

## Find a Mutant!

### Adaptive Evolutionary Models and Human Diseases

As described in the Chapters 3 and 4, the fundamental principle of genetic analysis is to find a mutant that alters the biological process being investigated. For most geneticists, finding a mutant means introducing mutations into genes of a laboratory species in order to identify individuals with unusual phenotypes. But another promising approach to find a mutant may be to take advantage of what has occurred during evolution. After all, every gene in every organism has been mutated at some time during evolution, so a mutation in the gene of interest has already occurred. Of course, most of these mutations either have no effect on the organism (that is, they are selectively neutral), in which case they are difficult to observe; or else they have a deleterious effect on the organism, in which case they are likely to have been eliminated by selection. But some mutations are adaptive and confer a selective advantage on a species, particularly in species living in unusual environments. These mutations have persisted in nature, and could form the basis of genetic analysis.

Analysis of these naturally occurring adaptive mutations is intellectually equivalent to finding a mutant in a laboratory model organism, as described in Chapter 3. As with a laboratory mutant, they can be used to understand a biological process, particularly in comparison to a laboratory model. They might also be used to provide naturally occurring models for some human genetic diseases. One key difference between adaptive mutations and laboratory mutations is that most of the species with such an adaptive mutation are not amenable to laboratory study, so some knowledge about the biological process in an appropriate laboratory model organism is needed to provide a starting point for the genetic analysis. In this text box we consider a few examples, drawn from different species of fish and discussed by Albertson *et al.*, to illustrate the potential usefulness of this approach.

**Antarctic icefish: anemia and bone density.** Species of fish that live in the Antarctic have numerous adaptations to their demanding environment, as compared to related species that swim in more temperate waters. There are 16 species known as white-blooded icefish that do not make red blood cells and apparently do not express many of the steps in the hematopoietic cell lineage common to all other vertebrates. By comparing cDNA from the hematopoietic tissues of white-blooded fish with that of related red-blooded

fish, a previously unknown gene called *bloodthirsty* was identified. *bloodthirsty* is expressed in red-blooded fish (including the laboratory model zebrafish) but not in the white-blooded Antarctic icefish. When expression of the *bloodthirsty* gene is knocked down using antisense technology in zebrafish (as described in the online Text Box for Chapter 7), the fish lack red blood cells. This, and other experiments, indicate the wild-type *bloodthirsty* gene plays a critical role in erythrocyte development in fish, possibly by shutting off genes that maintain hematopoietic cells in an undifferentiated state, as diagrammed in Figure 1 below. Because icefish do not express *bloodthirsty*, its target genes are not shut off, and stem cells remain in the undifferentiated state. The molecular mechanism by which *bloodthirsty* shuts off these target genes is not yet known, but this can be investigated using zebrafish. Humans and other vertebrates have orthologues of the *bloodthirsty* gene. Thus, this natural variant could provide insights into some human anemias.

Some species of Antarctic icefish have greatly reduced bone mineral density compared to related fish in other climates. In some icefish species, parts of the bony skeleton have been replaced by connective tissue, and the reduction in ossification results in greater buoyancy. Many humans experience a loss in bone mineral density, particularly associated with aging; effects include the increased likelihood of fractures and the overall deterioration of bone mass. While the genetic basis for the reduced bone mineral density in the icefish is not yet known, it is possible that it could provide a naturally occurring model for the human condition as well.

**Cavefish and retinal degeneration.** Most cave-dwelling species initiate the development of optic structures such as lenses and retinas, but these structures fail to develop fully and subsequently degenerate. The species are blind, but since they dwell in the low-light environment of caves, sight is not critical to survival. Analysis of the genetic basis for the blindness in cavefish and other cave-dwelling vertebrates could provide a natural model for some of the human retinal degeneration conditions.

Genes necessary for normal optic development have been identified in mice and humans. The best known of these is the evolutionarily conserved transcription factor PAX6, expressed in photo-sensing cells in both invertebrates and vertebrates. The Mexican tetra fish has both populations that live in surface waters and have vision, and populations that live in caves and are blind. This naturally occurring difference allows a

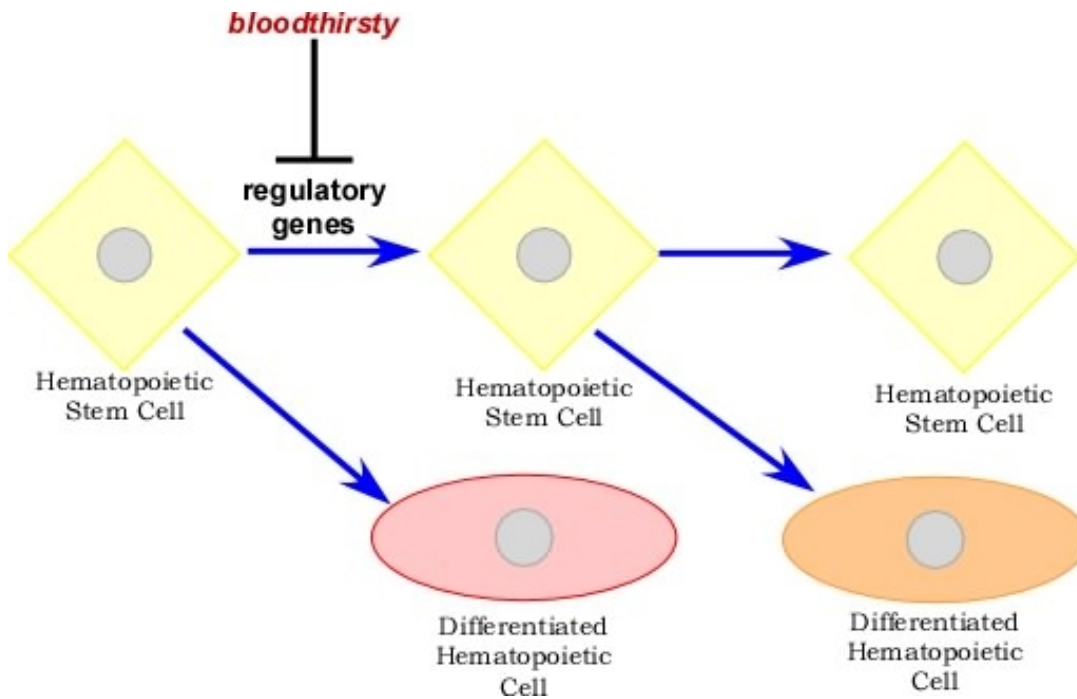
comparison between individuals of the same species. Notably, *pax6* expression is greatly reduced in the cave-dwelling populations. Genes of the *sonic hedgehog* gene family are known to be upstream regulators of *pax6* in many animals, and reduce *pax6* expression. Two members of the *sonic hedgehog* gene family have been experimentally over-expressed, resulting in the down-regulation of *pax6* expression in the surface-dweller fish. As expected from the known genetic pathway, these fish with reduced *pax6* expression have reduced development of the optic cup and are blind.

Although changes in the expression of the *sonic hedgehog* and *pax6* genes might contribute to blindness in cavefish, these do not appear to be the naturally occurring mutations in this species. While the altered genes have not yet been identified, these could define other, perhaps unknown, genes involved in *pax6* or *shh* regulation or even in some of the human retinal degeneration conditions.

**What makes a good evolutionary model?** The diversity of natural populations provides an attractive approach for understanding both evolutionary processes and human diseases. In their commentary article, Albertson *et al.* propose that this approach could be the most useful for genes that act relatively late in development and in only a few tissues—that is, genes that act at the terminal and differentiation steps of a developmental pathway. Mutations that affect genes with earlier times of action may have such pleiotropic effects that they are lethal to the organism. Thus, while these early-acting mutations have been found and characterized in laboratory model organisms, they may not survive well enough in nature to be evolutionarily significant. The evolutionary models should ideally affect biological processes that are reasonably well-studied in laboratory models, since most genetic techniques such as mapping, gene cloning, and assays for gene expression are not available in most species from nature. The commentary article also includes some other possible evolutionary models and some additional provocative ideas on what makes a good evolutionary model for human diseases.

#### **Literature cited**

Albertson *et al.*, 2009 *Trends in Genetics* 25: 74-81.



**Figure 1.** A postulated role for the *bloodthirsty* gene in hematopoiesis. In red-blooded fish, the *bloodthirsty* gene product somehow blocks regulatory genes needed to maintain the stem cell population, thus allowing the stem cell to differentiate. In icefish lacking *bloodthirsty*, the stem cell population is maintained but does not differentiate into hematopoietic cells.