Extra office hours
10-12 Tuesday
1-3 Wednesday
1-3 Thursday

Garriga review session
Sunday 11/2 4-6 PM
2060 VLSB

TA review session
Monday 11/3
120 Lattimore

RNA interference
Reading:
http://www.ambion.com/techlib/hottopics/rnai/
Lecture notes for this and next lecture.

Phenomena first observed in petunia
Attempted to overexpress chalone synthase (anthocyanin pigment gene) in petunia.
(trying to darken flower color)
Caused the loss of pigment.

Called co-suppression because suppressed expression of both endogenous gene and transgene.

Two mechanisms can explain this transgene-mediated gene silencing

Transcriptional gene silencing
Post Transcriptional Gene Silencing (PTGS)
mRNA is made, but then degraded

In 1995 Guo and Kemphues wanted to show that they had cloned the C. elegans par-1 gene (required for normal division of the zygote).
Used antisense RNA to prove.

Antisense par-1
Ssense par-1control
Injection produced mutant par-1 phenotype
Injection produced mutant par-1 phenotype
What?
In 1998 Andy Fire and Craig Mello showed that injections of double stranded RNA was more effective than single stranded RNA in generating mutant phenotypes.

**mRNA of endogenous gene was degraded**

<table>
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<tr>
<th>No probe</th>
<th><em>mex</em>-3 probe: wild type</th>
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**mRNA of endogenous gene was degraded**

**What is going on?**
Both genetic and biochemical approaches used to define mechanism

**RNA interference**

dsRNA more effective

mRNA degraded

Estimate that a few RNA molecules/cell can completely silence expression.

**But first, can introduce dsRNA by injection or feeding**

*pos-1* is an essential gene

<table>
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<tr>
<th>Inject <em>pos-1</em> dsRNA</th>
<th>Feed worms bacteria that express <em>pos-1</em> dsRNA</th>
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Embryos die

Embryos die

**Two approaches to identifying molecules involved in RNAi**

**Genetic**

**Biochemical**

Extracts of *Drosophila* S2 tissue culture cells will produce RNAI!
General mechanism of RNAi

In some organisms, RNA-dependent RNA polymerase amplifies the RNAi response.

WHY RNAi?

RNAi components also involved in:
- Transposon silencing
- Viral defense
- Gene regulation

RNAi screens

86% of 19,427 predicted genes expressed as dsRNA in E. coli

RNAi of nuclear genes involved in mitochondrial oxidative phosphorylation causes increases in lifespan.

Differences in RNAi between C. elegans and Drosophila.

C. elegans
- spreading
- dsRNA RNAi

Drosophila
- No spreading
- dsRNA RNAi
- dsRNA RNAi
Remember the bipartite GAL4-UAS system

- GAL4 is a transcriptional activator from yeast that recognizes a DNA sequence called the UAS (upstream activating sequence)
- We can use this to control expression of YFG in a tissue specific manner by using enhancer elements specific for the tissue we are interested in

Can express a inverted repeat representing sequences of any gene of interest.

RNAi can be used on many organisms where genetic analysis has been unavailable.