Chapter 4

Genetic Considerations

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Genetic Considerations

The safety questions raised by the planned introduction of genetically engineered organisms are not unique to such organisms. Yet the introduction of these organisms raises some safety questions that are quite different from the questions of physical containment on which previous discussions of the safety of recombinant DNA research have focused. And though the intimate interplay between the genes of an organism and the environmental parameters that govern the way the genes are expressed makes most separations of genetic and environmental factors difficult, such divisions make the issues easier to examine. This chapter focuses primarily on genetic issues, particularly as they relate to the potential for movement of engineered genetic material beyond the intended host. Most genetic factors are either important primarily as they relate to this potential, or are more clearly relevant in an ecological context, and are thus discussed in chapter 5.

The migration of genetic material, or horizontal transfer, is the passage of genetic material from one organism to another through a mechanism not involving specialized reproductive cells (i.e., nonsexual gene transfer). In bacteria, it is the transmission of genetic material from **one** contemporaneous bacterial cell **to** another, by **any of** several means. OTA assumes that genetic **material** introduced into **a** host in which **it does not** naturally occur has **some** finite probability of migrating **to a** nontarget organism. What **is** that probability? How **can the** movement of the genetic material be observed? What **are** the potential **consequences** of horizontal gene transfer? And what steps **can be** taken that would limit the frequency **or** mitigate the potentially adverse consequences of horizontal transfer?

An OTA workshop, convened **in** collaboration with the National Science Foundation, examined these and other questions surrounding genetic **issues** in the planned introduction of genetically altered organisms. This chapter **summarizes some of the factors that could influence the frequency of horizontal gene transfer after the planned introduction of recombinant organisms and examines technologies designed to affect horizontal transfer. Most of this chapter is relevant primarily to microbes and much less relevant to higher organisms such as plants or animals, unless specified.**

WHAT IS KNOWN ABOUT HORIZONTAL TRANSFER OF GENES?

Genetic material probably does not often move across large evolutionary distances, between organisms only remotely related. When gene transfer does occur, it appears to take place via a limited number of mechanisms. Different types of organisms, such as bacteria and plants, share some mechanisms for genetic transfer, while other mechanisms are specific or unique to particular organisms (2,14,15).

The different types and mechanical details of gene transfer have been discovered and examined primarily in controlled laboratory situations. Gene transfer between bacteria under laboratory conditions has been widely described, and there is evidence that transfer in natural ecosystems (e.g., in soil, in aquatic systems, or on plant surfaces) does occur (12). Horizontal transfer between plants has **not** been well studied, and **no** conclu**sive** data **exist to** indicate that **it occurs in nature. But nonsexual genetic exchange involving the insertion of bacterial material into plants is well documented. Gene transfer has been investigated and described in invertebrate systems, particularly insects. Evidence from the evolutionary record suggests that some rare horizontal transmission of genetic material has occurred between mammalian species. Although there is no firm evidence that genes are passing back and forth between diverse groups of organisms, there are instances that warrant further investigation.**

Although several specific systems of genetic transfer have been studied, they probably represent only a subset of what actually occurs in nature. In fact, since these systems have been

selected for **intensive research because of their ease of handling, the likelihood of producing rapid results, and their accessibility to existing research methodologies, their role in horizontal gene transfer in nature maybe overestimated. Indeed, many barriers to gene transfer exist in natural systems. Thus, many questions remain to be answered about gene transfer outside the laboratory, including:**

- How extensively do the gene transfer mechanisms **observed in the laboratory operate in nature?**
- What are the genetic and environmental conditions under which novel information could be incorporated **into a foreign genome and subsequently expressed?**
- Do populations of organisms limit **incursion of new genetic material, and if they do, by what means?**

WHAT GENETIC CONSIDERATIONS MUST BE EVALUATED IN PLANNED INTRODUCTIONS?

The planned introduction of a genetically engineered **organism raises three issues of immediate concern. First, if gene transfer does occur, will the new genetic information be maintained and expressed? Second, what is the potential extent of horizontal transfer of manipulated genetic material? And third, if the modified organism, or the inserted DNA it contains, moves beyond the point of introduction, how will it affect the surrounding populations or communities of plants, animals, and microbes? This issue, regarding ecological considerations, is discussed in chapter 5. The first two questions, of horizontal transfer and expression, are considered here.**

Some commentators have maintained that if the gene in question will not move to other organisms then there is no need to worry about potential consequences of its introduction. others maintain that if the modified organism or gene of interest would not cause problems even if it moved, then the exercise of estimating transfer probability is unnecessary. Both issues should be addressed in assessing potential consequences of a proposed introduction experiment (see ch. 6), although in some cases not enough is known about the life histories of organisms that could be involved to make such hazard estimates possible. But a very low probability of transfer multiplied by a moderate probability of expression and resultant hazard if transfer occurs is a different situation than if both probabilities are very low. By the same token, a significant probability of benefit could offset all or part of any potential risk.

Many factors **influence the magnitude, frequency, stability, and effects of horizontal gene transfer to nontarget organisms. Identifying these factors is necessary if scientists, corporate administrators, and government regulators are to evaluate environmental applications of genetically engineered organisms. Table 4-1 lists the minimum factors that must be considered, which are discussed in the next section.**

Predicting the Potential Effects of Horizontal Transfer

Can generic rules be discovered that would help distinguish a condition of low probability of hori-

Table 4-1.—Some Genetic Factors To Evaluate in Planned Introductions

Possible method of manipulation	Factor
Organism choice or design	Gene
	Vector
	Construct
	Host organism
	Recipient organism
Population manipulation	Survival of released organisms
	Population density of host
Other means	Presence of potential nontarget organisms
	Density of potential nontarget organisms
	Selection pressure

SOURCE" Office of Technology Assessment. 1988

zontal gene transfer from one of high probability? What types of accessible information yield likely estimates of the magnitudes of horizontal gene transfer? These questions are difficult to answer with precision.

An analysis of the magnitude of horizontal gene transfer **must include at least two components:**

- **an estimate of the frequency** of gene transfer from introduced to nontarget organisms, and
- an estimate of the **genetic distance** between the original organism and the nontarget species.

The consideration of both **intrinsic** and **extrinsic** factors can help assess the likely extent of horizontal gene transfer. Intrinsic factors, which are elements of molecular biology, include:

- **the host organism used in the application (e.g., plant or micro-organism);**
- **the gene being manipulated (e.g., gene conferring pesticide resistance or ice-nucleating activity);**
- **the vector introducing the gene into the host organism (e.g., a plasmid or virus); and**
- **the construct, or** final configuration of the **new** genetic material within the host organism, which will govern expression and sta**bility of the gene product.**

Extrinsic factors, which are elements of ecology, include:

- the **survival of the released host organism,**
- **the presence of potential nontarget recipients** of the gene and the evolutionary relationship between the host and potential nontarget organisms,
- the **population densities** of the engineered host and the potential nontarget recipients in the environment, and
- the **selection pressures to maintain the new** gene in either population.

By influencing the magnitude of horizontal gene transfer, these **extrinsic factors become integral to any examination of genetic considerations of environmental release.**

Intrinsic Factors

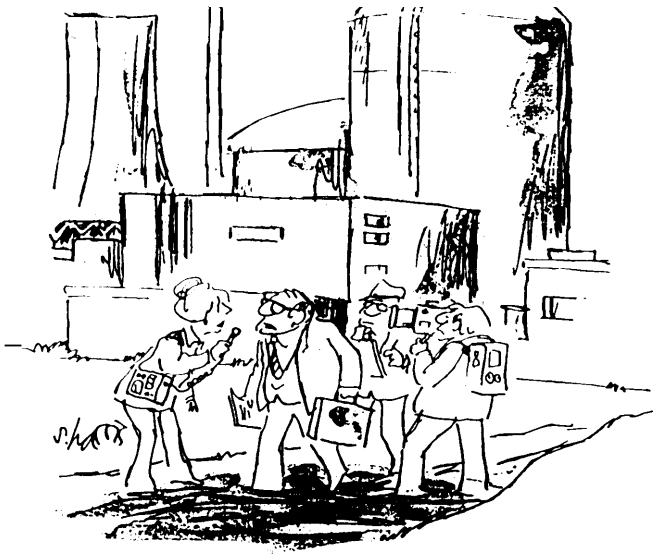
The impact of intrinsic factors on horizontal gene transfer cannot be measured by simple descriptive information about the host, gene, vector, and construct. A number of principles help explain and predict the behavior of genes. Before estimating the frequency of horizontal transfer in a system, the natural histories of each component must be understood. The information should include, but not be limited to, how the gene is expressed in different environments, both genetic and ecological; the behavior of the vector in different hosts; and the different life stages, if any, of the host.

Host

It is important that the micro-organism, virus, fungus, plant, or animal used as the host be well understood, and its life cycle well studied. Perhaps most important, the mechanism(s) by which the organism transfers genetic material in the laboratory should be identified. For instance, one class of bacteria (called gram-negative) usually uses plasmids or phages to facilitate genetic exchange. Another class (gram-positive) uses the direct exchange of DNA segments as an important mechanism of gene transfer.

Although the bacterium *Escherichia coli* is well understood, less is known about genetic exchange by bacteria outside the laboratory, especially in soil. In some instances, nondebilitated bacteria are being developed for planned introductions despite the paucity of information on host survival, genetics, and population structure. Some of these organisms may survive and function for long periods in their **new** environment (13). **Without a well-developed natural history of host organisms, it is impossible to evaluate the genetic and ecological implications of a planned introduction.** Substantial experience with past microbial introductions indicates, however, that even when introduced bacteria survive, they do not come to dominate the host community,

The presence of cryptic genetic material (e.g., cryptic plasmids) is a host characteristic that merits special consideration. Cryptic genetic ma-



@ 1986 by Sydney *Harris,* American Scientist *Magazine*

"There is no problem. Any damage caused by the nuclear accident can easily be remedied by genetic engineering. "

terial appears to have no assigned function and is often assumed to be inactive. But its function may depend on environmentally induced stimuli, a condition that could make it appear nonfunctional in the laboratory. A gene on a cryptic plasmid, for example, might be expressed only under starvation conditions, a common condition of bacteria in nature but not in the laboratory.

One species of *Yersinia* provides an example of differential gene expression. **This bacterium carries a plasmid that produces four or five important gene products only when the organism is growing within its natural environment. Another example can be seen in the difference between E.** *coli* **in the test tube and in its natural habitat, the gut. Some** E. *coli* **plasmids code for adhesion factors that allow the bacterium to colonize the gut. These plasmids are expressed only when the bacterium is in the gut. Thus, genetic material that appears to be cryptic in the laboratory could have important functions in nature. So it is theoretically possible that a seemingly dormant piece of** genetic material could provide the mechanism for an engineered gene to be transferred from the host to a nontarget organism. This possibility makes it important that the life history of the **of bacteria-carrying cryptic plasmids, at least in situations where it is imperative to avoid the possibility of gene transfer.**

On the other hand, the natural histories of many host organisms are well known. One such host is *Pseudomonas fluorescent.* **Scientists have proposed using this bacterium, altered to carry the delta-endotoxin gene from** *Bacillus thuringiensis,* **to protect corn roots from the black cutworm. The toxin kills the cutworm that feeds on the corn rootlets. The** *P. fluorescent* **host was chosen because it has been well studied and is easily identified. Since the toxin gene has been inserted into a new host organism, however, the probability and frequency of the gene being transferred out of the engineered** *Pseudomonas* **is a valid question. Do the mechanisms that 13.** *thuringiensis* **uses to exchange genetic material differ from those of** *P. fluorescent?* **Is the frequency of gene transfer different between the two, or do they typically exchange genes with the same or different species? Do restriction enzyme systems in potential nontarget recipients reduce the probability of transfer of intact DNA? Host-related questions such as these may be important in assessing genetic considerations of planned introductions.**

Gene

Ideally, planned introduction experiments would involve genes that have a well-understood natural history as well as host organisms that have been studied thoroughly. An extensive natural history would help determine whether potential new interactions between the gene and the environment could result from the gene's presence in a new host microenvironment. How specific should or could applications for approval of planned introduction field tests be about gene-environment interactions? Can any novel expression of phenotype occur? Unfortunately, these questions are impossible to answer, because they require scientists to predict and quantify the occurrence of rare and idiosyncratic events. Only gradually increasing experience will start to provide answers.

Again, the toxin gene of B. *thuringiensis* is a good example of a gene from an organism with a well-characterized natural history (l). The naturally occurring bacterium and derived materials have been used for decades. They are now available in over 410 different products, in 13 formulations (e.g., powder, pellet, or solution) to apply the toxin in garden, agricultural, and forestry settings (6). In many areas containers of B. *thuringiensis* spores can be purchased at garden stores. Tons of the bacterium have been applied to agricultural and forestry lands. Despite intensive searching, scientists have unearthed no evidence to date that either the endotoxin gene has escaped from the *Bacillus* and been expressed in other microbes, or that the toxin from this strain (var. *kurstaki)* has any effect on organisms other than Lepidoptera and closely related insects.

Vector

Vectors are the means by which genetic material is shuttled between organisms. Just as it is necessary to have a well-characterized host and gene, it is important to use a vector with well understood characteristics. Important factors include the vector's ability independently to initiate or sustain horizontal gene transfer, its need for outside help to move information, and its degree of mobility or the extent of its host range.

Construct

An important factor affecting the probability that an inserted gene might move from an altered host to a nontarget organism is the final configuration of the new gene in the host—i.e., the DNA structure at the site of gene insertion. For instance, inserting a gene into a chromosome minimizes subsequent gene movement, especially compared with inserting it into a plasmid. The source of the regulatory sequences controlling expression of the inserted material is also important and plays a major role in limiting the field of potential nontarget recipients.

Genetically engineered "ice-minus" bacteria also illustrate the importance of construct to the likelihood of horizontal gene transfer. This bacterium is created by removing a gene found naturally in *Pseudomonas, Erwinia,* and other bacteria, a construction that decreases the probability of horizontal gene transfer.

The transfer of a deletion—in this case, essentially a missing gene—to a nontarget organism cannot impart a new capability to the recipient in the same way that acquiring a novel structural gene can, as in the case of the B. *thuringiensis* toxin gene. So even if the altered genetic material is transferred beyond the host, it cannot add to the nontarget recipients the ability to produce a new gene product. Deletions can, however, alter the relationships of the host species to other organisms with which it interacts, a change that could be important under some circumstances.

Extrinsic Factors

The extrinsic factors that strongly influence the likelihood and magnitude of horizontal gene transfer are an integral part of the environment into which the engineered organism is introduced. The expression of the trait, the intended environment, and other environments that the engineered organisms could encounter must be analyzed for their possible impact.

Survival

A key determinant of potential horizontal transfer is the ability of the introduced organism to establish and reproduce itself in its new habitat, and to stably express the engineered trait, Unfortunately, little information exists on the potential survival, establishment, growth, and subsequent genetic transfer ability of engineered organisms placed as competitors to indigenous organisms in a natural environment (13), though most evidence suggests survival is most likely to be diminished. Laboratory conditions are artificial and differ significantly from those encountered by organisms in their native habitat. For example, the mean generation time for many bacteria in soil is about six months (although this figure varies widely for different soil organisms and with the season), compared to one hour or less under laboratory conditions. The time of year and the local qualities of individual introduction sites could also affect survival significantly.

In one experiment, naturally occurring *P. fluorescent* were isolated from corn roots and

given genetic markers to allow them to be detected at a later time. The organisms were then reinoculated onto the corn roots at a moderate density. During the following growing season it was difficult and in some cases impossible to re-isolate the marked organisms, Thus, a soil system (and perhaps other natural habitats) might not contain enough nutrients to allow measurable survival of laboratory-adapted microorganisms. Certainly for genetically engineered micro-organisms (and perhaps other organisms), the problem will likely be less one of persistence and gene transfer than of survival to perform the job for which they were designed.

Potential Nontarget Recipients

For horizontal gene transfer to take place, a compatible recipient must be available. The most likely recipient is an organism genetically similar to the engineered host. The probability of transfer generally declines as evolutionary relatedness decreases. Restriction enzyme systems that degrade evolutionarily unrelated "foreign" DNA are common among bacteria.

Information about the natural history of potential nontarget organisms in the environment, however, is scarce—less than for laboratory-engineered organisms. In the case of bacteria for agricultural applications, potential microbial recipients in soil are of interest. Yet only about 10 percent of the microbial species in soil can even be cultured in the laboratory.

Horizontal transfer of genetic material between higher organisms is less likely than that between simpler ones. However, gene transfer via sexual recombination among these organisms could be an important problem. In particular, genetic movement via natural sexual transfer from crop plants (e.g., engineered to be herbicide resistant) to related weedy species could occur. Such problems are neither new nor unique to engineered plants, however (see ch. 5), and the processes involved are understood.

Density

Important factors affecting the magnitude of horizontal gene transfer are the absolute densities of the introduced and recipient organisms. According to laboratory research with bacterial systems, the rate of transmission seems to be proportional to the product of the densities of the donors and recipients.

In the case of micro-organisms, it appears that the numbers of naturally occurring nontarget recipients in the environment (e.g., in soil or water) are low-considerably lower than the concentrations necessary for efficient gene transfer in the laboratory. For instance, among organisms that are well studied, the number of naturally occurring organisms in fertile soil is normally at least an order of magnitude lower than concentrations of bacteria necessary for horizontal transfer in the laboratory.

Density can also be affected by the method used to introduce an engineered organism. Additionally, the timing of the planned introduction can affect the density of both the engineered organism and potential nontarget recipients. But keeping introduction densities low to avoid gene transfer may not be consistent with an effective introduction, since high initial density and survival may be required for efficacy.

Selection Pressure

The probability that new genetic material will persist, be expressed, and increase in frequency in nontarget populations if transmitted is at least as important as the probability of horizontal gene transfer itself. Selection pressure is the major determinant. A low probability of positive selection —i.e., little likelihood of the persistence of the new material—is usually the desirable outcome.

Selection pressure is determined by a combination of factors, including the trait encoded by the engineered gene, the potential recipients, and the value of the trait in the introduction environment. Because environmental conditions are generally harsh and stringent (e.g., inadequate nutrients for growth, and suboptimum temperature conditions), selection pressure is crucial. Under usual conditions (i.e., the gene product does not confer a selective advantage), even a moderate amount of new DNA assimilated by an indigenous soil or water microorganism may impose enough of an energy drain that the organism will be selected against in competition with others that

do not carry additional DNA. However, different introductions will vary with respect to the important selection pressures, and they must be evaluated separately.

Even a low horizontal transfer rate can establish the trait in a new species if assisted by strong selection pressures. Although some individuals point to the rapid spread of antibiotic resistance in gonococcus as an example of the widespread problems that can occur when genes are horizontally transferred, it is important to realize that intense selection pressure exerted by indiscriminate and subtherapeutic antibiotic use, especially in foreign countries, was probably the overwhelming cause of this phenomenon. The development of penicillin resistance by gonococcus illustrates the power of selection pressure to overcome such seeming obstacles as low rate of transfer.

TECHNOLOGIES TO MONITOR HORIZONTAL GENE TRANSFER

Beyond the intrinsic and extrinsic factors that could affect the magnitude of horizontal gene transfer in environmental applications, it is important to examine risk management methods that could be used to monitor both the dispersal of altered organisms and the movement of genetic material. Because proposals to introduce genetically engineered organisms are still new, detection or tracking methods are not highly developed. Experience (e.g., with past introductions of rhizobial or plant pathogenic bacteria) suggests that although such tracking methods will be needed in the future, their current level of development presents more inconvenience than danger.

An important distinction in monitoring is the difference between tracking the organism and tracking the gene or construct of interest that the organism carries. Improved methods to do both have been identified as one of the major unmet research needs in this area (see ch. 6). Some tracking technologies are now available.

Selective Screening Methods

One tracking method is based on the ability of researchers to mark a host organism's chromosome with genetic characteristics, such as antibiotic resistance genes or nutritional markers, that will confer an advantage to the organism when placed under specific conditions in the laboratory. These selective methods, principally used with micro-organisms, increase the probability that an investigator can isolate the test organism from the environment if it has persisted.

While useful in the laboratory, markers that could confer an unintended selective advantage in the environment, either to the host organism or nontarget recipients, should be avoided if possible, and carefully evaluated when used. One study concluded that "it is essential to choose antibiotics which are not in use in humans or animals, since resistance to clinically useful antibiotics is a major public health problem" (10). The example of penicillin-resistant gonococcus, cited earlier, underscores this point. But even the large-scale introduction into the environment of genes for resistance to nontherapeutic antibiotics should be carefully evaluated. Some resistance genes could mutate to counter whole families of related antibiotics. The kanamycin resistance gene, for example, could acquire the ability to neutralize newer antibiotics derived from streptomycin.

Some individuals, however, argue that the introduction of resistance genesis unlikely to cause problems, especially inland applications. The argument is based on two considerations, both involving micro-organisms. First, many resistance genes are already present in soil micro-organisms. In fact, this background of resistance could hinder tracking efforts, a problem that will almost certainly require the use of multiple selective markers. Second, studies of root ecology have long involved the use of antibiotic resistance with no apparent adverse effects.

Technologies using selective methods to track the genetically engineered gene itself are under development and promising approaches have been designed. The antibiotic resistance strategy puts a resistance marker near the gene of interest. The antibiotic could be used to recover any cells containing the resistance gene. In most cases, the gene of interest—the inserted gene—would travel with

the antibiotic resistance marker. So obtaining and quantifying cells that are antibiotic-resistant would allow the measuring of horizontal gene transfer to nontarget species. Another approach avoids the use of antibiotics and employs a metabolic marker such as lac, which brings the capacity for metabolism of lactose, as a convenient, innocuous, but effective tracer gene. The km gene is inserted close to the gene of interest so that it may serve as a linked marker.

Biochemical Screening Methods

A different approach to the tracking problem employs gene probes constructed through recombinant DNA technology. A segment of DNA that is complementary to the gene, or DNA sequence, of interest serves as the probe. The segment is labeled with radioactivity, a specific dye, or other tag that can be easily detected in the laboratory. A sensitive method, this gene probe technique may identify both host and nontarget recipients of labeled material. Similarly precise identification is also possible with antibody probe analyses derived from monoclinal antibody technology.

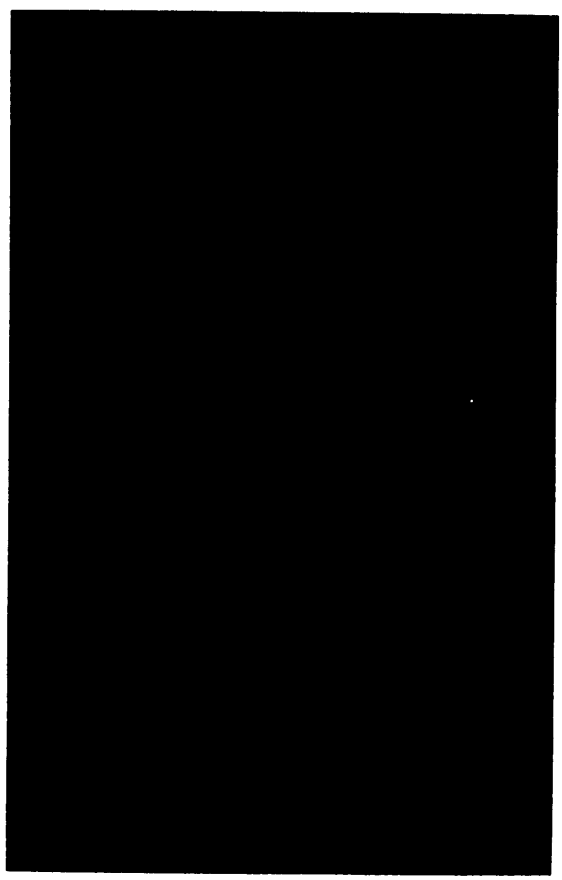
To apply these methods to bacteria or viruses, the organisms would be isolated from the environment and their DNA extracted and tested with the specific gene or antibody probe. With plants, leaves and other parts would be obtained and their DNA extracted and tested with the probe in the laboratory. By binding to an organism's DNA, the probe reveals that the organism carries the gene of interest. Such probes allow the detection of an inserted gene regardless of its position in the host's or recipient's genome. In contrast, the selective screening method just discussed becomes useless if the inserted gene becomes separated from a closely linked selection marker.

One disadvantage of the gene probe is that it provides no means of discriminating between samples that should be tested and those that should not. Everything that grows out of a sample (e.g., micro-organisms from the soil or a river, or plants from a wide area) must be screened. Furthermore, as mentioned earlier, many native micro-organisms cannot be cultured in the laboratory. Thus, these probe methods of tracking will probably be more useful to detect and monitor the presence of host organisms than to quantify horizontal gene transfer.

Research projects based on the gene probe concept are now being funded by the Environmental Protection Agency. The general applicability of biochemical monitoring appears promising. In the case of soil micro-organisms, extracted DNA must be purified sufficiently to meet laboratory conditions for the test to be accurate. In water applications, enormous volumes of water often must be processed to obtain test samples.

A related biochemical tracking method uses the luciferase gene cloned from fireflies. This gene codes for a light-emitting protein, luciferin, and has been inserted into plants and cultured plant cells that now "glow in the dark." Although the presence of the luciferase gene can be detected through the probe methods just described, it can also be detected easily through image intensifying video equipment or by contact exposure to photographic or x-ray film (see figure 4-1) (11).

Figure 4-l.—Luminescence From a Tobacco Plant Containing the Firefly Luciferase Gene



SOURCE: D.W. Ow, K.V. Wood, M. DeLuca, J.R.deWet, D.R.Helinski, and S.H. Howell, *Science* 234:856-859, 1986.

Using the firefly gene as a marker could be a fast, easy, and useful method to track genetically engineered organisms. It is very energy-intensive for the host organism, however, and its utility is therefore likely to be limited.

TECHNOLOGIES TO PREVENT OR REDUCE HORIZONTAL GENE TRANSFER

In addition to the technologies being developed to track host organisms and genes, methods are being developed that manipulate intrinsic factors so that introduced organisms have:

- **a lower probability of persisting after introduction,**
- **a lower probability of transferring genetic material, or**
- **both.**

Methods to prevent or reduce horizontal gene transfer are straightforward. The genetics and biochemistry of conjugal transfer have been well studied. By mutagenesis or genetic engineering, the capability for mobilizing DNA for transfer, and the genes specifying the necessary cellular apparatus for the transfer, can be removed from a plasmid. The plasmid can be further debilitated so that it is only poorly mobilized in the presence of another, potent plasmid. Such disabled plasmids are not capable of detectable horizontal gene transfer, and these disabled plasmids are commonly used at present in genetic engineering in bacteria.

Although the application of disabling methods may be an important component of planned introductions of genetically engineered organisms, the specific type of organism involved must be considered. Even the most active plant vectors, for example, probably have a lower likelihood of horizontal transfer than the least active bacterial vectors. Furthermore, it appears that genetically altered organisms that derive their utility by being deprived of a trait (i.e., a deletion) are less likely to be able to produce problems via gene transfer, although this might not always be the case (3,9).

Experience in working with recombinant DNA organisms in the laboratory provides some examples of success in restricting unintended gene movement by disarmament measures, These approaches, specifically the use of crippled bacteria and plasmids, have served as the starting point in developing ways to prevent or reduce horizontal gene transfer.

Debilitated Host Organisms

The degree to which a host organism should be debilitated will, again, depend on its intended application. In the case of a bacterium that will be used to degrade a toxic chemical, it might be prudent to use a self-destructing organism, since the bacterium only need persist as long as the pollutant is present. On the other hand, if the bacterium were designed to protect a plant from an insect pest, the organism persistence in the soil might be desirable (but see ch. 5).

One of the earliest attempts to construct a debilitated organism arose from the original questions surrounding the first uses of recombinant DNA technology. Although several studies had established that the organism initially used in recombinant DNA experiments (E. coli K-12) did not colonize the human intestinal tract (even after ingestion of billions of organisms by volunteers), a severely crippled strain of E. coli K-12, designated x^{1776}, was developed. This further debilitated derivative was, however, quite difficult to work with even in the laboratory. With experience, the original K-12 strain has proved to be an extremely successful and effective form of biological containment. The use of debilitated organisms in field tests, however, might compromise the value of the test, and therefore may not be a generally desirable approach.

Another approach sometimes suggested for reducing the chance of gene movement is to engineer restriction systems, common defense systems in naturally occurring bacteria, into a host bacterium. Restriction enzymes degrade unprotected DNA, so that foreign DNA from a donor is unable to infect the host. They are common enough in

natural populations of bacteria that they can be expected to play a significant role in deterring the transfer of genes from introduced engineered organisms. In addition, such naturally occurring restriction systems might be adapted to help inhibit transfer of the inserted gene out of the engineered host, or otherwise limit its function or persistence. At present, such systems are not well developed, but they hold substantial promise.

Disarmed or Nonmobile Vectors

In addition to engineering crippled host organisms, more stable vectors— those that would have little probability of facilitating genetic movement —are being developed. In particular, efforts are focused on obtaining microbial and viral vectors that are "escape-proof."

The concept of a debilitated host microorganism (e.g., E. coli K-12) was also applied to the development of a vector for that system. Plasmid pBR322 was isolated and has been used as a vector for transferring engineered genes in the laboratory. The plasmid is incapable of self-initiated transfer and is also poorly mobilizable. It is therefore considered safe; it has a low probability of being transferred to bacteria indigenous to natural habitats, including the human gastrointestinal tract. Similarly crippled vectors have been developed for use in insects and for mammalian genetic engineering.

Another precaution suggested by the gonococcal resistance example (see box A in ch. 1) is to **reduce or eliminate the use of mobile transfer elements in engineered organisms. Since some vectors are clearly more mobile than others, using disarmed versions of these vectors, or avoiding their use entirely, would reduce the probability of horizontal gene transfer.**

A disarmed vector (a transposable element) was the approach used in the insertion of the toxin gene into P. *fluorescent.* This technique appears to be successful, and the application to field test the organism is pending. Experiments show that it is unlikely that the nonmobile transposon will be excised (7,8).

Finally, an EPA research group is attempting to construct a "suicide" bacterium designed to persist in the environment only as long as it is needed. The organism is a bacterium that contains a vector (in this case a plasmid) that will self destruct in the absence of the toxic substance it has been designed to clean up. A better name for this technique might be "suicide plasmid," since the main purpose is to destroy the vector DNA before it transfers to another host. Other groups are also working on different means to similar ends (4).

However, the demonstrated ability of free DNA to sometimes maintain its integrity in soil or water creates a potential problem. If the cell were killed before the plasmid had self-destructed, and the plasmid with its inserted gene remained intact, it is possible that the plasmid could enter another cell.

SUMMARY AND CONCLUSIONS

The planned introduction of genetically engineered organisms (chiefly bacteria and plants) stands as the next research step in the anticipated biotechnological revolution. Although genetically altered organisms isolated through traditional genetic methods have been widely used in the environment for decades, the prospect of widespread application of genetically engineered organisms has heightened the concern of some that increased problems may arise. "The implication for R-DNA-engineered organisms is that large-scale or sustained applications might have consequences different from small-scale or single applications . . . the cumulative probability of undesirable effects resulting from repeated applications or frequent introductions must be considered" (5). Another potentially important question about the planned introductions concerns the possibility that an introduced organism might transmit its novel genetic material to nontarget hosts, resulting in unintended and possibly adverse consequences.

Most of what is known about horizontal gene movement has been discovered in laboratory studies. Little information is available on how the

phenomenon occurs in nature. There appear to be a limited number of gene transfer mechanisms; research has revealed that different types of organisms share some mechanisms for genetic. **transfer. Genetic material is not generally thought to transfer across large evolutionary distances, however, and there are numerous impediments to gene transfer, even between closely related species.**

For regulators to assess the potential genetic impact of an engineered application, several factors must be evaluated for their effect on horizontal gene transfer: intrinsic factors, such as the host organism, gene, vector, and construct, that are elements of the molecular techniques used to create the engineered organism; and various extrinsic factors that are elements of ecology, including survival, potential nontarget recipients, density, and selection pressure. Several methods can now be used not only to monitor survival of introduced organisms and genes, but also to reduce or prevent horizontal gene transfer.

Generic factors that can serve as a framework for regulation can be described. Technological advances exist or are being developed to protect the public and environment from any unintended consequences of introducing altered organisms. Some introductions merit closer scrutiny than others, and OTA finds that evaluation of proposed applications to introduce into the environment genetically engineered organisms which are believed to carry some element of risk should proceed on an adaptable, case-by-case basis, at least until knowledge has been accumulated to make more general reviews feasible. With an adaptable, case-by-case review of such planned introductions, not only the current spectrum of genetically engineered organisms, but kinds as yet unanticipated should be able to be tested in the environment without unreasonable risks. The current range of genetically engineered organisms seems to have a low probability of creating problems, particularly via the horizontal transfer of genetic information to nontarget recipients. However, this does not mean there are no risks at all. Careful regulation and enforcement can guard against potential environmental or public health problems and protect the biotechnology industry from the backlash and loss of credibility and confidence that a severe problem could precipitate.

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