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Retroviruses 2

Edited by P.K. Vogt and H. Koprowski

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Acute Transforming Feline Retroviruses

PETER BESMER

1 Introduction

The study of retroviruses associated with the domestic cat (*Felis catus*) has been of interest mainly because of the infective nature of these viruses in the domestic cat population and their association with neoplasms of an outbred mammalian species. The feline retroviruses can be divided into three groups: (a) the feline leukemia viruses (FeLVs), (b) the endogenous, xenotropic feline retroviruses, and (c) the acute transforming feline retroviruses (ATVs).

Feline leukemia viruses have been divided into subgroups according to their host range and interference properties (SARMA and LOG 1971, 1973; JARRETT 1980). All of them replicate in feline cells and they therefore are ecotropic viruses. FeLVs are horizontally transmitted in the cat population and are associated with a wide spectrum of neoplastic and degenerative blastopenic diseases: lymphosarcoma, erythremic myelosis, erythroleukemia, megakaryocytic leukemia, granulocytic leukemia, fibrosarcoma, erythroblastosis, pancytopenia, myeloblastopenia, glomerulonephritis and thymic atrophy (HARDY et al. 1973; JARRETT et al. 1973a; HARDY 1980). The FeLVs

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are chronic leukemia viruses, i.e., they induce neoplasms with a long latency period and do not transform fibroblasts in culture (RICKARD et al. 1969). Similar to other retroviruses, the integrated provirus of FeLV contains LTR sequences at both the 5′ and the 3′ end and the three structural genes gag, pol, and env, all of which are needed for the replication of the virus. DNA sequences homologous to FeLV are present in the genome of uninfected domestic cats (BENVENISTE et al. 1975). These endogenous FeLV-related sequences, about seven to nine copies per haploid genome, do not represent complete FeLV genomes, suggesting that FeLV is maintained in the cat population by exogenous infection. Nucleic acid sequence homology exists between FeLV and rodent retroviruses (BENVENISTE et al. 1975). It is, therefore, believed that FeLVs derive from an ancestral rodent retrovirus by cross-species infection.

The *xenotropic endogenous feline retroviruses*, which include the RD114 and the CCC viruses (MCALLISTER et al. 1972), constitute a separate class of feline retroviruses. They are distinct from FeLV by nucleic acid and by serological criteria as well as by host range and interference properties (EAST et al. 1973; TODARO et al. 1974; SHERR and TODARO 1974; HENDERSON et al. 1974). Sequences homologous to the CCC/RD114 virus are found in the genome of domestic cats (TODARO et al. 1974; BALUDA and ROY-BURMAN 1973). In contrast to the FeLV proviruses contained in the genome of uninfected cats, the endogenous CCC/RD114-related sequences represent complete viral genomes (LIVINGSTON and TODARO 1973). The viruses of the CCC/RD114 group are not oncogenic. Interestingly, the xenotropic feline retroviruses are related to the Baboon endogenous virus.

Acute transforming feline retroviruses (feline ATVs) were isolated from FeLV-associated feline multicentric fibrosarcomas. They induce neoplasms with a short latency period in animals and transform tissue culture cells in vitro. All feline ATVs are replication defective and need a helper virus for their propagation. Their genomes contain FeLV-related sequences and transformation-specific sequences (v-*onc* sequences) (FRANKEL et al. 1979). The v-*onc* sequences in these viruses are homologous with single-copy cellular genes (c-*oncs*), and ATVs therefore are thought to have arisen by recombination of FeLV with cat c-*onc* sequences (FRANKEL et al. 1979; SHERR et al. 1979). The oncogenic properties of ATVs are believed to be determined by the v-*onc*c sequences in these viruses. Until now, four different v-*onc* genes have been found in feline ATVs. One of the feline v-*onc* genes, v-*fms*, is unique. The other three, v-*fes*, v-*sis*, and v-*abl*, have also been identified in ATVs isolated from other species (WEISS et al. 1982).

The main purpose of this paper is to review the molecular biology of feline ATVs. Specifically, I will discuss the history of their isolation, their in vivo and in vitro transformation specificity, their genome structure, viral RNA and protein expression, and the viral oncogenes and their origins. Where appropriate I will compare feline ATVs with acute transforming viruses from other species with homologous v-*onc* genes. Since all feline ATVs derive by recombination from FeLV and cat cellular sequences, I will also briefly discuss the molecular biology of FeLV.

2 Isolation and In Vivo Transformation Specificities of Feline ATVs

Fibrosarcomas account for 6%–12% of all cat neoplasms (HARDY 1981). They are found mostly in older cats (mean age 10 years) and tend to be slow-growing solitary tumors. No ATVs have been isolated from old cats with solitary fibrosarcomas. Less frequently, multicentric fibrosarcomas are found in younger cats (mean age 3 years). These multicentric fibrosarcomas are often associated with FeLV infection. Feline acute transforming viruses have been obtained from the FeLV-associated multicentric fibrosarcomas of younger cats. Historically, sarcoma viruses were identified and isolated by induction of neoplasms in fetal or neonatal kittens with cell-free tumor extracts (SNYDER and THEILEN 1969). More recently, transforming agents have also been identified by focus induction in tissue culture cells with cell-free tumor extracts (HARDY et al. 1982; BESMER et al. 1983b).

The first isolation of a feline sarcoma virus (FeSV) was reported by Snyder and Theilen in 1969 (SNYDER and THEILEN 1969). Subcutaneous injection of cell-free tumor extracts from a cat with multiple subcutaneous fibrosarcoma and multiple metastasis was shown to induce fibrosarcomas in newborn kittens. This first acute transforming feline retrovirus, the Snyder-Theilen feline sarcoma virus, was designated ST-FeSV. The second feline ATV, the Gardner-Arnstein FeSV (GA-FeSV), was isolated from a Siamese cat with recurrent multiple subcutaneous fibrosarcoma (GARDNER et al. 1970). Cell-free tumor extracts again were found to induce fibrosarcoma in fetal and newborn kittens. When injected subcutaneously, the GA-FeSV induced melanomas and fibrosarcoma at the site of injection, while upon intraocular injection only melanomas were obtained (MCCULLOUGH et al. 1972; NIEDERKORN et al. 1980). The GA-FeSV therefore transforms cells of mesodermal and ectodermal origin. Experimental infection of species other than the cat with ST- and GA-FeSV demonstrated that fibrosarcomas can be induced in a wide range of different mammals, including guinea pigs, rabbits, dogs, goats, sheep, and a number of primates (reviewed in

Table 1. Characteristics of acute transforming feline retroviruses

Strain designation		Types of neoplasms induced in kittens	v-*onc*	Protein product of v-*onc*	Protein kinase activity
Snyder-Theilen	FeSV (ST-FeSV)	Fibrosarcoma	*fes*	P85 *gag-fes*	+
Gardner-Arnstein	FeSV (GA-FeSV)	Fibrosarcoma and melanoma	*fes*	P95 *gag-fes*	+
Hardy-Zuckerman 1	FeSV (HZ1-FeSV)	Fibrosarcoma	*fes*	P96 *gag-fes*	+
McDonough	FeSV (SM-FeSV)	Fibrosarcoma	*fms*	gp170 *gag-fms*	–
Parodi-Irgens	FeSV (PI-FeSV)	Fibrosarcoma	*sis*	P76 *gag-sis*	–
Gardner-Rasheed	FeSV (GR-FeSV)	Fibrosarcoma	?	P70 *gag-onc* ?	+
Hardy-Zuckerman 2	FeSV (HZ2-FeSV)	Fibrosarcoma	*abl*	P96 *gag-abl*	+

HARDY 1981). Transformation by ST- and GA-FeSV thus is not limited to the feline species. Murine and avian sarcoma viruses similarly have been shown to induce fibrosarcomas in other species. Pseudotypes of GA-FeSV with Moloney-MuLV and with amphotropic MuLV have been used to infect neonatal NFS mice, but no tumors were detected in the infected animals (EVEN et al. 1983). This is surprising since the same virus preparations easily induce transformation in murine cells in vitro.

The SM-FeSV was isolated in 1971 by MCDONOUGH and collaborators (MCDONOUGH et al. 1971). This virus was obtained from a $1^1/_2$ years old domestic short-hair cat with recurrent multiple subcutaneous fibrosarcoma. Another ATV strain, the Parodi-Irgens FeSV (PI-FeSV), was isolated in France (IRGENS et al. 1973). The PI-FeSV derived from a $1^1/_2$ year old cat with multiple subcutaneous fibrosarcoma with metastatic lesions in the lung and the peritoneum. This virus too induced sarcomas in newborn kittens. The Gardner-Rasheed FeSV (GR-FeSV) was isolated in 1982 (RASHEED et al. 1982). The fibrosarcoma from which the GR-FeSV was isolated contained a rhabdomyosarcomatous component, and upon intramuscular injection into kittens, neoplasms of the same type were obtained with metastasis to skeletal, visceral, and central nervous system tissues. In addition Snyder reported three transmissible fibrosarcomas: from a 14-month-old cat with multiple subcutaneous fibrosarcoma, from a 7-year-old cat with a solitary fibrosarcoma in the thorax with metastasis in the lung and the pleural cavity, and from a second 7-year-old cat with solitary fibrosarcoma in the deep fascia of the neck. Unfortunately the viruses isolated from these tumors have been lost (SNYDER 1971).

Two ATVs have recently been isolated in Hardy's laboratory. In contrast to earlier isolations of feline ATVs, the Hardy-Zuckerman strains of FeSV, HZ1-FeSV and HZ2-FeSV, were isolated by tissue culture procedures (SNYDER et al., in preparation; BESMER et al. 1983b). Both the HZ1- and the HZ2-FeSV derive from multicentric fibrosarcomas of pet cats. Upon injection of cell free tumor extracts into kittens, both viruses the HZ2-FeSV again induced fibrosarcomas.

All of the known feline ATVs induce tumors in vivo of the same type from which the virus originally was isolated. Only one, the GA-FeSV, was found to cause different types of neoplasms, namely fibrosarcomas and melanomas. No systematic studies have, however, been carried out, e.g., using different routes of inoculation in order to identify in vivo transformation targets other than those of the fibroblast cell lineage.

3 Molecular Biology of Feline ATVs

All the original isolates of feline ATVs were mixtures of ATV and FeLV. Very often the FeLV helper virus is present in large excess (100–1000-fold) (SARMA et al. 1971a; BESMER et al. 1983a). This situation has masked the replication-defective nature of the sarcoma viruses. The isolation of virus

nonproducer cells, i.e., cells that are infected by the ATV in the absence of helper virus, was therefore an important step in the analysis of the genomes and the transcriptional and translational products of these viruses (CHAN et al. 1974; HENDERSON et al. 1974; PORZIG et al. 1979). Transformed virus nonproducer cells can be obtained by low multiplicity infection of cells and isolation of foci of morphologically transformed cells. The most common cell lines used for the generation of virus nonproducer cells are the mink lung cell line (CCL 64), FRE and NRK rat cells, and NIH/3T3 mouse cells. For the propagation of the ATVs these can be rescued from the virus nonproducer cells by superinfection with replication-competent mammalian retroviruses such as the amphotropic MuLV, FeLV, or Moloney-MuLV. The amphotropic murine leukemic virus is particularly versatile for this purpose because of its broad host range (RASHEED et al. 1976) and because phenotypic mixing between FeLV and its derivative ATVs with murine retroviruses is a very efficient process (EVEN et al. 1983).

Of great importance in the study of feline ATVs was the demonstration of the recombinational origin of ATVs from FeLV and cat cellular sequences (FRANKEL et al. 1979). The techniques employed in these early studies were analogous to those used for the characterization of ATVs in other species at the time and involved the preparation of ATV-specific cDNA hybridization probes (STEHELIN et al. 1976). Since then the molecular structure and the genetic content of the genomes of the ST-, GA-, and SM-FeSV have been studied using recombinant DNA techniques. More recently, new isolates of feline ATVs have been obtained. Their characterization indicates that FeLV transduces a surprising variety of cellular oncogens in the pet cat population and that retroviruses of different species are able to transduce homologous cellular oncogenes.

3.1 Feline Leukemia Virus (FeLV)

Feline leukemia viruses have been divided into three subgroups, A, B, and C, according to their host range and interference properties (SARMA and LOG 1971, 1973; JARRETT et al. 1973b; JARRET (1980a, b). They are ecotropic retroviruses, that is, all of them replicate on cat cells. FeLV-A has the narrowest host range, growing preferentially on cat cells; FeLV-B and FeLV-C on the other hand replicate also on cells from other species such as mink, dog, and human (SARMA et al. 1975). All naturally occurring FeLV isolates contain FeLV-A, so that isolates of FeLV-B and FeLV-C are always contained in mixtures with FeLV-A (JARRETT 1980a, b).

By liquid nucleic acid hybridization techniques, the different subgroup FeLVs are highly related (ROBBINS et al. 1979). Using a more sensitive method, T1 oligonucleotide RNA fingerprinting, Rosenberg and Hazeltine found a remarkable amount of sequence identity between different FeLV strains (ROSENBERG et al. 1981). No oligonucleotide maps, however, were made from any of the analyzed FeLVs in order to determine whether differences observed in the various strains map to a particular region of the FeLV

genome. Two subgroup B FeLVs, the ST-FeLV and the GA-FeLV, have been molecularly cloned and analyzed. The ST-FeLV-B was cloned from unintegrated proviral DNA obtained after infection, and the integrated GA-FeLV-B genome was cloned from cellular DNA of GA-FeLV-infected human cells (SHERR et al. 1980a; MULLINS et al. 1981). The restriction maps of the two viral genomes indicate a very close relationship between the two viruses. For 10 restriction enzymes applied, 12 restriction enzyme sites are common to both viruses. 10 sites are unique for the ST-FeLV, and 1 is unique for the GA-FeLV.

Nucleic acid homology has been found between FeLV and murine retroviruses, indicating that FeLV and murine retroviruses have a common ancestor, and it has been proposed that FeLVs derive from an ancestral murine retrovirus by cross-species infection (BENVENISTE et al. 1975). In order to identify the regions of homology between FeLV and MuLV, SHERR and co-workers have analyzed in the electron microscope heteroduplexes formed under nonstringent hybridization conditions between FeLV DNA and Moloney murine sarcoma virus DNA. Two segments of homology, 1.5 kb and 0.5 kb in length, were identified. The 1.5-kb segment contains the U5 sequences of the 5′ LTR and sequences corresponding to the N terminus of the *gag* gene, and the 0.5-kb segment corresponds to sequences of the *pol-env* junction of the two viral genomes. SHERR and co-workers recently compared the nucleotide sequences of the LTR and the sequences corresponding to the N-terminus of the *gag* gene of the GA-FeLV with those of Moloney-MuLV and found extensive homology in agreement with the earlier EM-heteroduplex results (HAMPE et al. 1983). This indicates that the 5′-noncoding regulatory sequences of murine and feline retroviruses are highly conserved. Similarly, the coding sequences of the *gag* protein p15 of MuLV and FeLV are homologous.

The proteins which are coded by the FeLV genome show great structural similarity with those of murine retroviruses. The *gag* gene products are synthesized as a 65000-dalton polyprotein precursor, which is processed into the structural proteins p15, p12, p30, and p10 (OKAZINSKI and VELICER 1976, 1977). The arrangement of the *gag* proteins in the polyprotein precursor has been suggested to be NH2 p15-p12-p30-p10-COOH (KAHN and STEPHENSON 1977). Recent nucleic acid sequence analysis has confirmed this order, and the FeLV *gag* proteins therefore are analogous to those of the murine retroviruses (HAMPE et al. 1982). Similarly to MuLV-infected cells, FeLV-infected cells synthesize a glycosylated *gag* polyprotein. The size of the intracellular form of this protein was found to be 82000 daltons (NEIL et al. 1980). The FeLV-encoded reverse transcriptase has a molecular weight of 70000 daltons (SCOLNICK et al. 1972; RHO and GALLO 1979). The enzymatic activities of the reverse transcriptase protein of FeLV, however, have not been characterized extensively. The envelope glycoprotein of FeLV consists of a 70000-dalton component (gp70) which is linked by disulfide bonds to a second component, a nonglycosylated 15000-dalton protein (p15E), to form a 90000-dalton glycoprotein complex (VELICER and GRAVES 1974; PINTER and FLEISSNER 1979).

3.2 v-*fes* ATVs: ST- and GA-FeSV

The GA- and the ST-FeSV are replication-defective ATVs. Both of them transform fibroblastic and epithelioid cells in culture, and transformed virus nonproducer cell lines were derived in mink CCL64 and in rat NRK cells (HENDERSON et al. 1974; PORZIG et al. 1979). An initial understanding of the genetic composition of the genomes of the ST- and the GA-FeSV was obtained from liquid hybridization experiments such as those employed in the characterization of the v-*src* sequences of Rous sarcoma virus by STEHELIN and co-workers (STEHELIN et al. 1976; FRANKEL et al. 1979). A cDNA was synthesized from viral RNA of FeSV (FeLV) pseudotype virus. cDNA sequences specific to the FeSV genome were prepared by sequential adsorption with FeLV RNA and RNA isolated from FeSV nonproducer cells and hydroxyapatite chromatography. The genomes of the ST- and the GA-FeSV were found to be composed of sequences related to FeLV and sequences specific to both the ST- and the GA-FeSV (*fes* sequences). Sequences homologous to the *fes* cDNA were found in normal cat DNA at an abundance corresponding to a single copy gene. These experiments thus indicated the recombinational origins of the ST- and the GA-FeSV.

In 1980 Shibuya and collaborators found that the *fps* sequences of the Fujinami avian sarcoma virus are homologous with the *fes* sequences of the ST- and the GA-FeSV (SHIBUYA et al. 1980). This was the first demonstration that retroviruses from different species are able to transduce homologous cellular genes (c-*oncs*). The *fes* oncogene is the most prevalent oncogene found in acute transforming retroviruses. At present three feline ATVs and five avian ATVs are known to contain this oncogene.

More insight into the organization of the ST-FeSV genome was obtained from restriction mapping of unintegrated proviral DNA isolated from cells after infection and of molecularly cloned FeSV DNA genomes (SHERR et al. 1979, 1980a, b; FEDELE et al. 1981). The ST- and the GA-FeSV have a very similar genome organization; they have RNA genomes of 4.5 and 6.2 kb respectively. At the 5′ end both of them contain 1.3–1.4 kb of sequences related with FeLV and at the 3′ end the ST-FeSV contains 1.3–1.4 kb and the GA-FeSV 2.8 kb of FeLV-related sequences. The *fes* sequences in the middle consist of segments of 1.6 kb and 2.0 kb respectively. The genome structure in both the ST-FeSV and the GA-FeSV therefore appears to be 5′-*Δgag-fes-Δenv* 3′. This then predicts that the 85000- and 95000-dalton *gag*-polyproteins found in ST- and GA-FeSV-infected cells are *gag-fes* fusion proteins (STEPHENSON et al. 1977; SHERR et al. 1978; SNYDER et al. 1978). The genome organization of the ST- and the GA-FeSV is reminiscent of that of Abelson murine leukemia virus, the Fujinami, PRCII, and Y73 strains of avian sarcoma virus, and the avian acute transforming viruses, MC29, MH2, CMII, and E26, where helper virus *gag* sequences are fused to v-*onc* sequences to give rise to *gag*-v-*onc* fusion protein products (WEISS et al. 1982).

The nucleic acid sequences of the *gag-fes* genes of the ST- and the GA-FeSV and that of the Fujinami avian sarcoma virus (FSV) have recently

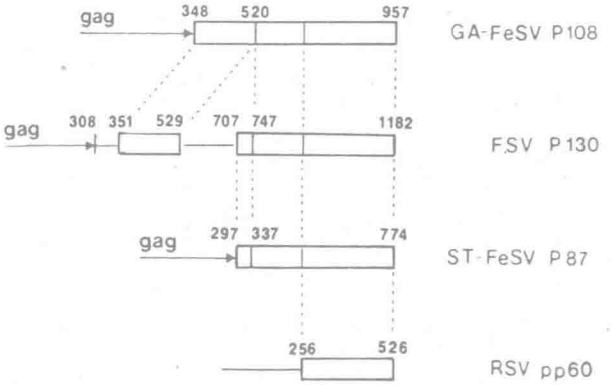

Fig. 1. Homologous amino acid domains in ST-FeSV, GA-FeSV, Fujinami sarcoma virus (FSV), and Rous sarcoma virus (RSV) transforming proteins. The *gag-onc* junctions in ST-FeSV, GA-FeSV, and FSV polyproteins are marked by *arrowheads*. Homologous domains are shown as *open rectangles* and are connected by *dotted lines*. (HAMPE et al. 1982)

been published (HAMPE et al. 1982; SHIBUYA and HANAFUSA 1982). Their comparison revealed interesting features of the related transforming proteins (Fig. 1). At the N-terminus the *gag-fes* proteins of GA- and ST-FeSV contain 348 and 297 amino acids each of the *gag* sequences p15, p12, and p30. The GA strain contains 151 amino acids of p30 sequences and the ST strain 100 p30 amino acids. v-*fes* of the ST and the GA strains encode 477 and 609 amino acids respectively. The C-terminal 437 amino acids of the ST and the GA *gag-fes* proteins are almost completely homologous. The 3′ ends of both the ST and the GA v-*fes* sequences terminate 51 nucleotides beyond the polyprotein coding sequences. Interestingly, the 3′ v-*fes* sequences are joined to identical FeLV sequences in both the ST- and the GA-FeSV. The FSV genome encodes a 130000-dalton *gag-fps* polyprotein. It contains 308 amino acids derived from the ALV *gag* genes p15, p10, and p27 and 871 amino acids derived from the *fps* sequences. Between the FSV, Ga-FeSV, and ST-FeSV *gag-fps* and *gag-fes* proteins there is an overall homology of approximately 70%. At the 5′ end the v-*fes* sequences of the GA- and the ST-FeSV strain differ. These strain-specific v-*fes* sequences of both the ST- and the GA-FeSV are represented in the FSV v-*fps* gene (Fig. 1). The ST-specific sequences are found in the middle of v-*fps* and those of the GA strain further upstream near the 5′ end of the FSV v-*fps*. With respect to v-*fps*, the GA-FeSV v-*fes* sequences therefore appear to contain an internal deletion (Fig. 1). It seems likely that the GA-FeSV derived by deletion from a progenitor similar to FSV upon passage of the virus in vitro or in vivo. This sequence comparison also suggests that the N-terminal 440 amino acids of v-*fps* may not be essential for the neoplastic properties of FSV. In the carboxyterminal domain the GA-FeSV, the ST-FeSV, and the FSV v-*fes* and v-*fps* sequences show extensive homology (85%). The extensive structural and functional relationship between v-*fps*

and v-*fes* indicates that the two viral oncogens derive from homologous cellular genes of cats and birds. Of considerable interest was the finding of an amino acid homology of about 40% between the C-terminal domains of the *gag-fes* protein (about 40%) and those of the transforming protein pp60*src* of Rous sarcoma virus and the transforming protein of the avian sarcoma virus Y73 (Fig. 1). The relationship between v-*fps*, v-*src*, and v-*yes*, all of them of avian origin, suggests that these genes have evolved from a common ancestral gene.

Cells infected by the ST- and the GA-FeSV synthesize only full-length genome RNA. No subgenomic RNAs have been reported (SHERR, personal communication). In agreement with the nucleotide sequence of the two viruses, *gag-fes* polyproteins of 85000 and 95000 daltons were identified in infected cells (BARBACID et al. 1980a; RUSCETTI et al. 1980). These polyproteins contain antigenic determinants of the FeLV *gag* proteins p15, p12, and p30. Two minor glycosylated forms of the *gag-fes* polyprotein have also been observed with electrophoretic mobilities of 80000 daltons and 100000 daltons (SHERR et al. 1980b). Similar to the transforming proteins of Rous sarcoma virus and Abelson murine leukemia virus, the *gag-fes* proteins exhibit a tyrosine-specific protein kinase activity in immunoprecipitates. Substrates for the in vitro phosphorylation include the *gag-fes* protein as well as casein. Phosphorylation of immunoglobulin heavy chain has been observed by some investigators but not by others (BARBACID et al. 1980b; VAN DE VEN et al. 1980a; SNYDER 1982). Very likely as a consequence of the viral protein kinase activity, cells transformed by the ST- and the GA-FeSV have elevated levels of proteins containing phosphotyrosine. The two viral *gag-fes* proteins have a single site for tyrosine phosphorylation (BLOMBERG et al. 1981). In analogy with Rous sarcoma virus, this single phosporylation site has been predicted to be in the C-terminal domain of the *gag-fes* proteins. This predicted position is in agreement with recent sequence analysis of phosphotyrosine peptides (PATCHINSKY et al. 1982).

In order to investigate the role of the *gag-fes* protein in the initiation and maintenance of cell transformation, transformation-defective mutants (td mutants) of ST-FeSV were isolated by two groups (DONNER et al. 1980; REYNOLDS et al. 1981b). Cells which harbor transmissible td FeSV variants exhibit a nontransformed phenotype: they do not grow in semisolid medium, they bind high levels of epidermal growth factor, and they express the *gag-fes* polyprotein. Cells containing the mutant virus, however, do not have elevated levels of proteins containing phosphotyrosine, and the *gag-fes* protein is inactive in the in vitro phosphorylation assay (BARBACID et al., 1981; REYNOLDS et al. 1981b). These observations suggest that the protein kinase activity which is associated with the *gag-fes* protein is required for the transformation induced by the virus. The sequences of the GA-FeSV genome which are required for transformation have recently also been defined by in vitro mutagenesis of the molecularly cloned GA-FeSV provirus. The 5′ LTR sequences which contain the signals for initiation of transcription and enhancer elements which regulate the efficiency of transcription as well as the coding region for the entire *gag-fes* protein were found to be necessary

for initiation and maintenance of transformation (BARBACID 1981; EVEN et al. 1983) Both the in vitro mutagenesis studies and the investigation of the td mutants have identified the *gag-fes* protein as the transforming protein of this virus.

3.3 v-*fms* ATVs: SM-FeSV

Like all other feline sarcoma viruses, the SM-FeSV is replication defective, and the isolation of transformed virus nonproducer cells made possible the characterization of the viral genome and it's gene products (PORZIG et al. 1979). Initial liquid hybridization experiments showed that the SM-FeSV genome is composed of FeLV-related and FeSV-specific nucleic acid sequences. The SM-FeSV specific sequences were designated v-*fms* and were shown to be homologous with a single-copy cat cellular gene (FRANKEL et al. 1979). These early studies indicated the recombinational origin of the SM-FeSV form FeLV and cat cellular sequences. The following structural analysis of a molecularly cloned integrated provirus provided more information about the organization of the SM-FeSV genome (DONNER et al. 1982). The virus has a 7.8-kb RNA genome. At the 5′ end it contains 2.1 kb of FeLV related sequences. The v-*fms* sequences are located on a 3.1-kb contiguous segment in the middle of the genome. Similar to GA-FeSV and ST-FeSV v-*fms* is joined to FeLV sequences 5′ of the *env* gene of FeLV. The gene order in the SM-FeSV thus was found to be 5′-*Δgag-fms-env*3′. By nucleic acid hybridization v-*fms* is not related to v-*fes*, v-*mos*, v-*abl*, v-*kis*, v-*has*, v-*sis*, and v-*src*. It is believed to be a unique oncogene. The sequence relationship between v-*fms* and the avian oncogenes v-*myc*, v-*erb*, v-*myb*, and v-*yes*, however, has not been reported yet.

Cells infected by the SM-FeSV synthesize full-length genome RNA and a subgenomic *env* mRNA. The following protein products which are specified by the SM-FeSV genome have been detected in infect cells: 170000-dalton gag polyprotein, a gp140, a gp120, and a *gag* p60 protein (BARBACID et al. 1980a; RUSCETTI et al. 1980). The gene order 5′ *gag-fms* predicts that the 170000-kilodalton *gag* polyprotein is a *gag-fms* fusion protein. Hyperimmune antisera which recognize v-*fms* sequences in the 170000-dalton *gag-fms* protein were reported some time ago (BARBACID et al. 1980; RUSCETTI et al. 1980; VAN DE VEN et al. 1980b). More recently monoclonal antibodies to epitopes of the v-*fms* sequences have been prepared by two groups (VERONESE et al. 1982; ANDERSON et al. 1982). The v-*fms* specific monoclonal antibodies reacted with three different proteins: the gp170 *gag-fms* polyprotein, a gp140 *fms*, and a gp-120 *fms* protein. Steady state analysis of these proteins in infected cells showed that the gp140 and gp120 species are the predominant products. In contrast to other transforming proteins, the v-*fms* protein products are extensively glycosylated. Upon tunicamycin treatment of infected cells a 155000-dalton unglycosylated *fms* containing protein was found, indicating that the primary translation product is a 155000-dalton *gag-fms* protein which is glycosylated to yield the gp170 *gag-fms* protein.

The gp170-protein possibly then is cleaved to yield the gp120 and gp140 *fms* proteins and the p60 *gag* protein. The v-*fms* containing protein products localize to the cytoplasm of infected cells and are quantitatively associated with sedimentable organelles. No significant tyrosine-specific protein kinase activity has been found to be associated with the *gag-fms* protein nor is this protein phosphorylated in vivo (REYNOLDS et al. 1981a). Similarly, no elevated levels of phosphotyrosine-containing proteins were detected in infected cells. When coprecipitated with the ST- or the GA-FeSV *gag-fes* proteins, a low level of gp170 *gag-fms* phosphorylation was observed and this phosphorylation was shown to be specific for tyrosine (VERONESE et al. 1982). Thus although the gp170 *gag-fms* protein lacks detectable protein kinase activity, it contains acceptor sites for tyrosine phosphorylation. From this evidence it seems probable that v-*fms* is not a member of the tyrosine kinase gene family. A comparison of the nucleotide sequence of v-*fms* with v-*fes*, v-*src*, v-*yes*, v-*abl*, and v-*mos* will, however, be necessary to confirm this prediction. No transformation-defective mutants have been isolated, nor have in vitro mutagenesis studies been reported to support the assumption that the *gag-fms* polyprotein and/or its cleavage products are the transforming protein of the SM-FeSV.

3.4 v-*abl* ATVs: HZ2-FeSV

The Hardy-Zuckerman 2 strain of FeSV (HZ2-FeSV), like all other FeSVs, derives from a cat with multicentric fibrosarcoma (BESMER et al. 1983b). The genome and the gene products of this replication-defective acute transforming retrovirus were analyzed in transformed virus nonproducer mink cells. The HZ2-FeSV provirus in the cellular DNA of mink virus nonproducer cells and the v-*onc* sequences contained in this virus were investigated by Southern blot analysis. It was found that restriction fragments which contained HZ2-FeSV sequences detected by an FeLV *rep* hybridization probe also hybridized with a v-*abl* hybridization probe, indicating that FeLV and v-*abl* sequences are linked in the HZ2-FeSV genome (BESMER et al. 1983b). It therefore appears that the v-*onc* sequences in the HZ2-FeSV are homologous with the v-*abl* sequences of the Abelson murine leukemia virus. The integrated provirus of the HZ2-FeSV has recently been cloned and analyzed in the author's laboratory. At the 5′ end it is homologous with 5′ FeLV sequences, including coding sequences of the FeLV *gag* proteins p15, p12, and p30, and at the 3′ end the viral genome contains FeLV *env* sequences (BERGOLD and BESMER, unpublished data). The size and the genetic composition of the cat v-*abl* insert is not precisely known yet. At the 5′ end v-*abl* is joined to FeLV *gag* sequences and at the 3′ end to FeLV sequences 5′ of the *gag-pol* junction. The gene order in the HZ2-FeSV therefore is 5′-*Δgag-abl-env*3′. Virus nonproducer cells synthesize 6.3-kb genome RNA and a 2.3-kb subgenomic RNA. The 6.3-kb RNA contains 5′ *gag*, *abl*, and *env* sequences but lacks *pol* sequences. The subgenomic RNA, on the other hand, contains only FeLV *env* sequences. This suggests

that the v-*abl* gene product in this virus is expressed from the 6.3-kb RNA and not from the subgenomic RNA. A 98000-dalton *gag* polyprotein is synthesized in the mink HZ2-FeSV nonproducer cells. It contains determinants of the FeLV *gag* proteins p15, p12, and p30. The genome structure discussed above predicts that this *gag* polyprotein is a *gag-abl* fusion protein. When assayed for in vitro kinase activity in immunoprecipitates containing the 98000-dalton protein, the 98000-dalton protein as well as immunoglobulin heavy chain was phosphorylated and phosphorylation was shown to be specific for tyrosine residues (LEDERMAN and SNYDER, unpublished data). No FeLV *env* gene products were detected in HZ2-FeSV infected cells, indicating that the *env* gene in this virus is defective.

Transformed mink CCL64 virus nonproducer cells were obtained by infection of mink cells with the HZ2-FeSV complex, which contains subgroup A and subgroup B FeLV helper virus. Transformed mink cells are round, refractile, and very loosely attached to the tissue culture plates. The HZ2-FeSV can be rescued with amphotropic MuLV. Transformation indistinguishable from Abelson-MuLV is obtained after infection of NIH/3T3, Balb/3T3, and FRE-3a rat cells with HZ2-FeSV (amph-MuLV) pseudotype virus. No transformation is observed upon infection of FLF-3 and Fea cat fibroblasts (ZUCKERMAN and HARDY, unpublished data). Upon injection of three 6-week-old kittens with cell-free tumor extract, one kitten developed multicentric fibrosarcoma. No in vivo experiments have, however, been done with the biologically cloned HZ2-FeSV.

Abelson murine leukemia virus was generated by recombination of Moloney murine leukemia virus and c-*abl* sequences of a Balb/c mouse (ABELSON and RABSTEIN 1970a, b; GOFF et al. 1980; ROSENBERG 1982; RISSER 1982). It is a replication-defective acute tranforming virus. The P160 strain of A-MuLV has been molecularly cloned and analyzed in detail (LATT et al. 1983). At the 5′ end A-MuLV contains 1.3 kb of Mol-MuLV sequences and at the 3′ end 0.7 kb of sequences homologous to the 3′ end of Mol-MuLV. The v-*abl* sequences in the middle of the genome consist of a contiguous 4.3-kb segment. The gene in this virus therefore is 5′-*Δgag-abl-Δ* env 3′. Toward the C-terminus the v-*src*, v-*fps*, v-*yes*, and v-*fes* protein sequences exhibit significant homology (SCHWARTZ et al. 1983; SHIBUYA and HANAFUSA 1982; KITAMURA et al. 1982; HAMPE et al. 1982). Homology exists also between these oncogene products and cyclic AMP-dependent protein kinase (BARKER and DAYHOFF 1982). Recently the nucleic acid sequence of v-*abl* has been found to display homology with the above-mentioned v-*oncs* (BALTIMORE et al., unpublished data). It appears, then, that *abl* is yet another member of the protein kinase gene family. Interestingly, however, in A-MuLV the homologous sequences are located toward the N-terminus of the v-*abl* sequences and not at the C-terminus as with v-*src*, v-*fps*, v-*yes*, and v-*mos*. It is possible that the additional C-terminal sequences of *abl* which are not found in other v-*onc* products contribute to the unique biological properties of the *gag-abl* gene product.

A-MuLV virus nonproducer cells synthesize a genome size virus-specific RNA and no subgenomic RNAs. An A-MuLV specific protein can be de-