

How to use these notes:

These notes are *not* intended to be a substitute for the lectures, and they do *not* present all of the information that was presented in the lectures.

What they are meant to do is to remind you of the main points of the lectures, and to present a framework that should help you relate the things you learned to each other, and relate the lectures to each other.

There are few pictures here, because you have access to the Impress files themselves at this website. It would probably be best if you used these notes with the Impress files available at the same time.

Please also note that the practical complements the lectures and is meant to help you become familiar with the developing mouse.

You can explore sections of mouse embryo on-line at <http://genex.hgu.mrc.ac.uk/Atlas/intro.html>

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Morphogenesis of the mouse 1 - overview

Website (slides, handouts, movies etc):

- go to <http://golgi.ana.ed.ac.uk/coursenotes/> and follow the links to db3.

Ex ovo omnia

- First, there is cleavage (mitosis with no growth).
- Then there is compaction (morula), followed by hollowing-out (blastula).
- The Inner Cell Mass forms inside the blastula (offset)
- This then hatches through the Zone Pellucida (and growth can start)
- The trophoblast invades the uterine lining to start making the placenta.
- The ICM divides into the hypoblast and epiblast (roots: *hypo*=under, *epi*=upon)
- The hypoblast migrates to make yolk sac
- The epiblast makes the embryonic epiblast and the amniotic ectoderm
- Embryonic epiblast cells stream to midline and pile up to form Hensen's node
- Gastrulation happens: cells migrate through the node and the streak left behind it and make endoderm and mesoderm.
- The embryo now has 3 layers, ectoderm, mesoderm and endoderm.
- Part of the endoderm (the bit that comes through the node itself) makes notochord, running along centre-line of the body.
- The ectoderm above the notochord thickens and invaginates to form the neural folds, that fuse to form the neural tube.
- Mesoderm to the sides of the neural tube form the somites, intermediate mesoderm and lateral mesoderm.
- Somites break down to make vertebrae, ribs, muscles and dermis
- Neural crest, at the dorsal-most part of the neural tube, scatters to form the PNS, adrenal medulla, facial tissues, melanocytes and tissues of various glands.
- The endoderm forms the gut tube, and its diverticula (lungs, liver, pancreas etc)
- The intermediate mesoderm forms gonads and kidneys (even their epithelial components).
- Mesodermal condensates differentiate into bones (via a cartilage intermediate) to produce the skeleton.

All of this is founded on about 10 basic mechanisms (see lecture 2 and 3).

-> Look at the website above for the pictures and movies.

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Morphogenesis of the mouse 2 – cell migration

Website (slides, handouts, movies etc):

- go to <http://golgi.ana.ed.ac.uk/coursenotes/> and follow the links to db3.

Cell migration is an important mechanism in the morphogenesis of vertebrates (including the mouse and human): it is so important, in fact, that Brian Hall has proposed that the migratory population known as the **neural crest** be considered a fourth germ layer (Pubmed ID 11256415), although this remains a heterodox view.

You learned in your second year MCI lectures that migratory cells produce a leading edge, consisting of a lamellipodium that is pushed forward by polymerisation of a network of actin microfilaments nucleated by ARP2/3, and often filopodia as well, which are also produced by actin polymerization (as bundles rather than a network). You also learned that different parts of the leading edge compete with each other to guide the cell forwards and that, presented with different substrates, one part will be more likely to 'win' and therefore guide the cell that way. Similarly, when presented with different concentrations of diffusible molecules that affect, indirectly, polymerisation and advance of actin, some parts of the leading edge will win and others lose, and the cell will therefore be able to make a choice of direction. If you have forgotten this, please look back at your MCI notes.

Within the embryo, navigation is achieved by navigating cells expressing receptors for molecules that are expressed by other cells, either surface (/matrix)-bound molecules expressed by cells along their path, or diffusible molecules expressed by their destination. An example is provided by the migration of primordial germ cells from the yolk sac, into the body, along the gut and into the gonad primordia. They are guided by 'general' adhesive matrix components such as laminin and fibronectin, and also by the surface-bound ligand SCF/Steel/Kitl that interacts with the Kit receptor tyrosine kinase expressed by the primordial germ cells themselves. Kit is also used by neural crest cells that are destined to make melanocytes in the skin; cells of the dermomyotome (part of the somite) express SCF/Steel/Kitl and, when these neural crest cells emerge, they express Kit and are attracted to the dermomyotome cells. The dermomyotome cells disperse to form the skin and switch off expression of SCF/Steel/Kitl mRNA, so the level of protein gently diminishes as the crest cells carry on migrating into the developing dermis. As the skin develops, the epidermis starts to express SCF/Steel/Kitl and the crest-derived cells migrate into it and settle down to form melanocytes.

The longest of the neural crest migrations is that which produces the enteric nervous system. Here neural crest cells express EDNRB and are attracted into the foregut by its ligand, EDN3 (which is expressed by the foregut cells). Once there, they also express Ret and are attracted by its ligand, GDNF, expressed first by the stomach and then further along the gut. They therefore migrate, as a wave of connected cells, caudally along the gut, dropping some cells off along the way: these cells later differentiate into the neurons and glia of the enteric nervous system. Mutations in these genes prevent the enteric nervous system from forming properly: in humans, Hirschsprung's disease – lack of peristalsis in the colon because there is no nervous system to drive it – is caused by mutations in Ret.

The wiring of the nervous systems (central and peripheral, including enteric) is achieved by the migration of growth cones that emerge from a neural cell body and leave the axon behind them (as a snail may leave a trail): the growth cone works in much the same way as other migratory cell, at least at the level of detail we need here. Growth cones can be guided either by attraction (chemotactic or haptotactic) or by repulsion. Repulsive influences, such as ephrins, cause the underlying cytoskeletal structures of growth cones to collapse; they do this by signalling via Eph receptors, via the small GTPases Rac, cdc42 and Rho, to the actin cytoskeleton. Repulsion can be used in an absolute way, for example by preventing retinal growth cones expressing EphB1 from crossing the ephrinB2-expressing midline of the CNS while growth cones not expressing EphB1 can cross. It can also be in a relative way, and this is used (again using Ephrins and Ephs) to sort out axons coming from the retina so that they map properly on to the optic tectum.

References for lecture 2:

- Davies JA (2005) *Mechanisms of Morphogenesis* section 3
- Gilbert S (2007) *Developmental Biology* – sections on the germ line and on the crest
- Heanue TA, Pachnis V (2007) *Enteric nervous system development and Hirschsprung's disease*. *Nature Reviews Neuroscience* 8: 466-480

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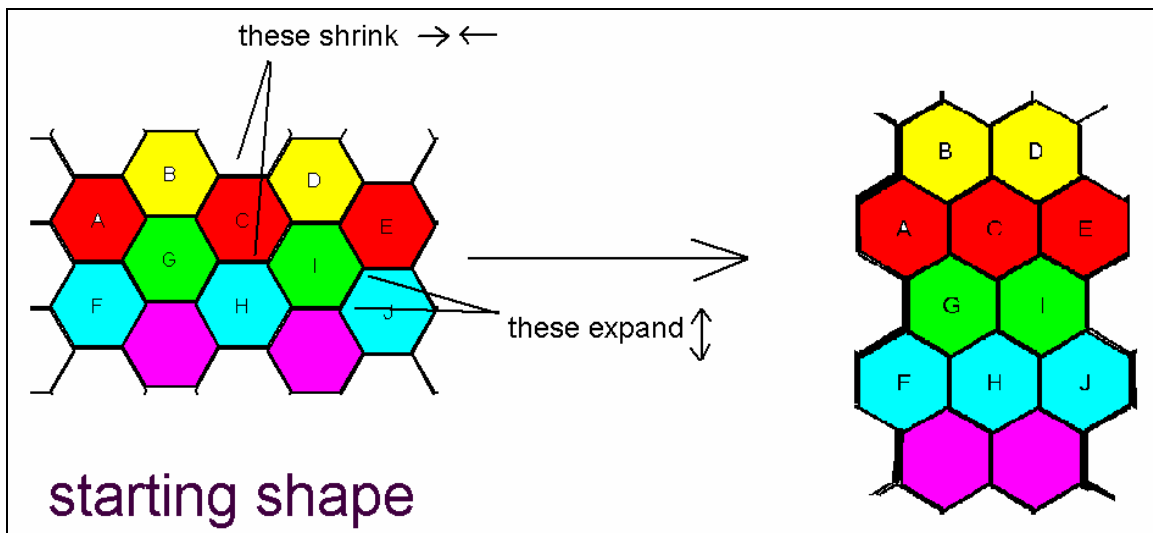
Morphogenesis of the mouse 3 – epithelial morphogenesis (rearrangement, folding, proliferation and death)

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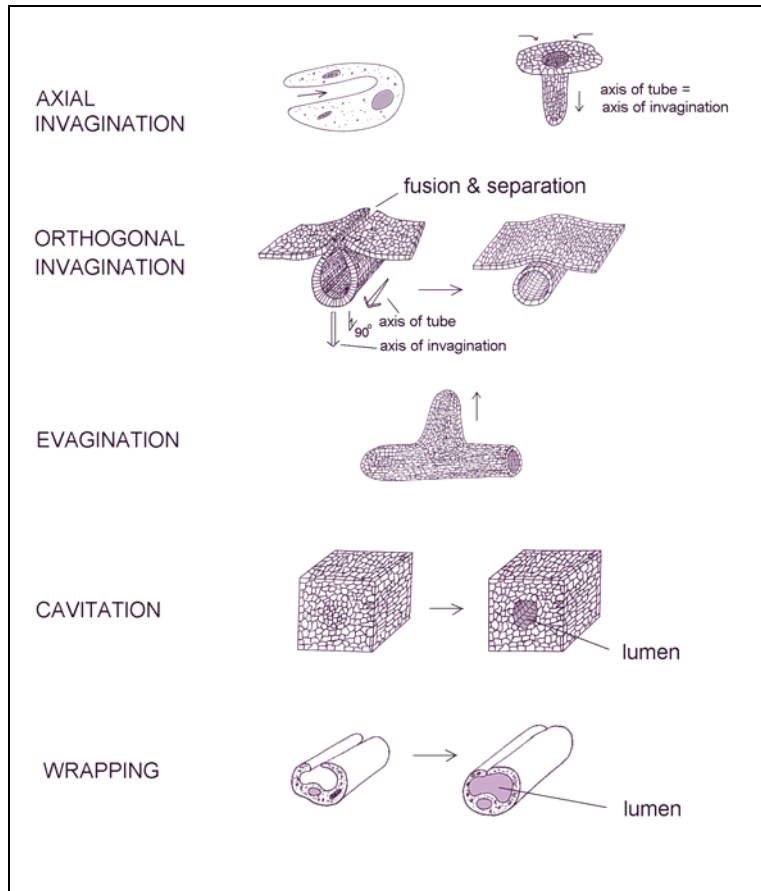
The last lecture focused on the role of guided cell migration in mammalian morphogenesis: this lecture concentrates more on epithelial cells that do not migrate independently but instead remain a sheet as they generate new shapes.

One common morphogenetic mechanism is convergent extension, in which cells in a sheet converge along one axis, causing the sheet to extend in the other (ie it goes from being short and fat to long and thin). Detailed time-lapse analysis of convergent extension in *Drosophila melanogaster* suggests that this works by the cell-cell boundaries that are arranged along the axis of convergence shrinking and those arranged along the axis of extension elongating, so that cells exchange neighbours;



Pic from Davies (2005) *Mechanisms of Morphogenesis* (Elsevier)

Tubes are generally formed either from sheets or pre-existing tubes, by cells becoming wedge-shaped so that their sheet undergoes tight local curvature. If this happens along an extended line, then a trough forms ('orthogonal invagination'), and this trough may become pinched off by epithelial fusion (see below), as happens in the formation of the neural tube and the penile urethra. If it happens in one place, then the result is the formation of a tube whose axis is in the direction of invagination (axial invagination). See diagram on next page. The driving force for cell wedging is unclear (it used to be thought to be contraction of the actin-myosin system at the apical end of the cell, but the observation that many systems invaginate perfectly even in the presence of actin destabilizing drugs now casts doubt on this. It may be that some proteins, such as Shroom, alter the spacing of cell-cell junctions and thus allow the apical end of the cells to shrink).



During mammalian morphogenesis, fusion of epithelia is a common event: it is used to create continuous tubes throughout the body (eg gut), to connect tubes to each other (eg excretory nephrons of the kidney to the urine collecting duct system, seminiferous tubules of the testis to the epididymis), to create separate tubes from orthogonal invaginations (eg neural tube and penile urethra) and to separate cavities (eg secondary palate). Fusion is achieved, as far as we know, by rearrangement of adhesive contacts and cell polarity. Its failure can cause common human congenital abnormalities such as spina bifida, cleft palate and hypospadias.

Epithelial branching happens by mechanisms similar to invagination, cells becoming wedge-shaped and causing a new tube to bulge out of an existing one. Endothelial branching to make capillaries can happen this way, but also happens by intussusceptive branching, in which the edges of a vessel invaginate inwards to meet and fuse, creating two small bores where one larger one existed before: doing this repeatedly makes lots of fine capillaries.

Orientated mitosis can be used to shape an epithelium: mitoses in the plane of the epithelium just make it grow, whereas those perpendicular to the plane create a two-layered structure. The inner one either falls away, as in the genesis of the inner cell mass of the early embryo, or stacks up as in the development of the rat endometrium under the control of oestrogen.

Elective cell death can be used to eliminate structures: an example is the 'webbing' between fingers. If this elective cell death in a chick leg is blocked, the chick gets webbed feet like a duck.