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Retrovirus Genes in Lymphocyte Function and Growth

Edited by E. Wecker and I. Horak

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With 8 Figures

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Preface

All but one* of the following articles represent comprehensive reports on a workshop held between 7 and 9 May 1981 at the Institute of Virology and Immunobiology, University of Würzburg, Federal Republic of Germany. The title of the workshop was "The Involvement of Endogenous Retroviruses in Normal Function and Pathological Growth of Lymphocytes."

Rather than collecting and printing manuscripts of the individual communications, the organizers asked selected participants to write, after the workshop, concise articles each comprising several contributions and discussions on major topics. In so doing, we hope to present to a larger audience a synopsis of the various information and views exchanged at the meeting.

Such a procedure seemed the more appropriate as the workshop was intended to bring together specialists from two rather diverse fields: RNA-tumor virology and immunobiology. While this created some initial problems of terminology, it was quite effective in making representatives of one field more aware of the significance and the contributions of the other. It also greatly contributed to realization of the complexity of the problems involved in virus-induced leukemogenesis.

Of course, the point of departure in such an enterprise had to be and indeed was a discussion of the viruses involved. Two sessions were devoted to this subject:

1. Classes of endogenous viruses and their origin (W. Rowe, H. Robinson, R. Mural, D. Steffen)

2. Structure of integrated retroviral genomes and their possible biological effects (P. Starlinger, G. Vande Woude, H. Robinson, R. Jaenisch, U. Rapp, H. Beug)

The recent findings of long terminal repeats (LTRs) at either end of all integrated proviruses have to be regarded as being of great significance and as having potential consequences. These sequences structurally resemble transposable elements and demonstrably possess promoter activity. Examples were given which strongly suggested that the insertion alone of such a viral promoter upstream of a critical onc-gene suffices to render the

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* Fleissner/Snyder, Oncoviral Proteins as Cellular Antigens

host cell malignantly transformed. The significance of retroviral genomes in malignant transformation may thus be reduced to the constitutive and nonregulated expression of (an) onc-gene(s), either cellular or viral, by virtue of a viral promoter.

Endogenous proviruses also possess LTRs at either end. Yet the expression of endogenous virus genes seems to be predominantly under negative cellular control which is released under certain conditions of cellular activation. This was borne out in session 3, "The expression of endogenous viral antigens" (E. Wecker, C. Moroni, B. Asjö, H.C. Morse III, E. Fleissner, M. Halpern). Depending on mouse strains, mitogenic or allogenic stimulation can lead to the production of infectious or defective endogenous C-type virus particles by lymphocytes. Moreover, the expression of viral envelope glycoproteins seems to be an universal marker of all mitogen- or antigen-activated lymphocytes in all mouse strains so far investigated. In some chicken strains, expression of viral envelope glycoprotein is also greatly increased by antigenic stimulation, as shown with B-lymphocytes. In other words, cells of the lymphoid origin display a striking correlation between cellular activation and activation of endogenous proviruses.

Lymphoid cells are also the prime target cells for malignant transformation by many retroviruses. Both virus replication and/or virus-dependent cellular transformation depend, however, on cell growth. Proliferation and differentiation of lymphocyte clones are, at the same time, basic features of every immune response. In addition, the host organism demonstrably reacts immunologically to retroviral antigens. The immune system and its cells thus seem to be rather centrally involved at several levels in the interaction between host organism and retroviruses which may finally lead to leukemia.

These aspects were discussed in the last session, "Regulation of lymphocyte proliferation and differentiation: effects of retroviral antigens" (A. Schimpl, J. Farrar, H. Cantor, J. Ihle, I. Weissmann, W. Schmidt, A. Coutinho). Lymphocyte proliferation and differentiation turn out to be regulated to a large extent via lymphokines. Moreover, lymphokines such as interleukin-2 provide a very useful tool in obtaining nontransformed, albeit also continuously proliferating and clonable, lymphocyte populations.

It was this last session and its very lively discussions which brought about the common realization of the remarkable complexity of events which are involved in retrovirus-related leukemias.

Regarding nonacute transforming leukemia viruses, the organizers record with pleasure that we may have caught a glimpse of a tentative and highly speculative novel view on the decisive

steps involved in these processes. Although we are fully aware of many remaining loopholes and the generally precocious nature of such an attempt, we venture to outline this vision in a severely abbreviated and oversimplified version and at our personal risk.

The envelope glycoproteins of retroviruses in particular are viral antigens which are immunologically recognized. B-lymphocytes, responding to this antigenic stimulus with proliferation, by the same token may become suitable host cells for infection and virus replication. The viral glycoprotein expressed on B-lymphocytes in association with H-2 antigens, especially Ia antigens, may lead to the activation of T-lymphocytes which respond with proliferation and production of lymphokines. These lymphokines in turn lead to the vastly enhanced proliferation (and differentiation) of other T-lymphocytes, possibly initially activated by the generally T cell mitogenic viral glycoprotein. All this would occur during the preleukemic phase, which is characterized by a strong proliferative activity of the lymphoid system, although still without any malignantly transformed cells. The final event in malignant transformation of chicken B-lymphocytes by leukosis viruses is very probably caused by the insertion of a viral promoter upstream of a critical cellular onc-gene. A similar mechanism would have to be suggested for the viral transformation of murine T-lymphocytes, although these cells have not yet been demonstrated as being truly infectable by retroviruses.

These speculations apply, of course, to exogenous, nonacute tranforming leukemia viruses. The situation with endogenous AKR-type may be similar. These viruses, in the long run, may represent the very important link between transforming viral sequences de novo introduced into a cellular genome and preexisting virus sequences which may play a role in the normal growth and/or differentiation of cells of lymphoid origin in particular.

The organizers gratefully acknowledge the readiness of all participants of the workshop to "audio et altera pars" and thereby to learn from one another. We would also like to express our thanks to all participants who, by their contributions, made this workshop an informative and successful meeting. We are particularly indebted to the colleagues who undertook the demanding task of summarizing the proceedings of the workshop in the following articles. The workshop was sponsored by the Federal Ministry of Youth, Familiy, and Health through the Cancer Committee of the Senate of the Deutsche Forschungsgemeinschaft.

Würzburg, 1981 Eberhard Wecker, Ivan Horak

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Endogenous Retroviruses of Mice and Chickens

DAVID L. STEFFEN* AND HARRIET ROBINSON*

1 Introduction

Endogenous retroviruses are retroviruses which are transmitted as proviruses in the germ line. Endogenous retroviruses are found in many animal species (*Aaronson* and *Stephenson* 1976). Some genetically transmitted proviruses lead to production of infectious virus, whereas others code for defective viruses or are unexpressed. Two species which have been intensively studied with respect to their endogenous proviral sequences are mice and chickens.

A single class of endogenous proviruses has been identified in chickens – that class being related to the avian leukosis viruses (ALVs) (*Robinson* 1978). Three to four classes of endogenous proviruses have been identified in *Mus musculus*. One class is related to the murine leukemia viruses (MLVs) (*Chattopadhyay* et al. 1974) and a second class to the mouse mammary tumor viruses (*Varmus* et al. 1972). The type A particle sequences (*Leuders* and *Kuff* 1980) represent a third class which is not related to an infectious virus of *Mus musculus*, but which is related to an infectious virus of *Mus cervicolor*. The VL30 sequences (*Keshet* et al. 1980) are not related to any known retrovirus. Their structure, however, is provirus-like, leading to the speculation that these represent a fourth class of endogenous proviruses.

This article deals predominantly with the genetically transmitted MLV proviruses of the standard American laboratory strains of mice and the genetically transmitted ALV proviruses of White Leghorn chickens. The ALV proviruses of chickens are present at

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0–5 copies per chicken genome. This relatively simple situation has allowed genetic isolation and individual characterization of these proviruses (*Astrin* et al. 1979). The MLV proviruses are present at 15 or more copies per mouse genome (*Chattopadhyay* et al. 1974), making characterization of individual proviruses much more difficult. This very complexity, however, coupled with the availability of a multitude of inbred strains of mice with well-documented histories, provides a unique resource for the study of the origins and evolution of endogenous proviruses. Thus, the endogenous ALV and MLV proviruses represent complementary systems for the study of endogenous viruses.

2 Families of Genetically Transmitted ALV and MLV Proviruses

The ALV proviruses that are genetically transmitted in White Leghorn chickens constitute one closely related family. The internal sequences of each of these proviruses has similar or identical sites for restriction endonuclease cleavage (*Hayward* et al. 1979; *Hughes* et al. 1981). Oligonucleotide fingerprints of RNAs transcribed from these proviruses reveal only 1%–2% nucleotide sequence divergene (*Conklin, Coffin* and *Robinson*, unpublished data).

In contrast, there are two or more distinct families of genetically transmitted MLV proviruses found among the standard American laboratory strains of mice. The first of these code for the endogenous ecotropic virus, AKV. AKV proviruses are present at 0–10 copies per mouse genome (*Rowe*, this meeting; *Steffen* et al. 1979; *Steffen,* this meeting).

A second class, identified by Southern blotting (*Steffen,* this meeting) and recombinant DNA technology (*Mural,* this meeting), actually consists of two closely related but distinguishable subfamilies. This family has not clearly been associated with an infectious MLV, but by restriction endonuclease mapping appears to be related to the xenotropic MLVs (*Roblin* et al., manuscript in preparation; *Chattopadhyay* et al. 1981). These two subfamilies have been provisionally named the 621-type and 14.1-type MLV proviruses. Together, these are presented at about 15 copies per mouse genome.

The endogenous MLV proviruses of mice have also been analyzed by a different approach; Southern blotting and hybridization to a series of MLV probes of differing specificities (*Rowe,* this meeting). An ecotropic virus specific probe detected 0–11 proviruses in the DNAs of different strains of mice. These presumably represent AKV proviruses. A xenotropic specific probe detected about 15 different proviruses in all strains of mice. These latter proviruses probably represent the 621/14.1 family of proviruses. A total MLV probe detected both the xenotropic and ecotropic proviruses, and perhaps a few additional proviruses. However, a probe specific for the long terminal repeats (LTRs) of the MLV provirus, which contains sequences that are highly conserved among different strains of MLV, detected 30–50 proviruses, suggesting the existence of additional families of MLV proviruses or, alternatively, the remnants of MLV proviruses left by legitimate recombination between proviral long terminal repeats (see Fig. 1, structure V).

3 The Structure of Endogenous Proviruses

Because there are relatively few copies of endogenous ALV proviruses in the DNA of any one chicken, it has been relatively easy to determine the structure of these proviruses

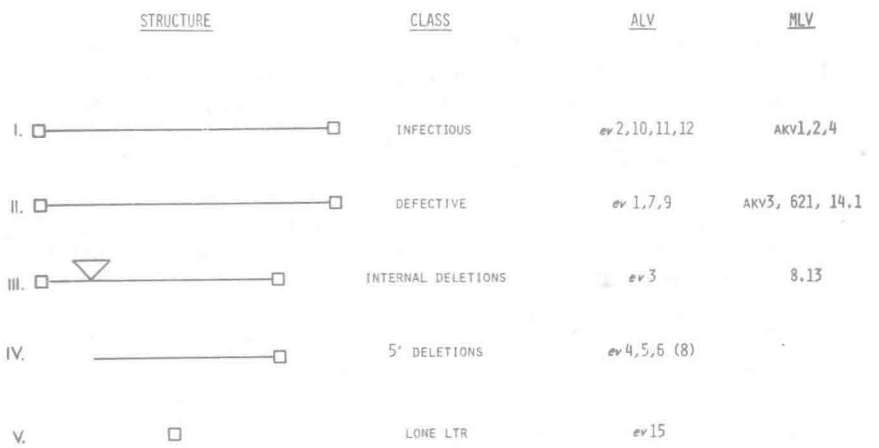

Fig. 1. Structures of endogenous ALV and MLV proviruses. Data for this figure comes from *Hughes* et al. 1981; *Mural,* this meeting; and *Steffen,* this meeting. The *lines* indicate the viral genome, the *boxes* indicate the long terminal repeats. To the *right* of each structure are listed ALV and MLV proviruses which have been shown to have the indicated structure

using restriction endonuclease digestion, the Southern technique, and cDNA probes for defined regions of the ALV genome. Consequently, the structure of a large number of such proviruses has been determined (*Hayward* et al. 1979; *Hughes* et al. 1981). These results are summarized in Fig. 1.

Because the DNA of a given mouse contains so many MLV proviruses, alternative strategies to that described above had to be developed. One approach was to isolate the viral DNA intermediates from acutely infected cells and to use this DNA to construct a restriction endonuclease cleavage map of the viral genome. This data was then used to search for specific sized fragments in mouse DNA. This approach was employed to analyze endogenous AKV proviruses (*Steffen* et al. 1979). A second approach was to use recombinant DNA technology to isolate endogenous proviruses. The 621/14.1 proviruses were so identified (*Lowy* et al. 1980; *Mural,* this meeting). The information thus derived was used to identify and analyze additional members of this provirus family (*Steffen*, this meeting). Both of the above approaches are relatively laborious; thus, relatively few endogenous MLV proviruses have been structurally analyzed. The data gathered to date is summarized in Fig. 1.

As is shown in Fig. 1, five structural classes of ALV proviruses have been identified. To date, representatives of three of these classes have been identified among endogenous MLV proviruses.

All of the proviral structures shown in Fig. 1 can be rationalized as having originated from infection. Structure I is the normal product of retrovirus infection. Structure II is the same as structure I, except that structure II proviruses do not express infectious virus. This lack of expression appears to result from mutations within the proviral genome affecting viral RNA or protein synthesis or function (*Baker* et al. 1981; *Conklin* et al., manuscript in preparation). Structure II proviruses have been observed among proviruses derived from infection (*Yoshimura* and *Yamamura* 1981). Structure III

proviruses contain internal deletions. Such deleted proviruses have also been observed among proviruses derived from infection (*Yoshimura* and *Yamamura* 1981; *Shields* et al. 1978). Structure IV proviruses have deletions of various lengths which all include the 5′ LTR. Since the 5′ LTR controls viral transcription, such proviruses are either unexpressed or under the control of cellular transcriptional control elements. This proviral structure has not been observed among proviruses derived from infection. It is possible that structure IV proviruses originated by deletion of structure I proviruses. In White Leghorn chickens, proviruses with structure IV are all found on chromosome 1, an unexplained phenomenon that may indicate that 5′ deleted proviruses have undergone amplification by a mechanism other than infection (*Tereba*, unpublished results). Structure V proviruses represent a single copy of the LTR. This structure is the expected product of legitimate recombination between the LTRs of proviruses with structures I, II, or III, which in turn presumably derived from germ line infection.

4 Site of Residence of Endogenous Proviruses in the Host Genome

Most of what is known about the sites in the host genome at which endogenous proviruses are found support the notion that these proviruses are derived from viral infections. Restriction endonuclease analysis indicates that endogenous proviruses reside at many sites in the host genome (*Steffen* and *Weinberg* 1978; *Astrin* et al. 1979). This is also observed for proviruses derived from infection (*Steffen* and *Weinberg* 1978; *Hughes* et al. 1979). Additionally, when different lineages of animals are examined, much more polymorphism is observed for proviruses than is seen for nonviral genes (*Hughes* et al. 1979), providing further support for the infectious source of endogenous proviruses. All proviruses except ALV proviruses with structure IV appear to be randomly distributed among the host chromosomes (*Jolicoer* et al. 1980; *Tereba*, unpublished results).

The strongest evidence for infection as the source of endogenous proviruses derives from *ev 1*. The cellular DNA adjacent to the *ev 1* provirus as well as DNA of the unoccupied site, derived from a chicken lacking *ev 1*, were sequenced (*Hishinuma* et al. 1980). A six nucleotide duplication of cellular sequences was found at the ends of the provirus – a feature characteristic of proviruses derived from infection.

5 Incrementation of Provirus Copy Number

A feature of the interaction between retroviruses and their hosts is that once a virus enters the germ line of an animal, the number of germ line proviruses coding for that virus can increase over time. In mice, reinfection of the germ line has been demonstrated to be the major, if not the sole, explanation for provirus incrementation.

Evidence that provirus incrementation comes from reinfection by existing endogenous viruses derives from a number of observations. First, lineages of mice that have an endogenous provirus coding for an infectious virus acquire additional proviruses over time (*Rowe* and *Kozak* 1980; *Steffen* et al., manuscript in preparation; *Herr*, manuscript in preparation). The second observation comes from comparing the spectrum of AKV proviruses genetically transmitted in different sublines of the inbred AKR strains of mouse (Fig. 2). The distribution observed is most readily explained if the sublines are ac-

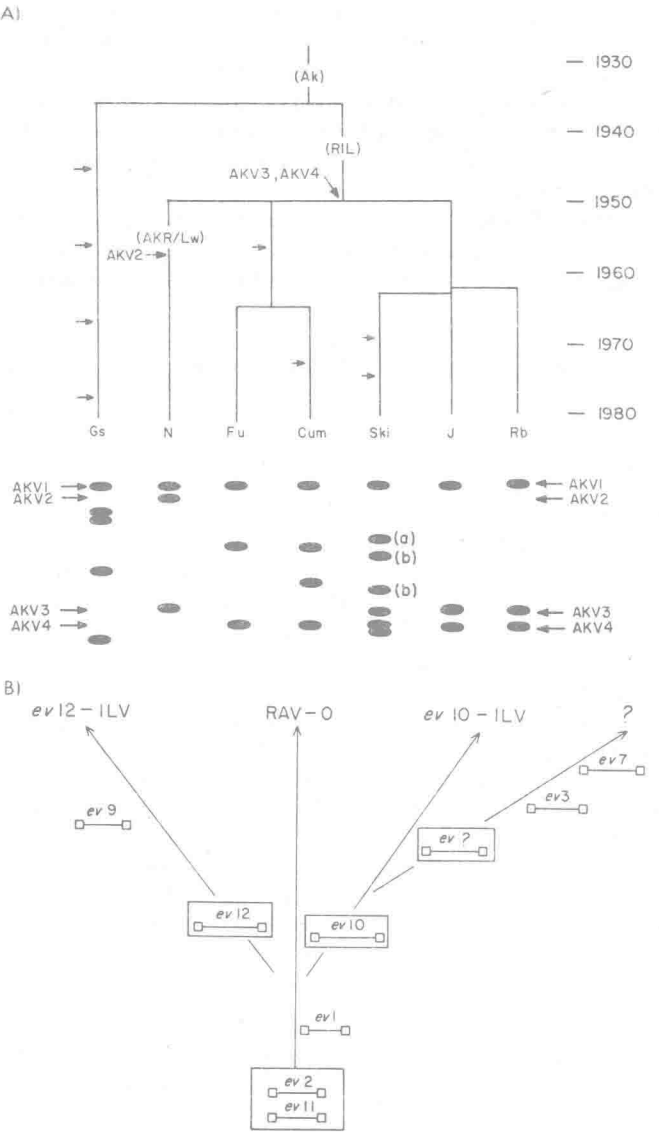

Fig. 2A, B. Genealogies of MLV and ALV proviruses. A) The genealogy of sublines of the AKR studies of mouse is shown in the *upper portion* of the figure. This is from published data (*Lynch* 1954). The *lower portion* of the figure diagrams the gel electrophoresis pattern of nuclease EcoR1-generated DNA fragments carrying AKV proviruses. Proviruses at different sites in the mouse genome are found in different sized fragments; proviruses at the same site in the mouse genome are found in the same sized fragment. Fragments labeled (*A*) or (*B*) are still segregating in the subline studied. *Arrows* at different points in the genealogy indicate when we believe AKV proviruses to have been inserted. B) This genealogy is derived from data presented by *Robinson* (this meeting). At the *top* of the figure are listed three infectious, endogenous ALVs believed to have given rise to

quiring proviruses over time. Thus, differences accumulate in sublines, beginning at the time of divergence. The role of infectious virus involvement in this process derives from comparison of viremic and nonviremic lineages of mice transmitting similar proviruses. Only the overtly viremic lineages display incrementation of provirus copy number.

A rather different sort of observation suggests that most endogenous chicken viruses have resulted from reinfection of the germ line by existing endogenous viruses. Comparison of the RNA fingerprints of the transcripts of a number of the *ev* loci reveal a small number of differences. These differences can be used to construct a genealogy of *ev* loci (Fig. 2B). If the various *ev* loci were acquired by multiple infections from a pool of horizontally transmitted viruses, one should not be able to construct such a genealogy.

6 Influences of Endogenous Proviruses on Virus Infection

Endogenous proviruses may genetically complement or recombine with an infecting virus of endogenous or exogenous origin. An example of complementation is the chf^+ phenotype coded for by the endogenous ALV proviruses *ev 3, ev 6,* and *ev 9*. These proviruses are capable of complementing a mutation in the envelope gene of avian retroviruses and thus allow growth of such mutant viruses.

Examples of recombination are found in both the avian and murine systems. The products of *ev 7* and *ev 1, ev 3,* or *ev 9* (all expressed, defective proviruses) recombine to give infectious virus. Recombination between exogenous ALVs of host ranges (subgroups) A, B, C, and D and endogenous ALV proviruses give rise to infectious, oncogenic viruses with the subgroup E host range.

Two different kinds of recombinant viruses have been identified in the murine system. Recombination between ecotropic MLVs with the $Fv\text{-}1^n$ phenotype (which determines which strains of mice the virus can infect) with endogenous xenotropic-like proviruses is believed to be responsible for ecotropic MLVs with the $Fv\text{-}1^b$ phenotype (*Benade* et al. 1978; *Robbins* et al. 1977). The second kind of recombinant also derives from ecotropic and xenotropic sequences. In this case, MCF or dualtropic viruses result (*Fischinger* et al. 1978). These viruses have the ability to infect both mouse and nonmouse cells, a host range which is the combination of the host ranges of their two parents. In addition, many MCF viruses appear to have an oncogenic potential which is greater than that of their parents (*Rowe,* this meeting).

7 Factors Regulating the Expression of Endogenous Proviruses

Regulation of the expression of endogenous proviruses which code for infectious and noninfectious viruses presents rather different problems. Although events governing initial expression of these two kinds of proviruses are presumably identical, expression of

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additional endogenous ALV proviruses by germ line infection. One additional infectious ALV whose existence is inferred, but which has not yet been found, is indicated by a *question mark*. *ev* loci in boxes code for infectious viruses, *ev* loci not in boxes code for defective viruses. This genealogy was constructed by comparing the oligonucleotide fingerprints of RNA transcripts of *ev* loci. Differences in the patterns could be explained as a series of single nucleotide changes. Changes occurring early would be shared by several *ev* loci, thus linking them in one branch of the genealogy

an infectious virus can lead to infection, integration of exogenous proviruses, and expression of these exogenous proviruses. In this section, we will consider factors that govern the initial expression of the provirus. Factors that affect virus spread as an infectious agent are discussed in the next section.

Few, if any, endogenous proviruses are expressed at high levels. Thus, the first question which arises concerns the mechanisms which limit provirus expression. The single most important factor that affects the level of expression of endogenous proviruses appears to be methylation of DNA. Growth of avian or murine cells in the presence of 5-azacytidine, a cytidine analog which is not capable of being methylated, results in 100- to 1000-fold increases in levels of virus expression (*Groudine* et al. 1981; *Conklin* et al., manuscript in preparation; *Eisenman* and *Robinson*, unpublished observations; *Hoffman* et al., manuscript in preparation). Thus, this inhibitor of DNA methylation is the most efficient known inducer for endogenous virus expression.

In contrast to proviruses that result from infection, all proviruses that pass through the germ line of an animal are heavily methylated (*R. Jaenish*, this meeting). Since proviruses that arise from infections are typically not heavily methylated, the dramatic difference in levels of expression of proviruses that are inherited from the germ line as opposed to those that arise from infection probably results from differences in levels of methylation of these two groups of viruses. What determines the state of methylation of a particular provirus is presently unknown.

Spontaneous expression of both infectious and noninfectious proviruses is very common. Often this expression is developmentally regulated. This has led to the suggestion that endogenous proviruses play a role in the normal growth and development of the host. Arguing against this hypothesis are the results of *Jaenish* (this meeting). He and his colleagues have introduced proviruses coding for the Moloney strain of MLV into the germ line of mice 13 independent times. Each of these proviruses is, as expected, present at a different site in the mouse genome. Some of these proviruses are expressed, and, quite interestingly, the expression of these proviruses is developmentally regulated. Each provirus is regulated differently. Thus, random introduction of proviruses into the germ line will lead, with relatively high efficiency, to proviruses which are developmentally regulated.

8 Host Resistance to Endogenous Viruses

Both chickens and mice exhibit resistance to infection by their endogenous viruses. One level of resistance blocks adsorption and penetration of the virus. All endogenous ALVs have the host range of subgroup E. Most chickens lack the cell surface receptor for subgroup E viruses, and are thus resistant to infection by endogenous ALVs. Similarly, all American laboratory strains of mice are resistant to xenotropic MLVs. We argued earlier that xenotropic proviruses represent a major class of endogenous MLV proviruses in mice. Thus, in both the avian and murine systems, the receptor for a major class of endogenous virus is widely distributed among closely related species, but is largely or entirely absent from the host species itself. This suggests that there has been specific evolutionary selection for loss of receptor activity for endogenous viruses.

In chickens, there are several observations which support the possibility described above. Whereas most gallinacious fowl have receptors for subgroup E viruses but not for

subgroup B viruses, most chickens have receptors for subgroup B viruses, but not for subgroup E viruses. Subgroup B and subgroup E receptors may be on the same molecule, since their genes reside at the same genetic locus and since subgroup B and subgroup E viruses exhibit cross interference. We suggest that the gene coding for subgroup E receptors in chickens is undergoing selection for loss of subgroup E virus-binding activity. This does not necessarily appear to result in loss of the receptor molecule (which may have an essential function), since loss of the ability to bind subgroup E viruses does not always result in loss of the ability to bind subgroup B viruses.

Many of those chickens which have subgroup E virus receptors are nonetheless resistant to subgroup E viruses because they carry defective endogenous proviruses that express high levels of the viral envelope glycoprotein. This protein interferes with adsorption and penetration of subgroup E virus and thus prevents infection. The protection provided by these endogenous ALV proviruses has been shown to be effective both in vitro and in vivo (*Robinson* et al. 1981). It is possible that a similar phenomenon may be partially responsible for the resistance of mice to xenotropic virus infection.

A level of resistance occurring after virus adsorption and penetration is evident in the murine system. The *Fv-1* gene of mice is, under appropriate conditions, capable of blocking infection at some point after virus penetration, although the mechanism of this resistance is unclear. Thus, the subset of mice carrying the $Fv\text{-}1^b$ allele are resistant to the endogenous ecotropic virus, AKV.

9 Conclusions

All evidence presently available supports the notion that endogenous ALV and MLV proviruses arose from infection of the germ line of the host animals. We argue that subsequent evolutionary pressure resulted in fixation of mutations, both in the viral and host genomes, that minimized deleterious effects of these viruses on their hosts. We further argue that the most evolved proviruses are the most widely distributed, have acquired the greatest number of defects, encounter the most resistance to infectious spread within their host, and are non-oncogenic. Based on these criteria, we suggest that the xenotropic MLV proviruses have undergone the most evolutionary selection, that the ALV proviruses have undergone an intermediate amount of selection, and that the endogenous ecotropic MLV (AKV) proviruses have undergone the least selection.

Developmental regulation of endogenous proviruses appears to result from the random integration of the provirus into a developmentally regulated region of the host genome. Jaenish and his colleagues have shown that newly introduced proviruses, which presumably have undergone random integrations into the genome, exhibit developmental regulation with relatively high frequency. Thus, observation of developmental regulation of endogenous proviruses cannot be taken as evidence for an essential role of this expression in normal growth and development.

The two groups of viruses considered here represent only a subset of the known endogenous retroviruses, which almost certainly represent only a subset of all the endogenous retroviruses. However, nothing that is known about other endogenous retroviruses conflicts with what is presented here. Thus, there is presently no reason to suppose that as yet unknown endogenous retroviruses will be fundamentally different from the endogenous MLV and ALV proviruses. If this is so, the interaction of endo-

genous proviruses with their host species can be best understood in terms of a host and its parasite.

Acknowledgments. This article is a summary of talks presented by W. Rowe, R. Mural, R. Jaenish, and ourselves at the conference on "The Involvement of Endogenous Retroviruses in Normal Function and Pathological Growth of Lymphocytes." Any errors in this manuscript, however, are our responsibility. D.L.S. acknowledges that the data presented by him include contributions by *S. Bird, D. Cowing, J. Ihle, J. Mielcarz, R. Mural, B. Taylor, R. Roblin, J. Young,* and *R. Weinberg.* H.R. acknowledges contributions from *J. Coffin, P. Tsichlis,* and *K. Conklin.*

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