Gilbert's Developmental Biology - Notes

Contents

Principles of Developmental Biology

The Anatomical Tradition

Questions

- differentiation: how cells become different?
- morphogenesis: how is development precisely ordered?
- growth: when to start and stop dividing?
- reproduction: how do you make the cells that produce an entire organism?
- evolution: what changes are possible given constraints of heritage?
- environment: how do organisms integrate context from around them into development?

Anatomical Approaches

- comparative embryology: between organisms
- evolutionary embryology: ancestry constrains types of development
- teratology: use birth defects to understand what is important
- mathematical modeling: use equations to predict

Evolutionary Embryology

How did "looking at embryos" help classify mysterious organisms?

- *Lancelet*: primitive "fish-like" chordate that diverged during Cambrian period
- *Tunicate*: immobile chordate (sea squirts)

Thompson - larval barnacles identical to larval crabs: barnacles are arthropods not mollusks Kowalevsky - Tunicate larvae have notochords + form neural tubes similar to amphioxus: therefore chordates and not mollusks.

Phylum Chordata:

- Subphylum Vertebrata
- Subphylum Cephalochordata (amphioxus, lancelets)
- Subphylum Urochordata (tunicates)

What characterizes chordata members:

- notochord (flexible rod of cells NOT vertebrae)
- dorsal hollow nerve chord (DNHC)
- pharyngeal
- post anal tail

DNHC: emerges from ectoderm, strip thickens (neural plate) and folds inwards to form tube *notochord*: forms from mesoderm, lies between DNHC and gut tube, acts as signaling center during development *pharyngeal slits* openings in throat area of the embryo *tail*: hmm

von Baer's laws: the more related an organism is, the later in its development the differences will appear.

Embryonic Homologies

Homology between gill support in jawless fish and vertebrate jaw.

Evidence for homology between gill support cartilage and vertebrate jaw:

1/ Bones derived from neural crest layer (almost all other come from mesoderm) 2/ Upper and lower portions with a hinge 3/ Support muscles seem homologous

Now look at progression of 1st and 2nd embryonic (pharyngeal) arches:

2/ Becomes *hyomandibular bone* (brace) that links jaw to cranium. Early vertebrates repurposed bone as good medium to transduce sound. As jaw fused to cranium, the linking function became unnecessary and the sound transduction function became primary. *Hyomandibular bone -> stapes bone*

1/ Becomes jaw element. The posterior pieces are responsible for articulation. Mammals developed articulation in another spot (), so these detached and formed two additional inner ear bones: *incus* and *malleus* bones. This is unique to mammals and lower vertebrates only have the ear bone derived from (2).

Primer on Embryonic Arches In week 4 of human development, small bulges separated by clefts that are responsible for certain structures.

1/ Mandibular (jaw and incus/malleus in humans) Meckel's cartilage 2/ Hyoid (staples in humans) Reichert's cartilage 3,4,6 / Laryngeal (swallowing, breathing) 5 has no function

They all share basic structure and plan:

1/ Ectoderm: skin 2/ Mesoderm: cranial nerve, aortic-arch arteries + cartilage thread through here 3/ Endoderm: gut

The temporary cartilaginous (eg. Meckel's) guide development and then mostly go away

Medical Embryology + Teratology

malformations: abnormalities caused by genetic events *syndromes*: many abnormalities running together

piebaldism as case study

Mutated KIT underexpressed in:

- neural crest cells - pigment, ear cells, gut neurons: underpigmentation, deafness, gut malformation
- blood: anemia
- germ: sterility

thalidomide

teratogens: Exogenous agents that cause abnormalities ("monster formers")

- Comes from latin root that means "demonstrate" / point out issue

Lenz and McBride demonstrated in 1961 that thalidomide caused limb defromities, heart defects, absence of ears, etc.

The window of susceptibility overlaps when actual organ is developing

- only during 34-50 post menstruation
- pharyngeal arch (30-34) form
- upper limb buds (36-40) form
- lower limb buds (42-46) form

This is where you get ear, upper + lower limb deformations respectively.

Math of Growth

Not much here.

- As volume doubles, length increases 1.27.
- Difference between allometric and isometric growth
- Understand the fixed
- isometric growth
- allometric growth

Life Cycles and Evolution

Stages of Animal Development

fertilization -> cleavage -> gastrulation ->

sperm + egg -> zygote -> blastula -> gastrula -> organs -> organism

Frog Development

https://www.youtube.com/watch?v=DfZ0b2M5Yaw https://www.youtube.com/watch?app=desktop&v=OPTmFxtivHI https://www.youtube.com/watch?v=VyNcgNlmcnU

- *Gastrulation*
- animal and vegetal pole
- blastocoel
- blastopore
- archenteron
- dorsal lip
- ventral lip
- bottle cells

Important Movements

- Invagination (folding inward)
- Involution (spreading within inside)
- Epiboly (spreading around outside)

Evolution in Unicellular Protists

Role of nucleus + transcriptional regulation

Acetabularia: 2-4 cm long *single cell* organism:

- Early demonstrations that nucleus contains organism instructions
- The instructions are transported elsewhere and there is a delay before they turn into structure

Sexual reproduction

Conjugation:

- Cytoplasmic bridge forms between oral apparatus
- 8 micronuclei form (two meiotic + one mitotic divisions)
- 7 degrade, 1 splits into migratory and stationary
- Halves migrate over, combine and divide mitotitcally
- Guys disengage

Multicellularity: Evolution of Differentiation

Volvovaceaens Volvocaceae

1/ ordered division of cells 2/ differentiation into types of cells

Dictyostelium A social amoeba (*myxamoeba*), though sometimes called "cellular slime mold".

Lifecycle

Cell adhesion

Differentiation:

- Broadly regulated by diffusable molecules
- cAMP propogation - circular pulses + cells migrating at different speed - inward spiral
- DIF -> prestalk + cAMP -> prespore

Sex + Individuality in Volvox Volvox simplest organism to exhibit division of labor between sex and body cell types.

- *inversion* movement resembles gastrulation of animal embryo
- juvenille Volvox release from hole and swim
- remaining somatic cells commit suicide

Sexual reproduction as a tool to survive weather cycles (pond drying up)

Antibodies in Dev Bio Correlative. How do we know glycoproteins are involved in adhesion? Antibodies stick right after division. Loss-of-function. Isolated Fabs actually stop the binding. Not causal: does not identify mechanism Gain-of-function.

Developmental Patterns in Metazoans

- dipoblasts
- protosomes
- deuterostomes

Facts about sponges:

- Can reproduce by passing single cells through a sieve
-
- Have no true mesoderm so lack organs
- Porifera (sponge)

Diploblasts - characterized by endoderm and ecotoderm but no true mesoderm:

- Cnidaria (jellyfish)
- Ctenophora (comb jellies)
- Cnidaria + Ctenophora characterized by radial symmetry: *Radiata*
- Protostomes or deuterostomes have bilateral symmetry: *Bilatera*
- Descended from some primitive flatworm, first to have true mesoderm
- Protostome means mouth first and deuterostome means mouth second
- Branches of protostomes: ecdysozoa + lophotrocozoa.
- *Branch* Ecdysozoa - animals that molt (*ecdysis*) - including *Arhtropoda* (phylum): arachnids, insects, mites, millipedes, crustaceans
- *Branch* Lophotrocozoa - annelids, molluscs, flatworms
- Intuitively, continuous vs staggered growth patterns

coelum (body cavity) formation

- protosome forms mesoderm from hollowing out single cavity: schizocoelous (literally "splitting" of mesoderm)
- deuterostome from pockets extending from gut: enterocoelous

four sacs:

- yolk: nutrients
- amniote: fluid
- allantois: waste products of metabolism accumulate
- chorion: outer layer, interacts with environment

Experimental Embryology

Environment on Embryo Dev

UVB

MAAs mycosporine-like amino acids

- convert UV light into heat energy + dissipated
- Without: absorbed direclty by nucleotides or biomolecules that become ROS
- effect on amphibians?

Internal Embryo Dev Programs

commitment (no phenotypic change) -> *differentiation* (phenotypic change)

commitment has two stages:

- specification (continues even when placed in neutral environment)
- determination (continues even when placed in another embryo region)

Important differentiated cell types

- *Keritinocyte* (epidermal Keratin): protect against abrasion, dessication; produces Keratin
- *Erythrocyte* (red blood): oxygen transport
- *Lens cell*: produces crystallin, transmits light
- *Melanocyte*: pigment production
- *Islet cells*: insuling; carbohydrage metabolism regulation
- *Leydig cell*: produce testosterone in males
- *Chondrocyte*: tendons and ligaments; produce cartilage and collagen
- *Osteoblast*: skeletal support; bone matrix
- *Myocyte*: actin + myosin; muscle contraction
- *Hepatocyte*: serum albumin, enzymes
- *Neuron*: transmit electrical impulses; produce neurotransmitters
- *Hen*:

Modes of Commitment Can define two "properties" of a blastomere: *potency*: cell types possible *fate*: cell types will form unaltered

- Conditional

Genes and Development

Cell-to-Cell Communication

Cell-to-Cell Communication

Summary

- protein-protein interactions are the basic building blocks
- Close (touching) interactions mediated by juxtacrine signaling
- Medium to far (diffuse secretion) signaling mediated by paracrine signaling
- Signal transduction is a chain of conformational changes in proteins that transmits information

Adhesion and Sorting

Organization of tissues largely explained by differential adhesion. Developing embryo is a function of the adhesion properties of different cell types. In a steady state until something changes.

1955: Townes and Holtfreter. Mixed amphibian embryo cells in alkaline solution. Demonstrated that tissue structure (eg. ecotoderm and mesoderm) and embryo structure emerge from mixture. *1964: Steinberg*. Proposed differential adhesion hypothesis.

Cadherins

What are the building blocks? *Cadherins* (calcium dependent adherins) Important to understand in context of the scaffolds they exist in:

- cadherin
- catenin
- actin framework

E-cadherin: embryo dev P-cadherin: placenta stick to uterus N-cadherin: CNS dev protocadherin: lack the catenins

Cadherin is actually 5 EC repeats that flop, degrade, etc. unless supported by a Ca2+.

With receptor fixed, the amount expressed can create many layers of tissue.

Zebrafish embryo epiboly

Epiboly, the spread of the epiblast (precursor to ectoderma) around the early embryo, is a key part of dev. This spread is basically controlled by gradients of cadherins that induce a movement called *radial intercalation*: cells squeezing from a thick block into layered sheets.

Knocking out E-cadherin creates "half baked" embryos with epiblast concentrated on one side never fully spreading.

The Extracellular Matrix as Source of Developmental Signals

ECM: collagen, proteoglycan, specialized glycoprotein (fibronectin, laminin)

Proteoglycan: long chain Glycoprotein: short chain

Proteoglycan

Key functional component: Glycoaminoglycan (GAG) polysaccharide:

- charge sequesters proteins
- osmotic swelling pressure (bind ions, water floods)
- mechanical tethers and load distributers
- organizes morphogens

"Sugar code":

- chain length
- domain
- N-Sulfation vs. N-acetylation composition
- epimerization (modifying stereocenter)
- how long the NS streches are (eg. NS islands)

Fibronectin

Large dimer glycoprotein (460 kDa) Pave the roads of cell migration "Lead germ cells to the gonads and heart cells to embryo midline"

Basal Lamina

Laminin (another glyco) -> basal lamina; epithelial Collagen IV -> reticular lamina; mesenchyme

But how do cells bind the glycoproteins?

Integrins connect ECM (like fibronectin) to internal structures (actin cytoskeleton).

[RGD AA motif] <-> BOTH talin + \alpha actin (bind to actin filaments) dual binding allows contraction of the actin to move against fixed ECM

Epithelial-Mesenchymal Transition

- cadherins (cell) and integrins (basal lamina) break
- release enzymes to break down basal lamina
- actin cytoskeleton rearrangement
- secretion of new mesenchyme ECM factors (?)

Cell Signaling

Big idea of dev bio: cell identity is defined by context.

inducer: tissue (or cells) that produces signals *responder*: tissue (or cells) being induced *competence*: ability to receive and respond to inductive signals

Building Vertebrate Eye

- Brain bulges out and induces head ectoderm
- Specification actually much earlier than this differentiation event

You want Otx2, Pax6, Sox2, L-Maf:

1/ First gut endoderm "enrualizes" ectoderm: Otx2 2/ Then mesoderm: Pax6 3/ FGF8 + BMP4 from brain bulge: L-Maf + Sox3

Paracrine Factors

Small toolkit of these factors, many of which morphogens, induce most organs.

morphogen: concentration gradients induce effects

Fibroblast Growth Factor (FGF)

- Broad developmental themes of factor
- 'Shape' of constituent pathways

Broadly delay differentiation (developmental brake) and promote proliferation. But also promotes *patterning* (distinct from differentiation) mostly in the concentration of end ERK.

RTK Far more 'graded' response + slower. Encodes more temporal + spatial information in response.

1/ RTKs dimerize and phosphorylate each other 2/ Adapter protein recognizes stuck receptors and recruits SOS a type of GEF (Guanine Exchange Factor) 3/ GEF removes GDP from Ras and allows GTP to swap in 4/ Ras-GTP acivates the Raf -> MEK -> ERK cascade (MAPKKK, MAPKK, MAPK) 6/ Eventually ERK makes its way into the nucleus and phosphorylates TF

The complexity, eg. three layers of MAP kinases (Raf -> MEK -> ERK) allow broader set of signals.

JAK/STAT Far more 'thresholded' + quick. Closer to digital signal.

1/ JAK bound to receptor dimerize + phosphorylate each other 2/ JAK phosphorylate their receptors 3/ STAT dock here + get phosphorylated 4/ STAT dimerize + regulate transcription directly

Generally negative feedback (output suppresses its own activity with inhibitor or similar)

Hedgehog

Named after loss of function phenotype in Drosophila (pointy spikes).

Vertebrates:

- Desert HH: spermatogenesis
- Indian HH: gut / cartilage and important for postnatal bone growth
- Sonic HH: all kinds of development

-> motorneurons come from ventral portion of neural tube -> only part of the somite forms vertebrate -> pinkies are always posterior digits

Secretion Lipids very important. C-terminus cleaved and => Palmitic acid - HH - cholesterol

1/ Needed for stable gradients. Diffuse too fast otherwise. 2/ Also needed to bind to Patched

Pathway *without HH*

- Patched inhibits Smoothened + Smoothened is degraded
- Ci/Gli stuck to microtubules.
- Part of Ci/Gli cleaved and this piece enters nucleus as transcriptional repressor

with HH

- HH binds to Patched and no longer inhibits Smoothened
- Smoothened released Ci/Gli from microtubules
- Full Ci/Gli protein enters nucleus and acts as activator

Double negative (doing something to an inhibitor to make activator)

Misc

- Some teratogens inhibit Smoothened and cause cyclopia.
- There are non Gli pathways that are targets of HH, eg. actin remodeling in pathfinding neurons

Wnt

- Cysteine rich glycoproteins
- At least 11 conserved across all vertebrates
- 19 genes in humans
- Name a combination of "wingless" and "integrated". Wingless from a forward genetic screen in Drosophila and "integrated" from vetrebrate homologue.

Discovered in the most "basal of extant metazoans"

Secretion

Negative feedback with Notum Wnt needs lipid to bind to Frizzled.

Notum is secreted when this happens and recognizes/removes this lipid, keeping it from binding to Frizzled continuously.

Canonical Pathway \beta-catenin is constantly getting degraded, primarily by phosphorylation by GSK3, causing recognition and destruction

1/ lipidated Wnt binds to Frizzled + LRP5/6, bringing together 2/ Linkage binds GSK3, preventing degradation of \beta-catenin. 3/ \beta-catenin enters nucleus and activates LEF/TCF TF (turning repressor into an activator)

Double negative (doing something to an inhibitor to make activator)

Non-canonical *Planar cell polarity*

- Frizzled paired with something else can activate *Rho GTPase*
- *Rho GTPase*, "master builder", can modulate all types of actin remodeling
- movement along a single plane
- ex: establishing germ layers, anterior-posterior axis

Wnt/calcium

Activates a phospholipase (PLC). Remember from T cell pathway biology? Releases calcium from smooth ER. Also movement?

TGB-\beta superfamily

- TGF-\beta family
- BMP
- Nodal/Activin
- (SMAD TFs)

TGF-\beta family

- ECM formation through stimulating collagen / fibronectin synthesis
- control eg. where/when epithelia branch in kdiney, lungs, salivary glands
- same family as those used to induce helper T cells

BMP

- originally named from role in bone formation
- chemically distinguished by 7 (not 9) conserved cysteines in polypeptide

BMP4/BMP7 broadly important in cell division, apoptosis, migration, differentiation; lets look for patterns later

Nodal / Activin

- Nodal creates left-right asymmetry of bilateral organisms
- right to left gradient created from beating cillia (!)

SMAD TFs These are TFs broadly ctivated by this paracrine factor superfamily

- Activin, Nodal, TGF-\beta activate Smad2,3
- BMP activate Smad1,2
- All dimerize with SMAD4 and enter nucleus

Auxin in plants Recall *Arabidopsis thaliana*:

- goes through octant -> globular -> heart embryo stages + develops two cotyledons (the 'cot' in dycot)
- auxin important paracrine factor mediated by active and passive transport
- polar expression of PIN on membranes guides *efflux transport*
- first from apical to basal, then recirculation pattern
- double negative activation as per usual
- goes back to first land plants (moss and liverworts), maybe even ancestral green algae

Cell Biology of Paracrine Signaling

FGF as case study 1/ Transcription rate and degradation 2/ Free diffusion 3/ Directed along HSPG fibers 4/ Sequestered by dense HSPG mesh 5/ Endocytosis by FGFR and degraded by lysosome

Heparan sulfate proteoglycans (HSPG)s

Dynamic membrane extensions

- *Filopidial cytonemes* reach out very long distances (100 uM) to present ligands or receptors
- First described in Drosophila
- *Primary cilium* are focal areas of membrane pushed out by microtubules
- With HH: Patched *on* the cilium and Smoothened away. When HH bound, Smoothened joins Patched on the cilium.

The microtubules are a scaffold for the motor proteins that move things around

Juxtacrine Signaling

1/ Notch <> Delta, Jagged, Serrate 2/ Cell adhesion (like cadherins) 3/ Eph receptors

Notch is essentially a TF tethered to the membrane

- Notch

Development and Evolution

Development and Evolution

Changes in anatomy come from changes in development. The 'components' of development provide (and focus) variation available for evolution.

Focus on 'arrival of the fittest' rather than 'survival of the fittest'.

- Changing 'enhancer' upstream of a gene can change where, when and how much of it is expressed
- Changing coding sequence of
- 'Eco-evo-devo': plasticity (incorporating environmental changes) and symbiosis

modularity and parsimony

How Genetics Drive Developmental Evolution

Developmental Structure of Genome

How can you get changes in morphology when development is so delicate? It seems like it should destroy the entire organism. Two properties of genetic underpinnings of development explain.

Modularity Genes are controlled by sets of enhancers. Adding or removing from this set (eg. by mutation) can change where and when a gene is expressed.

Pitx1 and Stickleback evolution

Marin sticklebacks lost pelvic spine when evolving into freshwater fish. The gene responsible, Pitx1, is identical in both. But a single enhancer element dysfunctional in freshwater and does not express in pelvis.

Recruiting functions in unfamiliar contexts

- Skeletogenic program in urchin larvae (unique amongst echinoderms)
- Many insects use *Apterous* to create forewing. Unique to beetles,*Apterous* turns on exoskeleton genes in dorsal wing while repressing in hindwing.

Parsimony "Small toolkit". Development all use the same basic groups of molecules. Evolution of enhancers and core pathways, especially Wnt + BMP, rapid. Jellyfish + flatworms use same kit as flies + vertebrates.

Examples:

- BMPs specify dorsal-ventral axis in all animals

- WNT + Hox genes specify posterior-anterior axis in all bilaterians
- Pax6 used to specify light sensing organs across mollusk, insect or primates
- Otx form heads in vertebrates and invertebrates
- tinman/Nkx2-5 forms insect + vertebrate hearts
- miRNA-124 in CNS of protostomes and deuterostomes
- miRNA-92 specify locomoter cells in protostomes and deuterostomes
- miRNA-12 found in most guts across animals

Homologous functions with homologous components called *deep homology*.

Chordin-BMP4 interaction same in vertebrates and invertebrates. BMP spreads upwards but Chordin blocks its effects and ectoderm at the top becomes the neurogenic ectoderm.

Can actually take

Duplication and Divergence Why do you get families of molecules: paracrine factors (Wnt, FGF), collagen, globin, Hox...

Copy and paste, preserving a functional copy while allowing another one to explore function space, is common pattern.

Molecules within a family are *paralogues* and come from descent from common anscestor instead of convergence.

Hox genes are a case study:

- 39 vertebrate Hox genes cluster only in four distinct clusters (in four different chromosomes)
- Deformed, Ultrabithorax, Antennapedia all emerged as duplications of same gene.
- Deformed (Dfd) can be swapped with Hoxb4 and function
- Each Drosophila Hox gene has a direct homologue in vertebrates. *Orthologues* as the homology is between individual genes and not families. They are even ordered identically on their chromosomes.

SRGAP2 duplicated twice and second duplication failed, producing ablated copy that suppresses original SRPGAP2 protein. This causes neurons to grow longer and coincided (2.4M years ago) with Austrolopithecus, increase in primate brain size and tool use.

How Evolution Uses this Structure

Protein encoding DNA between chimp and human almost identical. Evolution prefers to tinker with the netwwork of components rather than change composition of the component itself.

Heterotopy *Bat wings*. FGF suppresses BMP. BMP usually causes cells in digit webbing to undergo apoptosis. Bat wing webbing has *Fgf8*, keeping them alive and also promoting mitosis/growth.

Turtle shell. Turtle dermis has *Fgf10*: attracts ribs laterally (upwards) into dermis. Ribs produce BMP to undergo endochondral ossification (make bone). BMP in the dermis causes the skin to also become bone.

Flower petals. Recall angiosperm flowers are four concentric 'whirls': sepals, petals, stamen, carpel.

Rose: sepal {A, E} and petal {A, B, E} Tupal: petal/ {A, B} and petal {A, B}

Tulips drop the E that usually characterize sepals and differentiate from petals. They have identical classes in first two whirls, creating two rounds of 'tepal'

Heterochromy

- Neurons in human brain
- Jaws and forelimbs in marsupials (compared placental) so climb into maternal pouch
- Birds from growth of dinosaur skull
- Vertebrae + ribs in snakes. Oct4 from chromatin
- Prolonged Fgf8 responsible for long fingers in dolphin flipper

Heterometry

- Too much SHH in blind cavefish downregulates Pax6 (no eyes). Also increases jaw size and taste buds.
- class B gene determines sex in spinach plants (gender split into different organisms)

Finches. Ground vs Cactus (broad vs narrow beak). Neural-crest derived mesenchyme in the frontonasal section effected by timing and amount of Bmp4

Snakes. Actually make the limb buds but don't express the SHH to pattern it. Enhancer for SHH has deleted components and unable to react to eg. Hox to get expressed.

Heterotypy Why do insects only have 6 legs while most arthropods have many more?

Stretch of polyalanaine in *Ultrabithorax* (a Hox protein). This inhibits Distal-less transcription in the abdominal segments. Clade diagram with sequences super informative here.

Why did corn come from ancestral teosinte plants?

Single mutation in TF that usually creates a shell over the kernels.

Constraints on Development

We don't get arbitrary anatomy from evolution. Why is that?

1/ Physical 2/ Morphogenetic 3/ Pleiotropic

Don't think its very useful to frame Rather if models stop improving today, can you engineer your way to effective replacements. Assuming pre-training is fixed and existing post-training techniques are on the table.

Blood

HSC SP

self-renewal: ability of stem cell to produce a daughter cell that retains stem function

HSC Side Population Discovery

- 0.1% of marrow fraction 1000x enriched for repopulating HSC
- Both cycling and non cycling HSCs have self-renewal. Consensus at time was division "exhausts" self-renewal function

Definition of Clonal Hematopoiesis

https://www.nejm.org/doi/full/10.1056/NEJMoa1409405

Hans Clevers

- its hard to separate cancer and normal cells in organoid culture
- cancer cells are actually lousy proliferators but they won't stop

APC: regulates Wnt//beta-catenin signaling KRAS: GTPase that relays mitogenic signals. Downtream of receptor tyrosine. SMAD4: Mediator of TGF-\beta signaling. Destroy growth arrest effect of molecule while still expanding suppressive T_regs in TME TP53: encodes p53. DNA binding protein for repair,

You need the following growth factors in healthy cells but certain knockouts escape need:

Wnt: APC Noggin: SMAD4 EGF: KRAS

Cancer mutations provide clues as to the normal stem cell pathways that are hijacked

Story of epithelial organoids and BI

PhD thesis in Leiden. Hot wax molded against intenstine. Crypts: little pockets. Rapidly spit out daughter cells that exit hole in 2 days. Differentiate

- entero endocrine
- goblet
- absorptive cells

1989

\beta-catenin

1/ Cre-recombinase model that identified multi-potent properties of crypts

- Lgr5 (marker of
- LSL reporter (loxp-stop-loxp)
- Rosa26 locus

2/ Confetti mice

Function of Lgr5:

Wnt normally produces E3 ligases RNF43/ZNRF3 that add ubiquitins to the Wnt receptors (Frizzled + LRP5/6) LGR4/5 binds to R-spondin and sequesters RNF43/ZNRF3 from ubiquitnating

Wnt -> Frizzled + LRP5/6 -> \beta-catenin stabilization -> RNF43/ZNRF3

Paneth cells make essential growth factors for the stem cells. Needed to grow mini-gut.

- lysozymes + defensins (kill bacteria)
- WNT
- EGF
- Notch ligands
- Hepatocytes are hard to grow in culture
- But can show dramatic expansion when portion of liver is removed