DNA Technology

In continuation from the previous lesson, we are extending the discussion of genetic systems of microorganisms to the point whereby scientists are now able to manipulate the genetic makeup of particular organisms for the benefit of modern-day applications.

A. Genetic Engineering

Most notably, emergences in the world of microbial genetics have been incorporated into the science of genetic engineering, specifically processes involving recombinant (material produced by genetic engineering) DNA technology. The benefits these techniques hold is significant for they potentially have the ability to impact such diverse areas as medicine, agriculture, and industry.

Yet, use of these techniques is accompanied by a certain amount of risk, thus, the potential good to come out any of these procedures need to be weighed against the possible harm that can be done.

Therefore when we speak of genetic engineering, we need to first understand what it is- the ability to deliberately modify an organism's genetic information by directly changing its nucleic acid. We also need to know how Recombinant DNA technology--defined as the collection of methods used to accomplish genetic engineering--achieves this result.

B. Recombinant DNA Technology: Timeline

- **Late 1960s--**Arber and Smith discovered restriction endonucleases, which cleave (break apart) DNA at pre-set sequences.
- **1969--**Boyer is credited as being the first to isolate the restriction endonuclease EcoRI.
- **1970**--Baltimore and Temin, independent of one another, discovered reverse transcriptase; an enzyme which capable of being used to construct complementary DNA (cDNA) from any RNA molecule
- **1972--**Jackson, Symons, and Berg were all credited with producing the first recombinant DNA molecules made by fusing together DNA fragments.
- **1973**--Cohen and Boyer generated the first recombinant plasmid (vector). This was inserted into and replicated within a bacterial host
- **1975**--Southern, using radioactive DNA hybrid probes, developed a blotting procedure for detecting specific DNA fragments. This particular technique is now useful in isolating genes in need of additional study.

Note: non-radioactive, enzyme-linked probes (faster and safer though less sensitive) are now able to replace early-day radioactive probes.

- Late 1970s--Procedures for rapidly sequencing DNA molecules, synthesizing oligonucleotides, and expression of eucaryotic genes in bacteria had been developed

C. Recombinant DNA: Production Process

Synthesizing DNA

Short pieces of DNA or RNA, also known as Oligonucleotides, are able to be produced by building to adding onto a growing chain, one nucleotide at a time.

In site-directed mutagenesis, a small synthetic oligonucleotide carrying the desired sequence change is used as a primer for DNA polymerase. This then replicates the remainder of the target gene and produces a new gene copy with the desired mutation.

The Polymerase Chain Reaction (PCR)

The purpose of PCR is to synthesize large quantities of a DNA fragment without needing to clone it. PCR has demonstrated its validity and value within such scientific fields as: molecular biology, medicine (e.g., PCR-based diagnostic tests) and in biotechnology (e.g., use of DNA fingerprinting in forensic science)

How PCR works

Synthetic DNA molecules containing sequences identical to those bordering the target sequence are used as primers for DNA synthesis. Replication, carried out in successive cycles, use a heat-stabilized DNA polymerase.

Since being first introduced, PCR has become automated and improved. In fact, new procedures even allow RNA to be used as a template from which complementary DNA can be produced and amplify complementary DNA)

D. Recombinant DNA: Applications

After recombinant DNA is prepared (stages include: isolating and cloning of fragments), it can be either inserted into the organism's plasmid vector or stored for later use as part of a genomic library.

Desired genes may be selected and subsequently directly inserted into animal cells via microinjection. Should the genes result in producing fertilized eggs, the new organism produced is referred to as a transgenic animal.

Another related genealogical procedure is Electroporation. Electroporation entails exposing target cells mixed with DNA to high voltage. This type of procedure generally produces optimal results when applied to mammalian cells and plant cell protoplasts.

E. Recombinant DNA: Usages:

Within not only the medical world, but the world at large, manufactured (recombinant) DNA has been used for many positive (as well as negative) applications.

Foremost within medical arenas**,** the use of disease-causing organisms to develop vaccines, along with the widespread employment of beneficial health proteins, i.e., somatostatin, human growth hormone, human insulin (diabetes), interferon, and synthetic vaccines, etc., have all been formulated and refined for the purpose of treating specific diseases and conditions.

Still in development (and/or under investigation) are diagnostic probes for certain infectious diseases (HIV), genetic disorders (e.g., Alzheimer's with stem cell research) and somatic cell gene therapy for a subset of cancers.

Within the industrial sector, recombinant microbes have been used to manufacture protein products in an effort to improve bacterial, fungal, and mammalian cell strains used in certain bioprocesses. For instance, the implementation of bacterial degradation on petroleum products (to clean up oil spills) and other toxic materials has been in use for some time now.

Within the agricultural world, recombinant DNA strategies have contributed to the introduction of new desirable traits (e.g., increased growth rate) in farm animals and the transference of nitrogen fixation capabilities to non-legume (non-green) crop plants (this then helps to make them resistant to environmental stressors).

And, while the debate on whether MGO (modified genetic organisms) is good or bad or a non-issue, farmers throughout the US (and world) continue to capitalize upon the procedures to produce more voluminous crops, such as, corn, soybeans, cotton, canola, potato, squash, and tomato.

Further within the agricultural expanse, new techniques are continuously being investigated and experimented upon for protecting crops against frost damage and making plants unappealing (toxic) to insect pests.

Recombinant DNA Technology: Social Ramifications

Yet while there seem to be a wealth of positive applications for recombinant DNA technology, there also seems to be a questionable side whereas ethics and risks are concerned.

Take for example such safety concerns as the elicitation of widespread infections due to the release of recombinant strains of E. coli and the transference of genes form an instable gene to a sturdy one.

Fortunately, we as a population have not yet encountered such a global outbreak of disease or panic. This is on account of the governmental oversights in place to monitor practices involving use of such manufactured microbial practices. However, with many

researchers and microbiologists seeking to outdo one another there is never any telling as to when one may overstep the boundaries of acceptability.

With regard to cloning and reengineering existing genetic coding, there exists an atmosphere of moral dissonance as to whether these procedures go against fundamental ethical and religious beliefs.

And the question of whether science oversteps the bounds of what is right and wrong is most evident in cases where microorganisms have been used for offensive purposes (i.e., biological warfare agents aka weapons of mass destruction-WOMD).

Within these instances, the use of recombinant DNA technology--whereby a gene specifying a protein of interest for the removed from the genetic material of one organism to be added to the genetic material of the target organism--has made the creation and subsequent execution of biological weapons a frightening reality with which we must contend on a daily basis.

Within this context, it has been said that the science of microbiology by virtue of its own limitless potential contributes in fundamentally important ways to both defend and challenge national security and it even goes so far as to extend into the gathering of intelligence and supporting of espionage activities.

Further complicating the issue are the ecological concerns that can result from the usage of such biological warfare mechanisms, for once activated whereby destructive recombinant organisms are released into the environment, there exists the potential of disruption being done to an ecosystem.