

Genetic suppressors and enhancers provide clues to gene regulation and genetic pathways

Suppressor mutation: a second mutation results in a less severe phenotype than the original mutation

Suppressor mutations can be *intragenic* or *extragenic*

Enhancer mutation: a mutation in another gene results in a more severe phenotype than the original mutation

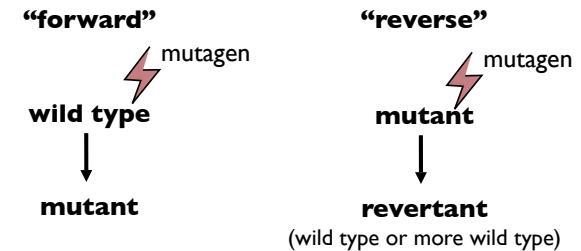
Phenotype ($m_1 + m_2$) > Phenotype (m_1) + Phenotype (m_2)



1

Screens for suppressors and enhancers are powerful tools to identify other genes that act in the same pathway/process as a known gene of interest.

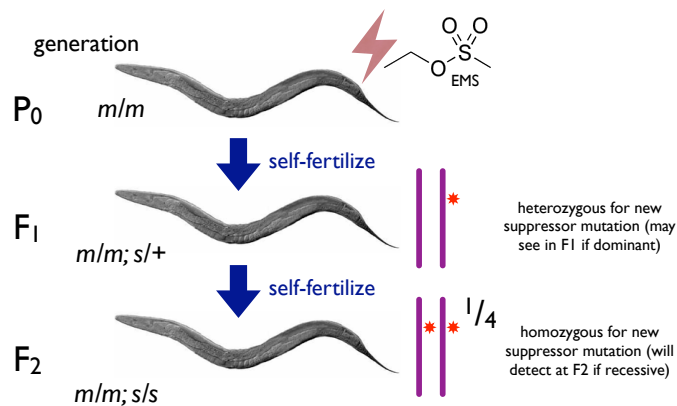
Suppressor screening in *C. elegans* is identical to a search for a revertant, or reverse mutation:



Note: don't confuse reverse mutations with reverse genetics

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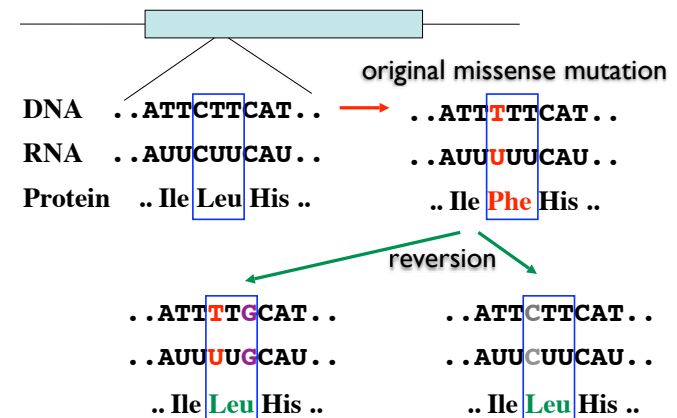
A "revertant" isolated in this manner may result from a new mutation in the same gene (in which case it's called a *revertant* or an *intragenic suppressor*), or a mutation in a different gene, in which case it's an *extragenic*, or *second-site*, suppressor



A dominant suppressor may have no phenotype on its own, or it may have a recessive phenotype

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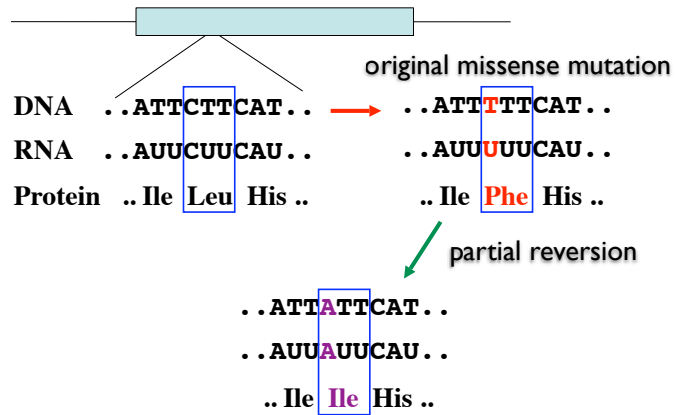
One class of intragenic suppressors: true revertants



Simplest case of reversion: restoration of the original protein sequence

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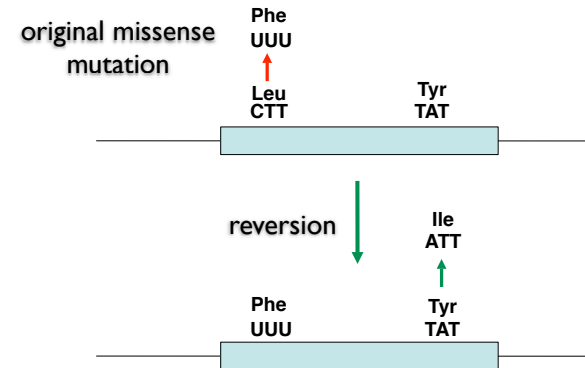
Another simple type of intragenic suppressor: partial revertants



Ile (isoleucine) is chemically more similar to leucine (Leu) than phenylalanine (Phe) and may be less disruptive of the protein's function

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Slightly more complicated intragenic suppressors: compensatory mutations



A second mutation offsets the damage
created by the first mutation

Here, an interaction in the 3D structure of the protein between leucine and tyrosine was disrupted by mutation of the leucine to phenylalanine, but the structure was restored by mutation of the tyrosine to isoleucine

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Extragenic suppressors

1. Interaction suppressors: allele specific, gene specific
These are compensatory mutations in a different gene
2. Informational suppressors: allele specific, gene nonspecific
(we'll also cover these today)

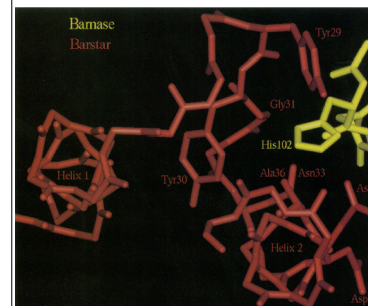
On Wednesday:

3. Bypass suppressors (parallel pathways): allele nonspecific, gene specific
4. Bypass suppressors (same pathway): allele nonspecific, gene specific (for example: *ced-3* or *ced-4* mutations suppress *ced-9* mutations)

ced-9 —| *ced-4* → *ced-3* → Apoptosis

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Interaction suppressors restore protein-protein interactions



Interaction suppressors can identify other important genes in a pathway of interest, and can tell you a lot about how proteins interact.

They are both *allele-specific* and *gene specific*

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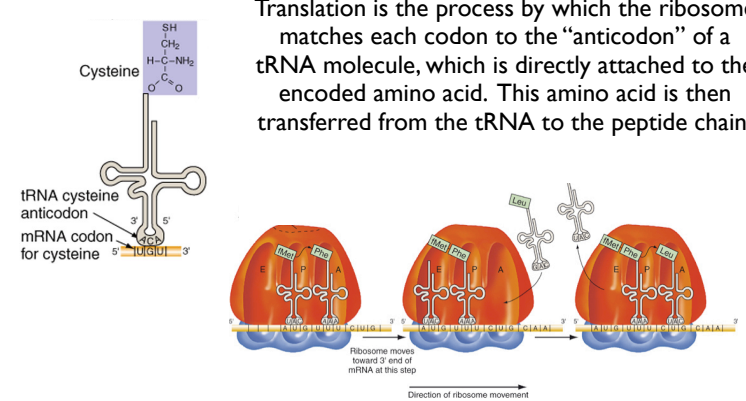
“Informational” suppressors are extragenic mutations that enable a mutated gene to function (usually only partially)

Two types that we will discuss:

nonsense suppressors
amber (UAG), *ochre* (UAA), *opal* (UGA)
smg suppressors

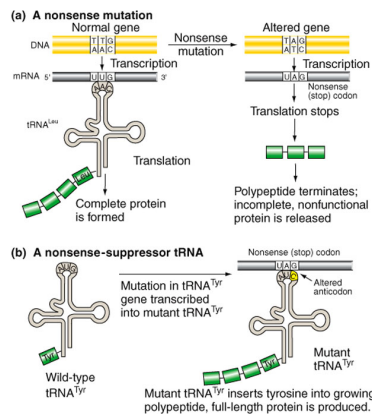
“Amber” mutations are nonsense mutations from any coding codon (often Tyr) to a UAG stop codon

To understand how nonsense suppressors work, we have to talk a little bit more about translation...



Translation is the process by which the ribosome matches each codon to the “anticodon” of a tRNA molecule, which is directly attached to the encoded amino acid. This amino acid is then transferred from the tRNA to the peptide chain.

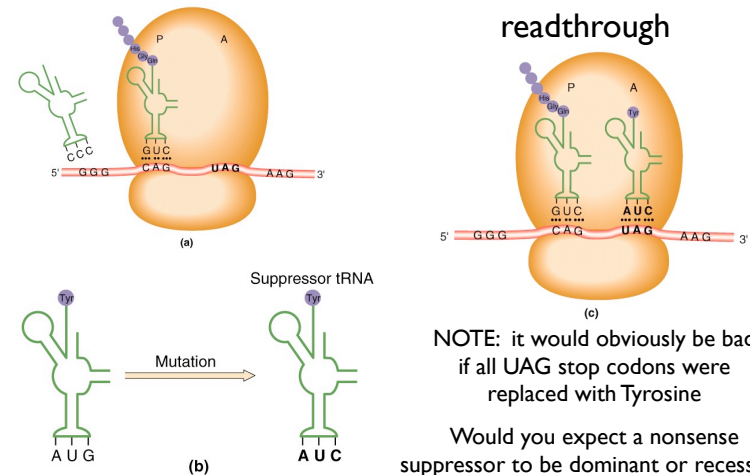
An “amber suppressor” is a mutation in a tRNA gene that enables the ribosome to put an amino acid at a UAG codon



NOTE: amber suppressor will suppress any amber mutation, but *not* other nonsense mutations (opal or ochre), missense mutations, frameshift mutations or deletions. Nonsense suppressors are therefore *allele specific* but *gene nonspecific* in their suppression.

An amber mutation in tRNA^{Tyr} is in the anticodon loop.

readthrough



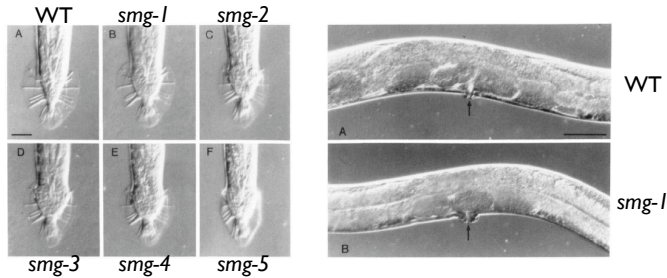
NOTE: it would obviously be bad if all UAG stop codons were replaced with Tyrosine

Would you expect a nonsense suppressor to be dominant or recessive?
 Read about this on pp. 290-91 (Ch. 8)

smg suppression

(Suppressors with morphological defects in the genitalia)

This class of suppressors was discovered in *C. elegans* and *S. cerevisiae* but they exist in all eukaryotes.



Mutations in seven different genes (*smg-1-7*) were isolated as allele-specific suppressors of *tra-2*, *lin-29*, and *unc-54*.

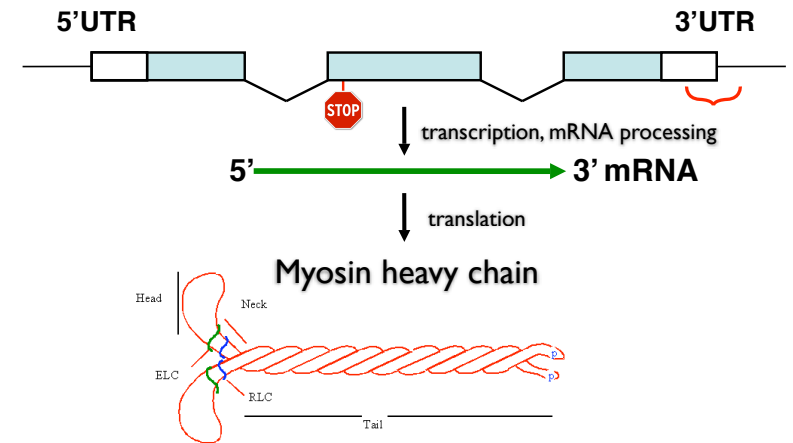
Like nonsense suppressors, these are informational suppressors they are *allele specific* and *gene nonspecific*

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The *unc-54* gene encodes muscle myosin

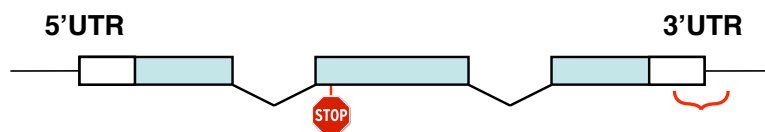
Homozygous *unc-54* mutants are paralyzed

Some mutations in *unc-54* mapped to the 3' UTR??

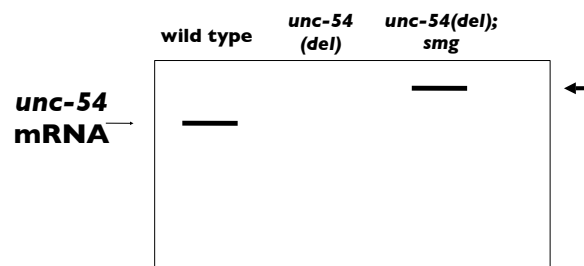


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Suppressors screens for *unc-54* suppressors identified a number of *smg* genes. Mutations in these genes stabilize the *unc-54* mRNA

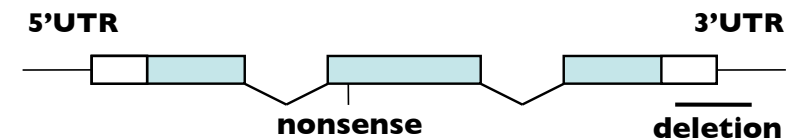


Northern blot probed with labeled *unc-54* DNA

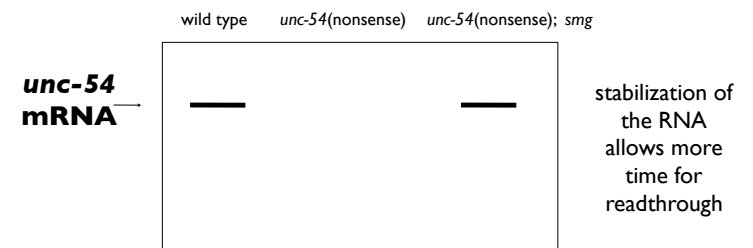


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Smg mutations can also suppress mRNA instability caused by nonsense mutations

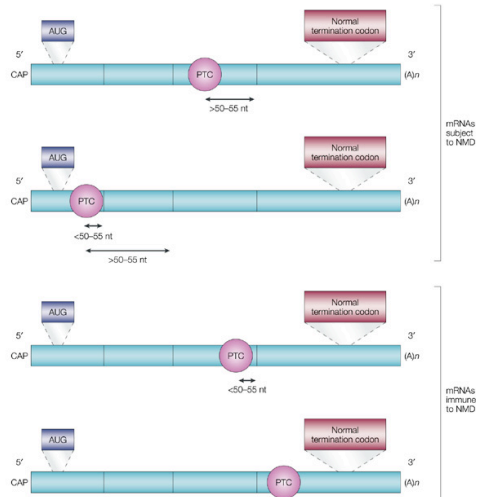


Northern blot probed with labeled *unc-54* DNA



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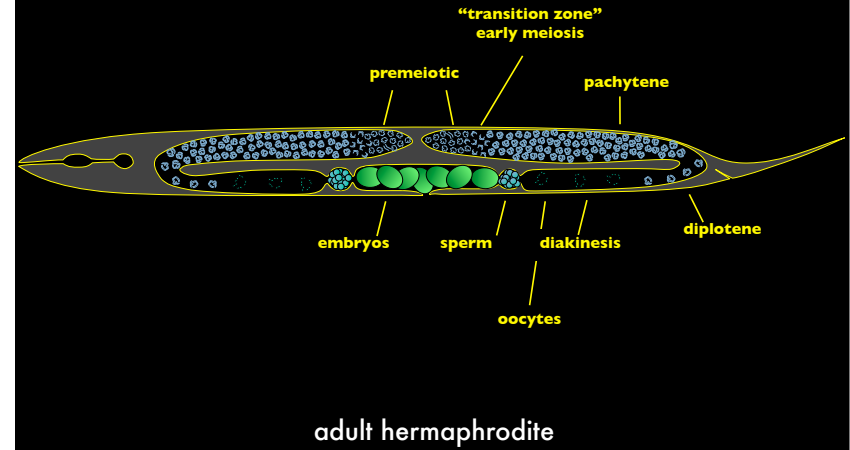
The *smg* genes encode components of a mechanism that degrades mRNAs with premature stop codons - "nonsense-mediated decay" (NMD)



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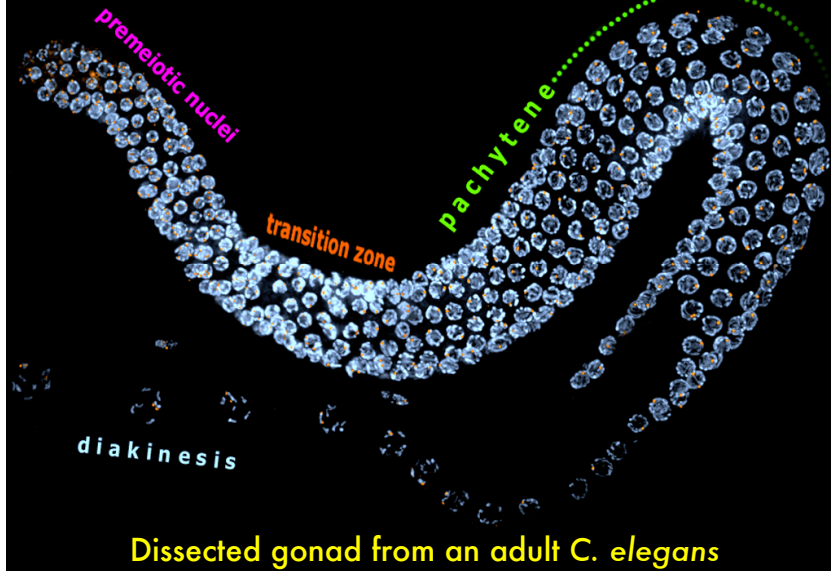
Meiosis in *C. elegans*



adult hermaphrodite

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All stages of meiosis can be seen in the microscope



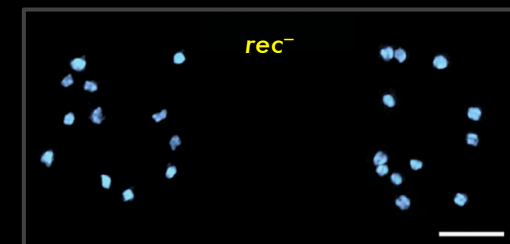
Dissected gonad from an adult *C. elegans*

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Lack of crossover recombination can be detected genetically or cytologically



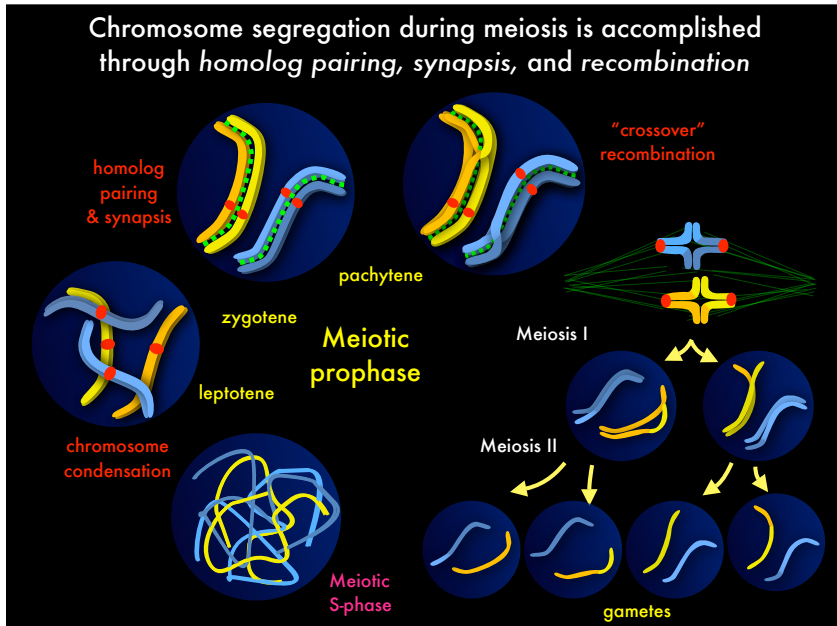
6
bivalents



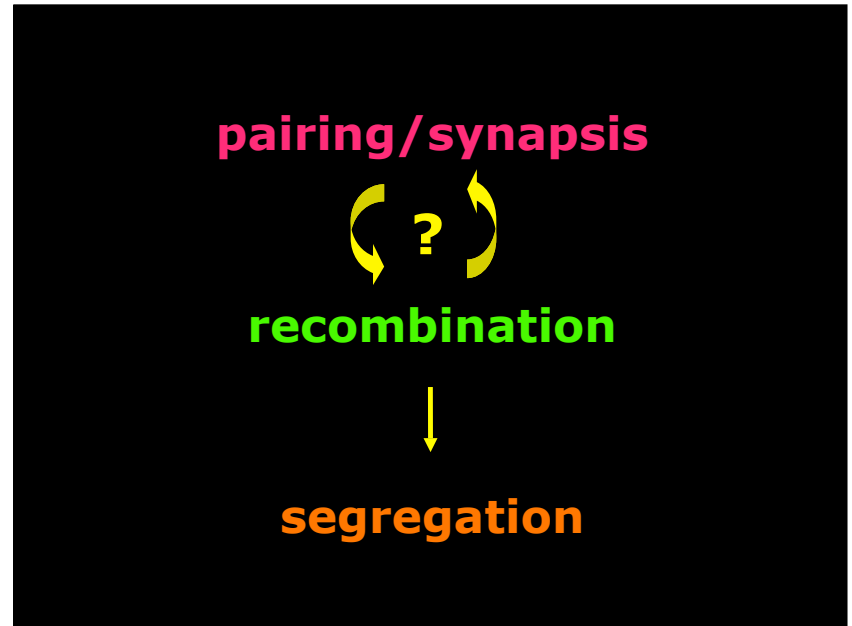
12
univalents

oocytes at diakinesis

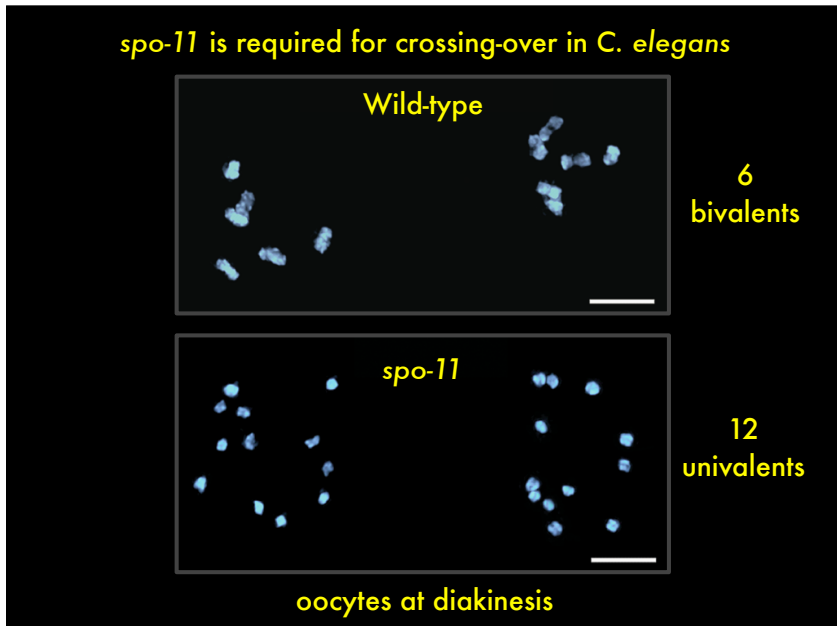
20



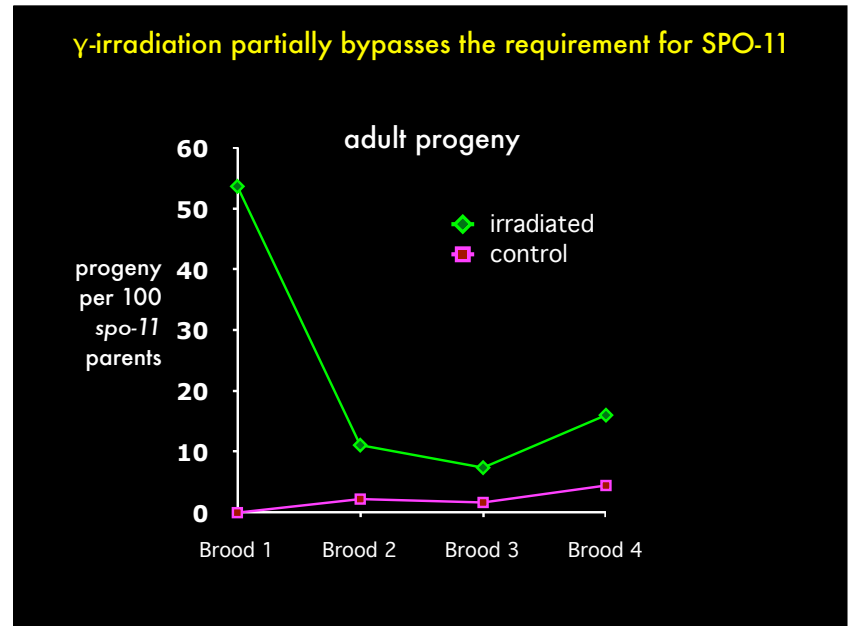
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Irradiation generates crossovers
in the *spo-11* mutant



Look for **Unc non-Dpy** and **Dpy non-Unc** recombinants
among the adult progeny:

♀ 19/93
recombinant

♂ 5/29
recombinant

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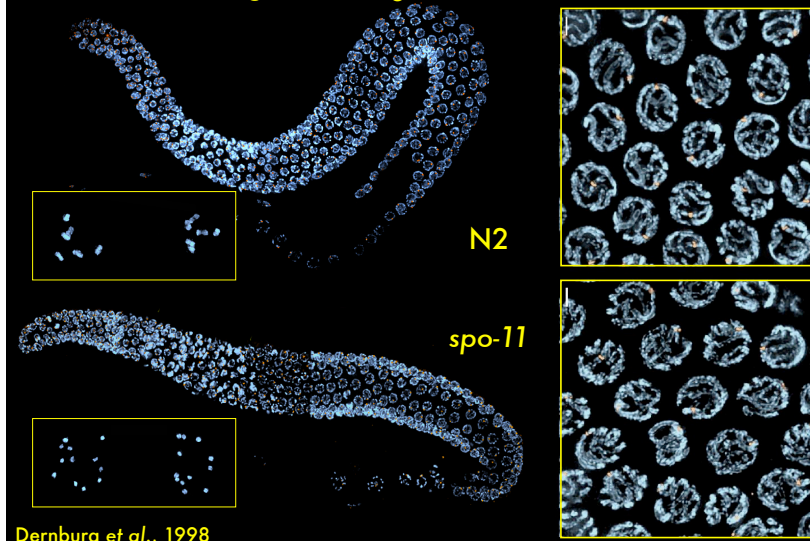
spo-11 is required for crossing-over in *C. elegans*

genotype	recombinant chromosomes*	total chromosomes	map distance (cM)	% of control map distance
+/+ or <i>spo-11</i> /+	503	1332	37.8	100
<i>spo-11</i> / <i>spo-11</i>	0	240	<0.5	<1

**dpy-3* - *unc-3* interval on X chromosome

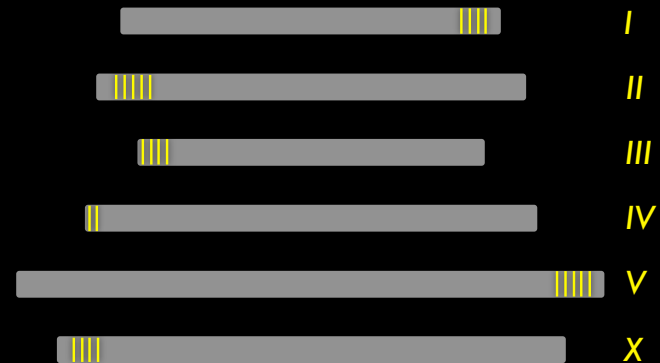
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Homolog pairing occurs normally in
C. elegans lacking double-strand breaks



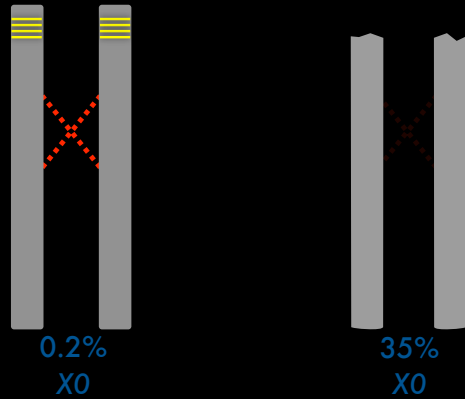
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Each of the 6 chromosomes in *C. elegans* has a special region
called a "Pairing Center"
that plays an important role in homologous recombination



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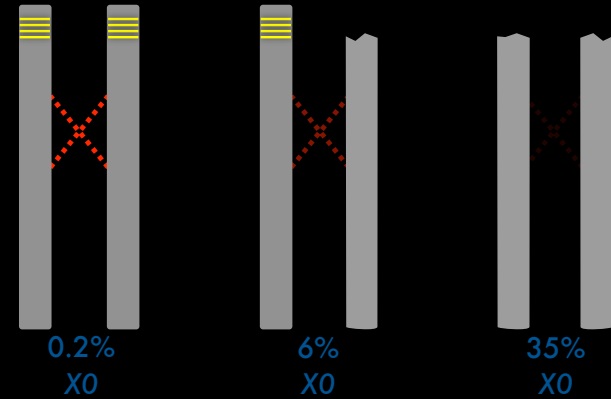
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Villeneuve (1994) *Genetics* 136, 887-902

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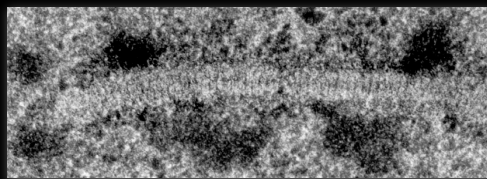
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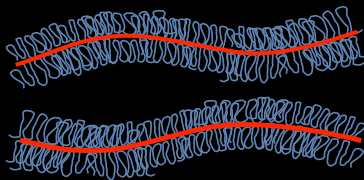
...but the determinants of homologous recognition are not restricted to this region of the chromosome

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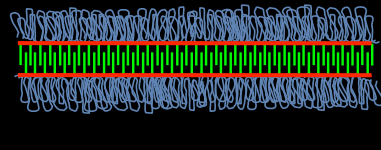
The Synaptonemal Complex (SC) is built in discrete steps



C. elegans
SC



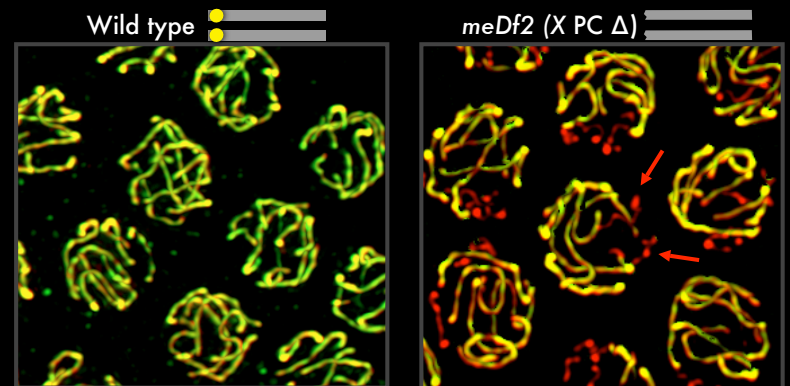
AXIAL ELEMENTS form between sisters prior to and independently of homolog pairing



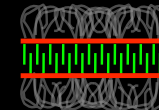
The CENTRAL ELEMENT normally polymerizes only after homolog pairing

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Central elements usually fail to polymerize between X chromosomes lacking Pairing Centers



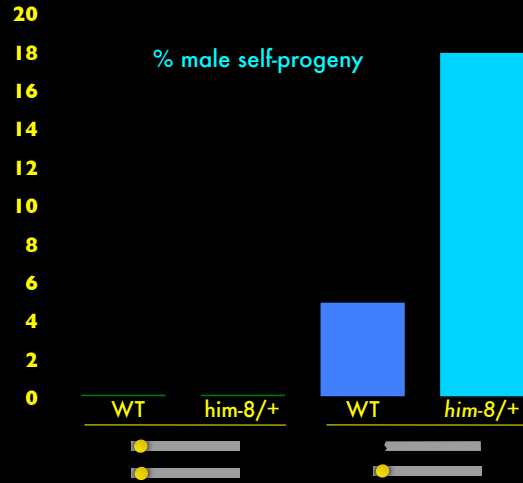
α -HTP-3
Axial
Element



α -SYP-1
Central
Element

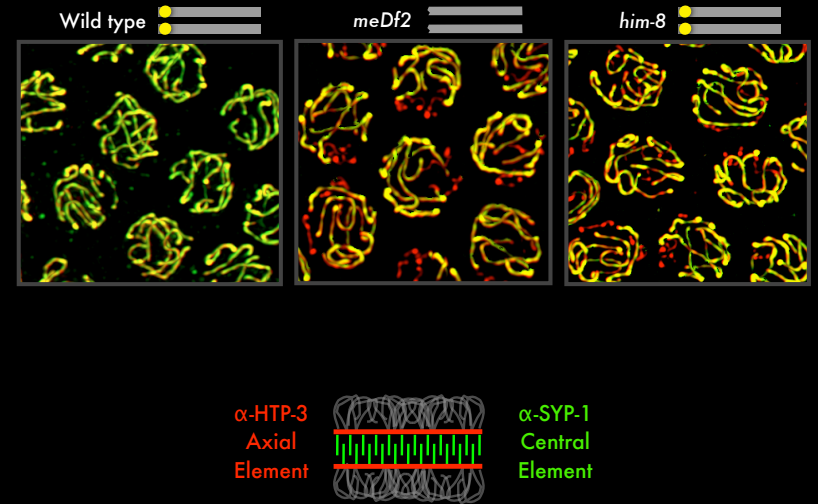
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him-8 mutations show dominant genetic enhancement of Pairing Center mutations



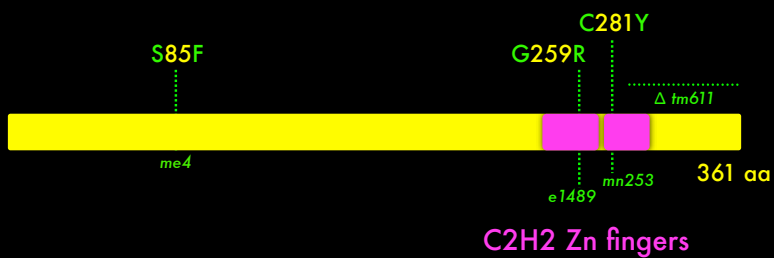
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Synapsis of the X chromosomes requires the X Pairing Center and the *him-8* gene



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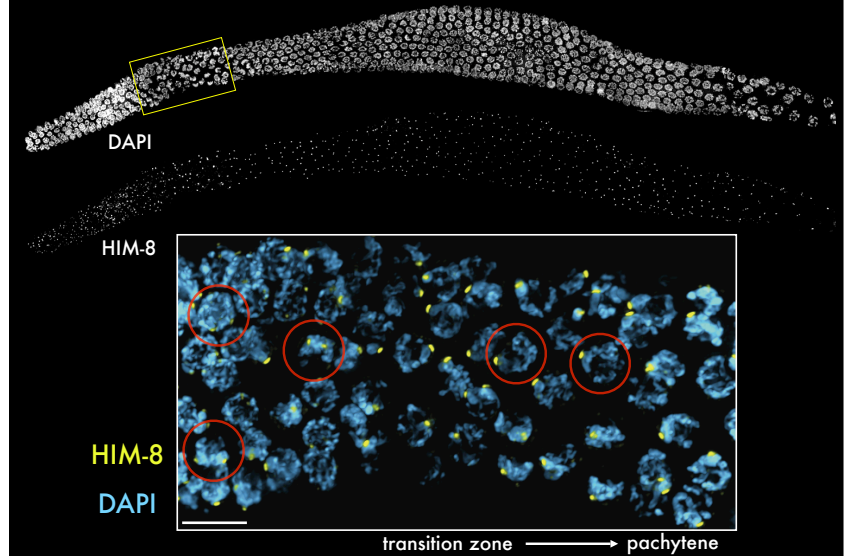
him-8 encodes a C2H2 zinc finger protein



Amino acid changes in mutant *him-8* alleles

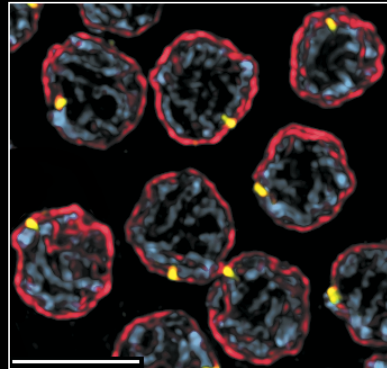
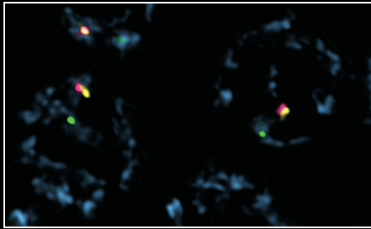
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HIM-8 localizes to chromosome foci



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HIM-8 localizes to the Pairing Center region of the X chromosome



α -HIM-8
DAPI

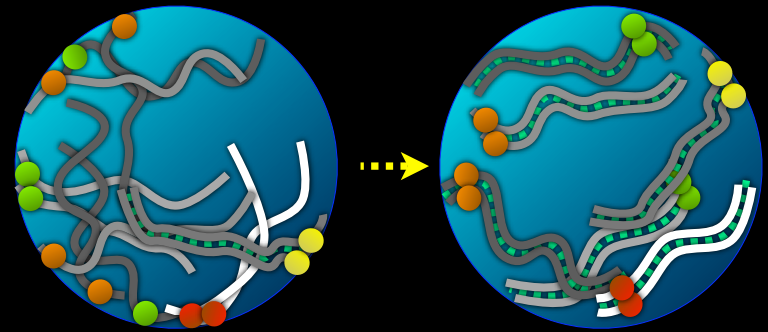
X-Left X-Center

and associates with the nuclear envelope

α -HIM-8 α -LMN-1 DAPI

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Each chromosome binds a specific HIM-8/ZIM protein to accomplish homologous pairing and synapsis



Transition zone

Pachytene

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