Up until this point we have focused on “Classical Genetics”: Starting with a biochemical, developmental, or other process, identify the genes involved and figure out how they work together...

FROM FUNCTION TO GENES

Starting in the early 90s, we knew about a lot of genes that were emerging from genome sequencing projects, but whose function was completely unknown.

“Reverse Genetics” - investigating the function of known genes by targeted disruption

FROM GENES TO FUNCTION
Gene disruption in mice is a long and laborious process... it sure would be nice to characterize genes of interest in a simpler organism before going to all this trouble!

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Mario Capecchi  Sir Martin J. Evans  Oliver Smithies

...for developing methods for gene disruption (a.k.a. gene targeting, or genetic knockouts) in mice

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Reverse genetics in Drosophila

In *Drosophila*, it is possible (but not trivial) to generate mutations in specific genes by “hopping” transposable elements around the genome and then sifting through the collection of resulting flies for individuals that have a transposon in the gene of interest. A transposon insertion can create a loss-of-function mutation, but sometimes it doesn’t (for example, transposons have a tendency to jump into introns rather than exons, in which case they can get spliced out of the messenger RNA). In these cases, you have to get the transposon to hop *out* of the gene and hope for an *imprecise excision* that deletes some of the gene.

A few different transposons are used to generate these insertion collections, because individual transposons have “hotspots” where they like to jump, and may never land in certain genes.

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In *C. elegans*, Craig Mello’s lab reported in 1997 that injection of “antisense” RNA could apparently reduce the function of a gene of interest...

It was *thought* that this effect occurred through a mechanism that blocks translation:
...but, there was some serious weirdness. They noticed that the “control” sense RNA could induce the same effect.

Andy Fire and Craig Mello figured out that the interference was due to small amounts of double-stranded RNA in the “sense” and “antisense” preparations.

Injection of purified sense or antisense RNA from the unc-22 gene into wild-type worms did not produce a mutant phenotype, but mixing the two strands did.
For this discovery, they were awarded the 2006 Nobel Prize in Physiology or Medicine

Andrew Fire  Craig Mello

Why was this simple finding so revolutionary?

Their experiments, along with follow-up work by their labs and others, uncovered the existence of an unknown mechanism in plants, animals, and many fungi (but not budding yeast) called “double-stranded RNA-mediated interference,” or RNAi.

This knowledge has radically changed experimental biology, and led to the possibility of RNAi-based therapeutics
RNAi probably evolved because double-stranded RNA is viewed as “toxic” by eukaryotic cells. A special RNAse enzyme called Dicer chops up dsRNA into small fragments. The resulting siRNAs (small interfering RNAs) are then bound by a protein complex (the RISC) complex, which leads to destruction of any complementary mRNA.
Worms that eat bacteria expressing dsRNA will undergo silencing of the corresponding gene

piece of worm gene coding sequence

bacterial cell
(E. coli)

T7 promoters
are turned on
when bacteria
are treated with IPTG

selectable marker (to maintain plasmid)
Worms that eat bacteria expressing dsRNA will undergo silencing of the corresponding gene

Worms eating bacteria

Control dsRNA

plg-1 dsRNA

wild-type phenotype

Plg phenotype
We previously discussed the idea of making a “transgene” that expresses a gene of interest from a tissue- or cell-type-specific promoter. The mec-7 gene is normally expressed in the ALM neurons. A different gene placed under the control of the mec-7 promoter will also be expressed at high levels in those neurons. The same strategy can be used to induce RNAi in specific cells.
Cells from other organisms (e.g., *Drosophila*) will undergo RNAi-mediated gene silencing if they are treated with dsRNA

RNAi by feeding or soaking has enabled many high-throughput (genome-wide) screens

**Figure 1: High-throughput Screen Protocol**

- Storage in 96 well plates of a set of dsRNAs that cover the entire genome (21,300) (quantity enough for >200 screens)
- Ready-to-screen 384 well plates
- 3-4 days incubation time to ensure protein depletion
- Automated Microscopy
- Fluorescent plate reader assays
- Luciferase plate reader assays
Advantages of RNAi-based screens

Every known gene in the genome can be tested

There is no need to clone a gene that gives an interesting phenotype - you already know what it is!

Hypomorphic (reduction-of-function) phenotypes can be identified for essential genes, since RNAi gene silencing is often incomplete

This makes RNAi particularly useful to identify genetic ENHANCERS of a particular mutation, since hypomorphic alleles are frequently good enhancers