

CELL BIOLOGY

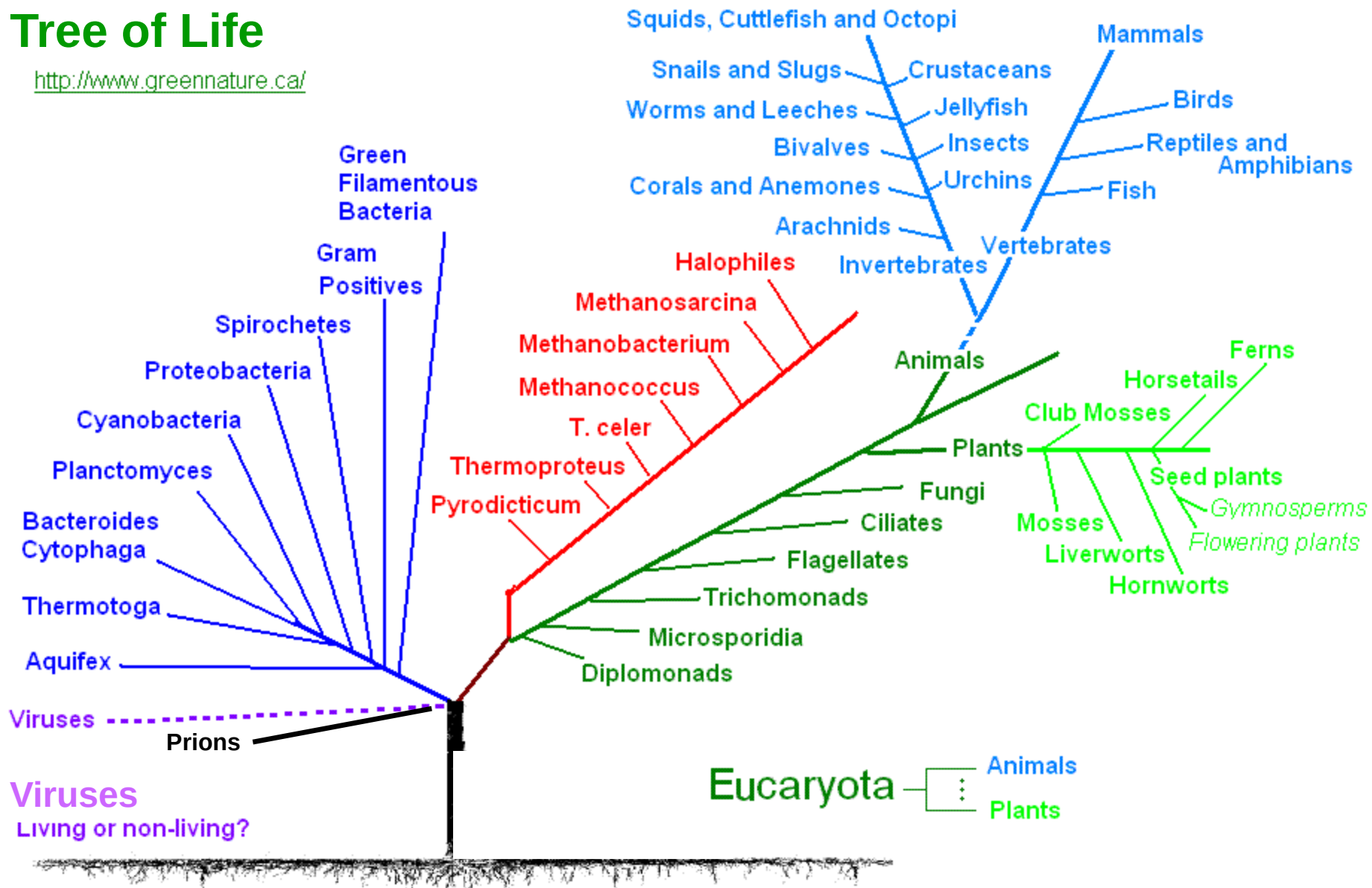
Eukaryotic cell

Department of Plant Physiology

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Tree of Life

<http://www.greennature.ca/>



Microscopy

Light (LM) – (bright field, ...)

Microscopy:

Light (LM) – (bright field, dark field, phase contrast, differentiation interference contrast = Nomarsky contrast)

Polarization (PM)

Fluorescent (FM)

Light sheet fluorescence microscopy (LSFM)

Ultraviolet (UVM)

Confocal laser scanning microscopy (CLSM)

Electron microscopy (EM) - (transmission - TEM, high-resolution transmission - HRTEM, scanning - SEM, ...)

Atomic force (AFM)

Tomography – scanning of beam numbers (röntgen, electron...)

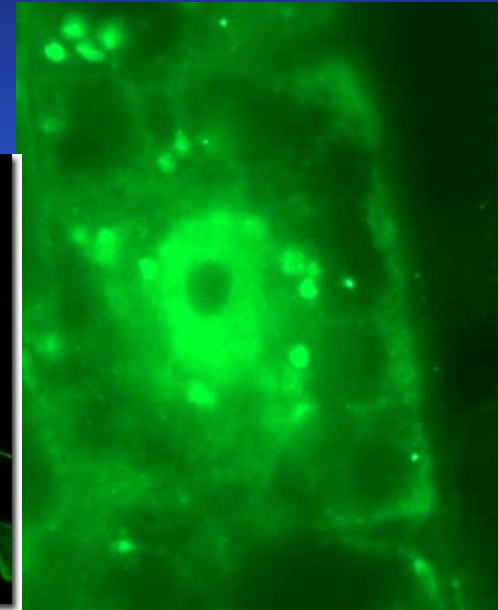
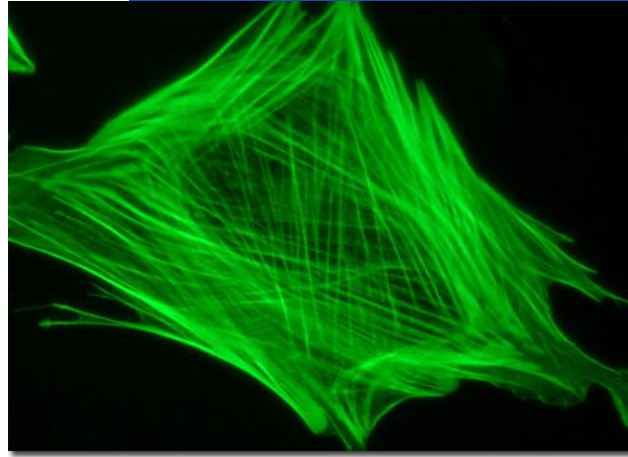
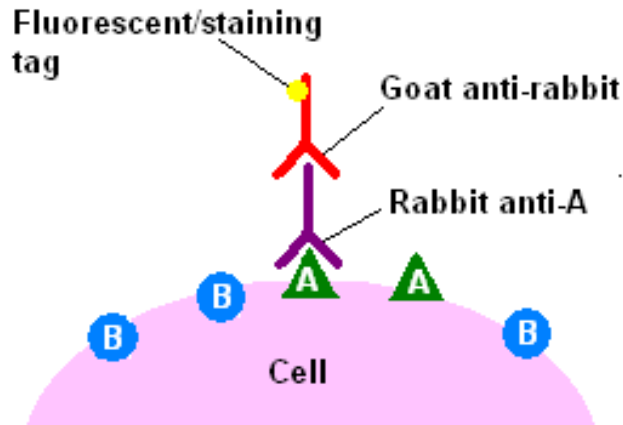
Holographic optical tweezer – sample localization by laser beam

Element analysis – energy-dispersive röntgen analysis (EDXA), electron energy loss spectrometry (EELS), energy-filtering transmission electron microscopy

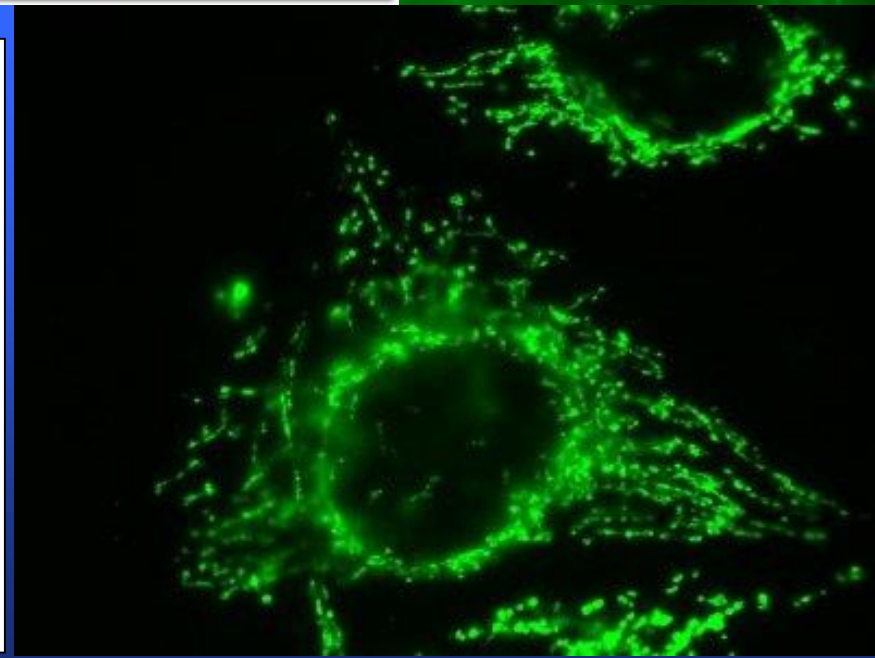
Spectral analysis, separations, ...

Visualisation of cell structures

Immunofluorescence microscopy



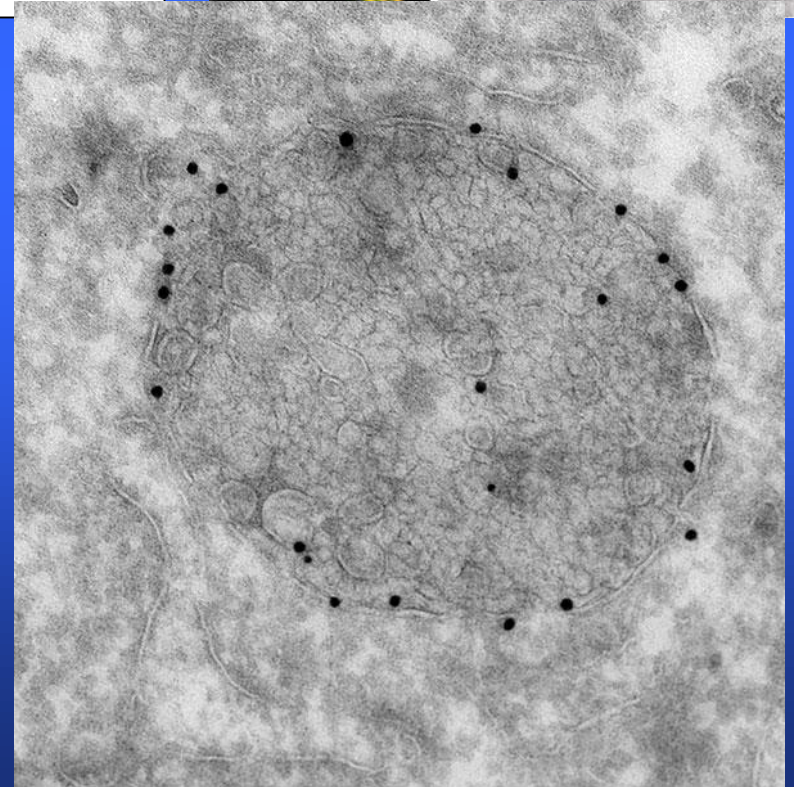
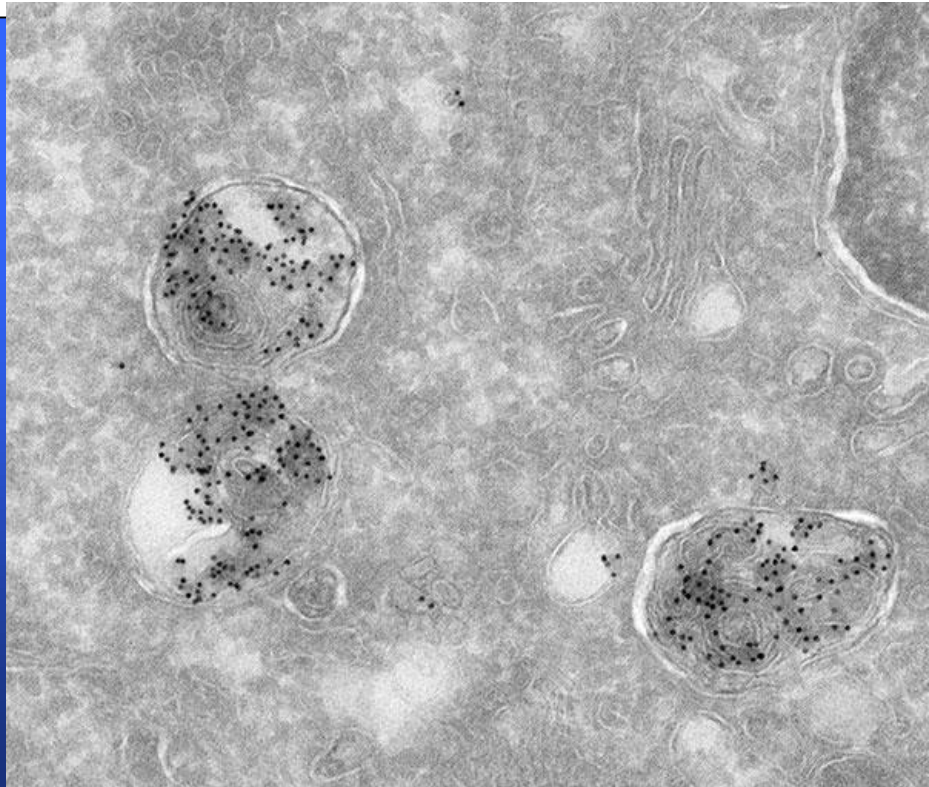
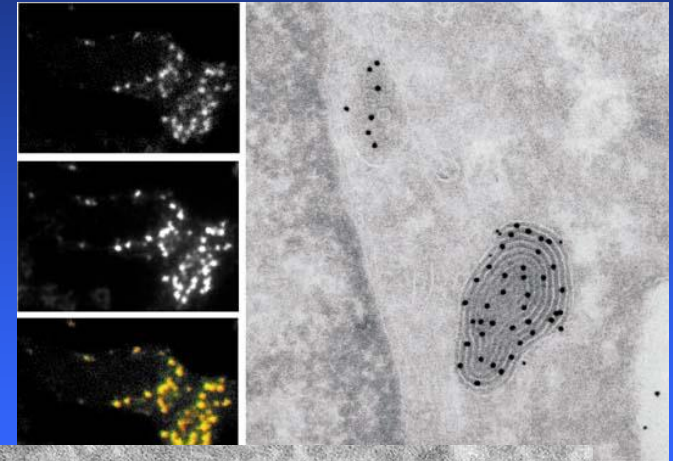
Primary antibody adheres to a specific protein or its specific part – **epitop**.
Secondary antibody (adheres to the primary antibody) can be conjugated with a **fluorescence marker** – it emits e. g. green signal in fluorescence microscope.



Visualisation of cell structures

„Immunogold“ electron microscopy

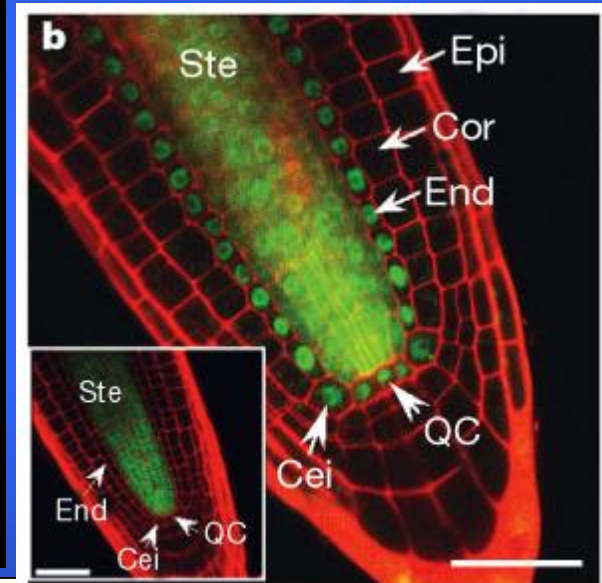
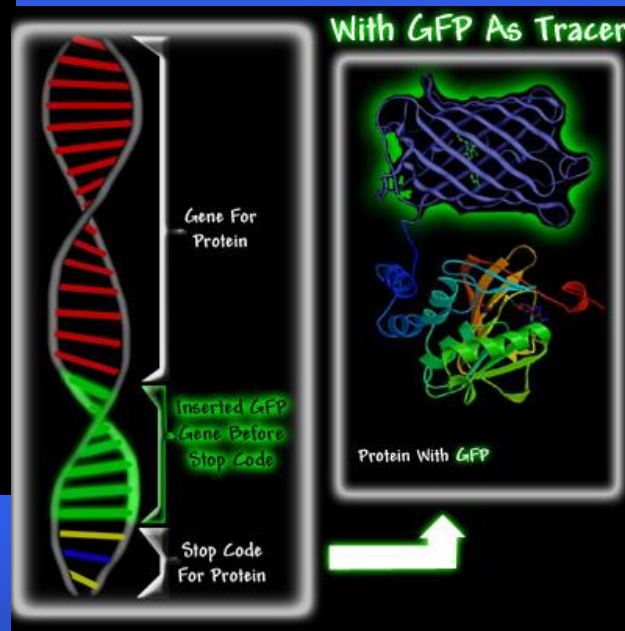
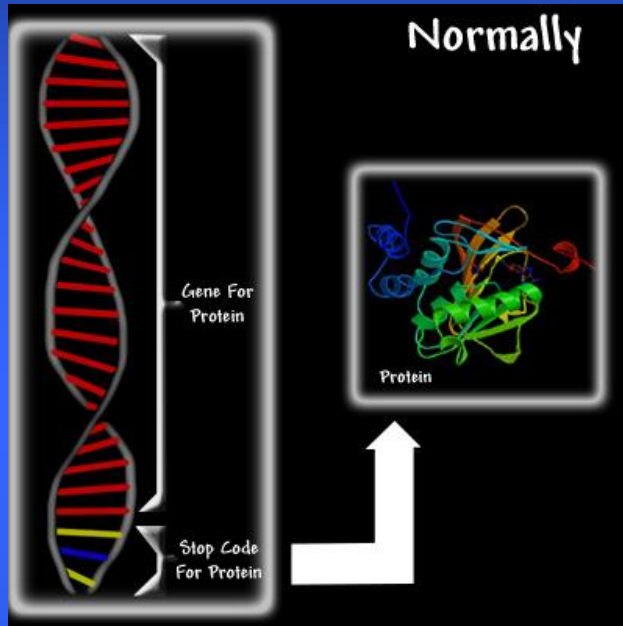
Secondary antibodies are in case of ImG-EM **conjugated with gold particles**, which are in transmission electron microscope electron-dense. ImG-EM possesses observing of specific protein **localisation on ultrastructure level**.



Visualisation of cell structures

GFP-fussion

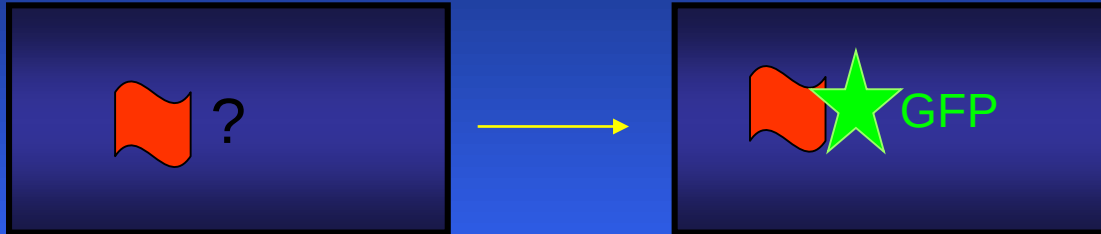
Technique requires DNA cloning and transformation of organism of interest – **transgenic organism**.



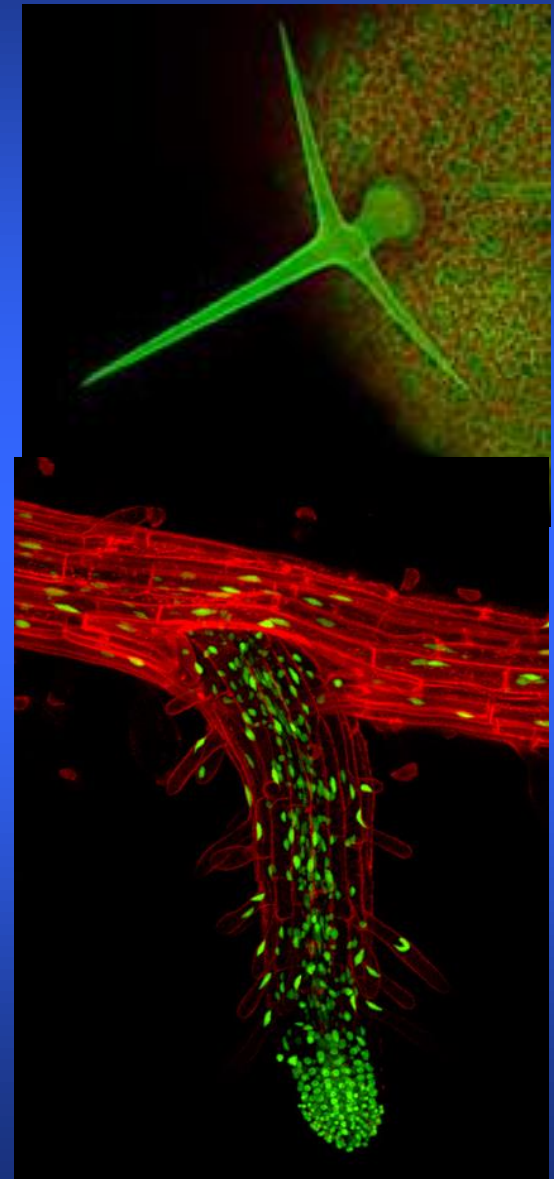
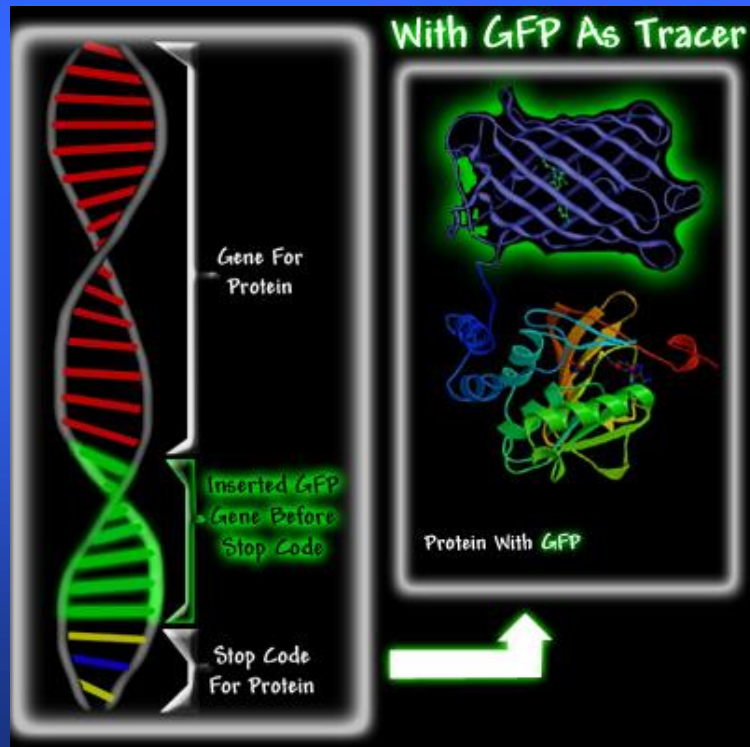
GFP = „*green fluorescence protein*“

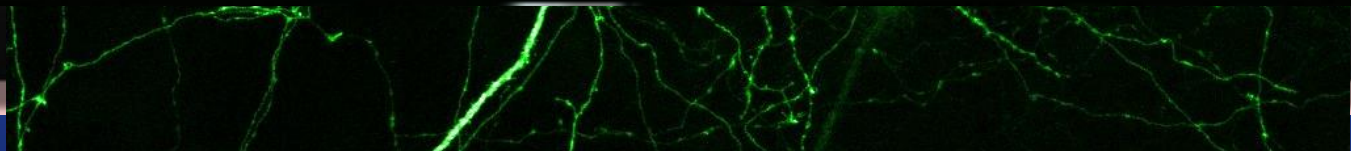
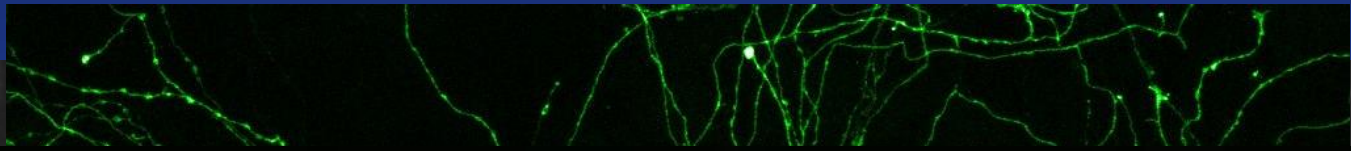
GFP is fused with a studied protein, which enables observation of its localisation and movement in living cells.

Fusions with GFP enables observation of localisation and movement of proteins (protein structures) in living cells



Protein with an unknown localisation Protein + GFP



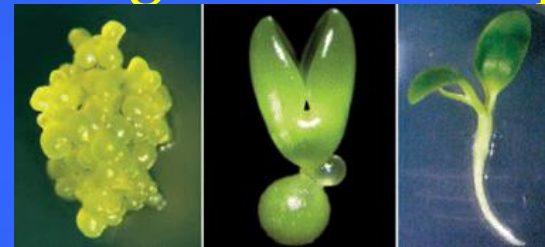


Introduction to formation of transgenic plants

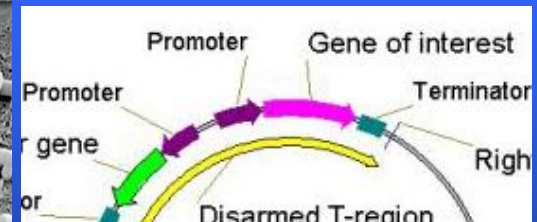
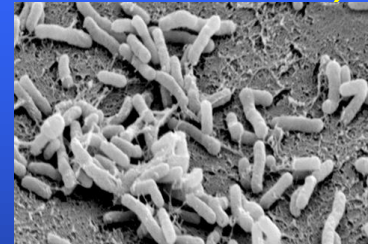
- DNA, gene, genetic code, gene expression



- Totipotence of plant cells, *in vitro* regeneration of plants

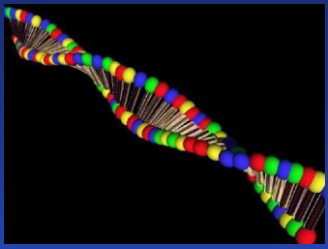


- How to insert new genes (and new features)

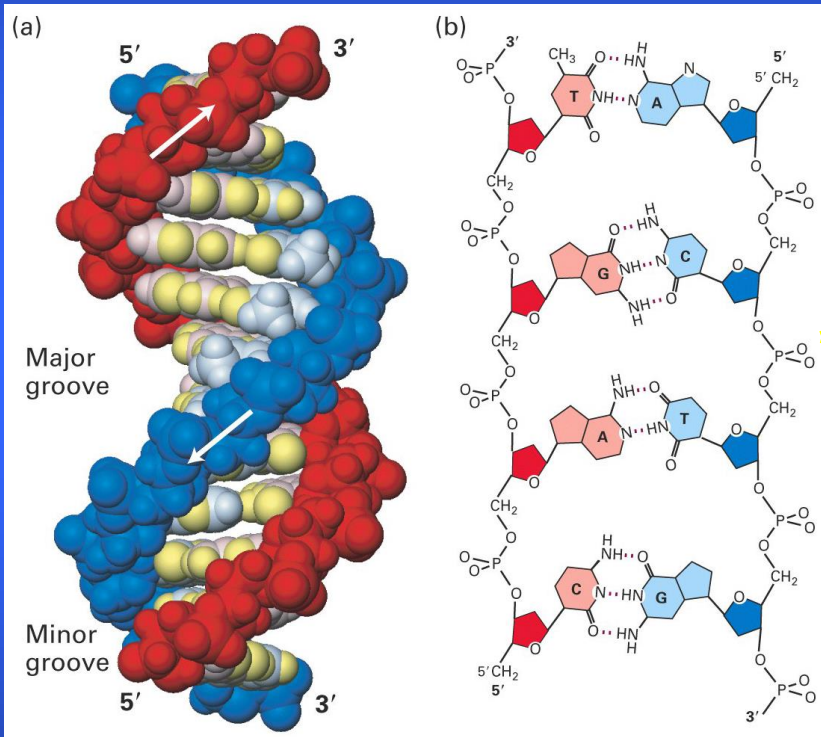
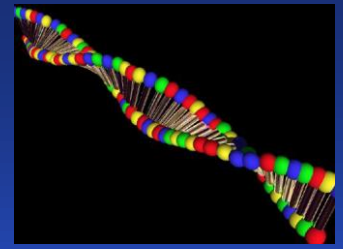


- Transgenic plants in the research and biotechnologies





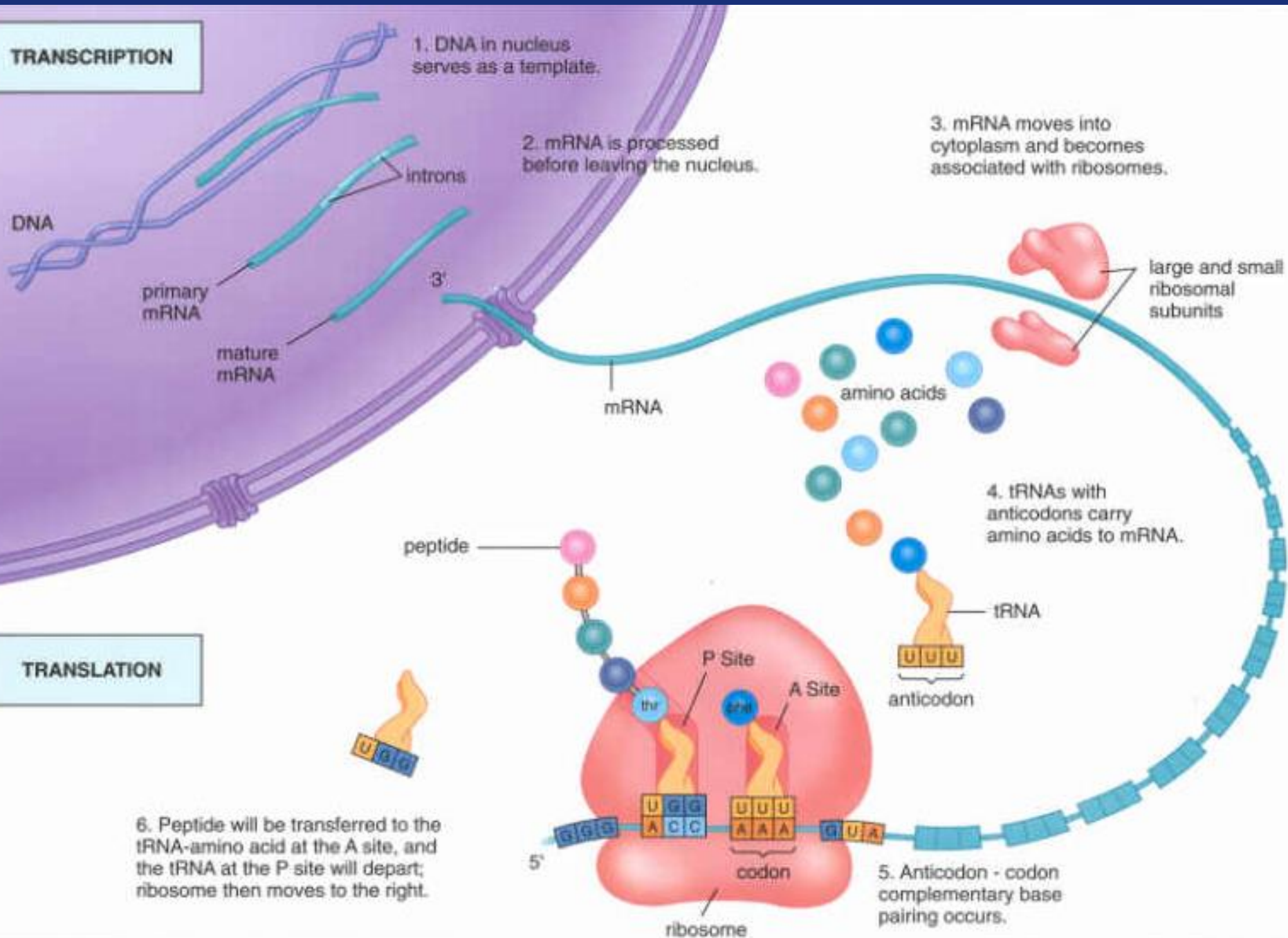
DNA in plant cells



DNA in nuclei
DNA in plastids
DNA in mitochondria

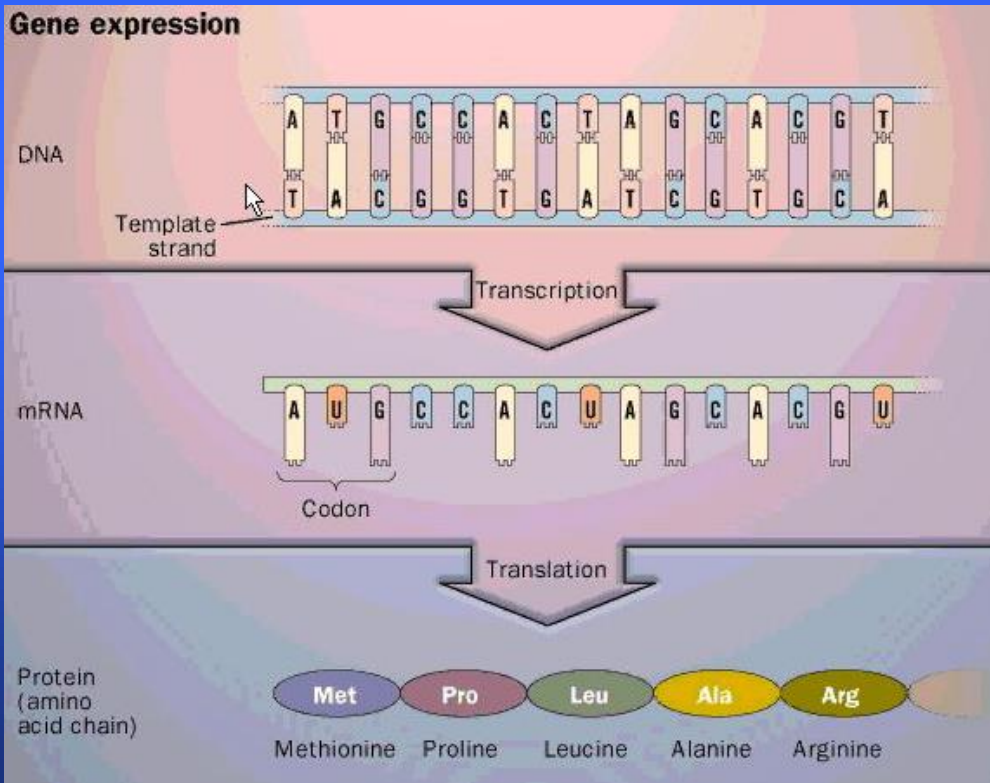
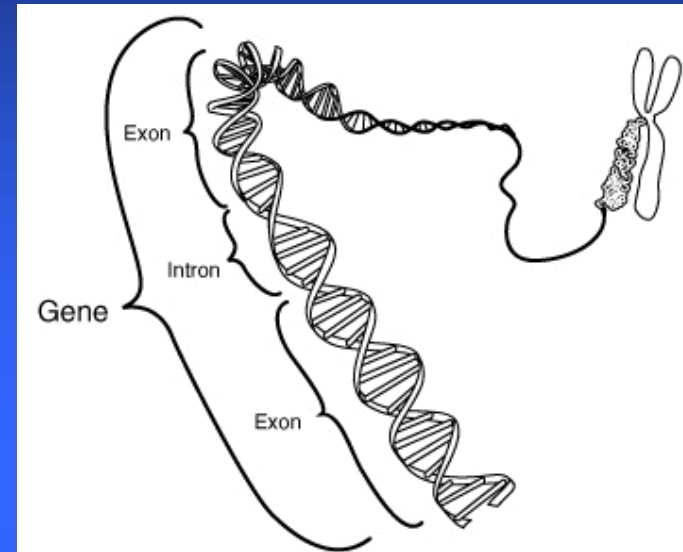
(chromosomal, nucleic DNA)
 (plastid, chloroplast cpDNA)
 (mitochondrion mtDNA)

TRANSCRIPTION



From genes to proteins... and from proteins to phenotypes

- Gene is a part of DNA coding a specific product
- Genes are coding: proteins
t-RNA
r-RNA, etc...



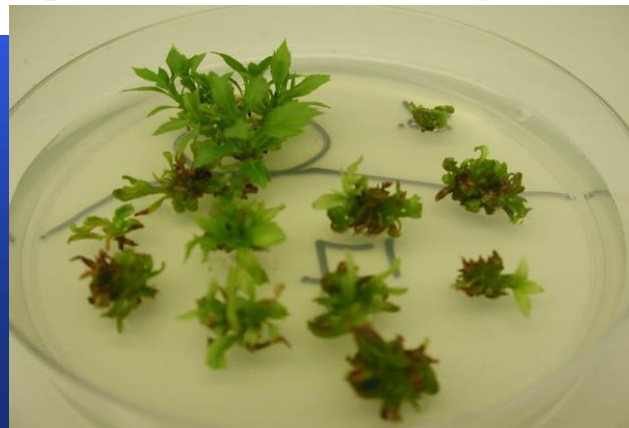
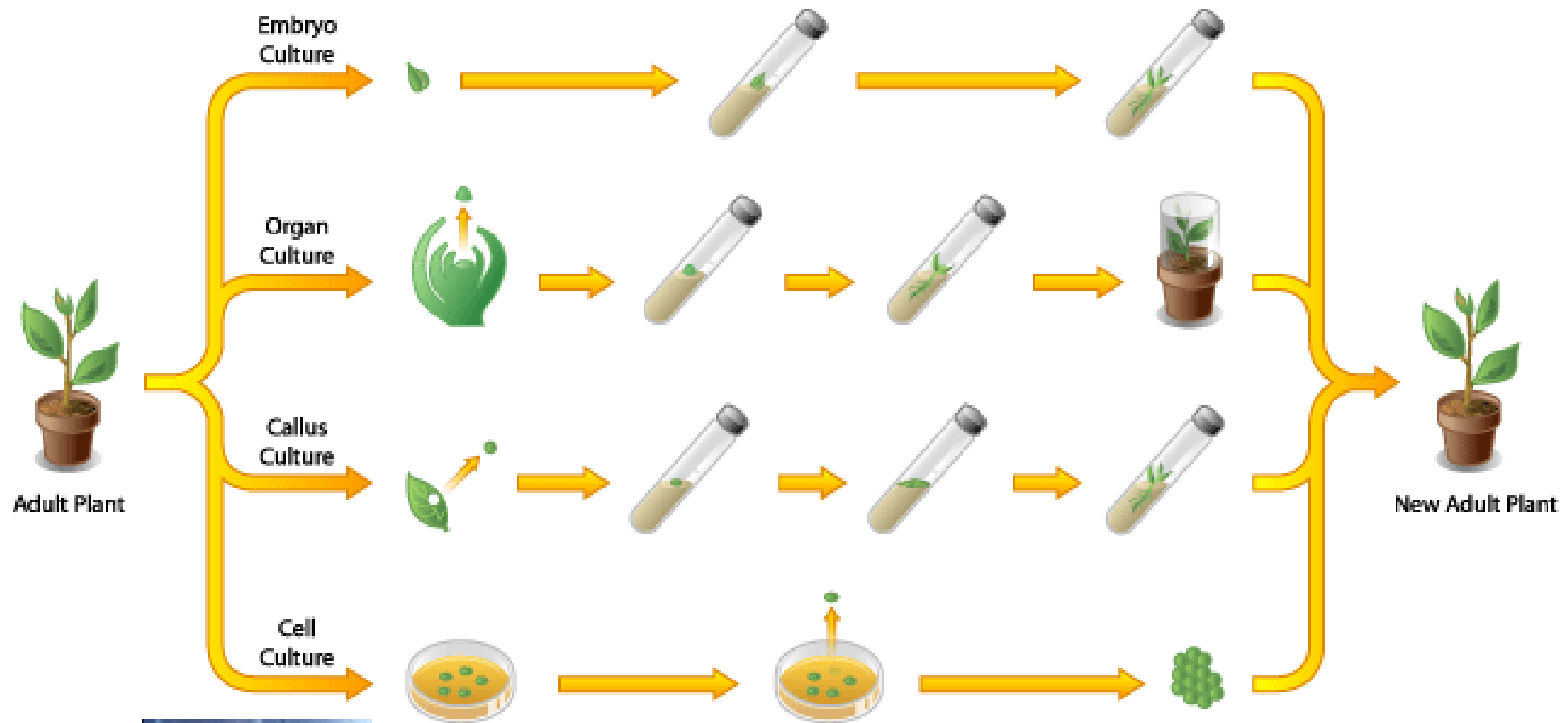
GENE → **PROTEIN (enzyme)**

ENZYME → **PIGMENT BIOSYNTHESIS**

GENE → **FLOWER COLOUR**

- Genetically modified plants possess specific modification in genome (short-time or permanent).
- GM-plants with genomic changes adopt new features or lose some original ones.

Plant tissue cultures and *in vitro* regeneration



From one cell to the whole plant



excised tissue
(explantate)



calus



regenerated
plants



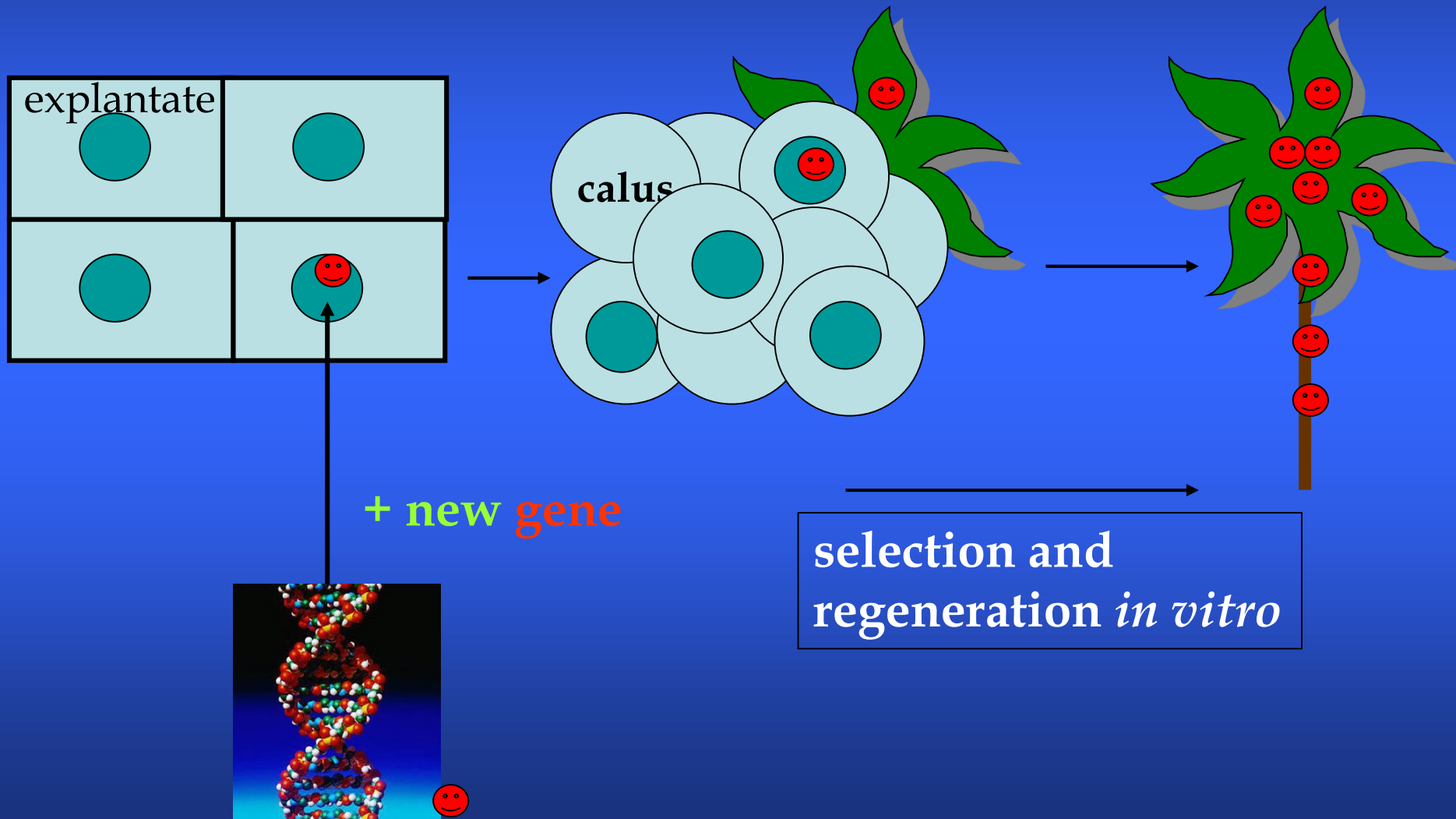
protoplast



somatic embryo



From transformed cell to the transformed plant



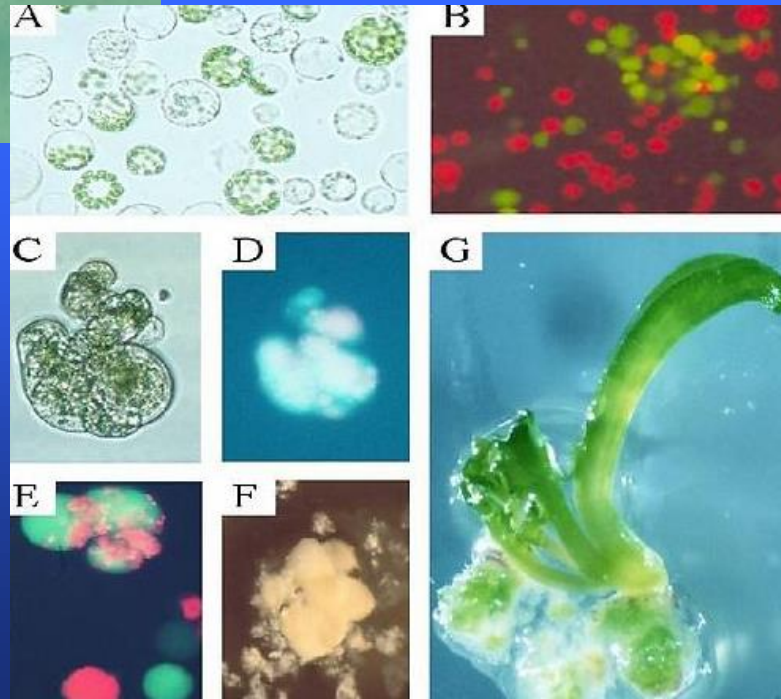
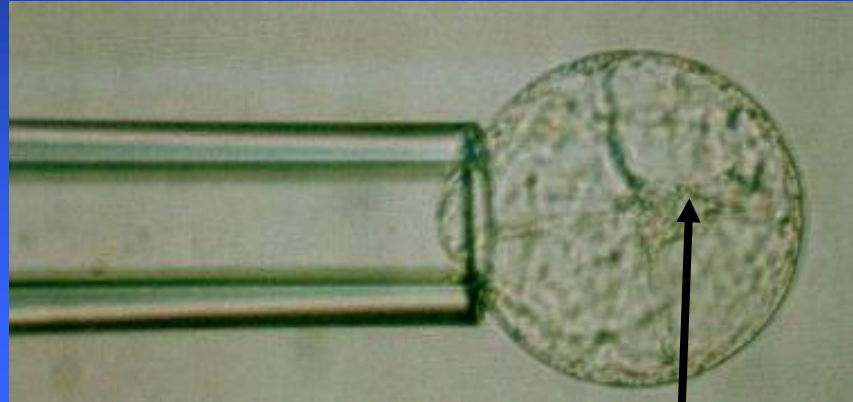
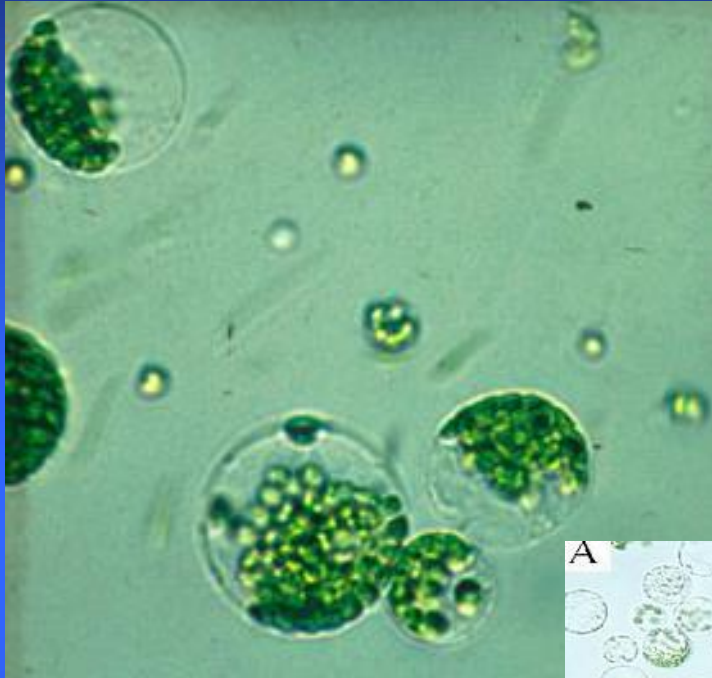
How to insert a new gene into the plant?

Direct DNA transfer into the protoplasts
(*PEG-mediated*, DNA transfection, injection)

DNA shooting (biolistic)
(*gene-gun*)

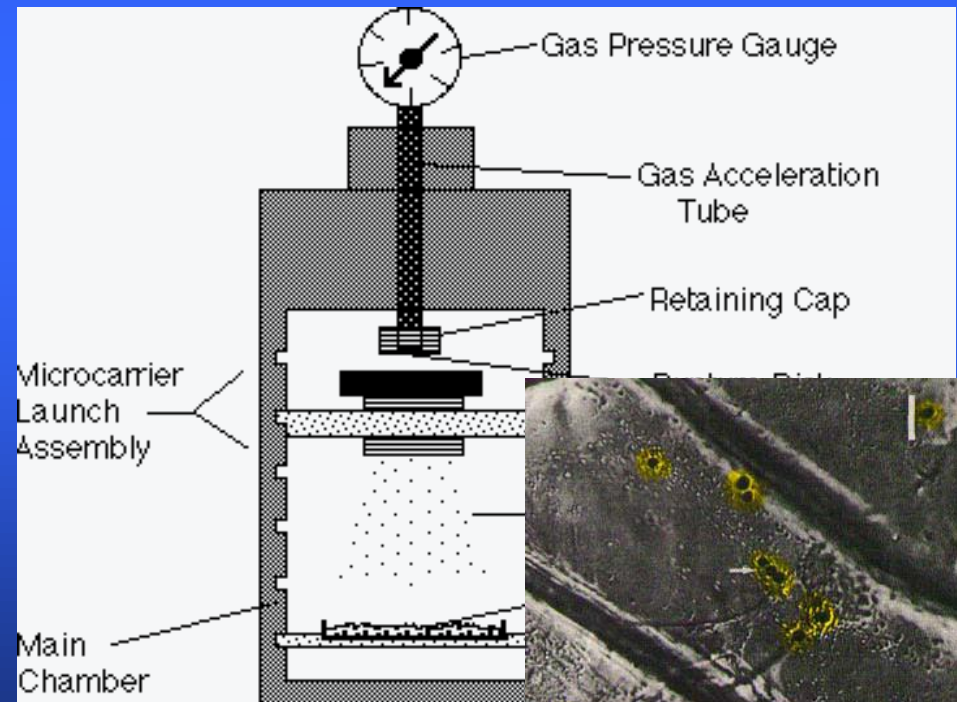
DNA insertion by bacteria
(*Agrobacterium-mediated*)

Direct DNA injection into protoplasts

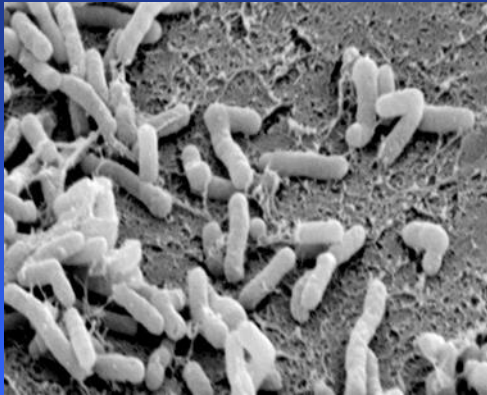


Gene shooting (biolistic)

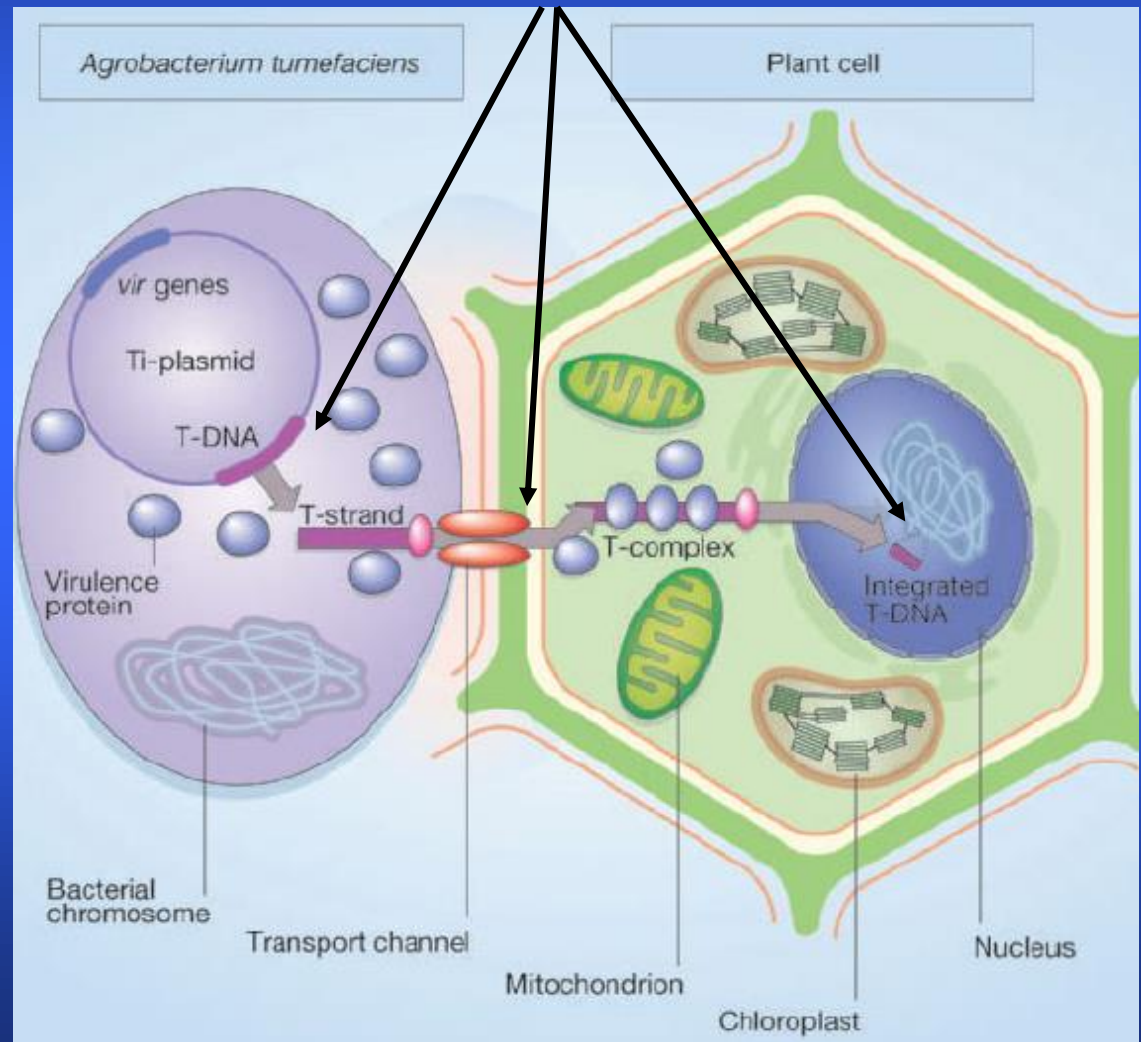
- DNA insert adheres on microprojectiles, that are shot by helium pressure into the plant tissue...



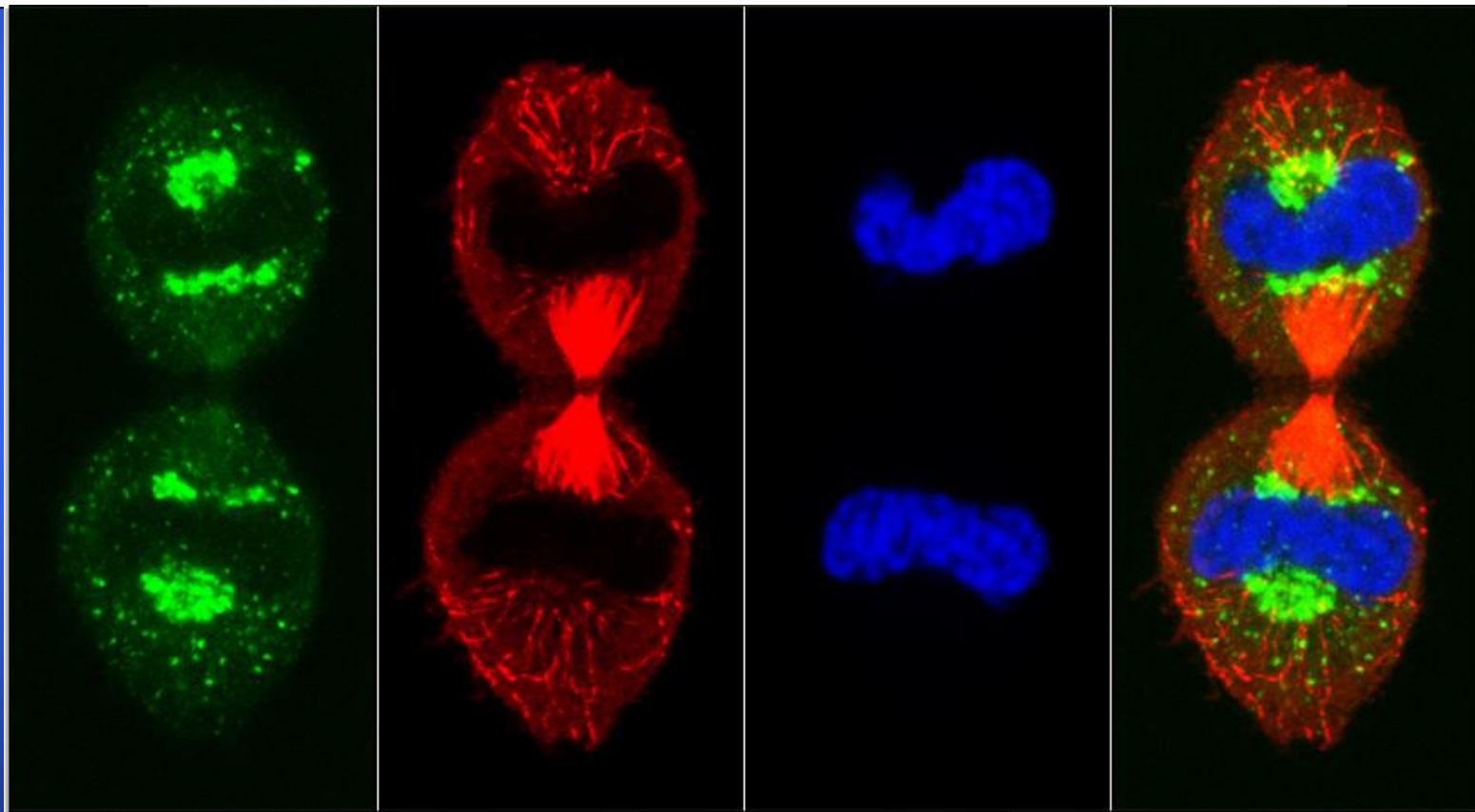
DNA insertion by *Agrobacterium tumefaciens*



- *Agrobacterium* is able to transport and integrate a part of its DNA (T-DNA) into the plant genome



There are know many proteins (molecules), which are present in a specific cell compartment – posses **specific localisation**. Antibodies against those molecules can be used as markers of such compartments / cell components. Instead of the antibodies and the GFP-fussions it is possible to use specific **fluorescence dyes** (e.g. DAPI – forms bound with nucleic acids) as a marker.



marker of
Golgi apparatus

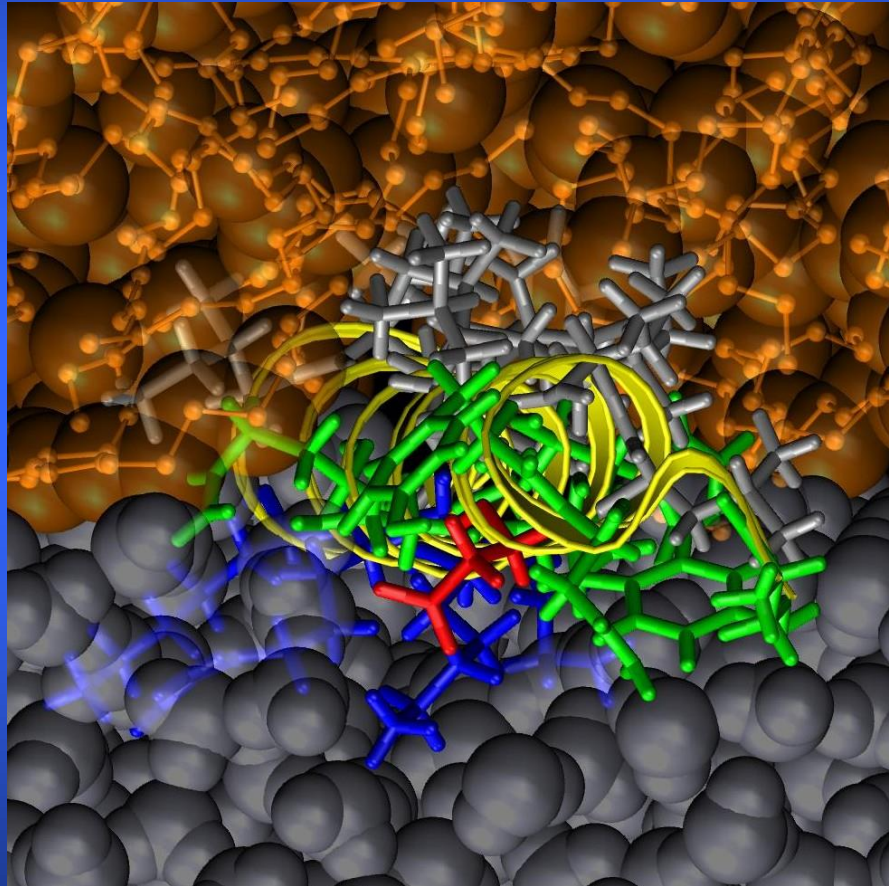
marker of
 β -tubulin

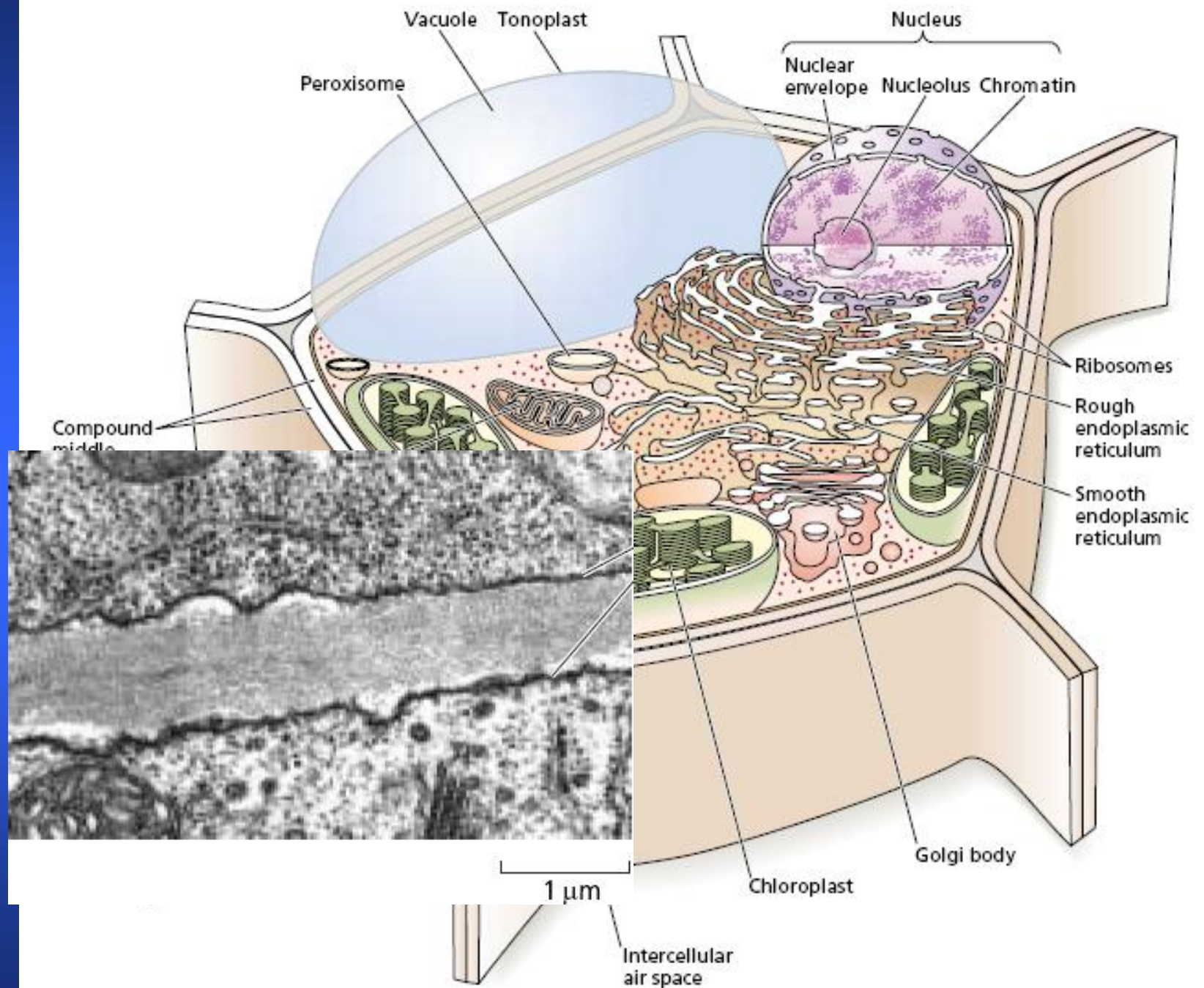
DAPI

Merge signal

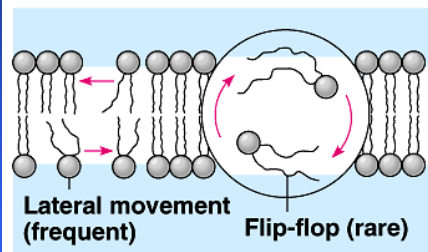
<http://www.nanoflight.info/nanoflight2.html>

BIOMEMBRANES

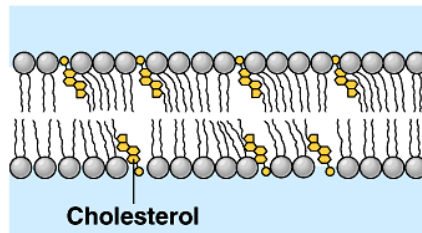
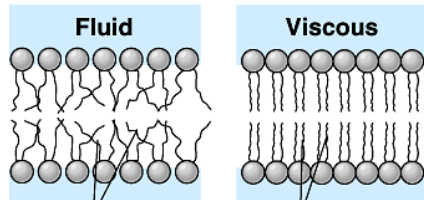




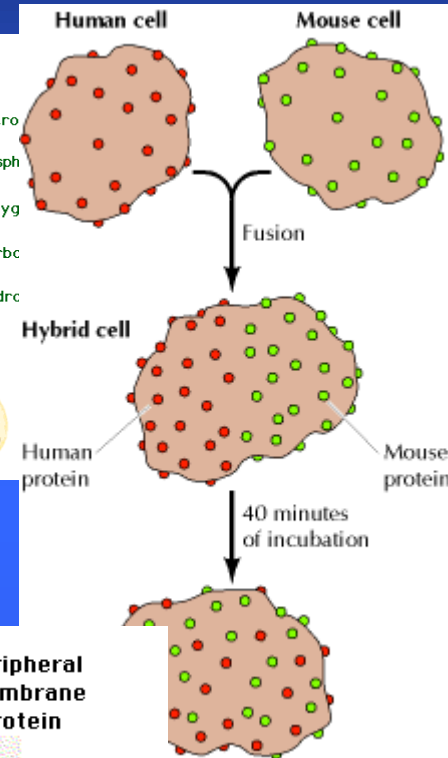
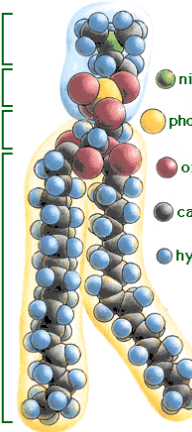
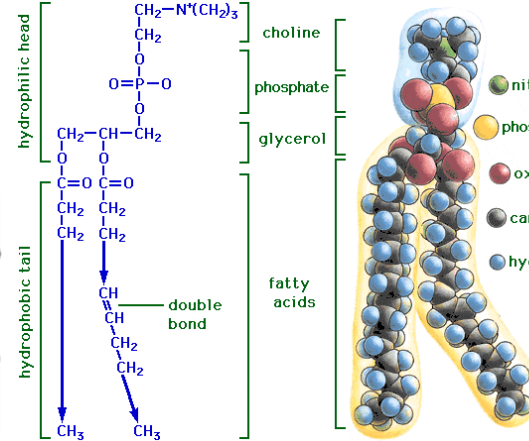
Biomembranes are composed of phospholipid bilayer. Phospholipids are amphipathic – contain both the hydrophilic and hydrophobic part.



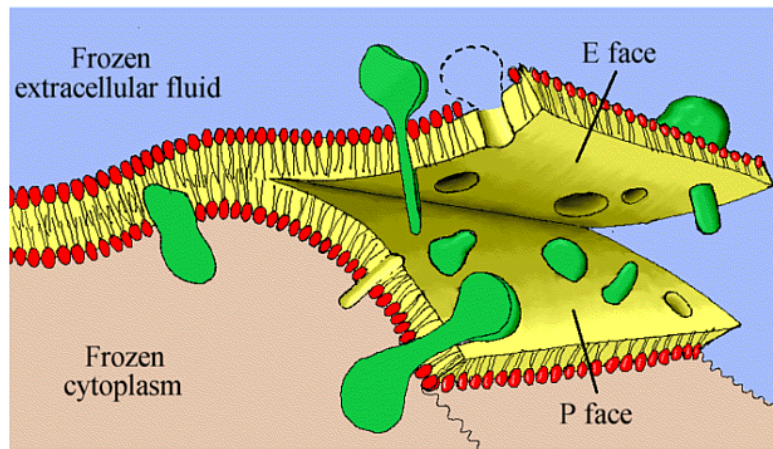
(a) Movement of phospholipids



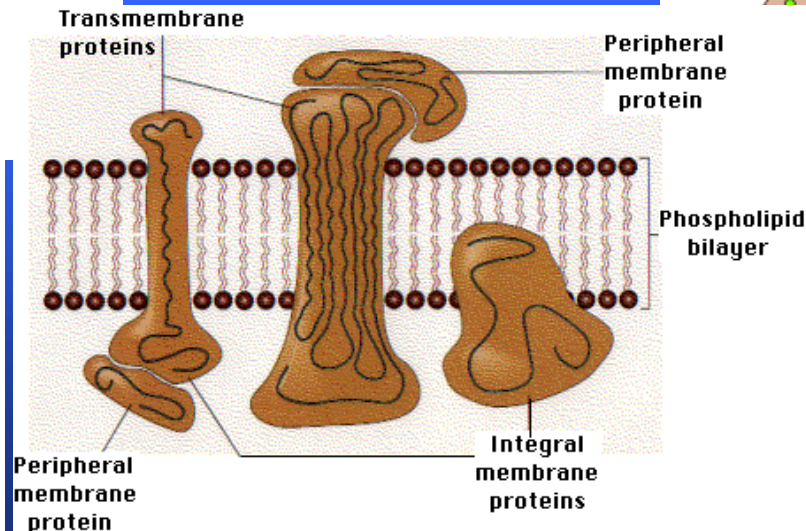
(c) Cholesterol within the membrane



Freeze-fracture of PM*

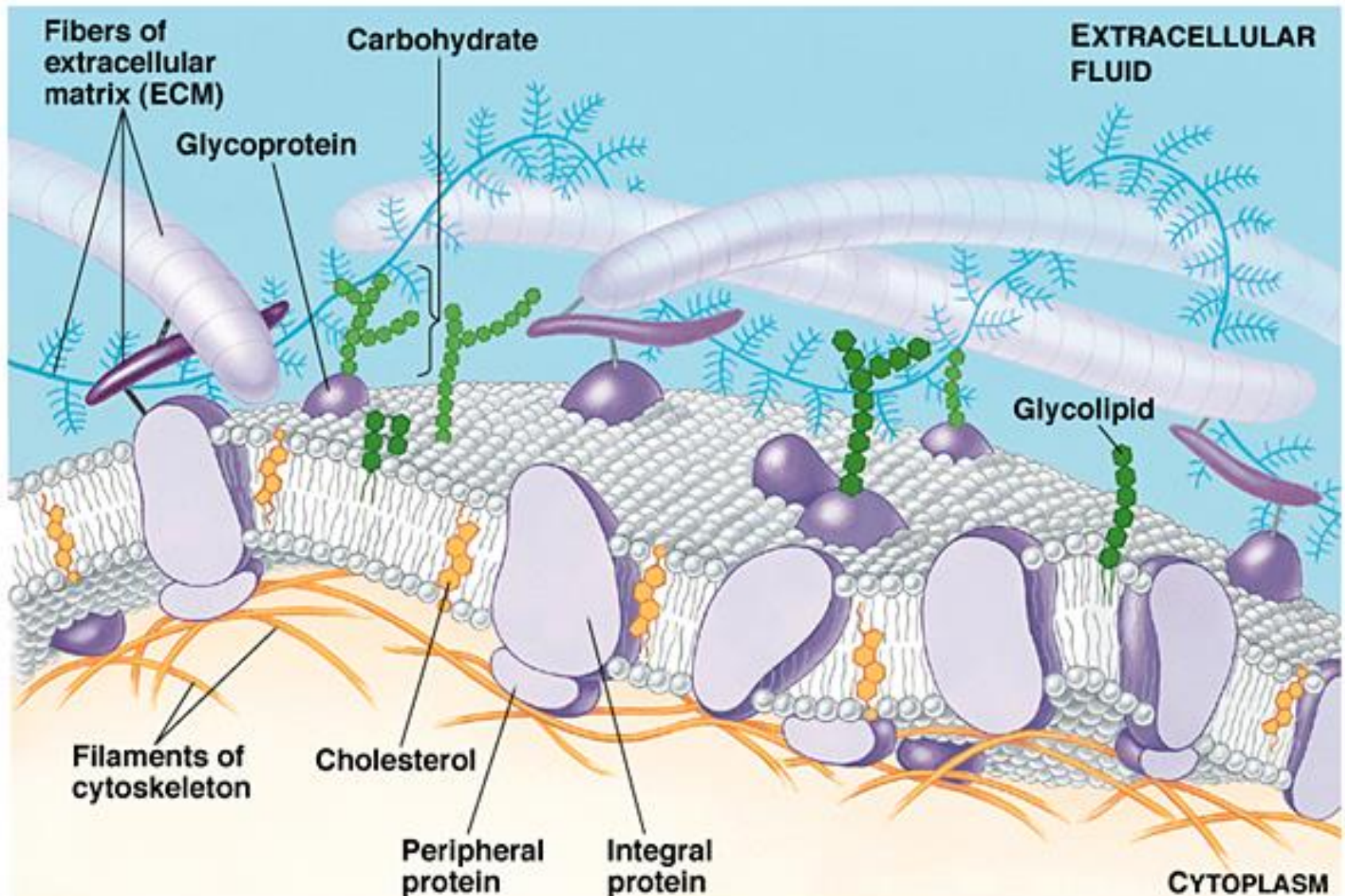


* Final product of FF is visualized as SEM of P/E face platinum shadows.



Up to date model of biological membrane

Model of fluid mosaic



Basic characteristics of bio-membranes:

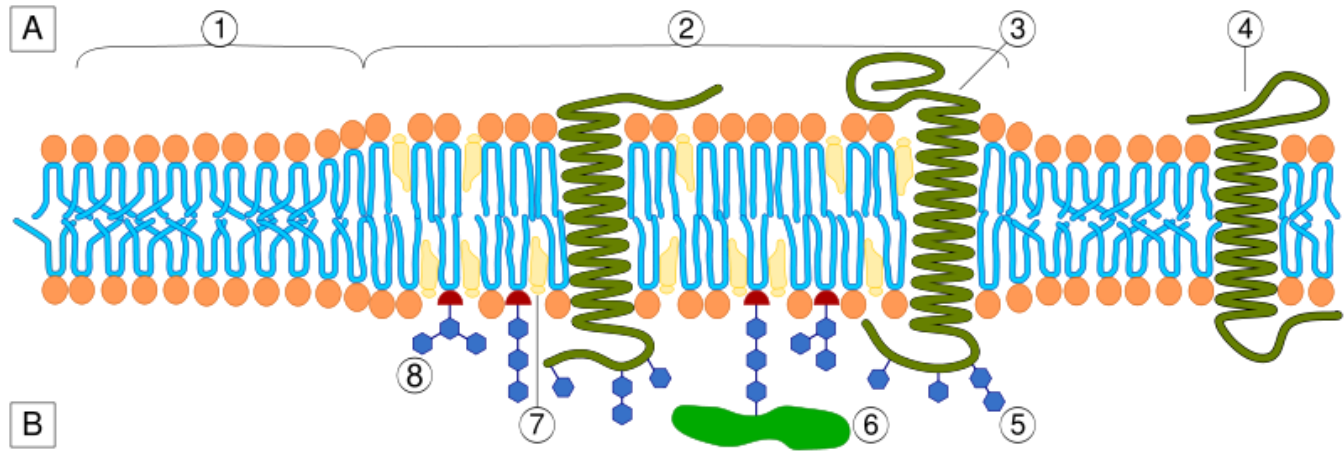
Membrane fluidity is temperature-dependent, but it depends also on the number of un-saturated bounds in hydrophobic part (fatty acids). The more of un-saturated bounds are the more fluid the membrane is.

Membrane fluidity depends on the amount of cholesterol, which is present in the membranes.

Membranes contain **proteins**: peripheral, integral (non-covalently bound and, interfering with the hydrophobic core of the lipid bilayer or passing through the whole lipid bilayer) and bind covalently to the membrane via any other molecule (anchored proteins).

Glycoproteins (contain molecules of saccharides) are formed in the cell ER and GA and into the membrane are transported by secretory pathway.

Membrane proteins fulfill various functions in the cell: transporters, channels, enzymes, receptors, mediate contact with cytoskeleton, possess signal function, maintain cell identity...

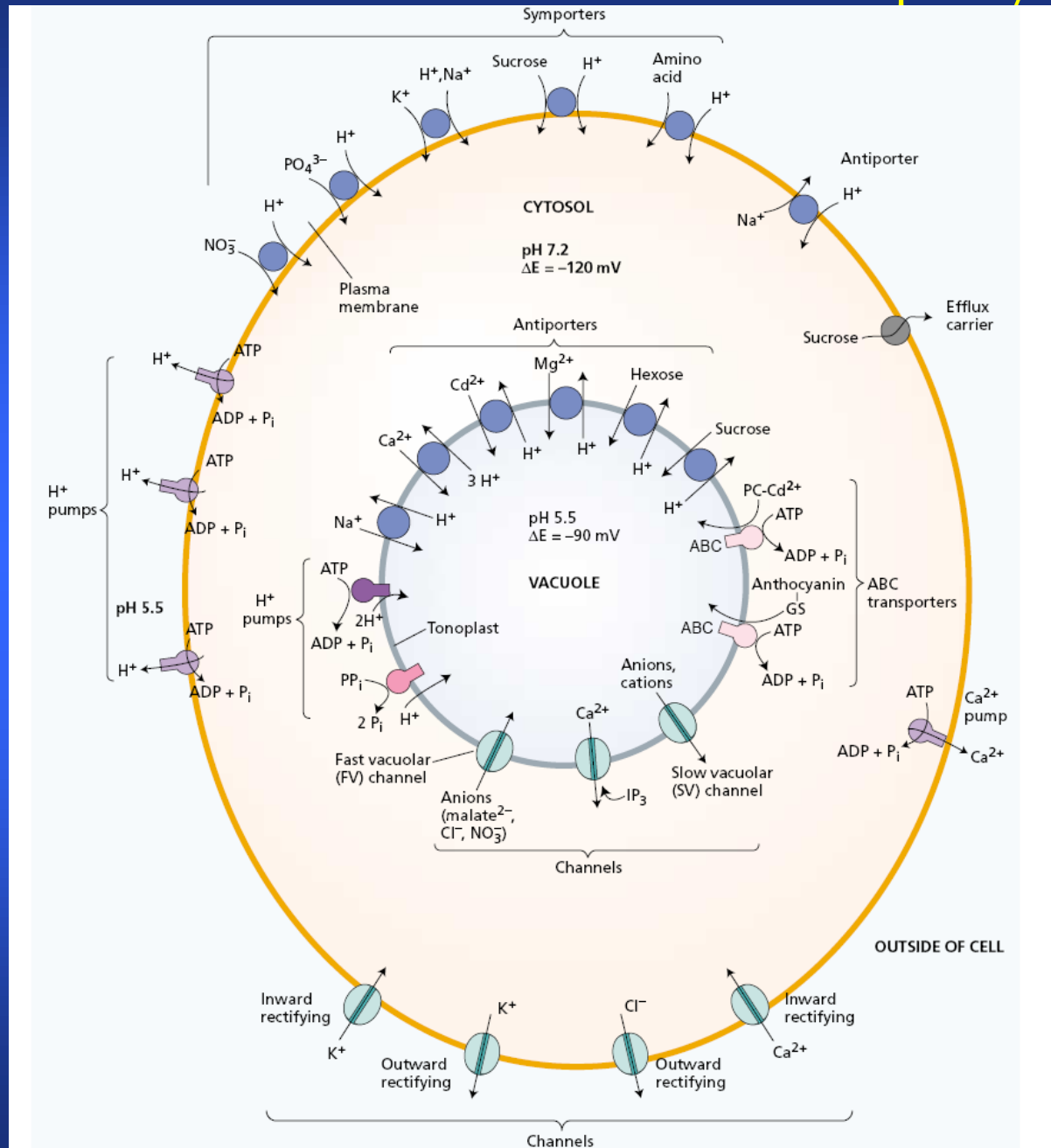


Membranes form specific functional areas (theory of **membrane rafts**).

Membrane features

- Mechanical support of cell and its components
- Substance exchange between the membrane separated sites
- Semipermeability – selective transparency
- Compartmentalisation – topological boundaries
- Surface increase
- Energy transformation
- Signalisation
- Communication
- Morphogenesis

Cytoplasmic membrane and substance import / export



Endocytosis – pinocytosis, fagocytosis, receptor-mediated endocytosis

