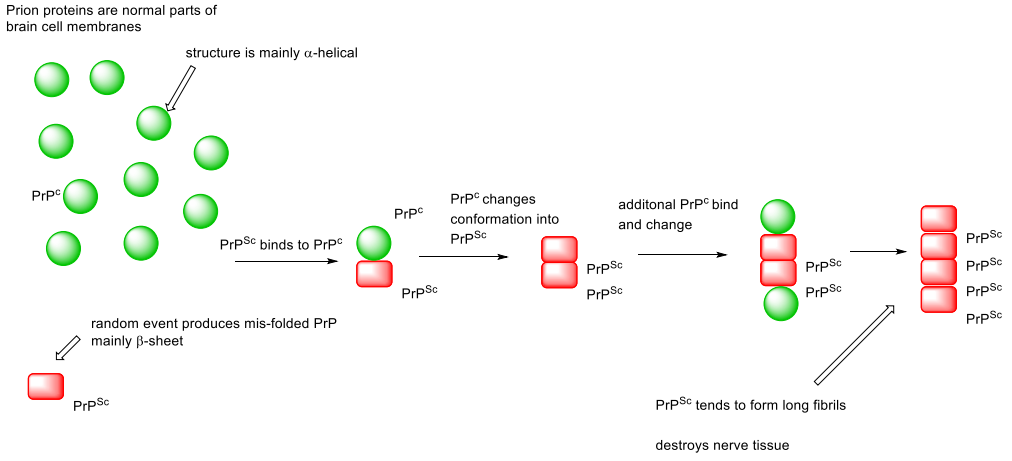
BPS 2110
Assignment 9 Answers

1. In 1985, a contamination problem in batches of human growth hormone caused serious illness that resulted in the FDA banning the use of the product.
    a. What was the source of the contaminated growth hormone?
    Human cadavers
    b. What disease did it cause?
    Creutzfeldt-Jakob or Transmissible Spongiform Encephalopathy
    c. What was the contaminant in the drug?
    Prions
    d. When did the contamination take place?
    1960's or 1970's (12+ years earlier)
    e. Why were so many people infected?
    Pituitaries processed in large batches. Single batch of hGH given to many patients. Any contamination in 1 pituitary will be included in the entire batch.

2. Transmissible spongiform encephalopathies are a family of degenerative brain diseases affecting various animals.
    a. What illnesses are classified as TSE's and what species do they infect?
    Scrapie (sheep)
    Kuru (Humans)
    Mad Cow disease or Bovine spongiform encephalopathy (cattle)
    Creutzfeldt-Jakob (humans)
    b. How do these diseases arise?
    Protein in brain tissue becomes mis-folded. This mis-folded protein induces other molecules of the same protein to mis-fold. Mis-folded proteins form long fibrils that disrupt cellular structure.



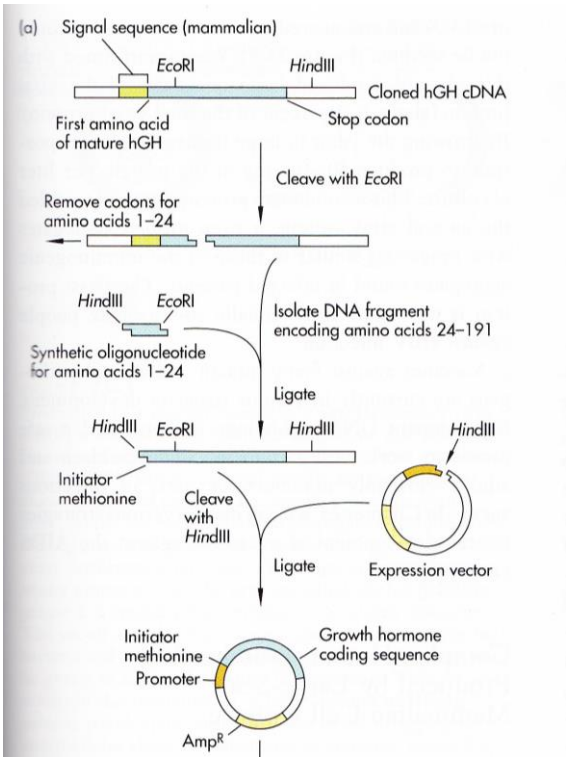
    c. What are the infectious agents called?
    Prions
    d. What are the structural characteristics of these agents?
    Mostly β-sheet conformations

3. During the 1990's, a serious outbreak of mad cow disease occurred in Europe.

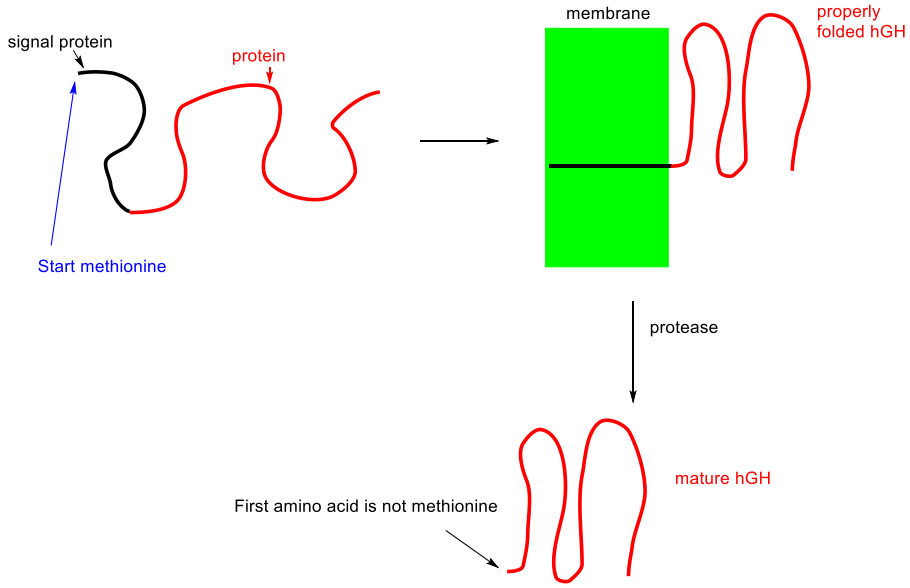
a. What was thought to be the main reason behind the outbreak?
   Use of meat and bone meal in cattle feed as a way of increasing protein content in feed. This product included brain tissue from cows. Any prions present would be exposed to large numbers of cattle.
b. Why was it important to incinerate the carcasses of affected animals?
   Prions are very robust molecules. Must ensure they are fully destroyed. Only way to do this is to burn the carcasses.

4. When cloning proteins, cDNA is sometimes used as a gene source.
   a. What is cDNA?
      Complimentary DNA. DNA containing only exons.
   b. Why is it necessary to use this DNA rather than simply cutting a gene out of a chromosome?
      Human genes contain lots of introns. Bacteria do not use introns and do not have the necessary enzymes to process them. Must remove all the introns from DNA before putting it into bacteria.
   c. In general, how is cDNA obtained?
      Isolate all the mature mRNA from cells. Purify the mRNA to isolate the desired mRNA. Use reverse transcriptase to convert mRNA into DNA.
   d. Why was it necessary to use pituitary tumors to obtain the cDNA to make human growth hormone?
      Need lots of cells to isolate mRNA. To do this is necessary to be able to grow pituitary cells. Normal pituitaries will not grow outside a living body, but pituitary tumors will.

5. The first recombinant human growth hormone included an extra amino acid (192 vs 191 in the native hormone)
   a. What was the extra amino acid and what position was it located on?
      Methionine at position 1 (N terminus)
   b. Why did this drug end up with an extra amino acid?
      hGH is normally expressed as a fusion protein in which a signal protein occurs just before the hGH sequence. The first amino acid of the signaling protein is Met (Start codon). The peptide is cut between hGH and the signal protein as part of maturation, which leaves hGH (Met is not the first amino acid.
      Because the signal protein had to be removed to express hGH in bacteria, it was necessary to add a Start codon to the beginning of the hGH sequence, and so the protein ended up with a Met at position 1 and was 192 amino acids long.



6. Other forms of human growth hormone were later marketed that were identical to native hGH (191 amino acids).
    a. How was the expression of this version of the drug carried out?
    DNA was created by splicing the cDNA of hGH onto a bacterial signal protein. After transformation, the peptides were transported outside the cells where proteases removed the signal proteins in the periplasmic space.
    b. Why was the extra amino acid, found in other versions, not present?
    The expression included a bacterial signal protein, which was removed as part of maturation. Because the Met (Start codon) was removed along with the signal protein, there was no extra Met on the hGH



c. What extra purification step was necessary when using this expression system?
Osmotic shock
d. How was this step carried out?
Grow the cells in a hypertonic solution (concentrated glucose). Then quickly switch the medium to a hypotonic one (low concentration of salts).
e. Other than the native amino acid sequence, what other advantage did this expression system provide?
Easier purification since the protein was secreted outside cells. Less extra protein to remove.

7. Describe how testing is done for the use of hGH in sports.
- Test for the amount of main isoform in blood.
- test for amounts of isoforms in blood.
- compare the ratios of the isoforms to the normal metabolic ratios.