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Sites of Synthesis of Viral Proteins in Avian Sarcoma Virus-Infected Chicken Cells

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We determined the sites of synthesis of avian sarcoma virus-specific proteins in infected chicken cells by immunoprecipitation of the products synthesized in vitro by free and membrane-bound polyribosomes; 85% of Pr76, the precursor of the viral internal structural proteins (group-specific antigens), was synthesized on free polyribosomes, and 15% was synthesized on membrane-bound polyribosomes. Pr92, the glycosylated precursor of the viral glycoproteins (gp85 and gp35), was synthesized exclusively on membrane-bound polyribosomes, which is consistent with its role as a membrane protein. When we investigated the site of synthesis of pp60^{src}, the product of the avian sarcoma virus *src* gene, we found that 90% was synthesized on free polyribosomes, whereas 10% was detected on membrane-bound polyribosomes. The implications of these results with respect to the subcellular location of pp60^{src} are discussed.

The genome of avian sarcoma virus (ASV) consists of a single-stranded 38S RNA molecule having a molecular weight of about 3.4×10^6. Shortly after infection of susceptible chicken cells by this virus, a double-stranded DNA copy of the RNA genome is synthesized by the virion-associated reverse transcriptase and becomes integrated into the host cell genome. This integrated proviral DNA then serves as a template for virus-specific messages, which in turn are translated into the viral proteins (1, 33).

The following four genes are generally recognized as being on the viral genome: a *gag* gene for the viral internal structural proteins, a *pol* gene for the reverse transcriptase, an *env* gene for the viral glycoprotein, and an *src* gene, which is required for the transformation of cells in vitro and tumor production in vivo. The gene order has been established as 5'-*gag-pol-env-src*-poly(A)-3' (36).

The group-specific antigens are synthesized as a high-molecular-weight precursor polypeptide Pr76 (molecular weight, 76,000), which is cleaved to form the mature internal structural proteins (34). The 38S RNA species can serve as a message for Pr76 (26, 29, 35). The polymerase protein is apparently synthesized as a 180,000-dalton readthrough product of the *gag* and *pol* genes (Pr180). The message for Pr180 is also present in the virions as a 38S RNA species (24, 25, 29).

In infected cells, one can identify a 92,000-dalton glycosylated polypeptide (Pr92), which is subsequently modified to form Pr90 (5, 10, 16); after insertion into the plasma membrane, it is acquired by the virus during the course of budding from the cell. This precursor is finally cleaved extracellularly into the mature forms of the viral glycoproteins gp85 and gp35, which are linked by disulfide bonds (16, 22). Recently, an unglycosylated precursor of Pr92 has been identified in infected cells as a 62,000-dalton polypeptide (8, 32). The active message for the *env* gene may be a 28S polyadenylylated RNA species which contains both *env* and *src* gene sequences (13, 37).

The product of the ASV *src* gene has been identified as a 60,000-dalton polypeptide (pp60^{src}), which can be translated from a 21S polyadenylylated subgenomic fragment of viral RNA, as well as from 21S polyadenylic acid-containing RNA from ASV-infected chicken cells (3, 28, 29; Purchio and Erikson, unpublished data).

Initial studies in our laboratory on the intracellular location of pp60^{src} indicated that this protein is present primarily in the cytoplasm of ASV-infected cells (4). These observations were based on radio-immunoprecipitation of pp60^{src} from detergent-fractionated cells, as well as on immunofluorescent staining of fixed cells. Rohrschneider also reported a cytoplasmic location of pp60^{src} in ASV-infected chicken and rat cells, based on immunofluorescent techniques (31). Lee et al. examined the distribution of ASV-specific mRNA's in infected chicken cells and found that essentially all of the *src* message was associated with free polyribosomes, suggesting that pp60^{src} is not a membrane protein (19).

However, an association of pp60^{src} with membranes has been reported. Using electron microscopic immunocytochemistry, Willingham et al. reported that pp60^{src} was concentrated on the inner portion of the plasma membrane (38). Experiments by Kamine and Buchanan indicated that a small 2,000-dalton segment could be cleaved from pp60^{src} which was synthesized in vitro by dog microsomes, suggesting that nascent pp60^{src} was thus associated with membrane-bound polyribosomes (15). More recently, Krueger et al. fractionated cell lysates by differential centrifugation and found that pp60^{src} cosedimented with cellular membranes (17).

As an alternative approach for determining the intracellular location of pp60^{src}, we investigated the site of synthesis of virus-specific polypeptides in infected chicken cells by immunoprecipitation of the products synthesized in vitro by free and membrane-bound polyribosomes, using antibodies against various viral proteins. Our results indicate that Pr76, Pr180, and pp60^{src} are synthesized mostly on free polyribosomes, whereas Pr92 is synthesized exclusively on membrane-bound polyribosomes.

MATERIALS AND METHODS

Cells and virus. Chicken embryo fibroblasts were prepared from 11-day-old embryos (Spafas, Inc., Roanoke, Ill.). The viruses used in these studies included the Schmidt-Ruppin strain of ASV, subgroup D (SRD) (originally obtained from J. Wyke), and Rous-associated virus 2 (RAV-2).

Production of antisera. Tumor-bearing rabbit (TBR) serum was obtained from New Zealand rabbits in which tumors had been produced by injection of purified Schmidt-Ruppin ASV, as previously described (3). Monospecific antibody against the virion internal structural protein p27 was prepared by injecting rabbits with p27 purified by sodium dodecyl sulfate gel electrophoresis. Antibody against gp85 was the generous gift of D. Bolognesi.

Polyribosome preparation. Cells were lysed in 10 mM Tris (pH 7.2)–1.5 mM $MgCl_2$–15 mM KCl by Dounce homogenization, and sucrose was then added to 30%. Nuclei were removed by centrifugation at 1,000 × *g* for 5 min, and the supernatants were fractionated on discontinuous sucrose gradients, as described by Morrison and Lodish (23). The pellet was taken as the free polyribosomes; the material between the 65 and 45% sucrose layers was taken as the membrane-bound polyribosomes and was further concentrated by centrifugation at 100,000 × *g* for 1.5 h in a Beckman SW41 rotor. The free and membrane-bound polyribosomes were suspended in 0.01 M Tris–1.5 mM $MgCl_2$ and used immediately for in vitro synthesis.

RNA preparation. Subgenomic polyadenylylated RNA was prepared from virions as described previously (29). RNA was extracted from membrane-bound polyribosomes three times with phenol–chloroform–isoamyl alcohol and chromatographed twice on oligodeoxythymidylic acid–cellulose, as described previously (29).

Cell-free synthesis. mRNA-dependent reticulocyte lysates were prepared as described previously (27). The reaction was carried out in a volume of 50 μl, and the reaction mixture contained 100 mM KCl, 20 mM Tris-hydrochloride (pH 7.4), 2 mM magnesium acetate, 6 mM 2-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 2 mg of creatinine phosphate per ml, 0.2 mg of creatinine phosphokinase per ml, unlabeled amino acids (200 μM) minus methionine, 25 μCi of [^{35}S]methionine (1,000 Ci/mmol; Amersham), 20 μM hemin, 2 μg of wheat germ tRNA, and either 0.3 μg of mRNA, 10 μl of free polyribosomes (absorbance at 260 nm, 54), or 10 μl of membrane-bound polyribosomes (absorbance at 260 nm, 63). The cell-free products were immunoprecipitated as described elsewhere (28), analyzed on sodium dodecyl sulfate-polyacrylamide gels (18), and fluorographed as described previously (6).

RESULTS

Immunoprecipitation of products synthesized by free and membrane-bound polyribosomes from RAV-2-infected chicken cells. To investigate the sites of synthesis of viral proteins in infected cells, polyribosomes were added directly to a messenger-dependent reticulocyte cell-free system. As a control, we first looked at the distribution of synthesis of viral proteins on free and membrane-bound polyribosomes from RAV-2-infected chicken cells. RAV-2 is a nontransforming leukosis virus whose genome consists of the *gag*, *pol*, and *env* genes. Figure 1A shows a polyacrylamide gel analysis of the polypeptides programmed by free and membrane-bound polyribosomes in our cell-free system; the molecular weights of the in vitro products ranged from 20,000 to 200,000. Immunoprecipitation of these products with monospecific antibody against p27 and subsequent polyacrylamide gel analysis indicated that the predominant immune-specific protein was Pr76, the product of the *gag* gene and the precursor of the viral internal structural proteins (Fig. 1B). We found that approximately 87% of Pr76 was synthesized on free polyribosomes and 13% was synthesized on membrane-bound polyribosomes. A small amount of Pr180, the joint product of the *gag* and *pol* genes, was also present in Fig. 1B, track 2.

A polyacrylamide gel analysis of the products synthesized in vitro and immunoprecipitated by antibody against the viral glycoprotein (anti-gp85 serum) is shown in Fig. 1C and D. A small amount of Pr76 was precipitated from the polypeptides synthesized by free polyribosomes due to some cross-reactivity of the serum with *gag* determinants (Fig. 1C, track 2). When we precipitated the products synthesized by the membrane-bound polyribosomes, we observed a diffuse immune-specific band with a molecular weight of 92,000 (Fig. 1D, track 3) which co-

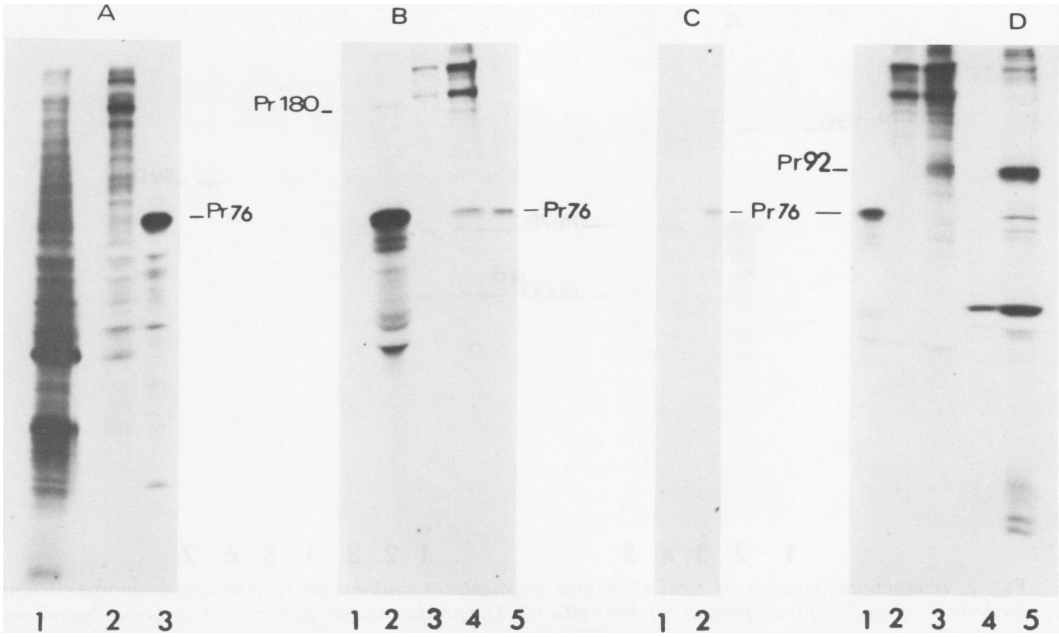

FIG. 1. *Immunoprecipitation of viral structural polypeptides synthesized by free and membrane-bound polyribosomes from RAV-2-infected chicken cells. (A) Free and membrane-bound polyribosomes were isolated from RAV-2-infected chicken cells. The products synthesized in vitro by these polyribosomes in message-dependent reticulocyte lysates were subjected to polyacrylamide gel electrophoresis. Track 1, Lysate plus free polyribosomes; track 2, lysate plus membrane-bound polyribosomes; track 3, Pr76 marker obtained by translation of RAV-2 virion 35S RNA. (B) The products made in vitro, as shown in (A), by membrane-bound and free polyribosomes were immunoprecipitated with monospecific antiserum against p27 and analyzed by polyacrylamide gel electrophoresis. Track 1, Free polyribosomes plus normal rabbit serum; track 2, free polyribosomes plus anti-p27 serum; track 3, membrane-bound polyribosomes plus normal rabbit serum; track 4, membrane-bound polyribosomes plus anti-p27 serum; track 5, RAV-2 Pr76 marker. (C and D) Analysis of products synthesized in vitro by membrane-bound and free polyribosomes, as shown in (A), and immunoprecipitated with anti-gp85 serum. (C) Track 1, Free polyribosomes plus normal rabbit serum; track 2, free polyribosomes plus anti-gp85 serum. (D) Track 1, Pr76 marker; track 2, membrane-bound polyribosomes plus normal rabbit serum; track 3, membrane-bound polyribosomes plus anti-gp85 serum; track 4, lysate from [^{35}S]methionine-labeled RAV-2-infected chicken cells plus normal rabbit serum; track 5, lysate from [^{35}S]-methionine-labeled RAV-2-infected chicken cells plus anti-gp85 serum.*

electrophoresed with a 92,000-dalton polypeptide immunoprecipitated directly from RAV-2-infected chicken cells (Fig. 1D, track 5). This 92,000-dalton band has been identified as the glycosylated precursor of gp85 and gp35, the two viral glycoproteins (5, 10, 16). We found that essentially all of the detectable Pr92 was synthesized by membrane-bound polyribosomes; no Pr92 was synthesized by free polyribosomes.

Immunoprecipitation of polypeptides synthesized by free and membrane-bound polyribosomes from ASV-transformed chicken cells with anti-p27 and anti-gp85 sera. A direct polyacrylamide gel analysis of the polypeptides synthesized by free and membrane-bound polyribosomes showed a complex pattern which was similar to that shown in Fig. 1A. Immunoprecipitation of these products with anti-p27 antibody indicated that the majority of Pr76 was synthesized on free polyribosomes, whereas a small amount was made on membrane-bound polyribosomes (Fig. 2A).

When anti-gp85 serum was used to immunoprecipitate the cell-free products, we again found that some Pr76 was precipitated from the products synthesized by the free polyribosomes; however, the major immune-specific polypeptide precipitated by anti-gp85 serum migrated as a diffuse band slightly slower than the Pr92 precipitated from SRD-infected chicken cells (Fig. 2B, tracks 5 and 7). This band was found only among the products synthesized by membrane-bound polyribosomes. The reason for the slight difference in electrophoretic mobilities between the in vivo and in vitro polypeptides may be due to differences in glycosylation which occur in vivo and in vitro. No Pr92 was synthesized by free polyribosomes; these results agree exactly with our observations on polyribosomes from RAV-2-infected chicken cells.

Immunoprecipitation of viral polypeptides from products programmed in vitro

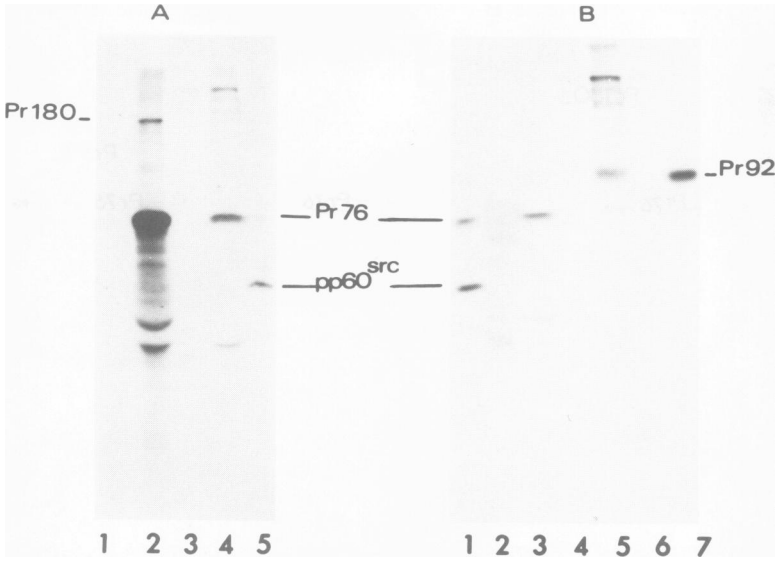

FIG. 2. *Immunoprecipitation of viral structural polypeptides synthesized by free and membrane-bound polyribosomes from SRD-transformed chicken cells. (A) The products made in vitro by membrane-bound and free polyribosomes which were isolated from SRD-transformed chicken cells were immunoprecipitated with anti-p27 serum and analyzed by polyacrylamide gel electrophoresis. Track 1, Free polyribosomes plus normal rabbit serum; track 2, free polyribosomes plus anti-p27 serum; track 3, membrane-bound polyribosomes plus normal rabbit serum; track 4, membrane-bound polyribosomes plus anti-p27 serum; track 5, pp60^{src} marker obtained by translation of virion 21S polyadenylated subgenomic RNA fragments (29). (B) Analysis of the products made in vitro by free and membrane-bound polyribosomes and immunoprecipitated with anti-gp85 serum. Track 1, pp60^{src} and Pr76 marker; track 2, free polyribosomes plus normal rabbit serum; track 3, free polyribosomes plus anti-gp85 serum; track 4, membrane-bound polyribosomes plus normal rabbit serum; track 5, membrane-bound polyribosomes plus anti-gp85 serum; track 6, lysate from [^{35}S]methionine-labeled SRD-infected chicken cells plus normal rabbit serum; track 7, extract from [^{35}S]methionine-labeled SRD-infected chicken cells plus anti-gp85 serum.*

by polyadenylic acid-containing RNA isolated from membrane-bound polyribosomes. The synthesis of Pr92 in vitro presumably results from the completion of polypeptides already initiated in vivo and from glycosylation by enzymes associated with the membrane-bound polyribosomal fraction. If this were the case, then translation of the mRNA's in this fraction should result in the synthesis of an unglycosylated precursor of Pr92, which could also be recognized by the anti-gp85 serum. Therefore, we isolated membrane-bound polyribosomes, extracted the polyadenylic acid-containing RNA, translated it in a reticulocyte cell-free system, and immunoprecipitated the products with anti-gp85 serum. Polyacrylamide gel analysis of the immunoprecipitates is shown in Fig. 3. The predominant immune-specific band was Pr76; no Pr92 was observed. However, a new band was detected, which had a molecular weight of 64,000 (Fig. 3, track 3) and was missing from the products synthesized in vitro by membrane-bound polyribosomes (Fig. 2B, track 5).

The markers used in Fig. 3, track 1, came from an in vitro translation programmed by a mixture of 39S, 28S, and 21 S polyadenylated subgenomic virion RNAs. The 39S species programs the synthesis of Pr76, whereas the 21S fragment serves as the message for pp60^{src} (25, 29, 35). The 64,000-dalton band in Fig. 3, track 1, was programmed by 28S polyadenylated subgenomic ASV RNA and co-electrophoresed with the 64,000-dalton polypeptide precipitated by anti-gp serum from the cell-free products programmed by polyadenylated RNA isolated from membrane-bound polyribosomes. We now have evidence that the 64,000-dalton polypeptide translated from 28S subgenomic viral RNA is indeed the unglycosylated precursor of Pr92 (P. Enrietto, A. F. Purchio, and R. L. Erickson, manuscript in preparation).

In the immunoprecipitation shown in Fig. 3, Pr76 was more heavily labeled than Pr64, whereas in Fig. 2B, track 5, Pr76 was barely detectable. This is because the antiserum used was raised against gp85 and is much less reactive toward unglycosylated Pr64. In addition, as judged by reverse transcriptase assays, viral cores and particles in the process of budding from cells concentrate on the 65% sucrose pad,

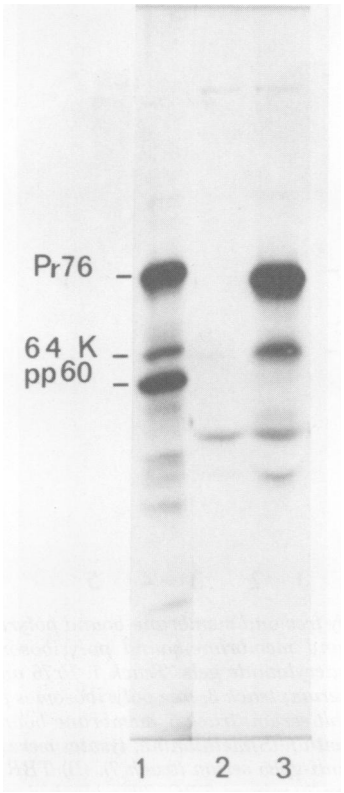

FIG. 3. *Immunoprecipitation of products programmed in vitro by polyadenylylated RNA from membrane-bound polyribosomes, using anti-gp85 serum. Polyadenylic acid-containing RNA was purified from membrane-bound polyribosomes and translated in a message-dependent reticulocyte cell-free system. The products of translation were immunoprecipitated with normal rabbit serum (track 2) or anti-gp85 serum (track 3) and analyzed on 10% polyacrylamide gels. Track 1 shows Pr76, pp60^{src}, and 64,000-dalton marker proteins obtained from the in vitro translation of 39S, 28S, and 21S polyadenylylated subgenomic RNAs, which were isolated from purified SRD virions.*

along with the membrane-bound polyribosomes (unpublished data); these particles would not have contributed to the products synthesized in vitro when polyribosomes were used in cell-free synthesis, but would have released their genomic RNA during sodium dodecyl sulfate–phenol extractions, thereby increasing the amount of Pr76 synthesized in the reticulocyte lysate when the RNA was used to program cell-free synthesis.

Immunoprecipitation of cell-free products by using TBR serum. Figure 4A shows an immunoprecipitation of the products synthesized by free and membrane-bound polyribosomes isolated from SRD-infected chicken cells when TBR serum was used. TBR serum contains antibody against the virion group-specific antigens, polymerase, and glycoprotein, as well as against pp60^{src} (2). TBR serum precipitates Pr76 from the products synthesized by both free and membrane-bound polyribosomes in the same ratio as does anti-p27 serum; Pr180 can also be seen in Fig. 4A, track 3. The membrane-bound polyribosomes also synthesized a diffuse high-molecular-weight band which was specifically precipitated by TBR serum and migrated just behind Pr92 which was obtained from SRD-infected chicken cells (Fig. 4A, track 5). This band was absent from the proteins precipitated from the in vitro products synthesized by free polyribosomes and presumably represented the glycosylated precursor of the viral glycoproteins. These results are the same as the results obtained with anti-P27 and anti-gp85 sera (Fig. 2).

In addition to Pr76, Pr92, and Pr180, TBR serum also precipitated pp60^{src} from the proteins synthesized by free polyribosomes and a small amount of pp60^{src} from the products synthesized by membrane-bound polyribosomes (Fig. 4A, tracks 3 and 5). To more clearly demonstrate the immunoprecipitation of pp60^{src}, we incubated the TBR serum with disrupted virus before the addition of the labeled in vitro products. This procedure blocked the precipitation of essentially all of the viral structural proteins but did not decrease the intensity of the pp60^{src} band (Fig. 4B).

Quantitation of Pr76, Pr92, and pp60^{src} synthesized on free and membrane-bound polyribosomes. To quantitate the amounts of the viral proteins synthesized on free and membrane-bound polyribosomes, we immunoprecipitated equal amounts of trichloroacetic acid-precipitable material from each in vitro synthesis with TBR serum and fractionated the precipitates on polyacrylamide gels. The appropriate bands were located by autoradiography, excised from the gel, and counted. The supernatants were re-precipitated to assure that there was an excess of antibody. Table 1 shows the averages of two independent determinations; 85% of Pr76 was synthesized on free polyribosomes, whereas 15% was made on membrane-bound polyribosomes. Pr92 was synthesized exclusively on membrane-bound polyribosomes; 90% of p60^{src} was made on free polyribosomes, whereas 10% was detected among the in vitro products synthesized by membrane-bound polyribosomes.

DISCUSSION

In this work we investigated the sites of synthesis of virus-specific proteins in ASV-infected chicken cells with regard to their locations on free and membrane-bound polyribosomes. The

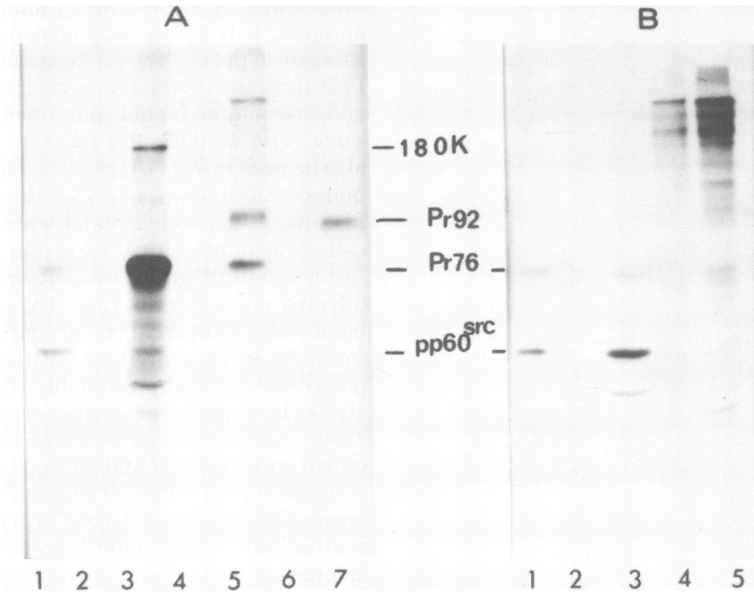

FIG. 4. *Immunoprecipitation of products synthesized in vitro by free and membrane-bound polyribosomes, using TBR serum. (A) The products made in vitro by free and membrane-bound polyribosomes were immunoprecipitated with TBR serum and analyzed on 10% polyacrylamide gels. Track 1, Pr76 and pp60^{src} marker proteins; track 2, free polyribosomes plus normal rabbit serum; track 3, free polyribosomes plus TBR serum; track 4, membrane-bound polyribosomes plus normal rabbit serum; track 5, membrane-bound polyribosomes + TBR serum. SRD-infected chicken cells were labeled with [^{35}S]methionine; lysates were prepared and immunoprecipitated with normal rabbit serum (track 6) or anti-gp85 serum (track 7). (B) TBR serum (3 μl) was incubated with 125 μg of Nonidet P-40-disrupted virus for 30 min at 37°C. This blocked serum was then used to immunoprecipitate the products made in vitro by free and membrane-bound polyribosomes which were isolated from SRD-infected chicken cells. Track 1, pp60^{src} marker; track 2, free polyribosomes plus normal rabbit serum; track 3, free polyribosomes plus blocked TBR serum; track 4, membrane-bound polyribosomes plus normal rabbit serum; track 5, membrane-bound polyribosomes plus blocked TBR serum.*

TABLE 1. *Quantitation of viral proteins synthesized on free and membrane-bound polyribosomes*[a]

Protein	Radioactivity (cpm)		% of total	
	Free	Membrane bound	Free	Membrane bound
Pr76	155,400	27,500	86	14
Pr92	Undetectable	2,500	0	100
pp60^{src}	3,960	450	90	10

[a] The products synthesized *in vitro* by free and membrane-bound polyribosomes were precipitated by using anti-p27, anti-gp85, and TBR sera and fractionated on 10% polyacrylamide gels. The appropriate bands were located by autoradiography, excised from the gel, and counted directly with a Packard liquid scintillation counter. The number of counts precipitated by normal rabbit serum was subtracted in each case as background. These results represent averages of two independent determinations; all supernatants were reprecipitated with the appropriate antiserum to assure that there was an excess of antibody.

majority of Pr76 (85%), the precursor of the viral internal structural proteins (34), was synthesized on free polyribosomes; approximately 15% of Pr76 was synthesized on membrane-bound polyribosomes. Although one normally thinks of Pr76 as a cytoplasmic protein, there have been reports that some Pr76 in cells is membrane associated (9). There may be two populations of Pr76 synthesized in infected cells, some of which is destined for transport to the membrane. Alternatively, some of these polyribosomes may attach to membranes during rupture of the cells. Pr180, the joint product of the *gag* and *pol* genes (24, 25, 29), was observed only in the products synthesized by free polyribosomes.

The major polypeptide precipitated by anti-gp85 serum migrated as a diffuse band with a molecular weight of about 92,000 (Fig. 1D and 2B) and was observed exclusively among the products synthesized in vitro by membrane-bound polyribosomes. In the case of RAV-2-infected chicken cells, the Pr92 synthesized in vitro comigrated with Pr92 immunoprecipitated from cells labeled with [^{35}S]methionine in vivo. In the case of ASV-transformed chicken cells, the Pr92 synthesized in vitro migrated slightly slower than the Pr92 labeled in vivo; this was most likely due to different glycosylation events

occurring in vitro and in vivo.

We also purified polyadenylated RNA from the membrane-bound polyribosomes, translated it in a reticulocyte cell-free system, and examined the products immunoprecipitable with anti-gp85 serum. No Pr92 was observed; instead, a new 64,000-dalton band appeared (Fig. 3), which was absent from the immunoprecipitates of the products synthesized in vitro by membrane-bound polyribosomes. Presumably, the glycosylating enzymes were removed during the process of phenol extraction, and the 64,000-dalton polypeptide represented the unglycosylated precursor of Pr92. Such a polypeptide has been identified in vivo (8, 32), and we now have evidence from the translation of virion 28S polyadenylated subgenomic RNA that the 64,000-dalton protein is related to Pr92 (Enrietto et al., manuscript in preparation).

There is evidence that membrane-bound polyribosomes are the site of synthesis of proteins which are secreted from cells or are inserted into the plasma membrane (12, 14, 30). Our observation that Pr92 is synthesized only on membrane-bound polyribosomes agrees well with the idea that the ASV glycoprotein is localized in the plasma membrane. These results parallel those of Morrison and Lodish, who found the mRNA which codes for the vesicular stomatitis virus glycoprotein exclusively on membrane-bound polyribosomes (23).

When we studied the site of synthesis of pp60^{src}, we found that 90% of this molecule was synthesized on free polyribosomes. Since proteins which remain in the cell sap are usually synthesized on free polyribosomes (12, 14, 30), this result is in agreement with previous observations, suggesting that pp60^{src} is primarily a cytoplasmic protein (4, 19, 31).

The significance of the 10% of pp60^{src} which was synthesized on membrane-bound polyribosomes is uncertain at this time. As mentioned above, a portion of these polyribosomes may attach nonspecifically to membranes during cell rupture, or the membrane-bound polyribosome preparation may be slightly contaminated with free polyribosomes. It is interesting to note that Morrison and Lodish found that the mRNA's which code for vesicular stomatitis virus nonmembrane proteins were located in both the membrane and free polyribosomal fractions (23). These authors suggested that only those proteins which are destined to become membrane bound (Pr62) may be able to be located with absolute specificity.

Recently, Willingham et al. determined that pp60^{src} is located on the inner surface of the plasma membrane at the electron microscopic level by using the ferritin bridge procedure (38). Krueger et al. also reported an association between pp60^{src} and membranes by showing that pp60^{src} fractionated with plasma membranes upon differential centrifugation of cell lysates (17). Although the results presented here suggest that pp60^{src} is not made on structures usually associated with the synthesis of membrane proteins, it must be recalled that the synthesis of membrane proteins on free polyribosomes has indeed been described previously (20, 21). It is still possible that a portion of pp60^{src} may become attached to the plasma membrane after translation. Further experiments to determine the subcellular location of pp60^{src}, as well as the site of action of the pp60^{src}-associated protein kinase (7, 11), are in progress.

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