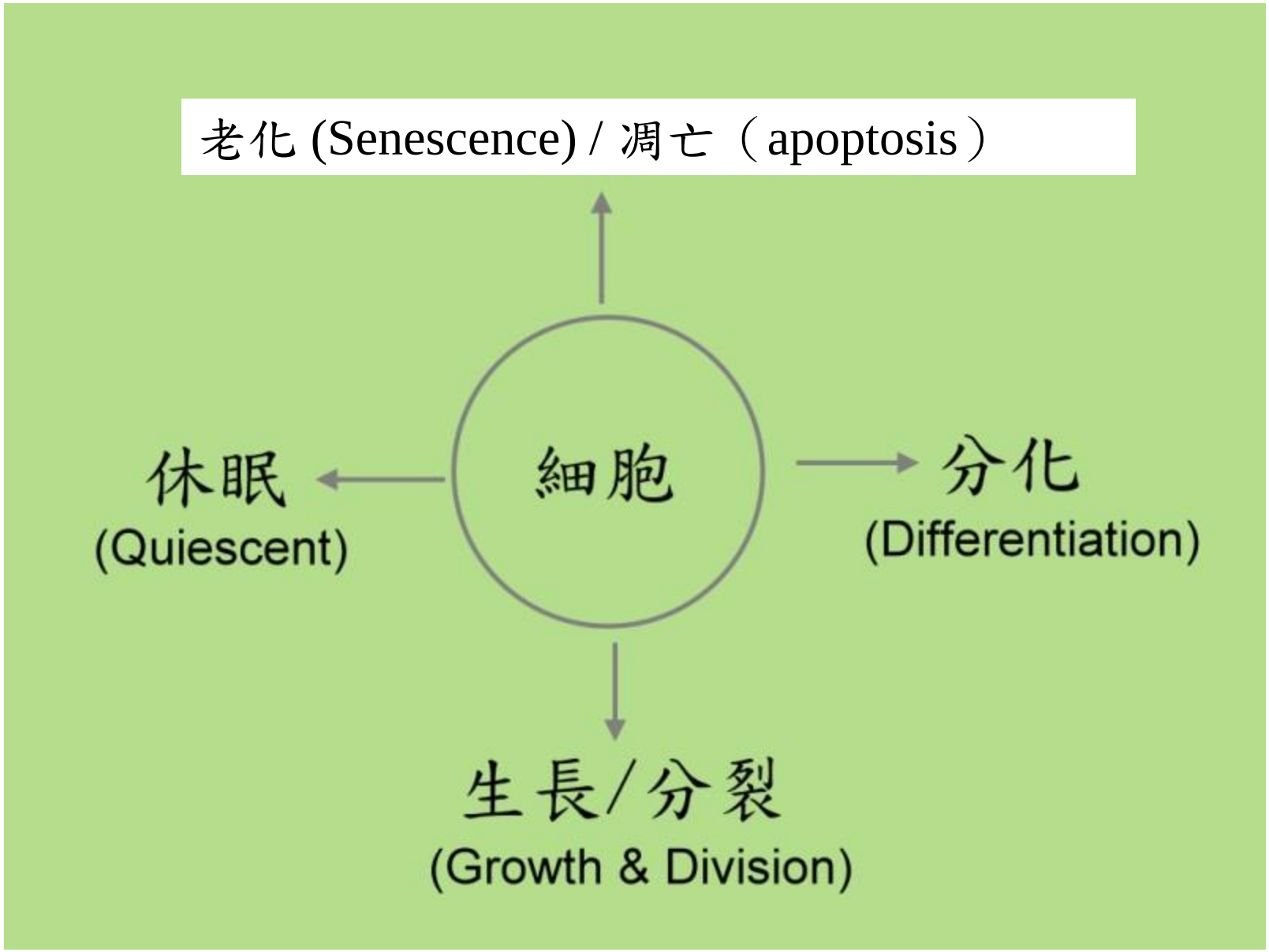
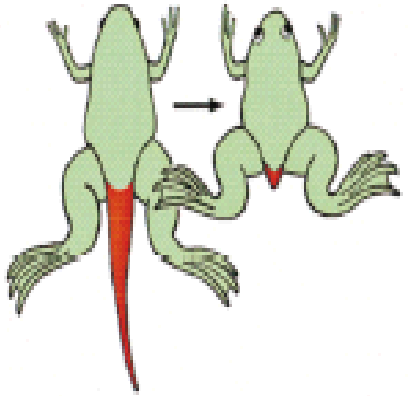
Cell apoptosis and senesence

2-22-2016

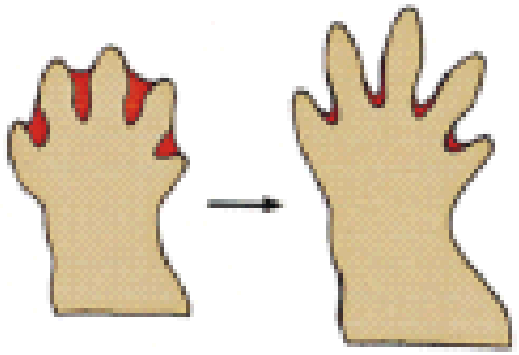

老化 (Senescence) / 凋亡（apoptosis）
休眠
(Quiescent)
細胞
分化
(Differentiation)
生長/分裂
(Growth & Division)

Normal functions of cell death

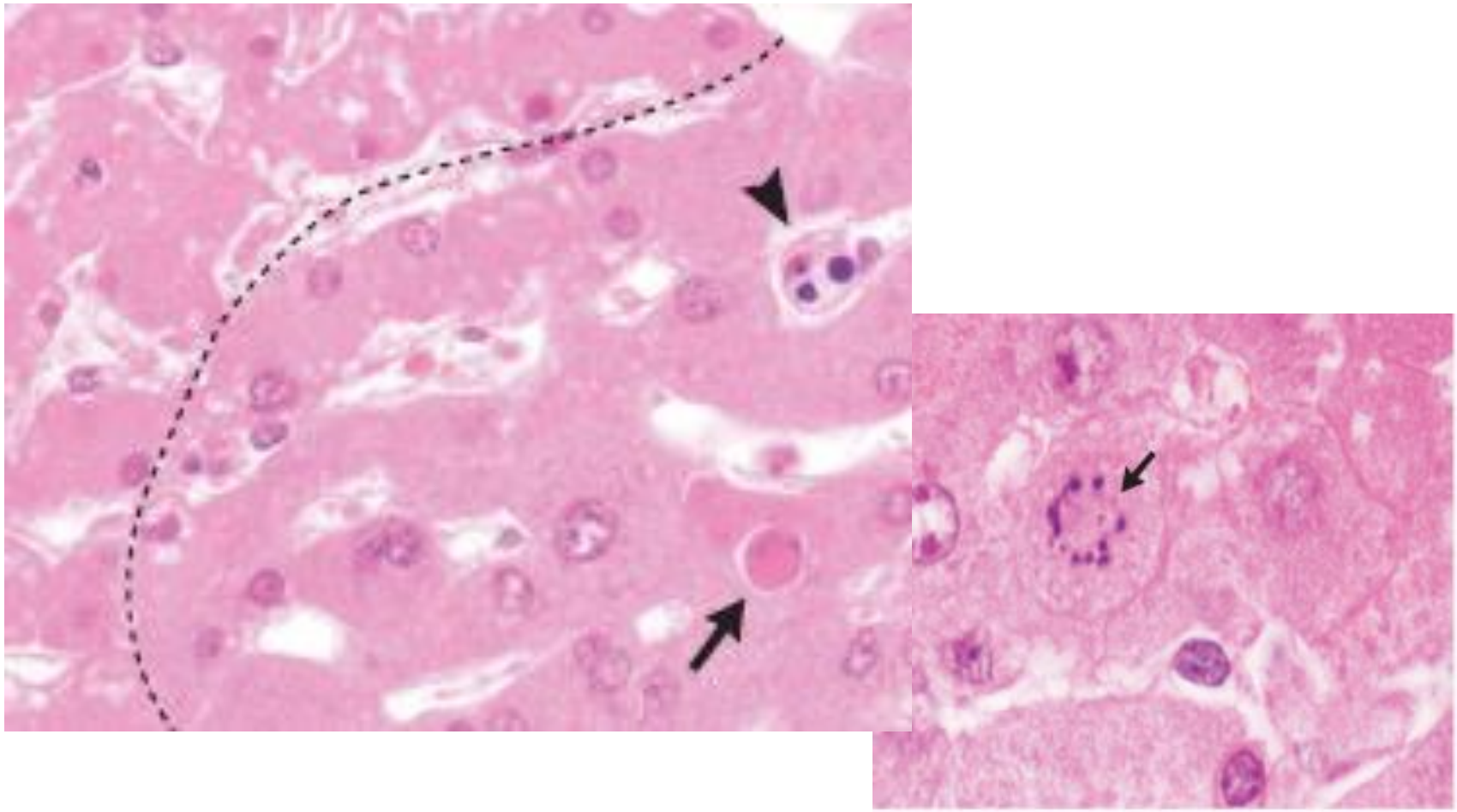
1) Deleting structures that are no longer required
   e.g. regression of the tadpole tail



2) Sculpting



Kerr, J. F., Wyllie, A. H. & Currie, A. R.
Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.
Br J Cancer 26: 239–57, **1972.**



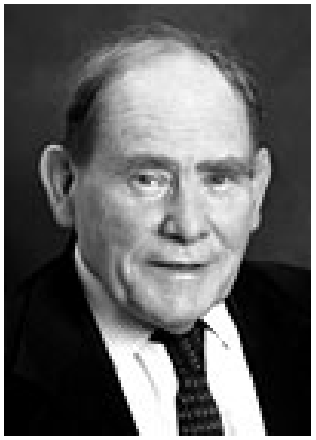
How to understand molecular mechanism of apoptosis?

Genetic approaches to identify genes control this process!

How?

The Nobel Prize in Physiology or Medicine 2002

"for their discoveries concerning 'genetic regulation of organ development and programmed cell death'"



Sydney Brenner

1/3 of the prize

United Kingdom

The Molecular Sciences Institute
Berkeley, CA, USA

b. 1927
(in Union of South Africa)

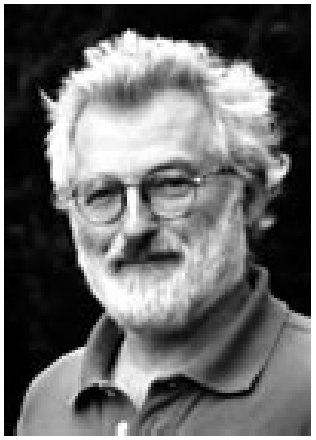
H. Robert Horvitz

1/3 of the prize

USA

Massachusetts Institute of Technology (MIT)
Cambridge, MA, USA

b. 1947



John E. Sulston

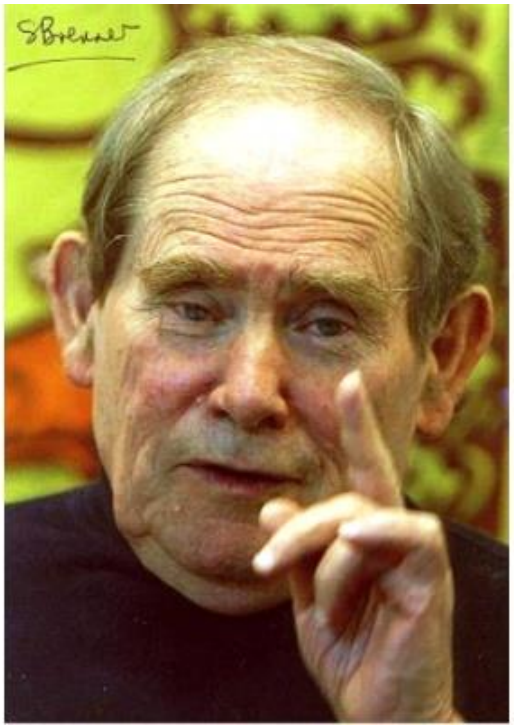
1/3 of the prize

United Kingdom

The Wellcome Trust Sanger Institute
Cambridge, United Kingdom

b. 1942

Sydney Brenner (1927 -)



- South African medical degree.
- D.Phil from Oxford.
- Work with F. Crick in molecular biology in Cambrige.

Discovery of mRNA!

Brenner S., Jacob F., Meselson M.
An unstable intermediate carrying information from genes to ribosomes for protein synthesis.

Nature 19: 576-581; 1961

Sydney Brenner
Letter to Max Perutz on 5 June, 1963

Dear Max:

"It is now widely realized that nearly all the "classical" problems of molecular biology have either been solved or will be solved in the next decade."

"I have long felt ***that the future of molecular biology*** lies in the extension of research to other fields of biology, ***notably development and the nervous system*** ....."

"As a more long term possibility, I would like to tame a small metazoan organism to study development directly. My ideas on this are still fluid and ***I cannot specify this in greater detail at the present time***."

An one page proposal to Max Perutz on 26 September, 1963

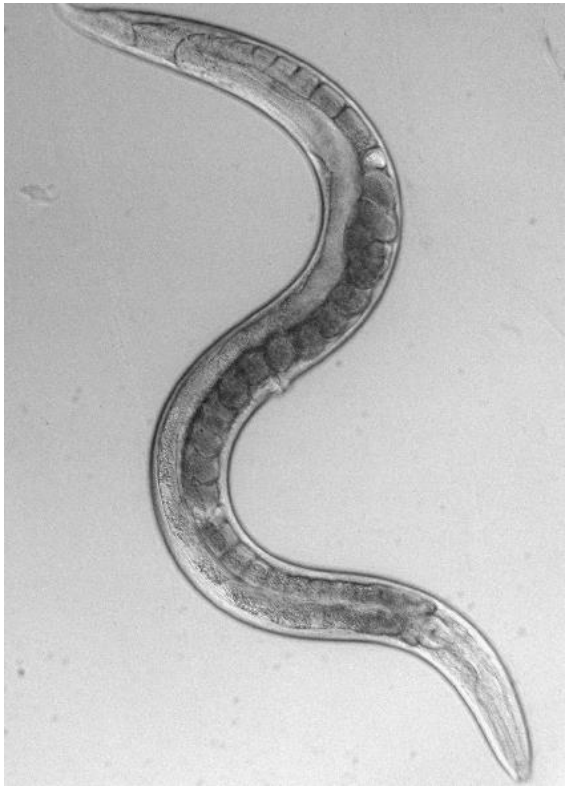
Part of the success of molecular geneics was due to the use of extremely simple organisms which could be handled in larg numbers: bacteria and bacterial viruses…….

Thus we want a multicellular organism which has a short life cycle, can be easily cultivated, and is samll enough to be handled in large numbers. It should have relatively few cells, so that exhausive studies of lineage and patterns can be made, and should be amenable to genetic analysis.

We think we have a good candidate in the form of a small nematode worm, Caenorhabditis briggsae, which has the follwing properties…

To start with we propose to identify every cell in the worm and trace lineages. We shall also investigate the constancy of development and study its control by looking for mutants.

http://elegans.swmed.edu/Sydney.html



秀麗線蟲

成蟲全身959個細胞；

身長約0.5-1mm。

以吞食洋菜膠上的細菌為生。

攝氏20度下可正常存活約20天。

在攝氏35度下可存活約

10-14小時。

照片由長庚大學生命科學系羅時成教授提供！

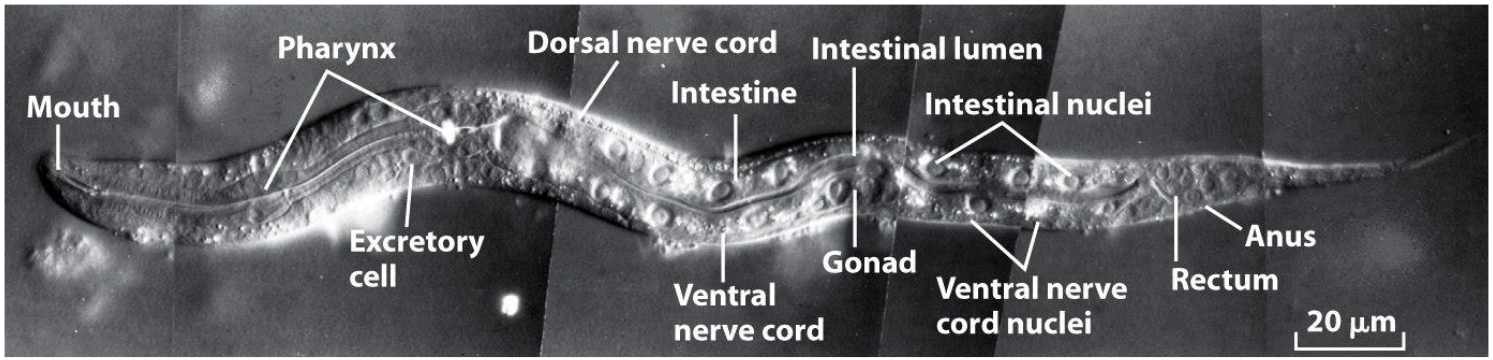


Figure 21-4
Molecular Cell Biology, Sixth Edition

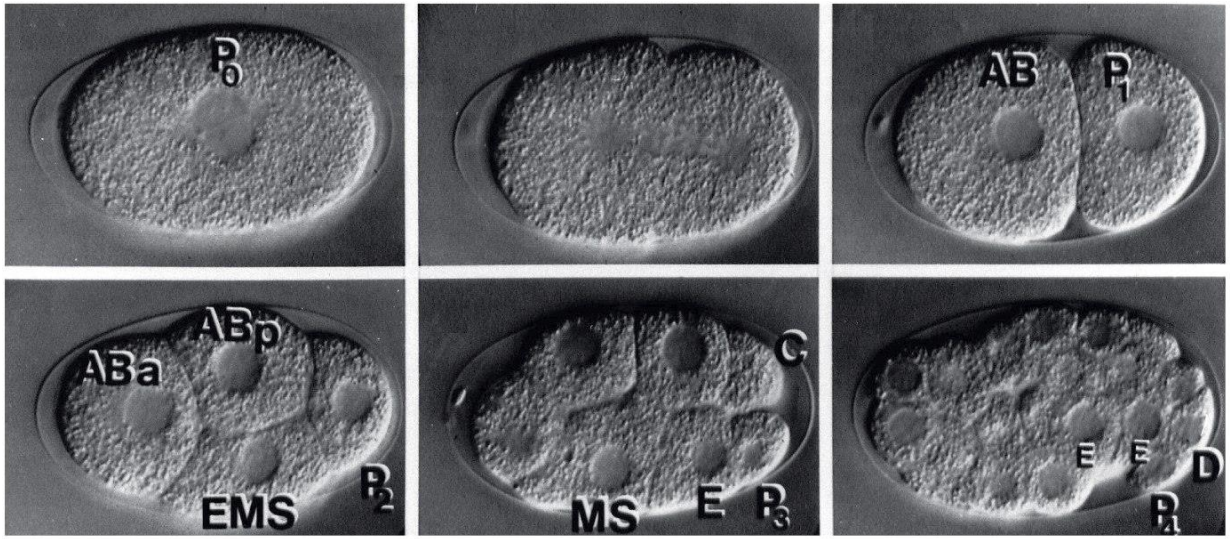


Figure 21-5b
Molecular Cell Biology, Sixth Edition

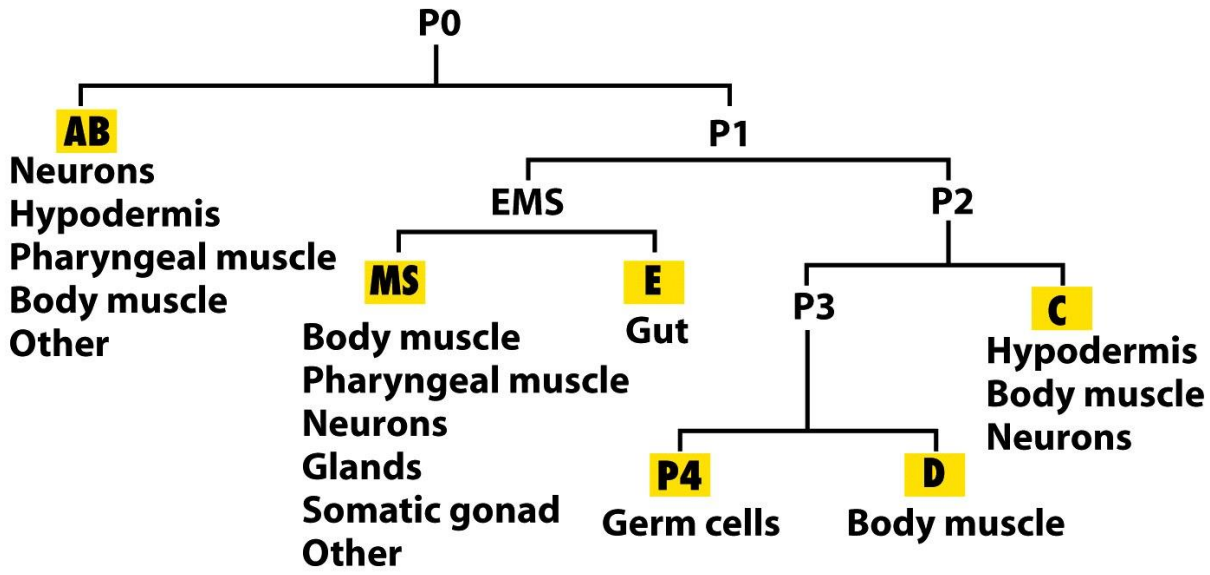
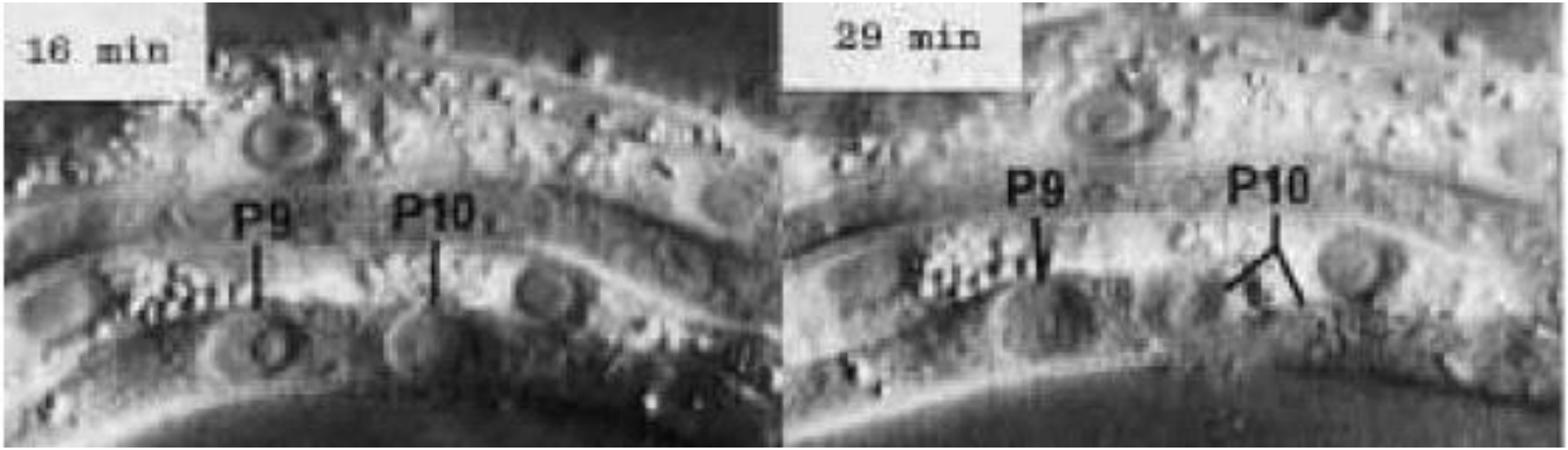
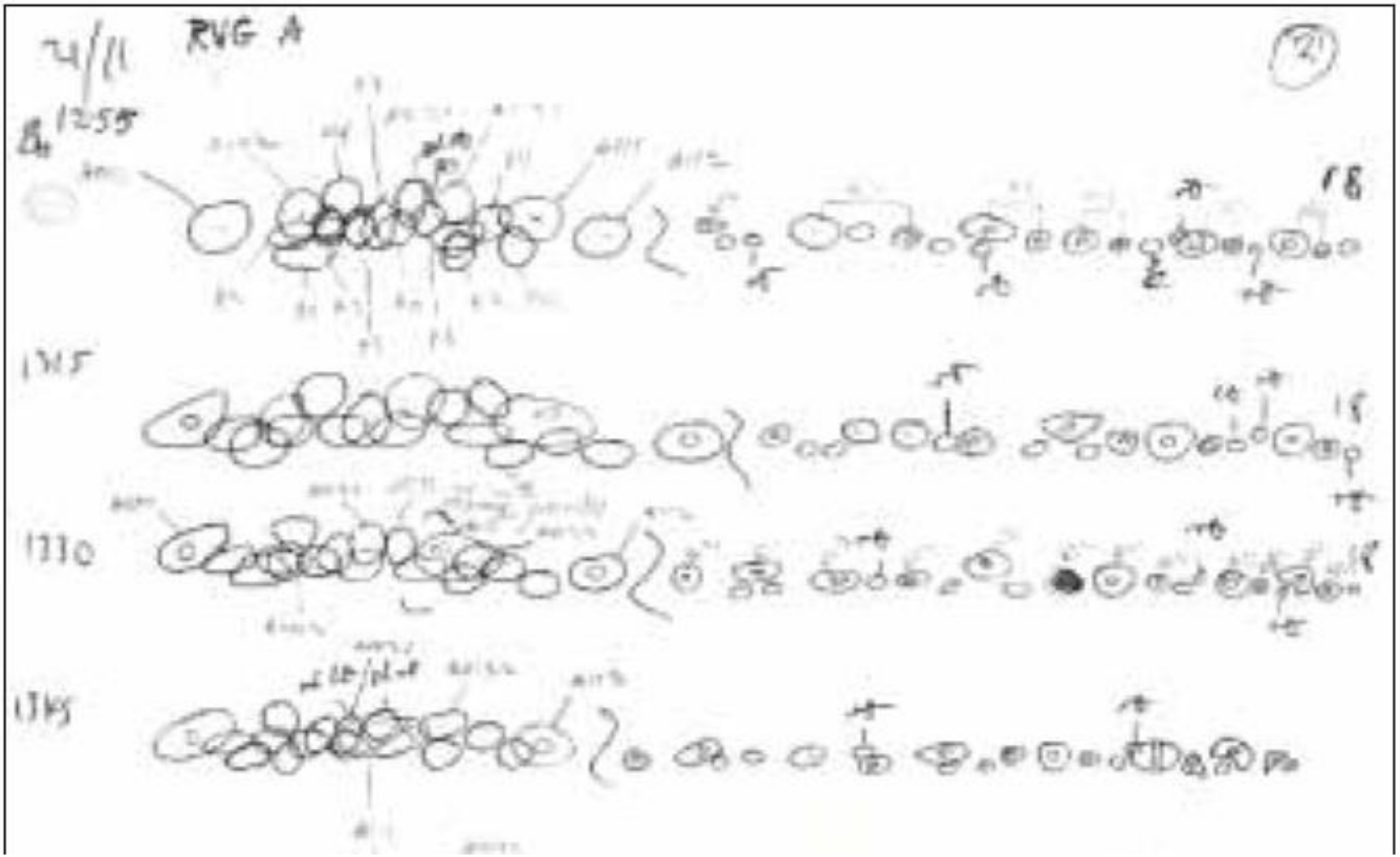


Figure 21-5a
Molecular Cell Biology, Sixth Edition



The first division of two of the ventral cord neuroblasts, viewed by Nomarski optics in a living animal.

[Sulston and Horvitz, 1977].



Drawings recording neuroblast divisions over a two hour period in the anterior ventral cord and retrovesicular ganglion, 1974

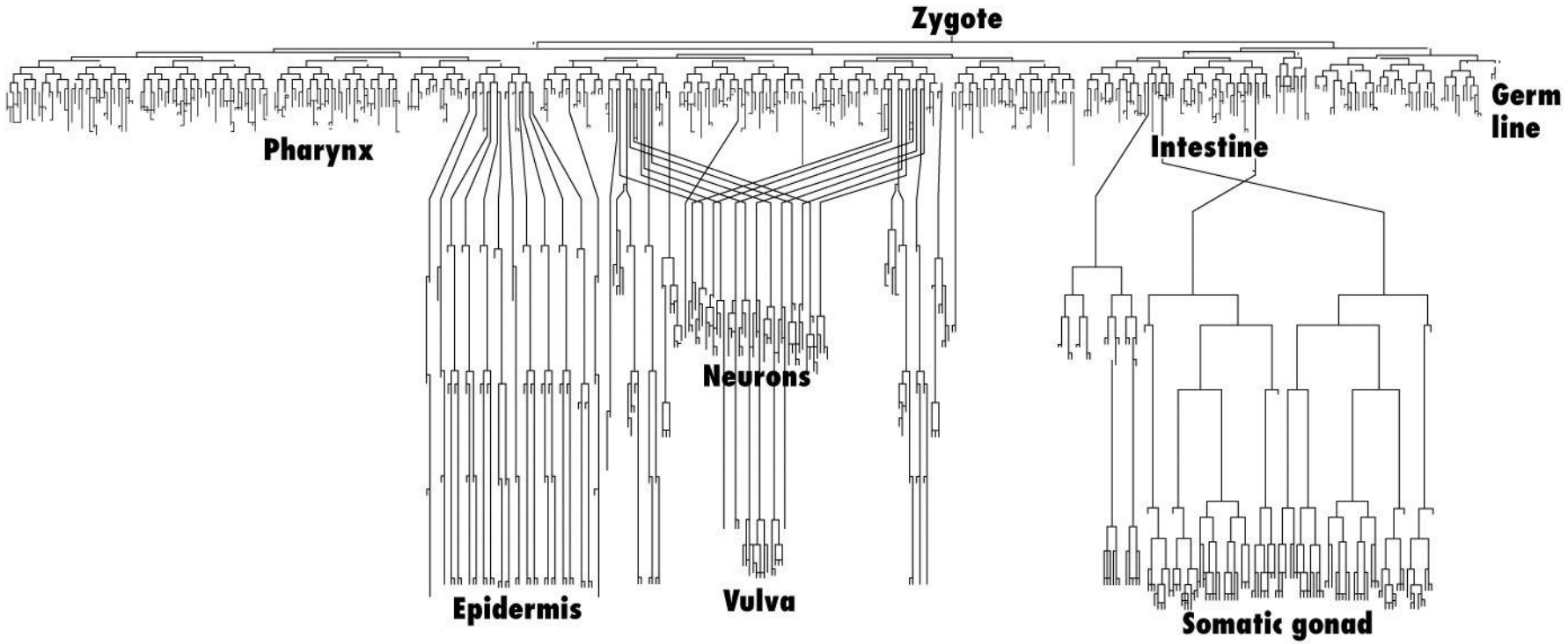
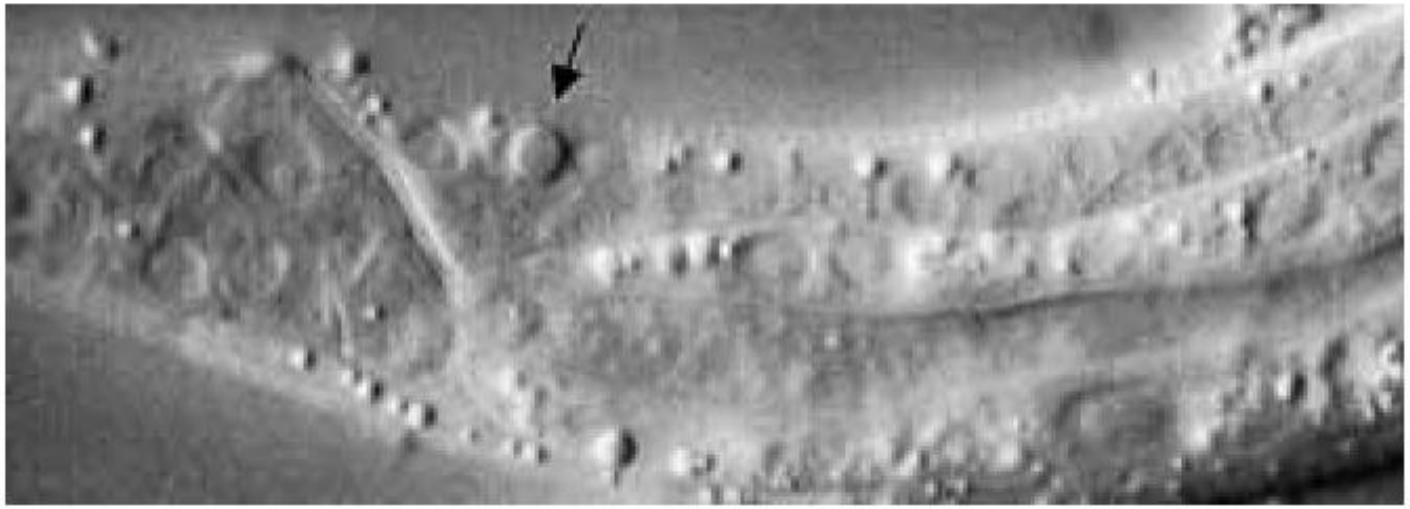
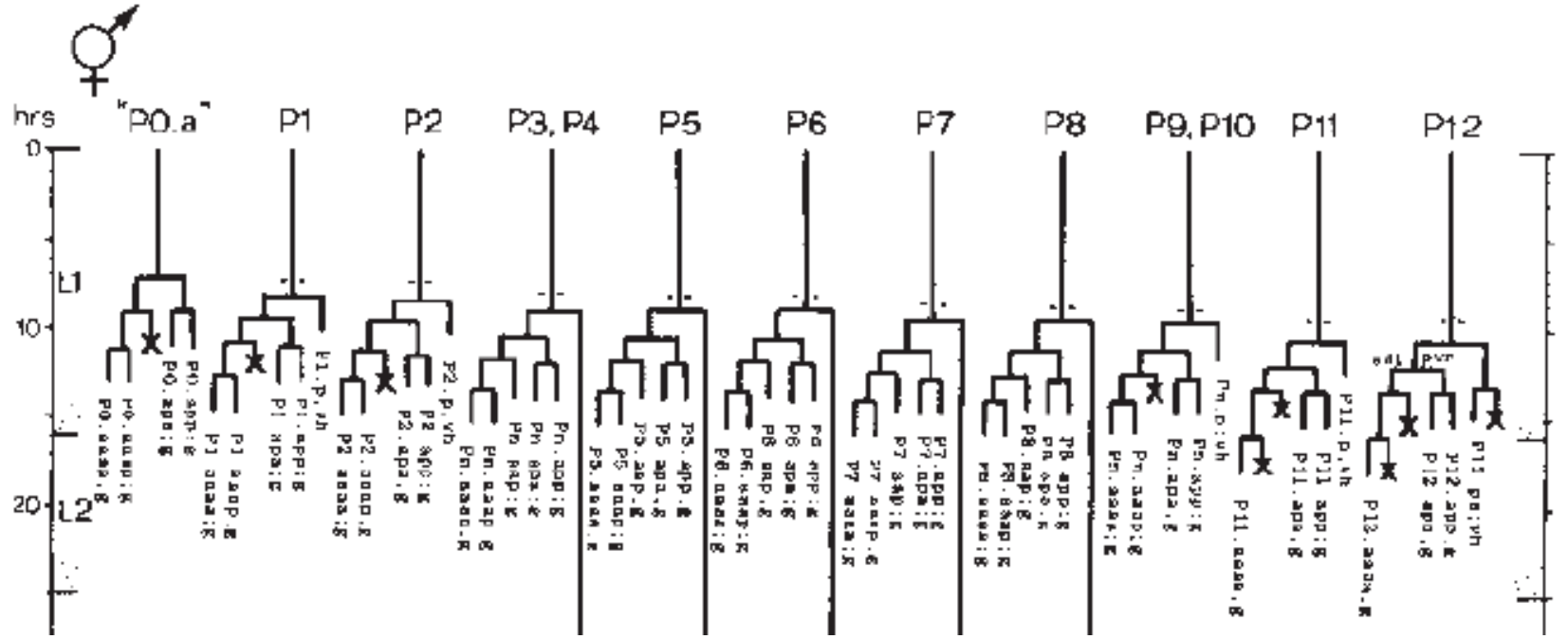


Figure 21-5c
Molecular Cell Biology, Sixth Edition

In addition to the 959 cells generated during worm development and found in the adult, another 131 cells are generated but are not present in the adult!



The arrow points to a programmed cell death as it passes through its peak refractivity; posterior end of the cord, Nomarski optics.



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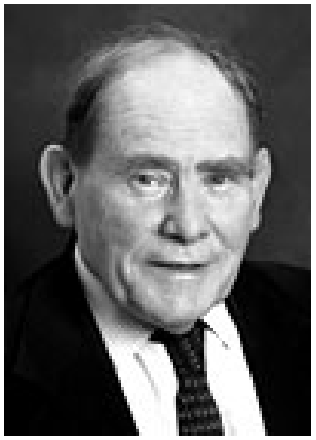

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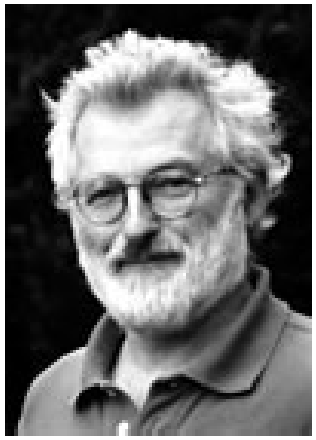

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1/3 of the prize

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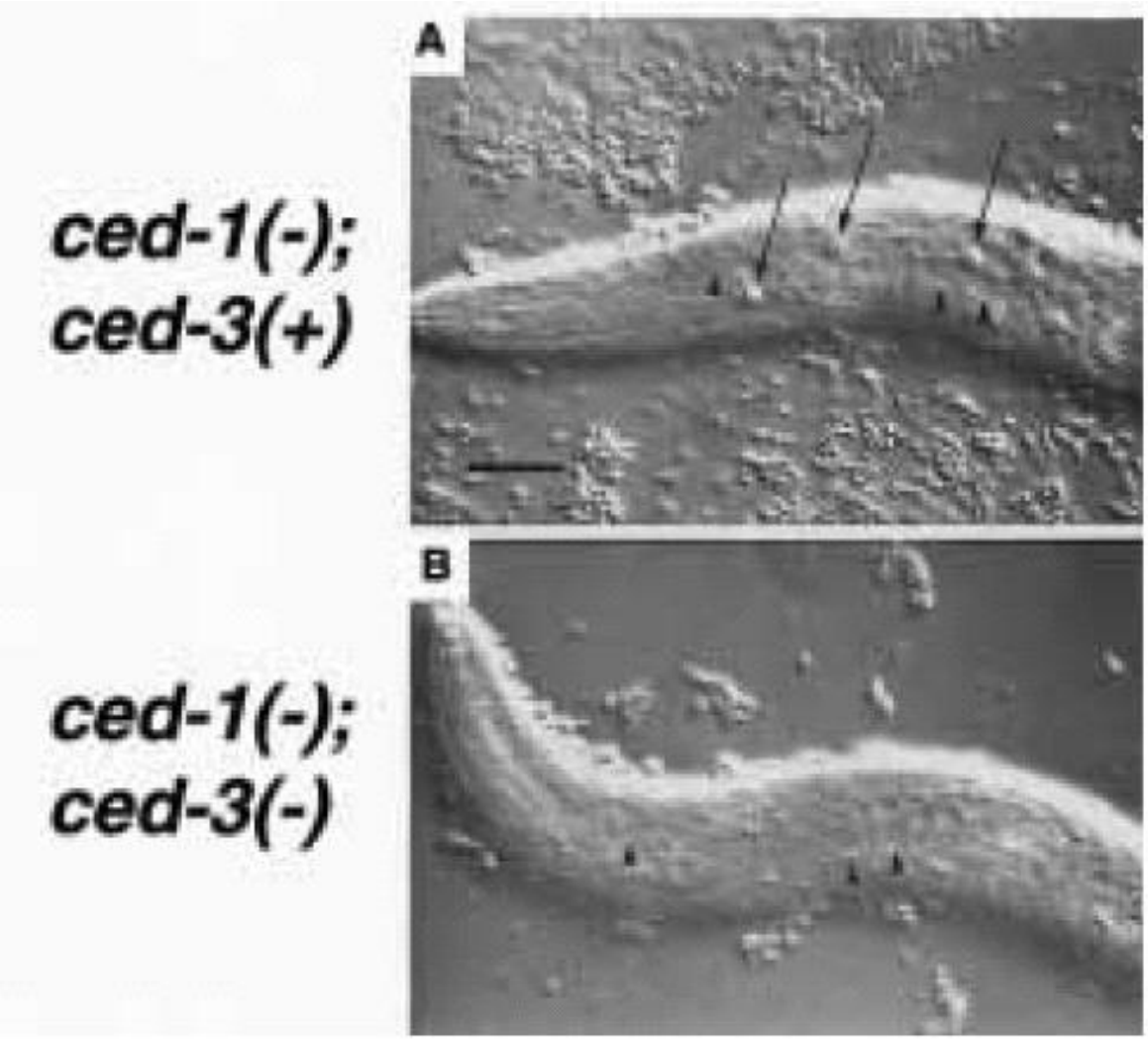
The Wellcome Trust Sanger Institute
Cambridge, United Kingdom

b. 1942

Programmed cell death was already known,
but now in the worm it was
both *visible and predictable*.

Is this a gene regulated phenomenon?

Discovery of ced-3 as the first gene known to actually control cell death!



Ellis, H. M. & Horvitz, H. R. **1986.** Genetic control of programmed cell death in the nematode *C. elegans*. ***Cell*** 44: 817–829**.**

If *ced-3* activity is reduced or eliminated by mutation, essentially all 131 cells that normally die instead survive!
What is *ced-3* doing during cell death?

However, ced3 is a novel gene
until…

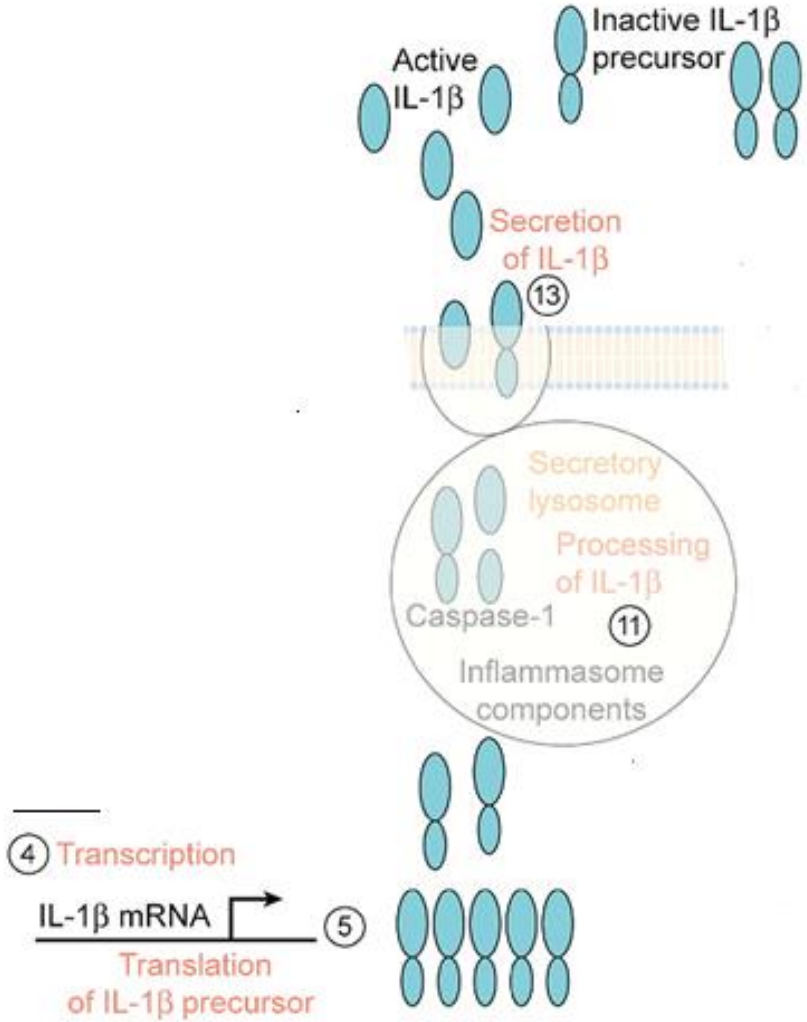
```
Query=  TITLE CED-3_PROTEIN.TXT;2
        (503 residues)

Database:  Non-redundant SwissProt+PIR+GenPept+GPUpdate, 4:54 AM EDT Apr 27,
           1992
           65,370 sequences; 18,314,537 total residues.
Searching.....................................................done
```

Sequences producing High-scoring Segment Pairs:		High Score	Smallest Poisson Probability P(N)	N
GPU:HUMIL1BCE_1	Homo sapien interleukin-1 beta convertase...	97	1.4e-14	2
GP:VE2_HPV47	E2 PROTEIN. >PIR:W2WL47 E2 protein - Huma...	56	0.019	2
PIR:A35419	*Neutrophil protein - Pig (fragment) >GP:...	58	0.021	2
SP:PDXJ_ECOLI	PYRIDOXAL PHOSPHATE BIOSYNTHETIC PROTEIN ...	66	0.28	1
GP:CHKPR264_1	G.gallus PR264 mRNA >GPU:CHKPR264_1 G.gal...	62	0.72	1
GP:HUMPR264_1	H.sapiens PR264 mRNA >GPU:HUMPR264_1 H.sa...	62	0.72	1
GPU:HUMSC35A_1	Human splicing factor SC35 mRNA, complete...	62	0.72	1
SP:POLG_SVDVH	GENOME POLYPROTEIN (COAT PROTEINS VP1 TO ...	56	1.0	1
SP:POLG_SVDVU	GENOME POLYPROTEIN (COAT PROTEINS VP1 TO ...	56	1.0	1
GP:SVDSVDV_11	Swine vesicular disease virus complete ge...	56	1.0	1

```
>GPU:HUMIL1BCE_1 Homo sapien interleukin-1 beta convertase (IL1BCE) mRNA,
           complete cds.
           Length = 404
```

Steps in the processing and release of IL-1 induced by IL-1.



Charles A. Dinarello Blood 2011;117:3720-3732

IL-1 converting enzyme (ICE)
Caspases (**c**ysteine-dependent **asp**artate-directed prote**ases**)

Searching for more regulatory genes of apoptosis

	HSN present?	Egg-laying defective?
wild type	yes	no
egl-1	no	yes
ced-3; egl-1	yes	no

A very efficient way to isolate more ced-3-like mutants:

Look for mutations that suppress the egg-laying defect of egl-1 mutants.

Ced-4 was isolated!

Ced4 is also a novel gene!

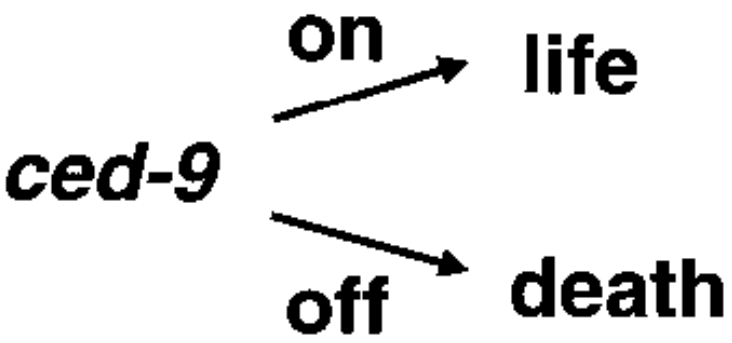
More mutants were isolated, most of them are defective in *ced-3* or *ced-4*.

But one (ced-9) was different.

It's a gain of function mutation!

C. elegans cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene bcl-2.

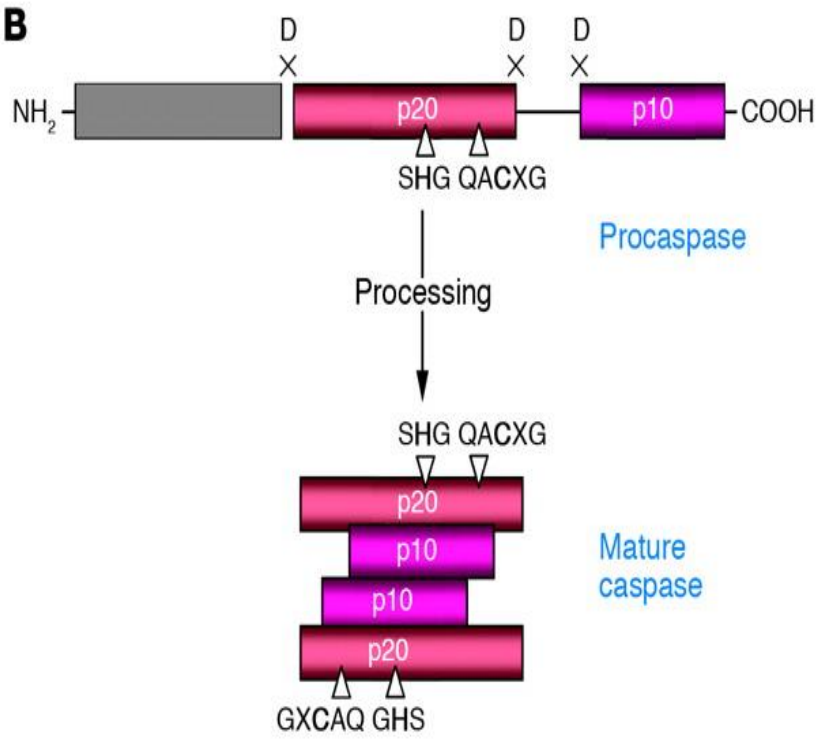
Cell 76: 665–676; 1994.

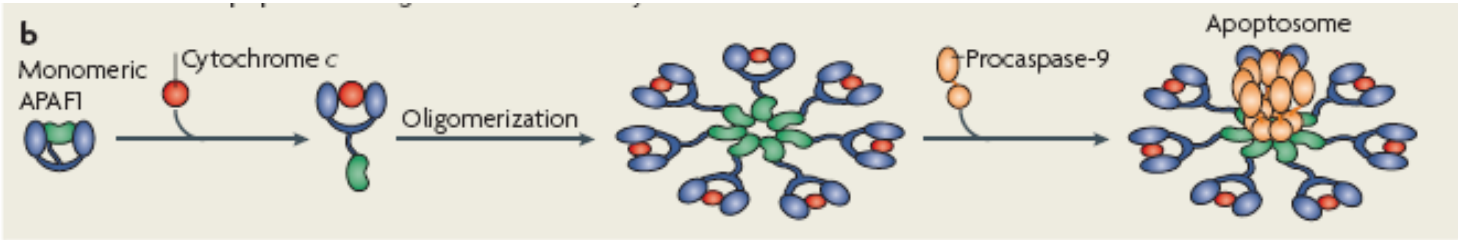


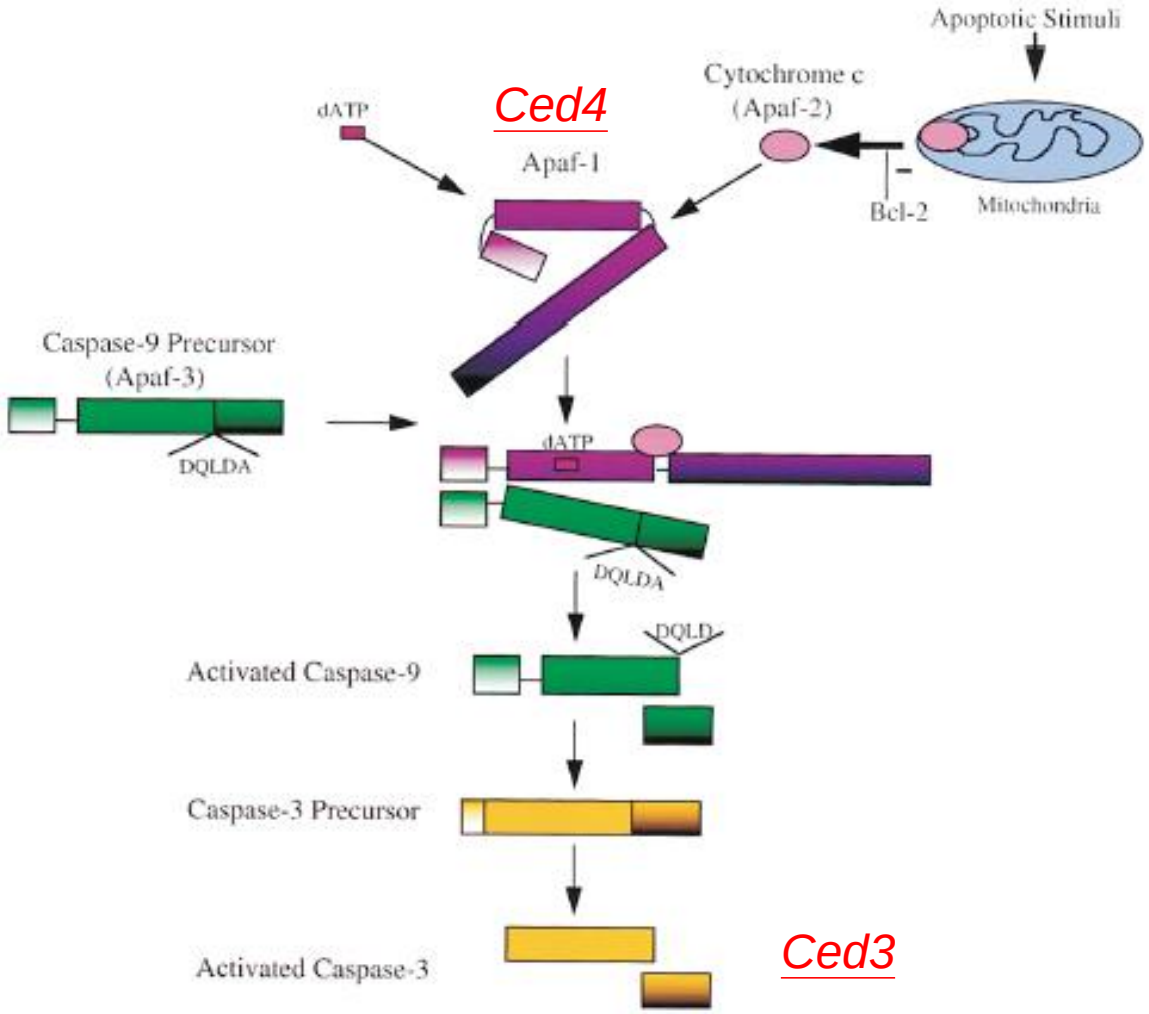
Caspases (Cysteine-dependent ASPartyl-specific proteASE) requires zymogen activation to become active.

What cellular signal to initiate caspase activation and the process of apoptosis?

Set an assay to isolate cellular component can activate Ced3 in vitro (Wang's work)




b
Monomeric
APAF1
Cytochrome c
Oligomerization
Procaspase-9
Apoptosome


Apoptotic Stimuli
Cytochrome c
(Apaf-2)
dATP
Ced4
Apaf-1
Bcl-2
Mitochondria
Caspase-9 Precursor
(Apaf-3)
DQLDA
dATP
DQLDA
DQLD
Activated Caspase-9
Caspase-3 Precursor
Activated Caspase-3
Ced3

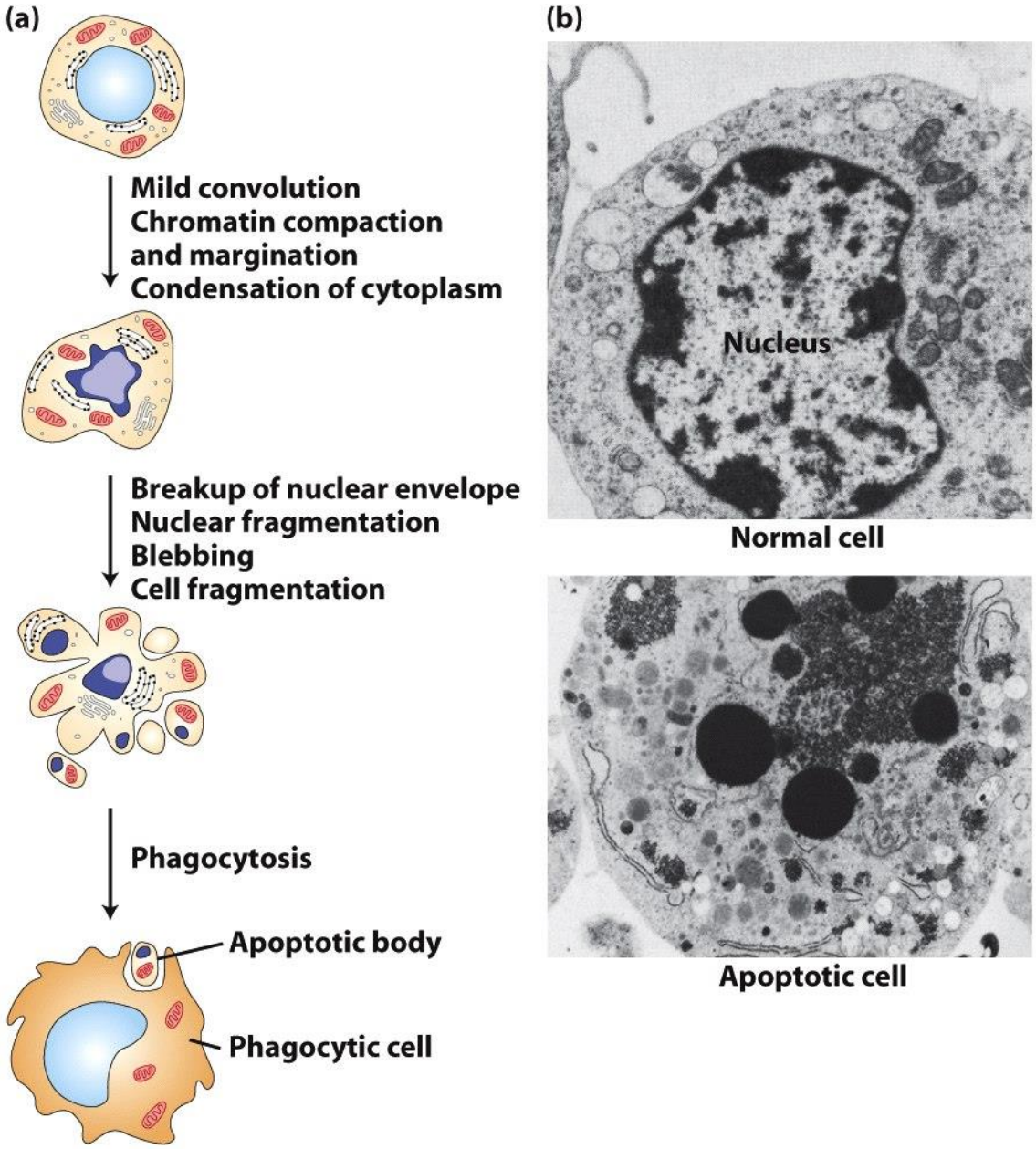
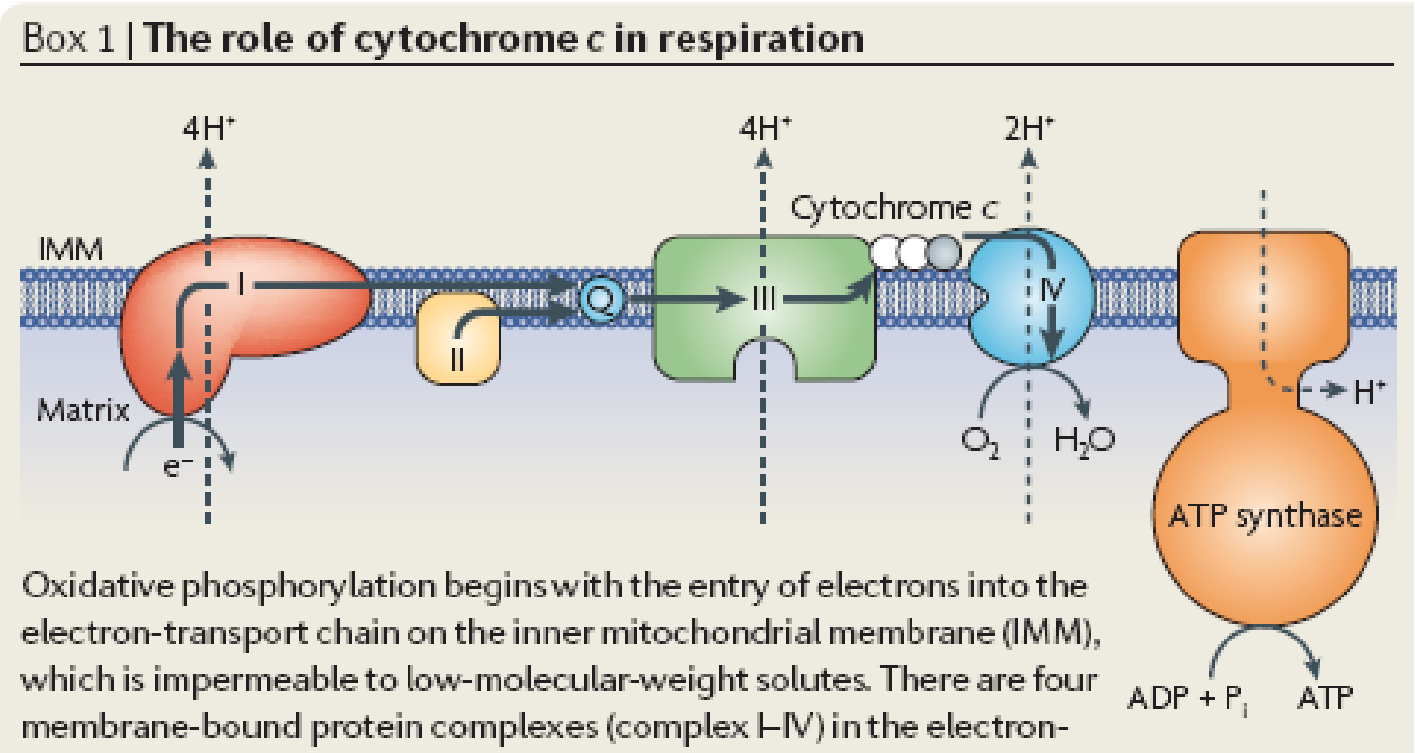
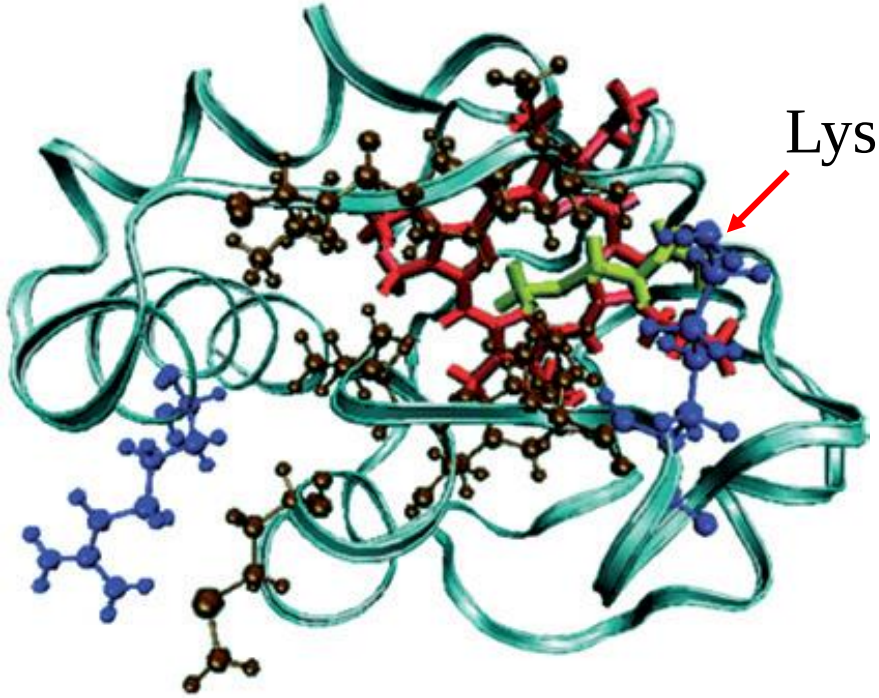
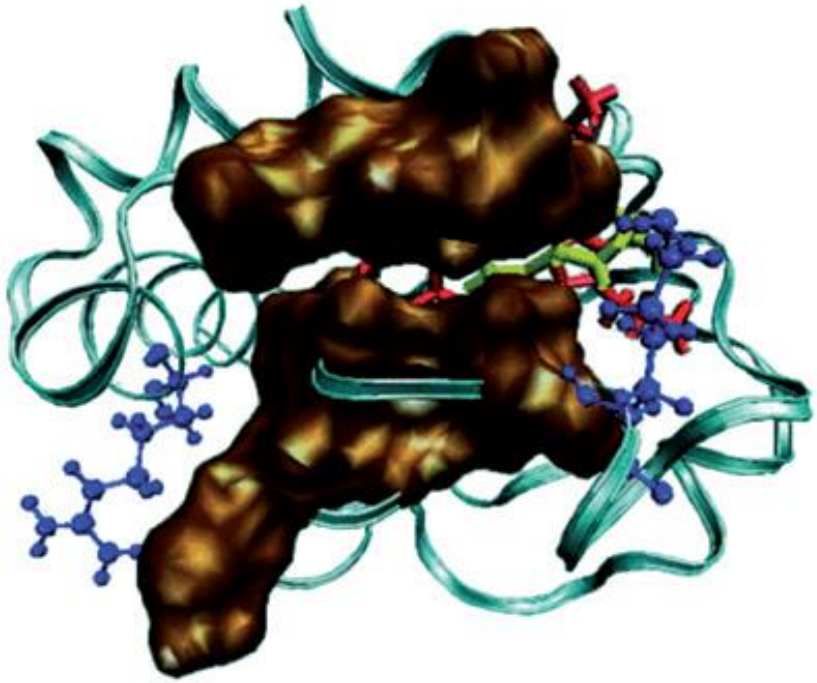


Figure 21-33
Molecular Cell Biology, Sixth Edition

How to demonstrate that the cytochrome C indeed plays a key role in apoptosis?





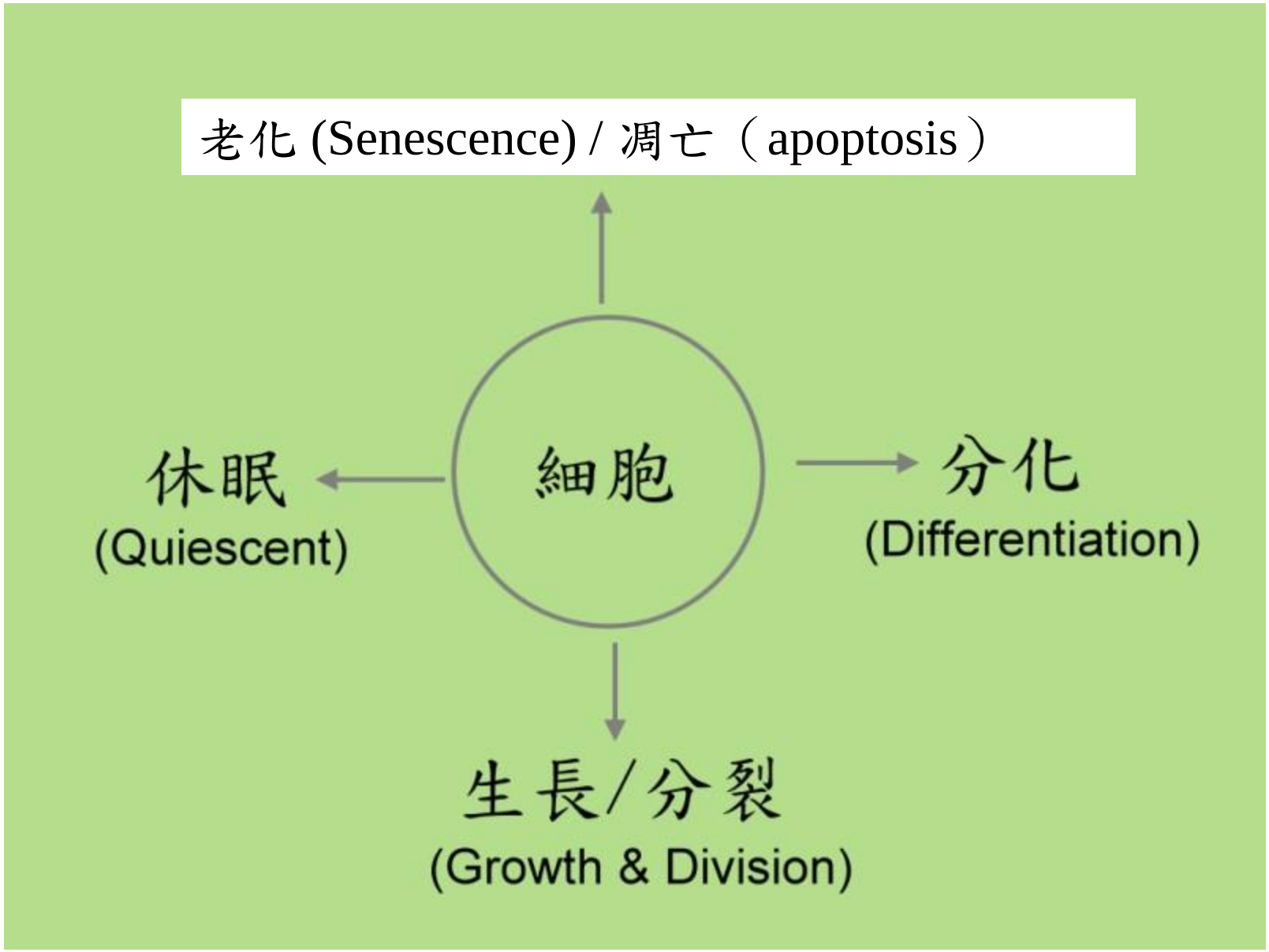


Lys72 is essential for the stability of the cytochrome *c*–APAF1 interaction, but is not essential for it's electron transport ability.

.

To mutate lys72 in cytochrome C and make a knock-in mouse carries such mutated cytochrome C

Specific Ablation of the Apoptotic Functions of Cytochrome c Reveals a Differential Requirement for Cytochrome c and Apaf-1 in Apoptosis
Cell *121*, 579–591; 2005


老化 (Senescence) / 凋亡（apoptosis）
休眠
(Quiescent)
細胞
分化
(Differentiation)
生長/分裂
(Growth & Division)

細胞會老嗎？

給予充分的營養，細胞在體外能無限期的生長分裂嗎？

Experimental Cell Research **25**, *585–621 (1961)*

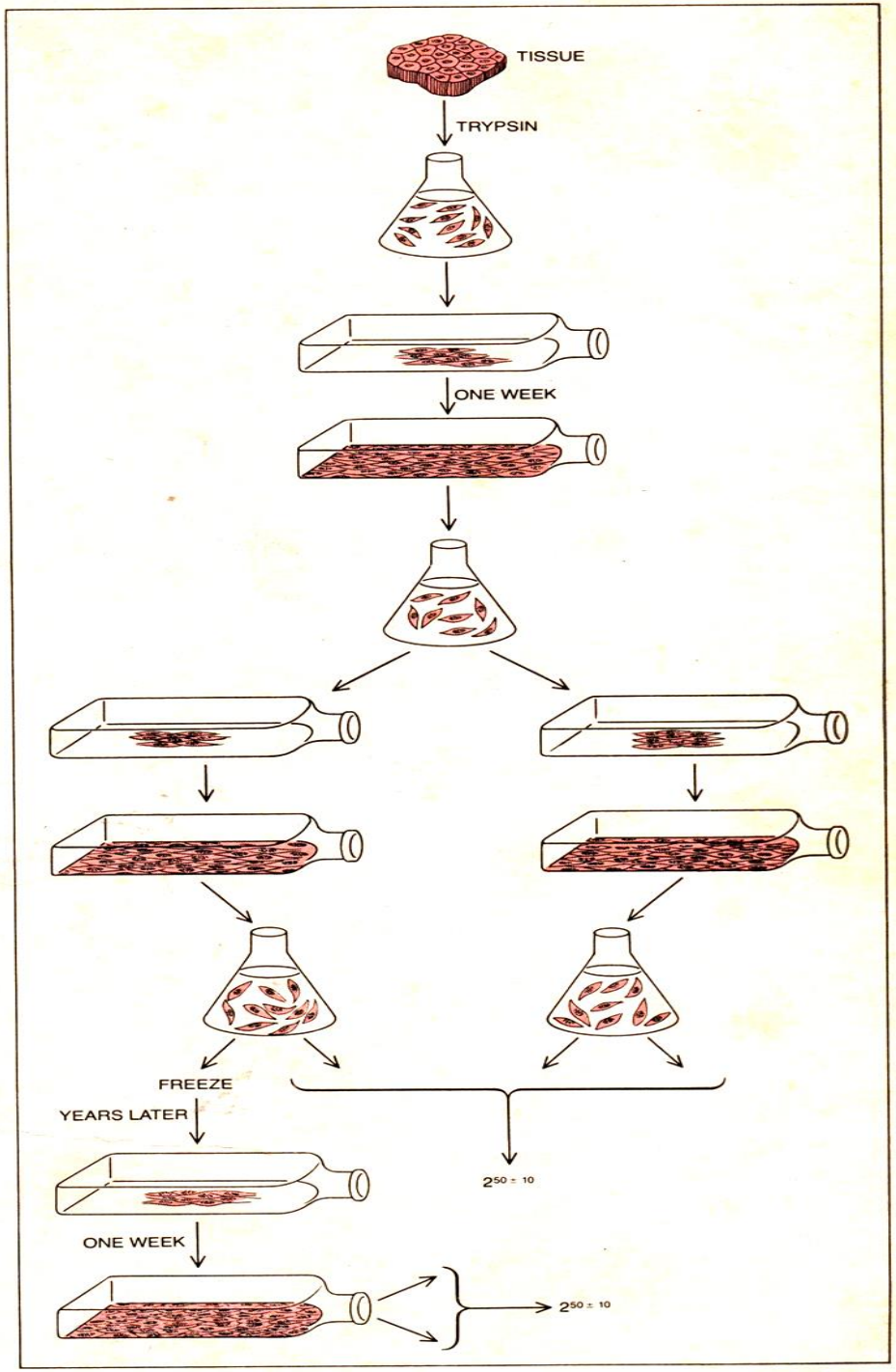
THE SERIAL CULTIVATION OF HUMAN DIPLOID CELL STRAINS[1]

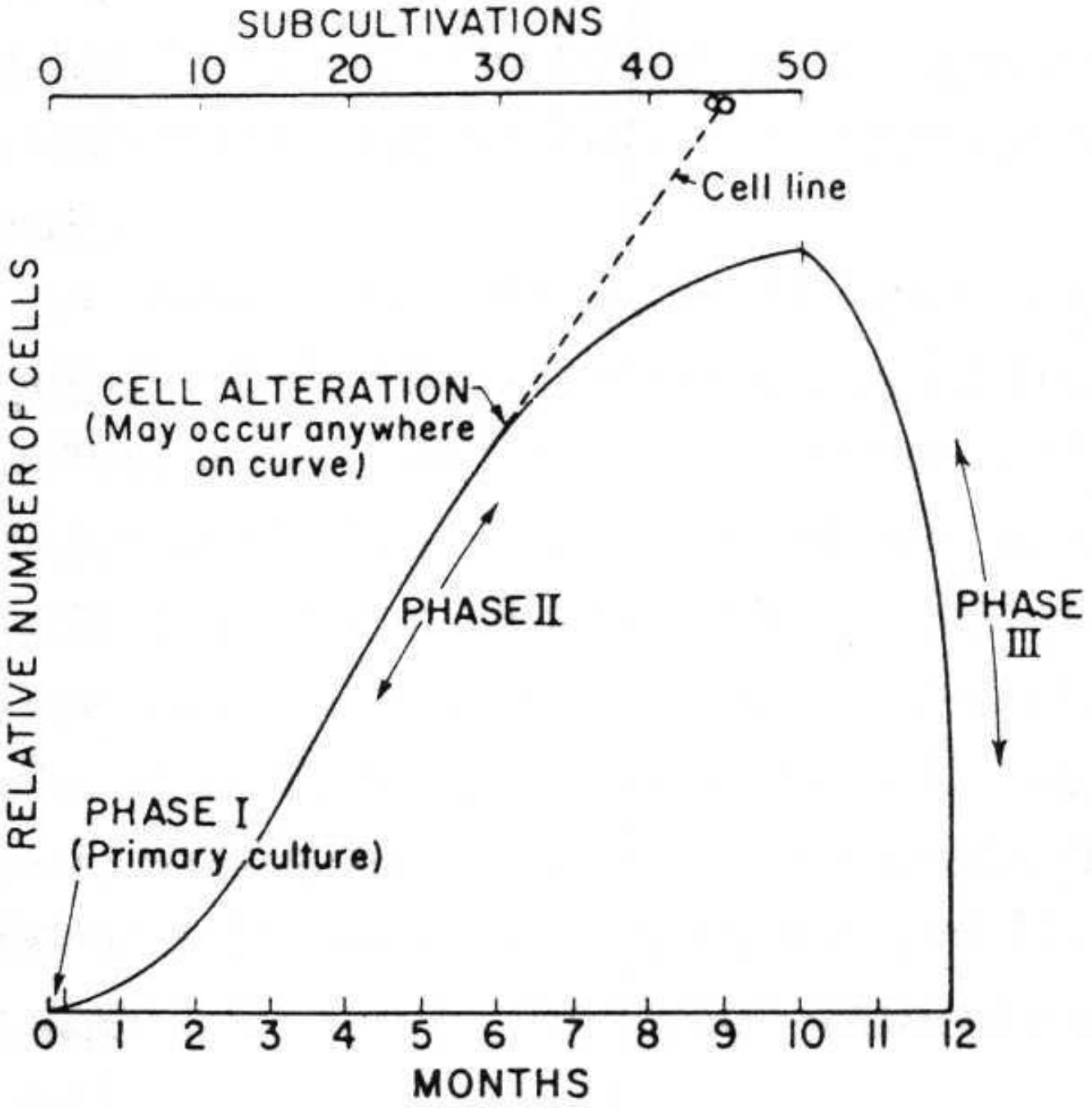
L. HAYFLICK and P. S. MOORHEAD

Wistar Institute of Anatomy and Biology, Philadelphia, Pa., U.S.A.

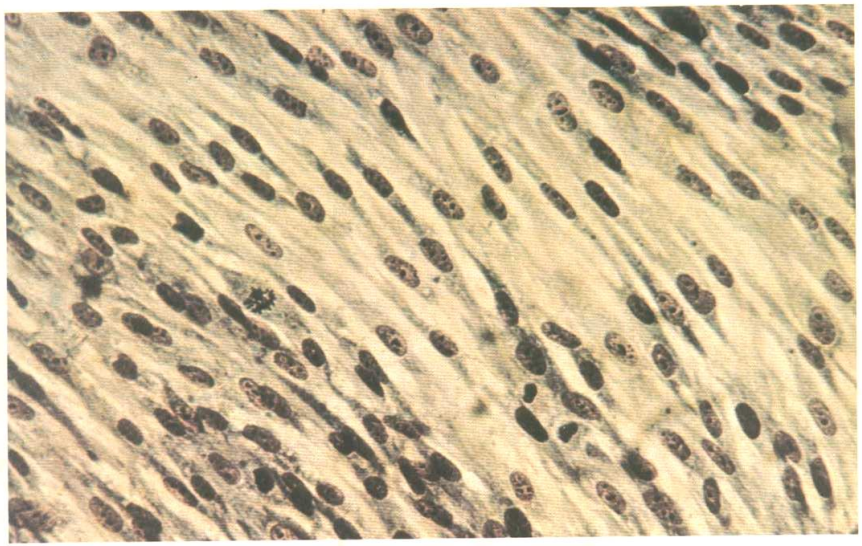
Cellular senescence

Human diploid fibroblast has limited replicative life span, termed replicative senescence or cellular senescence.
Human diploid fibroblasts can divide ~60-80 times.




SUBCULTIVATIONS
0
10
20
30
40
50
∞
Cell line
RELATIVE NUMBER OF CELLS
CELL ALTERATION
(May occur anywhere
on curve)
PHASE II
PHASE
III
PHASE I
(Primary culture)
0
1
2
3
4
5
6
7
8
9
10
11
12
MONTHS

體外培養的細胞會老。



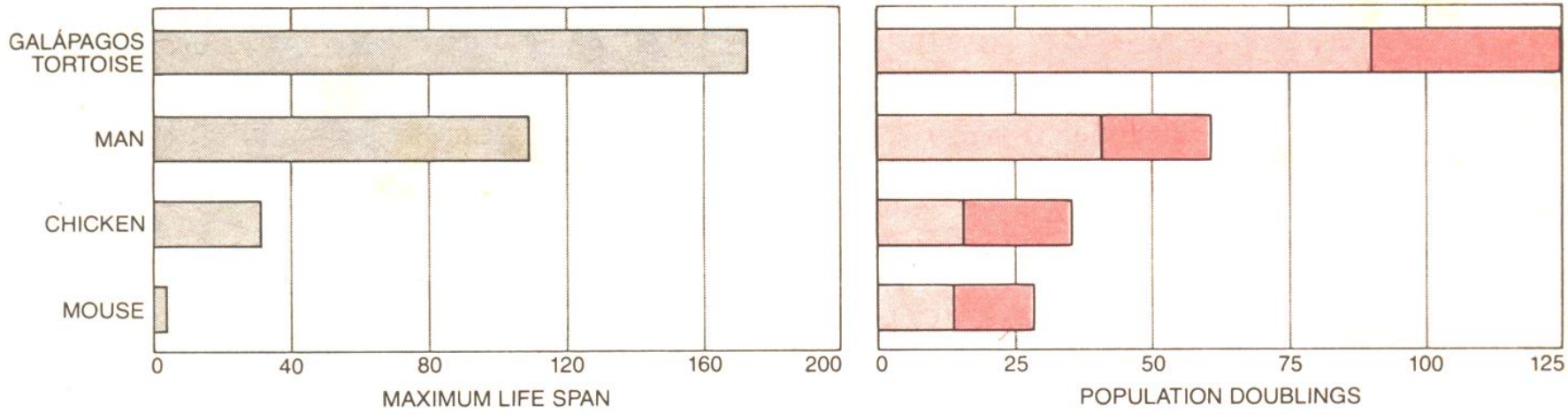
"YOUNG" FIBROBLASTS (connective-tissue cells) obtained from human fetal tissue carpet the surface of a culture dish in this photomicrograph. The spindle-shaped cells were fixed and stained during a period of active proliferation. Reddish objects are cell nuclei.

SENESCENT FIBROBLASTS were stained during the loss of division capacity that follows approximately 50 population doublings in culture. The cells undergo a variety of degenerative changes and then die. This observation suggests that aging is an innate property of normal living cells. Micrographs were made in the author's laboratory at the Children's Hospital Medical Center of Northern California.

Is cellular senescence the same as biological aging?

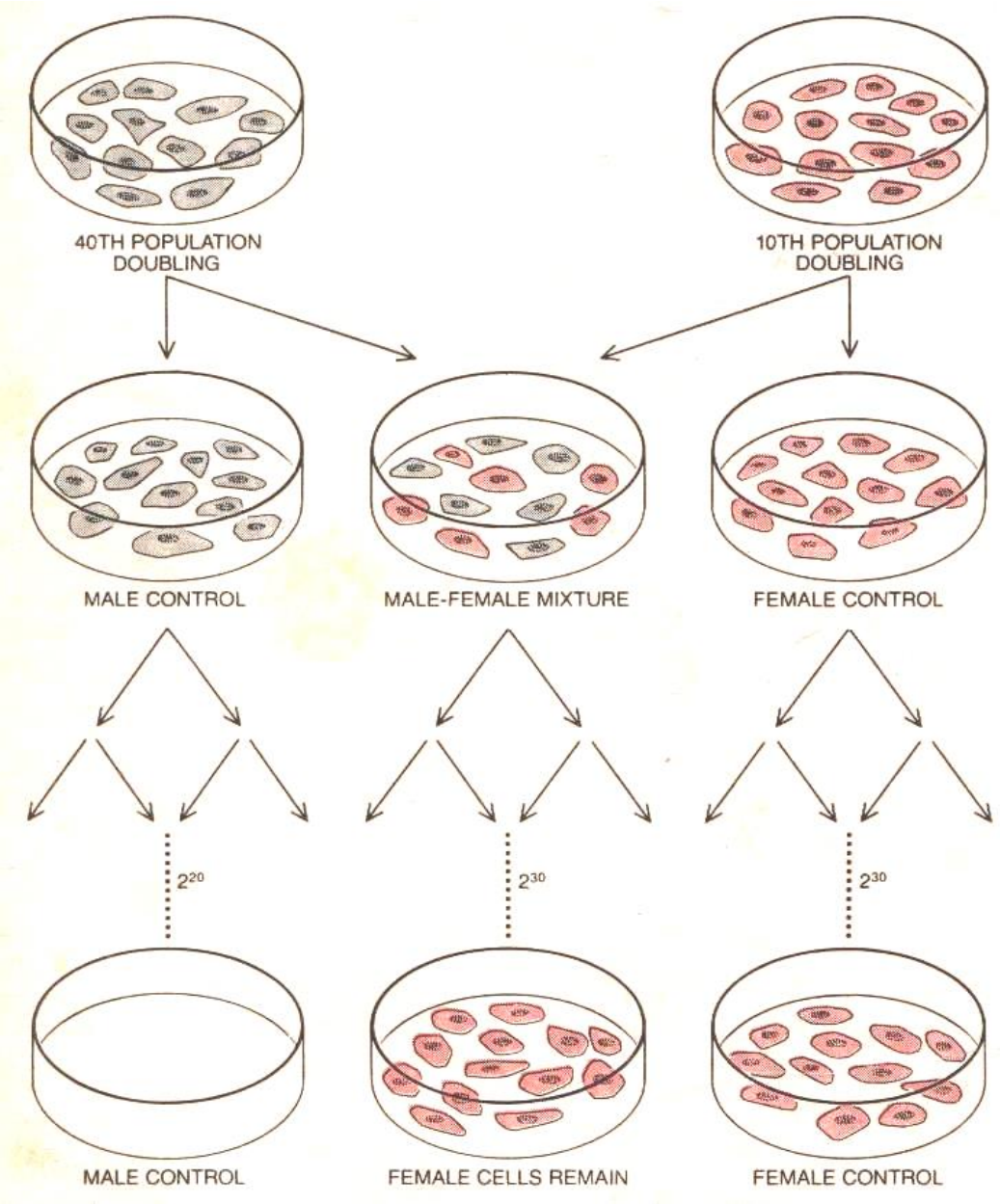
- Donor age
- Cells cultured from old donors tend to senesce after fewer population-doublings than cells from young cell.
- Interspecies comparison suggests that cell replicative life span and biological life span is genetically related.

Cells from long-lived species are capable of replicating more times



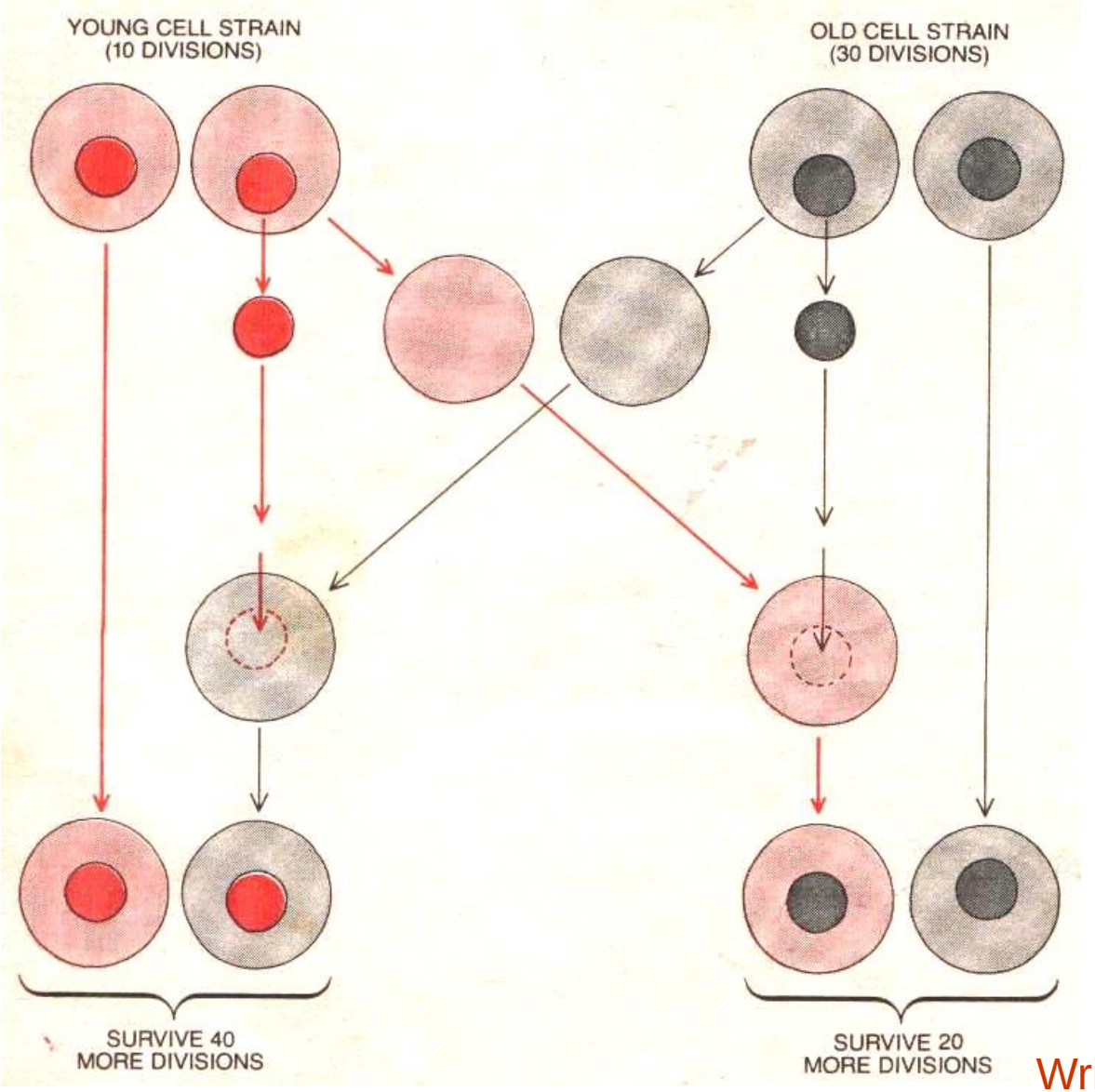
REPLICATIVE ABILITY of fibroblasts from the fetus (from the newborn animal in the case of the tortoise) and grown in culture is proportional to life span of species. Observation suggests that limited replicative ability of cultured normal cells may be correlate of aging.

Aging is an autonomous property of normal cells



Hayflick and Moorhead

Nucleus determines cellular aging

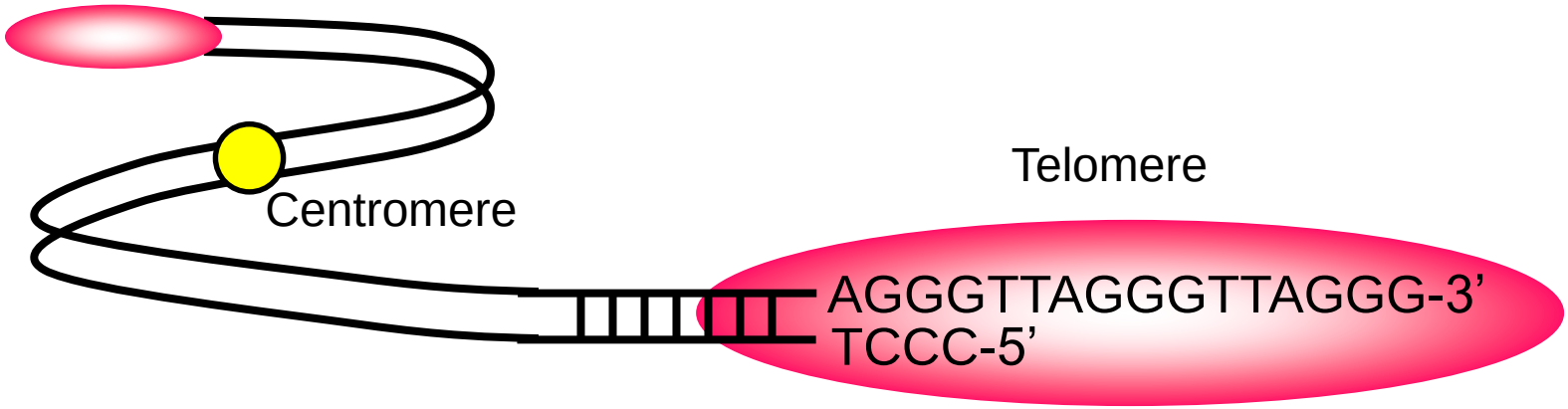


Wright and Hayflick

Why cell age (senescence) ?

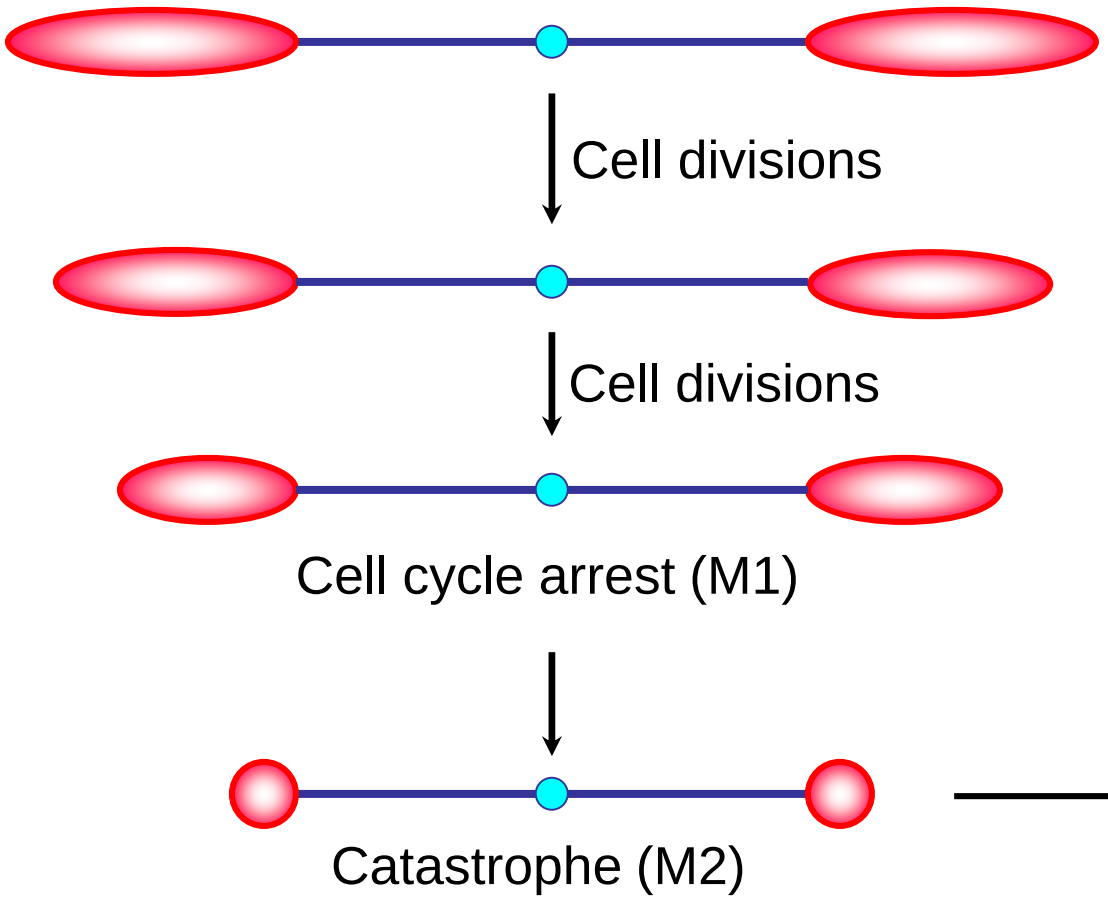
- Accumulation of damages
- Accumulation of mutations
- Shortening telemere during each replication
- Turn on a specific genetic (differentiation) program induced by “shortening telemere”

染色體端粒子(telomere)：由端粒酵素(telomerase)製造的一個特殊結構來保護染色體的端點。而端粒酵素在正常細胞中很早就消失了！



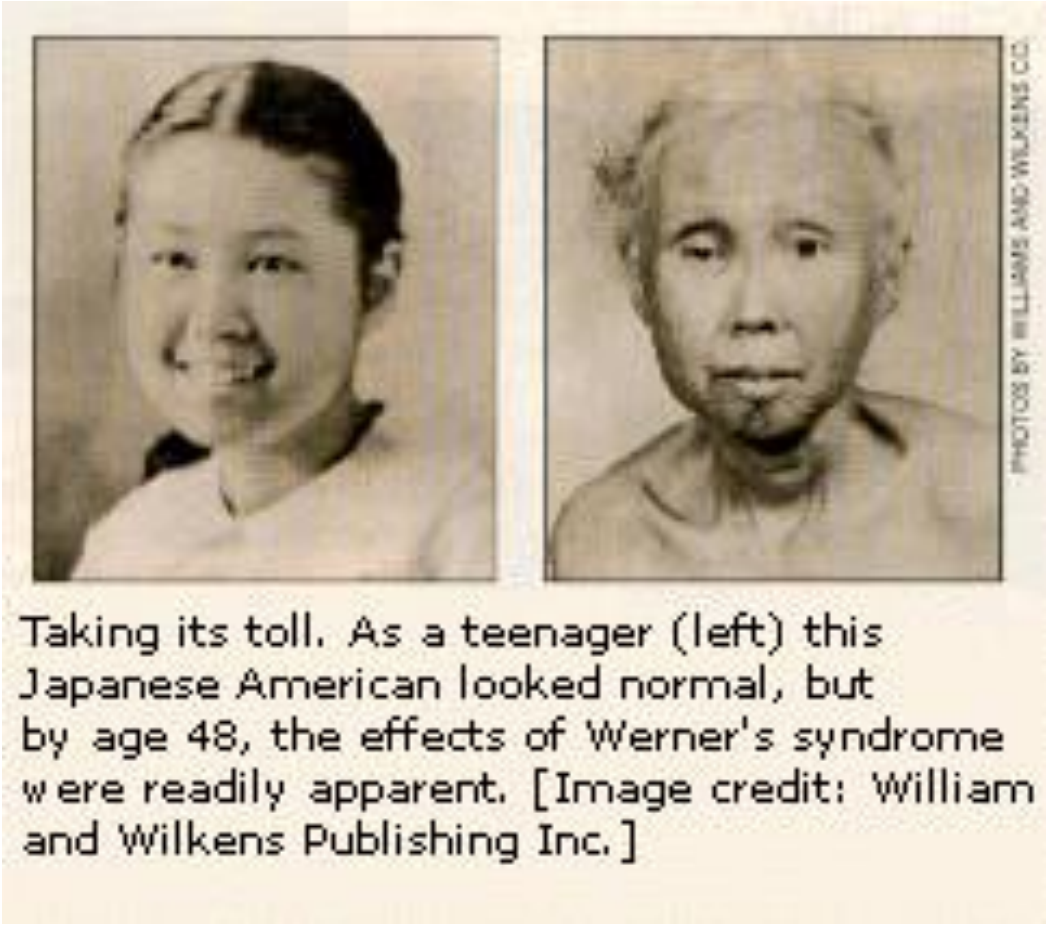
(In Greek, Telos means end; meros, part)

染色體端粒子會隨著正常細胞分裂而消耗掉

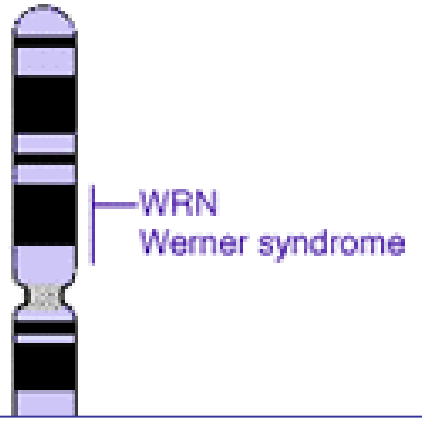


細胞逐漸失去分裂的能力(老化)，終致走向死亡！

Werner Syndrome:遺傳性的早衰症



Taking its toll. As a teenager (left) this Japanese American looked normal, but by age 48, the effects of Werner's syndrome were readily apparent. [Image credit: William and Wilkens Publishing Inc.]



nature genetics • volume 24 • january 2000 16

Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts

Fiona S. Wyllie[1]*, Christopher J. Jones[1]*, Julia W. Skinner[1], Michele F. Haughton[1], Corrin Wallis[2], David Wynford-Thomas[1], Richard G.A. Faragher[2] & David Kipling[1]

[1]Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff, UK. [2]School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft Building, Brighton, UK. Correspondence should be addressed to R.G.A.F.

它是誰？

What is the cause of Dolly's death?

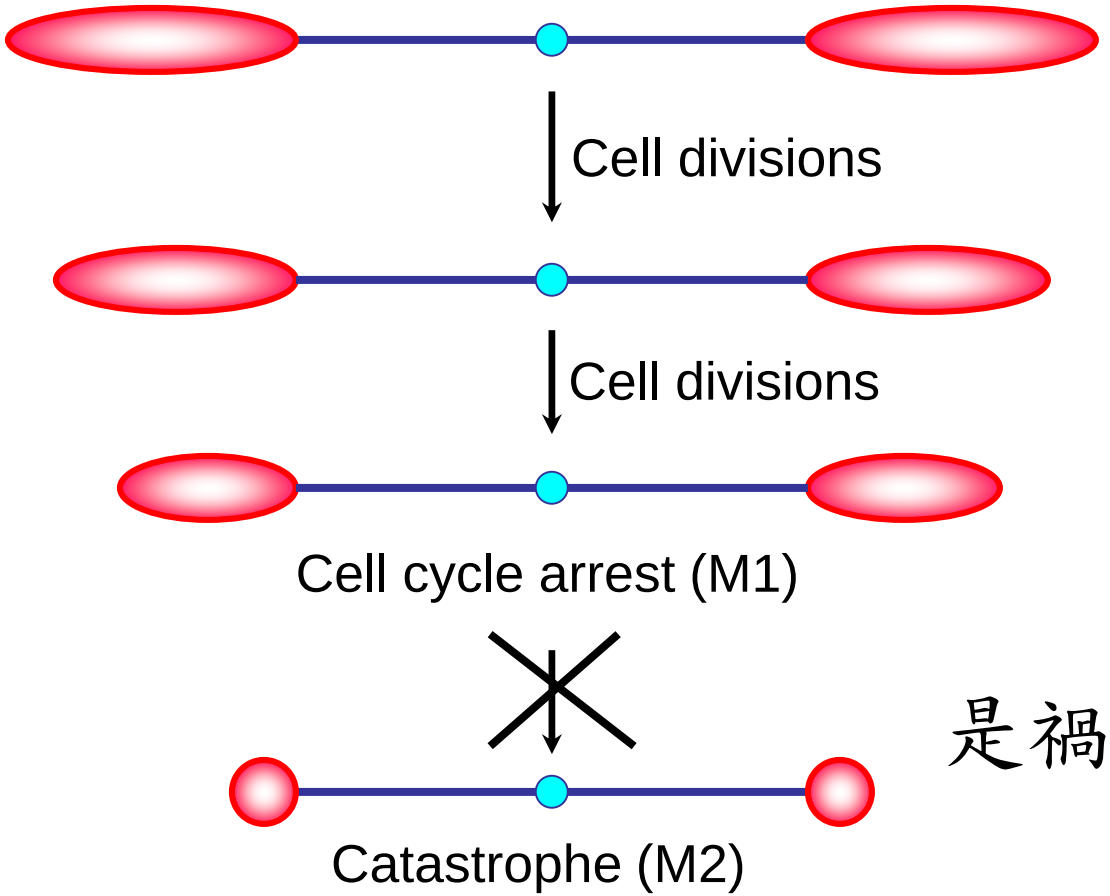
Dolly, 1997-2003

- Dolly was cloned by transferring of a nucleus from a six-year-old sheep mammary epithelial cells in 1997.
- Analysis of telomere lengths in Dolly indicates that Dolly's telomere length is similar to normal six-year-old sheep (1999).
- Dolly die on 14 Feb, 2003

Cellular Senescence

Why cell needs senescene?

細胞永生的祕密：重新活化端粒酵素?!



端粒酵素的
重新活化

是禍害？還是福氣？

Telomerase is expressed in immortal and cancer cells

SCIENCE • VOL. 266 • 23 DECEMBER 1994

Specific Association of Human Telomerase Activity with Immortal Cells and Cancer

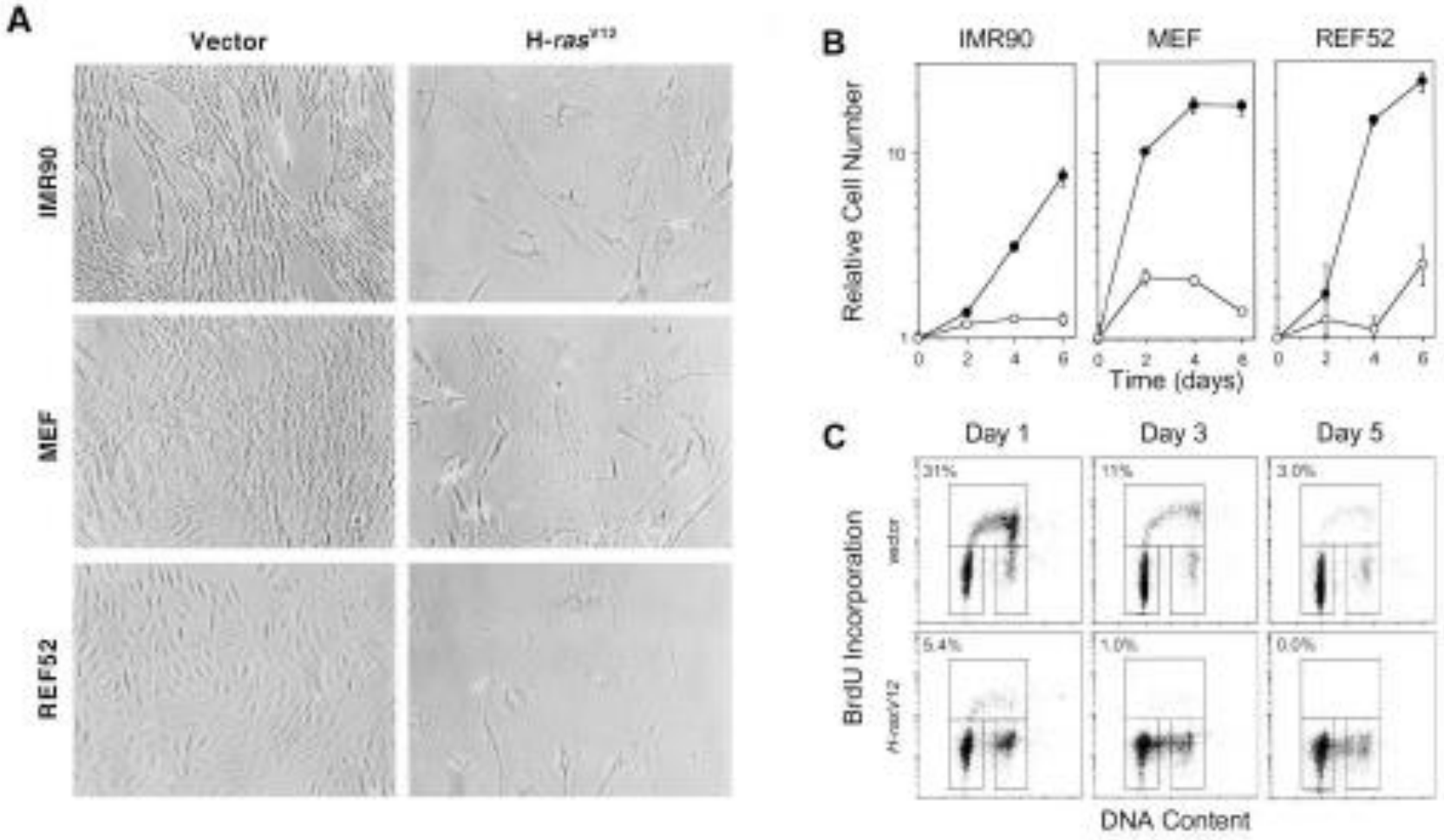
Nam W. Kim,* Mieczyslaw A. Piatyszek,* Karen R. Prowse, Calvin B. Harley, Michael D. West, Peter L. C. Ho, Gina M. Coviello, Woodring E. Wright, Scott L. Weinrich,*† Jerry W. Shay*†

Tissue type	Telomerase activity (no. positive/ no. tested)	Tissue type	Telomerase activity (no. positive/ no. tested)
Fetal testis	2/2	Normal breast tissue (from noncancer patients)	0/8
Adult testis	1/1	Prostate cancer	2/2
Fetal ovary	2/2	Prostatic intraepithelial neoplasia type 3	3/5
Ovarian follicle	1/1	Benign prostatic hyperplasia	1/10
Gastrointestinal malignancies		Normal prostatic tissue	0/8
Hepatocellular carcinoma*	1/1	Neuroblastoma	5/5
Colon cancer	8/8	Brain tumors	6/8
Adjacent colonic tissue	0/7	Lung small-cell carcinoma	4/4
Colonic tubular adenoma	0/1	Rhabdomyosarcoma	1/1
Colonic polyp	0/1	Leiomyosarcoma	3/3
Squamous cell carcinoma (head and neck)	14/16	Leiomyoma (fibroids)	0/11
Adjacent tissue	6/16	Normal myometrium	0/10
Wilms tumor	6/6	Hematological malignancies	
Adjacent kidney tissue	2/6	Acute lymphocytic leukemia	14/16
Breast cancer (ductal and lobular, node positive)	18/20	Chronic lymphocytic leukemia	2/2
Breast cancer (axillary node negative)	1/4	Lymphoma (adult)	5/5
Adjacent tissue	2/20		

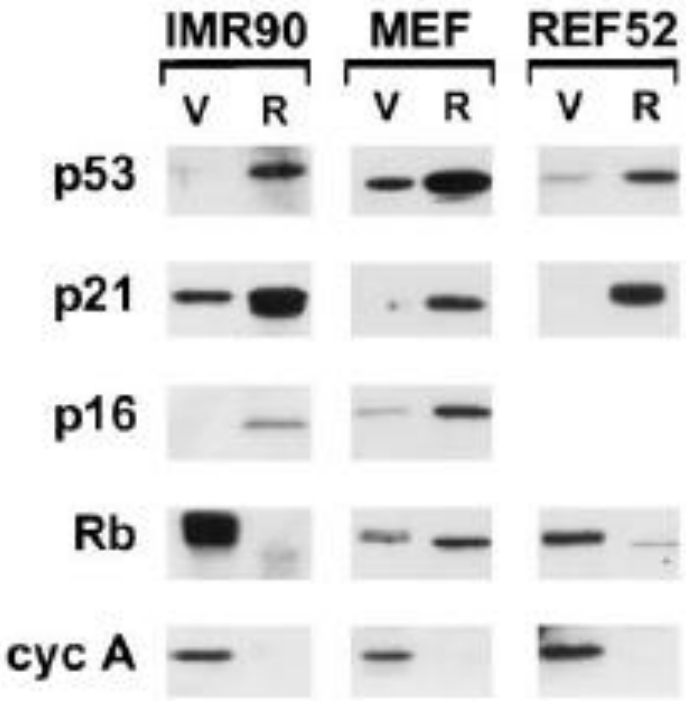
*Needle biopsy, frozen 3 months.

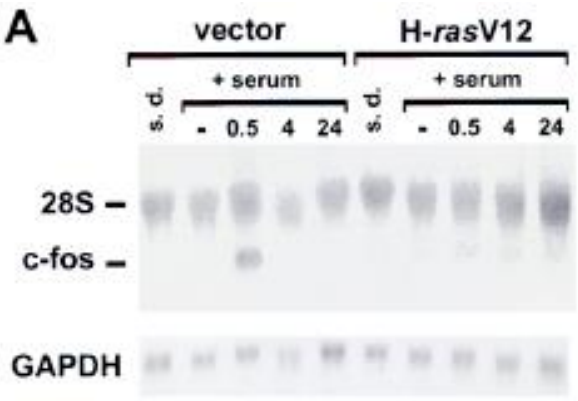
Table 1. Telomerase activity in normal and immortal cells (*29*).

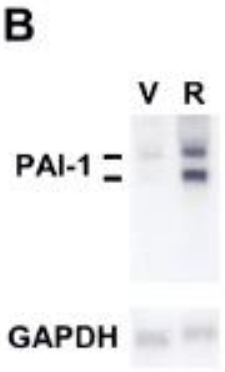
Tissue of origin	Cell type	Telomerase activity (no. positive/ no. tested)
Skin	Tumor	8/8
Skin	Normal	0/5
Connective	Tumor	1/1
Joint	Normal	0/1
Adipose	Tumor	1/1
Breast	Tumor	22/22
Breast	Normal	0/8
Lung	Tumor	18/18
Lung	Transformed	2/3
Lung	Normal	0/3
Stomach	Tumor	1/1
Pancreas	Tumor	3/3
Ovary	Tumor	5/5
Cervix	Tumor	3/3
Cervix	Normal	0/1
Uterus	Normal	0/1
Kidney	Tumor	8/8
Kidney	Transformed	1/1
Bladder	Tumor	3/3
Bladder	Normal	0/1
Colon	Tumor	7/7
Prostate	Tumor	2/2
Prostate	Transformed	0/1
Prostate	Normal	0/2
CNS	Tumor	3/3
Retina	Transformed	1/1
Blood	Tumor	9/9

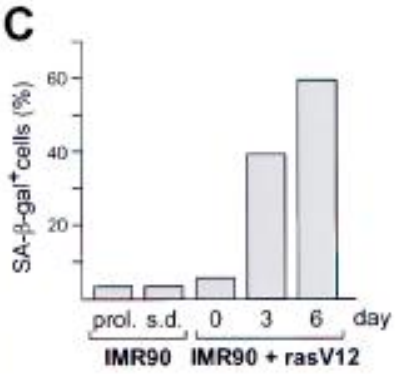


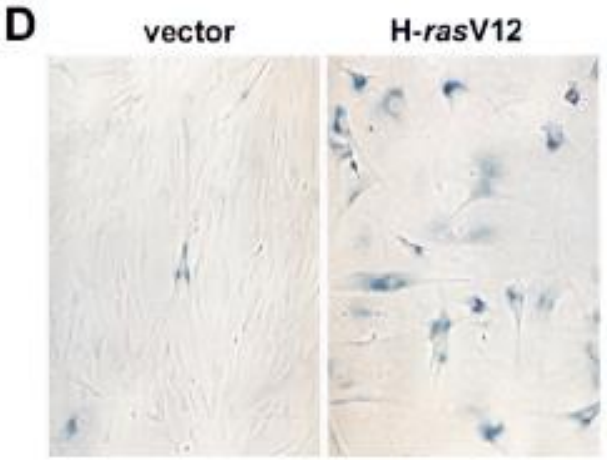
Oncogenic *ras* Provokes Premature Cell Senescence Associated with Accumulation of p53 and p16INK4a. Cell *88*, 593-602 (1997)


IMR90
MEF
REF52
V
R
V
R
V
R
p53
p21
p16
Rb
cyc A


A
vector
H-rasV12
+ serum
+ serum
s.d.
-
0.5
4
24
s.d.
-
0.5
4
24
28S
c-fos
GAPDH


B
V
R
PAI-1
GAPDH


C
SA-β-gal+cells (%)
60
40
20
prol.
s.d.
0
3
6
day
IMR90
IMR90 + rasV12


D
vector
H-rasV12

The NEW ENGLAND JOURNAL of MEDICINE

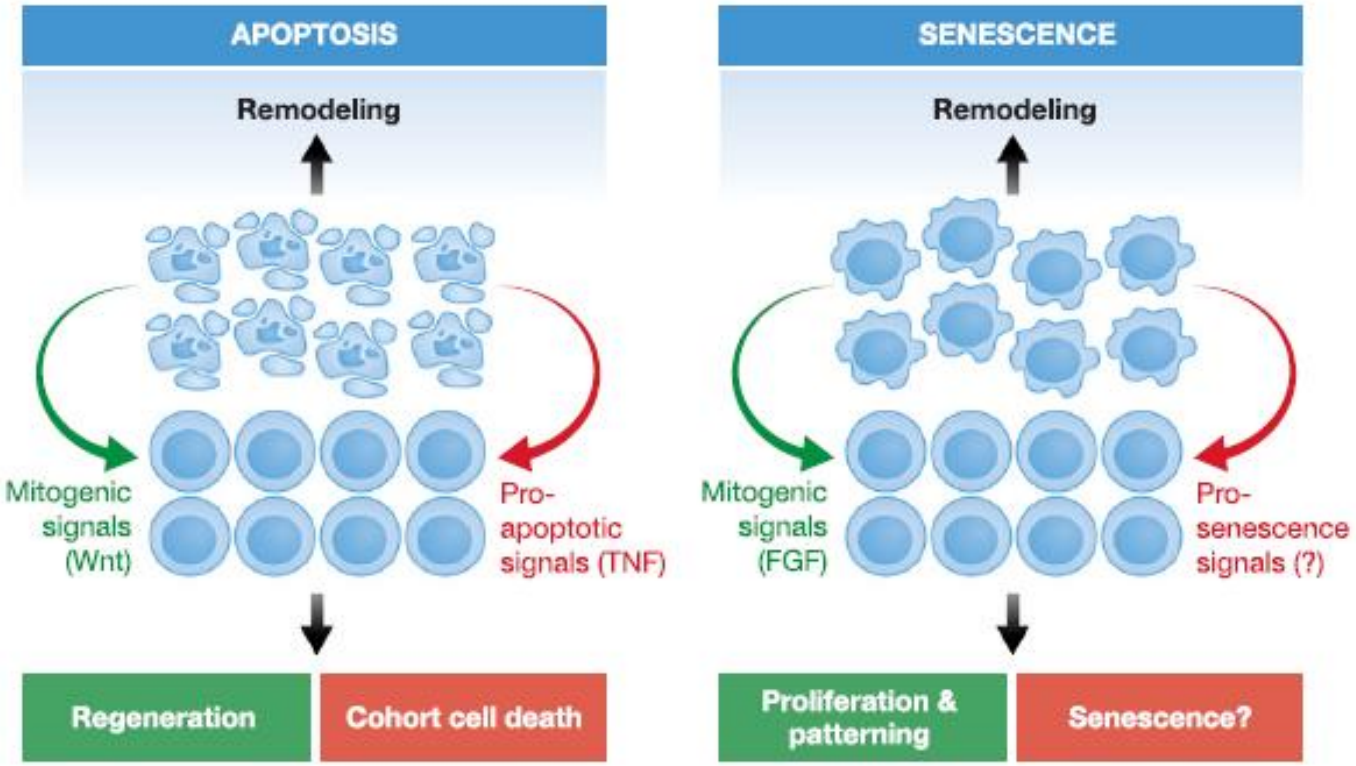
REVIEW ARTICLE

MECHANISMS OF DISEASE

Oncogene-Induced Cell Senescence — Halting on the Road to Cancer

W.J. Mooi, M.D., and D.S. Peeper, Ph.D.

New England J. of Med. P1037-46 September 7, 2006



Mu~noz-Espın D, et al (2013) Programmed cell senescence during mammalian embryonic development. Cell 155: 1104 – 1118

Storer M, et al (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell 155: 1119 – 1130

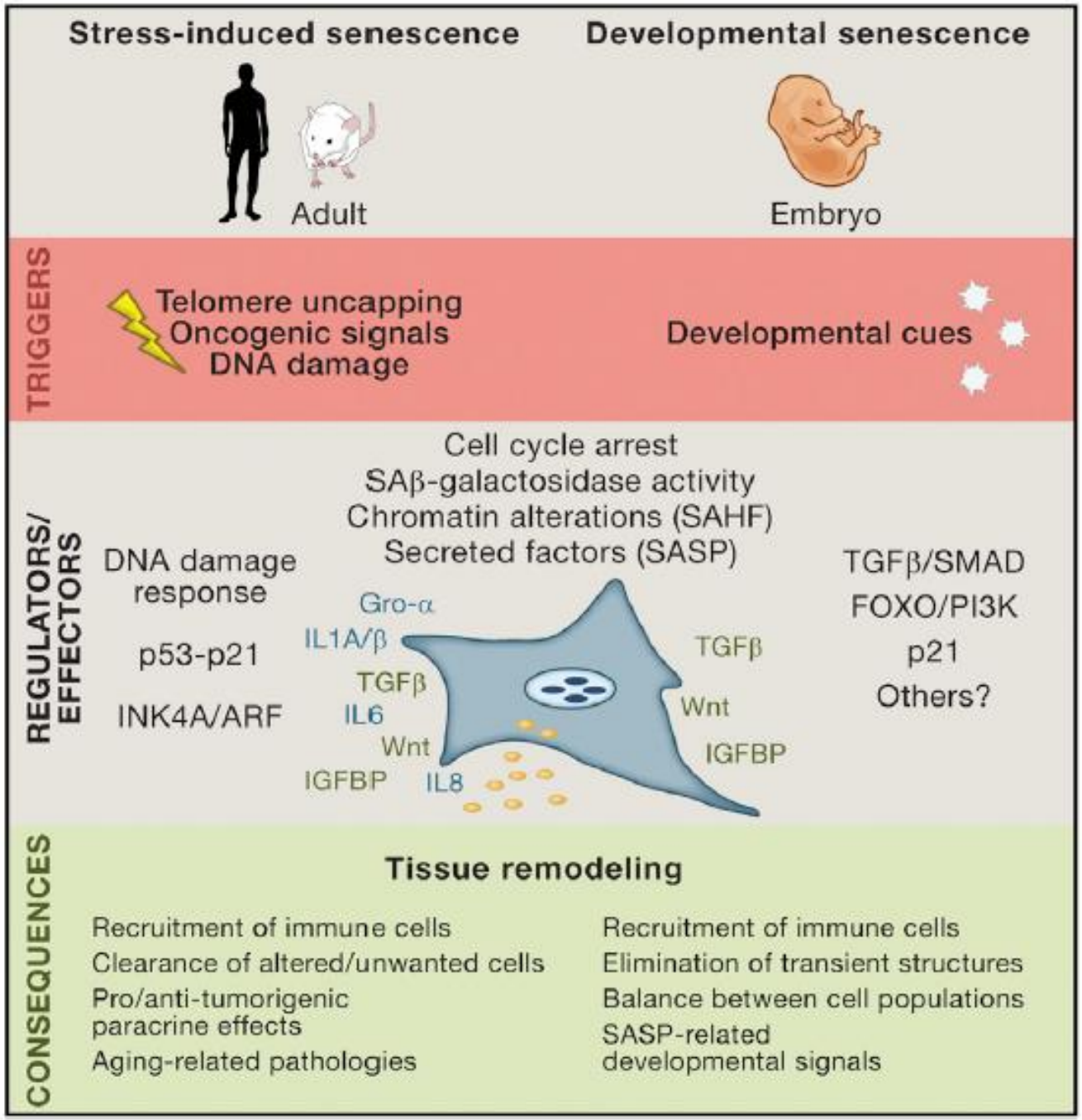

Stress-induced senescence
Developmental senescence
Adult
Embryo
TRIGGERS
Telomere uncapping
Oncogenic signals
DNA damage
Developmental cues
REGULATORS/ EFFECTORS
Cell cycle arrest
SAβ-galactosidase activity
Chromatin alterations (SAHF)
Secreted factors (SASP)
DNA damage response
p53-p21
INK4A/ARF
Gro-α
IL1A/β
TGFβ
IL6
Wnt
IGFBP
IL8
TGFβ
Wnt
IGFBP
TGFβ/SMAD
FOXO/PI3K
p21
Others?
CONSEQUENCES
Tissue remodeling
Recruitment of immune cells
Clearance of altered/unwanted cells
Pro/anti-tumorigenic paracrine effects
Aging-related pathologies
Recruitment of immune cells
Elimination of transient structures
Balance between cell populations
SASP-related developmental signals

Figure 21.33 **Evolutionary conservation of apoptosis pathways.**

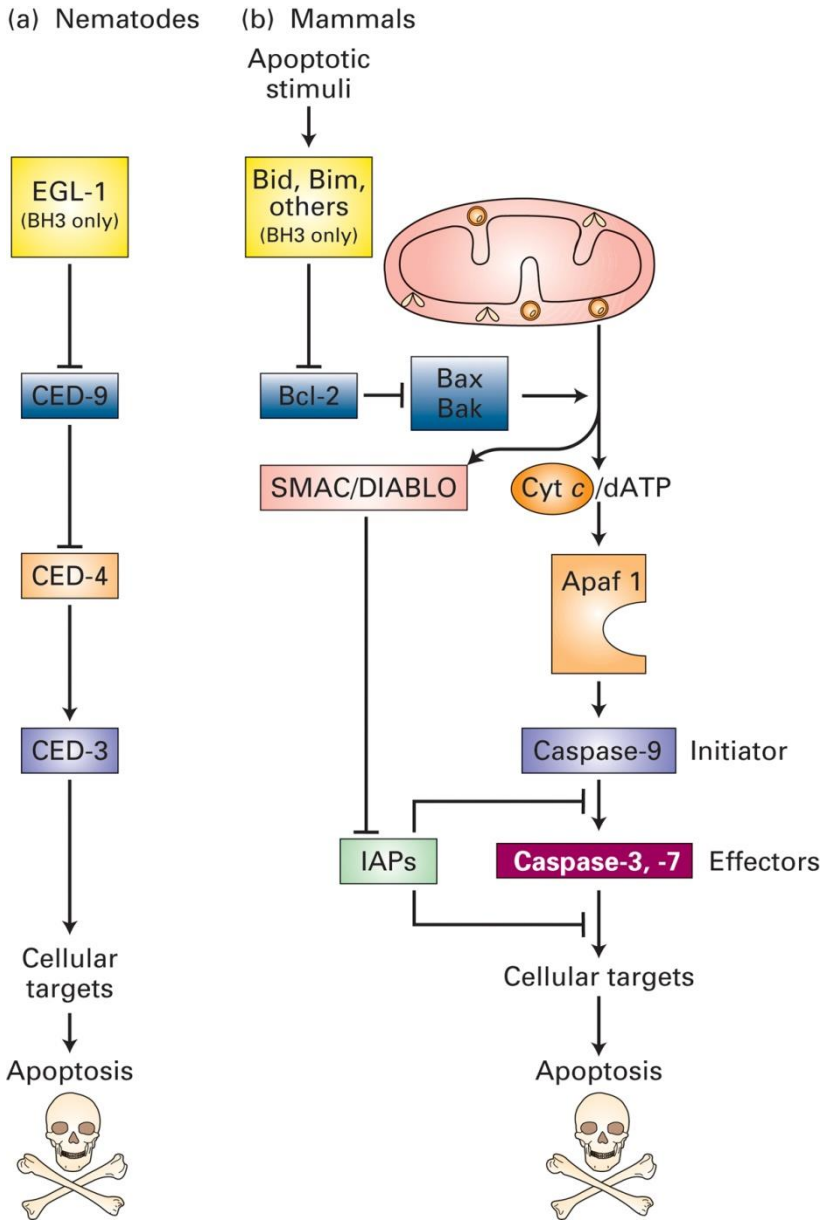


Figure 21.40 **Cell murder: the extrinsic apoptosis pathway.**

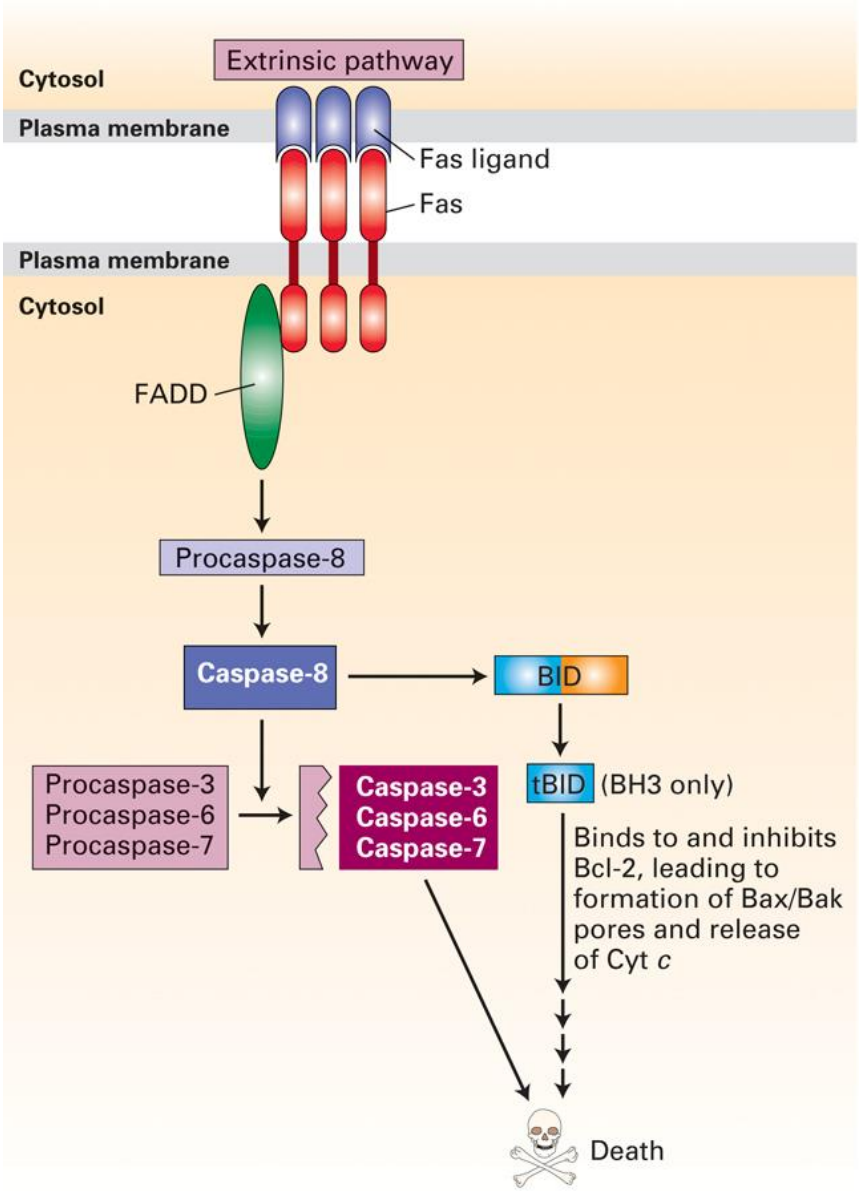
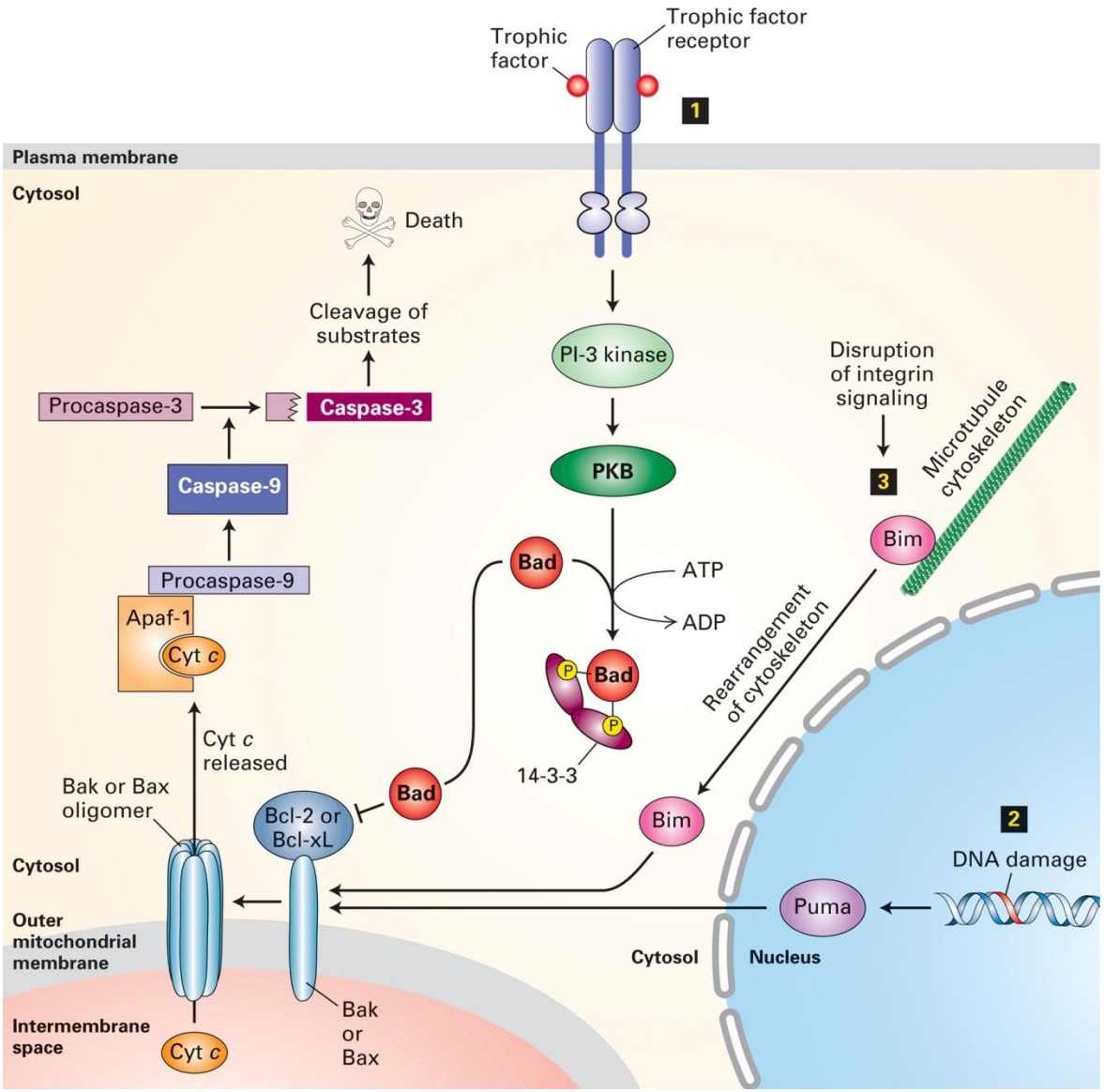
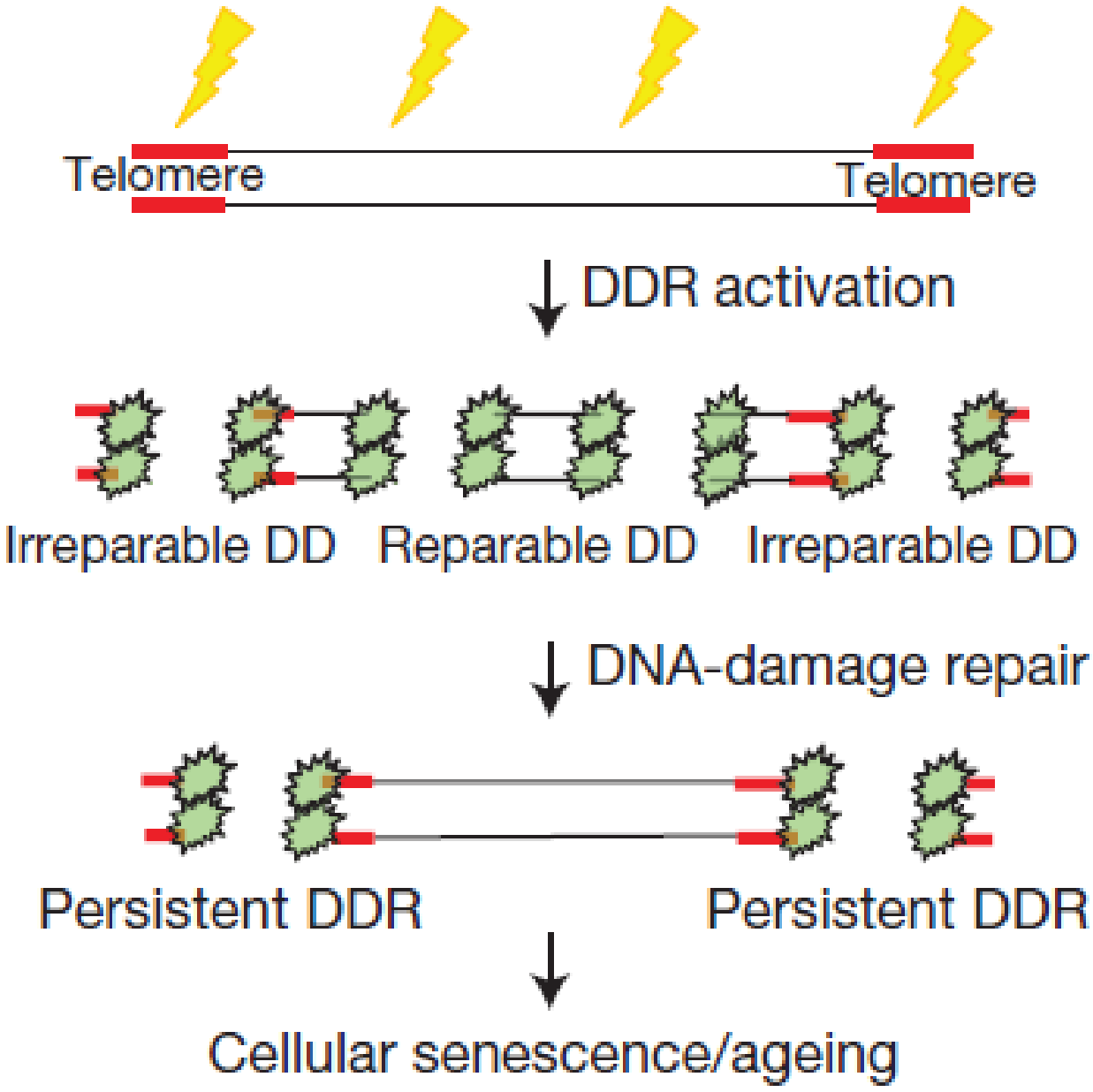
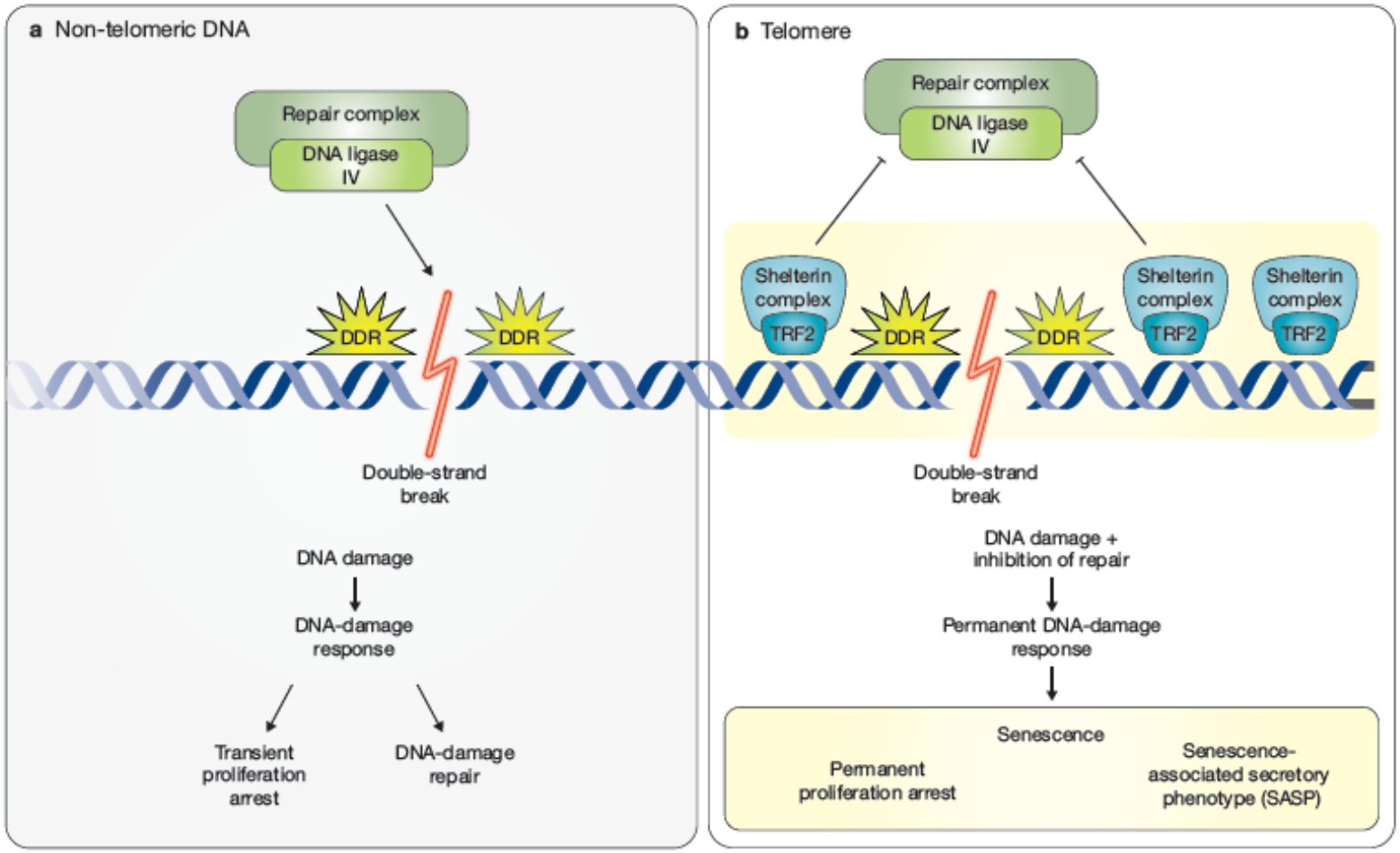


Figure 21.38 **Integration of multiple signaling pathways in vertebrate cells that regulate mitochondrial outer membrane permeability and apoptosis.**



What signals in the normal fibroblasts were induced by oncogenic ras which turn on cellular senescence program ?


Telomere
Telomere
DDR activation
Irreparable DD
Reparable DD
Irreparable DD
DNA-damage repair
Persistent DDR
Persistent DDR
Cellular senescence/ageing



Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nature Cell Biology 14:355-365; 2012