

# Transformation and Oncogenesis

Lecture 18

Biology 3310/4310

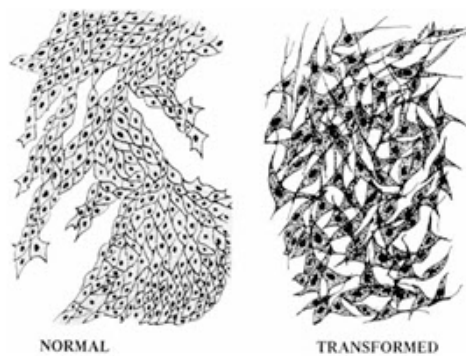
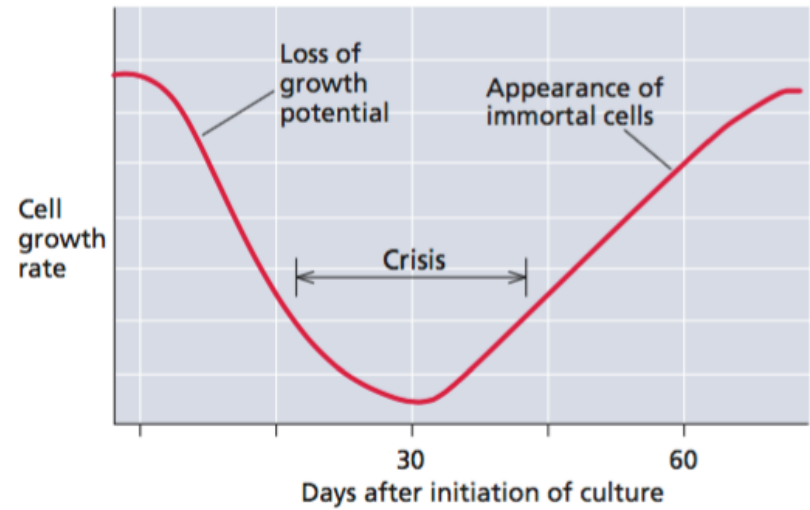
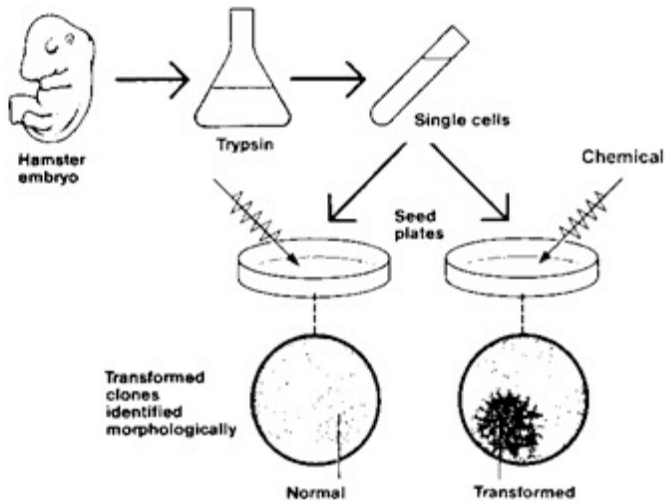
Virology

Spring 2017

*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.*

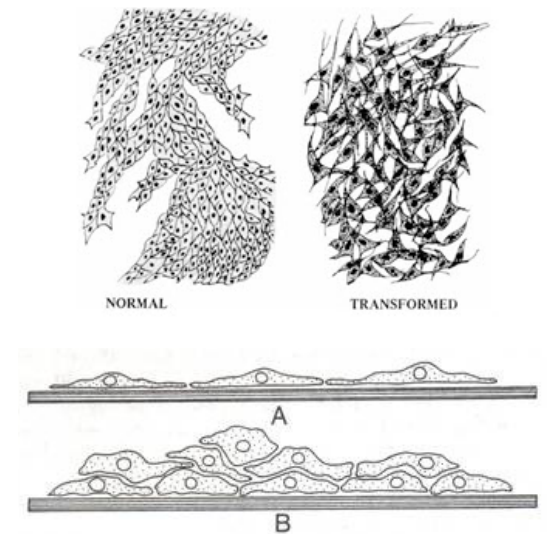
RALPH WALDO EMERSON

# Transformation



# The puzzling properties of transformed cells in the laboratory

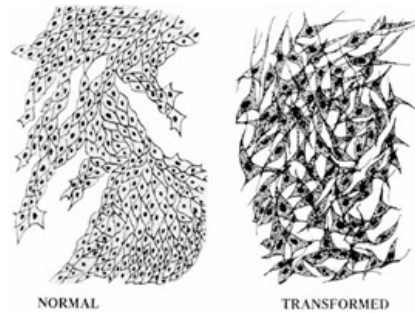
- Immortal: Grow indefinitely (HeLa)
- Loss of anchorage dependence
- Loss of contact inhibition
- Colony formation in semi-solid media
- Decreased requirements for growth factors (serum)



# 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



NORMAL

TRANSFORMED

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

**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!*



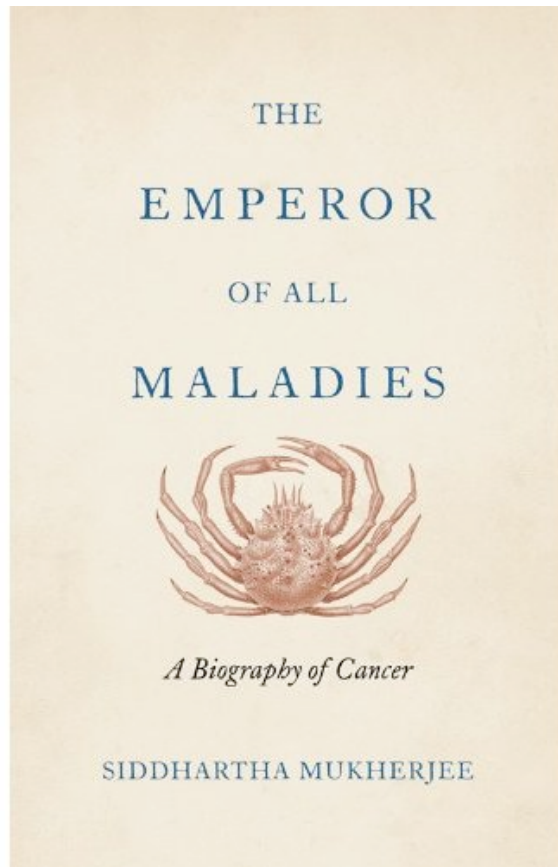


***It took the world almost 50 years to accept this idea***

**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.

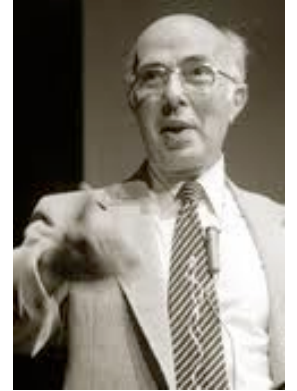
The virologists, lead by Rous, claimed that viruses caused cancer, although no such virus had been found in human studies.

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



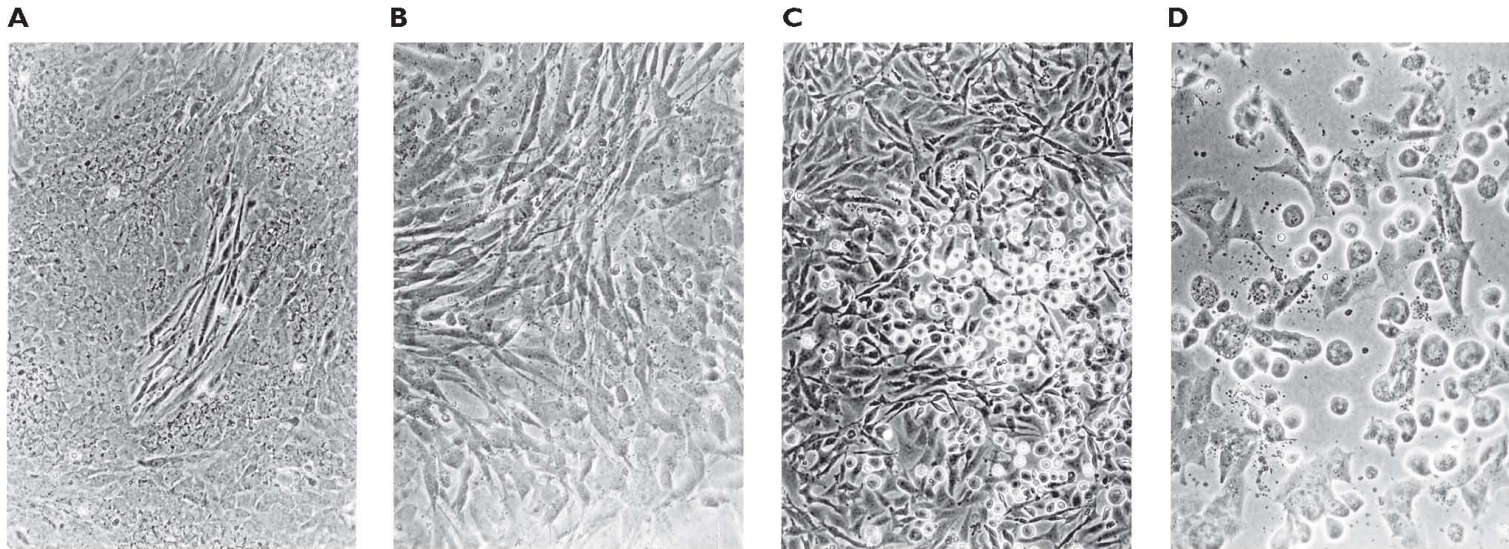
In 1951, a young virologist named Howard Temin arrived at Cal Tech to study the genetics of fruit flies. Restless and imaginative, he soon grew bored with fruit flies. Switching fields, he chose to study Rous sarcoma virus in Renato Dulbecco's laboratory.

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.

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



**Fusiform**

**Round, refractile**

# 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 virions*
- The cell must continue to divide
  - *It becomes immortal*



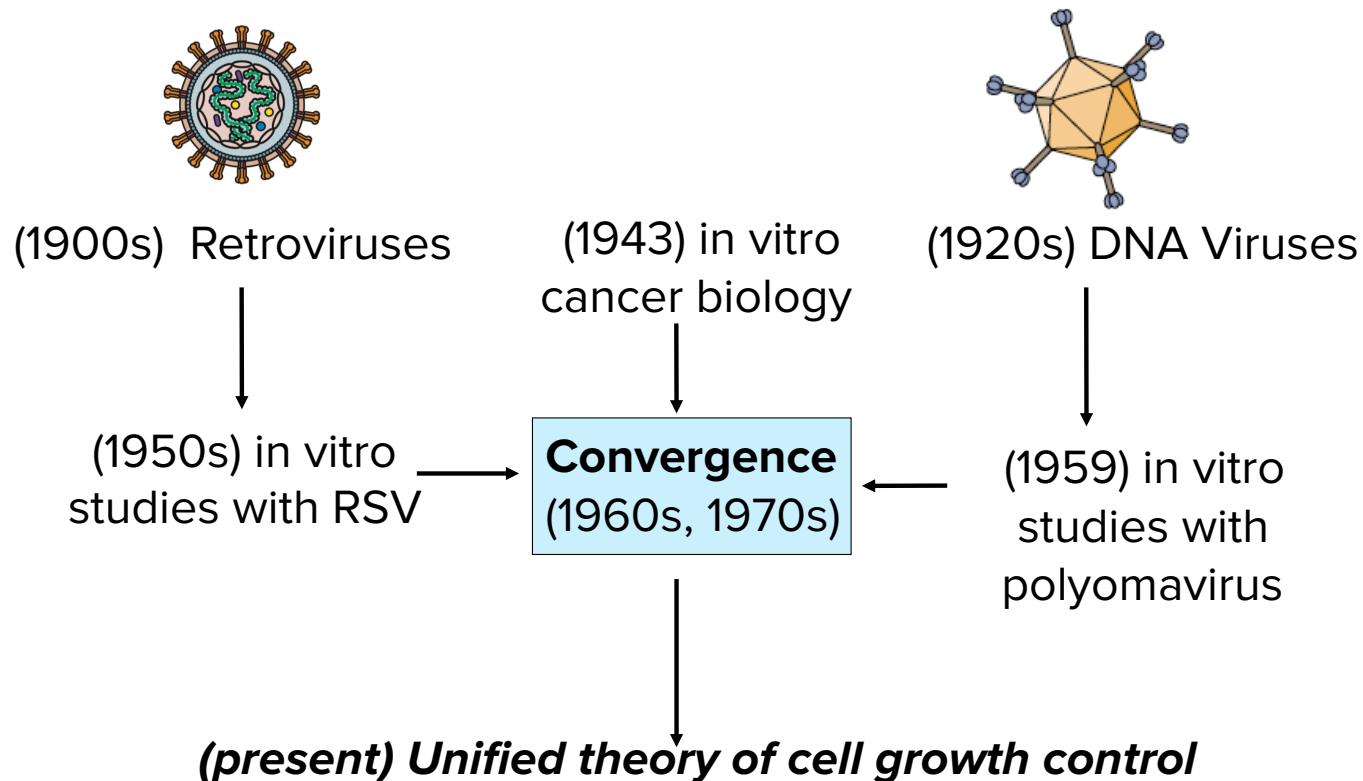
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room number: virus

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

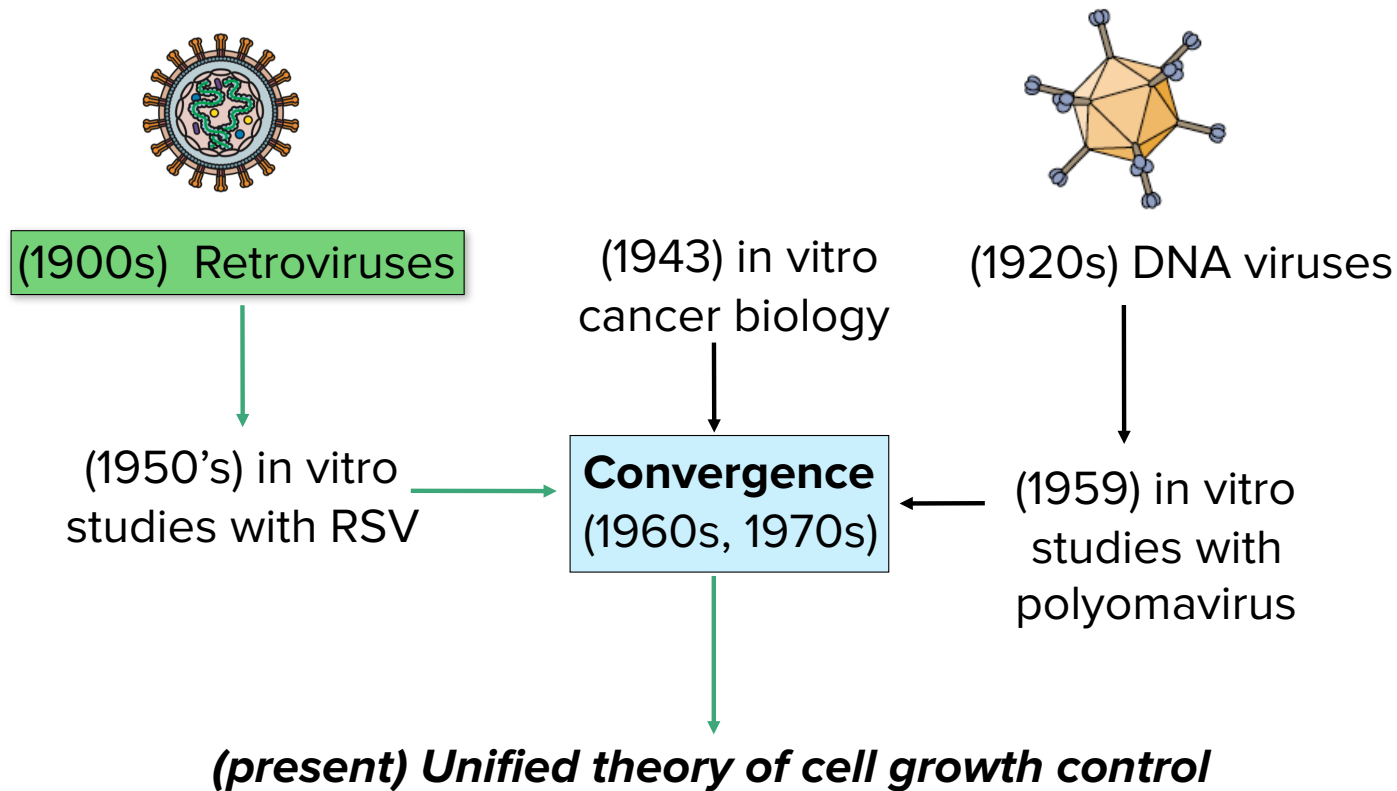
- A. Increased requirements for growth factors
- B. Immortality
- C. Loss of anchorage dependence
- D. Loss of contact inhibition
- E. Colony formation in semi-solid media

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

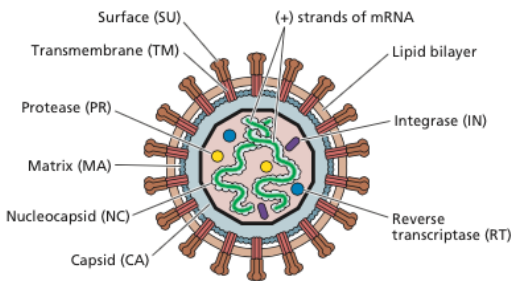
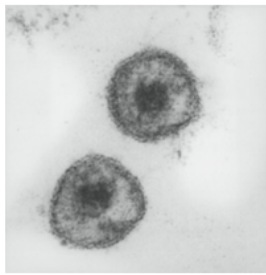




# How does Rous sarcoma virus cause tumors in chickens and transform cells *in vitro*?



# 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

# 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?

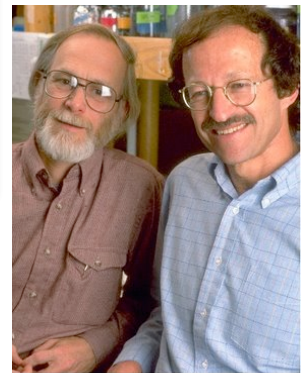
- Key finding: the viral genomes from solid tumors were recombinants!
- A piece of the ALV genome is replaced with a *segment of host DNA* called an *oncogene*

**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*

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

<https://youtu.be/frbMV-YGgQU>



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

## 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**

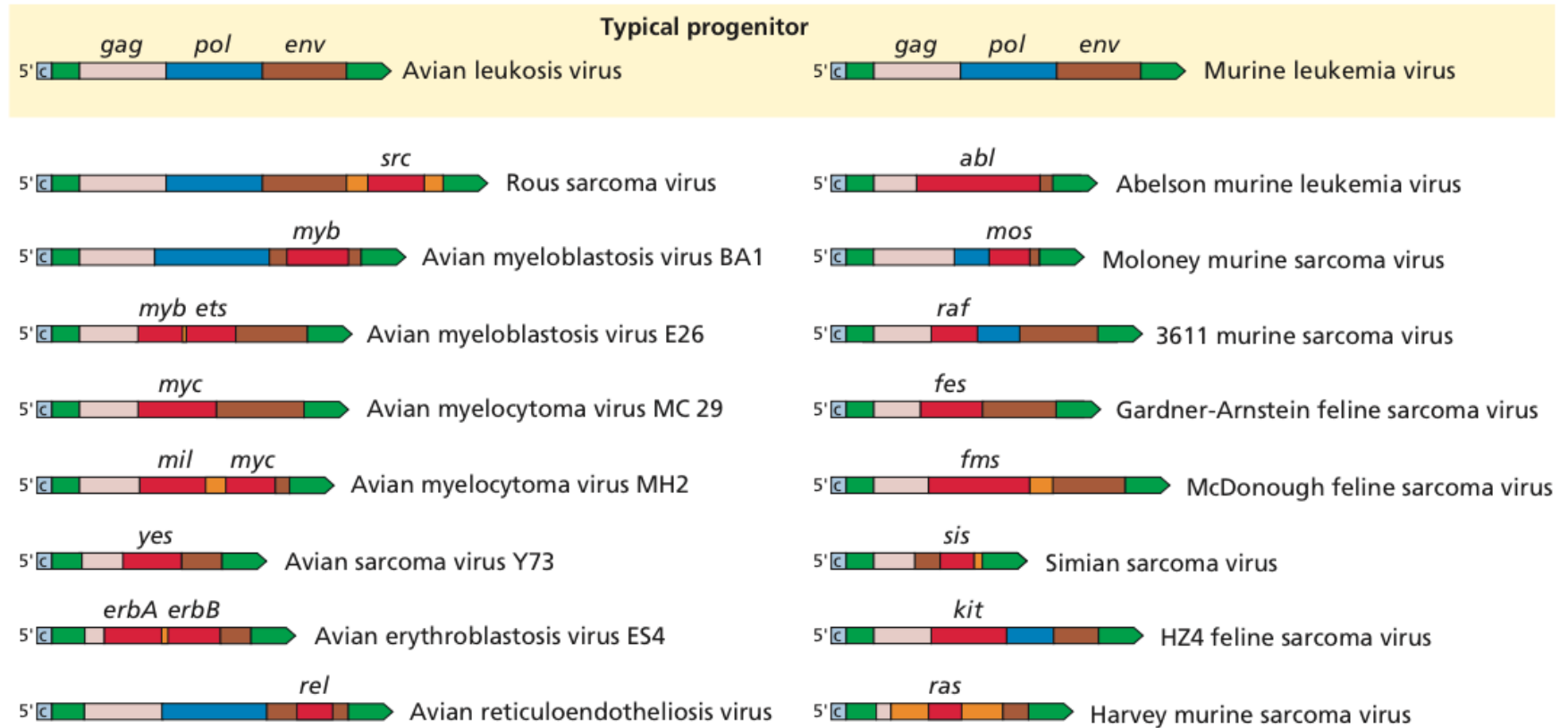
*Each new DNA segment had a novel chicken oncogene*

*A gold mine for molecular oncology*

# Genomes of transducing retroviruses

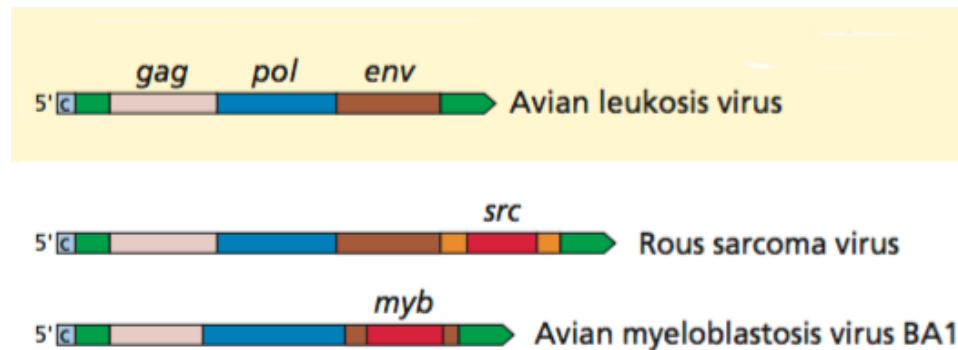
## Avian transducing retroviruses

## Mammalian transducing retroviruses

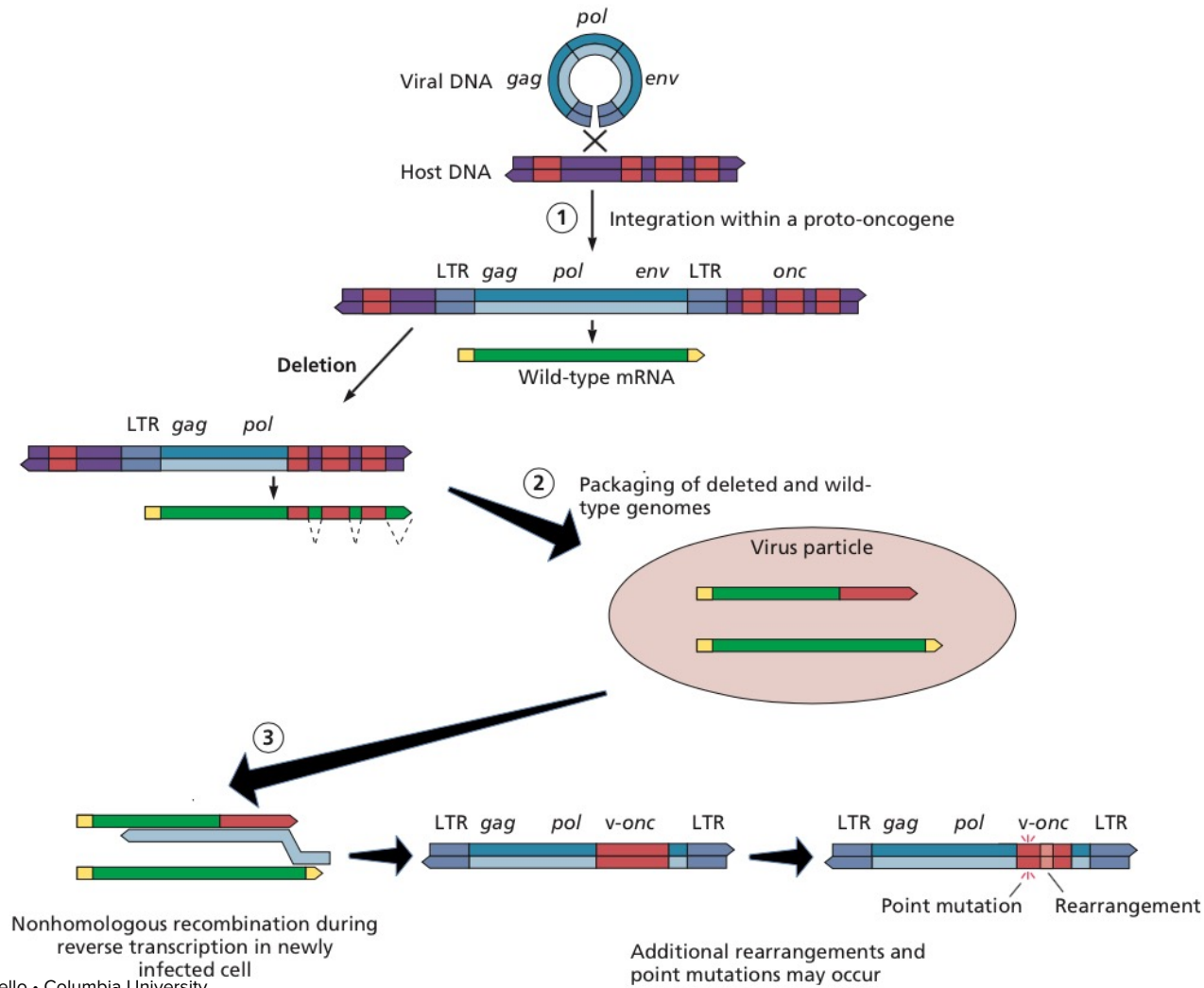


# Defective vs non-defective retroviruses

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



# Mechanism for oncogene capture

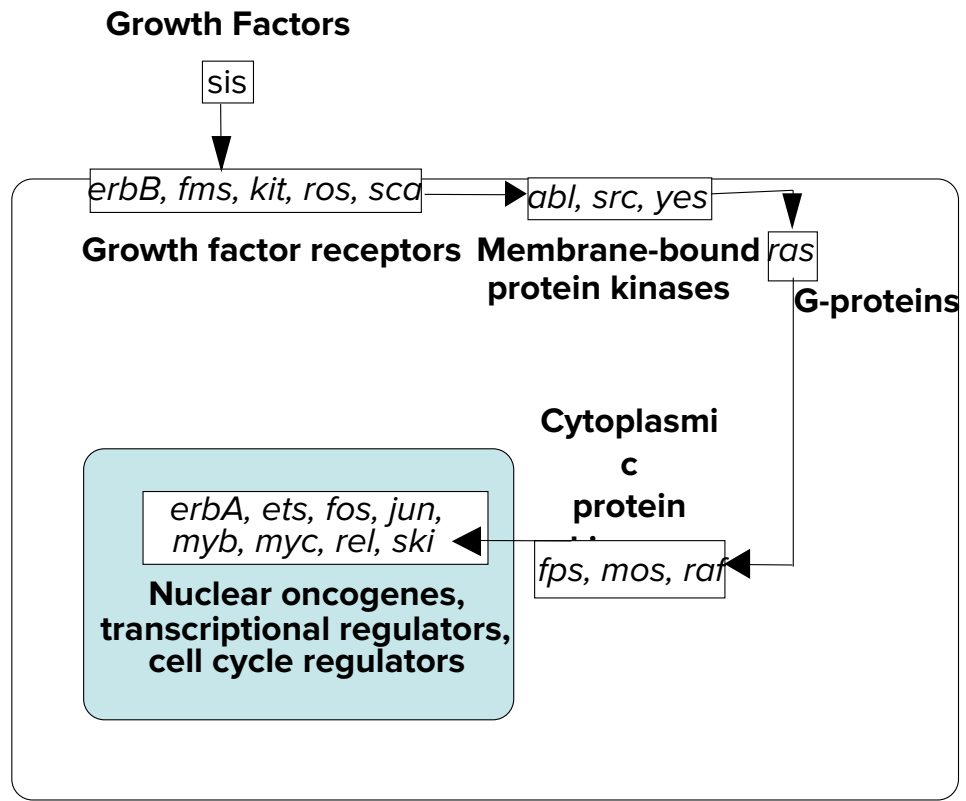




# Proto-oncogenes

- >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

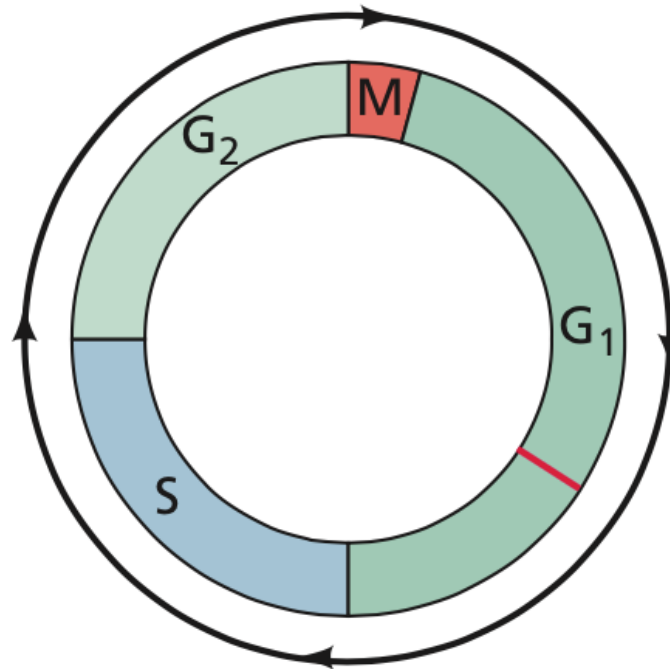
# The cell cycle

Proto-oncogenes



Go

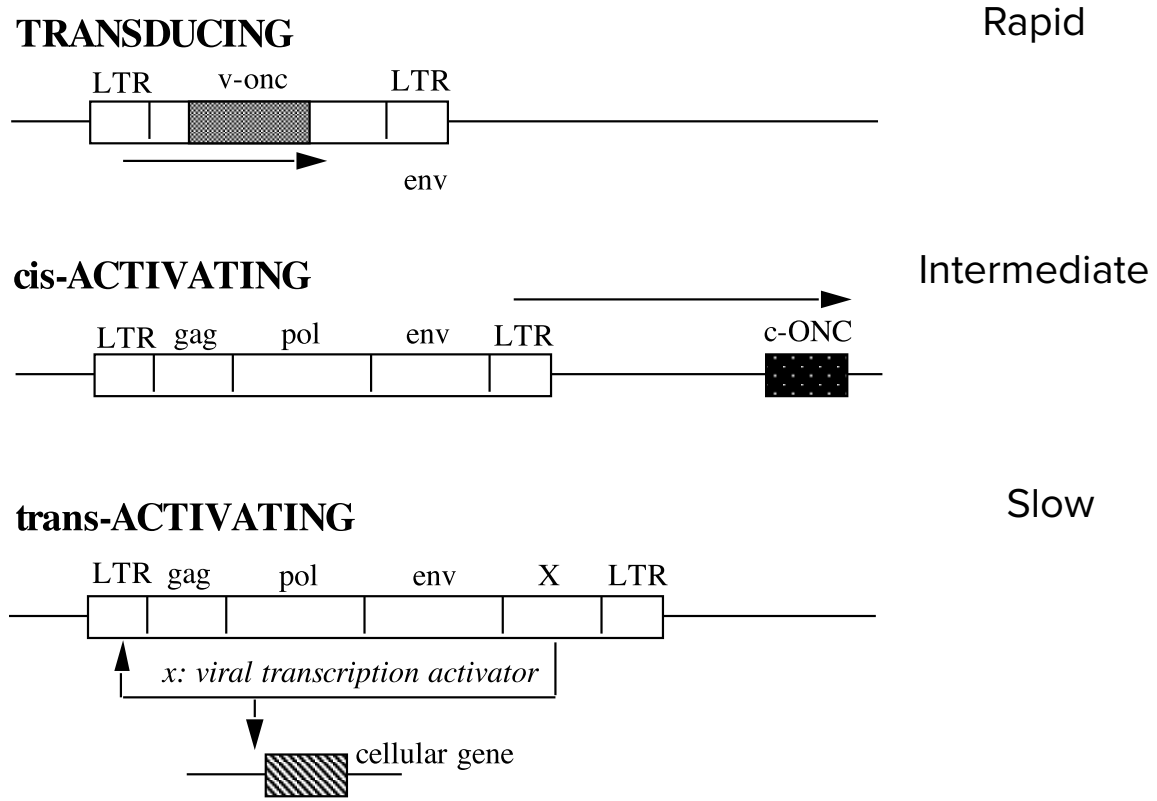
Mitogenic signals revealed  
by studying  
*transforming retroviruses*  
*Dominant oncogenes*



# 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*

# Provirus 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 - they must integrate their DNA
- No obvious viral requirement for transformation or for oncogenesis



*Walleye dermal sarcoma virus*

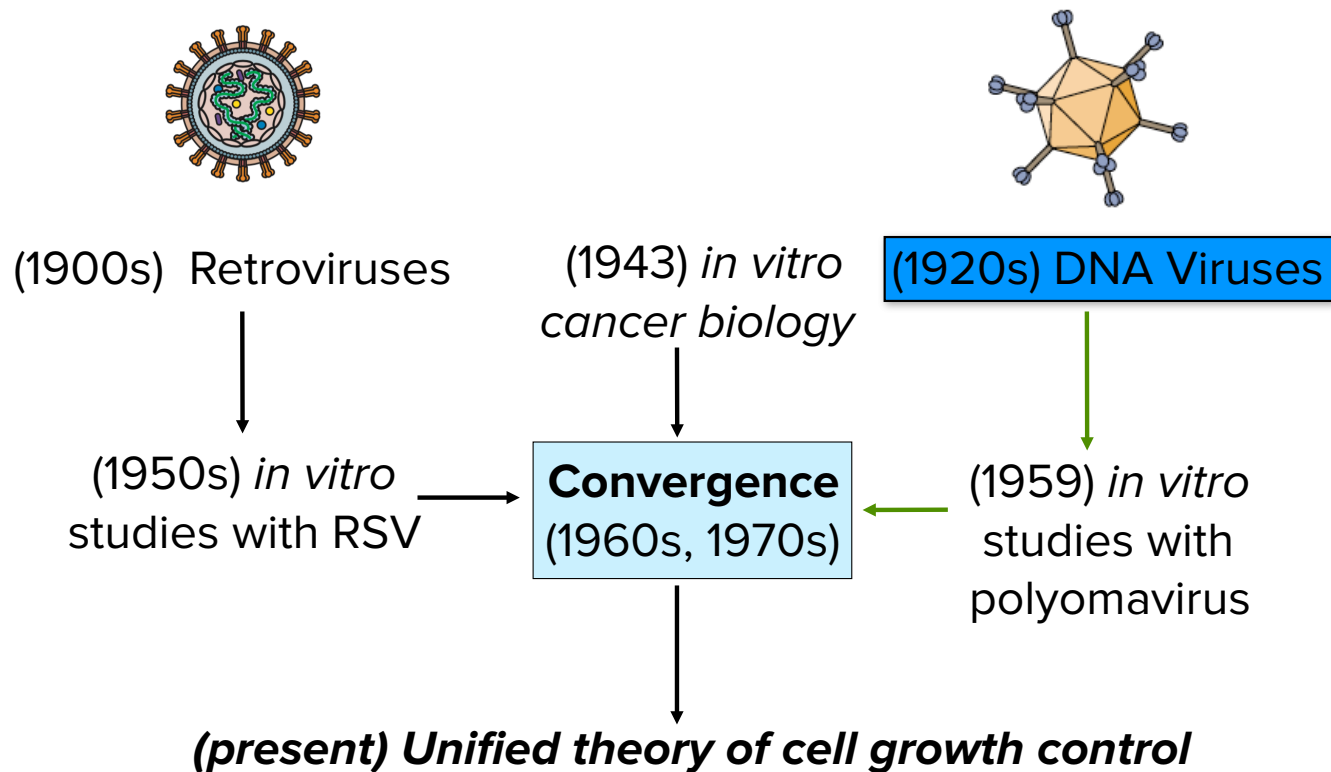
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room number: virus

**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

# How study of DNA virus transformation revealed how the cell cycle is regulated



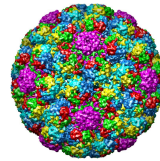


## DNA tumor viruses

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

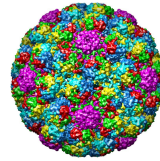


## DNA tumor viruses: *Polyomaviridae*



- Ludwig Gross discovered murine polyomaviruses in 1953
- Caused **rare** tumors under certain conditions
  - Natural host is the mouse
  - Ubiquitous 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*
  - *Causes no tumors in monkeys*
  - *Does not transform monkey cells in culture*

## Response of different cells to infection

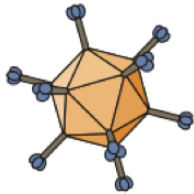
Species	SV40	Mouse polyomavirus
Monkey	<i>Permissive</i>	Non-permissive
Mouse	Non-permissive	<i>Permissive</i>
*Hamster	Semi-permissive	Semi-permissive
*Rat	Semi-permissive	Semi-permissive

\* Tumors

# Polyomaviral transformation of cultured cells is rare

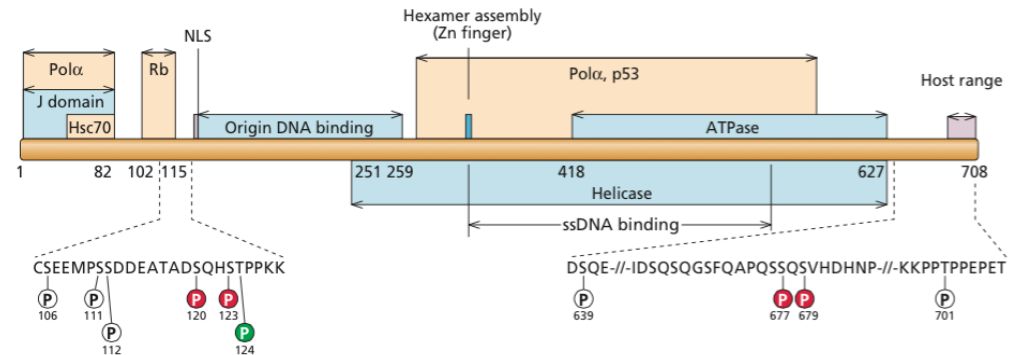
- 1 transformed cell per 100,000 infected cells
- Why is it so rare?
- How does this property relate to rare tumor formation in animals?

# ***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

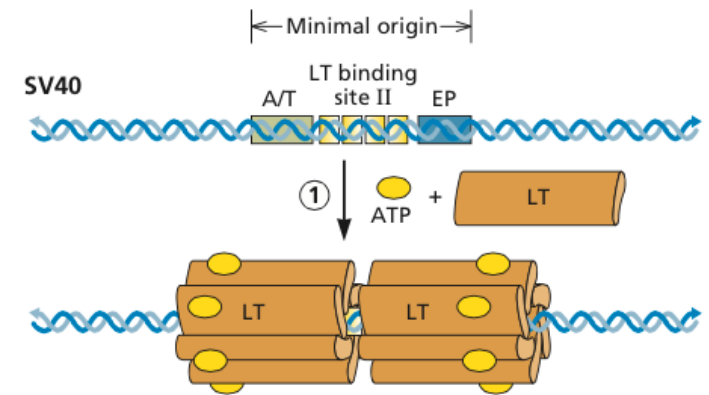


- SV40: Large T, small T
- Polyomaviruses: Large T, middle T, small T
- Papillomaviruses; T encoded by E5, E6, E7 genes
- Adenoviruses: T antigens are E1A, E1B

*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



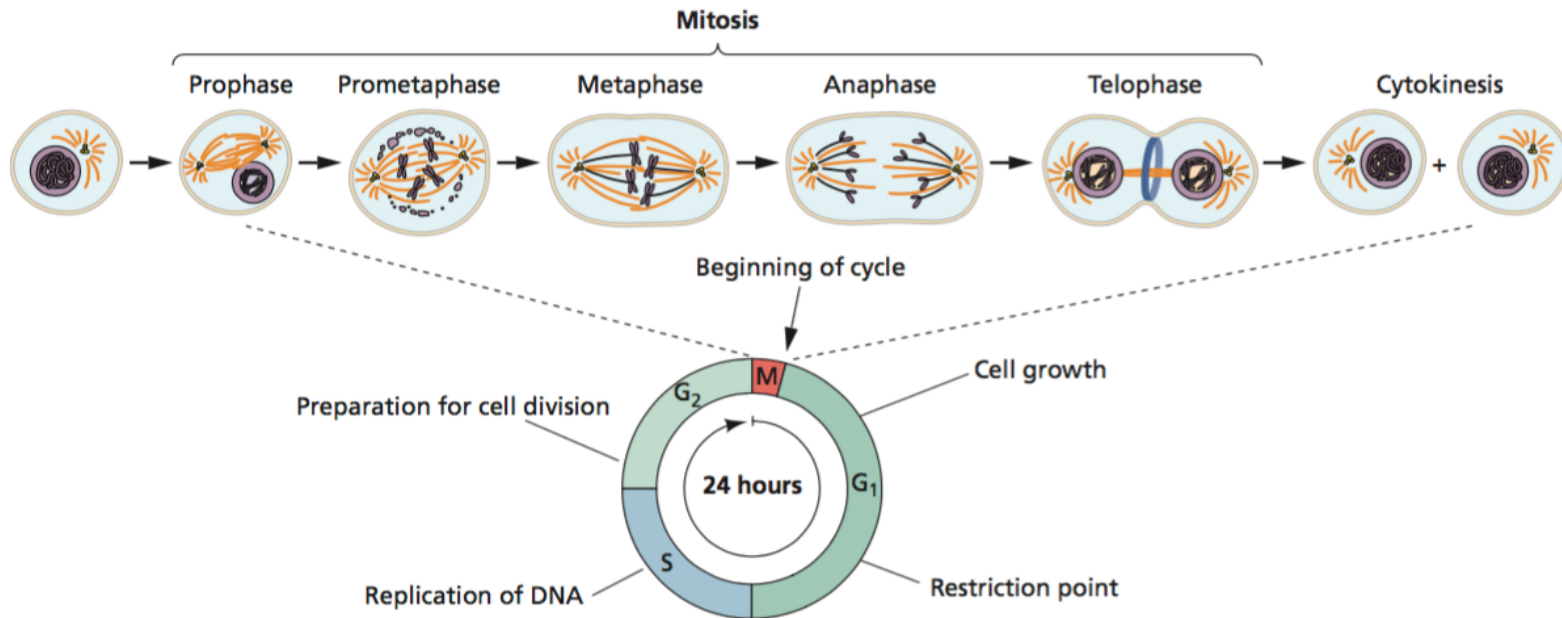


## Three seemingly unconnected discoveries in DNA virus biology were critical to understanding the link between viruses, transformation, and the cell cycle

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**

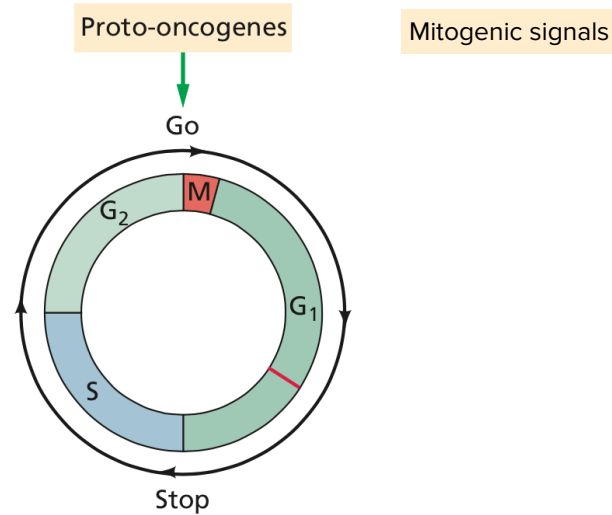
# The cell cycle



**G is for “gap” Resting cells are in “G zero”**

*When stimulated to divide, cells enter the G<sub>1</sub> phase and then into S where they replicate their DNA and prepare for cell division*

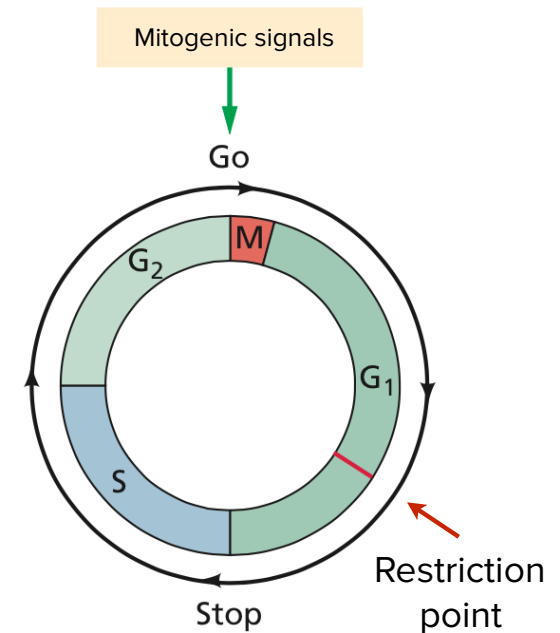
# A go/no go decision is determined by nutrient concentration and growth factors



- 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

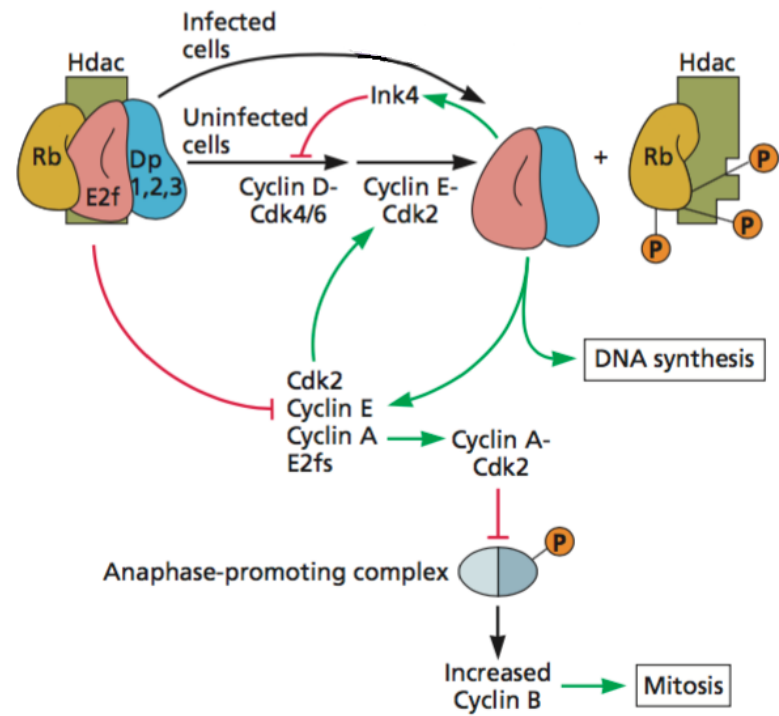
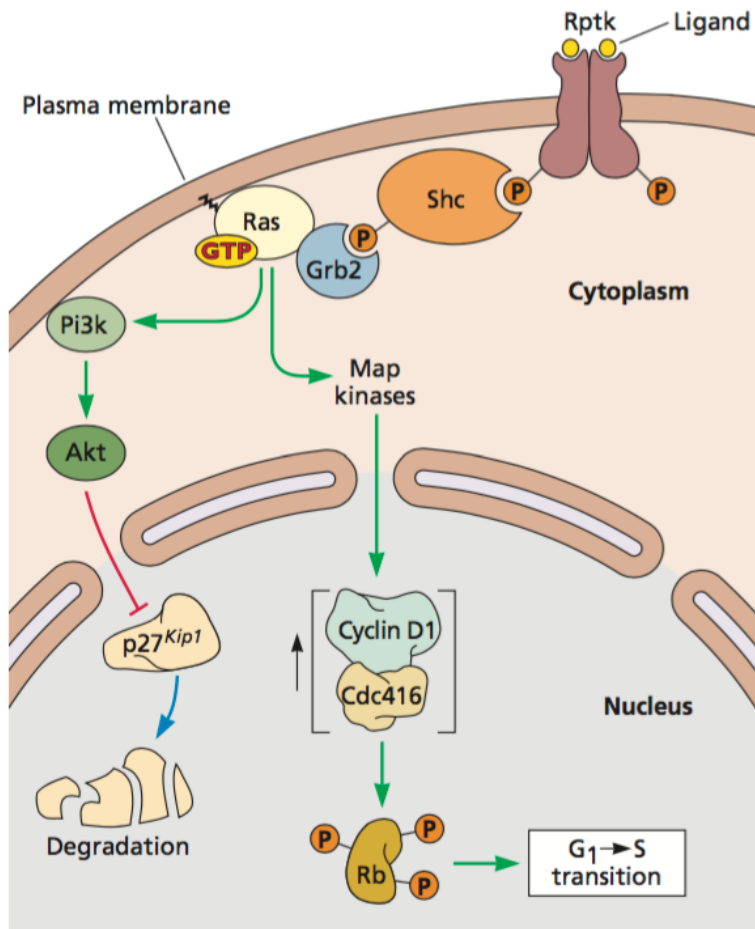
# If conditions are not right, cell cycle pauses at *restriction point*

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

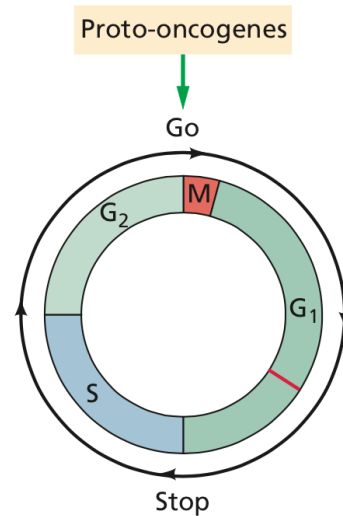


*Retinoblastoma protein (Rb) – if both copies of the Rb gene are lost, develop retinal tumors of retinoblasts that form retina - these cells are gone by age 5*

***It is a recessive oncogene (the wild type protein is a tumor suppressor)***

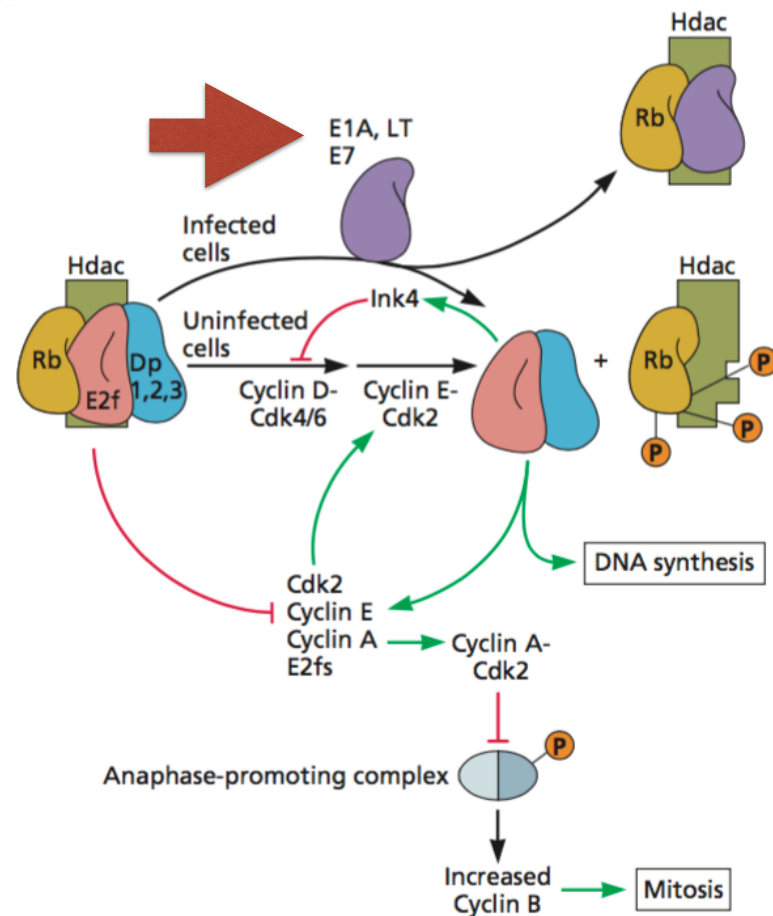


# But DNA viruses need cells in S phase so they can replicate their DNA



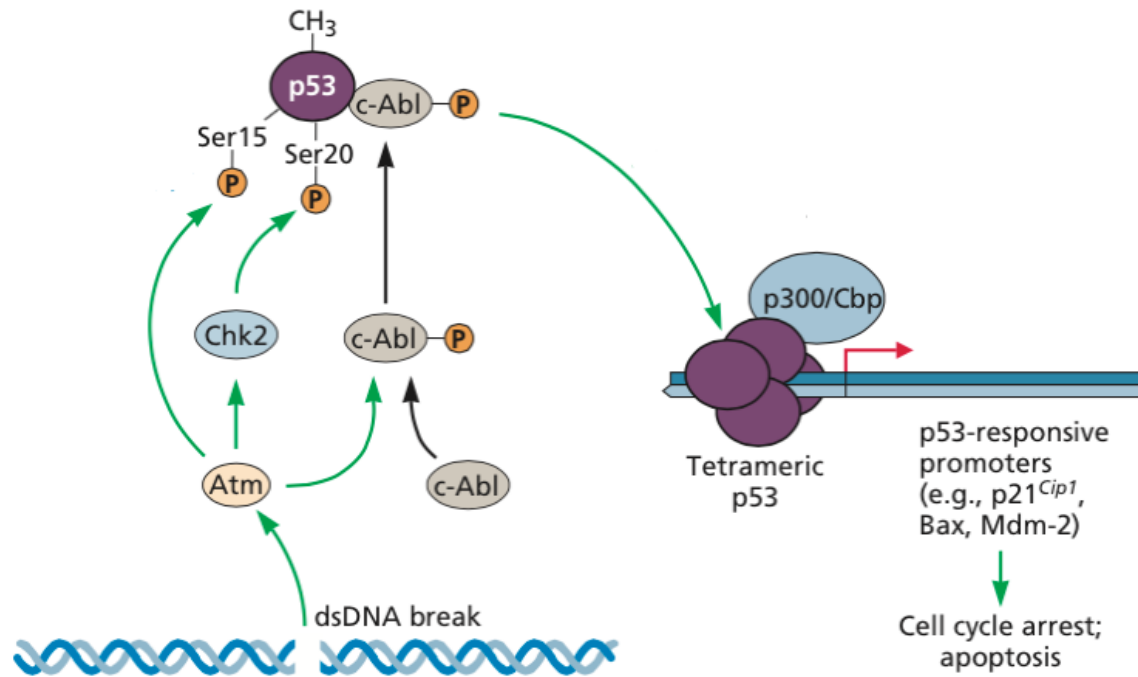
*T antigens kick quiescent cells into S phase!!*

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



(Ad transcription also requires E2f)

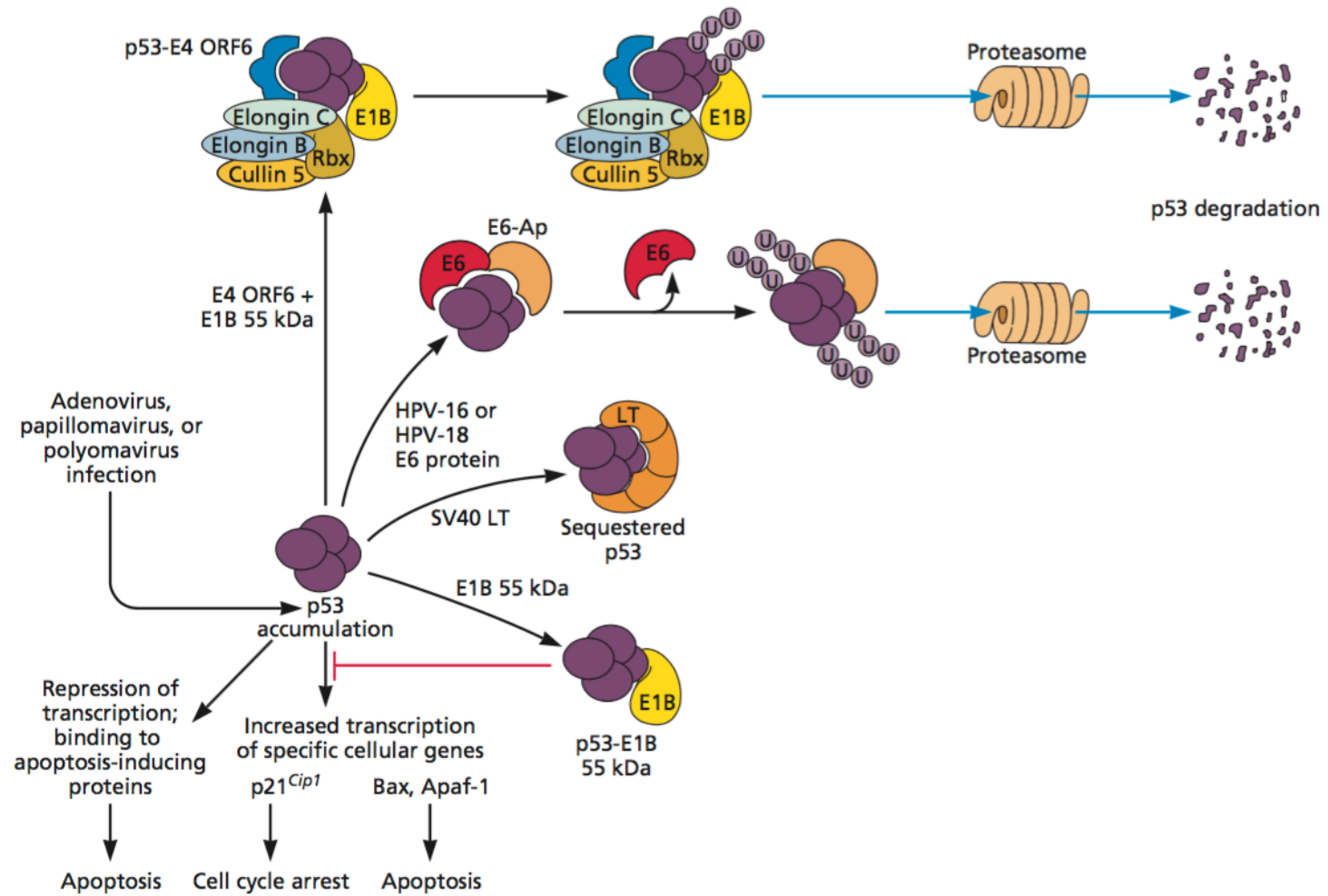
# The entry into S decision is under MORE control



- 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?



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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, polyoma, 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 life cycle or transmission**

*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*

