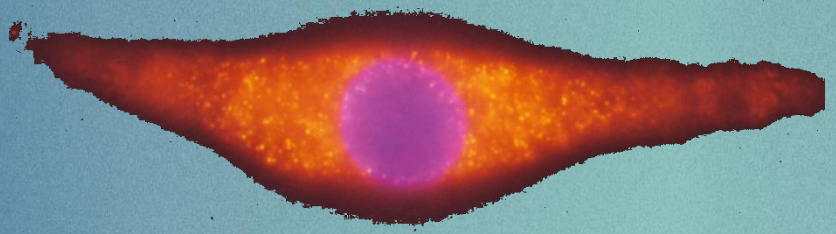
THE UNIVERSITY OF MELBOURNE
Faculty of Veterinary and Agricultural Sciences



Lecture 13
Receptors and signalling pathways 2: G protein coupled receptors, enzyme receptors and intracellular receptors

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VETS30015 / VETS90121

Intended learning objectives

- Describe the main signal transduction pathways involved in cell signalling
    1. Ligand-gated ion channels
    2. G-protein coupled receptors,
    3. Receptor enzymes (i.e., tyrosine kinase),
    4. Nuclear receptors (class I and class II)
        - ➢ Discuss the mechanism of nuclear receptor activation
    - Be able to give an example of each.

- Describe how different types of G proteins (Gs, Gi ,Gq, Gt signalling) can be linked to different intracellular signalling pathways
- Describe how endothelial cells can be stimulated to produce nitric oxide, causing dilation of smooth muscle.
- Describe how enzyme-linked receptors, such as growth factor receptors and the insulin receptor, activate downstream effector proteins.
- Understand how some hormones act through intra-cellular receptors, leading to changes in gene transcription via hormone response elements.

G protein families

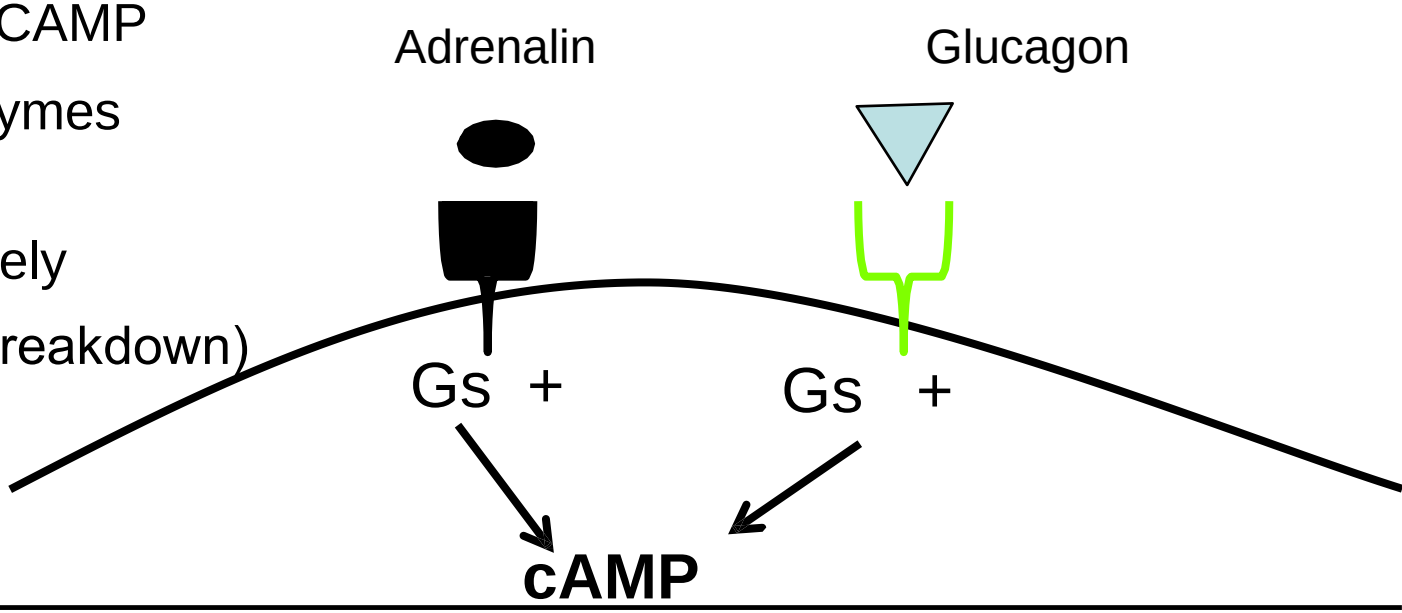
G protein	Receptor	Signalling pathway
Gs	β-adrenergic receptors, glucagon, histamine, serotonin	Stimulatory, increase cAMP
Gi	α_2-adrenergic receptors	Inhibitory, decrease cAMP
Gq	α_1-adrenergic receptors, some muscarinic receptors; histamine	IP_3, DAG Increase cytoplasmic Ca^{++}
Gt	Light receptors in eye	Transducin, Increase cGMP phosphodiesterase (catalytic) Decrease cGMP

Important regulatory control points

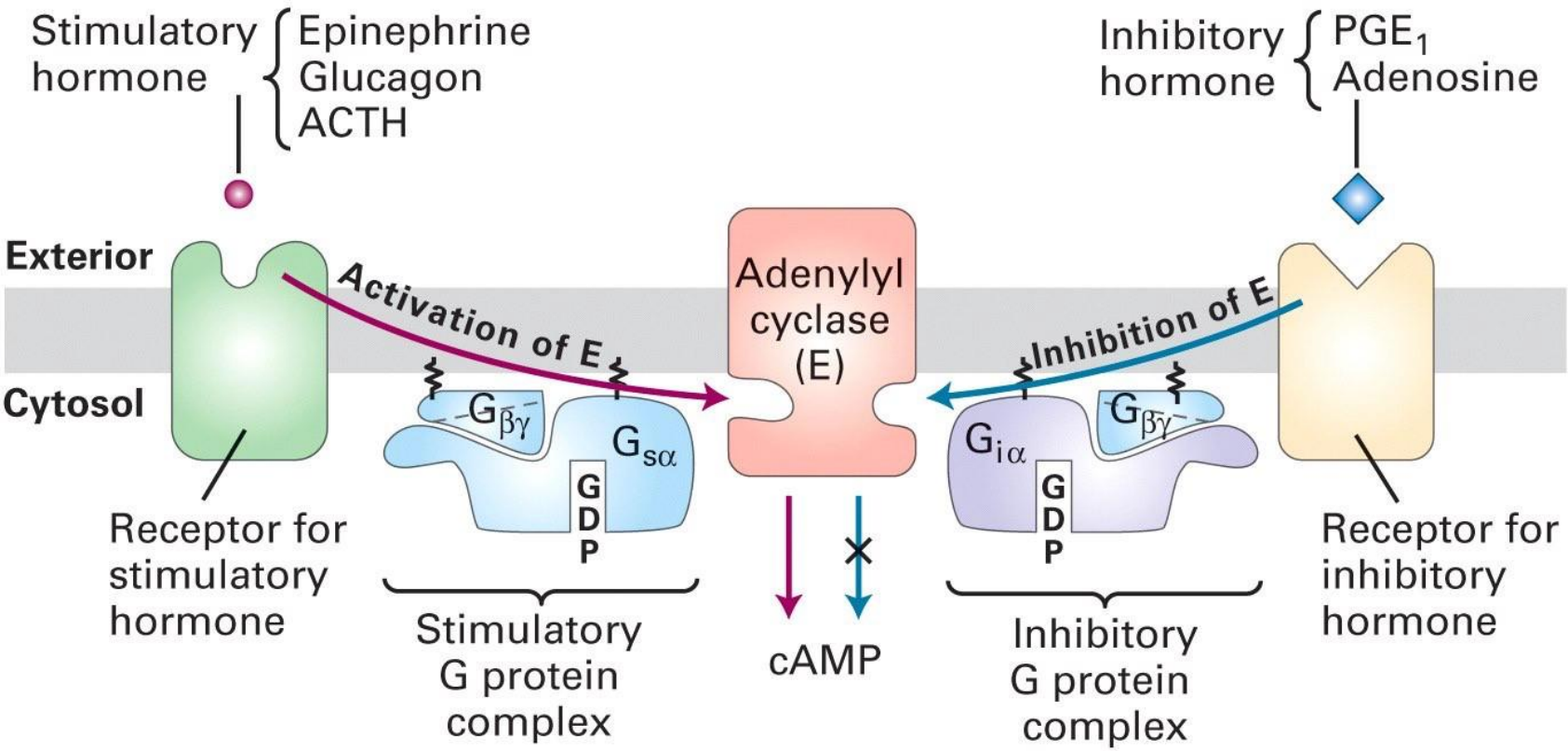
- Example of two hormones acting additively - Use same second messenger, cAMP
- Glucagon & Adrenalin, acting on the liver
  - Both acts via GS proteins
  - Increase adenylate cyclase

    → increase CAMP
  - Same enzymes activated
  - Act additively

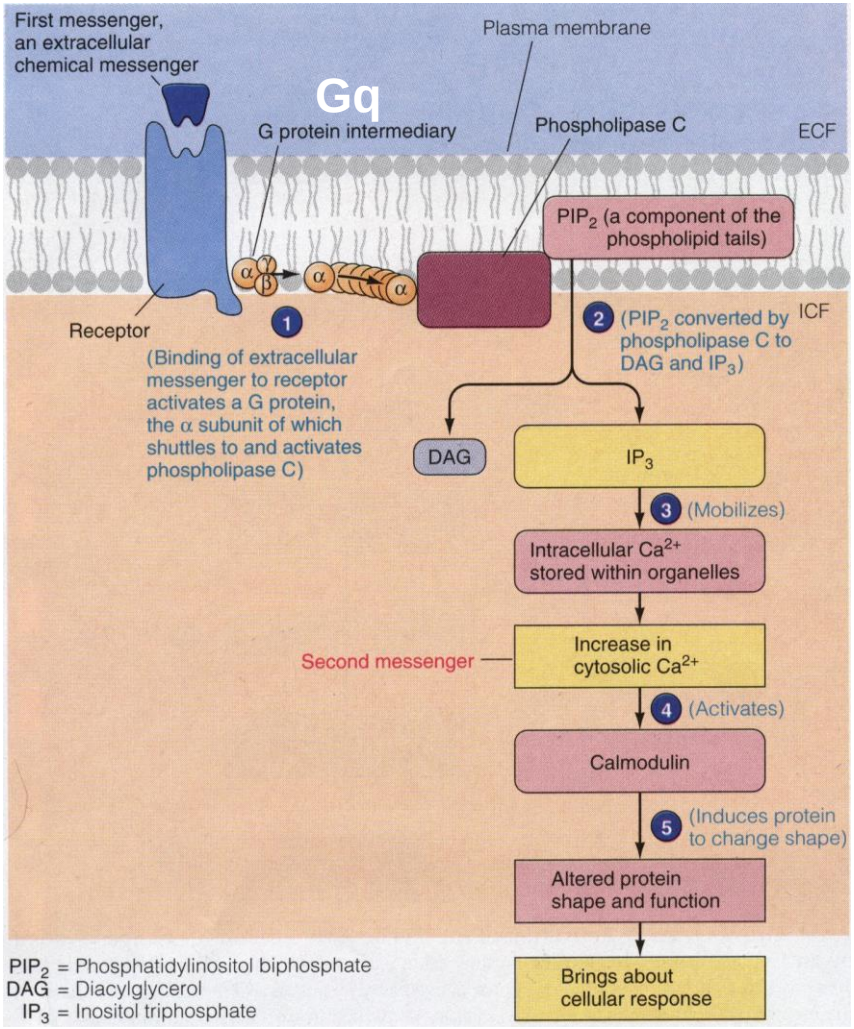
(↑ glycogen breakdown)

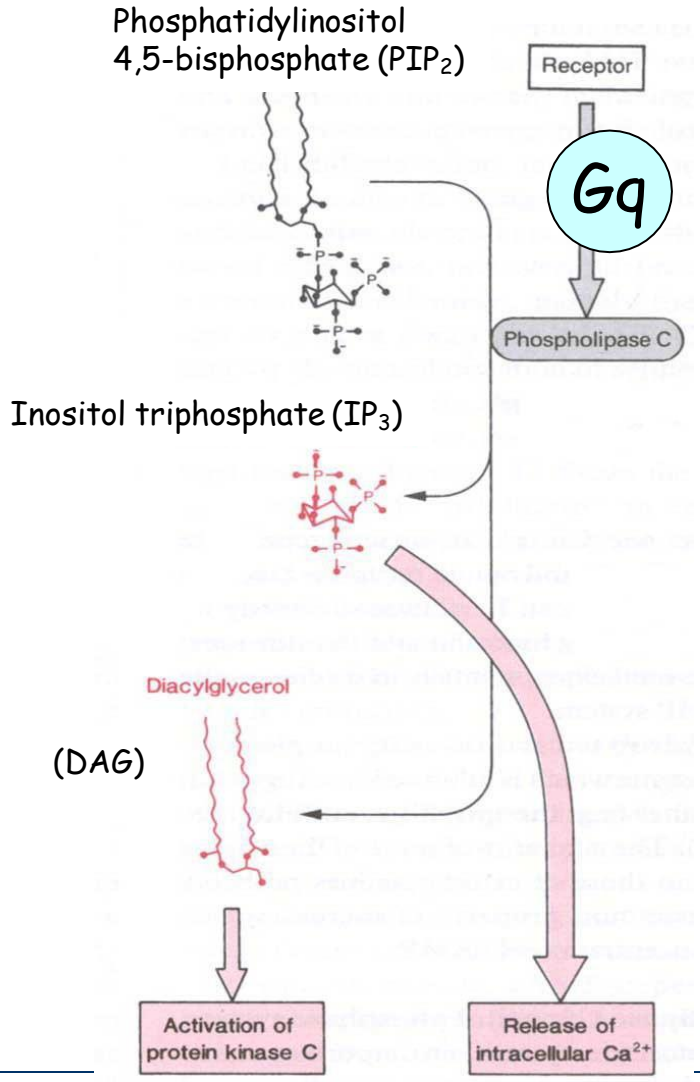


Important regulatory control points Gs & Gi proteins



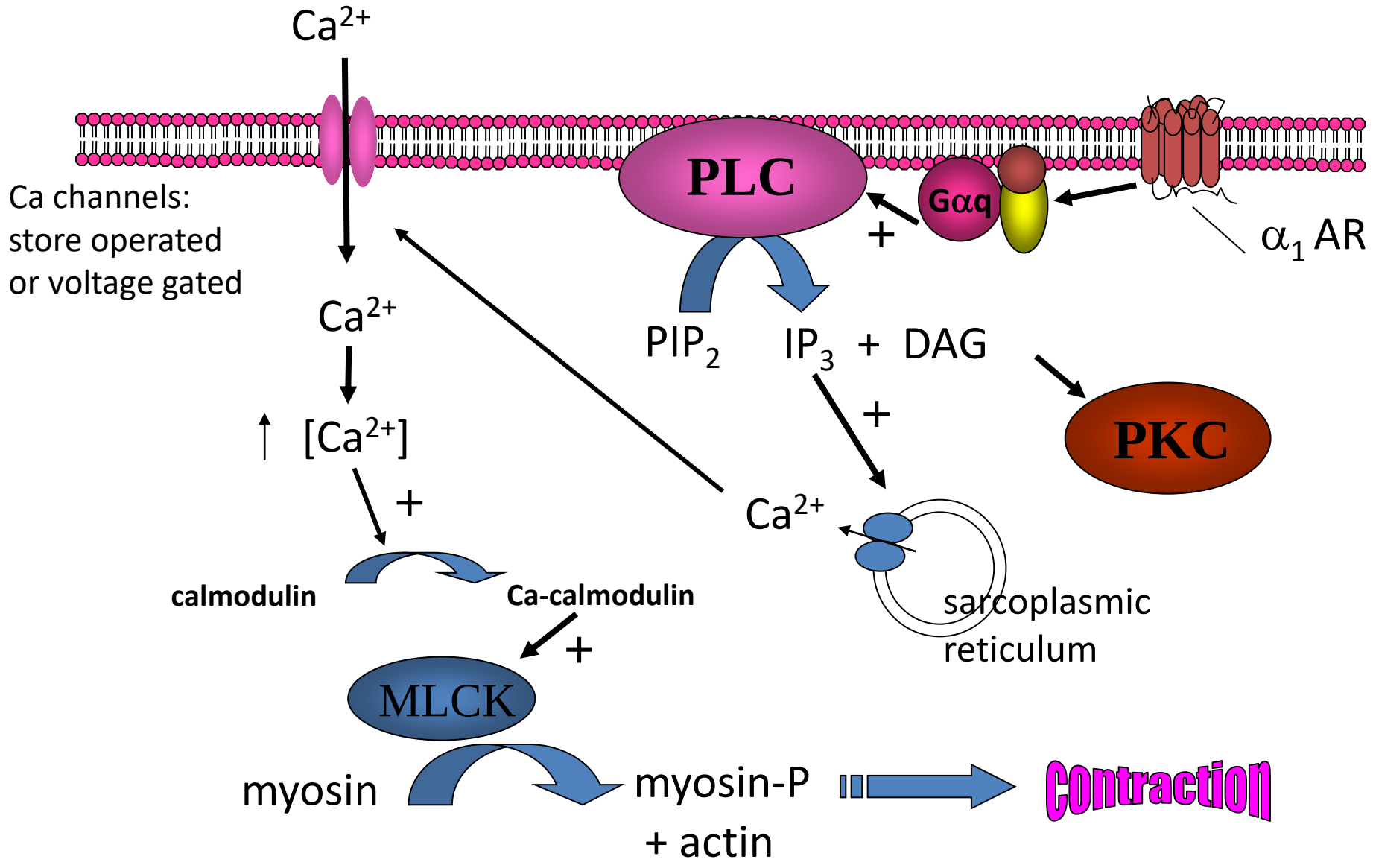
Gq proteins & phospholipase C activation



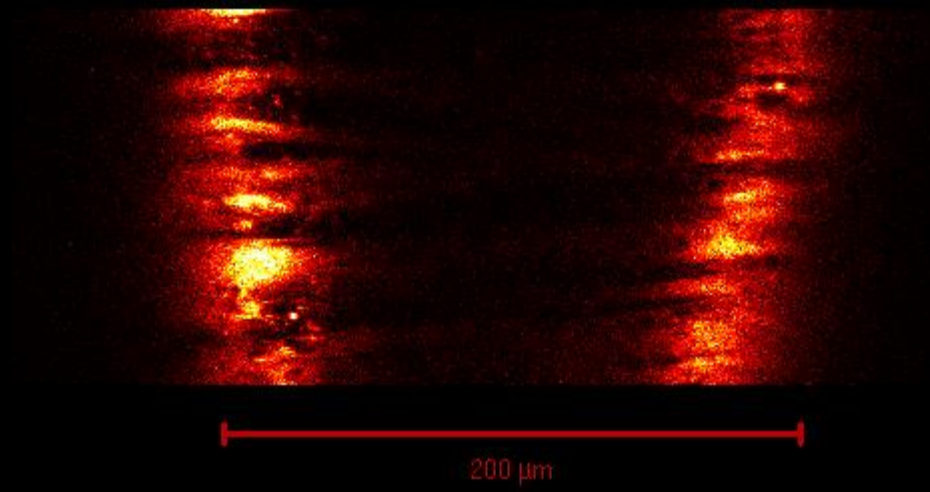


Sherwood 1997

α_1 adrenoceptor-mediated constriction of smooth muscle

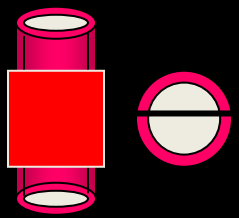


Calcium influx in mouse tail arteriole



Ca^{2+} activated fluorescent dye

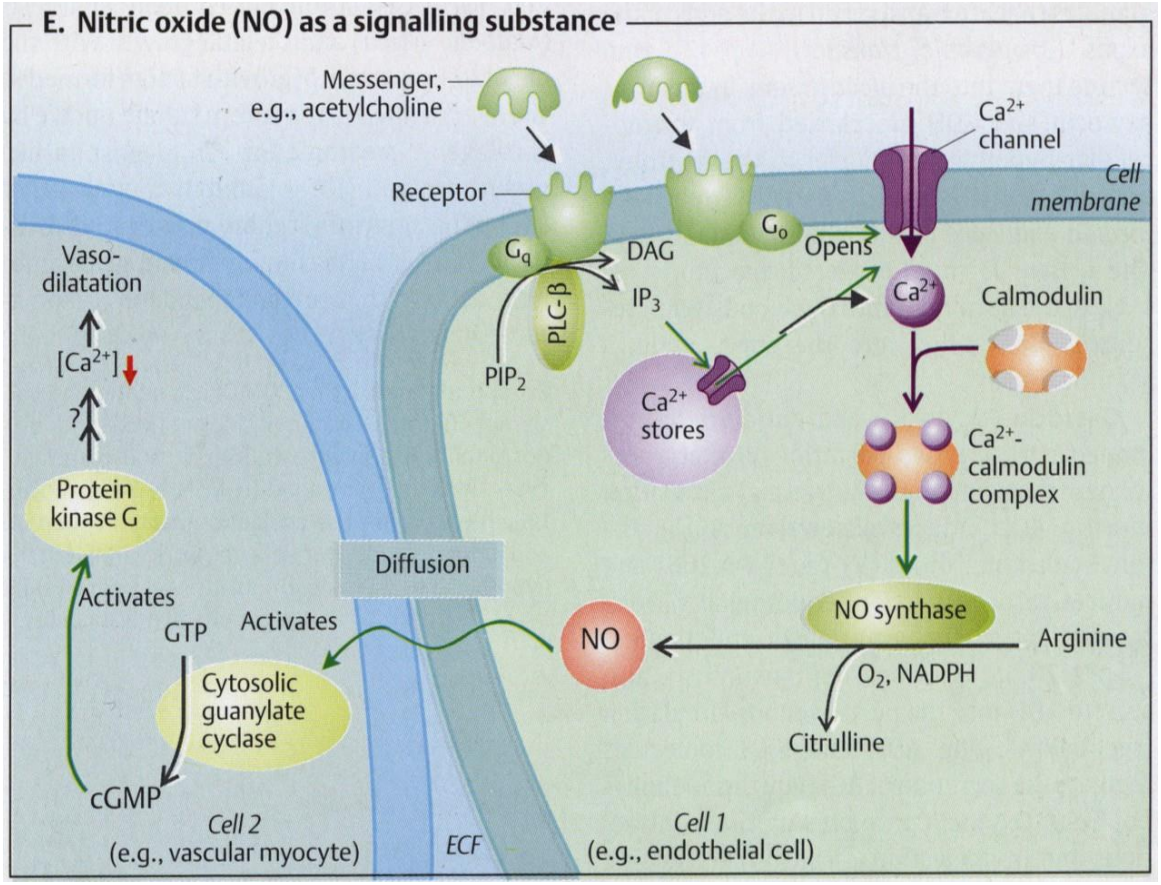
α_1 adrenoceptors stimulated with NA mimetic



Nitric oxide signalling

Nitric oxide (NO):

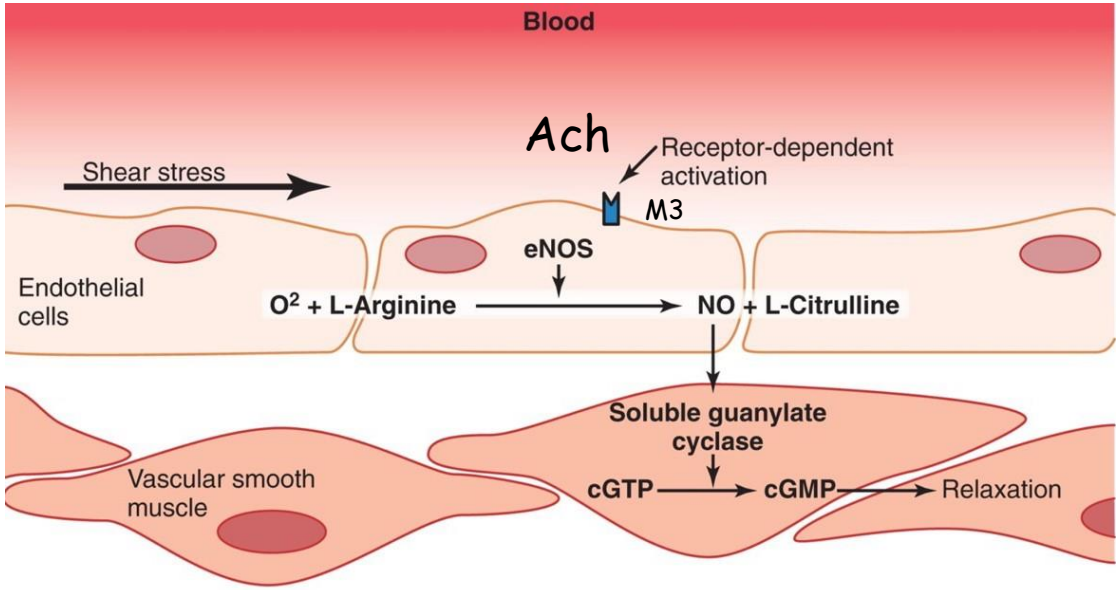
- Labile (short-lived) gas molecule
- Produced by cardiovascular endothelial cells
- Causes vasodilation
- NO donor compounds used in the treatment of congestive heart failure



Despopoulos & Silbernagel 2003

Nitric oxide signalling in blood vessels

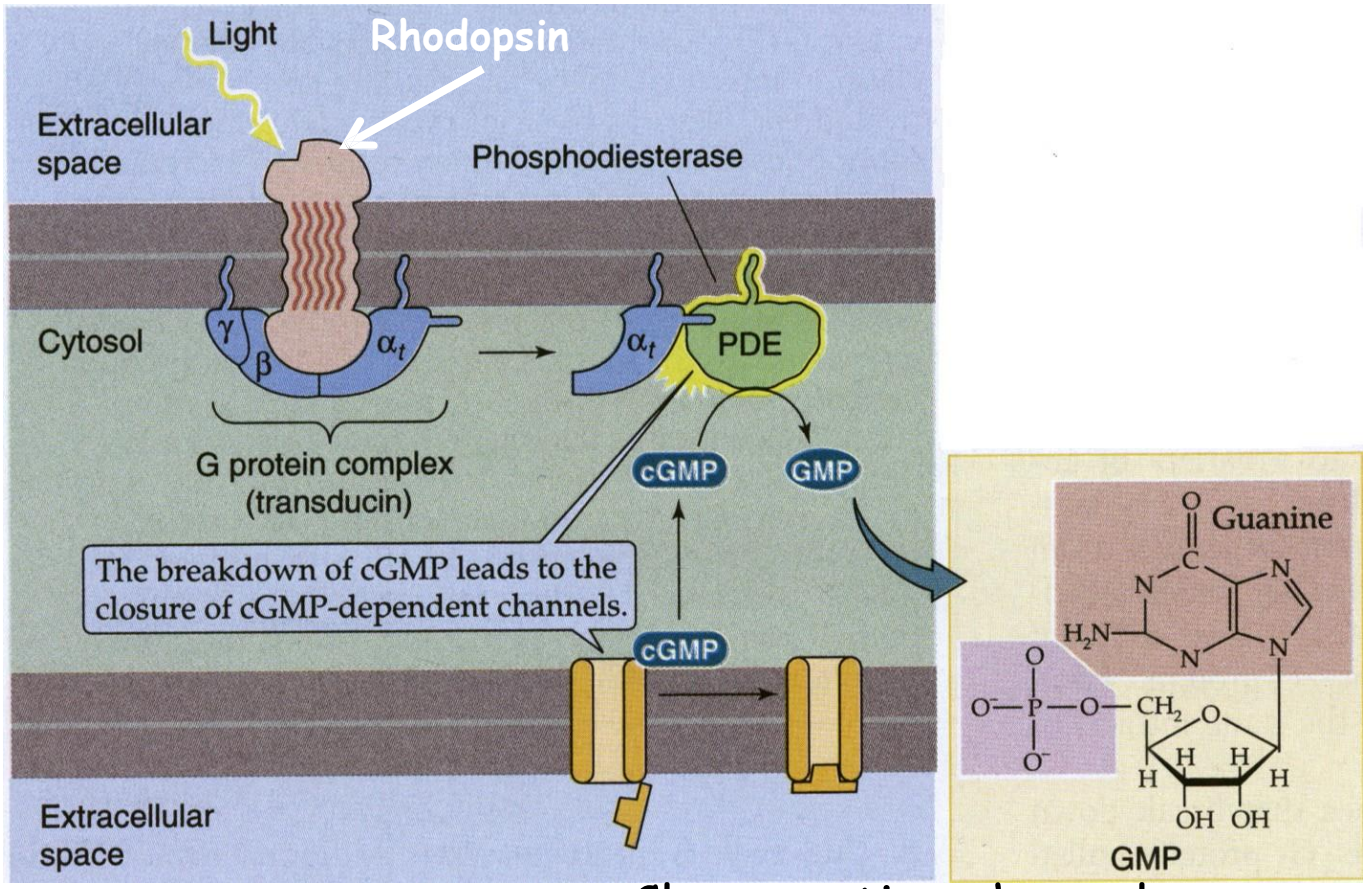
- Small, toxic gas
- Acts locally
- Short ½ life of 5-10 sec
- Endogenous NO release stimulated by bradykinin, acetylcholine, adenine nucleotides



- Example
  1. Ach binds Muscarinic M3 receptor linked to Gq protein on endothelial cell
  2. Causes cascade that activates NO synthase (eNOS)
  3. Arginine converted to citrulline + NO
  4. NO diffuses into adjacent smooth muscle cells
  5. NO activates cytosolic guanylyl cyclase
  6. cGTP is converted to cGMP
  7. cGMP activates protein kinase G which phosphorylates muscle proteins to induce muscle relaxation – more blood flow

Guyton & Hall, 2011

$G\alpha_t$ proteins & phosphodiesterase Phototransduction in the retina

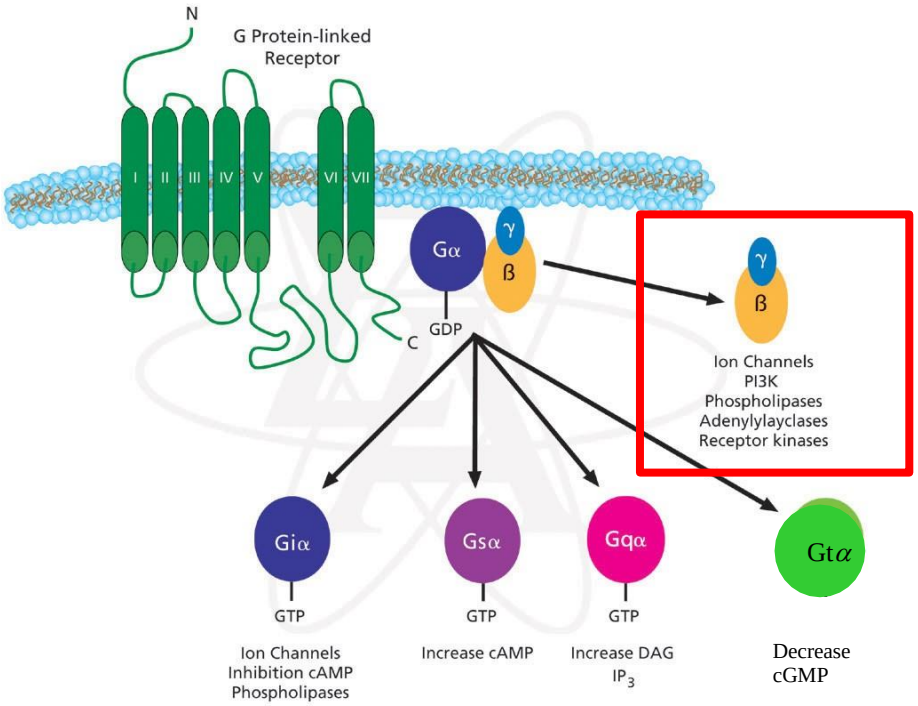


Closes cation channels

Na^+ & Ca^{2+}

- Boron & Boulpaep 2012

G-protein families + βγ signalling



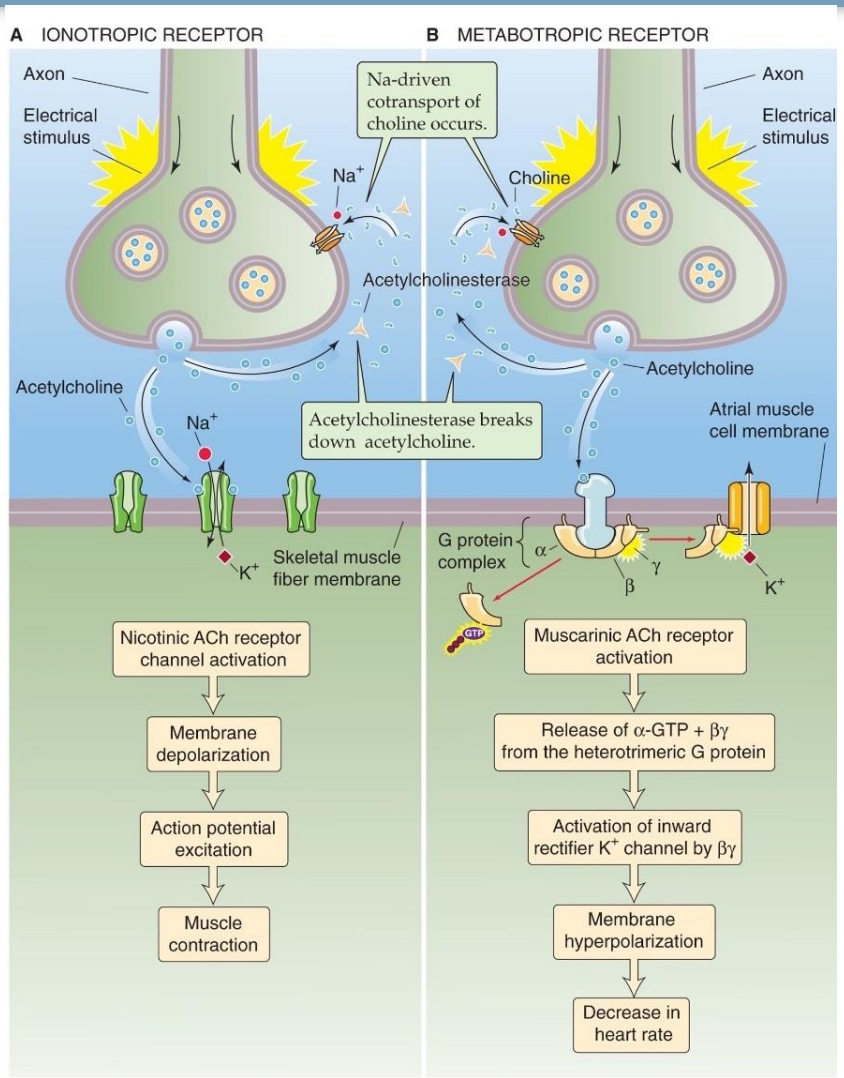
Diversity of

G Protein-Coupled receptor signal transduction pathways

Examples – ion channel vs G protein transduction βγ signalling

- Ionotropic receptor
  - ➢Nicotinic Ach receptor
  - ➢Opens Na+ ion channel
  - Ø Membrane depolarization
  - Ø Action potential produced
  - Ø Muscle contraction

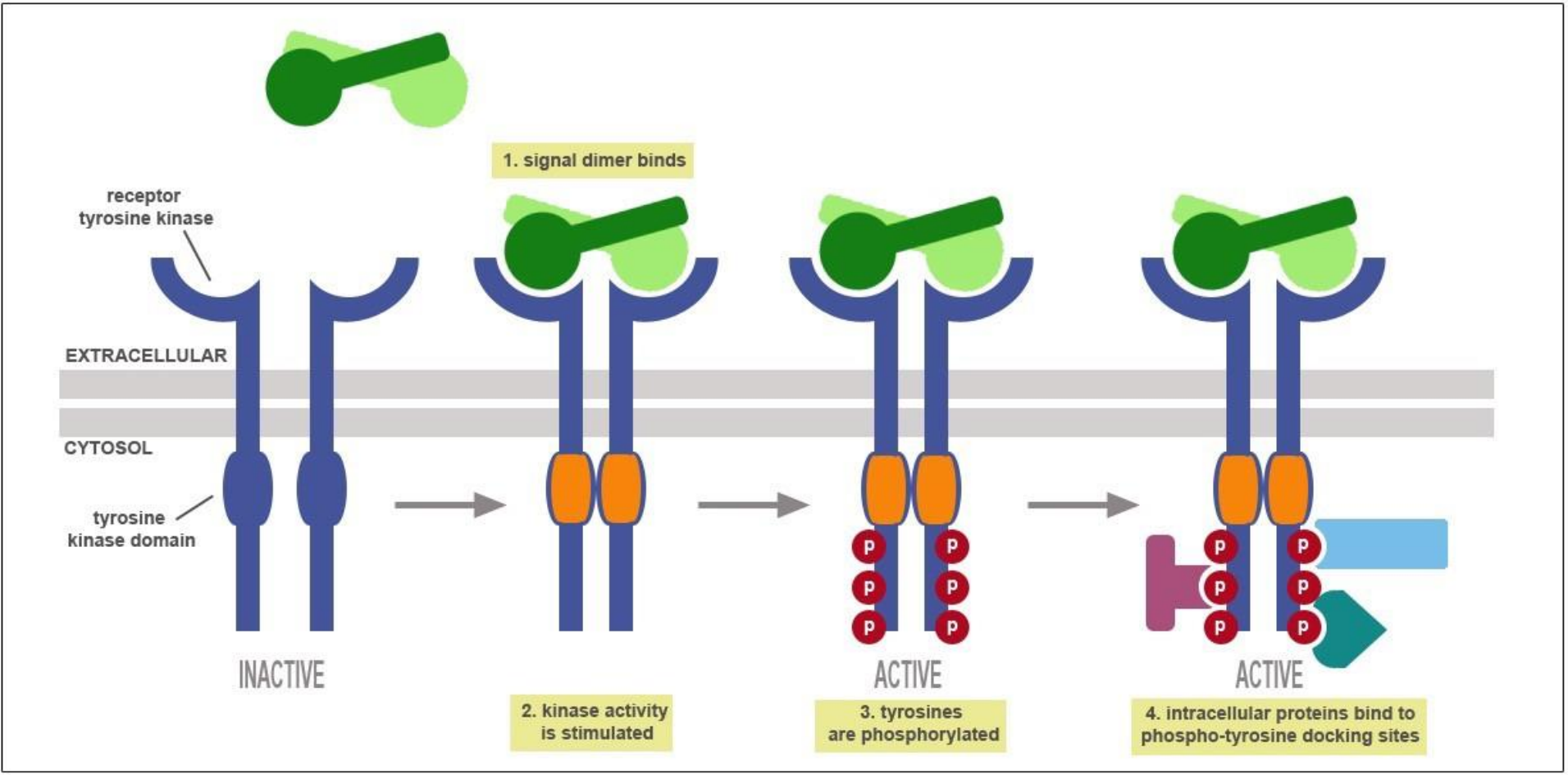
  Skeletal muscle
- Metabotropic receptor
  - ➢ Muscarinic receptor
  - ➢ G protein activated
  - ➢ $\beta\gamma$ subunit activates K^+ channel
  - ➢ Opens K^+ ion channel
  - ➢ Membrane hyperpolarization
  - ➢ Decreased heart rate
  - Cardiac muscle



G protein-coupled receptors 2nd messengers

Ligand or signal	Effector enzyme	2nd messenger
Adrenaline (epinephrine) Thyroid stimulating hormone (TSH) Angiotensin II (epithelial cells) Catecholamine (β receptors) Acetylcholine (ACh, via a muscarinic receptor) Adrenocorticotropic hormone (ACTH) Corticotrophin-releasing hormone (CRH) Follicle stimulating hormone (FSH) Glucagon Vasopressin (V_2 receptor - epithelial cells)	Adenylyl cyclase	cAMP
Angiotensin II (vascular smooth muscle) Catecholamine (α receptors) Gonadotropin-releasing hormone (GnRH) Growth hormone-releasing hormone (GHRH) Oxytocin Thyrotropin releasing hormone (TRH) Vasopressin (V_1 receptor - vascular smooth muscle)	Phospholipase C	Inositol (1,4,5) trisphosphate (IP_3) & Diacylglycerol (DAG)
Nitric oxide Atrial natriuretic hormone Light	Guanylyl cyclase (+) Phosphodiesterase (-)	cGMP

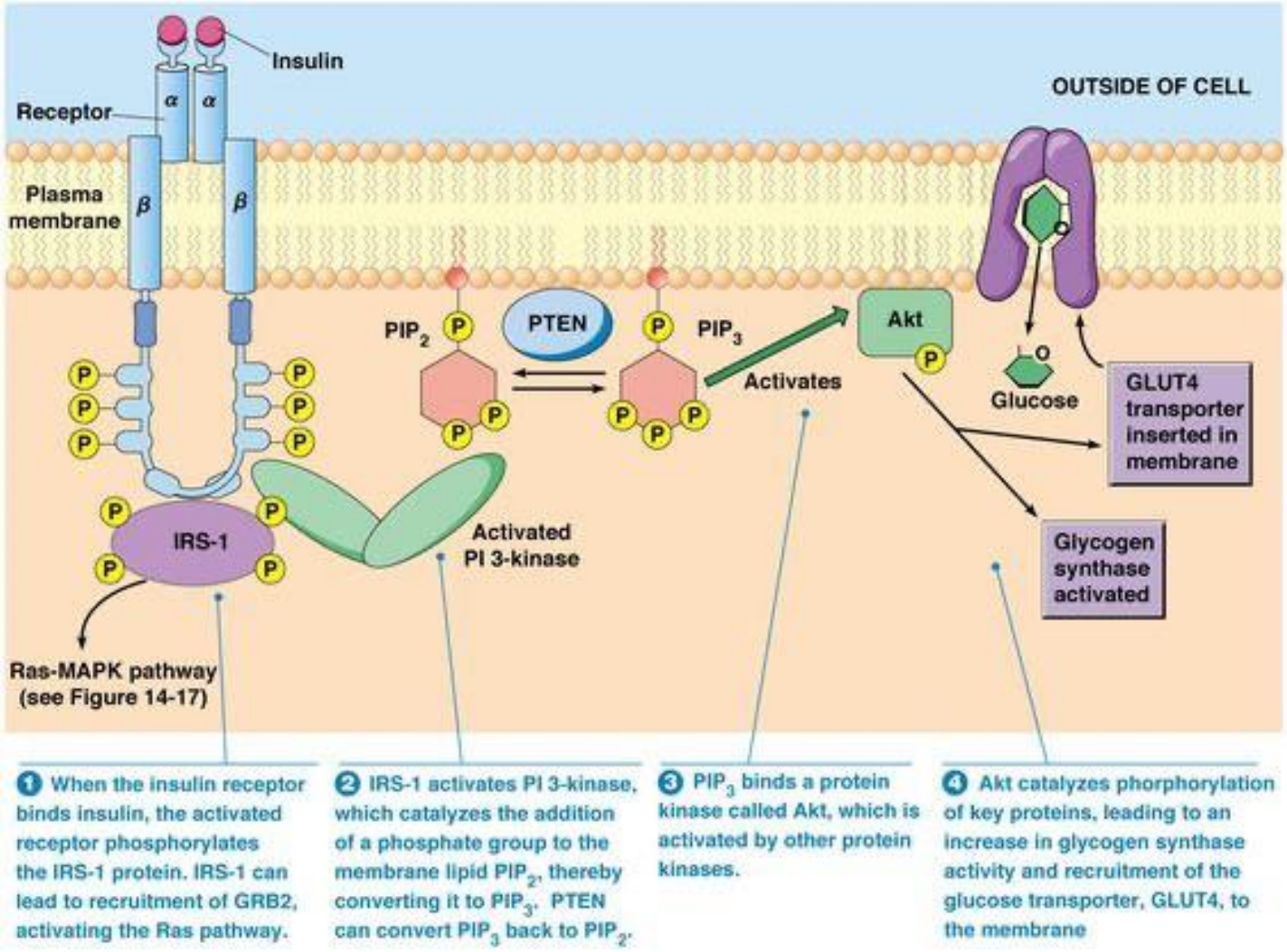
Activation of receptor kinase



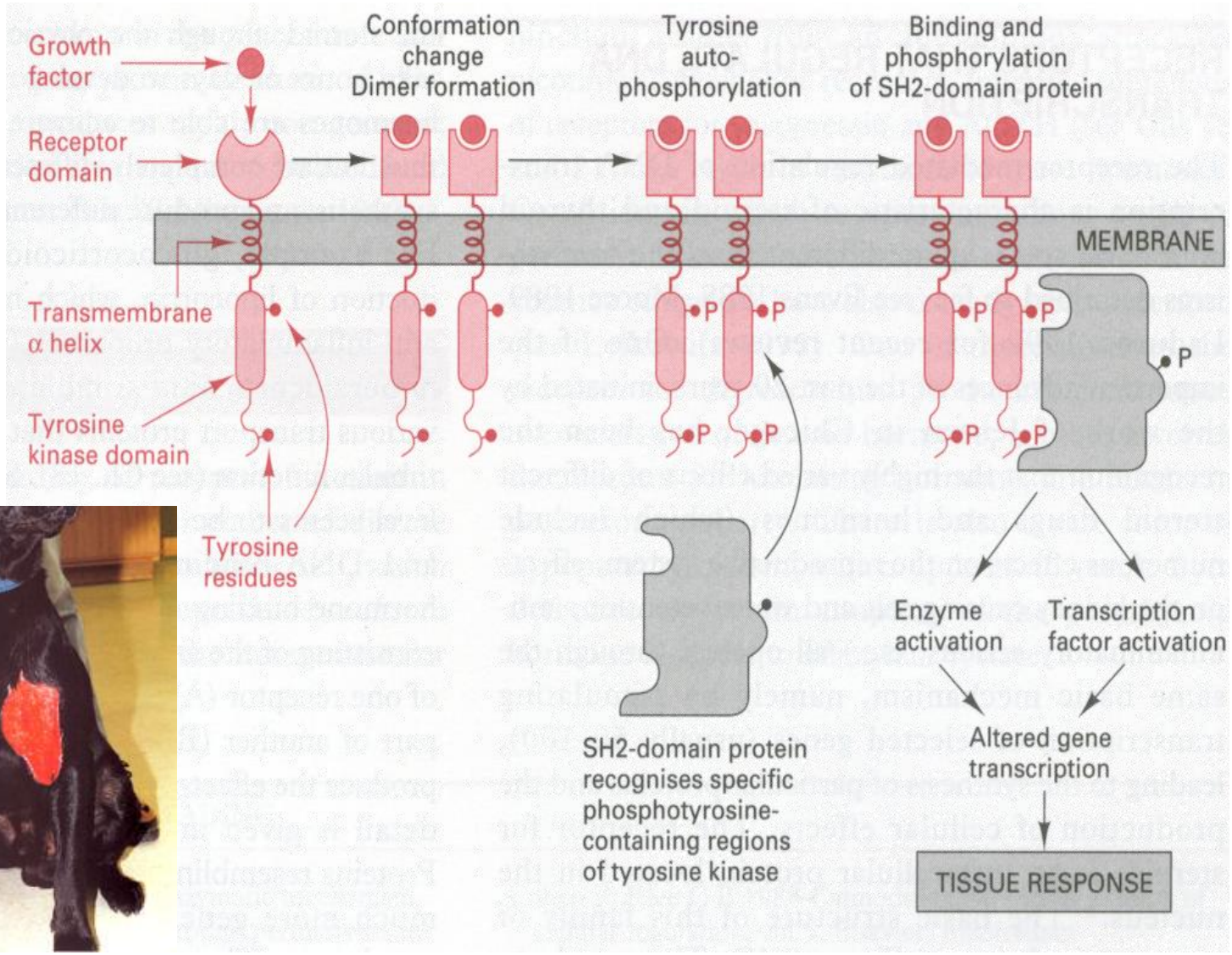
http://membranereceptors.com/Test2/wp-content/uploads/2013/07/Enzyme-linkedreceptors1.jpg

Mechanism of action of insulin

- Activates insulin receptor substrates (IRS) via tyrosine kinase receptor



Growth factor receptors



Functional classes of hormones

- **Water soluble**
  - Amines (other than thyroid hormones)
  - Peptides & proteins, Eicosanoids

1. First messenger (hormone) binds to membrane receptor
2. Activated receptor sets off cascade that activates an enzyme
3. Enzyme reaction produces a second messenger (eg cAMP)
4. Second messenger produces response in cell

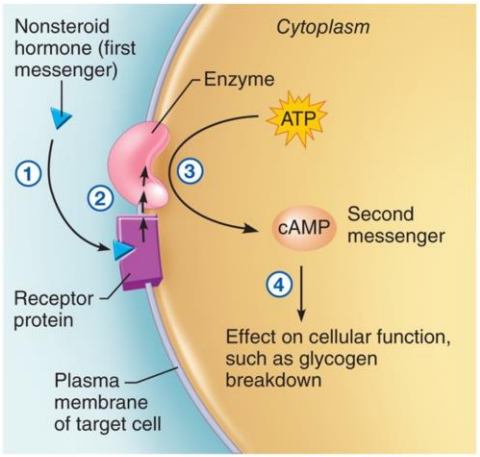
- **Lipid soluble**
  - Steroids
  - Thyroid hormones (amines), Nitric oxide

1. Diffuse through plasma membrane (except thyroid hormones transported)
2. Bind to specific receptors in cytoplasm **or** nucleus
3. Hormone & receptor bind to DNA
4. Gene is transcribed or deactivated

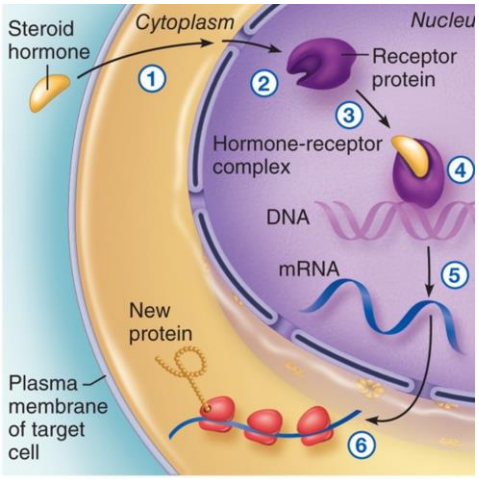
Receptors (>80 identified) include those for:

- Corticosteroids (glucocorticoid, mineralocorticoid) cytoplasm
- Sex hormone receptors (estrogen, testosterone)
- Thyroid hormone receptors (nuclear)

Transduction differences



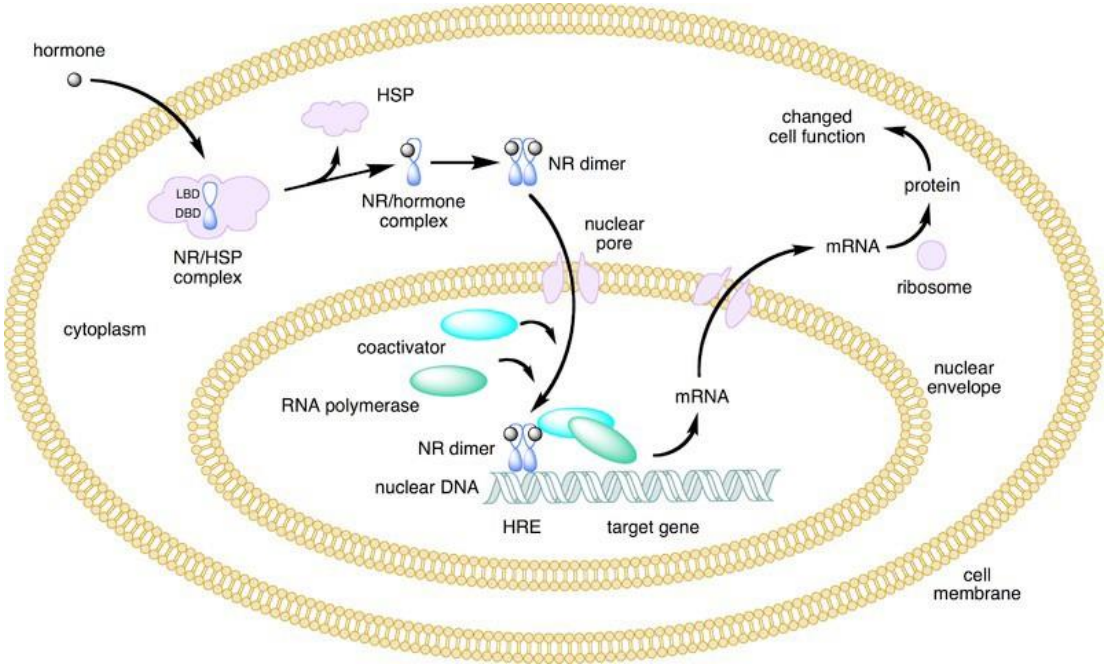
(b) Nonsteroid hormone action



(a) Steroid hormone action

Class I Nuclear Receptors -Steroids

1. Ligand binding to type I nuclear receptors (NR) in the cytosol
2. Dissociation of inhibitory heat shock proteins (HSP).
3. Receptors homodimerization
4. Translocation to nucleus (active transport)
5. Binding to specific DNA (HRE)
6. Activate RNA polymerase or inhibit certain genes.

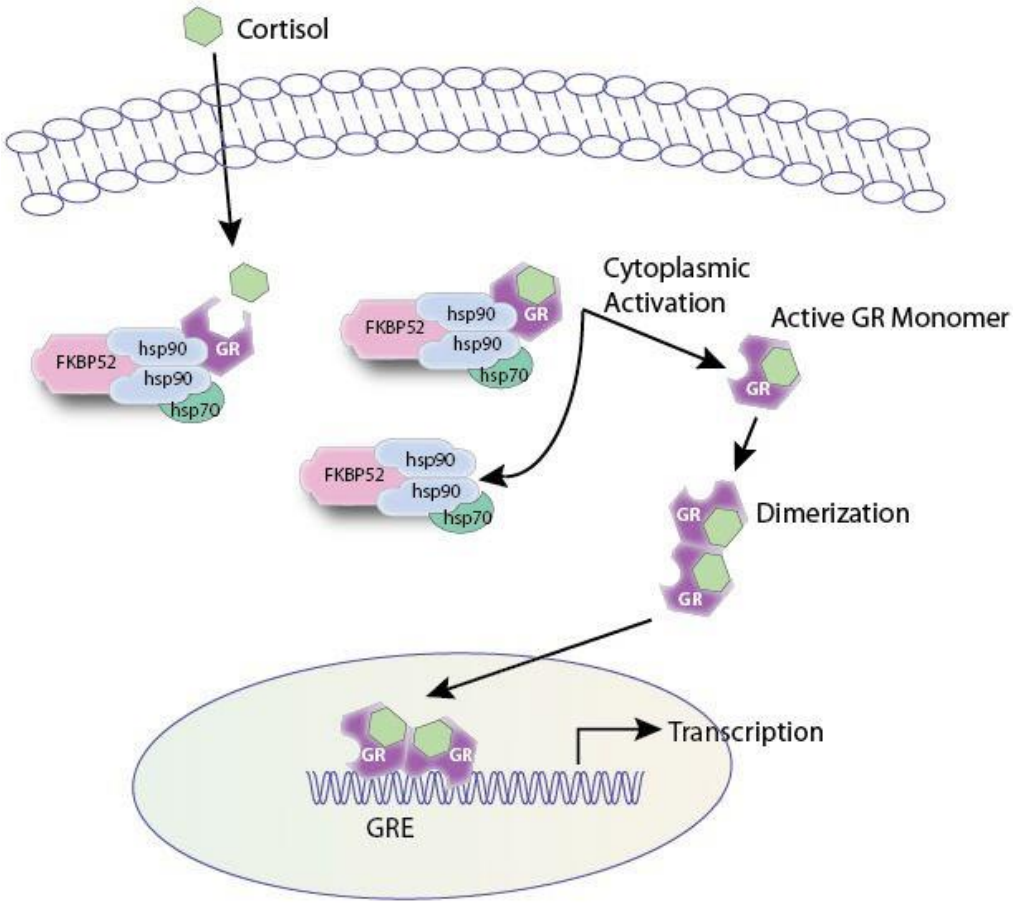


For example, cortisone and aldosterone

http://human.freescience.org/htmx/nuclear_receptor.php

Glucocorticoid receptor signaling

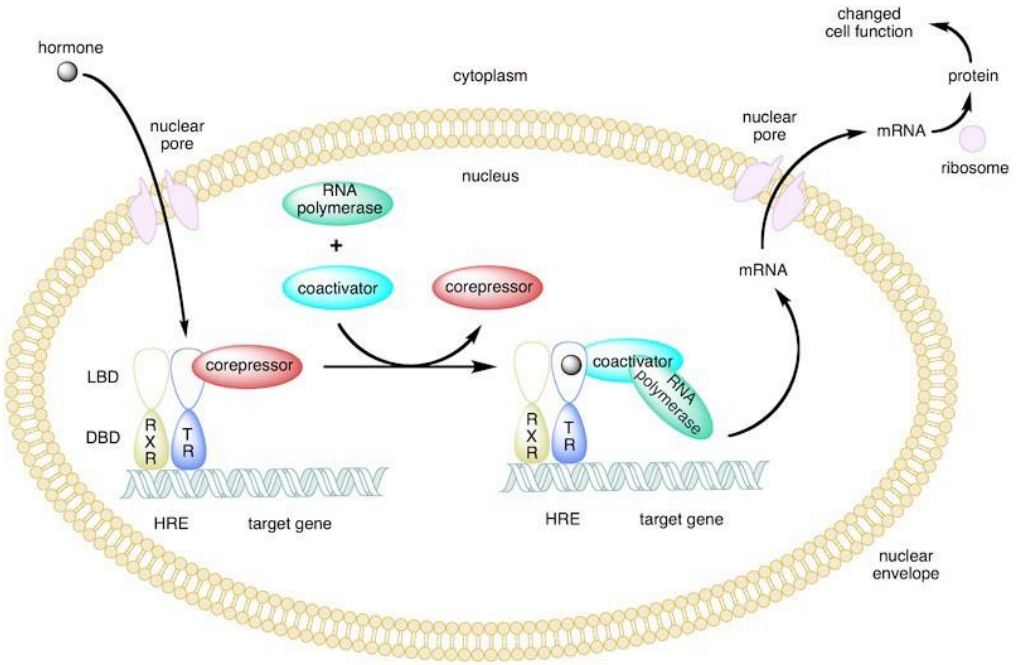
- Cortisol passes through cell membrane
- Binds glucocorticoid receptor (GR)
- Forms non-DNA-binding oligomer
- Oligomer dissociates to form active GR monomer
- GR monomers form DNA-binding homodimers
- These pass through nuclear pores
  - Bind to glucocorticoid response elements (GRE) to activate transcription



http://denikrisna.wordpress.com/2011/01/06/transduksi-sinyal/

Class II Nuclear Receptors
Thyroid & Retinoic acid

1. NR located in nucleus
2. Thyroid hormone receptor (TR) heterodimerized to the retinoic acid receptor RXR.
3. TR is bound to corepressor protein. OFF
4. Ligand binding to TR causes a dissociation of corepressor and recruitment of coactivator protein ON
5. Activate RNA polymerase that are responsible for transcription



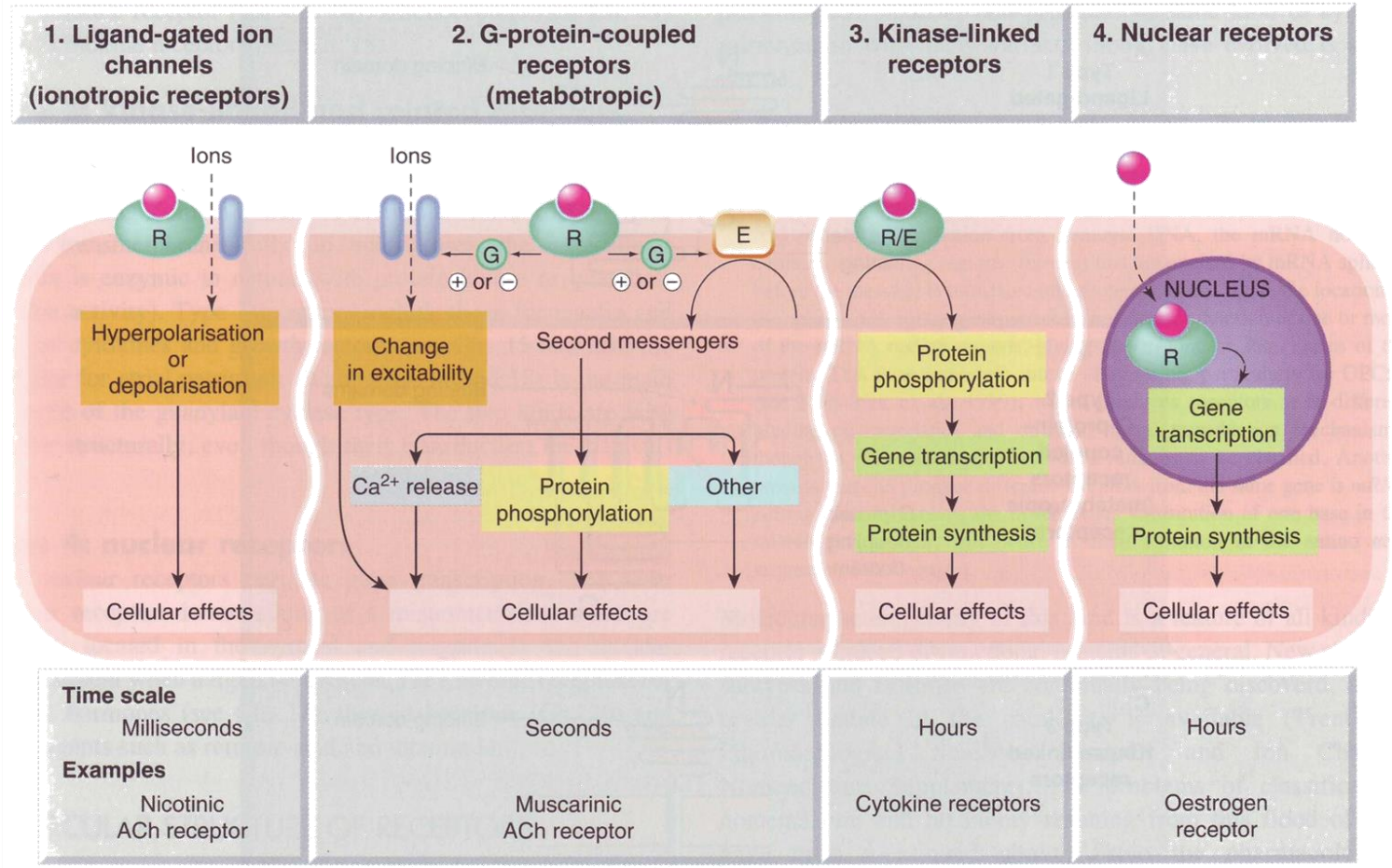
For example, Thyroid hormones

http://human.freescience.org/htmx/nuclear_receptor.php

Key aspects of hormone signalling

1. **Specificity:**
   - A high receptor ligand specificity
   - Non-covalent interactions
2. **Amplification**
   - First messengers are often short-lived and in low concentrations
   - Induce key intracellular signalling proteins to behave as a molecular switch.
   - Amplification proceeds usually via enzyme cascades
3. **Integration**
   - Cells frequently receive multiple signals
   - Must be coordinated with integrated cellular response
4. **Rapid decay**
   - Hormones give short-term, reversible messages so system must decay relatively rapidly.
5. **Desensitization**
   - Often achieved by a feedback loop
   - The target may be the receptor – affinity, activity or expression

Summary: receptor-linked transduction pathways



[R = receptor, G = G protein, E = enzyme]