.** 1977. The genetics of RNA tumor viruses, p. 341-455. *In* H. Fraenkel-Conrat and R. Wagner (ed.), Comprehensive virology, vol. 9. Academic Press Inc., New York.
27. **Vogt, V. M., R. Eisenman, and H. Diggelmann.** 1975. Generation of avian myeloblastosis virus structural proteins by proteolytic cleavage of a precursor polypeptide. J. Mol. Biol. **96**:471-493.
28. **Wang, L.-H., P. H. Duesberg, K. Beemon, and P. K. Vogt.** 1975. Mapping RNase T_1-resistant oligonucleotides of avian tumor virus RNAs: sarcoma-specific oligonucleotides are near the poly(A) end and oligonucleotides common to sarcoma and transformation-defective viruses are at the poly(A) end. J. Virol. **16**:1051-1070.
29. **Wang, L.-H., P. H. Duesberg, P. Mellon, and P. K. Vogt.** 1976. Distribution of envelope-specific and sarcoma-specific nucleotide sequences from different parents in the RNAs of avian tumor virus recombinants. Proc. Natl. Acad. Sci. U.S.A **73**:1073-1077.
30. **Weiss, S. R., H. E. Varmus, and J. M. Bishop.** 1977. The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukosis viruses. Cell **12**:983-992.