1. Avery, MacCleod and McCarty first reported that DNA purified from a smooth strain of "pneumococcus" could "transform" avirulent rough strains to virulent smooth strains.

Approximately when were these observations reported? 1940s (44)

Many were not convinced by their conclusion. Why?

Most thought DNA with just 4 "bases" could not convey enough information to make protein with 20 amino acids, in part because DNA had been reported to contain 25% of each base, in a repeating order such as (AGCT). Also, there was real doubt that all the proteins were eliminated from the DNA preparation, and that those still bound would be the genes.

2. a) Identify the base or area represented by each number:

1  A
2  G
3  T
4  C
5  H-bonds
6 deoxyribose

b) which numbers are purines?
   1 & 2 (note that pair with bonds is GC, so the double ring is G, etc.

c) label the 5' and 3' ends
3) Check each of the following that is an amino acid encoded in mRNA:

- tryptophan
- tyramine
- thiamin
- thymine
- glycine
- glucose
- ornithine
- pyrolysine
- histamine
- histone
- phenylalanine

4) Amino acid 20 in the normal tryptophan synthase β-subunit from *E. coli* is isoleucine. A mutant with no enzyme activity was recovered. Revertants with full or partial activity were recovered and found to have ile, ser, gly or thr.

![Amino acid structure diagram]

- ile AUA
- mutant AGA (arg)
- revertants AUA AGC/U GGA ACA

a) So far as possible show the most likely codon for each blank.

b) What type of change occurred in the DNA to cause the inactive mutant and revertants 2, 3 and 4?

- transversion (U to G)
- transversion (A to Py)
- transition
- transversion (A to G)
- transversion (G to C)

5) In honor of Erwin Chargaff (he died just last year!) explain his contribution to our knowledge of DNA structure.

- That the amounts of A, T, G and C in DNA is species-specific but that in all cases the amount of A = T and G= C.

6) Is there any significance to the fact that "ori" regions are AT rich? Justify your answer.
Yes, AT base pairs are easier to "melt" since they only contain 2 hydrogen bonds and being able to separate the strands is a key to starting replication.

7) Draw a very simplified (no loops required) picture of a DNA replication fork showing the various component proteins of the replication complex; list the function of each component.

Your picture should include:
- helicase to unwind the strands,
- Topoisomerase to relieve supercoiling,
- SSBs (single stranded binding proteins to protect unwound DNA
- primase to insert RNA primers
- DNA polymease III or equivalent to extend the primers
- DNA polymerase I or equivalent to replace RNA bases with DNA bases
- Ligase to connect the Okazaki Fragments that occur in the lagging strand

8) Track the sources of energy required for each peptide bond formed during translation.

ATP is converted to AMP in order to connect each amino acid to its tRNA in a high energy bond (two phosphates are used)
- 1 GTP is spent getting each tRNA onto the ribosome
- 1 GTP is spent moving the mRNA etc. after each peptide bond is formed

9) When Holley first deduced the sequence of a tRNA (tRNA<sub>ala</sub>) in 1965, what is now clearly the anticodon loop was not so clear then because it contained an I, instead of one of the standard bases. What is the significance of an I in an anticodon, show where is it and tell how (in general) it got there.

The I occurs in the third (Wobble) codon/anticodon pairing position where it can pair with U, C or A. This means for example that 1 tRNA can decode all the isoleucine codons.

It was put there by post-transcriptional modification. (an Adenosine deaminase converts A to I)

10) How does initiation of translation on prokaryotic ribosomes compare to that on cytoplasmic ribosomes in eukaryotic organisms?
A sequence in the leader of prokaryotic messages (Shine-Delgarno sequence) base pairs with a part of the 16s rRNA to get the start codon in position at the P site once the whole ribosome is complete (3 IF factors needed). In eukaryotes, the CAP-binding protein, one of 9-11 eIFs required for initiation, attaches, scans the leader for a conserved sequence that includes the AUG start and aligns the start codon at the A site. A GTP is spent, moving it and the met-tRNAi to the P site.

Although both use a special initiator met-tRNA, the met is only changed to F-met in prokaryotes.

11) Diagram a "typical" eukaryotic gene; label the key components.

12) What features of *Neurospora* made it the organism of choice for Beadle and Tatum?

- it grows on a chemically defined "minimal" medium
- it produces millions of genetically identical *haploid* spores asexually
- controlled crosses could be made, even reciprocal crosses

13) Classic Hemophilia in humans results from defective clotting factor VIII. Although the gene is large (over 186,000 base pairs in which 26 exons code for a protein 2,351 amino acids long) the specific defect has been identified in well over a thousand patients. About half of the changes are single base changes, the most common of which are cases
where the C in CpG dinucleotides (CG/GC) is replaced by a T (becoming TG/AC).

a) What type of change is this and why is it not surprising that these were the most common changes found?
1) it is a transition and these can occur from tautomers of normal bases
2) oxidative deamination of C gives U which creates base-pair mismatch
3) C next to G often methylated and deamination of 5MeG gives T, a normal base. In this case, repair could only "guess" at the correction procedure

b) There are great differences in clotting efficiency among patients. Four different alleles are described below. For each describe the effect of the mutation on transcription/translation and predict if it will be lead to mild or severe hemophilia:

1) allele .0069: codon 5 in exon 1 changes from CGA to TGA
creates nonsense mutation; severe

2) allele .0079: the last A in intron 4 is replaced by G
alters normal splice site; severe (note these did come from real patients, so did cause hemophilia, which might not be the case if the base change in the intron did not a splice junction)

3) allele .0027: TAT altered to TGT at codon 2105 in exon 22
missense mutation, Tyr to Cys; severe to mild possible, mild actually

4) allele 0075: AA deleted from codon 48 in exon 2
frameshift mutation, alters all remaining codons; severe.

c) List a different chemical mutagen that could induce each mutation.
1. EMS, NG, 2AP, 5BU etc
2. same list
3. same list
4. acridine, EtBr, ICR170
14) An Hfr strain with markers met−, his+, leu+ and trp+ of unknown order is mated to a female with opposite markers, although the his− allele is temperature sensitive so that histidine is required only at 42°C, not 37°C. The met− gene is transferred very late compared to the others. After mating for 30 minutes, the mating was disrupted, the mixture was diluted and plated on 4 types of media with the supplements and results shown when incubated at 42°C:

<table>
<thead>
<tr>
<th>Media Supplements</th>
<th># of colonies</th>
<th>GENOTYPE(s) of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>histidine and tryptophan</td>
<td>250</td>
<td>met+, his+-, trp+-/- leu+</td>
</tr>
<tr>
<td>histidine and leucine</td>
<td>50</td>
<td>met+, his+-, trp+, leu+/-</td>
</tr>
<tr>
<td>leucine and tryptophan</td>
<td>500</td>
<td>met+, his+, trp+-/- leu+/-</td>
</tr>
<tr>
<td>histidine only</td>
<td>10</td>
<td>met+, his+-/-, trp+, leu+</td>
</tr>
</tbody>
</table>

a) What was the reason for including the met− marker in the Hfr strain? 

*to kill the donor males making it possible look for recombination that occurred in partially heterozygous recipients*

b) Fill in the potential genotypes of the colonies (use +/- if either allele may be present)

c) Which genes entered first, second and third? his+; leu+, trp+

15) Proline auxotrophs 1-4 gave the following responses when tested for growth on a series of potential intermediates (+ = growth, - = no growth)

<table>
<thead>
<tr>
<th>Mutant</th>
<th>GSA</th>
<th>GA</th>
<th>GP</th>
<th>Proline</th>
</tr>
</thead>
<tbody>
<tr>
<td>prol-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>prol-2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>prol-3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>prol-4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

a) Use these data to make a pathway for proline synthesis; show where each mutant blocks the pathway.

b) Give 2 explanations for the similar responses of mutants 1 and 4 (they are not alleles).

1) unknown intermediate between GSA and proline 2) 2 polypeptides/1 enzyme
15. Give a human example of a mutation that prevents normal DNA repair and in 1-5 words describe the consequences.

Examples include:
xeroderma pigmentosum; lack of excision repair enzyme
*p53* mutations lack *p53* functions for divide versus apoptosis
*BRC2A*; interacts with Rad51
e tc

In all cases, there is a high risk of cancer.