Early Genetics

- biochemical group first thought to contain genetic information =	proteins
Griffith bacterium experiment~	
smooth strain (S)	outer capsule; pathogenic
rough strain (R)	NO capsule; NOT pathogenic
Conclusion~	
R cells combined w/ killed S cells transformed into living S cells	
Avery bacterium experiment~	
- deactivated parts of dead S cells to find what transformed the cells	
Conclusion~	
DNA transforms the bacteria	
Hershey & Chase DNA experiment~	
phages reproduced in presence of DNA (not proteins)	
Conclusion~	
DNA is the genetic material	
Chargaff nucleotide experiment~	
Conclusions~	

Early Genetics (cont)

base composition varies between each species (diff. % nucleotides)
of nitrogenous bases equaled (A=T G=C)

DNA Structure

- x-ray crystallography images of DNA by:	Rosalind Franklin
↳ DNA is a helical shape	
- construction of the double helix model by:	Watson & Crick
- purines (2 rings)	A & G
- pyrimidines (1 ring)	T & C
- A pairs with T by...	2 H bonds
- C pairs with G by...	3 H bonds
- base pairs present in 1 helix turn =	10
antiparallel:	subunits run in opposite directions

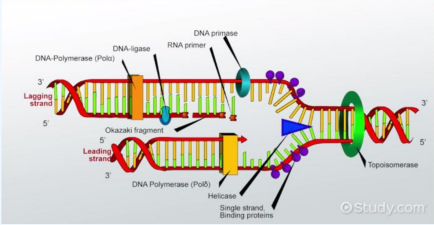
DNA Replication Experiment

- experiment done by:	Meselson & Stahl	
Prediction		
replication style	**# bands 1st rep.**	**# bands 2nd rep.**
conservative	2	2
semiconservative	1	2
dispersive	1	1
Results		
# bands 1st rep.	1	
# bands 2nd rep.	2	
conclusion =	semiconservative	

Replication Process

origin of replication:	site where the replication of DNA molecules begins
replication fork:	Y-shaped region on the replicating DNA molecule
- E. coli	
↳ 1 replication origin	
↳ 500 nucleotides/sec	
- human	
↳ 100s-1000s of replication origins	
↳ 50 nucleotides/sec	
- 2 items required to start replication:	
1. primer	2. DNA template strand
- how added nucleotides bring energy:	
↳ nucleotides carried by triphosphate	
↳ DNA polymerase catalyzes triphosphate	
↳ 2 phosphates are released	
- DNA polymerase adds to the...	3' end
	(elongates from 5' to 3')
- lagging strand created from series of...	Okazaki fragments

Replication



helicase: enzyme that unwinds & separates the DNA strands
topoisomerase: enzyme that breaks, swivels, & rejoins the DNA
primase: enzyme that synthesizes RNA primers
primer: a short sequence of RNA that starts Okazaki fragments
polymerase III: enzyme that adds nucleotides
polymerase I: enzyme that removes the primer and replaces the nucleotides
ligase: enzyme that forms the final bonds between the fragments and nucleotides

Errors in DNA

- as replication occurs, DNA polymerase finds & corrects any mistakes ---- *reducing the error rate*

- change in the DNA nucleotide is *permanent/mutation* when ---- *the pair is replicated*

- changes in DNA nucleotides due to...

↳ **1.** replication errors **2.** chemicals **3.** x-rays **4.** spontaneously

telomeres: multiple repetitions of a short nucleotide sequence at the end of a chromosome

↳ buffer zone to delay erosion of the genes as they get replicated

telomerase: enzyme that catalyzes the lengthening of telomeres (restore original length)

histone: protein responsible for the first level of packing of chromosomes

nucleosome: segment of DNA wound around a protein unit

Gene Expression Background

gene:	region of DNA expressed to produce a functional product (polypeptide/RNA molecule)
transcription:	synthesis of RNA from DNA template
translation:	synthesis of proteins from encoded mRNA
primary transcript:	initial RNA transcript from any gene (pre-mRNA)
codon:	3 nucleotide sequence that specifies a particular amino acid
- eukaryotes~	transcribe DNA to pre-mRNA
	from nucleus to ribosome
- prokaryotes~	transcribe DNA to mRNA
	from cytoplasm to ribosome

Transcription

RNA polymerase:	enzyme that controls the transcription of DNA to RNA
↳ pries DNA strands apart & joins the RNA nucleotides	
↳ moves 3' to 5'	(strand formed 5' to 3')
↳ attaches at the **promoter**	

3 STAGES OF TRANSCRIPTION

1. Initiation

- transcription factors:	protein that allows for polymerase to attach to DNA and transcribe
- 3 items to make up transcription initiation complex =	transcription factors, RNA polymerase, & promoter
- TATA box:	promoter that is 20-25 nucleotide from the starting point

* prokaryotes have NO transcription factors

2. Elongation

a. *10-20* nucleotides exposed at a time

b. nucleotides added to the *3' end* of the RNA molecule

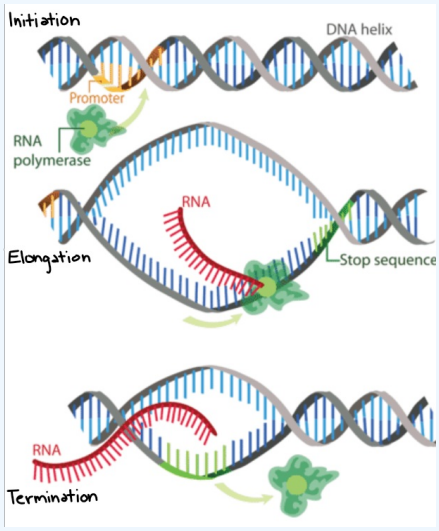
- difference between RNA & DNA nucleotides =	different sugars
- nucleotide RNA that DNA doesn't have...	*uracil*
- RNA & DNA nucleotides held together by...	*hydrogen bonds*

3. Termination

a. transcription of the *polyadenylation signal* adds nucleotides of AAUAAA to RNA

b. protein cuts the pre-mRNA from polymerase = end of process!

Transcription Diagram



Pre-mRNA Modification

- 5' end receives **5' cap**

- 3' end receives **poly-A tail** (enzyme adds 50-250 more A nucleotides)

↳ *facilitate export* from nucleus

↳ *protect mRNA* from hydrolytic enzymes

↳ *help ribosomes attach* to end of mRNA

RNA splicing:	process of removing RNA sections from pre-mRNA
- introns:	noncoding sequences of pre-mRNA
- exons:	sequences of pre-mRNA used for translation

- 3 benefits of introns:

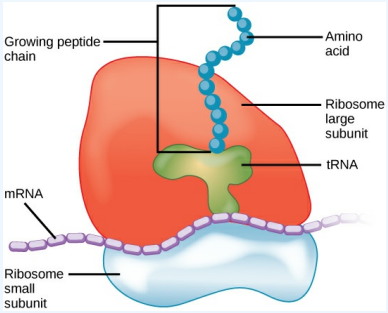
↳ make many differnet polypeptides

↳ discrete structural/functional regions

↳ increase exon shuffling (new protein function)

ribozymes:	RNA molecule that functions as an enzyme
	(create 3D structure; contain functional groups; H bond w/ DNA or RNA)

Ribosome Structure



tRNA: transfers amino acids from cytoplasm to ribosomes (& contain anticodon)

↳ **anticodon:** nucleotide triplet on tRNA molecule

↳**'wobble':** flexible base pairing at the 3rd codon position

- # of amino acids used= *20*

-makeup of a ribosome:

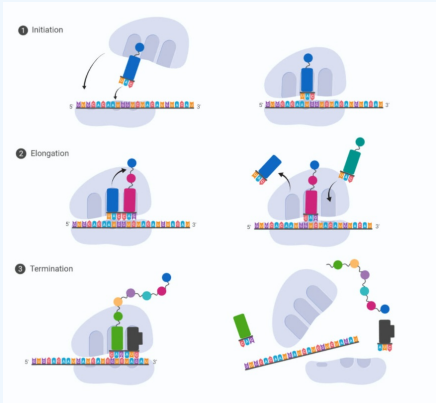
- *large & small subunit*~ made of proteins and rRNAs (eukaryotes in nucleolus & prokaryotes in cytoplasm)

Translation

3 STAGES OF TRANSLATION

1. Initiation	
a. small subunit binds to mRNA & initiator tRNA	
b. *translation initiator complex=*	attachment of large subunit (& initiation factors)
2. Elongation	
a. codon recognition-	anticodon of tRNA pairs w. mRNA codon
b. peptide bond formation-	removes polypeptide from tRNA by forming peptide bond
c. translocation-	empty tRNA released
* ribosome moves 5' to 3'	
3. Termination	
a. stop codon-	"release" factor accepted
b. hydrolysis of bond-	freeing polypeptide
c. subunits dissociate-	mRNA can be used again

Translation Diagram

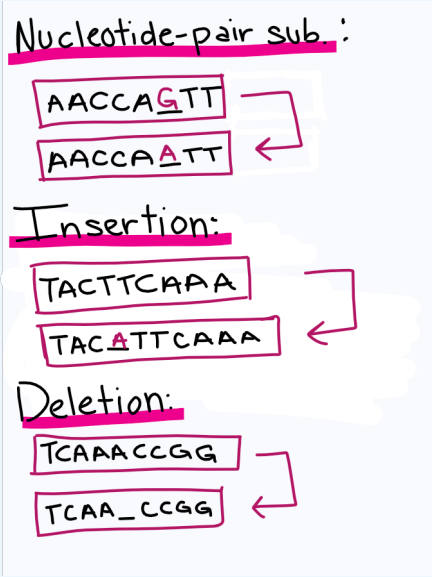


polyribosomes: series of ribosomes moving over an mRNA at the same time

chaperone protein: proteins that assist polypeptides in forming 3D structures

signal peptides: sequence of amino acids at beginning of polypeptide tagging it to where it will go

Nucleotide Mutations



point mutation: change in a single nucleotide

frameshift mutation: change in nucleotide # to not be a multiple of 3

↳ may still code for same amino acid

↳ may code for stop codon early

↳ may result in protein not functioning properly

Regulation of Gene Expression

- responds to changes in environmental conditions

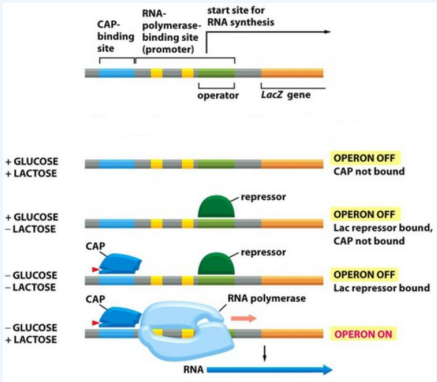
- either adjusts **activity** of enzymes present or **production** of enzymes

- 3 things to make up an *operon*:	operator; promoter; genes
operator:	segment of DNA within promoter that controls the access of RNA polymerase to the genes
repressor:	protein that binds to operator to block attachment of RNA polymerase

↳ made by activity of *regulatory gene*

repressible operon:	transcription is inhibited by small molecule binding to regulatory protein
inducible operon:	stimulated when small molecule binds to regulatory protein

Lac Operon



- *high lactose* = allolactose bind to repressor to change shape & no longer attach
- *low glucose* = high levels of cAMP combine with CAP

Differential Gene Expression

- differential gene expression = different cell types

- 3 processes of development:

1. cell division **2.** cell differentiation **3.** morphogenesis

cytoplasmic determinants: substances in the egg that influence the course of early development

induction: embryonic cells influence the development of another (change in gene expression)

homeotic genes: genes that control pattern formation as an organism develops

Biotechnology

Gel electrophoresis

- separates DNA by size and charge

- DNA negatively charges

↳ smaller segments = farther to bottom

Polymerase Chain Reaction (PCR)

- create many copies of DNA segment

↳ DNA denatured → primers added → DNA replicated

Recombinant DNA

- DNA segment put into plasmid to be reproduced

DNA Sequencing

- establish the order of nucleotides

↳ labeled with dye