To the Graduate Council:

I am submitting herewithin a dissertation written by Amanda Carr Sozer entitled "Ecotropic Retroviruses of Nonirradiated and Irradiated C57BL/10.F Mice." I have examined the final copy of this dissertation for form and content and recommend that it be accepted for partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biomedical Sciences.

Raymond A. Popp, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

ECOTROPIC RETROVIRUSES OF NONIRRADIATED AND IRRADIATED C57BL/10.F MICE

A Dissertation

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ABSTRACT

Mus musculus strains C57BL/10 and C57BL/10.F are congenic. Both strains carry a germline murine leukemia provirus, *emv-2*, on chromosome 8 but differ in the expression of ecotropic MuLV. Southern analysis of restriction endonuclease digested DNA and hybridization with a 400 base pair ecotropic virus specific probe were used to detect ecotropic proviruses. Restriction fragment length polymorphisms were used to identify unique proviral sequences, to determine partial restriction maps, and to examine the frequency and location of proviral integration of ecotropic MuLVs in the somatic cell DNA of these strains of mice.

DNA from the spleens of six-month-old male B10.F mice contained multiple copies of ecotropic proviruses that were not detected in the B10 mice. Every B10.F mouse examined contained a provirus different from *emv-2*; this provirus was designated B10Fv. In addition to *emv-2* and B10Fv, a variety of other novel proviruses were found in the DNA of B10.F splenocytes. These

proviruses were integrated into many different sites in the DNA at an average frequency of two copies per cell. These novel proviruses may be recombinant viruses generated in these highly viremic animals. DNA from spleens and lymph nodes contained more copies of these proviruses than did DNA from nonlymphatic tissues (e.g. liver and kidney).

To study the effects of radiation on the integration and appearance of novel acquired proviruses in the C57BL/10.F mice, two-month-old male mice were exposed to a single dose of 1, 2, or 3 Gy of X-rays. Four months after the 2 and 3 Gy exposure there was an increase in the number of mice with enlarged spleens, incidence and severity of histopathological changes in the spleens, clonal expansion of cells containing a common provirus, and the number of spleens containing novel acquired proviruses.

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INTRODUCTION

Retroviruses are RNA-containing viruses that infect vertebrate cells. They are unusual among known viruses because of two distinct characteristics of their life cycle: 1) the flow of information is reversed (RNA is a template for DNA synthesis), and 2) there is an obligatory step in which the DNA intermediate inserts into the host genome (Freifelder, 1983). A provirus is a DNA copy of a retrovirus that has integrated into chromosomal DNA of the host and is inherited by the daughter cells when the cell replicates.

Retroviruses are known to infect a wide range of vertebrate hosts from fish to man and cause a variety of diseases (Bendinelli et al., 1985; Weiss, 1986). Because of the association of retroviruses in a wide variety of diseases that include neoplasias, autoimmunity and immunodeficiency, it is of particular

interest to understand the role that retroviruses play in the development of these abnormalities.

Discovery of Retroviruses

Retroviruses were discovered more than 80 years ago as filterable agents that caused cancer in chickens (Rous, 1911). Evidence for oncogenic viruses in mammals was demonstrated by Bittners' observation in 1936 of the transmission of murine mammary carcinoma by a factor in milk (Bittner, 1942). Experiments by Gross in 1951 on the induction of tumors following the inoculation of C3H mice with lymphoma filtrate led to the first isolation of murine leukemia virus (Gross, 1951). Since then, many different viruses have been isolated (Friend, 1957; Moloney, 1960; Rauscher, 1962). The development of quantitative assays for viruses in cultured cells aided in the detailed characterization of these isolates (Temmin and Rubin, 1958). The discovery of the inheritance of proviruses in germ-cells (Huebner and Todaro, 1969), reverse transcriptase (Temmin and Mizutani, 1970), and the cellular progenitors of retroviral oncogenes (Selten et al.,

1976) led to great advances in the study of retroviruses. More recently, the isolation of human immunodeficiency virus type 1 (HIV-1) (Barré-Sinousse et al., 1983) and its causal association with acquired immune deficiency syndrome (AIDS) (Gallo et al., 1984) has resulted in an increased research effort in the study of retroviruses.

Biology of Retroviruses

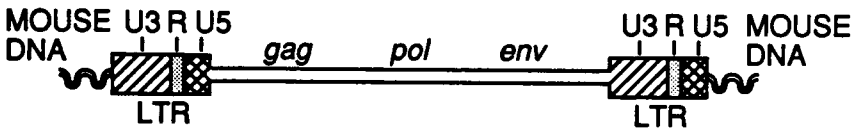
Virion structure and genome organization

The structure of the virion and the genome organization of retroviruses have been reviewed in great detail (Coffin, 1979; White and Fenner, 1986; Zijlstra and Melif, 1986; Varmus, 1988). The virion is a three layered structure approximately 1000 Å in diameter. The genome-nucleoprotein complex incorporates the enzyme reverse transcriptase, has helical symmetry, and is enclosed within an icosahedrial capsid. The capsid is surrounded by a host-derived envelope which has virus-coded glycoprotein projections ("spikes" surmounted by "knobs") (Lowy, 1986). The retroviral genome is

uncommon among mammalian viruses in that it is diploid and composed of two identical positive-sense single stranded RNAs that possess a capped 5' structure and a polyadenylated 3' end. The two RNA subunits are bound noncovalently to each other near their 5' end. A molecule of cellular $tRNA_{Pro}$ is bonded near the 5' end of the genome and serves as a primer for initiation of DNA synthesis (Lowy, 1986).

A typical integrated murine leukemia provirus is generally colinear with the RNA genome and shares the common genomic organization 5'-LTR-gag-pol-env-LTR-3' (see Figure 1.1). The gag region codes for the core proteins p15, p12, p30, and p10. The pol region codes for p14, p80 and p46. The env region codes for the two major glycoproteins gp70 and p15E. Leis et al. (1988) have suggested that general terms be used to describe the products of a retrovirus; p15 should be referred to as MA, matrix protein; p30 as CA, capsid protein; p10 as NC, nucleocapsid protein; p14 as PR, protease; p80 as RT, reverse transcriptase; p46 as IN, integrase; gp70 as SU, surface protein; and p15E as TM transmembrane protein. These descriptive terms will be used in this dissertation.

Figure 1.1. Structure of a typical murine leukemia provirus. The provirus is composed of three coding regions, gag, pol and env, which are flanked on both sides by long terminal repeats (LTRs). See text for further detail.


MOUSE
DNA
U3 R U5
gag
pol
env
U3 R U5
MOUSE
DNA
LTR
LTR

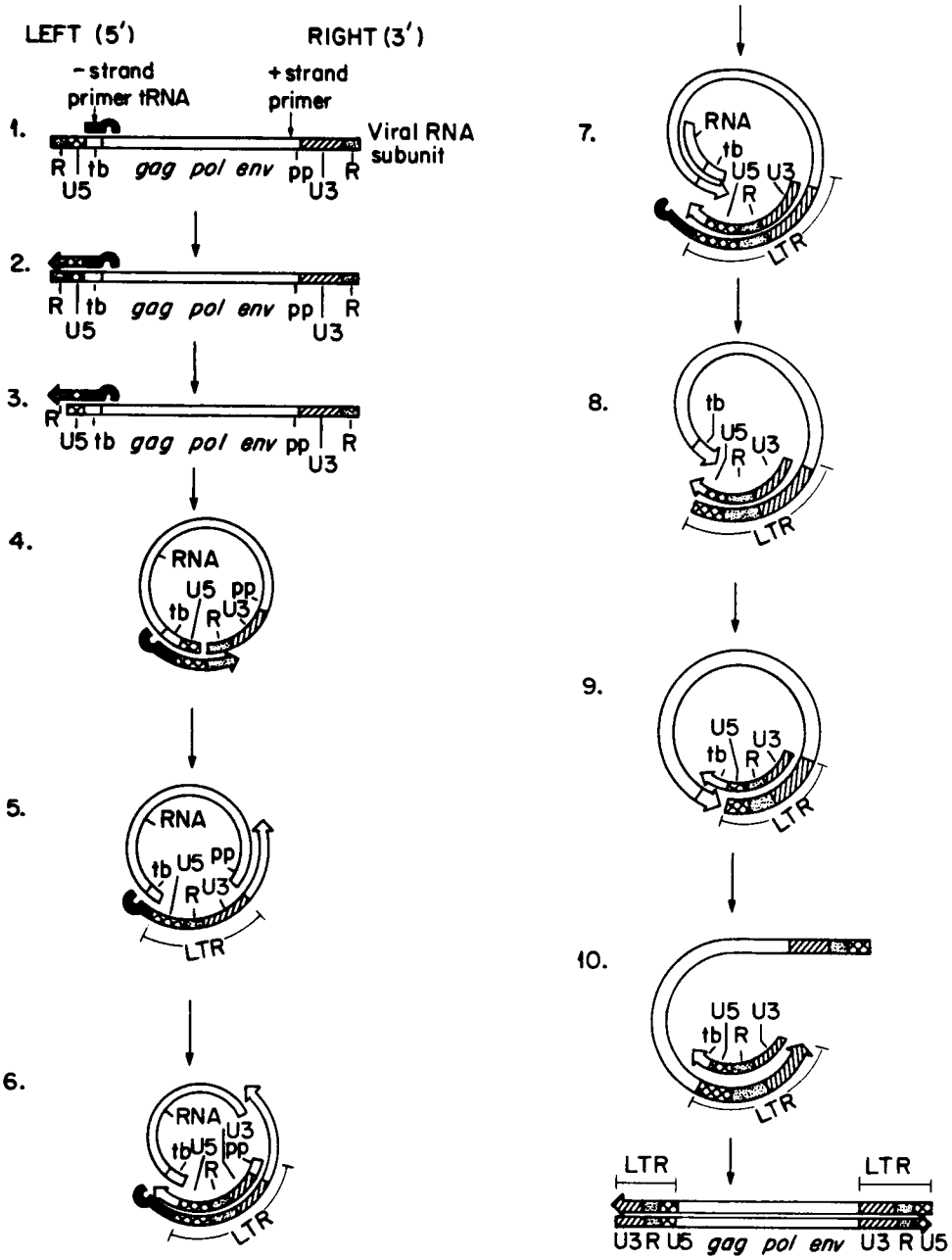
An important feature of the proviral DNA is the presence of identical long terminal repeat (LTR) sequences at both ends of the provirus. The LTR sequences generated during the reverse transcription reaction contain sequences unique to the 5' end of the viral RNA called U5, a repeated sequence called R, and sequences unique to the 3' end of the RNA genome called U3. The U3-LTR sequences contain the 'TATAA' and CCAAT-like regulatory signals important in transcription, enhancer regions, and transcription termination signals (Datta et al., 1983; Weiss, 1987; Celender and Haseltine, 1987). Specific sequences in the LTRs also influence tissue tropism and leukemogenesis (DesGroseillers et al., 1983; Wellinger et al., 1986; Rassart et al., 1988).

Retrovirus replication

Replication of the retrovirus, as outlined by Friefelder (1983) and Lowy (1986), involves the following steps (see Figure 1.2):

1. Reverse transcriptase extends one of the tRNA molecules from its 3'-OH terminus to the 5' end of the template, resulting in a copy of R-U5.

Figure 1.2. Steps in the replication of a single retrovirus genome from RNA to free double-stranded linear DNA. tp refers to the tRNA binding site and sequences complimentary to it; pp is the site of the positive-strand primer and sequences complimentary to it. (adapted from Lowy, 1984).


LEFT (5')
RIGHT (3')
- strand primer tRNA
+ strand primer
1.
Viral RNA subunit
R
tb
gag pol env
pp
R
U5
U3
2.
3.
4.
RNA
U5
pp
tb
R
U3
5.
LTR
6.
7.
8.
9.
10.
LTR
LTR
U3 R U5 gag pol env U3 R U5

2. The RNA bases corresponding to the R-U5 sequences are removed by the RNAseH activity of reverse transcriptase which renders the complementary sequence R'-U5' in the newly synthesized DNA available for hydrogen bonding. The 5' cap and 3' poly(A) tail are also assumed to be removed at this point.

3. The newly synthesized DNA binds to the other end of the RNA forming hydrogen bonds between R and R'.

4. Reverse transcriptase adds nucleotides to the 3' terminus of the DNA.

5. RNAseH activity of the reverse transcriptase degrades the RNA from the RNA:DNA hybrid.

6. Reverse transcriptase again adds nucleotides to the 3' terminus of the RNA, completing the duplex and forming the first LTR.

7. The synthesis of the first plus strand of DNA is completed.

8. RNAseH completes degradation of the RNA from RNA:DNA.

9. The first LTR is melted and the short fragment forms a duplex at the opposite end of the molecule. This pairing generates a template for the formation for the second LTR.

10. A DNA polymerase adds nucleotides to the second minus strand forming a complete duplex terminated at each end by a LTR.

Integration of viral DNA

The mechanism of formation of the provirus is not known in detail; however, it is thought that integration is similar to prokaryotic transposition (Fujiwara, 1988). The integration of viral DNA into the host genome requires the virally encoded integrase and cellular DNA synthesis. The DNA breakage and joining steps in the integration reaction are coupled, and no high energy cofactor is needed (Brown et al., 1987; Varmus et al., 1987). During the process of viral integration into the host genome, 4-9 base pairs of cellular DNA are duplicated at the integration site and short inverted repeats at the ends of each LTR are produced (VanBeveren et al., 1982; Risser, 1983).

Some viruses, such as mouse mammary tumor virus, a variety of mink cell focus-forming (MCF) viruses, and Moloney murine leukemia virus (MMuLV) recombinant constructs appear to integrate at preferred sites in the genome; for example, near proto-oncogenes such as pim-1 and Evi-1 or in areas that are transcriptionally

active (Nusse and Varmus, 1982; Cuypers et al., 1984; Barklis et al., 1986; Morishita et al., 1988). However, integration can also occur at many sites in the DNA (Lowy et al., 1980; Rosomondo and Meruelo, 1986; Horowitz et al., 1987; Shih et al., 1988). Once the virus has integrated into the host DNA, the presence of the provirus does not usually inhibit cell division and daughter cells inherit the integrated virus.

Expression of proviral DNA

The transcription and expression of proviral sequences have been reviewed (Freifelder, 1983; Lowy, 1986; White and Fenner, 1986). Expression of a given provirus depends on a variety of host and viral factors. These factors include the physiological state of the cell, the site of integration, and the specific nucleotide sequences in the LTR. Transcription of the provirus depends entirely on enzymes encoded by the host's genome.

The U3 sequence within the 5' LTR contains a promoter which directs initiation of transcription and an enhancer which elevates transcription (Luciw et al., 1983; Wood et al., 1983). Transcription is initiated

at the 5' end of the R sequence in the 5' LTR. Host RNA polymerase II transcribes RNA from the proviral DNA. A single transcript (designated 38S RNA) is formed. Each RNA transcript is polyadenylated at the 3' end of R in the 3' LTR. Approximately 50% of the RNA that is transcribed from the provirus is incorporated into the virion (Eisenman and Vogt, 1978). The remainder serves as mRNA which is processed and translated. The protein products are then phosphorylated and glycosolated to form the final gag, pol, and env proteins. The gag products give rise to the core proteins, the functions of which are not well characterized. The pol protein products have been discussed in the sections on retrovirus replication and integration. The env protein products are located on the outermembrane of the virion. The TM (p15E) is linked by disulfide bonds to the SU (gp70) which in the electron microscope look like "spikes" surmounted by "knobs". The SU (gp70) determines the hostrange, interference pattern, and neutralization properties of the virus (Lowy, 1986).

Retrovirus-like elements in the genome of the mouse

The DNA of laboratory mice contains hundreds of retrovirus-related sequences that account for a few tenths of one percent of the total DNA (Stoye and Coffin, 1985). Kozak (1985a) has reviewed the retrovirus-like elements found in the mouse. There are four major classes of endogenous mouse retrovirus-related sequences: virus-like 30S sequences (VL30s), intracisternal A particles (IAPs), the type B mouse mammary tumor viruses (MMTVs), and the type C mouse leukemia viruses (MuLVs). Unless otherwise specified, this dissertation will be concerned with the type C MuLVs.

Murine Retroviruses

Laboratory mice have been used to study retroviruses for two main reasons: 1) laboratory mice are easy to manipulate, and 2) recombinant inbred and congenic strains are unique resources for studying the genetics of virus expression leading to disease.

Host-range classes of MuLV

The type C MuLvs are the best characterized of the endogenous mouse retroviruses. These viruses can most easily be distinguished on the basis of their host range which is determined by the surface protein (gp70) (Teich, 1982; Cloyd et al., 1985). The ecotropic MuLVs only infect and replicate in mouse cells. The xenotropic MuLVs cannot infect mouse cells but infect and replicate in several other species, such as cat, rabbit, mink or man. The wild-mouse-derived amphotropic MuLVs are infectious for both mouse and certain non-mouse species. The dualtropic or polytropic mink cell focus-forming (MCF) MuLVs are capable of infecting and replicating in mouse cells as well as cells of some unrelated species.

Genetic basis of strain susceptibility

The ecotropic viruses fall into two important host-range subclasses, N-tropic and B-tropic, which are defined by their ability to replicate in different inbred mouse strains (Hartley et al., 1970). The *Fv-1* locus on chromosome 4 of the mouse codes for a product which inhibits the integration of restricted viral DNA into the cellular genome (Meruelo and Bach, 1983). The

product of the Fv-1 gene interacts with the capsid protein (p30), encoded in the gag region of the virus, and thereby inhibits proviral integration (DesGroseillers and Jolicoeur, 1983). Inhibition occurs when the Fv-1 gene product either blocks circularization of linear viral DNA directly or promotes synthesis of a defective linear DNA which can not circularize (Jolicoeur and Rassart, 1981). At least three Fv-1 alleles are known in inbred mice (Meruelo and Bach, 1983). Fv-$1^{n/n}$ mice are permissive for the N-tropic virus strains and Fv-$1^{b/b}$ mice are sensitive to B-tropic viruses. Heterozygotes, Fv-$1^{n/b}$, are resistant to both N- and B-tropic viruses, indicating that resistance to these viruses is a dominant trait. The Fv-$1^{nr/nr}$ allele is carried by a few inbred mouse strains; it confers partial resistance to N- and complete resistance to B-tropic viruses (Risser et al., 1983; Kozak, 1985b).

Other host genes also play a role in the infection, integration, and expression of specific ecotropic viruses. These include Gv-1, Rcmf, Fv-2, Fv-4, inc-1, inb-1, and H-2 (McCubrey and Risser, 1982; Levy et al., 1985; Ruscetti et al., 1985; Zijlstra et al., 1986).

Strain distribution of ecotropic proviruses

On the basis of their viral expression, inbred strains of mice can be placed in one of three categories; high, low, and negative (Chattopadhyay et al., 1974; Rowe, 1976; Jenkins et al., 1982; Risser et al., 1983). High virus strains include AKR and C58. These strains have high incidences of spontaneous lymphomas and express high titers of virus early in life. An ecotropic-specific env probe has been used to establish that their germ-line DNA contains multiple copies of genome-length ecotropic proviruses located on different chromosomes (Chattopadhyay et al., 1974). Low-virus expressing strains, such as BALB/c and C57BL, develop leukemia late in life and carry a single ecotropic provirus in their genome (not at the same location in the two strains) (Jenkins et al., 1982). Virus-negative strains, such as NIH and SWR, do not carry ecotropic proviruses in their genomes. However, it has been demonstrated that NIH embryos, newborns and weanlings express group-specific antigens of the C-type RNA tumor viruses in one or more of their tissues (Huebner et al., 1970).

Mode of transmission of retroviruses

Retroviruses can be transmitted from one mouse to another either horizontally or vertically. Many retroviruses are acquired by horizontal spread, as are most other infectious agents (Teich et al., 1982; Lowy, 1986). Horizontally transmitted viruses are called exogenous and are most commonly ecotropic in their host range. Endogenous retroviruses are transmitted vertically as heritable proviruses and usually posses an ecotropic or xenotropic host range. In addition to congenital transmission, viruses can be passed vertically through milk transmission or by intrauterine infection (Bittner, 1942; Melief et al., 1975; Zijlstra et al., 1983 Morse et al., 1985).

Origin of variant retroviruses by recombination and mutation

Formation of altered MuLVs by recombination and errors in viral replication has been reviewed (Hunter, 1978; Stoye and Coffin, 1985; Coffin, 1986; Temmin, 1989). Even among closely related retrovirus isolates, major structural differences can arise by point mutations, deletions, insertions, or substitutions of specific sequences into the viral genome (Kozak,

1985a). As many as half of the viral DNA molecules that have been extensively characterized have undergone some alteration (Shimotohno and Temin, 1982). Retroviruses have a very high rate of mutation, 1.4 x 10^{-4} mutations per nucleotide per replication cycle (Leider et al., 1988). Many of the mutations are the result of errors made by reverse transcriptase, a highly error-prone enzyme (Battula and Loeb, 1974; Leider et al., 1988). The ability of reverse transcriptase to change templates while elongating the DNA molecule (an essential feature of retroviral replication) would allow frequent rearrangement and recombination of viral genomes. Recombination of viral genomes could take place as a result of the copackaging of viral transcripts (Coffin, 1986).

Dualtropic or polytropic MCF MuLVs are generated through recombination between ecotropic and endogenous xenotropic-like sequences (Rowe et al., 1980; Chattopadhyay et al., 1981; Rassart et al., 1983; Thomas, 1986). A model for the generation of polytropic viruses has been proposed by Evans and Malik (1987) whereby two nonecotropic substitutions are derived from different endogenous sequences by stepwise recombination. A substitution in the 3' p15/U3 region

is derived by recombination of an ecotropic MuLV with an endogenous xenotropic MuLV, while the gene that codes for the 5' SU (gp70) sequence is derived by recombination with other endogenous retroviral species. Genetic recombination could explain substitutions both within the gp70-coding region of *env* and within the p15E/U3 region (Stoye and Coffin, 1987).

Germ-line mutations associated with retrovirus integration

Exogenous viruses integrate into the DNA of the host during virus replication. Integrations leading to mutations can occur in somatic cells or germ-cells (Cohn et al., 1979). Four specific integrations of ecotropic MuLVs are associated with germ-line mutations; dilute coat color mutation (*d*) (Jenkins et al., 1981), A^y lethal yellow (Copeland et al., 1983a; Copeland et al., 1983b), recessive lethal (*Mov-13*) mutation (Janeish et al., 1983), and the hairless (*hr*) mutation (Stoye et al., 1988).

Tumor induction by retrovirus integration into somatic cell DNA

The effects of viral integration into cellular DNA are not limited to germ-line mutations. Infectious MuLVs are associated with a variety of neoplasms in the laboratory mouse. Murine leukemia viruses contain no oncogene and are considered to be slow-transforming retroviruses (White and Fenner, 1986). Tumorigenesis caused by these viruses may require a period of viremia and involves rare but specific events of viral and host genetic components (Kozak 1985a). Several different theories have been postulated to explain the mechanisms of retroviral-induced lymphomagenesis:

1) Retroviral integration into one cell type can cause the proliferation of another cell type. In humans the infection of CD4+ cells by HIV causes the production of B cell stimulatory factor (IL-6) which plays an essential role in the polyclonal activation of noninfected B cells (Nikajima et al., 1989). The involvement of CD4+ T cells and the proliferation of B cells has also been described in the pathogenesis of murine acquired immunodeficiency syndrome (Pattengale et al., 1982; Klinken et al., 1988; Yetter et al., 1988).

2) Retroviral-induced immunosuppression may promote oncogenesis. Benidilli et al. (1985) have reviewed evidence that retroviral-induced immunodepression is a prerequisite for induced leukemias. The mechanism of immunosuppression may depend on a T-cell-dependent pathway of B-lymphocyte activation (Mosier et al., 1985).

3) Insertion of retroviruses adjacent to cellular oncogenes may induce tumors. Many tumors are associated with retroviral insertions in the vicinity of cellular oncogenes (Zijlstra and Melif, 1986; White and Fenner, 1986). The presence of a strong retroviral promoter and enhancer in the vicinity of a cellular oncogenes can lead to increased transcription of the oncogene (Boone et al., 1986; Shenin et al., 1987). As reviewed in Zijlstra and Melif (1986), somatically acquired proviruses are found in the vicinity of pim-1 (approximately 50%) and/or c-myc (approximately 45%) in early MMuLV-induced tumors. Specific viral integration sites which are not associated with known oncogenes have also been found in tumors, suggesting that sequences in these alternate areas may also contribute to tumorigenesis (Kozak, 1985a; Schuermann and Michalides, 1987; Mucenski et al., 1988).

4) Chromosomal aberrations, including trisomy and reciprocal translocations, are characteristically found in certain types of virus-induced lymphomas and leukemias (Zijlstra and Melif, 1986). The most common chromosomal aberration in murine T-cell lymphomas is trisomy of chromosome 15 (Wiener et al., 1982). Trisomy 15 has been detected in both virally and X-ray induced T-cell lymphomas (Chang et al., 1977; Wiener et al., 1978). Lymphomas with translocations involving chromosome 15 have also been reported (Webb et al., 1984). In these tumors, the breakpoint on chromosome 15 maps to the same chromosome band as c-myc (Webb et al., 1984).

5) MCF viruses have been isolated from naturally arising tumors and from radiation-induced tumors (Teich et al., 1982). As stated previously, MCF viruses are not inherited as complete proviruses; they are generated de novo by recombination between ecotropic and endogenous viruses (Chattopadhyay et al., 1981). These recombinant retroviruses along with recombinant ecotropic viruses have been implicated in tumor formation (Herr and Gilbert, 1982; Teich et al., 1982; Lenz and Haseltine, 1983; Risser, 1983; Villemur et al., 1983). Many recombinant MCF and ecotropic viruses

that have been isolated from tumors have alterations in the 3' env and/or LTR regions (DesGroseillers et al., 1983; Holland et al., 1985). Many of these isolates are highly oncogenic (DesGroseillers et al., 1983; Holland et al., 1985). Results obtained with recombinant MuLVs suggest that U3 sequences in the LTR could play a critical role in determining whether a given virus will be oncogenic (Coffin, 1984; DesGroseillers et al., 1983). This oncogenic property might be the result of variations in the promoter and enhancer elements in the U3 region of the LTR (Lowy, 1986).

Viral Appearance in X-Irradiated Mice

Background

Ionizing radiation, the most extensively studied of all environmental agents, was first discovered in the 1890s by Roentgen. In the early 1900s the first neoplasms attributed to radiation were reported. These neoplasms were epidermoid carcinomas on the hands of pioneer radiographers who repeatedly exposed their hands while focusing their fluoroscopic equipment

(Frieben, 1902). Within a decade of the first observations of radiation-induced cancer in humans, studies on the experimental induction of tumors in laboratory animals were reported by Marie and coworkers (cf Upton, 1973). The field of experimental leukemogenesis, initiated by Furth and Furth (1936), and intensively investigated subsequently by many research teams, has made important contributions to our understanding of radiation-induced neoplasms. Neoplasms of virtually every type have been induced experimentally by radiation in test animals such as chickens, dogs, guinea pigs, rabbits, rats, and mice (Upton, 1968; Fry and Storer, 1987). Upton (1973) described the following conclusions from the vast array of experimental studies on radiation carcinogenesis: 1) Any type of neoplasm can be induced under the appropriate conditions. 2) Irradiation may influence the pathogenesis of neoplasia through a variety of changes both directly on the tumor-forming cell itself and also indirectly by influencing other cells or organs. 3) Radiations of high linear energy transfer (LET), such as alpha particles, are more effective in inducing neoplasms than radiations of low LET, such as X-rays. 4) Events in addition to initiation by

radiation also contribute to the induction of cancer. 5) Carcinogenic effects fail to be expressed fully at high dose levels because of side effects or excessive injury to the animal. 6) Viruses are implicated in certain experimentally induced neoplasms.

Age and strain related effects of radiation

The age at the time of the first exposure to radiation is an important determinant in radiation leukemogenesis (Haran-Ghera and Peled, 1979; Covelli et al., 1984). The most extensive studies on age-related susceptibility to radiation leukemogenesis have been reported by Kaplan in 1948 using C57BL mice. He found that the susceptibility of mice to fractionated whole-body irradiation was maximal at 2-5 weeks of age and decreased rapidly thereafter (Kaplan, 1948). The reduction in susceptibility to radiation leukemogenesis with increase in age might be attributed to natural build-up of immunity to radiation-induced viruses (Haran-Ghera, 1976). Additionally, the lack of a suitable thymus microenvironment (where T cells undergo differentiation) might also inhibit T-cell lymphoma development (Haran-Ghera and Peled, 1979).

The induction of neoplasms in mice exposed to radiation is also strain dependent (Kirschbaum et al., 1947; Storer, 1973; Meruolo and Rossomando, 1986; Schmidt et al., 1988). The difference between strains in susceptibility to neoplasia is genetically controlled (Fry and Storer, 1987). Induction of lymphomas in C57BL/6 and BALB/c strains of mice by radiation and radiation leukemia virus is affected by two different genes on chromosome 2 (Meruelo et al., 1983). The incidence of induced tumors may be altered by genes that suppress expression of oncogenes, restrict integration of transposed sequences, regulate immune responsiveness, or code for repair enzymes (Haran-Ghera and Peled, 1979; Fry and Storer, 1987).

The role of radiation in tumor induction

Ionizing radiation has long been recognized as a potent carcinogen. Single doses of low LET radiation given whole body in the range of 0.25 - 3.0 Gy increase the frequency of malignant and benign tumors in irradiated animals (Fry, 1981). Lymphomas and leukemias are the most common of all the radiation-induced neoplasms in experimental animals; however, the precise mechanisms of radiation-induced carcinogenesis

are not understood (Niwa and Sugahara, 1979). The carcinogenic effects of ionizing radiation are attributed to their production of physical and chemical changes in the macromolecular components (for example, the DNA) of cells in which ionizing particles are absorbed. Changes in DNA caused by ionizing radiation include; base alteration, base destruction, sugar-phosphate bond cleavage, chain breakage (single strand and double strand), crosslinking of strands (intrastrand and interstrand) and degradation (Kanazir, 1969). The final mutagenic or carcinogenic event is the result of a sequence of events. The outcome depends not only on the initial lesion but also on the nature of attempted repair of the lesion (Rauth, 1987).

Ionizing radiation produces a number of different aberrations in the DNA of cells, which suggests that several different DNA lesions may initiate the process of carcinogenesis. Mutation of one specific gene does not seem to induce the neoplastic process; target theory calculations indicate that the size of a target for transformation <u>in vitro</u> is 30 - 100 times the size of a gene (Goodhead, 1984). Genes may become activated through chromosome breakage, faulty repair, or

translocation. For a review of activation of oncogenes, see Mindem (1987).

Radiation and viral induction

The activation of endogenous murine leukemia viruses in vitro and in vivo by radiation has been the subject of numerous studies (Kaplan, 1967; Liberman et al., 1976; Kaplan, 1977; Ellis et al., 1980; Okumoto et al., 1983). Radiation-induced viral activation is dependent on many factors including the dose rate and the genetics of the mouse strain (Niwa and Sugahara, 1979; Schmidt et al., 1988). Expression of virus after irradiation is transient, requires cell division, and requires special cell culture techniques for detection (Delceve et al., 1976; Tennant et al., 1976; Haas, 1977). Nonendogenous ecotropic and recombinant proviruses have been documented in radiation-induced tumors (Jolicour et al., 1983; Astier-Gin et al., 1986; and Boone et al., 1986). Three interpretations have been proposed from these studies relating the role of radiation-induced retroviruses in the development of neoplasms: 1) Viral integrations observed in lymphoma cells are epiphenomena and not involved with radio-leukemogenesis. 2) Integration of the viruses

activates some cellular genes by inserting new promoter and enhancer sequences near critical sites of the cellular genome which could cause uncontrolled cell growth. 3) The initial cell growth is not malignant but rather a hyperplastic proliferation under the mitogenic stimulus of a product from cells infected with murine leukemia virus. These same interpretations could also account for the role of retroviruses (in the absence of radiation) in the development of neoplasms.

Cocarcinogenic effects of viruses and radiation in viremic mice

The involvement of murine leukemia viruses in the leukemogenic action of radiation is evidenced by the coleukemogenic action of subleukemogenic doses of radiation and weakly oncogenic viruses. Haran-Ghera (1969) observed that direct inoculation of a weakly oncogenic virus into the thymus of adult mice followed by a low dose of X-rays was more effective in causing the development of leukemia than either treatment given alone. Astier-Gin et al. (1986) reported that the combined treatment of a subleukemogenic dose of X-ray and the injection of a nonpathogenic retrovirus caused thymic lymphomas to develop more readily than the

additive effects of virus and radiation alone. They also reported a correlation between the combined treatment of virus and radiation and the appearance of recombinant proviruses in the chromosomal DNA. Legrand et al. (1986) have also found that the frequency of thymic lymphosarcomas increased above the additive effect when mice were injected with a weakly oncogenic virus before radiation exposure. Radiation may act as a coleukemogenic agent by enhancing the production of cells which are sensitive to the oncogenic effects of the virus (Haran-Ghera, 1976; Galliay et al., 1986).

Effects of radiation-induced viruses on tumor induction

Liberman and Kaplan (1959) have shown that C57BL mice develop neoplasms if they were inoculated at birth with cell-free filtrates from radiation-induced C57BL thymic lymphomas (Liberman and Kaplan, 1959). It was later shown that these cell-free filtrates contained a radiation-induced virus (Carnes et al., 1966). A review of the radiation-induced leukemia virus (RadLV) can be found in Kaplan (1967). Other RadLV variants have been isolated from radiation-induced neoplasms (Haran-Ghera, 1971; Mistry and Duplan, 1973; Haran-Ghera, 1977). Several RadLV variants have been

isolated from C57BL mice and propagated *in vivo* for many years. The variants affect different target cells and express different leukemogenic activities, for a review see Haran-Ghera and Peled (1979). Various subpopulations of the RadLVs have been derived and their properties examined (Astier-Gin et al., 1977; Grymes et al., 1983). When some RadLVs are continually passaged, their properties change; for example, initial viral isolates from C57BL/Ka mice are mainly N-tropic, but, after several passages *in vivo* or *in vitro*, the RadLVs recovered were distinctly B-tropic (Liberman et al., 1976). This suggests that the initial RadLV population is a mixture of N- and B-tropic viruses. Guillemain et al. (1977), Astier-Gin et al. (1977), and Grymes et al. (1983) have indicated that other RadLV variants consist of at least two separate viral populations. Utilizing mapping studies, Grymes et al. (1983) postulated that the RadLV viruses have evolved by one or more recombinational events between endogenous viruses and represent novel lymphomagenic viruses not found in the germ-line DNA.

Characteristics of C57BL/10 and C57BL/10.F Mice

The use of inbred and congenic strains of mice to study virus susceptibility and cancer

Mice are called inbred if the strain has been maintained by brother-sister matings for 20 or more consecutive generations (Green, 1968). Inbred strains are more than 99 percent homozygous and the alleles at each locus are genetically uniform except for the inherent dimorphism associated with the X and Y chromosomes (Roderick and Schlager, 1968). A large number of inbred strains have been developed to study the genetic factors influencing cancers (Staats, 1968). Congenic strains are strains that are genetically identical to an inbred strain except for a difference at a single genetic locus and are derived by repeated selective backcrossing between inbred and donor strains (Snell and Stimpfling, 1968). Congenic strains were first developed by Snell (1948) to study the phenomena associated with acceptance and rejection of tumors.

Inbred and congenic strains have been used extensively to study the effects of genetic background and specific genes on the restriction and expression of retroviruses. Mice that differ at certain single

genetic loci often differ in their susceptibility to and expression of retroviruses (Datta et al., 1978; Colombatti et al., 1979; Moll et al., 1979; Mowat and Bernstein, 1983; Morse et al., 1985). The most commonly used inbred strains of mice for studying the properties of retroviruses include AKR, BALB/c, DBA/2, and C57BL. The C57BL/10 (B10) congenic strains have been used to study the role of alleles at the major histocompatibility locus (H-2) in the susceptibility to retroviruses in mice. Several congenic strains that have been used for these studies include B10.A (Melief et al., 1975; Vlug et al., 1981), B10.Y (Colombatti et al., 1979), B10.BR (Moll et al., 1979), and B10.F (Morse et al., 1985; Popp et al., 1986).

The C57BL/10, C57BL/10.F congenic pair

The C57BL/10.F (B10.F) strain of mice is theoretically identical to its congenic partner C57BL/10 (B10) except for a portion of the genome adjacent to the H-2 region on chromosome 17. The B10 carries the $H\text{-}2^b$ haplotype and the B10.F has the $H\text{-}2^p$ haplotype. The $H\text{-}2^p$ haplotype of B10.F mice, designated $H\text{-}2^n$ in many earlier publications, was inherited from the F/St donor strain. The B10.F strain

was originally produced by Dr. D. B. Amos (D. M. Popp, personal communication) to study properties of the H-2 locus. Many studies have been performed to compare the biological properties of the B10 and B10.F mice. The two mice differ in several of their biological characteristics (Popp, 1978; Popp, 1979a; Popp, 1979b; Popp, 1982; Morse et al., 1985; Popp et al., 1986), as described below.

B10.F mice exhibit premature age changes and have a shorter lifespan than mice of the inbred partner strain B10 (Popp, 1978). The B10.F mice gray early (at 2 - 4 months of age), have an early onset of hair loss, display severe weight loss, and the skin becomes thin and fragile (Popp, 1978). The mean ages at death of B10 and the B10.F mice are 706 +/- 14 days and 456 +/- 15, days, respectively (Popp, 1978). The B10.F also has reduced basal serum IgA levels compared with the B10. Popp (1979a) showed that there is a three-fold difference in the basal levels of IgA between the two strains. Strain B10 has a serum IgA level of 256 +/- 18 mg/dl, whereas strain B10.F had a serum IgA level of 63 +/- 3 mg/dl. Other inbred strains, such as BALB/c, C3H, and SEC, have serum IgA levels very similar to the level found in the B10 mouse (Popp, 1979a). The strain

of the maternal parent influenced the serum IgA levels of its offspring (Popp, 1979b). (B10.F X B10)F_1 progeny had low serum IgA levels like that of the maternal B10.F parent; (B10 X B10.F)F_1 progeny had high levels of serum IgA, and the trait was not sex-linked (Popp, 1979b). A study of genetic factors that regulate the lifespans of the B10 and B10.F mice also showed that the strain of the maternal parent influenced the survival of its progeny (Popp, 1982).

Graying in B10.F mice was studied by Morse et al. (1985). Strain B10.F and B10A congenic strains of mice exhibit significant graying and also express high levels of infectious ecotropic and MCF MuLVs whereas B10 mice do not gray and express little virus, and then only late in life. Electron microscopy demonstrated that large accumulations of MuLV were present in the melanocytes from gray mice. These results suggested that the graying with age resulted from melanocyte dysfunction. Morse et al. (1985) were also able to demonstrate a maternal influence on both appearance of graying and virus expression.

Viruses in C57BL/10 mice

C57BL substrains C57BL/6 and C57BL/10 are closely related sharing many virological properties. Ecotropic virus are only sporadically detected in the tissues of old C57BL mice (Mistry and Duplan, 1973). However, the virus can be efficiently induced from cultured cells with 5-iododeoxyuridine (Kozak and Rowe, 1980). C57BL mice have a single endogenous ecotropic provirus, designated emv-2, which has been mapped to chromosome 8 (Kozak and Rowe, 1980; Jenkins et al., 1982). Using a molecular probe specific for endogenous ecotropic viruses, Horowitz and Risser (1982) studied the relationship between induction loci and ecotropic virus structural genes in the C57BL/6 mouse. A dominant gene, termed Inb-1, located near emv-2, is responsible for virus induction.

The emv-2 provirus is N-tropic (Liberman et al., 1976; Benade et al., 1978), but it is a defective provirus due to a mutation in its reverse transcriptase gene (King et al., 1987). The mutation causes an alanine to proline substitution that inactivates the reverse transcriptase in the tether region, five amino acids away from the carboxyl-terminal end of the polymerase domain. MCFs and recombinant ecotropic

MuLVs have been recovered from C57BL mice. The ecotropic viruses could arise by recombination between envelope region sequences of the defective endogenous provirus (emv-2) and gag regions derived from endogenous xenotropic-like sequences (Benade and Ihle, 1980; Thomas and Coffin, 1982).

The lymphocytes of C57BL/10 mice have a low expression of infectious xenotropic and MCF MuLV (Morse and Hartley, 1982; Yetter et al., 1983; Morse et al., 1985). DNAs of all inbred mouse strains contain multiple copies (18-28 copies per haploid mouse genome) of endogenous xenotropic-like MuLV-related sequences, which are detectable by Southern analysis with MuLV probes (Stephen et al., 1982; Coffin, 1984). Wejman et al. (1984) classified the endogenous nonecotropic viruses of the C57BL/10 mouse into eight structural variants. Some of these structural variants may map to chromosomal regions that encode mouse lymphocyte antigens. In addition to the numerous endogenous xenotropic proviruses, there are xenotropic virus-inducing loci in C57BL mice. A xenotropic virus-inducing locus, Bxv-1, in C57BL/10 is located on chromosome 1 closely linked to the Dip-1 isozyme locus (Kozak and Rowe, 1978; Kozak and Rowe, 1980).

In addition to the MuLVs in the C57BL/10 genome, there are also multiple incomplete viral genomes that hybridize with selective portions of viral probes. Two of these retrovirus-like sequences have been localized to the TL region of the major histocompatibility complex (MHC) (Meruelo et al., 1984). One of the retroviral-like sequences, TLev2, hybridizes with MuLV probes derived from the pol region. The other sequence, TLev1, hybridizes with MuLV gag, pol and env region probes (Pampeno and Meruelo, 1986).

Viruses in C57BL/10.F mice

C57BL/10.F mice have the same single endogenous ecotropic provirus on chromosome 8 as found in C57BL/10. However, unlike B10, the B10.F mice express high levels of ecotropic B-tropic MuLV early in life (Yetter et al., 1983; Morse et al., 1985; Popp et al., 1986). Maternal transmission via transplacental infection and exposure to milk-borne virus contributes to the high virus phenotype of B10.F mice (Morse et al., 1985; Popp et al., 1986). The B-tropic ecotropic MuLVs expressed in B10.F mice infect somatic cells and integrate into the chromosomal DNA of the host (Langdon et al., 1984; Morse et al., 1985; Popp et al., 1986).

Langdon et al. (1984) have cloned B10.F(ash), an infectious B-tropic ecotropic MuLV from B10.F. Because Emv-2 is a defective N-tropic virus, the infectious B-tropic virus in the B10.F could have been an exogenous virus transmitted from mother to offspring or it could have arisen through recombination between the Emv-2 virus and nonecotropic MuLVs.

B10.F mice have a high level of expression of infectious xenotropic and MCF MuLVs (Yetter et al., 1983; Morse et al., 1985). The high level of expression of xenotropic viruses may be controlled by Cxv-1, the xenotropic virus-inducing locus, that is closely linked to the H-2 locus in the F/St mouse. B10.F mice might carry the Cxv-1^s allele from the F/St mouse (Morse and Hartley, 1982; Yetter et al., 1983).

F/St mice show a high level of spontaneous expression of infectious xenotropic viruses (Morse and Hartley, 1982; Morse et al., 1982; Yetter et al., 1983). The expression of xenotropic virus in F/St mice behaves as a recessive trait. F/St is the only inbred strain that produces high levels of infectious ecotropic as well as xenotropic MuLV (Morse et al., 1982). The high ecotropic virus phenotype of F/St mice is governed by three or more independently

assorting loci (Morse et al., 1982). Using molecular hybridization techniques, Morse et al. (1982) demonstrated that F/St mice have the largest number of germline MuLV integrations among inbred mice. It is possible that the high titers of ecotropic virus in B10.F mice result from congenital transmission of ecotropic viruses from the viremic F/St mother in the original F/St X C57BL/10 cross to establish the B10.F congenic mice.

Research Objectives

C57BL/10.F is a unique strain of mice. Unlike other high viremic strains, B10.F mice do not develop a high incidence of neoplasia or lymphomas that commonly accompany high viremia (Popp, in press). The purpose of this dissertation was to study the characteristics (structure, minimal number, multiplicity of integration, and copy number) of the ecotropic retroviruses in somatic cell DNA of the C57BL/10 and C57BL/10.F mice. In addition, the effects of radiation on the structure of the ecotropic retroviruses in the

B10.F mouse and the relationship between specific viruses, viral integrations and tissue hyperplasia were also studied.

This dissertation describes some of the restriction endonuclease polymorphisms found among different ecotropic proviruses, the effect of radiation on the frequency of viral integrations into somatic cell DNA, clonal expansion of cells bearing a common viral integration, and the minimal number of viral variants in control and X-irradiated B10.F mice.

CHAPTER II

CHARACTERIZATION OF ECOTROPIC PROVIRUSES IN SOMATIC CELLS OF C57BL/10 AND C57BL/10.F MICE

Introduction

Biology of the C57BL/10.F mouse

The C57BL/10.F (B10.F) strain of mice is congenic to C57BL/10 (B10). The two strains differ around the H-2 region on chromosome 17. The B10 carries the $H\text{-}2^b$ haplotype and the B10.F has inherited the $H\text{-}2^p$ (originally designated $H\text{-}2^n$) haplotype from the F/St donor strain. The B10 and B10.F strains differ in several of their biological characteristics (Popp, 1978; Popp, 1979; Popp, 1982; Morse et al., 1985; Popp et al., 1986). Compared with B10, B10.F mice exhibit premature age-related changes; B10.F mice gray early (at 2 - 4 months of age), have an early onset of hair loss, display an earlier weight loss, and the skin

becomes thin and fragile (Popp, 1978). B10.F mice have a decreased life span; the mean ages at death of B10 and B10.F mice are 706 +/- 14 days and 456 +/- 15 days, respectively (Popp, 1978). Compared with B10, B10.F mice have reduced basal serum IgA levels (Popp, 1979a). Studies of genetic factors that regulate life spans and basal serum IgA levels of the B10 and B10.F mice showed that the strain of the maternal parent influences the phenotype of the progeny (Popp, 1979b; Popp, 1982). The premature graying, decreased lifespan and low IgA levels exhibited by the B10.F mouse are associated with the spontaneous expression of high levels of infectious murine leukemia viruses (Yetter et al., 1983; Morse et al., 1985; Popp et al., 1986). The B10 mouse, which does not display these premature age-related changes, expresses little virus and only late in life.

Viruses in C57BL/10.F mice

Both B10 and B10.F mice carry emv-2, a defective N-tropic germ-line provirus, and the ecotropic virus induction locus, Inb-1, on chromosome 8 (Benade et al., 1978; Kozak and Rowe, 1980; Horowitz and Risser 1982; King et al., 1987). However, unlike the B10, the B10.F mouse expresses high levels of infectious B-tropic

ecotropic, MCF, and/or xenotropic MuLVs early in life (Yetter et al., 1983; Morse et al., 1985; Popp et al., 1986). Maternal transmission via transplacental infection and exposure to milk-borne virus contribute to the high virus phenotype of the B10.F mice (Morse et al., 1985; Popp, 1986). Because the emv-2 provirus is defective and N-tropic, the infectious B-tropic ecotropic MuLVs in B10.F mice could be recombinants between the Emv-2 virus and nonecotropic MuLVs or they could be other exogenous viruses unrelated to emv-2. The viruses in our colony of B10.F mice are probably transmitted successively from mother to offspring.

This chapter describes some of the characteristics of the ecotropic proviral sequences in the somatic cell DNA of B10 and B10.F mice.

Materials and Methods

A detailed description of all experimental procedures can be found in the Appendix.

Animals

Male mice of strain C57BL/10 (B10) and C57BL/10.F (B10.F) were obtained from the colony of Raymond A. Popp, Oak Ridge National Laboratory, Oak Ridge Tennessee. The original B10.F mice obtained in 1970 from Dr. Jack Stimpfling (The McLaughlin Research Institute, Great Falls, Montana) were derived from (F/St X C57BL)F_2 $\underline{H\text{-}2^{n/n}}$ homozygotes produced by D. B. Amos (Popp, 1982). The B10 and B10.F mice used in this study have been further inbred at Oak Ridge since 1970. Mice were raised under conditions approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). At six months of age, eight B10 and 51 B10.F (A1-A51) mice were sacrificed, and the internal organs were inspected, removed and weighed. Pieces of spleen, liver, kidneys, lymph nodes, thymus and whole sternum were fixed for histological examination in buffered formalin, and stained with hematoxylin and eosin. Portions of the liver, spleen, lymph nodes and kidneys were frozen at -20^0 C for later DNA extraction.

DNA preparation

High molecular weight DNA was separated by centrifugation in a CsCl gradient as previously described by Popp et al. (1981) or by a rapid small scale isolation method described below. Approximately 75 mg of frozen tissue was homogenized in disruption buffer (50 mM Tris pH 8.0; 100 mM EDTA; 100 mM NaCl; 1.0% SDS; 127 ug/ml Proteinase K, BRL; 50 ug/ml RNase). The homogenate was incubated overnight at 65^0 C. Protein was removed by extracting with equal volumes of phenol and chloroform, and DNA was spooled onto a glass rod in the presence of ethanol. The DNA was dried, dissolved in TE containing 0.1 ug/ml RNase, and stored at -20^0 C.

Restriction enzyme analysis, electrophoresis, DNA transfer and hybridization

High molecular weight somatic cell DNAs (5 or 10 ug/lane) were digested to completion under conditions recommended by the supplier of the restriction endonucleases (BRL). The digested DNA was electrophoresed through 0.7-0.8% agarose gels and transferred to BA85 nitrocellulose (Schliecher and

Schuell) using a modification of the protocol described by Maniatis et al (1982).

Ecotropic specific probe and hybridization

The probe used to identify viral DNA sequences in the genomic DNA was a 400 base pair SmaI/SmaI fragment derived from the 5' end of the Akv env gene (Chattopadhyay et al., 1980). The probe is specific for ecotropic env sequences and does not cross-react with MCF or xenotropic env sequences under the stringent washing conditions used in this study (Chattopadhyay et al., 1980). The nitrocellulose filters were hybridized with 2.0 X 10^6 cpm of ^{32}P-labeled probe, washed and autoradiographed as previously described (Popp, 1981).

Results

Structure of the endogenous emv-2

The pattern of restriction fragments derived from the defective endogenous provirus, emv-2, was determined in order to use restriction endonucleases to identify and study the acquired ecotropic proviral

sequences in somatic cell DNA of B10 and B10.F mice. The patterns of restriction fragments of emv-2 in B10 and B10.F mice were identical (Figure 2.1, 2.2 and 2.3). The restriction enzymes used were known to cut within the prototypic ecotropic murine leukemia virus AKV, a virus found in the AKR mouse.

When the splenic DNA of B10 mice was cut with PvuII, an enzyme which cuts twice within the pol region of the AKV provirus, three restriction fragments containing proviral DNA were produced. The probe hybridized to a 5.2 kb junction fragment that was composed of 3.0 kb of 3' proviral DNA and 2.2 kb of flanking mouse genomic DNA (Figure 2.3). The size of the junction fragment was dependent upon the position of the PvuII restriction site in the flanking mouse genomic DNA. Since emv-2 is located in the same site in the genome of every cell, only one PvuII restriction fragment was detected. When the splenic DNA from B10 mice was digested with PstI, which cuts characteristically within each LTR, an 8.3 kb fragment hybridized with the ecotropic virus-specific probe. This restriction fragment was composed entirely of proviral DNA. The size of this fragment indicated the relative positions of the PstI sites in emv-2. When

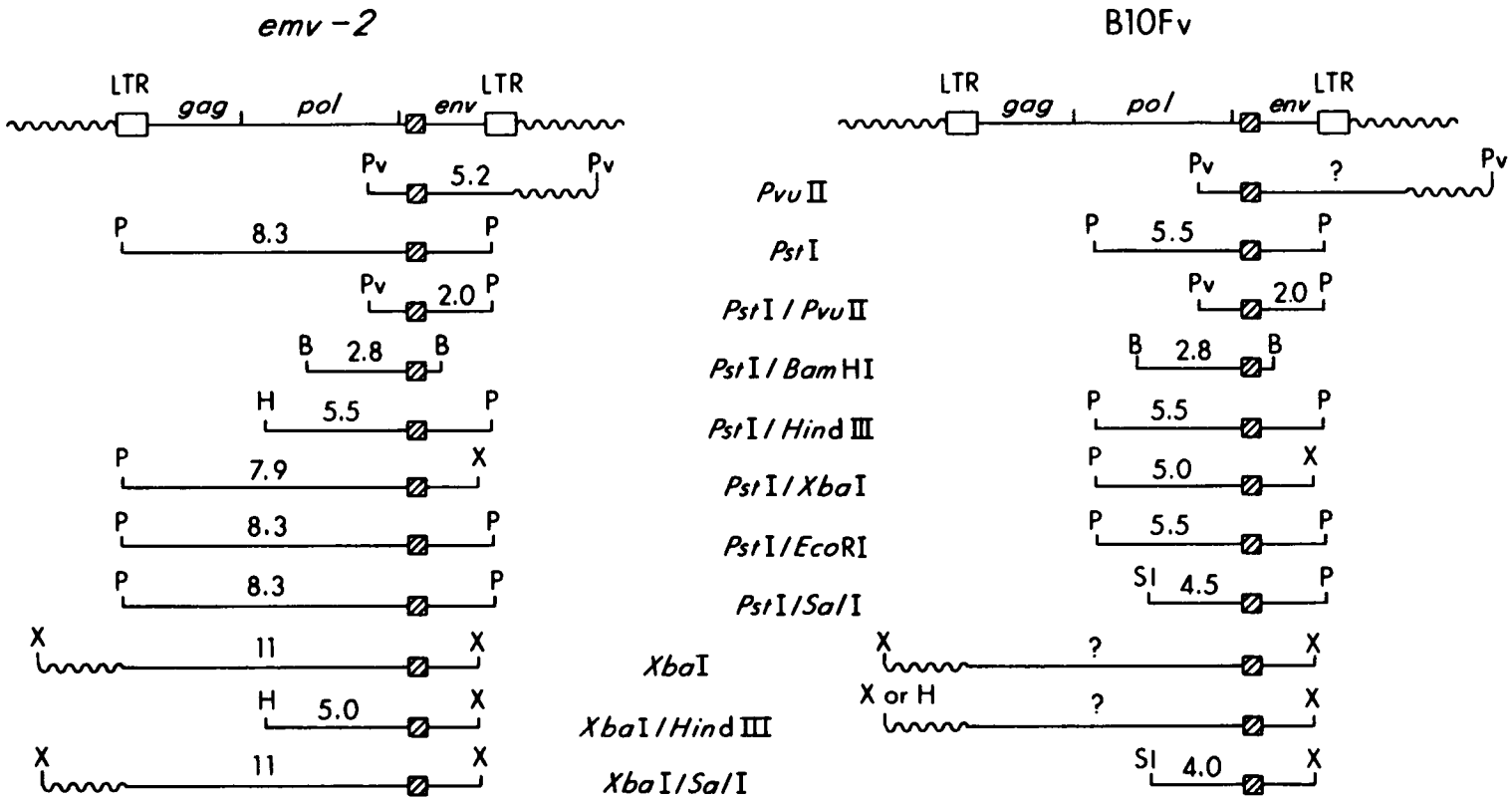
Figure 2.1. Southern blot of the ecotropic MuLV sequences in splenic DNA of the B10 mouse. HindIII digested Lambda DNA fragments were used as DNA size standards. The single restriction fragment in each lane indicates that there is only one ecotropic provirus in the genome of the B10 mouse and that it was located at the same site in every cell.

origin
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
kb
9.4
6.7
4.4
2.3
2.0

Figure 2.2. Southern blot of the ecotropic MuLV sequences in splenic DNA of a typical six-month-old B10.F mouse. Restriction fragments in addition to those attributed to the endogenous emv-2 observed when the DNA was digested with PstI, PstI/XbaI, PstI/EcoRI, PstI/SalI, XbaI/HindIII, and XbaI/SalI are characteristic of B10Fv. Formation of common restriction fragments when the DNA was cut with PstI/PvuII, PstI/BamHI, and PstI/HindIII indicates that emv-2 and B10Fv have identical PvuII, BamHI and HindIII restriction sites in the pol region, identical XbaI sites in the env region and identical PstI sites in the 3' LTR.

origin
kb
9.4
6.7
4.4
2.3
2.0
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI

Figure 2.3. Relative positions of restriction fragments generated from emv-2 and B10Fv. Restriction endonuclease maps were generated from the data in Figures 2.1 and 2.2. The hatched area in the env region represents the site of hybridization of the 400-base-pair ecotropic virus-specific probe. Abbreviations used for restriction endonucleases; B, BamHI; H, HindIII; P, PstI; Pv, PvuII; Sl, SalI; X, XbaI.

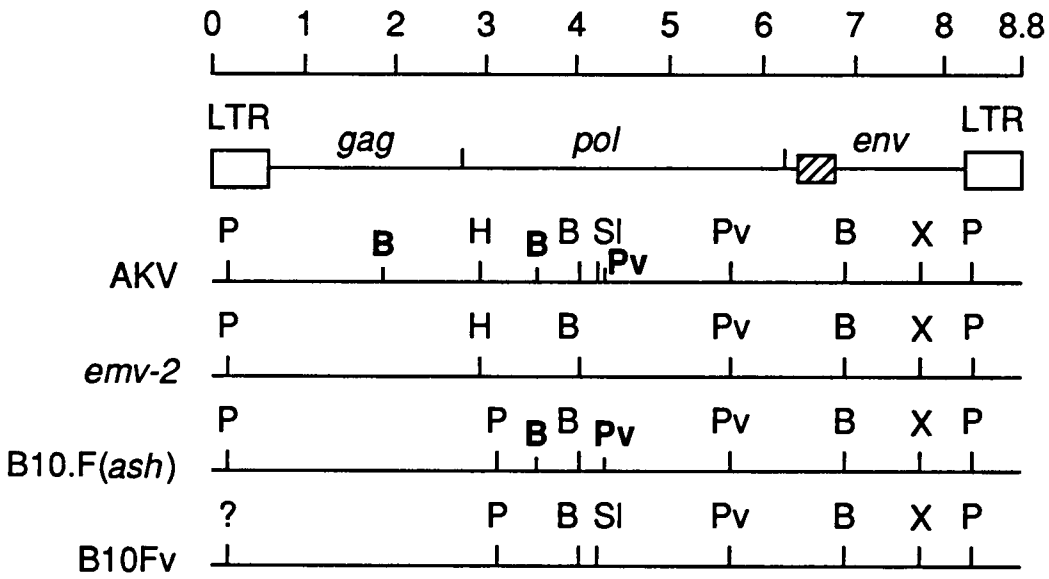

emv-2
LTR
gag
pol
env
LTR
Pv
5.2
Pv
P
8.3
P
Pv
2.0
P
B
2.8
B
H
5.5
P
P
7.9
X
P
8.3
P
P
8.3
P
X
11
X
H
5.0
X
X
11
X
PvuII
PstI
PstI / PvuII
PstI / BamHI
PstI / HindIII
PstI / XbaI
PstI / EcoRI
PstI / SalI
XbaI
XbaI / HindIII
XbaI / SalI
B10Fv
LTR
gag
pol
env
LTR
Pv
?
Pv
P
5.5
P
Pv
2.0
P
B
2.8
B
P
5.5
P
P
5.0
X
P
5.5
P
SI
4.5
P
X
?
X
X or H
?
X
SI
4.0
X

the B10 splenic DNA was digested with PstI/PvuII a single fragment hybridized with the probe. This fragment was 2.0 kb in length and represented a restriction fragment that had a 5' PvuII end in the pol region and a 3' PstI end in the 3' LTR. The size of the PstI/PvuII restriction fragment indicated the distance between the PvuII and PstI sites in emv-2 was 2.0 kb. When the splenic DNA from the B10 mice was digested with PstI/BamHI, a 2.8 kb restriction fragment hybridized with the probe. The BamHI sites were placed in positions homologous to the BamHI sites in AKV; the 5' BamHI end was placed in the pol region and the 3' BamHI end was placed in the env region. After digestion with PstI/HindIII, a 5.5 kb 5' HindIII 3' PstI restriction fragment hybridized with the probe. This allowed the positioning of a characteristic HindIII site in the 5' end of the pol region. After digestion of the splenic DNA from the B10 mice with PstI/XbaI, a 7.9 kb restriction fragment was detected indicating that an XbaI site was located in the 3' end of the env region. When the splenic DNA from the B10 mice was digested with PstI/EcoRI or with PstI/SalI an 8.3 kb PstI/PstI fragment was detected, indicating the absence of internal EcoRI and SalI sites within the

emv-2 provirus. When the DNA was digested with XbaI alone, an 11 kb junction fragment hybridized with the probe. This junction fragment contained approximately 8.0 kb of 5' proviral DNA and 3.0 kb of 5' flanking mouse DNA. Analogous with the PvuII digestion, the size of the XbaI fragment depended upon the position of the XbaI site in the flanking mouse genomic DNA 5' to emv-2. After digestion of the splenic DNA from B10 mice with PstI/HindIII, a 5.5 kb fragment was detected and a HindIII site was placed in the pol region. After digestion with XbaI/SalI, an 11 kb restriction fragment was detected. The size of this fragment was consistent with the absence of a SalI site in emv-2.

The restriction enzymes which used were selected so that a limited restriction map of emv-2 could be determined (Figure 2.4). The purpose of these digestions was to characterize emv-2 sufficiently so that proviruses in the B10.F mouse that possess restriction sites that differ from those in emv-2 could be detected. The hybridization of the probe with different sized restriction fragments composed entirely of proviral DNA can be used to indicate the relative number of proviruses with altered restriction endonuclease sites in the host genome. Likewise, the

Figure 2.4. Restriction endonuclease maps of emv-2 and B10Fv. Restriction endonuclease maps were generated from the data in Figures 2.1 and 2.2. The restriction sites found in the N-tropic AKV and B-tropic B10.F(ash) (Langdon et al., 1984) are shown for comparison. There are no internal EcoRI sites in any of the viruses. Because of the presence of the internal PstI site in the pol region of B10Fv, the presence or absence of the characteristic PstI site in the 5' LTR was not analyzed. The hatched area in the env region represents the site of hybridization of the 400-base-pair ecotropic virus-specific probe. Abbreviations used for restriction endonucleases; B, BamHI; H, HindIII; P, PstI; Pv, PvuII; Sl, SalI; X, XbaI. The bold type indicates sites that exist in AKV and B10.F(ash) that could not be detected in this study in emv-2 or B10Fv.


0
1
2
3
4
5
6
7
8
8.8
LTR
gag
pol
env
LTR
AKV
P
B
H
B
B
Sl
Pv
Pv
B
X
P
emv-2
P
H
B
Pv
B
X
P
B10.F(ash)
P
P
B
B
Pv
Pv
B
X
P
B10Fv
?
P
B
Sl
Pv
B
X
P

hybridization of the probe with different sized junction fragments can be used to indicate the randomness of integrations of proviruses in the genome of the host.

The use of restriction fragment length polymorphisms to identify B10Fv in the splenic DNA of B10.F mice

The information on the restriction sites in the endogenous emv-2 provirus in DNA from B10 and B10.F mice was used to identify other ecotropic virus-related sequences in the genome of the B10.F mice. Splenic DNA from B10.F mice was digested with restriction enzymes that cut at least twice within the provirus to identify ecotropic virus-specific restriction fragments that differed from those which could be attributed to emv-2. Splenic DNA from every B10.F mouse had ecotropic provirus in addition to emv-2 (Figure 2.2, page 52). When splenic DNA from B10.F mice was digested with PstI, a 5.5 kb restriction fragment in addition to the 8.3 kb emv-2 PstI restriction fragment was detected with the ecotropic virus-specific probe. An additional restriction fragment was also detected when the DNA was digested with PstI/XbaI, PstI/EcoRI, PstI/SalI, and XbaI/SalI. Because B10.F mice in this colony are

viremic (Popp et al., 1986), the additional band that is detected in the splenic DNA of every B10.F mouse is most probably a portion of a complete ecotropic provirus. Additionally, it has been shown that B10.F mice from other colonies shed an ecotropic virus that is structurally distinct from emv-2 and at least one such virus, B10.F(ash), has a 5.5 kb internal PstI fragment (Morse et al., 1982; Langdon et al., 1984). The ecotropic provirus that is detected in addition to emv-2 in the splenic DNA of every B10.F mouse examined has been termed B10Fv.

Because only one restriction fragment was detected when the splenic DNA from a B10.F mouse was cut with PstI/PvuII, both emv-2 and B10Fv must have the same PvuII and PstI sites in the sequence detected by the virus-specific probe (Figure 2.2 and 2.3, pages 52 and 54). Because one of the PstI sites is located in the 3' LTR, the second PstI site must be located 5.5 kb 5' in the 5' end of the pol region. Only one fragment was detected when B10.F splenic DNA was digested with PstI/BamHI, indicating that emv-2 and B10Fv have common BamHI sites. When the DNA was digested with PstI/HindIII one band, 5.5 kb in length, was detected. It was concluded that this band represented a 5.5 kb

PstI/HindIII fragment derived from emv-2 and a 5.5 kb PstI/PstI restriction fragment derived from B10Fv because when the DNA was digested with XbaI/HindIII one band and a smear were detected. The 5.0 kb band corresponded to emv-2; the junction fragments formed from B10Fv were of large and varying sizes and were detected as a smear at the top of the lane. This smear occurred in every B10.F splenic DNA sample digested with XbaI or XbaI/HindIII and was not detected in XbaI/SalI digests, indicating that the smear was not due to incomplete digestion with XbaI. Digestion of splenic DNA from B10.F mice with PstI/XbaI produced a 5.0 kb restriction fragment, indicating that emv-2 and B10Fv share an XbaI site in the env region and that an additional PstI site is present in the pol region of B10Fv. Digestion with PstI/EcoRI established that B10Fv does not have any internal EcoRI sites. Digestion with PstI/SalI demonstrated that a SalI site is located 4.5 kb 5' to PstI, adjacent to the BamHI site in the pol region of B10Fv. The restriction fragments detected after digestion with XbaI/SalI were consistent with the presence of a SalI site in the pol region of B10Fv.

The use of restriction enzymes which cut within the provirus allowed the detection of B10Fv in the splenic DNA of every B10.F mouse examined. B10Fv was not detected in the DNA of the B10 mice. B10Fv differed from *emv-2* at the *Hin*dIII and *Pst*I sites in the 5' end of the *pol* region and also at the *Sal*I site near the common *Bam*HI site in the *pol* region. B10Fv varied from B10.F(*ash*), only at its *Sal*I site. The structure of B10Fv has been compared with other common proviruses, AKV, *emv-2*, and B10F(*ash*), in Figure 2.4.

Location of B10Fv in the splenic DNA of B10.F mice

The number of different integration sites was investigated by digesting the DNA with restriction enzymes which produced provirus-cell DNA junction fragments. The sizes of the restriction fragments which hybridized with the probe were dependent upon the distance from the integration site of the provirus to the adjacent restriction site in the cellular DNA. When the splenic DNA from B10.F mice was digested with *Pvu*II or *Xba*I, restriction enzymes which produce provirus-cell DNA junction fragments, only one restriction fragment was detected in each digest. These restriction fragments were derived from the

junction of endogenous emv-2 and genomic DNA. The B10Fv viruses had integrated randomly into the genome and the B10Fv-genomic junction fragments were of varying sizes. No specific sized restriction fragment was present in sufficient quantity to be detected with the probe. If B10Fv were endogenous or had integrated into the same site in many cells then the B10Fv-genomic junction fragments would be the same size and in sufficient quantity to be detected.

Additional PvuII restriction fragments could also be detected when a cell containing a provirus at a unique site had proliferated sufficiently for detection. The intensity of hybridization with the additional PvuII fragments could indicate the proportion of cells in the spleen which contain the specific integration. If an additional PvuII band is 25% the intensity of the endogenous PvuII band (2 copies per diploid genome), approximately one half of the cells would contain that particular provirus.

Additional acquired proviruses in the splenic DNA of the B10.F mouse

When the splenic DNA from four B10.F mice; A7, A17, A42 and A46 was cut with restriction enzymes which

produced fragments that were composed entirely of proviral sequences, a number of novel restriction fragments were produced from proviruses in addition to those attributable to emv-2 and B10Fv (Table 2.1). These novel restriction fragments indicated the presence of acquired proviruses in addition to B10Fv.

Four restriction fragments were detected when splenic DNA from A7 was digested with PstI/SalI; two restriction fragments, 2.9 and 4.9 kb in length, were not derived from emv-2 and B10Fv, which produce 8.3 and 4.3 kb fragments, respectively (Figure 2.5). The presence of the two additional restriction fragments indicated that at least four different proviruses were present in the splenic DNA of mouse A7. The 8.3 kb restriction fragment corresponded to emv-2, the 4.3 kb fragment corresponded to the acquired B10Fv and the 4.9 and 2.9 kb restriction fragments corresponded to acquired proviruses with altered PstI/SalI restriction sites. The two PstI/PvuII restriction fragments detected in addition to the 2.0 kb emv-2 and B10Fv restriction fragments also indicated that a minimum of four structurally different proviruses were present in the splenic DNA from mouse A7. A population of proviruses in the DNA of this mouse had PstI sites that

TABLE 2.1

DNA PROFILES OF B10 AND B10.F MICE

Animal	No. *Pst*I[a]	No. *Pvu*II[a]	No. *Pst*I/*Pvu*II[a]	Minimum no. provirus[b]
All B10	1(8.3)	1(5.2)	1(2.0)	1(P)
Typical B10.F	2(8.3) (5.5)	1(5.2)	1(2.0)	2(P)
A7	3(8.3) (5.5) (4.8)	1(5.2)	3(3.2) (2.0) (0.75)	4(P/S) (8.3) (4.5) (4.2) (2.9)
A17	3(8.3) (5.5) (5.1)	2(5.2) (4.5)	1(2.0)	3(P)

TABLE 2.1 (Continued)

Animal	No. PstI[a]	No. PvuII[a]	No. PstI/PvuII[a]	Minimum no. provirus[b]
A42	4(8.3) (5.5) (4.9) (3.1)	7(8.5) (7.5) (6.0) (5.2) (4.5) (4.0) (2.8)	3(3.1) (2.0) (1.1)	6(X/S)(11) (7.8) (6.5) (5.3) (4.9) (4.0)
A46	2(8.3) (5.5)	3(5.2) (3.4) (2.8)	1(2.0)	3(Pv)

[a]Number of bands observed when digested with restriction enzymes; kb sizes of bands are given in parenthesis.

[b]Restriction enzymes used to determine the minimum number of different proviruses in the splenic DNA; P is PstI, Pv is PvuII, X is XbaI, S is SalI.

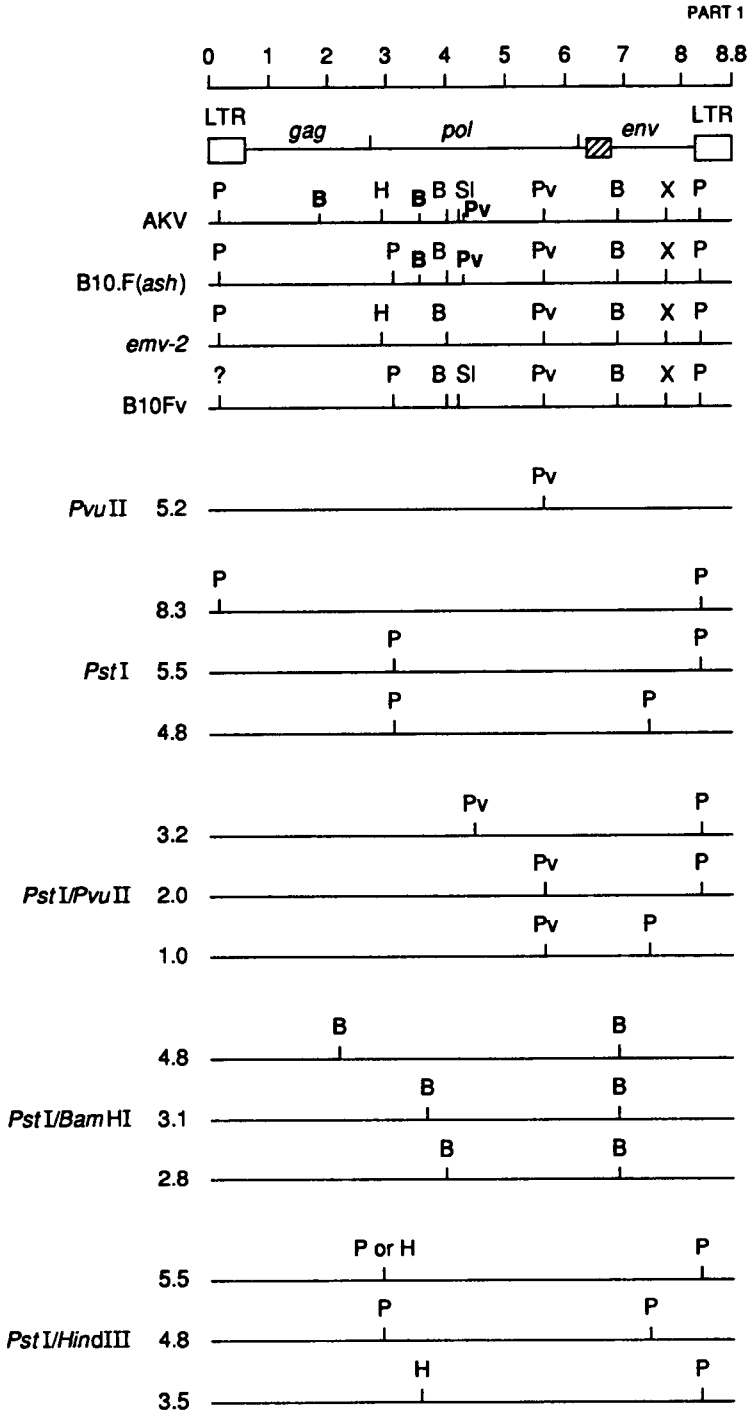
Figure 2.5. Southern blot of ecotropic MuLV sequences in addition to emv-2 and B10Fv found in the splenic DNA of mouse A7. Novel restriction fragments in addition to those attributed to emv-2 and B10Fv were observed when the DNA was digested by each restriction enzymes except PvuII. Double digestion with PstI/PvuII (lane 2) generated three restriction fragments (3.2, 2.0 and 0.75 kb) and four restriction fragments (8.5, 4.9, 4.2 and 2.9 kb) were found in PstI/SalI double digests (lane 8). These data indicate that the splenic DNA of mouse A7 had at least four structurally different ecotropic proviruses. There were two proviral populations with internal EcoRI sites. One population (generating a 0.3 kb EcoRI fragment) had an EcoRI site within the region of env that hybridizes with the 400 bp ecotropic specific probe.

origin
kb
9.4
6.7
4.4
2.3
2.0
PvuII
PstI / PvuII
PstI
PstI / BamHI
PstI / HindIII
PstI / XbaI
PstI / EcoRI
PstI / SalI
XbaI
XbaI / HindIII
XbaI / SalI

were 4.9 kb apart. It was also apparent from the restriction digests that this population of proviruses did not contain BamHI, HindIII or SalI sites between the PstI sites. However, the provirus contained internal XbaI and EcoRI sites. The 0.3 kb PstI/EcoRI restriction fragment that was detected with the probe indicated that a population of proviruses contained an EcoRI restriction site within the 400 base pair SmaI/SmaI region that hybridized with the probe and another EcoRI restriction site in the env region. The absence of additional restriction fragments when the DNA was digested with PvuII indicated that the acquired proviruses integrated randomly in the splenic DNA of mouse A7. The restriction maps and restriction fragments generated from the ecotropic proviruses in the splenic DNA of mouse A7 are illustrated in Figure 2.6.

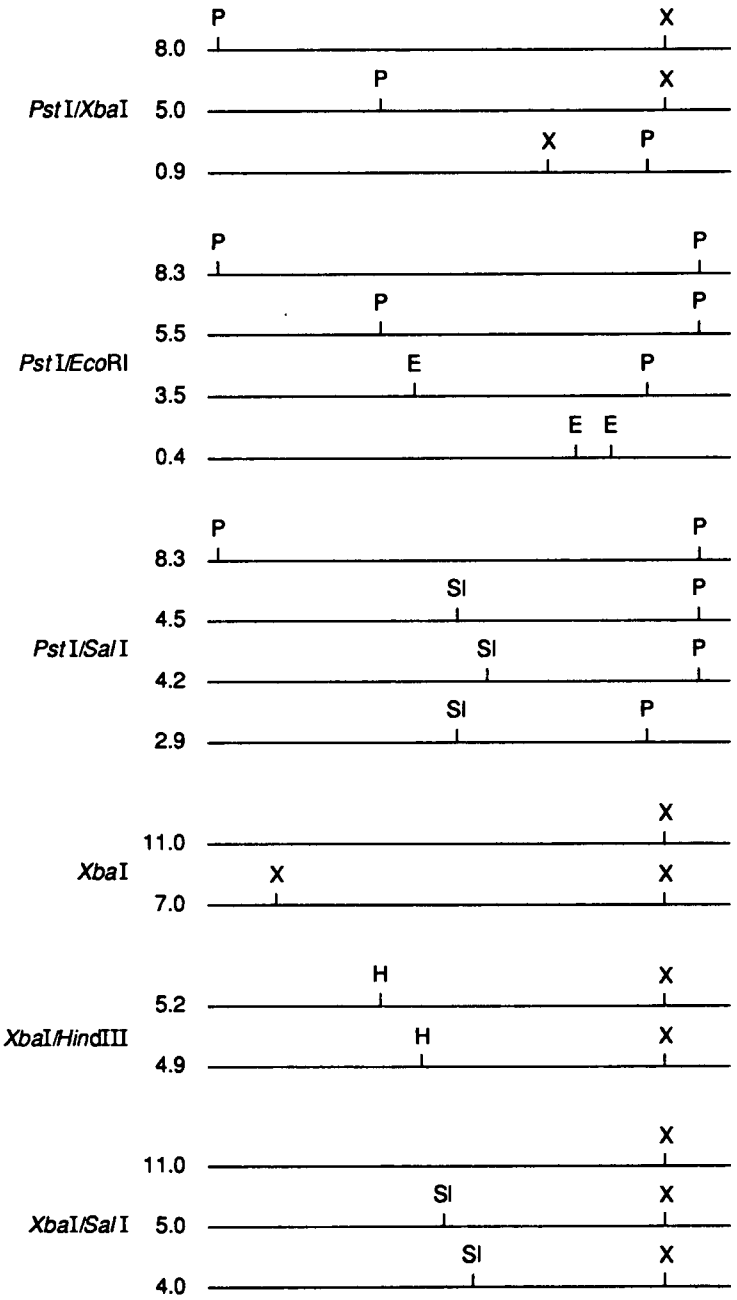
When splenic DNA from mouse A17 was cut with PstI, three restriction fragments hybridizing with the probe were generated (Table 2.1). A 5.1 kb fragment was found in addition to the 8.3 kb emv-2 and the 5.5 kb B10Fv fragments, indicating that at least three proviral populations existed in the splenic DNA of this mouse. The 4.5 kb PvuII/PvuII fragment that was

Figure 2.6. Restriction maps and restriction fragments generated from the ecotropic proviruses in the splenic DNA of mouse A7. Restriction endonuclease maps were generated from the data in Figure 2.5. The restriction endonuclease sites in AKV, B10.F(ash), emv-2 and B10Fv are shown for comparison. The restriction endonucleases, restriction fragment sizes and relative positions of the restriction endonuclease sites are shown. The hatched area in the env region represents the site of hybridization of the 400 base pair ecotropic specific probe. The symbols used are given in the legend to Figure 2.4.


PART 1
0 1 2 3 4 5 6 7 8 8.8
LTR gag pol env LTR
AKV
B10.F(ash)
emv-2
B10Fv
PvuII 5.2
PstI 8.3 5.5 4.8
PstI/PvuII 3.2 2.0 1.0
PstI/BamHI 4.8 3.1 2.8
PstI/HindIII 5.5 4.8 3.5
P or H

(continued)

PART 2


PstI/XbaI
8.0
5.0
0.9
PstI/EcoRI
8.3
5.5
3.5
0.4
PstI/SalI
8.3
4.5
4.2
2.9
XbaI
11.0
7.0
XbaI/HindIII
5.2
4.9
XbaI/SalI
11.0
5.0
4.0

observed indicated that a large portion of the cells in the spleen contained a provirus integrated into the same location in the genome.

Southern blots of DNA from mouse A42 demonstrated that the splenocytes contained proviruses in addition to emv-2 and B10Fv. These additional proviruses were detected by the presence of extra restriction fragments that hybridized with the probe when the DNA was digested with every enzyme (Figure 2.7). The minimum number of viruses that was present in the splenic DNA was determined by counting the number of restriction fragments detected after digestion with XbaI/SalI. Six restriction fragments were detected; the sizes ranged from 11 kb (corresponding to emv-2) to 4.0 kb (corresponding to B10Fv). The detection of six restriction fragments indicated the presence of least six different viral populations with different XbaI/SalI restriction sites. In addition, one proviral population had PstI sites similar to B10Fv but did not contain a SalI site.

Digestion of the splenic DNA from mouse A42 with PvuII yielded seven restriction fragments that were detected with the ecotropic gene specific probe. The sizes of the fragments ranged from 8.5 to 2.8 kb (5.5,

Figure 2.7. Southern blot of ecotropic MuLV sequences in addition to emv-2 and B10Fv found in the splenic DNA of mouse A42. Novel restriction fragments in addition to those attributed to emv-2 and B10Fv are observed when the DNA was digested with all the restriction enzymes. These additional restriction fragments represent novel acquired proviruses. Digestion with both XbaI and SalI produced six restriction fragments of 3.9, 4.9, 5.5, 6.5, 7.8 and 11 kb in length. The appearance of these six restriction fragments indicates that there were at least six structurally different ecotropic proviruses in the splenic DNA of mouse A42. Also note the six PvuII restriction fragments detected with the ecotropic probe. Based on the relative intensities of the additional PvuII restriction fragments and the restriction fragments corresponding to B10Fv, the majority of cells undergoing proliferation probably contained B10Fv.

origin –
PvuII
PstI/PvuII
PstI
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI
kb
9.4–
6.7–
4.4–
2.3–
2.0

7.5, 6.0, 5.2, 4.5, and 2.8) in length. Histological examination of the enlarged spleen from this animal showed the presence of a few predominant cell types, which is consistent with the hypothesis that the multiple PvuII restriction fragments result from the clonal expansion of one or a few cells containing ecotropic proviruses. The correspondingly high intensities of hybridization of the additional PvuII restriction fragments compared with the high intensities of hybridization of B10Fv restriction fragments in the other lanes indicated that the majority of clonal expansion occurred in cells containing B10Fv and not other acquired proviruses. It was concluded that the 2.8 kb PvuII/PvuII restriction fragment represented a proviral population with a new PvuII structure. The restriction fragment size was less than 3.0 kb, which is the distance from the PvuII site in the pol region to the 3' end of the LTR in both emv-2 and B10Fv (Figure 2.8).

Restriction endonuclease maps of the additional acquired proviruses found in mice A7, A17, A42 and A46 were generated (Figures 2.9 and 2.10).

The number of proviruses with different PstI/SalI restriction fragments in the B10.F population was

Figure 2.8. Restriction endonuclease maps of the PvuII generated restriction fragments in the splenic DNA of mouse A42. PvuII restriction endonuclease maps were generated from the data in Figure 2.7. Digestion with PvuII produced a virus-cell junction fragment. As shown in this figure, the size of the PvuII restriction fragment depended upon the location of the virus in the genome. The distance from the PvuII site in the env region to the 3' end of the provirus is approximately 3.0 kb. Therefore, PvuII restriction fragments less than 3.0 kb represent provirus-related sequences with an internal PvuII site. It is also possible that the PvuII site is not in the 3' LTR; the restriction fragments less than 3.0 kb could represent defective proviruses with a small deletion in the 3' end of the provirus. A provirus containing a deletion might be defective and detected in a cell that has undergone proliferation. It could possibly reinfect cells through the use of helper viruses.

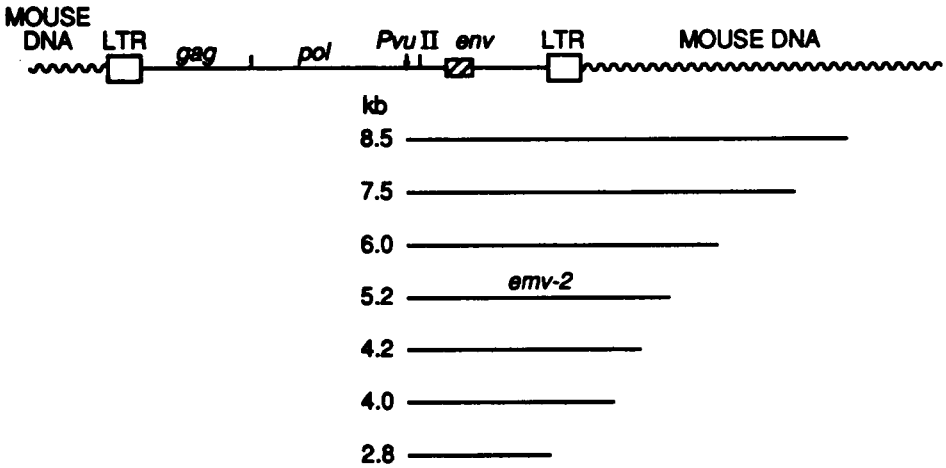

MOUSE DNA
LTR
gag
pol
PvuII
env
LTR
MOUSE DNA
kb
8.5
7.5
6.0
emv-2
5.2
4.2
4.0
2.8

Figure 2.9. Composite restriction endonuclease map of acquired ecotropic proviruses present in the splenic DNA of the B10.F population. Restriction sites for acquired proviruses that differed from B10Fv were generated from Southern blots of splenic DNA from 50 mice digested with PstI, PvuII and PstI/PvuII and from 2 mice digested with PvuII, PstI, PstI/PvuII, PstI/BamHI, PstI/HindIII, PstI/XbaI, PstI/EcoRI, PstI/SalI, XbaI, XbaI/HindIII and XbaI/SalI (Figures 2.5 and 2.6, and Table 2.1). For the composite map, restriction endonuclease sites shown above the line indicate the absence of sites that are present in B10Fv. Restriction endonuclease sites shown below the line indicate sites that are not present in B10Fv but are present in other proviruses found in the splenic DNA of B10.F mice. The numbers shown as superscripts next to the sites indicate the number of times (greater than one) these variants were observed. The corresponding restriction sites in AKV, B10.F(ash), emv-2 and B10Fv are shown for comparison. The symbols used are given in Figure 2.4.

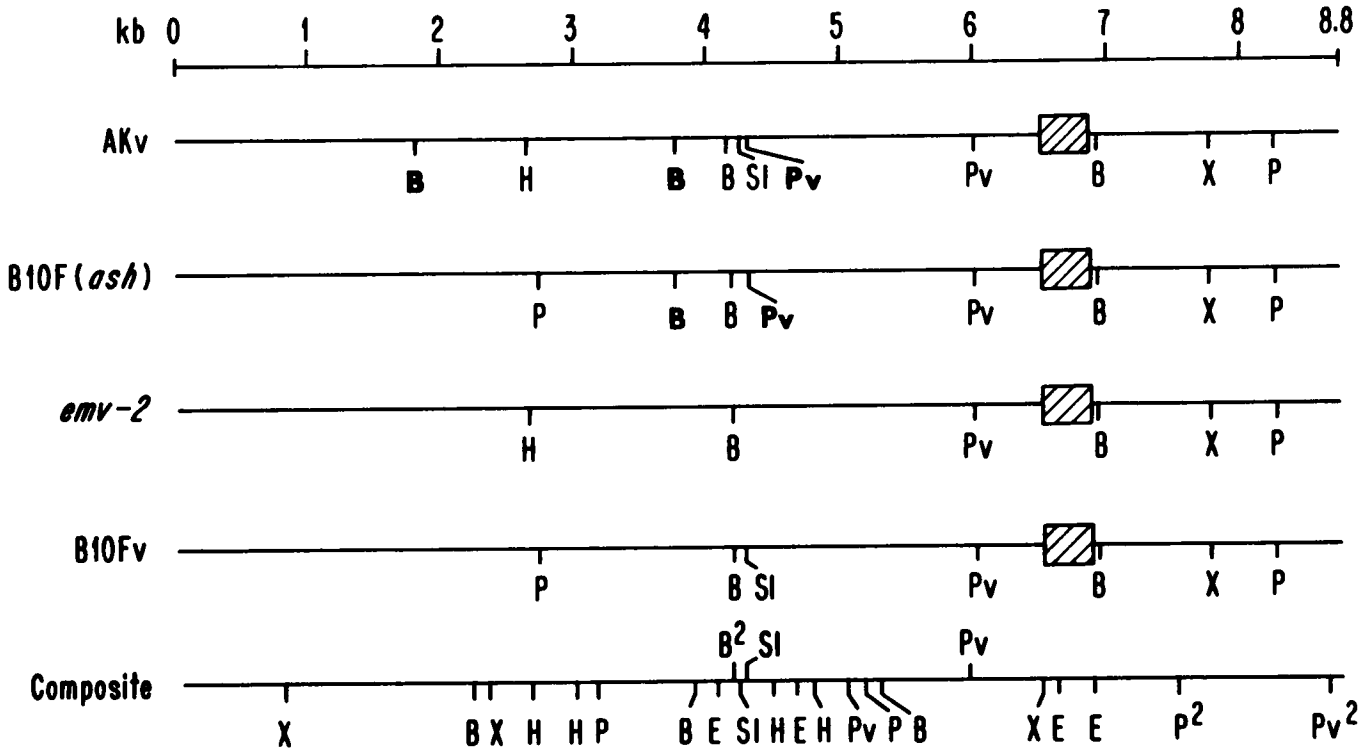
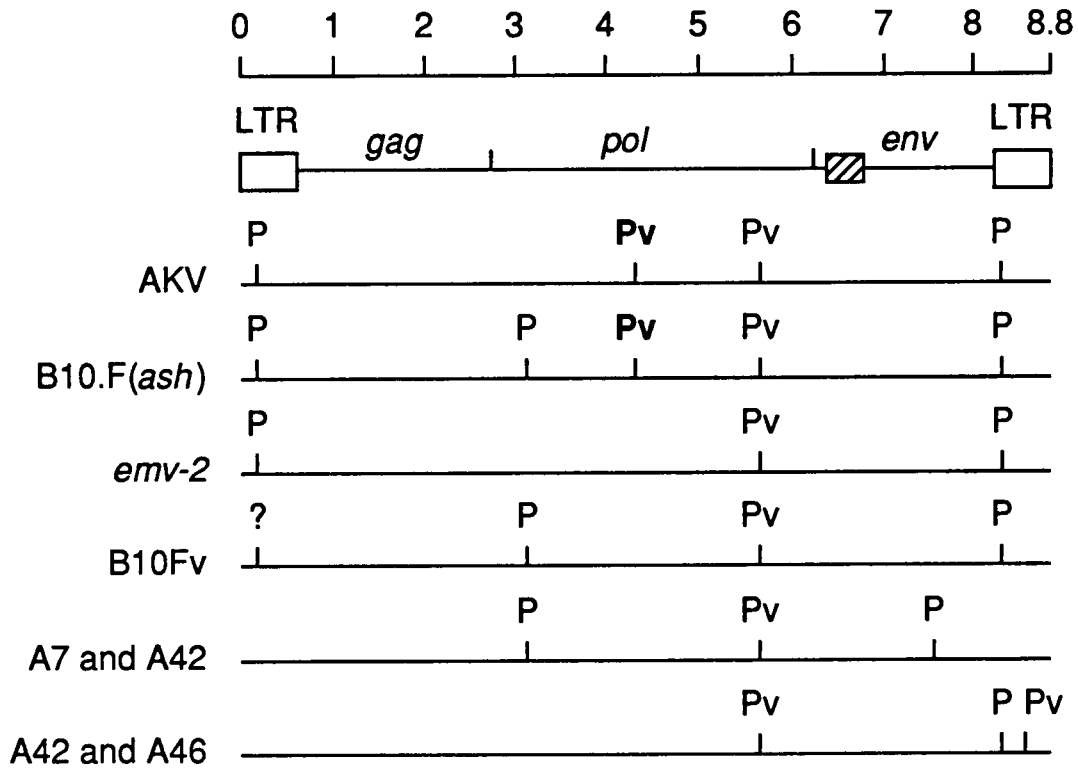

kb 0 1 2 3 4 5 6 7 8 8.8
AKv
B H B B Sl Pv Pv B X P
B10F (osh)
P B B Pv Pv B X P
emv-2
H B Pv B X P
B10Fv
P B Sl Pv B X P
Composite
B2 Sl Pv
X B X H H P B E Sl H E H Pv P B X E E P2 Pv2

Figure 2.10. Maps of PstI and PvuII restriction endonuclease sites in the common proviruses found in the B10.F population. Data were generated from Figures 2.5 and 2.6, and Table 2.1. The 4.8 kb PstI restriction fragment detected in the splenic DNA of both A7 and A42, and the 2.8 kb PvuII restriction fragment detected in the splenic DNA from both A42 and A46, are compared with the corresponding restriction endonuclease sites in AKV, B10.F(ash), emv-2, and B10Fv.


0
1
2
3
4
5
6
7
8
8.8
LTR
gag
pol
env
LTR
AKV
P
Pv
Pv
P
B10.F(ash)
P
P
Pv
Pv
P
emv-2
P
Pv
P
B10Fv
?
P
Pv
P
A7 and A42
P
Pv
P
A42 and A46
Pv
P Pv

determined from PstI/SalI double digests of the four DNA samples that had restriction fragment variations. When the DNAs were cut with PstI/SalI and run side by side in the same gel a total of six fragments with different sizes were detected. The sizes of these fragments were 3.1, 3.5, 4.2, 4.5, 5.5, and 8.3 kb (Figure 2.11). The maps of the PstI/SalI restriction fragments generated from the ecotropic proviruses in the B10.F population are illustrated in Figure 2.12.

Approximate copy number of acquired ecotropic proviruses in the DNA of B10.F mice

When the splenic DNA from B10 mice was cut with PstI, only the 8.3 kb emv-2 fragment was present. However, digestion of splenic DNA from all B10.F mice yielded a 5.5 kb PstI restriction fragment in addition to the 8.3 kb emv-2 fragment. The intensity of the restriction fragments characteristic of the somatically acquired proviruses varied from mouse to mouse. In order to determine the copy number of acquired proviruses in the B10.F mice, the intensity of the endogenous provirus PstI band, which represents two copies per diploid genome, and the intensity of the acquired provirus PstI bands were compared. The ratio

Figure 2.11. Estimation of the number of proviruses with different PstI/SalI restriction fragments in the B10.F population. The four splenic DNA samples that showed restriction fragment variations from the other B10.F mice were digested (10 ug/lane) with both PstI/SalI and run side by side in an agarose gel. A total of six different restriction fragments were observed (indicated by the dots). The sizes of the restriction fragments were 2.9, 3.1, 4.2, 4.5, 5.5, and 8.3 kb. The two fragments, 4.5 kb and 8.3 kb, seen in every lane were the fragments derived from B10Fv and emv-2, respectively. The other fragments represent additional proviruses. There were a minimum of six proviruses possessing different PstI/SalI restriction endonuclease sites in the splenic DNA of the B10.F population examined.

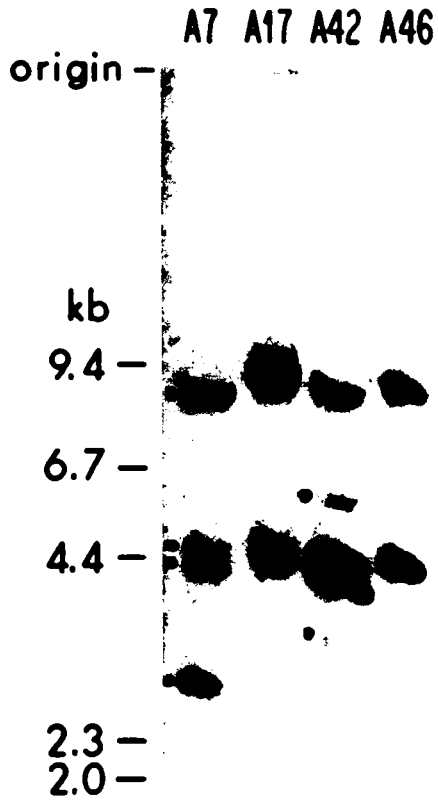
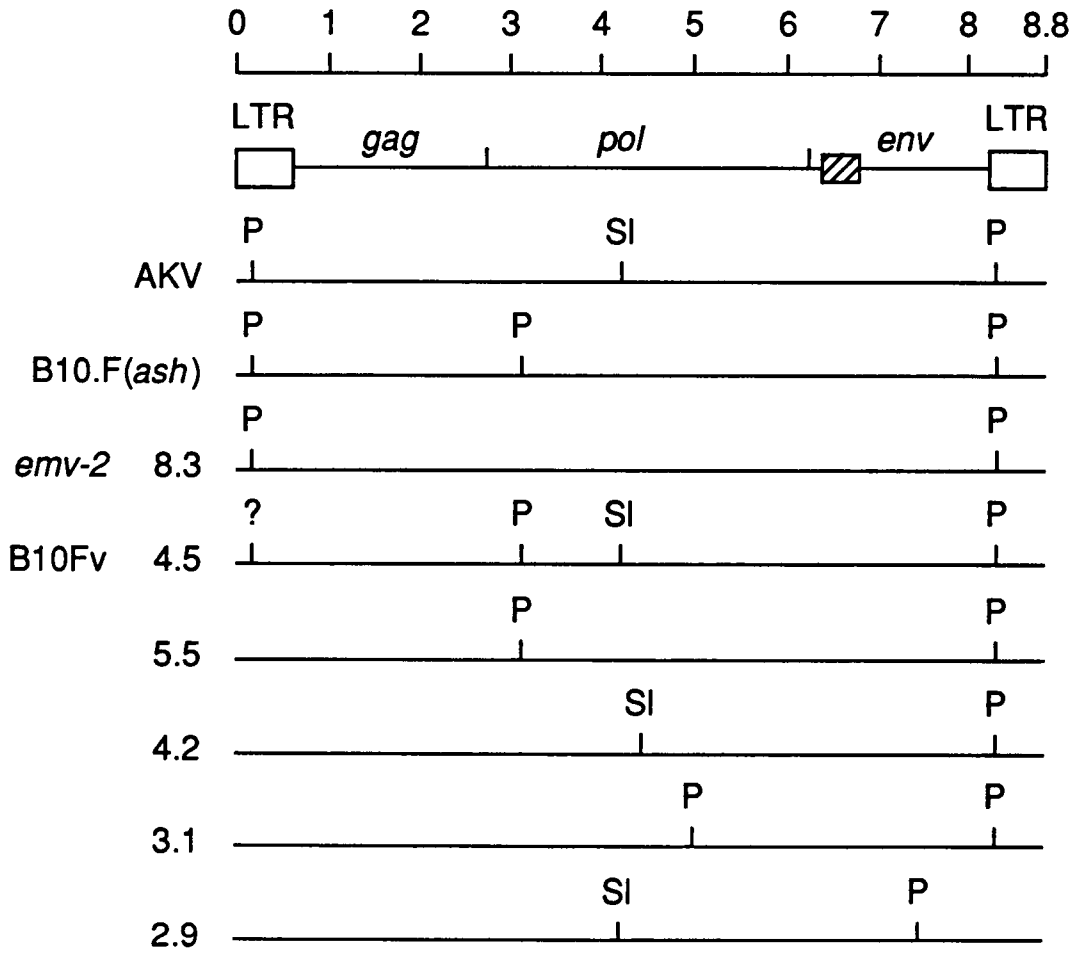

A7
A17
A42
A46
origin
kb
9.4
6.7
4.4
2.3
2.0

Figure 2.12. Maps of PstI/SalI restriction fragments generated from the ecotropic proviruses in the B10.F population. Data were generated from Figure 2.9. The corresponding restriction sites in AKV and B10.F(ash) are shown for comparison. In addition to emv-2 and B10Fv, four other PstI/SalI restriction fragments were found, indicating the presence of four additional proviruses with different PstI/SalI restriction endonuclease sites. The symbols used are described in Figure 2.4.


0
1
2
3
4
5
6
7
8
8.8
LTR
gag
pol
env
LTR
AKV
P
SI
P
B10.F(ash)
P
P
P
emv-2
8.3
P
P
B10Fv
4.5
?
P
SI
P
5.5
P
P
4.2
SI
P
3.1
P
P
2.9
SI
P

of the intensity of hybridization of the nonendogenous bands to the intensity of hybridization of theendogenous band was estimated visually. Generally, the intensity of the emv-2 fragment and the fragment(s) from the acquired proviruses were nearly equal; thus, on the average, the B10.F splenocytes contained at least two acquired ecotropic proviruses per diploid genome (Figure 2.13).

Tropism of viruses found in B10.F mice

Restriction endonucleases were used to digest the DNA isolated from liver, spleen, lymph nodes and kidney of four B10.F mice to determine whether acquired proviruses were present in other tissues. When the DNA was digested with PstI all DNA samples contained emv-2 as expected. However, only in the DNA from the spleens and lymph nodes was the 5.5 kb B10Fv restriction fragment apparent in all mice. In the digested DNA from the liver and kidney of two mice there was some evidence of the 5.5 kb B10Fv PstI restriction fragment (Figure 2.14).

Figure 2.13. Frequency of appearance of acquired proviruses in the B10.F population. The hybridization intensity of the nonendogenous bands was visually compared to the hybridization intensity of the endogenous emv-2 band. The ratios of hybridization intensity of the acquired proviruses to the endogenous provirus ranged from <0.5 to >1.5 with a mean of 1.1.

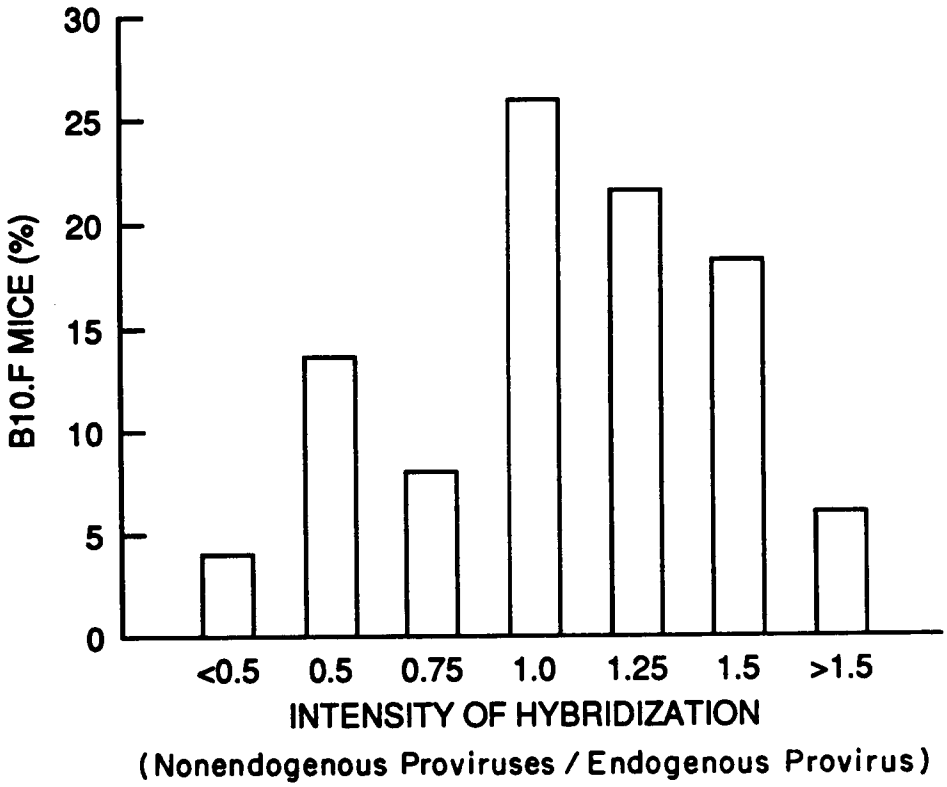
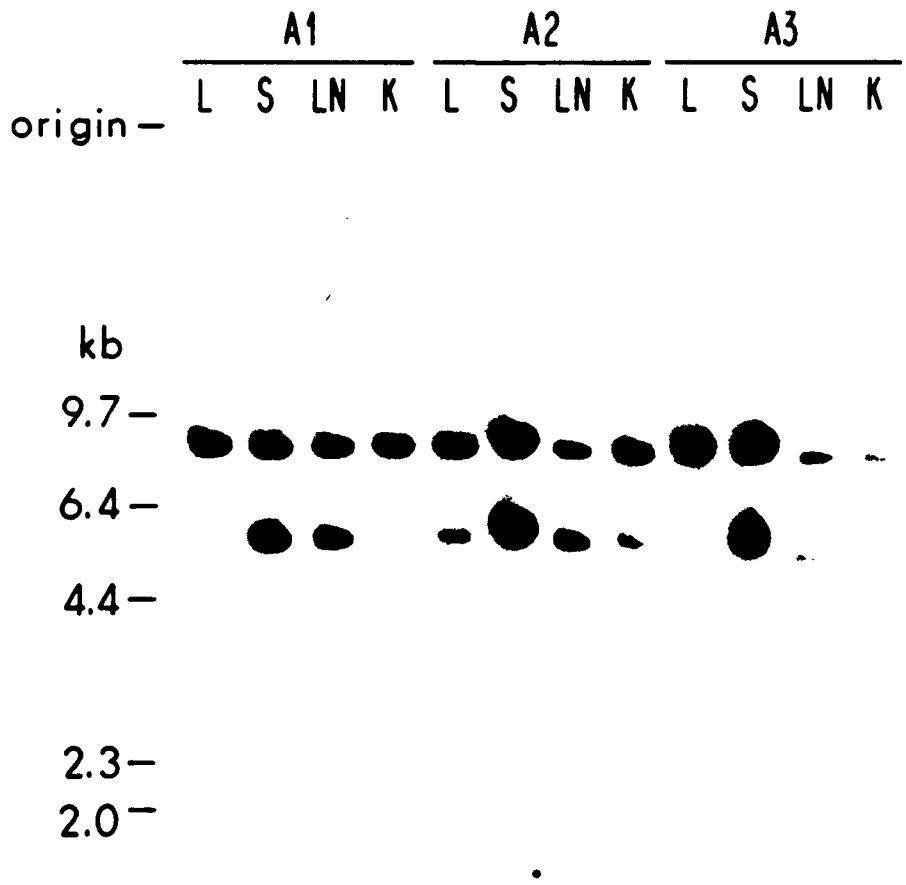

30
25
20
15
10
5
0
B10.F MICE (%)
<0.5
0.5
0.75
1.0
1.25
1.5
>1.5
INTENSITY OF HYBRIDIZATION
(Nonendogenous Proviruses / Endogenous Provirus)

Figure 2.14. Tissue specificity of virus. DNA from the liver, spleen, lymph nodes, and kidney of three typical six-month-old male B10.F mice (A1, A2, and A3) was digested to completion with PstI (5 ug/lane) and analyzed by Southern blotting for ecotropic proviral sequences. The intensity of hybridization in the 5.5 kb PstI restriction fragment derived from the acquired proviruses, was less for DNA isolated from the liver (L) and kidney (K) than from DNA isolated from spleen (S) and lymph nodes (LN).


A1
A2
A3
L S LN K L S LN K L S LN K
origin
kb
9.7
6.4
4.4
2.3
2.0

Discussion

Previous studies have shown that the B10.F mouse is highly viremic (Morse et al., 1985; Popp et al., 1986). This study has shown that the highly viremic B10.F mouse contains multiple copies of acquired ecotropic provirus in the DNA of the splenocytes. One provirus, B10Fv, was found integrated into many different sites and at an average frequency of two copies per diploid genome in the splenic DNA of every B10.F mouse examined. B10Fv was also found to a lesser extent in the DNA of nonlymphatic organs such as liver and kidney. Additional acquired proviruses were also present in the splenic DNA of some of the B10.F mice.

Structure and origin of proviruses in the B10.F mouse

The structure of the endogenous and acquired proviruses can be distinguished through the use of restriction endonuclease analysis. B10 and B10.F mice both carry the endogenous emv-2. The restriction pattern of emv-2 in the B10 and the B10.F mouse is identical. The sizes of the restriction fragments generated by digestion of emv-2 in splenic DNA with

PvuII, XbaI and PstI were consistent with those reported by Jenkins et al. (1982). The restriction maps of emv-2 and the prototypic virus AKV are very similar except that emv-2 is not cleaved with SalI. This apparent lack of a SalI site may be attributed to the sensitivity of SalI to 5-methylcytosine. Two to seven percent of the cytosine residues in mammalian cells can be methylated and DNA methylation is one of the key elements that govern vertebrate gene function (Razin and Riggs, 1980). The absence of methyl groups is associated with gene expression (Razin and Riggs, 1980). It is possible that emv-2 does contain a SalI site but it is not cleaved because the cytosine residues in the SalI site are methylated. PvuII is also sensitive to methylation and does cleave emv-2. Therefore, it is most likely that emv-2 does not contain a SalI site.

The spleens of B10.F mice contain multiple copies of nonendogenous proviruses that are not present in the B10 mice. PstI digestion of DNA facilitates the detection of new viral DNA sequences even when the proviruses are positioned at many sites throughout the cellular genome. This is possible because the digestion products that hybridize with the probe are

comprised entirely of proviral DNA. B10Fv is the most commonly acquired provirus that was identified through restriction endonuclease analysis. B10Fv, like B10.F(ash), has a PstI site in the pol region. However, unlike the B10.F(ash), B10Fv contains a SalI site in the pol region (Figure 2.4, page 58).

Comparison of the intensities of hybridization of the restriction fragments from acquired proviruses to the intensity of hybridization of the restriction fragment from the endogenous provirus indicated that on the average, the DNA of spleen cells of B10.F mice had two copies of B10Fv provirus (Figure 2.14, page 92).

These results are consistent with the observation that B10.F mice, unlike B10, express high levels of ecotropic B-tropic MuLV early in life (Yetter et al., 1983; Morse et al., 1985; Popp et al., 1986). The appearance of virus in B10.F, but not in B10, mice may be attributed to one or more factors: 1) High titers of virus in the B10.F mice result from congenital transmission of ecotropic viruses from viremic mothers to offspring. F/St is a highly viremic strain of mice and F/St mothers were used in the original F/St X C57BL/10 cross to establish the B10.F congenic mice (Morse et al., 1982; Diana M. Popp, personal

communication). Continued maternal transmission via transplacental infection and exposure to milk-borne virus could contribute to the high virus phenotype of B10.F mice (Morse et al., 1983). 2) Additionally, B10.F mice might carry the *Cxv-1*^{s} allele which is closely linked to the *H-2* locus. This allele controls the high level of expression of xenotropic viruses (Yetter et al., 1983). A high expression of xenotropic viruses may in turn cause a high expression of ecotropic viruses by providing the defective N-tropic *emv-2* with many targets available for recombination. 3) The two strains may have different susceptibilities to retroviral infection. B10 mice that are foster nursed onto B10.F females acquire the B10.F phenotype. However, after two or three generations this phenotype is lost (D. M. Popp, personal communication). B10 mouse carries two retrovirus-like sequences, *Tlev1* and *Tlev2*, within the Tl locus of the MHC (Pampeno and Meruelo, 1986). B10 and B10.F are congenic at the *H-2* locus of the MHC and may differ at the *Tlev1* and *Tlev2* loci as well. The expression of the Tlev antigens on the surface of the B10 cells may confer resistance to retroviral infection (Pampeno and Meruelo, 1986). If B10.F mice do not express this antigen, they would be

expected to be more susceptible to infection by retroviruses.

In addition to the acquired B10Fv provirus found in the B10.F mice, a variety of other acquired proviruses were found through restriction endonuclease analysis. The structures of these acquired proviruses differed at one or a few sites from the structures of AKV, B10.F(ash), and emv-2 and B10Fv (Figure 2.9, page 80). Restriction fragment polymorphism established that a minimum of six proviruses with different PstI/SalI restriction sites were present in the splenic DNA of the 50 B10.F mice examined (Figures 2.11 and 2.12, pages 85 and 87). Proviruses with different structures may also exist because alterations could be present at sites which were not examined or exist in an insufficient quantity to be detected.

B10Fv was probably present in the highly viremic F/St mother of the original F/St X C57BL/10 mating made to establish the B10.F congenic mouse and has been congenitally transmitted from mother to offspring in utero or via milk transmission ever since (Zijlstra et al., 1983; Morse et al., 1982; Popp et al., 1986). Moreover, B10Fv most probably contributes to the formation of other proviruses found in the B10.F

through errors in replication, for example misincorporation of bases and deletions, and through recombination with other proviral sequences in the mouse genome via copackaging of viral transcripts (Coffin, 1986; Temin, 1989). The recombination could occur with xenotropic-like sequences or the defective ecotropic emv-2 provirus in the cell. Nucleotide sequence analysis of the acquired proviruses might distinguish between alternative mechanisms for their origin.

There is also a possibility that the acquired proviruses are generated de novo by recombination of Emv-2 with xenotropic-like sequences in the genome. It has been proposed that the ecotropic MuLVs recovered from C57BL mice are recombinant viruses with env regions derived from the endogenous ecotropic provirus and gag regions derived from endogenous xenotropic-like sequences (Benade and Ihle, 1980; Rassart et al., 1983; Rassart et al., 1986). In order for emv-2 to replicate in B10.F, the endogenous ecotropic virus would have to be altered in both the gag and the pol regions. The alterations must include the capsid protein (CA;p30) determining the N-tropism and the mutation in the tether region of the reverse transcriptase 3' to

HindIII and 5' to the SalI site (King et al., 1987). Recombinants involving emv-2 would be very similar in structure to B10Fv. However, for B10Fv to be generated de novo rather than passed from mother to offspring, the recombinations involving emv-2 would have to occur at the same position in every mouse, which is highly unlikely.

Among the proviruses examined, the majority of the structural variation occurred 5' to the env region of the provirus (Figure 2.9, page 80). However, in at least two DNA samples (A42 and A46), alterations in the 3' region of the virus were detected when the DNA was cut with PvuII (Figure 2.8, 2.9 and 2.10, pages 78, 80 and 82). PvuII digestion of the splenic DNA samples from these two mice produced restriction fragments that were less than 3.0 kb in length. Restriction fragments smaller than 3.0 kb may be the result of additional PvuII sites in the 3' LTR or deletions in the 3' end of the provirus. Recombination in the 3' end of ecotropic viruses has been reported (Nowinski and Hays, 1978; Lenz et al., 1982; Lenz and Haseltine, 1983; Thomas, 1986). In the present study, recombinants in the 5' region were preferentially identified because the probe is specific for the 3' region; recombinations that

occur between ecotropic and xenotropic viruses that alter the env region, specifically within the region that hybridizes with the probe, may lead to viruses that do not react with the ecotropic specific probe, i.e. MCF viruses, and therefore would not be detected.

The splenic DNA from some animals gave similar length proviral restriction fragments. The splenic DNA from mice A7 and A42 contained ecotropic proviral sequences with PstI restriction sites that were approximately 4.8 kb apart (Table 2.1, page 66). Analysis of the restriction fragments detected after digestion with several restriction endonucleases placed a PstI site in the 5' end of the pol region (analogous to B10Fv) and an additional PstI site 4.8 kb 3' in the 3' end of the env region (Figures 2.6, 2.9 and 2.10, pages 71, 80 and 82). Additionally, the splenic DNA from mice A42 and A46 both contained proviral sequences with PvuII restriction fragments that were 2.8 kb in length, indicating the presence of common proviruses with alterations in the 3' end of the provirus (Figure 2.9 and 2.10, pages 80 and 82). This commonality among the additional acquired proviruses indicates that a subpopulation of acquired proviruses might be present among the B10.F mice. Alternatively, these proviruses

may only share a few common sites (like emv-2 and B10Fv) and in fact differ in other sites that were not examined.

Clonal expansion of cells containing unique viral integrations

The site of retrovirus integration in the DNA may have profound consequences on the cell (White, 1986; Zijlstra and Melif, 1986). Detection of proviruses through the use of restriction enzymes that cut at least twice within the viral sequence established that the acquired proviruses in the B10.F mouse were integrated at many different sites in the DNA. However, the analysis of integration sites could not distinguish between random integration and integration into a large number of preferred sites in the host genome. Shih et al. (1988) produced a collection of clones comprised of integrated provirus (Rous sarcoma virus containing a bacterial suppressor tRNA gene) with host flanking sequences and analyzed them for the specificity of integration sites. They estimated that there were 500 - 1000 preferred target sites for retroviral integration. Even though the actual site of integration might be random, the area of integration

may be of biological significance. Digesting the DNA with restriction endonucleases that cut the DNA infrequently might produce common restriction fragments that contain the acquired proviruses. This would indicate an area of biological significance rather than a specific site.

Although the integration sites of the nonendogenous ecotropic retroviruses were randomly located throughout the genome, DNA from three mice contained additional PvuII virus-host DNA junction restriction fragments. This observation indicates that a portion of the tissue was derived from one or a few cells that contained an acquired provirus, or less likely that the virus has integrated independently into the same site of many cells. The detection of a unique viral integration in a tissue, such as the spleen, comprised of many different cell populations could be explained in three ways: 1) MuLV can integrate into the genome of a mouse as early as the 4 - 8 cell stage of development (Jaenisch et al., 1975). If a virus integrates into the DNA of a cleavage stage embryo, a large number of the cells in the mouse will contain the unique viral integration. 2) Viral integration into the DNA of a differentiated cell may cause the cell containing the

integration to undergo abnormal clonal expansion (McGrath and Weissman, 1979). The presence of a strong retroviral promoter in the vicinity of a cellular oncogene can increase transcription of this cellular oncogene, and the increased transcription could result in transformation of the cell (Sheinin et al., 1987). 3) A cell that contains a specific integration may be stimulated to proliferate for reasons unrelated to the integration of the provirus in that cell (Nakajima et al., 1989). Each of these three possibilities could account for the detection of unique viral integrations in the splenic DNA of the B10.F mouse.

Tissue specificity of virus

DNA from lymphatic organs contained more nonendogenous proviruses than did DNA from nonlymphatic tissues. This difference could be due to three alternate factors: 1) Cells of the liver and kidney of the B10.F mouse may be resistant to infection by B10Fv. The low level of exogenous proviruses found in the liver and kidney could come from DNA of lymphocytes that carry the provirus. 2) The liver and kidney are resistant to B10Fv but not to other acquired viruses that are able to integrate at a lower frequency. These

viruses may have different LTRs which enable them to infect and replicate in the liver and kidney (Rassart et al., 1988). An altered tropism might allow the virus to infect and replicate in the liver and kidney. 3) The exogenous virus may infect and integrate into fewer cells of the liver and kidney, possibly only in cells that are undergoing cell division (Varmus, 1987).

CHAPTER III

THE EFFECT OF X-IRRADIATION ON THE ECOTROPIC PROVIRUSES AND THE RELATIONSHIP TO TISSUE HYPERPLASIA IN THE C57BL/10.F MOUSE

Introduction

Radiation has been long recognized as a potent carcinogen. Lymphomas and leukemias are the most common of all radiation-induced neoplasms (Niwa and Sugahara, 1979). The carcinogenic effects of ionizing radiations are attributed to their deposition of energy in certain targets, e.g., the DNA of cells, causing molecular changes (Kanizir, 1969). Ionizing radiation produces a number of different lesions in the DNA of cells. Such lesions include base alterations, single and double strand breaks, crosslinking of strands and degradation of DNA (Kanizar, 1969). The variety of lesions produced suggests that several different

mechanisms may be involved in the process of carcinogenesis.

Experimental evidence links the appearance of certain radiation-induced tumors with viral activation (Liberman et al., 1976; Kaplan, 1977). Induction of viruses does not appear to be a general requirement of radiation-induced tumorigenesis, but it does play a role in selected systems.

Radiation-induced viral activation in C57BL mice has been the subject of numerous studies (Kaplan, 1967; Liberman et al., 1976). The activation of retroviruses is dependent on the dose rate, age of the animal, genes that affect the expression of viruses, and the immune responsiveness of the animal (Kaplan, 1967; Niwa and Sugura, 1979; Yang et al., 1980; Meruelo et al., 1986; Fry and Storer, 1987). Nonendogenous ecotropic and recombinant proviruses have been observed in radiation-induced tumors (Jolicour et al., 1983; Astier-Gin et al., 1986; Boone et al., 1986). The induction of disease by subleukemogenic doses of radiation combined with infection by weakly oncogenic viruses is further evidence for the involvement of murine leukemia viruses in the leukemic action of radiation (Haran-Ghera, 1969; Aster-Gin et al., 1986; Legrand et al., 1986).

This study was undertaken to study the effects of X-irradiation on the detection of somatically acquired ecotropic proviruses in B10.F mice and the possible relationship between specific proviruses and tissue hyperplasia. This chapter describes the gross anatomy and histology, the structure and number of proviruses, and the proliferation of somatic cells that contain proviruses in irradiated B10.F mice.

Materials and Methods

Two-month-old male B10.F mice were exposed (whole body) to a single dose of 1 (52 mice; B1-B7, B16-B60), 2 (54 mice; C1-C54) or 3 (53 mice; D1-D53) Gy of X-rays. Fifty one mice served as nonirradiated controls (A1-A51, discussed in Chapter II). A Philips X-ray machine was operated at 20 ma and 300 kVp, with 1.0 mm inherent Be filtration and 3.0 mm added Al filtration, giving a dose rate of approximately 0.75 Gy per minute at a target to object distance of 100 cm. At six months of age the mice were sacrificed and analyzed as described in Chapter II pages 45-48.

Results

Gross and histological examination of mice and tissues

In order to assess the effect of X-rays on tissue hyperplasia, the animals and their organs were weighed. The mean whole body weights and organ weights for the animals are presented Table 3.1. The whole body, liver and kidney weights were similar for each group of mice. However, the 2 and 3 Gy groups had a larger number of mice with enlarged spleens as shown in the distribution profile in Figure 3.1.

Histological sections of liver, spleen, kidneys, and lymph nodes from the irradiated animals were examined. Morphological changes were observed primarily in the spleens of the irradiated mice; specific changes in histology were designated as Types I, II, III, IV, and V. The histological characteristics of these patterns are summarized in Table 3.2. Spleens with Type I histology had intact architecture of the lymphatic follicles with well organized red pulp (composed primarily of myeloid cells) and white pulp (composed of lymphocytes). Hematopoiesis in the red pulp was normal with the presence of granulocytes, megakaryocytes and

TABLE 3.1

CHARACTERISTICS OF B10.F MICE EXPOSED TO IONIZING RADIATION

Treatment (Gy)	No. of mice	Body wt (g)[a]	Liver wt (mg)[a]	Kidney wt (mg)[a]	Spleen wt (mg)[a]	No. of enlarged spleens[b]
0	51	28.3±0.4	1646±31	425±6.6	131±7.7	2
1	52	28.7±0.4	1663±40	405±7.3	124±3.9	1
2	54	27.9±0.3	1723±63	426±30.8	275±86.2	9
3	53	26.7±0.8	1584±51	415±20.4	191±31.8	7

[a]Average value ± standard error of the mean.
[b]Spleens greater than 200 mg in weight.

Figure 3.1. Distribution of spleen weights among B10.F mice exposed to 0, 1, 2, and 3 Gy of ionizing radiation. Two-month-old B10.F male mice were exposed (whole body) to a single dose of X-ray and sacrificed at six months of age.

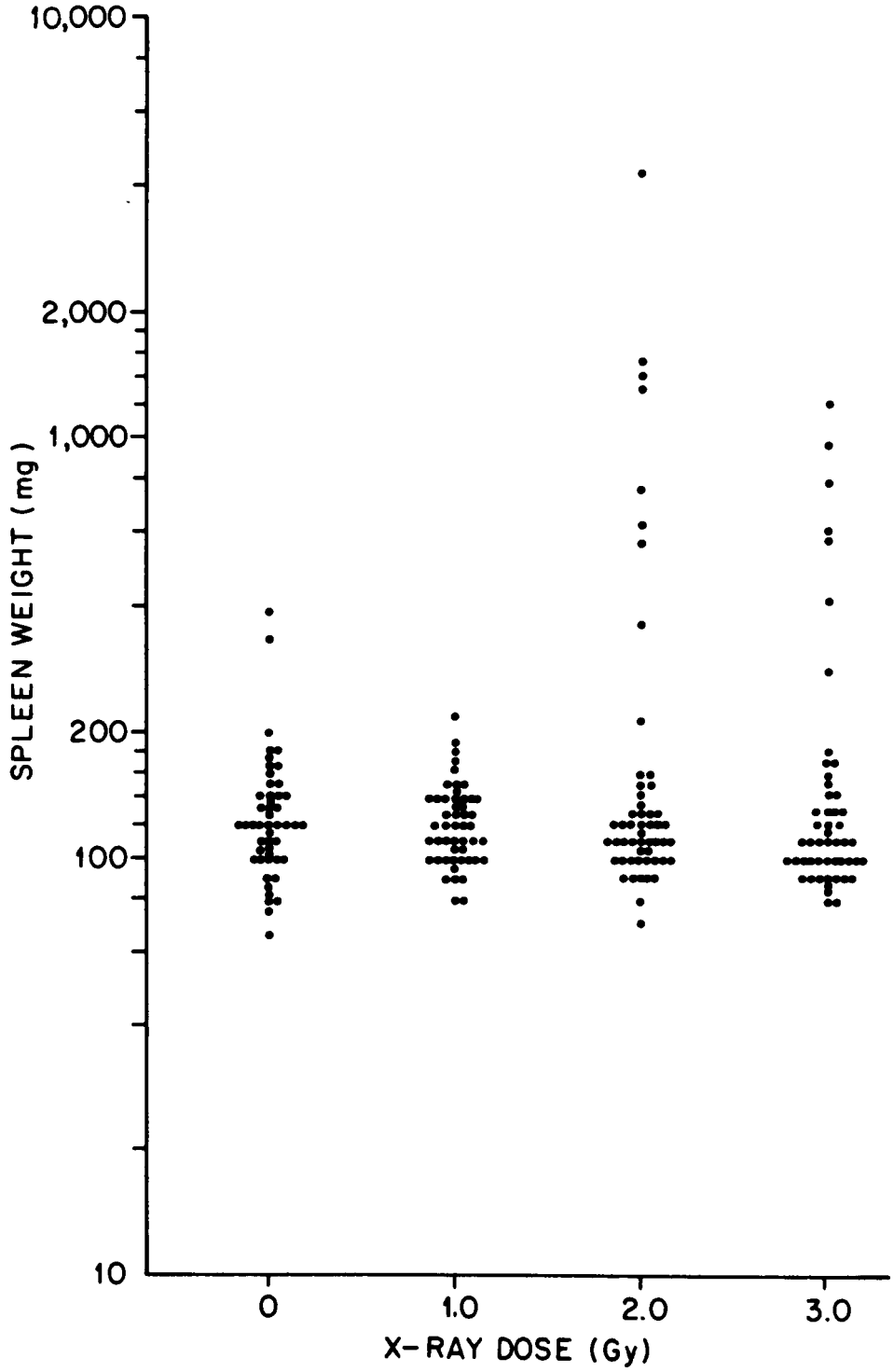

10,000
2,000
1,000
200
100
10
SPLEEN WEIGHT (mg)
0
1.0
2.0
3.0
X-RAY DOSE (Gy)

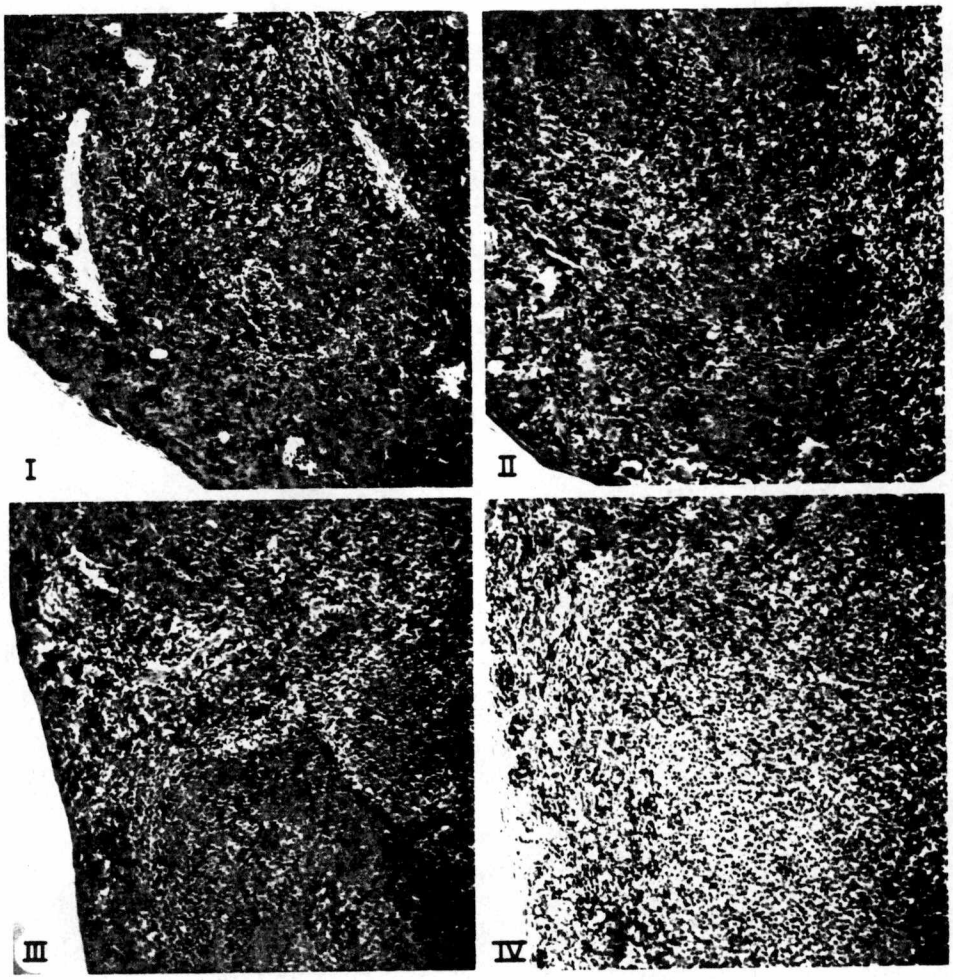
TABLE 3.2

HISTOLOGICAL CHARACTERISTICS OF SPLEENS
FROM IRRADIATED B10.F MICE

Type	Histopathological Characteristics
I	Well organized white and red pulp Normal hematopoiesis with: granulocytes megakaryocytes erythrocytes
II	Well organized white and red pulp Secondary follicles in white pulp Increased hematopoiesis
III	Loss of discrete germinal center zones Expansion of white pulp
IV	No discrete germinal center zones Decreased red pulp Focal expansion of one or a few cell types in white pulp Hyperplastic state without metastasis
V	Predominance of one or a few cell types Metastasis

erythrocytes near the capsule of the spleen. The architecture was still intact in spleens with Type II histology; however, secondary follicles were found within the discrete areas of white pulp and the red pulp showed increased hematopoiesis. In spleens with Type III histology the white pulp had expanded with onset of follicular atrophy and loss of discrete germinal center zones. Due to the focal expansion of one or a few cell types in the white pulp, the architecture of spleens with Type IV histology was markedly disturbed. Discrete lymphatic follicles were not found and lymphopoiesis had spread into the red pulp. Spleens with Type V histologies displayed gross changes in cellular organization and were characterized by the predominance of one or a few cell types. In addition there was evidence of metastasis of cells from the Type V spleen to other organs. The five morphological patterns are shown in Figure 3.2. The majority of the mice had spleens with Type II histology (Table 3.3). Nine of the 51 mice exposed to 2 Gy and five of the 53 mice exposed to 3 Gy of X-ray had histopathological changes characteristic of Type V (Table 3.3).

Figure 3.2. Representative histologies of Types I-V. Spleens and liver were fixed in formalin and stained with hematoxylin and eosin. Note the discrete white and red pulp in the Type I spleen. In the Type II spleen there were secondary follicles in the white pulp. Note the germinal center zones and the expansion of the white pulp in the Type III spleen. In the Type IV spleen there was an absence of discrete germinal center zones, decreased red pulp and the focal expansion of one or a few cell types in the white pulp. Note the loss of lymphatic follicles and the predominance of a few cell types. VL shows metastasis of cells from the Type V spleen to the liver. Magnification X 100.


I
II
III
IV

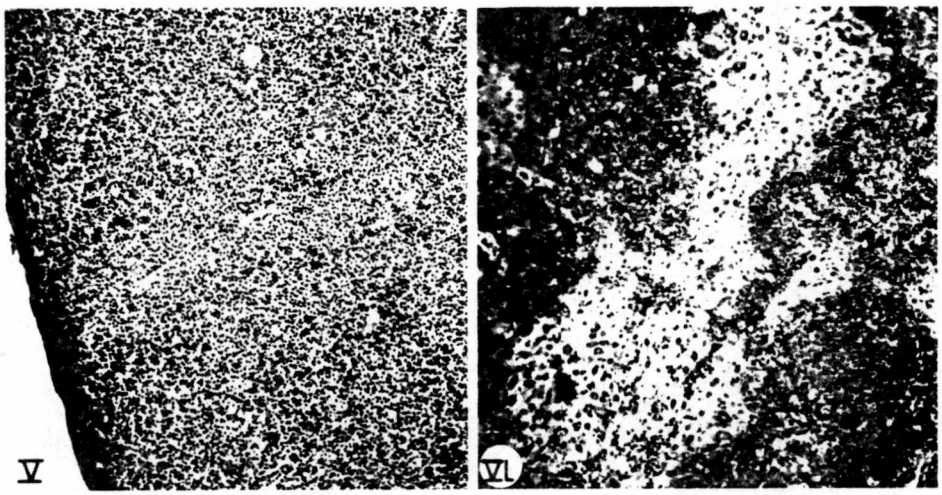

V
VI

TABLE 3.3

HISTOLOGICAL CHARACTERISTICS OF SPLEENS FROM MICE EXPOSED TO IONIZING RADIATION

Treatment (Gy)	No. of spleens	Percent of spleens with histologies of Types				
		I[a]	II[a]	III[a]	IV[a]	V[a]
0	47	8.5	70.2	12.8	8.5	0
1	51	3.9	84.0	9.8	2.0	0
2	51	2.0	61.0	19.6	2.0	15.6
3	53	1.9	71.7	16.9	0	9.4

[a]Histopathological characteristics as described in Table 3.1.

Identification of acquired proviruses in splenic DNA of B10.F mice exposed to ionizing radiation

Splenic DNA from the irradiated B10.F mice was assayed with restriction endonucleases for the presence of acquired ecotropic proviruses as described in Chapter II. Splenic DNA from eight of the 52 mice exposed to 1 Gy of ionizing radiation yielded novel ecotropic virus-specific restriction fragments in addition to the restriction fragments generated from emv-2 and B10Fv when their DNA was cut with PstI or a combination of PstI/PvuII (Table 3.4). When splenic DNA from animal B55 was cut with PstI, four different restriction fragments were detected: 8.3 (emv-2), 5.5 (B10Fv), 3.9 and 0.6 kb in size indicating that the DNA contained at least two proviruses in addition to B10Fv and the endogenous emv-2. B36, B45, B48 and B49 each had one additional ecotropic provirus as detected by the formation of PstI restriction fragments 3.4, 3.5, and 3.8 kb in length, respectively. When splenic DNA from B25, B26, and B31 was digested with PstI/PvuII a novel restriction fragment 4.0 kb in length was detected (Figure 3.3).

TABLE 3.4

B10.F MICE EXPOSED TO 1 Gy OF X-RAYS AND DISPLAYING ATYPICAL DNA PROFILES

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
B1	135	II	1.5	2(8.3) (5.5)	2(6.3) (5.2)	1(2.0)	3(P/X) (8.0) (5.6) (5.0)
B25	100	II	1.25	2(8.3) (5.5)	1(5.2)	2(4.1) (2.0)	3(P/Pv)
B26	100	II	1.25	2(8.3) (5.5)	1(5.2)	2(4.1) (2.0)	3(P/Pv)
B31	180	II	2.5	2(8.3) (5.5)	3(5.2) (4.2) (3.3)	2(4.0) (2.0)	3(P/Pv)
B36	110	II	1.25	3(8.3) (5.5) (3.4)	2(5.2) (3.4)	2(2.0) (1.6)	3(P)
B45	95	II	1.25	3(8.3) (5.5) (3.5)	2(5.2) (2.5)	1(2.0)	3(P)

TABLE 3.4 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
B48	110	II	1.25	3(8.3) (5.5) (3.8)	2(5.2) (2.5)	1(2.0)	3(P)
B49	100	II	1.0	3(8.3) (5.5) (3.8)	2(5.2) (2.5)	1(2.0)	3(P)
B55	145	II	1.5	4(8.3) (5.5) (3.9) (0.6)	3(5.2) (3.0) (2.2)	3(2.0) (1.1) (0.6)	4(P)
B59	130	II	2.0	2(8.3) (5.5)	3(5.2) (3.8) (3.0)	1(2.0)	2(P)

[a]Histopathological characteristics as described in Table 3.1.
[b]Hybridization intensity of acquired proviruses/emv-2.
[c]Number of bands observed when digested with restriction enzymes; kb sizes of bands are given in parenthesis.
[d]Restriction enzymes used to determine the minimum number of proviruses in the splenic DNA; P is PstI, X is XbaI, Pv is PvuII.

Figure 3.3. Southern blot of the ecotropic MuLV sequences in splenic DNA of Mouse B26. The 4.1 kb band in the PstI/PvuII lane indicated the presence of a provirus in addition to emv-2 and B10Fv. This provirus differed from emv-2 and B10Fv only at its PvuII site in the pol region because only the expected bands for emv-2 and B10Fv were observed in the other lanes. The provirus had lost the PvuII site that is common to both emv-2 and B10Fv near the 3' end of the pol region enabling the detection of a PvuII site near the 5' end of the pol region.

origin
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI
kb
9.4
6.7
4.4
2.3
2.0

Restriction fragments in addition to those attributed to B10Fv and emv-2 were detected in splenic DNA digests from twelve of the 54 mice exposed to 2 Gy of X-rays (Table 3.5). When splenic DNA from C1 was cut with PstI/HindIII, six different restriction fragments were detected. The sizes of these fragments were 7.0, 5.5 (emv-2 and B10Fv), 4.5, 3.7, 3.4, and 0.95 kb in length. The additional restriction fragments indicate that the DNA had at least five proviruses in addition to emv-2 and B10Fv (Figure 3.4). Splenic DNA from C41 produced five restriction fragments when digested with PstI/PvuII. The sizes of the fragments were 5.3, 4.8, 2.0 (emv-2 and B10Fv), 1.8, and 1.1. The DNA from the spleen of C30 produced four restriction fragments of sizes 5.0 (emv-2), 6.9, 7.8, and 9.5 (cell-virus junction fragments) kb when digested with XbaI/HindIII. Splenic DNA from C22 also produced four ecotropic-specific restriction fragments when digested with PstI. The sizes of the PstI restriction fragments were 8.3 (emv-2), 5.5 (B10Fv), 4.0, and 2.2 kb. DNA from the spleens of mice C10, C20, C25, C27, C44, C49, C52 and C53 all produced three

TABLE 3.5

B10.F MICE EXPOSED TO 2 Gy OF X-RAYS AND DISPLAYING ATYPICAL DNA PROFILES

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
C1	1530	V	1.25	3(8.3) (7.5) (5.5)	3(5.2) (4.3) (3.5)	3(3.1) (2.0) (1.0)	7(P/H) (7.0) (5.2) (4.5) (3.7) (3.4) (0.95)
C10	4240	IV	1.0	2(8.3) (5.5)	3(5.2) (4.0) (3.0)	1(2.0) (1.7)	3(P/Pv)
C19	120	ND[e]	1.0	2(8.3) (5.5)	2(5.2) (3.2)	1(2.0)	2(P)
C20	100	II	1.0	2(8.3) (5.5)	2(5.2) (1.7)	2(2.0) (1.3)	3(P/Pv)
C21	105	III	1.25	2(8.3) (5.5)	2(5.2) (2.9)	1(2.0)	3(Pv)
C22	110	III	2.0	4(8.3) (5.5) (4.0) (2.2)	4(5.2) (4.0) (3.2) (2.7)	1(2.0)	4(P)

TABLE 3.5 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
C25	120	II	2.0	3(8.3) (5.5) (4.5)	4(6.5) (5.2) (4.5) (3.0)	1(2.0)	3(P)
C27	1320	V	1.5	2(8.3) (5.5)	4(6.5) (5.2) (4.5) (3.0)	1(2.0)	3(P/X) (8.0) (4.9) (4.2)
C30	360	V	1.0	2(8.3) (5.5)	1(5.2)	2(2.0) (1.4)	4(X/H) (9.5) (7.8) (6.9) (4.5)
C34	100	ND[e]	2.0	2(8.3) (5.5)	3(5.2) (5.0) (4.0)	1(2.0)	2(P)
C41	755	V	1.25	3(8.3) (5.5) (4.8)	3(5.8) (5.2) (3.4)	5(5.3) (4.8) (2.0) (1.8) (1.1)	6(P/Pv)

TABLE 3.5 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
C43	100	II	1.5	2(8.3) (5.5)	2(5.2) (3.0)	1(2.0)	2(P)
C44	105	III	1.25	2(8.3) (5.5)	3(5.2) (3.2) (2.3)	1(2.0)	3(Pv)
C47	140	III	1.5	2(8.3) (5.5)	2(5.2) (3.1)	1(2.0)	2(P)
C48	120	V	2.0	2(8.3) (5.5)	2(5.2) (4.0)	1(2.0)	2(P)
C49	160	II	1.0	2(8.3) (5.5)	2(5.2) (2.3)	1(2.0)	3(Pv)

TABLE 3.5 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ *emv-2*[b]	No. *Pst*I[c]	No. *Pvu*II[c]	No. *Pst*I/*Pvu*II[c]	Minimum no. provirus[d]
C52	610	V	1.0	2(8.3) (5.5)	1(5.2)	1(2.0)	3(X/H) (8.5) (7.5) (5.0)
C53	560	V	0.75	2(8.3) (5.5)	2(5.2) (3.8)	1(2.0)	3(X/H) (9.0) (7.9) (5.0)

[a]Histopathological characteristics as described in Table 3.1.
[b]Hybridization intensity of acquired proviruses/*emv-2*.
[c]Number of bands observed when digested with restriction enzymes; kb sizes of bands are given in parenthesis.
[d]Restriction enzymes used to determine the minimum number of viruses in the splenic DNA; P is *Pst*I, X is *Xba*I, Pv is *Pvu*II, H is *Hin*dIII, S is *Sal*I.
[e]Not determined.

Figure 3.4. Southern blot of the ecotropic MuLV sequences in splenic DNA of mouse C1. Note the appearance of novel restriction fragments in each lane. The five restriction fragments seen in the PstI/HindIII digest (7.0, 5.2; emv-2 and B10Fv, 4.5, 3.7, 3.4 and 0.95 kb in length) suggests that the DNA contained at least five proviruses in addition to emv-2 and B10Fv. It is most likely that the majority of the novel proviruses differ at the HindIII site because there is conservation in the PstI site as indicated by the number of restriction fragments seen in the PstI lane. Note the population of proviruses with PstI sites 7.5 kb apart and no internal HindIII, EcoRI nor SalI sites. There is also a population of proviruses which contained an EcoRI site in the pol region of their genome. The three PvuII restriction fragments indicate that there has been a clonal expansion of one or two cell types containing proviruses.

origin
kb
9.4
6.7
4.4
2.3
2.0
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI

restriction fragments when cut with specific endonucleases (Table 3.5).

Splenic DNA from eight of the 53 mice exposed to 3 Gy of X-rays gave proviral restriction fragments in addition to those that could be attributed to B10Fv and emv-2 (Table 3.6). PstI/PvuII restriction endonuclease digestion of splenic DNA from mouse D10 yielded a total of five restriction fragments which indicated the presence of at least four proviruses in addition to emv-2 and B10Fv. The sizes of the additional restriction fragments were 3.1, 2.5, 1.6, and 1.05 kb. When splenic DNA from D15 was digested with PstI/EcoRI, five different restriction fragments were detected by the ecotropic virus-specific probe. The sizes of the restriction fragments were 8.3 kb (emv-2), 5.5 kb (B10Fv) and 3.5 kb, 1.75 kb and 1.5 kb derived from novel acquired proviruses (Figure 3.5). In six DNA samples (D1, D6, D11, D13, D52 and D53), three restriction fragments were detected after digestion with PstI or PstI/PvuII (Table 3.6). Figure 3.6 illustrates composite restriction endonuclease maps of acquired ecotropic proviruses present in the splenic DNA of the B10.F mice exposed to 1, 2 or 3 Gy of ionizing radiation.

TABLE 3.6

B10.F MICE EXPOSED TO 3 Gy OF X-RAYS AND DISPLAYING ATYPICAL DNA PROFILES

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
D1	80	I	2.0	2(8.3) (5.5)	4(7.0) (5.2) (4.7) (4.2)	2(2.8) (2.0)	3(P/Pv)
D6	404	III	0.5	3(8.3) (5.5) (4.8)	1(5.2)	1(2.0)	3(P)
D10	280	III	2.0	4(8.3) (5.5) (4.5) (2.8)	9(9.5) (6.5) (5.5) (5.2) (4.1) (3.8) (3.1) (2.9) (1.6)	5(3.1) (2.5) (2.0) (1.6) (1.1)	6(P/Pv)
D11	1200	II	1.0	2(8.3) (5.5)	1(5.2) (1.6)	2(2.0) (1.6)	3(P/Pv)

TABLE 3.6 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
D13	180	II	1.0	2(8.3) (5.5)	1(5.2)	2(2.0) (1.6)	3(P/Pv)
D15	600	V	1.0	3(8.3) (5.5) (4.5)	2(5.2) (4.5)	5(3.1) (2.0) (1.5) (1.0)	5(P/Pv)
D21	100	II	1.0	2(8.3) (5.5)	2(6.8) (5.2)	1(2.0)	2(P)
D25	130	II	1.0	2(8.3) (5.5)	2(7.0) (5.2)	1(2.0)	2(P)
D31	785	V	1.0	2(8.3) (5.5)	2(5.2) (3.6)	1(2.0)	2(P)

TABLE 3.6 (Continued)

Animal	Spl. wt. (mg)	Spl. hist.[a]	Acquired/ emv-2[b]	No. PstI[c]	No. PvuII[c]	No. PstI/PvuII[c]	Minimum no. provirus[d]
D52	570	V	0.5	3(8.3) (5.5) (4.0)	2(5.2) (2.5)	2(2.0) (1.3)	3(P)
D53	100	III	1.0	2(8.3) (5.5)	1(5.2)	2(2.0) (1.6)	3(P/Pv)

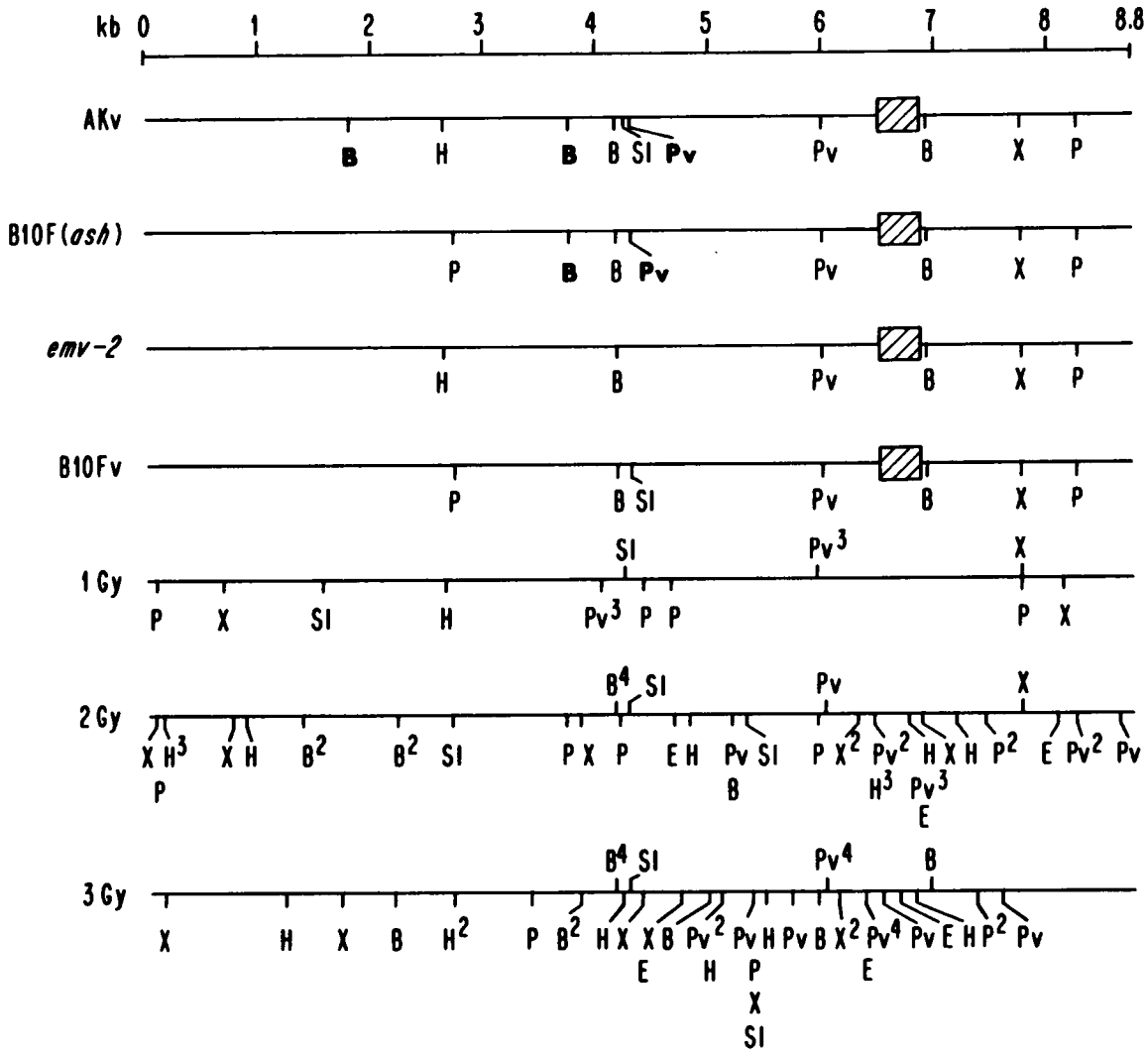
[a]Histopathological characteristics as described in Table 3.1.
[b]Hybridization intensity of acquired proviruses/emv-2.
[c]Number of bands observed when digested with restriction enzymes; kb sizes of bands are given in parenthesis.
[d]Restriction enzymes used to determine the minimum number of viruses in the splenic DNA; P is PstI, Pv is PvuII, E is EcoRI.

Figure 3.5. Southern blot of the ecotropic MuLV sequences in splenic DNA of mouse D15. Note the appearance of novel restriction fragments in each lane. Five restriction fragments were seen when the DNA was cut with PstI/EcoRI; there sizes were 8.3 (emv-2), 5.5 (B10Fv), 3.5, 1.75, and 1.5 kb in length indicate that there are at least three additional novel acquired proviruses in addition to emv-2 and B10Fv. Note that these novelly acquired ecotropic proviruses have internal EcoRI restriction sites, which are not common to ecotropic viruses.

origin –
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
kb
9.4 –
6.7 –
4.4 –
2.3 –
2.0 –

15

Figure 3.6. Composite restriction endonuclease maps of acquired ecotropic proviruses present in the splenic DNA of the B10.F mice exposed to 1, 2 or 3 Gy of ionizing radiation. Partial restriction maps of acquired proviruses that differed from B10Fv were generated from Southern blots of splenic DNA from 52 mice exposed to 1 Gy, 54 mice exposed to 2 Gy and 53 mice exposed to 3 Gy of X-rays and digested with PstI, PvuII and PstI/PvuII and Southern blots of splenic DNA from 5 mice exposed to 1 Gy, 7 mice exposed to 2 Gy and 6 mice exposed to 3 Gy of X-rays and digested with PvuII, PstI, PstI/PvuII, PstI/BamHI, PstI/HindIII, PstI/XbaI, PstI/EcoRI, PstI/SalI, XbaI, XbaI/HindIII and XbaI/SalI. For each of the 1, 2, and 3 Gy composite maps, restriction endonuclease sites shown above the line indicate the absence of sites that are present in B10Fv but are present in other proviruses found in splenic DNA of B10.F mice exposed to X-rays. Restriction endonuclease sites shown below the line indicate sites that are not present in B10Fv. The numbers shown as superscripts next to the sites indicate the number of times (greater than one) these variants were observed. The corresponding restriction sites in AKV, B10.F(ash), emv-2 and B10Fv are shown for comparison. The symbols used are given in Figure 2.4.


kb 0 1 2 3 4 5 6 7 8 8.8
AKv
B H B B Sl Pv Pv B X P
B10F(ash)
P B B Pv Pv B X P
emv-2
H B Pv B X P
B10Fv
P B Sl Pv B X P
1 Gy
Sl Pv3 X
P X Sl H Pv3 P P P X
2 Gy
B4 Sl Pv X
X H3 X H B2 B2 Sl P X P E H Pv Sl P X2 Pv2 H X H P2 E Pv2 Pv
P B H3 Pv3 E
3 Gy
B4 Sl Pv4 B
X H X B H2 P B2 H X X B Pv2 Pv H Pv B X2 Pv4 Pv E H P2 Pv
E H P E
X
Sl

Five of the mice exposed to radiation had enlarged spleens but had no detectable proviruses other than emv-2 and B10Fv (Table 3.7, Figure 3.7, and Figure 3.8).

Comparison of the proviruses found in the splenic DNA of the irradiated B10.F mice

In order to estimate the minimum number of structurally different proviruses in the DNA of the spleens, DNA samples were digested with several restriction endonucleases to search for modification of restriction sites within the proviral DNA. The number and structure of proviruses in the splenic DNA of three mice will be discussed. The wide variety of different restriction fragments were typical of results obtained with DNA from other mice.

The splenic DNA from mouse B26 contained a minimum of three proviruses. The novel acquired provirus were distinguished from B10Fv and emv-2 when the DNA was digested with PstI/PvuII (compare Figure 2.2 page 52 and Figure 3.3, page 122). The extra PstI/PvuII restriction fragment indicated that the novel acquired provirus lacked a PvuII site common to emv-2 and B10Fv near the 3' end of the pol region and had a PvuII restriction site near the 5' end of the pol region near

TABLE 3.7

CHARACTERISTICS OF MICE WITH SPLEENS GREATER THAN 200 MG AND HAVING ONLY B10Fv ACQUIRED PROVIRUSES

Animal	Graying	Spleen weight (mg)	Spleen histology[a]	Aquired/ emv-2[b]
B53	2.5	220	II	1.25
C12	2.0	1400	V	0.75
C23	2.5	210	II	1.5
D31	2.5	785	III	1.0
D41	2.5	995	III	0.75

[a]Histopathological characteristics as described in Table 3.1.
[b]Hybridization intensity of acquired proviruses/emv-2.

Figure 3.7. Southern blot of the ecotropic MuLV sequences in splenic DNA from mouse C12. Note that B10Fv is the only acquired proviral sequence in the DNA.

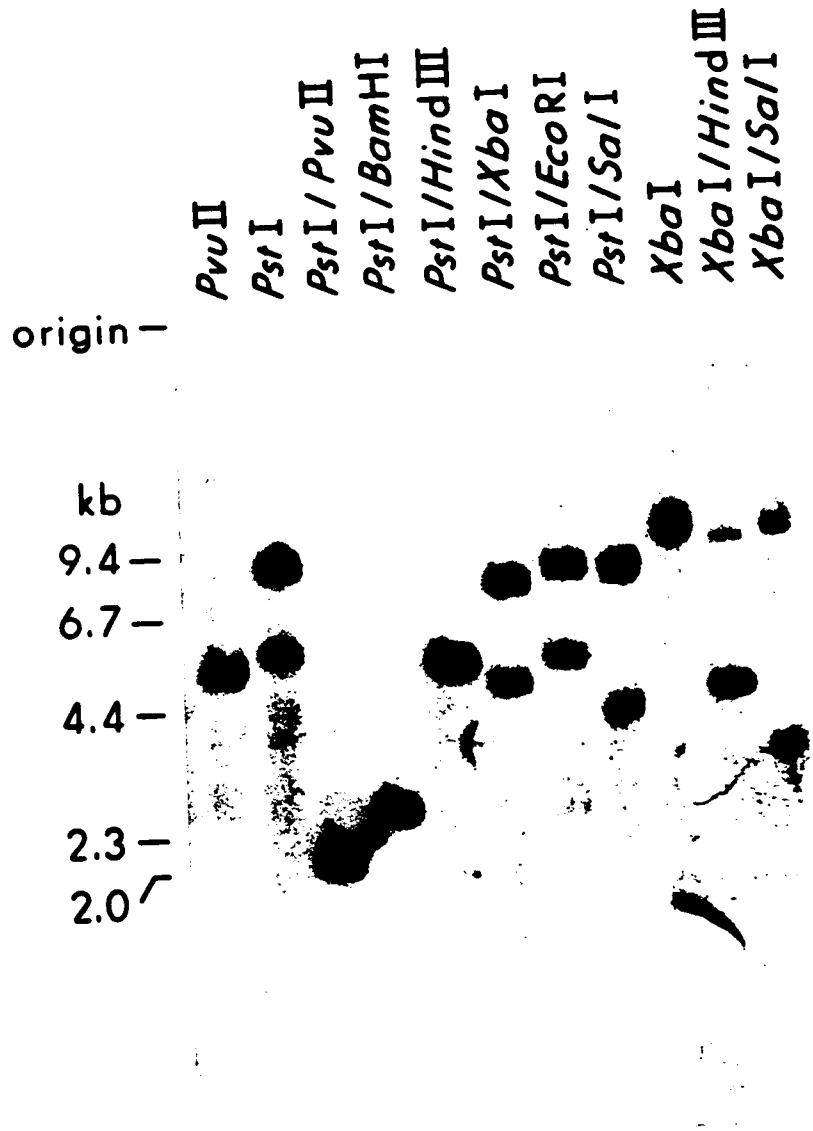

PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI
origin
kb
9.4
6.7
4.4
2.3
2.0

Figure 3.8. Southern blot of the ecotropic MuLV sequences in splenic DNA of mouse D31. Although digestion with PvuII generated an additional junction fragment, B10Fv was the only acquired provirus in the DNA.

origin
PvuII
PstI
PstI/PvuII
PstI/BamHI
PstI/HindIII
PstI/XbaI
PstI/EcoRI
PstI/SalI
XbaI
XbaI/HindIII
XbaI/SalI
kb
9.4
6.7
4.4
2.3
2.0

the SalI site in B10Fv. This PvuII site in the 5' region of pol is common to both AKV and B10Fv (Figure 2.3 page 54). The available data do not establish whether the novel acquired provirus has the same PstI, SalI, or HindIII sites as emv-2, B10Fv, or a combination of the two. However, all three of the proviruses do share the same BamHI and XbaI sites shown by the single restriction fragment when the DNA was digested with PstI/BamHI and the expected PstI/XbaI restriction fragments.

When the splenic DNA from mouse C1 was digested with PstI/HindIII, six restriction fragments were produced (Figure 3.4, page 129). The sizes of the fragments were 7.0, 5.2 (emv-2 and B10Fv), 4.5, 3.7, 3.4, and 0.95 kb. The detection of five new restriction fragments indicated the presence of five proviruses that have PstI/HindIII restriction sites different from emv-2 and B10Fv. Little restriction fragment variability was observed when the DNA was digested with PstI alone; this suggests that the variation in the PstI/HindIII restriction fragments were due to alterations in HindIII sites rather than PstI sites. A population of proviruses, which have PstI sites 7.5 kb apart and no internal HindIII, EcoRI

or SalI sites, was also apparent. Additionally, there was a population of proviruses that contained an internal EcoRI restriction site. The 3.5 kb size of the PstI/EcoRI fragment indicated that the EcoRI site is located with in the pol region.

A minimum number of three novel acquired proviruses were detected in the splenic DNA from mouse D15 (Figure 3.5, page 135) because digestion with PstI/PvuII generated four restriction fragments (1.0, 1.5, 2.0 (emv-2 and B10Fv), and 3.1 kb). Three of these restriction fragments could not be derived from emv-2 or B10Fv. In addition, the results of the PstI/EcoRI digestion indicated the presence of a minimum number of three novel acquired proviruses which had restriction fragment sizes of 1.5, 1.75 and 3.5 kb. These restriction fragments could not be attributed to emv-2 or B10Fv. The same conclusion was drawn from the PstI/XbaI double digest. The 4.7 kb PstI restriction fragment could represent a population of proviruses that had a PstI site 1.0 kb 5' to the PstI site in the 3' LTR of B10Fv, very near to the XbaI site. This new PstI site gave rise to the PstI/PvuII, PstI/HindIII and PstI/SalI restriction fragments that were all approximately 1.0 kb smaller than the fragment that

could be attributed to B10Fv. It appears that this population of proviruses did contain EcoRI and XbaI sites; this is evidenced by the absence of a 4.7 kb band after digestion with PstI/EcoRI and PstI/XbaI. Restriction fragment polymorphism was also detected when the DNA was digested with the other enzymes.

EcoRI sites are not common in ecotropic viruses; however, ten of the 18 splenic DNA samples that were digested with EcoRI contained at least one population of proviruses with internal EcoRI sites.

The multiplicity of the proviruses in the splenic DNA of the 159 irradiated B10.F mice was estimated from the number of restriction site variations among the proviruses detected by the ecotropic virus-specific probe. A minimum of 20 different proviruses were present in the splenic DNAs of the irradiated mice. Analogous to the ecotropic proviruses in the nonirradiated B10.F mice described in Chapter II, several of the unique proviruses were found in more than one of the irradiated mice (Figure 3.6, page 137).

Clonal expansion of cells containing proviruses

If a cell containing an acquired provirus had proliferated to form a population of identical cells, a

novel restriction fragment corresponding to the location of the acquired provirus would be detected when the DNA was digested with restriction enzymes that produce virus-cell junction fragments (Table 3.8).

Of the 52 mice exposed to 1 Gy of X-rays, PvuII DNA digests from eight mice (B1, B31, B36, B45, B48 B49, B55, and B59) contained virus-host junction fragments that hybridized to the ecotropic-specific probe (Table 3.4, page 120). B1 had one additional restriction fragment 6.3 kb in length indicating that the provirus integrated 3.3 kb 5' to a PvuII restriction site. There were two additional PvuII restriction fragments observed in the splenic DNAs from mice B31, B55 and B59. The sizes ranged from 2.2 to 4.2 kb. The splenic DNA from mice B36, B45, B48, and B49 each had one additional restriction fragment of 3.4, 2.5, 2.5 and 2.5 kb in length, respectively.

Splenic DNA from 16 of the 54 mice exposed to 2 Gy of X-rays had more than one restriction fragment when the DNA was digested with PvuII (Table 3.5, page 125). C22, C25 and C27 each had four restriction fragments when the DNA was digested with PvuII. DNA samples from five mice (C1, C10, C34, C41 and C44) each had three restriction fragments. Only one restriction fragment

TABLE 3.8

EFFECTS OF RADIATION ON SPLEEN ENLARGEMENT AND ACQUIRED PROVIRUSES IN C57BL/10.F MICE

Treatment (Gy)	Number of mice with			
	enlarged spleens[a]	spleens of Type V histology	novel acquired proviruses	additional PvuII fragments[b]
0	2/51	0/47	4/50	3/50
1.0	1/52	0/51	8/52*	5/52
2.0	9/54+	8/51**	13/54+	12/54**
3.0	7/53	5/53*	8/53	7/53

[a]Spleens greater than 200 mg in weight.
[b]PvuII restriction fragments greater than 3.0 kb in length.
+p = 0.05
*p = 0.10 - 0.05
**p < 0.05

in addition to emv-2 was detected in the endonuclease digests of the splenic DNA from C19, C20, C21, C43, C47, C48, C49 and C53. The sizes of the additional PvuII restriction fragments found in the DNA of the mice exposed to 2 Gy of ionizing radiation ranged from 1.7 to 6.7 kb in length. The intensity of hybridization of the additional PvuII restriction fragments was quite variable.

Splenic DNA from seven of the 53 mice exposed to 3 Gy of X-rays had additional PvuII restriction fragments (Table 3.6, page 132). D10 had a total of nine PvuII restriction fragments ranging in size from 9.5 to 1.5 kb in length. D1 had four PvuII restriction fragments that were 7.0, 5.2, 4.7, and 4.2 kb in length. Five mice (D15, D21, D25, D31, and D52) had one PvuII fragment in addition to the endogenous 5.2 kb emv-2 fragment; their sizes were 4.5, 6.8, 7.0, 3.6 and 2.5 kb, respectively.

Nine of the splenic DNA samples in which additional PvuII restriction fragments were detected weighed more than 200 mg; however, additional PvuII fragments were not detected with the ecotropic-specific probe in the DNA from eight of irradiated mice with enlarged spleens (Table 3.9).

TABLE 3.9

CHARACTERISTICS OF MICE WITH SPLEENS GREATER THAN 200 MG AND NO ADDITIONAL PVUII RESTRICTION FRAGMENTS

Animal	Graying	Spleen weight (mg)	Spleen histology[a]	Acquired/ emv-2[b]	Minimum no. proviruses[c]
A7	0.5	332	IV	0.5	4(P/S)
B53	2.5	220	II	1.25	2(P)
C12	2.0	1400	V	0.75	2(P)
C23	2.5	210	II	1.5	2(P)
C30	2.0	360	V	1.0	4(H/X)
C52	3.5	610	V	1.0	3(X/H)
D6	2.0	404	III	0.5	3(P)
D11	2.0	1200	II	2.0	3(P/Pv)
D41	2.5	995	V	0.75	2(P)

[a]Histopathological characteristics as described in Table 3.1.
[b]Hybridization intensity of acquired proviruses/emv-2.
[c]Restriction enzymes used to determine the minimum number of proviruses in the splenic DNA; P is PstI, Pv is PvuII, S is SalI, X is XbaI, H is HindIII.

Discussion

Radiation is known to cause a variety of different deleterious effects in experimental animals (Rauth, 1987). Radiation-induced neoplasms have been associated with viral activation (Liberman et al., 1976; Kaplan, 1977). This study demonstrates that a single whole body dose of 2 Gy of ionizing radiation causes an increase in the number of six-month-old C57BL/10.F male mice with enlarged spleens, histopathological changes in the spleens, spleens containing unique integrations of proviruses and spleens containing novel acquired proviruses.

The effects of X-Rays on B10.F mice

There was a significantly larger number of enlarged spleens in mice exposed to 2 Gy than in mice that were not exposed to X-rays ($p=0.05$). Moreover, the number of mice exposed to 2 Gy of X-rays having spleens with Type V histopathologies was significantly greater than the number of control mice with spleens having Type V histopathologies ($p<0.05$). Spleens with Type V

histopathology are not unique to the irradiated B10.F mice, but were not observed among the 51 nonirradiated mice in the present study. Spleens with Type V histopathology are found in older B10.F mice (D. M. Popp, personal communication).

Two of the 51 six-month old B10.F mice examined had enlarged spleens and histopathological changes in the absence of irradiation. There is a latent period between radiation treatment and detection of neoplasia. Thus, the detection of 12 spleens with Type V histopathology in irradiated six-month-old B10.F mice (four months after irradiation) may represent an earlier onset of neoplasia rather than an absolute increase in the incidence of neoplasia.

Effects of X-irradiation on acquired proviruses in the B10.F mouse

Identification of acquired proviruses in splenic DNA by restriction fragment length polymorphism. The use of restriction endonuclease analysis to differentiate between the endogenous *emv-2* and acquired proviruses in B10.F mice was described in Chapter II. Like the nonirradiated B10.F mice, the splenic DNA from

all irradiated mice had on the average a minimum of two copies of acquired proviruses per diploid genome. B10Fv, the most commonly acquired provirus detected in the nonirradiated B10.F mice, was also detected in all irradiated mice. Additional proviruses were also detected. In the control group, four of the 50 mice examined had acquired proviruses in addition to B10Fv. In the irradiated group, eight of the 52 mice exposed to 1 Gy, thirteen of the 54 mice exposed to 2 Gy and eight of the 53 mice exposed to 3 Gy of ionizing radiation had at least one acquired proviruses in addition to B10Fv. A 2 x k contingency test, which verifies heterogeneity (Zar, 1974), showed that the increase in the number of mice with novel acquired proviruses was significantly higher ($p=0.05$) among B10.F mice exposed to 2 Gy of ionizing radiation than among the nonirradiated control group of mice (Table 3.8)

Radiation has long been noted for its mutagenic properties. X-rays may have caused direct mutations in B10Fv proviruses or in free viruses in the cell through deletions or base alterations. These alterations in the proviruses or free viruses would represent actual DNA damage by radiation.

Viral activation induced by radiation (Kaplan, 1967; Ellis et al., 1980; and Okumoto et al., 1983) can lead to an increased opportunity for the formation of new viruses through viral recombination and alterations in viral replication by the error-prone reverse transcriptase (Coffin, 1986). The altered proviruses are then able to reinfect cells and can be detected when they reach sufficient copy number in the tissue.

Clonal expansion of cells containing proviruses. The majority of the ecotropic acquired proviruses in the splenic DNA of the irradiated B10.F mice were randomly located throughout the genome. However, PvuII restriction fragments were detected in the splenic DNA of a number of mice. As stated in Chapter II (Figure 2.8, page 78), the production of PvuII restriction fragments greater than 3.0 kb in length usually indicates a portion of the tissue was derived from one or a few cells that contained an acquired provirus.

The number of mice with additional PvuII restriction fragments (greater than 3.0 kb in length) in the control group was compared to the number of mice in the treated groups using a 2 x k contingency test. The number of mice with additional PvuII fragments is

significantly increased in mice exposed to 2 Gy of radiation ($p<0.05$) (Table 3.8, page 149). This increase could be attributed to an increase in frequency of integration of virus into the same site in many cells or to the increase in clonal expansion of cells containing proviruses. An increased frequency of proviruses at the same site should produce a common PvuII restriction fragment which was not observed. Thus, it is most likely that radiation caused an increase in the proliferation of cells containing proviruses. Translocations are induced by radiation and extensive cell proliferation can occur when translocations are adjacent to oncogenes (Mindem, 1987; Rauth, 1987). In addition, ionizing radiation causes cell death; after an exposure of 1-3 Gy of ionizing radiation, there is a decrease in spleen weight followed by splenic regeneration (Mauyama and Feola, 1987). The few progenitor cells that proliferate will give rise to the majority of the cells in the repopulated spleen. If the progenitor cell contains a unique proviral integration it will be detected in the DNA of the repopulated spleen.

Viral integration and tumor induction

The sizes of the restriction fragments in digests of DNAs from enlarged spleens were compared to determine if a common site of integration or common provirus could be correlated with tissue hyperplasia. Specific viruses and sites of viral integration have been correlated with splenic hyperplasia and metastasis (Lowy, 1984; Zijlstra and Melif, 1986).

The sizes of PvuII restriction fragments derived from ecotropic proviruses in the DNA of mice with enlarged spleens varied over a wide range but the increased spleen size was not correlated with a specific PvuII fragment of a specific size or a unique provirus detected by restriction endonuclease analysis. Additionally, when internal cutters were used to assay for proviruses or when PvuII was used to assay for clonal expansion, the DNA from several mice with enlarged spleens did not have any detectable restriction fragments in addition to those which could be correlated to B10Fv and emv-2 (Table 3.7 and 3.9, pages 140 and 151) .

The lack of correlation between specific novel acquired proviruses and tissue hyperplasia may be due to one or a combination of the following possibilities:

1) Unlike B10Fv, the novel acquired proviruses that are detected in the DNA of some of the enlarged spleens are innocuous and do not contribute to the abnormal growth of the cells. 2) Retroviral infection into one cell type leads to the proliferation of another cell type, as seen with the infection of CD4+ cells causing polyclonal B-cell activation in HIV-infected lymphocytes (Nikajima et al., 1989). The cell that has undergone clonal expansion may or may not contain nonendogenous proviruses. 3) X-irradiation is responsible for spleen enlargement. A single dose of 0.25-3 Gy of low LET radiation given whole body can increase the frequency of malignant and benign tumors in irradiated animals (Fry, 1981, Rauth 1987). The cell or a group of cells affected by the radiation may or may not contain nonendogenous proviruses. Because the DNA from enlarged spleens of the nonirradiated control mice also contained a variety of proviruses, the tissue hyperplasia in the irradiated B10.F mice probably was induced both by virus and by radiation.

The enlarged spleens that did not have additional PvuII restriction fragments may be composed of cells derived from many different progenitors each having proviruses at different locations in the genome.

Alternatively, it is possible that the majority of cells in the enlarged spleens do not contain nonendogenous proviruses. In fact, in some of the enlarged spleens the restriction fragment corresponding to emv-2 (Table 3.4, 3.5, and 3.6 pages 120, 125 and 132, respectively) hybridized more intensely with the ecotropic virus-specific probe than the restriction fragments derived from the acquired proviruses. In the B10.F mouse we have not found a pattern of proviral integration that can be correlated with tissue hyperplasia.

Dose response relationship

The spleen is a component of the perpherial lymphoid organs and is composed of a variety of cell types including a heterogenous population of lymphocytes. The radiation response of different types of lymphocytes appears to be complex and heterogeneous; T and B cells appear to have about the same sensitivity to radiation (LD_{50} values of 1 Gy) with reticulum cells being more resistant (Mauyama and Feola, 1987). The cell death and subsequent regeneration of the cells in the spleen could have profound effects on the histopathological changes and viremia seen in the

spleens of the irradiated B10.F mice. Only after an exposure to 2 Gy of ionizing radiation was there a significant increase in the number of mice exhibiting enlarged spleens, an increase in disturbance of splenic architecture, splenic DNA with novel acquired proviruses and clonal expansion of splenocytes containing proviruses (Table 3.8, page 149). This dose response parallels a bell shaped relationship and could be compatible with the model of the dose response relationship for low-LET single, high dose-rate exposures (Fry and Storer, 1987). The dose-response curves for the radiation-induced transformation of cells in vitro, based on the transformation rate per cell exposed, are bell-shaped (Rauth, 1987; Fry and Storer, 1987). The "bending over" (decline in effect with increasing dose) of dose response curves for radiation-induced cancer in experimental animals, as well as humans, has been recognized for many years (Fry and Storer, 1987) and is due to radiation-induced cell killing (Han et al., 1980). The relative radiosensitivities of the spleen and lymphohemopoietic system have been extensively reviewed by Maruyama and Feola (1987). The spleen, a complex organ, is quite sensitive to the radiation doses received in these

studies (Maruyama and Feola, 1987). Radiation-induced cell killing may explain the absence of a significant increase in the incidence of enlarged spleens, disturbance in splenic architecture, additional acquired proviruses in the splenic DNA, and clonal expansion of cells containing proviruses in the mice exposed to 3 Gy of radiation.

CHAPTER IV

SUMMARY AND RECOMMENDATIONS

Summary

DNA from the splenocytes of C57BL/10.F mice contain acquired ecotropic proviruses that are not present in B10 mice. Splenic DNA from every B10.F mouse examined contained at least one acquired provirus. This virus, designated B10Fv, was not detected in the splenic DNA of B10 mice. Unlike emv-2, the endogenous provirus carried by both B10 and B10.F mice, B10Fv had PstI and SalI restriction endonuclease sites in the pol region but lacked a HindIII restriction endonuclease site in the pol region. In addition to emv-2 and the B10Fv provirus found in the splenic DNA of B10.F, some mice contained other proviruses. These proviruses were detected because they possess different restriction endonuclease cleavage sites. The acquired proviruses

found in the splenic DNA from six-month-old male B10.F mice were integrated into many different sites at an average frequency of two copies per diploid genome. The DNA from the lymphatic organs showed a higher copy number of the acquired ecotropic proviruses than did nonlymphatic tissue.

B10.F mice, unlike B10, express high levels of ecotropic B-tropic MuLV early in life (Yetter et al., 1983; Morse et al., 1985; Popp et al., 1986). These ecotropic viruses are congenitally transmitted from mother to offspring via the placenta and milk. Cesarean-derived B10.F mice still express ecotropic virus but at lower titers than B10.F controls. In addition, B10 mice foster nursed on B10.F females also express ecotropic virus and develop the phenotypes (e.g. graying and low IgA levels) associated with the viremia in the B10.F mouse (Morse et al., 1985; Popp et al., 1986). The viruses in B10.F mice most probably came from congenital transmission of ecotropic viruses present in the viremic F/St mother used in the original mating of F/St X C57BL/10 mice made to establish the B10.F congenic mice (Morse and Hartley, 1982; Yetter et al., 1983). The continued maternal transmission via transplacental infection and exposure to milk-borne

B10Fv contributes to the high-virus phenotype of the B10.F. B10Fv can be a source of the other novel acquired proviruses in the B10.F mouse through mutation and genetic recombination of B10Fv with other virus-related sequences in the genome (Coffin, 1986; Clavel et al., 1989; Temin, 1989).

When two-month-old B10.F male mice were exposed to ionizing radiation and examined at six months of age, there was an increase in the number of mice with enlarged spleens, histopathological changes in the spleens (both in the presence and absence of novel acquired proviruses), spleens containing unique integrations, and spleens containing novel acquired proviruses. The number of mice with these altered characteristics was dependent on the dose of radiation received. The effects were greatest after a single dose of 2 Gy.

Lymphatic organs, such as the spleen, are sensitive to cell killing and to induction of leukemias by radiation (Fry, 1981; Maruyama and Feola, 1987). However, the spleen is an organ with extensive regenerative capability and can reconstitute itself from a small pool of surviving stem cells (Maruyama and Feola, 1987). In addition, there is also rapid splenic

growth in response to antigenic stimulation (Maruyama and Coleman, 1978). Therefore, splenocytes that have been exposed to radiation as well as antigenic stimulation (e.g. infection by MuLV) are most likely undergoing cell proliferation. This cell proliferation may allow for the detection of specific proviral integrations found in the DNA of the irradiated mice.

Radiation is also known to activate viruses both *in vivo* and *in vitro*. The *in vivo* studies have demonstrated that activation of virus by radiation is dose dependent and viruses have been found in mice after exposure to irradiation but prior to the onset of leukemia (Haas, 1977; Niwa, 1979; Legrand et al., 1986; Schmidt et al., 1988). B-tropic ecotropic retroviruses are frequently activated in C57BL/6 mice exposed to irradiation (Galliay et al., 1986). These viruses are observed in the pre-leukemic period (Sankar-Mistry and Jolicoeur, 1980). In the experiments described in this dissertation the increase in the number of irradiated mice with novel acquired proviruses was probably due to the effects of the radiation exposure. A higher copy number of viruses induced by radiation followed by retroviral mutation during viral replication would be expected to result in the appearance of the large

variety of novel proviruses (Okumoto et al., 1983; Coffin, 1986).

This dissertation reports the results of studies to establish the profile of ecotropic retroviruses through analysis of integrated proviral sequences in B10.F mice. B10Fv is not oncogenic in all B10.F mice and permits the study of perpetual viremia in an animal without the development of a high incidence of malignancies (Popp, in press). The B10 and B10.F congenic pair of mice is a model system suited to study genetic variation among novel acquired proviruses, the infection of subsets of lymphocyte populations by retroviruses, the effect of viral infection on the host, and the resistance of the host genome to viral infection. These congenic mice can be used for basic studies on viral diseases without the risk of viral infection to the investigator. The genetics of the congenic mice is well defined, the time required to observe the results of viral infection is reasonable, and several experimental parameters can be controlled at will. The results of basic studies on viruses in experimental animals are expected to have a direct application to understanding human viruses such as HIV. Moreover, the description of the proviral content of

the B10.F mouse establishes basic information for future studies.

Recommendations

The identification of a large variety of restriction site differences among the proviruses in the B10.F mouse raises some very interesting questions on the origin of the variant viruses that integrate into the somatic cell DNA, the effects of individual viruses to induce nonneoplastic hyperplasia and neoplasia, and the cell or type of cells in the spleen that are infected by these viruses. In order to answer these questions the viruses that correspond to the acquired proviruses must be isolated. Once the viruses have been isolated and molecular clones prepared, their structure can be characterized in detail through the use of restriction enzymes and nucleotide sequence analysis. The structures of the various viruses can be compared with one another to determine the possible origin of the novel acquired proviruses.

A virus-free B10.F mouse would also aide in understanding the effects of the host's genome on viral

infection and the role of the viruses in the histopathological changes observed in the spleens of the B10.F mice. Presently, in the laboratory of R. A. Popp, virus free B10.F mice (B10.F V-) are being derived through blastocyst transfer. Neonatal B10 and B10.F V- mice could be given injections of isolated B10Fv and other acquired viruses to study the infectivity of individual viruses in mice with the different H-2 haplotypes of the congenic mice.

The relationship between the H-2 haplotype of the B10.F host and the extent of viral infection by B10Fv is of fundamental interest. If B10 mice are foster nursed onto B10.F females, the B10 mice acquire ecotropic virus and develop viremia (Morse et al, 1985; Popp et al., 1986). However, the viremic phenotype in progeny of the foster nursed B10 animals is lost after two or three generations (D.M. Popp, personal communication). A variety of chromosomal loci, such as Fv-1, inc-1, inb-1, Cvx-1 and H-2 play a role in the infection, integration and expression of specific ecotropic viruses (McCubrey and Risser, 1982; Levy et al., 1985; Ruscetti et al., 1985; Zijlstra et al., 1986). It is possible that the decreased immunocompetence of the B10.F mouse (Popp, 1979)

permits the continued infection and replication of B10Fv and other proviruses in the B10.F population. These viruses may be less infective in the congenic B10 mice which may have an increased ability to respond immunologically to exogenous virus.

B10Fv is associated with histological changes observed in the spleens of the B10.F mice. However, several novel acquired proviruses were often found in the DNA of enlarged spleens of B10.F mice. The role that each viruses plays in tissue hyperplasia could be investigated. Some of the novel acquired proviruses may be oncogenic by themselves or only in combination with other acquired viruses. Individual and/or combinations of the biologically cloned viruses could be introduced into B10 and B10.F V- mice and the oncogenic potential of each virus or combination of viruses determined. If tumors are formed, their DNA could be examined for proviral content and site of integration. A specific virus may be oncogenic because it integrates at a higher frequency adjacent to cellular oncogenes.

The isolation and further structural characterization of B10Fv and other novel proviruses found in the B10.F mouse should indicate whether the

variety of ecotropic retroviruses are always transmitted from mother to offspring or are generated de novo through mutations of a family of viruses that are highly homologous to B10Fv. Injection of different clones of virus into the B10 and B10.F V- mice should provide a more complete understanding of the host-virus relationship and its effects on viral infection and integration.

LITERATURE CITED

LITERATURE CITED

Astier-Gin, T., M. Janowski, B. Guillemian, L. Baugnet-Mahieu, J.R. Maisin, and J.F. Duplan (1977) Physico-Chemical Properties of Two Viral Sub-Populations Derived From a C57BL Radiation Induced Leukemia. In J.F. Duplan (ed): Radiation-Induced Leukemogenesis and Related Viruses. Amsterdam: North-Holland Publishing Company, pp. 311-317.

Astier-Gin, T., M. Galiay, E. Legrand, D. Moynet, N. Rebeyrotte, A. Artus, B. Guillemain, and J.F. Duplan (1986) Murine Thymic Lymphomas after Infection with a B-Ecotropic Murine Leukemia Virus and/or X-Irradiation: Proviral Organization and RNA Expression. Leukemia Research 10: 809-817.

Barklis, E., R. Mulligan, and R. Jaenish (1986) Chromosomal Position or Virus Mutation Permits Retrovirus Expression in Embryonal Carcinoma Cells. Cell 47: 391-399.

Barre-Sinoussi, F., C.J. Cherman, F. Rey, M.T. Nucman, C. Dauget, and C. Axed-Ble (1983) Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Aquired Immune Deficiency Syndrome (AIDS). Science 220: 868-870.

Battula, N., and L. Loeb (1974) The Infidelity of Avian Myeloblastosis Virus Deoxyribonucleic Acid Polymerase in Polynucleotide Replication. The Journal of Biological Chemistry 249: 4086-4093.

Benade, L., J. Ihle, and A. Decleve (1978) Serological Characterization of B-Tropic Viruses of C57BL Mice: Possible Origin by Recombination of Endogenous N-Tropic and Xenotropic Viruses. Proceedings of the National Academy of Science 75: 4553-4557.

Benade, L.E., and J.N. Ihle (1980) Different Serotypes of B-Tropic Murine Leukemia Viruses and Association with Endogenous Ecotropic Viral Loci. Virology 106: 374-386.

Bendinelli, M., D. Matteucci, and H. Friedman (1985) Retrovirus-Induced Acquired Immunodeficiencies. In G. Klein and S. Weinhouse (eds): Advances in Cancer Research. Orlando: Academic Press, pp. 125-179.

Bittner, J.J. (1942) The Milk-Influence of Breast Tumors in Mice. Science 95: 2470-2471.

Boone, L.R., G.S. Boone, C.L. Innes, W.K. Yang, and R.W. Tennant (1986) Hematopoietic Neoplasias of the RFM/Un Mouse Contain Somatic Re-Integration of the Restriction Endogenous Ecotropic Provirus. Carcinogenesis 7: 529-534.

Brown, P.O., B. Bowerman, H.E. Varmus, and J.M. Bishop (1987) Correct Integration of Retroviral DNA In Vitro. Cell 49: 347-356.

Carnes, W.H., M. Lieberman, M. Marchildon, and H.S. Kaplan (1966) Electron Micrograph Demonstration of C57BL Mouse Lymphoma Virus (RLV). Federation Proceedings 25: 478.

Celander, D., and W.A. Haseltine (1987) Glucorticoid Regulation of Murine Leukemia Virus Transcription Elements is Specified by Determinants within the Viral Enhancer Region. Journal of Virology 61: 269-275.

Chang, T.D., J.L. Bielder, E. Stockert, and T.D. Old (1977) Trisomy 15 in X-Ray-Induced Mouse Leukemia. Proceedings of the American Association of Cancer Research 18: 225.

Chattopadhyay, S.K., M.R. Lander, S. Gupta, E. Rands, and D. Lowy (1981) Origin of Mink Cytopathic Focus Forming (MCF) Viruses: Comparison with Ecotropic and Xenotropic Murine Leukemia Virus Genomes. Virology 113 : 465-483.

Chattopadhyay, S.K., M.R. Lander, E. Rands, and D. R. Lowy (1980) Structure of Endogenous Murine Leukemia Virus DNA in Mouse Genomes. Proceedings of the National Academy of Sciences 77: 5774-5778.

Chattopadhyay, S.K., D.R. Lowy, N.M. Teich, A.S. Levine, and W.P. Rowe (1974) Qualitative and Quantitavive Studies of AKR-type Murine Leukemia Virus Sequences in Mouse DNA. Cold Spring Harbor Symposium on Quantitative Biology 39: 1085-1101.

Clavel, F., M.D. Hoggan, R.L. Willey, K. Strebel, M.A. Martin, and R. Repaske (1989) Genetic Recombination of Human Immunodeficiency Virus. Journal of Virology 63: 1455-1459.

Cloyd, M.W., M.M. Thompson, and J.W. Hartley (1985) Host Range of Mink Cell Focus-Inducing Viruses. Virology 140: 239-248.

Coffin, J. (1984) Endogenous Viruses. In R. Weiss, N. Teich, H. Varmus, and J. Coffin (eds): RNA Tumor Viruses, 2nd edition. Cold Spring Harbor: Cold Spring Harbor Laboratory, pp. 1109-1204.

Coffin, J.M. (1979) Structure, Replication, and Recombination of Retrovirus Genomes: Some Unifying Hypotheses. Journal of General Virology 42: 1-26.

Coffin, J.M. (1986) Genetic Variation in AIDS Viruses. Cell 46: 1-4.

Cohen, J.C., P.R. Shank, V.L. Morris, R. Cardiff, and H.E. Varmus (1979) Integration of the DNA of Mouse Mammary Tumor Virus in Virus Infected Normal and Neoplastic Tissue of the Mouse. Cell 16: 333-345.

Colombatti, A., A. Dux, A. Berns, P. Demant, and J. Hilgers (1979) H-2-Dependent Regulation of the High Level of Expression of Ecotropic Murine Leukemia Virus. Journal of the National Cancer Institute 63: 869-873.

Copeland, N.G., K.W. Hutchison, and N.A. Jenkins (1983) Excision of the DBA Ecotropic Provirus in Dilute Coat-Color Revertants of Mice Occurs by Homologous Recombination Involving LTRs. Cell 33: 379-387.

Copeland, N.G., N.A. Jenkins, and B.K. Lee (1983) Association of the Lethal Yellow (A^y) Coat Color Mutation with an Ecotropic Murine Leukemia Virus Genome. Proceedings of the National Academy of Sciences 80: 247-249.

Covelli, V., V. DiMajo, B. Bassani, S. Rebessi, M. Coppola, and G. Silini (1984) Influence of Age on Life Shortening and Tumor Induction after X-Ray and Neutron Irradiation. Radiation Research 100: 348-364.

Cuypers, H.T., G. Selten, W. Quint, M. Zijlstra, E. R. Maandag, W. Boelens, P. vanEezenbeek, and C. Melief (1984) Murine Leukemia Virus-Induced T-Cell Lymphomagenesis: Integration of Proviruses in a Distinct Chromosomal Region. Cell 37: 141-150.

Datta, S.K., C.Y. Thomas, J.A. Nicklas, and J.M. Coffin (1983) Thymic Epithelial Genotype Influences the Production of Recombinant Leukemogenic Retroviruses in Mice. Journal of Virology 47: 33-45.

Datta, S.K., P. Tsichlis, R.S. Schwartz, S.K. Chattophadhyay, and C.J.M. Melief (1978) Genetic Differences Unrelated to H-2 in H-2 Congenic Mice. Immunogenetics 7: 359-365.

Decleve, A., O. Niwa, E. Glemann, and H.S. Kaplan (1976) Radiation Activation of Endogenous Leukemia Viruses in Cell Culture: Acute X-Ray Irradiation. In J.M. Yuhas, R.W. Tennant, and J.D. Regan (eds): Biology of Radiation Carcinogenesis. New York: Raven Press, pp. 217-225.

DesGroseillers, L., and P. Jolicoeur (1983) Physical Mapping of the Fv-1 Tropism Host Range Determinant of BALB/c Murine Leukemia Viruses. Journal of Virology 48: 685-696.

DesGroseillers, L., E. Rassart, and P. Jolicoeur (1983) Thymotropism of Murine Leukemia Virus is Conferred by it's Long Terminal Repeat. Proceedings of the National Academy of Sciences 80: 4203-4207.

Eisenman, R.N., and V.M. Vogt (1978) The Biosynthesis of Oncovirus Proteins. Biochemica et Biophysica Acta 473: 187-239.

Ellis, R.W., E. Stockert, and E. Fleissner (1980) Association of Endogenous Retroviruses with Radiation-Induced Leukemias of BALB/c Mice. Journal of Virology 33: 652-660.

Evans, L.H., and F. Malik (1987) Class II Polytropic Murine Leukemia Viruses (MuLVs) of AKR/J Mice: Possible Role in the Generation of Class I Oncogenic Polytropic MuLVs. Journal of Virology 61: 1882-1892.

Frieben, A. (1902) Demonstration Lines Cancroids de Rechten Handruckens das Sich Nach Langdauernder Einwirkung von Rotgenstrahlen Entwickelt Hatte. Fortschr. Geb. Rotgenstr. 6: 106.

Friefelder, D. (1983) Molecular Biology. Boston: Jones and Bartlett Publishers, pp. 868-877.

Friend, C. (1957) Cell-free Transmission in Adult Swiss Mice of a Disease Having the Character of a Leukemia. Journal of Experimental Medicine 105: 307-318.

Fry, R.J.M. (1981) Experimental Radiation Carcinogenesis: What Have We Learned?. Radiation Research 87: 224-239.

Fry, R.J.M., and J.B. Storer (1987) External Radiation Carcinogenesis. In J.T. Lett (ed): Advances in Radiation Biology. San Diego: Academic Press, pp. 31-90.

Fujiwara, T., and K. Mizuuchi (1988) Retroviral DNA Integration: Structure of an Integration Intermediate. Cell 54: 497-504.

Furth, J., and O.B. Furth (1936) Neoplastic Diseases Produced in Mice by General Irradiation with X-Rays. American Journal of Cancer 28: 54-65.

Galliay, M., E. Legrand, T. Astier-Gin, B. Guillemain, C. Rollet, and J.F. Duplan (1986) Induction of Thymic Lymphosarcomas in C57BL/6 Mice After Inoculation of Weakly Oncogenic Viruses Associated with a Sub-Leukemogenic Radiation Exposure (1.75 Gy X 2). International Journal of Cancer 38: 223-228.

Gallo, R.C., S.Z. Salahudden, and M. Popovx (1984) Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. Science 224: 500-502.

Goodhead, D.T. (1984) Deductions from Cellular Studies of Inactivation, Mutagenesis, and Transformation. In J.D. Boice and J.F. Fraumeni (eds): Radiation Carcinogenesis: Epidemiology and Biological Significance. New York: Raven Press, pp. 369-385.

Green, E.L. (1968) Breeding Systems. In E.L. Green (ed): Biology of the Laboratory Mouse. New York: Dover Publications Inc., pp. 11-22.

Gross, L. (1951) "Spontaneous" Leukemia Developing in C3H Mice Following Inoculation, in Infancy, with AK-Leukemic Extracts, or AK-Embryos. Proceedings of the Society for Experimental Biological Medicine 76: 27-32.

Grymes, R.A., M.L. Scott, J.P. Kim, K.E. Fry, and H.S. Kaplan (1983) Molecular Studies of the Radiation Leukemia Virus (RadLV) and Related Retroviruses of C57BL/Ka Mice. Progress in Nucleic Acid Research and Molecular Biology 29: 53-73.

Guillemain, B., R. Mamoun, T. Astier, J.P. Portail, E. Legrand, and J.F. Duplan (1977) Studies of Radiation Leukemia Virus (Duplan Isolate) Released by a Cell Line Derived from the Leukemic Spleen of a C57BL Mouse. In J.F. Duplan (ed): Radiation Induced Leukemogenesis and Related Viruses. Amsterdam: North-Holland Publishing Company, pp. 297-309.

Haas, M. (1977) Transient Virus Expression During Murine Leukemia Induction by X-Irradiation. Journal of the National Cancer Institute 58: 251-257.

Han, A., C.k. Hill, and M.M. Elkind (1980) Repair of Cell Killing and Neoplastic Transformation at Reduced Dose Rates of ^{60}Co Gamma-Rays. Cancer Research 40: 3328-3332.

Haran-Ghera, N. (1969) The role of Immune Impairment in Viral Leukaemogenesis. In L. Severi (ed): Immunity and Tolerance in Oncogenesis. Peruga: Division of Cancer Research, pp. 585-597.

Haran-Ghera, N. (1971) Influence of Host Factors on Leukemogenesis by the Radiation Leukemia Virus. Isreal Journal of Medical Sciences 7: 17-25.

Haran-Ghera, N. (1976) Pathways in Murine Radiation Leukemogenesis-Coleukemogenesis. In J.M. Yuhas, R.W. Tennant, and J.D. Regan, (eds): Biology of Radiation Carcinogenesis. New York: Raven Press, pp. 245-260.

Haran-Ghera, N. (1977) Target Cells Involved in Radiation and Radiation Leukemia Leukemogenesis. In J.F. Duplan (ed): Radiation-Induced Leukemogenesis and Related Viruses. Amsterdam: North-Holland Publishing Company, pp. 79-89.

Haran-Ghera, N., and A. Peled (1979) Induction of Leukemia in Mice by Irradiation and Radiation Leukemia Virus Variants. In G. Klein and S. Weinhouse (eds): Advances in Cancer Research. New York: Academic Press, pp. 45-87.

Hartley, J.W., W.P. Rowe, and R.J. Huebner (1970) Host-Range Restrictions of Murine Leukemia Viruses in Mouse Embryo Cell Cultures. Journal of Virology 5: 221-225.

Herr, W. and W. Gilbert (1982) Germ-line MuLV Reintegration in AKR/J Mice. Nature 29: 805-807.

Holland, C.A., J.W. Hartley, W.P. Rowe, and N. Hopkins (1985) At Least Four Viral Genes Contribute to the Leukemogenicity of Murine Retroviruses MCF 247 in AKR Mice. Journal of Virology 53: 158-165.

Horowitz, J.M., G.D. Holland, S.R. King, and R. Risser (1987) Germ Line Integration of a Murine Leukemia Provirus into a Retrovirus Like Sequence. Journal of Virology 61: 701-707.

Horowitz, J.M., and R. Risser (1982) A Locus That Inhances the Induction of Endogenous Ecotropic Murine Leukemia Viruses is Distinct from Genome-Length Ecotropic Proviruses. Journal of Virology 44: 950-957.

Huebner, R.J., and G.J. Todaro (1969) Oncogenes of RNA Tumor Viruses as Determinants of Cancer. Proceedings of the National Academy of Sciences 64: 1087-1094.

Huebner, R.J., G.J. Kelloff, P.S. Sarma, W.T. Lane, H.C. Turner, R.V. Gilden, S. Oroszlan, H. Meier, D.D. Myers, and R.L. Peters (1970) Group-Specific Antigen Expression During Embryogenesis of the Genome of the C-Type RNA Tumor Virus: Implications for Oncogenesis. Proceedings of the National Academy of Sciences 67: 366-376.

Hunter, E. (1978) The Mechanisms for Genetic Recombination in the Avian Retrovirus. Current Topics in Microbiology and Immunology 79: 295-309.

Jaenisch, R., H. Fan, and B. Croker (1975) Infection of Preimplantation Mouse Embryos and of Newborn Mice with Leukemia Virus: Tissue Distribution of Viral DNA and RNA and Leukemogenesis in the Adult Animal. Proceedings of the National Academy of Science 72: 4008-4012.

Jaenisch, R., K. Harbers, A. Schnieke, J. Loher, I. Chumakov, D. Jahner, D. Grotkopp, and E. Hoffman (1983) Germline Integration of Moloney Murine Leukemia Virus at the Mov13 Locus Leads to Recessive Lethal Mutation and Early Embryonic Death. Cell 32: 209-216.

Jenkins, N.A., N.G. Copeland, B.A. Taylor, and B.K. Lee (1981) Dilute (d) Coat Colour Mutation of DBA/2J Mice is Associated with the Site of Integration of an Ecotropic MuLV Genome. Nature 293: 370-374.

Jenkins, N.A., N.G. Copeland, B.A. Taylor, and B.K. Lee (1982) Organization, Distribution, and Stability of Endogenous Ecotropic Murine Leukemia Virus DNA Sequences in Chromosomes of Mus musculus. Journal of Virology 43: 26-36.

Jolicoeur, P., and E. Rassart (1981) Fate of Unintegrated Viral DNA in Fv-1 Permissive and Resistant Mouse Cells Infected with Murine Leukemia Virus. Journal of Virology 37: 609-619.

Jolicoeur, P., E. Rassart, and P. Sankar-Mistry (1983) Strong Selection for Cells Containing New Ecotropic Recombinant Murine Leukemia Virus Provirus After Propagation of C57BL/6 Radiation-Induced Thymoma Cells In Vitro or In Vivo. Molecular and Cellular Biology 3: 1675-1679.

Kanizar, D.T. (1969) Radiation-Induced Alterations in the Structure of Deoxyribonucleic Acid and their Biological Consequences. Progress in Nucleic Acid Research and Molecular Biology 9: 117-222.

Kaplan, H.S. (1948) Influence of Age on Susceptibility of Mice to the Develpoment of Lymphoid Tumors After Irradiation. Journal of the National Cancer Institute 9: 55-56.

Kaplan, H.S. (1967) On the Natural History of the Murine Leukemias: Pressidental Address. Cancer Research 27: 1325-1340.

Kaplan, H.S. (1977) Interaction Between Radiation and Viruses in the Induction of Murine Thymic Lymphomas and Lymphatic Leukemias. In J.F. Duplan (ed): Radiation-Induced Leukemogenesis and Related Viruses. Amsterdam: North-Holland Publishing Company, pp. 1-18.

King, S.R., B.J. Berson, and R. Risser (1987) Mechanism of Interaction between Endogenous Ecotropic Murine Leukemia Viruses in (BALB/c x C57BL/6) Hybrid Cells. Virology 161:

Kirschbaum, A., and H.W. Mixer (1947) Induction of Leukemia in Eight Inbred Stocks of Mice Varying in Susceptibility to the Spontaneous Disease. Journal of Laboratory Clinical Medicine 32: 720-731.

Klinken, S.P., T.N. Fredrickson, J.W. Hartley, R.A. Yetter, and H.C. Morse III (1988) Evolution of B Cell Lineage Lymphomas in Mice with a Retrovirus-Induced Immunodeficiency Syndrome, MAIDS. Journal of Immunology 140: 1123-1131

Kozak, C.A. (1985a) Retroviruses as Chromosomal Genes in the Mouse. In G. Klein and S. Weinhouse (ed): Advances in Cancer Research: New York: Academic Press, 44: 295-336.

Kozak, C.A. (1985b) Analysis of Wild-Derived Mice for Fv-1 and Fv-2 Murine Leukemia Virus Restriction Loci: a Novel Wild Mouse Fv-1 Allele Responsible for Lack of Host Range Restriction. Journal of Virology 55: 281-285.

Kozak, C.A., and W.P. Rowe (1978) Genetic Mapping of Xenotropic Leukemia Virus-Inducing Loci in Two Mouse Strains. Science 199: 1448-1449.

Kozak, C.A., and W.P. Rowe (1980) Chromosomal Mapping of Ecotropic and Xenotropic Leukemia virus-Inducing Loci in the Mouse. In (ed): Animal Virus Genetics. New York: Academic Press, pp. 171-175.

Langdon, W.Y., T.S. Thedore, C.E. Buckler, J.H. Stimpfling, M.A. Martin, and H.C. Morse (1984) Relationship between a Retroviral Germ Line Reintegration and a New Mutation at the Ashen Locus in B10.F Mice. Virology 133: 183-190.

Legrand, E., M. Galiay, T. Astier-Gin, C. Rollet, B. Guillemain, and J.F. Duplan (1986) Thymic Lymphosarcomas Obtained by X-Rays in Association with a Wealky Leukemogenic Virus: Cellular Studies. Leukemia Research 7: 791-795.

Leider, J.M., P. Palese, and F.I. Smith (1988) Determination of the Mutation Rate of a Retrovirus. Journal of Virology 62: 3084-3091.

Leis, J., D. Baltimore, J.M. Bishop, J. Coffin, E. Fleissner, S.P. Goff, S. Oroszlan, and H. Robinson (1988) Standardized and Simplified Nomenclature for Proteins Common to all Retroviruses. Journal of Virology 62: 1808-1809.

Lenz, J., R. Crowther, S. Klimenko, and W. Haseltine (1982) Molecular Cloning of a Highly Leukemogenic, Ecotropic Retrovirus from an AKR Mouse. Journal of Virology 43: 943-951.

Lenz, J., and W.A. Haseltine (1983) Localization of the Leukemogenic Determinants of SL3-3, an Ecotropic, XC-Positive Murine Leukemia Virus of AKR Mouse Origin. Journal of Virology 47: 317-328.

Levy, D.E., R.A. Lerner, and M.C. Wilson (1985) The Gv-1 Locus Coordinately Regulates the Expression of Multiple Endogenous Murine Retroviruses. Cell 41: 289-299.

Liberman, M., H.S. Kaplan, and A. Decleve (1976) Anomalous Viral Expression in Radiogenic Lymphomas of C57BL/Ka Mice. In J.M. Yuhas, R.W.Tennant, and J.D. Regan (eds): Biology of Radiation Carcinogenisis. New York: Raven Press, pp. 237-244.

Lieberman, M., and H.S. Kaplan (1959) Leukemogenic Activity of Filtrates from Radiation-Induced Lymphoid Tumors of Mice. Science 130: 387-388.

Lowy, D.R. (1986) Transformation and Oncogenesis: Retroviruses. In B.N. Fields and D.M. Knipe (eds): Fundamental Virology. New York: Raven Press, pp. 235-263.

Lowy, D.R., E. Rands, S.K. Chattopadhyay, C.F. Garon, and G.L. Hager (1980) Molecular Cloning of Infectious Integrated Murine Leukemia Virus DNA from Infected Mouse Cells. Proceedings of the National Academy of Science 77: 614-618.

Luciw, P.A., J.M. Bishop, H.E. Varmus, and M.R. Capecchi (1983) Location and Function of Retroviral and SV40 Sequences that Enhance Biochemical Transformation after Microinjection of DNA. Cell 33: 705-716.

Maniatis, T., Fritschef, and J. Sambrook (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor: Cold Spring Harbor Laboratory.

Maruyama, Y., and M.S. Coleman (1978) Induction of Spleen Cell Growth and DNA Polymerase Activity by Corynebacterium parvum. Cancer Research 38: 1617-1620.

Maruyama, Y., and J.M. Feola (1987) Relative Radiosensitivities of the Thymus, Spleen, and Lymphohemopoietic Systems. In J.T. Lett and K. I. Altman (eds): Advances in Radiation Biology; Relative Radiation Sensitivities of Human Organ Systems. Orlando: Academic Press, pp. 1-82.

McCubrey, J., and R. Risser (1982) Genetic Interactions in the Spontaneous Production of Endogenous Murine Leukemia Virus in Low Leukemic Mouse Strains. Journal of Experimental Medicine 156: 337-349.

McGrath, M.S., and I.R. Weissman (1979) AKR Leukemogenesis: Identification and Biological Significance of Thymic Lymphoma Receptors for AKV Retroviruses. Cell 17: 65-75.

Melief, C.J., S. Louie, and R.S. Schwartz (1975) Ecotropic Leukemia Viruses in Congenic C57BL Mice: Natural Dissemination by Milk-Borne Infection. Journal of the National Cancer Institute 55: 691-698.

Meruelo, D., R. Kornreich, A. Rossomando, C. Pampeno, A.L. Mellor, E.H. Weiss, R.A. Flavell, and A. Pellicer (1984) Murine Leukemia Virus Sequences are Encoded in the Murine Major Histocompatibility Complex. Proceedings of the National Academy of Science 81: 1804-1808.

Meruelo, D., and R. Bach (1983) Genetics of Resistance to Virus-Induced Leukemias. Advances in Cancer Research 40: 107-188.

Meruelo, D., M. Offer, and A. Rossomando (1983) Induction of Leukemia by Both Fractionated X-Irradiation and Radiation Leukemia Virus Involves Loci in the Chromosome 2 Segment H-30-A. Proceeding of the National Academy of Sciences 80: 462-466.

Meruelo, D., and A. Rossomando (1986) A Molecular and Genetic Approach to Understanding the Mechanism by Which Fractionated X-Irradiation Induces Leukemia in Mice. Leukemia Research 10: 819-832.

Mindem, M.D. (1987) Oncogenes. In I.F. Tannock and R.P. Hill (eds): The Basic Science of Oncology. New York: Pergamon Press, pp. 72-88.

Mistry, P.B., and J.F. Duplan (1973) Properietes Biologiques d'un Virus Isole d'une Radioleucemie C57BL. Bullitin du Cancer 60: 287-300.

Moll, B., J.W. Hartley, and W.P. Rowe (1979) Introduction of B-Tropic and N-Tropic Murine Leukemia Virus From B10.BR/SgLi Mouse Embryo Cell Lines by 5-Iodo-2'-Deoxyuridine. Journal of the National Cancer Institute 63: 213-217.

Moloney, J.B. (1960) Biological Studies on a Lymphoid-Leukemia Virus Extracted from Sarcoma 37 I. Origin and Introductory Investigations. Journal of the National Cancer Institute 24: 933-951.

Morishita, K., D.S. Parker, M.L. Mucenski, N.A. Jenkins, N.G. Copeland, and J.N. Ihle (1988) Retroviral Activation of a Novel Gene Encoding a Zinc Finger Protein in IL-3-Dependent Myeloid Leukemia Cell Lines. Cell 54: 831-840.

Morse, H.C., and J.W. Hartley (1982) Expression of Xenotropic Murine Leukemia Viruses. Current Topics in Microbiology and Immunology 104: 17-26.

Morse, H.C., C.A. Kozak, R.A. Yetter, and J.W. Hartley (1982) Unique Features of Retrovirus Expression in F/St Mice. Journal of Virology 43: 1-7.

Morse, H.C., R.A. Yetter, J.H. Stimpfling, O.M. Pitts, T.N. Fredrickson, and J.W. Hartley (1985) Greying with Age in Mice: Relation to Expression of Murine Leukemia Viruses. Cell 41: 439-448.

Mosier, D.E., R.A. Yetter, and H.C. Morse (1985) Retroviral Induction of Acute Lymphoproliferative Disease and Profound Immunosupression in Adult C57BL/6 Mice. Journal of Experimental Medicine 161: 766-784.

Mowat, M., and A. Bernstein (1983) Linkage of the Fv-2 Gene to a Newly Reinserted Ecotropic Retrovirus in Fv-2 congenic Mice. Journal of Virology 47:471-477.

Mucenski, M.L., B.A. Taylor, J.N. Ihle, J.W. Hartley, H.C. Morse, N.A. Jenkins, and N.G. Copeland (1988) Identification of a Common Ecotropic Viral Integration Site, Evi-1, in the DNA of AKXD Murine Myeloid Tumors. Molecular and Cellular Biology 8: 301-308.

Nakajima, K., O. Martinez-Maza, T. Hirano, E. Breen, P. Nishanian, J.F. Salazar-Gonzalez, J.L. Fahey, and T. Kishimoto (1989) Induction of IL-6 (B Cell Stimulatory Factor-2/INF-B2) Production by HIV. The Journal of Immunology 142: 531-536.

Niwa, O., and T. Sugahara (1979) Radiation Induction of Endogenous Type C Virus from Mouse Cells Transformed In Vitro by Murine Sarcoma Virus. Journal of the National Cancer Institute 62: 329-335.

Nowinski, R.C., and E.F. Hays (1978) Oncogenicity of AKR Endogenous Leukemia Viruses. Journal of Virology 27: 13-18.

Nusse, R., and H. Varmus (1982) Many Tumors Induced by the Mouse Mammary Tumor Virus Contain a Provirus Integrated in the Same Region of the Host Genome. Cell 31: 99-109.

Okumoto, M., R. Nishikawa, Y. Takamori, Y. Iwai, and M. Iwai (1983) Expression of Type-C Virus in NFS Mice with Radiation-Induced Leukemia. In J.J. Broerse, G.W. Barendsen, H.B. Kal, and A.J. Van der Kogel (eds): Radiation Research: Somatic and Genetic Effects. Amsterdam: Martinus Nijhoff, pp. 6-13.

Pampeno, C.L., and D. Meruelo (1986) Isolation of a Retrovirus like Sequence from the TL Locus of the C57BL/10 Murine Major Histocompatibility Complex. Journal of Virology 58: 296-306.

Pattengale, P.K., C.R. Taylor, T. Twomey, S. Hill, J. Johnosn, T. Beardsley, and M. Hass (1982) Immunology of B-cell Lymphomas Induced in C57BL/6 Mice by Dualtropic Murine Leukemia Virus (MuLV). American Journal of Pathology 107: 362-377.

Popp, D.M. (1978) Use of Congenic Mice to Study the Genetic Basis of Degenerative Disease. Birth Defects 14: 261-279.

Popp, D.M. (1979a) Basal Serum Immunoglobulin Levels. I. Evidence for Genetic Control in B10 and B10.F Mice. Immunogenetics 9: 125-135.

Popp, D.M. (1979b) Basal Serum Immunoglobulin Levels. II. Interaction of Genetic Loci and Maternal Effect on Regulation of IgA. Immunognetics 9: 281-292.

Popp, D.M. (1982) An Analysis of Genetic Factors Regulating Life-Span in Congenic Mice. Mechanisms of Aging and Development 18: 125-134.

Popp, D.M. (in press) Lymphocyte Subsets and Abnormalities in Retrovirally Infected Mice. CRC Reviews in Immunology. CRC Press, Boca Ratan, FL.

Popp, D.M., J.M. Otten, and R.A. Popp (1986) Interaction of H2 Genotype and Basal Serum Immunoglobulin A Level Influences Longevity. Mechanisms of Aging and Development 36: 79-93.

Popp, R.A., P.A. Lalley, J.B. Whitney and W.F. Anderson (1981) Mouse Alpha-Globin and Alpha-Globin-Like Pseudogenes are not Syntenic. Proceedings of the National Academy of Sciences 78: 6362-6366.

Rassart, E., Y. Paquette, and P. Jolicoeur (1988) Inability of Kaplan Radiation Leukemia Virus to Replicate on Mouse Fibroblasts is Conferred by Its Long Terminal Repeat. Journal of Virology 62: 3840-3848.

Rassart, E., P. Sankar-Mistry, G. Lemay, L. DesGroseillres, and P. Jolicour (1983) New Class of Leukemogenic Ecotropic Recombinant Murine Leukemia Virus Isolated from Radiation-Induced Thymomas of C57BL/6 Mice. Journal of Virology 45: 565-575.

Rassart, E., M. Shang, Y. Boie, and P. Jolicoeur (1986) Studies on Emerging Radiation Leukemia Virus Varients in C57BL/Ka Mice. Journal of Virology 58: 96-106.

Rauscher, F.J. (1962) A Virus-Induced Disease of Mice Characterized by Erythropoiesis and Lymphoid Leukemia. Journal of the National Cancer Institute 29: 515-543.

Rauth, A.M. (1987) Radiation Carcinogenesis. In I. F. Tannock and R.P. Hill (eds): The Basic Science of Oncology. New York: Pergamon Press, pp. 106-124.

Razin, A., and A. Riggs (1980) DNA Methylation and Gene Function. Science 210: 604-610.

Risser, R., J.M. Horowitz, and J. McCubrey (1983) Endogenous Mouse Leukemia Viruses. Annual Review of Genetics 17: 85-121.

Roderick, T.H., and G. Schlager (1968) Multiple Factor Inheritance. In E.L. Green (ed): Biology of the Laboratory Mouse. New York: Dover Publishers Inc., pp. 151-164.

Rossomando, A., and D. Meruelo (1986) Viral Sequences are Associated with Many Histocompatibility Genes. Immunogenetics 23: 233-245.

Rous, P. (1911) A Sarcoma of the Fowl Transmissible by an Agent Separable from the Tumor Cells. Journal of Experimental Medicine 13: 397-411.

Rowe, W.P. (1976) Leukemia Virus Genomes in the Chromosomal DNA of the Mouse. Harvey Lectures 72: 173-192.

Rowe, W.P., M.W. Cloyd, and J.W. Hartley (1980) Status of the Association of Mink Cell Focus-forming Viruses and Leukemogenesis. Cold Spring Harbor Symposia on Quantitative Biology 44 : 1265-1268.

Ruscetti, S., R. Matthai, and M. Potter (1985) Susceptibility of BALB/c Mice Carrying Various DBA/2 Genes to Development of Friend Murine Leukemia Virus-Induced Erythroleukemia. Journal of Experimental Medicine 162: 157-1587.

Sankar-Mistry,P., P. Jolicoeur (1980) Frequent Isolation of Ecotropic Murine Leukemia Virus After X-Ray Irradiation of C57BL/6 Mice and Establishment of Producer Lymphoid Cell Lines from Radiation Induced Lymphomas. Journal of Virology 35: 270-275.

Schmidt, J., A. Luz, and V. Erfle (1988) Endogenous Murine Leukemia Viruses: Frequency of Radiation-Activation and Novel Pathogenic Effects of Viral Isolates. Leukemia Research 12: 393-403.

Schuermann, M., and R. Michalides (1987) A Rare Common Integration Site of Proviruses of the Mouse Mammary Tumor Virus in P-Type Tumors of Mouse Strain GR. Virology 156: 229-237.

Selten, H., H.T. Cuypers, M. Zijlstra, C. Melief, and A. Berns (1986) Involvement of c-myc in MuLV-Induced T cell Lymphomas in Mice: Frequency and Mechanisms of Action. The EMBO Journal : 3215-3222.

Sheinin, R., T.W. Mak, and S.P. Clark (1987) Viruses and Cancer. In I.F. Tannock and R.P. Hill (eds): The Basic Science of Oncology. New York: Pergamon Press, pp. 52-71.

Shih, C., J.P. Stoye, and J.M. Coffin (1988) Highly Preferred Targets for Retroviral Integration. Cell 53: 531-537.

Shimotohno, K., and H. Temin (1982) Spontaneous Variation and Synthesis in the U3 Region of the Long Terminal Repeat of an Avian Retrovirus. Journal of Virology 41: 163-171.

Snell, G.D. (1948) Methods for the Study of Histocompatibility Genes. Journal of Genetics 49: 87-108.

Snell, G.D., and J.H. Stimpfling (1968) Genetics of Tissue Transplantation. In E.L. Green (ed): Biology of the Laboratory Mouse. New York: Dover Publishers Inc., pp. 457-492.

Staats, J. (1968) The Laboratory Mouse. In E.L. Green (ed): Biology of the Laboratory Mouse. New York: Dover Publishers Inc., pp. 1-10.

Steffen, D.L., R. Mural, D. Cowing, J. Mielczar, J. Young, and R. Roblin (1982) Most of the Murine Leukemia Virus Sequences in the DNA of NIH/Swiss Mice Consist of Two Closely Related Proviruses, Each Repeated Several Times. Journal of Virology 43: 127-135.

Storer, J.B. (1973) Radiation Carcinogenesis. In F.F. Becker (ed): Cancer a Comprehensive Treatise. New York, Plenum Press, 2: 453-483.

Stoye, J., and J. Coffin (1985) Endogenous Retroviruses. In R. Weiss, N. Teich, H. Varmus, and J. Coffin (eds): RNA Tumor Viruses. Cold Spring Harbor: Cold Spring Harbor Laboratory, pp. 357-403.

Stoye, J.P., and J.M. Coffin (1987) The Four Classes of Endogenous Murine Leukemia Virus: Structural Relationships and Potential for Recombination. Journal of Virology 61: 2659-2669.

Stoye, J.P., S. Fenner, G.E. Greenoak, C. Moran, and J.M. Coffin (1988) Role of Endogenous Retroviruses as Mutagens: The Hairless Mutation of Mice. Cell 54: 383-391.

Teich, N. (1982) Taxnonomy of Retroviruses. In R. A. Weiss, N. Teich, H.E. Varmus, and J.M. Coffin (eds): Molecular Biology of RNA Tumor Viruses. Cold Springs Harbor: Cold Springs Harbor, pp. 785-998.

Teich, N., J. Wyke, T. Mak, A. Bernstein, and W. Hardy (1982) Pathogenesis of Retrovirus Induced Disease. In R. Weiss, N. Teich, H. Varmus, and J. Coffin (eds): RNA Tumor Viruses. Cold Spring Harbor: Cold Spring Harbor Laboratory, pp. 785-998.

Temin, H.M. (1989) Is HIV Unique of Merely Different? Journal of Acquired Immune Deficiency Syndromes 2: 1-9.

Temin, H.M., and S. Mizutani (1970) RNA-Dependent DNA Polymerase in Virions of Rous Sarcoma Virus. Nature 226: 1211-1213.

Temin, H.M., and H. Rubin (1958) Characteristics of an Assay for Rous Sarcoma Virus and Rous Sarcoma Cells in Tissue Culture. Virology 6: 669-688.

Tennant, R.W., J.A. Otten, J.M. Quarles, W. Yang, and A. Brown (1976) Cellular Factors That Regulate Radiation Activation and Restriction of Mouse Leukemia Viruses. In J.M. Yuhas, R.W. Tennant, and J.D. Regan (eds): Biology of Radiation Carcinogenesis. New York: Raven Press, pp. 227-236.

Thomas, C.Y. (1986) AKR Ecotropic Murine Leukemia Virus SL3-3 Forms Envelope Gene Recombinants In Vivo. Journal of Virology 59: 23-30.

Thomas, C.Y., and J.M. Coffin (1982) Genetic Alterations of RNA Leukemia Viruses Associated with the Development of Spontaneous Thymic Leukemia in AKR/J mice. Journal of Virology 43: 416-426.

Upton, A.C. (1968) Radiation Carcinogenesis. In Busch (ed): Methods in Cancer Research. New York: Academic Press, pp. 53-82.

Upton, A.C. (1973) Physical Carcinogenesis: Radiation-History and Sources. In F.F. Becker (ed): Cancer A Comprehensive Treatise. New York: Plenum Press, pp. 387-403.

VanBeveren, C., E. Rands, S.K. Chattpoadhyay, D.R. Lowy, and I.M. Verma (1982) Long Terminal Repeat of Murine Retroviral DNAs: Sequence Analysis, Host-Proviral Junctions, and Preintegration Site. Journal of Virology 41: 542-556.

Varmus, H. (1988) Retroviruses. Science 240: 1427-1435.

Varmus, H.E., T. Padgett, S. Heaslet, G. Simon, and J.M. Bishop (1987) Cellular Functions are Required for the Synthesis and Integration of Avian Sarcoma Virus-Specific DNA. Cell 11: 307-319.

Villemur, R., E. Rassart, L. DesGroseillers, and P. Jolicoeur (1983) Molecular Cloning of Viral DNA from Leukemogenic Gross Passage A Murine Leukemia Virus and Nucleotide Sequence of Its Long Terminal Repeat. Journal of Virology 45: 539-546.

Vlug, A., H.J. Schoenmakers, and C.J.M. Melief (1981) Genes of the H-2 Complex Regulate the Antibody Response to Murine Leukemia Virus. Journal of Immunology 126: 2355-2360.

Webb, E., J.M. Adams, and S. Cory (1984) Variant (6;15) Translocation in a Murine Plasmacytoma Occurs Near an Immunoglobulin k Gene but Far From the myc Oncogene. Nature 312: 777-779.

Weiss, R.A. (1986) Molecular and Cellular Aspects of Retrovirus Pathogenesis. W.C. Russell and J.W. Almond(ed):In Molecular Basis of Virus Disease. Cambridge:Cambridge Press, pp. 167-192.

Wejman, J.C., B.A. Taylor, N.A. Jenkins, and N.G. Copeland (1984) Endogenous Xenotropic Murine Leukemia Virus-Related Sequences Map to Chromosomal Regions Encoding Mouse Lymphocyte Antigens. Journal of Virology 50: 237-247.

Wellinger, R.J., M. Garcia, A. Vessaz, and H. Diggelmann (1986) Exogenous Mouse Mammary Tumor Virus Proviral DNA Isolated from a Kidney Adenocarcinoma Cell Line Contains Alterations in the U3 Region of the Long Terminal Repeat. Journal of Virology 60: 1-11.

White, D.O., and F. Fenner (1986) Medical Virology. Orlando: Academic Press, Inc., pp. 217-246.

Wiener, F., S. Ohono, J. Spira, N. Haran-Ghera, and G. Klein (1978) Chromosome Changes (Trisomy #15 and 17) Associated with Tumor Progression in Leukemias Induced by Radiation Leukemia Virus. Journal of the National Cancer Institute 61: 227-233.

Wiener, F., J. Spira, M. Babonits, and G. Klein (1982) Non-Random Duplication of Chromosome 15 in T-Cell Leukemias Induced in Mice Heterozygous for Reciprocal and Robertsonian Translocations. International Journal of Cancer 30: 479-487.

Wood, T.G., M.L. McGeady, D.G. Blair, and G.F. Vande Woude (1983) Long Terminal Repeat Enhancement of v-mos Transforming Activity: Identification of Essential Regions. Journal of Virology 46: 726-736.

Yetter, R.A., R.M.L. Buller, J.S. Lee, K.L. Elkins, D.E. Mosier, T.N. Fredrickson, and H.C. Morse III (1988) CD4+ T Cells are Required for Development of a Murine Retrovirus-Induced Immunodeficiency Syndrome (MAIDS) Journal of Experimental Medicine 168: 623-635.

Yang, W.K., J.O. Kiggans, D. Yang, C. Ou, R. Tennant, A. Brown, and R. Bassin (1980) Synthesis and Circularization of N- and B-Tropic Retroviral DNA in Fv-1 Permissive and Restrictive Mouse Cells. Proceedings of the National Academy of Sciences 77: 2994-2998.

Yetter, R.A., J.W. Hartley, and H.C. Morse (1983) H-2-linked Regulation of Xenotropic Murine Leukemia Virus Expression. Proceedings of the National Academy of Sciences USA 80: 505-509.

Zar, J.H. (1974) Biostatistical Analysis. Englewood Cliffs: Prentice-Hall, Inc., pp. 63-65.

Zijlstra, M., R.E. deGoede, H.J. Shoenmakers, A.H. Schinkel, W.G. Hesselink, J.L. Portis, and C.J. Melief (1983) Naturally Occuring Leukemia Viruses in H-2 Congenic C57BL Mice. III. Characterization of C-Type Viruses Isolated From Lymphomas Induced by Milk Transmission of B-Ecotropic Virus. Virology 125: 47-63.

Zijlstra, M., and C.J.M. Melief (1986) Virology, Genetics and Immunology of Murine Lymphomagenesis. Biochimica et Biophysica Acta 865: 197-231.

Zijlstra, M., W.L.E. Vasmel, T. Radaskiewicz, E. Matthews, and C.J.M. Melief (1986) The H-2 Complex Regulates Both the Susceptibility to Mouse Viral Lymphomagenesis and the Phenotype of the Virus-Induced Lymphomas. Journal of Immunogenetics 13: 69-76.

APPENDIX

EXPERIMENTAL PROCEDURES

Preparation of Genomic DNA

Two methods were used to obtain genomic DNA from B10 and B10.F mice in order to examine the ecotropic proviral content of spleens and other organs. The large and small scale isolation procedures are described separately.

Large scale isolation of DNA

Isolation of genomic DNA has been described (Popp et al., 1981). Whole tissues, or up to 1 gram, were quick frozen in liquid nitrogen and homogenized in a smooth glass homogenizer with a loosely-fitted teflon plunger in 20 ml of calcium, magnesium-free phosphate buffered saline (PBS), pH 7.4. The homogenate was spun at 1500 x g for 10 minutes and the supernate was decanted. The pellet was resuspended and washed in 20 ml of PBS until the supernatant was clear. This procedure removed much of the cellular debris (proteins, membranes, mitochondria, and microsomes) from the intact nuclei in the pellet.

Nuclei were ruptured by dissolving the pellet in 2.7 ml of 6.0 M guanidine hydrochloride (BRL) and vortexing for 30 seconds. To dissociate lipids, 6.0 ml Sarkosyl (4% in 0.1M Tris/HCL pH 8.0) was added and the mixture was vortexed vigorously for 10 minutes. To denature proteins, the preparation was acidified by adding 0.3 ml of 2 M potassium acetate, pH 5.0.

DNA was separated from RNA, proteins, and lipids, in a cesium chloride-density gradient. A volume of 1.6 ml of 5.6 M CsCl (Chemetall) in 0.1 M EDTA, pH 7.0 was placed in the bottom of a polyallomer centrifuge tube (Beckmann # 331372) to form a dense cushion. Solid cesium chloride, 3.6g, was dissolved in the DNA preparation and the mixture was carefully layered above the cushion. The samples were centrifuged (SW41Ti rotor) for 48 hours in a L5-50B Beckman ultracentrifuge at 30,000 RPM at 25° C. DNA localized above the dense CsCl cushion, was removed with a pasteur pipette and placed in cellulose dialysis tubing (VWR Scientific) to remove CsCl salt by dialysis against 1 X SSC (0.15 M NaCl : 0.15 M Na citrate, pH 7.0) for three days at 12° C with three changes of SSC.

Contaminating RNA in the DNA solution was removed by RNase digestion: 0.125 mg RNase per ml of dialyzed DNA solution was incubated for one hour in a 37° C water bath.

RNase and other contaminating proteins were removed from the DNA solution by extraction with phenol. Equal volumes of the RNase-treated DNA and a 1:1 mixture of phenol (water saturated and neutralized with an equal volume of 0.1 M Tris, pH 9.0) and chloroform were thoroughly mixed (by inversion) in a Corex tube and the phases were separated by centrifugation at 2000 x g for 10 minutes. The DNA, in the top aqueous layer, was transferred to a clean Corex tube. Potassium acetate (3 M) was added to a final concentration of 0.2 M to facilitate DNA precipitation in ethanol. Two volumes of cold absolute ethanol were added to each tube, solutions mixed, and DNA was allowed to precipitate overnight at -20° C. The DNA was pelleted at 10,000 rpm using a JA-20 rotor in a Beckman J2-21 centrifuge at -20° C for 60 minutes. The ethanol was decanted from the tube and the DNA pellet was dissolved in 1.5 ml of TE buffer (0.01 M Tris in 1.0 mM EDTA) and dialyzed in cellulose dialysis tubing against three changes of TE buffer to remove ethanol. After dialysis the DNA solution was stored at 4° C.

Alternatively, DNA was isolated following the addition of potassium acetate by gently layering two volumes of cold absolute ethanol over the DNA solution. The tip of a pasture pipette was placed just below the interface between the DNA solution and the alcohol. The pipette was moved in a circular motion around the tube with increasing speed. When the top layer turned from clear to white and back to clear, the pipette was twirled down to the bottom of the tube several times until all DNA had adhered to the pipette. The DNA on the pipette was rinsed in 95% cold ethanol to remove any excess salt and was allowed to air dry. The air dried DNA was rehydrated in 1.5 ml of 1X TE and stored at 4° C.

The quality and quantity of the DNA solution was determined from the OD_{260}/OD_{280} readings. OD_{260}/OD_{280} values between 1.7 and 1.9 were acceptable. The concentration of DNA in the solution was determined by the following calculation: one OD_{260} unit is approximately equivalent to 50 ug DNA, so that 50 x OD_{260} x dilution = concentration of DNA (ug).

Rapid small scale isolation of DNA

Tissues were previously frozen at -70° C. Approximately 75 mg of splenic tissue was placed in 375 ul 2X disruption buffer (100 mM Tris pH 8.0: 200 mM EDTA: 200mM NaCl) and 300 ul water. The spleen was disrupted by gently pushing the tissue through a 1 cc tuberculin syringe (without needle) against the bottom of an Eppendorf tube. Membranes, proteins, and RNA were disrupted by adding SDS (10%), proteinase K (50 mg/ml, BRL), and RNase (2 mg/ml), to give final concentrations of 1%, 127 ug/ml, and 50 ug/ul, respectively. The cell suspension was incubated at 65° C overnight.

Proteins were removed by extracting with equal volumes of phenol, phenol/chloroform, and chloroform. A volume of 750 ul of phenol (saturated with STE (TE + 100 mM NaCL)) was added to the solution, vortexed for 10 minutes, and spun at 10,000 rpm in a table top Eppendorf centrifuge for 15 minutes. The top aqueous layer was removed and placed in a clean Eppendorf tube, leaving the interface behind. The DNA solution was extracted with 750 ul PCA (24 phenol: 24 chloroform: 1 isoamyl alcohol), vortexed for 10 minutes, and spun in the Eppendorf centrifuge for 10 minutes at 10,000 rpm. The top aqueous layer was removed, placed in a clean Eppendorf tube, 750 ul chloroform was added, vortexed for 10 minutes, and spun as above for 10 minutes. The clear aqueous layer was removed and placed in a clean Eppendorf tube.

DNA was removed from the solution by spooling it onto a glass rod in the presence of ethanol. One end of a 25 ul glass micropipette was flamed shut and cooled. One volume of absolute ethanol was slowly added to the DNA solution. The closed end of the glass micropipette was slowly twirled just below the interface. The micropipette was worked around the interface with increasing speed. When the top phase went from clear to white then back to clear the micropipette was twirled down to the bottom of the Eppendorf tube several times. The DNA on the glass micropipette was rinsed in 95% cold ethanol to remove excess salt. The rod was inverted and the alcohol was allowed to evaporate in air for 5-10 minutes. The glass micropipette with the DNA was then inverted into a clean tube containing 150 to 250 ul TE (depending on yield of DNA) and 0.1 ug/ml RNase. The dissolved DNA solutions were stored at -20° C.

Restriction Endonuclease Digestion of Genomic DNA

High-molecular-weight DNAs from the mouse tissues were digested to completion under conditions recommended by the supplier of the restriction endonucleases (BRL). Isolated genomic DNA was placed in a clean Eppendorf tube containing 2.5 or 3.5 ul BRL REact 2 buffer, 2.5 or 3.5 ul 0.02 mg/ml RNase, and water to bring the volume to 25 or 35 ul, respectively. A total of 25 units of PvuII (BRL), PstI (BRL), or PvuII and PstI were added to 5 ug DNA for routine analysis of viral structure and copy number. A total of 35 units of PvuII, PstI, BamHI, HindIII, or XbaI (BRL) was added to 10 ug of DNA for determination of restriction maps. The reaction mixture was incubated overnight at 37° C. If the DNA was also to be digested with SalI or EcoRI the reaction tube was placed at 68° C for 10 minutes to inactivate the enzyme, 3.5 ul of 0.5 M Nacl was added to bring the salt concentration to 100 mM, and 17.5 units of SalI or EcoRI (BRL) were added and the reaction mixture placed at 37° C for an additional 5-7 hours.

Electrophoretic Separation of Restriction Endonuclease Digested DNA Fragments in Agarose Gel

Agarose gel electrophoresis was used to separate the endonuclease digested DNA fragments by size. A 0.7-0.8% agarose gel made in TBE buffer (0.089 M Tris-borate : 0.089 M boric acid : 0.002 M EDTA) was poured in a 11 x 14 cm gel tray (BRL model H3) and allowed to cool. Five volumes of digested DNA were mixed with one volume of 6X gel loading buffer (0.25% bromophenol blue : 15% Ficoll type 400 in H_2O) and the DNA samples were heated at 68° C for 10 minutes before being loaded onto the gel. Size markers (Lambda cut with HindIII, BRL) were also loaded onto the gel. Horizontal gel electrophoresis was done at a constant 45 volts in TBE buffer overnight at 4° C.

Visualization of DNA by Staining with Ethidium Bromide

Following electrophoresis, the fluorescent dye ethidium bromide was used to visualize the DNA. The gels were placed in a glass pan, marked for orientation, and immersed in water. Ethidium bromide (10 mg/ml : BRL) was added to a final concentration of

0.5 ug/ml. The gel was allowed to stain for 15 minutes and DNA was visualized under UV light. A photograph was taken of the gel using a yellow photographic filter and type 52 film (Polaroid).

Transfer of DNA from Agarose Gel to Nitrocellulose Paper

The protocol used for the transfer of DNA from agarose gel to nitrocellulose paper was adapted from Maniatis et al. (1982). To improve the efficiency of transfer of high-molecular-weight fragments to the nitrocellulose paper, the DNA in the gel was depurinated with two 170 ml washes of 0.25 M hydrochloric acid during a 15 minute period. After a brief rinse in distilled water, the DNA contained in the gel was denatured for 45 minutes in two 170 ml washes of 0.5 N NaOH in the presence of 1.5 M NaCl. The gel was briefly rinsed before it was neutralized for 45 minutes in two 170 ml washes of 1 M Tris.Cl (pH 8.0) in 1.5 M NaCl. Three pieces of Whatman 3MM paper were wrapped around a piece of glass. The wrapped support was placed inside a large baking dish that contained 20X SSC. The paper was allowed to completely wet and all air bubbles were removed by rolling them to the edge with a pipette. The gel was inverted and placed on the damp 3MM paper. Air bubbles between the gel and the 3MM paper were removed. The very edges of the gel were wrapped in plastic wrap to ensure efficient blotting through the gel. An 11 x 14 cm nitrocellulose filter (Schliecher and Schuell, Frankfort) was soaked in 2 X SSC and placed on top of the gel. All air bubbles were removed. Two sheets of Whatman 3MM paper, cut to the same size as the gel, were soaked in 2X SSC and placed above the nitrocellulose. A 5 - 8 cm high stack of paper towels was placed on top of the 3MM papers. The paper towels were weighted down with a 500 g weight. Approximately 500 - 1000 ml of 20X SSC was placed in the tray and, by capillary action, was drawn up through the paper stack; during this process the DNA was transferred from the agarose gel to the nitrocellulose filter. After approximately 48 hours the nitrocellulose was removed, marked for orientation, soaked for 5 minutes in 6X SSC to remove any agarose debris, and allowed to air dry. The agarose gel was restained to confirm transfer of DNA from the gel. The nitrocellulose paper was wrapped

loosely in aluminum foil and baked at 80° C for two hours *in vacuo*.

Ecotropic Specific DNA Probe

The probe used to identify ecotropic viral DNA sequences in the mouse genomic DNA was a 400 base pair *Sma*I-*Sma*I fragment derived from the 5' end of the Akv *env* gene (Chattopadhyay et al., 1980). The probe was originally obtained from Eva Eicher, Bar Harbor. The 400 base pair *Sma*I fragment was subcloned from pBR322 into pBS(+/-), a 3 kb phagemid derived from pUC19 that contains a M13 origin of replication.

Radioactive Labeling of the Probe

The ecotropic specific probe was radioactively labeled by a modification of the method recommended by the supplier of the Klenow fragment (BRL). One half ug of probe (10 ul), 4 pmoles (8 ul) of M13 Primer (USB) and 8 ul of water were placed in a clean Eppendorf tube, heated in a boiling waterbath for 4 minutes, then allowed to cool to room temperature. The slow cooling allowed annealing of the M13 primer to the probe. After the mixture had cooled, it was placed on ice. Volumes of 11 ul of ^{32}P-dCTP (110 uCi : DuPont), 5ul 10X polymerase buffer (0.5 M TE; 0.5 M NaCl; 0.1 M MgCl), 2 ul 0.5 mM dATP (BRL), 2 ul 0.5 mM dGTP (BRL), and 2 ul 0.5 mM dTTP (BRL) were added to the cooled DNA solution to give a total volume of 48 ul. The reaction mixture was mixed and 2 ul of Klenow fragment (BRL) was added. The mixture was incubated at 25° C for approximately two hours. A measurement of ^{32}P-dCTP incorporation was made by TCA precipitation of a 2 ul aliquot of the reaction and a specific activity of 2-4 x 10^7 cpm/ug was usually obtained. Unincorporated nucleotides were removed by precipitating the DNA. The reaction mixture volume was diluted to 150 ul with TE, and 75 ul of 7.5M ammonium acetate, 5 ug of sonicated salmon sperm carrier DNA, and 2 volumes of cold ethanol were added. The mixture was placed at -70° C for 15 minutes and spun in a table top Eppendorf centrifuge for 10 minutes to pellet the DNA. The ethanol was poured off and the pellet was rinsed in 95% cold ethanol and dissolved in 150 ul TE. The DNA was reprecipitated by adding 75 ul 7.5 M ammonium acetate and 450 ul of ethanol, then freezing the mixture at

-70° C for 15 minutes, centrifuging the DNA and decanting the ethanol. After the second precipitation the DNA was dissolved in 100 ul of TE and stored at -20° C. The labeled probe was used within 5 days.

Hybridization of Genomic DNA on Southern Blot

Techniques for the hybridization of genomic DNA on Southern blot are outlined in Maniatis et al. (1982). The nitrocellulose blot was prepared for hybridization by soaking the filter in 6X SSC for 5 minutes and placing it in a heat sealable plastic bag (Scotchpak Kapak 4.5 mil) with 0.2 ml prehybridization solution per cm^2 nitrocellulose. The hybridization solution consisted of 5X SSC , 0.1% Ficoll, 0.1% polyvinylpyrrolidone (PVP), 0.1% bovine serum albumin (BSA), 0.50 ug/ml sheared, single-stranded salmon sperm DNA and 0.1% SDS. The nitrocellulose was incubated in the prehybridization mixture in a 65° C water bath with constant agitation. After two hours the prehybridization buffer was removed, hybridization buffer (50 ul/cm^2 of 5X SSC, 0.1% Ficoll, 0.1% PVP, 0.1% BSA, 0.50 ug/ml sheared, single-stranded salmon sperm DNA, 0.5% SDS, 10% dextran sulfate, and 0.05 ug radioactive probe) were added, the bag was sealed, and the nitrocellulose was incubated at 65° C with constant agitation overnight.

Nonspecifically bound probe was removed from the nitrocellulose filter by washing. The filter was removed from the hybridization solution and immediately placed in a tray containing 2X SSC and 0.05% SDS at room temperature. After 5 minutes, the filter was transferred to a fresh tray containing 2X SSC and 0.01% SDS. The filter was washed for 15 minutes at room temperature with gentle agitation. The filter was then transferred to a flat-bottom plastic box containing a solution of 0.1X SSC and 0.5% SDS and washed in a 65° C waterbath for 2 hours with gentle agitation. The buffer was changed and the filter was washed for an additional 5 minutes.

Autoradiography

The wet nitrocellulose filter was wrapped in plastic wrap and placed in a cassette with Kodak X-Omat AR X-ray film and X-Omatic intensifying screens for

12 - 96 hours at -70° C before developing the autoradiogram in a Kodak X-OMAT M20 film processor.

VITA

Amanda Suzanne Carr was born in Somerville, New Jersey on June 3, 1960. She was educated in the public school system of Bridgewater New Jersey and was graduated from Bridgewater Raritan High School East in June 1978. The following September she entered Beaver College in Glenside Pennsylvania where she studied for two years. In September of 1980 she transferred to Rutgers University where she received a Bachelor of Science Degree in Biology in 1982. In the Fall of 1982 she became a graduate student at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and received the Doctor of Philosophy degree in August 1989.

The author is a member of Phi Kappa Phi Honor Society and is presently seeking employment.