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5. The Infectious Spread of Engineered Genes

INTRODUCTION

The deliberate release of genetically engineered organisms into the environment may provide a variety of economic and social benefits (see Chapter 1). Possible environmental applications of biotechnology include bacteria that have been engineered to degrade toxins, such as pesticides, that are contaminating soil and water (Kellogg et al. 1981; Broda et al. 1981; Chatterjee et al. 1981); pathogens that have been engineered to control pests, like weeds and insects, that are damaging forests and agricultural crops (Paul 1981; Yoder 1983; Pimentel 1985; Watrud et al. 1985); and crop plants that have been engineered for greater productivity, or to resist insects, disease, and even herbicides used to control competing weeds (Barton and Brill 1983; Hahlbrock et al., 1984; Goodman and Newell 1985; Hardy 1985). But attendant with these potential benefits are also potential risks associated with unanticipated consequences of the release of genetically engineered organisms into the environment (see Chapters 4 and 6).

Until recently, concerns over the safety of research with genetically engineered organisms were allayed by appropriate containment procedures (see Chapters 2 and 3). These procedures have limited not only the opportunities for physical transport of engineered organisms outside of research laboratories, but also the biological potential for survival and replication of these organisms should they accidentally escape physical containment. This "biological" containment has been accomplished by the use of organisms that grow well only under restricted conditions that are unlikely to be met outside research laboratories (Curtiss 1976; Curtiss et al. 1977).

But for applications requiring deliberate release, genetically engineered organisms must be chosen for their ability to survive and reproduce in natural environments, at least to an extent sufficient for them to carry out their intended functions. Thus, it is imperative to evaluate any possible risks associated with the release of genetically engineered organisms into the environment. As has been emphasized elsewhere, this evaluation should proceed on a case-by-case basis, because the complexity of ecological systems makes generalizations concerning possible risks of genetically engineered organisms difficult, if not impossible (Brown et al. 1984; Simberloff and Colwell 1985; Regal 1986). The primary foci of these evaluations, broadly stated, must be to predict the fate of the genetically engineered organism after its release into the environment (Sharples 1983; Stotzky and Babich 1984) and to anticipate the effects of the genetically engineered organism on populations of other organisms in the environment, and on important ecosystem processes, including nutrient cycling and productivity (Vitousek 1985; Flanagan 1986).

These evaluations are complicated by the possibility that an engineered organism may disappear (or otherwise have no adverse effects), but the DNA introduced during the course of its engineering may be transferred to some other organism present in the environment, where it has a different fate (or produces different effects). Although the infectious spread of an engineered gene to another organism is in some sense secondary to the direct effects of the engineered organism, it could potentially be very significant.

In this chapter, I provide several hypothetical cases where the infectious transfer of engineered genetic material results in adverse environmental consequences that would otherwise not arise. I then describe some of the mechanisms by which DNA can be infectiously transmitted from one organism to another, and I present a short review of our current understanding of the importance of infectious gene transfer in nature, drawing especially on data for bacteria. Finally, I provide a general framework which describes the information necessary to evaluate fully the likelihood of infectious transfer of an engineered gene.

HYPOTHETICAL EXAMPLES OF ADVERSE CONSEQUENCES ARISING FROM THE INFECTIOUS SPREAD OF ENGINEERED GENES

The scenarios that follow are provided only to illustrate the sorts of complications that could arise as a result of the transfer of genetic material from an engineered organism that has been deliberately released to another organism already present in the environment. They are not intended to be directly relevant to any specific applications currently being considered, nor are they intended to represent "worst case" scenarios.

Case One

Let us consider a bacterium that has been genetically engineered to degrade completely a pollutant that is present in some environment. The engineered bacteria derive energy from the degradative process, and so can use the pollutant as an ecological resource. However, the engineered bacteria are unable to compete successfully with the indigenous bacteria for naturally occurring resources. Thus, once the engineered bacteria have completed their intended biodegradative function, they should disappear from the environment without further consequence.

But let us imagine that one of the genes involved in this biodegradative process is infectiously transmitted to an indigenous bacterium. This gene permits the conversion of the pollutant into an intermediate compound. Unfortunately, the intermediate compound that is produced by the indigenous recipient of the engineered gene is even more toxic than the original pollutant.

Perhaps the engineered organism can use the highly toxic intermediate as a resource, and thus complete the intended degradation of the pollutant with no untoward consequences. This will depend on the details of the biochemistry, physiology, and ecology of the bacteria. The critical point is that it becomes necessary to answer many more questions. How many steps are involved in the degradative process, and how many of these yield net energetic gains for the bacteria? Are any of the biochemical intermediates toxic, and can bacteria take up these intermediates and complete their biodegradation? Can any or all of the engineered genes whose products are involved in the degradative process be transmitted to the indigenous bacteria?

Case Two

Now consider an insect that is a forest pest. There occurs naturally a highly specialized virus which can infect this pest, but it normally has little impact because

the insect has evolved a defense against this type of infection. The virus is genetically engineered, however, to bypass the insect's defense. This engineered virus can then be used successfully as a biological control agent, thereby avoiding the need to use chemical pesticides.

However, if the trait which permits the virus to overcome the insect pest's defense can be transmitted to other viruses that infect a broad array of insects, then many beneficial insect species might be harmed. It is conceivable that the deleterious effects of other insect pests, released from control by their beneficial insect predators, could be even more harmful than the effects of the pest that was originally targeted by the engineered virus. In fact, situations that are very similar to this have been documented for chemical insecticides (see DeBach 1974). Once again, the possibility that the engineered genetic material could spread to other species, each with its unique ecological relationships, greatly increases the number of questions that must be addressed in order to ascertain any possible risks associated with deliberate release (see Pimentel 1985).

Case Three

Finally, let us consider a crop plant that has been engineered to be resistant to herbicides. Herbicides can be applied in higher doses without adversely affecting the crop plant, thereby permitting higher yields. One might reasonably view crop plants as particularly safe organisms for genetic engineering and introduction into the environment, since agricultural systems are not natural, but highly simplified and tightly managed. Crop plants are adapted to rapid growth in environments that are tailored to their needs, but they are unlikely to fare well in other settings. Once again, consider the possible effect if the engineered herbicide resistance could be infectiously transmitted to other plant species, including weeds.

In order to evaluate the likelihood of this complication, we would again need to address many more questions than would otherwise be necessary. Can infectious transfer of the resistance trait, perhaps mediated by plant viruses, occur in nature? What is the range of host plants that these viruses can infect? What is the source of the genetic material used to engineer the herbicide resistance in the crop plant, and could it be transmitted directly from this source to the weed species in nature?

MECHANISMS OF INFECTIOUS TRANSFER OF GENES, AND THEIR ECOLOGICAL AND EVOLUTIONARY SIGNIFICANCE

Mutation and recombination generate variability within biological populations. This variability provides the genetic basis for natural selection, which is responsible for the diversification of organisms and their adaptation to the environment. The traditional view of evolution, which has been based largely on higher organisms, is that genes follow vertical lineages, or lines of descent. Sexuality, as manifest by Mendelian segregation and reassortment, provides a mechanism for the formation of new gene combinations in conjunction with production of new individuals. Sexual recombination of genes normally occurs only within the confines of a single species. In fact, this traditional view is embodied in the most widely accepted definition of biological species: "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1970). Hybridization between closely related species may occur under exceptional circumstances, but the traditional view of evolutionary processes gives no consideration to the movement of genes between organisms that is independent of the production of new individuals.

In procaryotes (which include bacteria and blue-green algae), the situation is dramatically different from this traditional view. First of all, procaryotes reproduce in a clonal manner, without sex and the resulting genetic recombination. One might therefore imagine that mutation is the sole source of genetic variability in populations of procaryotes, but this is not true (Reanney 1976; Slater 1984). Intensive genetic studies over the last several decades have identified a variety of mechanisms that can result in gene exchange, especially among bacteria. In fact, these mechanisms are utilized by molecular biologists in their efforts to engineer the recombinant organisms intended for biotechnological applications.

Gene exchange in bacteria operates independently of the production of new individuals, and hence is termed horizontal gene transfer. Mechanisms of horizontal gene transfer are sometimes also called infectious, because they can be mediated by semiautonomous extrachromosomal genetic elements, which behave to varying degrees as molecular parasites. For example, transduction refers to horizontal gene transfer that is mediated by viruses. During the course of replication and packaging of their own DNA, viruses may pick up sequences from their host's genome, which

can then be transmitted to other hosts during subsequent infections. Certain viruses can actually integrate their genetic material into the host's genome, and such viruses are especially likely to transmit host genes. The phylogenetic distance over which virus-mediated exchange of host genes can occur is determined by the host range of the virus, which may be quite broad. For example, the virus P1 can infect cells belonging to several different genera of bacteria (Reanney 1976).

Another process by which bacteria can exchange genes is mediated by extrachromosomal elements called conjugative plasmids (Falkow 1975; Broda 1979; Day 1982; Hardy 1986). Plasmids differ from viruses in that they have no extracellular particle state. However, many plasmids are able to promote their own transfer from one bacterium to another. This process, referred to as conjugation, requires physical contact between two cells. Transfer of the plasmid is facilitated by a special structure, termed a pilus, that is produced by the plasmid-bearing cell (Bradley 1981). As with viruses, certain plasmids may become integrated into the host genome, increasing the likelihood that chromosomal genes will be transmitted along with the genes of the extrachromosomal element. Conjugative plasmids may also mobilize the transmission of other nonconjugative plasmids, which do not encode pilus formation and are not otherwise transmitted horizontally. Once again, the phylogenetic extent of plasmid-mediated recombination depends on the host range of the plasmid. This may be extremely broad, especially when one allows for indirect transmission occurring through intermediates. For example, Barkay, Fouts, and Olson (1985) have demonstrated homology in the plasmid-encoded mercury resistance genes for diverse gram-negative bacteria.

Bacteria may also exchange genes via a process known as transformation. Unlike transduction and conjugation, which are mediated by semiautonomous genetic entities, transformation involves the exchange of free DNA between cells. For some bacteria, including the widely studied Escherichia coli, transformation occurs only under restricted conditions that are unlikely to be met outside the laboratory. For other bacterial species, however, transformation may be an important form of recombination under natural conditions, as suggested by the work of Graham and Istock (1979) on Bacillus subtilis.

Genes may also be exchanged between chromosomes and extrachromosomal elements via a mechanism known as transposition. Transposons (also sometimes called mobile genetic elements or "jumping genes") contain special regions of nucleotide bases at their extremities that promote recombination with sequences elsewhere in the genome (Campbell 1983; Shapiro 1983; Levy 1985; Hardy 1986). Viruses themselves may recombine if two different types infect the same host. Similarly, plasmids may recombine when two or more forms occur together in a host cell. In a sense, these semiautonomous elements can undergo a sort of "modular" evolution, at the same time mediating the acquisition of new functions in their bacterial hosts (Reanney 1976; Botstein 1980; Muster et al. 1981).

At present, it is not clear how important analogous processes are in eucaryotes. Within the confines of a single species, sexually mediated recombination is certainly far more important than horizontal gene transfer. But there are at least occasional opportunities for infectious transmission of genes involving eucaryotic species. The Ti plasmid of Agrobacterium tumefasciens can become incorporated into the genome of infected plants, and can also be used to mediate the interspecific transfer of genetic material (Zambryski et al. 1983; Depicker et al. 1984; Horsch et al. 1984; Schell et al. 1984). Also, it has been hypothesized that the mitochondria and chloroplasts found in eucaryotic cells are themselves derived from ancient symbiotic procaryotes (Margulis 1970), which suggests additional possibilities for gene transfer between distantly related taxa (Zambryski et al. 1983; Timmis and Scott 1984; Price et al. 1986). Processes comparable to viral transduction in procaryotes can almost certainly occur in eucaryotes, and there are several instances of possible interspecific transfer of genetic material that might be explained in this way (Krieber and Rose 1986). However, it seems unlikely that horizontal gene transfer has had the evolutionary significance in eucaryotes that it has had in procaryotes.

In bacteria, genetic traits that are commonly transmitted by plasmids and viruses include resistance to antibiotics and to heavy metals, the ability to produce certain toxins, and the ability to metabolize various substrates (Reanney 1976; Day 1982; Hardy 1986). These horizontally transmitted traits typically can generate a profound

selective advantage for the host bacterium under appropriate environmental conditions (e.g., in the presence of an antibiotic), and they allow a bacterium to acquire a complex function without the combination of improbable mutations that would be necessary to evolve that function de novo.

In the absence of the appropriate selective regime, however, carriage of plasmids or integrated viruses which encode these functions may have no benefit to the host bacterium, and instead may be so much "excess baggage," owing to the added costs of producing the extra DNA and proteins. Bacteria may lose plasmids and viruses via segregation of their genetic material during cell replication. These bacterial segregants may then out-compete those bacteria-carrying plasmids or viruses that provide no direct benefit. Accordingly, the relationship between bacteria and their plasmid and viral vectors of horizontal transfer can be viewed on a continuum from mutualism to antagonism (Levin and Lenski 1983). On the one hand, these vectors provide bacteria with new and useful functions that would otherwise be difficult to evolve, thereby benefiting their hosts. On the other hand, these vectors of horizontal gene transfer are truly parasites, utilizing the resources and machinery of their bacterial hosts to ensure the replication and infectious transmission of their own "selfish" DNA.

This duality has led to some academic disagreement and perhaps confusion concerning whether horizontal gene transfer should be viewed as an integral feature of the bacterial life cycle, or alternatively as an indirect consequence of the infectious transmission of subcellular parasites. This duality also may affect how we view the potential significance of infectious transmission of recombinant DNA from genetically engineered organisms that are deliberately released into the environment. Is infectious transmission of genetic material so pervasive that we can assume that any recombinant engineered in the laboratory has already been tried in nature, so that we have nothing new with which to concern ourselves? (See also Regal 1986.) Or is infectious gene transfer so rare that we can effectively ignore it in our considerations of the possible risks associated with the release of genetically engineered organisms?

Despite these academic arguments, there are few biologists who would not agree with the statement that rates of infectious gene transfer vary considerably. For example, genes that are found on the vectors themselves are far more likely to be transferred than are genes normally found in the host chromosome. Thus, although the spread of plasmid

borne antibiotic resistance in enteric bacteria gives ample evidence for the potential for rapid exchange of extrachromosomal genes (Falkow 1975; Broda 1979; Koch 1981; Hughes and Datta 1983; Levy 1985; Hardy 1986), detailed studies of the patterns of linkage among chromosomal variants in *E. coli* indicate that horizontal transmission of these genes is a rather rare occurrence (Selander and Levin 1980; Caugant et al. 1981; Selander and Whittam 1983). Similar results are obtained for the pathogens *Haemophilus influenzae* (Musser et al. 1985) and *Legionella pneumophila* (Selander et al. 1985), whereas the patterns of linkage among genetic variants in *Neisseria gonorrhoeae* suggest a much higher degree of horizontal transfer of chromosomal genes (Musser 1986). This variability may reflect differences in the nature of the mechanisms of gene exchange operating between taxa; differences in the susceptibility of various taxa to infection by certain types of vectors; and differences in the biological, physical, and chemical properties of the environment, which influence the degree of cell contact and the population dynamics of vectors and hosts (Stotzky and Krasovsky 1981; Freter 1984).

The significance of this variability is that there are no simple answers to the questions regarding the likelihood of infectious transfer of recombinant DNA. Perhaps the only broad generalization to be made is that infectious gene transfer is neither so rare that we can ignore the possibility of its occurrence, nor so common that we can assume its consequences to be trivial. Therefore, it is prudent that the evaluation of the likelihood of infectious gene transfer be undertaken on a careful case-by-case basis when considering the deliberate release of recombinant organisms into the environment.

A GENERAL FRAMEWORK FOR EVALUATING THE RISKS ASSOCIATED WITH INFECTIOUS GENE TRANSFER

In the preceding section, we saw that a variety of processes could give rise to the infectious transfer of genetic material, and I claimed that no broad generalization could be reached concerning the importance of these processes in nature. What, then, would we need to know for a particular case in order to evaluate fully any potential risks associated with the infectious spread of the engineered genetic material?

The hypothetical scenarios presented earlier in this chapter demonstrate that we need to understand as much

basic biology as possible about the recombinant organism and about the natural community of organisms into which it is to be released. Only then will it be feasible to identify the potential risks, and seek to minimize their likelihood and their impact. It is therefore essential that we endeavor to bring as much basic biological expertise as possible into the risk assessment process.

What follows now is a general framework for assessing the likelihood of horizontal gene transfer, and evaluating alternative measures for minimizing this likelihood. It presumes that the basic biology of the engineered organism and the natural community into which it is to be deliberately released are sufficiently well understood that one can identify particular routes of infectious gene exchange that might produce undesirable consequences.

First, it is important to recognize that the likelihood of an adverse consequence resulting from the deliberate release of a recombinant organism into the environment is dependent upon scale (Levin and Stewart 1977; Alexander 1985a). That is, an adverse consequence arising from a low rate of infectious spread of an engineered gene is not likely to occur if the recombinant organisms are released into the environment in very small numbers. However, with the release of many recombinants, the same low level of horizontal gene transfer may be significant. This scale effect is especially relevant because we are considering biological entities, which are self-replicating.

The effect of scale is well illustrated by the rise of resistance to penicillin in the bacterium N. gonorrhoeae. For many years, the gonococcus was treated efficaciously by penicillin, and only occasional low-level resistance was observed. However, in 1976, this pathogen acquired a gene that encodes the enzyme beta-lactamase, which enables a cell to cleave the penicillin molecule, and resistance by the gonococcus rapidly became a serious clinical problem. It appears that a plasmid encoding this function was transferred from an enteric bacterium to the gonococcus, a very rare event (Roberts et al. 1977). Had penicillin therapy of gonorrhea been conducted on a much smaller scale, clinically significant resistance might never have arisen. Unfortunately, one difficulty with risk assessment procedures that are based solely on direct observation is that they can tell us nothing about rare events, which might still have extremely important consequences. Thus, we should be willing to include in our considerations

any reasonable inferences that are based on parallels with similar systems where we have greater experience.[1]

In addition to absolute scale, the dynamics of horizontal gene transfer are dependent on the local population densities of the donor and the recipient. In particular, the rate of infectious spread of an engineered genetic molecule can be expected to be proportional to the product of these densities, multiplied by some factor that indicates the intrinsic rate of gene transfer (Stewart and Levin 1977; Levin et al. 1979; Levin and Rice 1980; Evans 1986). The magnitude of this intrinsic rate will depend on the properties of the particular recombinant organism and the natural community into which it is to be released. Some of the important properties will include whether an engineered gene in the recombinant is located on a chromosome or on an extrachromosomal element; the propensity of the vector to integrate into its host's genome; the susceptibility of the recipient population to infection by the vector; and the physical and chemical properties of the environment that affect the survival of the vectors, the accessibility of donors and recipients, and so on.

Levin and Stewart (1977) examined the dynamics of infectious gene transfer that would result from the hypothetical release of a recombinant organism into the environment.[2] In their calculations, they identified two critical variables. The first is the ecological fitness of the donor recombinant organism, which is expressed as the rate at which the recombinants are lost from the community. The second variable is a measure of the rate of infectious transfer of the engineered genetic material to indigenous organisms in the community. This rate is expressed so that it is independent of the densities of the donor and recipient populations. In addition to these variables, their computations require the initial density for the donor population, as well as the density for the recipient population, which is assumed to remain constant through time. One can then derive a simple integral to compute the expected number of events resulting in infectious transfer of the engineered gene prior to the extinction of the donor recombinant population. In particular, it can be demonstrated that the likelihood of infectious spread of an engineered gene is as sensitive to the fitness of the recombinant donor as it is to the rate of horizontal gene transfer. If the recombinant organism does not persist in the environment, then there can be some reasonable expectation

of avoiding any adverse consequence associated with a low level of infectious transfer. But if the recombinant organism persists indefinitely, then there is an ever-increasing likelihood of eventual transfer of the engineered genetic material to an indigenous population, with its attendant risks. Thus, the fate of the recombinant organism, of obvious importance in its own right, is also inextricably linked to the likelihood of infectious spread of its engineered genes to other organisms.

What factors determine the fate of the recombinant organism? Once again, answering this question requires a detailed understanding of the biology of the recombinant organism and of the natural system into which it is to be released, which can only be attained by careful study on a case-by-case basis. However, let me repeat a widely stated explanation for why a population of recombinant organisms might fail to persist after its deliberate release. According to this argument, the introduction of foreign genes into an organism, although providing some function of biotechnological utility, is intrinsically maladaptive to the recombinant organism. That is because the engineered genes are presumed to represent the same sort of "excess baggage" associated with DNA replication and protein synthesis that was discussed when considering the parasitic nature of vectors of horizontal gene transfer (see also Regal 1986). Thus, it is assumed that natural selection will purge these ecological "misfits" from communities after they have performed their intended biotechnological function. If true, this argument should go a long way towards allaying not only those concerns about risks associated with the direct ecological effects of the recombinant organism, but also those concerns arising from the indirect effects of horizontal gene transfer.

Unfortunately, there is a dilemma to be faced in the engineering of a recombinant organism for deliberate release into the environment. On the one hand, ecologically unfit recombinants reduce the possibility of adverse consequences associated with their release. On the other hand, many of the applications of recombinant organisms require that they be able to survive and replicate in the environment in order to perform their intended biotechnological functions. Thus, there is a fine line between recombinants that are too fit and those that are not fit enough (see also Simberloff and Colwell 1985). Whether this balance can indeed be attained is likely to depend on the specificity of the traits that are engineered into recombinant organisms. That is, a high specificity of ecological function

should allow a recombinant trait to be maintained only where it is needed.

Evaluating this specificity is especially difficult, however, when one considers the infectious transfer of an engineered trait from one organism to another. In each of the hypothetical examples presented early in this chapter, the engineered trait in the recombinant organism satisfied the criterion of a high degree of ecological specificity. Yet in each case, the expression of this trait in another organism had dramatically different ecological consequences. This is because the fitness effects associated with carriage of a particular gene may depend on the genetic background, as well as on the environment, in which it is found (see also Dykhuizen and Hartl 1983; Hartl 1985).

Thus, even knowing the likelihood of horizontal gene transfer tells us very little about the fate of the recombinant organisms that may arise in situ. An analogy can be made between horizontal transfer of a gene between organisms and dispersal of an organism between habitats. Dispersal of an organism to a new habitat is unlikely to have any lasting effect if it is poorly adapted to the conditions in its new habitat and cannot become established. Similarly, infectious transfer of engineered genetic material is likely to be of consequence only if it provides a selective advantage to the recipient (see also Alexander 1985b; Hartl 1985). If the engineered genetic material allows the recipient to utilize an additional resource, or permits the recipient to become less sensitive to the effects of some agent of control, then it is much more likely that the horizontal transfer will have a significant ecological impact. In fact, one can extend the analysis of the dynamics of infectious transfer of an engineered gene to incorporate the effect of the selective advantage, if any, accruing to the recipient. This analysis indicates that the likelihood that a recipient of an engineered gene will become established in its natural community is directly proportional to its selective advantage (see similar analyses by Haldane 1927; Feller 1957; and Lenski and Levin 1985a).[3]

It is sometimes assumed that ecological niches are "full." (See exchanges between Davis 1984 and Simberloff and Colwell 1985, and between Brill 1985a, b, and Colwell et al. 1985.) If valid, this would suggest that horizontal transfer of engineered genetic material is unlikely to provide the recipient with a truly novel selective advantage that allows it to proliferate with unanticipated consequences. This view ignores, however, two important

considerations. First, natural communities are constantly undergoing change, and few, if any, are invulnerable to invasion by introduced species (see Chapter 4; also Sharples 1983; Simberloff 1981, 1985). Second, there are constraints on evolutionary processes that limit the ability of organisms to become "perfectly" adapted, including the simple lack of appropriate genetic material (Gould and Lewontin 1979; Lenski and Levin 1985a; Regal 1986). One simply cannot assume that organisms have long ago "solved" all the problems of their adaptation.

It has also been argued that the introduction of foreign genes is no more likely to have unintended consequences than traditional practices used in agriculture. (See exchanges between Brill 1985a, b, and Colwell et al. 1985; and among Hardy and Glass 1985, Lenski and Levin 1985b, and Regal 1985.) In fact, there are examples in which crop varieties produced by traditional means have become weeds, and there are instances of apparently very simple genetic changes in natural populations that have had major ecological effects (Alexander 1985b; Colwell et al. 1985; Simberloff 1985). Moreover, this argument ignores the primary advantage of new recombinant techniques over traditional selective breeding programs employed for many centuries: recombinant techniques enable one to move genetic material across taxonomic barriers where natural gene exchange either does not occur or else occurs only very rarely. Therefore, we should not exclude the possibility that the transfer of engineered genes in situ may provide novel genetic variation and new ecological benefits to the recipient organisms.

We must know the following in order to predict accurately the likelihood of an adverse consequence arising from the infectious spread of engineered genetic material. First, we must understand the biology of the recombinant organism and the ecology of the community into which it is to be deliberately released in sufficient depth so that we can identify the horizontal gene transfer events that could reasonably be expected to produce potentially adverse effects. Without this knowledge, the complexity of ecological communities and the number of possible routes of gene exchange make a comprehensive survey infeasible. Second, we must be able to quantify the likelihood that such horizontal transfer will occur and give rise to these potentially adverse effects. This likelihood is equally sensitive to three critical factors: the ecological fitness of the deliberately released recombinant organism, which will

determine its persistence in the environment and hence its opportunity to serve as donor of an engineered gene; the intrinsic rate of transfer of an engineered gene, which will depend upon the specific characteristics of the donor, vector, recipient, and environment; and the effect of an engineered gene on the fitness of the potential recipient, which will determine whether the recipient that arises in situ can become established in the ecological community.

Finally, it must also be emphasized that one cannot assume that the various factors that are important in determining the likelihood of infectious spread of engineered genetic molecules will remain constant. While the laws of chemistry and physics are constant, the relationships between organisms and even between genes within an organism are constantly changing (Lenski and Levin 1985b; Regal 1986). For example, there may be genetic changes in recombinant organisms that modify the rates at which engineered genes are transmitted. A gene may transpose from a chromosome or a nonconjugative plasmid to a conjugative plasmid, greatly increasing its ability to be infectiously transmitted (Levy 1985; Slater 1985). A plasmid that is repressed for the production of conjugative pili may become derepressed, allowing that vector to be maintained in a population where it could otherwise not persist (Lundquist and Levin 1986). Similarly, genetic changes may be favored which compensate for the "cost" of carrying an engineered trait. This may occur, for example, as the result of mutations that alter the regulation of gene expression, so that some gene product is no longer overproduced when it is not beneficial (Dykhuizen and Hartl 1983; Moyed and Bertrand 1983). The effect of such changes will be to increase the stability of the engineered trait in the recombinant organism, and thereby enhance the persistence of the recombinant organism in the environment, which in turn increases the opportunity for subsequent infectious spread of an engineered gene (see also Roberts et al. 1977; Lenski and Levin 1985b). Thus, the heterogeneity of ecological systems in space and time, and the ability of biological populations to undergo evolutionary change, introduce further variables that must be considered.

SUMMARY

The deliberate release of genetically engineered organisms into the environment may provide a variety of

economic and social benefits. Evaluating the possible risks associated with the release of genetically engineered organisms into the environment is difficult because of the complexity of ecological systems. This risk assessment is further complicated by the possibility that an engineered gene may be transmitted to another organism. It is possible to construct plausible scenarios wherein the spread of an engineered gene to an indigenous organism has deleterious consequences, even though the engineered organism itself causes no direct adverse effects.

According to traditional views in genetics and evolutionary biology, recombination is limited to Mendelian segregation and reassortment, which occurs during sexual reproduction and is confined within species boundaries. Procaryotes (including bacteria) reproduce asexually, yet they can undergo genetic recombination by a variety of mechanisms, some of which are mediated by subcellular parasites (including viruses). These mechanisms are variously termed infectious or horizontal gene transmission, and they form the technological basis for genetic engineering. Infectious gene exchange may occur even between rather distantly related taxa, as witnessed by the spread of antibiotic resistance genes in bacteria.

Many factors influence the importance of infectious transmission as a source of genetic variation in natural populations. There is no broad generalization that can be made regarding the likelihood of infectious spread of engineered genes. Infectious gene transfer is neither so rare that we can ignore the possibility of its occurrence, nor so common that we can assume its consequences to be trivial. Thus, it seems prudent to evaluate any possible risks associated with the infectious spread of engineered genes on a case-by-case basis. One difficulty in evaluating these risks arises because horizontal transmission may often be a very rare event that nonetheless produces important effects, owing to the self-replicating nature of biological entities. Hence, the likelihood of observing the infectious spread of an engineered gene will be highly dependent on the scale of the deliberate release of the recombinant organism.

In this chapter, I have argued that we must have the following information in order to predict accurately the likelihood of infectious spread of an engineered gene. First and foremost, we must understand the biology of the recombinant organism and the ecology of the community into which it is to be deliberately released in sufficient

detail that we can identify those avenues of horizontal gene transfer that could produce potentially adverse environmental effects. Second, we must be able to quantify the likelihood that such horizontal transfer will occur, and this requires answers to several questions: How long will the deliberately released recombinant organism persist in the environment, and hence be a potential donor of the engineered gene? At what rate is the engineered gene infectiously transmitted to the potential recipient? And how will the engineered gene affect the ecological fitness of the potential recipient, and thus its ability to proliferate in the environment? Also, the heterogeneity of ecological systems in space and time, and the ability of biological populations to undergo evolutionary change, introduce further variables that must be considered.

It should be apparent that evaluating any possible risks associated with the infectious transmission of engineered genes requires an interdisciplinary effort (see Chapter 7; also Regal 1985; Flanagan 1986). Molecular biologists must investigate the expression and stability of engineered traits, and they must understand how these are affected by the genetic background in which the traits are found. Population geneticists must measure the rates of gene transfer in natural populations, and evolutionary biologists must determine the effects of engineered traits on the fitness of organisms. Ecologists must understand the structure of natural communities in order to evaluate changes in the selective pressures resulting from genetic modification of organisms, and they must be able to use this information to predict the effects of genetic modification on such diverse processes as the dynamics of populations and the cycling of nutrients in ecosystems. In the coming years, ecologists and evolutionary biologists may learn a great deal by observing the effects that result from the careful modification of specific genetic traits, and from this, they may be able to offer suggestions regarding how to engineer organisms that are not only safer, but also more effective in their intended biotechnological applications.

NOTES

1. It is of interest to note in this regard that Falkow and colleagues used such reasoning to suggest the possibility of plasmid-mediated penicillin resistance in Neisseria gonorrhoeae before it was actually observed.

2. In the 1970s, concerns focused on the accidental release of engineered organisms from laboratory containment. The mathematical analysis of the fate of deliberately released engineered organisms is identical.

3. It is also possible that a gene that is selectively neutral or even disadvantageous for the recipient could become established, provided that the rate of infectious transmission of that gene is sufficiently high (Levin and Stewart 1977). However, the conditions for this to occur are much more restrictive and, in my opinion, are generally less applicable.

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6. Evaluating Environmental Risks from Biotechnology: Contributions of Ecology

INTRODUCTION

The history of technology development is replete with examples of well-meant introductions of new technologies, followed by unexpected adverse consequences. Nuclear power plants, pesticide manufacture in Third World countries, and some Green Revolution plant varieties provide a few contemporary examples. One lesson to be gained from our past experience with new technologies is that they carry the risk of unforeseen deleterious side effects. Biotechnology represents a rapidly developing area, as yet free from such catastrophes; thus we are fortunate in having the opportunity to anticipate potential problems and to minimize potential social risks.

Biotechnology, as used in this chapter, refers to a class of techniques that can be distinguished from conventional methods of genetic manipulation such as selective breeding, crop rotation, introduction of exotic organisms, hybridization, and isolation of microbial pesticides. These technologies generally involve whole, intact organisms. Biotechnology extends these to include "genetic engineering" technologies based on the suborganismic level--for example, tissue culture, cell culture, and genetic transformation, the movement of genes from one organism to another by recombinant DNA (rDNA) techniques. These new techniques are in many respects quite similar to the more conventional genetic and breeding techniques, but they are distinguished by two characteristics: the potential to transfer genetic material between widely disparate organisms, and the potential to do this with precision.

Biotechnology has a large potential to improve the human condition, and to engender important advances in

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