•Eukaryotic cells are about 1000 times larger than bacteria cells and also have a membrane enclosed nucleus containing their DNA, and several other internal structures known as organelles.

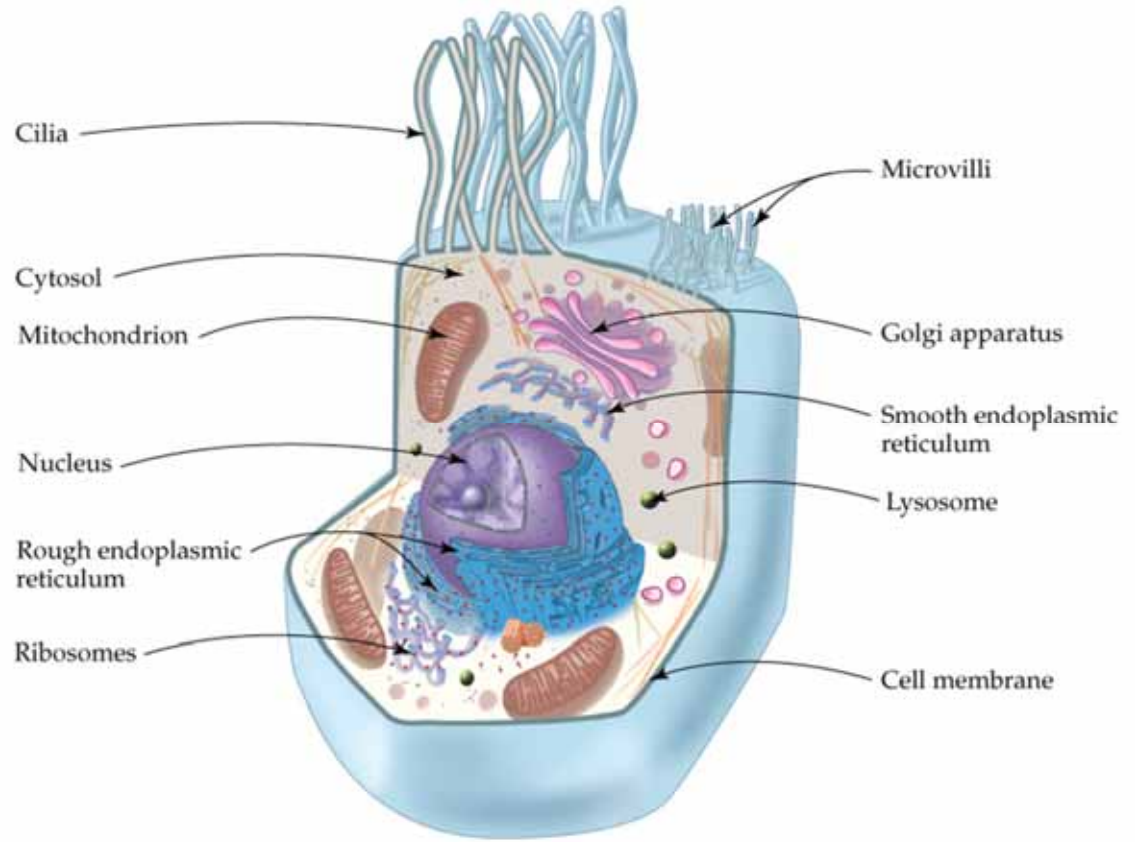
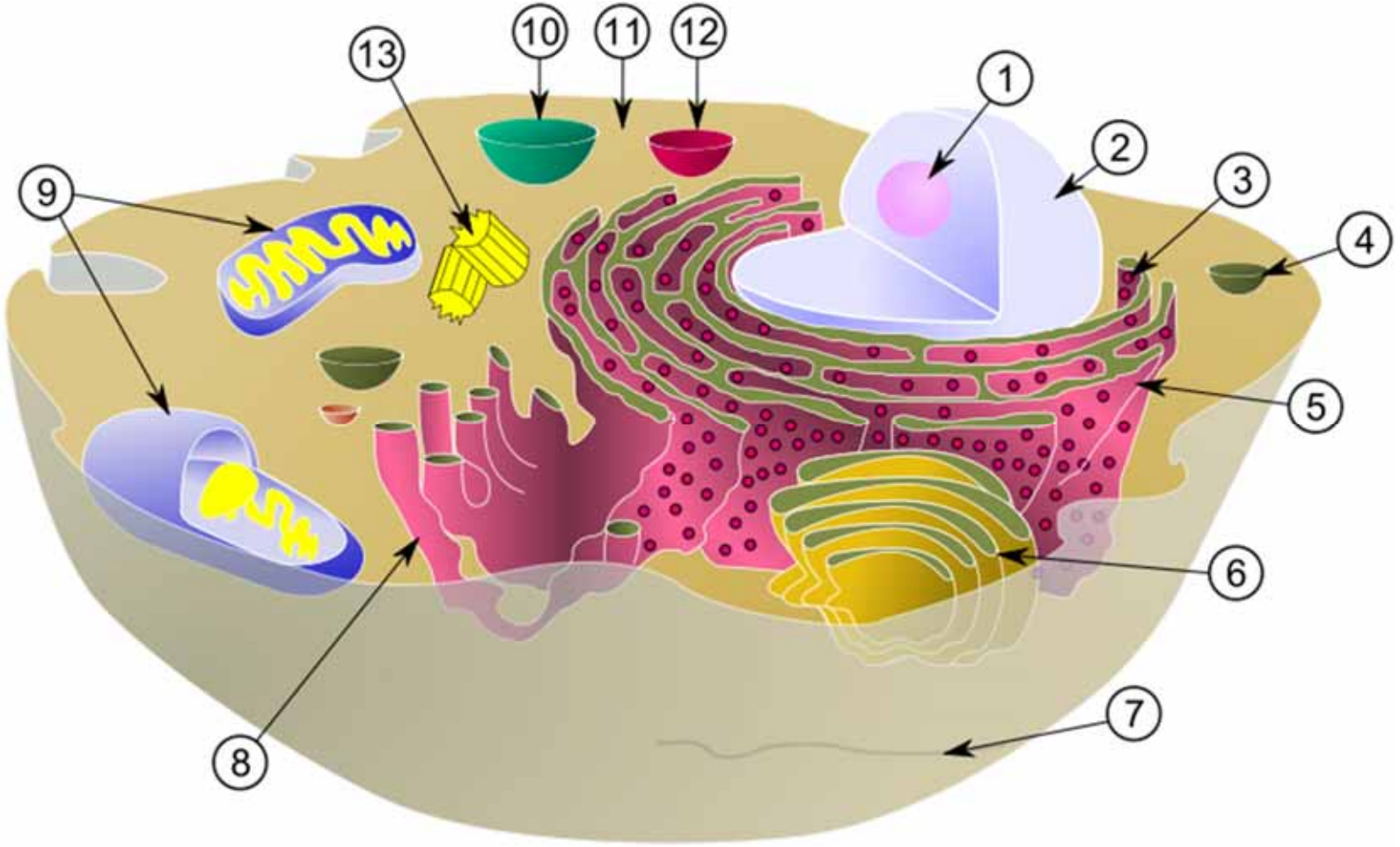
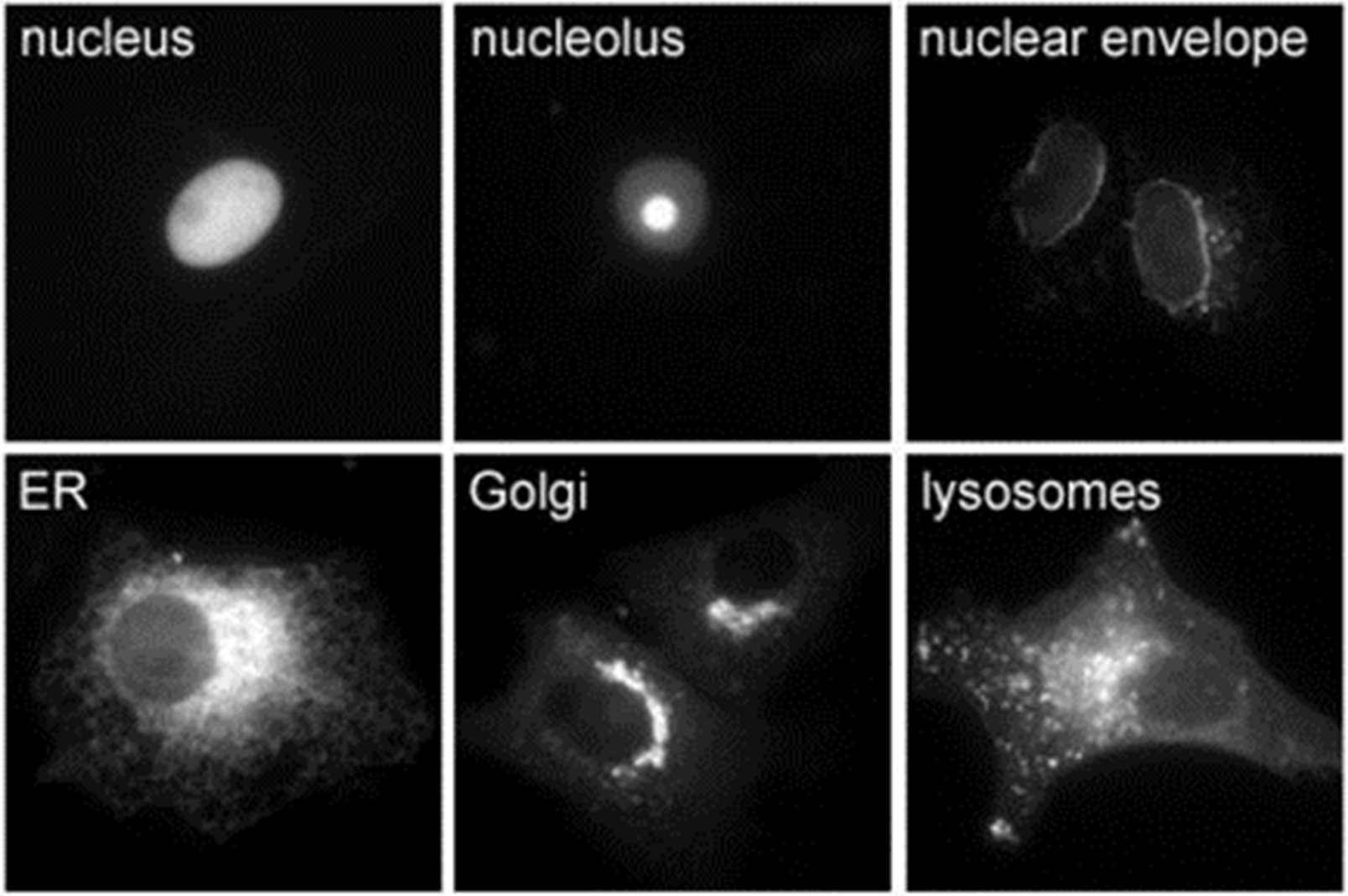
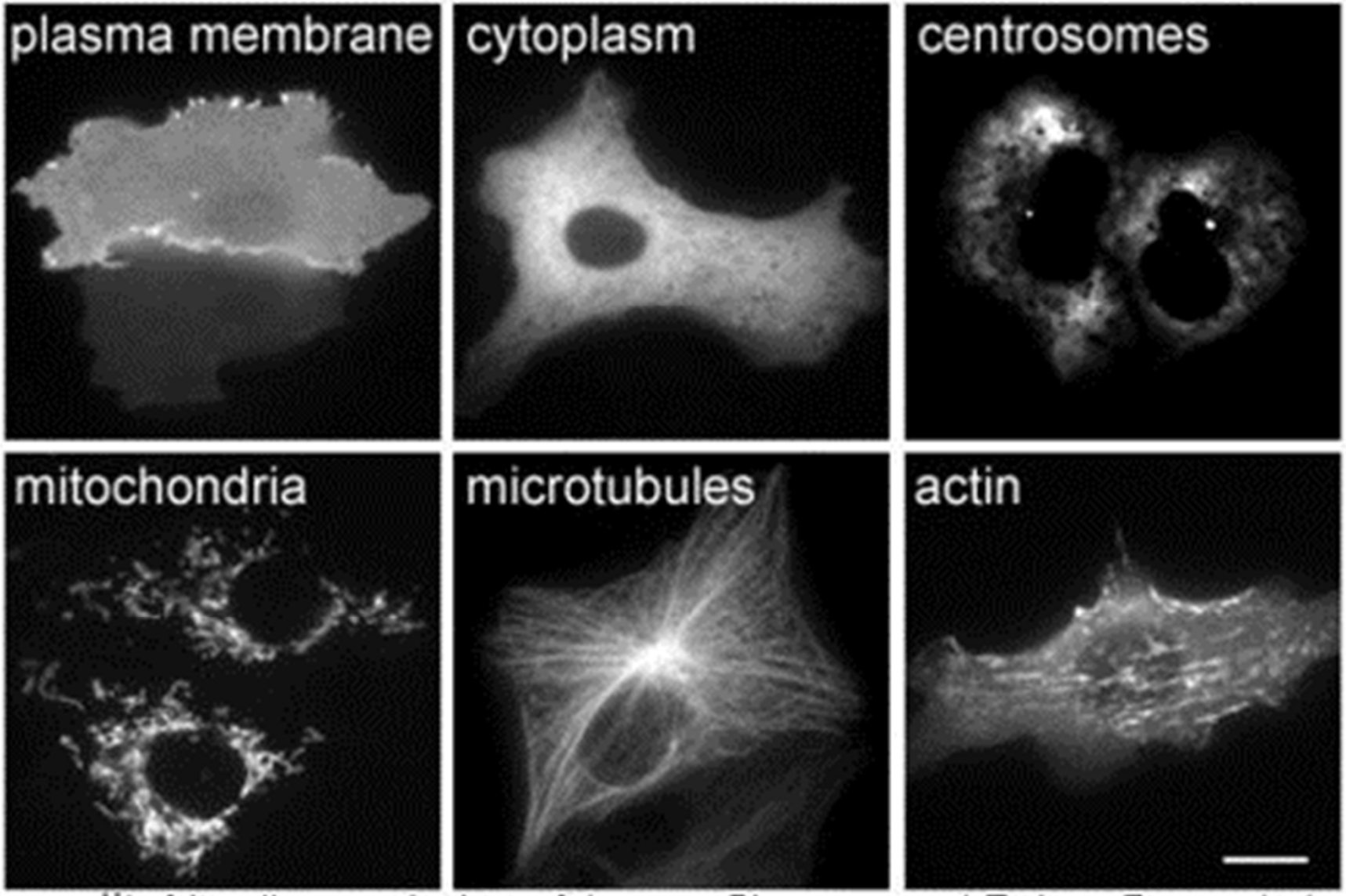



Fig 21.3 A generalized eukaryotic cell.



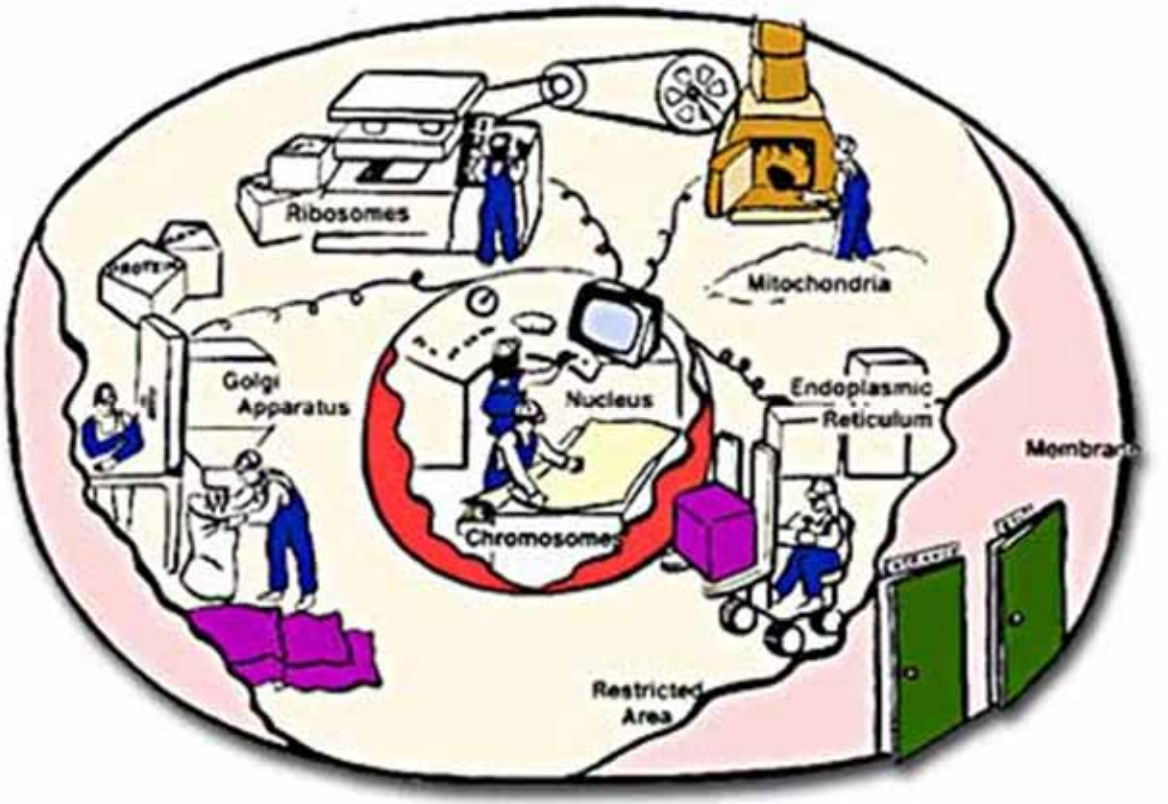

•Schematic showing the cytoplasm, with its components (or *organelles*), of a typical animal cell. Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (6) Golgi apparatus (7) cytoskeleton (8) smooth endoplasmic reticulum (9) mitochondria (10) vacuole (11) cytosol (12) lysosome (13) centriole.


nucleus
nucleolus
nuclear envelope
ER
Golgi
lysosomes



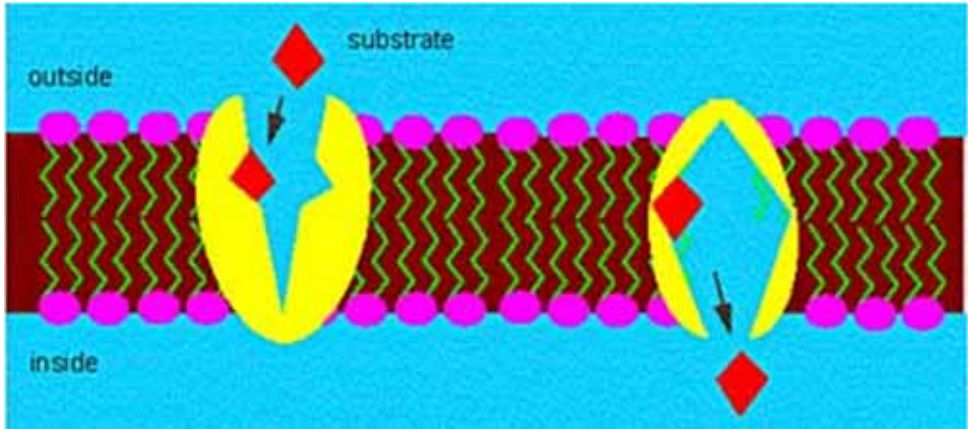

with friendly permission of Jeremy Simpson and Rainer Pepperkok

# A Busy Factory

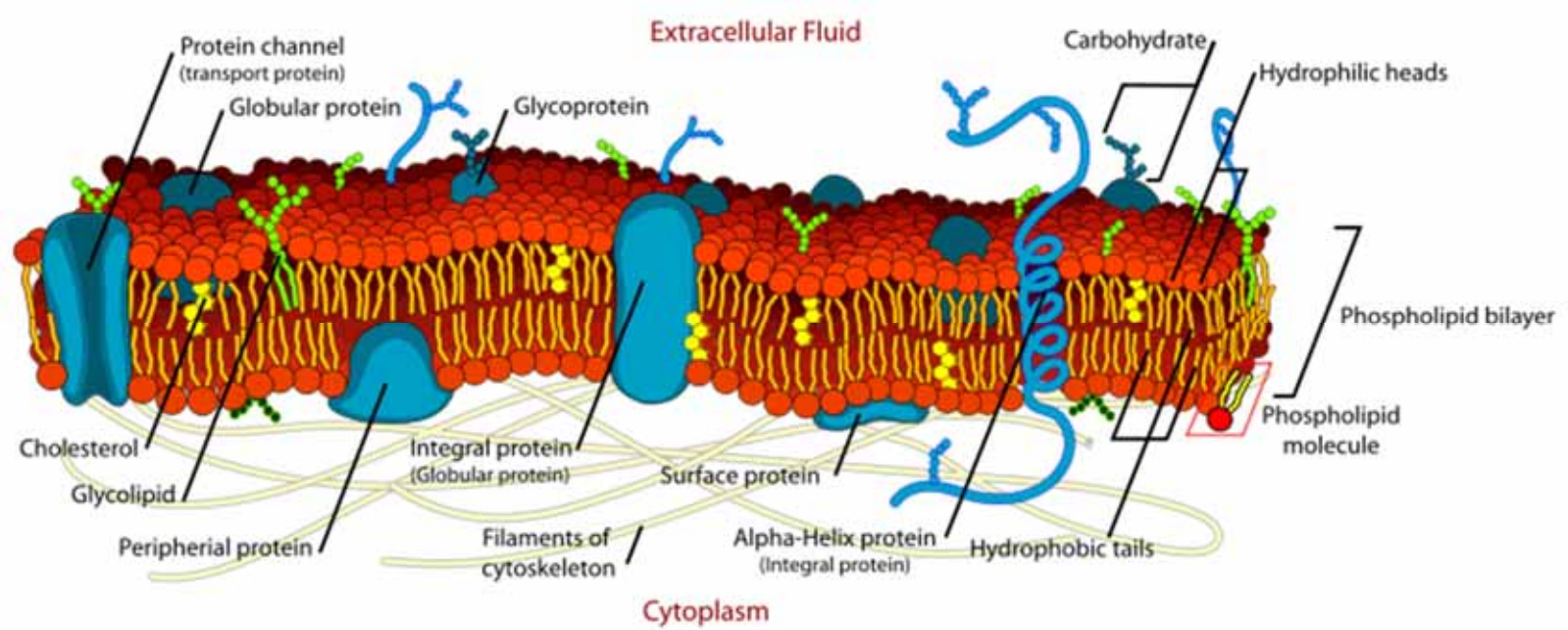



A cell can be thought of as a "factory," with different departments each performing specialized tasks.

# The Plasma Membrane



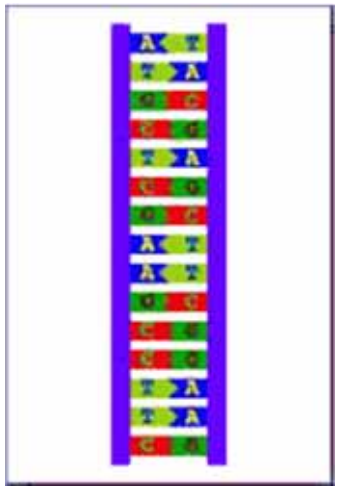
# Cell Membrane




Characteristic diffusivities

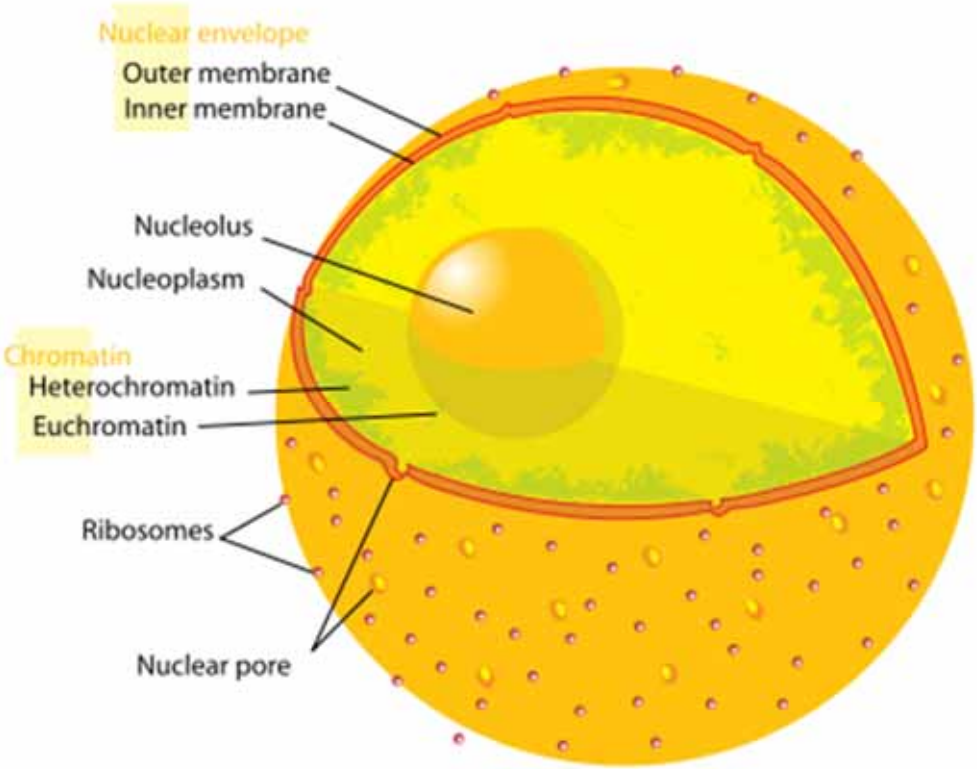
| Particle | Typical size | Diffusion constant |
|---|---|---|
| Solute ion | $10^{-1}$ nm | $2 \times 10^3$ $\mu m^2/s$ |
| Small protein | 5 nm | 40 $\mu m^2/s$ |
| Virus | 100 nm | 2 $\mu m^2/s$ |
| Bacterium | 1 $\mu m$ | 0.2 $\mu m^2/s$ |
| Mammalian/human cell | 10 $\mu m$ | 0.02 $\mu m^2/s$ |

# The Nucleus



The cell factory contains a large inventory of blueprints dating all the way to its founding. Some of these blueprints are out of date, and some are for parts and products that are no longer made. Part of your job would entail sorting through everything, finding the correct blueprints, copying them, and sending the copies out to the assembly line at the correct time.

# Nucleus

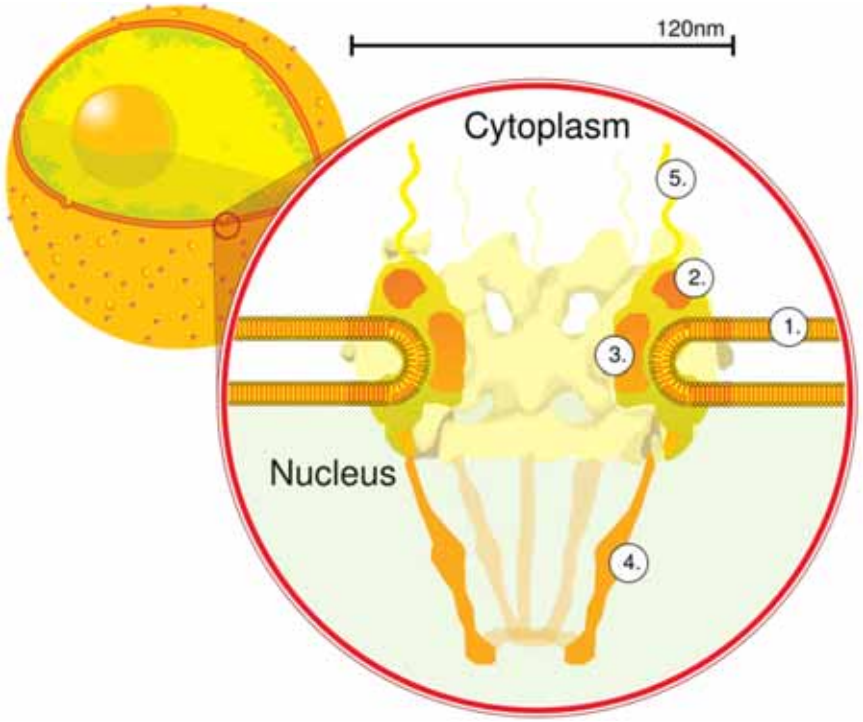



•In cell biology, the **nucleus** is a membrane-enclosed organelle found in most eukaryotic cells. It contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins such as histones to form chromosomes. The genes within these chromosomes make up the cell's nuclear genome. The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression.

In cell biology, the **nucleolus** (plural *nucleoli*) is a "sub-organelle" of the cell nucleus, which itself is an organelle. A main function of the nucleolus is the production and assembly of ribosome components

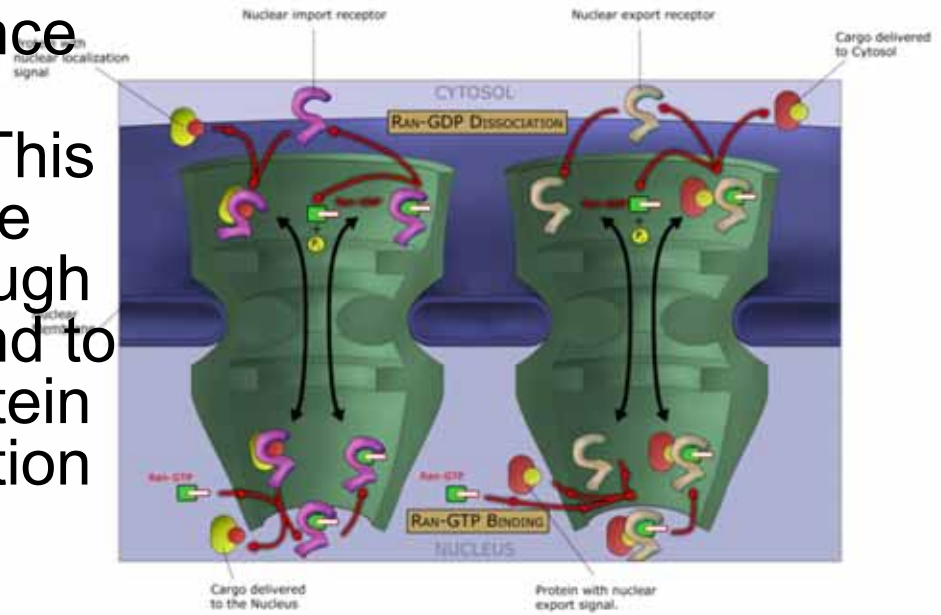
# Nuclear pores

Nuclear pores, which provide aqueous channels through the envelope, are composed of multiple proteins, collectively referred to as nucleoporins. The pores are 100 nm in total diameter; however, the gap through which molecules freely diffuse is only about 9 nm wide, due to the presence of regulatory systems within the center of the pore. This size allows the free passage of small water-soluble molecules while preventing larger molecules, such as nucleic acids and proteins, from inappropriately entering or exiting the nucleus. These large molecules must be actively transported into the nucleus instead. The nucleus of a typical mammalian cell will have about 3000 to 4000 pores throughout its envelope

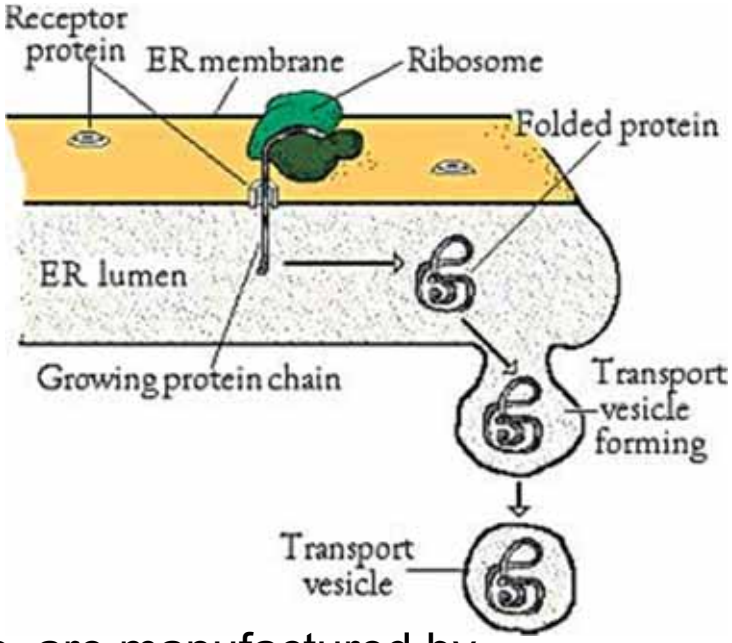


# Nuclear localizing sequence (NLS)

- A **nuclear localizing sequence** (**NLS**) is an amino acid sequence which acts like a 'tag' on the exposed surface of a protein. This sequence is used to confine the protein to the cell nucleus through the **Nuclear Pore Complex** and to direct a newly synthesized protein into the nucleus via its recognition by cytosolic nuclear transport receptors. Typically, this signal consists of a few short sequences of positively charged lysines or arginines. Typically the NLS will have a sequence (NH2)-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-(COOH).



# The Ribosomes and the ER

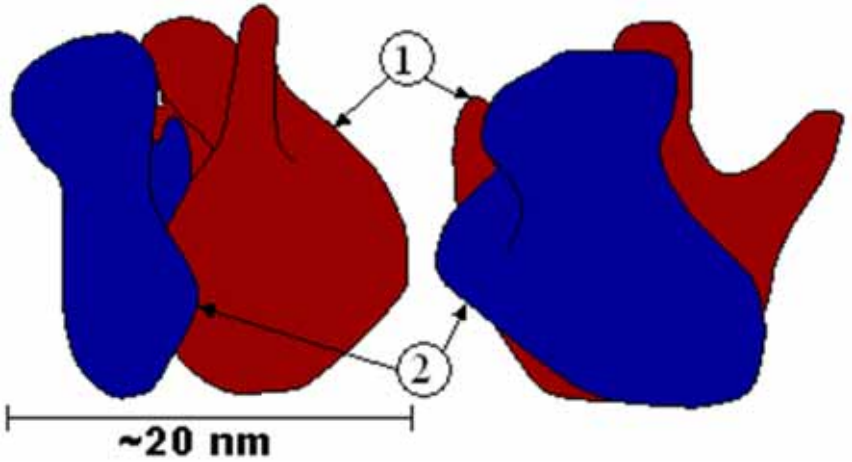



Ribosomes, the workers that build proteins, are manufactured by the nucleolus. They consist of two separate subunits: a large, lower subunit and a small, upper subunit. Ribosomes attach to the rough ER . Now let's take a look at how final processing occurs

The cell has its own assembly line and workers. Within the cytoplasm is a series of large, flattened membranes that fold back and forth on each other and have a very large surface area. This collection of membranes is called the **ENDOPLASMIC RETICULUM**, or**ER**.

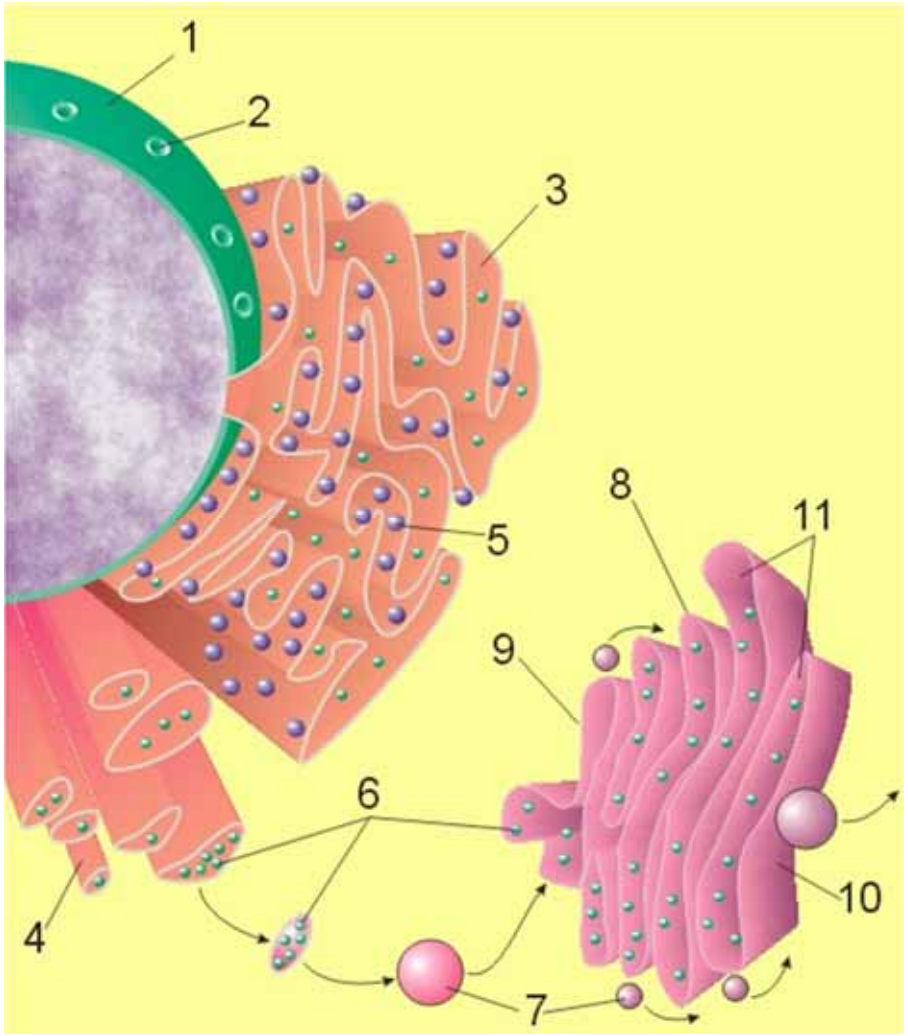
# Ribosome

A **ribosome** is a small, dense organelle in cells that assembles proteins. Ribosomes are about 20nm in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins (known as a Ribonucleoprotein or RNP). It translates messenger RNA (mRNA) to build a polypeptide chain (e.g., a protein) using amino acids delivered by Transfer RNA (tRNA). It can be thought of as a giant enzyme that builds a protein from a set of genetic instructions. Ribosomes can float freely in the cytoplasm (the internal fluid of the cell) or bound to the endoplasmic reticulum, or to the nuclear envelope.



# Endoplasmic Reticulum

The **endoplasmic reticulum** or **ER** is an organelle found in all eukaryotic cells that is an interconnected network of tubules, vesicles and cisternae that is responsible for several specialized functions: Protein translation, folding, and transport of proteins to be used in the cell membrane (e.g., transmembrane receptors and other integral membrane proteins), or to be secreted (exocytosed) from the cell (e.g., digestive enzymes); sequestration of calcium; and production and storage of glycogen, steroids, and other macromolecules.[1] The endoplasmic reticulum is part of the endomembrane system. The basic structure and composition of the ER membrane is similar to the plasma membrane.



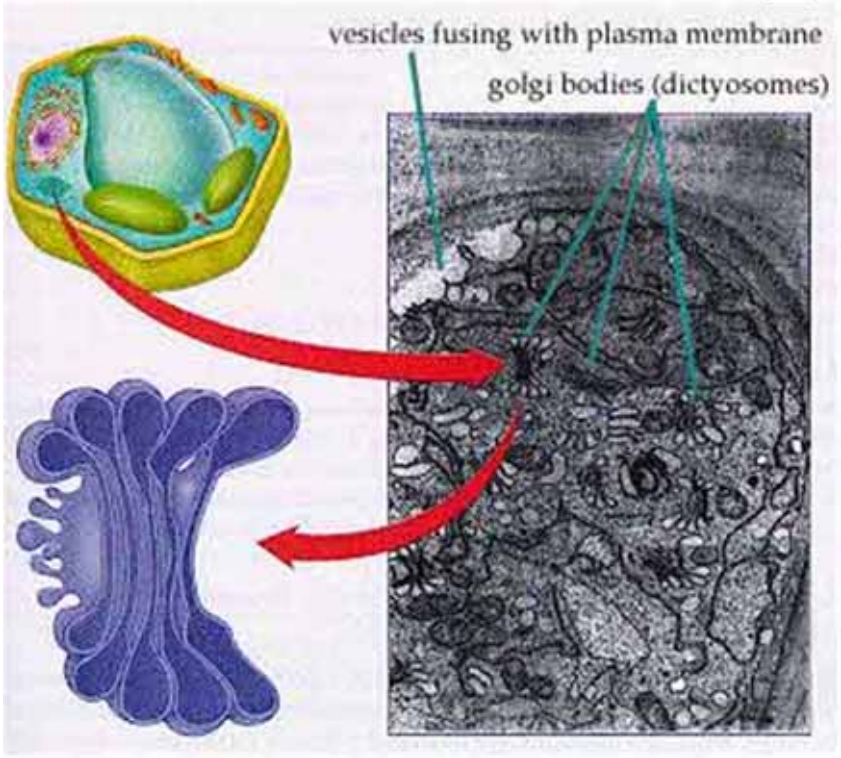
# Rough endoplasmic reticulum

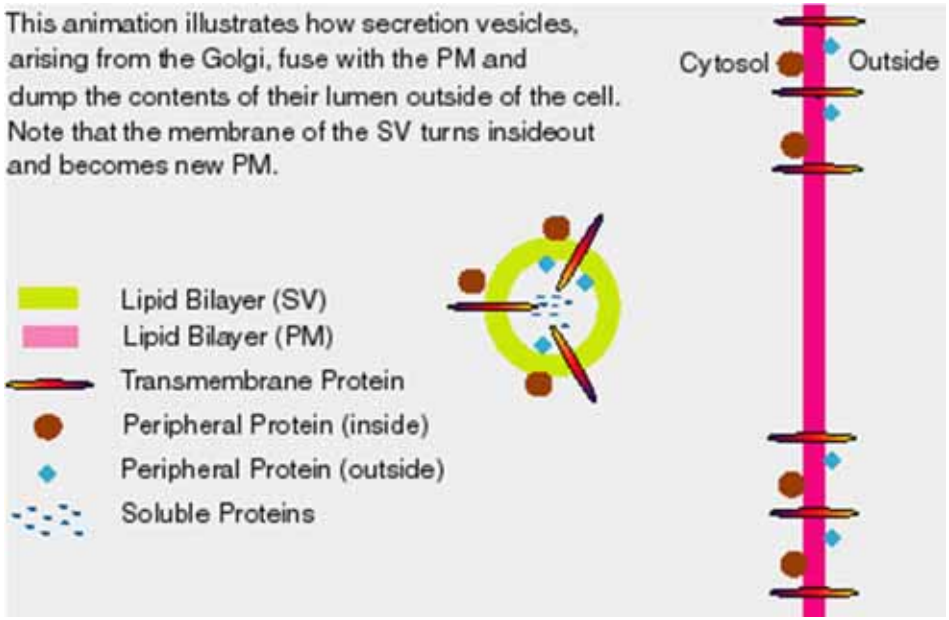
- The surface of the rough endoplasmic reticulum is studded with protein-manufacturing ribosomes giving it a "rough" appearance. But it should be noted that these ribosomes are not resident of the endoplasmic reticulum incessantly. The ribosomes only bind to the ER once it begins to synthesize a protein destined for sorting. The membrane of the rough endoplasmic reticulum is continuous with the outer layer of the nuclear envelope. Although there is no continuous membrane between the rough ER and the Golgi apparatus, membrane bound vesicles shuttle proteins between these two compartments. The rough endoplasmic reticulum works in concert with the Golgi complex to target new proteins to their proper destinations

# Smooth endoplasmic reticulum

- The smooth endoplasmic reticulum has functions in several metabolic processes, including synthesis of lipids, metabolism of carbohydrates and calcium concentration, and attachment of receptors on cell membrane proteins. It is connected to the nuclear envelope. Smooth endoplasmic reticulum is found in a variety of cell types (both animal and plant) and it serves different functions in each. It consists of tubules and vesicles that branch forming a network. In some cells there are dilated areas like the sacs of rough endoplasmic reticulum. The network of smooth endoplasmic reticulum allows increased surface area for the action or storage of key enzymes and the products of these enzymes. The smooth endoplasmic reticulum is known for its storage of calcium ions in muscle cells.

# The Golgi Apparatus

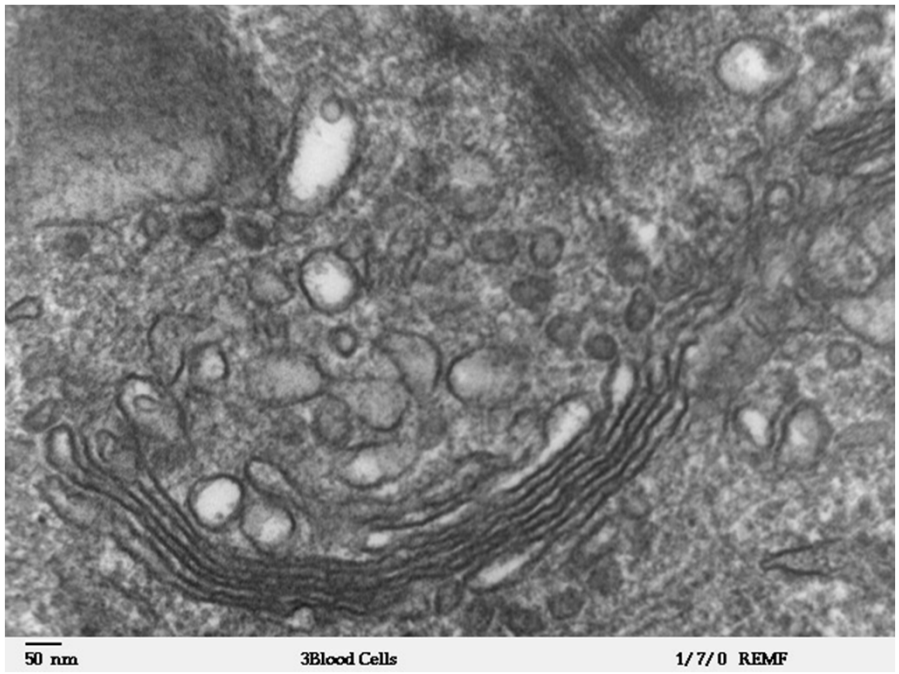


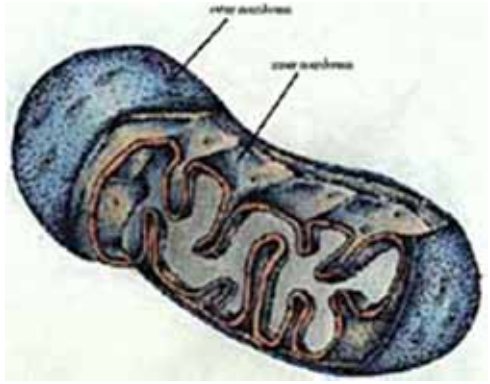

The Golgi apparatus is analogous to the finishing and packing room in a factory. Once the ribosome finishes manufacturing a protein in the rough ER, the protein needs to be prepared for use or export. Special enzymes will trim off any extra amino acids, and then the unfinished protein moves through channels in the smooth ER.

# Golgi apparatus

The **Golgi apparatus** (also called the **Golgi body**, **Golgi complex**, or **dictyosome**) is an organelle found in typical eukaryotic cells. It was identified in 1898 by the Italian physician Camillo Golgi and was named after him. The primary function of the Golgi apparatus is to process and package macromolecules synthesised by the cell, primarily proteins and lipids. The Golgi apparatus forms a part of the endomembrane system present in eukaryotic cells.



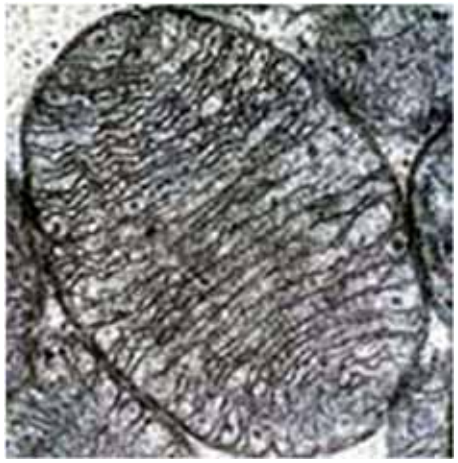
# Mitochondria



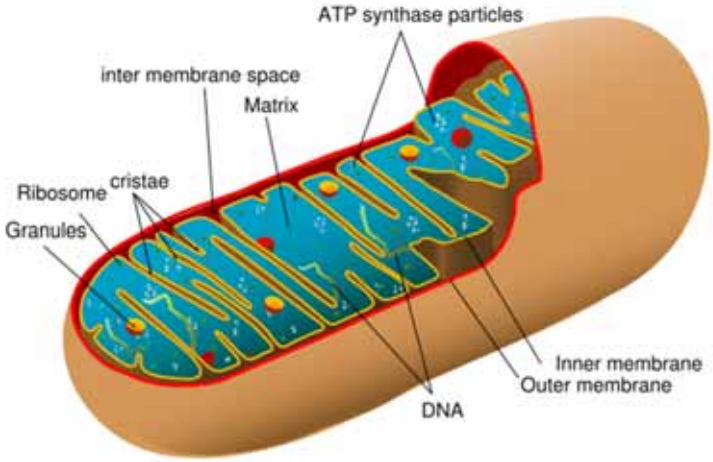
**- ATP -**
**a Source of**
**ENERGY**

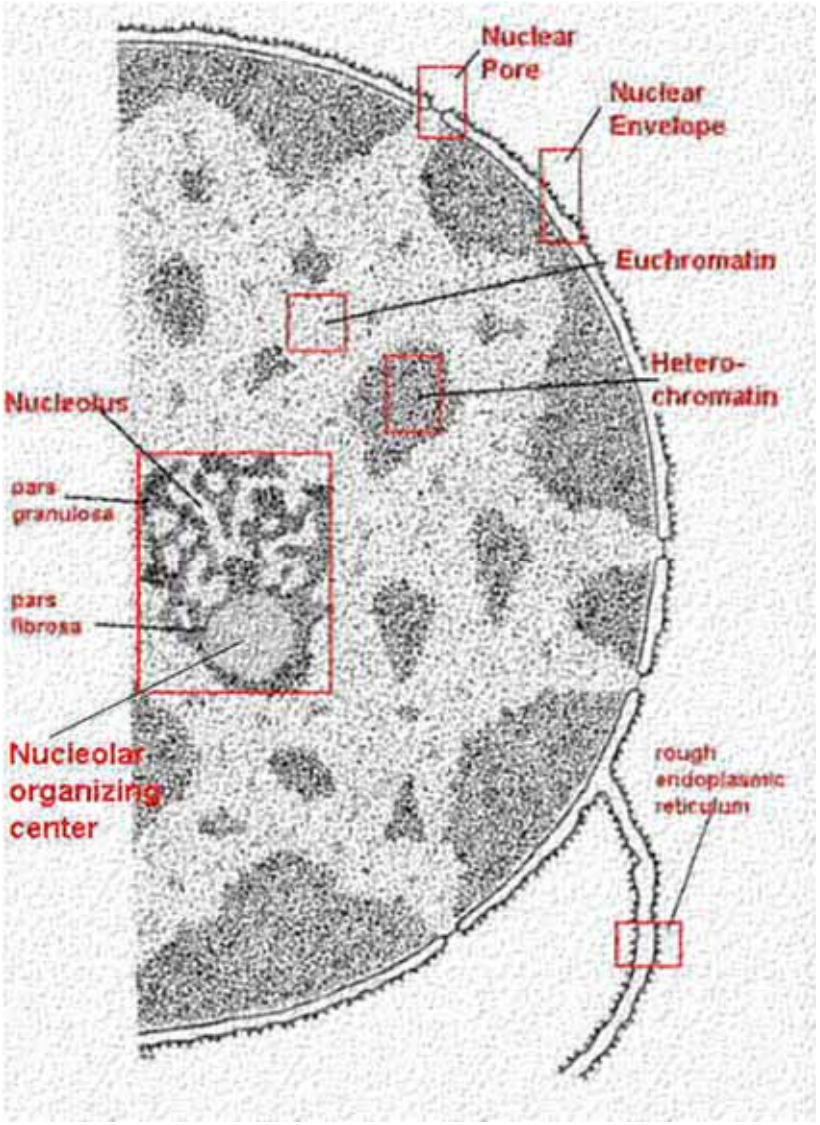
Like our factory's power plant, mitochondria and chloroplasts transform one form of energy to another. Remember that nearly all the energy used by living things on Earth comes from the Sun. This section discusses how energy is made available for cell processes.

# Mitochondrion



- In cell biology, a **mitochondrion** is a membrane-enclosed organelle, found in most eukaryotic cells.Mitochondria are sometimes described as "cellular power plants," because they convert NADH and NADPH into energy in the form of ATP via the process of oxidative phosphorylation. A typical eukaryotic cell contains about 2,000 mitochondria, which occupy roughly one fifth of its total volume. Mitochondria contain DNA that is independent of the DNA located in the cell nucleus. According to the endosymbiotic theory, mitochondria are descended from free-living prokaryotes.





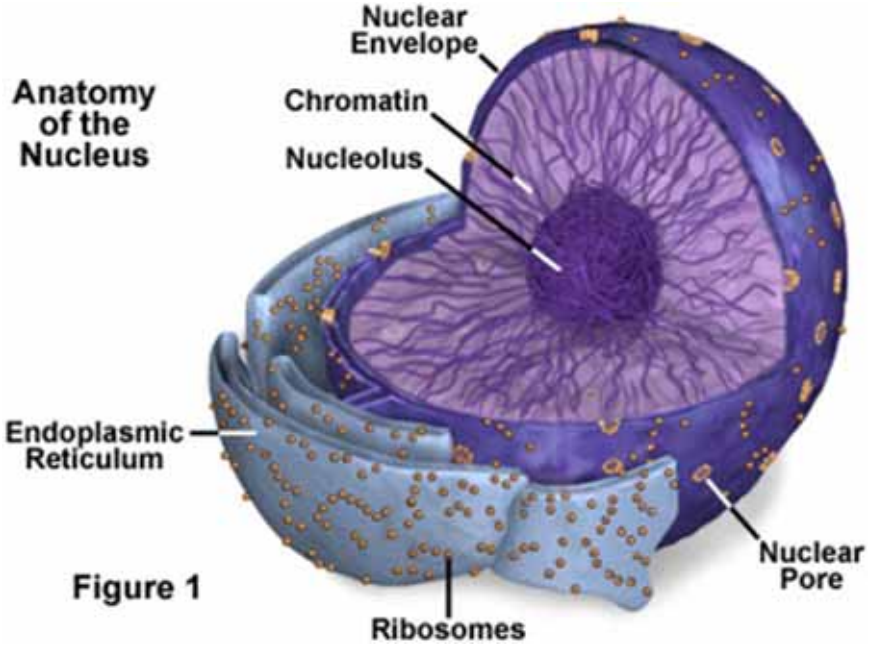




Figure 1

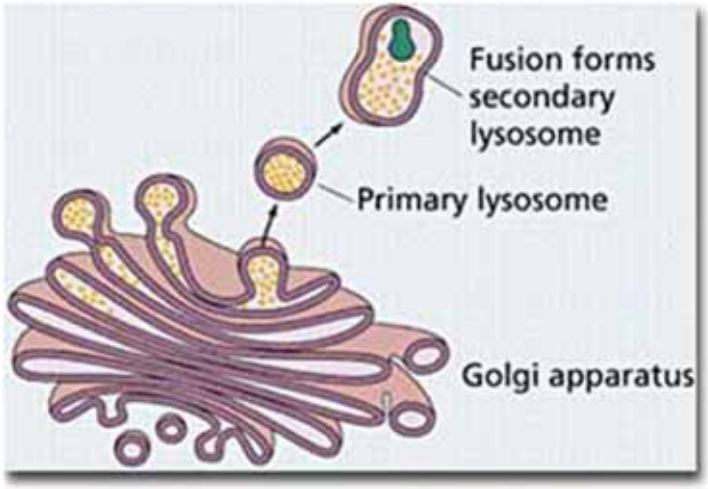
The main roles of the nucleolus are to synthesize rRNA and assemble ribosomes

The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle

# Lysosomes

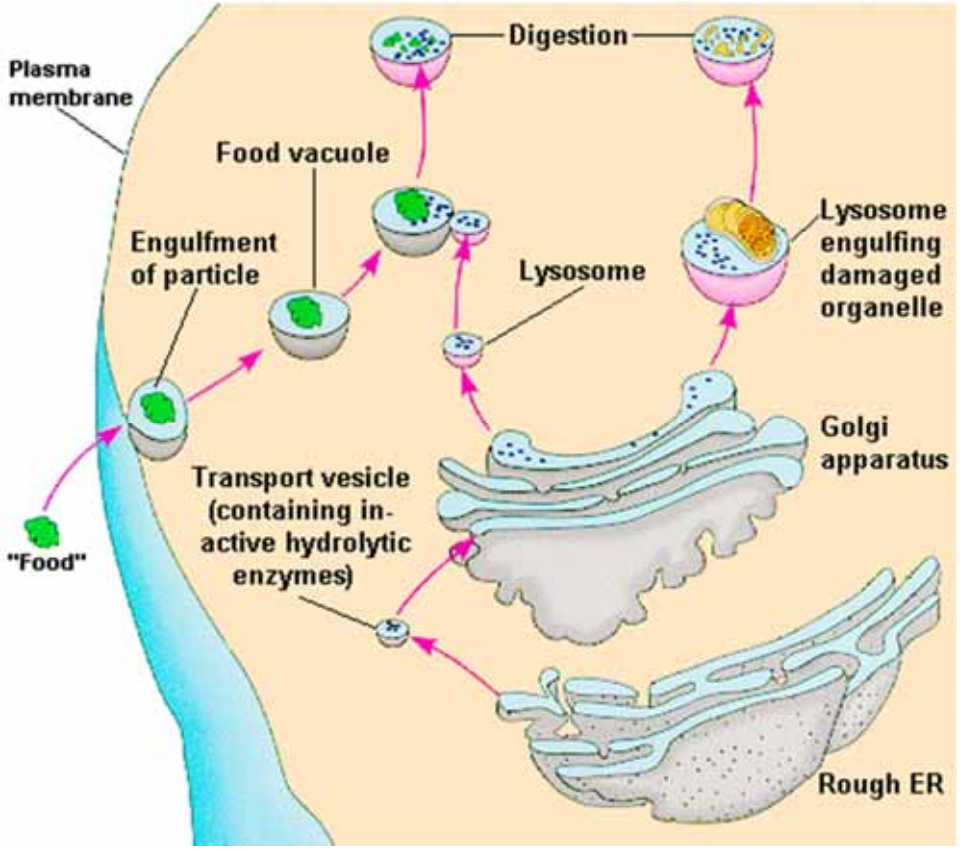
- **Lysosomes** are organelles that contain digestive enzymes (acid hydrolases). They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria. The membrane surrounding a lysosome prevents the digestive enzymes inside from destroying the cell. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are built in the Golgi apparatus. The name *lysosome* derives from the Greek words *lysis*, which means dissolution or destruction, and *soma*, which means body. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their role in autolysis.

# Lysosomes




Lysosomes are responsible for the breakdown and absorption of materials taken in by the cell. Often, a cell engulfs a foreign substance through **ENDOCYTOSIS**, another form of active transport. During endocytosis, the cell membrane puckers up, forms a pouch around materials outside the cell, and pinches off to become a vesicle. If the contents need to be destroyed, lysosomes combine with the vesicle and release their enzymes.

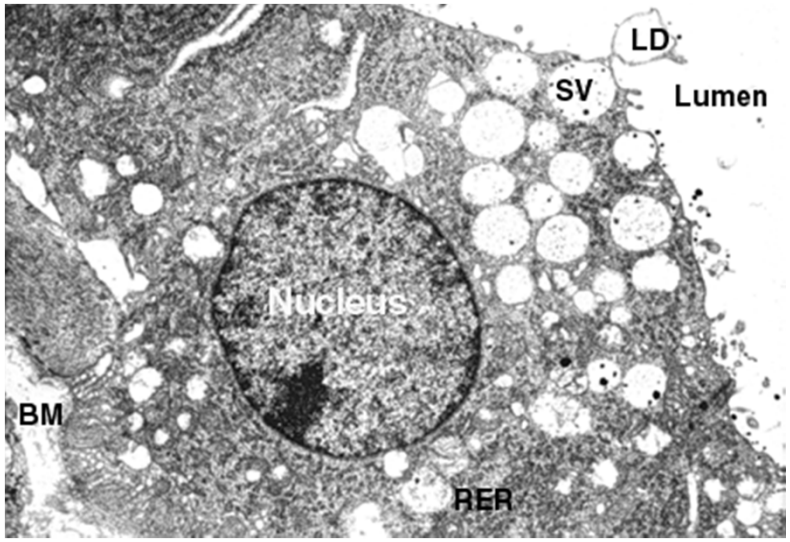
# Lysosome

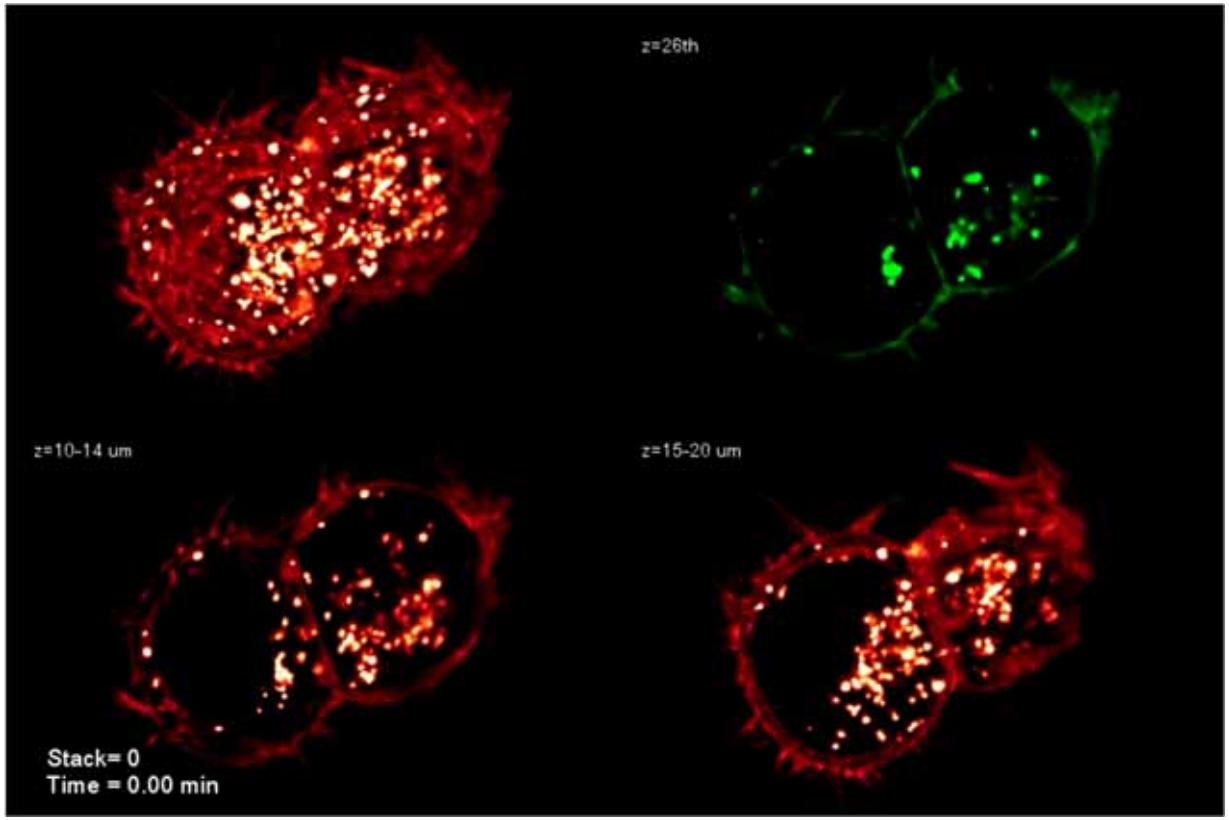

Digestion
Plasma membrane
Food vacuole
Engulfment of particle
Lysosome
Lysosome engulfing damaged organelle
Golgi apparatus
Transport vesicle (containing in-active hydrolytic enzymes)
"Food"
Rough ER

# Vesicle

In cell biology, a **vesicle** is a relatively small and enclosed compartment, separated from the cytosol by at least one lipid bilayer. If there is only one lipid bilayer, they are called *unilamellar* vesicles; otherwise they are called *multilamellar*. Vesicles store, transport, or digest cellular products and waste.

This biomembrane enclosing the vesicle is similar to that of the plasma membrane. Because it is separated from the cytosol, the intravesicular environment can be made to be different from the cytosolic environment. Vesicles are a basic tool of the cell for organizing metabolism, transport, enzyme storage, as well as being chemical reaction chambers. Many vesicles are made in the Golgi apparatus, but also in the endoplasmic reticulum, or are made from parts of the plasma membrane.

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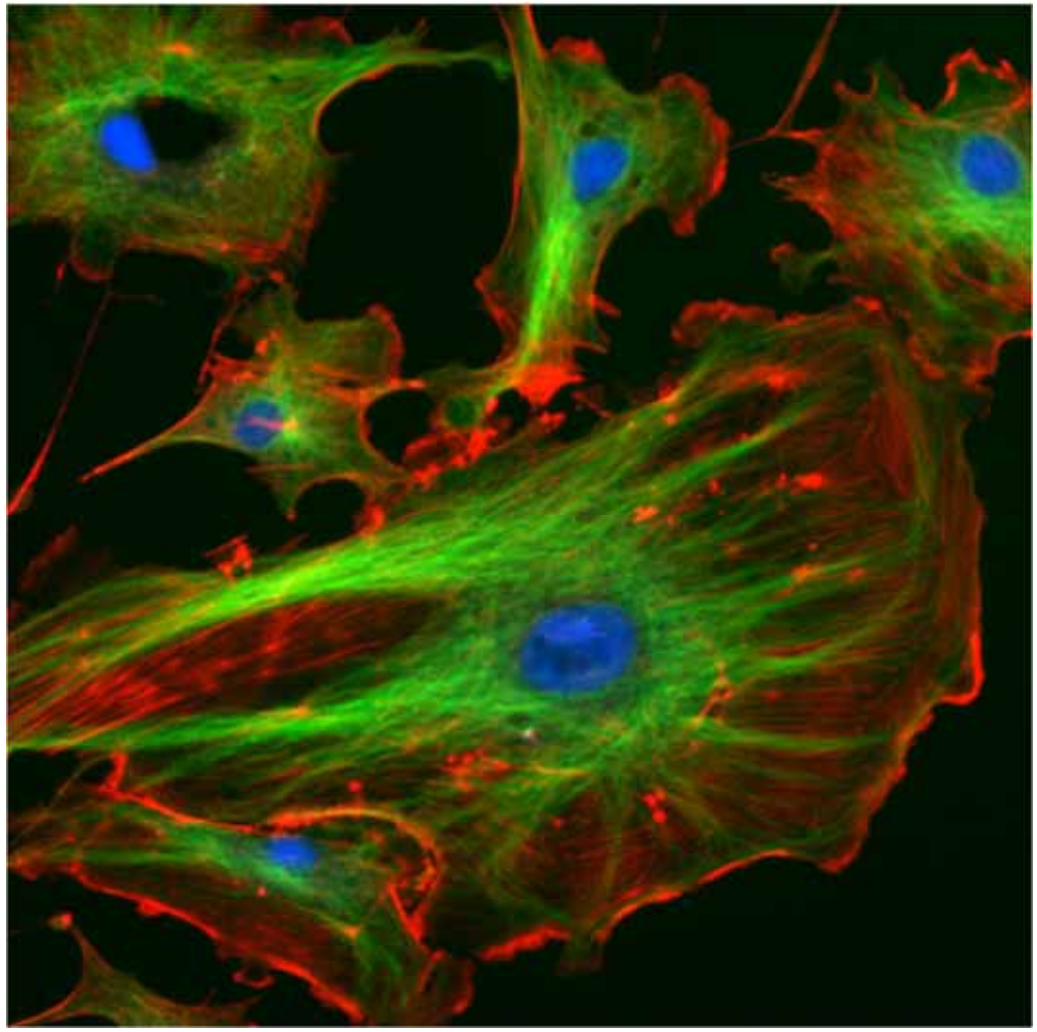
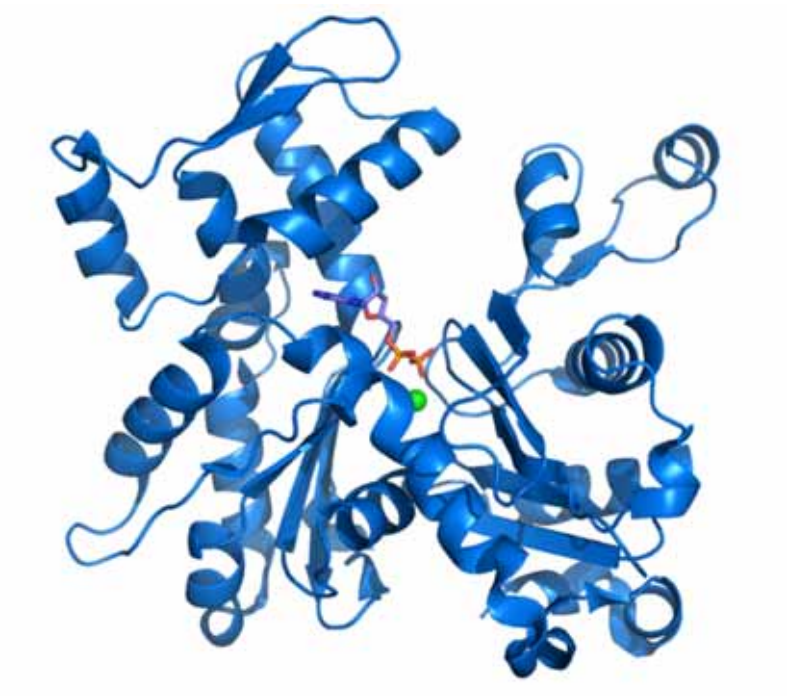
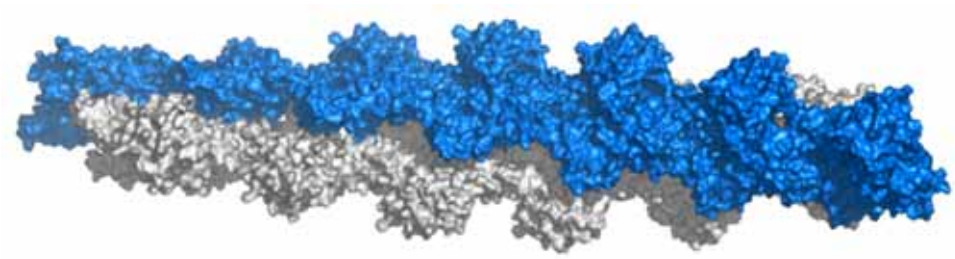
# Cytoskeleton



The eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei are in blue.

# Actin

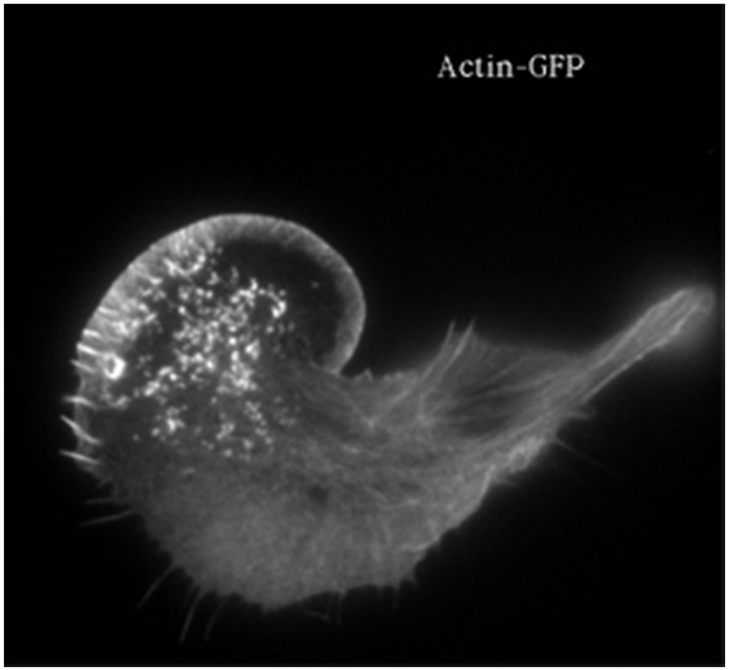


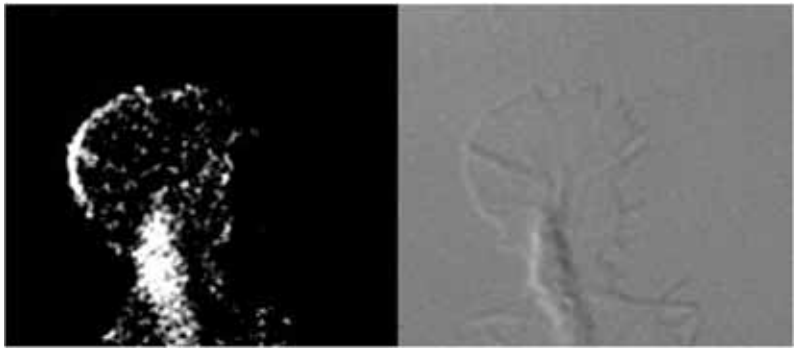


- **Actin** is a globular structural, 42 kDa, protein that polymerizes in a helical fashion to form **actin filaments** (or **microfilaments**). These form the cytoskeleton, a three-dimensional network inside the eukaryotic cell. Actin filaments provide mechanical support for the cell, determine its shape, and enable movement of the cell through lamellipodia, filopodia, or pseudopodia. Actin filaments, along with myosin, have an essential role in muscular contraction. In the cytosol, actin is predominantly bound to ATP, but can also bind to ADP. An ATP-actin complex polymerizes faster and dissociates slower than an ADP-actin complex.

# Lamellipodia

- The **lamellipodium** is a cytoskeletal actin projection on the mobile edge of the cell. It contains a two-dimensional actin mesh; the whole structure pulls the cell across a substrate. Within the lamellipodia are ribs of actin called microspikes, which, when they spread beyond the lamellipodium frontier, are called filopodia (Small, et all, 2002). The lamellipodium is born of actin nucleation in the plasma membrane of the cell (Alberts, et al, 2002) and is the primary area of actin incorporation or microfilament formation of the cell. Lamellipodia range from 1µm to 5µm in breadth and are approximately 0.2µm thick.Lamellipodia are found primarily in very mobile cells, crawling at a speeds of 10-20µm/minute over epithelial surfaces..
- The tip of the lamellipodium is the site where exocytosis occurs in migrating mammalian cells as part of their clathrin-mediated endocytic cycle.

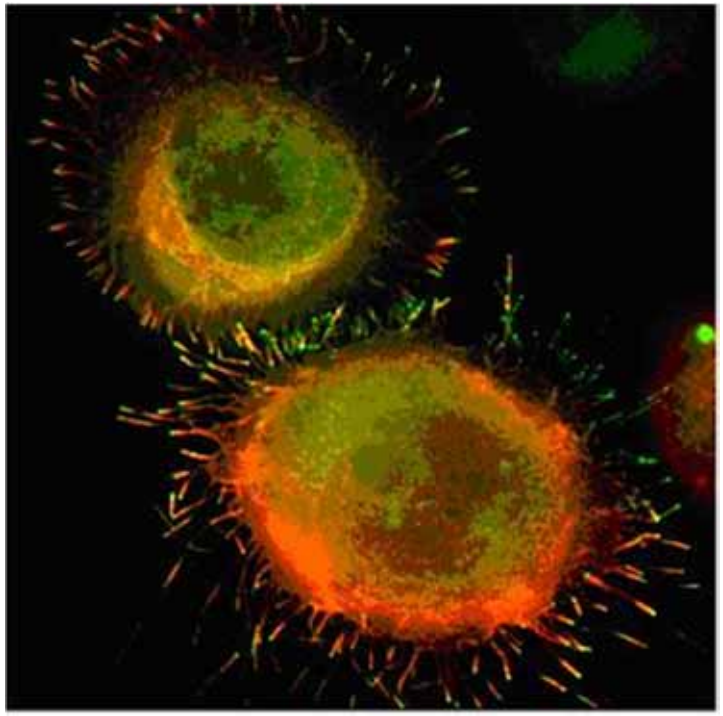


http://www.microscopyu.com/moviegallery/livecellimaging/3t3/t1/3t3-dslwmp1.html

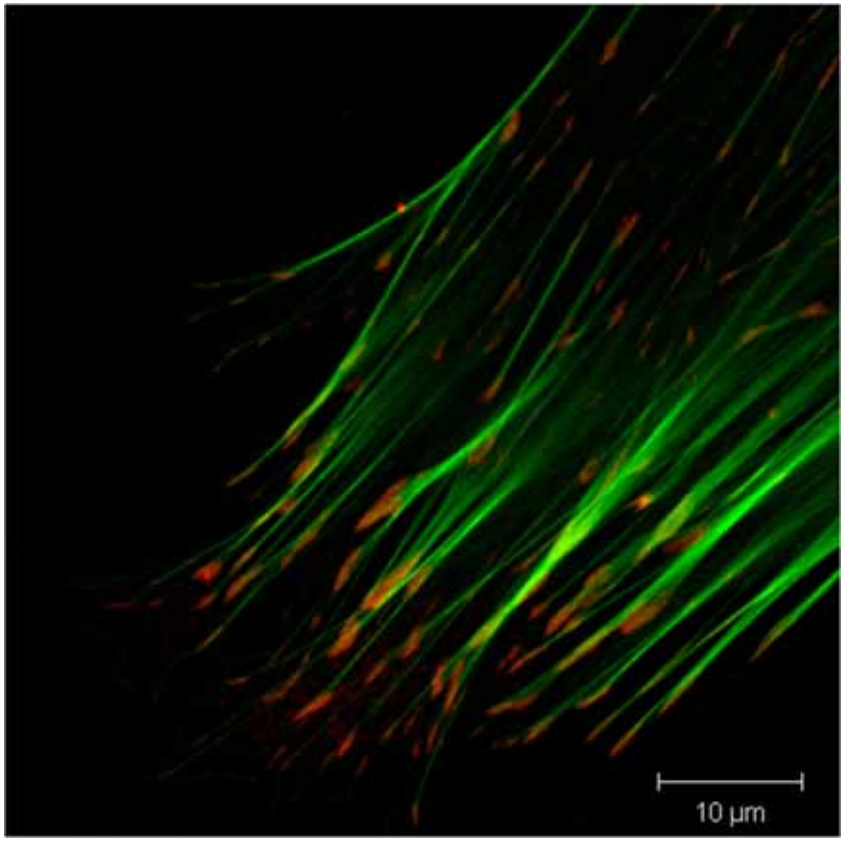
# Filopodia

The **filopodia** are slender cytoplasmic projections, similar to lamellipodia, which extend from the leading edge of migrating cells. They contain actin filaments cross-linked into bundles by actin-binding proteins, e.g. fimbrin. Filopodia form focal adhesions with the substratum, linking it to the cell surface. A cell migrates along a surface by extending filopodia at the leading edge. The filopodia attach to the substratum further down the migratory pathway, then contraction of stress fibres retracts the rear of the cell to move the cell forwards.



# Focal adhesion

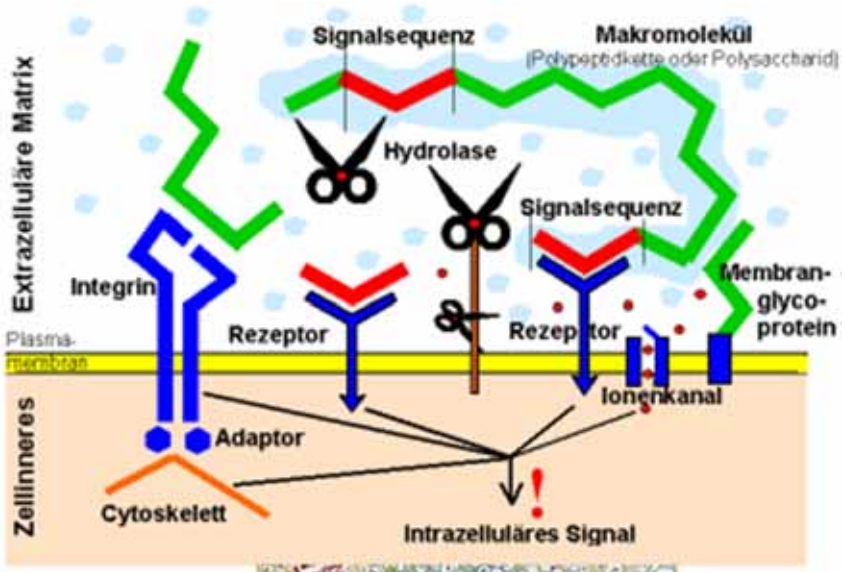
- In cell biology, '**Focal Adhesions'** are specific types of large macromolecular assemblies through which both mechanical force and regulatory signals are transmitted. More precisely, **FAs** can be considered as sub-cellular macromolecules that mediate the regulatory effects (e.g. cell anchorage) of extracellular matrix (ECM) adhesion on cell behavior.



# Extra Cellular Matrix

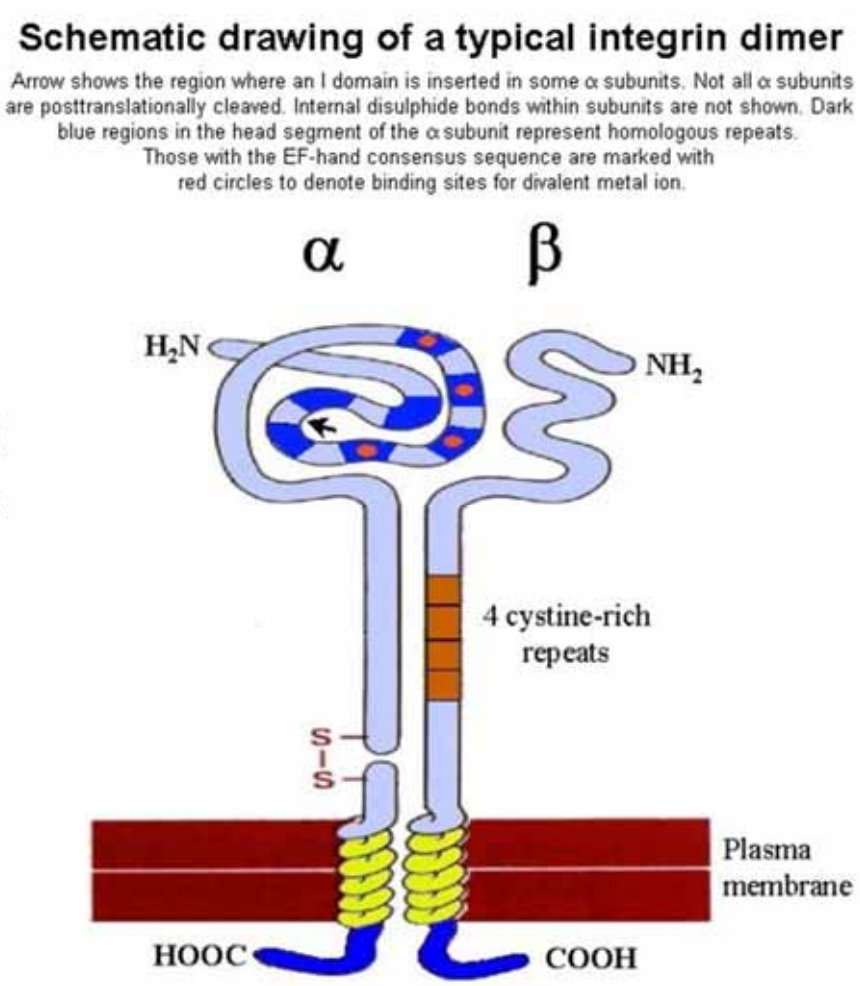
The ECM's main components are various glycoproteins, proteoglycans and hyaluronic acid. In most animals, the most abundant glycoproteins in the ECM are collagens.

ECM also contains many other components: proteins such as fibrin, elastin, fibronectins, laminins, and nidogens, and minerals such as hydroxylapatite, or fluids such as blood plasma or serum with secreted free flowing antigens.

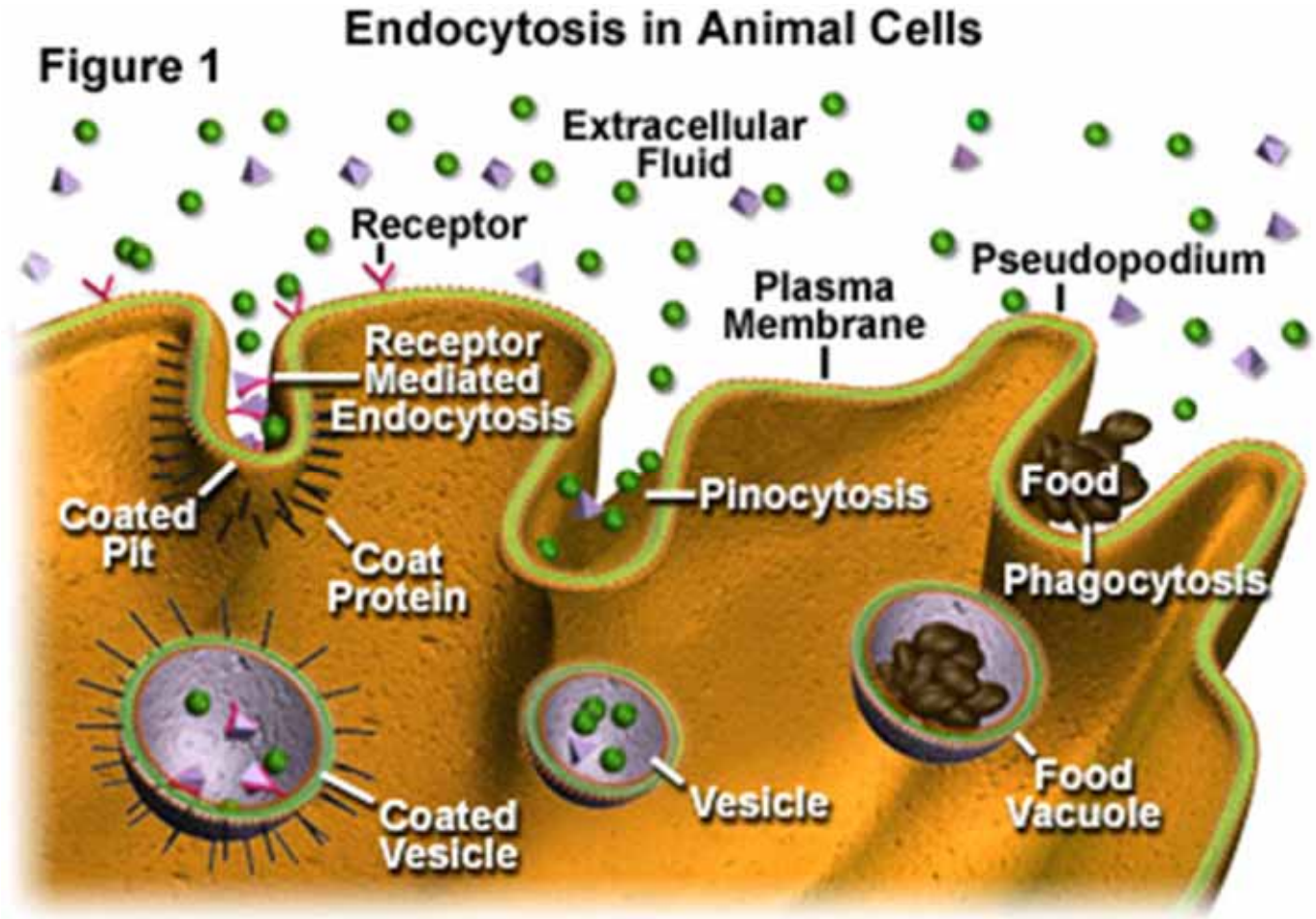



# Integrin

An **integrin**, or **integrin receptor**, is an integral membrane protein in the plasma membrane of cells. It plays a role in the attachment of a cell to the extracellular matrix (ECM) and to other cells, and in signal transduction from the ECM to the cell. There are many types of integrin, and many cells have multiple types on their surface. Integrins are of vital importance to all metazoans, from humans to sponges.



# Endocytosis



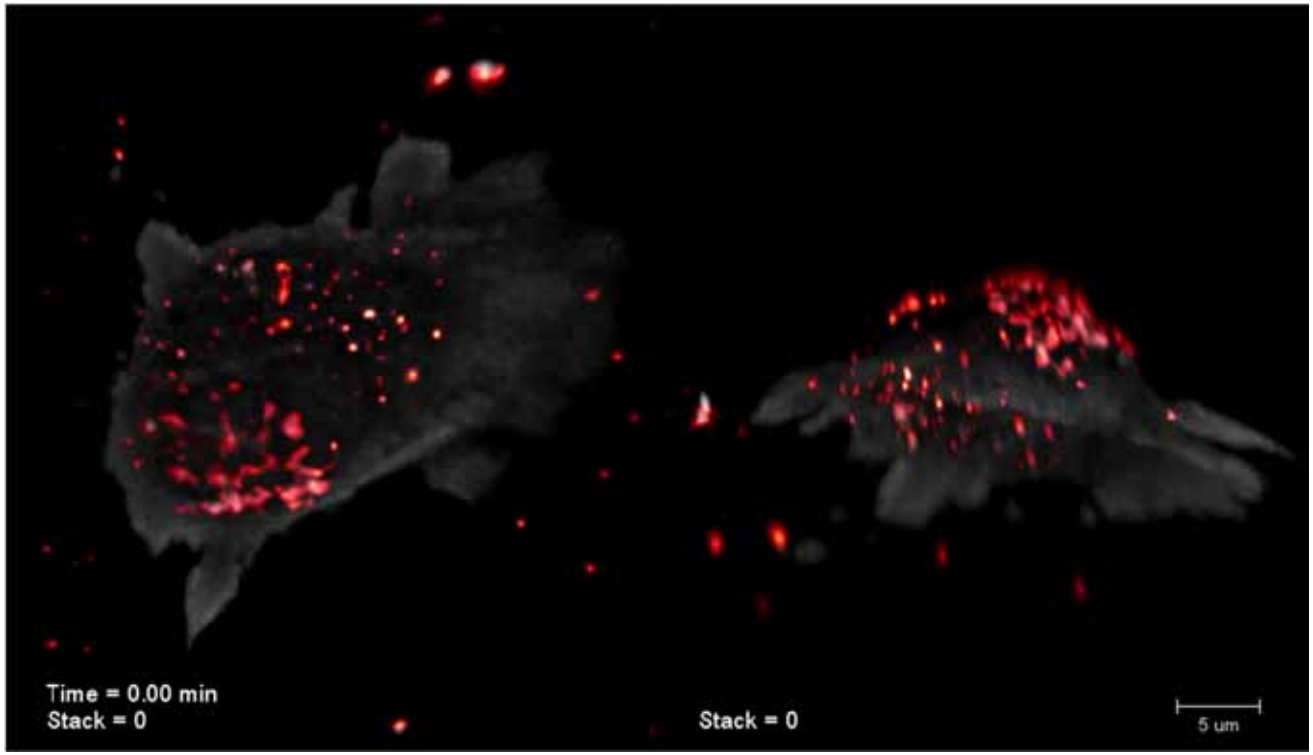
# Endocytosis

- Phagocytosis is the process by which cells ingest large objects, such as cells which have undergone apoptosis, bacteria, or viruses. The membrane folds around the object, and the object is sealed off into a large vacuole known as a phagosome.
- Pinocytosis is a synonym for endocytosis. This process is concerned with the uptake of solutes and single molecules such as proteins.
- Receptor-mediated endocytosis is a more specific active event where the cytoplasm membrane folds inward to form coated pits. These inward budding vesicles bud to form cytoplasmic vesicles.

http://highered.mcgraw-hill.com/olc/dl/120068/bio02.swf

# Endocytosis pathways

- Macropinocytosis is the invagination of the cell membrane to form a pocket which then pinches off into the cell to form a vesicle filled with extracellular fluid (and molecules within it). The filling of the pocket occurs in a non-specific manner. The vesicle then travels into the cytosol and fuses with other vesicles such as endosomes and lysosomes.
- Clathrin-mediated endocytosis is the specific uptake of large extracellular molecules such as proteins, membrane localized receptors and ion-channels. These receptors are associated with the cytosolic protein clathrin which initiates the formation of a vesicle by forming a crystalline coat on the inner surface of the cell's membrane.
- Caveolae consist of the protein caveolin-1 with a bilayer enriched in cholesterol and glycosphingolipids. Caveolae are flask shaped pits in the membrane that resemble the shape of a cave (hence the name caveolae). Uptake of extracellular molecules are also believed to be specifically mediated via receptors in caveolae.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Stack = 0
5 um

# Endocytic pathway in mammalian cells

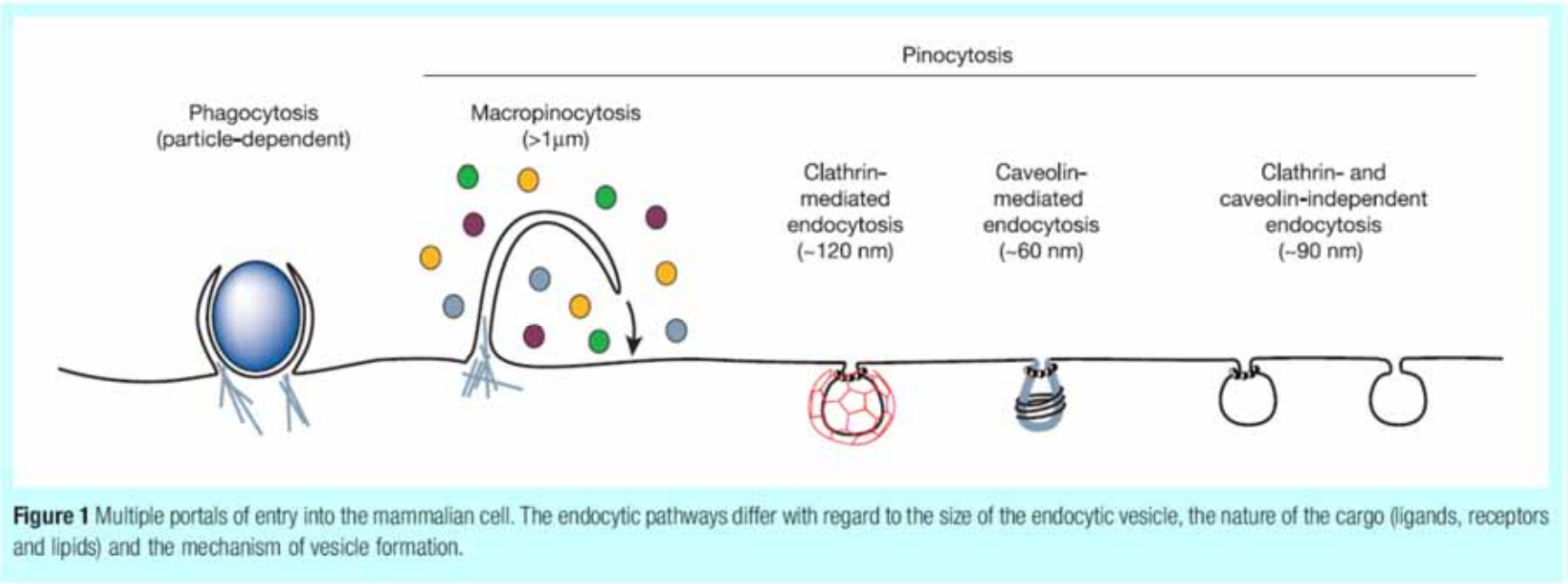



**Figure 1** Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

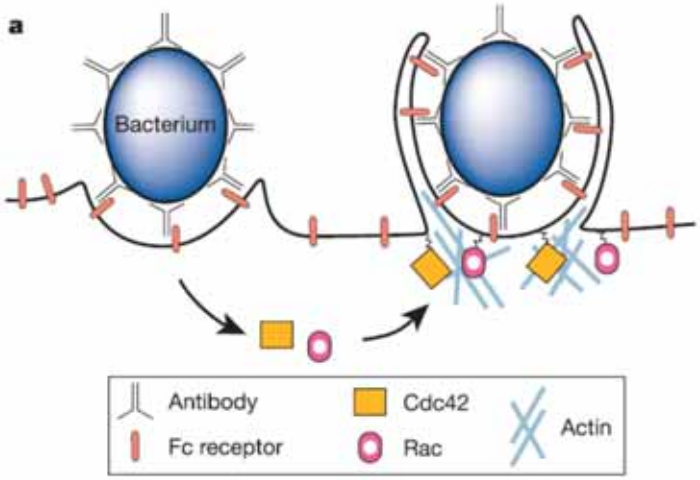



**Figure 2** Cargo-stimulated signalling pathways induce uptake by phagocytosis and caveolae. **a**, Fc receptors on the surface of macrophages are activated by immunoglobulin-$\gamma$ molecules bound to a bacterium. A signalling cascade that involves Rac, Cdc42 and downstream kinases triggers actin rearrangements, protrusion of the membrane around the bacterium, and its engulfment into a phagosome. **b**, Albumin binds to and presumably clusters its receptor, gp60, in caveolae to activate $G_{i\alpha}$ and Src kinases, triggering caveolae endocytosis.

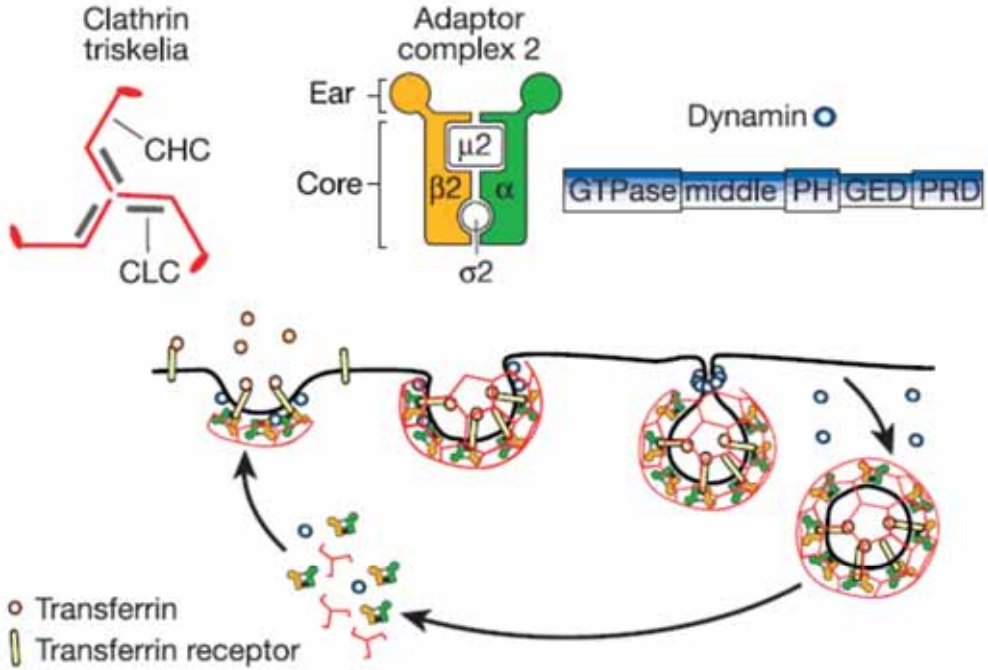
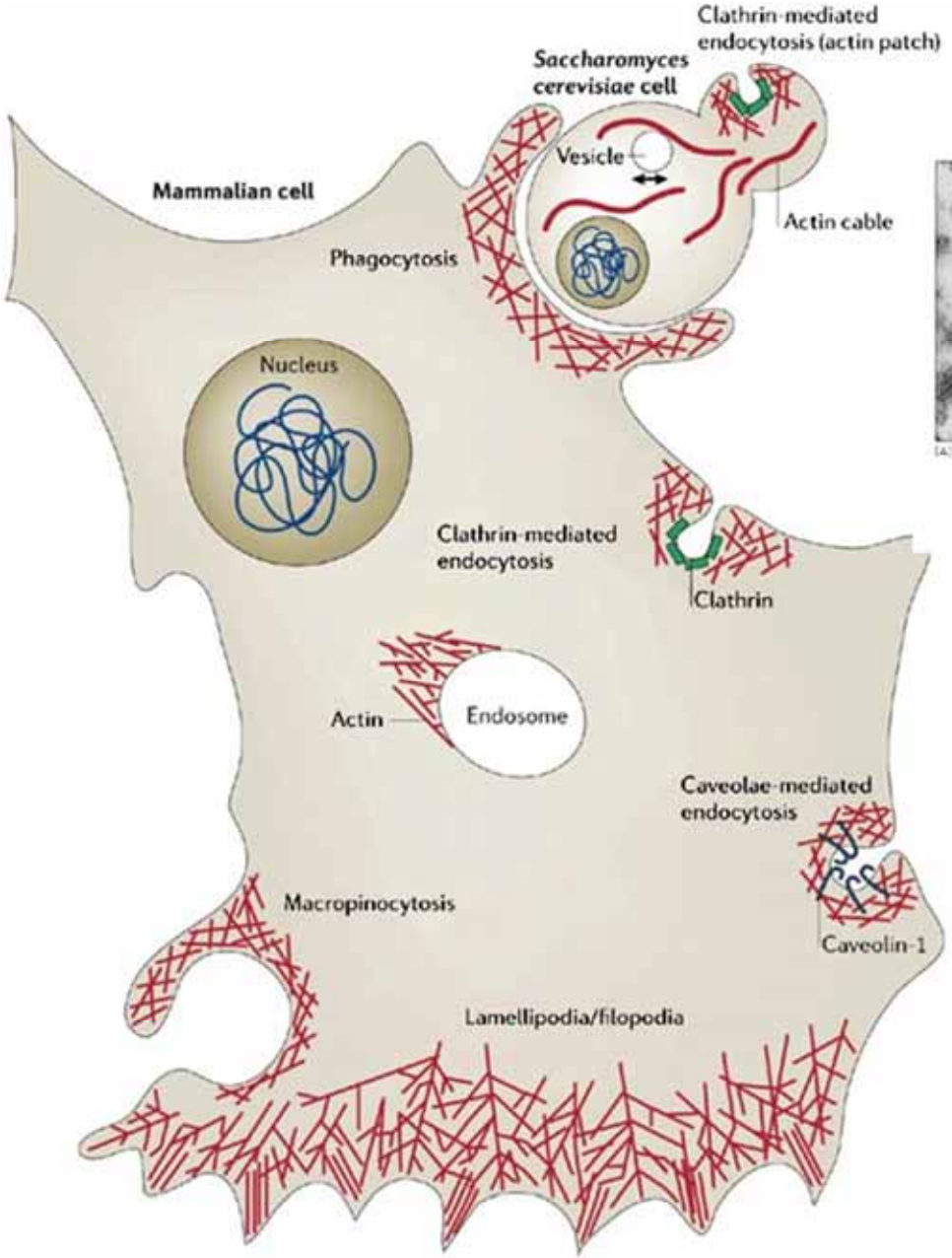
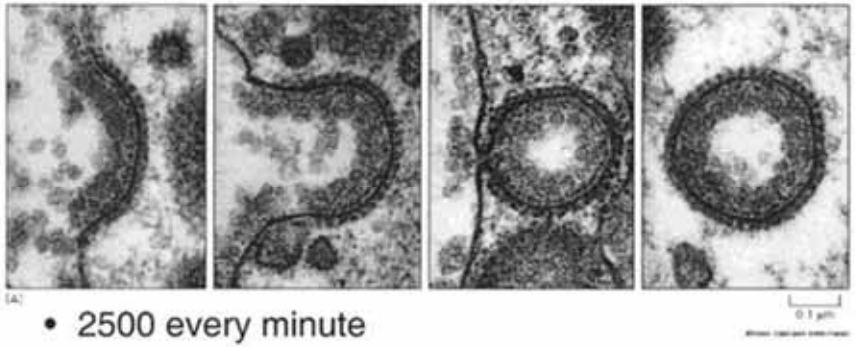



**Figure 3** Core components of the machinery driving clathrin-mediated endocytosis. Clathrin triskelions, composed of three clathrin heavy chains (CHC) and three tightly associated light chains (CLC), assemble into a polygonal lattice, which helps to deform the overlying plasma membrane into a coated pit. Heterotetrameric AP2 complexes are targeted to the plasma membrane by the $\alpha$-adaptin subunits, where they mediate clathrin assembly through the $\beta$2-subunit, and interact directly with sorting motifs on cargo molecules through their $\mu$2 subunits. Dynamin is a multidomain GTPase that is recruited to the necks of coated pits, where it can assemble into a spiral or 'collar' to mediate or monitor membrane fission and the release of CCVs (see text for details). A subsequent uncoating reaction recycles the coat constituents for reuse.



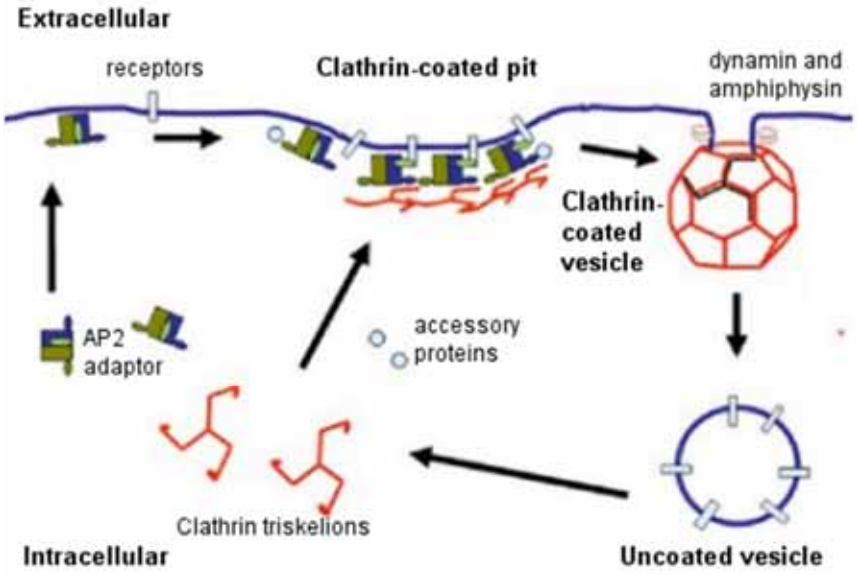


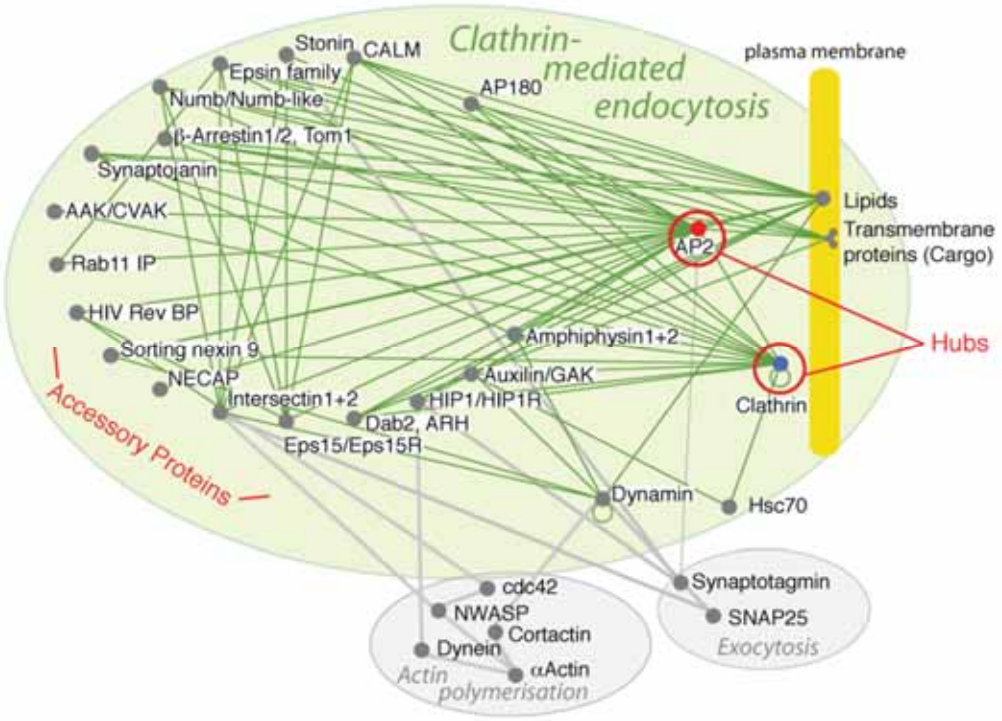

# Formation of Clathrin-Coated Vesicles



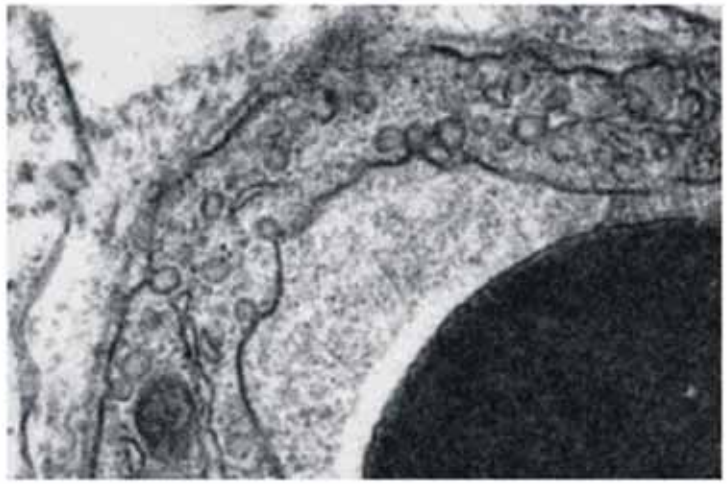
- 2500 every minute
- CCV uncoat within seconds

# Clathrin-mediated endocytosis

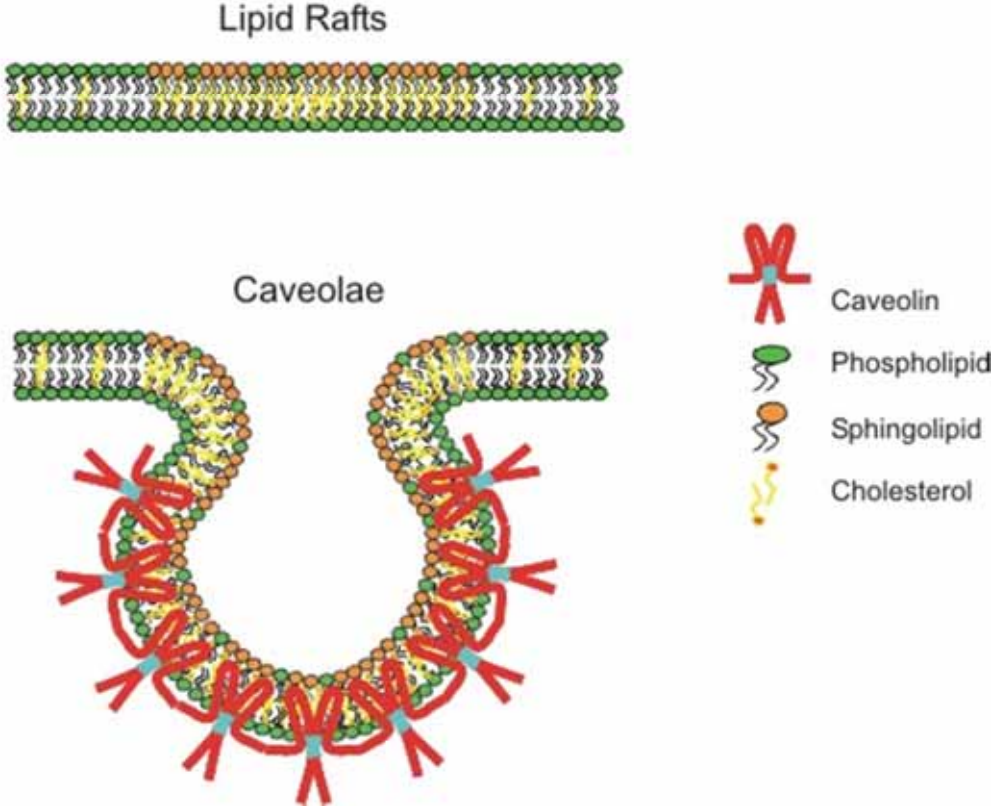


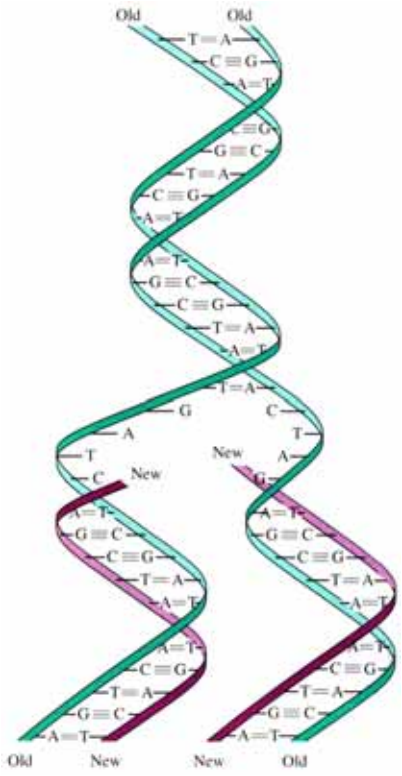
A

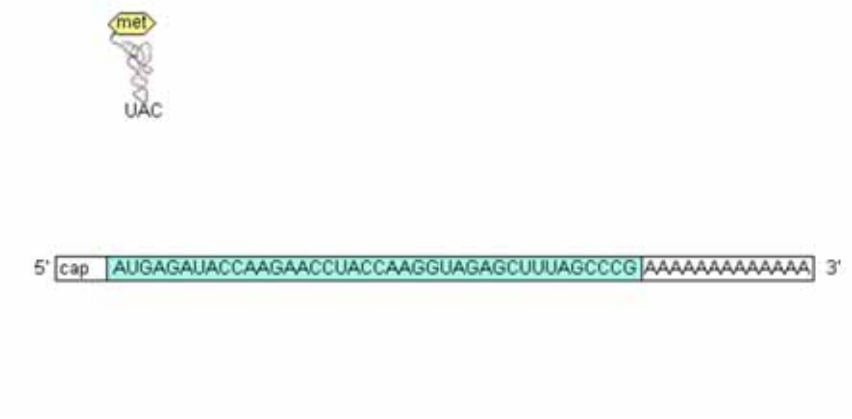


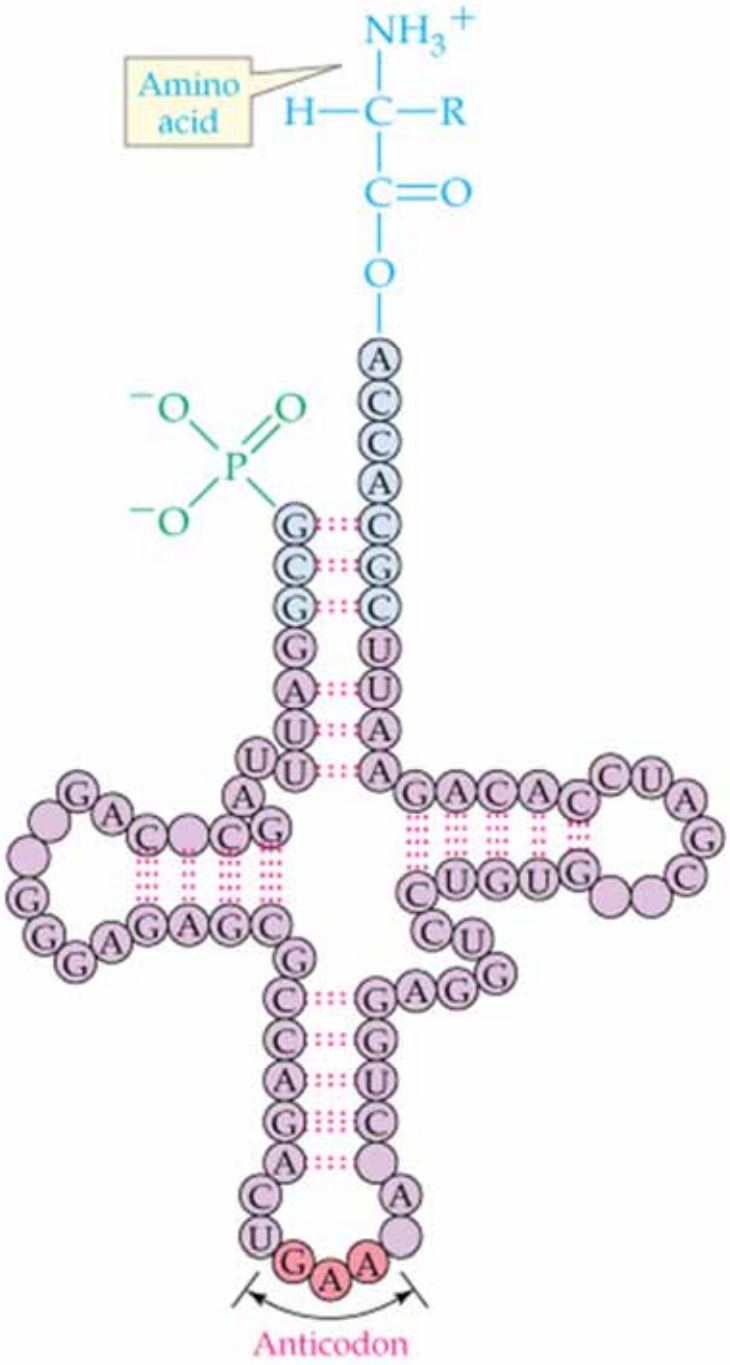
B


Lipid Rafts
Caveolae
Caveolin
Phospholipid
Sphingolipid
Cholesterol

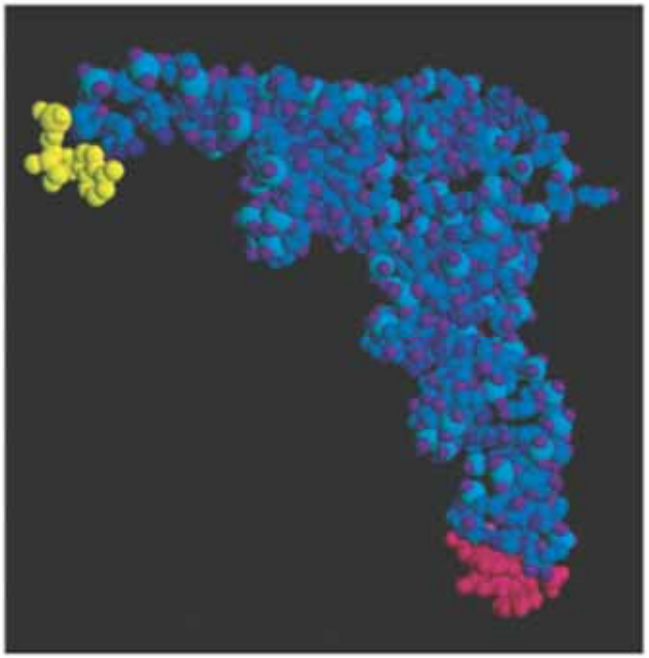
# Self-Assembly Process in Nature



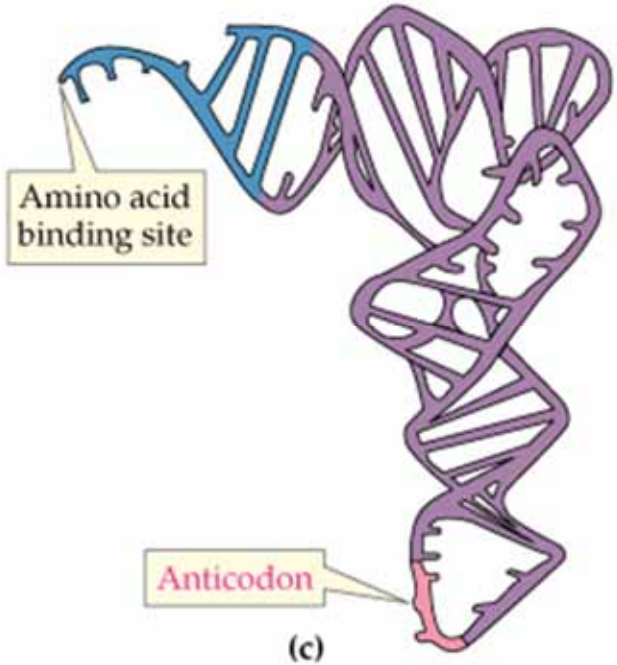




Amino acid
$NH_3^+$
H—C—R
C=O
O
$^-O$
O
P
$^-O$
Anticodon

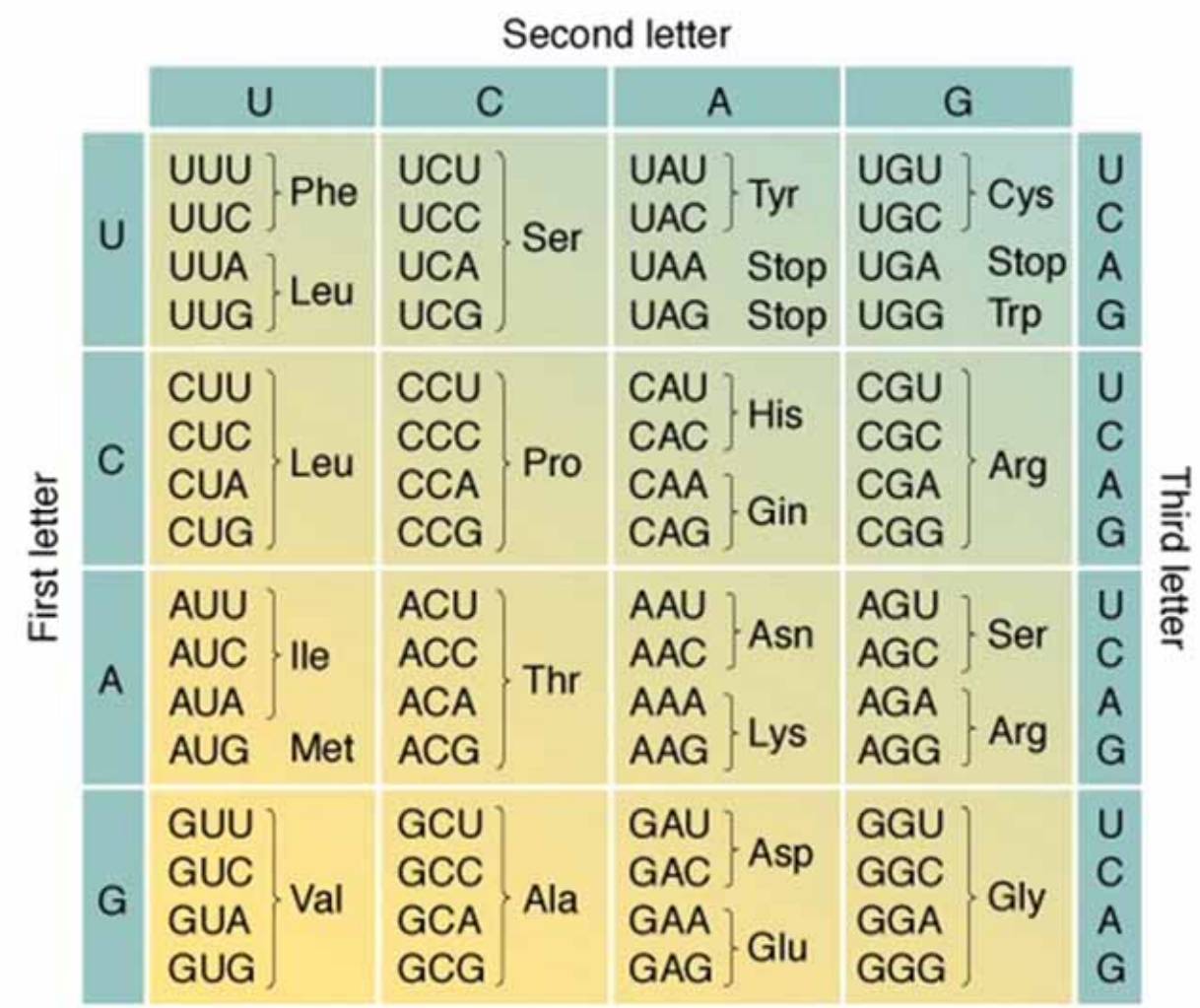
(a)



(b)

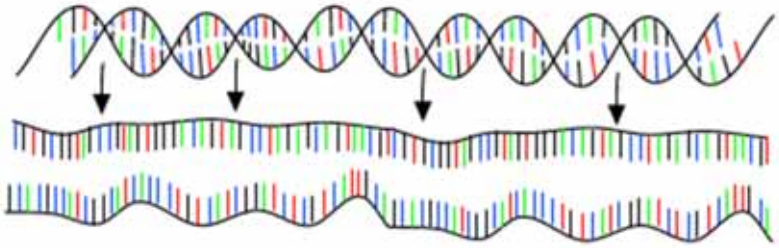

Amino acid binding site
Anticodon

(c)


Second letter
U
C
A
G
First letter
Third letter
U
UUU Phe
UUC
UUA Leu
UUG
UCU Ser
UCC
UCA
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
C
CUU Leu
CUC
CUA
CUG
CCU Pro
CCC
CCA
CCG
CAU His
CAC
CAA Gln
CAG
CGU Arg
CGC
CGA
CGG
A
AUU Ile
AUC
AUA
AUG Met
ACU Thr
ACC
ACA
ACG
AAU Asn
AAC
AAA Lys
AAG
AGU Ser
AGC
AGA Arg
AGG
G
GUU Val
GUC
GUA
GUG
GCU Ala
GCC
GCA
GCG
GAU Asp
GAC
GAA Glu
GAG
GGU Gly
GGC
GGA
GGG

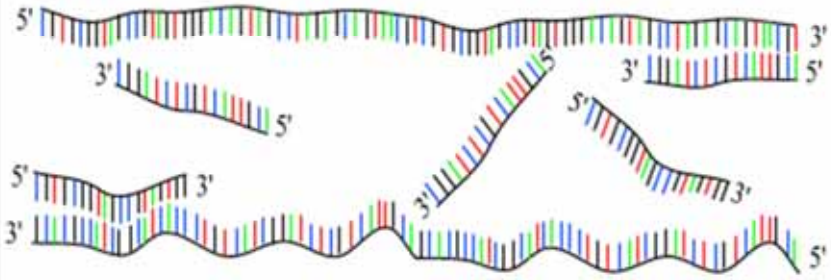
# PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



**Step 1 : denaturation**

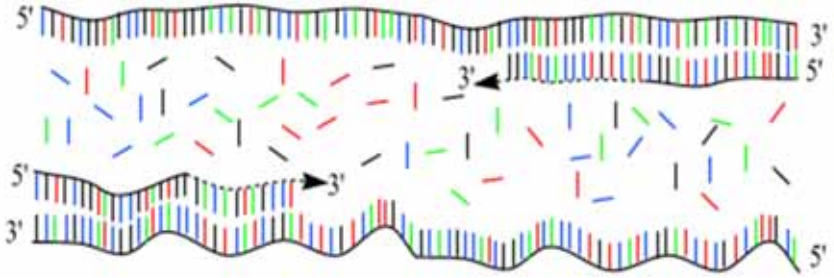
1 minut 94 °C




**Step 2 : annealing**

45 seconds 54 °C

forward and reverse primers !!!

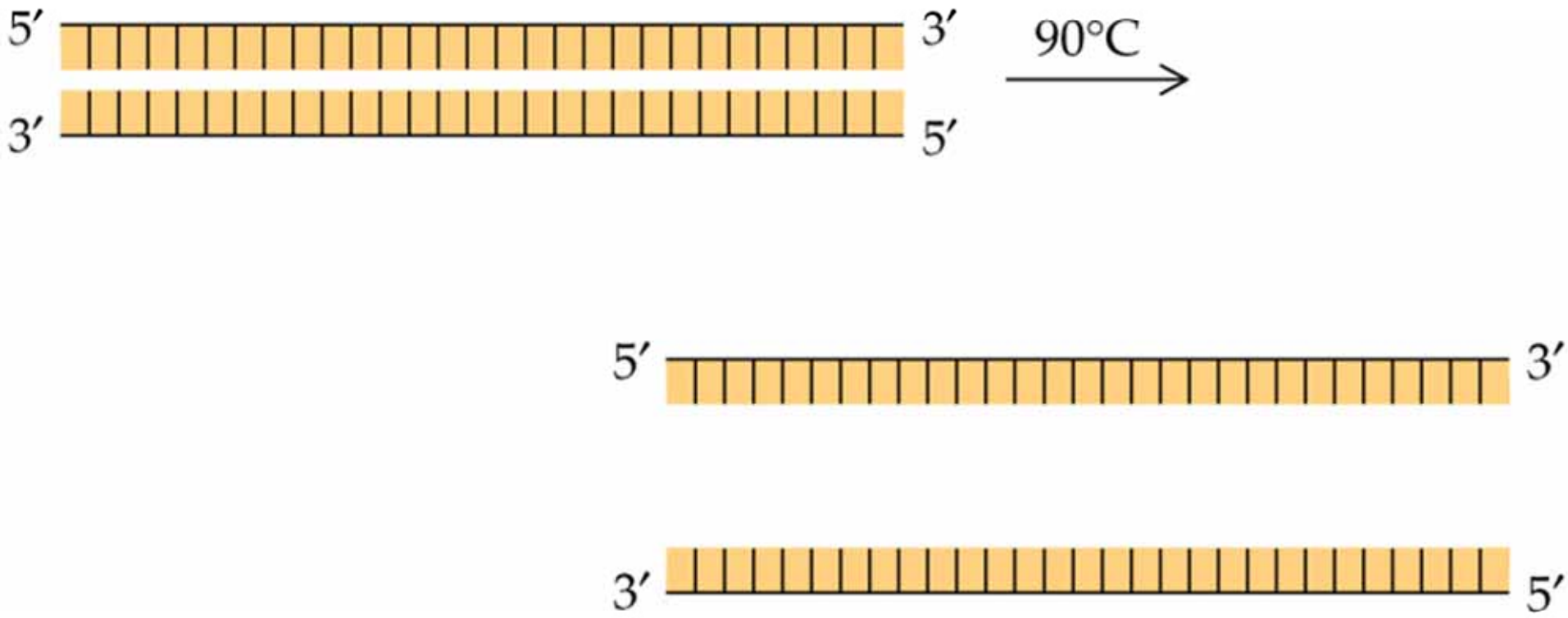


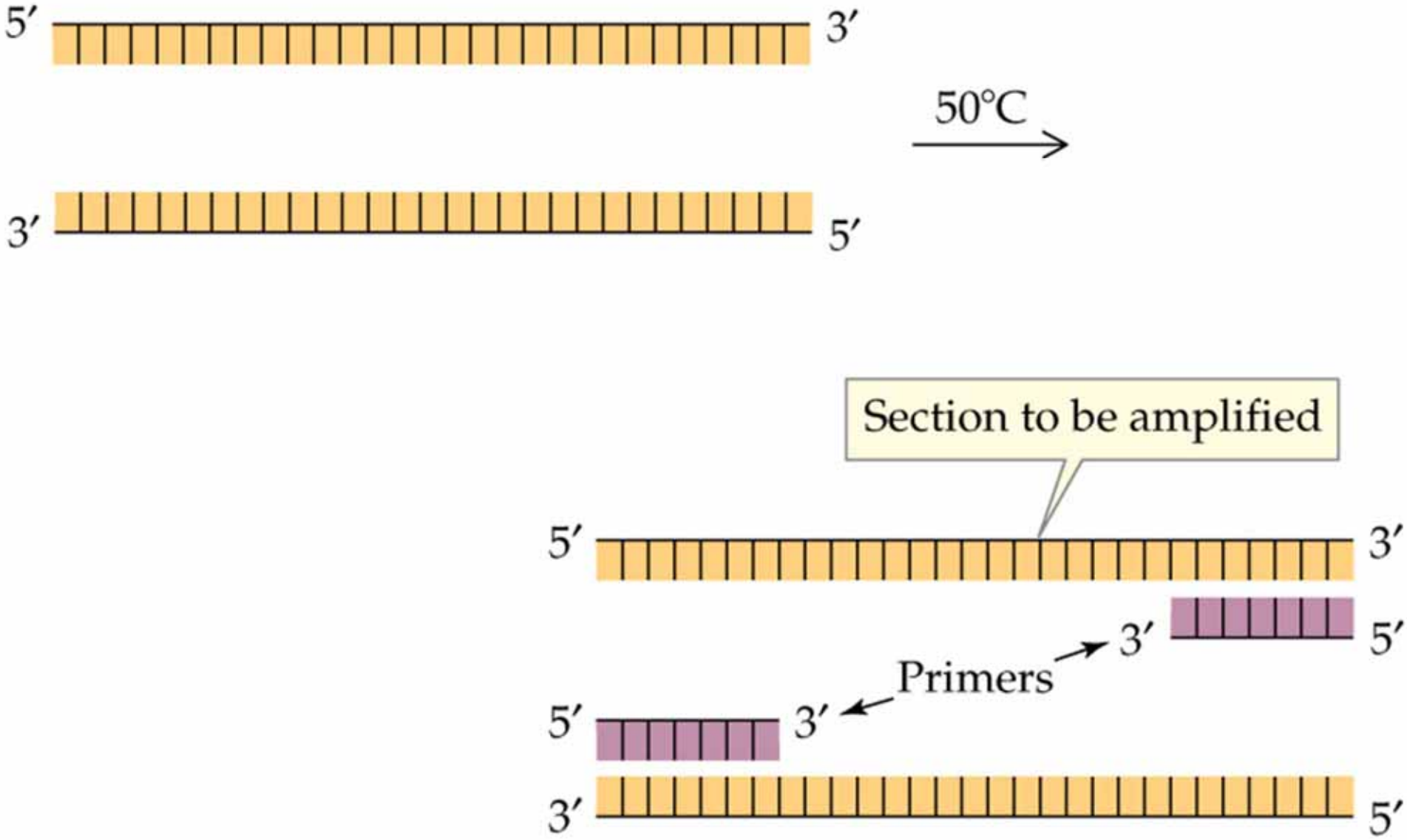

**Step 3 : extension**

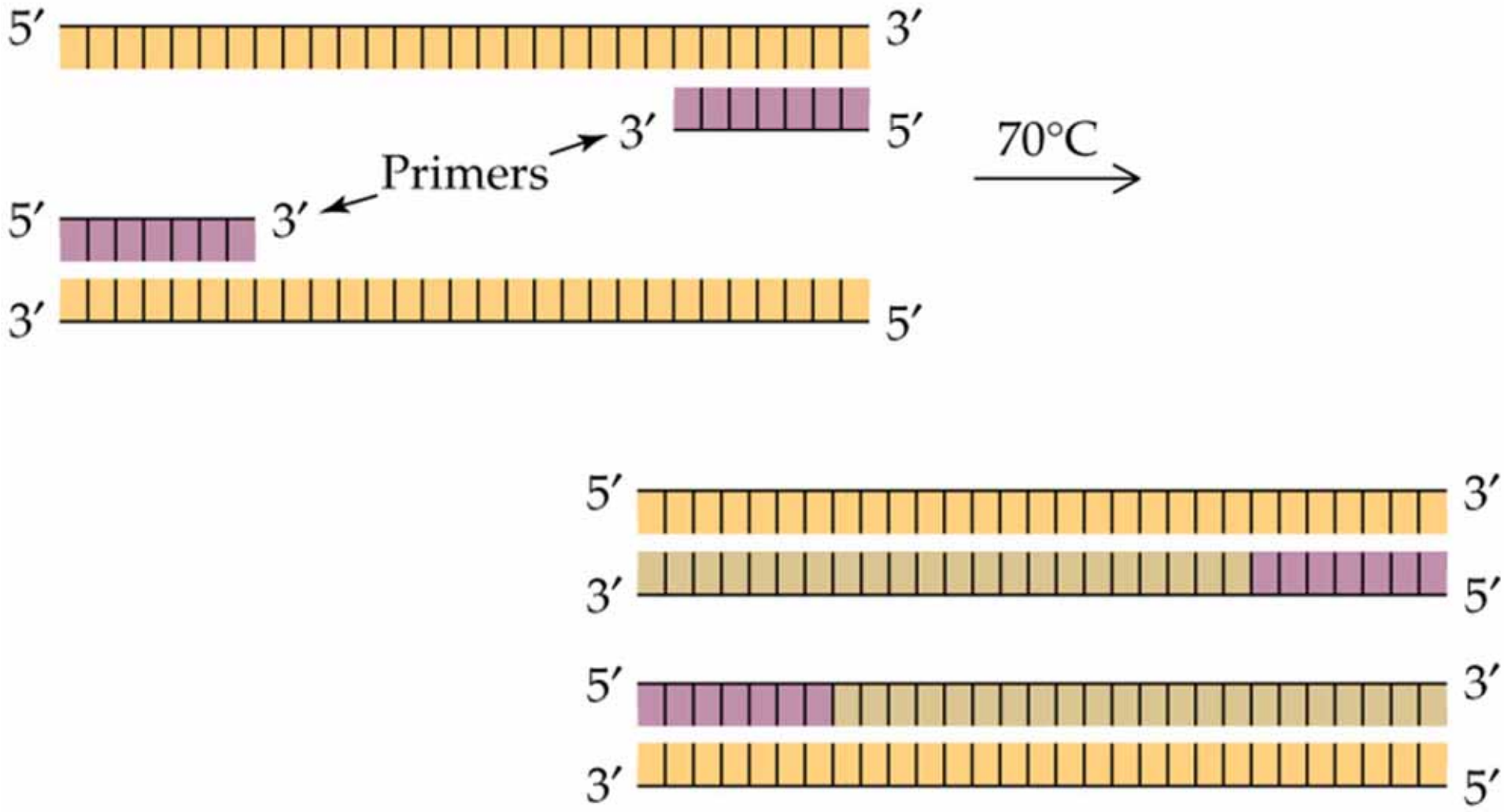
2 minutes 72 °C

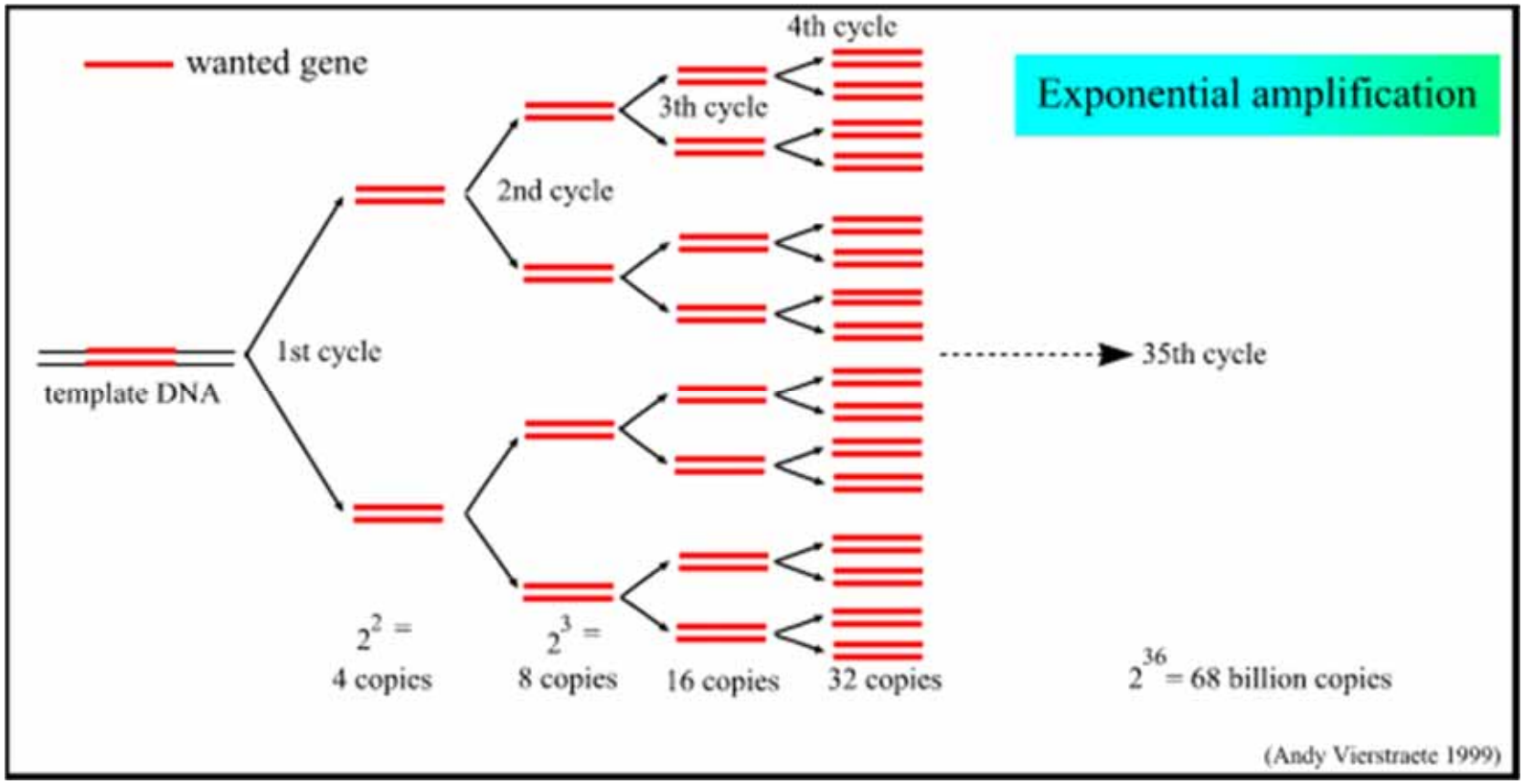
only dNTP's

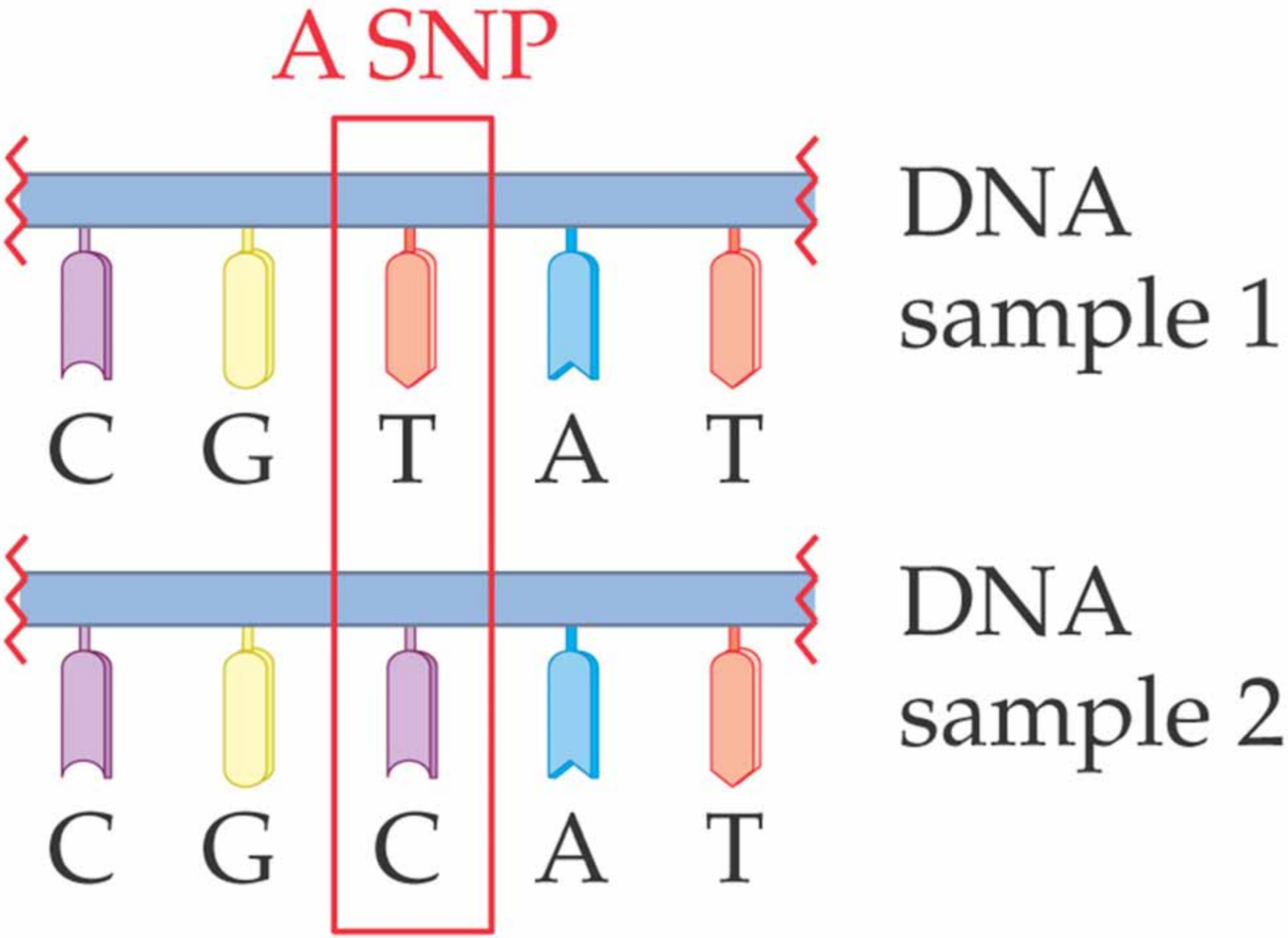
(Andy Vierstraete 1999)

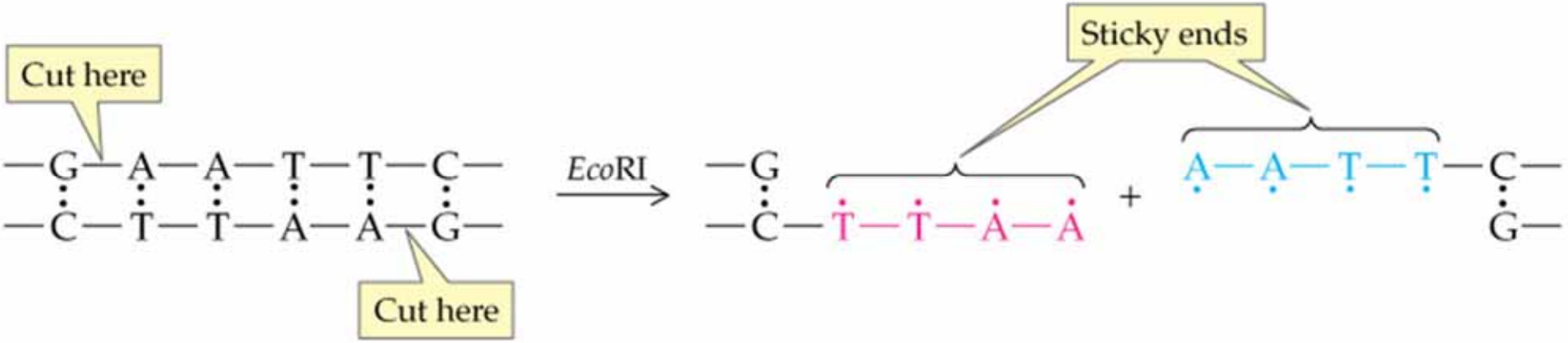

5′
3′
90°C
3′
5′
5′
3′
3′
5′

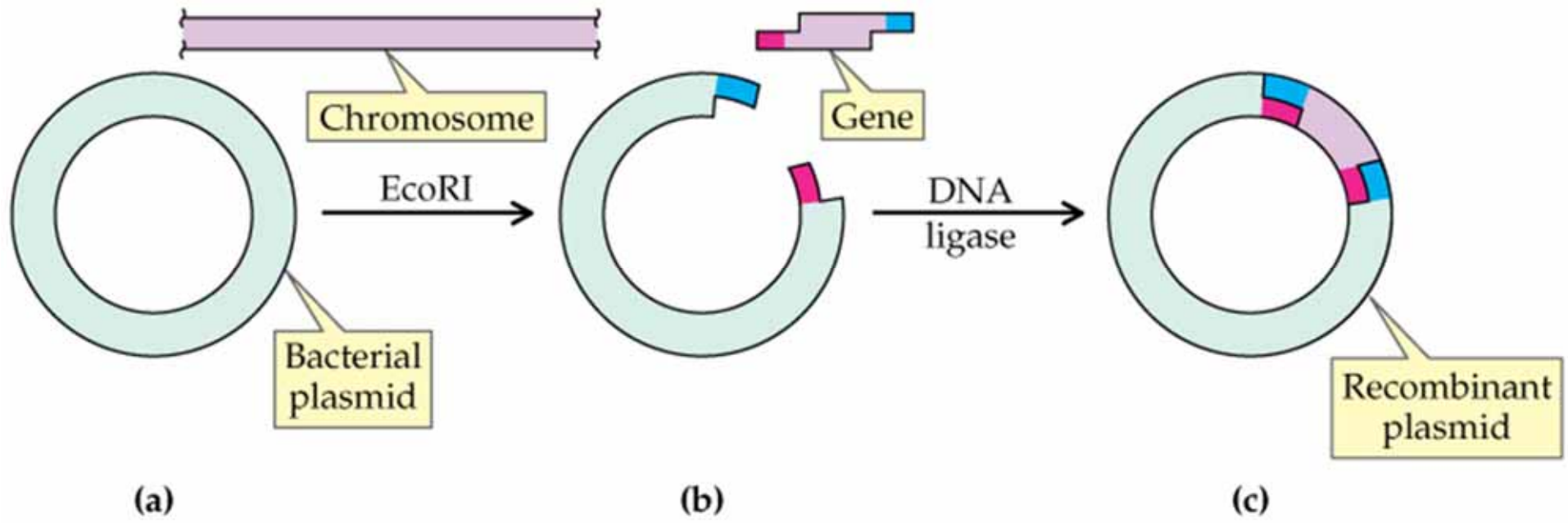

5′
3′
50°C
3′
5′
Section to be amplified
5′
3′
3′
5′
Primers
5′
3′
3′
5′


5′
3′
3′
5′
Primers
70°C
5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
3′
5′


wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
Exponential amplification
35th cycle
$2^2$ =
4 copies
$2^3$ =
8 copies
16 copies
32 copies
$2^{36}$ = 68 billion copies
(Andy Vierstraete 1999)

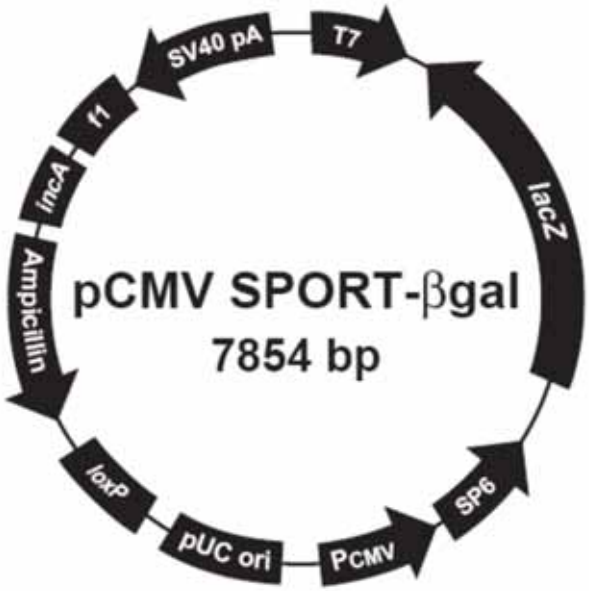

A SNP
C G T A T
DNA sample 1
C G C A T
DNA sample 2

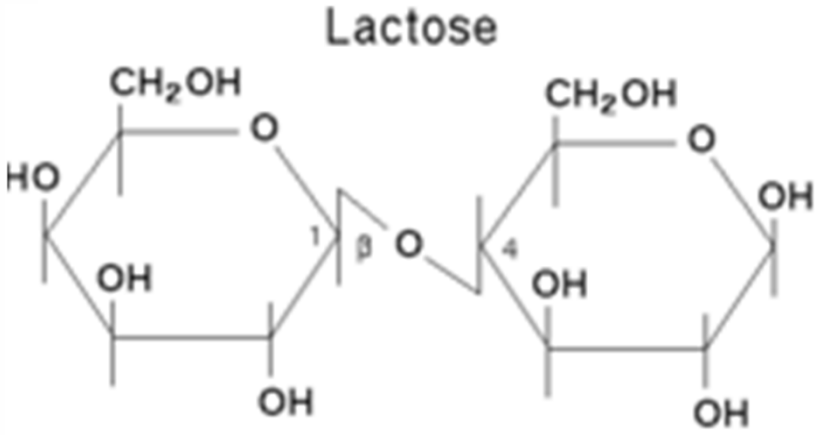

Cut here
—G—A—A—T—T—C—
—C—T—T—A—A—G—
Cut here
EcoRI
—G
—C—T—T—A—A
+
Sticky ends
A—A—T—T—C—
G—


Chromosome
Gene
EcoRI
DNA
ligase
Bacterial
plasmid
Recombinant
plasmid
(a)
(b)
(c)

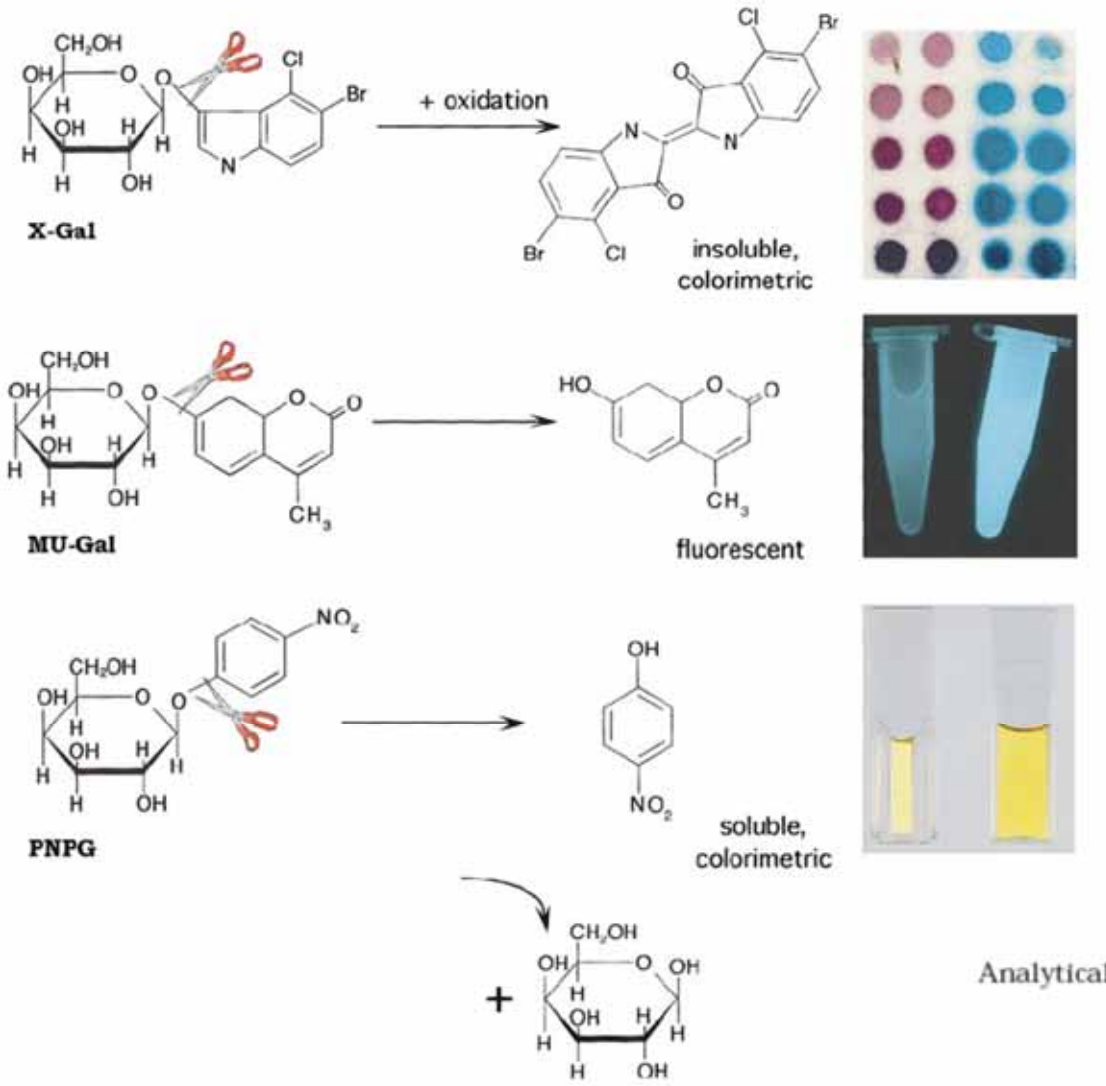
# β-Galactosidase

The enzyme that splits lactose into glucose and galactose. Coded by a gene (lacZ) in the lac operon of Escherichia coli.



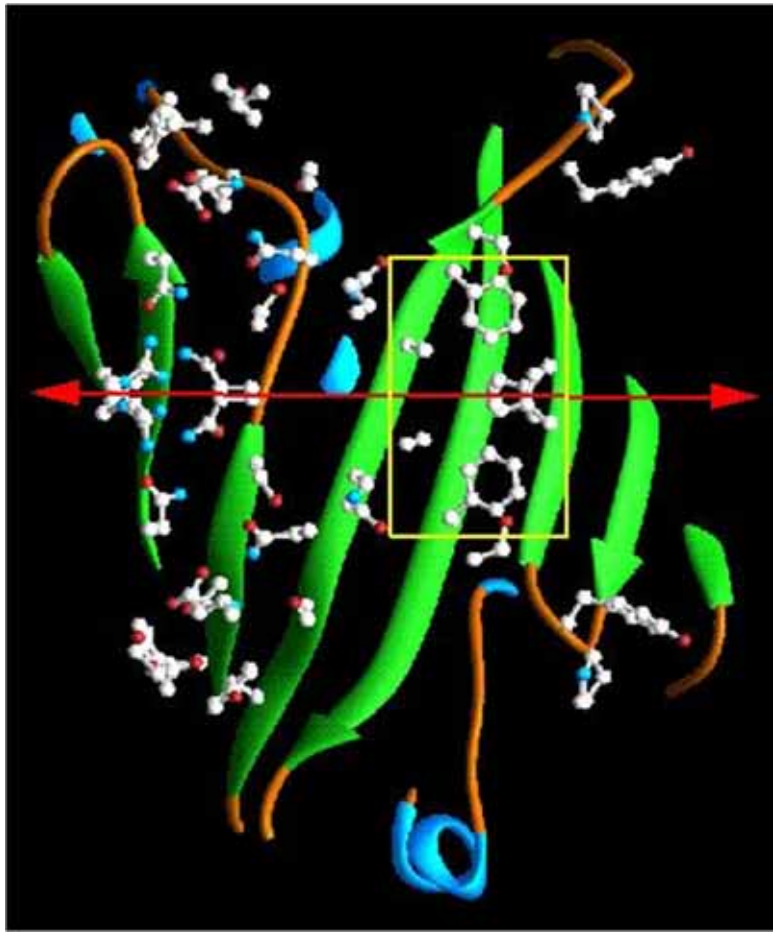

PUC is a family of plasmids that have an ampicillin resistance gene and more importantly a *lacZ* gene. A functional lacZ gene will produce the protein β - galactosidase. Bacterial colonies in which β - galactosidase is produced, will form blue colonies in the presence of the substrate 5 - bromo - 4 - chloro - 3 - indolyl - b - D - galactoside or as it is more commonly referred to, X-gal.



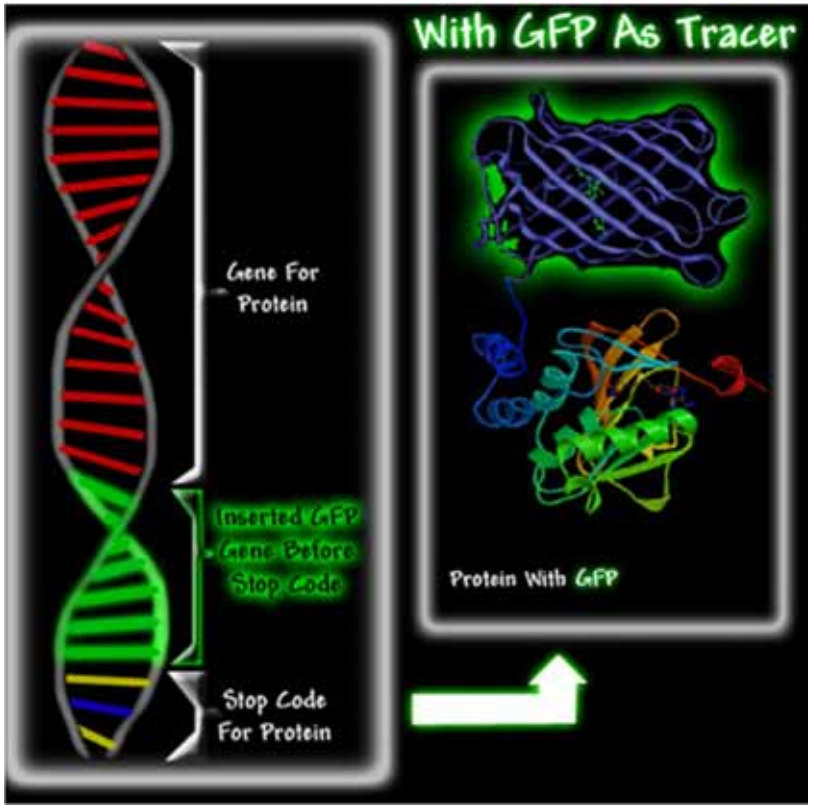

Analytical Biochemistry **285**, 1–15 (2000)

**FIG. 1.** Enzymatic function of β-galactosidase in cleaving indicator substrates. β-gal cleaves β-D-galactoside containing substrates with a diverse range of aglycone groups, targeting between the glycosyl oxygen and anomeric carbon as indicated (scissors). Substrates shown indicate commonly used indicators for assays on β-gal function on plates (X-Gal) or for liquid assay by measure of fluorescence (MU-Gal or MUG) or color (ONPG). Top left, X-Gal is 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and when cleaved and oxidized produces the insoluble dye 5-bromo-4-chloro-indigo, as described previously (22). Right panel, top, yeast colonies expressing β-gal and exposed to X-Gal (right half) or the closely related compound Magenta-Gal (left half, see Biosynth, Inc., or Diagnostic Chemicals Limited). Middle left, MUG is methylumbelliferyl-β-D-galactoside, and when cleaved by β-gal produces the fluorescent product methylumbelliferone (first described in (102)). Right panel, middle, shows yeast lysates expressing β-gal exposed to MUG, under long-wave UV. Bottom left, PNPG and ONPG are closely related nitrophenol-β-D-galactosides with similar assay properties, e.g., (103), whose cleavage releases the yellow product nitrophenol (right panel, bottom); PNPG is shown.

# Green Fluorescent Protein (GFP)

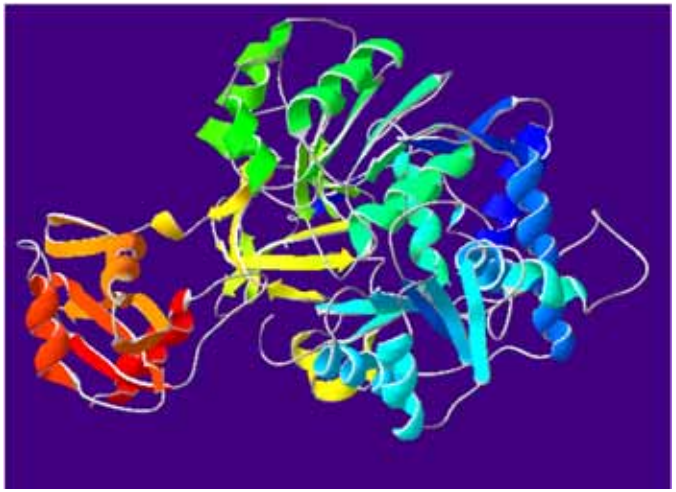


The **green fluorescent protein** (**GFP**) is a protein from the jellyfish *Aequorea victoria* that fluoresces green when exposed to blue light.



# GFP Rats

# Luciferase

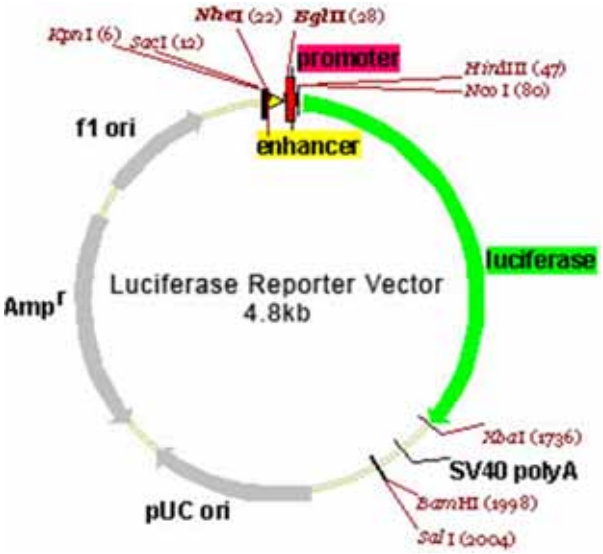


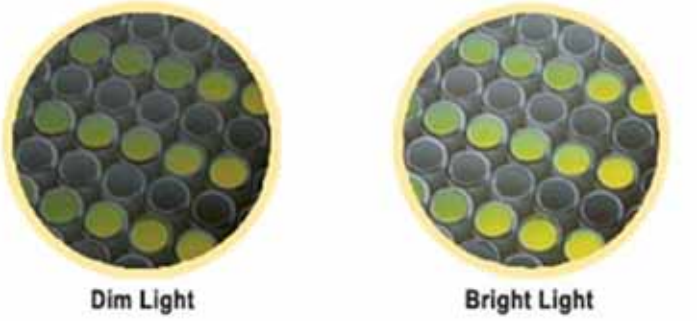

**Luciferase** is a generic name for enzymes commonly used in nature for bioluminescence. The name itself is derived from *Lucifer*, which means *light-bearer*. The most famous one is firefly luciferase from the firefly *Photinus pyralis*. In luminescent reactions, light is produced by the oxidation of a luciferin (a pigment), sometimes involving Adenosine triphosphate (ATP). The rates of this reaction between luciferin and oxygen are extremely slow until they are catalyzed by luciferase, often mediated by the presence of calcium ions (an analog of muscle contraction). The reaction takes place in two steps:

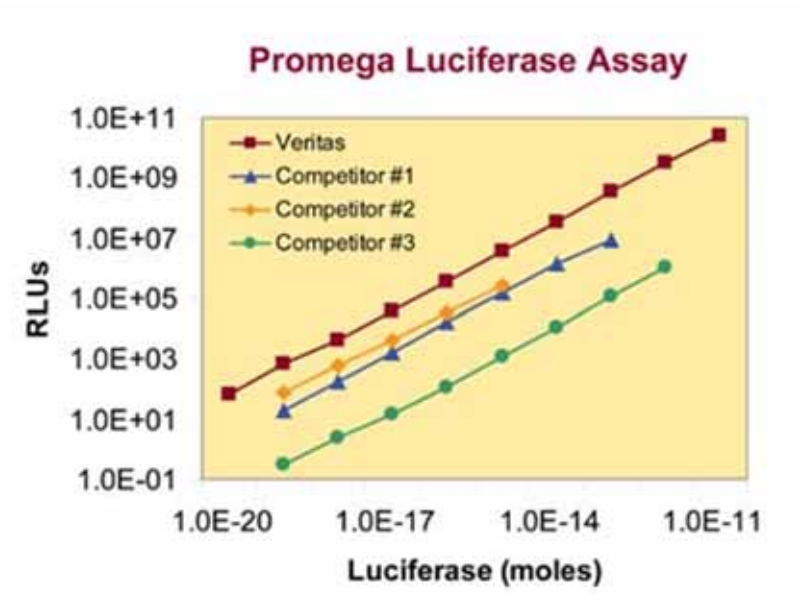
luciferin + ATP → luciferyl adenylate + PPi

luciferyl adenylate + O2 → oxyluciferin + AMP + light

# Luciferase


Nhe I (22)
Bgl II (28)
Kpn I (6)
Sac I (12)
promoter
Hind III (47)
Nco I (80)
f1 ori
enhancer
luciferase
Luciferase Reporter Vector
4.8kb
Amp^r
Xba I (1736)
SV40 polyA
BamHI (1998)
pUC ori
Sal I (2004)


Dim Light
Bright Light


Promega Luciferase Assay
1.0E+11
1.0E+09
1.0E+07
1.0E+05
1.0E+03
1.0E+01
1.0E-01
RLUs
Veritas
Competitor #1
Competitor #2
Competitor #3
1.0E-20
1.0E-17
1.0E-14
1.0E-11
Luciferase (moles)

# DNA Origami

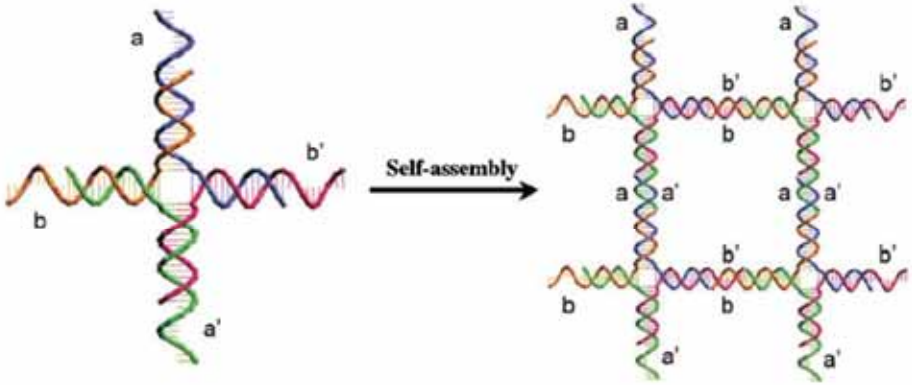
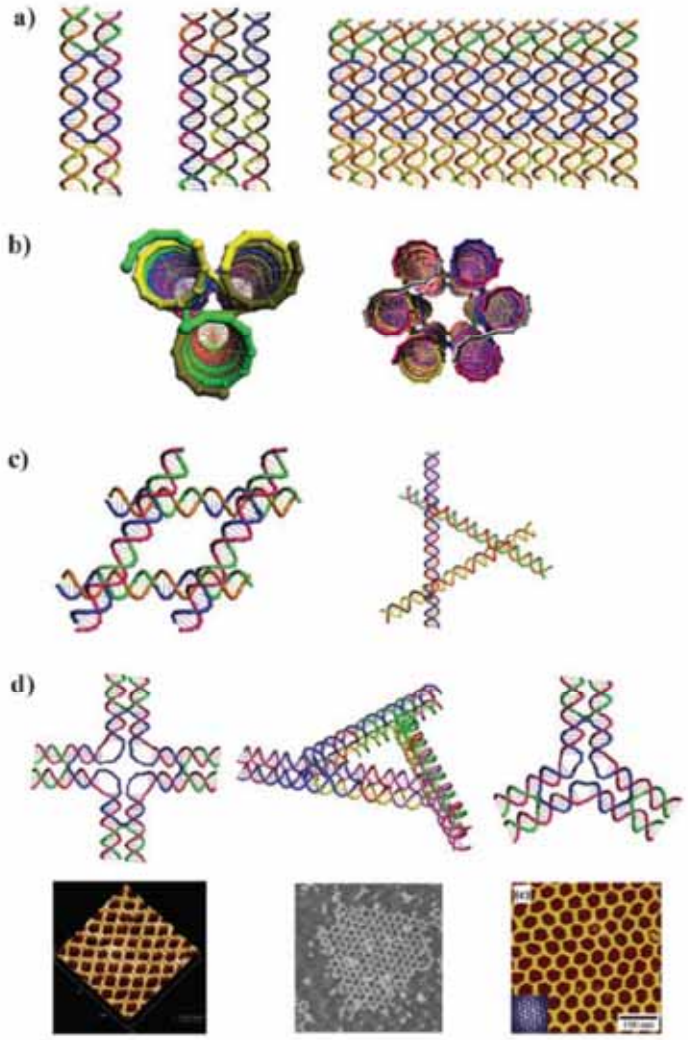


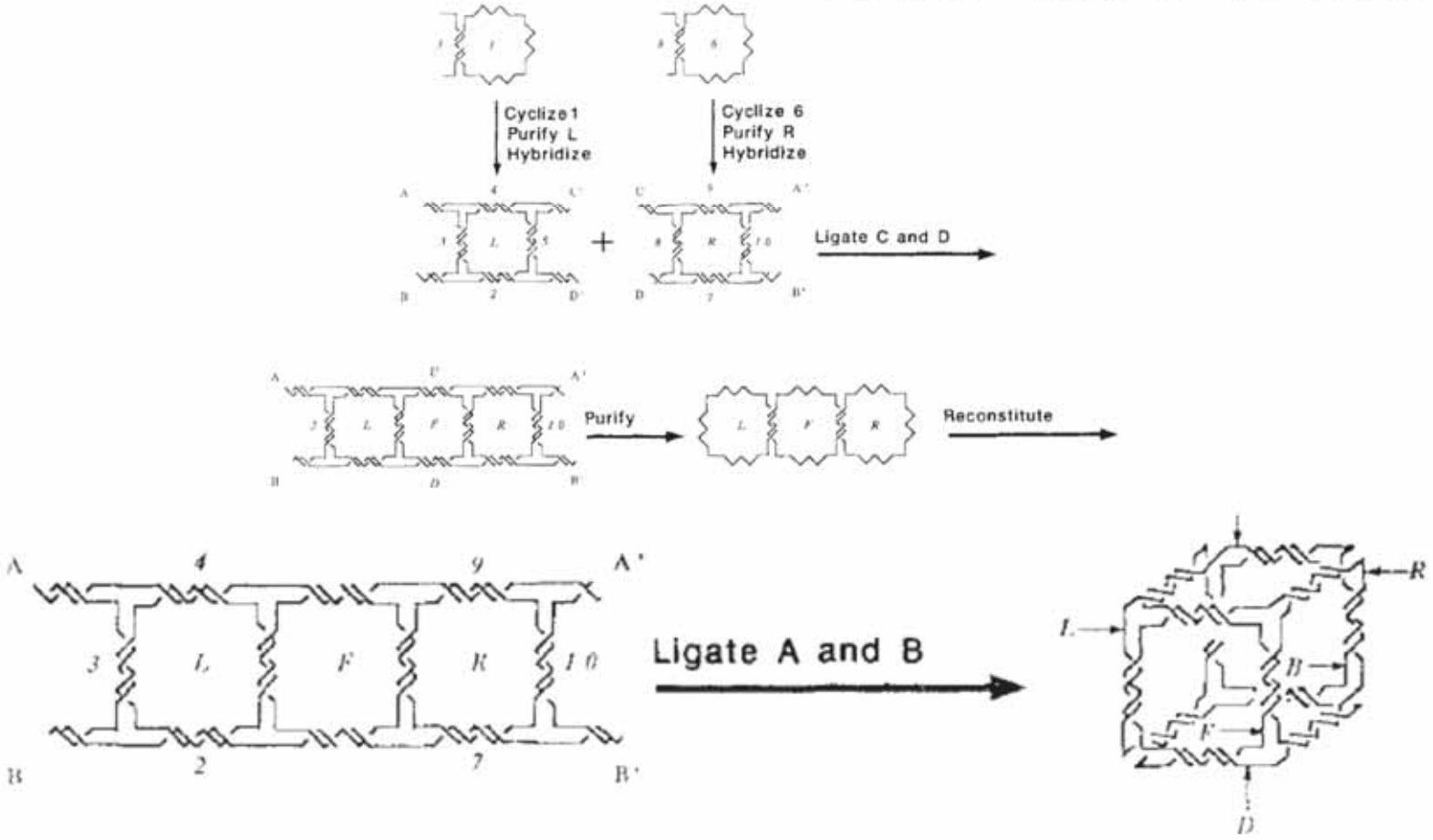

**Figure 1.** Key concept of DNA tile based self-assembly: combining branched DNA junction with sticky-end associations (e.g. a–a′ and b–b′ pairings) to self-assemble 2D lattices (adapted from ref. [1]). The DNA model was rendered using the Strata program (www.strata.com).



# Synthesis from DNA of a molecule with the connectivity of a cube

**Junghuei Chen & Nadrian C. Seeman**

NATURE · VOL 350 · 18 APRIL 1991



# Folding DNA to create nanoscale shapes and patterns

NATURE|Vol 440|16 March 2006

Paul W. K. Rothemund[1]

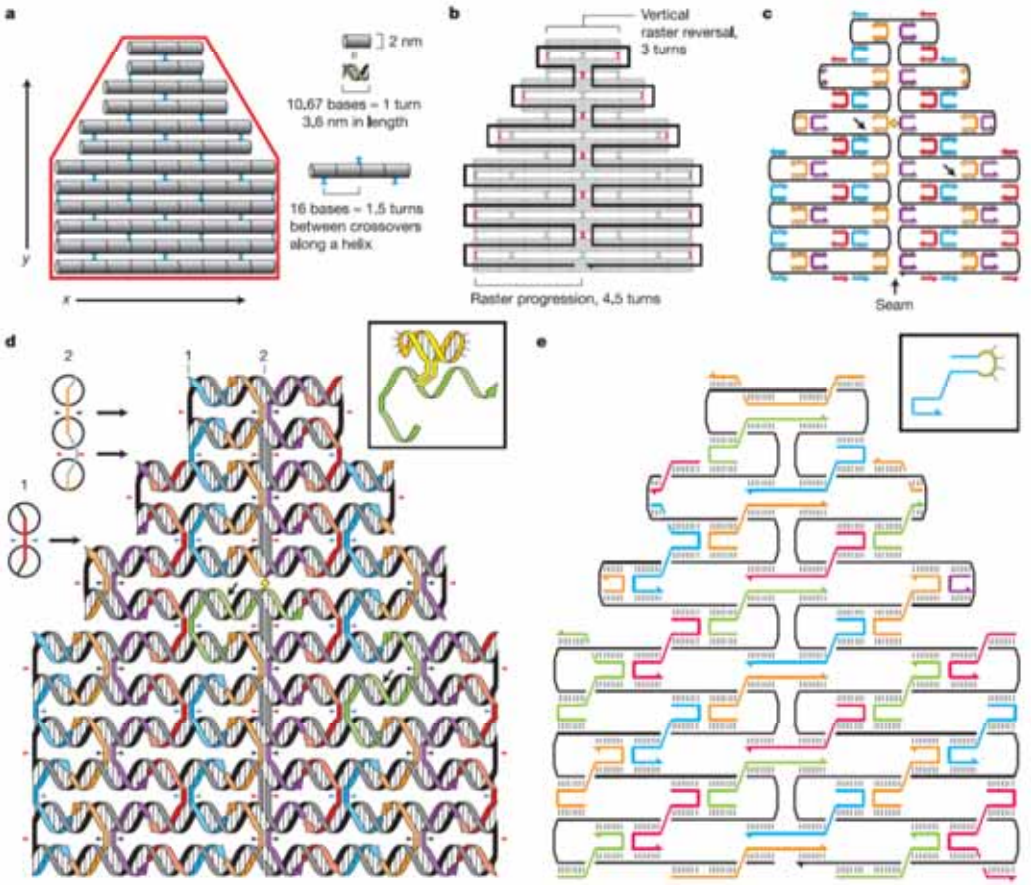
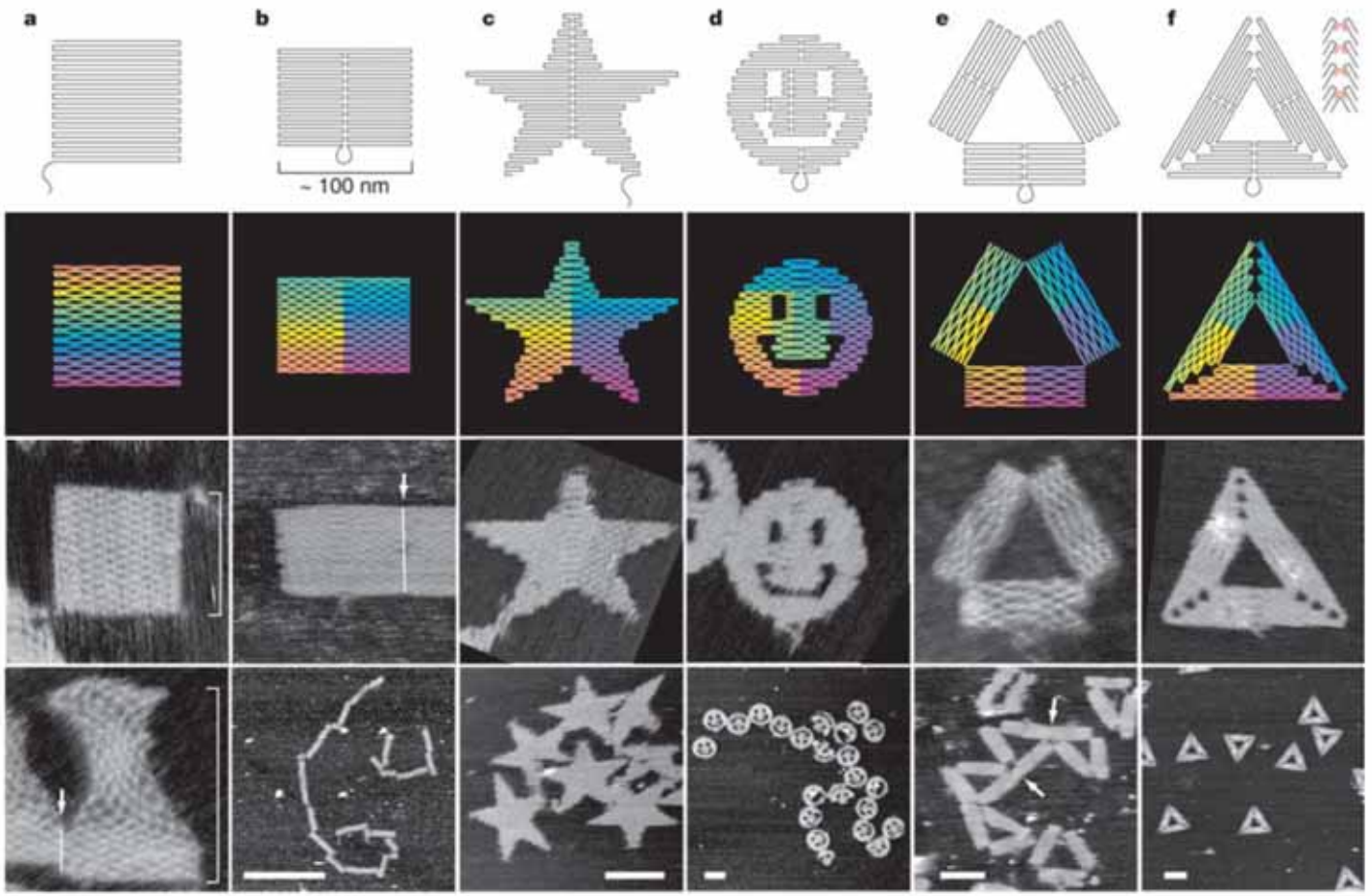
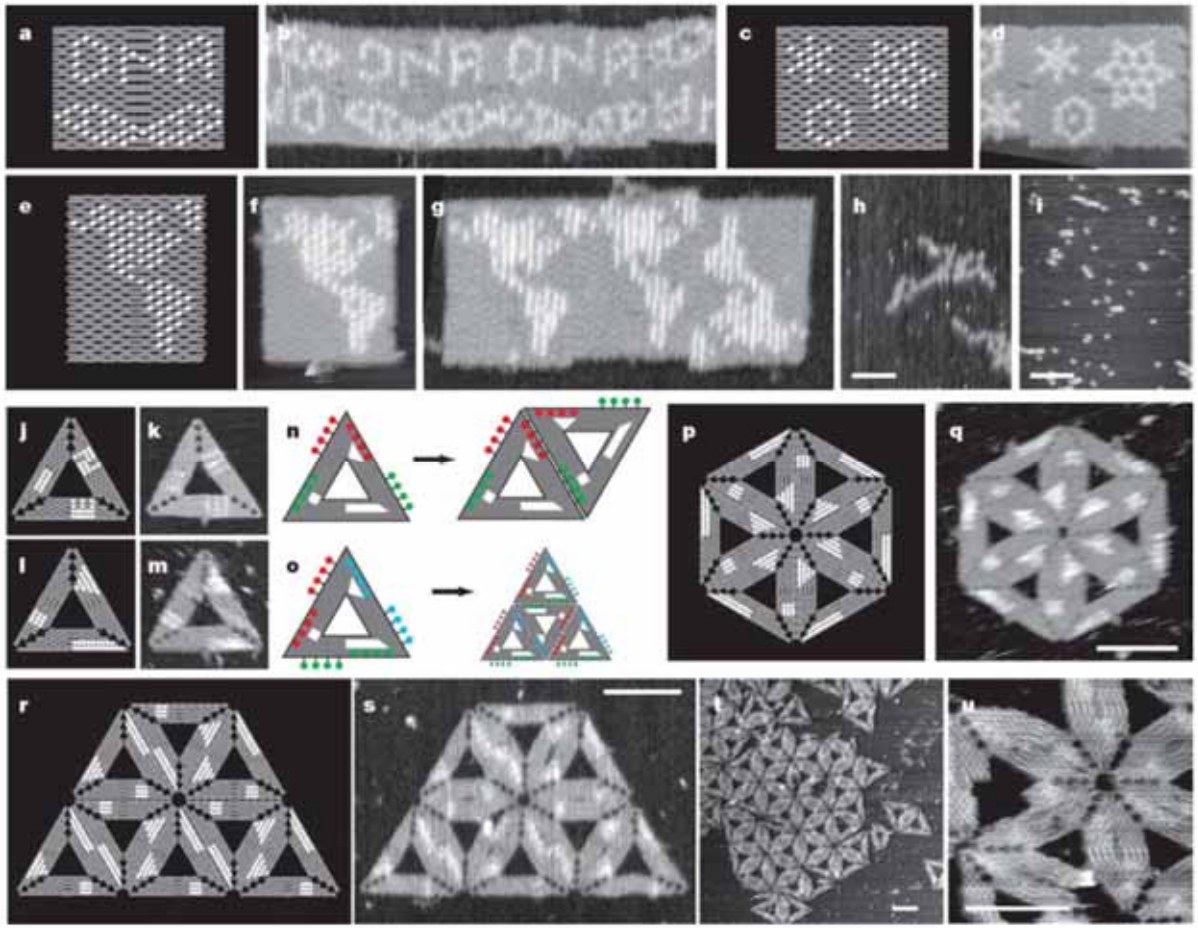



**Figure 1 | Design of DNA origami.** **a**, A shape (red) approximated by parallel double helices joined by periodic crossovers (blue). **b**, A scaffold (black) runs through every helix and forms more crossovers (red). **c**, As first designed, most staples bind two helices and are 16-mers. **d**, Similar to **c** with strands drawn as helices. Red triangles point to scaffold crossovers, black triangles to periodic crossovers with minor grooves on the top face of the shape, blue triangles to periodic crossovers with minor grooves on bottom. Cross-sections of crossovers (1, 2, viewed from left) indicate backbone positions with coloured lines, and major/minor grooves by large/small angles between them. Arrows in **c** point to nicks sealed to create green strands in **d**. Yellow diamonds in **c** and **d** indicate a position at which staples may be cut and resealed to bridge the seam. **e**, A finished design after merges and rearrangements along the seam. Most staples are 32-mers spanning three helices. Insets show a dumbbell hairpin (**d**) and a 4-T loop (**e**), modifications used in Fig. 3.


a
b
c
d
e
f
~ 100 nm


a
b
c
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e
f
g
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j
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l
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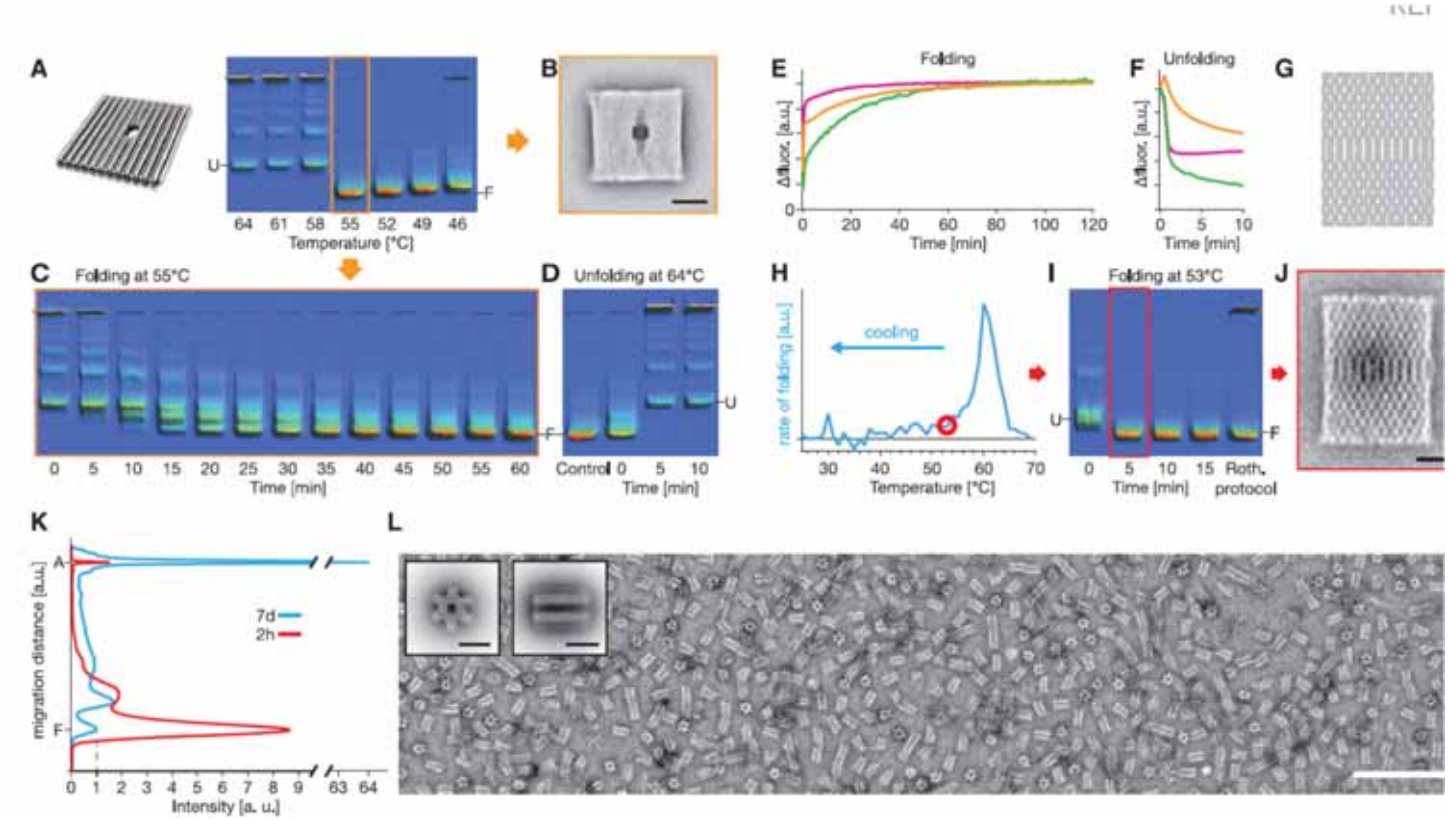
u

# Rapid Folding of DNA into Nanoscale Shapes at Constant Temperature

Jean-Philippe J. Sobczak *et al.*

*Science* **338**, 1458 (2012);
DOI: 10.1126/science.1229919



# DNA Origami as a Carrier for Circumvention of Drug Resistance

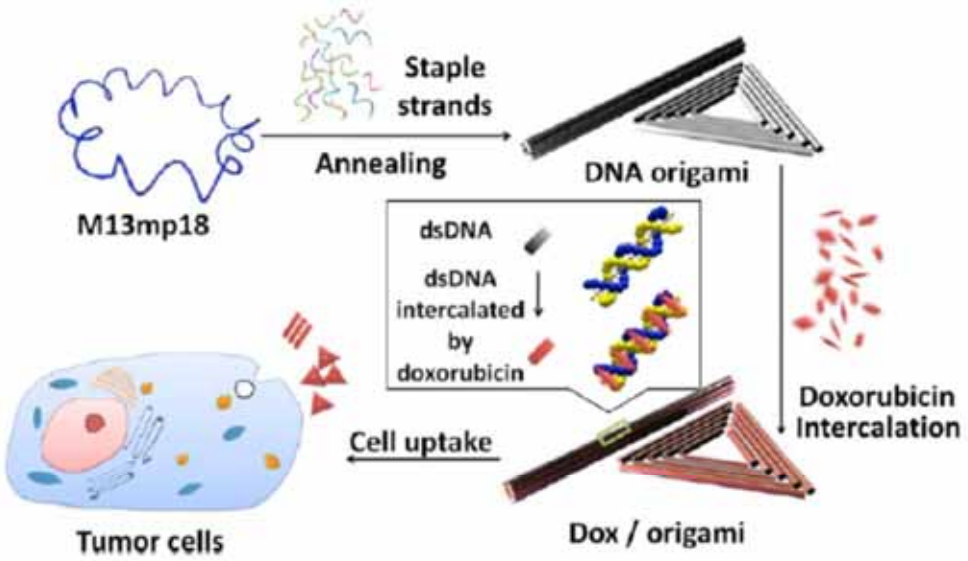
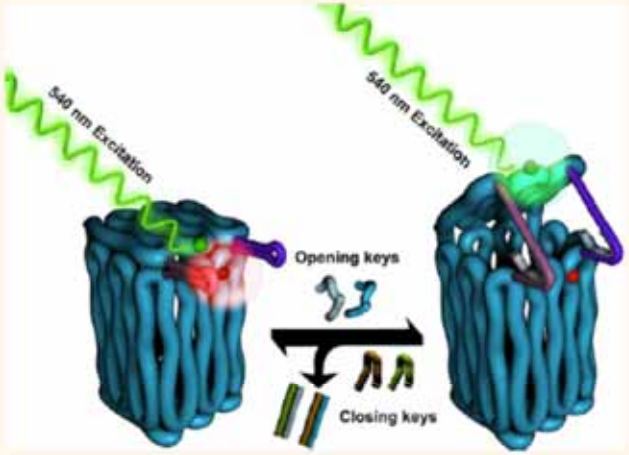



Figure 1. DNA origami and doxorubicin origami delivery system assembly. The long single-strand M13mp18 genomic DNA scaffold strand (blue) is folded into the triangle and tube structures through the hybridization of rationally designed staple strands. Watson–Crick base pairs in the double helices serve as docking sites for doxorubicin intercalation. After incubation with doxorubicin, the drug-loaded DNA nanostructure delivery vessels were administered to MCF 7 cells, and the effects were investigated.

dx.doi.org/10.1021/ja304263n | *J. Am. Chem. Soc.* 2012, 134, 13396–13403

# Construction of a 4 Zeptoliters Switchable 3D DNA Box Origami

**ABSTRACT** The DNA origami technique is a recently developed self-assembly method that allows construction of 3D objects at the nanoscale for various applications. In the current study we report the production of a 18 × 18 × 24 $nm^3$ hollow DNA box origami structure with a switchable lid. The structure was efficiently produced and characterized by atomic force microscopy, transmission electron microscopy, and Förster resonance energy transfer spectroscopy. The DNA box has a unique reclosing mechanism, which enables it to repeatedly open and close in response to a unique set of DNA keys. This DNA device can potentially be used for a broad range of applications such as controlling the function of single molecules, controlled drug delivery, and molecular computing.

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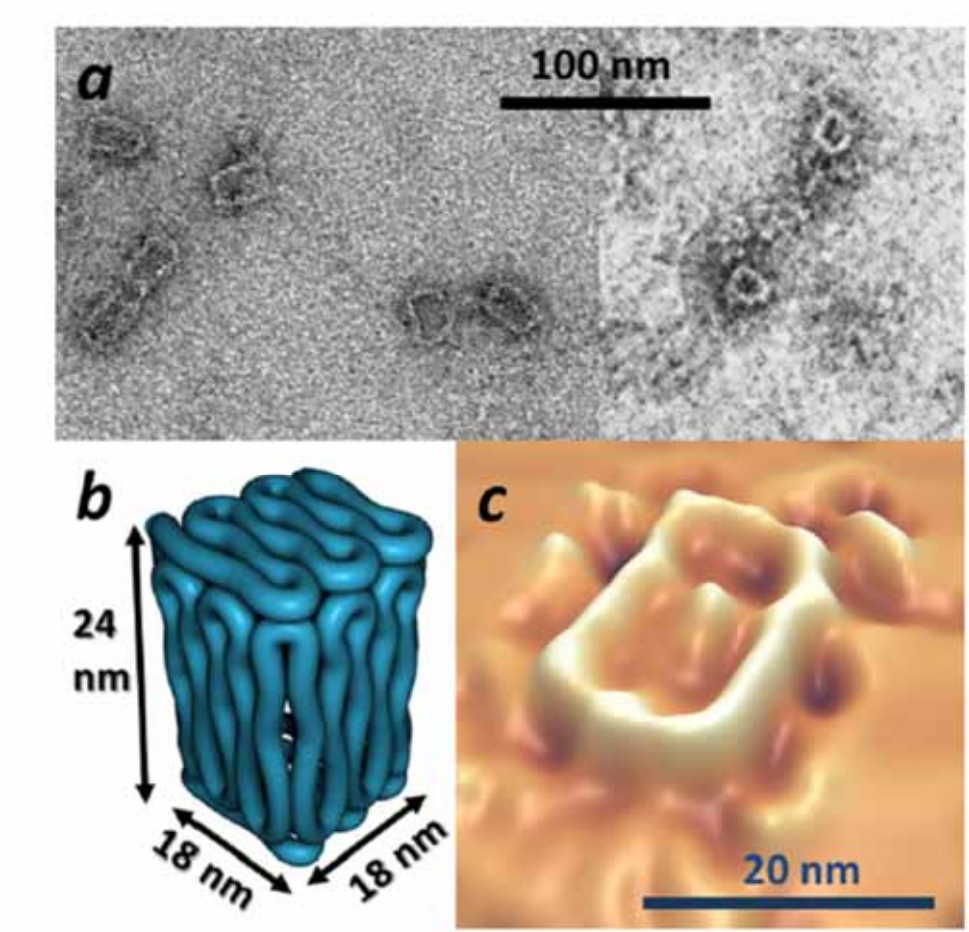
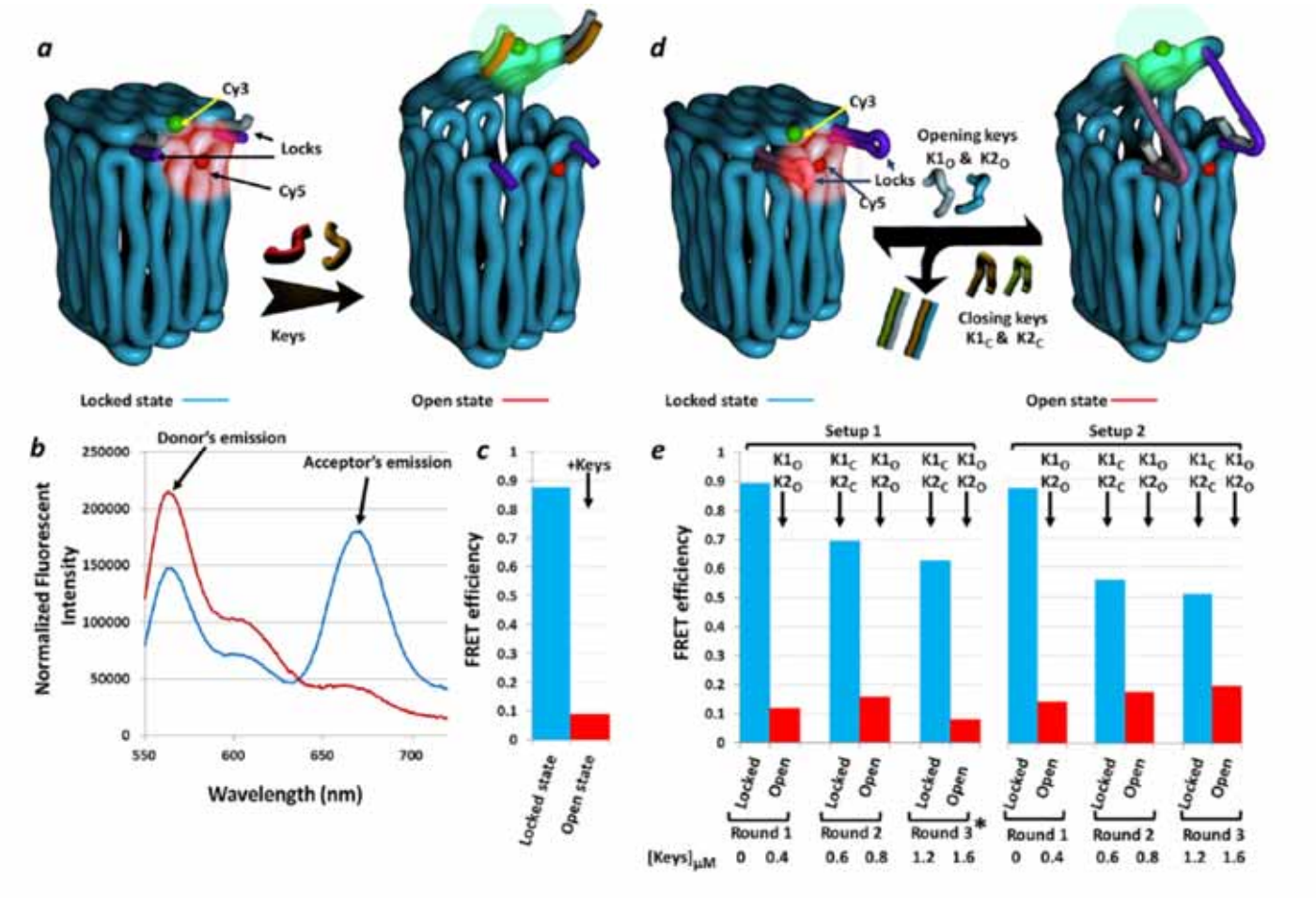

a
100 nm
b
24
nm
18 nm
18 nm
c
20 nm


a
Cy3
Locks
Cy5
Keys
Locked state
Open state
d
Cy3
Locks
Cy5
Opening keys
K1O & K2O
Closing keys
K1C & K2C
Locked state
Open state
b
Donor's emission
Acceptor's emission
Normalized Fluorescent Intensity
250000
200000
150000
100000
50000
0
550
600
650
700
Wavelength (nm)
c
+Keys
FRET efficiency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Locked state
Open state
e
Setup 1
Setup 2
K1O K2O
K1C K2C
FRET efficiency
Locked
Open
Round 1
Round 2
Round 3 *
[Keys]μM
0 0.4 0.6 0.8 1.2 1.6
0 0.4 0.6 0.8 1.2 1.6

# Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
  - Biocompatible
  - Non-immunogenic
  - Stable in blood stream
  - Protect DNA during transport
  - Small enough to extravagate
  - Cell and tissue specific

# Gene Therapy

- Gene therapy is a technique for correcting defective genes responsible for disease development. Researchers may use one of several approaches for correcting faulty genes:
  - **A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.**
  - **An abnormal gene could be swapped for a normal gene through homologous recombination.**
  - **The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.**
  - **The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.**

# How Gene Therapy Works?

- In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.
- Target cells such as the patient's liver or lung cells are infected with the viral vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

# Gene Delivery

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