BIOLOGIE CELULARA SI MOLECULARA

LP04. Culturi de celule. Diviziunea celulara. Ciclul celular. Controlul activitatii celulare.

Biologie Celulara si Moleculara

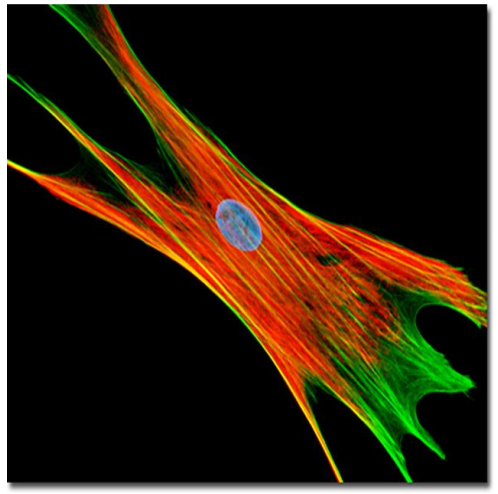
Modul Biologie celulara:
Notiuni microscopie
Evaluare organite celulare;
Culturi de celule;

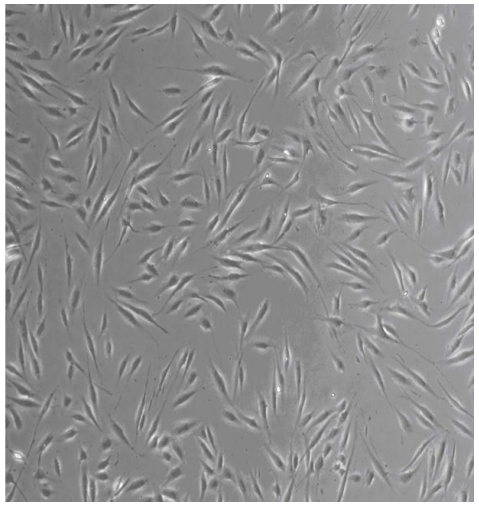
Modul Biologie moleculara:
Izolarea ADN şi ARN;
Amplificarea ADN/ARN
Electroforeza
Tehnici de detectare a mutaţiilor:
- ASO.
- RFLP.
- DGGE.
- Real-Time PCR.
- Secvenţiere.

ADN şi ARN non-self.

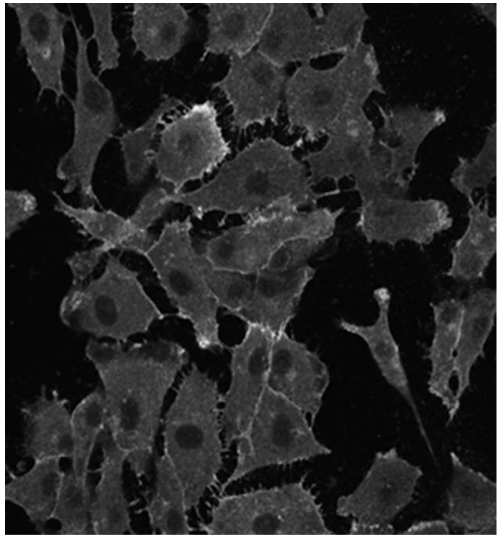
Culturi de celule. Diviziunea celulara. Ciclul celular. Controlul activitatii celulare

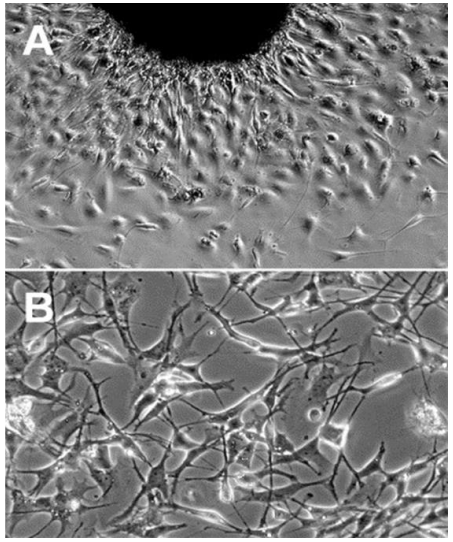
Tipuri de culturi de celule



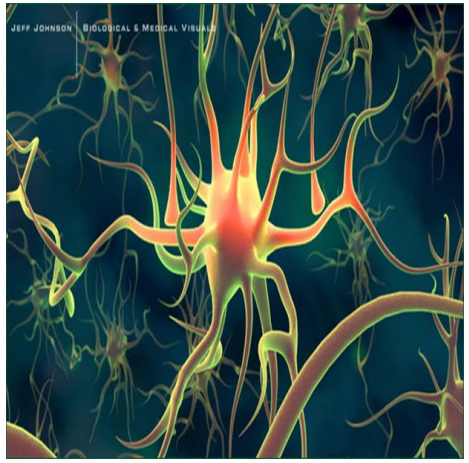


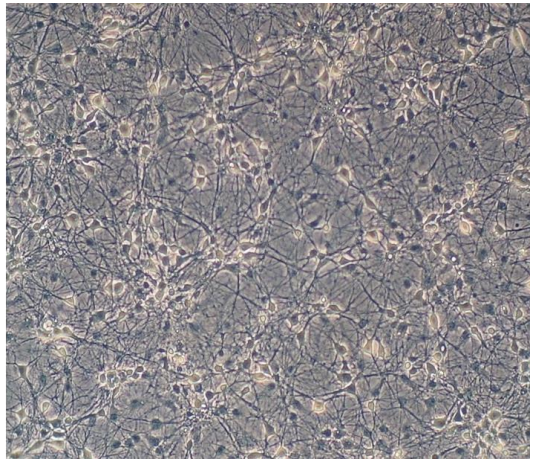
Fibroblaste





Celule tumorale



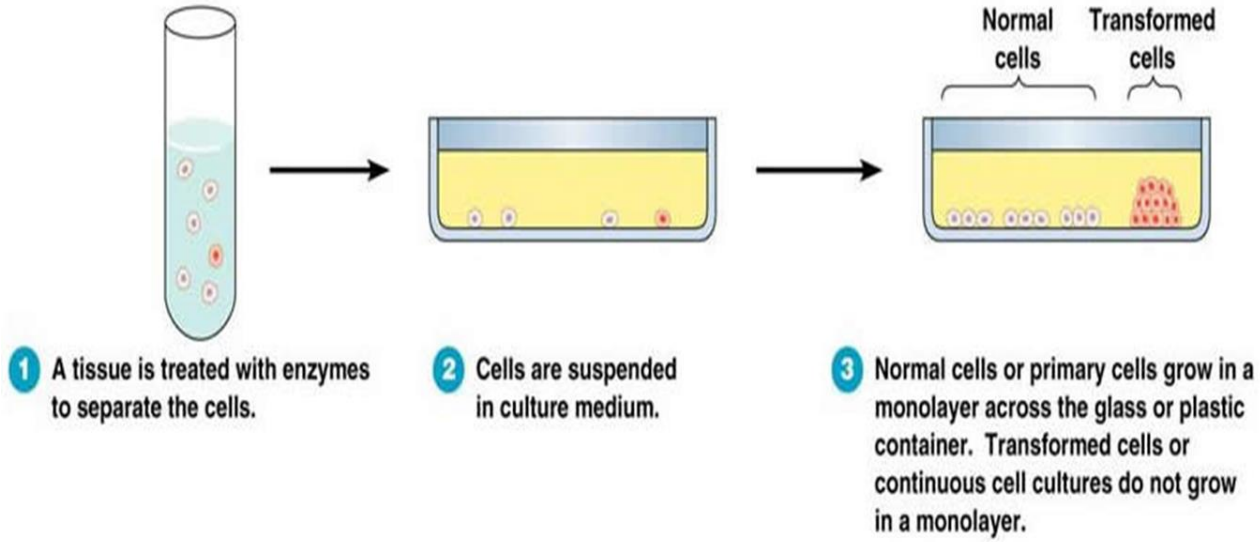


Neuroni

Etapele obtinerii unei culture celulare

- **Disocierea celulelor:**
- “digerarea” matricei extracelulare cu enzime proteolitice (tripsina, colageneza)
- tratamentul fragmentelor de tesut cu anumiti agenti chelatori (EDTA) care leaga ionii de calciu, de care depinde foarte mult adeziunea intercelulara.

- **Multiplicarea celulelor de un anumit tip in culturi celulare**



Disocierea celulelor – sortator de celule activate fluorescent

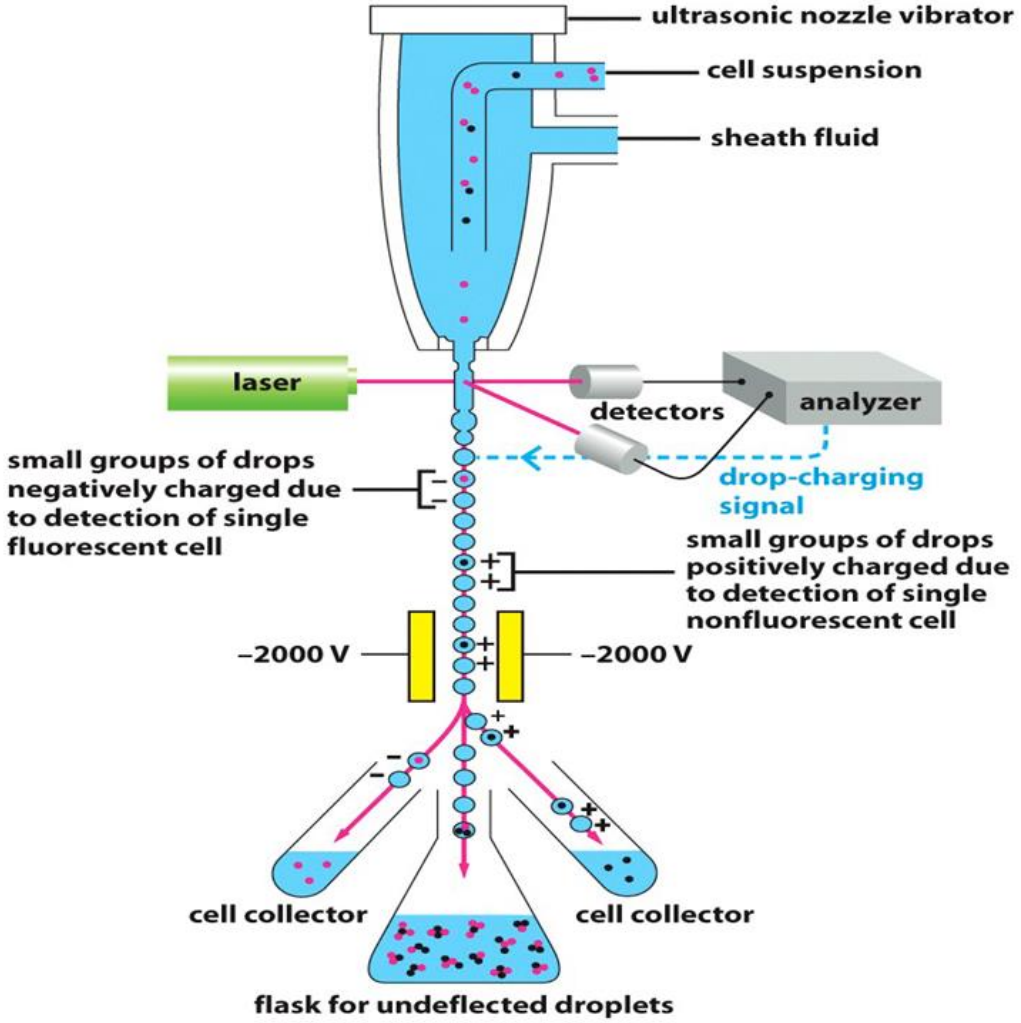


Figure 8-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Disocierea celulelor – microdisectie cu laser

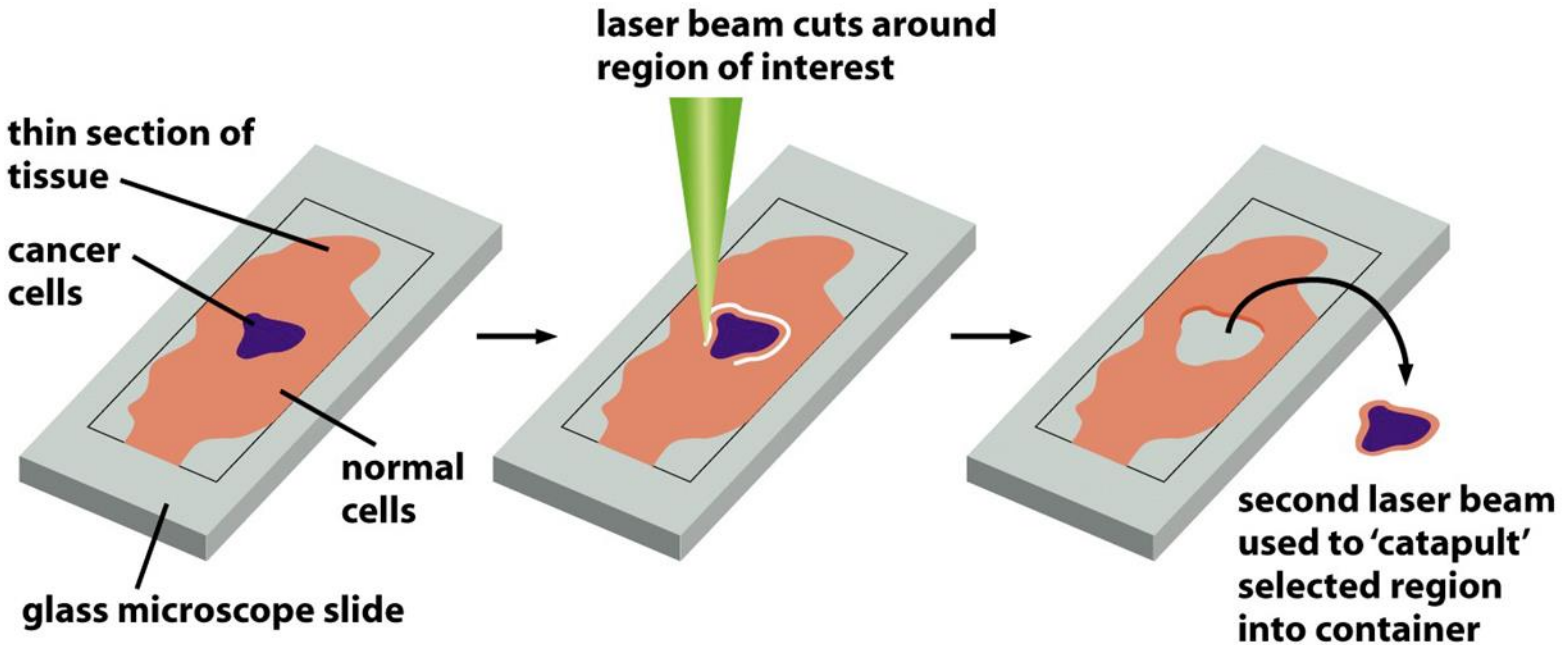


Figure 8-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Multiplicarea celulelor de un anumit tip in culturi celulare

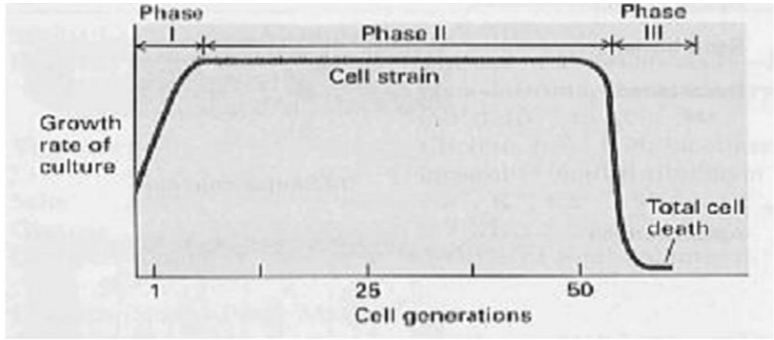
Celulele animale, pentru a creşte in cultura, au anumite ***necesitati bine definite:***

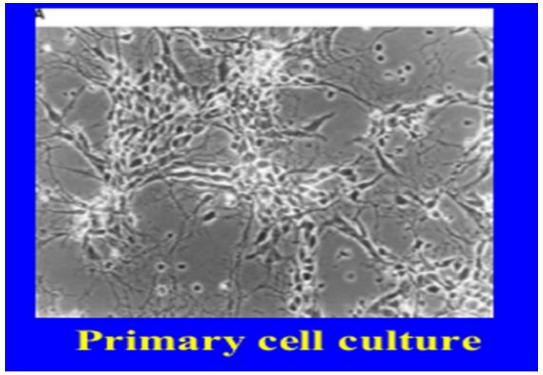
- aminoacizii esentiali (arginina, histidina, izoleucina, lizina, metionina, fenilalanina, treonina, triptofanul si valina) din mediul in care traiesc deoarece nu si-i pot sintetiza
- cisteina, glutamina si tirozina, deoarece acesti aminoacizi pot fi sintetizati doar de celule specializate din organismul animal (tirozina – ficat, glutamina – ficat si rinichi)
- vitaminele
- sarurile
- glucoza
- serul

Tipuri de culturi celulare

- **Culturi primare**
- **Culturi secundare**
- Culturi transformate

- Culturile realizate direct din celule ce provin dintr-un tesut prelevat dintr-un organism poarta numele de ***culturi primare.***
- Acestea pot fi realizate cu sau fara o etapa prealabila de separare a diferitelor tipuri celulare. In majoritatea cazurilor, aceste culturi pot fi indepartate din vasul initial de cultura si recultivate de mai multe ori, realizand asa-numitele ***culturi secundare****.*
- Astfel, o cultura poate fi *subcultivata (pasata)* de mai multe ori pentru cateva saptamani sau luni.





Tipuri de culturi celulare

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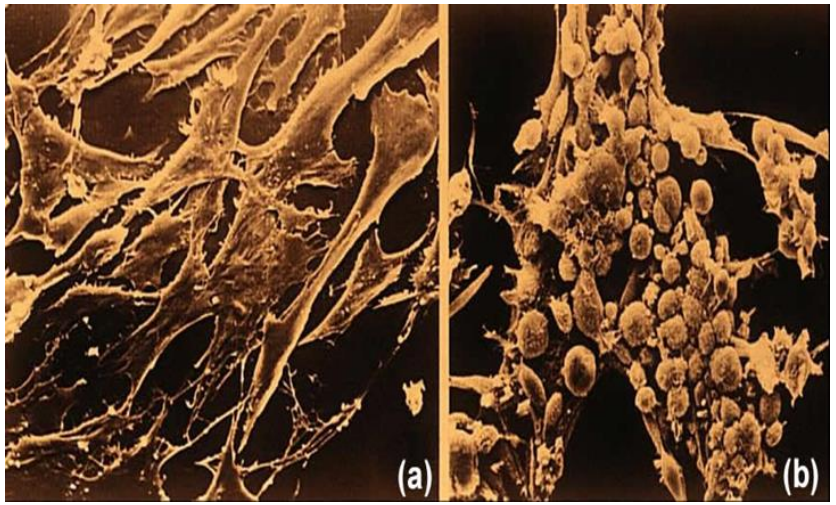
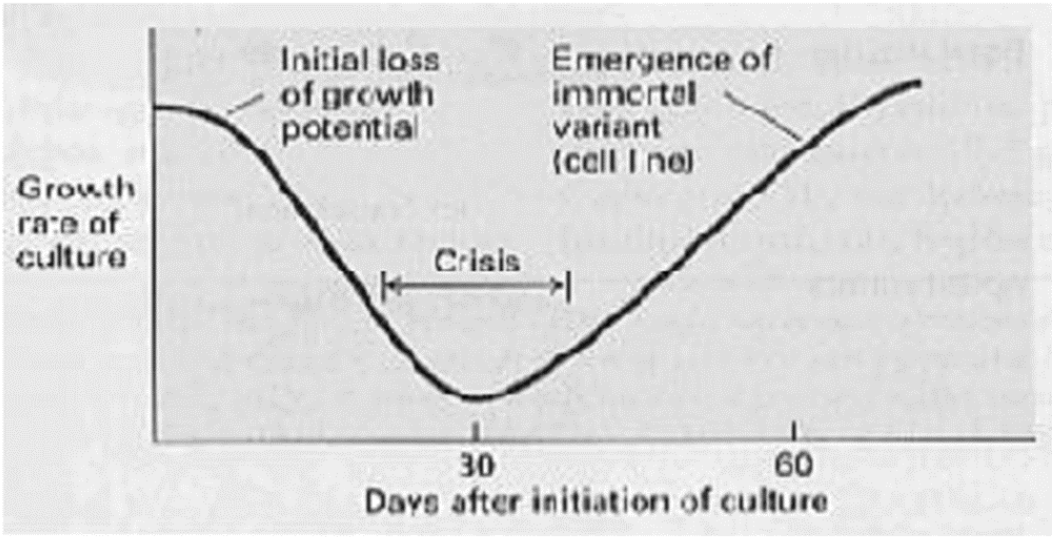
- Culturi secundare
- **Culturi transformate**



Uninfected cells in culture form a monolayer

Cells infected with vesicular stomatitis virus round up and pile up on top of each other



In cultura, celulele si pot pastra unele proprietati particulare ale tesutului din care provin:

- fibroblastele continua sa secrete colagen,
- celulele derivate din muschiul scheletic embrionar fuzioneaza pentru a forma fibrele musculare care se contracta spontan in flacoanele de cultura,
- celulele nervoase isi extind axonii care isi pastreaza excitabilitatea electrica si intra in sinapsa cu celulele vecine;
- celulele epiteliale formeaza un strat cu proprietati similare epiteliului intact.

- Celulele din culturile primare cresc bine cand sunt plasate in flacoane de cultura, multiplicandu-se timp de 50-100 de generatii, dupa care intra in “criza”, cand isi incetinesc cresterea, pentru ca in cele din urma sa se opreasca din diviziune si sa moara.
- Fibroblastele provenite de la organisme tinere rezista in cultura mai multe generatii decat cele provenite de la un organism adult.
- Capacitatea limitata de proliferare este rezultatul unei scurtari progresive a ***telomerelor*** (secvente repetitive de ADN si proteine situate la capetele cromozomilor).
- Celulele somatice umane diferentiate si-au pierdut capacitatea de a produce enzima ***telomeraza*** care mentine integritatea telomerelor; din acest motiv telomerele se scurteaza la fiecare diviziune celulara.

- Fibroblastele pot fi adesea modificate astfel incat sa prolifereze nelimitat prin introducerea genei care codifica subunitatea catalitica a telomerazei; in acest caz, ele pot fi propagate ca si linii celulare “imortalizate”.
- Exista tipuri celulare nu pot fi imortalizate astfel. La acestea din urma, desi nu se mai scurteza telomerele, diviziunile celulare inceteaza dupa un anumit interval deoarece se
- activeaza *mecanismele de control a ciclului celular (cell cycle check-point mechanisms).*
- Pentru imortalizarea acestor culturi este necesar, pe langa introducerea telomerazei, si de inactivarea acestor mecanisme de control a ciclului celular.
- Acest lucru poate fi realizat prin introducerea unor

  oncogene, precum cele derivate din virusurile tumorale.

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The Nobel Prize in Physiology or Medicine 2009

Elizabeth H. Blackburn
Prize share:
1/3

Carol W. Greider
Prize share:
1/3

Jack W. Szostak
Prize share:
1/3

The Nobel Prize in Physiology or Medicine 2009 was awarded jointly to Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak *"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"*.

- Liniile celulare pot fi mai usor generate din celule canceroase; aceste culturi difera esential de cele generate din celule normale si sunt denumite ***culturi transformate.***
- Liniile celulare transformate cresc adesea fara a se atasa de suprafata vasului de cultura, si pot prolifera pana la o densitate celulara superioara comparativ cu celulele normale.
- Liniile celulare transformate pot produce tumori daca sunt injectate la un animal de experienta susceptibil.
- Atat culturile celulare primare, cat si cele transformate pot fi stocate timp nelimitat in azot lichid, la -196°C si raman viabile atunci cand sunt dezghetate, putand fi cultivate din nou.

Linii celulare

Linia celulara	Tipul celular si originea
3T3	fibroblast (soarece)
BHK21	fibroblast (hamster sirian)
MDCK	celule epiteliale (caine)
HeLa	celule epiteliale (om)
L6	mioblasti (sobolan)
COS	rinichi (maimuta)
CHO	ovar (hamster chinezesc)
H1, H9	celule stem embrionare (om)
S2	celule macrofag-like (*Drosophila*)

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Culturile de celule stem

- Cel mai promitator domeniu al culturilor de celule este acela al cultivarii celulelor stem embrionare (ES).
- Aceste celule, au fost prima data recoltate din masa interna de celule a embrionului de soarece si pot prolifera nelimitat in cultura.
- Replasate in mediul embrionar, aceste celule din cultura pot da nastere oricarui tip celular din organism, inclusiv celule ale liniei germinale .

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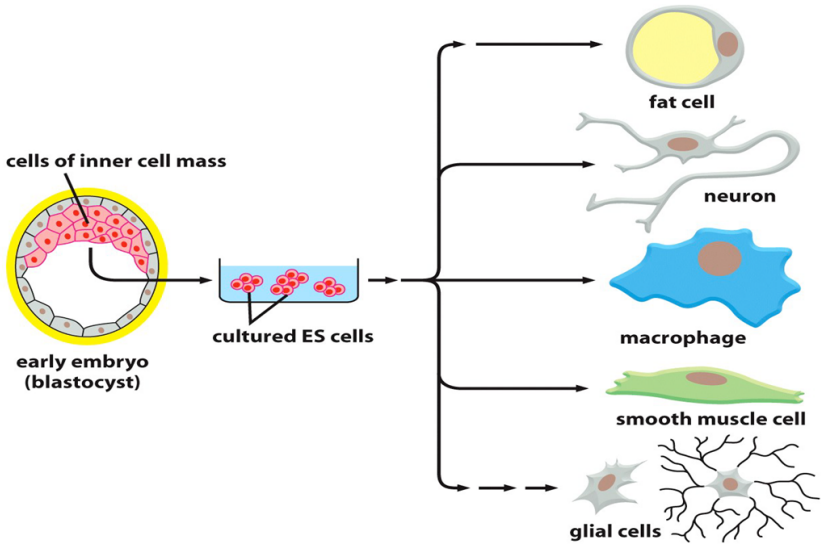


Figure 8-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

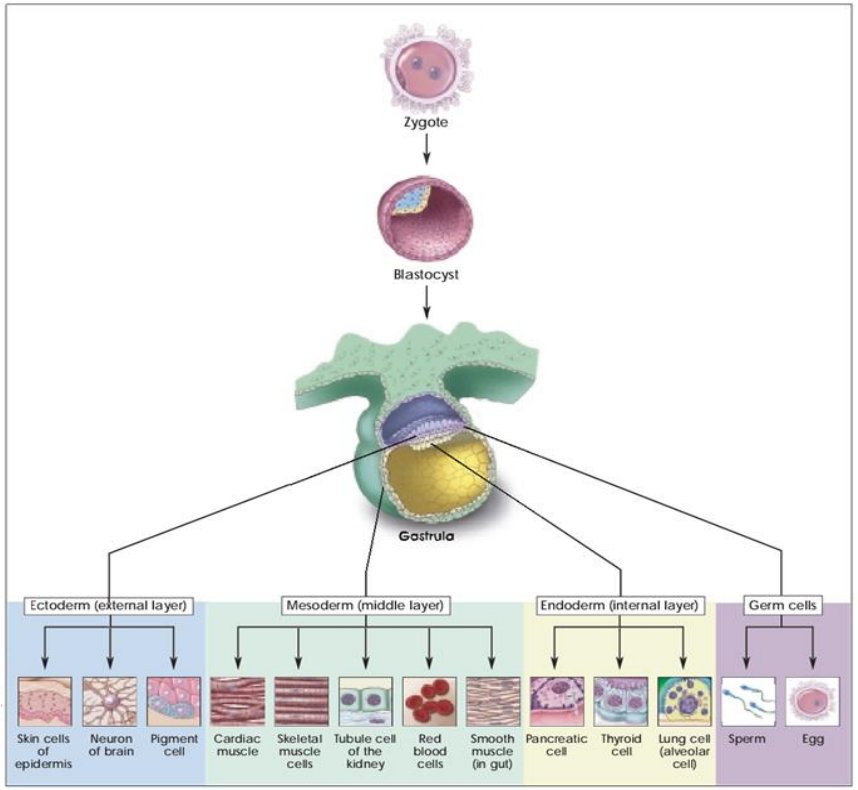
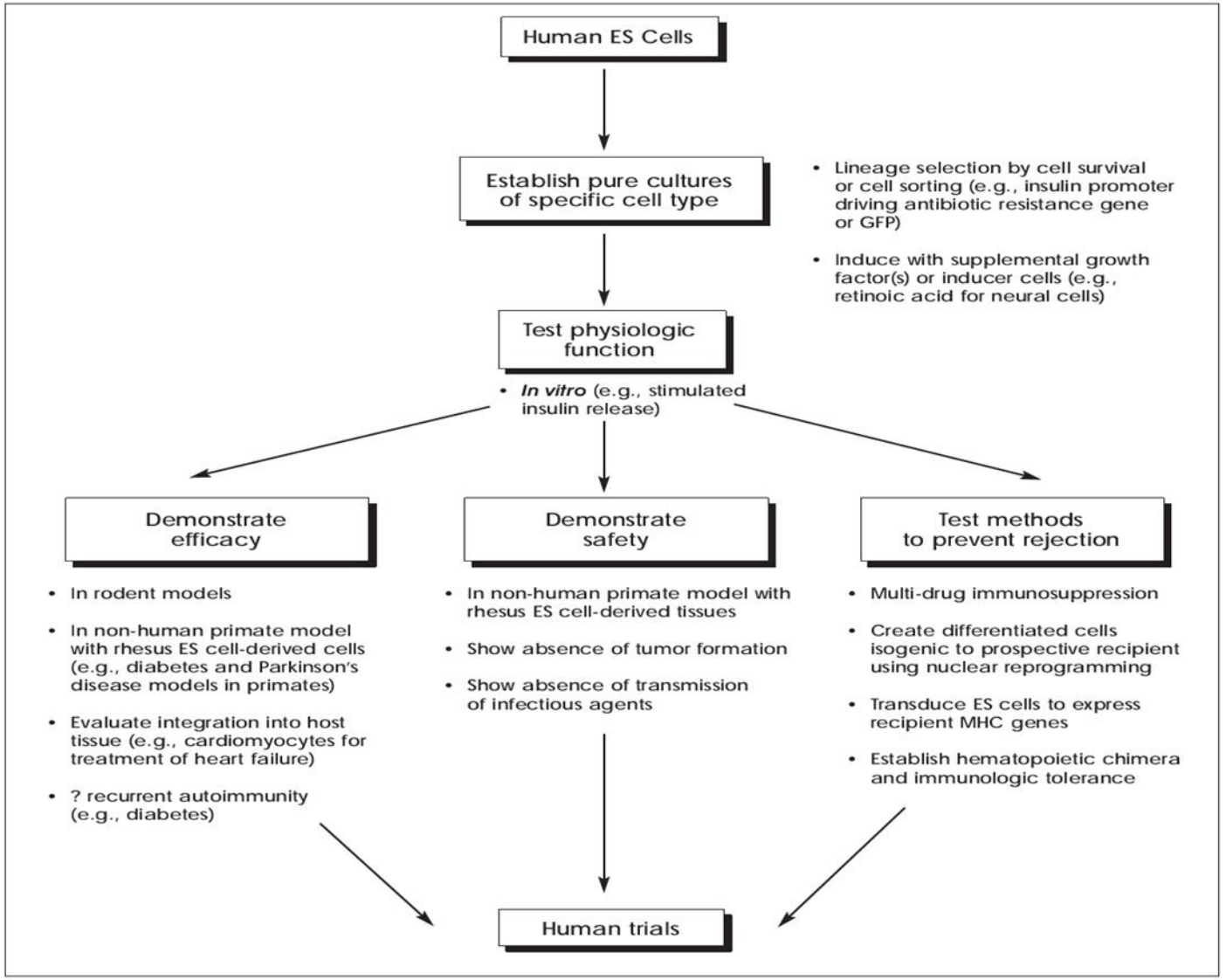


Figure 1.1. Differentiation of Human Tissues.

Celulele stem embrionare

- Celule cu proprietati similare cu celulele ES de soarece pot fi derivate si din embrioni umani, generand o rezerva potential nelimitata de celule care pot fi utilizate pentru a repara sau inlocui tesuturi umane mature.
- Experimentele pe celule ES de soarece sugereaza faptul ca va fi posibila utilizarea celulelor ES pentru a genera celule specializate utilizate pentru terapie: pentru inlocuirea fibrelor musculare degenerate la pacientii cu distrofie musculara, a celulelor nervoase la pacienti cu boala Parkinson, celulelor producatoare de insulina la pacientii cu diabet zaharat de tip I, sau a miocardului la pacientii care au suferit un infarct.
- Celulele ES nu se transplanteaza ca atare in organismele adulte, deoarece pot produce un tip particular de tumori denumite **teratoame.**

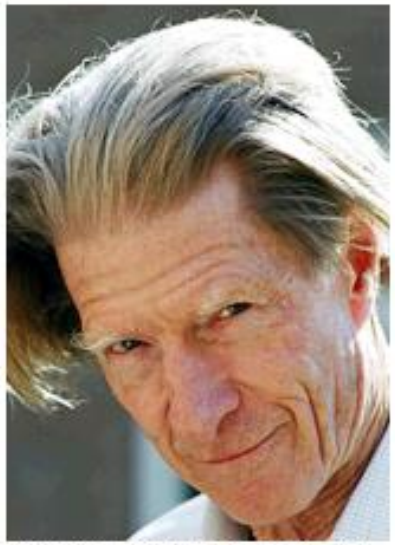


Celulele stem pluripotente

The Nobel Prize in Physiology or Medicine 2012

Sir John B. Gurdon

Shinya Yamanaka



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Sir John B. Gurdon

Shinya Yamanaka

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"*

Page 1 of 1 Go Sort by: Publication Date -- newest to oldest

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1. Title: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors**

Author(s): Takahashi, Kazutoshi; Yamanaka, Shinya

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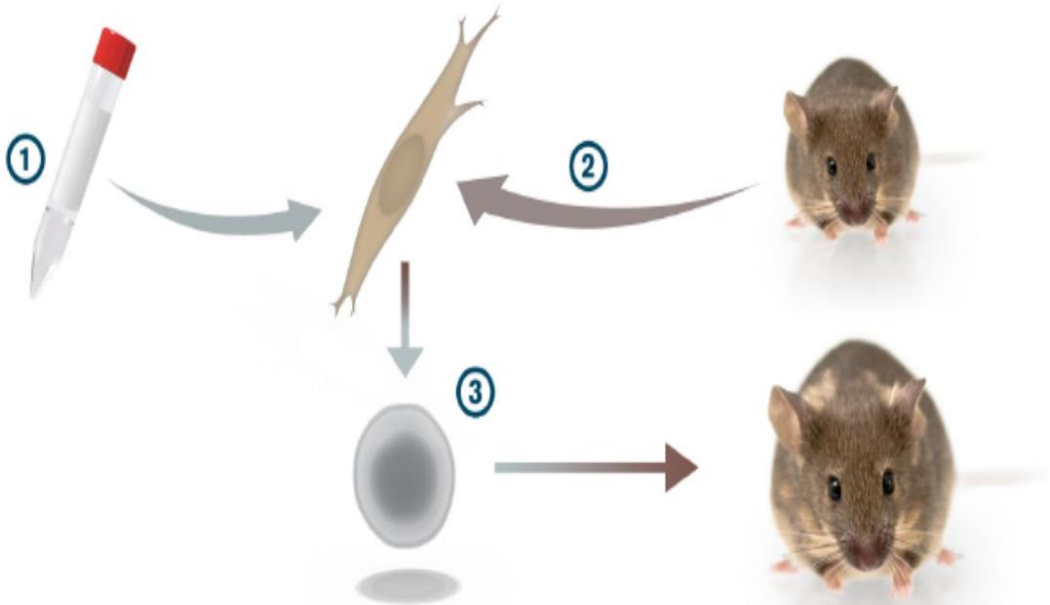
S·F·X [View abstract]



Shinya Yamanaka

Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
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DOI 10.1016/j.cell.2006.07.024

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

INTRODUCTION

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Evans and Kaufman, 1981; Martin, 1981). Human ES cells might be used to treat a host of diseases, such as Parkinson's disease, spinal cord injury, and diabetes (Thomson et al., 1998). However, there are ethical difficulties regarding the use of human embryos, as well as the problem of tissue rejection following transplantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from the patients' own cells.

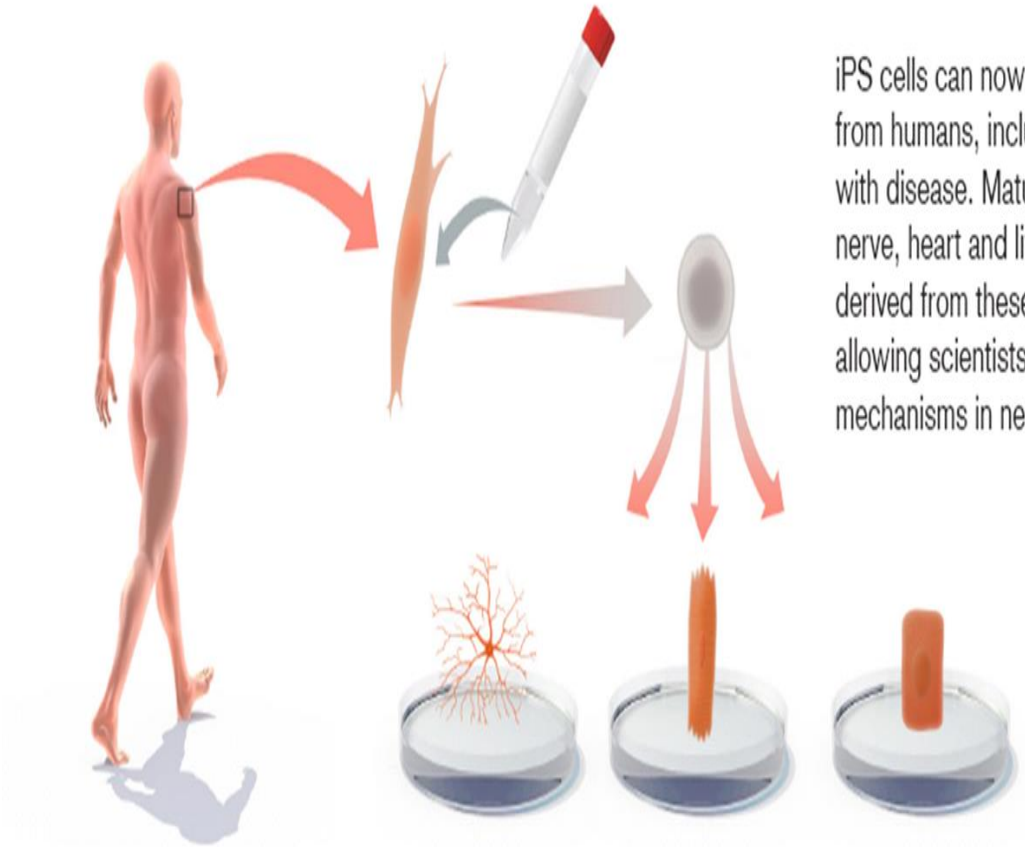
Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997) or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and *β-catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

RESULTS

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β-catenin, c-Myc, and Stat3, we used active forms, S33Y-β-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2ΔSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.



iPS cells can now be generated from humans, including patients with disease. Mature cells including nerve, heart and liver cells can be derived from these iPS cells, thereby allowing scientists to study disease mechanisms in new ways.

Illustration and layout: Mattias Karlén

CICLUL CELULAR. CONTROLUL ACTIVITATII CELULARE

- ***Ciclu celular* = *c***elulele se multiplica prin realizarea unei secvente ordonate de evenimente in care isi duplica continutul si apoi il imparte in doua parti egale.
- **Speciile unicelulare** (precum bacteriile si drojdiile)= fiecare diviziune celulara produce un organism complet nou.
- **Speciile multicelulare**= sunt necesare secvente lungi si complexe de diviziuni celulare pentru a produce un nou organism.

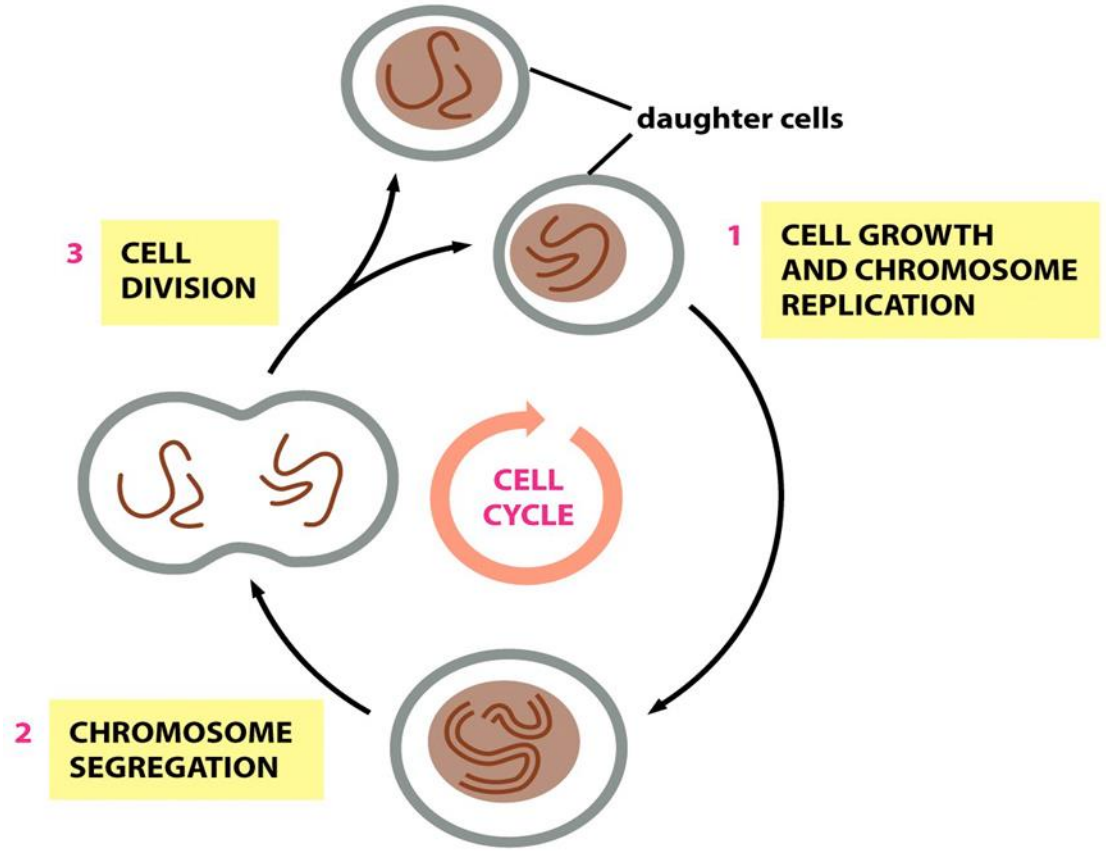


Figure 17-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Etapele ciclului celular

- Duplicatia cromozilor se produce in faza S (S de la sinteza ADN), etapa care se realizeaza in 10-12 ore si reprezinta aproximativ 50% din durata ciclului celular la o celula tipica de mamifer.
- Dupa faza S, segregarea cromozomilor si diviziunea celulara se realizeaza in faza M (M de la mitoza), care se realizeaza in mai putin de o ora la celulele de mamifer.

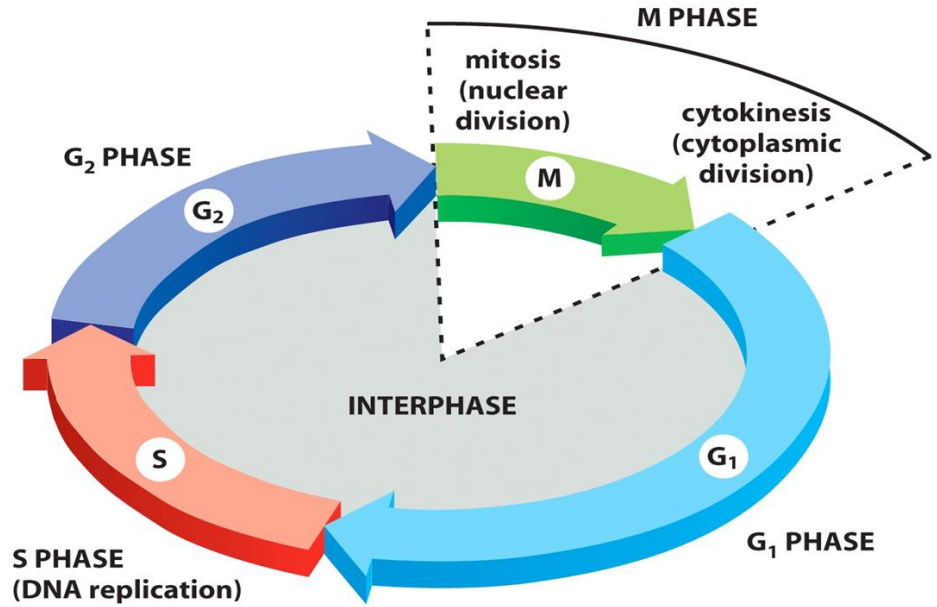


Figure 17-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

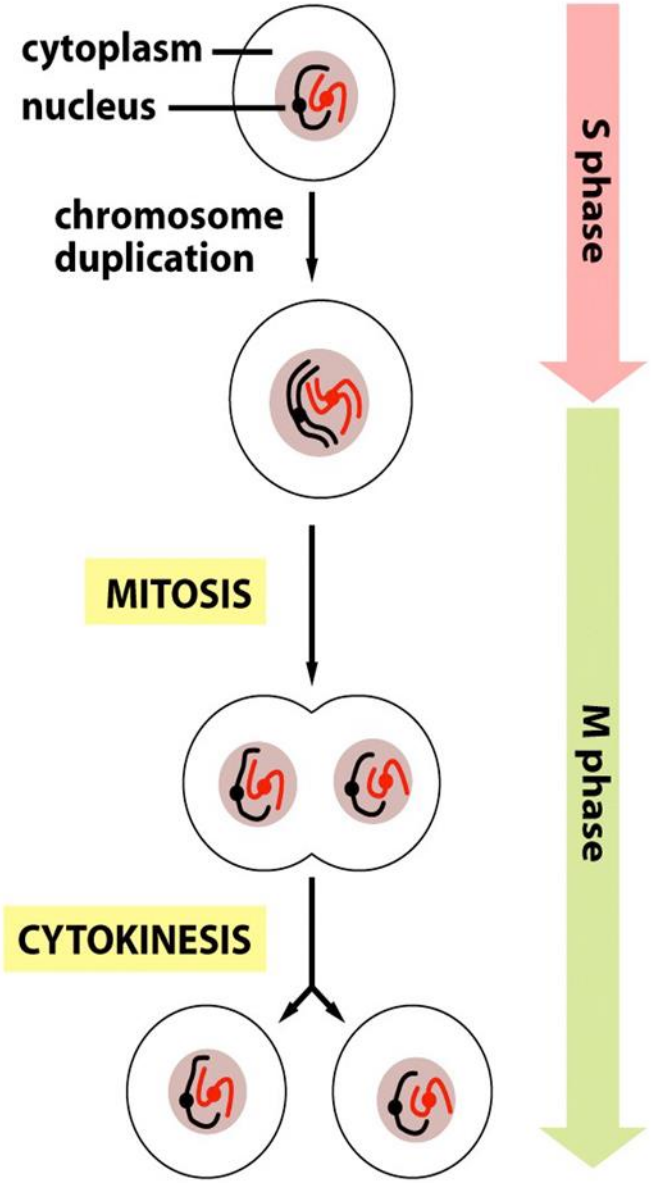


Figure 17-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Diviziunea celulara

Faza M cuprinde doua evenimente majore:

- diviziunea nucleara, sau mitoza, in timpul careia cromozomii sunt distribuiti celor doua celule fiice;
- diviziunea citoplasmatica, sau citokineza, care presupune impartirea celulei in doua.

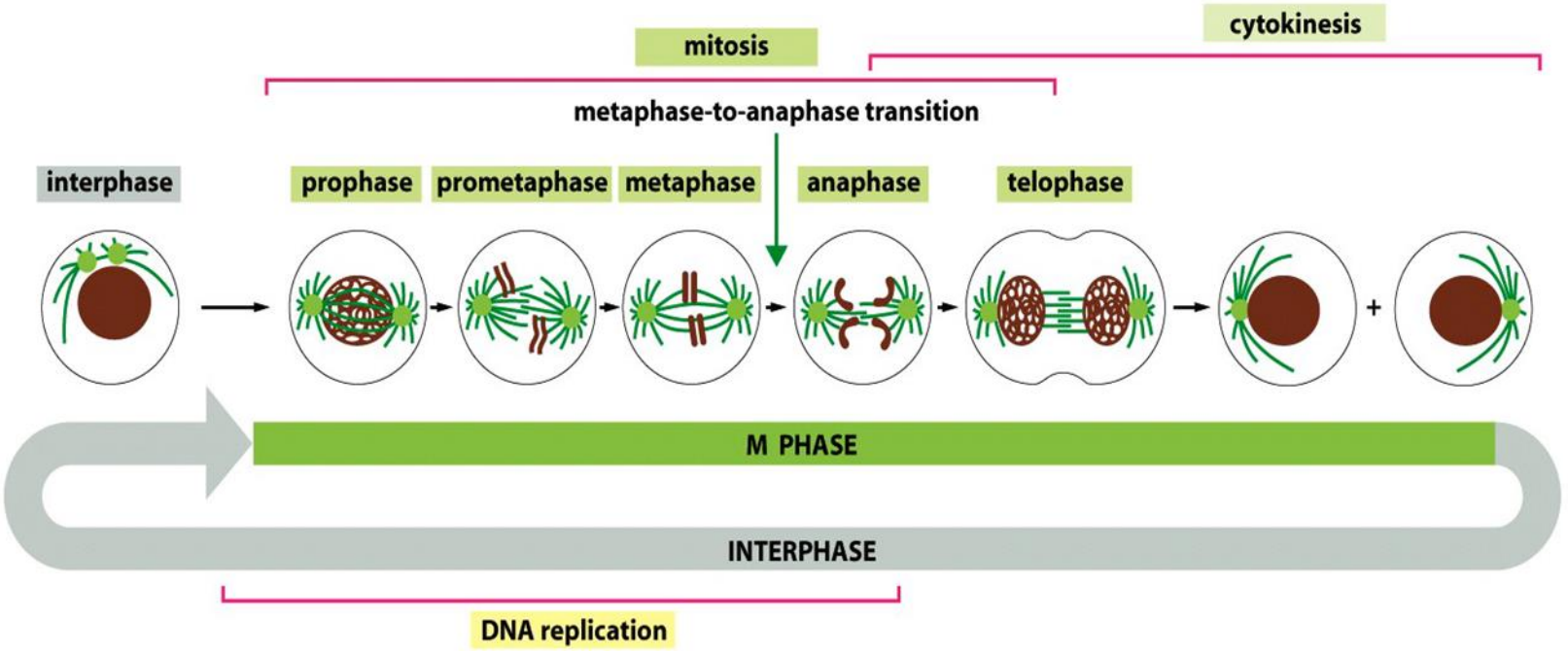


Figure 17-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Controlul ciclului celular

- Sistemul de control al ciclului celular dirijeaza progresia ciclului celular in 3 puncte majore de tranzitie, denumite puncte de control (checkpoints) .
- Primul checkpoint este punctul de restrictie de la finalul fazei G1, cand celula se dedica duplicarii cromozomilor.
- Al doilea punct este punctul G2/M, unde sistemul de control dirijeaza evenimentele precoce din mitoza care conduc la aranjarea cromozomilor la fusul de diviziune.
- Al treilea punct este tranzitia metafaza-anafaza, cand sistemul de control stimuleaza separarea cromatidelor surori, conducand la finalizarea mitozei si citokinezei.
- Sistemul de control blocheaza progresia prin fiecare din aceste puncte daca sunt detectate probleme in interiorul sau in exteriorul celulei.

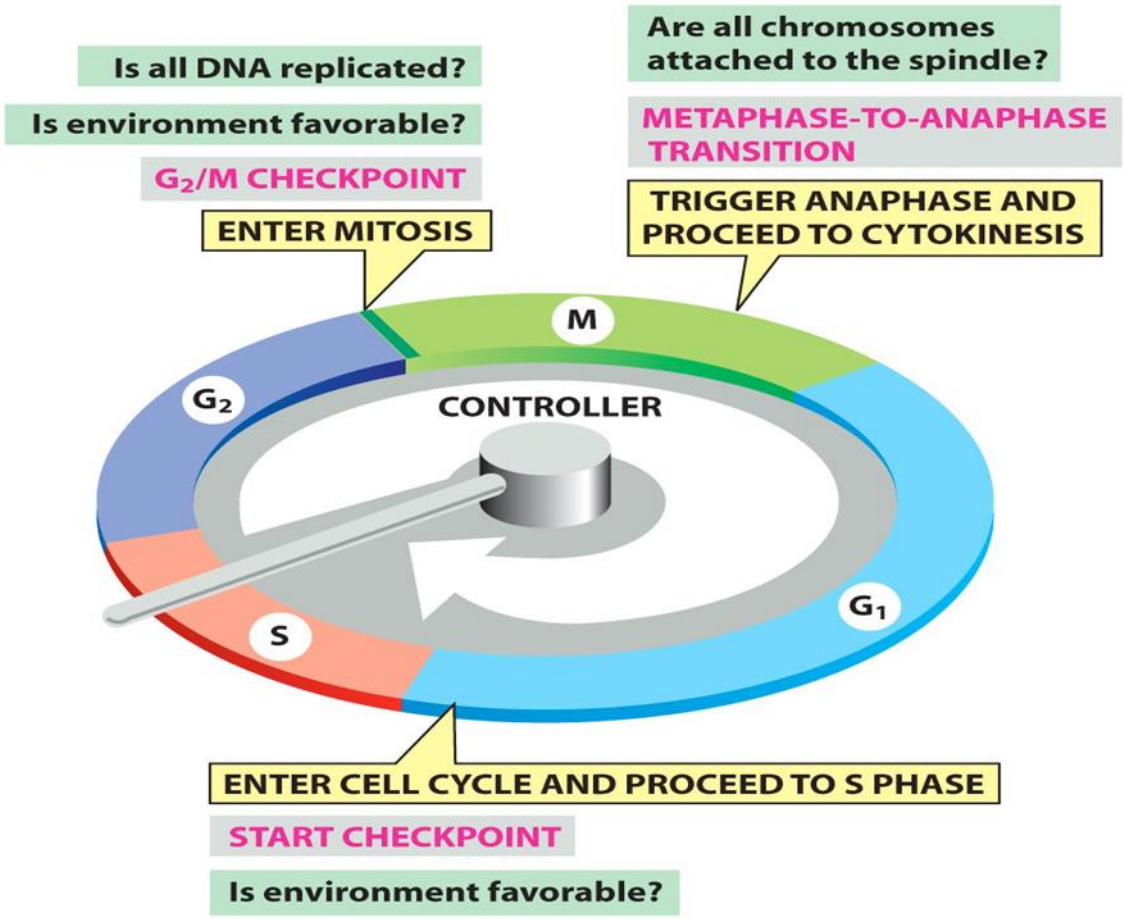


Figure 17-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

DISCUȚII