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2010 Workshop on the Development of Behaviour: Emergent Properties of Nervous Systems

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Early development

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Development: The Construction of Multicellular Organisms

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Introduction

This part of the Biology of Cells course deals with the mechanisms that allow cells to cooperate in groups to form multicellular organisms. We shall be considering both animals and plants and the two principle questions that underlie the development of all multicellular organisms:

Cell **differentiation** depends on cell specific patterns of gene expression - how are genes switched on and off in different cell types?

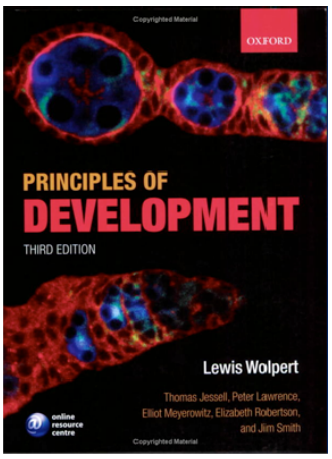
How does the right cell form at the right place - in other words how is cell differentiation organised so that **patterns** of cells are formed?

Lectures

The six lectures will cover the following topics:

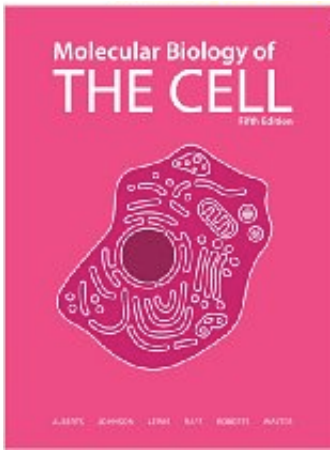
1. Early embryogenesis, early experiments, fate maps and how to clone a frog.
2. Regulating and initiating gene expression. How much information in an egg cell?
3. Induction cell signalling and positional information
4. How to make a limb. Fore limbs, hind limbs and homeotic genes
5. Plant development
6. Embryonic and adult stem cells

Suggested Reading

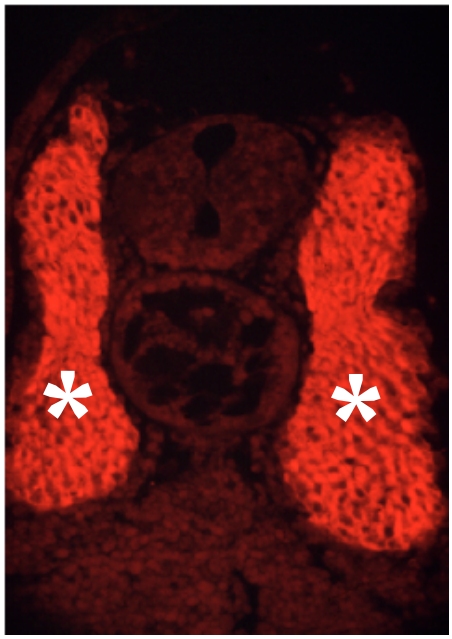


Principles of Development (OUP) by Lewis Wolpert and colleagues covers much of the material in these lectures

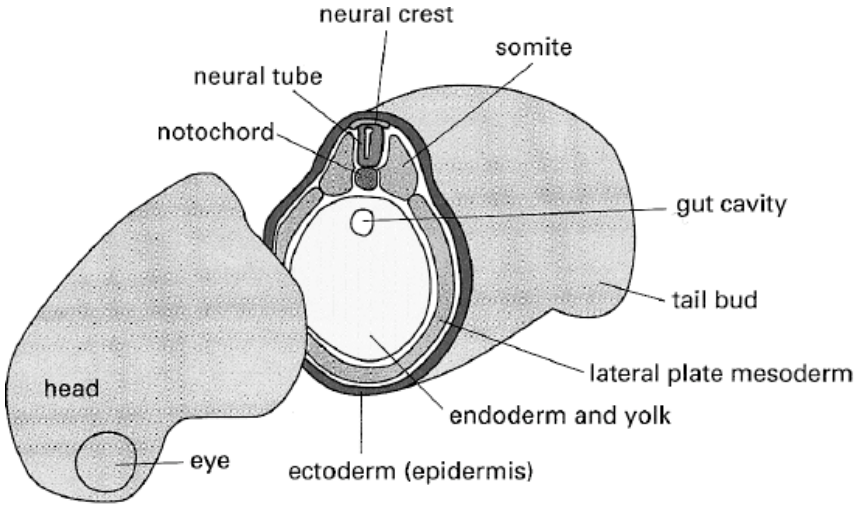
Chapter 22 of *Molecular Biology of the Cell* (Alberts, 5th Edition) provides an excellent summary of both animal and plant development. Many of the pictures and diagrams used in these lectures are taken from these two texts



An example of cell differentiation:

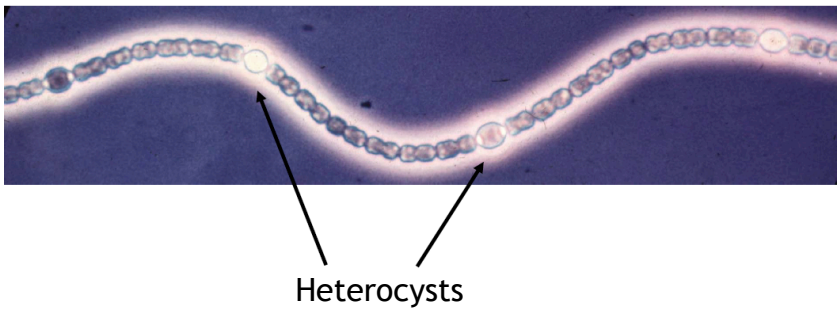


Cells of the frog somite (asterisks, left) express the gene for muscle myosin but their immediate neighbours in the skin and neural tube do not. How is this selective expression of genes by some cells in an organism but not others achieved?



Two simple examples of pattern formation:

At regular intervals, the blue green alga *Anabaena* forms cells called heterocysts, specialised for the fixation of nitrogen. How is this spacing controlled?



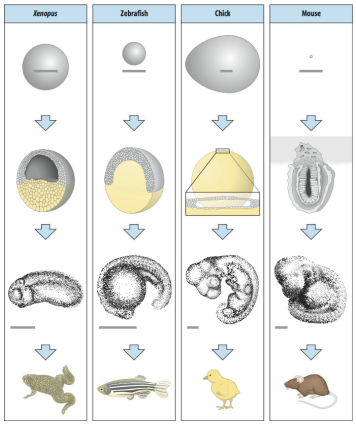
The coelenterate *Hydra* has a head with tentacles and a mouth for ingesting prey organisms. At the other end of its body is a foot for attaching to the substrate.

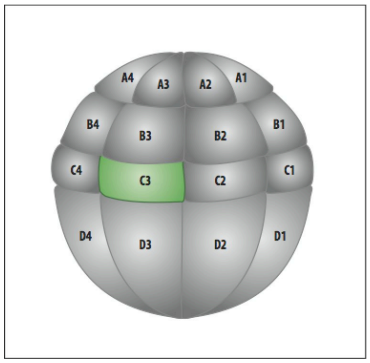
If *Hydra* is cut in half, the cut face of the foot end regenerates a head, while the immediately neighbouring cells that are now the cut face of the head end form a new foot.

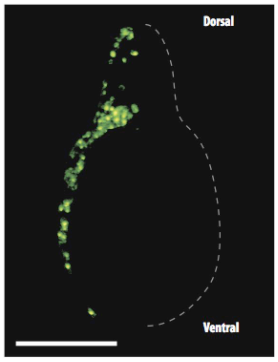
How do the cells "know" where they are in the two halves and thus form appropriate new structures?

Fate Maps: how an embryo organises its development

Eggs and embryos come in many different shapes and sizes but they all have one thing is common: the sequence and pattern of development is very regular.In the case of a frog (left hand column in panel) the egg cell divides to produce a ball of cells sometimes called a *blastula*

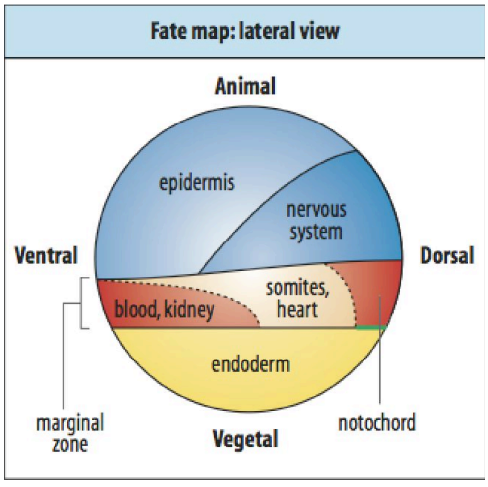






We can follow the fate of single cells and their descendants as they divide and ask what they contribute to the developing embryo. For example, by injecting a single cell in a frog blastula with a fluorescent dye we can find out which cells are labelled in the developing frog - in this case cell C3 and its progeny appear to have contributed to muscle and skin

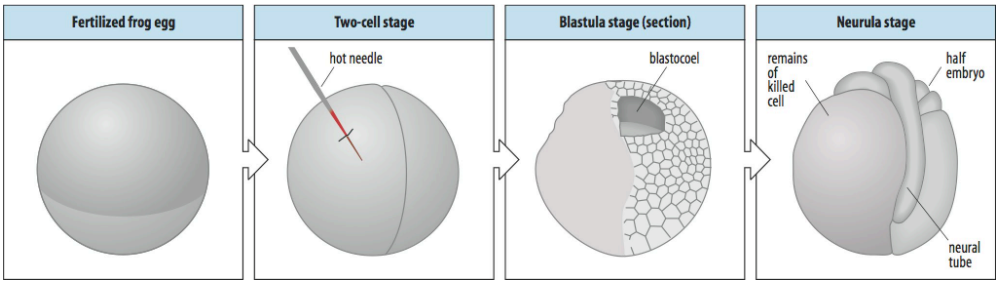
Because development is a regular process we can use many such experiments to build up a complete picture of what each cell will contribute to the final structure and map it back onto the early embryo.Such a map is called a **fate map** and it predicts the developmental fate of each cell in the early embryo. For example we can predict that in normal development cells of the upper dorsal part of the blastula will make the future nervous system



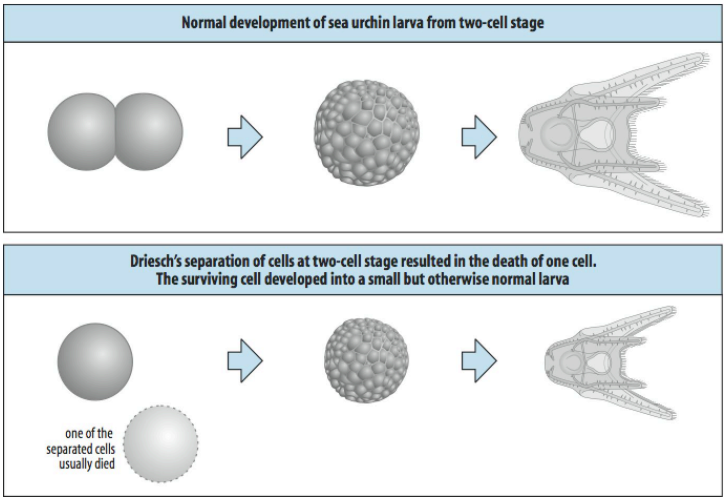
The key question of course is how do the cells “know” what to do? Where does the *information* come from?

The first experiments

The first serious attempt to answer this question was carried out by **Wilhelm Roux** – in the late 19th century. He used a hot pin to kill one of the two cells formed by the first division of a frog's egg. The results of the experiment appeared to be clear cut – if the left hand cell was killed, then only the right half of the embryo developed.



Roux concluded that the *information* necessary to make the embryo was partitioned at each cell division – in this case the information for the left half had been destroyed, so only the right half could form. But his experiment and his conclusions were flawed – by the fact that he hadn't removed the dead cell, which blocked the normal development of the embryo on that side.



A much better experiment was done shortly afterwards by **Hans Driesch** – who used the embryo of a sea urchin. Driesch *separated* the first two cells formed by the dividing egg (or the first four cells formed by two divisions) and cultured them individually. To his amazement he found that any of these cells on its own was capable of forming an entire sea urchin embryo and larva.

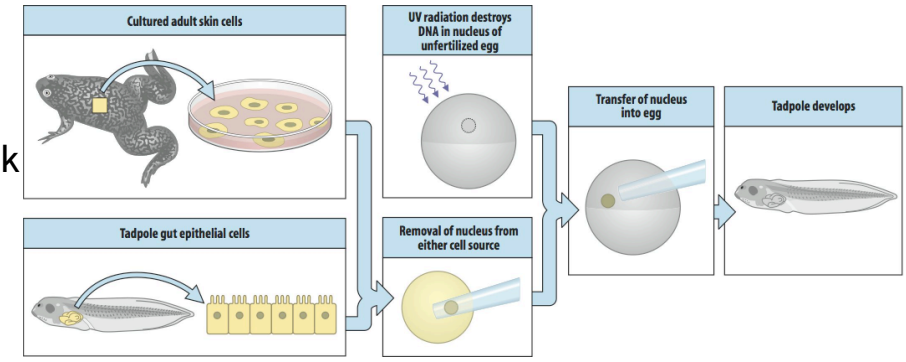
Driesch was baffled by his findings. The early embryo seemed to have extraordinary properties: a self organising system and furthermore a system that could organise and reorganise its own construction even if it was cut in half.

It is this remarkable property of multicellular organisms that developmental biologists seek to understand. Driesch would certainly have enjoyed what has been discovered in the hundred or so years since his experiments and even more the fact that there is still much that we don't understand.

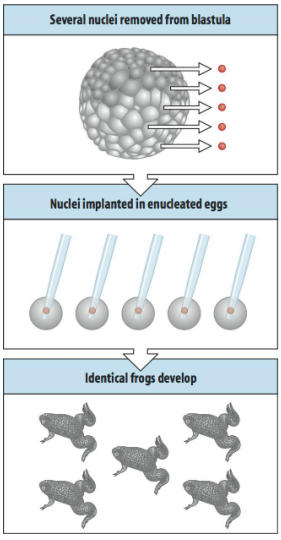
Nuclear transplantation, or how to clone a frog

We now know that all the information necessary to build an organism is inherited and present in the DNA of the original egg cell. The first question we should ask therefore is whether any of this information is lost as embryos develop. Do skin cells for example specialise in making skin specific proteins rather than those of the liver, because they lose genetically encoded information necessary to make other cell types?

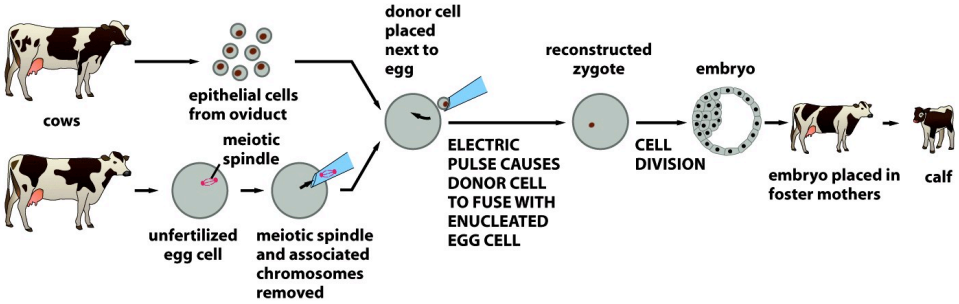
The best way to answer this question is to take the nucleus of a differentiated cell and ask it to programme the whole of development by putting it back into an enucleated egg cell.



Experiments carried out by John Gurdon show that the nucleus of a differentiated cell such as a skin cell is capable of orchestrating the complete development of a tadpole - so genetically encoded information is clearly not lost as cells differentiate and undertake specialised programmes of gene expression.



With techniques like these it's possible to make a *clone* of genetically identical frogs or indeed to clone a cow from the nucleus of an adult cell



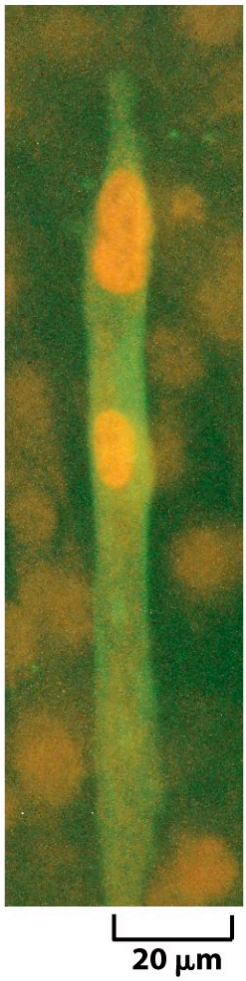
All such experiments lead to the same general conclusion: there is no permanent loss of genetically encoded information from differentiated cells. *Cell differentiation therefore depends on the selective expression of parts of a full set of genes.*

Nucleus and cytoplasm

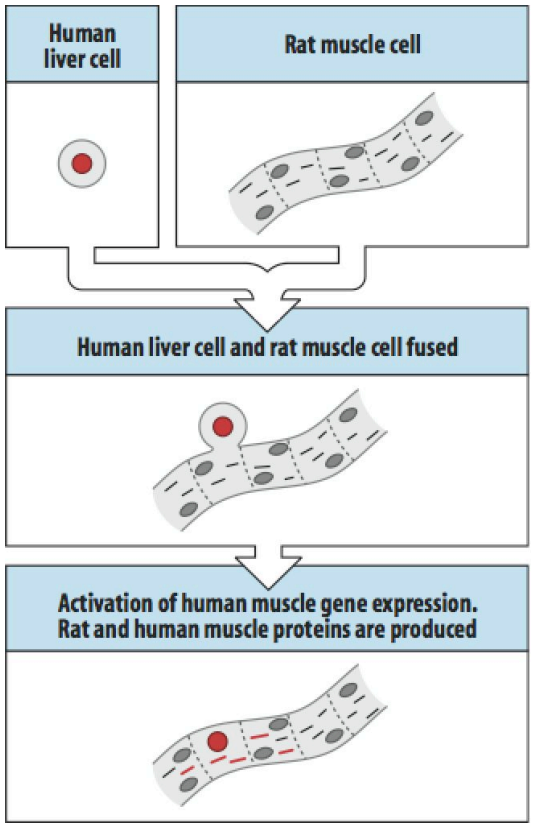
To understand the selective expression of genes in embryonic development we start by considering the exchange of information between nucleus and cytoplasm. The relationship between genetically encoded information in the nucleus and regulatory molecules in the cytoplasm that surrounds it is a key to understanding cell differentiation and its control.

As we would predict from the nuclear transplant experiments, exposing a differentiated cell nucleus (eg skin) to the cytoplasm of a foreign cell (eg an egg) can change its pattern of gene expression.

Nuclei can be exposed to foreign cell cytoplasm by experiments in which different cell types are caused to fuse by exposure to chemicals or viral suspensions. For example chick red blood cells have nuclei that are completely inactive transcriptionally, but if fused with a human cancer cell, the blood cell nucleus enlarges and chick specific proteins begin to be synthesised in the fused cell: clearly factors transferred through the cytoplasm have reversed the inactivity of the nucleus and transcription of the chick genome is reinitiated



In a similar but more specific way, if a human liver cell is fused with a rat muscle cell (a large multinucleate cell, left), the expression of human liver specific genes is turned off and the transcription of human muscle specific genes is switched on. The expression of both human and rat muscle proteins can be detected in the fused cell (right).

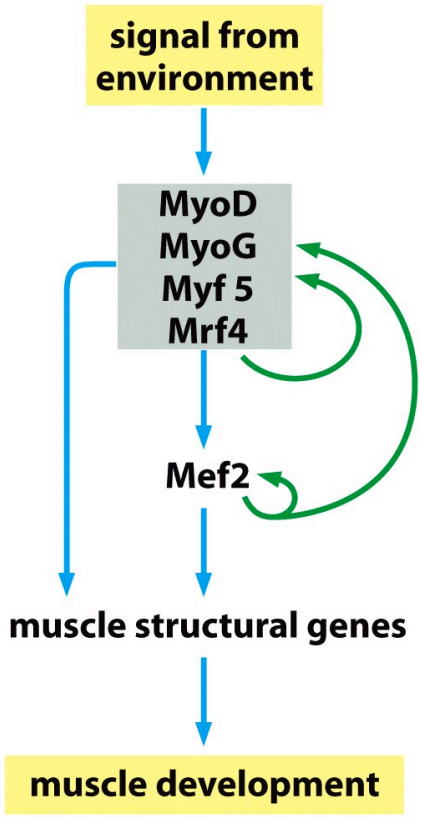


MyoD and gene regulatory networks

These results and other like them show that gene expression is regulated by factors present in the cytoplasm of differentiated cells. What are these factors?

Weintraub and colleagues set out to answer this question for muscle by transfecting cells with cDNAs made from mRNA taken from proliferating myoblasts. (They reasoned that some of these muscle specific mRNAs would be for regulatory proteins controlling muscle cell differentiation)

What they found was rather striking: a single cDNA (for a gene they named MyoD - **myo**genic **d**ifferentiation gene)if transfected into differentiated fibroblasts would cause these cells to switch fate and become muscle cells. MyoD encodes a DNA binding protein - a transcription factor that binds to the upstream sequences of muscle specific genes and activates them. It also activates its own transcription - so once the MyoD gene is activated, the cytoplasmic synthesis of MyoD protein ensures that a stable feed back loop operates to maintain the expression of muscle specific genes.



As you can see (left) we know a great deal more now about the regulation of muscle gene expression than when Weintraub did his experiments (1987)

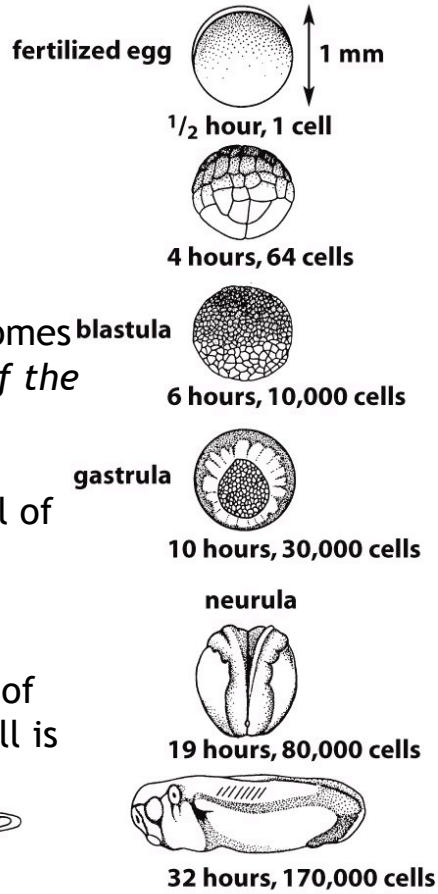
There are many more transcription factors involved than just MyoD and the combined effect of these factors and other genes downstream of them is to create a complex gene regulatory network. Analogous networks regulate selective gene expression in other differentiated cells. The presence of multiple feedbacks means that the differentiated state, once established, is very stable.

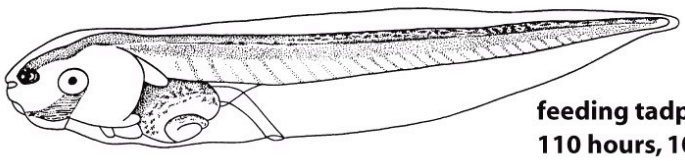
What we have to understand is how such stabilising loops are *initiated* as the embryo develops. In the case of MyoD, a signal leads to the onset of muscle differentiation and we will touch on this in a later lecture, but for now we need to go back to the beginnings of development and see how the relation between nucleus and cytoplasm begins

Cleavage and the mid blastula transition: the beginnings of embryonic development

Fertilisation releases a programme of rapid cell division and DNA replication known as *cleavage*. In both *Xenopus* and the fly *Drosophila* cleavage results in each newly replicated set of chromosomes becoming associated with a *different fraction of the original egg cell cytoplasm*. The process is significantly different in the two organisms-in *Xenopus* the egg cell is simply divided into a ball of cells. In *Drosophila* nuclei are first replicated without cell division - they then migrate to the surface of the egg where each nucleus becomes surrounded by a cell membrane. The end result of fractionating the original contents of the egg cell is the same however.

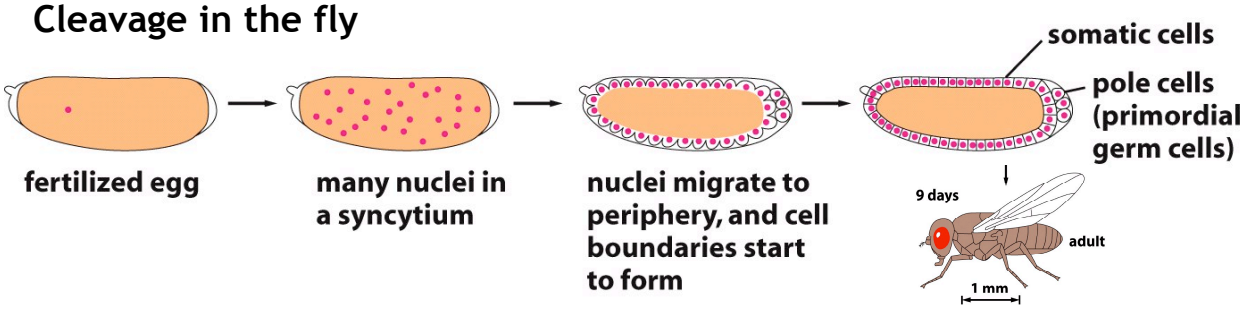
Cleavage in the frog



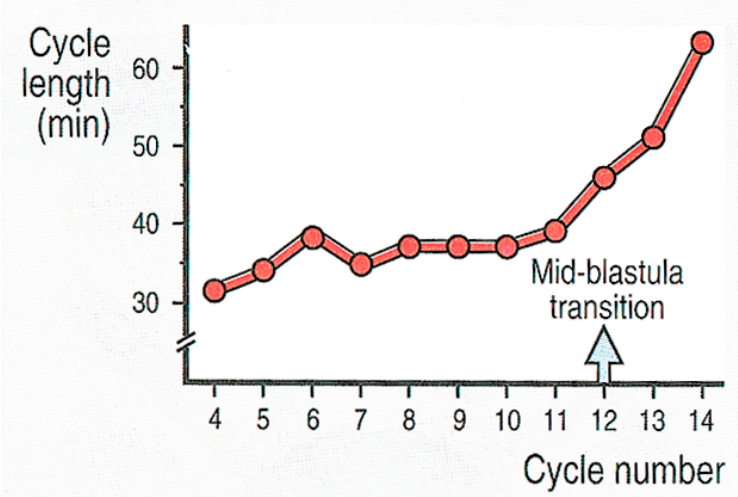


feeding tadpole
110 hours, 10^6 cells

Cleavage in the fly

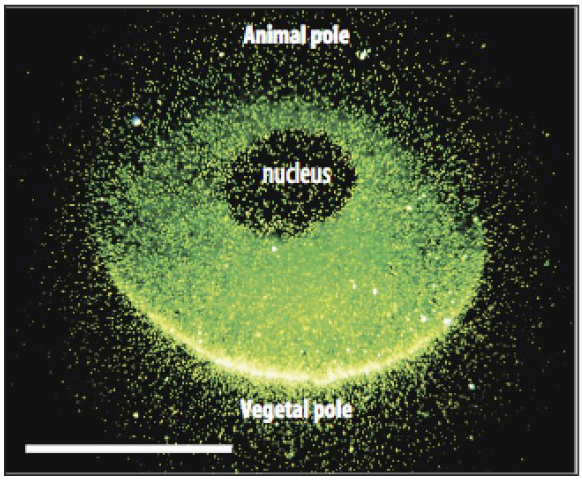


The whole process of cleavage is programmed into the unfertilised egg cell in the form of *maternal* gene products put into the egg by the mother. These products (RNAs and proteins) are sufficient to carry the embryo through the early rounds of cell division and nuclear replication.

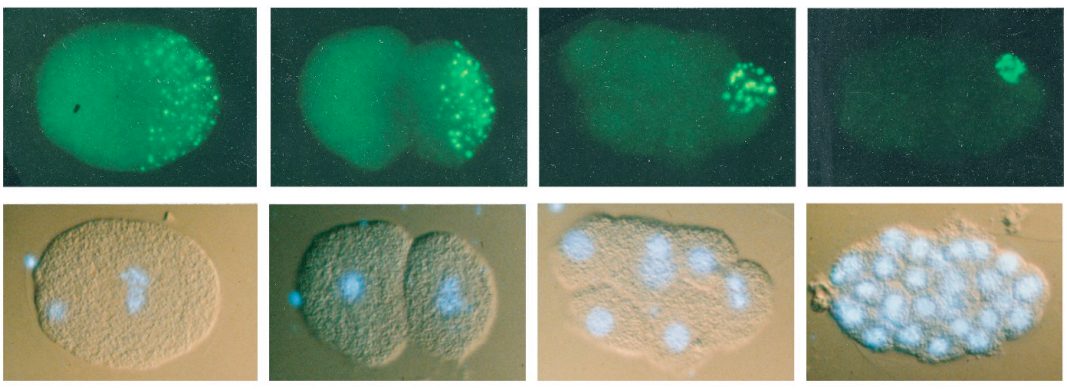


Transcription of the embryonic (*zygotic*) genome doesn't begin until cell cycle 12 in *Xenopus*. This event, when the embryo begins to take charge of its own development is know as the **mid blastula transition**. Not only does transcription begin but the cell cycle lengthens to include a growth phase rather than repeated cycles of replication and division. A similar event occurs in *Drosophila* and many other embryos

It's not hard to show - using antibodies and in situ hybridisation that proteins and RNAs are unequally distributed in the egg cell and partitioned by cleavage. For example, Vg1 transcripts are localised to the vegetal pole of the frog egg (bright fluorescence).

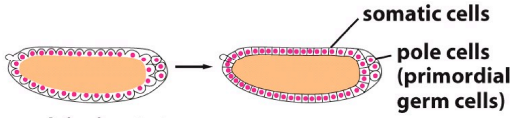


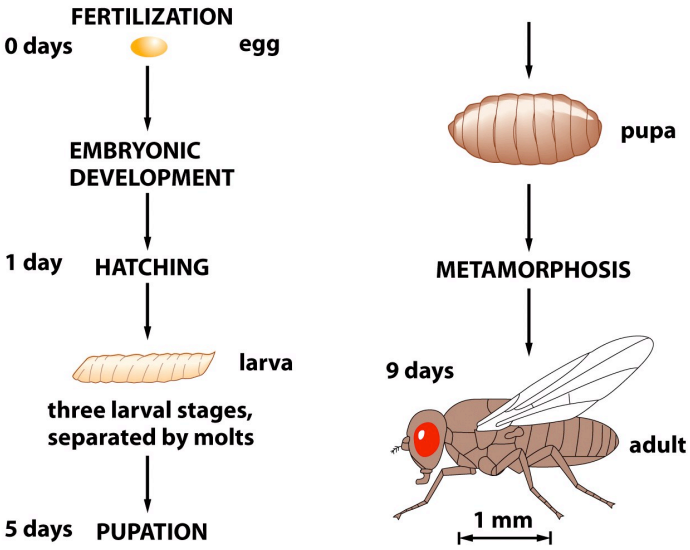
And in the nematode *C.elegans*, there is a progressive segregation of protein granules (green, upper panels) into the germ cells, as the egg cell divides (DNA, blue, lower panels) and this begins with an asymmetric distribution of the granules in the egg cell before it begins to divide.



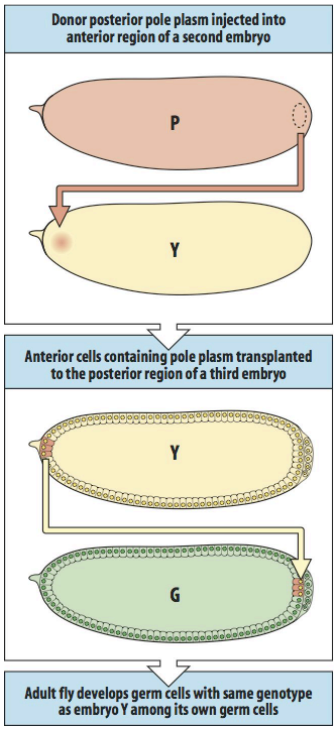
Cytoplasmic determinants in egg cells

Drosophila (full life cycle shown right) provides us with a simple test of the notion that the unequal distribution of proteins and RNAs in the egg cytoplasm might be a critical determinant of the fates of the cells generated as this cytoplasm is subdivided by cleavage. Notice (below) that, as in the nematode, the germ cells in the fly are produced by cells at the posterior pole of the embryo.





Uniquely, these “pole” cells include in them the cytoplasm of the most posterior part of the egg. Is there something special about this part of the egg cytoplasm that actually dictates the formation of germ cells rather than any other cell type? The experiment illustrated on the right shows that there is. Posterior cytoplasm is transplanted to an anterior position in another egg . Nuclei at this position normally contribute to head structures but as the embryo cellularises these anterior cells include posterior egg cytoplasm. Do they now make germ cells rather than head structures? Yes, they do as the experiment in the bottom panel shows. This experiment shows clearly that there is something in posterior egg cytoplasm that dictates germ cell production when it is included in forming cells We now know that this is the product of a gene called oskar. You should think about the controls (which were done!) that would required to make this a convincing interpretation. For the moment however we need to think about how we can find out what (apart from a germ cell determinant, which we now know is the product of a gene called *oskar*) is put into the forming egg cell by the maternal genome and how we can identify these factors molecularly.

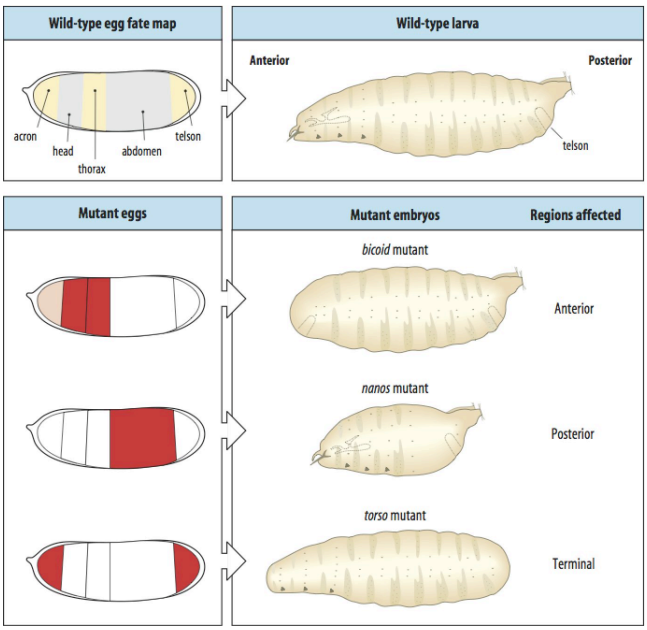


Maternal Effect Mutations and the Information Content of the Egg Cell

The best way of finding out about what is present in egg cells is based on a simple premise that turns out to be very powerful: Whatever the mother puts into her egg cells must be encoded in the maternal genome. Therefore if we systematically create mutations in the maternal genome and screen for mutations that lead to the production of defective eggs and embryos we will identify all the genes concerned and we can show how their products operate in egg cells.

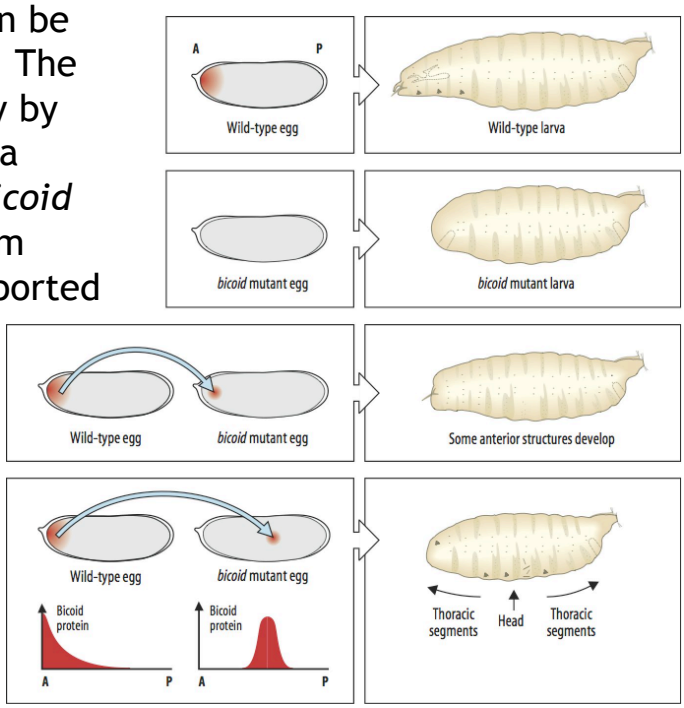
Notice that what we are looking for here are so-called **Maternal Effect Mutations** - that is mutations that have their effect *only* when they are present in the mother but have no effect if they are carried by the father. So:

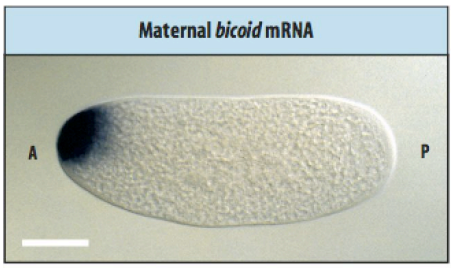
Female		**Male**		**Femal**		**Male**
Mutation/Mutation	X	Wild type/Wildtype		Wild type/Wildtype	X	Mutation/Mutation
Mutation/ Wildtype ***Defective*** Embryo				Mutation/ Wildtype ***Normal*** Embryo		

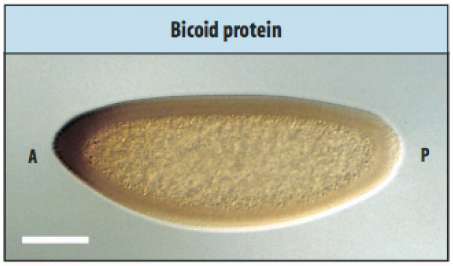


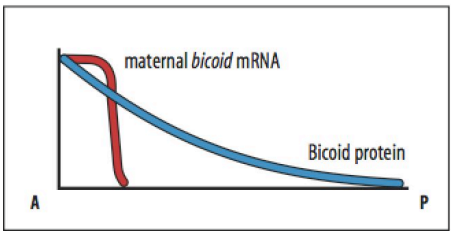
On the left you can see examples of mutant phenotypes recovered in a screen for maternal effect mutations in *Drosophila*. In this case the genes encode products required for the normal development of structures along the antero-posterior axis. *bicoid*: anterior structures, *nanos* posterior structures and *torso* for the terminal parts of the embryo. In each case, the embryos produced by mutant mothers lack these particular structures

The technique of cytoplasm transfer can be used to show how genes like *bicoid* act. The *bicoid* phenotype can be rescued simply by adding wild type anterior cytoplasm to a *bicoid* mutant egg. And the idea that *bicoid* encodes something in anterior cytoplasm necessary for anterior structures is supported by the finding that if the anterior cytoplasm is transferred to the middle of a *bicoid* mutant egg then head structures form there. As the gradients in the diagram suggest, the active substance is the Bicoid protein, synthesised from maternal *bicoid* transcripts localised in the anterior part of the egg cell. This idea is confirmed by the visualisation (below) of bicoid RNA and protein in the egg cell - nicely localised to the anterior pole and diffusing away from it in a graded fashion.







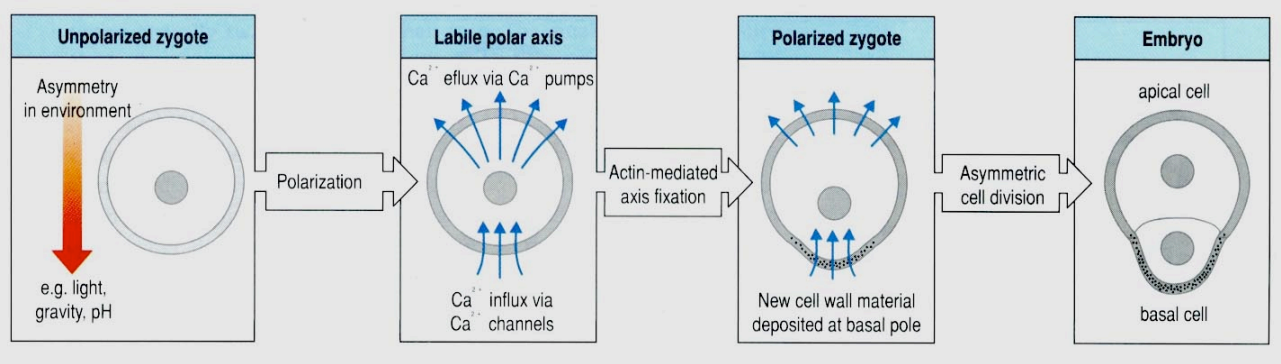


So, if Bicoid is sufficient to trigger the formation of anterior structures, how many other such factors does the maternal genome encode and put into the egg? Or to put it another way, how much information is there in the egg cell? Does it contain a fine grained map of determinants for all the future structures of the larva that will hatch from the egg cell? The screen for mutations answers this question clearly and decisively: there is no such map. Mutations of just four classes are recovered: causing defects a) in the formation of germ cells (eg *oskar*) b) the anterior-posterior axis (eg *bicoid*) c) the dorso-ventral axis(eg *dorsal*) and d) the termini of the embryo

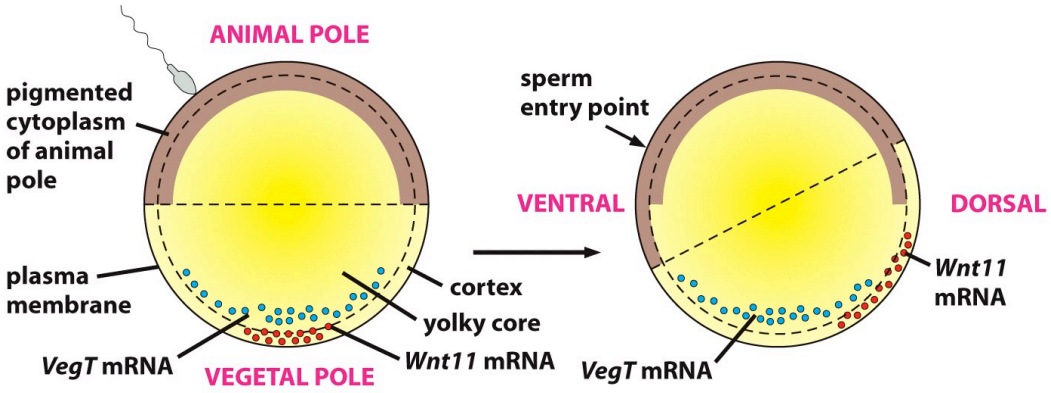
It seems therefore that the information content of the egg cell is quite low. The maternal genome simply sets the coordinates of future development by laying out the anterior-posterior and dorso-ventral axes. The fine grained details (ie the assignment of cells to form particular structures along these axes will be filled in later as the zygotic genome becomes active. For the moment we need to ask how you can put an axis in an egg cell?

Putting axes into egg cells: laying out the coordinates of the embryo

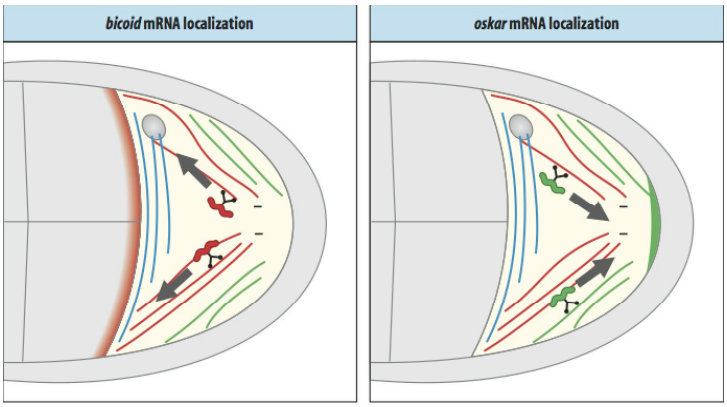
How do you polarise an egg cell? It's an interesting question because it really asks how do you start to get the singularities in an otherwise uniform cell from which all subsequent differences will unfold. How does it begin?

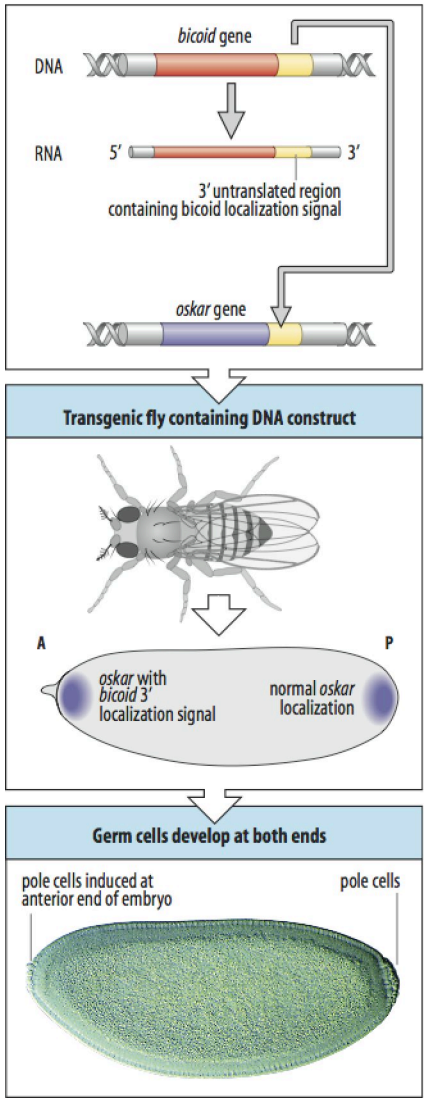


The common sea weed *Fucus* gives us one answer. *Fucus* sheds eggs into sea water which are then fertilised. The fertilised egg is a uniform sphere for the first few hours but then polarises so that at the first division one cell is apical and will develop to produce the frond of the sea weed whereas the other is basal and will make the holdfast. The apico-basal polarity is set as the cell responds to environmental cues by redistributing pumps and leaks for Ca^{++}, so that the formerly uniform flux of Ca^{++} across the membrane becomes focussed as a current flowing in the apicobasal axis.

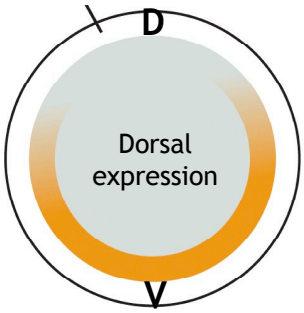


The two axes of the frog's egg are set differently. The *animal vegetal* axis is probably set by gravity: as the egg floats in the water, the yellow yolk inevitably falls to the lower pole of the egg, leaving less yolky cytoplasm towards the animal pole. Transcripts like the VegT RNA are associated with the yolk at the vegetal pole. Others such as Wnt11 are closer to the plasma membrane in the cortical cytoplasm. The dorso-ventral axis of the egg is set in response to the entry of the sperm: as the sperm enters, it triggers a mechanism in the egg that rotates cortical cytoplasm so that vegetally located transcripts such as Wnt11 are shifted away from the entry point. This asymmetric redistribution of egg cell contents sets the future dorso-ventral axis of the embryo.

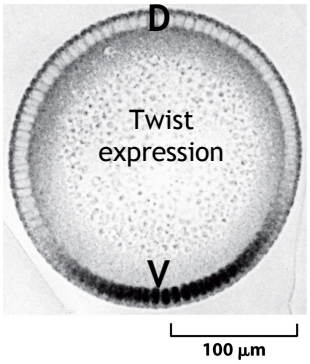




The Bicoid and Oskar proteins segregate to opposite ends of the developing egg cell because they are transported in opposite directions along the microtubular skeleton of the oocyte: Bicoid to the anterior, Oskar to the posterior. The specificity of this interaction depends on the 3' untranslated regions of the transcripts. The experiment on the right shows Oskar localises with Bicoid if the Oskar 3' UTR is replaced with that of Bicoid and, as expected, in this case pole cells are induced to form at the anterior end of the embryo.



Cross sections through Drosophila egg

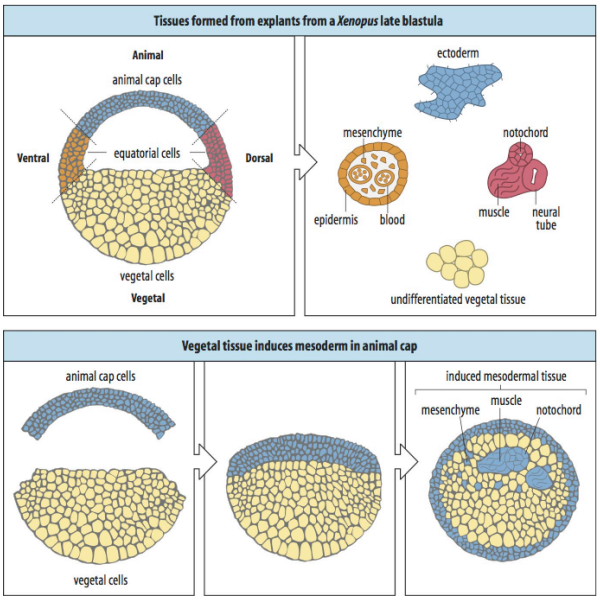


Many of the early maternal products in the *Drosophila* egg are transcription factors, so they regulate gene expression in the cells in which they are included. So, for example, the graded distribution of the maternally derived transcription factor Dorsal in the dorso-ventral axis (high ventrally **V**, low dorsally **D** upper left panel) leads to the activation of the zygotic gene *twist* (lower left panel)in the nuclei of the most ventral cells. Expression of *twist* causes ventral cells to form mesoderm.

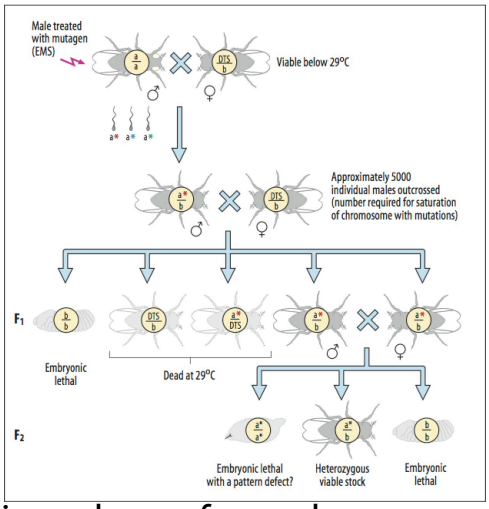
Filling in the details: cell/cell interactions and induction

Although the axes of the egg cell can be set by distributing maternally encoded transcription factors that will activate gene expression in the cells that include that fraction of the egg cytoplasm, the details of the embryonic pattern are filled in only once the embryo has cellularised and at this point cell/cell interactions become crucial in dictating patterns of cell differentiation.

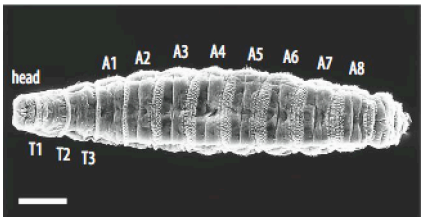
For example the amphibian fate map tells us that mesoderm in the frog embryo forms from a strip of cells running round the equator between the animal and vegetal halves.How do these cells get uniquely allocated to this fate?

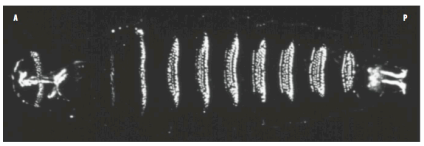


The experiment diagrammed on the left shows that it certainly isn't because mesodermal "determinants" are distributed in this equatorial band of the egg cell. Animal and vegetal halves of the embryo exclusively form ectodermal and endodermal structures if cultured separately. Mesodermal cell types are formed only where the two halves of the embryo come into contact. Cells of the most animal pole can be **induced** to form mesoderm if they are experimentally placed in contact with vegetal cells. Thus vegetal cells are the source of a signal(s) that "tells" animal cells to make mesoderm. There are in fact several such signals, one of which is Vg1, which we earlier saw localised as a transcript in the vegetal half of the undivided egg cell.Vg1 encodes a signal of the TGFb family of (which you should encounter in the next set of lectures).

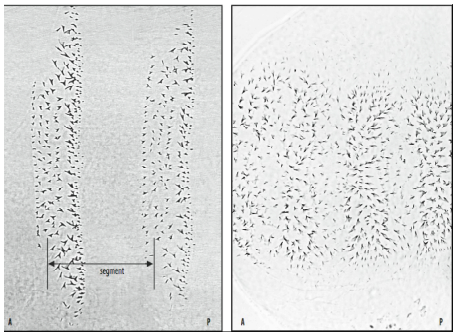


One way of finding all the elements of the machinery necessary to pattern the developing embryo (ie all the different signalling pathways and regulators of gene expression) is once again to carry out a genetic screen. We have already seen how a screen for maternally acting genes revealed the machinery for aptterning an egg cell. Now we need to screen the zygotic genome for genes required for normal embryonic development ie we need to look for genes that block development and cause embryonic lethality. On the right is acrossing scheme for such a screen. The people who did this won a Nobel Prize - on the next page you'll see why their work was so important.



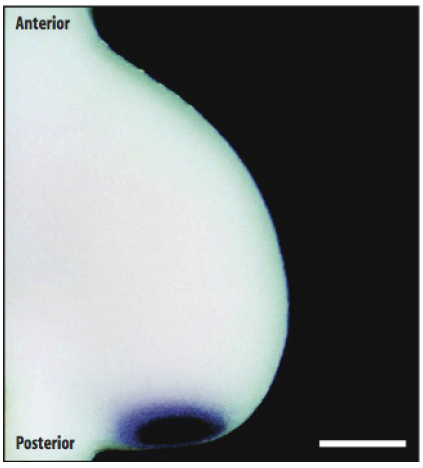


The researchers who did this work used the regular pattern of denticles that develops on the underside of each segment of the *Drosophila* larva as a simple way to look for aberrations in the normal pattern of development.The larva uses the denticle bands as "feet" which engage with the substrate as it crawls. On the left you can see the denticle pattern in a scanning micrograph (upper panel) and a dark field image (lower panel). By systematically screening for mutations that disrupt this pattern they discovered many of the genes needed for normal development and they could then show that these genes encoded elements of signalling pathways and regulatory networks controlling gene expression. For example on the left you see a normal pattern of denticles and on the right the denticle pattern in a gene mutant for the Wingless signalling pathway. They had found many of the factors required to build a maggot. But why a Nobel Prize for a maggot!?



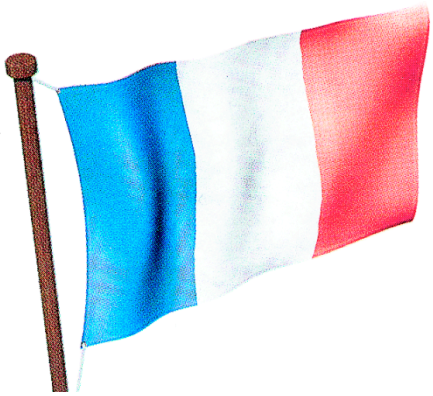
Developmental mechanisms are conserved in evolution

The answer is simple but really important: the basic toolkit for building an organism is largely conserved across the animal kingdom: the same genes that are used to build a fly or a worm are also used to build a fish, a mouse and you and me. So by studying a fly they had found many of the basic mechanisms needed to construct a human being.

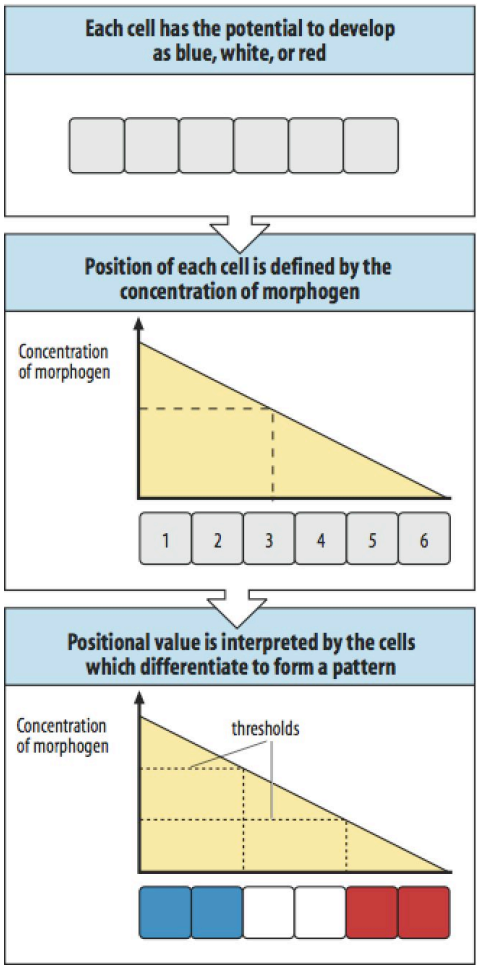


On the left you can see a striking example of this point. One of the genes found in *Drosophila* was called *hedgehog* because when mutant it produced dead embryos with a prickly pattern of denticles. Once the gene was discovered, it could be shown that it encoded a diffusible signalling molecule important for patterning the segment. By homology, the same gene was found in vertebrates and called *sonic hedgehog*. Sonic hedgehog is a key signalling molecule for the normal development of many structures in vertebrate embryos, including the central nervous system and the limbs. The figure shows the developing limb growing out from the flank of chick embryo (it could equally well be a mouse or a human). An in situ hybridisation (dark staining) shows the location of Sonic hedgehog transcripts specifically expressed by cells at the posterior margin of the limb bud and from here Sonic diffuses anteriorly as a graded signal, which as we shall see is critical for the patterning of the limb in this axis.

Positional Information and Pattern Formation



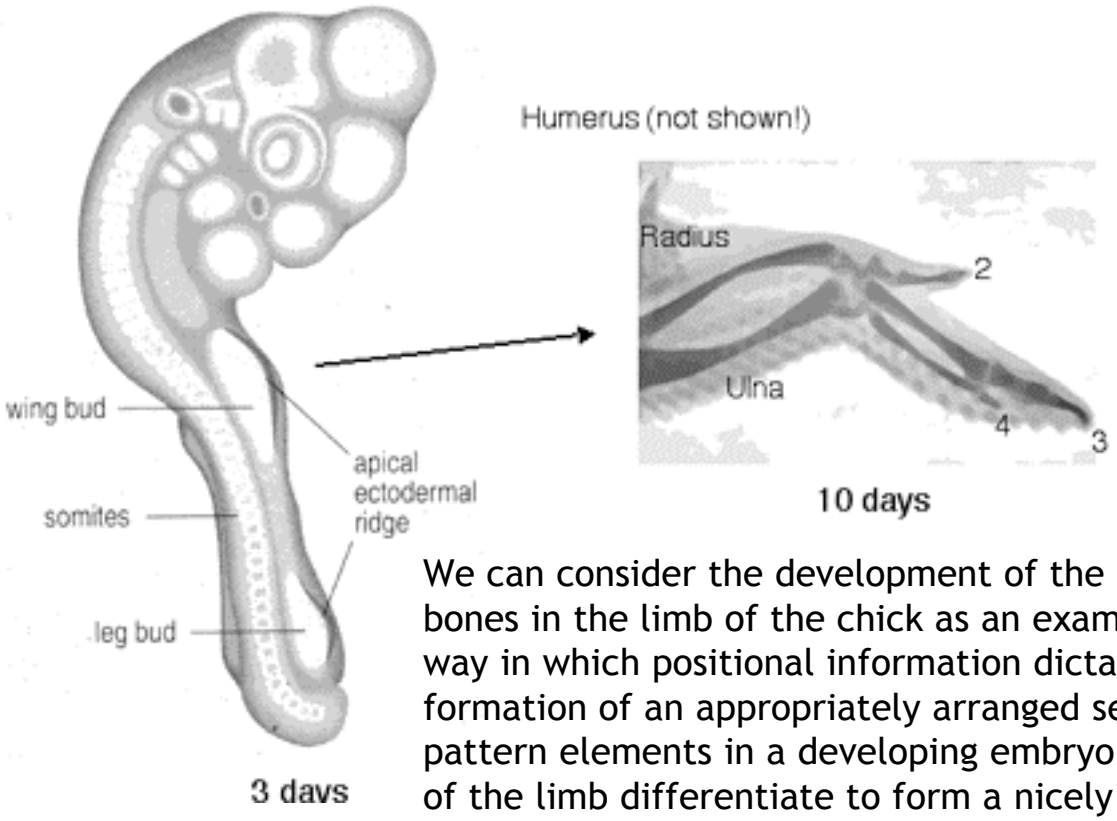
As we have seen, there is information in the cytoplasm of egg cells which regulates the expression of the zygotic DNA. However this information is sufficient only to lay out the ground plan of the embryo, principally by setting the coordinates within which cells will differentiate. The details of the embryonic pattern have to be filled in by additional mechanisms which assign cells to particular pathways of differentiation by their relative position in the developing organism. Genetic screens in the fly have revealed the details of many of these mechanisms - but how do they operate to produce the myriad patterns of cell differentiation we actually find in developing organisms?



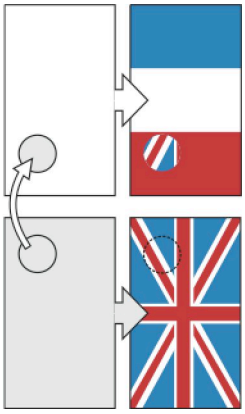
Lewis Wolpert introduced a very useful way of thinking about this problem (left). He suggests that as organisms develop they subdivide themselves into groups of cells (fields) separated by boundaries. Each field will be dedicated to making a part of the final structure such as an arm or a leg. Such fields generate information about position, perhaps in the form of a gradient of a diffusible substance established between the boundaries. Cells assess their position by reference to the local level of the gradient and differentiate accordingly. An important aspect of the model is that all fields generate the same information, but the cells of each field have access to a different part of the genetic programme (eg to make a hand or to make a foot). A thought experiment would be to imagine cells in a field with the genetic programme to make the French Flag. At levels 4 and 5 the cells read the gradient and make white and blue respectively. Another group of cells is programmed to make the US flag - they generate an identical gradient, but at levels 4 and 5, the cells do something different - they make stripes

Of course we are not only going to have to find evidence that this positional information exists, but we will also need convincing evidence that cells are indeed assigned in groups to make limited parts of the final organism. We'll begin by considering as an example the development of limbs in vertebrate embryos

The chick limb as an example of pattern formation

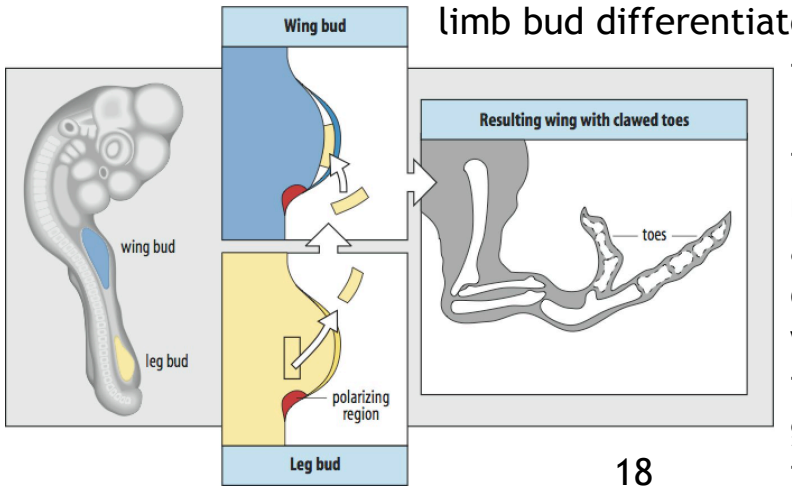


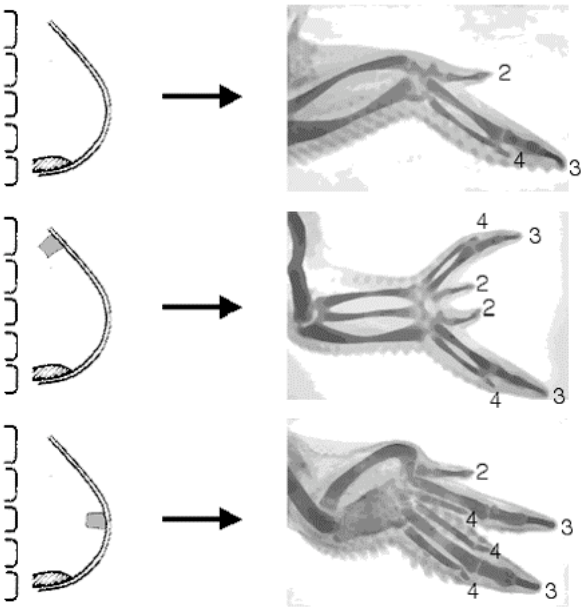
We can consider the development of the pattern of bones in the limb of the chick as an example of the way in which positional information dictates the formation of an appropriately arranged set of pattern elements in a developing embryo. The cells of the limb differentiate to form a nicely demarcated set of pattern elements: the bones of the limb in a proximo-distal sequence (humerus, radius/ulna,wrist, digits and the antero-posterior sequence of digits (2,3,4)



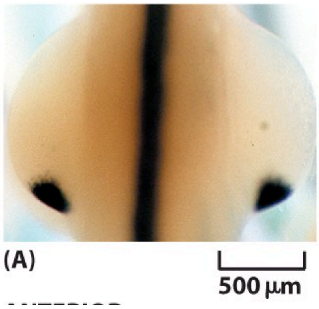
Wolpert's French Flag model predicts that two fields of cells making different flags will have the same positional information. Therefore cells from one group transplanted to the other (left) will be able to "read" their new position and differentiate appropriately but following the programme to which they have access.

Remarkably, this is exactly what happens when cells are transplanted between limb buds: hind limb cells in a fore limb bud differentiate according to their position in the limb bud but they make hind limb structures. This indicates that two important aspects of the model hold in this case - cells are assigned in groups to make different parts of the organism - wing or leg (How? See later) and the positional information in each group is the same, so how is this information generated?

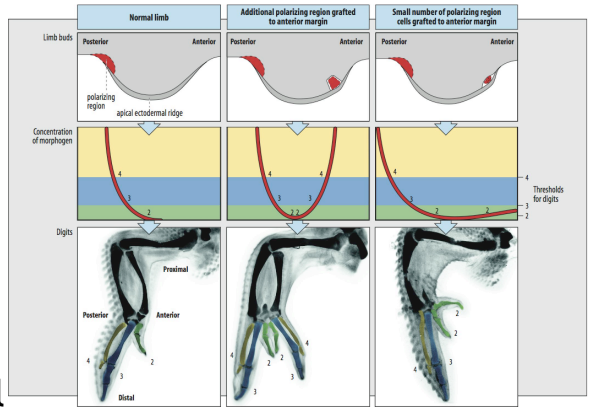


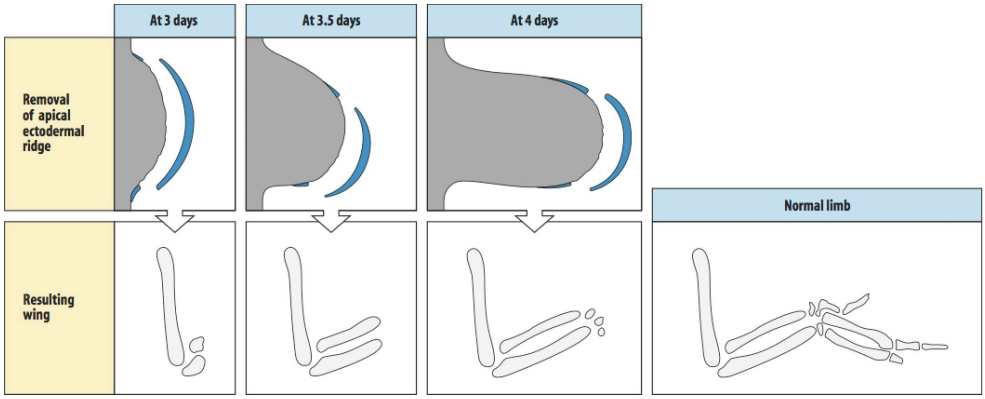


Early experiments involving transplants showed that cells measure their position in the antero-posterior axis of the limb bud by reference to a posterior region of the limb known as the *zone of polarising activity*. The zpa generates a gradient of a substance which diffuses across the limb and cells respond according to their level in the gradient (cf Wolpert's model). Transplanted zpas interact with the existing zpa to alter the level of the gradient - if an additional zpa is grafted to the anterior margin, cells differentiate to make a mirror image duplication of the posterior digits

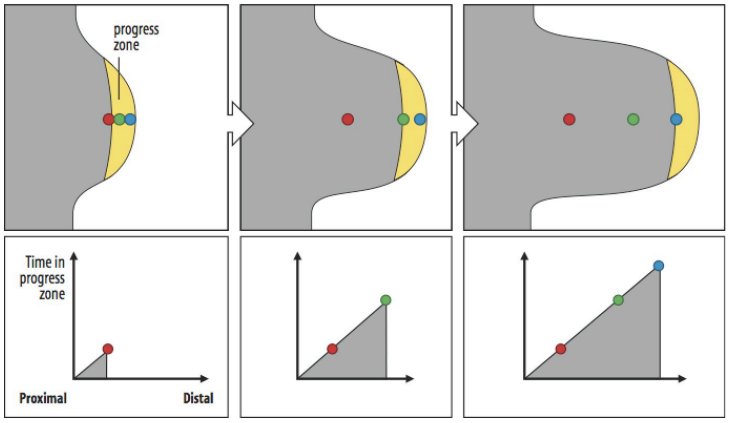


We now know of course that the graded signal diffusing from the zpa is Sonic hedgehog synthesised by the cells in this region. On the left you can see the expression of Sonic hedgehog in the limb buds and along the midline of the developing spinal cord. On the right the gradient model.





In the proximo-distal axis, cells appear to assess their position by measuring the time that they spend in a region of dividing cells at the tip of the limb known as the *progress zone*. The dividing cells lie beneath a distal layer of ectodermal cells called the apical ectodermal ridge, the AER. If the AER is removed, then division ceases and cells differentiate prematurely. In normal development as the limb extends cells leave the progress zone stop dividing and differentiate laying down elements of the pattern in a proximo-distal sequence.



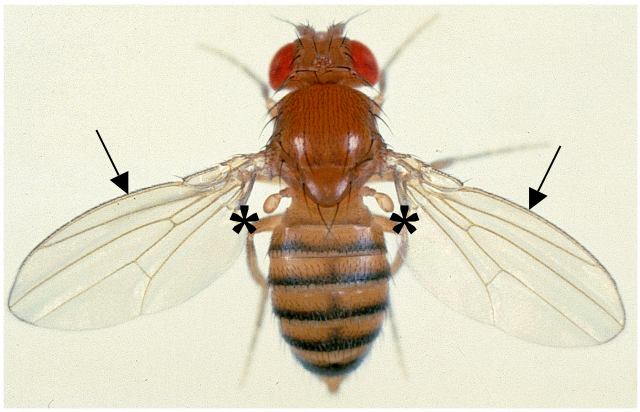
A model for the operation of the progress zone. We know know that the signals emanating from the apical ectodermal ridge are fibroblast growth factors (FGFs) which are sufficient to elicit cell division if the AER is removed.

Thus two signalling centres organise the pattern of limb development in two axes, much as Wolpert's model suggests. However while one acts as a graded signal the other promotes cell division and extension of the limb and time is the critical determinant of what each cell does in the proximo-distal axis.

Homeotic genes and the body plans of flies and vertebrates

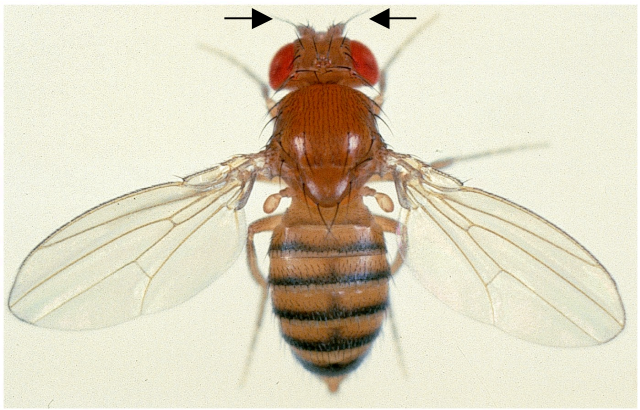
Interactions between cells mediated by signals such as *hedgehog* and their receptors lead to the formation of spatially organised patterns of cell differentiation in structures such as the vertebrate limb bud or the developing segment of an insect. Transplants indicate that this system of generating "positional information" is repeated in different units (segments in insects, limbs in a vertebrate embryo), just as Wolpert predicted. But different units (anterior versus posterior segments, fore limbs as against hind limbs) form different specialised structures appropriate to their position within the embryo. How do cells get assigned to these different tasks – how do they gain access to one specific subroutine of the available genetic programme?

Once again the answer comes from studying flies but the findings are of almost universal significance for understanding how animals (including ourselves) develop.



The clue comes from flies like those shown in the right hand panels above and below - these two flies carry mutations which are said to be **homeotic**, that is to say they cleanly transform one body part into another. Naturalists have collected variants of animals with homeotic changes to their structure for many years as natural oddities. But the important thing here is that these flies carry heritable changes in individual genes which are therefore called *homeotic genes and mutations*. The two left hand flies are normal - they have two wings and a pair of antennae on the head (arrows). But the flies on the right are mutant: top right is a mutation in the gene *Ultrabithorax* and the phenotype of this mutation is the transformation of the cells that would normally make halteres (asterisks - balancing organs) into a second pair of wings giving a remarkable four-winged fly.

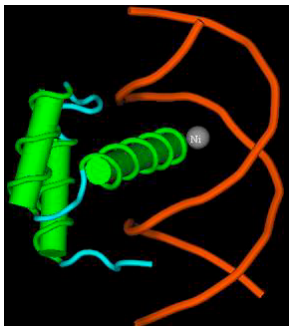
Meanwhile the right hand fly below has a mutation in another gene, *Antennapedia* and the effect of this is to cause cells that would normally make an antenna to make, not antennae, but another pair of legs sprouting out of the head!

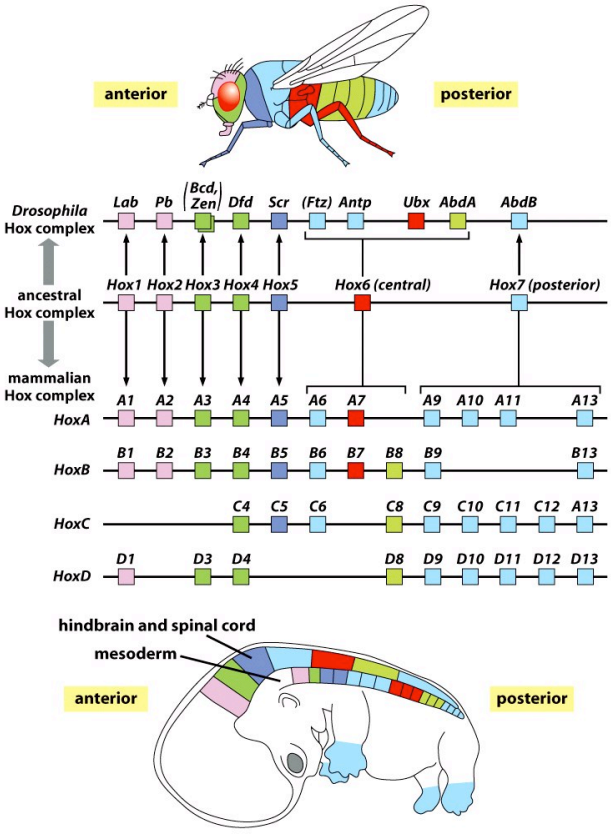


In both cases cells in one part of the developing fly have been assigned to make a structure that is inappropriate to their position. In both cases the transformation is remarkably clean and localised - the genes concerned appear to be acting as developmental switches between alternative coherent subroutines of the genetic programme: make leg or make antenna or make wing or make haltere.

Not surprisingly, the homeotic gene class that this kind of mutation reveals encode transcription factors. Just as MyoD organises the development of a complex cell type, muscle, by regulating the expression of many other genes, so we might expect a gene that can orchestrate the development of a structure such as a limb would coordinate the expression of the many different genes required to build a limb - and this is exactly what the homeotic genes do - activating the expression of some genes, repressing the activity of others, all within a localised region of the embryo.

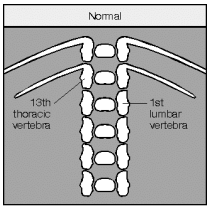
Among the many remarkable properties of the **homeotic** genes is that they all encode the same type of transcription factor known as a **homeodomain** protein from the characteristic motif of the DNA binding domain (right). This particular region of the protein is encoded by a characteristic DNA sequence know as the **homeobox**. It is for this reason that you will often see the homeotic genes abbreviated to **Hox** genes.

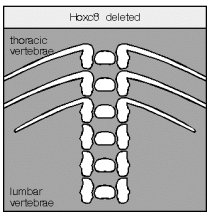




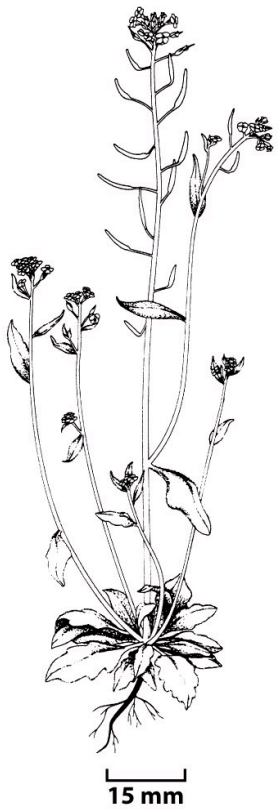
In *Drosophila* the Hox genes are present as two clusters on the 3rd chromosome. Different members of the clusters are expressed at different positions along the body axis. Interestingly the sequence of genes along the chromosome matches the antero-posterior pattern of expression along the embryo.

Although the Hox genes were first found in flies, they have since been found in many organisms including vertebrates (and humans of course). Homologues of individual fly genes are found in mammals (left) and, amazingly, although the genes have been duplicated in the course of evolution and form four clusters, the sequence of genes along the chromosomes is conserved, together with the anterior to posterior pattern of expression in the embryo as well!





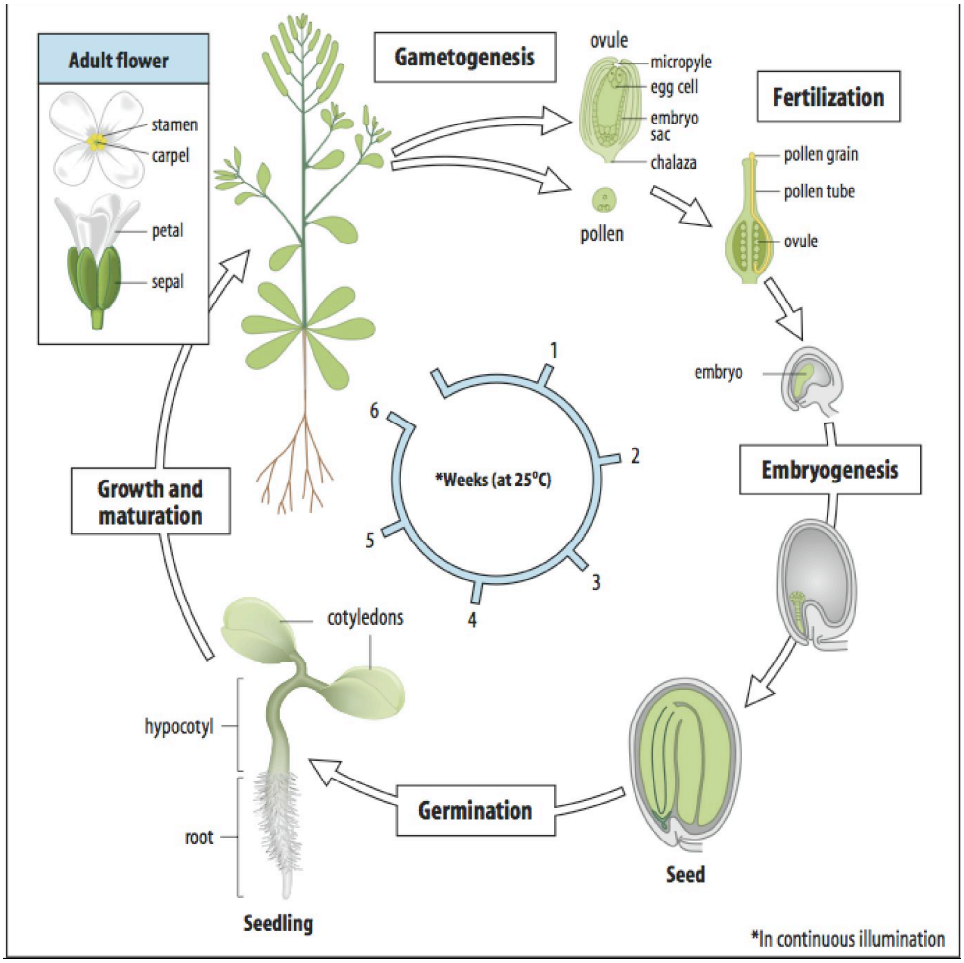
As in *Drosophila*, mutations in the mammalian Hox genes produce characteristic alterations to structure along the antero-posterior axis as shown on the left.
We can imagine that the original forms of the Hox genes would have switched cells between different fates in our ancient, perhaps worm-like ancestors



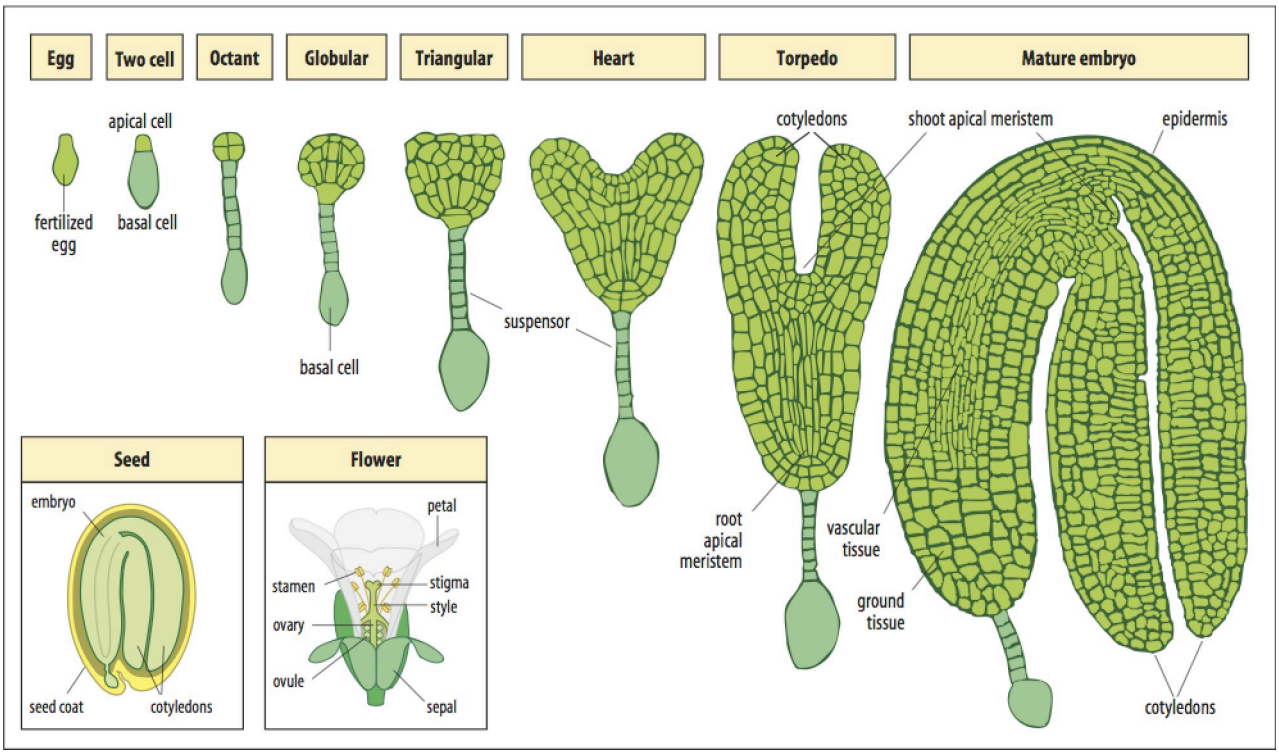
The development of plants

Plant development is intrinsically interesting and different from that of animals. It is likely that plants and animals evolved multicellularity independently from each other, diverging from common ancestors something like 1.6 billion years ago. Nonetheless there are common problems to be faced in organising the development of any multicellular organism and sometimes the solutions are remarkably similar although the genes involved are quite different.

For the plant scientist the equivalent organism to *Drosophila* is a small flowering weed called *Arabidopsis* (left and life cycle below).



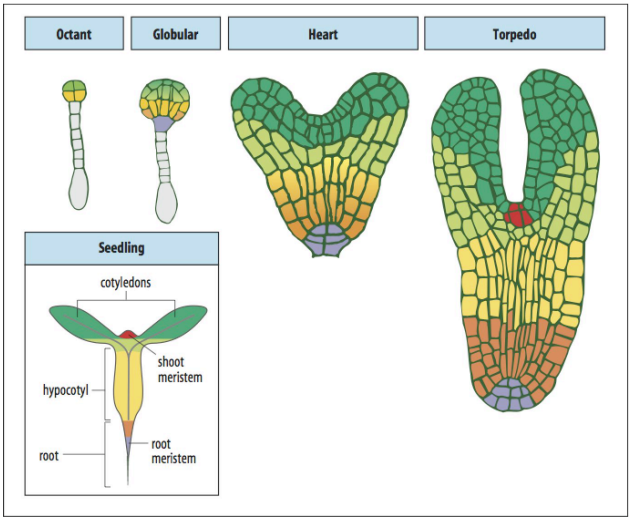
Arabidopsis can be grown in the lab. in large numbers and produces thousands of offspring per plant. It also has one of the smallest plant genomes known and is well suited to cellular as well as molecular and genetic analysis



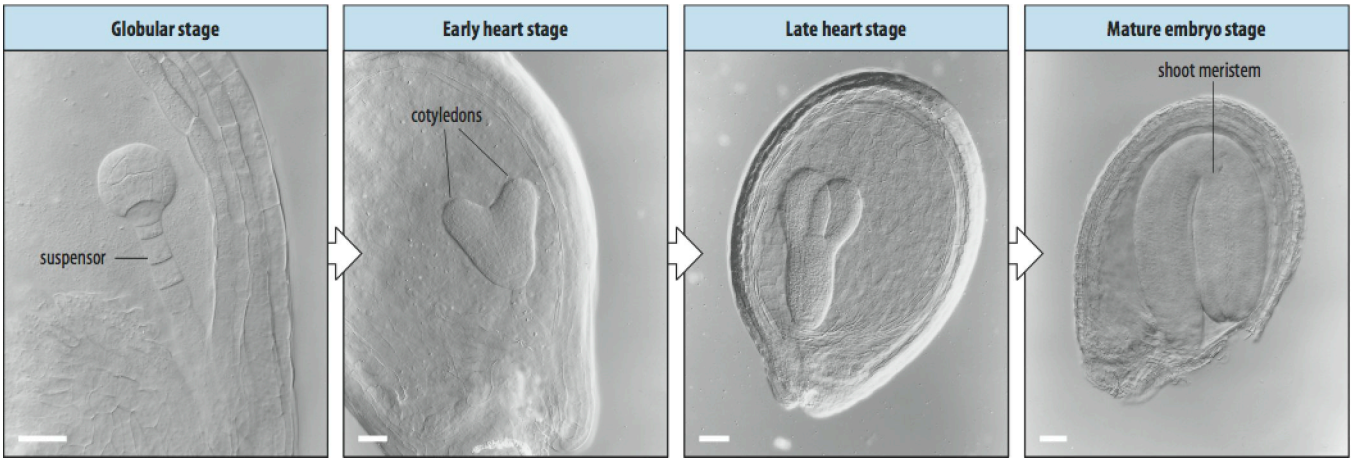
The development of a new *Arabidopsis* plant begins with the fusion of pollen and egg cell. Two haploid pollen nuclei reach the ovule: one fertilises the egg cell the other fuses with two other nuclei in the ovule to form the 3N storage tissue or endosperm that provides nutrients for embryonic development. The sequence of embryonic development following fertilisation is shown above.

The outcome of embryogenesis is a small seedling which has two sets of stem cells set aside at opposite ends of the shoot/root axis: the shoot apical *meristem* that will generate the shoot, leaves and flowers of the plant and the root apical *meristem*. You should remember hearing about the self renewing stem cell population of the apical meristem from Ron Laskey's lectures last term.

Thus the fundamental axis for all future development of the plant has been set during embryogenesis. Unlike animal embryos which are generally immature versions of adult forms, the mature plant embryo largely consists of two distinct sets of stem cells from which all the structures of the plant will unfold as it grows and develops postembryonically.



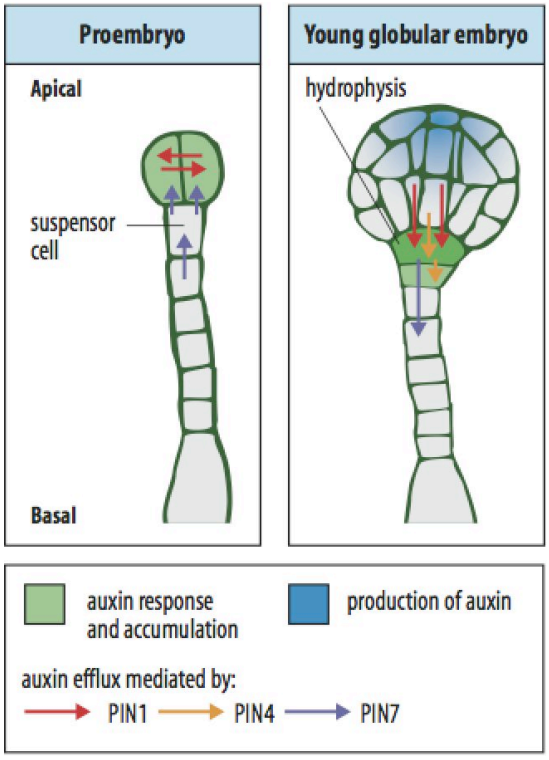
Because cell division in the embryo is rather regular, it's possible to trace back the origin of the root/shoot axis to the very earliest stages of development, when the egg cell divides to form an apical and a basal cell. The seedling is largely formed from the apical cell while the basal cell forms the suspensor cells that link the embryo to nutrient tissue. However the uppermost suspensor cell contributes to the root meristem.

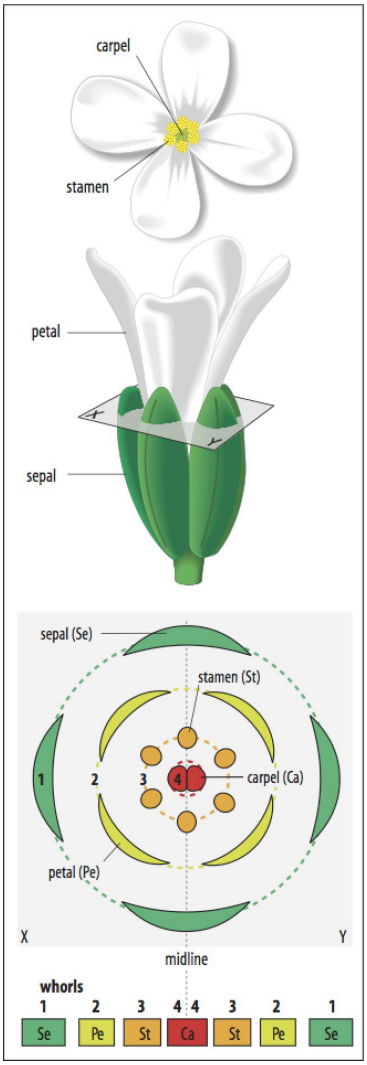


What sets the apico-basal axis of the embryo?

Essentially this is exactly the same question we asked of animal egg cells - how do you lay out the coordinates of future development? There the maternal genome was critical, here there is little or no maternal contribution to patterning the embryo and the axis is set by gradients of the signalling molecule auxin (indoleacetic acid or IAA)

Immediately after the first division of the egg cell, auxin is actively transported from the basal to the apical cell and there it is required for the specification of apical characteristics. This flux continues until the globular stage when apical cells begin to synthesise auxin and the flow reverses. Auxin now accumulates in the basal cells which will form the root meristem. The auxin fluxes are mediated by a special class of membrane proteins responsible for auxin efflux - the PIN proteins. The changing distribution of these proteins in the plasma membranes of the embryonic cells switches the polarity of auxin flow as shown (right).



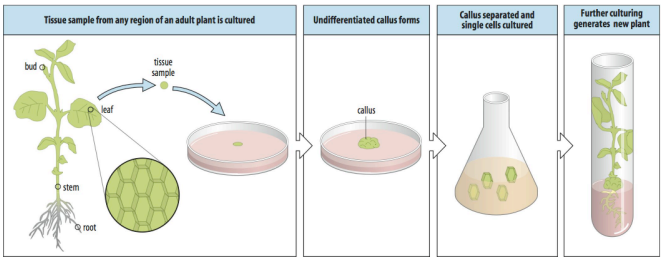


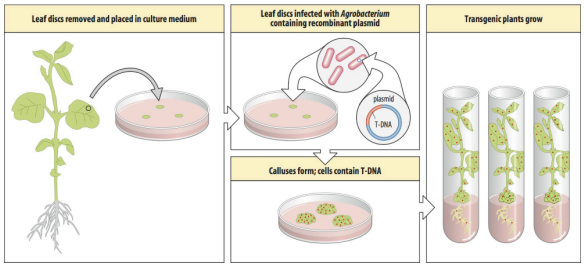
As plants develop, and environmental conditions elicit the formation of structures like flowers (left), cells formed in the floral meristem have to be assigned to form elements of the final structure - just as animal cells are assigned in groups to form parts of the complete organism. The genetic solution to accessing different subroutines in the genome for particular structures is remarkably similar to that found in animals - with the result that although the genes are completely different, plants exhibit very dramatic homeotic structural transformations - many of which are prized by horticulturalists.

The structure of the flower can be reduced to a so-called floral diagram(left) representing a series of concentric whorls, each forming a specialised structure: outer sepals (leaf like protective covering), petals, stamens (cells undergo meiosis to from haplod pollen) and the central carpel (contains ovaries within which cells undergo meiosis and form an embryo sac containing the egg cell.

The formation of these different primordia depends on overlapping patterns of transcription factor expression in the floral meristem: the overlaps defining four different domains of expression from which the four different structures will be formed. In mutants where elements of the pattern of transcription are lost there are consequent homeotic transformations of floral structure - some prettier than others, for example *agamous*, where petals are over produced. Clearly the genes concerned are homeotic but they don't encode homeodomain proteins. Instead several of these floral identity genes have a characteristic DNA binding motif known as the MADS-box

Stem cells and pluripotency

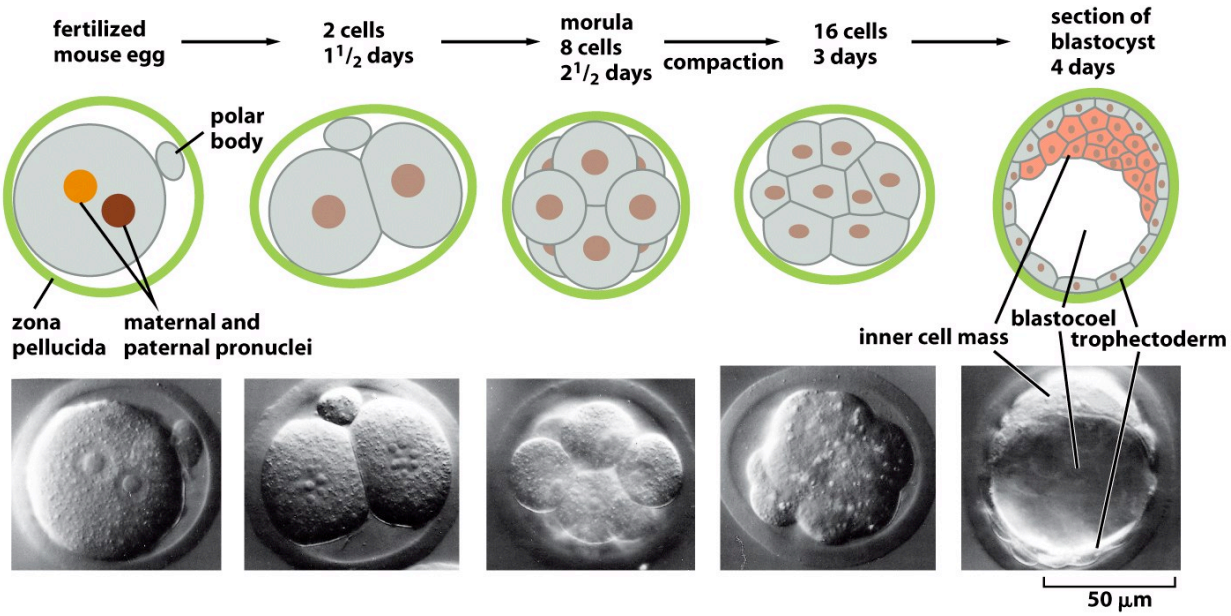




The fact that whole plants can be grown from cultured single plant cells shows us that these cells are pluripotent: they have the capacity to differentiate into many different cell types under appropriate conditions. This is extremely useful practically, because it means that genetic contructs can readily be introduced into every cell in a plant grown from such a cell.

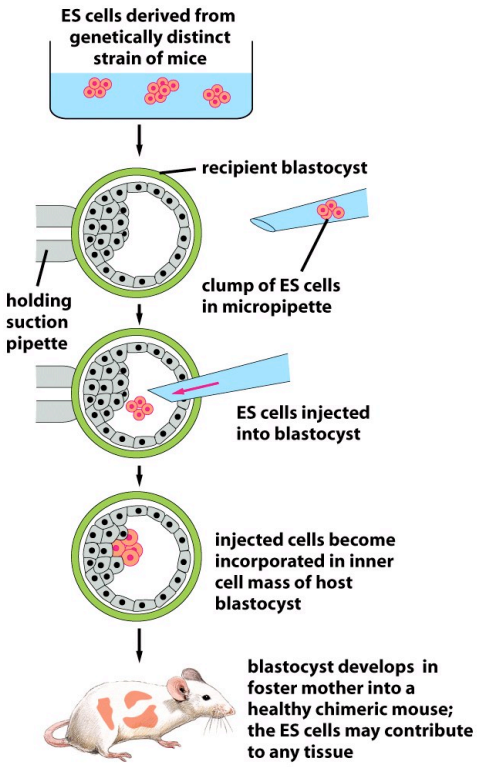
As these lectures have repeatedly emphasised animal *cells* do not have this capacity - the differentiated state is highly stable. However the *nuclei* of differentiated cells remain pluripotent, so the information for other cell types is retained, but inaccessible except in highly unusual circumstances such as a nuclear transplant.

Early mouse embryogenesis



Here you can see diagrams and micrographs illustrating the early stages of embryogenesis in a mouse. Notice that a key stage is the separation of cells into two different lineages: the inner cell mass (ICM from which the mouse embryo will develop) and the trophectoderm that gives rise to extra embryonic tissue.

The early cells of the ICM have a remarkable property: if cultured with appropriate “feeder” cells they can form a self renewing population of stem cells without differentiating.



Furthermore these cultured cells retain their pluripotent state - that is they can still contribute to the formation of any tissue in the mouse and that is shown by the sort of experiment shown on the left.

Embryonic stem (ES) cells are injected into a recipient blastocyst of a different genotype, which then develops in the uterus of a surrogate mother to form a chimaeric mouse consisting of cells of two different genotypes - that of the ES cells and that of the host blastocyst.

Since the ES cells can contribute to the germ line, it is possible to generate mouse strains carrying whatever genotype gave rise to the ES cells originally.

The fact that stem cells can be caused to differentiate into any cell type means that they may be the key to regenerative therapies in medicine. More fundamentally, because it is possible to engineer the genome of ES cells (for example by creating gene knockouts) it means that genetic analyses that could formerly only be carried out in flies and worms can now be done in mutant strains of mice derived from these cells.

Like other cell states, the transient pluripotency of the cells of the ICM, which is captured in cultured ES cells depends on a regulatory gene network that operates to maintain this characteristic until the cell are caused to differentiate.

The recent discovery (see diagram right) that adult cells can be transformed into a pluripotent state by transvecting them with the regulatory genes required for the ES cell state opens up the very exciting possibility of generating stem cells from adult cells rather than from embryos.