BT 2123 - Systems Biology

Introduction to Biological Systems

BI 2123 (E) - Semester 3

01/09

Lecture 01

The goal is to understand the complexity in biology, and to develop a systems approach in thinking.

Integrative Levels of Organisation in Biology — Novikoff, 1945

Biosphere
Ecosystem
⋮
Organism
⋮
Cell
⋮
Atom

- Each of the higher levels are built on the properties of lower ones.
- New functions arise in higher levels, so understanding lower levels doesn't mean you'll get the subsequent higher one i.e. NO ontological preference
- The course will cover Molecule to Organism

Features of Organization

1. All levels are highly organised across length & time scales
2. Lot of organisation appears spontaneously
3. These systems are able to perform low error functions (rather accurately) and computations — even in environment with thermal fluctuations & dense soup of molecules
   Eg: A cell translates mRNA, responds to stimuli specifically & so on while its packed with 40-100 billion proteins.
   So there are local inhomogenities & other fluctuations that affect equilibrium
4. Systems are optimized to achieve objectives efficiently
5. They have evolved over time & continue to evolve

Premise

Biological systems can be understood from a perspective of unifying Design Principles which can be represented in mathematical framework.

These design principles are —

→ Optimized to efficiently achieve objectives:
- Robust * (stable even with lot of external perturbations)
- Specific *
- Sensitive *

→ Shaped by evolution.

What is a 'Complex System'?

[It is a group of parts that comes together to form a more complex unit.] → interacting & interdependent

None of the individual parts possess the properties of the whole system, but when integrated, they display special properties called Emergent Properties that arise from the multiplicity of relatively simple interactions.

Eg: Glass sphere + Tungsten wire → Incandescent light bulb

These emergent properties cannot be reduced to the individual properties of the parts. So they're Irreducible.

What is Systems Biology?

It is the study of an organism viewed as an integrated and ~~integrating~~ interacting network of its constituents — majorly the genes, proteins, biochemical reactions & biophysical processes that has given rise to life

Systems Bio has emerged in the last ~25~ years
- Its associated with the identification of parts that make up the system and their interaction which gives rise to emergent properties & putting them in an analytical framework.
- This has been possible because many high throughput methodologies have arisen that allows us to study and identify the components of the system.
- The genomic and other -omic projects made possible the accumulation of a critical mass of information
- Development and application of analytical & computational methods that can help us make sense of the data.

Associated identification / quantification methodology —
* Transcriptomics : gene expression
* Proteomics : protein expression pattern
* Phospho-/Glycoproteomics : post-translational protein modification
* Metabolomics : analysis of metabolites
* Glycomics : carbohydrates

These have accelerated the growth of Systems biology.

Associated analytics platform
Ability to study the flux and dynamics of various components in a cell —

* Interactomics : identifying & mapping interactions (genetic & protein)
* Fluxomics : changes of molecule dynamics over time

Objective : Figure out the design principles over various levels of organisation.

In the course, we will study the integration & organisation in —

1. Cells - Gene regulation & regulatory networks
   Signal transduction
2. Organisms - Early development & pattern formation
   Themes in development
3. Physiology - Immune & Nervous System.

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Lecture 02
Gene Regulation & Transcriptional Networks
This is in relation to responding appropriately to its (cell's) external & internal environment.
We'll look at a system & identify it's design parameters — to understand how it works and how it evolved.

Cells emerge from interaction of thousands of proteins.
Humans have ~30k protein coding genes & with this relatively small no. of proteins a cell has to constantly respond accurately to its environment, by regulating production of proteins, to survive.

Transcription Factors (are proteins) which regulate the rate of production of other proteins.

Environmental stimulus
↓
Signal Transduction
↓
Transcription Factors
↓
Target Genes

- Tx factors need to switch on & off fairly quickly in response to stimulus.

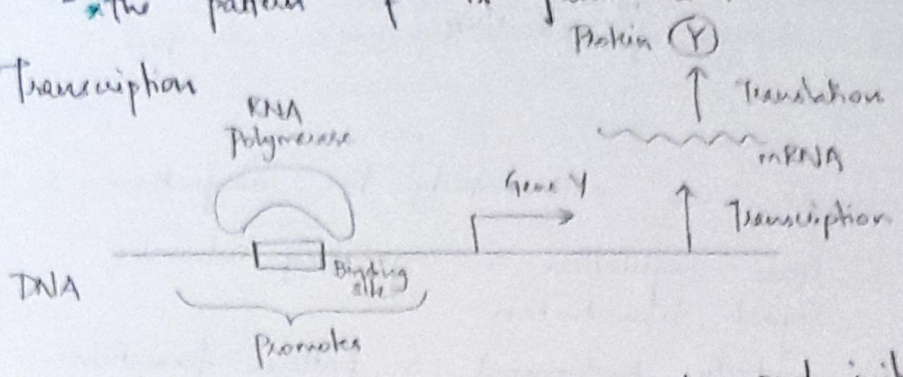
⇒ Transcription factor activities are an internal representation of the environment in the cell

Uri Alon and Young, 2002

Alon 2007

Information processing activity involves both the -
- Signal processing system that relays signals to Tx factors; and
- the pattern of Tx factors activities.

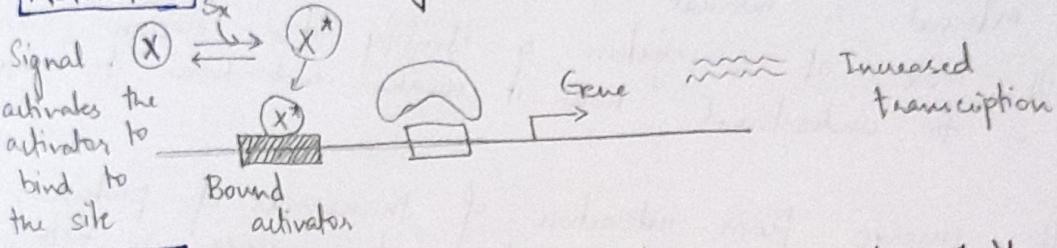
Alon 2020



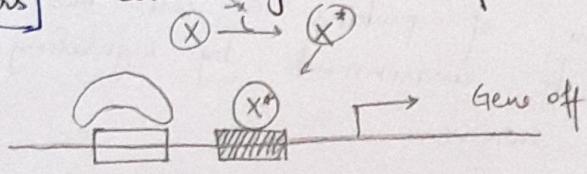
RNA polymerase bind to the promoter and initiate transcription.
Tx factors regulate the rate at which RNA polymerase initiates transcription. They're designed to rapidly switch on and off.

Types of Tx factors.

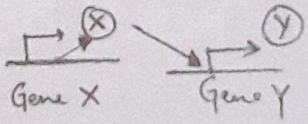
1. (+) **Activators** increase gene transcription $X \rightarrow Y$



2. (−) **Repressors** decrease gene transcription $X \dashv Y$



Transcriptional Networks
Tx factors are proteins themselves and coded by genes
⇒ They're regulated by other Tx factors
This set of interactions form transcription networks.
It's through these networks that different proteins are regulated to respond accurately to stimulus.



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The transcription networks are complex and interrelated.
Nodes : proteins (or genes encoding them)
Edges : Tx regulation

- Some nodes are populous (regulate many other genes) and they're called Party Hubs
- These networks are dynamic – they change over time. Those nodes that shift and switch are called Date Hubs

⇒ Tx Networks are <u>dynamical systems</u> –
- Input signal
- Change in Tx factor ~~regulation~~ activities
- Change in protein production rates
- Some are Tx factors & regulate other genes
- Other proteins carry out cellular function
- There are diff. feedback loops

Collectively, the change in <u>protein regulation</u> (1° (primary) output) causes change in behaviour of cell (terminal output) and these have <u>feedback system</u> which act as signal inputs.

⇒ Strong seperation of timescale :
* Input signal changing Tx factor ~ 1ms
* Tx factor binding to DNA ~ 1s
* Transcription & translation ~ 5 min
* Accumulation of protein products ~ mins to hours

⇒ Tx Network show modularity of their components
- One can take DNA (gene) from one organism & express it in another.
  Eg: Insulin gene put in E.coli to mass produce insulin
  Green fluorescent protein (GFP) from jelly fish can be expressed in many other cells & tissues.
- Any promoter can be chosen for these genes.
  ⇒ Promoters & genes are modular & interchangeable.

This modularity/interchangeability <u>makes Tx networks plastic</u> during networks evolution – it can incorporate new genes and regulations.

Thus these Networks evolve rapidly – edges evolve on a faster timescale than coding regions (genes)

Order of evolution in bacteria – $\sim 10^{-9}$ per base per replication event
⇒ A population of 10^{10} bacteria (from a single spore in a day) will have 10 mutations in any base pair.

In order to maintain the network, the edges are constantly selected for. [∵ of mutations Tx networks have tendency to evolve and get randomized]

Shen-Orr 2002

Network Motifs

They are * patterns of connectivity in "real networks". (exptally determined)
They are the building blocks of networks.
They are encountered in networks in statistically higher numbers (enriched) (than expected if they were randomized) ⇒ they've been selected through evolution.

If we can understand dynamics of motifs, we can attempt to understand networks.

Alon 2007 ⇒

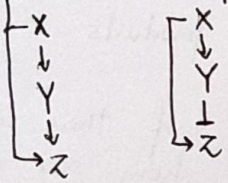
How to find network motifs?

We *compare 'real' and 'randomized' networks. We compare experimentally determined networks with randomized networks (i.e. same no. of nodes & edges but random connections)

Erdos-Renyi (?)

Network motifs are patterns that occur more significantly (statistically) in real than randomized network.
They're likely motifs preserved in 'real' networks.

Examples: Feedforward Loops



Mutations can randomly add or remove edges. Network motifs are patterns conserved through evolution.

Pg 33 - Network motifs are patterns of connectivity that occur more often than chance because they've been conserved through evolution

Randomized Erdos-Renly network

Lecture 03

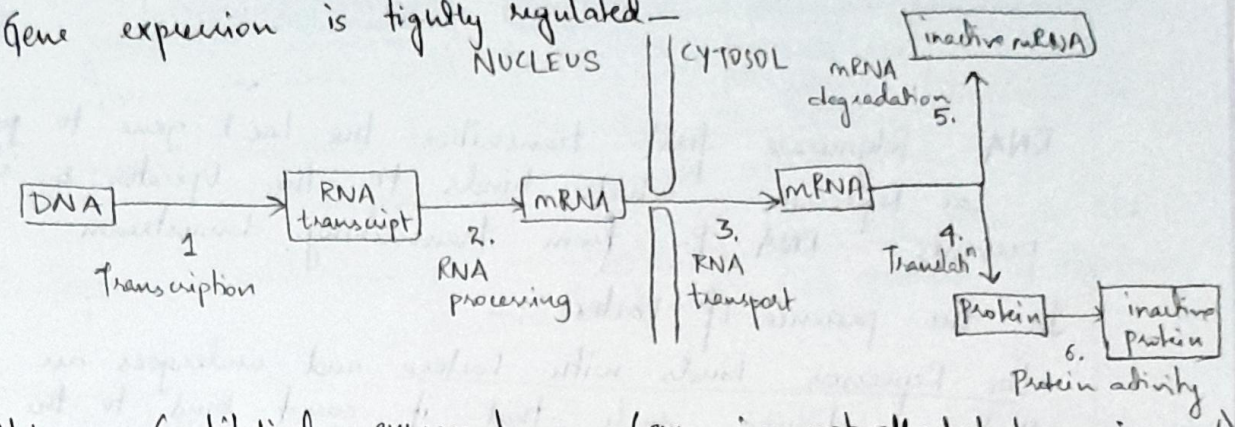
Gene Regulation and Operon

Importance of regulation of gene expression — examples :-

1. Differentiation of cells — in structure and function
2. Metamorphosis : morphology of caterpillar and butterfly

So even though they have the same genome, they have different
- physiology
- proteomes
- expression pattern

Gene expression is tightly regulated —



Note : Constitutively expressed gene (expression not affected by environment)
Regulated genes — inducible or repressible

Lac Operon in E. coli

E. coli prefers glucose but can use Lactose. To break it down

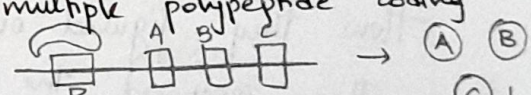
Lactose $\xrightarrow{\beta\text{-galactisidase}}$ Glucose + Galactose

G+ , L− : trace amt. of β-gal
G− , L+ : 10% of protein weight is β-gal

} Environment sensing system.

Lactose requires a channel protein : Lactose permease to enter the bacterial cell

Operon : Regulatable stretch of DNA that produces single mRNA transcript that has multiple polypeptide coding regions. (Polycistronic)

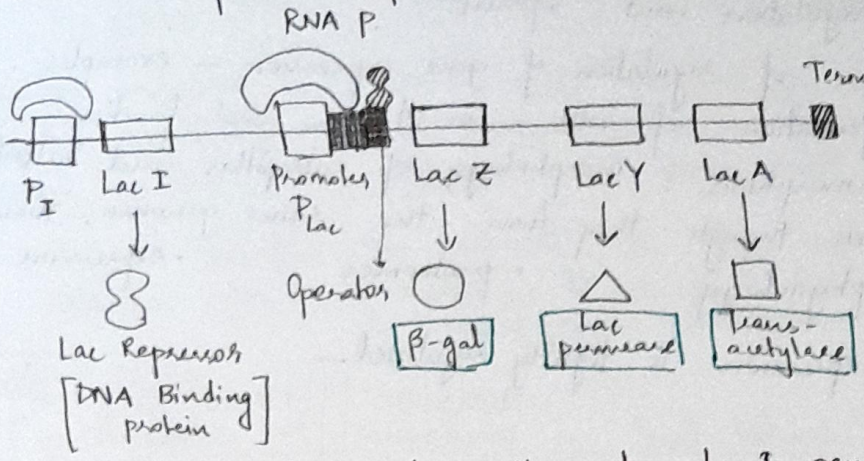


This is useful if all gene products are required for same biochemical activity.

Operons are mostly found in bacteria & contains regulated genes.

All coding regions are collectively "operated" on

Structure of Lac Operon :-



RNA Polymerase first transcribes the Lac I gene to produce Lac Repressor which binds to the Operator & sterically prevents RNA P. from transcribing downstream.

In the presence of Lactose :-

Lac Repressor binds with Lactose and undergoes an allosteric change such that it can't bind to the Operator. DNA

⇒ Lactose itself triggers the induction of operon by binding to the repressor so the genes can be transcribed

∴ + L → ↑ β-gal

− L → trace β-gal

The steric block is not an absolute binary switch; its probabilistic ⇒ there's always trace amounts of β-gal and Lac permease. Hence some Lactose can come in to induce the Lac Operon.

It needs to sense not only presence of Lactose but also the absence of Glucose.

How they figured out :-

They compared the system when provided with analogs of Lactose -

	Inducer	Substrate
Lactose	✓	✓
IPTG	✓	×
X-Gal	×	✓ (blue)

(09) Jacob and Monod subjected the bacteria to mutations (Radiation) and checked to see if β-gal was produced anywhere ~~without~~ in the absence of Lactose.

They did this by giving X-gal as nutrient source.

⇒ In wild types E.coli, there would be no blue colour unless IPTG was added to the petridish.

In mutated colonies, there would be blue coloration even without adding IPTG. They were called Constitutive Mutants (∵ they made β-gal without regulation)

There mutants could of two types –

1. Operator Constitutive [O^c] : Mutation in Operator sequence so that the repressor can't bind to it.
2. Repsessor Constitutive [I^c] : Mutation in LacI gene so the structure / conformation of protein is affected.

	Genotype	- IPTG	+ IPTG	Arbitrary nos. indicating the amount of β-gal
1.	$I^+ O^+ Z^+$ (wild type)	1	1000	
2.	$I^+ O^c Z^+$	1000	1000	
3.	$\frac{I^+ O^+ Z^+}{I^+ O^c Z^+}$ Diploid	1001	8000	
4.	$\frac{I^+ O^+ Z^+}{I^+ O^c Z^-}$	1	1001	
5.	$\frac{I^+ O^+ Z^-}{I^+ O^c Z^+}$	1000	1000	[cis-arranged]

These experiments made Jacob and Monod postulate that the operator sequence was on the same DNA strand as the Lac Z gene (& that it was infact a gene)

6	$I^c O^+ Z^+$	1000	1000	* The repressor produced by the wild type gene will bind to the operator of I^c DNA *
7. ★	$\frac{I^+ O^+ Z^+}{I^c O^+ Z^+}$	1	1000	
8. ★	$\frac{I^+ O^+ Z^-}{I^c O^+ Z^+}$	1	1000	

(*) This allowed them to hypothesize that the repressor was a protein that need not be produced by the same DNA sequence.

(**) This made them say that the repressor would work if in a trans-arrangement with LacZ but the operator sequence had to be in cis-arrangement.

Super important (*)(**)

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Discussion

→ Increasing rate of transcription means increasing the frequency of binding of RNA-Polymerase to the promoter.

→ Having 2 operons in a bacteria ⇒ 2 operons on two molecules of DNA – circular chromosome & plasmid. This is called a mero-diploid (half-diploid).

→ During / Due to recombination, the internal system & transcript^{n} network of the bacteria is messed up. So while theoretically any gene can be expressed in a bacteria, there are many practical problems and a lot of optimisation has to be done so that we get enough of the product but not end up killing the organism

→ Genes on a Tx Network neednot be physically close to each other.

→ Operons are mostly found in bacteria and eukaryotic cells like yeast, C-elegans, fruitfly and so on. In mammalian and most other cells, gene regulation is more nuanced – methylation and acetylation of histones.

→ Regulation of mitochondrial genes is majorly done through the nucleus' Tf but some part of it is regulated by mitochondrial Tf as well. → (made exclusively for mitochondria)

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Lecture 04

Gene Regulation and Operon II

Recall : Lac Operon – inducible operon.

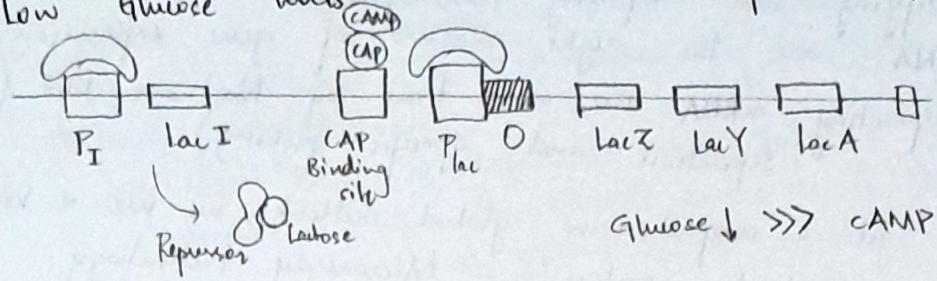
Even when the repressor isn't bound to the Operator, the binding of RNA-P to the promoter is weak. So, weak transcription occurs.

So, even the presence of lactose (which binds to repressor) doesn't do much to increase efficiency of operator.

When cell is glucose-starved —

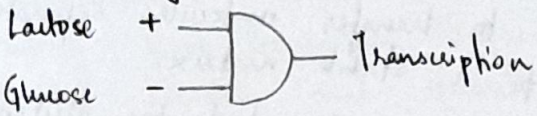
- Through some metabolic pathways, it increases the production of cAMP
- When levels of cAMP is high, it binds to Catabolite Activator Protein (CAP) and forms a complex, which can now bind to a specific sequence of DNA which happens to be upstream of P_{lac}
- This brings about a change in conformation of DNA (bends it) and allows RNA-P to bind to P_{lac} efficiently.

∴ Low Glucose levels enhance the rate of Transcription.



So, Lac Operon works best in presence of lots of Lactose and absence of Glucose.

This creates a genetic AND gate —

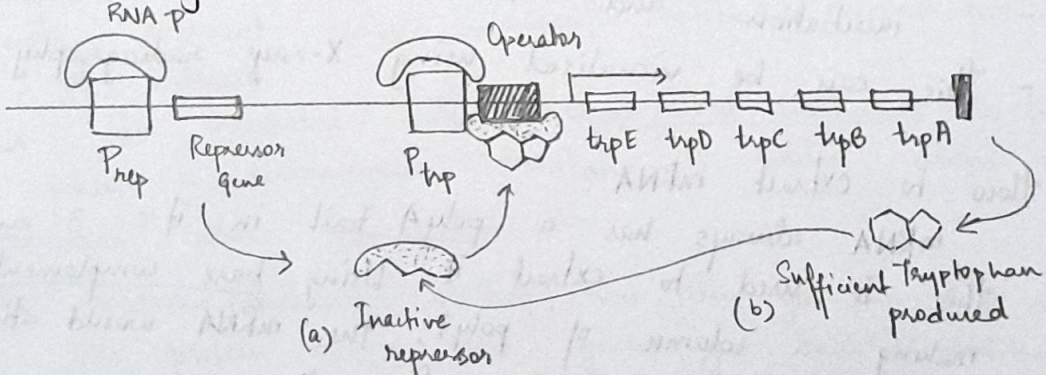


Repressible Operon

This operon keeps expressing and when it receives a cue it stops expressing it. An example is —

Trp Operon

It synthesizes proteins that help in synthesis of tryptophan.



(a) In the absence of tryptophan, the operon keeps producing proteins that help synthesize tryptophan. Here, repressor can't bind to Operator as it's activated.

(b) Once enough tryptophan is produced, it activates the repressor which can now bind to operator and hinders RNA-P thus a phenomelting rate of transcription rates.

Evaluating Transcriptomics and Proteomics.

→ Quantifying and Comparing Transcriptomes

mRNA are the right measure of gene expression.

1. Seperating mRNA one at a time by Northern Blot (mass seperation and specific probing)
2. To get a comprehensive, global outlook, we use a kind of 'molecular sorting' - Microarray technology
3. Comprehensive quantitative sequencing (for mRNA of a cell) - RNA sequencing.

▲ Blotting - technique to transfer molecules seperated on gels to a stable matrix.

- After extraction, RNA is seperated by electrophoresis (on basis of size) in agarose gel. But this gel is fragile and difficult to handle.
- So, mRNA is transferred onto a stable matrix, like a nitrocellulose membrane. RNA is fixed to membrane with UV or heat
- The required fragments are identified by labelled probes (i.e membrane is hybridised with incubation and detection.
- This can be visualised using X-ray radiography.

How to extract mRNA?

mRNA always has a polyA tail in it's 3' end and this is used to extract it. Using base complementation i.e making a column of polyT, the mRNA would stick to the column while others flow off.

(13)

Alt Bio of the cell pg-530

Microarray Scheme

- Here, the probe is bits of DNA from genes we're interested in. After PCR amplification and purification, each probe is put in small well in a slide (using robotic pipetting)
- The mRNA is (sometimes) converted to its cDNA and labelled with fluorophore probes (say, red for test and green for reference).
- The microarray is incubated with cDNA and hybridisation is allowed to occur. Then its washed to remove cDNA that's not tightly bound
- The position in microarray in which labelled DNA fragments have attached is identified by an automated scanning electron microscope.
- If a particular RNA is expressed more relative to the reference sample its colored red; & if its decreased relatively, its colored green. If its equal to reference sample, the color is yellow

RNA - sequencing

Its a quantitative analysis that sequences every single mRNA using Next Generation Sequencing (NGS). This reveals the identity and quantity of RNA in a sample at any given time

→ Quantifying and Comparing Proteomics

Drawback :-
It doesn't distinguish between two proteins of same molecular weight

Western Blot

- Protein extracts are seperated using SDS - PAGE electrophoresis and its transferred to a membrane and incubated with relevant probes - specific antibody against protein of interest.
- Then its incubated with <u>enzyme liked antibodies</u>; so that when a chromogenic substrate is added, colour is produced, where the proteins are.

Like Southern / Northern blotting, here also you can only detect one protein at a time, given you have its antibody.

Intensity of colour shows the amount of protein present

2D Gel electrophoresis

This increases the resolution that SDS PAGE lacks.

1D – isoelectric focusing (seperation on basis of pI)

2D – gel electrophoresis (on basis of size).

pH at which the charge on protein is 0. So it stops moving based on electric field.

Again, label, isolate, identify

Difference Gel Electrophoresis (DiGE)

- Proteins are extracted from cells in different states; as different cells. Proteome #1 is labelled with green dye and #2 is labelled red.
- They're then mixed and 2D gel is run & scanned for florescence. Yellow ⇒ expressed equally in both
  Green/Red ⇒ Greater in one, lesser in another

This allows us to compare proteomes.

Mass spectroscopy helps identify proteins of interest.

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Lecture 05

Signal Transduction I

The environmental stimuli is relayed to the Tx factors by a network of signalling factors through process called cellular signaling.

- The size of genome or number of genes donot correlate with the complexity of an organism.
- But the number of proteins involved in signalling is positively correlated with the complexity – i.e. a richer network of proteins allows organisms to perceive the environment in a nuanced way.

What is Signal Transduction?

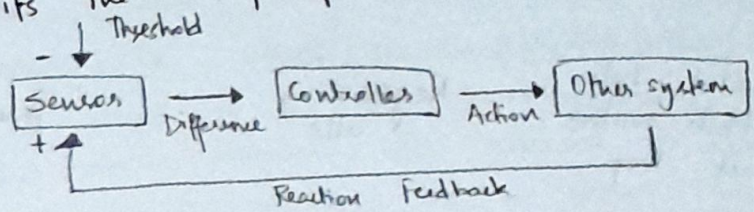
Essentially information processing – receiving, interpreting, processing and amplifying (so the response is robust).

It's the process of converting chemical & physical (mechanical forces, temperature, light, osmolarity etc). signals into responses through a series of molecular events.

Cybernetics

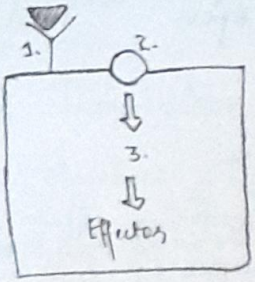
A cybernetic loop is a regulatory information processing systems with feedback. first used in electrical circuits.

It was applied to living systems in 1960s. there, its the study of control and communication in animals



Signal transduction is conversion of information from one form to another. Components –

Signal → 1. DISCRIMINATOR ⇒ 2. TRANSDUCER ⇒ 3. AMPLIFIER → Response



Martin Rodbell applied this analogy to signal transduction in cells.

- Receptor proteins present at membrane are the signal discriminators.
- They transduce the signal to the internal through a series of changes in protein conformation.
- Then, they activate secondary messengers (like cAMP and Ca^{2+}) which act as amplifiers.
- They act on their target proteins which directly or indirectly act as effectors, affecting the Tx network.

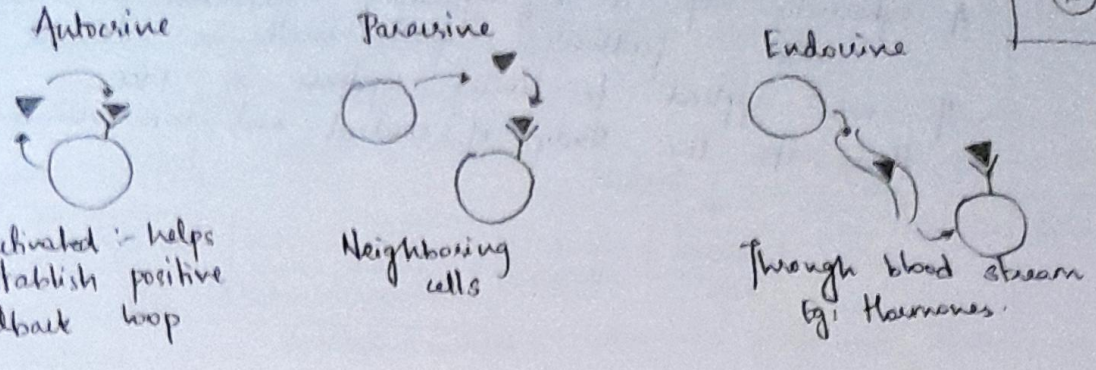
The Secondary messengers are amplifiers because they're small and hence, can be produced in large numbers and they can diffuse throughout the cell very quickly.

Types of Cellular Signals –

i) Cell-Surface receptor – Molecules that are too large or too hydrophilic to cross the cell membrane – they rely on surface receptors and secondary messengers.

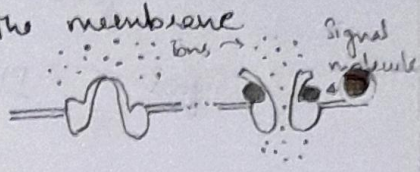
ii) Intracellular receptors – Molecules that are small or hydrophobic can easily cross the cell membrane and attach to the receptors which are inside or they can directly activate intracellular enzymes.
eg: NO, steroids

Secreted molecules - mediated signalling

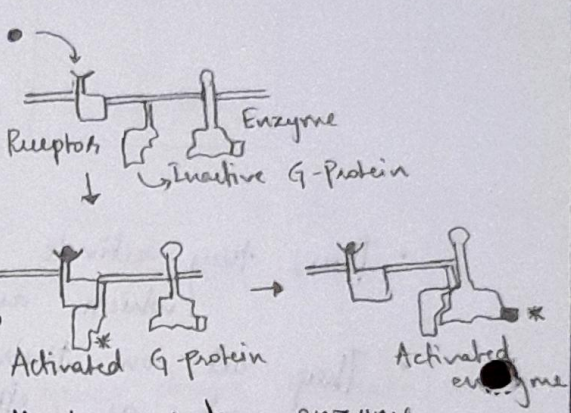


Cell Surface Receptors are of 3 types -

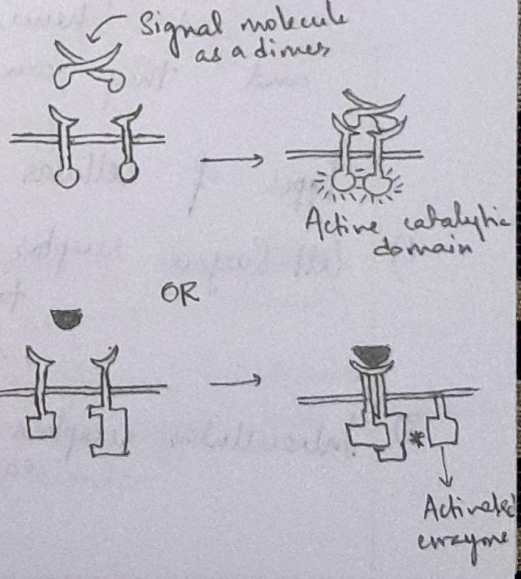
1. Ion-channel linked receptors.
   Ion channel can transfer ions across the membrane
   They're generally closed, but when signal molecule binds to them, they open and allow ions to go through



2. G-Protein linked Receptors
   They're the largest class of receptors.
   - These receptors on their intracellular side are coupled with proteins that involve hydrolysis of GTP.
   - When the receptor binds to signalling molecule, it also alters the conformation of G-protein, activating it.
   - The activated G-protein in turn activates another enzyme that transduces the signal



3. Enzyme-linked Receptors.
   These are receptors, which on their intracellular side have a catalytic activity site (inactive).
   When a signal molecules binds to them, their catalytic site is activated.



IP3 - inositol-3,4,5-triphosphate
PI - phosphoinositides

Secondary Messengers (Amplifiers)

They're - non-protein, small molecules → can be made in large quantities
- can be generated quickly
- can diffuse quickly through cytosol to reach their target

These features make them good amplifiers

Eg: cAMP, Ca^{2+}, cGMP, 1,2-diacylglycerol (DAG), IP3, PI

Note ▸ Ca^{2+} is stored in ER and isn't generally found in cytosol. But when required, its released in large amounts

▸ cAMP is rapidly produced by an enzyme Adenine nin-cyclase which converts ATP to cAMP.

▸ Eg: Epinephrine (10^{-10} M) - primary messenger
cAMP $10^{4}\times$ (10^{-6} M) - Second messenger.

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Lecture 06
Signal Transduction II

Basic structure of Signaling network -

Signaling molecule → Receptor → Components of signal transduction → Tx factors → Transcription

Mitogen Activated Protein kinase (MAP K) Pathway

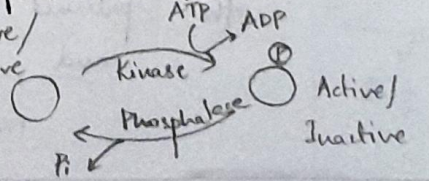
- Mitogens are factors that promote mitosis of cell
- Kinases are proteins the phosphorylate stuff (its substrate)
- MAP K is only one tier of a complex multi-tiered pathway which is a relay of phosphorylation events.
- Each tier has multiple kinds of proteins.
- The MAP K signalling cascade can process multiple kinds of signals and give very specific, robust responses.

From this relatively simple biochemical pathway, how does the system achieve
- specificity
- sensitivity
- reliability (robustness)
- pleiotropy

* or acetylation

Proteins activities can be regulated by post-translational modifications like phosphorylation.

Both forward & backward reactions are energetically favourable but enzimatically regulated

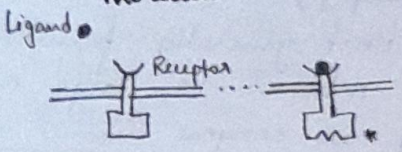


Typically, one state of protein is enzymatically active
Switch in activity states allow logical operations

Allosteric Regulation

Regulation of protein activity by binding of an effector molecule at a site other than protein's active site

This ensures SPECIFICITY.



Common modes of regulation — Activation or Inhibition of —

i) Active site opening / closing
ii) Formation / distortion of active site
iii) Enhancement / Inhibition of Dimerization

Refer Lec 06 - 16:30 mins

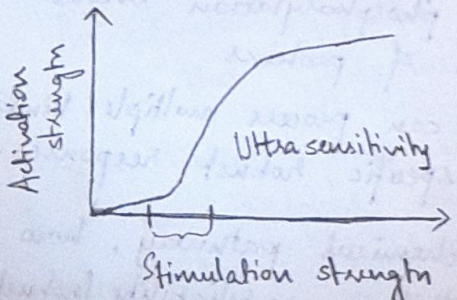
In the MAPK Signaling Cascade,

* There are 3 tiers involved
* for full activation of MAPK and MAPKK, they need to be phosphorylated twice

Signal → MAPKKK → MAPKK → MAPK → Response

For this blueprint to be preserved through evolution, they need to confer some advantages.

i) Dual Phosphorylation

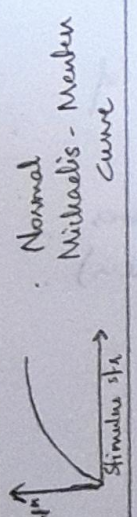


*a) Ultrasensitivity - there is a sharp change in activation strength for a small range of stimulus — this allows it to act like a switch.

*b) Coincidence detection - One phosphorylation can happen due to some 'background noise' signal. But for both phosphorylations to happen (in the presence of phosphotases) the signal must be strong and definite. This ensures reliable activation.

Robust

Substrate binding changes substrate affinity: cooperativity

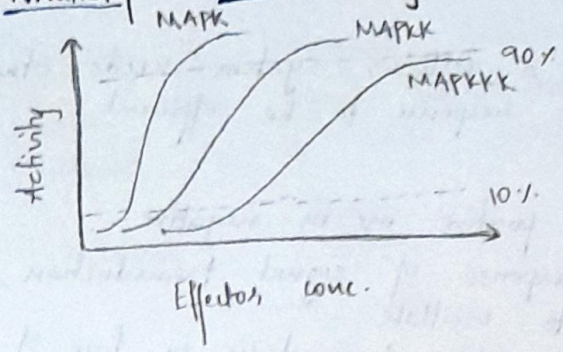


(19) ii) Three-tiered structure — i. Signal amplification (modest)

- Having multiple tiers allows it to amplify the signal (although its modest when compared to cAMP & Ca^{2+})

(ii) More regulatory interfaces - The network can be regulated by phosphorylations, feedback loops, crosstalk between parallel cascades etc. This gives the network larger regulation computational power.

(iii) Multistep Ultrasensitivity



MAPK gets activated rapidly at low effector conc. as compared to MAPKKK

This extended reaction cascade ends up enhancing sensitivity in each consequent tier.

★ This allows the network to filter noise - by using switch-like response for small range of filtered stimuli.

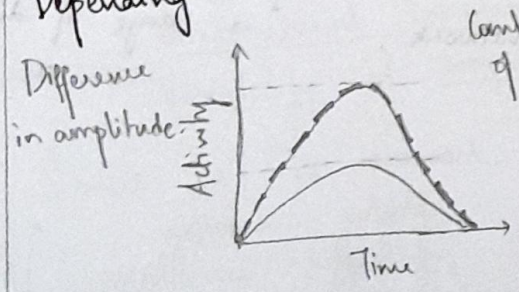
i.e. ★ MAPK is activated by small range of filtered stimuli only if MAPKKK remains activated for a long range

★ There is significantly more regulation than just dual phosphorylation - many more factors are involved.

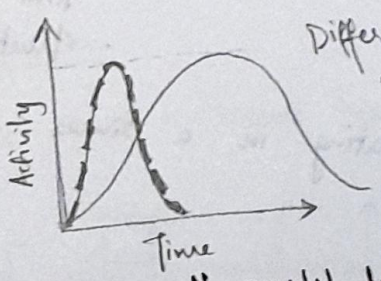
★ Phosphatases reset activities. This activity decides how long the activated MAK(KK) lasts. (i.e how long it goes on after stimuli is gone). And these phosphatases are also regulated.

(?)

★ Depending on the kind of stimuli, MAPK activity differes -



Combinations of the two



So if the activity of MAPK was of low ~~off~~ amplitude and for short duration, only the substrate with most affinity would get phosphorylated

Scaffold Proteins

They help in localizing the signalling pathway by -

- assembling signal complexes
- regulate selection of components → Arrange proteins in each ties, so that signal transduct^n is maximised.
- increase fidelity
- localise signal complexes

i.e. regulates the selection of proteins

— Missed a discussion.

29/9

Lecture 07
Signal Transduction III

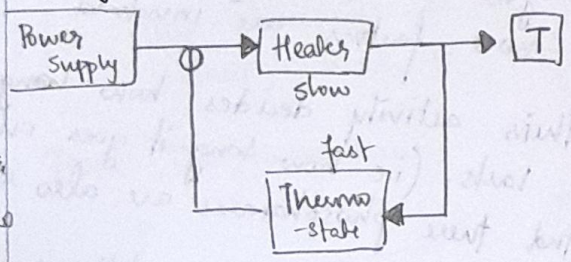
The dynamic plasticity of a cellular system needs other features for the response to be efficient.

One of them is -

Feedback loops : can be positive ~~or~~ or negative

* Negative : stabilise response of signal transduction, cause it to oscillate
  It helps maintain homeostasis in face of fluction
* Positive : amplify signals, create graded, analog (switch-like) signals.

Simple example -



Nguyen & Khalodenko 2016

This uses fast control on slow devices.

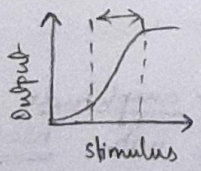
If the response time of detector was large, it would create oscillations because of the lag.

So based on
- strength of feedback
- time delay
- structure of network,

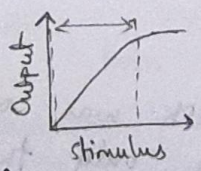
feedback can create a range of dynamics

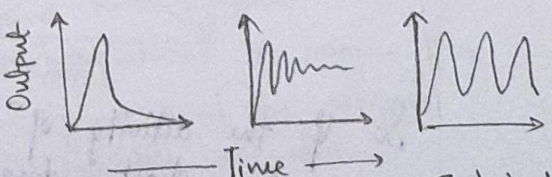
ROBUSTNESS

Operating in a linear range



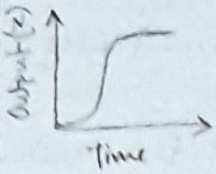
Negative Feedback



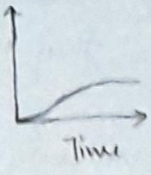


Negative feedback can also increase stability or perturbation tolerance.

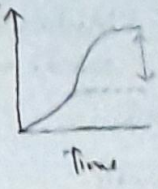
With positive feedback –



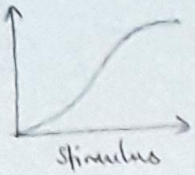
Switch-on Dynamic



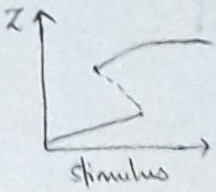
Positive ⇒ Feedback



* Amplifying input signal



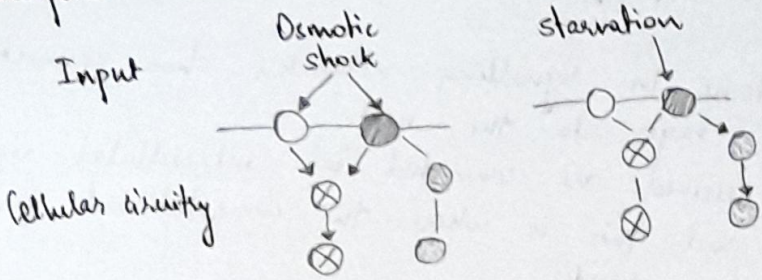
Positive feedback



* Create bistable response

Pleiotropy of biological function.

The same signal transduction pathway can detect and respond to a wide variety of signals.



This optimises the system because its not possible to have a unique receptor or signal transduction unit for every input. Thus, there networks are largely coupled and interconnected.

feasible

What differentiates each signal –

- Amplitude & duration of signal flux
- Combinatorial integration of network crosstalk
- Versatality of component function.

Similar to Tx network, we can draw a signal transduction network where –

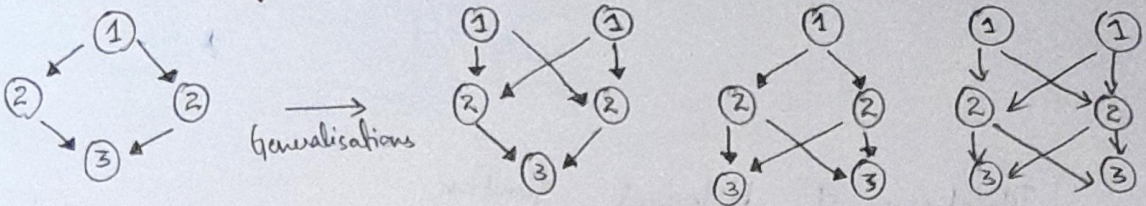
Nodes are signalling proteins.

Edges are directed interactions i.e. covalent modification of another protein.

Here too, we can observe "network motifs" – patterns of connectivity which are enriched/over-represented in experimentally determined (real) networks.

Thus network motifs are patterns conserved through evolution. Mutations can randomly change edges — to prevent randomization, they need to be constantly selected.

Diamond motif — seen uniquely in signal transduction network

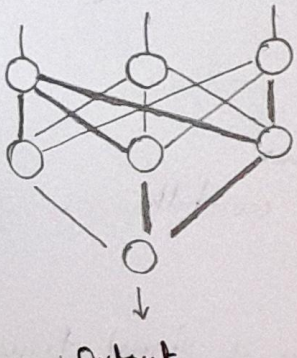


A small set of proteins in a S.T. network can code and bring out specific output for a large variety of input stimuli.

Multilayer patterns in signalling networks show connections from one layer to the next.

The stimuli received are converted into intracellular signalling pathways and this is where the computational power of the network resides.

To understand the working of these networks, we compare it to patterns in AI and Artificial Neural networks —



Thickness → strength of connection weight

The activity of components in hidden layer depends on —

1. Input they receive, called Connection Weights, whose strength may vary.
2. Mathematical formula it uses to integrate all the inputs to generate its output.

Neural networks can be trained to recognize specific input patterns & generate corresponding specific output pattern.

(23) → How do we 'train' neural networks?

First, we give it a set of inputs and its correct outputs. Depending on how the network performs, you give it an error score – 0 if all outputs are correct.

After each set of tests, the connection weights are randomly changed. The network path with minimum error rate is selected over others.

One the network is sufficiently trained, it can solve the new inputs itself.

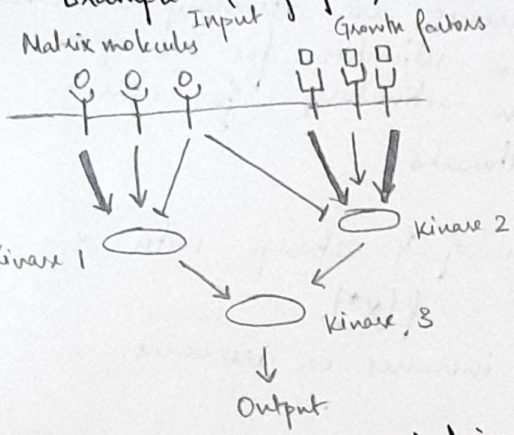
→ A similar analog to this is the signalling networks.

Each node is a protein and the connection weights are post-translational modifications like phosphorylation.

Just like neural network, this can be trained to recognise a pattern of inputs and give a specific output.

Here, the 'training' will occur over evolution.

Example (simplified) —



A result of this highly interconnected network is **stability** — tolerance to perturbations in the environment or a change in one of the components i.e. it won't collapse

This is called Robustness.

That is, it can maintain function despite internal and external perturbations.

Robustness is essential for – Appropriate communication
Generating apt. response
Preventing cellular malfunction.

Lecture 08

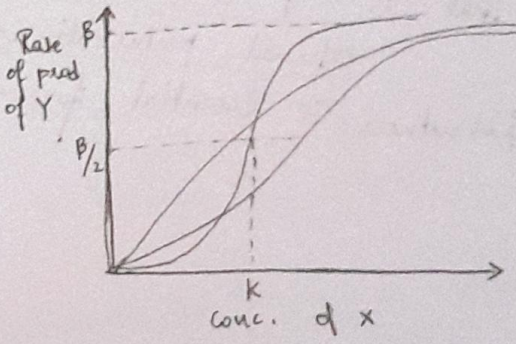
Transcription Networks

- A single E.coli has 4,500 different kinds of proteins (300 are TF) and 10^6 of them. It responds to stimuli by changing the conc. of proteins
- It does this by regulating Transcription by Tx factors
  A network of Tx factors regulate the production of other Tx factors and proteins.
- Thus they represent the internal & external state.
  They represent this in a vector of 300 dimensions [like how any colour can be represented in 3D space of Red-Blue-Green].
- Tx factors can be of two types –
  - ⊕ Activator $X \rightarrow Y$
  - ⊖ Repressor $X \dashv Y$

Dale's Principle : The out arrows are strongly correlated.
i.e. if X is an activator for one gene then its an activatory for others.
Also true for neural networks.

- How does rate of production of Y change with X?
  Rate of production of Y = $f(X^*)$
  $f(X^*)$ is monotonic – increases or decreases.

X-activator



Hill's Function

$$f(X^*) = \frac{\beta X^{*n}}{K^n + X^{*n}}$$

K : Units of conc.

If $X^* = K \Rightarrow f(X^*) = \beta/2$

$X^* \to \infty \Rightarrow f(X^*) = \beta$

β is the ~~saturating~~ saturated rate of prod. of Y
K is the conc. of X at which rate of production is β/2
Also known as "Activation coefficient"

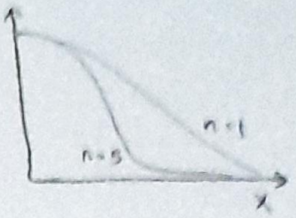
* Value of K is determined by binding affinity between X and the binding site

n : Hill's coefficient - gives a measure of the steepness of the curve

For very high n, the curve looks like a step function.

* Hill's function for Repressor

$$f(x^*) = \frac{\beta k^n}{k^n + x^{*n}}$$: Monotonically decreasing function.



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Discussion

" Mathematics is Biology's next microscope, only better;
Biology is Mathematic's next physics, only better."

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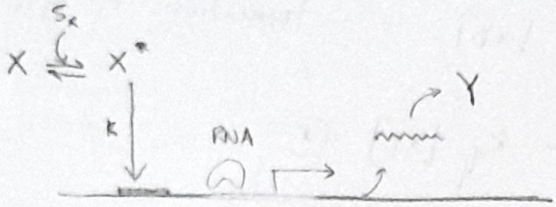
Lecture 09



$X \rightarrow Y$

In this process, we can model each step at a time -

01. $X \underset{S_x}{\rightleftharpoons} X^*$

The activator is switching back and forth between its active & inactive states.

happens in μs.

02. Binding of X^* to Promoter : This happens at rate of seconds.

03. Transcription & Translation : Minutes

04. Change in conc of protein : ~ 1 hours

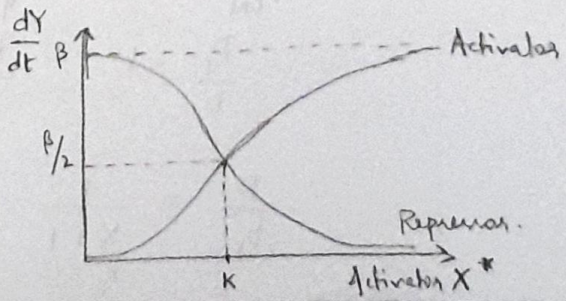
This is known as seperation of time scales.

This has to be factored in while calculating $\frac{d[Y]}{dt}$ as a function of X.

Recall : Hill's Function.

$$f(x^*) = \frac{\beta x^{*n}}{k^n + x^{*n}}$$ for activator

$$f(x^*) = \frac{\beta k^n}{k^n + x^{*n}}$$ for repressor.



To get around the separation of time scales

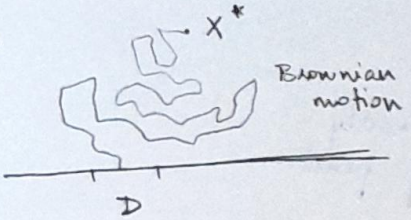
Case : Repressor binding to the promoter region

X : Repressor

D : Binding site of X^*

D is unbound by X^*

$P(X^* \text{ binds to } D)$: Probability that X^* binds to promoter.



$$\frac{dY}{dt} \propto P\left(\underset{D \text{ is unbound}}{X^* \text{ binds to } D}\right)$$

D_T : Total conc of binding sites D : Free site [XD] : Bound sites

$D_T = D + [XD]$: Conservation Eq^n $\therefore$ D_T is a constant.

Say there was one binding site per cell. Then,

$\mu m^3 = 10^{-15}$ L

$D_T = 1/\text{cell volume} \simeq \frac{1}{\mu m^3} \sim 1$ nM (?)

Looking at the collision rate of X^* and D -

$$X + D \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [XD]$$: Formation of Spittan

$$\frac{d[XD]}{dt} = k_{on} \cdot X \cdot D - k_{off}[XD] \quad (*)$$

k_{on} : $\frac{1}{\text{conc} \times \text{time}}$

k_{off} : $\frac{1}{\text{time}}$

} To maintain dimensions

* k_{ON} : No. of collisions of X & D that occur per unit time per unit conc of protein

It is entirely dependent on <u>diffusion</u> - how often X^* comes close to D.

* k_{off} : It depends on the <u>strength of interaction</u> b/w X and D.

Let's assume the above eq^n (*) has reached an equilibrium

$$\Rightarrow \frac{d[XD]}{dt} = 0 \quad \Rightarrow k_{on} \cdot X \cdot D = k_{off}[XD]$$

Using the conservation equation - $[XD] + D = D_T$

$$k_{ON} \cdot X \cdot D = k_{off}(D_T - D)$$

$$\frac{D_T}{D} - 1 = \frac{k_{on} \cdot X}{k_{off}}$$

$$\therefore \frac{D}{D_T} = \frac{1}{\frac{X}{k_d} + 1}$$

$$k_d = \frac{k_{off}}{k_{on}}$$: Dissociation constant.

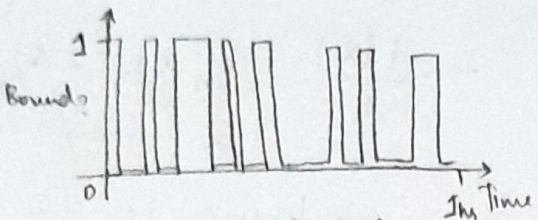
$\frac{D}{D_T} = \frac{1}{1 + X/K_d}$: Hill's function for $n = 1$ (↳ repressor with

$\frac{D}{D_T}$ = P(D is unbound)

We know that. $k_{off} \approx 1\ s^{-1}$

$Y \rightarrow 1$ hour

Consider a binary plot (0 & 1) of 'Bound?' v/s time ~ 1 hour,



$\frac{D}{D_T}$ averaged over multiple binding & unbinding events

Here, we can interpret the probability as the percentage of time D remains free

∴ We are averaging over a looong period of time

If the site is free, the rate of transcription i.e the number of mRNA ~~s~~ transcribed per second : β

i.e. Promoter activity = $\beta \cdot \left(\frac{D}{D_T}\right) = \frac{\beta}{1 + X/K_d}$

This gives us the no. of proteins produced per unit time
Also represents the Hill's function with $n = 1$.

To model things realistically : We could have a system where n transcription factors have to bind to D. (or) n binding sites on promoters

$\Rightarrow nX + D \rightleftharpoons [nXD] \Rightarrow \frac{d[nXD]}{dt} = k_{ON} \cdot DX^n - k_{off}[D_T - D]$

Here, $[nXD] + D = D_T$

We can derive $f(X)$ similar to D/D_T —

$\frac{D}{D_T} = \frac{X^n}{X^n + K^n}$ (activator) $\qquad \frac{D}{D_T} = \frac{K^n}{X^n + K^n}$ (repressor)

When we multiply by β, we get the rate of protein production i.e Hill's function, with $n > 1$.

Multidimensional Input functions & Logic Approximations.

Logic Approx is used to get some mathematical clarity and glean some important features of Tx network.
When applied to the whole system, its called Boolean Network.

Many proteins are regulated by their interaction with several Tx factors (inhibitory or excitatory).

Recall: $X + D \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} [XD] \Rightarrow \frac{d[XD]}{dt} = \underbrace{k_{ON} \cdot X \cdot D}_{\text{comes from Law of mass action}} - k_{off} \cdot [DX]$

We had assumed that [XD] was in equilibrium i.e. conc. of complex doesn't change over time

$\Rightarrow \frac{d[XD]}{dt} = 0 \Rightarrow X \cdot D = \frac{k_{off}}{k_{on}} \cdot [XD]$ where $k_d = \frac{k_{off}}{k_{ON}}$

From Law of conservation eqⁿ: $D_T = D + [XD]$

we get, $\frac{D}{D_T} = \frac{1}{1 + \frac{X}{k_d}}$

We can consider $\frac{D}{D_T}$ as P(D unbound)

If the site had to be unbound by n. inhibitors,

$\Rightarrow \frac{D}{D_T} = \frac{1}{1 + \frac{X^n}{k_d^n}}$ Hill's function

Multidimensional Input Functions

Consider a promoter which is bound/unboundy by Tx factors activator X and repressor Y.

There are 4 cases —

D : Unbound

D_Y : Bound only by Y

D_X : Bound only by X

D_{XY} : Bound by both X & Y.

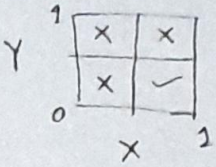
Consider a situation where transcription occurs only when promoter is bound by X i.e. D_X.

Here, $P(Y \text{ not bound}) = \dfrac{1}{1 + \frac{Y}{K_2}}$ K_2 dissociation const. of Y

$P(X \text{ bound}) = 1 - P(X \text{ not bound})$

$= \dfrac{X/K_1}{1 + X/K_1}$

Full repression by Y

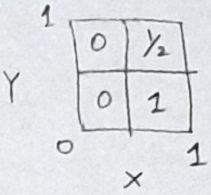


$P(D_X) = P(X \text{ bound } \underline{\text{AND}} \text{ Y not bound})$

$= P(X \text{ bound}) \cdot P(Y \text{ is not bound})$

$P(D_X) = \dfrac{\frac{X}{K_1}}{1 + \frac{X}{K_1} + \frac{Y}{K_2} + \frac{XY}{K_1 K_2}} = f(X,Y)$

Partial repression by Y

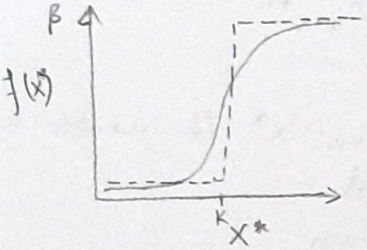


In most cases, we look at experimental data and try to fit the function as best as we can.

Logic Approximation.

Here, the Hill's function is reduced to an even simpler function – Boolean. i.e the gene is either on or off.

Consider: Hill's fn for $n = 4$



For an activator,

$$f(x^*) = \beta \cdot \theta(x^* > K)$$

θ indicates step function –

$\theta = 1$ if $x^* > K$

$\theta = 0$ if $x^* < K$

For an inhibitor, (repressor)

$$f(x^*) = \beta \cdot \theta(x^* < K).$$

Philip Kaufmann (1969) first used the Boolean functions to describe the evolution of Tx networks.

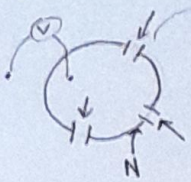
Consider: State of Tx network $[X_1, X_2, \dots X_N]$ in form of $[1, 0, 1 \dots 1, 1]$ etc

$\Rightarrow$ There are 2^N possible states.

Certain states are attractors i.e. when slightly disturbed, the system returns to these particular states.

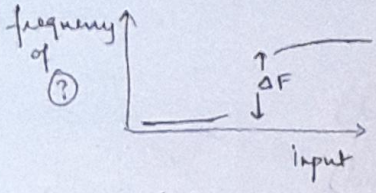
It also shows a varied dynamics eg. cycles.

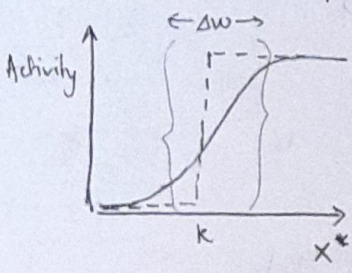
Discussion



The activity of ion channels, regulated by neurotransmitter is in the form of a Hills function.

If we put a voltmeter across the membrane and measure the voltage difference,





The logic approximation is valid when $x^* \gg K$ and $x^* \ll K$.

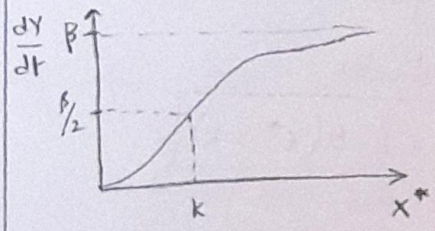
In the transition state, its not very close

The width of this regime, Δw is a function of n (Hills coefficient)

Lecture 11.

Dynamics of Simple Regulation.

$X \rightarrow Y$



The production of Protein Y is regulated by Tx factor X.

$$f(x^*) = \frac{\beta x^{*n}}{k^n + x^{*n}}$$

If the conc. of Y only depended on x^*, it would keep increasing. It has to be removed -

But it wouldn't! Only till β

- Degradation : Proteins are broken down

$\Rightarrow \alpha_{deg} \cdot Y$ where α_{deg} : Probability that it gets degraded

β: Rate of Production NOT [Y]

- Dilution : Due to increase in volume of cell, the conc. of protein decreases as -

$\Rightarrow \alpha_{dil} \cdot Y$

Considering this,

$$\frac{dY}{dt} = \frac{\beta x^{*n}}{k^n + x^{*n}} - (\alpha_{deg} + \alpha_{dil}) \cdot Y$$

when x^* is an activator.

While solving the equation, we assume that the system has been active for a while, i.e. $x^* \gg K$

$\Rightarrow \frac{\beta x^{*n}}{K^n + x^{*n}} \rightarrow \beta : \frac{conc}{t}$ Also, $\alpha_{deg} + \alpha_{dil} = \alpha : \frac{1}{t}$

$\Rightarrow \boxed{\frac{dY}{dt} = \beta - \alpha . Y}$

1. Steady state

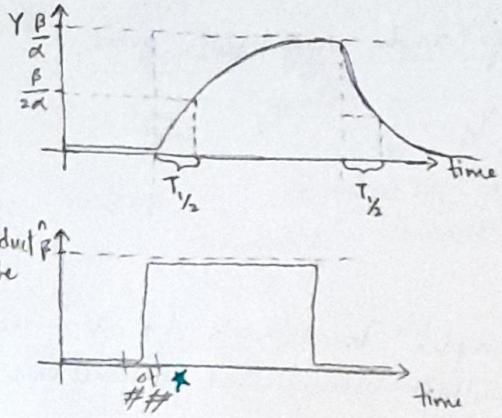
i.e. $\frac{dY}{dt} = 0 \Rightarrow Y_{st} = \frac{\beta}{\alpha}$

\# When x^* goes to 0, why doesn't $[Y]$ decrease linearly? $\because \frac{dY}{dt} = -\alpha Y$

$\because \int_{Y_0}^{Y(t)} \frac{1}{Y} . dY = \int_0^t -\alpha \, dt$

$\Rightarrow Y(t) = Y_0 e^{-\alpha t}$ *facepalm*

2. Trajectory



Consider a situation where the system is activated at some time, and the switched off after a long amount of time.

$\Rightarrow$ The conc of Y increases, reaches its steady state maximum i.e. β/α and when switched off, conc decreases. #

$T_{1/2}$: Time taken for conc of Y to go from 0 to $\frac{\beta}{2\alpha}$ or from $\frac{\beta}{\alpha} \rightarrow \frac{\beta}{2\alpha}$: Gives a measure of $\frac{dY}{dt}$

VERIFY

But how can it be same? It comes for first $T_{1/2}$, but only one for second one

Assume, $Y = Y_{st}$ and suddenly, $\beta = 0$. $T_{1/2} = ?$

$\Rightarrow Y(t=0) = Y_{st} = \beta/\alpha$

$\frac{dY}{dt} = 0 - \alpha Y \Rightarrow \therefore \boxed{Y(t) = \frac{\beta}{\alpha} e^{-\alpha t}}$

So, when $Y = \frac{\beta}{2\alpha}$ at $T_{1/2}$, $\frac{\beta}{2\alpha} = \frac{\beta}{\alpha} e^{-\alpha T_{1/2}}$

$\Rightarrow e^{\alpha T_{1/2}} = 2$

$\therefore \boxed{T_{1/2} = \frac{\ln 2}{\alpha}}$

** Wrt, rate of production of Y, we can consider that x^* saturates instantaneously i.e. $\Delta t \approx 0$

$\therefore T_{1/2}$ depends only on α!

Turns out, $T_{1/2}$ is order of magnitude of ~1 cell generation because we're only considering α_{dil} $\because \alpha_{deg}$ is too small since proteins are quite stable.

If $T_{1/2}$ is so large, then it takes so long for protein to be produced, that the daughter cells reap the benefit of its parent cells' response to change in stimulus.

So one way of decreasing $T_{1/2}$ is increasing α by increasing α_{deg}. BUT since $Y_{st} = \frac{\beta}{\alpha}$, increasing α would decrease Y_{st}. To maintain high Y_{st}, β should also be increased. *

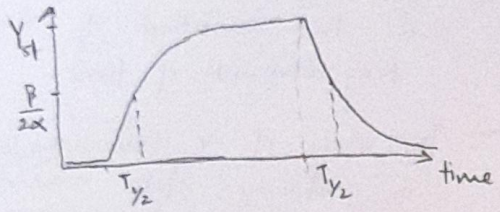
This leads to a cycle of high production rate and high rate of degradation, which is very wasteful.

5/11/20

Lecture

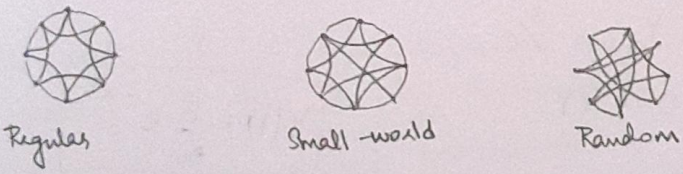
Recall: $X \rightarrow Y$ - Dynamics of simply regulated gene

$$Y_{st} = \frac{\beta}{\alpha} \qquad T_{1/2} = \frac{\log 2}{\alpha}$$



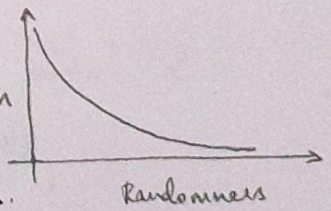
But we need to model a complex Tx network (E.coli ~ 424 nodes, 519 edges) which has multiple, complicated interactions.

Watts and Strogatz - 1998 described a set of networks called Small World networks - a kind of network that's in between Regular and Random networks.



Same no. of nodes and edges

$p = 0$ ——— Increasing Randomness ———→ $p = 1$



Surprisingly, its noticed that path length of what? decreases drastically as randomness increases.

* 6 degrees of seperation, Erdgich numbers.

Challenges to modelling a complex system -

- It has a huge dimensionality (no. of nodes & edges) → study network motif.
- Non-linear functions. → simplify function

To model such dynamics, we consider and study a part of the network and hope that it gives you a sufficient understanding.

We can do it by -

i) Simplify dynamics - Eg: Simplifying Hills function to Boolean.

ii) Identify special subnetworks i.e. network motif - patterns of connectivity that occur more often than chance.
⇒ They've been preserved through evolution

To identify these network motifs, several real networks were considered - predator-prey, internet, power grid etc.

The patterns in real network is statistically compared to Randomized Erdos-Renly network, and if some pattern occurs significantly more times, then they're considered motifs.

* same no of nodes & edges

So, instead of stimulating the whole network, these motifs are stimulated because they must serve a purpose

13/11

LECTURE 12

Negative Auto-regulation (NAR)

This is a phenomenon where the gene product represses the gene. Its represented by -

Recall: Ecoli has N = 424 E = 519

No. of self-regulating nodes: $N_{self} = 40$ → 34 NAR, 6 others.

In NAR, gene is regulated by its own protein product where it represses its own production.

Is NAR a network motif? i.e is $N_{self} = 40$ much more than chance?

In a randomized network, what's the probability that an edge connects to itself is -

$$P_{self} = \frac{1}{N}$$

What's the probability that K edges out E are self edges?

Similar to asking: probability of K heads when a coin is tossed E times - binomial distribution.

$$P(K) = \binom{E}{K} P_{self}^{K} (1 - P_{self})^{E-K}$$

Binomial distribution Expectation = n.p = E.1/N

Average no. of self edges for this distribution is = $E \cdot P_{self} = \frac{E}{N}$

⇒ For a random network with 424 nodes and 519 edges, the avg no. of self edges we'd get is –

$$\frac{E}{N} = \frac{519}{424} \approx 1.2$$

But no. of self edges in real network = 40

To know if this value is significantly higher, we need to know the std deviation –

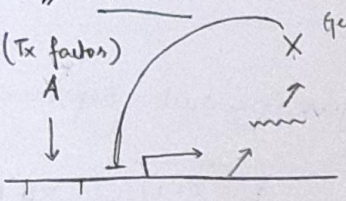
$$\sigma = \sqrt{\frac{E}{N}} \approx 1.1$$

To see how far away is the observed value from expected, we calculate the Z score –

$$Z = \frac{N_{self,real} - \langle N_{self,rand} \rangle}{\sigma_{rand}} = \frac{40 - 1.2}{1.1} \approx 32$$

∴ *The observed value is 32σ away from expected mean! *

2.32σ is in the 99th percentile.

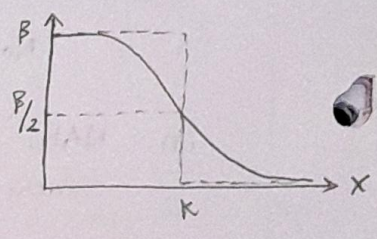


$$\frac{dx}{dt} = \underbrace{f(x)}_{\text{production}} - \underbrace{\alpha x}_{\text{degradation + dilution}}$$

For a repressor, $f(x) = \frac{\beta K^n}{x^n + K^n}$: Hill's fn.

Logic approximation, $f(x) = \beta$ for $x < K$

$= 0$ for $x > K$

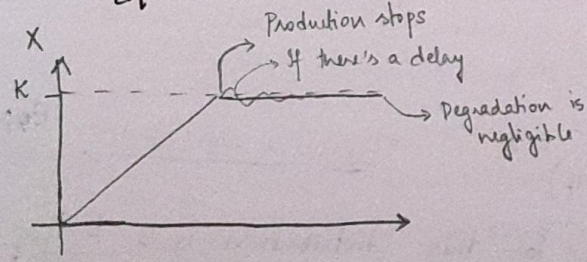


We assume that we're working in a system with $x \leq K$.

$$\Rightarrow \frac{dx}{dt} = \beta - \alpha x$$

In such a system if we assume α to be very small as well, the we can ignore the term 'αx'. $\because x \ll \frac{\beta}{\alpha}$

$$\Rightarrow \frac{dx}{dt} = \beta \Rightarrow \boxed{X(t) = \beta t}$$



$X_{st} = K$

$T_{1/2}$: time taken to reach $\frac{X_{st}}{2}$

$$\Rightarrow \frac{K}{2} = X_{st} = \beta T_{1/2}$$

$$\therefore \boxed{T_{1/2} = \frac{K}{2\beta}}$$

Lecture 13

13/11

Comparing simple regulation and NAR,

$X \to Y$ $\quad Y(t) = \frac{\beta}{\alpha} e^{-\alpha t}$ $\qquad$ $X \circlearrowright$ $\quad X(t) = \beta t \leftarrow \frac{dY}{dt} = \beta$

Steady state: $X_{st}^{S} = \frac{\beta_s}{\alpha_s}$ $\quad \frac{dY}{dt} = \beta - \alpha Y$ $\qquad$ $X_{st}^{NAR} = k$

$T_{1/2}$: $T_{1/2}^{S} = \frac{\log 2}{\alpha_s}$ $\qquad$ $T_{1/2}^{NAR} = \frac{k}{2\beta_{NAR}}$

★ To decrease $T_{1/2}^{S}$, i.e. make system faster we've to increase α_s i.e increase the rate of degradation
This happens because X_{st} also decreases. If we want to produce the same amount of protein, we'll have to increase rate of production ⇒ vicious cycle.

★ With NAR, to decrease $T_{1/2}^{NAR}$, we just have to increase β_{NAR} i.e the rate of production. This doesn't affect X_{st}^{NAR}

Assgn Compare $T_{1/2}^{S}$ and $T_{1/2}^{NAR}$ where $X_{st}^{S} = X_{st}^{NAR}$ $\quad \alpha_s \to 0$ in NAR

$X_{st}^{S} = X_{s}^{NAR} \Rightarrow \frac{\beta_s}{\alpha_s} = k$

Find $\frac{T_{1/2}^{NAR}}{T_{1/2}^{S}} = \frac{\frac{k}{2\beta_{NAR}}}{\frac{\log 2}{\alpha_s}} = \frac{\beta_s}{\alpha_s} \cdot \frac{1}{2\beta_{NAR}} \cdot \frac{\alpha_s}{\log 2} = \frac{\beta_s}{2\log 2 \cdot \beta_{NAR}}$ $\quad 2\log 2 \approx 1.4$ (take e.)

If $\beta_s = \beta_{NAR}$, $T_{1/2}^{NAR} = \frac{T_{1/2}^{S}}{1.4}$

19/11

Lecture 14 (Live)

Recall: While describing NAR, we said $\frac{dY}{dt} = \beta$, assuming that —

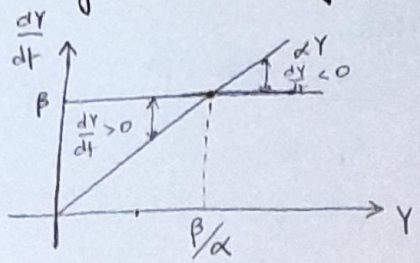
- Hill's function is a step-function, where $\beta = \beta$ if $X < K$
- We assumed that $\alpha Y \ll \beta \Rightarrow Y \ll \frac{\beta}{\alpha}$ so 'αY' is negligible

In Simple Regulation —

$\frac{dY}{dt} = f(X) - \alpha Y$ where $f(X) = \beta$ if X is sufficiently large i.e. $X \gg K$.

$\frac{dY}{dt} = \beta - \alpha Y$

Looking at this geometrically — Phase-Space Plot:



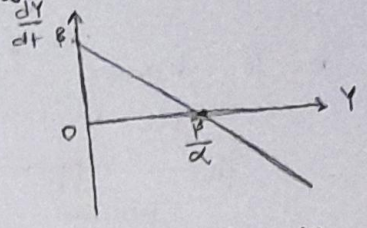
At the point of intersection,

$\beta = \alpha Y \Rightarrow \frac{dY}{dt} = \beta - \alpha Y = 0$

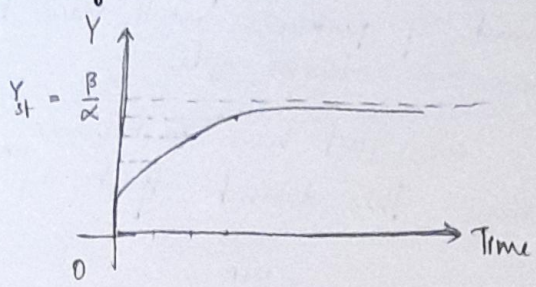
This is called a Stationary / steady state

This is a point of stable equilibrium i.e. if its disturbed (small change in initial condition) it returns to this stable point.

Components wise

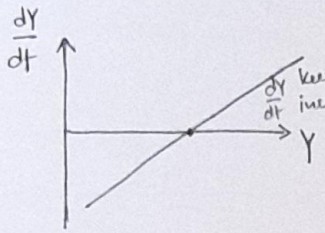


Plotting one of Y vs. time —



If $Y_{initial} < \frac{\beta}{\alpha}$, the conc. of Y increases until it reaches Y_s $\frac{\beta}{\alpha}$

Also as Y increases, the rate of production $\left(\frac{dY}{dt}\right)$ decreases.



Unstable steady state

⇒ System rolls away when perturbed.

From a geometric solution —

i) We can identify stationary states

ii) We can figure out if its a stable or unstable steady state.

(iii) It gives a qualitative picture of the time evolution of the system.

$\frac{dY}{dt} = \beta - \alpha Y$

$Y = Y_{st}\, e^{-\alpha t}$

$Y = Y_{st}\left(1 - e^{-\alpha t}\right)$

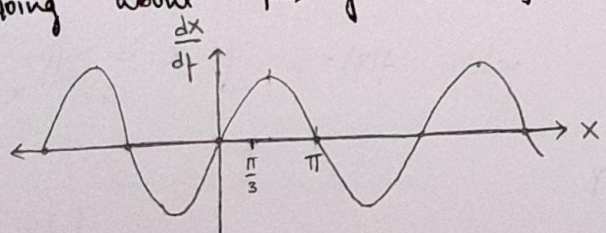
} Analytically

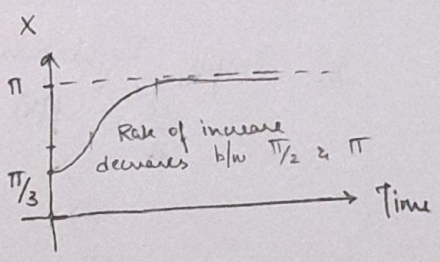
⇒ Example : $\frac{dx}{dt} = \sin x$: Non-linear, analytically solvable

$\int dt = \int dx \cdot \operatorname{Cosec} x \quad \Rightarrow \quad t = \ln \left| \frac{\operatorname{Cosec} x_0 + \cot x_0}{\operatorname{Cosec} x + \cot x} \right|$

Given $x_0 = \pi/3$, plot x vs time

Going about this geometrically —





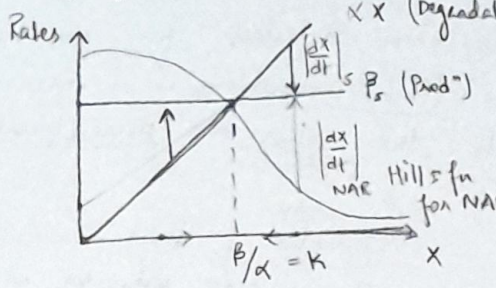
Lecture 14

Robustness

With the knowledge of previous lecture, we will try to prove –

1. $T_{1/2}^{NAR} < T_{1/2}^{S}$

Employing Geometric solutions –



* Simple reg: $\frac{dx}{dt} = \beta_s - \alpha x$

$x_{st} = \frac{\beta_s}{\alpha}$ where $\frac{dx}{dt} = 0$

This is a stable fixed point
System will asymptotically settle to $x \to \beta/\alpha$.

* NAR: $\frac{dx}{dt} = \frac{\beta_{NAR} K^n}{x^n + K^n} - \alpha_{NAR} \cdot x$

We want to make a mathematically 'controlled' comparison

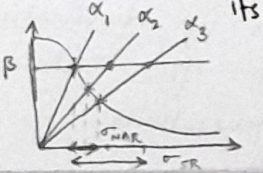
$\Rightarrow x_{st}^{S} = x_{st}^{NAR}$ $\quad x_{st}^{NAR} = K = \frac{\beta_s}{\alpha}$

- When both systems are at a point away from x_{st}, how fast they reach the stable state is a function of $\left|\frac{dx}{dt}\right|$
- In both cases, ($x < x_{st}$ & $x > x_{st}$), the $\left|\frac{dx}{dt}\right|$ for NAR is greater than that of SR $\Rightarrow$ it'll head to x_{st} with greater velocity $\Rightarrow \therefore T_{1/2}^{NAR} < T_{1/2}^{SR}$
- How do we know $\left|\frac{dx}{dt}\right|_{NAR}$ is always greater than $\left|\frac{dx}{dt}\right|_{SR}$?
  NAR is a monotonically decreasing function — Hence ↑ How (?)

2. NAR systems are robust to noise

To see this, we compare x_{st}. $\quad x_{st}^{SR} = \frac{\beta}{\alpha}$ $\quad x_{st}^{NAR} = K$

* From experiments, WKT, the value of β varies from cell-cell by about 10%, & it can last n generation time at any Tx fluctn
* In comparison, K_{NAR} is determined by the binding of (X) to its DNA site, which is the same across all cells.
* value of α can also vary with different cells



For a given set of αs, the steady states of SR have much greater deviation than x_{st} of NAR $\Rightarrow$ NAR is more robust.

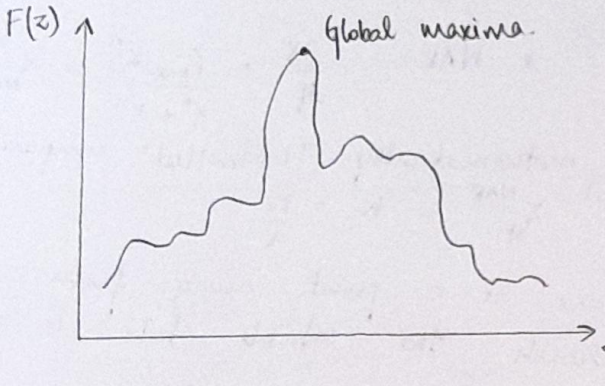
Optimality in design of gene circuits

Recall: NAR is a network motif that has been selected for. [320]

It provides certain advantages —

1. Speed up the production of certain proteins
2. Makes the system robust — insulates against noise.

When we claim that it has been selected for, we have to talk about its Fitness function — a measure of the probability with which the genes are passed onto the next generation.



Examples of trait —
No. of NAR networks in E.coli
Length of beak/plumage and lung capacity in birds.

Usually, some kind of proxy is chosen to get a measure of fitness.
For bacteria, its growth rate proxy

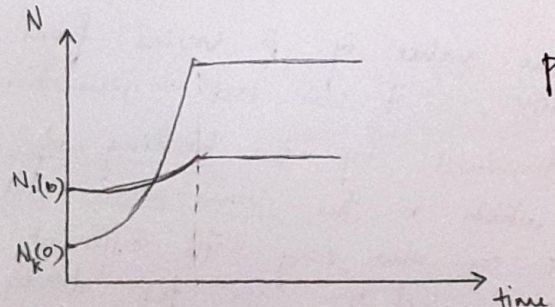
Consider 'k' different kinds of E.coli in a petri dish with growth rates subsequently increasing —

$$F_1 < F_2 < F_3 \dots < F_k$$

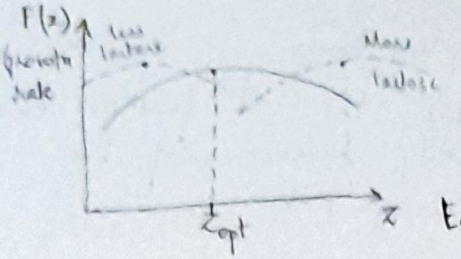
$$N_i(t) = N_i(t=0)\, e^{F_i t}$$

Formula that gives the number of certain kind of E.coli given its initial no. and growth function/rate.

It increases exponentially, but as the density in petridish increases, the number stops at some point.



Once they reach this stationary phase, the no. of i type of E.coli peters out.
Even with $N_k(t=0) < N_1(t=0)$, Since $F_k \gg F_1$, the 'k' kind of E.coli ends up dominating the the petri dish after certain no. of generations.



The trait could be the no. of proteins produced. At some value of the trait, the fitness function is most optimal.

Eg: In wild type of E.coli, 60,000 copies of LacZ protein are produced — presumably, the system has settled to this optimal value for a particular kind of environment, over an evolutionary scale

Recall Lac Operon - Pg. 9.

LacZ gene codes for β-galactosidase which directly contributes to growth rate by breaking up lactose

But there's a cost to making these proteins.

Fitness = Benefit - Cost

Lac Z = 60,000

We consider that fitness function is maximum at Lac Z = 60,000 i.e. its the optimum amount.

This value can change based on environmental conditions

Eg: If there was even more lactose, then the optimum or value of z also increases.

Dekel and Alon, 2005

This group conducted experiments to describe optimality in Lac Operon

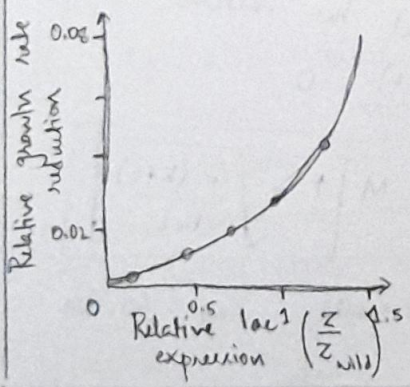
Cost term

If a protein is produced more than it's needed or when its not needed, this hampers the growth rate.

So if Lac-Z is produced when lactose is not present —

Using: IPTG - a gratuitous inducer

It mimics lactose and activates the operon, but it can't be used by the cell.



Relative growth rate reduction

$$= \frac{F(z_{wild}) - F(z)}{F(z_{wild})}$$

A function that fits this well is:

$$c(z) = \frac{c_0 z}{1 - \frac{z}{M}}$$

When $z \ll z_{wild}$,

$C(z) = C_0 z$ i.e. it's more or less linear

But after a point M ($\approx 2 z_{wild}$), the cost function blows up exponentially to infinity because of the cost incurred to produce so many proteins

→ Benefit function.

This is considered as the relative growth rate increase not relative lac expression.

This is proportional to how quickly lactose is broken down, which depends on no. of proteins & conc of lactose

$$B(z, L) = B_0 \frac{zL}{K + L}$$

L: conc of lactose

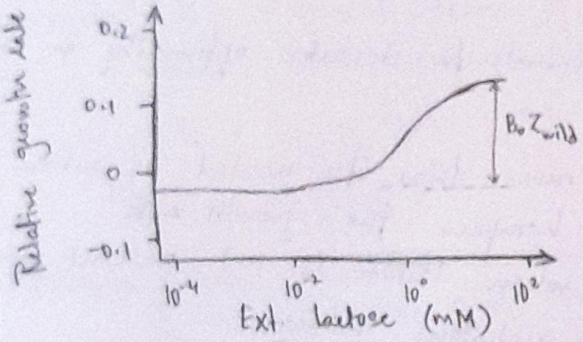
K: Michaelis menten constant

B_0: Proportionality constant.

B_0 is the maximum benefit value per each protein molecule, at saturated lactose conc

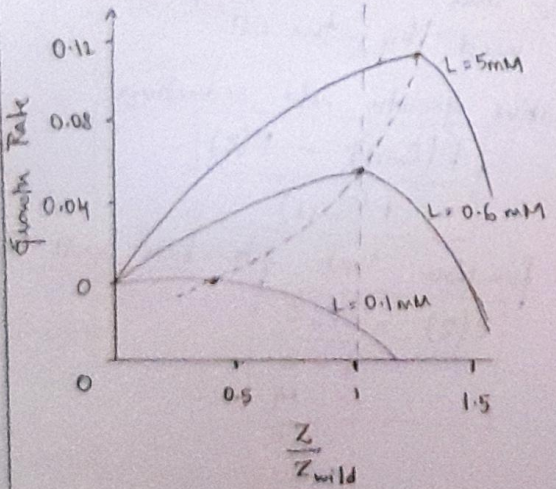
The benefit function is determined by activating the lac genes through IPTG, so that system is maximally induced

Then, the external lactose conc is gradually increased.



$$F(z, L) = B(z, L) - C(z)$$

$$F(z, L) = \frac{B_0 zL}{K + L} - \frac{C_0 z}{1 - \frac{z}{M}}$$



* As the conc of lactose increases, the optimal value of z also increases *

To find the maxima, i.e. optimal value,

$$\frac{dF(z, L)}{dz} = 0$$

$$z_{opt} = M \left[1 - \sqrt{\frac{C_0 (K + L)}{B_0 L}} \right]$$

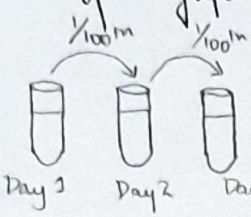
At $L = 0.6$ mM, $z_{opt} = 60,000$

Lecture 16

14/12

The shift of Z_{opt} was observed through serial dilution.

The system is maximally induced through IPTG, and a certain amount / conc of lactose is added in a medium of glycerine which is the backup carbon source.

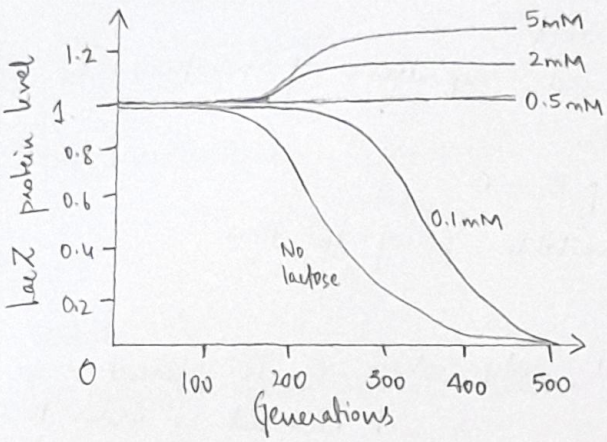


After 1 day, 1/100th of the solution is added to a testtube with same lactose conc. level

⇒ Everyday, the bacteria grow 100-fold ≃ 6.6 generations

This is carried on for several months, with different set-ups for different conc of lactose (0 to 4 mM), and everyday, the amount of protein (Z) is measured.

When the conc of protein lacZ is plotted v/s generations —

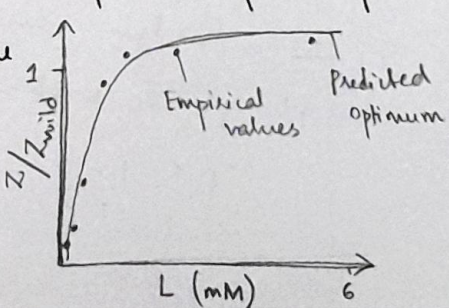


* for about 100 generations, there's no change in amt of lacZ produced i.e its ~60,000
* If there's no lactose in the system, then the amt of lacZ produced falls rapidly i.e. the bacteria that take over the test tube are ones that don't produce lacZ.

* Similarly, other lines can also be explained as the conc. of lactose increases.
* Remarkably, over ~100 generations, the bacteria have heritably changed their expression of lacZ protein

The Optimality theory i.e the fitness formula predicts a certain optimal amount of Z that is expected from the system. The final values match the predicted optimum very well.

This proves that bacteria indeed evolve to the optimum fitness value over several hundred generations.

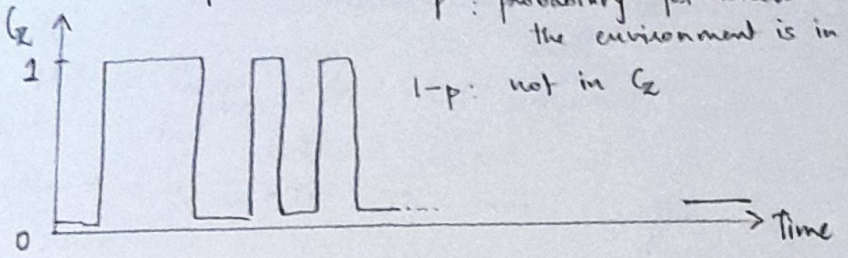


Evolution is constantly reshaping the expression of
* all the proteins as a function of the environment.
A function that is n-dimensional

But, in the experiments, the environment was kept constant to minute details. Consider that a gene product that is beneficial in an environment C_z.

P : probability for which the environment is in C_z

1-p : not in C_z



Eg. C_z : presence of lactose
$C_z = 0$ if $L = 0$
$C_z = 1$ if $L = 0.6$ mM

If C_z is changing over time, then when should the cell express the gene? To explore the strategy employed, consider 3 cases –

1. z is constitutively expressed
i.e. z is always expressed, regardless of whether C_z is present or not.

Fitness function : $F_1 = p \cdot B - C$

The benefit is only available 'p' of the time.

2. z is regulated
Protein z is produced only when C_z is present –

$$F_2 = p \cdot B - \underbrace{p \cdot C - r}_{\text{cost}}$$

r : cost incurred in order to regulate

3. z is turned off
It has no benefit and no cost. (?) Isn't there a cost to not producing z at C_z

$F_3 = 0$.

When will Organism (2) take over?

$F_2 > F_1$ and $F_2 > F_3$

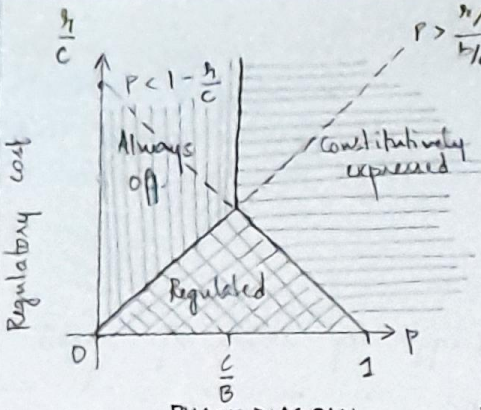
$pB - pC - r > pB - C$

$\Rightarrow p < 1 - \frac{r}{C}$

$pB - pC - r > 0$

$\Rightarrow p > \frac{r}{B-C}$

X axis - Demand for Z
Y axis - Relative cost



PHASE DIAGRAM

$\frac{h/c}{\frac{B}{c}-1} < P < 1 - \frac{h}{c}$

When the system is in the bound/shaded region, its beneficial for z to be regulated

Outside, its not regulated

Its constitutively expressed when -

$F_1 > F_2$

$P > 1 - \frac{h}{c}$

$F_1 > F_3$

$P > \frac{c}{B}$

The bacteria that live inside aphids live in a near-constant environment — such organisms lose virtually all their regulatory systems. Out of their 600 genes, either all are expressed or not expressed. (?)

Why even have it?

* The methodology developed over the course actually applies to a wide variety of systems & scenarios.

17/12/20

Lecture 17

Development and Pattern Regulation

↳ Emergence of form and organised structure from an initial single fertilised egg

Factors involved in development of multicellular organism -

1. Gene regulation
   Genes control development by regulating when, where & how much of proteins are synthesized in a cell

2. Networks
   Gene activity establishes intracellular networks of interaction b/w genes & proteins (TF network), and among proteins. This gives cells their properties.

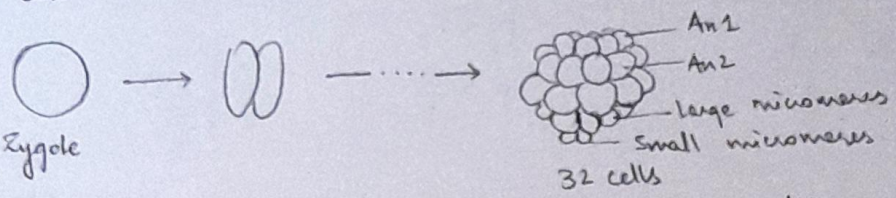
3. Cell-cell interaction
   Key cellular property is to communicate and coordinate activities with other cells.

Key processes — Cleavage division
Pattern formation
Morphogenesis
Cell differentiation
Growth

These processes overlap & influence each other
Don't necessarily occur in this order

→ Cleavage divisions

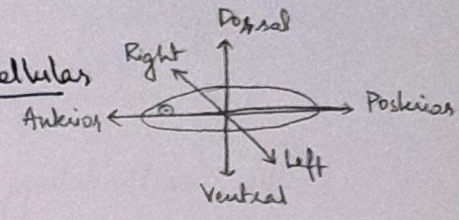
Development starts with a trigger of series of rapid cell divisions, where there's no increase in cell mass



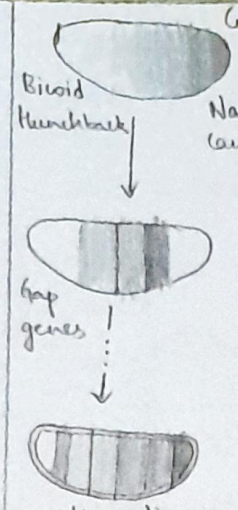
There cells in 16-32 cell stage may have differing gene expression

⇒ Pattern formation

- Organizing spatial & temporal pattern of cellular activities that allow a well ordered structure to develop.



- Anterior-posterior axis : Rostrocaudal axis
  Dorsal - ventral axis
- The developing individual can be superimposed on these orthogonal axes so that each coordinate point is coded to express certain genes so that a very well-organized yet heterogenous structure can be formed
- Early patterning helps establish the overall body plan
  Basic patter of body plan and form of embryo is laid down on small scale
- Pattern formation and cell differentiation are closely connected — the former instructing the cells to have appropriately varying gene expression patterns & forming an embryo with a pattern specific to that organism



Maternal Effect genes → Gap genes → Homeotic genes

* Drosophila embryo is not homogenous
It has different kinds of proteins enriched at opposite ends
⇒ There's a gradient achieved by anchoring the RNA of these proteins to one pole

* This gradient sets up the next level of gene expression pattern where certain genes (Gap genes) are highly expressed in certain regions of the embryo
i.e. Groups of cells in a segmented form express Gap genes.

* Through further processes, the embryo is further segmented into ~~and~~ expression patterns of Homeotic or Hox genes, which specify the fate of each of these segments
* Hox genes influence at the ~~anterior~~ cells at the anterior to change their gene expression pattern and interactions so that it develops into a head.
Hox genes = master regulators
* This pattern is also observed in mammals. Distinctly at the stage of development of mid brain, hindbrain & spinal cord. The relevant genes (orthologs) are seen in the same order in mouse as seen in drosophila.

∴ Pattern formation drives cells in certain segments to express Hox genes which modify gene expression to produce characteristics specific to that segment.

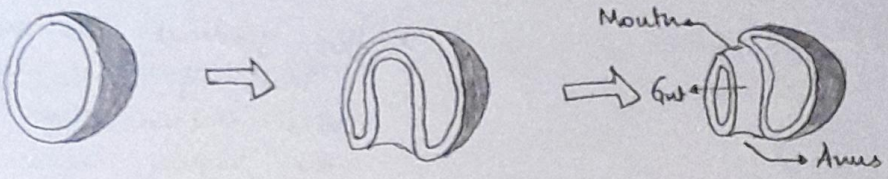
Eg:- When Hox gene of leg-forming segment is artificially expressed in the head region, the head grows a pair of legs

- Bithorax mutant : One segment of thorax produces wings & the one below that produces halteres. If we mutate the Hox gene specifying halteres, then we get a drosophila with two pairs of wings

→ Morphogenesis

Changes in three-dimensional form – origins of shape and structure

Dramatic example in early stages of development – Gastrulation



★ After several rounds of cleavage, the embryo becomes a hollow sphere of cells. During gastrulation, one part of it invaginates to meet the other and form a tube that opens to the surface. It later forms the digestive tract.

★ This also causes a physical rearrangement – bringing cells closer that were far apart before. This also rearranges the mechanism of communication and form distinct layers – endoderm, mesoderm & ectoderm

★ These germ layers go on to form various tissue of the body –

Ectoderm – epidermis, nervous system

Endoderm – gut & derivatives like liver & lungs

Mesoderm – skeleto-muscular system, internal organs – heart & kidney

★ Gene expression pattern is relatively homogenous before invagination, which physically rearranges & brings cells closer leading to signalling process which bring about different cell expression patterns in cells, hence forming the layers.

★ Other example: cells that give rise to facial structures have migrate there from other parts of embryo

In response to signalling

→ Cell differentiation

Structural and functional changes in cells due to changes in gene expression patterns resulting in distinct cell types.

Patterning lays out what each cell in the coordinate space of body axes should differentiate into – groups of cells start specialising & collectively the embryo changes shape & cells form tissue identity

Example: Differentiation of blood cells from stem cells.
Depending on the signals it receives, stem cell changes gene expression to go into different lineages of specialisation, which after several steps gives rise to blood cell

→ Growth

Basic pattern of body plan and form of embryo is laid down very early on a small scale. So, the rest of the development just involves growth — increase in cell size, cell number, extracellular depositions etc which eventually increase the volume

Growth is not uniform, its allometric — head of a baby is much larger in ratio than an adult's head

Gene expression → Cell behaviours → Developmental processes

- Past & current patterns of gene activity in a cell define its identity, which is reflected in its molecular organisation, and in turn, in its behavior & fate.
- Changing pattern of gene activity, driven ~~for~~ by pattern formation, is essential for —
  Cell identity → determine future behavior → leads to final differentiation
- Cells influence each other, so embryo develops in coordinated way
- Cell movement generates physical forces that bring about morphogenesis
- Development is progressive (occurs sequentially) & fates of cells are determined in a temporal sequence.

Embryo contains a **generative** rather than a **descriptive** programme

Set of instructions, which when executed sequentially results in the final form

Essentially a detailed blueprint of the final structure of organism

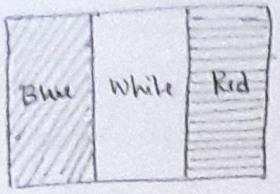
Analogy: Origami — instructions to make a swan don't include descriptions of final structure.

∴ Genome encodes a series of progressive instructions (generative programme) to build the organism

Pattern formation

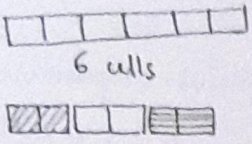
Allows a group of relatively homogenous cells to organize and specialize in a coordinated manner to build an organized structure of an animal

French flag analogy



1/3 space for each color along 1 axis
Can come in many sizes but same pattern

* Consider a row of 6 cells, each has the potential to develop as Red, blue or white



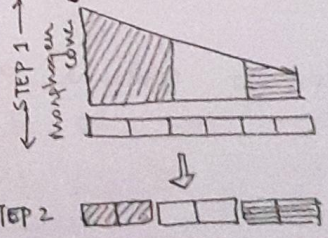
* Solution is for each cell to acquire a positional info followed by its interpretation by differentiating according to their genetic programme.

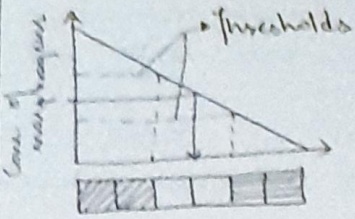
Two distinct stages –

i) Acquiring positional value specified w.r.t. some boundary

ii) Interpretation

* Interpretation depends on particular genetic instructions active in the group of cells
* As its positional value & interpretation are independent, in different circumstances, same set of positional values can be used to generate a different pattern (Irish flag)
* Simplest position specification: gradient of a substance conc. of chemicals in any cell along the line specifies the position of cell w.r.t. the boundary
* Morphogen: chemical showing concentration gradient or variation involved in patterning.
  - Things that end up giving structure.





Position of each cell is defined by conc. of morphogen

If positional value is interpreted in a thresholded manner, then we get a pattern

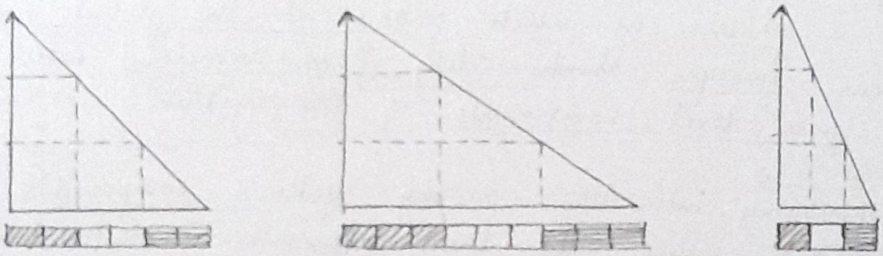
i.e. Above a particular conc, cells turn blue. Below this, they turn white & even longer lower threshold conc. turns them red

Thresholds could work in following way —

- Amount of morphogen that must bind to receptors to activate a signal transduction system
- Conc of Tx factors required to activate certain genes.

The system is "regulative"

- Even if length of line varies, system regulates and generates the pattern correctly as long as boundary conditions are correctly defined, keeping the conc. constant at each end
- System could regenerate original pattern if cut in half, provided boundary conditions are reestablished.



Re-establishing boundary conditions.

This sort of system is what is seen in embryo of drosophila. — Pg. 45

There are two gradients, based on which, cells interpret this function, creating a sharp segmentation of embryo

→ This called a pre-pattern

Patterns in biology can emerge spontaneously by self-organization without a pre-pattern — Example —

- Dictostelium — myxomycete — social amoeba

When starved, these single cell organisms (that live in soil) produce waves of cAMP and attract neighbors along

those waves, creating an aggregation, forming mounds, then slug and a structure with base, stalk and fruiting body which produces spores.

These are self organised spiral waves. ~ 33:20 mins that arise spontaneously and organise individual dictostelium into a multicellular structure.

- Beating of a heart
  The contraction of atria & ventricles are correlated (?)

- Knee-jerk reflex network
  A neural network that causes the leg to jerk when the knee is given a sharp tap.
  The sensor neurons take the info to spinal cord, some computation happens & two motor neurons simultaneously cause the quadriceps to contract and the hamstring to relax

The direction of information in neurons (as an electrical pulse) is unidirectional. This is an example of pattern formation when it could be bidirectional

Self-organisation
  Its a process in which pattern at the global level of system emerges ~~slowly~~ solely from numerous interactions among the lower level components of the system

Rules specifying interactions among system's components are executed using only local information, without reference to the global pattern.

How do spontaneous organisation & patterns emerge from a disordered system?

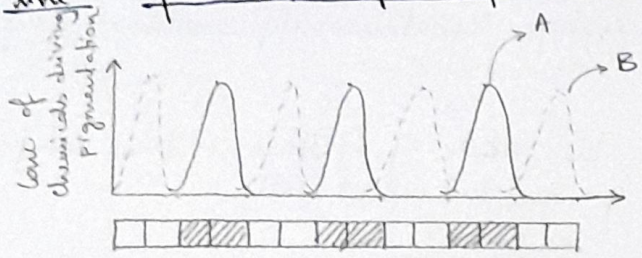
Belnosov-Zhabotinsky reactions (~1950s)
  Its a homogenous mixture of chemicals that changes colors from purple to blue to back again over certain time
  i.e the chemical reactions are oscillating
  This cannot be explained by equilibrium because they are non-equilibrium reactions.

When these chemicals are taken in a dish, we observe that patterns start to emerge spontaneously.
A sheet of cells can be modelled based on this

The reactions are autocatalising which oscillate b/w oxidative and reductive reactions. There is a difference in rate of diffusion of products formed

Many patterns in biology - parking of cells, tiger stripes, cheetah's spots, butterfly wings, human hand etc. - arise spontaneously.

To model this sheet of cells showing pattern, we can consider a grid of cells (like sudoku) or even simply, a line of cells expressing a periodic pattern.



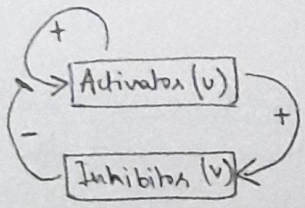
If we can manage to achieve this spatial diffusion & conc of chemicals, then we could develop such patterns.

But how?

Turing's Reaction Diffusion System
Paper: Chemical basis of Morphogenesis (1952)

Two morphogens ~~can~~ that can react with each other and diffuse at varying rates -

- If diffusion rates are not equal, diffusion can be destabalizing - the reaction rates at any given point may not be able to adjust quickly enough to reach equilibrium
- If conditions are right, a small ~~spatial~~ spatial disturbance can become unstable & a pattern begins to grow

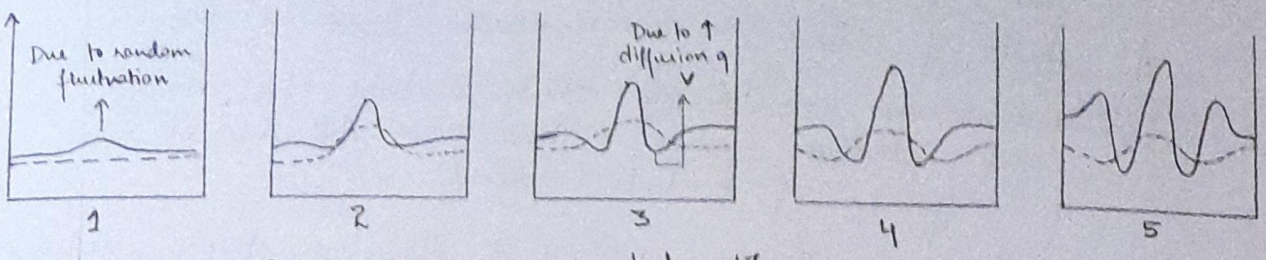


Kondo & Miura 2010

$Diff_v \gg Diff_u$

Consider these two morphogens - u and v, given the conditions.

U —— V -----



Refer 1:11:40 in Lecture 17

Turing pattern: a kind of non-linear standing wave pattern maintained by dynamic equilibrium of the system

The wavelength is determined by ⊙ molecular interactions & ⊙ diffusion rates

Such patterns can emerge spontaneously, without any pre-pattern.

For a sheet of cells, the scale of pattern is large compared with diameter of an individual cell.

By making the system more complex, increasing no. of morphogens, their interaction etc. we can create an infinite set of patterns.

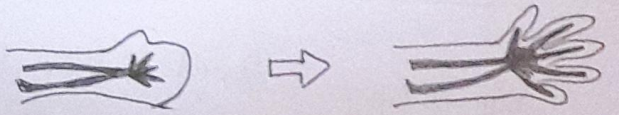
Simulations have achieved similar patterns, that of real biology.

Again, this is also regulatory — the pattern is retained with scaling of size

The wavelength changes if we modify —

- reaction speed
- diffusion rates
- tissue length scale

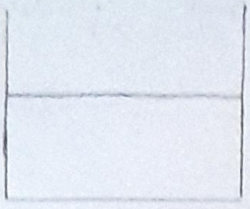
Development of digits

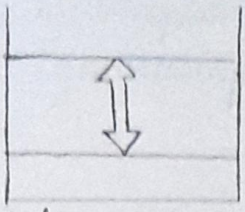


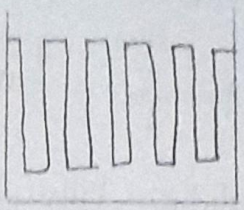
In the process of development, a flat paw forms a hand This is done by retaining the tissue around the digits but killing off the tissue in between

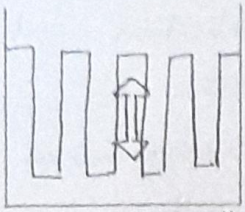
If we know the morphogens that generate digits, then by tweaking their interactions, the wavelength observed in the tissues would change & we would expect to see different no. of digits.
This was observed dramatically in mice – Sheth et al 2012

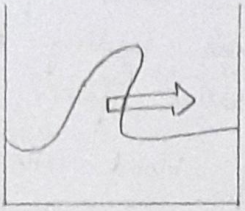
Turing's differential equation can result in Six stable states.

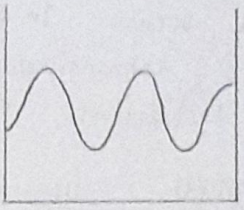

Uniform, stationary (No pattern)


Uniform, oscillating
Eg: Beating heart


Eg: One cell inhibiting neighbours (?)
Stationary waves with very short wavelength


Oscillatory cares with very short wavelength

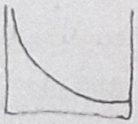

Oscillatory cares with finite λ
Eg: spirals in Dictyostelium aggregation

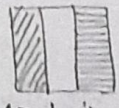

Stationary waves with finite wavelength
Eg: Turing pattern

Summary.

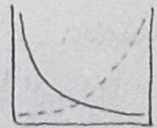
A, B: Prepattern dependent

A: 1 morphogen :


Gradient


1D horizontal

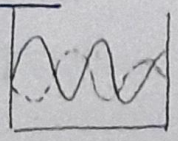
B: 2 morphogens :


Gradient


2D pattern

C: Prepattern independent-

Morphogen Interaction :




Turing pattern

"reaction" – acts as local source of morphogen

Stem cells are not only important for development but also for the maintainance of adult tissue i.e. tissue homeostasis and replacement; and in therapeutic approaches.

Stem cells are unspecialised cells that can give rise to other specialised cells types to carry out particular functions in the body.

Fertilised egg is the ultimate stem cell. Gives rise to an initial group of unspecialised cells that specialise through cell differentiation

Cellular Differentiation

- Process of generating specialised cells from an initial population of equivalent, unspecialised cells.
- It occurs through a series of progressive changes in gene expression patterns leading to specialisation and restriction of potential fates.
- Example : Generation of blood cells from unspecialised precursor known as hematopoietic stem cell.
  Upon receiving different signals, it becomes a specialised cell & its restricted to that - can't turn back.
  The specialised WBCs, RBCs form progressively
- Differentiation occurs progressively over suessive cell generations with organised change in gene expression patterns (new features are acquired and potential fates restricted)
- Differentiated cells serve very specialized funtions and usually reach a terminally differentiated state and rarely divide furthur
- Even in initial stages of division, the gene expression patterns are changing in the cells (though they look similar), so they're on different differentiation lineages.
- Cell fate map of C. elegans : Males - 1031 cells
  Hermaphrodite - 959 cells.

- In C. elegans, except for the germline, other cells don't get replaced. But in most other organisms, stem cells remain throughout their lifespan and replace the tissue

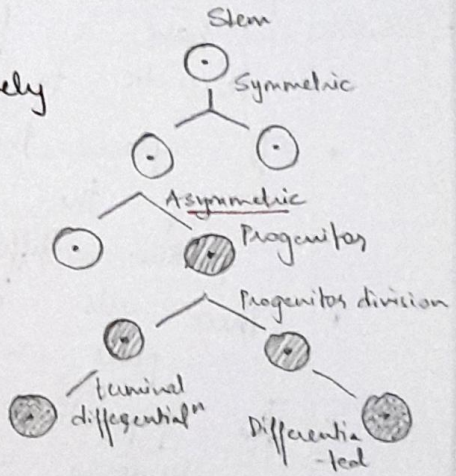
- Avg adult human : ~ 37.2 billion cells
  2^n mitotic divisions would be required to form N cells
  n : no. of divisions
  44 - 45 mitotic events result in this (~37 billion) no. of cells
  Yet 10^{16-17} mitotic events occur in a lifetime.
  This suggests there's a massive cellular turnover & cell death.
  # Even bones undergo 10% turnover.

Defn #2
Stem cells are unspecialised cells that can potentially reproduce themselves (self-renewal) & generate more-specialised cells (potency) indefinitely.

Stem cells are able to —

* Self-renewal — reproduce themselves indefinitely by symmetric division

* divide asymmetrically to produce one identical daughter cell and one that is different and usually of more restricted potential



Types of stem cells —

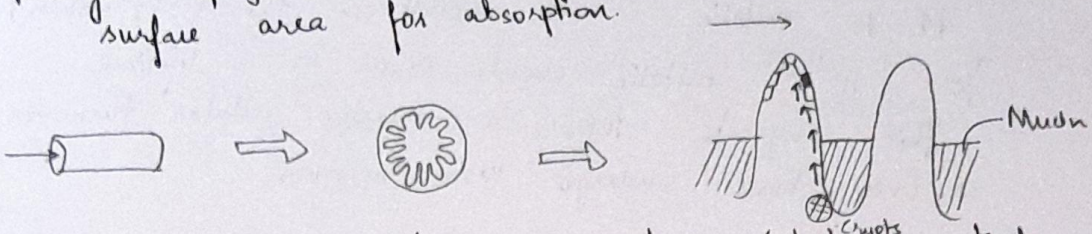
1. Totipotent : can differentiate into all embryonic & extra-embryonic cell types
   Eg: cells produced by first few divisions of fertilised egg
   Plant cells
2. Pluripotent : slightly more restricted — they can differentiate into cells derived from any of the 3 germ layers
   Eg: inner cell mass of developing embryos
3. Multipotent : produce only cells of a closely related family of cells
   Eg: Hemopoietic cells
4. Unipotent : produce only one cell type. They have the property of self renewal Eg: Muscle stem cells.

Stem cells are important for growth, homeostasis and repair of many tissues.

⇒ Example – Tissue maintenance.

1. Lumen of the intestine

* The lining of the gut is specialised to absorb nutrients through fingerlike projections called 'villi' that increase the surface area for absorption.



* Partially digested food contains many toxic substances that the villi are exposed to. The cells may become cancerous due to these oncogenic substance.
* To control this, the cells of the intestinal epithelium ~~are~~ have a finite lifespan of 4-7 days. They're programmed to die to prevent the accumulation of mutation.
* To replace these cells, the stem cells present at the base of the villi (crypts) undergo asymmetrical division to produce differentiated cells → Specialised cells that ultimately give rise to differentiated ones
* These cells migrate along the ~~the~~ epidermis to reach the place where the cell has died.
* The population of stem cell is maintained because they undergo asymmetrical division. They're protected from the toxic substances by extracellular secretion – mucin – which covers and protects these cells. (sugar based)

2. Epidermis

The outermost layer of cells in epidermis constantly die and are shed off. They are replaced by skin cells produced from a group of stem cells in the dermis.

3. Haematopoesis

Haematopoietic stem cells produce different kinds of blood cells.

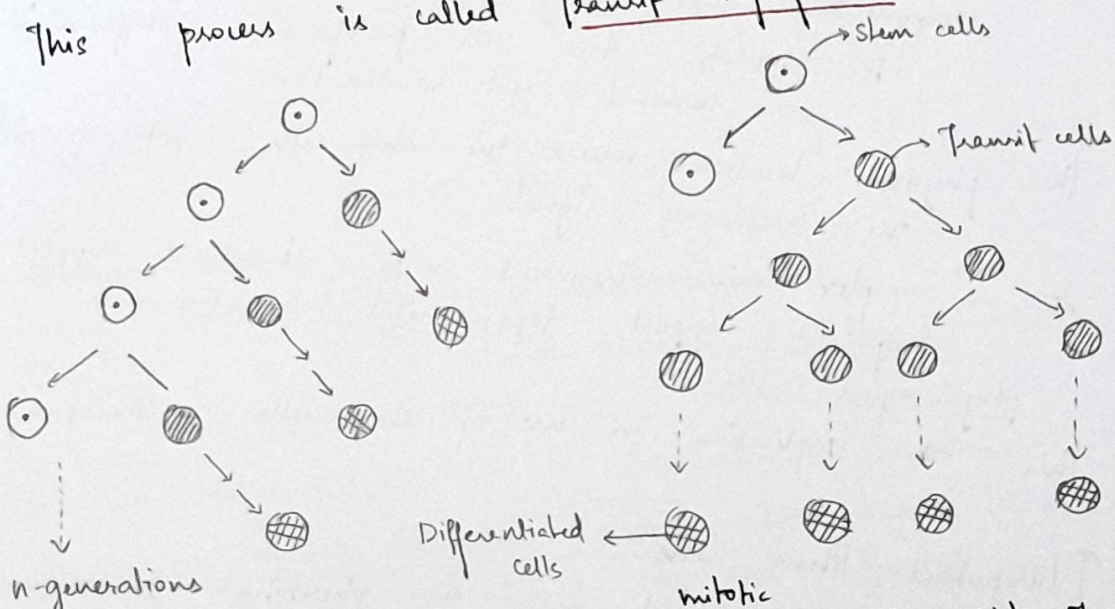
(57)

Stem cells are risky —

- Indefinite mitotic potential
  This is necessary to be able to replenish themselves throughout the lifetime of an organism. WKT mutations commonly occurs during cell division.
  Except for stem cells and germline cells, others have a definite potential to contain the possibility of cancer causing mutations.
- can easily be nudged to form aberrant cell types in the wrong place.

Thus, the stem have a risk of tumerogenesis.

Thus, stem cells have adapted mechanisms to control this risk.
We assume that to replace lost tissue, stem cells have to divide frequently. But in reality, stem cells themselves divide only a few times. Instead, the progenitors (transit) cells undergo bulk of the division (& they have finite potential) and undergo differentiation to replace the lost tissue
⇒ Stem cells divide rarely / infrequently.
This process is called Transit amplification.

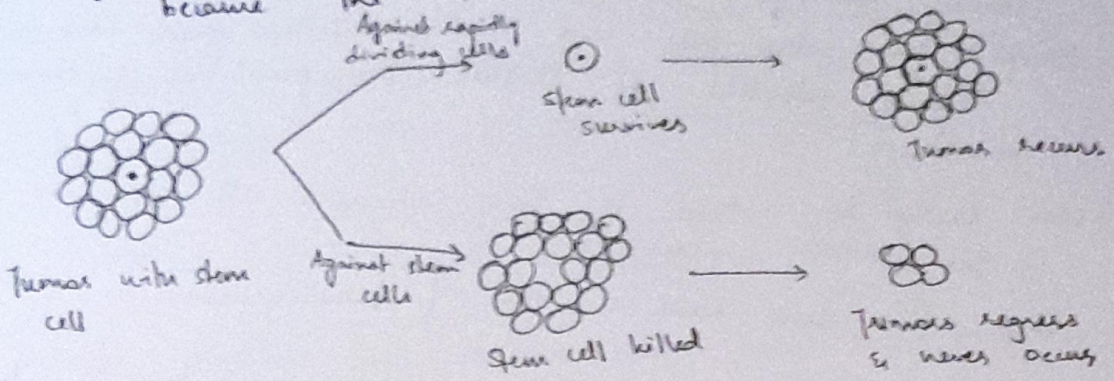


Since transit cells have a finite mitotic potential, the risk of tumerogenesis is greatly reduced

Transit amplification: Process where the bulk of the division & amplification occurs through transit cells, thus reducing the no. of times the stem cells have to divide

Cancer stem cells – therapeutic implication

It has been noted that many tumors are actually caused by aberrant stem cells. So therapies directed against rapidly developing dividing cells don't work because the stem cell survives



Stem cell Niche

The maintenance and survival of stem cells is regulated by inputs from their local microenvironment, referred to as stem cell niche

Niche – An anatomical structure, including cellular and acellular components, that integrates local and systemic factors to regulate stem cell proliferation, differentiation, survival and localization.

The physical location where the stem cells reside provides the necessary signals.

Its a complex microenvironment with structural support, trophic support, topographical information and physiological cues.

This has implication in use of stem cells in therapies.

Pluripotent Stem Cells

Cells of Inner cell mass are pluripotent. In mammals, they are 100-150 cells found inside the blastula (hollow spherical stage in embryonic development).

They can be coaxed to form any of the cell found in the organism.

Embryonic stem cells —
→ are pluripotent
→ are derived from inner cell mass of blastula
→ can be maintained in culture
→ can integrate into developing blastula
↳ Experiment : • ES cells from pigmented strain were introduced into the blastula of an albino mice
• They incorporated with the inner cell mass of the host
• This blastocyst developed in a foster mother into a healthy chimeric mouse – with patches of pigmented / marked cells in each tissue

This method is used to derive stem cells and make transgenic animals.

⇒ Applications – in regenerative medicine

1. Transplantational biology – heart, cornea, bone marrow
2. Cell replacement therapy – diabetes (β cell of pancreas), neurodegenerative pathologies
3. Cancer treatment – cancer stem cells
4. Reversal of ageing – aging related disorders

⇒ Sources of stem cells
- Surplus embryos in IVF clinics
- Aborted embryos
- Embryos created exclusively to derive stem cells

There are a lot of ethical concerns regarding where we get them from.

So, can differentiated cells be reprogrammed to stem cell fate?

→ Somatic cell nuclear transplant (SCNT) – John Gurdon (1958)
* Gurdon took an unfertilised egg and destroyed its nucleus by UV light – so its enucleated
* He grew a culture of skin cells in a petridish. He isolated their nucleus & injected this diploid nucleus (from a terminally differentiated cell) into the enucleated egg.
* This embryo developed and formed a tadpole. This showed that genome of a terminally differentiated cell could be

reprogrammed (by factors in the cytoplasm) to become totipotent

Similarly, Wilmut / Campbell (1997) managed to clone a mammal through a similar process - Dolly the Sheep.

Dolly didn't have facial pigmentation, even though the cytoplasmic donor & surrogate did have them.

This is because the nucleus donor, from whose mammary cells the nucleus was taken, had no facial pigmentation.

→ Transdifferentiation of adult stem cell

Multipotent stem cells isolated from adults can be induced to change lineage by changing its niche

Haematopoietic stem cells can form bone, muscles etc

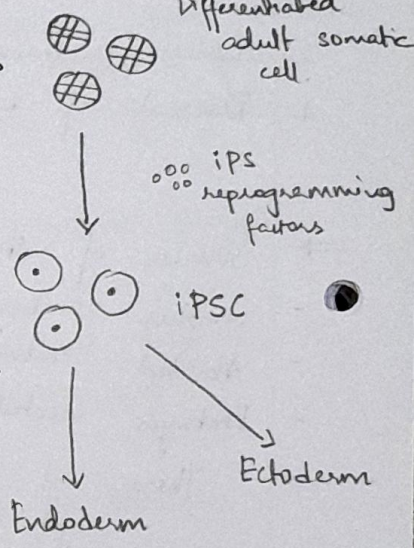
⇒ Gene expressions are plastic and can be reprogrammed to produce different kinds of cell

Induce switching of lineage - transdifferentiation.

→ Induced Pluripotent stem cells (iPSC) - Yamanaka (2006)

Here, adult differentiated cells were made to artificially express 4 Tx factors known as Yamanaka factors so that the entire gene expression pattern now changed to that of stem cells.

When given the right kind of chemical cues, these stem cells could enter differential lineages and form all kinds of cells.



29/12/20

Lecture 19

Logic of the immune System

Discuss the organisation and integration at the level of physiology

"Immunis" - Latin : exempt, keep out

Immunology - mechanisms used by the body as protection against infections.

Infection: invasion of an organisms body tissue by disease causing agents, their multiplication, depletion of resources, and reaction of host tissues to the infectious agents and toxins they produce

Unusual combination of features —

- Not anatomically well defined or circumscribed by some organs
- Is resting in the ideal state i.e needs to be activated
- Following activation, it undergoes maturation — takes some time to respond
- DNA needs to be actively rearranged during the system's development and maturation

Necessity —

- Immune system turns out to be a necessary precondition of multicellularity in metazoans, because true multicellularity necessitates • keeping the component cells integrated, and • division of labours i.e specialisation
- But when an organism specializes too much, many groups of cells can't remain free-living, and require support.
- To keep these cells working efficiently, one needs to provide controlled internal environment rich in nutrients
- This attracts freeloaders (other organisms, rogue cells) which want the nutrients but don't contribute to the body's working
- So, there's a need to generate mechanisms that defends the body against infections.

multicellularity dictates

Example — Guinea worm disease

- Guinea worm is a parasitic worm. Its life cycle is completed in water
- It infects the person by entering through abrasions when the person touches contaminated water.
- It reproduces and grows inside the body upto 1m. It punctures the skin and comes out of the host body to complete its life cycle.

- It has managed to evade the immune system for so long because they're slow growing, cause relatively less damage to the tissue and they're not as aggressively infectious as other pathogens.
- So the immune system actually fights an 'infection' and not a "foreign substance."

As the infection can enter from anywhere, the immune system has to be everywhere too
⇒ not a well circumscribed system

And its only needed when an infection occurs – so its not a continuous function i.e inducible
⇒ its at rest when there's no infection.

How to deal with infections?

Detection and Action

★ Simplest – immune cell with a special receptor, which upon activation (triggers) results in necessary action

★ There are a huge variety of infectious agents, so one tag won't work for all pathogens
⇒ There exist an array of receptors to detect many pathogens

★ Similarly, since there are a wide range of evasion strategies, just one response will fail. So there needs to be diversity in responses too.

★ There could be pathogens with same tag but different evasion strategies – So detection & response has to be decoupled

A – The detection & action should be decoupled This allows you to select from a repertoire of detection strategies and couple them with the appropriate response

How to generate an array of receptors?

1. Clonal uniformity (innate immunity)
In the first kind of receptors, all cells in a population are copies of each other
- They all bear the same receptors.

- They recognise molecules that are identified as belonging to 'expected' pathogens
Macrophages bear such receptors
- They have limited and fixed range, and recognise a few conserved, frequently occurring legends
- They must have come about through evolutionary history. coded in genes Its 'innate' immunity.
- Eg: Ability to detect lipopolysaccharides (found in cell wall of bacteria and fungi) would be very useful & evolutionarily preserved

Bal & Roth 1997

Immunity given by cloned array of receptors is called innate immunity.

- Problems – * stop making as change tag sufficiently to avoid recognition – false negative
potentially * removes commensal bacteria on our skin & gut – false positive
- Works best: in relatively constant environment (less diversity of pathogens) like sedentary organisms
hard shell
restricted range of potential invaders

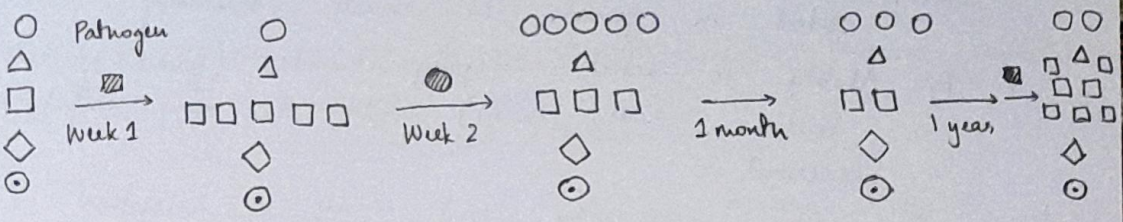
2. Clonal diversity (adaptive immunity)
All cells descend from parent cell but express unique receptors.
- bear unique receptors, recognizing an extremely large diversity of unique molecules shapes
- only a small subset of these cells will be triggered upon engaging with specific target.
- Therefore – has to proliferate so that there's enough to deal with the infection ⇒ resting system that's activated
- But they take time to mature and mount a strong response
⇒ Price of having a diversity of receptors is the time delay in having an effective response

Challenges: • How to generate this large repertoire of receptors?
We don't have enough genes

- Also not enough cells – only few cells will express a particular receptor. Not enough to control the infection

As only a few are triggered, there's a need to proliferate so that there's enough to deal with the infection.

Unique receptors –



Receptors

Following an infection, the activated cell type is now slightly expand and more sensitive (for sometime after infection is gone) – immune memory / immunity

If it encounters the pathogen again, it will be able to suppress it much faster and more efficiently, because these cells are now more sensitive

Features – of adaptive immunity –

- triggers only a small subset of immune cells
- will require amplification i.e cell division
- effector cells need activation & then maturation
- this allows for memory.

Parasite Niche

The next challenge faced by detection & action strategies ~~are~~ is where the pathogens are located –

→ Extracellular niche
Inside and outside blood vessels, between cells
Eg: Pneumococcus – pneumonia

→ Intracellular niche – Vesicular or endosomal
Eg: Mycobacterium – TB, Salmonella – typhoid

Cytoplasmic
Eg: Viruses, shigella – dysentary.

If pathogen is engulfed by phagocytosis, its enveloped in a vesicle / endosome which fuses with a lysosome (contains enzymes to digest) and it is normally destroyed.

(38 - 30) But pathogens have evolved ways of avoiding this fate - they "tickle" (molecular scratch) a nearby cell, so they get engulfed but prevent the lysosome from forming, thus escaping the immune cells.

Different responses -

Extracellular niche

Free molecules help tag extracellular invaders to be eaten by macrophages or neutrophils. It may also directly destroy them.

Vesicular (Intracellular) niche

Helper cells, when they detect an infected cell, they signal it to increase its activity and kill intracellular parasites lurking in vesicles.

Cytoplasmic niche

Killer cells signal cells infected with intracellular parasites in their cytoplasm to undergo cell death.

Site of action

The immune system has to be present everywhere because pathogens are present everywhere.

Immune cells are present in the tissue or interstitial fluid (that bathes all the cells of the body) which drains into the lymphatic system or lymph nodes, which is connected to the circulatory system, so the fluid is continually recycled.

Travels of an immune cell

WBCs are formed in bone marrow - mature (thymus for T-cell) - move to lymph nodes or circulating between blood vessels, tissue fluids and back.

Dealing with pathogens in the extracellular milieu -

Innate immunity

- Recognition by dendritic cells like macrophages
- Circulating free molecules (complement system, creative proteins)
  They recognise molecular features of common pathogens, bind to them and kill them in a variety of ways (eg by making pores in the membrane of the pathogen)
- Strategy: common molecular shapes found in pathogens are recognised.

PAMPs: Pathogen associated molecular patterns

Adaptive immunity
It detects and acts against pathogens through circulating free molecules called Antibodies - produced by B cells

PAMPs are small molecular motifs consistently found on pathogens, so through evolutionary history, all metazoans have evolved the ability to recognise them by
Pattern Recognition Receptors (PRR)

Consequence of PAMP: PRR interaction -
- Elicit other components of immune system by -
  activation of immune cells
  activation of the cell's own degradation machinery

- Toll-like receptors (TLR) - major PRR family. They can recognise many features commonly present in pathogens - lipoproteins, flagellin, DNA, ssRNA, dsRNA etc
- They're found on on surface of cells and within endosomes of cells
- They work in combinations

→ Phagocytosis - engulfing the pathogen, inv forming endosomes and the lysosome (containing hydrolytic enzymes) is fused with this to form a phagosome & digest the pathogen - its remains are thrown out of the cell.

(67)

Complement mediated lysis

~ 20 interacting soluble proteins, which can detect expected pathogen motifs and form protein aggregates which destroys the pathogen by * lysis, * tagging it so its easily detectably by phagocytes and * triggering other components of the immune system

* by pore formation

C-reactive protein: Recognises pathogen motifs, binds and recruits the complement system

Its significantly upregulated when there's an infection, so diagnostic tests check for CRP levels.

Circulating antibodies

They are produced by B cells. They're free floating molecules and they're like receptors. Its protective mechanisms -

1. Agglutination - clumps the pathogens, increasing phagocytosis & reduces the no. of infectious units to be dealt with
2. Activation of complement leading to lysis
3. Opsonisation - coating pathogen with antibodies enhances phagocytosis
4. Neutralisation - blocks bacteria and virus from entering the cells & blocks the active site of toxins
5. Inflammation - disruption of cell by complement/CRP attracts phagocytic and other defensive immune cells
6. Antibody dependent cell-mediated cytotoxicity.

Dealing with pathogens in intracellular niche.

Adaptive immunity - recognition & action by T cells

Innate immunity - natural killer (NK) cells.

Action of T-cells

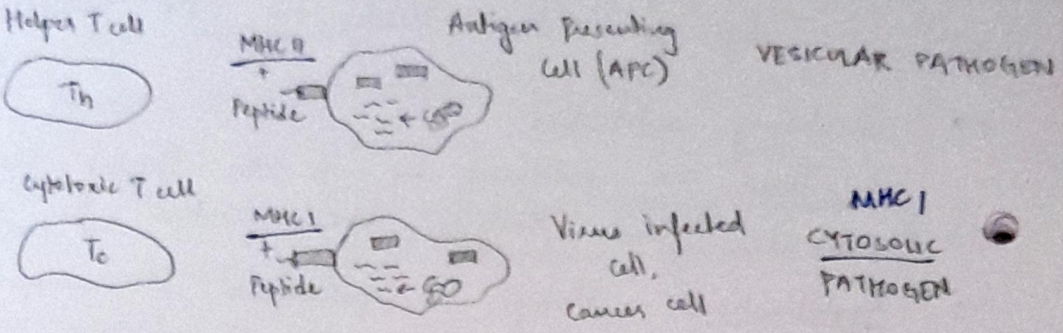
They must recognize infected cells and if pathogen is in -

- Vesicles - send signals to infected cell & activate them increasing their ability to fuse lysosomes (HELPER T-CELLS)

- Cytoplasm - instruct the infected cell to die
  KILLER T CELLS

When pathogens are inside the cell, these T cells must recognise infected cells as targets and not free molecules

T-cells can recognise antigens associated with certain molecules (MHC) which are expressed on surface of cells
i.e it has to detect PAMP together with cellular markers to know that a cell has been infected



- Proteins in the cell are constantly recycled, and a part of the cycle is that a cellular complex MHC presents a piece of the protein (a peptide) on the cell's membrane
- When Helper t-cell recognises a pathogen peptide in conjuction with MHC II, it signals the cell to increase its lysosomic activity → Resides in vesicles
- When cytotoxic T-cell recognise an pathogen peptide, it encourages MHC 1 presenting a the cell to die
  Present in cytoplasm.

(~ 1:05:00)

Innate immunity also responds to intracellular pathogens. One way is through -

Natural killer (NK) cells
They recognise molecular patterns common to infected cells
(∵ they have lesser diversity so its not feasible to recognise pathogens) and induce death
One way it detects a sick cell - reduced levels of certain proteins on their cell surface - NK cells recognise these

Lecture 20

2/1/2024

Components of Innate immunity

1. Anatomic – skin as a mechanical barrier
   mucus trapping of microbes
   ciliar propulsion of epithelia
2. Physiological – low pH
   secretion of lysozyme, antibiotic peptides
   producing interferons & complent system
3. Inflammatory – vasodilation
   increased vascular permeability
   kinins, histamine
   coagulation factors
4. Phagocytic – phagocytes engulf and destroy pathogens
   NK cells kill virus-infected or tumor cells

Interferons

They are signalling molecule released by virus infected cells

It binds to the receptors of neighbouring cells –

- promotes macrophage function & apoptosis of infected cells
- triggers synthesis of enzymes
  - destroying viral RNA or DNA
  - to inhibit viral proteins

The inflammatory response –

Could be local or systemic ie fever

Rubor – Redness – due to increased blood flow

Calor – Heat – due to increased blood flow & metabolic activity

Tumor – Swelling – increase in fluid loss from capillaries to interstitial space – because capillaries become more permeable due to histamine & other chemicals.

→ by sending a signal to hypothalamus to increase body T

- Dolor – Pain – stimulation of pain receptors from compression of interstitial fluid, chemical irritation by kinins, prostaglandins, microbe substances
  It serves as an indicator & brings attention to the infected area
- Loss of local function – usually temporary.

Good inflammatory response is acute - local, non-specific response, typically lasting 8-10 days

Sometimes it persists systemically as chronic inflammation.

Process

- Tissue damage causes mast cells to release chemotactic and vasoactive factors - triggering increase in local blood flow and capillary permeability. This provides a suitable gradient for immune cells to migrate
- This allows an influx of fluid and cells (vasodilation and capillary permeability)
- Phagocytes migrate to the site of inflammation thru chemotaxis, which destroys the bacteria.

Immediate, local, non-specific response -

Step 1: secreting factors like histamine, leukotrienes, prostaglandins and chemotactic factors.

Step 2: Vasodilation & increased capillary permeability

Step 3: Phagocytic cells are recruited from circulatory system through chemotaxis to remove pathogens

Innate immunity	Adaptive immunity
• Recognize a class of molecules typical of commonly encountered pathogens (clonal uniformity)	• High degree of specificity (clonal diversity)
• Less specific and acts as the first line of defense	• Once activated, gives a strong and ~~fast~~ response long-lasting
• Since they're encoded in the genes, they're ~~triggered only after~~ present before the onset of infection	• Triggered only after antigen challenge but retains immunological memory.
• Perfect self/non-self discrimination	• Imperfect discrimination
• All metazoans	• Only vertebrates (gnathostomata onwards)
• Constitutive level of response	• Inducible response

Innate and adaptive immunity are a continuum.

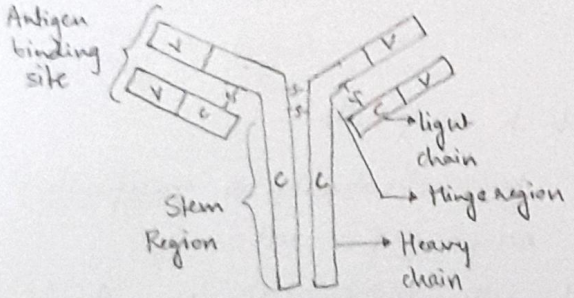
T-lymphocytes detect peptides / part of pathogen through antigen presenting cells, whereas B lymphocytes have receptors on their surface to detect pathogens directly. B-cells proliferate and release their receptors as soluble factors known as antibodies which neutralise / eliminate the pathogen.

The ability of Adaptive immunity to recognise a large no of pathogens and mount an attack is because of large repertoire of clonally diverse receptors on T and B cells.

Components of adaptive immune response -
- Cell mediated immunity - T cells
- Humoural immunity - Antibodies (Ab) produced by B-cells

Antigenic determinants (Epitopes)
Antigen - antibody generators - any substance capable of eliciting an adaptive immune response.
Antibodies recognize and react with epitopes

Antibody structure.



V-region - between heavy & light chain dimer - antigen binding site
Its Variable.

C-region - Constant.

Ig G - Immunoglobulin G

Also, IgA. IgE. IgG. IgM (dimer) (pentamer)

This feature of antibodies - variability in Antigen binding site - allows them to bind to a wide region of antigens / epitopes.

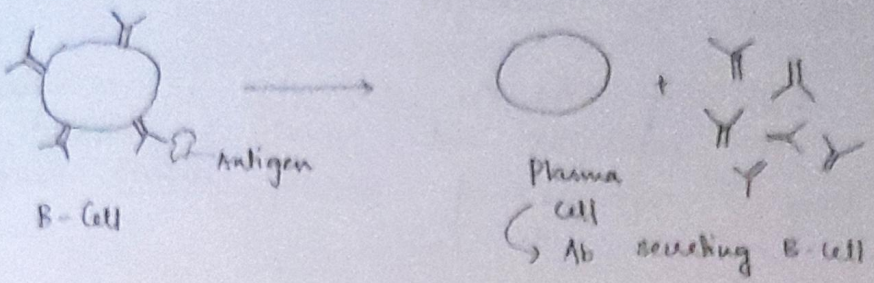
How is such a wide variety of V-regions coded from the limited no. of genes we have.

Function of Antibodies - Refer Pg. 67.

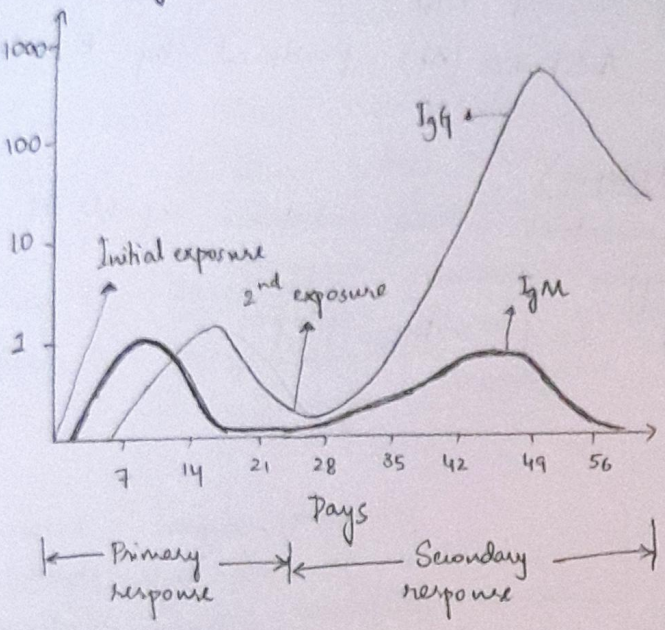
Formation of antibodies

Ab start off as surface bound receptors of B-cells

When a B-cell recognizes its antigen, it produces more and more of the receptors and releases them into the blood stream to circulate as soluble antibodies.



Antibody titer



Upon initial exposure, it takes time for B-cells to mature and mount an attack.

But once that's managed, any subsequent attack is dealt with much more strongly and effectively because the no. of mature B-cells doesn't go down to 0.

Immune system should be able to deal with -

Recognize trillions of possible Ag, individually & specifically

Use only one kind of receptor per cell

To have sufficient no. of cells specific for the Ag in question to mount an effective response.

Ultimate problem : There's not enough space

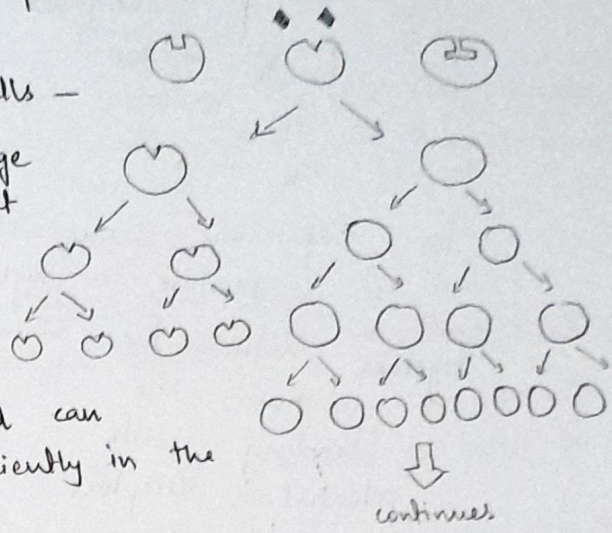
Clonal selection

Immune system has an extremely low frequency of cells specific for each of trillions of possible specificities

Refer Pg. 64 for diagram

* There cells continuously recirculate – ie if a cell doesn't "meet" its correct antigen within 2-3 days, it dies.
* When a cell encounters its Ag, it replicates extensively – turning 5-10 cells into millions of progeny.
* When the infection is dealt with, the no. of cells go down – but not as low as its initial level

When stimulated with a certain antigen, the B-cell replicates extensively to produce its clones to deal with the infection



It divides into 2 classes of cells –

- Plasma cells: produced in very large no. they are clonal cells that produce a lot of antibodies and release them
- Memory cells: produced in smaller amount, they remain behind in the body (unlike plasma cell) and can be activated quickly and efficiently in the second exposure.

B-lymphocyte activation requires –

* Given Ag find the B-cell expressing a specific receptors
* This cell multiplies to provide enough cells to be effective
* An 'off' signal once the Ag is removed to limit pathology

Generating adaptive diversity of Abs for a large no. of possible antigens is done by shuffling immunoglobu gene segments.

WKT IgG has heavy chain and light chains

→ Mouse heavy chain gene expression.

theavy and light chains are formed by subgenes of Ig gene

V, D & J subgenes form the variable region whereas C subgenes make up the constant region.

Kinds of subgenes –

$L_H - V_H \sim 300$ $\quad J_H \sim 4$ $\quad V_H d_H j_H = V_H$

$D_H \sim 10-50$ $\quad C_K = 8$

During the process of maturation of B cell, only 1 V (out of 300), 1 D (out of 20) and 1 J (of 4) are randomly selected and rest others are removed

After splicing, the V, D, J subgenes are combined with one of C_κ thus forming a heavy chain.

→ Now, light chain gene expression
there too similar to heavy chain there are many kinds of subgenes –

L_κ - V_κ ~ 300
J_κ ~ 5
C_κ ~ 1

$V_\kappa J_\kappa = V_\kappa$

for expression, one of each is randomly chosen to produce a light chain.

Together with the variations in light and heavy chain, they give rise to a large no. of diverse antigen binding site – makes up a large repertoire of potential receptors

But there exist a larger no. of receptors due to –
- "Errors" in recombination at V-D-J / V-J junctions - random mutations in genetic code.
- Affinity for antigen can be increased during maturation by process like "~~hypermutation~~" "Somatic hypermutation".
- mutation occurs at a much higher rate in the variable region so that slightly different variations of receptors are produced ⇒ increased affinity.

Affinity maturation – this process selects for better-binding receptors / antibodies.

Similar mechanisms help in creating a diverse array of receptors on T-cells too.

Self - Non self recognition

The price for producing a large repertoire of receptors is the risk of the receptor binding to a self molecule and initiating autoimmune responses.

Eg: Rheumatoid arthritis, Multiple sclerosis & so on

Self - Non-self discrimination is very important for components of Adaptive immunity. Some methods -

* If something is ubiquitous and not associated with functional hallmarks of an invader, its likely to be self.
* Ubiquitous targets are likely to be encountered almost immediately after B & T cells are born. They can be trained to recognise ubiquitous targets and not attack them. If any cells recognise 'self' antigens and attack them, then those cells are removed.

Eg: T cells mature in the Thymus. Immature T cells keep sampling the epitopes on Antigen Presenting Cells. If they respond to 'self' antigens, they're removed.

This method is not very efficient.

* So, another method is to make sure that its not associated with functional hallmarks of invaders (context) to determine self-antigen

Co-stimulatory information is required for B and T cells to mount a response.

When this system fails it results in autoimmune disorders.

Acquired immunity

	Active	Passive
Natural	Results from initial infection	Transplacental or via colostrum
Artificial	Injection of Ag (vaccine) System retains memory of Ag	Injection of antibody Eg: Plasma therapy

Lecture 21

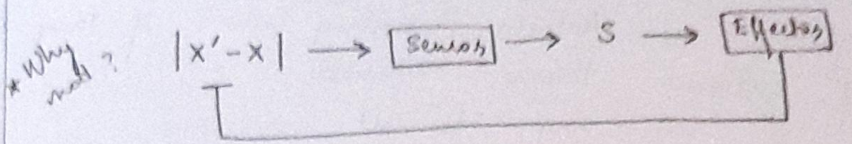
Design Principles of Immune System Response & Homeostasis

- Physiological processes operate only under a narrow range of conditions (temp, pH, salinity etc)

For the functional stability of the system, homeostatic processes actively maintain these conditions against internal and external perturbations

Homeostasis is a critical feature of life

Homeostatic circuit.



x : Homeostatic variable x' : set point value for x

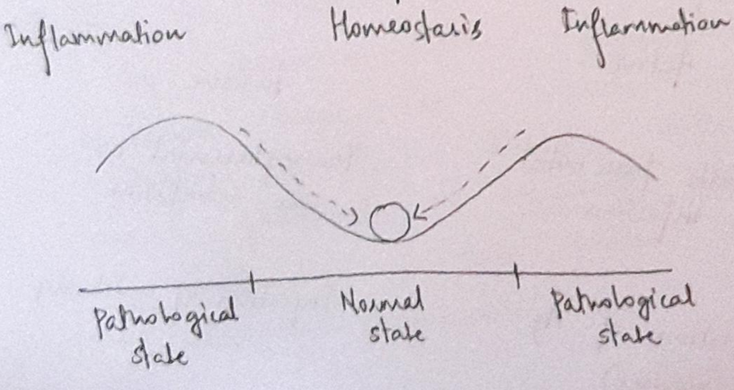
Sensor : Monitors x and compares it with x'

S - signal generated by the sensor that changes effector activity

Effector : changes value of x and gets it to approach x'.

Eg : Similar to a thermostat.

Maintaining blood glucose levels (~ 5 m mol) - antagonistic action of insulin & glucagon.



Inflammation is induced when perturbations exceed the homeostatic capacity of the system.

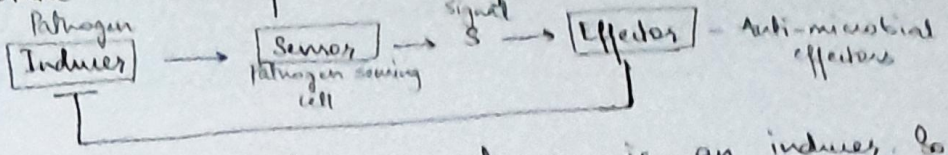
To restore homeostasis, we need an acute response – inflammation.

Infections cause a major perturbation

Pathogen → Structural feature recognition —Defense response→ Inflammation

Pathogen ↓ Loss of Homeostasis —Stress response→ Inflammation

Homeostatic circuit of immune system -



For the immune system, a pathogen is an inducer. So it has evolved to detect pathogens and generate a protective response to remove the infection.

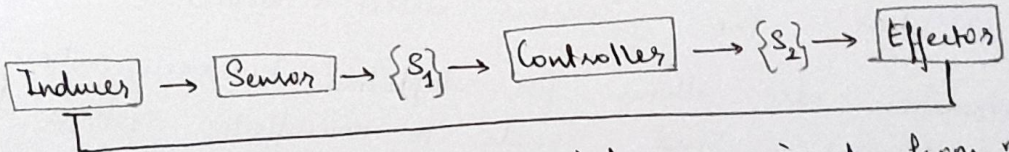
Sensor cells can release X signals that can -

- Chemokines : recruit effector cells to the infection site
- Cytokines : activate effector cells to generate a defense response.

Macrophages and Mast cells detect pathogens and release chemokines and cytokines. They are increase local circulation and help in vasodilation, which helps the effector cells. (n 13:10)

Inducers	Sensors	Mediators	Effectors
Pathogens	Macrophages	TNF, IL-1, IL-6	Liver
Tissue damage	Mast cells	CCL2, CXCL8	Immune cells
Loss of homeostasis	Sensory neurons	Histamine	Epithelial response
		Bradikinin	Changes in local circulation
		Eicosanoids	

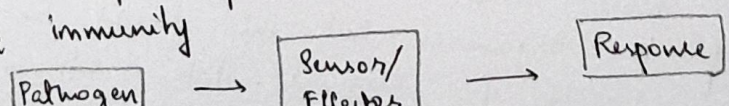
Typically, the immune system has more elaborations. So this is a better representation -



Controller : a component that integrates signals from many sensors and then sends appropriate signals to effectors

Design Principles of Immune response

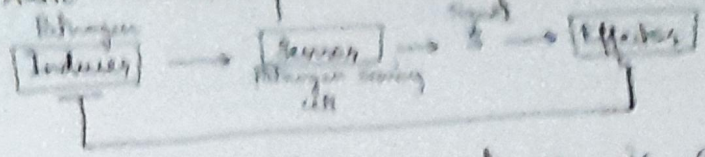
Innate immunity



Pathogen → Detected and phagocytosed by macrophages → Pathogen eliminated.

(11)

Homeostatic circuit of immune system —



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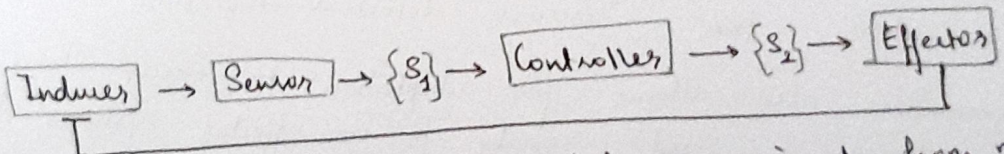

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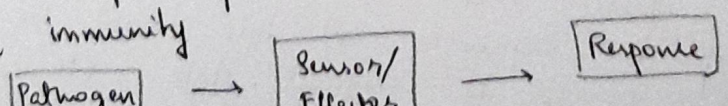
Design Principles of Immune response

Innate immunity



Pathogen → Detected and phagocytosed by macrophages → Pathogen eliminated.

2. Pathogen → Sensor → {1st order cytokine} → Effectors → Response

→ Detected by macrophages → IL-8 → Recruit neutrophils → Neutrophils phagocytose and eliminate pathogen

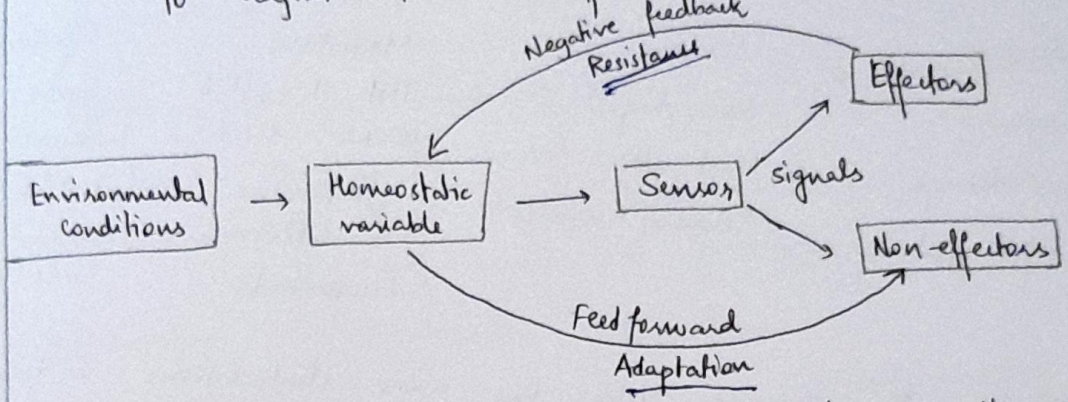
Adaptive immunity or Innate immunity (depends on lymphocyte type)

3. Pathogen → Sensor → {1st order cytokine} → Lymphocyte → {2nd order cytokine} → Effector

↳ Detected by macrophages → IL-12 → B / T-cells / NK cells (Adaptive / Innate) → IFN-γ → Activates macrophages → to kill pathogen

If inflammatory ~~and~~ responses are not able to return the system to homeostatic state, then it means certain death for the organism.

Adaption is a feature of inflammation allowing the system to adjust to a different set point.



- Effectors work by trying to return the state to its set point when the sensors detect a deviation
- Inflammation also allows for adaptation to occur when the sensors send signals to non-effector systems too. Rather, this enables the system to adapt to a new state, where the set point has shifted
- This is through a feed-forward loop.
- This new stable state would prevents the catastrophic fate of the organism. But this should be resolved and system returned to its original state. Otherwise, this gives rise to chronic inflammation which is the underlying cause of systemic disorders like obesity, diabetes, cardiovascular etc.

Effectors – Livers, immune cells
Non-effectors – Lungs, brain

When inflammatory mediators talk to non effectors, they're preparing them for a different set point until the pathogen load is brought down – adaptation

Classic example – Fever (~ 31:00 mins)