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79

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Table of Contents

Expression of RNA Tumor Viruses at Translation and Transcription Lebels

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I. Introduction

RNA tumor viruses have been studied extensively in the last 15 years. Some of these viruses cause well-defined neoplasms, and a detailed study of viral infection may elucidate the process involved in malignant transformation. In addition, other RNA tumor viruses are transmitted as normal host cell genes and can be expressed during normal development and differentiation of the animal. Therefore these viruses also provide an interesting model system for studying the regulation and expression of normal cellular genes. Much of the regulation of expression for both exogenous and endogenous RNA tumor viruses occurs at the level of virus-specific protein and RNA synthesis, which is the subject of this review.

A. Scope of the Review

Several excellent and comprehensive reviews of RNA tumor viruses have been published, and for a general view the reader is referred to reviews by *Baltimore* (1974) and *Vogt* (1977). Particular aspects of RNA tumor viruses have been reviewed by authors cited below.

This review deals with the translation and transcription of RNA tumor virus-specific RNA in infected and uninfected cells. Since a review on the synthesis and processing of RNA tumor virus proteins is being written concurrently (*R. Eisenman,* in preparation), this Review will emphasize synthesis of virus-specific RNA. Virus-specific protein synthesis will be discussed primarily to provide an understanding of virus-specific messenger RNA; a detailed analysis of viral protein metabolism will not be attempted.

The experiments discussed here will generally involve avian or murine C-type RNA tumor viruses because they have been used for most studies on intracellular virus-specific RNA and protein synthesis. In some cases other RNA tumor viruses will also be discussed, but a comprehensive review of all RNA tumor viruses will not be attempted.

B. Virus Structure

1. Viral Proteins

RNA tumor viruses are enveloped viruses that contain three classes of structural proteins, i.e., internal structural proteins, envelope glycoproteins, and reverse

transcriptase. These proteins together comprise approximately 250000 daltons and require approximately 7500 nucleotides of single strand nucleic acid coding capacity. With the exception of the sarcomagenic ("sarc") information of sarcoma viruses (see below), the viral structural proteins appear to be the only virus-coded proteins (*Baltimore*, 1974).

a) Internal Structural Proteins

The core of the RNA tumor virus particle appears to be an icosahedron (*Nermut* et al., 1972) that is comprised of genomic RNA and internal structural proteins. The internal structural proteins are relatively small (10000–30000 daltons) and are present in approximately 5000 copies per virus particle (*Stromberg* et al., 1974). The proteins are identified by the prefix "p" (for protein) followed by a number signifying the molecular weight of the protein in thousands of daltons (*August* et al., 1974). Avian RNA tumor viruses contain five internal structural proteins (p27, p19, p15, p12, and p10), and murine viruses contain four (p30, p15, p12, and p10).

b) Reverse Transcriptase

The virus core also contains reverse transcriptase — an enzyme which transcibes the viral RNA into DNA (*Baltimore*, 1970; *Temin* and *Mizutani*, 1970; reviewed by *Verma*, 1977). Reverse transcriptase consists of two proteins of approximately 100000 and 70000 daltons in avian viruses, and one protein subunit (80000 datons) in murine viruses. In avian reverse transcriptase, the small subunit contains peptides present in the large subunit and may be derived from the larger subunit by proteolytic cleavage (*Gibson* and *Verma*, 1974). Each virus particle contains approximately 50–100 molecules of reverse transcriptase (*Panet* et al., 1975).

c) Envelope Glycoprotein

The envelope of RNA tumor viruses contains glycosylated virus-specific proteins. Avian viruses contain two glycoproteins gp85 and pg35 ("glycoprotein" of 85000 daltons and 35000 daltons), and murine viruses contain two glycoproteins gp70 and gp45 (*August* et al., 1974). Low molecular weight envelope proteins [p15(E) and p12(E) in murine viruses] are also present. Every virus particle contains 200–500 molecules of each envelope glycoprotein (*Stromberg* et al., 1974).

2. Viral RNA

Virus particles contain RNA of both low and high molecular weight (reviewed by *Beemon*, 1977). Low molecular weight RNA consists of host-derived 4S transfer RNA and 7S RNA. The high molecular weight RNA has a sedimentation value of approximately 70S and consists of two 35S subunits complexed with host cell tRNA. The 35S subunits contain identical nucleic acid sequences,

so the total information capacity of the RNA tumor virus genome corresponds to one 35S RNA molecule (approximately 10000 nucleotides). The 35S RNA molecules have several properties characteristic of host cell messenger RNA (mRNA) [e.g., contain poly(A) at the 3′ end, have a 5′ terminal methylated "cap" structure, and have internal adenosine residues that are methylated].

Certain strains of RNA tumor viruses morphologically transform fibroblasts in vitro and cause sarcomas in vivo (sarcoma viruses). The sarcomagenic property of these viruses is associated with additional RNA sequences ("sarc" sequences), corresponding to approximately 1500 nucleotides in avian viruses.

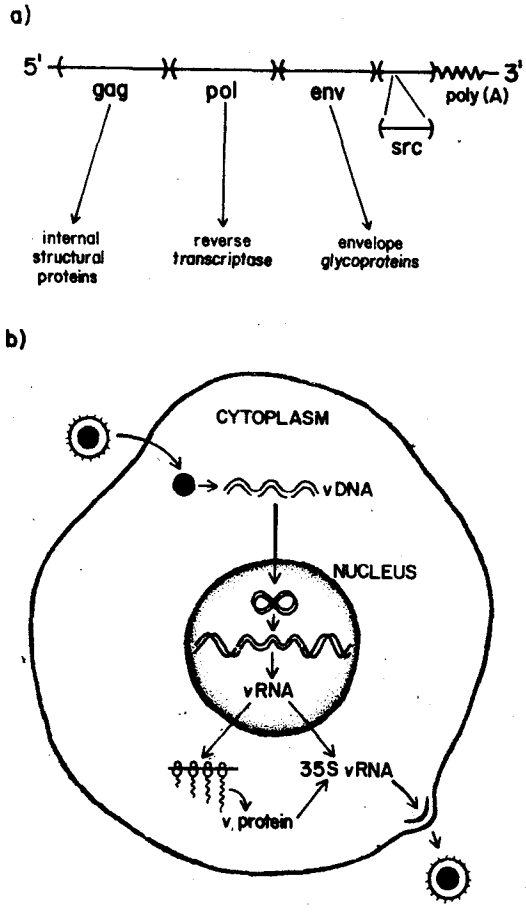

Fig. 1a and b. Gentic map and life cycle of RNA tumor viruses. (a) Gentic map. Gene order of viral genes on RNA tumor virus 35S RNA is shown (for a detailed analysis see *Beemon*, 1977). Sarcoma viruses contain additional sequences responsible for sarcomagenic potential of viruses (*scr* gene), and location of these additional sequences is also shown. (b) Life cycle of RNA tumor virus infection. A brief description of this process is in Section A-IV

C. The Genetic Map

Recently, the genetic map of avian RNA tumor viruses has been determined by deletion mapping of RNA oligonucleotides (reviewed by *Beemon*, 1977; see also *Duesberg* et al., 1976; *Joho* et al., 1976). The genetic order on the 35S RNA from 5′ to 3′ is *gag*, (coding for the internal structural proteins or "group-specific antigens"); *pol* (coding for reverse transcriptase); *env*, (coding for the envelope glycoproteins); and *src*, (coding for the sarcomagenic information) in the case of sarcoma viruses (see *Baltimore*, 1974 for nomencalture of the genes). Fig. 1a shows a schematic diagram of the genome.

D. The Infection Cycle

Fig. 1b is a schematic diagram of RNA tumor viruses infection cycle. Virus particles absorb to the cell surface, and penetrate into the cytoplasm. In the cytoplasm, the viral RNA is transcribed into virus-specific DNA (*Varmus* et al., 1974; reviewed by *Weinberg*, 1977). The cytoplasmic virus-specific DNA is transported to the nucleus, where some covalently closed circular DNA is found, and is subsequently integrated into the host chromosomal DNA (*Varmus* et al., 1974). Virus-specific RNA is then synthesized from the integrated viral DNA, transported to the cytoplasm, and translated into viral protein. In addition, some of the cytoplasmic virus-specific RNA is packaged with viral proteins into virus particles, which bud from the cell surface.

Infection with RNA tumor viruses is not a lytic event. Productively infected cells continue to grow (sarcoma viruses may even grow faster) and continuously shed virus into the extracellular medium. Virus-producing cultures can be maintained indefinitely.

E. Endogenous Viruses

Many animal species carry genetic information for RNA tumor virus(es) as a normal host cell gene (for a detailed analysis of one system, see *Chattopadhyay* et al., 1974). A variety of chemical treatments can induce virus expression (*Lowy* et al., 1971; *Aaronson* et al., 1971; *Aaronson* and *Dunn*, 1974); the resultant viruses, in general, are competent to carry out a complete infection cycle in the appropriate host cell. In some species, e.g., mice, multiple copies of endogenous viruses are present in the genome (*Stephenson* et al., 1974), which may complicate analysis.

II. Synthesis of Viral Proteins

A. Synthesis in Infected Cells

Viral protein synthesis in cells infected with RNA tumor viruses has generally been studied by pulse labeling infected cells and immunoprecipitating virus-specific proteins with antiserum directed against virion proteins. This method

is necessary because host protein synthesis is not shut off during RNA tumor virus infection. Proteins related to particular classes of viral proteins have been identified by using antisera monospecific for individual viral proteins, as well as tryptic peptide analysis of the immunoprecipitated proteins.

1. Internal Structural (Gag) Proteins

Virion internal structural protein synthesis was originally studied by immunoprecipitating pulse-labeled infected cell extracts with antiserum directed against group-specific (*gs*) antigenic determinants. These *gs* antigenic determinants are predominantly located on the virion internal structural proteins. Early experiments (*Shanmugam* et al., 1972) indicated that labeled viral proteins of the same size as virion structural proteins were present after a 10 min amino acid label of mouse cells infected with murine sarcoma (MSV) and murine leukemia (MuLV) virus. However, *Vogt* and *Eisenman* (1973) demonstrated that immunoprecipitation of pulse-labeled avian myeloblastosis virus (AMV)-infected chick myeloblast extracts by avian RNA tumor virus *gs*-antiserum revealed a virus-related protein of 76000 daltons. This protein (termed pr76, for "precursor of size 76000 daltons") contained the tryptic peptides of several structural proteins. Pulse-chase experiments indicated this protein was a precursor to the AMV internal structural proteins p27, p19, and p12. This situation is reminiscent of poliovirus protein synthesis, where the entire poliovirus genome is translated as a single polyprotein followed by cleavage to yield the viral structural and nonstructural proteins (*Jacobson* and *Baltimore*, 1968). All of the poliovirus proteins are by necessity synthesized in equimolar amounts. Although equimolar amounts of all internal structural proteins are synthesized, equimolar amounts of the other classes of viral proteins, e.g., polymerase and glycoprotein are not synthesized. Therefore, although the internal structural proteins are synthesized by way of a high molecular weight precursor, it is not clear whether all of the RNA tumor virus-specific proteins are derived from an even higher molecular weight precursor.

Vogt et al. (1975) further studied the precursors for AMV internal structural proteins and found 66000, 60000, and 32000 dalton intermediates in the cleavage of the pr76 precursor molecule. The order of the internal structural proteins in the pr76 polypeptide was found to be p19, p27, and p15 from N-terminal to C-terminal. The relative position of the p12 protein could not be determined.

Similar experiments to study the synthesis of MuLV internal structural proteins have also been performed (*Naso* et al., 1975; *van Zaane* et al., 1975; *Shapiro* et al., 1976). A major protein of 65000 datons (pr65) could be immunoprecipitated from pulse-labeled infected cells, and chase experiments indicated it was a precursor to the internal structural proteins (*van Zaane* et al., 1975; *Shapiro* et al., 1976). However, in addition to the pr65 protein, virus-related proteins of higher molecular weight (180000 and 80000 daltons) were also detected. There proteins could be immunoprecipitated with antiserum monospecific for the internal structural protein p30 (*Naso* et al., 1975; *Arlinghaus* et al., 1976; *Arcement* et al., 1976). Tryptic peptide analysis indicated that all three of these high molecular weight proteins shared amino acid sequences with the

internal structural proteins (*Arcement* et al., 1976). It was proposed that they represent different cleavage intermediates of a very large precursor protein (*Arlinghaus* et al., 1976). Labeling kinetics, however, do not completely support this hypothesis, because after a relatively short pulse label, the majority of radioactivity is in the pr80 protein rather than the pr180 protein.

Radioactivity, furthermore, appears to chase efficiently from pr80 into pr65 and then into the *gag* proteins, but chase from the pr180 is very slow. An explanation of the above data could be that the major translation product of the *gag* gene is the pr80 protein, which is rapidly cleaved to the pr65 precursor, and more slowly cleaved into the internal structural proteins. The pr180 protein, which is synthesized in lower amounts, might represent a separate translation product of both the *gag* and *pol* genes (see below). This protein, however, might not be an obligate precursor for the internal structural proteins.

2. Reverse Transcriptase (Pol)

The pr180 from MuLV infected cells (see above) is also immunoprecipitated by an antiserum directed against MuLV reverse transcriptase (*Arlinghaus* et al., 1976), and likely represents a molecule that contains both the polymerase and internal structural protein sequences. Treatment of cells with protease inhibitors builds up pr180 protein, indicating that it might normally be cleaved to produce polymerase and internal structural proteins. However, the pr180 protein is unusually stable during a pulse-chase experiment. This fact raises the possibility that the protein is not a physiologic precursor to either the polymerase or internal structural proteins. An additional difficulty is that no reverse transcriptase (80000 datons) appears on a chase with unlabeled amino acids. This anomaly may be because the pulse label experiments were performed with S^{35} methionine. Murine reverse transcriptase may have nor or very few methionine residues, because the methionine peptides of the pr180 and pr80 are essentially identical (*Arlinghaus* et al., 1976; *Kerr* et al., 1976), with no peptides that correspond to the extra (polymerase) protein apparent. This hypothesis also raises the possibility that other methionine-poor precursor proteins for reverse transcriptase might have been missed in experiments that used methionine as the only labeled amino acid. Similar results for RSV-infected chick cells have also indicated presence of a pr180 protein that contains determinants for both internal structural proteins and avian reverse transcriptase (*M. Hayman*, personal communication).

In summary, a large protein of 180000 daltons that contains peptides of both internal structural proteins and reverse transcriptase may be the precursor to intracellular reverse transcriptase. However, the pulse-chase kinetics for these experiments are somewhat equivocal, and the fact that only methionine labels were used leaves the possibility that other precursor molecules to the reverse transcriptase may have been missed.

3. Envelope Glycoprotein (Env)

By the same methods described above, a higher molecular weight precursor to the envelope glycoproteins gp69/71 and p15(E) of MuLV has been detected,

with molecular weight of 82000 daltons (*van Zaane* et al., 1975) or 90000 daltons, (*Arcement* et al., 1976; *Arlinghaus* et al., 1976; *Shapiro* et al., 1976; *Famulari* et al., 1976). This precursor is glycosylated and can be labeled with radioactive glucosamine, but appears to lack the terminal fucose residues of the oligosaccharide chains (*Naso* et al., 1976). Processing this precursor to yield gp69/71 and p15(E) is accompanied by adding the fucose to the mature gp69/71 protein (*Naso* et al., 1976). Inhibition of glycosylation by incubating cells in the presence of 2-deoxyglucose during the labeling period causes the synthesis of a 70000–dalton protein, which is immunoprecipitable with anti-gp69/71 (*Shapiro* et al., 1976). This protein may represent an unglycosylated (or extremely underglycosylated) form of the pr90 glycoprotein precursor. The 70000–dalton protein is not converted to pr90 or gp69/71 during a chase without 2-deoxyglucose and appears to be completely degraded. These phenomena may reflect the fact that certain glycosylation events can occur only on growing nascent polypeptide chains (*Sefton*, 1977).

A similar higher molecular weight precursor (95000 daltons) for avian virus gp85 has been reported for RSV-infected chick cells (*M. Hayman*, personal communication). In addition, a precursor for the envelope glycoprotein of smaller size (70000 daltons) has also been reported (*Halpern* et al., 1974). This smaller protein contains some sugar residues (evidenced by glucosamine labeling), but lacks the terminal fucose residues. The exact relationship between the 70000–dalton precursor, pr95, and gp85 is presently unclear.

Membrane-bound proteins that are secreted from both host cell and viral origin appear to be synthesized on membrane-bound polyribosomes (*Cioli* and *Lennox*, 1973; *Rolleston*, 1974; *Morrison* and *Lodish*, 1975; *Devillers-Thiery* et al., 1975). This phenomenon also may be true for the glycoproteins of RNA tumor viruses. Virus-specific mRNA has been detected in both membrane-bound and free polyribosomes from MuLV-infected cells (*Gielkens* et al., 1974). The membrane-bound virus-specific mRNA (consisting predominantly of subgenomic sized mRNA) is likely to code for the envelope glycoproteins (Sec. CIII-1).

4. Sarcomagenic Information (Src)

No biochemical identification of the protein or proteins that are responsible for morphologic transformation of fibroblasts by sarcoma viruses (a product of the *src* gene) have yet been reported. Persuasive genetic evidence exists, however, that sarcoma viruses indeed encode such a protein. Mutants of RSV exist, which are temperature sensitive for the ability to transform fibroblasts morphologically, but are nondefective for the establishment and maintenance of virus production (*Martin*, 1970; *Kawai* and *Hanafusa*, 1971; *Bader* and *Brown*, 1971; *Biquard* and *Vigier*, 1972; *Wyke*, 1973). Cells that have been infected by these temperature sensitive mutants have transformed phenotype at the permissive (low) temperature, and, reversibly, revert to normal phenotype when shifted to high temperature. The search for the RNA tumor virus sarcomagenic protein is currently a field of intense investigation.

Recently, a protein of 60000 daltons has been identified by immunoprecipitation of Rous sarcoma virus (RSV) transformed cell extracts (*Brugge, J., Erickson, R.L.*, personal. communic.) which may represent the sacromagenic protein.

5. Extremely Large Polyproteins

Very minor amounts of extremely high molecular weight virus-specific proteins (250000–350000 daltons) have been detected in cells infected with MuLV (*Naso* et al., 1975; *Shapiro* et al., 1976). These extremely large polyproteins contain antigenic determinants for more than one class of viral protein, and are immunoprecipitable with antisera monospecific for both internal structural proteins and envelope glycoprotein (*Shapiro* et al., 1976). Considering the genetic map of virion 35S RNA, these large proteins may represent a translation product of the entire 35S RNA molecule. At the present time however, it is unclear whether these large polyproteins have any physiologic function.

B. In Vitro Translation of Viral RNA

1. Virion RNA

a) Full-Length 70S or 35S RNA

Full-length genomic RNA of both avian and murine RNA tumor viruses has been translated in cell-free protein synthesizing systems. An initial report (*Siegert* et al., 1972) indicated that translation of AMV 35S RNA in an *E. coli* cell-free system resulted in proteins the same size as the virion internal structural proteins – a somewhat surprising result in that these are derived in vivo from a pr76 precursor. More recently, *von der Helm* and *Duesberg* (1975) reported that in vitro translation of RSV RNA in a *Krebs* ascites cell-free system results in synthesis of a 76000-dalton protein. Immunoprecipitation and tryptic peptide analysis of this protein indicated that it was similar or identical to the pr76 precursor for the internal structural proteins. No major virus-coded proteins of larger size were seen. Three important conclusion can be drawn from these results:

(1) The RSV genome is "positive" stranded at least for the *gag* gene – that is, the nucleic acid sequences of the genomic 35S RNA are identical rather than complementary to those of the virus-specific mRNAs.

(2) Considering the recently deduced gene order for RSV, the fact that *gag* is the 5′ terminal gene on the 35S RNA implies that a strong signal for termination of protein synthesis (or a strong signal for nascent protein cleavage) must exist between the end of the *gag* gene and the other RSV genes on the 35S RNA.

(3) Because a strong termination site is present at the end of the *gag* gene, initiation sites for synthesis of the 5′ distal genes are possibly present on the 35S RNA, but these sites are inactive (closed) in full-length 35S RNA.

Other workers (*Salden* and *Bloemendal*, 1976; *Pawson* et al., 1976; *K. Beemon* and *T. Hunter*, 1977) using various avian tumor virus genomic RNAs and other cell-free systems have confirmed the results of *von der Helm* and *Duesberg*. In general, translation systems derived from mammalian cells have been more efficient in producing full-length pr76 protein than cell-free systems derived from *E. coli* or wheat germ.

Murine RNA tumor virus 35S RNA has also been translated in cell-free systems. Major bands corresponding to the pr65 and pr80 precursors of the internal structural proteins were again detected (*Naso* et al., 1975; *Salden* et al., 1976; *Kerr* et al., 1976). In addition, minor amounts of higher molecular weight proteins were also observed (*Naso* et al., 1975; *Kerr* et al., 1976; *K. Beemon, T. Hunter, S. Edwards* and *H. Fan*, unpublished). In particular, a 180000-dalton protein with peptides in common with the pr65 and pr80 precursors was detected (*Kerr* et al., 1976; see Fig. 2), which may correspond to the pr180 protein of MuLV-infected cells (*Arlinghaus* et al., 1976). This protein presumably arises by low level suppression of the protein synthesis termination signal at the end of the *gag* gene, resulting in readthrough into the *pol* gene. The low level suppression may provide a mechanism by which different amounts of the two gene products can be synthesized using one protein synthesis initiation signal. For the reasons cited above, however, it is unclear if the pr180 protein has any physiologic function. Recent experiments (*K. Beemon, T. Hunter* and *S. Edwards*, unpublished) also indicate similar high molecular weight translation products from avian virus 35S RNA, as shown in Fig. 2. The higher molecular weight translation products containing both *gag* and *pol* gene products, therefore, are not restricted to murine viruses. It is thus more likely that the weak suppression of the *gag* gene termination signal is universal for RNA tumor viruses, and has a physiologic function.

In vitro translation of full-length 35S genomic RNA has not resulted in proteins with the appropriate size for the glycoprotein precursor molecules. Immunoprecipitation of RSV primed translation products with antiserum monospecific for the RSV gp85 protein did not reveal any envelope-specific products (*Pawson* et al., 1976). Therefore the initiation site for *env* gene translation on 35S virion RNA is likely closed. Another less likely possibility (for reasons, see C-I) is that the nucleic acid sequences for the *env* gene in virion 35S RNA are not identical to, but rather, complementary to the mRNA sequences, which code for envelope glycoprotein.

b) Subgenomic RNA

Because initiation sites for certain RNA tumor virus genes appear to be closed in virion RNA, cleavage and/or processing of the 35S RNA may be necessary to activate these sites. To test this hypothesis, partially degraded virion 35S RNA has been studied. *Purchio* et al. (*A.F. Purchio* and *R.L. Erickson*, personal communication) obtained partially degraded AMV 35S RNA by incubating virions at 37° C, and poly(A)-containing RNA was separated according to size on a sucrose gradient. Translation of the different sized RNA classes in a wheat germ cell-free system indicated that small RNA molecules (containing sequences

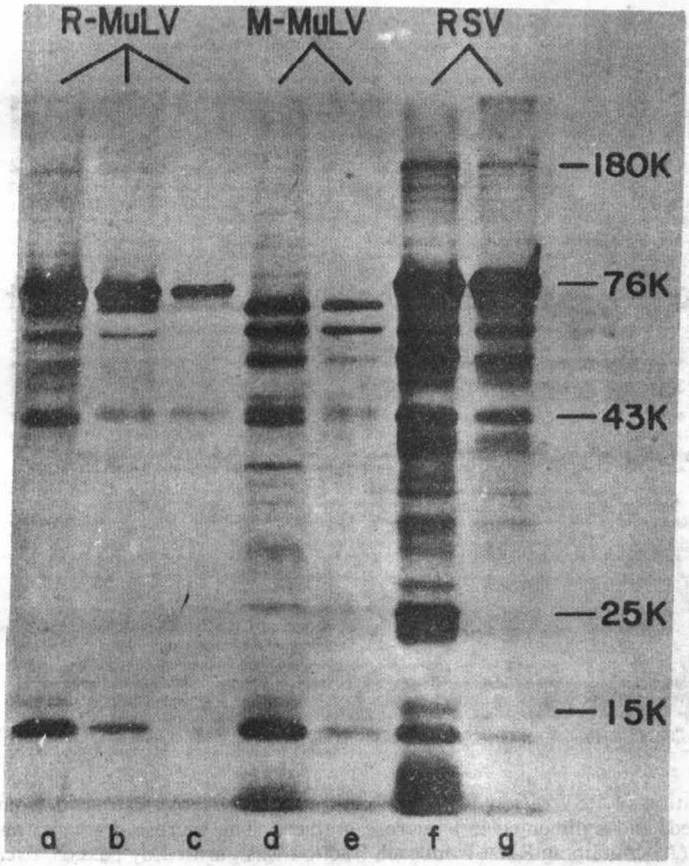

Fig. 2. In vitro translation of MuLV and RSV genomic RNA. RNA was extracted from *Rauscher* and *Moloney* MuLV as well as PRAGUE B strain RSV virions. RNA was denatured by brief heat treatment and translated in a messenger dependent reticulocyte cell-free protein synthesizing system. Reaction mixtures contained S^{35} methionine to label proteins. Reaction products were analyzed by electrophoresis in a polyacrylamide slab gel, and an autroadiograph of dried gel is shown. Channels *a–c*: *R-MuLV* RNA translated at 30° (*a*), 36° (*b*) and 39° (*c*); *d* and *e*: *M-MuLV* RNA translated at 30° (*d*) and 36° (*e*); *f* and *g*: *RSV* RNA translated at *f* 30° and *g* 36°. Molecular weight positions of 180000, 76000, 43000, and 25000 daltons for RSV trasnlation products were determined from similar gel by parallel electrophoresis of marker proteins. All three viruses program synthesis of major protein corresponding to 76000-dalton protein for RSV, but exact sizes of proteins differ between viruses. A higher molecular weight band series between 180000 and 76000 dalton is also present

close to the 3′ end of the 35S RNA molecule) were extremely efficiently translated. These proteins were not efficiently precipitated by antibody to whole virus, which suggests that they might represent nonstructural virion proteins, but further analysis has not yet been reported.

Beemon and *Hunter* (1977) have performed similar experiments with RNA smaller than 35S from RSV virions, using a messenger-dependent reticulocyte

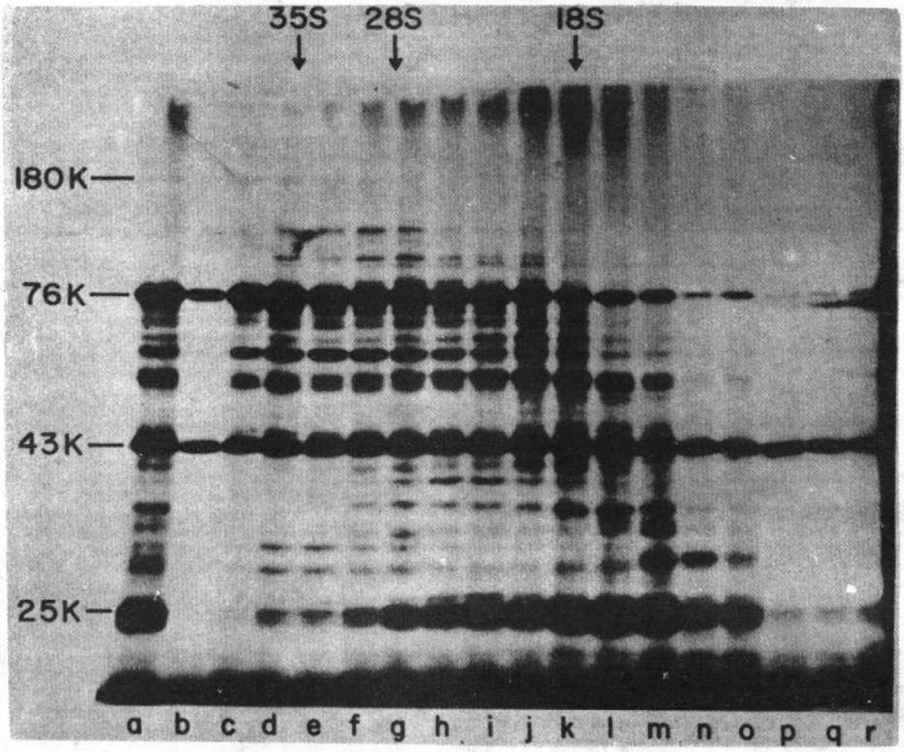

Fig. 3. In vitro translation of RSV virion RNA different sizes. RNA was extracted from RSV virions, denatured, and sedimented in a sucrose gradient. The sucrose gradient was fractionated, and poly(A)-containing RNA from each fraction was purified by passage over an oligo (dT) cellulose column. RNA from equal amounts of each fraction was translated as in Fig. 2, and slab gel electrophoretic analysis is shown. Channel *a* represents translation of total virion RNA. Channels *b–r* represent RNA from the different sucrose gradient fractions, from bottom to top. The sedimentation positions of *35S* viral RNA and *28S* and *18S* ribosomal RNA are indicated. *35S* RNA codes for the 76000 (*76K*) and 180000 (*180K*) dalton proteins, while 14-18S RNA codes for sarcoma virus-specific proteins of 25000 (*25K*), 23000 (*23K*), 15000 (*15K*), and 13000 (*13K*) daltons (see Fig. 4). In this gel the 15000- and 13000-dalton proteins were run off the gel

Fig. 4a and b. In vitro translation of RSV and *td* RSV virion RNA. (a) Virion RNA from Pr-B RSV and transformation defective (*td*) Pr-B RSV was translated as in Fig. 2 in presence (*b* and *d*) and absence (*a* and *c*) of M^7-GTP (analog for methylated 5′ cap structure of mRNAs). *a* and *b*: *RSV* RNA; *c* and *d*: *td-RSV* RNA. (b) An in vitro translation product of RSV RNA was immunoprecipitated with rabbit antisera monospecific for internal structural protein p27 and envelope glycoprotein gp85. Channels *b* and *f*, total in vitro product; *c*, immunoprecipitation with anti-p27; *d*, immunoprecipitation with anti-gp85; *e*, immunoprecipitation with normal serum. Channels *a* and *g* show lighter exposures of channels *b* and *f* from a second autoradiogram of same gel. Immunoprecipitation with anti-p27 selectively precipitates 76000 (*76K*) dalton protein. Immunoprecipitation with anti-gp85 may precipitate a small amount of protein of 50000–60000 daltons. Neither antiserum specifically precipitates 23000–25000 (*23K–25K*), 13000–15000 (*13K–15K*) dalton proteins