

CELL FATE

CONDITIONAL SPECIFICATION
Environment influences cell fate.

Environment regulates cell signaling
is dependent on:

- INDUCTION** - environmental cue
- cell / tissue / molecule induces fate of a tissue / cell
- ligand

COMPETENCE - ability to sense environmental cue
A tissue or cell can respond to an induced signal
Having a receptor

TRANSCRIPTION FACTOR INTERPLAY
A = activator R = repressor

1) lots of variability in how tightly TF bind to DNA
2) lots of diff ways TFs can interact w/ each other.

ID TRANSCRIPTION FACTORS → 3 letter gene w/ an "x" at the end. ex: Hox gene

HOW DO ENHancers & TFs ALLOW FOR TISSUE SPECIFICITY → TFs can be tissue specific

HOW DO WE KNOW IF A SEQUENCE IS AN ENHancer OR REPRESSor?

BETA-GAL REPORTER ASSAY (for example lacZ)

- B-D-gal cuts lactose but x-gal looks very alike so it'll cut it too.
- When it cuts it, it produces a blue precipitate.

If an activator binds to our reporter gene: blue fluorescence

- can isolate an enhancer & see if there's expression of that gene

Dmrt3 methylates all C (CGG & no CGG)
Dmrt1: methylates CpGs methylated by Dmrt3 (replicates Cts, maintains it)

DNA methylation → condenses (recruits H3K9me3 & H3K27me3)
Histone Acetylation → decondenses
HATs → condenses

HISTONES

METHYLATION CAN REGULATE TXN
Some methylation makes things too compact & txn can't occur b/c RNA pol can't get in to bind promoter

which Lysine on that histone is getting methylated recruits different types of proteins

Methylation recruits:
Chromatin Remodelers will either open or close chromatin
if it opens the chromatin: TF can bind

Types of gene: As R_s

Developmental gene default state

- Genes involved in mass producing cells
- Rapidly divide
- This genes tend to not get methylated @ CpGs, they're pretty open (decondensed)

Mature cell specific gene default state (cell-type specific)

- Genes specific to a certain cell type (make insulin, neurotransmitters)
- This genes tend to be on a default methylated STATE, they're OFF b/c you don't want or muscle cells to produce gastric acid for example.
- They're off until they need to be on, until they're on the correct mature cell when it's not anymore

Cells go from developmental to mature stage in response to environmental stimuli.

PRE-TXNAL GENE EXPRESSION
Whether a gene gets expressed is dependent on:

- TF availability in a cell
↳ This is cell-type specific & timing specific DIFFERENTIALLY EXPRESSED!
- Whether the gene is accessible
↳ whether chromatin is condensed or decondensed

Proteins CTCF & Cohesin

CTCF: Repressive TF
Cohesin: Ring that loops DNA & keeps it in close proximity
↳ brings enhancer in close proximity to promoter & allows mediator to make bridge connections

2 ways in which chromatin loop formation can occur by CTCF & Cohesin

- Have Cohesin in location where it brings enhancer & promoter closely together
- If have Cohesin in location where it can't bring enhancer & promoter together. CTCF regulates this b/c it determines where Cohesin stops.

CHROMATIN REMODELING + TXNAL CONTROL OCCURS WHILE CELLS ARE DIVING.
Overtime early genes stop being expressed. Over cell division, the genes expressed win a population of cells start changing. Early genes can't continue to be expressed b/c the momentum is going →. Early genes code for some staff/late genes for others.

Once cohesin starts moving there's less resistance in that direction b/c if it goes back there's coiled DNA that tenses so it wants to go the relaxed direction (→). CTCF also prevents it from backing up.

Enhancers are used at diff. times in development

How do you get a LOF mutation?

- Cow single base insertion
- Human: single base substitution
- Dog: Single base substitution
- Sheep: Single base substitution

① Frameshift causing nonsense mutation
② Alternative splicing
↳ the spliceosome wouldn't recognize that spot to cut & now the intron would be also translated b/c it wasn't spliced

③ Non-conservative Missense or nonsense mutation (substitution)

④ Option 1: forms an miRNA binding site
Option 2: Polyadenylation won't happen & transport would be impacted.

MUT PATHWAYS

Inactivating Mutations: If a key component of the signaling pathway (such as a receptor, kinase, or transcription factor) is inactivated by a mutation, the signal may not reach the nucleus, preventing activation or repression of genes needed for cellular function. For example, a mutation in a receptor may prevent it from recognizing a signal (like a hormone), causing the cell to behave as if the signal isn't present, even when it is.

Gain-of-Function Mutations: These mutations can make components of a signaling pathway permanently active, even in the absence of a signal. For example, a mutation in a growth factor receptor may lead to constant activation of pathways promoting cell division, which can result in unregulated cell proliferation and potentially cancer.

**Mutation might affect org. development if there's not enough As to start txn.
whole embryo won't be made**

AUTONOMOUS SPECIFICATION
what's in the cell determines cell fate (won't change no matter the env, doesn't need induction & competence)

Molecules regulate it: mRNA, miRNA

ALTERNATIVE POLYADENYLATION

↳ Polyadenylation occurs @ specific nucleotide sequences
In 3' end there are "magical sequences" that can:

- bind miRNA (miRNA can bind anywhere in the gene but they really like to bind to 3' end)
- be bound by other proteins that take RNA to diff parts of cell

Purpose: (of polyA)

- helps stabilize mRNA
- involved in translating mRNA

Early in development: Short 3' UTR
↳ can't get degraded by miRNA

Don't want genes that allow cell to grow to get degraded

Mature cells: Longer 3' UTR
↳ can get degraded by miRNA
Want to be able to control expression

RNA PROCESSING

Purpose of alternative splicing
↳ Alt splicing occurs b/c the spliceosome recognizes more variety of proteins we can produce from our genome diff splice sites
↳ Produce distinct tissue versions of proteins.

Tissue specific protein functions
Timing specific protein fns

ALTERNATIVE SPlicing CAN BE REGULATED IN A TEMPORAL MANNER

Produce different versions of proteins at different times
Needed for elasticity as babies spliced b/c as adults how we need structure (rigid) & support connecting STH head limb

if STH is inhibited, head is more impacted, limb is used to receive more signal

CELLULAR LOCALIZATION

Assay: Immunofluorescence w/ anti-SOX1
Control: 2° antibody NO 1
Independent variable: diff proteins being detected

E Nestin SOX1 Merge

1) Assay → Reporter Assay
2) Control → control basic
3) Independent variable length of Promoter
4) Results → medium = expression of reporter gene

DROSOPHILA AXIS PATTERNING THROUGH MATERNAL EFFECT & GAP GENES

syncretic specification
Early fly embryos are called syncytium

Maternal Effect Genes
they have lots of nuclei in one really large cell

- proteins a parent encoded by the mom's genome that get deposited into the oocyte prior to fertilization
- ↳ they regulate the initial stages of embryogenesis
- ↳ they provide embryos w/ the initial steps necessary to determine axis specification

Gap Genes - Tf's

- Drosophila larvae have 14 segments
each segment corresponds to a specific body region
- Specify body planning
- They're expressed in large chunks
- Mutations in these genes result in lots of a large part of embryo
- They set up other genes in a segment specific way
- Gap genes encode for Tf's
- They bind in combination to diff enhancer sites (or silencers) on many genes
- The combination that they bind in matters b/c some repress & some activate txn.
- Too many R = no txn
Too little amount of R = no txn
↳ Enhancers, Tf's & Histone acetylation can be diff in diff tissues w/in the same org.
- Gap genes are the Tf's of pair-rule genes
- Gap genes regulate pair-rule genes
- Exist in strips
- ↳ have multiple enhancers (bound by multiple Tf's)
- ↳ So even though the combination of Gap genes changes across the embryo, the pair-rule genes are expressed at precise intervals along the anterior-posterior axis
- Location of pair rule gene expression is determined by:

 - multiple enhancers & available Tf's to bind enhancers
 - Gap gene & maternal gene proteins that bind to these sites

- Technique we can use to determine what enhancer is used for a given region?
B-gal reporter assay
↳ isolate an enhancer from rest of gene & put it in combination w/ lacZ reporter & we can see where lacZ is expressed (where enhancer is)
- If we want to know if a mutant enhancer has an impact on gene expression:
Make mutation on enhancer, put it under lacZ & see how it impacts
↳ Control = mutated enhancer
↳ If mutated has any change compared to control = there's impact

EXPERIMENTAL METHODS TO ID MOLECULES

To know HOW MUCH THERE IS OR IF THERE IS ANY PRESENT?

DNA

- EDNA sequencing ↳ add heat to ID mutation (FISH)
- RNA ↳ RT-PCR ↳ tells us how much RNA

PROTEIN

- Western Blot ↳ tells size & amount of protein
↳ running protein gel, use antibodies to detect specific proteins
↳ faster = bigger protein
↳ slower = smaller protein
- Immunohistochemistry / Immunofluorescence ↳ instead of probe we use antibody to ID protein
↳ tells where in the cell the protein is localized

EXPERIMENTAL METHODS TO ID PROCESSES

TXN

- Reporters Genes ↳ in-situ hybridization ↳ determines which tissues the enhancers are in
- RT-PCR ↳ Reporters Genes (run gel & look @ RNA)

TRANSLATION

- Reporters genes + Western Blots ↳ GFP/green fluorescent protein, mcherry (red) ↳ Detect Color
↳ If requires translation

PROTEIN ACTIVITY

- Immunohistochemistry / Immunofluorescence (is there activity or not?)
↳ Co-immunoprecipitation
↳ What binds protein? Is protein binding how it should?
↳ Enzyme assays / kinase assay (kinase kinase act.)
↳ a phosphoprotein sees if it phosphorylates other proteins

Cell-cell communication: Juxtacrine signaling, extracellular matrix & some paracrine signaling

DIFFERENTIAL ADHESION HYPOTHESIS

Cell sorting occurs because the strength of their adhesions differ.

① strong adhesions are central

Weaker adhesions are periphery
more cadherins = central

② Consistent hierarchy: cells always rearrange to maintain correct germ layer position.

What are adhesions?

Transmembrane molecules called Cadherins

They're calcium dependent adhesion molecules

N, P, E - cadherin bind to actin through catenins & are critical for epithelial sheet movement.

② Protocadherins

Important for interacting w/ cells expressing same protocadherin & repelling cells that express diff. protocadherin

Cell Types based on their Movement

- Epithelial

↳ They move together as a sheet

• have strong adhesions to basal lamina

• have a lot of integrin

• have protocadherins that allow them to connect to neighboring cells that have same type of cadherin molecules

- Mesenchymal

↳ They move individually

• weakly associates w/ fibronectin (allows cells to move around)

• loose protocadherins b/c they don't want to bind to other cells

• loose integrin to move quickly & independently

• degrade proteins that connect them to basal lamina or to other cells

Extracellular Matrix (ECM)

↳ Space outside the cell

Provides structure to cells & allows cell to move

• made up of basal lamina

↳ collagen + lamina

Extracellular Matrix Proteins

↳ Interact w/ Fibronectin & Proteoglycans

collagen → glycoprotein (has sugar modification)
• it's an intermediate adhesive molecule (not very strong)

↳ this allows cells to move around parts of ECM, cells can grab along & move, unbind & bind to next fibronectin, ads w/ integrin

Receptors for ECM → allows cells to move & be involved in signal transduction

↳ CONNECT CELL TO CYTOSKELETON MATRIX
Anchor to cytoskeleton when integrin binds to matrix, it forces things forward, No grip = No advancing forward

Proteoglycans → glycoprotein helpful for cell signaling proteins

• add in paracrine signaling
• helps ligand or hormone bind receptor
• can slow down rate @ when it's moving which captures

• helps get into cell

• in ECM or cell membranes

• capture paracrine signals

• interact w/ ligand & maintain [ligand]

Cell-Cell Signaling Mechanisms

Juxtacrine Signaling
• cells are near to each other

• ligand is attached to cell so only way to get signal is if cells are together

↳ name bc creates borders in fly wings

6 signaling Pathways

1 NOTCH SIGNALING - Juxtacrine

Receptor: Notch

Ligand: Delta

TFs: (activation)

- CSL
- P300
- Notch (cut section)

① Repression is bound to Activator & prevent it from sending activation signals to RNA pol.

② Delta binds Notch &

causes protease to cut Notch which leaves a piece of Notch hanging around, bumps off P &

& how activator can send signals.

2 FIBROBLAST GROWTH FACTOR PATHWAY

LIPID INSOLUBLE, Paracrine

↳ FGFR ligand dimer

↳ Homodimer of two FGFRs

↳ Ligand: FGF (1-10, 16-23)

↳ Receptors: In nucleus

• Ligand binds receptors

• Receptors phosphorylate each other

• RAS activates kinases

• TFs end up phosphorylated

• Gene activation

↳ Retinoblastoma many diff ligands: which can become a problem if ligand is mutated bc it would keep mutating all receptors. To stop this:

make a nonsense mutation in binding site of specific ligand to make it non-functional but receptor would still work.

If ligand is mutated & it binds to receptor & can cause a lot in receptor

↳ Ligand: G protein

↳ Ligand: GTP

↳ Ligand: Ras

↳ Ligand: Raf

↳ Ligand: MEK

↳ Ligand: ERK

↳ Ligand: Transcription factor

↳ Ligand: Expression of ETS-domain transcription factors

↳ Ligand: Lipid

↳ Ligand: Protein

↳ Ligand: Nucleus

↳ Ligand: DNA

↳ Ligand: RNA

↳ Ligand: Protein

↳ Ligand: Lipid

↳ Ligand: Protein