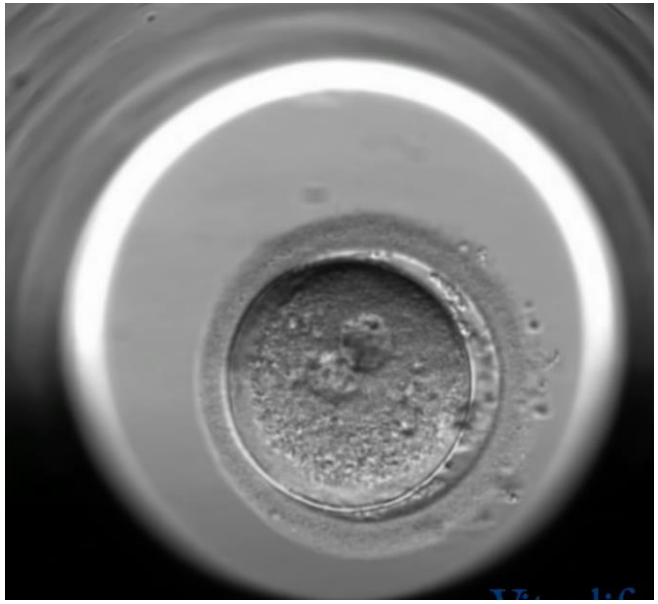
Biologie živočišné buňky

ŽIVOTNÍ CYKLUS ŽIVOČIŠNÉ BUŇKY – BUNĚČNÝ CYKLUS, DIFERENCIACE A BUNĚČNÁ SMRT

Karel Souček

Růst a replikace - esence života většiny buněk



Salamander Grow From a Single Cell
https://www.youtube.com/watch?v=SEejivHRIbE

Biologie živočišné buňky

The Nobel Prize in Physiology or Medicine 2001

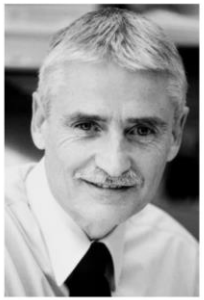


Photo from the Nobel Foundation archive.
Leland H. Hartwell
Prize share: 1/3

Photo from the Nobel Foundation archive.
Tim Hunt
Prize share: 1/3

Photo from the Nobel Foundation archive.
Sir Paul M. Nurse
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2001 was awarded jointly to Leland H. Hartwell, Tim Hunt and Sir Paul M. Nurse "for their discoveries of key regulators of the cell cycle."

The Nobel Prize in Physiology or Medicine 2012

© The Nobel Foundation. Photo: U. Montan
Sir John B. Gurdon
Prize share: 1/2

© The Nobel Foundation. Photo: U. Montan
Shinya Yamanaka
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent."

The Nobel Prize in Physiology or Medicine 2002

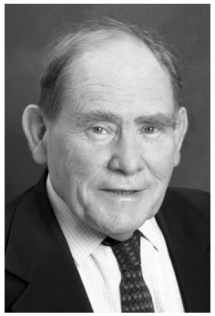


Photo from the Nobel Foundation archive.
Sydney Brenner
Prize share: 1/3

Photo from the Nobel Foundation archive.
H. Robert Horvitz
Prize share: 1/3

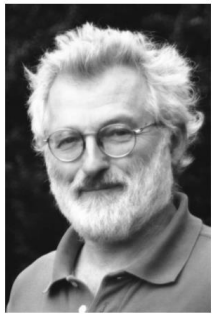


Photo from the Nobel Foundation archive.
John E. Sulston
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2002 was awarded jointly to Sydney Brenner, H. Robert Horvitz and John E. Sulston "for their discoveries concerning genetic regulation of organ development and programmed cell death'."

The Nobel Prize in Physiology or Medicine 2016

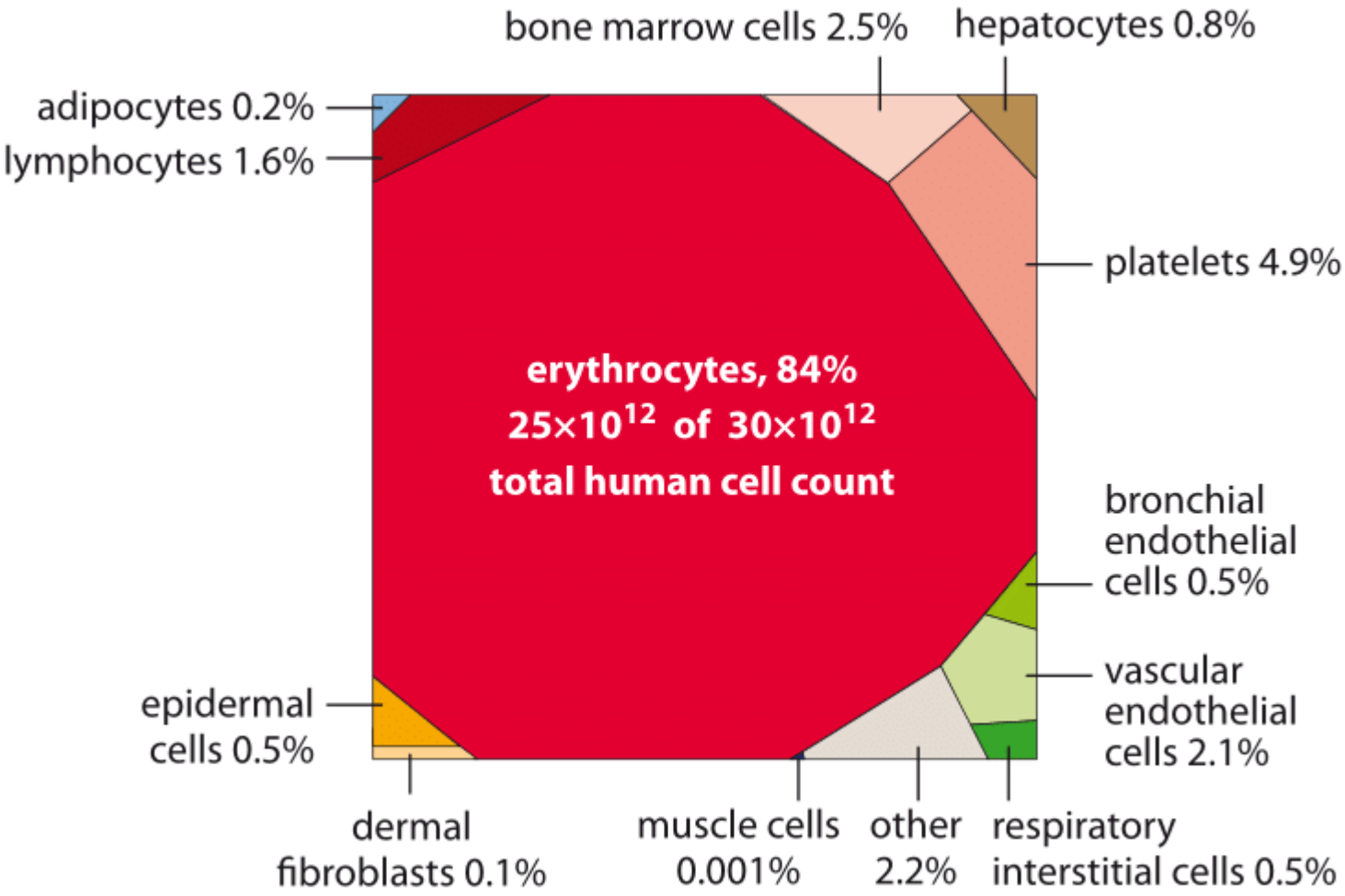
© Nobel Media AB. Photo: A. Mahmoud
Yoshinori Ohsumi
Prize share: 1/1

The Nobel Prize in Physiology or Medicine 2016 was awarded to Yoshinori Ohsumi "for his discoveries of mechanisms for autophagy."

100 000 000 000

- V dospělém lidském těle zemře denně ~100 mld. buněk a je nahrazeno novými (~ 1 milion/s)
- Hmota buněk ztracených ročně se tak blíží váze celého těla *(Developmental Biology. 6th edition)*
- Vlas se prodlužuje ~ 1 cm za měsíc, nehet ~ 0.3 za měsíc = stejná rychlost jakou se pohybují tektonické desky a vzdaluje se Severní Amerika a Evropa (*https://hypertextbook.com/facts/1997/ZhenHuang.shtml*)
  - *http://book.bionumbers.org*

Jaký podíl našeho těla tvoří různé typy buněk?

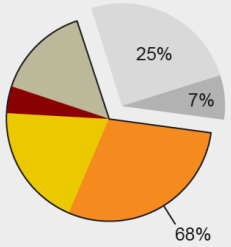


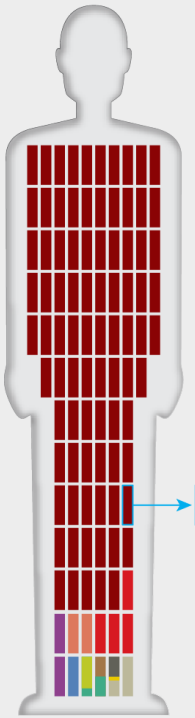
http://book.bionumbers.org/

Cells in the Body

Number* A 70-kilogram male has roughly 30 trillion human cells. Fat and muscle cells are large—72 percent of cellular mass—but are only 0.1 percent of the total number. About 87 percent, by number, are erythrocytes—red blood cells—which are extremely small.

Mass About 25 percent of body mass is fluid outside of cells, such as plasma; another 7 percent is solids, such as minerals. That leaves 68 percent made of human cells.





Cell Type

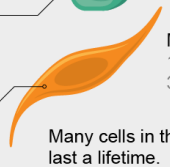
Blood
- Erythrocytes
- Lymphocytes
- Neutrophils
- Monocytes

- Endothelial (vessels)
- Lung
- Hepatocytes (liver)
- Gastrointestinal lining
- Skin
- Brain
- Adipocytes (fat)
- Myocytes (muscle)
- Other

Little Cells Rule
Large cells tend to live long, so daily turnover is dominated by plentiful, small cells with very short life spans.

Erythrocytes
Mass: 0.1 nanogram (ng)
Life span: 120 days

Colon epithelial cells
1 ng
3–5 days



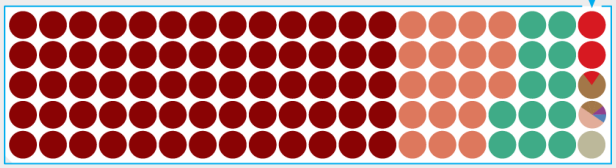
Muscle
1,000–10,000 ng
30–70 years

Many cells in the heart, eyes and brain last a lifetime.

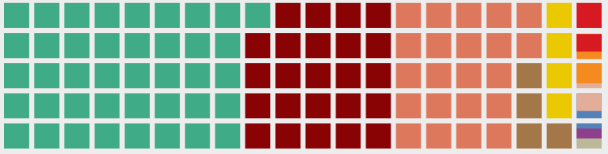
Each rectangle represents 1 percent of all 30 trillion cells. That's roughly equivalent to the number of cells the body sheds and produces every day.

Cell Turnover per Day

By Number† Roughly 330 billion cells (+/–20 billion) turn over every day. About 86 percent are blood cells, and 12 percent are gut cells. Other cells are replaced very slowly.



By Mass About 49 percent are blood cells, 41 percent are gut cells, with skin making up 4 percent, fat 4 percent and muscle 1 percent. Daily mass turnover is 80 grams (+/–20).



**This research is rooted in a standard reference person, which historically has been defined as a male, age 20 to 30, weighing 70 kilograms. Cells lost or gained resulting from menstruation were not taken into account. Negligible percentages are not shown.*

†Our bodies harbor another 38 trillion bacteria and many more viruses, but they weigh only 200 to 300 grams (seven to 11 ounces) and are not counted as human.

https://www.scientificamerican.com/article/our-bodies-replace-billions-of-cells-every-day/

Cell renewal rates in different tissues of the human body.

cell type	turnover time	BNID
small intestine epithelium	2-4 days	107812, 109231
stomach	2-9 days	101940
blood Neutrophils	1-5 days	101940
white blood cells Eosinophils	2-5 days	109901, 109902
gastrointestinal colon crypt cells	3-4 days	107812
cervix	6 days	110321
lungs alveoli	8 days	101940
tongue taste buds (rat)	10 days	111427
platelets	10 days	111407,111408
bone osteoclasts	2 weeks	109906
intestine Paneth cells	20 days	107812
skin epidermis cells	10-30 days	109214, 109215
pancreas beta cells (rat)	20-50 days	109228
blood B cells (mouse)	4-7 weeks	107910
trachea	1-2 months	101940
hematopoietic stem cells	2 months	109232
sperm (male gametes)	2 months	110319, 110320
bone osteoblasts	3 months	109907
red blood cells	4 months	101706, 107875
liver hepatocyte cells	0.5-1 year	109233
fat cells	8 years	103455
cardiomyocytes	0.5-10% per year	107076, 107077, 107078
central nervous system	life time	101940
skeleton	10% per year	109908
lens cells	life time	109840
oocytes (female gametes)	life time	111451

http://book.bionumbers.org/how-quickly-do-different-cells-in-the-body-replace-themselves/

Buněčný cyklus

The Nobel Prize in Physiology or Medicine 2001
Leland Hartwell, Tim Hunt, Sir Paul Nurse

- Duplikace chromozomální DNA a její segregace do dvou identických kopií dceřiných buněk
- S fáze (DNA synthesis) – 10-12h, představuje cca ½ délky celého buněčného cyklu
- M fáze – segregace a rozdělení buněk, < 1 hodinu
  - Mitóza – rozdělení jádra
  - Cytokineze – rozdělení cytoplazmy a oddělení buněk

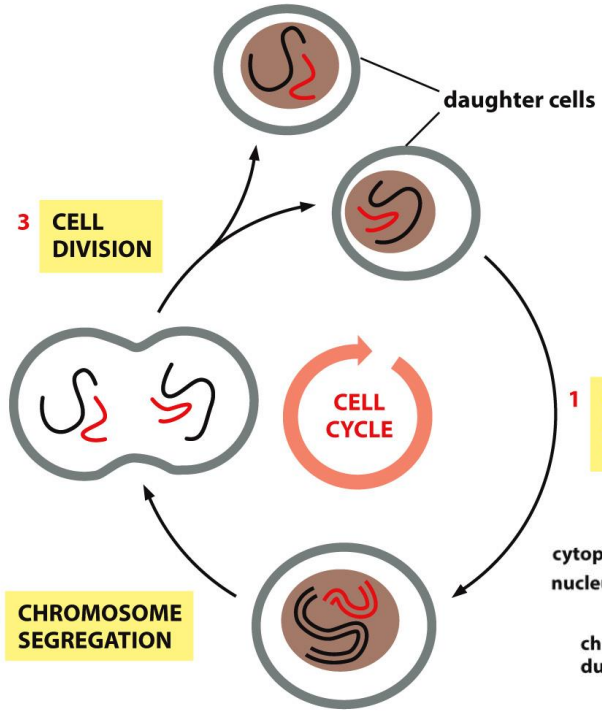


Figure 17-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

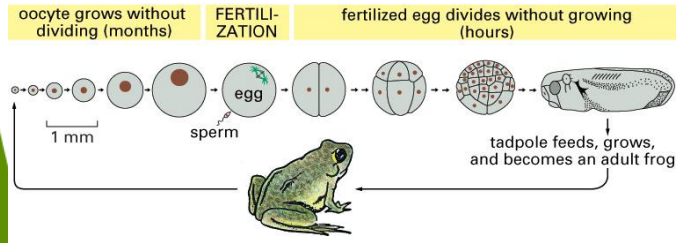
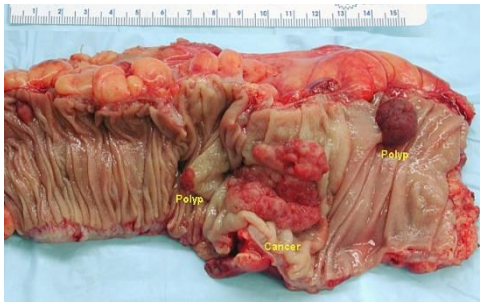


Figure 17–8. Molecular Biology of the Cell, 4th Edition.



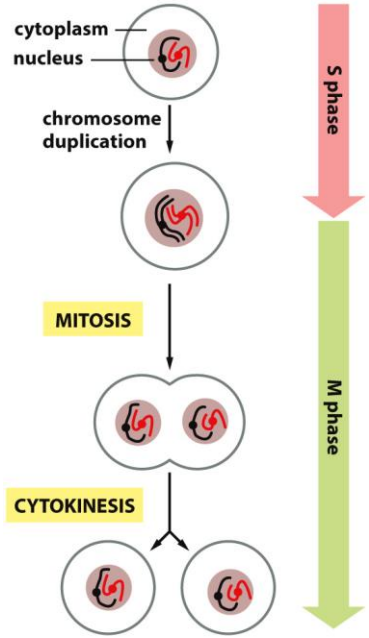


Figure 17-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Mitóza

- Profáze – sesterské chromatidy, zformované mitotické vřeténko, intaktní jaderná membrána
- Prometafáze – rozpad jaderné membrány, asociace chromozomů s mikrotubuly
- Metafáze – seřazení chromozómů
- Anafáze – oddělení sesterských chromatid
- Telofáze – dekondenzace chromatinu, formování jaderných membrán, počátek oddělování cytoplazmy
- Cytokineze – oddělení cytoplazmy dceřiných buněk

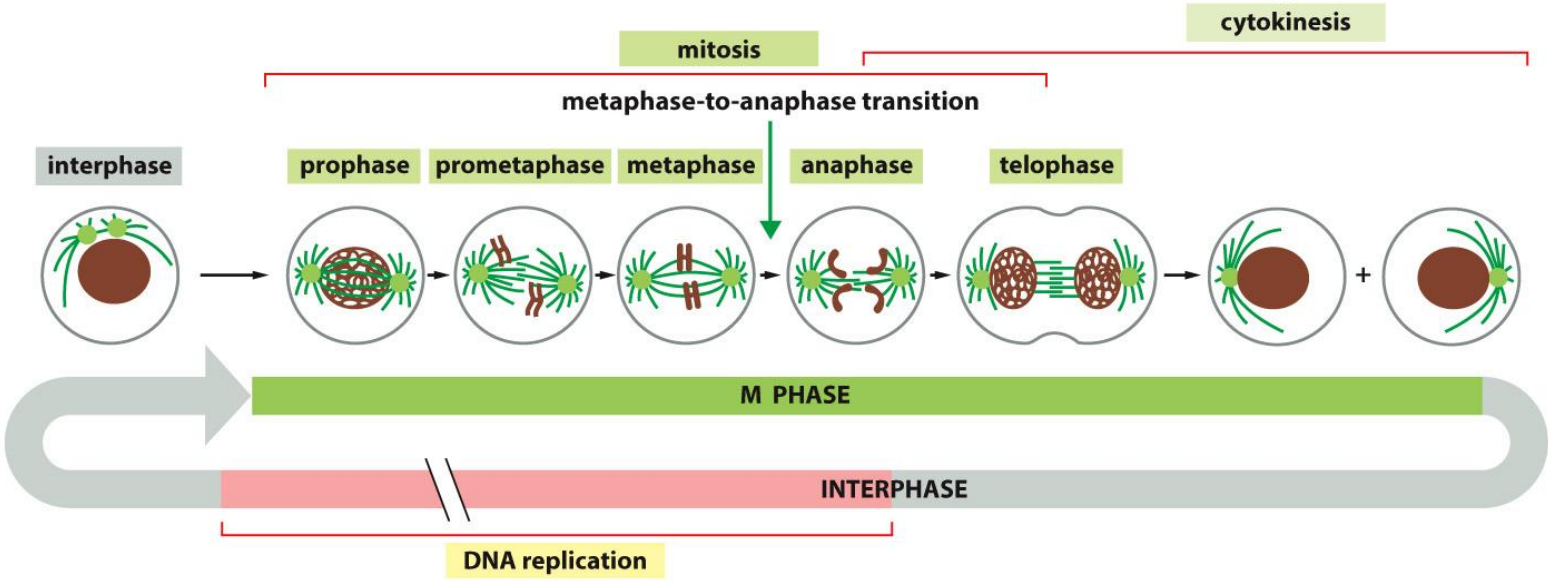


Figure 17-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

Fáze buněčného cyklu

- G1 – gap fáze mezi M a S fází
- G2 – gap mezi S a M fází
  - Poskytují potřebný čas pro monitorování vnějšího a vnitřního stavu mikroprostředí, délka záleží na vnějších podmínkách a signálech od okolních buněk
- G0 – klidová fáze (ve smyslu buněčného dělení), dny až roky
- Restrikční bod, restriction point
  - např. na konci G1 – za ním dochází k replikaci DNA i po odejmutí mitogenních stimulů

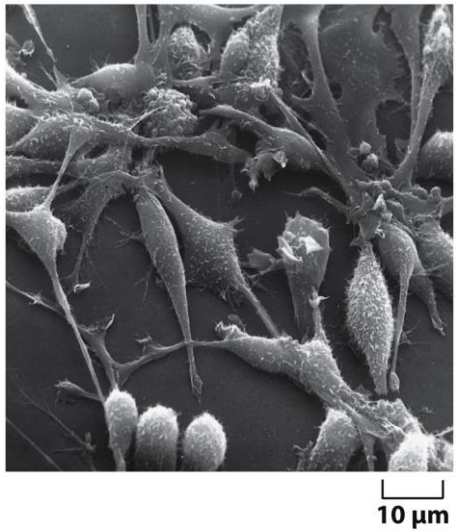


Figure 17-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

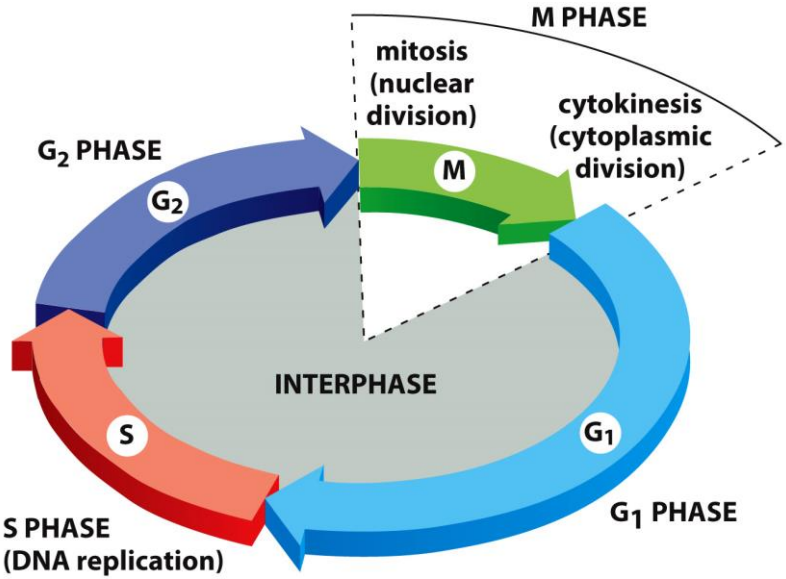
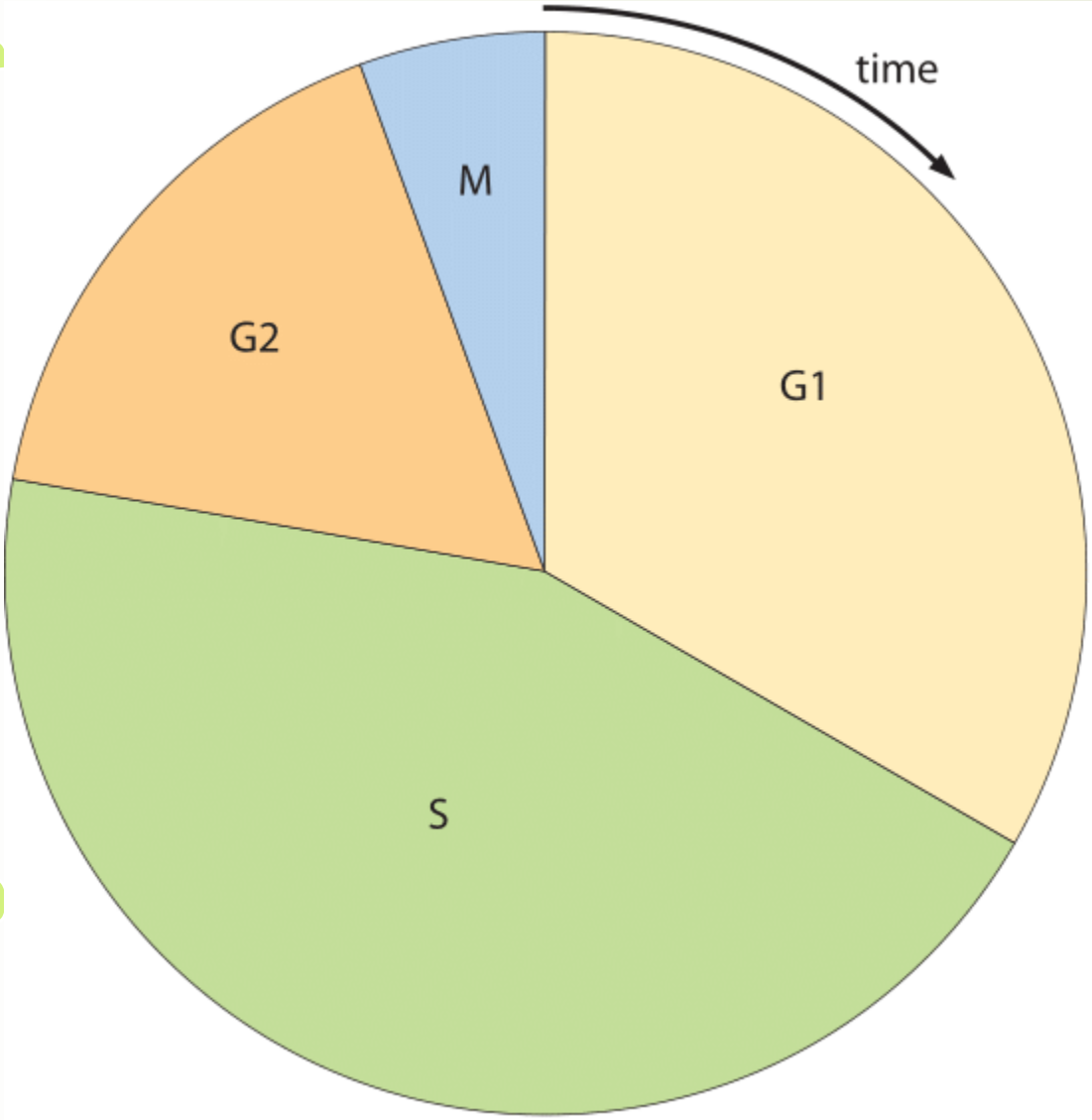
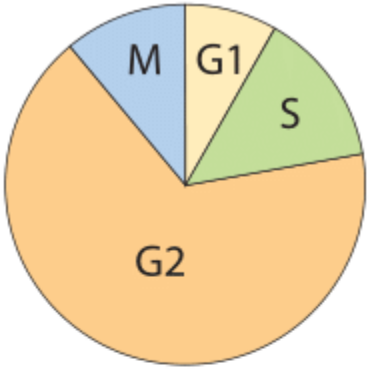


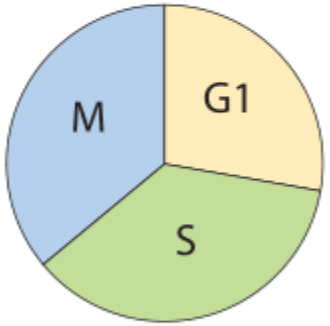
Figure 17-4 Molecular Biology of the Cell 6e (© Garland Science 2015)



human cell line (20 hr)



fission yeast (2 hr)

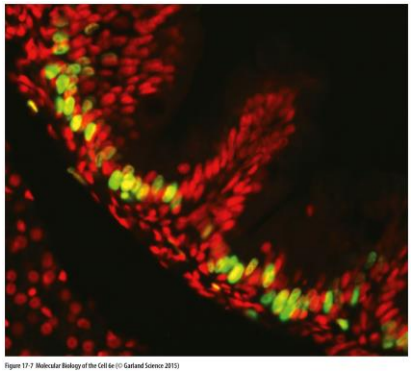


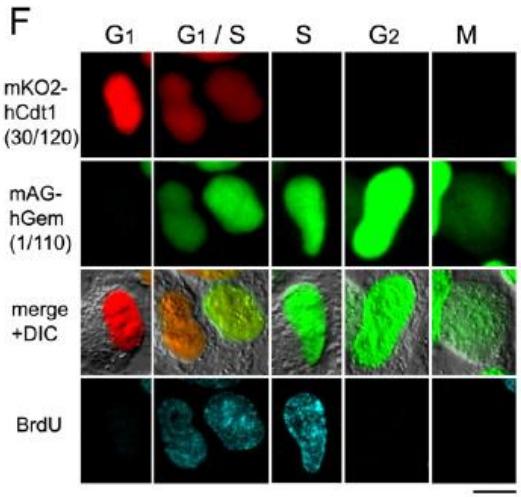
budding yeast (1.5 hr)

Drosophila embryo (8 min)

Adapted from “The Cell Cycle – Principles of Control” by David Morgan.)

Studium buněčného cyklu





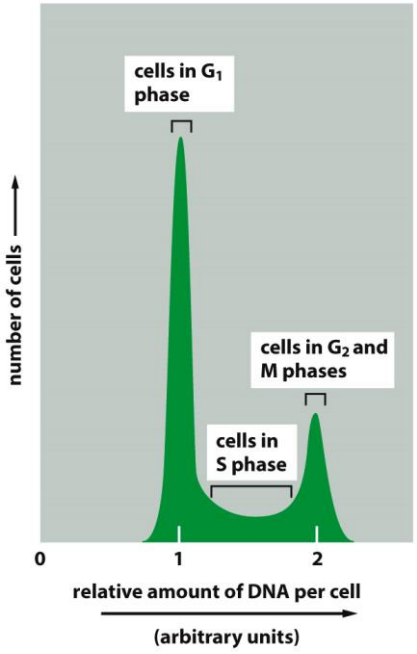
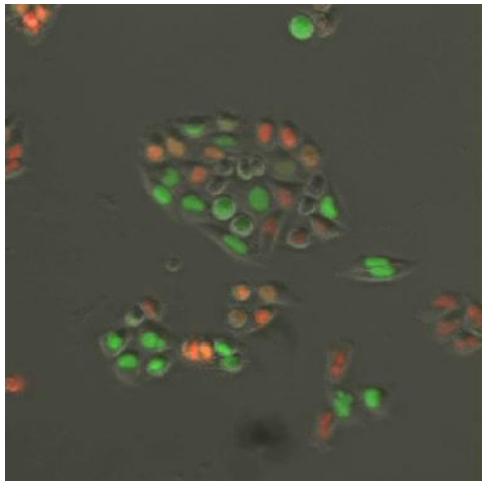
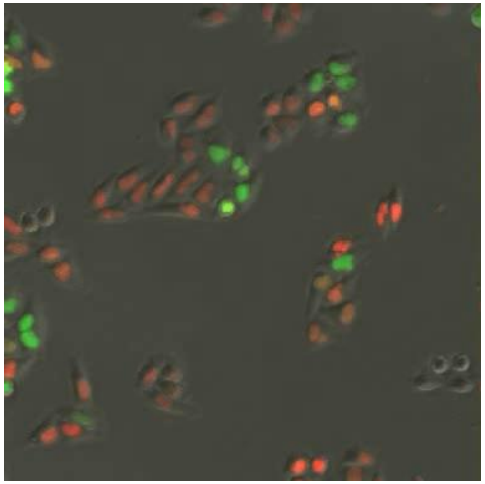


Figure 17-8 Molecular Biology of the Cell 6e (© Garland Science 2015)





Kontrola buněčného cyklu

- Propojená série biochemických přepínačů iniciujících konkrétní události v průběhu buněčného cyklu
  - binární – ano/ne
  - nereverzibilní – další se spouští až po úplném dokončení předchozího kroku
  - spolehlivé – zálohované
  - adaptibilní – různé typy buněk v různém tkáňovém mikroporostředí
- tři hlavní kontrolní/přechodové body
  - G1 -> S, vnější prostředí OK?
  - G2 -> M – DNA, replikovaná, nepoškozená?
  - M: metafáze -> anafáze – všechny chromozómy připojeny k dělícímu vřeténku?

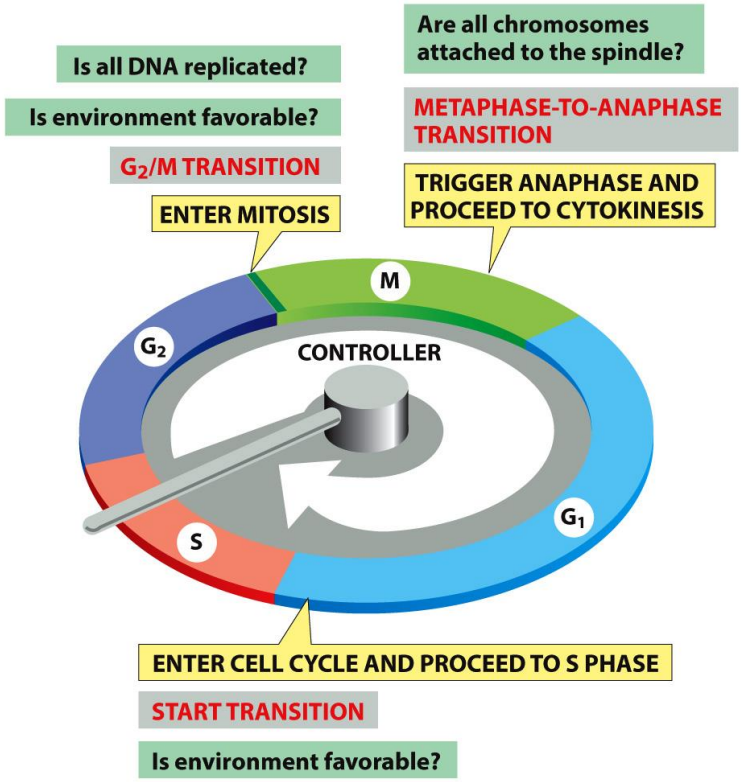


Figure 17-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

Cyklin dependentní kinázy (Cdks) a cykliny

- rodina proteinových kináz
- jejich kinázová aktivita stoupá a klesá v průběhu buněčného cyklu -> cyklické změny ve fosforylaci intracelulárních cílových proteinů – iniciace a regulace událostí spojených s cyklem
- nejvýznamnějším kontrolorem jejich aktivity jsou cykliny
- hladiny cyklinů se mění, hladina Cdks je konstatní
- cyklování hladiny cyklinů v průběhu buněčného cyklu ovlivňuje sestavení a aktivaci komplexu cyklin-Cdk v konkrétních fázích cyklu

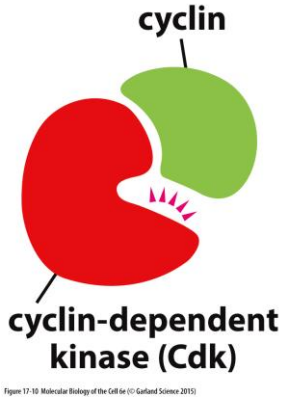


Figure 17-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

Cyklin dependentní kinázy (Cdks) a cykliny

- 4 skupiny cyklinů
  - G1 cykliny – napomáhají řídit aktivitu G1/S cyklinů
  - G1/S cykliny – aktivují Cdks v pozdní G1, vstup do buněčného cyklu, hladina klesá v S fázi
  - S cykliny – vážou se na Cdks ihned po vstupu do cyklu, hladina udržovaná až do mitózy
  - M- cykliny – aktivují Cdks regulující vstup do G2/M přechodu, pokles uprostřed mitózy

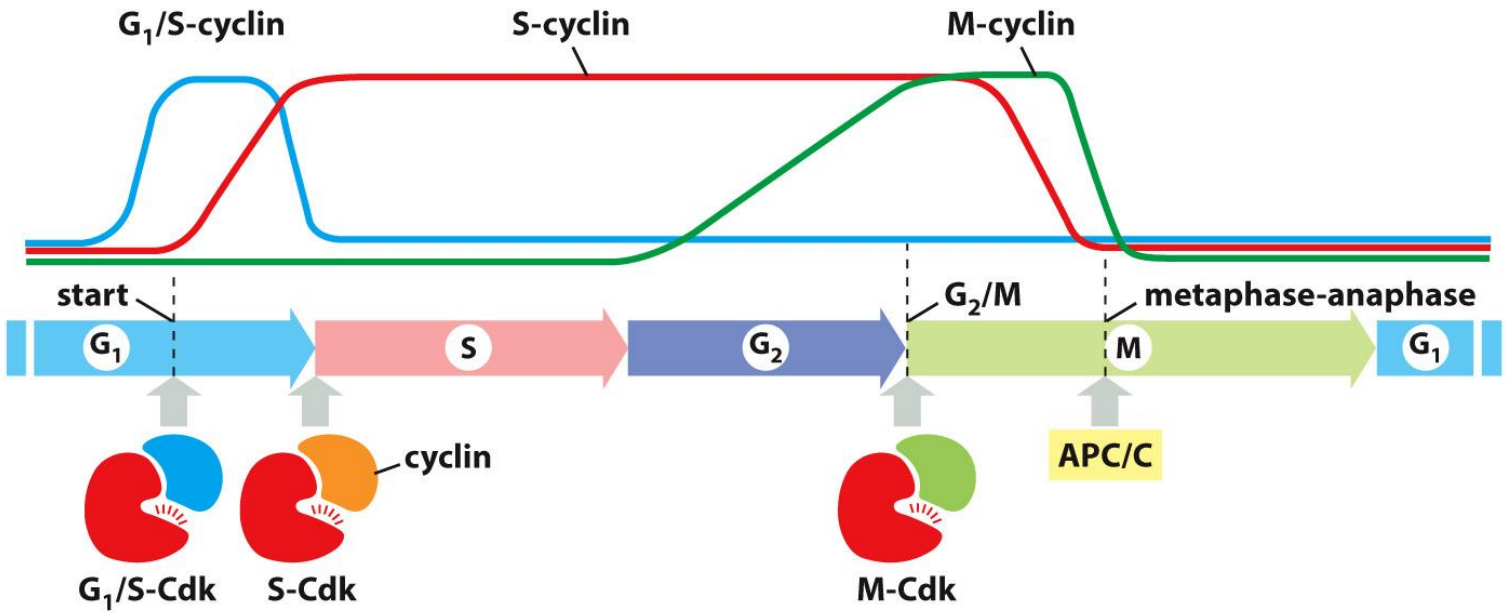


Figure 17-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

Cyklin dependentní kinázy (Cdks) a cykliny

TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

	Vertebrates		Budding yeast	
Cyclin–Cdk complex	**Cyclin**	**Cdk partner**	**Cyclin**	**Cdk partner**
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Table 17-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

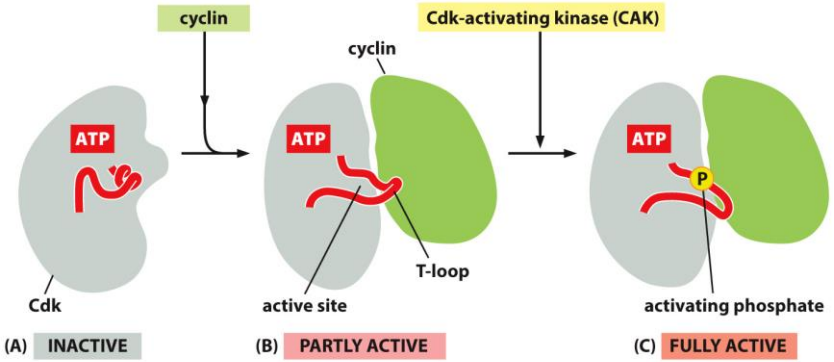


Figure 17-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

Inhibice Cdk aktivity

- Fosforylace v aktivním místě Cdk inhibuje aktivitu cyclin-Cdk komplexu
  - Wee1 kináza
- Naopak jeho defosforylace aktivitu zvyšuje
  - Cdc25 fosfatáza
- Vazba inhibitorů Cdk (Cdk inhibitor proteins, CDKs) inaktivuje komplexy cyklin-Cdk

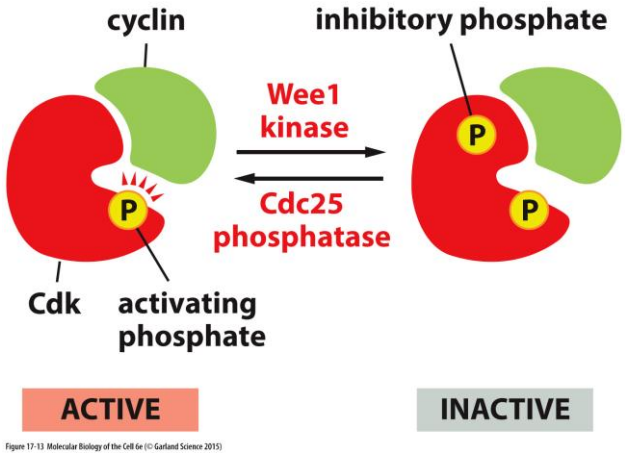


Figure 17-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

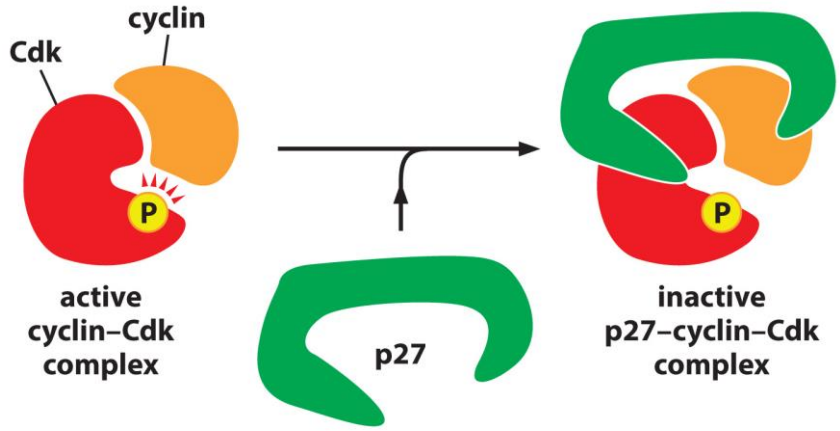


Figure 17-14 Molecular Biology of the Cell 6e (© Garland Science 2015)

Řízená proteolýza v regulaci buněčného cyklu

- APC/C (anaphase promoting complex, cyclosome)
  - komplex určující vstup do anafáze
  - Ubiquitin ligáza
    - securin (chrání spojení sesterských chromatid)
    - S- a M- cykliny
- SCF (Skp, Cullin, F-box containing complex
  - multi-protein E3 ubiquitin ligase
    - CKI, G1/S cykliny
    - konstantní aktivita

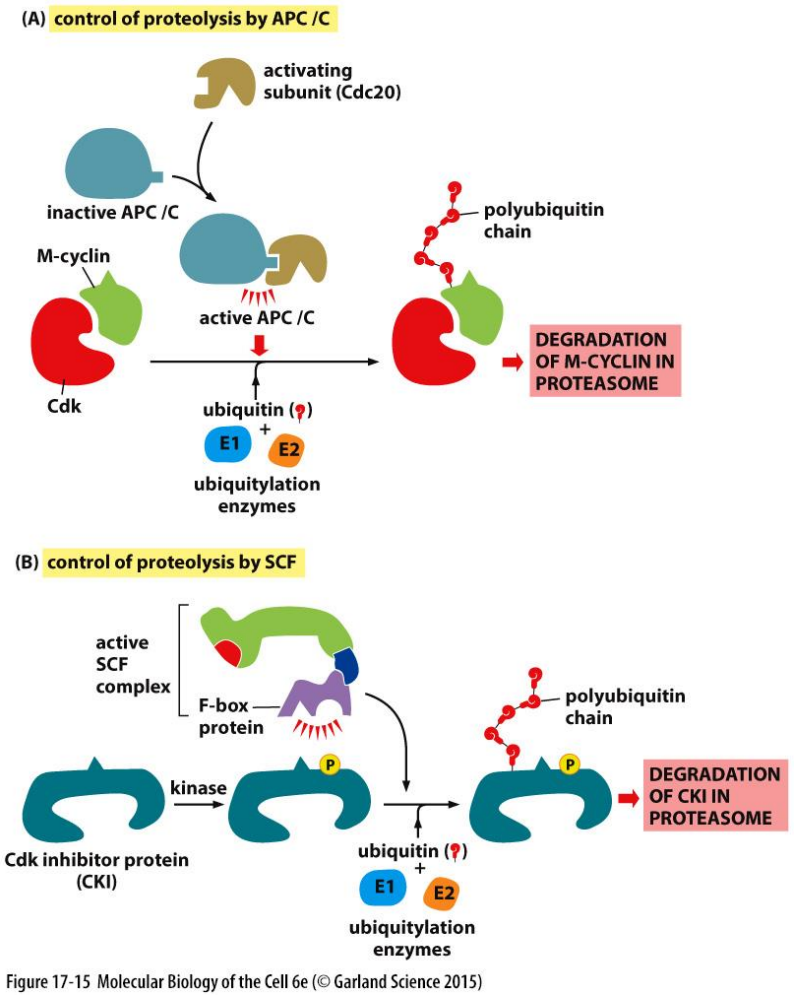


Figure 17-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

Přehled hlavních regulátorů buněčného cyklu

TABLE 17–2 Summary of the Major Cell Cycle Regulatory Proteins

General name	Functions and comments
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	Phosphorylates an activating site in Cdks
Wee1 kinase	Phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	Removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	Suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	Suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	Catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits Cdc20 or Cdh1
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	Catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity

Table 17-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Přehled systému řízení buněčného cyklu

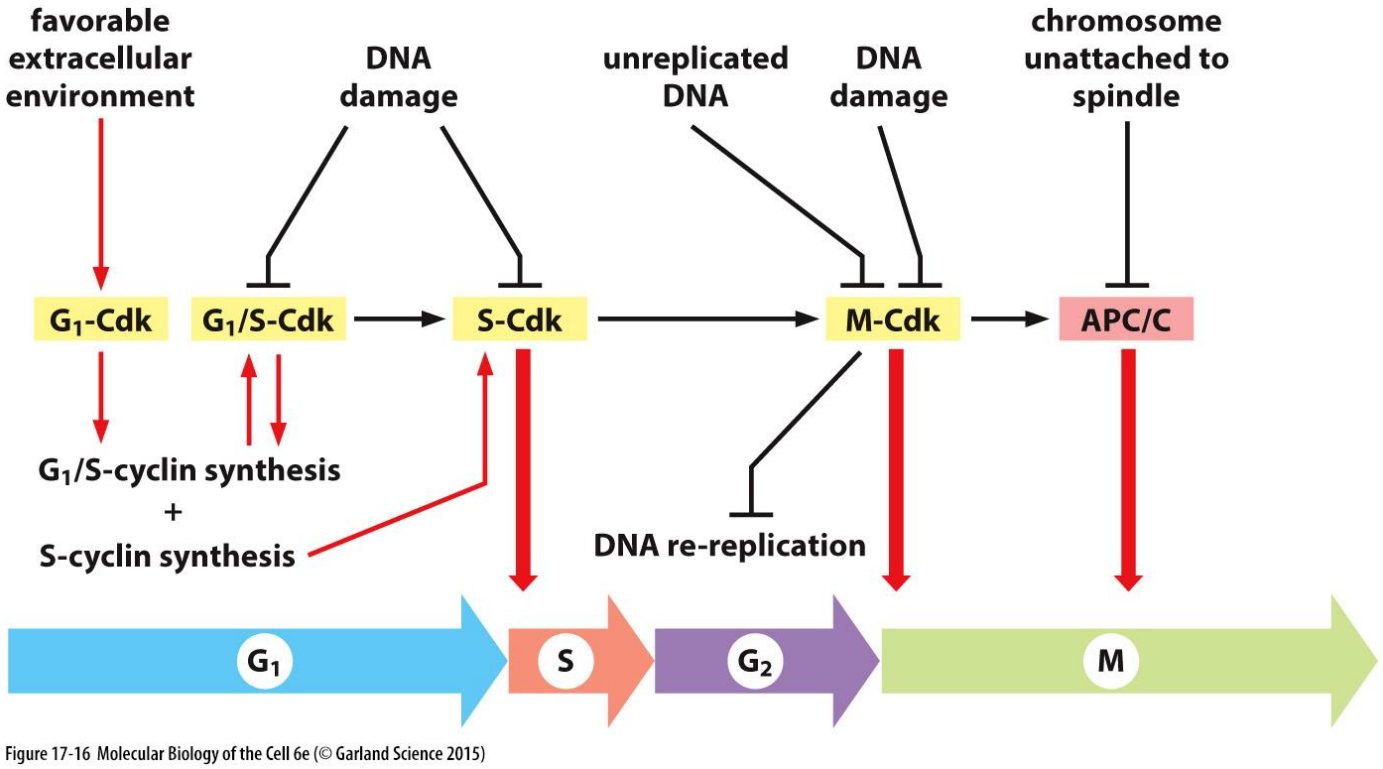


Figure 17-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

Kontrola buněčného dělení a růstu

- Buněčný růst, dělení a přežívání buňek jsou klíčové procesy ovlivňující velikost orgánů a celých organismů (např. myš vs. člověk)
- Vliv extracelulárních signálů
  - Mitogeny – stimulují buněčné dělení, G1/S-Cdk aktivita
  - Růstové faktory – stimulují růst buněčné hmoty, indukce syntézy proteinů a dalších makromolekul, inhibice jejich degradace
  - Faktory přežití „survival factors“ – inhibují buněčnou smrt, apoptózu

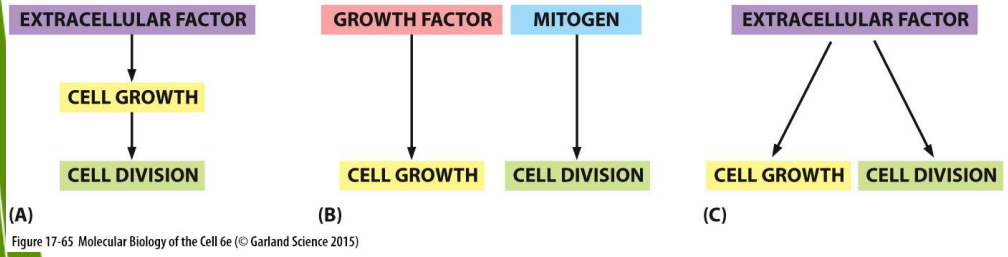


Figure 17-65 Molecular Biology of the Cell 6e (© Garland Science 2015)

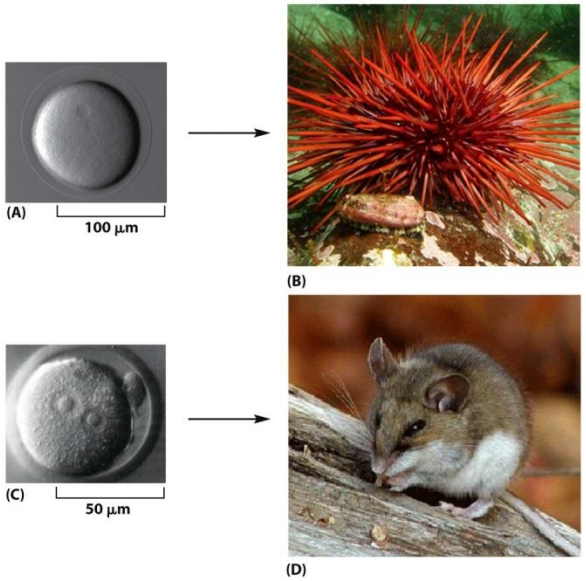


Figure 20-33 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Mitogeny

- Extracelulární signál mitogenních faktorů vede k překonání vnitřního mechanismu blokujícího buněčné dělení
- PDGF – platelet-derived growth factor, jeden z cca 50-ti známých mitogenních faktorů
- V řadě tkání jsou přítomny extracelulární proteiny inhibující působení mitogenů např. TGF-β1
- Mitogeny stimulují G1-Cdk a G1/S-Cdk aktivitu
- vybrané klíčové molekuly:
  - Ras – monomeric GTPase
  - MAPs – mitogen activated protein kinases – časná odpověď
  - Myc, E2F – transkripční faktory
  - Rb protein - pocket protein (nádorový supresor)

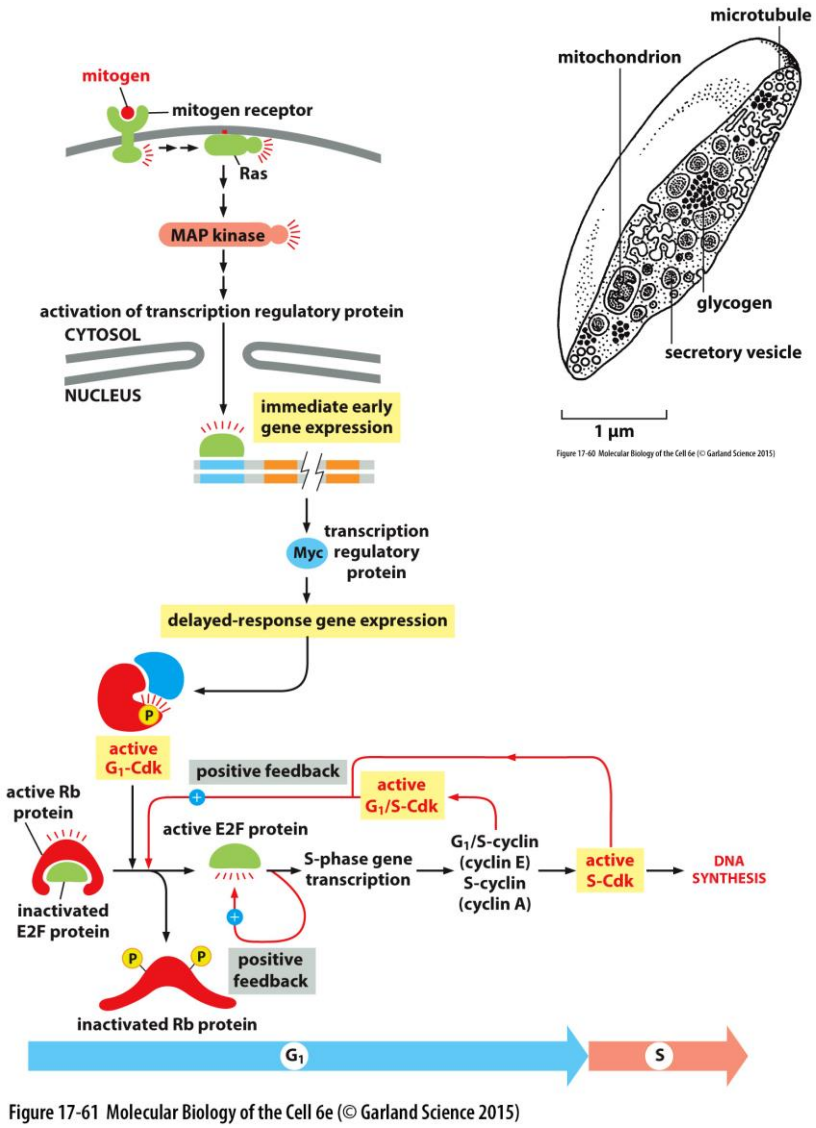


Figure 17-61 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 17-60 Molecular Biology of the Cell 6e (© Garland Science 2015)

Regulace buněčného cyklu po poškození DNA

- Oprava poškozené DNA před její replikací je esenciální
- V případě detekce poškození, zástava proliferace před počátkem S fáze nebo před vstupem do mitózy
- vybrané klíčové molekuly:
  - ATM (ataxia-telangiectasia mutated), ATR (ATM- and Rad3-Related) – kinázy
  - Chk1, Chk2 - kinázy
  - p53 – transkripční faktor
  - MDM2 – regulátor stability p53
  - p21 – CDK inhibitor

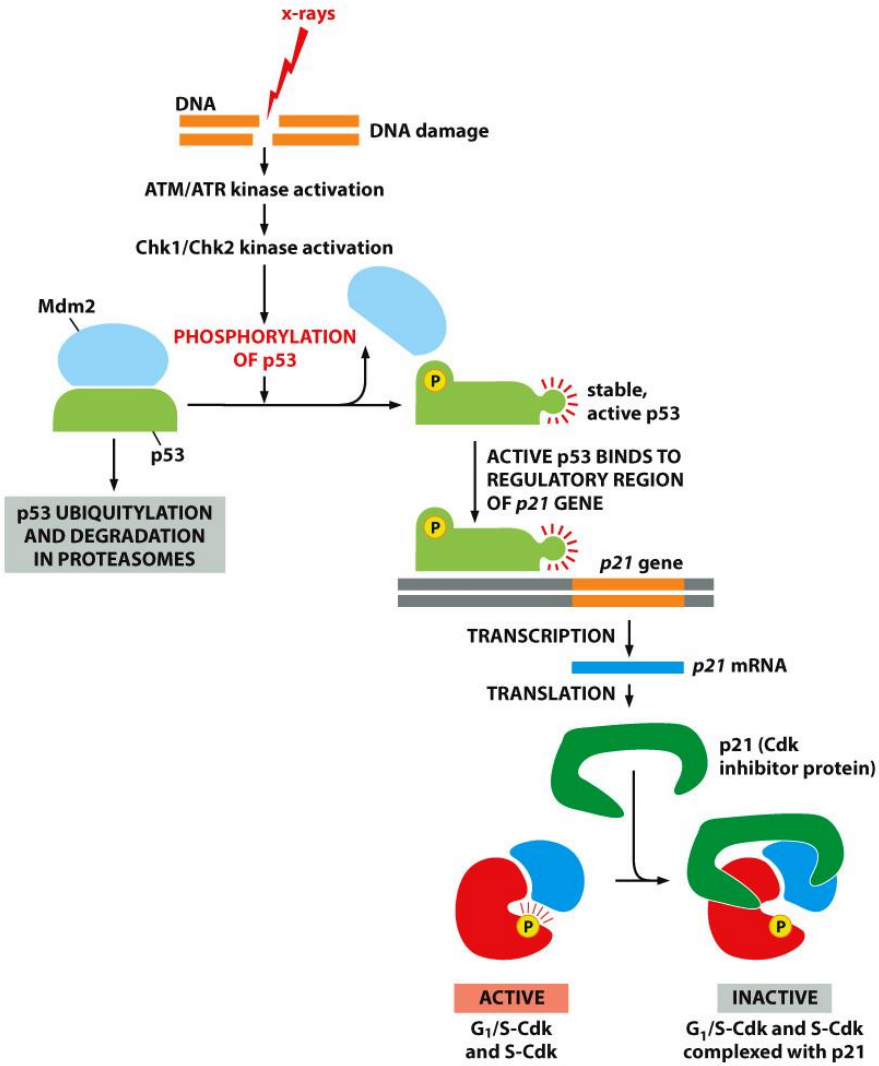


Figure 17-62 Molecular Biology of the Cell 6e (© Garland Science 2015)

Zástava buněčného cyklu v případě nadměrné mitogenní stimulace

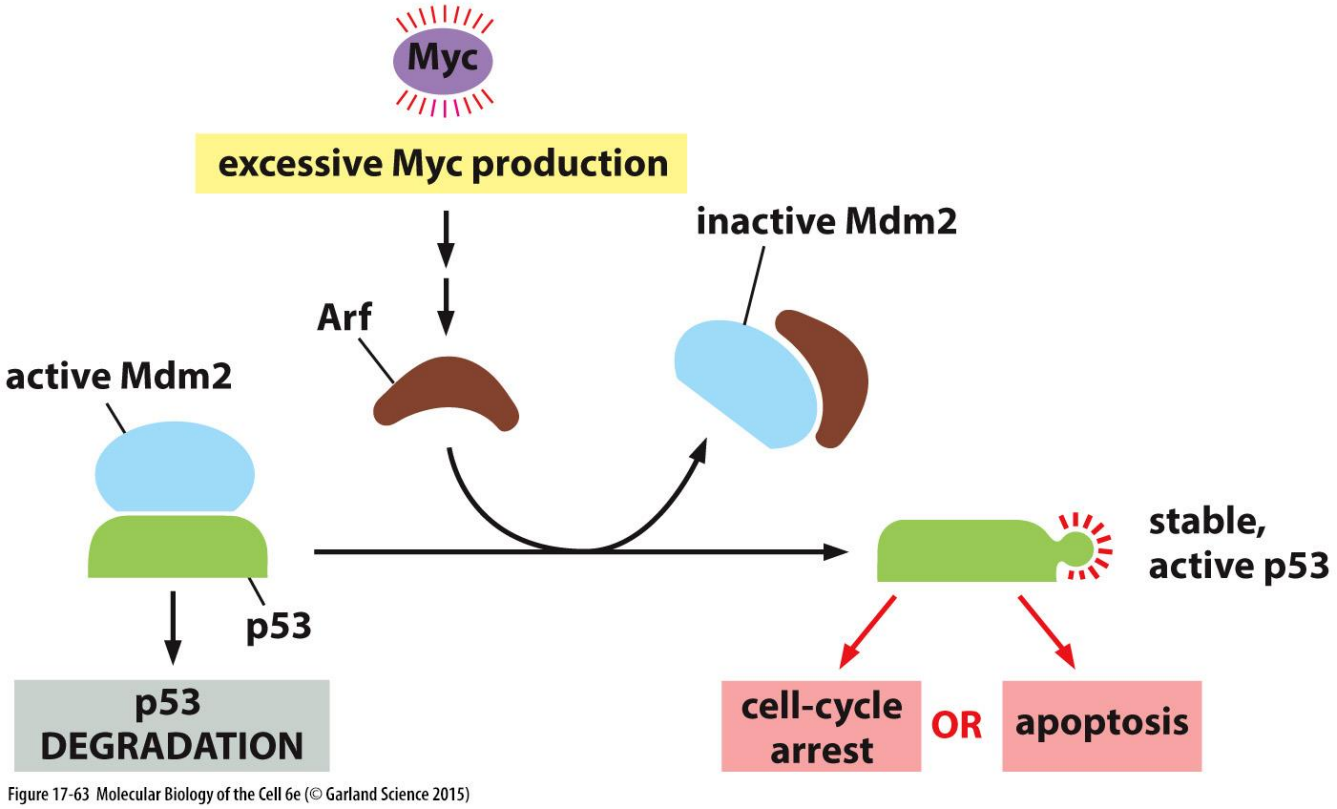


Figure 17-63 Molecular Biology of the Cell 6e (© Garland Science 2015)

Buněčná proliferace je doprovázena buněčným růstem

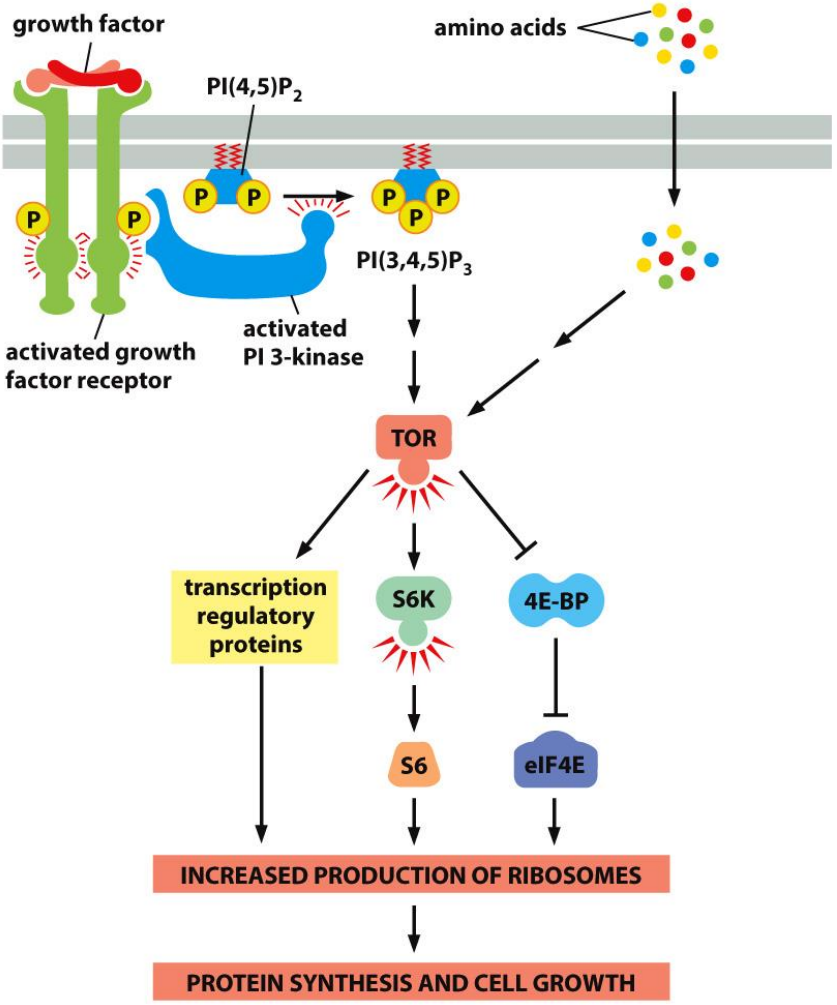


Figure 17-64 Molecular Biology of the Cell 6e (© Garland Science 2015)

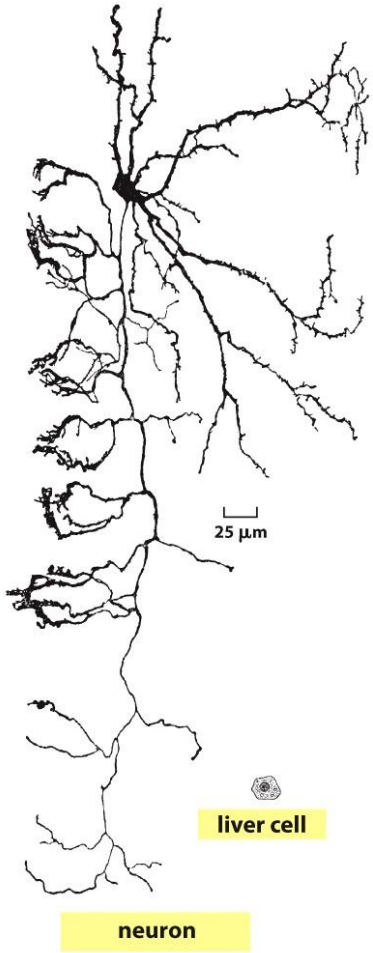
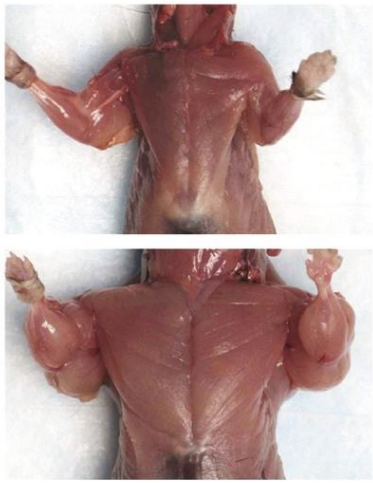
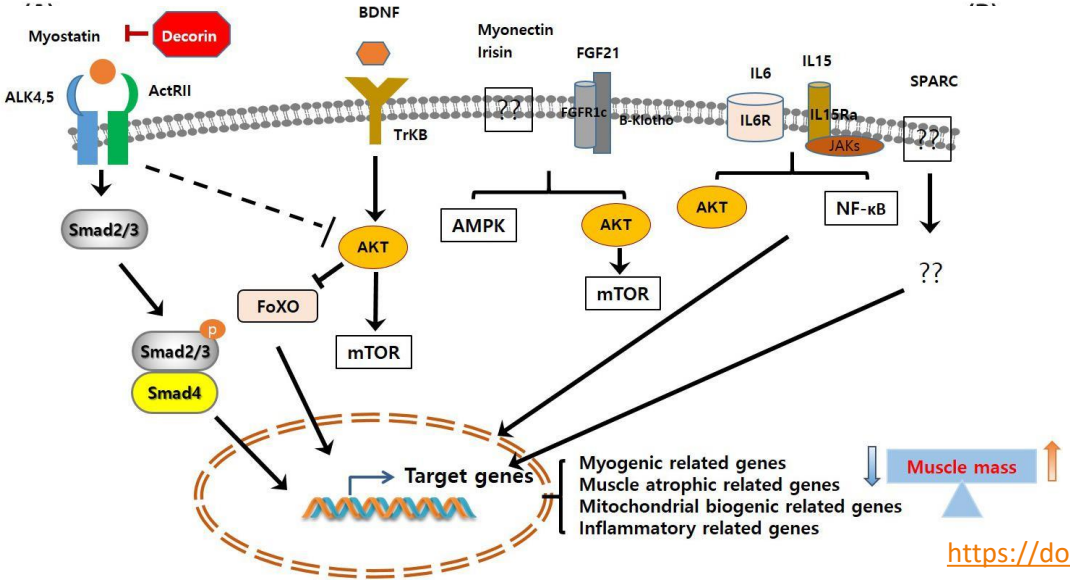


Figure 18-44 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Buněčná proliferace je doprovázena buněčným růstem

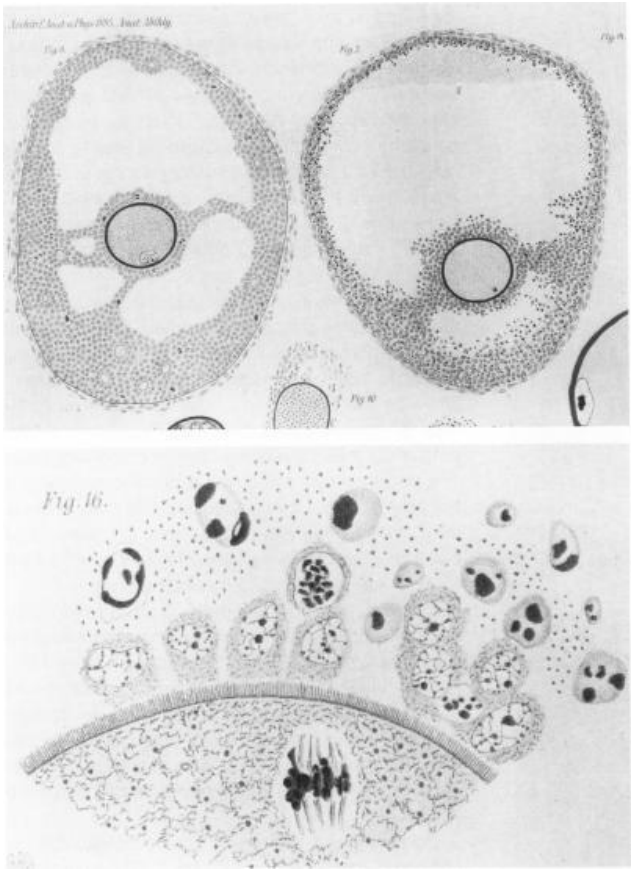
Mutovaný gen myostatin

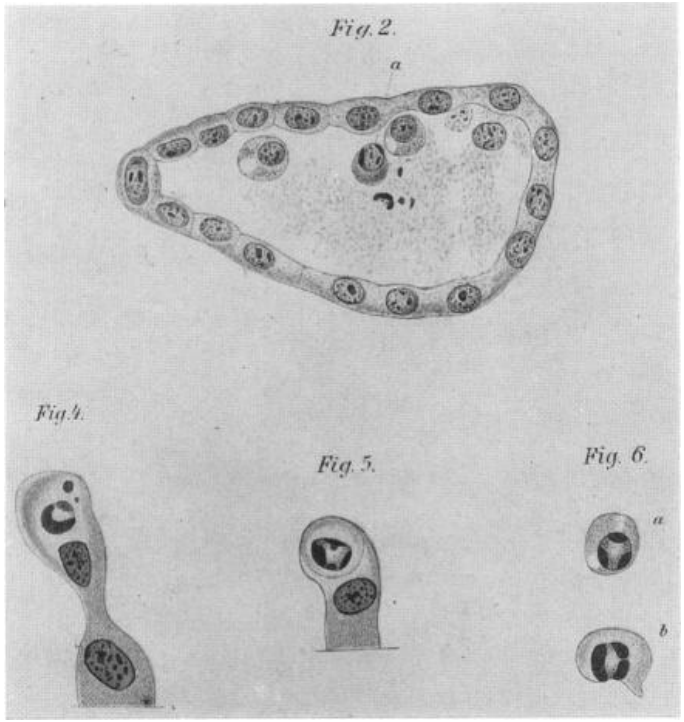




https://doi.org/10.3389/fphys.2019.00042

Programovaná buněčná smrt, apoptóza, (Greek ἀπόπτωσις, apóptōsis, "falling off„)





Apoptosis as seen in 1886 by a German medical student, Franz Nissen, in the lactating mammary gland

Apotosis as observed in 1885 by Flemming, who called it chromatolysis.

Sydney Brenner, Robert Horvitz a John E. Sulston, 2002

Buněčná smrt

- Vývoj, růst, regenerace a udržování homeostázy mnohobuněčných organizmů vyžaduje mechanismy umožňující řízenou destrukci nežádoucích buněk
  - Apoptóza – řízený způsob smrti
  - Nekróza – neřízená odpověď na akutní poškození
  - Nekroptóza – forma řízené buněčné smrti v odpovědi na specifické stimuly

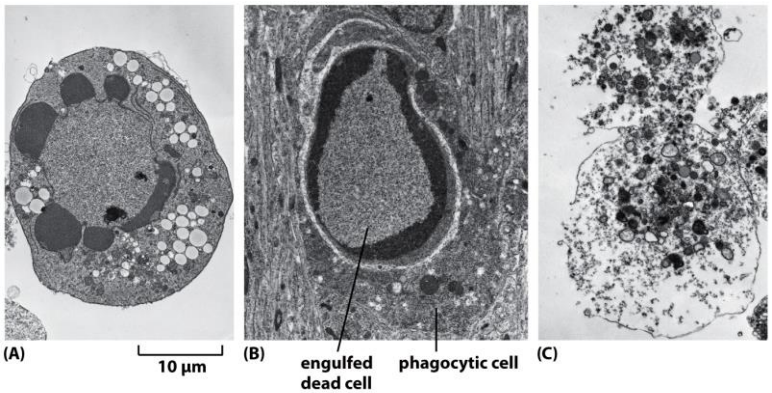


Figure 18-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

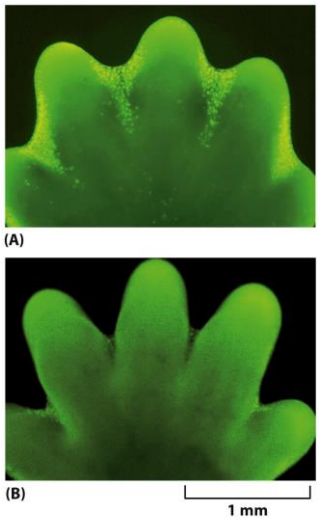


Figure 18-2 Molecular Biology of the Cell 6e (© Garland Science 2015)


N Engl J Med 2013; 369:e1

DEVELOPMENTAL BIOLOGY 56, 110-156 (1977)

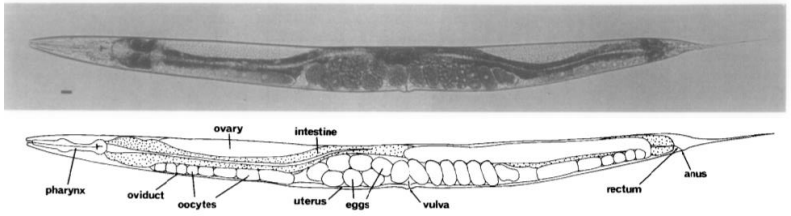
Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*

J. E. SULSTON AND H. R. HORVITZ

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England

Received August 23, 1976; accepted November 4, 1976

The number of nongonadal nuclei in the free-living soil nematode *Caenorhabditis elegans* increases from about 550 in the newly hatched larva to about 810 in the mature hermaphrodite and to about 970 in the mature male. The pattern of cell divisions which leads to this increase is essentially invariant among individuals; rigidly determined cell lineages generate a fixed number of progeny cells of strictly specified fates. These lineages range in length from one to eight sequential divisions and lead to significant developmental changes in the neuronal, muscular, hypodermal, and digestive systems. Frequently, several blast cells follow the same asymmetric program of divisions; lineally equivalent progeny of such cells generally differentiate into functionally equivalent cells. We have determined these cell lineages by direct observation of the divisions, migrations, and deaths of individual cells in living nematodes. Many of the cell lineages are involved in sexual maturation. At hatching, the hermaphrodite and male are almost identical morphologically; by the adult stage, gross anatomical differences are obvious. Some of these sexual differences arise from blast cells whose division patterns are initially identical in the male and in the hermaphrodite but later diverge. In the hermaphrodite, these cells produce structures used in egg-laying and mating, whereas, in the male, they produce morphologically different structures which function before and during copulation. In addition, development of the male involves a number of lineages derived from cells which do not divide in the hermaphrodite. Similar postembryonic developmental events occur in other nematode species.



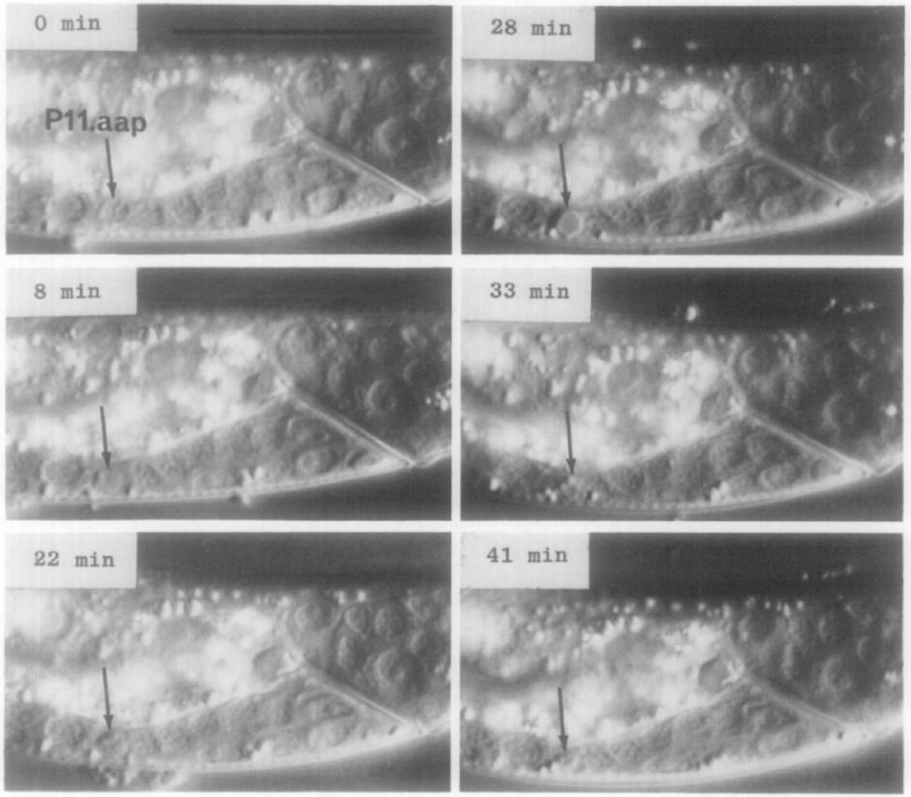


FIG. 8. Cell death. Sequential photographs of an L1 hermaphrodite, lateral view; Nomarski optics. The arrow points to the dying cell, P11.aap. Bar = 20 μm.

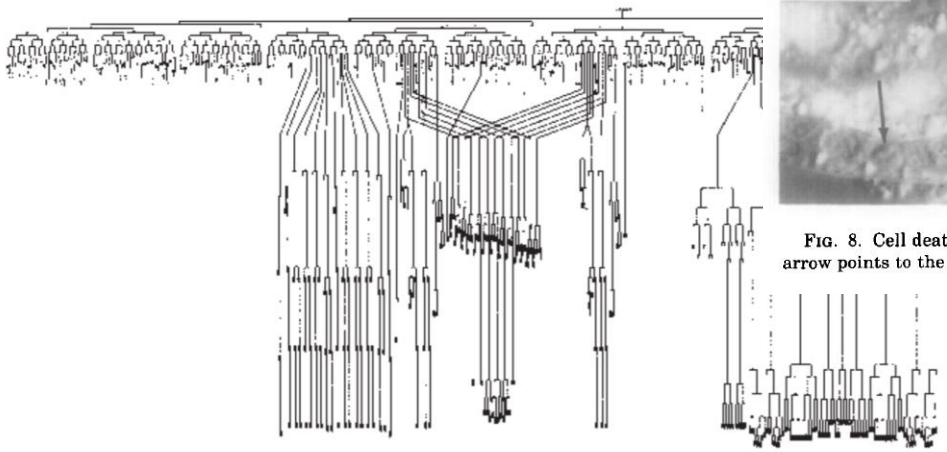


Figure 3. The complete cell lineage of *C. elegans*.

Cell, Vol. 44, 817–829, March 28, 1986, Copyright © 1986 by Cell Press

Genetic Control of Programmed Cell Death in the Nematode C. elegans

Hilary M. Ellis,* and H. Robert Horvitz
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

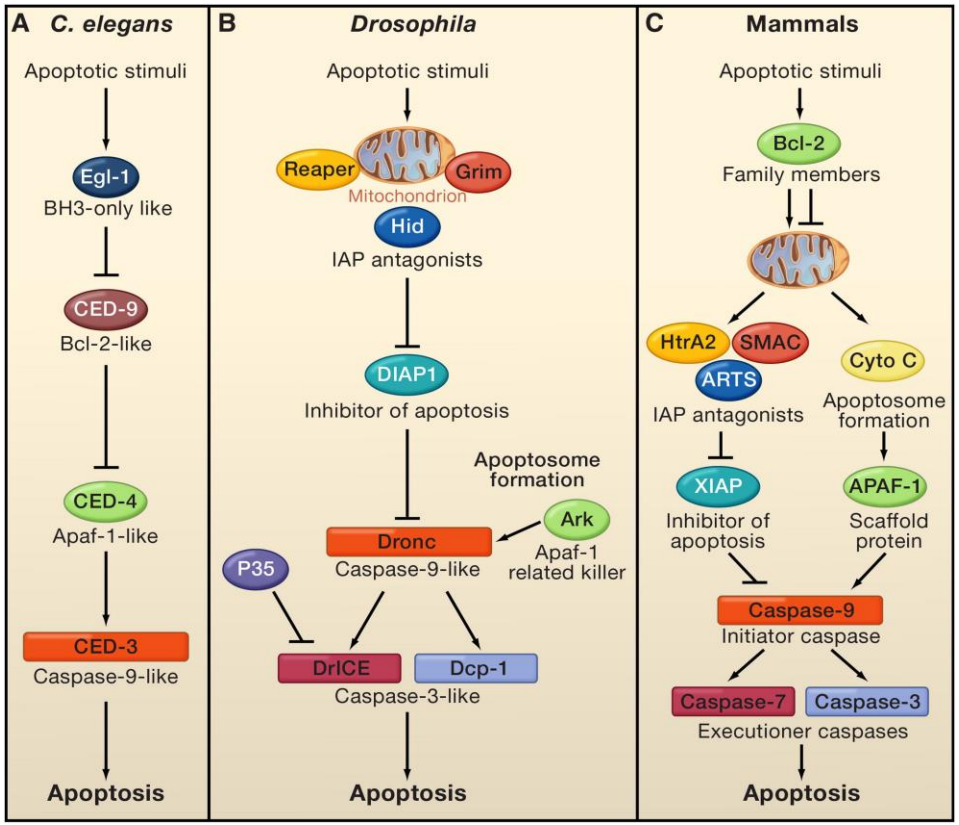
Summary

The wild-type functions of the genes *ced-3* and *ced-4* are required for the initiation of programmed cell deaths in the nematode Caenorhabditis elegans. The reduction or loss of *ced-3* or *ced-4* function results in a transformation in the fates of cells that normally die; in *ced-3* or *ced-4* mutants, such cells instead survive and differentiate, adopting fates that in the wild type are associated with other cells. *ced-3* and *ced-4* mutants appear grossly normal in morphology and behavior, indicating that programmed cell death is not an essential aspect of nematode development. The genes *ced-3* and *ced-4* define the first known step of a developmental pathway for programmed cell death, suggesting that these genes may be involved in determining which cells die during C. elegans development.

The phenomenon of programmed cell death raises a number of questions. Why are cells generated only to die? By what mechanisms do they die? How is it determined during development which cells die? C. elegans is well suited for studies that attempt to answer these questions. This nematode has fewer than 1000 somatic cells, and fixed patterns of cell divisions, migrations, and deaths generate individuals of invariant anatomy (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). Thus, specific developmental events can be examined reproducibly and at the resolution of single cells. In addition, the short generation time (3 days at 20°C) and large brood size of C. elegans facilitate genetic manipulations (Brenner, 1974; Herman and Horvitz, 1980).

We describe here the isolation and characterization of mutations that prevent the initiation of programmed cell death in C. elegans, causing cells that would normally die to survive instead. These mutations define two genes, *ced-3* and *ced-4*, that may be involved in determining which cells express the fate of programmed cell death.

Results



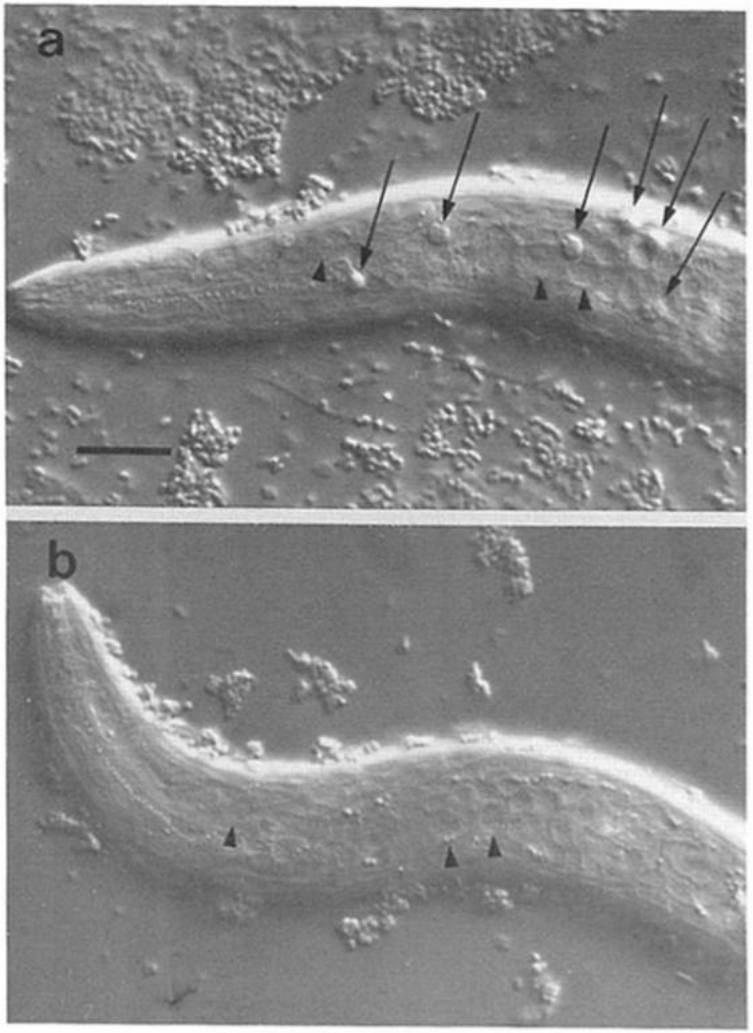


Figure 1. Absence of Cell Deaths in *ced-3* Animals

(a) Nomarski photomicrograph of a newly hatched *ced-1* larva. Arrows indicate dying cells. (b) Nomarski photomicrograph of a newly hatched *ced-1; ced-3* larva. Plane of focus is approximately that shown in (a). Arrowheads indicate several of the nuclei that can be seen in both (a) and (b). No cell deaths are seen in the *ced-1; ced-3* larva. Bar = 10 μ.

Základní mechanismus apoptózy

- Aktivace intracelulárních proteáz – kaspáz (caspases)
  - Vnitřní nebo vnější cestou
- Syntetizovány jako inaktivní prekurzory
  - Iniciační
    - Aktivují exekuční kaspázy
  - Exekuční
    - štěpí řadu substrátů (cytoskelet, proteiny jaderného obalu, atd.)
- Fragmentace DNA
  - Vznik apoptotických tělísek

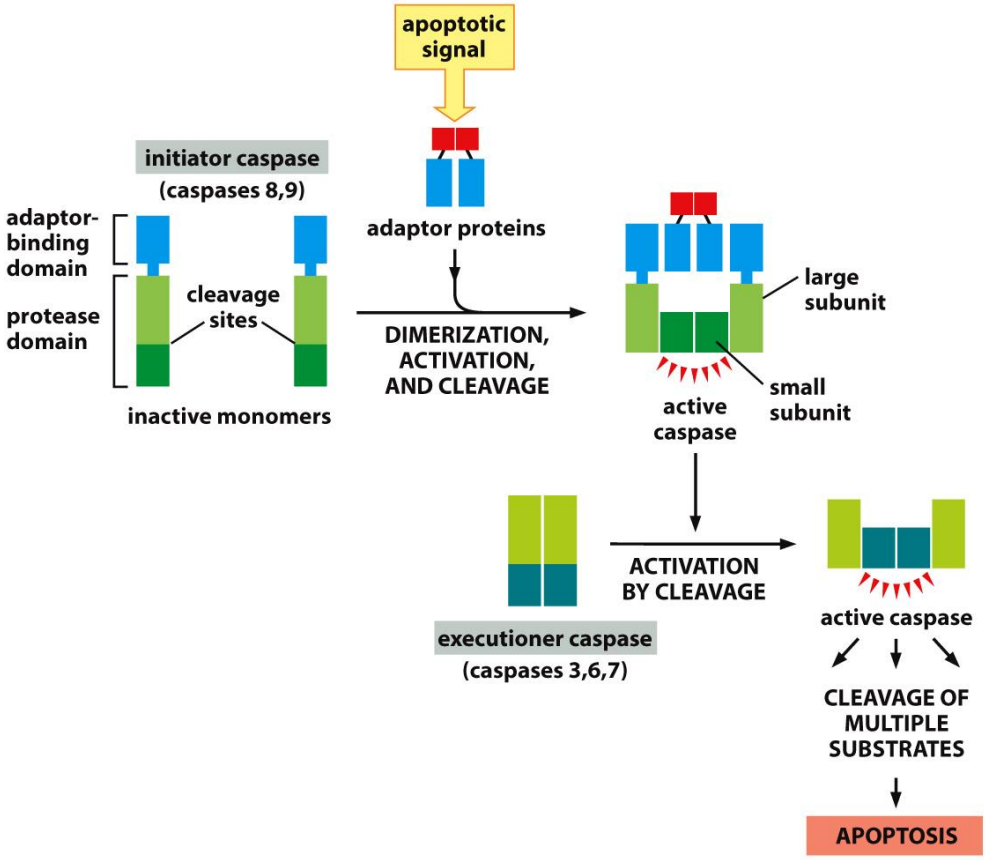


Figure 18-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

Fragmentace DNA během apoptózy

- CAD (Caspase-activated Dnase) endonukleáza je u intaktních buněk neaktivní díky asociaci se svým inhibitorem iCAD
- Exekuční kaspázy štěpí iCAD a dochází k aktivaci CAD
- Štěpení DNA mezi nucleosomy vede ke vzniku fragmentů DNA

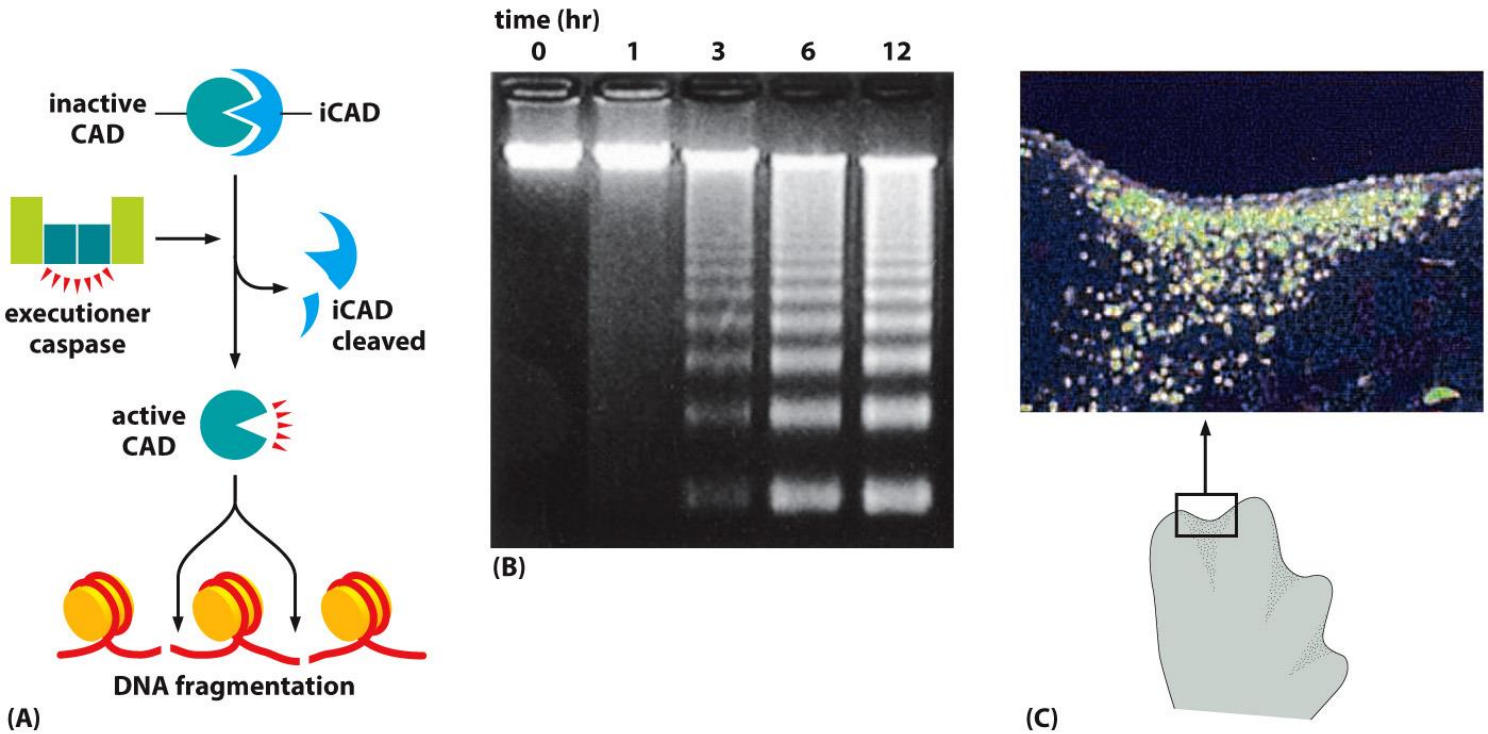


Figure 18-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

Vnější aktivace apoptotické dráhy

- receptory smrti (dead receptors), homotrimery
  - Ligand vázající extracelulární doména, intracelulární dead doména
- rodina cytokinů Tumor Necrosis Factor-α
  - TNF-α, FasL, TRAIL
- death-inducing signaling complex (DISC)
  - Kaspáza-8, FADD, death doména receptoru

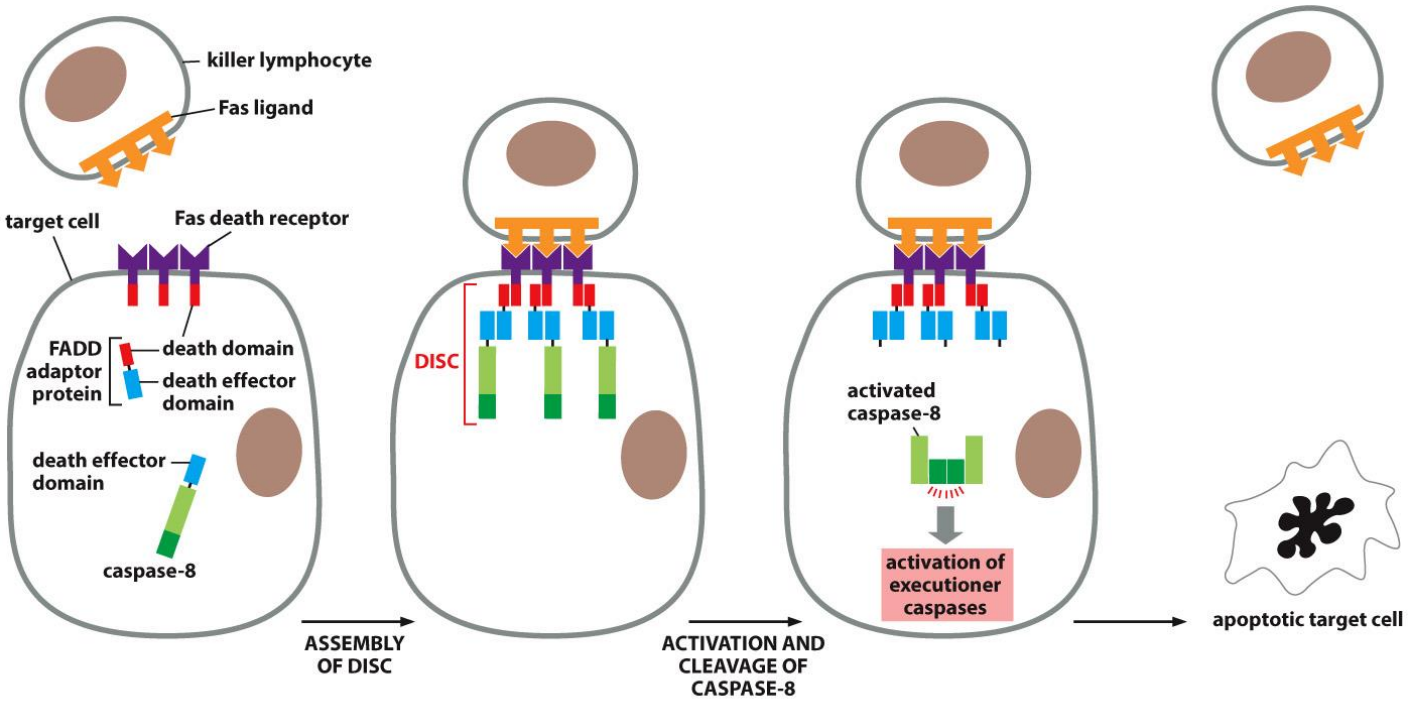


Figure 18-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

Role mitochondrií ve vnitřní dráze apoptózy

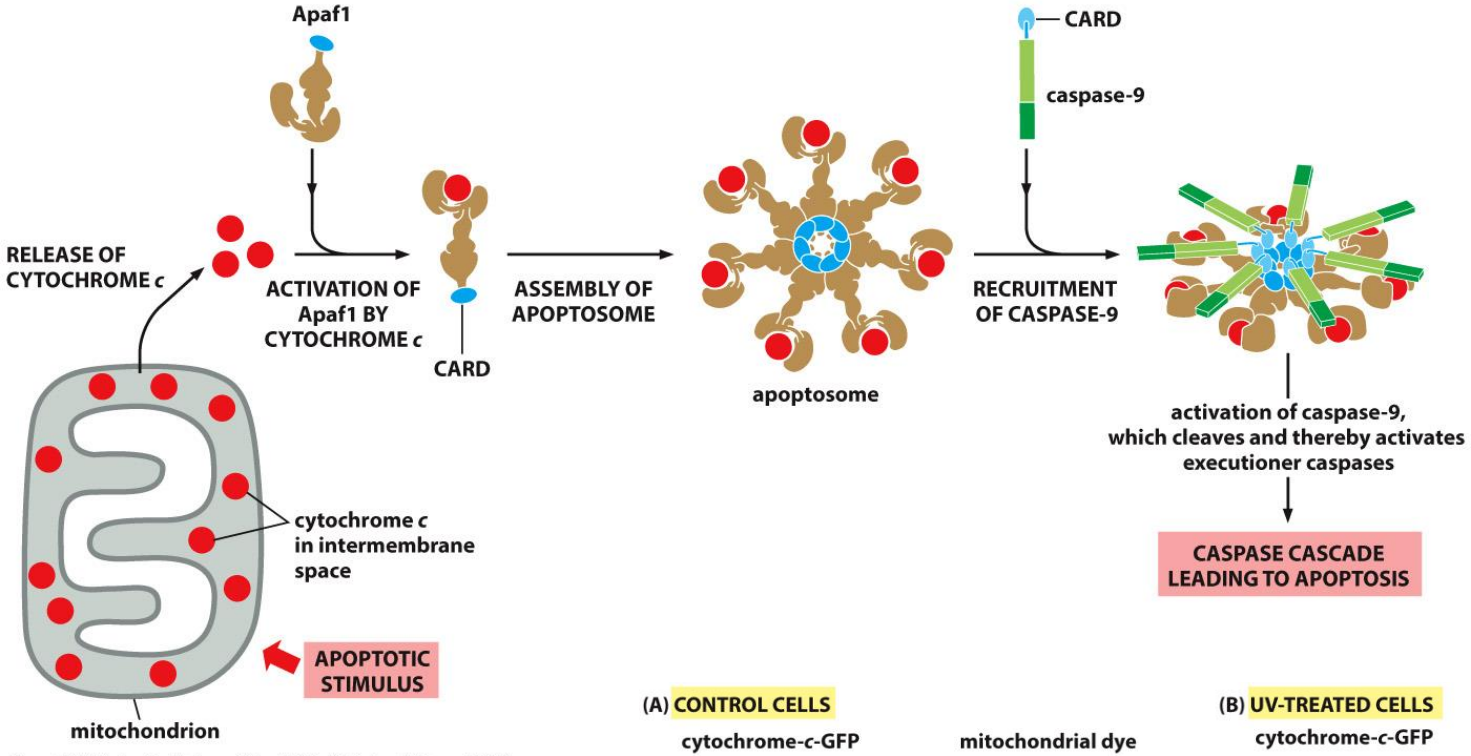


Figure 18-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

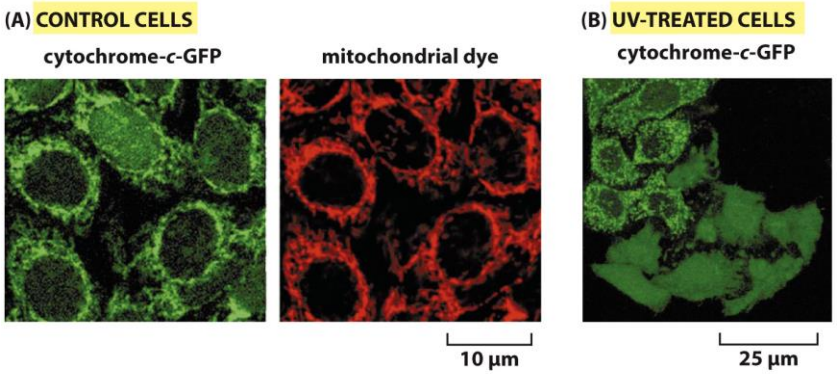


Figure 18-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

Bcl-2 rodina

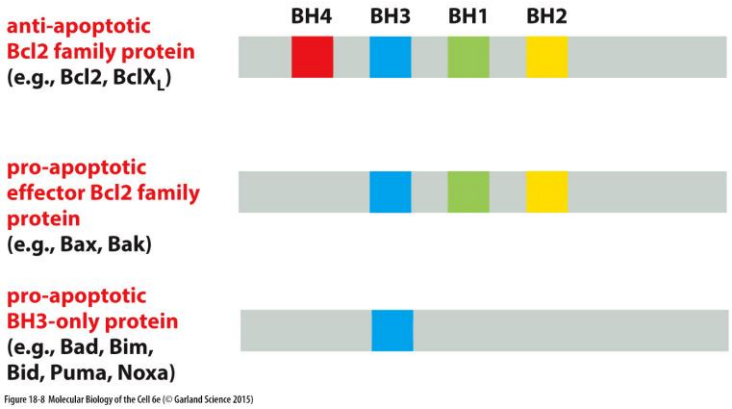


Figure 18-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

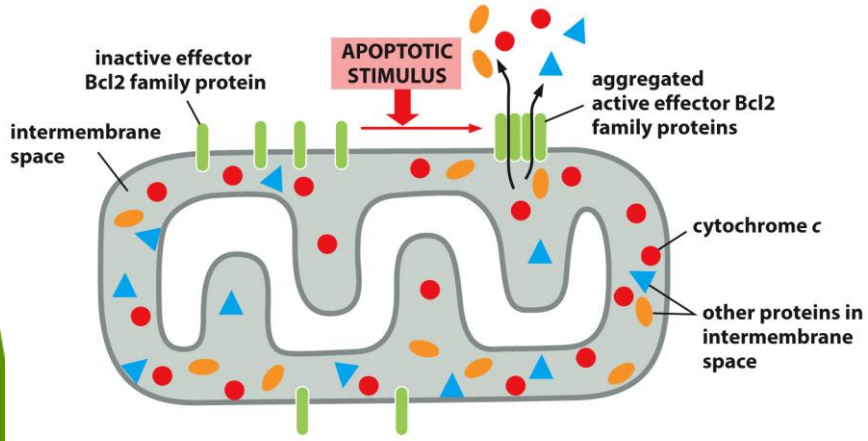


Figure 18-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

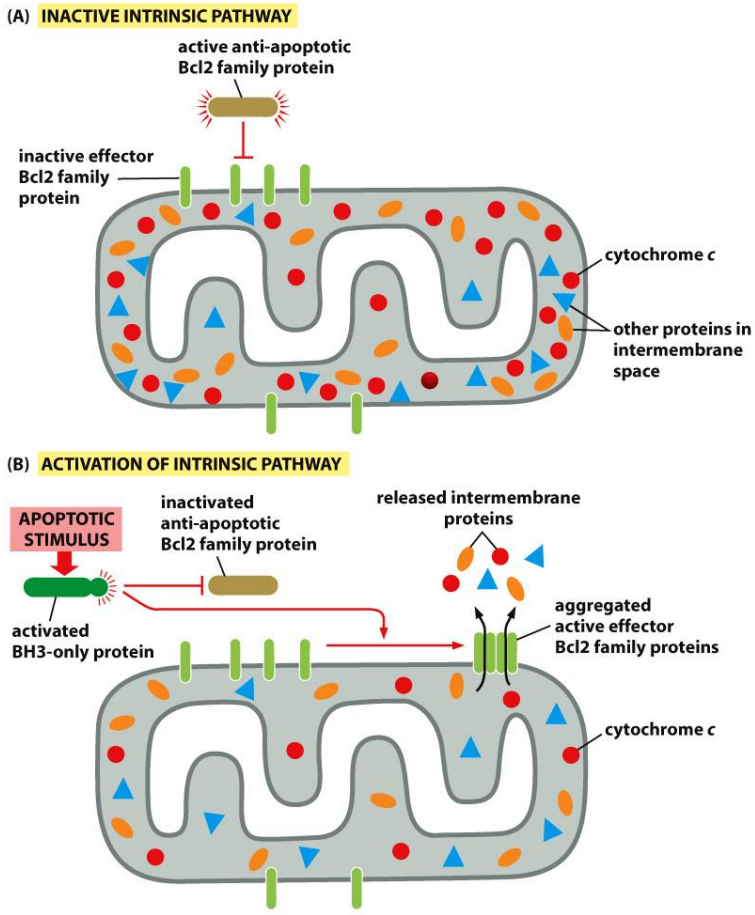


Figure 18-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

Role extracelulárních „survival“ faktorů v inhibici apoptózy

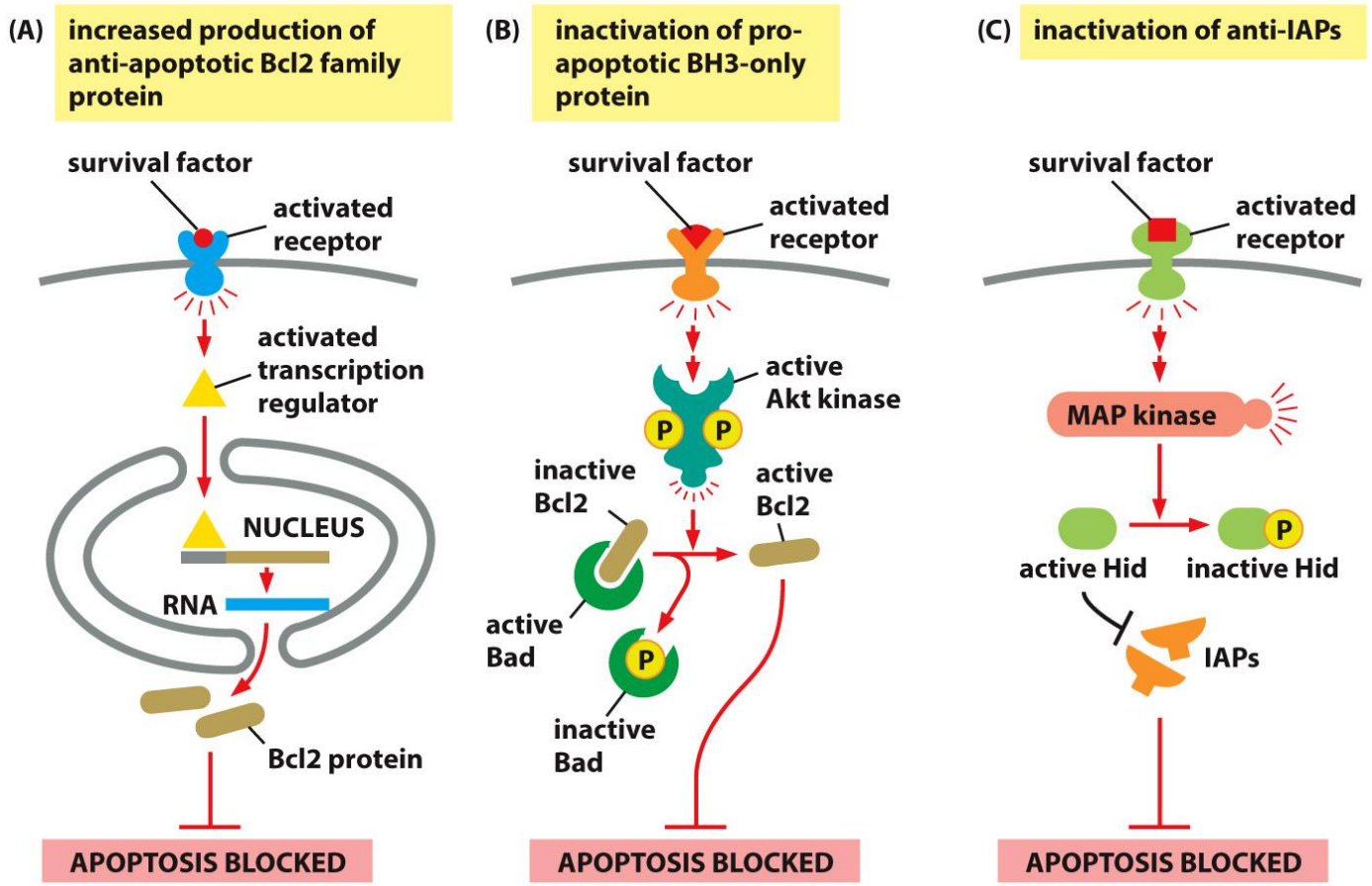
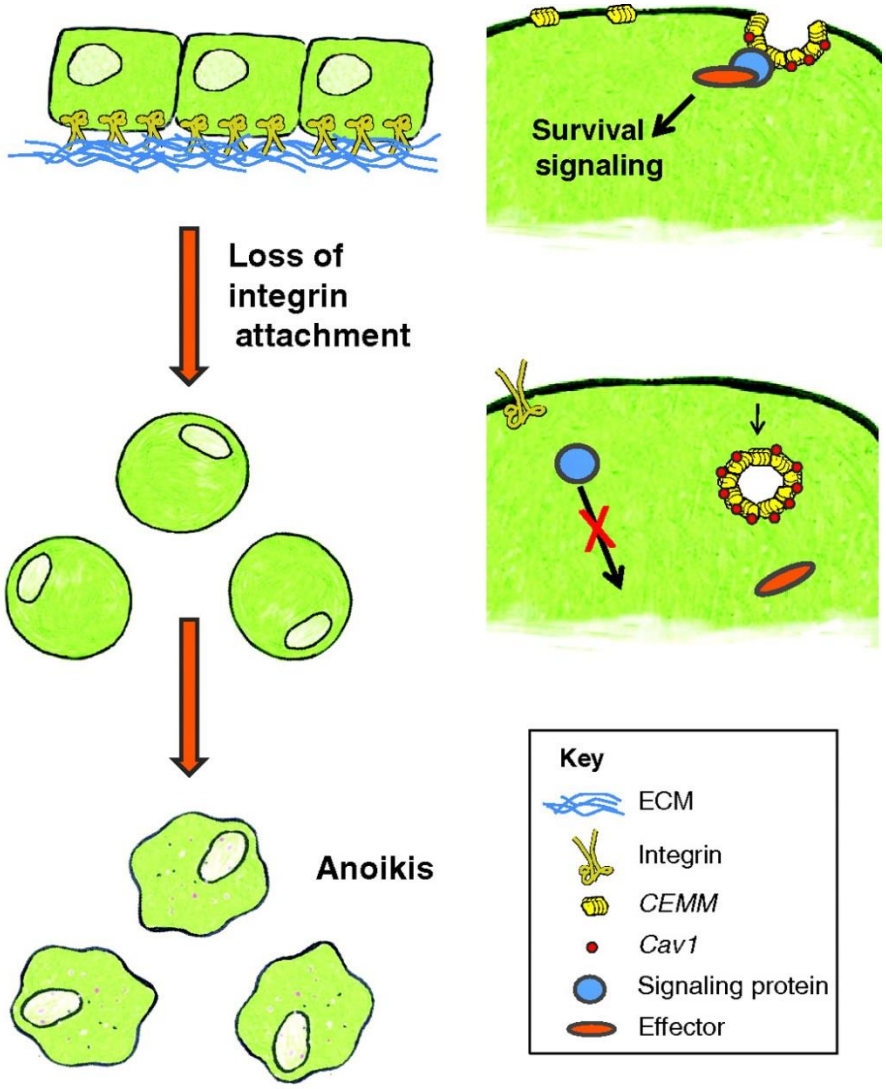


Figure 18-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

Anoikis (cell-detachment-induced apoptosis, Frisch and Francis, Journal of Cell Biology in 1994, Greek ἀν- "without", οἰκ- "house", and the suffix -ις.)



Marta C. Guadamillas et al. J Cell Sci 2011;124:3189-3197

Autofagie (Greek αὐτόφαγος autóphagos, "self-devouring"and κύτος kýtos, meaning "hollow“)

- Intracelulární recyklační systém
- Degradační proces – normální během vývoje, difereciace (restrukruralizace), adaptace na stres (hladovění, infekce)
- Neselektivní vs. selektivní (např. mitofagie)

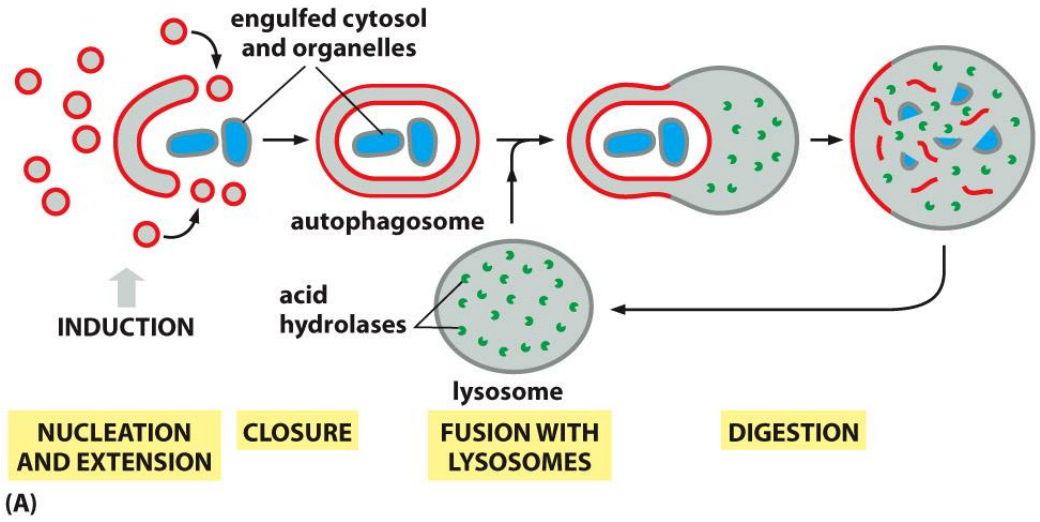
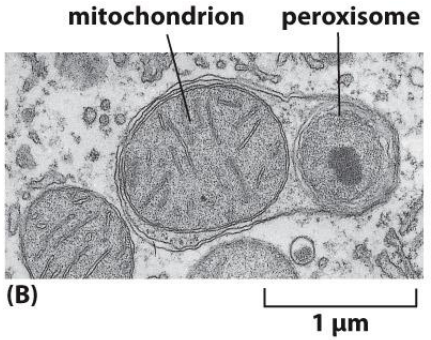


Figure 13-43 Molecular Biology of the Cell 6e (© Garland Science 2015)

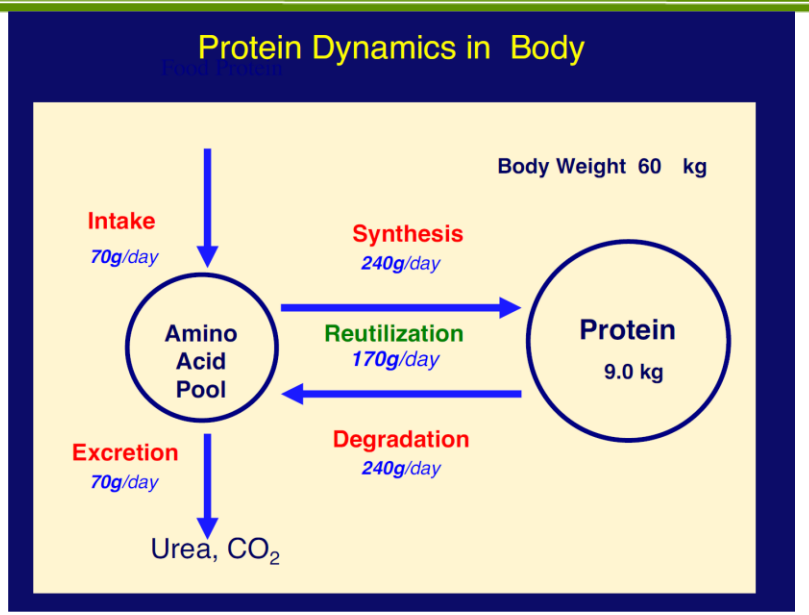


Yoshinori Ohsumi, 2016

Autofagie

Yoshinori Ohsumi, 2016

- Červené krvinky 3x10^6/s
- Hemoglobin 1x10^15/s
- Život je v rovnováze mezi syntézou a degradací proteinů
- Recyklace je esenciální proces pro život
  - Schopnost adaptace na hladovění jako kritický selekční faktor během evoluce



Using genetics to understand autophagy

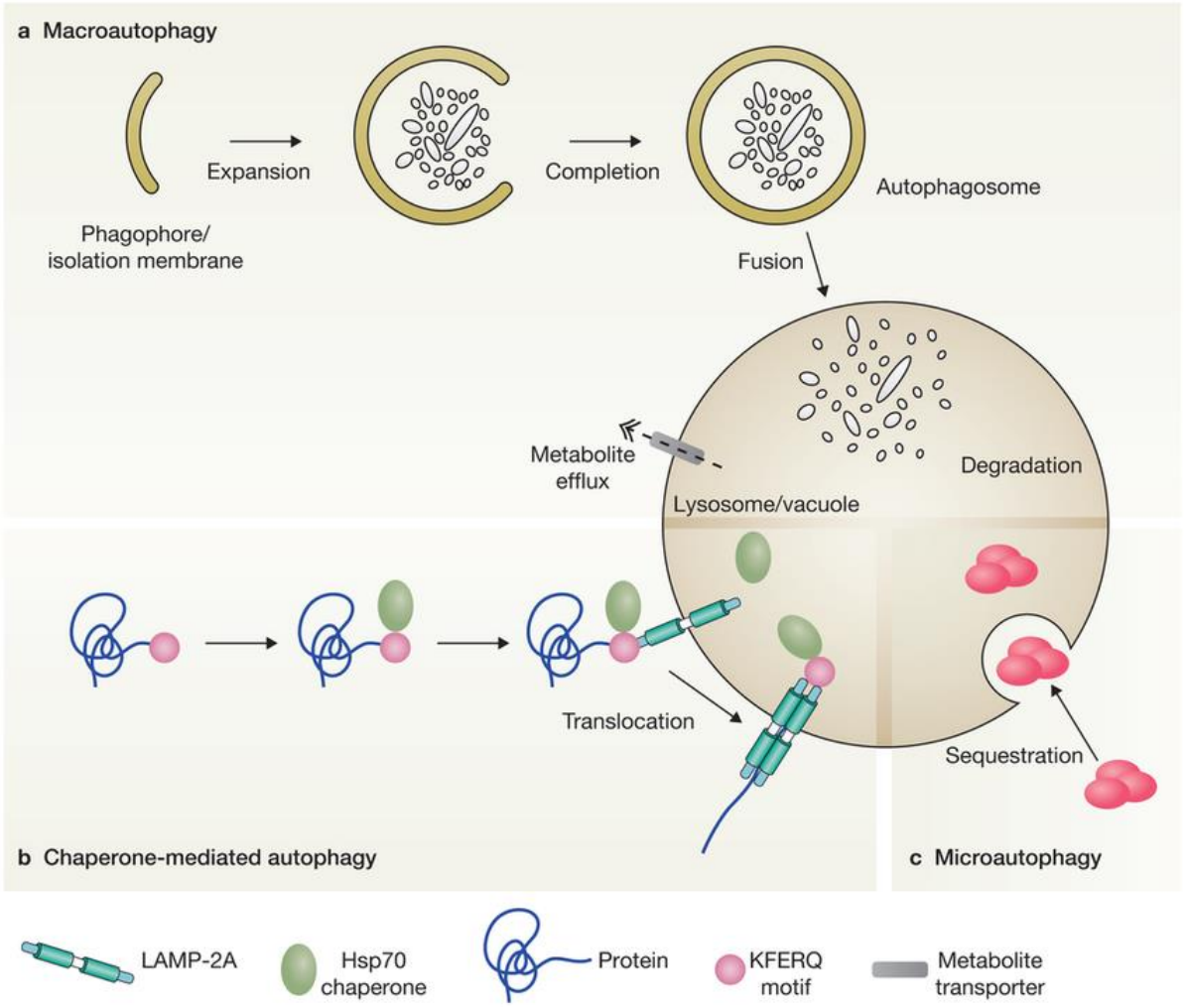
cdc mutants : cell cycle
By Lee H. Hartwell, Nobel Prize 2004
sec mutants: secretory pathway
By Randy W. Schekman, Nobel Prize 2013

Isolation of autophagy-defective mutants

What is the phenotype of the mutants?
Microscopic screen : no accumulation of autophagic bodies in the vacuole

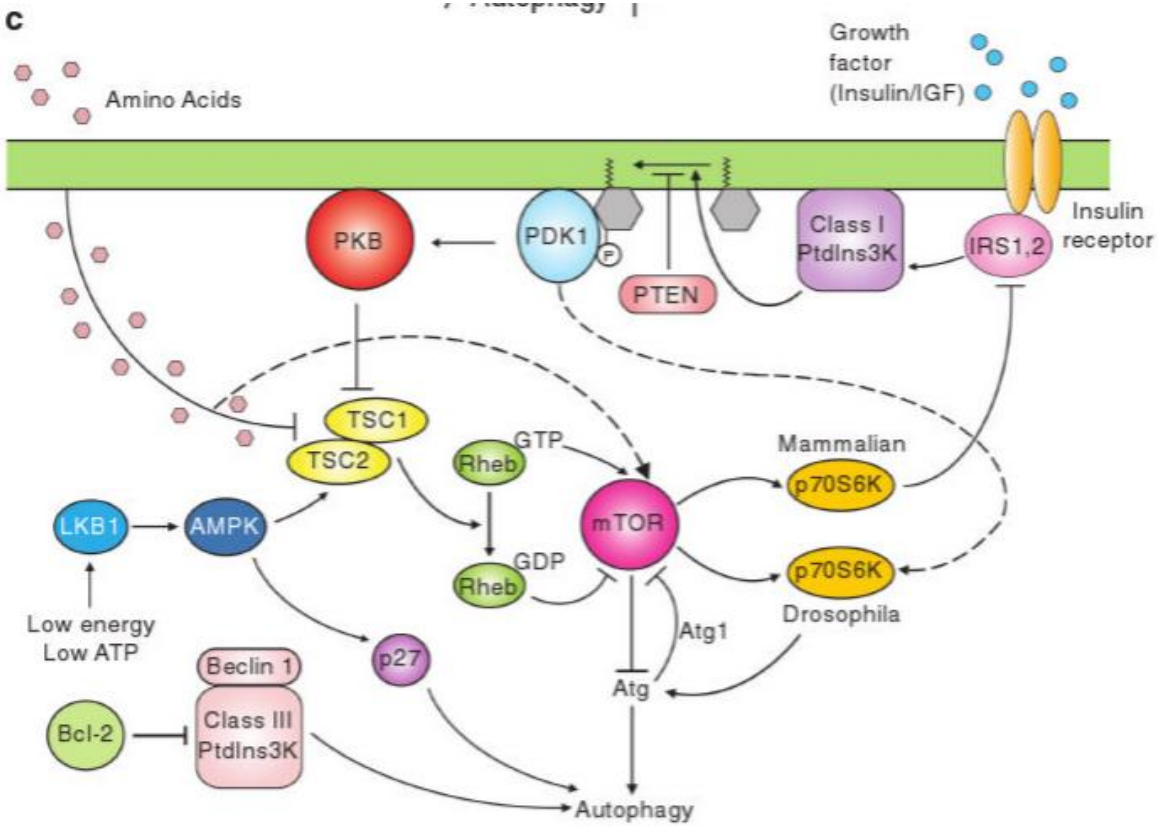
Only one mutant, *apg1*-1 (*atg1*)

Typy autofagie



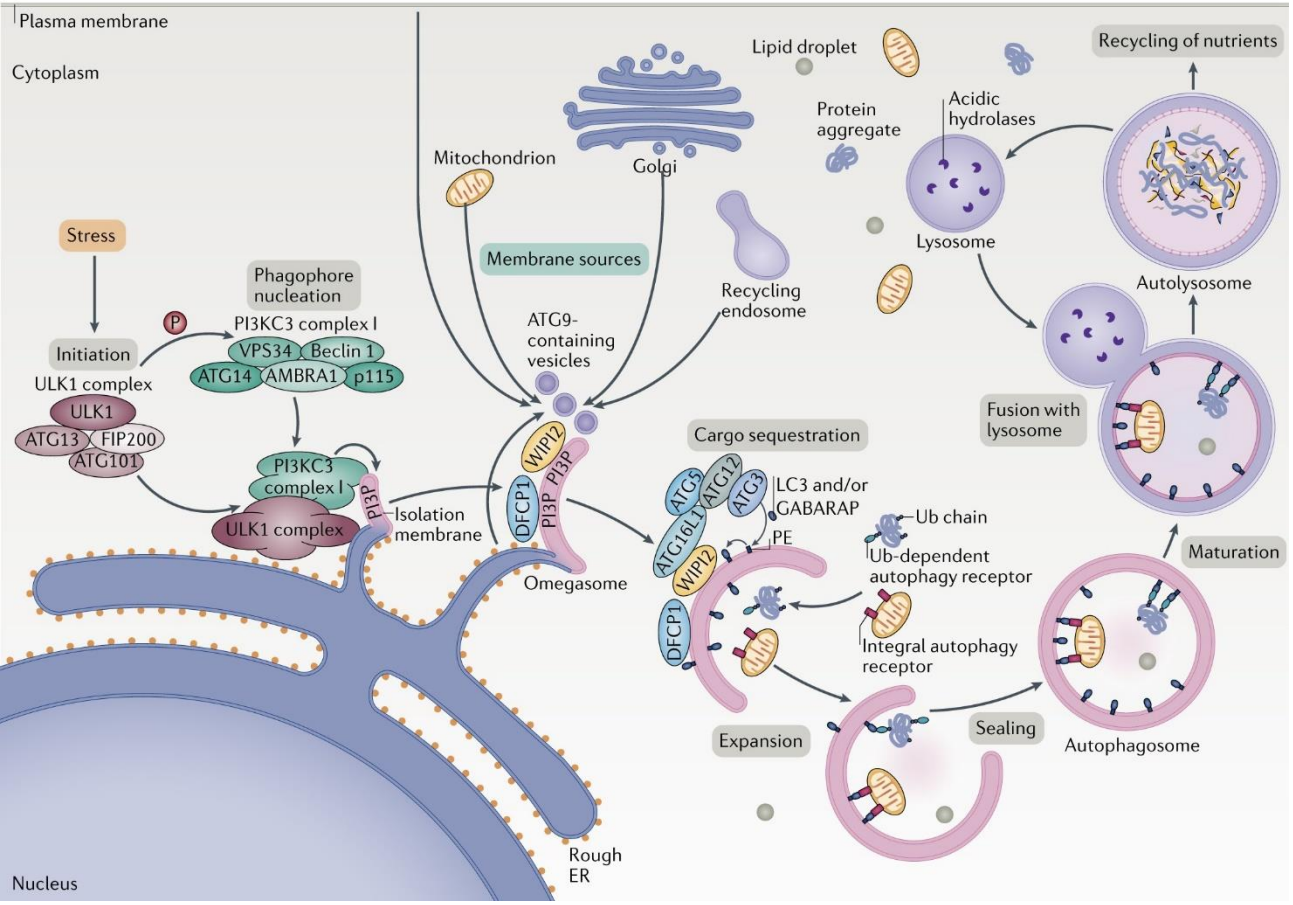
Jul 2013 · Nature Cell Biology

Mechanismus autofagie



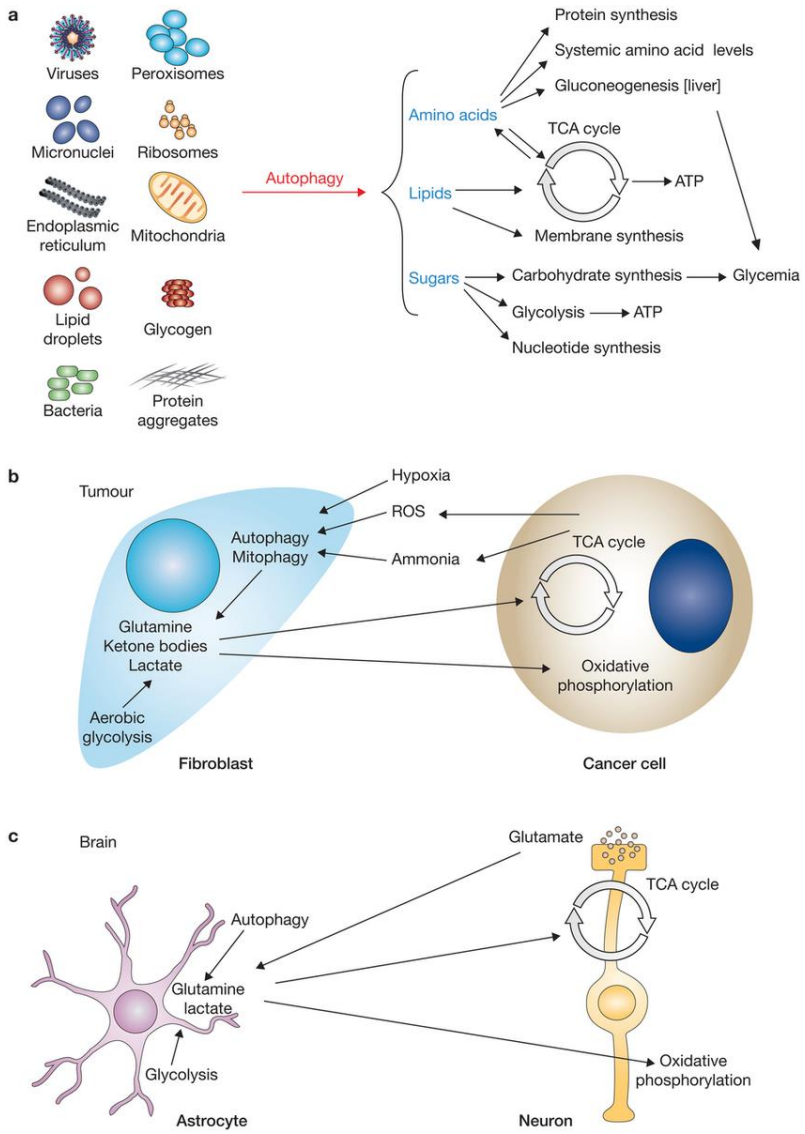
- **mTOR, mammalian target of rapamycin – esenciální Ser/Thr kináza, „pro-survival“ signál**
- **Rheb – malá GTPáza (hormony)**
- **Rag– malá GTPáza (aminokyseliny)**

Mechanismus autofagie



Iniciace phagophoru
- ULK1, ATG, Beclin
Expanze phagophoru
- ATG
Sequestrace karga
- Ubiquitin, cardiolipin, LC3
Uzavření membrány
- LC3
Maturace Autophagozomu
- ATG
Fúze s lysozomem
- LC3

Autofagie a metabolismus



Jul 2013 · Nature Cell Biology

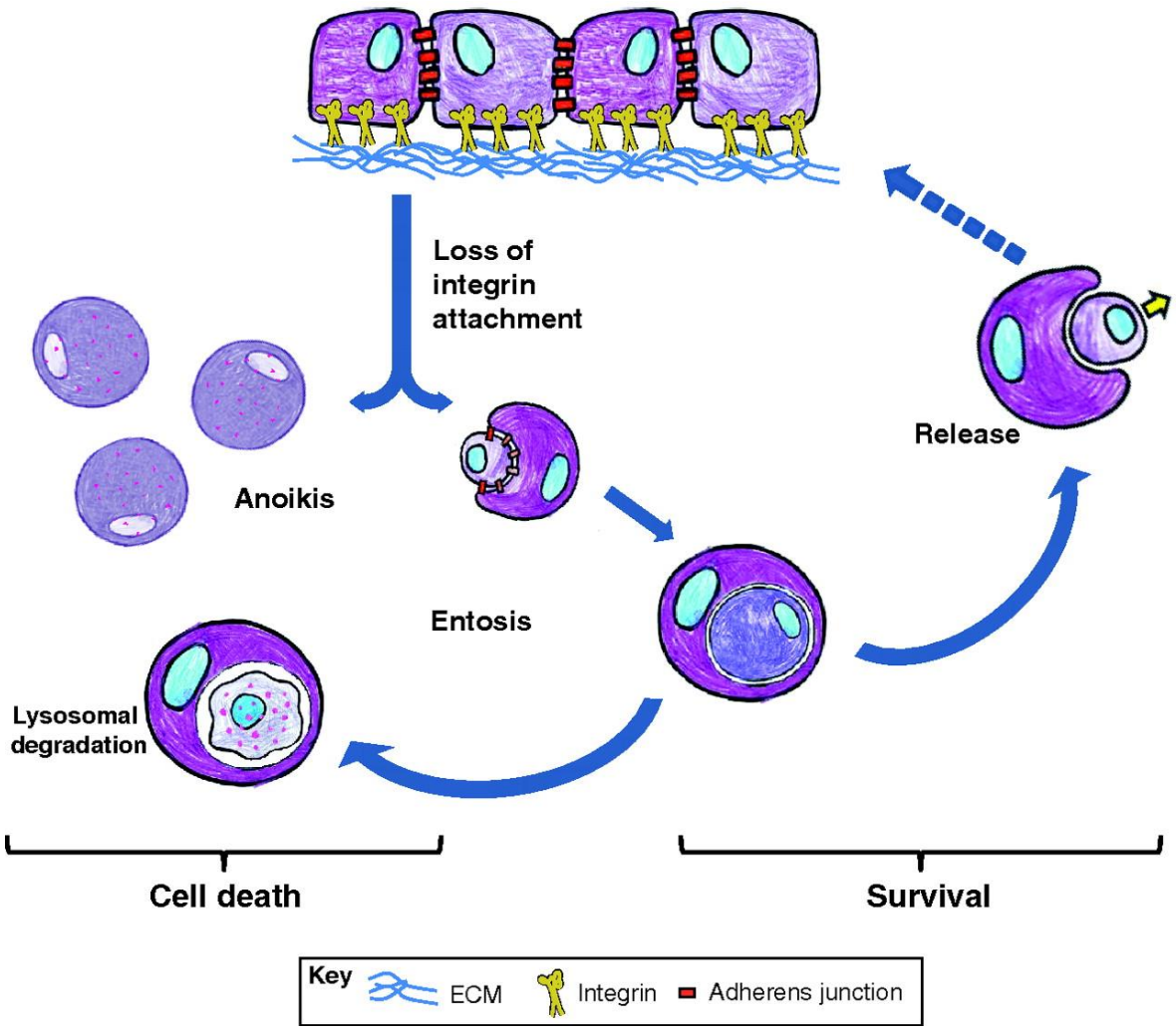
Onemocnění spojená s poruchami autofagie

Table 1 Examples of human diseases associated with defective autophagy[a]

	Primary autophagy defects	Secondary autophagy defects	Both
Organ-specific	**Lung** Asthma (*ATG5*) **Heart** Ischemia/reperfusion **Intestine** Crohn's disease (*ATG16L1*, *NOD2*, *IRGM*) Ulcerative colitis (*SMURF1*) **Bone** Paget's disease of bone (*SQSTM1*) **Central nervous system** Static encephalopathy of childhood with neurodegeneration in adulthood (SENDA) (*WDR45*)	**Central nervous system** Huntington's disease Tauopathies Stroke **Heart** Cardiomyopathy **Skeletal muscle** Muscle atrophy Autophagic vacuolar myopathies Collagen VI–related myopathies Inclusion body myositis **Liver** α1-Antitrypsin deficiency Nonalcoholic fatty liver disease (NAFLD)	**Central nervous system** Alzheimer's disease (*PSEN1*) Parkinson's disease (*PINK1*, *PARK2*) Amyotrophic lateral sclerosis (*SQSTM1*)
Multisystemic	**Cancer** Breast (*BECN1*) Ovarian (*BECN1*) Prostate (*BECN1*) Brain (*PARK2*) Lung (*PARK2*) Gastric (*UVRAG*, *PARK2*) **Immune disease** Vici syndrome (*EPG5*) *Mycobacterium tuberculosis* infection (*IRGM*) *Mycobacterium leprae* infection (*NOD2*) **Lysosomal disease** Danon disease (*LAMP2*)	**Cancer** Carcinoma Sarcoma **Immune disease** Infection **Metabolic dysfunction** Type II diabetes Metabolic syndrome Obesity **Vascular disease** Ischemia/reperfusion **Lysosomal disease** Pompe disease Gaucher disease	**Immune disease** Lupus erythematosus (*ATG5*)

Annu. Rev. Pharmacol. Toxicol. 2017. 57:375–98

Entóza (Greek ἐντός entos, "within" and -ωσις -osis, "disease")



Marta C. Guadamillas et al. J Cell Sci 2011;124:3189-3197

Diferenciace, obnova tkání, kmenové buňky

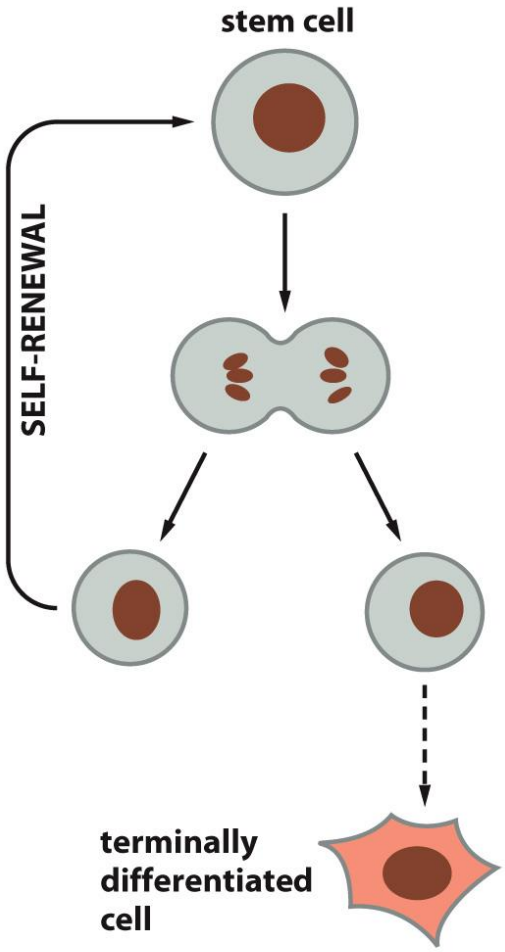


Figure 22-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

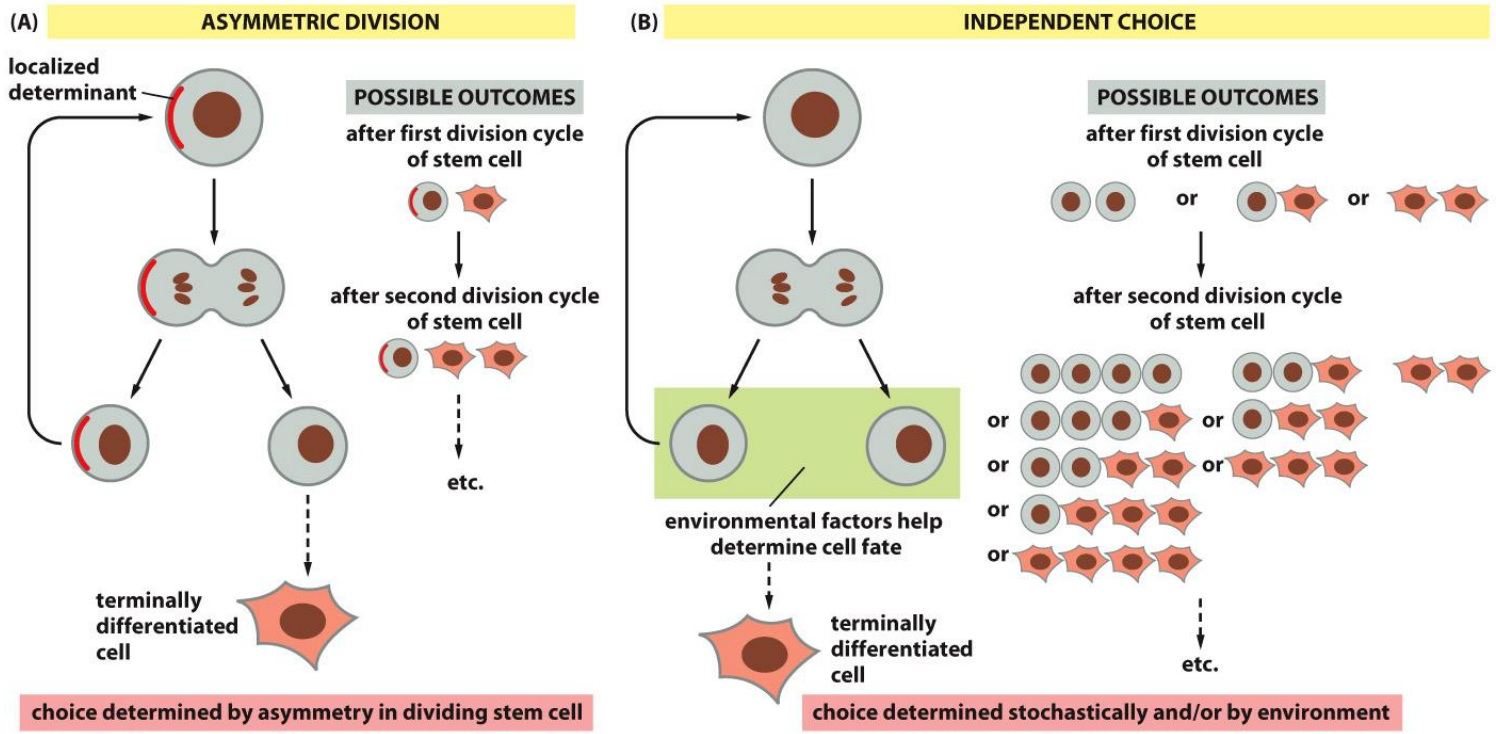


Figure 22-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

Střevní epitel

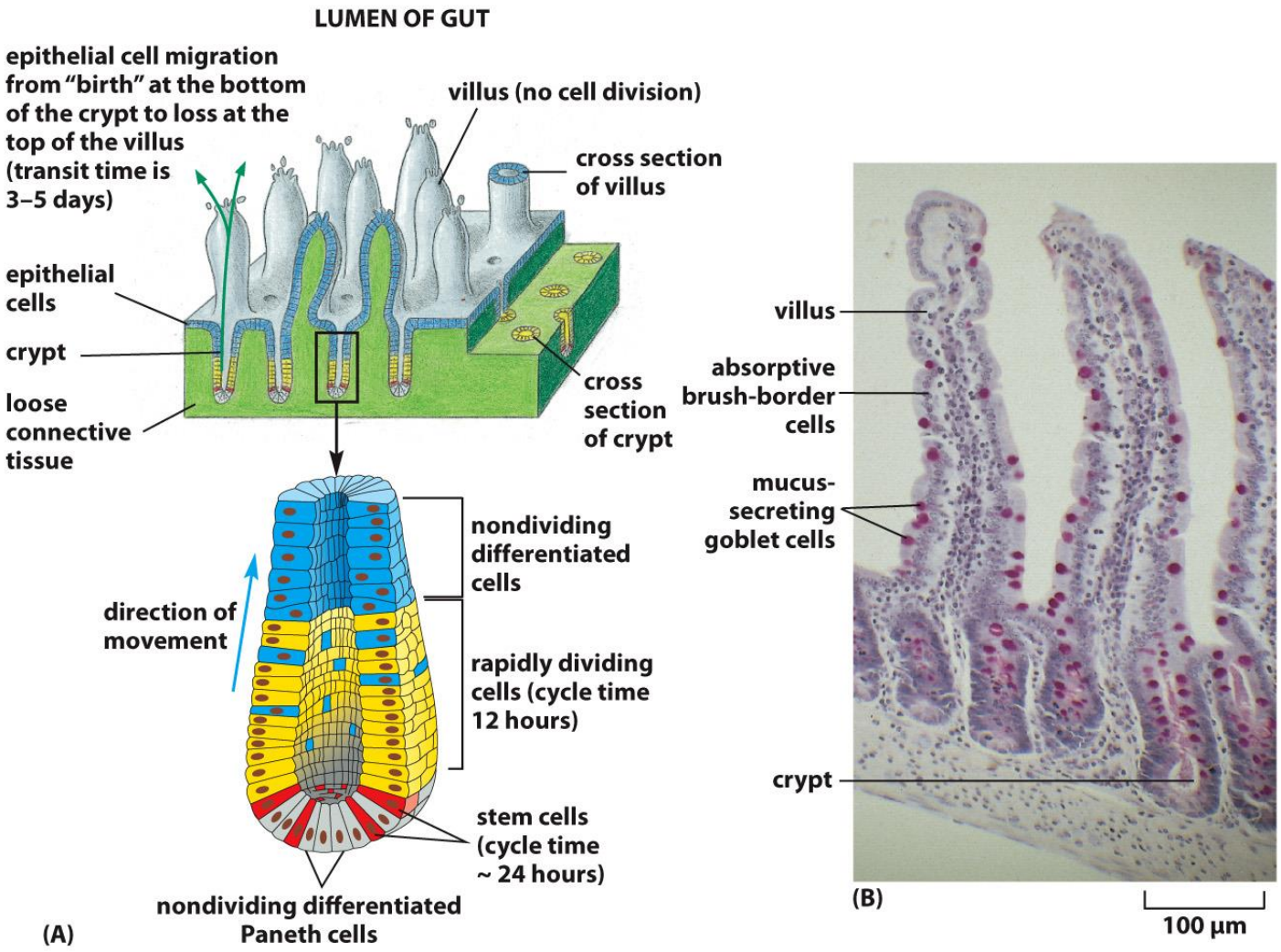


Figure 22-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

Hlavní buněčné typy střevního epitelu

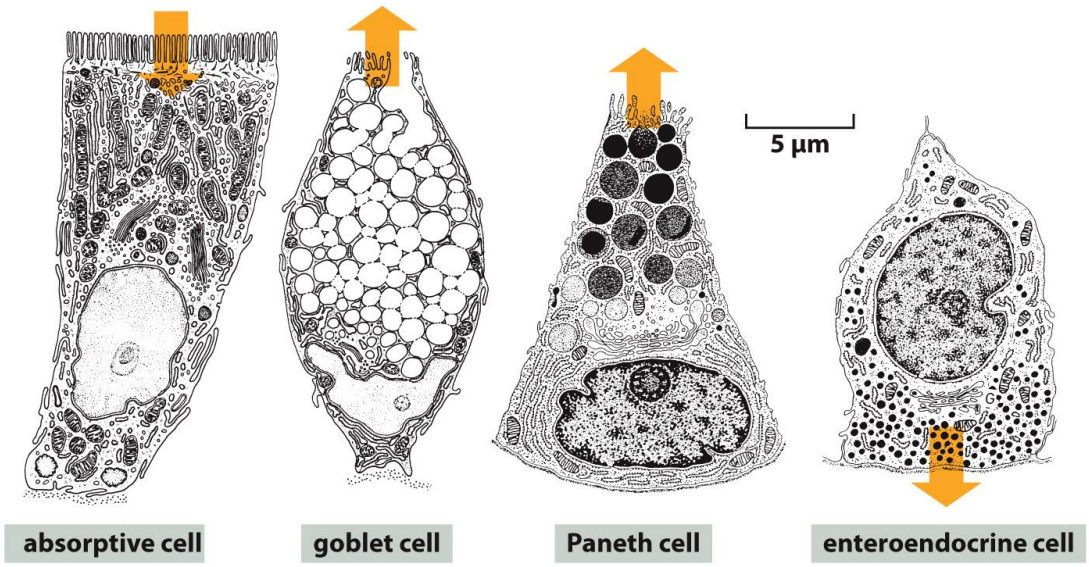


Figure 22-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

absorptive cell	goblet cell	Paneth cell	enteroendocrine cell
Největší počet – 10x Plocha 30x	Sekretují hlen	Sekretují cryptdin (defensin)	Sekretují cholescystokinin

Clevers group: The intestinal Crypt

- https://www.youtube.com/watch?v=NlT1VYqMzgc

Hans Clevers (Hubrecht I., UU) 1: Discovery and Characterization of Adult Stem Cells in the Gut
https://www.youtube.com/watch?v=HgVivkoA7UA

Stem cells - Dr Jekyll or Mr Hyde: Hans Clevers at TEDxAmsterdam
https://www.youtube.com/watch?v=oMEihKffBJI

Notch & Wnt signálizace udržuje kmenovost a řídí diferenciaci střevního epitelu

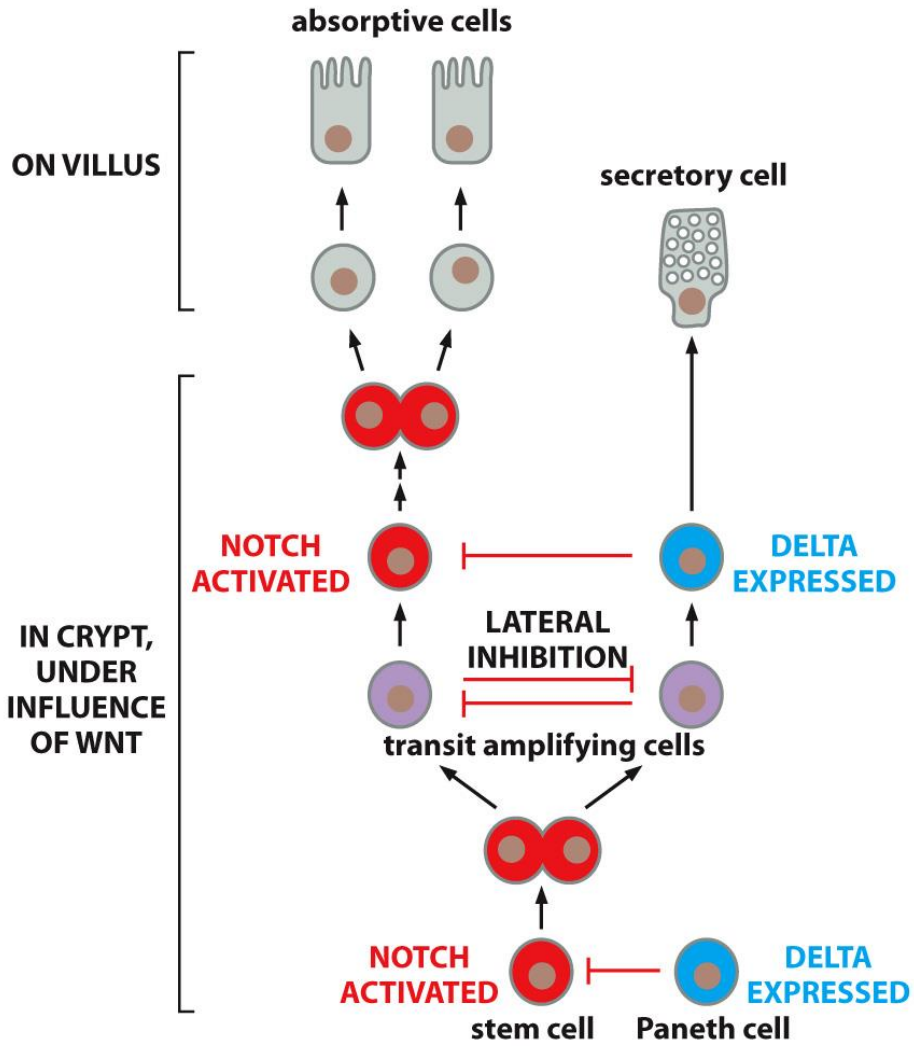
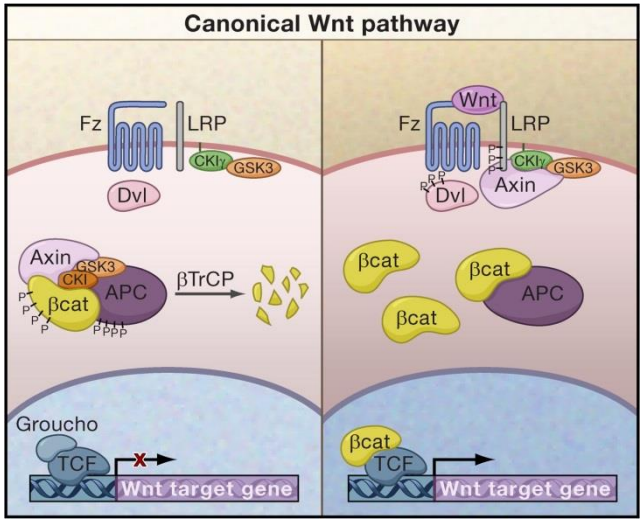
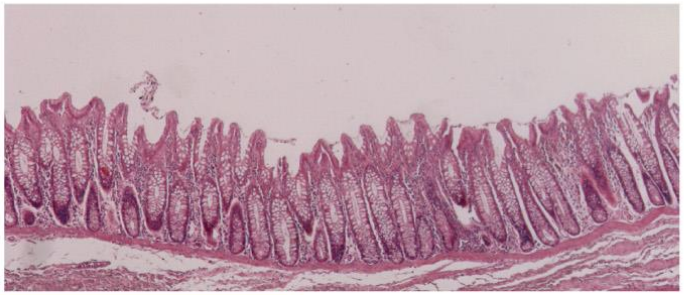


Figure 22-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

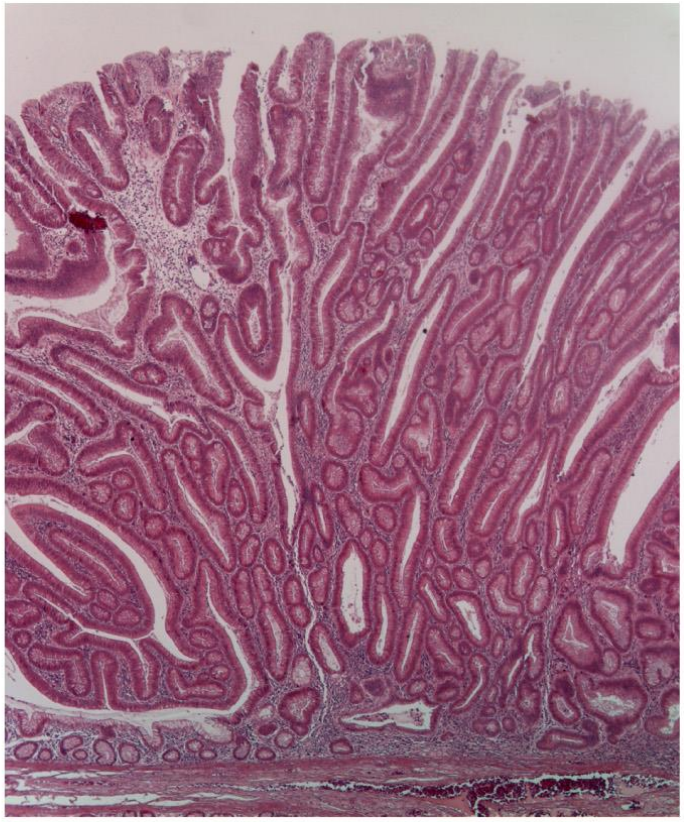
Adenom střeva

- Pacient s mutací v APC (adenomatous polyposis coli)- > pernamentní aktivace Wnt





NORMAL COLON



ADENOMA

200 µm

Figure 22-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

GASTROENTEROLOGY 2007;132:628–632

The Intestinal Wnt/TCF Signature

LAURENS G. VAN DER FLIER,* JACOB SABATES-BELLVER,‡ IRMA OVING,* ANDREA HAEGEBARTH,* MARIAGRAZIA DE PALO,§ MARCELLO ANTI,§ MARIELLE E. VAN GIJN,* SASKIA SUIJKERBUIJK,* MARC VAN DE WETERING,* GIANCARLO MARRA,‡ and HANS CLEVERS*

*Hubrecht Institute, Netherlands Institute for Developmental Biology and Centre for Biomedical Genetics, Utrecht, The Netherlands; ‡Institute of Molecular C Research, University of Zurich, Zurich, Switzerland; and §the Gastroenterology Unit, Belcolle Hospital, Strada Sammartinese, Viterbo, Italy

Background & Aims: **In colorectal cancer, activating mutations in the Wnt pathway transform epithelial cells through the inappropriate expression of a TCF4 target gene program, which is physiologically expressed in intestinal crypts.** ***Methods:*** **We have now performed an exhaustive array-based analysis of this target gene program in colorectal cancer cell lines carrying an inducible block of the Wnt cascade. Independently, differential gene-expression profiles of human adenomas and adenocarcinomas vs normal colonic epithelium were obtained.** ***Results:*** **Expression analyses of approximately 80 genes common between these data sets were performed in a murine adenoma model. The combined data sets describe a core target gene program, the intestinal Wnt/TCF signature gene set, which is responsible for the transformation of human intestinal epithelial cells.** ***Conclusions:*** **The genes were invariably expressed in adenomas, yet could be subdivided into 3 modules, based on expression in distinct crypt compartments. A module of 17 genes was specifically expressed at the position of the crypt stem cell.**

lowed by transient promoter assays. DNA array t ogy allows the assessment of differential messeng (mRNA) expression on a genome-wide scale.

Materials and Methods

Cell Culture

CRC cell lines LS174T and DLD1, stably exp inducible dominant-negative (dn)TCF1 or dnTCF generated as previously described.[8] The Wnt pathw ity in the CRC cells was determined as described pre using the optimized TCF reporter pTopGlow and i tive control pFopGlow, constructed in our laborat

Oligonucleotide Microarray Analysis of Cell Lines

RNA was isolated after 10 and 20 hours indu the dnTCFs. RNA quality was assessed using capi electrophoresis (BioAnalyzer; Agilent Technologies plementary DNA (cDNA) synthesis and labeling w formed according to Affymetrix (Santa Clara, CA lines. cRNA was synthesized and labeled with Aff One-cycle Target Labeling kit, and hybridized on Aff

Table 1. Target Genes Down-Regulated in All 4 CRC Cell Lines on Over Expression of dnTCFs

			LS174T dnTCF1		LS174T dnTCF4		DLD1 dnTCF1		DLD1	
Gene symbol	References	Affymetrix ID	10 h	20 h	10 h	20 h	10 h	20 h	10 h	20 h
ASCL2	18	229215_at	−4.3	−24	−2.3	−15	−2.8	−7.5	−3.0	−4.0
AXIN2	14–16	222696_at	−2.5	−4.9	−2.3	−2.6	−2.8	−3.5	−2.8	−3.7
BMP4[a]	21	211518_s_at	−2.0	−2.5	−2.3	−4.9	−3.0	−3.2	−1.6	−2.3
C1orf33[a]		220688_s_at	−1.7	−3.2	−2.3	−2.8	−1.7	−2.0	−1.6	−2.0
HIG2	20	1554452_a_at	−1.7	−4.0	−2.1	−3.2	−1.4	−2.6	−1.9	−2.0
HSPC111		203023_at	−1.5	−3.0	−2.3	−2.5	−1.4	−1.6	−1.9	−1.7
HSPC111		214011_s_at	−1.6	−3.0	−2.3	−2.3	−1.5	−1.9	−1.6	−1.6
KITLG[a]		226534_at	−2.6	−3.0	−2.3	−2.3	−4.3	−2.6	−1.6	−2.8
LGR5[a]	19	213880_at	−4.3	−9.8	−2.3	−3.5	−7.5	−7.5	−2.1	−3.2
MYC[a]	17	202431_s_at	−2.1	−2.3	−2.3	−1.6	−2.8	−3.0	−2.1	−2.3
NOL1		214427_at	−1.7	−2.8	−2.3	−2.1	−1.3	−1.9	−1.6	−1.6
PPIF		201490_s_at	−1.4	−1.6	−2.3	−2.0	−1.5	−1.9	−1.5	−1.5
SOX4	22	201416_at	−1.3	−2.1	−2.3	−2.1	−3.0	−3.2	−2.1	−2.0
WDR71		218957_s_at	−2.8	−17	−2.3	−2.8	−1.9	−3.7	−2.0	−3.7
ZIC2		223642_at	−1.7	−2.3	−2.3	−2.3	−1.4	−2.1	−1.7	−1.9
ZNRF3[a]		226360_at	−4.6	−3.2	−2.3	−2.5	−3.5	−3.2	−2.1	−2.8

NOTE. Fold changes of genes down-regulated in all 4 CRC cell lines on over expression of dnTCFs are shown.
[a]Genes also have been identified in the Stanford array experiment.[8]

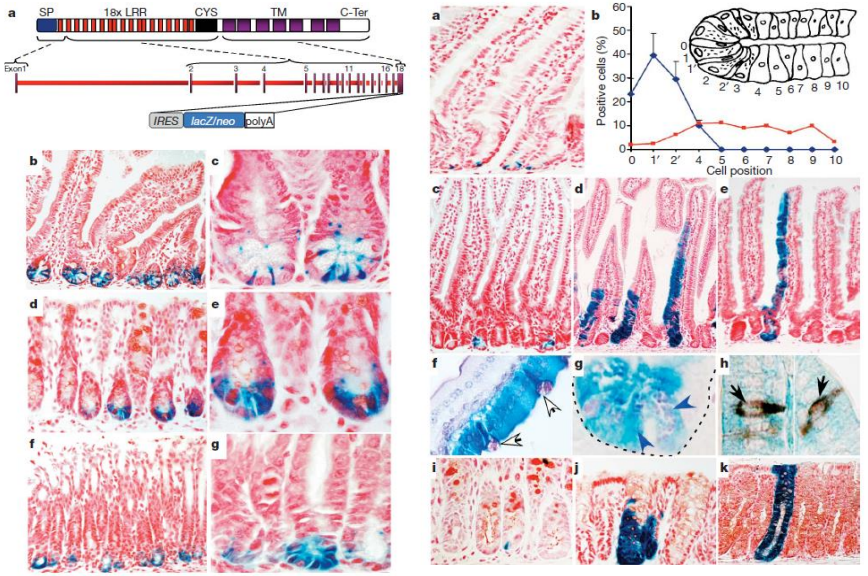
Vol 449|25 October 2007|doi:10.1038/nature06196 nature

ARTICLES

Identification of stem cells in small intestine and colon by marker gene *Lgr5*

Nick Barker[1], Johan H. van Es[1], Jeroen Kuipers[1], Pekka Kujala[2], Maaike van den Born[1], Miranda Cozijnsen[1], Andrea Haegebarth[1], Jeroen Korving[1], Harry Begthel[1], Peter J. Peters[2] & Hans Clevers[1]

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. It is currently believed that four to six crypt stem cells reside at the +4 position immediately above the Paneth cells in the small intestine; colon stem cells remain undefined. *Lgr5* (leucine-rich-repeat-containing G-protein-coupled receptor 5, also known as *Gpr49*) was selected from a panel of intestinal Wnt target genes for its restricted crypt expression. Here, using two knock-in alleles, we reveal exclusive expression of *Lgr5* in cycling columnar cells at the crypt base. In addition, *Lgr5* was expressed in rare cells in several other tissues. Using an inducible Cre knock-in allele and the *Rosa26-lacZ* reporter strain, lineage-tracing experiments were performed in adult mice. The Lgr5-positive crypt base columnar cell generated all epithelial lineages over a 60-day period, suggesting that it represents the stem cell of the small intestine and colon. The expression pattern of *Lgr5* suggests that it marks stem cells in multiple adult tissues and cancers.



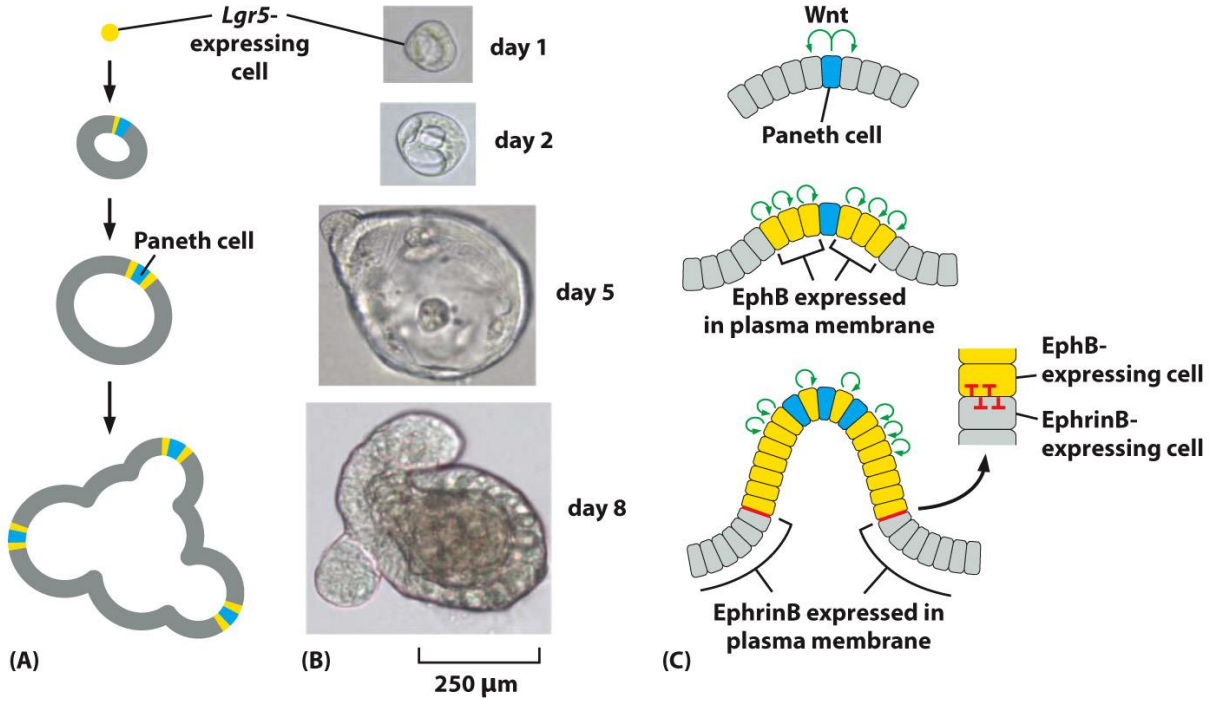


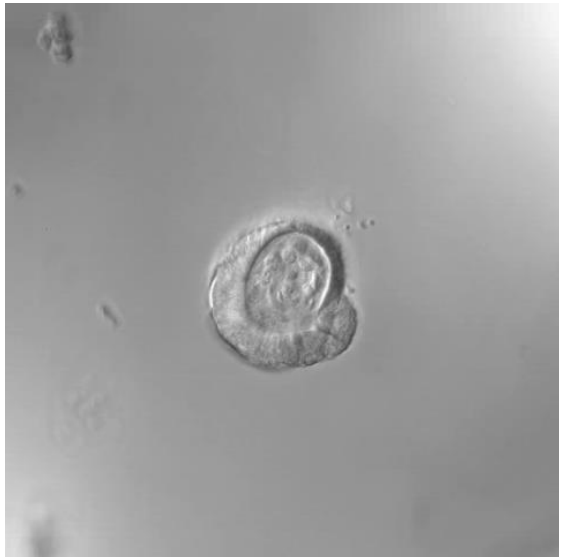
Figure 22-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

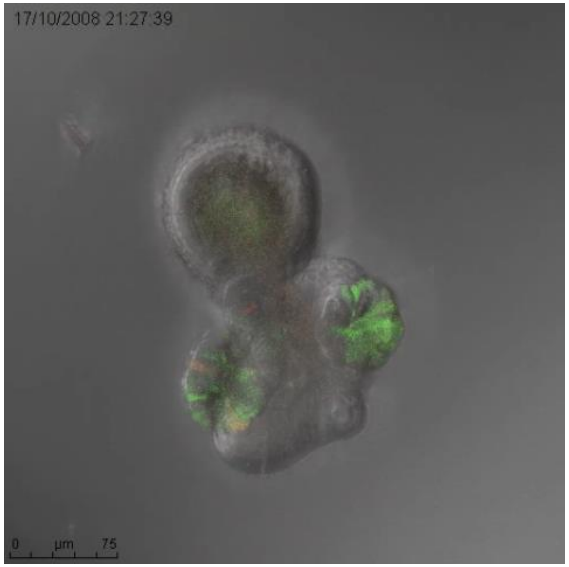
nature Vol 459|14 May 2009|doi:10.1038/nature07935

LETTERS

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]





https://www.youtube.com/watch?v=XddaxQcFOs8

Hematopoéza – hierarchický systém

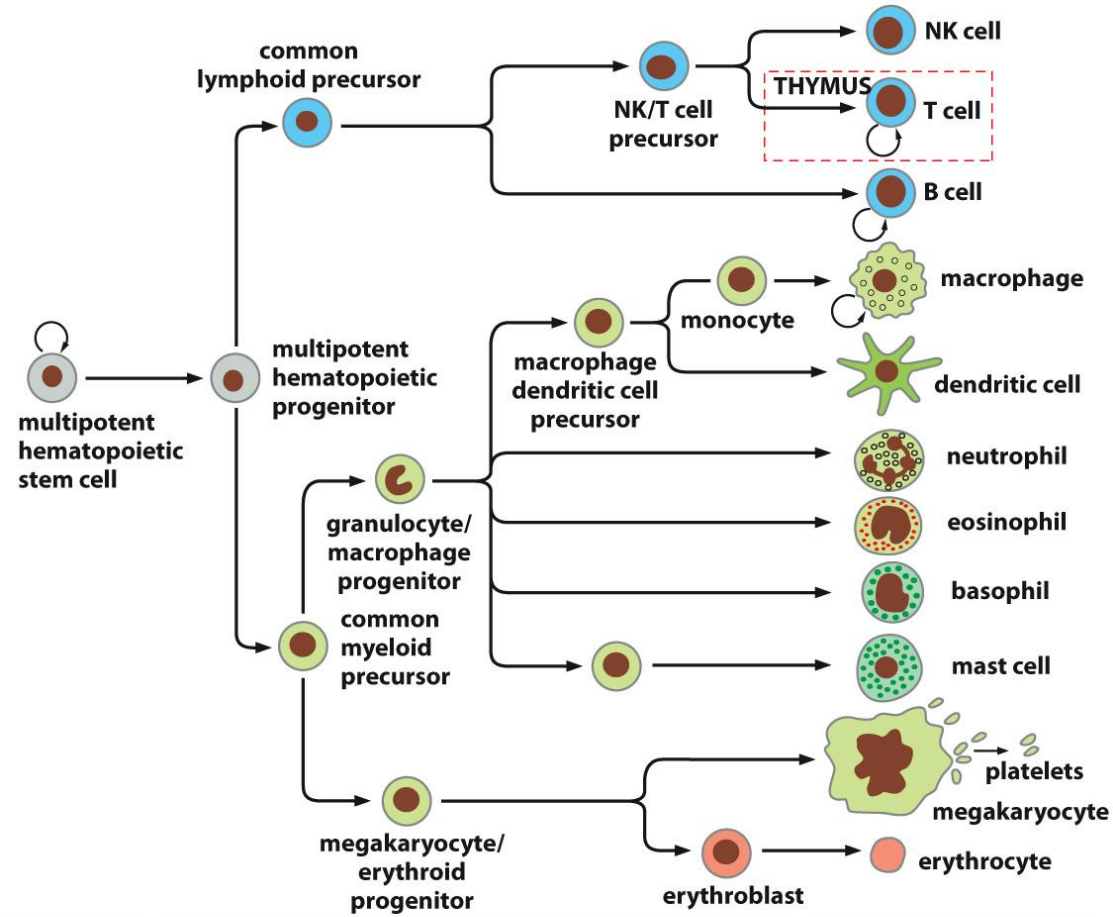
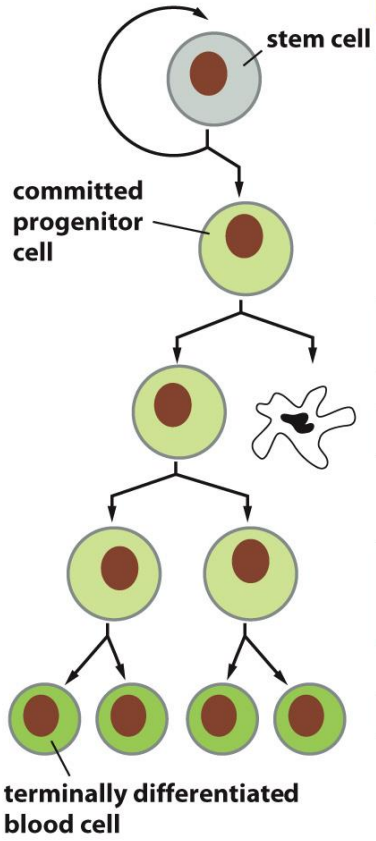


Figure 22-31 Molecular Biology of the Cell 6e (© Garland Science 2015)

Regulace



CONTROLLABLE PARAMETER

1. Frequency of stem-cell division
2. Probability of stem-cell death
3. Probability that stem-cell daughter will become a committed progenitor cell of the given type
4. Division cycle time of committed progenitor cell
5. Probability of progenitor-cell death
6. Number of committed progenitor-cell divisions before terminal differentiation
7. Lifetime of differentiated cells

Figure 22-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

Závislost kmenové buňky na kontaktu se stromatem

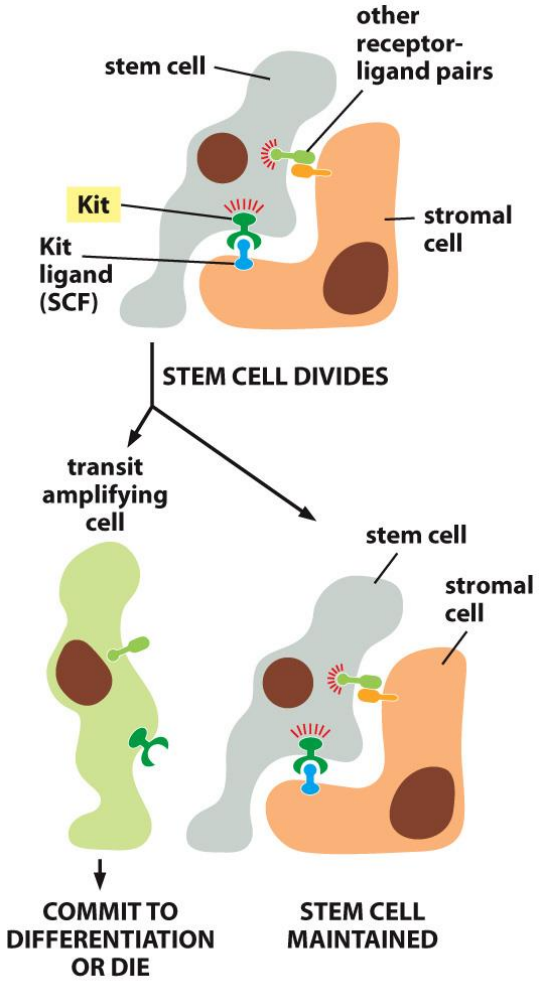
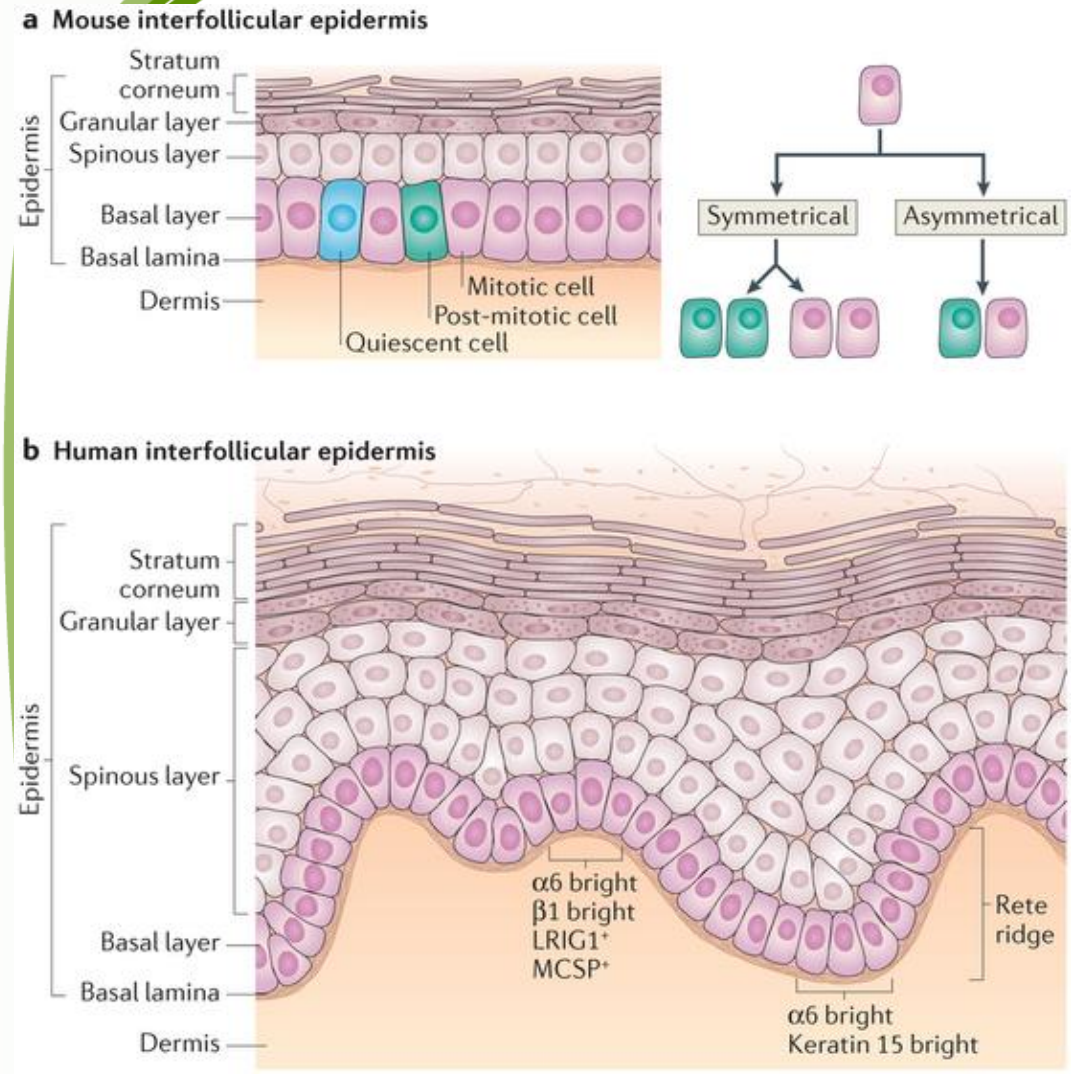


Figure 22-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

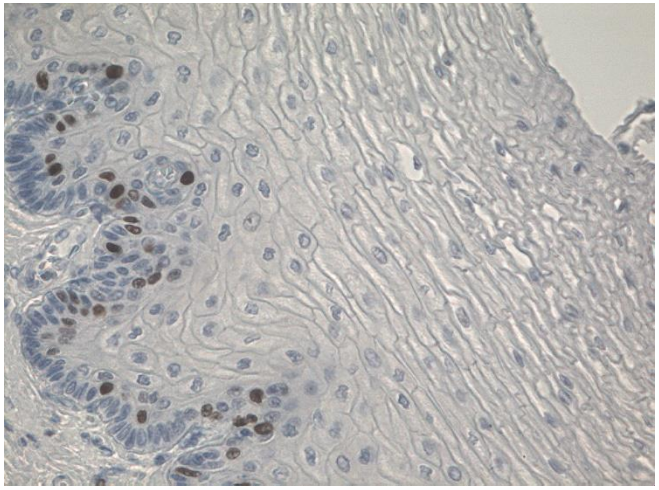
c-kit receptor – CD117, receptorová tyrosin kináza

SCF – stem cells factor, cytokin

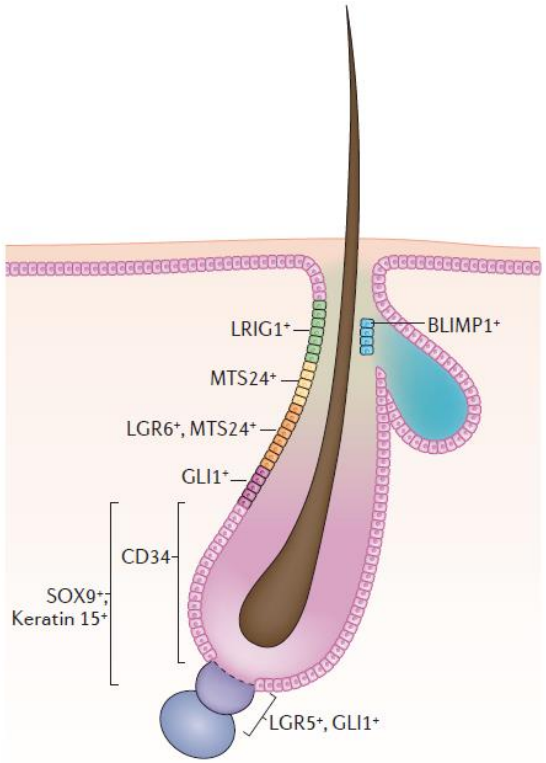
Obnova epidermis

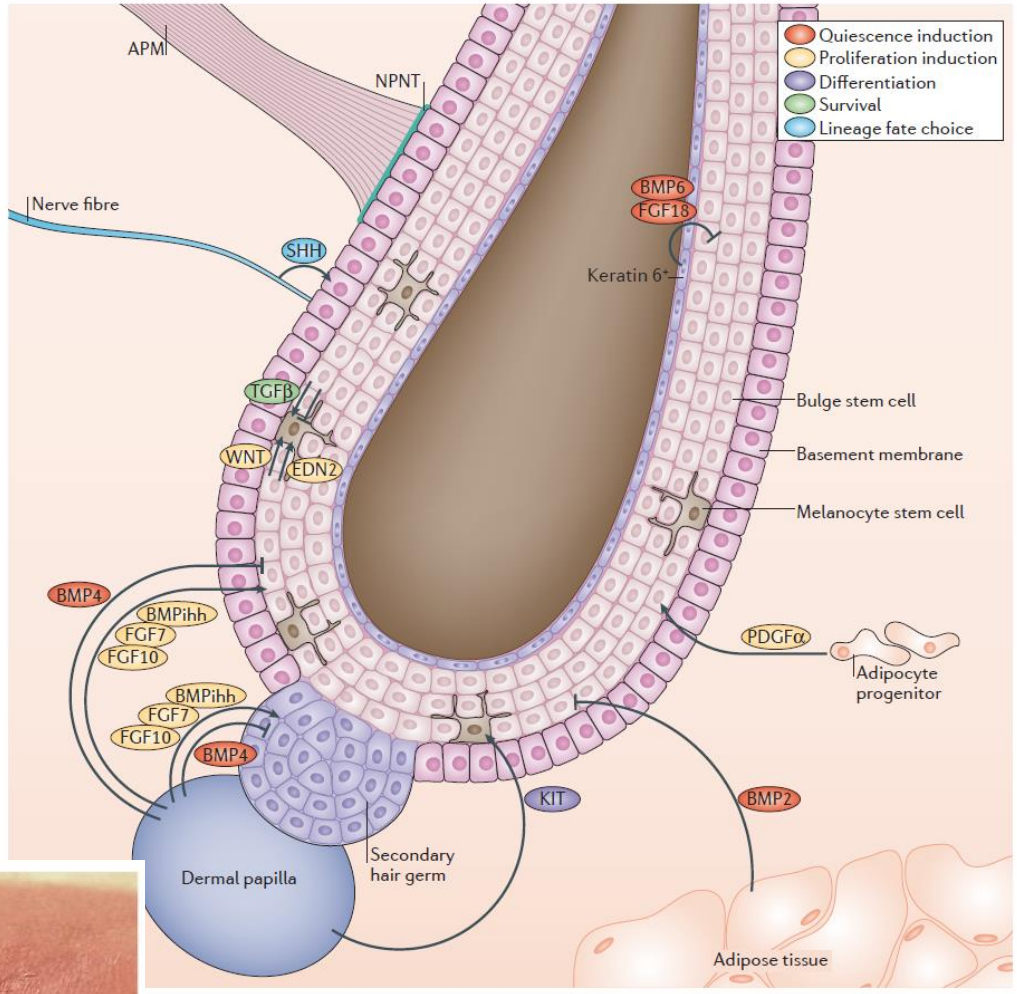


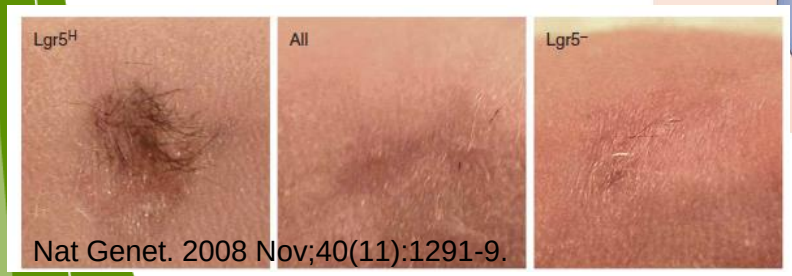
Ki67+ = proliferace



Kmenová buňka vlasového folikulu



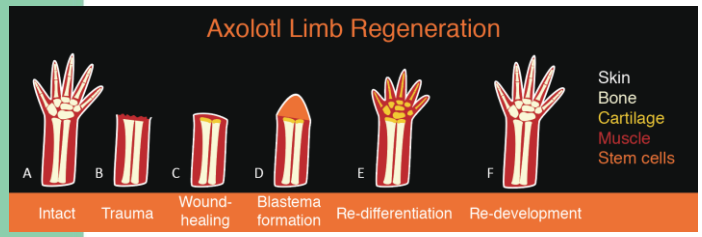




Nat Genet. 2008 Nov;40(11):1291-9.

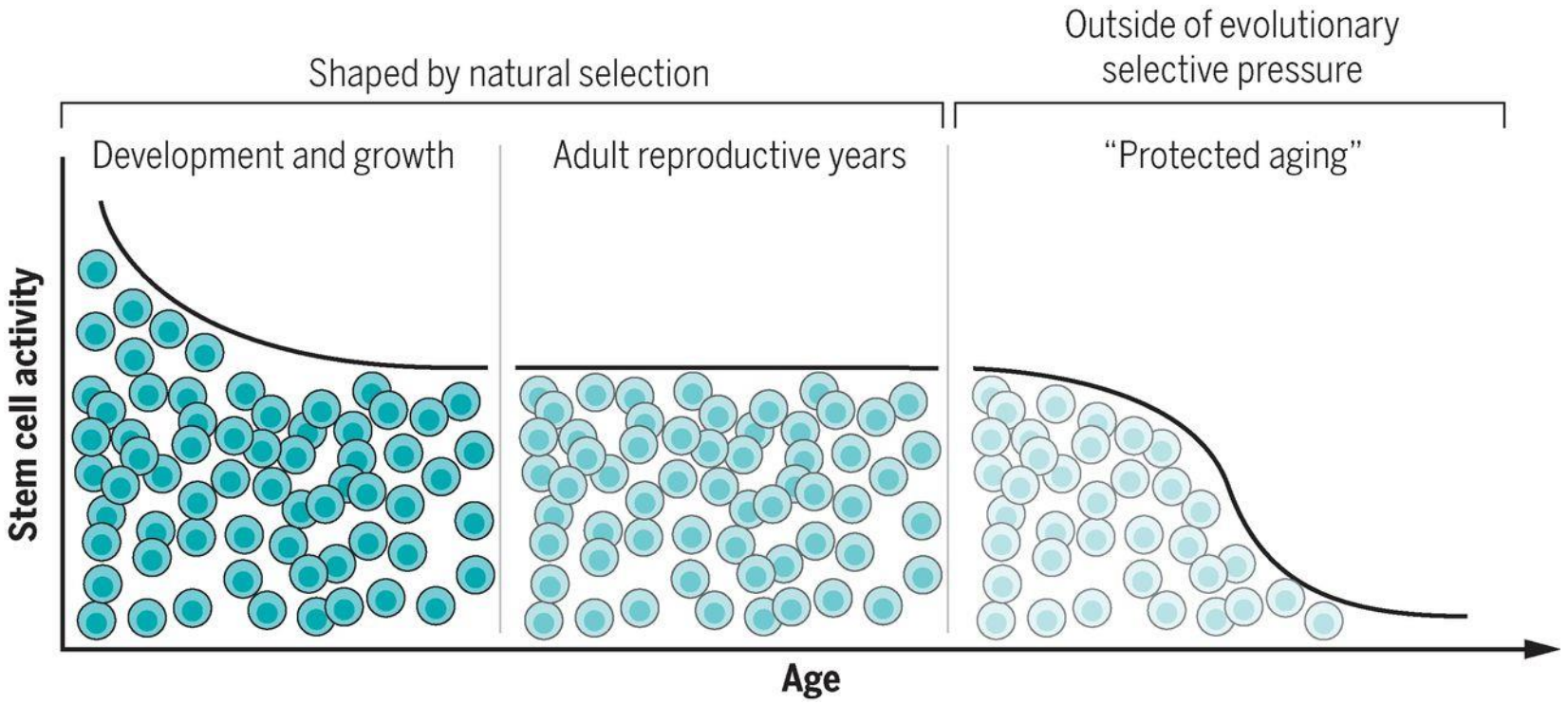
Regenerace

	Animal		What they regenerate
Invertebrates	Flatworm		Any part of their bodies, including their heads!
	Sea star		Limbs and even their whole bodies if their central nerve ring is intact.
Vertebrates	Axolotl		Limbs and spine.
	Frog		Tadpoles can regenerate limbs but lose this ability in adulthood.
	Human		Adult human regeneration is largely limited to skin and liver cells.



http://sitn.hms.harvard.edu/flash/2018/regeneration-axolotl-can-teach-us-regrowing-human-limbs/

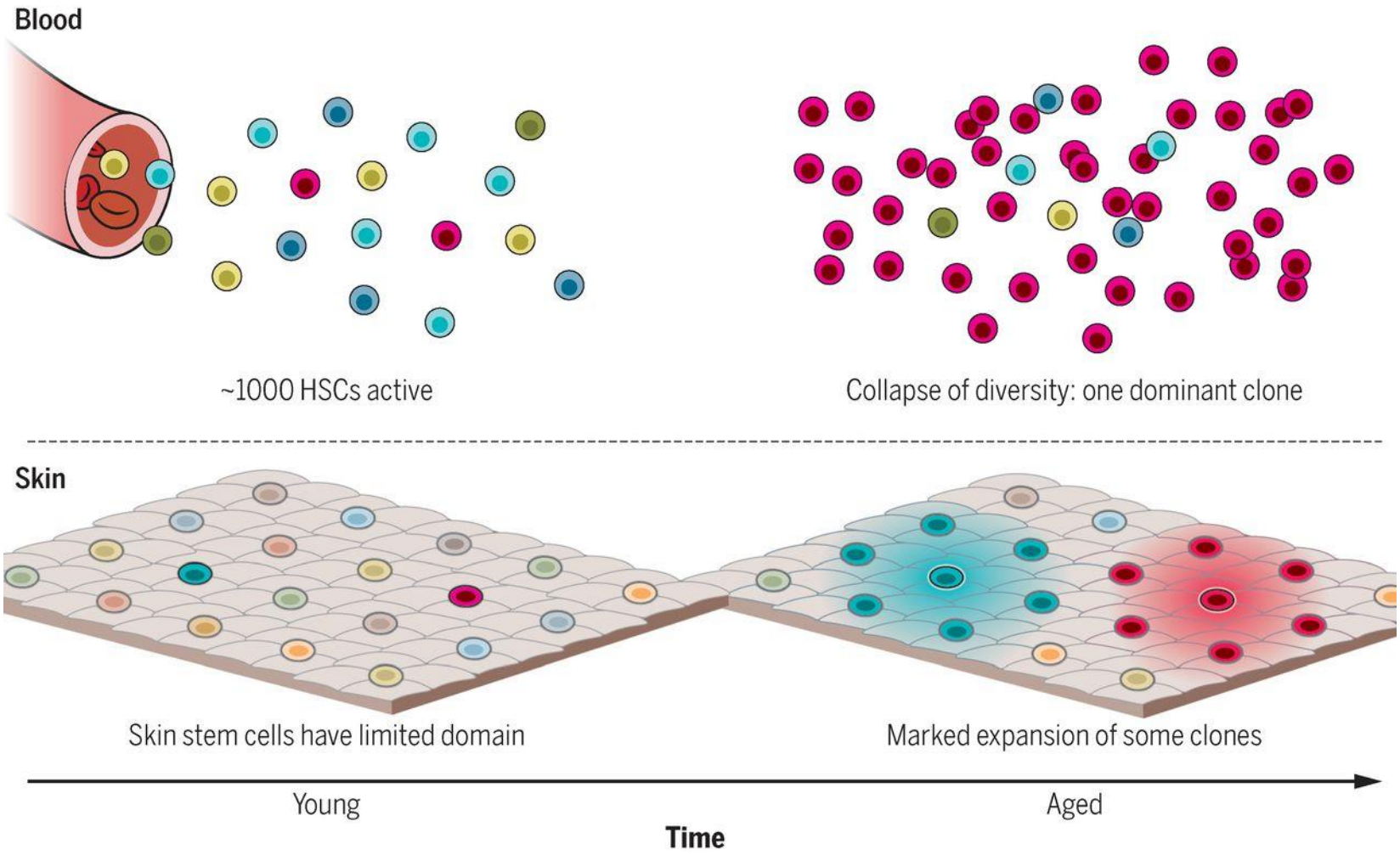
Model of stem cell use over the life span.



Margaret A. Goodell, and Thomas A. Rando Science 2015;350:1199-1204

Science AAAS

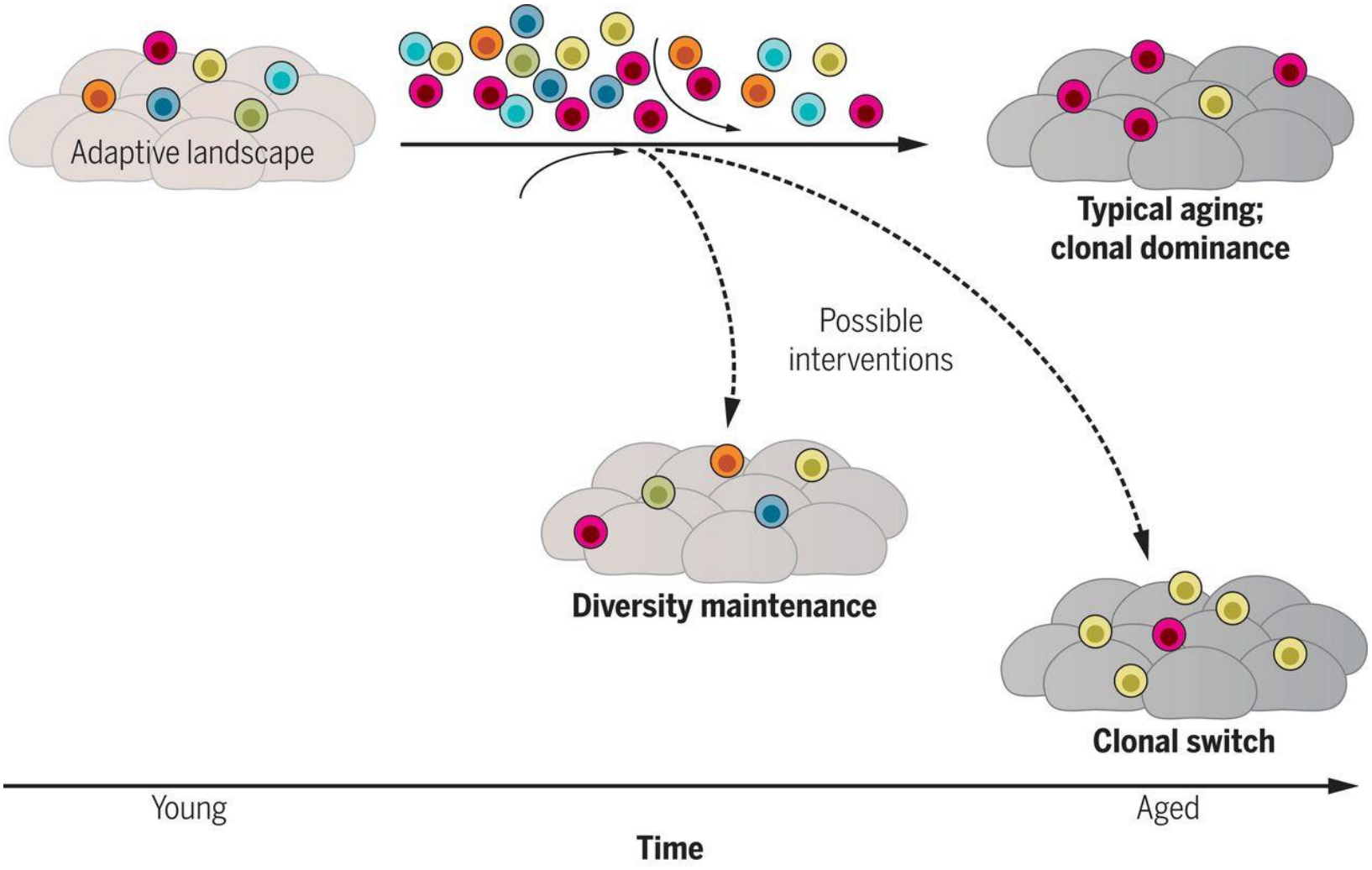
Fig. 2 Stem cell diversity and dynamics with age.



Margaret A. Goodell, and Thomas A. Rando Science 2015;350:1199-1204

Science AAAS

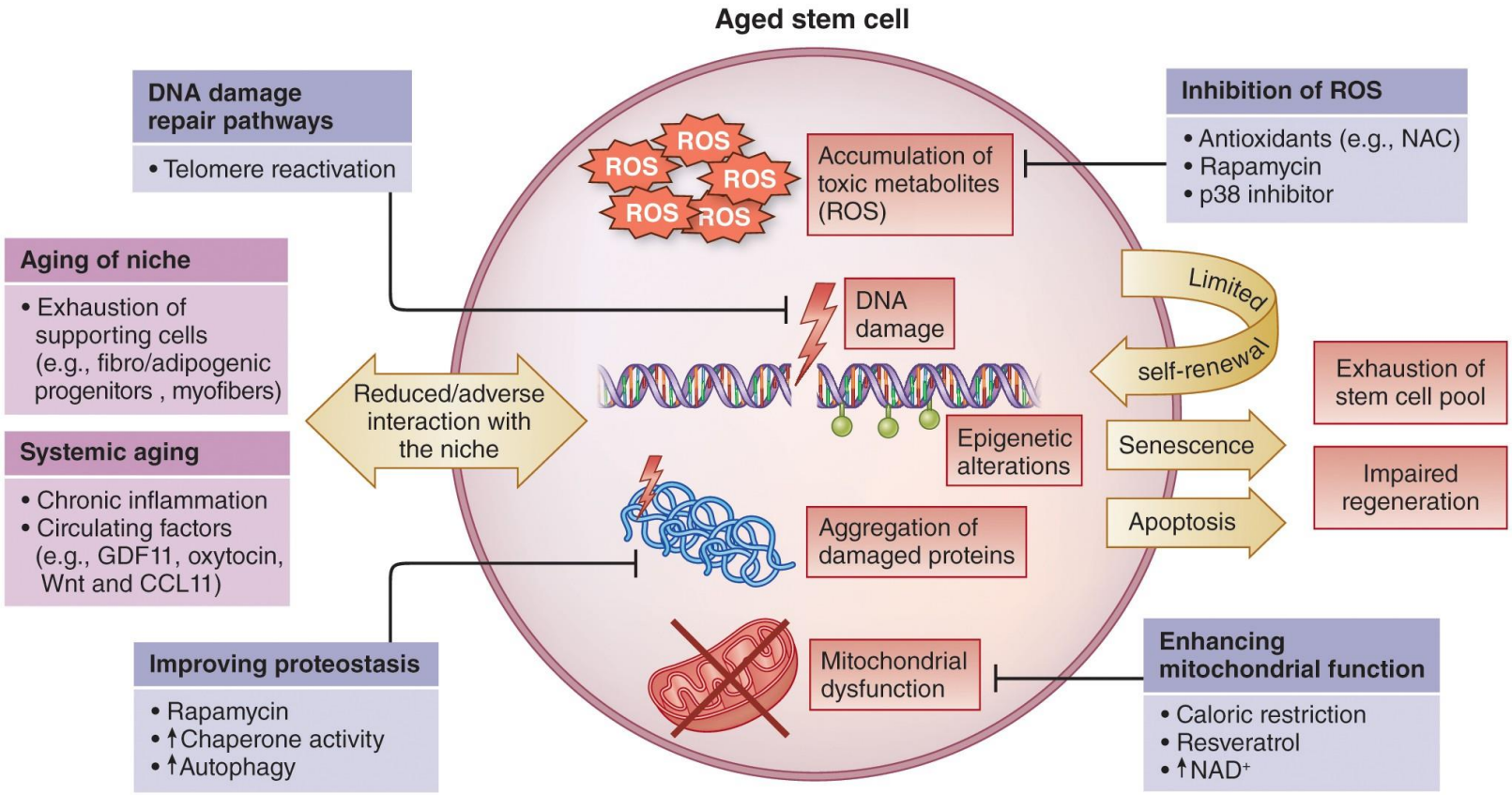
Model of age-related selection for stem cells with new characteristics and potential outcomes.



Margaret A. Goodell, and Thomas A. Rando Science 2015;350:1199-1204

Science
AAAS

Stárnutí kmenových buněk



Diferenciace *in vitro*

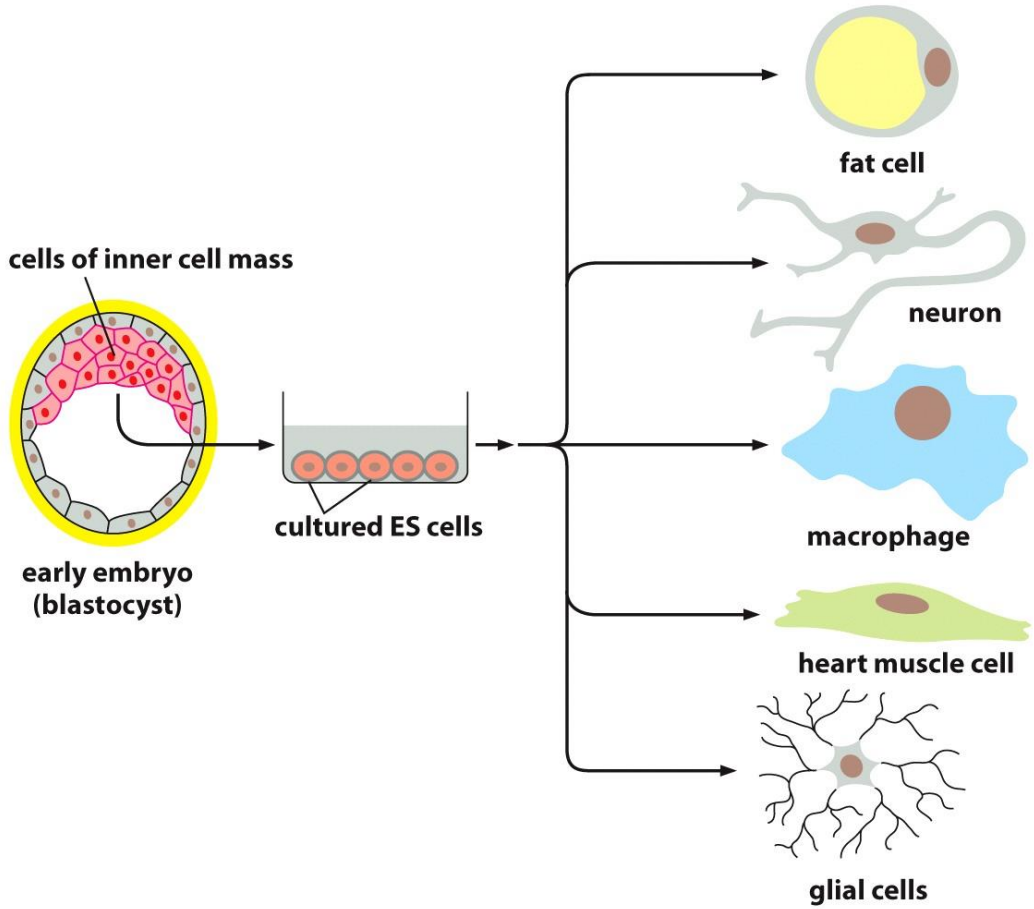


Figure 20-41 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Přeprogramování

The Nobel Prize in Physiology or Medicine 2012
Sir John B. Gurdon, Shinya Yamanaka

Cell *131*, 861–872, November 30, 2007 ©2007 Elsevier Inc.

Cell

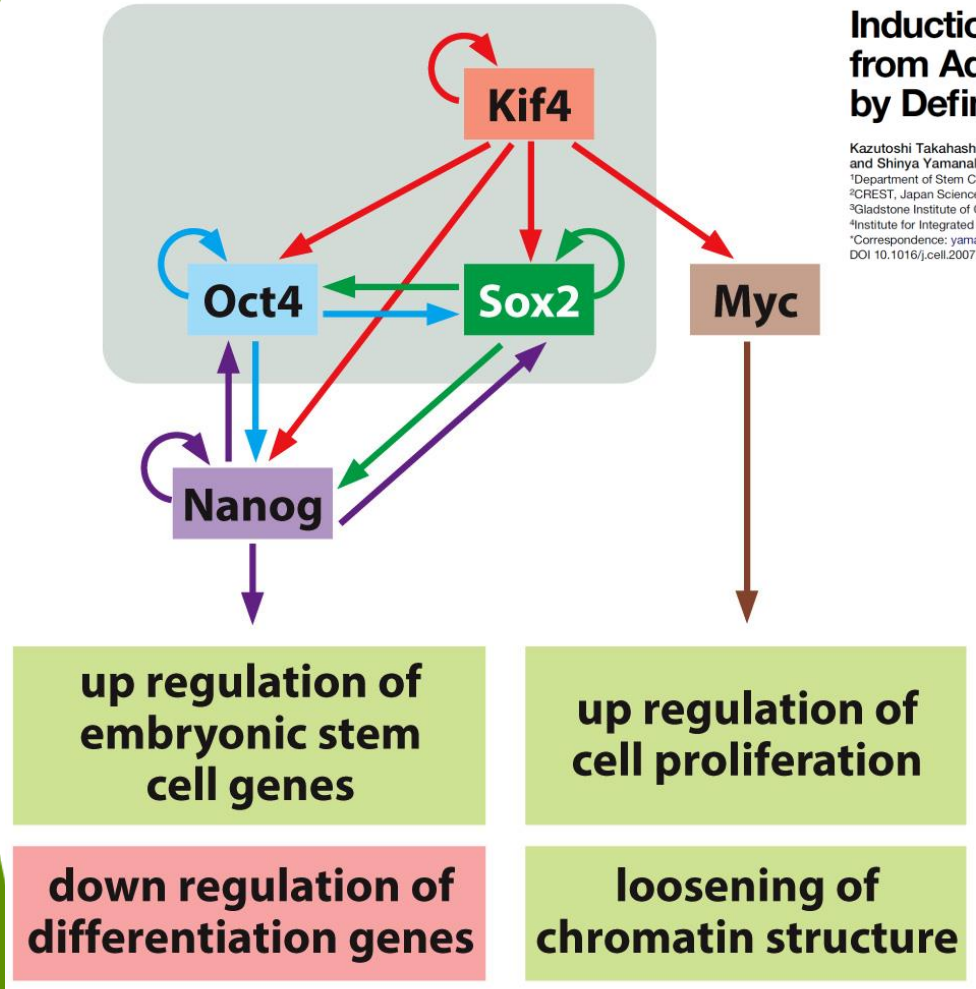


Figure 22-41 Molecular Biology of the Cell 6e (© Garland Science 2015)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

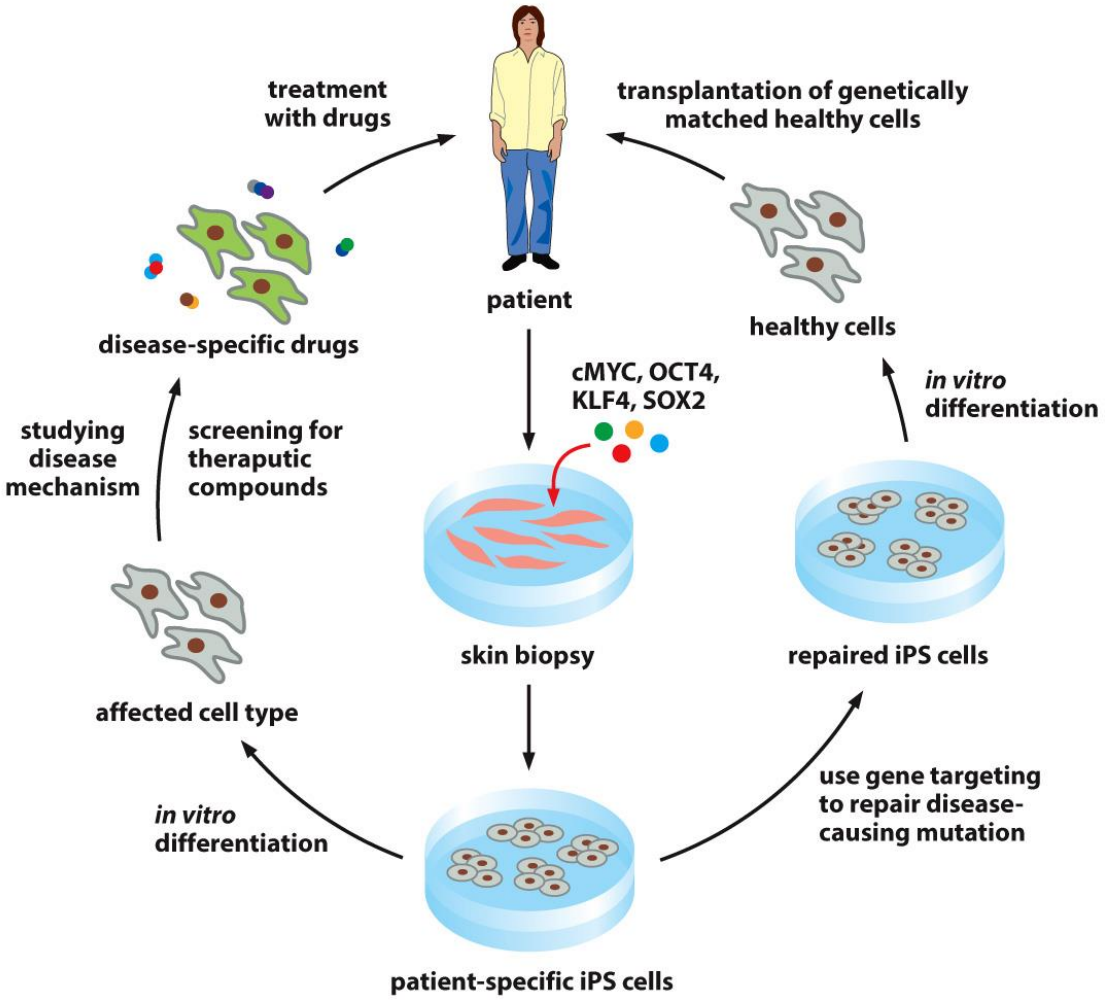


Figure 22-47 Molecular Biology of the Cell 6e (© Garland Science 2015)

Deregulace proliferace a buněčné smrti

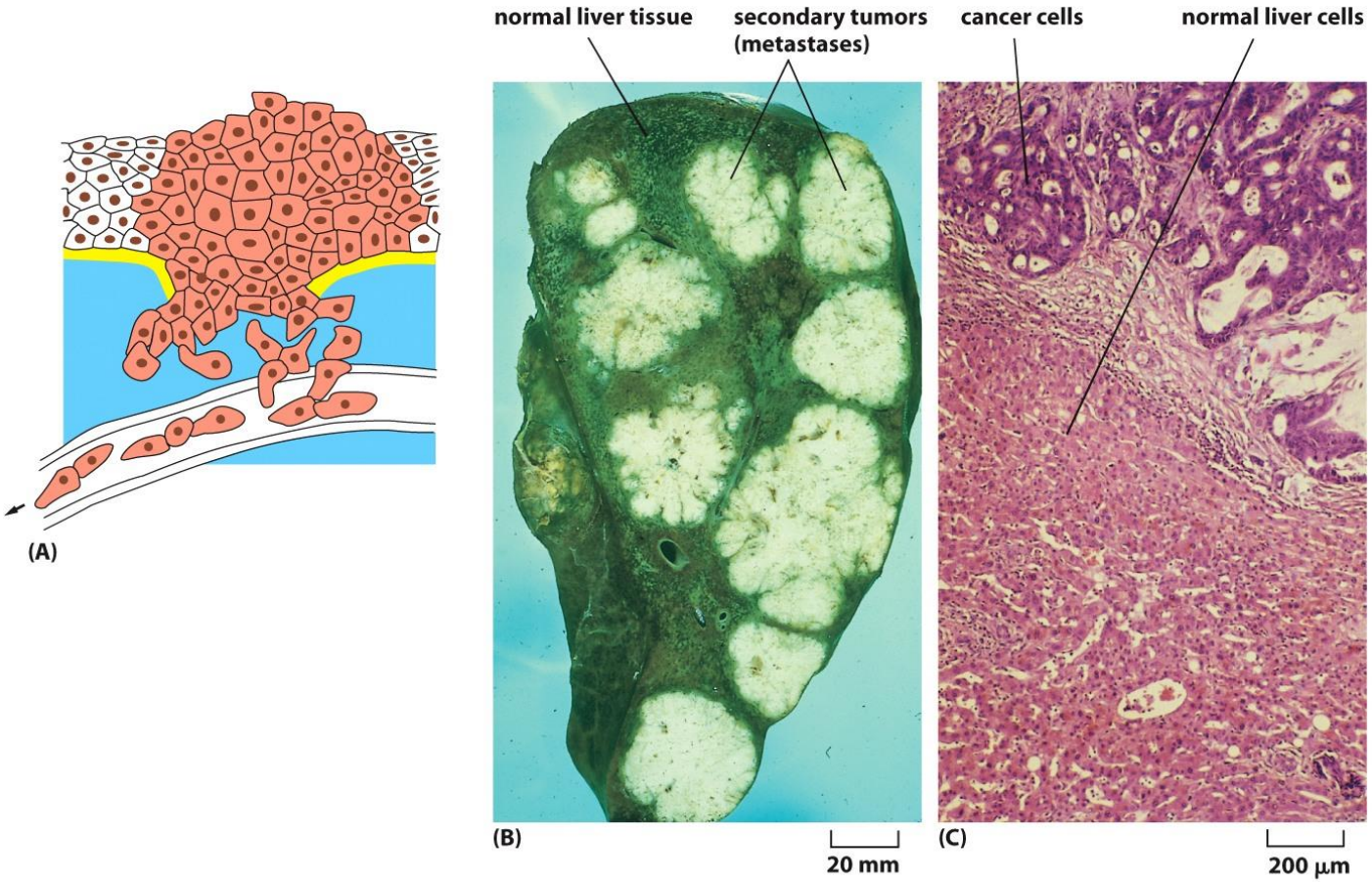
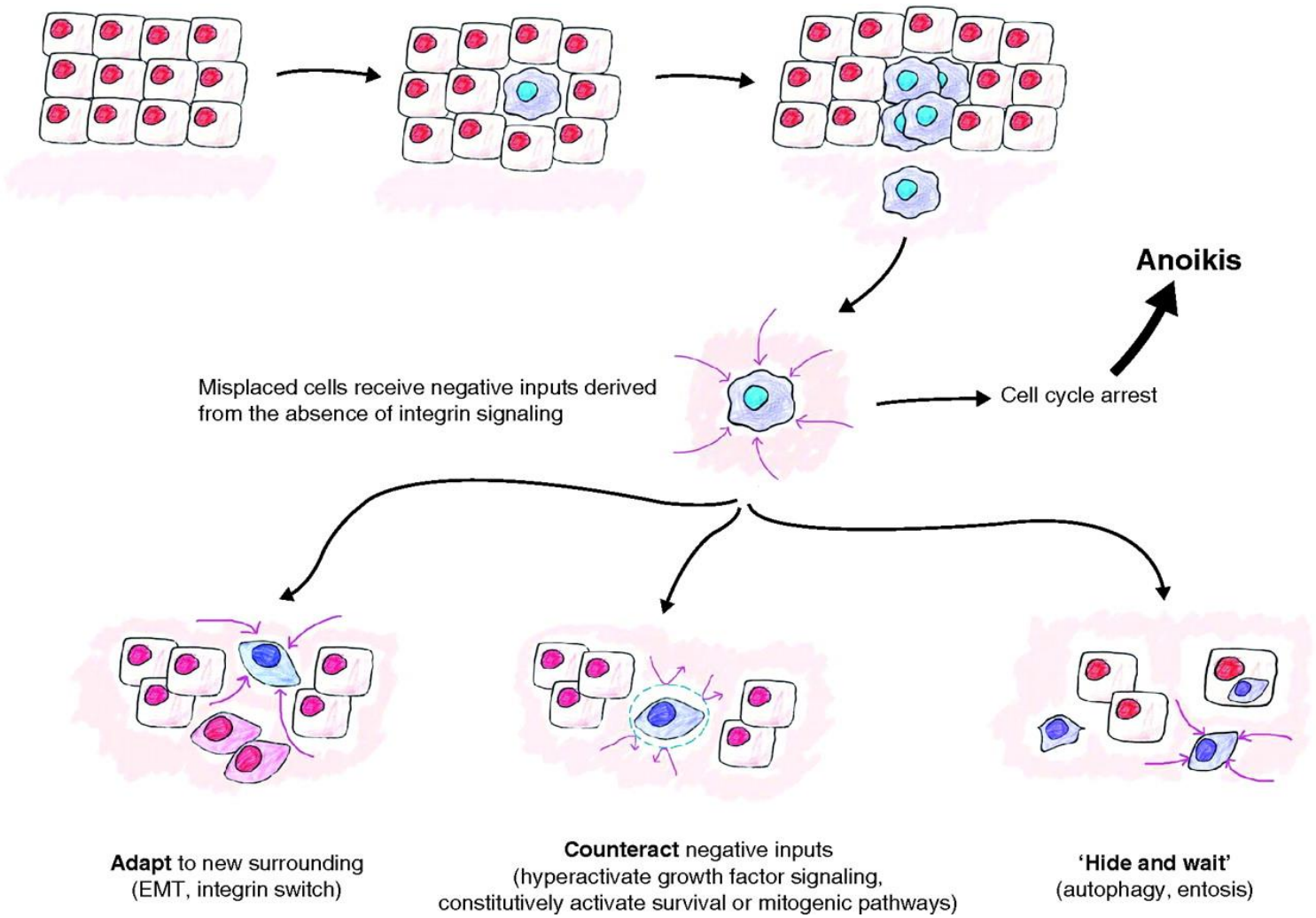


Figure 20-45 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Strategie úniku transformované buňky



Marta C. Guadamillas et al. J Cell Sci 2011;124:3189-3197

Deregulace proliferace a buněčné smrti

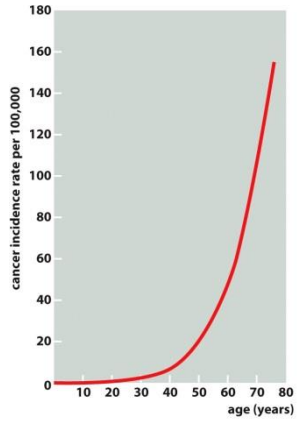


Figure Q20-16 Essential Cell Biology, 4th ed. (© Garland Science 2014)

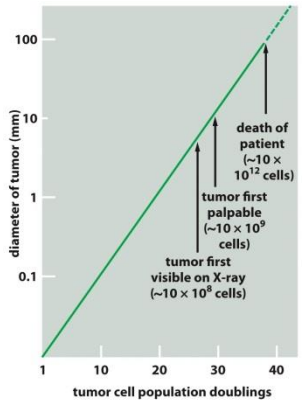


Figure 20-54 Essential Cell Biology, 4th ed. (© Garland Science 2014)

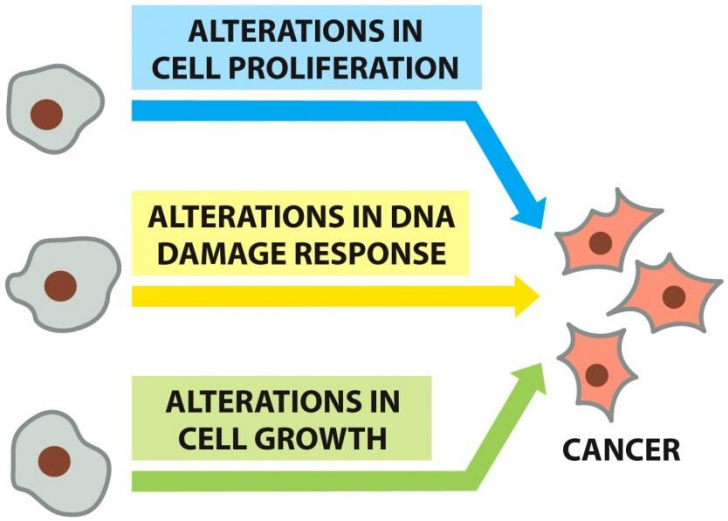


Figure 20-51 Essential Cell Biology, 4th ed. (© Garland Science 2014)

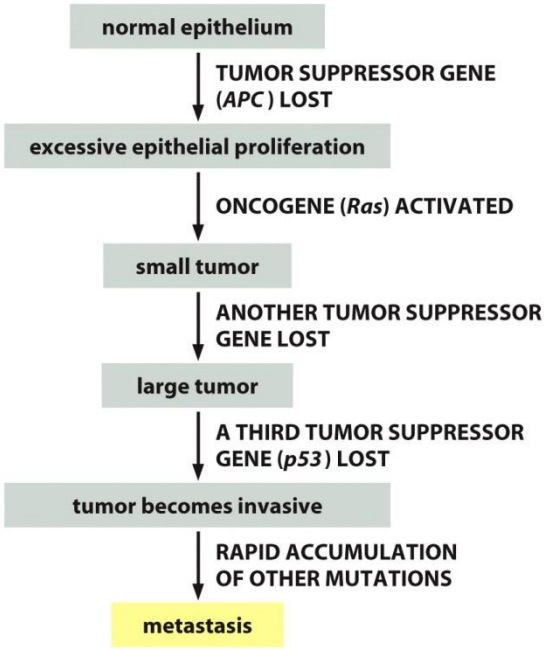


Figure 20-53 Essential Cell Biology, 4th ed. (© Garland Science 2014)

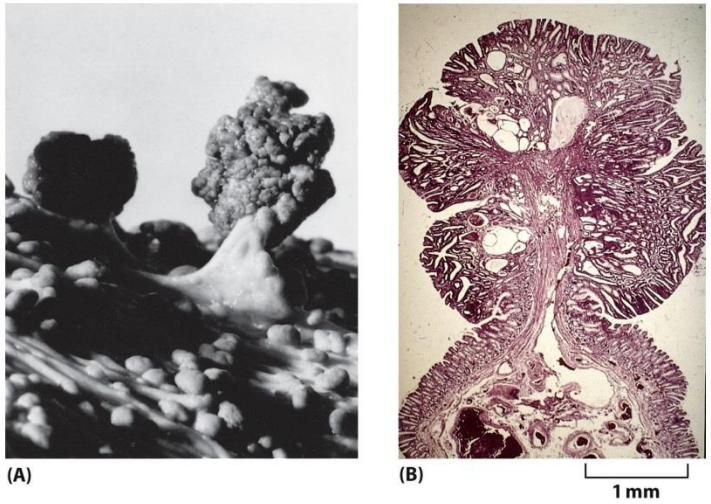


Figure 20-52 Essential Cell Biology, 4th ed. (© Garland Science 2014)