A T coding or sense strand

mRNA

RNA polymerase

template strand
The triplet code
3 bases = 1 amino acid

Punctuation: sta AUG = methionine, the rt: first amino acid in (almost) all proteins sto UAA, UAG, and UGA.

p:

NOT an amino aci
The triplet code
3 bases = 1 amino acid

Punctuation: sta AUG = methionine, the rt: first amino acid in sto (almost) all proteins p: UAA, UAG, and UGA.

overlapping etc.
mRNA
The Genetic Code: Who is the interpreter? Where’s the dictionary? What are the rules of grammar?

tRNA = transfer RNA

amino acid

met

3’

tRNA

charged tRNA

anticodon

5′UAC3′ mRNA
The genetic code
The **ribosome**: mediates translation

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

After the 1st two tRNAs have bound...
the ribosome breaks the Met-tRNA bond; Met is instead joined to the second amino acid

\[ \text{ribosome} \]

\[ 5' \ldots \text{AUAUGACUUCAGUAACCAUCUAA} \ldots 3' \]
the ribosome breaks the Met-tRNA bond; Met is instead joined to the second amino acid and the Met-tRNA is released... 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.
5' ...AUUAGACUUCAGUAACCAUCUAAC... 3'
5’ ...AUUGACUUCAGUAACCCCAUCUAACA... 3’
When the ribosome reaches the Stop codon... termination

5’...AUAUGACUUCAGUAACCAUCUAACA...3’
The finished peptide!

\[ \text{NH}_3^+ \text{Met} \text{Thr} \text{Ser} \text{Val} \text{Thr} \text{Phe} \text{COO}^- \]

\[ 5' \ldots \text{UAUGACUCUUCAGUAACCAUCUAAACA} \ldots 3' \]
Coupling of transcription and translation

... in prokaryotes, like E. coli.

mRNAs covered with ribosomes

DNA
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 the first AUG encountered.

Start counting AUG triplets from this base:

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
- what might the presence of introns do to our virtual translation?
Identifying ORFs in DNA sequence

Coding or sense strand of DNA

Template strand of DNA

mRNA

...GGATATGACTTCAGTAACCATCTA
...CCTATACTGAAGTCATTGGTAGAT

Template strand of DNA

mRNA

...GGATATGACTTCAGTAACCATCTA
...CCTATACTGAAGTCATTGGTAGAT

STOP

STOP

STOP
Looking for ORFs

Practice question
Practice questions

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

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

cbdryloiaucahjdhtheflybitthedogbutnotthecatjhhajctipheq
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.

![DNA diagram]

(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
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 Scbolt-Leopold*1

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

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

\[\text{human} \text{ cervical cancer cells use } \text{Ca}^{2+} \text{ signalling, protein}\]
\[\text{protein tyrosine phosphorylation and MAP kinase in regulatory}\]
\[\text{volume decrease}\]

Hyperexpression of Mitogen-activated Protein Kinase in Human Breast Cancer


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

Maria T. Abreu-Martín, † Ajai Chari, ‡ Andrew A. Palladino, † Noah A. Craft, ‡§
and Charles L. Sawyers ‡§
Some commonly used model organisms

- Escherichia coli
- Budding yeast — *Saccharomyces cerevisiae*
- Round worm — *Caenorhabditis elegans*
- Fruit fly — *Drosophila melanogaster*
- Zebrafish — *Danio rerio*
- Mouse — *Mus musculus*
- 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?

How would a geneticist approach this question?

Science, 16 Dec 2005
Linking genotype & phenotype: model organisms

Mutant identified Human pedigree in a model organism segregating a trait

Protein acting in a biological process

Association studies

Sequence analysis

```
945  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009 GGA CCT TTA CGG CTT 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 TTG 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
```
A genetic approach...

Pick a model organism

Find mutant(s) with “interesting” phenotypes

Rebecca Lamason et al., Science, 16 Dec 2005
Their model organism... zebrafish

Wild type (i.e. mutant

Melanosomes in EM
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 human gene!
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 segregating a trait

Protein acting in a biological process

Association

Sequence analysis

945 ATT GTC TGT 9GC 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 TCC CTT CTT CAC CAT
1324 TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
Pick a model organism

Find mutant(s)

Map the gene that has been mutated (HD)

Identify genes in the region

Find which of these genes is the “culprit”
Pick a model organism (mouse)

Make mutant(s)

Map the gene in humans that has been mutated (HD)

Identify genes in the region

Find which of these genes is the “culprit”

what can we learn
The disease is caused by an expansion in the number of CAG codons:

- (CAG)$_{19-35}$
- (CAG)$_{36-41}$
- (CAG)$_{42-240}$

Huntingtin protein with expansion of glutamines
Huntington’s Disease

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

(CAG)\textsubscript{19-35}
(CAG)\textsubscript{36-41}
(CAG)\textsubscript{42-240}

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

Elements of cell division
- Cell growth
- Chromosome duplication
- Chromosome segregation

Mitosis (many, many times!)
Tissue differentiation

Zygote
2N

Meiosis

1N
Sperm

Egg
1N

Diploid
2N

Chromosomes condensed
Chromosomes condensed
Chromosomes condensed
Chromosome structure: coils of coils of coils…

nucleosome (histone octa)

Local unpacking of chromatin…to allow gene expression & replication

at mitosis
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)

Before | After replication

| Short arm (p) | Centromere | Long arm (q) |

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 $\rightarrow$ banding patterns (when stained)
karyotype: picture of human chromosome set from 1 individual

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

DNA replication

Mitosis

G₂

G₁

S phase
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 b
  
  Will a normal haploid cell undergo meiosis? No
Mitosis

...segregating replicated chromosomes in somatic cells

Good

Bad!

or any outcome where each daughter cell does not have exactly one copy of each homologue pair.
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
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)

Correct attachment

sister chromatids

separate inactive

no tension

kinetochore

cohesins
Monitoring correct attachment to spindle (cont’d)

Anaphase begins!
The anaphase entry checkpoint

- Unattached kinetochore
  - separase active!
  - cohesins
  - Sister chromatid separation

- No tension
- Cohesins
- Sister chromatids
- Kinetochore
The anaphase entry checkpoint—genetic analysis

separate (non-functional) mutation*... phenotype?

cohesin (non-functional) mutation*... phenotype?

Double mutant phenotype?

*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
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.