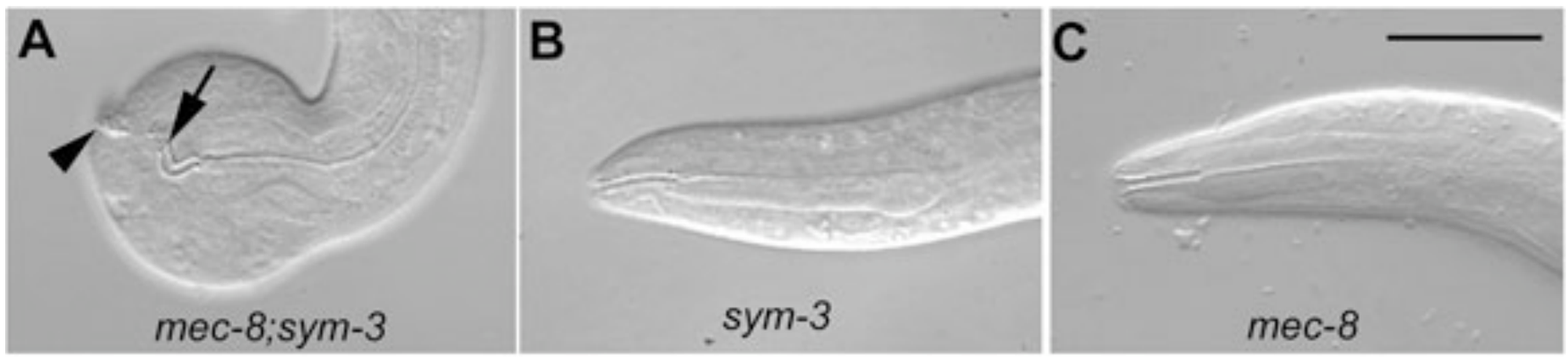
Genetic suppressors and enhancers provide clues to gene regulation and genetic pathways

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)



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:

"forward"

mutagen

wild type

mutant

"reverse"

mutagen

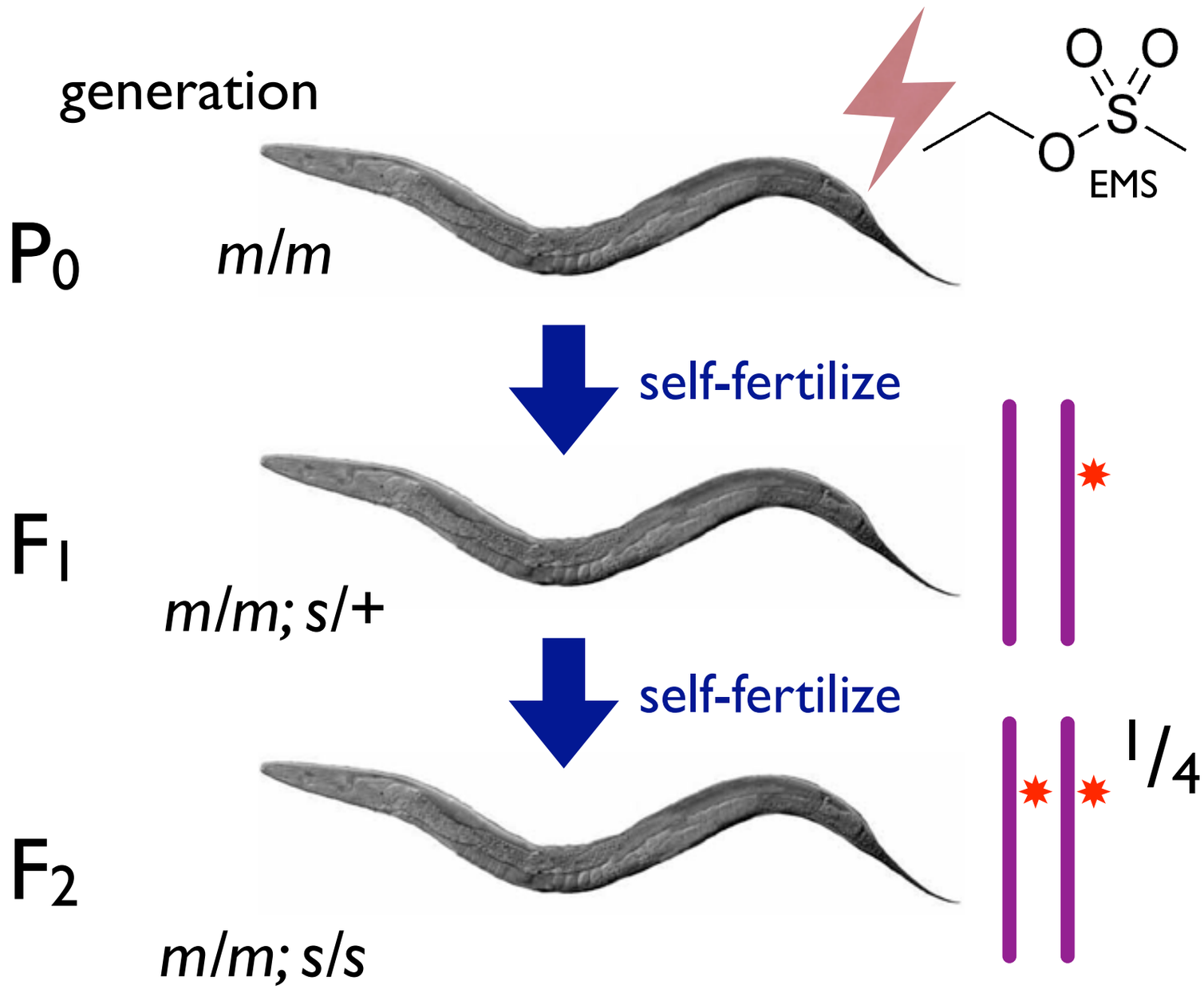
mutant

revertant

(wild type or more wild type)

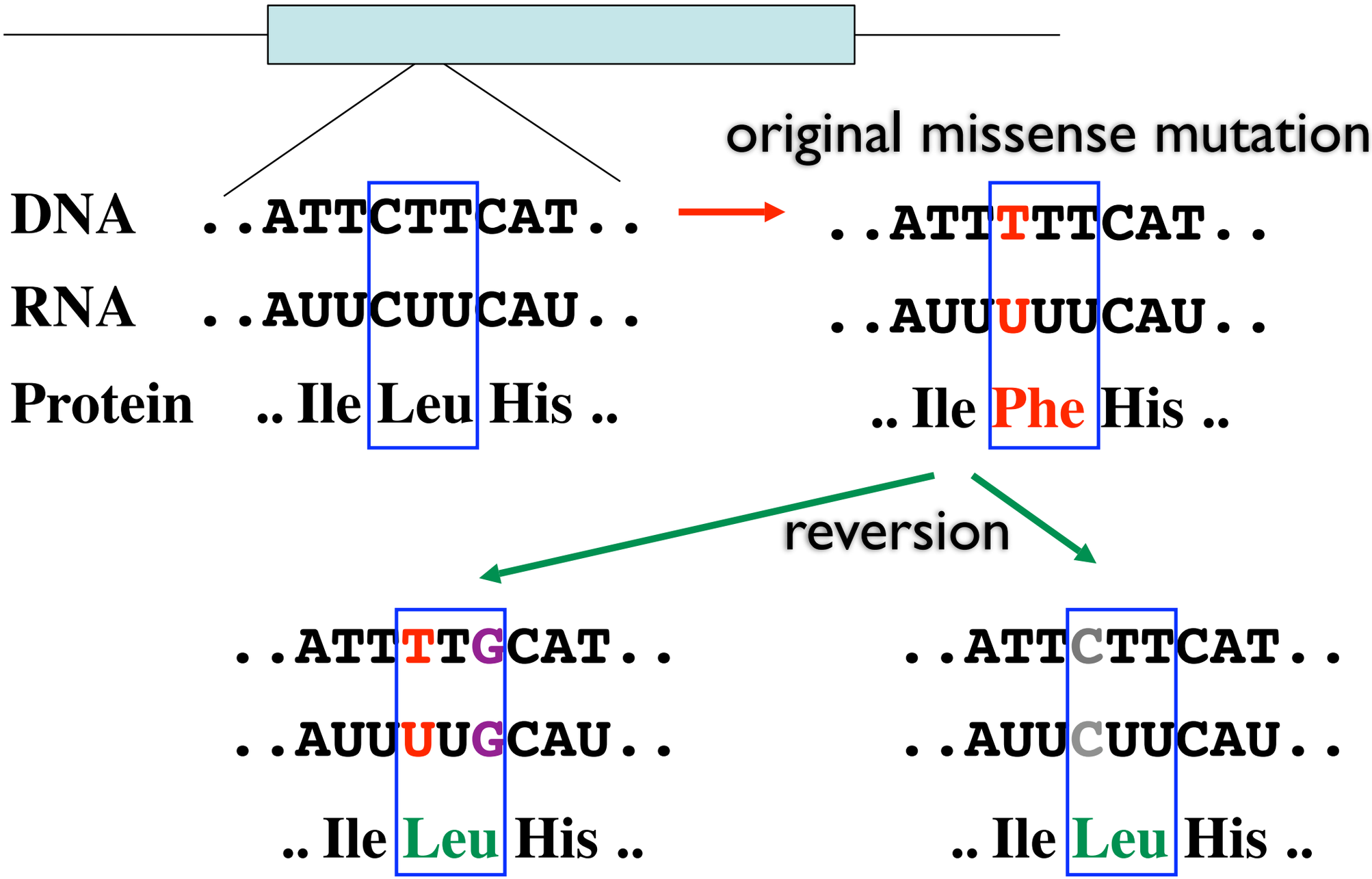
Note: don't confuse *reverse mutations* with *reverse genetics*

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



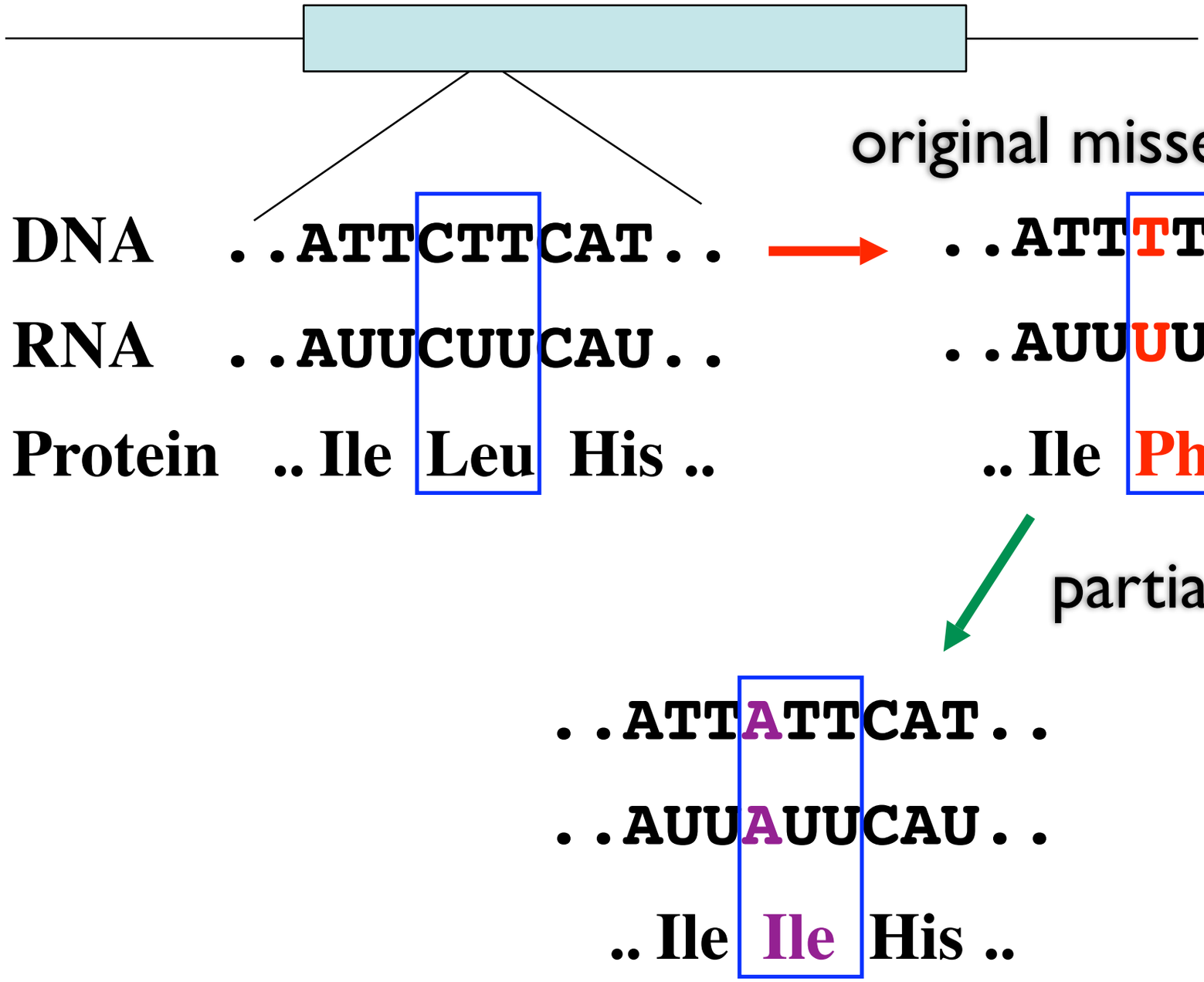
A dominant suppressor may have no phenotype on its own, or it may have a recessive phenotype

One class of intragenic suppressors: true revertants



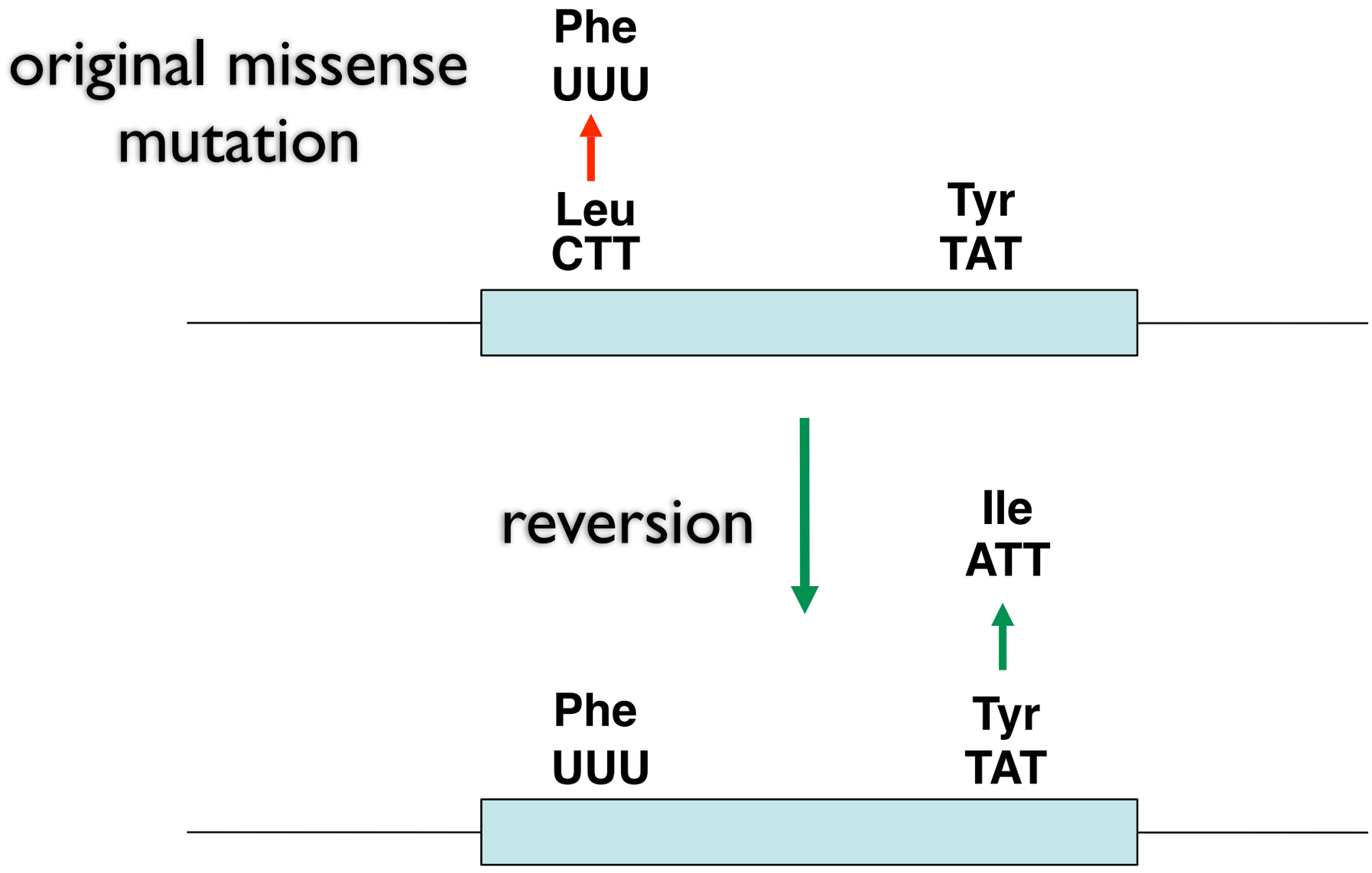
Simplest case of reversion: restoration of the original protein sequence

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

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

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)

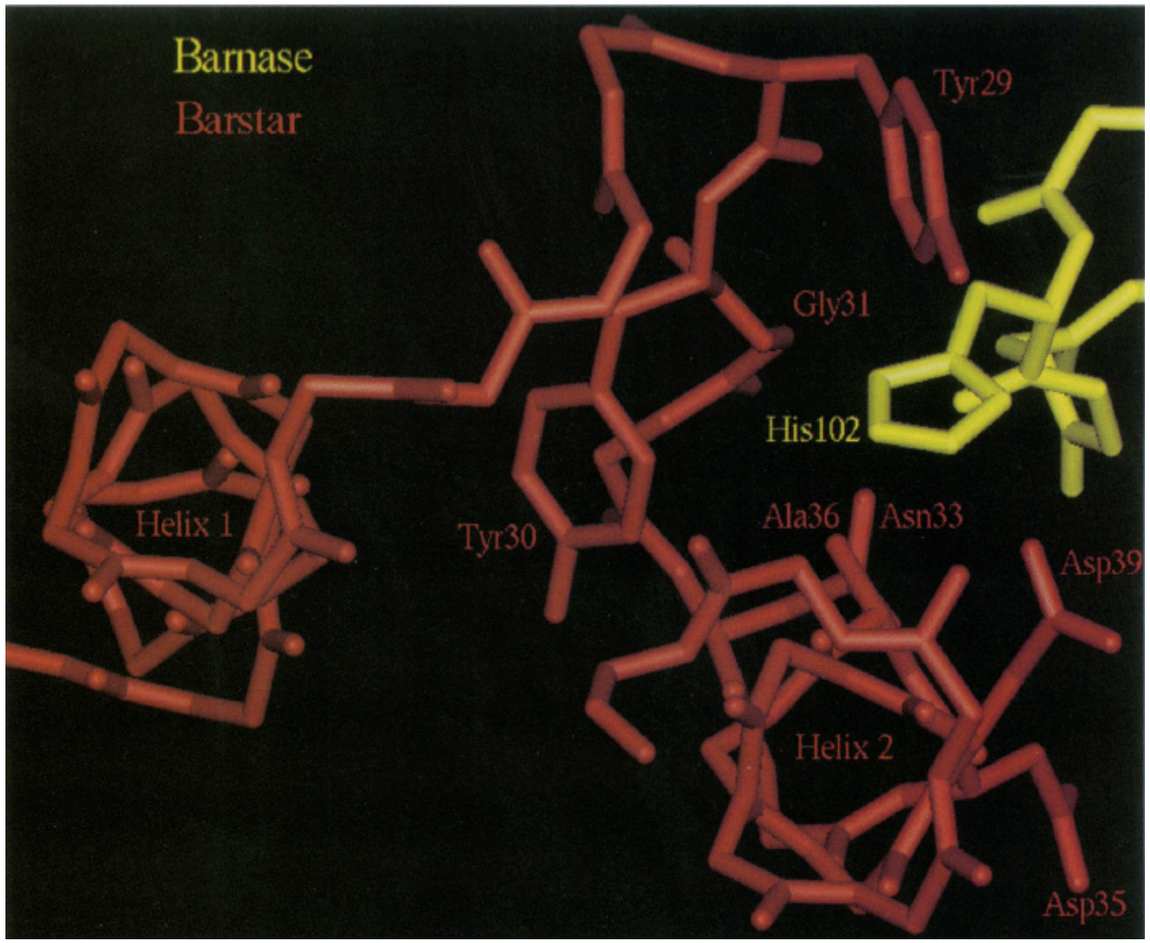
Interaction suppressors restore protein-protein interactions

protein B

protein A

missense mutation in *gene A* disrupts interaction

missense mutation in *gene B* restores interaction



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*

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

<u>Two types that we will discuss:</u>

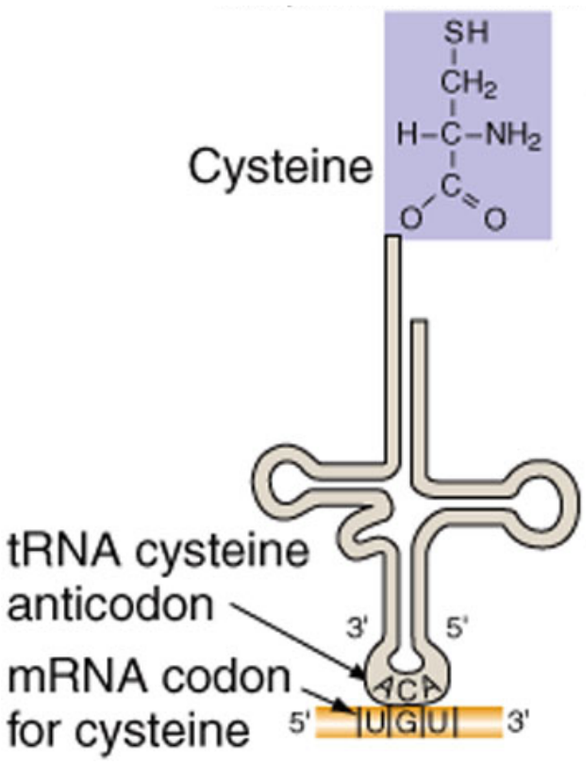
nonsense suppressors

amber (UAG), *ochre* (UAA), *opal*(UGA)

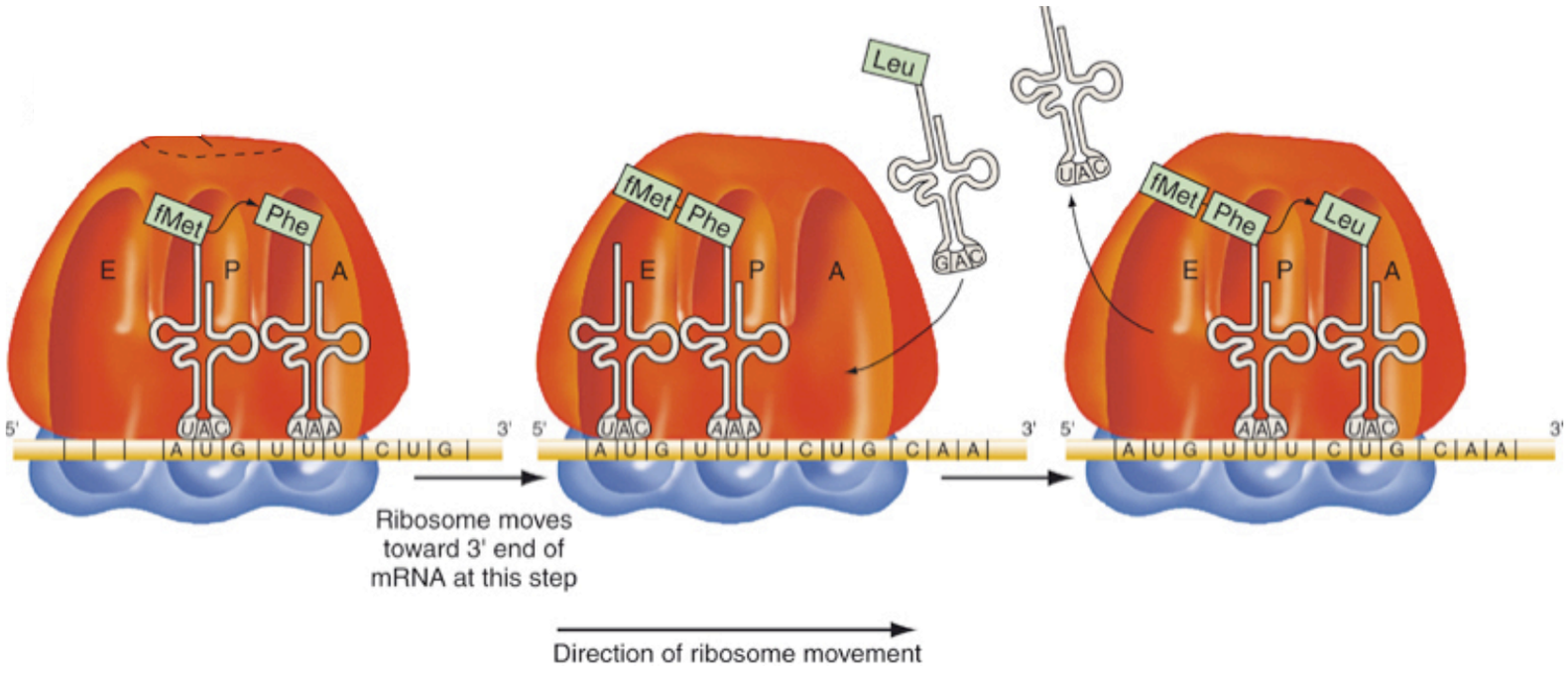
smg suppressors

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

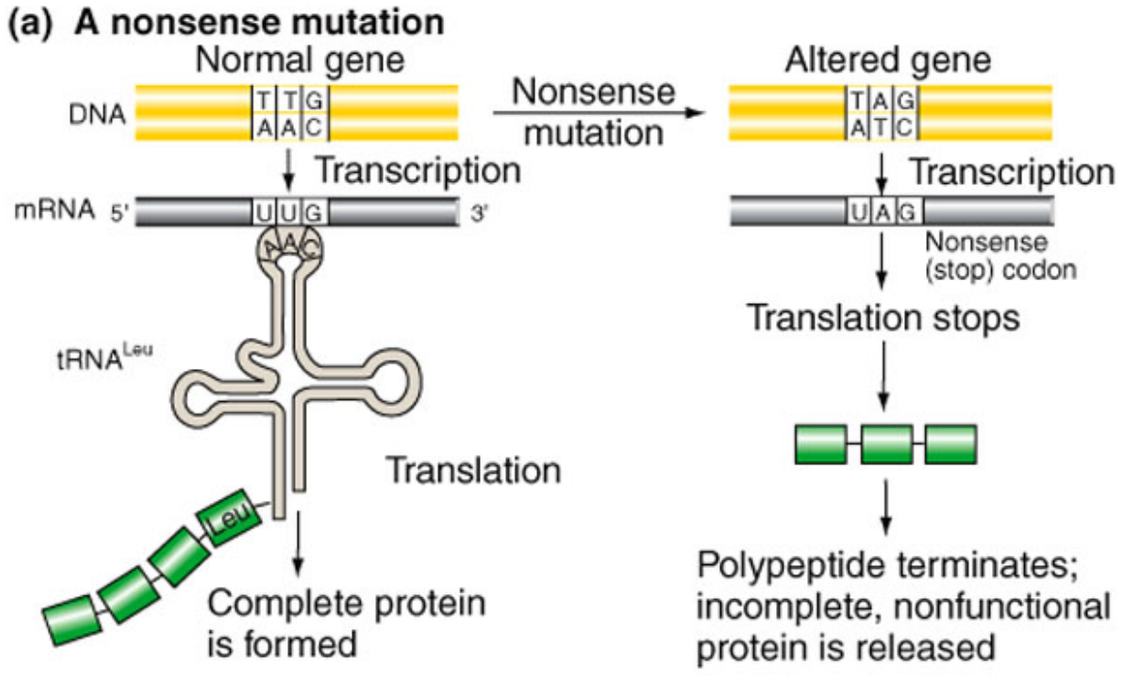
To understand how nonsense suppressors work, we have to talk a little bit more about translation...

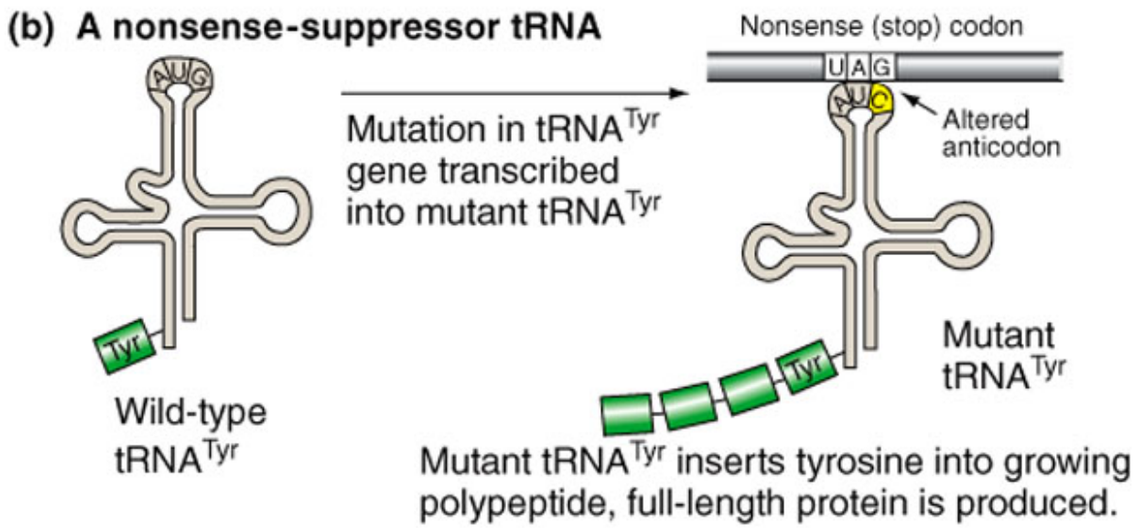


Translation is the process by which the ribosome matches each codon to the "anticodon" of a tRNA molecule, which is directly attached to the encoded amino acid. This amino acid is then transferred from the tRNA to the peptide chain.



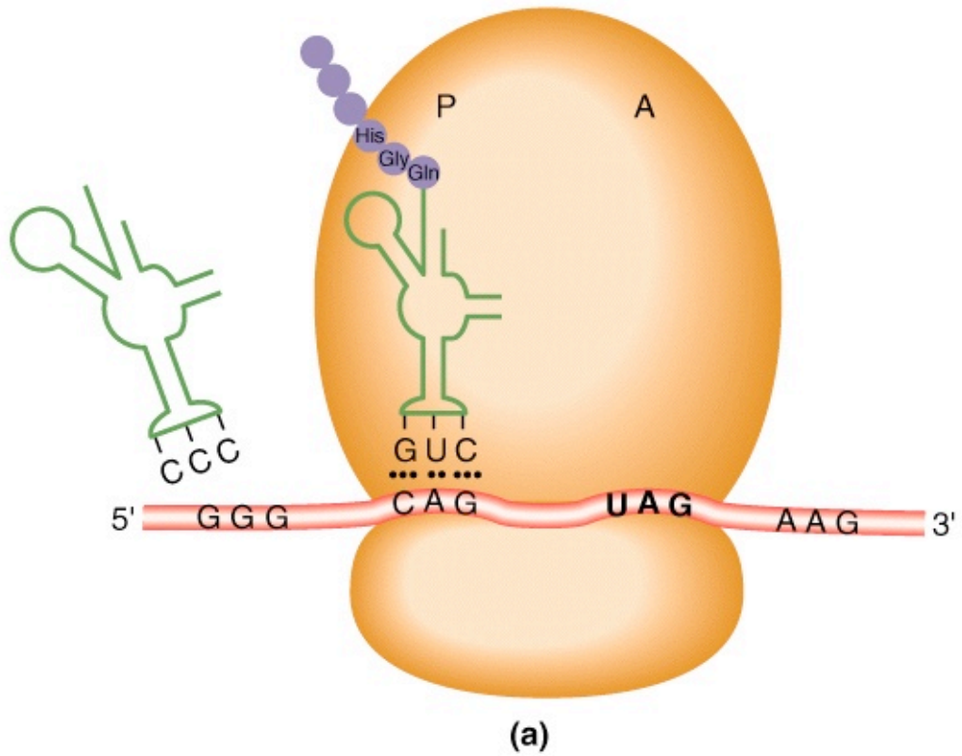
An "amber suppressor" is a mutation in a tRNA gene that enables the ribosome to put an amino acid at a UAG codon



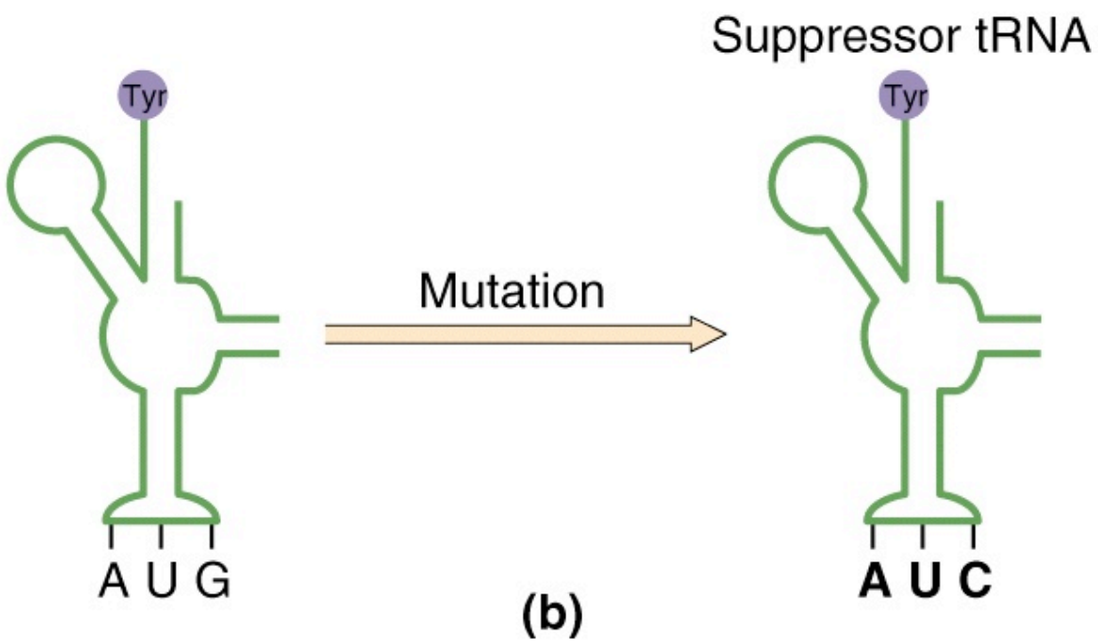


NOTE: amber suppressor will suppress any amber mutation, but *not* other nonsense mutations (opal or ochre), missense mutations, frameshift mutations or deletions. Nonsense suppressors are therefore *allele specific* but *gene nonspecific* in their suppression.

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

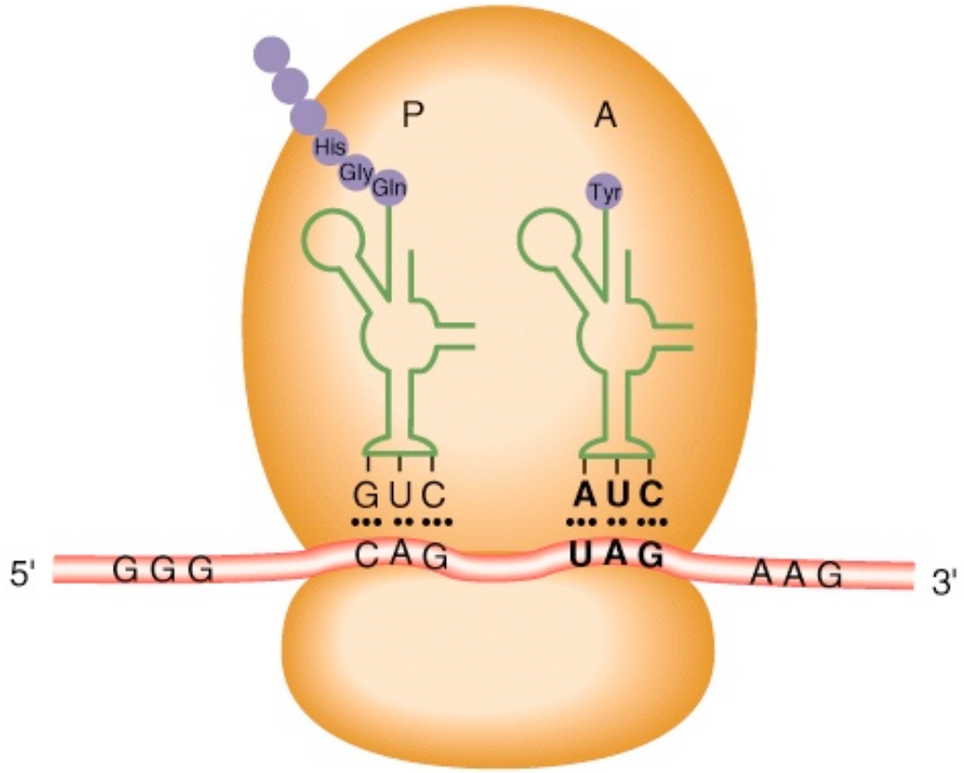


(a)



(b)

readthrough



(c)

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

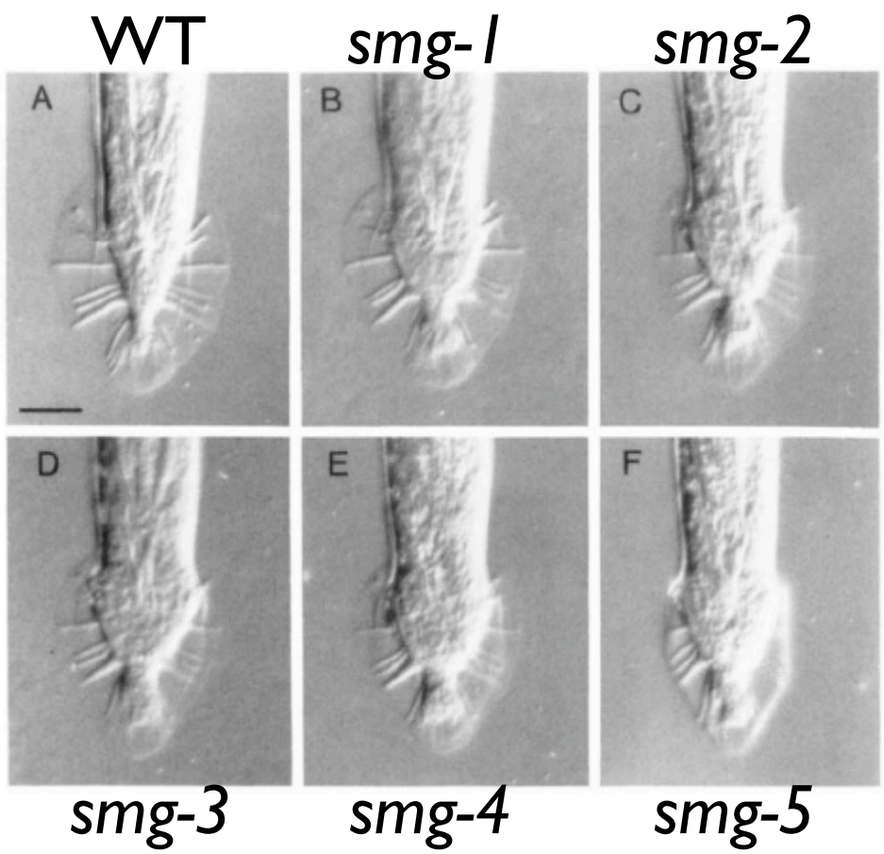
Would you expect a nonsense suppressor to be dominant or recessive?

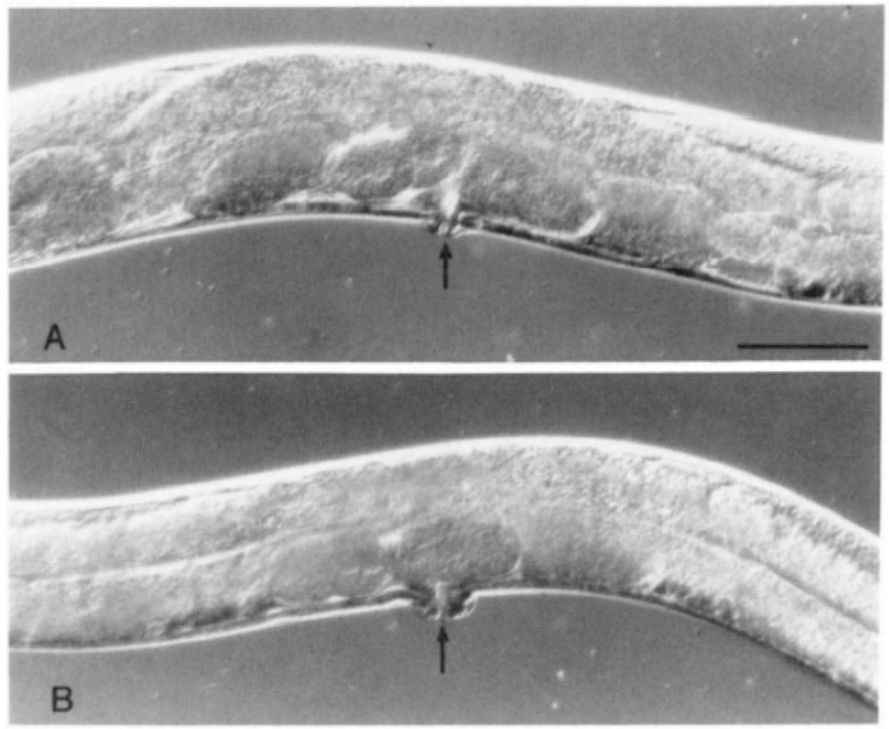
Read about this on pp. 290-91 (Ch. 8)

smg suppression

(Suppressors with *m*orphological defects in the genitalia)

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





WT

smg-1

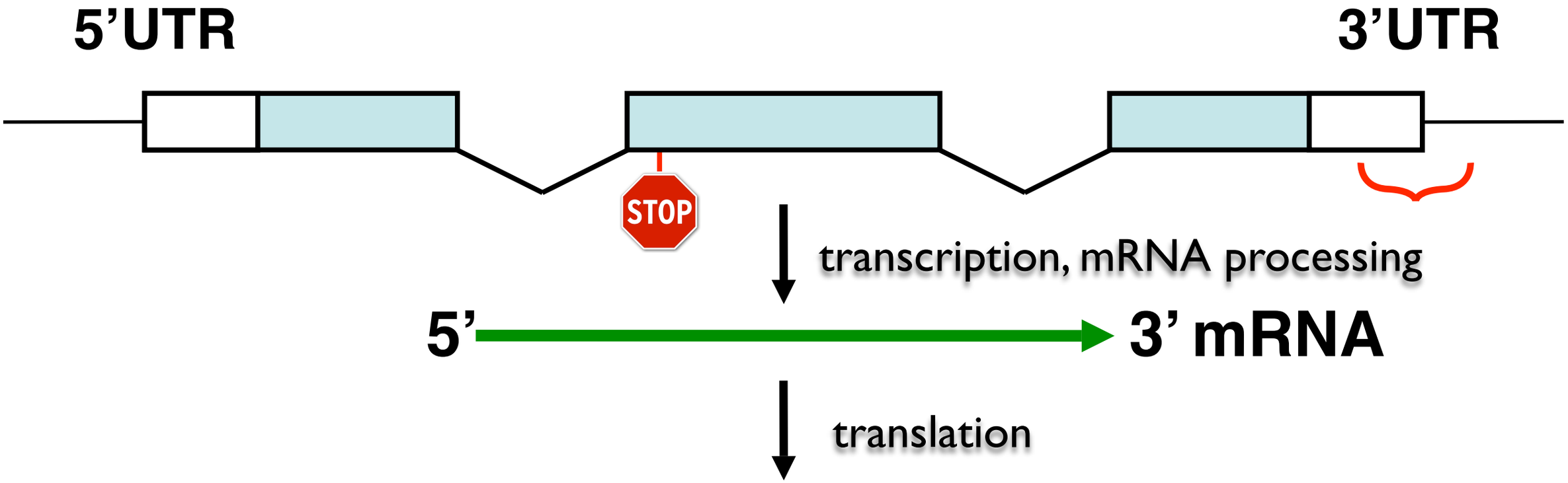
Mutations in seven different genes *(smg-1-7)* were isolated as allele-specific suppressors of *tra-2*, *lin-29*, and *unc-54*.

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

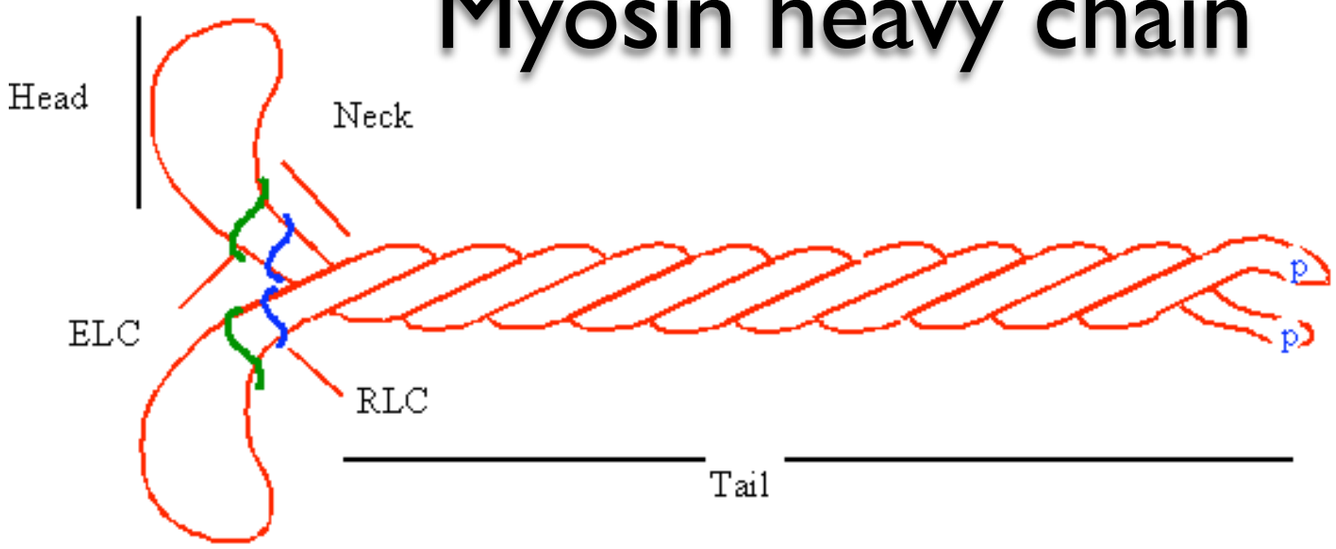
The *unc-54* gene encodes muscle myosin

Homozygous *unc-54* mutants are paralyzed

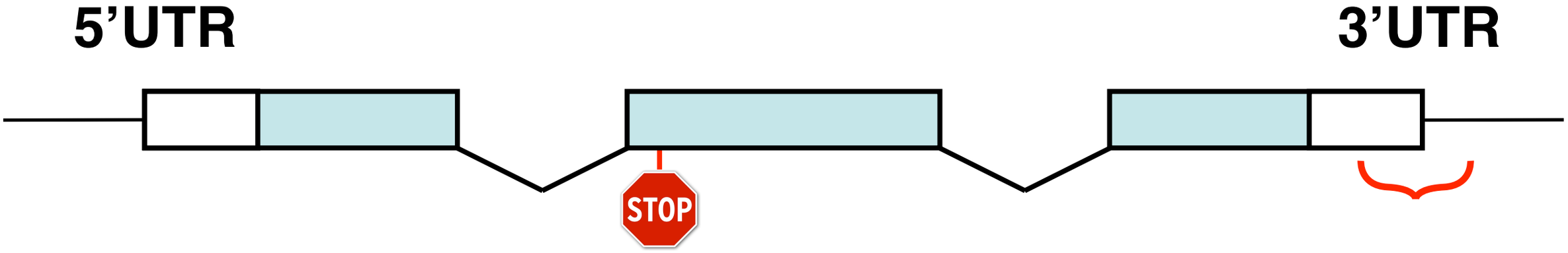
Some mutations in unc-54 mapped to the 3' UTR??



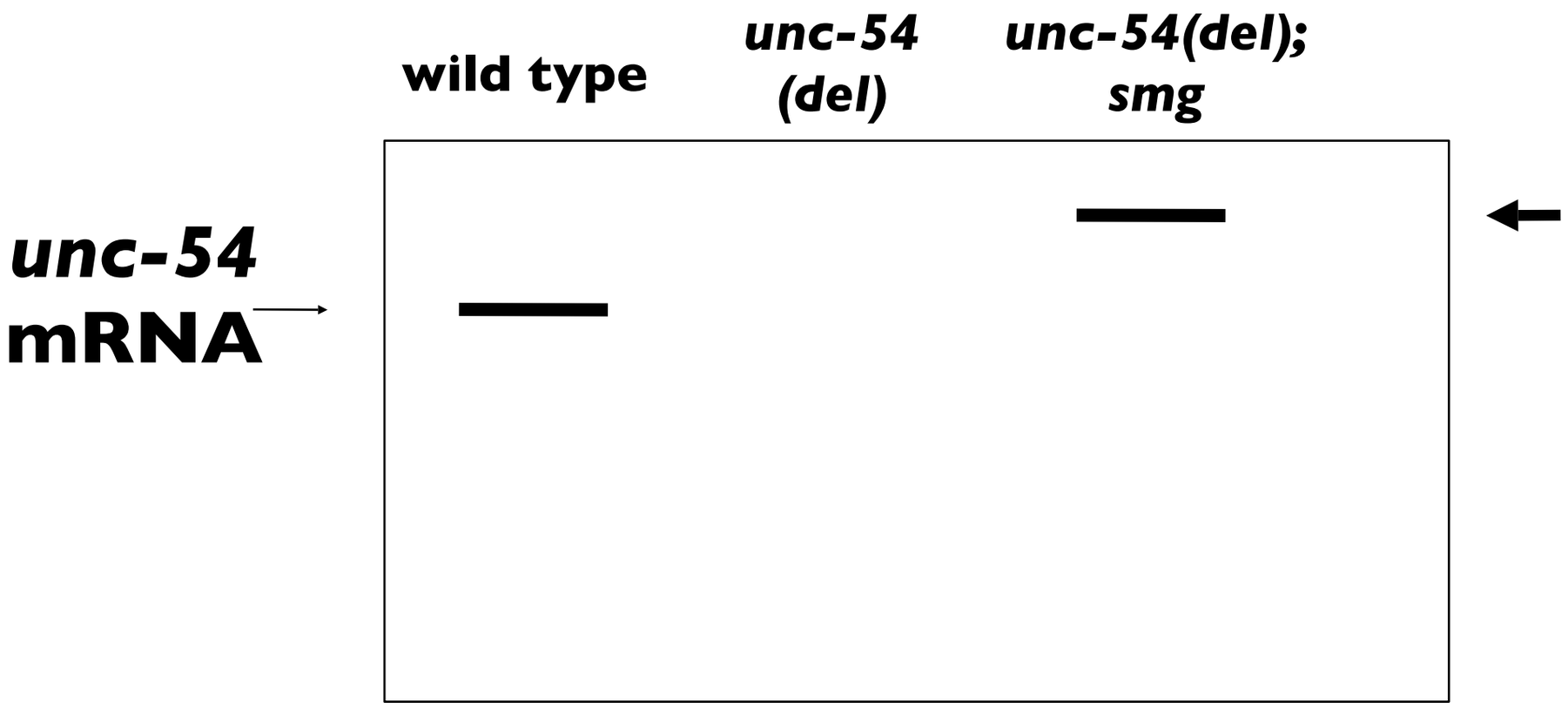
Myosin heavy chain



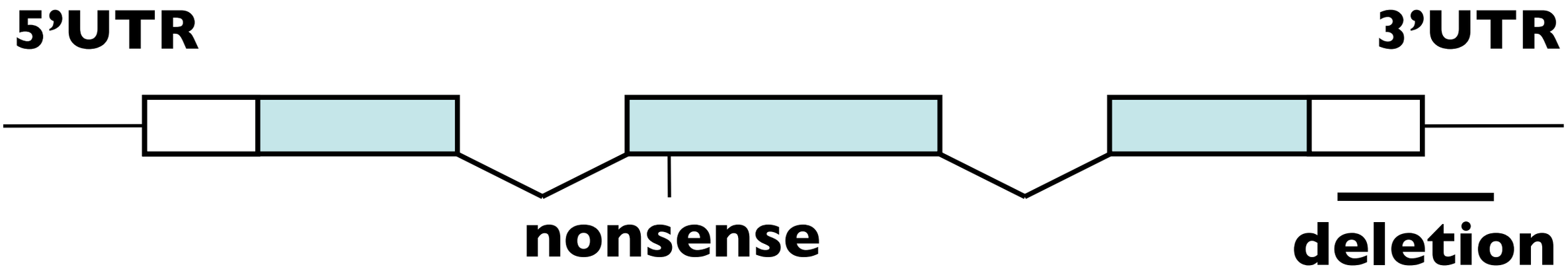
Suppressors screens for *unc-54* suppressors identified a number of *smg* genes. Mutations in these genes stabilize the *unc-54* mRNA



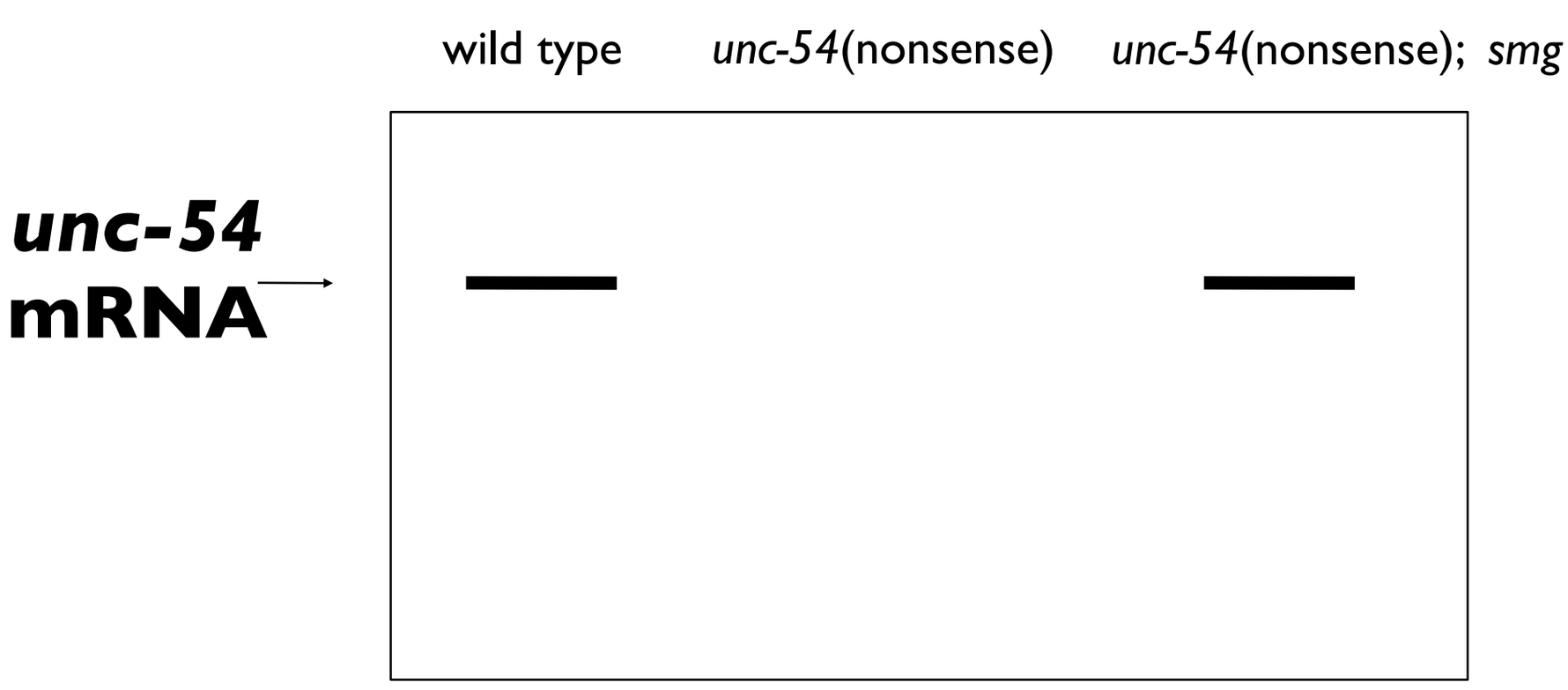
Northern blot probed with labeled *unc-54* DNA



Smg mutations can also suppress mRNA instability caused by nonsense mutations

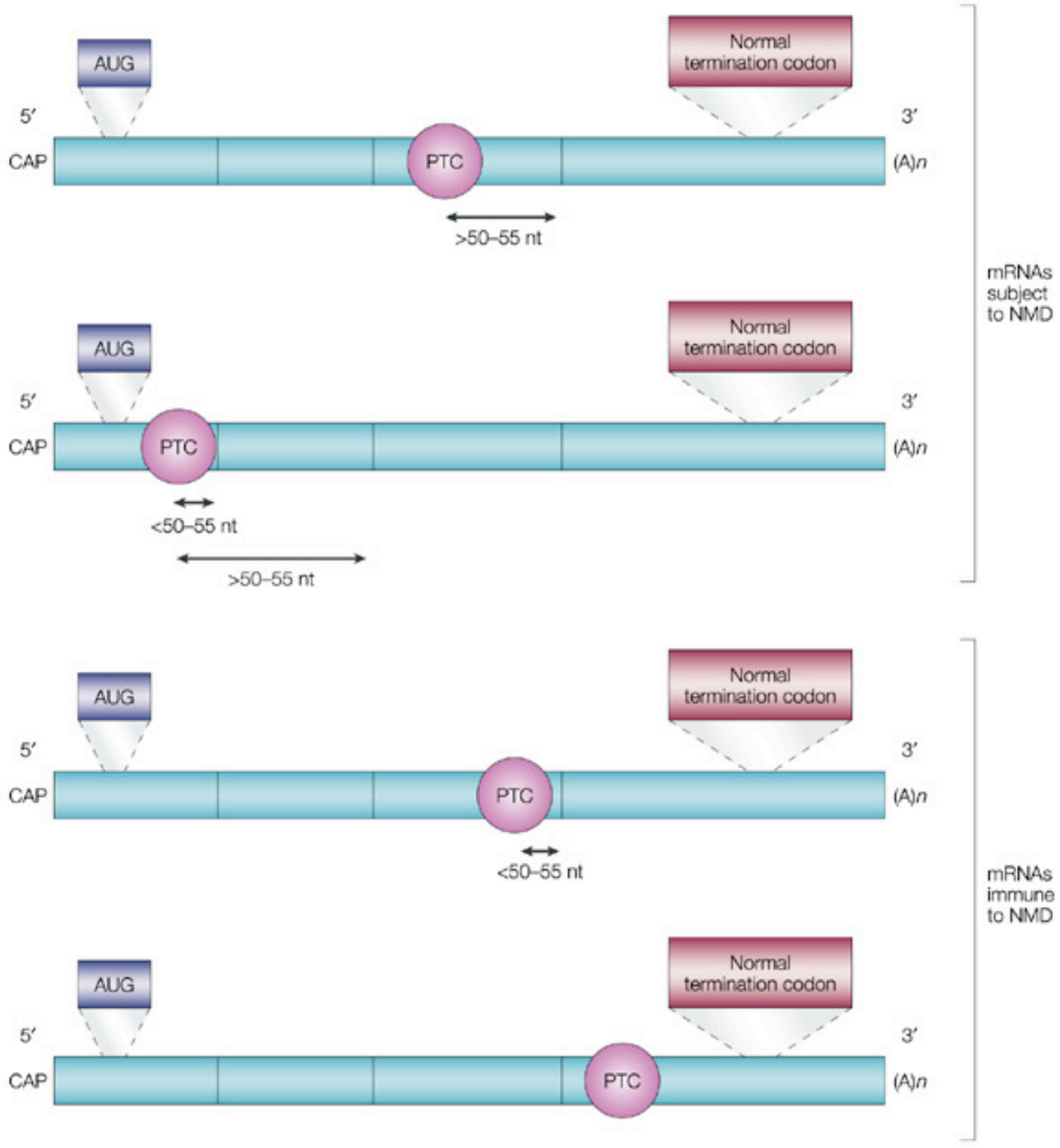


Northern blot probed with labeled *unc-54* DNA



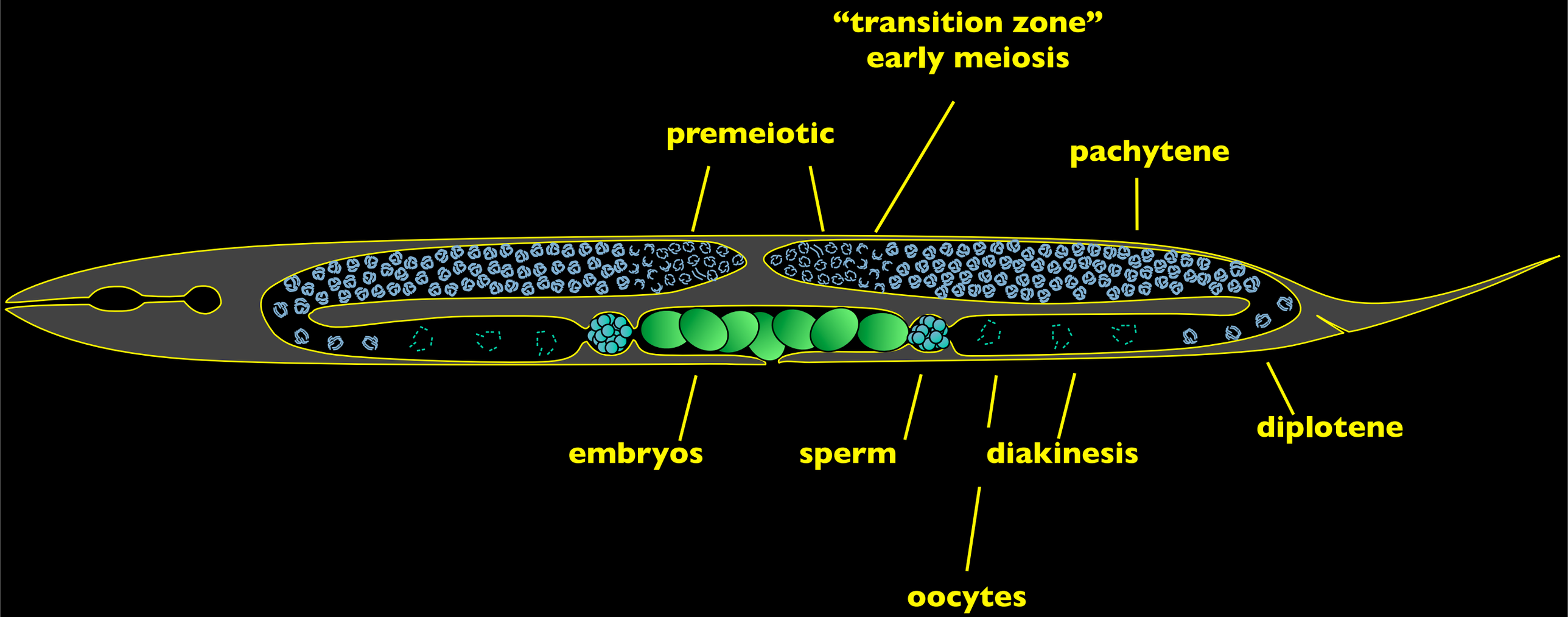
stabilization of the RNA allows more time for readthrough

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



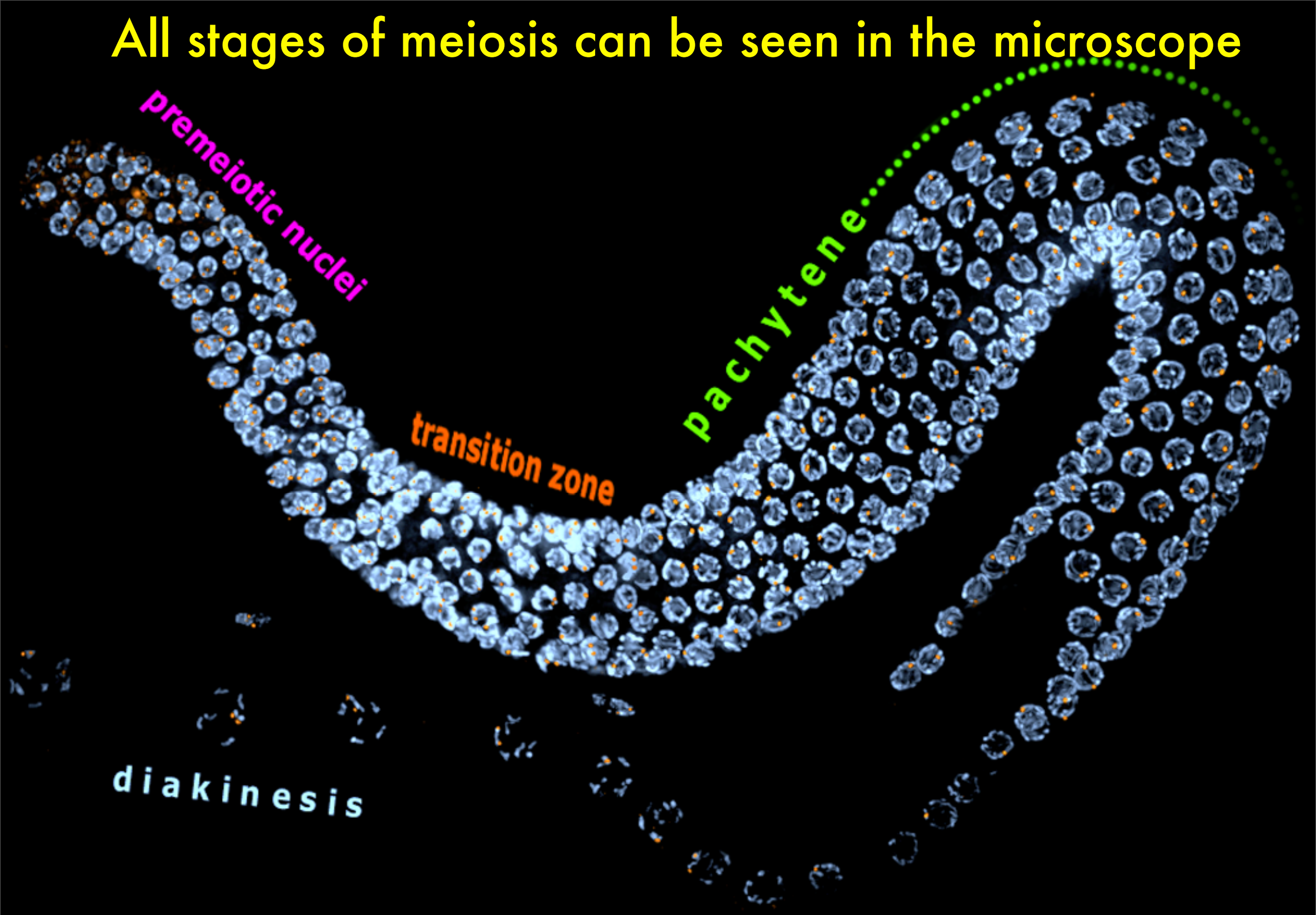
17

Meiosis in C. *elegans*



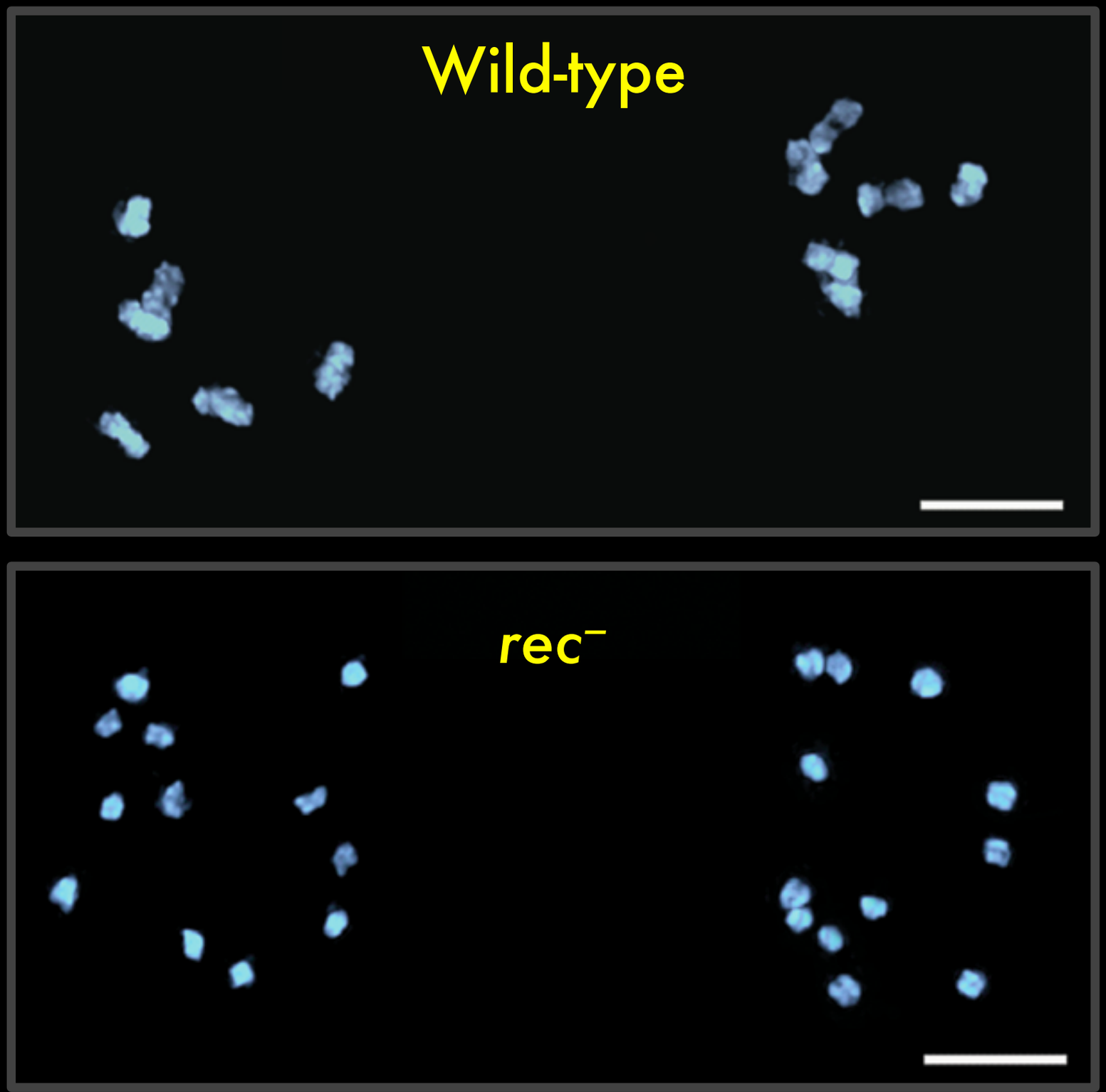
adult hermaphrodite

All stages of meiosis can be seen in the microscope



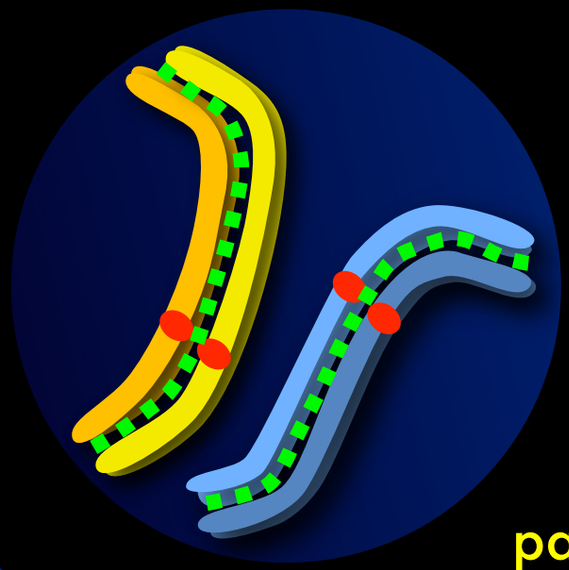
Dissected gonad from an adult C. *elegans*

Lack of crossover recombination can be detected genetically or cytologically

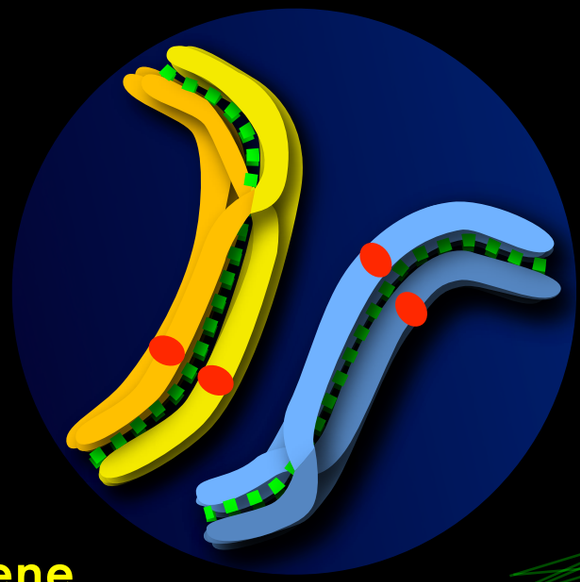


oocytes at diakinesis

Chromosome segregation during meiosis is accomplished through *homolog pairing*, *synapsis*, and *recombination*

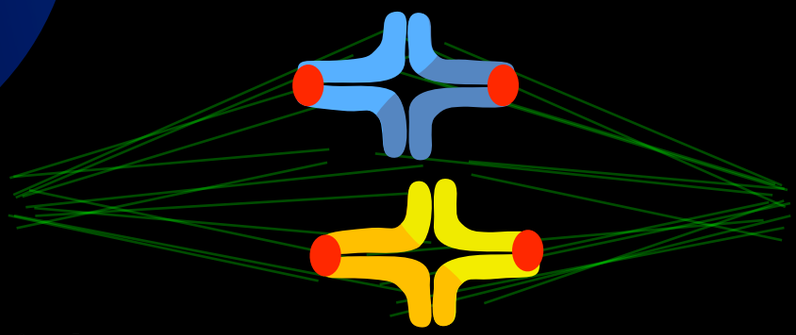


homolog pairing & synapsis



"crossover" recombination

pachytene

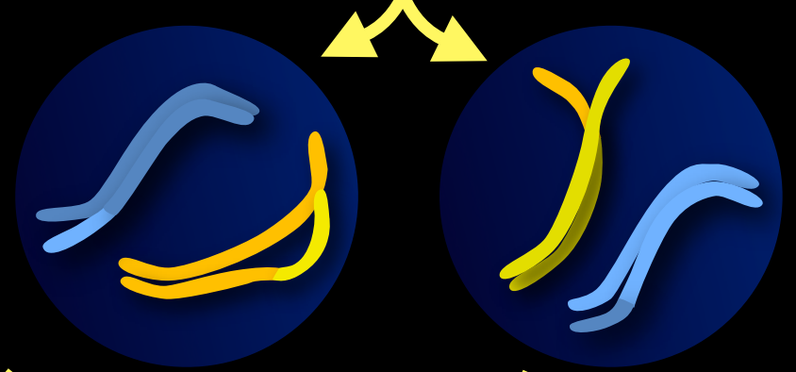


zygotene

Meiotic prophase

Meiosis I

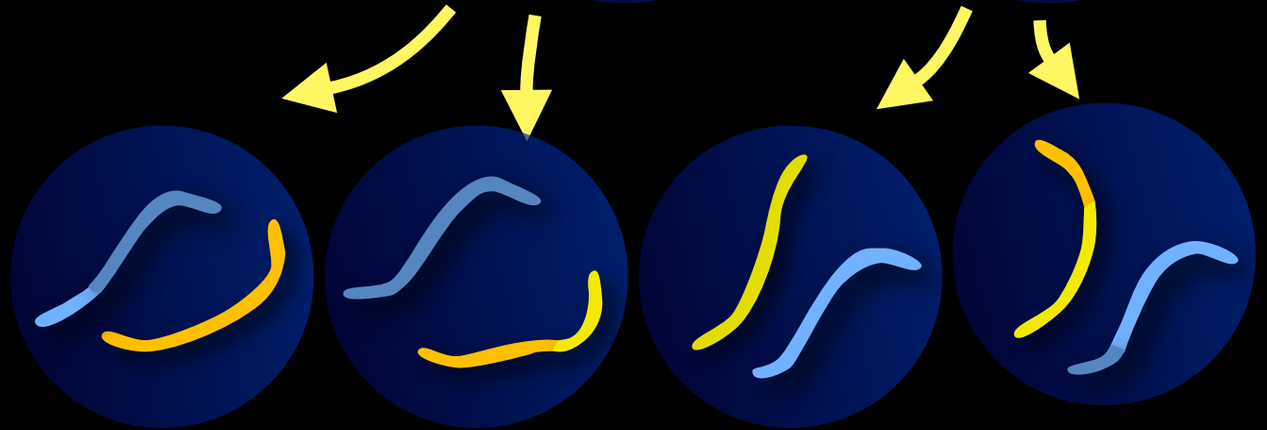
leptotene



chromosome condensation

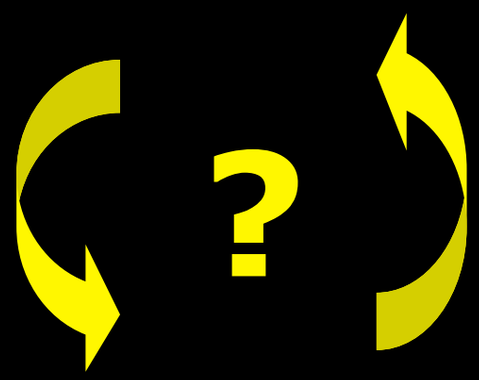
Meiosis II

Meiotic S-phase

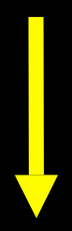


gametes

pairing/synapsis

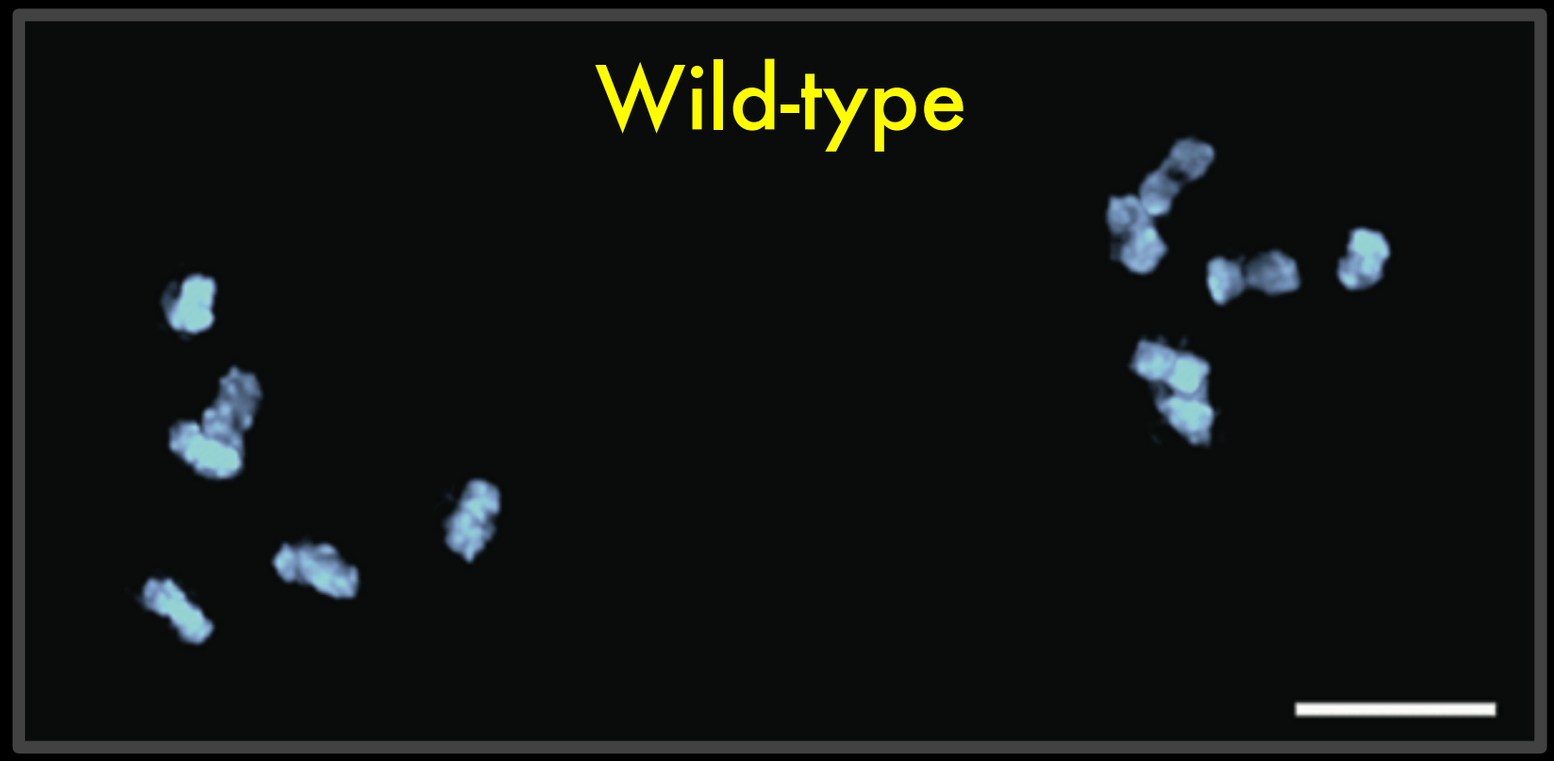


recombination

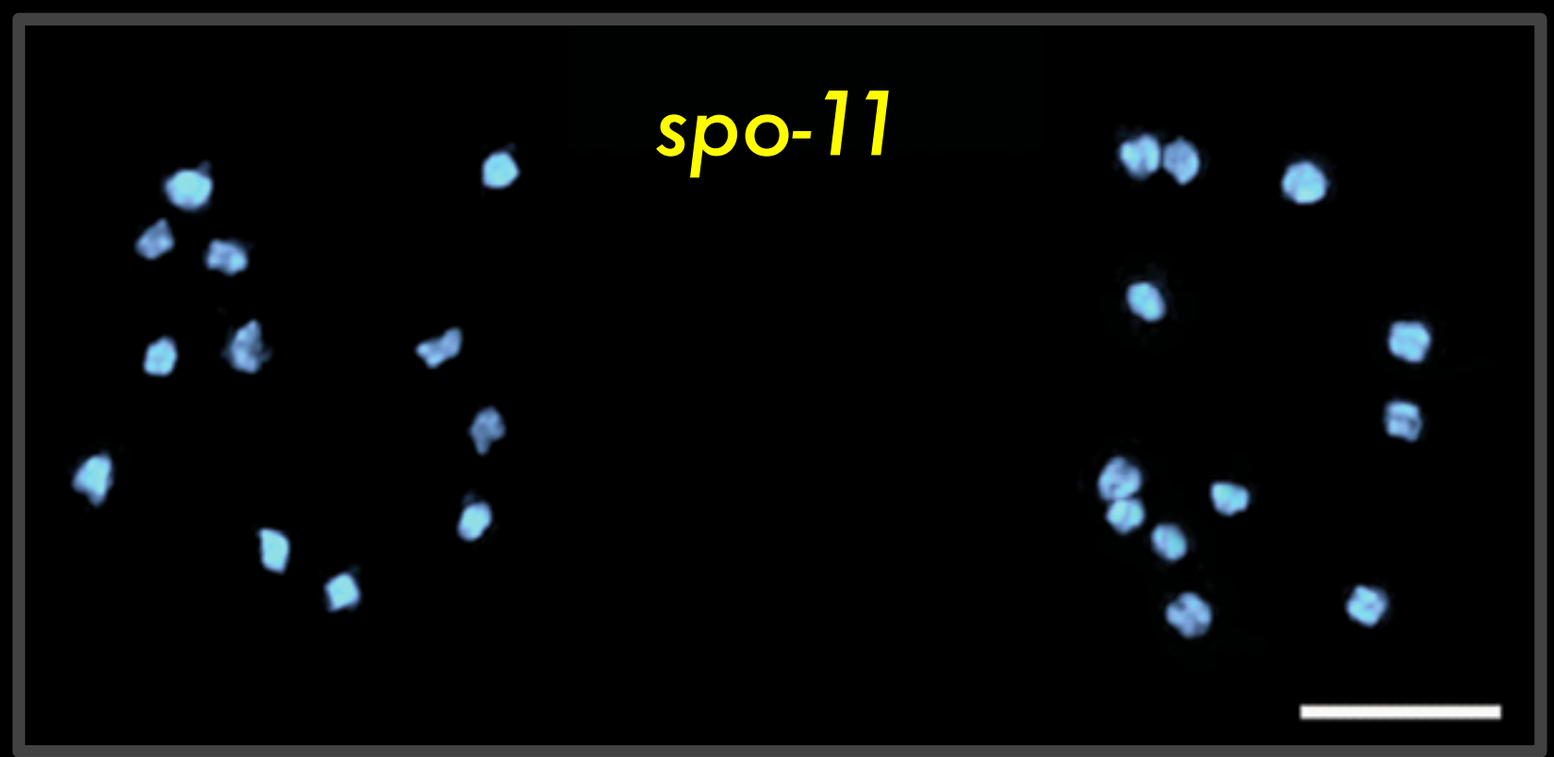


segregation

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



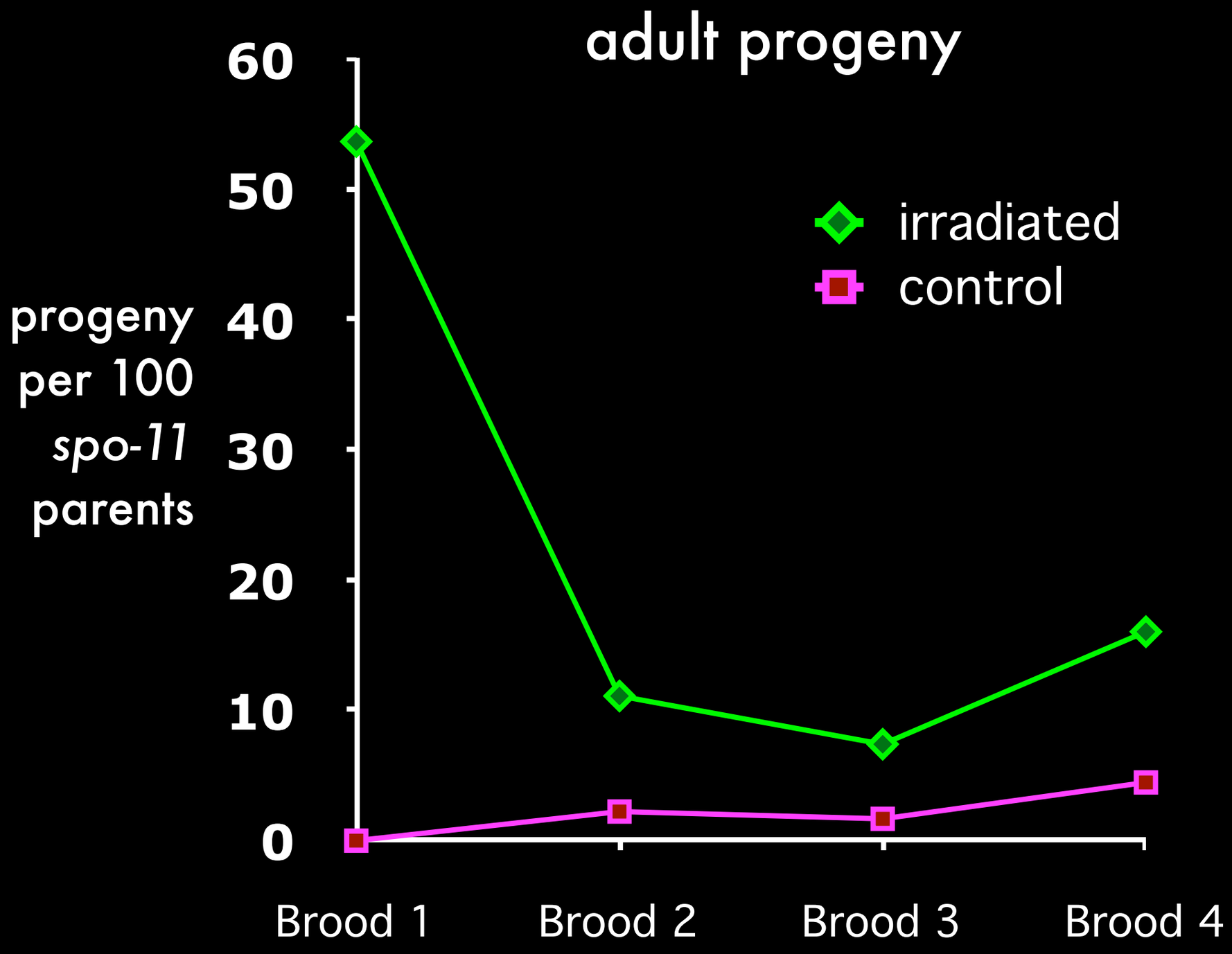
6
bivalents



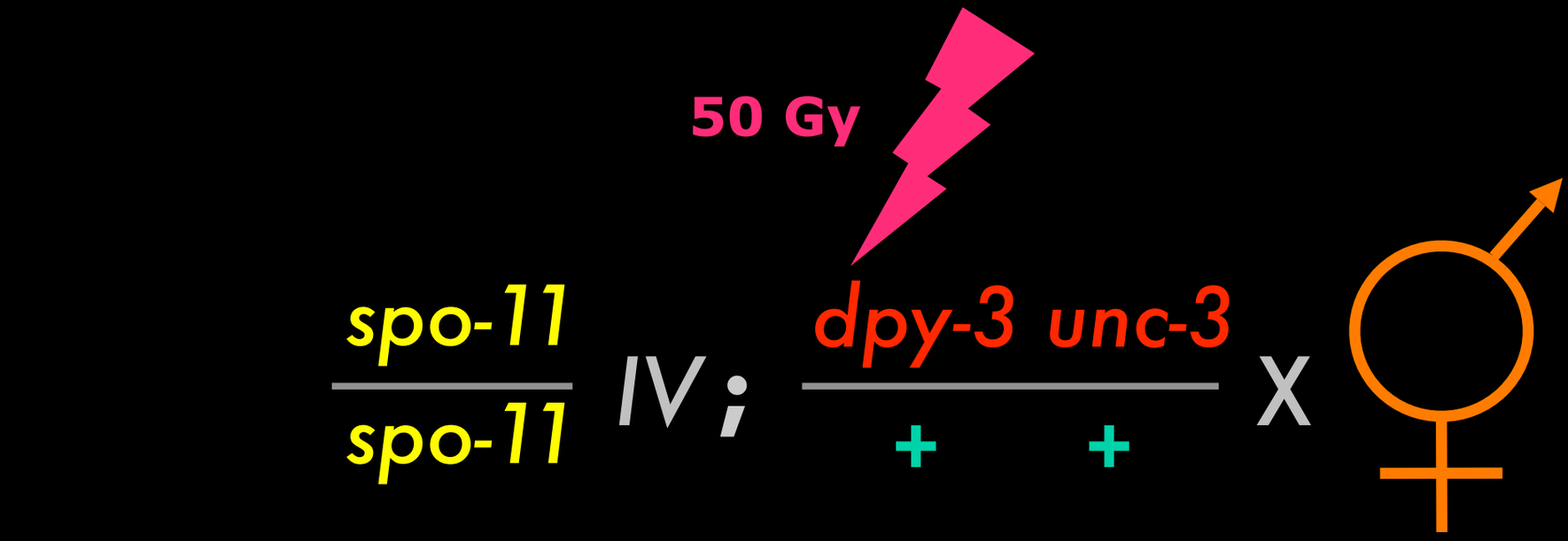
12
univalents

oocytes at diakinesis

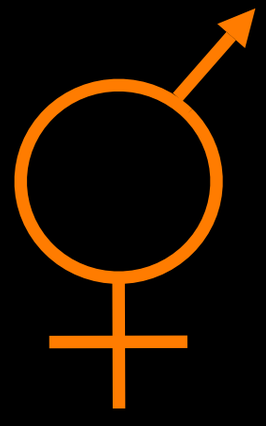
γ-irradiation partially bypasses the requirement for SPO-11



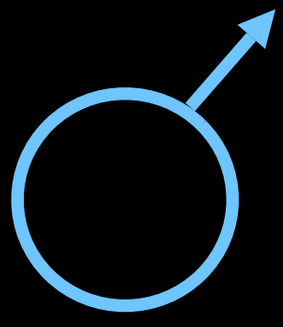
Irradiation generates crossovers in the *spo-11* mutant



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



19/93
recombinant



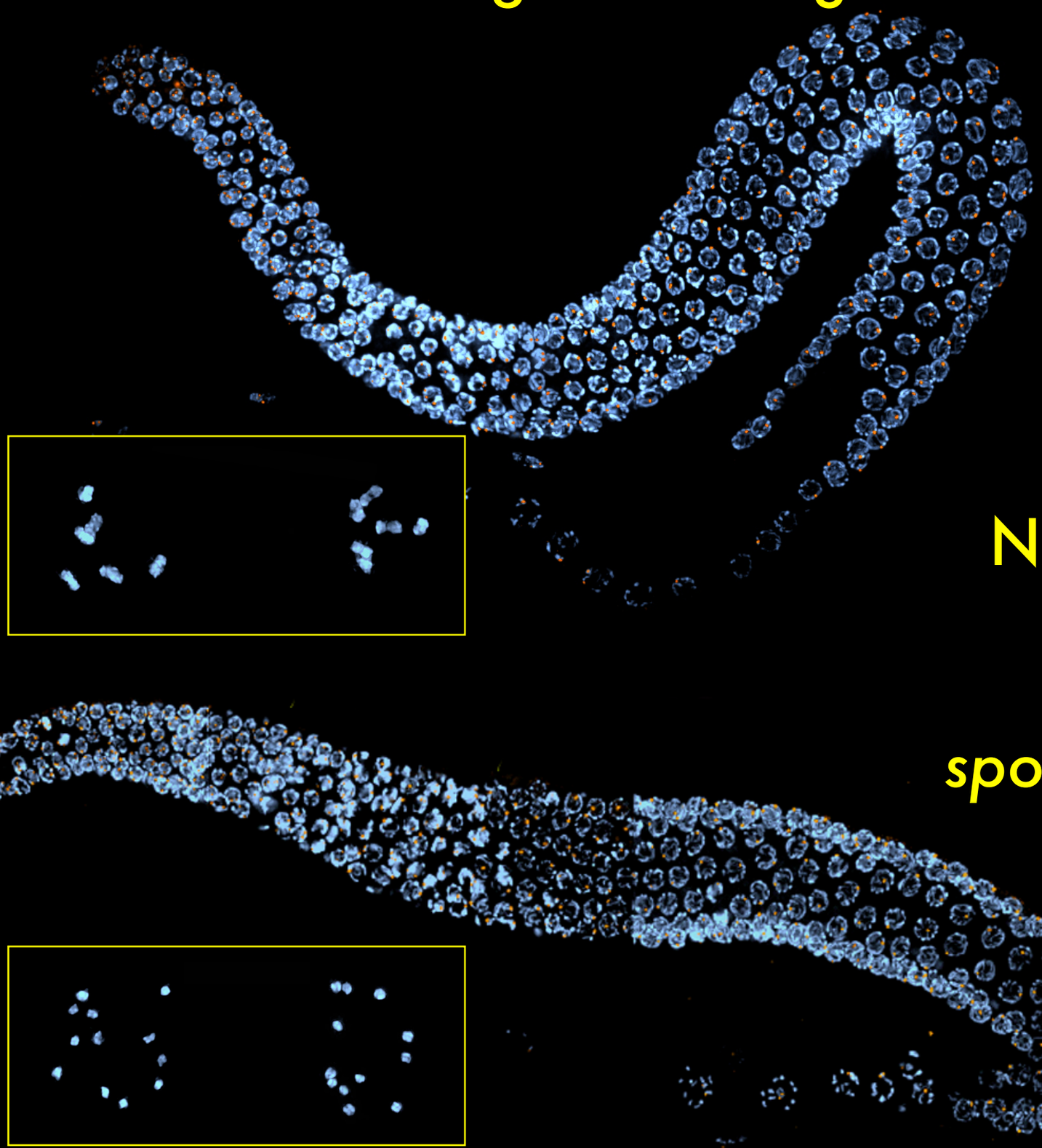
5/29
recombinant

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

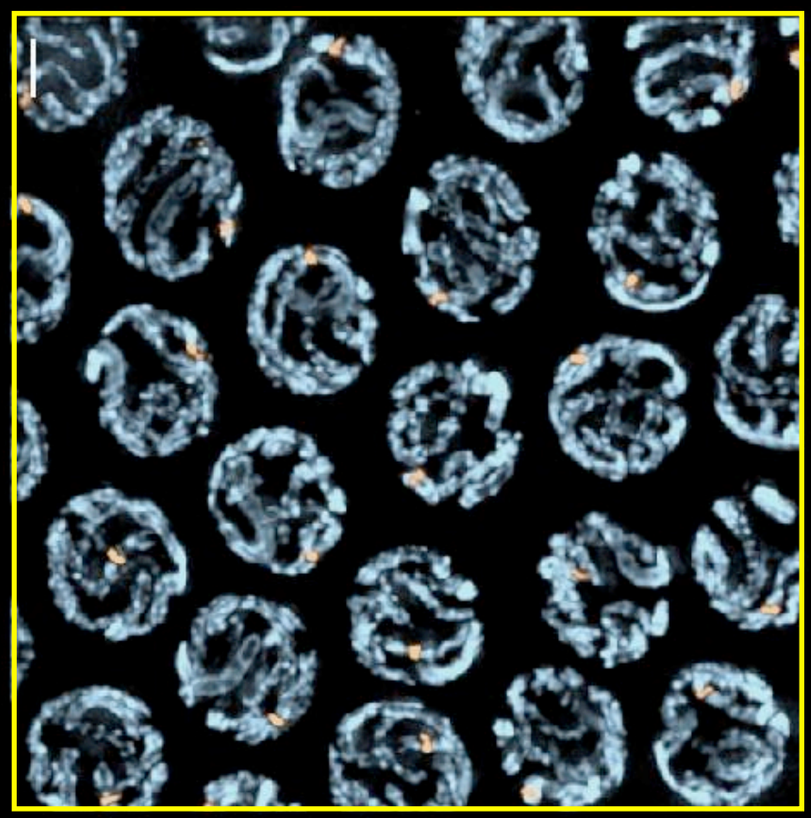
genotype	recombinant chromosomes*	total chromosomes	map distance (cM)	% of control map distance
+/+ or *spo-11*/+	503	1332	37.8	100
spo-11/*spo-11*	0	240	<0.5	<1

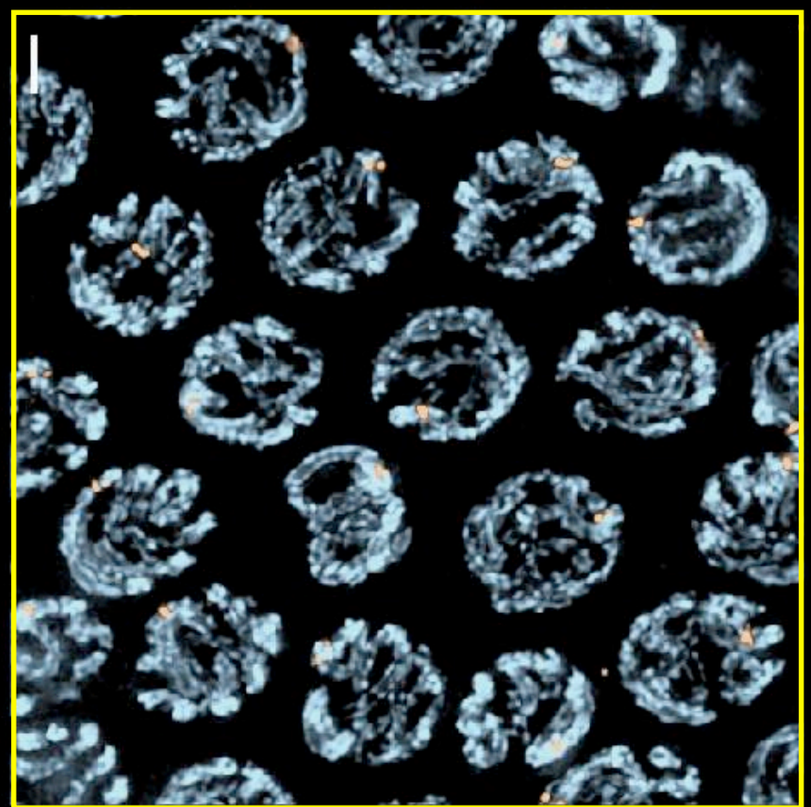
**dpy-3 – unc-3* interval on *X* chromosome

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



N2

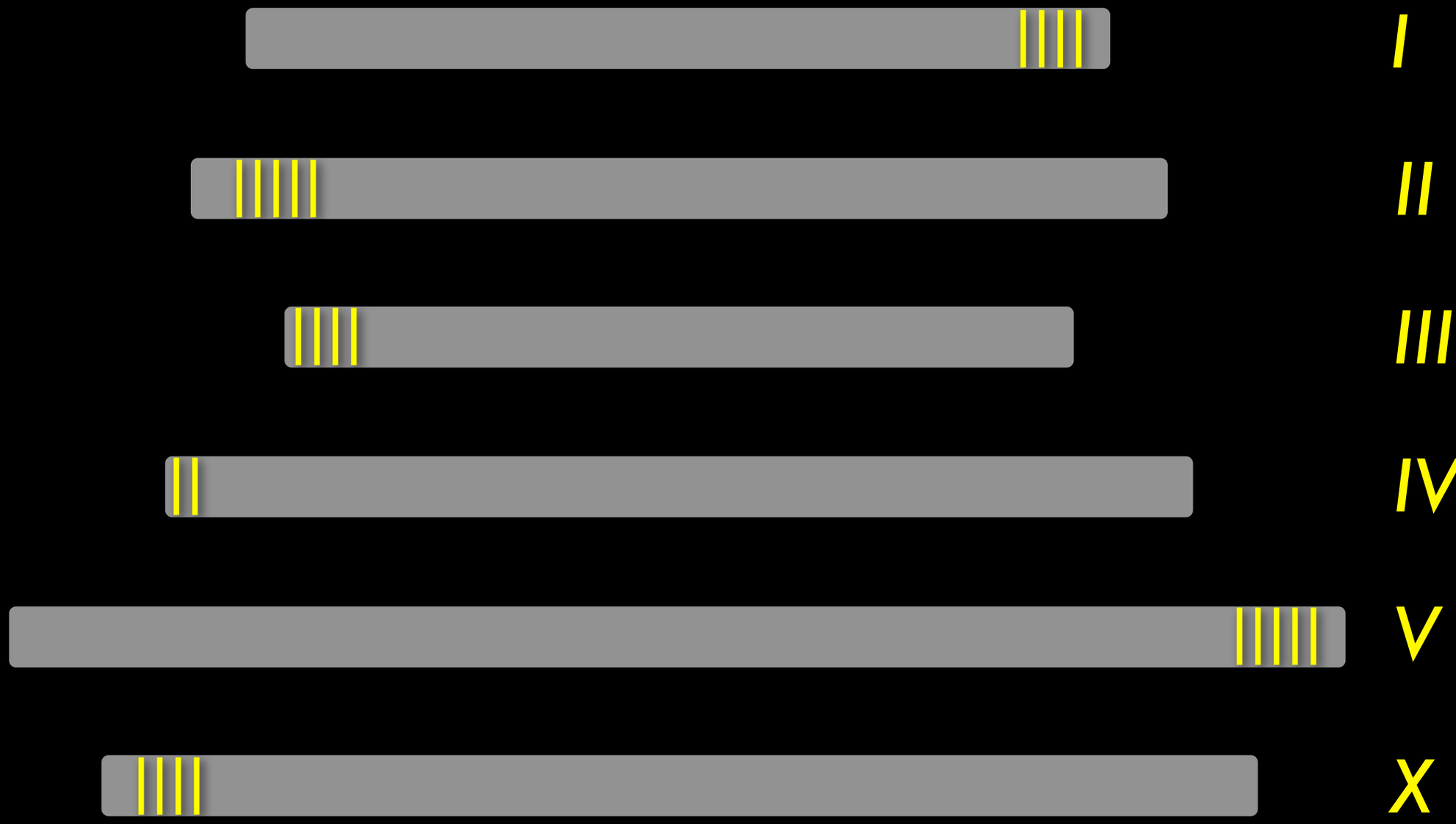




spo-11

Dernburg *et al.*, 1998

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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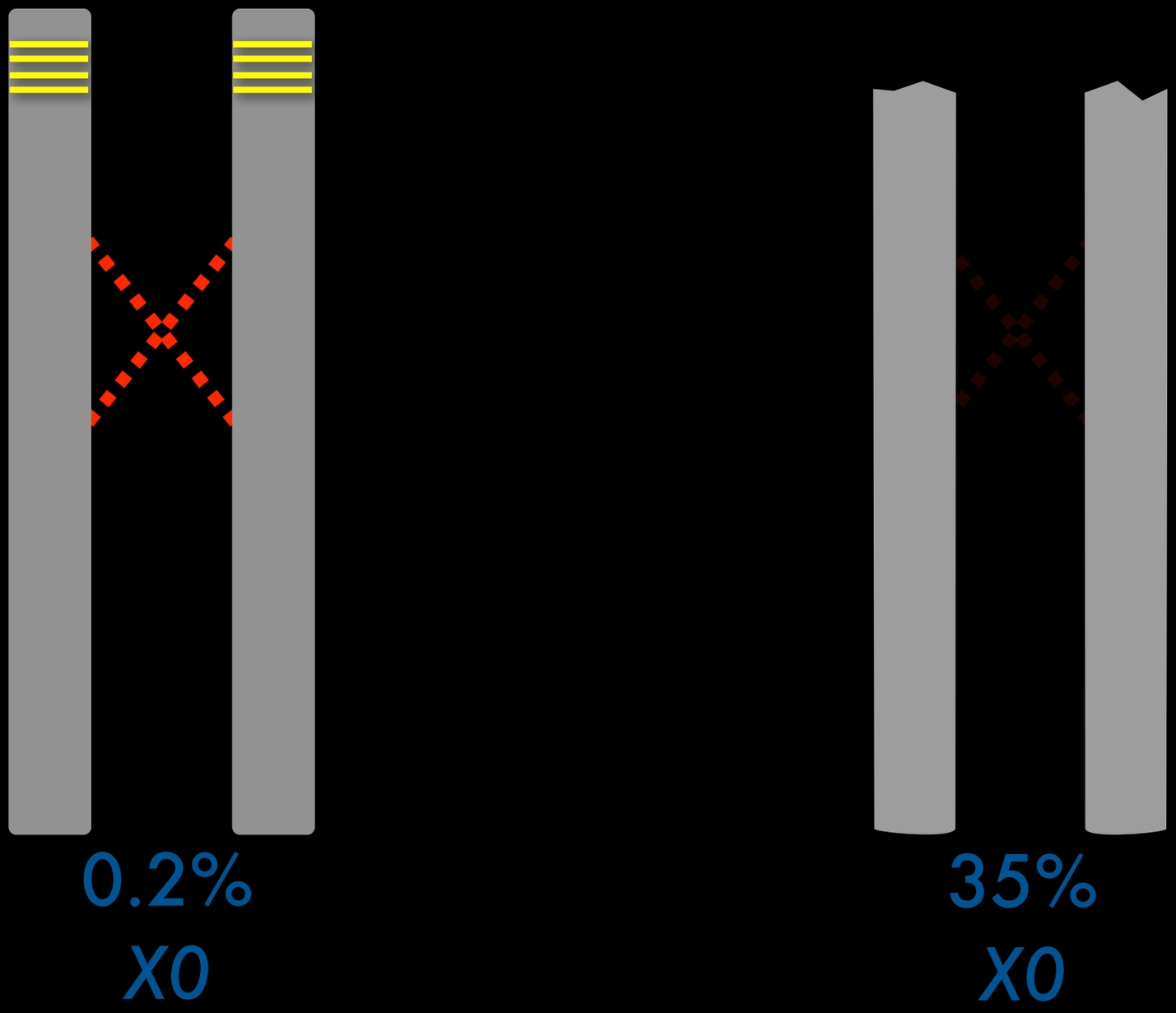
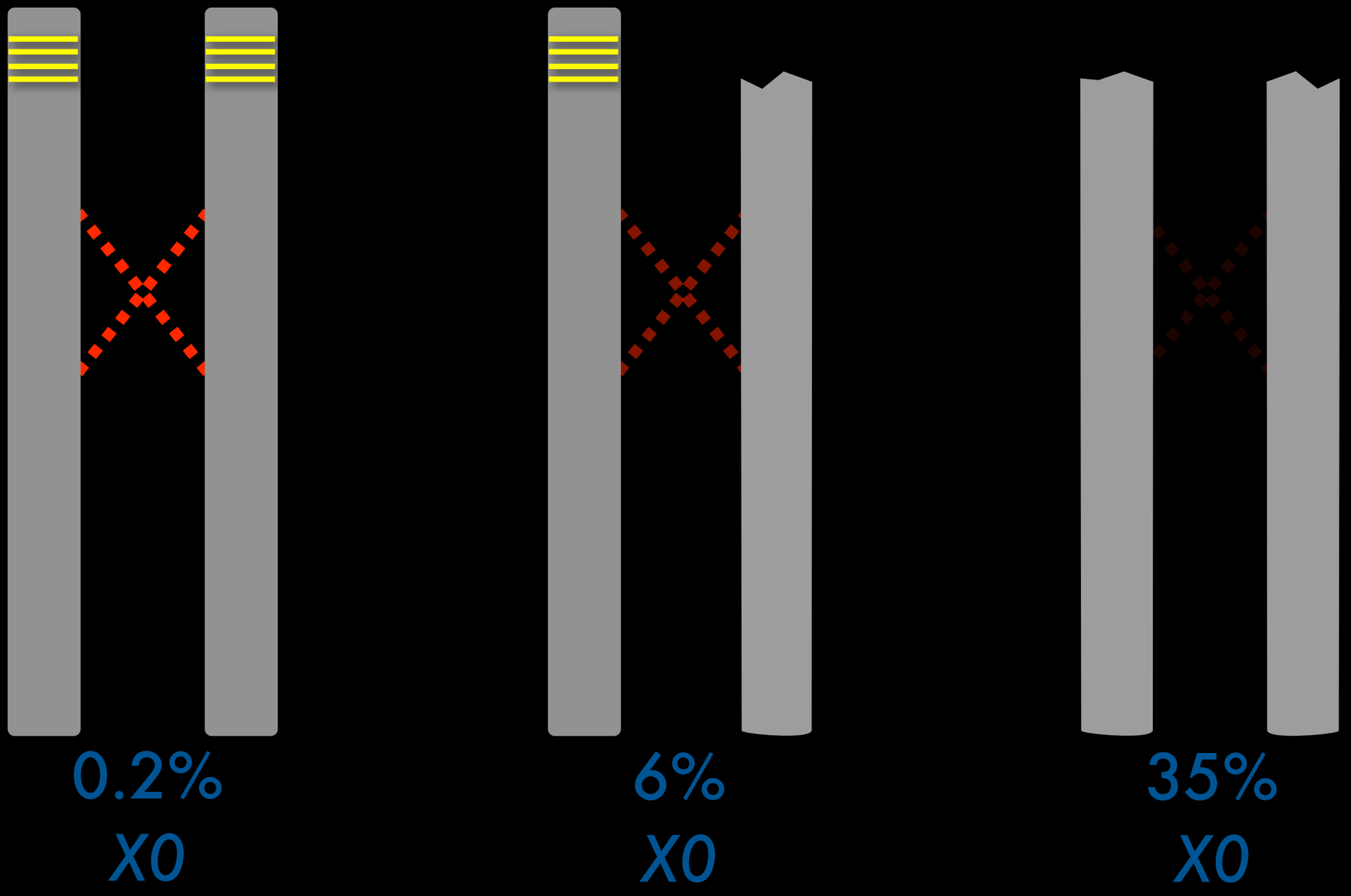


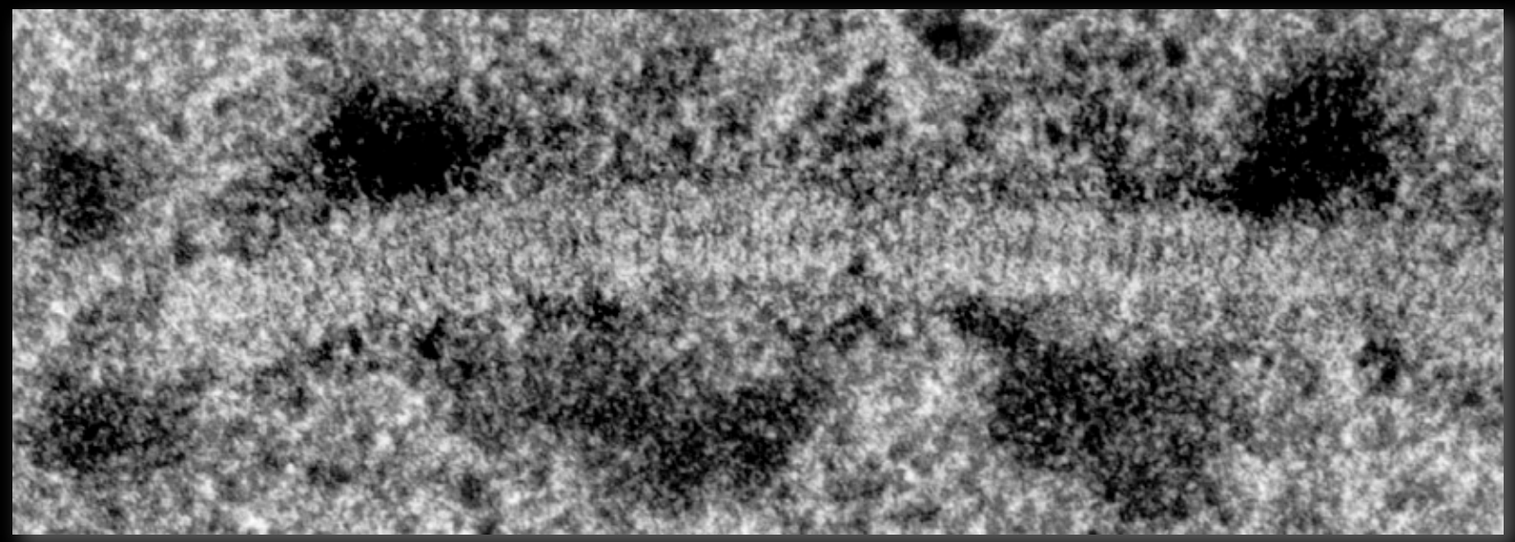
Villeneuve (1994) *Genetics* 136, 887-902

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

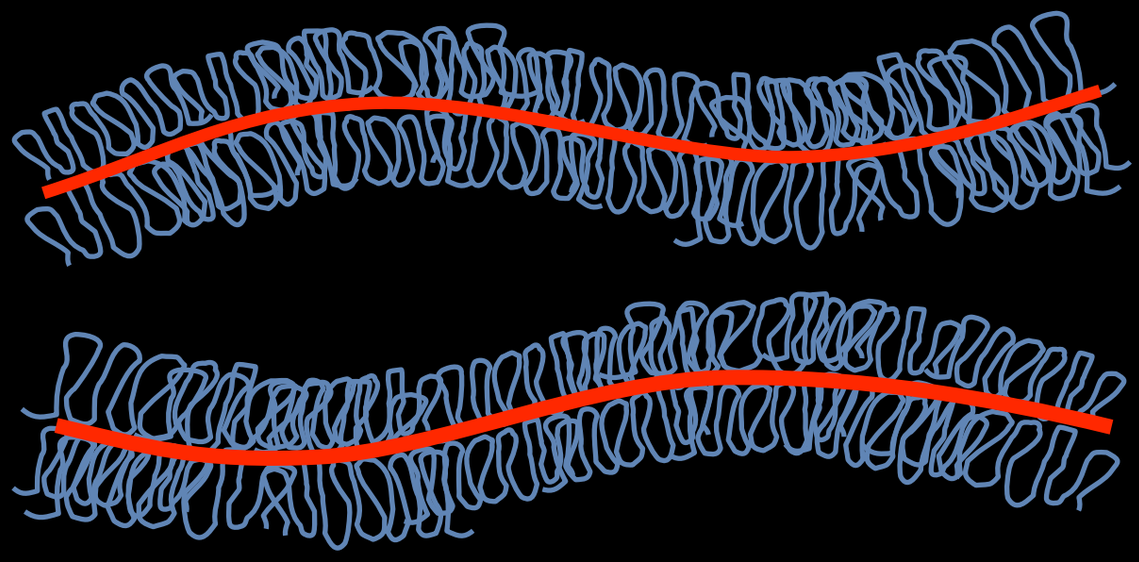


...but the determinants of homologous recognition are not restricted to this region of the chromosome

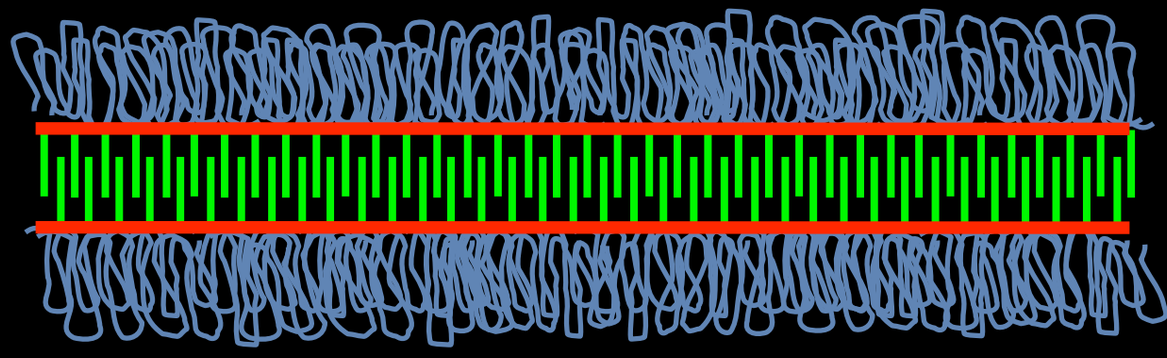
The Synaptonemal Complex (SC) is built in discrete steps



C. elegans SC



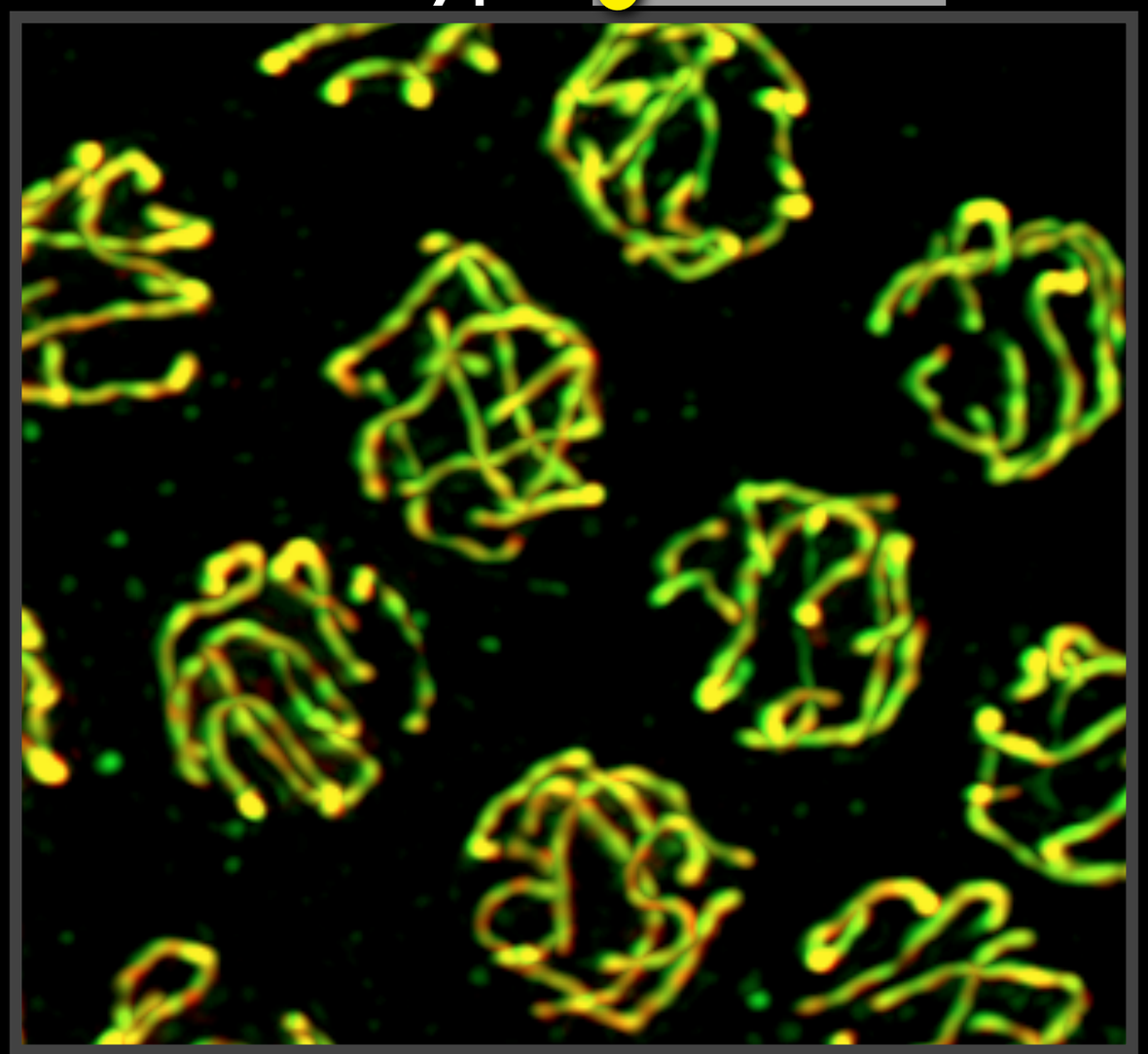
AXIAL ELEMENTS form between sisters prior to and independently of homolog pairing



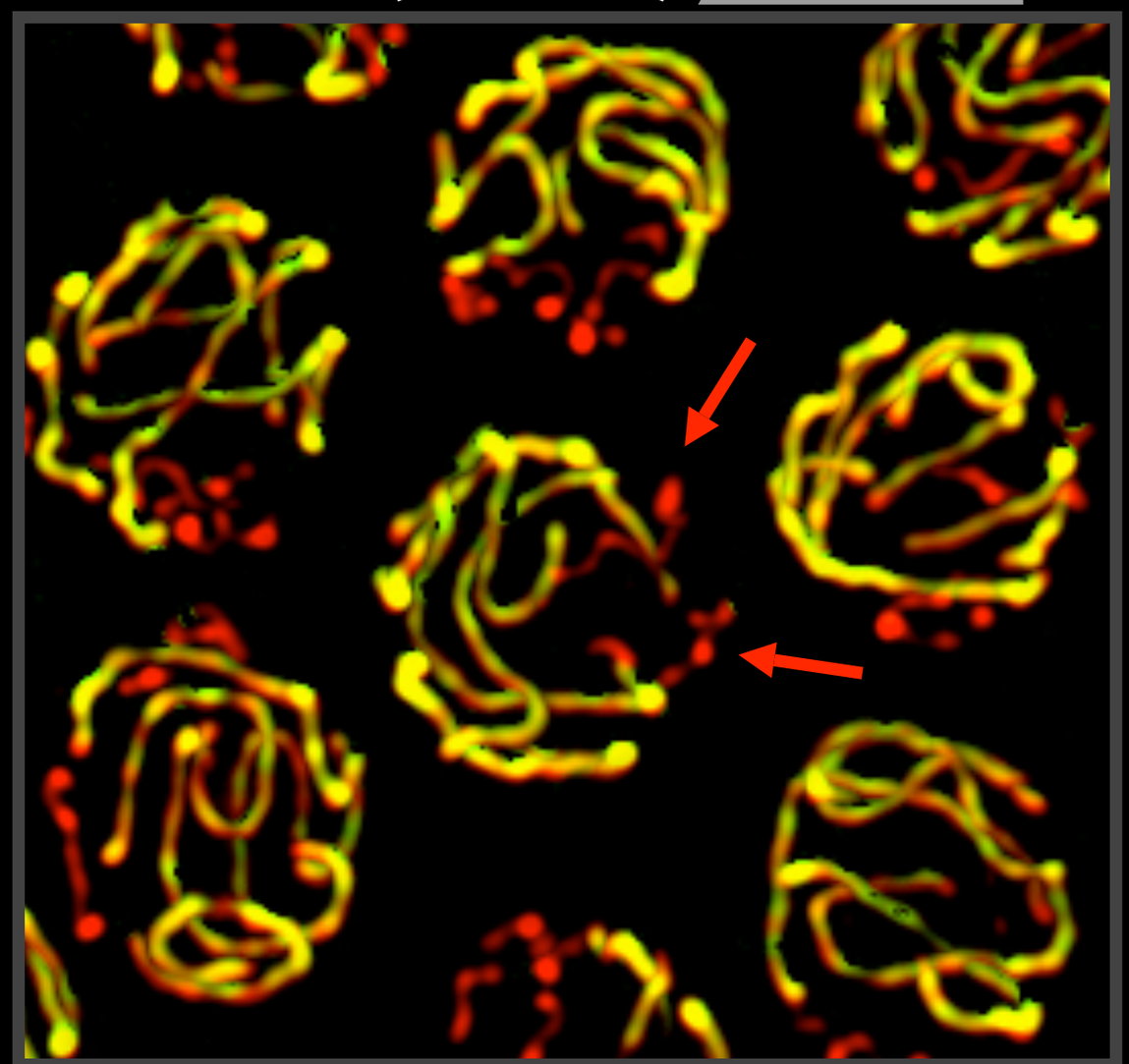
The CENTRAL ELEMENT normally polymerizes only after homolog pairing

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

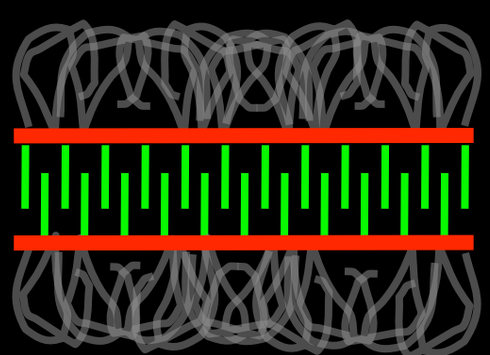
Wild type



meDf2 (X PC Δ)



α-HTP-3
Axial
Element

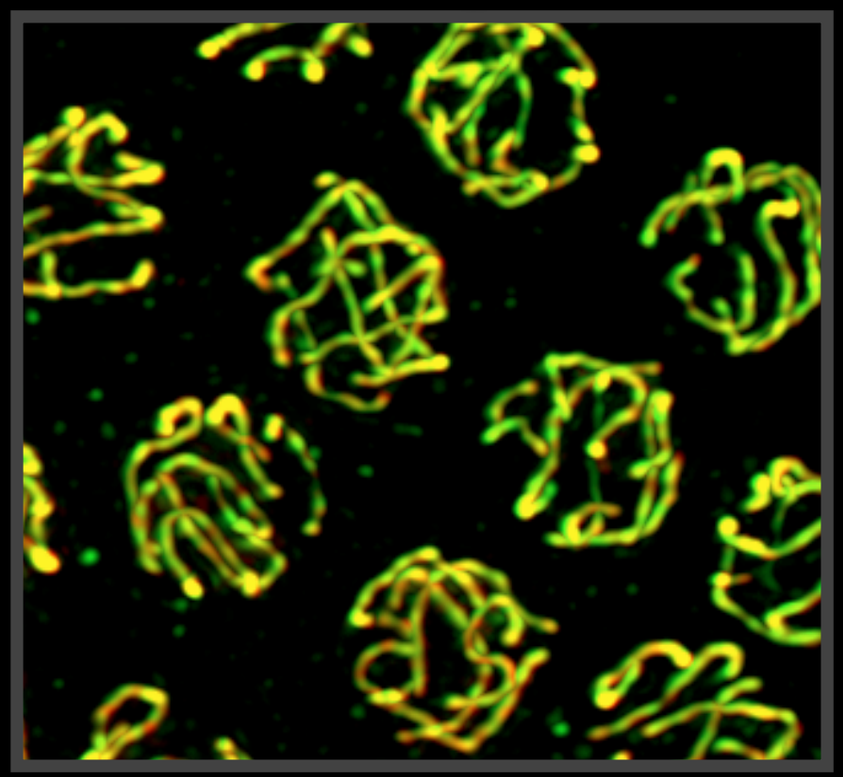


α-SYP-1
Central
Element

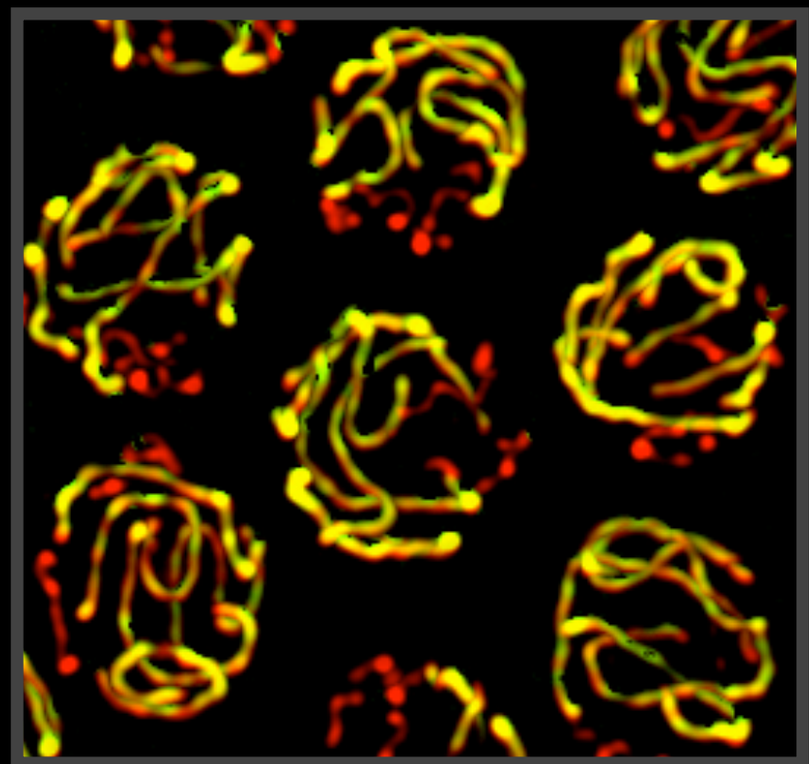
him-8 mutations show dominant genetic enhancement of Pairing Center mutations

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

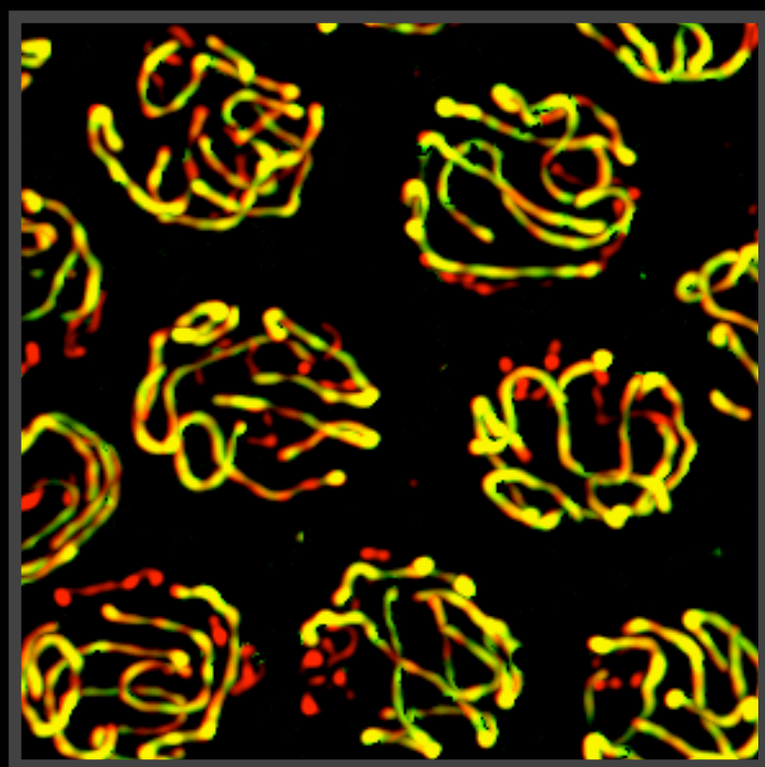
Wild type



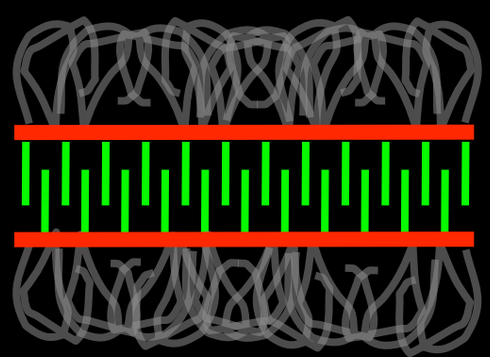
meDf2



him-8



α-HTP-3
Axial
Element



α-SYP-1
Central
Element

him-8 encodes a C2H2 zinc finger protein

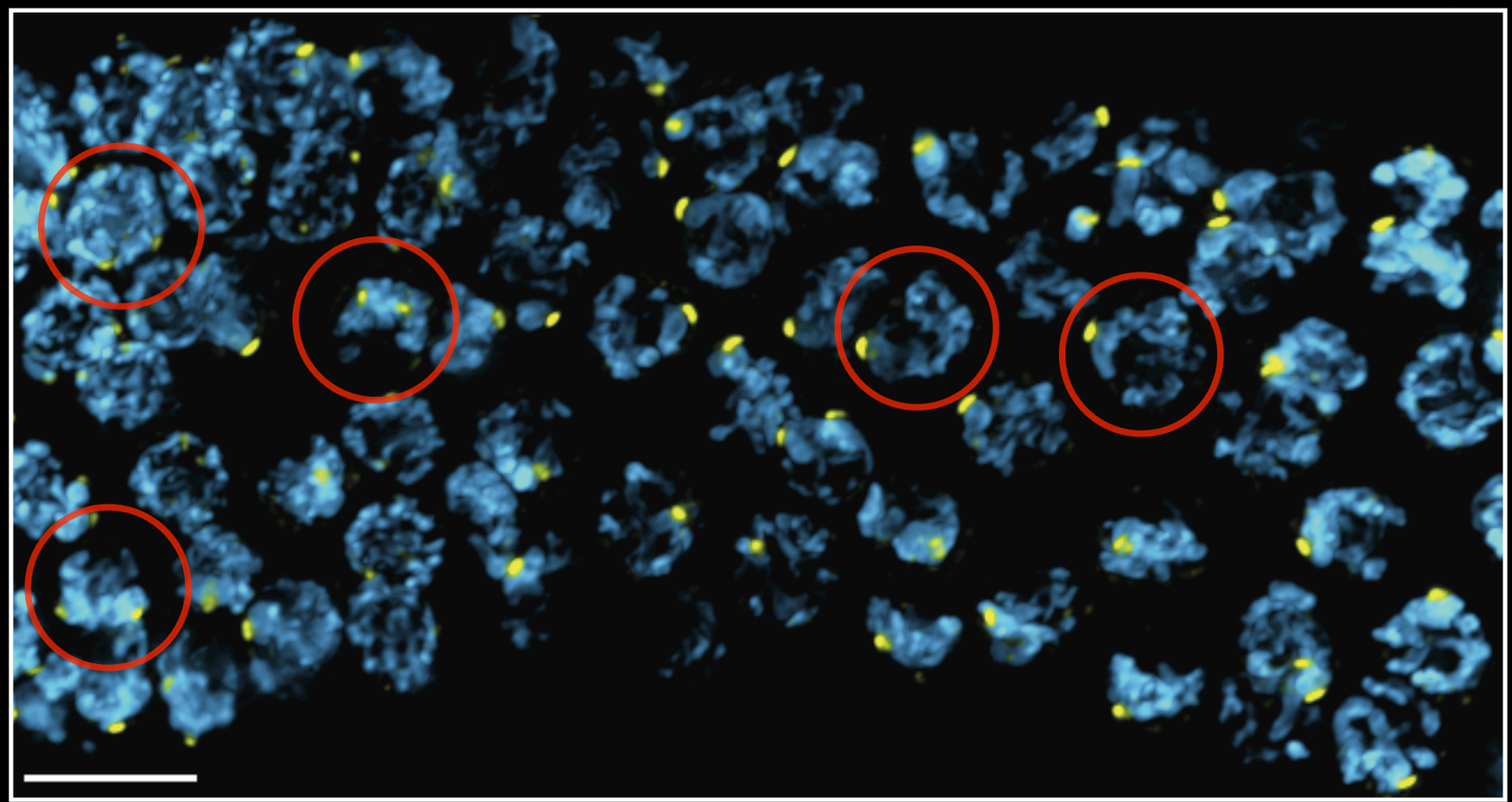
C2H2 Zn fingers

Amino acid changes in mutant *him*-8 alleles

HIM-8 localizes to chromosome foci

DAPI

HIM-8

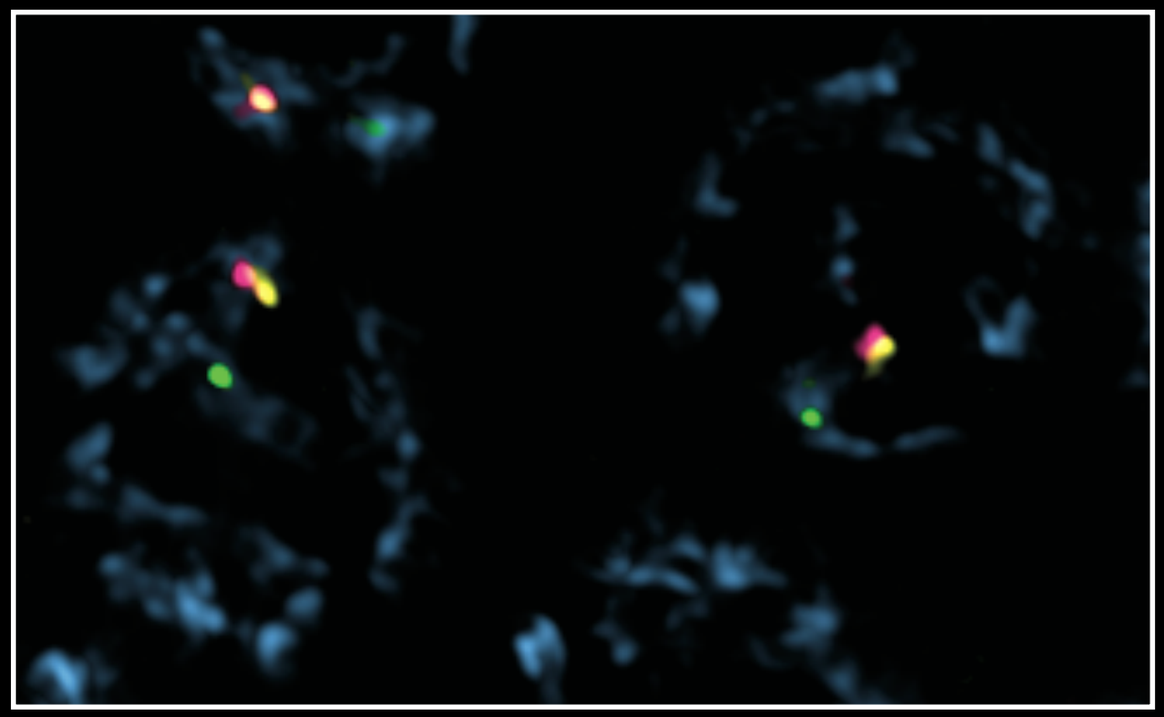


HIM-8

DAPI

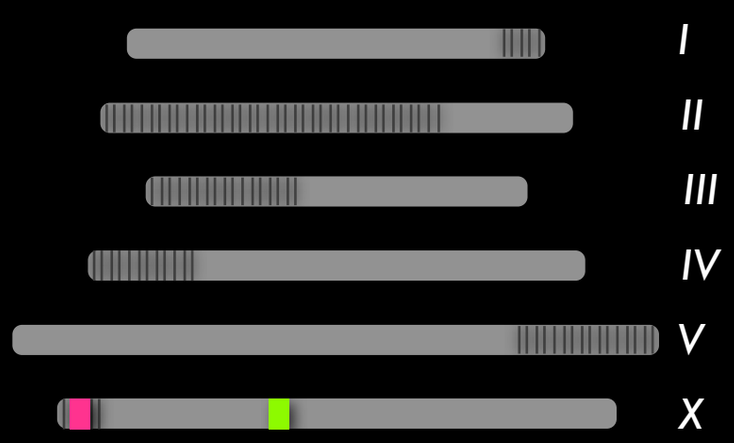
transition zone ⟶ pachytene

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

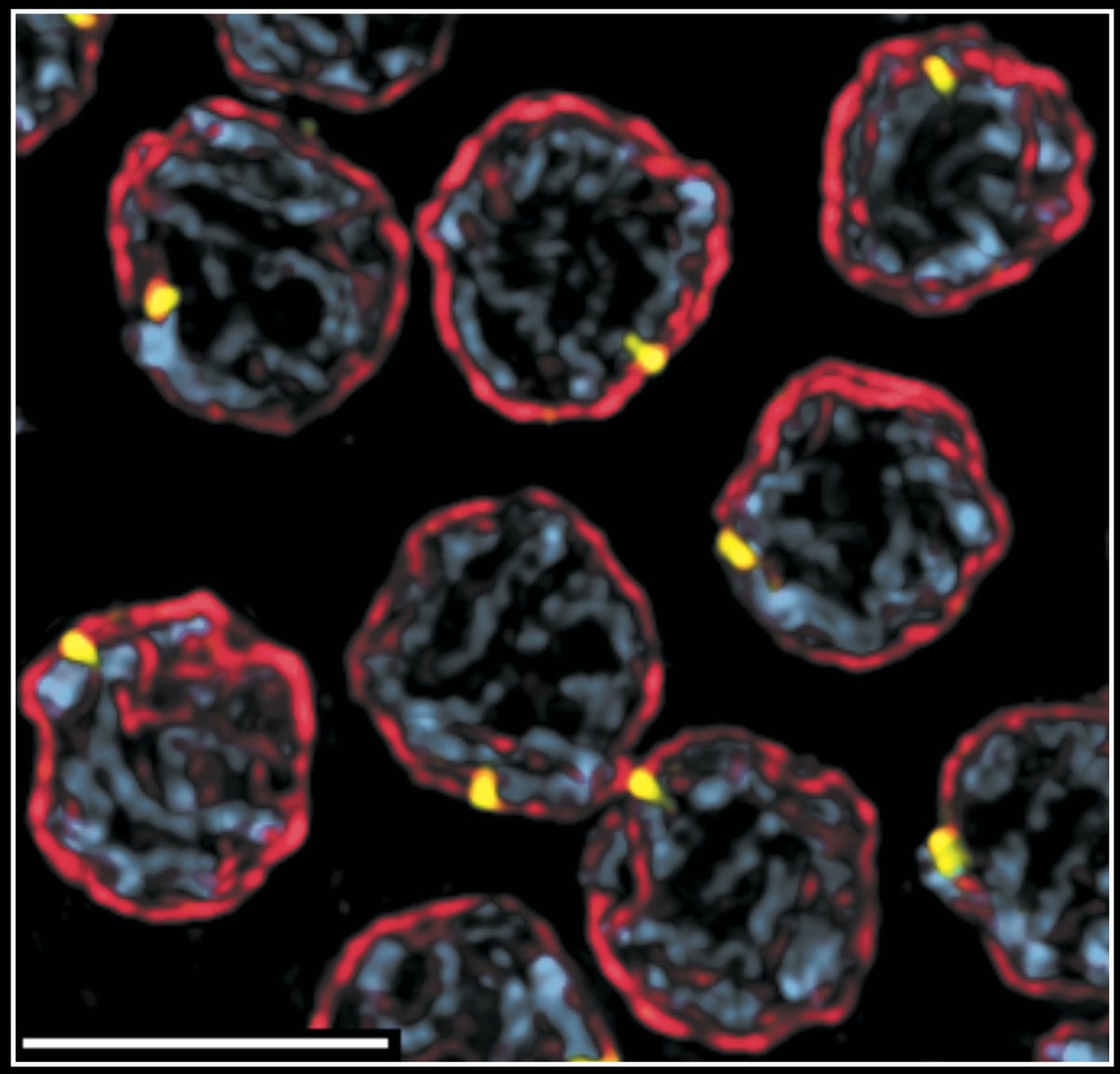


α–HIM-8

DAPI



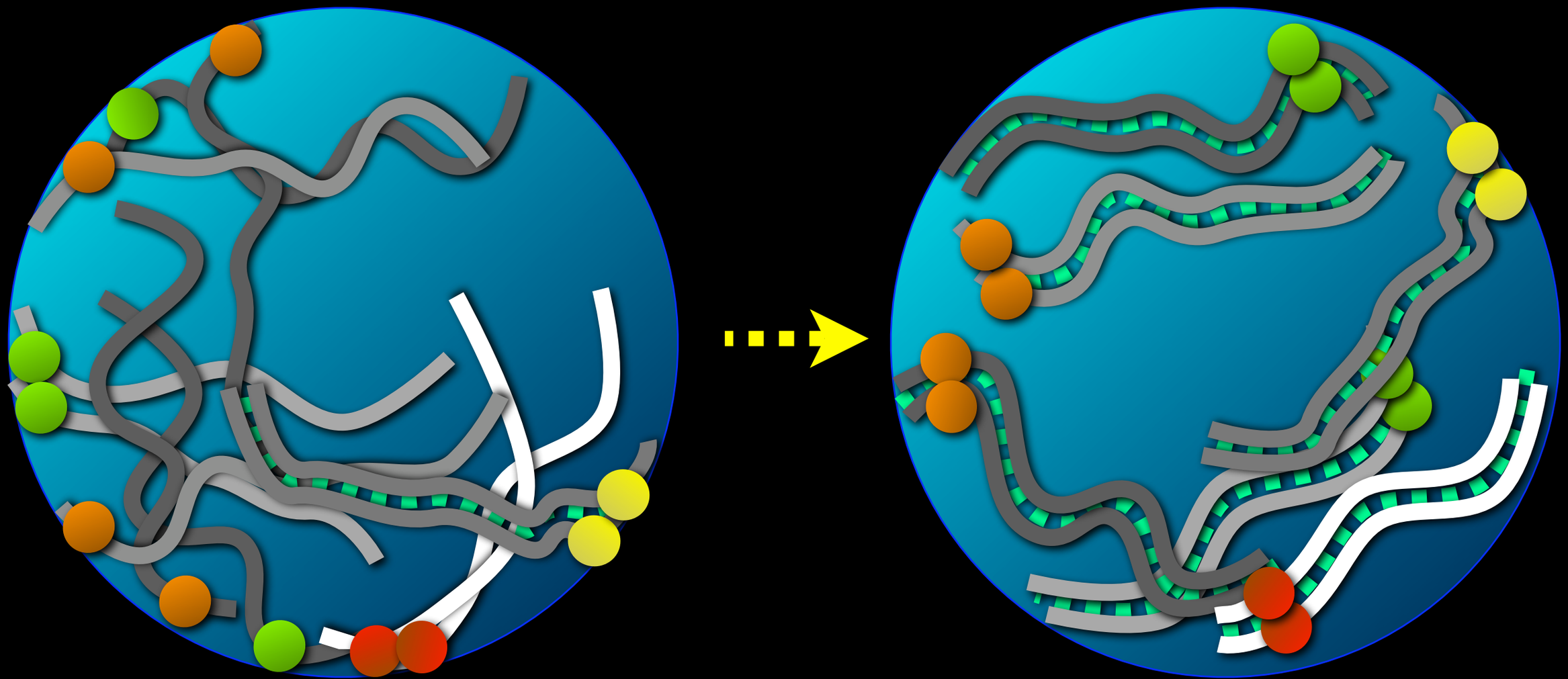
X-Left *X*-Center



α–HIM-8 α–LMN-1 DAPI

and associates with the nuclear envelope

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



Transition zone

Pachytene