Extracellular concentrations (EM, blood) are 2 mM, and levels in cytoplasmic vesicles and the ER can reach up to 10mM.

Baseline cytosolic Ca\(^{2+}\) concentration is around 100 nM in resting cells. Only 0.1% of the cellular Ca\(^{2+}\) is ionized (Hodgkin & Keynes 1957: radioactive Ca\(^{2+}\) shows different voltage-gradient mediated diffusion rates in solution than after microinjection into giant squid axon --> conclusion: ionized Ca\(^{2+}\) has to be less than 100 nM!!)

<table>
<thead>
<tr>
<th>Conc in mM</th>
<th>ECF</th>
<th>ICF</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>4.5</td>
<td>160</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>144</td>
<td>7</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>114</td>
<td>7</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>2.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

High gradient makes this a very fast a sensitive signaling system since only slight changes in membrane permeability will result in dramatic changes in the concentration of \([\text{Ca}^{2+}]\)_i.

Low level of \([\text{Ca}^{2+}]_i\) is also a necessity to facilitate the phosphate oriented cellular metabolism (high calcium and high phosphate concentrations are incompatible!!)

**Evolutionary challenge: Maintain calcium gradient !!!**
Evolvement of proteins that bind Ca\(^{2+}\) with high affinity, but reject magnesium!

Two classes of Ca-binding proteins:
- membrane-integrated (unlimited capacity --> transporter systems: Ca-channels, calcium pumps)
- non-membranous (limited capacity --> not only buffering, but processing of signal through conformational changes that enable interaction with target proteins: Calmodulin, Troponin C ...)

**********************************************************
"Where to get it from" (Ca-ON):
"Channels and stores"

*) Extracellular compartment: (predominantly in nerve cells, cardiac cells; smooth muscle cells)
Two types of plasma-membrane localized calcium channels:
- Voltage operated calcium channels:
  Action potential depolarizes plasma membrane, which results in the opening of "voltage" dependent calcium channels (channels can be opened by increase in extracellular K⁺).

Three types:

<table>
<thead>
<tr>
<th>Property</th>
<th>Channel type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td>Activation voltage range</td>
<td>More positive than −70 mV</td>
</tr>
<tr>
<td>Inactivation voltage range</td>
<td>−100 to −60 mV</td>
</tr>
<tr>
<td>Single channel conductance</td>
<td>8.10 pS</td>
</tr>
<tr>
<td>Ca⁺⁺ current kinetics</td>
<td>Long latency, brief current, moderate rate of current decrease</td>
</tr>
<tr>
<td>Blocked by dihydropyridines</td>
<td>No</td>
</tr>
<tr>
<td>Blocked by cadmium</td>
<td>No</td>
</tr>
<tr>
<td>Blocked by cobalt</td>
<td>Strongly</td>
</tr>
</tbody>
</table>

Opening of channel by depolarization
Each channel protein has four homologous domains, each containing six membrane spanning $\alpha$-helices (the fourth one is thought to be the “voltage” sensor.

- **Ligand gated calcium channels:**
  Calcium channels opened after ligand binding to the receptor (e.g. glutamate/NMDA receptor; ATP receptor; alsoNicotinic ACh receptors are coupled to ligand-gated cation channels ( muscarinic ACh receptors signal through G-Proteins-->slower), prostaglandin receptors
Store operated calcium channels:
Activated by emptying of intracellular stores, exact mechanism unknown

Intracellular stores: (predominantly in muscle cells)
Calcium stored in mM concentrations in endo/sarcoplasmatic reticulum bound to Calsequestrin. Previously mitochondria were thought to play an important role as Ca$^{2+}$-stores, but more recent evidence showed that the uptake rate is 10x lower than of the ER/SR.

Calcium released from the ER/SR is regulated by two different calcium channels in the ER/SR membrane:
- Ryanodine receptor controlled calcium channels (RYR):
  Activity potentially regulated by cyclic ADP ribose as a second messenger (?).
  Ryanodine: plant alkaloid, irreversible inhibitor
  Caffeine: potent reversible activator of RYRs
- IP3-sensitive calcium channels (IP3R):
  Inositol-1,4,5-triphosphate is produced through the activity of receptor activated phospholipases--diffuses through cytoplasm and binds IP3R on the ER/SR.
“How to make it go away” (Ca-OFF):

or

“Pumps, buffers and sensors”

*) **Ca**^{2+} - pumps:
Activity of these pumps is induced by increases in cytosolic calcium.

-) **Plasma membrane Na^+/**Ca^{2+} exchanger** (mainly in excitable cells, e.g. cardiac cells)
  three Na^{+}-ions are exchanged for one Ca^{2+}-ion
  Digitalis alkaloids: Na^{+}/K^{+}-ATPase inhibitors => intracellular Na^{+} raises=>< Na^{+}/Ca^{2+} exchange less efficient => Ca^{2+} intracellular increases=> stronger contractions

-) **Plasma membrane Ca^{2+}-ATPase** (PMCA)
  two Ca^{2+}- ions are transported per ATP molecule hydrolyzed
  become phosphorylated on aspartate during ion transport (causes the conformational change that propells the Ca^{2+}-ions to the ECF)
  regulated by CaM, PKA or PKC

-) **SR/ER Ca^{2+}-ATPase** (SERCA): 80% (!) of integral membrane protein of SR target of thapsigargin (=>Ca^{2+}-release from intracellular stores)

*) **Ca**^{2+}-Buffers:
Low affinity (!) but high capacity (50-100 Ca^{2+}-ions/molecule)
-) Calsequestrin (very acidic, 37% of aa are aspartic and glutamic acid), calreticulin, parvalbumin

*) **Ca**^{2+}-Sensors:
-) **Annexins** (low affinity):
  Family of proteins w/ common feature that they interact w/ membranes in a Ca^{2+}-dependent manner.
  Low affinity for Ca2+-ion s restricts action to membrane proximity (high local Ca^{2+} conc.!). implicated in the regulation of PLA2, cytoskeletal (re)organization and vesicle movement
- **EF-hand proteins** (high affinity):
  named after the shape created by the E and F $\alpha$-helices of the Ca$^{2+}$-binding domain

![Diagram of EF-hand proteins](image)

**Calmodulin**: ubiquitous expression; binds 4 Ca$^{2+}$-ions; acts through stimulation of either protein kinases (CaMKs) or protein phosphatases (Calcineurin). also activates cAMP phosphodiesterase

**Troponin C**: restricted expression, regulates contraction of skeletal and heart muscle
“What is it doing?”

*) Muscle contraction:
  -) Skeletal muscle:
  Contraction (=actin-myosin interaction) controlled by proteins on actin filaments
(tropomyosin w/ troponin)
  Troponin I inhibits formation of *cross-bridges* between actin and myosin =>
muscle relaxed.
  Troponin C combines with Ca\(^{2+}\)-ions and blocks the action of Troponin I =>
muscle contracted

- Smooth muscle:
  Contraction controlled by proteins on either actin....
  NO Troponin=>regulation occurs through the CaM binding *Caldesmon* :
  Low Ca2+-conc.: Caldesmon forms complex with actin and tropomyosin=>
  access of myosin to actin is restricted=>muscle relaxed.

....OR myosin filaments:

  phosphorylation of *myosin light chain* (MLC) by *MLC kinase* (MLCK):
  phosphorylated myosin is able to interact w/ actin=>contraction
MLCK is regulated through interaction w/ calmodulin:

Additional modulation of smooth muscle response through inhibitory phosphorylation by PKC (activated by 1,2-diacylglycerol):
+ mediated through phosphorylation of MLC on inhibitory site
+ mediated through phosphorylation that inhibits the function of caldesmon
*) **Neuronal excitibility and secretion:**
Increase of Ca$^{2+}$-concentration induces fusion of the synaptic vesicles with the plasma membrane => this causes exocytosis of neuro-transmitters into the synaptic cleft.

*) **Glycogen hydrolysis:**
Glycogen hydrolysis is mediated by glycogen phosphorylase, which in turn is activated by glycogen phosphorylase kinase (GPK). GPK consists of multiple subunits, one of which ($\delta$) is calmodulin. Phosphorylation of the $\alpha$ and $\beta$ subunits by cAMP dependent kinase increases affinity of calmodulin for Ca$^{2+}$-ions.

*) **Immune response:**
TCR stimulation => Ca$^{2+}$-conc. Increases => activates Calcinurin => dephosphorylates NFATc on ser/thr => NFATc translocates to nucleus where it combines w/ NFATn and induces transcription of IL2 gene => T cell proliferation

Cyclosporin A and FK506:
bind immunophilins : the drug/immunophilin complex binds then to calcineurin, blocking its catalytic activity => no IL2 => immunosuppression
“How to measure it”

*) **$^{45}$Ca:**
   Influx only!

*) **Microelectrodes:**
   Expensive, requires micromanipulators

*) **Aequorin:**
   21 kDa protein from *Aequorea forskalea* (jellyfish):
   protein emits light in an ATP dependent manner when bound to Ca$^{2+}$-ions.
   Very sensitive, but requires introduction of the protein into cells.

*) **Fluorescence indicators:**
   Derivatives of EGTA (=>Ca$^{2+}$-ions chelators)
   QUIN, INDO, FURA, FLUO...
   Compounds change their excitation/emission spectra when binding Ca$^{2+}$-ions.

Very hydrophilic=> introduced into cells as acetoxy methyl esters which are then in the cytoplasm hydrolyzed to generate ionic, Ca$^{2+}$-ion binding form.
SIGNALING THROUGH LIPID- AND INOSITOL DERIVED SECOND MESSENGERS
Mostly derived from the glycerophospholipids phosphoinositol:

>TABLE 11-2. THE COMMON CLASSES OF GLYCEROPHOSPHOLIPIDS

<table>
<thead>
<tr>
<th>Name of X — OH</th>
<th>Formula of — X</th>
<th>Name of Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>— H</td>
<td>Phosphatidic acid</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>— CH₂CH₂NH₃⁺</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>Choline</td>
<td>— CH₃CH₂N(CH₃)₂⁺</td>
<td>Phosphatidylcholine (lecithin)</td>
</tr>
<tr>
<td>Serine</td>
<td>— CH₃CH(NH₃⁺)COO⁻</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>M₁₀-Inositol</td>
<td></td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>Glycerol</td>
<td>— CH₂CH(OH)CH₂OH</td>
<td>Phosphatidylglycerol</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>— CH₂CH(OH)CH₂OH — CH₂CH(OH)CH₂OH</td>
<td>Diphosphatidylglycerol (cardiolipin)</td>
</tr>
</tbody>
</table>

Second messenger released through the hydrolysis by phospholipases or generated through the actions of lipid kinases:

- **Phospholipases**
=) PLA2:  
+) Cytoplasmic form (90 kDa) is regulated through nM Ca\(^{2+}\) (Annexins) and phosphorylation; AA specific => signaling function
+) Secreted form (pancreas, 14 kDa) is also Ca\(^{2+}\) dependent (mM range)=> digestive function

=) PLC:  
coupled to a variety of (growth factor) receptors:
+) PLC\(\beta\) is activated through G-protein (G\(_{q/\alpha}\)) => binding enhances its catalytic activity and the GTPase activity of G\(_{q/\alpha}\) (similar to GAP function in ras signaling)
+) PLC\(\gamma\) couples with its SH2 domains directly to growth factor receptors (EGF, PDGF) or tp the TCR, where it is activated through tyrosine phosphorylation (TCR: activation through src-kinases)

Both phospholipases yield finally arachidonic acid (see below), in addition, PLC activity also produces DAG and IP3:

=) DAG:  
remains membrane bound; diacylglycerol kinase phosphorylates DAG to generate phosphatidic acid which functions as a substrate for PLA2. Together with phosphatidyl-serine (PS) and Ca\(^{2+}\), DAG activates PKC on the plasma membrane

=) IP3:  
see Ca\(^{2+}\) signaling !!
**Glucocorticoids:** inhibit PLA2 by transcriptionally inducing *Lipocortin*, a protein which binds to PLA2 and blocks its activity.

**Phorbol esters:** strongest known tumor promotors; mimic DAG and bind PKC and activate it. Also potent activator of Ca$^{2+}$ influx, MAPK pathway etc.

- **Lipid kinases:**

  =) **PI-3-Kinase:** also a serine kinase
      +) binds to and becomes tyrosine phosphorylated in response to activation of growth factor receptors;
      +) 85 kDa regulatory subunit (pY) and a 110 kDa catalytic subunit
      +) regulatory subunit contains SH2 and SH3 domains

  =) PI-3-phosphates can bind to the pleckstrin homology (PH) domain of *Akt*
     => Akt activation => phosphorylation of BAD, which dissociates from the antiapoptotic protein bcl-2 => inhibition of apoptosis

**Wortmannin:** fungal metabolite, potent, irreversible inhibitor of PI3Kinase
**Ly290004:** synthetic compound, blocks ATP binding site of PI3Kinase
Arachidonic acid metabolism:

**Eicosanoids**: collective name for derivatives of arachidonic acid (=5,8,11,14-eicosatetraenic acid)
AA is mainly generated through the action of PLA2 and DAG-lipase. Rapidly metabolized by cyclooxygenase and lipoxygenase into prostaglandins and leukotrienes.

Prostaglandins:

- First observed in seminal fluid => name.
- Structure of cyclopentane ring defines letter
  - Double bonds in side chains account for number
  - Greek letter refers to the spatial position of the OH group at C-9.

+) Initial step in PG synthesis catalyzed by PGH-synthase which has dual enzymatic activity:
  - Cyclooxygenase (closes ring => PGG2) and peroxidase (=> 15-OH)

+) PG signal through cell surface receptors => Ca$^{2+}$, PIP and cAMP
### Biological functions:

<table>
<thead>
<tr>
<th>Function</th>
<th>Action</th>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular tone</td>
<td>Relaxation:</td>
<td>PGs $E_1$, $E_2$, $F_2\alpha$ and $I_2$</td>
</tr>
<tr>
<td></td>
<td>Constriction:</td>
<td>PGs $F_2\alpha$, $TxA_2$</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>Increase:</td>
<td>PGs $E_1$, $TxA_2$</td>
</tr>
<tr>
<td></td>
<td>Decrease:</td>
<td>PGs $E_2$, $I_2$</td>
</tr>
<tr>
<td>Uterus tone</td>
<td>Increase:</td>
<td>PGs $E_1$, $E_2$, $F_1\alpha$</td>
</tr>
<tr>
<td>Bronchial muscle</td>
<td>Constriction:</td>
<td>PGFs</td>
</tr>
<tr>
<td></td>
<td>Relaxation:</td>
<td>PGEs</td>
</tr>
<tr>
<td>Gastric secretion</td>
<td>Inhibition:</td>
<td>PGs $E_1$, $E_2$, $I_2$</td>
</tr>
<tr>
<td>Temperature and pain</td>
<td>Increase</td>
<td>PGEs</td>
</tr>
</tbody>
</table>

**NSAIDs:** **Non-steroidal Antiinflammatory Drugs:**

Aspirin, Ibopufren, Diclofenac etc. Act all on cyclooxygenase $\Rightarrow$ prevent PG synthesis $\Rightarrow$ fever sinks, pain relief

Inhibit blood coagulation $\Rightarrow$ protect against myocardial infarct

Cause ulcers!!

- **Leukotrienes:**
  
  + First found in leucocytes; contain 3 conjugated double bonds

![Chemical structures of Leukotrienes and Prostaglandins](image-url)
Biological functions:

+ LTC₄, D₄ and E₄ mediate allergic reaction: **Slow Reacting Substance of Anaphylaxis (SRS-A)** => mediates anaphylactic shock
  10,000 fold more potent than histamine!!! => constrict bronchi, dilate blood vessels

+ LT are the cause for aspirin mediated asthma attacks!

+ LTB₄ is a strong chemoattractant for macrophages
SIGNALING THROUGH GTP BINDING-PROTEINS
(cNMP and PDEs)

*) **G-Proteins**: Guanine nucleotide binding proteins that participate in reversible, GTP mediated macromolecular interactions.

*) **Common features**:

- bind GDP and GTP with high affinity, but adopt different structure depending on the bound nucleotide.
- GTP-bound complex has high affinity for other proteins ("acceptor"), affecting their enzymatic activity
- possess intrinsic GTPase activity that is usually activated by interaction with an acceptor proteins
- covalent attachment of various lipids (myristoylation, palmitoylation,...) is responsible for membrane targeting

*) **Additional control exerted through**:

- **GTPase Activating Proteins** (GAPs): function on small GTP binding proteins
- **Guanine-nucleotide Exchange Factors** (GEFs): promote GDP release
- **Regulators of G-protein Signaling** (RGSs): similar to GAPs, but act on heterotrimeric G-Proteins

*) **Two major groups**:

- "Small GTP binding proteins" (act downstream of receptor: ras, rac etc.) => see growth factor receptor signaling

- "Heterotrimeric G-proteins" (directly coupled to receptor and enzyme):
  + Coupled to 7 transmembrane spanning receptors: (β-adrenergic R, PG-R)
All members are heterotrimeric, consisting of \( \alpha \), \( \beta \) and \( \gamma \) subunits:

- **\( \alpha \)-subunit** (23 distinct forms): contains the GTP/GDP binding site, is responsible for the identity and function

- **\( \beta \) (5 isoforms) and \( \gamma \) (12 isoforms) subunits:** are identical or very similar; interchangeable in vitro; most of them are ubiquitously expressed; membrane anchored through prenylation of \( G_\beta \)

**Table:**

<table>
<thead>
<tr>
<th>( G_\alpha ) Subclass*</th>
<th>Effect</th>
<th>Associated Effector Protein</th>
<th>2nd Messenger</th>
</tr>
</thead>
<tbody>
<tr>
<td>( G_q )</td>
<td>↑</td>
<td>Adenylyl cyclase</td>
<td>cAMP</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>( Ca^{2+} ) channel</td>
<td>( Ca^{2+} )</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>( Na^+ ) channel</td>
<td>Change in membrane potential</td>
</tr>
<tr>
<td>( G_i )</td>
<td>↓</td>
<td>Adenylyl cyclase</td>
<td>cAMP</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>( K^+ ) channel</td>
<td>Change in membrane potential</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>( Ca^{2+} ) channel</td>
<td>( Ca^{2+} )</td>
</tr>
<tr>
<td>( G_q )</td>
<td>↑</td>
<td>Phospholipase C</td>
<td>IP_3, DAG</td>
</tr>
<tr>
<td>( G_o )</td>
<td>↑</td>
<td>Phospholipase C</td>
<td>IP_3, DAG</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>( Ca^{2+} ) channel</td>
<td>( Ca^{2+} )</td>
</tr>
<tr>
<td>( G_t )</td>
<td>↑</td>
<td>cGMP phosphodiesterase</td>
<td>cGMP</td>
</tr>
</tbody>
</table>

- \( G_q \) and \( G_{olf} \) (expressed only in olfactory cells) are coupled to PLC

- \( G_T \) (=Transducin) is coupled to a cGMP phosphodiesterase and is expressed only in the rod cells of the retina (these cells are INactivated by light!)

  Photons hit Rhodopsin \( \rightarrow \) activated opsin is generated \( \rightarrow \) facilitates GTP loading of \( G_T \) \( \rightarrow \) activates cGMP phosphodiesterase \( \rightarrow \) cGMP (keeps \( Na^+ \) and \( Ca^{2+} \) channels open to cause membrane depolarization \( \rightarrow \) neurotransmitter release) converted to 5’GMP (inactive \( \rightarrow \) channels closed) \( \rightarrow \) membrane polarization \( \rightarrow \) NO neurotransmitter release)
G_s and G_i are coupled to ion channels (see Calcium signaling) and to Adenylate-cyclase:

- Two repeats of six transmembrane \( \alpha \)-helices and two catalytic domains that convert ATP into cAMP.

- Activated or inhibited by G-proteins (a brain specific isoform is also activated through activated CaM)
- GTP-bound $G_{s\alpha}$ activates AC, GTP-bound $G_{i\alpha}$ inhibits activity

*) Main target for cAMP is the cAMP-dependent protein kinase PKA:

- Consists of four subunits: two regulatory and two catalytic subunits: $\Rightarrow$ after cAMP binding to the regulatory subunits the catalytic subunits dissociate and translocate to the target substrates.

-) First identified process regulated by PKA was glycogenolysis (PKA phosphorylates glycogen phosphorylase kinase which in turn activates glycogen phosphorylase $\Rightarrow$ release of glucose)

-) PKA also phosphorylates transcription factors: CREB

CRE (cAMP response element) in the promoter of cAMP responsive genes
CREB becomes phosphorylated by PKA that translocated to the nucleus
CREB can also be phosphorylated by CaM activated PKC
- **Forskolin**: direct activator of AC $\Rightarrow$ cAMP increase

- **Cholera-toxin**: causes ADP-ribosylation of $G_{s\alpha}$ $\Rightarrow$ release of GTP inhibited $\Rightarrow$ $G_{s\alpha}$ trapped in active form. CAMP regulates secretion of fluid into the intestine $\Rightarrow$ enormous loss of liquid and electrolytes $\Rightarrow$ death!

- **Pertussis-toxin**: causes ADP-ribosylation of $G_{i\alpha}$, release of GDP inhibited $\Rightarrow$ $G_{i\alpha}$ locked in its inactive form $\Rightarrow$ can not inhibit AC!
**Attenuation of G-protein/AC coupled receptors derived signals**

*) **Phosphodiesterases:** convert cAMP to 5’AMP

several families; activation creates feed-back loops

<table>
<thead>
<tr>
<th>ISOENZYME FAMILY</th>
<th>REGULATORY CHARACTERISTICS</th>
<th>KNOWN FUNCTIONAL EFFECTS OF ISOENZYME INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ca^{2+}, calmodulin-regulated with different $K_m$ values for cGMP and cAMP hydrolysis</td>
<td>CNS modulation; vasorelaxation</td>
</tr>
<tr>
<td>II</td>
<td>cGMP-stimulated cAMP hydrolysis with high $K_m$ for cAMP</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>cGMP-inhibited cAMP hydrolysis; low $K_m$ for cAMP and cGMP</td>
<td>Positive inotropism; vascular and airway dilation; inhibition of platelet aggregation; stimulation of lipolysis; inhibition of cytokine production*</td>
</tr>
<tr>
<td>IV</td>
<td>Low $K_m$ for cAMP hydrolysis</td>
<td>Airway smooth muscle relaxation; inhibition of inflammatory mediator release; CNS modulation; gastric acid secretion</td>
</tr>
<tr>
<td>V</td>
<td>High and low $K_m$ isoforms for cGMP specific hydrolysis</td>
<td>Platelet aggregation inhibition</td>
</tr>
<tr>
<td>VI</td>
<td>Activity regulated by interaction with transducin</td>
<td>Photoreceptor phosphodiesterase</td>
</tr>
<tr>
<td>VII</td>
<td>Low $K_m$ for cAMP hydrolysis</td>
<td>Abundant in skeletal muscle; present in heart and kidney</td>
</tr>
</tbody>
</table>

- **Phosphodiesterase inhibitors:**
  
  +) **Methylxanthines:** Caffeine, theophylline => enhance and prolong the signals originating from adrenergic receptors

*) **Receptor desensitization:**

- PKA phosphorylates all G-protein coupled receptors so they can not interact with G-proteins => until phosphorylation is removed, receptor remains blocked (=heterologous desensitization)

- β adrenergic receptor kinase (BARK): phosphorylates β adrenergic receptor selectively (homologous desensitization)