Regulation and signaling

Overview

Cells need to **regulate** the amounts of different proteins they express, depending on

- cell development (skin vs liver cell)
- cell stage
- environmental conditions (food, temperature, mechanical stimuli)

Regulation affects **gene expression**

Regulation is in response to various intracellular and extracellular **signals**, which are usually chemical entities (specific molecules)

The principles of **recognition** enable a specific response → however, many recognition events are often put together to build more robust **signaling cascades or networks**

Control of gene expression

Cells can target many parts of the protein production pathway for control

- transcription
- RNA processing
- RNA transport
- translation
- mRNA degradation
- protein activity

Transcriptional regulation

Common regulatory mechanism targets transcription itself

Transcription requires initiation with a **promoter** sequence → proteins that bind to these sequences, **transcription regulators**, can modify transcription rates

- **repressor** – prevents binding of RNA polymerase

- **activator** – facilitates binding of RNA polymerase

In eukaryotes, **chromatin structure** also affects expression → can be controlled by regulating concentrations of **chromatin-remodeling protein**

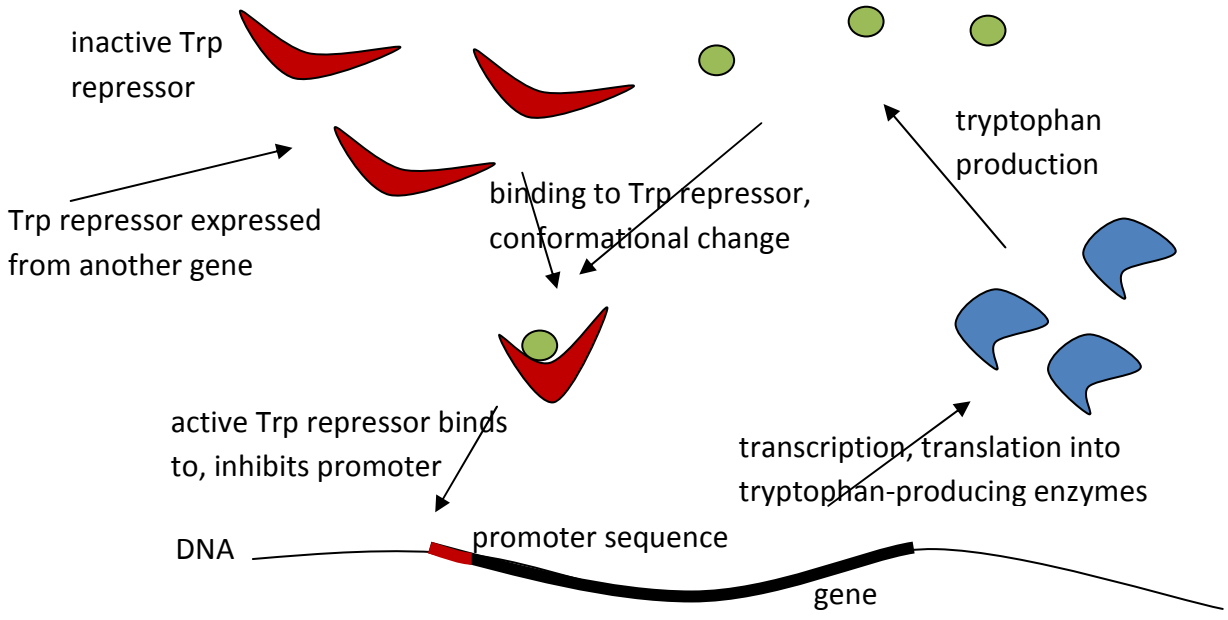
Logic gates

Multiple activators and a repressors can control the same gene. Consider two:

repressor	activator	gene expression
OFF	OFF	OFF
ON	OFF	OFF
ON	ON	OFF
OFF	ON	ON

Feedback control can be initiated using allosteric principles.

Example:control of tryptophan concentration in E. coli



Complex regulatory signals in eukaryotes

Because transcription requires the assembly of many factors at the initiation site in eukaryotes, gene expression can result from the combination of many different signals

Different genes require different initiation complexes

Lack of key proteins or other ligands in the assembled complex at the initiation site will result in low gene expression levels

The same molecule may be key to controlling many different genes because it participates in multiple initiation complexes, for different genes → we call this the same **signal**.

Gene expression controls the **differentiation** of cells into different cell types

Gene expression can be inherited

- **positive feedback loop** – transcription regulator also activates its own production
- **chromatin structure**
- **DNA methylation –** turns off genes

RNA post-transcriptional control

Riboswitches

- mRNA molecules that encode for a protein can also bind to small molecules and change conformation, thus terminating transcription
- example of switching off gene expression in response to concentrations of particular metabolites, etc
- most prevalent in prokaryotes
- simplest mechanism of regulation, another indication of the RNA world hypothesis

Covalent modification of mRNAs to inhibit translation

microRNAs – small bits of RNA that bind to mRNA and inhibit translation

Signal transduction

Cells send signals through various chemical components → interconversion is called **signal transduction**

Signals are able to effect highly specific action because of binding to and **recognition** by various proteins → typical $K_D \sim 1nM$

Binding and recognition induces conformational changes that affect protein activity

Activated/deactivated proteins then can affect or send signals to other proteins in complex chemical reaction networks or **cascades**

These signals affect

- growth, differentiation, movement and shape
- expression of genes
- metabolism
- secretion

Variations in response speed

- fast – change in protein conformation and hence activity (e.g., enzyme activity)
- moderate – vesicle fusion (due to protein conformational changes)
- slow – gene expression (hours)

Signals can be processed much like electrical logic gates can, using multiple binding sites on the same protein or binding-induced protein assembly

- **signal relay** – protein "passes" the message to another protein, e.g., by binding to it and activating it, or by catalyzing a reaction that produces or inhibits other signal molecules
- **signal distribution** – similar to relay, but message then activates or inhibits multiple other protein
- **signal amplification** – protein increases the strength and distribution of the message, e.g., by catalyzing a reaction to produce many more signal molecules or by increasing gene expression of other proteins
- **signal integration** – protein(s) takes two signals and produces one output, e.g., two signal molecules can bind to the protein regulating its activity, either by activation or inhibition; or multiple proteins can regulate transcription (previous discussion)

The last component enables complex networks of different proteins to yield very sophisticated cell responses to **combinations** of signals

Modeling cell response to signals is highly challenging due to the intricacy of signaling pathways → many proteins, signals, and binding and reaction events underlie a single cell response

Importantly, signals can be **spatially localized** → gives rise to asymmetric cell/tissue development or migration of cells in a particular direction (e.g., capillaries)

Extracellular pathways

Signals sent from cell to cell involve

- signal reception at the extracellular cell surface
- transduction to the interior of the plasma membrane (either by direct passage of signal through the plasma membrane or by a conformational change in a protein **receptor)**
- transduction to other components in the cell

How do chemical messages get sent from cell to cell?

- **endocrine** – chemicals called **hormones** sent through blood stream or sap (e.g., insulin)
- **paracrine** – chemicals called **local mediators** sent in the extracellular space and diffuse to adjacent cells (e.g., clotting factors)
- **neuronal** – specialized fast signaling along long distances to specific receptor cells
- **contact-dependent** – extracellular proteins in adjacent cells directly interact (e.g., embryonic development)

Two basic kinds of signal molecules

- small molecules with enough hydrophobicity to pass through the plasma membrane: **steroid hormones, thyroid hormones, NO**
- hydrophilic or large molecules that bind to a **receptor** protein on the cell surface

Most signals function through the latter mechanism, by interaction with **receptor proteins**

- highly specific binding interactions
- many kinds on cell surface
- binding induces conformation changes / activity changes that can propagate to the intracellular side

Intracellular pathways

Kinds of receptors that transduce signals to the intracellular space

- ion-channel-coupled – binding induces change in ion channel state
- enzyme-coupled – binding induces enzyme activity on the cell interior
- G-protein-coupled – affects a cascade of interactions through G-proteins

G-protein-coupled receptors

- largest family of cell-surface receptors – over 700 in humans
- major drug targets
- transmembrane
- activate membrane-associated **G-proteins** on the cytosolic side by triggering binding of GTP an unbinding of GDP
- eventually the G-protein hydrolyzes GTP to GDP and becomes inactive

When active, **G-protein complexes** dissociate and the alpha subunit can then regulate many kinds of other proteins by binding to them

- ion channels
- membrane-bound enzymes

Once the message is sent to the interior of the cell, a frequent mechanism by which it is passed is using **molecular switch** proteins that can be toggled between active and inactive states

Common mechanisms of switching

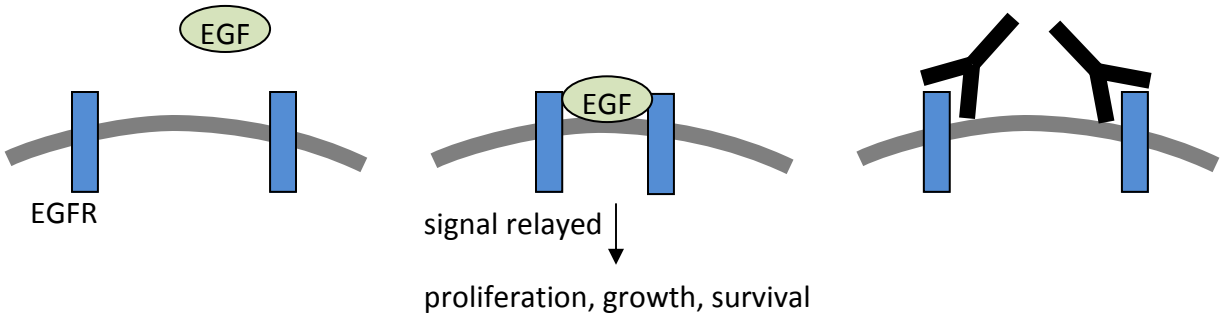
- **phosphorylation** by **kinases, de-phosphorylation** by **phosphatases**
- **GTP**-**binding** and subsequent hydrolysis

Many kinases control other kinases, e.g., when a kinase is phosphorylated, it becomes active and phosphorylates another kinase → common mechanism for signal **amplification**

Modeling signaling: a case study

Goal: predict how tumor cells will respond to a therapeutic or growth factor.

Case study: Anti-EGFR monoclonal antibodies bind to receptor and inhibit interaction with ligand → reduces "growth" signal



The response of the cell is a complex function of the chemical inputs

$$[\text{response of cell}] = f(\text{signal inputs})$$

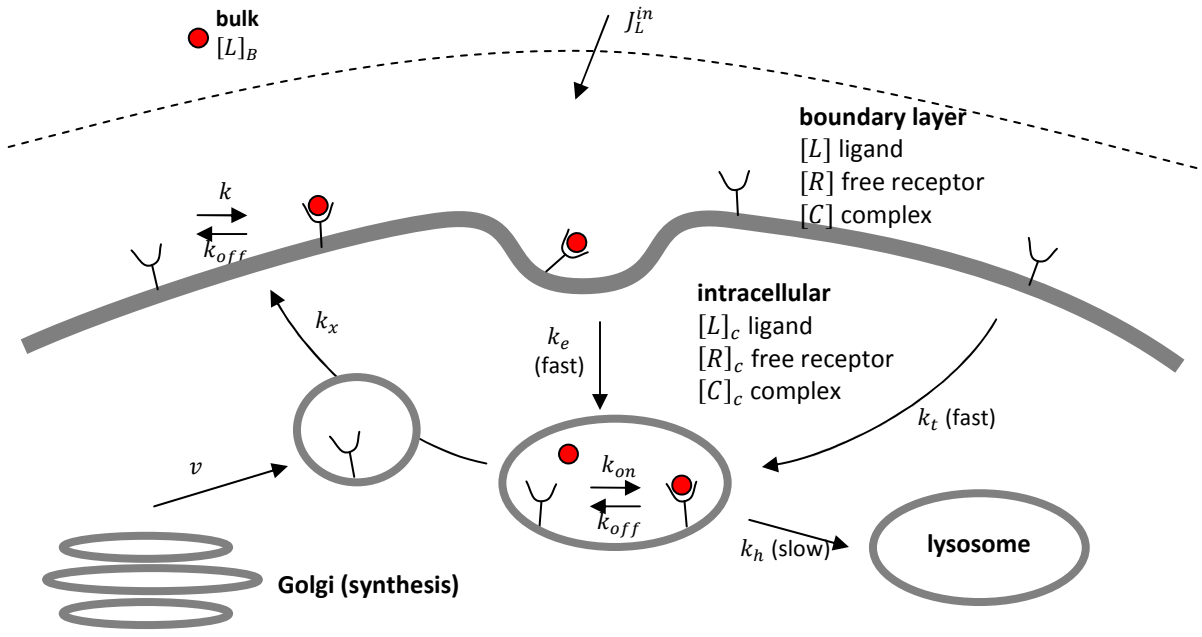
We can write the response to EGF a proportional to the number of bound receptors

$$[\text{response to EGF}] \propto [EGF - (EGFR)_2]$$

Key questions for a model

- How much $EGF - (EGFR)_2$ exists in the tumor?
- How much can the antibody block complex formation?

Basic picture of receptor binding and transport:



Model the changes in concentrations due to binding and transport using mass balances

Boundary layer:

$$\frac{d[C]}{dt} = IN - OUT + GEN - CONS$$
$$= 0 - k_e[C] + k_{on}[L][R] - k_{off}[C]$$

$$\frac{d[R]}{dt} = -k_{on}[L][R] + k_{off}[C] - k_t[R] + k_x[R]_c$$

$$\frac{d[L]}{dt} = -k_{on}[L][R] + k_{off}[C] + J_L^{in}$$

Intracellular:

$$\frac{d[C]_c}{dt} = k_e[C] + k_{on}[R]_c[L]_c - k_{off}[C]_c - k_h[C]_c$$

$$\frac{d[R]_c}{dt} = k_t[R] + k_{off}[C]_c - k_{on}[R]_c[L]_c - k_x[R]_c - k_h[R]_c + v$$

$$\frac{d[L]_c}{dt} = k_{off}[C]_c - k_{on}[R]_c[L]_c - k_h[L]_c$$

Total of six differential equations for six unknown concentrations with time

Cellular response proportional to number of complexes at any time t

$$response \propto [C] + [C]_e \approx [C]$$

Model can predict

- rate of growth as function of $[L]$
- recycling upon signaling and synthesis

What if we introduce a L-binding antibody?

$$\frac{d[L]}{dt} = -k_{on}[L][R] + k_{off}[C] + J_L^{in} - k'_{on}[L][A] + k'_{off}[LA]$$

Want a high k'_{on}, low k'_{off} for the antibody