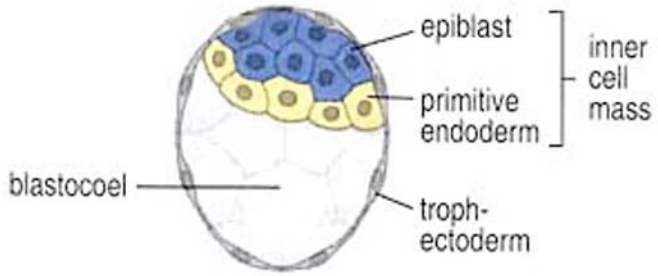
Pre-implantation mammalian development and derivation of embryonic stem cells (HS lecture 1)

The mouse is not an immediately obvious choice of model system for developmental biology. Its embryos are small, develop relatively slowly and are inaccessible to experimental manipulation because most of development must take place in utero. However, mice are at least as easy to study as any other eutherian (placental - not marsupial) mammal and, being mammals ourselves, we have a particular interest in understanding mammalian development. The early stages of mammalian development are quite different from those of other vertebrates such as Xenopus and so need to be studied in a mammal. Moreover, mouse genetics is comparatively well developed (lots of mutants) and techniques exist for manipulating gene function (knocking genes out and turning them on), which are not available in any other model vertebrate.

Early mouse development

The fertilized egg divides and develops as it travels along the oviduct to the uterus. In mice this process takes 4-5 days.The egg has a protective membrane, the zona pellucida, which stops it from implanting in the oviduct wall. By the time it reaches the uterus the egg has undergone many cell divisions to form a blastocyst, which hatches from the zona to implant into the uterine wall. Pre-implantation mouse development can be studied in culture - prior to implantation embryos can survive and can develop in liquid media.



One reason mammalian early development is different from that of other vertebrates is that the extra-embryonic membranes (amnion and chorion) play a much more important role (they generate much of the placenta). Much of early development involves deciding which cells will give rise to these extra-embryonic membranes and which give rise to the embryo proper. Just before it implants, the blastocyst is essentially an asymmetric hollow ball of cells surrounding a fluid filled cavity - the blastocoel. Cells in the outer layer and the inner mass have different fates. The outer layer of cells is called the trophectoderm and will give rise to the chorion. The inner cell mass or epiblast gives rise to the embryo proper. Inner cell mass cells in contact with the blastocoel have a distinct identity. They form the primitive endoderm and will give rise to the amnion.

The process by which cells become committed to become part of the embryo rather than of the extra-embryonic membranes have been well characterized. Up to the eight cell stage of development all cells are developmentally equal and totipotent (able to give rise to any type of cell, embryonic or extra-embryonic). The evidence for this comes from two types of experiment:-

1) From experiments isolating cells from two-, four- or eight-cell embryos to see if each can give rise to a complete individual (this can happen spontaneously to produce identical twins, quads and, in sheep, octuplets).

2) From experiments where two eight-cell embryos are fused and give rise two normal mice. The mice are chimeras, meaning that they are composed of cells derived from two genetically distinct individuals. If the cells had already committed to a particular fate you might expect to produce a double embryo/placenta.

The fact that cells remain totipotent up to this late stage makes mouse development very different from that of other vertebrates and from *Drosophila*. In fish, amphibians (like *Xenopus*) and birds localized maternal determinants direct the cells that inherit them to follow different developmental pathways from very early stages just as bicoid and nanos direct the formation of the A-P axis in *Drosophila*. In mice, by contrast, there is very little evidence that maternal transcripts play any significant role in development.

The process by which blastocyst cells acquire their different fates begins at the eight-cell stage with a phenomena called compaction. During compaction the cells become polarised and tight junctions form between them. Compaction can be reversed by antibodies to E-cadherin so presumably this cell-adhesion molecule plays a role in the process. The net effect of compaction is that cells can now be exposed to two different types of microenvironment depending on their position within the embryo. Cells on the outside are exposed to the medium surrounding the embryo while cells on the inside are not. Cells on the outside give rise to trophectoderm and those on the inside to inner cell mass (ICM). After compaction the future trophectoderm cells begin to secrete fluid, creating a cavity, which will eventually form the blastocoel. ICM cells exposed to this fluid (effectively this forms another different microenvironment) will form the primitive endoderm. The remaining ICM cells form the embryo.

By the blastocyst stage, although they have lost the capacity to form extra-embryonic membranes, any ICM cell can contribute to any embryonic tissue - they are still totipotent with respect to embryonic development (shown by generating chimeras by injecting ICM cells from one strain of mice into a blastocycst from another).

Derivation of embryonic stem (ES) cells

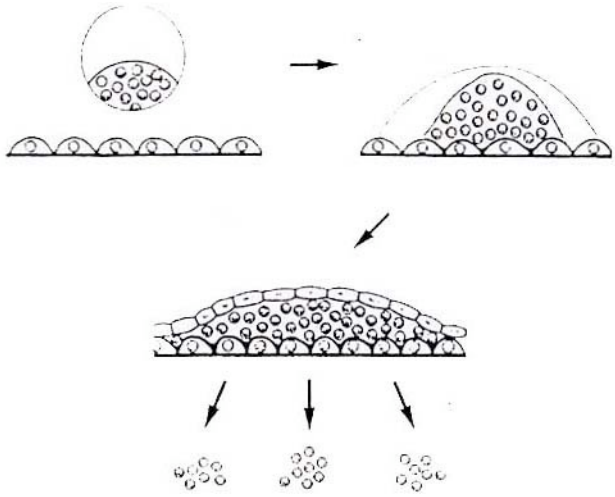
Targeted mutations in mice are performed by manipulating the genome of cultured cells derived from the ICM - Embryonic stem (ES) cells.

Cultured cells can be derived by the following process:-

1) Dissociation and plating. Most tissues, if dissociated into single cells, can be plated out on specially treated surfaces to which the cells become attached.

2) Attachment growth and differentiation. When allowed to grow under liquid medium. Some of the cells differentiate and lose the capacity to divide, others continue to divide.

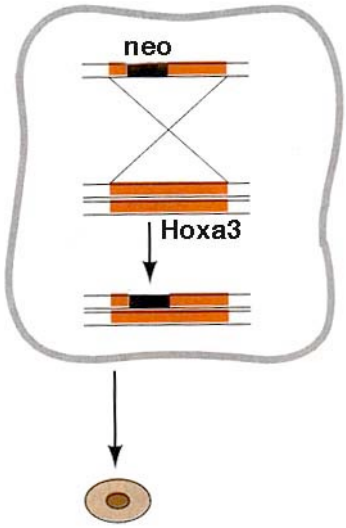
3) Dissociation and replating. Dividing cells can be selected and re-plated. This time a larger proportion of the cells will retain the ability to divide.



4) Repeating this process eventually selects for cells, which are able to divide indefinitely, unless exposed to media containing factors which force them to differentiate. These cells can be used to establish a cultured cell line. Most cell lines can differentiate to produce the cell type from which they were originally derived muscle to muscle cells, nerve to nerve cells. Cultured ES cells derived from the ICM can give rise to any cell type. Moreover, like ICM cells ES cells injected into blastocyst embryos can contribute to all tissues of the resulting chimeric adult including the germ cells -sperm or eggs.

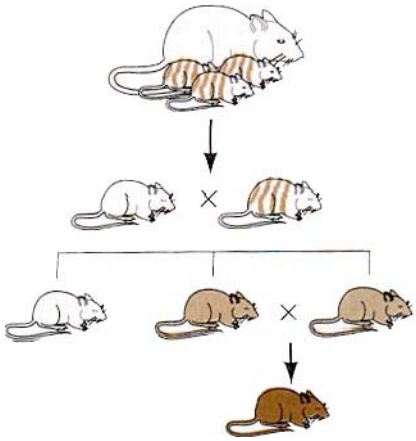
Targeted mutagenesis

ES cells can be induced to take up foreign DNA e.g. by electroporation. If this DNA includes sequences with homology to endogenous mouse sequences it can be incorporated into the ES cell genome by homologous recombination replacing the endogenous sequence. This can be used to generate mice in which the coding sequence of a particular gene (e.g. *Hoxa3*) have been replaced by sequences coding for a selectable marker such as the gene for neomycin resistance. Cells in which this has happened (usually to only one copy of the gene) will be the only ones to survive adding neomycin to the culture medium.



These cells, which are *Hoxa3/neomycin* heterozygous mutant for the deletion/replacement can be injected into a wild type blastocyst and re-

implanted in a foster mother. The mice derived from embryos that have undergone this procedure will be chimeric, composed of a mixture of mutant ES cell and wild type host cells. However, if the ES cells have contributed to the germ line and given rise to mutant sperm or eggs, F1 offspring of the chimeras can be fully heterozygous mutant. Crossing these F1 heterozygotes to each other can produce homozygous *Hoxa3* mutant mice whose phenotype can be analyzed to determine the effects of developing in the absence of *Hoxa3* protein.



Transgenesis

Transgenic mice can be generated by simple microinjection of DNA into the nuclei of fertilized eggs. Injected eggs are re-implanted in a foster mother (similar to IVF in humans) and usually about 10% of the surviving offspring will carry an insertion of the injected transgene in their chromosomal DNA. Transgenes insert at random locations in the genome (non-homologous recombination).

Transgenesis is used in research :-

* to assess the function of tissue specific regulatory sequences
* to analyze the effect of misexpression or overexpression
* for insertional mutagenesis

Insertional mutagenesis occurs when the transgene inserts into an endogenous mouse gene and disrupts its function. Even if the insertion has no dominant phenotypic effect, heterozygote carriers can be identified by testing for the presence of the transgene.

References

Wolpert (2nd edition) pp 41-44 and pp 87-89

Scott Gilbert (8th edition) Chapter 11 pp 348-358

Scott Gilbert 8e-supplement (http://8e.devbio.com/index.php)
Chapter 11 The Cell Surface and the Mechanism of Compaction