

Chapter 1

Overview of Retrovirology

Naomi Rosenberg

Abstract In the 100 years since their discovery, retroviruses have played a special role in virology and in molecular biology. These agents have been at the center of cancer research and shaped our understanding of cell growth, differentiation and survival in ways that stretch far beyond investigations using these viruses. The discovery of retroviral oncogenes established the central paradigm that altered cellular genes can provide a dominant signal initiating cancer development. Their unique replication mechanism and their integration into cellular DNA allow these viruses to alter the properties of their hosts beyond the life span of the infected individual and contribute to the evolution of species. This same property has made retroviral vectors an important tool for gene therapy. Indeed, the impact of retrovirus research has been far-reaching and despite the amazing progress that has been made, retroviruses continue to reveal new insights into the host – pathogen interaction.

Keywords Oncogene • Endogenous virus • Retrovirus replication • Retrovirus classification • Insertional mutagenesis

Introduction

Studies of retroviruses have shaped our knowledge of cancer, development, differentiation, and gene regulation for over a century. Indeed, the impact that retrovirus research has had on modern molecular biology and oncogenesis cannot be overstated. Our knowledge of the ways in which cellular genes can be corrupted and can contribute to cancer derive their fundamental underpinnings from studies of these agents. The concept that a cellular gene can become an oncogene was validated by research conducted using retroviruses, and many genes that participate in human tumor development were first isolated as retroviral genes or targets of retroviral

N. Rosenberg (✉)
Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine,
136 Harrison Avenue, Boston, MA 02111-1800
e-mail: naomi.rosenberg@tufts.edu

insertional mutagenesis. Studies of retrovirus-mediated oncogenesis have led to broader insights as well. Perhaps better than any other virus group, retroviruses illustrate how studies directed at understanding fundamental virological mechanisms reveal insights into basic cellular process. Novel ways to disrupt normal cell function, to regulate gene expression, and to transfer genetic information from one type of nucleic acid to another have all emerged from study of these viruses.

The ability of some retroviruses to induce tumors has been known since the turn of the 20th century. In 1908, Ellerman and Bang described a chicken erythroleukemia that was caused by a retrovirus followed by isolation of Rous sarcoma virus from a chicken fibrosarcoma by Peyton Rous (Rosenberg and Jolicoeur, 1997). These discoveries marked the beginning of experimentation that led to our current understanding of retroviruses as cancer-causing agents. Subsequent studies extended the general paradigm to mammalian hosts. The discoveries of Bittner and Gross revealed that retroviruses were associated with mammary tumors and thymic lymphomas in mice. The list of animals affected by oncogenic retroviruses expanded as the 20th century progressed to include cats, cows, rats, sheep and goats, koalas, several primates, and some fish (Rosenberg and Jolicoeur, 1997). Predictably, the isolation of human T-cell leukemia virus (HTLV) marked the discovery of a retrovirus that caused malignant disease in humans (Poiesz et al., 1980). The strong tools developed for retrovirus research and associated understanding of the biology of these agents provided a strong foundation that almost certainly facilitated the isolation of human immunodeficiency virus (HIV). Although HIV is not an oncogenic virus, the critical importance of HIV to human health made retrovirus research a major national priority and has contributed to a broader understanding of all retroviruses as well as the immune response (see also chapter on Retrovirus-Induced Immunodeficiency and Cancer).

Retrovirus Structure

Retroviruses are enveloped viruses that have an irregular spherical to conical capsid (Coffin, 1992) (Fig. 1.1). The envelope contains a lipid bilayer derived from cellular membrane by the budding process, which occurs when a newly formed virus particle is released from the cell. The virus Env proteins, SU (surface) and TM (transmembrane), exist as a heterotrimer in the bilayer with the SU protein protruding from the surface of the virion. Structural proteins associated with the protein shell include CA (capsid, the major component of the shell), MA (matrix, a protein on the inner surface of the cell membrane), and NC (nucleocapsid, a protein that is condensed in the core of the particle in association with the RNA genome). The viral enzymes – protease (PR), reverse transcriptase (RT), and integrase (IN) – are also packaged in the virion. The viral genome exists as a dimer of two single-stranded positive-sense RNAs. In addition to these components, small amounts of cellular RNAs (Rulli et al., 2007) and proteins are packaged in the virion. For example, cellular tRNAs are specifically bound to viral RNAs for priming reverse transcription. Members of the APOBEC family of cellular proteins, which may affect retrovirus replication, also may be packaged (Huthoff and Towers, 2008) (see also chapter on Genetics of Host Resistance to Retroviruses and Cancer).

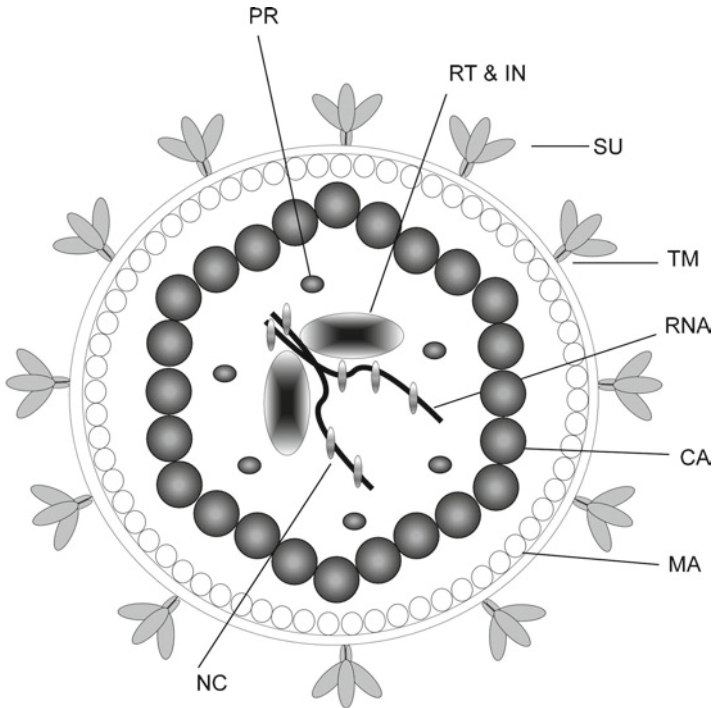


Fig. 1.1 Virion Structure. The cartoon illustrates a retrovirus virion. Virion proteins and the RNA genome are illustrated. The NC proteins completely encapsidate the packaged viral RNA. Additional information on the different proteins is found in the text. Some retrovirus particles incorporate additional cellular and host proteins. The drawing is not to scale

Retrovirus Classification

Retroviruses are members of the family *Retroviridae* and are enveloped RNA-containing viruses that utilize reverse transcription of their genome as an obligate step in virus replication (Linial et al., 2005). These viruses are further divided into two subfamilies: *Orthoretrovirinae* and *Spumaretrovirinae* (Table 1.1) based on differences in morphology, pattern of gene expression, and processing of viral proteins. *Spumaretrovirinae* virions contain a large amount of reverse transcribed DNA, a feature that is distinctive to this subfamily. All oncogenic retroviruses are members of the *Orthoretrovirinae*, which contains six genera that are distinguished based on virion morphology, genome and protein structure, and sequence relationships. Among these, only the genus *Lentivirus* lacks oncogenic members.

All retroviruses contain four genes, *gag*, *pro*, *pol*, and *env*, all of which encode proteins required for replication (Linial et al., 2005). The *gag* gene encodes virion structural proteins, the *pro* domain encodes a protease contained within the virion necessary for maturation of the virus particle, the *pol* gene encodes RT and IN (enzymes required for reverse transcription and integration of the genome), and

Table 1.1 Orthoretrovirinae Associated with Malignancies

Genus	Properties	Disease(s)	Host(s)	Representative Virus(es)
<i>Alpharetrovirus</i>	<i>gag, pol, pro, env</i> genes (some with <i>v-onc</i> genes); C-type morphology; plasma membrane assembly; endogenous and exogenous members	malignancies, wasting and osteopetrosis	chickens other birds	Avian leukosis virus Rous sarcoma virus
<i>Betaretrovirus</i>	<i>gag, pol, pro, env</i> genes (some with accessory genes; no <i>v-onc</i> genes); B- or D-type morphology; cytoplasmic assembly; endogenous and exogenous members	malignancies immunodeficiencies	mice primates sheep	Mouse mammary tumor virus Jaagsiekte sheep retrovirus Mason-Pfizer monkey virus
<i>Gammaretrovirus</i>	<i>gag, pol, pro, env</i> (some with <i>v-onc</i> genes); C-type morphology; plasma membrane assembly; endogenous and exogenous members	malignancies immunosuppression neurologic disease	mice primates guinea pigs pigs cats birds snakes	Murine leukemia virus Reticuloendotheliosis virus Abelson murine leukemia virus Feline leukemia virus
<i>Deltaretrovirus</i>	<i>gag, pol, pro, env, tax, rex, HBZ</i> ; C-type morphology; plasma membrane assembly; no endogenous members	malignancies neurologic disease	humans other primates	Bovine leukemia virus Human T-lymphotrophic virus
<i>Epsiloretrovirus</i>	<i>gag, pol, env, pro</i> , accessory genes; no endogenous members	malignancies	fish	Walleye dermal sarcoma virus
<i>Lentivirus</i>	<i>gag, pol, env, pro</i> , accessory genes; no endogenous members; distinctive morphology compared to other <i>Orthoretrovirinae</i>	immunodeficiency malignancies neurologic disease arthritis	humans other primates cats sheep goats horses	Human immunodeficiency virus Feline immunodeficiency virus Visna/maedi virus Equine infectious anemia virus

For additional information, see Linial, M. L., H. Fan, B. Hahn, R. Löwer, J. Neil, S. Quackenbush, A. Rethwilm, P. Sonigo, J. Stoye and M. Tristem. 2005. Retroviridae, p421–440. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.

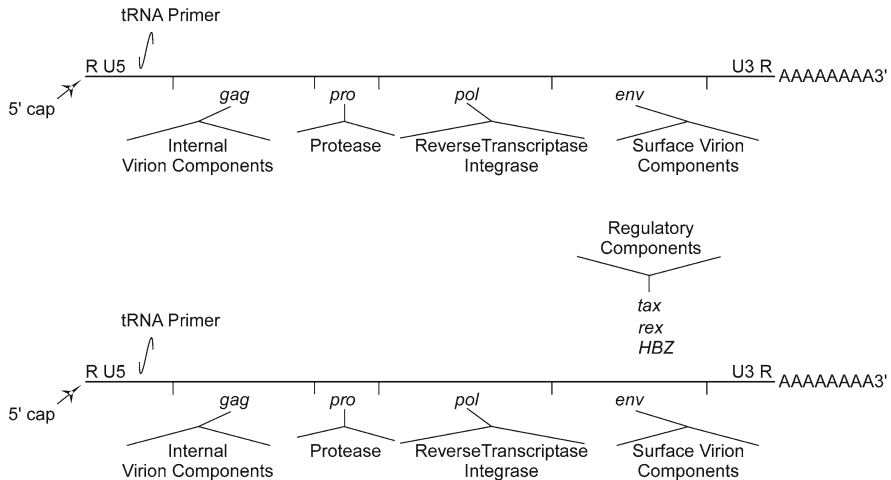


Fig. 1.2 Retrovirus Genome. The upper diagram illustrates the organization of the RNA genome of a simple retrovirus. The lower diagram shows the additional genes specified by a typical onco-genic complex retrovirus (HTLV). The cellular tRNA bound to viral RNA at the primer-binding sites varies for different retroviruses. The drawing is not to scale

the *env* gene encodes SU and TM (proteins that interact with cellular receptors and mediate entry and early steps in infection) (Fig. 1.2). Some members of the Betaretrovirus, Deltaretrovirus, Epsilonretrovirus and Lentivirus genera also contain additional genes that influence viral and host gene expression as well as viral pathogenesis. In addition, some members of the Alpharetrovirus and Gammaretrovirus genera contain oncogenes, sequences derived from normal cellular genes that have been captured and stably incorporated into the retroviral genome. With the exception of Rous sarcoma virus, all retroviruses that contain oncogenes are replication defective due to the absence of complete coding sequences for at least one of the four retrovirus genes required for replication. The overview presented here will focus on the *Orthoretrovirinae* genera that contain oncogenic viruses.

Retrovirus Replication

Infection by retroviruses begins with binding of the Env glycoprotein to a cellular receptor (Fig. 1.3). This interaction [please specify what interaction] is the major determinant of virus host range. Many receptors for retroviruses have been identified in the past fifteen years, and these molecules participate in a number of normal cellular functions. For example, the receptor for viruses like Moloney murine leukemia virus (MuLV) and related murine viruses is the cationic amino acid transporter (Kim et al., 1991), whereas the receptor for subgroup B avian leukosis

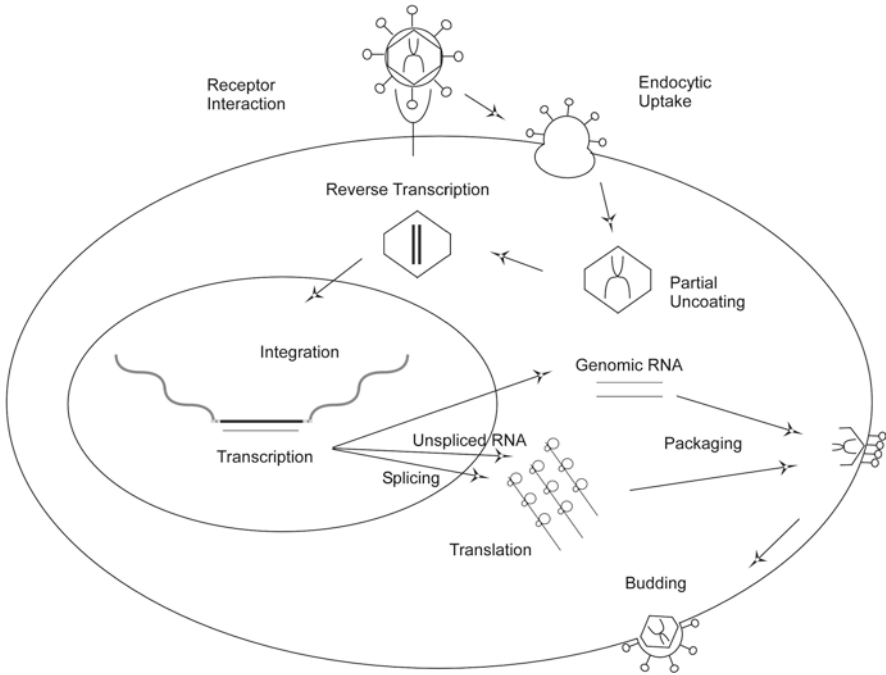


Fig. 1.3 Retrovirus Replication. Replication begins with the interaction of the retrovirus virion and the virus receptor. After entry, partial uncoating occurs to generate the PIC. Reverse transcription of the viral RNA genome generates a double-stranded DNA copy of the genome with direct repeats (LTRs) at both ends. This structure integrates randomly into the cellular DNA. Transcription and translation utilize cellular machinery. The specific site of assembly varies depending upon the specific virus genus; many retroviruses assemble at the plasma membrane as shown in the figure. Budding and release of the newly formed virion completes the replication cycle. Additional details are found in the text

viruses is a member of the tumor necrosis factor (TNF) family (Bates, Young and Varmus, 1993), and the receptor for mouse mammary tumor virus is transferrin receptor I (Ross et al., 2002). In addition to performing an array of functions, the receptors have a range of structures. For example, the subgroup A avian leukosis viruses (ALVs) use a single transmembrane spanning protein as a receptor, whereas the cationic ion transporter is a multiple transmembrane spanning molecule, as are the receptors for all known gammaretroviruses.

Virus entry involves the fusion of viral and cellular membranes, a process similar to that employed by a wide range of enveloped viruses (Barnard, Elleder and Young, 2006; Marsh and Helenius, 2006). Fusion involves juxtaposition of viral and cellular membranes through a series of conformational changes that expose a virus-encoded fusion protein. The retroviral Env proteins are class I fusion molecules similar to the fusion proteins of orthomyxo- and paramyxoviruses as well as those of filoviruses and coronaviruses. Env contains an N-terminal SU subunit that

interacts with the receptor and a C-terminal TM subunit that orchestrates membrane fusion (Barnard, Elleder and Young, 2006). Conformational changes allow interaction with cellular receptors to expose the fusion peptide at the N-terminus of TM and allow insertion of the peptide into the cellular membrane. Additional conformational changes lead to the first step of membrane fusion, called hemifusion, which is followed by further conformational alterations that complete the process and allow virus delivery into the cell.

The triggers that mediate viral entry differ depending on the retrovirus. For most, including murine gammaretroviruses like Moloney MuLV (McClure et al., 1990), MMTV (Redmond, Peters and Dickson, 1984; Ross et al., 2002), alpharetroviruses (Mothes et al., 2000) and Jaagsiekte sheep retrovirus (JSRV) (Bertrand et al., 2008), a pH-dependent step is required. The amphotrophic MuLV 10A1 (McClure et al., 1990; Nussbaum, Roop and Anderson, 1993) and HIV (McClure, Marsh and Weiss, 1988) use a pH-independent pathway. The pH-dependent pathway involves endocytic uptake, an event that follows interaction with the receptor. Exposure to low pH in the endosome initiates hemifusion. However, different viruses vary with respect to the precise details of these events. For example, alpharetroviruses can remain in a receptor-primed state for an extended period of time before the entry process is completed by low pH exposure (Barnard, Elleder and Young, 2006; Mothes et al., 2000). Dynamin, a molecule important for clathrin-mediated endocytosis, has been implicated in the entry of several of these viruses (Bertrand et al., 2008; Brindley and Maury, 2008; Lee, Zhao and Anderson, 1999), suggesting that endocytic uptake is mediated via these organelles. Lipid rafts are involved in the entry of others (Diaz-Griffero, Jackson and Brojatsch, 2005; Narayan, Barnard and Young, 2003).

Once internalized, uncoating of the virion and replication of the RNA genome begins (Goff, 2001; Suzuki and Craigie, 2007), although the details of uncoating are unknown. Reverse transcription begins at this stage and is primed by a tRNA that is packaged in the virion (Telesnitsky and Goff, 1997; Wilhelm and Wilhelm, 2001). RT has two required activities, a polymerase, which uses either RNA or DNA as a template for DNA synthesis, and a nuclease called RNase H. The polymerase copies the genome while the RNase H activity degrades RNA associated with newly synthesized DNA. Both strands of the genome are used in a process that involves two strand transfers to generate a double-stranded DNA copy (provirus) (Fig. 1.4a). Similar to replication of all RNA viruses, reverse transcription is highly error-prone (Svarovskaia et al., 2003). The enzyme lacks a proof-reading function, and the strand transfer mechanism critical to copying of the genome can be imprecise, resulting in deletions or sequence duplications. This replication process is of central importance to the generation of retroviruses containing oncogenes and also facilitates variations in SU and the LTR, a feature critical for leukemogenesis of some viruses that lack oncogenes.

Completion of reverse transcription generates 5' and 3' direct repeats known as long terminal repeats (LTRs) (Telesnitsky and Goff, 1997; Wilhelm and Wilhelm, 2001). This structure contains U3 sequences derived from a unique region at the 3' end of the RNA genome, R sequences repeated at both the 3' and 5' ends of the RNA

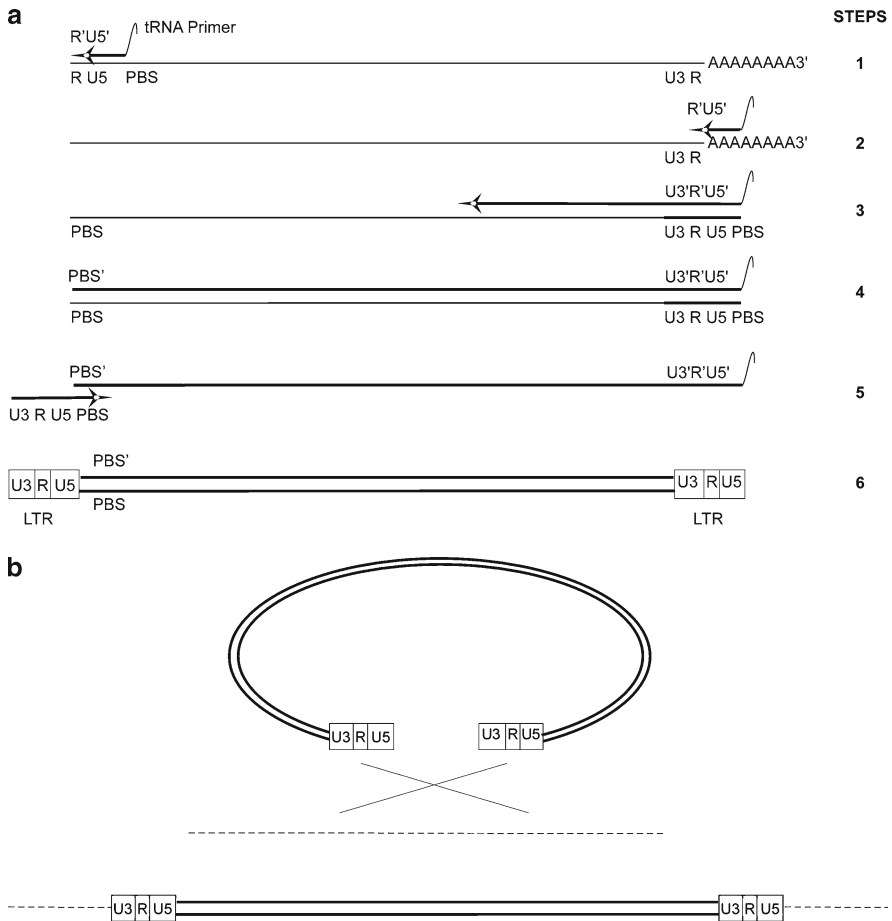


Fig. 1.4 (a) Reverse Transcription. Reverse transcription generates a double-stranded DNA copy of the viral genome and involves six steps: 1. synthesis of minus strand DNA using tRNA as a primer from the primer binding site (PBS) to the 5' end of the RNA genome; 2. transfer of the newly synthesized DNA to the 3' end of the RNA and continued synthesis of the minus strand; 3. synthesis of a small plus strand using RNase H-resistant RNA fragments as a primer; 4. completion of minus-strand synthesis; 5. transfer of the short plus-strand DNA to the 5' end; 6. completion of plus-strand synthesis, leading to the synthesis of LTRs at both ends of the genome. Arrows indicate the direction of synthesis. **(b) Integration.** The newly synthesized double-stranded linear DNA integrates into the cellular genome using the viral IN protein. Integration is random with respect to cellular sequences, but precise with respect to virus sequences. Integrase mediates the initial steps of the integration reaction; cellular repair molecules complete the integration process

genome, and U5 sequences derived from unique sequences at the 5' end of the genome (Fig. 1.4b). The proviral DNA is associated with some capsid derived proteins and IN in the pre-integration complex (PIC) (Goff, 2001; Suzuki and Craigie, 2007). Although PIC composition is not identical or even characterized for all types of retroviruses, entry of viral DNA into the nucleus and its integration into the host

genome requires this structure. Although not yet fully understood, integration of the provirus is essential for subsequent virus gene expression.

The double-stranded linear form of the provirus is the substrate for integration into the host DNA, an event that is mediated by IN (Brown, 1997; Katz et al., 1990). Integration is specific with respect to retrovirus sequences, and inverted repeats at the ends of the LTR are required. Integrase first removes two (or in some instances three) bases from the ends of the LTRs to expose a 3'OH on each end of the linear double-stranded DNA. The exposed OH groups attack phosphodiester bonds on the cellular DNA in a staggered fashion, with four to six bp (depending on the virus) separating the points of attack on each of the cellular DNA strands. The viral sequences are then joined to the cellular sequences in a transesterification reaction using the energy generated by breakage of the phosphodiester bonds (Bushman, Fujiwara and Craigie, 1990; Craigie, Fujiwara and Bushman, 1990; Engelman, Mizuuchi and Craigie, 1991). Cellular enzymes complete the integration reaction to generate a direct repeat of cellular sequences flanking the integration site. The length of this repeat is characteristic of the virus and reflects the spacing of the bases involved in the transesterification reaction. These events ensure that integration is specific with respect to viral sequences and that the integrated provirus preserves the order of elements in the retrovirus genome (Brown, 1997).

In contrast to the specificity with respect to viral sequences, integration into host sequences is influenced by sequence and structural features that exhibit only weak specificity at the nucleotide level (Bushman et al., 2005). Different types of virus have a propensity to integrate in particular types of sequences. For example, gammaretroviruses tend to integrate near the promoter regions of transcriptionally active genes (Hematti et al., 2004; Wu et al., 2003), whereas lentiviruses tend to integrate into transcription units without a preference for introns or exons (Mitchell et al., 2004; Schroder et al., 2002). In contrast, alpharetroviruses integrate in a more random fashion (Barr et al., 2005; Mitchell et al., 2004; Narezkina et al., 2004). The features that control these differences are not fully understood, but both IN and Gag play a role (Lewinski et al., 2006). In addition, cellular factors that promote the interaction of the PIC with chromatin are important (Bushman et al., 2005). For example, LEDGF/p75 (Engelman and Cherepanov, 2008), a member of the hepatoma-derived growth factor family, has been implicated in targeting integration to regions transcription units (Ciuffi et al., 2005). Other features that may influence targeting include chromatin structure and cell-cycle differences (Bushman et al., 2005). This latter idea originates in the observation that gammaretrovirus PICs and those produced by most other retroviruses typically enter the nucleus during mitosis, whereas lentivirus PICs can enter the nucleus in the absence of cell division (Suzuki and Craigie, 2007). Promoter trap assays that monitor activation of a promoterless marker gene inserted into a retrovirus gene is monitored reveal that these genes are activated more frequently in the setting of MuLV compared to HIV (De Palma et al., 2005). These data suggest that integration near promoters is important for full expression of MuLV viral genes, and that this feature influenced the evolution of viral integration patterns.

Integration is an obligate step that is required for expression of viral proteins by the *Orthoretrovirinae*. Expression of these sequences is mediated by host cell machinery using sequences within the LTR to guide and regulate the process

(Rabson and Graves, 1997). RNA polymerase II and cellular factors mediate transcriptional initiation at the first base of R. Polyadenylation of the transcripts requires cis-acting sequences in the 3' LTR. Enhancer sequences located primarily in the U3 region of the 5' LTR are recognized by host transcription factors and regulate levels of proviral expression. Such interactions affect not only virus replication, but also have a strong impact on pathogenicity, influencing both the ability of the virus to induce disease as well as the target cell. For example, these differences influence the oncogenicity of viruses derived from *Akv1* such as the SL3 series of MULVs (Lenz et al., 1984; Morrison, Soni and Lenz, 1995) and affect the erythroid cell tropism of Friend MULV (Chatis et al., 1983).

Transcription of the provirus generates a minimum of two mRNAs. The unspliced RNA encodes the Gag, Pro and Pol proteins, and also serves as the genome (Rabson and Graves, 1997). A singly spliced *env* mRNA contains 5' sequences identical to those in the unspliced RNA upstream of a donor splice site in the untranslated region and is joined to a downstream acceptor to eliminate the *pol* gene. For viruses that contain oncogenes, the strategy of expression generally reflects the genome structure and the relationship of the oncogene to viral sequences. Some viral oncogenes are expressed from unspliced mRNA, whereas others use spliced mRNAs, often using the same signals that generate the *env* mRNA.

Some retroviruses of the Betaretrovirus, Deltaretrovirus and Epsilonretrovirus genera encode additional proteins that are expressed from spliced mRNAs. The functions of these molecules vary and range from regulatory molecules that affect viral and cellular gene expression to molecules that stimulate immune cells. MMTV expresses the Sag protein, a superantigen that stimulates immune cells and is important for transmission of infection in host animals (Acha-Orbea and MacDonald, 1995; Ross, 2008). This virus also encodes Rem, a molecule that influences export of unspliced MMTV RNAs from the nucleus (Indik et al., 2005; Mertz et al., 2005) as well as virus expression (Mertz, Lozano and Dudley, 2009). HTLV expresses the regulatory proteins Tax, Rex, and HBZ. Tax interacts with cellular transcription factors, including CREB, to increase HTLV transcription and functions in a similar fashion to influence expression of a number of cellular genes involved in cell proliferation, migration, cell cycle, apoptosis, and transformation (Giam and Jeang, 2007; Legros et al., 2009; Wycuff and Marriott, 2005). Rex facilitates the nuclear export and expression of incompletely spliced viral RNAs leading to increased virus replication (Legros et al., 2009; Younis and Green, 2005), whereas HBZ is expressed from an anti-sense transcript and influences pathogenicity (Matsuoka and Green, 2009; Satou et al., 2006) (see also chapter on Retroviral Regulatory/Accessory Genes). Walleye dermal sarcoma virus (WDSV), an Epsilonretrovirus, encodes several additional proteins, including a viral cyclin (Holzschu, Lapierre and Lairmore, 2003), but the expression mechanisms have not been completely elucidated (see also chapter on Cancers Induced by Piscine Retroviruses).

Viral proteins are translated on cellular ribosomes, primarily by a cap-dependent mechanism (Bolinger and Boris-Lawrie, 2009; Rabson and Graves, 1997; Swanstrom and Wills, 1997). However, some evidence for IRES-mediated mechanisms has been presented for several viruses including RSV (Deffaud and Darlix, 2000) and MULV

(Berlioz and Darlix, 1995; Vagner et al., 1995). Gag, Pro and Pol are produced from a genome-length transcript using several strategies. Most retroviruses use one or two ribosomal frameshifts to produce Pro and Pol (Falk et al., 1993; Jacks and Varmus, 1985; Mador, Panet and Honigman, 1989), yet some viruses, including MuLV and FeLV use termination codon readthrough (Hatfield et al., 1992; Yoshinaka et al., 1985a, b). Ribosome frameshifting involves ribosome pausing produced by a combination of repeat or “shifty” sequences and stable secondary structure that causes a change in reading frame. Termination codon readthrough in MuLV involves the interaction of virally encoded RT and eRF1 (Orlova et al., 2003). This interaction enhances readthrough and allows self-regulation of RT production. These mechanisms are critical for production of different optimal amounts of the various viral proteins from a single genomic mRNA (Swanstrom and Wills, 1997).

For all Orthoretrovirinae except Betaretroviruses, the virion proteins assemble at the plasma membrane using Gag proteins in a cytoplasmic complex after interaction with Env and cellular proteins associated with the membrane. Betaretroviruses assemble in the cytoplasm. Packaging sequences (ψ) usually located near the 5' end of the viral genome, are required for insertion of the genome into the nascent virion (D'Souza and Summers, 2005; Mann, Mulligan and Baltimore, 1983). Packaging signals do not share a common primary sequence; their function appears to be mediated by a complex structure adopted during the packaging process. The NC portion of the Gag polyprotein, in concert with the Ψ sequences, select the sequences that will be inserted into the nascent virion (Berkowitz et al., 1995; Zhang and Barklis, 1995). The genome is packaged as a dimer containing two copies of the RNA genome and dimerization, which is influenced by RNA structure and the chaperone activity of NC, is also important for packaging (Greatorex, 2004; Housset et al., 1993; Paillart et al., 2004). Viruses that contain mutations limiting or abolishing dimerization display reduced infectivity or are non-infectious. The newly formed virus buds from the cell, leaving the host cell intact. Depending on the virus, final processing steps mediated by Pro occur as the virion buds from the cell or soon thereafter, producing the mature virion (Swanstrom and Wills, 1997). The absence of virally-mediated cell lysis by most retroviruses and failure of the immune system to recognize infected cells may ensure life-long persistence that promotes viral oncogenesis (see also chapter on Immune Response to Oncogenic Retroviruses).

Retrovirus Replication and Effects on the Host

The retrovirus replication strategy allows virus propagation, but aspects of the life cycle, especially the ability of the virus to integrate and to associate its DNA permanently with the host cell, have a major impact on the outcome of infection. This feature [specify what feature] is linked to the ability of many retroviruses to induce tumors as well as their ability to alter host cell gene expression. The intimate and stable association with the cellular genome allows retroviral elements to influence the genetic composition of host cells and, in many cases, the

evolution of entire species. Furthermore, because integration preserves the structure of the viral genes and because infectious retroviruses can be produced after all gene products are supplied in trans, these viruses have been useful as vectors for gene therapy. These and other unique features of the replication cycle explain the far-reaching impacts of retroviral research on eukaryotic molecular biology and cell biology.

Endogenous Viruses

As noted above, a unique feature of retrovirus replication is the obligate requirement for integration into the host genome for efficient expression. Integrated proviral DNA remains a stable part of the cellular genome. Even though reverse transcription is an error-prone process with high potential for alteration of the provirus, integration fixes its position and sequence since further replication occurs through the cellular machinery. If a germ cell is infected, then the retrovirus becomes permanently part of the genetic makeup of offspring resulting from that cell. Although germline infections are rare, such insertions have occurred many times throughout mammalian evolution, indicating that retroviral insertions are stable and ubiquitous. These viruses, referred to as endogenous viruses, become normal components of the genome (Jern and Coffin, 2008; Stoye, 2001) (see also chapter on Endogenous Retroviruses and Cancer).

Because of their stability, endogenous viruses have been used as genetic markers to determine evolutionary relationships. Despite this inherent stability, endogenous virus LTRs, which are direct repeats, have been particularly susceptible to recombination events. While such recombination events are extremely rare in the lifetime of individual cells, evolution has produced many events that result in the deletion of all retroviral sequences except a single or “solo” LTR (Hughes and Coffin, 2001). For example, human endogenous retroviruses comprise about 8% of the human genome, but most of these sequences represent solo LTRs (Jern and Coffin, 2008). Nonetheless, as illustrated by their large number in the human genome, such sequences contribute greatly to the overall genetic makeup of an organism.

Although many endogenous viruses have been silenced by mutations occurring in the millions of years since germline introduction, some remain active or are activated under certain circumstances. Many endogenous viruses, such as *Akv1*, a mouse endogenous gammaretrovirus, contribute by recombination to replication-competent viruses that directly cause tumor development (Coffin, Stoye and Frankel, 1989). Components of other endogenous viruses regulate susceptibility of the host to particular viruses by encoding proteins that block cellular receptors, or interfere with other steps in infection. Indeed, certain retroviral genes and their functions were identified before their association with endogenous viruses became evident (Jern and Coffin, 2008). The Sag proteins encoded by endogenous MMTVs (*Mtvs*) and the *Fv4* locus, actually an endogenous

murine gammaretrovirus, are salient examples. The open reading frame that encodes Sag was identified through sequence analysis of MMTV (Donehower, Huang and Hager, 1981) some years before the significance of the coding region was understood. The significance of the open reading frame was revealed through studies of host products that influenced the T-cell response in particular strains of mice (Abe and Hodes, 1989; Janeway et al., 1989), a property known long before the discovery of its function as a superantigen. Understanding these seemingly disparate features helped advance our understanding of MMTV transmission (Acha-Orbea and MacDonald, 1995). In a similar fashion, *Fv4* was identified as a locus that restricted infection by MuLV (Kai et al., 1976) prior to the discovery that an endogenous virus-encoded Env protein (Ikeda and Sugimura, 1989) functioned through super-infection resistance mechanisms to block the incoming virus (Taylor, Gao and Sanders, 2001).

Other Consequences of Integration

Retrovirus integration disrupts cellular sequences at the point of integration and can affect the expression of cellular genes over long distances. These changes can occur through the effects of viral enhancer sequences located primarily within the LTR or through mechanisms that allow transcription beginning in viral sequences to proceed into cellular sequences. In addition, integration affects the relationship of normal cellular regulatory and coding sequences by disrupting the positioning of these elements within a gene. All of these events may upregulate expression of cellular genes and, in rare instances when the integration occurs near a proto-oncogene, cell growth can be altered (Rosenberg and Jolicoeur, 1997). In some cases, integration can disrupt a gene, resulting in a truncated protein product that lacks regulatory sequences and functions independently of normal cellular cues. Such events are believed to be required for oncogenesis by retroviruses that lack oncogenes (see also chapter on Retroviruses as Tools to Identify Oncogenes and Tumor Suppressor Genes).

Tumor induction is not the inevitable consequence of retrovirus regulation of a neighboring gene. Some integrations result in altered gene expression in ways that do not induce disease, but influence host phenotype in other ways. One classical example is illustrated by the effects of a gibbon ape leukemia virus (GaLV)-derived endogenous virus. GaLV integrated into an early primate that produced Old World monkeys and great apes. As a consequence, these animals express pancreatic amylase gene in the parotid gland (Samuelson et al., 1990; Ting et al., 1992), a modification that may have influenced the preference for starchy foods displayed by these primates and their relatives today.

Although upregulation of cellular gene expression is one consequence of retroviral integration, these events can occasionally result in loss of gene expression. Loss of expression, like activation, is often associated with oncogenesis caused by infection with retroviruses that lack oncogenes. Because of their relatively random nature, integrations that inactivate gene expression typically affect only one of two

copies of a somatic cell gene. Thus, many inactivation events have minimal consequences to the cell compared to gene activations. Nevertheless, integrations resulting in haploinsufficiency of genes that encode tumor suppressors have been reported. For example, integration into the gene encoding the p53 tumor suppressor has been documented in Friend MuLV-induced tumors (Ben-David and Bernstein, 1991; Ben-David et al., 1988). Integrations affecting *NFI*, a gene that encodes a Ras pathway regulatory protein (GAP) also have been reported (Buchberg et al., 1990; Cho et al., 1995). Similar to the situation with gene activation, integrations that lead to loss of gene expression are not invariably associated with tumor induction. Furthermore, an endogenous MuLV integrated in the *Hrs* locus of HRS/J mice is responsible for disrupting the gene, causing the hairless phenotype of these mice (Stoye et al., 1988).

The products of endogenous viruses also affect host function and development. A particularly striking example is syncytin-1 and syncytin-2, molecules involved in placenta morphogenesis (Mi et al., 2000). These molecules are products of the endogenous *Env* genes; syncytin-1 is encoded by the *env* gene of the ERVWE1 human endogenous retrovirus (HERV-W) locus and syncytin-2 is encoded by HERV-FRD (Blaise et al., 2003; Mallet et al., 2004; Mangeney et al., 2007). Syncytins are specifically expressed by trophoblasts, cells that form the boundary between the mother and the fetus in the placenta. These proteins are important for fusion of cytotrophoblasts, an important step in the development of the placenta. The molecule also has immunosuppressive properties that may allow immunologic tolerance of the placenta, a structure that expresses both maternal and fetal antigens (Mangeney et al., 2007).

The ability of retroviruses to integrate stably and express genes other than those encoding viral proteins led to their use as tools to manipulate gene expression and function. The ability to remove coding sequences from the retrovirus genome and maintain infectious, but non-replicating, virus particles paved the way for the development of retrovirus vectors. At the outset, experiments used retroviruses to over-express non-viral genes of interest and study their effects on cellular processes. These experiments were rapidly extended by development of retrovirus vectors designed to introduce genes that have the potential to stably correct genetic deficiencies (Thomas, Ehrhardt and Kay, 2003).

Perhaps the most striking example that highlights both the tremendous benefits and risks inherent in these vectors have been demonstrated by investigators who used gammaretroviral vectors to treat human subjects suffering from X-linked severe combined immunodeficiency (X-SCID) (Santilli et al., 2008). Individuals with this disorder fail to express a functional membrane protein called the common γ chain, a critical component of cytokine receptors required for the development of immune cells. As a consequence of the mutation, these individuals are profoundly immunodeficient and typically succumb to their disease by their teenage years or earlier (De Ravin and Malech, 2009). When gammaretroviral vectors expressing the common γ chain were introduced into these subjects, immune function was restored in dramatic fashion, a change that allowed the individuals to resume a normal lifestyle (Hacein-Bey-Abina et al., 2002). Unfortunately, five of the 20 subjects developed leukemia as

a consequence of integration properties that made the retroviral vector an attractive approach for gene transfer (Hacein-Bey-Abina et al., 2003; Howe et al., 2008; Neven et al., 2009). In four of the five affected subjects, the retroviral vector had inserted in the vicinity of the *LMO2* gene (Nam and Rabbitts, 2006). *LMO2* functions in concert with the SCL and E47 transcription factors during hematopoiesis. Thus, these tumors arose by insertional mutagenesis, a mechanism long known to be involved in tumor induction by many oncogenic retroviruses that do not encode oncogenes (see also chapter on Mechanisms of Oncogenesis by Retroviruses).

As our understanding of integration patterns of particular retroviruses has emerged, strategies using lentivirus-based vectors, which lack the propensity to integrate near promoters, are being tested. Such vectors have not yet shown the high frequency of oncogene activation that characterizes gammaretrovirus-based vectors (Cattoglio et al., 2007). In addition, strategies including the use of insulator sequences or self-inactivating (SIN) vectors that maintain expression of the “payload” gene that is designed to correct a genetic deficiency, but prevent or minimize activation of cellular genes are being explored (Howe et al., 2008; Montini et al., 2009; Montini et al., 2006; Thornhill et al., 2008). This work both builds on our deep knowledge of retrovirus replication extends this knowledge through study of the properties of different vectors.

Factors Influencing Infection

As noted earlier, the ability of retroviruses to infect cells is mediated by interactions that involve the virion protein SU and a cellular receptor (Hunter, 1997). Thus, expression of the appropriate cellular receptor is a major determinant of susceptibility to infection. For example, most murine leukemia viruses that infect mouse and other rodent species do not infect human cells, which lack proteins that function as receptors because of sequence differences. Alpharetroviruses use a variety of receptors and their ability to infect different types of chicken and other avian cells is largely controlled at the level of host cell receptor expression. Host range restrictions are important for limiting mixed infections that allow exchange of retrovirus information, creating a new virus with higher fitness or an enhanced ability to spread. Such restriction could also limit the types of cells that are susceptible to infection. The expression patterns of CD4, the receptor for HIV, control the interaction of this virus with host cells, although similar examples for oncogenic retroviruses have not been described.

Although interactions between the SU protein and the cellular receptor play a central role in determining host range, other factors can affect virus entry. Env proteins encoded by endogenous viruses can restrict infection by blocking the ability of the receptor to interact with virus encountering the surface of the cell. This phenomenon, called superinfection resistance, has been documented in mice, chickens and sheep (Jern and Coffin, 2008). As noted earlier, the *Fv4* locus, first identified as a cellular gene that restricted infection with some MuLVs (Kai et al.,

1976), was later revealed to be an endogenous provirus that retained the ability to encode an Env protein that blocks the receptor needed for MuLV infection (Ikeda and Sugimura, 1989; Taylor, Gao and Sanders, 2001). A similar phenomenon occurs in some chickens that are resistant to ALVs (Weiss, 1993).

In addition to restriction mediated by Env gene products, Gag-related molecules can also restrict infection. One example involves an endogenous JSRV that encodes a mutant Gag that prevents release of virions containing normal Gag proteins in a dominant fashion (Arnaud, Murcia and Palmarini, 2007). Association between the normal and mutant forms inhibits the normal trafficking of the function Gag protein to restrict infection. The *FvI* locus is a second example. This locus was originally identified by its ability to partially restrict replication of different types of MuLVs in mice and in tissue culture cells (Lilly, 1967). Restriction is influenced by CA sequences encoded by the viruses (Boone et al., 1988; Kozak and Chakraborti, 1996) and is not absolute. Replication is affected after reverse transcription, but before entry into the nucleus and integration (Jolicoeur and Baltimore, 1976; Pryciak and Varmus, 1992). Although the mechanism by which *FvI* orchestrates its effects remain poorly understood, the gene responsible is related to a retroviral *gag* gene with similarity to the ERV-L family of endogenous retroviruses (Best et al., 1996).

In addition to the receptor, other cellular gene products may also influence virus replication (Wolf and Goff, 2008). Some cytidine deaminases of the APOBEC family of proteins. Some members of this family interfere with replication during reverse transcription by promoting A to G mutations through deamination of cytidines to deoxyuracils (Harris and Liddament, 2004; Huthoff and Towers, 2008; Wolf and Goff, 2008). This change causes guanine to adenine substitutions when DNA is generated during reverse transcription. APOBEC proteins are incorporated into virions of some types of retroviruses when virus is released and exert their effects following infection of cells. In addition to their role in editing, these molecules may affect infection and tumorigenesis by MMTV and MuLVs using additional mechanisms (Ross, 2009).

An important mechanism by which retroviral infection is restricted relates to the cell cycle status of the cell. As noted earlier, unlike the PIC of lentiviruses, gammaretrovirus PICs and perhaps those encoded by other retroviruses, enter the nucleus much more efficiently when the nuclear membrane has broken down during the mitotic phase of the cell cycle (Suzuki and Craigie, 2007). This feature restricts infection with these viruses to dividing cells. This requirement likely has a strong influence on the phenotypes of different tumors induced by these viruses, many of which cause hematopoietic cancers. Cell replication is tightly controlled during the differentiation events that give rise to these cells; end-stage or fully differentiated cells and the earliest stem cells that give rise to hematopoietic cells are usually not in cycle, whereas many cells in intermediate stages are dividing. Intermediate stages of hematopoietic cell differentiation are more susceptible to infection and tumor development (Rosenberg and Jolicoeur, 1997).

Types of Oncogenic Viruses

Although oncogenic retroviruses belong to five different genera based on taxonomic classification, these agents can be further divided based on their ability to replicate independently (Rosenberg and Jolicoeur, 1997). Many oncogenic viruses contain a full complement of functional replication genes and induce tumors by affecting the expression of cellular genes as a consequence of integration and insertional mutagenesis. Other oncogenic retroviruses are defective for replication after recombination with cellular sequences and loss of structural genes. Viruses of this type were initially isolated as mixed stocks that contained both the replication-competent retrovirus that participated in the recombination event and the replication-defective virus. These viruses usually contain oncogenes that induce tumors rapidly because the oncogene product plays a dominant and direct role in tumor induction (see also chapter on *Deregulation of Signal Transduction Pathways by Oncogenic Retroviruses*).

Replication-Competent Viruses and Tumor Induction

Most retrovirus-induced tumors arise following infection with a replication-competent virus that expresses the four basic genes found in all retroviruses. A wide range of gammaretroviruses and alpharetroviruses have oncogenic capacity (Maeda, Fan and Yoshikai, 2008). As noted earlier, tumors arise when one of these viruses integrates in the vicinity of a cellular gene and alters the expression of that gene (Rosenberg and Jolicoeur, 1997). Insertional mutagenesis usually involves up-regulation of the cellular gene by one of several mechanisms (Mikkers and Berns, 2003; Rosenberg and Jolicoeur, 1997). The most flexible mechanism involves the effect of the LTR enhancer sequences on cellular sequences. Because enhancers are relatively independent of position with respect to transcriptional orientation and can reportedly act over >100 kilobases, proviral integration requires little precision to affect a neighboring gene. Perhaps not surprisingly, the majority of insertional mutagenesis events that activate cellular genes appear to use this mechanism.

In addition to enhancer-mediated effects, two other mechanisms increase gene expression in situations where integration has occurred upstream of the cellular gene. The first mechanism involves transcripts that readthrough the normal transcription termination and polyadenylation signals in the 3' LTR to include cellular sequences. Depending on the virus, such readthrough transcripts may represent 15% of viral RNA (Herman and Coffin, 1986). These transcripts can then be processed to yield hybrid RNAs that encode at least a portion of the cellular gene. In second mechanism, downstream transcripts are generated. These transcripts initiate in the viral 3' LTR to direct synthesis of a hybrid RNA containing flanking cellular sequence. Both of these mechanisms are well-documented but, for unknown reasons, tend to be associated with particular virus and tumor combinations. For example, readthrough transcripts

are often involved in ASLV-induced erythroleukemia (Fung et al., 1983; Mähle et al., 1988) and downstream transcripts are particularly prominent in ASLV-induced bursal lymphoma (Hayward, Neel and Astrin, 1981).

A wide range of genes are activated by retroviral insertional mutagenesis, often targeting several genes in each tumor. In most cases, insertional mutagenesis affects genes that encode proteins. However, in at least one instance involving ASLV, insertion influences expression of the *bic* locus which encodes an miRNA (Clurman and Hayward, 1989; Eis et al., 2005; Tam, Ben-Yehuda and Hayward, 1997; Tam et al., 2002). The advent of modern genomics has facilitated rapid analysis of integration sites in a large number of tumors (Du et al., 2005; Lund et al., 2002). These studies have revealed an extensive list of genes involved in growth, survival and differentiation that contribute to tumorigenesis (see also chapters on Retroviruses as Tools to Identify Oncogenes and Tumor Suppressor genes as well as Mechanisms of Oncogenesis by Retroviruses). Many of these genes are implicated in non-retroviral tumors and, like other studies of oncogenic retroviruses, their identification in retroviral models made significant contributions to our understanding of gene activation and its role in spontaneous human tumors.

Although insertional mutagenesis is a key step in tumor induction by replication-competent retroviruses, other events are also required. In several mouse model systems, the generation of recombinant viruses, with changes in both the *env* gene and the LTR, is necessary for tumorigenesis (Stoye, Moroni and Coffin, 1991). Endogenous retroviruses in the host play important roles in the recombination process. Tumorigenesis then selects for recombinant viruses with enhanced transcription and with an extended host range (see also chapters on Endogenous Retroviruses and Cancer as well as Emerging Oncogenic Retroviruses).

Replication-Defective Viruses that Lack Oncogenes

Although the majority of retrovirus-induced tumors involve replication-competent viruses, a number of tumors arise after infection with viruses that have lost the capacity to replicate in the absence of helper viruses. As discussed below, the majority of these retroviruses contain oncogenes that have been captured from cellular proto-oncogenes. The spleen focus-forming virus (SFFV) originally isolated in combination with Friend MuLV is the hallmark of this type of oncogenic agent (Lee et al., 2003). SFFV induced an erythroid proliferation in mice that leads to massive splenomegaly and death of the animal. The key viral gene product responsible for disease induction is a deleted Env protein that cannot function as a virion component (Kabat, 1989). This molecule interacts with the receptor for erythropoietin, a molecule expressed by erythroid precursors that normally initiates proliferative signals after binding the hormone erythropoietin (Ferro et al., 1993; Nishigaki et al., 2001; Wang et al., 1993). Indeed, although the SFFV Env protein and erythropoietin do not interact with the receptor in identical ways, the result of the interaction is the same, leading to a signaling cascade and initiation of proliferation (see also chapter on Deregulation of Signal Transduction Pathways by Oncogenic Retroviruses).

Viruses Containing Oncogenes

Nearly 100 different retrovirus isolates contain oncogenes that were derived from cellular proto-oncogenes. These genes, referred to as *v-onc* genes, are responsible for the oncogenic properties of their respective viruses (Rosenberg and Jolicoeur, 1997). These retroviruses have advanced our understanding of the ways in which altered gene expression contributes to tumor development, but they are not generally associated with naturally occurring tumors. Each agent arose in a single animal and their recognition and subsequent study provided tools to understand oncogenesis.

Consistent with the role that replication-competent retroviruses play in the generation of replication-defective agents, each of the viruses arose spontaneously in a host that is infected either naturally or in a laboratory setting with replicating retroviruses. Chickens (naturally infected with ALVs) and domestic cats (naturally infected with FeLVs) are two common sources of these viruses (Rosenberg and Jolicoeur, 1997). The importance of chickens as a food source and the role of cats as human companions enhance the chances that a retrovirus-induced tumor arising in one of these animals will be recognized by veterinarians and scientists. Mice are the third major source of these viruses and a common experimental model used to study retrovirus biology. The careful observation of disease patterns in these animals led to the identification of many oncogene-containing retroviruses. Indeed, among this group of viruses, only the primate-derived simian sarcoma virus originated in another animal.

Oncogene-containing retroviruses arose through recombination between viral and cellular sequences, a phenomenon called oncogene capture. The rarity of these events prevented study of this phenomenon in the natural setting. Nonetheless, comparisons of *c-onc* and *v-onc* structure and modeling conducted using vectors that mimic some steps in the process has suggested that oncogene capture occurs in a multi-step process (Fig. 1.5). Integration of a retrovirus upstream of an oncogene is the first step in the capture event (Telesnitsky and Goff, 1997). Because retrovirus transcription bypasses the normal stop signals in the 3'LTR as much as 15% of the time (Herman and Coffin, 1986), hybrid transcripts that contain both viral and cellular sequences are generated. Some readthrough transcripts are incorporated into nascent virions, which can then infect another cell (Swain and Coffin, 1989, 1992). Because retroviruses package two copies of their genome, a fraction of virions will contain a wild-type copy of the replication-competent virus and a copy of the hybrid transcript. When these viruses infect another cell, template switching during reverse transcription completes the "recombination" that was initiated by readthrough transcription to incorporate the cellular sequences into the viral genome. Although these events are believed to occur at an extremely low frequency, the ability of the newly acquired *v-onc* product to stimulate cell growth provides a strong selective advantage for virus-producing cells. Evidence suggests that additional mutation occurs at this early stage as the virus continues to replicate in the developing tumor (Vennstrom et al., 1994).

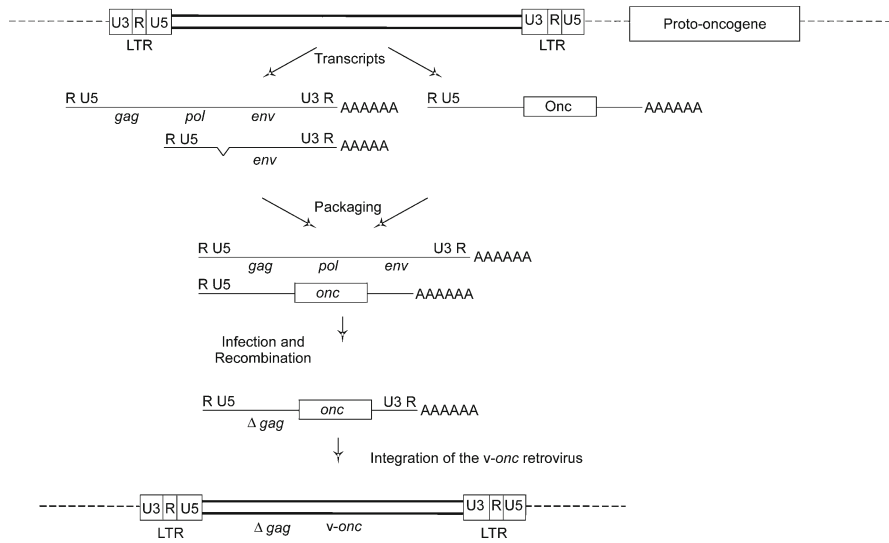


Fig. 1.5 Oncogene Capture. A model describing oncogene capture is illustrated. A retrovirus integrates near a cellular proto-oncogene. Transcription generates the expected retrovirus transcripts, but also reads through into the cellular DNA to generate a transcript containing both viral and cellular sequences. Some virions produced by this cell package a copy of the normal viral genome and a copy of the readthrough transcript. Recombination between these molecules can occur during reverse transcription when a virion carrying these two transcripts infects another cell. As illustrated, the recombination event may lead to incorporation of the proto-oncogene sequences into the retrovirus

A hallmark of *v-onc* gene containing retroviruses is their ability to induce tumors that appear rapidly (within several weeks of infection), a property immediately evident for viruses that arose in a laboratory setting. Development of tumors with unexpected phenotypes was a second hallmark displayed by many of these agents. For example, the Abelson MuLV (Ab-MuLV) was isolated from a mouse that had been infected with Moloney MuLV (Mo-MuLV) (Abelson and Rabstein, 1970), a virus that induces thymic tumors after a long latency (several months). One mouse inoculated with Mo-MuLV was treated with corticosteroids to ablate the thymus, the normal target tissue of the virus. The non-thymic tumor that developed several weeks after infection allowed the isolation of Ab-MuLV, the causative agent.

The different genes acquired by *v-onc*-containing viruses encode proteins of diverse function, yet virtually all are members of protein groups that regulate key pathways controlling cell growth and survival (Rosenberg and Jolicoeur, 1997), including growth factors, growth factor receptors, intracellular kinases, G-proteins, adaptor proteins, and transcription factors (Table 1.2). Many of these proteins and their respective signaling cascades were first discovered through the study of these retroviruses. The impact of this work on our understanding of human tumor biology cannot be underestimated.

Table 1.2 Representative *v-onc* Genes

Function	Representative Gene(s) ^a	Representative Virus(es) ^a	Host	Disease
Growth factors	<i>v-sis</i>	SSV	wooly monkey	sarcoma
		PI-FeSV	cat	sarcoma
Growth factor receptors	<i>v-fms</i> <i>v-erbB</i>	SM-FeSV	cats	sarcoma
		AEV	chickens	erythroblastosis
		HZ4-FeSV	cats	sarcoma
Non-receptor tyrosine kinases	<i>v-kit</i> <i>v-abl</i>	Abelson MULV	mice	lymphoma
		HZ-2 FeSV	cats	sarcoma
		Rous sarcoma virus	chickens	sarcoma
		GR-FeSV	chickens	sarcoma
Adaptor proteins	<i>v-src</i> <i>v-fgr</i>	ASV-1	chickens	sarcoma
		Cas NS-1	mice	lymphoma
E3 ligase	<i>v-cbl</i>	AKT8	mice	lymphoma
		Mo-MSV	mice	sarcoma
Serine/threonine kinases	<i>v-akt</i> <i>v-mos</i> <i>v-raf</i>	3611-MSV	mice	sarcoma
		Ha-MSV	rat	erythroleukemia; sarcoma
		MC-29	chickens	myelocytomatosis
G-proteins	<i>v-ras</i> [†]	AMV	chickens	myeloblastosis
		AEV-ES24	chickens	erythroblastosis
		FBJ-MSV	mice	osteosarcoma

^aRepresentative viral oncogenes and some of the viruses that carry them are listed.

Despite the wide range of encoded proteins, the *v-onc*-containing viruses induce a more restricted set of tumors compared to the spectrum of spontaneous cancers. Most tumors induced by these retroviruses have a mesenchymal origin, and many induce sarcomas. Others induce leukemias or lymphomas, tumors involving hematopoietic cells that affect cells of the B and T lymphocyte, myeloid and erythroid lineages (Rosenberg and Jolicoeur, 1997). Carcinomas, which are tumors of epithelial origin and the most common type of human cancer, are not typically associated with these viruses. Despite this variety, a particular virus is strongly associated with a specific tumor type. The mechanistic basis for this association remains poorly understood, but likely reflects properties of the *v-onc*-encoded protein as well as replication and host requirements of the virus. For example, the *v-abl* oncogene was first isolated in Ab-MuLV, an agent that induces an early B-lymphocyte tumor (Abelson and Rabstein, 1970). HZ-2 virus, which induces feline sarcomas, also contains the *v-abl* oncogene (Besmer et al., 1983a). Although the precise structure of *v-abl* differs in the two viruses, the tumor type reflects host differences because expression of the feline isolate in mice recapitulates the tumor induction pattern displayed by the murine virus. In contrast, several *v-onc* containing viruses, isolated independently from different species that carry the same oncogene induce similar types of tumors. For example, 3611 MSV (Rapp et al., 1983) and MH2 (Jansen, Patschinsky and Bister, 1983), isolated from mice and chickens, respectively, express the murine and avian homologs of the *raf* oncogene to induce sarcomas.

Viruses that contain *v-onc* genes also generally alter the growth of tissue culture cells, i.e., cause cell transformation. Infection stimulates aberrant and disorganized patterns of growth in cell monolayers of chick embryo fibroblasts or immortalized rodent cell lines, such as NIH3T3 or Rat-1 cells (Rosenberg and Jolicoeur, 1997). Many of the viruses associated with hematopoietic tumors immortalize cells that were phenotypically similar to the original tumor cells. Typically in these cases, transformation correlated with the ability to grow continuously in culture in the absence of growth factors or cytokines necessary for normal cell proliferation. Viral transforming properties proved extremely useful for identifying viruses that contain *v-onc* genes as well as the ability to clone and study the viruses before molecular approaches were available. In addition, study of the mechanisms of transformation in cultured cells revealed important insights into the function of the virus-encoded oncoproteins.

Despite the array of functions that characterize the *v-onc* products, these proteins share several common features. Their incorporation into a virus allows expression in every infected cell. Since expression of many *v-onc* genes is restricted to particular cell types or differentiation stages, aberrant expression in new cell types may prevent normal regulatory mechanisms and can lead to dramatically altered growth. The Moloney murine sarcoma virus (Mo-MSV), which contains the *v-mos* oncogene, illustrates this phenomenon. The cellular *c-mos* proto-oncogene regulates oocytes during meiosis (Wu and Kornbluth, 2008) and is not expressed in most somatic cells; high levels of virus-directed *v-mos* expression in other cell types is sufficient to transform cells (Blair et al., 1981).

Most *v-onc* genes differ in sequence from their cellular counterparts, leading to mutations that compromise normal regulatory features of the oncoproteins. Loss of regulatory domains, either through deletion or point mutation, renders these molecules constitutively active in infected cells. For example, the Ras proteins found in Harvey MSV and several other MSVs, are constitutively active because point mutations render the active, GTP-bound state of these proteins more stable (Dhar et al., 1982; Rasheed, Norman and Heidecker, 1983; Tsuchida, Ohtsubo and Ryder, 1982). In a similar fashion, the *v-Src* protein has lost C-terminal regulatory residues that modulate the tyrosine protein kinase activity associated with this protein (Cartwright et al., 1987; Kmiecik and Shalloway, 1987; Piwnica-Worms et al., 1987). Although changes in protein structure occur more commonly, loss of regulatory sequences such as those located in the 3' untranslated region of the *c-onc* gene also contribute to the oncogenic properties of *v-onc* gene products. This type of altered regulation is exemplified by the *v-fos* gene (Verma, Mitchell and Sassone-Corsi, 1986).

In addition to this type of mutations, many *v-onc* gene sequences are expressed as fusion proteins that contain portions of viral sequence. A particularly common structure involves fusion between Gag sequences and *v-onc*-encoded sequences. In many of these cases, the virus-derived sequences contribute to the function of the protein. For example, in Ab-MULV, the *v-Abl* protein is fused to Gag residues, a feature that mediates localization to the inner face of the plasma membrane and is important for transformation (Rosenberg and Witte, 1988). This situation contrasts with the normal localization of the *c-Abl* protein, which shuttles between the

cytoplasm and the nucleus in response to a variety of molecular cues. Other *v-onc* genes are fused to different portions of coding sequence and, in some instances, the same oncogene is fused to different parts of the genome in different viruses (Rosenberg and Jolicoeur, 1997). For example the *v-sis* gene is expressed as an *env* fusion in simian sarcoma virus, but expressed as a *gag* fusion in a feline sarcoma virus (Besmer et al., 1983b).

Some *v-onc* containing viruses have acquired two oncogenes. AEV-ES4, a virus that induces erythroblastosis in chickens has both the *v-erbA* and *v-erbB* genes, whereas E26-AMV, an avian myeloblastosis virus carries both *v-myb* and *v-ets*. In each case, independently isolated, oncogenic avian viruses that express only *v-myb* or only *v-erbB*, have also been isolated. However, the presence of both oncogenes affects the disease patterns observed in infected birds, reflecting the ability of the gene combination to alter growth and differentiation of the cells differently than cells infected with viruses expressing only *v-myb* or *v-erbB* (Rosenberg and Jolicoeur, 1997). These observations are similar to findings that demonstrate oncogene cooperativity after infection of oncogene-expressing transgenic mice with retroviruses that lack oncogenes or spontaneous tumors that acquire sequential mutations to allow tumor progression (see also chapter on Retroviruses as Tools to Identify Oncogenes and Tumor Suppressor Genes).

Conclusions

Retroviruses have provided tremendous insights into the growth and differentiation of eukaryotic cells and the de-regulation of these processes that occurs during cancer induction and progression. These agents allowed the identification of oncogenes, both through their incorporation into viral genomes and their effects on cellular gene expression following proviral integration into host chromosomes. The ability of retroviruses to stably affect cellular gene expression has provided both opportunities and liabilities for their use as gene therapy vectors. Their mutagenic nature also continues to serve as a source of novel agents that induce disease and the evolution of multiple animal species, including humans. These features are further explored in subsequent chapters of this volume.

Acknowledgments I am grateful to John Coffin for helpful discussions and assistance with the figures and to the NCI for support.

References

- Abe, R., and Hodes, R. J. 1989. T-cell recognition of minor lymphocyte stimulating (MIs) gene products. *Annu. Rev. Immunol.* 7:683–708.
- Abelson, H. T., and Rabstein, L. S. 1970. Lymphosarcoma: virus-induced thymic-independent disease in mice. *Cancer Res.* 30:2213–2222.

- Acha-Orbea, H., and MacDonald, H. R. 1995. Superantigens of mouse mammary tumor virus. *Annu. Rev. Immunol.* 13:459–486.
- Arnaud, F., Murcia, P. R., and Palmarini, M. 2007. Mechanisms of late restriction induced by an endogenous retrovirus. *J. Virol.* 81(20):11441–11451.
- Barnard, R. J., Elleder, D., and Young, J. A. 2006. Avian sarcoma and leukosis virus-receptor interactions: from classical genetics to novel insights into virus-cell membrane fusion. *Virology* 344(1):25–29.
- Barr, S. D., Leipzig, J., Shinn, P., et al. 2005. Integration targeting by avian sarcoma-leukosis virus and human immunodeficiency virus in the chicken genome. *J. Virol.* 79(18):12035–12044.
- Bates, P., Young, J. A., and Varmus, H. E. 1993. A receptor for subgroup A Rous sarcoma virus is related to the low density lipoprotein receptor. *Cell* 74(6):1043–1051.
- Ben-David, Y., and Bernstein, A. 1991. Friend virus-induced erythroleukemia and the multistage nature of cancer. *Cell* 66:831–834.
- Ben-David, Y., Prideaux V R Chow V, et al. 1988. Inactivation of the p53 oncogene by internal deletion or retroviral integration in erythroleukemic cell lines induced by Friend leukemia virus. *Oncogene* 3(2):179–185.
- Berkowitz, R. D., Ohagen, A., Hoglund, S., et al. 1995. Retroviral nucleocapsid domains mediate the specific recognition of genomic viral RNAs by chimeric Gag polyproteins during RNA packaging in vivo. *J. Virol.* 69(10):6445–6456.
- Berlitz, C., and Darlix, J. L. 1995. An internal ribosomal entry mechanism promotes translation of murine leukemia virus gag polyprotein precursors. *J. Virol.* 69(4):2214–2222.
- Bertrand, P., Cote, M., Zheng, Y. M., et al. 2008. Jaagsiekte sheep retrovirus utilizes a pH-dependent endocytosis pathway for entry. *J. Virol.* 82(5):2555–2559.
- Besmer, P., Hardy, W. D., Jr, Zuckerman, E. E., et al. 1983a. The Hardy-Zuckerman 2-FeSV, a new feline retrovirus with oncogene homology to Abelson-MuLV. *Nature* 303:825–828.
- Besmer, P., Snyder, H. W., Jr, Murphy, J. E., et al. 1983b. The Parodi-Irgens feline sarcoma virus and simian sarcoma virus have homologous oncogenes, but in different contexts of the viral genomes. *J. Virol.* 46(2):606–613.
- Best, S., Le Tissier, P., Towers, G., et al. 1996. Positional cloning of the mouse retrovirus restriction gene Fv1. *Nature* 382(6594):826–829.
- Blair, D. G., Oskarsson, M., Wood, T. G., et al. 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. *Science* 212(4497):941–943.
- Blaise, S., de Parseval, N., Benit, L., et al. 2003. Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. *Proc. Natl. Acad. Sci. U S A* 100(22):13013–13018.
- Bolinger, C., and Boris-Lawrie, K. 2009. Mechanisms employed by retroviruses to exploit host factors for translational control of a complicated proteome. *Retrovirology* 6:8.
- Boone, L. R., Glover, P. L., Innes, C. L., et al. 1988. Fv-1 N- and B-tropism-specific sequences in murine leukemia virus and related endogenous proviral genomes. *J. Virol.* 62(8):2644–2650.
- Brindley, M. A., and Maury, W. 2008. Equine infectious anemia virus entry occurs through clathrin-mediated endocytosis. *J. Virol.* 82(4):1628–1637.
- Brown, P. (Year) Integration. In: J. M. Coffin, S. H. Hughes and H. E. Varmus (eds). *Retroviruses*, pp. 161–203. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Buchberg, A. M., Bedigian, H. G., Jenkins, N. A., et al. 1990. Evi-2, a common integration site involved in murine myeloid leukemogenesis. *Mol. Cell Biol.* 10(9):4658–4666.
- Bushman, F., Lewinski, M., Ciuffi, A., et al. 2005. Genome-wide analysis of retroviral DNA integration. *Nat. Rev. Microbiol.* 3(11):848–858.
- Bushman, F. D., Fujiwara, T., and Craigie, R. 1990. Retroviral DNA integration directed by HIV integration protein in vitro. *Science* 249(4976):1555–1558.
- Cartwright, C. A., Eckhart, W., Simon, S., et al. 1987. Cell transformation by pp60c-src mutated in the carboxy-terminal regulatory domain. *Cell* 49(1):83–91.
- Catoggio, C., Facchini, G., Sartori, D., et al. 2007. Hot spots of retroviral integration in human CD34+ hematopoietic cells. *Blood* 110(6):1770–1778.

- Chatis, P. A., Holland, C. A., Hartley, J. W., et al. 1983. Role for the 3' end of the genome in determining disease specificity of Friend and Moloney murine leukemia viruses. *Proc. Natl. Acad. Sci. U S A* 80(14):4408–4411.
- Cho, B. C., Shaughnessy, J. D., Jr, Largaespada, D. A., et al. 1995. Frequent disruption of the Nf1 gene by a novel murine AIDS virus-related provirus in BXH-2 murine myeloid lymphomas. *J. Virol.* 69(11):7138–7146.
- Ciuffi, A., Llano, M., Poeschla, E., et al. 2005. A role for LEDGF/p75 in targeting HIV DNA integration. *Nat. Med.* 11(12):1287–1289.
- Clurman, B. E., and Hayward, W. S. 1989. Multiple proto-oncogene activations in avian leukosis virus-induced lymphomas: evidence for stage-specific events. *Mol. Cell Biol.* 9(6):2657–2664.
- Coffin, J. M. (Year) Structure and classification of retroviruses. In *The Retroviridae*, ed. J. Levy, pp. 19–50. New York: Plenum Press.
- Coffin, J. M., Stoye, J. P., and Frankel, W. N. 1989. Genetics of endogenous murine leukemia viruses. *Ann. N Y Acad. Sci.* 567:39–49.
- Craigie, R., Fujiwara, T., and Bushman, F. 1990. The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration in vitro. *Cell* 62(4):829–837.
- D'Souza, V., and Summers, M. F. 2005. How retroviruses select their genomes. *Nat. Rev. Microbiol.* 3(8):643–655.
- De Palma, M., Montini, E., Santoni de Sio, F. R. 2005. Promoter trapping reveals significant differences in integration site selection between MLV and HIV vectors in primary hematopoietic cells. *Blood* 105(6):2307–2315.
- De Ravin, S. S., and Malech, H. L. 2009. Partially corrected X-linked severe combined immunodeficiency: long-term problems and treatment options. *Immunol. Res.* 43(1–3):223–242.
- Deffaud, C., and Darlix, J. L. 2000. Characterization of an internal ribosomal entry segment in the 5' leader of murine leukemia virus env RNA. *J. Virol.* 74(2):846–850.
- Dhar, R., Ellis, R. W., Shih, T. Y., et al. 1982. Nucleotide sequence of the p21 transforming protein of Harvey murine sarcoma virus. *Science* 217(4563):934–936.
- Diaz-Griffero, F., Jackson, A. P., and Brojatsch, J. 2005. Cellular uptake of avian leukosis virus subgroup B is mediated by clathrin. *Virology* 337(1):45–54.
- Donehower, L. A., Huang, A. L., and Hager, G. L. 1981. Regulatory and coding potential of the mouse mammary tumor virus long terminal redundancy. *J. Virol.* 37(1):226–238.
- Du, Y., Spence, S. E., Jenkins, N. A., et al. 2005. Cooperating cancer-gene identification through oncogenic-retrovirus-induced insertional mutagenesis. *Blood* 106(7):2498–2505.
- Eis, P. S., Tam, W., Sun, L., et al. 2005. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc. Natl. Acad. Sci. U S A* 102(10):3627–3632.
- Engelman, A. and Cherepanov, P. (2008) The lentiviral integrase binding protein LEDGF/p75 and HIV-1 replication. *PLoS Pathog.* 4(3):e1000046.
- Engelman, A., Mizuuchi, K., and Craigie, R. 1991. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. *Cell* 67(6):1211–1221.
- Ferro, F. E., Jr, Kozak, S. L., Hoatlin, M. E., et al. 1993. Cell surface site for mitogenic interaction of erythropoietin receptors with the membrane glycoprotein encoded by Friend erythroleukemia virus. *J. Biol. Chem.* 268(8):5741–5747.
- Fung, Y. K., Lewis, W. G., Crittenden, L. B., et al. 1983. Activation of the cellular oncogene c-erbB by LTR insertion: molecular basis for induction of erythroblastosis by avian leukosis virus. *Cell* 33(2):357–368.
- Giam, C. Z., and Jeang, K. T. 2007. HTLV-1 Tax and adult T-cell leukemia. *Front. Biosci.* 12:1496–1507.
- Greatorex, J. 2004. The retroviral RNA dimer linkage: different structures may reflect different roles. *Retrovirology* 1:22.
- Hacein-Bey-Abina, S., Le Deist, F., Carlier, F., et al. 2002. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N. Engl. J. Med.* 346(16):1185–1193.

- Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., et al. 2003. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302(5644):415–419.
- Harris, R. S., and Liddament, M. T. 2004. Retroviral restriction by APOBEC proteins. *Nat. Rev. Immunol.* 4(11):868–877.
- Hatfield, D. L., Levin, J. G., Rein, A., et al. 1992. Translational suppression in retroviral gene expression. *Adv. Virus. Res.* 41:193–239.
- Hayward, W. S., Neel, B. G., and Astrin, S. M. 1981. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leucosis. *Nature* 290(5806):475–480.
- Hematti, P., Hong, B. K., Ferguson, C., et al. 2004. Distinct genomic integration of MLV and SIV vectors in primate hematopoietic stem and progenitor cells. *PLoS Biol.* 2(12):e423.
- Herman, S. A., and Coffin, J. M. 1986. Differential transcription from the long terminal repeats of integrated avian leukosis virus DNA. *J. Virol.* 60(2):497–505.
- Holzschu, D., Lapiere, L. A., and Lairmore, M. D. 2003. Comparative pathogenesis of epsilon-retroviruses. *J. Virol.* 77(23):12385–12391.
- Housset, V., De Rocquigny, H., Roques, B. P., and Darlix, J. L. 1993. Basic amino acids flanking the zinc finger of Moloney murine leukemia virus nucleocapsid protein NCp10 are critical for virus infectivity. *J. Virol.* 67(5):2537–2545.
- Howe, S. J., Mansour, M. R., Schwarzwaelder, K., et al. 2008. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J. Clin. Invest.* 118(9):3143–3150.
- Hughes, J. F., and Coffin, J. M. 2001. Evidence for genomic rearrangements mediated by human endogenous retroviruses during primate evolution. *Nat. Genet.* 29(4):487–489.
- Hunter, E. (2008) Viral entry and receptors. In J. M. Coffin, S. H. Hughes, and H. E. Varmus, pp 71–120. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Huthoff, H., and Towers, G. J. 2008. Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha. *Trends Microbiol.* 16(12):612–619.
- Ikeda, H., and Sugimura, H. 1989. Fv-4 resistance gene: a truncated endogenous murine leukemia virus with ecotropic interference properties. *J. Virol.* 63(12):5405–5412.
- Indik, S., Gunzburg, W. H., Salmons, B., et al. 2005. A novel, mouse mammary tumor virus encoded protein with Rev-like properties. *Virology* 337(1):1–6.
- Janeway, C. A., Jr, Yagi, J., Conrad, P. J., et al. 1989. T-cell responses to MIs and to bacterial proteins that mimic its behavior. *Immunol. Rev.* 107:61–88.
- Jansen, H. W., Patschinsky, T., and Bister, K. 1983. Avian oncovirus MH2: molecular cloning of proviral DNA and structural analysis of viral RNA and protein. *J. Virol.* 48(1):61–73.
- Jern, P., and Coffin, J. M. 2008. Effects of retroviruses on host genome function. *Annu. Rev. Genet.* 42:709–732.
- Jolicœur, P., and Baltimore, D. 1976. Effect of Fv-1 gene product on proviral DNA formation and integration in cells infected with murine leukemia viruses. *Proc. Natl. Acad. Sci. U S A* 73(7):2236–2240.
- Kabat, D. 1989. Molecular biology of Friend viral erythroleukemia. *Curr. Top. Microbiol. Immunol.* 148:1–42.
- Kai, K., Ikeda, H., Yuasa, Y., et al. 1976. Mouse strain resistant to N-, B-, and NB-tropic murine leukemia viruses. *J. Virol.* 20(2):436–440.
- Katz, R. A., Merkel, G., Kulkosky, J., et al. 1990. The avian retroviral IN protein is both necessary and sufficient for integrative recombination in vitro. *Cell* 63(1):87–95.
- Kim, J. W., Closs, E. I., Albritton, L. M., et al. 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. *Nature* 352(6337):725–728.
- Kmieciak, T. E., and Shalloway, D. 1987. Activation and suppression of pp60c-src transforming ability by mutation of its primary sites of tyrosine phosphorylation. *Cell* 49(1):65–73.
- Kozak, C. A., and Chakraborti, A. 1996. Single amino acid changes in the murine leukemia virus capsid protein gene define the target of Fv1 resistance. *Virology* 225(2):300–305.
- Lee, C. R., Cervi, D., Truong, A. H., et al. 2003. Friend virus-induced erythroleukemias: a unique and well-defined mouse model for the development of leukemia. *Anticancer Res.* 23(3A):2159–2166.

- Lee, S., Zhao, Y., and Anderson, W. F. 1999. Receptor-mediated Moloney murine leukemia virus entry can occur independently of the clathrin-coated-pit-mediated endocytic pathway. *J. Virol.* 73(7):5994–6005.
- Legros, S., Boxus, M., Dewulf, J. F., et al. 2009. Protein-protein interactions and gene expression regulation in HTLV-1 infected cells. *Front. Biosci.* 14:4138–4148.
- Lenz, J., Celander, D., Crowther, R. L., et al. 1984. Determination of the leukaemogenicity of a murine retrovirus by sequences within the long terminal repeat. *Nature* 308(5958):467–470.
- Lewinski, M. K., Yamashita, M., Emerman, M., et al. 2006. Retroviral DNA integration: viral and cellular determinants of target-site selection. *PLoS Pathog.* 2(6):e60.
- Lilly, F. 1967. Susceptibility to two strains of Friend leukemia virus in mice. *Science* 155(761):461–462.
- Linial, M. L., Hahn, B., et al. (Year) In *Virus taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, eds. C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball, pp. 421–440. San Diego: Elsevier Academic Press.
- Lund, A. H., Turner, G., Trubetskoy, A., et al. 2002. Genome-wide retroviral insertional tagging of genes involved in cancer in Cdkn2a-deficient mice. *Nat. Genet.* 32(1):160–165.
- Maeda, N., Fan, H., and Yoshikai, Y. 2008. Oncogenesis by retroviruses: old and new paradigms. *Rev. Med. Virol.* 18(6):387–405.
- Maihle, N. J., Raines, M. A., Flickinger, T. W., et al. 1988. Proviral insertional activation of c-erbB: differential processing of the protein products arising from two alternate transcripts. *Mol. Cell Biol.* 8(11):4868–4876.
- Mallet, F., Bouton, O., Prudhomme, S., et al. 2004. The endogenous retroviral locus ERVWE1 is a bona fide gene involved in hominoid placental physiology. *Proc. Natl. Acad. Sci. U S A* 101(6):1731–1736.
- Mangeney, M., Renard, M., Schlecht-Louf, G., et al. 2007. Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc. Natl. Acad. Sci. U S A* 104(51):20534–20539.
- Mann, R., Mulligan, R. C., and Baltimore, D. 1983. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. *Cell* 33(1):153–159.
- Marsh, M., and Helenius, A. 2006. Virus entry: open sesame. *Cell* 124(4):729–740.
- Matsuoka, M., and Green, P. L. 2009. The HBZ gene, a key player in HTLV-1 pathogenesis. *Retrovirology* 6(1):71.
- McClure, M. O., Marsh, M., and Weiss, R. A. 1988. Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. *EMBO J.* 7(2):513–518.
- McClure, M. O., Sommerfelt, M. A., Marsh, M., et al. 1990. The pH independence of mammalian retrovirus infection. *J. Gen. Virol.* 71(Pt 4):767–773.
- Mertz, J. A., Lozano, M. M., and Dudley, J. P. 2009. Rev and Rex proteins of human complex retroviruses function with the MMTV Rem-responsive element. *Retrovirology* 6:10.
- Mertz, J. A., Simper, M. S., Lozano, M. M., et al. 2005. Mouse mammary tumor virus encodes a self-regulatory RNA export protein and is a complex retrovirus. *J. Virol.* 79(23):14737–14747.
- Mi, S., Lee, X., Li, X., et al. 2000. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403(6771):785–789.
- Mikkers, H., and Berns, A. 2003. Retroviral insertional mutagenesis: tagging cancer pathways. *Adv. Cancer Res.* 88:53–99.
- Mitchell, R. S., Beitzel, B. F., Schroder, A. R., et al. 2004. Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS Biol.* 2(8):E234.
- Montini, E., Cesana, D., Schmidt, M., et al. 2009. The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *J. Clin. Invest.* 119(4):964–975.
- Montini, E., Cesana, D., Schmidt, M., et al. 2006. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. *Nat. Biotechnol.* 24(6):687–696.

- Morrison, H. L., Soni, B., and Lenz, J. 1995. Long terminal repeat enhancer core sequences in proviruses adjacent to c-myc in T-cell lymphomas induced by a murine retrovirus. *J. Virol.* 69(1):446–455.
- Mothes, W., Boeger, A. L., Narayan, S., et al. 2000. Retroviral entry mediated by receptor priming and low pH triggering of an envelope glycoprotein. *Cell* 103(4):679–689.
- Nam, C. H., and Rabbitts, T. H. 2006. The role of LMO2 in development and in T cell leukemia after chromosomal translocation or retroviral insertion. *Mol. Ther.* 13(1):15–25.
- Narayan, S., Barnard, R. J., and Young, J. A. 2003. Two retroviral entry pathways distinguished by lipid raft association of the viral receptor and differences in viral infectivity. *J. Virol.* 77(3):1977–1983.
- Narezkina, A., Taganov, K. D., Litwin, S., et al. 2004. Genome-wide analyses of avian sarcoma virus integration sites. *J. Virol.* 78(21):11656–11663.
- Neven, B., Leroy, S., Decaluwe, H., et al. 2009. Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. *Blood* 113(17):4114–4124.
- Nishigaki, K., Thompson, D., Hanson, C., et al. 2001. The envelope glycoprotein of friend spleen focus-forming virus covalently interacts with and constitutively activates a truncated form of the receptor tyrosine kinase Stk. *J. Virol.* 75(17):7893–7903.
- Nussbaum, O., Roop, A., and Anderson W F. 1993. Sequences determining the pH dependence of viral entry are distinct from the host range-determining region of the murine ecotropic and amphotropic retrovirus envelope proteins. *J. Virol.* 67(12):7402–7405.
- Orlova, M., Yueh, A., Leung, J., and Goff, S. P. 2003. Reverse transcriptase of Moloney murine leukemia virus binds to eukaryotic release factor 1 to modulate suppression of translational termination. *Cell* 115(3):319–331.
- Paillart, J. C., Shehu-Xhilaga, M., Marquet, R., et al. 2004. Dimerization of retroviral RNA genomes: an inseparable pair. *Nat. Rev. Microbiol.* 2(6):461–472.
- Piwica-Worms, H., Saunders, K. B., Roberts, T. M., et al. 1987. Tyrosine phosphorylation regulates the biochemical and biological properties of pp60c-src. *Cell* 49(1):75–82.
- Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., et al. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. U S A* 77(12):7415–7419.
- Pryciak, P. M., and Varmus, H. E. 1992. Fv-1 restriction and its effects on murine leukemia virus integration in vivo and in vitro. *J. Virol.* 66(10):5959–5966.
- Rabson, A. B., and Graves, B. J. (Year) Synthesis and Processing of Viral RNA. In *Retroviruses*, eds J. M. Coffin, S. H. Hughes, and H. E. Varnus. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Rapp, U. R., Goldsborough, M. D., Mark, G. E., et al. 1983. Structure and biological activity of v-raf, a unique oncogene transduced by a retrovirus. *Proc. Natl. Acad. Sci. U S A* 80(14):4218–4222.
- Rasheed, S., Norman, G. L., and Heidecker, G. 1983. Nucleotide sequence of the Rasheed rat sarcoma virus oncogene: new mutations. *Science* 221(4606):155–157.
- Redmond, S., Peters, G., and Dickson, C. 1984. Mouse mammary tumor virus can mediate cell fusion at reduced pH. *Virology* 133(2):393–402.
- Rosenberg, N., and Jolicoeur, P. (Year) Retrovirus Pathogenesis. In *Retroviruses*, eds J. M. Coffin, S. E. Hughes, and H. E. Varnus, pp. 475–586. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Rosenberg, N., and Witte, O. N. (Year) The viral and cellular forms of the Abelson (*abl*) oncogene. In *Advances in Virus Research*, ed. A. Shatkin, pp. 39–81. New York: Academic Press.
- Ross, S. R. 2008. MMTV infectious cycle and the contribution of virus-encoded proteins to transformation of mammary tissue. *J. Mammary Gland Biol. Neoplasia* 13(3):299–307.
- Ross, S. R. 2009. Are viruses inhibited by APOBEC3 molecules from their host species? *PLoS Pathog.* 5(4):e1000347.
- Ross, S. R., Schofield, J. J., Farr, C. J., et al. 2002. Mouse transferrin receptor 1 is the cell entry receptor for mouse mammary tumor virus. *Proc. Natl. Acad. Sci. U S A* 99(19):12386–12390.

- Rulli, S. J., Jr, Hibbert, C. S., Mirro, J., et al. 2007. Selective and nonselective packaging of cellular RNAs in retrovirus particles. *J. Virol.* 81(12):6623–6631.
- Samuelson, L. C., Wiebauer, K., Snow, C. M., et al. 1990. Retroviral and pseudogene insertion sites reveal the lineage of human salivary and pancreatic amylase genes from a single gene during primate evolution. *Mol. Cell Biol.* 10(6):2513–2520.
- Santilli, G., Thornhill, S. I., Kinnon, C., et al. 2008. Gene therapy of inherited immunodeficiencies. *Expert. Opin. Biol. Ther.* 8(4):397–407.
- Satou, Y., Yasunaga, J., Yoshida, M., et al. 2006. HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. *Proc. Natl. Acad. Sci. U S A* 103(3):720–725.
- Schroder, A. R., Shinn, P., Chen, H., et al. 2002. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 110(4):521–529.
- Stoye, J. P. 2001. Endogenous retroviruses: still active after all these years? *Curr. Biol.* 11(22):R914–916.
- Stoye, J. P., Fenner, S., Greenoak, G. E., et al. 1988. Role of endogenous retroviruses as mutagens: The hairless mutation of mice. *Cell* 54:383–391.
- Stoye, J. P., Moroni, C., and Coffin, J. M. 1991. Virological events leading to spontaneous AKR thymomas. *J. Virol.* 65(3):1273–1285.
- Suzuki, Y., and Craigie, R. 2007. The road to chromatin - nuclear entry of retroviruses. *Nat. Rev. Microbiol.* 5(3):187–196.
- Svarovskaia, E. S., Cheslock, S. R., Zhang, W. H., et al. 2003. Retroviral mutation rates and reverse transcriptase fidelity. *Front. Biosci.* 8:d117–134.
- Swain, A., and Coffin, J. M. 1989. Polyadenylation at correct sites in genome RNA is not required for retrovirus replication or genome encapsidation. *J. Virol.* 63(8):3301–3306.
- Swain, A., and Coffin, J. M. 1992. Mechanism of transduction by retroviruses. *Science* 255(5046):841–845.
- Swanstrom, R., and Wills, J. W. (Year) Synthesis, Assembly, and Processing of Viral Proteins. In *Retroviruses*, eds. J. M. Coffin, S. H. Hughes, and H. E. Varmus. Cold Spring Harbor NY: Cold Spring Harbor Laboratory Press.
- Tam, W., Ben-Yehuda, D., and Hayward, W. S. 1997. bic, a novel gene activated by proviral insertions in avian leukemia virus-induced lymphomas, is likely to function through its noncoding RNA. *Mol. Cell Biol.* 17(3):1490–1502.
- Tam, W., Hughes, S. H., Hayward, W. S., et al. 2002. Avian bic, a gene isolated from a common retroviral site in avian leukemia virus-induced lymphomas that encodes a noncoding RNA, cooperates with c-myc in lymphomagenesis and erythroleukemogenesis. *J. Virol.* 76(9):4275–4286.
- Taylor, G. M., Gao, Y., and Sanders, D. A. 2001. Fv-4: identification of the defect in Env and the mechanism of resistance to ecotropic murine leukemia virus. *J. Virol.* 75(22):11244–11248.
- Telesnitsky, A., and Goff, S. P. (Year) Reverse Transcriptase and the Generation of Retroviral DNA. In J. M. Coffin, S. H. Hughes and H. E. Varmus (eds). *Retroviruses*, pp. 121–160. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Thomas, C. E., Ehrhardt, A., and Kay, M. A. 2003. Progress and problems with the use of viral vectors for gene therapy. *Nat. Rev. Genet.* 4(5):346–358.
- Thornhill, S. I., Schambach, A., Howe, S. J., et al. 2008. Self-inactivating gammaretroviral vectors for gene therapy of X-linked severe combined immunodeficiency. *Mol. Ther.* 16(3):590–598.
- Ting, C. N., Rosenberg, M. P., Snow, C. M., et al. 1992. Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene. *Genes. Dev.* 6(8):1457–1465.
- Tsuchida, N., Ohtsubo, E., and Ryder, T. 1982. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. *Science* 217(4563):937–939.
- Vagner, S., Waysbort, A., Marena, M., et al. 1995. Alternative translation initiation of the Moloney murine leukemia virus mRNA controlled by internal ribosome entry involving the p57/PTB splicing factor. *J. Biol. Chem.* 270(35):20376–20383.

- Vennstrom, B., Raynoscheck, C., Jansson, L., et al. 1994. Retroviral capture of *c-erbB* proto-oncogene sequences: rapid evolution of distinct viral genomes carrying mutant *v-erbB* genes with different transforming capacities. *Oncogene* 9:1307–1320.
- Verma, I. M., Mitchell, R. L., and Sassone-Corsi, P. 1986. Proto-oncogene *fos*: an inducible gene. *Princess Takamatsu Symp.* 17:279–290.
- Wang, Y., Kayman, S. C., Li, J. P., et al. 1993. Erythropoietin receptor (EpoR)-dependent mitogenicity of spleen focus-forming virus correlates with viral pathogenicity and processing of env protein but not with formation of gp52-EpoR complexes in the endoplasmic reticulum. *J. Virol.* 67(3):1322–1327.
- Weiss, R. A. (Year) Cellular Receptors and Viral Glycoproteins Involved in Retrovirus Entry. In J. A. Levy (ed). *The Retroviridae*, pp. 1–108. New York, NY: Plenum.
- Wilhelm, M., and Wilhelm, F. X. 2001. Reverse transcription of retroviruses and LTR retrotransposons. *Cell Mol. Life Sci.* 58(9):1246–1262.
- Wolf, D., and Goff, S. P. 2008. Host restriction factors blocking retroviral replication. *Annu. Rev. Genet.* 42:143–163.
- Wu, X., Li, Y., Crise, B., and Burgess, S. M. 2003. Transcription start regions in the human genome are favored targets for MLV integration. *Science* 300(5626):1749–1751.
- Wycuff, D. R., and Marriott, S. J. 2005. The HTLV-I Tax oncoprotein: hyper-tasking at the molecular level. *Front. Biosci.* 10:620–642.
- Yoshinaka, Y., Katoh, I., Copeland, T. D., and Oroszlan, S. 1985a. Murine leukemia virus protease is encoded by the gag-pol gene and is synthesized through suppression of an amber termination codon. *Proc. Natl. Acad. Sci. U S A* 82(6):1618–1622.
- Yoshinaka, Y., Katoh, I., Copeland, T. D., et al. 1985b. Translational readthrough of an amber termination codon during synthesis of feline leukemia virus protease. *J. Virol.* 55(3):870–873.
- Younis, I., and Green, P. L. 2005. The human T-cell leukemia virus Rex protein. *Front. Biosci.* 10:431–445.
- Zhang, Y., and Barklis, E. 1995. Nucleocapsid protein effects on the specificity of retrovirus RNA encapsidation. *J. Virol.* 69(9):5716–5722.