Základy bunkovej biológie

N-bCBI-303

ZS 2016/2017

Prednáška:	**utorok**	**9.50 – 11.20**
Seminár:	**štvrtok**	**15.40 – 17.10; 17.20 – 18.50**
Miestnosť:	**CH1-222**	

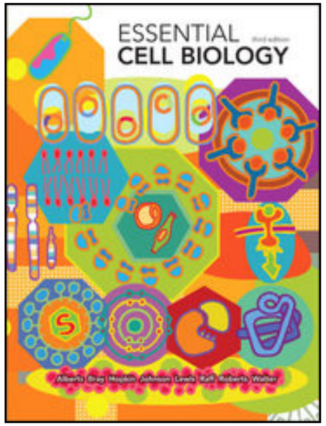
Syllabus ZS 2016/2017

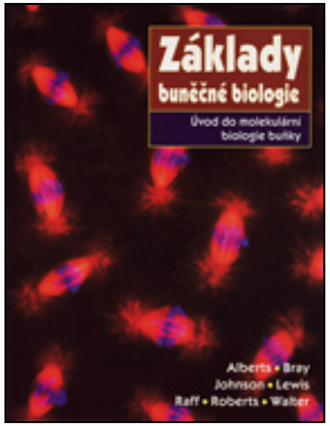
1. **Vnútorná organizácia buniek a ich pôvod v evolúcii**
2. **Bunkové jadro: štruktúra a dynamika chromozómov**
3. **Mechanizmy opravy poškodenej DNA**
4. **Transkripcia a úlohy RNA v bunke**
5. **Syntéza a distribúcia proteínov v bunkách**
6. **Princípy kontroly expresie génov**
7. **Úloha biologických membrán v eukaryotickej bunke**
8. **Mitochondrie a chloroplasty**
9. **Endoplazmatické retikulum a Golgiho aparát**
10. **Vakuoly, lyzozómy a peroxizómy**
11. **Cytoskelet ako dynamická štruktúra**
12. **Od jednotlivých buniek k tkanivám a mnohobunkovým organizmom**

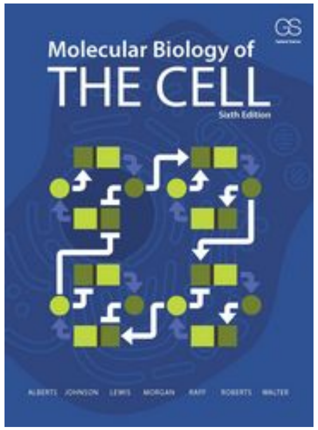
Hodnotenie: ústna skúška, predpokladom pripustenia ku skúške je zisk minimálne 50% bodového hodnotenia zo seminára

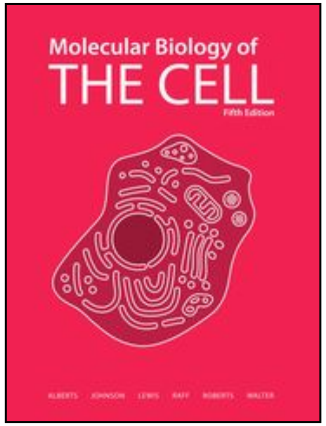
Odporučená literatúra:

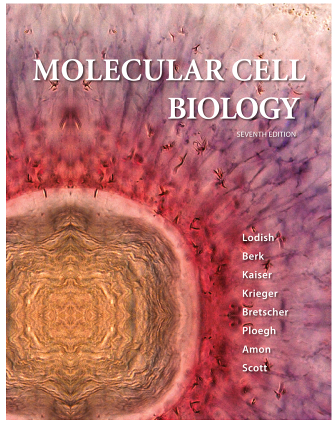
1. Alberts B. *et al.* (2014) Molecular Biology of the Cell. 6th edition. Garland Publishing Inc. http://www.ncbi.nlm.nih.gov/books/NBK21054/
2. Lodish H. *et al.* (2012) Molecular Cell Biology. 7th edition. W. H. Freeman & Co. http://www.ncbi.nlm.nih.gov/books/NBK21475/
3. Alberts B. *et al.* (2009) Essential Cell Biology. 3rd edition. Garland Publishing Inc.
4. Alberts B. a kol. (2005) Základy buněčné biologie: Úvod do molekulární biologie bunky. 2. vydanie Espero Publishing.
5. Cooper G.M. & Hausman R.E. (2009) The Cell: A Molecular Approach. 5th edition. Sinauer Associates. http://www.ncbi.nlm.nih.gov/books/NBK9839/











Prednášky PDF: http://moodle.uniba.sk

Prednáška 1:

Vnútorná organizácia buniek a ich pôvod v evolúcii

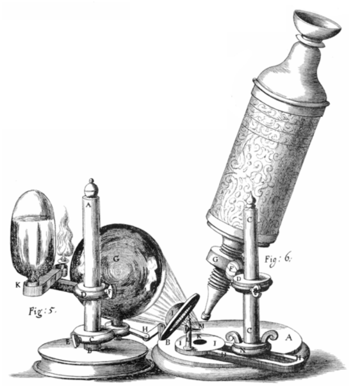
- História a kľúčové objavy bunkovej biológie
- Bunková teória
- Porovnanie prokaryotických a eukaryotických buniek
- Komplexná organizácia eukaryotickej bunky
- Význam intracelulárnej kompartmentalizácie a vnútrobunkový dialóg
- Vznik buniek v evolúcii
- Pôvod komplexnej (eukaryotickej) bunky

Cytológia
Biochémia
Genetika
Molekulárna biológia
Fyziológia
Evolučná biológia

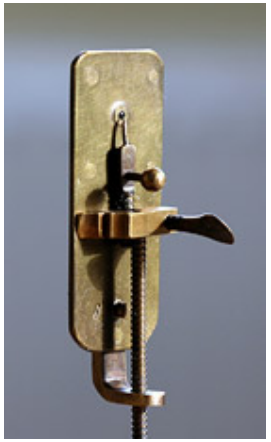
Prvé pozorovania buniek…

Robert Hooke
(1635-1703)

Antonie van Leeuwenhoek
(1632-1723)

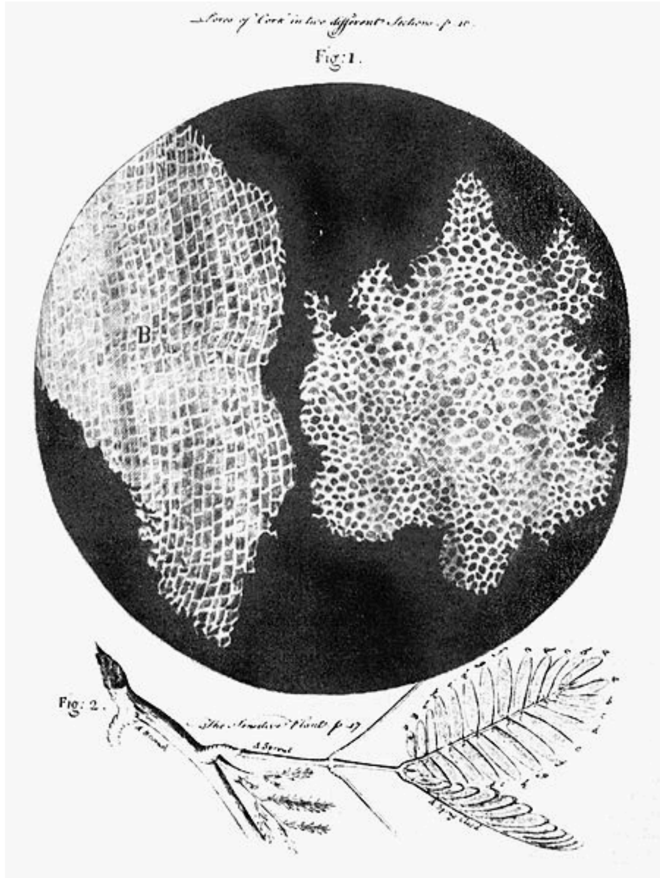


Micrographia (1665)
- termín „bunka” (angl. *cell*)
- organizmy pozostávajú z buniek



http://www.history-of-the-microscope.org/

- riasy, prvoky, kvasinky, erytrocyty, spermie, baktérie, vakuoly, …



Bunky v pletivách *Quercus suber*
(dub korkový)

Bunková teória (1839-1858)

Theodor Schwann
(1810-1882)

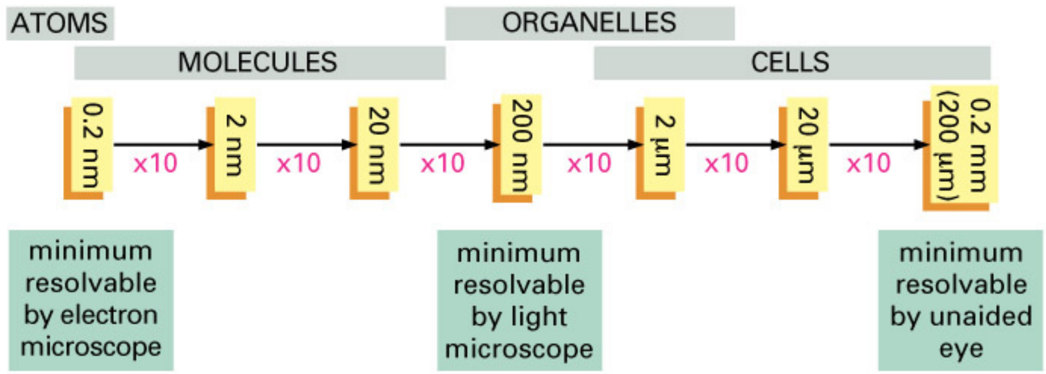
Matthias J. Schleiden
(1804-1881)

Robert Remak
(1815-1865)

Rudolf L.K. Virchow
(1821-1902)

- Živé organizmy pozostávajú z jednej alebo viacerých buniek
- Bunky sú základné štruktúrne a funkčné jednotky živých systémov (organizmov)
- Bunky vznikajú len delením preexistujúcich buniek
- Všetky bunky majú v podstate rovnaké (podobné) chemické zloženie
- Bunky predstavujú chemický systém, v ktorom prebieha premena energií a metabolické (biochemické) reakcie
- Genetický materiál buniek (genóm) tvoria molekuly DNA

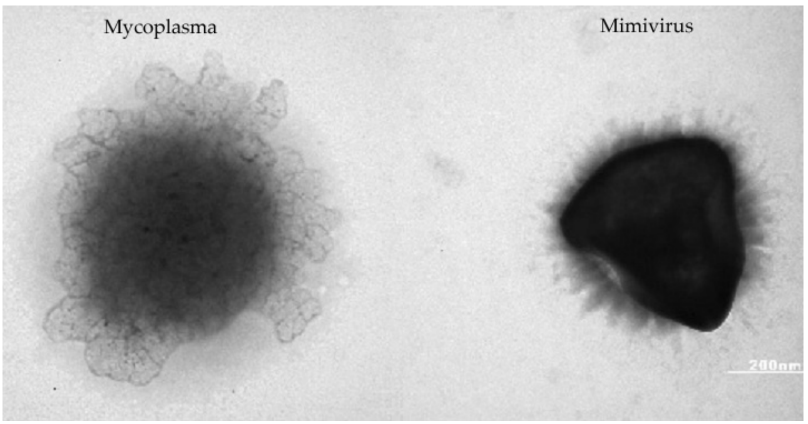
Veľkosť buniek



$$1\ \text{m} = 10^3\ \text{mm} = 10^6\ \mu\text{m} = 10^9\ \text{nm}$$

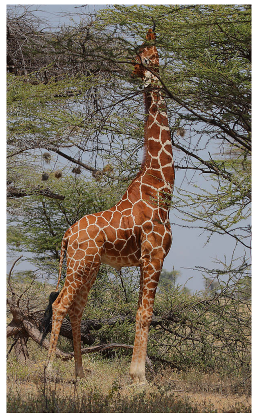
Figure 1-6 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Vírusy: min. 30 nm (*picornavirus*) - 250 nm (*paramyxovirus*); *mimivirus* (0.3-0.8 µm); max. *pithovirus* (1.5 µm)
Prokaryoty: min. *Mycoplasma* (0.3 µm) … max. *Epulopiscium fishelsoni* (0.7 mm)
Eukaryoty: 9 µm (erytrocyt) - 800 µm (améby) … max. neurón v žirafom krku (až 3 m)
pštrosie vajce (15x13 cm, až 1,5 kg)



http://www.giantvirus.org/

pštrosie vajce



neurón žirafy

Philippe *et al.* (2013) *Science* 341: 281-286.

Pandoraviruses: Amoeba Viruses with Genomes Up to 2.5 Mb Reaching That of Parasitic Eukaryotes

Nadège Philippe,[1,2]* Matthieu Legendre,[1]* Gabriel Doutre,[1] Yohann Couté,[3] Olivier Poirot,[1] Magali Lescot,[1] Defne Arslan,[1] Virginie Seltzer,[1] Lionel Bertaux,[1] Christophe Bruley,[3] Jérome Garin,[3] Jean-Michel Claverie,[1]† Chantal Abergel[1]†

Ten years ago, the discovery of Mimivirus, a virus infecting Acanthamoeba, initiated a reappraisal of the upper limits of the viral world, both in terms of particle size (>0.7 micrometers) and genome complexity (>1000 genes), dimensions typical of parasitic bacteria. The diversity of these giant viruses (the Megaviridae) was assessed by sampling a variety of aquatic environments and their associated sediments worldwide. We report the isolation of two giant viruses, one off the coast of central Chile, the other from a freshwater pond near Melbourne (Australia), without morphological or genomic resemblance to any previously defined virus families. Their micrometer-sized ovoid particles contain DNA genomes of at least 2.5 and 1.9 megabases, respectively. These viruses are the first members of the proposed "Pandoravirus" genus, a term reflecting their lack of similarity with previously described microorganisms and the surprises expected from their future study.

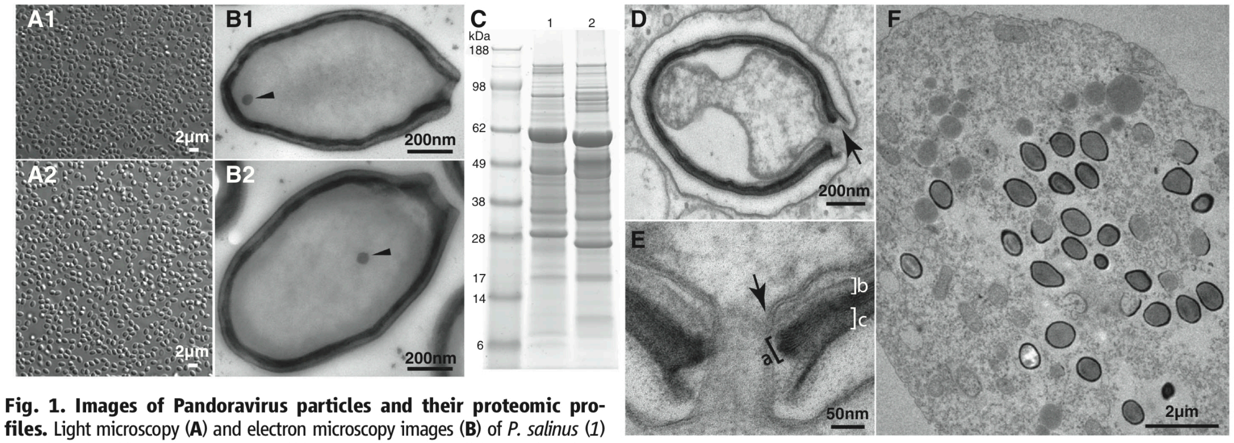


Fig. 1. Images of Pandoravirus particles and their proteomic profiles. Light microscopy (**A**) and electron microscopy images (**B**) of *P. salinus* (*1*) and *P. dulcis* (*2*) purified particles. (**C**) Electrophoresis profiles of *P. salinus* (lane 1) and *P. dulcis* (lane 2) extracted proteins. (**D**) Internalized *P. salinus* particle in the host vacuole. Once fused with the vacuole membrane (arrow), the virion internal membrane creates a continuum with the host cytoplasm. The particles are wrapped into a ~70-nm-thick tegument-like envelope consisting of three layers. (**E**) Magnified image of the opened ostiole-like apex: from the inside out, a layer of light density of unknown composition (~20 nm, marked "a"), a dark layer comprising a dense mesh of fibrils (~25 nm, marked "b"), and an external layer of medium density (~25 nm, marked "c"). This tegument-like envelope is interrupted by the ostiole-like pore measuring ~70 nm in diameter. Inside the particle, the lipid membrane encloses a diffuse interior devoid of visible substructure, except for a spherical area of electron-dense material (50 nm in diameter, arrowhead) seen episodically but in a reproducible fashion. (**F**) Ultrathin section of an Acanthamoeba cell filled with *P. salinus* at various stages of maturation.

Morfologická variabilita buniek

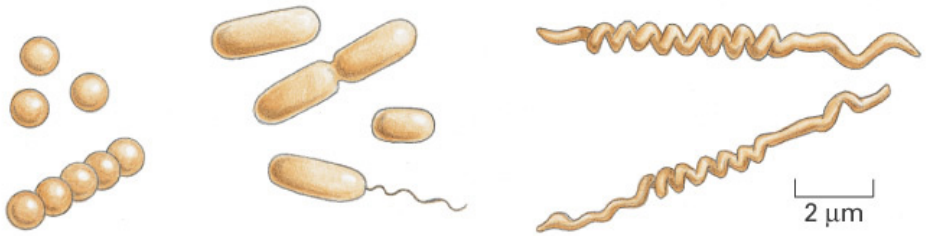


Figure 1-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

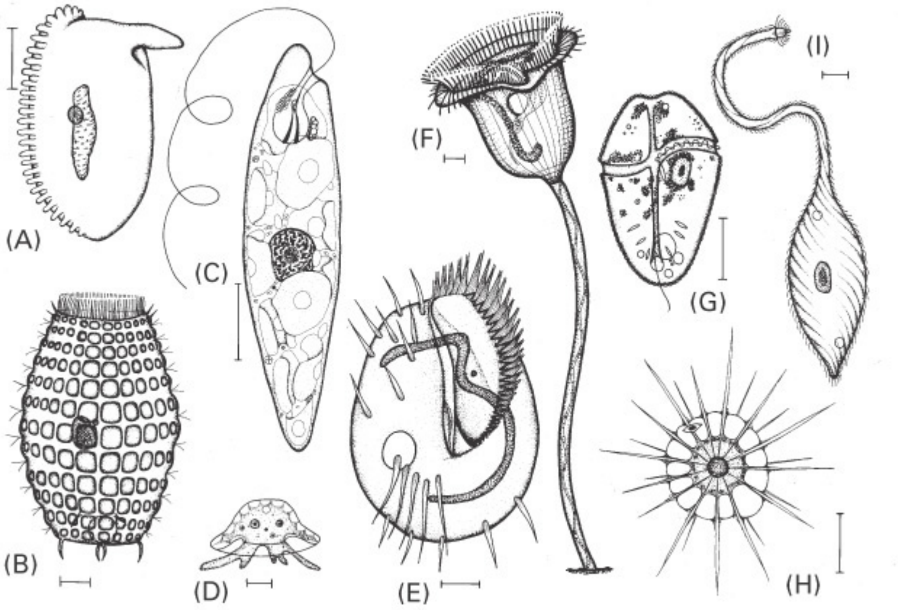


Figure 1-31 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Morfologická variabilita ako dôsledok diferenciácie z jednej bunky (zygoty)

(človek - cca 3,72 x10^{13} buniek)

Figure 2-46. Molecular Biology of the Cell. 4th edition.

fibroblasty

erytrocyty

Figure 10-27. Molecular Biology of the Cell. 4th edition.

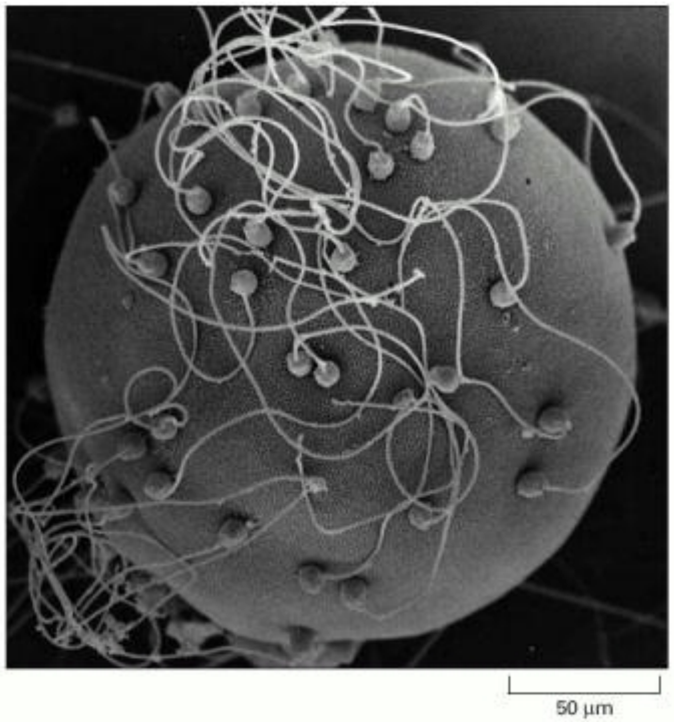


Figure 20-4. Molecular Biology of the Cell. 4th edition.

oocyt a spermie

neurón

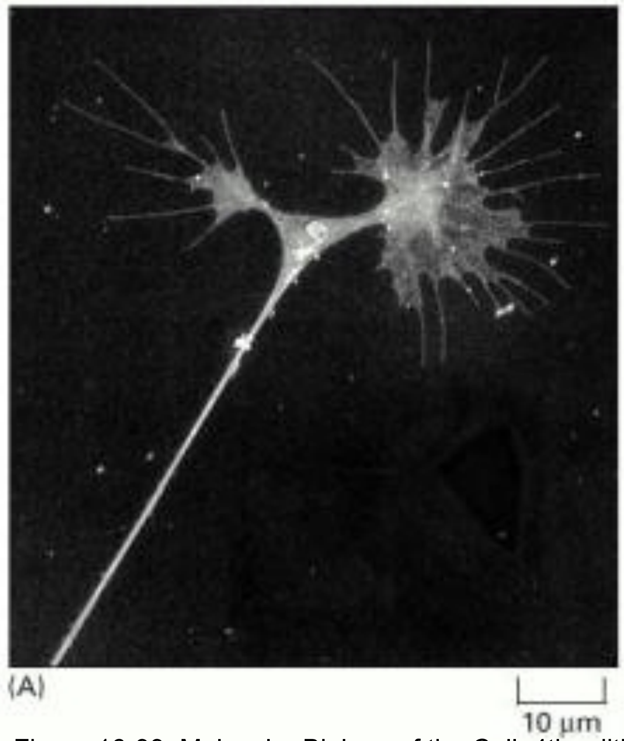


Figure 16-99. Molecular Biology of the Cell. 4th edition.

Spoločenstvá jednobunkových organizmov sú jednoduchými modelmi diferenciácie

Kolónie zelených rias

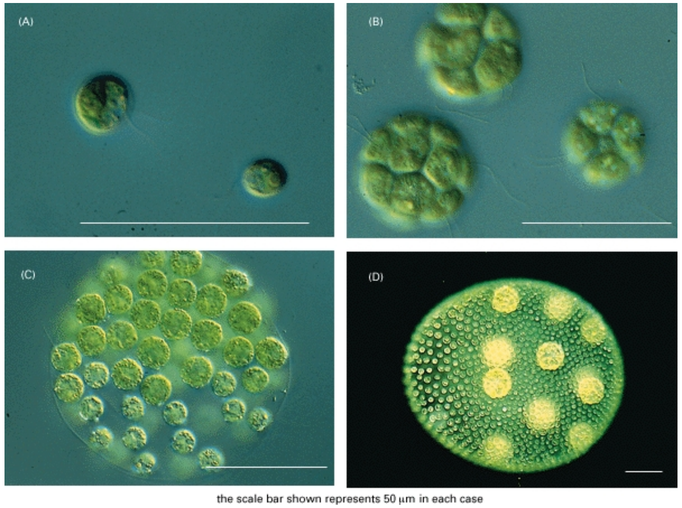


Figure 1-32. Molecular Biology of the Cell. 3rd edition

myxobacterium *Chondromyces crocatus*

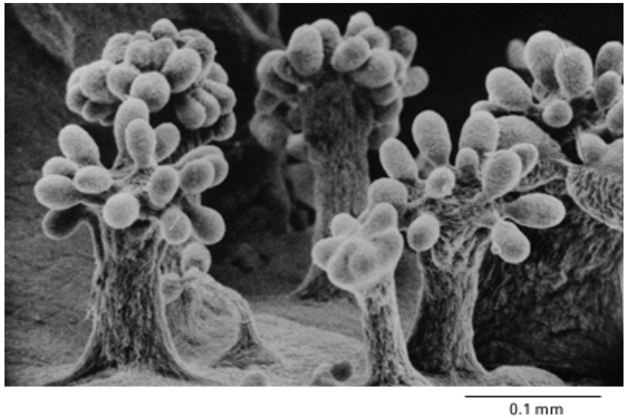
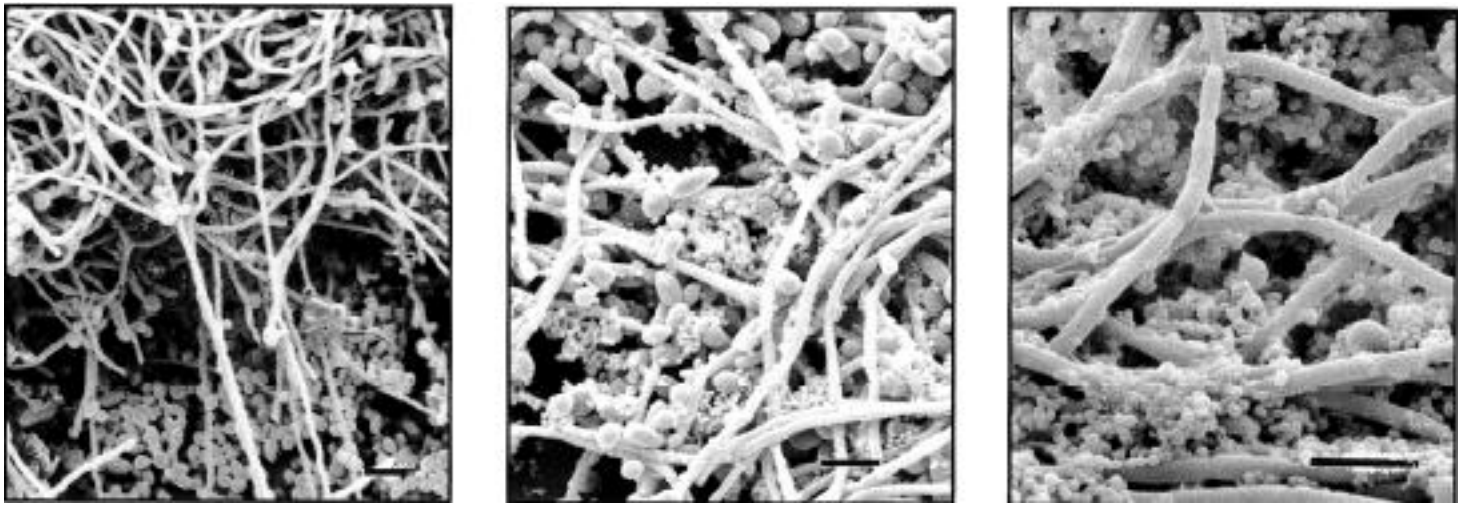


Figure 1-31. Molecular Biology of the Cell. 3rd edition

Biofilmy



Brogden KA & Guthmiller JM (2002). Polymicrobial Diseases. ASM Press.

Všetky bunky majú podobné chemické zloženie - bunky sú chemické systémy

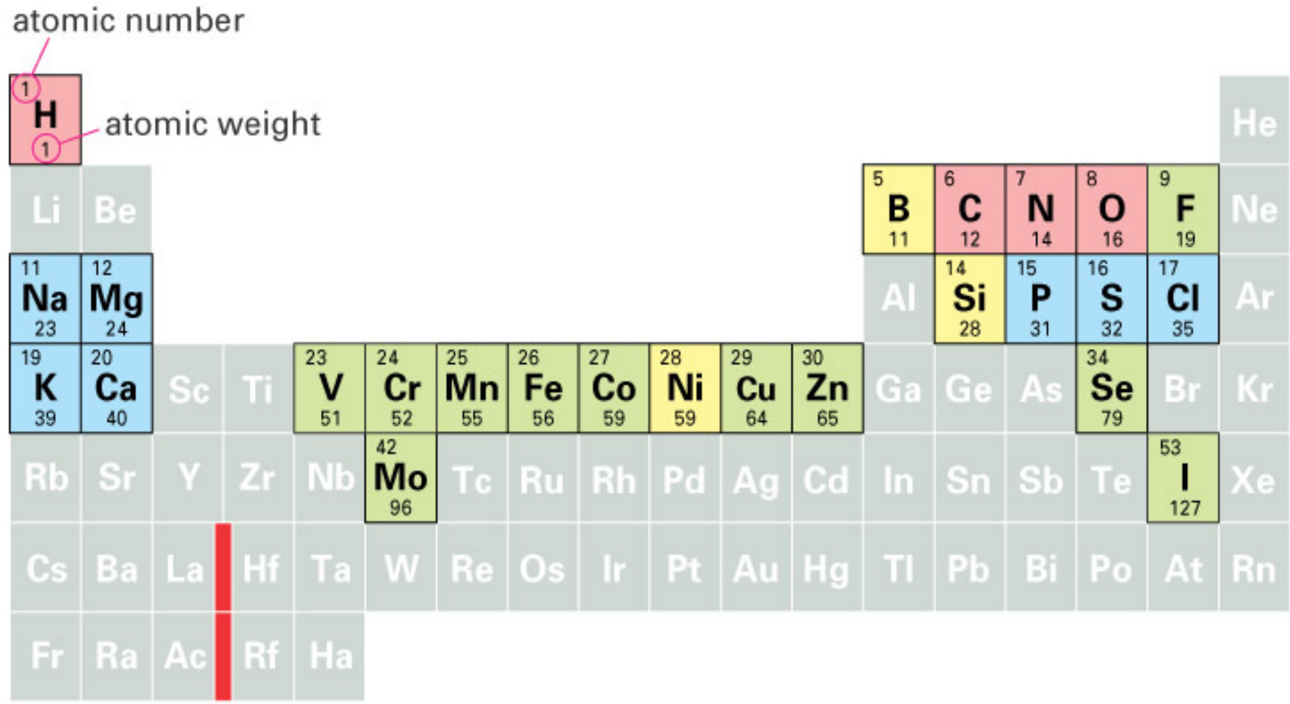


Figure 2-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

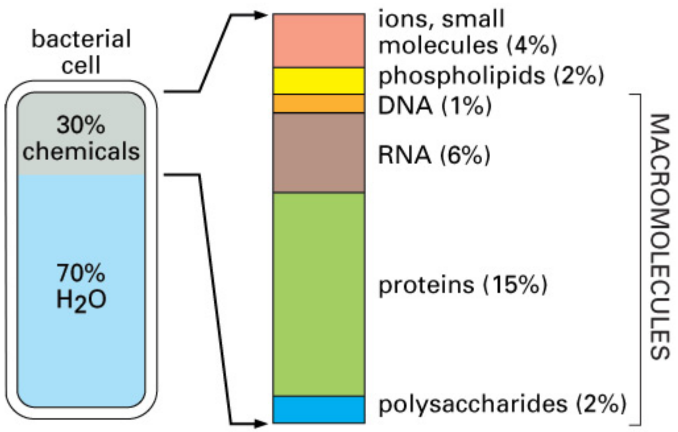


Figure 2–29. Molecular Biology of the Cell, 4th Edition.

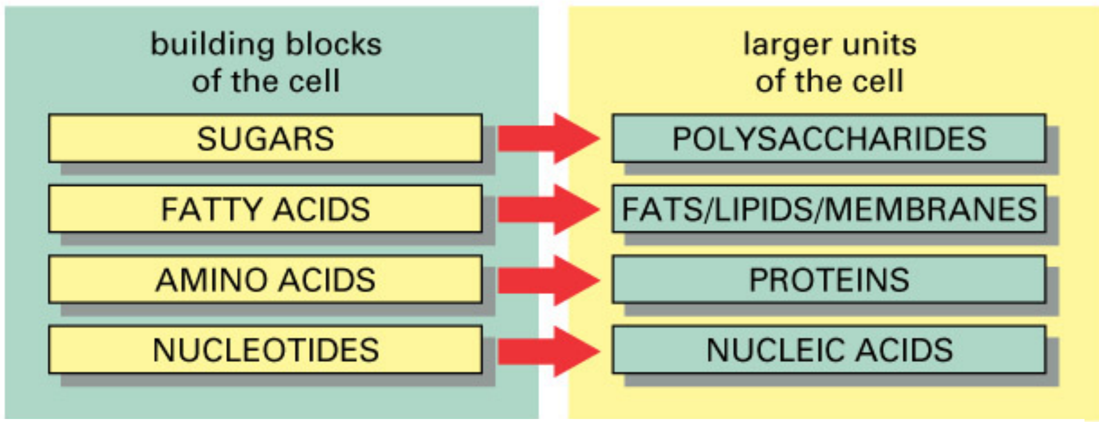


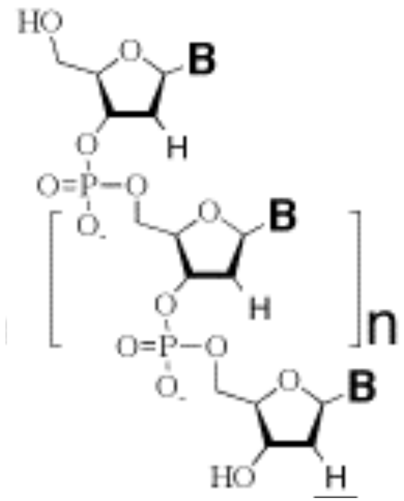
Figure 2-15 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Wolfe-Simon F. et al. (2011) *Science* 332: 1163-1166.

A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus

Felisa Wolfe-Simon,[1,2*] Jodi Switzer Blum,[2] Thomas R. Kulp,[2] Gwyneth W. Gordon,[3] Shelley E. Hoeft,[2] Jennifer Pett-Ridge,[4] John F. Stolz,[5] Samuel M. Webb,[6] Peter K. Weber,[4] Paul C. W. Davies,[1,7] Ariel D. Anbar,[1,3,8] Ronald S. Oremland[2]

Life is mostly composed of the elements carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus. Although these six elements make up nucleic acids, proteins, and lipids and thus the bulk of living matter, it is theoretically possible that some other elements in the periodic table could serve the same functions. Here, we describe a bacterium, strain GFAJ-1 of the Halomonadaceae, isolated from Mono Lake, California, that is able to substitute arsenic for phosphorus to sustain its growth. Our data show evidence for arsenate in macromolecules that normally contain phosphate, most notably nucleic acids and proteins. Exchange of one of the major bio-elements may have profound evolutionary and geochemical importance.



P → As
(?)

Erb T.J. *et al.* (2012) *Science* 337: 467-469.

GFAJ-1 Is an Arsenate-Resistant, Phosphate-Dependent Organism

Tobias J. Erb,[1]*† Patrick Kiefer,[1]* Bodo Hattendorf,[2] Detlef Günther,[2] Julia A. Vorholt[1]†

The bacterial isolate GFAJ-1 has been proposed to substitute arsenic for phosphorus to sustain growth. We have shown that GFAJ-1 is able to grow at low phosphate concentrations (1.7 μM), even in the presence of high concentrations of arsenate (40 mM), but lacks the ability to grow in phosphorus-depleted (<0.3 μM), arsenate-containing medium. High-resolution mass spectrometry analyses revealed that phosphorylated central metabolites and phosphorylated nucleic acids predominated. A few arsenylated compounds, including C6 sugar arsenates, were detected in extracts of GFAJ-1, when GFAJ-1 was incubated with arsenate, but further experiments showed they formed abiotically. Inductively coupled plasma mass spectrometry confirmed the presence of phosphorus in nucleic acid extracts, while arsenic could not be detected and was below 1 per mil relative to phosphorus. Taken together, we conclude that GFAJ-1 is an arsenate-resistant, but still a phosphate-dependent, bacterium.

Reaves M.L. *et al.* (2012) *Science* 337: 469-473.

Absence of Detectable Arsenate in DNA from Arsenate-Grown GFAJ-1 Cells

**Marshall Louis Reaves,[1,2] Sunita Sinha,[3] Joshua D. Rabinowitz,[1,4]
Leonid Kruglyak,[1,5,6] Rosemary J. Redfield[3]***

A strain of *Halomonas* bacteria, GFAJ-1, has been claimed to be able to use arsenate as a nutrient when phosphate is limiting and to specifically incorporate arsenic into its DNA in place of phosphorus. However, we have found that arsenate does not contribute to growth of GFAJ-1 when phosphate is limiting and that DNA purified from cells grown with limiting phosphate and abundant arsenate does not exhibit the spontaneous hydrolysis expected of arsenate ester bonds. Furthermore, mass spectrometry showed that this DNA contains only trace amounts of free arsenate and no detectable covalently bound arsenate.

Prokaryotické a eukaryotické bunky

Figure 1–18 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

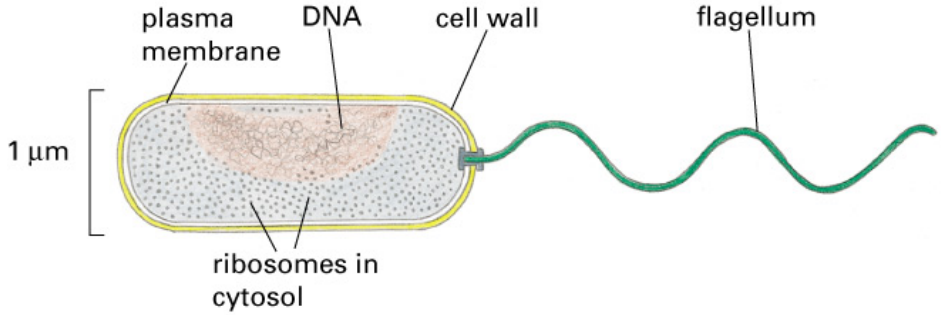


Figure 1–18 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

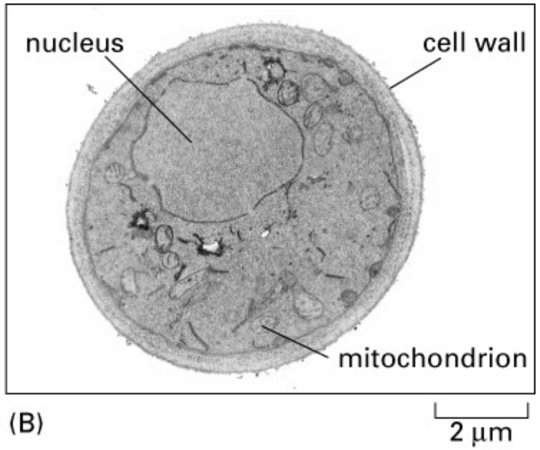


Figure 1–43. Molecular Biology of the Cell, 4th Edition.

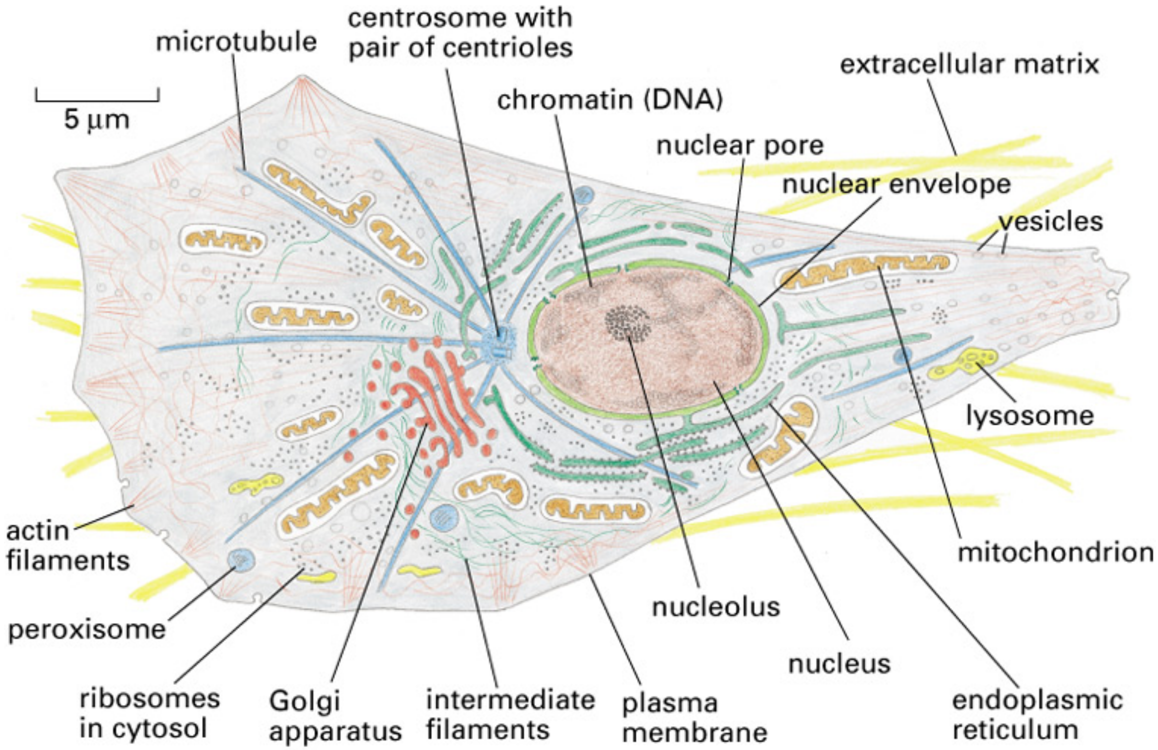


Figure 1–31. Molecular Biology of the Cell, 4th Edition.

Vnútrobunková kompartmentalizácia

Kompartment - priestor oddelený od ostaných častí (bunky):

- membránou
    - endomembránový systém bunky
    - vnútrobunkový symbiont
- proteínmi (komplexom proteínov)

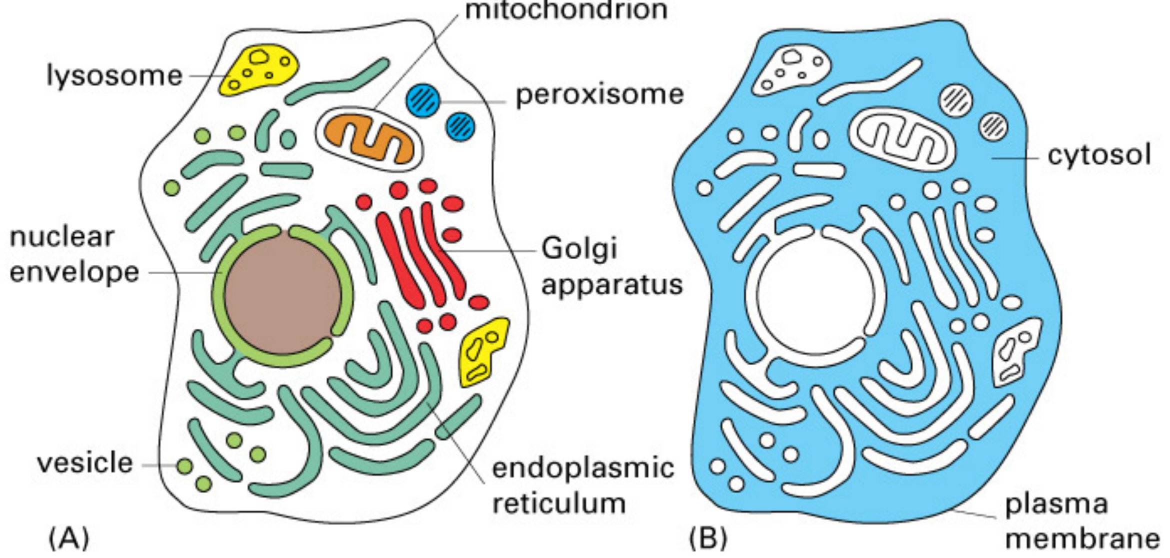


Figure 1-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Vnútrobunkové kompartmenty eukaryotickej bunky, ktoré sú oddelené membránami:

- jadro
- mitochondrie, hydrogenozómy
- plastidy (rastlinné bunky)
- endoplazmatické retikulum
- Golgiho aparát
- lyzozómy, vakuoly
- peroxizómy
- cytosol

Aj v bunkách baktérií a *Archaea* sa nachádzajú kompartmenty:

- **plynové vesikuly (*gas vesicles*)**
- **karboxyzómy – fixácia CO_2**
- **acidokalcizómy – osmoregulácia**
- **chlorozómy – fotosyntéza**
- **magnetozómy – magnetotaxia**
- **endosymbionty**
- **…**

Diekmann & Pereira-Leal (2013) Evolution of intracellular compartmentalization. *Biochem. J.* 449: 319–331.

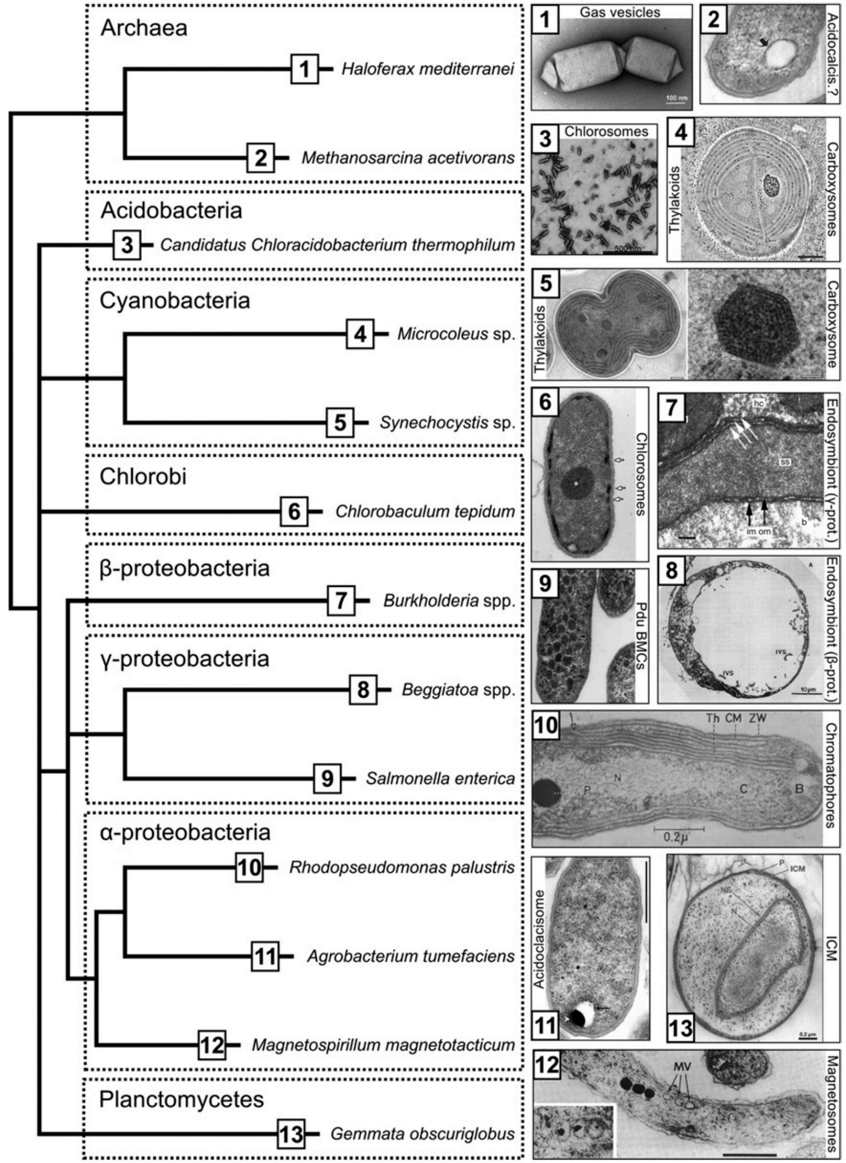


Figure 2 Ultrastructure of selected prokaryotic compartments

Illustrations of the structural and taxonomic diversity of prokaryotic intracellular compartments. 1, Archaeal gas vesicles. Taken from Springer, *Complex Intracellular Structures in Prokaryotes*, 2006, 115–140, Gas vesicles of archaea and bacteria by Pfeifer, F., Figure 4(A), © 2006 Springer-Verlag, Berlin, with kind permission from Springer Science and Business Media. 2, An archaeal granule which has been proposed to be homologous with acidocalcisomes (Acidocalcis., uncertainty indicated by question mark). The arrow indicates the electron-dense volutin granule. Reproduced with permission from Seufferheld, M.J., Kim, K.M., Whitfield, J., Valerio, A. and Caetano-Anolles, G. (2011) Evolution of vacuolar proton pyrophosphatase domains and volutin granules: clues into the early evolutionary origin of the acidocalcisome. Biol. Direct **6**, 50. 3, Isolated chlorosomes (photosynthetic membranes) from Acidobacteria. From Bryant, D.A., Costas, A.M., Maresca, J.A., Chew, A.G., Klatt, C.G. et al. (2007) *Candidatus* Chloracidobacterium thermophilum: an aerobic phototrophic Acidobacterium. Science **317**: 523–526. Reprinted with permission from AAAS. 4, Thylakoid membranes with perforations, as well as carboxysomes. Scale bar, 500 nm. Reprinted by permission from Macmillan Publishers Ltd: *EMBO J.*, Nevo, R., Charuvi, D., Shimoni, E.,

Theodosius Dobzhansky
(1900-1975)

Nothing in biology makes sense except in the light of evolution (*1973*).

"… What do these biochemical or biologic universals mean? They suggest that life arose from inanimate matter only once and that all organisms, no matter now diverse, in other respects, **conserve the basic features of the primordial life**. (It is also possible that there were several, or even many, origins of life; if so, the progeny of only one of them has survived and inherited the earth.)…

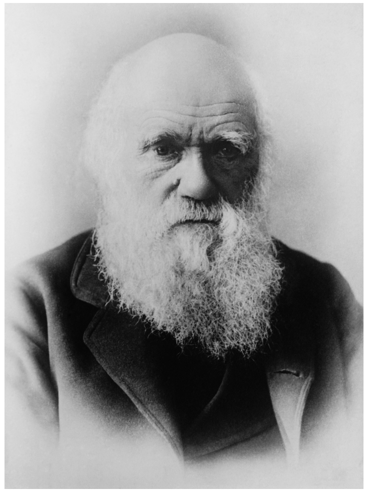
… Seen in the light of evolution, biology is, perhaps, intellectually the most satisfying and inspiring science. **Without that light it becomes a pile of sundry facts some of them interesting or curious but making no meaningful picture as a whole**…"

François Jacob
(1920-2013)

Le jeu des possibles (*1981*).

"... **L'évolution ne tire pas ses nouveautés du néant.** Elle travaille sur ce qui existe déjà, soit qu'elle transforme un système ancien pour lui donner une fonction nouvelle, soit qu'elle combine plusieurs systèmes pour échafauder un autre plus complexe. ... **L'évolution biologique est ainsi fondée sur une sorte de bricolage moléculaire, sur la réutilisation constante du vieux pour faire du neuf** ..."

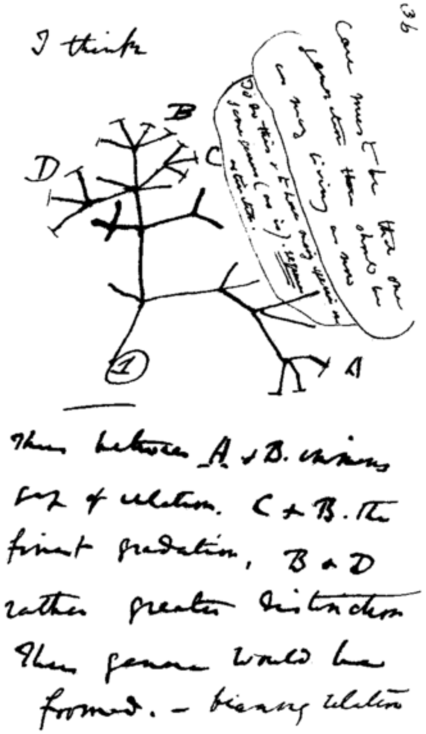
O pôvode druhov



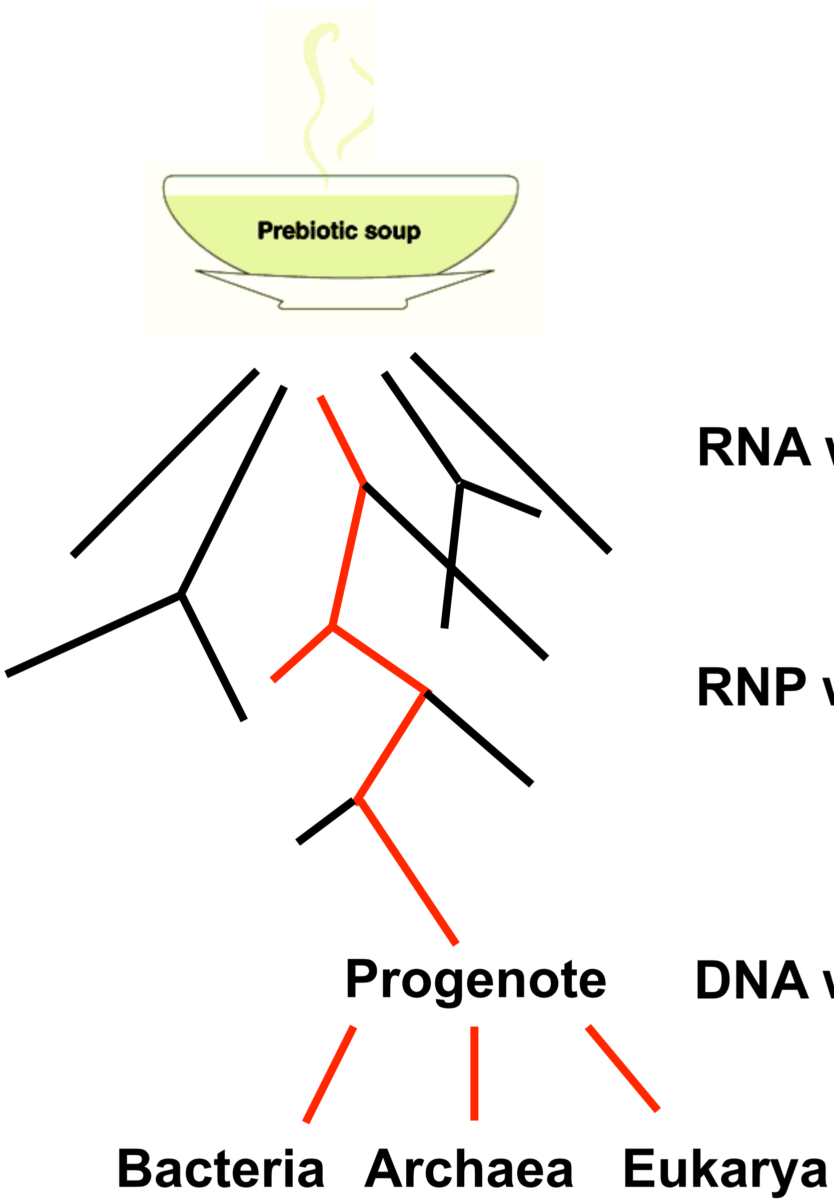
Charles R. Darwin
(1809-1882)

***On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* (1859).**

…probably all of the organic beings which have ever lived on this earth have descended from some one primordial form...



Prebiotic evolution



RNA world

RNP world

DNA world

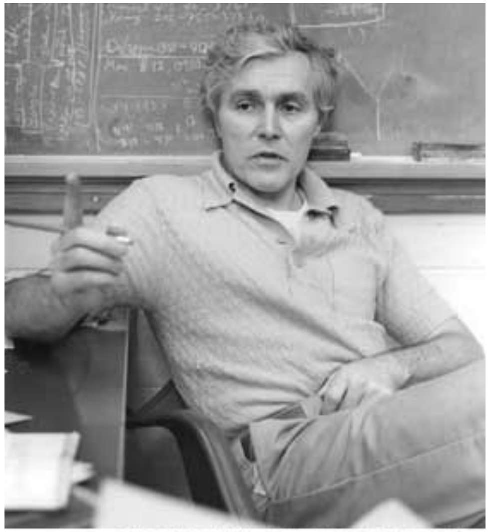
RNA
genotype + phenotype

RNP
genotype + phenotype

DNA genotype

protein phenotype

Tri domény živých organizmov: *Archaea*, *Bacteria*, *Eukarya*


NARA/U. of Illinois 306-PS-E-77—5743

Carl R. Woese
(1928-2012)

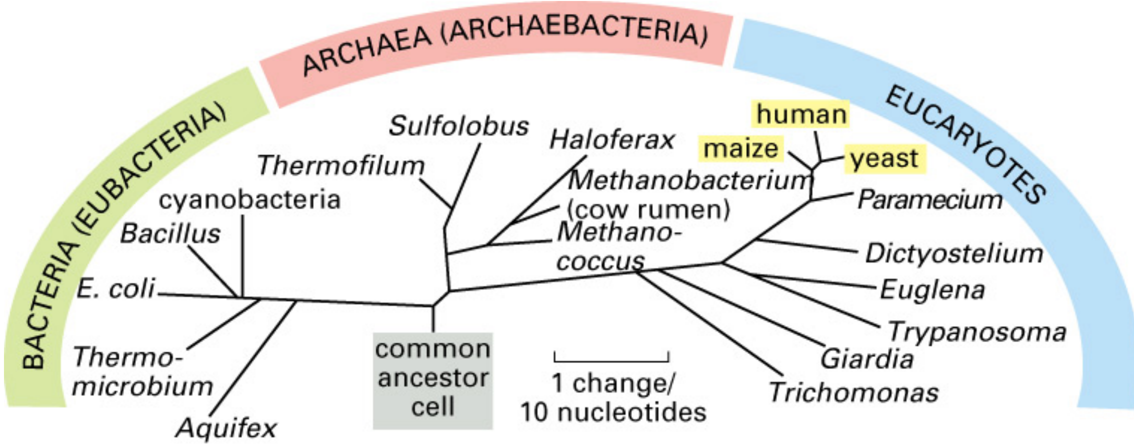


Figure 1–21. Molecular Biology of the Cell, 4th Edition.

Woese CR & Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci USA* **74**: 5088-5090

Woese CR *et al*. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* **87**: 4576-4579

Woese *vs.* Darwin

Mushegian AR & Koonin EV (1996) A minimal gene set for cellular life derived by comparison of complete bacterial genomes. *Proc Natl Acad Sci USA* **93**: 10268-10273

… the last common ancestor of the three primary kingdoms had an RNA genome …

Woese CR (2002) On the evolution of cells. *Proc Natl Acad Sci USA* **99**: 8742-8747

.. Extant life on Earth is descended not from one, but from three distinctly different cell types. However, the designs of the three have developed and matured, in a communal fashion, along with those of many other designs that along the way became extinct…

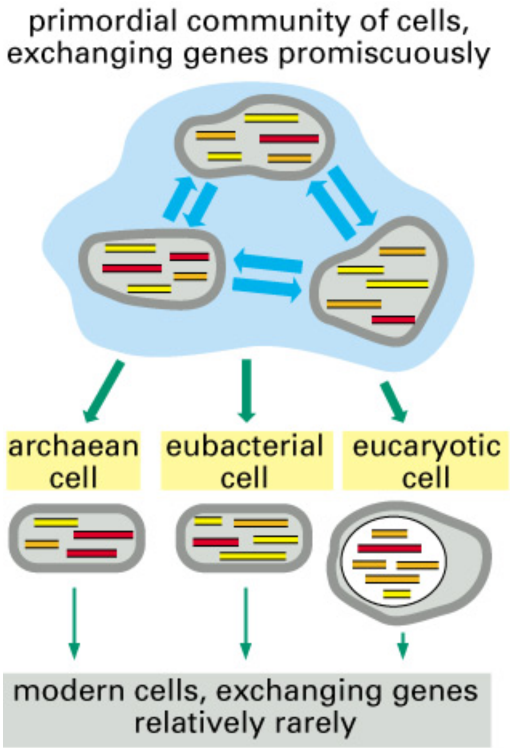


Figure 1–28. Molecular Biology of the Cell, 4th Edition.

Horizonálny transfer génov (HGT, *horizontal gene transfer*)

Darwinovský prah (*Darwinian Treshold*)

RNA svet

↓

RNP svet (supramolekulové agregáty,translácia)

↓

Bunkové formy

↓ ↓ ↓

Archaea*, *Bacteria*, *Eukarya

Vznik komplexnej (eukaryotickej) bunky: Endosymbiotická teória

Richard Altman (1890) - bioblasty pripomínajúce baktérie
Konstantin Merežkovskij (1905) - teória symbiogenézy
↓
Lynn Margulis (1967) - endosymbiotická teória

Lynn Margulis
(1938-2011)

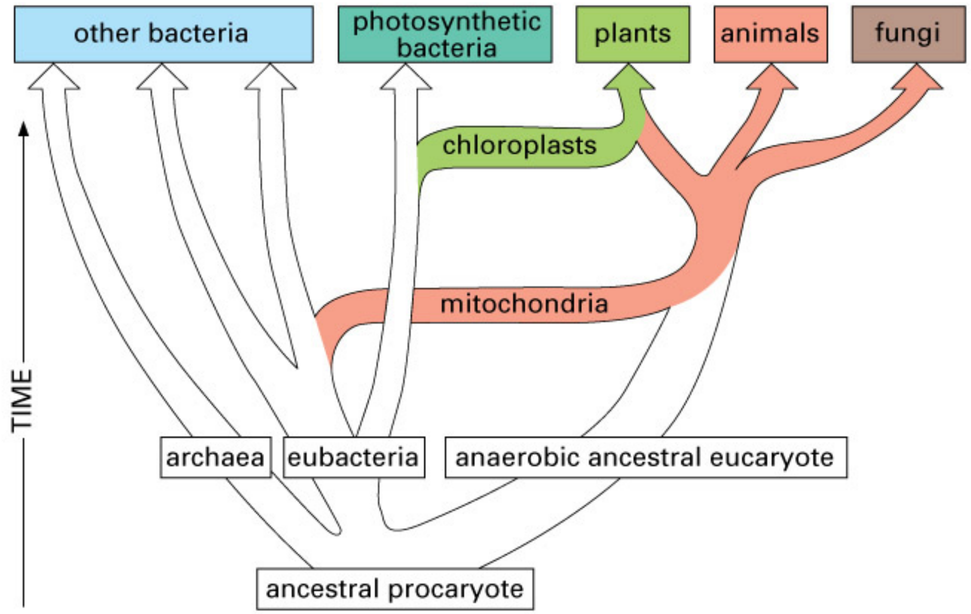
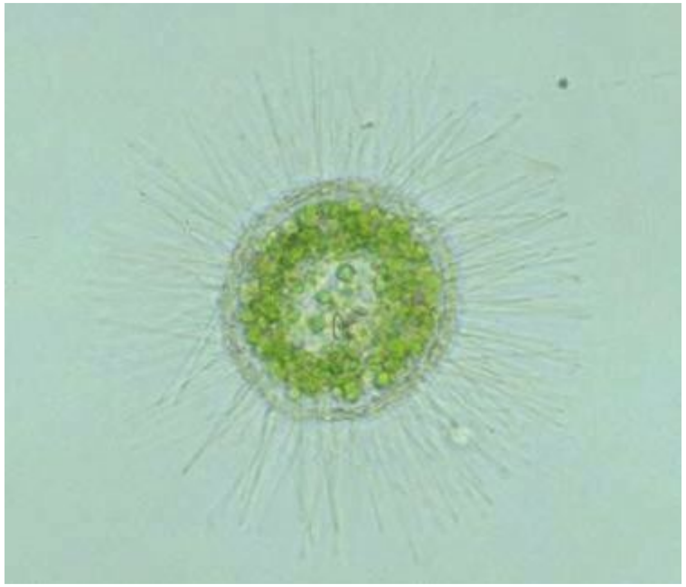


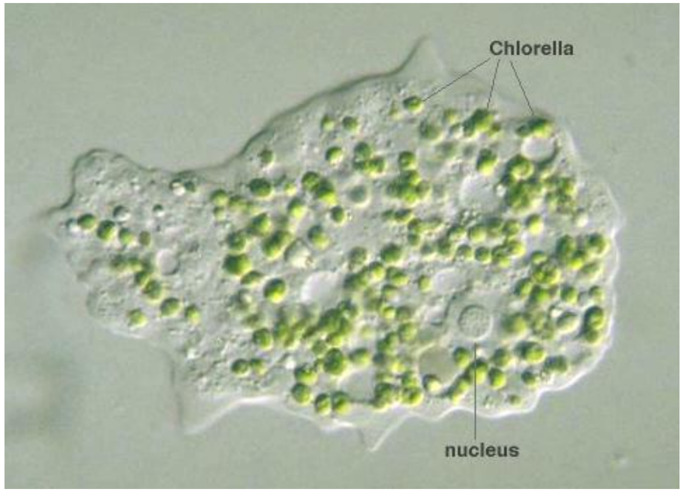
Figure 1-29 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Vnútrobunkové symbiotické organizmy (endosymbionty)

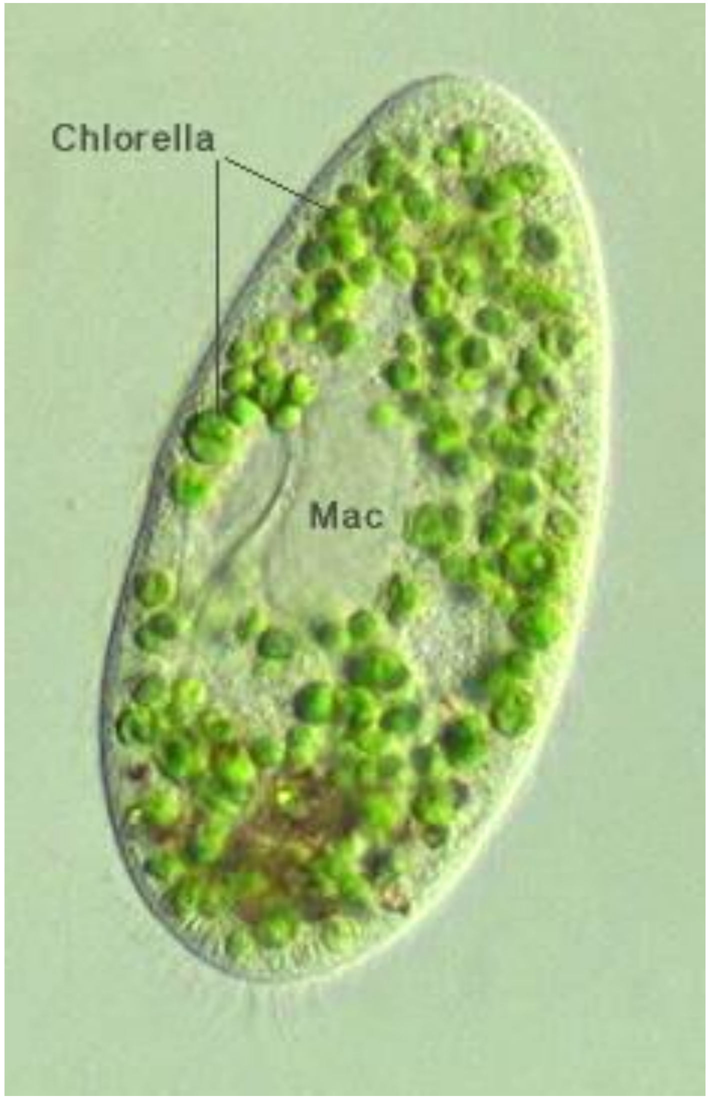
Endosymbiotické riasy (*Chlorella*)

Acanthocystis





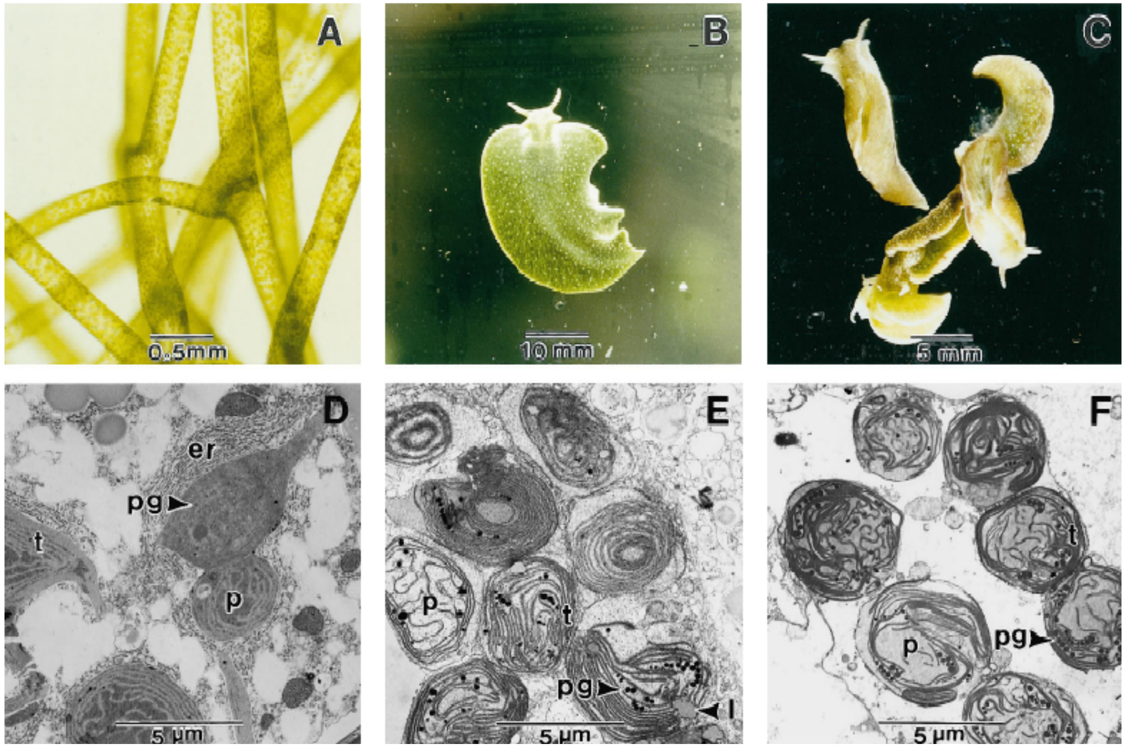
Mayorella



Paramecium

Vnútrobunkové symbiotické organizmy (endosymbionty)

Elysia chlorotica* + *Vaucheria litorea



Mujer C.V. *et al.* (1996) *Proc Natl Acad Sci USA* 93: 12333-12338.

Vnútrobunkové symbiotické organizmy (endosymbionty)

'*Solar (photosynthesizing) salamander*' *Ambystoma maculatum*
s vnútrobunkovou symbiotickou riasou *Oophila amblystomatis*

Kerneya R. *et al.* (2011) Intracellular invasion of green algae in a salamander host. *Proc Natl Acad Sci USA* 108: 6497–6502.
Petherick A. (2011) A solar salamander. Nature doi:10.1038/news.2010.384

Pôvod mitochondrií

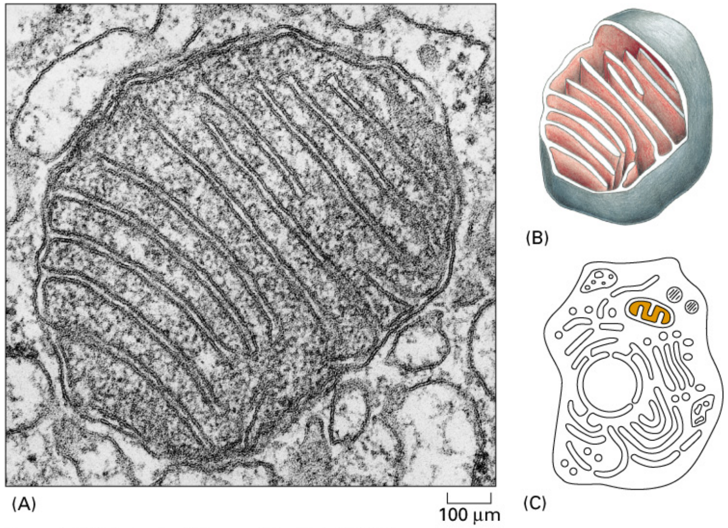


Figure 1–34. Molecular Biology of the Cell, 4th Edition.

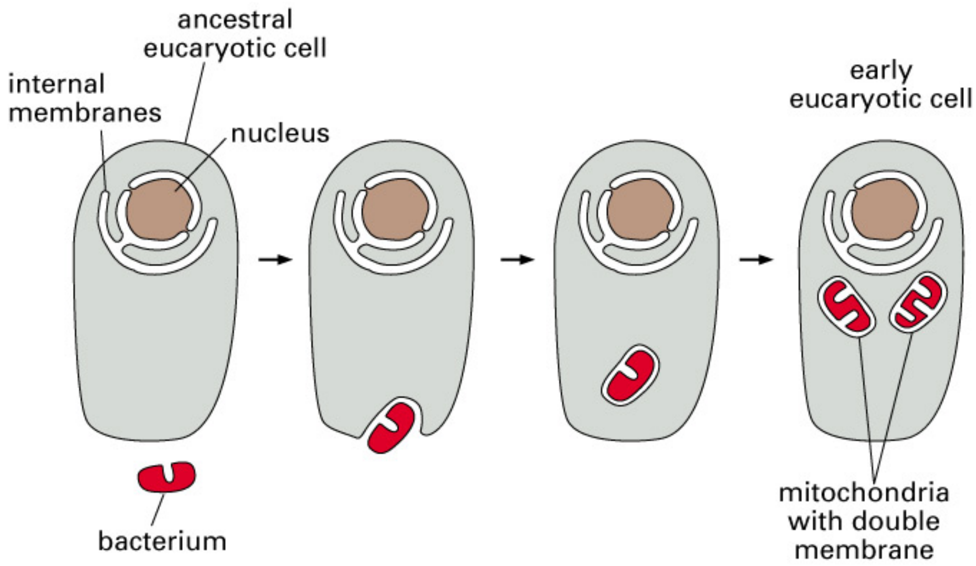


Figure 1–35. Molecular Biology of the Cell, 4th Edition.

Pôvod plastidov

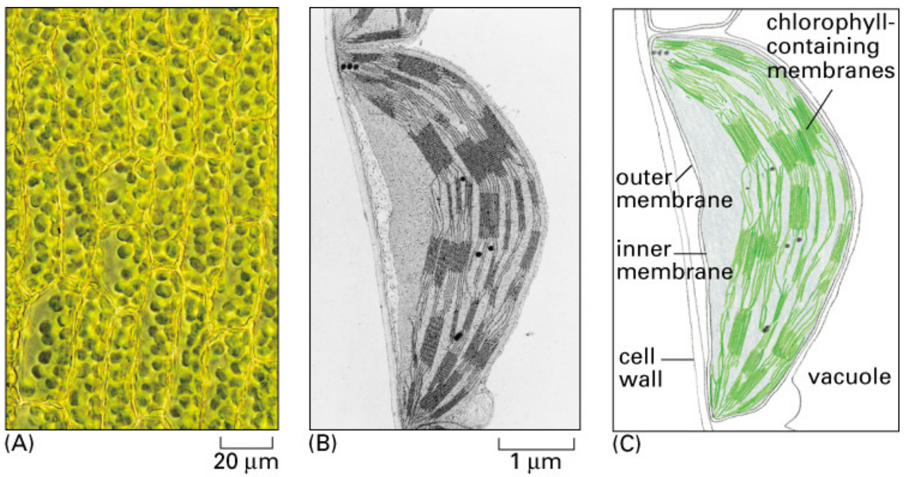


Figure 1–36. Molecular Biology of the Cell, 4th Edition.

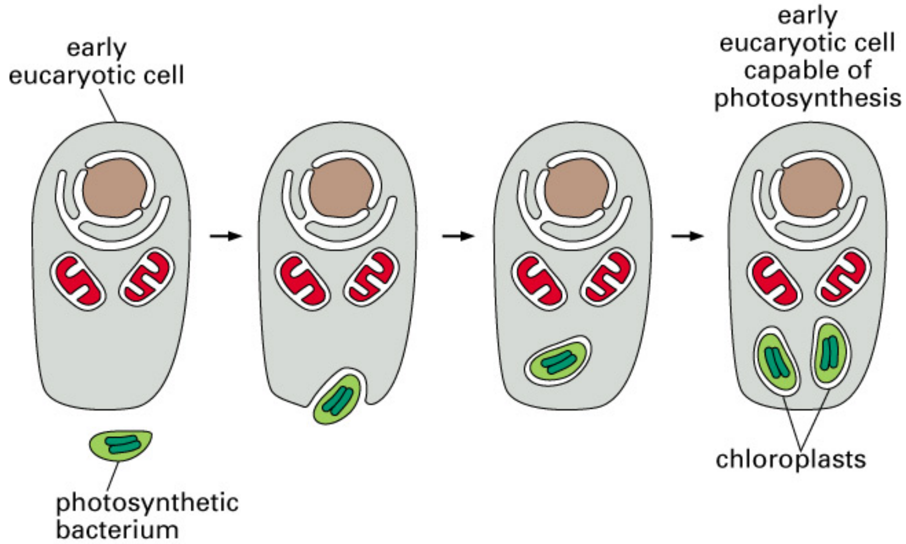
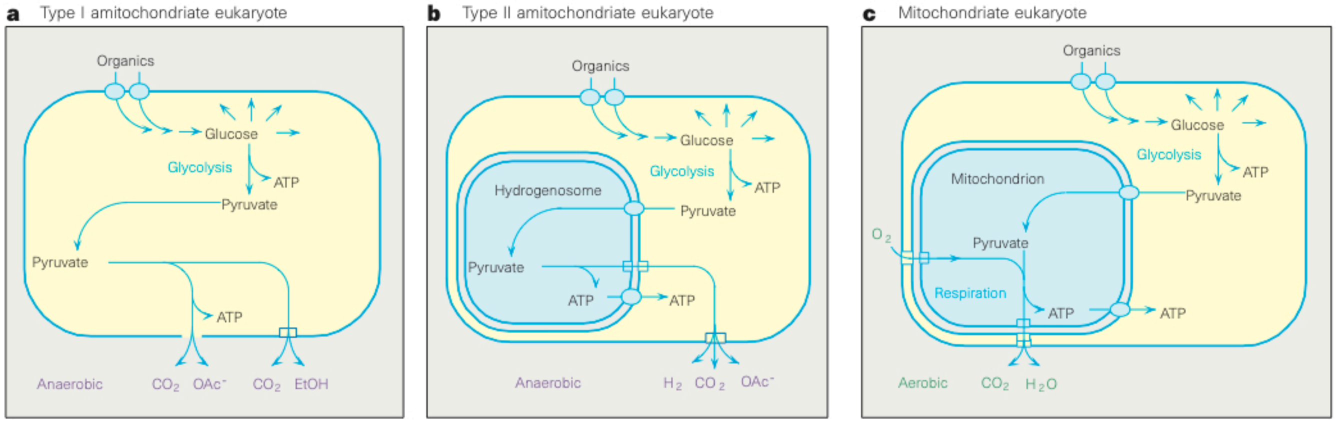


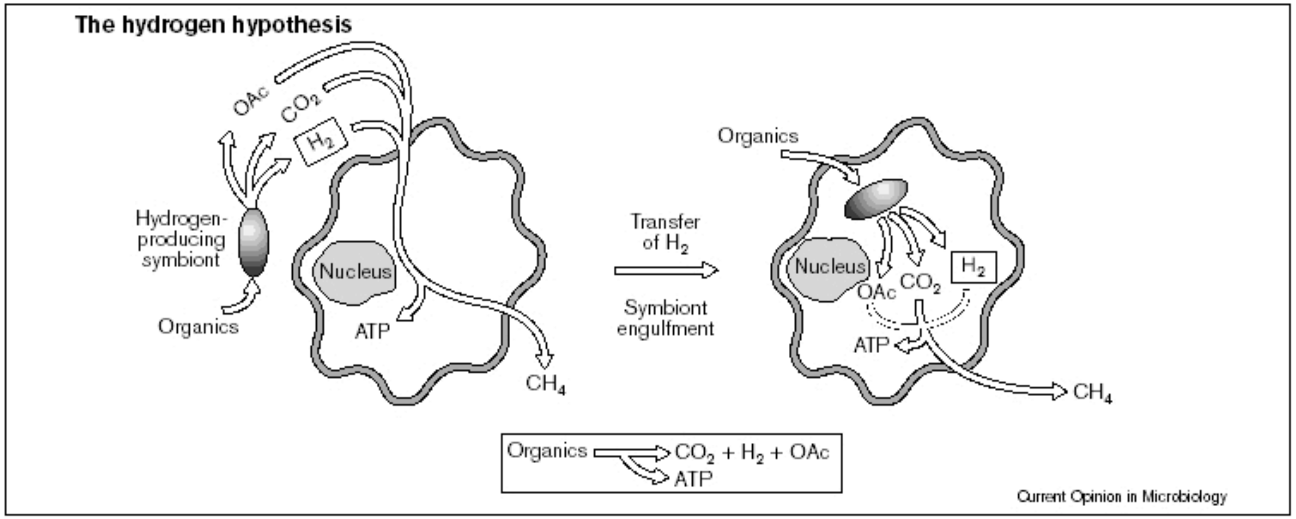
Figure 1–37. Molecular Biology of the Cell, 4th Edition.

Vodíková hypotéza vzniku eukaryotickej bunky

Martin W. & Müller M. (1998) The hydrogen hypothesis for the first eukaryote. *Nature* **392**: 37-41
Embley T.M. & Martin W. (2006) Eukaryotic evolution, changes and challenges. *Nature* **440**: 623-630.

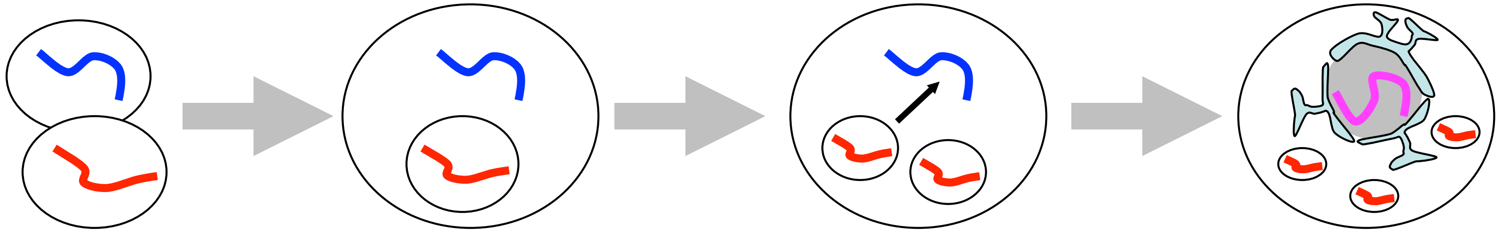
William F. Martin
(1957 -)





Andersson SGE & Kurland CG (1999) Origins of mitochondria and hydrogenosomes. *Curr Opin Microbiol* **2**: 535-541

Genóm modernej eukaryotickej bunky je zložitou evolučnou mozaikou



Jadrový genóm: derivovaný z genómov *archaea* a *bacteria*
Mitochondriálny genóm: redukovaný genóm α*-proteobacteria*
Plastidový genóm: redukovaný genóm *cyanobacteria*

Reduktívna fáza evolúcie:
- strata podstatnej časti genómu (génov) pôvodného endsymbionta
- transfer časti génov endosymbionta do jadra (EGT) a ich adaptácia
- redukcia mitochondriálneho genómu

Expanzívna fáza evolúcie:
- vznik nových génov (molekulárny *tinkering*)
- prokaryotické gény získané HGT (nepochádzajú z pôvodného endosymbionta)
- konverzia endosymbionta na organelu exportujúcu ATP

Veľkosť a komplexita genómov nezodpovedá morfologickej komplexite organizmu ani jeho phylogenetickému postaveniu

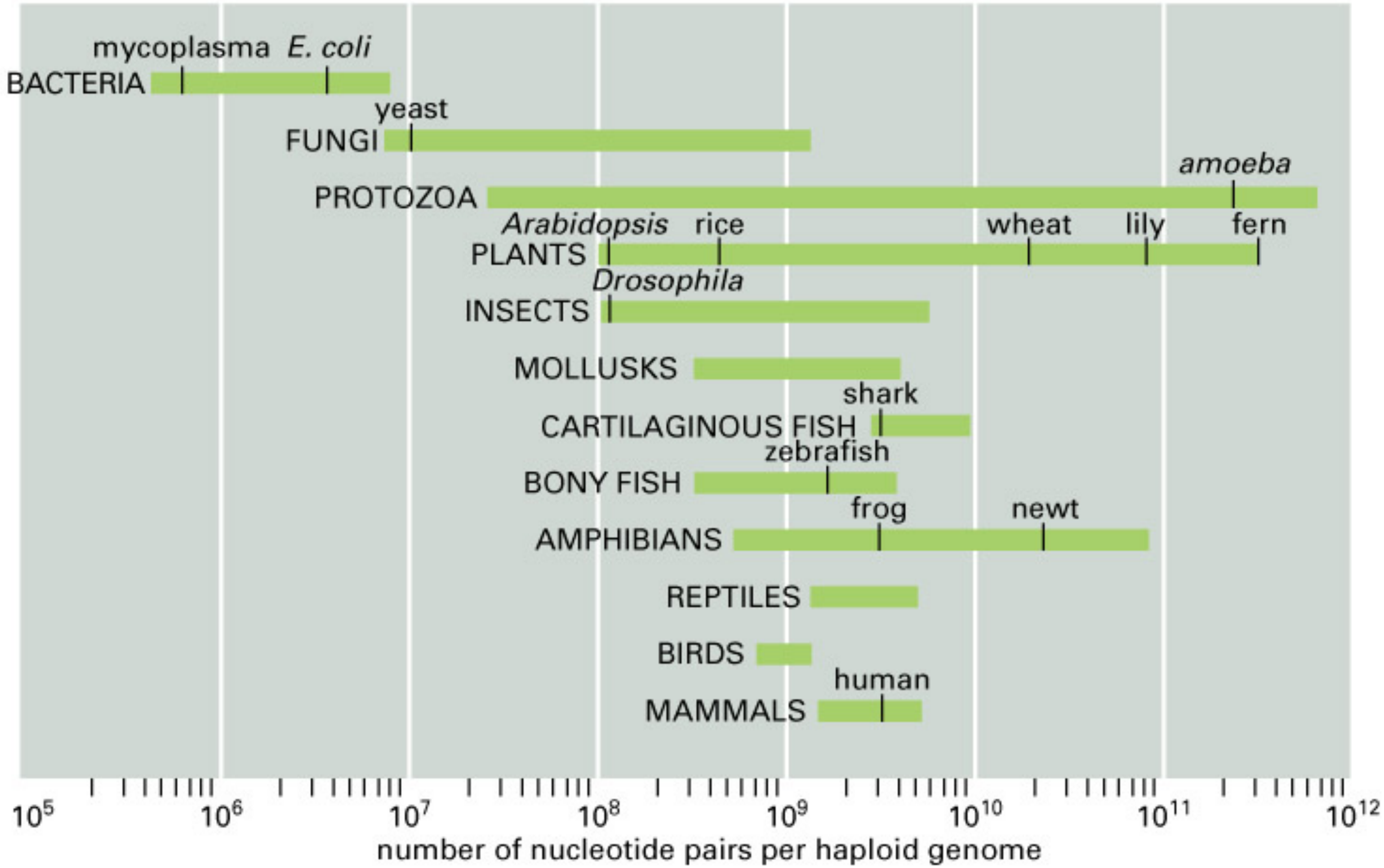


Figure 1-40 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Nabudúce:

2. Bunkové jadro: štruktúra a dynamika chromozómov:

- Prokaryotické, eukaryotické a organelové chromozómy.
- DNA a proteínové komponenty chromozómov.
- Distribúcia chromozómov pri delení buniek.
- Objav úlohy DNA.
- Replikačné stratégie DNA.
- Experimenty Meselsona a Stahla. Semikonzervatívny mechanizmus syntézy DNA.
- Iniciácia, elongácia a terminácia replikácie (replikačné počiatky, replikačné bubliny. Okazakiho fragmenty, *leading* a *lagging* vlákno).
- Replizóm.