Transformation and Oncogenesis

Session 18

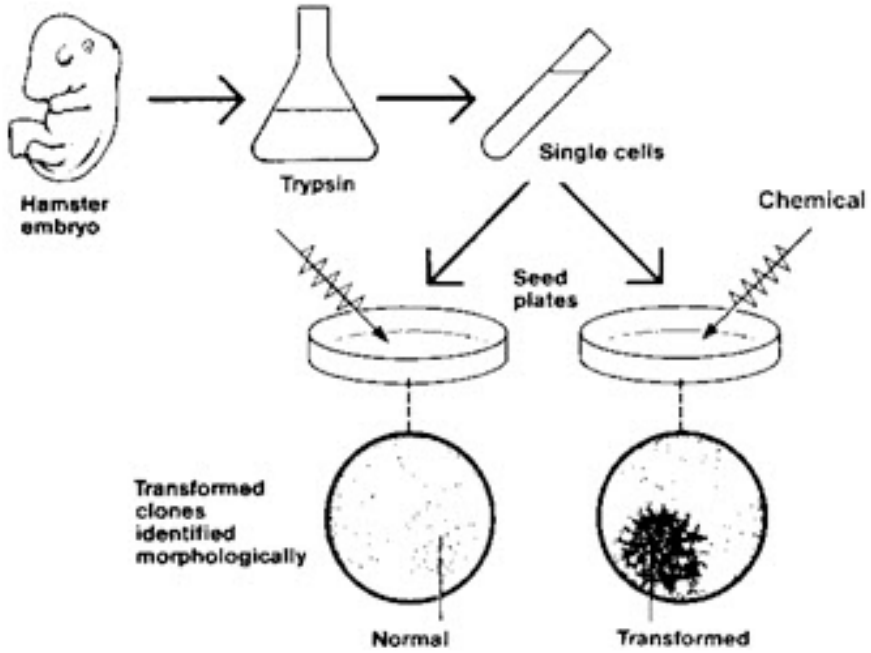
Virology Live

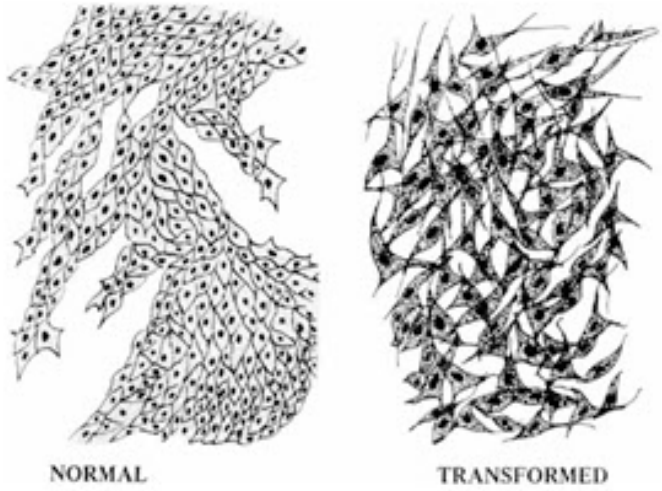
Fall 2021

Cause and effect, means and ends, seed and fruit, cannot be severed; for the effect already blooms in the cause, the end pre-exists in the means, the fruit in the seed.

Ralph Waldo Emerson

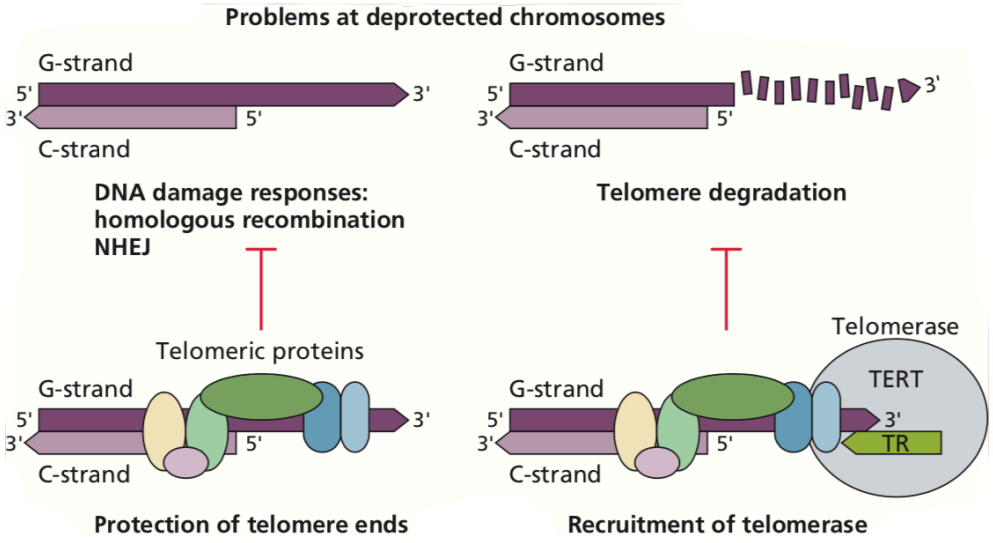
Transformation





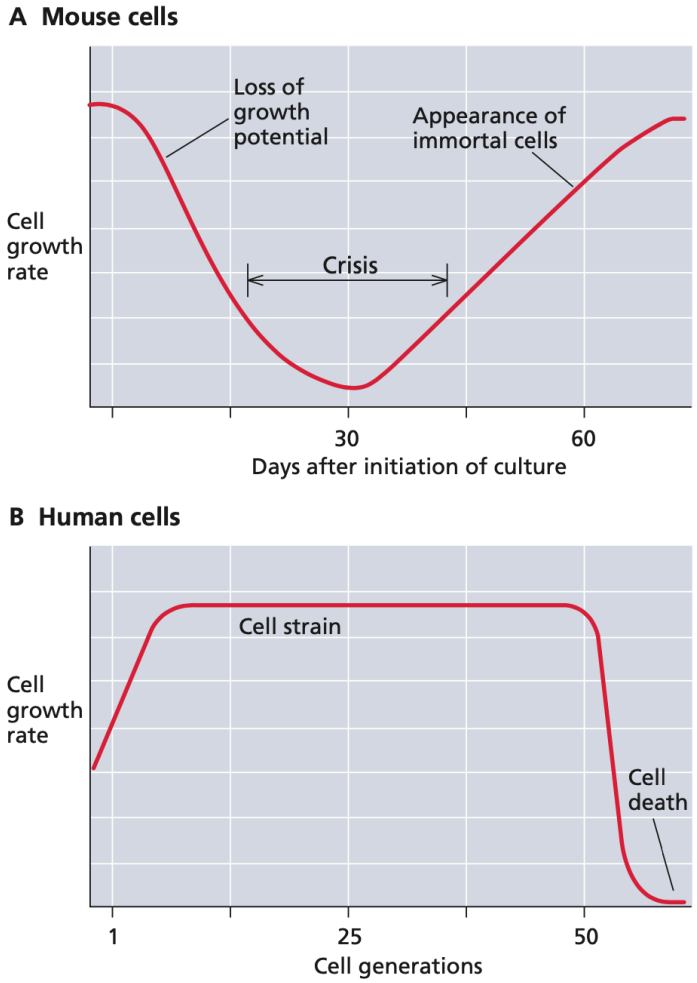
Telomeres, telomerase, and cellular immortality

- Telomerase active in all cells during early embryonic development
- Most mammalian somatic cells do not produce TERT
- In culture, telomeres shorten with each cell division, at ~4 kbp cells die; fibroblasts only survive 50 divisions
- Mouse telomeres are longer, fibroblasts in culture can proliferate more generations, increasing the chance of spontaneous mutations leading to immortality (e.g. telomerase production)



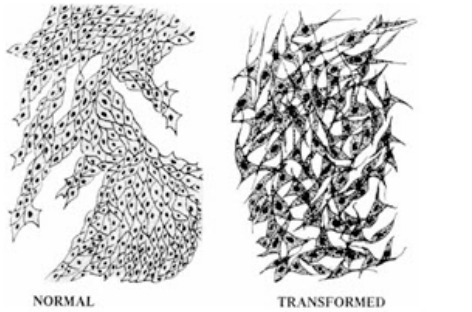
Principles of Virology, ASM Press

Stages in the establishment of a cell culture



The puzzling properties of transformed cells in the laboratory

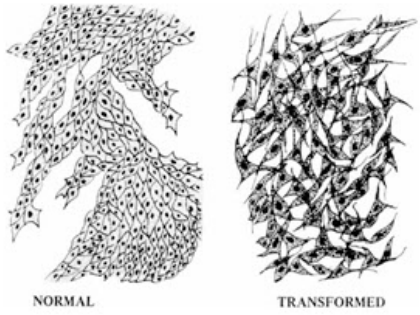
- Immortal: Grow indefinitely (HeLa)
- Loss of anchorage dependence
- Loss of contact inhibition
- Colony formation in semi-solid media
- Decreased requirements for growth factors (serum)



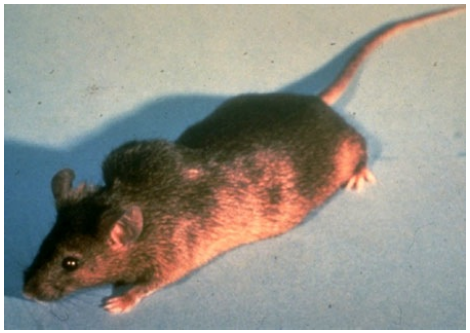
Oncogenesis

- Development of cancer
  - Tumor: swelling caused by abnormal growth of tissue, benign or malignant
- Cancer is a genetic disease
- 8.2 million deaths/yr developed countries
- Mutations (~12) affect signal transduction pathways that govern cell proliferation, survival, determination of cell fate, maintenance of genome integrity
- Mutations may be inherited, caused by DNA damage, environmental carcinogens, infectious agents including viruses

Transformation and oncogenesis are distinct



May not be oncogenic



Requires additional genetic changes

- Studying virus-transformed cells provides insight into molecular events that establish oncogenic potential
- *No virus can do it all*

Human cancer viruses

Virus	Cancer
RNA viruses	
Human T-lymphotropic virus-1	Adult T cell leukemia
Human immunodeficiency virus-1	Many tissues and organs
Hepatitis C virus	Hepatocellular carcinoma
DNA viruses	
Epstein-Barr virus	Burkitt's lymphoma
Kaposi's sarcoma herpesvirus	Kaposi's sarcoma Primary effusion lymphoma Multicentric Castleman's disease
Hepatitis B virus	Hepatocellular carcinoma
Human papillomavirus	Cervical, penile, anogenital, head and neck cancers
Merkel cell polyomavirus	Merkel cell carcinoma

Contributing factor in ~20% of human cancers

Virus-induced cancer

Transformation and oncogenesis is not required for replication of any virus*

except Walleye dermal sarcoma virus

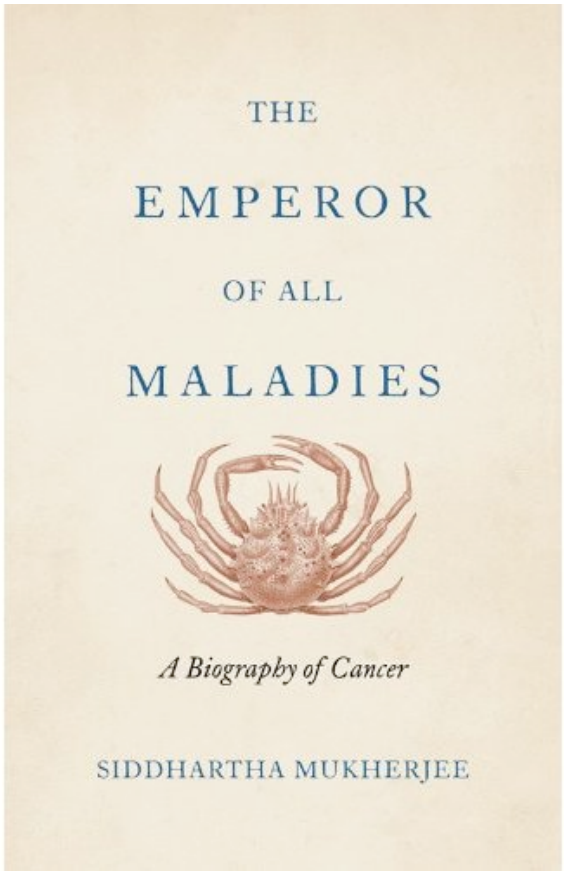
On October 1, 1909, Dr. Peyton Rous removed a tumor from an English hen and injected a cell-free filtrate from the tumor into another healthy chicken, which later developed the same type of tumor

Cancer could be caused by a viral infection!

It took the world almost 50 years to accept this idea

Dr. Rous lived long enough to be awarded the Nobel Prize for Physiology and Medicine in 1966 for his research

His legacy: RSV; Rous Sarcoma Virus, a key player in two more Nobel Prizes



Scribner, 2011

"By the 1950s, cancer researchers had split into three feuding camps.

The virologists, lead by Rous, claimed that viruses caused cancer, although no such virus had been found in human studies.

Epidemiologists...argued that exogenous chemicals caused cancer, although they could not offer a mechanistic explanation.

The third camp possessed weak, circumstantial evidence that genes internal to the cell might cause cancer...

Howard Temin

In 1951, a young virologist named Howard Temin arrived at Cal Tech to study the genetics of fruit flies. Restless and imaginative, he soon grew bored with fruit flies. Switching fields, he chose to study Rous sarcoma virus in Renato Dulbecco's laboratory.

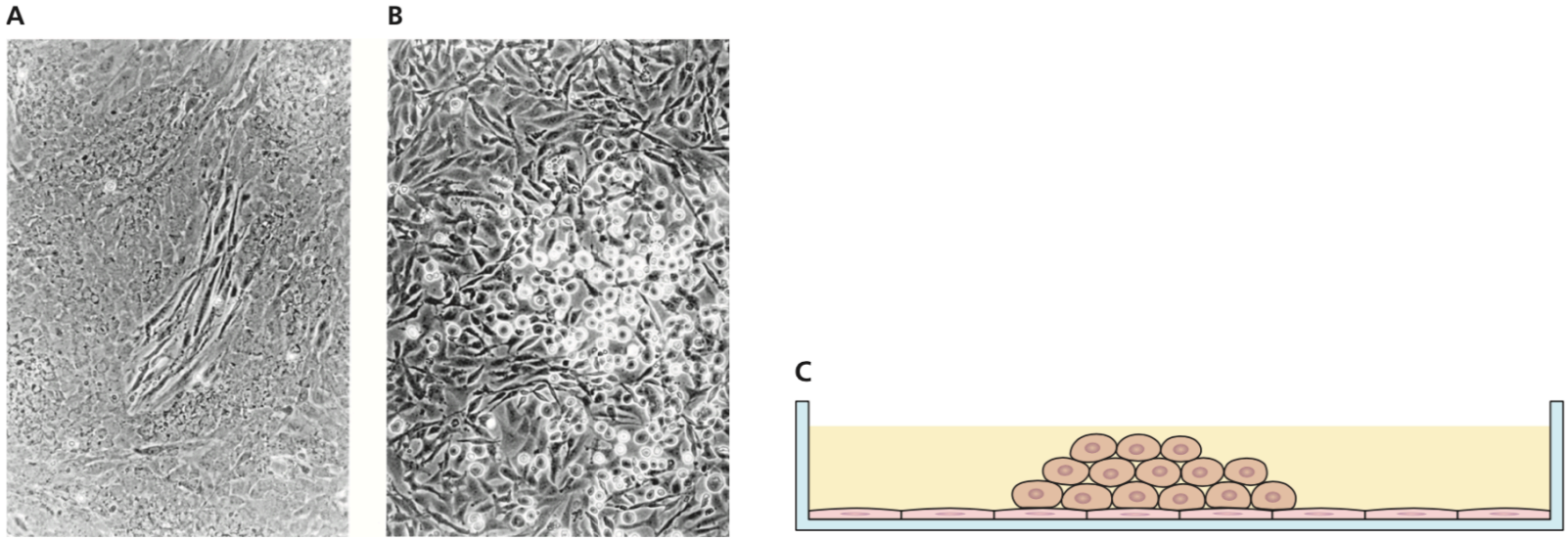
Until the late fifties, Rous sarcoma virus had been shown to cause tumors only in live chickens. Temin imagined creating *cancer* in a petri dish. In 1958, in his seventh year in Dulbecco's lab, Temin succeeded.

Scribner, 2011

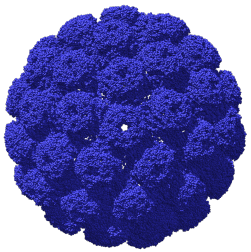
He added Rous sarcoma virus to a layer of normal cells in a petri dish. The infection of the cells incited them to grow uncontrollably, forcing them to form tiny distorted heaps containing hundreds of cells that Temin called foci. The foci, Temin reasoned, represented cancer distilled into its essential, elemental form: cells growing uncontrollably, unstoppably - pathological mitosis.

Temin went on to discover RT in RSV

Avian cells transformed by RSV



Principles of Virology, ASM Press



Transformation of cells by viruses

- 1962: After infection with polyomavirus, rare BHK21 cells changed shape, kept growing
- 1964: After infection of Swiss 3T3 cells with SV40, rare cells grew as colonies

Most of the infected cells died, but rare cells did not

They were *"transformed"*

How can a viral infection transform a cell?

- Cytopathic effects must be reduced or eliminated
  - *The infected cell does not die*
- Viral replication must be reduced or eliminated
  - *Transformed cells do not produce virus particles*
- The cell must continue to divide
  - *It becomes immortal*

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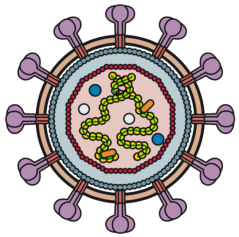
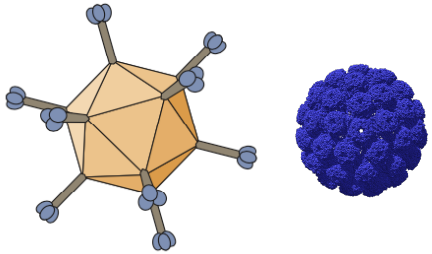
room number: virus

Which of the following is not a property of transformed cells?

A. Increased requirements for growth factors
B. Immortality
C. Loss of anchorage dependence
D. Loss of contact inhibition
E. Colony formation in semi-solid media

Route to understanding viral transformation of cells in culture and relationship to cancer was convoluted





(1900s) Retroviruses

(1943) in vitro cancer biology

(1920s) DNA Viruses

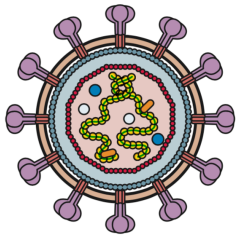
(1950s) in vitro studies with RSV

Convergence (1960s, 1970s)

(1959) in vitro studies with polyomavirus

(present) Unified theory of cell growth control

How does Rous sarcoma virus cause tumors in chickens and transform cells *in vitro*?



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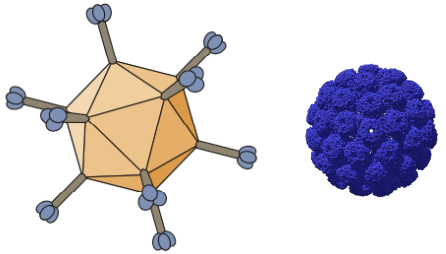

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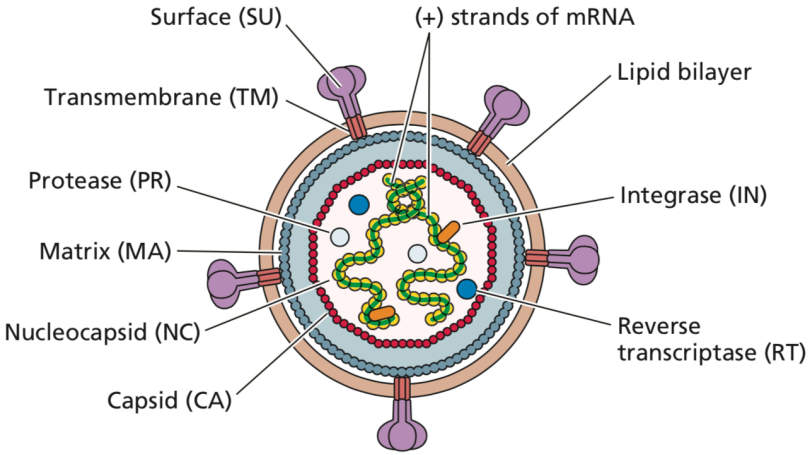
Avian leucosis retroviruses (ALV) are endemic in virtually all chicken flocks



- Ellerman & Bang 1908
- Most chickens infected with ALV within a few months of hatching
- Leucosis (leukemia) occurs sporadically in infected birds >14 wk old (3%)
- 97% of birds have transient viremia, become immune, don’t get leukemia

Principles of Virology, ASM Press

Infected birds develop other cancers as they age

Rous was lucky!

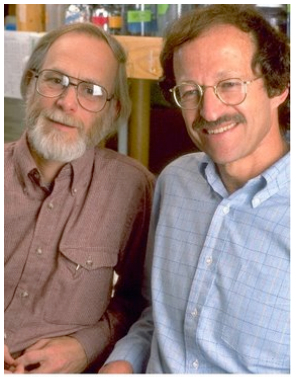
- Connective tissue tumors or sarcomas (solid tumors)
- Viruses isolated from these solid tumors rapidly cause sarcomas, not leucosis
- Rous isolated one of these viruses: Rous sarcoma virus, RSV
- Most of these sarcoma viruses are *defective*

How does RSV, but not ALV, cause sarcomas?

- Key finding: the viral genomes from solid tumors were recombinants!
- A piece of the ALV genome is replaced with a *segment of host DNA* called an *oncogene*

J. Michael Bishop and H. Varmus identified the oncogene (v-SRC) carried by Rous sarcoma virus in 1976

Nobel Prize to both in 1989 for this discovery



J. Michael Bishop (1936 -)
Harold E. Varmus (1939 -)

http://www.microbe.tv/twiv/twiv-400/

https://youtu.be/frbMV-YGgQU

Major insight

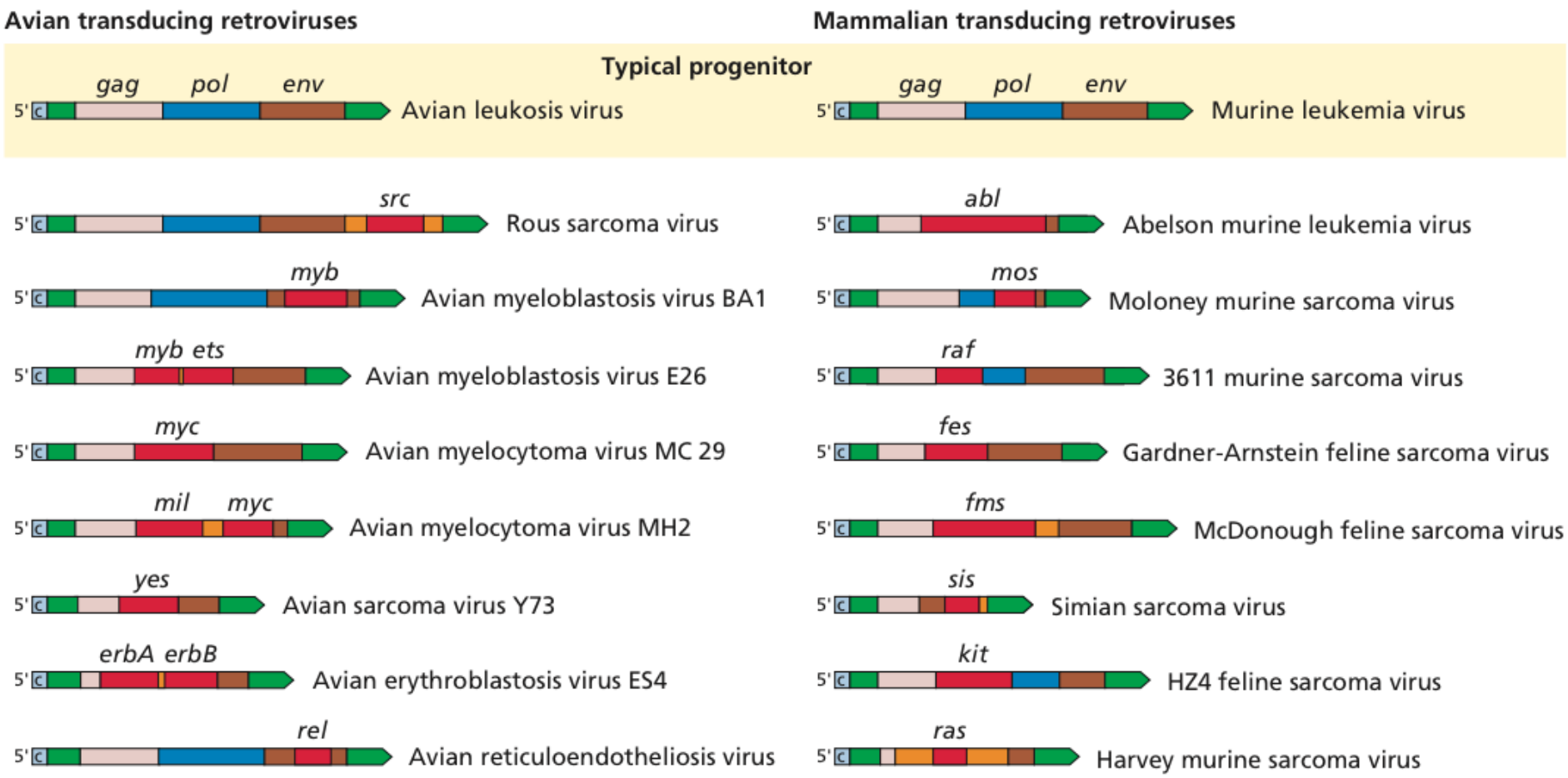
- ALV infected birds came down with a variety of tumors
- These rare tumors all contained retroviruses derived from ALV, but most were defective and all were different
- Rous was lucky - *his RSV isolate was not defective*

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

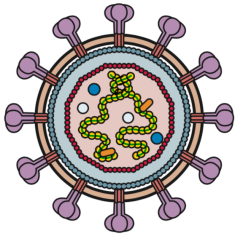
A gold mine for molecular oncology

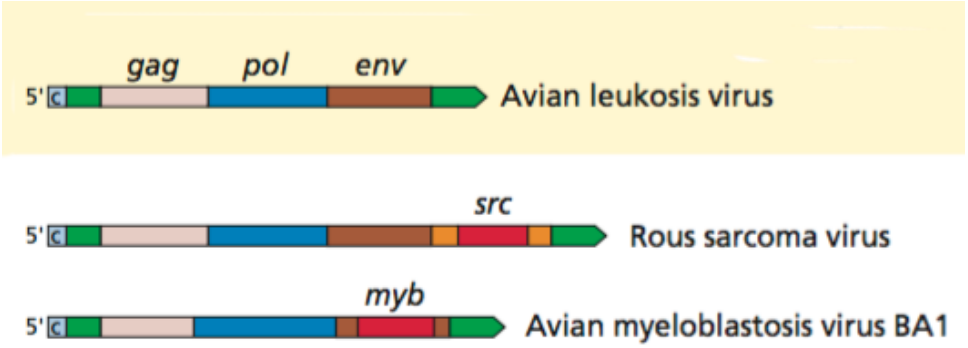
Genomes of transducing retroviruses



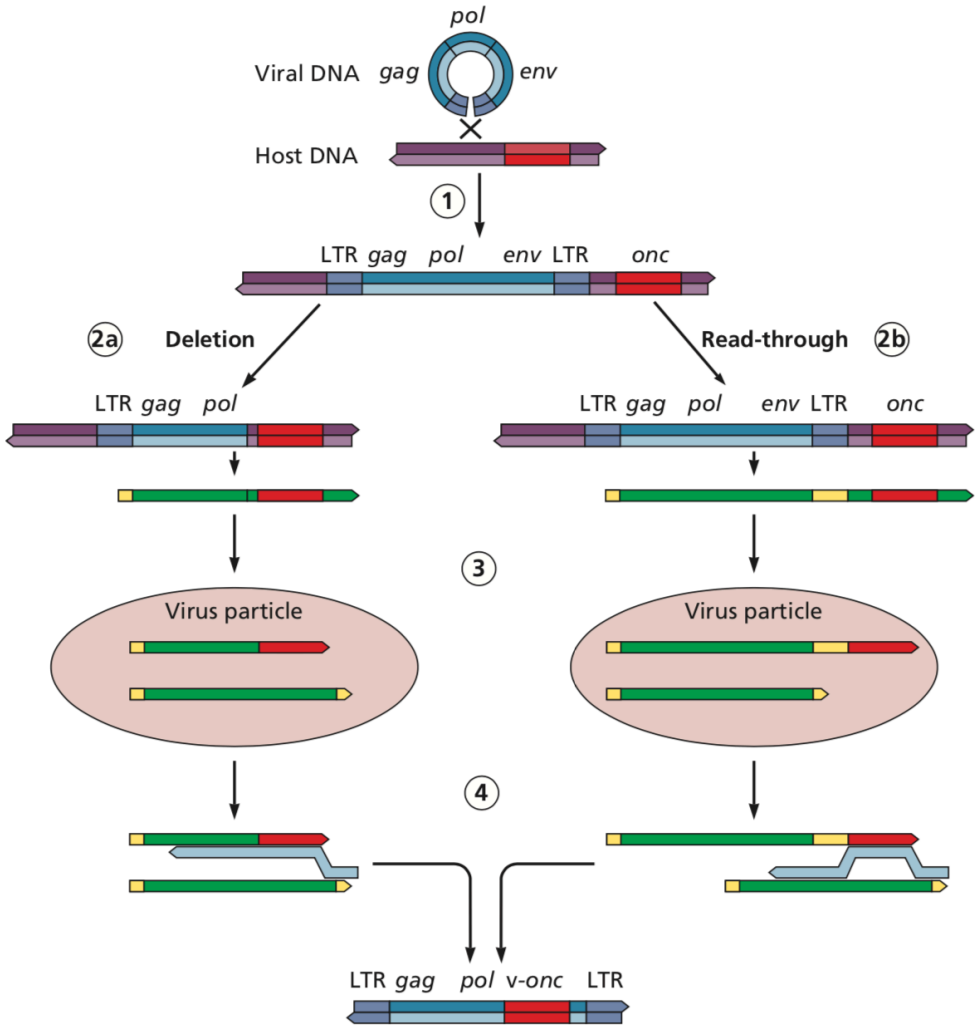
Defective vs non-defective retroviruses

- Defective viruses require helper virus to produce more virus
- Usually missing envelope proteins
- Envelope genes deleted during oncogene capture



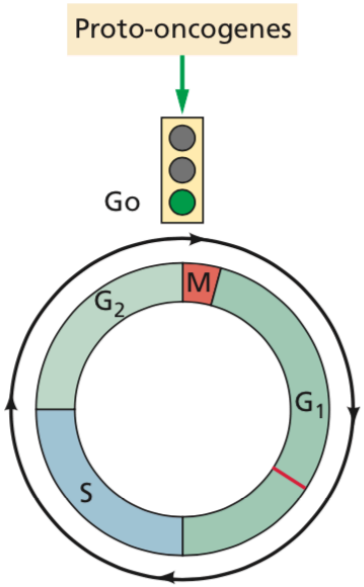


Mechanism for oncogene capture



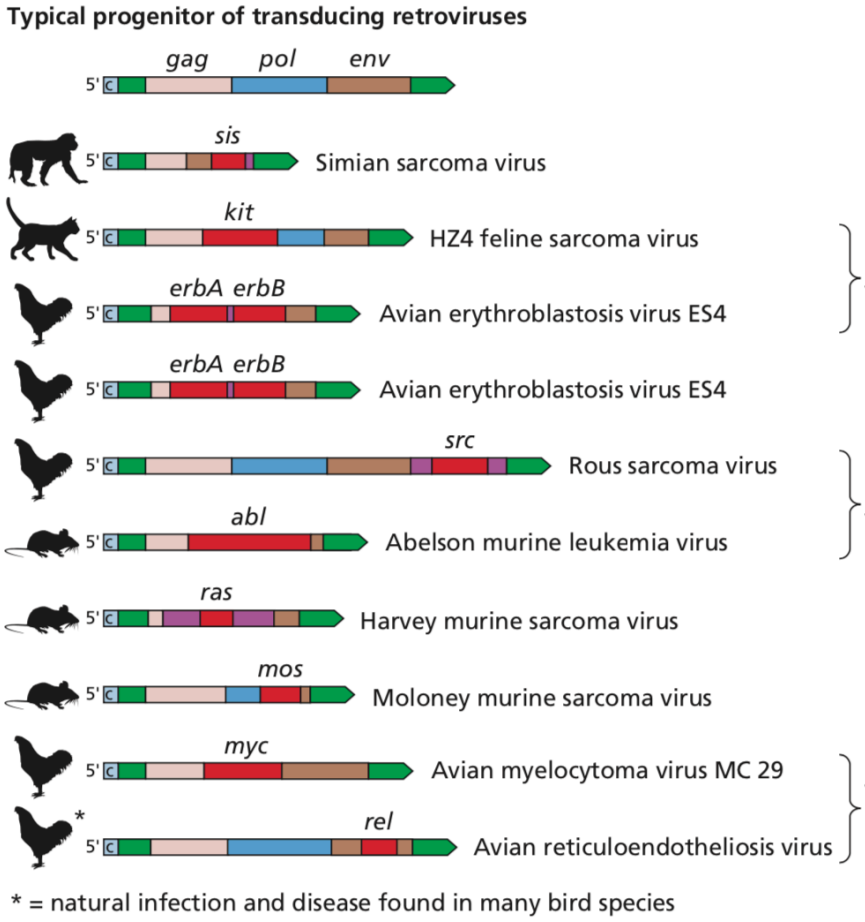
Principles of Virology, ASM Press

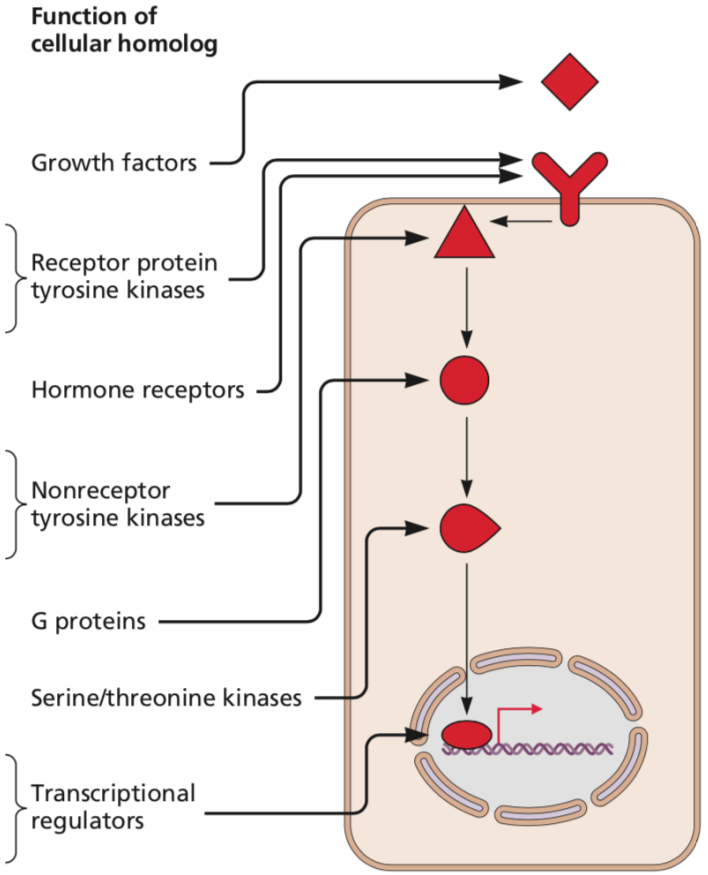
Proto-oncogenes



- >60
- In all cells, control cell growth
- Highly regulated
- Normal cellular genes abbreviated as c-ONCS, eg c-SRC, c-MYC, c-MOS, C-RAS
- Certain retroviruses isolated from tumors carry altered copies of c-ONCS abbreviated as v-ONCS, eg v-SRC, v-MYC, v-MOS, v-RAS

Subcellular location of major classes of oncoproteins



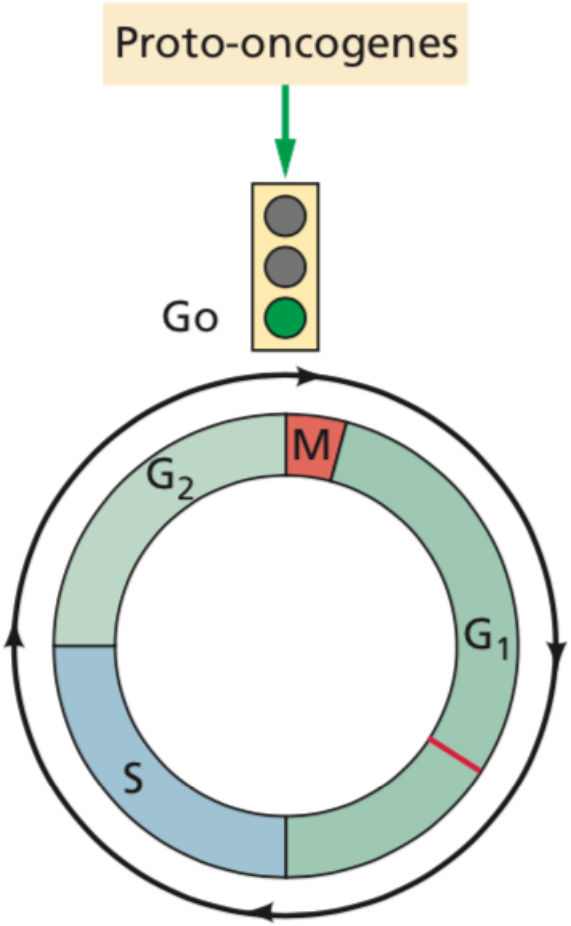


Original phenotype in cultured cells:

TRANSFORMATION

Uncontrolled cell growth

The cell cycle

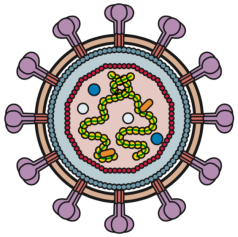


Mitogenic signals revealed by studying

transforming retroviruses

Dominant oncogenes

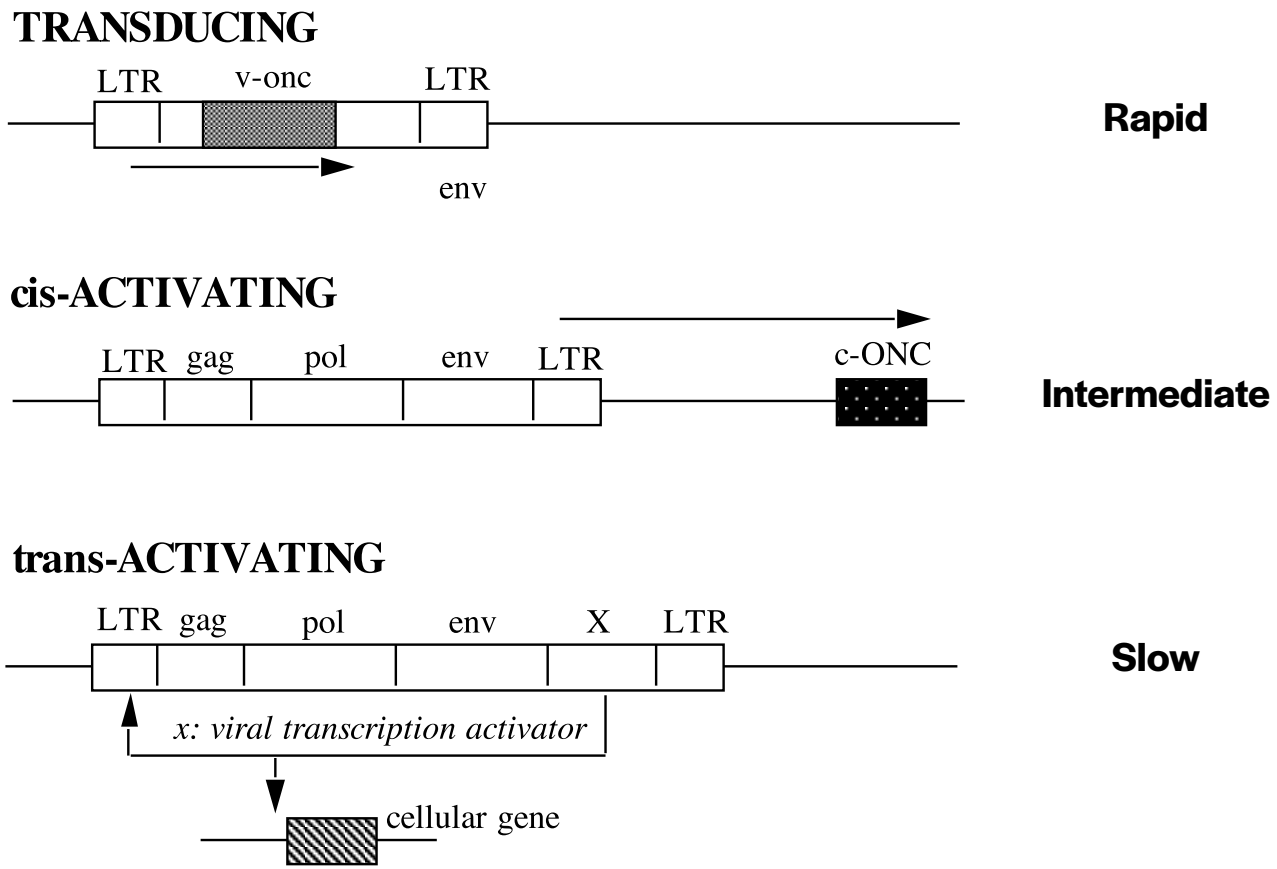
Principles of Virology, ASM Press



Retroviruses transform cells by three mechanisms

- Rapid tumor formation: eg RSV; 2 weeks
  - *RSV has activated dominant oncogene in genome (v-SRC)*
  - *Protein produced immediately when virus replicates*
- Intermediate kinetics of tumor formation: eg ALV; months
  - *ALV carries no dominant v-ONC gene*
  - *cis-activation: provirus turns on expression of endogenous oncogene*
- Slow kinetics of tumor formation; eg HTLV; years
  - *HTLV carries no dominant v-ONC gene*
  - *Does not cause cis-activation of local oncogenes*
  - *A viral regulatory protein activates oncogenes by trans-activation*

Proviruses with different transforming potential



e.g., IL2 and the IL2 receptor

Mammalian transforming retroviruses

- Retroviruses transform cells as a mistake or byproduct of their life cycle - *their DNA must integrate into the host cell chromosome*
- No obvious viral requirement for transformation or for oncogenesis

Walleye dermal sarcoma virus

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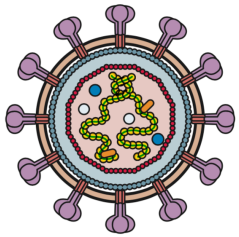
room number: virus

Which of the following allows Rous sarcoma virus to transform cells?

A. Presence of the env gene
B. Presence of a pol gene
C. Presence of a *src* gene
D. Presence of LTRs
E. None of the above

The study of DNA virus transformation also revealed how the cell cycle is regulated



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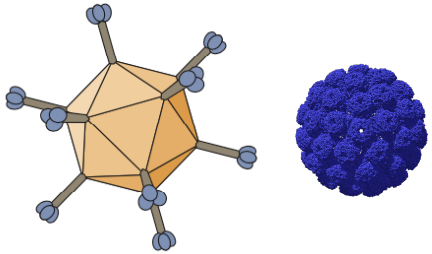

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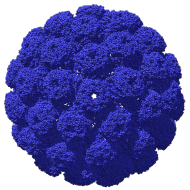
(1943) *in vitro cancer biology*

(1950s) *in vitro* studies with RSV

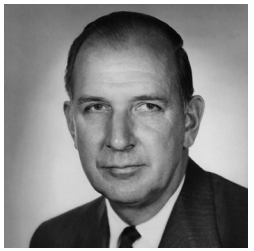
Convergence (1960s, 1970s)

(1959) *in vitro* **studies with polyomavirus**

(present) Unified theory of cell growth control

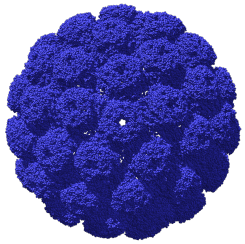


DNA tumor viruses: *Papillomaviridae*

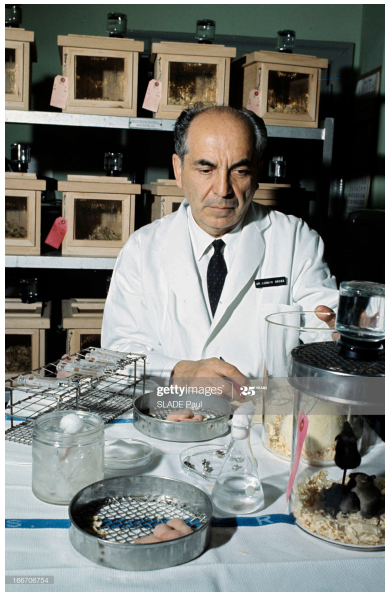


First oncogenic DNA virus discovered was *papillomavirus* that causes warts (papillomas) in cottontail rabbits - Richard Shope, 1933

Jackalope?

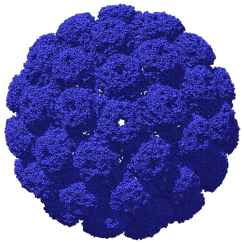


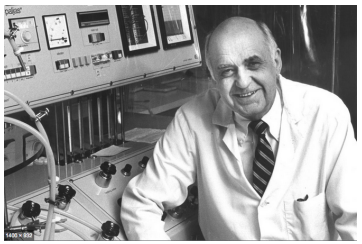
DNA tumor viruses: *Polyomaviridae*



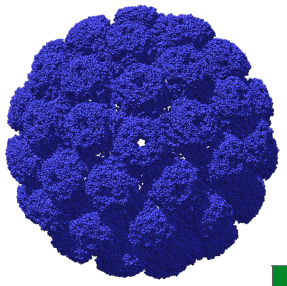
- Ludwig Gross discovered murine polyomaviruses in 1953
- Caused **rare** tumors under certain conditions
  - Natural host is the mouse
  - Ubiquitious in mice; no role in mouse cancer
  - Makes tumors of many tissues (poly-oma) in infant hamsters, rats, rabbits

DNA tumor viruses: *Polyomaviridae*





- Eddy and Hilleman showed that SV40, a contaminant of early poliovirus vaccines, induced **rare** tumors in newborn hamsters - 1962
- Several million Americans were infected with SV40 by poliovirus immunization
  - *Natural host is monkey (simian virus 40)*
  - *Causes no tumors in monkeys*
  - *Does not transform monkey cells in culture*

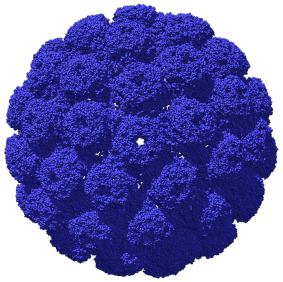


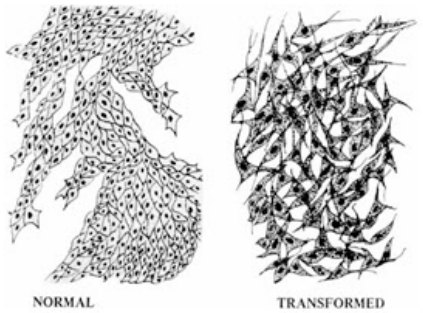
Response of different cells to infection

Species	SV40	Mouse polyomavirus
Monkey	*Permissive*	Non-permissive
Mouse	Non-permissive	*Permissive*
* Hamster	Semi-permissive	Semi-permissive
* Rat	Semi-permissive	Semi-permissive

* Tumors

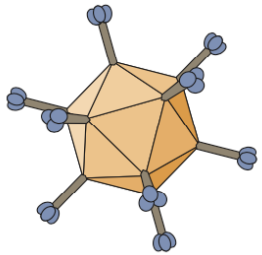
Polyomaviral transformation of cultured cells is rare





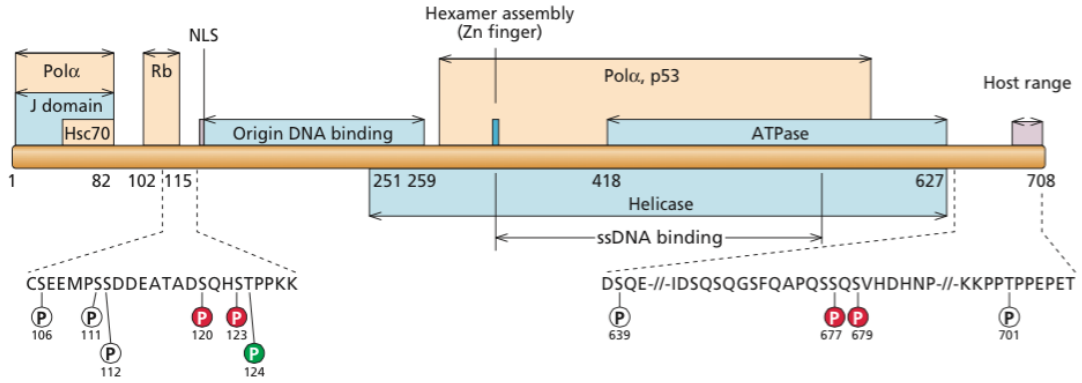
- 1 transformed cell per 100,000 infected cells
- Why is it so rare?
- How does this property relate to rare tumor formation in animals?

Adenoviridae: Another family of transforming DNA viruses



- Many human serotypes, do NOT cause cancer in humans
- Ad 12-18 tumors in hamsters
- Ad 7-11 poorly tumorigenic in hamsters
- Tumors and transformation of cells: like polyomaviruses and papillomaviruses, very rare events

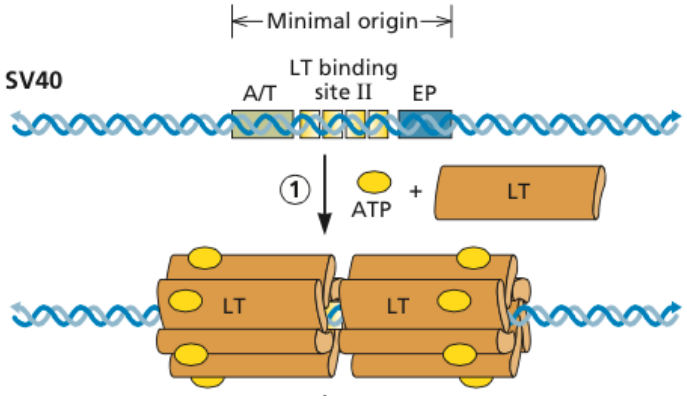
Key finding: Viral T antigens in tumors and transformed cells



- SV40: Large T, small T
- Polyomaviruses: Large T, middle T, small T
- Papillomaviruses; T encoded by E6, E7 genes
- Adenoviruses: T antigens are E1A, E1B

All different proteins!

T antigens are encoded by essential viral genes



- Required for replication
- Activate viral transcription
- Required for viral DNA synthesis
- Only viral genes *always* retained in tumor cells or transformed cells
- T antigen alone can transform cultured cells

Three seemingly unconnected discoveries in DNA virus biology were critical to understanding the link between viruses, transformation, and the cell cycle

1. 53 kDa cell protein binds SV40 T antigen

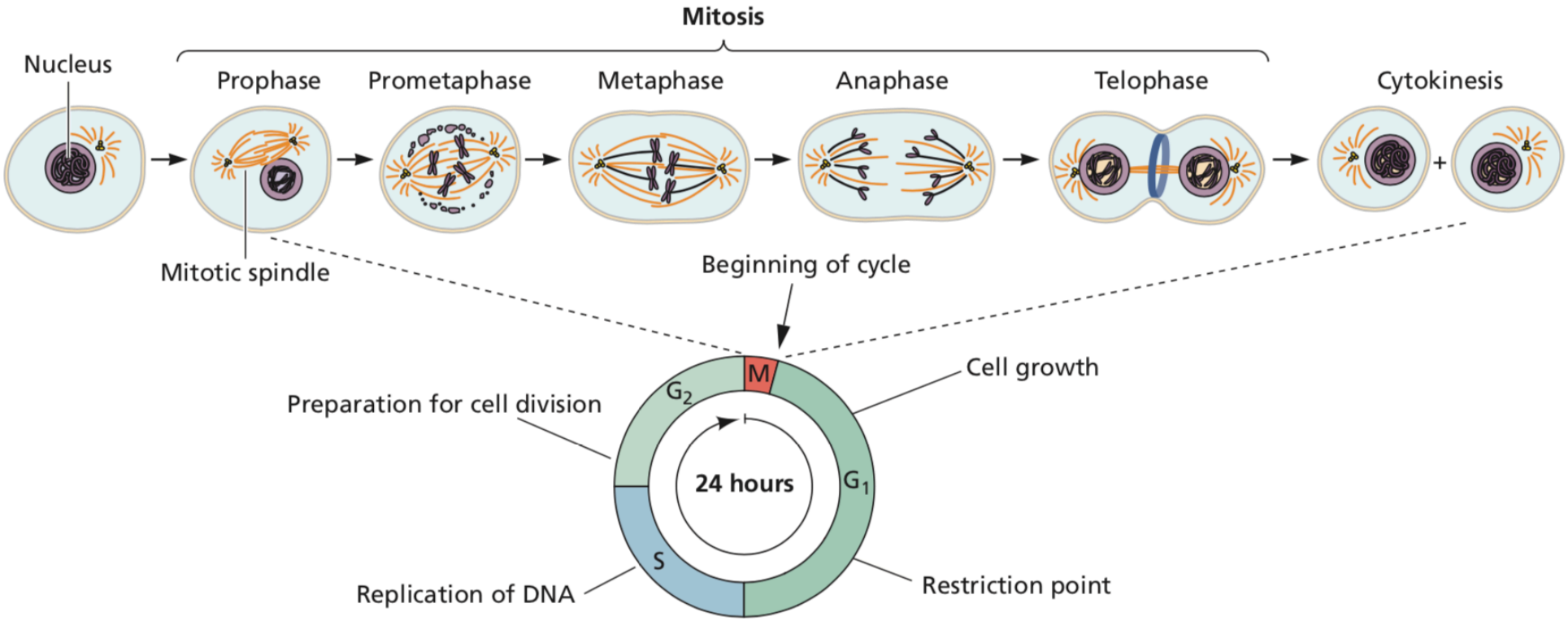
2. Transcription of a set of adenovirus early genes (the E2 gene cluster) requires cell protein E2f (E2 factor)

   - *Now a family of proteins called the E2f family*

3. E2f found to bind a cellular protein called Retinoblastoma protein (Rb)

p53, Rb and E2F were subsequently discovered to be critical players in control of the normal cell cycle

The cell cycle

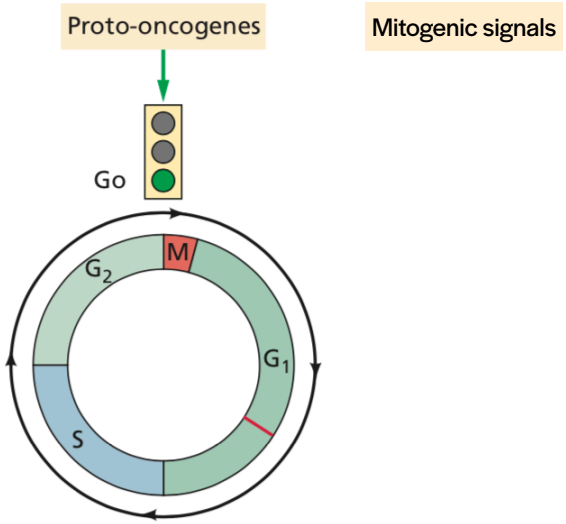


G is for "gap" Resting cells are in "G zero"

When stimulated to divide, cells enter the G1 phase and then into S where they replicate their DNA and prepare for cell division

Principles of Virology, ASM Press

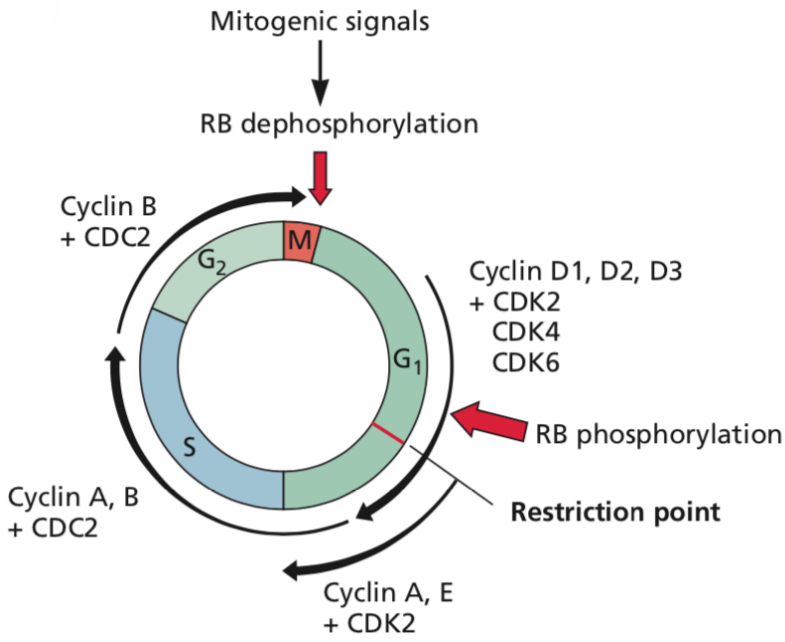
A go/no go decision is determined by nutrient concentration and growth factors



- Is the outside world rich enough to replicate the cell?
- Remember: Detectors and signaling proteins for growth were discovered as oncogenes carried by transforming retroviruses

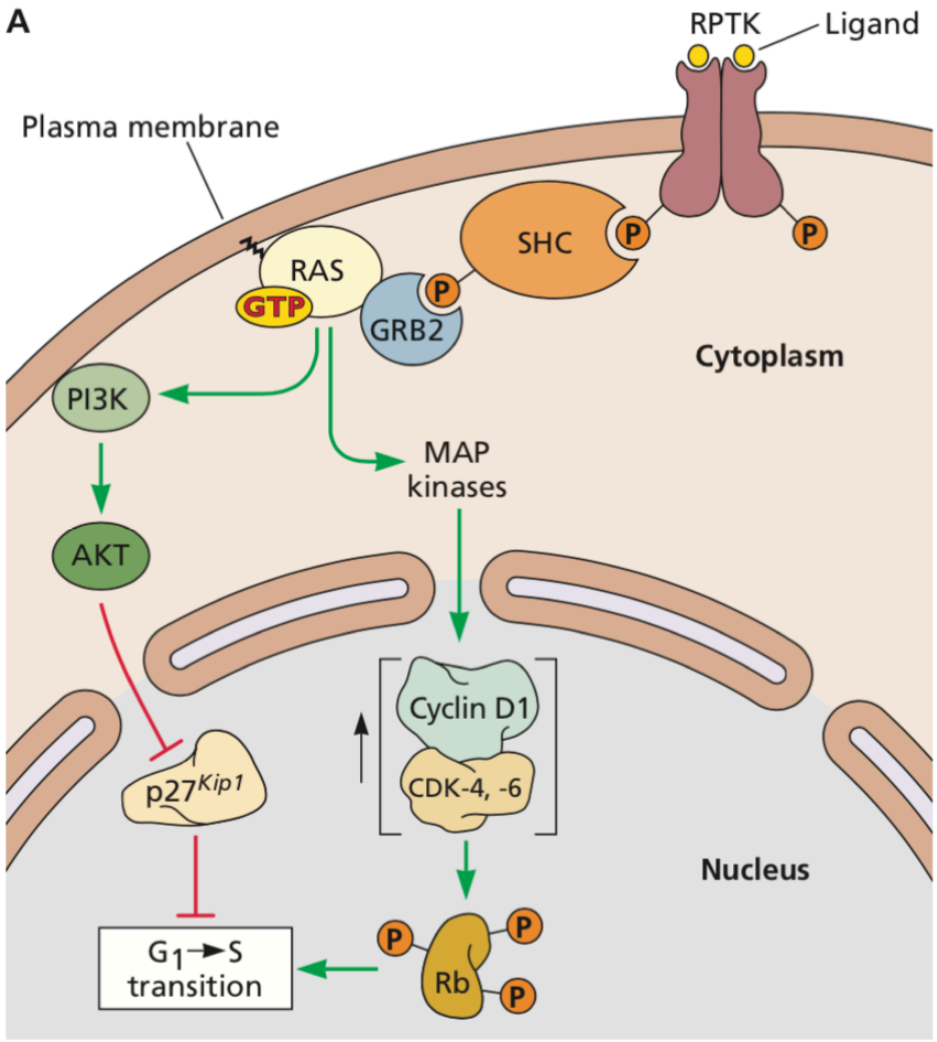
If conditions are not right, cell cycle pauses at *restriction point*

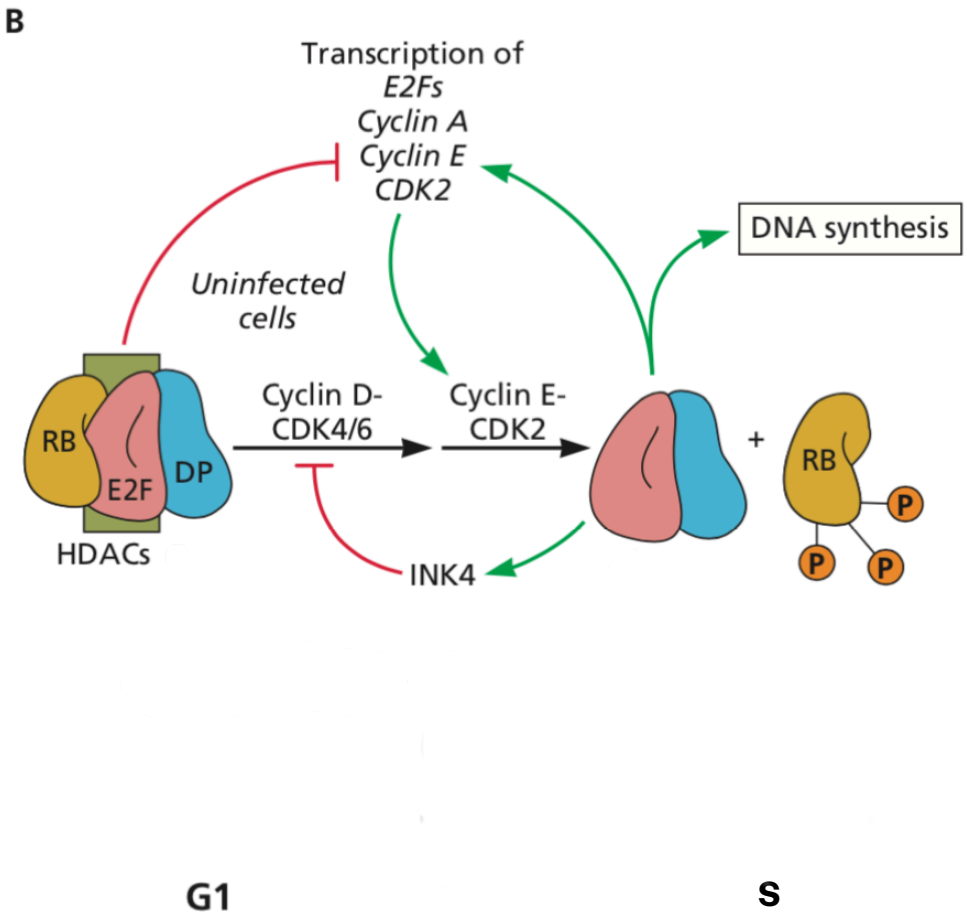
- No DNA synthesis, no cell division
- The protein that regulates the restriction point decision is *Rb*



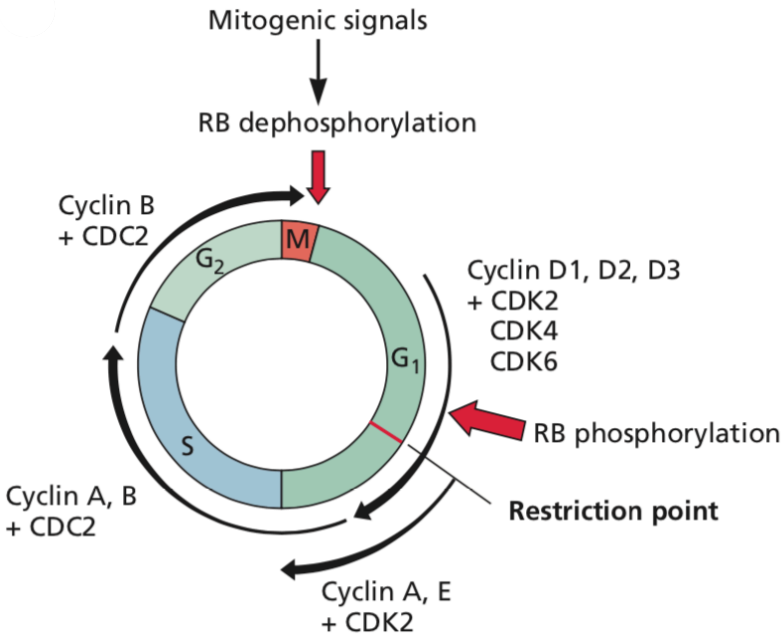
Retinoblastoma protein (Rb) – if both copies of the Rb gene are lost, develop retinal tumors of retinoblasts that form retina - these cells are gone by age 5
It is a recessive oncogene (the wild type protein is a tumor suppressor)

Principles of Virology, ASM Press


A
RPTK
Ligand
Plasma membrane
SHC
P
P
RAS
GTP
GRB2
P
Cytoplasm
PI3K
MAP
kinases
AKT
Cyclin D1
CDK-4, -6
p27Kip1
Nucleus
G1→S
transition
P
P
Rb
P
P


B
Transcription of
E2Fs
Cyclin A
Cyclin E
CDK2
DNA synthesis
Uninfected
cells
RB
E2F
DP
HDACs
Cyclin D-
CDK4/6
Cyclin E-
CDK2
+
RB
P
P
P
INK4
G1
S

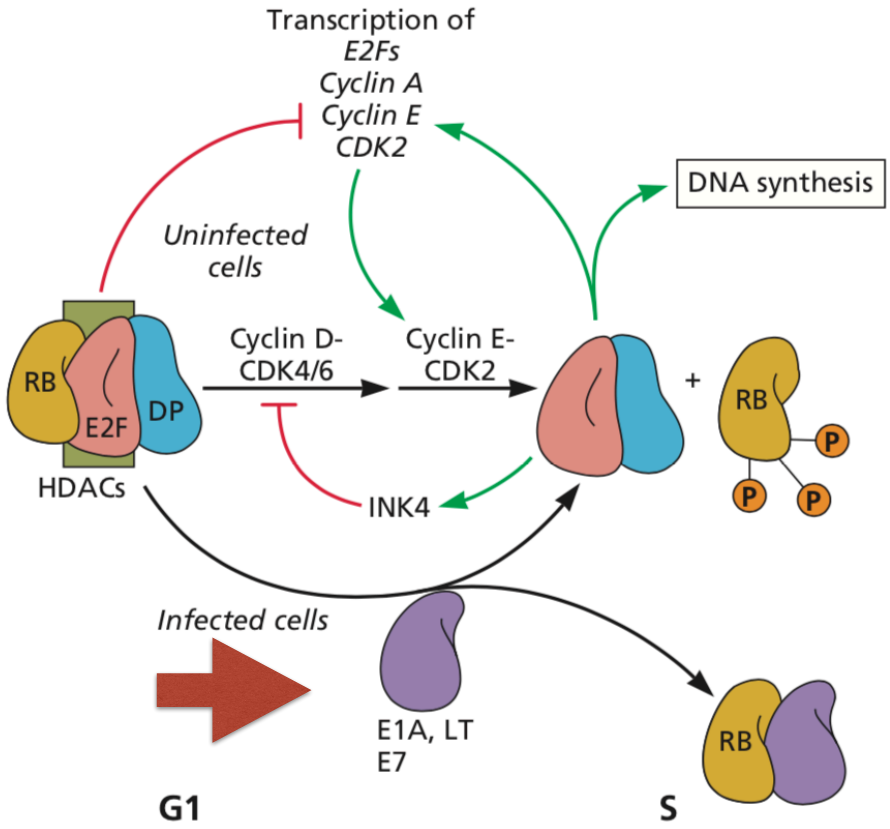
But DNA viruses need cells in S phase so they can replicate their DNA



T antigens kick quiescent cells into S phase!!

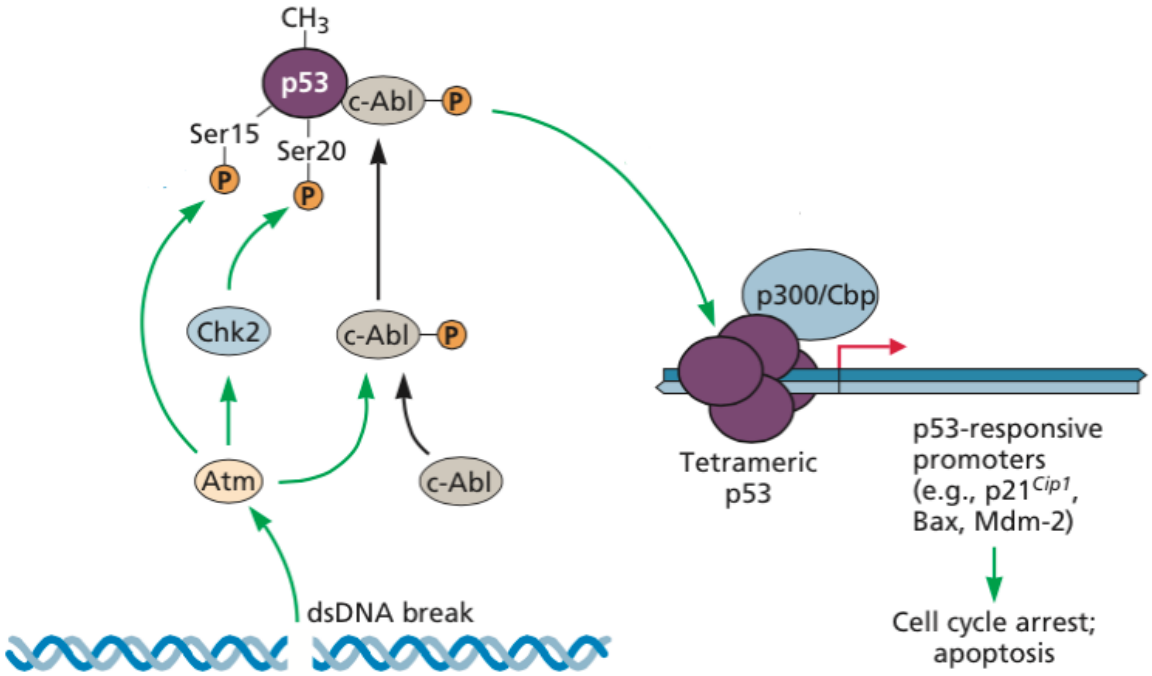
Principles of Virology, ASM Press

When viral T antigens bind to Rb, E2f proteins are released and initiate S phase transcription



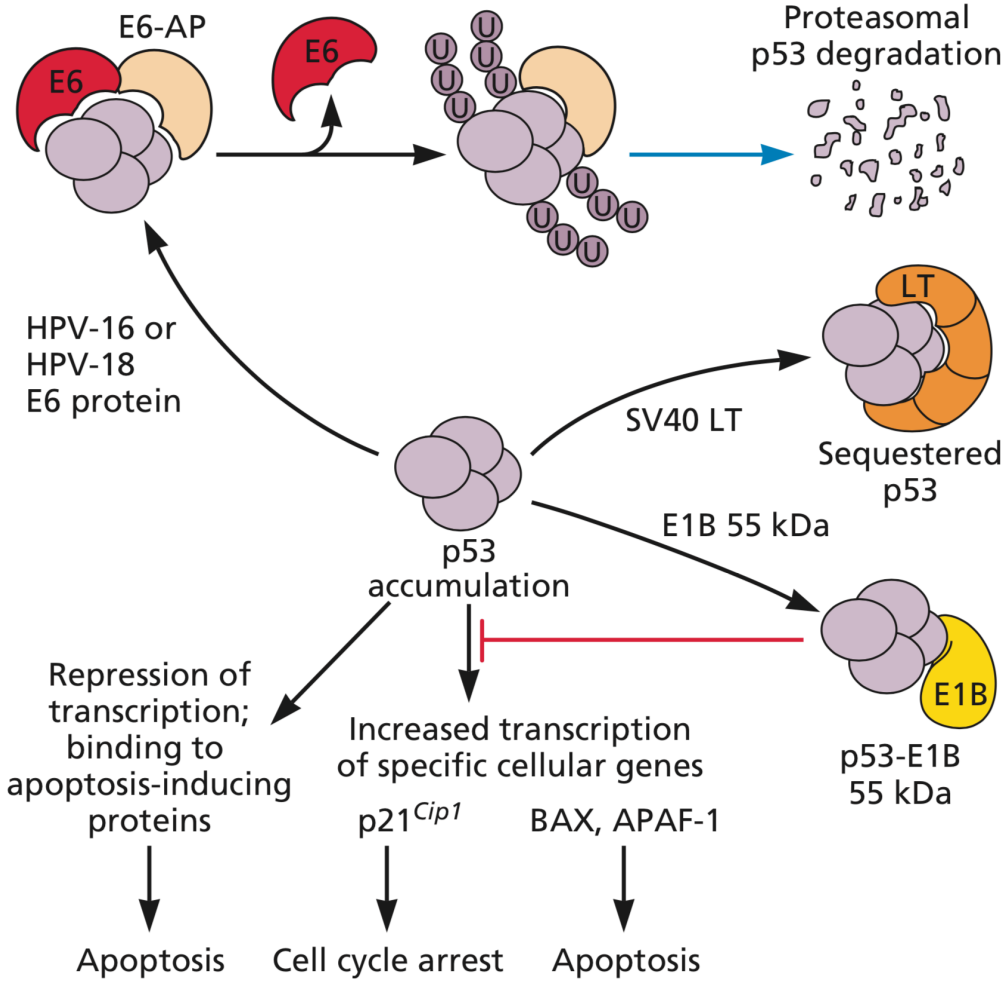
(Ad transcription also requires E2f)

The entry into S decision is under MORE control



- DNA damage or unscheduled DNA synthesis is monitored by p53
- Don’t duplicate damaged genetic information!
- Viruses must counter p53

How do viruses counter p53?



Go to:

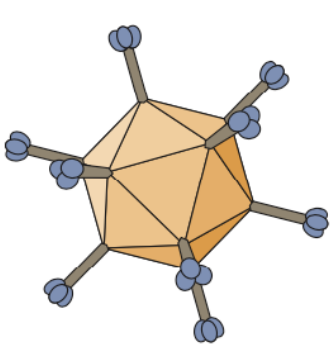
b.socrative.com/login/student

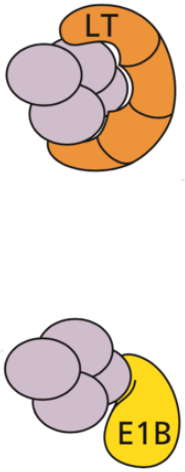
room number: virus

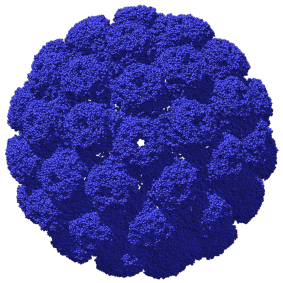
T antigens are:

A. Encoded by viral genes that are essential for replication
B. Present in tumors and transformed cells
C. Encoded by viral genes that have been incorporated into the cell genome
D. Antagonists of cell cycle checkpoint proteins
E. All of the above

Two more mysteries remain



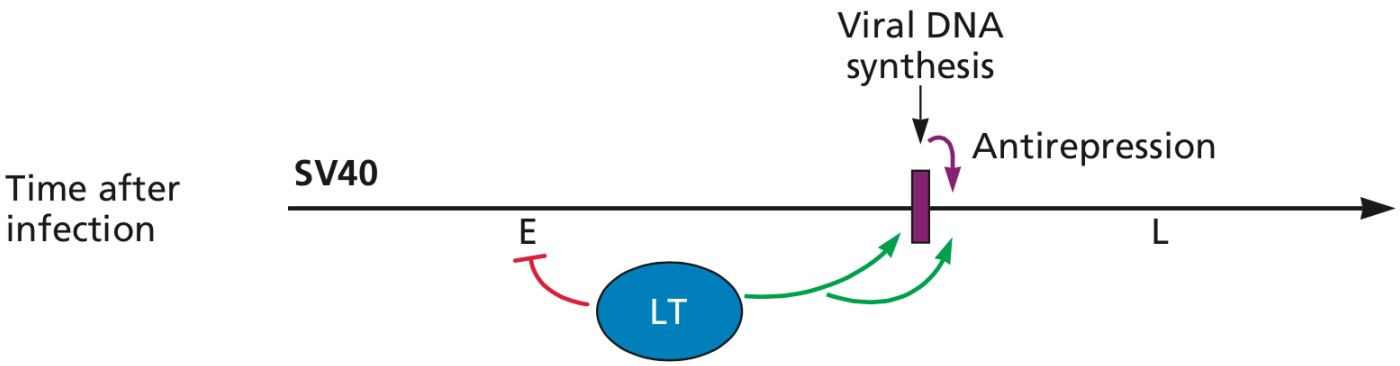




1. Why are all viral genes EXCEPT T-antigen encoding genes deleted or turned off in SV40, polyma, and adenovirus- transformed cells?

2. Why is transformation by these viruses so rare?

Transformation is rare because two low probability events are required

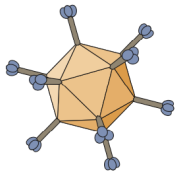


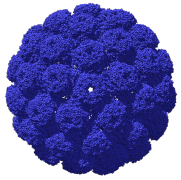
1. Lethal late genes must not be expressed
   - *Rare spontaneous deletion of late genes*
   - *Infection of semi-permissive cells; late gene expression blocked*
2. T antigen must be on constitutively and transmitted to every cell
   - *Viral DNA encoding T antigen must be integrated into the host DNA*

Transformation and tumor formation are abnormal, epigenetic processes for these DNA “tumor” viruses

These events are not required for the normal viral reproduction cycle or transmission

They were discovered by infecting the ‘wrong’ hosts



We now understand that even in the natural host, these rare events may occur, leading to tumorigenesis (e.g. HPV)

Transformation is an epiphenomena* of a unique reproductive cycle

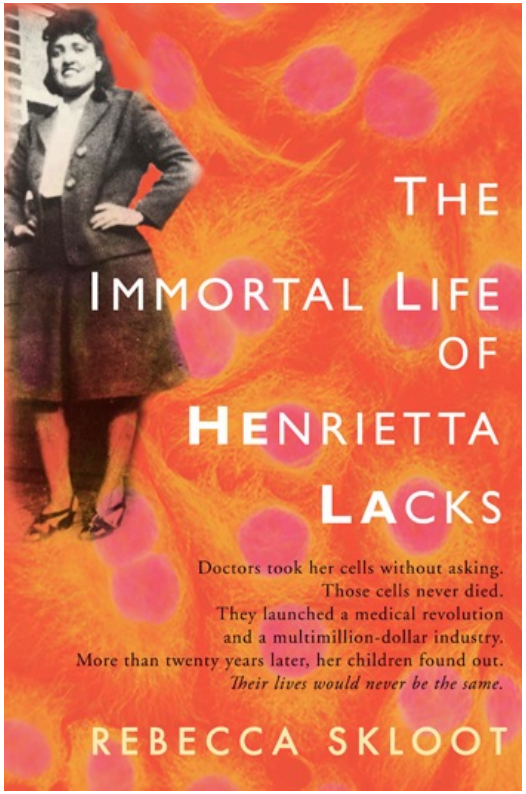
- DNA tumor viruses must start the cell synthetic machinery to make DNA
- T antigens turn on the cell cycle to start the G1 to S phases through inactivation of normal inhibitor (Rb)
- Inactivation of p53 blocks apoptosis

If lytic events are blocked, cells making T antigens continue to divide **– they are transformed**

They are on their way to becoming cancer cells

*a secondary effect that arises from but does not causally influence a process

Henrietta Lacks' cervical cancer and Hela cells explained

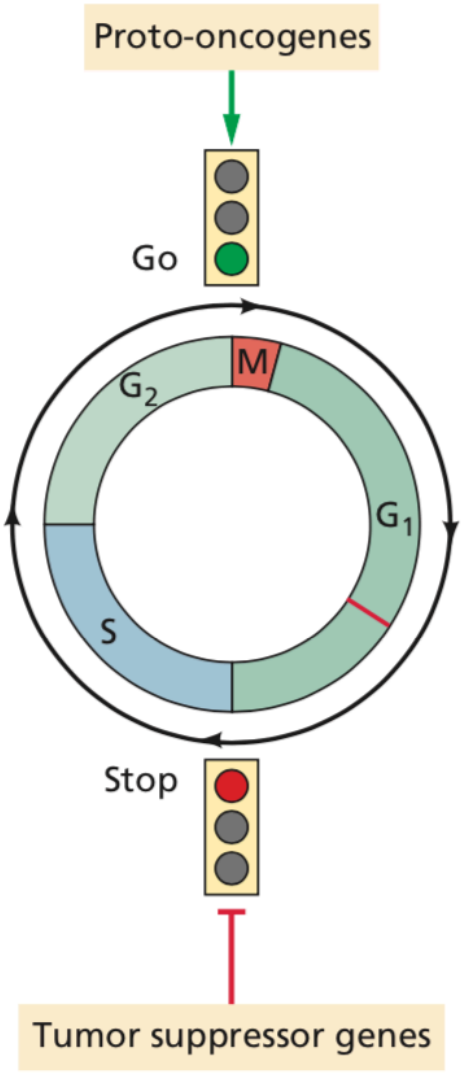


- Genome sequence of Hela cells reveals integration of HPV 18 in chromosome 18
- Only E6 (binds Rb), E7 (degrades p53) intact genes present
- Integration of viral DNA rare event in infected individuals

Transformation by RNA and DNA tumor viruses are epiphenomena of a unique reproductive cycle

RNA tumor viruses: integration into host DNA

DNA tumor viruses: turn on cell cycle



Revealed by studying transforming retroviruses

Dominant oncogenes

Revealed by studying DNA tumor viruses

Recessive oncogenes

Next time: Vaccines