11.12: Forward and Reverse genetics

The Zebrafish

The zebrafish, *Danio rerio*, has become another popular "model" organism with which to study fundamental biological questions. It is a small (1–1.5 inches) (2.5–3.8 cm) freshwater fish that grows easily in aquaria (it is available at many pet stores). Some of its advantages for biologists:

- It breeds early and often (daily).
- It is a vertebrate, like us, and thus can provide clues to human biology that invertebrates like *Drosophila* and *Caenorhabditis elegans* may not.
- Its embryos, like those of most fishes, develop outside the body where they can be easily observed (unlike mice).
- Its embryos are transparent so defects in development can be seen easily.
- Individual cells in the embryo can be labeled with a fluorescent dye and their fate followed.
- Embryonic development is quick (they hatch in two days).
- They can absorb small molecules, such as mutagens, from the aquarium water.
- Individual cells — or clusters of cells — can be transplanted to other locations in the embryo (as Mangold did with newt embryos).
- They can be forced to develop by parthenogenesis to produce at will homozygous animals with either:
  - a male-derived or
  - female-derived genome.
- They can be cloned from somatic cells.
- They can be made transgenic (like mice and *Drosophila*).
• Its genome ($1.4 \times 10^9$ base pairs) has been sequenced revealing 26,606 protein-coding genes.

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## Forward Genetics

Since Mendel's time, most genetics has involved observing an interesting phenotype and tracking down the gene responsible for it. So this "forward" genetics proceeds from phenotype -> genotype. Some examples in these pages:

• Mendel's work
• RFLP analysis of large families
• the one gene - one enzyme theory

These methods have been called "forward" genetics to distinguish them from a more recent approach, which has become an urgent priority with the successes of genome sequencing.

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## Reverse Genetics

Rapid methods of DNA sequencing has generated a vast amount of data. Thousands of suspected genes have been revealed (e.g., finding open reading frames — ORFs), but the function of many of them is still unknown. But now with a knowledge of the DNA sequence of a gene of unknown function, one can use methods for suppressing that particular gene ("knockdown"), and then observe the effect on the phenotype.

So this "reverse" genetics proceeds from genotype -> phenotype. Reverse genetics has been applied successfully to plants; mice; C. elegans; and can also be used with the zebrafish. For example, the function of a mysterious gene sequence in *Danio* can be studied by

• synthesizing a short antisense oligonucleotide complementary to a section of the gene.
• The oligonucleotide is chemically-modified to make it more stable than a fragment of RNA.
• Binding to its complementary sequence on the messenger RNA (mRNA) produced by transcription of the animal's gene, blocks ("knocks down") gene expression by preventing translation or disrupting normal splicing of the mRNA.

Because we share so many similar gene sequences (orthologous genes) with *Danio*, if one can discover the function of the gene in *Danio*, then we have a better idea of the role of its ortholog in humans.

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## Contributors and Attributions

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