1. The search for genetic material lead to DNA

- Once Morgan’s group showed that genes are located on chromosomes, the next question was’”

- Is it the DNA or the proteins in chromosomes that is the “stuff of life”?
Explain how contributions from each of the following scientists led to an understanding of DNA structure and function:

- Griffith
- Avery McCarty & McLeod
- Hershey & Chase
- Erwin Chargaff
- Watson, Crick, Franklin, & Wilkins
• Frederick Griffith in 1928 started us on the right track.
• Here’s what he did.
• Griffith called this phenomenon transformation, but he never determined what the “stuff” was.

• Give me a quick interpretation of the results of each situation below. Justification?
• In 1944 Avery, MacLeod, and McCarty performed transformation experiments using live, harmless bacteria and extracts from virulent bacteria treated with various enzymes. They used Proteases and RNAases to rule out protein and RNA as the transforming factors.

• Still, many biologists were skeptical.

• Why, do you figure?
In 1952, Alfred Hershey and Martha Chase at Cold Spring Harbor showed that DNA was the genetic material by using a neat looking virus.
• Watch here to see what they did.

• Elegant, eh?
• Here it is in diagram form.

**Fig. 16.2b**

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• By 1947 (before Hershey and Chase), Erwin Chargaff figured out this significant tidbit about DNA:

• A=T and C=G
• This peculiar regularity in the ratios of nucleotide bases is known as Chargaff’s rule.

• Human DNA, for example, is 30.9% adenine, 29.4% thymine, 19.9% guanine and 19.8% cytosine.

• Why aren’t they exactly the same?
Politics and Science often mix.

- By the beginning of the 1950’s, the race was on to figure out the structure of DNA.
- Everyone knew there was a dynamite prize for the winner.
  - Among the scientists working on the problem were Linus Pauling, in California, and Maurice Wilkins and Rosalind Franklin, in London, at the Cavendish Lab at Cambridge University.
  - Cold war politics kept Pauling from becoming a bigger star.
DNA has two main jobs:

- What do you figure they are?
- Any structure these researchers proposed, then, must be one that is able to perform these functions.
Essential knowledge 3.A.1: DNA, and in some cases RNA, is the primary source of heritable information.

Evidence of student learning is a demonstrated understanding of each of the following:

1. Genetic information is stored in and passed to subsequent generations through DNA molecules and, in some cases, RNA molecules.
• Diagram a molecule of DNA and explain how its features allow for both heredity and protein synthesis.
• Explain the relationship between DNA, RNA, Protein, Cells and the Organism.

Diagram the process of DNA replication. Discuss all inputs, processes, and outputs. Explain the roles of all pertinent enzymes.
• Rosalind Franklin took this famous picture which stirred up quite an ethical storm.
Here’s a bit of what the picture means

• The gap between the second and third bands from the center told them it was a *double* helix, not triple like they and Linus Pauling had thought.

• The angles between the bars told them the diameter, the distance from side to side; this was a key clue.

• I learned this from Dr. Hughson at Princeton, a smart guy who does this kind of stuff in his lab.
Watson and his colleague Francis Crick began to work on a model of DNA with two strands, the double helix.

Much of their brainstorming sessions took place at their favorite lab, The Eagle, with their colleague, The Abbot.
THE EAGLE

Our History

The site was bequeathed to Corpus Christi College in 1525. The inn was first mentioned as a commercial activity (then called 'Eagle & Child') in 1667. The Rutland Club founded in 1728 by John Mortlock, set up its headquarters in The Eagle in the 18th. During their research into 'DNA' in the early 1950's, Watson & Crick used The Eagle as a place to relax & discuss their theories whilst refreshing themselves with ale. In 1988 a major restoration was carried out by Corpus Christi & Greene King, & opened as it is now in 1992. Special interest is the 'RAF Bar' ceiling covered with the names & squadron no's of RAF & USAF airmen in World War II.
This is the famous scale model they built to literally solve the puzzle.
Latest (2012) image of DNA taken by Enzo DiFabrizio in Italy. This is a few strands.
• The key breakthrough came when Watson put the sugar-phosphate chain on the outside and the nitrogen bases on the inside of the double helix.

• The sugar-phosphate chains of each strand are like the sides of a rope ladder.

• Pairs of nitrogenous bases, one from each strand, form rungs.

• Notice how we use the term “strand” in a different way than when describing chromosomes. Don’t be confused!!!
Fig. 16.5
Knowing the basic structure of the bases, they concluded these bonding arrangements.

This finding explained Chargaff’s rules.
Other Details of DNA Structure That Someone May Ask You At, Like, A Party Or Something…

• purine -pyrimidine pairs. Which bases are which?
• A nucleotide is ?????
• The bases are connected to the ?????, not the ????
• What are phosphodiester bonds, or 3’- 5’ linkages?
• And just what is anti-parallel?
• But watch out for this Biologist pick up line - I wish I was adenine, then I could get paired with U.
• The sequence of the four bases can be varied in countless ways, meeting the requirement of carrying large amounts of information.

• Base pairing allowed for a way of another requirement, accurate replication, as we will see.

• In April 1953, Watson and Crick published a succinct, one-page paper in Nature reporting their double helix model of DNA.

• Do we have time to read it and figure out the structure from it?

• How about a neat paper model? See it here.

1. Both RNA and DNA have three components — sugar, phosphate and a nitrogenous base — which form nucleotide units that are connected by covalent bonds to form a linear molecule with 3' and 5' ends, with the nitrogenous bases perpendicular to the sugar-phosphate backbone. iv. The two DNA strands in double-stranded DNA are antiparallel in directionality.
iii. DNA is usually double stranded, RNA is usually single stranded.
iv. The two DNA strands in double-stranded DNA are antiparallel in directionality.

3. Both DNA and RNA exhibit specific nucleotide base pairing that is conserved through evolution: adenine pairs with thymine or uracil (A-T or A-U) and cytosine pairs with guanine (C-G).

i. Purines (G and A) have a double ring structure.

ii. Pyrimidines (C, T and U) have a single ring structure.
1. During DNA replication, base pairing enables existing DNA strands to serve as templates for new complementary strands

- In a second paper Watson and Crick published their hypothesis for how DNA replicates.
  - All of DNA’s functions revolve around complementary base pairing: only A to T and C to G.
  - What does “template” mean?
When a cell copies a DNA molecule, each strand serves as a template for the making of the other.
• Watson and Crick’s model, *semiconservative replication*, meaning what????

• Other competing models, the conservative model and the dispersive model, were also proposed.

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**Fig. 16.8**

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• Experiments (more of those elegant ones) in the late 1950s by Matthew Meselson and Franklin Stahl supported the semiconservative model, proposed by Watson and Crick, over the other two models.

• Watch now
Fig. 16.9
Did you watch this guy last night??
• Diagram a molecule of DNA and explain how its features allow for both heredity and protein synthesis.
• Explain the relationship between DNA, RNA, Protein, Cells and the Organism.

Diagram the process of DNA replication. Discuss all inputs, processes, and outputs. Explain the roles of all pertinent enzymes.
2. A large team of enzymes and other proteins carries out DNA replication

- More than a dozen enzymes and other proteins participate in DNA replication. Let’s watch these two, in order, and then come back to notes. Watch now

The names of the steps and particular enzymes involved, beyond DNA polymerase, ligase, RNA polymerase, helicase and topoisomerase, are outside the scope of the course for the purposes of the AP Exam.
DNA replication ensures continuity of hereditary information.

i. Replication is a semiconservative process; that is, one strand serves as the template for a new, complementary strand.

ii. Replication requires DNA polymerase plus many other essential cellular enzymes, occurs bidirectionally, and differs in the production of the leading and lagging strands.
• The replication of a DNA molecule begins at a specific sequence of nucleotides called origins of replication.

• Enzymes separate the strands, forming a replication bubble.

And https://www.youtube.com/watch?v=ef6EyTcD_pUis one shows it more as it really happens (1 min.) – all enzymes are part of a non-moving complex called a replisome. This would be very hard to show in a diagram, hence the traditional bubble. 6 min. one

Now back to the notes.
In eukaryotes, there may be hundreds or thousands of origin sites per chromosome.

At the origin sites, the DNA strands separate forming a replication “bubble” with replication forks at each end.

The replication bubbles elongate and eventually fuse as the DNA is replicated.
The raw nucleotides are nucleoside triphosphates.

- They are like bricks that carry with them the energy source to cement them to other bricks to make a wall.
• The exergonic hydrolysis of pyrophosphate to two inorganic phosphate molecules drives the polymerization of the nucleotide to the new strand.
What does the antiparallel thing have to do with it?
• Ever heard of the little train that could?
• Let’s draw a whole bubble.
• What are primers?? Primase???
Primase joins RNA nucleotides into primer.

Single-stranded region of parental DNA

Primase

DNA polymerase adds DNA nucleotides to primer.

RNA primer

Newly made DNA

A different DNA polymerase replaces the RNA with DNA.

Newest DNA

DNA polymerase

This daughter strand is now complete.
• Review the enzymes involved.

Fig. 16.15
Oops! Let’s fix the twisting problem.

- [https://www.youtube.com/watch?v=ef6EyTcDPUR](https://www.youtube.com/watch?v=ef6EyTcDPUR) pUr’s how that works.

- So add topoisomerase (gyrase) to that list of enzymes involved in replication.
3. Enzymes proofread DNA during its replication and repair damage in existing DNA

- What if there is a mistake???
• Reactive chemicals, radioactive emissions, X-rays, and ultraviolet light can change nucleotides in ways that can affect encoded genetic information.

• Each cell continually monitors and repairs its genetic material, with over 130 repair enzymes identified in humans.  

• This feature gives both the molecule and the message stability.
4. The ends of DNA molecules are replicated by a special mechanism

• 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.
Fig. 16.18

Parental DNA

RNA priming and DNA synthesis

Leading strand

RNA primer

Lagging strand

Removal of primers and filling of gaps with DNA where a 3′ end is available

Gap remains unfilled

Further rounds of replication

Shorter and shorter daughter molecules
• 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.

Fig. 16.19a
What do telomeres do?

- They protect the chromosomes.
- They separate one chromosome from another in the DNA sequence.
- Without telomeres, the ends of the chromosomes would be "repaired", leading to chromosome fusion and massive genomic instability.
Telomere function, cont’.

- Telomeres are also thought to be the "clock" that regulates how many times an individual cell can divide. Telomeric sequences shorten each time the DNA replicates.
Think of it like this…. 

- Telomeres effectively "cap" the end of a chromosome in a manner similar to the way the plastic on the ends of our shoelaces "caps" and protects the shoelaces from unraveling.
How are telomeres linked to aging?

- Once the telomere shrinks to a certain level, the cell can no longer divide. Its metabolism slows down, it ages, and dies.
Healthy human cells are mortal because they can divide only a finite number of times, growing older each time they divide. Thus cells in an elderly person are much older than cells in an infant.

• 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.

![Diagram of telomere extension](Fig. 16.19b)

1. Telomerase extends the 3’ end of a DNA strand.

2. The other strand is extended in the usual way by primase, DNA polymerase, and ligase.

3. The result is a longer telomere with a 3’-end "overhang."

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• 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 is pretty good.
  • This overcomes the progressive shortening that would eventually lead to self-destruction of the cancer.