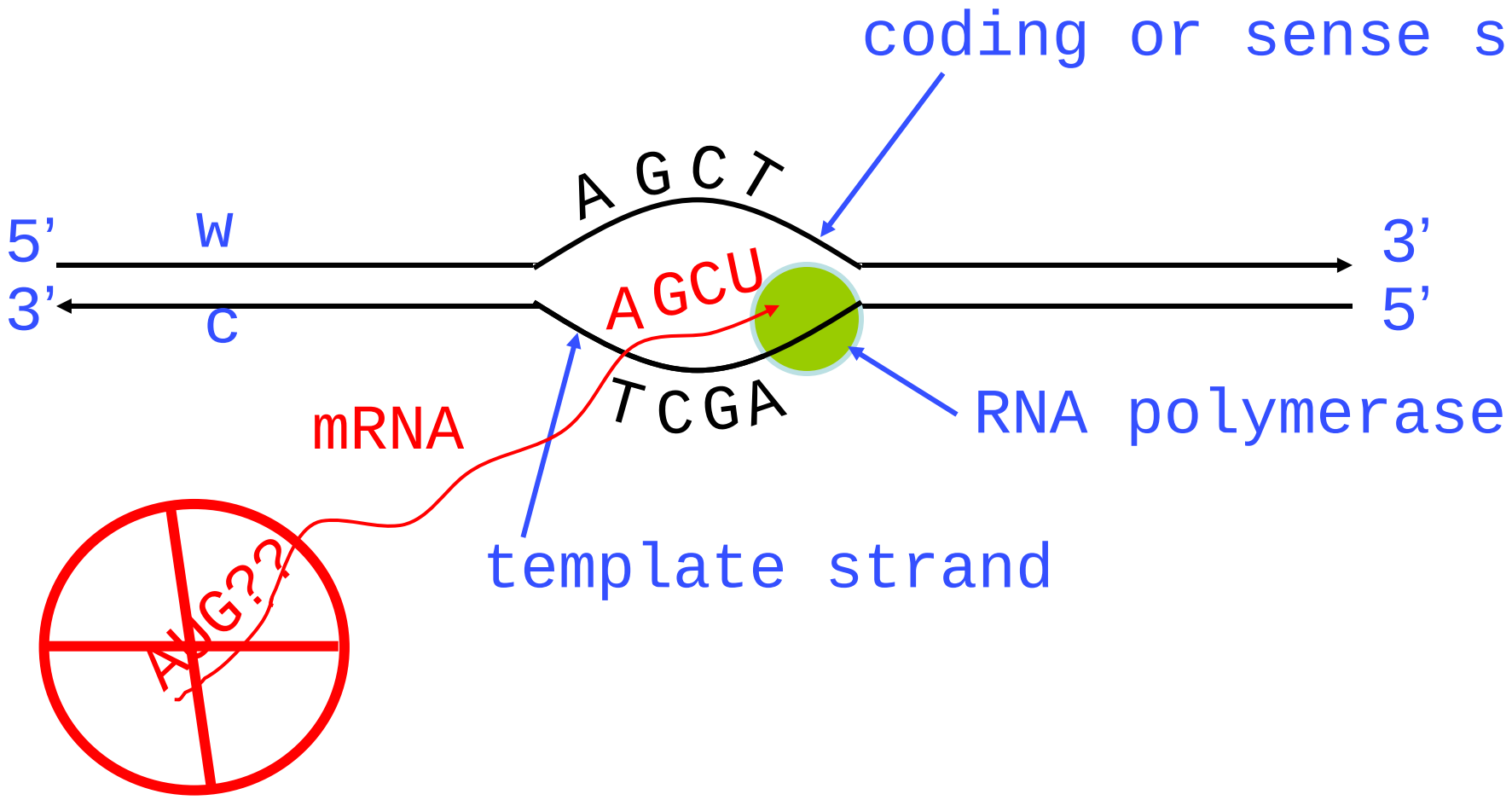

coding or sense s
A G C T
5’
W
3’
3’
C
5’
A GCU
T C G A
mRNA
RNA polymerase
template strand
AAG??

The triplet code

3 bases = 1 amino acid

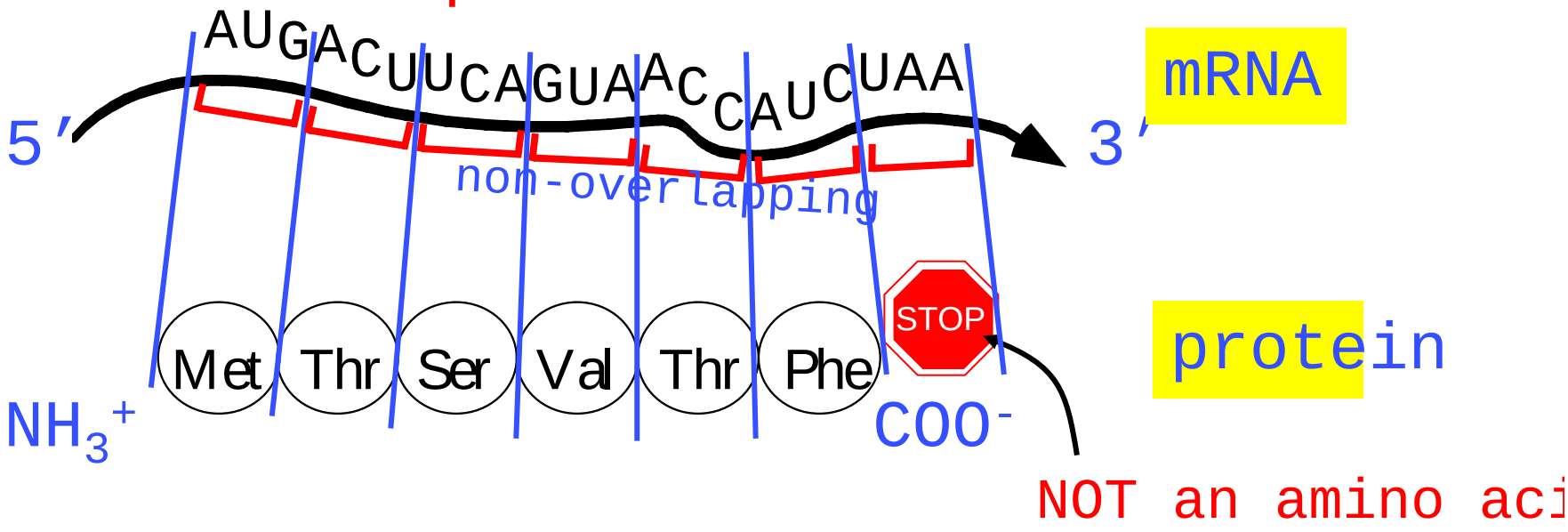
Punctuation:

sta AUG = methionine, the first amino acid in (almost) all proteins

rt:

sto UAA, UAG, and UGA.

p:



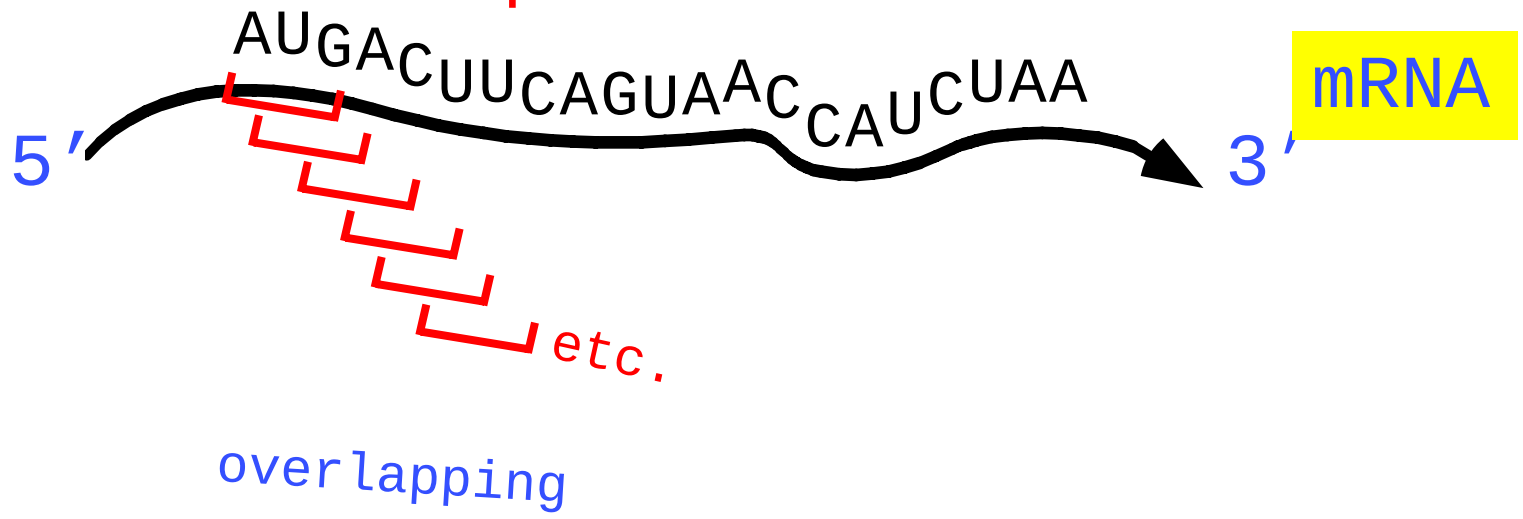
The triplet code

3 bases = 1 amino acid

Punctuation:

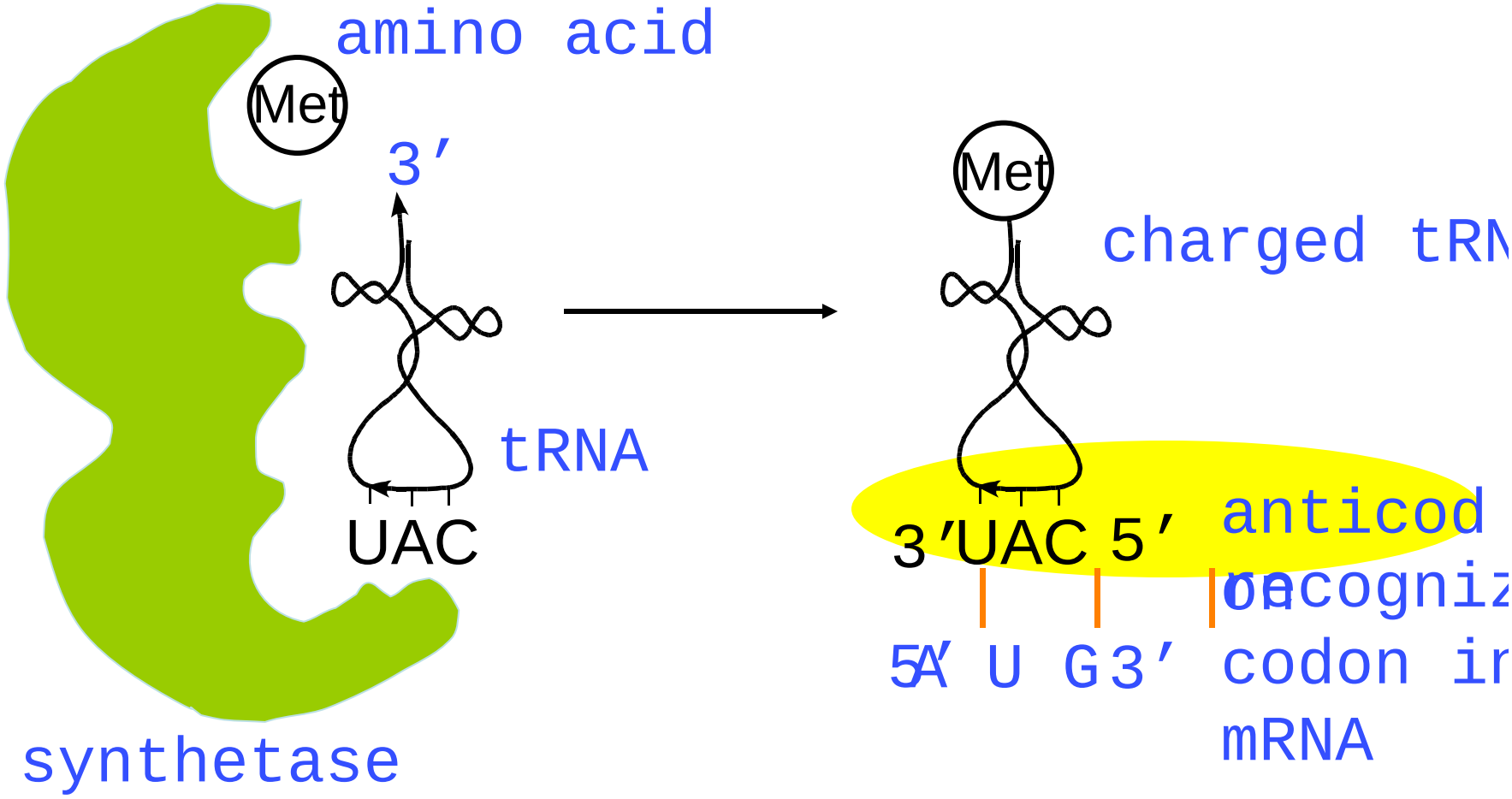
start: AUG = methionine, the first amino acid in (almost) all proteins

stop: UAA, UAG, and UGA.

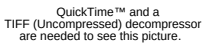


The Genetic Code: Who is the interpreter? Where's the dictionary? What are the rules of grammar?

tRNA = transfer RNA

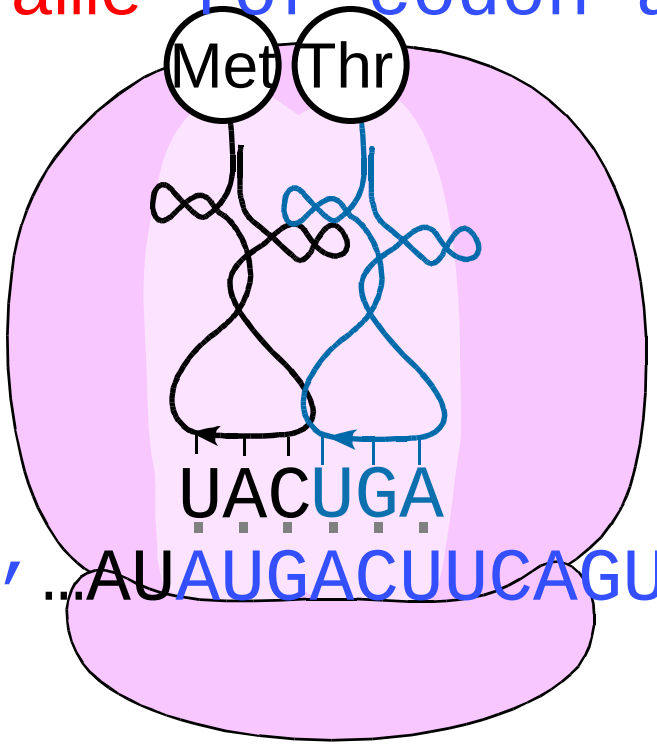


The genetic code



The **ribosome**: mediates translation

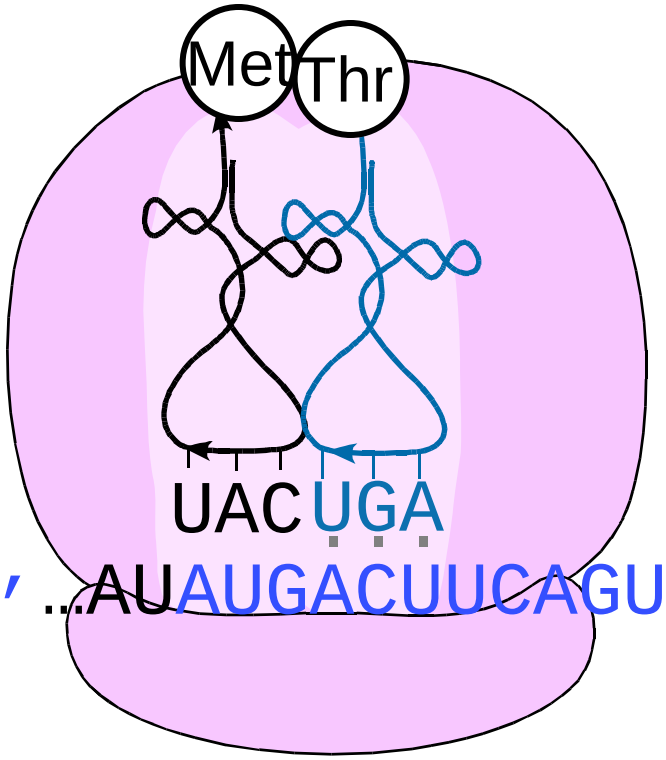
Locates the 1st AUG, sets the reading frame for codon-anticodon base-pairing



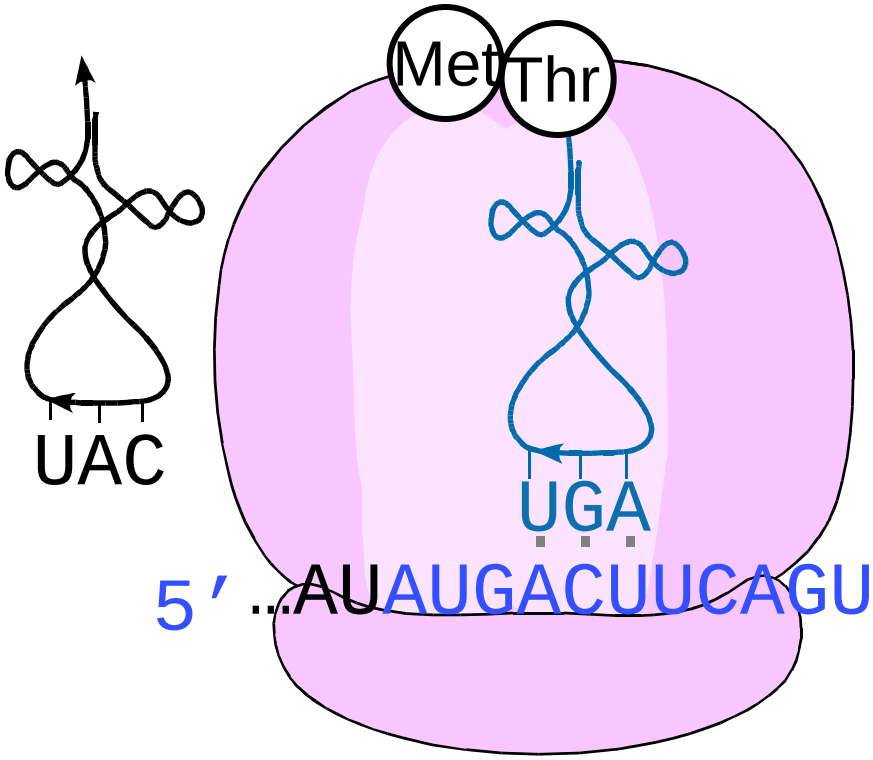
ribosome

After the 1st two tRNAs have bound…

the ribosome breaks the Met-tRNA bond; Met is instead joined to the second amino acid



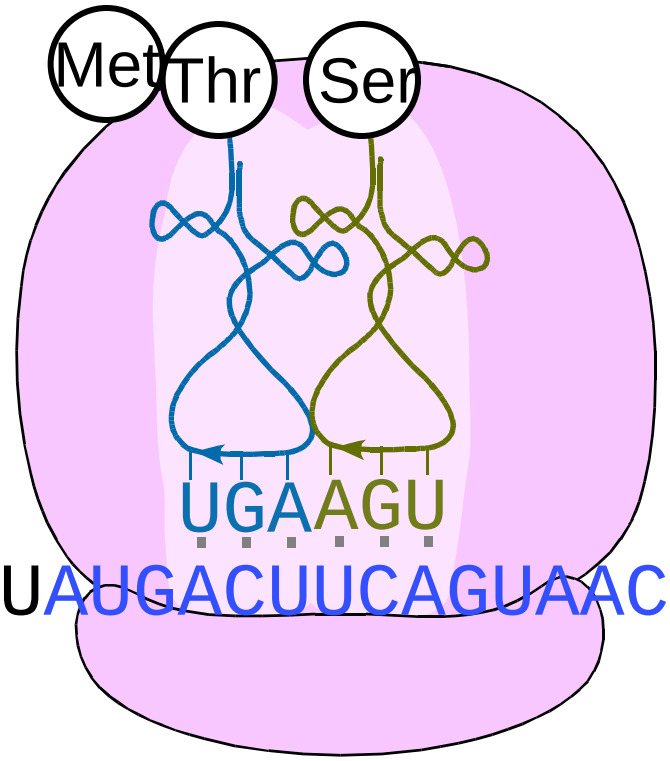
the ribosome breaks the Met-tRNA bond; Met is instead joined to the Thr-tRNA



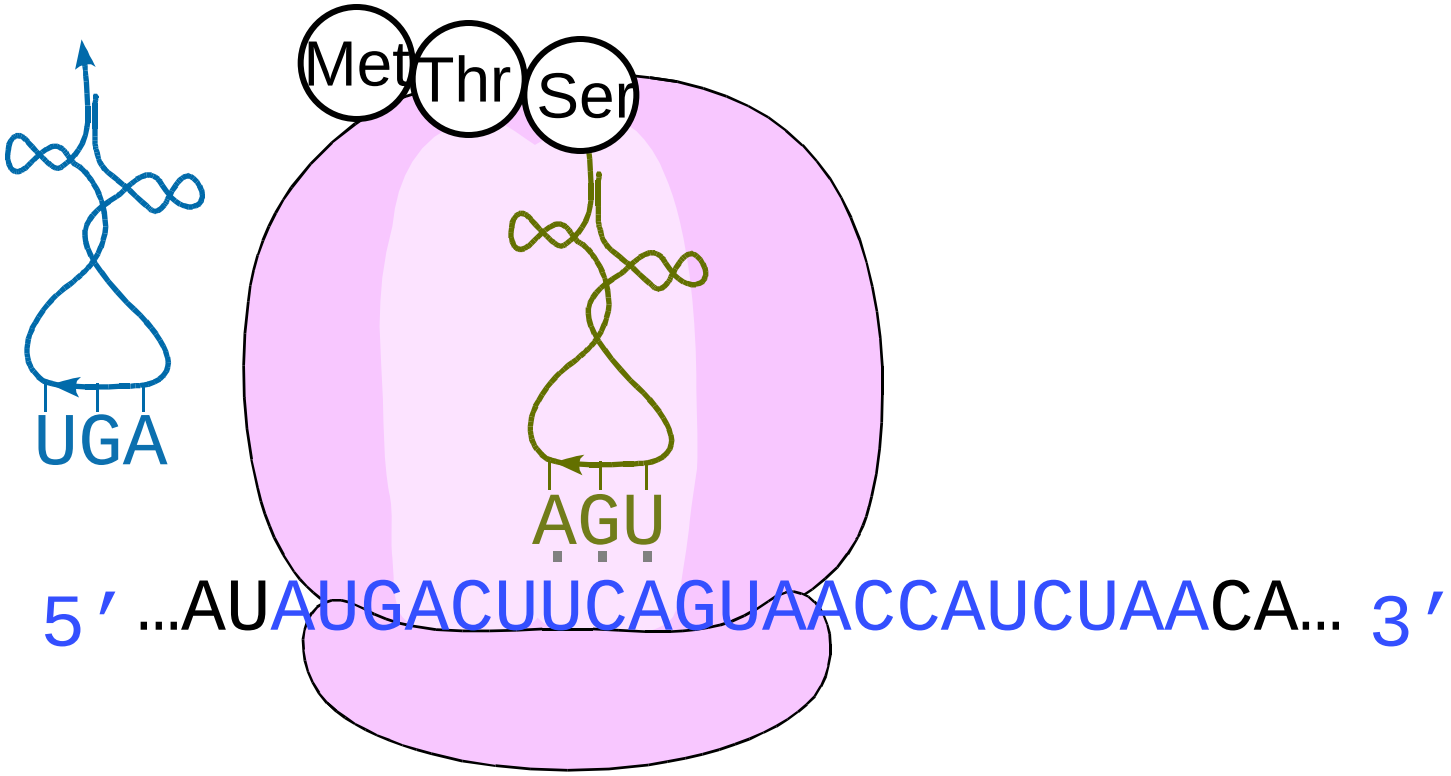
ribosome

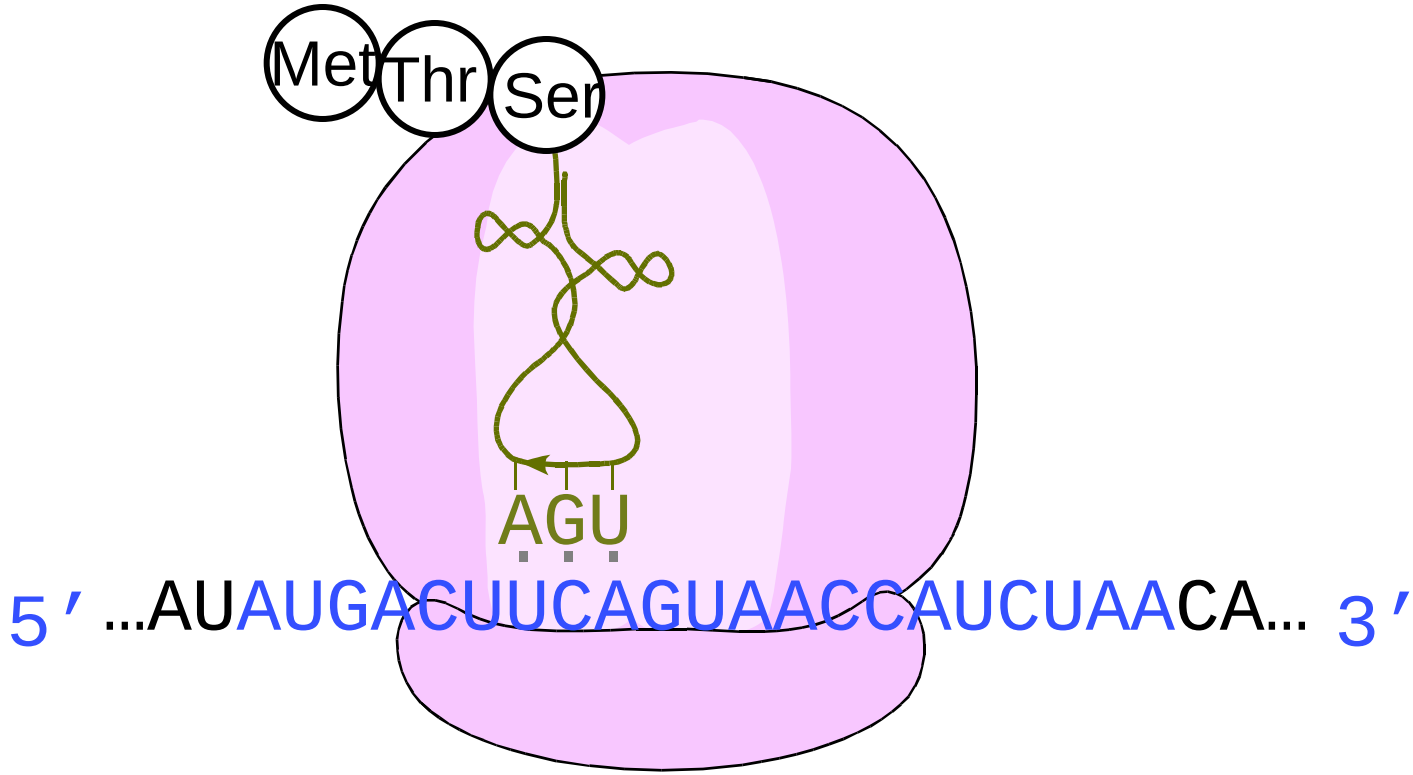
…then ribosome moves over by 1 codon in the 3’ direction

and the next tRNA can bind, and the process repeats

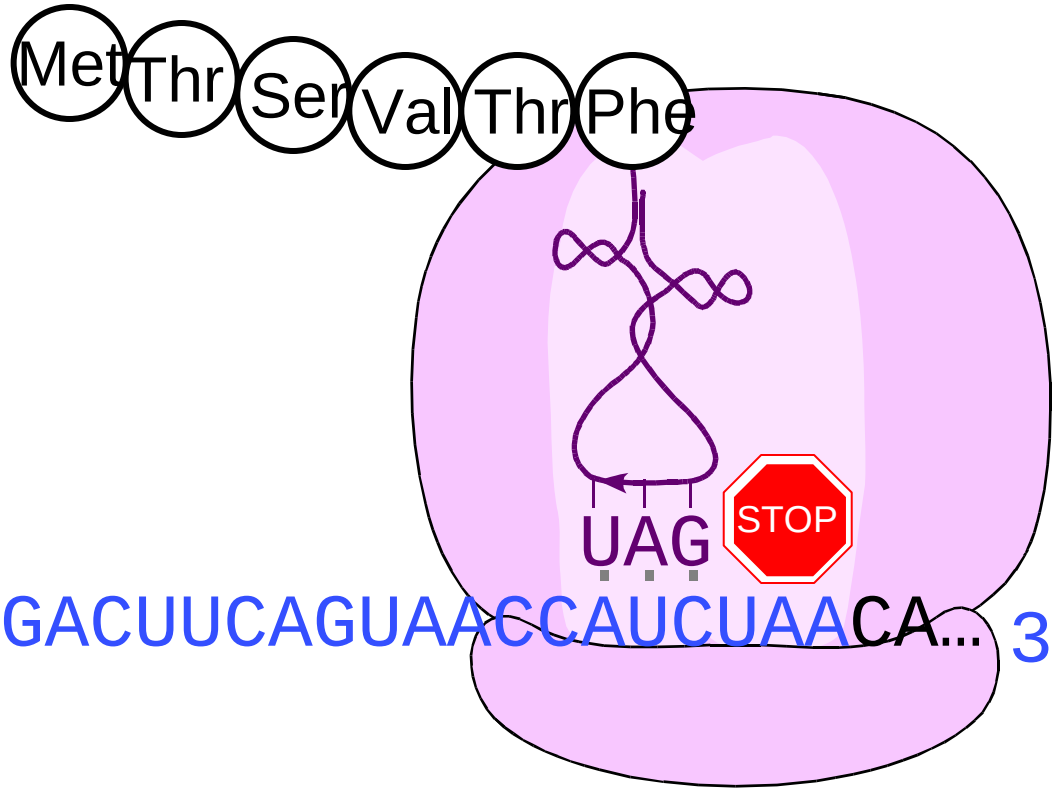


…then ribosome moves over by 1 codon in the 3’ direction

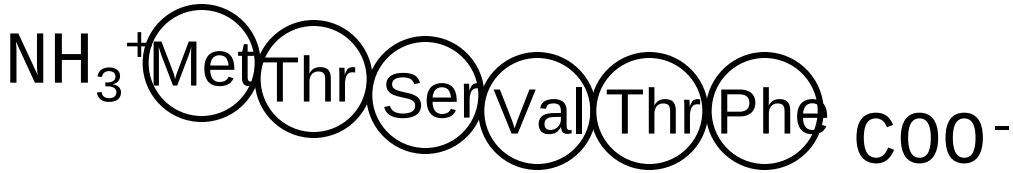

Met
Thr
Ser
UGA
AGU
5′ …AUAUGACUUCAGUAACCAUCUAACA… 3′


Met
Thr
Ser
AGU
5′ …AUAUGACUUCAGUAACCAUCUAACA… 3′

When the ribosome reaches the Stop codon… termination



The finished peptide!

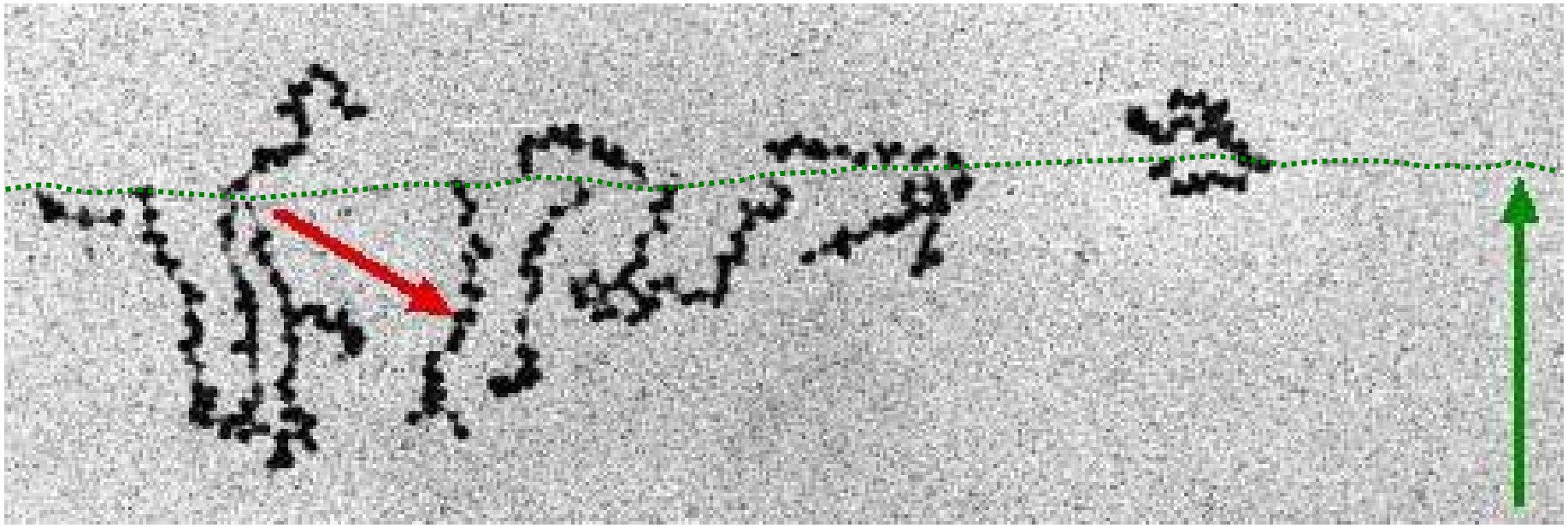


5′ …AUAUGACUUCAGUAACCAUCUAACA… 3′

Practice questions… homework

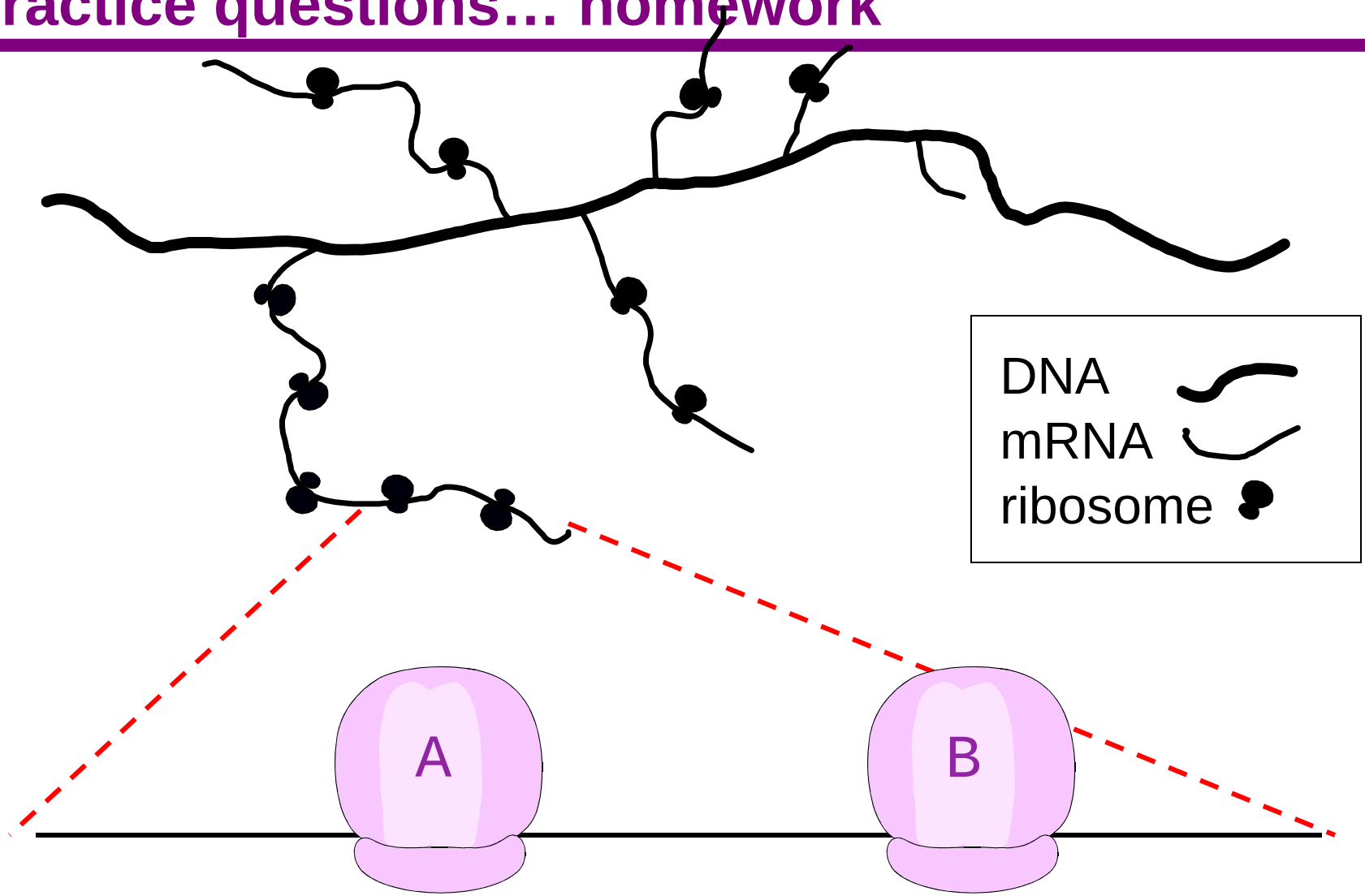
Coupling of transcription and translation

. . . in prokaryotes, like E. coli.



mRNAs covered
with ribosomes

Practice questions… homework



1. Label the 5’ and 3’ ends of the mRNA, then answer the following questions:

2. Which way (to the right or to the left) are ribosomes A and B moving?
3. Toward which end (left or right) is the AUG start codon?
4. Which ribosome (A or B) has the shorter nascent polypeptide?
5. Which end of the polypeptide (amino or carboxy) has not yet been synthesized?

Reading Frame: the ribosome establishes the grouping of nucleotides that correspond to codons by Start counting triplets from this base AUG ed.

5′…AUAUGACUUCAGUAACCAUCUAACA… 3′

Open Reading Frame: ORF: from the first AUG to the first in-frame stop. The ORF is the information for the protein.

More generally: a reading frame with a stretch of codons not interrupted by stop

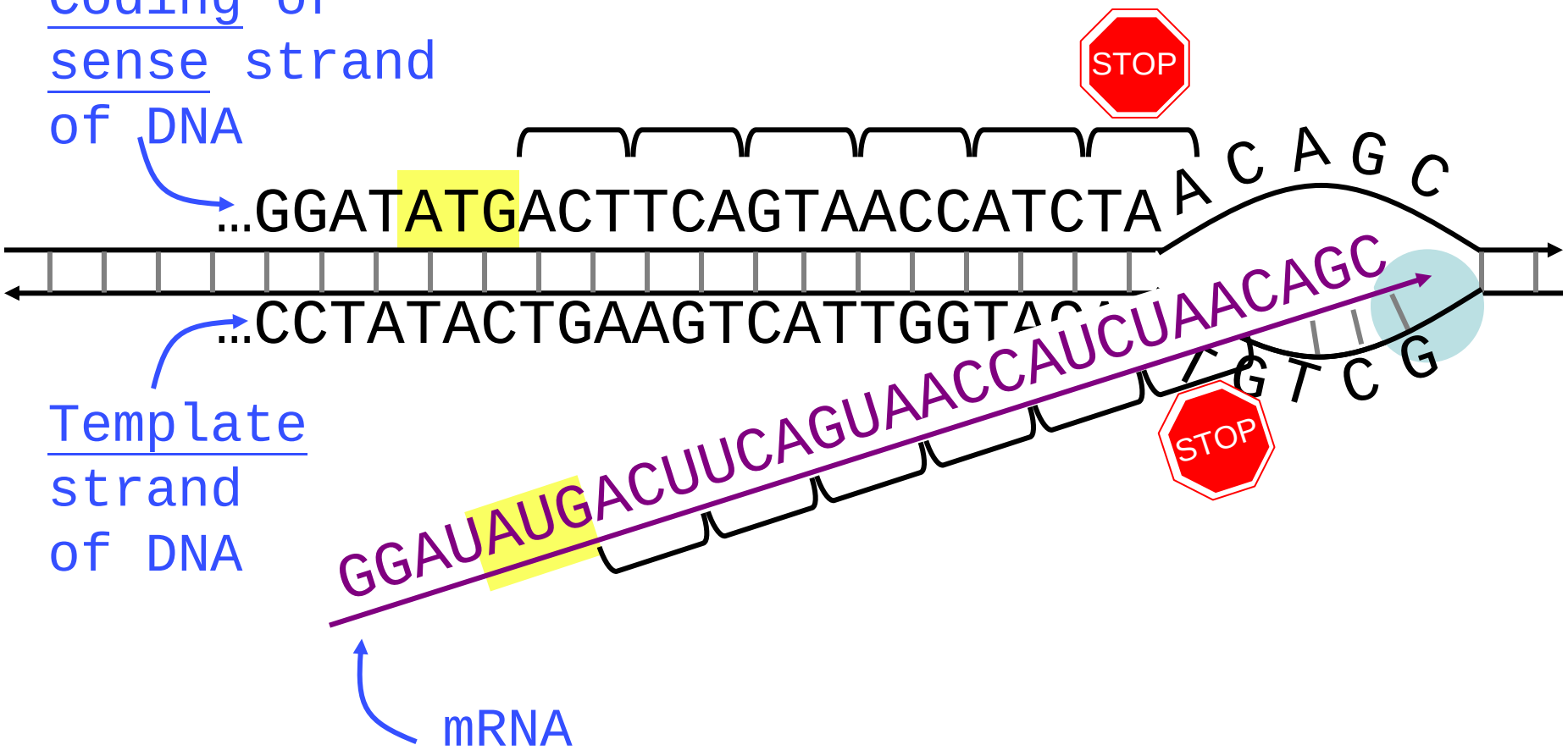
Looking for ORFs

- read the sequence 5′ → 3′, looking for stop
- try each reading frame
- since we know the genetic code—can do a virtual translation if necessary

Something to think about…

- what might the presence of introns do to our virtual translation?

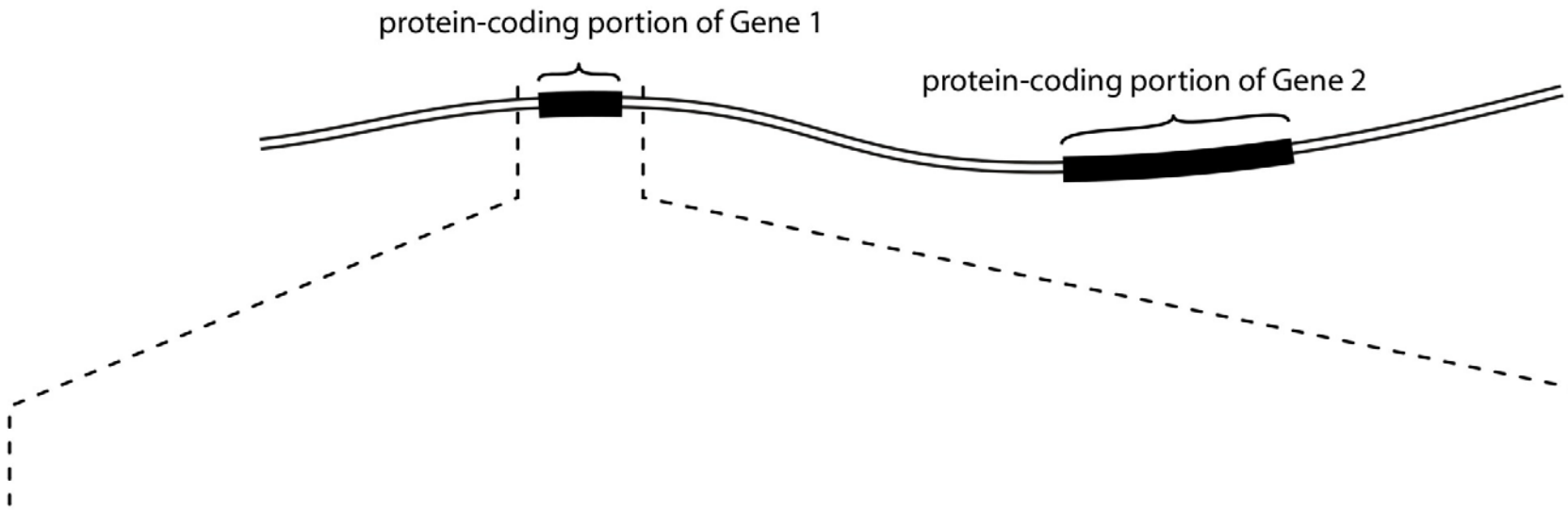
Identifying ORFs in DNA sequence



Looking for ORFs

Practice question

Practice questions



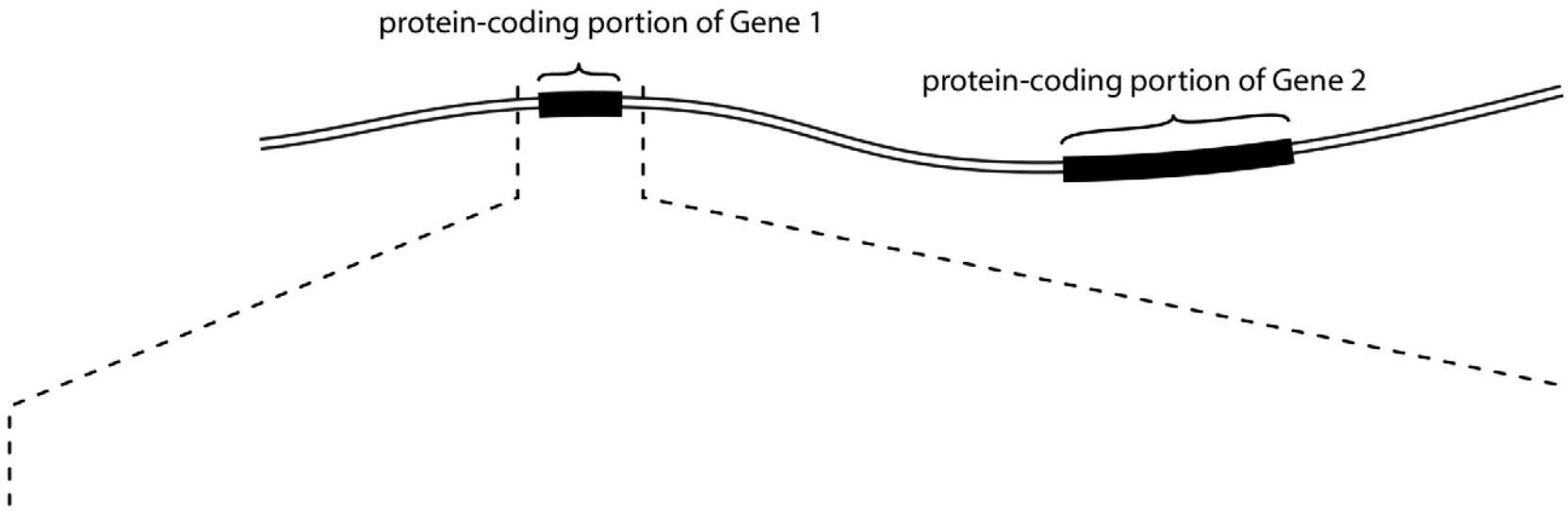
5'-GATTAAAAGAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCATAAGCCCGACT-3'
3'-CTAATTTTCTTACTCTAAAGGAAGTTAAAAACTGCAGTAAAATAGCGTATTCTTAATG-5'

transcription

5'-AAAAGAAUGAGAUUUCCUUCAAUUUUUACUGCAGUUUUAUUCGCAUAAGCCCGACU-3'

1. Which strand on the DNA sequence is the coding (sense) strand? How can you tell?

Practice questions



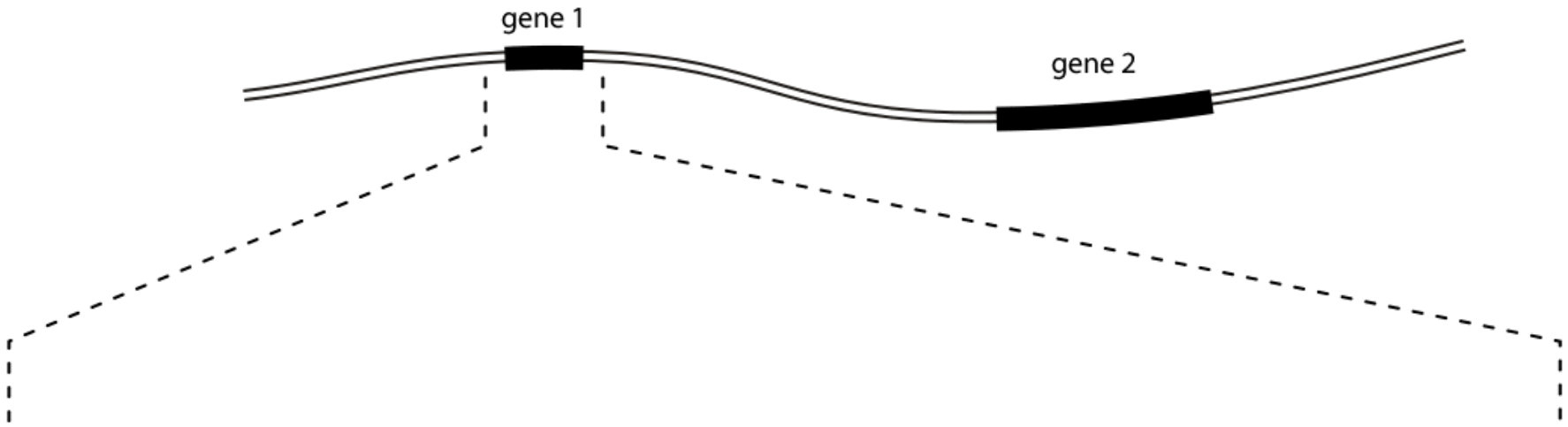
5'-GATTAAAAGAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCATAAGCCCGACT-3'
3'-CTAATTTTCTTACTCTAAAGGAAGTTAAAAACTGCAGTAAAATAGCGTATTCGGGCTGA-5'

transcription

5'-AAAAGAAUGAGAUUUCCUUCAAUUUUUACUGCAGUUUUAUUCGCAUAAGCCCGACU-3'

2. On the DNA sequence, circle the nucleotides that correspond to the start codon.

Practice questions



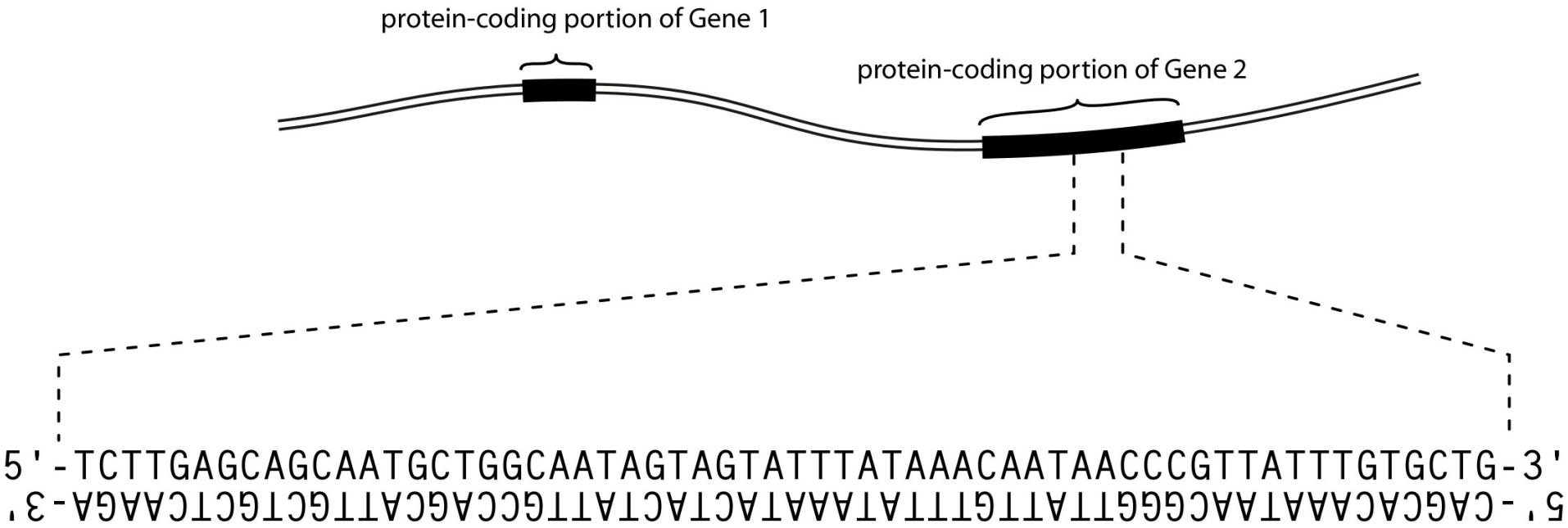
5'-GATTAAAAGAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCATAAGCCCGACT-3'
3'-CTAATTTTCTTACTCTAAAAGGAAGTTAAAAACTGCAGTAAAAATAGCGTTCCGGGCTA-5'

transcription

5'-AAAAGAAUGAGAUUUCCUUCAAUUUUUACUGCAGUUUUAUUCGCAUAAGCCCGACU-3'

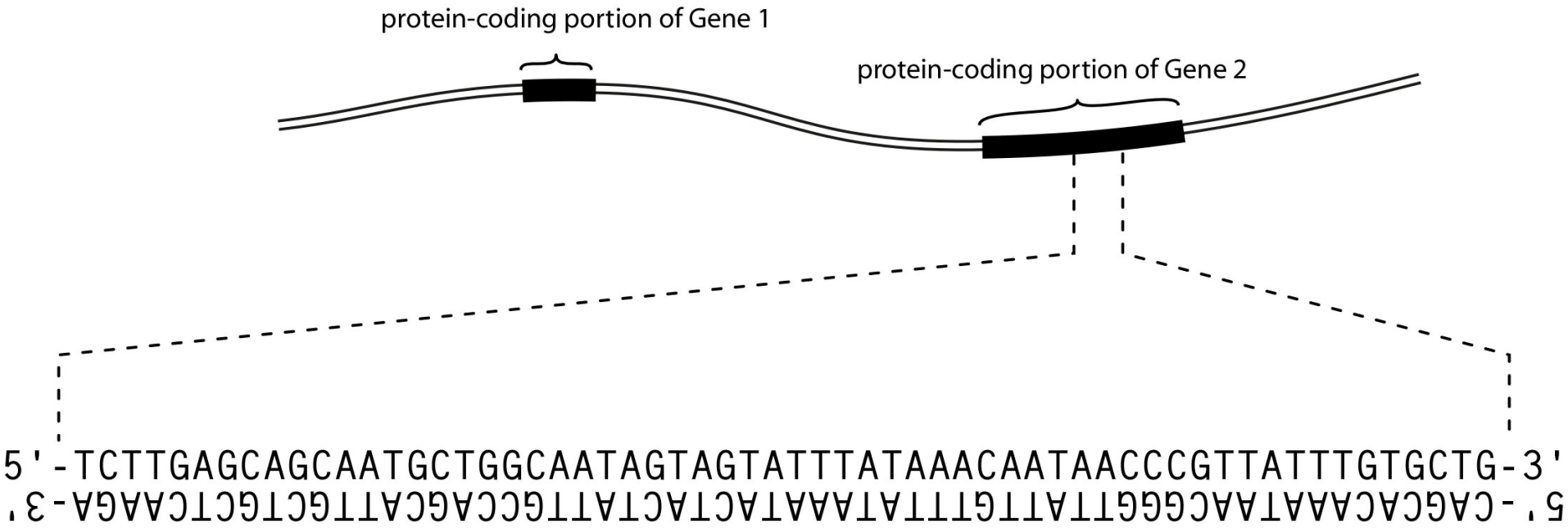
3. How many amino acids are encoded by this gene?

Practice questions



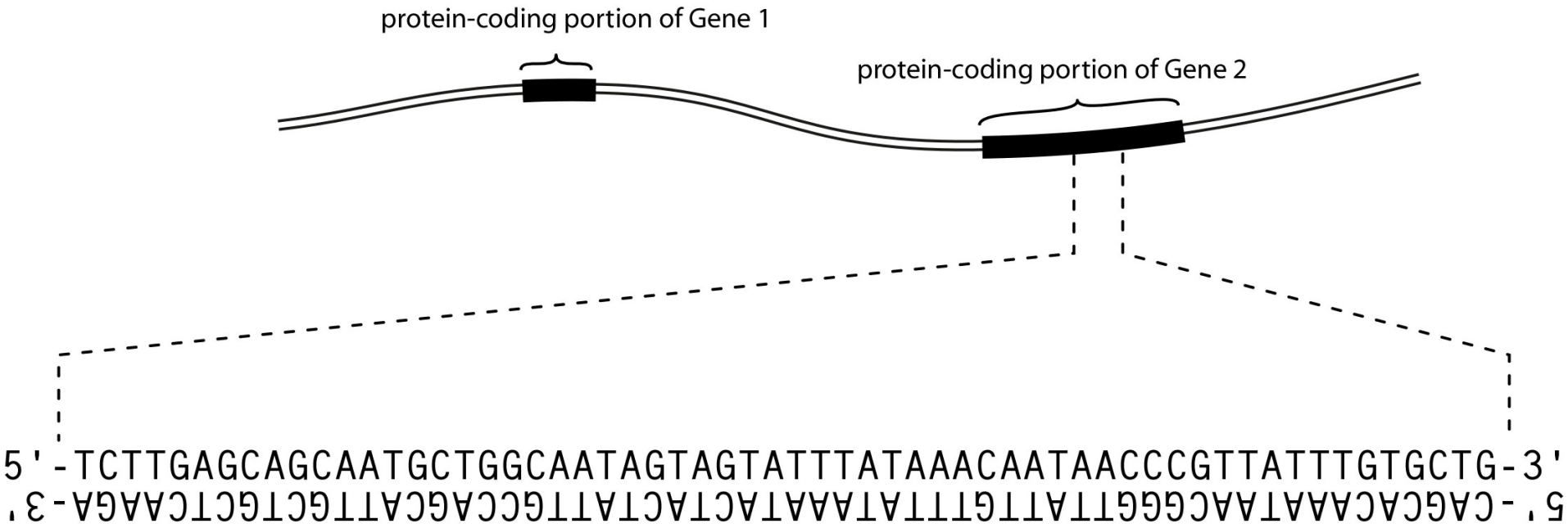
1. Do you expect the start and stop codons of gene 2 to be represented in the DNA sequence that is shown?

Practice questions



2. How many potential reading frames do you think this chunk of DNA sequence contains? How did you arrive at your answer? Would the answer be the same if you didn't know that this sequence came from the middle of a gene?

Practice questions



3. On the appropriate strand, mark the codons for the portion of gene 2 that is shown.

Finding genes in DNA sequence

Given a chunk of DNA sequence…

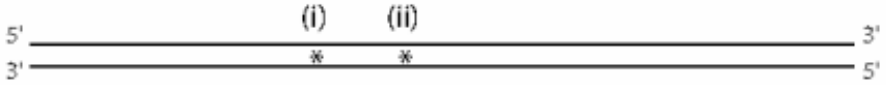
- Open reading frames (termination codons?)
- Splicing signals
- Promoters & transcriptional termination sequences
- Other features
- Computer programs build models of each organism's genes and scan the genome

How do you find out if it contains a gene? How do you identify the gene?

Finding sense in nonsense

cbdryloiaucahjdhtheflybitthedogbutnotthecatjhnhajctipheq

The diagram below represents the region of cat genomic DNA that contains the *tyrosinase* gene (needed for fur pigment production). The asterisks marked (i) and (ii) show the locations of two mutations that have been found in this gene (in separate cats). Mutation (i) causes fur pigmentation to be much more intense than normal, but no amino acid changes were found in the tyrosinase protein in this mutant. Mutation (ii) is a TCA→TGA change that results in a truncated, non-functional protein.

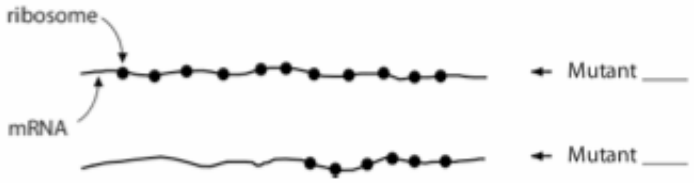


(a) Based on what you have been told about mutation (i), suggest a hypothesis to explain the altered fur phenotype.

(b) Mark the start codon of the tyrosinase gene in the diagram above by drawing a small circle at its approximate location on the **coding strand**. Your answer here should not contradict your answer in (a).

(c) In the close-up representation of a transcription bubble in the tyrosinase gene (below), mark the coding (sense) and template strands... again, consistent with your answer in (a). Draw a circle to mark the location of the RNA polymerase and draw a short RNA transcript with its 5' and 3' ends marked. Is the promoter to the left or to the right? Circle one: Left Right

(d) The picture below represents electron micrographs of tyrosinase mRNAs from the two mutants (i and ii) as they are being translated by ribosomes. [The proteins being made are not shown.] Both mRNAs are in the same orientation (i.e., both have their 5' ends on the same side). Identify which mRNA is from which mutant. Then mark the 5' and 3' ends on one of the mRNAs and put a box around the approximate location of the start codon.



Chromosome segregation (mainly)

- Model organisms in genetics

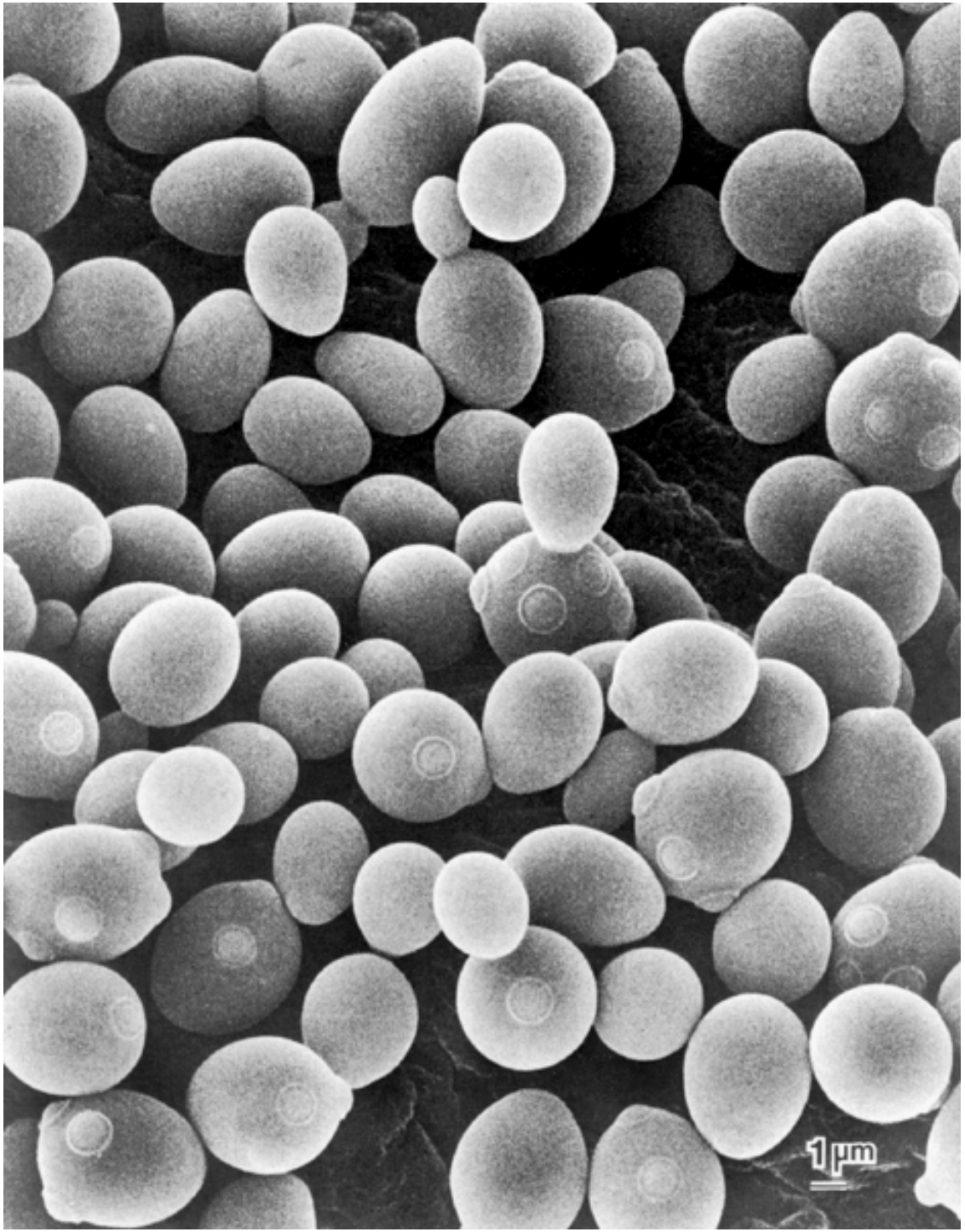
- Chromosomes and the cell cycle
- Mitosis
- (Meiosis)

Quiz Section 1 — The Central Dogma

One way of identifying genes in DNA sequence

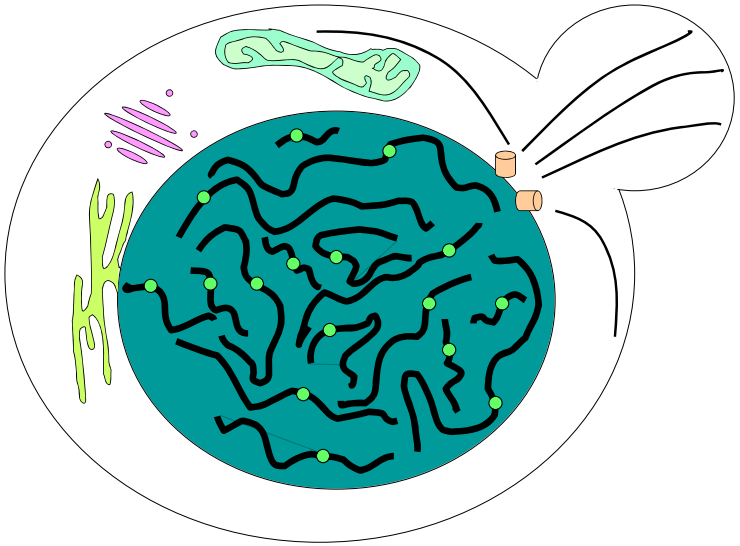
Getting familiar with gene structure, transcription, and translation

…using Baker's yeast genome

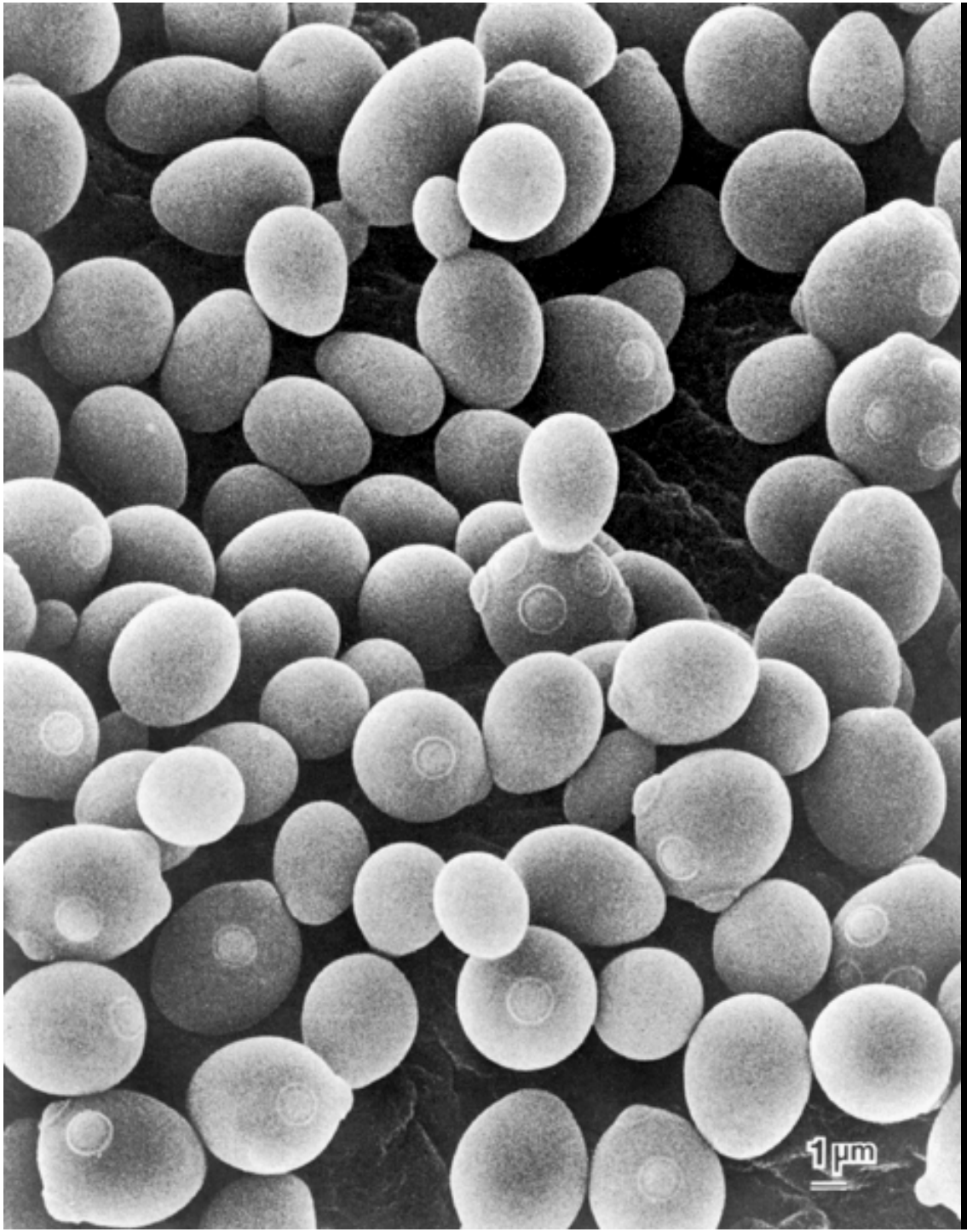


Baker's yeast =
budding yeast =

Saccharomyces cerevisiae



- Yeast is a eukaryote
- 16 chromosomes
- ~6000 genes
- Very few introns



Why yeast?

The use of model organisms

What is a model organism?

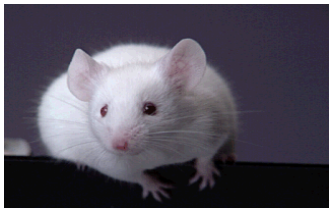
A species that one can experiment with to ask a biological question

Why bother with model organisms?

- Not always possible to do experiments on the organism you want
- If the basic biology is similar, it may make sense to study a simple organism rather than a

Features of a good model organism?

- Small, easy to maintain
- Short life cycle
- Large numbers of progeny
- Well-studied life cycle, biology
- Appropriate for the question at hand
- Has a genome sequence available



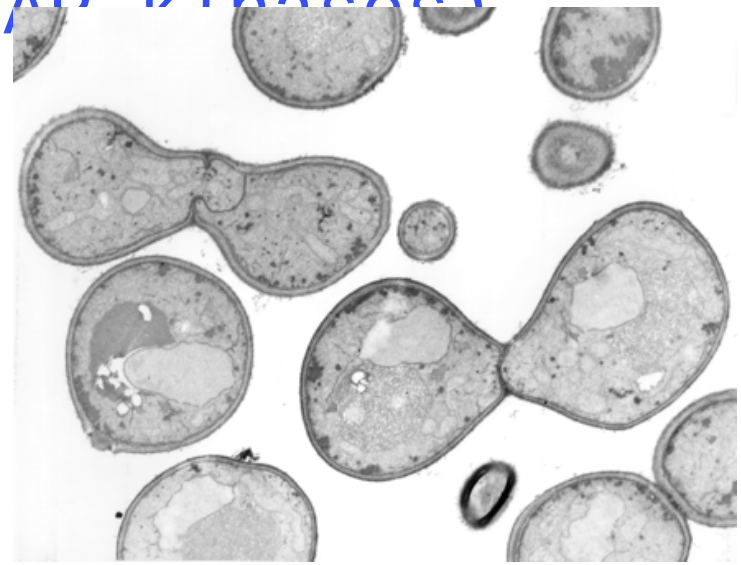
Using model organisms… Example 1

February 1988:

Yeast *STE7*, *STE11*, and *STE12* Genes Are Required for Expression of Cell-Type-Specific Genes

STANLEY FIELDS,[1] DEBORAH T. CHALEFF,[2]†* AND GEORGE F. SPRAGUE, JR.[3]

Analysis in yeast of the role of genes encoding a cascade of protein kinases (MAP kinases)



Development of anticancer drugs targeting the MAP kinase pathway

Judith S Sebolt-Leopold*,[1]

Targeting the EGF receptor in ovarian cancer with the tyrosine kinase inhibitor ZD 1839 ('Iressa')

...M Sewell[1], KG Macleo... ...rth[1] and SP Langdon*,[1]

Human cervical cancer cells use Ca^{2+} signalling, protein tyrosine phosphorylation and MAP kinase in regulatory volume decrease

...Chen *†, Cheng-Yang Chou †, Joseph A. Browning *, ... and J. Clive Ellory *

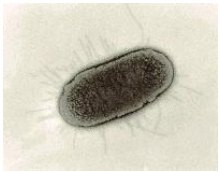
Hyperexpression of Mitogen-activated Protein Kinase in Human Breast Cancer

Vimala S. Sivaraman,* Hsien-yu Wang,‡ Gerard J. Nuovo,§ and Craig C. Malbon‖

Mitogen-Activated Protein Kinase Kinase Kinase 1 Activates Androgen Receptor-Dependent Transcription and Apoptosis in Prostate Cancer

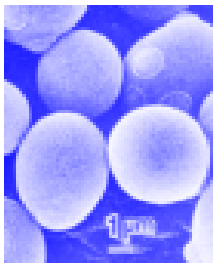
MARIA T. ABREU-MARTIN,[1] AJAI CHARI,[2,3] ANDREW A. PALLADINO,[1] NOAH A. CRAFT,[2,3] AND CHARLES L. SAWYERS[2,3]*

Some commonly used model organisms

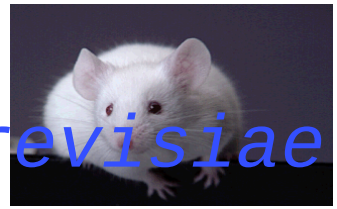


Escherichia co

Zebrafish – *Danio rerio*



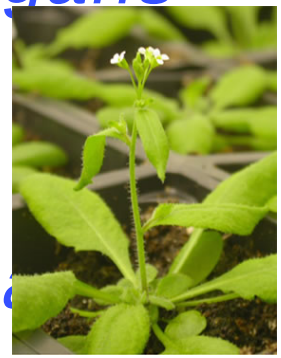
Budding yeast – *Saccharomyces cerevisiae*



Mouse – *Mus musculu*

Round worm – *Caenorhabditis elegans*

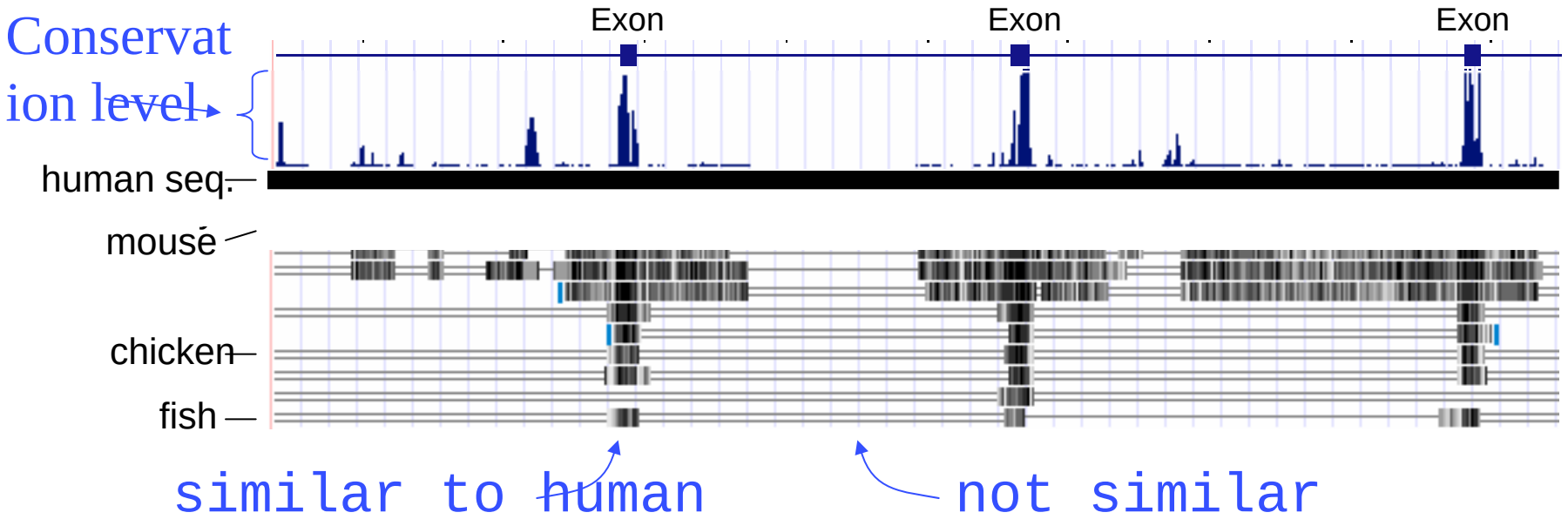
Fruit fly – *Drosophila melanoga*



Thale cress – *Arabidopsis thaliana*

Sequence conservation across species

Comparison of human sequences to those of other organisms:



Even for yeast:
~50% of yeast genes have at least one similar human gene;
~50% of human genes have at least one similar yeast gene

Using model organisms… Example 2

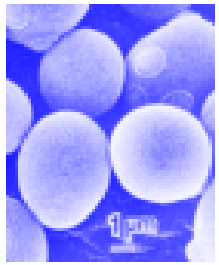
What is the basis of human skin color differences?

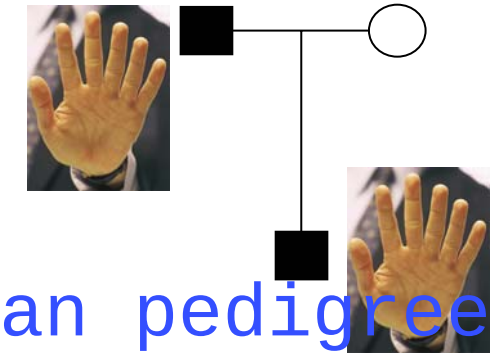
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Science, 16 Dec 2005

How would a geneticist approach this question?

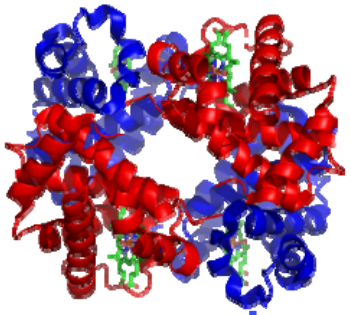
Linking genotype & phenotype: model organisms

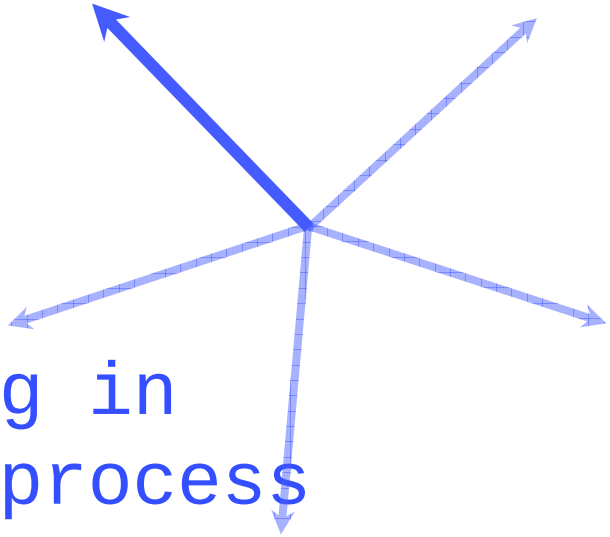


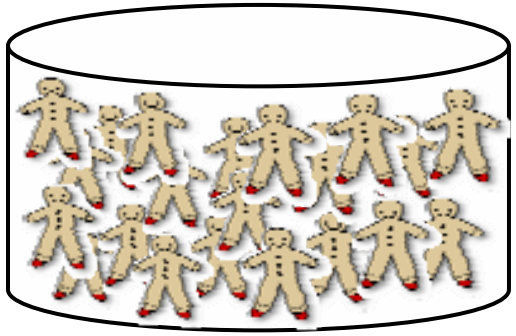


Mutant identified in a model organism

Human pedigree segregating a trai







Protein acting in a biological process

Association s

```
 946  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009  GGA CCT TTA CGG CGT AAT CCT GGA AAC CAT GAC AAA
1072  GCT GAT GTA GAA TTT TGC CTG AGT TTG ACC CAA TAT
1135  AAT TTC AGC TTT AGA AAT ACA CTG GAA GGA TTT GCT
1198  TCT CAA AGC AGC ATG CAC AAT GCC TTG CAC ATC TAT
1261  GGA TCT GCC AAC GAT CCT ATC TTC CTT CTT CAC CAT
1324  TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
```

Sequence analysi

A genetic approach…

Pick a model organism

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

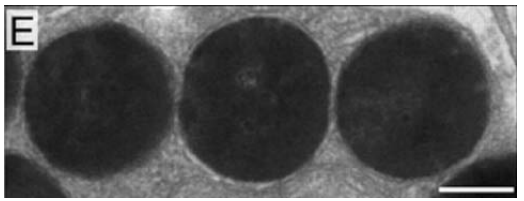
Find mutant(s) with “interesting” phenotypes

Rebecca Lamason et al., Science, 16 Dec 2005

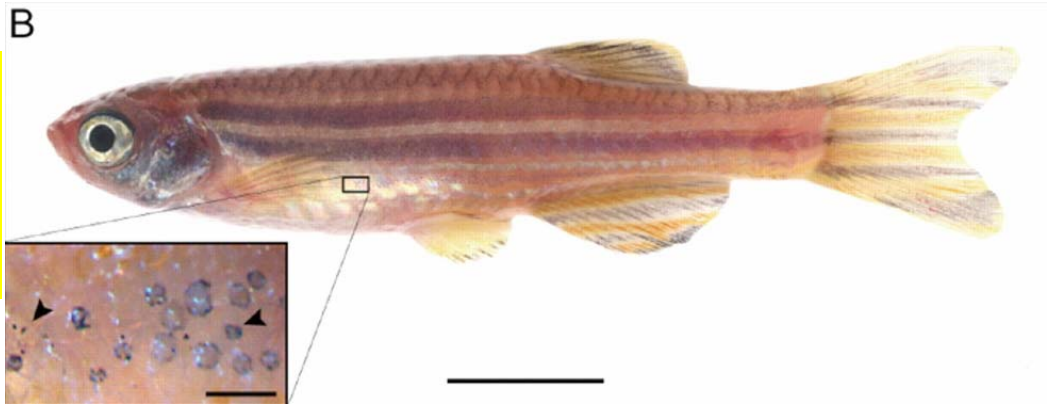
wild type (i.e

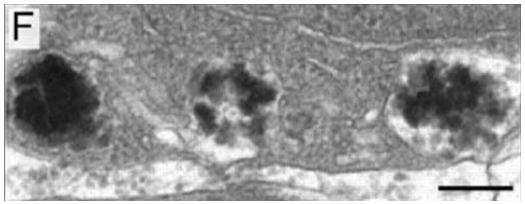
Their model organism… zebrafish

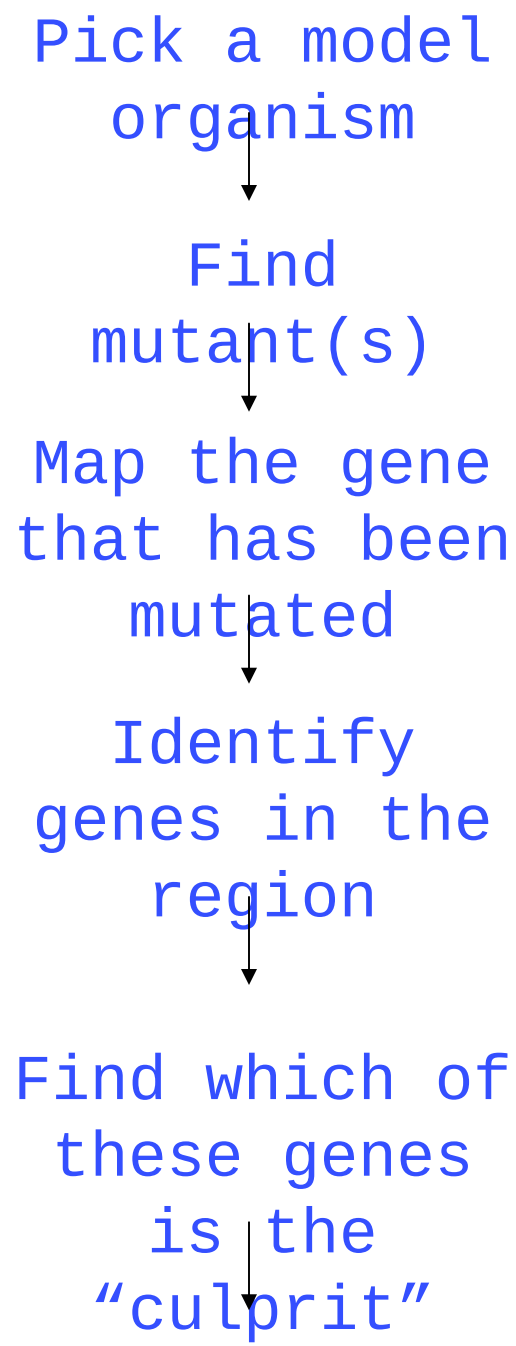
Melanosomes in EM



mutant






Pick a model organism
Find mutant(s)
Map the gene that has been mutated
Identify genes in the region
Find which of these genes is the “culprit”

But what does it have to do with humans?

Find which of these genes is the "culprit"

- Do humans have a similar gene?
- If so, does the human version also control pigmentation?
- Are there different alleles corresponding to different

mutant

mutant + human gene!

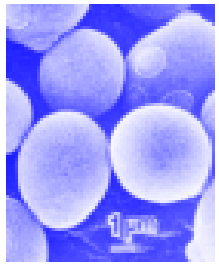
A somewhat different path… Example 3

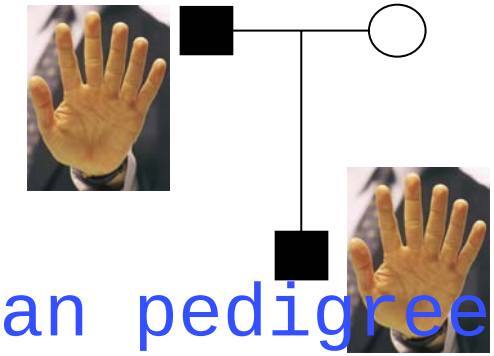
Huntington Disease (HD)

A neurodegenerative disorder

- movement disorder (“chorea”)
- lack of coordination
- cognitive changes
- invariably lethal
- no known cure

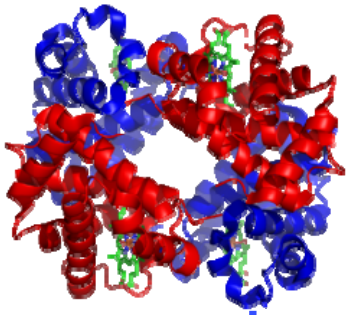
Linking genotype & phenotype: human pedigrees

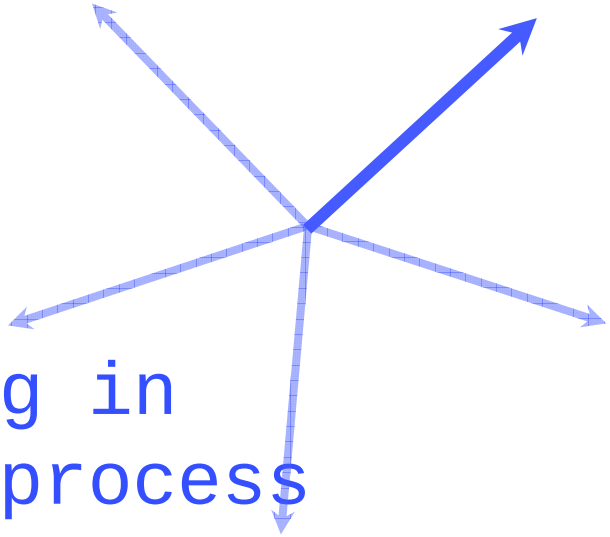


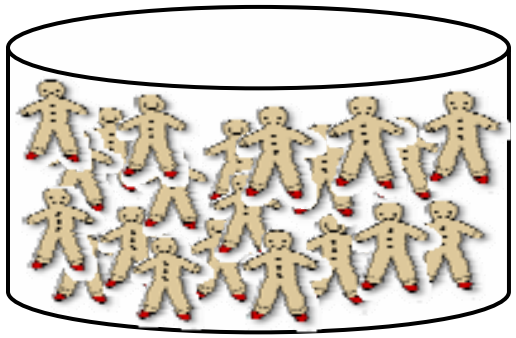


Mutant identified in a model organism

Human pedigree segregating a trai





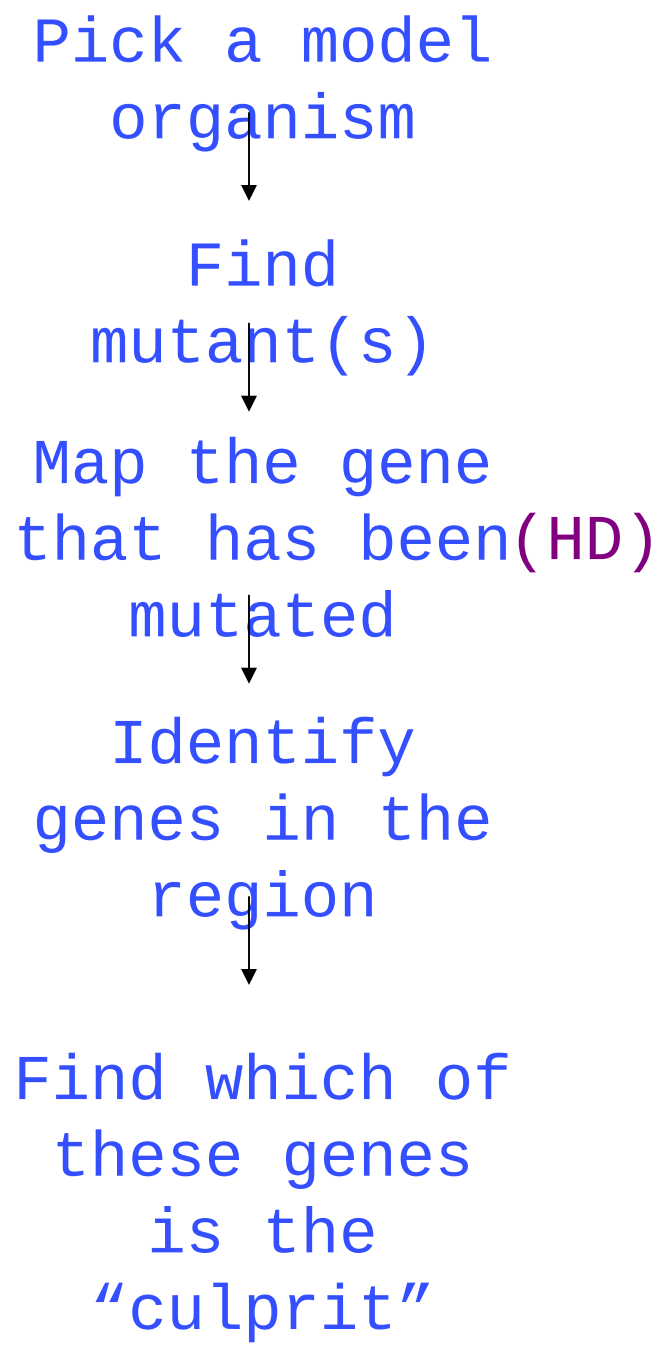


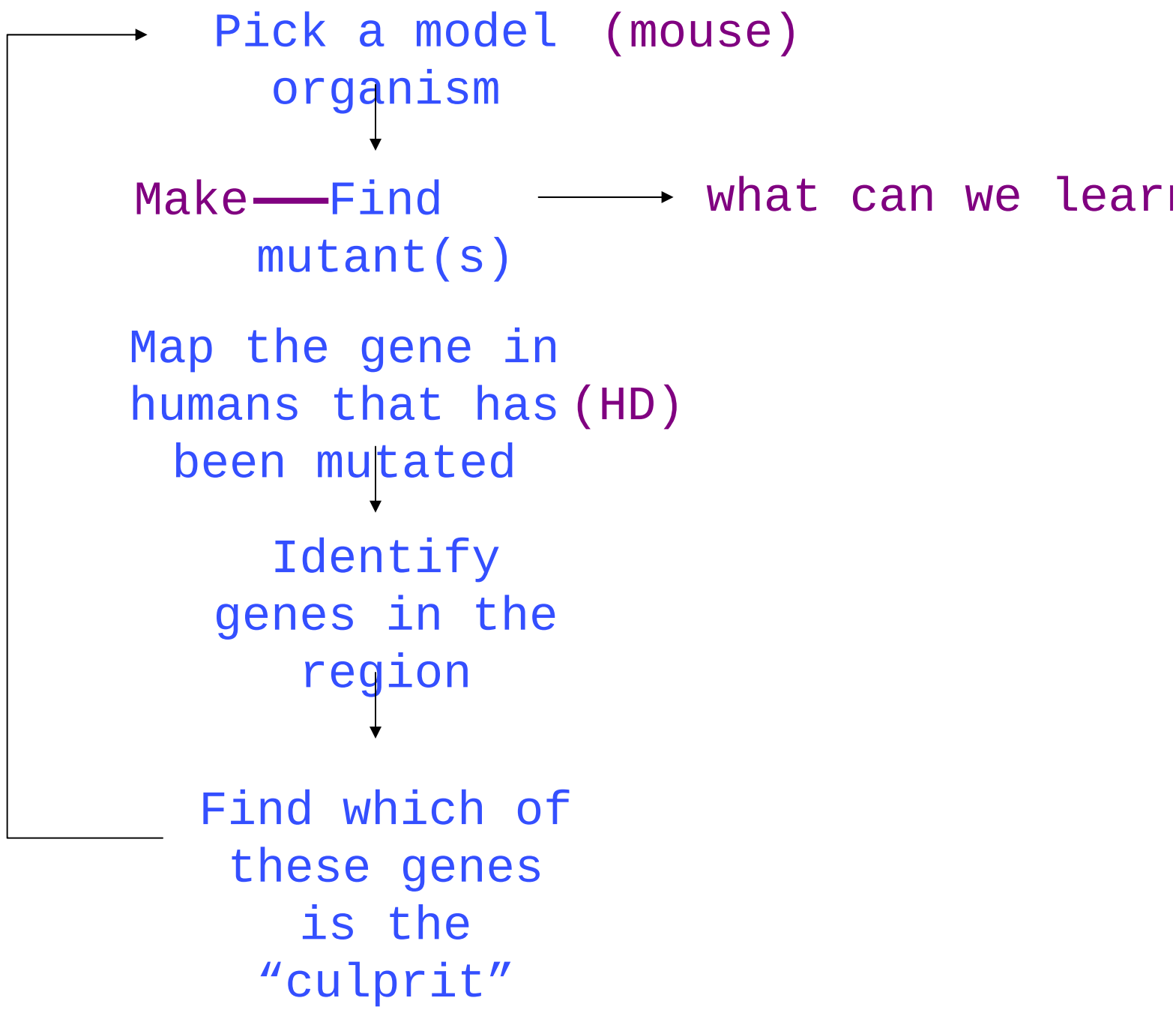
Protein acting in a biological process

Association s

```
 946  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009  GGA CCT TTA CGG CGT AAT CCT GGA AAC CAT GAC AAA
1072  GCT GAT GTA GAA TTT TGC CTG AGT TTG ACC CAA TAT
1135  AAT TTC AGC TTT AGA AAT ACA CTG GAA GGA TTT GCT
1198  TCT CAA AGC AGC ATG CAC AAT GCC TTG CAC ATC TAT
1261  GGA TCT GCC AAC GAT CCT ATC TTC CTT CTT CAC CAT
1324  TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
```

Sequence analysi

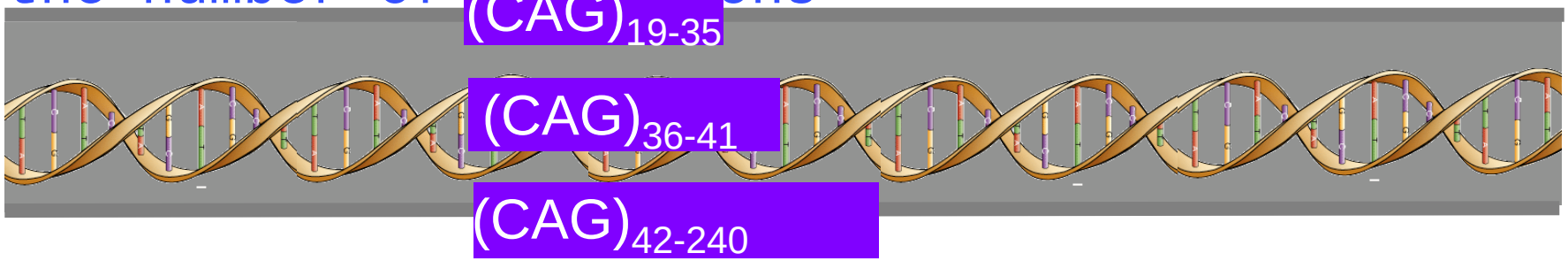

Pick a model organism
Find mutant(s)
Map the gene that has been(HD) mutated
Identify genes in the region
Find which of these genes is the “culprit”


Pick a model (mouse) organism
Make
Find mutant(s)
what can we learn
Map the gene in humans that has been mutated (HD)
Identify genes in the region
Find which of these genes is the "culprit"

Beyond the Basics

Huntington Disease

The disease is caused by an expansion in the number of CAG codons

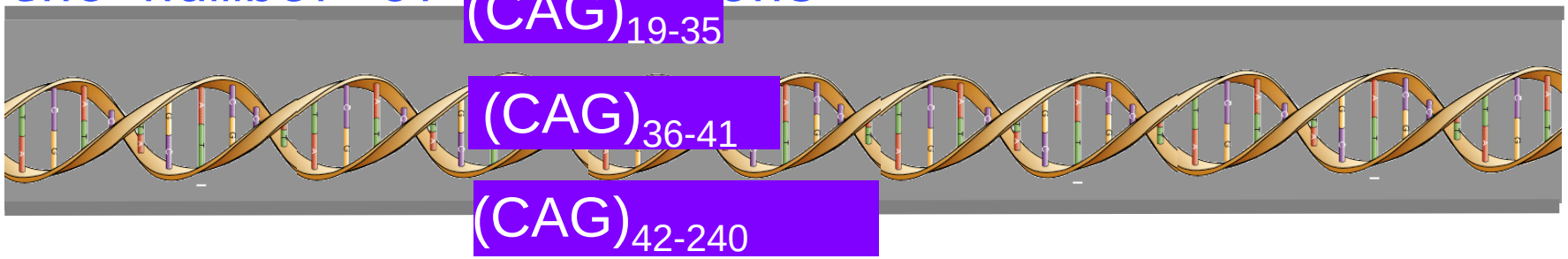


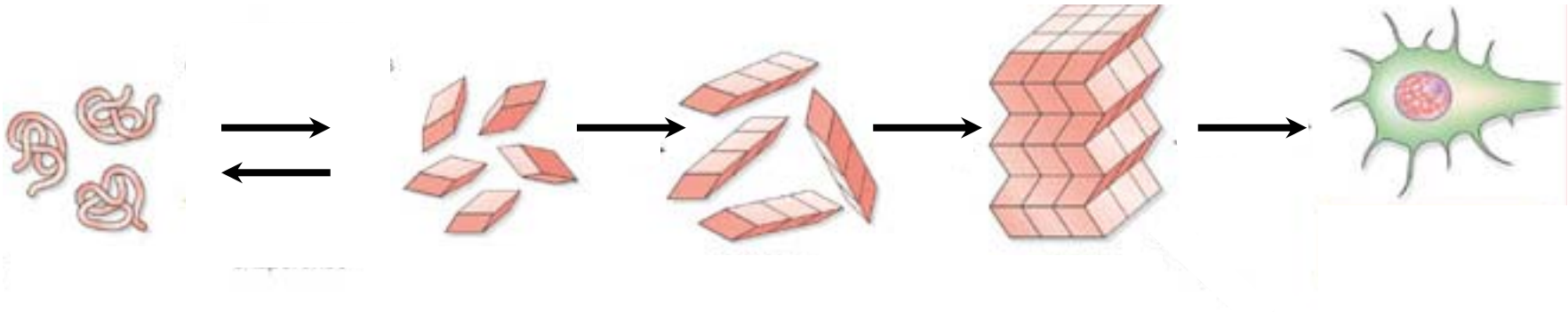
Huntingtin protein with expansion of glutamines

Beyond the Basics

Huntington's Disease

The disease is caused by an expansion in the number of CAG codons





Native protein | **Misfolded proteins** | **Oligomers** | **Fibrils** | **Intranuclear inclusion (poly-gln expansion)**

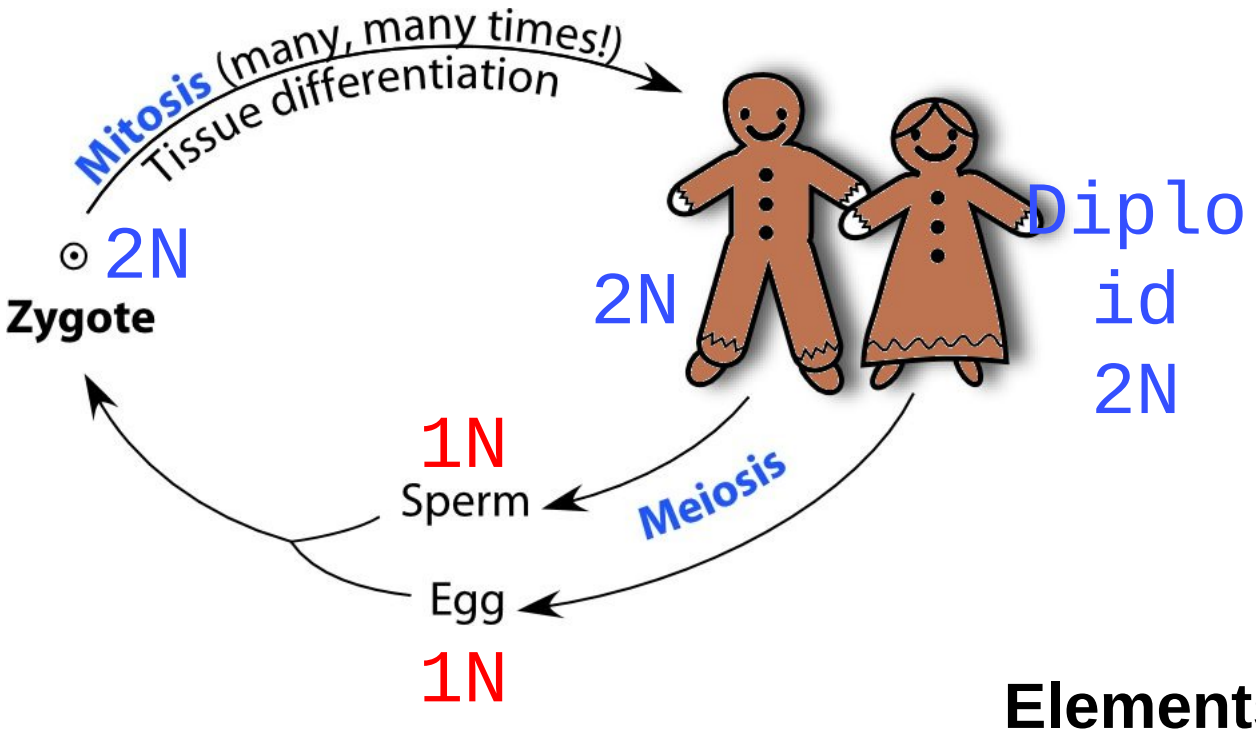
»Model organisms in genetics

»Chromosomes and the cell cycle

»Mitosis

»(Meiosis)

Cell division and the life cycle



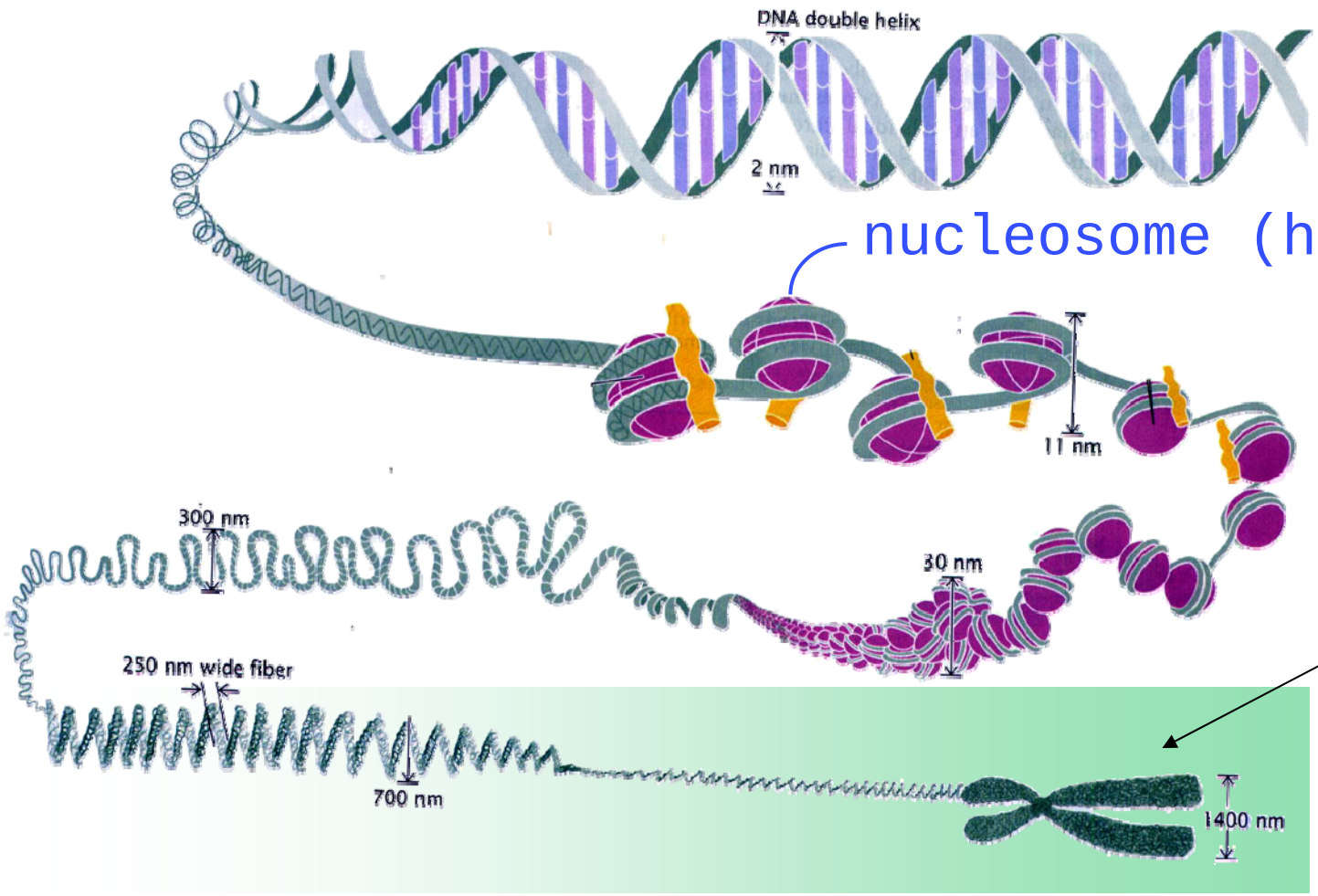
Chromosomes decondensed

Chromosomes condensed

Elements of cell division

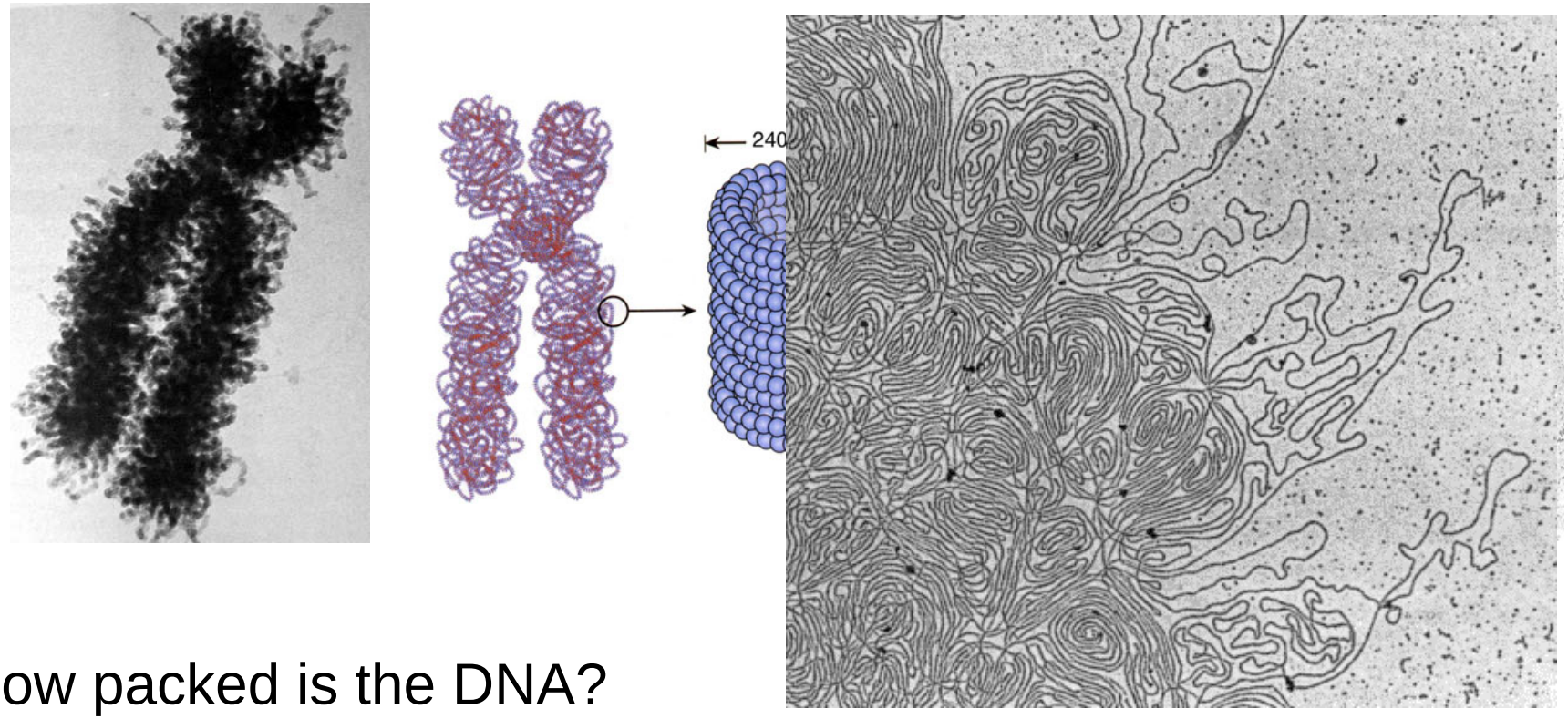
- Cell growth
- Chromosome duplication
- Chromosome segregation

Chromosome structure: coils of coils of coils…



Local unpacking of chromatin…to allow gene expression & replication

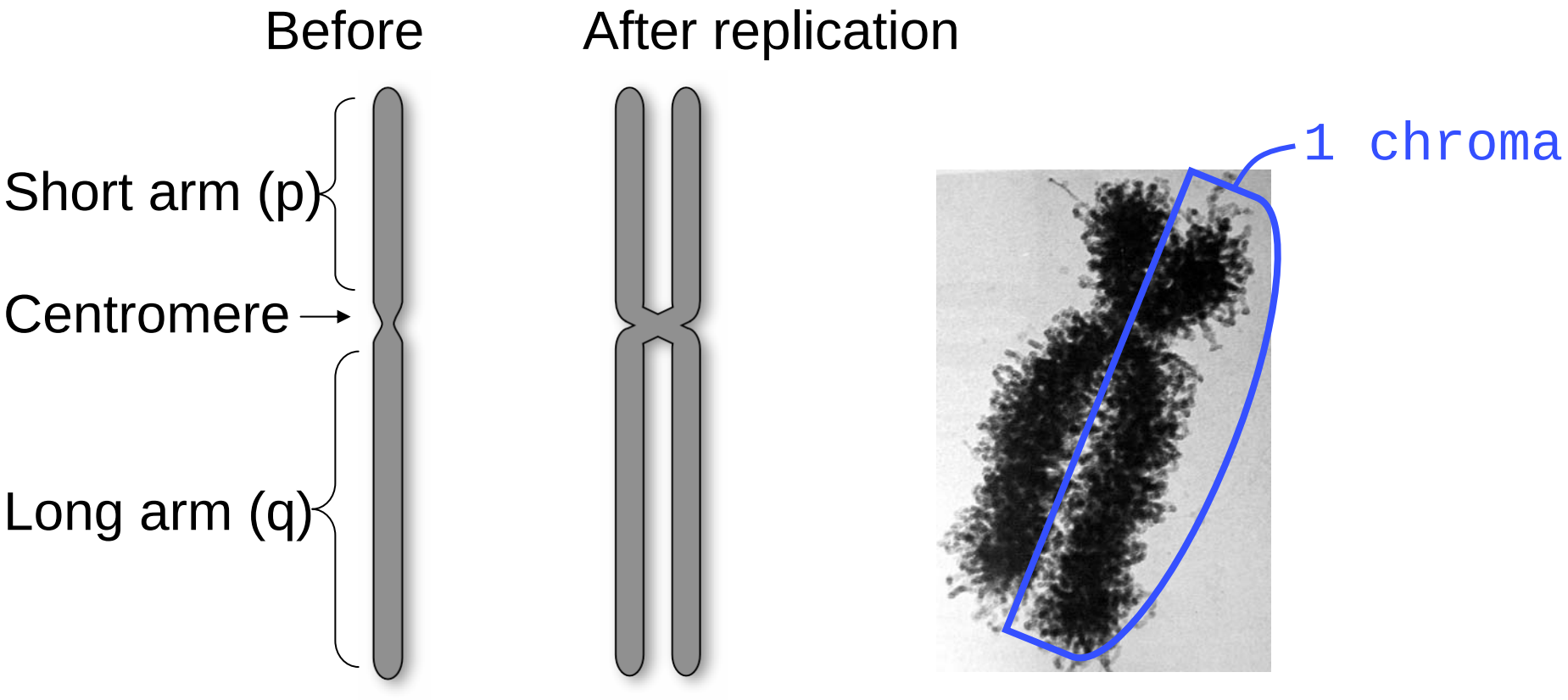
Chromosome structure: coils of coils of coils…



How packed is the DNA?

- 1 human cell has ~ 2 meters of DNA
- 1 average human body: DNA length equivalent to ~600+ round-trips to the sun!

Chromosome structure (cont'd)



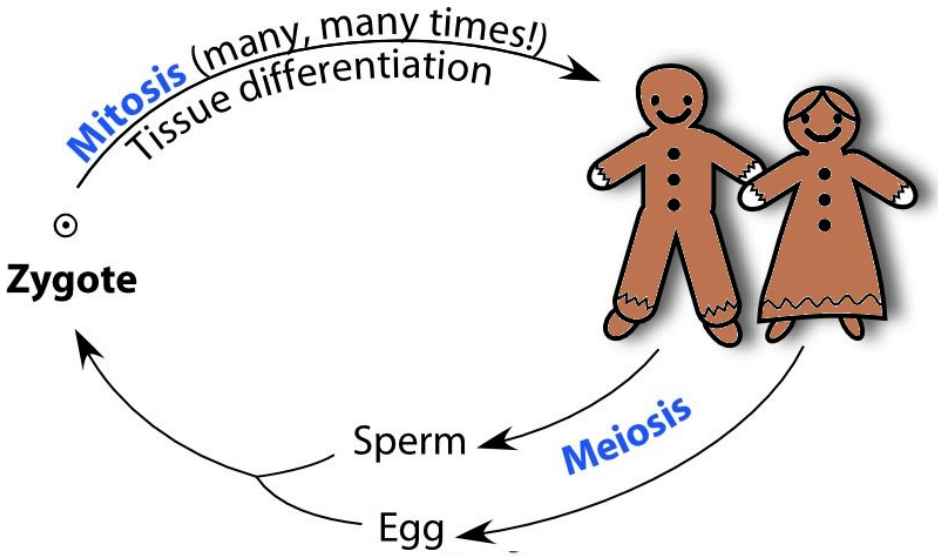
After a chromosome is replicated but before the two copies are separated… **(sister) chromatids**

Chromosome folding pattern is reproducible

Each chromosome has its own characteristic folding pattern…

variations in level of folding → banding patterns (when stained)

karyotype: picture of human chromosome set from 1 individu

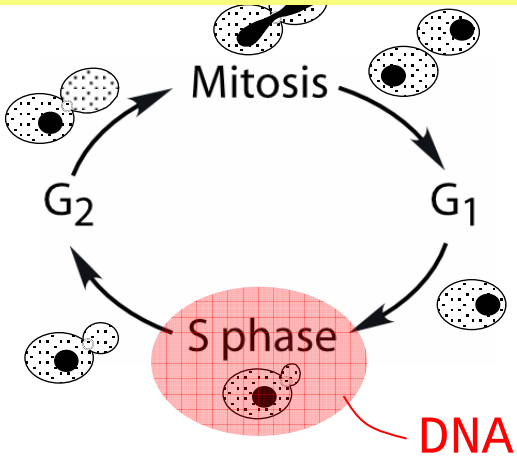


Two copies of each chromosome type…
homologous pairs

The cell division cycle

Elements of cell division

- Cell growth
- Chromosome duplication
- Chromosome segregation



Cell cycle genetics— originally from yeast mutants

- cell and nuclear morphology reflect cell cycle stage
- haploid life style → recessive phenotypes revealed
- *temperature-sensitive* mutants relatively easy to find

Segregating the replicated chromosomes

What happens to the replicated chromosomes?
… depends on the goal of the division

- to make more “vegetative” cells: mitosis

  daughter cells’ chromosome set should be identical to parental cell’s

- to make gametes: meiosis

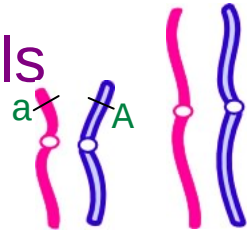
  each daughter cell should have half the number of chromo

  If parental cell was diploid (2N)… daughters should be

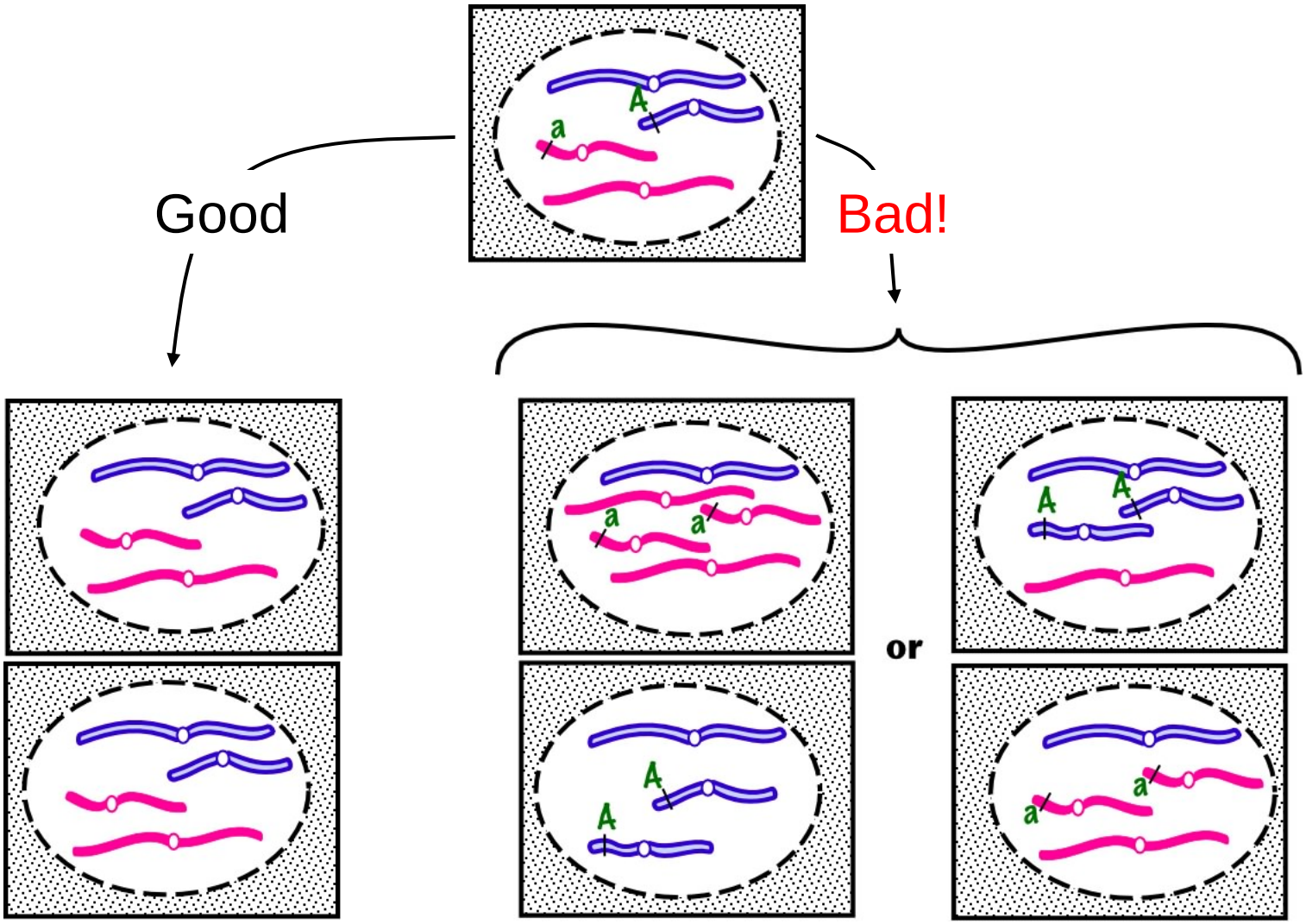
  Will a normal haploid cell undergo meiosis? No

Mitosis

…segregating replicated chromosomes in somatic cells



Diploid cell… homologue pairs



or any outcome where each daughter cell does not have exactly one copy of each

Mitosis (cont'd)

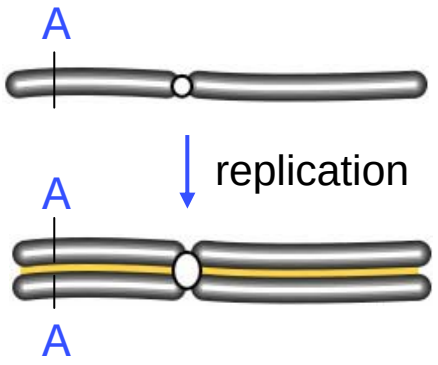
The problem

Partitioning replicated chromosomes so that each daughter cell gets one copy of each chromosome

The solution

After replication of a chromosome…

- hold the two sister chromatids together
- target them to opposite poles
- **then** separate the sisters



Mitosis (cont'd)

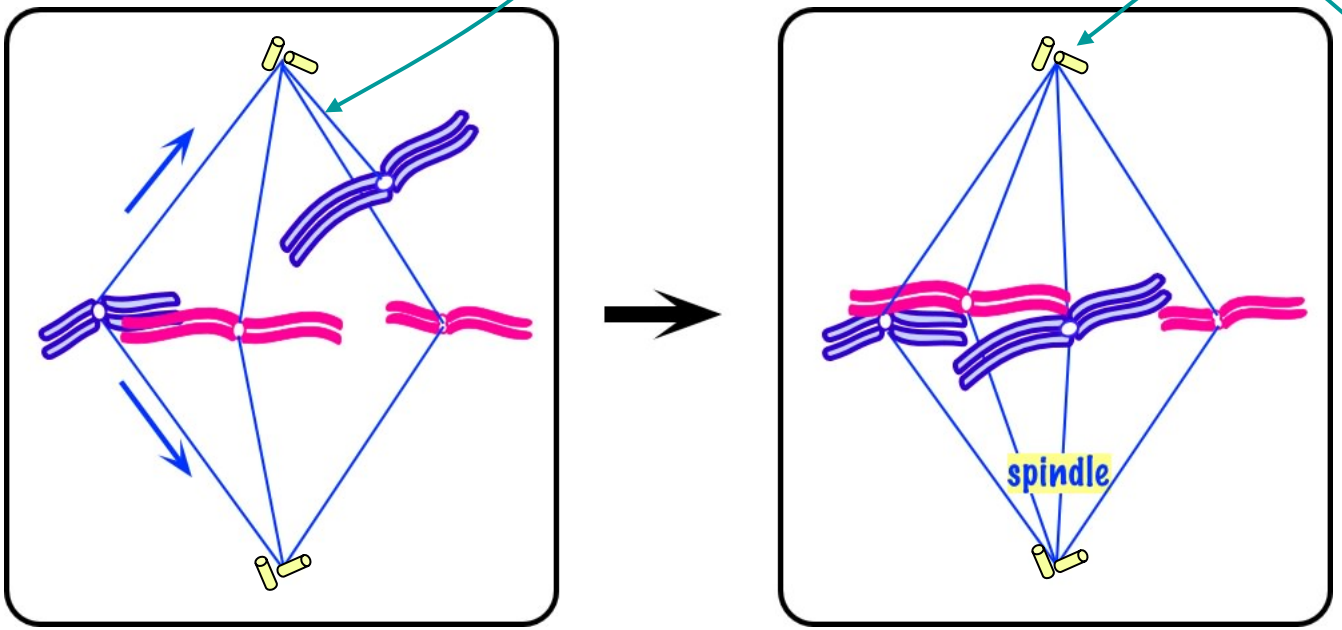
At Metaphase . . .

Chromosomes line up at cell's "equatorial plate"

Mechanism? Spindle fibers exerting tension on kinetochores

kinetochore

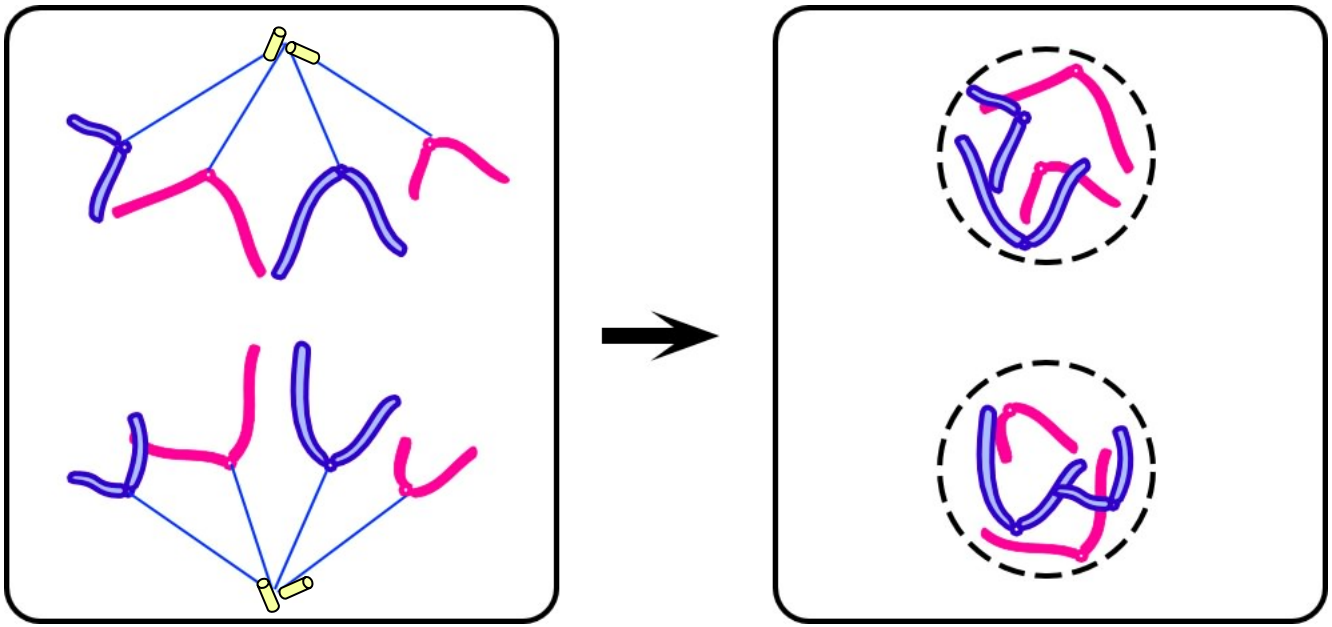
Centromere: DNA sequence on which kinetochore is built



Centriole = Spindle pole body (yeast) = MTOC (microtubule organizing center)

Mitosis (cont'd)

At anaphase… cohesion between sister chromatids dissolved, sisters pulled to opposite poles



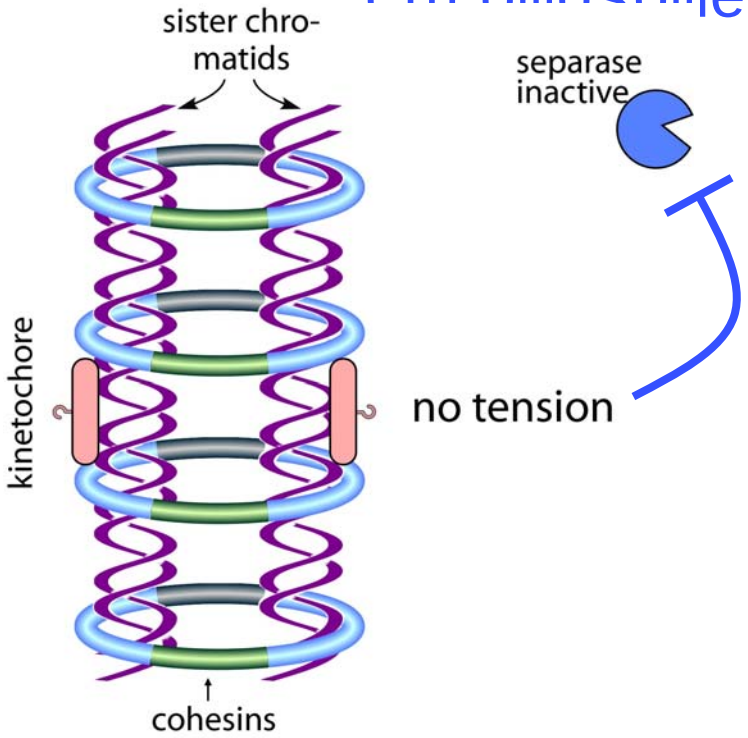
Anaphase Telophase

Monitoring correct attachment to spindle

Sister chromatids are held together by **cohesin** proteins…

Any kinetochore not experiencing tension → block destruction of cohesins

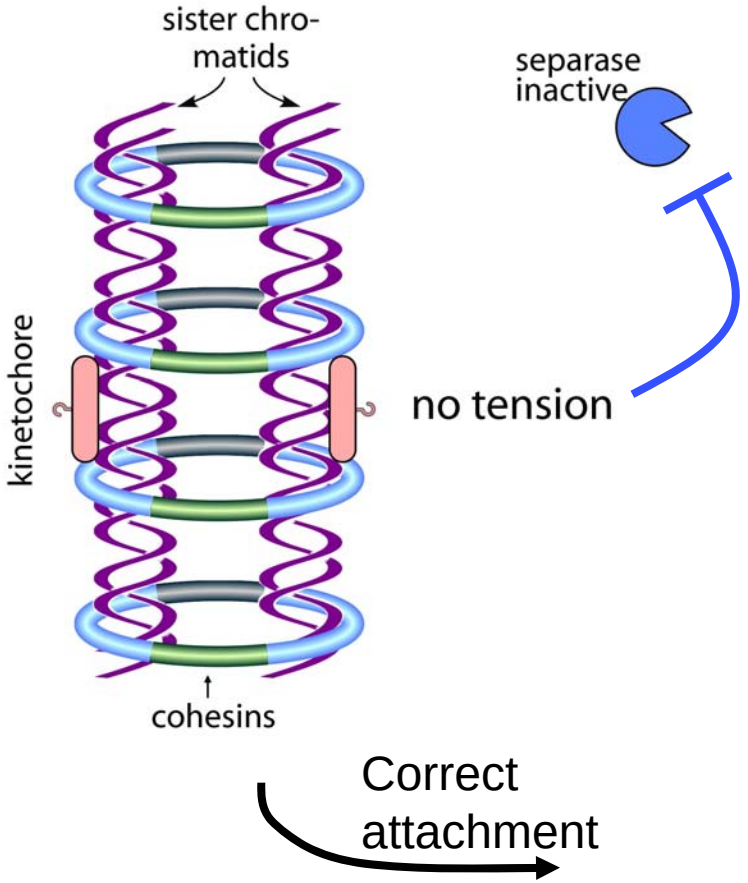
So, no sister separation until all chromosomes are ready!



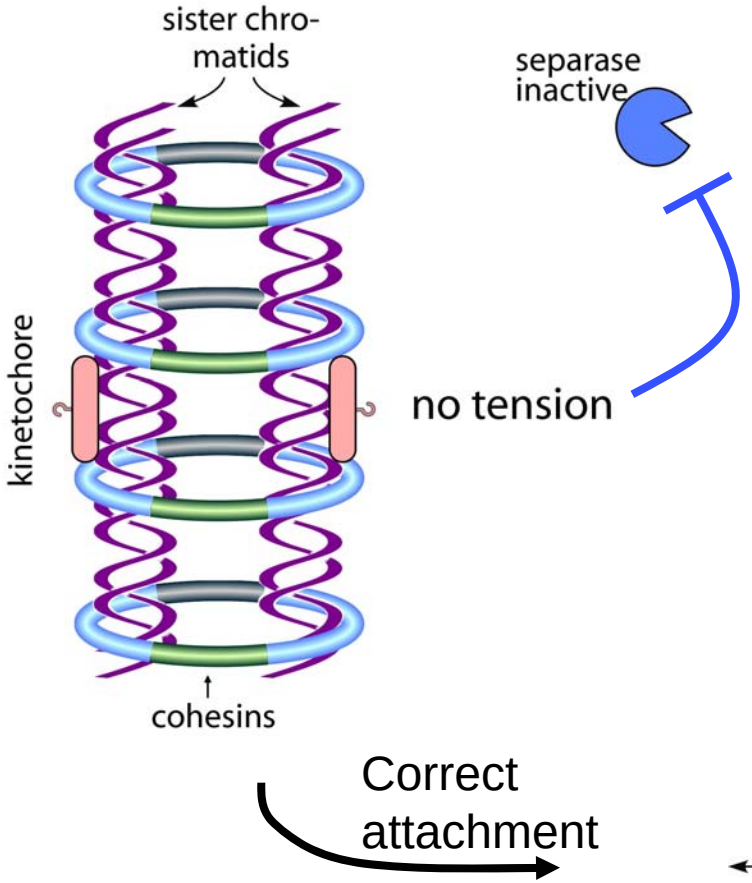
Separase: can destroy cohesins

Unattached kinetochore: blocks separase

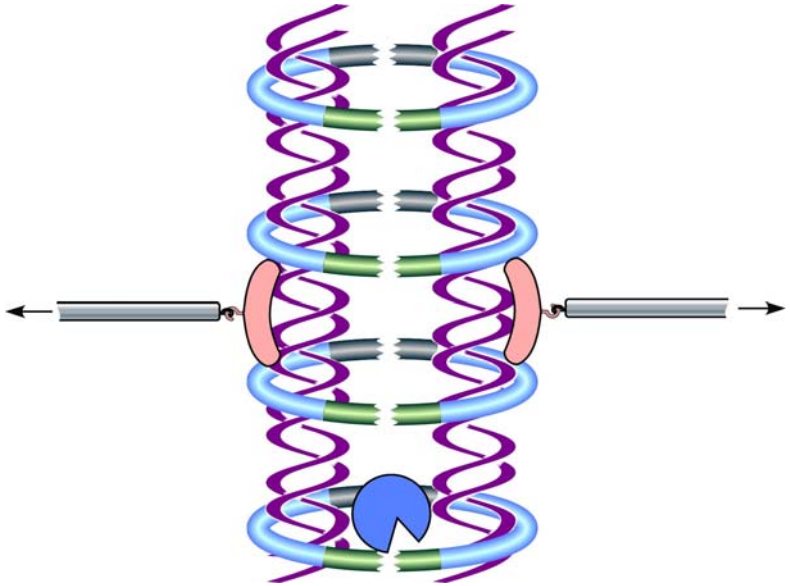
Monitoring correct attachment to spindle (cont'd)



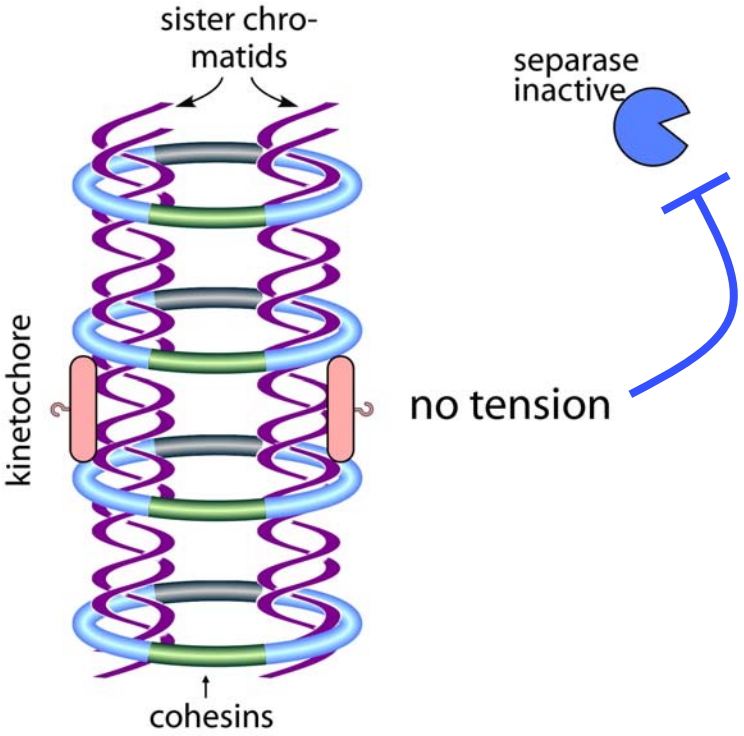
Monitoring correct attachment to spindle (cont'd)



Anaphase begins!



The anaphase entry checkpoint



Unattached kinetochore

⊥

separase **active!**

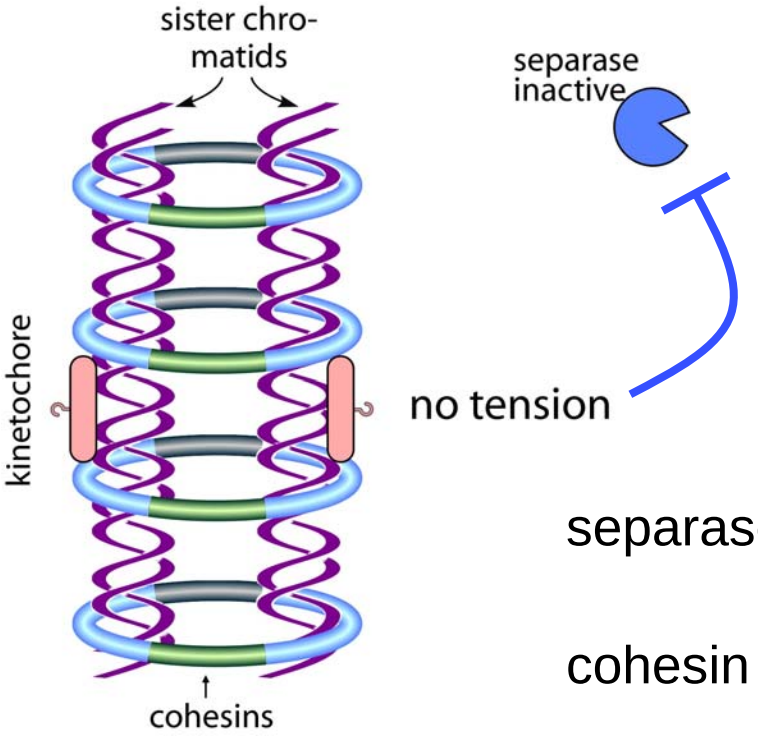
⊥

cohesins

⊥

Sister chromatid separation

The anaphase entry checkpoint—genetic analysis



separase (non-functional) mutation*… phenotype?

cells stuck in metaphase

cohesin (non-functional) mutation*… phenotype?

premature sister separation

Double mutant phenotype?

premature sister separation!

*how to keep the strains alive? …use temperature sensitive mutants

Checkpoints

Cellular surveillance systems to monitor the integrity of the genome and of cellular structures

Enforce the correct order of execution of cellular events.

Examples:

- Chromosomes not attached to spindle → block onset of anaphase
- DNA is damaged → halt the cell cycle to allow repair
- Irreparable DNA damage → trigger cell death

A tiny practice question

The haploid chromosome number in honey bees is 16. Male honey bees are haploid while females are diploid. A single cell isolated from a bee's body was found to have 32 double-stranded DNA molecules. Was the cell from a male, a female, or is it not possible to make a definite conclusion from the information given? Explain BRIEFLY.