Retroviruses with simple genome 2

Some retroviruses contains extra genes

"typical retrovirus"

R U5 GAG POL ENV U3 R

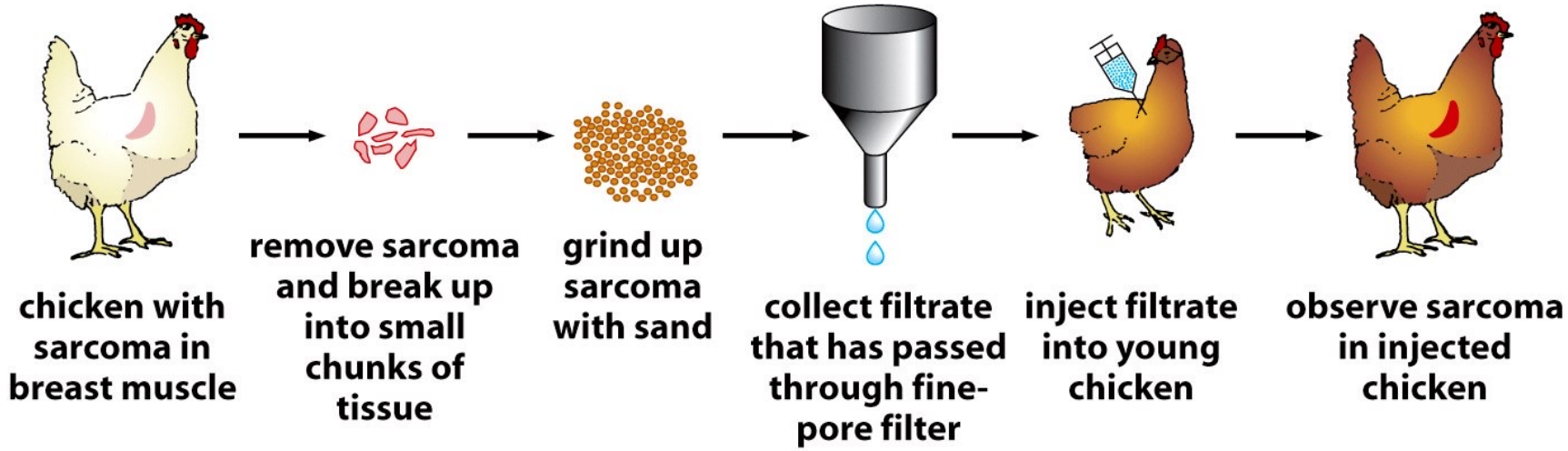
Rous Sarcoma Virus

R U5 GAG POL ENV SRC U3 R

Peyton Rous and the hen that launched modern cancer research

Francis Peyton Rous began his work in 1910 that led to the discovery of Rous sarcoma virus (RSV) (left). More than 50 years later (1966), he received the Nobel Prize in Medicine and Physiology for this seminal work—a tribute to his persistence and longevity (right).

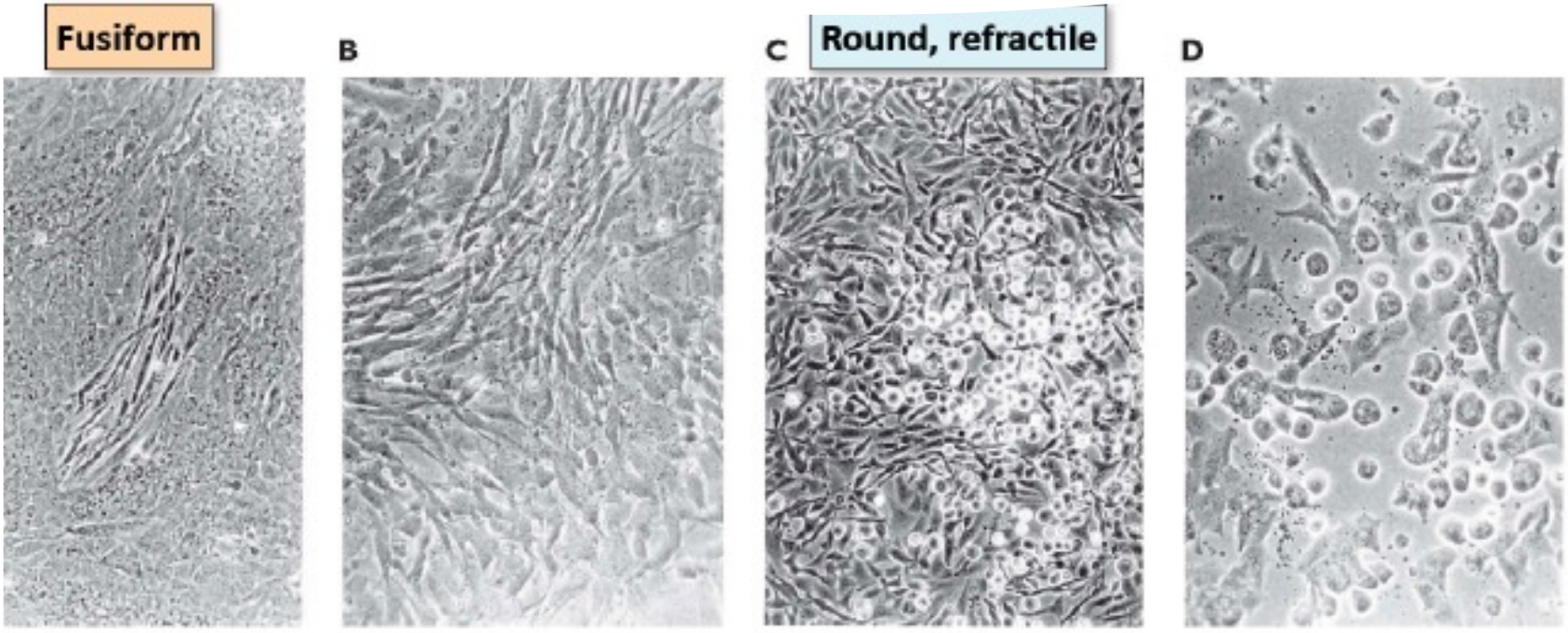
1911 Peyton Rous discovers a chicken Sarcoma Virus, called Rous Sarcoma Virus (RSV). Nobel Prize 1966



Rous's protocol for inducing sarcomas in chickens. Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus produced sarcomas far more rapidly than the original viral isolate. The molecular nature of the virus and the means by which it multiplied would remain mysteries for more than half a century after Rous's initial discovery.

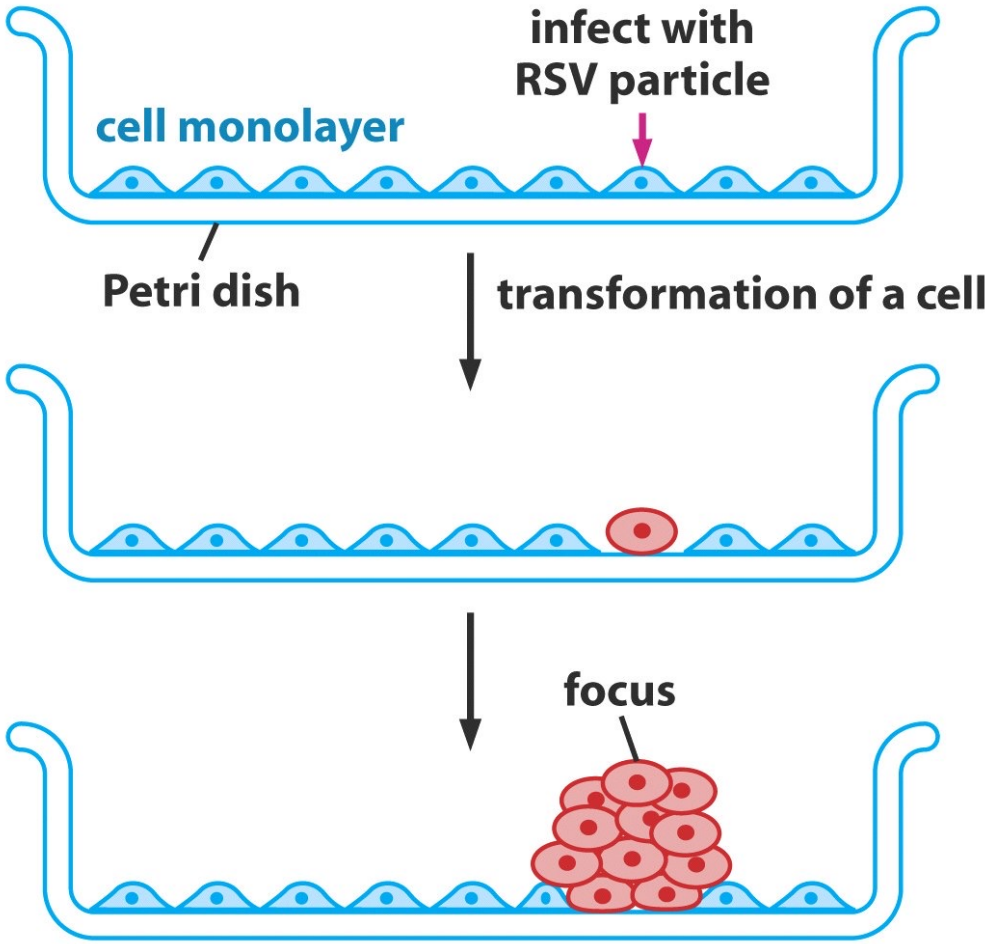
Rous sarcoma virus is discovered to transform infected cells in culture

The rebirth of Rous sarcoma virus research began largely at the California Institute of Technology in Pasadena, in the laboratory of **Renato Dulbecco**. Dulbecco's postdoctoral fellow **Harry Rubin** found that when stocks of RSV were introduced into Petri dishes carrying cultures of chicken embryo fibroblasts, **the RSV-infected cells survived, apparently indefinitely. It seemed that RSV parasitized these cells, forcing them to produce a steady stream of progeny virus particles for many days, weeks, even months.** Most other viruses, in contrast, were known to enter into host cells, multiply, and quickly kill their hosts; the multitude of progeny virus particles released from dying cells could then proceed to infect yet other susceptible cells in the vicinity, repeating the cycle of infection, multiplication, and cell destruction. Most important, the RSV-infected cells in these cultures displayed many of the traits associated with cancer cells. Thus**, foci** (clusters) of cells appeared after infection. Under the microscope, these cells strongly resembled the cells isolated from chicken sarcomas, exhibiting the characteristic rounded morphology of cancer cells.



RSV induces cell trasformation in vitro

Transformed cells forming foci
(A) Normal chicken embryo fibroblasts (blue) growing on the bottom of a Petri dish form a layer one cell thick—a monolayer. This is formed because these cells cease proliferating when they touch one another—the behavior termed "**contact inhibition**." However, if one of these cells is infected by RSV prior to reaching confluence, this cell (red) and its descendants acquire a rounded morphology and lose contact inhibition. As a consequence, they continue to proliferate in spite of touching one another and eventually accumulate in a multilayered clump of cells (**a focus**) visible to the naked eye.



The Biology of Cancer (© Garland Science 2014)

Re-infection could not explain the stable transmission of RSV genome

How could RSV succeed in transmitting its genome through many generations within a cell lineage? The genome of RSV is made of single-stranded RNA, which clearly could not be integrated directly into the chromosomal DNA of an infected cell. Still, RSV succeeded in transmitting its genetic information through many successive cycles of cell growth and division.

The transmission of RSV genomes to the descendants of initially infected cells could be explained, in principle, by a mechanism in which the virus-infected cells continually release virus particles that subsequently infect daughter cells. Such repeated cycles of reinfection can ensure that an RSV genome can be perpetuated indefinitely in a population of cells without the need for chromosomal integration of viral genetic information—the strategy used by SV40 virus. **However, stable transformation of cell populations by RSV was also observed using strains of RSV that could infect and transform a cell but were unable to replicate in that cell**. Such observations effectively ruled out re-infection as the means by which RSV perpetuated itself in lineages of cells over multiple cell generations

Retroviral genomes become integrated into the chromosomes of infected cells

This puzzle consumed **Howard Temin** in the mid- and late-1960s and caused him to propose a solution so unorthodox that it was ridiculed by many. **Temin argued that after RSV particles (and those of related viruses) infected a cell, they made double-stranded DNA (dsDNA) copies of their RNA genomes**. It was these dsDNA versions of the viral genome, he said, that became established in the chromosomal DNA of the host cell. Once established, the DNA version of the viral genome—which he called a provirus—then assumed the molecular configuration of a cellular gene and would be replicated each time the cell replicated its chromosomal DNA. In addition, the proviral DNA could then serve as a template for transcription by cellular RNA polymerase, thereby yielding RNA molecules that could be incorporated into progeny virus particles or, alternatively, could function as messenger RNA (mRNA) that was used for the synthesis of viral proteins

Evidence for a DNA intermediate in retroviral replication

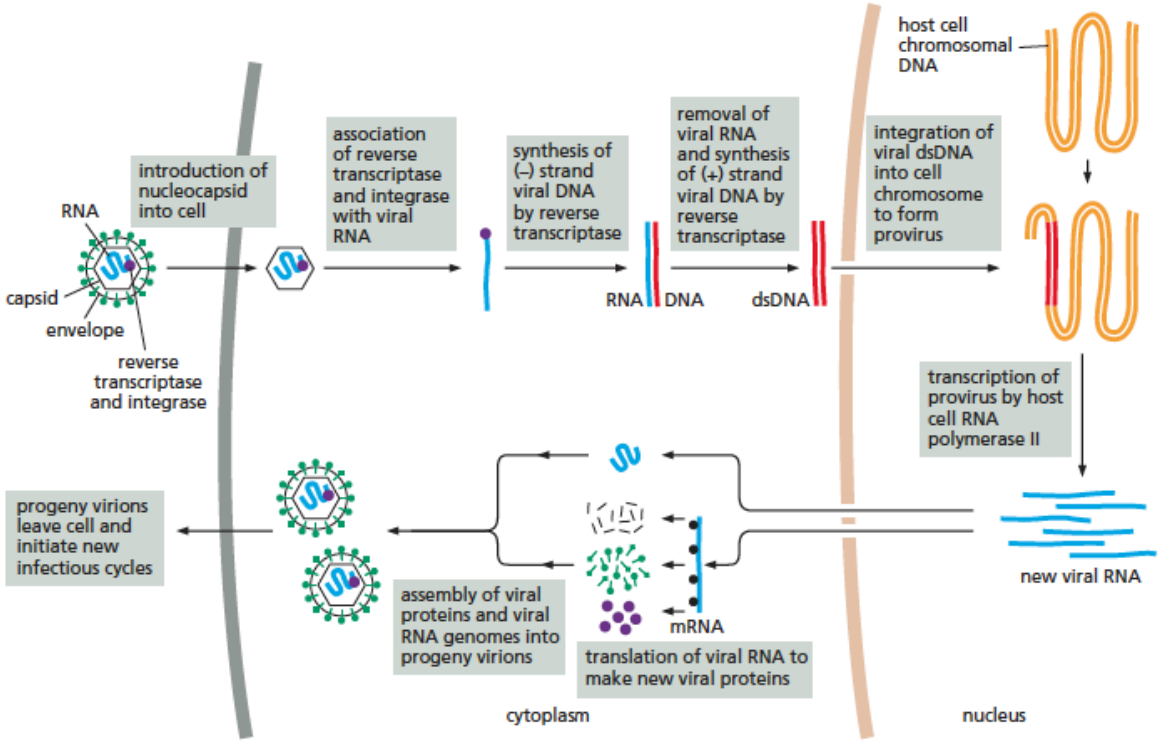
- Infection can be prevented by inhibitors of DNA synthesis added during the first 8–12 hours after exposure of the cells to the virus, but not later.

- Formation of virions is sensitive to actinomycin D, an inhibitor of host RNA polymerase II, which uses DNA as a template for RNA synthesis.

- Infection of cells by Rous sarcoma virus* confers stably inheritable changes to the cells' appearance and growth properties The details of these changes are virus-strain specific, indicating heritability of viral genetic information in the cells, a DNA property.

*Rous sarcoma virus infection is not cytolytic so the cells do not die from the infection.

1970 discovery of the reverse transcriptase, Temin e Baltimore Nobel Prize 1975



The process of reverse transcription that Temin proposed—making DNA copies of RNA—was without precedent in the molecular biology of the time, which recognized information flow only in a single direction, specifically, DNA→RNA→proteins. But the idea prevailed, receiving strong support from **Temin's and David Baltimore's simultaneous discoveries in 1970 that RSV and related virus particles carry the enzyme reverse transcriptase.** As both research groups discovered, this enzyme has the capacity to execute the key copying step that Temin had predicted—the step required in order for RSV to transmit its genome through many cycles of cell growth and division.

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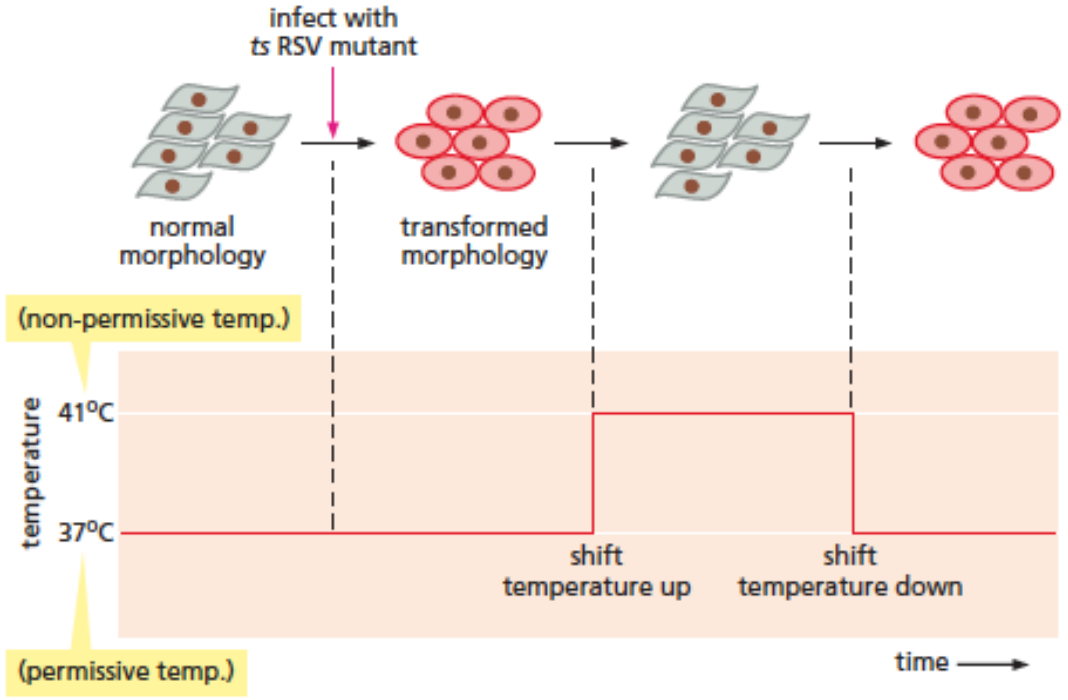
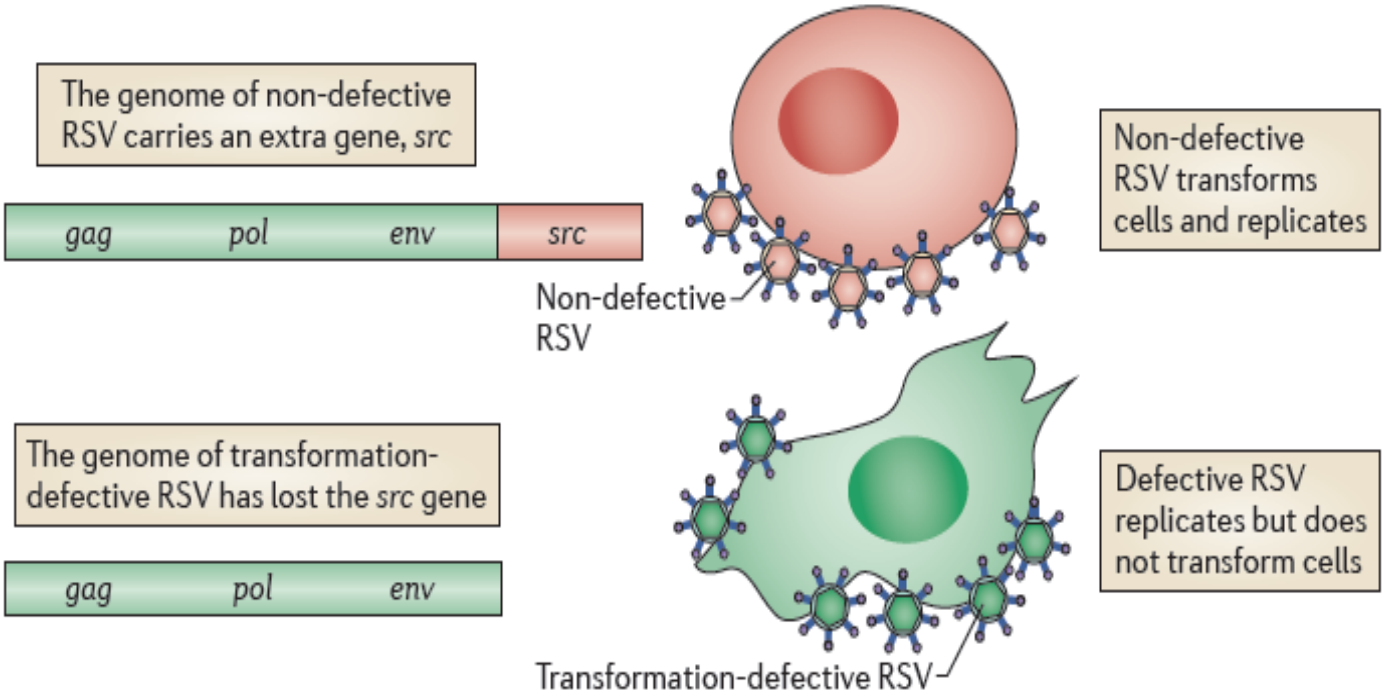
The continued presence of RSV (i.e transforming function) is needed to maintain transformed phenotype



Temperature-sensitive mutant and the maintenance of transformation by RSV When chicken embryo fibroblasts were infected with a temperature-sensitive mutant of RSV and cultured at the permissive temperature (37°C), the viral transforming function could be expressed and the cells became transformed. However, when the cultures containing these infected cells were shifted to the nonpermissive temperature (41°C), the cells reverted to a normal, non-transformed morphology. Later, with a shift of the cultures back to the permissive temperature, the cells once again exhibited a transformed morphology. The loss of the transformed phenotype upon temperature shift-up demonstrated that the continuous action of some temperature-sensitive viral protein was required in order to maintain this phenotype. The reacquisition of the transformed phenotype after temperature shift-down many days later indicated that the viral genome continued to be present in these cells at the high temperature in spite of their normal appearance.

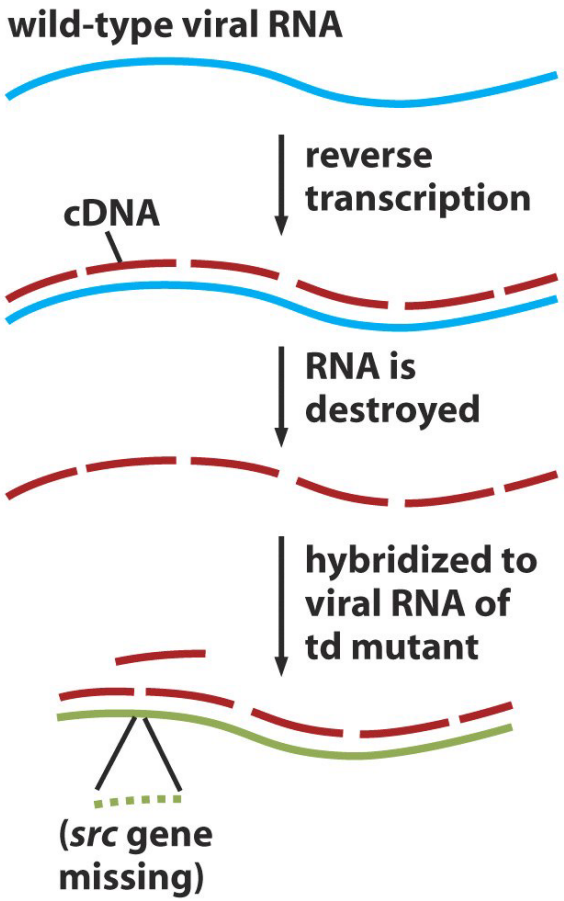
1976 The discovery of the transforming gene v-*src* (pronunciation: sark) and the cellular homologous c-*src*. Varmus e Bishop Nobel Prize 1989



The use of RSV mutants revealed that the functions of viral replication (including reverse transcription and the construction of progeny virions) required one set of genes, while the function of viral transformation required another. Thus, some mutant versions of RSV could replicate perfectly well in infected cells, producing large numbers of progeny virus particles, yet such mutants lacked transforming function. Conversely, other mutant derivatives of RSV could transform cells but had lost the ability to replicate and make progeny virions in these transformed cells.

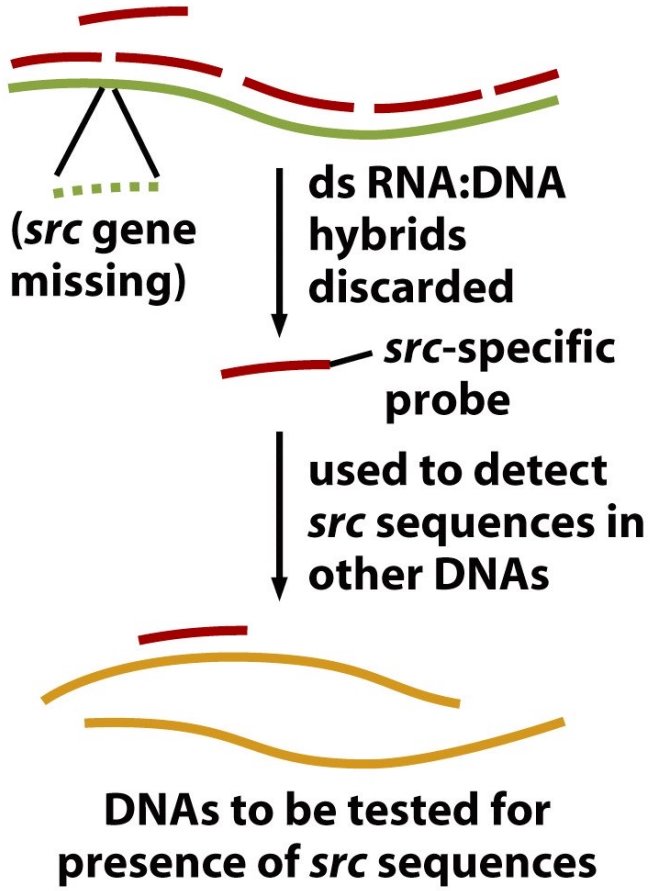
P. Vogt, NATURE REVIEWS | **CANCER** (2012)

1976 The discovery of the transforming gene v-*src* (pronunciation: sark) and the cellular homologous c-src. Varmus e Bishop Nobel Prize 1989



The construction of a src-specific DNA probe
Wild-type (wt) RSV RNA (blue) was reverse-transcribed under conditions where only a single-stranded (ss) complementary DNA molecule (a cDNA; red) was synthesized. The wt single-stranded viral DNA was then annealed (hybridized) to viral RNA (green) of a transformation-defective (td) RSV mutant, which had lost its transforming function and apparently deleted its src gene.

1976 The discovery of the transforming gene v-src (pronunciation: sark) and the cellular homologous c-src. Varmus e Bishop Nobel Prize 1989



The resulting ds RNA:DNA hybrids were discarded, leaving behind the ssDNA fragment of the wtDNA that failed to hybridize to the RNA of the td mutant. This surviving DNA fragment, if radiolabeled, could then be used as a src-specific probe to detect src-related sequences in various cellular DNAs (orange).

The retrovirus genomes isolated from each new solid tumor had different host DNA, NOT the v-SRC gene found in RSV

Each new DNA segment had a novel chicken oncogene

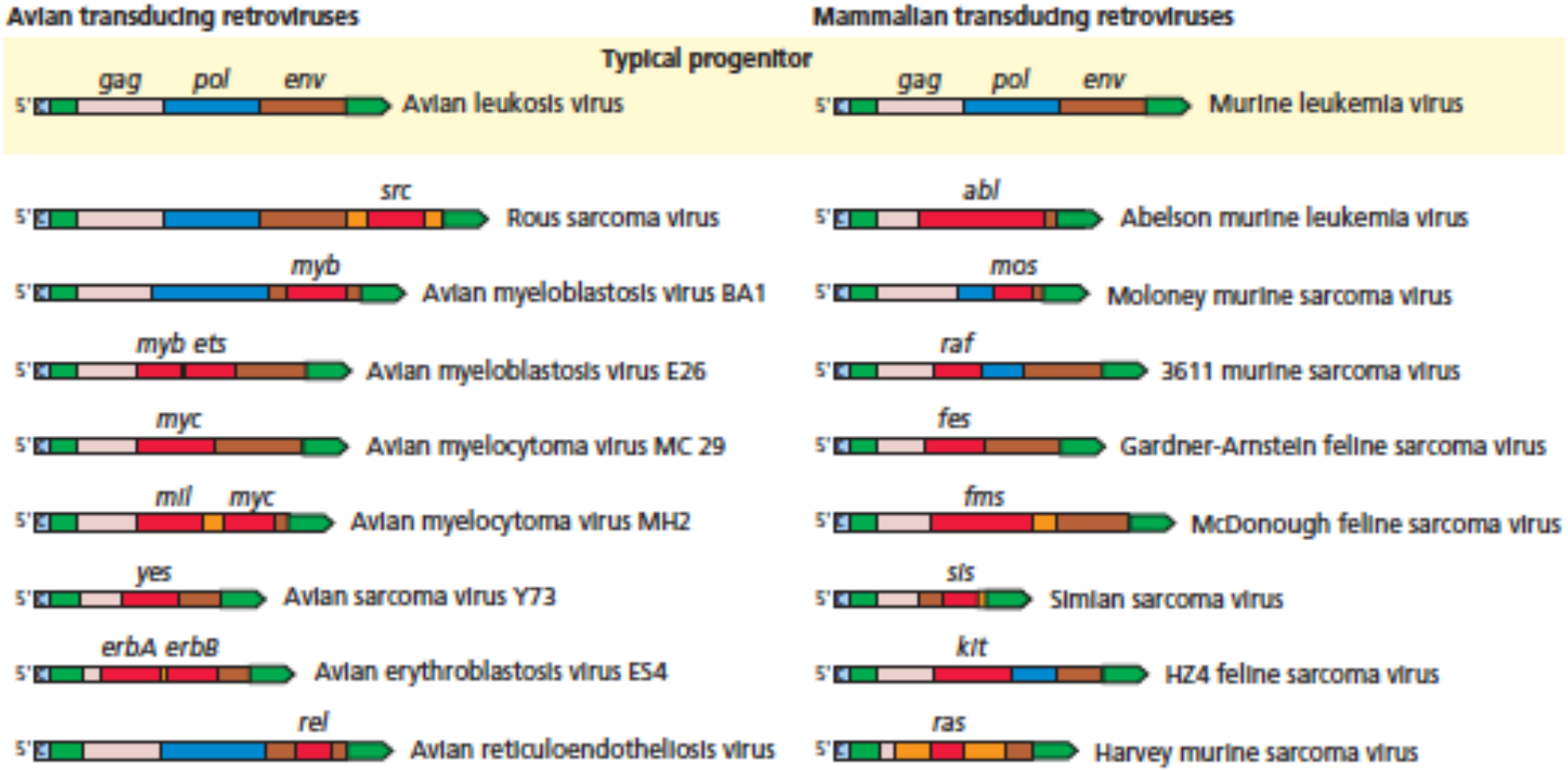
A gold mine for molecular oncology

Table 7-3 Nomenclature Conventions*

Virus	Retroviral Gene		Corresponding Human Gene†	
	Oncogene*	Protein**	Proto-oncogene*	Protein**
Rous sarcoma virus	v-*src*	v-Src	*SRC*	Src
Avian myelocytomatosis virus	v-*myc*	v-Myc	*MYC*	Myc

*Gene names are always in italics (lowercase letters for viral genes and usually all capital letters for human genes).
**Protein names begin with a capital letter and are not in italics.
†Sometimes the names of the human genes and proteins are preceded with a "c" for "cellular" (for example, c-*SRC* gene and c-Src protein, or c-*MYC* gene and c-Myc protein).

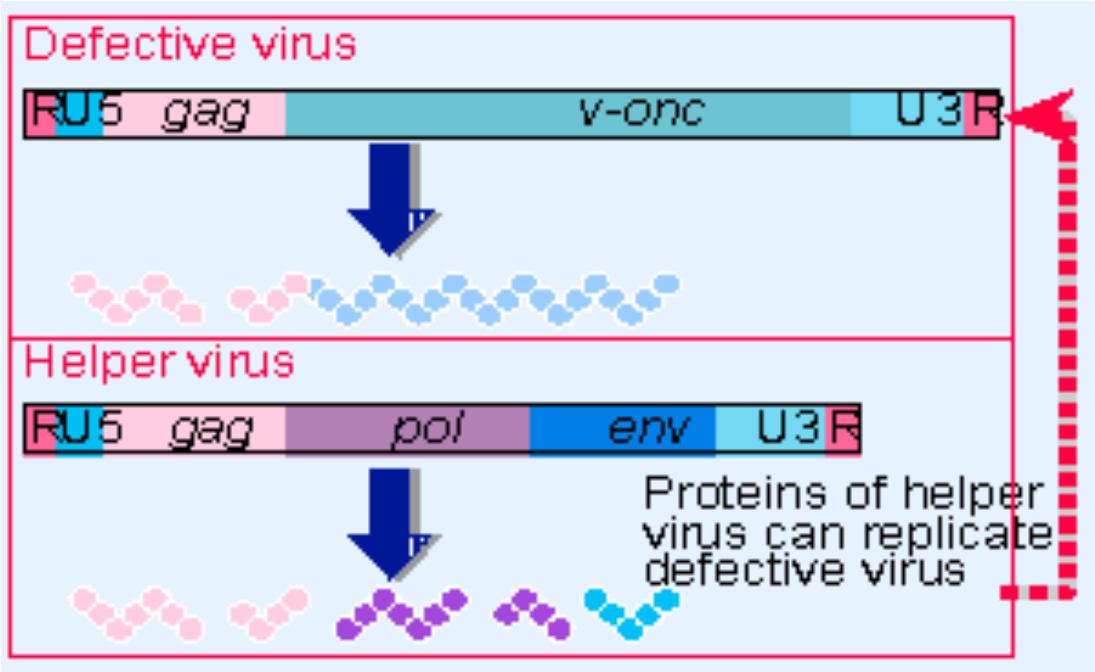
Genome maps of avian and mammalian transducing retroviruses



In Rous sarcoma virus, the oncogene src is added to the complete viral genome. In all other avian and mammalian transducing retroviruses, some of the viral coding information is replaced by cell-derived oncogene sequences (red). Consequently, such transducing viruses are defective in replication. The majority of the transducing retroviruses carry a single v-oncogene in their genomes, but some include more than one (e.g., erbA and erbB in avian erythroblastosis virus ES4). In such cases, one is sufficient for transformation, while the second accelerates this process. In some cases, additional cellular DNA sequences (orange) were also captured in the viral genome.

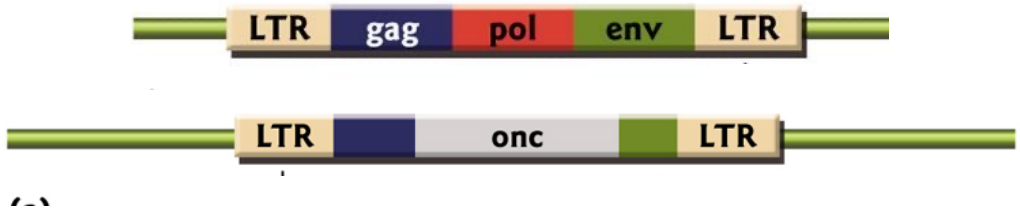
From Flint et al. *Principles of Virology* (2015), ASM Press

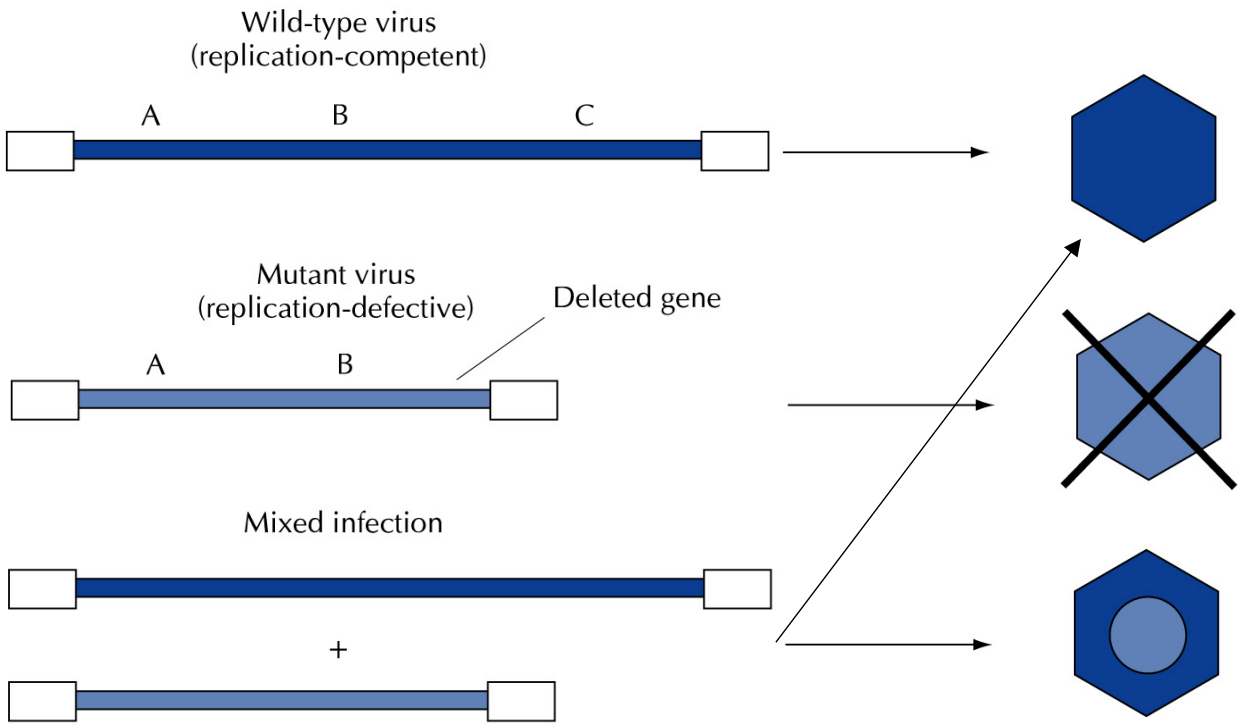
Defective retroviruses require helper virus to produce more virus particles



Replication-defective transforming viruses have a cellular sequence substituted for part of the viral sequence. The defective virus may replicate with the assistance of a helper virus that carries the wild-type functions.

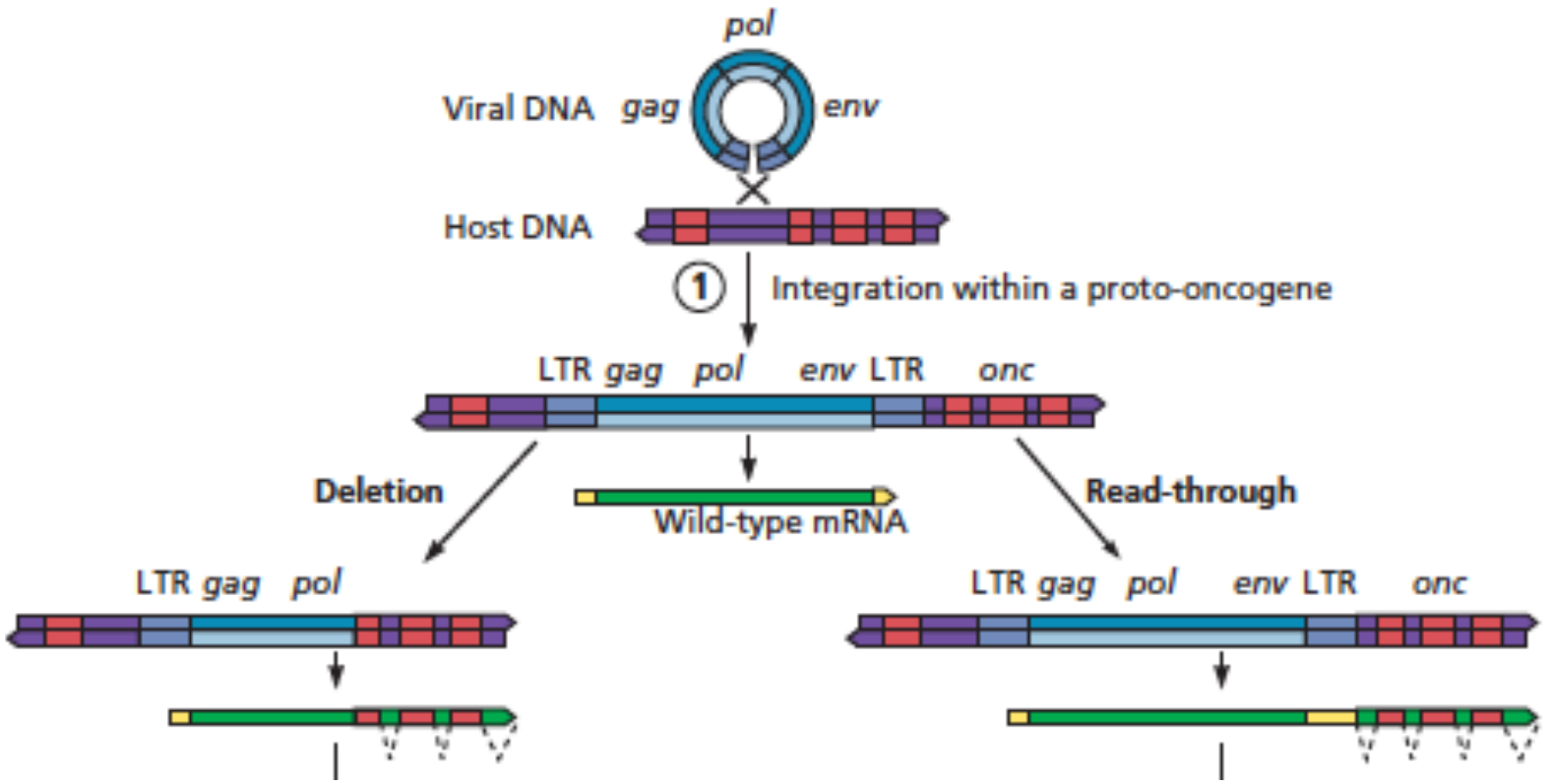
Complementation





From Cann *Principles of molecular virology* (2001). Academic Press

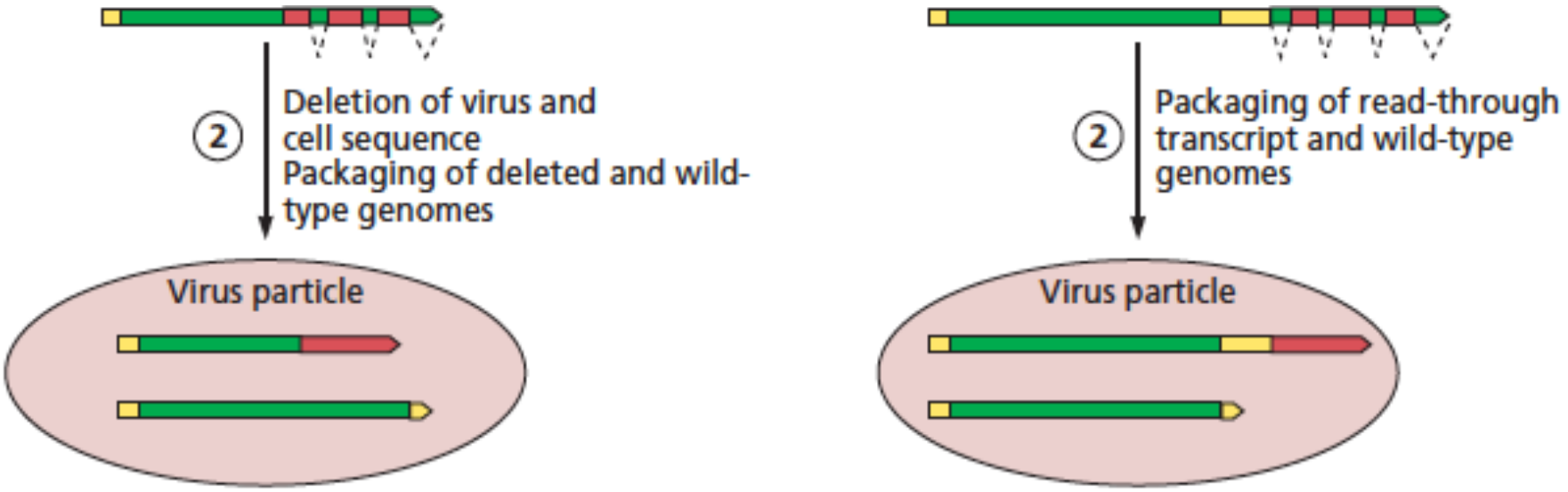
Possible mechanisms for oncogene capture by retroviruses



The first step in each of two mechanisms shown is integration of a provirus in or near a cellular gene (onc). The deletion mechanism (left) requires removal of the right end of the provirus, thereby linking cellular sequences to the strong viral transcriptional control region in the left LTR. The first recombination step in this mechanism therefore takes place at the DNA level. It leads to synthesis of a chimeric RNA, in which viral sequences from the left end of the provirus are joined to cellular sequences. Chimeric RNA molecules that include the viral packaging signal can be incorporated efficiently into viral particles with a wild-type genome produced from another provirus in the same cell. The read-through mechanism (right) does not require a chromosomal deletion. Viral transcription does not always terminate at the 3' end of the proviral DNA, but continues to produce transcripts containing cellular sequences. Such chimeric transcripts can then be incorporated into virus particles together with the normal viral transcript.

From Flint et al. *Principles of Virology* (2015), ASM Press

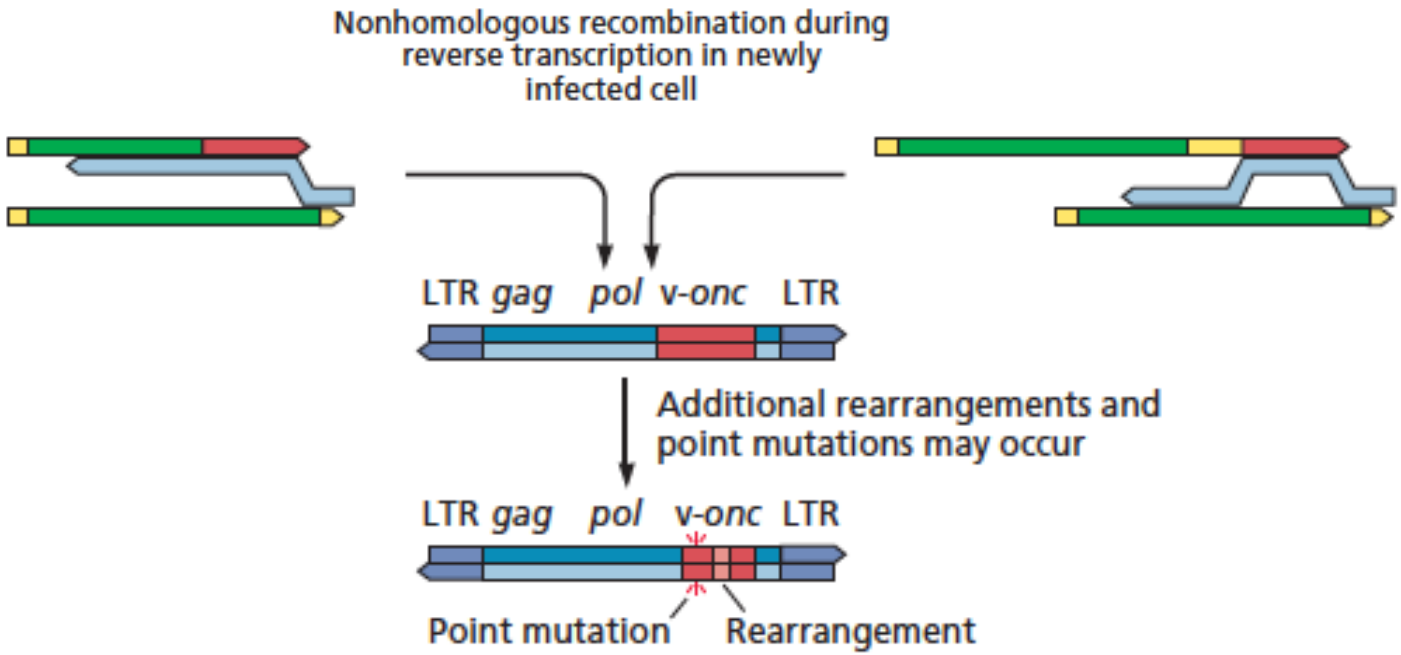
Possible mechanisms for oncogene capture by retroviruses



Chimeric RNA molecules that include the viral packaging signal can be incorporated efficiently into viral particles with a wild-type genome produced from another provirus in the same cell.

From Flint et al. *Principles of Virology* (2015), ASM Press

Possible mechanisms for oncogene capture by retroviruses



A second recombination reaction, during reverse transcription is then required to add right-end viral sequences to the recombinant. At a minimum, these right-end sequences must include signals for subsequent integration of the recombinant viral DNA into the genome of the newly infected host cell, from which the transduced gene is then expressed. Important additional mutations and rearrangements probably occur during subsequent virus replication.

From Flint et al. *Principles of Virology* (2015), ASM Press

Table 1 | **Functional classes of retroviral oncoproteins**

Functional class	Examples	Source virus
Growth factor	Sis (PDGFB)	Simian sarcoma virus
Receptor tyrosine kinase	ErbB (EGFR)	Avian erythroblastosis virus
Hormone receptor	ErbA (THRA)	Avian erythroblastosis virus
G protein	Ha-ras, a GTPase	Harvey sarcoma virus
	Ki-ras, a GTPase	Kirsten sarcoma virus
Adaptor protein	Crk, a modular signalling link	CT10 avian sarcoma virus
Non-receptor tyrosine kinase	Src, a signalling protein kinase	Rous sarcoma virus
	Abl, a signalling protein kinase	Abelson murine leukemia virus
Serine/threonine kinase	Akt, a signalling protein kinase	Akt8 murine thymoma virus
	Mos, a signalling protein kinase	Moloney murine sarcoma virus
Transcriptional regulator	Jun, a component of the AP1 complex	Avian sarcoma virus 17
	Fos, a component of the AP1 complex	Finkel–Biskis–Jinkins murine sarcoma virus
	Myc, a transcription factor	Avian myelocytomatosis virus MC29
Lipid kinase	Pi3k	Avian sarcoma virus 16

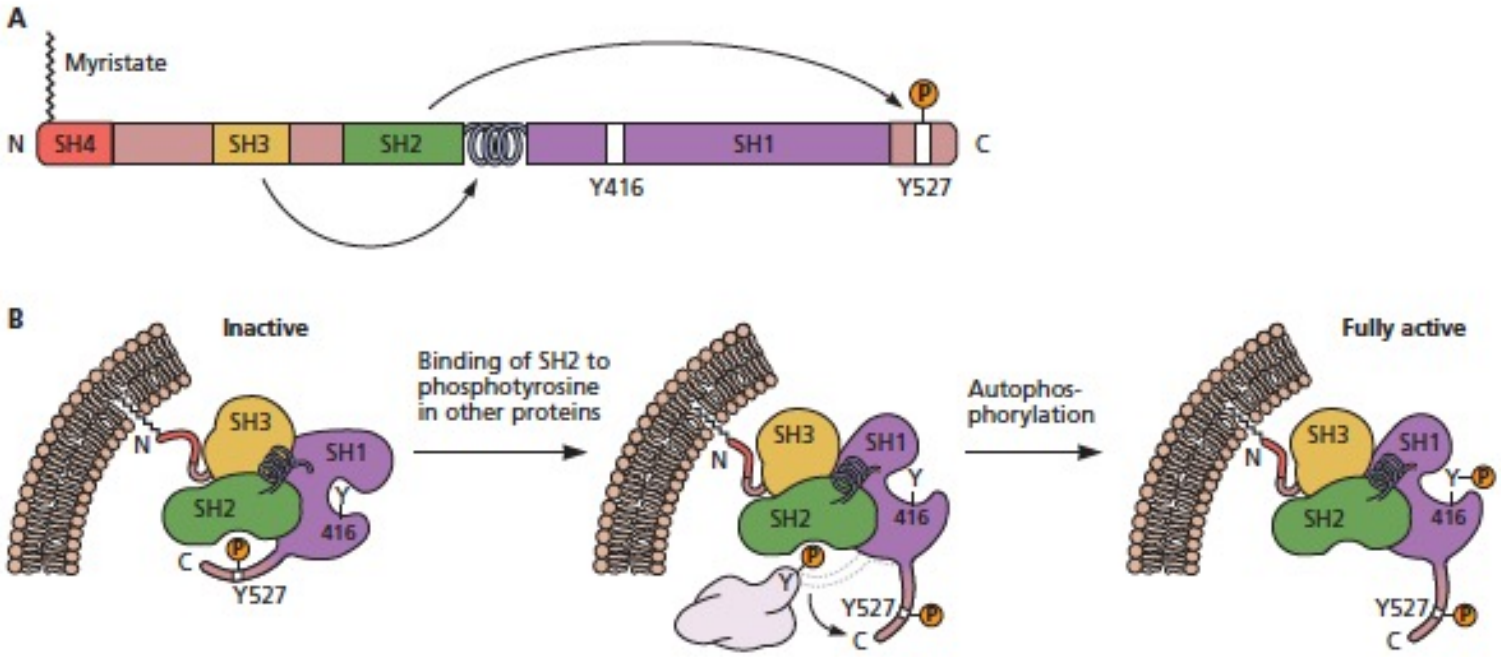
AP1, activator protein 1; EGFR, epidermal growth factor receptor; Ha-ras, Harvey-ras; Ki-ras, Kirsten-ras; PDGFB, platelet-derived growth factor-β; THRA, thyroid hormone receptor-α.

P. Vogt, NATURE REVIEWS | **CANCER** (2012)

Mechanisms of activation of proto-oncogenes to oncogenes as a consequence of retroviral transduction

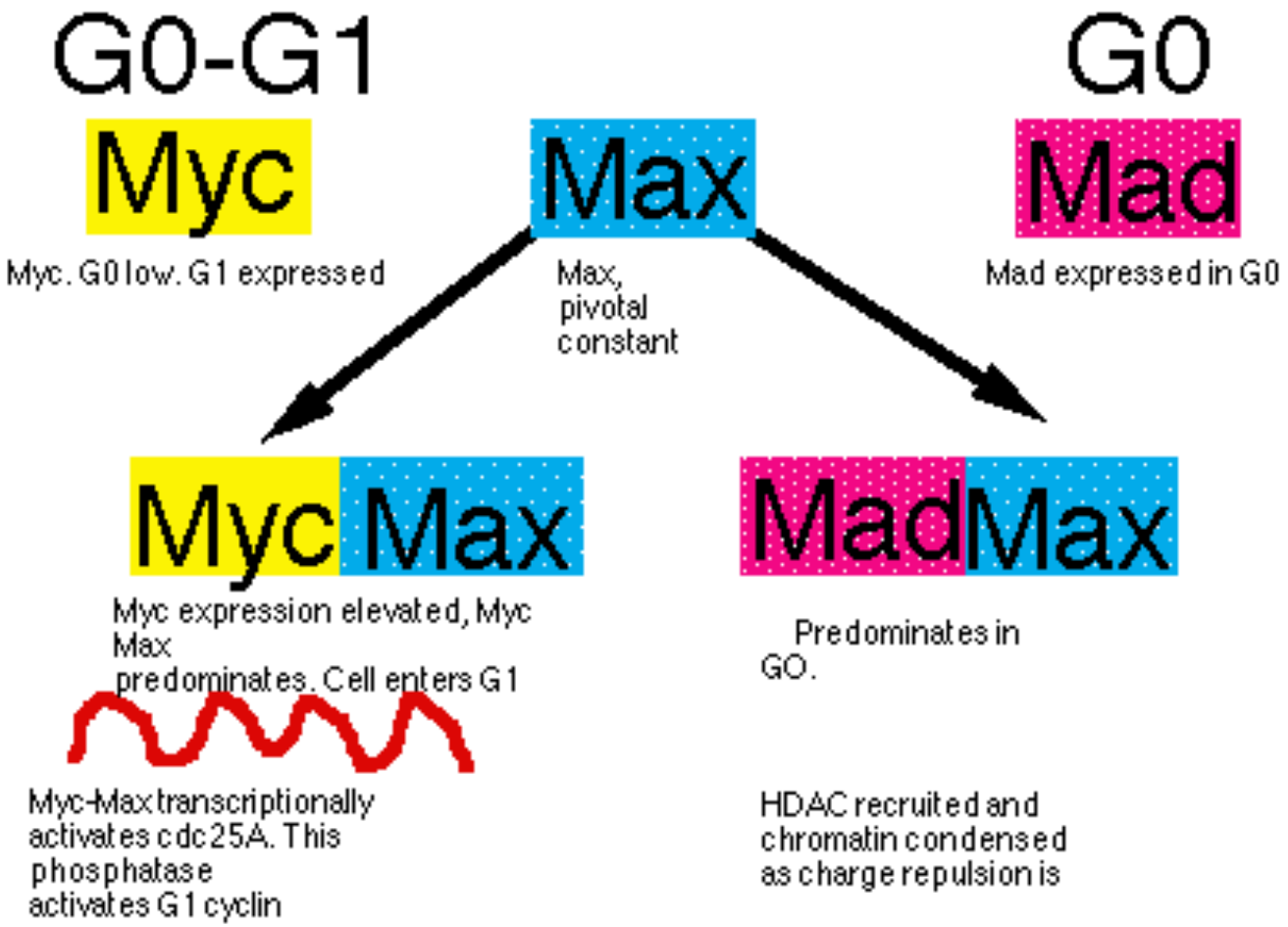
Lack of introns
May be truncated e.g. v-src
May contain point mutation e.g. v-ras
Expressed at abnormal high and/or constitutive levels under LTR control
Frequently found fusion between a viral gene and the oncogene e.g. gag-onc or env-onc

Regulation of c-src



The v-Src protein is not subjected to such autoinhibition, because the sequence encoding the C-terminal regulatory region of c-Src was deleted during transduction of the cellular gene.

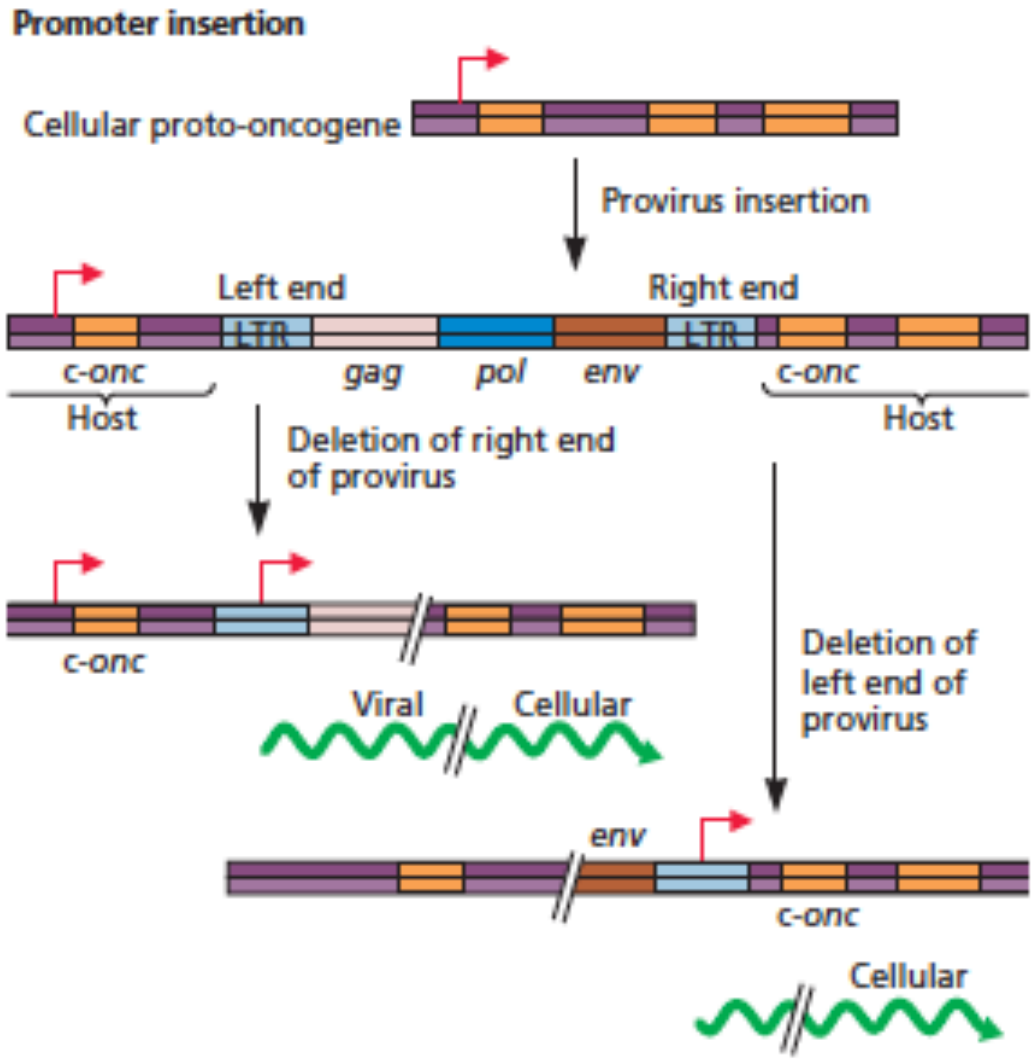
From Flint et al. *Principles of Virology* (2015), ASM Press


G0-G1
Myc
Myc. G0 low. G1 expressed
Max
Max, pivotal constant
G0
Mad
Mad expressed in G0
Myc Max
Myc expression elevated, Myc Max predominates. Cell enters G1
Myc-Max transcriptionally activates cdc25A. This phosphatase activates G1 cyclin
Mad Max
Predominates in GO.
HDAC recruited and chromatin condensed as charge repulsion is

“Now, a third of a century later, we realize that these transforming retroviruses provided cancer researchers with a convenient window through which to view the cellular genome and its cohort of proto-oncogenes. Without these retroviruses, the discovery of proto-oncogenes would have been exceedingly difficult. By fishing these genes out of the cellular genome and revealing their latent powers, these viruses catapulted cancer research forward by decades.”

Robert Weinberg *The Biology of Cancer* (© Garland Science 2014)

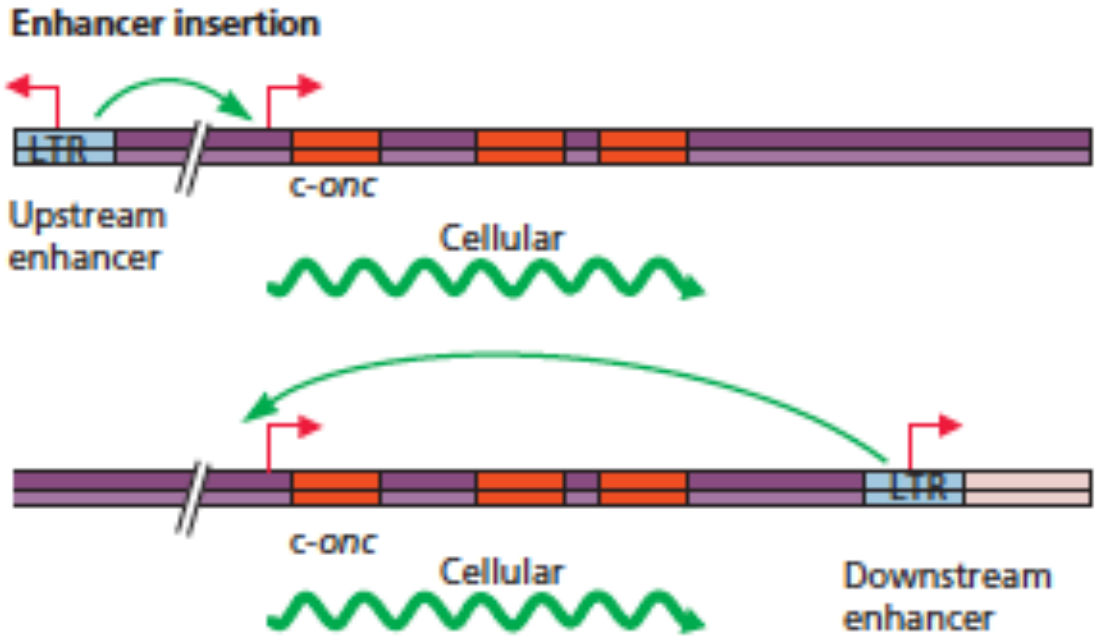
Slowly transforming retroviruses: insertional mutagenesis



From Flint et al. *Principles of Virology* (2000), ASM Press

The first mechanism, promoter insertion, results in production of a chimeric RNA in which sequences transcribed from the proviral LTR are linked to cellular proto-oncogene sequences. If transcription originates from the left-end LTR, some viral coding sequences may be included. However, transcription from the right-end LTR seems to be more common, and in these cases the proviral left-end LTR has usually been deleted. Proviral integration often occurs within the cellular proto-oncogene, truncating cellular coding sequences and eliminating noncoding domains that may include negative regulatory sequences. Some chimeric transcripts formed in this way are analogous to the intermediates that give rise to oncogene capture by the transducing retroviruses. Indeed, it has been possible to isolate newly generated, oncogene-transducing retroviruses from tumors arising as a result of promoter insertion.

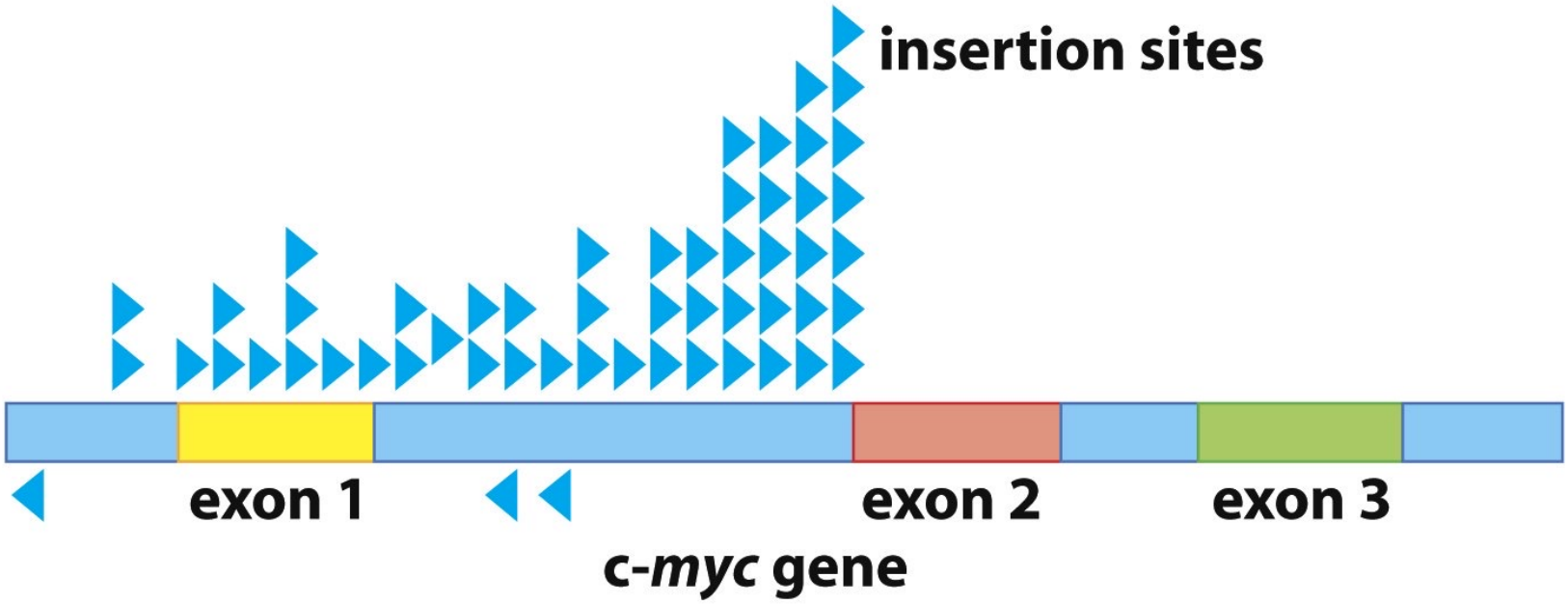
Slowly transforming retroviruses: insertional mutagenesis



In the second type of insertional activation, enhancer insertion, viral and cellular transcripts are not fused. Instead, activation of the cellular gene is mediated by the strong viral enhancers, which increase transcription from the cellular promoter. Because enhancer activity is independent of orientation and can be exerted over long distances, the provirus need not be oriented in the same direction as the proto-oncogene, and may lie downstream of it.

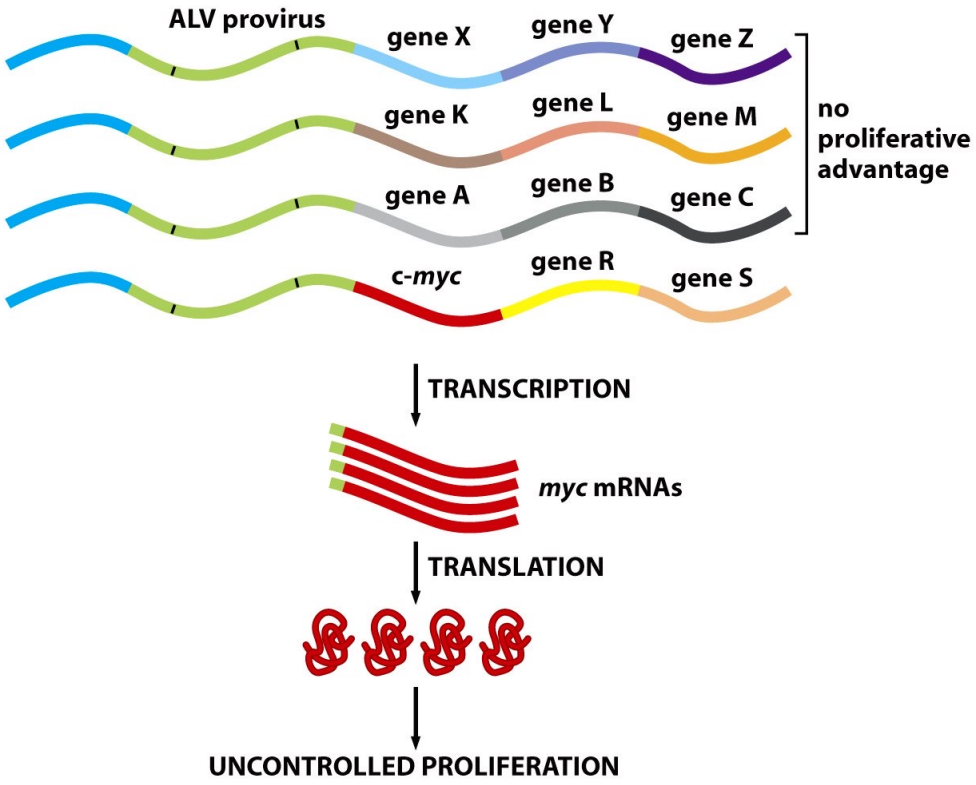
From Flint et al. *Principles of Virology* (2000), ASM Press

Slowly transforming retroviruses: insertional mutagenesis (ALV)



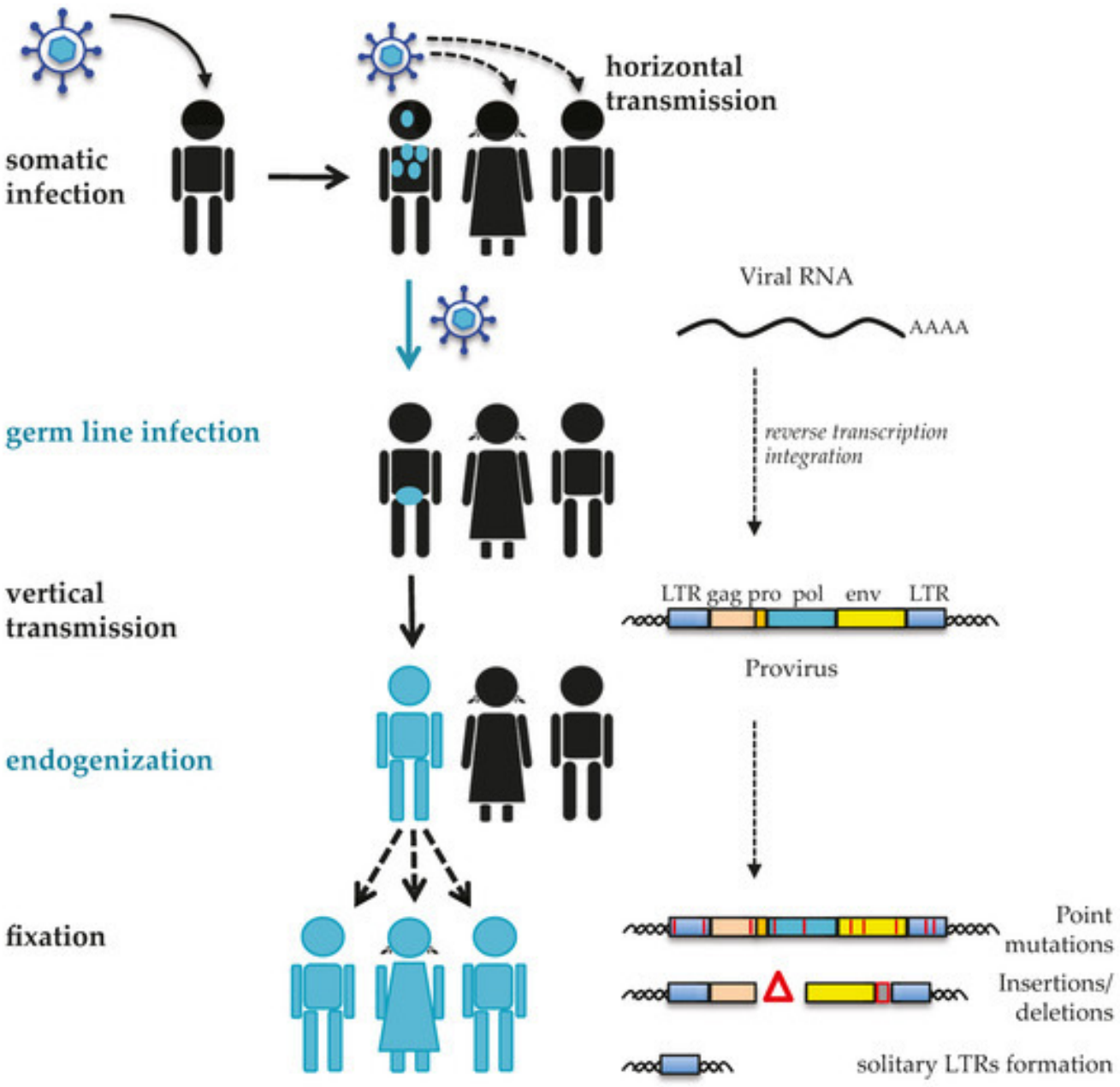
Analysis of numerous B-cell lymphomas that were induced by ALV revealed that a large proportion of the ALV proviruses were integrated (filled triangles) into the DNA segment carrying the c-*myc* proto-oncogene, usually in the same transcriptional orientation as that of the c-*myc* gene; the majority were integrated between the first noncoding exon of c-*myc* and the second exon, in which the *myc* reading frame begins.

Slowly transforming retroviruses: insertional mutagenesis (ALV)

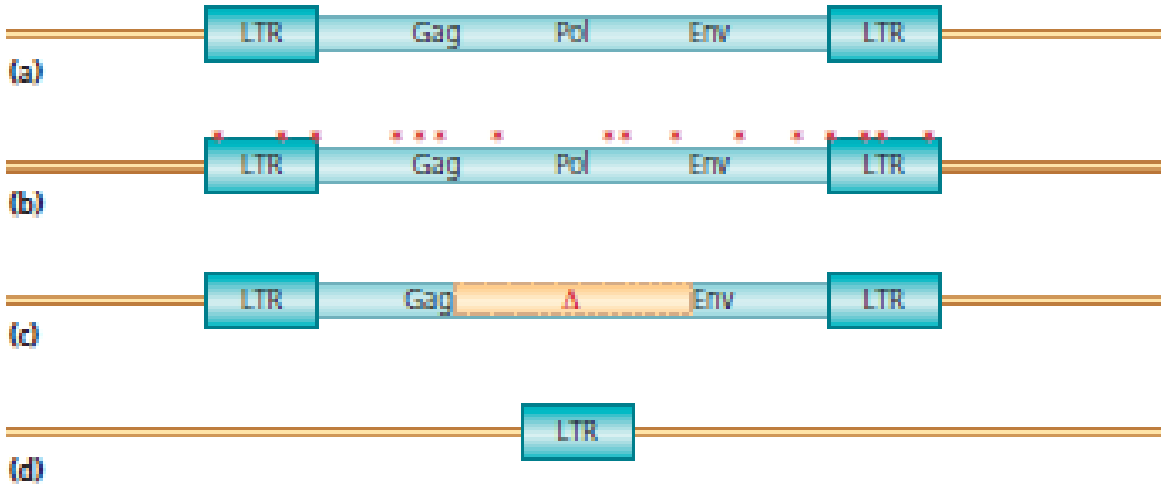


ALV's oncogenic behavior could be rationalized as follows. In the course of ALV infection of chicken lymphocytes, ALV proviruses (green) become integrated randomly at millions of different sites in the chromosomal DNA of these cells. (Chromosomal DNA maps of only four are illustrated schematically here.) On rare occasions, an ALV provirus becomes integrated (by chance) within the c-myc proto-oncogene (red). This may then cause transcription of the c-myc gene to be driven by the strong, constitutively acting ALV promoter. Because high levels of the Myc protein are potent in driving cell proliferation, the cell carrying this particular integrated provirus and activated c-myc gene will now multiply uncontrollably, eventually spawning a large host of descendants that will constitute a lymphoma.

Endogenous retroviruses (ERV)



Endogenous retroviruses (ERV)



Endogenous retroviral genomes decay by mutation. (a) The initial product of integration following horizontal infection of a germ cell; (b–d) possible organizations of the endogenous virus sequence after many generations within the host germline. (b) Random point mutations can degrade the protein-coding capacity of the genome and/or alter the function of the two LTRs. (c) Large random deletions within the coding region can remove some or all of the genes. (d) Homologous recombination between the two LTRs of the original integrated copy can precisely excise the entire coding region to leave a single LTR embedded in the genome.