

Oncogenes I and II

Readings for both lectures:

The Biology of Cancer (2nd edition, 2014)

by Robert A. Weinberg

- ◆ Chapters 3, 4, 10, and 11

Oncogenes I

■ Organization of today's lecture

- mammalian cell culture
- telomere hypothesis
- properties of malignantly-transformed cells
- retroviruses
- mechanisms of retroviral-induced tumorigenesis

Use of cell culture to study the malignant process

■ advantages:

- the properties of cells from normal tissues and from tumors can be compared under controlled conditions.
- cells from normal tissues can be experimentally “transformed” into cells with a more malignant phenotype.
- *in vitro* cell transformation can be measured using quantitative and reproducible assays.
- the phenotypic differences between normal cells and their transformed counterparts can be correlated with their ability to form tumors *in vivo*.

■ disadvantages:

- cell culture cannot recapitulate the entire malignant process

Acquired properties of cancer cells

- Inappropriate cell proliferation
- Inappropriate resistance to cell death
- Failure of cellular differentiation
- Invasiveness
- Metastatic potential
- Angiogenic capacity

Culturing normal mammalian cells

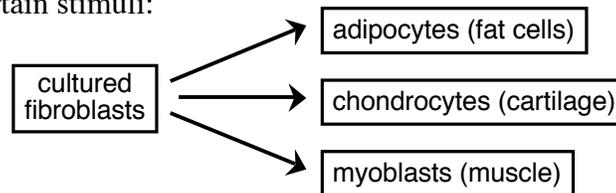
- to culture mammalian cells *in vitro*:
 - tissue explants are dissociated to single cells (e.g., with trypsin)
 - cells are incubated in a petri dish with a sterile aqueous medium containing:
 - ◆ amino acids
 - ◆ salts
 - ◆ glucose (energy source)
 - ◆ serum (growth factors, survival factors, etc.)
 - cells adhere to the plate, and some may proliferate.
- “primary culture”

Passaging cells *in vitro*

- cells are incubated for a defined period.
 - (e.g., 3 days)
- the spent medium is removed, and the adhered cells are dissociated (trypsin).
- cells are counted, diluted in fresh media, and re-plated at a specific density (e.g., 3×10^5 cells/plate).
 - secondary culture
- “3 times 3” (3T3) passaging
- cells can be maintained in culture for long periods by continual passaging.

Cultured fibroblasts

- If the explant is derived from a complex tissue (e.g., skin biopsies) it will contain a mix of epithelial and mesenchymal cells.
- Within a few passages, fibroblasts overgrow the culture
- “cultured fibroblasts”
 - more immature than tissue fibroblasts
 - often exhibit properties of mesenchymal stem cells; e.g., some cultured fibroblasts can differentiate *in vitro* in response to certain stimuli:



In vitro culture of epithelial cells

- need to remove fibroblasts from the culture
 - physical isolation of epithelial cells
 - ◆ dissection of explant
 - ◆ partial trypsinization of explant
 - preferential viability of epithelial cells
 - ◆ grow in a serum-free medium
 - ▲ remember: serum is especially rich in PDGF and FGFs
 - ◆ provide other sources of epithelial growth factors (such as recombinant proteins)

human mammary epithelial cells (HMECs)

explant – surgical discard from reduction mammoplasties

- ◆ cut away fatty material
- ◆ digest with collagenase and hyaluronidase
- ◆ collect semi-pure epithelial clumps (“organoids”) on a 100-mm filter (filtrate contains mainly fibroblasts)

epithelial organoids

- ◆ seed onto a petri dish
- ◆ incubate in serum-free medium supplemented with:
 - rEGF, insulin, hydrocortisone
 - bovine pituitary extract

HMEC culture

adherent vs. non-adherent cells

■ fibroblasts and epithelial cells are adherent

- anchorage on a solid substratum is essential for growth
- adherent cells secrete matrix proteins:
 - ◆ fibronectins, laminins, collagen
- proper adherence is detected by signal pathways that report to the central cell cycle regulatory pathways

■ hematopoietic cells can often grow in suspension

- *in vivo*, - blood cells are non-adherent
 - spleen and bone marrow cells are semi-adherent

Life span of human fibroblasts in culture

- human fetal fibroblasts undergo “replicative senescence” or “early crisis” after ~ 60 population doublings (PDs).
- properties of senescent cells:
 - cells exist in a state of permanent growth arrest.
 - ◆ resembles but distinct from quiescence (the G₀ state)
 - cells are viable and metabolically active.
 - large cytoplasm (“fried egg” appearance).
 - express β-galactosidase (stain blue with Xgal).
 - express the p16^{INK4a} tumor suppressor (Rb pathway).
 - display shortened telomeres.
 - otherwise, senescent cells have a stable genome (diploid).
- mortal cell cultures = “cells strains”
- immortal cells never emerge spontaneously from cultured strains of human fibroblasts.

Life span of human fibroblasts in culture

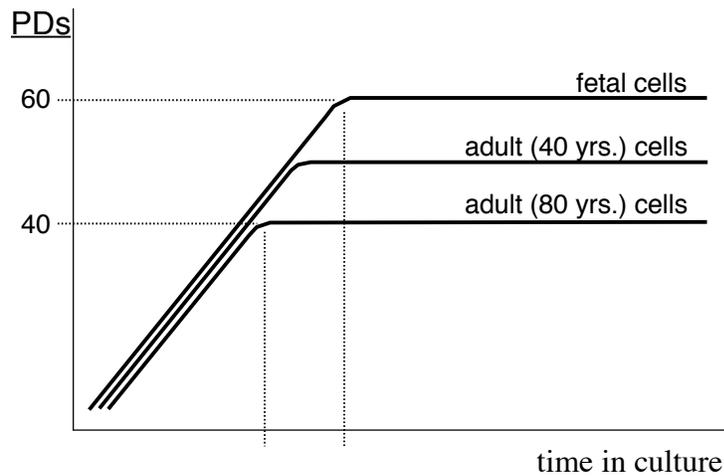
- the lifespan of a cell strain correlates inversely with the age of the donor:

fetus —————> 60 PDs

40 yrs. —————> 50 PDs

80 yrs. —————> 40 PDs

Life span of human fibroblasts in culture



Life span of human fibroblasts in culture

→ Therefore, the lifespan of a cell strain is not dependent solely on the number of cell divisions in culture.

Instead, it reflects the number of cell divisions both *in vivo* and *in vitro*.

- Is there a mechanism that “counts” the number of cell divisions from conception ?

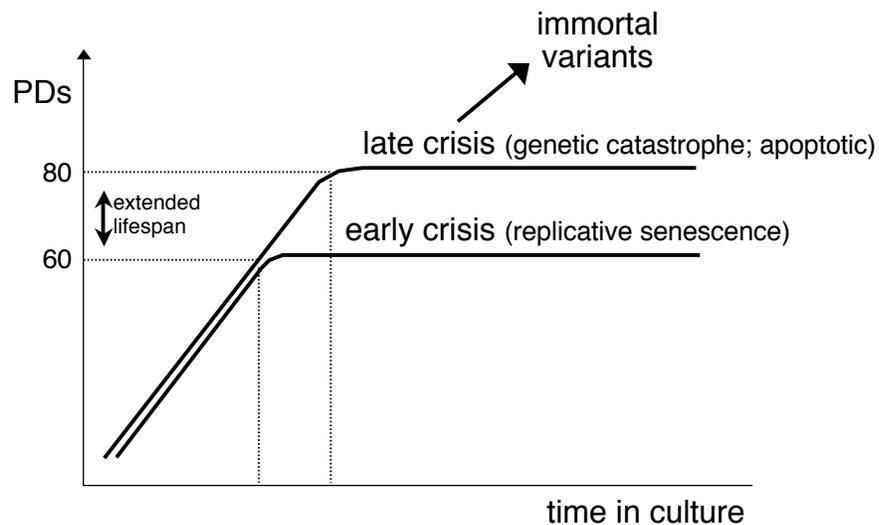
Experimental immortalization of human fibroblast strains

- disruption of Rb and p53 tumor suppressor pathways
 - infection with DNA tumor viruses or transfection with the transforming genes of these viruses:
 - ◆ SV40 polyoma virus encodes: large T antigen (targets Rb and p53)
 - ◆ adenovirus encodes: E1A protein (targets Rb)
E1B protein (targets p53)
 - ◆ human papilloma virus (HPV) encodes: E7 protein (targets Rb)
E6 protein (targets p53)
- virally-transformed cells bypass replicative senescence
- continued proliferation for ~ 20 PDs.
- cells experience a “genetic catastrophe” (late crisis).

Relevant aspects of genetic catastrophe

- during the extended life span, proliferating cells undergo further telomere erosion
- gross genomic instability occurs:
 - chromosome rearrangements
 - aneuploidy (changes in chromosome number)
- massive cell death ensues
- rare immortal variants can emerge...
 - giving rise to permanent “cell lines”.

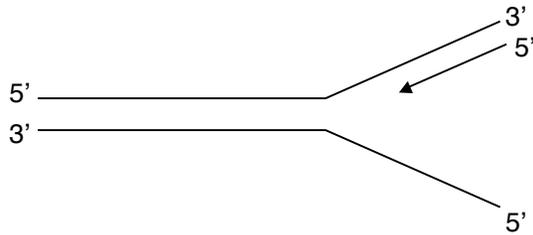
Immortalization of human fibroblast



Cell lifespan and telomere function

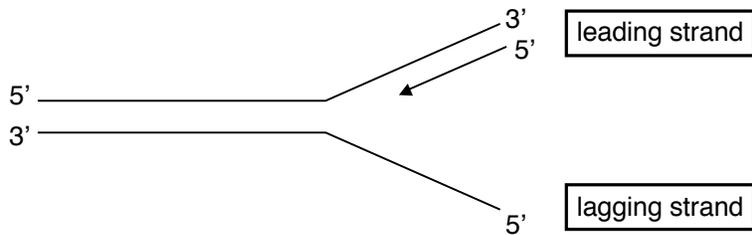
- The telomere hypothesis may explain the behavior of human fibroblasts in culture
- telomeres: specialized structures at the ends of all linear chromosome
- the functions of telomeres:
 - solve the “end replication problem” of DNA replication.
 - masks chromosome ends from cellular pathways that recognize and repair double-strand DNA breaks.
 - protect linear chromosomes from illegitimate recombination (e.g., formation of end-to-end fusions).

End replication problem



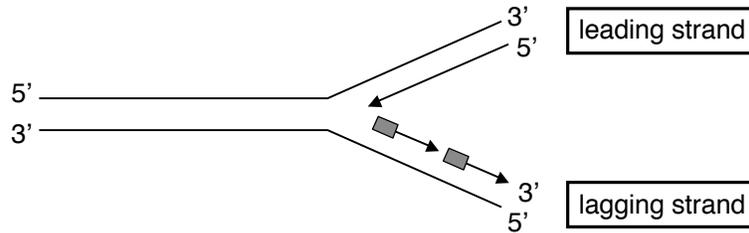
- DNA polymerization occurs $5' \rightarrow 3'$ (with respect to the nascent strand).

Leading strand synthesis



- leading strand synthesis
 - occurs continuously ($5' \rightarrow 3'$) toward replication fork
- lagging strand synthesis
 - cannot occur continuously

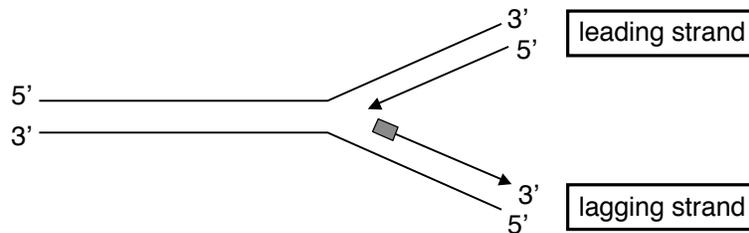
Lagging strand synthesis



■ lagging strand synthesis

- occurs discontinuously in short spurts of 5' → 3' synthesis
- each spurt is newly primed with an RNA oligonucleotide
- generates an “Okazaki fragment” of DNA (200-400 bps)
- as the replication fork progresses, multiple RNA-primed Okazaki fragments are produced.

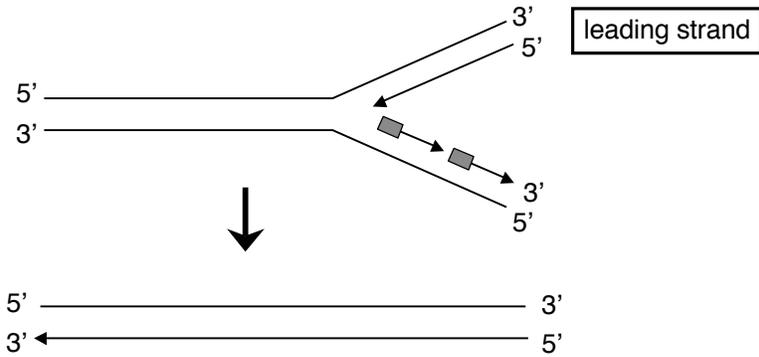
Lagging strand synthesis



■ lagging strand synthesis

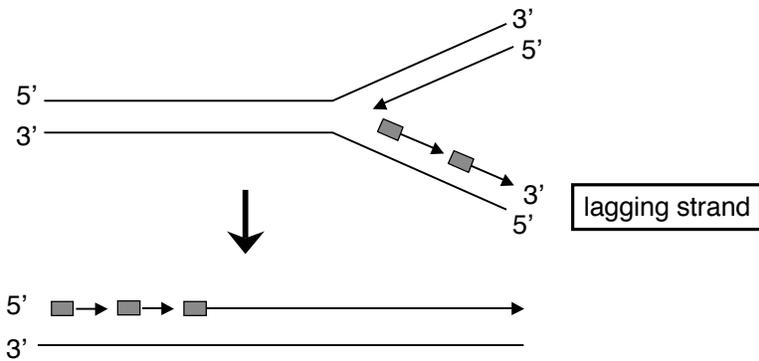
- DNA synthesis of new Okazaki fragment displaces the RNA primer of the previous fragment.
- RNA primer degraded
- adjacent Okazaki fragments are ligated together

End replication problem



- leading strand synthesis proceeds to end of template DNA

End replication problem



- lagging strand synthesis proceeds by consecutive ligation of Okasaki fragments.

Telomere length

- note: the terminal DNA sequences lost at each cell division are comprised entirely of telomere repeats
- telomere length shortens during *in vitro* culture of human fibroblasts:
 - early passage fibroblasts = 18-25 kilobases (kb)
 - at replicative senescent = 8-10 kb
 - at genetic catastrophe = 1-2 kb
- telomere attrition also occurs *in vivo*.
- how is telomere length restored in each new generation ?
 - Telomerase: an enzymatic complex that extends telomeres.
 - since telomerase is highly active in germ cells, chromosomes of the germline retain full telomere length.

Telomerase

- an enzymatic complex consisting of...
 - TERT (telomerase reverse transcriptase): a catalytic subunit that uses an RNA template to synthesize tandem DNA copies of the telomere repeat sequence.
 - TR (telomerase RNA): ssRNA molecule of 451 nucleotides
 - ◆ contains a sequence that is complementary to the telomere repeat:
5'-CUAACCCUAA-3'
 - ◆ provides the template for synthesis of the telomere repeat.
- Telomerase adds telomere repeat units (5'-TTAGGG-3') to the 3'-ssDNA overhang.
- in humans, TERT expression is largely restricted to the germline and to certain stem cells.

Telomere hypothesis

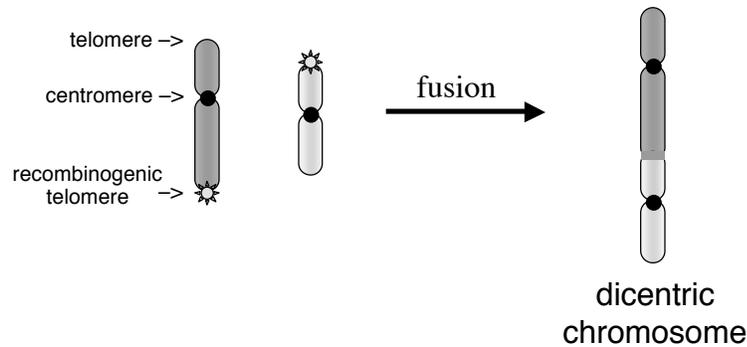
- Replicative senescence (early crisis)...
 - is induced by telomeric shortening below a threshold level (8-10 kb in human fibroblasts)
 - enforced by the p53 and Rb checkpoints
- Genetic catastrophe (late crisis)...
 - occurs when disruption of p53/Rb checkpoint (e.g., by viral oncogenes) allows continued proliferation despite impending telomere dysfunction.
 - further telomere erosion → telomere dysfunction
 - loss of telomere function → genetic catastrophe
 - ◆ genomic instability
 - ◆ massive cell death
 - ◆ emergence of immortal variants

Short telomeres & genetic instability

- when short telomeres become dysfunctional...
 - chromosome ends behave like dsDNA breaks
 - ◆ highly recombinogenic
 - ◆ induce the cellular DNA damage response
 - “breakage-fusion-bridge” cycles are initiated
 - ◆ recombinogenic telomeres of two chromosome ends fuse to form a dicentric chromosome
 - ◆ during metaphase, the same chromatid of a dicentric can attach to both spindle poles
 - ◆ this chromatid may break during anaphase
 - chromosome fragmentation (further rounds of breakage-fusion-bridge)
 - non-reciprocal chromosome translocations
 - loss of genetic material

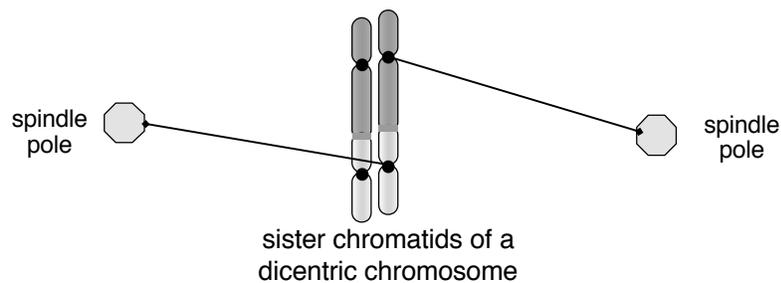
“breakage-fusion-bridge” cycles I

- the recombinogenic telomeres of two chromosomes fuse to form a dicentric chromosome...



“breakage-fusion-bridge” cycles II

- during metaphase, the same chromatid of a dicentric can attach to both spindle poles ...



- As the chromosomes segregate, this chromatid will form an “anaphase bridge” (Figure 10.16B), and will eventually break.
- The break sites of the two resulting broken chromatids will also be recombinogenic.

“breakage-fusion-bridge” cycles - III

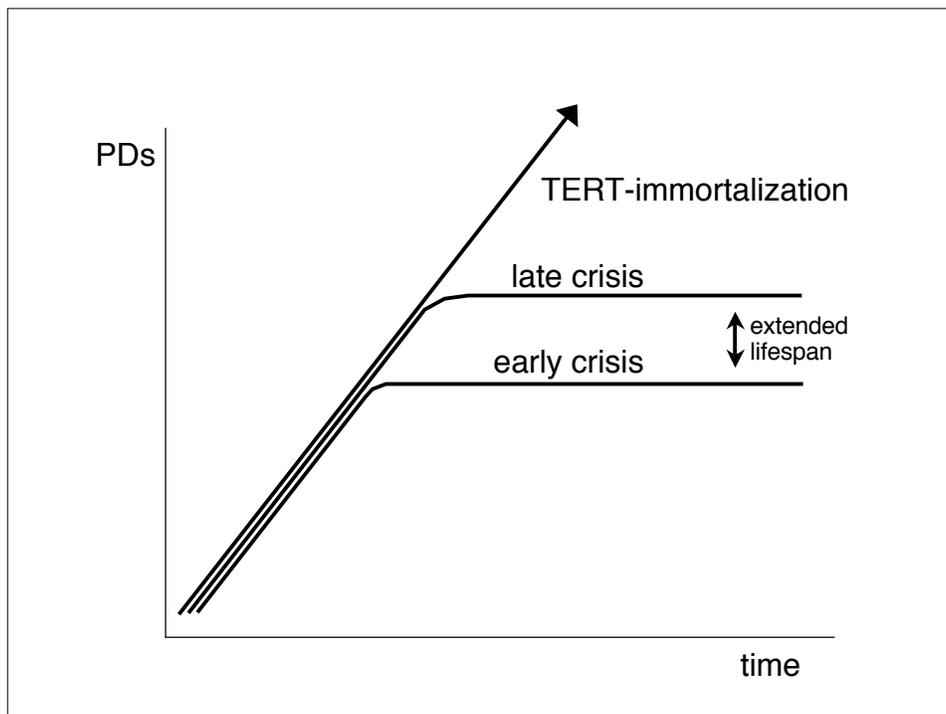
- The previous two slides show a breakage-fusion-bridge (BFB) cycle that is initiated by fusion between the recombinogenic telomeres of two different chromosomes.
- Note: BFB cycles can also be initiated by fusion between recombinogenic telomeres of the two chromatids of the same chromosome (in G2 phase). This is illustrated in Figure 10.15 of Weinberg (2007).

Immortalization

- immortal variants arise by acquiring a genetic change that stabilizes telomere length:
 - ectopic expression of TERT
 - activation of ALT (Section 10.8)
- once telomere length has been stabilized, telomere function and genomic stability are restored
- yet, these variants have sustained lasting genetic lesions
 - karyotypic level: aneuploidy & rearranged chromosomes
 - genetic level: functional alterations of unknown genes ?
 - telomere-induced genomic instability is an important aspect of human tumorigenesis.
- immortal variants emerge from human fibroblasts at a reasonable rate (10^{-5} - 10^{-6})

Immortalization with TERT

- stably transfect early passage (“pre-crisis”) human fibroblasts with an expression vector encoding TERT...
 - telomere length is stabilized.
 - p53/Rb checkpoints are not activated.
 - replicative senescence and genetic catastrophe are averted.
 - cell are rendered immortal.
 - TERT immortalized cells have a stable diploid genome.
- strong evidence that, in human fibroblasts, replicative senescence and genetic catastrophe are telomere-dependent events



Telomere hypothesis explains...

- Replicative Senescence and Genetic Catastrophe
- limited lifespan of cells in culture and *in vivo*
 - ◆ provides a counting mechanism for each somatic cell
- the effect of donor age on lifespan of cultured cells
- the species-dependent behavior of cells in culture

But, why not express telomerase constitutively ?

- telomere-induced replicative senescence
 - a very useful mechanism to suppress tumor development, especially in large and long-lived animals.
- Suppose that...
 - at least 20 cell divisions required to yield an oncogenic mutation
 - at least 5 independent mutations are needed for malignancy:
 - at least 100 cell divisions necessary to produce a single malignant cell.
 - telomere-induced senescence will severely limit the neoplastic development of pre-malignant cells.
 - consequently, telomere-induced senescence is a useful barrier to cancer development in humans.
- telomerase activation occurs in almost all human tumors

Telomere maintenance in the mouse

- murine cells have much longer telomeres
 - ~ 50 kb (compared to 10-20 kb in human cells)
- somatic expression of TERT is much more widespread in mice than humans
- telomere erosion is not a major barrier to the *in vitro* culture of mouse cells

In vitro culture of mouse fibroblasts

- after ~20 PDs, mouse embryo fibroblasts (MEFs) undergo a “crisis” that resembles the replicative senescence of human fibroblasts
 - cells arrest in senescent state (metabolically active)
 - senescent MEFs maintain a stable diploid genome
 - senescence is established by the p53 checkpoint pathway
 - senescence is averted by disabling the p53 pathway
 - yet, after only ~20 PDs, telomeres are not significantly eroded
 - What triggers the p53/RB checkpoint ? Not known for certain.
 - ◆ inadequate culture conditions; oxidative stress ?

mouse fibroblast cell lines

- unlike human fibroblasts, MEF cultures routinely give rise to immortal variants
 - rare immortal variants arise during crisis (presumably due to mutations in the p53 pathway), and these variants overgrow the culture
 - “cell lines”
 - MEF cell lines are diploid and have reasonably stable genomes
 - the source of NIH-3T3 cells

Culturing malignant cells

- cell lines derived from...
 - explants of natural tumors
 - *in vitro* transformation of normal cells in culture

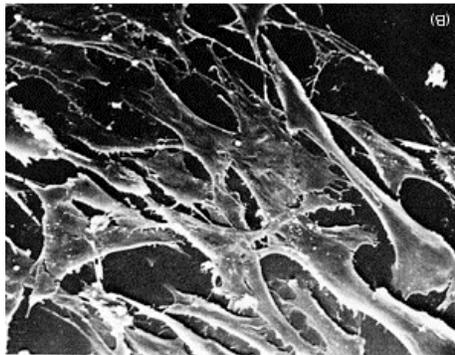
in vitro properties of malignant cells

- immortality
- decreased growth factor requirements
- altered morphology
- loss of contact inhibition
- loss of dependence on anchorage for cell growth

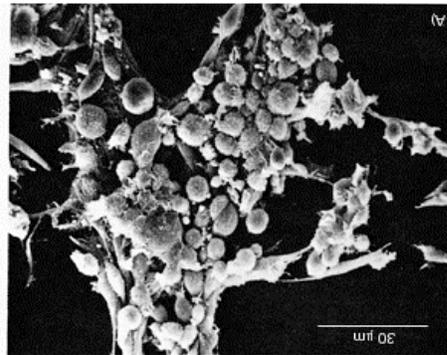
altered morphology

- rounded appearance (vs. the elongated, spindly shape of normal fibroblasts)
- increased nuclear volume

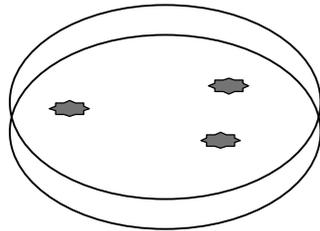
normal CEF



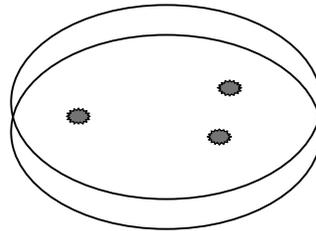
RSV-transformed CEF



loss of contact inhibition



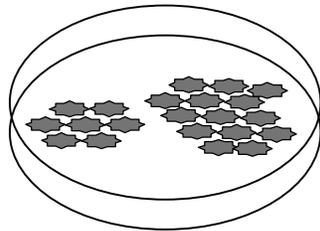
normal cells



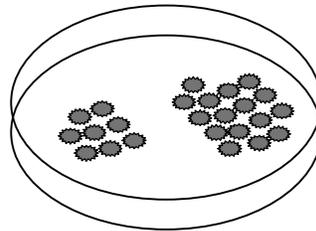
malignant cells

- seed cells onto petri dish at low dilution
- cells attach to dish and start to proliferate

loss of contact inhibition



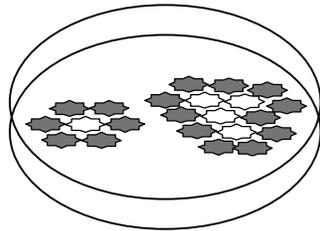
normal cells



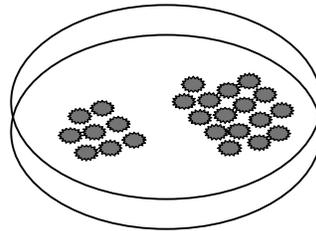
malignant cells

- growing culture begins to form a monolayer
- some cells attain cell-cell contacts on all sides
- these cells normally experience “contact inhibition”

loss of contact inhibition



normal cells

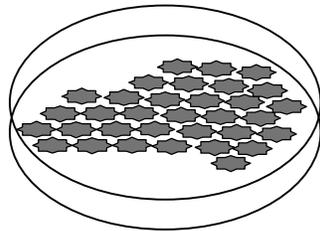


malignant cells

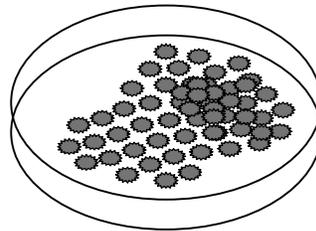
 = contact-inhibited cell

- in normal cells, contact inhibition causes cell cycle arrest.
- malignant cells are not affected by contact inhibition.

anchorage independence



normal cells



malignant cells

- normal cells will only grow if attached to petri dish.
 - form a monolayer
- malignant cells will grow on top of one another.
- anchorage-independence is an especially important parameter of *in vivo* tumorigenicity

in vitro assays of cell transformation

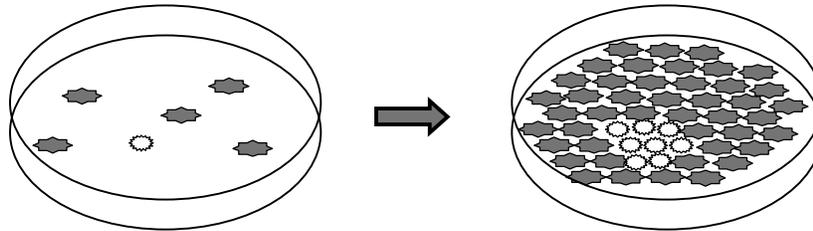
- what happens to normal cultured cells that are treated with agents that induce tumors in animals?
 - e.g., viruses, radiation, chemicals
- they acquire some properties of malignant cells
- “*in vitro* cell transformation” can be measured using quantitative assays:
 - focus formation
 - colony formation in soft agar

focus formation assay

- based on several features of transformed cells:
 - altered morphology
 - loss of contact-inhibition
 - loss of anchorage dependence
- treat normal cells with oncogenic agent
- plate cells at low dilution
- progeny of rare transformed cells give rise to a “focus” that can be visibly distinguished from the monolayer of normal cells.

focus formation assay

- treat normal cell culture with tumorigenic agent
- plate cells at low dilution

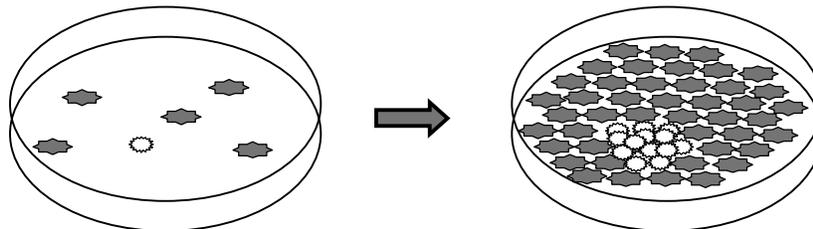


○ = transformed cell

- if transformed cells acquire...
 - ▲ altered morphology
- visible as a focus (light microscope at low magnification)
 - see Figure 3.5

focus formation assay

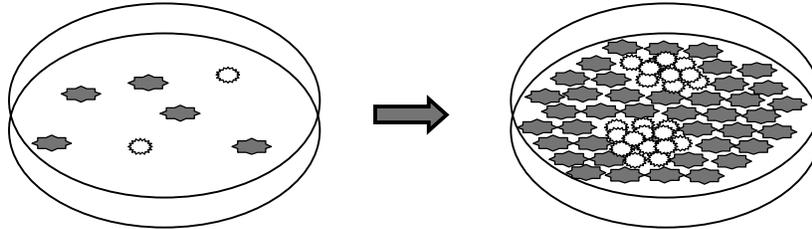
- treat normal cell culture with tumorigenic agent
- plate cells at low dilution



- if transformed cells acquire...
 - ▲ loss of contact inhibition
 - ▲ loss of anchorage dependence
- visible as a focus (more obvious - can see by eye!)

focus formation assay

- the focus formation assay is ideal for quantitation
- each original transformation event yields a single focus



- also, each focus can be individually picked and re-cultured
- allows the isolation of a cell clone containing the progeny of the original transformed cell.

colony formation assay

- based on:
 - loss of anchorage dependence
- treat normal cells with oncogenic agent
- dilute cells in “soft agar” (0.3% agar)
- pour agar onto petri dish
 - cells are suspended in agar
 - normal cells will not divide (insufficient anchorage)
- progeny of rare transformed cells give rise to a “colony” of growing cells (visible by eye or under low magnification)
- colonies can be counted and picked

in vivo tumorigenesis

- inject cultured cells into animals...
- look for and count tumors.
- common hosts:
 - adult mice
 - fetal/newborn mice → immature immune system
 - “nude” mice → no T cell immunity
 - SCID mice → no B or T cell immunity
- correlations between *in vitro* properties and *in vivo* tumorigenicity of transformed cells are not absolute.

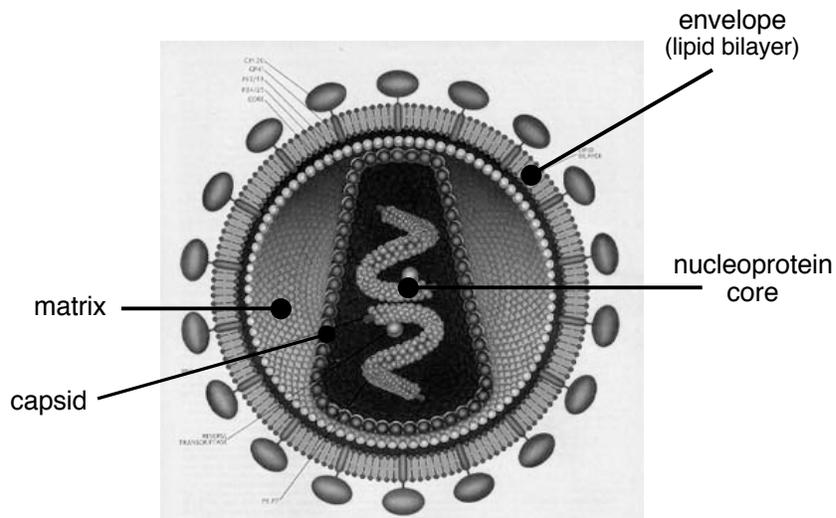
Retroviruses

- Key features of all retroviruses:
 - an enveloped virion
 - ssRNA genome
 - all retroviral genomes have *gag*, *pol*, and *env* genes
 - all encode a reverse transcriptase
- Let's consider Avian Leukosis Virus (ALV) as a prototype of an oncogenic retrovirus

Retroviral proteins

- Each retroviral gene encodes a single polyprotein that is subsequently processed by proteolysis to form mature viral proteins.
 - *gag* encodes – matrix protein (MA)
 - capsid protein (CA)
 - nucleocapsid protein (NC)
 - *pol* encodes – reverse transcriptase (RT)
 - integrase (IN)
 - protease (PR)
 - *env* encodes – surface protein (SU)
 - transmembrane protein (TM)

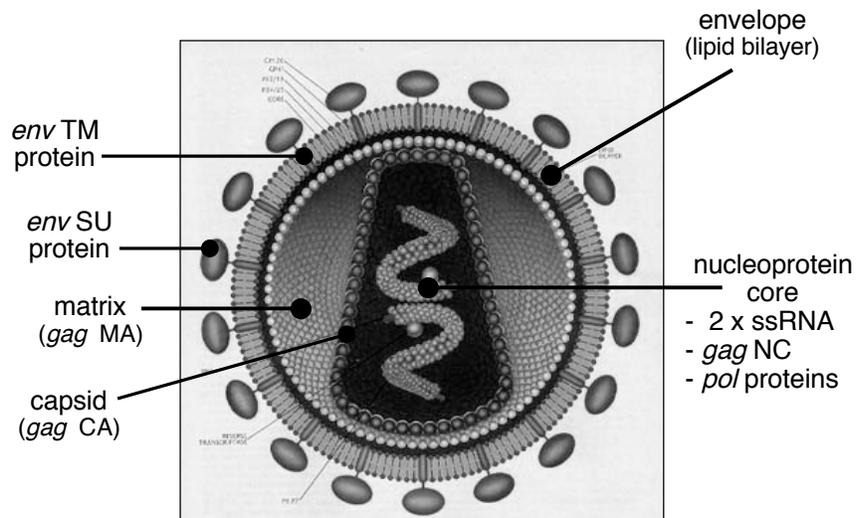
Retroviral virion



Retrovirus Virion

- The virion of a retrovirus contains...
 - envelope: a lipid bilayer containing viral *env* proteins
 - matrix: a lattice of *gag* MA proteins attached to the inner surface of envelope
 - capsid: an icosahedral assembly of *gag* CA proteins
 - nucleoprotein core:
 - ◆ 2 copies of the ssRNA genome (~9000 nucleotides)
 - ◆ the *gag* NC protein
 - ◆ the *pol* gene products (reverse transcriptase and integrase)
 - ◆ various host proteins and host tRNAs

Retroviral virion

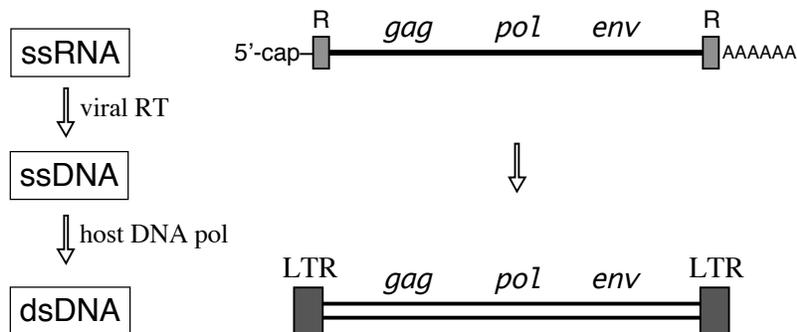


retroviral infection

- host range of a retrovirus
 - determined principally by the *env* protein
 - species specificity: ALV infects chickens
 - tissue specificity: ALV infects most cell types
- early stages of infection
 - *env* binds specific receptors on host cell membrane
 - fusion of viral envelope and host cell membrane
 - viral proteins enter cytoplasm

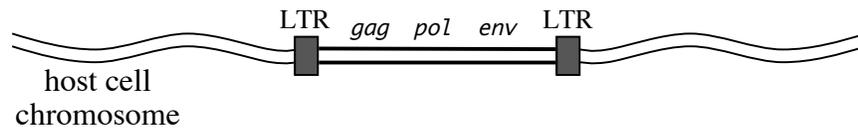
Reverse transcription

- Reverse transcription occurs in the cytoplasm
 - generates a dsDNA genome
 - flanked by LTRs (long terminal repeats)



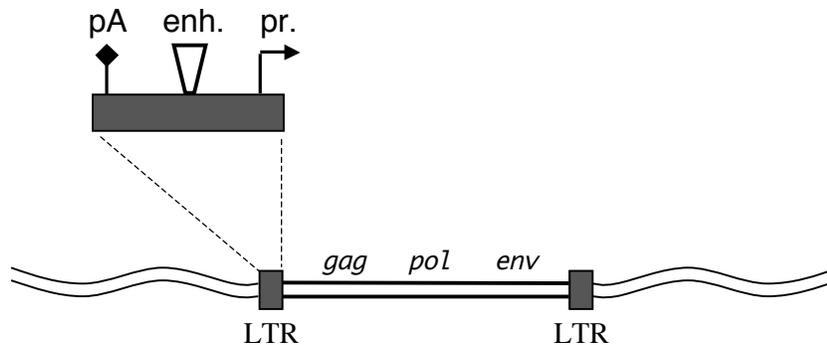
Proviral integration

- viral dsDNA migrates into the cell nucleus
- circularization of dsDNA
- integration of dsDNA into host genome
 - catalyzed by viral integrase protein
 - site-specific with respect to the viral genome
 - random with respect to the host genome
 - generates a “provirus”



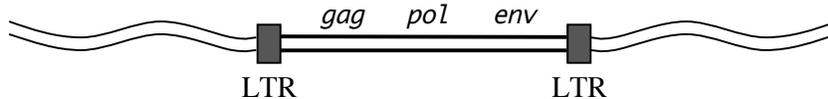
Proviral transcription

- the provirus is an active transcription unit
 - each LTR contains
 - promoter
 - enhancer
 - polyA signal



Proviral transcription

- transcription products of the provirus
 - full-length RNA
 - ◆ mRNA to encode gag and pol polypeptides
 - ◆ genomic RNA for next generation of virions
 - spliced RNA (excises *gag* and *pol* sequences)
 - ◆ mRNA for the env polyprotein



Proviral replication

- During cell division, the provirus is replicated along with the host genome
 - all daughter cells inherit the provirus
- Retroviruses can also infect germline cells (e.g., oocytes or spermatocytes).
 - provirus then becomes part of the genetic material of the species
- Endogenous retrovirus
 - constitute > 0.1% of mouse genome
 - usually transcriptionally inactive

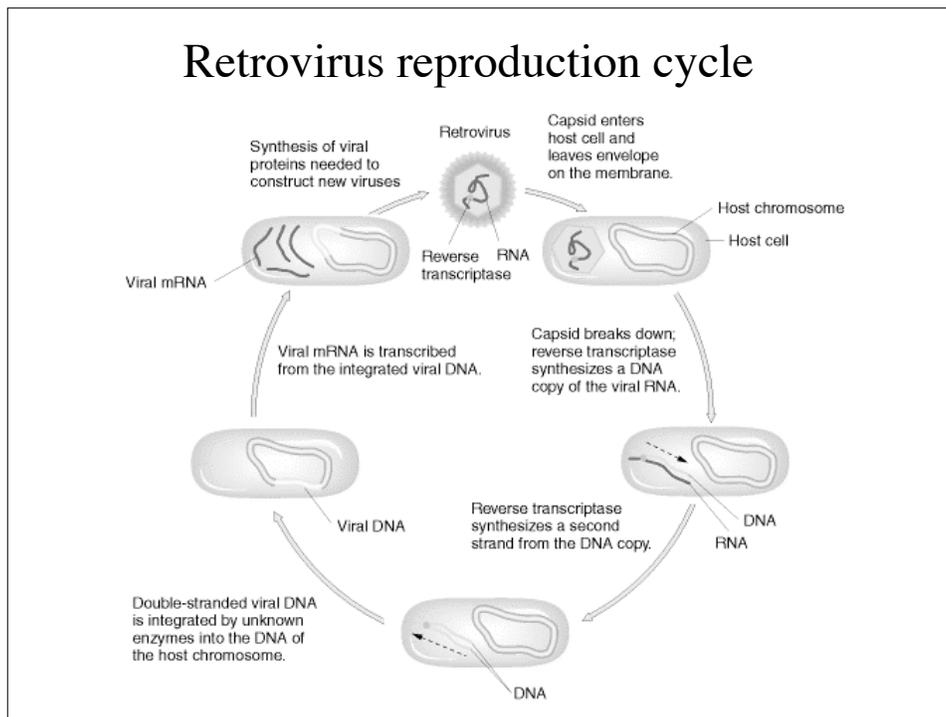
Virion production - I

- *env* proteins
 - processed through the secretory pathway and inserted into host cell membrane.
- *pol* proteins (RT, integrase, protease)
 - associate with ssRNA genome to form the nucleoprotein core (in cytoplasm).
- *gag* proteins
 - associate with the nucleoprotein to form a capsid (in cytoplasm).

Virion production - II

- the capsid buds from the host cell membrane
- while the capsid buds out, a segment of the *env*-impregnated cell surface encircles it and forms the viral envelope.
- ALV replication is not cytopathic
 - Infected cells are difficult to distinguish from uninfected cells. To do so may require...
 - ◆ serological analysis to detect viral antigens
 - ◆ electron microscopy to visualize intracytoplasmic capsids and budding virions.

Retrovirus reproduction cycle



Transmission of retroviruses

- Horizontal transmission (from another animal)
- Vertical transmission (from parents)
 - Genetic transmission
 - ◆ animal inherits endogenous provirus.
 - ◆ rarely significant in oncogenesis (except in genetically susceptible strains, such as AKR mice).
 - Congenital infection
- Experimental transmission (by a scientist)

Horizontal transmission (ALV)

- re. - ALV infects cells from a broad spectrum of chicken tissues.
- if chick is more than a few days old (post-hatching) at infection:
 - transient viremia develops (virions in bloodstream)
 - chick produces neutralizing antibodies
 - viremia clears; chick immune to further infection
 - chick does not develop virally-induced lymphoma

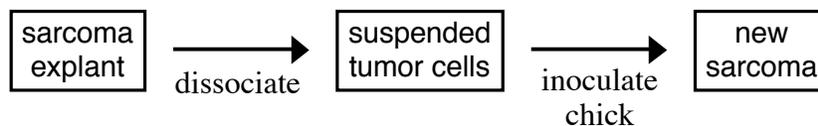
Congenital Infection (ALV)

- mother infects her offspring
 - chickens - infection of egg while in the female reproductive organs
 - mammals - transmission via placenta or milk
- consequences of congenital infection with ALV:
 - viremia occurs during embryonic development
 - chick develops immunological tolerance to ALV
 - viremia continues
 - lymphomas develop during adulthood

Acutely Transforming Retroviruses

Acutely Transforming Retroviruses

- Peyton Rous (Rockefeller University)
 - observed a spontaneous sarcoma in a chicken
 - in retrospect, we know that this chicken was from a flock congenitally infected with ALV
- Rous propagated the sarcoma cells by passaging from chicken to chicken.



Rous Sarcoma Virus (RSV)

- In 1911, Rous prepared a cell-free filtrate from one of his sarcoma explants
 - passed the dissociated explant through filter paper, removing bacterial & eukaryotic cells
- inoculated chicks with the filtrate
- chicks developed sarcomas at site of injection!!
- Rous Sarcoma Virus (RSV)



RSV is a powerful transforming agent

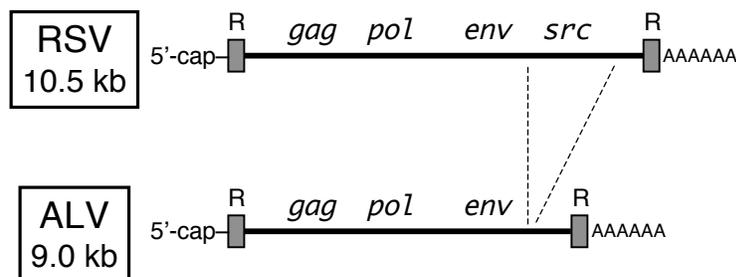
- induces visible sarcoma in chicks within 2 weeks (ALV does not).
 - “acutely” transforming
- even induces sarcomas in immuno-competent adult chickens (ALV does not)
- causes focus formation in cultured chick fibroblasts (ALV does not)

Why is RSV so tumorigenic ?

- especially compared with ALV
 - RSV: “acutely-transforming retrovirus”
 - ALV: “slowly-transforming retrovirus”
- RSV and ALV: – structurally similar
– infect similar cells

The *v-src* gene

- The RSV genome harbors additional sequences of ~ 1,500 nucleotides (*v-src*, for “viral src”).



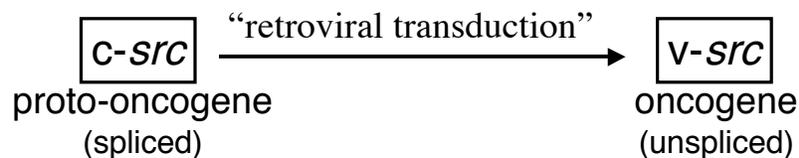
- Is *v-src* responsible for the increased malignant potential of RSV ?

The origin of *v-src* sequences

- *v-src* is absent from almost all other retroviral genomes. Thus, where did it originate ?
- in 1977, Bishop & Varmus reported that genomic DNA from normal, uninfected chicken cells harbors a close homolog of *v-src*
 - *c-src* (for “cellular src”)
 - the *c-src* gene is conserved phylogenetically
 - *c-src* functions in normal animal development

Retroviral transduction

- RSV originally appeared in a spontaneous sarcoma from an ALV-infected chicken
- thus, it was proposed that RSV arose by incorporation of *c-src* sequences into ALV
- acquisition of *c-src* converted a slowly-transforming retrovirus (ALV) into an acutely-transforming retrovirus (RSV).



- other acutely transforming retroviruses have been identified in chickens, cats, mice, and rats (> 100 independent isolates).

<u>retrovirus</u>	<u>species</u>	<u>tumor</u>	<u>v-onc</u>
Y73	avian	sarcoma	v-yes
MC29	avian	myelocytomatosis	v-myc
AMV	avian	myeloblastosis	v-myb
Harvey	mouse	sarcoma	v-H-ras
Kirsten	mouse	sarcoma	v-K-ras
Abelson	mouse	leukemia	v-abl

> 40 different v-onc genes identified; all derived from cellular (c-onc) genes by retroviral transduction.

How are transduced oncogenes rendered malignant?

- Quantitative effects:
 - inappropriate expression - the transduced gene is regulated transcriptionally by the LTR.
 - elevated expression - LTR is a strong promoter.
- Qualitative effects:
 - oncoprotein can be expressed in a truncated or fused form.
 - transduced oncogenes can acquire point mutations during viral replication (due to low fidelity of RT).

Slowly-transforming retroviruses

Slowly-transforming retroviruses

- chicks congenitally infected with ALV
or
- inoculate eggs or young chicks with ALV



- immunological tolerance
- persistent viremia

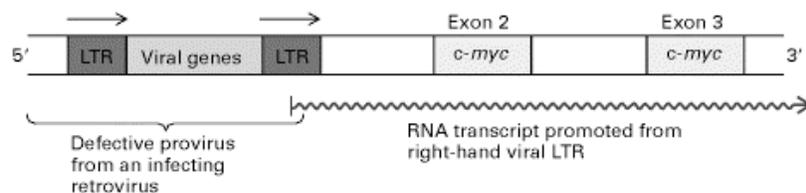
- all adults develop enlarged livers and spleens, infiltrated with B-lymphoblasts
- B cell lymphomas
 - tumor cells contain integrated ALV sequences

Patterns of ALV proviral integration

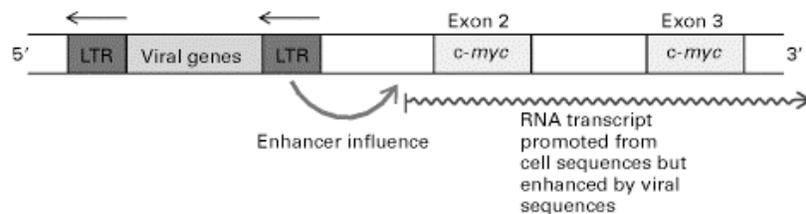
- All cells within a particular tumor have the same pattern of proviral insertion (i.e., each tumor is monoclonal)
 - same # of proviruses inserted
 - same integration sites within the chicken genome
- Common integration sites were found in independent tumors from different chickens.
- Hypothesis: ALV transforms cells by insertional mutagenesis of a specific host gene.
- molecular cloning → ALV integration near *c-myc* in 80% of ALV-induced lymphomas!!
 - provirally altered *c-myc* alleles are transcribed at high rates (due to integrated LTR sequences).

Activation of c-Myc by proviral insertion

(a) Promoter insertion



(b) Enhancer insertions



Contrasting ALV- and RSV-induced transformation

- insertional mutagenesis can explain differences in the natural history of ALV- and RSV-induced lymphomas
 - long latency of ALV-induced tumor formation
 - ◆ ALV provirus integrates randomly in host genome
 - ◆ only in a rare cell, does ALV integrate adjacent to a proto-oncogene in a manner that activates its malignant potential
 - the monoclonality of ALV-induced lymphomas
 - the inability of ALV to transform cells *in vitro*

Other tumors induced by ALV

- chickens congenitally infected with ALV can also develop other forms of cancer as adults.
 - these forms usually develop later than lymphomas
 - these forms become prominent in bursectomized chickens (in which the bursa has been surgically removed).
- ALV-induced nephroblastomas:
 - proviral integration at the *c-H-ras* proto-oncogene
- ALV-induced erythroleukemias:
 - proviral integration at the *c-erbB* proto-oncogene

Slowly-transforming retroviruses in other species

- chickens
 - Avian Leukosis Virus (ALV)
- mice
 - Murine Leukemia Virus (MuLV)
 - Mouse Mammary Tumor Virus (MMTV)
- cats
 - Feline Leukemia Virus (FeLV)
- humans
 - none?

Contrasting ALV- and RSV-induced transformation

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Retroviral oncogenesis

Two major mechanisms identified in animals...

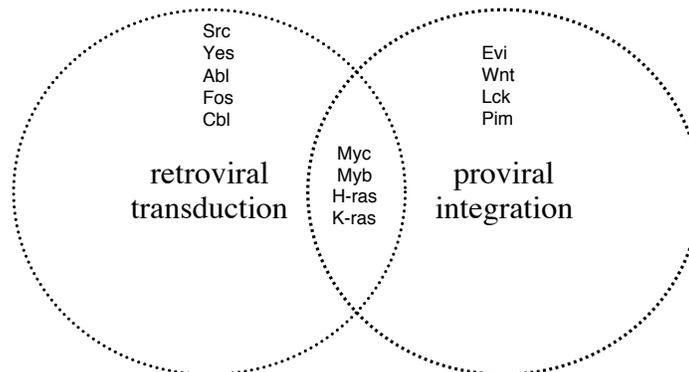
■Retroviral Transduction

- mediated by the “acutely-transforming retroviruses”
 - ◆ Abelson Murine Leukemia Virus (A-MuLV)
 - ◆ Rous Sarcoma Virus (RSV)
- these retroviruses carry a transduced oncogene

■Proviral Integration

- mediated by the “slowly-transforming retroviruses”
 - ◆ Murine Leukemia Virus (MuLV)
 - ◆ Mouse Mammary Tumor Virus (MMTV)
 - ◆ Avian Leukosis Virus (ALV)
- normal retroviral genome (e.g., gag, pol, env)

Many proto-oncogenes identified in studies of retroviral tumorigenesis in animals



→ Are these proto-oncogenes involved in human cancer?