In the beginning, there were restriction enzymes, plasmids, and foreign DNA… an intro to genetic engineering

Chem 210

3/9/00

What is genetic engineering?

- Introduction of genes from one organism into another.
- Genetic engineering using molecular techniques is related (historically) to more traditional genetic manipulation of organisms.
  - Selective breeding
  - Cross breeding of species
  - Fermentation--use of micro-organisms to produce a material
- Genetic engineering by molecular techniques allows one to create larger genetic changes at a much more rapid pace.

Why do genetic engineering?

- Introduce genes into micro-organisms--bacteria can be used as “factories” to produce proteins from more complex organisms (humans, plants, other animals).
- These proteins are valuable for lab studies.
  - The proteins can be changed/altered by *mutagenesis*.
  - Proteins which have therapeutic value (such as insulin) can be biosynthesized.
- Introduce genes into plants--create new agricultural products.
  - Increase nutritional content of plants.
  - Make plants resistant to synthetic pesticides.
  - Design plants with inherent pest resistance.
- Introduce genes into animals
  - Use animals as factories for protein production.
  - Gene therapy--alter the genetic make up of humans

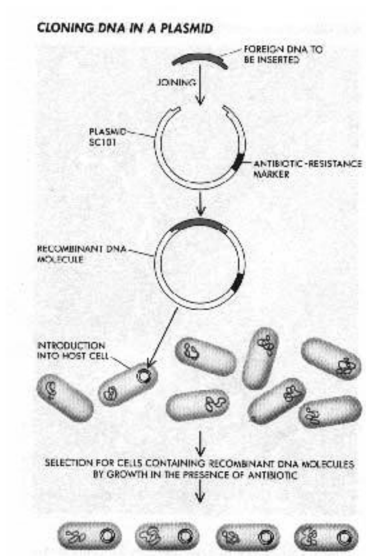
Genetic engineering raises many ethical, social, and political questions

- Is the new technology dangerous?
- How will genetically altered foods affect our health?
- Can “life” be patented?
- Do we have the right to

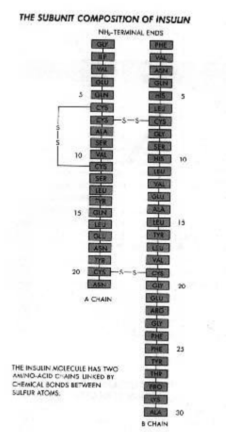
Modern genetic engineering began in the early 1970s

- The discovery of *restriction endonucleases* and *DNA ligase* allow molecular biologists to cut and paste DNA.
  - Possible to take DNA from two different sources and link them together.
  - Create *recombinant DNA*--DNA combined from different sources.
- First recombinant DNA experiments performed by Paul Berg (molecular biologist from Stanford) in 1973.
  - Inserted genes from a virus SV40 into a plasmid, transformed into *E. coli*.
  - SV40 is a virus shown to produce tumors in some animals (but not humans).
  - These experiments raised huge alarms--what are the biohazards of recombinant DNA technology?
- In 1973, Berg calls for a voluntary halt to recombinant DNA experiments.

Foreign DNA with “sticky ends” can be inserted into a plasmid



Insulin was the first commercially produced recombinant protein



- Insulin is an important hormone which regulates sugar metabolism.
- An inability to produce insulin results in a form of diabetes; this diseases can be treated by daily injections of insulin.
- Historically, insulin from pigs or cows used--produces immune reactions in some patients.
- Challenge: how to make human insulin to be used as a drug?

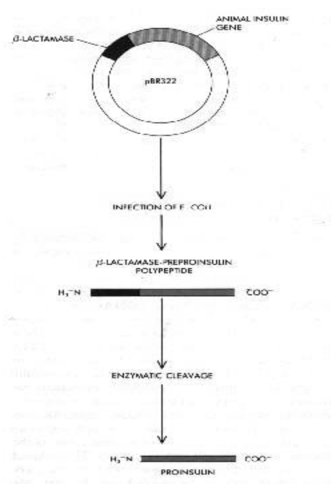
Recombinant insulin overcomes many of these problems

- Idea: take the gene for human insulin, clone into a plasmid, introduce the plasmid into *E. coli*, and use the *E. coli* as “Factories” for insulin production.
- Amino acid sequence identical to that of the “natural” human protein--shouldn’t cause immune reactions.
- Can be produced in large amounts in bacterial cultures.
- Much more economical than attempts to produce insulin be chemical synthesis.
- So, how to do this?

Challenges to expression of human proteins in bacteria

- Human genes have introns which can't be removed in bacteria--need to use cDNA to clone into *E. coli*.
- In eukaryotes, polypeptides are processed (or modified) after synthesis on the ribosome.
  - N-terminal methionine is removed--doesn't happen to E. coil soluble proteins.
  - Occasionally, the polypeptide is cleaved or digested.
- Human insulin is synthesized as a single polypeptide chain (proinsulin); later cut into two strands to form the active protein.

Strategy for producing recombinant human insulin



Strategy for producing recombinant human insulin