



PART V

Introduction to biotechnology

16. Biosafety and recombinant DNA technology

Recombinant DNA technology involves combining genetic material from different sources thereby creating genetically modified organisms (GMOs) that may have never existed in nature before. Initially there was concern among molecular biologists that such organisms might have unpredictable and undesirable properties that could represent a biohazard if they escaped from the laboratory. This concern became the focus of a scientific conference held in Asilomar, CA, USA, in 1975 (45). At that meeting, safety issues were discussed and the first guidelines for recombinant DNA technology were proposed. The subsequent 25+ years of research experience have demonstrated that genetic engineering may be conducted in a safe manner when an appropriate risk assessment is performed and adequate safety measures are used.

Recombinant DNA technology or genetic engineering was first used to clone DNA segments in bacterial hosts in order to overexpress specific gene products for further studies. Recombinant DNA molecules have also been used to create GMOs such as transgenic and “knock-out” animals and transgenic plants.

Recombinant DNA technology has already had an enormous impact on biology and medicine, and will probably have an even greater influence now that the nucleotide sequence of the entire human genome is available. Tens of thousands of genes of yet unknown functions will be studied using recombinant DNA technology. Gene therapy may become a routine treatment for certain diseases, and new vectors for gene transfer are likely to be devised using genetic engineering techniques. Also, transgenic plants produced by recombinant DNA technology may play an increasingly important role in modern agriculture.

Experiments involving the construction or use of GMOs should be conducted after performing a biosafety risk assessment. The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized. The properties of the donor organism, the nature of the DNA sequences that will be transferred, the properties of the recipient organism, and the properties of the environment should be evaluated. These factors should help determine the biosafety level that is required for the safe handling of the resulting GMO, and identify the biological and physical containment systems that should be used.

Biosafety considerations for biological expression systems

Biological expression systems consist of vectors and host cells. A number of criteria must be satisfied to make them effective and safe to use. An example of such a biological expression system is plasmid pUC18. Frequently used as a cloning vector in combination with *Escherichia coli* K12 cells, the pUC18 plasmid has been entirely sequenced. All genes required for expression in other bacteria have been deleted from its precursor plasmid pBR322. *E. coli* K12 is a non-pathogenic strain that cannot permanently colonize the gut of healthy humans or animals. Routine genetic engineering experiments can safely be performed in *E. coli* K12/pUC18 at Biosafety Level 1, provided the inserted foreign DNA expression products do not require higher biosafety levels.

Biosafety considerations for expression vectors

Higher biosafety levels may be required when:

1. The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the GMO
2. Inserted DNA sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms
3. Gene products have potential pharmacological activity
4. Gene products code for toxins.

Viral vectors for gene transfer

Viral vectors, e.g. adenovirus vectors, are used for the transfer of genes to other cells. Such vectors lack certain virus replication genes and are propagated in cell lines that complement the defect.

Stocks of such vectors may be contaminated with replication-competent viruses, generated by rare spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification. These vectors should be handled at the same biosafety level as the parent adenovirus from which they are derived.

Transgenic and “knock-out” animals

Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards.

Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species. If such animals escaped from the laboratory and transmitted the transgene to the wild animal population, an animal reservoir for that particular virus could theoretically be generated.

This possibility has been discussed for poliovirus and is particularly relevant in the context of poliomyelitis eradication. Transgenic mice expressing the human poliovirus

receptor generated in different laboratories were susceptible to poliovirus infection by various inoculation routes and the resulting disease was clinically and histopathologically similar to human poliomyelitis. However, the mouse model differs from humans in that alimentary tract replication of orally administered poliovirus is either inefficient or does not occur. It is therefore very unlikely that escape of such transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus. Nevertheless, this example indicates that, for each new line of transgenic animal, detailed studies should be conducted to determine the routes by which the animals can be infected, the inoculum size required for infection, and the extent of virus shedding by the infected animals. In addition, all measures should be taken to assure strict containment of receptor transgenic mice.

Transgenic plants

Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world. The discussions focus on the food-safety of such plants, and on the long-term ecological consequences of their cultivation.

Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products. A risk assessment should determine the appropriate biosafety level for the production of these plants.

Risk assessments for genetically modified organisms

Risk assessments for work with GMOs should consider the characteristics of donor and recipient/host organisms.

Examples of characteristics for consideration include the following.

Hazards arising directly from the inserted gene (donor organism)

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins
2. Cytokines
3. Hormones
4. Gene expression regulators
5. Virulence factors or enhancers
6. Oncogenic gene sequences
7. Antibiotic resistance
8. Allergens.

The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

Hazards associated with the recipient/host

1. Susceptibility of the host
2. Pathogenicity of the host strain, including virulence, infectivity and toxin production
3. Modification of the host range
4. Recipient immune status
5. Consequences of exposure.

Hazards arising from the alteration of existing pathogenic traits

Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive).

1. Is there an increase in infectivity or pathogenicity?
2. Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?
3. Does the foreign gene encode a pathogenicity determinant from another organism?
4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?
5. Is treatment available?
6. Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?
7. Is eradication of the GMO achievable?

Further considerations

The use of whole animals or plants for experimental purposes also requires careful consideration. Investigators must comply with the regulations, restrictions and requirements for the conduct of work with GMOs in host countries and institutions.

Countries may have national authorities that establish guidelines for work with GMOs, and may help scientists classify their work at the appropriate biosafety level. In some cases classification may differ between countries, or countries may decide to classify work at a lower or higher level when new information on a particular vector/host system becomes available.

Risk assessment is a dynamic process that takes into account new developments and the progress of science. The performance of appropriate risk assessments will assure that the benefits of recombinant DNA technology remain available to humankind in the years to come.

For further information see references (17) and (46–48).