Cancer Biology

Lecture 4-Tumor Viruses

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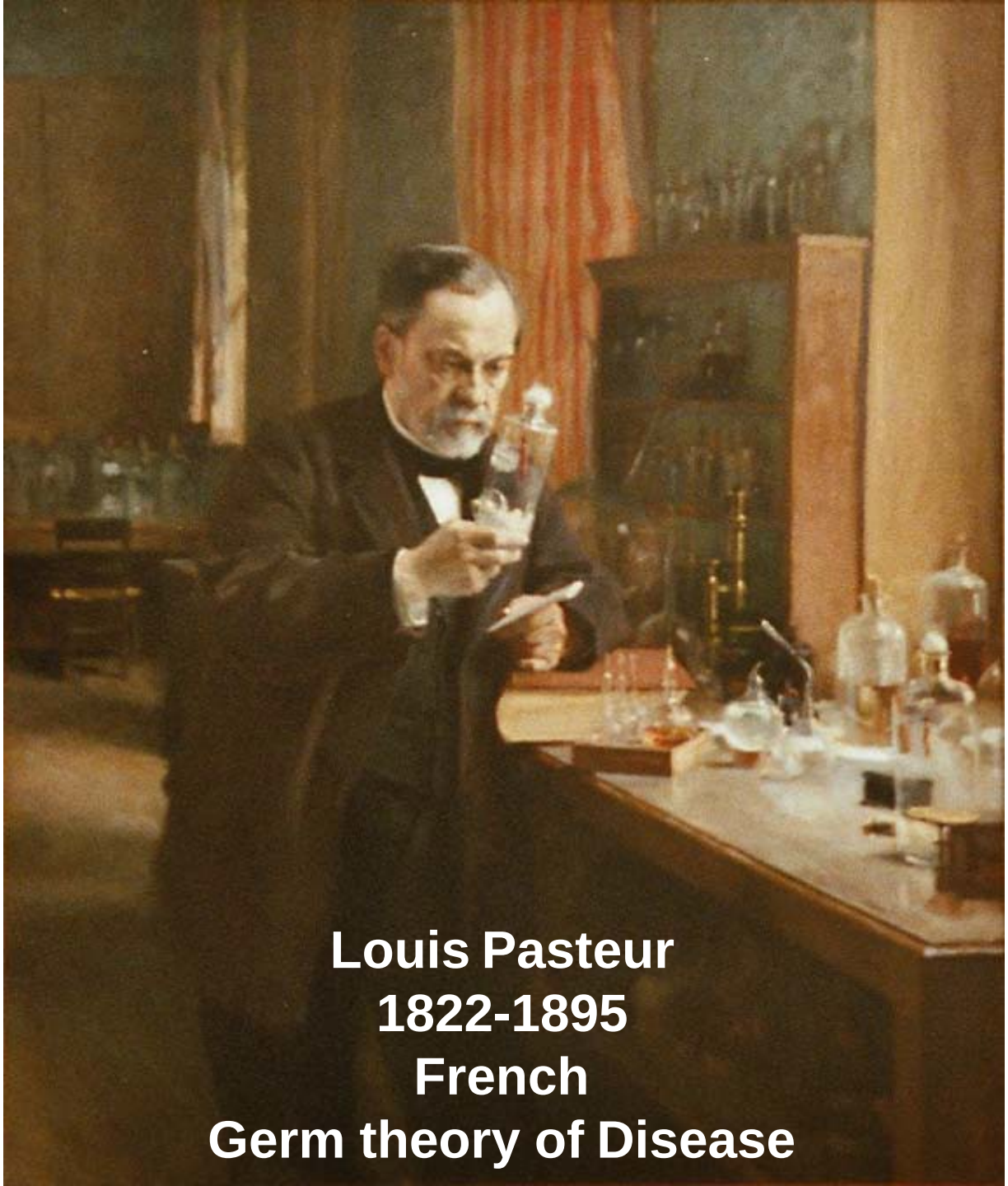
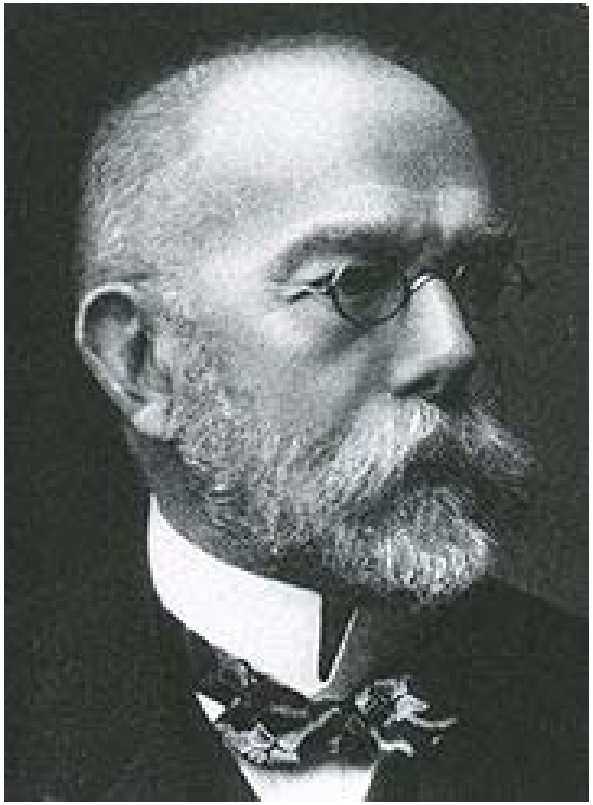
Introduction

- What is cancer?
  - Normal cells go bad.
- What causes cancer?
  - Carcinogens-chemicals
  - **Infection-Virus**
- 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

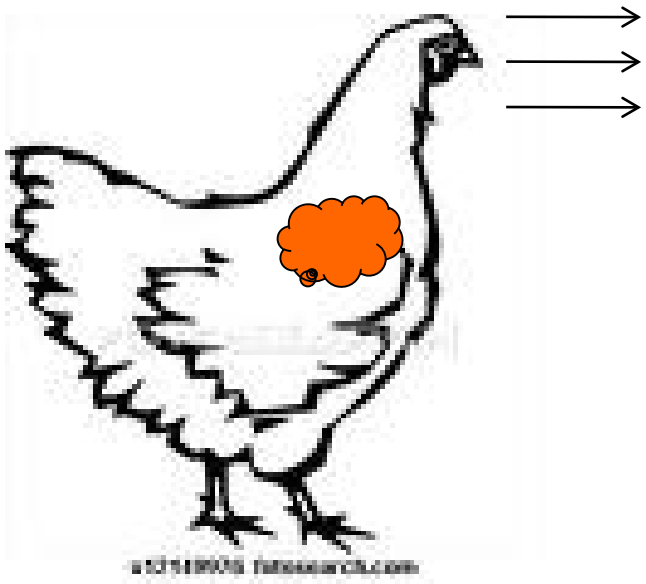
Cancer and infection

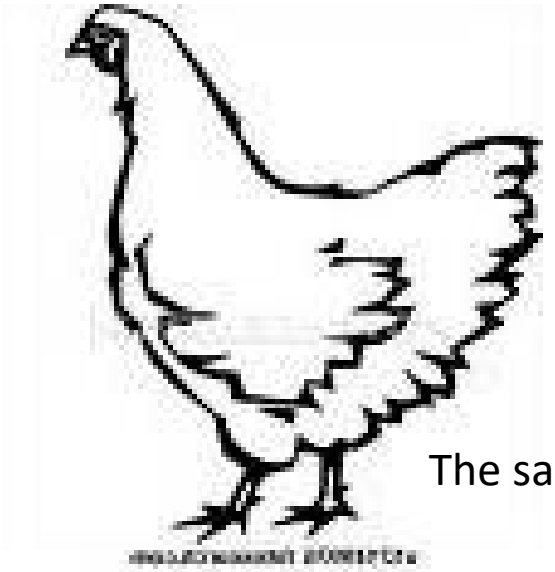
Chicken tumor

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

1879-1970

Rous experiment #1

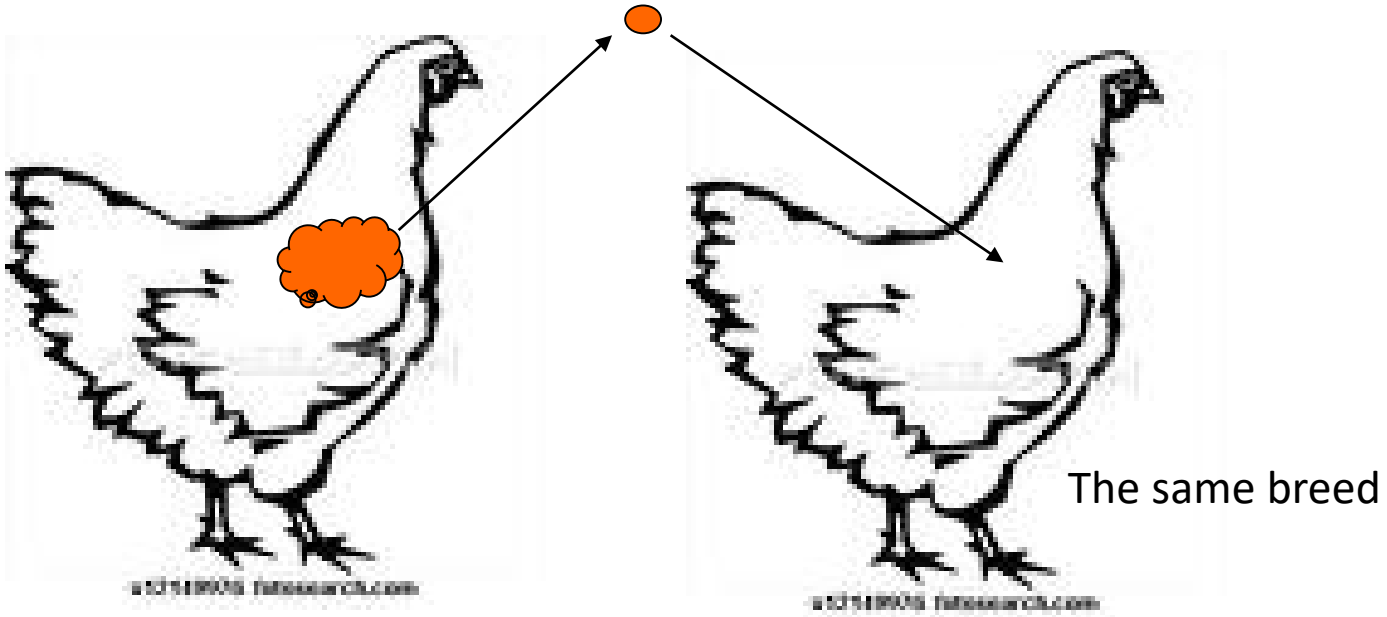




The same breed

Conclusion ?

Rous experiment #2

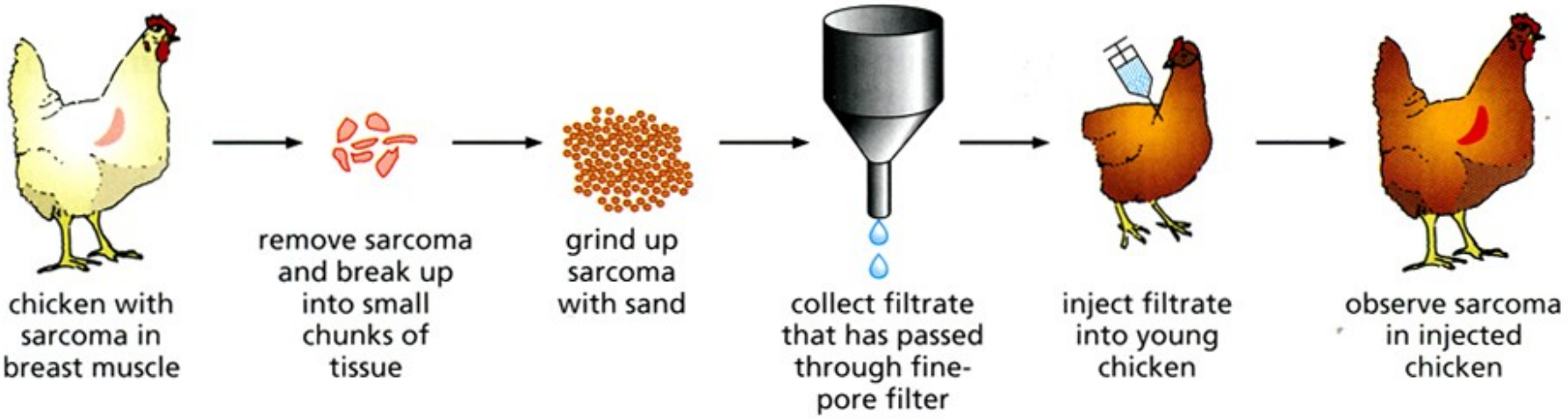


The same breed

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)

The keys for Rous's Experiments

In 1909, Rous began his study of a sarcoma that had appeared in the breast muscle of a hen. In initial experiments, Rous succeeded in transmitting the tumor by implanting small fragments of it into other birds of the same breed. Later, as a variation of this experiment, he ground up a sarcoma fragment in sand and filtered the resulting homogenate (**Figure 3.2**). When he injected the resulting filtrate into young birds, they too developed tumors, sometimes within several weeks. He subsequently found that these induced tumors could also be homogenized to yield, once again, an infectious agent that could be transmitted to yet other birds, which also developed sarcomas at the sites of injection.

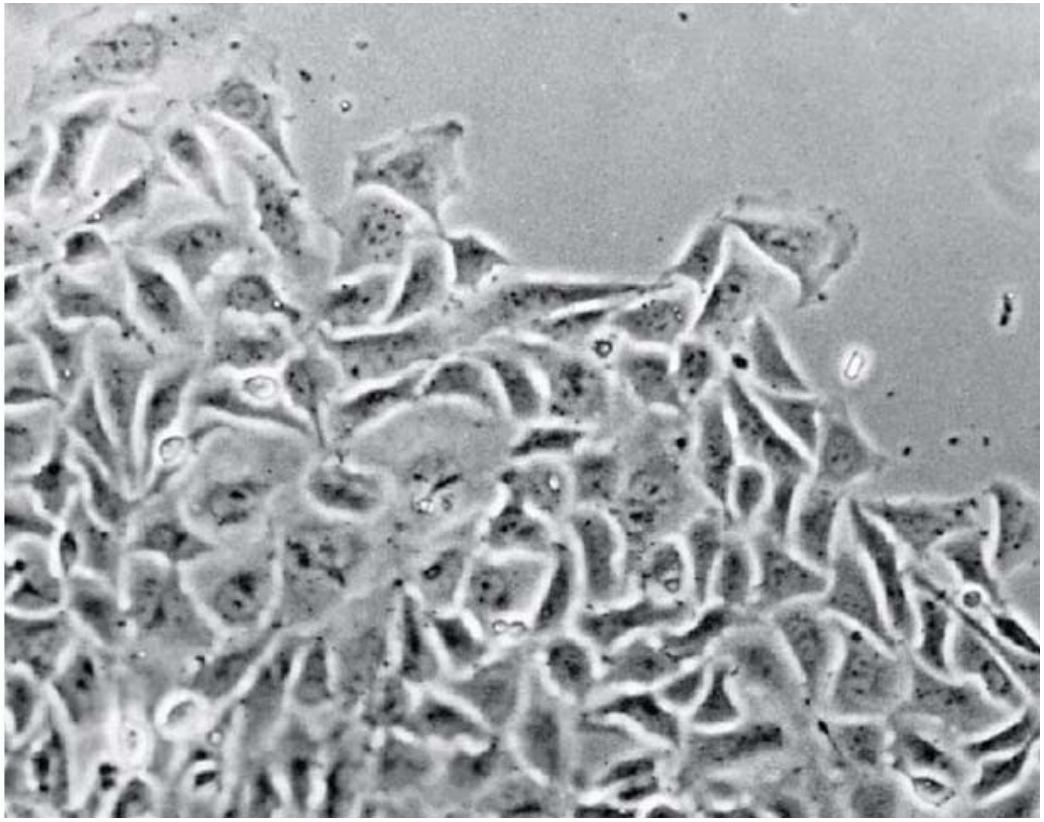
Victory of infectious theory

- 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?

Chicken embryonic fibroblasts



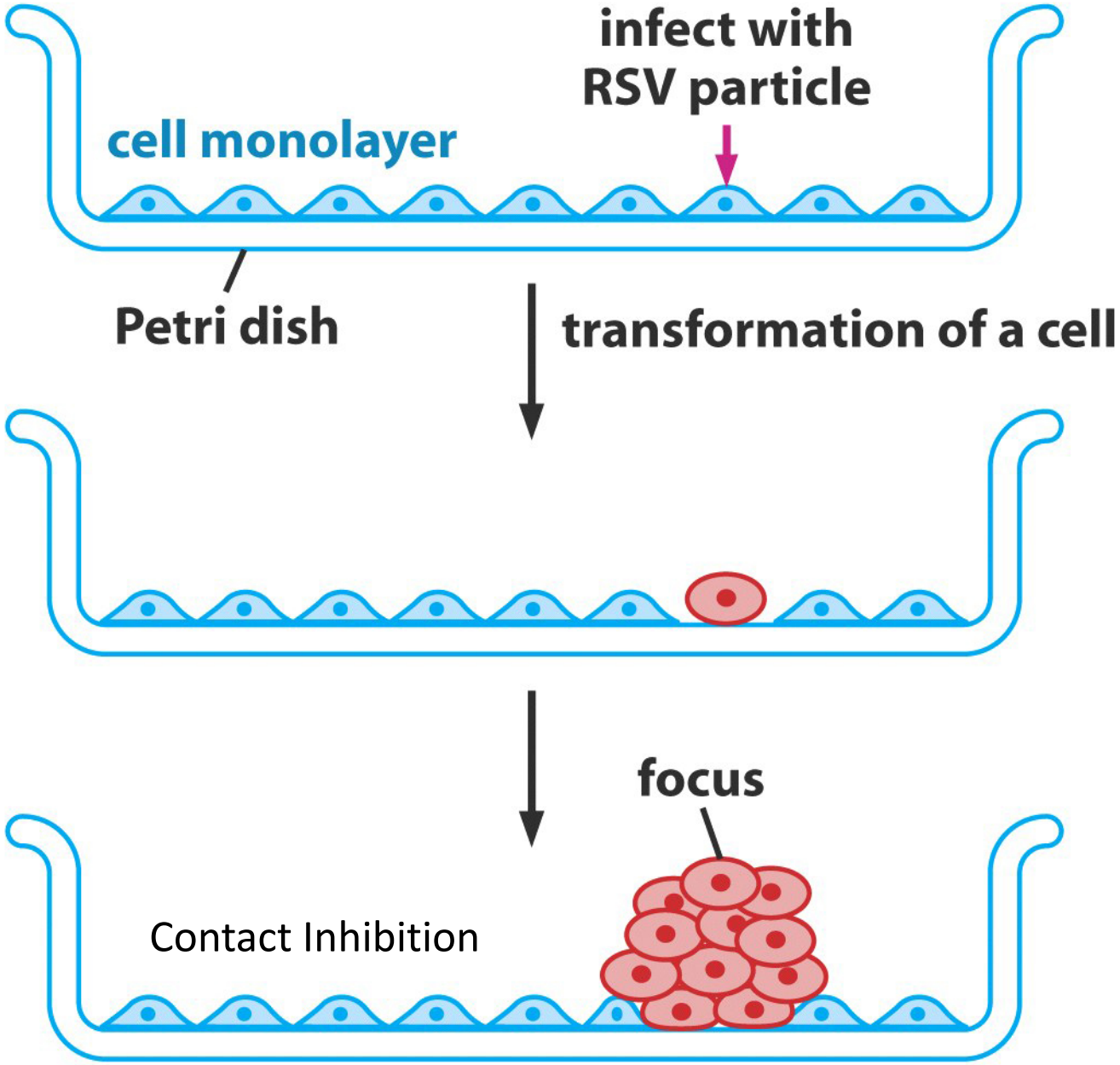
+ RSV

→ **Cells survive !**

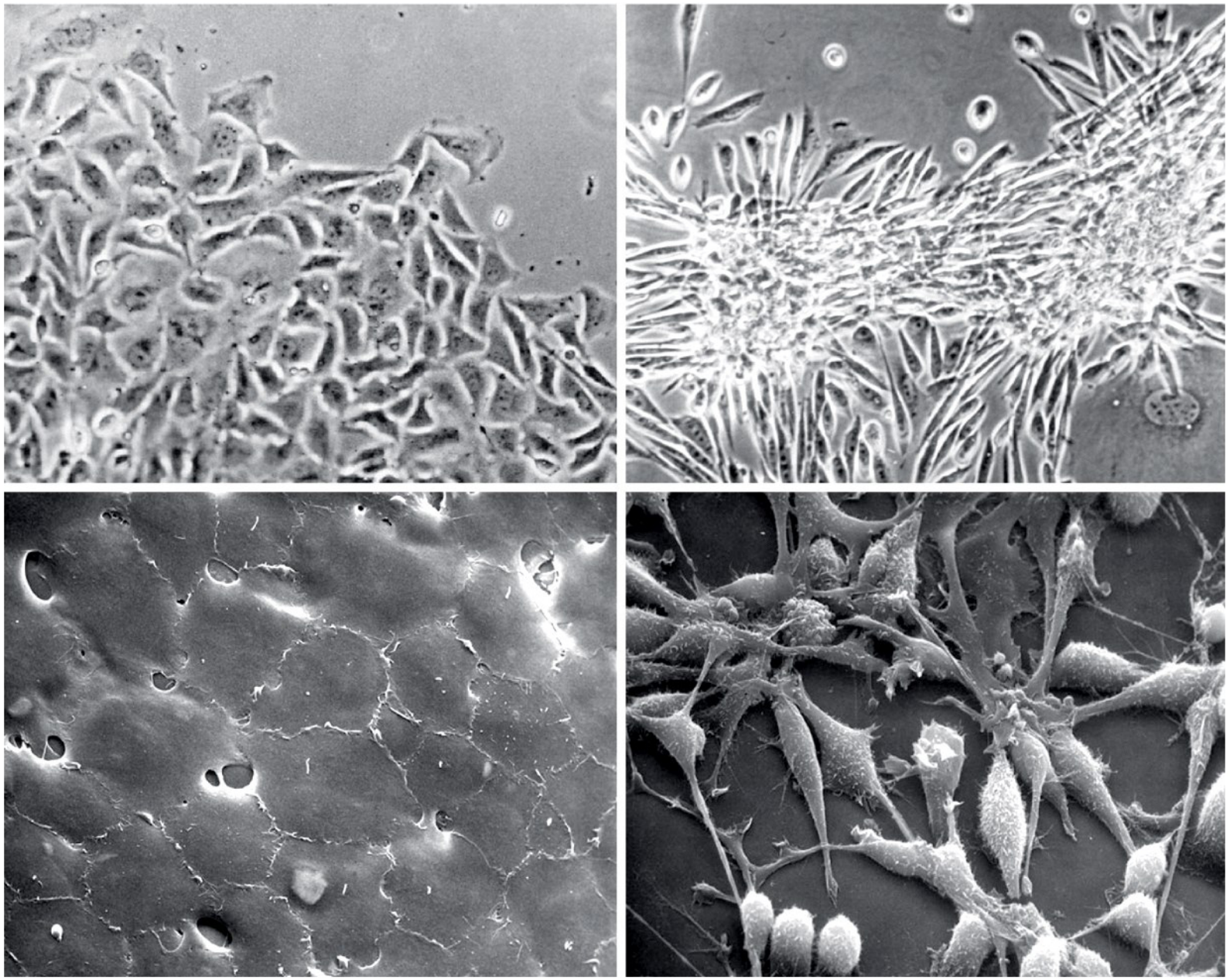
They look like sarcoma cells…

What is cancer?
at cellular level…

- Properties of transformed cells


infect with
RSV particle
cell monolayer
Petri dish
transformation of a cell
focus
Contact Inhibition

+RSV



Transformed cells

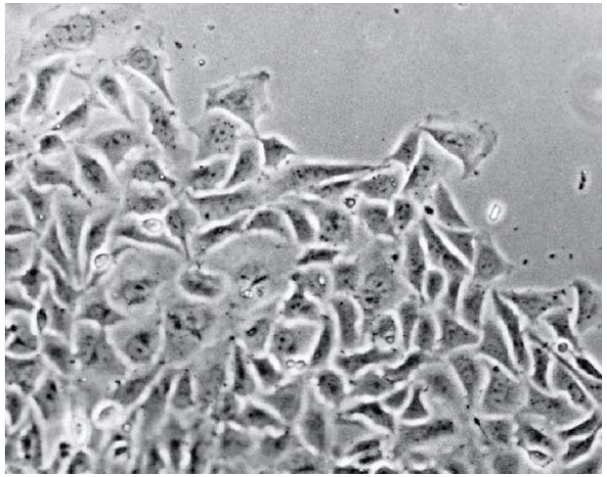
Change in cell shape
Loss of contact inhibition
Proliferate indefinitely

→ Your evaluation of the transformed cells:

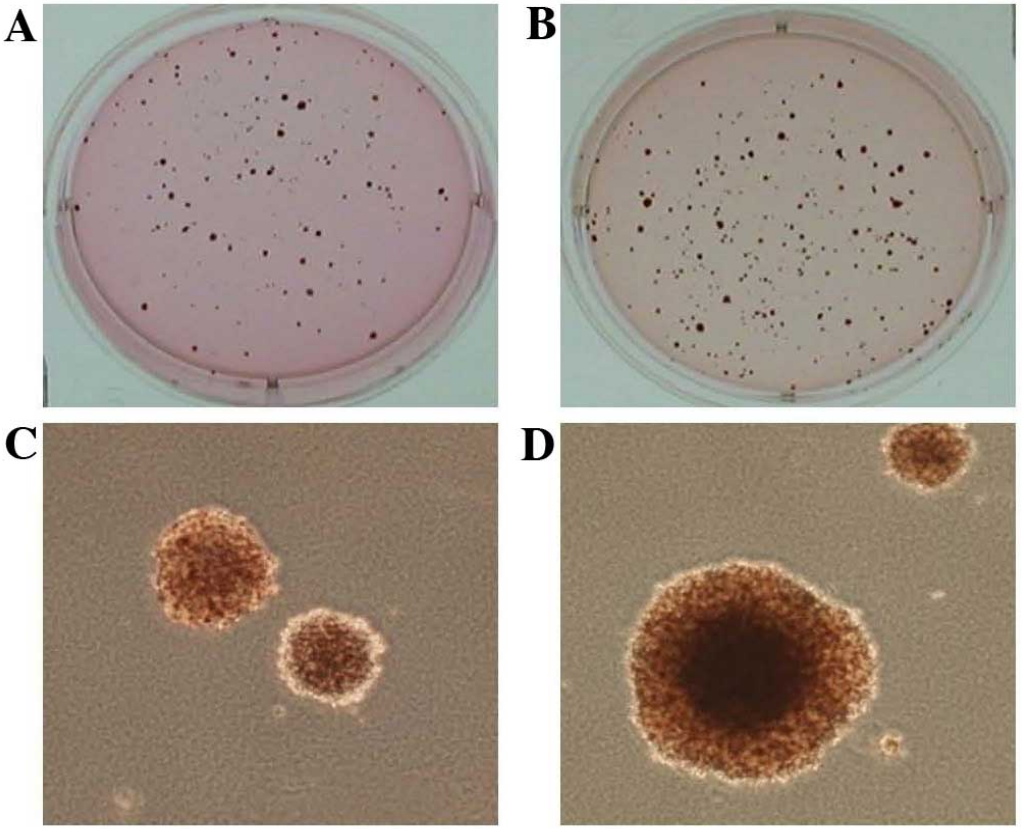
Neoplasia
Metastatic tumor

Assay 1 for Transformed cells

Ability to grow without attachment



Foci formation on soft agar dish

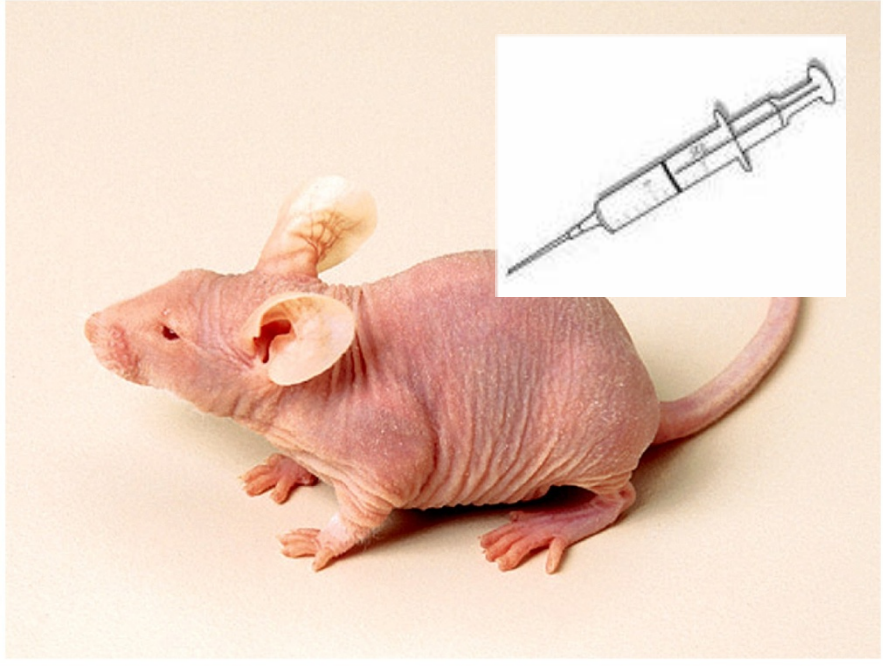


Assay 2 for Transformed cells

Nude mice

1. Lacks thymus
    - Immuno-compromised
    - Accept grafts from unrelated sources, such as human cells

2. No hair: Easy to see tumors



How bad are the cells?
2 functional assays

1. Foci formation on soft agar

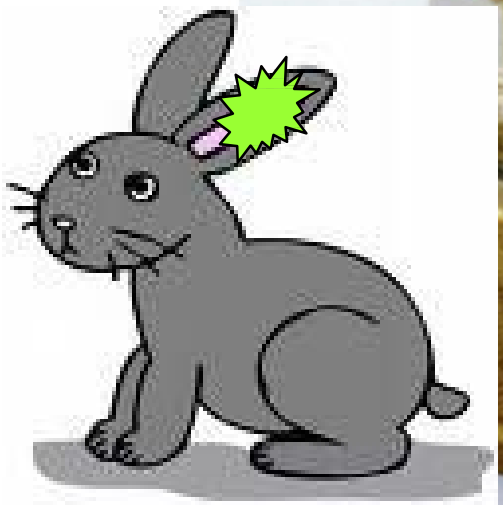
2. Tumor formation in Nude mice

Carcinogen test

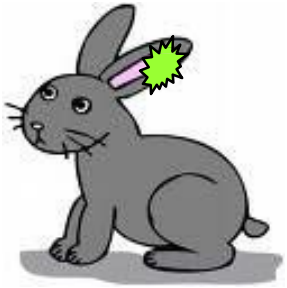
Katsusaburo Yamagiwa, 1915
Japan

660 days
Coal Tar
(焦油)

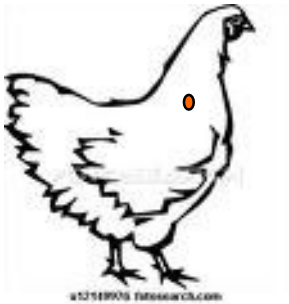
Skin
carcinoma



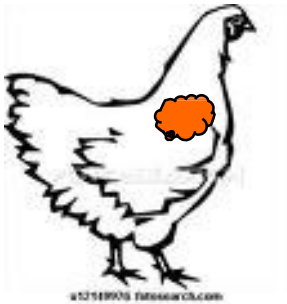
A new study system



1. Months,years

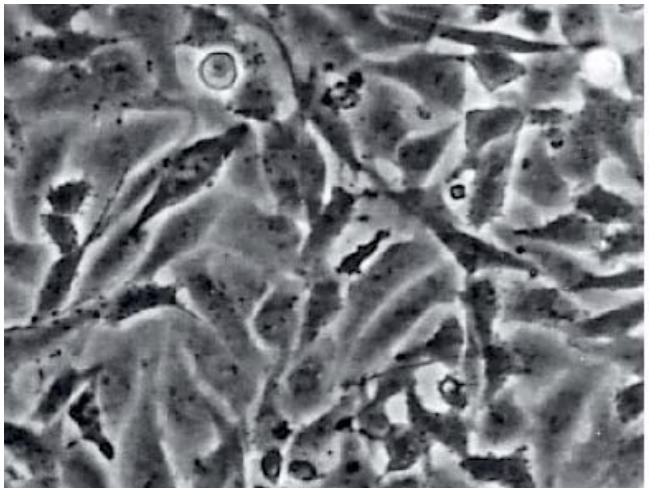


2. Several weeks

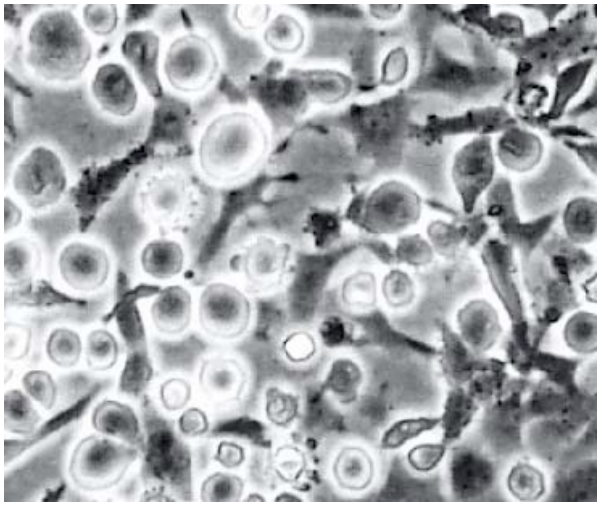


3. Several days

Transformation



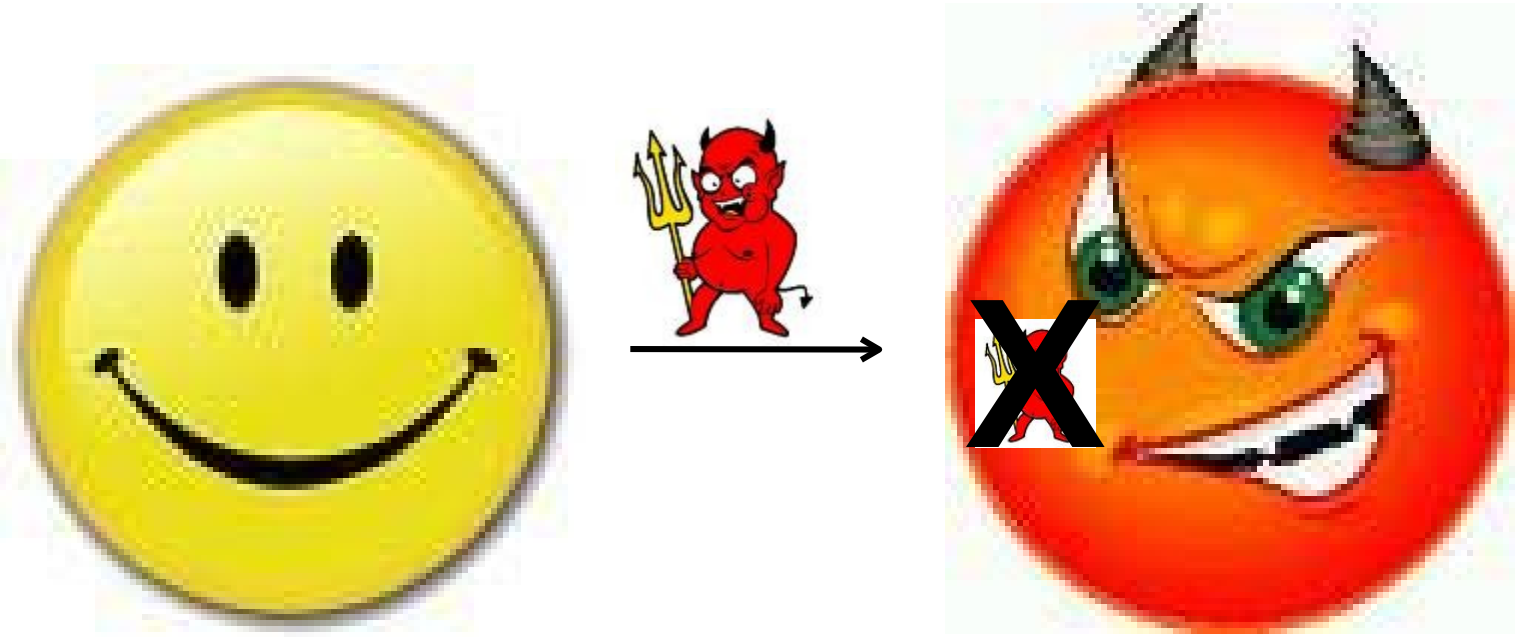
+RSV



Other viruses can also induce cancer

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

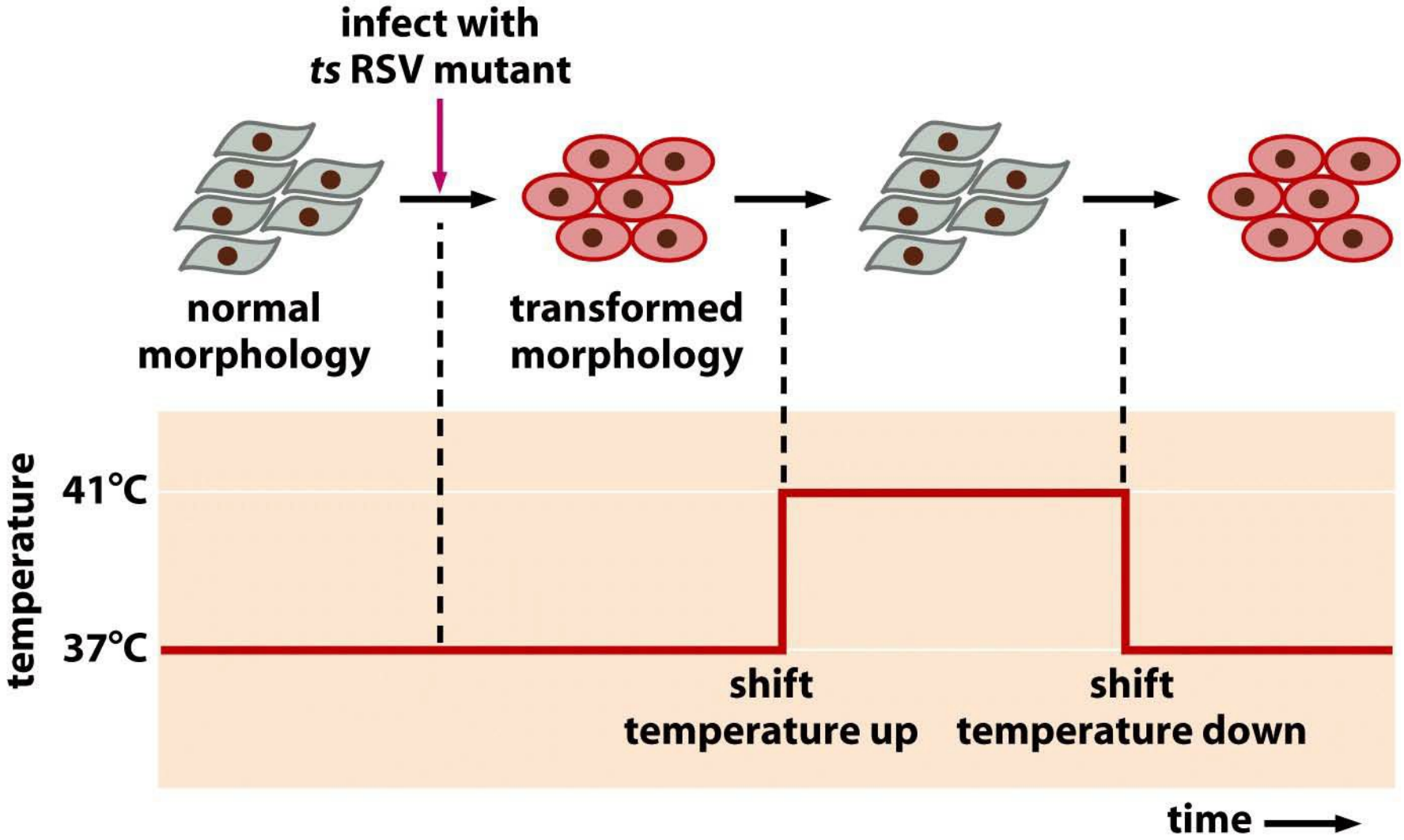
Hit & Run, or dependence on virus?



Normal cell

Tumor cell

Need RSV continuously?



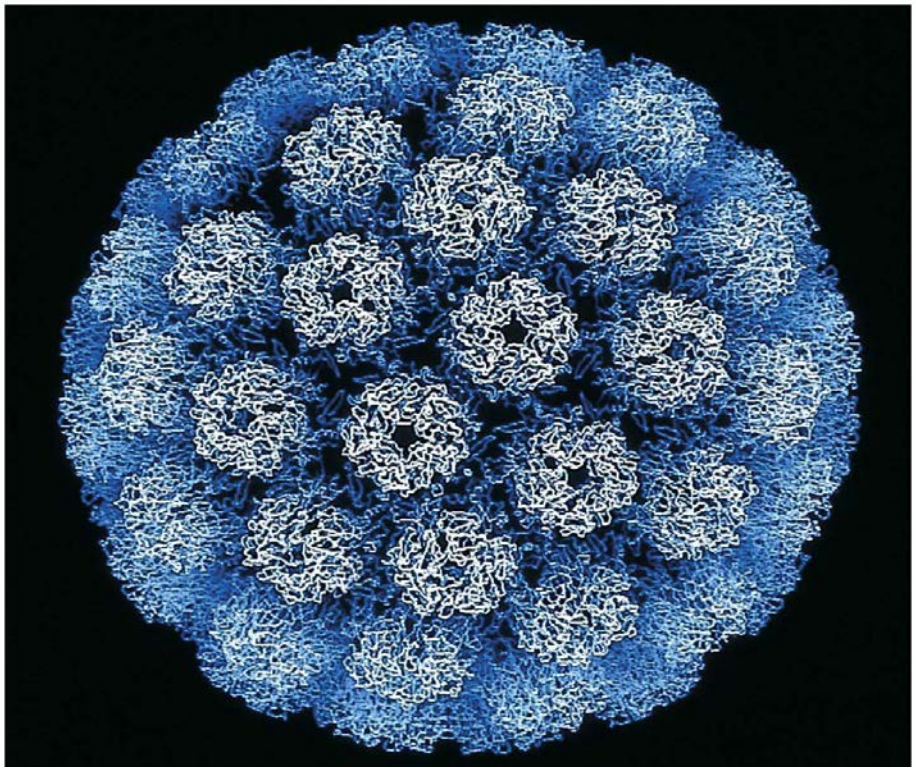
Summary

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.

How do viruses cause cancer? lineage?

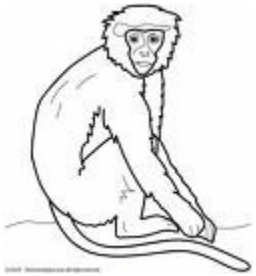
How could Virus succeed in Transmitting its genome through many generation within a cell lineage?

SV40 virus



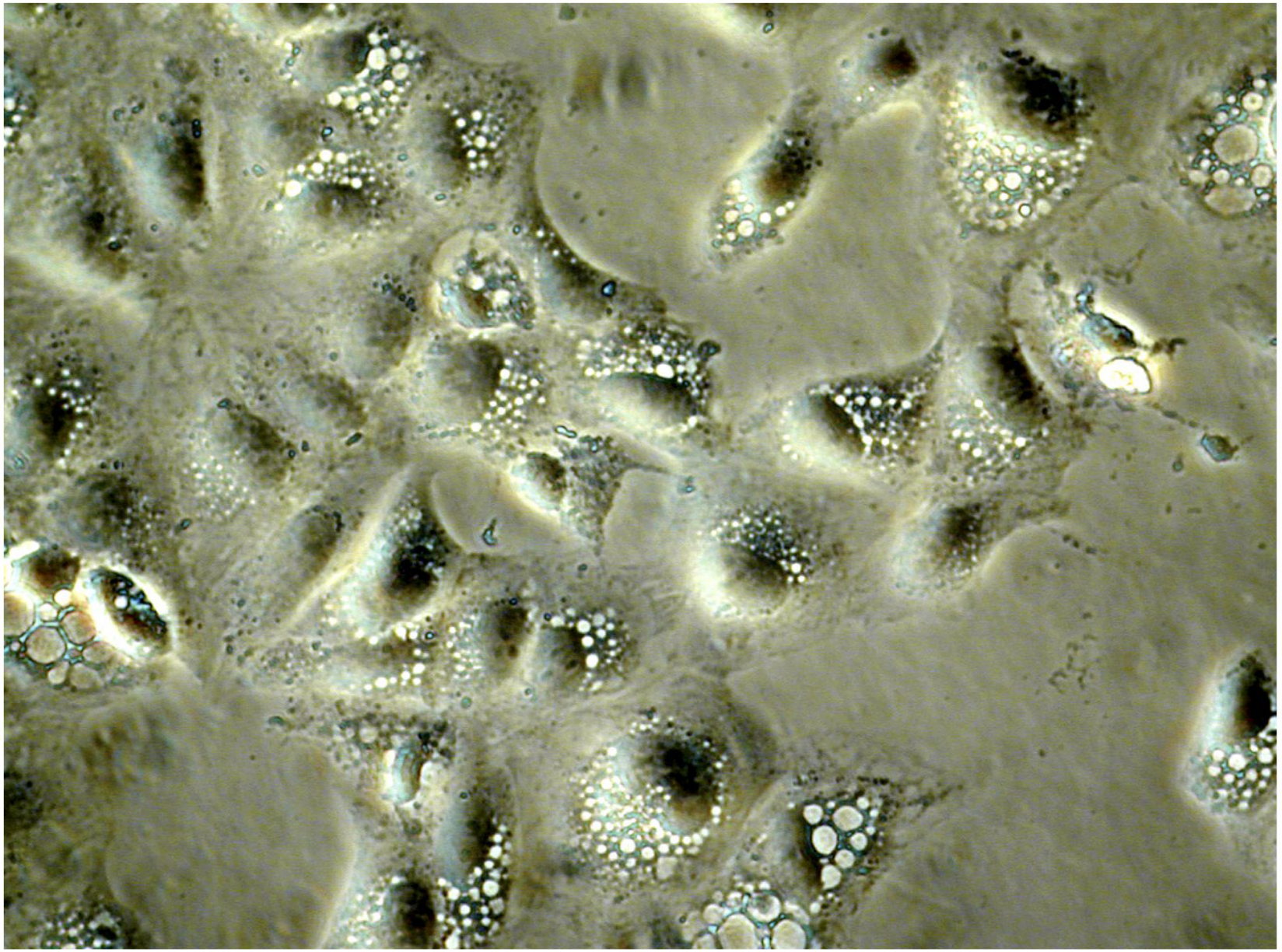
SV40 infection

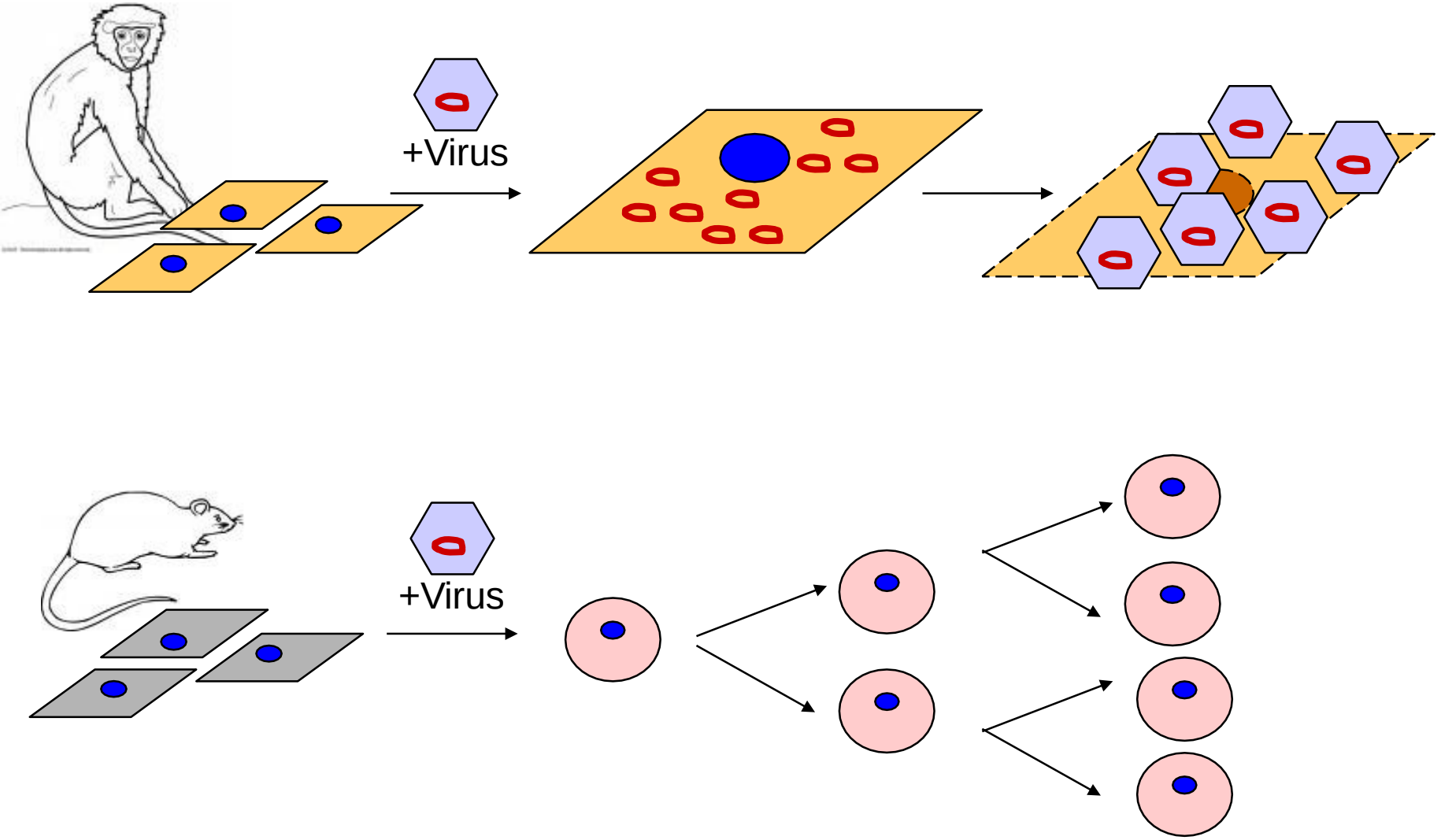
- Monkey cell lines → replicate →Kill cells



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

SV40 infected monkey cells

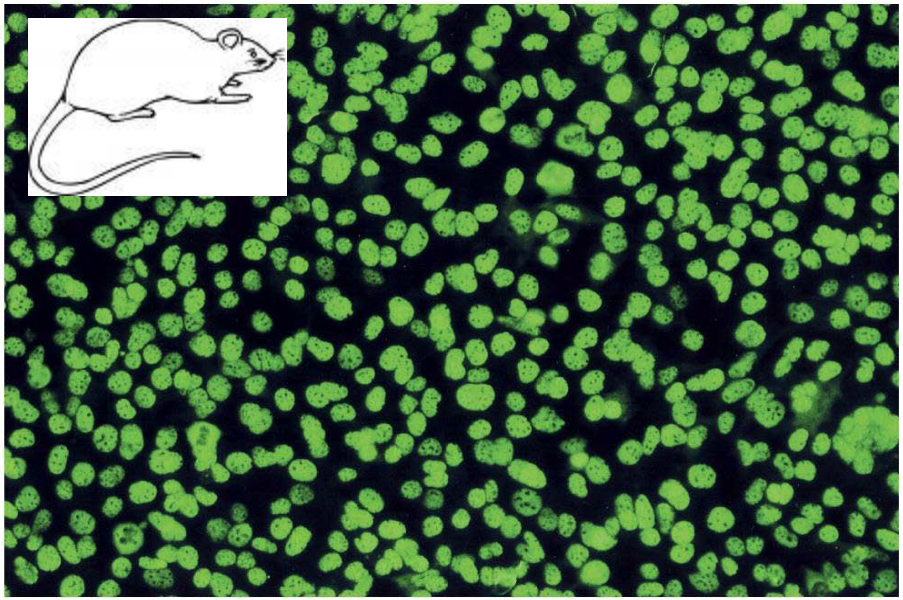


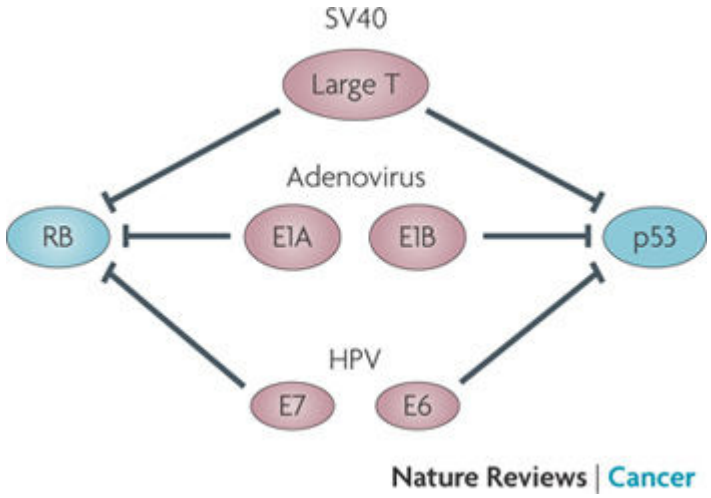


- How does the virus continue in daughter cells?

SV40 is required for tranformation

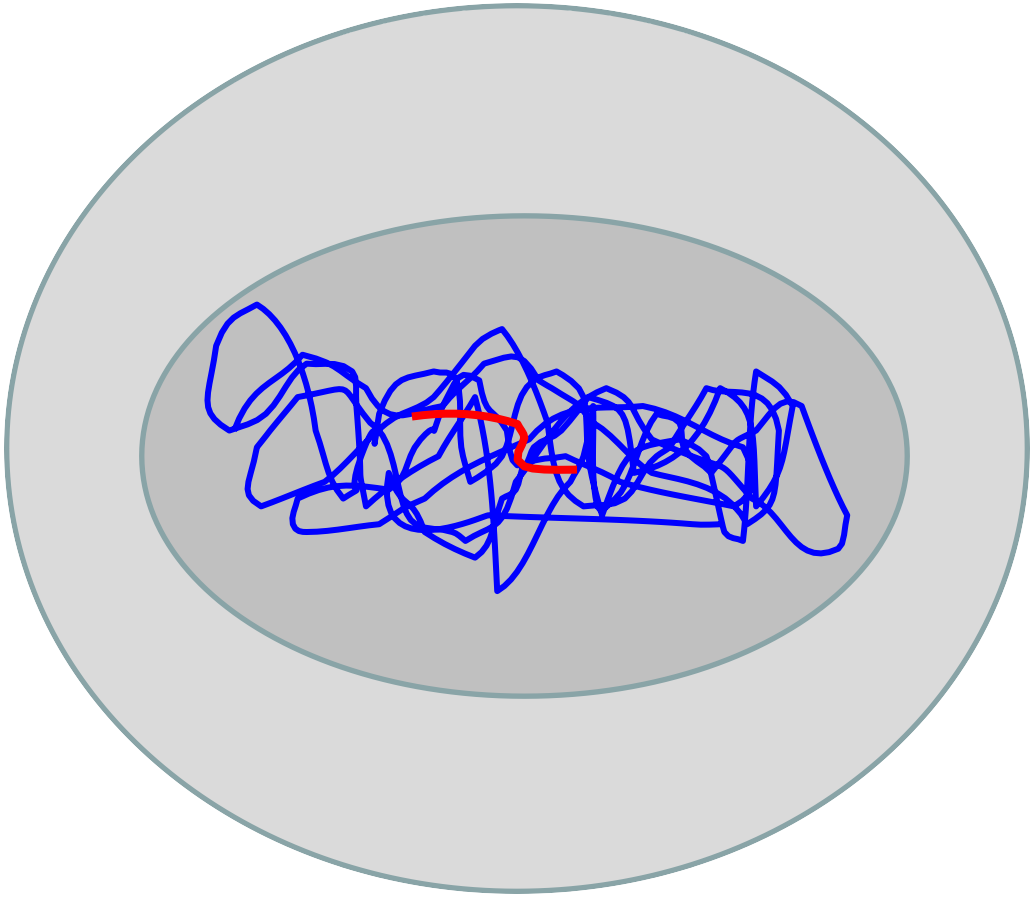
SV40 T antigen in mouse cells



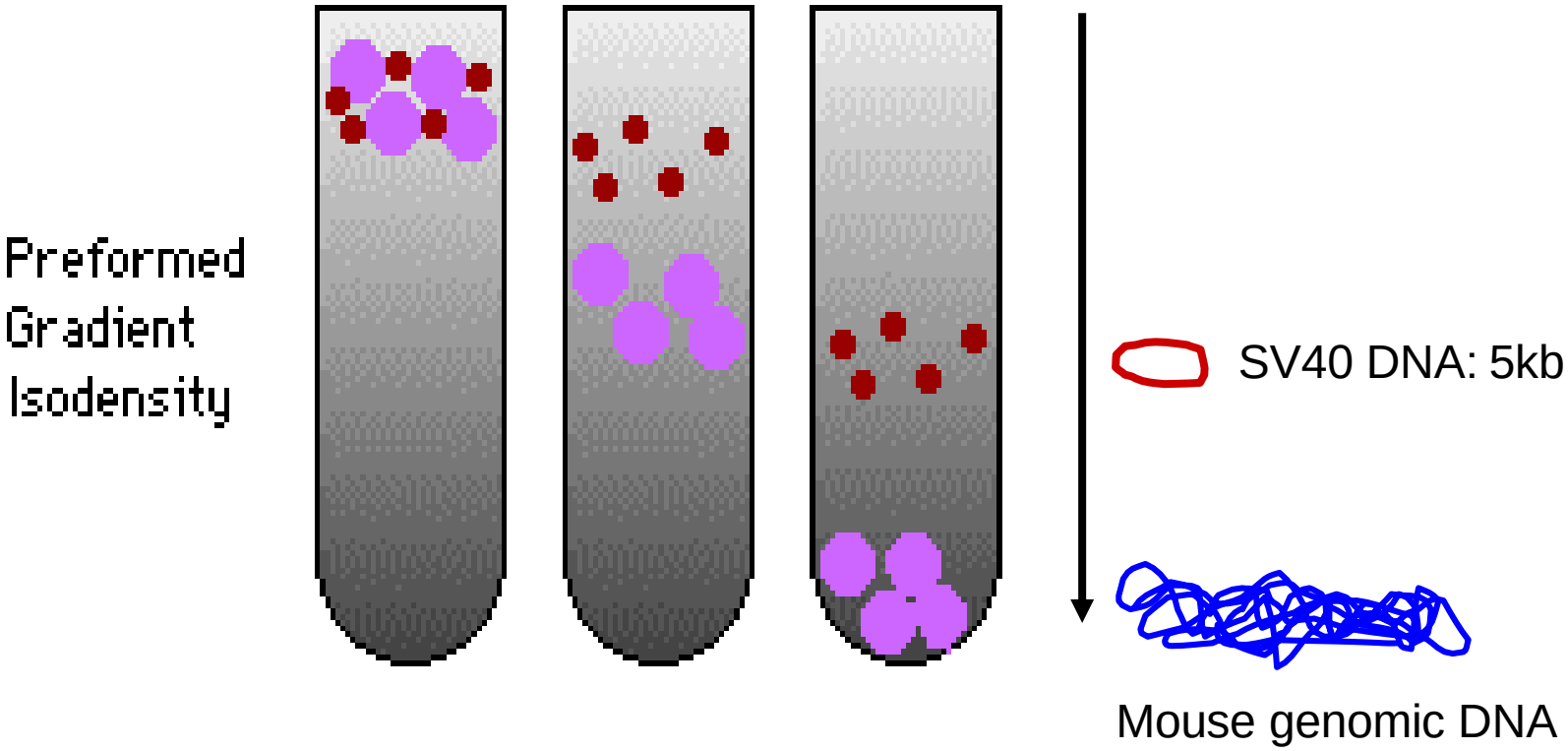


SV40 DNA

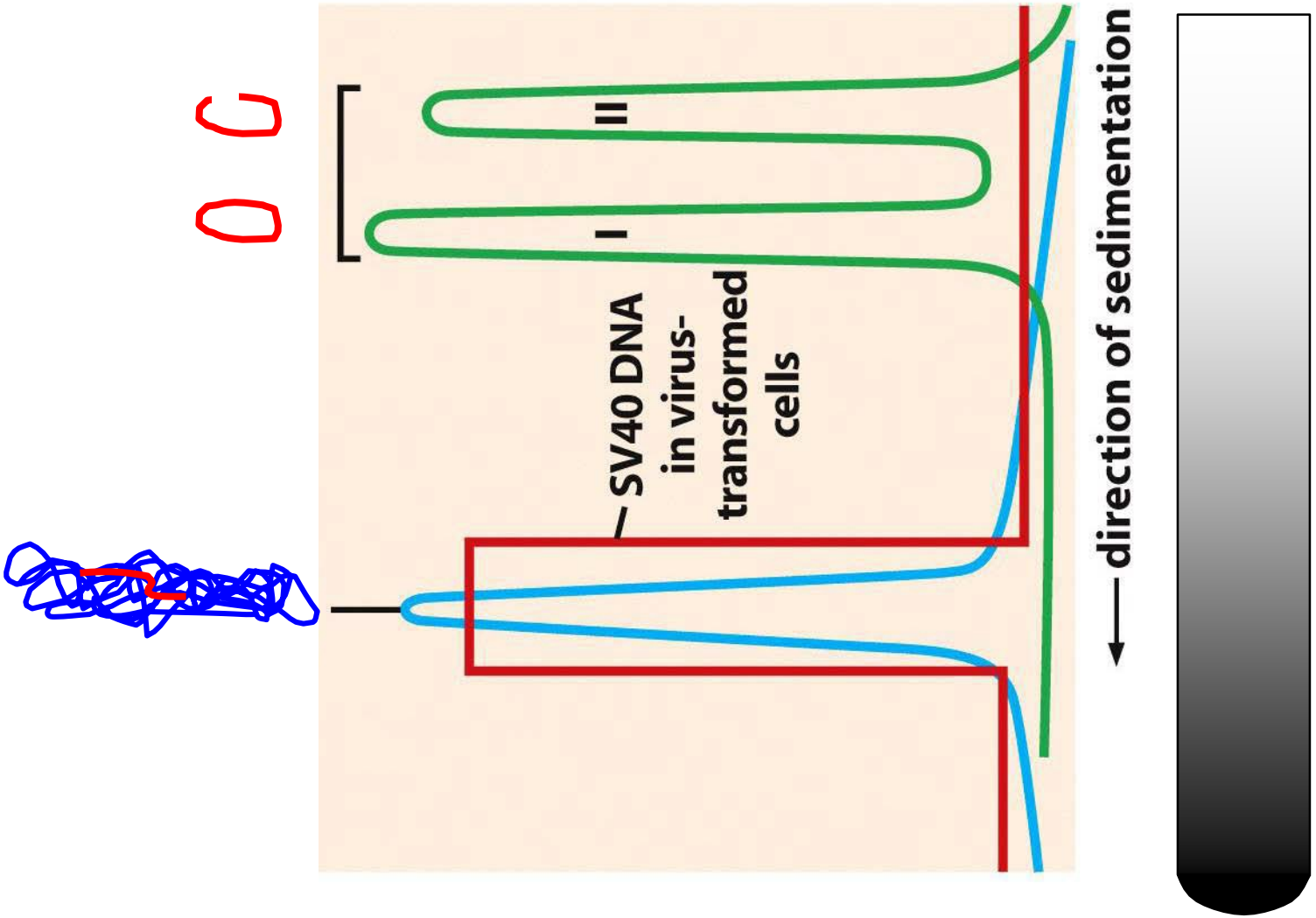
Integrated viral DNA→ tumor?



SV40 DNA in mouse cells



Sambrook, 1968



Integration

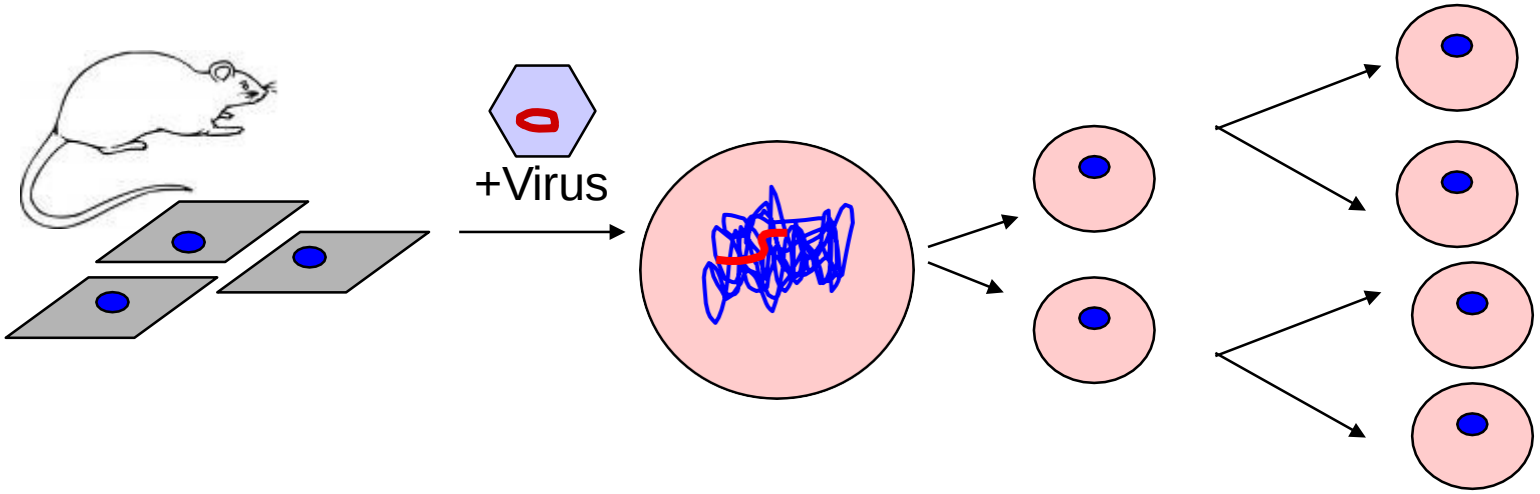
Alkaline pH

High temperature

……

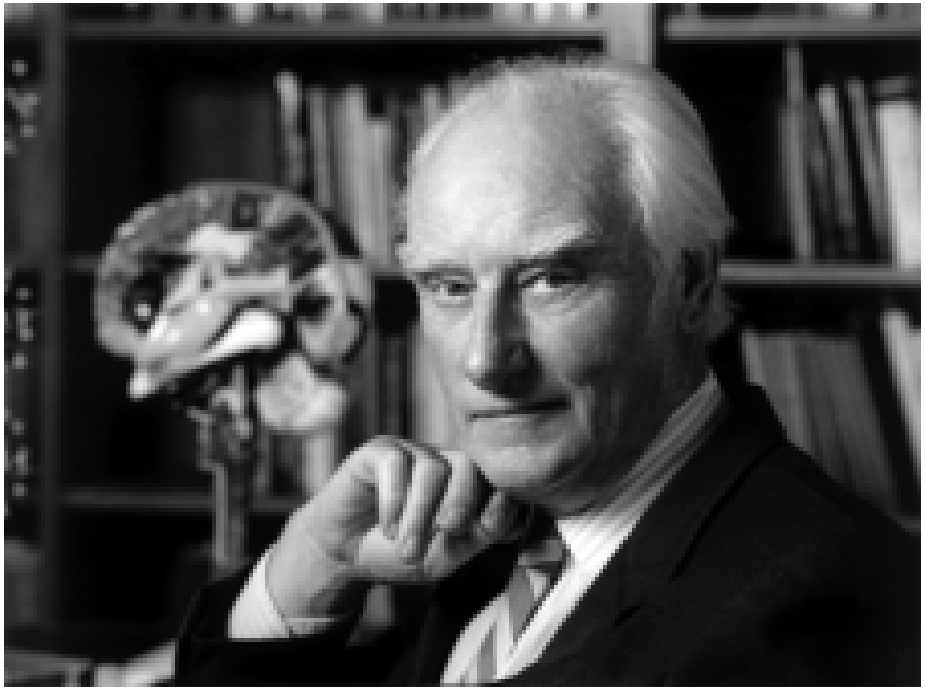
Resistant to chemical separation!

Viral DNA integration



How do viruses cause cancer? lineage?

How could RSV(RNA Virus) succeed in Transmitting its genome through many generation within a cell lineage?



Francis Crick

Nobel award

Central Dogma:
1958

DNA

↓

RNA

↓

Protein

How can RNA integrate into DNA?

Howard Temin

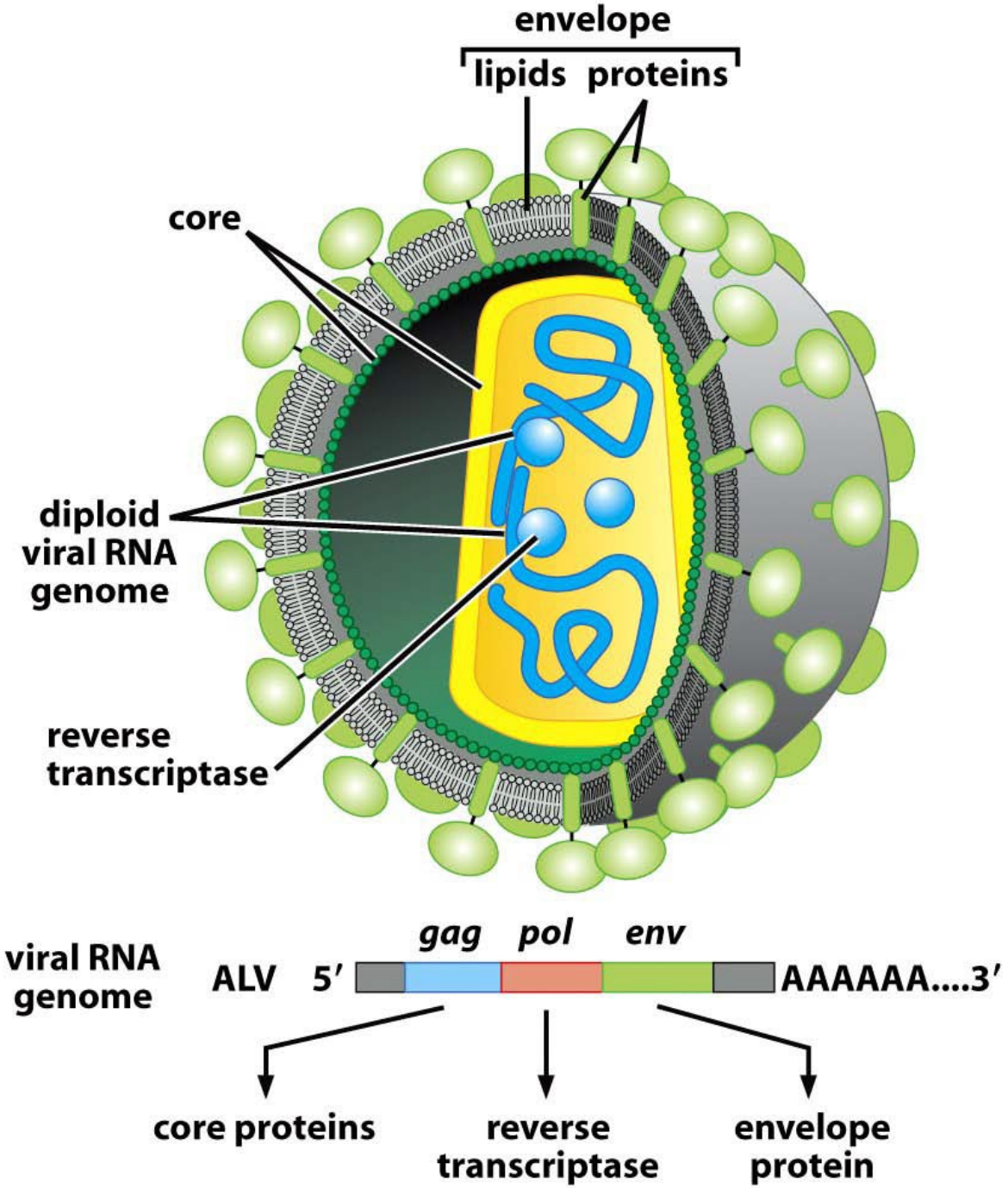
Ph.D 1959 @ age 25
Idea of provirus @ age 30

Temin's idea

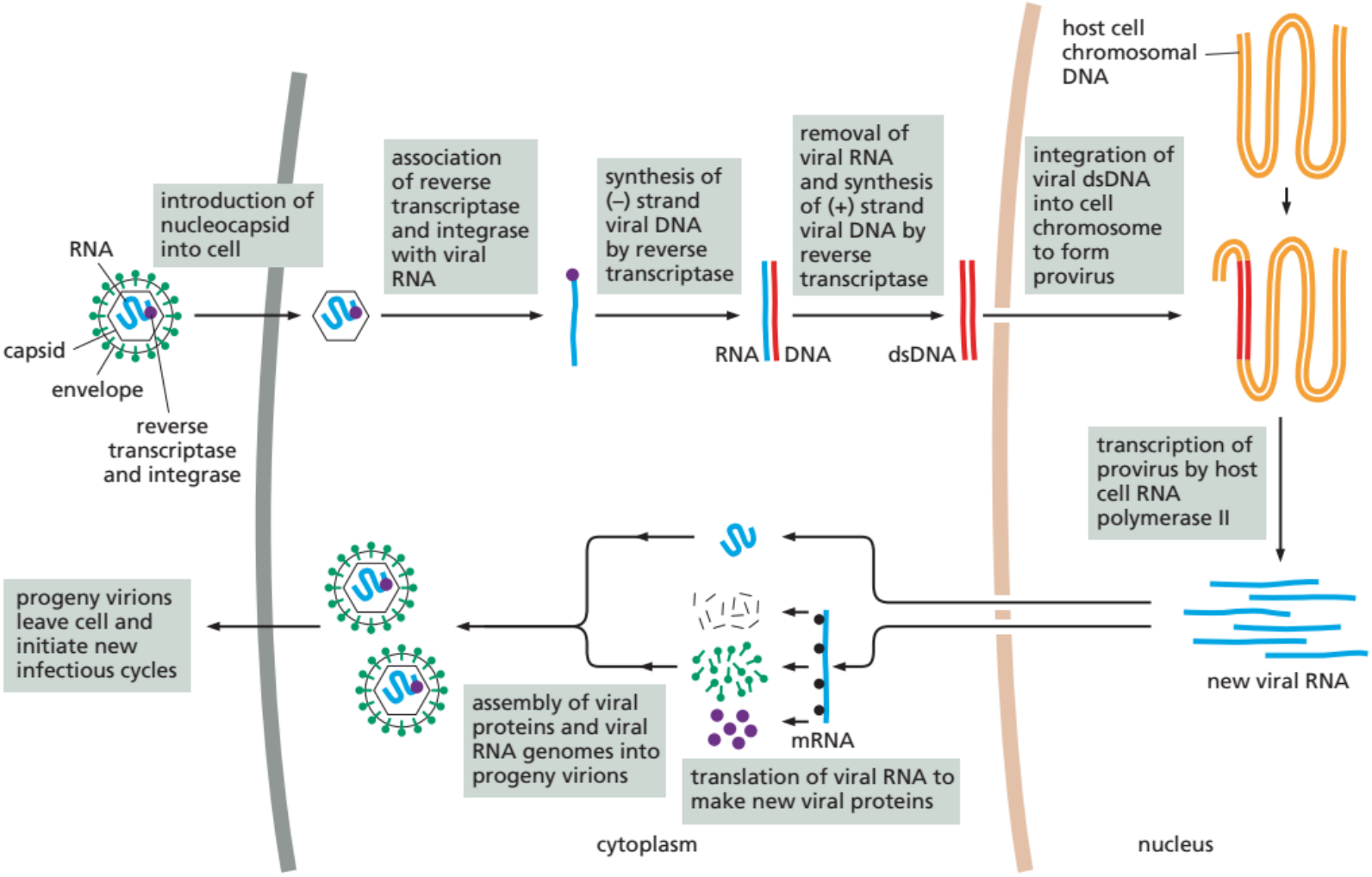
1964

1975

- RNA virus
  - RSV



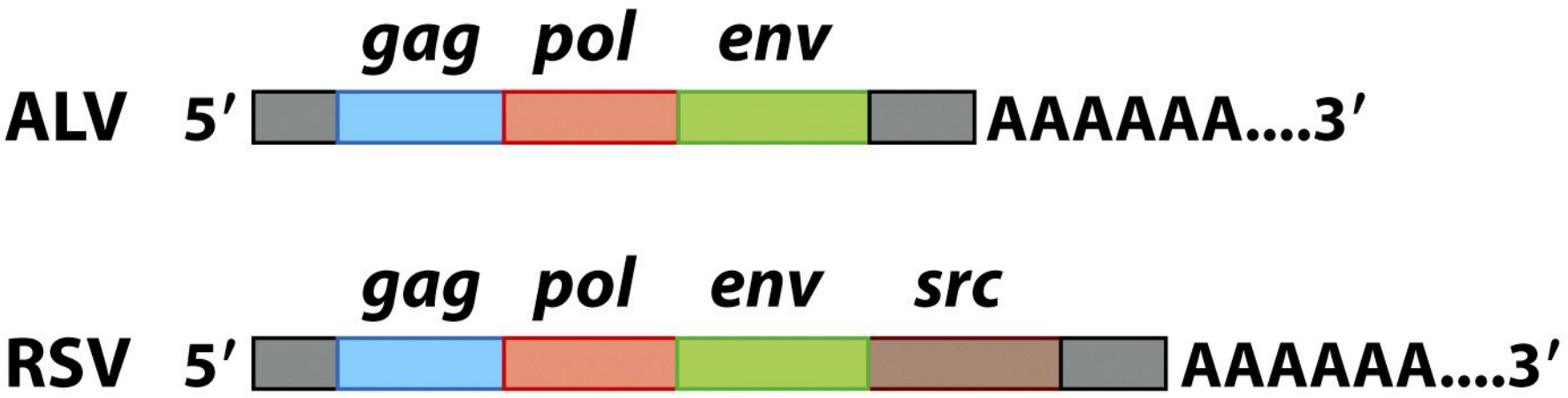
Life cycle of RNA virus



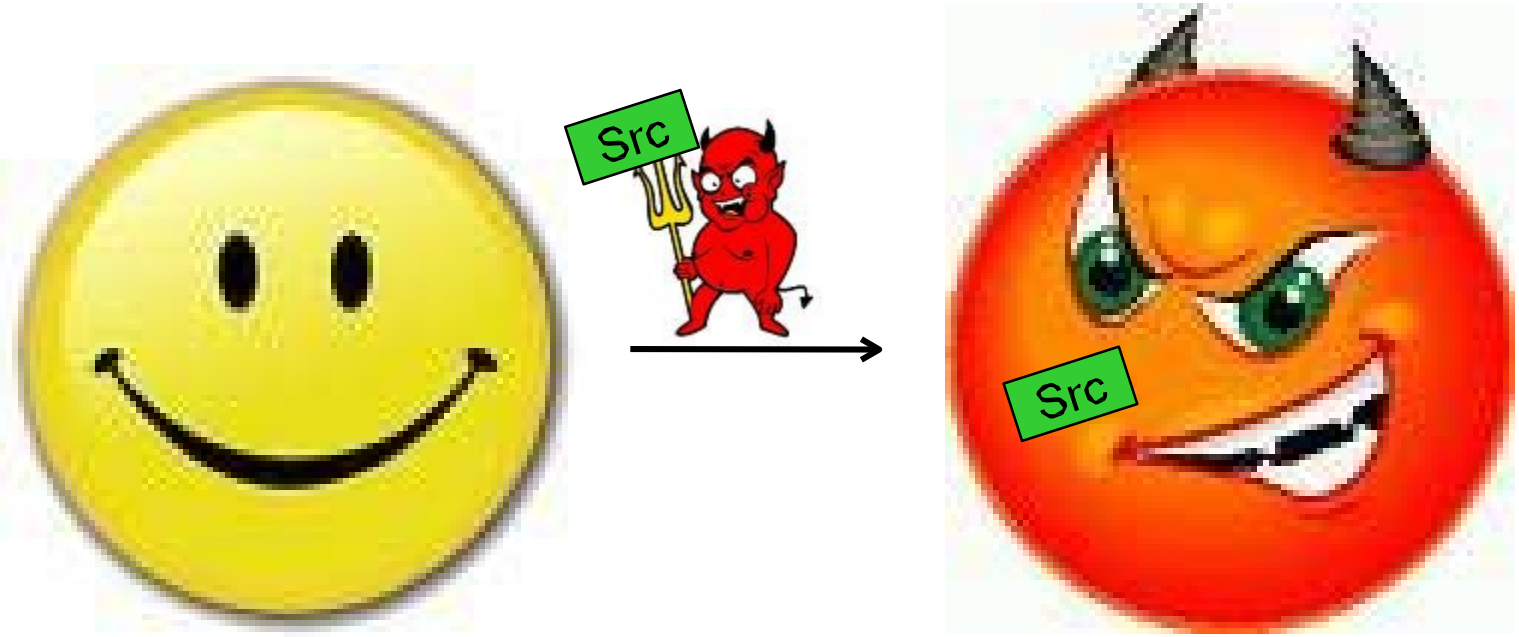
Virus and Cancer

- How does virus cause cancer?
  - Molecular mechanisms

Avian Leukosis Virus: Retrovirus（白细胞组织增生)



The first oncogene-Src



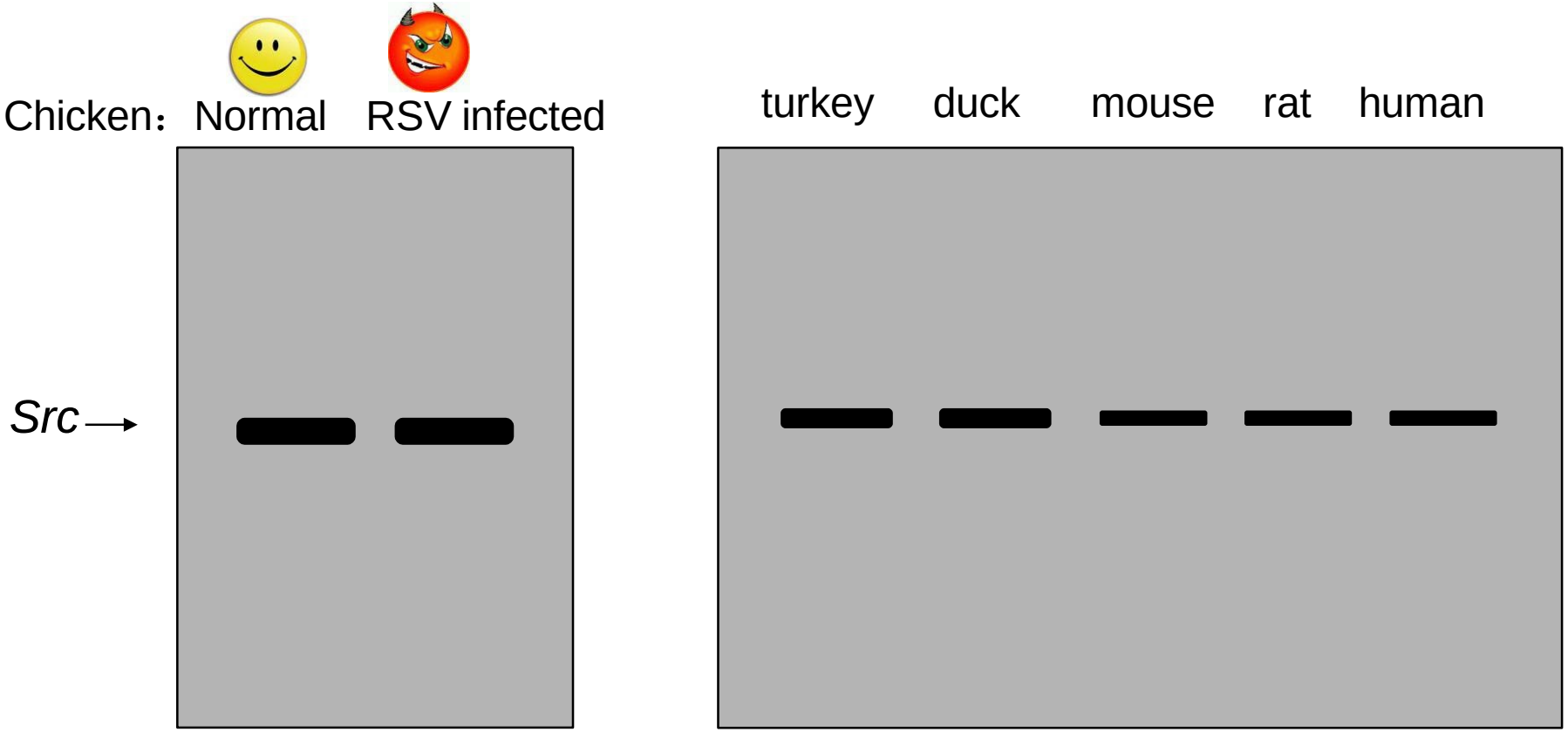
Normal cell

Tumor cell

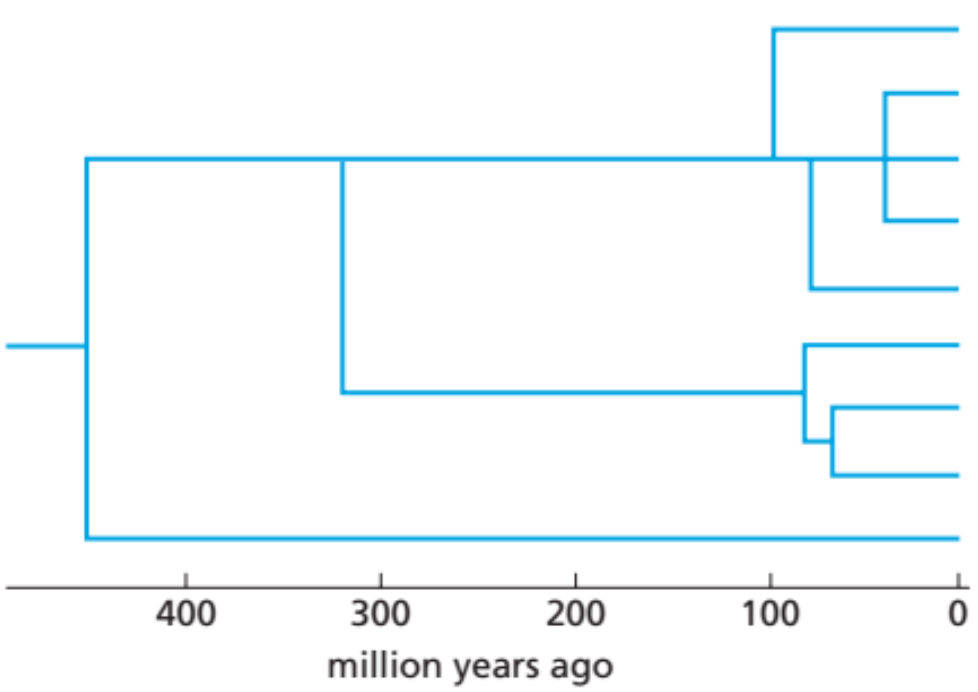
When src is activated, it promotes survival, angiogenesis, proliferation and invasion pathways. It also regulates angiogenic factors and vascular permeability after focal cerebral ischemia-reperfusion,[19][20] and regulates matrix metalloproteinase-9 activity after intracerebral hemorrhage。

Detect *Src* in sarcoma cells

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



Evolutionary tree of the *src* gene

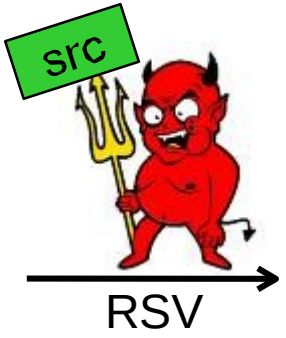


	extent of hybridization	melting temp. (°C)
RSV-rat	70%	82°
emu	16%	75.5°
quail	48%	78°
chicken	54%	80°
turkey	53%	78°
duck	46%	76°
calf	24%	65°
mouse	28%	65.5°
human	29%	66.5°
salmon	19%	66.5°

Src in normal cells

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

Normal cell



Tumor cell

Does Src cause tumor?

Src : cellular origin of retroviral oncogenes

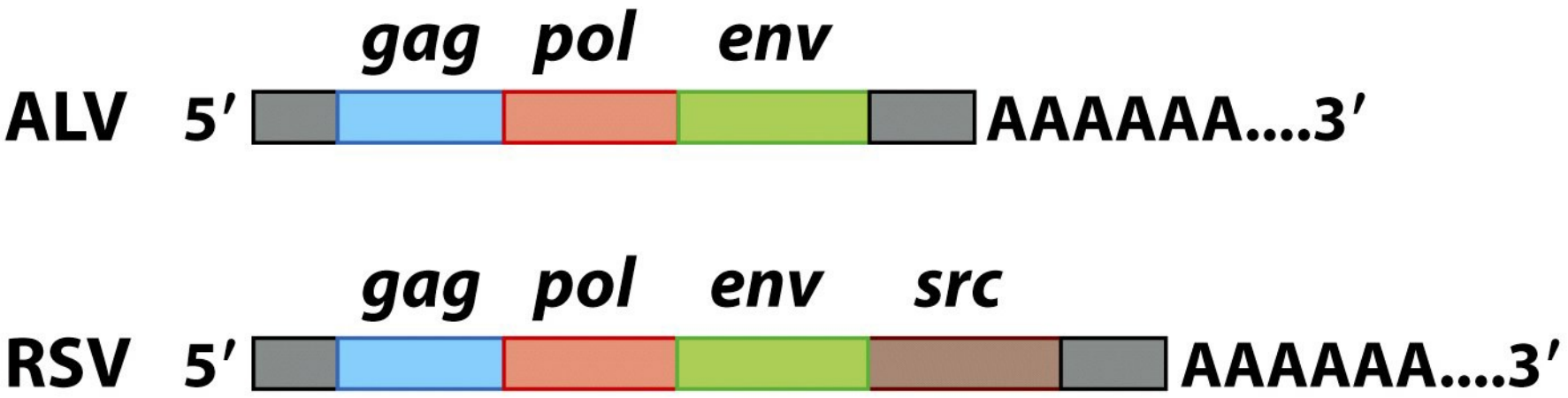
J. Michael Bishop

1989

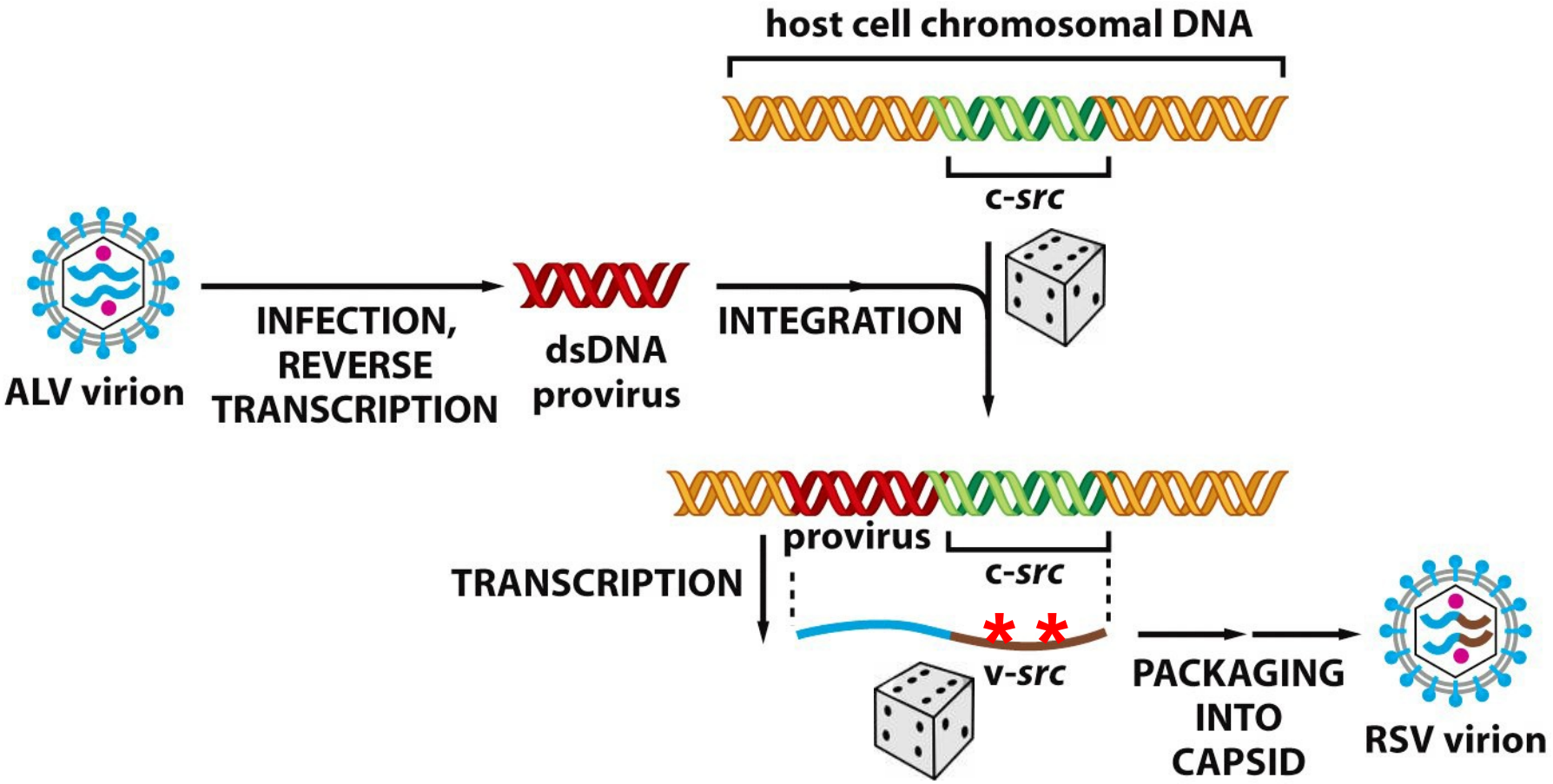
Harold E. Varmus

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

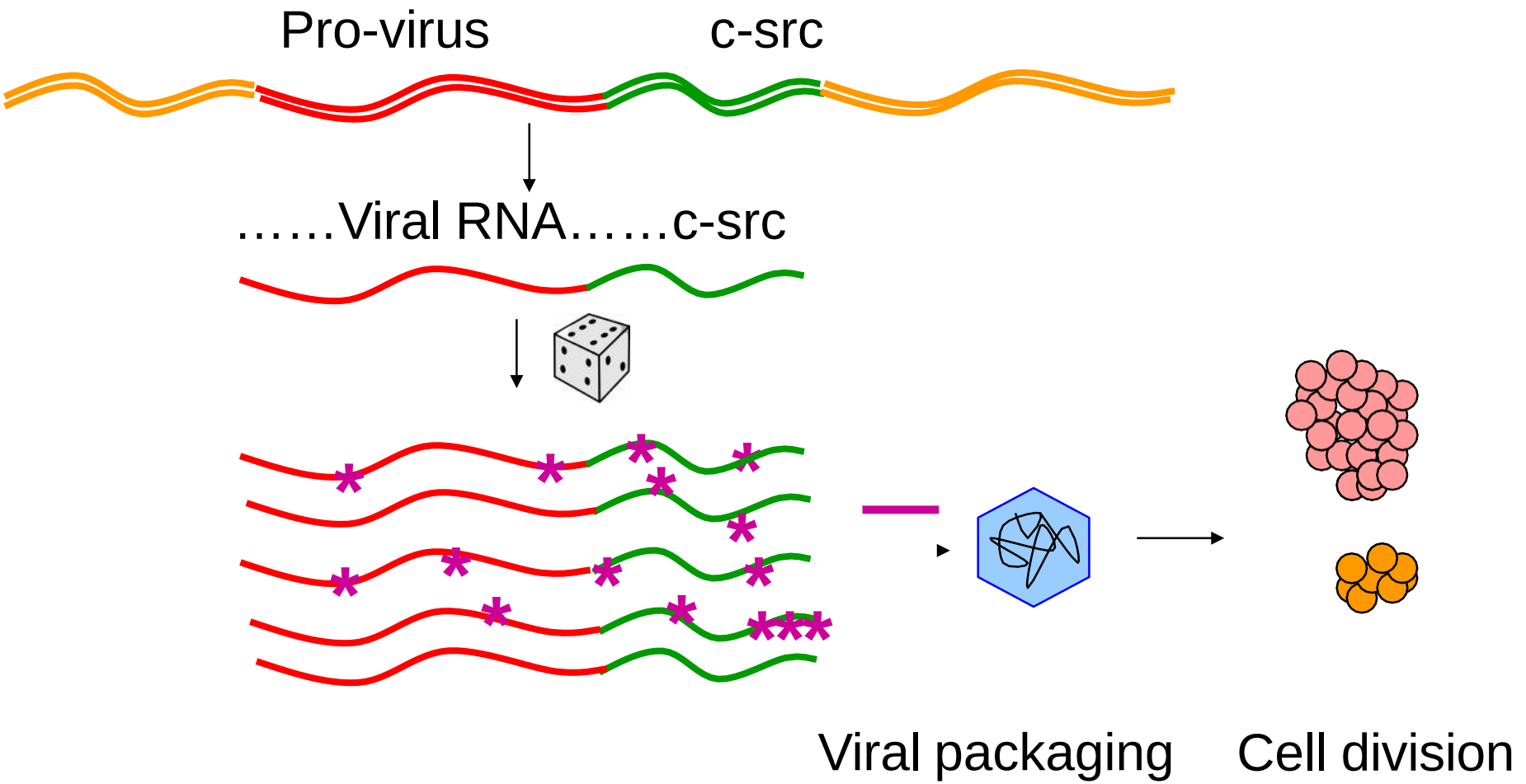
Avian Leukosis Virus: Retrovirus



Origin of *v-src*



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 Tirm	Sarcomas (monkeys)
COLIAI-PDGFB	PDGF	Chromosomal transk>cation	Fibrosarcoma
2. Receptors			
v-erb-b	EGF receptor	Avian emhroblastosisvirus	Leukemia (chickens)
RET	Ret receptor	Point mutation, chromosomal translocation	Thyroid
TRK	Nerve growth factor receptor	DKA rearrangement (im·ersion)	Thyroid
ERBB2	ErbB2 receptor	Amplification	Breast, ovary
v-mpl	Thrombopoietin receptor	Myeloproliferatn--eleukemia virus	Leukemia (mice)
3. Plasmamembrane G proteins			
v-K-ras	Ras	Han-e, sarcoma virus	Sarcomas (rats)
v-H-ras	Ras	Kirsten 5arcoma virus	Sarcomas (rats)
KRAS	Ras	Point mutation	Pancreas, colon, lung, others
HRAS	Ras	Point mutation	Bladder
NRAS	Ras	Point mutation	Leukemias
4. Intracellular proteink.inases			
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

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

Mapping of ALV in chicken genome

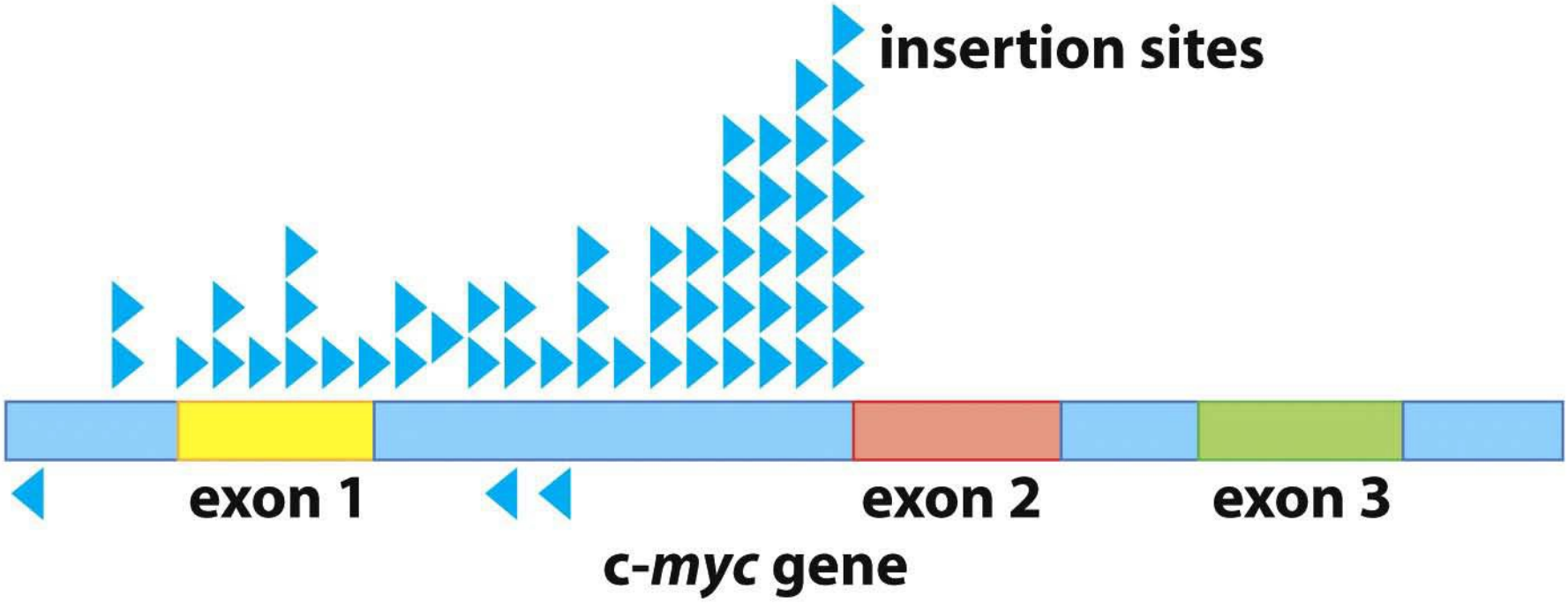
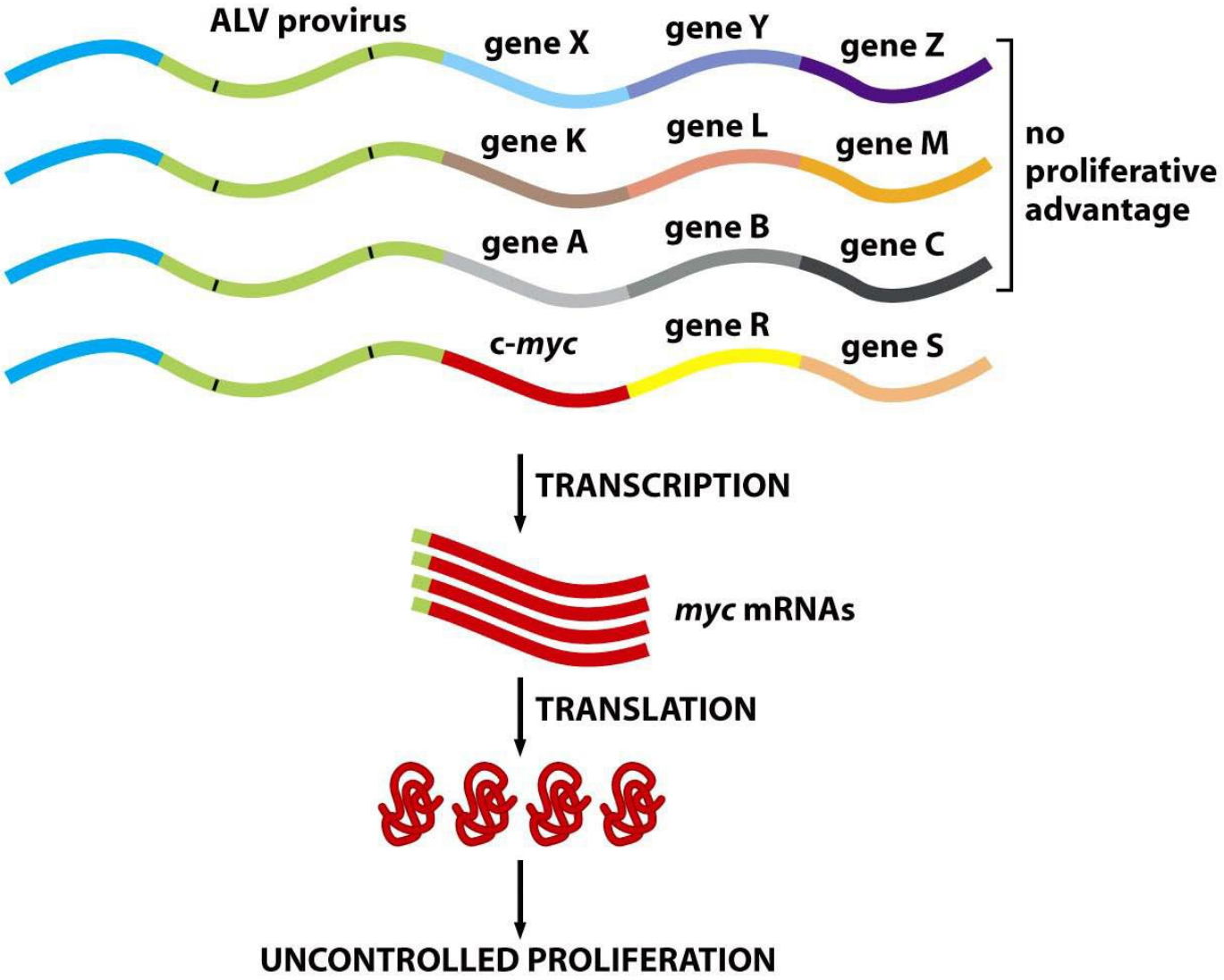


Figure 3-23a The Biology of Cancer (© Garland Science 2007)

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

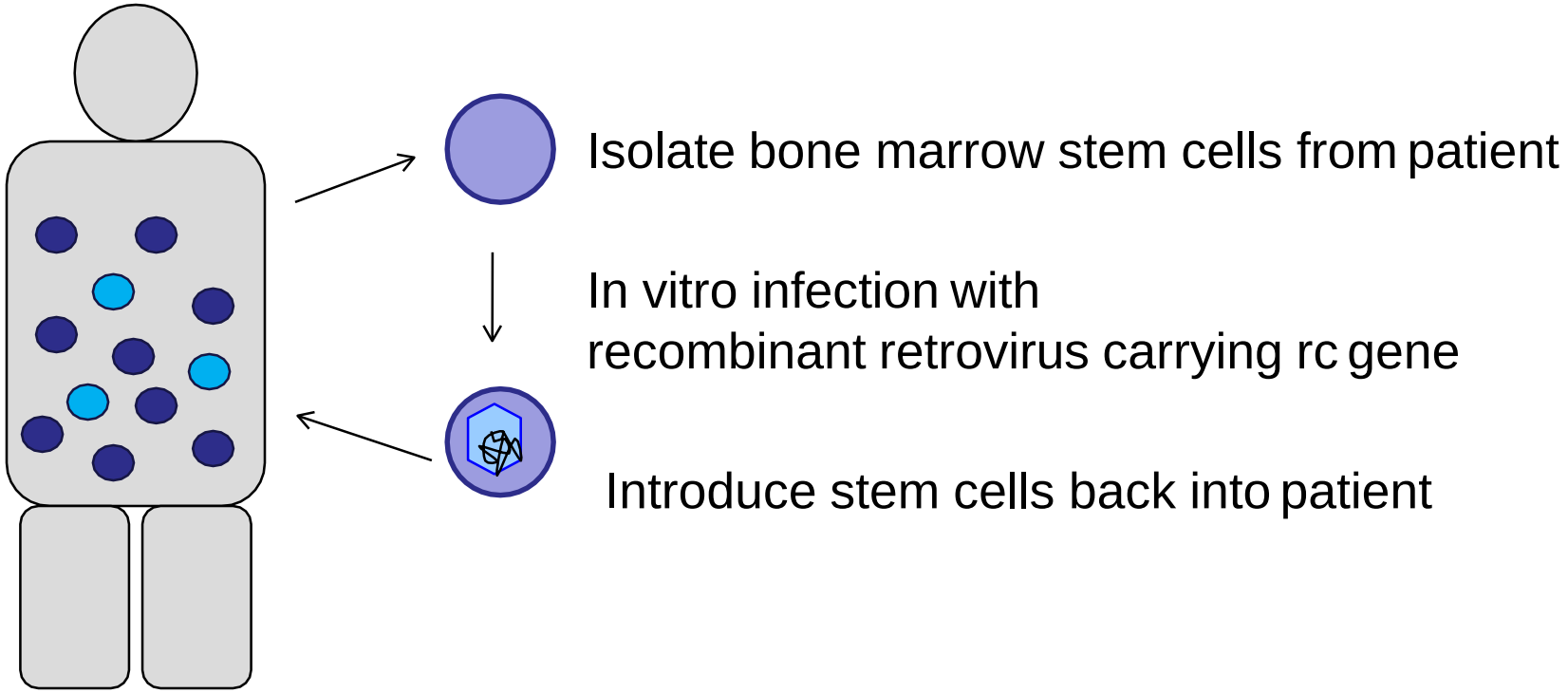
Int-1	MMTV	Mouse mammary carcinoma	Growth factor=Wnt-1
Int-2	MMTV	Mouse mammary carcinoma	Growth factor=FGF
Int-3	MMTV	Mouse mammary carcinoma	Receptor=Notch related
Erb-B	ALV	Chicken erythroblastosis	Growth factor receptor=EGF-R
K-ras	F-MLV	Mouse T cell lymphoma	Small G protein
Cyclin-D1	F-MLV	Mouse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

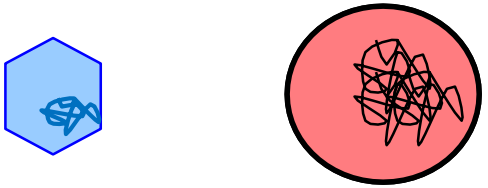
- Germ-line mutation that affects hematopoietic cells
- Expressing missing gene by retrovirus vector



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

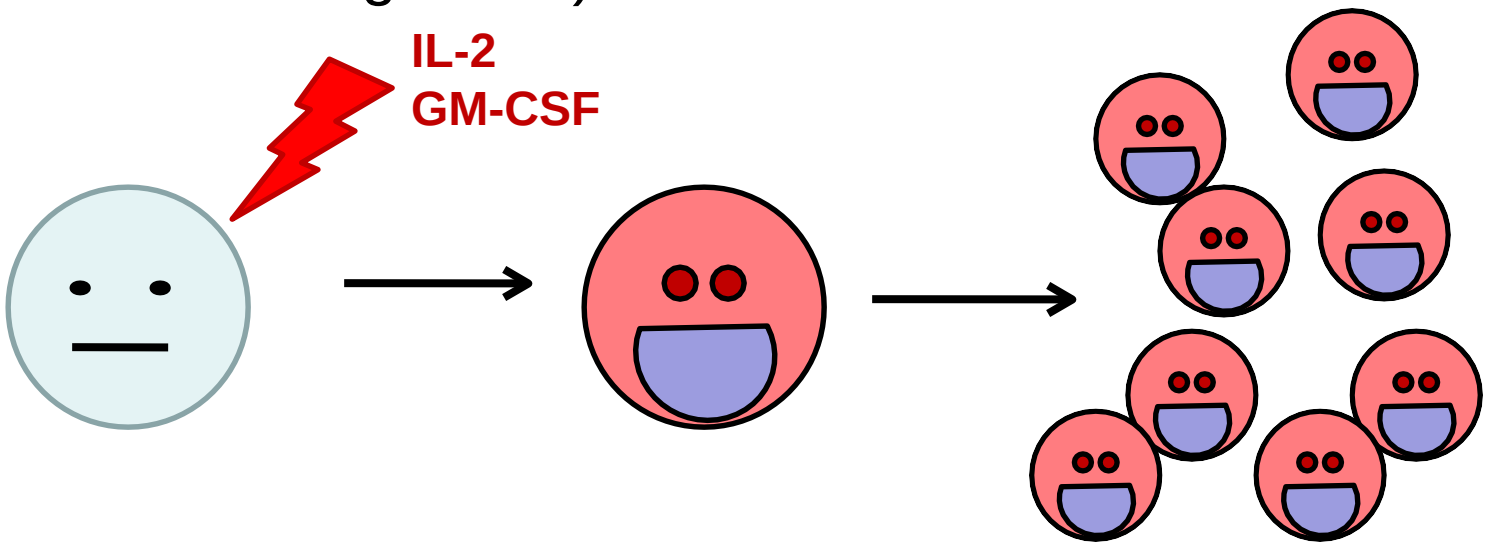
3rd mechanism

- 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*

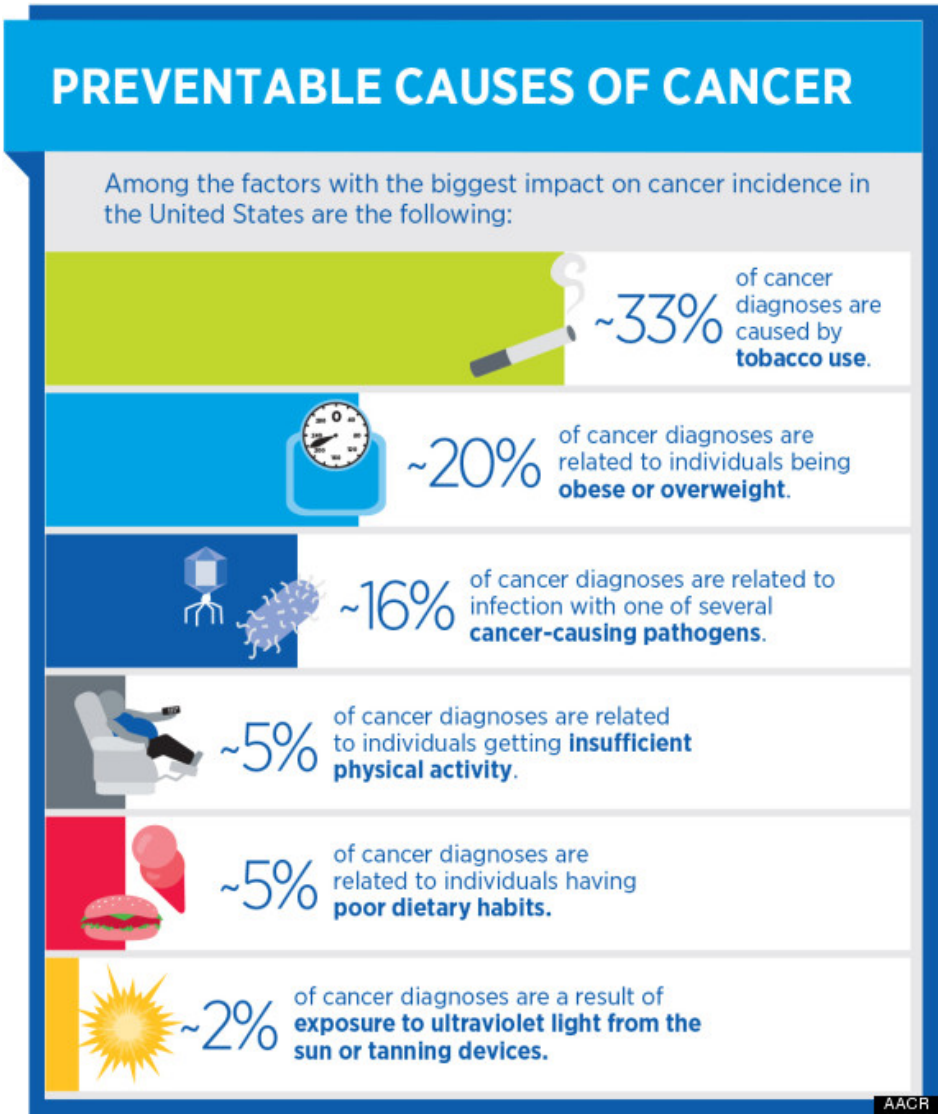
3. Virus gene acts as oncogene: HTLV-I *tax*

Viruses and Human Cancer

Viruses account for about 20% of total human cancer cases，
many viruses can cause various tumors in animals, only seven of them are associated with human cancers and are currently considered oncogenic viruses

Virus	Human Cancer
hepatitis B virus (HBV),	80% of hepatocellular carcinoma (HCC)
hepatitis C virus (HCV),	
human papillomavirus (HPV),	cervical cancer、head and neck tumors.
Epstein Barr virus (EBV),	nasopharyngeal carcinoma, Hodgkin's & Burkitt's lymphoma
human herpes virus 8 (HHV8),	Kaposi's sarcoma（ often found in AIDS patients）
Merkel cell polyomavirus (MCPyV),	Merkel cell carcinoma
HTLV-1	T-cell lymphoma.

Summary: Causes of Cancer & Prevention



References

The Biology of Cancer (Second Edition, chapter 3,)

additional reading

1. Kung HJ, Boerkoel C & Cater TH (1991) Retroviral mutagenesis of cellular oncogenes: a review with insights into the mechanisms of insertional activation. Curr. Top. Microbiol. Immunol. 171, 1–15.

2. Martin D & Gutkind JS (2008) Human tumor-associated viruses and new insights into the molecular mechanisms of cancer. Oncogene 27, S31–S42.

3. Javier RT, Butel JS. (2008). The history of tumor virology. Cancer Research. 68(19):7693-706.

4. Moore PS & Chang Y (2010) Why do viruses cause cancer? Highlights of the first century of human tumor virology. Nat. Rev. Cancer 10, 878–889.