



Analyzing Genes Involved in Eye Development: On-Line Self-Study

Goals of this exercise

- Review material covered in a previous exercise
- To see how mutants have been generated to analyze the genes involved in eye development

Manipulating Gene Expression

Gene Knockouts and Knock-ins

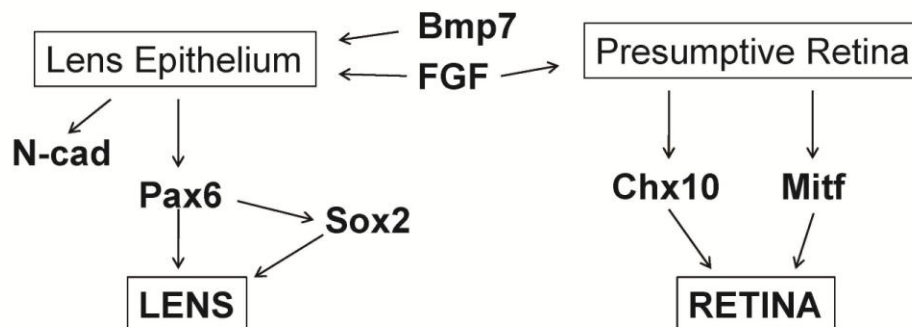
- Genes of interest can be deleted (gene knockouts) or changed to a mutant form (knocked in) through gene targeting after which researchers can: determine the effects on development in a diversity of ways
- The production of transgenic mice is discussed in a subsequent tutorial

Knock-down experiments

- embryonic cells can be injected with morpholinos (stabilized mRNAs) or RNAi (interfering double-stranded RNA) which decrease gene expression rather than completely blocking it.
- The genetically manipulated embryos are then studied using the diversity of techniques open to cell and developmental biologists.

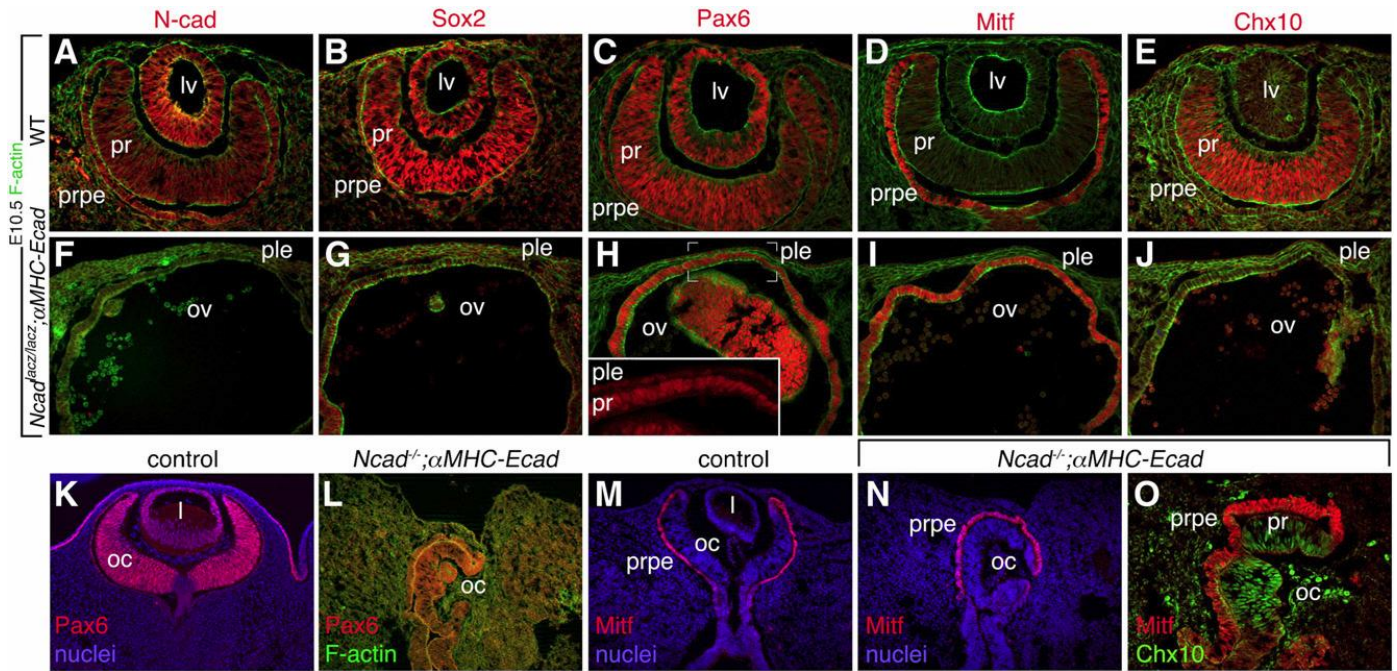
Example: Genes Involved in Eye Development

Here, we'll look at the expression of some of the genes involved in lens induction a topic covered in lecture later in the course. N-cad is the calcium-dependent cell adhesion molecule neural-cadherin. Sox2 is a Sry family member transcription factor involved in lens formation. The transcription factor Pax6 is essential for lens development being induced by fibroblast growth factor (FGF) and bone morphogenetic protein 7 (bmp7). Chx10 is a retinal homeobox gene. Mutations in this gene are linked to anophthalmia (absence of an eye) and microphthalmia (small eye within the orbit) that have a combined birth prevalence of approximately 30 per 100,000 population. The microphthalmia-associated transcription factor (Mitf) regulates the development of various cell types including the retinal pigment epithelial cells of the eye.



A short summary of the sequence of genetic events of eye development

Experimental Techniques



A failure of lens development in N-cadherin mutants. The confocal micrographs show the eye region of E10.5 (A–J) or E12.5 (K–O) embryos of the indicated genotypes labeled for the markers as color-coded above or on the panels. The absence of lens development is apparent at E10.5 examples (F–J) and E12.5 (L, N, O). lv—lens vesicle, pr—presumptive retina, prpe—presumptive retina pigmented epithelium, ple—presumptive lens ectoderm, ov—optic vesicle, oc—optic cup. Figure 2 from Smith et al, 2010.

Be sure to make note of the expression patterns for each of the genes in the parental strain (WT) and N-cad mutants.

While the above-mentioned techniques are the major tools of the embryologist and developmental biologist they are not the only ones used. Today any cell or molecular technique can be adapted to the study of embryogenesis including western blotting, molecular isolation and identification, DNA arrays, etc.

References

Ackermann et al, 2010. CaMKII α interacts with multi-PDZ domain protein MUPP1 in spermatozoa and prevents spontaneous Acrosomal exocytosis. *J. Cell Sci.* 122: 4547-4557.

Smith et al, 2010. Which FGF ligands are involved in lens induction? *Developmental Biology* 337: 195-198.

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