Development

Positional information & limb development

Introduction

Lewis Wolpert's model
- Cytoplasmic inheritance and simple cell-to-cell contact aren't enough to describe how cells specialise
  - e.g.: In anabaena, has **nitrogen fixing cells**, and have some mechanism to ensure that they appear at particular intervals
- Cells develop in two stages
  - Find where they are in the cell
  - Use this **positional information** to specialise accordingly
- How do we get this positional information?
  - "Flagpole" releases a **morphogen**, and cells differentiate based on the concentration of morphogen
  - The cells have thresholds

Evidence
- Mesoderm induction
  - Prospective **ectodermal cells** treated with activin
  - *High doses of activin* --> **goosecoid**
  - *Intermediate doses* --> **Brachyury**
  - *Low doses* --> **keratin**
  - Sharp threshold (check with RNA)
- If we want to see whether it works in an established conc. gradient
  - Sandwich agarose bead soaked in activin in between two such cells
  - Sets up conc. gradient

Problems
- How can cell distinguish between different levels of activin (increasing it by as little as 50% can make a change)
- How does activin diffuse through a field of cells
  - Could be that it just works its way along
  - Can make tagged activin with GFP by introducing the GFP gene before the activin gene
  - Take two cells and label them with different colour activin - seems to diffuse *in between* cells

The chick-limb

- Studies mostly in chick
  - Accessible
  - Develops quickly
  - Invertebrate
- Method
  - Egg taken from incubator, and little window cut
  - Two little bulges at the bottom of the embryo
  - Will become wing and leg
  - At the very tip of each, we have the **apical ectodermal ridge**
- Parts
  - Radius and ulna are in the forearm
  - Humerus is upper arm
  - Three digits, 2 3 and 4

Antero-posterior axis
- Thumb to little finger
- How is it that 4 is always POSTERIOR (bottom), 3 next and 2 is ANTERIOR
- Work in John Saunders in the late 60s
- If take special region from **posterior part** and transplant it to **anterior part** of other embryo, developing wing has another finger pattern
  - As mirror image of before
  - Also affect ulna and radius
- If graft in the **middle**, we 4 3 4 4 3 2 (from posterior to anterior)
- Explanation - bit near posterior part which releases a morphogen
  - 4 if high conc.
  - 3 if lower conc.
  - 2 if lowest conc.
  - Nothing if 0 conc.
- Other test
  - Graft region anteriorly in each case
  - Affect it with chemicals (progressively more) - radiation, actinmysin D, etc...
  - Find that the fingers move as strength of signal
    - 432234
    - 43223
    - 4322
    - 432
  - Still need chemical to make 2
- Raft only sends signals
- **Sonic hedgehog**
  - Its expression overlaps especially with cells in the ZPA
  - (ZPA = zone of polarising activity) / polarising zone
  - Once the ability to produce morphogen dissapear, sonic hedgehog dissapears
  - Cells persuaded to produce it or beads producing it have the same effect
- Retinoic acid does the same thing

Proximo-distal axis
- Shoulder to hand
- Nothing to do with morphogens - works in completely different way
- The longer in time you stay behind the apical ectodermal ridge, the more distance you become
- Provided in experiments removing the apical ectodermal ridge
  - If there for very short, get a humerus, etc...
- Progress zone model
  - A E R has cells in it
  - Cells are forced out at different times
  - The longer you are in there, the more distal you are
  - Apical ectodermal ridge proved to be a source of FGF4 in any case
  - So if remove A E R and replaced it with eads soaked in FGF4, get limb that looks reasonably OK

Wing or leg?
- The difference between wing and leg isn't the signal, it's what's expressed - the signals (sonic hegehog and FGF4 are the SAME in wing and leg)
- They look different because wing and leg cells react differently to the signal
- so if implant THIGH into region of wing that will become distal, they'll become distal, and do it in a leg way, and become a toe!
- But what IS the different between wing and leg
  - Some genes expressed different
  - Tbx6 expressed in wing and not in leg
  - Tbx4 expressed in leg and not in wing
  - Could be something to do with that

Homeosis and axis formation

Fly structure
- However, respond to the same positional information, even though they change their cell type
- There are lots of genes that regulate these cell types - 7 in drosophila
  - Different mutatins change body parts in drosophila
- All on chromosome 3
- Set up axis formation in the fly
- The things genes express are 1/2 a segemtn out of phase
- Genes define **parasegments** that are 1/2 segment out of phase with the ones eventually produced
- Look at the larva under dark-field-microscopy at the shape of the bristles - they tell us what segment's what
- Also express spatial pattern of expression
- 3 means early expressed in head
  - Cumulative - earlier ones also expressed in later ones
  - Therefore, if knock some out, go partway across the fly
  - Amazing how much one can learn without knowing what the products are
  - Order of the genes along chromosomes represent order in which they are expressed
- Were called **homeotic genes**
- The conserved sequence was **homeobox - hox gene**
  - Each gene had a particular motif
  - Conserved sequence is homeobox
  - Binds DNA
- When cloning genes out of mouse, found that
  - mice have four complexes
  - humans also
  - Can match up genes in mouse to those in the fly
  - Arranged in the same order as in fly
  - Some complexes don't always have all of those like in mice
  - Again, expressed in a temporal order as expected by order of chromosome
  - Similarly, 3' expressed anterior, and 5' posterior
  - Again, array of genes expressed tells us what part is made
  - Know that from overexpression and knockout experiments
  - Less dramatic than that in the fly because of redundancy
  - In addition, more position-expressed genes tend to inhibit anterior genes
- All animals have anterior-posterior patterns specified by hox genes
  - c. Elegans also has a hox cluster, even though it's not very segmented

- Retionic acid is a derivative of vitamin A
- Can affect the function profoundly
- If soak xenopus embryo in retinoic acid, express Hoxb9 anteriorly (usually, just posteriorly)
- Retionic acid induces hox genes in concentration-dependent fashion in cell structure
- Gradient of rationoic aicid in the mouse embryo
- Can see how it's expressed by hooking up regulatory sequence to reporter gene rather than hox gene
- HoxB3 also activated by Krox20

Cloning and gene regulation
Subtopic

How target genes
- If have the same binding domain, assume all affect similar genes
- Work going on on CONNECTIN (getting axons in right place) and SPALT (encodes transcription factor) in drosophila
- Through microarrays is one main way it done

Identified through various approaches
- labile genes?
- Challenge is to fit them together in a network - what do they all do?
- Systems biology

Hox genes specificity
- Antennapedia has a glutamine
- Bicoid has a lysine
- Sequences highly conserved (bund the sequence ATTA), but one slight variation at position 50 (helix 3)
- Bind GGATTA and CCATTA (inverse reversibility)
- This AA seems to make all the difference
- This is the **recognition helix**

Introduction

- Mechanisms of development are exceptionally well conserved

In-situ hybridisation
- We want to know which genes are expressed in a given embryo
- Best way to do that is to see whether there is mRNA for that gene in that embryo
- All we do is syntheses a strand of cRNA for this segment and tag it
- We then add it to extracted DNA from our embryo. RNA-RNA hybrids are very strong
- Wash and detect the tag (using an antibody, for example)

Organisms
- **Mouse**
  - A mammal
  - Very powerful genetics
  - Can "knock out" any gene of interest and observe
  - Expensive
  - Inconvenient - the embryo is inside the animal and we can't just look at it
- **Chick**
  - Not a mammal, but an **amniote**
  - Convenient and inexpensive to work on (just get an egg)
  - Good for micro-manipulations
  - Subtopic
  - Genetics not a strength
- **Frog**
- **Fish**
- **Fly**
- **Worm**
- **Aradidopsis**

Cleavage
- *Xenopus*
  - Fertilisation of an amphibian egg triggers a programme of rapid DNA replication and subdivision of the egg cell cytoplasm
  - This is known as **cleavage**
  - It subdivides the egg *without growth* - the DNA content increases but the amount of cytoplasm remains constant
  - These cell cycles have no G1 and G2 phases and are synchronised throughout the embryo
  - When a certain DNA:Cytoplasm ratio is obtained, the **Mid Blastula Transition** occurs
    - Appears to depend on the titration of a certain cytoplasmic regulatory factor against the increasing DNA content in the embryo
    - Evidence
      - Delayed by 1 cycle in haploid cells
      - Can be made earlier by injecting more DNA
    - Usually about 12 cycles
  - The cell cycle lengthens and becomes asynchronous - transcription of zygotic DNA is initiated, and the cell becomes susceptible to apoptosis
- *Drosophila*
  - Nuclei divide rapidly before the egg cell partitions its cytoplasm
  - The nuclei migrate to the surface where the cytoplasm is *then* subdivided
  - **Imaginal disks** in the drosophila larvae are disks of cells set aside in specific regions of the embryo which eventually differentiate in response to hormonal signals into the components of the adult fly

Fate maps
- This is a description of what each part of the embryo at a particular stage will go on to form
- Mark cells in one way at an early stage, and see what they go on to form at a later stage
- Examples of markers
  - Nile blue sulphate
  - Fluorescent markers attached to large molecules that cannot cross the cell membrane
  - Inject with something like horseradish peroxidase whose activity can later be detected through a specific reaction
- We can prepare a solution comprising NaCl KCl and divalent cations and a buffer - **neutral medium**
- We then allow the embryo to grow in such a medium, and what grows is what the cells are **specified** to form
- The cell however, might not know itself what it'll become!
  - The state of specification of a cell is not necessarily the same as its eventual fate
    - The fate and specification maps of Xenopus shortly before the onset of gastrulation differ!
    - For example, of all the cells specified to be epidermis, some become the nervous system
    - Those specified to become blood also become various other things (heart, kidney)
    - The cell has to receive some **further developmental instructions**

Determination
- A cell that is specified to form a certain structure in neutral solution may be persuaded to form a different tissue in a different environment
- If, however, it can resist this, it is said to be **determined**
- Clearly, this is an operational test
  - Take a cell from one part of an embryo and label it
  - Move it to another part of the embryo
  - See what happens

Determinant and induction

- How do cells acquire different states of specification and determination?

Cytoplasmic determinants
- Simplest way is through differential inheritance at cleavage of molecules that cause cells to adopt particular fates
- If the molecule is distributed unevenly in the parent, it'll be shared unequally in the daughter cells
- **Bicoid and the antero-posterior axis of the fly**
  - Flies lacking **bicoid gene** do not form **anterior** (head) structures
  - Evidence that this is due to some anteriorly localised determinant
    - Injecting anterior cytoplasm into a mutant, get head
    - Flanked by thoratic structure
    - Whenever we put it
    - Clearly, this is due to a gradient of bicoid proteins whose high points define "head" and lower concentrations define "thorax"
  - This was confirmed by the cloning of the bicoid gene
    - Bicoid RNA is localised in the anterior region of the drosophila
      - Particular sequence in the 3' untranslated region of mRNA
      - Interacts with microtubules and causes anterior translocation
    - Bicoid protein therefore creates a gradient
    - The **hunchback** gene is controlled by bicoid, and is only formed past a certain bicoid threshold
      - We find that it forms in roughly 1/2 the cell, with a sharp dividing line
- **Pole cell determination**

Embryonic induction
- One group of cells influences the development of another
- Usually, cytoplasmic determinants used for major differences (front or back), but finer control is needed otherwise
- The best way to infer inductive interaction - fate maps vs. specification maps
- **Mesoderm induction**
  - One of the first discrepancies concerns prospective mesoderm
    - Layer of cells that lies between ectoderm and endoderm
    - Goes on to form muscle and blood, amongst other things, in fate map
  - In specification map at **early blastula** stage, it's **ectoderm** (skin)
  - **Nieuwkoop** showed that it forms from **equitorial cells of the embryo** in response to a signal derived from the vegetal hemisphere
  - Inducers include members of the FGF-BETA family, Vg1, activin and derrières
  - Posterior mesoderm is induced by members of the FGF (fibroplast growth factor) family
  - It's been confirmed by inhibiting their functions by mean of "dominant negative receptors"
    - Allow cell to grow normally
    - Flood it with lots of an inactivated unit of the dimer protein
- **Dorsalisation and neural induction**
  - Cells fated to form somite and the cells fated to form neural tissue 5/6 not specified at the early gastrula stage
  - Recent results suggest that the inducers are the same