**Dr. Youngman’s DNA lectures for Exam 1**

**Chromosomes**

*Defining a gene*

- units of biological information

- segments of DNA along the length of a chromosome

- responsible for phenotypes

- exits in different versions because of differences in sequence

• different versions of the same gene = alleles

• some rare alleles produce mutant phenotypes

*Anatomy of a chromosome*

- centromere

• repetitive DNA sequence at the constriction point between arms

• bound by kinetochore proteins

• where microtubules of the spindle apparatus attach

- telomeres

• repetitive DNA sequence at the ends of chromosomes

• shorten with each cell division

- p arm

• short arm

• above centromere

- q arm

• short arm

• below centromere

- band

• segment of chromosome containing many genes revealed by staining

• Giemsa staining (G staining) reveals dark bands of condensed heterochromatin

and light bands of open euchromatin

- each chromosome has a characteristic banding pattern

- light and dark bands are numbered to create a map of the chromosome

• physical position of genes can be described in terms of the band

in which they are found

• note that bands may be on either strand of DNA and may be

overlapping

*Additional chromosome terminology*

- cytogenetics: study of DNA structure inside cell nucleus

- karyotype: also called a “chromosome spread”; a means of visualizing condensed

chromosomes isolated from a cell nucleus and then stained or fluorescently

labeled. Usually karyotypes of diploid species show homologous chromosomes as pairs in numerical order.

• allows for the number of chromosomes to be determined

- sex chromosomes: in humans these are X and Y chromosomes that determine gender

- autosomes: all chromosomes other than sex chromosomes

*Chromosome number (Ploidy)*

- different species have different number of chromosomes

• related species have similar number of chromosomes

- number of versions of each chromosome (homologues) present in each cell is their

“ploidy”

• e.g. diploid = 2 versions; tetraploid = 4 versions

- n = haploid chromosome number

• how many chromosomes a cell would contain if it had a single version of every

chromosome

- humans: somatic cells are 2n; germ cells are n

- more than two complete sets of chromosomes = polyploid

- abnormal number of chromosomes = aneuploid

• occurs because of nondisjunction—failure of homologous chromosomes or

sister chromatids to separate during meiosis

• homologues separate at meiosis I

• sister chromatids separate at meiosis II

• basis for some genetic disorders, e.g. Down Syndrome

*Chromosome structure*

- bacterial chromosomes are circular

- for linear chromosomes, shape is determined by centromere position

• metacentric: centrally located centromere = arms of equal length

• sub-metacentric: centromere closer to one end = p arm length < q arm length

• acrocentric: centromere nearly at one end = p arm is very short an pinched;

creates “satellites” at the ends of chromosomes

• telocentric: centromere is at the end; no p arm

- chromosome architecture is dynamic

• chromosomes can become more or less compact, condensed

- structure is determined in part by packaging proteins that bind to DNA

• chromosomes + proteins that package them = chromatin

• eukaryotic DNA is wrapped around complexes of histone proteins

• bacterial chromosome compacts into loops

• eukaryotic chromatin can be open (euchromatin) or closed (heterochromatin)

- can switch states

- euchromatin = genes expressed; tight winding of DNA around histones

- heterochromatin = low or no gene expression; loose association

between DNA and histones

- Four key functions of chromatin

1) compacts DNA

2) stabilizes and protects DNA

3) promotes and regulates chromosome dynamics

4) regulates gene expression and DNA replication

NOTE: Armada Model of inheritance is for your reference only and will NOT be covered on the exam.

**DNA is the Genetic Material**

*Chromosome theory of inheritance*

- Sutton’s observations of grasshopper chromosomes

• visualized chromosomes during meiosis by microscopy

• put forth ideas of paternal and maternal chromosomes as well as pairing of homologous chromosomes at meiosis

- chromosomes condense and are physically manipulated during mitosis and meiosis

- chromosomes are linkage groups—subsets of genes are found on the same

chromosome and alleles may be inherited together when they are tightly linked to each other

- chromatin helps to compact DNA

• chromatin = DNA + proteins

• histone + DNA wrapped around it = nucleosome

• nucleosomes can be bunched together or spread apart to change chromatin

structure

*Discovery of DNA*

- not by Watson and Crick!

- Swiss biochemist Miescher in 1869

• isolated nuclei from white blood cells (leukocytes)

• discovered “nuclein”, a substance that was chemically distinct from proteins

*Experimental evidence suggesting that DNA is the genetic material*

- Griffith’s *S. pneumoniae* transformation

• combined heat-killed smooth, virulent S cells with rough, avirulent R cells

• S= has polysaccharide coating, R= no coating

- type II and type III S cells are possible

- type IIR can mutate to type IIS and vice versa

- no interconverting between type II and type III

• heat killed S mixed with R causes lethal infection in mice

- concludes that heat killed S “transformed” R into virulent type

- heat completely denatures proteins, so functional proteins are unlikely

to be “Transforming principle” (aka genetic material)

- Avery-MacLeod-McCarty’s systematic analysis of individual components of S cell

lysates

• homogenized heat-killed S cells

• systematically treated homogenate with enzymes targeting individual

biomolecules (e.g. lipids, proteins, RNA, DNA)

- addressed argument of whether sequences of 4 nucleotides (instead of

20 amino acids) could provide sufficient variation to account for

biodiversity

• asked if enzyme-treated cell lysate could still transform R cells (S. pneumoniae)

• DNase-treated homogenate cannot transform R cells—therefore DNA is likely

the transforming principle

- Hershey-Chase’s radiolabeling of bacteriophage

• bacteriophages are viruses that infect bacteria

- inject genome into bacterial cells

• used 35S to label bacteriophage proteins (methionine and cysteine residues)

• used 32P to label bacteriophage DNA (phosphate in backbone)

• infected bacteria with either 35S- or 32P-labeled phage then removed phage

“ghosts” by mixing samples in a blender

• asked whether radiolabel was found inside infected cells or if it was removed

by blending (so not contained within infected cells)

• found 32P but not 35S is within infected cells, suggesting that viruses

transferred their DNA to bacterial cytoplasm during infection

**DNA Replication 1**

*Chemical composition of nuclein*

- nuclein consists of nucleotides

• nucleotide = base + sugar (deoxyribose in DNA) + phosphate

- different bases

• pyrimidines = cytosine, uracil (in RNA only), thymine

- acronym = CUTE

- smaller, only one ring with 6 members

• purines = arginine, guanine

- “pure agriculture”

- larger, with 2 rings fused to each other

• other structural differences between bases include what groups are attached

to atoms that make up the ring(s)

- may be: carbonyl oxygens, amino groups, methyl groups

- nucleotides are the building blocks of DNA

*Key experiments leading to determination of DNA structure*

- Chargaff’s ratios

• based on knowledge that “nuclein” is made up of four nucleotides

• discovered that amount of A = amount of T and amount of G = amount of C

• purines are present in equal ratio to pyrimidines

- Franklin’s x-ray diffraction pattern

• irradiated crystallized DNA with x-rays

• detected X-like shape made of dashed lines

• diffraction pattern is reminiscent of a coil (e.g. spring shape)

- Watson and Crick used information from Chargaff and Franklin to propose double helix

structure for DNA

*The basis of base pairing in DNA*

- hydrogen bond donors and acceptors within nitrogenous bases

• both acceptors and donors are electronegative atoms (N or O)

• acceptors have lone pairs associated with them

• donors are covalently bonded to at least on H atom

• donors and acceptors must be physically close to each other

- common hydrogen bond donors and acceptors in biological molecules

• Donors: O-H, N-H

• Acceptors: O=C, N(R)3, some others (e.g. C-O-C)

- 3 hydrogen bonds in G-C base pairs

- 2 hydrogen bonds in A-T base pairs

*Chemistry of synthesizing a new strand of DNA*

- features of DNA structure

• double helix held together by hydrogen bonds

• two strands have opposite directionality (are “antiparallel”)

• nucleotides are covalent linked by phosphodiester bonds to create sugar-

phosphate backbone

- when added to a growing strand of DNA, nucleotides are in tri-phosphate version

(dNTPs)

• total of three phosphates attached to deoxyribose

• deoxyribose has a 3’-OH (hydroxyl group) that is capable of “attacking” alpha

phosphorous of dNTPs

- forms a phosphodiester bond

- pyrophosphate is the leaving group

- this reaction is catalyzed by DNA polymerase

*DNA polymerases*

- evolutionarily conserved

- common structural features

- different names but similar functions between polymerases in prokaryotes and

eukaryotes

- require primers to begin synthesizing new strand of DNA

- require dNTPs (high energy precursor)

- polymerase activity is directional: 5’🡪3’

- proofreading feature erases mistakes in 3’🡪5’ direction = exonuclease activity

*Features of DNA replication*

- occurs during Synthesis (S) phase of the cell cycle

• after G1 and before G2

- product of replication is sister chromatid

- pulling template apart creates a replication bubble

- two replication forks within the replication bubble

- DNA replication proceeds bidirectionally

• replication machinery can proceed from opposite directions beginning at the

Origin

• produces a “theta” structure during replication of bacterial chromosomes

**DNA Replication 2**

*Features of DNA replication (continued)*

- special cases: unidirectional replication

• mitochondrial DNA

• characteristic D-loop structure formed during replication

- both strands of the original DNA molecule are used as templates

• each template pairs with a newly made strand

• since two new “daughter” molecules of DNA each have one original strand

(that was the template) and one newly made strand, DNA replication is “semi-conservative”

- begins at an origin of replication

• one in bacterial chromosomes

- defined by consensus sequence of repeats of 13-mer and 9-mer

• many (thousands?) in eukaryotic chromosomes

• bacterial origins are made up of repeating consensus sequences of nucleotides

• eukaryotic origins are more difficult to define

• origins are recognized (bound by) DNA helicases

*Mechanism of DNA replication*

- DNA is denatured (unwound, unzipped, etc.)

• denatured by Helicase

- unwound DNA strands must be kept separated by single strand binding proteins (SSB)

- DNA synthesis begins with RNA primers made by Primase

• primers base pair with each template strand

• primer sequence is complementary to template

• primers are short

• primers are in opposite orientation to the template (as defined by 5’ and 3’

ends)

• direction of 3’ end (with 3’OH) determines direction of synthesis on each

Strand

- DNA polymerase catalyzes the formation of phosphodiester bonds

• this is DNA pol III in prokaryotes

• incoming nucleotides add to the 3’end of new DNA strand

• always proceeds in 5’🡪3’ direction with new nucleotides added to a 3’OH of

deoxyribose

• 3’OH of deoxyribose attacks phosphorous of a dNTP

slipping off of the template

• DNA pol III “processivity” is enhanced by clamp proteins that help keep it from

- Leading strand is synthesized continuously

• single RNA primer oriented with 3’OH toward direction of moving replication

fork is all that is required for synthesis of the entire strand

• synthesis follows the direction of fork movement

• new nucleotides incorporated into new strand as soon as more template is

revealed as the fork progresses

- Lagging strand is synthesized discontinuously

• synthesis of this strand proceeds opposite the direction of fork movement

• 3’OH of primers points away from replication fork

• only short segments of DNA can be made at a time since new RNA primers are

laid down by Primase only as new template sequence is revealed by the

progression of the replication fork

- short segments of DNA are Okazaki fragments

- DNA polymerase I removes RNA primers and replaces them with DNA

• same enzymatic activity as DNA pol III (5’🡪3’ polymerase and 3’🡪5’

exonuclease

• cannot ligate Okazaki fragments

- DNA ligase connects Okazaki fragments

• catalyzes formation of phosphodiester bond

*Additional points about DNA replication*

- Symmetry about the origin of replication

•  bidirectional replication means that synthesis of new strands begins in

opposite

directions, beginning from the origin of replication

• RNA primers anneal to DNA on either side of the origin

• two sets of DNA replication machinery (the “replisome”) function at the same

time

- unwinding DNA results in kinks

• kinks = “supercoiling”

• supercoils are relieved by topoisomerase

- topo cuts one segment of DNA and allows another segment to pass

through the gap

- topo cleaves phosphodiester bond in the DNA backbone

- cut DNA is resealed once other segment is allowed to pass through

- end replication problem at the end of chromosomes

• once the last RNA primer is removed from the lagging strand there is no 3’OH

is available to begin reaction to fill missing nucleotides

• neglecting to copy this portion of the chromosome leads to loss of length of

the DNA molecule with each replication cycle

• telomeres are repetitive sequences at the ends of chromosomes that protect

from loss of genetic information

- evolutionarily conserved

- many species have similar telomere sequences

• telomerase is an enzyme the extends the lagging strand

- recognizes telomere sequence through an RNA template that it carries

- RNA template anneals to overhang in lagging strand and telomerase

lengthens the template strand

- RNA primer can now anneal to extended template strand and DNA

polymerase can make the final Okazaki fragment of the lagging strand

**Mutation and DNA Repair 1**

*Role of mutation in biology*

- random, spontaneous changes in DNA sequence are possible

- responsible for genetic and phenotypic variation

- may produce traits that confer fitness advantage

- new traits are subjected to natural selection and adopted over time

- some mutations produce detrimental phenotypes associated with loss of fitness and

disease

• fitness = ability to produce viable progeny

• inherited diseases are due to mutations that are present in all cells, including

the germline

• mutations in non-germline cells (somatic cells) are not heritable

*Base mispairing*

- one base is substituted for another

- “point mutations”

- spontaneous during DNA replication

- attributable to tautomerization of bases

- tautomers = transient, rare forms of bases where a hydrogen atom switches positions

• nitrogen in ring of A or C gain a hydrogen from an amino group

• carbonyl oxygen gains a hydrogen to become hydroxide in G and T

- tautomerization leads to non-standard (non-Watson-Crick) base pairing

• e.g. tautomer of C base pairs with A

- leads to transitions or transversions

• transition: when a purine is switched for another purine or a pyrimidine is

switched for another pyrimidine

• transversion: when a purine is switched for a pyrimidine or vice versa

*Insertions and deletions arise from looping out/slipped mispairing*

- typically occurs in stretches with single or double nucleotide repeats

- looping out of nucleotide in daughter strand during DNA replication = insertion

- looping out of one or more nucleotides in template strand during DNA replication =

deletion

*Consequences of base mispairing and indel mutations*

- substitutions may or may not lead to change in amino acid sequence of protein for

which gene codes

• depends on where in the codon mutation occurs (i.e. first, second, or third

nucleotide of the codon)

• silent mutation: no change in amino acid

• missense mutation: different amino acid is incorporated into protein

• nonsense mutation: results from a amino acid codon that is converted to a

STOP codon, causing ribosome to stop translating mRNA = shorter protein (truncation) produced

- insertions or deletions may lead to a frameshift

• changes “window” of triplet nucleotides (codons) read by the ribosome

• results in changing all amino acids in protein past where the insertion/deletion

occurred in coding sequence of gene

*Spontaneous chemical changes to DNA*

- depurination: base is cleaved from deoxyribose

• creates abasic site

- deamination: amino group cleaved from base

• may change identity of base (e.g. deaminated C becomes a U)

- bases may also be methylated or oxidized

• 5-methylcytosine is intentional—created by DNA methyltransferase enzyme

- modifications may affect hydrogen bond donors, acceptors and influence base pairing

**Mutation and DNA Repair 2**

*Role of intentionally created mutations in biology*

- use chemical or physical means to create random mutations in DNA in animals or cells

of interest

- look for phenotype of interest among progeny of mutagenized organisms

• forward genetic screen

• “wreck and check”= break the genes and see what happens

- isolate mutants with phenotype of interest then determine their genotype

• e.g. fruit flies with different eye colors (brown, purple, etc.)

• in what gene(s) were mutations made?

• which mutation(s) account for the phenotype of interest?

*Chemical mutagens*

• base analogs

- structurally similar to normal bases

- induce mispairing

- e.g. 5-bromouracil (5-BU)

• incorporated as a T and base pairs with A

• can tautomerize and base pair with G

- e.g. 2-aminopurine base pairs with T or C

• base modifying agents

- alkylating agents

- e.g. ethylmethane sulfonate (EMS)

• adds ethyl group to G or T

• intercalating agents

- skinny, flat multi-ringed molecules

- sit in between base pairs of DNA

- alter DNA structure

- many absorb and emit light (e.g. ethidium bromide)

*Physical mutagens*

- ionizing radiation

• x-rays

• causes breaks in DNA backbone

• causes abasic sites

- non-ionizing radiation

• UV light

• creates covalent bonds between sequential pyrimidines in the same strand of

DNA

*Cellular response to DNA damage*

- initiate programmed cell death through apoptosis

- continue cell cycle without attempting to repair damage

- arrest cell cycle

• allows for time to repair damage

- many types of repair, specific to type of damage

- common themes of DNA repair pathways: recognize damage, cut and

remove nucleotides, re-synthesize missing nucleotides, re-ligate backbone

*DNA repair pathways* (**from 1.31 and beginning of 2.2 lectures**)

*\* Make note of enzymes involved in each pathway and look for common themes*

- Base excision repair (BER)

• repairs damaged bases

• only the damaged base is removed

• “precise, surgical, like extracting a tooth”

• glycosylase enzyme flips bases out of helix

- recognizes damaged bases

- cuts damaged base off of deoxyribose

- Nucleotide excision repair (NER)

• repairs bulky damage that distorts helix

• repairs damage to more than one base (e.g. pyrimidine dimers)

• involves helicase to remove segment of one strand

• “remove the tumor + some healthy tissue”

• deficiency in this pathway associated with human disease

- e.g. xeroderma pigmentosum and Cockayne syndrome

- Mismatch repair (MMR)

• MutS recognizes incorrect base pairing

• similar mechanism to NER

- Single strand break repair (SSBR)

• similar to BER mechanism

- Non-homologous end joining (NHEJ  
 • to repair double strand breaks

• when there is NOT a sister chromatid available

• incompatible ends are chewed by exonuclease (removes nucleotides)

• no filling-in of missing nucleotides

• ligase reseals backbone

- Synthesis-dependent strand annealing (SDSA)

• to repair double strand breaks

• when there IS a sister chromatid available

• exonuclease removes nucleotides from end of DNA on either side of break

• signature feature is strand invasion: one strand from broken DNA molecule

base pairs with complementary strand of undamaged sister chromatid

- allows missing nucleotides to be filled in by synthesis

*Failure to repair double strand breaks results in chromosomal block mutations*

- chromosomal block mutations are large rearrangements of chromosome segments

- four major types

1) duplication: region of chromosome is repeated

2) deletion: region of chromosome is omitted

3) inversion: some segments of chromosome are in reversed order

4) translocation: piece of one chromosome is spliced on to another

- associated with transformation = cell becomes cancerous

- may result in aneuploidy

- more common in cells with deficient DNA repair

**PCR and Recombinant DNA 1**

*Tools of DNA replication and repair are used in genetic engineering*

- used to produce recombinant DNA = modifying a DNA molecule to contain a new

sequence that it would not normally have, including transgenes = genes that are not part of the organism’s natural genome

- introducing recombinant DNA into cells/organisms makes them transgenic

• new DNA is in vectors (plasmids) or incorporated into chromosome

*Cloning genes*

- necessary to generate quantities required for genetic engineering

- in vivo approach

• restriction enzymes randomly fragment chromosome(s)

• chromosome fragments inserted into plasmids

• plasmids inserted into bacteria for replication

• sort through plasmids to find gene of interest

- in vitro approach

• synthesize many copies of gene of interest in a reaction with purified DNA

polymerase

• uses polymerase chain reaction (PCR) to precisely and efficiently copy a

defined segment of DNA

*Polymerase Chain Reaction (PCR)*

- three portions to a cycle that is repeated 30-40 times

1) denature: high temperature melts DNA template

2) anneal: forward and reverse primers base pair to template at a lower

temperature

3) extension: DNA polymerase builds off of primers and synthesizes new strands

of DNA complementary to the template

- primers define which sequence is amplified

• short oligonucleotides

• complementary to template

• flank region to be amplified

• forward primer anneals to one template strand; revers primer anneals to other

template strand

*Restriction enzymes facilitate a cut-and-paste mechanism of inserting (cloned) DNA into vectors*

- restriction enzymes are endonucleases

- restriction enzymes recognize specific 6- or 4- base pair sequences (“restriction sites”)

• palindromic = same sequence 5’🡪3’ on both strands of DNA

- make cuts in DNA backbone between same two bases on both strands

- each enzyme has its own restriction sites

• many enzymes = many restriction sites to choose from

- isolated from bacteria

• name based on species they come from

- restriction sites may be engineered into PCR primers to yield products that can be cut

by restriction enzymes

**PCR and Recombinant DNA 2**

*Restriction enzymes facilitate a cut-and-paste mechanism of inserting (cloned) DNA into vectors (continued)*

- cutting both vector and DNA to be inserted into it with same restriction enzyme makes

for compatible ends that can base pair with each other

• cutting with two enzymes allows for directional insertion = eliminates possibility of inserted DNA pasting in to vector backwards

*Recombinant DNA in plasmid vectors is amplified in bacteria*

- features of prokaryotic plasmids used for cloning genes

• origin of replication: facilitates replication

• antibiotic resistance gene: forces bacterial cells to maintain the plasmid under

selective pressure of growth in medium containing antibiotic

• multiple cloning site: segment of plasmid with many restriction sites where

gene of interest will be inserted

• marker gene: to tell if gene of interest has been inserted

- e.g. gene that codes for beta-galactosidase is disrupted by insertion of

gene of interest = enzyme no longer functional

*in vivo cloning*

- restriction enzyme(s) used to cut chromosome into fragments

- fragments inserted into vectors (plasmid)

- plasmids introduced into bacteria for amplification (replication)

• each bacterial cell should take up only one plasmid, so has only one

chromosomal fragment

- requires screening to find bacterial colony with the correct DNA sequence

- screening may use labeled DNA probes that are complementary to sequence of

interest inserted into plasmid

*Example applications of creating transgenic organisms*

- transgenic bacteria used to produce insulin

• human insulin inserted into bacterial plasmid

• gene encoding insulin is expressed in bacteria

• bacterial-produced insulin can be isolated and purified

• basis for Humulin = first approved drug to use recombinant DNA

- use of green fluorescent protein (GFP) to study gene expression and protein function

• GFP is a natural gene in genome of A. Victoria jellyfish

• GFP coding sequence inserted into vectors

• can be used for reporter constructs or translational fusions

- reporters: promoter of gene of interest drives GFP expression

- translational fusion: full length gene encoding a protein placed in front

of GFP to create a fusion protein (protein “tagged” with GFP)

• can determine where and when protein of interest is expressed

*Creating transgenic organisms through genome editing using CRISPR*

- chromosome itself is altered

- DNA sequence is added to or removed from endogenous genetic locus

- makes use of Cas9, a programmable endonuclease