Cancer Biology
Lecture 3

Fall 2018

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Based on clinical effect on patients

- Benign:
  - Grows locally
  - Without invading of adjacent tissues
- Malignant
  - Invade nearby tissues
  - Spawned metastasis

Clinical effect of Benign tumor

- Secrete high levels of hormones
- Grow only in one place, cannot spread or invade other parts
- Be dangerous if they press on vital organs

Clinical effect of Malignant tumor

- 90% cancer deaths are due to metastasis

Cancer classification

I. Carcinoma ← epithelia
   1. Adenocarcinoma ← gland epithelia
   2. Squamous cell carcinoma ← protective sheets
   3. Other types of epithelia
II. Sarcoma ← Mesenchymal cells of connective tissue
III. Leukemia and lymphoma
IV. Neuroectodermal tumors
V. Can't fit in the above 4 / origin unknown

How cells become cancerous

- Normal cell
- Immortalized cell
- Transformed cell
- Metastatic cells

Cell growth homeostatis in a multicellular animal. What's normal?

To exist in an orderly, developmentally regulated tissue, cells generally have some common characteristics:

- **Limited proliferation capacity:** somatic cells are subject to the Hayflick limit (Hayflick, *Exp. Cell Res.* **37** 614 (1965) and are limited to 40-50 divisions before undergoing senescence and death.
- **Anchorage dependence:** proliferation requires binding via integrins to extracellular matrix **(ECM)** components. Specific integrins recognize specific ECM molecules.
- **Contact inhibition:** contact with **like** cell types inhibits cell movement and proliferation, quiescent G0 cells, monolayers in cell culture. Contact with **unlike** cells allows motility and hence spontaneous cell sorting.
- **Growth factor dependence:** proliferation depends on availability of tissue-type specific growth factors. In many cases factor withdrawal leads to apoptosis. Growth in serum-rich or conditioned media (autocrine factors, plating density dependence of growth).

Transformation

- **Immortalization and aneuploidy:** survival and continuous growth beyond normal limits involves changes at the telomere that frequently result in major chromosomal rearrangements.
- **Partial or complete loss of growth factor dependence:** growth on less rich serum, or at lower initial cell density.
- **Loss of contact inhibition:** overgrowth of monolayers.
- **Loss of anchorage requirement:** growth on soft agar or in suspension.

Tumorigenicity is not solely loss of proliferation control; loss of contact and anchorage dependence leads to the motility and invasiveness of malignant tumor cells.

cancer progression

Normal cells

↓

Hyperplasia

↓

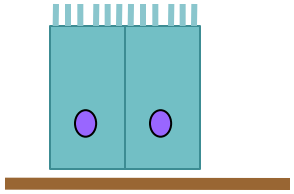
Dysplasia (Metaplasia)

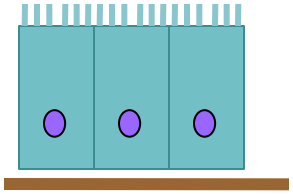
↓

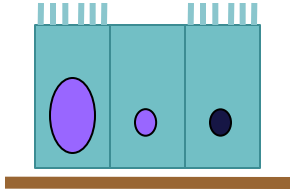
Neoplasia

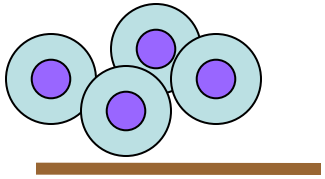
↓

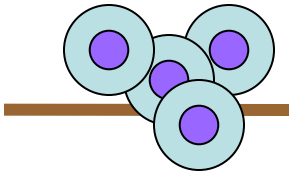
Metastasis











Cancer Epidemiology

Population studies

- Some cancers have similar risk/rates in all populations

- Others have very different rates in different populations

→identification of risk factors

1) Genetic
2) Environmental

Introduction

- What is cancer?
- **What causes cancer?**
  - **Chemicals**
  - **Virus**
- How do we deal with cancer?

Introduction

- What is cancer?
  - Normal cells go bad.
- What causes cancer?
  - Carcinogens
  - **Infection?**
- How do we deal with cancer?

Carcinogen

- 1775: chimney sweep and scrotal (阴囊）carcinoma.

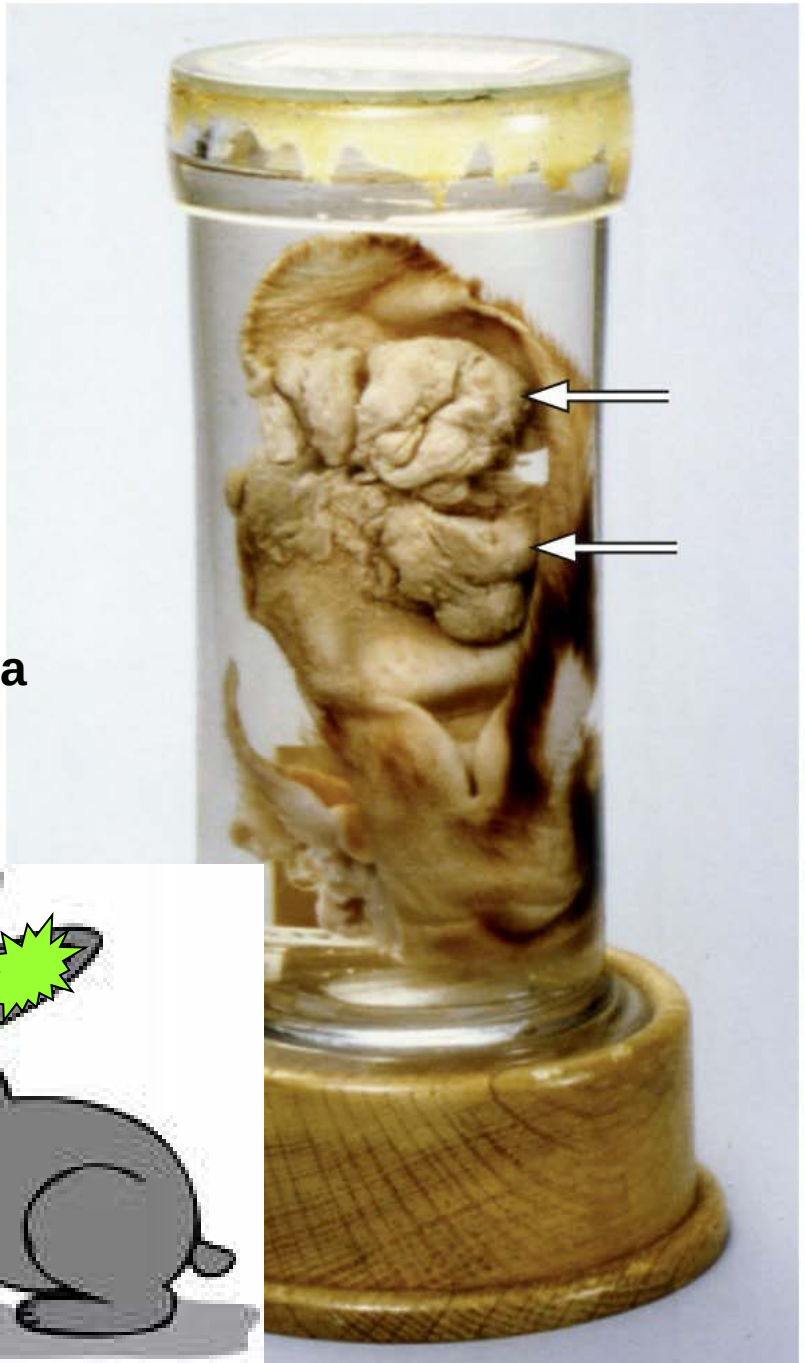
Sir Percivall Pott (1714-1788)

Carcinogen test

Katsusaburo Yamagiwa, 1915
Japan

660 days
Coal Tar
(焦油)

Skin
carcinoma



Carcinogens in tar

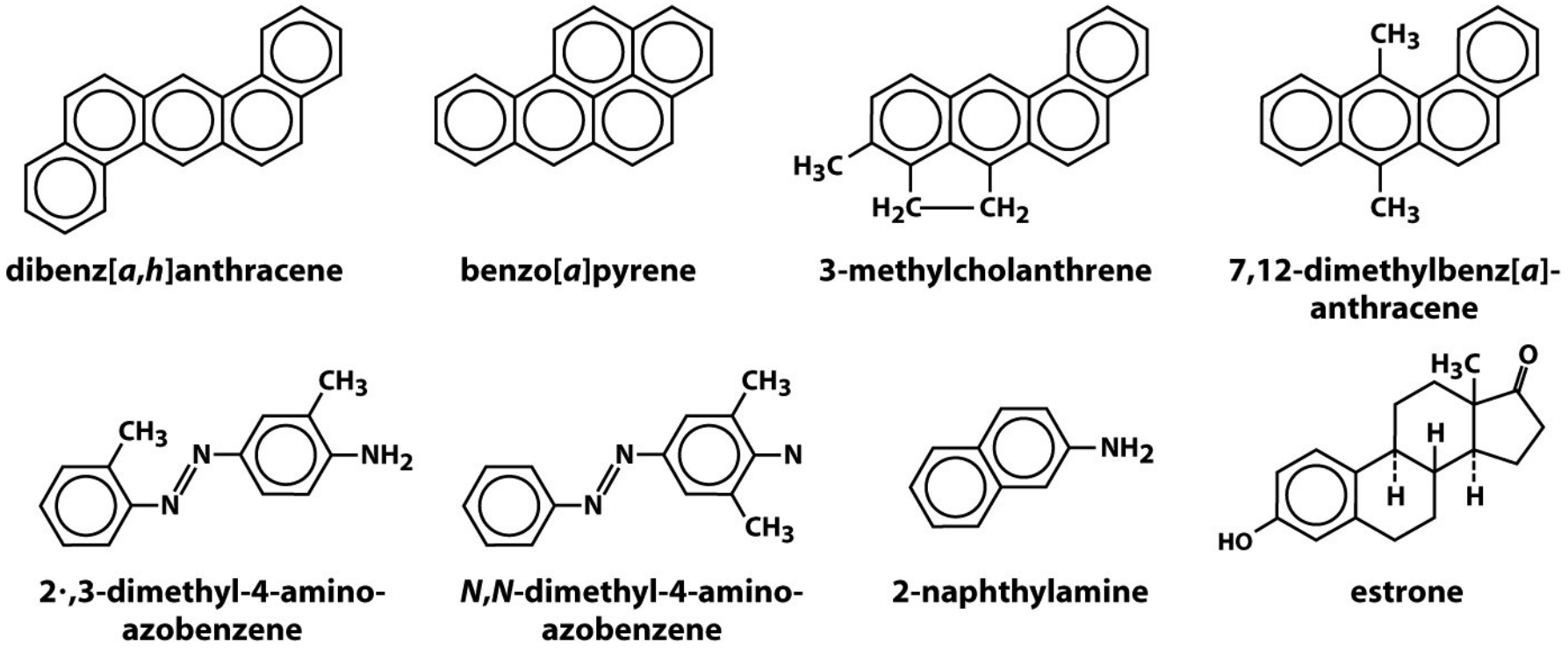


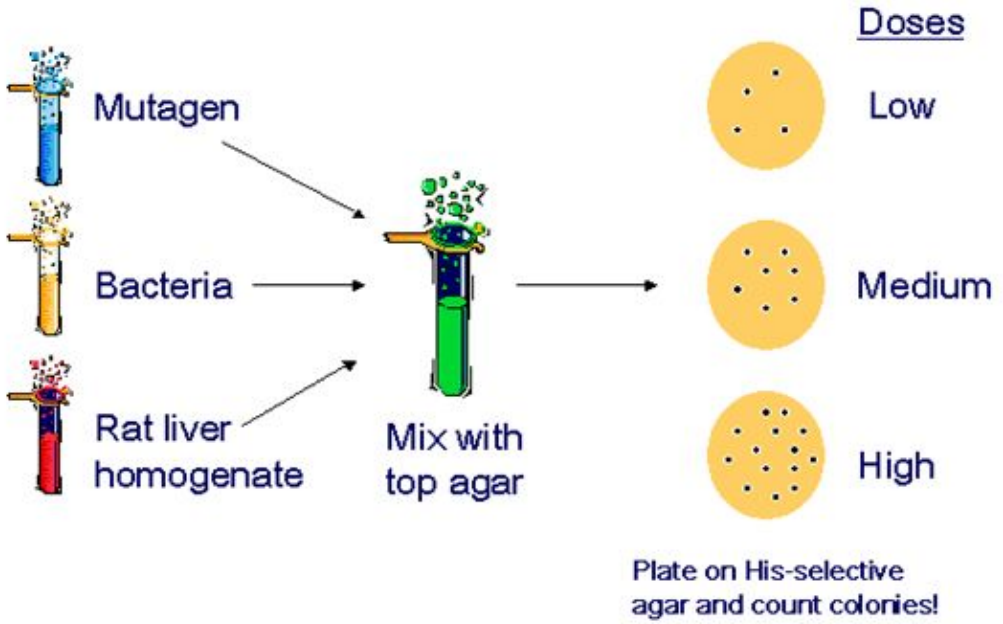
Figure 2-22 The Biology of Cancer (© Garland Science 2007)

How to test for carcinogens?

How do we know if a Compound is Carcinogenic?

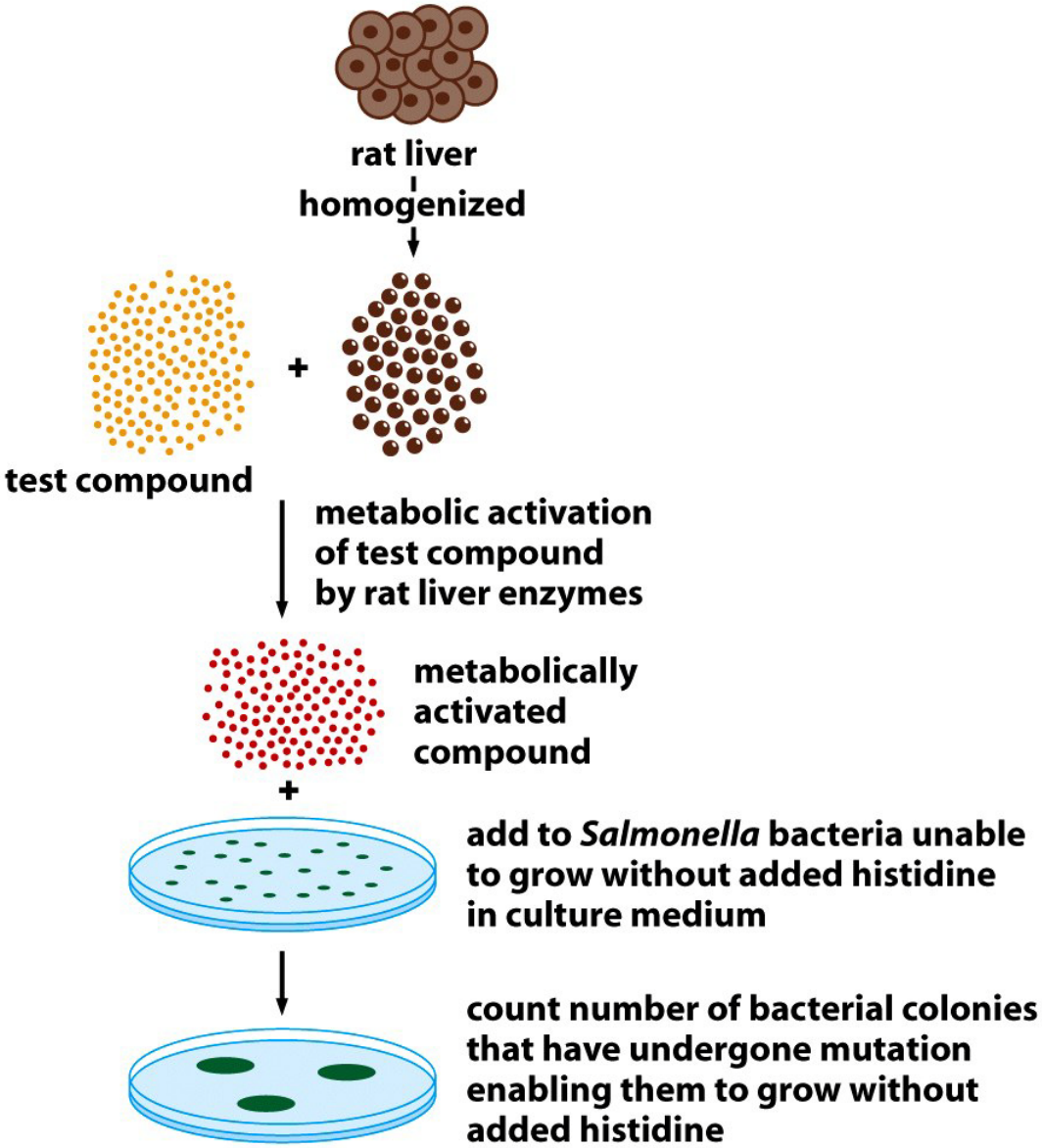
- Epidemiology: do the study of correlation, incidence of cancer and exposure to the compound
- Animal testing: does the same compound give similar cancer?
- Ames test
  - Short-term assay for mutagenicity
  - Test compound for ability to induce reversion in *Salmonella typhimurium* strains
  - $his^- \rightarrow his^+$; base substitution or frameshift
  - Liver microsomal extracts used for activation
  - Senstive, fast, inexpensive

The Standard Ames Assay



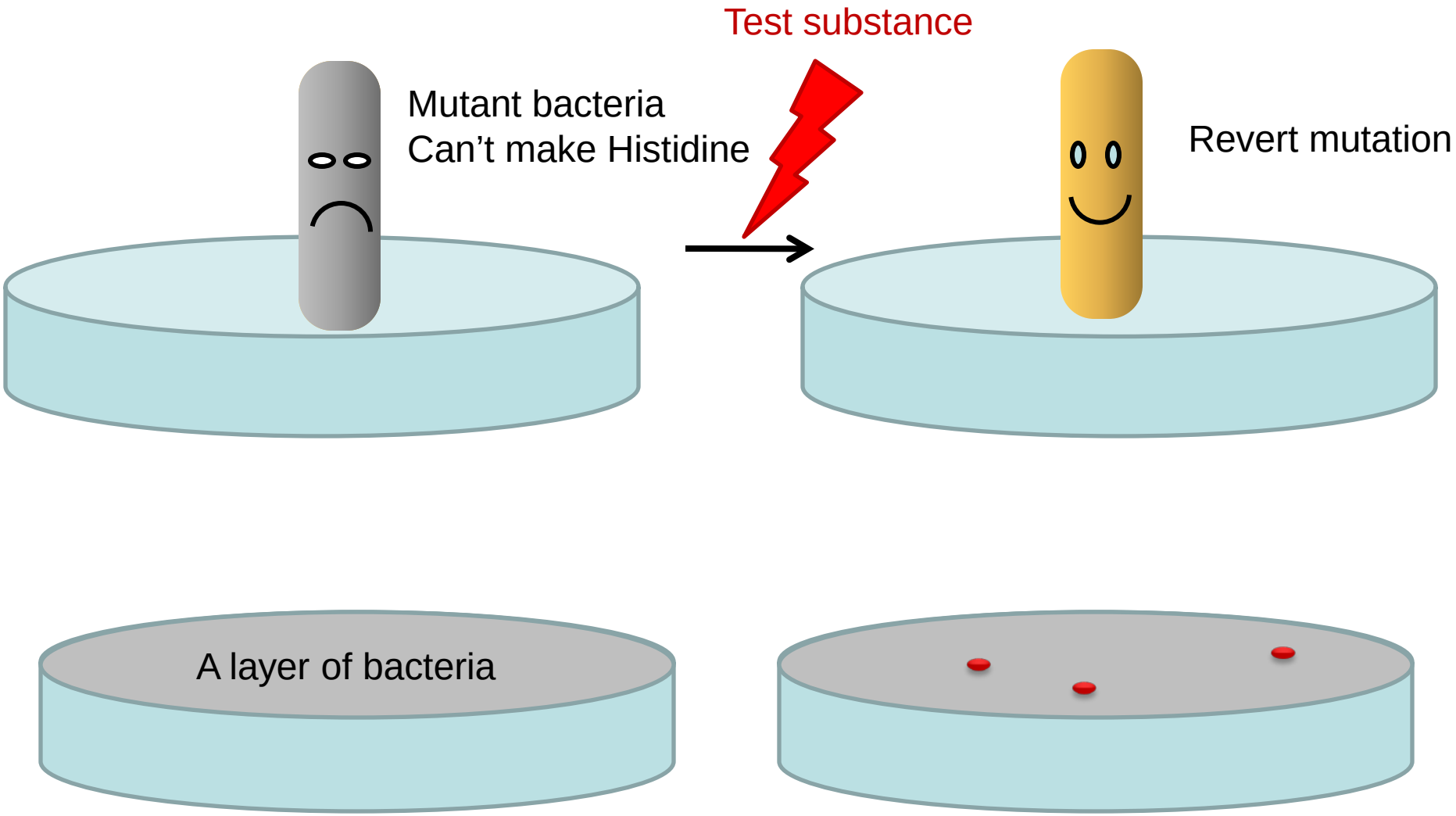
Prof. Dr. Bruce Ames
DoB 16th Dec, 1928
Biochem Mol Biol
UC Berkeley, USA

Ames test: Metabolic activation



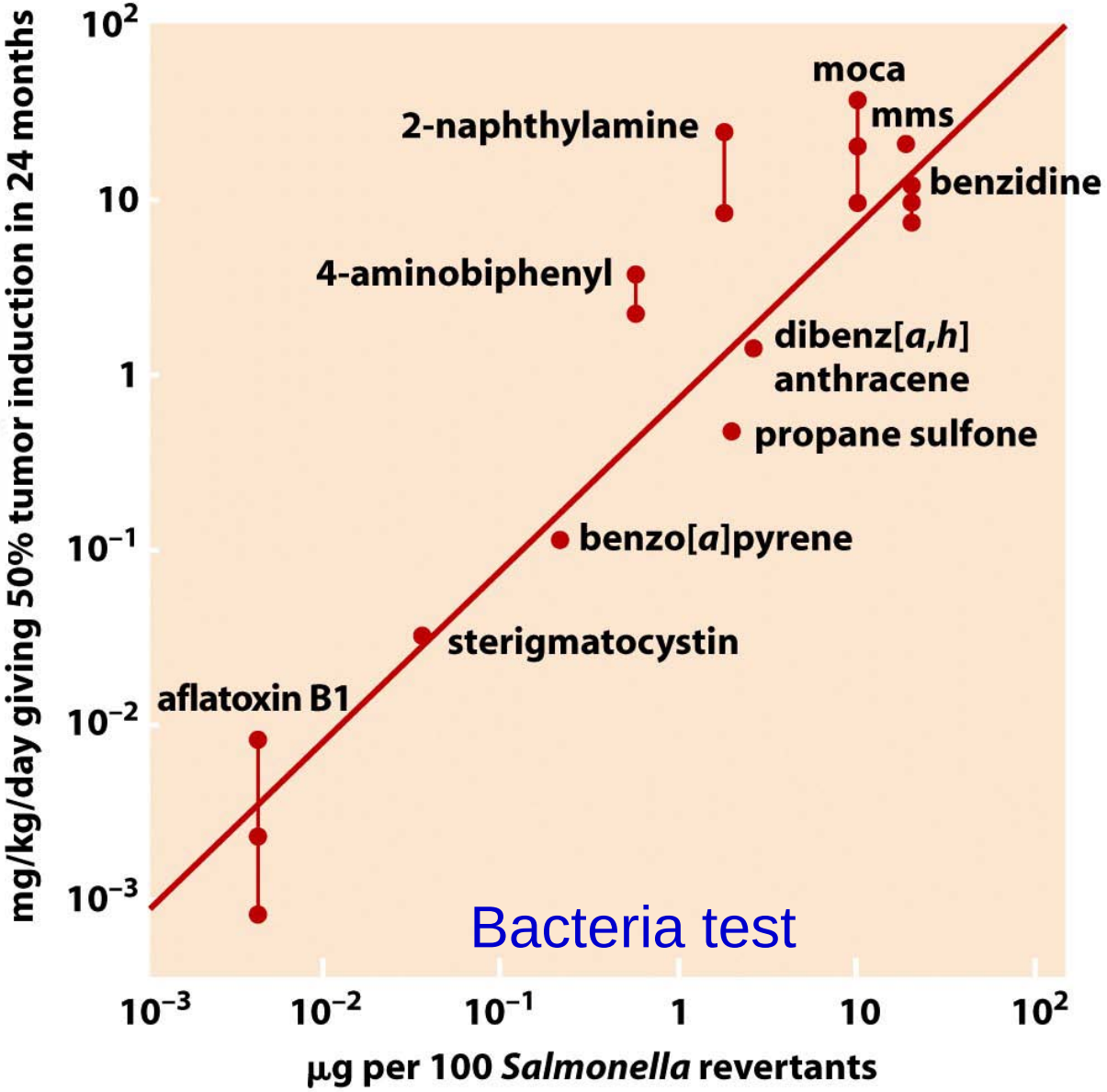
Ames Test

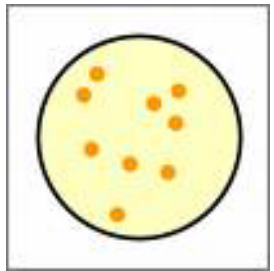
(1970s by Bruce Ames)



Mutagen ~ Carcinogen

黄曲霉毒素





Limitation of tests

	In vitro Ames test	*In vivo* Animal model	**Epidemiology study**
Tobacco smoke			
Asbestos 石棉			
Saccharin 糖精			
Phenobarbital 催眠药			
Isoniazid 抗结核药			

Chemical Carcinogen Models

- **1775- Sir Percival Pott,** a London surgeon, noticed that chimney sweepers frequently developed a peculiar form of scrotal cancer. He ascribed it to frequent, direct contact with coal tar. This launched 125 years of research in to the chemical basis of cancer.
- **Treatment of mice with carcinogens** is the basis of numerous mouse models of cancer.

Skin –	7,12-dimethylbenz[α]anthracene (DMBA) + 2-O-tetradecanoylphorbol-13-acetate (TPA)
Lung –	Nitrosamines
Liver –	vinyl chloride
Breast –	N-Nitroso-N-methylurea (NMU)
Colon –	dimethylhydrazine (DMH) Nitrosamines
Bladder –	Aromatic Amines

DMBA/TPA

Br J Dermatol 149(5):960-967, 2003

Cancer News

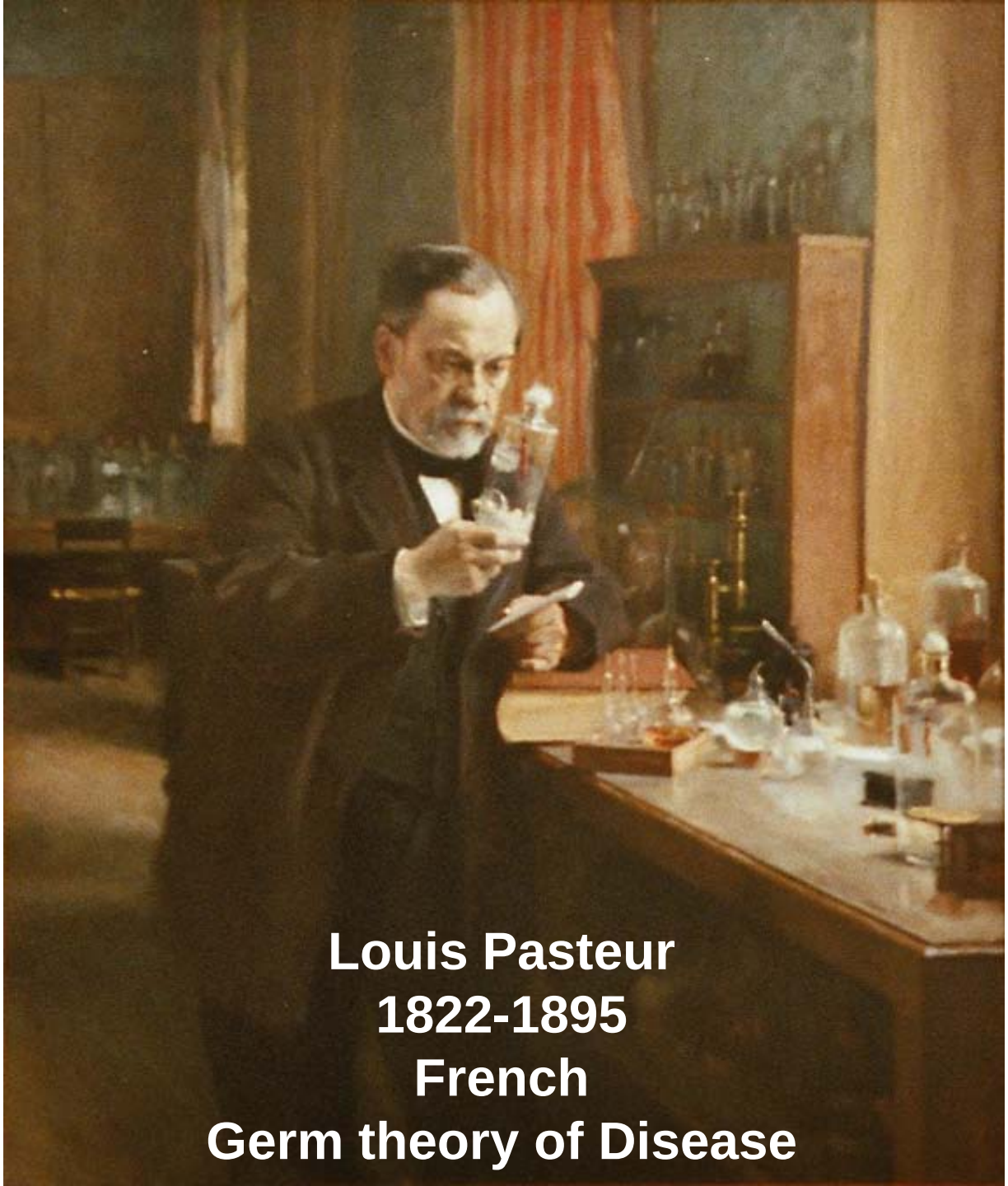
Why the discrepancy?

- Model system (Bacteria, rabbit) ≠ Human
- Dose
- Indirect Mechanism:

  Asbestos → Lung damage → Inflammation

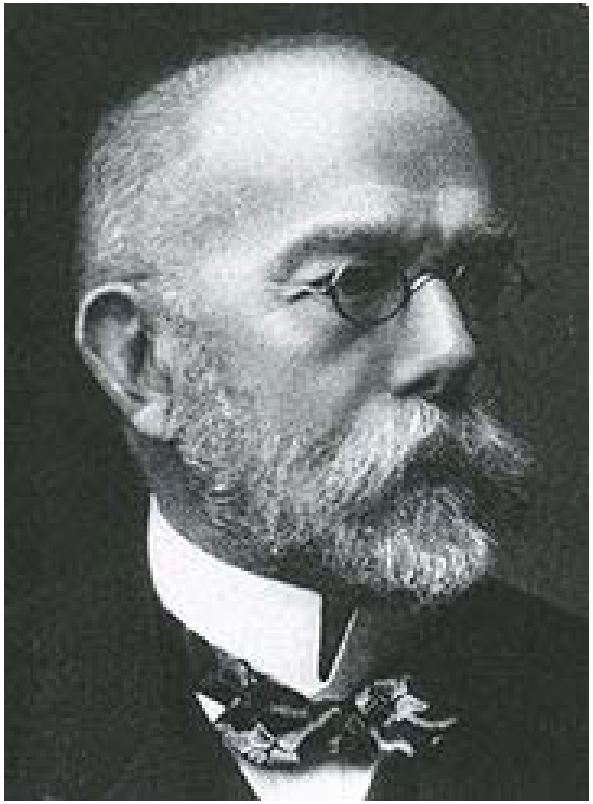
Introduction

- What is cancer?
  - Normal cells go bad.
- What causes cancer?
  - Carcinogens
  - **Infection?**
- How do we deal with cancer?



Louis Pasteur
1822-1895
French
Germ theory of Disease

Disease and infection



Robert Koch (1843 –1910)
German physician

- Isolated

  anthrax (炭疽菌),
  tuberculosis (结核）,
  cholera （霍乱 ）

- Koch Postulates:

  Germs → Diseases

1905

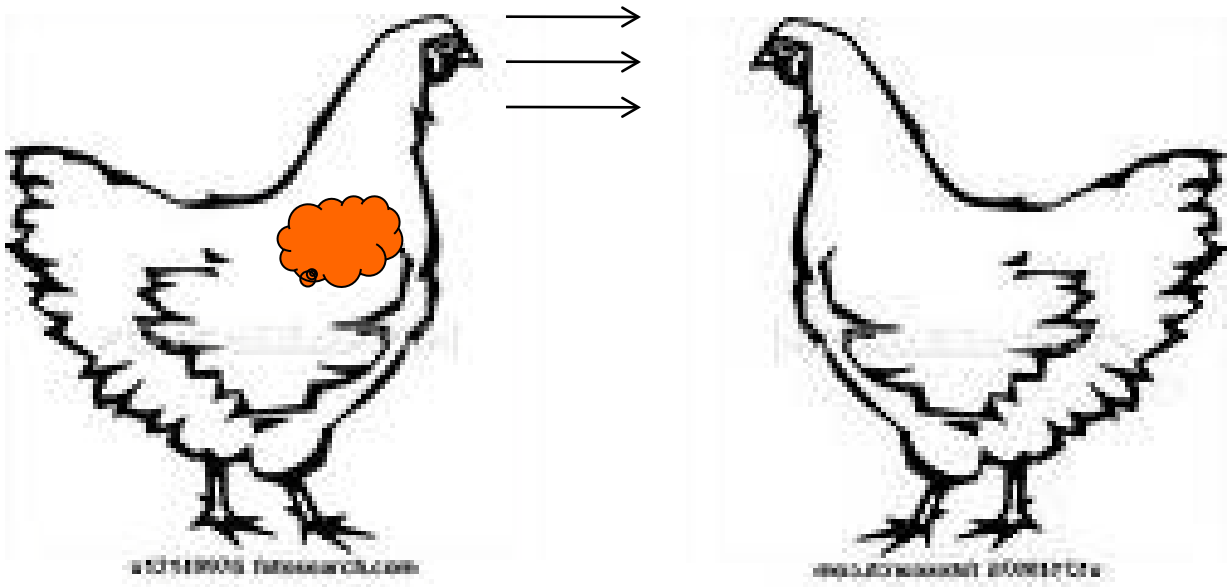
Cancer and infection

Chicken tumor

- 1909 Peyton Rous , Long island, New York
- A farmer with sick chicken

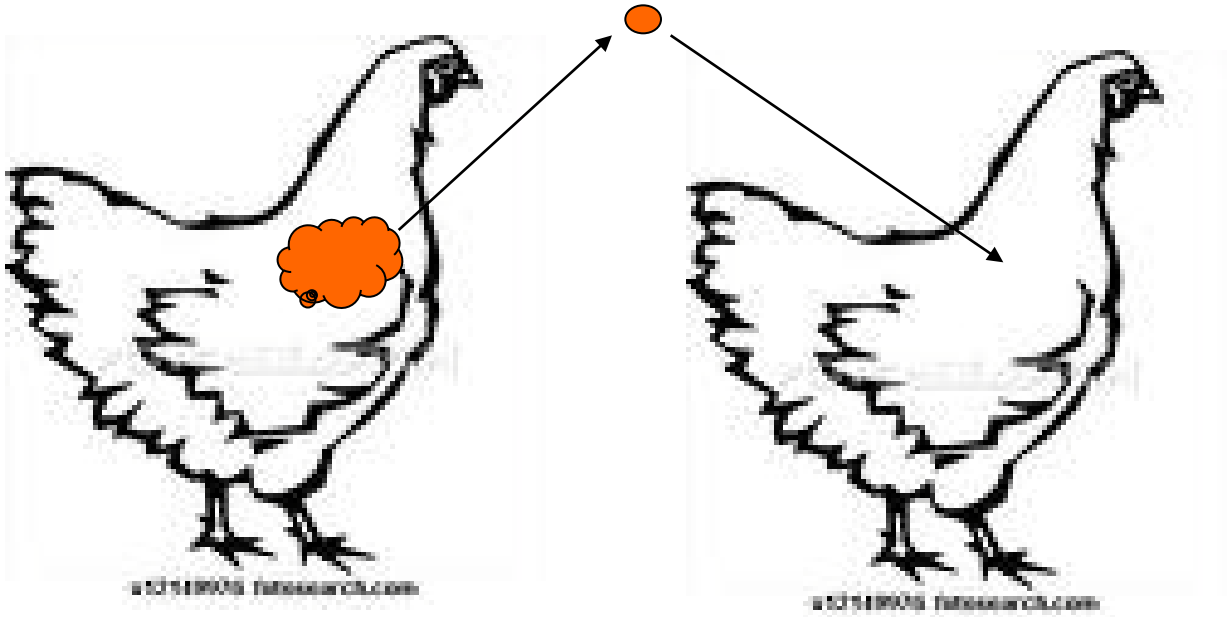
1879-1970

Rous experiment #1



Conclusion ?

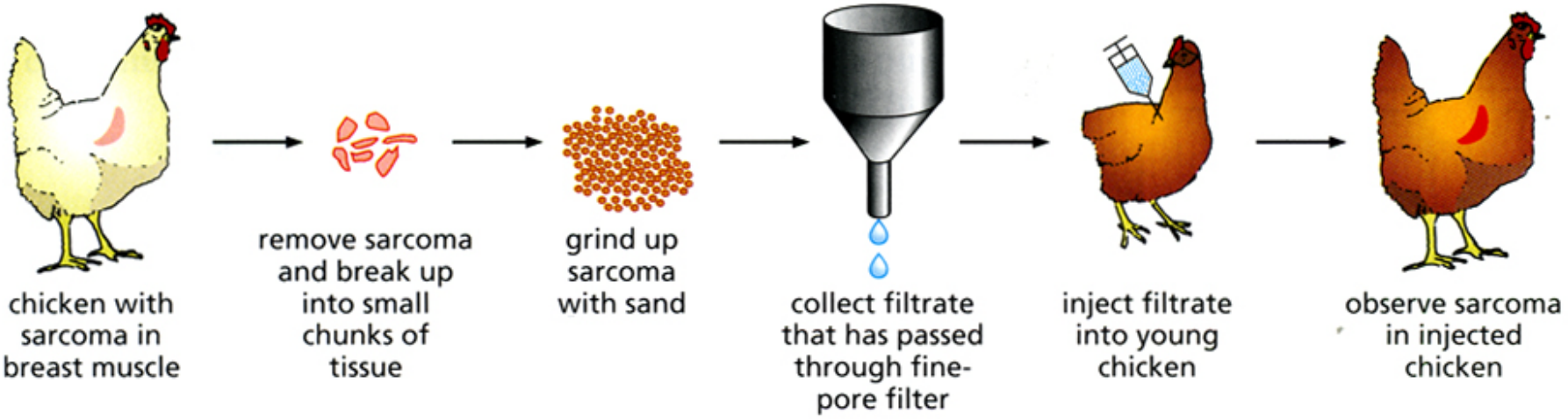
Rous experiment #2



Conclusion ?

NEXT ?

Rous experiment #3



Conclusion:

1. Tumor develops in a few weeks→good model for research

2. It can be passed again and again→ Something alive

3. It is very small→ Virus (Rous Sarcoma Virus: RSV)

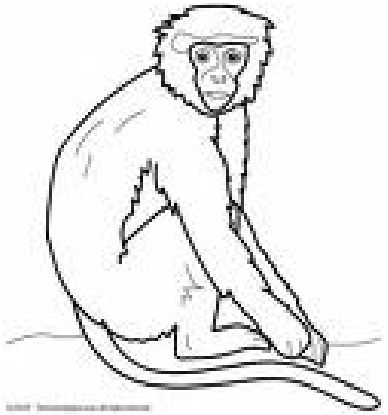
Victory of infectious theory

- 1911 Rabbit virus→ Myxomas 黏液瘤
- Rous and Collaborators: （1930-1970）
  2 more chicken viruses→ papilloma

Rous sarcoma virus, now known to be a retrovirus

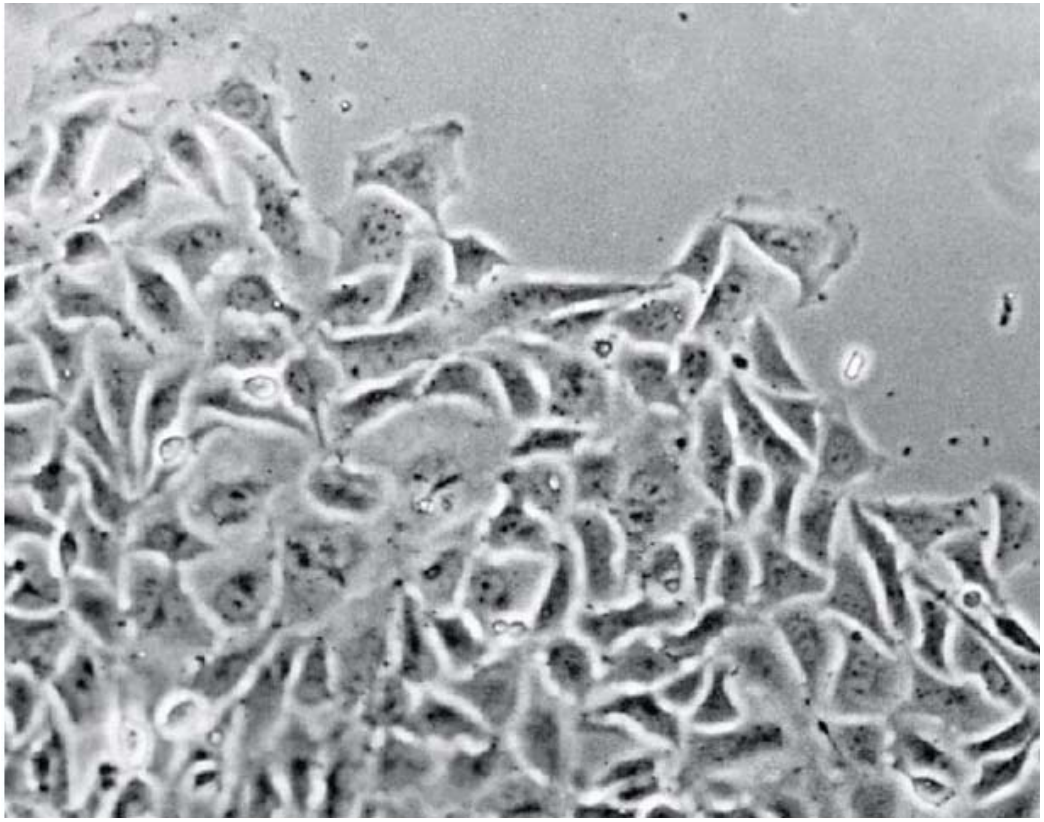
Cancer = Infectious disease

SV40 virus



- Monkey cell lines → replicate → Kill cells
- Lytic life cycle

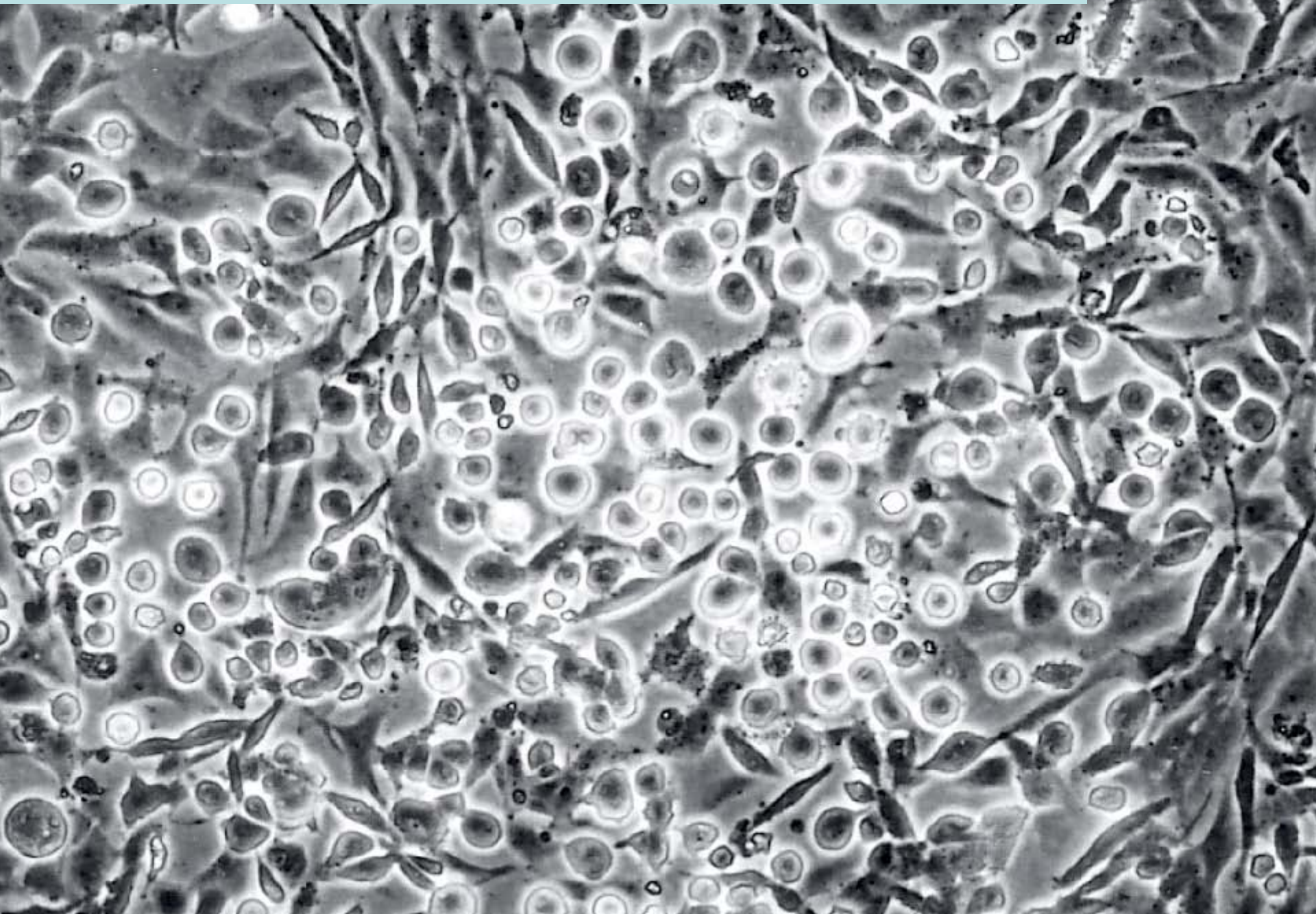
Chicken embryonic fibroblasts



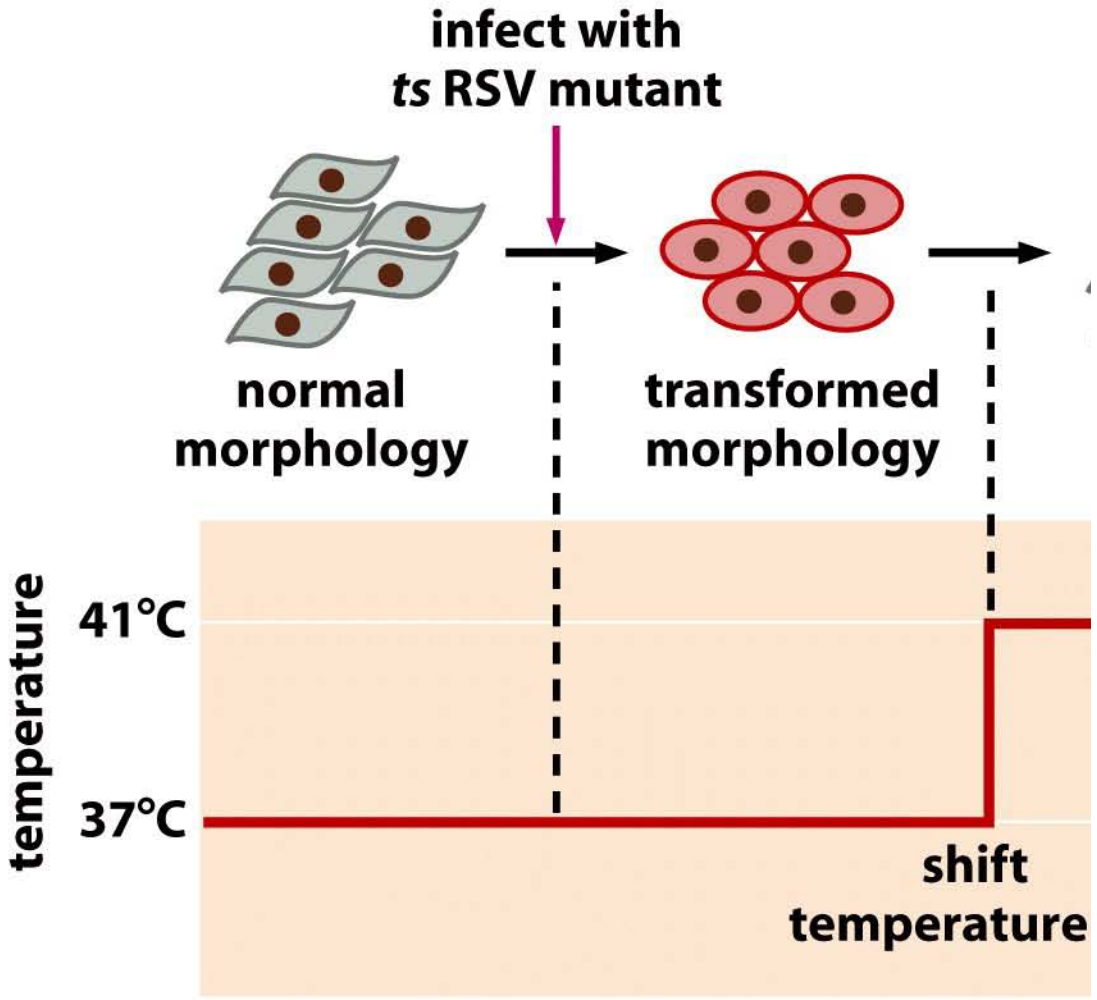
+ RSV

→ **Cells survive !**

They look like sarcoma cells…



Need RSV continuously?



Transformed cells

- Change in cell shape
- Loss of contact inhibition
- Proliferate indefinitely

- → Your evaluation of the transformed cells:
  - Neoplasia
  - Metastatic tumor

How bad are the cells?
2 functional assays

1. Foci formation on soft agar

2. Tumor formation in Nude mice

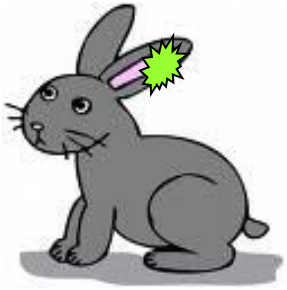
Other viruses can also induce cancer

- Richard Shope: rabbit papillomas (Warts) DNA virus
- SV40 – the 40th simian virus
- MMLV- mouse mammary tumor virus (RNA)

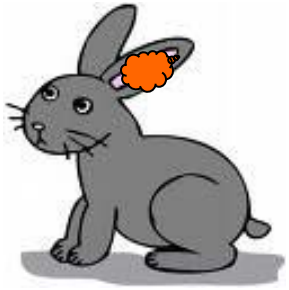
Summary

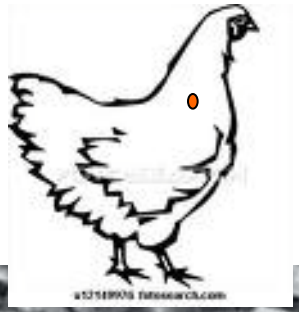
1. Some viruses can cause tumor.
2. The infected cells are **transformed** to tumor-like cells.
   - Loss of **contact inhibition**
   - Independent of **matrix attachment**
   - Change in cell shape
   - **Immortalized**
3. The transformation requires the **continuous** presence of the virus.

A new study system

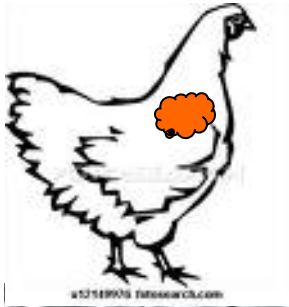


1. Months,years



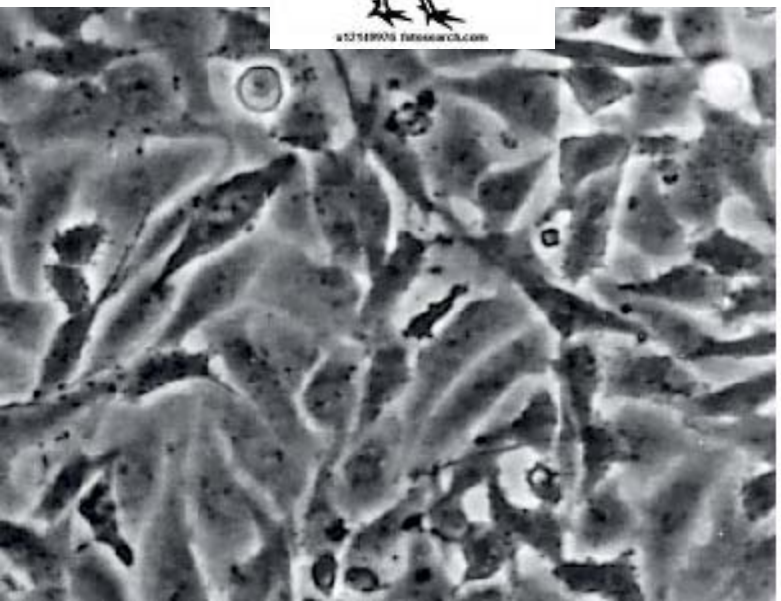


2. Several weeks

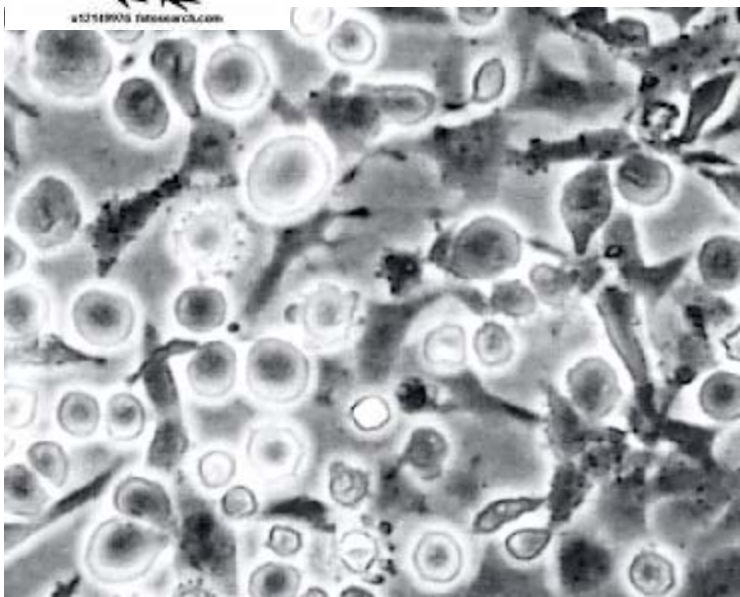


3. Several days

Transformation



+RSV



Victory of infectious theory

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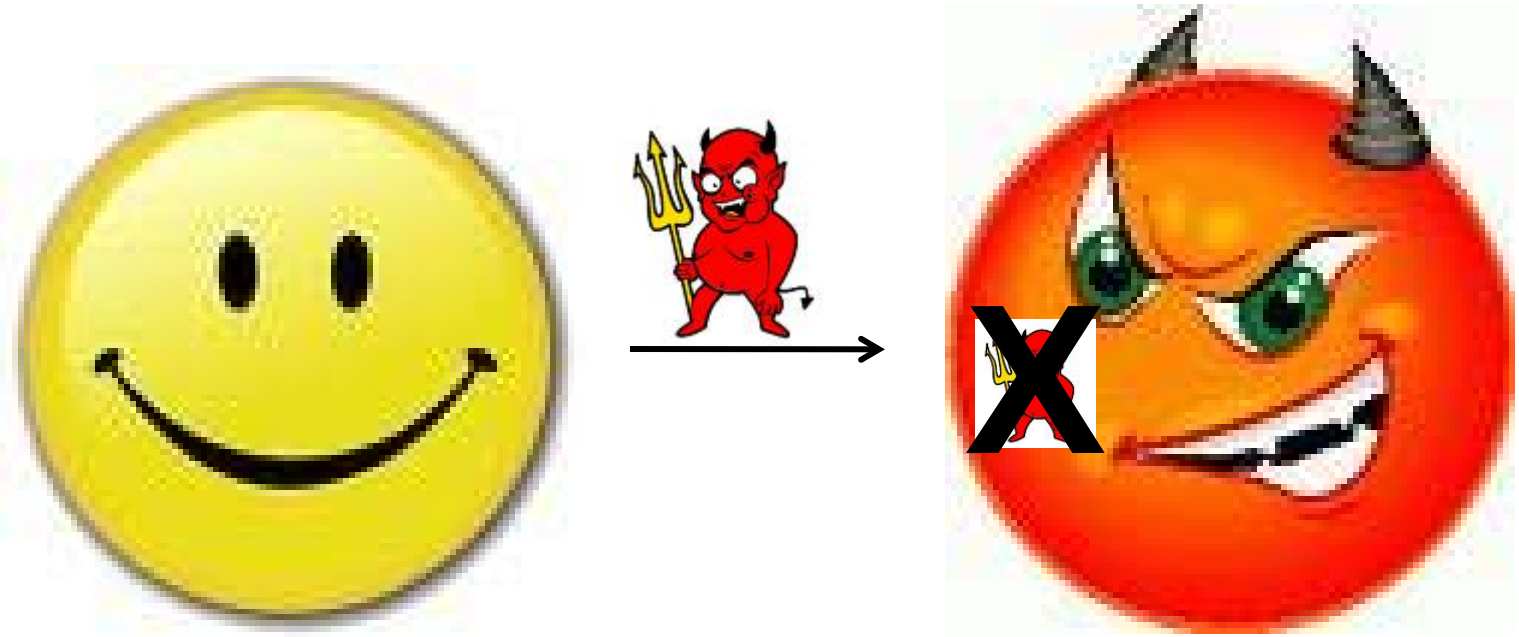
Rous sarcoma virus, now known to be a retrovirus

Cancer = Infectious disease

Hit & Run, or dependence on virus



Normal cell

Tumor cell

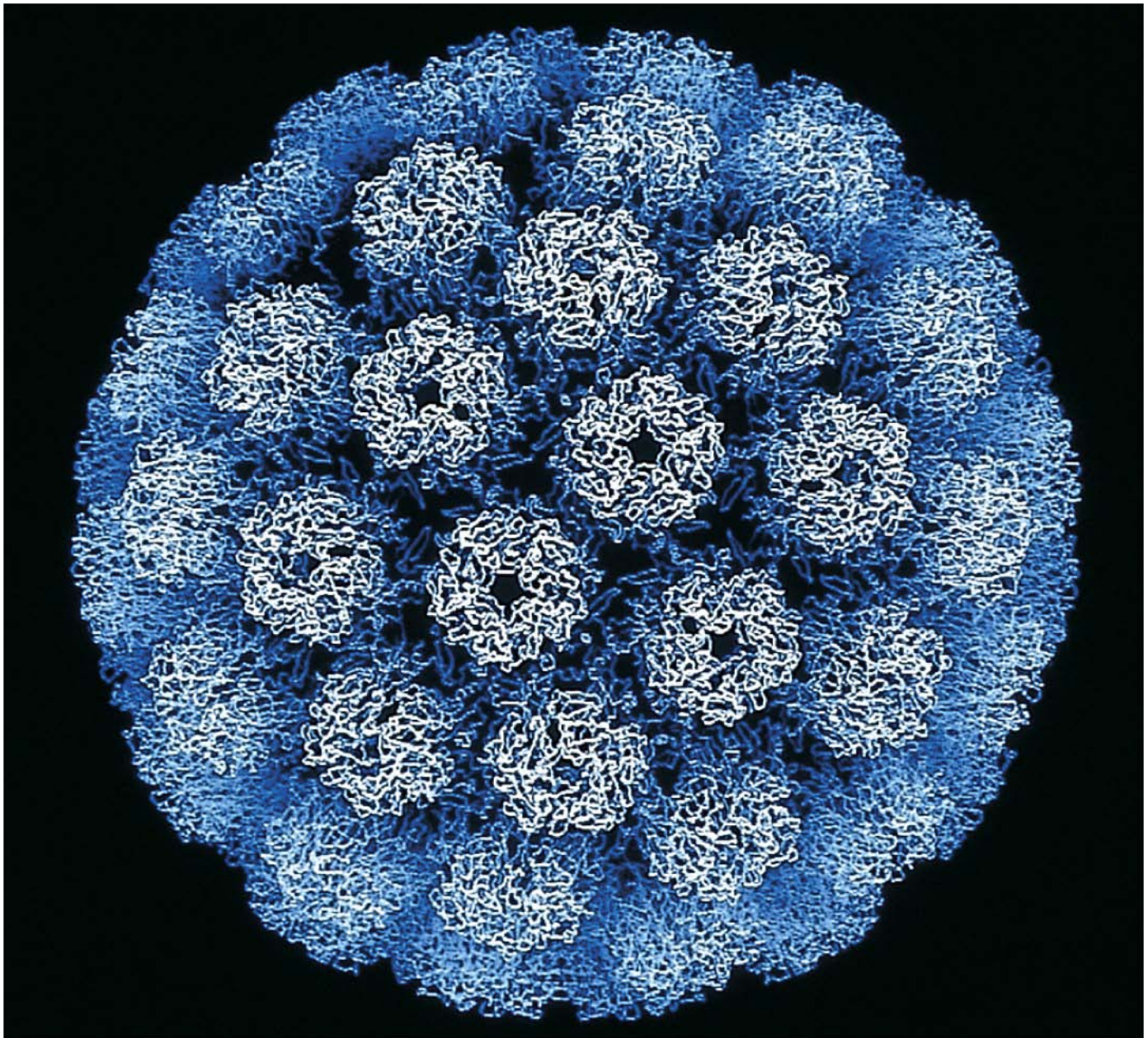
How do viruses cause cancer?

SV40 infection

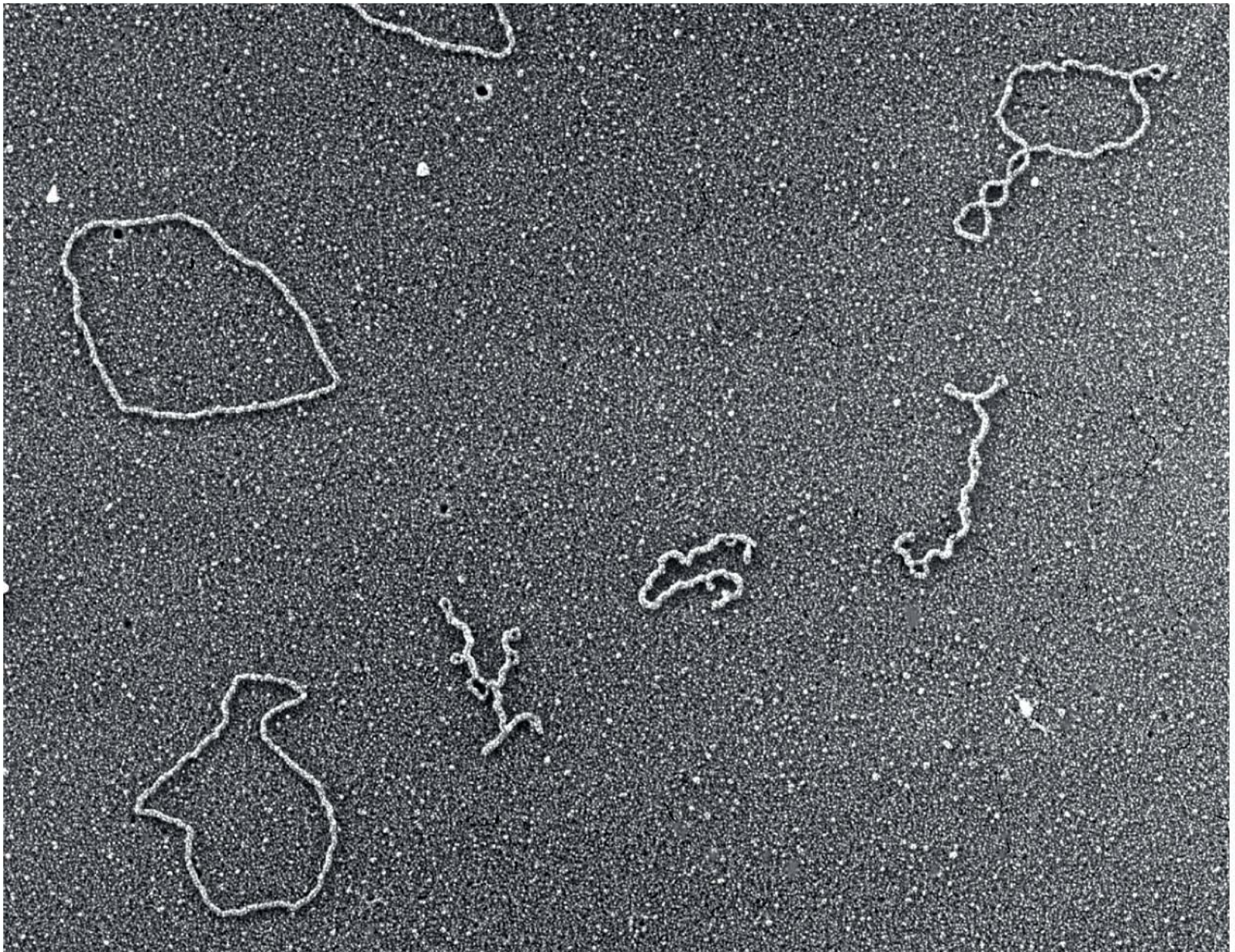
- Monkey cell lines → replicate →Kill cells

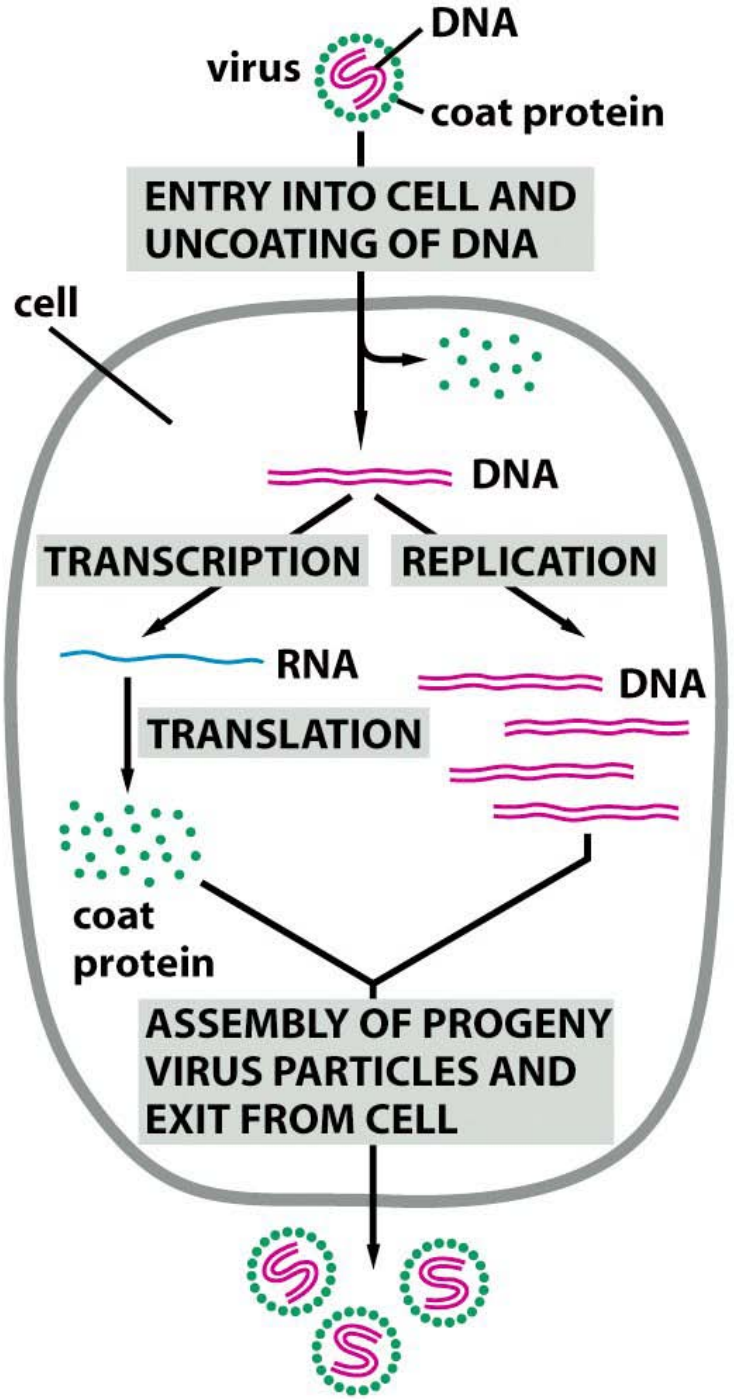
- Mouse or rat cell lines → non-permissive
  - Low frequency: transformed cells

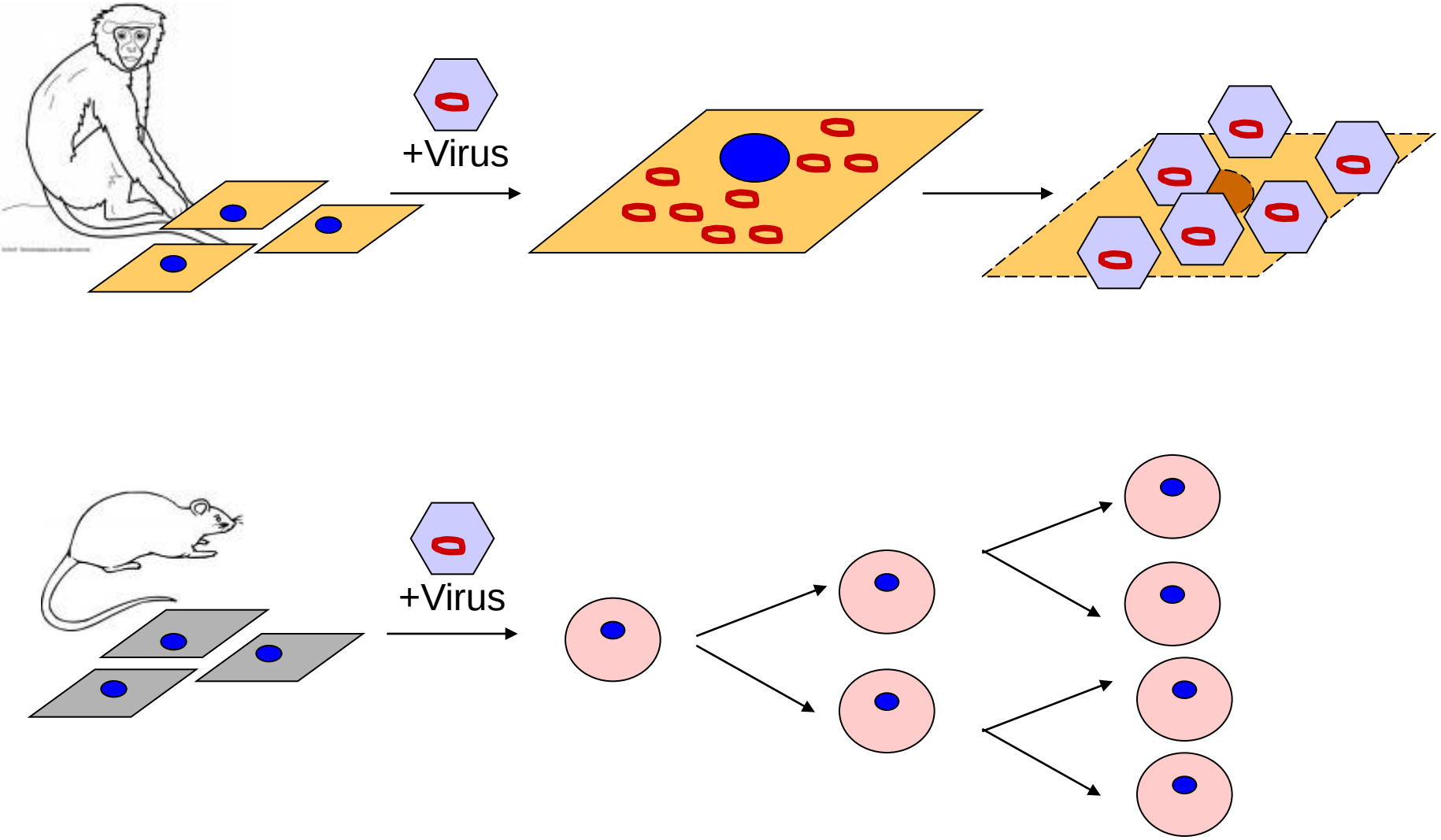
SV40 virus



SV40 DNA

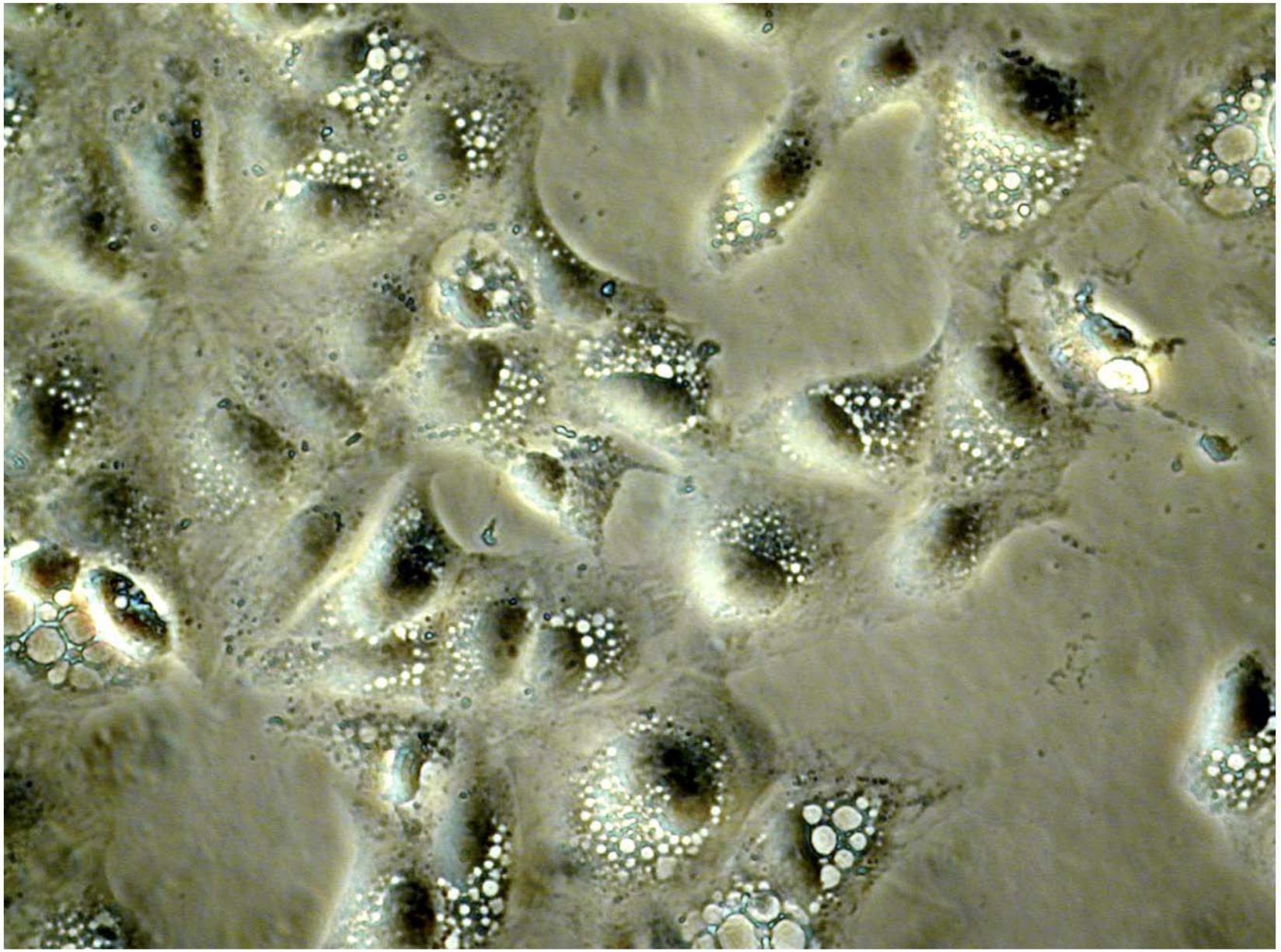



DNA
virus
coat protein
ENTRY INTO CELL AND
UNCOATING OF DNA
cell
DNA
TRANSCRIPTION
REPLICATION
RNA
DNA
TRANSLATION
coat
protein
ASSEMBLY OF PROGENY
VIRUS PARTICLES AND
EXIT FROM CELL



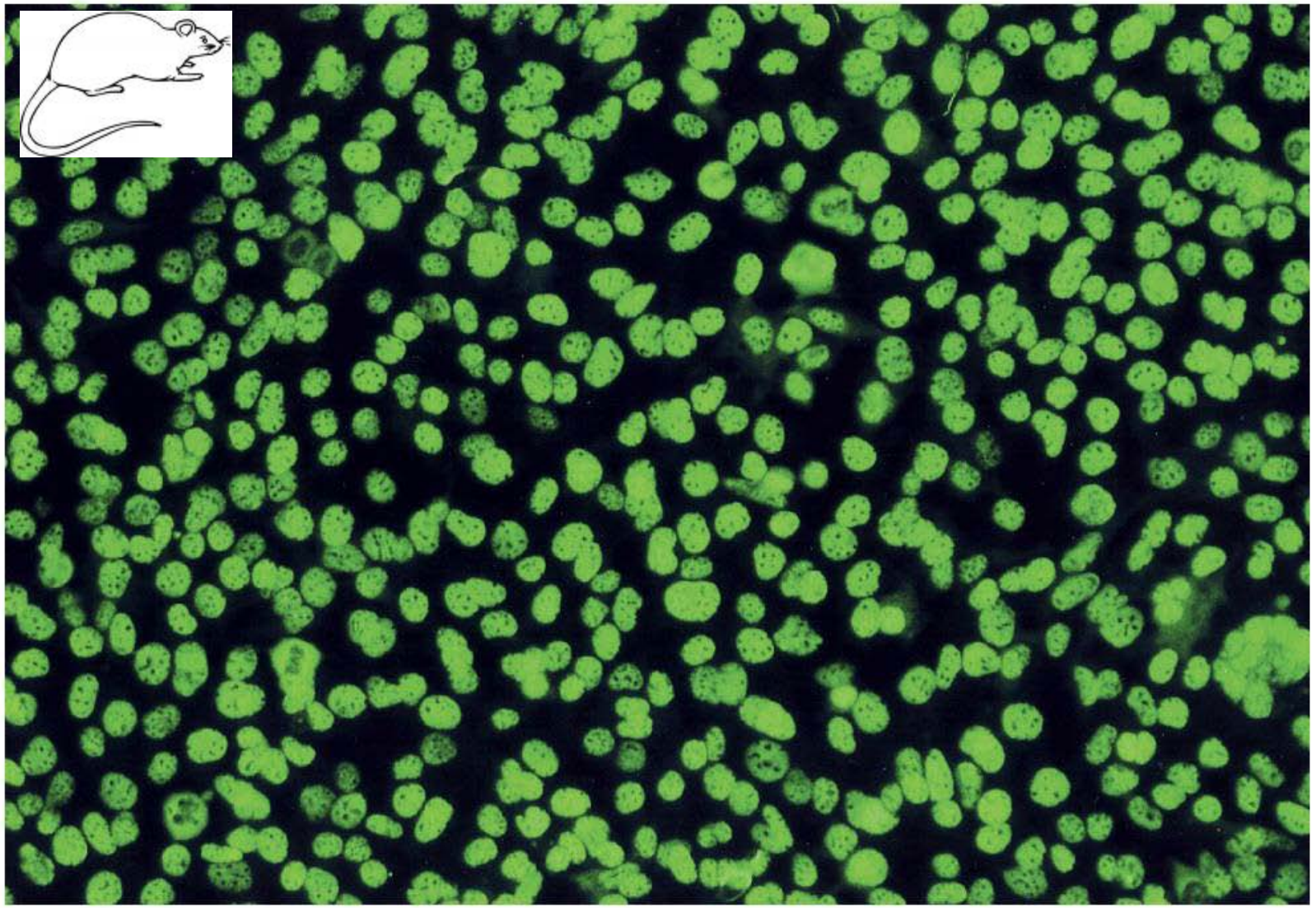
- How does the virus continue in daughter cells?

SV40 infected monkey cells

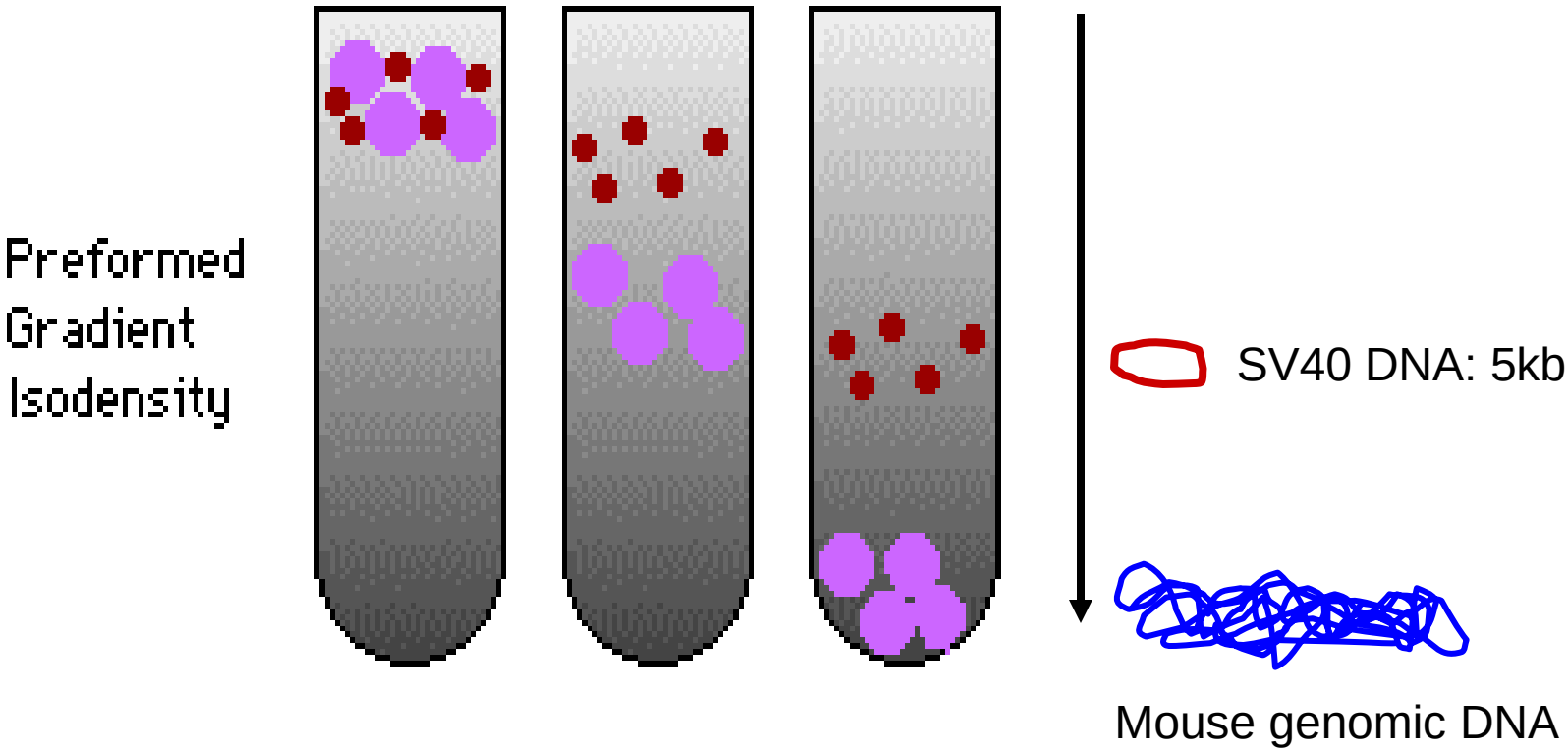


SV40 is required for tranformation

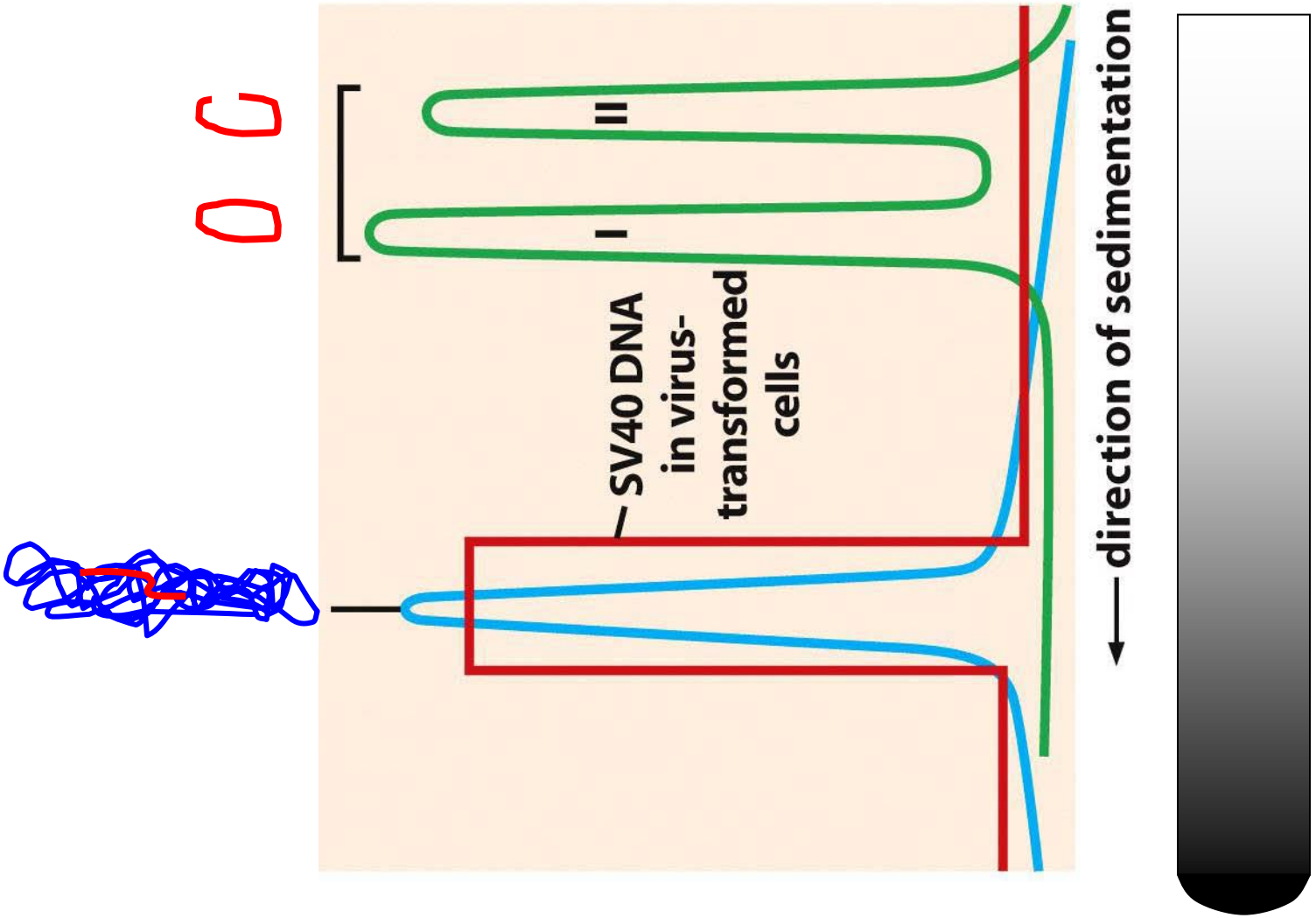
SV40 T antigen in mouse cells



SV40 DNA in mouse cells



Sambrook, 1968



Integration

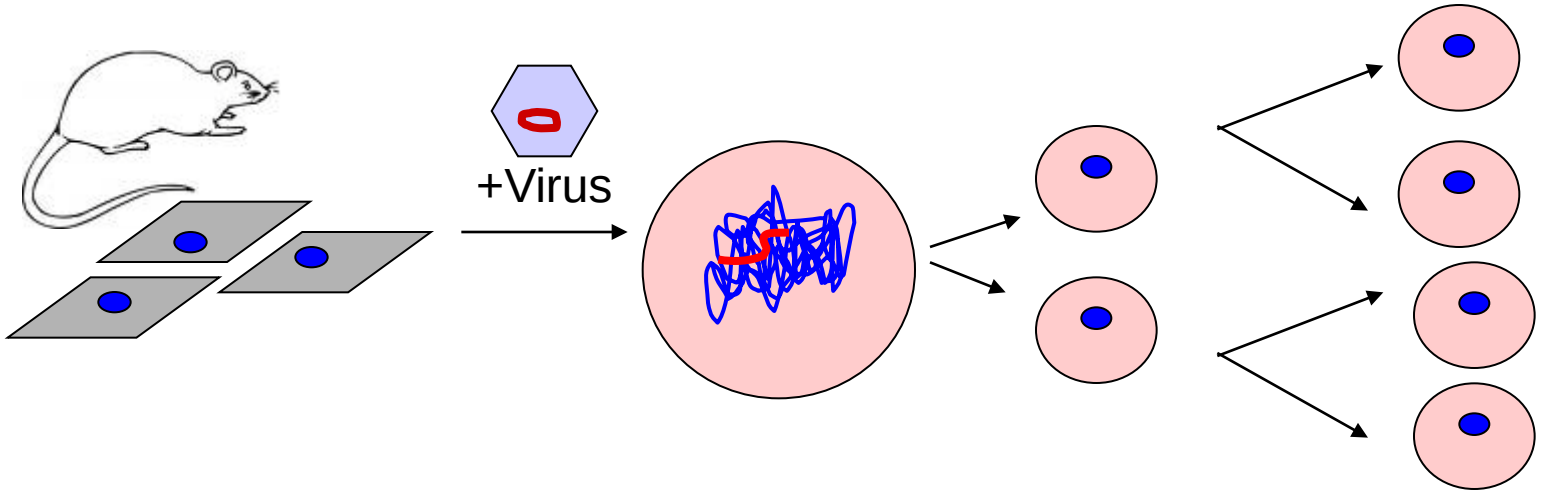
Alkaline pH

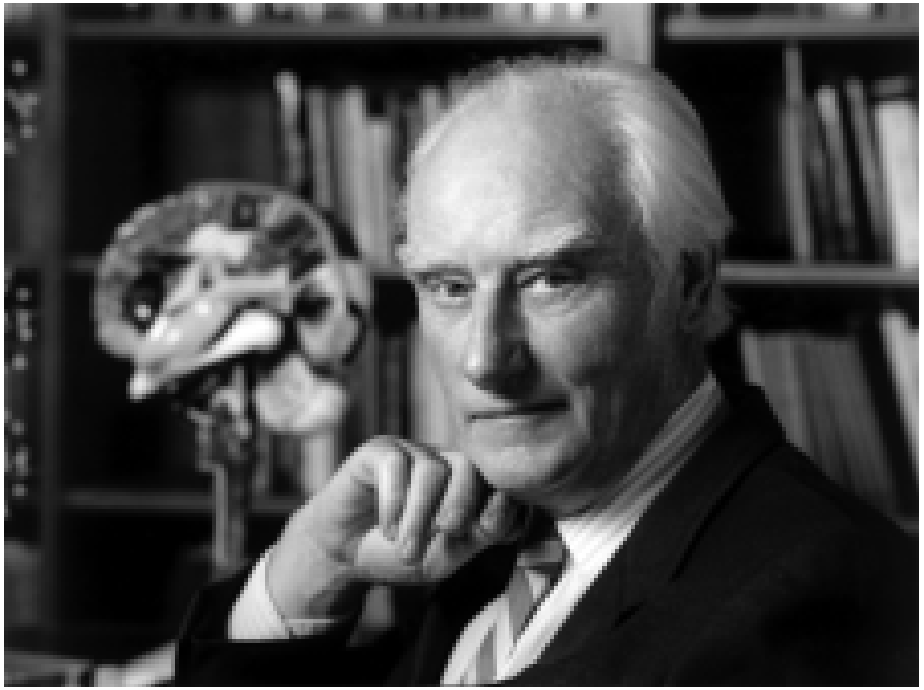
High temperature

……

Resistant to chemical separation!

Viral DNA integration





Francis Crick

Nobel award

Central Dogma:
1958

DNA

↓

RNA

↓

Protein

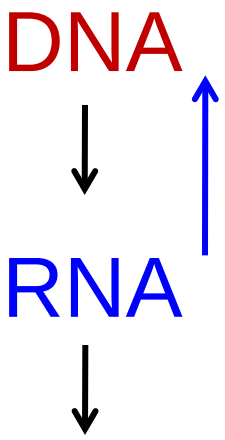
How can RNA integrate into DNA?

Howard Temin

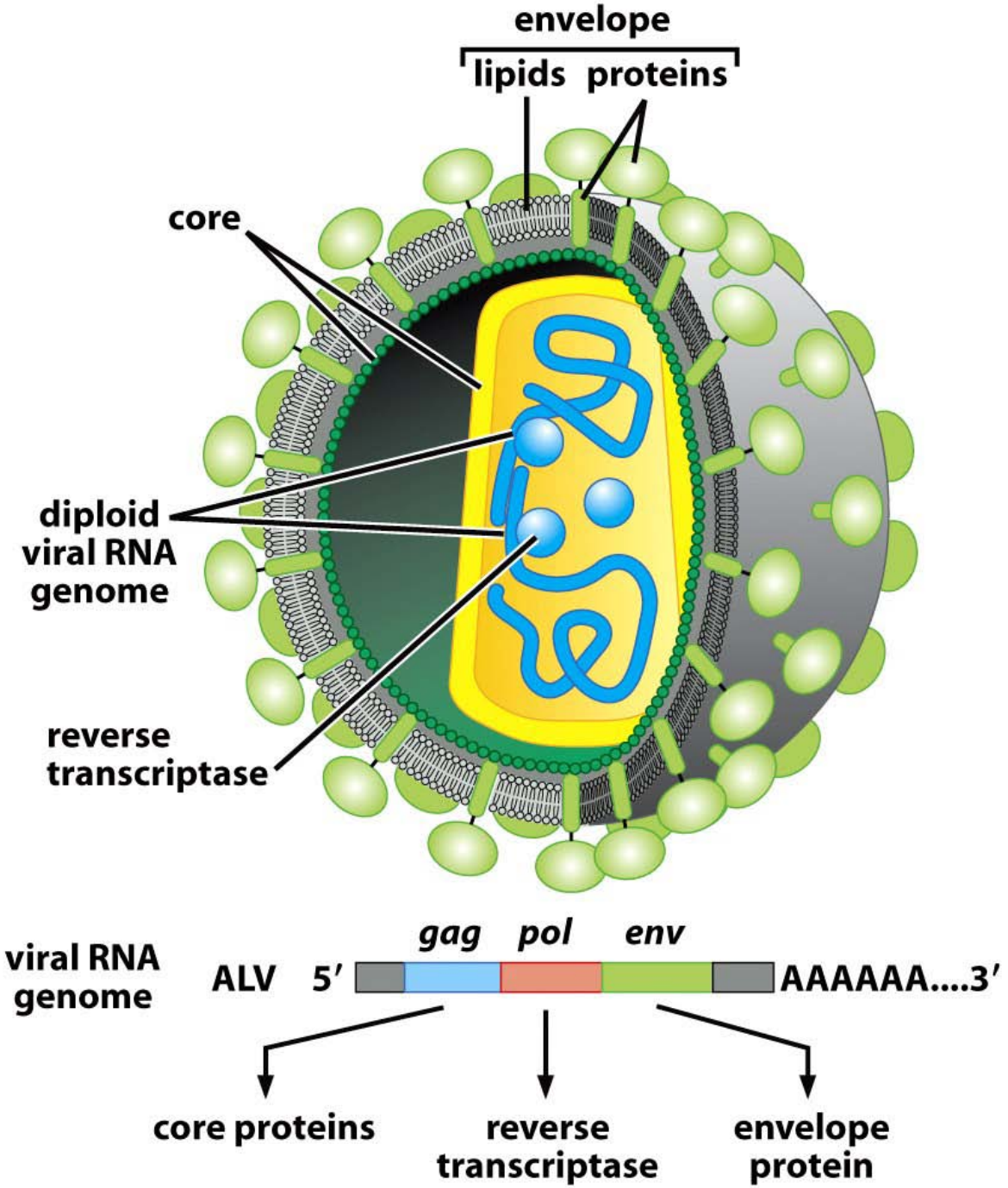
Ph.D 1959 @ age 25
Idea of provirus @ age 30

Temin's idea

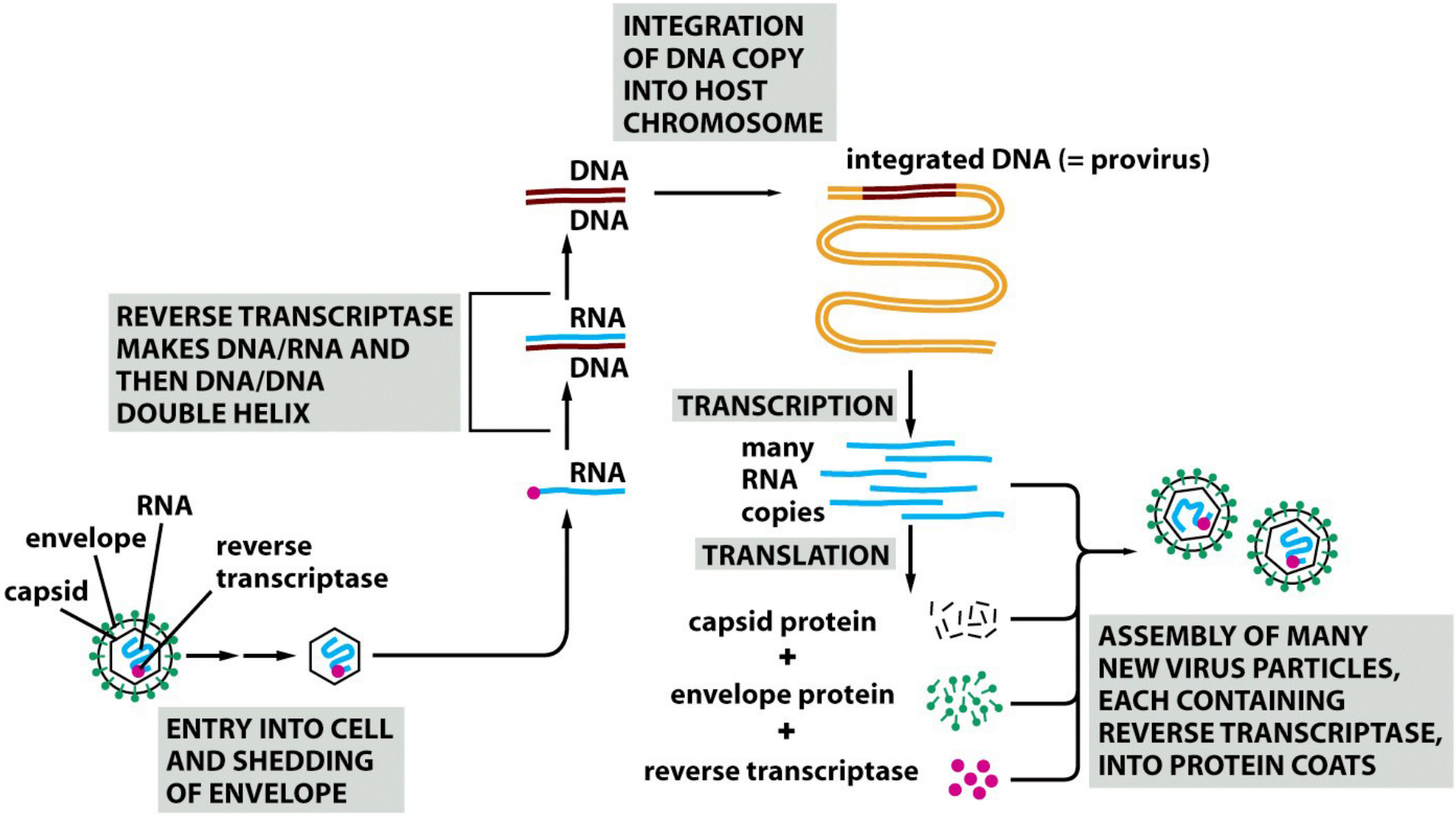
1964



- RNA virus
  - RSV



Life cycle of RNA virus



How can RNA integrate into DNA?

Howard Temin

Ph.D 1959 @ age 25
Idea of provirus @ age 30

Temin's idea

1964

DNA

↓

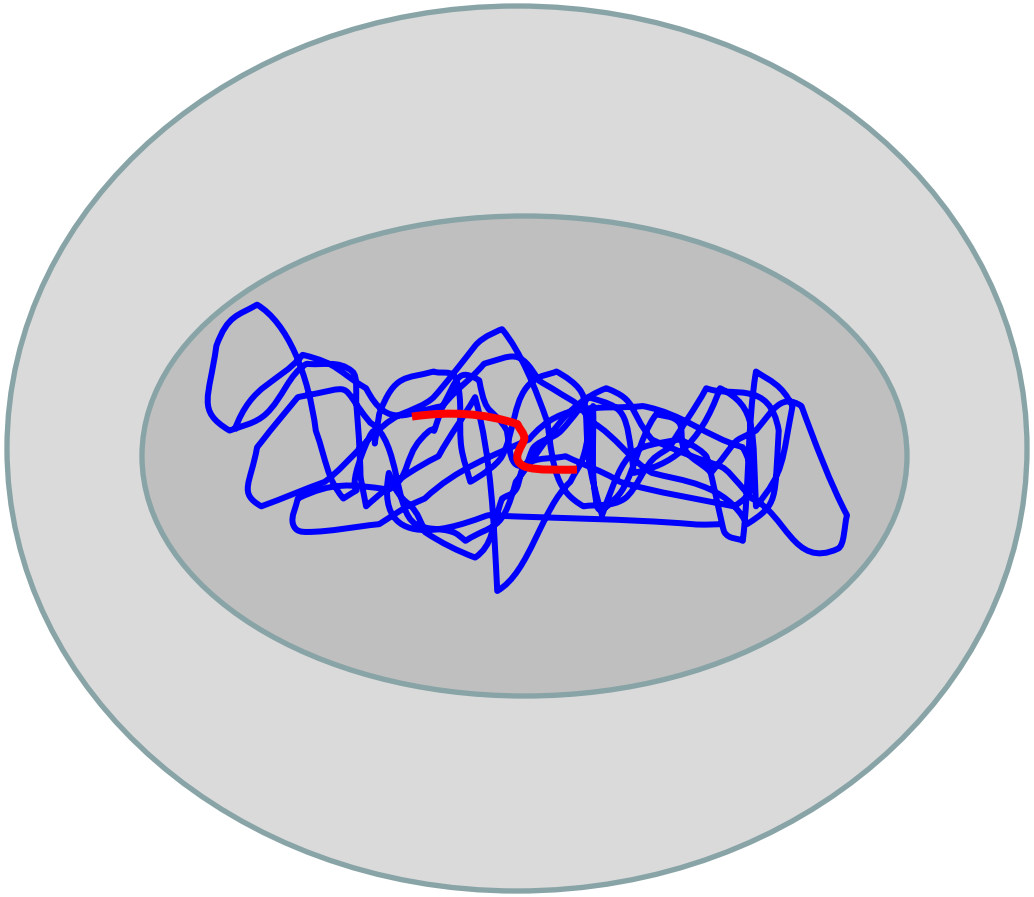
RNA

↓

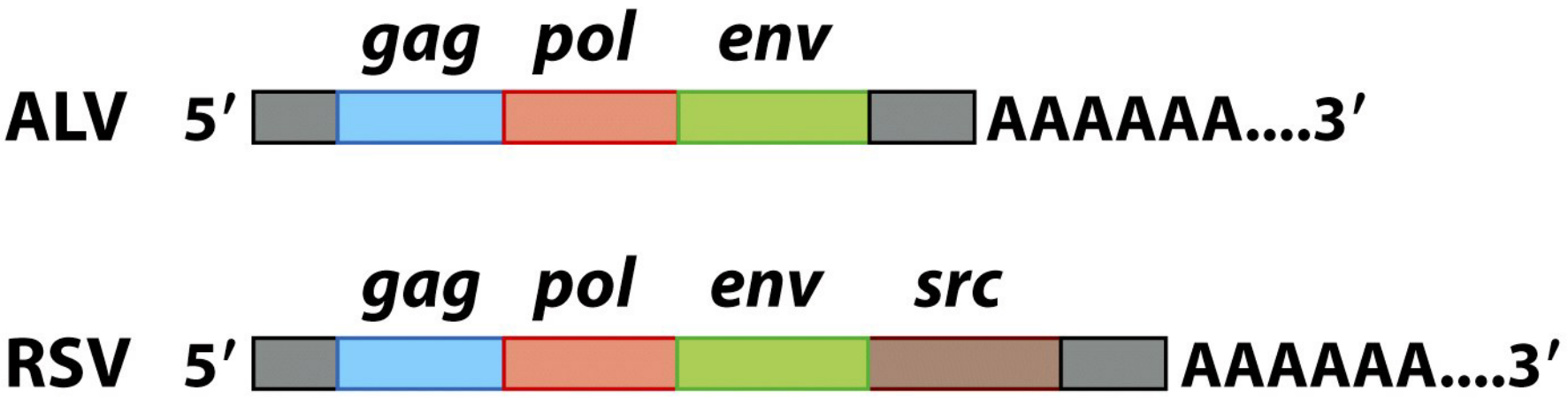
Protein

1975

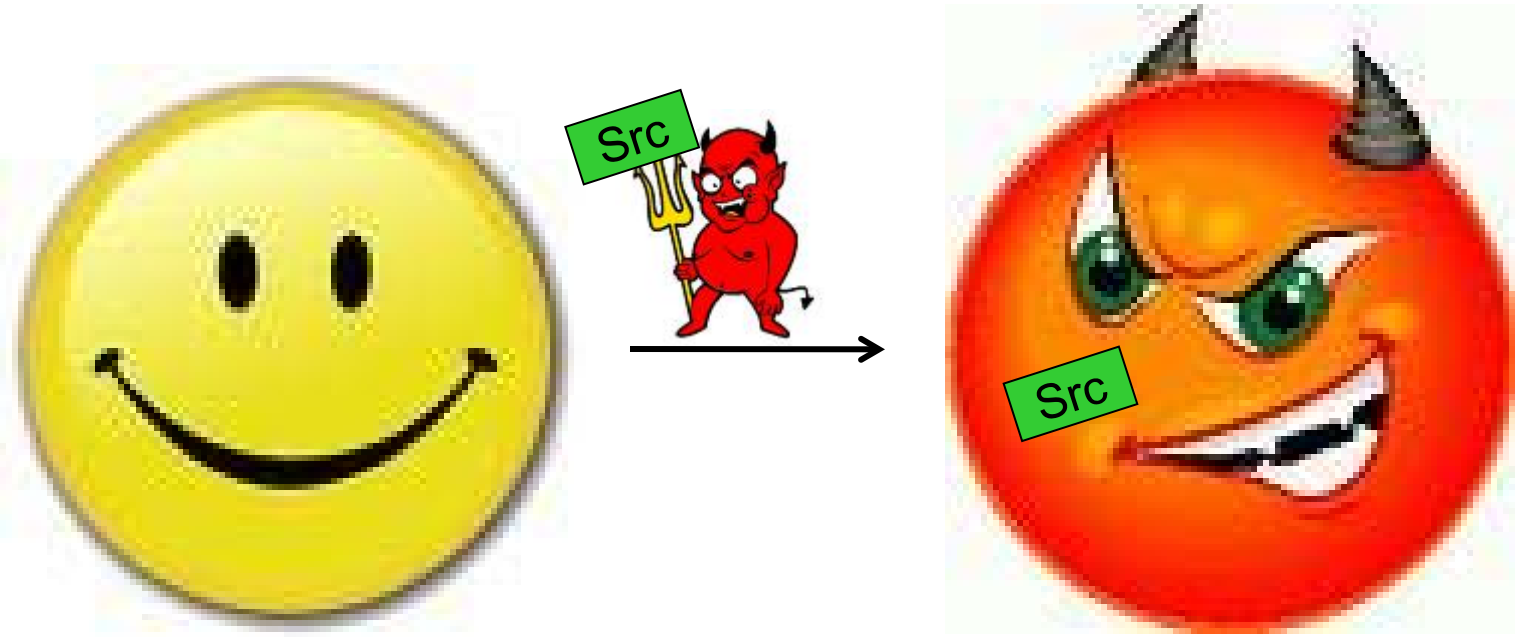
Integrated viral DNA→ tumor?



Avian Leukosis Virus(禽类白血病病毒)



The first oncogene-Src

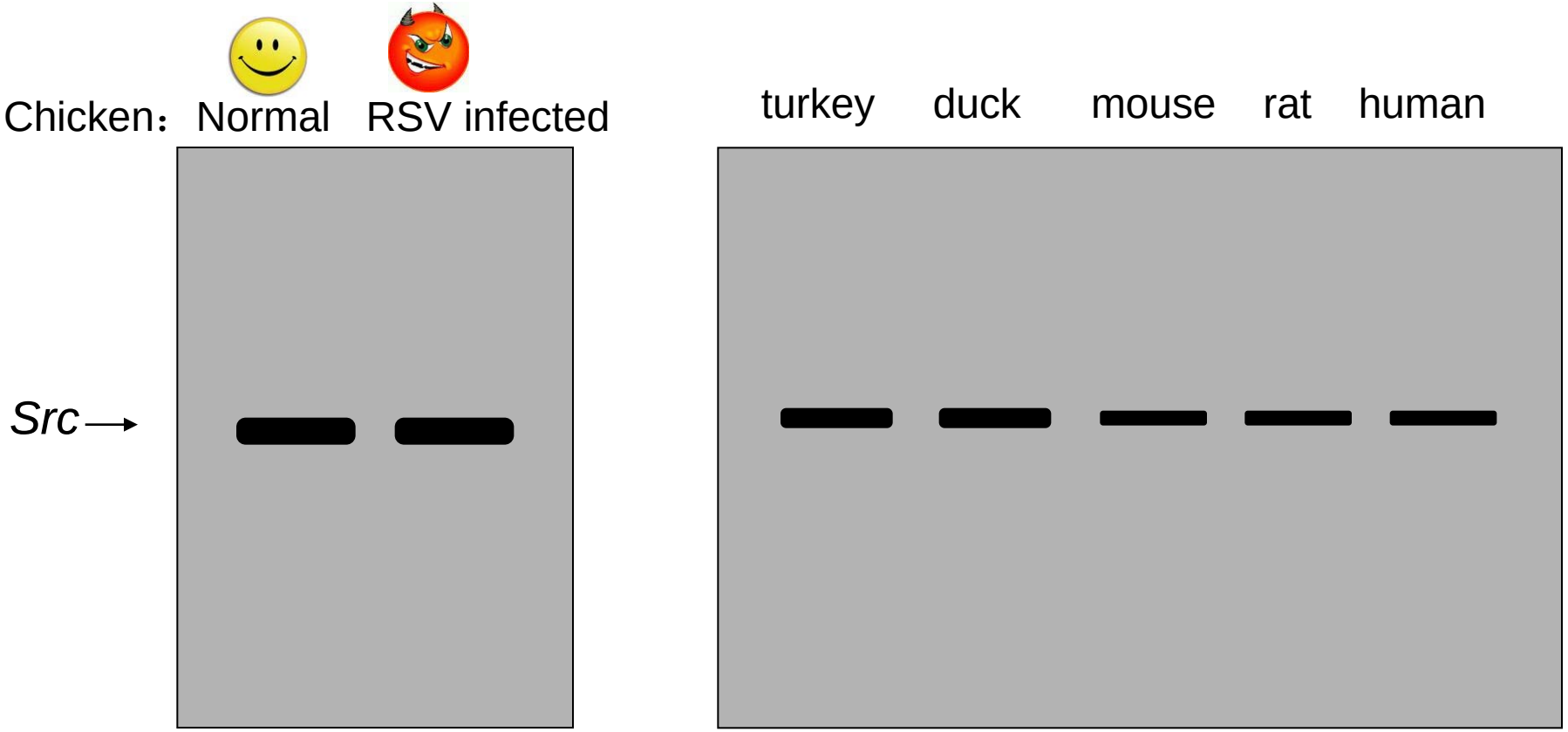


Normal cell

Tumor cell

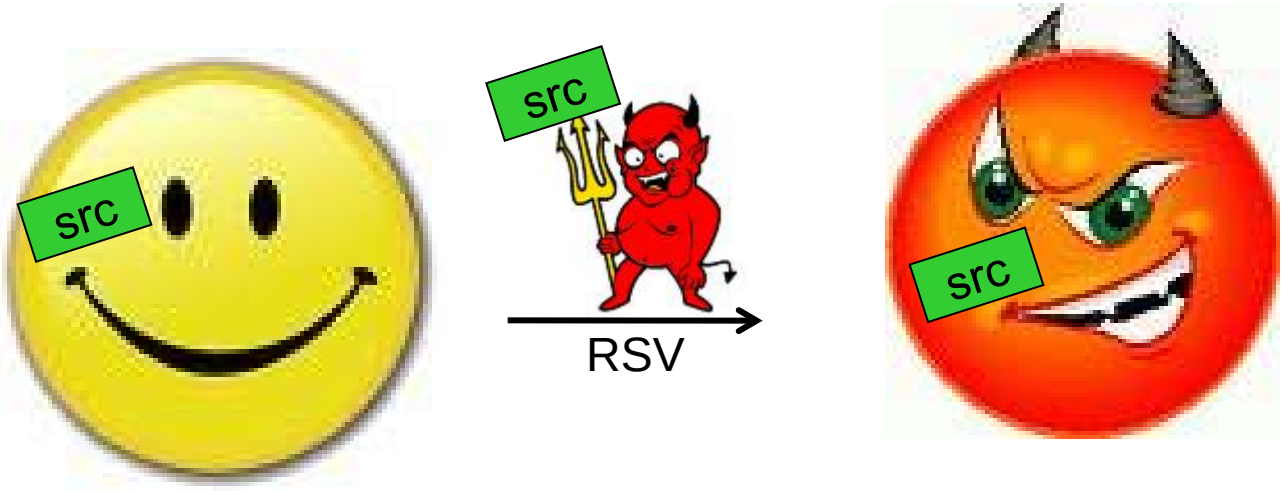
Detect *Src* in sarcoma cells

- Michael Bioshop and Harold Varmus, 1974, University of California, San Francisco



Src in normal cells

- Src gene is present in normal genome.
- Conserved sequences



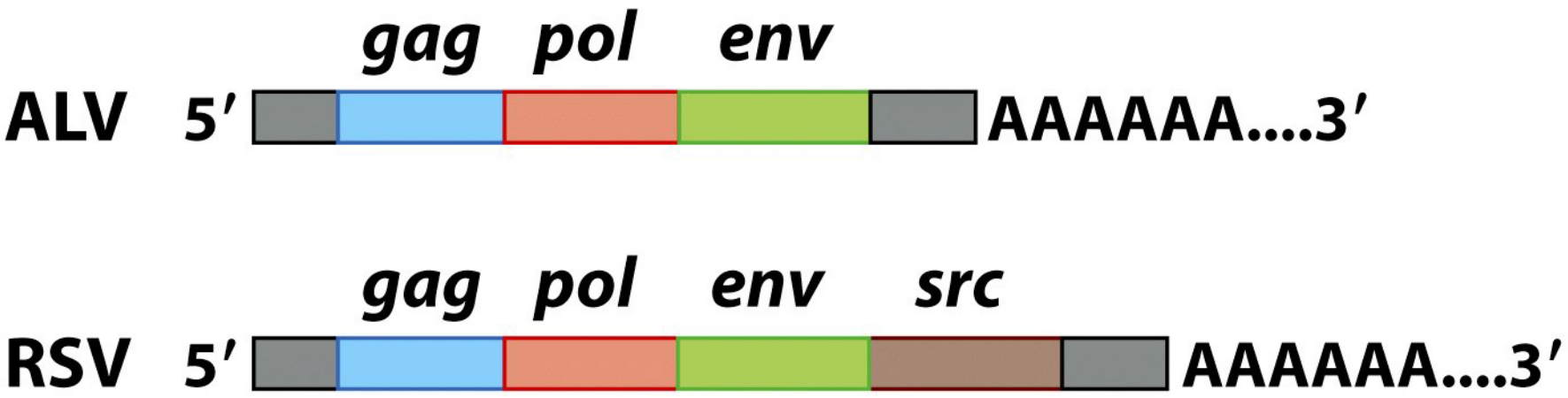
Normal cell

Tumor cell

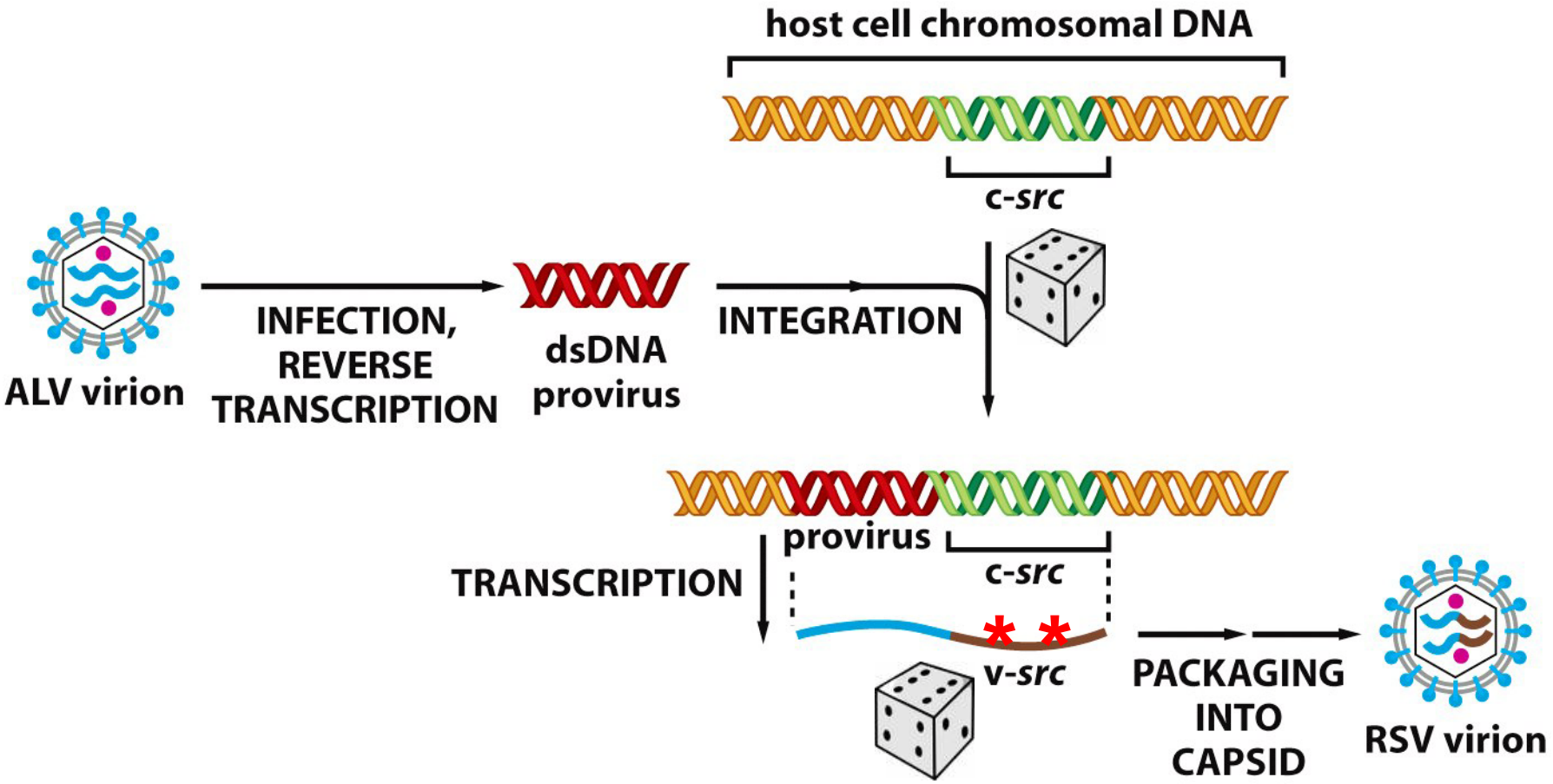
Does Src cause tumor?

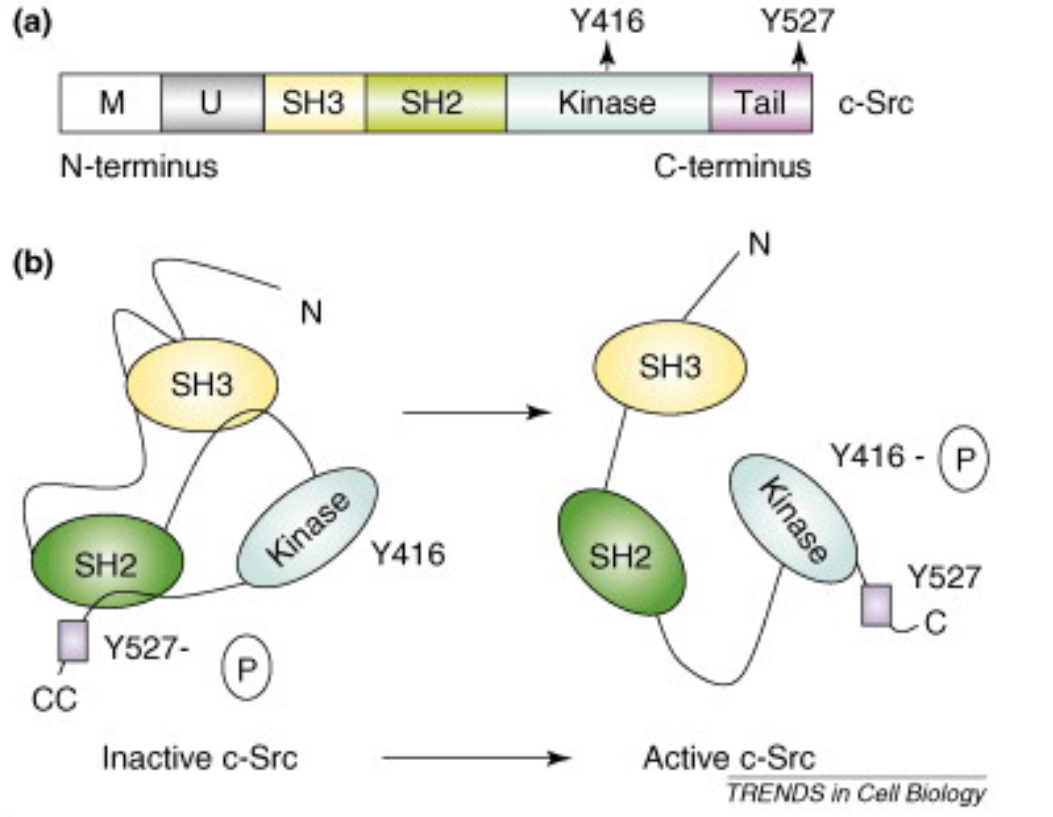
V-src	C-src
Viral (RSV)	Cellular
Small difference in sequences	
Oncogene 癌基因	Proto-oncogene 原癌基因

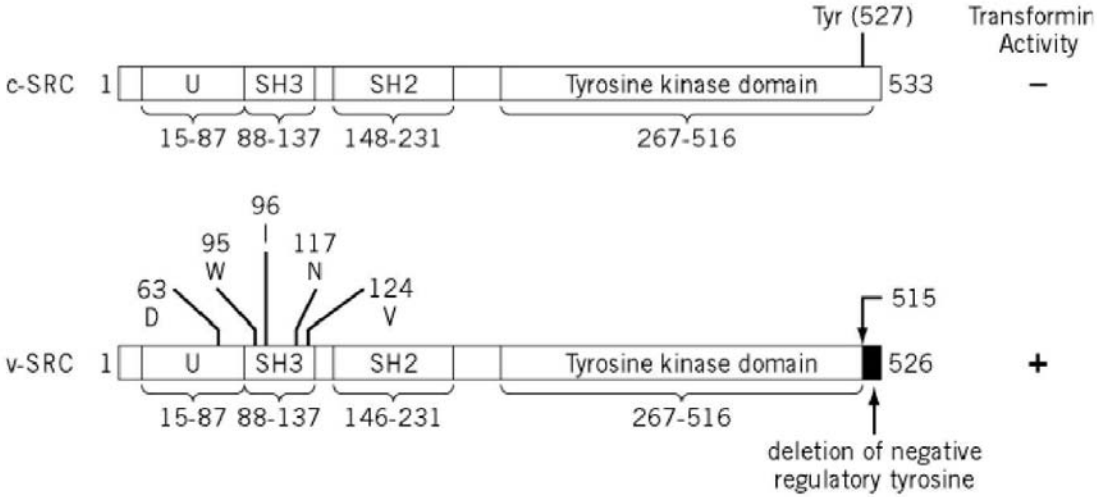
Avian Leukosis Virus: Retrovirus



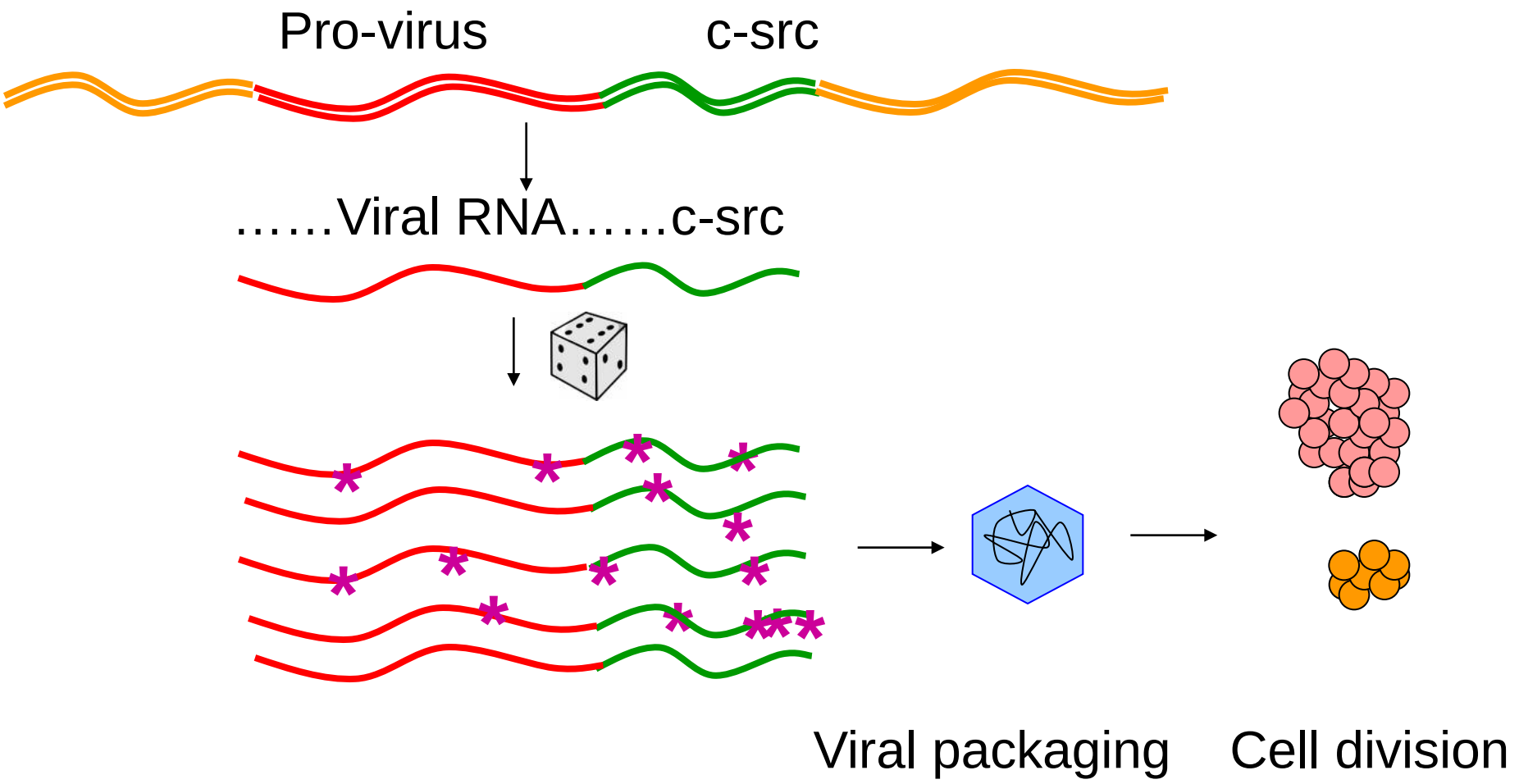
Origin of *v-src*




(a)
Y416
Y527
M
U
SH3
SH2
Kinase
Tail
c-Src
N-terminus
C-terminus
(b)
N
SH3
SH2
Kinase
Y416
Y527-
P
CC
Inactive c-Src
N
SH3
Y416 -
P
SH2
Kinase
Y527
C
Active c-Src
TRENDS in Cell Biology


Tyr (527)
Transformin
Activity
c-SRC
1
U
SH3
SH2
Tyrosine kinase domain
533
–
15-87
88-137
148-231
267-516
96
95
W
117
N
63
D
124
V
515
v-SRC
1
U
SH3
SH2
Tyrosine kinase domain
526
+
15-87
88-137
146-231
267-516
deletion of negative
regulatory tyrosine

Why did random mutation produce a v-src that could induce tumor???



src → 3 ideas (1976)

1. Mutation of a **cellular gene** → oncogene

2. A **single oncogene** is capable of change the fate of a normal cell to cancer cell.

3. Other tumor viruses may point to **additional genes** for oncogenesis

Table 9-1 Selected Oncogenes Grouped Together by Protein Function

Oncogene Name	Protein Produced	Oncogene Origin	Common Cancer Type*
1. Growth factors			
v-*sis*	PDGF	Simian sarcoma virus	Sarcomas (monkeys)
COL1A1-PDGFB	PDGF	Chromosomal translocation	Fibrosarcoma
2. Receptors			
v-*erb-b*	EGF receptor	Avian erythroblastosis virus	Leukemia (chickens)
RET	Ret receptor	Point mutation, chromosomal translocation	Thyroid
TRK	Nerve growth factor receptor	DNA rearrangement (inversion)	Thyroid
ERBB2	ErbB2 receptor	Amplification	Breast, ovary
v-*mpl*	Thrombopoietin receptor	Myeloproliferative leukemia virus	Leukemia (mice)
3. Plasma membrane G proteins			
v-*K-ras*	Ras	Harvey sarcoma virus	Sarcomas (rats)
v-*H-ras*	Ras	Kirsten sarcoma virus	Sarcomas (rats)
KRAS	Ras	Point mutation	Pancreas, colon, lung, others
HRAS	Ras	Point mutation	Bladder
NRAS	Ras	Point mutation	Leukemias
4. Intracellular protein kinases			
BRAF	Raf kinase	Point mutation	Melanoma
v-*src*	Src kinase	Rous sarcoma virus	Sarcomas (chickens)
SRC	Src kinase	DNA rearrangement	Colon
TEL-JAK2	Jak kinase	Chromosomal translocation	Leukemias
BCR-ABL	Abl kinase	Chromosomal translocation	Chronic myelogenous leukemia

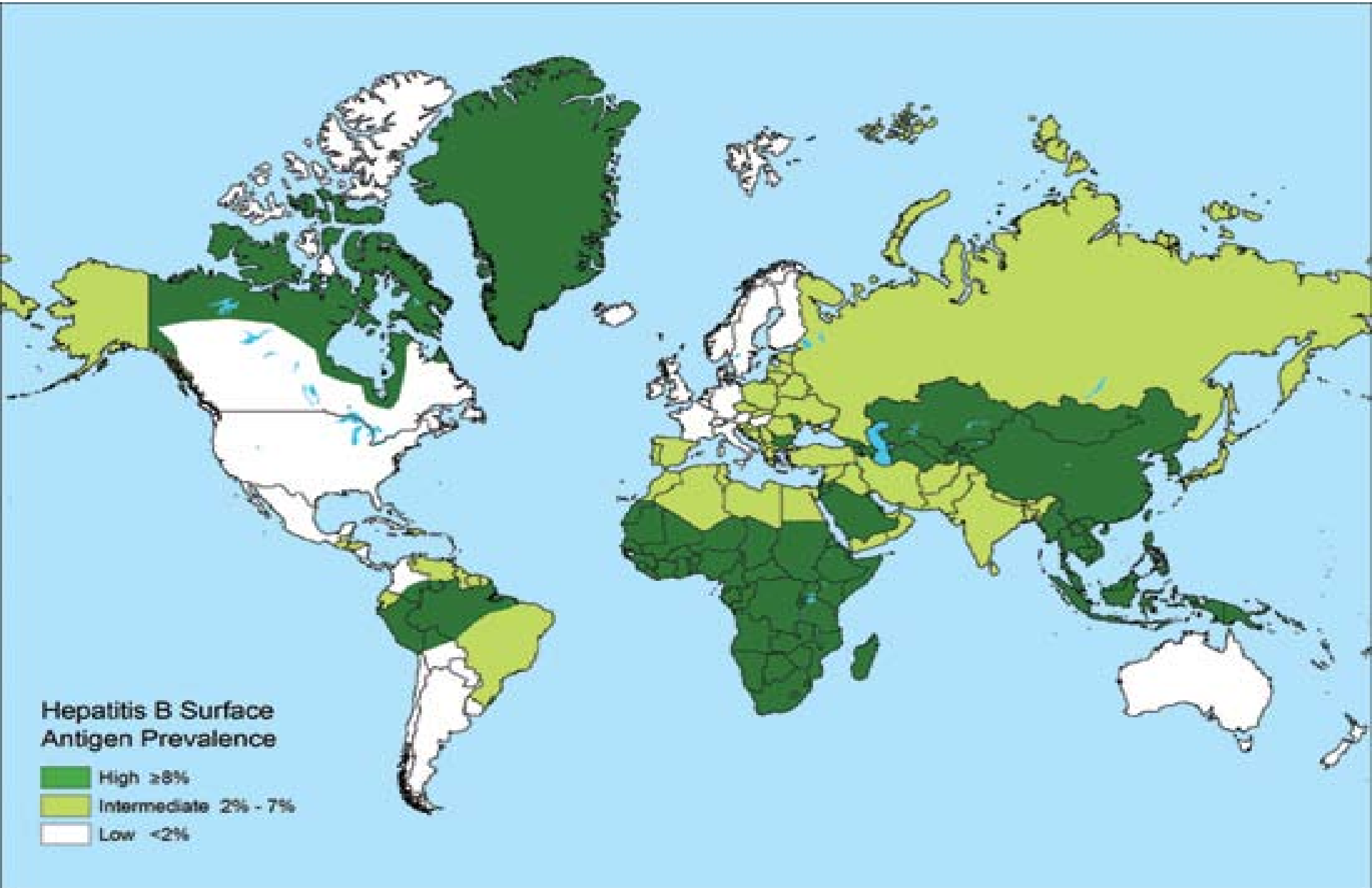
Src : cellular origin of retroviral oncogenes

J. Michael Bishop

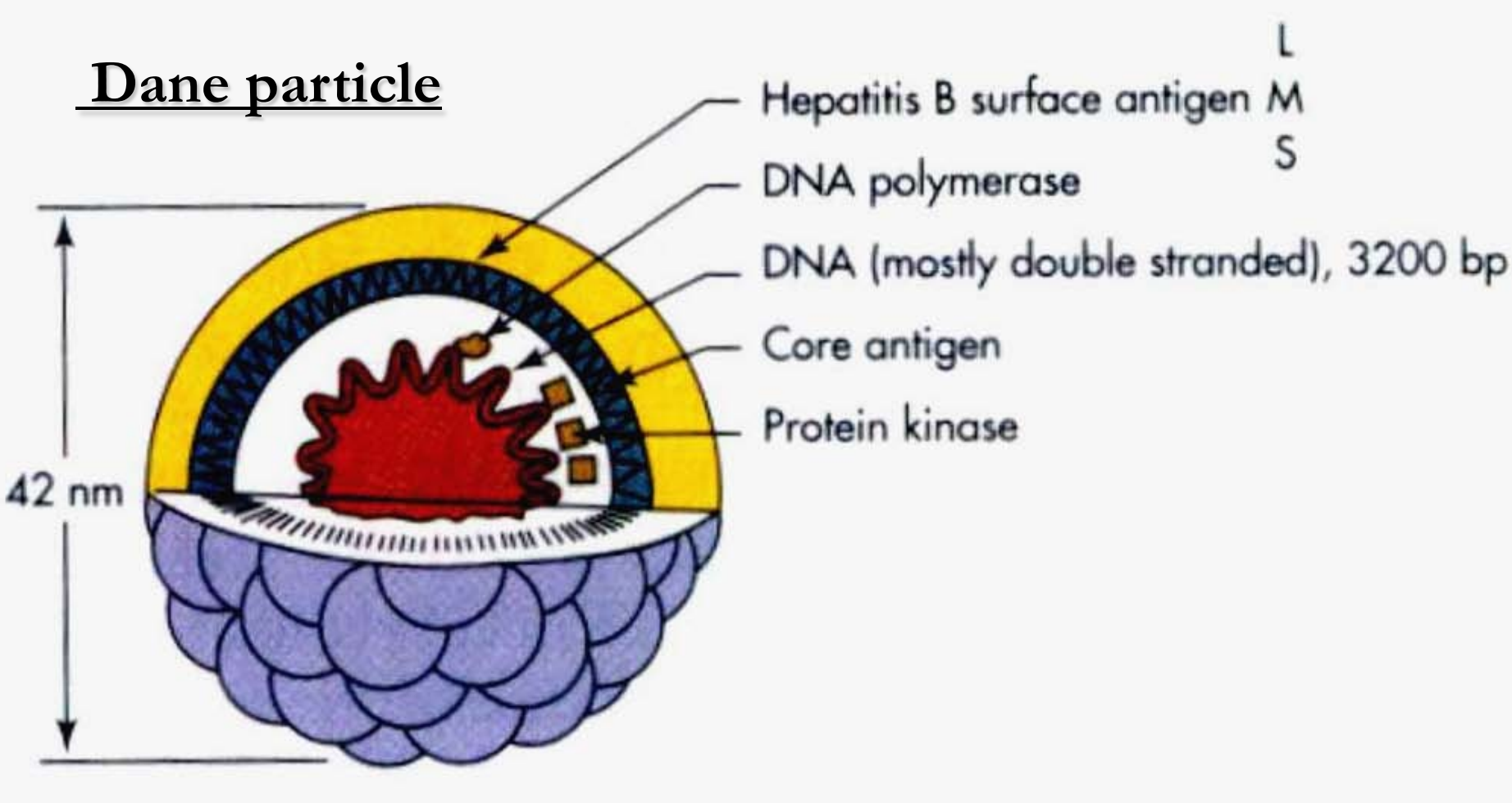
1989

Harold E. Varmus

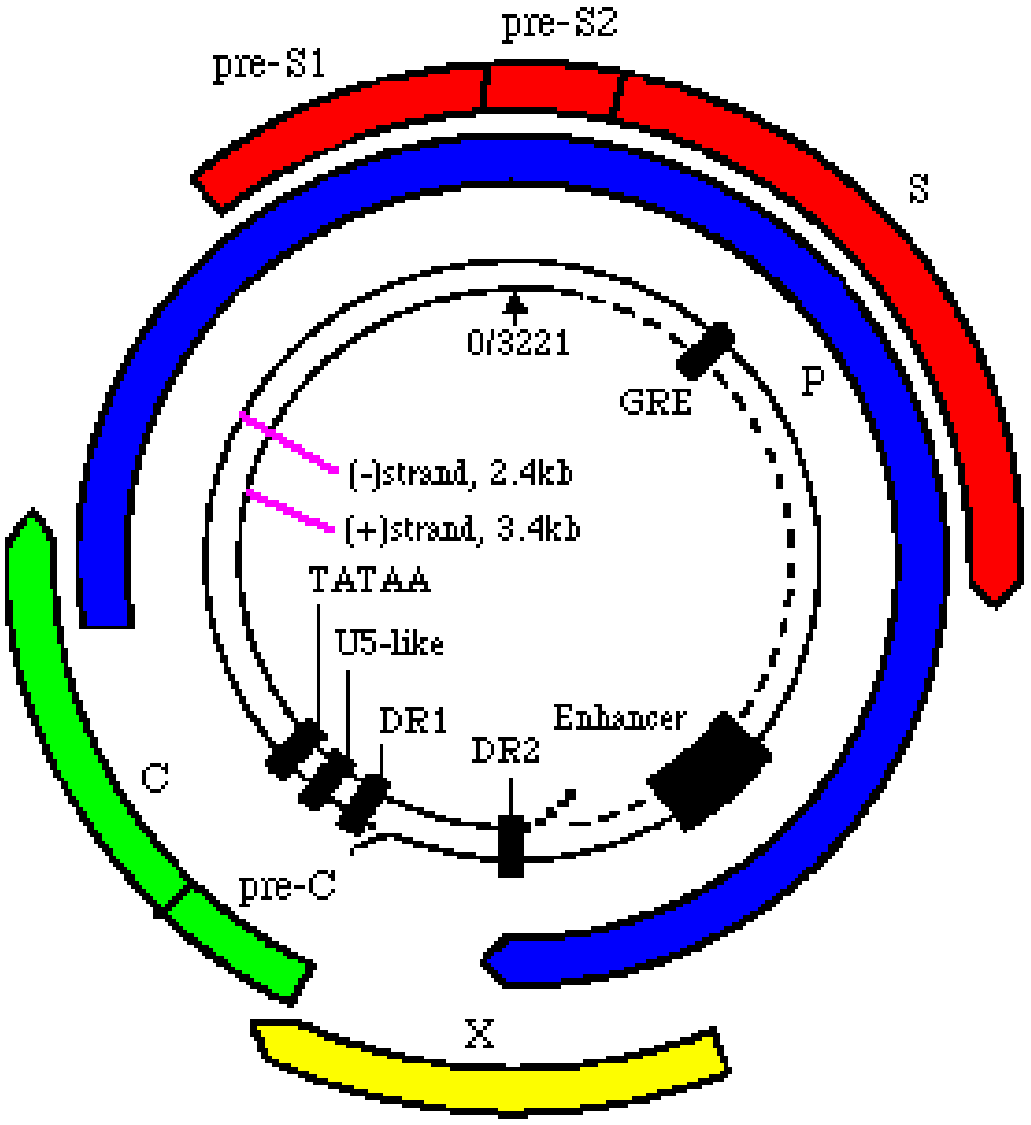
Hepatitis B Prevalence



HBV Structure & Antigens



GENOME


pre-S2
pre-S1
S
0/3221
GRE
P
(-)strand, 2.4kb
(+)strand, 3.4kb
TATAA
U5-like
DR1
DR2
Enhancer
C
pre-C
X

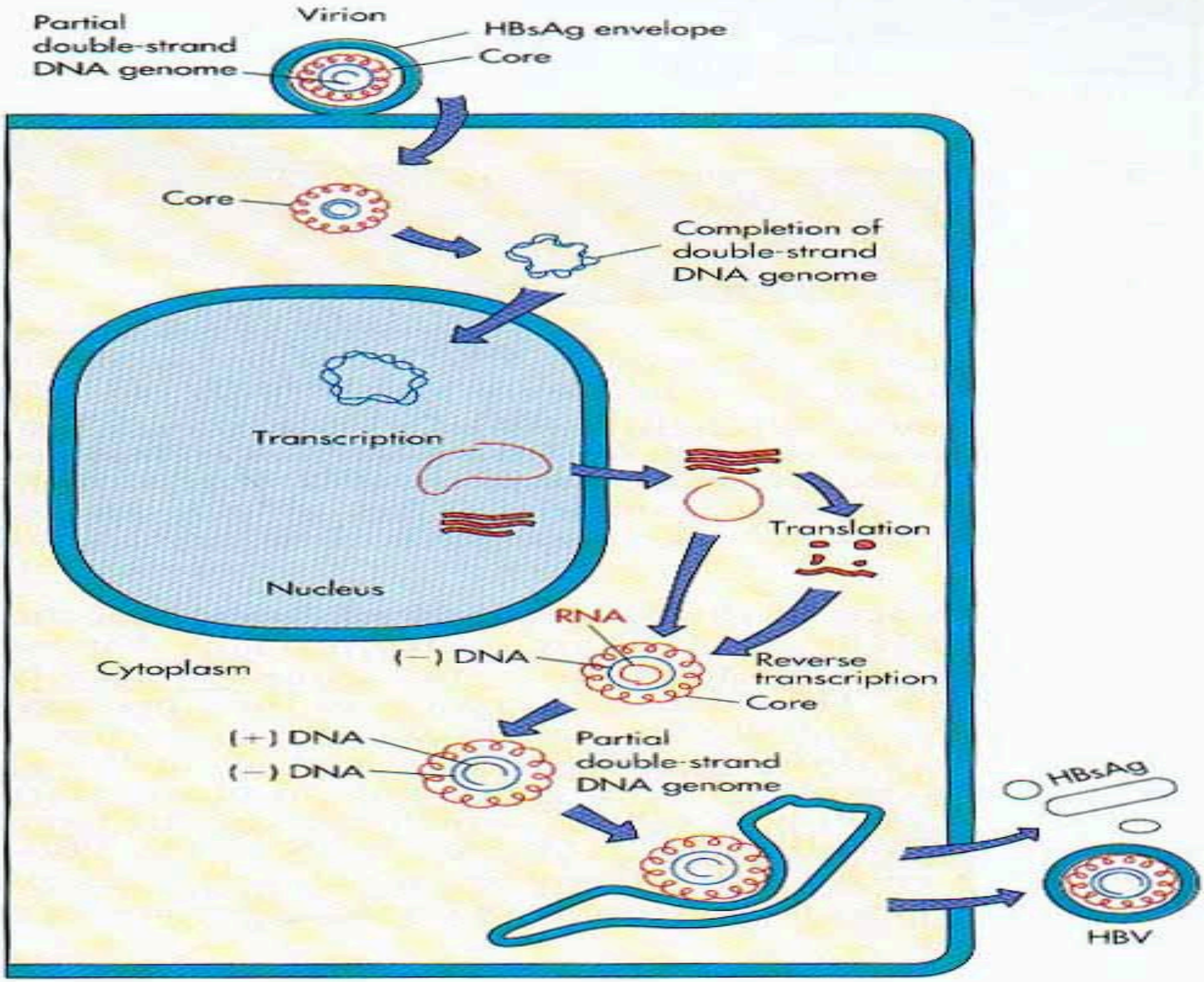
Open Reading Frames

There are 4 **open reading frames** derived from the same strand (the incomplete + strand)

- **S -** the 3 polypeptides of the surface antigen (**preS1, preS2 and S -** produced from alternative translation start sites.
- **C -** the core protein
- **P -** the polymerase
- **X -** a transactivator of viral transcription (and cellular genes?). HBx is conserved in all mammalian (but not avian) hepadnaviruses. Though not essential in transfected cells, it is required for infection in vivo.

HBV: Replication

- **Reverse transcription:** one of the mRNAs is replicated with a reverse transcriptase making the DNA that will eventually be the core of the progeny virion
- **RNA intermediate**: HBV replicates through an RNA intermediate and produces and release antigenic decoy particles.
- **Integration:** Some DNA integrates into host genome causing carrier state

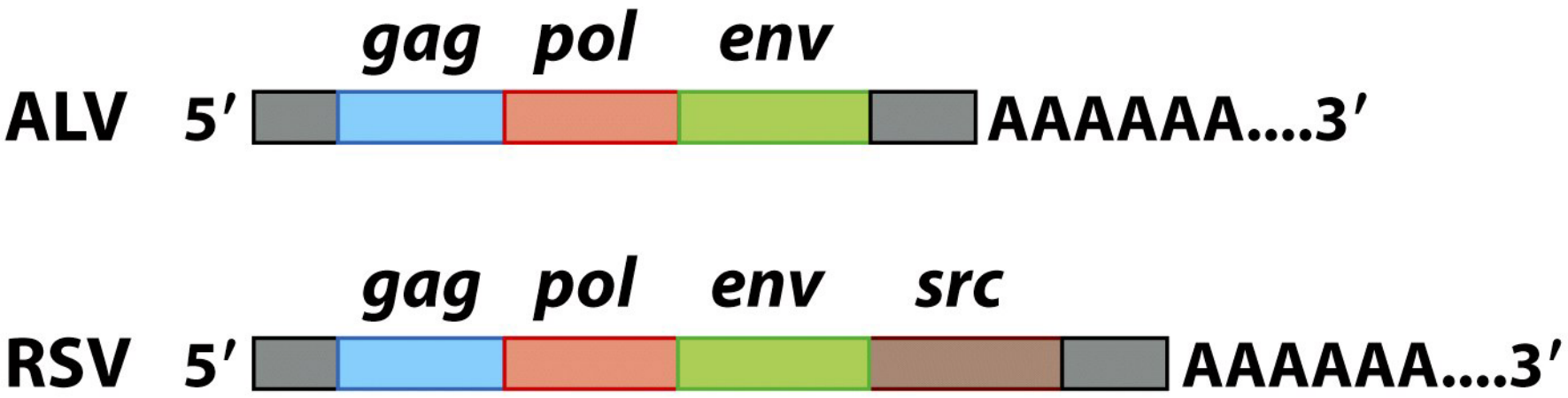

Partial
double-strand
DNA genome
Virion
HBsAg envelope
Core
Core
Completion of
double-strand
DNA genome
Transcription
Nucleus
Translation
Cytoplasm
RNA
(−) DNA
Reverse
transcription
Core
(+) DNA
(−) DNA
Partial
double-strand
DNA genome
HBsAg
HBV

Virus and Cancer

- How does virus cause cancer?
  - Molecular mechanisms

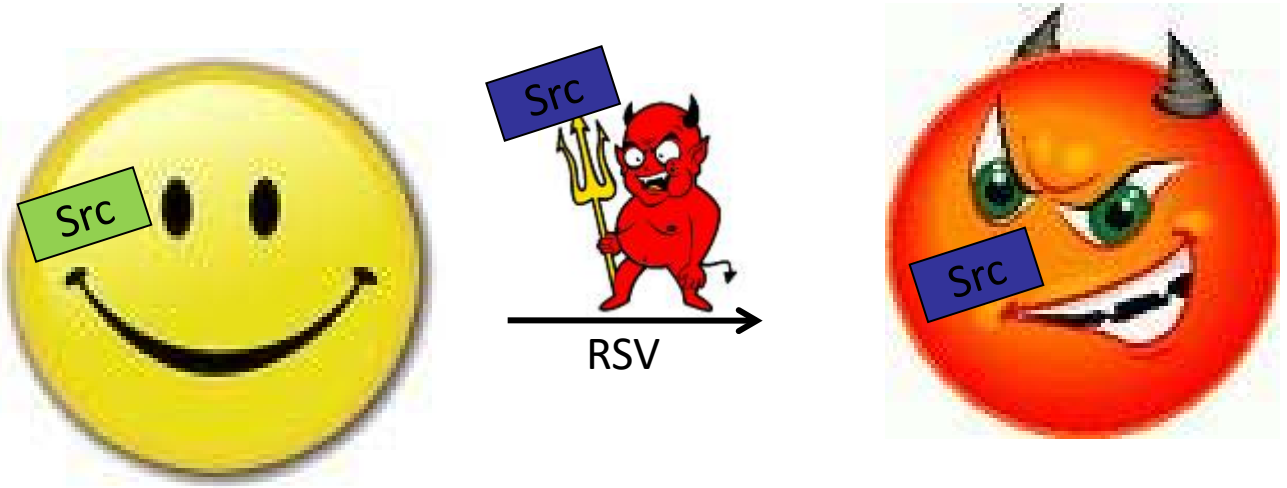
Cancer and infection

Avian Leukosis Virus: Retrovirus



Src in normal cells

- Src gene is present in normal genome (**c-Src**).
- **V-Src** contains mutations



Normal cell

Tumor cell

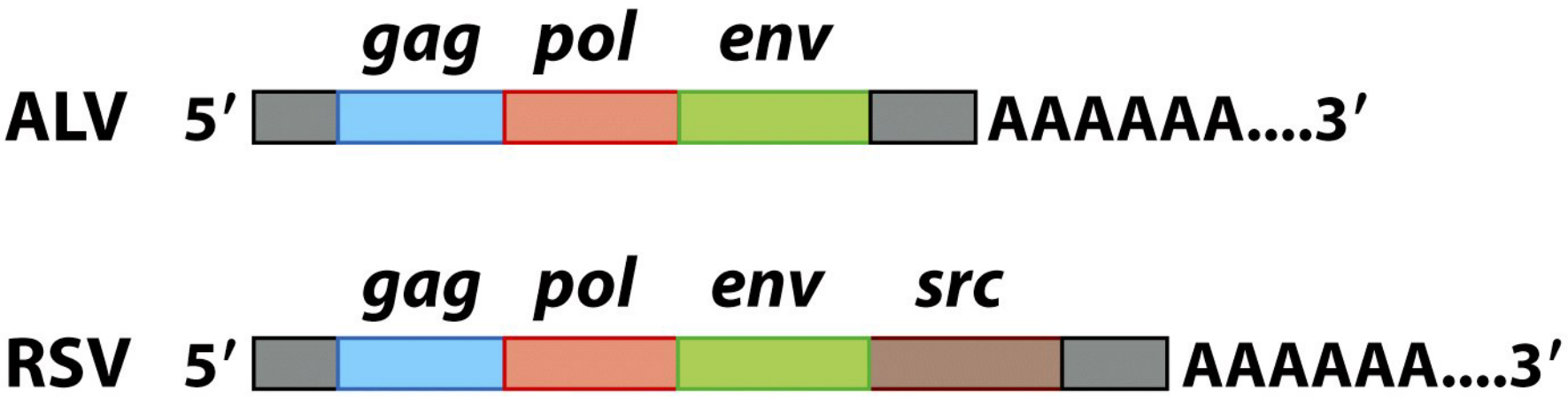
Acutely transforming retrovirus

Type of virus	Viral oncogene	species	Major disease	Nature of oncoprotein
Rous Sarcoma	src	chicken	sarcoma	Non-receptor TK
Myelocytomatosis 29	myc	chicken	Myeloid leukemia	Transcription factor
Avian myeloblastosis E26	myb	chicken	Myeloid leukemia	Transcription factor
E26	ets	chicken	Myeloid leukemia	Transcription factor
Avian Erythroblastosis ES4	erbA	Chicken	Erythroleukemia	Thyroid hormone receptor
ES4	erbB	chicken	Erythroleukemia	EGF RTK
Harvey murine sarcoma	H-ras	Mouse, rat	sarcoma	Small G protein
Kirsten murine sarcoma	K-ras	mouse	sarcoma	Small G protein

Slow-acting retrovirus

- RSV: induce sarcoma in a few weeks
  - Src→ tumor
- ALV: Do not contain oncogene
  - Can not induce tumor quickly
  - But they are found in chicken leukemia

Avian Leukosis Virus: Retrovirus



Mapping of ALV in chicken genome

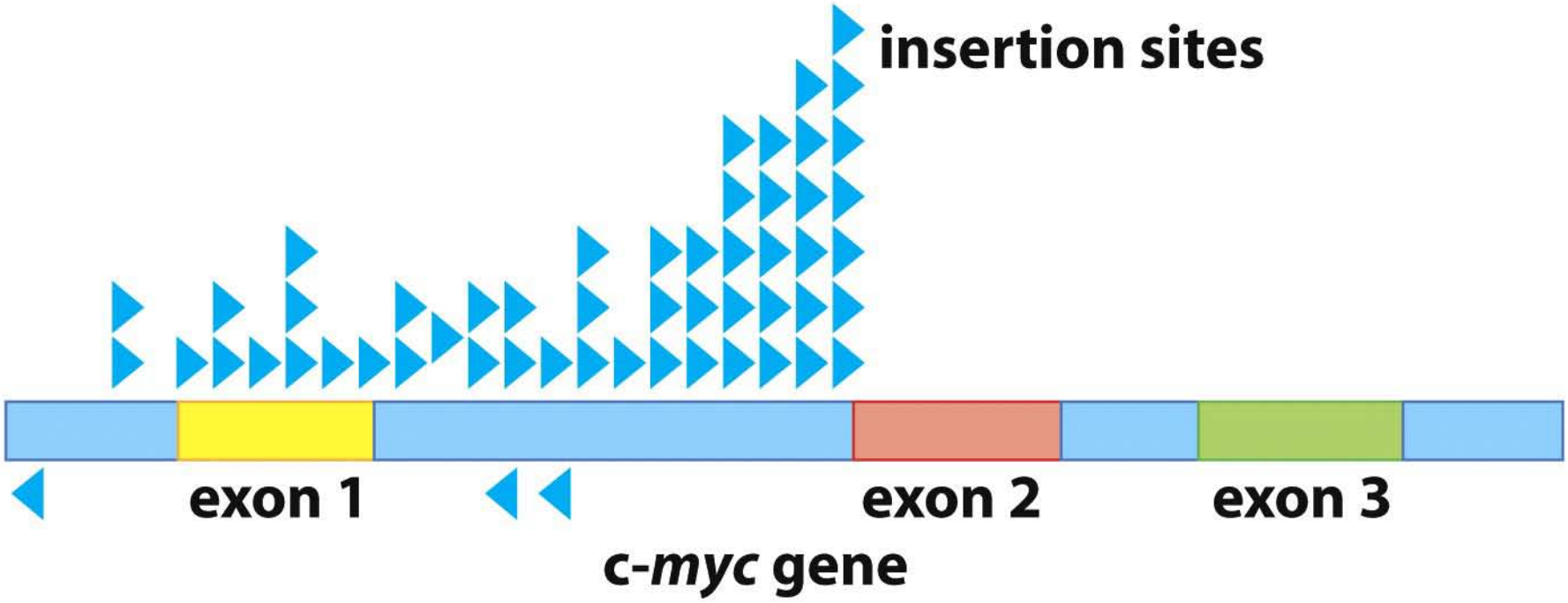
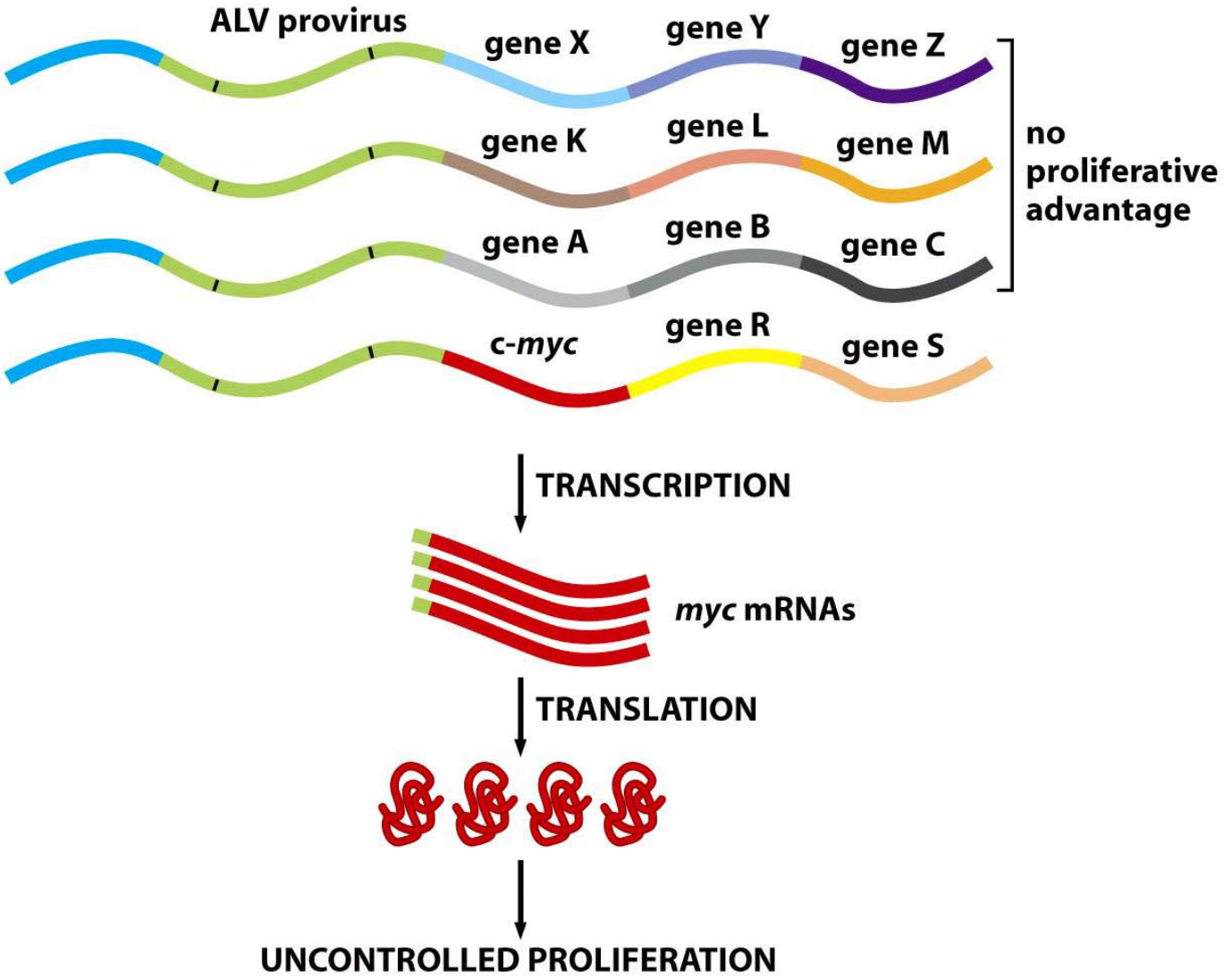


Figure 3-23a The Biology of Cancer (© Garland Science 2007)

2nd mechanism: slow kinetics



2 mechanisms

1. Virus hijacked cellular genes (RSV-Src)
2. Insertional mutagenesis: ALV-myc

   Powerful way of identifying proto-oncogenes

Genes activated by insertional mutation

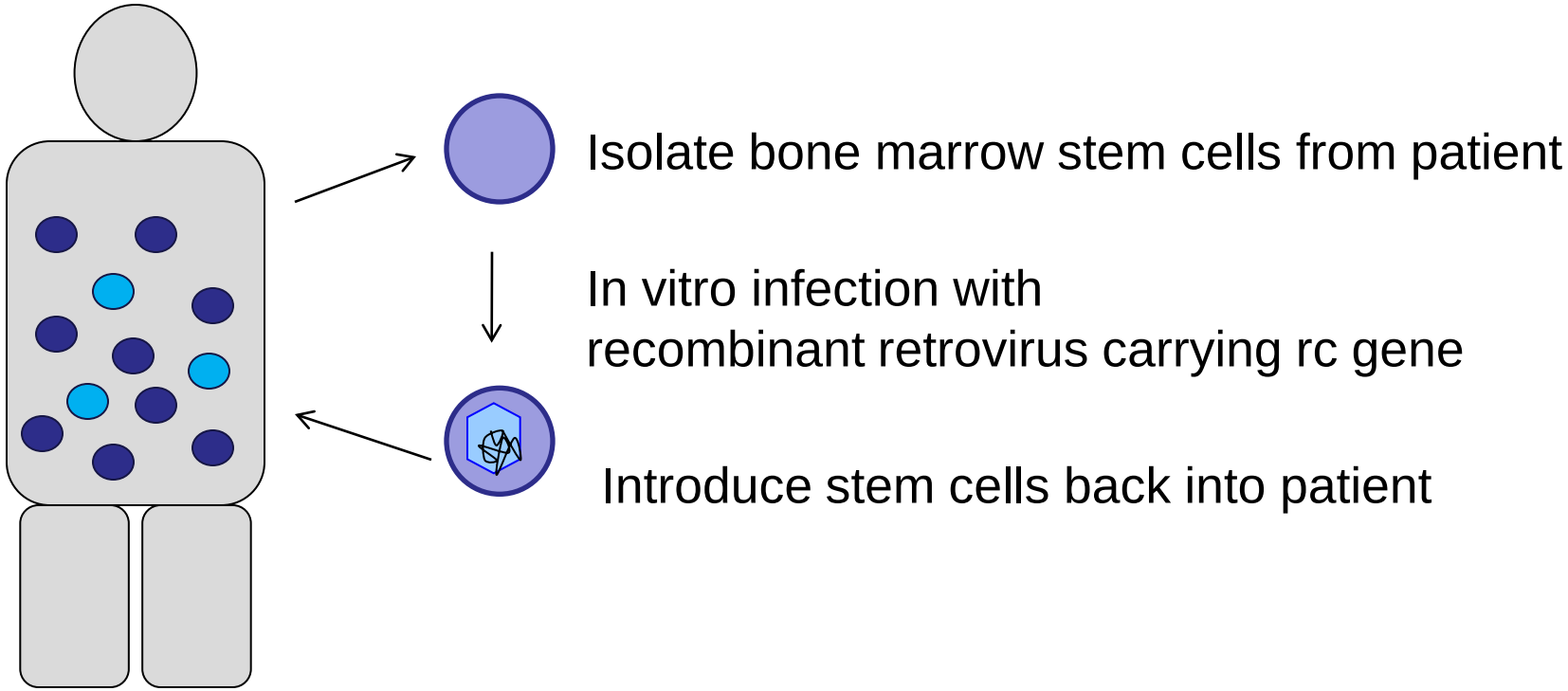
Int-1	MMTV	Mouse mammary carcinoma	Growth factor=Wnt-1
Int-2	MMTV	Mouse mammary carcinoma	Growth factor=FGF
Int-3	MMTV	Mouse mammary carcinoma	Receptor=Notch related
Erb-B	ALV	Chicken erythroblastosis	Growth factor receptor=EGF-R
K-ras	F-MLV	Mouse T cell lymphoma	Small G protein
Cyclin-D1	F-MLV	Mouse T cell lymphoma	G1 cyclin
Cyclin-D2	F-MLV	Mouse T cell lymphoma	G1 cyclin

Genes activated by insertional mutation

mos	IAP	Mouse plasmacytoma	Ser/thr kinase
nov	ALV	Chicken nephroblastoma	Growth factor
IL-2	GalV	Gibbon ape T cell lymphoma	Cytokine
GM-CSF	IAP	Mouse myelomonocytic leukemia	Growth factor
P53	Mo-MLV	Mouse T cell leukemia	Transcription factor

Gene therapy

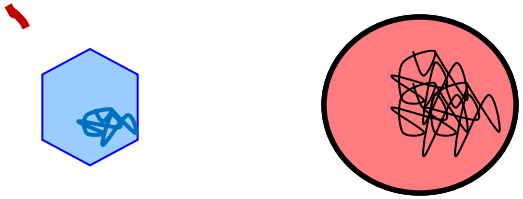
- Germ-line mutation that affects hematopoietic cells
- Expressing missing gene by retrovirus vector



SCID: severe combined immunodeficiency

Gene therapy 2003

- Fourteen children with X-SCID
  - 10 in France
  - 4 in London
- Moloney murine leukemia virus (MLV) derived vector



France, 2003

- Dramatic reconstitution of immune function

- 3 cases of T cell leukemia 3 years later
  - Insertion site: upstream of ***LMO2*** (proto-oncogene)
  - Known to be activated in T cell leukemia

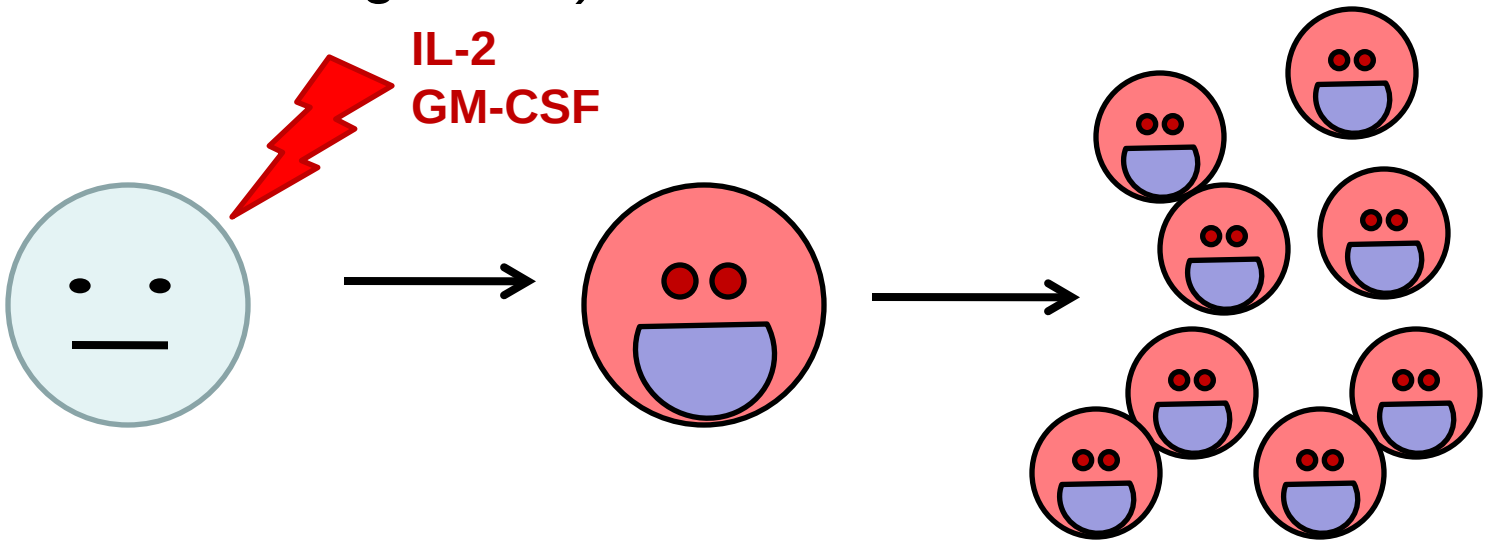
3rd mechanism

- Human T cell leukemia virus (HTLV-I)
- 1% infection rate in Kyushu, Japan
- 3-4% risk of developing adult T-cell leukemia
- Milk born (Mother → infant)

1. No genes of cellular origin
2. Insertional sites: not clustered

Viral oncogene

- Viral tax gene:
  - For activating viral transcription
  - Also activates transcription of 2 cellular genes:
    - IL-2 (interleukine-2)
    - GM-CSF (granulocyte macrophage colony stimulating factor)



3 mechanisms

1. Virus hijacked cellular genes (RSV *src*)

2. Insertional mutagenesis: ALV *myc*

3. Virus gene acts as oncogene: HTLV-I *tax*

Role of viral infection in human cancer

- About 20% human cancers world wide are due to key role of viral infection
- Hepatitis B and C (HBV and HCV)→ liver cancer
- Human papillomavirus (HPV) → cervical cancer

Summary

1. **Integration**: oncogenic viral genomes are transmitted by integration.

2. **RT (Reverse transcription)** : is a key step in retroviral life cycle.

3. Hajack of cellular gene
   - **proto-oncogene → oncogene**