1 ## Molecular Basis of Inheritance

Chapter 16

2 ## Search for Genetic Material

- Looking for a molecule that could be specific and show great variation
- Molecule needs to be abundant
- Needs to be able to be copied precisely
- What is your guess based on these requirements?

3 ## Evidence of Genetic Material

- Griffith looking for vaccine against Streptococcus pneumoniae
- 2 strains: S-smooth colonies; R-rough
- S are encapsulated with polysaccharide coat
- Alternative phenotypes (S and R) are inherited

4 ## Griffith Experiment

- Injected live S strain into mice: mice died of pneumonia (S is pathogenic)
- Injected live R strain into mice: mice healthy (R is nonpathogenic)
- Mice injected with heat killed S: mice healthy
- Mice injected with heat killed S mixed w live R cells: mice died
- Blood samples from dead mice contained live S cells: R cell acquired from dead S cells ability to make coats TRANSFORMATION

5 ## Transformation

6 ## Implications

- Transformation: assimilation of external genetic material by a cell
- Not a protein-heat denatures proteins but heat did not destroy the transforming ability of the genetic material in the heat killed S cells
- Later Avery, McCarty, and MacLeod discovered transforming agent was DNA

7 ## Evidence of Viral DNA

- Bacteriophage (phage): virus that infects bacteria
- Alfred Hershey & Martha Chase DNA genetic material of phage T2
- Virus was DNA and a protein coat
- Protein tagging: T2 and E. coli were grown
- DNA tagging: T2 and E. coli were grown in media w 32 P

8 ## Phage structure

9 ## Hershey and Chase

- Protein labeled infected E. coli
- DNA labeled infected separate E. coli
- Mixtures were agitated to break loose phage coats from bacteria
- Mixtures were centrifuged; cells in the pellet; viruses in the supernatant

- S labeled in supernatant
- P labeled in the pellet
- Bacteria P labeled released viruses w P

10 ## Hershey and Chase's Method

11 ## Conclusions Hershey & Chase

- Viral proteins remain outside the host cell
- Viral DNA injected into host cell
- Injected DNA molecules cause cells to produce additional viruses w more viral DNA and proteins
- Nuclei acids not proteins are hereditary material

12 ## Chargaff's Experiment

- Analyzed DNA of different organisms
- DNA composition is species specific: amount and ratios of nitrogenous bases vary from one species to another
- Adenine residues equaled number of thymines; cytosines equaled number of guanines
- Chargaff's rules A=T; G=C
- This molecular diversity supports DNA as hereditary material

13 ## Circumstantial Evidence for DNA

- Eukaryotic cell doubles DNA content prior to mitosis
- During mitosis, the doubled DNA is equally divided btwn 2 daughter cells
- Organism's diploid cells have 2x DNA as haploid gametes

14 ## Watson, Crick, & Franklin

- Working on 3D structure
- Wilkins fed Watson and Crick Franklin's X ray of DNA crystal
- Watson and Crick deduced:
- Helix w uniform width of 2 nm
- Purine and pyrimidine bases stacked .34 nm apart
- Helix makes 1 full turn 3.4 nm
- There are 10 layers of bases in ea turn

15 ## DNA Structure

- Tried sugar phosphate chains on inside no go
- On outside, hydrophobic interactions of nitrogenous bases pushed them to inside
- Ladder twisted into a spiral
- 2 sugar phosphate backbones of the helix are antiparallel; they run in opposite directions

16 ## One strand of DNA

17 ## DNA rungs

- Pair of nitrogenous bases
- Purine must pair w pyrimidines to get .34 nm
- W Chargaff, A purine + T pyrimidine
- G purine + C pyrimidine
- Suggests mechanisms for DNA replication
- Sequences of bases highly variable allowing specificity for genetic coding
- Hydrogen bonds and van der waals stabilize DNA

18 ## DNA Replication

- Watson & Crick proposed genes on original DNA strand are copied by specific pairing of complementary bases, creating a complementary strand
- Complementary strand can function as template to produce a copy of original strand
- 2 strands separate each acts as template for complementary strand
- Enzymes link nucleotides together at sugar-phosphate groups

19 ## 3D models

20 ## Meselson and Stahl

- 3 hypotheses
- Conservative: parental double helix remain intact and second DNA molecule entirely new molecule
- Semiconservative: each DNA molecules should be composed of one original & one new strand
- Dispersive: both strands of newly produced DNA molecules should contain mix of old and new DNA

21 ## Meselson & Stahl Experiment

- Grew E coli on medium w 15N (heavy nitrogen)
- Transferred to medium w 14N
- 1st generation DNA extracted from E coli after on generation of growth in light medium
- 2nd generation DNA extracted from E coli after 2 replications in light medium
- Isolated DNA was mixed w CsCl & centrifuged
- Centrifugal force created CsCl gradient w >conc at bottom; DNA moved to place density matched density of CsCl

22 ## Meselson & Stahl Method

23 ## Results Meselson & Stahl

- Parents: 1 distinct band / tube
- 1st generation 1 distinct band near center
- 2nd generation 2 bands one near center other light

24 ## Conclusions: Meselson & Stahl

- 1st generation all hybrid: semiconservative model
- 1st generation eliminated conservative, but not dispersive
- 2nd generation eliminated dispersive; only one band would have occurred if dispersive replication

25 ## Semiconservative Replication

26 ## DNA Replication

- Helical molecule must untwist (helicase) while it copies its two antiparallel strands simultaneously
- Requires 2 dozen enzymes and other proteins
- Prokaryotes: 500 nucleotides/sec
- Few hours to copy 6 billion bases of single human cell
- Accurate: 1 in a billion nucleotides is incorrectly paired

27 ## Enzymes for Replication

28 ## Origins of Replication

- DNA replication begins at sites called origins of replication that have a specific sequence of nucleotides
- Specific proteins required to initiate replication bind to origin
- DNA double helix opens at origin and replication forks spread in both directions away from point form replication bubble
- Prokaryotes one origin; eukaryotes thousands

29 ## Elongating a new strand

- Helicases are enzymes which catalyze unwinding of parental double helix
- Single strand binding proteins keep strands apart and stabilize the unwound DNA until new strand can be synthesized
- DNA polymerases catalyze synthesis of a new DNA strand
- New nucleotides align on template of old
- DNA polymerase links nucleotides to growing strand; only grow from 5' to 3' only add to 3'

30 ## Replication is endergonic

- Requires energy
- Nucleoside triphosphate is source
- Covalently linked to 5' carbon of pentose
- Nucleoside triphosphate lose 2 phosphates form covalent linkages to the growing chain
- Hydrolysis of phosphate bond drives synthesis of DNA

31 ## Antiparallel

- Continuous synthesis of both DNA strands is not possible due to the antiparallel construction
- Can only elongate from 5′ to 3′
- Continuous synthesis occurs on the leading strand which is 5′ to 3′
- The lagging strand (complementary strand) has discontinuous synthesis
- Lagging strand produced as a number of short segments called Okazaki fragments

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Replication of antiparallel strands

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Okazaki Fragments

- Synthesized in 5′ to 3′ direction
- Fragments are 1000-2000 nucleotides in length in bacteria and 100-200 nucleotides long in eukaryotes
- Fragments are ligated by DNA ligase, linking enzyme that catalyzes formation of a covalent bond between the 3′ end of each new fragment and the 5′ end of the growing chain

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primer

- Primer is a short RNA segment that is complementary to DNA segment & that is necessary to begin DNA replication
- Primers are polymerized by an enzyme called primase
- Portion of parental DNA serves as template for primer w a base sequence that is about 10 nucleotides long in eukaryotes
- Primer formation must precede DNA replication, DNA polymerase only add nucleotides to a poly nucleotide that is already correctly base-paired w complementary strand

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primers

- Only one is needed for leading strand
- Thousands are needed for lagging strand
- RNA primer must initiate the synthesis of each Okazaki fragment
- Fragments are ligated in 2 steps to produce a continuous DNA strand
- DNA polymerase removes the RNA primer and replaces it w DNA; DNA ligase catalyzes linkage
- Between 3′ end of each fragment & 5′ of chain

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Enzymes repair damage

- Initial pairing errors occur at a frequency of 1 in 10K
- DNA can be repaired as it is being synthesized: mismatch repair DNA polymerase proofreads each newly added nucleotide against its template; if incorrect removes and replaces it (eukaryotes have proteins too to proofread)
- Excision repair: accidental changes in DNA can result from exposure; 50 different DNA repair enzymes; one excises and gap filled by base-pairing by DNA polymerase and DNA ligase

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Mismatch repair

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Repair Significance

- The importance of proper function of repair enzymes is clear from the inherited disorder xeroderma pigmentosum.
  - These individuals are hypersensitive to sunlight.
  - In particular, ultraviolet light can produce thymine dimers between adjacent thymine nucleotides.
  - This buckles the DNA double helix and interferes with DNA replication.
  - In individuals with this disorder, mutations in their skin cells are left uncorrected and cause skin cancer.

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Telomere replication

- Limitations in the DNA polymerase create problems for the linear DNA of eukaryotic chromosomes.
- The usual replication machinery provides no way to complete the 5′ ends of daughter DNA strands.
  - Repeated rounds of replication produce shorter and shorter DNA molecules

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Telomere

41

- The ends of eukaryotic chromosomal DNA molecules, the **telomeres**, have special nucleotide sequences.
  - In human telomeres, this sequence is typically TTAGGG, repeated between 100 and 1,000 times.
- Telomeres protect genes from being eroded through multiple rounds of DNA replication.

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- Eukaryotic cells have evolved a mechanism to restore shortened telomeres.
- **Telomerase** uses a short molecule of RNA as a template to extend the 3′ end of the telomere.
  - There is now room for primase and DNA polymerase to extend the 5′ end.
  - It does not repair the 3′-end "overhang," but it does lengthen the telomere.

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Telomerase

- Telomerase is not present in most cells of multicellular organisms.
- Therefore, the DNA of dividing somatic cells and cultured cells does tend to become shorter.
- Thus, telomere length may be a limiting factor in the life span of certain tissues and the organism.
- Telomerase is present in germ-line cells, ensuring that zygotes have long telomeres.
- Active telomerase is also found in cancerous somatic cells.
  - This overcomes the progressive shortening that would eventually lead to self-destruction of the cancer.