

# Signal transduction in biology

Oliver Sturm

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## Overview

1. Crash course in biology
2. What is cell signalling
3. MAPK pathway as an example for a signalling pathway
4. BPS project: Modelling signal transduction
  - PC12 cell differentiation as a model system for MAPK signalling
  - Experimental techniques to provide data on signal transduction

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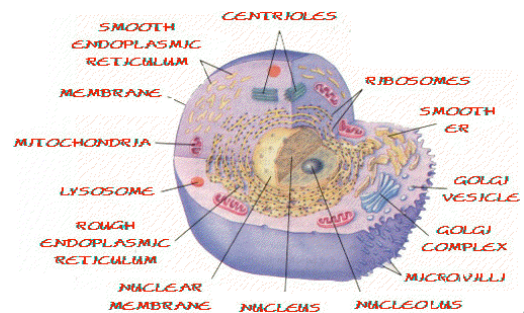
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## 1. Crash course in biology

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## Overview of a cell



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## What are cells made of?

- Water, Ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ )
- Proteins (chains of amino acids with complicated structures)
- Lipids (cell membrane is a lipid bilayer)
- Nucleic acids (DNA, RNA)
- Small molecules (ATP, GTP, metabolites, etc..)
- Polyglycosides, etc...

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## Some key words explained ..

- Protein: A molecule that is basically a string of amino-acids with a complicated 3-dimensional structure
- Enzyme: A protein that enables a chemical reaction by catalysis.
- Receptor: A trans-membrane protein that has an intracellular and extracellular domain and a section that is embedded in the cell membrane
- Amino acid: There are 20 amino acids which are structurally similar and can react with on another to form a chain. When this happens you get a peptide or a protein.
- Cytoplasm: The space between the plasmamembrane and the nucleus of the cell.
- Phosphorylation: A chemical reaction during which a phosphate group ( $-\text{PO}_4^{3-}$ ) is attached to a protein. This changes the 3-dimensional structure of the protein.
- G-Protein: GDP/GTP binding protein. This protein is activated by a GDP/GTP exchange factor, which catalyzes the removal of GDP from it's binding site. As soon as GDP is removed, GTP (more abundant in the cytoplasm) bind to the G-Protein and activates it. Active G-Protein binds Raf and activates it.
- Kinase: A protein that catalyzes the phosphorylation of other proteins
- Phosphatase: A protein that catalyzes the de-phosphorylation of other proteins
- Antibodies: Proteins produced by the immunosystem which specifically bind to a particular protein. Antibodies against specific targets are nowadays available from companies or can be custom-made to order.
- Fluorophore: A molecule that emits light when it is excited by light. It is very useful when it is used

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## 2. What is cell signalling?

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## Why do cells need signalling?

1. Cells need to sense their environment.
2. Cells need to receive orders on what they have to do
  - Live or Die
  - Proliferate or Differentiate
  - Perform a specific task
3. Cells need to keep track of time (Circadian clock)

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Different forms of life need different levels of signalling ...

### Single cells organisms

vs.

### Multi-cellular organisms

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## Single cell organism:

- What kind of signalling does it need?
  - Sense the direction of food → swim to it (chemotaxis)
  - Adjust to the environment ( e.g. stress response: the cell adjusts to extremes in temperature, osmolarity, radiation, toxins)
  - Sense and react to pathogens ( bacteria, viruses, fungi)
- Where does the signalling take place?
  - Receptors in the cell membrane act as chemical sensors - they bind molecules and transduce the information into the cell
  - Inside the cell cascade of signal transduction events

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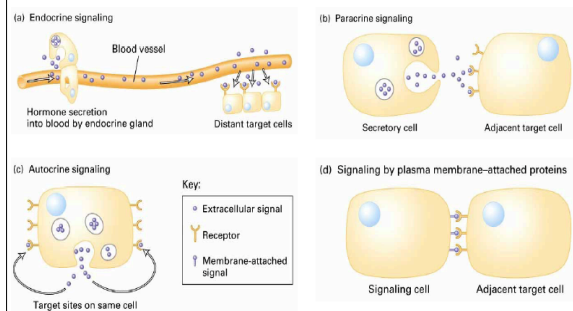
## Multi-cellular organism

- What kind of signalling does it need?
  - Development (fertilized egg → homo sapiens):
  - Homeostasis (food intake to energy expenditure, blood sugar level, hormonal cycles, ...)
  - Response to pathogens (virus, bacteria,

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## Signalling in multicellular organisms - Where does the signalling take place



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## What are the signals made of

### Extracellular space:

1. Small molecules: Adrenaline
2. Peptides/Proteins: Insulin, NGF
3. Electromagnetic field (Depolarised membrane, ion fluxes through membrane)

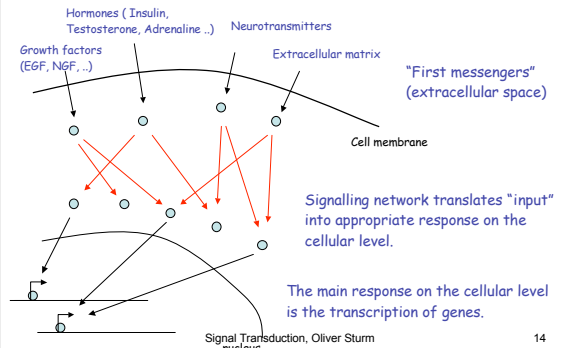
### Intracellular space:

1. Proteins binding to other proteins
2. Phosphorylation of proteins
3. Small molecules: DAG
4. Ions: Calcium  $Ca^{2+}$

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## Cell signalling is information processing



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## Signalling molecules

- 1<sup>st</sup> messengers (extracellular):
  - "Hormones": Adrenaline, Insulin, Glucagon, Oxytocin, ..
  - "Growth factors": nerve growth factor (NGF), EGF, ..
  - Neurotransmitters (Serotonin, ..)
- 2ndary messengers (intracellular):
  - cAMP (intracellular)
  - $Ca^{2+}$  (intracellular)
  - Kinases (Enzymes which attach Phosphate on other proteins ...)
  - Phosphatase (enzymes which remove phosphate from proteins)
  - many others..

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## 3. Example of a signalling pathway in mammals

### Mitogen Activated Protein Kinase Pathway "MAPK Pathway"

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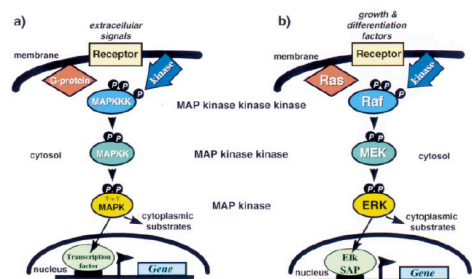
## MAPK pathway

- Mitogen Activated Protein Kinase pathway (MAPK)
- Conserved in all organisms from yeast upwards to mammals
- Controls growth in all its facets:
  - Proliferation (rate of cell division)
  - Differentiation (transformation of cells into a specialized cell type)
  - Survival / Apoptosis (programmed cell death)
- Involved in cancer
  - Defects in MAPK pathway components frequently found in tumours
- 6 "versions" of the MAPK pathway are currently known in

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## Structure of the MAPK pathway



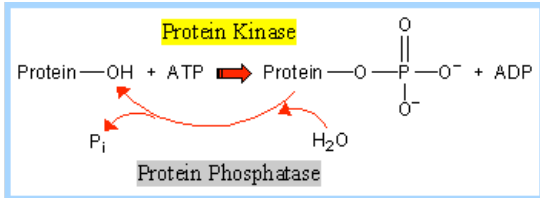
a. General schema

b. A example of a specific MAPK pathway:  
The Raf/MEK/ERK pathway

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A kinase is an enzyme that "phosphorylates" other proteins

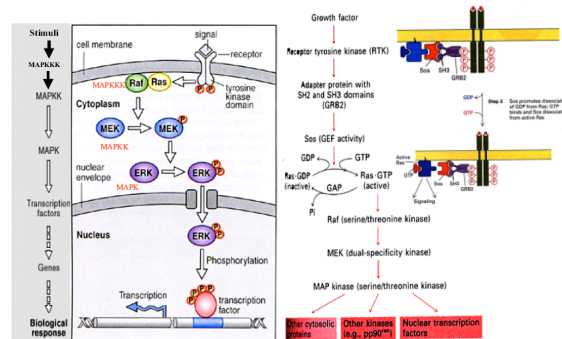


A phosphatase is an enzyme that "dephosphorylates" other proteins

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### MAPK Activation (ERK)



### Signalling along the MAPK pathway - Activation

1. A growth factor binds to the extracellular domain of a receptor. The receptor is a protein that traverses the membrane and contains extra- and intracellular domains
2. The receptor is activated by dimerisation and phosphorylates itself on tyrosines (an amino acid) on the intracellular domain (autophosphorylation)
3. SH2 and SH3 domain docking proteins (Grb2, Sos) bind to the phosphorylated tyrosines on the receptor and activate a protein called a GTP-binding exchange factor (Sos)
4. A small G-protein (Ras, Rap1) binds to this exchange factor (Sos). The exchange factor (Sos) catalyzes the exchange of a GDP to a GTP in the G-Protein (Ras). This results in activation of the G-Protein (Ras).
5. The G-protein (Ras) binds and activates Raf (belongs to the MAPKKK protein family).
6. Raf phosphorylates MEK1/2. MEK1/2 is activated and acts now as a kinase.
7. MEK1/2 phosphorylates and activates ERK1/2.
8. ERK1/2 phosphorylates and activates over 80 targets in the cytosol and nucleus of the cell. Many of the targets are transcription factors which regulate gene

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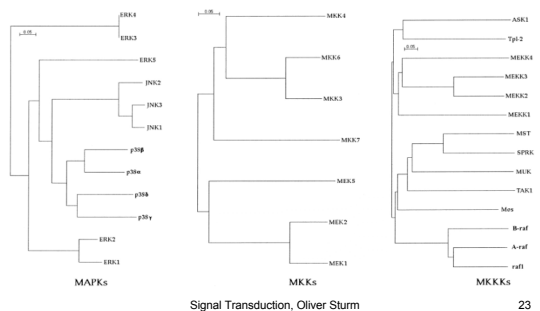
### Signalling along the MAPK pathway - Deactivation

1. The receptor - adaptor complex falls apart because activated ERK phosphorylates adaptor molecules. The receptor itself is degraded by endocytosis. That means the receptor is transported into the cell and digested.
2. The GTP-binding exchange factor (Sos) is deactivated as soon as it is feedback phosphorylated and no longer binds to the receptor complex.
3. The small G-protein (Ras) catalyzes the hydrolysis of GTP into GDP. This reaction is catalyzed by GTPase activating protein (GAP).
4. Raf is deactivated by feedback phosphorylation from ERK
5. MEK and ERK are dephosphorylated by phosphatases

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MAPK, MAPKK (MKK) and MAPKKK (MKKK) represents a protein family (structurally related proteins)



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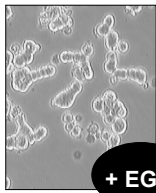
### 4. BPS project: Modelling signal transduction

- PC12 cell differentiation as a model system for MAPK signalling
- Experimental techniques to provide data on signal transduction

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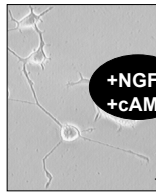
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## The PC12 cell model of neuronal differentiation



+ EGF

Proliferation



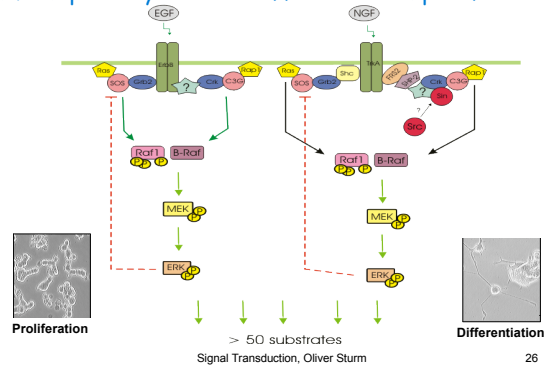
+NGF  
+cAMP

Differentiation

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## MAPK pathway in PC 12 differentiation/proliferation



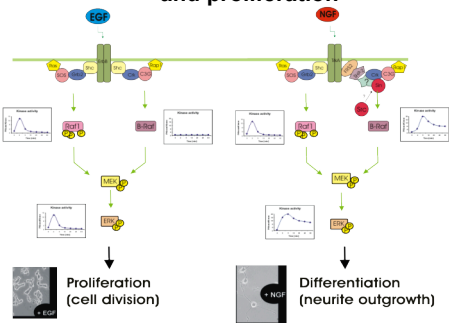
Proliferation

> 50 substrates  
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Differentiation

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## PC12 cells switch between neuronal differentiation and proliferation



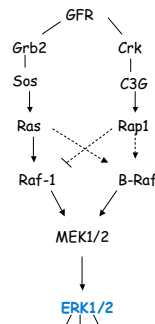
Proliferation  
(cell division)

Differentiation  
(neurite outgrowth)

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## How to measure the activation of ERK?



ERK is active when it is phosphorylated, therefore we have to find out how much is phosphorylated compared to how much is not phosphorylated.

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## How to measure the activation of ERK - Treatment of PC12 cells in culture and lysis

PC12 cells in cell culture plates

Treat the same amount of PC12 cells with NGF (100 ng/ml)  
Lyse the cells with a detergent after certain time (0, 5, 10 min)

Cell lysates, one for each timepoint

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## How to measure the activation of ERK - Separation of the proteins in the lysate by electrophoresis

Cell lysates

SDS -PAGE

Cell lysates separated on a gel

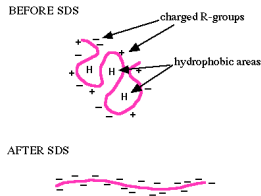
Image file of the gel that can be evaluated

Immunoblotting

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## SDS-PAGE and Immunoblotting 1



SDS (a chemical) is added to protein to denature it and charge it negatively proportional to the size of the protein

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## SDS-PAGE and Immunoblotting 2

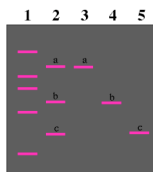


The SDS - treated protein lysate is loaded on top of a gel. An electric potential is applied to the gel and the proteins are pulled through the gel. The gel acts like a sieve that separates proteins according to size. This technique is called polyacrylamid gel electrophoresis (PAGE).

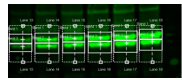
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## SDS-PAGE and Immunoblotting 3



Detection of a protein (f.i. ERK) by antibody, scanning of blot



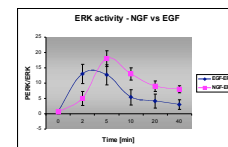
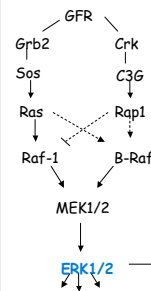
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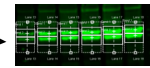
After the protein have been separated according to size, we need a method to visualize them. This can be done by staining them with a dye.

A better method is to use antibodies to determine where they are. Antibodies are proteins produced by the immune system which bind specifically to a target structure. The antibodies are "labelled" with a fluorophore (i. e. a molecule that emits light is chemically attached to them). This property is used to detect the bands with a scanner.

## Measurement of ERK activity by SDS - PAGE and Immunoblotting



Ratio of phospho-ERK to ERK is plotted against time and we get a measurement of ERK activity.



Amount of ERK and phospho-ERK is quantified with the scanner.

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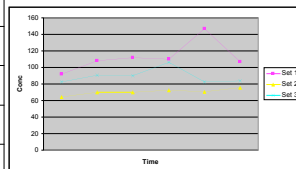
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## Experimental observations...

Time	Set 1	Set 2	Set 3
0	92.06901	64.25505	82.57557
2	108.3974	69.72303	90.80861
5	111.9357	70.16582	90.28703
10	110.2452	72.76562	106.1241
20	146.861	70.43287	82.39227
40	106.9155	75.45979	83.9535
Average	112.7373	70.46703	89.35684

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## Take home message...

- Basic definition of cell signalling
- There is an analogy of signalling in organisms and signal processing in machines (circuits)
- Overview of MAPK pathway in general and in the shape of the PC12 cell model.
- Example of how we perform experiment in biochemistry (SDS-PAGE and immunoblotting).
- Data on cell signalling is
  - like to contain errors
  - relative (you compare things, these are not absolute measurements)
  - sparse (it is time consuming and difficult to obtain information about cellular processes)

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