ADVANCES IN CANCER RESEARCH

GEORGE KLEIN
SIDNEY WEINHOUSE

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ADVANCES IN CANCER RESEARCH

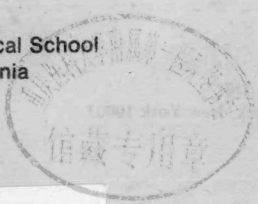
Edited by

GEORGE KLEIN

Department of Tumor Biology
Karolinska Institutet
Stockholm, Sweden

SIDNEY WEINHOUSE

Fels Research Institute
Temple University Medical School
Philadelphia, Pennsylvania



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CONTRIBUTORS TO VOLUME 27

Numbers in parentheses indicate the pages on which the authors' contributions begin.

J. I. BREWER, *Department of Obstetrics and Gynecology and the Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611* (89)

C. DEAN BUCKNER, *The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 and The Department of Medicine, Division of Oncology, University of Washington School of Medicine, Seattle, Washington 98195* (269)

SUSHILKUMAR G. DEVARE, *Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland 20014* (1)

ALEXANDER FEFER, *The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 and The Department of Medicine, Division of Oncology, University of Washington School of Medicine, Seattle, Washington 98195* (269)

B. HALPERN, *Department of Obstetrics and Gynecology and the Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611* (89)

RONALD B. HERBERMAN, *Laboratory of Immunodiagnosis, National Cancer Institute, Bethesda, Maryland 20014* (305)

HAROLD B. HEWITT, *Department of Morbid Anatomy, King's College Hospital Medical School, London S.E.5., England* (149)

HOWARD T. HOLDEN, *Laboratory of Immunodiagnosis, National Cancer Institute, Bethesda, Maryland 20014* (305)

B. D. KAHAN,* *Department of Surgery, Northwestern University Medical School, Chicago, Illinois 60611* (89)

MARTIN LIPKIN, *Memorial Sloan-Kettering Cancer Center, New York, New York 10021* (281)

PAUL E. NEIMAN, *The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 and The Department of Medicine, Division of Oncology, University of Washington School of Medicine, Seattle, Washington 98195* (269)

* Present address: Divisions of Organ Transplantation and Immunology, Departments of Surgery and Biochemistry, University of Texas Medical School at Houston, Houston, Texas 77030.

FRED H. REYNOLDS, JR., *Viral Oncology Program, Frederick Cancer Research Center, Frederick, Maryland 21701* (1)

JOHN ROBOZ, *Department of Neoplastic Diseases, Mount Sinai School of Medicine, The City University of New York, New York, New York 10029* (201)

C. R. STANHOPE, *Department of Obstetrics and Gynecology and the Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611* (89)

JOHN R. STEPHENSON, *Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland 20014* (1)

RAINER STORB, *The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 and The Department of Medicine, Division of Oncology, University of Washington, Seattle, Washington 98195* (269)

E. DONNALL THOMAS, *The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 and The Department of Medicine, Division of Oncology, University of Washington, Seattle, Washington 98195* (269)

E. E. TOROK, *Department of Obstetrics and Gynecology and the Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611* (89)

ALICE S. WHITTEMORE, *Department of Environmental Medicine, New York University Medical Center, New York, New York* (55)

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John R. Stephenson, Sushilkumar G. Devare, and Fred H. Reynolds, Jr.

Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland
and Viral Oncology Program, Frederick Cancer Research Center, Frederick, Maryland

I. Introduction

The existence of oncornavirus genetic sequences in a naturally integrated state within the cellular genome of a broad spectrum of vertebrate species is well established (Lieber and Todaro, 1975; Aaronson and Stephenson, 1976). Release of infectious virus particles, while generally repressed, can occur both spontaneously (Hartley *et al.*, 1969; Aaronson *et al.*, 1969; Stephenson and Aaronson, 1972b; Lieber *et al.*, 1973) and following treatment with chemicals (Lowy *et al.*, 1971; Aaronson *et al.*, 1971b). Following activation, infectious virus may be transmitted horizontally both between individual animals of the same species (Hardy *et al.*, 1973; Jarrett *et al.*, 1973) as well as to other species (Benveniste and Todaro, 1976). The association of infectious oncornavirus particles with lymphoid tumors of many species has also been demonstrated (Gross, 1959; Lilly *et al.*, 1975; Essex, 1975). Moreover, there is accumulating evidence that elevated endogenous virus expression may be an important determinant of host susceptibility to neoplastic transformation (Niman *et al.*, 1977).

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Studies of the translational products of oncornaviruses have been initiated in efforts to gain insight into the role that these viruses may have both in normal cellular processes and in the etiology of tumors of their natural hosts.

As early as 1958, Bernhard proposed a classification scheme for the diverse group of RNA viruses now included under the general term "oncornavirus". According to this system, RNA tumor viruses are designated as type-A, type-B, type-C (Bernhard, 1958, 1960), or type-D (Dalton *et al.*, 1974) primarily on the basis of morphologic criteria. Intracellular virus-like particles occurring in a variety of mouse tumors have been designated type-A (Dalton *et al.*, 1961). These are distinguished from other oncornaviruses mainly by virtue of their association with the endoplasmic reticulum rather than plasma membrane (Dalton, 1962). The second class of oncornaviruses, designated as type-B, have eccentrically located nucleoids and their envelope possesses characteristic projections or spikes (Sarkar *et al.*, 1972). While mouse mammary tumor virus (MMTV), the prototype virus of this group, has been studied extensively, much less information is currently available regarding type-B viruses of other species of origin, such as the guinea pig (Opler, 1967; Nadel *et al.*, 1967) and domestic cow (Miller *et al.*, 1969; Van Der Maaten *et al.*, 1974). The possibility that type-B particles may represent maturational products of intracytoplasmic type-A particles has been suggested on the basis of apparent similarities in the immunologic properties of their major structural proteins (Sarkar and Dion, 1975; Tanaka, 1977).

The most extensively studied class of oncornaviruses are the type-C RNA tumor viruses. This group of viruses is characterized by their centrally located nucleoid and a pattern of virion assembly which occurs as a budding process at the plasma membrane (Sarkar *et al.*, 1972). Type-C oncornaviruses can be distinguished from either type-B and type-D viruses on the basis of both morphologic criteria (Bernhard, 1958; Dalton *et al.*, 1974) and the divalent cation preference of their RNA-dependent DNA-polymerase (Scolnick *et al.*, 1970; Howk *et al.*, 1973; Abrell and Gallo, 1973). In addition, many type-C virus structural proteins can be readily distinguished from those of type-B and type-D viruses. Moreover, a number of structural proteins of all type-C oncornavirus isolates examined to date have been found to share cross-reactive interspecies antigenic determinants (Gilden, 1975; Stephenson *et al.*, 1977b), and the major structural proteins of type-C isolates of several species have been shown to exhibit extensive regions of primary structure homology (Oroszlan *et al.*, 1975, 1976). Another characteristic property of type-C RNA tumor viruses is their unique

ability to provide helper functions for replication-defective sarcoma viruses (Hartley and Rowe, 1966; Huebner, 1967; Sarma *et al.*, 1970; Aaronson and Rowe, 1970).

In view of the fact that type-C viruses represent the major emphasis of the present review, the origins of many of the presently available isolates are summarized in Table I. It should be noted that in several instances endogenous type-C viruses of one species were transmitted to and became stably associated with the germ line of a second species (Benveniste and Todaro, 1974, 1975b). In fact, the majority of type-C virus isolates can be traced back to two main lineages of ancestral viruses, one of rodent origin and the second, endogenous to primates. An understanding of the relatedness of different type-C virus isolates is important in the evaluation of much of the currently available information regarding properties of their structural proteins.

Type-C viruses of a number of mammalian species, including endogenous viruses that have existed within the pig genome for millions of years (Benveniste and Todaro, 1975b), as well as a group of infectious horizontally transmitted isolates of gibbon apes (Kawakami *et al.*, 1972) and a woolly monkey isolate (Theilen *et al.*, 1971) are all related to known endogenous mouse type-C virus isolates and appear to be

TABLE I
MAMMALIAN TYPE-C ONCORNAVIRUSES

Species of origin	Prototype virus isolate	Ancestral origin
Rodent		
Mouse *Mus musculus*	R-MuLV, AKR-MuLV, etc.	Rodent
Mus caroli	CERO CI	Rodent
Mus cervicolor	CERV CI, CII	Rodent
Rat *Rattus norvegicus*	RT 21C, SF-1, RMTDV	Rodent
Hamster *Cricetulus griseus*	CCL 14.1	Rodent
Carnivores		
Cat *Felis catus*	RD114	Primate
	FeLV	Rodent
Felis sylvestris	FS-1, WCV-1	Primate
Artiodactyls		
Pig *Sus scrofa*	CCL-33, PK(15)	Rodent
Deer *Odocoileus hemionus*	DKV	Unknown
Primates		
Baboon *Papio cynocephalus*	M7, M28, BAB8-K	Primate
Papio hamadryas	BILN	Primate
Gelada *Theropithecus gelada*	TG-1-K	Primate
Woolly monkey *Lagothrix spp.*	SSAV-1	Rodent
Gibbon ape *Hylobates lar*	GALV	Rodent

evolutionarily related to ancestral mouse viruses (Lieber *et al.*, 1975b). While other endogenous rodent viruses, such as those of hamster (Graffi *et al.*, 1968; Kelloff *et al.*, 1970) and rat (Bergs *et al.*, 1970) origin, have not been as well studied, these also appear to constitute a highly related group (Benveniste and Todaro, 1975a). In addition, feline leukemia virus (FeLV), a horizontally transmitted type-C virus of cats, has been shown to possess significant nucleic acid sequence homology with, and was apparently derived from an endogenous rodent virus (Benveniste and Todaro, 1975a).

Endogenous type-C viruses of Old World monkeys, apes, and possibly man constitute the second major lineage of mammalian type-C viruses. While isolation of infectious viruses of this group have been limited to baboon species of the genus *Papio* (Todaro *et al.*, 1976; Stephenson and Aaronson, 1977), the presence (Benveniste and Todaro, 1976) and partial expression (Stephenson and Aaronson, 1977) of related nucleic acid sequences within the genomes of a much broader range of Old World primates has also been demonstrated. A class of endogenous feline viruses, the prototype of which is designated RD114 (McAllister *et al.*, 1972), are apparently of primate origin, having entered the germ line of an ancestral cat 20–30 million years ago (Benveniste and Todaro, 1974). In addition, there is suggestive evidence that a less well-characterized group of type-C viruses, endogenous to ungulates may be somewhat more closely related to primate than to rodent viruses (Aaronson *et al.*, 1976; Tronick *et al.*, 1977).

The fourth major group of oncornaviruses, designated as type-D (Dalton *et al.*, 1974) were described subsequent to the original oncornavirus classification proposed by Bernhard. These particles are somewhat larger in size than type-B or type-C viruses and have pleomorphic bullet-shaped nucleoids. The prototype isolates of this class include the Mason-Pfizer monkey virus (MPMV) (Chopra and Mason, 1970; Kramarsky *et al.*, 1971) and a recently reported endogenous virus of the langur (Todaro *et al.*, 1977a). A number of oncornavirus isolates of squirrel monkey origin have also been tentatively classified as type-D viruses (Heberling *et al.*, 1977; Todaro *et al.*, 1978).

In addition to the four classes of oncornaviruses summarized above, there is a category of RNA tumor viruses generally known as RNA sarcoma viruses. These are replication-defective, transforming viruses which appear to have arisen as a result of genetic recombination between type-C viral and host cell genetic sequences (Scolnick *et al.*, 1973, 1975; Frankel and Fischinger, 1977). Mammalian sarcoma isolates studied to date, while competent for transformation, have invariably been found to require type-C leukemia helper viruses for their

replication (Hartley and Rowe, 1966; Huebner, 1967; Aaronson and Rowe, 1970). Isolates of this group of viruses have been restricted to four mammalian species; these include two rodents, mouse (Moloney, 1966; Levy *et al.*, 1973) and rat (Harvey, 1964; Kirsten and Mayer, 1967), one carnivore species, cat (Snyder and Theilen, 1969; Gardner *et al.*, 1971), and one primate, woolly monkey (Wolfe *et al.*, 1971).

II. Type-C Viral Genome Structure and Complexity

The single-stranded type-C viral genomic RNA has a sedimentation coefficient of about 70 S, corresponding to an estimated molecular weight of approximately 1.2×10^7 (Robinson *et al.*, 1965; Duesberg, 1968; Montagnier *et al.*, 1969). In addition, smaller RNA species with sedimentation values of 4 S and 7 S are found within the virion (Bishop *et al.*, 1970a,b). Denaturation of the 70 S genomic RNA leads to production of two 35 S RNA subunits (Duesberg, 1968). On the basis of electrophoretic mobility (Duesberg and Vogt, 1973) and end-group analysis (Keith *et al.*, 1974), a molecular weight of about 3×10^6 was derived for each 35 S subunit. That the viral genome is polyploid and all 35 S subunits are similar in their sequence has been demonstrated by oligonucleotide fingerprinting analysis using ribonuclease T_1 (Billeter *et al.*, 1974; Duesberg *et al.*, 1974; Coffin and Billeter, 1976), size measurements of infectious DNA (Hill and Hillova, 1974) as well as by molecular hybridization (Baluda *et al.*, 1974). Moreover, the recent application of heteroduplex mapping techniques to studies of type-C viruses have indicated the viral RNA to consist of two 35 S monomeric subunits, attached near their 5′ ends in a dimer linkage structure (Kung *et al.*, 1975, 1976; Bender and Davidson, 1976). The 4 S virion-associated RNA species has been shown to represent tRNA (Erikson and Erikson, 1971; Bonar *et al.*, 1967; Travnicek, 1968) and has been identified as $tRNA^{Trp}$ for avian type-C viruses (Dahlberg *et al.*, 1974b; Harada *et al.*, 1975) and $tRNA^{Pro}$ in the case of some but not all mammalian type-C virus isolates (Peters *et al.*, 1977).

Studies on Rous sarcoma virus (Furuichi *et al.*, 1975; Keith and Fraenkel-Conrat, 1975) and the Moloney strain of murine leukemia virus (MuLV) (Stoltzfus and Dimock, 1976; Bondurant *et al.*, 1976; Rose *et al.*, 1976) have shown that the 5′ end of the viral RNA is capped by the structure $m^7G(5')ppp(5')N^mpNp$. Such capping structures are common among eukaryotic mRNAs and may act to protect the RNA from attack by phosphatases and other nucleases and in addition may promote initiation of translation (Shatkin, 1976). The 3′ terminus of each 30–35 S RNA has a poly(A) sequence of about 200

nucleotides (Lai and Duesberg, 1972; Ross *et al.*, 1972; Keith *et al.*, 1974; Wang *et al.*, 1975). A tRNA molecule is associated with the viral 35 S RNA and functions as the primer for RNA-directed DNA-polymerase (RDDP), initiating synthesis of complementary DNA at a unique site located within 150–200-nucleotide residues from the 5′ terminus of the viral genome (Faras *et al.*, 1974; Taylor and Illmensee, 1975; Cashion *et al.*, 1976; Haseltine *et al.*, 1976).

Recently, the sequence of the first 101 bases beginning at the 5′ end of the Prague RSV-C genome has been determined (Haseltine *et al.*, 1977; Shine *et al.*, 1977). These studies have resulted in the identification of a possible initiation triplet (AUG) for protein synthesis located 85 bases from the 5′ cap structure. Moreover, a sequence of 21 nucleotides immediately preceding the 3′ poly(A) of a prototype avian type-C virus, PrRSV-C, has been identified as: 5′GCCAUUUU-ACCAUUCACCA poly(A) 3′ (Schwartz *et al.*, 1977). The fact that this sequence is identical to that of the first 21 nucleotides located at the 5′ end of the 35 S RNA indicates that the viral genome is terminally redundant. This possibility has recently been confirmed (Coffin and Haseltine, 1977). Independent evidence for terminal redundancy was derived from restriction endonuclease mapping of DNA sequences complementary to the Moloney sarcoma virus genome (Canaani *et al.*, 1977). This terminal redundancy provides for the possibility of circularization of the viral genome prior to integration into host cellular DNA. In fact, circular structures have been visualized by electron microscopy heteroduplex analysis and a replication mechanism involving a circular intermediate has been proposed (Junghans *et al.*, 1977).

III. Proteins of Type-C RNA Tumor Viruses

In view of the above findings indicating the complexity of the type-C viral genome to be of the order of $2–3 \times 10^6$, the maximum size of the translational product for which it can code is about 300,000. Studies on type-C virus-coded proteins have now led to identification and characterization of a sufficient number of proteins to essentially account for this entire coding capacity. These consist of a protein with RNA-dependent DNA-polymerase enzymatic activity as well as structural components of the virion, including a 70,000 molecular weight envelope glycoprotein and several low molecular weight nonglycosylated proteins. In addition, a number of type-C virus isolates are known to have acquired transformation-specific sequences by recombination with host cell genes. Such recombinant viruses, which in general are replication-defective, apparently code for one or more pro-

teins associated with malignant transformation. In the following sections, currently available information regarding the properties of type-C viral translational products, with emphasis on possible functions, is reviewed. In addition, an attempt has been made to define and genetically map regions of the viral genome coding for individual translational products.

A. RNA-Dependent DNA-Polymerase

The RNA-dependent DNA-polymerase (RDDP), also known as "reverse transcriptase," has the capacity to use both, polyribonucleotides and polydeoxyribonucleotides as template to synthesize complementary DNA (Baltimore, 1970; Temin and Mizutani, 1970; Baltimore and Smoler, 1971; Spiegelman *et al.*, 1970a,b; Temin and Baltimore, 1972; Verma, 1977). The purified RDDP also exhibits ribonuclease activity "RNase H" which can selectively degrade the RNA moiety of RNA-DNA hybrids (Moelling *et al.*, 1971; Baltimore and Smoler, 1972; Keller and Crouch, 1972; Leis *et al.*, 1973). Analysis of mutants of avian and mammalian type-C viruses, characterized by temperature-sensitive lesions in their RNA-dependent DNA-polymerase, DNA-dependent DNA-polymerase and RNase H activities convincingly demonstrated these activities to be virus-coded and essential for integration of the viral genome into the cellular DNA (Linial and Mason, 1973; Mason *et al.*, 1974; Verma *et al.*, 1974, 1976; Tronick *et al.*, 1975).

Most of the viral RDDP requires a primer such as transfer RNA and some metal ions for activity (Dahlberg *et al.*, 1974b; Hasteline and Baltimore, 1976; Grandgenett, 1976b). Thus, the type-C viral enzyme prefers Mn^{2+} ions while type-B and type-D viruses prefer Mg^{2+} ions for their activity (Scolnick *et al.*, 1970; Howk *et al.*, 1973; Abrell and Gallo, 1973; Michalides *et al.*, 1975). In addition to transcription of their natural template, all viral polymerases faithfully copy synthetic template-primers, such as poly(A) · oligo(dT), poly(C) · oligo(dG), to various extents (Spiegelman *et al.*, 1970a,b; Mizutani *et al.*, 1970; Riman and Beaudreau, 1970). Recently, optimal conditions for reverse transcription of complete copy of the viral genome *in vitro* have been described (Rothenberg and Baltimore, 1977). Under conditions of limiting Mg^{2+} ion concentration, full length, apparently infectious (Rothenberg *et al.*, 1977) complementary DNA copies of the viral RNA can be synthesized.

The RDDP from the murine leukemia viruses has been shown to consist of a single polypoptide of about 70,000 molecular weight (Ross *et al.*, 1971; Tronick *et al.*, 1972; Gerwin and Milstein, 1972;

Hurwitz and Leis, 1972). In contrast, the avian type-C viral reverse transcriptase contains two subunits, α (70,000) and β (110,000) (Temin and Baltimore, 1972; Verma *et al.*, 1974; Gibson and Verma, 1974; Kacian *et al.*, 1971; Grandgenett *et al.*, 1973). The α subunit exhibits both polymerase and nuclease activities while the β subunit apparently enhances the binding of α to the template or substrate (Verma *et al.*, 1974; Gibson and Verma, 1974; Grandgenett and Green, 1974; Moelling, 1974; Grandgenett, 1976a). Pulse-labeling of the Rauscher (R)-MuLV infected mouse cells has indicated that RDDP is initially synthesized in the form of a large precursor protein of about 200,000 molecular weight (Naso *et al.*, 1975; Arlinghaus *et al.*, 1976). Post-translational cleavage of this high molecular weight precursor gives rise to an 80,000 *gag* gene-coded precursor and the viral RDDP of about 75,000 molecular weight (Naso *et al.*, 1975; Arlinghaus *et al.*, 1976).

The reverse transcriptase also provides a useful antigentic marker for the identification and characterization of type-C viruses of diverse origin (Aaronson *et al.*, 1971a; Scolnick *et al.*, 1972a). Antisera prepared against the enzyme of a given mammalian type-C virus most strongly inhibits the activity of the homologous enzyme and to a lesser degree enzymes of type-C virus isolates of other mammalian species (Aaronson *et al.*, 1971a; Scolnick *et al.*, 1972a; Parks *et al.*, 1972). However, antisera to mammalian type-C viral enzymes do not inhibit the reverse transcriptases of avian type-C viruses or of mammalian oncornaviruses that are not type-C viral in origin (Aaronson *et al.*, 1971a; Scolnick *et al.*, 1972a). Recently, radioimmunoassays for the RNA-dependent DNA-polymerases of avian (Panet *et al.*, 1975; Reynolds and Stephenson, 1977) and mammalian (Krakower *et al.*, 1977) type-C viruses have been described. By use of homologous competition assay for R-MuLV it was possible to distinguish R-MuLV enzyme from that of other murine viruses while in heterologous more broadly reactive assays, a number of mammalian type-C viruses showed immunologic cross-reactivity (Krakower *et al.*, 1977). Application of competition immunoassays for the viral reverse transcriptase to studies of intracellular RDDP expression have led to the demonstration that translation of the type-C viral genome must involve more than one initiation site (Reynolds and Stephenson, 1977).

B. Structural Proteins

Mammalian type-C viruses of diverse species of origin exhibit marked similarities in their structural components. Thus, it has been

possible to identify functionally analogous structural proteins of different type-C RNA viruses on the basis of their biochemical and immunologic properties. Type-C viral structural proteins can be separated into two groups on the basis of the map positions at which they are coded within the viral genome. One group, which includes the major envelope glycoprotein (gp70) and a nonglycosylated 15,000 molecular weight protein (p15E) are synthesized in the form of a common precursor coded for by a viral gene generally referred to as *env*. The remaining viral proteins are characterized by molecular weights in the 10,000 to 30,000 range and are synthesized as a 65,000 molecular weight precursor protein coded for by a region of the viral genome designated *gag*. These latter proteins are nonglycosylated and are generally thought to be located in the nucleoid or core of the virion. The biochemical and immunologic properties and post-translational processing of *env* and *gag* coded structural proteins are considered below.

1. Env *Gene-Coded Proteins*

There is accumulating evidence from a number of laboratories consistent with the possibility that the major envelope glycoprotein, gp70, and a lower molecular weight, nonglycosylated virion structural protein, p15E, are initially synthesized in the form of a common precursor. This is indicated by the demonstration of an 85,000–90,000 molecular weight glycoprotein in R-MuLV infected cells which is precipitable by anti-gp70 and anti-p15(E) sera and which by pulse chase experiments gives rise to cleavage products of around 70,000 and 15,000 molecular weights, respectively. Methionine-labeled peptide sequences analogous to those of gp70 and p15(E) within this precursor have been identified by tryptic digest analysis (Arcement *et al.*, 1976; Shapiro *et al.*, 1976; Van Zaane *et al.*, 1976; Famulari *et al.*, 1976). Inhibition of glycosylation of the primary *env* gene product by use of 2-deoxy-D-glucose or cytochalasin B leads to formation of a 70,000 molecular weight nonglycosylated protein (Shapiro *et al.*, 1976; W. J. M. van de Ven, personal communication) which presumably represents *env* gene translational product prior to glycosylation. The product-precursor relationships between these various *env* gene coded proteins have been confirmed by *in vitro* protein synthesis studies (Gielkens *et al.*, 1974; Van Zaane *et al.*, 1977). For instance, in a rabbit reticulocyte cell-free system, 22 S mRNA, isolated from R-MuLV infected cells, was shown to code for synthesis of a 70,000 nonglycosylated protein containing antigenic determinants in common with gp70 (Gielkens *et al.*, 1974). Injection of the same mRNA