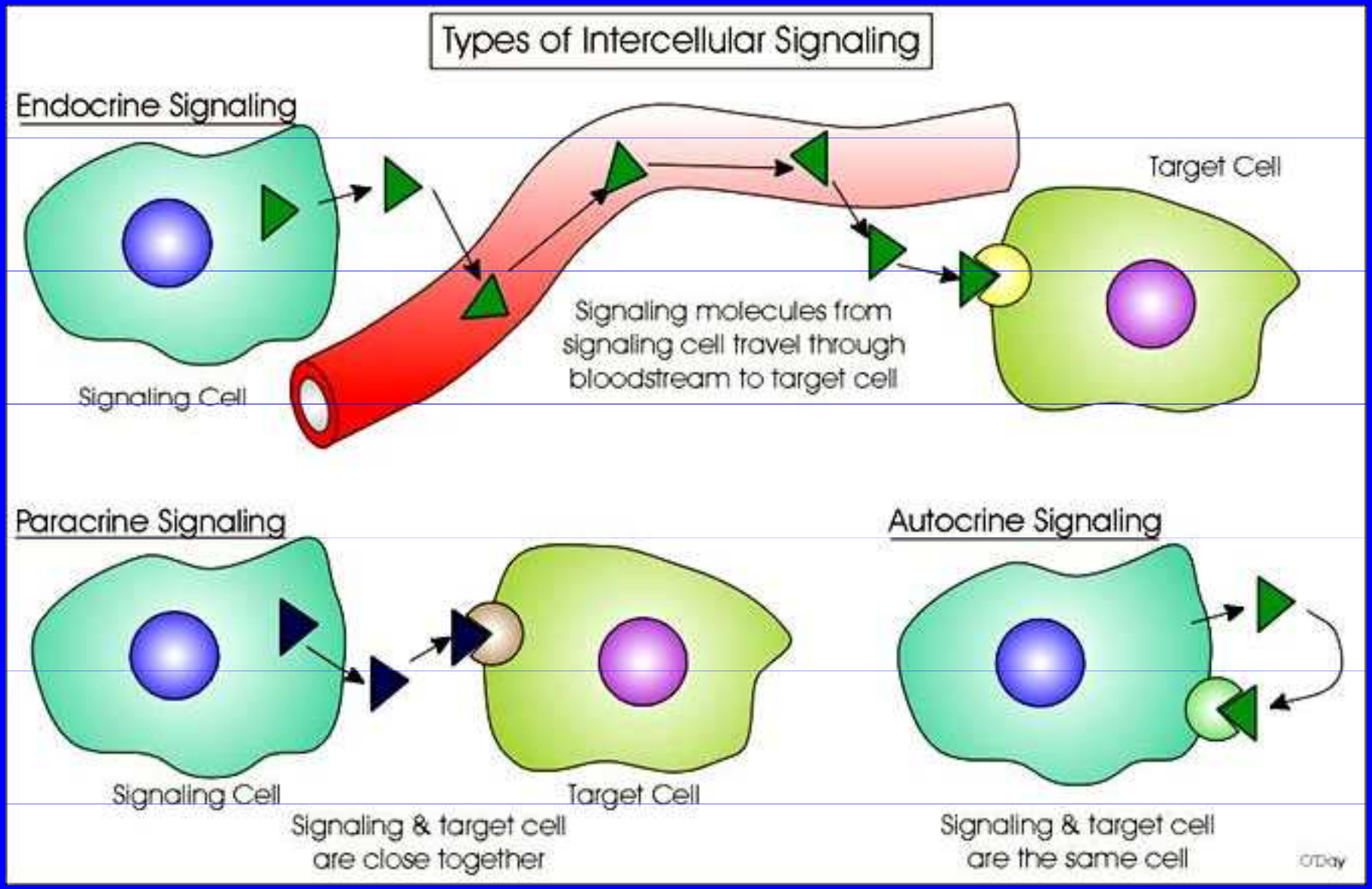
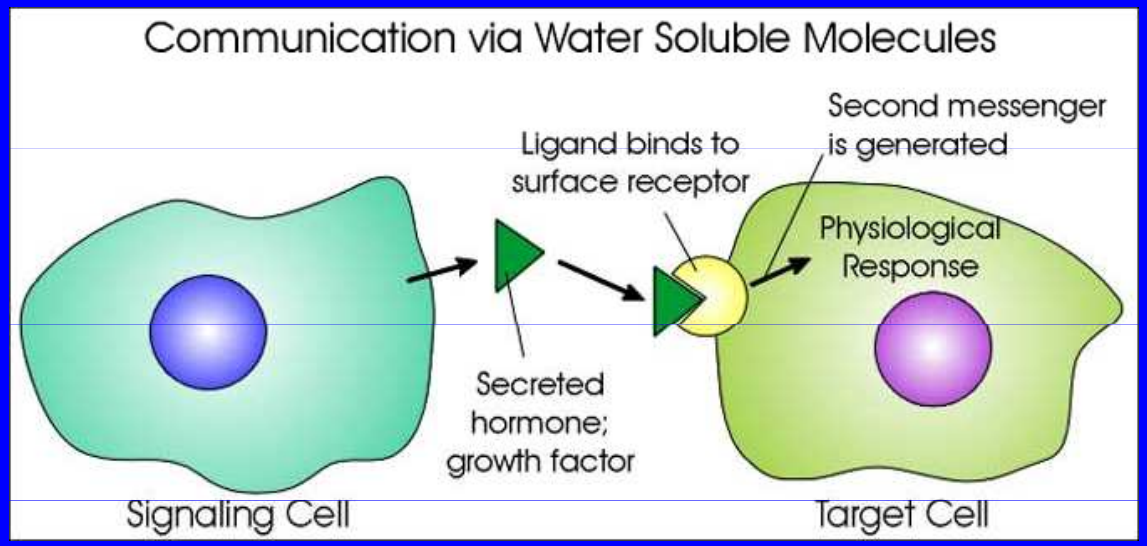
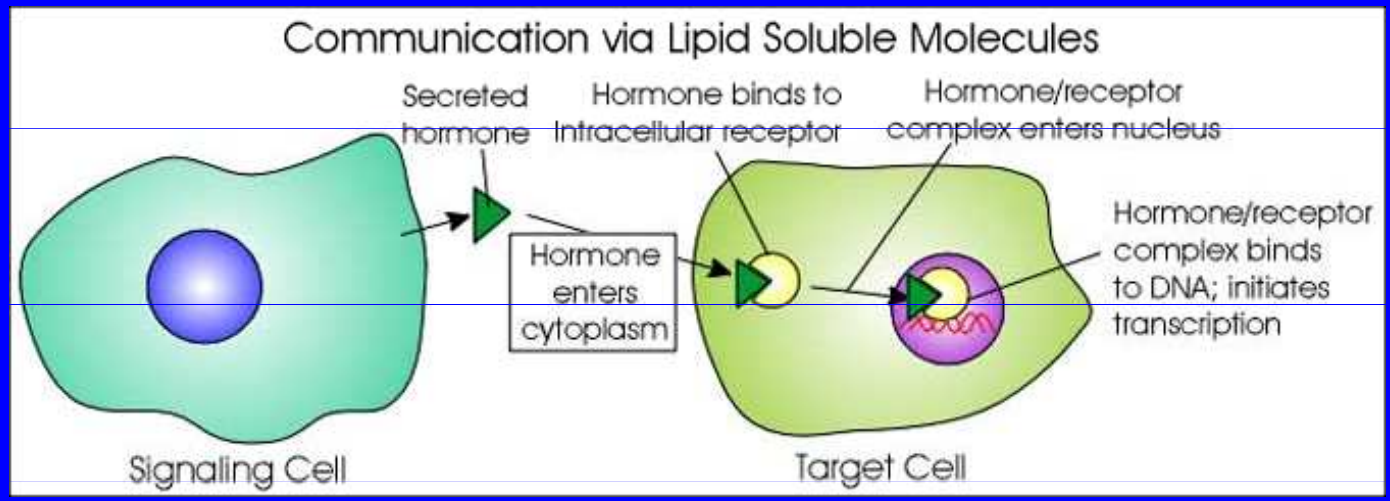
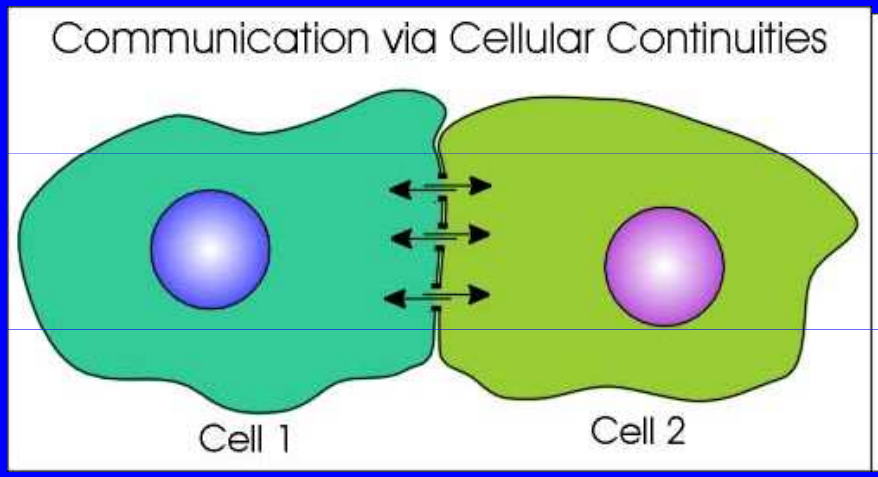
Cell communication & regulation - target of toxicants

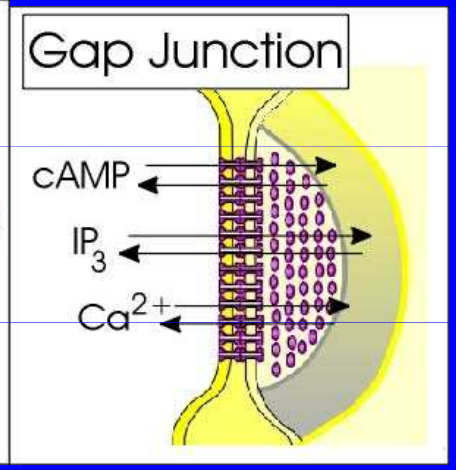


Cell communication









Signal transduction - target of toxicants

- Regulation of cell life / death (apoptosis)

- metabolism
- proliferation
- differentiation
- death (apoptosis)

- Signalling

- "network" of general pathways
- similar in all cells / different cell-specific effects

Signalling disruption

- Consequences of signalling disruption

- unwanted changes in proliferation/differentiation/apoptosis

-> cell transformation (carcinogenicity)

-> embryotoxicity

-> immunotoxicity

-> reproduction toxicity

.... *other chronic types of toxicity*

Signal transduction - principles

: major processes

– protein-(de)phosphorylation **(PKinases, PPases)**

- secondary messengers (cAMP / IP3, PIP2, DAG, Ca2+, AA)

1: Membrane receptors (G-protein, kinases)

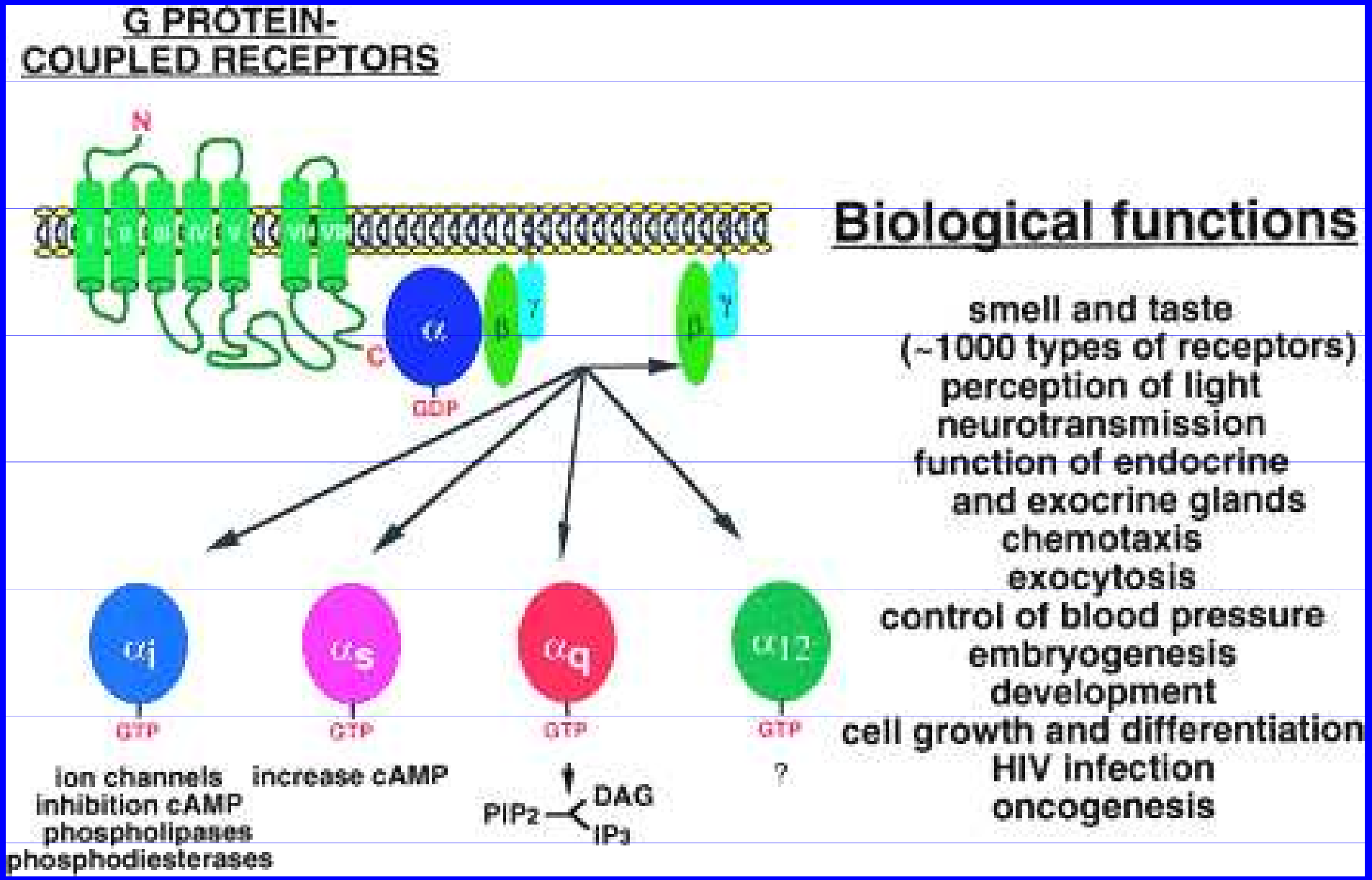
-> PKA activation: cAMP

2: Membrane receptors -> PLC / PKC activation

-> PKC activation: IP3, PIP2, DAG, Ca2+, AA

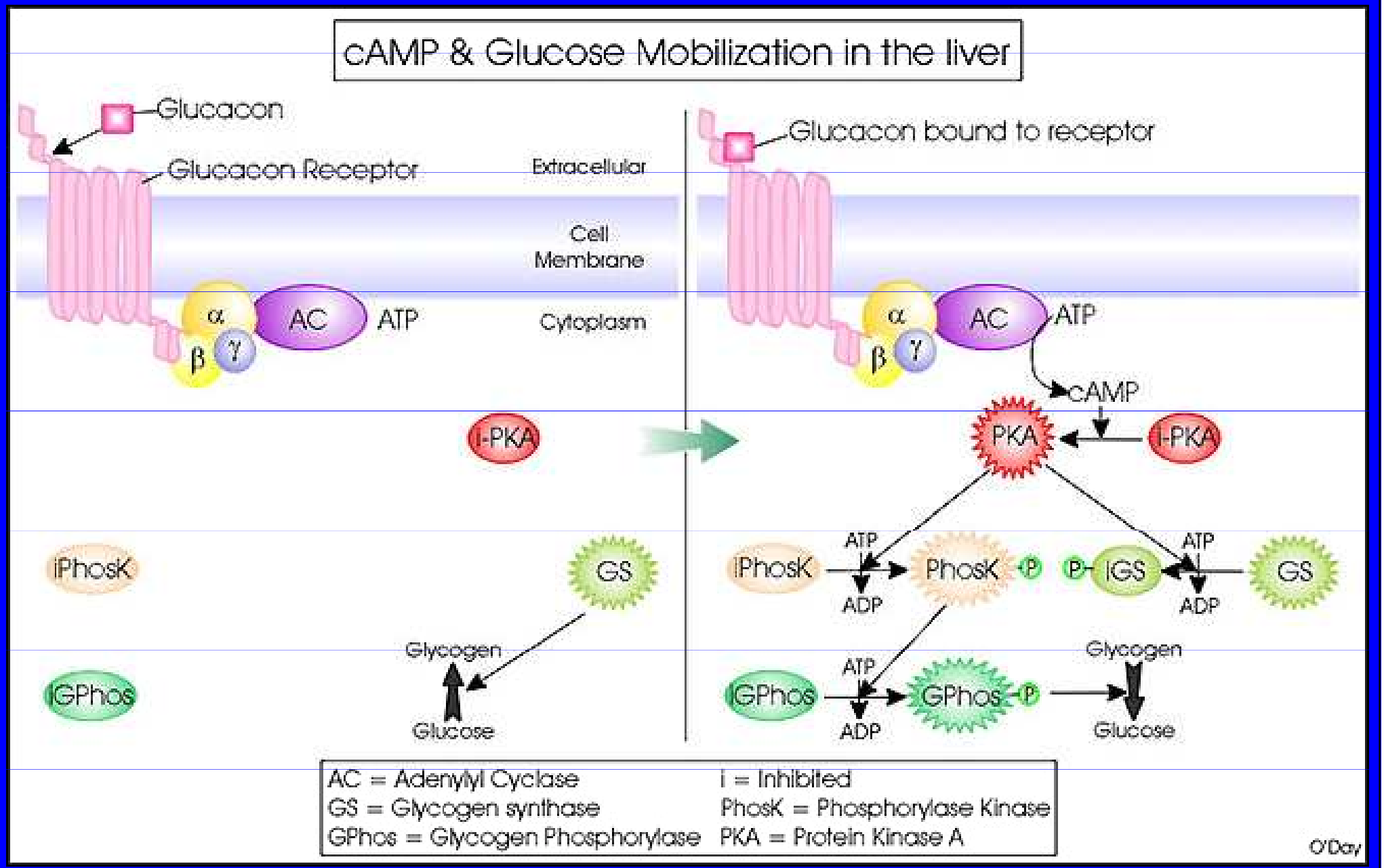
3: Cytoplasmic (nuclear) receptors

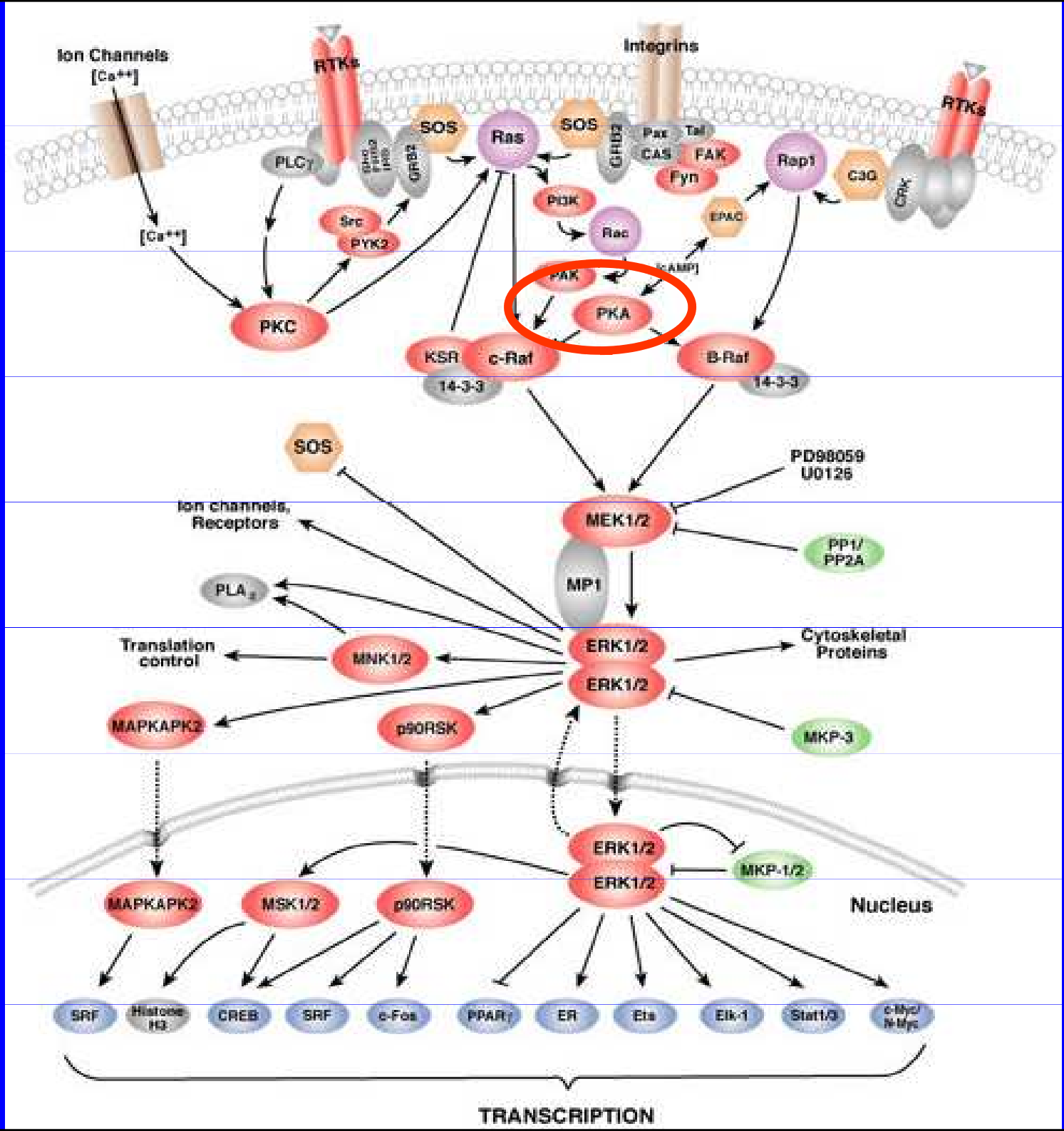
Membrane receptors (PKs): G-proteins



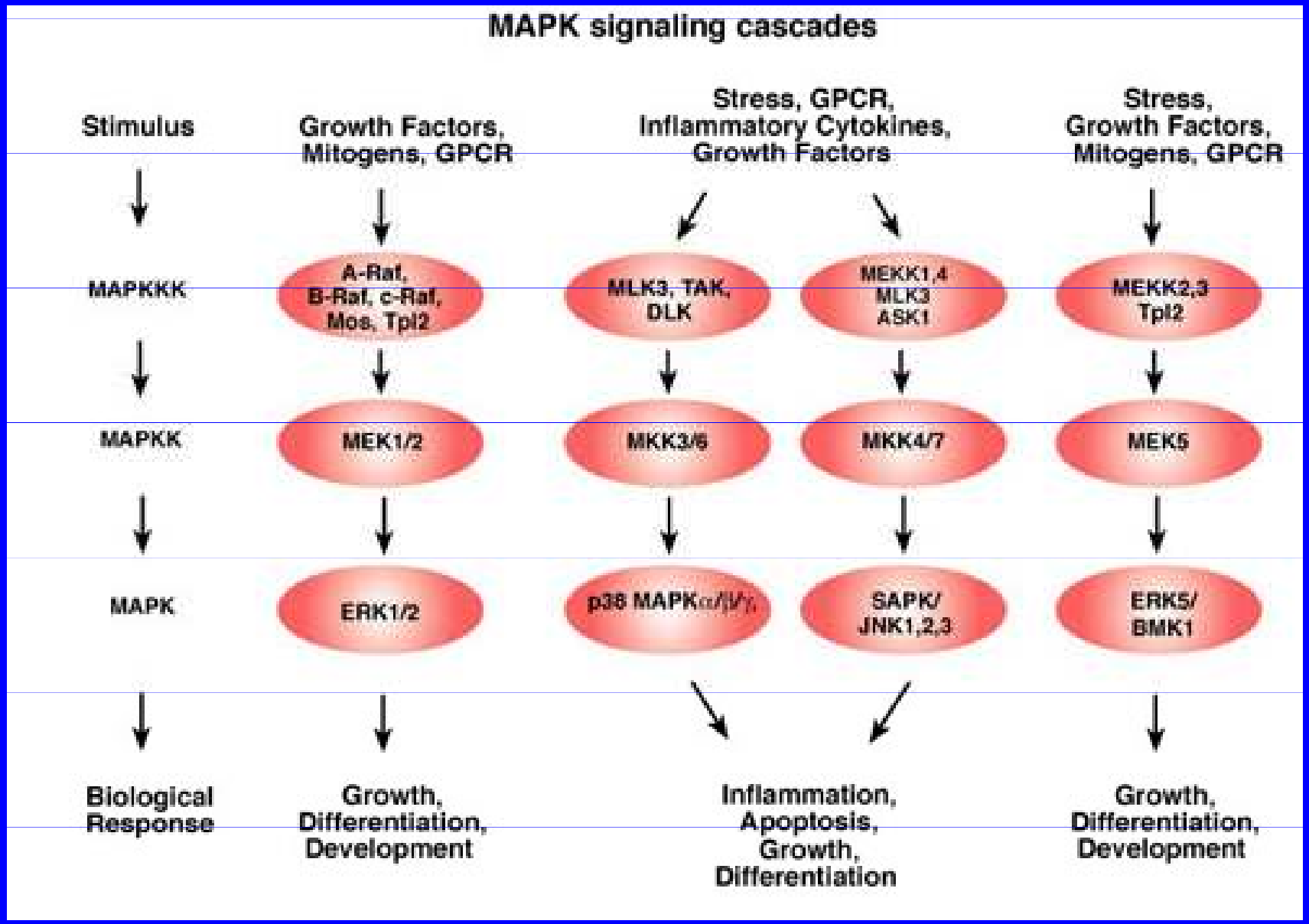
1: Membrane receptors (PKs)

-> Adenylate cyclase -> cAMP -> PKA – modulation




Ion Channels
[Ca++]
RTKs
Integrins
RTKs
SOS
Ras
SOS
PLCγ
GRB2
GRB2
Pax
Tal
CAS
FAK
Fyn
Rap1
C3G
CRK
PI3K
Rac
EPAC
Src
PYK2
[Ca++]
[cAMP]
PAK
PKA
PKC
KSR
c-Raf
B-Raf
14-3-3
14-3-3
SOS
PD98059
U0126
Ion channels,
Receptors
MEK1/2
PP1/
PP2A
MP1
PLA2
Translation
control
MNK1/2
ERK1/2
ERK1/2
Cytoskeletal
Proteins
MAPKAPK2
p90RSK
MKP-3
ERK1/2
ERK1/2
MKP-1/2
MAPKAPK2
MSK1/2
p90RSK
Nucleus
SRF
Histone
H3
CREB
SRF
c-Fos
PPARγ
ER
Ets
Elk-1
Stat1/3
c-Myc/
N-Myc
TRANSCRIPTION

Mitogen Activated Protein Kinases (MAPK) – dependent effects

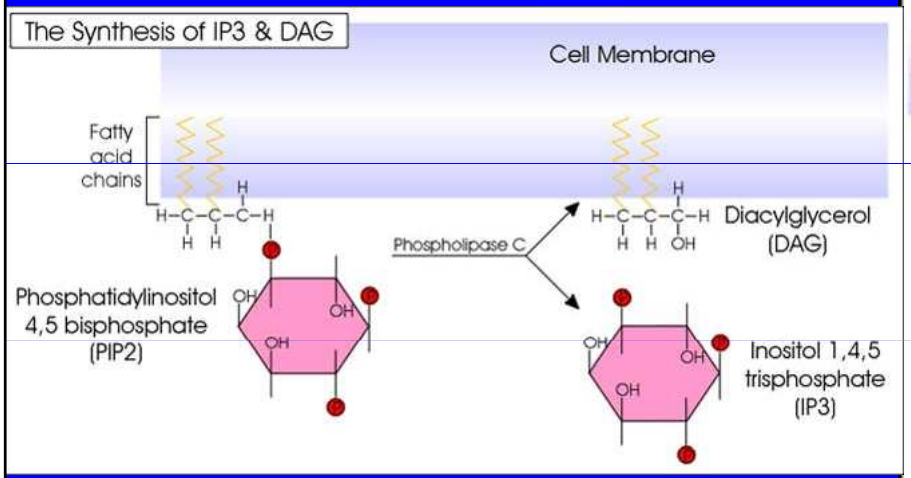


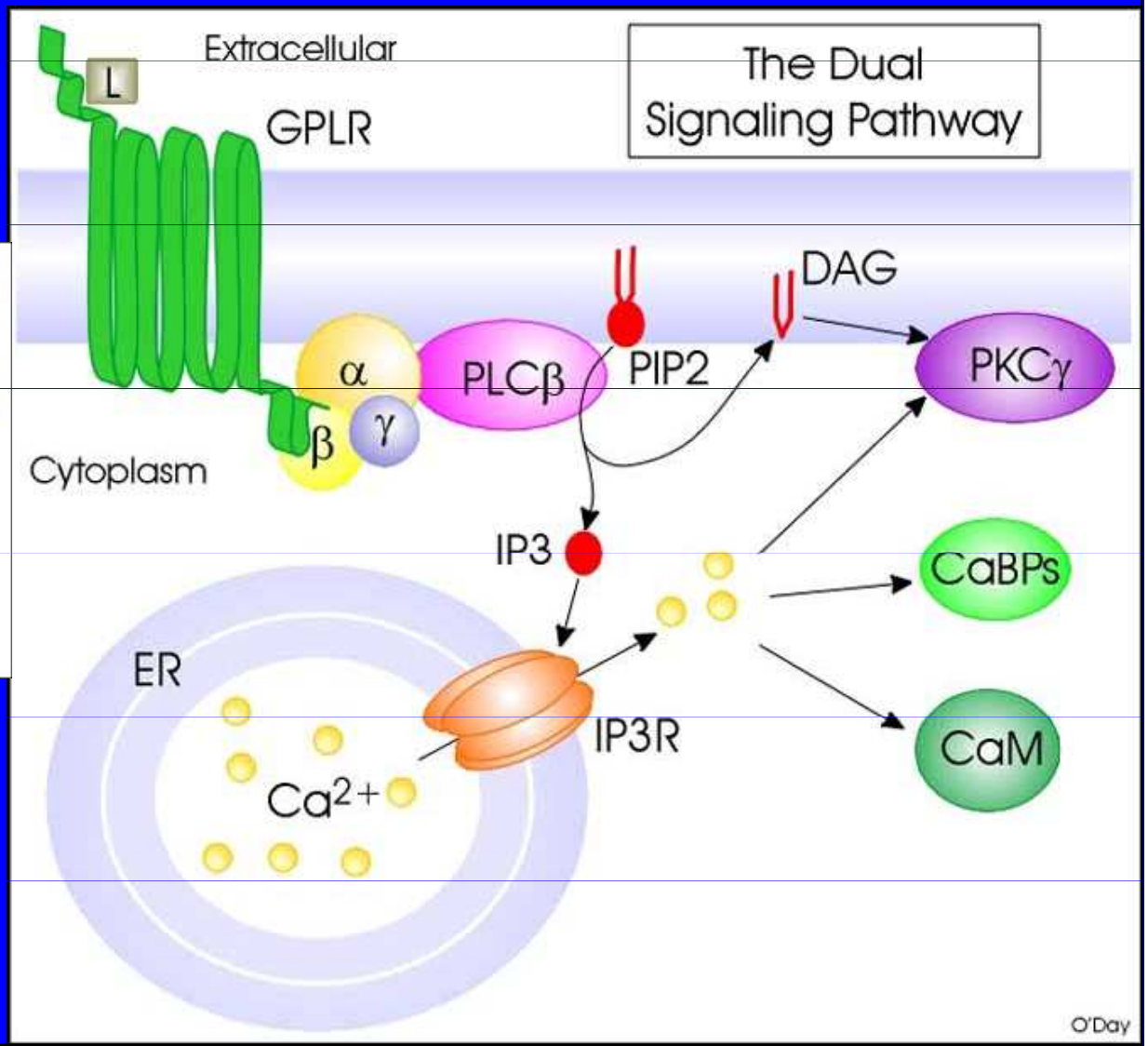
2: Membrane receptors

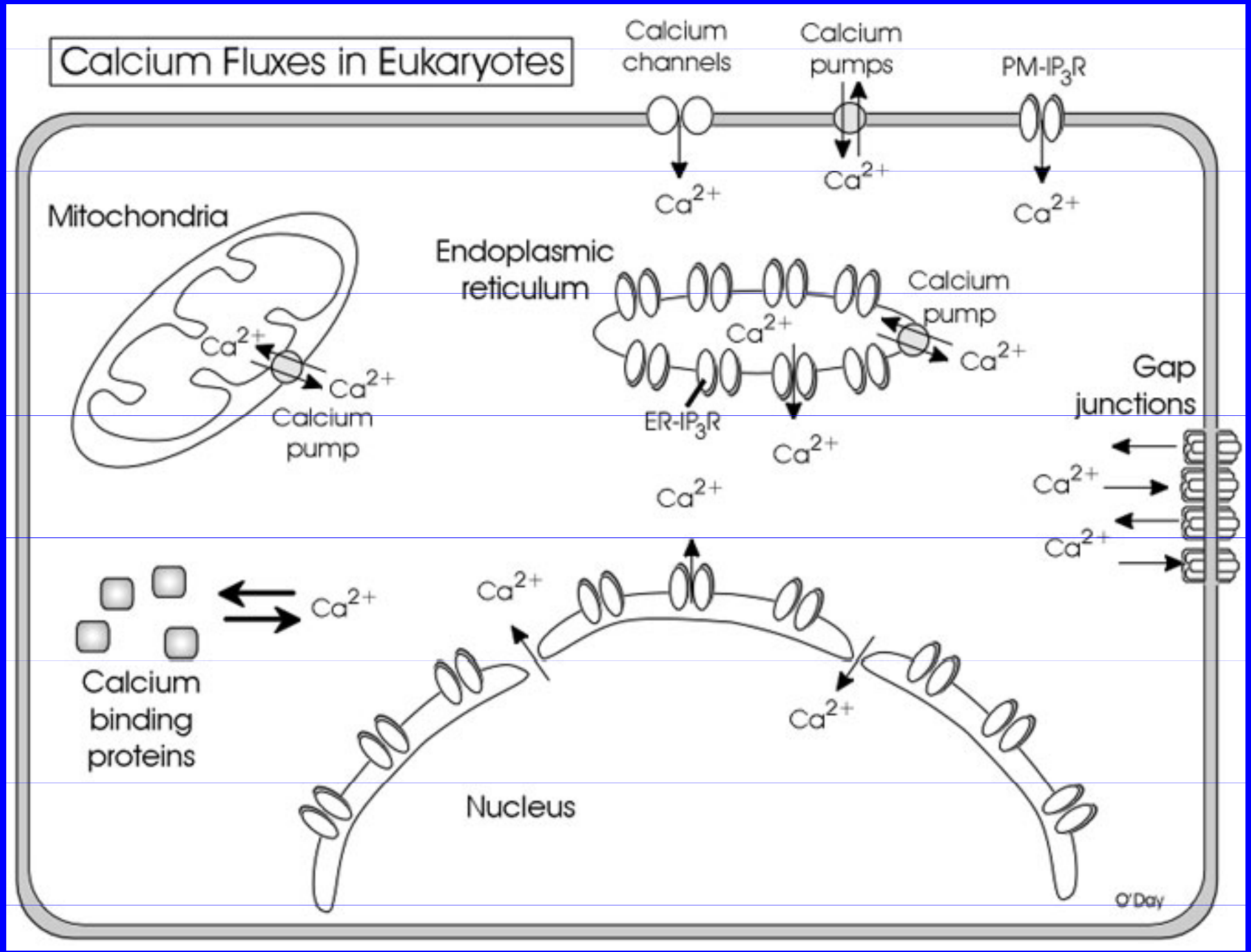
-> Phospholipase C:

PIPs -> DAG -> PKC / arachidonic acid

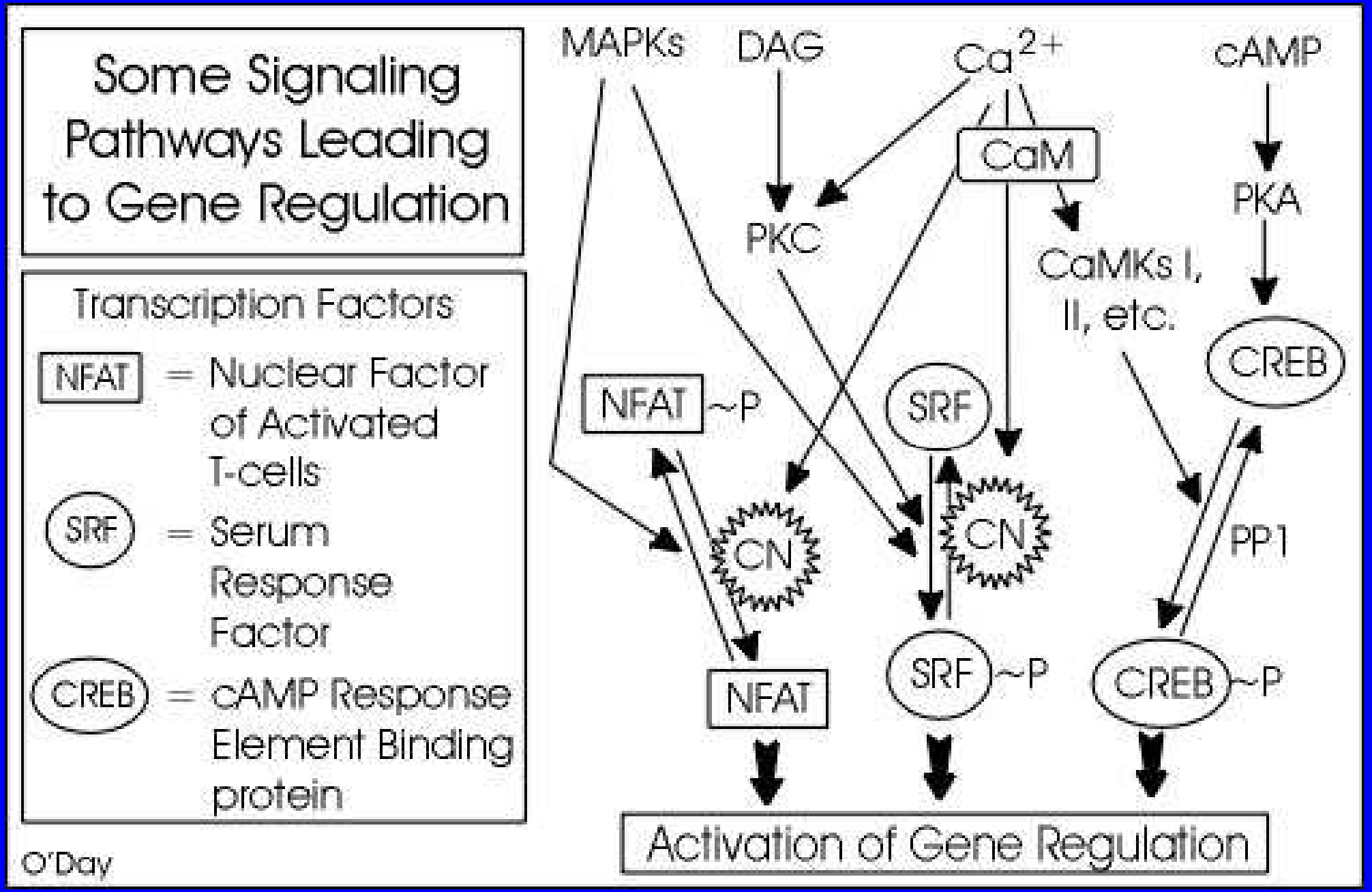
+ IP3 -> Ca^{2+}






Calcium Fluxes in Eukaryotes
Calcium channels
Calcium pumps
PM-IP3R
Ca2+
Ca2+
Ca2+
Mitochondria
Ca2+
Ca2+
Calcium pump
Endoplasmic reticulum
Calcium pump
Ca2+
Ca2+
ER-IP3R
Ca2+
Ca2+
Gap junctions
Ca2+
Ca2+
Ca2+
Calcium binding proteins
Ca2+
Ca2+
Ca2+
Nucleus
O'Day

Crosstalk


Some Signaling Pathways Leading to Gene Regulation
Transcription Factors
NFAT = Nuclear Factor of Activated T-cells
SRF = Serum Response Factor
CREB = cAMP Response Element Binding protein
O'Day
MAPKs
DAG
Ca 2+
cAMP
CaM
PKC
PKA
CaMKs I, II, etc.
CREB
NFAT ~P
SRF
CN
CN
PP1
NFAT
SRF ~P
CREB ~P
Activation of Gene Regulation

Examples

ER-dependent estrogenicity (DDE) [other lecture]

xenoestrogenicity, binding to ER + activation

ER-independent estrogenicity (PAHs)

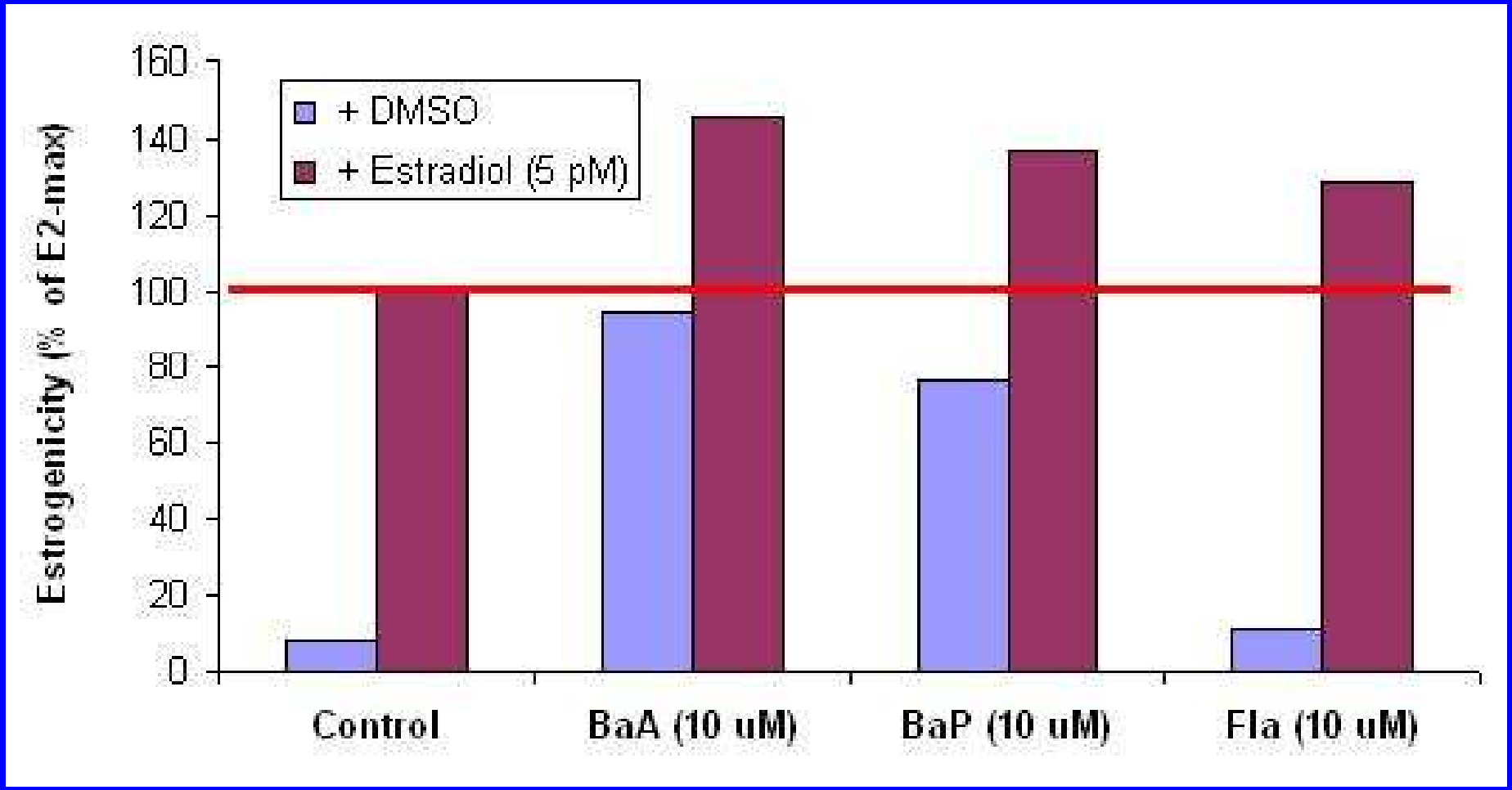
modulation of PKs/PPases: phosphorylation
-> activation of ER-dependent genes

AhR-dependent anti-estrogenicity, retinoid toxicity

modulation of estrogen / retinoid levels
[other lectures]

AhR -> CYPs -> steroid-metabolism
PAHs/POPs -> inhibition of Aromatase (CYP19)

PAHs significantly potentiate the effect of 17β-estradiol (*via increased phosporylation of ER)*



Vondráček et al. 2002 Toxicol Sci 70(2) 193

Examples

Microcystins -> liver tumor promotion

inhibition of PPases [other lecture]

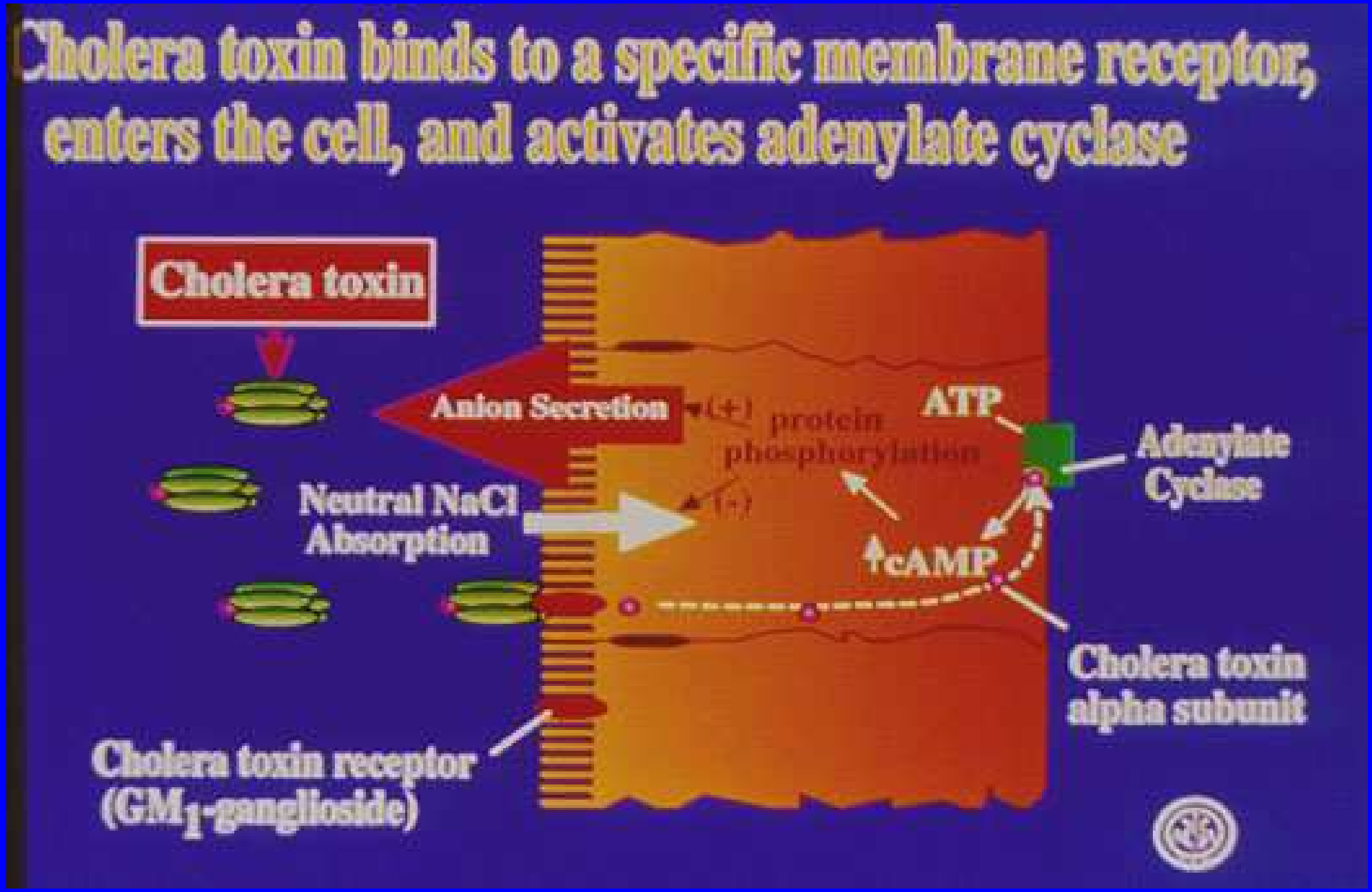
Immunotoxicity

- (Cyano)bacterial lipopolysaccharides, heavy metals ...
- Cholera toxin
  - AC: cAMP -> effects

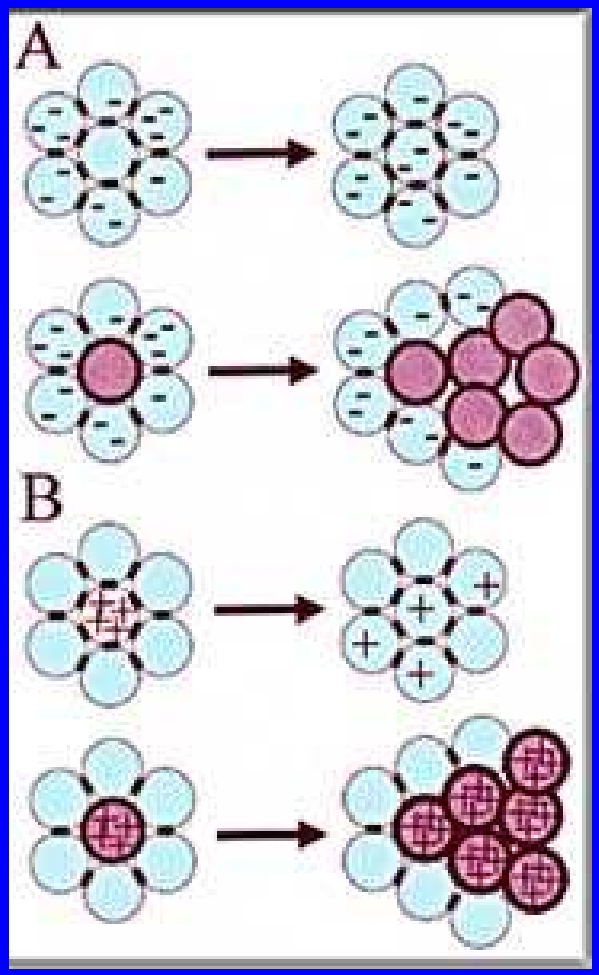
PAHs -> Inhibition of Gap-junctions

- Gap-junctional intercellular communication

Cholera toxin binds to a specific membrane receptor, enters the cell, and activates adenylate cyclase



Inhibition of GJIC - biomarker of tumor promotion

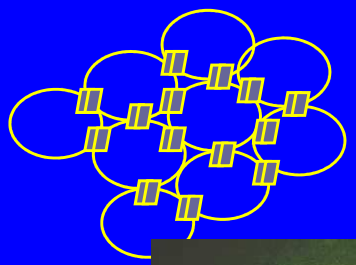


- gap-junctional intercellular communication (GJIC) - transfer of signalling molecules via protein channels (*gap junctions)*
- regulation of proliferation, differentiation, apoptosis
- inhibition of GJIC -> proliferation ~ tumor promotion
- **relevance: tumors *in vivo* have inhibited gap-junctions**

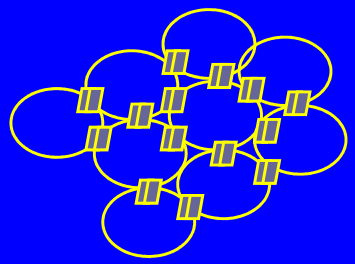
from Trosko and Ruch 1998, Frontiers in Bioscience 3:d208

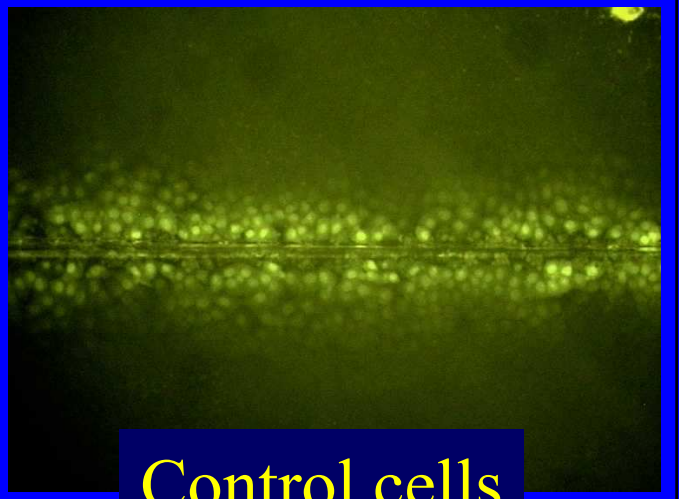
Scrape loading / dye transfer assay (GJIC inhibition)

Rat liver WB-F344 (normal stem-like cells)

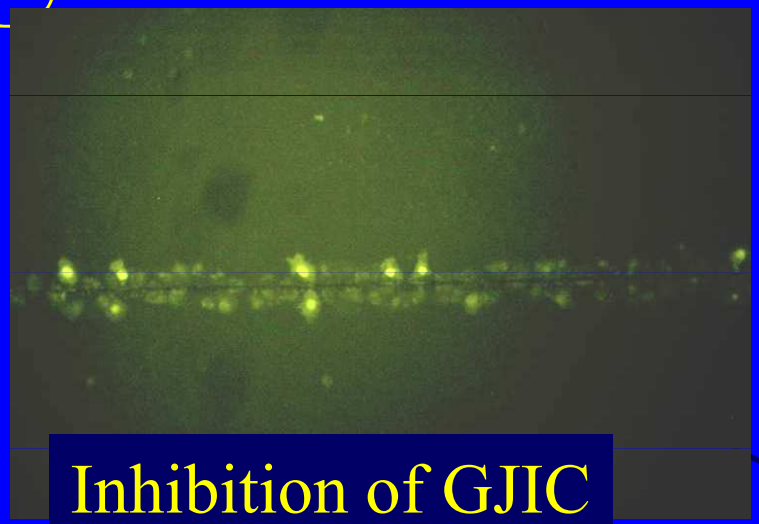


Exposure (30 min)
standards, samples



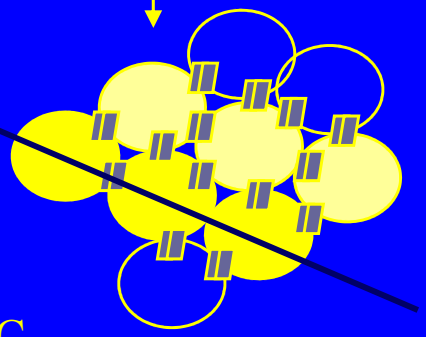


Control cells



Inhibition of GJIC

- Wash
- Fluorescence dye (lucifer yellow)
- Scrape of the cells with the razor blade

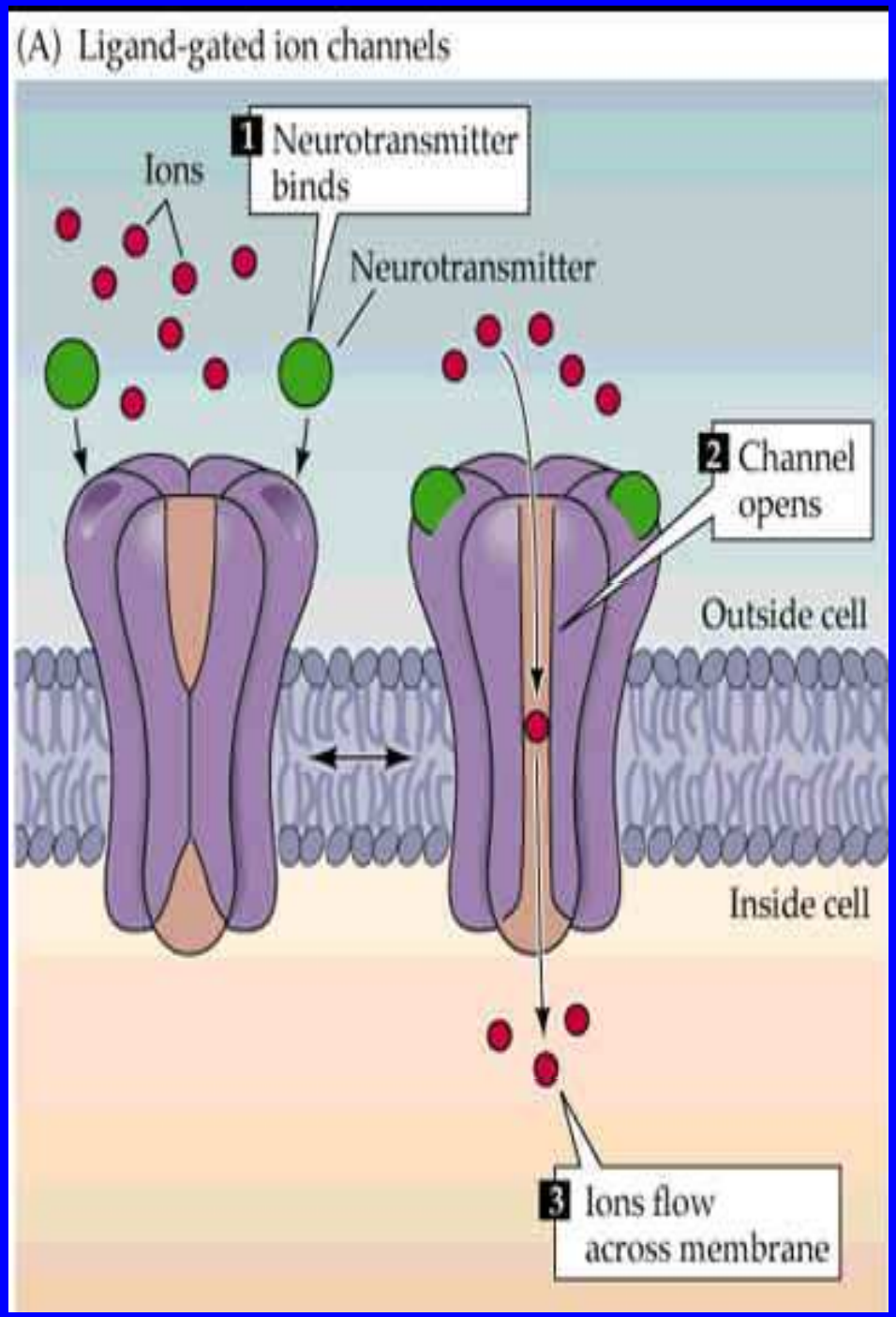


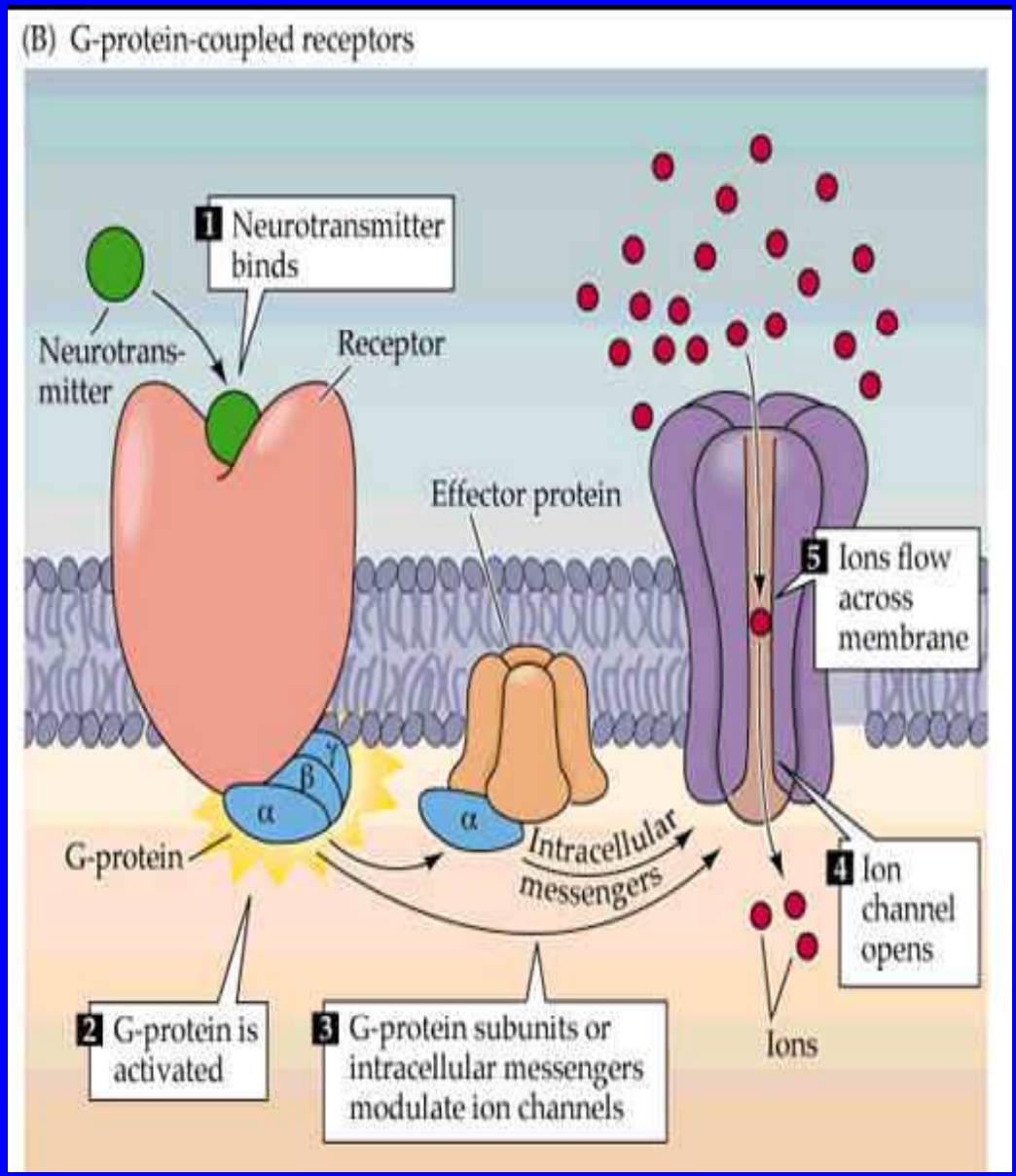
Fluorescence microscopy
diffusion length ~ level of GJIC

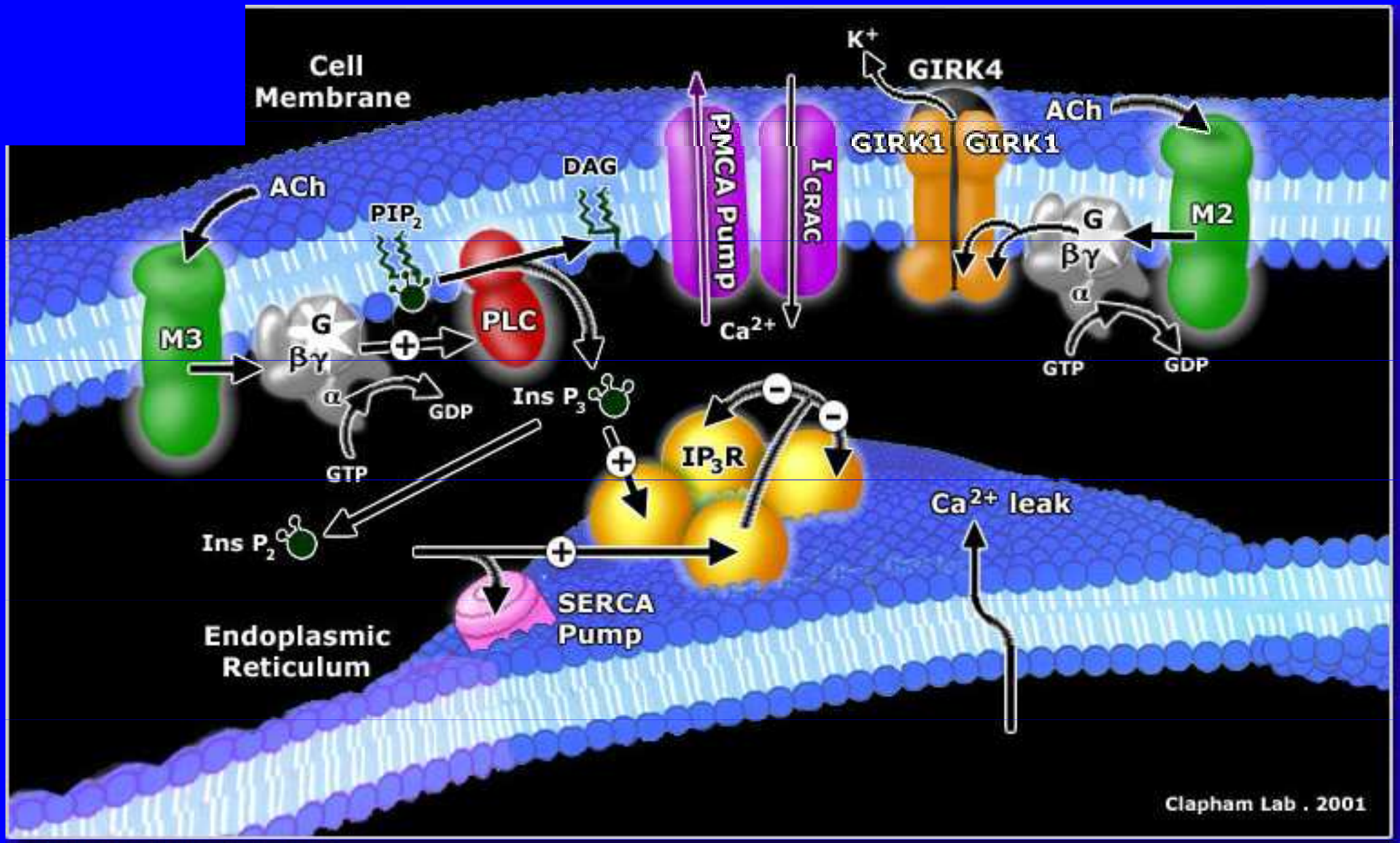
Toxicity to membrane gradients and transport

- **Semipermeability of membranes:**
  several key functions
  - **cytoplasmic membrane:**
    signalling, neural cells Na+/K+ gradient
  - **mitochondrial membrane:**
    electrone flow -> ATP synthesis
  - **endoplasmatic reticulum**
    Ca^{2+} signalling

- **Membrane fusion / transport**
  neurotransmitter release


(A) Ligand-gated ion channels
Ions
1 Neurotransmitter binds
Neurotransmitter
2 Channel opens
Outside cell
Inside cell
3 Ions flow across membrane

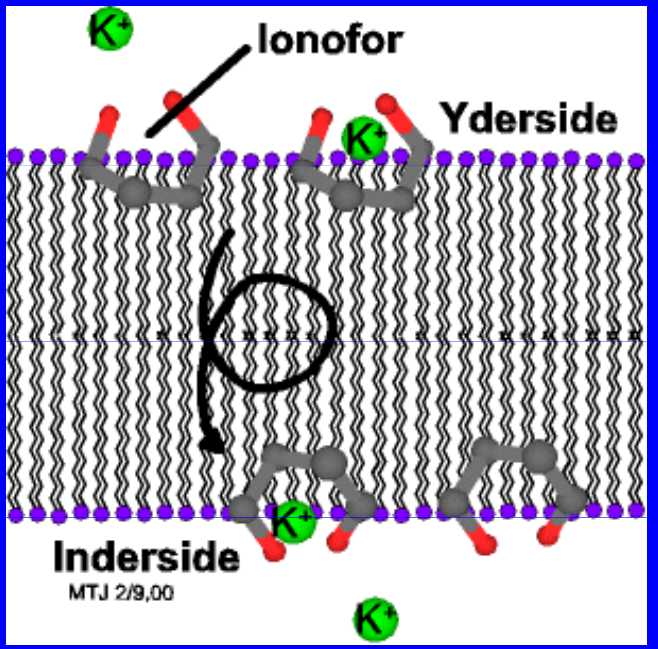

(B) G-protein-coupled receptors
1 Neurotransmitter binds
Neurotrans-mitter
Receptor
Effector protein
5 Ions flow across membrane
γ
β
α
α
G-protein
Intracellular messengers
4 Ion channel opens
2 G-protein is activated
3 G-protein subunits or intracellular messengers modulate ion channels
Ions

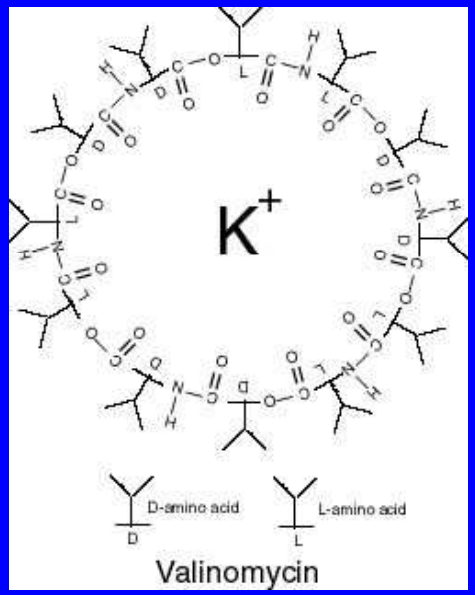

Cell Membrane
ACh
M3
PIP2
DAG
G
βγ
α
GDP
GTP
PLC
Ins P3
Ins P2
PMCA Pump
ICRAC
Ca2+
K+
GIRK4
GIRK1
GIRK1
ACh
M2
G
βγ
α
GTP
GDP
IP3R
Ca2+ leak
SERCA Pump
Endoplasmic Reticulum
Clapham Lab . 2001

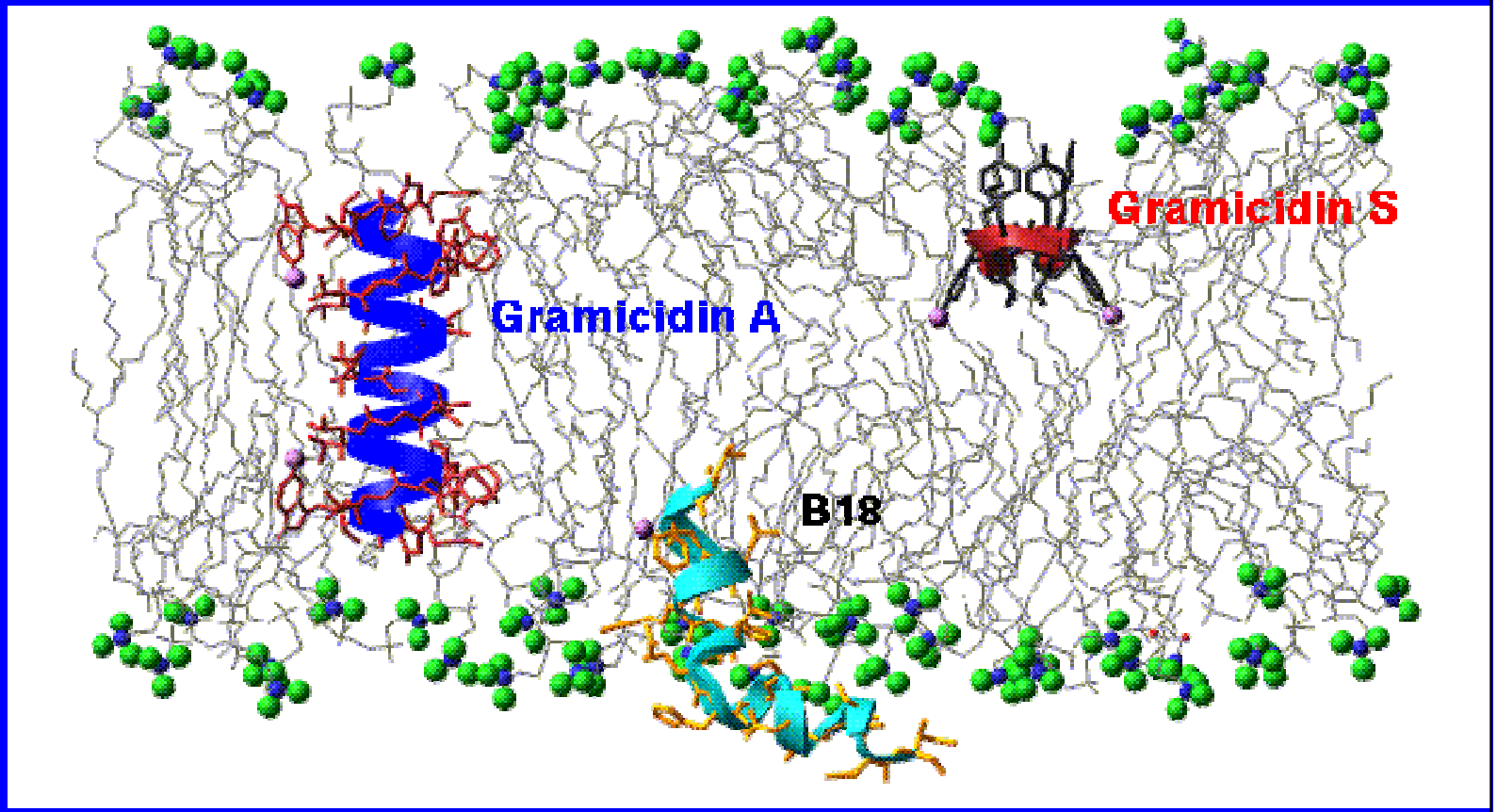
Membrane gradient disruption

Ion transfer ("ionofors")

antibiotics
(K+, Ca2+, Mg2+)

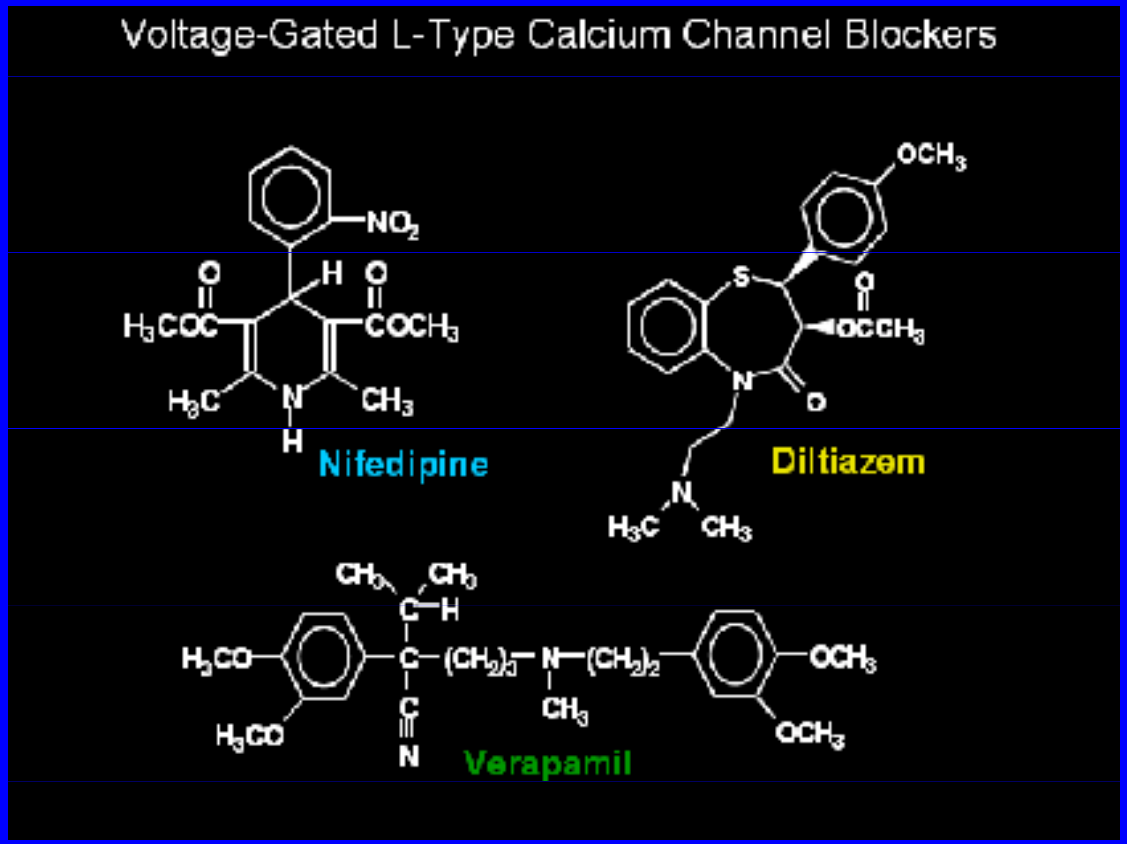




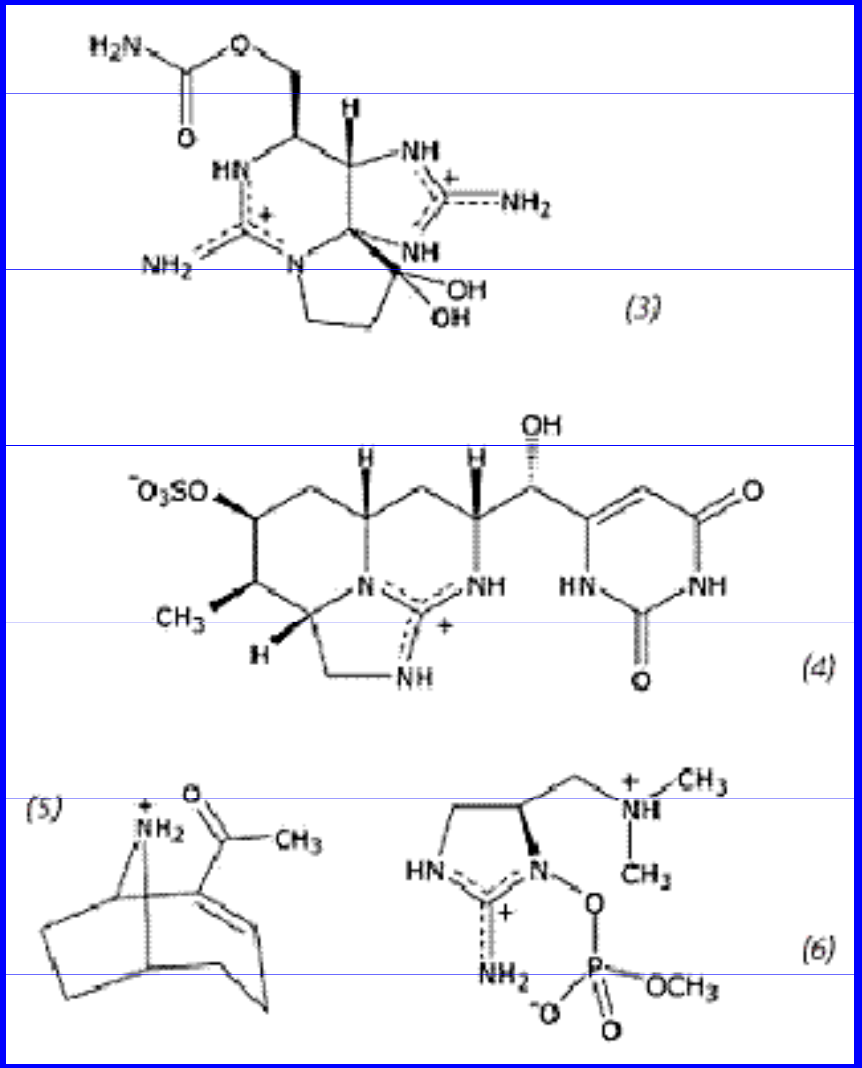


Ion Channel BLOCKERS / ACTIVATORS

Neuromodulators (drugs)

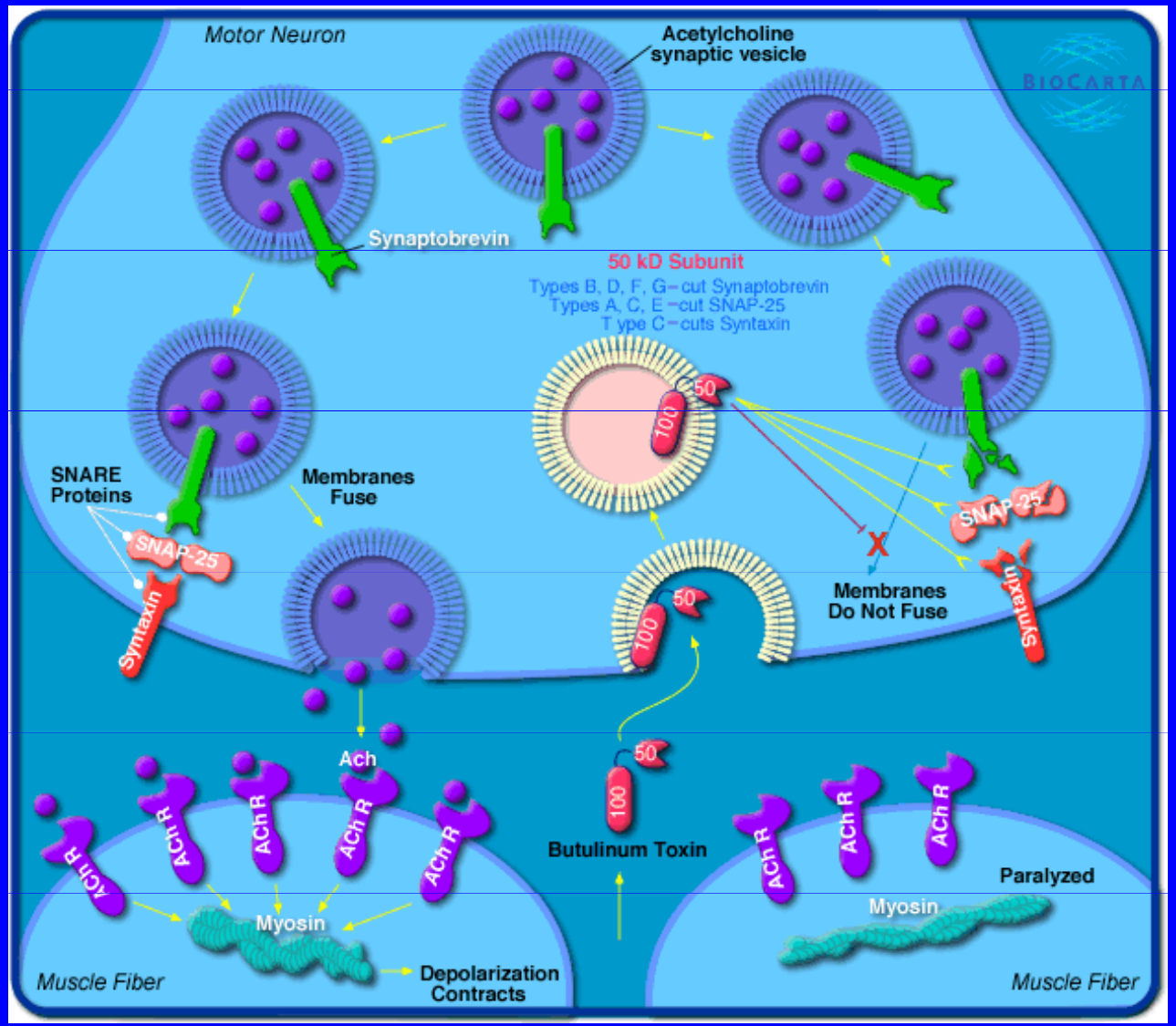


Neurotoxins (cyanobacterial)

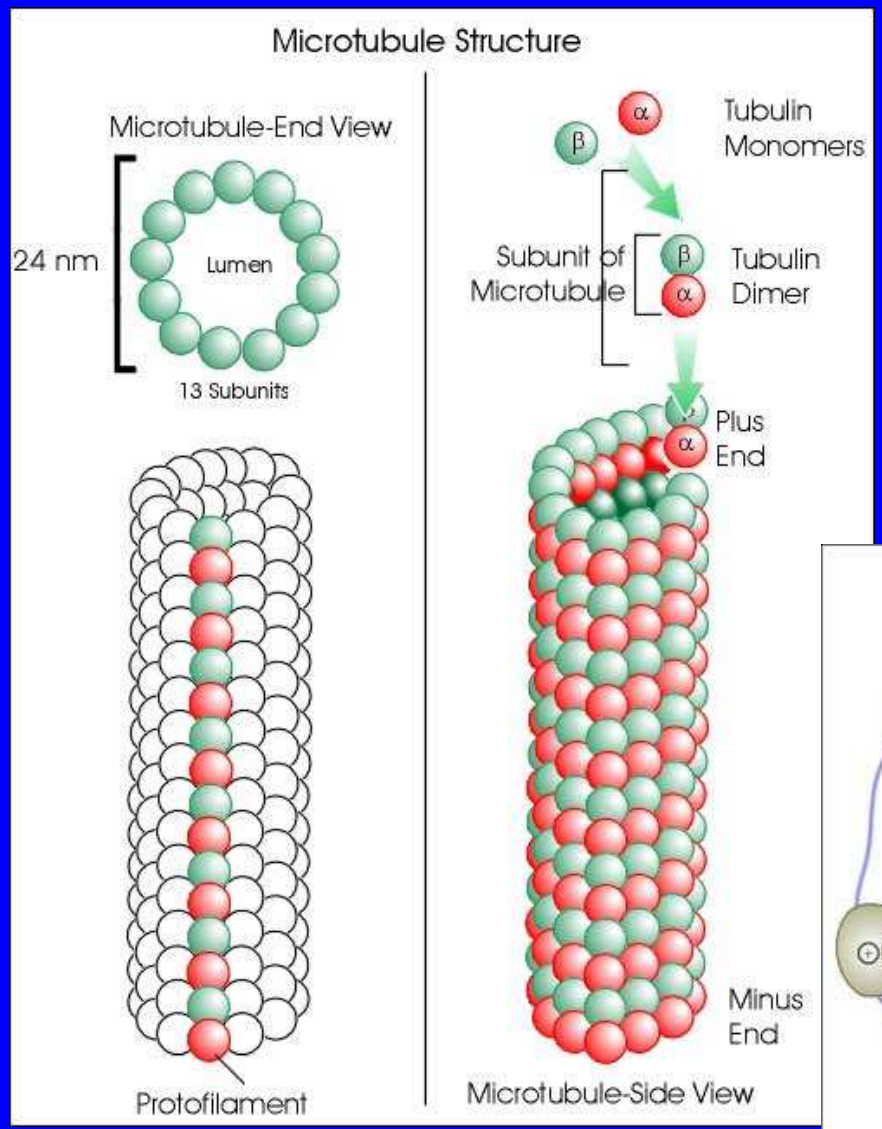


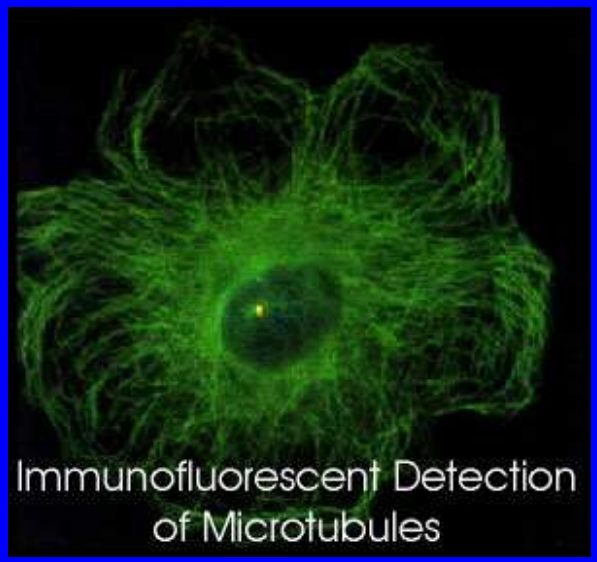
Botulotoxin, Tetanotoxin

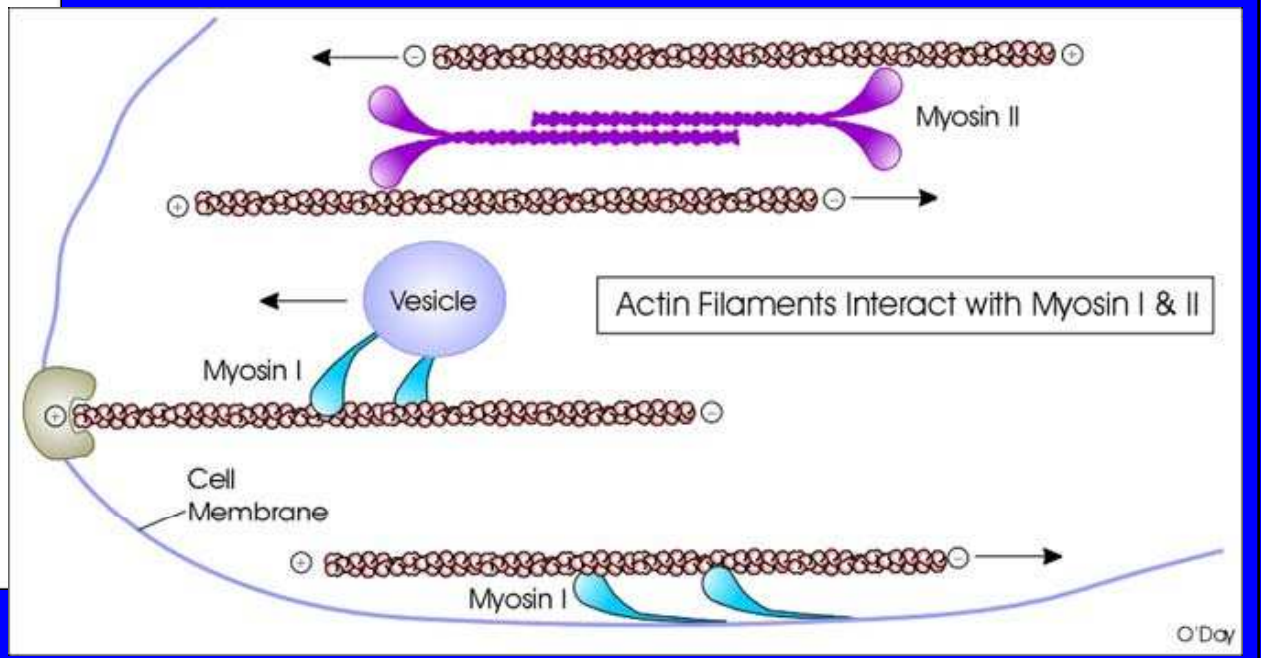
- proteases (!)
- selective inhibition of neutrotransmitter release (membrane vesicles)



Cytoskeleton as target of toxicants
microtubules / actin-myosin

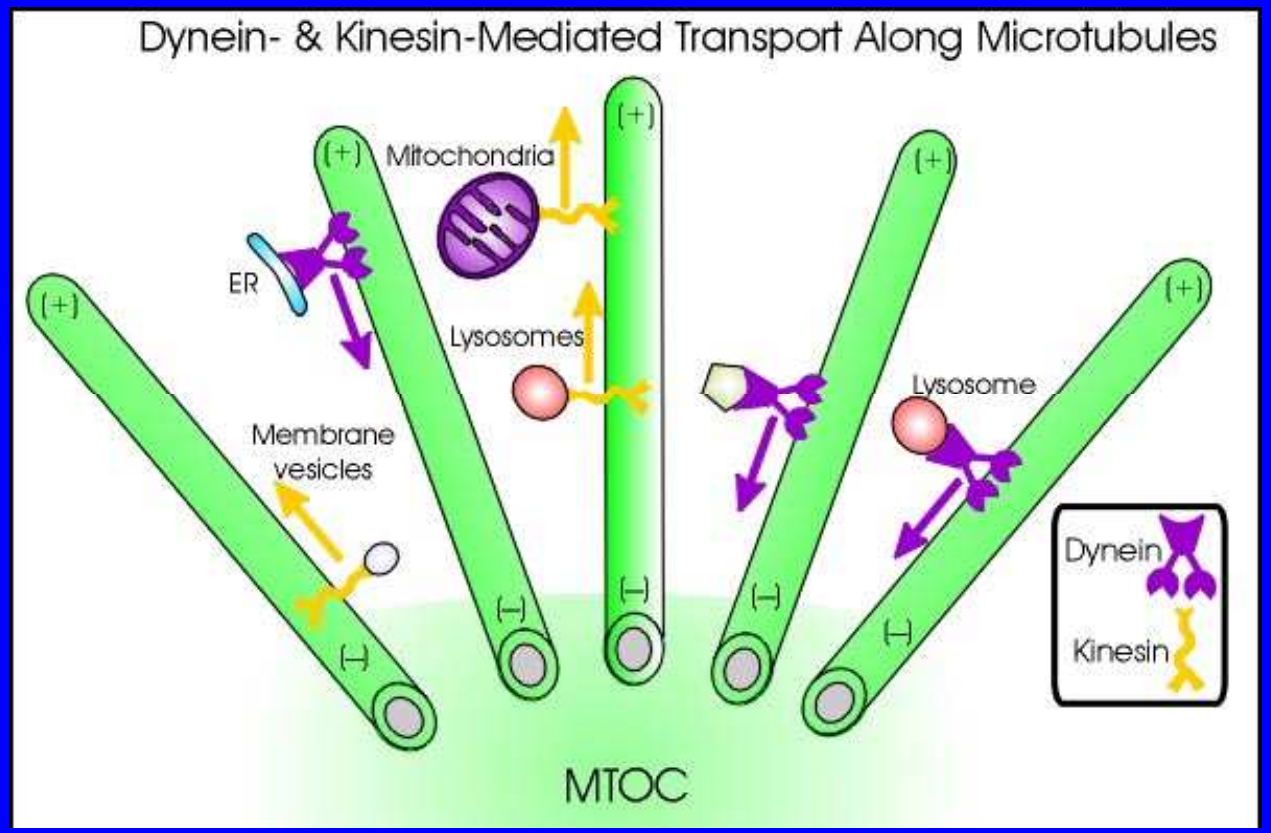




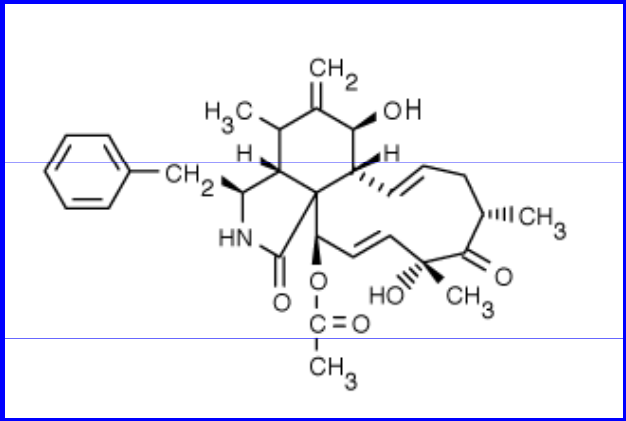


Cytoskeleton – function

- intracellular transport
- cell replication and division (mitotic poisons)
- muscle movement
- membrane (vesicles) fusion

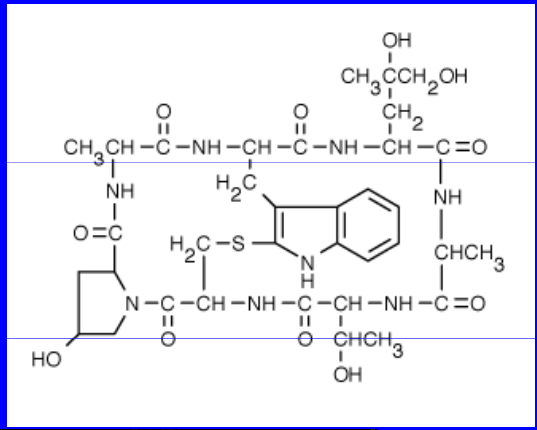


TOXINS: effects on (DE)POLYMERIZATION



cytochalasin D

phalloidin



Effects of Inhibitors on Actin Filaments

Cytochalasins

Polymerization

G-Actin ⇌ (⊗) Depolymerization

Actin filaments disappear due to depolymerization

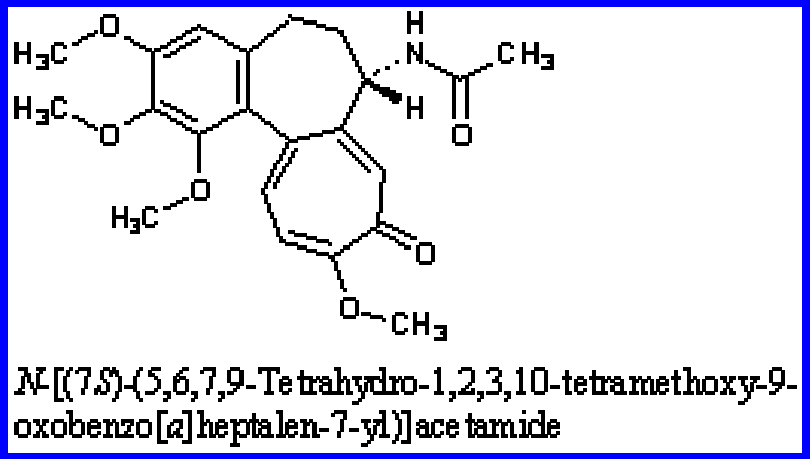
G-Actin → (⊗) Depolymerization

Polymerization

Actin filaments are stabilized in existing pattern

Phalloidin

TOXINS: effects on (DE)POLYMERIZATION



colchicine

taxol