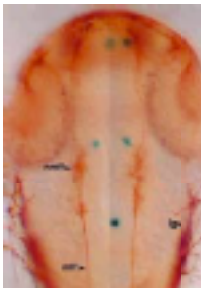
Transgenesis in zebrafish and mice

Method
The most efficient method of introducing DNA to vertebrate eggs is microinjection. Linear DNA is injected into fertilised oocytes at the one cell stage before cleavage divisions begin. Zebrafish ooctes are very yolky making it difficult to see the nucleus so DNA is injected at high concentration into the cytoplasm. Mouse oocytes are relatively yolk free making it possible to inject directly into the nucleus (and use lower concentrations of DNA).

In the nucleus the injected DNA can integrate into the fish or mouse chromosomes by a mechanism which is not fully understood. Integration sites are random and bear no relationship to the sequence injected (the transgene). Often multiple copies of the injected sequence insert together at the same site and rearrangement or deletion of the sequence can occur.

Another problem is that integration may not take place immediately and, particularly in the rapidly dividing zebrafish embryo, can happen after the egg has divided. In these cases not all cells of the embryo will carry the insertion - the embryo and eventually the adult is mosaic. Mosaicism can include the germ line in which case the potential founder animal may not be able to transmit the transgene to offspring to set up a stable line of transgenics. Founders must be outcrossed to normal animals and the F1 offspring tested (e.g. by PCR analysis of non-essential tissue, usually tail).



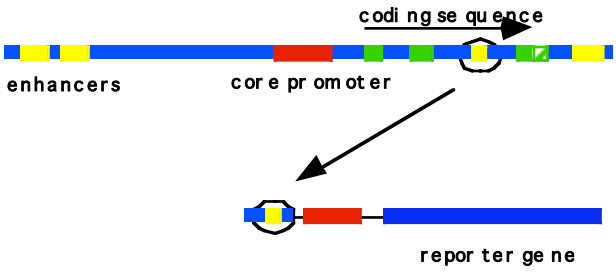
Mosaic expression of a lacZ transgene in a small proposrtion of cells

Applications
Transgenic animals have both research and commercial/biomedical applications:-

1. **Identification of regulatory sequences**
2. **Cell and tissue specific markers**

Much research with transgenic animals is focused on the identification of stage and tissue specific regulatory sequences. Some examples of this are given in the later half of the lecture.

Regulatory sequences can be identified by looking at the expression of transgenes in which segments of the genomic sequence flanking a gene have been cloned adjacent to a reporter gene.



Reporter genes produce proteins the expression of which can easily be detected.. The most common are β-galactosidase, activity of which converts the colourless substrate X-gal to a blue compound and Green Flourescent Protein (GFP). This is derived from jellyfish and fluoresces under UV illumination. GFP fluorescence can be observed in living transgenic animals, to stain with X-gal animals have to be killed and fixed. Transgenes which successfully recapitulate the cell or tissue specific expression of the endogenous gene can be used to mark those tissues in subsequent experiments.

One important point about using transgenics to identify regulatory elements. Transgenes can become rearranged, or insert at locations where they are inactivated (e.g. heterochromatin) or come under the control of endogenous regulatory elements (position effects). It is, therefore, essential to check the expression of any transgene in several different lines before drawing conclusions about the regulatory effects of any sequences included.

3. **Targeted, misexpression and overexpression of transgenes**
4. **Functional complementation of mutants**

Once cell stage or tissue specific regulatory elements have been isolated they can be used to drive the expression of genes other than the one from which they were derived. Transgenes can also be used to supplement the expression of endogenous genes, simulating the effects of chromosomal duplications. Transgenes which mimic the expression of endogenous genes can also be crossed into a mutant background to see if they can rescue the effects of the mutant (functional complementation).

5. **Generating animal models of human diseases**
6. **Pharmaceutical production**
7. **Improved strains of food animals/plants**

Transgenic mice are used to model diseases. Mice which overexpress parts of the α-amyloid protein precursor show Alzheimers-like symptoms and are used to test the effects of drugs that could alleviate this condition. Transgenic mice can also be used to model the effects of overexpression of myelin proteins observed in congenital neurological disorders such as Charcot Marie-Tooth disease.

Mice with transgenes coupled to the β-lactoglobulin promoter express the transgenes in their milk. This promoter has been used to make transgenic sheep which produce α-antitrypsin (used to treat cystic fibrosis) in their milk - sheep being easier to milk on a commercial scale than mice.

More controversial is the recent development of transgenic salmon with added growth factor. There are arguments against the licensing of such fish for general consumption on environmental, safety and welfare grounds.

Examples

<u>Checking the zebrafish α-actin promoter for muscle specific activity</u>

α-actin is restricted to skeletal muscle. A 3.6kb ftragment from the 5' end of the fish a-actin gene was fused to GFP coding sequences and injected into eggs at the 1-cell stage.

α-actin GFP transgene

10% of injected fish which expressed GFP were able to to transmit the transgene. GFP expression was restricted to skeletal muscle in most (but not all) lines.

Similar GFP lines have been generated using promoter sequences from neural and glial specific genes. These lines are extremely useful to developmental biologists as they allow the development of these cells to be followed in living embryos, including mutant embryos.

Identifying conserved floorplate specific regulatory elements of the Shh gene

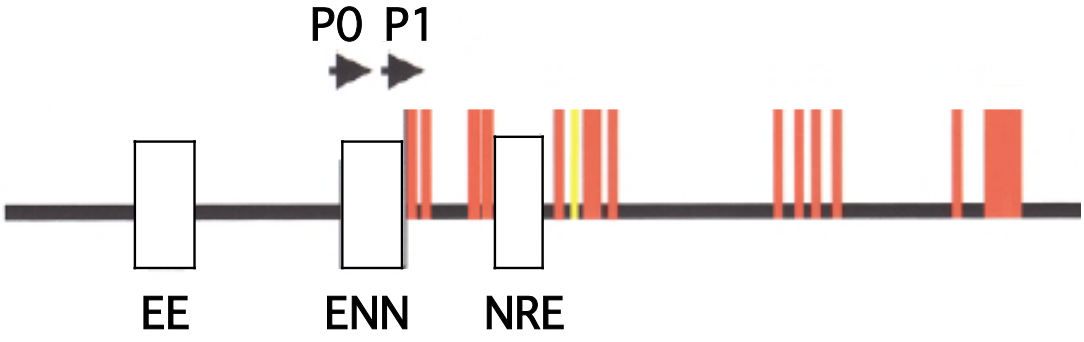
Shh is initially expressed in the floor plate and later in other tissues such as the limb buds. The pattern is similar in all vertebrates. Promoter sequences fused to β-galactosidase coding sequences do not express β-galactosidase in floor plate. Adding a genomic segment covering introns 1 and 2 of the gene

produces transgenic fish with mosaic expression of β-galactosidase in floor plate cells. The same construct injected into mouse eggs also shows floor plate specific expression. Comparing the sequences of zebrafish and mouse introns 1 and 2 allows the region driving this floor plate expression to be narrowed down even further.

Mapping long range regulatory elements of the Mouse/human Pax6 (Aniridia) gene

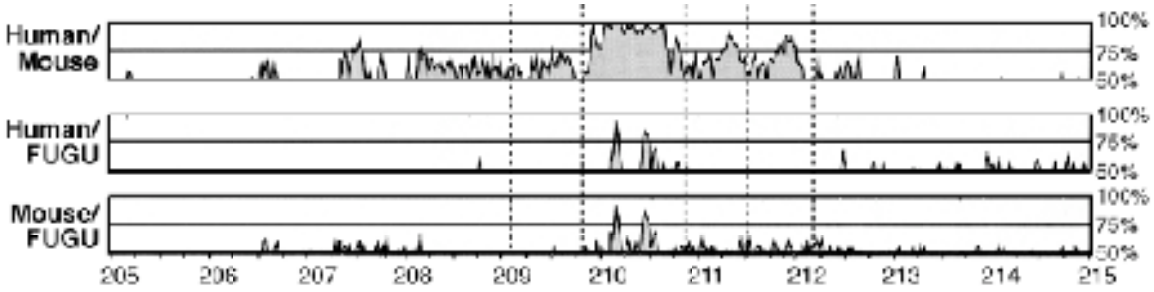
Pax6 is a highly conserved transcription factor with a complex and dynamic expression pattern in the developing eye brain, olfactory system spinal cord and pancreas. In humans mutations in *PAX6* cause the dominantly inherited eye defects Aniridia and Peter's anomaly and may also be associated with some cases of mental retardation.

Pax6 is a large gene with 13 exons covering a 22 kb region. The ATG lies in exon 4, there is one alternatively spliced exon (5a) and two promoters, P0 and P1.



Standard transgenic analysis of the promoter region and the more upstream introns has uncoverd three regions (EE, ENN and NRE) with enhancer activity in the lens and pancreas (EE), the forebrain, hindbrain and spinal cord (ENN) and peripheral retina(NRE).

Advantage has been taken of the availability of the complete genome sequences for humans, mice and fugu fish to identify conserved non-coding regions downstream of *Pax6*.



Above is a section of the trace produced by a computer programme that searches for regions of high sequence similarity when two genomes are aligned. Two regions show both hich levels of sequence conservation and enhancer activity in transgenic assays. HS234 is a 4.5kb region >150 kb downstream of the Pax6 P0 promoter which drives transgene expression in early eye development. C117 Box123 is a 2.9 kb region ~77kb downstream of the Pax6 P0 promoter which drives transgene expression in the olfactory system, central retina and forebrain. Interestingly, transgenes made using the Box123 region often exhibit ectopic expression in the midbrain. This result suggests that enhancers that drive midbrain expression are present in this region, but that *Pax6* expression in the midbrain is normally inhibited by suppressor elements, which lie elsewhere in the genome.

References

Making transgenic mice
(http://genome.wellcome.ac.uk/doc%5Fwtd021044.html)

Gene regulation in "Molecular Cell Biology" Chapter 7 pp 276-286

Transgenic animals and food production
http://www.fda.gov/fdac/features/2001/101_fish.html