

## Cell-to-Cell Signaling Second Messengers



### Why do we need second messengers?



- If it can't get in it can't work
- Receptor on the surface can "pass" the signal to intracellular second messengers
- Second because hormone (ligand for the receptor) is the first messenger

### Second messengers

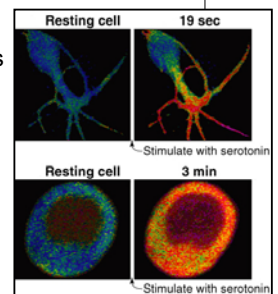


- Short lived intracellular signaling molecules
- Elevated concentration of second messenger leads to rapid alteration in the activity of one or more cellular enzymes
- Removal or degradation of second messenger terminate the cellular response
- Message is encoded by concentration
  - Speed of making
  - Speed of breaking

### Second messengers



- Rate or frequency of increases and decreases in concentration
- Localization (solubility, size)



### Four classes of second messengers



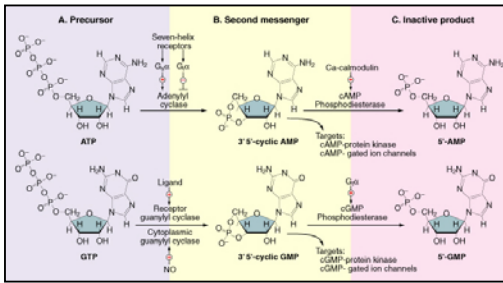
- Cyclic nucleotides
- Membrane lipid derivatives
- $\text{Ca}^{2+}$
- Nitric oxide/carbon monoxide

### Cyclic nucleotides



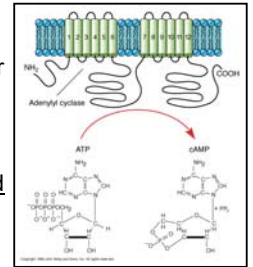
- cAMP, cGMP
- Act by binding reversibly to specific target proteins
- Diffuse freely in cytoplasm
- **Made by** cyclases
- **Broken by** phosphodiesterases (break cyclic nucleotides to 5' monophosphates)

## Cyclic nucleotides



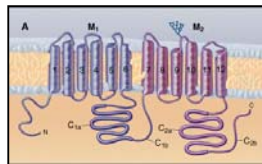
## cAMP

- Generated from ATP by adenylyl cyclase
- Popular second messenger of **G protein coupled receptors**
- cAMP doesn't function in signaling pathways initiated by receptor tyrosine kinases



## Adenylyl cyclase

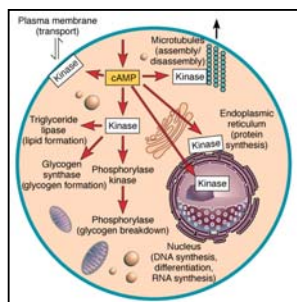
- Membrane bound enzyme
- Has two catalytic domains on the cytosolic face of the membrane
  - Catalytic domains bind ATP and break it produce cAMP and inorganic phosphate



## Targets of cAMP

- Kinases
  - cAMP-dependent protein kinases (protein kinase A = PKA)
- Cyclic nucleotide gated ion channels

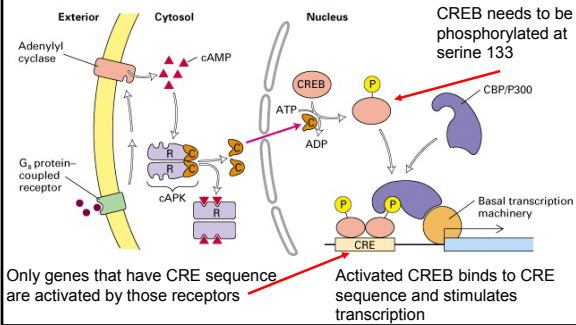
## Cellular functions regulated by cAMP



## Cellular functions regulated by PKA

- Phosphorylation of cellular proteins and change of their activity
  - ex: lipase, glycogen phosphorylase, cholesteryl esterase - activation
  - glycogen synthase - inhibition
- Regulation of transcription factors – affect protein expression
  - CREB - cyclic AMP Response Element Binding protein

## Regulation of transcription by cAMP kinase



## CREB links cAMP signals to transcription

- Only genes that have CRE sequence are activated by those receptors
- CREB needs to be phosphorylated at serine 133
- Interacts with a co-activator CBP/P300
- Activated CREB binds to CRE sequence
- CBP/P300 links CREB to transcription factors
- and stimulates transcription

## Hormones that act through cAMP mediated mechanism

- Epinephrine and norepinephrine ( $\beta$ -adrenergic receptors)
- Glucagon
- ACTH
- Vasopressin
- Luteinizing hormone LH
- Thyroid stimulating hormone TSH

## Specificity of cAMP signaling

- Effects of cAMP and cAPKs are localized to specific regions
- cAMP kinase associated proteins (AKAPs) anchor inactive cAPKs to specific subcellular localizations

## Why do the doctors on ER always give traumatized patients epinephrine?

- Epinephrine increases cAMP levels
- Activates PKA
- PKA activates MLCK (myosin light chain kinase)
- Increased cardiac muscle contractility

## Guanylyl cyclase

- Similar to adenylyl cyclase
- Two forms
  - Soluble – enzyme in the cytoplasm
  - Transmembrane receptor (receptor with intrinsic enzymatic activity)
- Induced respectively by NO (soluble) and peptide hormones (transmembrane ex: atrial natriuretic factor)

## cGMP signaling

- Regulates the activity of cGMP activated protein kinases
- Directly binds to and regulates the ion channels in rods and cones of retina (vision)

## NO signaling

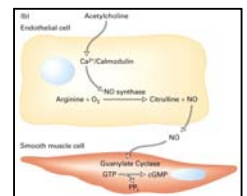
- Free radical gas
- Diffuses rapidly
- Goes also through membranes
- Produced by nitric oxide synthase (NOS)
- Inactivated by  $O_2$  or binding to hemoglobin

## NO synthase

- Converts L-arginine and  $O_2$  to citrulline and NO
- Two forms
  - Inducible (in macrophages stimulated by bacterial toxins)
  - Constitutive (epithelial and neuronal)
- Regulated by  $Ca^{2+}$ /calmodulin and phosphorylation

## Targets

- NO kills microorganisms directly
- Activates guanylyl cyclase and cGMP production
  - Relaxes smooth muscles



## Cellular effects mediated by cGMP

- Regulation of blood flow and blood pressure
  - Epithelial Derived Relaxation Factor (EDRF)
  - NO is a product of nitroglycerine
- Penis erection
  - Viagra inhibits phosphodiesterase and cGMP breakdown

## Lipid derived second messengers

- Another class of second messengers used by seven helix receptors
- Activated by different families of G proteins than cAMP signaling
- Products of membrane phospholipid breakdown
- 3 primary substrates
  - Phosphatidylinositol
  - Phosphatidylcholine
  - Sphingomyelin



## PIP<sub>2</sub> breakdown

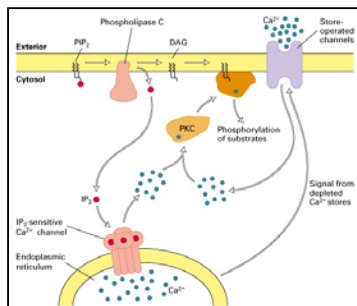
- Second messenger system for Gq family of G proteins
- Gq activates phospholipase C (PLC)
- Phospholipase C (PLC) is membrane bound enzyme
- PLC β breaks phosphatidylinositol 4,5 bisphosphate (PIP<sub>2</sub>) to IP<sub>3</sub> and DAG

## PIP<sub>2</sub> breakdown

- Both IP<sub>3</sub> and DAG are second messengers
- IP<sub>3</sub> increases intracellular calcium levels via the release from intracellular stores
- DAG activates protein kinase C (PKC)



## IP<sub>3</sub> induces the release of Ca<sup>2+</sup> from the ER



## Protein kinase C (PKC) signaling

- Serine/threonine kinase
- Activated by DAG
- Phosphorylates various cellular effectors
- Activates transcription factors AP-1 (c-fos and c-jun are both protooncogenes)

## Calcium as a second messenger

- Ca<sup>2+</sup> versatile second messenger
- All eukaryotes use Ca<sup>2+</sup> signaling
- Regulates many processes
  - Synaptic transmission
- Levels controlled by release and removal (not metabolism)

## Calcium as a second messenger

- Two forms of Ca<sup>2+</sup> release channels
  - IP<sub>3</sub> receptors
  - Ryanodine receptors
- Removal by pumps and calcium sequestering proteins (!!!)

## IP<sub>3</sub> receptor

- Responds to IP<sub>3</sub> by release of Ca<sup>2+</sup> from endoplasmic reticulum
- Responds rapidly
- Local feedback loop
  - Positive for low calcium
  - Negative for high
- This gives a self-limited Ca<sup>2+</sup> pulses

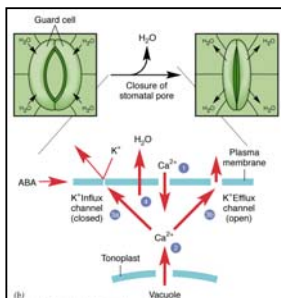


## Targets

- Direct effects
  - Cell motility
  - Contraction of muscle cells
  - Secretion
  - Activation of regulatory enzymes
  - Ca<sup>2+</sup> activated K<sup>+</sup> channels
- Effects through calmodulin
  - Activation of phosphorylase kinase
  - Activation of cAMP phosphodiesterase
- CaM kinase II

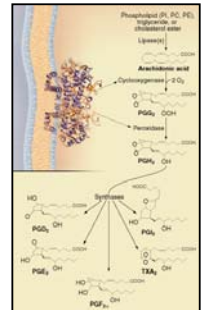


## Calcium acts as a second messenger in guard cell closure



## Lipid derived second messengers for intercellular signaling

- Eicosanoids
  - Arachidonic acid derivatives
- Prostaglandins
- Thromboxanes
- Leukotrienes
- Lipoxins



## Lipid derived second messengers for intercellular signaling

- Escape the cell and participate in intercellular signaling
- Sensitize normal physiological responses
- Important as local inflammatory mediators

