1. The cell nucleus

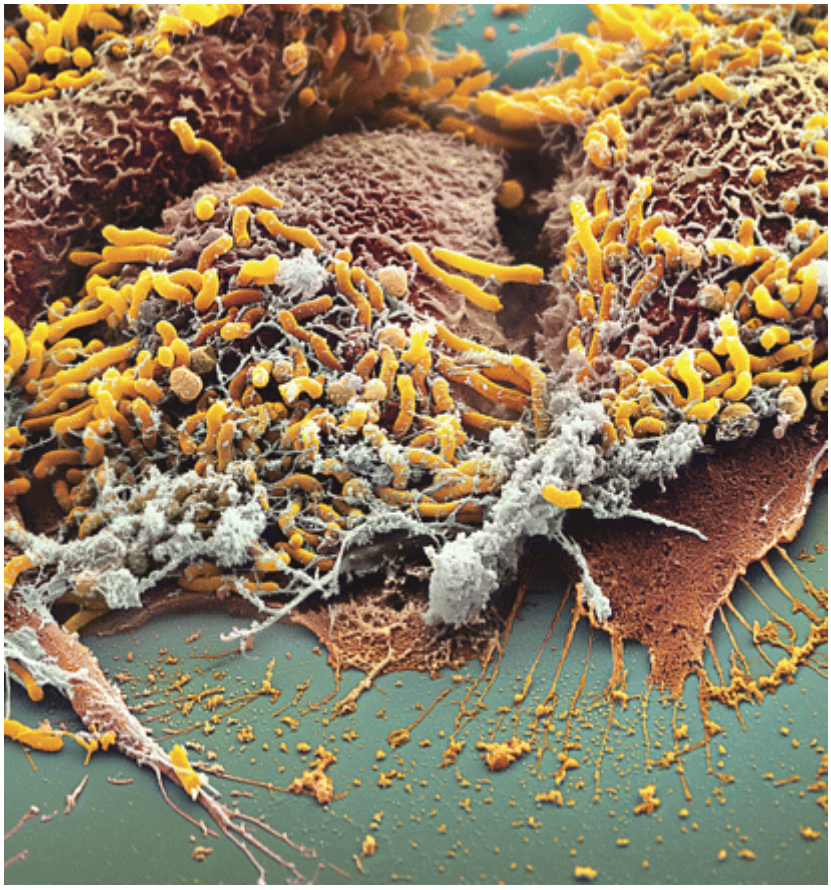
Neutrophil phagocytosis of bacterial cells.

(scanning elektronmicroscope, digital coloring)

http://www.pnas.org/content/100/19.cover-expansion

Which type of bacterial cells based on the shape do these bacteria belong to?

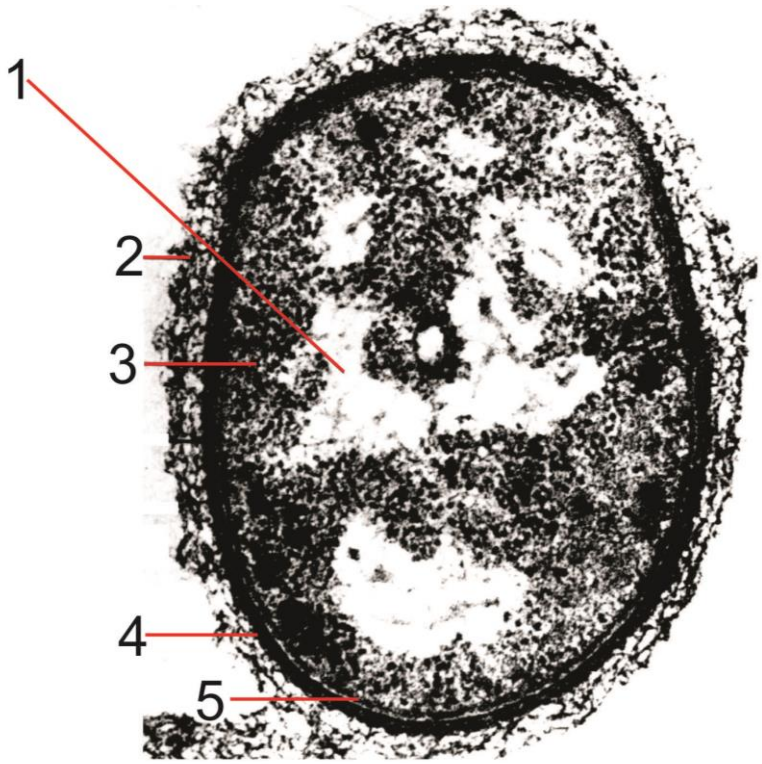
Gastric epithelial cells infected with Helicobacter pylori bacteria (scanning elektronmicroscope, digital coloring)



http://www.pnas.org/content/108/36.cover-expansion

Which type of bacterial cells based on the shape do these bacteria belong to?

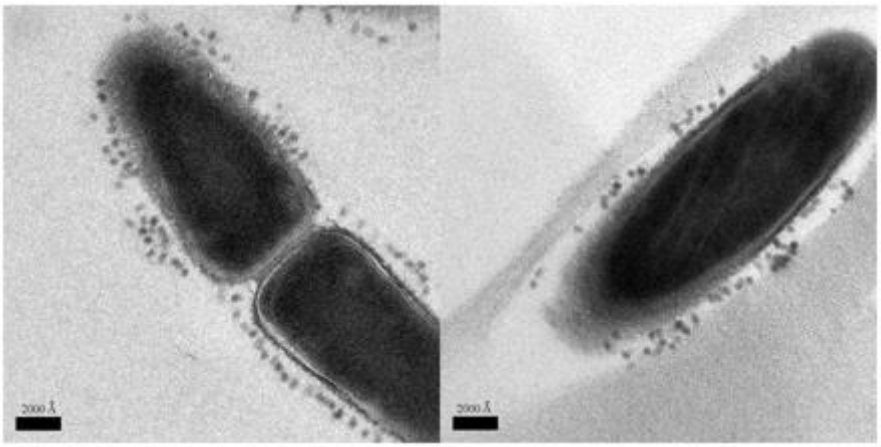
Transmission electronmicrograph of a bacterial cell



1. nucleoid, 2. capsule, 3. protoplasm, 4. cell wall, 5. cell membrane

What is the approximate magnification of this image?

Infection by bacteriophages (transmission electronmicroscope)

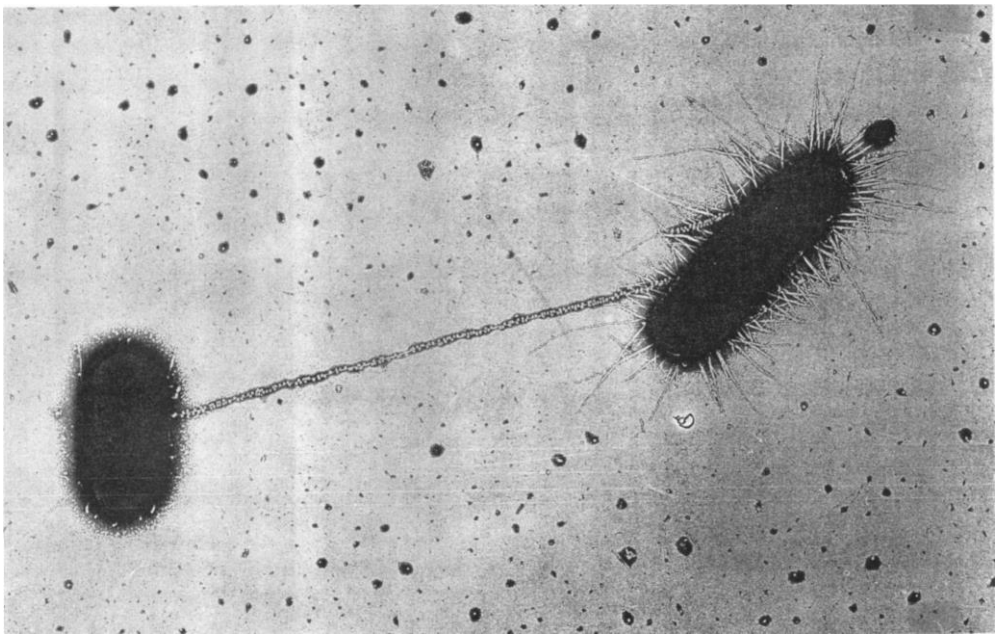


http://www.pnas.org/content/108/12/4806/F5.expansion.html

1. What is the significance of the bacteriophages in molecular biology?
2. What is the a magnification of this image?

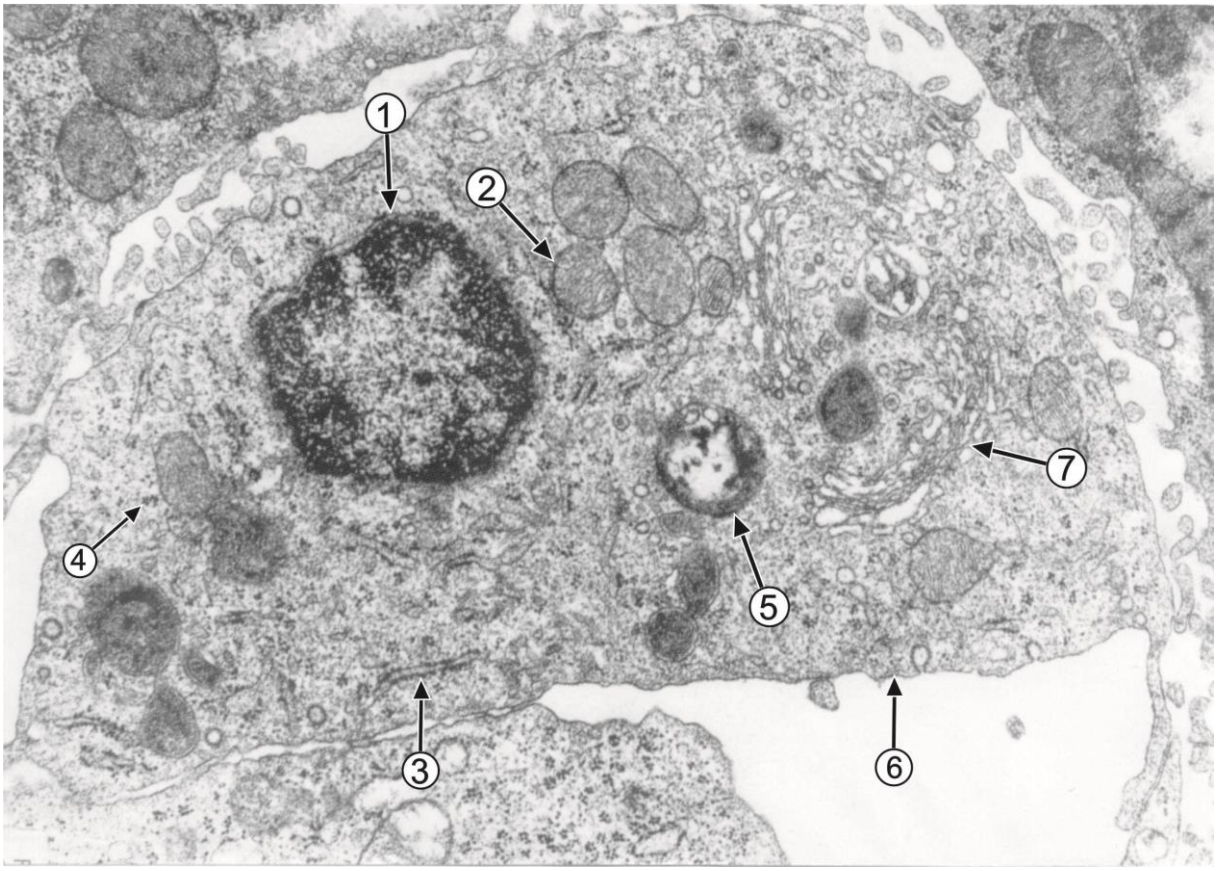
Transmission of genetic information between E. coli cells by conjugation.

(transmission electronmicroscope)



What is the significance of the conjugation in biology?

Transmission electronmicrograph of a eukaryotic cell



1. cell nucleus, 2. mitochondrion, 3. rough endoplasmic reticulum, 4. free ribosomes, 5. secunder lysosome, 6. cell membrane, 7. Golgi complex

Name the cellular organelles seen in this image but are not present in prokaryotic cells!

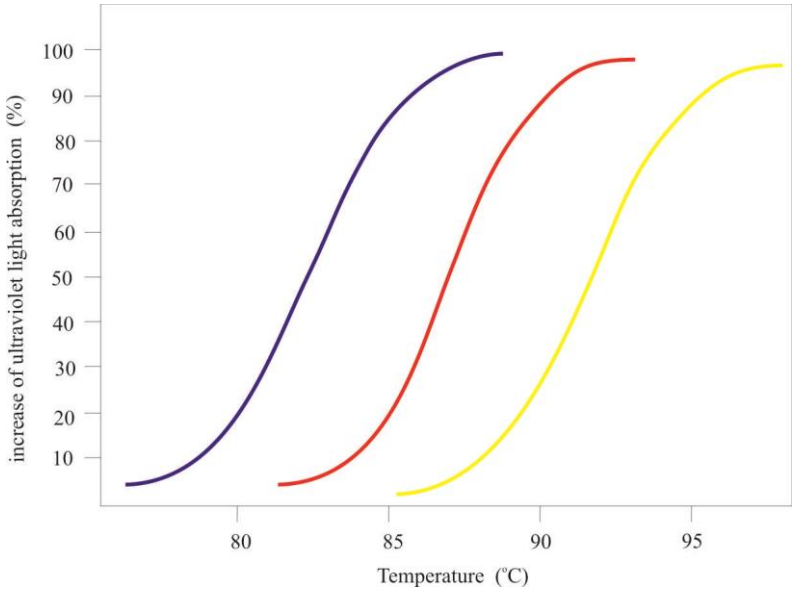
Cytoplasm of a eukaryotic cell (transmission electronmicroscope)

1. rough endoplasmic reticulum, 2. free ribosomes, 3. secunder lysosome, 4. transport vesiculum, 5., 6., 7. Golgi complex, 8., 9., 10. secretory vesicules

Name the cellular organelles seen in this image but are not present in prokaryotic cells!

2.DNA

Hyperchromicity curve of DNA



Medical Biology Department, Medical School, Pécs University

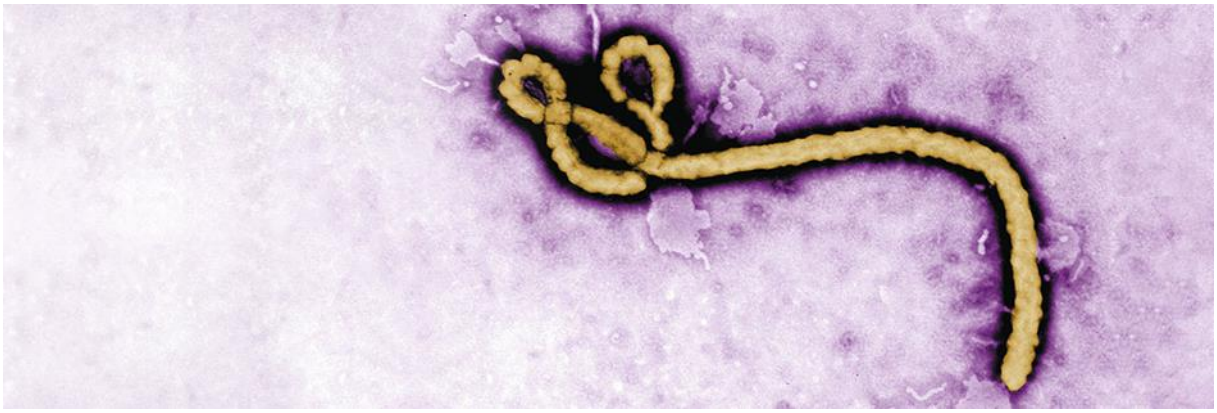
1. Wat kind of alteration in the structure of DNA results in this phenomenon?
2. How can the melting point of DNA be determined in this graph?
3. What explaines the difference between the melting points of the different DNA molecules?

SV40 virus DNA by rotary shadowing (transmission electronmicroscope)

1. How is the contrasting done in the case of rotary shadowing?
2. Name the 3 shown structural forms of SV40 DNA!
3. What kind of enzymatic activities can cause the transitions between the shown forms of DNA?

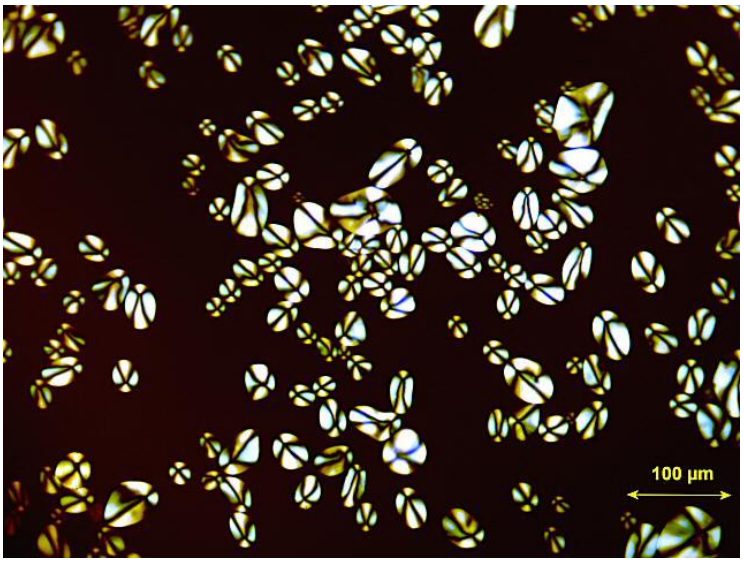
3.Microscopy

Transmission electronmicroscopic image of Ebola virus by negative contrasting (digital colouring)



How is the negative contrasting technique performed?

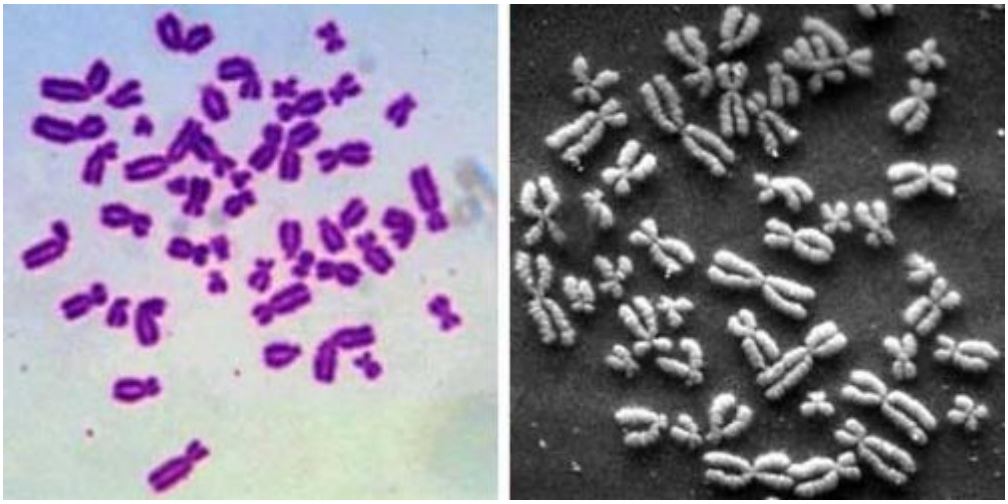
Polarization microscopic image of starch grains



1. Which type of structures can be studied with a polarization microscope?
2. What is the difference between the construction of a polarization microscope and that of a regular light microscope?

Microscopic images of human chromosomes

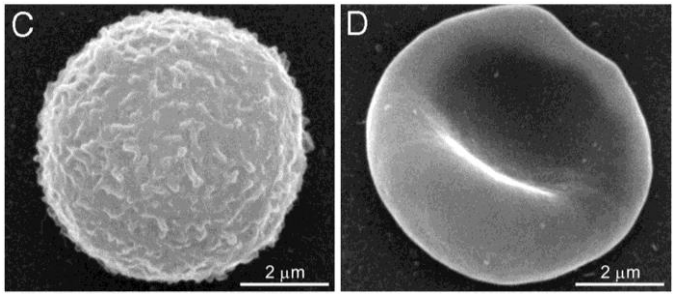
(light microscope and scanning electronmicroscope)



1. How were the contrasting procedures done in the case of the above images?
2. What are the maximum magnifications and resolutions in the case of the above microscopic techniques?

Scanning electronmicrographs of blood cells

(white blood cell and red blood cell)



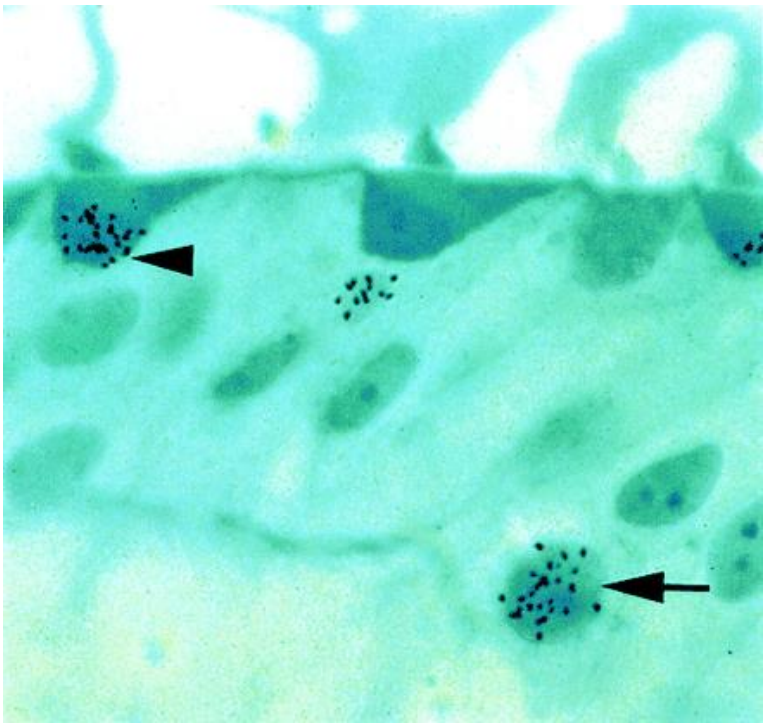
What is the a magnification of this image?

Phase contrast microscopic imageof PC12 cells

J. Bátor (Medical Biology Department, Medical School, Pécs University)

Which type of structures can be studied with a phase contrast microscope?

^{3}H-thymidine labeling (light microscopic autoradiography)

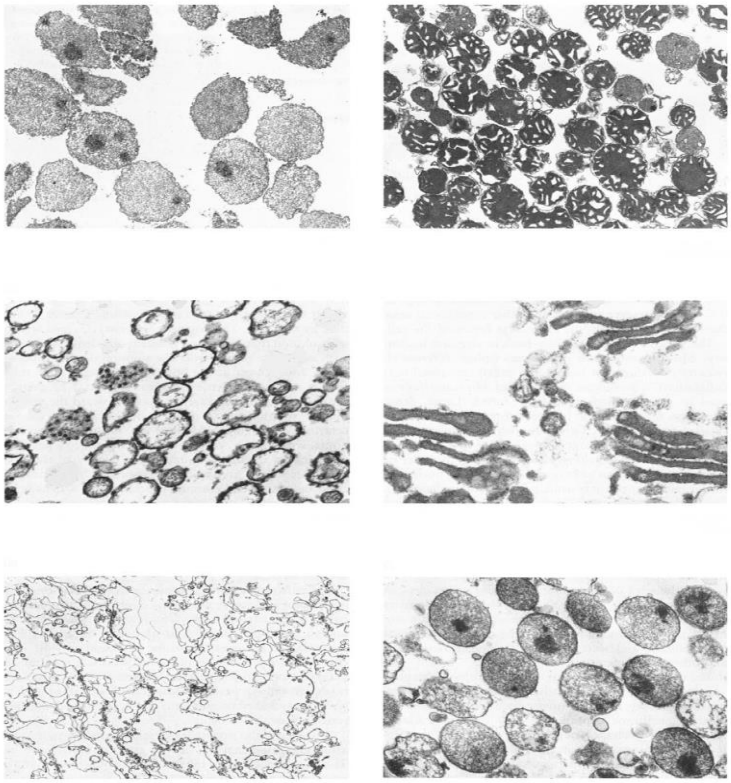


http://www.pnas.org/content/97/22/11714/F1.expansion.html

1. What are the steps of light microscopic autoradiography?
2. What are the small black dots?

4.Separation methods

Fractions of cell fractionating differential centrifugation (transmission electronmicroscope)

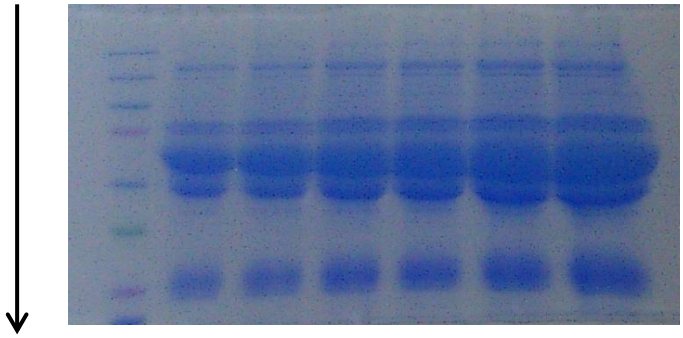


J.Darnell, H. Lodish, D. Baltimore: Molecular cell biology

1. nuclei 2. rough endoplasmic reticulum, 3. plasma membrane, 4. mitochondria, 5. Golgi complex, 6. peroxisomes

What is the order of the fractions during this type of centrifugation?

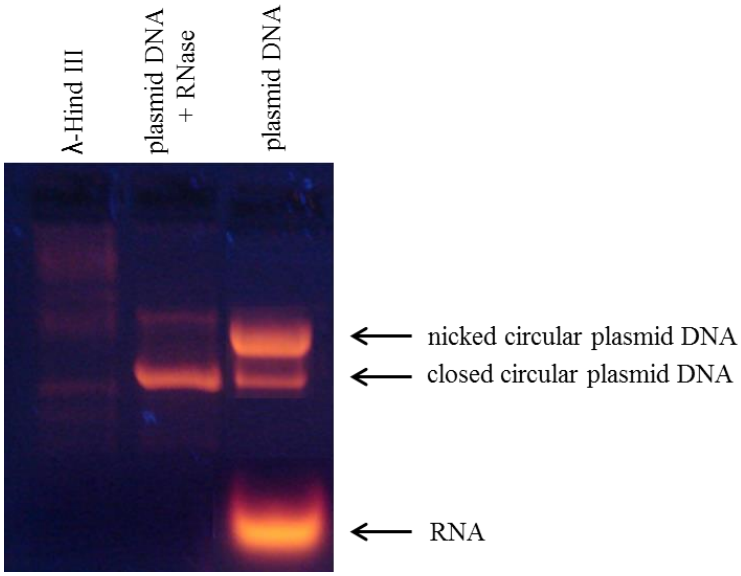
SDS polyacrilamide gel electrophoresis (Commassie Brilliant Blue staining)



R. Schipp (Medical Biology Department, Medical School, Pécs University)

1. Which type of molecules can be separated by this technique?
2. What is the basis of the separation in this tecnique?

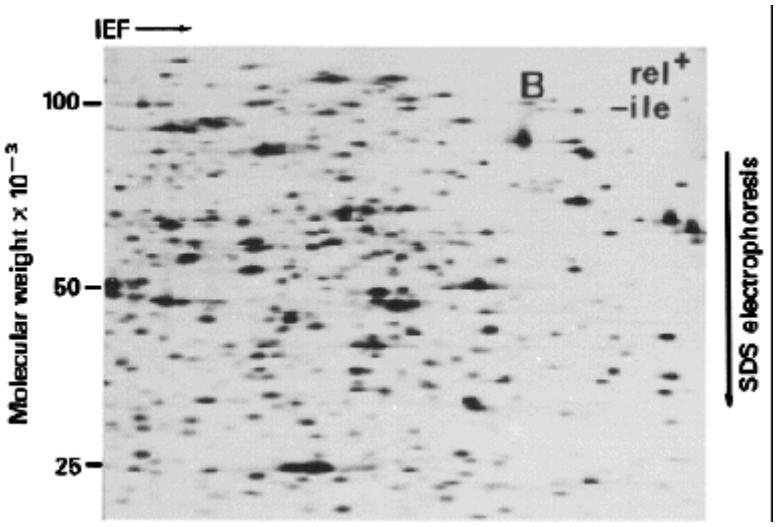
Agarose gel electrophoresis (ethidiumbromide staining)



R. Schipp (Medical Biology Department, Medical School, Pécs University)

1. What is the basis of the separation in this tecnique?
2. How can the plasmid DNA be isolated from bacterial cells?
3. What is the significance of RNase treatment?

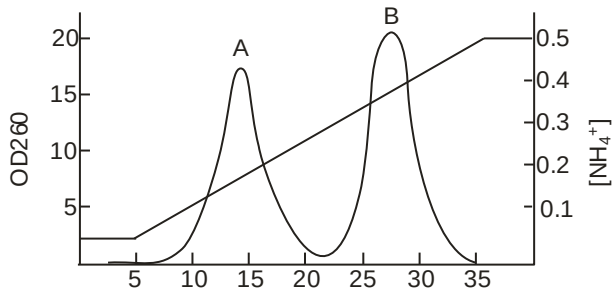
Two-dimensional gelelectrophoresis



http://www.sci.sdsu.edu/

1. In which case is this thechnique useful to separate proteins?
2. What is the basis of the separation in this tecnique?

Ion-exchange chromatography



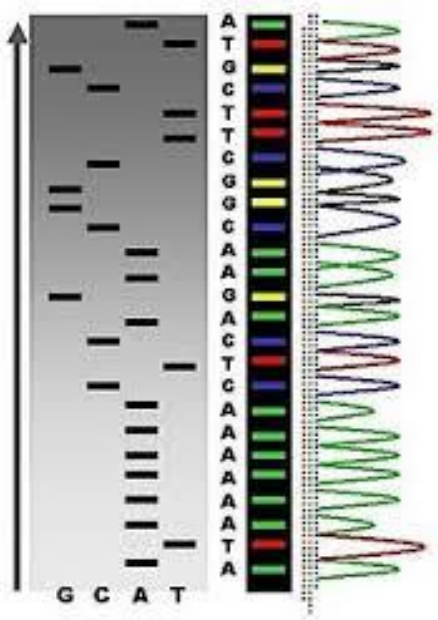
Medical Biology Department, Medical School, Pécs University

1. Which types of ion exchange chromatography do you know?
2. How is the elution performed in the case of ion exchange chromatograpy?
3. Which type of ion exchange chromatography resulted in the above demonstrated chromatogram?
4. Which molecules were separated during the above demonstrated chromatography?
5. What is the difference between the molecules separated from each other (molecules A and B) during the above demonstrated chromatography?

5.Methods of molecular biology

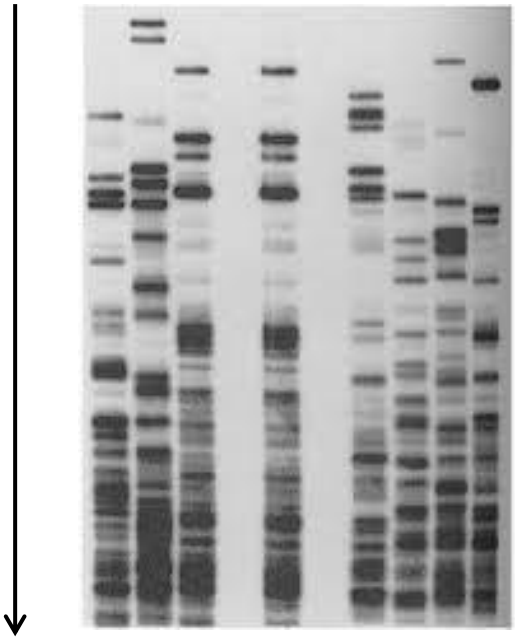
Sanger's chain termination DNA sequencing analysis

- **left side: regular sequence analysis**
- **right side: automated sequence analysis (fluorescently labeled ddNTPs, capillary gelelectrophoresis**)



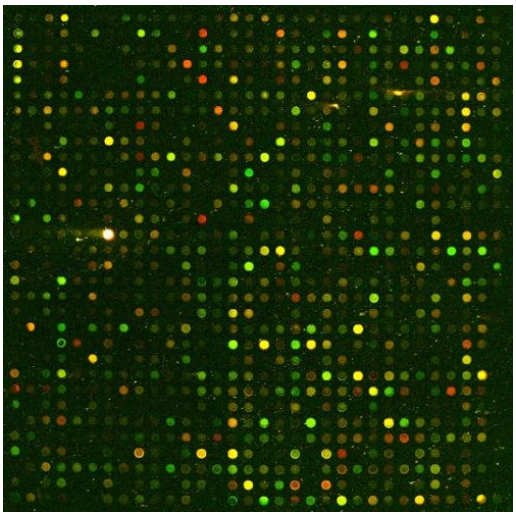
1. What do the reaction mixtures of this method contain?
2. How are the reaction products labeled in the regular type of sequencing?
3. What is the material of the sequencing gel?

DNA fingerprint analysis



1. Which molecular biological method is this analysis based on?
2. What is the medical significance of this method?

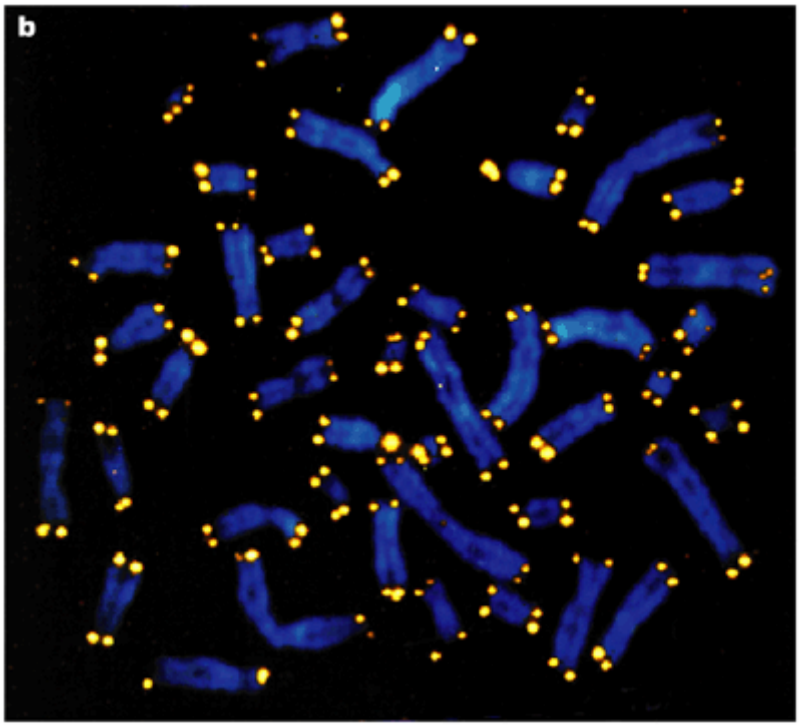
DNA microchip/ microarray



https://worldwide.promega.com/resources/product-guides-and-selectors/protocols-and-applications-guide/expression-analysis

1. What is the molecular biological basis of this analysis?
2. Which molecule is labeled in this analysis?
3. How is the result of this analysis detected?
4. What is the difference between the DNA-microchip and the cDNA-microchip techniques?

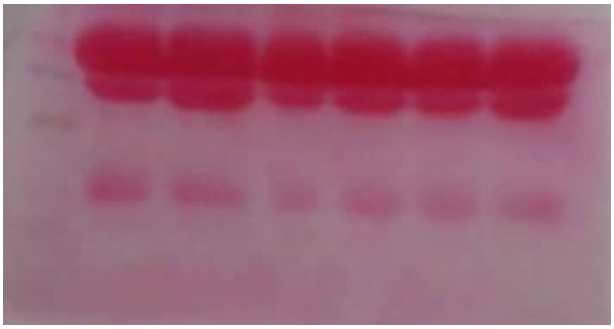
Fluorescence in situ hybridization (FISH) analysis of telomere DNA sequences



http://www.nature.com/nri/journal/v2/n9/box/nri890_BX2.html

1. What is the molecular biological basis of this analysis?
2. Name a fluorescence DNA dye!

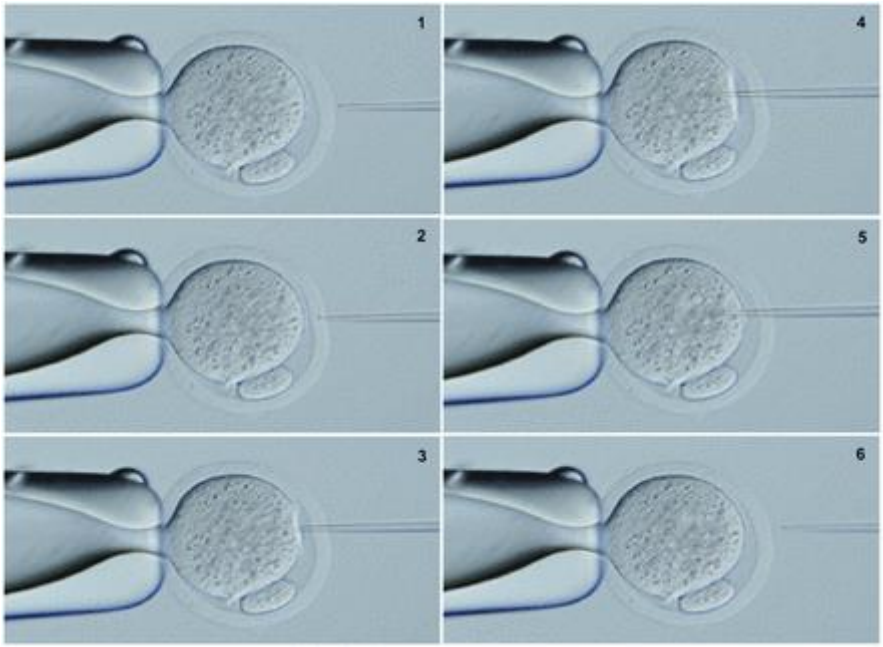
Western bloting; proteins transfered to nitrocellulose membrane (Ponceau staining)



R. Schipp (Medical Biology Department, Medical School, Pécs University)

1. Name the steps of Western bloting (immunobloting)?
2. How are the proteins transfered from the gel to the membrane?

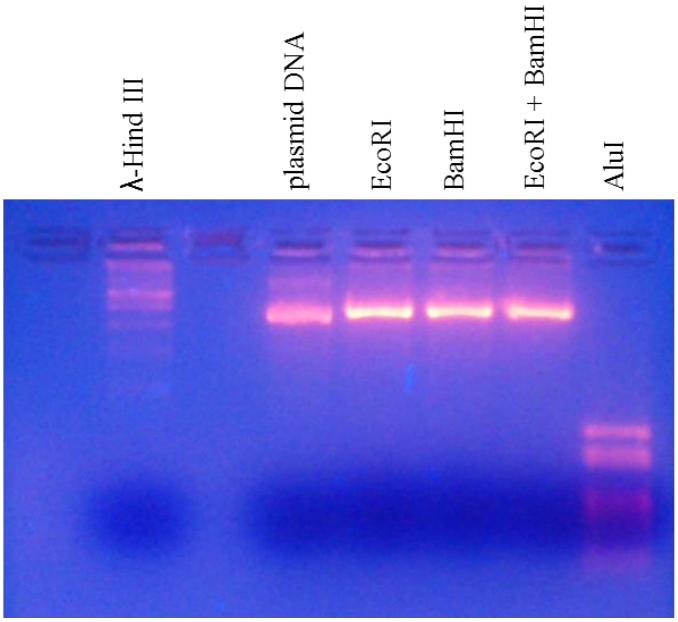
Microinjection of plasmid DNA into a one-cell-embryo



http://www.pnas.org/content/109/47/19184/F2.expansion.html

1. Which other types of gene transfer techniques beside the microinjection do you know?
2. What is the other way of the introduction of a transgene into an embryo?
3. What is the difference between a real transgenic animal and a chimeric animal?
4. How can the presence of the transgene be detected in a transgenic embryo or animal?

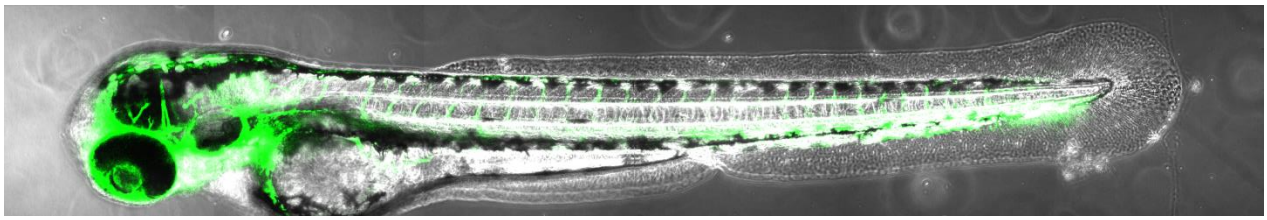
Restriction endonuclease digestion (ethidium bromide staining)



R. Schipp (Medical Biology Department, Medical School, Pécs University)

1. How do the restriction endonuclease recognition sites look like?
2. Which molecular biological methods require restriction endonuclease digestion?
3. Whish type of electrophoresis is demonstrated by this figure?
4. What is the role of the λ-Hind III sample in this method?

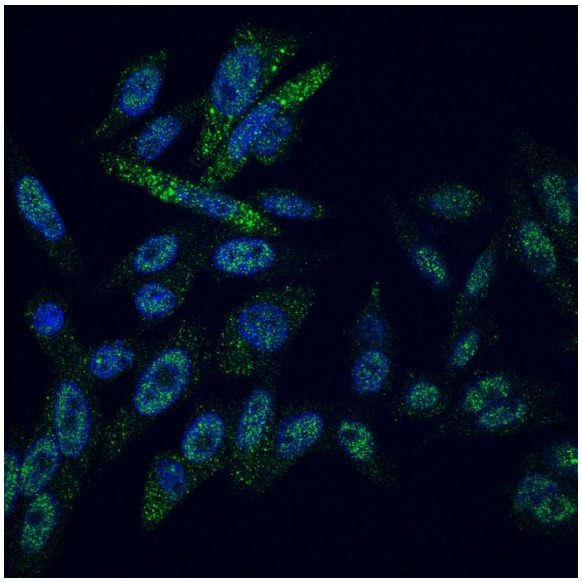
Transgenic zebrafish (confocal laser scanning fluorescence microscope; transfection of the gene of green fluorescence protein)



S. Varga (Medical Biology Department, Medical School, Pécs University)

1. How can a real transgenic animal be created?
2. How can the tissue secific expression of a transgene be obtained?

Immunocytochemistry (fluorescence microscope, green: cytoplasmic protein; blue: Hoechst DNA dye)

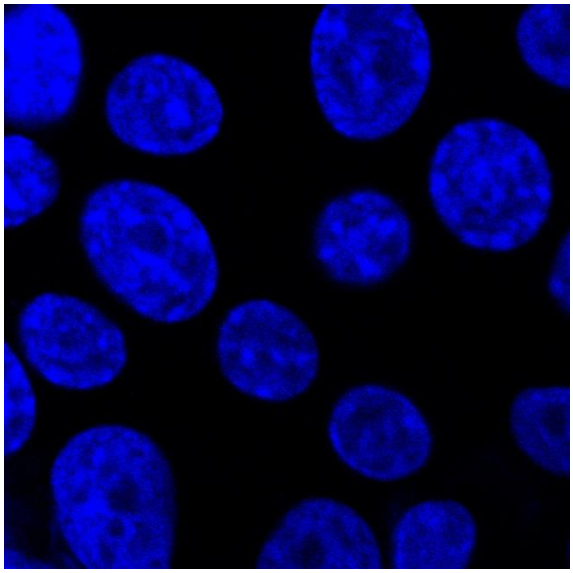


Zs. Fekete (Medical Biology Department, Medical School, Pécs University)

1. What is the difference between the direct and indirect immuncytochemical tecniques?
2. Which other ways of antibody labeling beside fluorescence labeling do you know in the case of immunocytochemistry?

6.The cell nucleus

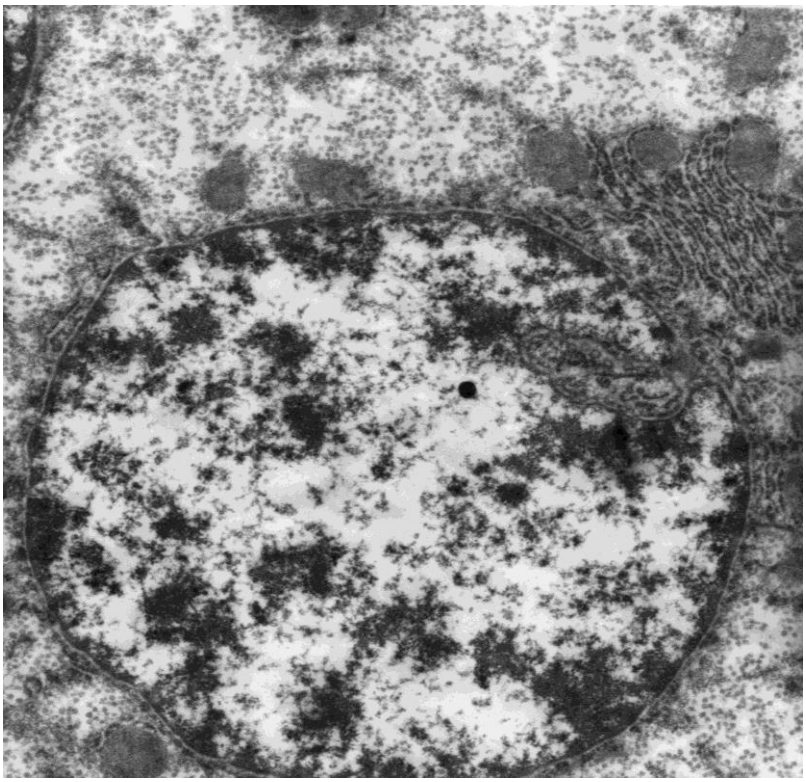
Fluorescence microscopic image of cell nuclei (Hoechst DNA dye)



K. Kiss (Medical Biology Department, Medical School, Pécs University)

1. Which other ways of light microscopic nuclear staining techniques do you know?
2. What is the average size of a cell nucleus?

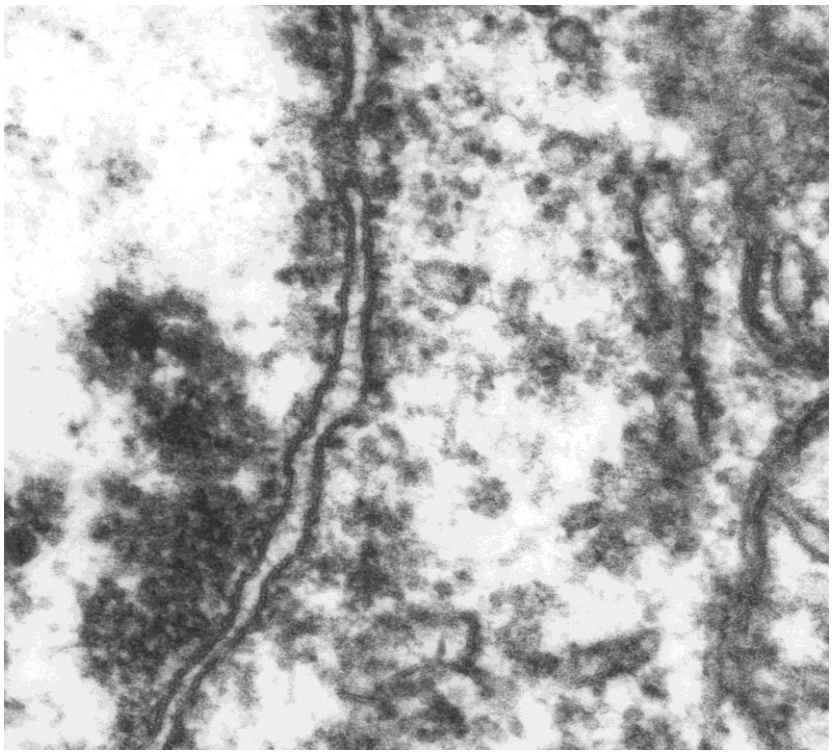
Transmission electronmicrograph of cell nucleus



H. Ábrahám (Central Electronmicroscopic Laboratory, Medical School, Pécs University)

1. Name the main components of the cell nucleus!
2. What is the differrence between the transmission electronmicroscopic appearances of heterochromatin and euchromatin areas?

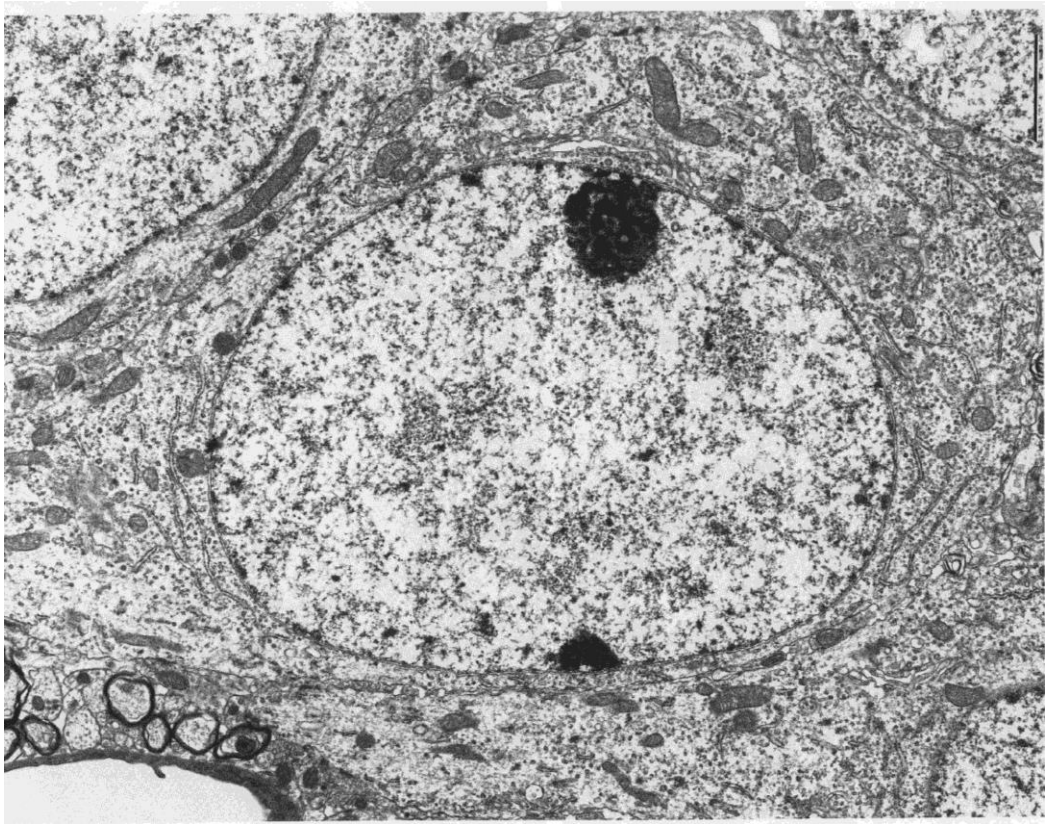
Transmission electronmicrograph of a nuclear pore



H. Ábrahám (Central Electronmicroscopic Laboratory, Medical School, Pécs University)

1. What are the components of a nuclear pore complex?
2. Which types of molecules are transported through the nuclear pore?
3. How is the nucleocytoplasmic transport of proteins performed?

Transmission electronmicrograph of cell nucleus (neuronal tissue)

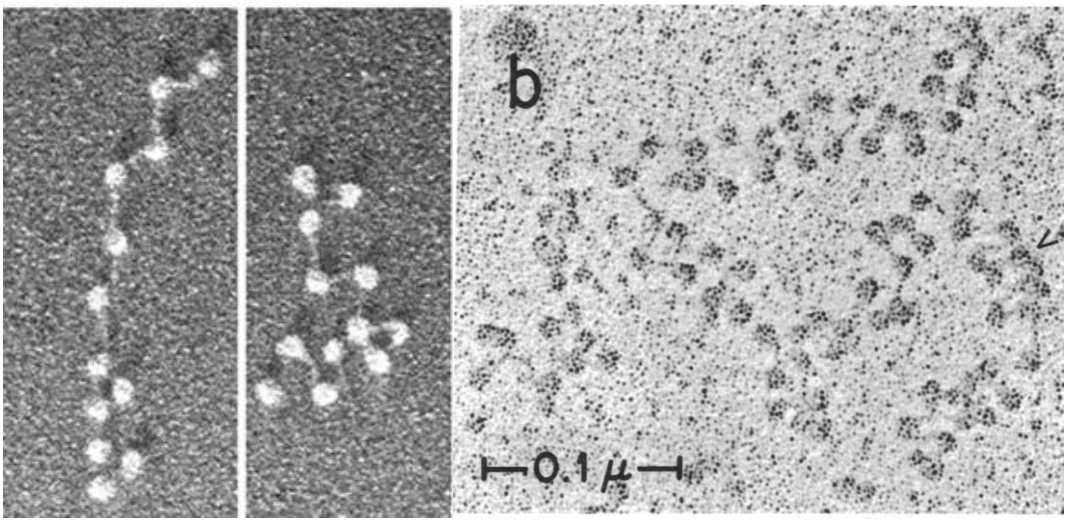


H. Ábrahám (Central Electronmicroscopic Laboratory, Medical School, Pécs University)

How can you explain the presence of the enlarged euchromatin area (euchromatinisation)?

7. THE ORGANISATION OF CHROMATIN

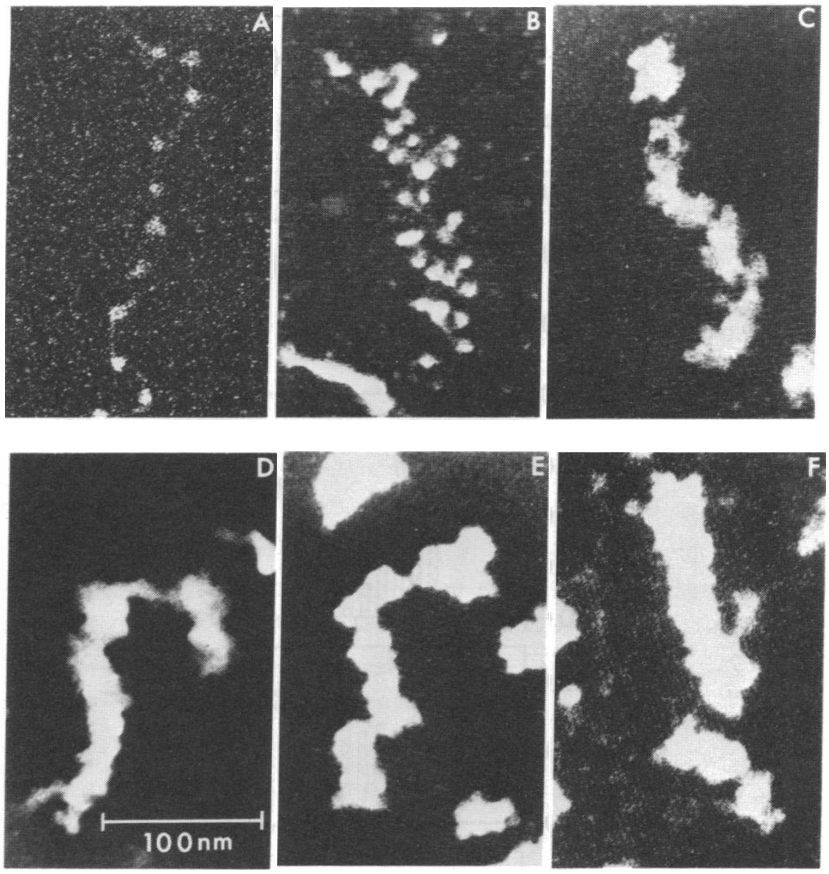
Beads-on-string structure of DNA



PNAS _ August 11, 2009 _ vol. 106 _ no. 32 _ 13317–13322**,**
Proc. Nati. Acad. Sci. USA Vol. 78, No. 3, pp. 1461-1465, March 1981

1. Which microscopic methods are suitable to produce these pictures?
2. Which proteins are present in the visualized objects?

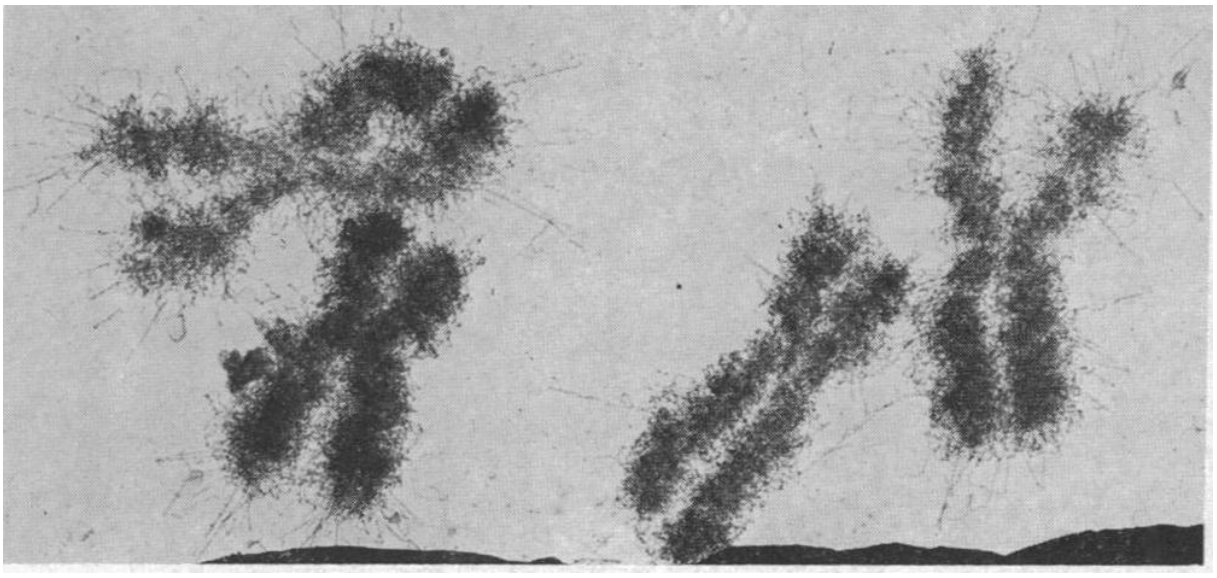
Formation of solenoid structure



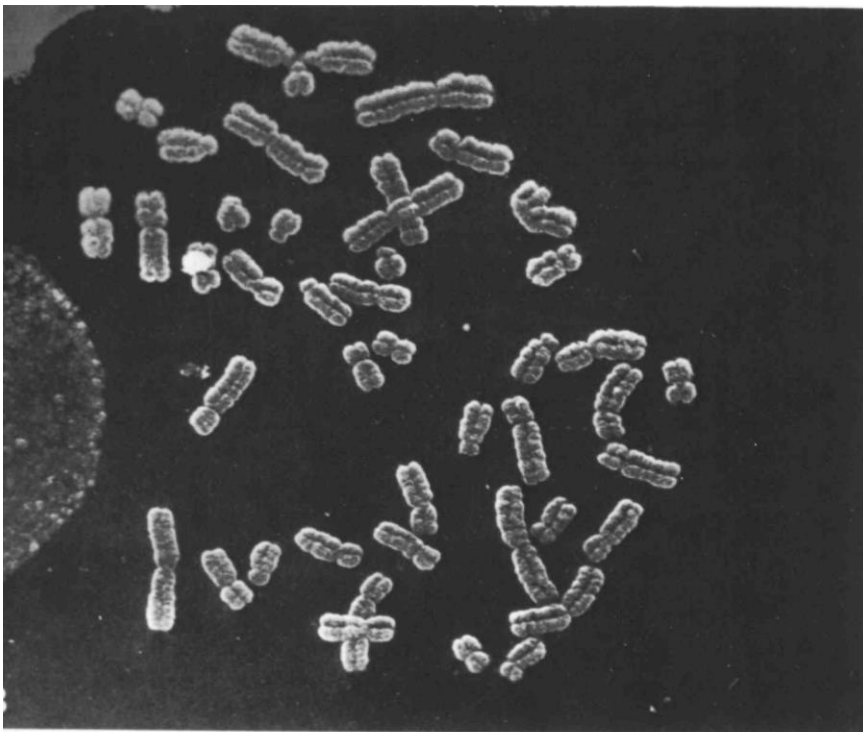
Proc. Nati. Acad. Sci. USA, Vol. 84, pp. 7802-7806, November 1987

1. Which process is shown by the pictures?
2. Where are structures „A” and „F” localized within the nucleus?

Metaphase chromosomes



Proc. Nat. Acad. Sci. USA, Vol. 68, No. 4, pp. 726-730, April 1971



J. Cell Sd. 77, 143-153 (1985)

1. In which phase of the cell cycle are the cells which can be used to isolate the chromosomes visible on pictures?
2. Which proteins could be detected between the chromatids?

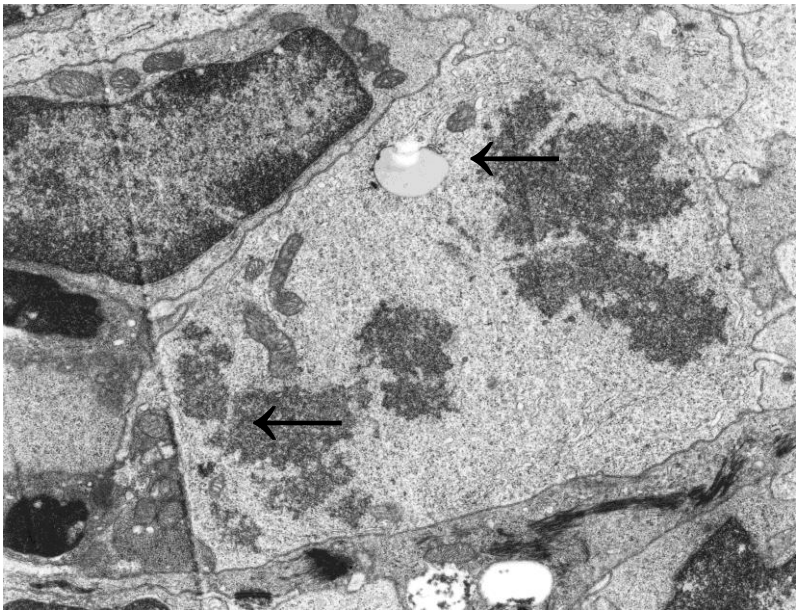
Electron micrograph of chromosomes digested with DN-ase

PNAS Vol.74,No.1,pp.4937-4941,November1977 Biochemistry

1. Name the structure seen in this figure!
2. What type of macromoelcule(s) is/are made of it?

8. CELL DIVISION

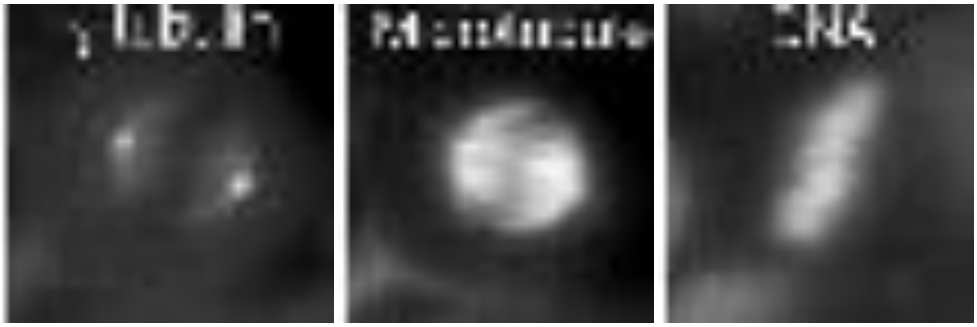
Divinding cell



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. On which structures do the arrows point at?
2. Name several proteins, which are sure present in the structures labelled with arrows!

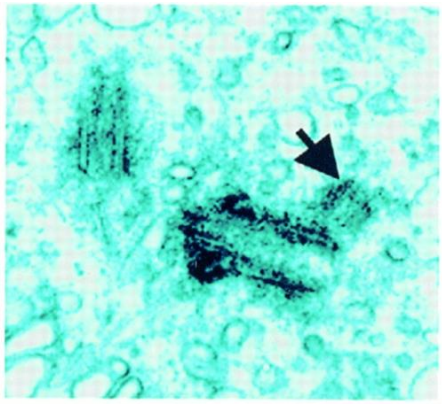
Mitotic spindle



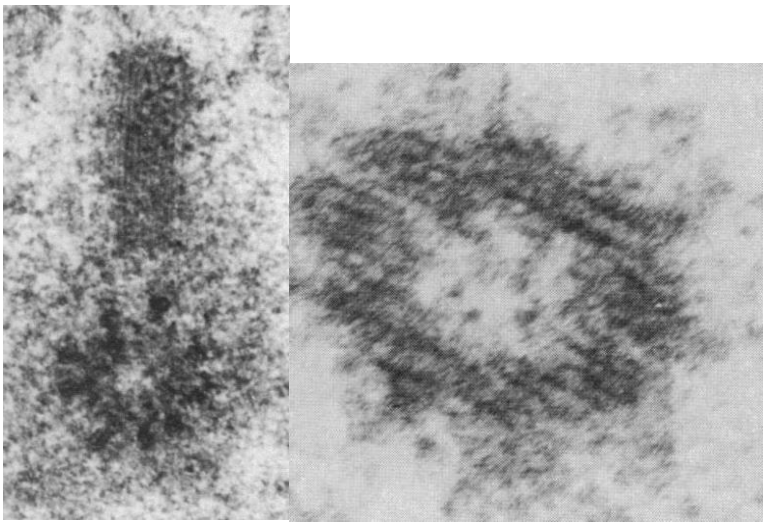
PNAS 2009 106 (17) 6998-7003

1. In which phase of the cell cycle is the cell shown on the pictures?
2. Where is the γ-tubulin located according to the pictures?
3. How were the microtubules and γ-tubulin visualized?

Centrioles



PNAS, August 29, 2000, vol. 97, no. 18, 10003



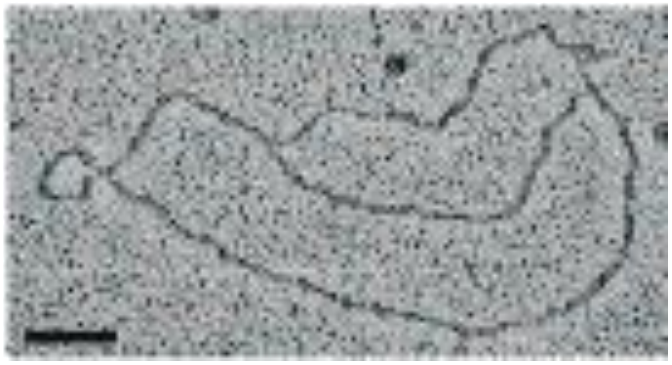
Proc. Natl. Acad. Sci. USA, Vol. 88, pp. 4806-4810, June 1991

1. What is the role of centrioles?
2. What can be seen on the pictures?
3. What can be shown by the arrow (the beginning of which process)?

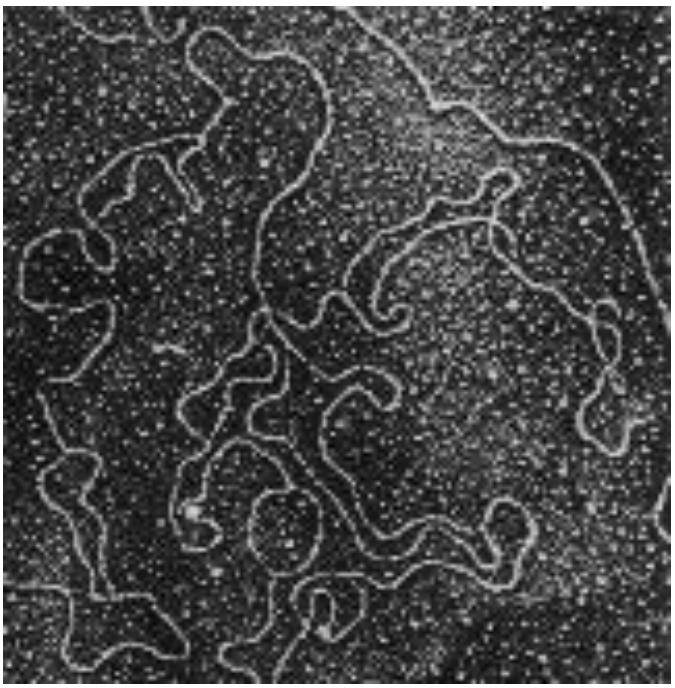
9. REPLICATION

Electron micrographs showing isolated DNA molecules

A



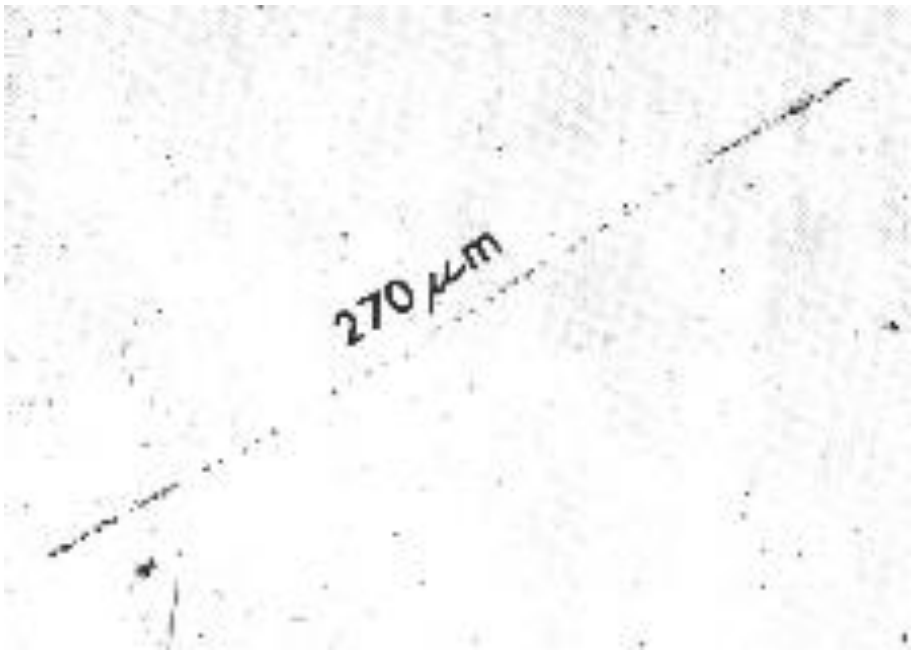
B



PNAS Vol. 104, pp.1500-1505, 2007
PNAS Vol. 71, No. 1, pp. 135-139, January 1974

1. Which picture represents the prokaryotic, and which one is the eukaryotic replication?
2. What are the main differences between prokaryotic and eukaryotic replication?
3. What types of methods were used for the visualization of the samples?

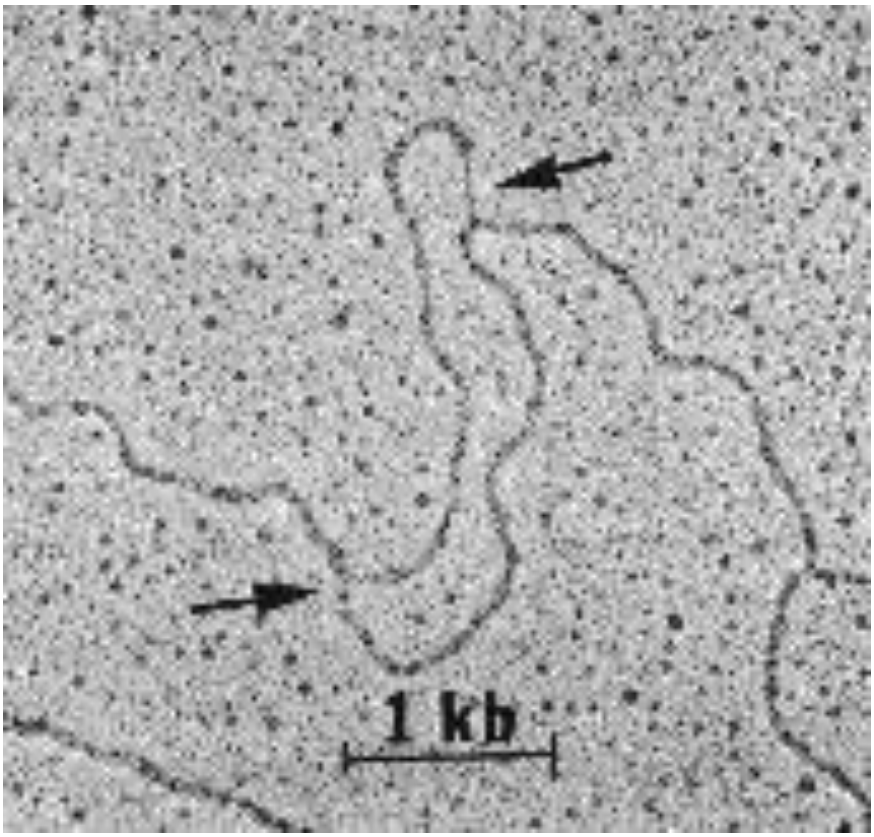
Bacterial DNA replication demonstrated by fiber autoradiography



PNAS Vol. 69, No. 10, pp. 2842-2845, October 1972

1. The arrows point at two parts of the replication bubble. Explain why these parts are thinner!

DNA replication bubble



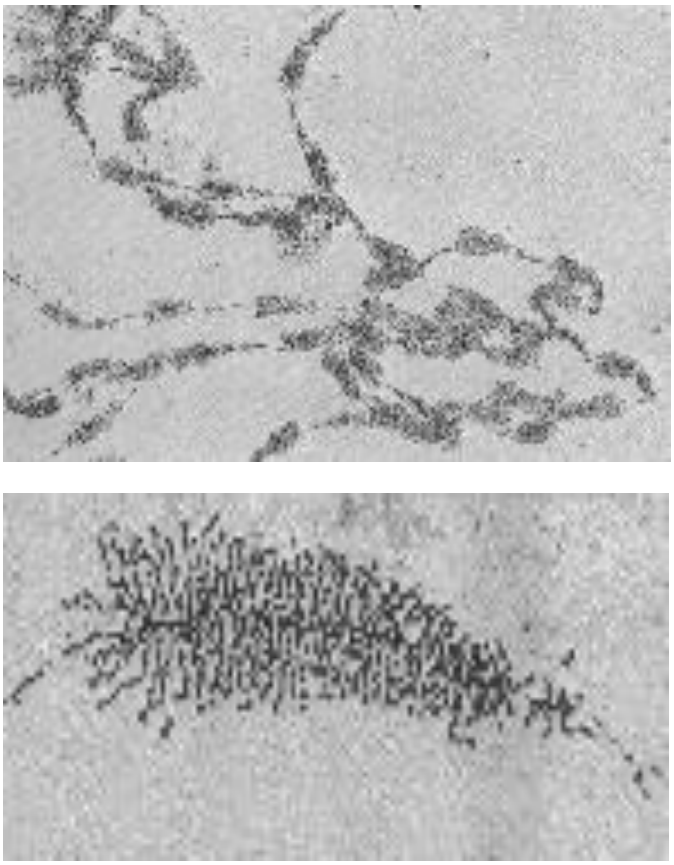
PNAS Vol. 71, No. 1, pp. 135-139, January 1974

1. The arrows point at two parts of the replication bubble.

2. Explain why these parts are thinner!

10. RNA TRANSCRIPTION AND PROCESSING

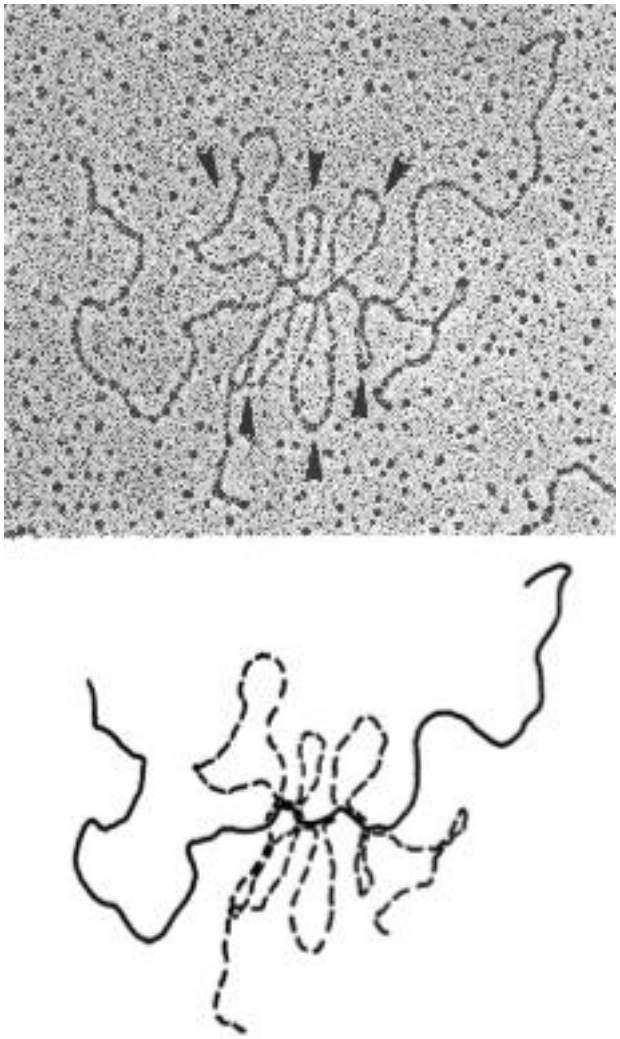
Electron micrograph of spread nucleolar material



PNAS Vol. 71, No. 9, pp. 362S-3630, September 1974

1. What type of process can be seen on this picture?
2. Name the parts of the formation seen on picture 1.B!
3. Name the main steps of the technique used for the visualization of this process!

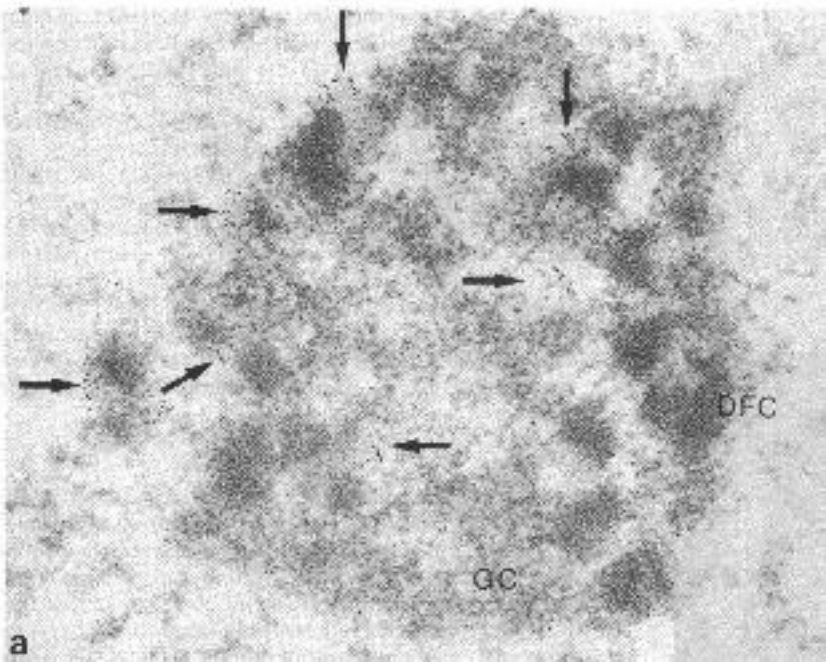
Electron micrograph and interpretative representation of a heteroduplex between human procollagen cDNA and human genomic DNA clone



PNAS Vol. 83, pp. 1568-1572, March 1986 Biochemistry

1. Explain the reason for the different length of the two component of the heteroduplex!
2. What do the arrows point at on the electron micrograph?

Localization of RNA polymerase I within the nucleolus using immunogold technique

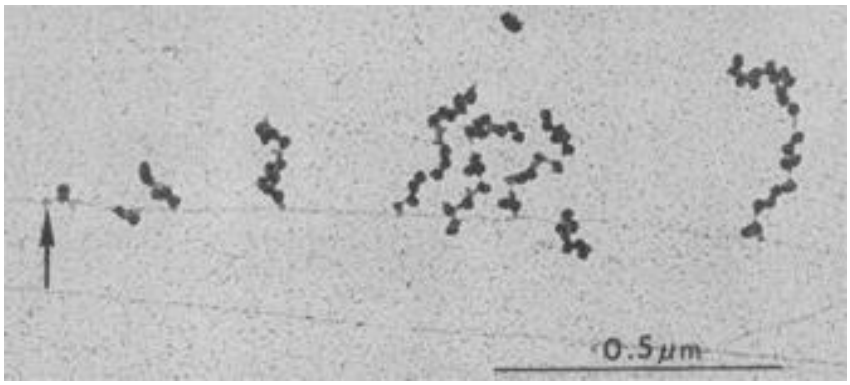


PNAS Vol. 81, pp. 1431-1435, March 1984 CellBiology

1. What are the main steps of the immunogold technique?
2. What is the function of RNA polymerase I?
3. Explain the exact localization of RNA polymerase I within nucleolus!

11. PROTEIN SYNTHESIS

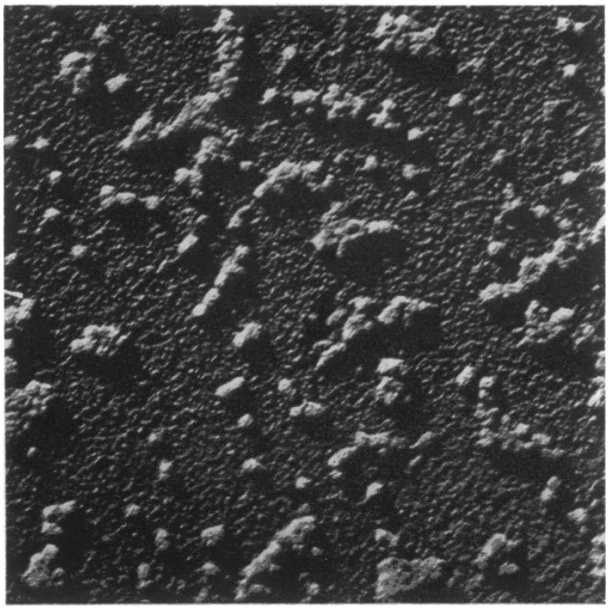
Electron micrograph of a chromosome-polysome complex



Science Vol.169, pp. 392-395, 1970

1. In what type of organism can this process be visualized?
2. Identify the structures seen in this the picture!
3. What does the arrow point at?

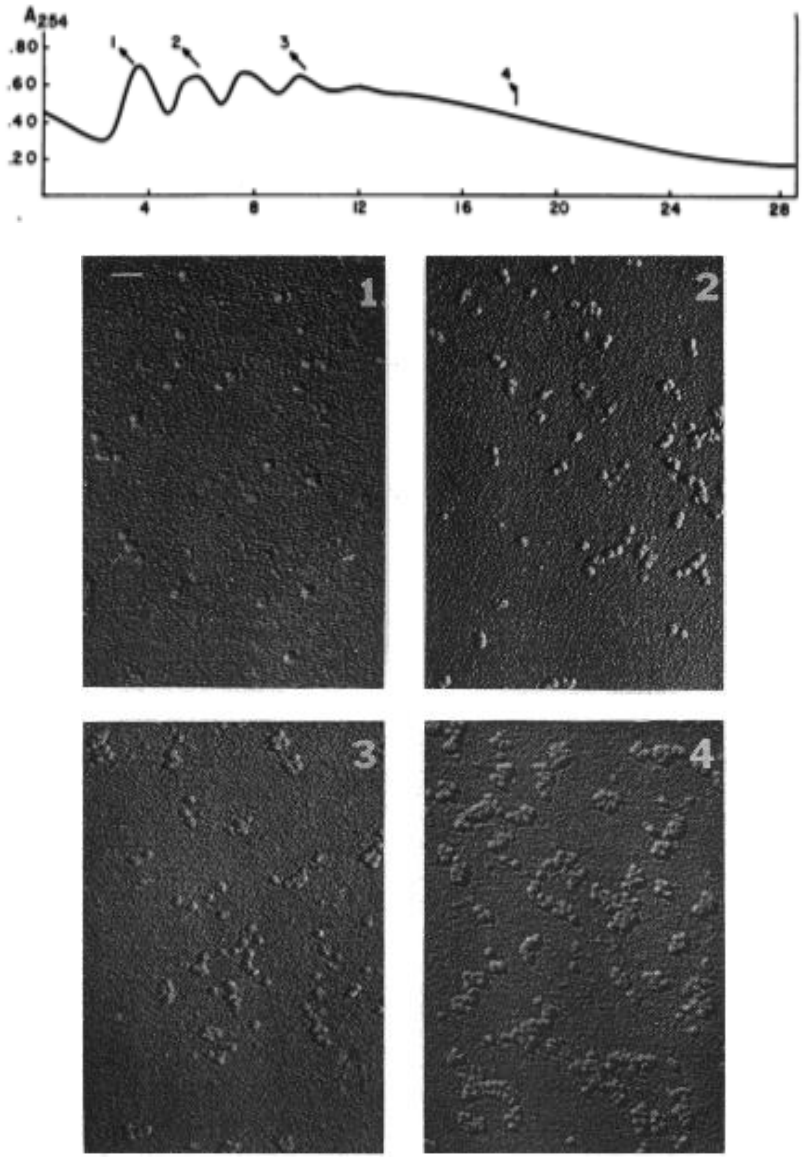
Isolated polysomes



PNAS, Vol 60, 1968

1. What are polysomes made of? What is the function of polysomes?
2. Which microscopic procedure was used to produce this picture?

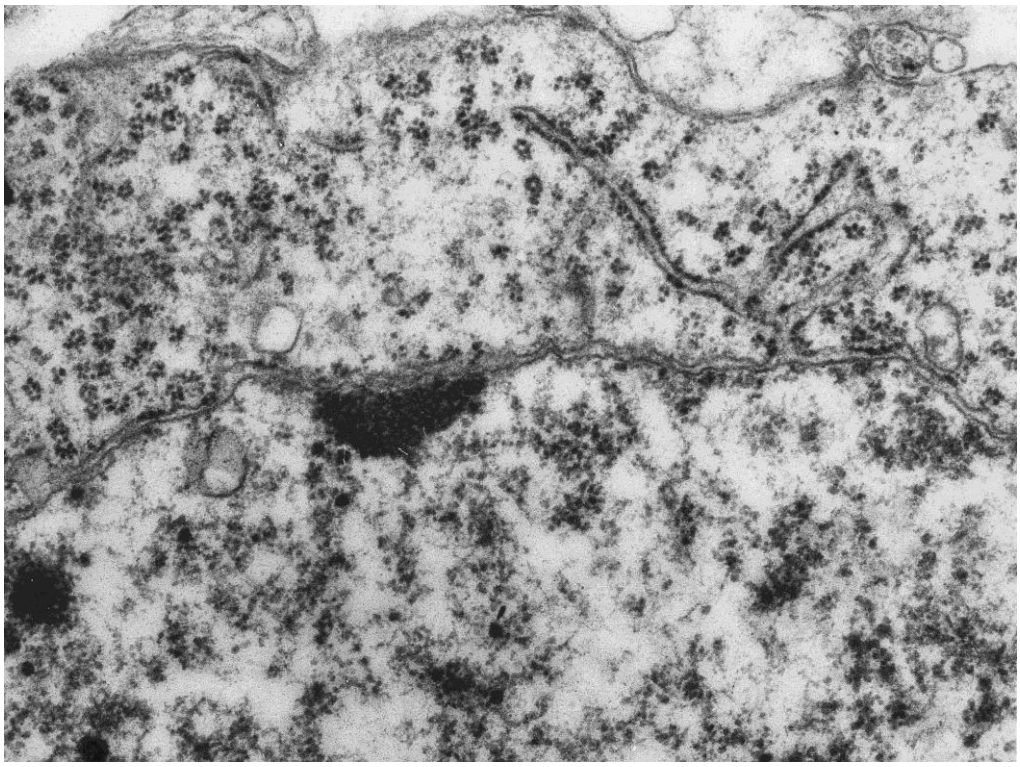
Electron micrographs of mouse brain polysomes. The isolated polysomes were subjected to sucrose density gradient centrifugation then fractions 1-4 were collected for electron microscopy



PNAS Vol. 61, pp. 606-613, 1968

1. What is the difference between polysomes found in different fractions?
2. Which fraction can be found at the closest position to the bottom of the centrifugation tube and why?

GER and free ribosomes

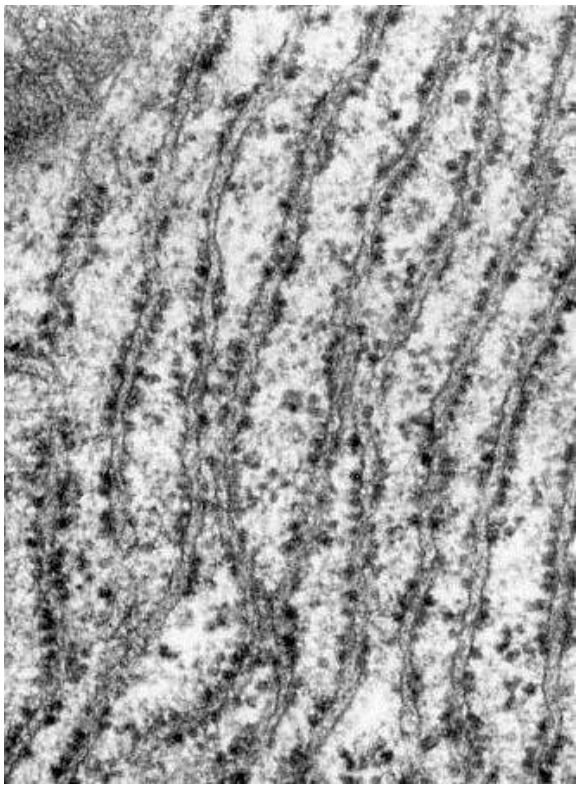


Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. Which proteins are synthesized on bounded and on free ribosomes?
2. Which other cell organelle(s) are visible on the picture?

12. Vesicular transport: secretion and endocytosis

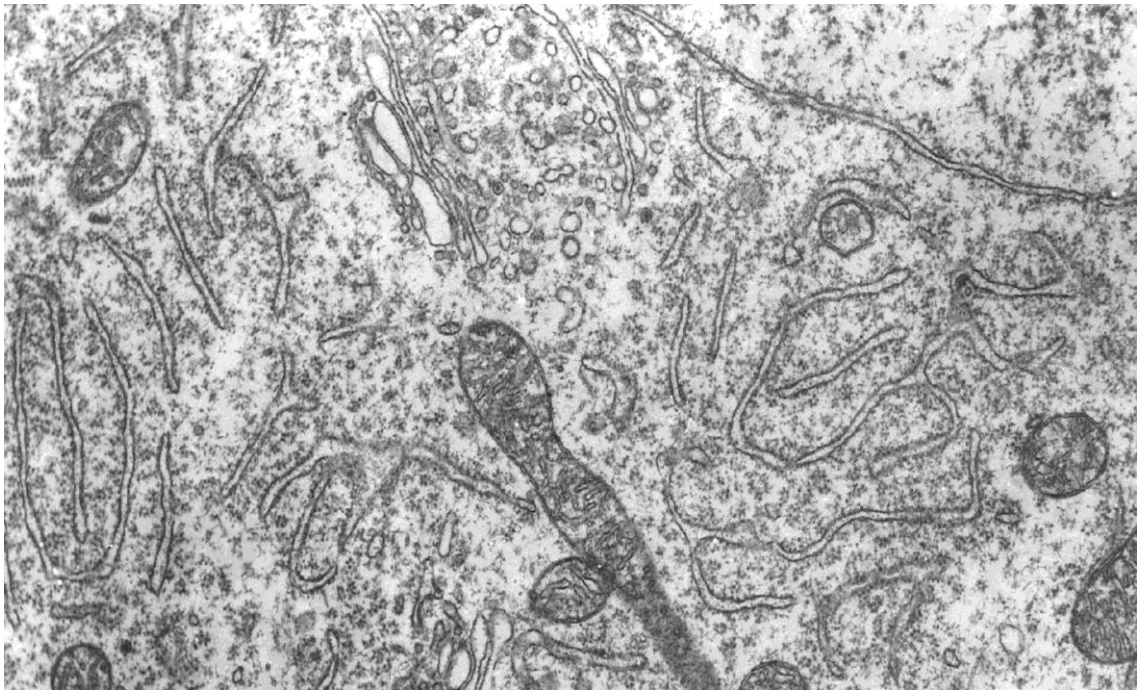
Rough endoplasmic reticulum (RER), Transmission Electron Microscope (TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. What is the role of the rough endoplasmic reticulum in the formation of familial hypercholesterolemia?
2. Name three proteins found in the rough endoplasmic reticulum!

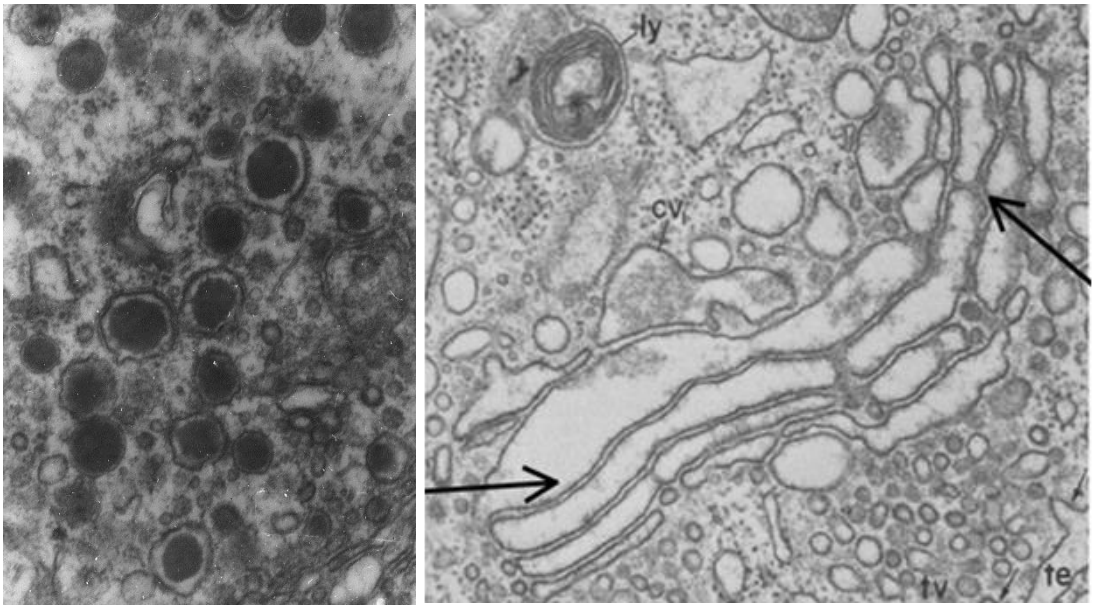
Rough endoplasmic reticulum (TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. Which protein types are synthetized on the bound ribosomes?
2. How are the proteins, synthesized on bound ribosomes, transported into the lumen of RER?

Golgi apparatus (cross and longitudinal section), TEM

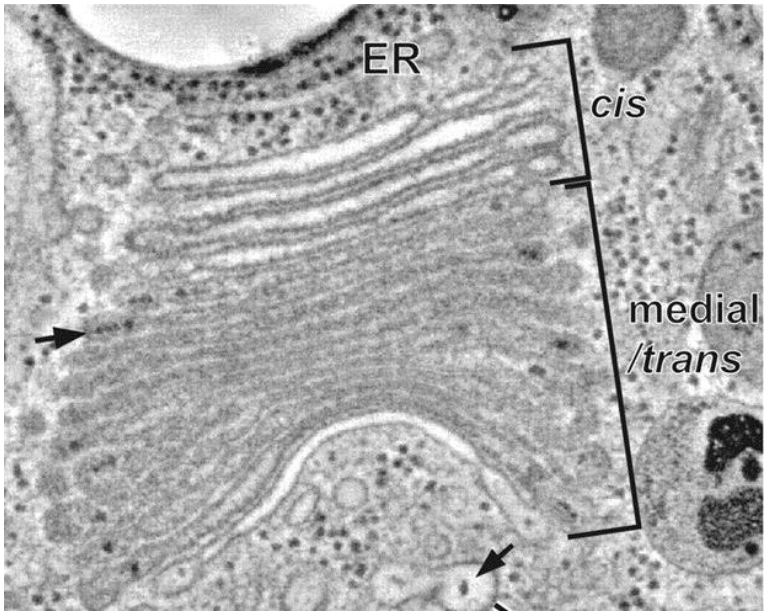


Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory (cross section)

Pfeffer SR PNAS (2010); 107:19614-19618 (longitudinal section)

1. Which posttranslational modification of proteins occurs in the Golgi apparatus?
2. What is the fate of proteins leaving the trans-Golgi reticulum?

Golgi apparatus, longitudinal section, TEM

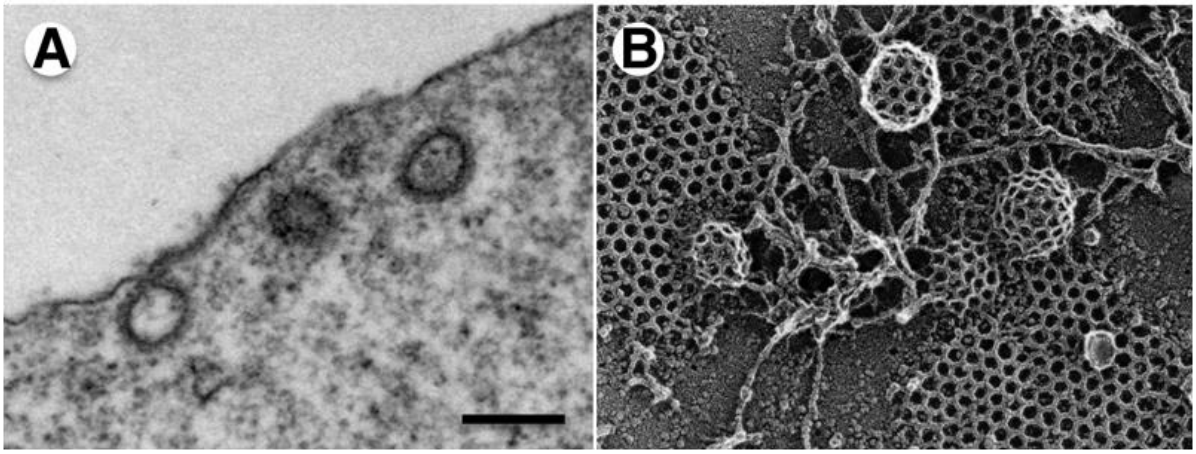


Donohoe BS et al., PNAS (2007); 104:163-168

1. What does the term, cisternae maturation mean?
2. Which part of the Golgi apparatus are the mannose-6-phophate-receptors found in and what is their function?

Clathrin-coated pits on the cell membrane and budding vesicles (A)

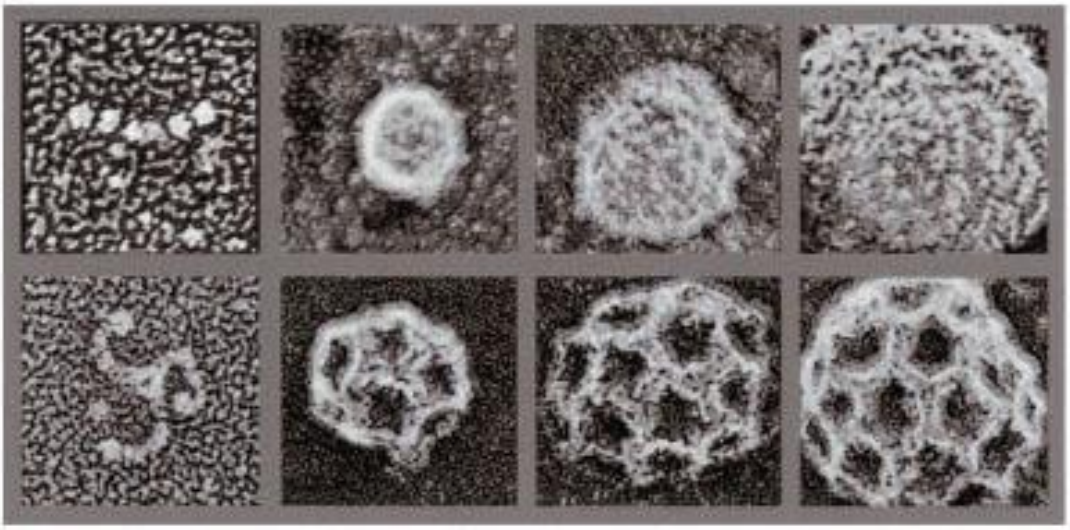
Clathrin-coated vesicles (B)



Krijnse LJ and Schmid SL, PLoS Biol (2013); 11(8): e1001639

1. What methods were used to visualize the structures seen on figures A and B?
2. What is the fate of the clathrin-coat after shedding off the vesicle during receptor-mediated endocytosis?

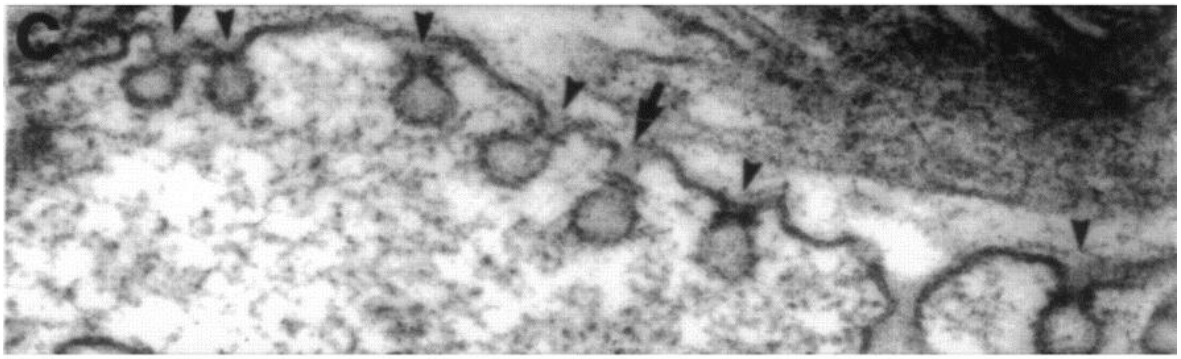
COPII-coated (first row) and clathrin-coated vesicles (second row) (TEM)



Matsuoka K et al., PNAS USA (2001); 98(24): 13705-9

1. Name a process, in which the COPII-coated vesicles play a role!
2. During which processes are the clathrin-coated vesicles formed?

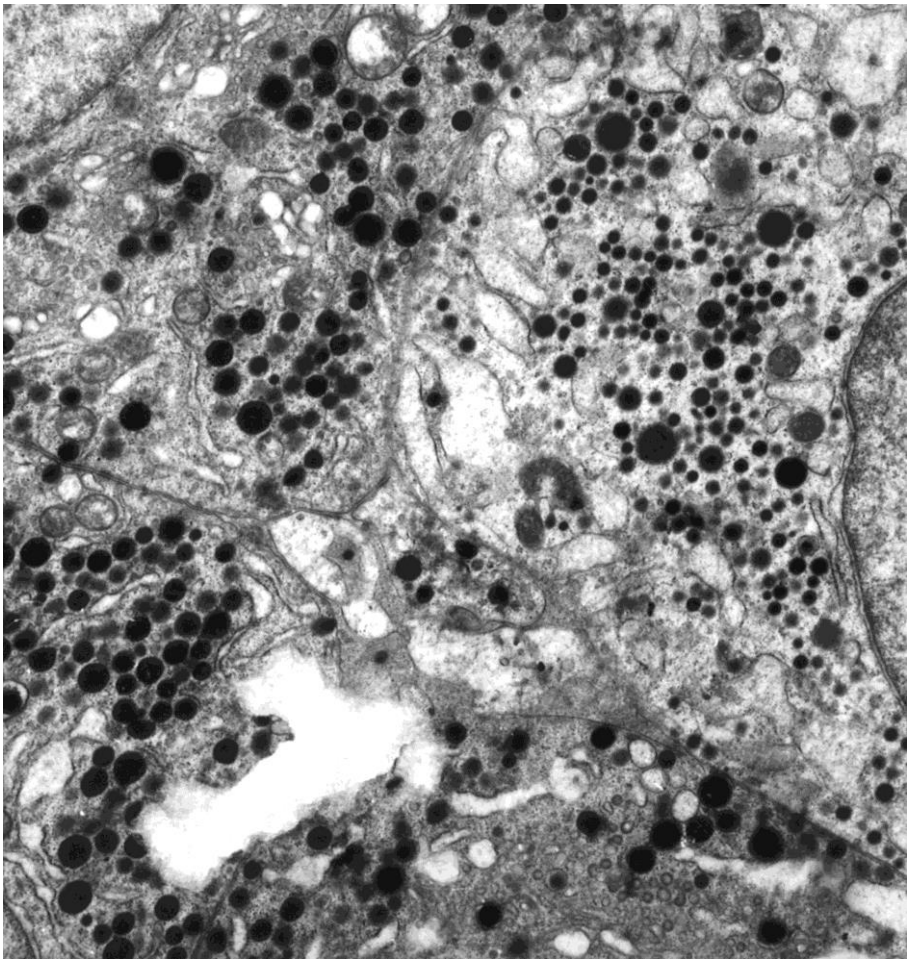
Endocytosis, TEM



Urrutia et al., PNAS USA (1997); 94: 377-384

1. What can be the fate of the endocytotic vesicles?
2. What kind of molecule is taken up by receptor-mediated endocytosis?

Secretory granules (TEM)

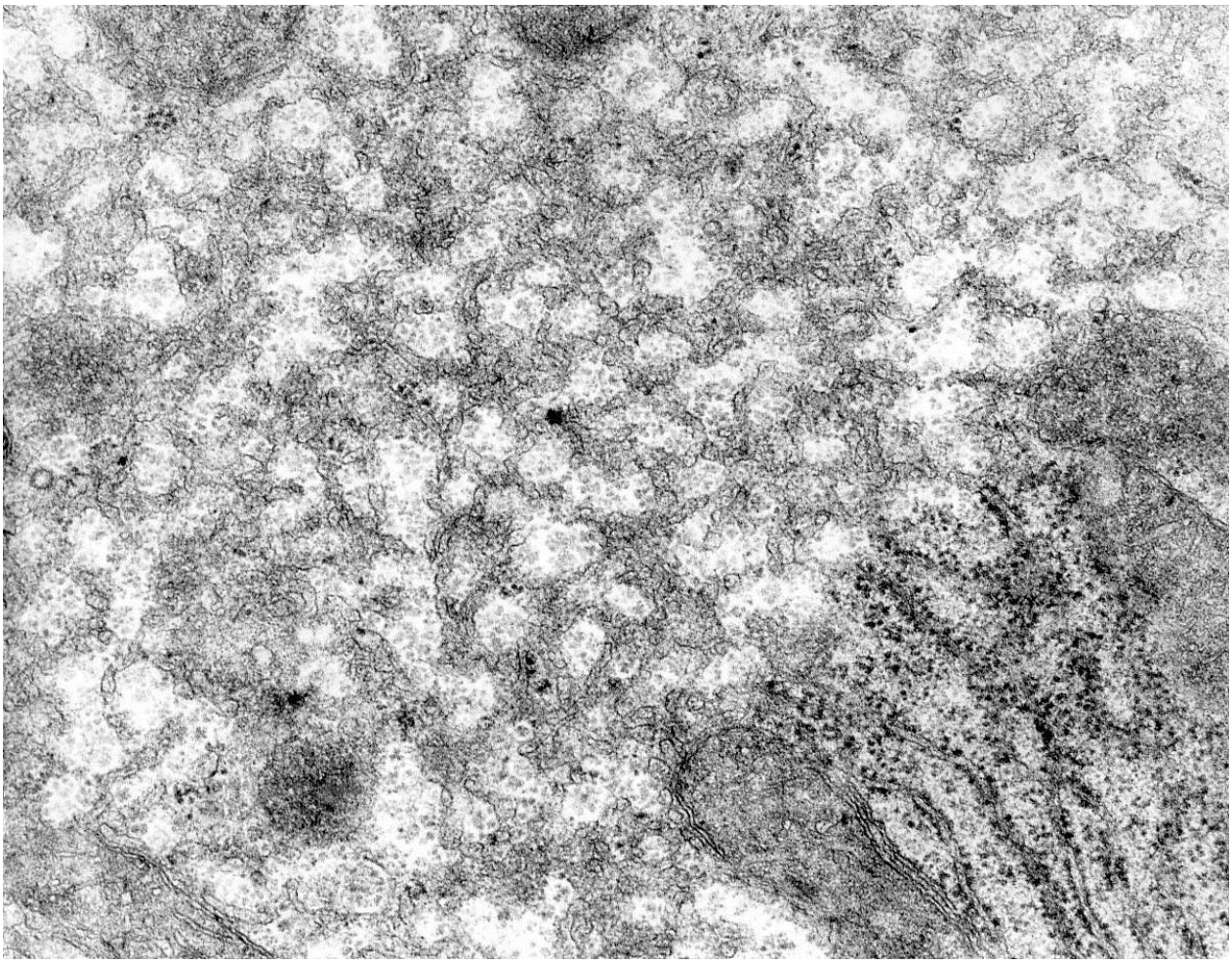


Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. How are the secretory proteins able to leave the cells?
2. How could you explain it that the secretory vesicles are darker than their environment on the electronmicrograph?

13. Cell defense

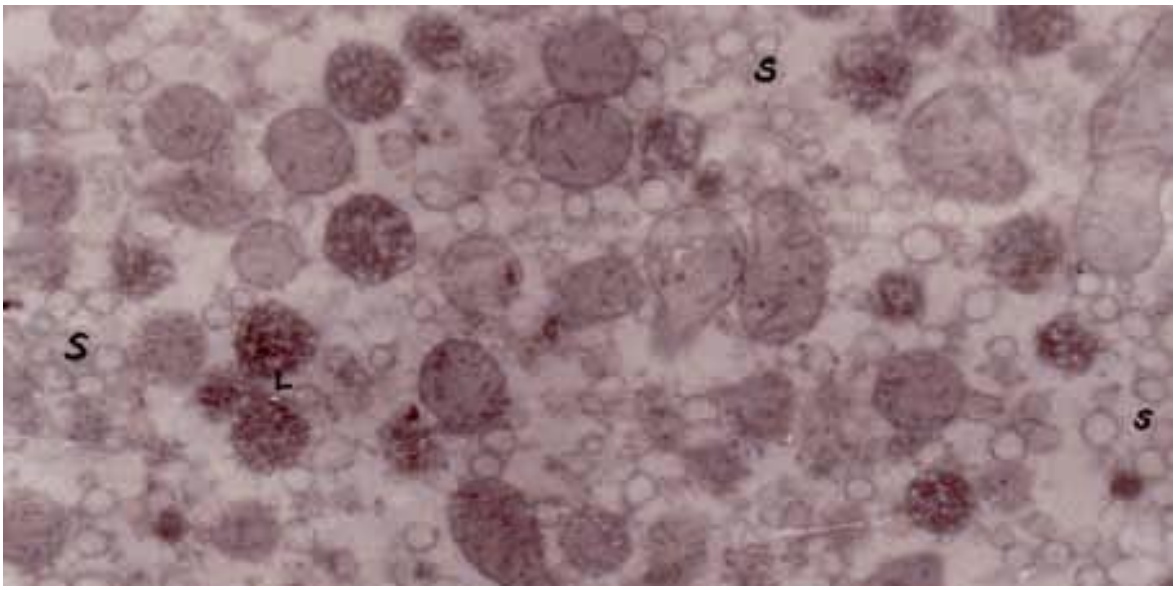
Smooth endoplasmic reticulum (SER) (Transmission Electron Microscope, TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. What are the three main functions of smooth endoplasmic reticulum?
2. Which fraction is found the SER after cell fractionation in?

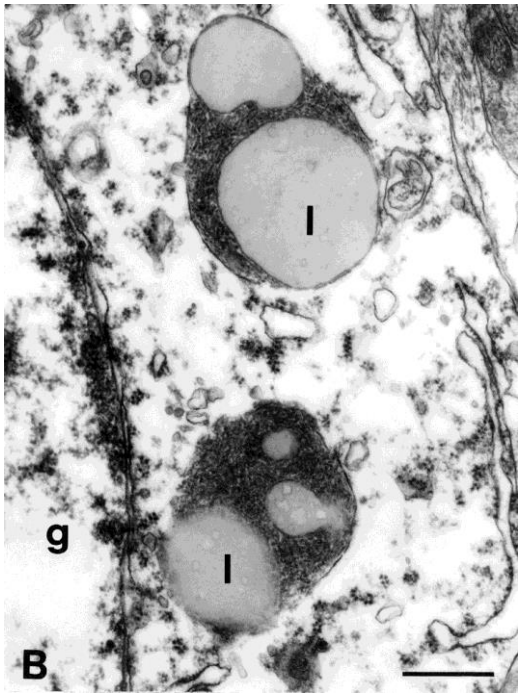
Smooth endoplasmic reticulum (S) and secondary lysosomes (L), TEM



Baric I et al., PNAS (2004) 101(12):4234-9

1. Which lipids are synthesized on the surface of SER?
2. Which signal helps the lysosomal proteins to enter/find the lysosome?

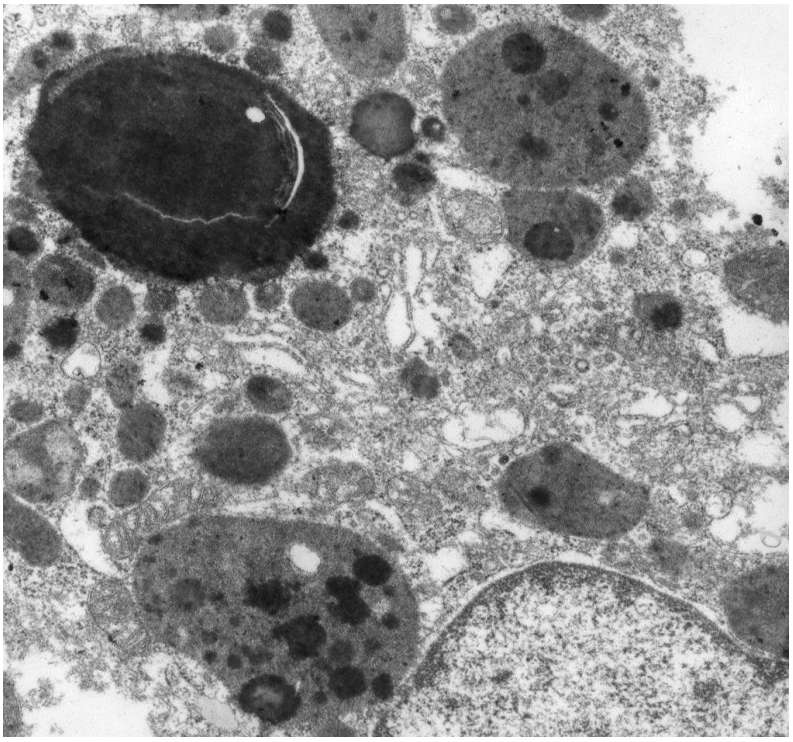
Secondary lysosomes (TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. What kind of molecules are accumulated in the lysosomes in Tay-Sachs disease?
2. Which process and what protein is responsible for the low pH in the lysosomes?

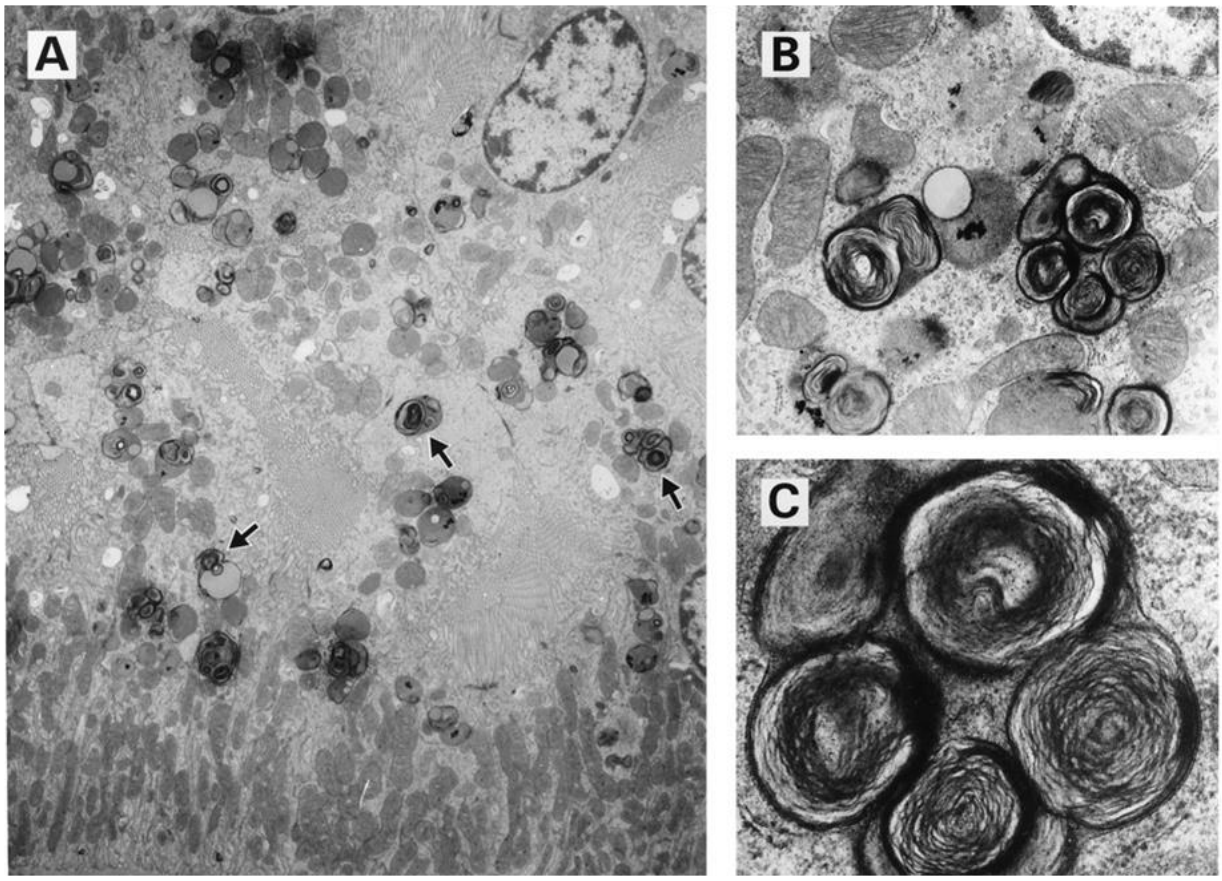
Secondary lysosomes (TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. What is the difference between primary and secondary lysosomes?
2. What is the common name of the lysosomal proteins?

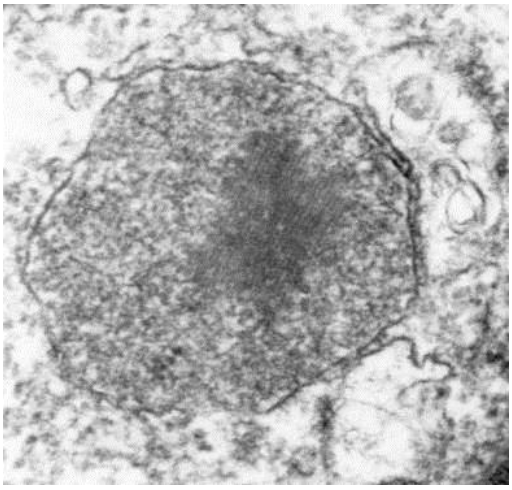
Lysosomal storage diseases (TEM)



Ohshima T et al. PNAS (1997); 94:2540-2544

1. What is able to cause lysosomal storage diseases?
2. What are the characteristics of these diseases?

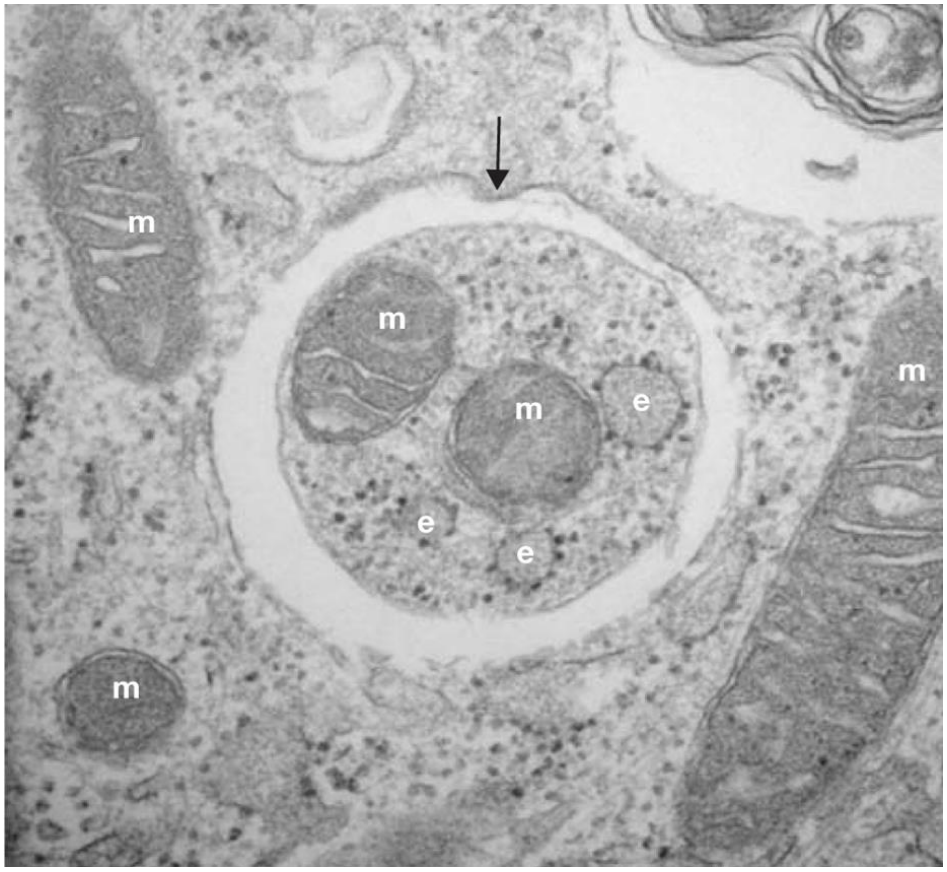
Peroxysome (TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. Which free radical is generated and broken down in peroxysomes?
2. Name two enzymes found in the peroxysomes!

Autophagy (TEM)

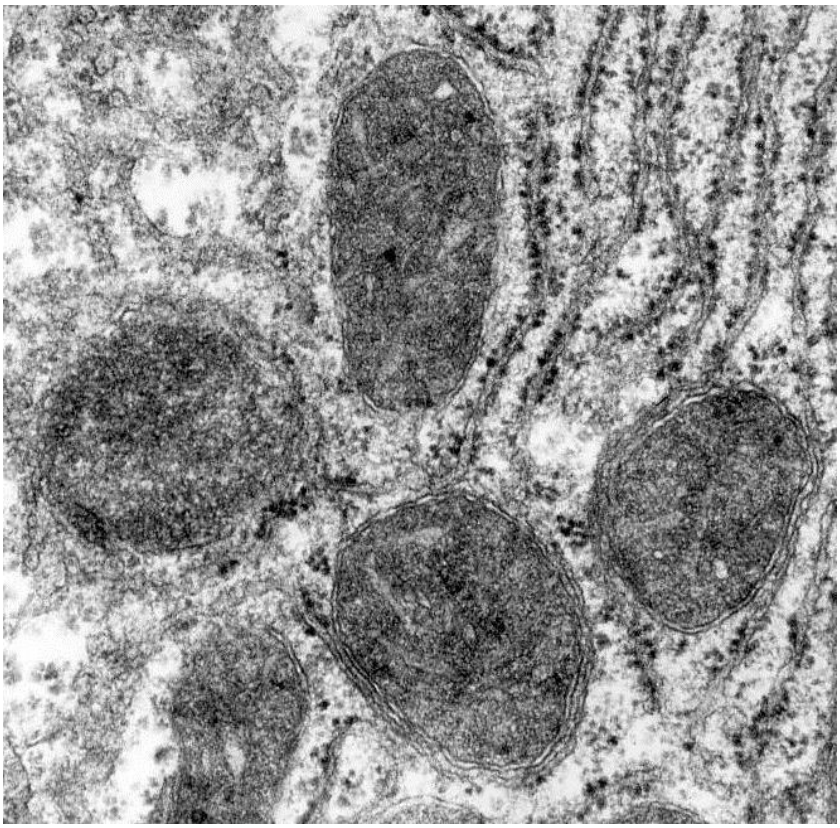


Mizushima N and Levine B, Nat Cell Biol. (2010); 12(9):823-30

1. What is labelled with the letters „m" and „e" on the figure?
2. What is the fate of organelles engulfed by autophagosomes?

14. Mitochondrion

Mitochondria (Transmission Electron Microscope, TEM)



Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

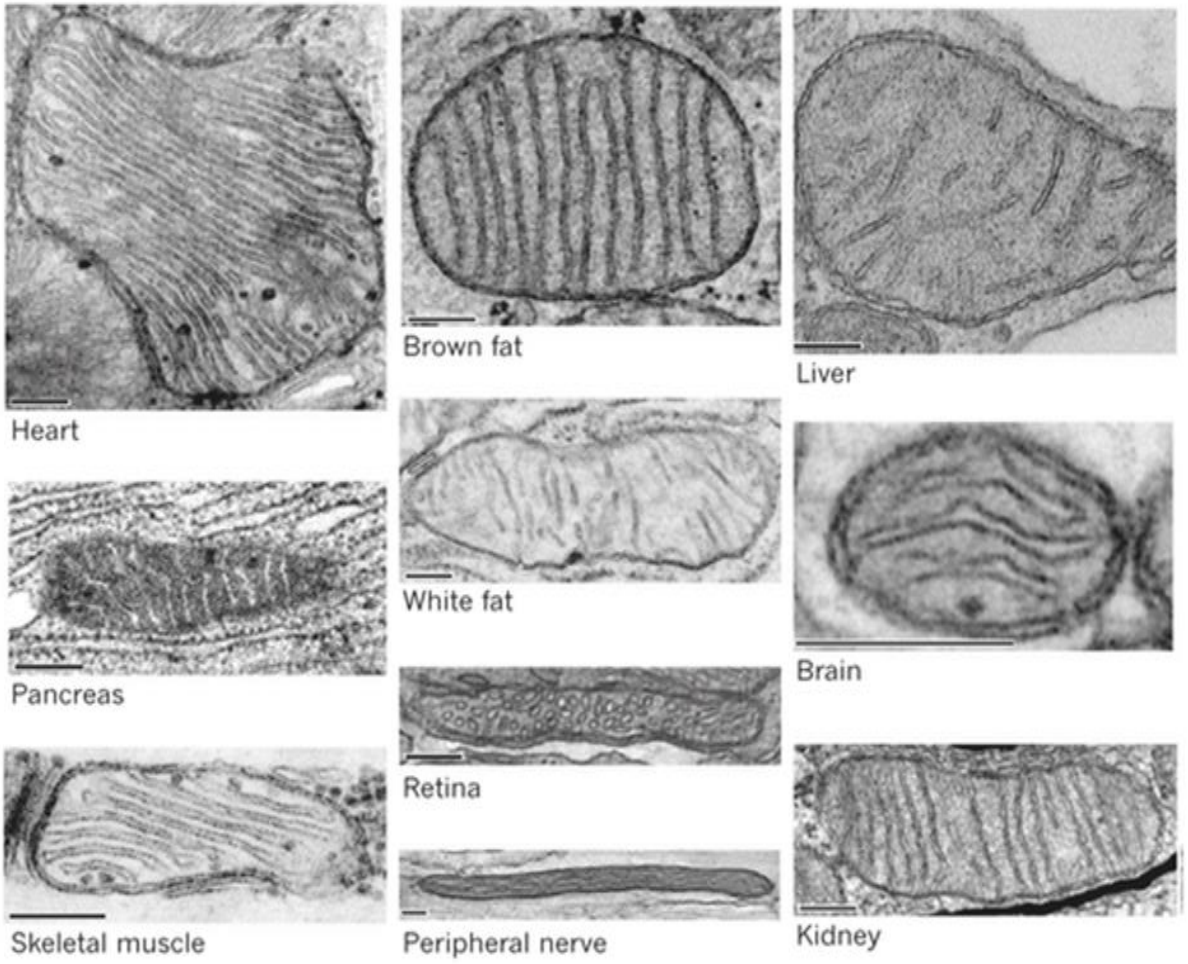
1. Which tissue types contain high amount of mitochondria?
2. Where are the most mitochondrial proteins synthetized?

Mitochondria (TEM)

Hajnalka Ábrahám, University of Pécs, Medical School, Central Electron Microscope Laboratory

1. Which steps of the biological oxidation occur in the mitochondria and where exactly?
2. Which method can be used to isolate mitochondria from eukaryotic cells?

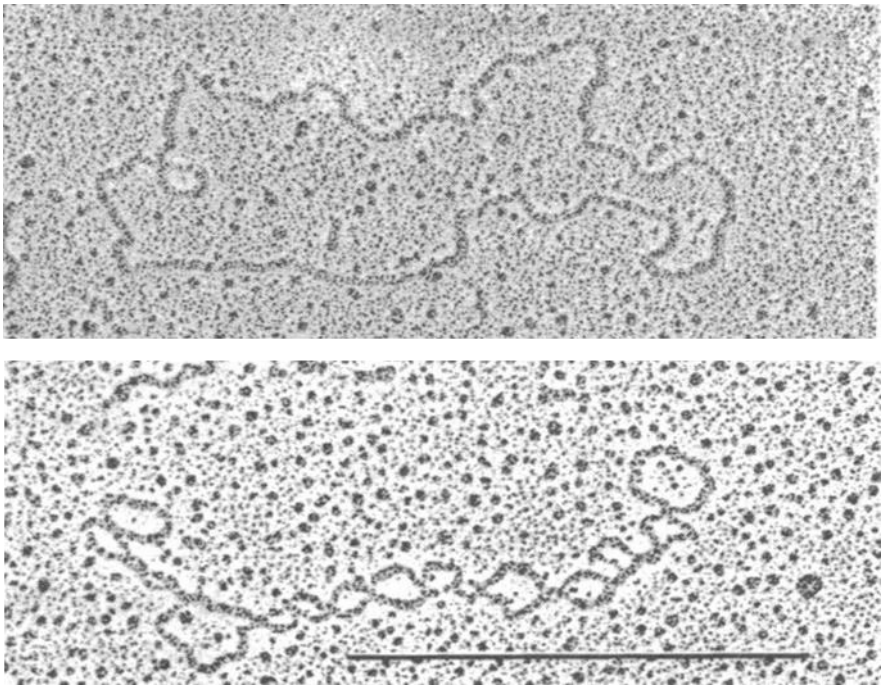
Mitochondria in different cell types (TEM)



Vafai SB and Mootha VK, Nature (2012); 491(7424):374-83

1. What is the basis of the permeability difference between the outer and the inner membrane of the mitochondria?
2. Which transport proteins are found in the inner membrane of the mitochondria?

Mitochondrial DNA (mtDNA) (TEM)



Ryan R et al., PNAS U S A. (1978); 75(7):3268-72.

1. What is the difference between the two DNA molecules seen on the figure?
2. Which separation method can be used to separate the two strands of mitochondrial DNA?

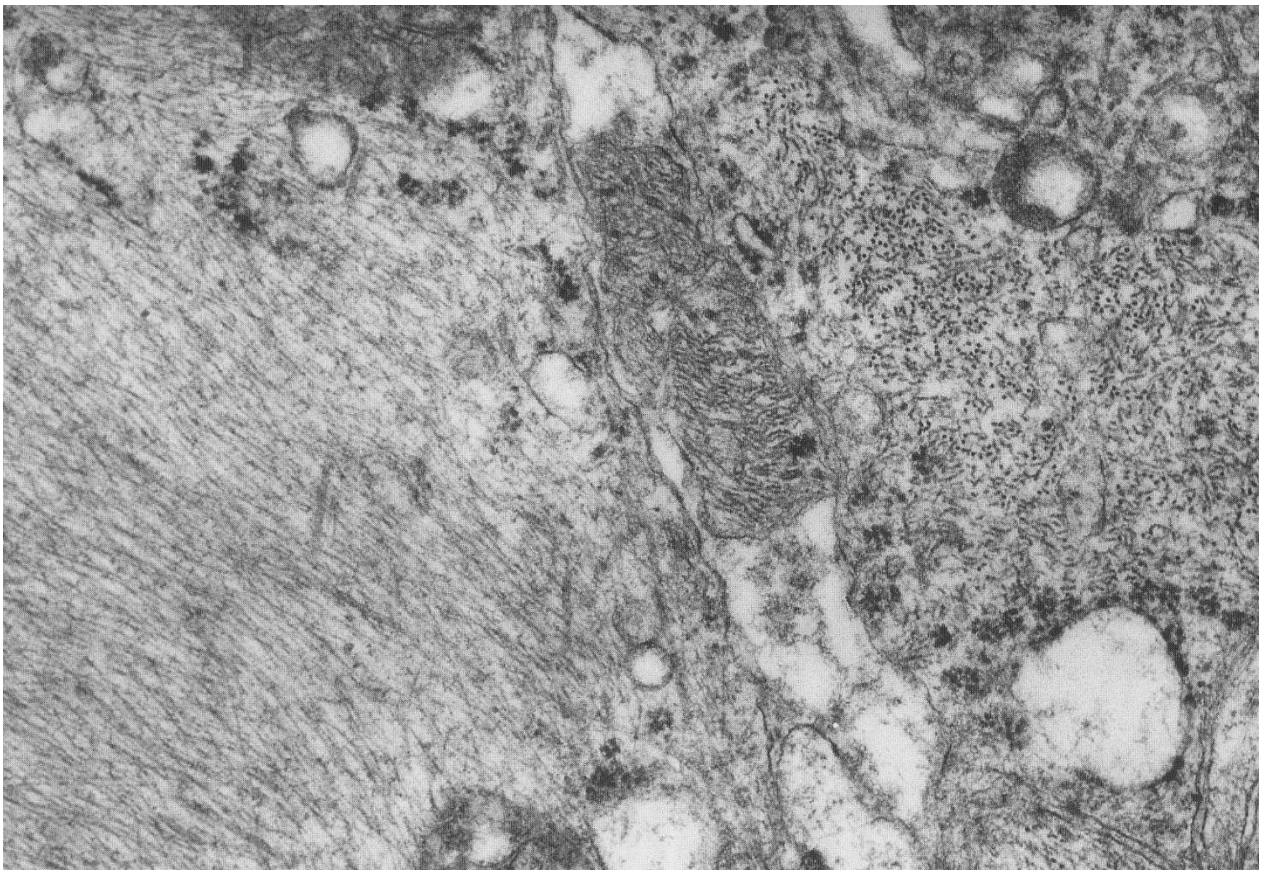
Mitochondrial DNA (Scanning Electron Microscope, SEM)

Hudson, B. et al. Nature. (1967); 216(5116):647-52

1. Which DNA polymerase takes part in the mitochondrial DNA synthesis?
2. What does the term, heteroplasmy mean?

15. Cytoskeleton

Intermediate filaments (Transmission Electron Microscope, TEM)



Hajnalka Ábrahám (University of Pécs, Medical School, Central Electron Microscope Laboratory)

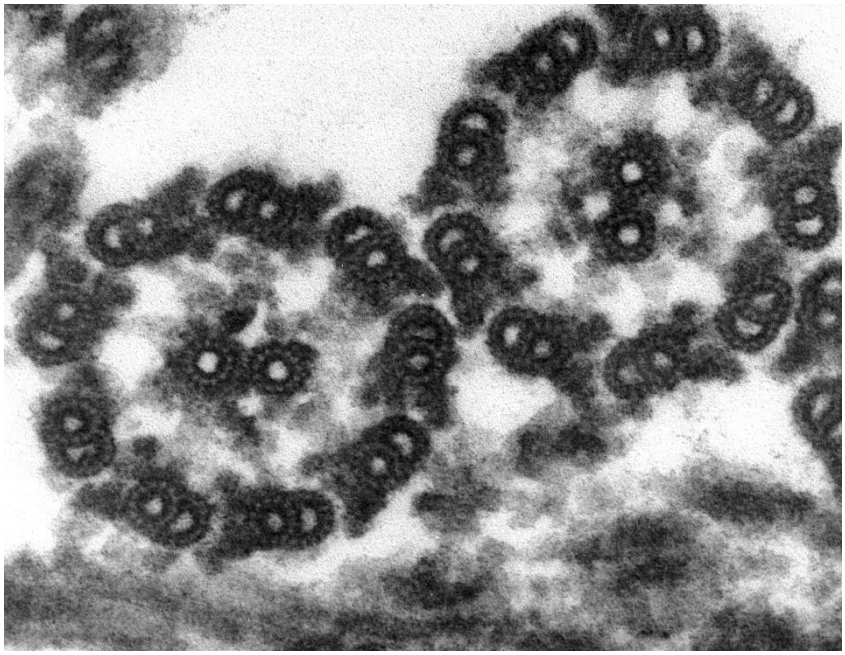
1. Which cell-cell and cell-matrix junctions contain intermediate filaments?
2. Which intermediate filament type is present in all eukaryotic cells?

Microtubules (TEM)

Hajnalka Ábrahám (University of Pécs, Medical School, Central Electron Microscope Laboratory)

1. What are the functions of microtubules?
2. Name two agents inhibiting the polymerization of microtubules!

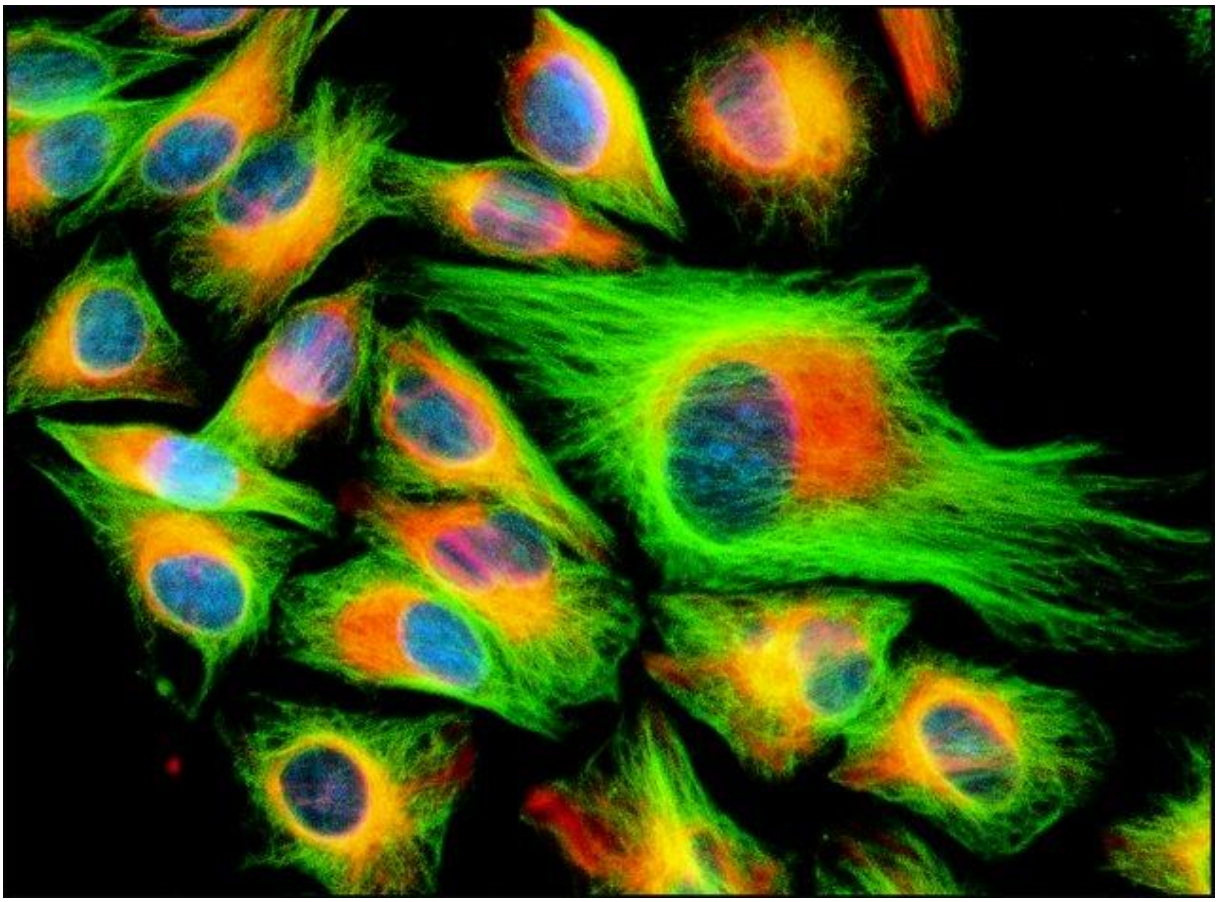
Cross section of the Chlamydomonas flagella (TEM)



http://remf.dartmouth.edu/imagesindex.html

1. Name the types and the functions of microtubules in the mitotic spindle!
2. Which motor proteins are able to move along the microtubules? What is the direction of the movement?

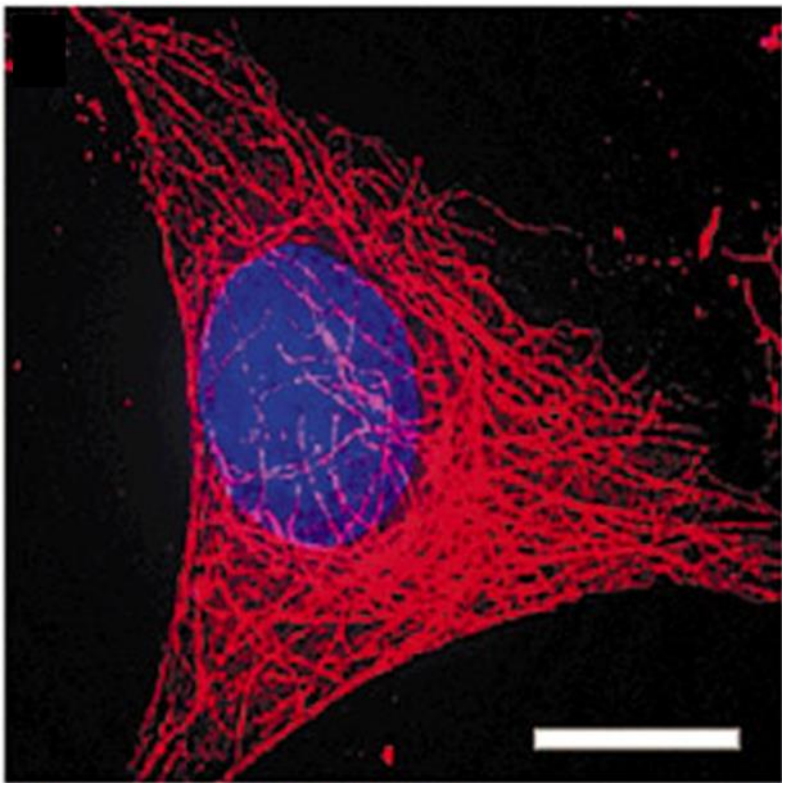
Cytokeratin (green), vimentin (red), nucleus (blue) (Fluorescence microscopy)



Sigma Catalogue (http://www.sigmaaldrich.com/catalog/product/sigma/v5255?lang=hu&region=HU)

1. What kind of cells contain cytokeratin and vimentin?
2. Describe the aim and the steps of immunocytochemistry!

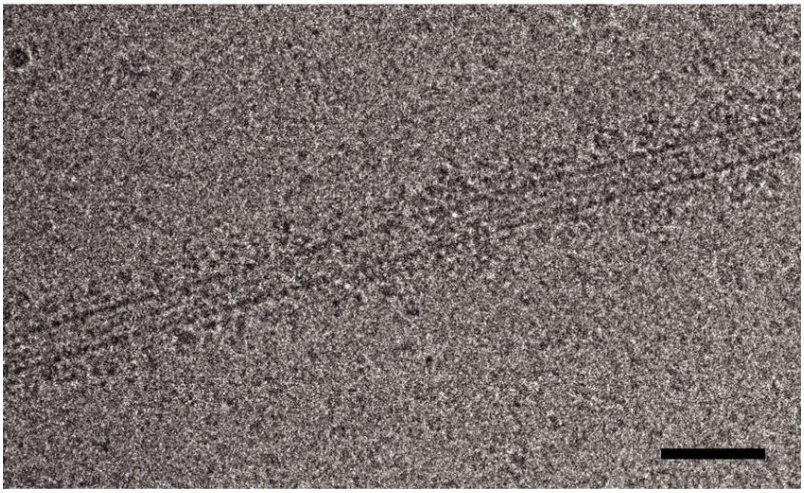
Microtubules, Fluorescence microscopy

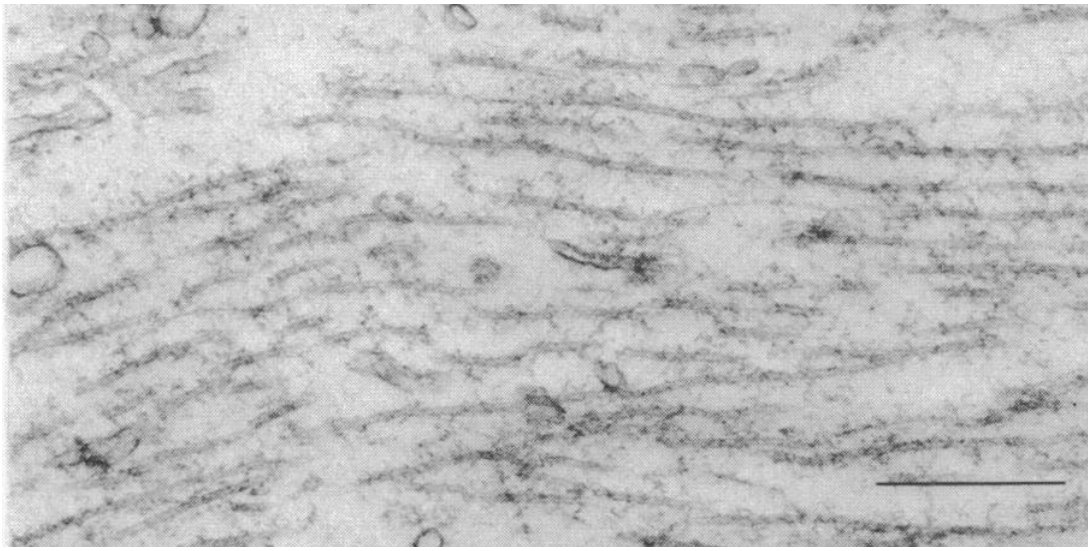


Wu X et al., Nat Biotechnol. (2003); 21(1):41-6

1. What is the energy source of microtubule polymerization?
2. What is the MTOC, where can it be seen on the figure?

Microtubules (TEM)



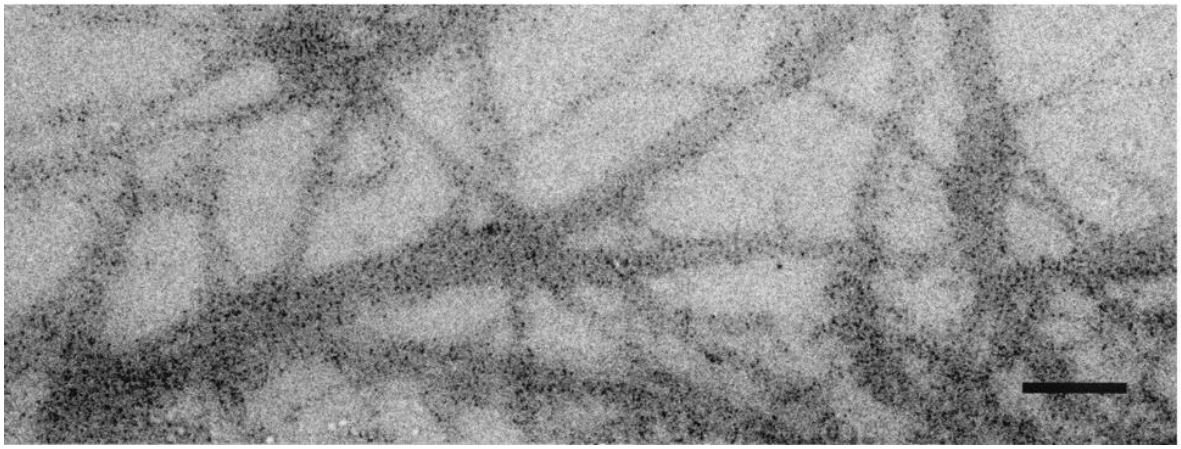


Mizuno N et al., PNAS (2007); 104:20832-20837

O'Connor TM et al., PNAS (1974); 71(10):4198-202

1. Where are the tubulin molecules synthetized?
2. What is the effect of colchicine on microtubules?

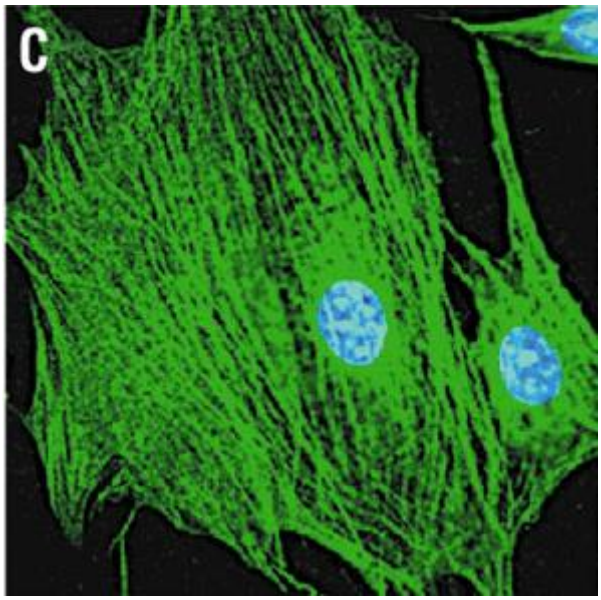
Actin filaments with myosin, labelled by gold-particles, (TEM)



Sugi H et al., PNAS (1997); 94:4378-4382

1. What is the effect of phalloidine on microfilaments?
2. Describe the treadmilling mechanism!

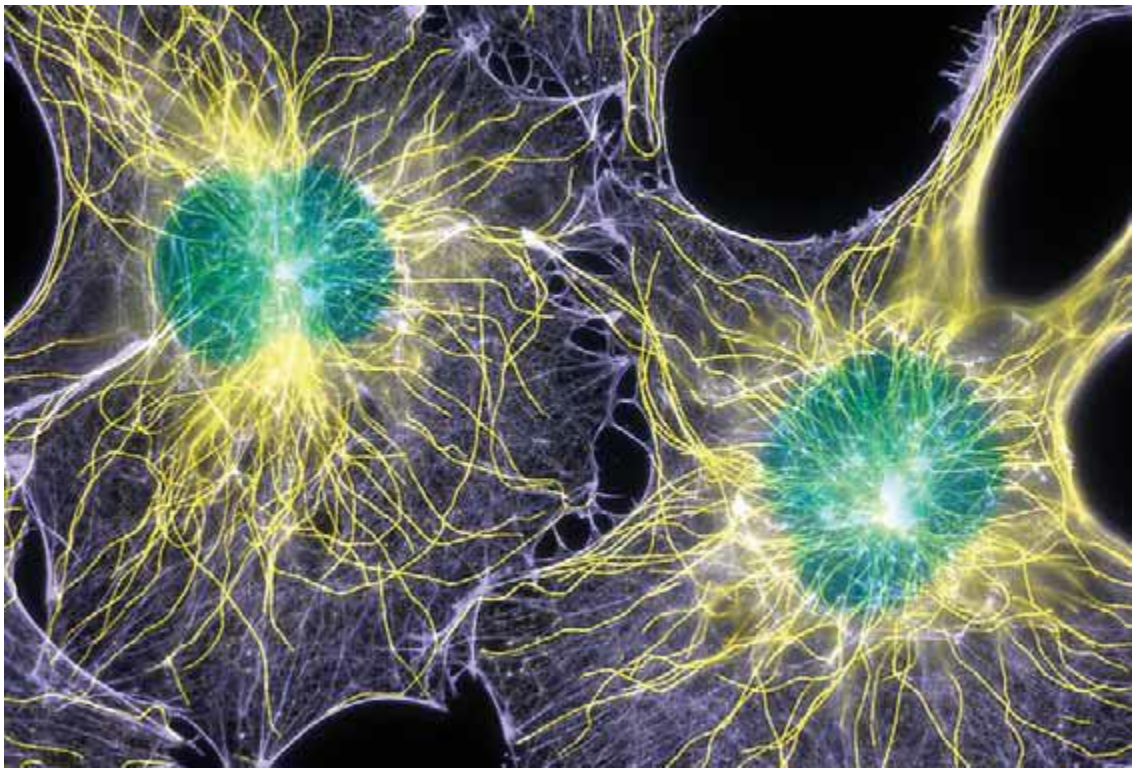
Actin filaments, Fluorescence microscopy



Wu X et al., Nat Biotechnol. (2003); 21(1):41-6

1. What kind of motor proteins are moving along the microfilaments? What is their function?
2. What kind of structures are formed by microfilaments?

Actin filaments (white) and microtubules (yellow)

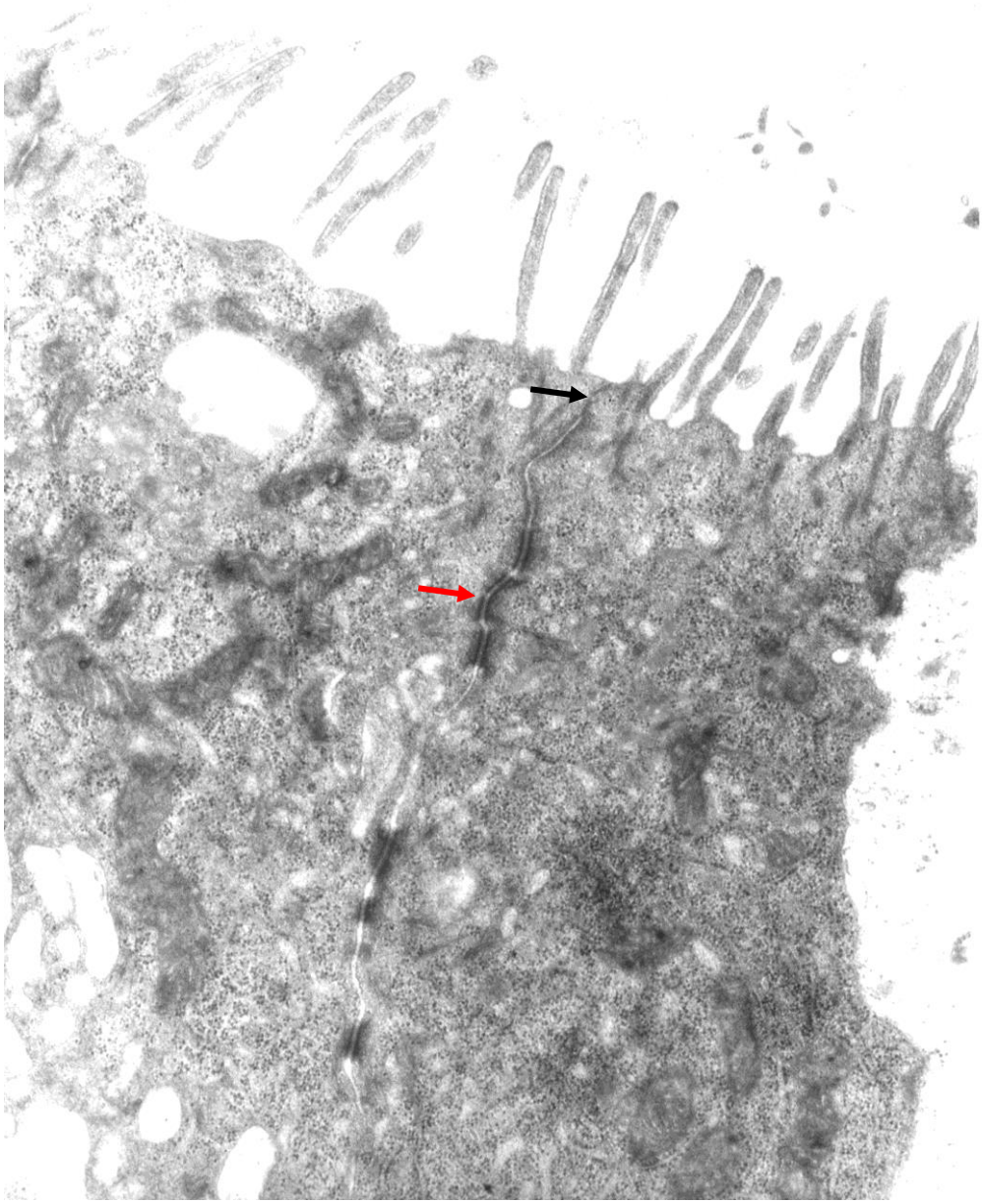


http://publications.nigms.nih.gov/insidethecell/

1. What is the name of the monomers building up the microtubules?
2. What is the energy source of actin polymerization?

16. Cell membrane, cell junctions, extracellular matrix

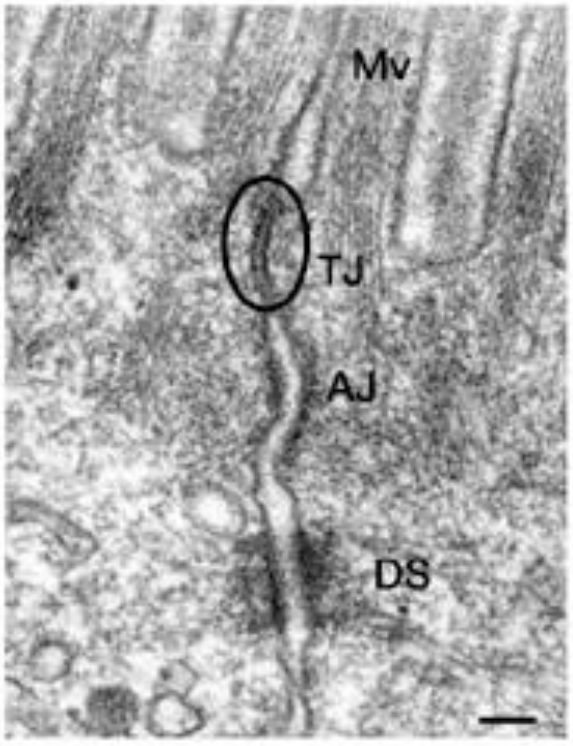
Cell junctions between intestinal epithelial cells (TEM)



Hajnalka Ábrahám (University of Pécs, Medical School Central Electron Microscope Laboratory)

1. Name the structure shown by the black arrow! Name its function!
2. Which cell junction is labelled with the red arrow? Which cytoskeletal element is present in this cell junction?

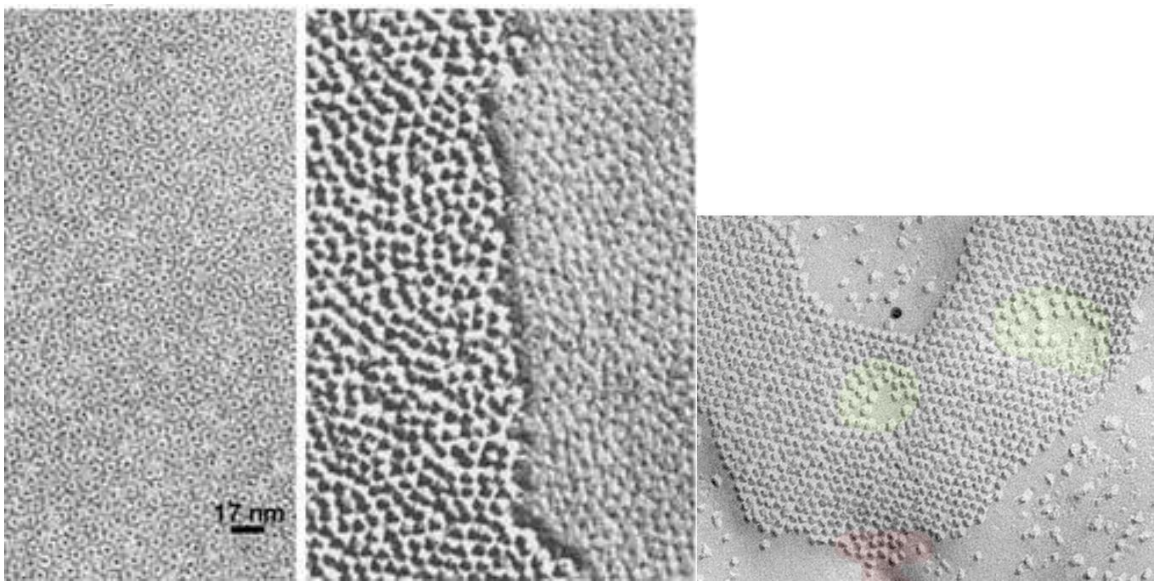
Cell junctions between two epithelial cells (TEM)



Tsukita S et al., Nat Rev Mol Cell Biol. (2001); 2(4):285-93

1. Which cell junctions are shown by the abbreviations on the figure?
2. Which cytoskeletal element is present in the microvilli?

Gap junction, negative staining (left), freeze fracture (right) (TEM)

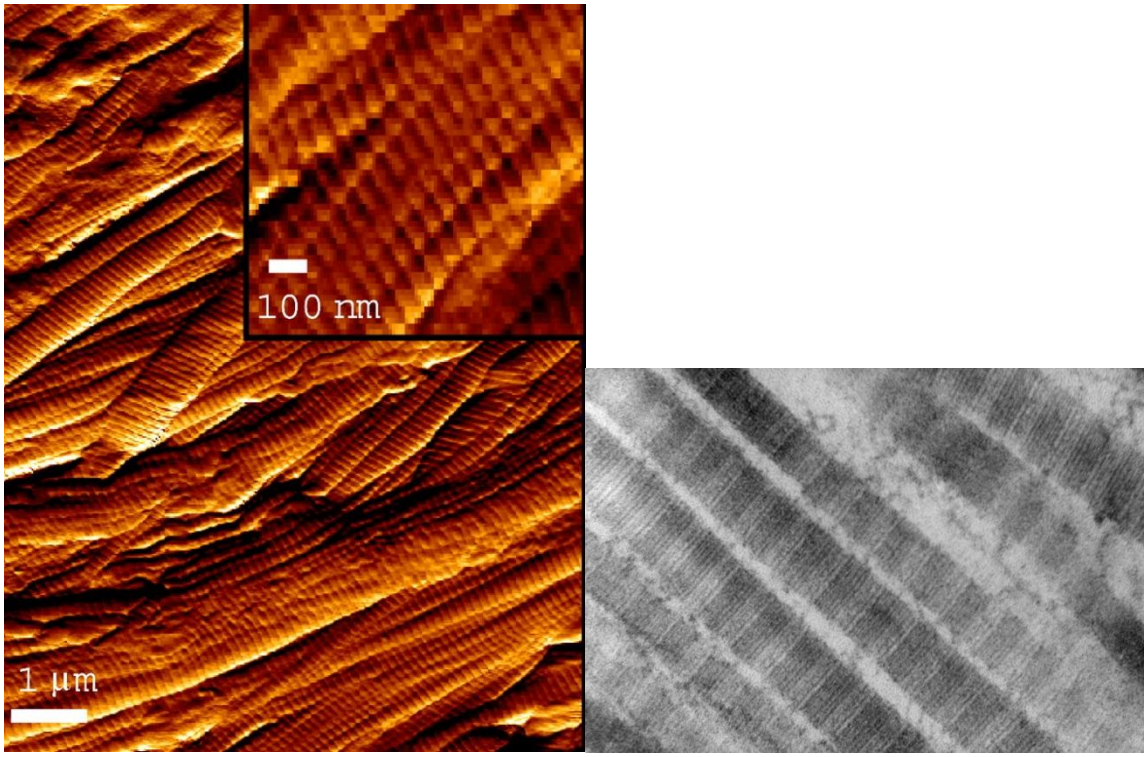


Goodenough DA and Paul DL, Nat Rev Mol Cell Biol. (2003); 4(4):285-94

Flores CE et al., PNAS (2012); 109:3211-3212

1. What proteins are the gap junction made of?
2. What kinds of diseases are formed by the gene mutation of these proteins?

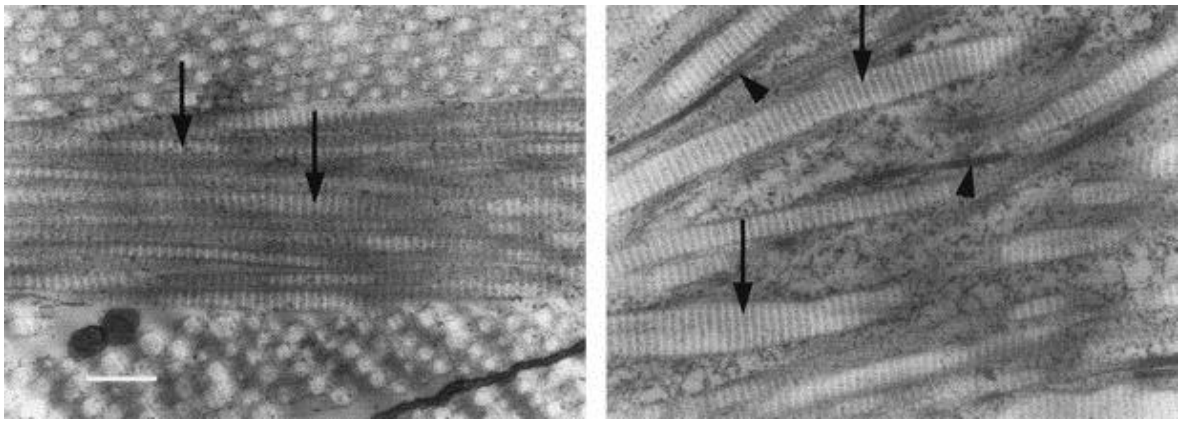
Collagen (Atomic force microscope: left, TEM: right)



Berenguer de la Cuesta F et al., PNAS (2009); 106:15297-15301

http://commons.wikimedia.org/wiki/File:Fibers_of_Collagen_Type_I_-_TEM_.jpg

Collagen (TEM)



Liu X et al. PNAS (1997); 94:1852-1856

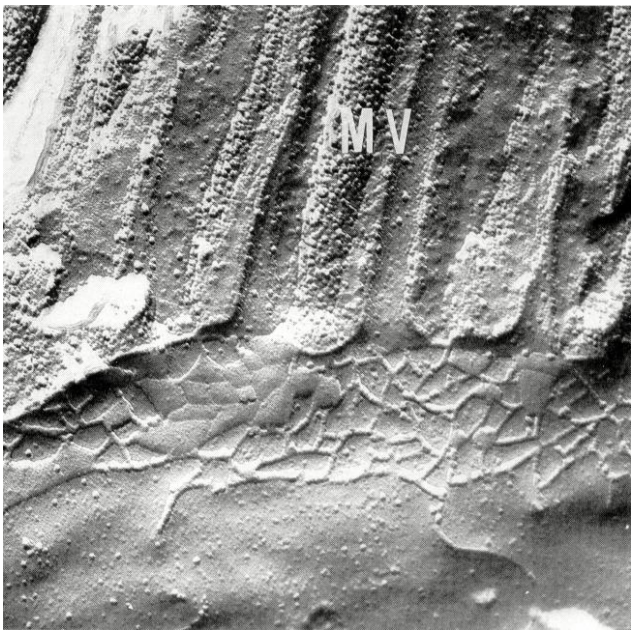
1. What is the molecular background of scurvy caused by decreased vitamin C uptake?
2. Name the steps of collagen synthesis!

Belt desmosome (zonula adherens) (TEM)

Harris TJC and Tepass U, Nat Rev Mol Cell Biol (2010); 11: 502-514

1. Which cytoskeletal element is responsible for the structure of belt desmosome formation?
2. Why is this cell junction called belt desmosome?

Tight junction and microvilli (TEM, freeze-fracture)



Schulzke JD et al., Pediatr Res. (1998) 43, 435–441

1. How is the freeze-fracture technic performed?
2. What molecules are the tight junctions made of?