# Cell communication & regulation: a target for toxicants

Any sensitively regulated process is susceptible to toxicants

! REGULATIONS & SIGNALLING

Hierarchy

- systems: neuronal <----> endocrine

- cell-to-cell
  - hormonal & neuronal signal transmission
  - contact channels

- intracellular signal transduction

# HORMONES - fate

1. **Biosynthesis** of a particular hormone in a particular tissue

2. Storage and **secretion** of the hormone

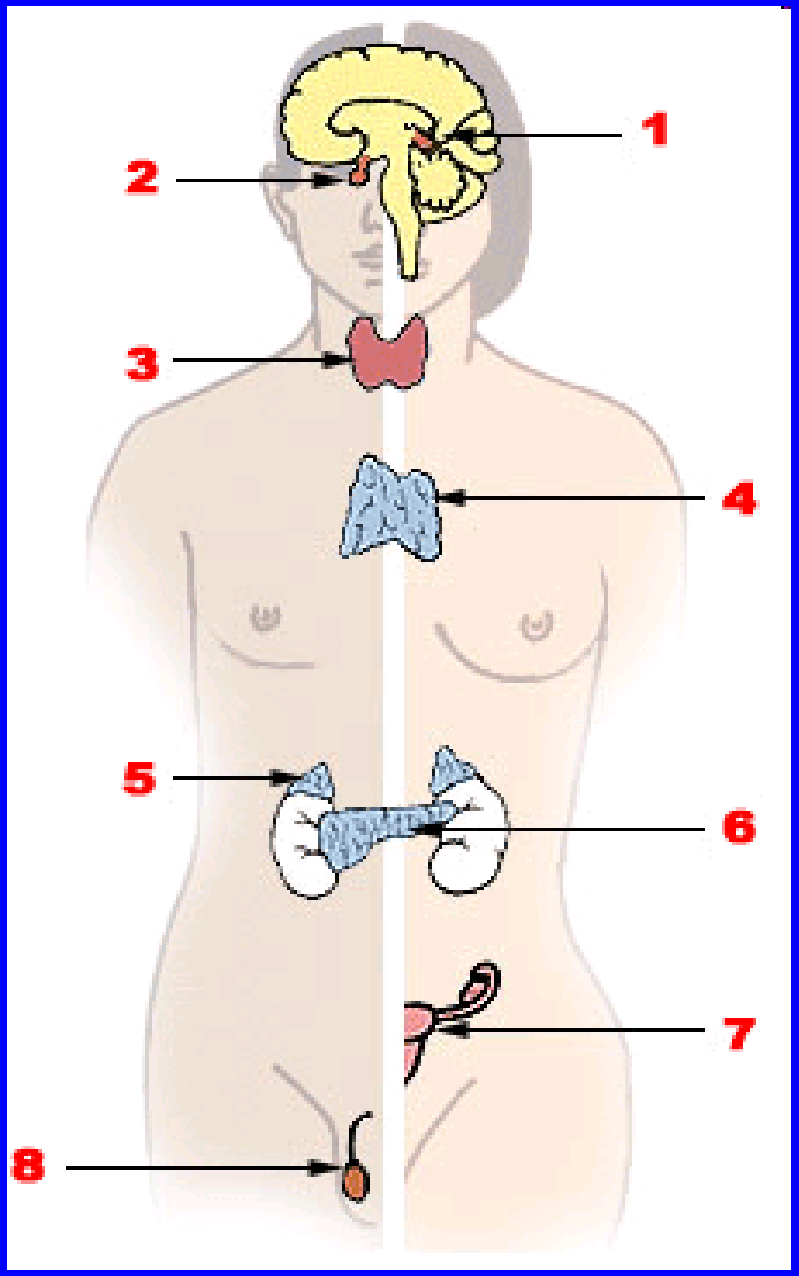
3. **Transport** of the hormone to the target cell(s)

4. **Recognition of the hormone** by an associated cell membrane or intracellular receptor protein.

5. Relay and **amplification of the received hormonal signal** via a signal transduction process -> cellular response.

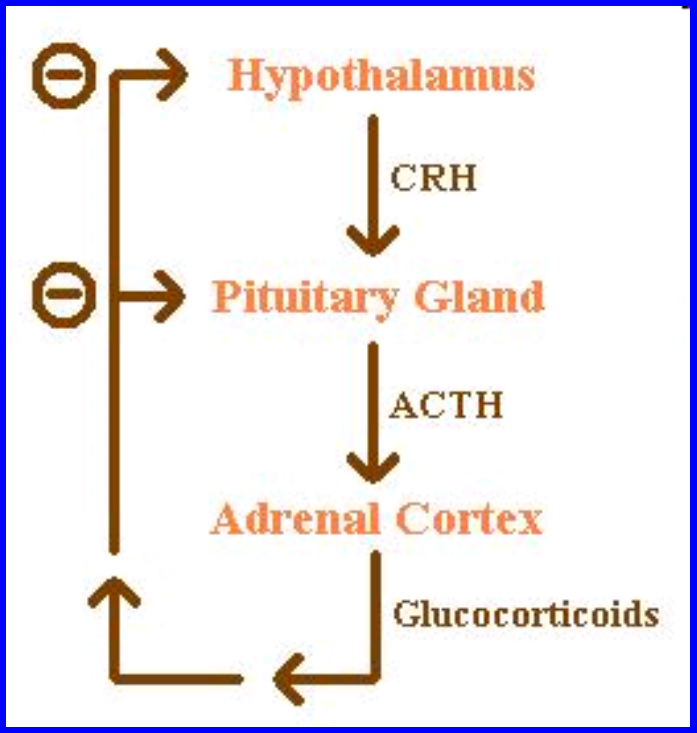
6. The reaction of the target cells is recognized by the original hormone-producing cells (**negative feedback loop**)

7. **Degradation and metabolism** of the hormone




**Endocrine system:**

1. Pineal gland, 2. Pituitary gland, 3. Thyroid gland, 4. Thymus, 5. Adrenal gland, 6. Pancreas, 7. Ovary, 8.Testis




**Example:** feedback loop

# HORMONES - actions and controls

* stimulation or inhibition of growth
* mood swings
* induction or suppression of apoptosis
  (programmed cell death)
* activation or inhibition of the immune system
* regulation of metabolism
* preparation for fighting, fleeing, mating …
* preparation for a new phase of life
  (puberty, caring for offspring, and menopause)
* control of the reproductive cycle

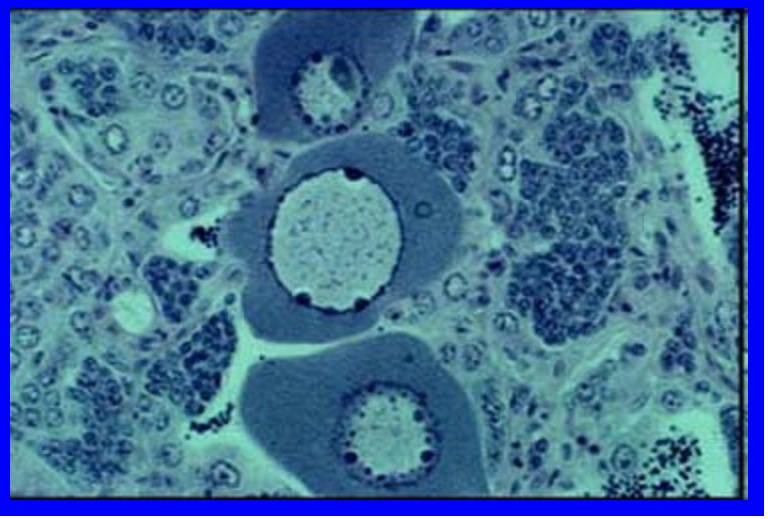
# TOXICITY TO HORMONAL ACTION = ENDOCRINE DISRUPTION

ED & EDCs - major problem in environmental toxicology

- Effects at **all levels of hormonal action**
  (*synthesis, transport, action ….)*

- **Multiple effects** (! Not only „xenoestrogenicity“ & feminization)
  (*immunotoxicity, reproduction …*)

*(WILL BE DISCUSSED FURTHER)*



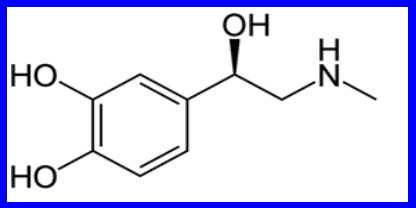
Intersex roach testis
containing both oocytes and spermatozoa,
caused by exposure to environmental oestrogens

# **HORMONES - chemicals** (vertebrates)

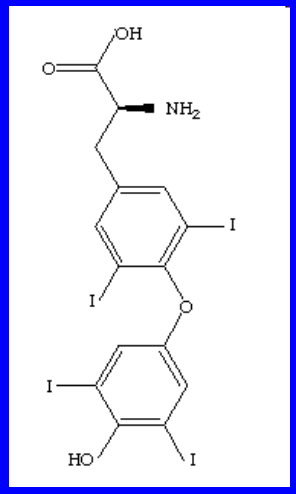
* **Amine-derived hormones** are derivatives of the amino acids tyrosine and tryptophan. Examples are catecholamines and thyroxine.

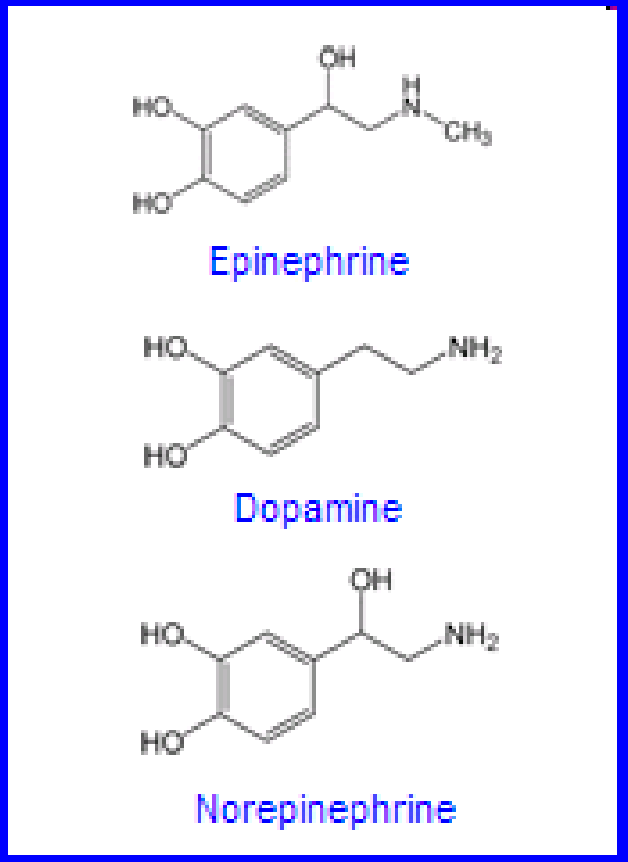
*(small molecules - similar to organic toxicants - TOXIC EFFECTS)*

Adrenalin




Thyroxin




Further:

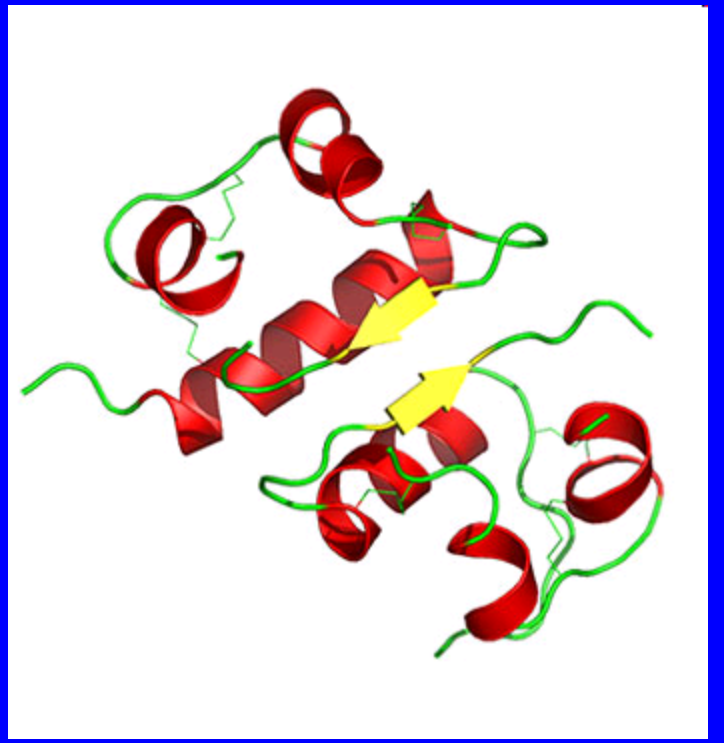
* *Peptide hormones*
* *Lipid and phospholipid-derived hormones*

# **HORMONES - chemicals** (vertebrates)

* **Peptide hormones** chains of amino acids. - small: TRH and vasopressin; proteins: insulin, growth hormone, luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone).

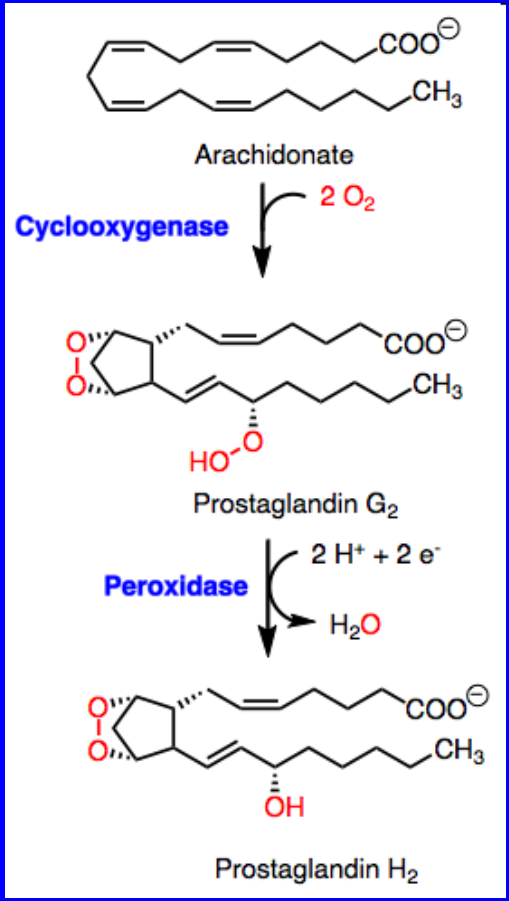
*Large molecules; receptors on surfaces of the cells*
*(Interactions with toxic chemicals **less likely**)*

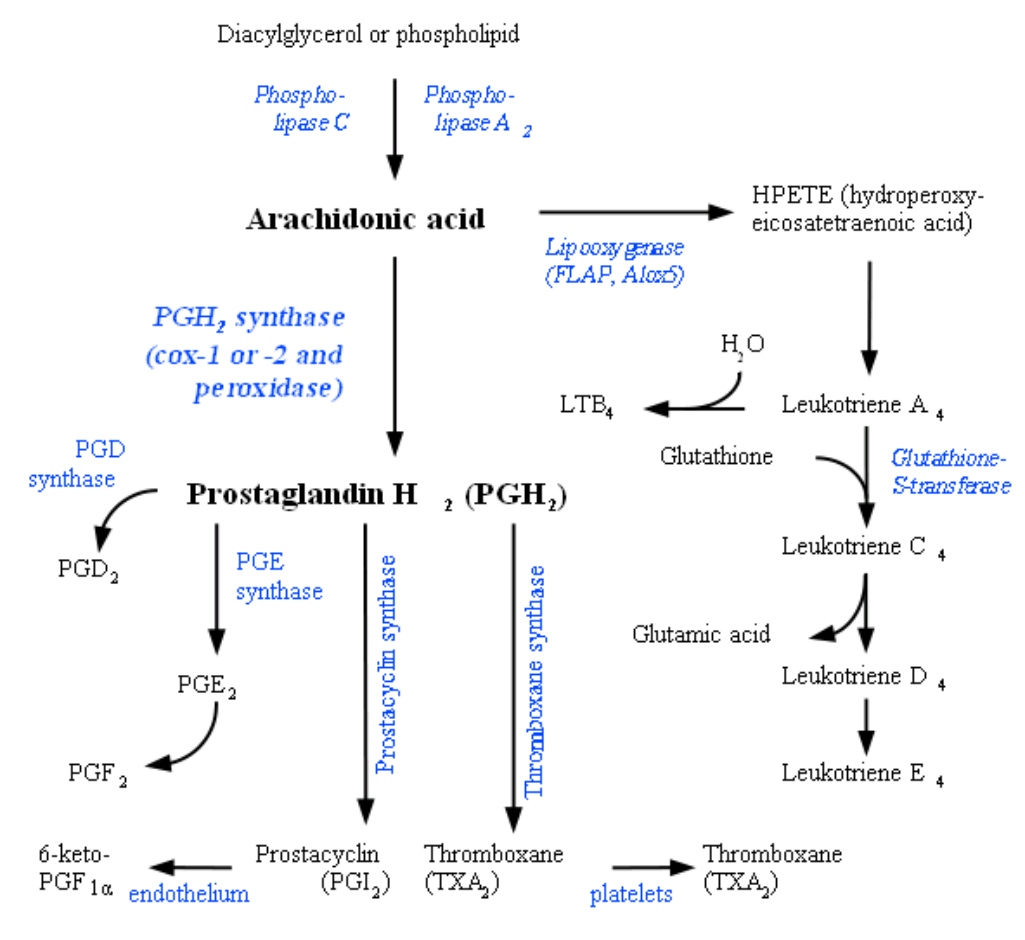
Example - insulin



# HORMONES - chemicals (vertebrates)

**Lipid derived hormones** (1) (from linoleic acid, arachidonic acid)
- prostaglandins



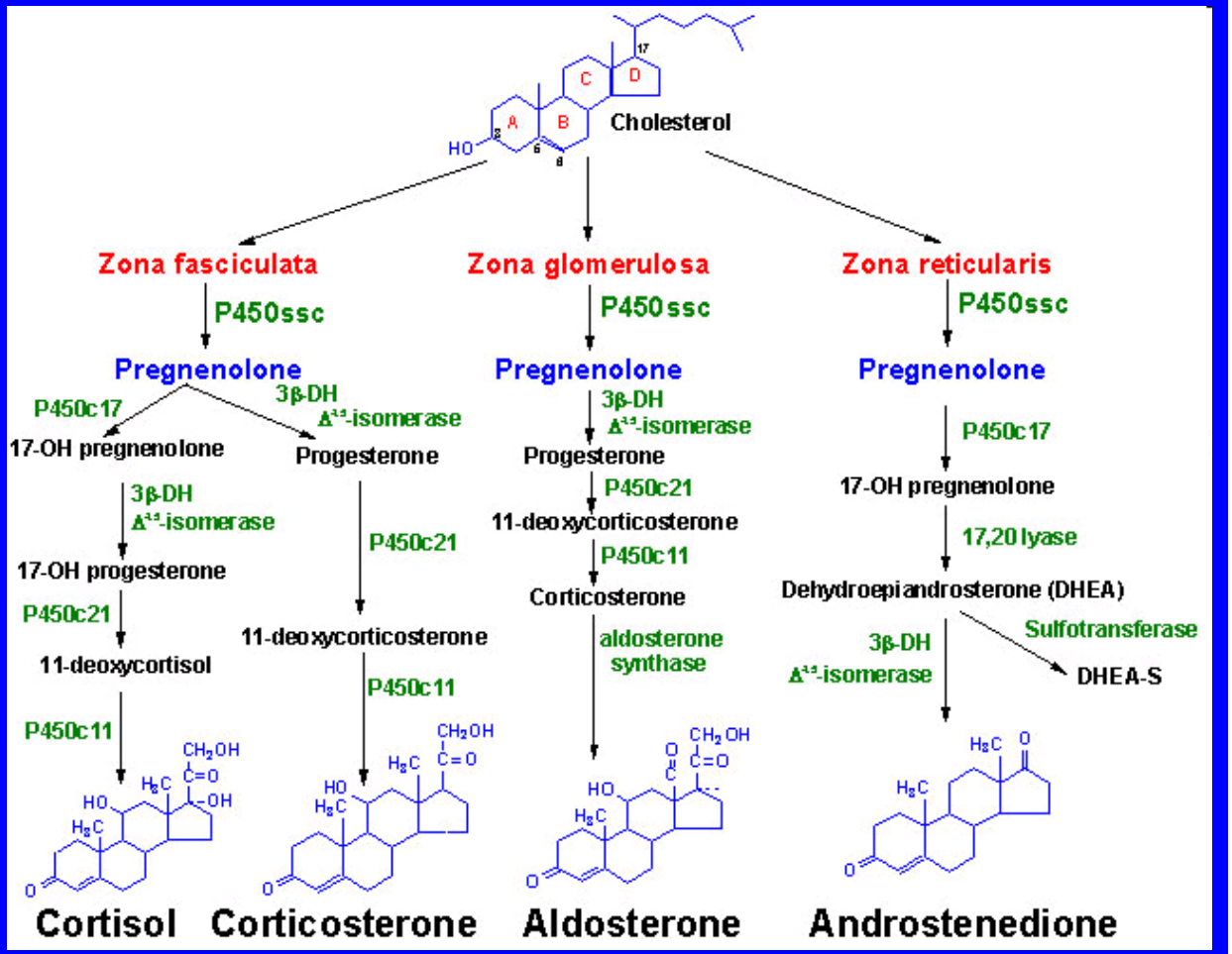
# **HORMONES - chemicals** (vertebrates)

**Lipid derived hormones** (2)

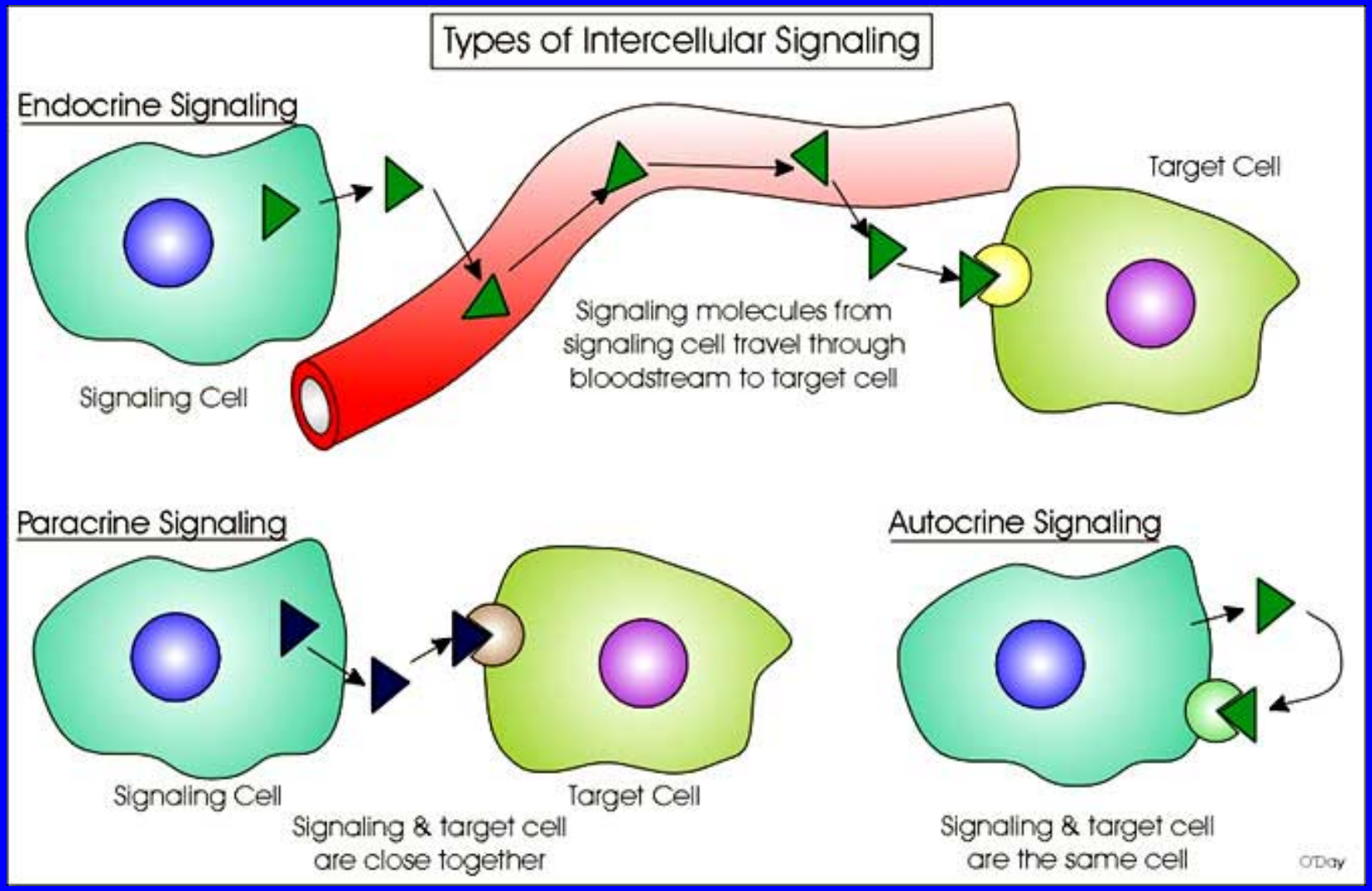
*(small molecules - similar to organic toxicants - TOXIC EFFECTS)*

**- steroid hormones**
(from cholesterol)

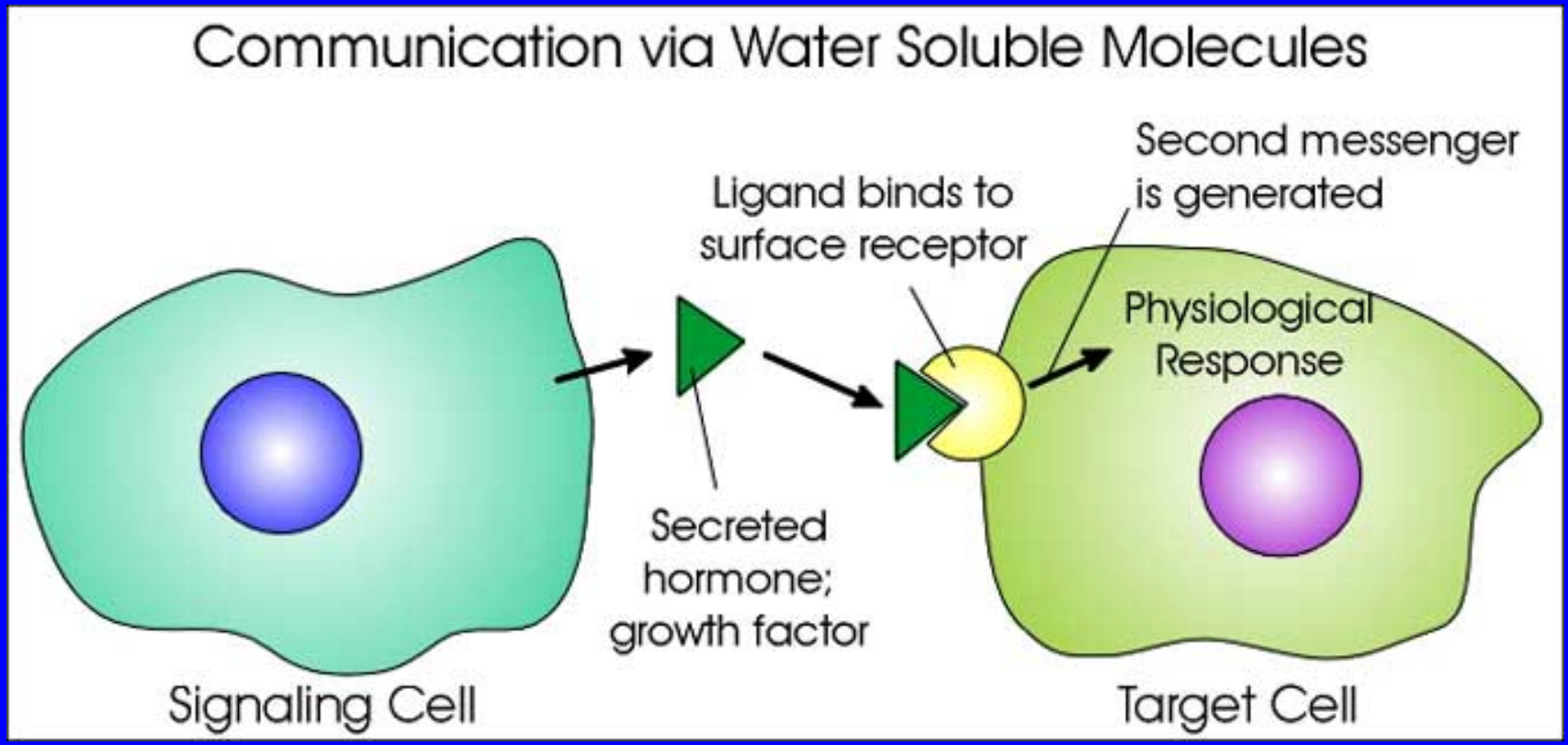
testosterone,
cortisol,
estradiol …



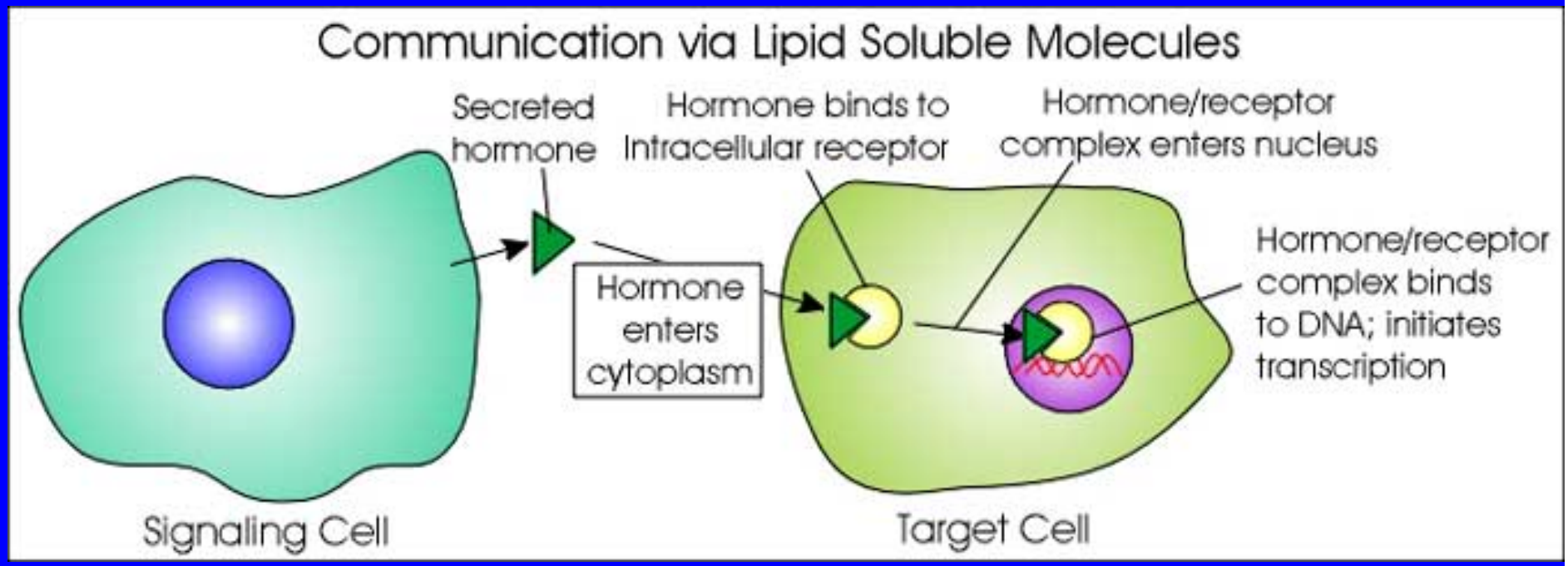
# Cell communication & regulation: a target for toxicants



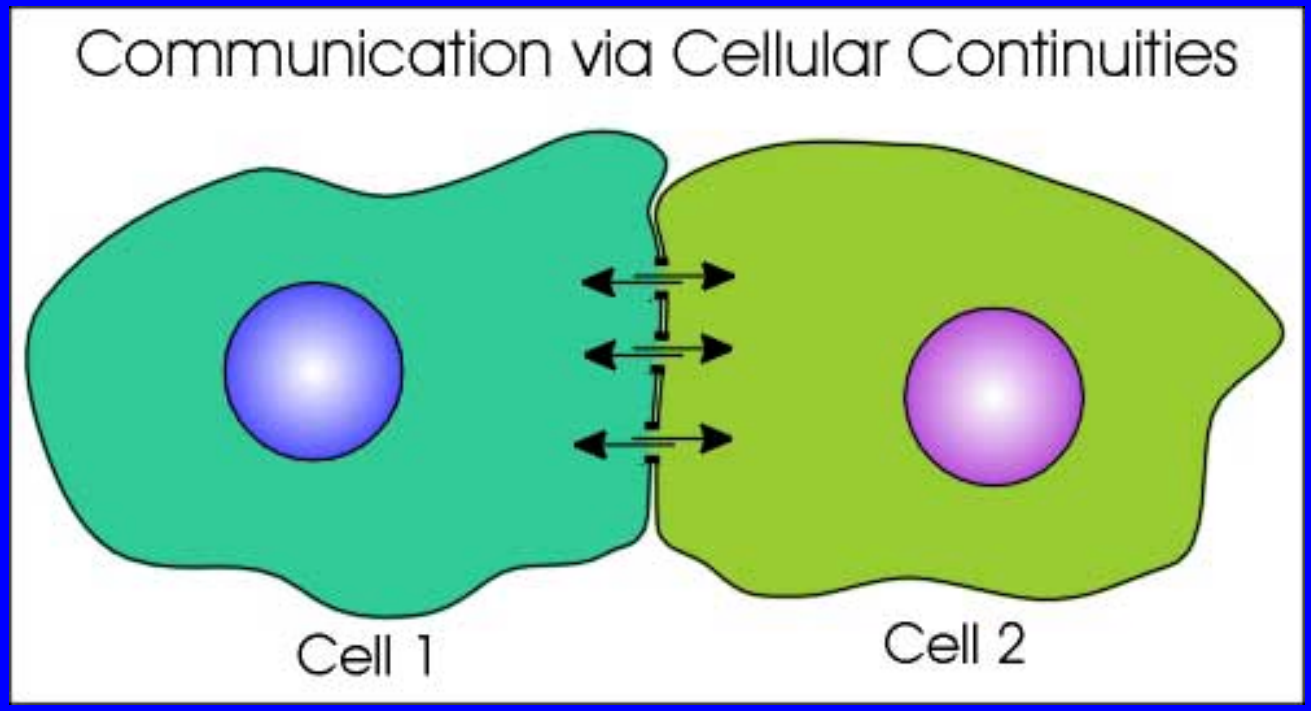
# Cell communication (1)

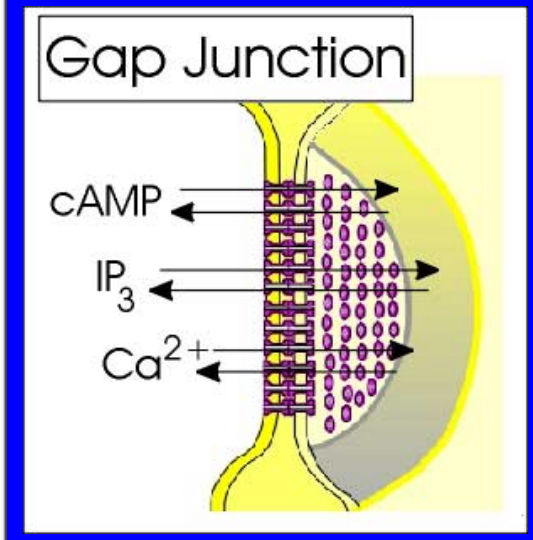


# Cell communication (2)



# Cell communication (3)



# 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)**

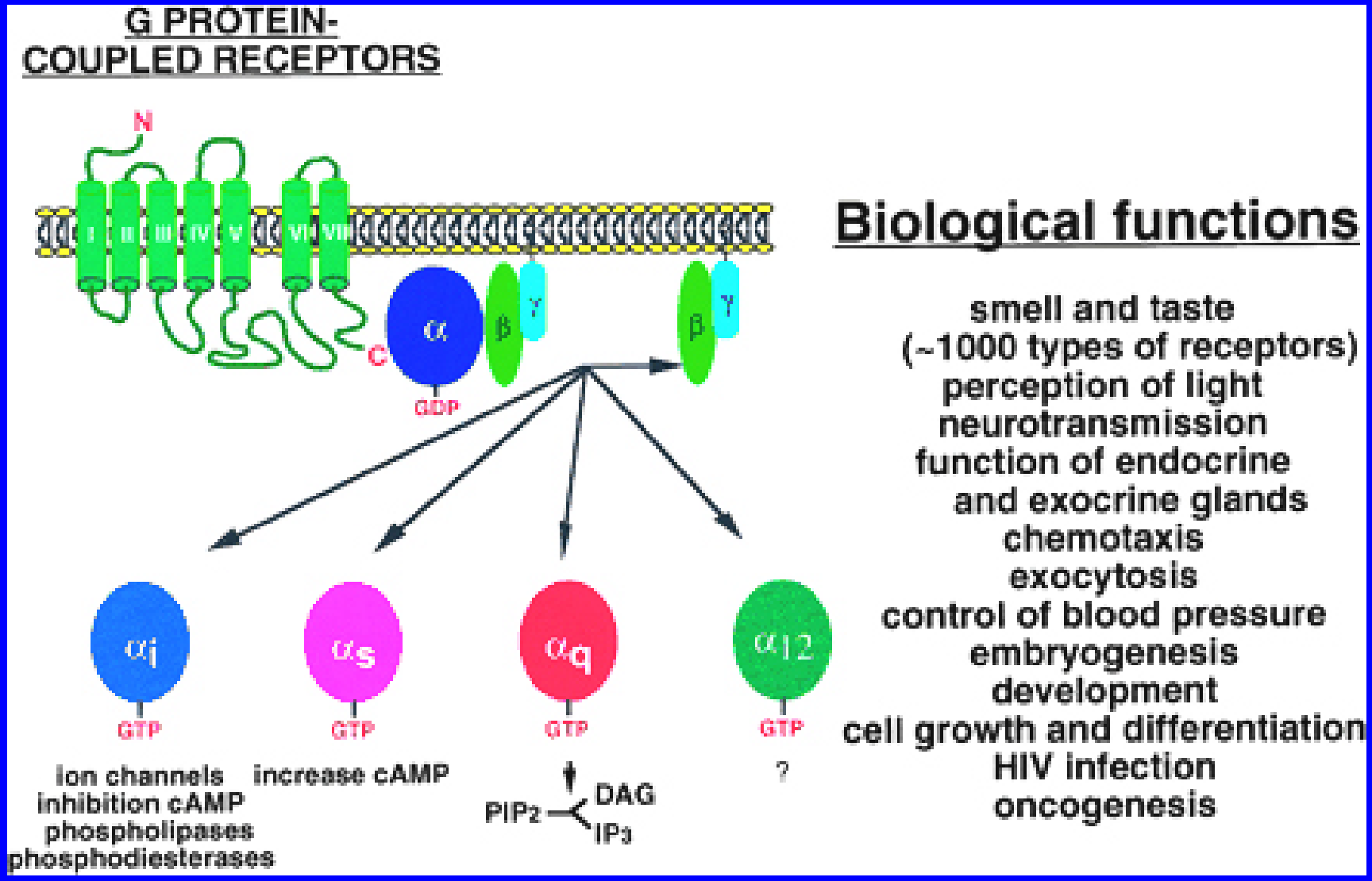
**-> PKA activation:** cAMP

**2: Membrane receptors -> PLC / PKC activation**

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

**3: Cytoplasmic (nuclear) receptors**

# Membrane receptors (PKs): G-proteins (GPCRs)

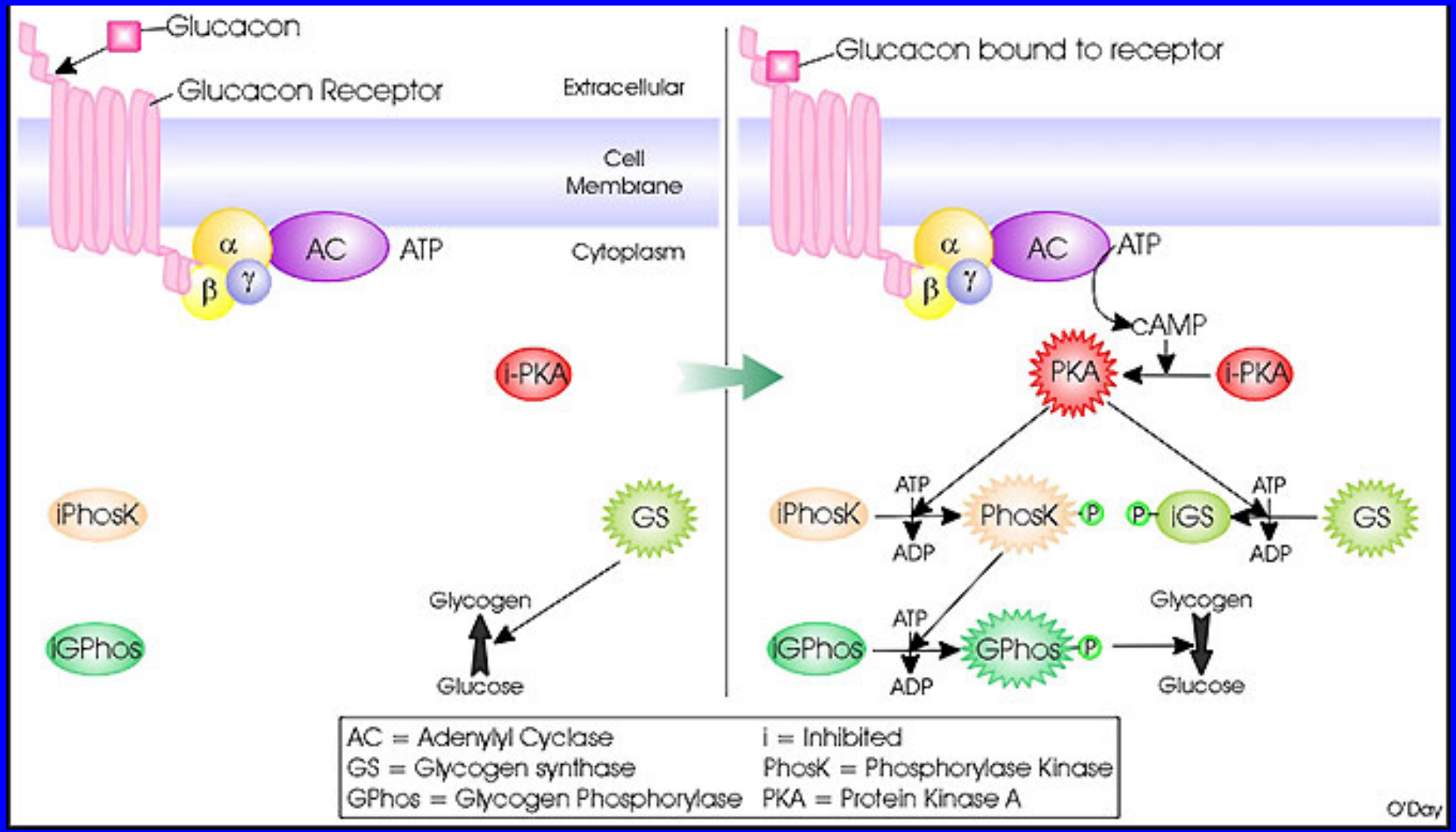


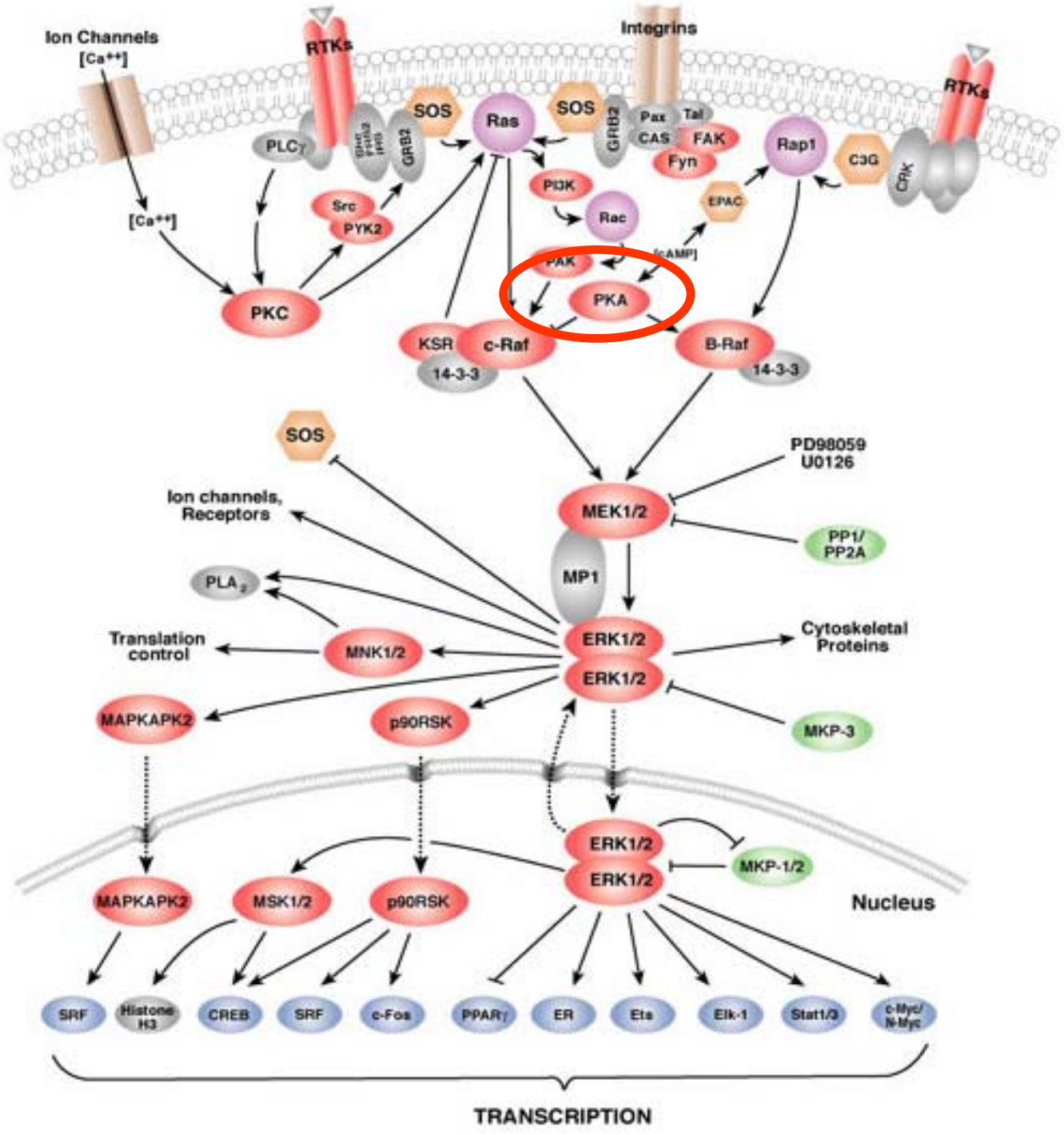

## Biological functions

smell and taste
(~1000 types of receptors)
perception of light
neurotransmission
function of endocrine
and exocrine glands
chemotaxis
exocytosis
control of blood pressure
embryogenesis
development
cell growth and differentiation
HIV infection
oncogenesis

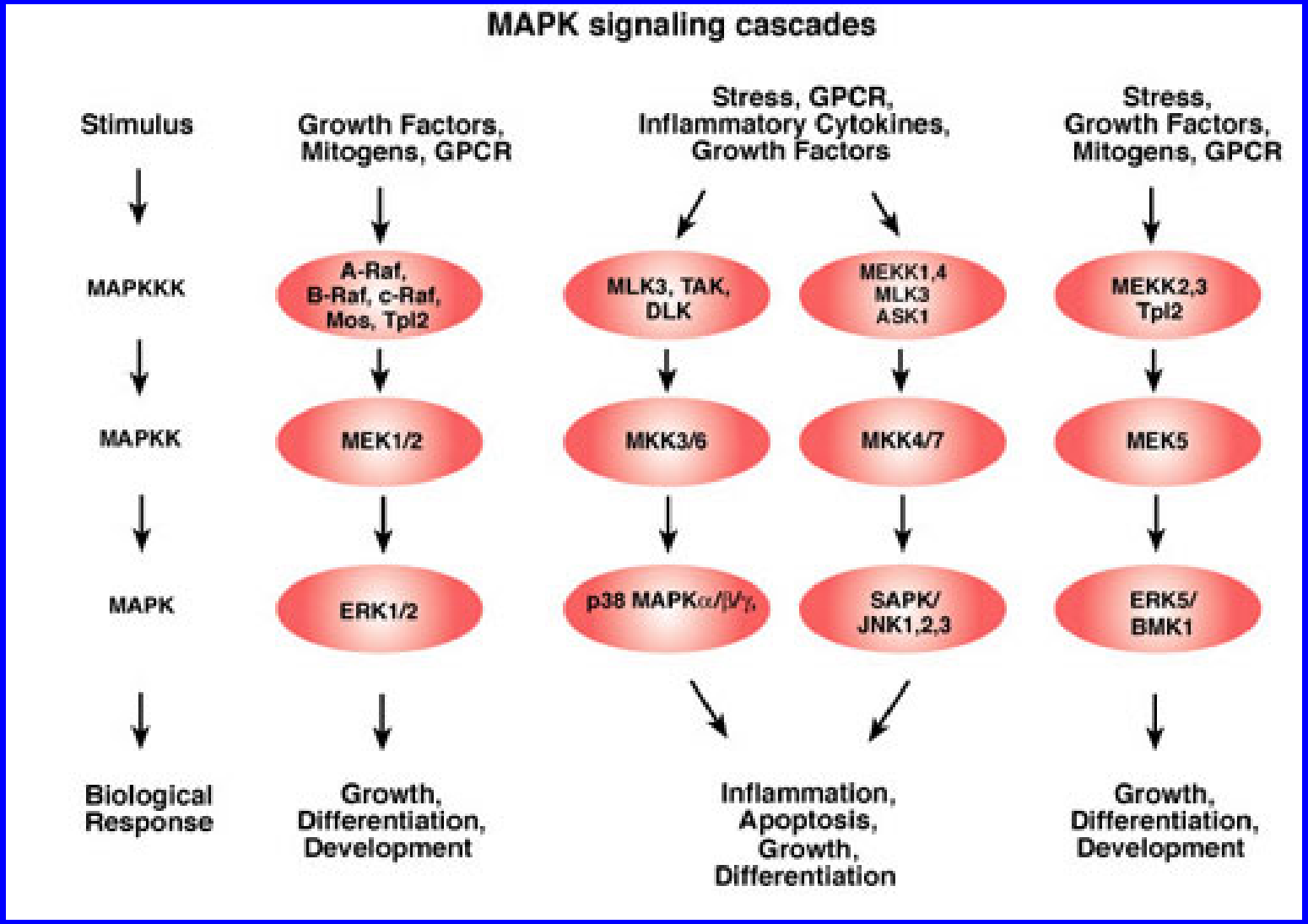
# 1: Membrane receptors (PKs)

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



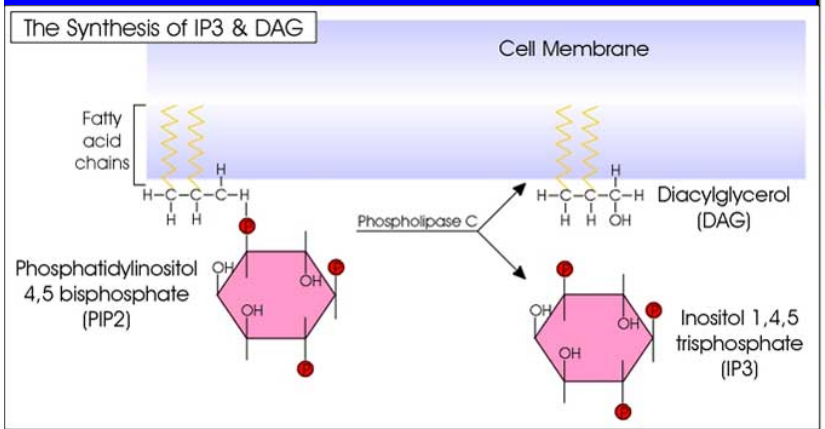

Ion Channels
[Ca++]
RTKs
Integrins
RTKs
SOS
Ras
SOS
GRB2
Pax
Tal
CAS
FAK
Fyn
Rap1
C3G
CRK
PLCγ
GRB2
PI3K
Rac
EPAC
[cAMP]
Src
PYK2
[Ca++]
PAK
PKA
PKC
KSR
c-Raf
14-3-3
B-Raf
14-3-3
SOS
PD98059
U0126
Ion channels,
Receptors
MEK1/2
PP1/
PP2A
MP1
PLA2
Translation
control
MNK1/2
ERK1/2
ERK1/2
Cytoskeletal
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

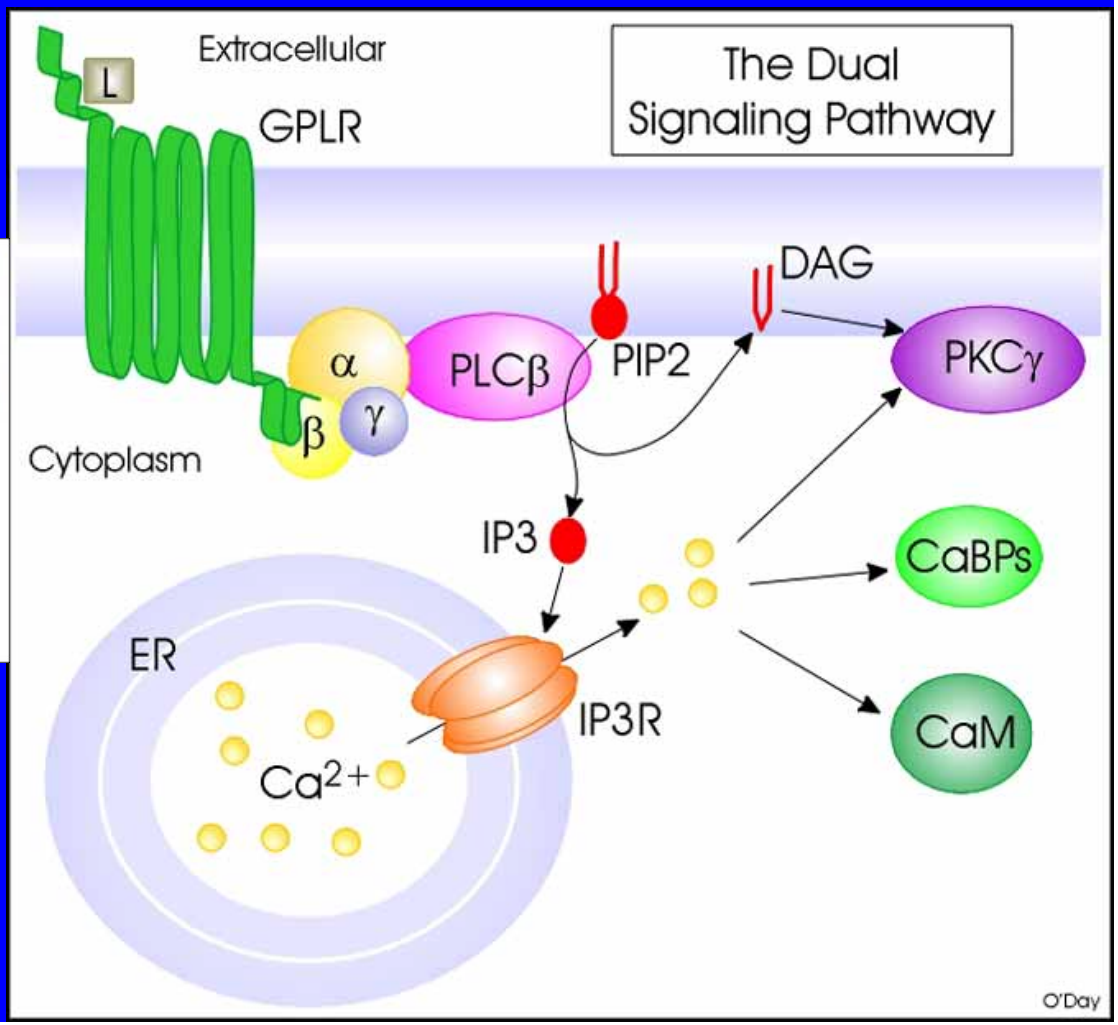
# (!!!) Mitogen Activated Protein Kinases (MAPK) – dependent effects

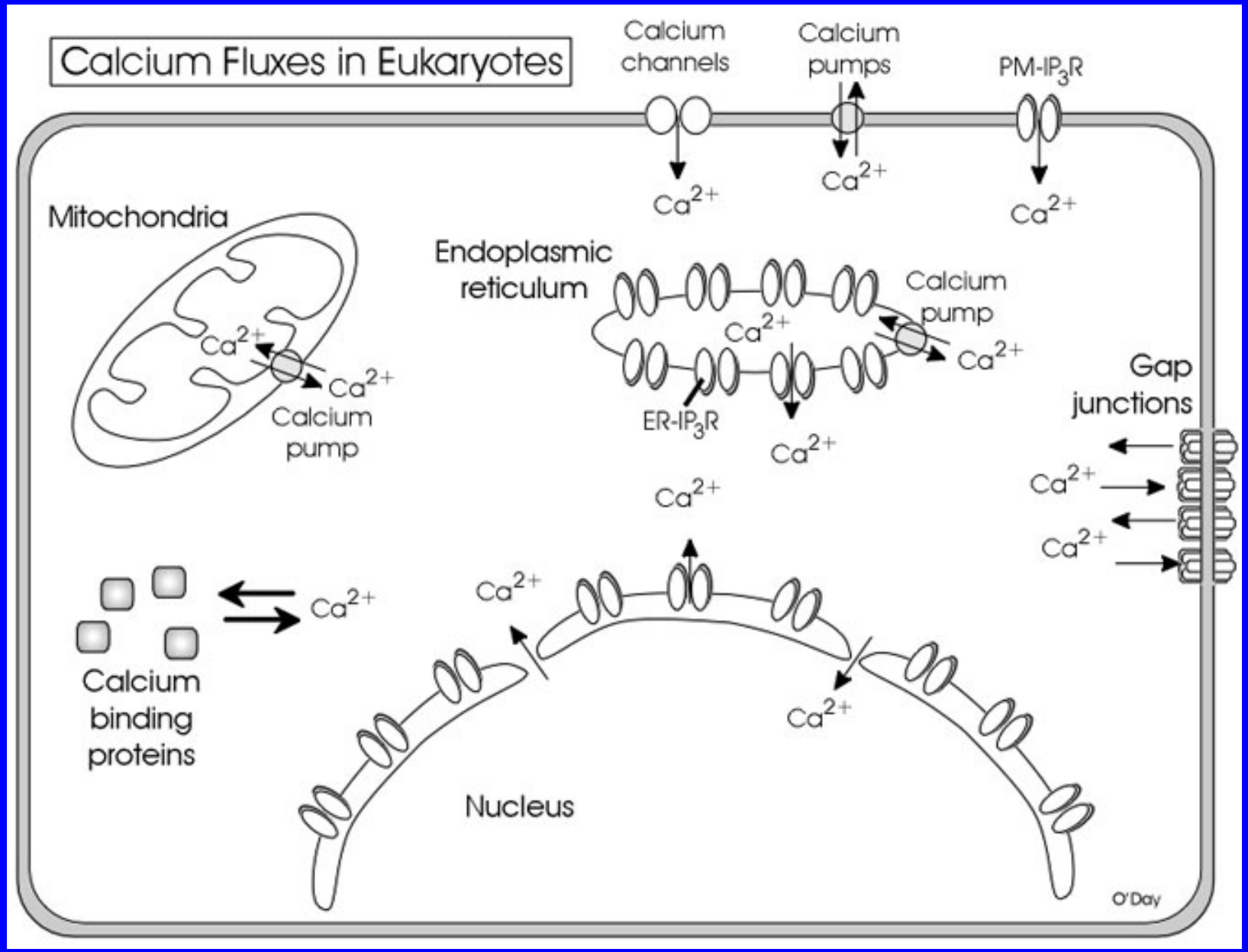


## 2: Membrane receptors
### -> Phospholipase C:

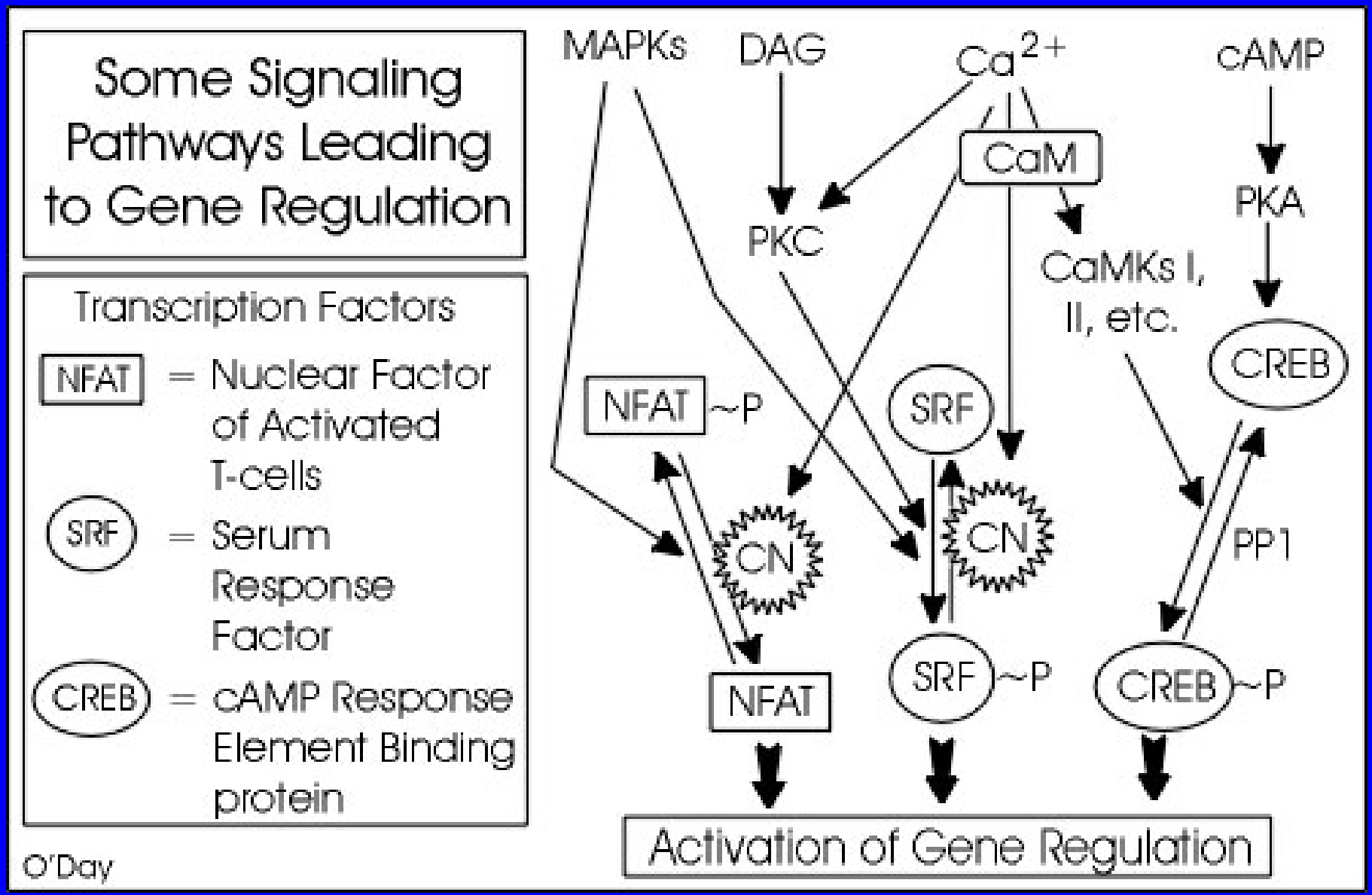
**PIPs -> DAG -> PKC / arachidonic acid**
**+ IP3 -> $Ca^{2+}$**




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+
Gap junctions
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Calcium binding proteins
Nucleus
O'Day

# Signalling crosstalk

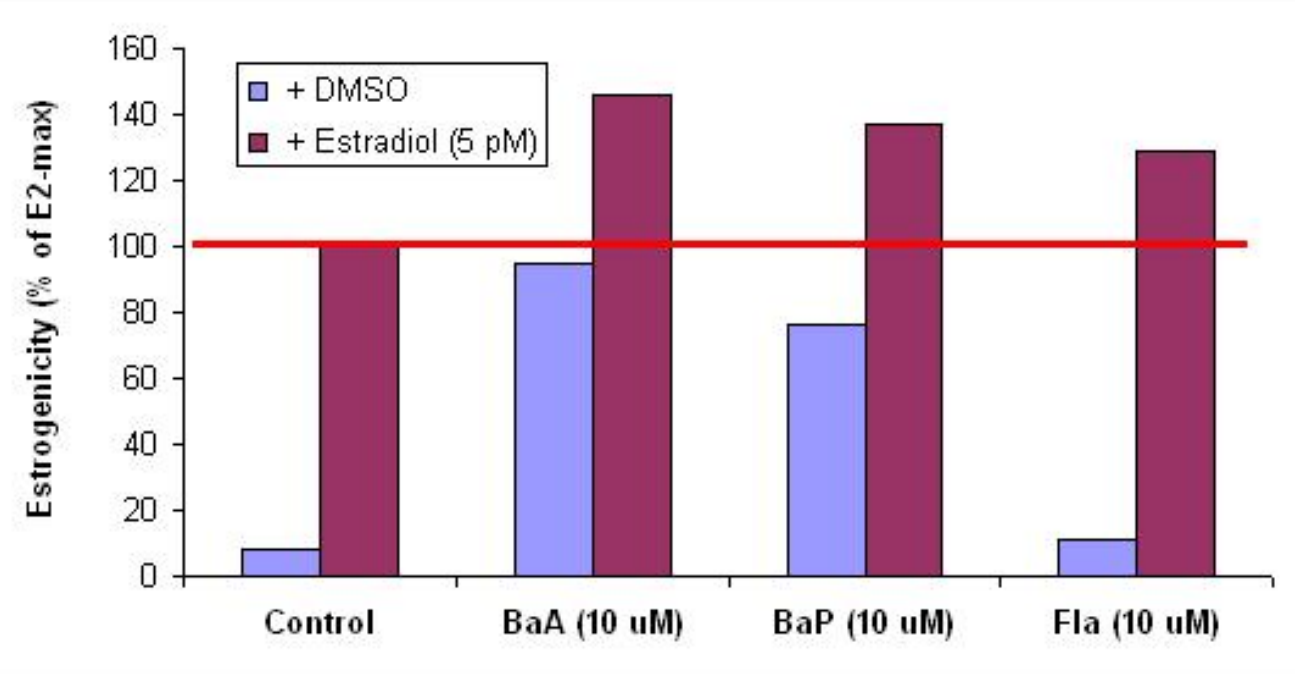


# Examples

## ER-independent estrogenicity (PAHs)

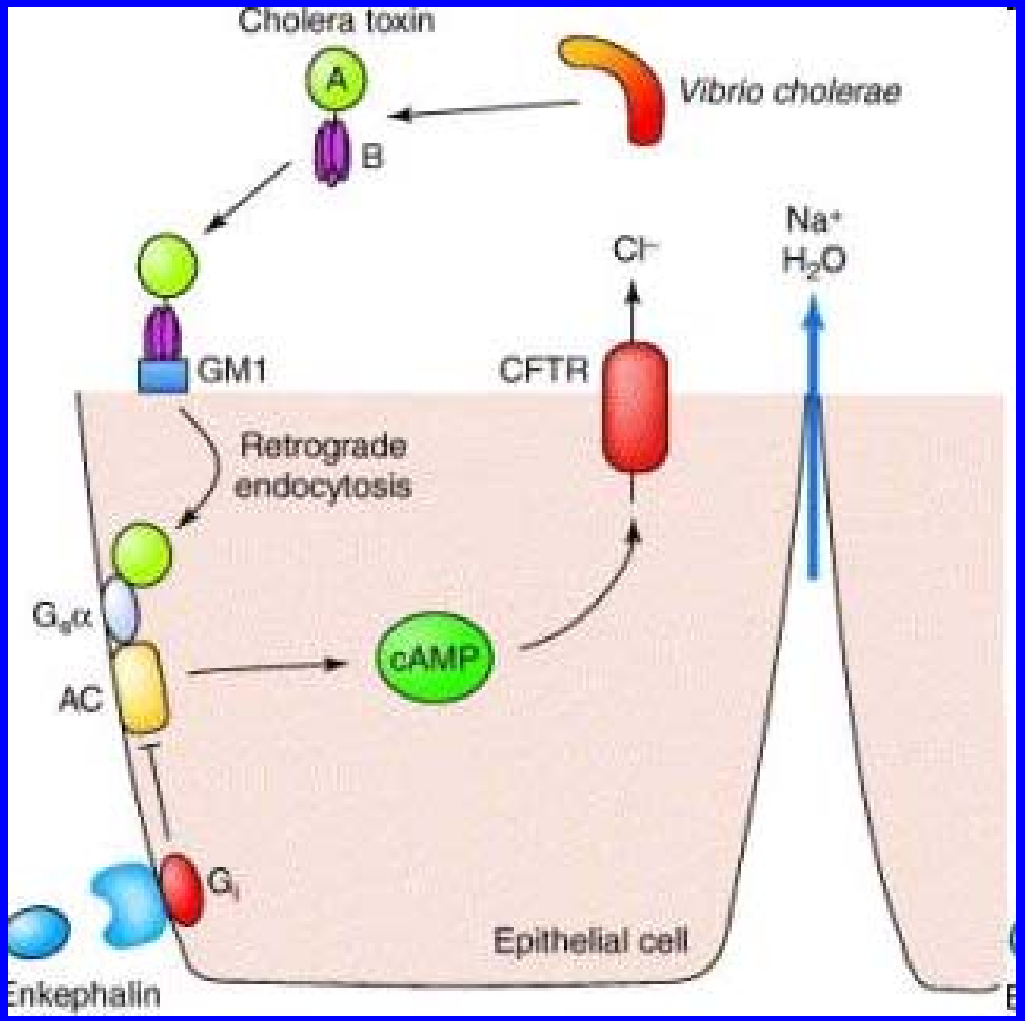
modulation of PKs/PPases: phosphorylation
-> activation of ER-dependent genes

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

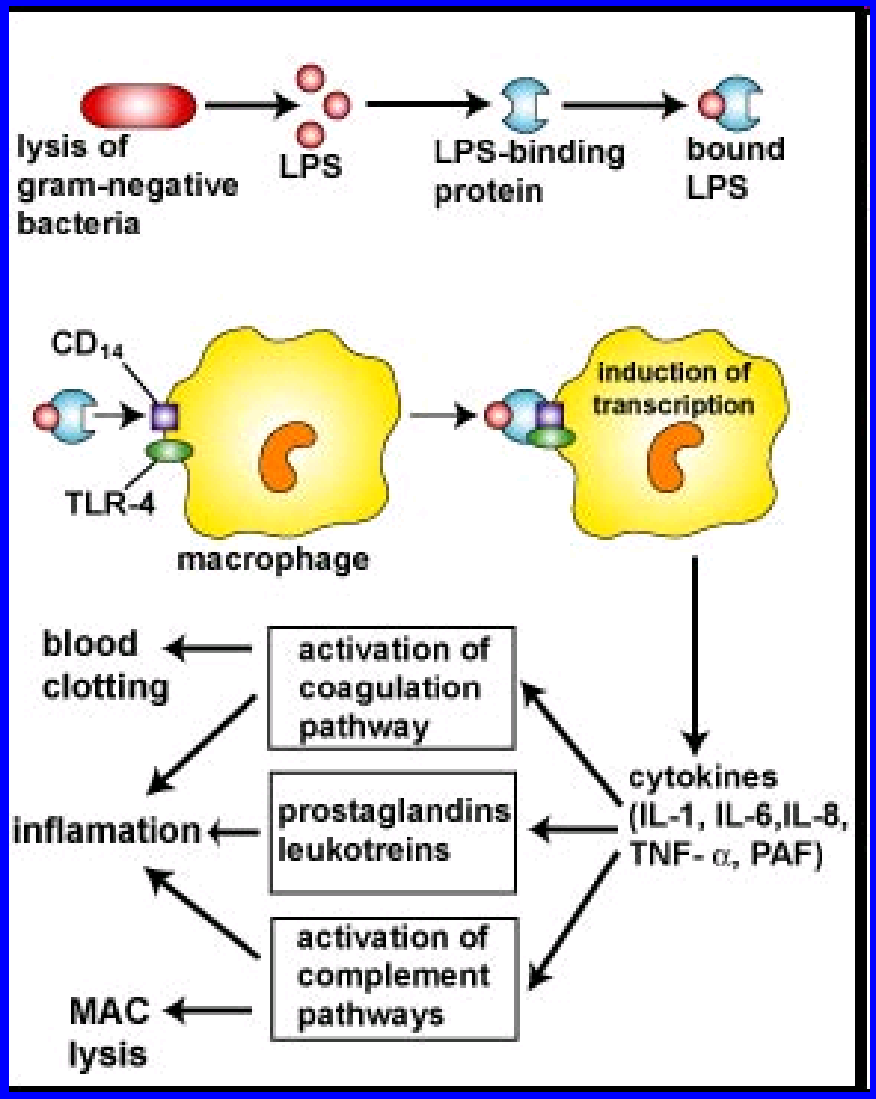



*Vondráček et al. 2002 Toxicol Sci 70(2) 193*

# Cholera toxin - activation of adenylate cyclase



# Lipopolysaccharide (bacteria) - immunotoxicity



# Examples - other lectures

**ER-dependent estrogenicity (DDE)** [other lectures]

xenoestrogenicity, binding to ER + activation

**AhR-dependent anti-estrogenicity, retinoid toxicity,** modulation of estrogen / retinoid levels [other lectures]

AhR -> CYPs -> steroid-metabolism

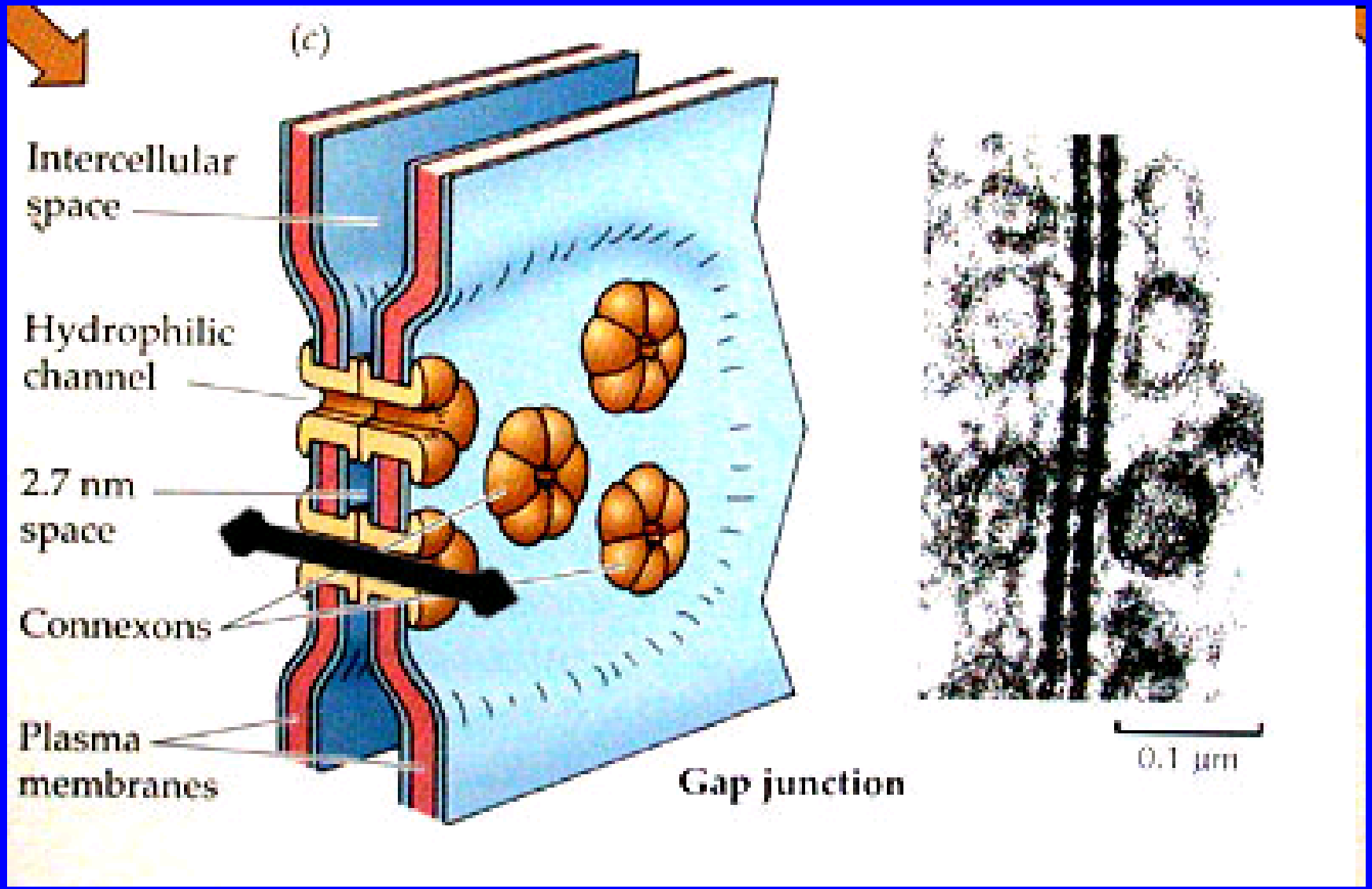
PAHs/POPs -> inhibition of Aromatase (CYP19)

**Microcystins -> liver tumor promotion**

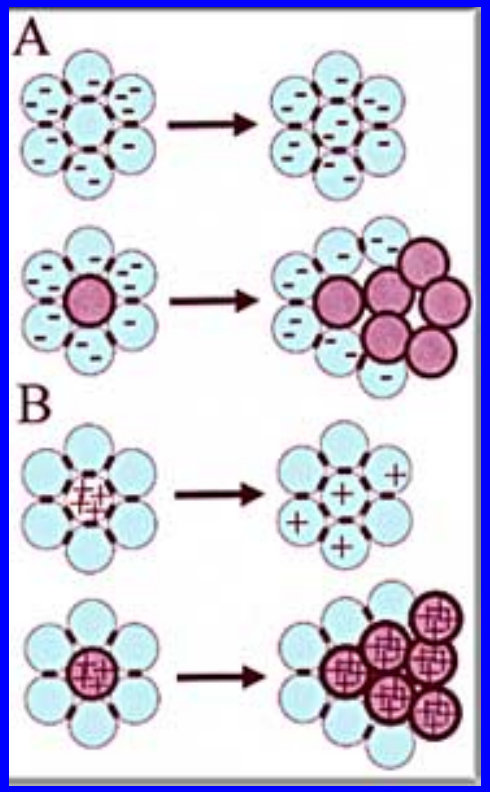
inhibition of PPases [other lecture]

# Gap junctions and cellular continuum

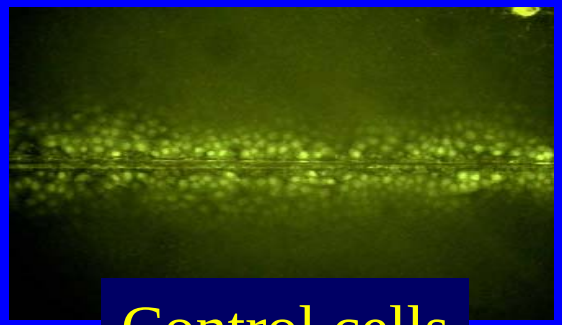
(Gap Junctional Intercellular Communication - GJIC)



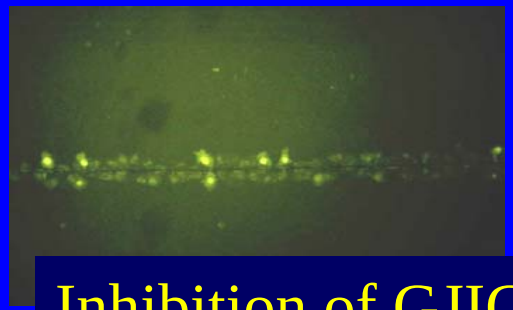
# **Inhibition of GJIC** - mechanism of tumor promotion




- gap-junctional intercellular communication (GJIC) - transfer of small 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**

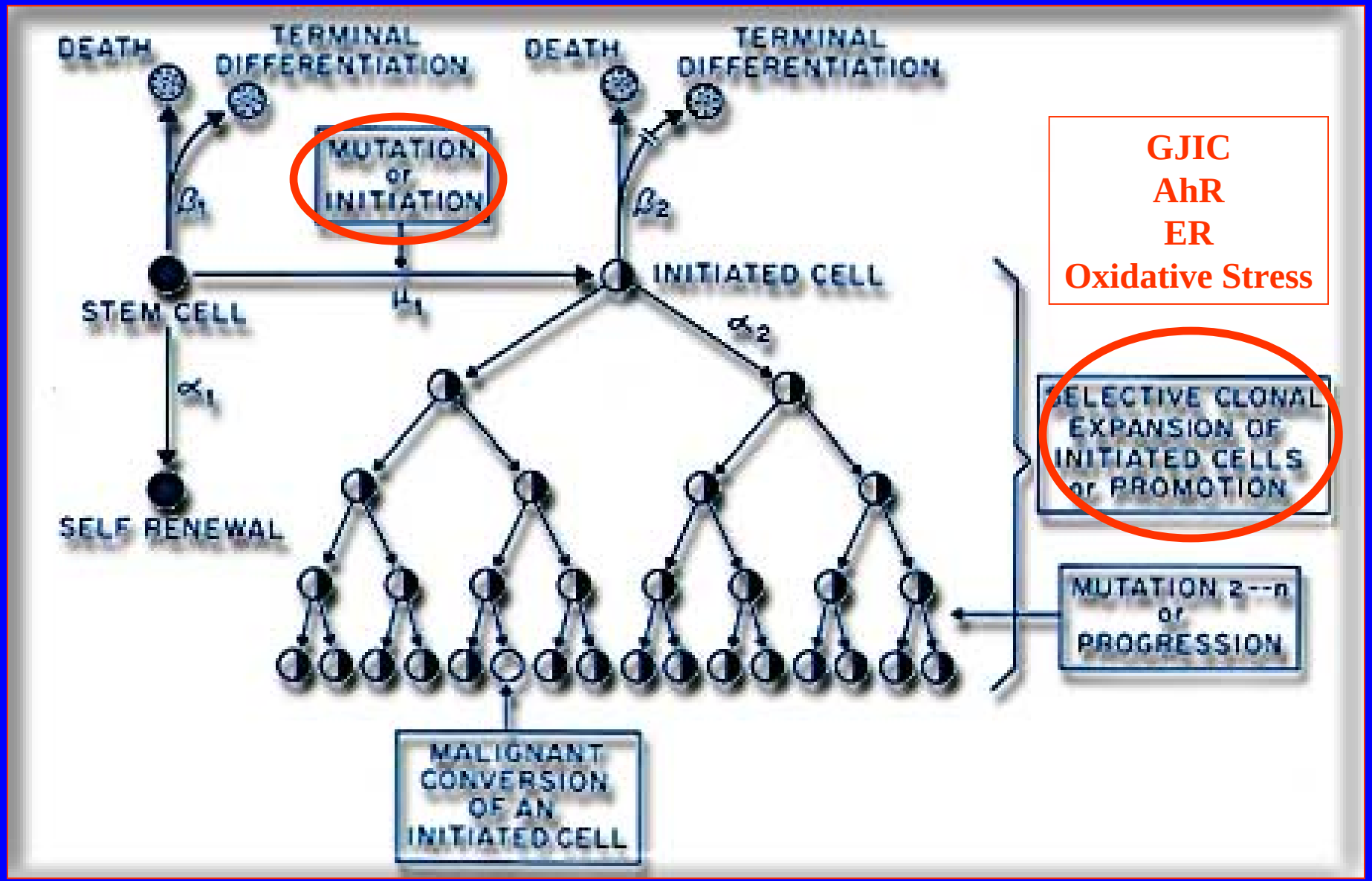


Control cells

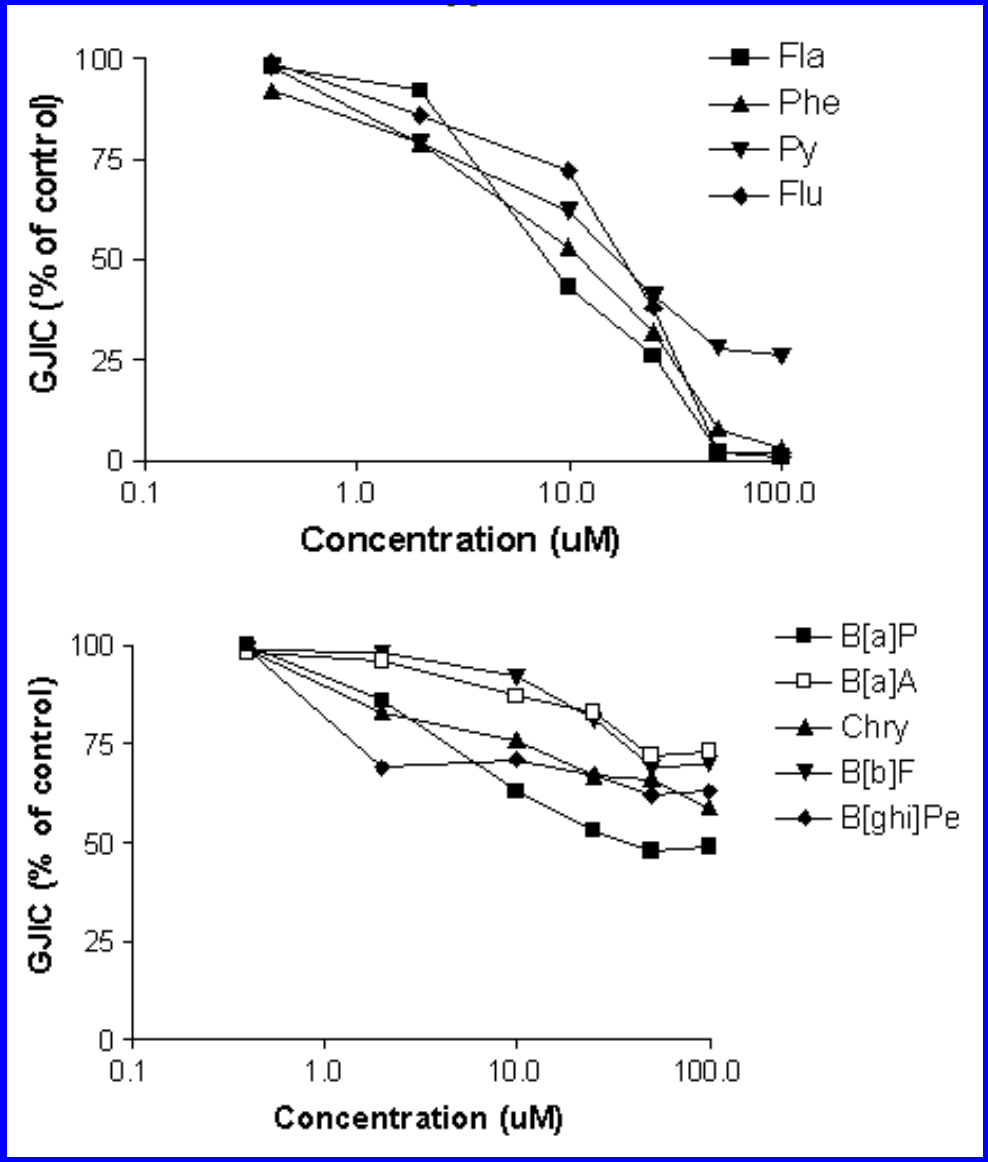


Inhibition of GJIC

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


DEATH
TERMINAL DIFFERENTIATION
DEATH
TERMINAL DIFFERENTIATION
MUTATION or INITIATION
$\beta_1$
$\beta_2$
INITIATED CELL
STEM CELL
$\mu_1$
$\alpha_2$
$\alpha_1$
SELF RENEWAL
SELECTIVE CLONAL EXPANSION OF INITIATED CELLS or PROMOTION
MUTATION 2--n or PROGRESSION
MALIGNANT CONVERSION OF AN INITIATED CELL
GJIC
AhR
ER
Oxidative Stress

# PAHs as tumor promoters
## - inhibition of GJIC -

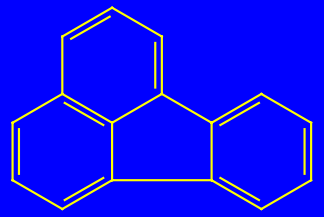



**- Several PAHs inhibits GJIC within 30 min exposure ($IC_{50}$ ~ 10-40 μM)**

**- Low MW and bay/bay-like regions promotes the effect**

**-Fluoranthene**

:non-mutagenic
:non-AhR-inducing
:tumor promoter *in vivo* (!)



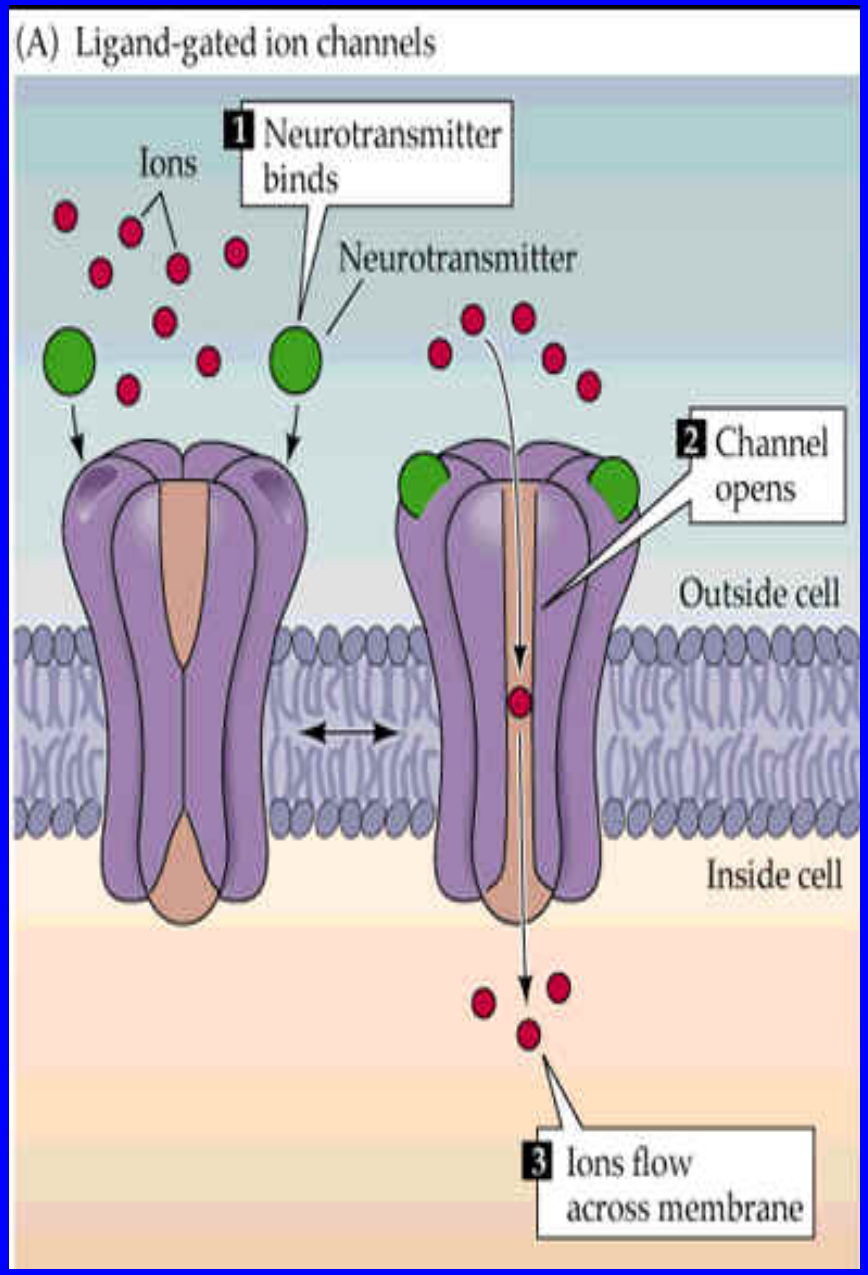
*Bláha et al. 2002 Toxicol Sci 65: 43*

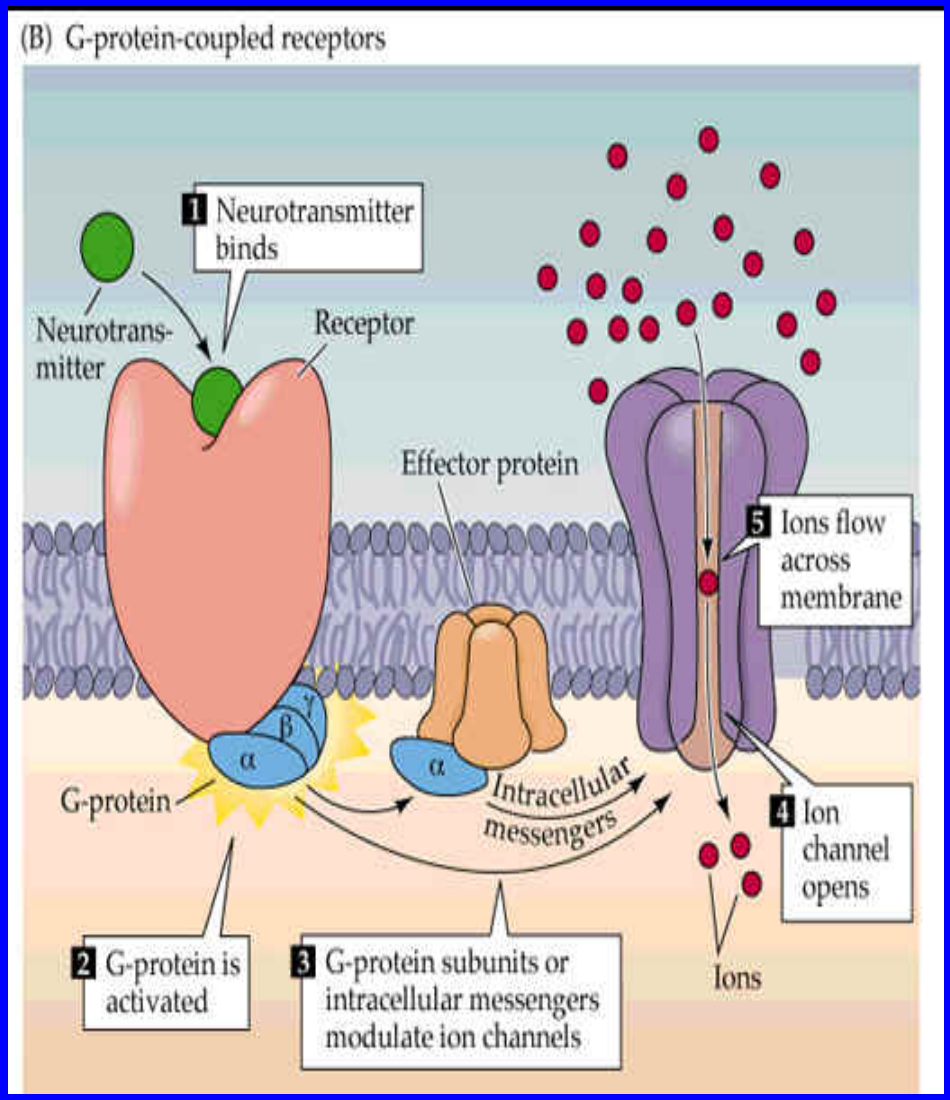
# Toxicity to membrane gradients and transport

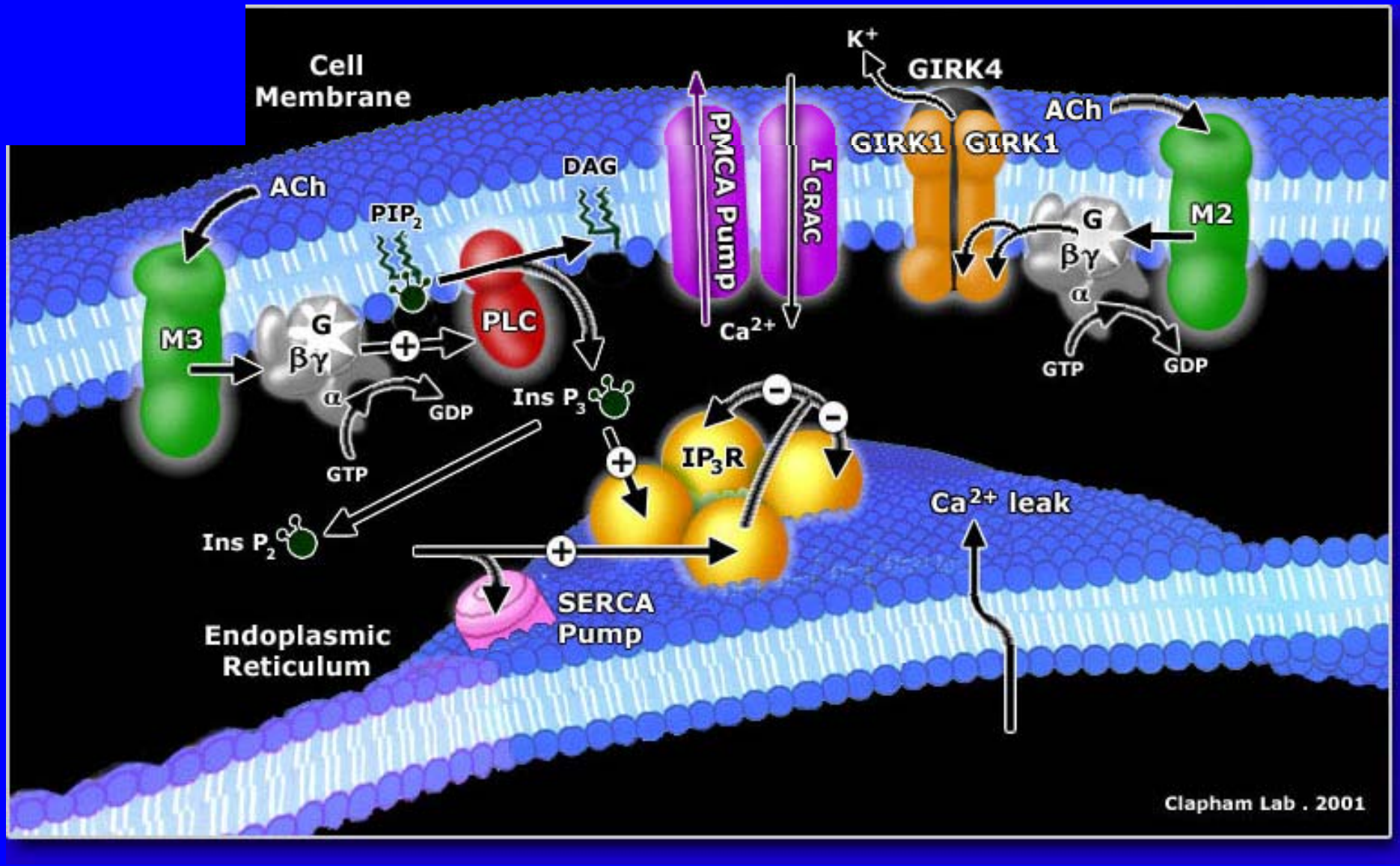
**- Semipermeability of membranes:**
**several key functions**

**- 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

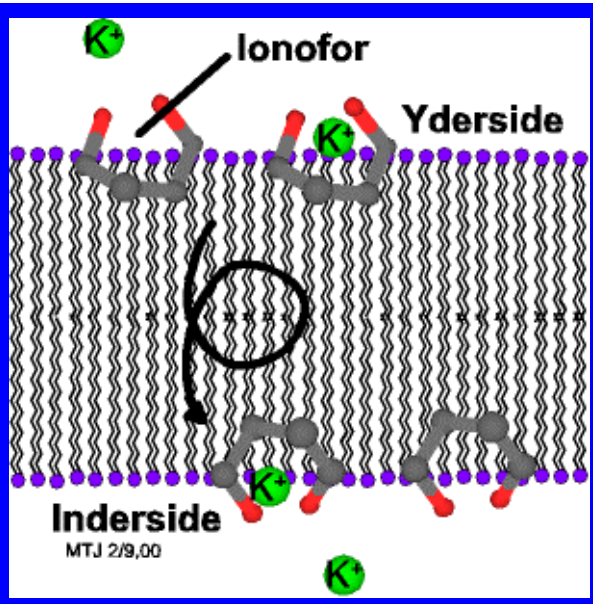

(B) G-protein-coupled receptors
1 Neurotransmitter binds
Neurotrans-mitter
Receptor
Effector protein
5 Ions flow across membrane
α
β
γ
α
G-protein
Intracellular messengers
4 Ion channel opens
Ions
2 G-protein is activated
3 G-protein subunits or intracellular messengers modulate ion channels

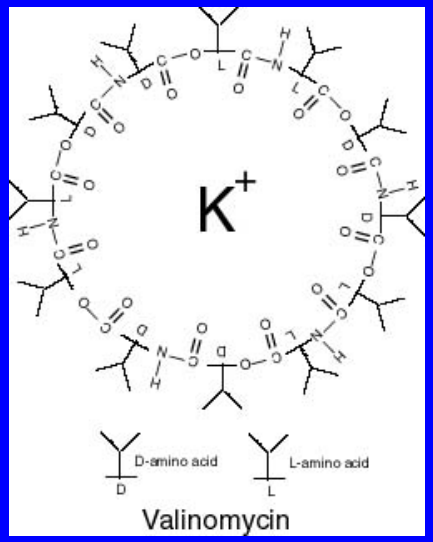

Cell Membrane
K+
GIRK4
ACh
GIRK1
GIRK1
DAG
PMCA Pump
ICRAC
ACh
PIP2
M2
G
βγ
α
M3
G
βγ
PLC
Ca2+
GTP
GDP
Ins P3
α
GDP
GTP
IP3R
Ca2+ leak
Ins P2
SERCA Pump
Endoplasmic Reticulum
Clapham Lab . 2001

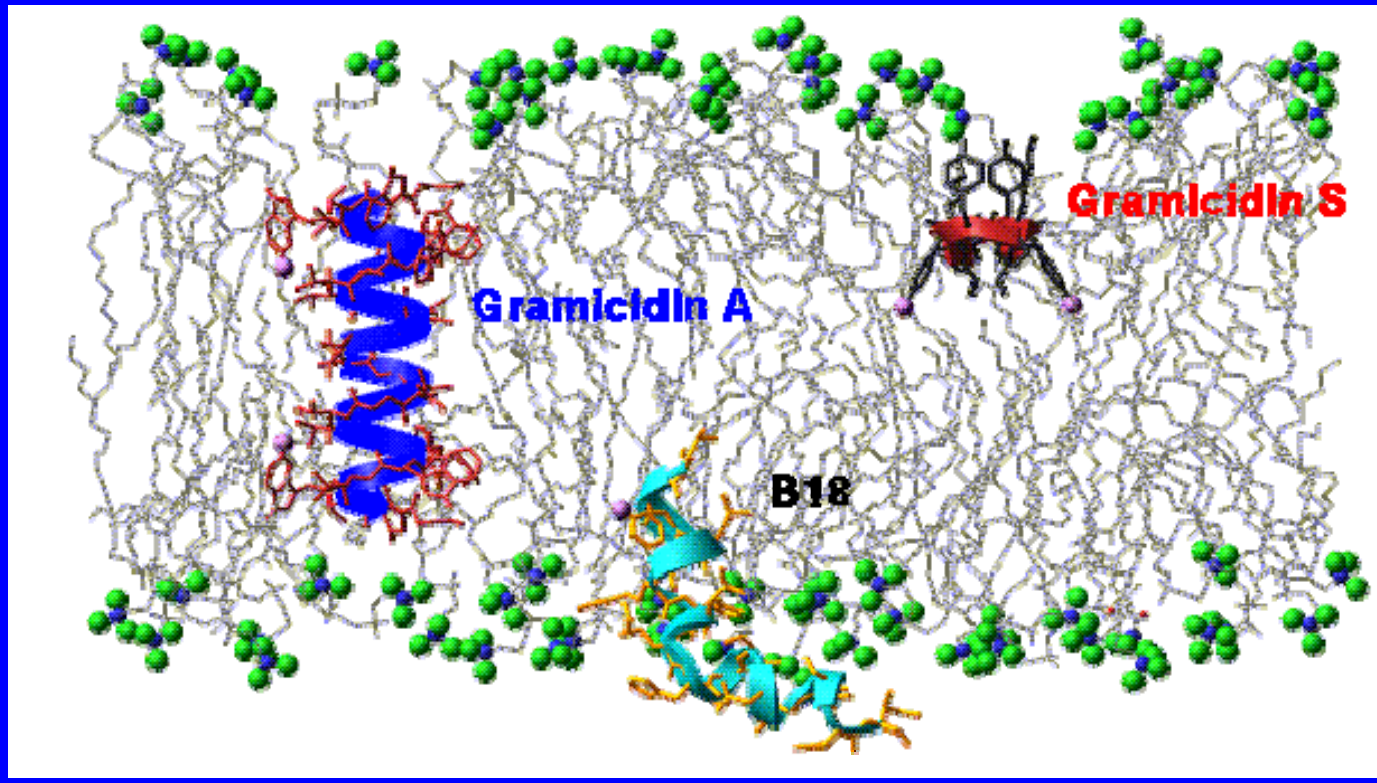
# Membrane gradient disruption

## Ion transfer ("ionofors")

antibiotics
(K+, Ca2+, Mg2+)

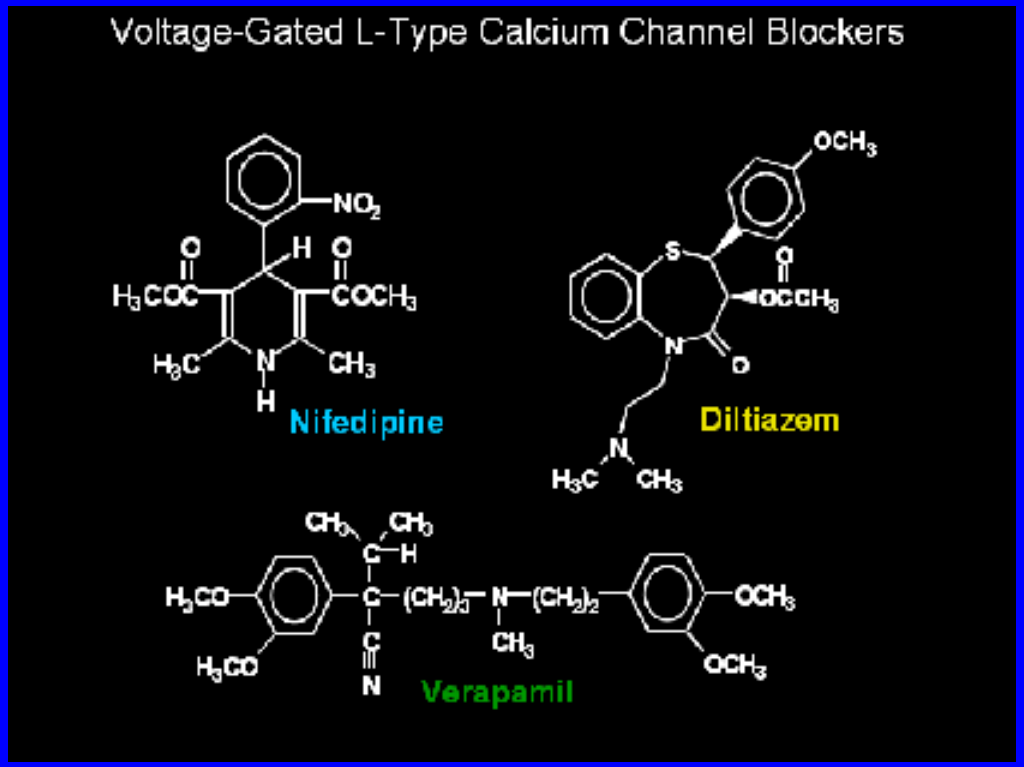


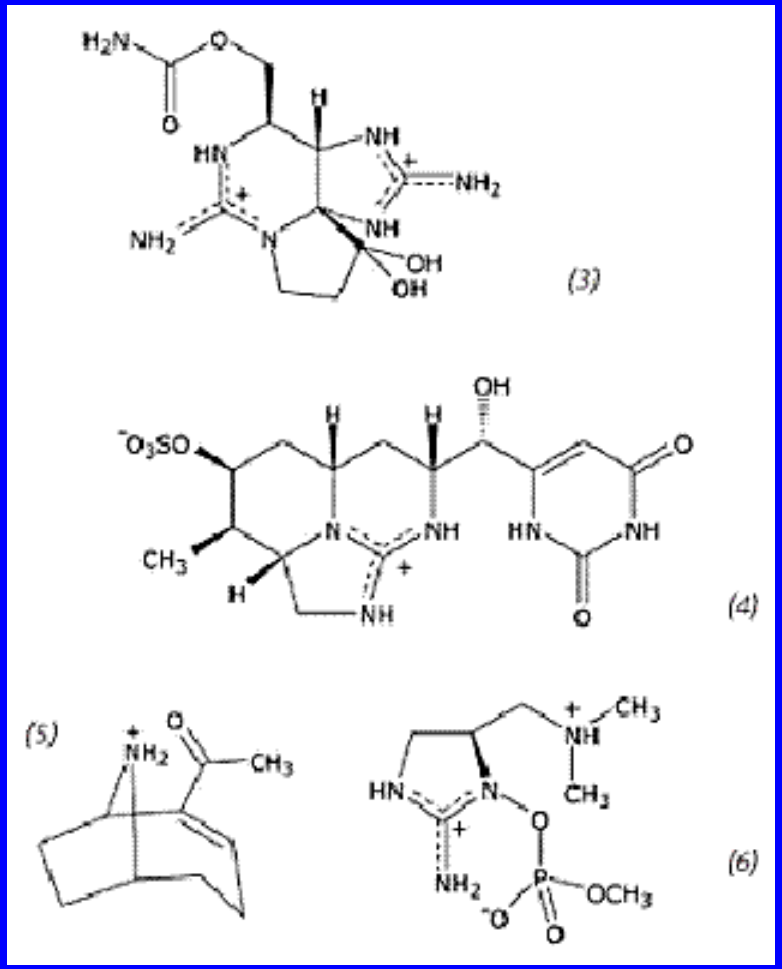



# Ion Channel BLOCKERS / ACTIVATORS

## Neuromodulators (drugs)




## Neurotoxins (cyanobacterial)



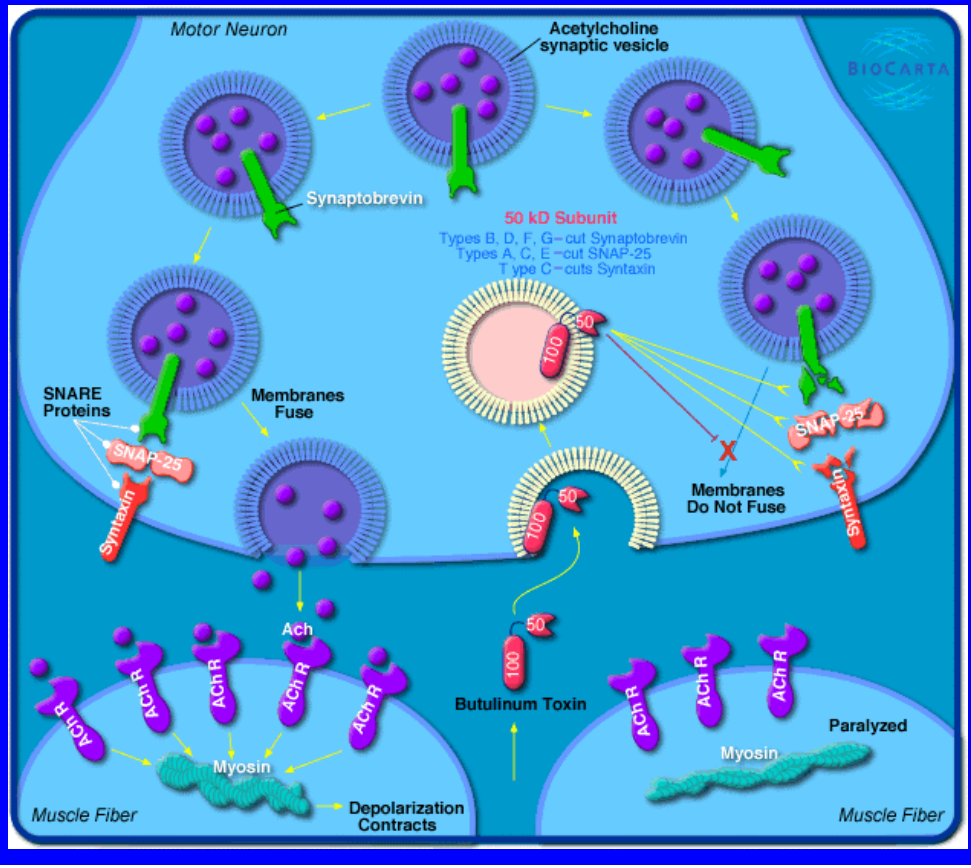
# Botulinum and Tetanus toxins

(Clostridium botulinum, Clostridium tetani)

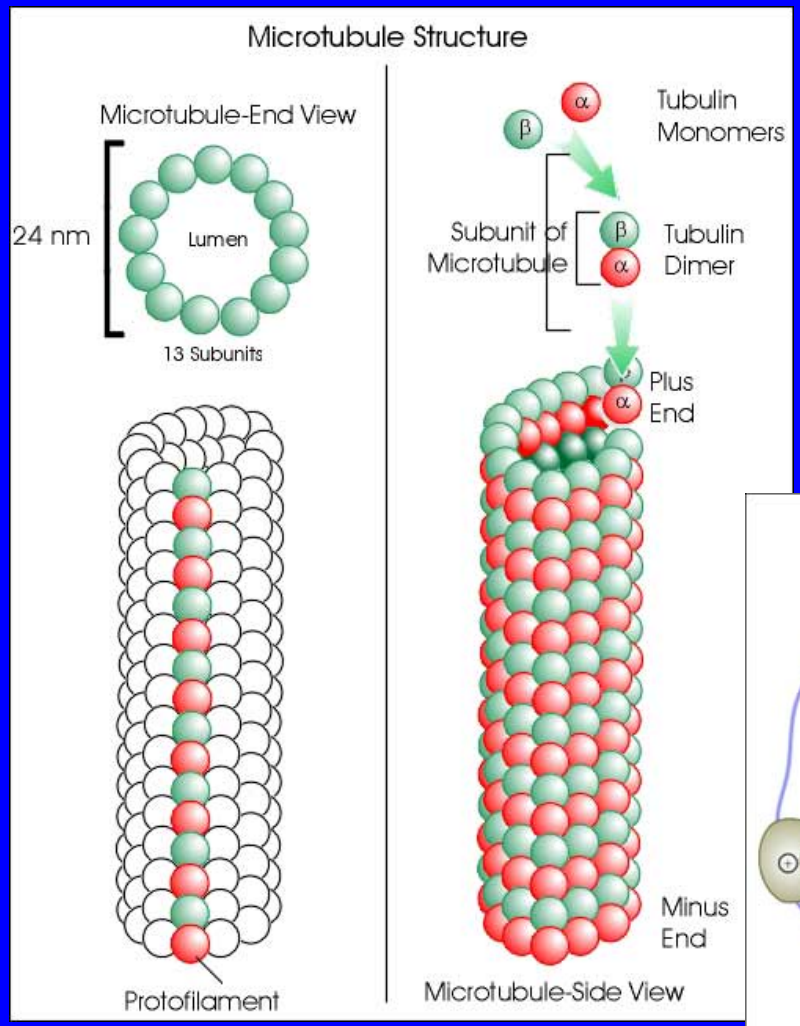
**Toxins = enzymes** - proteases (!)

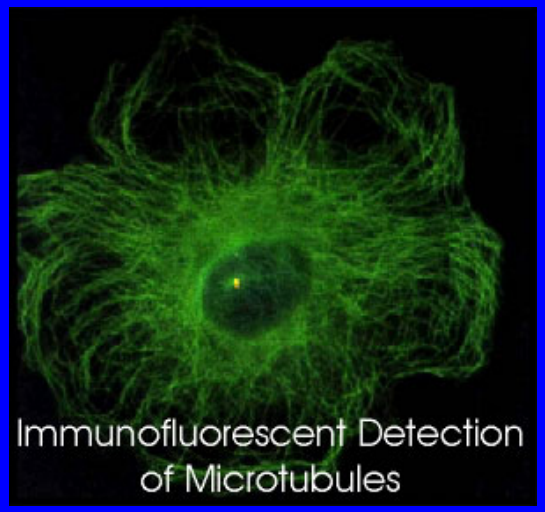
- cleavage of proteins involved in vesicle formation
- selective inhibition of neutrotransmitter release

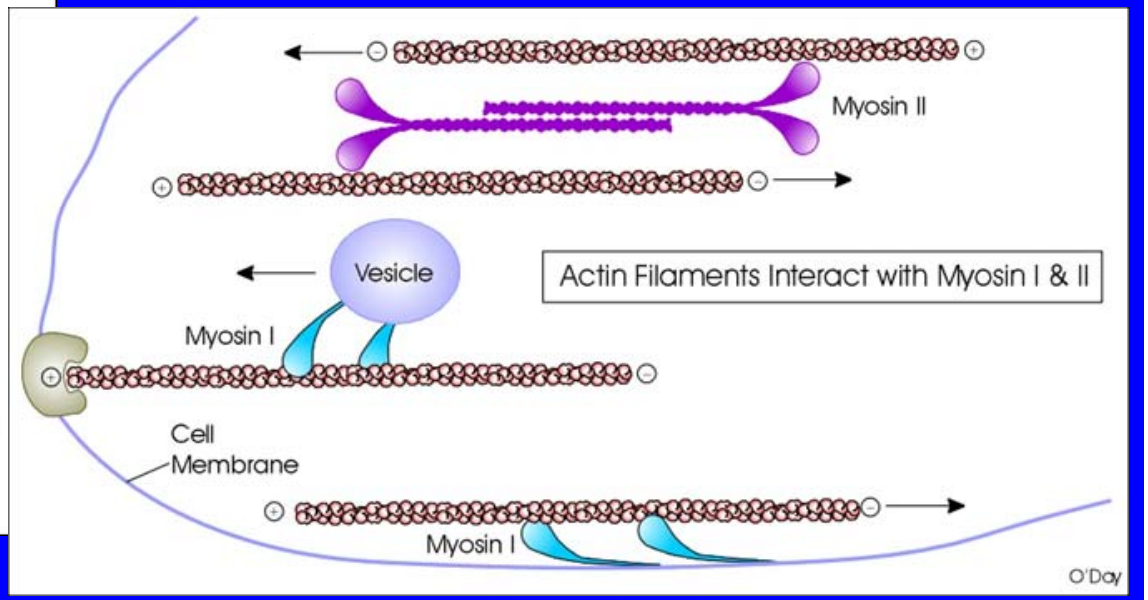
*neurotoxicity*



# Cytoskeleton as target of toxicants
## microtubules / actin-myosin

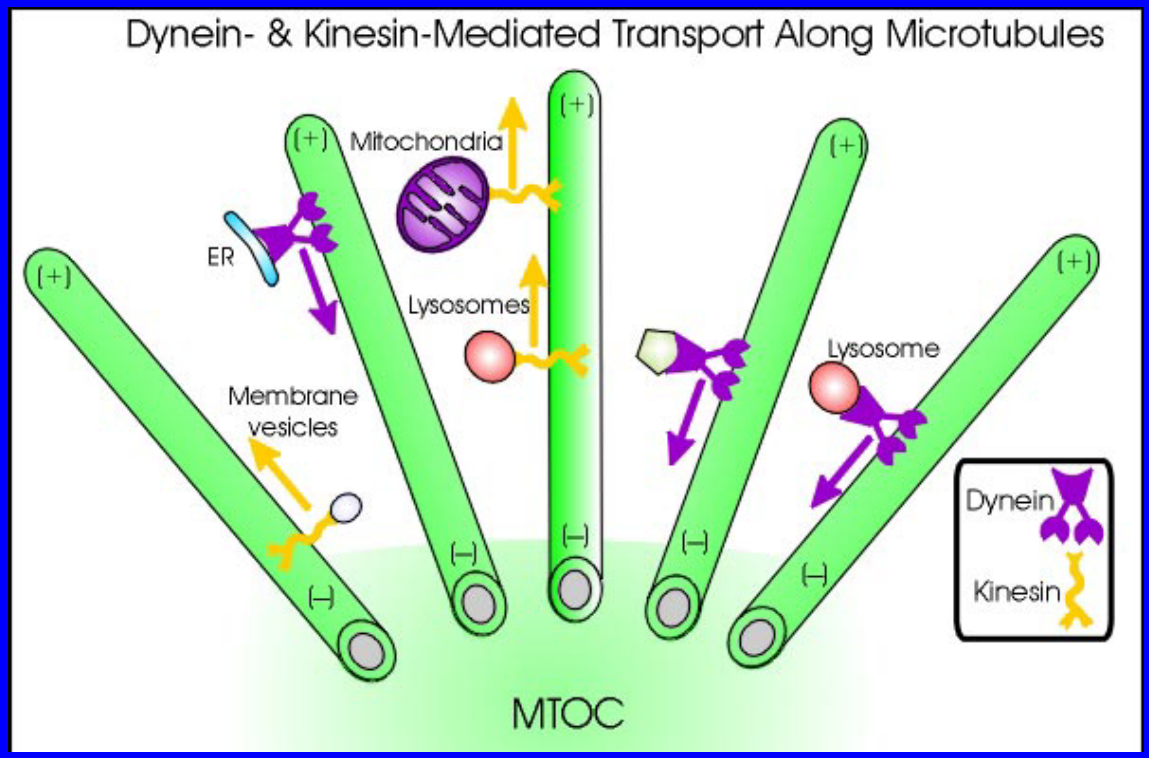


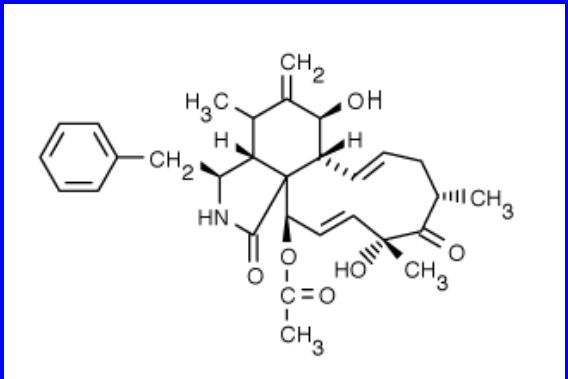



# Cytoskeleton – function

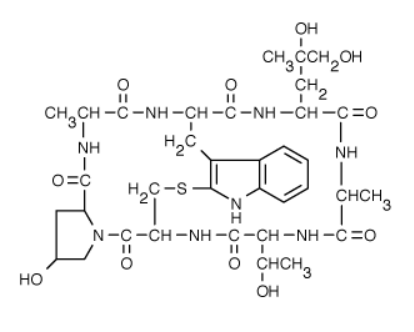
- intracellular transport
- cell replication and division (mitosis:chromosomes)
- muscle movement
- membrane (vesicles) fusion



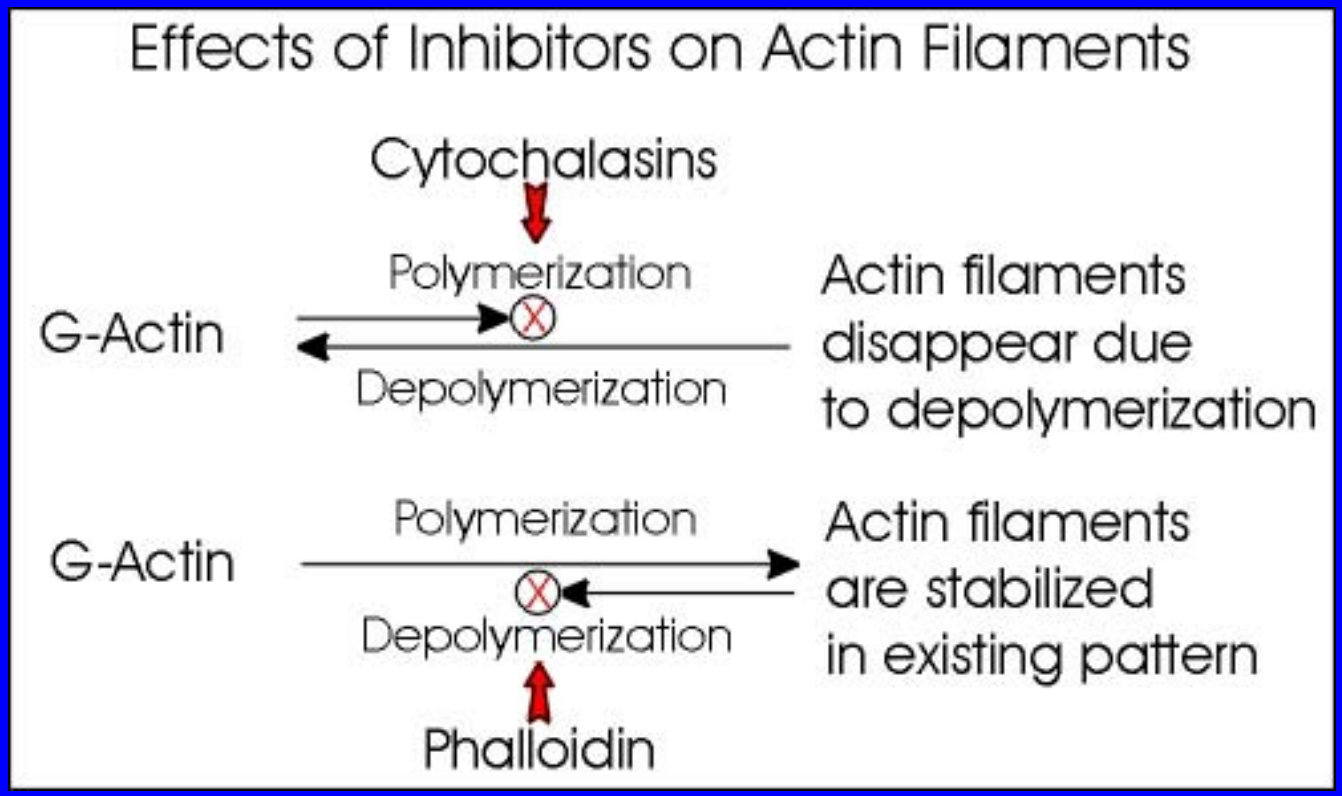
# TOXINS: effects on (DE)POLYMERIZATION



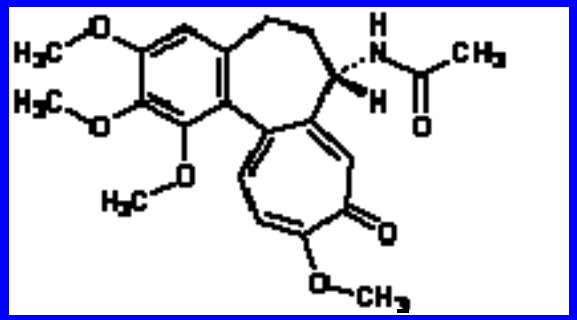
cytochalasin D
(fungal toxin)



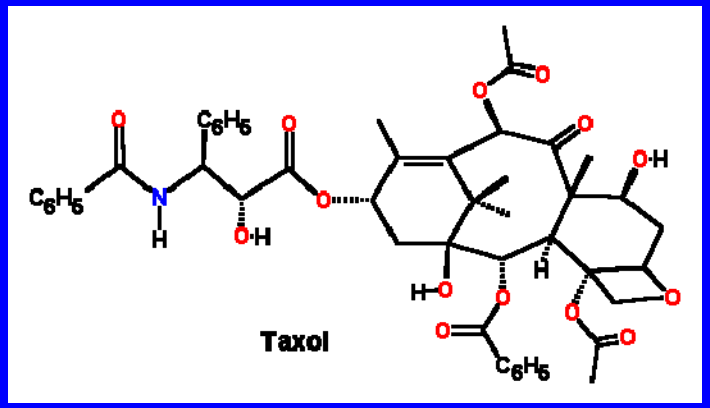
Phalloidin
(death cap - Amanita phalloides)

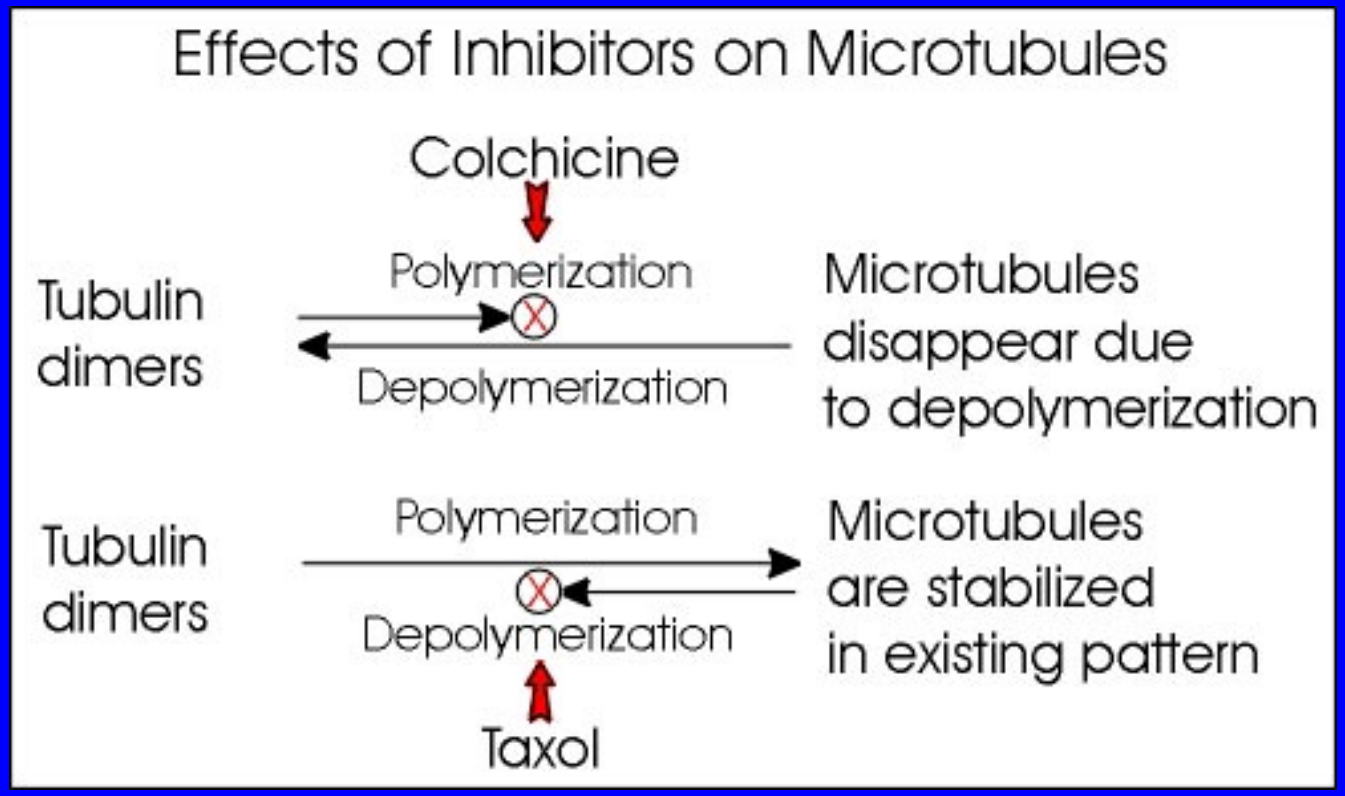


# TOXINS: effects on (DE)POLYMERIZATION



Colchicine




taxol