INTRO TO DEVELOPMENTAL GENETICS: ZEBRAFISH AS A MODEL SYSTEM

Reading: Skim Chapter 20. Slides from lecture are attached.
Optional Supplementary reading: Two review articles will also be posted - one on the history of zebrafish as a model system and one on zebrafish genetic "tricks" and how these are used for mapping. This reading is not required, but available if you'd like to supplement the lectures.
Problems: Problem set (and solutions) posted on website

Today we'll talk about how mutagenesis strategies can be used to study vertebrate development. You do not need to memorize things like developmental biology terms and landmarks, but you should understand how haploid and gynogenetic diploids can be made in zebrafish, and how these genetic "tricks" can be used to facilitate genetic screens and mapping.

DEVELOPMENTAL GENETICS

Definition: Study of how the fertilized egg of a multicellular organism becomes an adult. Most developmental geneticists use simplier model organisms to uncover genes that are important in human development and disease.

Your book describes several model organisms: *Saccharomyces cerevisiae* (yeast), *Arabidopsis thaliana* ("mouse ear cress", a weed), *Caenorhabditis elegans* (a nematode worm), *Drosophila melanogaster* (fruit fly), and *Mus musculus* (the house mouse). Today, we'll highlight another model organism, *Danio rerio* (the zebrafish). Zebrafish are freshwater vertebrates that share many of the features that make the other model organisms so amenable to genetic research. In addition, they have a few of their own little idiosyncracies (the ability to make haploid and gynogenetic diploid embryos, the optical clarity of the embryos) that set them apart.

First, let's review the features that make model organisms useful for genetic analyses.
(1) Easy to cultivate
(2) Short generation time (reproduce rapidly)
(3) Easy to house many individuals (small size)
(4) Specific characteristics that make it particularly suitable for a given developmental process.
(5) Accumulated knowledge, e.g., large numbers of mutations, stock centers, genomic tools (including dense genetic maps, genome sequencing projects)

WHAT FEATURES OF ZEBRAFISH MAKE IT USEFUL AS A MODEL GENETIC ORGANISM?

First, zebrafish embryos develop outside the mother and are thus easily accessible for observation and experimentation. In an embryo that is about 18 hours old, you can see that it is already quite well developed, with an eye, ear, and developing brain at the anterior, and segmenting muscle and tail posteriorly. The embryo is wrapped around a large yolk, which nourishes the embryo until it can feed a few days later.

******SLIDE: FIGURE OF 18-HOUR OLD ZEBRAFISH EMBRYO******

A **second** feature is that the zebrafish embryo is optically clear. In a close-up view of the trunk of a zebrafish, we can see virtual transparency of zebrafish embryonic cells even more clearly. In a high power view of the trunk region of a zebrafish embryo at about one day of development,

we can easily see structures that lie deep within the embryo. For example, we can see every forming notochord cell in the embryonic midline, as well as each floor plate cell of the neural tube that lies directly above it. At even higher power, we can pick out the cell bodies of individually identifiable neurons in the spinal cord. Thus, the clarity of zebrafish embryos allows us to follow individual cells during development, as well as to easily recognize developmental mutants in genetic screens simply by viewing them under a microscope.

******SLIDE: HIGH MAG VIEW OF 24-HOUR ZEBRAFISH TRUNK******

A **third** feature is that zebrafish develop rapidly. The blastula period lasts only 3 hours, and gastrulation is completed in about 5 hours. For those of you that haven't taken developmental biology, the gastrula period of development is a time in which major cell rearrangements occur to transform the embryo into something that looks much more like an embryo to our eyes. Mesodermal segmentation, in which the mesoderm of the body is separated into a series of reiterated compartments, occurs shortly afterwards, so that by 24 hours, segmentation is completed and many primary organ systems have formed. By 72h, the embryo has hatched from its eggshell, and within the next 2 days, will begin to actively hunt for food. In a period of just four days, the embryo has rapidly become a small version of the adult. This rapid development obviously simplifies developmental and genetic studies. For comparison, a human zygote has only divided one time to make a two-cell embryo in the first 24 hours after fertilization.

******SLIDE: ZEBRAFISH STAGING SERIES******

A **fourth** advantage is that zebrafish adults reach sexual maturity quickly (for a vertebrate), having a generation time of about 10 weeks. In addition, a single pair of zebrafish can lay more than a hundred eggs at weekly intervals. Zebrafish are very hardy fish and very easy to raise.

A **fifth** advantage is that there is an extensive <u>genetic map</u> of all 25 zebrafish chromosomes. In addition, there is a large effort to sequence zebrafish <u>expressed sequence tags</u> (ESTs). The EST database is a "bank" of mRNAs that are expressed during development. Sequencing of the entire zebrafish genome is underway and should be near completion soon.

INTERMISSION: Looked at 3 movies of early zebrafish development

Recently, two large scale genetic screens for zebrafish developmental mutants have been performed. These screens, combined with several smaller scale screens, have identified hundreds of genes involved in embryonic development. Most of these mutants were identified strictly using morphological criteria. To give you a flavor for the kinds of mutants that can be identified, I showed a sampling of zebrafish developmental mutations at one day of development. *no tail* mutants lack a tail and a notochord, whereas *cyclops* and *one-eyed pinhead* embryos are both cyclopic and deficient in head mesoderm. From complementation tests, we know that *cyclops* and *one-eyed pinhead* are different genes. A more subtle phenotype is revealed by a mutation in the *no isthmus*, or *noi*, gene. At higher power, one can see that *noi* mutant embryos lack the isthmus, a structure which demarcates the division between the midbrain and hindbrain, and later becomes the cerebellum. *no tail*, *cyclops*, *one-eyed pinhead*, and *no isthmus* have now also been identified at the molecular level, in large part with help of the zebrafish genetic map.

******SLIDE: EXAMPLES OF ZEBRAFISH MUTANTS******

HOW DO WE SCREEN FOR ZEBRAFISH MUTANTS?

******SLIDES: ZFISH TRADITIONAL VS HAPLOID SCREENING STRATEGIES******

Traditional approach:

In the parental (P) generation, we mutagenize males (their spermatogonia) and mate to wild-type females. The F1 progeny are carriers of mutations induced in their father. To generate families of fish that are carriers of a particular mutation, F1 fish are crossed to wild-type and the resulting F2 family is kept separate from all the other F2 families. Sibling matings in F2 families can now reveal the recessive mutant phenotype. If both the male and female are carriers, then approximately 25% of their progeny will reveal the mutant phenotype. Using the traditional approach, we need three generations to "uncover" the recessive mutation.

Haploid screening approach:

We can streamline the screening process because we can generate haploid zebrafish embryos. Zebrafish haploids do not survive past three days, so unlike yeast cells, they cannot propagate in the haploid state. However, many developmental processes do occur in haploid embryos, and we can use haploids to screen for mutations that disrupt these processes. Because haploid technology exists, we can do a genetic screen in two, not three, generations. In the P generation, adult males are treated with a chemical mutagen and then mated with wild-type females. As in the traditional screen, the F1 progeny of this cross are carriers of mutations that were induced in their father's sperm. Here's where the process differs. Eggs are gently expelled from F1 females and mixed with sperm that has been UV-irradiated to destroy its genetic material. The irradiated sperm triggers development of the egg, but does not contribute any genetic material to the developing haploid embryo. If the mother carries a recessive mutation that affects embryonic development, the mutant phenotype will be observed in about 50% of her haploid progeny. If a mutant phenotype is observed, the mother is outcrossed to a wild-type male to generate a line from which to recover the mutation. This approach is useful as it saves time and space. However, genes involved in some developmental process, especially those that function later in development, cannot be screened for in this manner.

Expression screening approach:

Genetic screens in zebrafish have also been done using expression of particular genes as an assay, instead of just morphological criteria. This is a useful approach if one is interested in a developmental process that might not result in an overt morphological phenotype. An example might be to screen for neuronal cell types that express a particular neurotransmitter. A mutation that eliminated neurotransmitter function may have drastic effects on physiology, but minor effects on actual morphology of the fish. Here are two examples of genes identified by mutation based upon expression strategies.

******SLIDE: EXPRESSION SCREENING EXAMPLE #1******

(These embryos have been fixed and processed to detect a variety of transcripts; the blue color indicates where each gene is expressed. You can see that the embryo on the right has a defect in the second *krox20* stripe. A morphological defect in the mutant embryo hindbrain is present, although it is difficult for the untrained eye to see. By contrast, even an untrained eye can pick out mutants from their wild-type siblings using gene expression patterns!) Photo courtesy of C. Moens.

******SLIDE #7: EXPRESSION SCREENING EXAMPLE #2******

(These embryos have been fixed and processed to detect a variety of gene transcripts using in situ hybridization [brief description on p. 661 of your book]; the blue color indicates where each gene is expressed. You can see that the embryo on the right expressed most of the genes in the right place and pattern, but that part of the pattern is scrambled.)

You can screen for virtually anything as long as you have an assay! Zebrafish mutations in genes required for gastrulation, tissue and organ formation, motor neuron development and pathfinding, behavior, etc. etc, have been isolated. We watched three movies: two showed how transgenic zebrafish expressing a fluorescent proteins driven by heart or blood vessel specific promoters can give a lot of detail about the cells that form those structures if we follow them over time in living embryos; one showed the zebrafish startle response.

Once interesting genes have been identified by mutation, one goal is to identify the gene molecularly. Is it a transcription factor? a cell surface receptor? a secreted signaing molecule? The first step to molecular characterization is to map the mutation (place it on the genetic map).

HALF TETRAD ANALYSIS IN ZEBRAFISH: ONE WAY TO MAP MUTATIONS

Although tetrad analysis is an extraordinarily powerful mapping method in yeast, it has not been used extensively in plants and animals. In part, this is because often only one of four female meiotic products survives and because male meiotic products rarely remain together.

In zebrafish, meiotic **half-tetrads** (or gynogenetic diploids) can be routinely made by blocking the second meiotic division using the application of "early-pressure" (EP). The zebrafish ova are mixed with genetically inactivated sperm to activate "fertilization" and then are subjected to pressure to block the second meiotic division. (The sister chromatids do eventually separate, but not into two different cells). The resulting EP gynogenetic diploids develop normally, unless there is a lethal mutation in the genetic background.

If there are no crossovers between a gene an its centromere (both genes on the same chromosome, then a PD half-tetrad results. If a single crossover occurs, then a T half-tetrad results. The only double crossover that gives NPD progeny is the four-strand double crossover, which is very rare since zebrafish exhibit high interference.

We can use half-tetrad analysis to map zebrafish mutations. If a recessive mutation is close to the centromere, you will see 50% mutant progeny and 50% wild-type progeny, because very few recombination events occur. If a single crossover does occur, then all of the heterozygous progeny will appear wildtype; thus the number of wild-type progeny will increase with increasing distance from the centromere. However, we can't distinguish the wild-type embryos of the PD class from the T class, so the useful information is the number of mutant embryos.

F1 female	-----> First meiotic division	-----> Half-tetrads
Mm	-----> (no crossovers)	-----> Wildtype MM (p) + Mutant mm (q)
Mm	-----> (single xover)	-----> Wildtype Mm (r) + wildtype mM (s)

The recombination frequency of half-tetrads is r + s / total. But we can't tell the difference between r and s and p, since they all look wildtype. Since (p = q) and (p + q + r + s) = total, we can say that **(r + s) = total - 2q.**

But we need to know the frequency of recombinant chromatids. Because only one of the two chromatids in each half-tetrad resulting from a single crossover event is a recombinant chromatid, we must figure that the frequency of recombinant chromatids is half the frequency of recombinant half-tetrads, which is **1/2 x (total-2q) / total**. Thus, the distance of the gene to the centromere is **[1/2 x (total-2q) / total] x 100**. Not only can this mapping technique be used to locate a gene from its centromere, but we can also use it to map genes relative to each other (linkage mapping). As with tetrad analysis in yeast, zebrafish PD half-tetrads will outnumber NPD half-tetrads when two genes are linked.

Practice Problem (more on the handout):

(A) You have a female carrying a recessive mutation for albinism. You make gynogenetic diploids (by blocking the second meiotic division of eggs fertilized with UV-irradiated sperm) from the carrier and score the resulting 100 embryos. You find 25 albinos and 75 wildtype fish. How far is *albino* from its centromere?

(B) Mutations in a different gene called *oyster* also cause albinism (recessive trait). This time, when you make gynogenetic diploids from a heterozygous carrier you observe 50 wildtype and 50 *oyster* embryos. Is *oyster* very close or very far away from its centromere?

Answers:

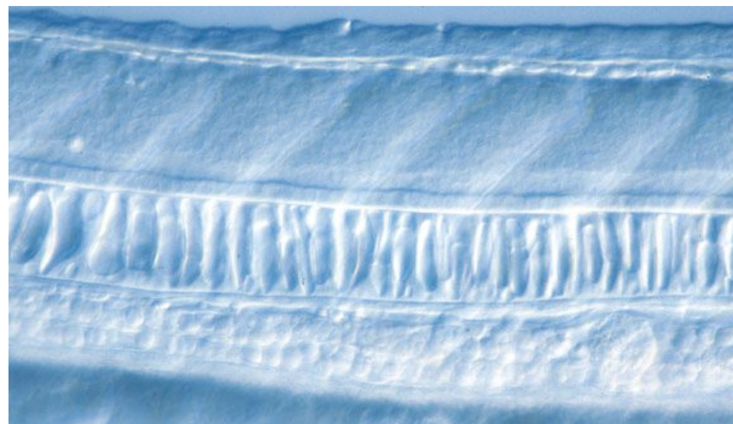
(A) The number of mutants is half the non-recombinant class of half-tetrads, so the number of non-recombinant half-tetrads is 50. That means that the recombinant half-tetrads is total – 50, or 50. (If it helps, you can think of the recombinant half-tetrads like yeast tetratype tetrads, except that they don't undergo the second meiotic division to generate haploids. However, just like we had to divide yeast tetratype tetrads in half to get the # of single crossover chromatids, we also divided the zebrafish half-tetrads in half for the same reason.) Thus,

[1/2 (total – 2q)/total] x 100 = [1/2 (100 – 50)/100] x 100 = 1/2[50] = 25 map units

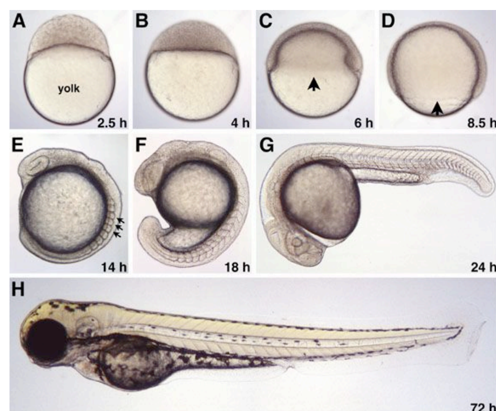
(B) The number of mutants (50) is half the total number of gynogenetic diploids (100). Since the number of mutants (q) is half the total embryos scored, there must be no recombinants. All the wildtype embryos must be non-recombinant (p=q) – because q=50, p must also be 50. Thus, the *oyster* gene is so tightly linked to its centromere that there are no recombination events between them.

18-hour zebrafish embryo

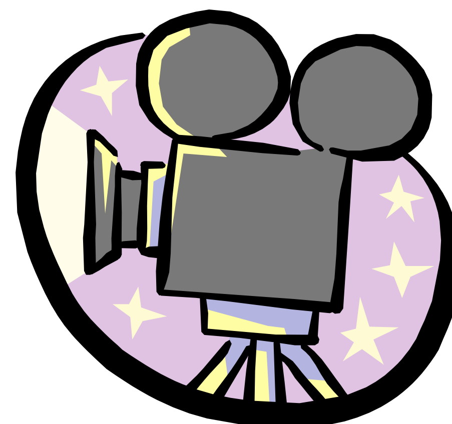
Close-up of the zebrafish trunk



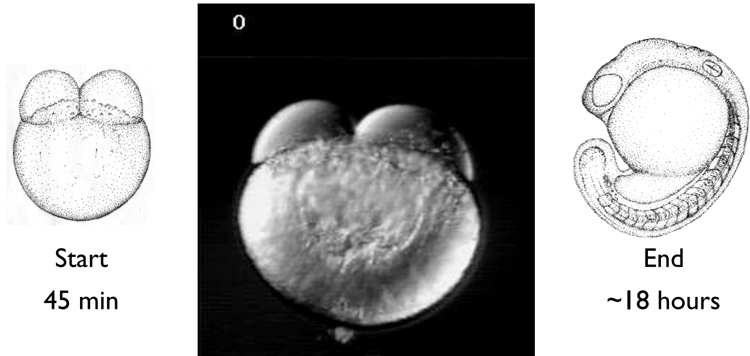
Zebrafish Staging Series



INTERMISSION



Zebrafish Development (2-cell to 18-somite)

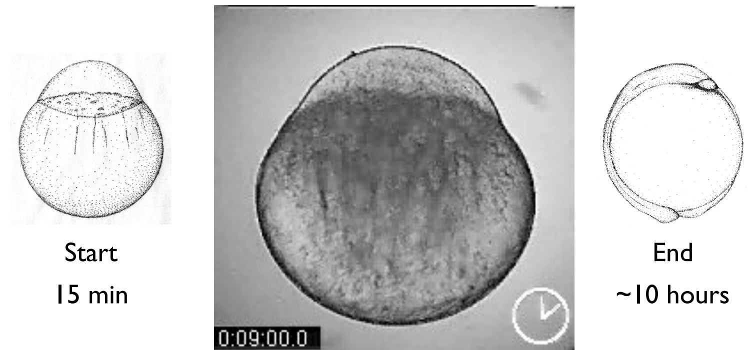


Start
45 min

End
~18 hours

Karlstrom and Kane, 1996

Zebrafish Development (1-cell through gastrulation)

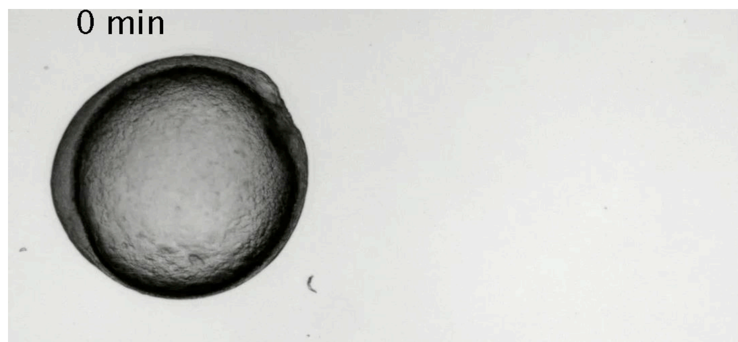


Start
15 min

End
~10 hours

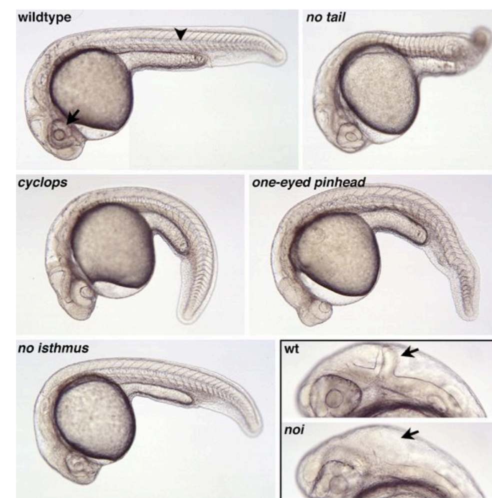
P.Z. Myers

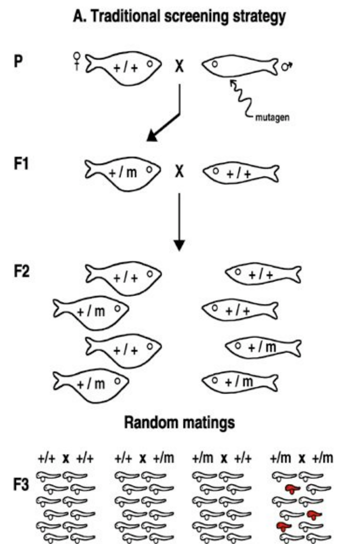
Making somites (11-24 hours)

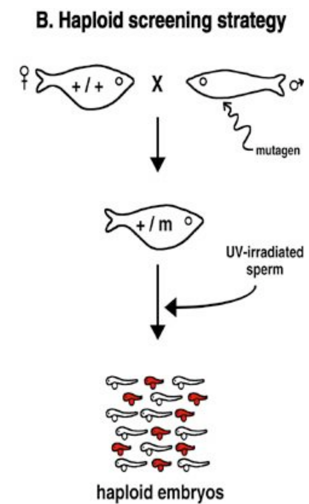


Shröter et al. (2008)

Zebrafish Mutants

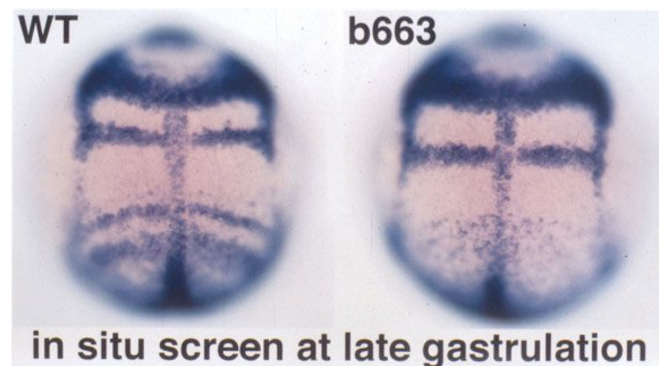




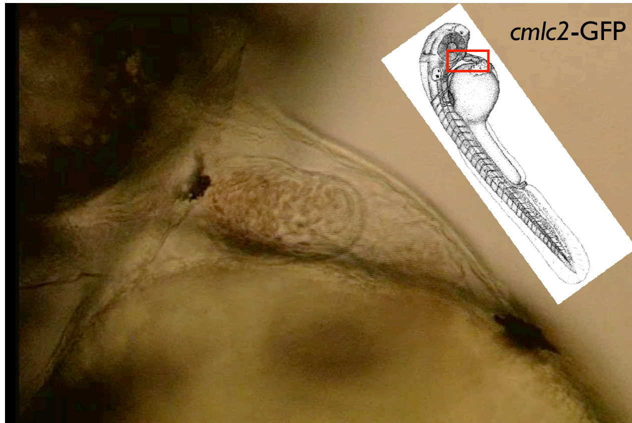


Screening for gene expression #1

Screening for gene expression #2

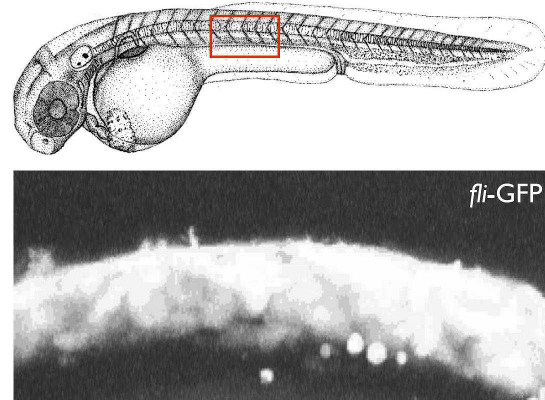


Watching Heart Function



Auman et al., 2007 *PLoS Biology* (Yelon Lab)

Angiogenesis



Brant Weinstein

Startle Response - Behavior

Granato Lab