Regulation of Products from Biotechnology

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PROPONENTS OF BIOTECHNOLOGY OFTEN ASSERT THAT THE safety of "genetically engineered" organisms has been established because adverse effects have yet to be documented after handling the organisms in contained facilities for a decade. But the past is not necessarily a reliable guide to the future. Although the absence of effects on the health of workers in biotechnology laboratories is admirable, it is not particularly relevant to the question of whether uncontained uses of modified organisms will be equally harmless. For various reasons, the concerns for environmental applications of biotechnology products are fundamentally different from those for laboratory uses.

Environmental Applications Versus Laboratory Uses

First, in environmental applications, it is the myriad of nonhuman species in an ecological community that will be exposed to released organisms. Second, the spectrum of potential effects is not restricted to pathogenicity, although this, too, is certainly a significant concern. Additions of nonindigenous organisms can influence the structure (population size and species diversity) and function (energy and material dynamics) of ecological communities through a variety of mechanisms that sometimes displace or destroy indigenous species. Such events are copiously documented in the literature of ecology (*1*), and experience with the ecological dislocations and economic losses that sometimes result when organisms are introduced into environments where they are not normally found is too abundant to be trivialized or ignored.

Third, the degree of control afforded by experiments conducted in containment differs from that involved in releases in the field. Once released, modified organisms that find suitable habitats may not only reproduce and spread, but can be expected to evolve in ways that are beneficial to their own survival. The evolutionary process can allow modified organisms to escape constraints imposed by debilitating them before their release, so that both physical and biological containment may be nullified outside the laboratory. Fourth, differences of scale become important as the transition from research to commercial products is made. It is one thing for trained experimenters to apply novel organisms to a 0.2-acre field under close supervision. It will be quite another matter to market commercial products for widespread use by applicators whose major qualification for using them is possessing the cash to acquire them. To

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Bacterial Domestication: Underlying Assumptions

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OVER THE PAST DECADE THE RECOMBINANT DNA ADVISory Committee (RAC) of the National Institutes of Health (NIH) has outgrown the initial assumption that all recombinant bacteria are dangerous until proved otherwise. Its current restrictions on laboratory research are essentially limited to experiments involving pathogens. Building on this experience, in the recent notice of a coordinated framework for the regulation of biotechnology (*1*), the Food and Drug Administration and the U.S. Department of Agriculture (USDA) announced that they plan to regulate genetically engineered microbes no differently from strains obtained by traditional techniques. The Environmental Protection Agency (EPA), however, adopted a different position; although this was an improvement on its initial, "process-based" proposal (*2*) (which even considered declaring DNA a toxic substance), it still presented highly restrictive and elaborate regulations, accompanied by an extensive exegesis on the hypothetical dangers of engineered organisms.

The conflicting regulations of the different agencies will create administrative problems. However, I will focus here on arguments—most of them presented during the debate over recombinant bacteria a decade ago (*3*)—against some of the underlying scientific assumptions. In addition, I will emphasize that the use of modified microbes is not entirely novel but is an extension of the old process of domestication of wild organisms—including the selection of microbial variants to make bread, wine, antibiotics, or vaccines. Finally, I shall argue that in trying to assess the potential dangers, the experience of ecologists with transplanted higher organisms is less pertinent than are the insights of fields closer to the specific properties of engineered microorganisms: population genetics, bacterial physiology, epidemiology, and the study of pathogenesis.

Not only are the present regulations quixotic, but the problem continues to receive much attention in the news media, and some legislators are proposing more restrictive new laws. Although there have been individual efforts [for example (*4*)] to counter demagogic attacks against this field and the resulting widespread misconceptions, they have been limited. Yet more than the ability of biotechnical industries to engage in field testing is at stake.

Accidental Release Versus Deliberate Introduction

The most basic question in the current debate is how much we are thrashing over issues that have already been settled in the delibera-

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assert, therefore, that we are merely thrashing over issues that were laid to rest years ago is to ignore all these important differences.

Evolution and Selections of New Organisms

Biologists in the molecular and ecological fields disagree in the application of "evolutionary principles" to arguments about safety issues. All biologists are, to some degree, "evolutionary biologists" in the sense that all scientists who study living systems receive schooling in the basics of evolutionary theory. Because evolution deals with changes in the genetic structures of populations of organisms, both those whose primary interest is in genes and those whose focus is on whole organisms and the higher systems of which they are a part (populations, communities, and ecosystems) can lay claim, if they choose, to the title of "evolutionary biologist." In fact, many scientists whose research interests are in such subdisciplines as "ecological genetics" prefer to call themselves "evolutionary biologists" rather than ecologists. In short, no one discipline in the biological sciences corners the market on the use and interpretation of "evolutionary principles." Both major factions in the biotechnology controversy can support their cases with evolutionary arguments.

Take, for example, the question of whether a novel organism is likely to survive and spread after release. It is frequently argued that "genetically engineered" organisms will not have superior ability to survive in the environment because the addition of the engineered genetic material is likely to disrupt the coadaptation of the natural genomes of the organisms or because the added genetic material is likely to pose a physiological burden and thus be a handicap, or both. Given the evolutionary principle that a "new" organism must have an advantage in order to survive and spread in the environment, and interpreting engineered modifications as disadvantages, it is not difficult to deduce that engineered organisms should not survive. But there is more to it than this relatively superficial view suggests.

The fundamental premise of evolutionary theory is that natural selection, the dominant force responsible for adaptations of organisms to their environments, operates on genetic alterations or novelties—mutations, rearrangements, and acquired accessory elements, such as plasmids—to produce evolutionary change. It follows that at least some genetic alterations improve the abilities of organisms to survive, reproduce, compete for resources, or invade new habitats. A general assertion that genetic alterations, be they natural or man-made, always lower the fitness of organisms is therefore not warranted and runs counter to basic evolutionary principles.

Some kinds of genetic alterations may be more apt to lower the fitness of organisms than others (*2*). In each of the major categories mentioned, some kinds of alterations probably do consistently lower fitness. Simple mutations that disrupt the production of necessary enzymes should, for example, certainly produce serious disadvantages. But what of mutations that do not affect essential proteins or that do not disrupt protein function? The existence of high levels of allelic diversity for many different proteins in many kinds of organisms is interpreted by some evolutionary biologists to mean that many simple mutations are not "perceived" by natural selection. And some simple mutations are clearly advantageous. Slight modifications in only one or a few genes are implicated or clearly documented in many phenomena involving changes in environmentally important phenotypes in all manner of organisms. Examples include expansions of the host ranges of insect and microorganism pests or parasites and acquisition of resistance to chemical control agents in insects and bacteria (*3*).

Mutations may also be associated with abilities of organisms to overcome natural limiting factors. Such changes may allow organisms to invade new habitats, which, in turn, may produce concomitant changes in their surrounding ecological communities. An example involves cheatgrass, a plant once restricted to moderately moist habitats. The Agricultural Research Service recently reported that because of a mutation that must have occurred about 10 years ago, cheatgrass is now able to colonize rangelands with dry sandy soils in which it was previously unable to survive (*4*). The overall result has been that millions of acres of western rangeland that were once considered unburnable are now subject to wildfires that destroy valuable grazing resources. Many of the engineered organisms being considered for environmental use also will have been purposely designed to overcome natural limiting factors such as low nitrogen, low temperatures, or predation by insects. Such changes, although accomplished with minor genetic modifications, can nevertheless be expressed as major shifts in properties of ecological significance.

Genomic rearrangements and chromosomal abnormalities are often associated with disease conditions, particularly in humans. Yet changes in the number and organization of chromosomes (for example, polyploidy) have frequently been exploited by breeders to produce superior plants and have clearly also been important in many major evolutionary events. Although rearrangements may often have catastrophic effects, this is not the inevitable result. Many genes found in plasmids or other accessory elements code for resistance to sources of stress such as antibiotics and heavy metals. Their role in ensuring survival of microorganisms under many kinds of extreme selection is well understood. Yet even in the absence of clearly identified selection factors, accessory elements are often maintained in microbial populations (*5*). The explanation for this may be that selection is operating on some cryptic gene with an unknown function. But it is not inevitable that the presence of an accessory element will serve as a handicap to survival in all environments.

Furthermore, the extent to which a handicap actually does reduce fitness can depend on the environmental context in which an organism finds itself (*2*). Suppose, for example, that there is an ecological system with two resources and one consumer organism. This organism efficiently uses one resource and leaves the other unexploited. Suppose, then, that a mutant form of the organism arises that is marginally equipped to use either resource. It cannot compete with its efficient parent for the resource the parent favors, but since the second resource is also available, the mutant has it all to itself. Despite its genetic handicap, the mutant form may be sufficiently fit to survive on the unexploited resource. If it can survive long enough, selection may subsequently increase its efficiency and its fitness. The inefficient exploitation of an abundant resource for which there is no competition may, in fact, be as effective as is efficient exploitation of a resource that is being rapidly depleted by competitors. In short, even handicapped organisms may find profitable strategies for survival, and it is not difficult to postulate circumstances that would allow this to occur. In microorganisms, engineered genes for the degradation of xenobiotics could result in such a situation. In general, there is no reason to assume that man-made changes will be any more or less likely to alter fitness than are naturally occurring genetic modifications. Particular modifications must be evaluated for specific organisms in particular environmental settings to arrive at valid determinations of the likelihood and effects of increasing or decreasing fitness.

Another favorite argument for dismissing concerns about the environmental products of biotechnology deals with "domesticated" species. Domesticated plants and animals are supposed to be familiar and their behavior predictable; unable to survive in the wild as a

result of artificial selection for traits of use only to their "masters"; and, above all, harmless. Most of the agricultural and horticultural plant species common in the modern western world provide reasonable examples of just these characteristics. If the benign successes of modern agriculture furnished the only experiences on record, the conclusion one would draw is that domesticated species are not capable of inflicting ecological harm. Such is not the case, however. Feral populations of domesticated animals, particularly goats and rabbits, have repeatedly been responsible for massive damage to natural vegetation in both island and continental settings all over the world. Domestic cats are associated with dozens of cases of harmful predation on other animals, including more than 30 cases of complete extinction (*6*), in places where cats have been released by humans. To claim that all domesticated species are debilitated and harmless is simply incorrect. In addition, the assertion that the products of biotechnology can be construed as the equivalent of domesticated species is dubious, at best. Among bacteria, probably only two or three taxa, such as the human gut commensal, *Escherichia coli*, and the various species of nitrogen-fixing symbionts in the genus *Rhizobium*, have been studied well enough to qualify for the "domesticated" label. If these familiar organisms represented the limits of biotechnology's horizons, there might indeed be little cause for concern, but they do not. The spectrum of organisms suitable for genetic engineering is already broad and, as technical capabilities continue to develop, may eventually include almost any organism deemed to have useful properties worth manipulating. It is not uncommon these days for genetic engineering efforts to begin on bacterial species that have only just been described and even before their basic physiological properties have been determined (*7*). In addition, most of what is known of the basic biological properties of bacteria is information that has been determined from laboratory work with single-species cultures. Knowledge of the biotic and abiotic interactions of most species in mixed populations in natural ecological systems is extremely limited. Currently the unknowns far outweigh the knowns where the ecological properties of microbes are concerned.

Harmful Natural and Engineered Modifications

Finally, there is the argument that no ecological harm will result from any man-made modifications that merely duplicate genotypes that already occur in nature. The basic premises are usually (i) that something must be truly "unique" (that is, not found in nature) to have potential for harm; and (ii) that in the 3 to 4 billion years over which life has evolved, nature herself has no doubt already produced organisms with all possible gene combinations. Since most of these have already failed the test of survival, there is no reason to be concerned that their man-made duplicates will be any more apt to survive and be harmful. Premise (ii) serves, in effect, as a neat "catch-22" for (i).

The premise that all possible gene combinations have already been tested in nature cannot be true. It has been estimated that there are 10^{70} atoms in the universe, whereas a single organism that is heterozygous at only 232 structural gene loci can produce 10^{70} different kinds of gametes (*8*). The sudden appearance of the virus that causes acquired immune deficiency syndrome should serve to convince us that nature occasionally does produce something with "new" and unanticipated properties. Equating "natural" with "harmless" makes no more sense than equating "artificial" or "man-made" with "harmful." Nature is full of harmful phenomena that would not be to mankind's benefit to duplicate or promote. And genetic modifications may be only one element contributing to an ecologically "unique" situation. Frequency-dependent effects and the influences of shifting environmental contexts are also important. To assert, for example, that the number of organisms released is not relevant to the magnitude of potential effects is to ignore a great deal of evidence to the contrary from both epidemiology and ecology. A basic principle of epidemiology is that the spread of an epidemic is dependent on, among other things, the size of the source pool of pathogens (*9*)—the larger the source pool, the more effective the transmission of the disease agent. Ecologists have repeatedly observed threshold effects in the abilities of populations to survive. Large and concentrated numbers of organisms above critical population sizes may gain footholds where small populations cannot. To state that the scale of an introduction or application is only important for chemicals, but not for organisms, is absurd. Chemicals are invariably diluted, and are often degraded, as they disperse among various environmental compartments (*10*). A population of released organisms that finds itself in a suitable environmental setting, however, may reproduce, evolve, and transfer genetic material to other organisms in the environment. Mistakes, therefore, can have permanent consequences.

Risk Assessment

Environmental scientists regard the safety of engineered organism products as a genuine concern that requires evaluation of associated risks. Regulation of biotechnology products is a means of ensuring that adequate consideration is given to risk assessment. This situation does not differ from that which pertains to new chemicals and drugs. Regulation of biotechnology products is justified and should be supported.

The Coordinated Framework for Biotechnology Regulation, issued by the Office of Science and Technology Policy on 26 June 1986, announced an overall federal policy for review of biotechnology products and dealt with a number of regulatory issues (*11*). First, it clarified the roles of the various regulatory agencies with statutory jurisdiction over the broad spectrum of product types that biotechnology offers. Second, it defined levels of review and regulation that are intended to reflect degrees of risk. As a first cut, the provisions of the framework were fundamentally reasonable with respect to review requirements. It is easy to agree that the limited resources available for risk assessment should not be wasted on innocuous products but rather should be concentrated where the probability of negative effects is greatest. It is more difficult, however, to agree with all of the particulars set out in the framework for distinguishing between the innocuous and the potentially harmful.

The underlying logic of the regulatory scheme is that organisms that are "new" require closer scrutiny than organisms that are not new because new organisms are more likely to have unique properties and their behavior in the environment will therefore be more difficult to predict. "New" organisms are then defined as those that are "deliberately formed to contain an intergeneric combination of genetic material." A number of problems with both the logic and the definition exist here.

First, the assertion that gene transfers between species in the same genus will always represent less risk than gene transfers between organisms in two different genera is highly suspect. The presumption is that congeners are genetically similar, and therefore the intrageneric transfer of a gene is unlikely to produce unusual changes in behavior. But classification schemes originated long before an understanding of a genetic basis for taxonomic relationships was possible, and congeneric genomes may not be highly similar. In addition, intrageneric combinations might present genuine risks, such as transfer of a rare gene for degradation of an

important substrate (for example, lignin) to a congener with broad environmental tolerances. If intrageneric transfers are to be routinely subject only to lower levels of regulatory review, the flexibility to elevate cases that present special risk factors to higher levels of review should be built into the policy framework. There is, in fact, already such an exception for intrageneric transfers between obligate pathogens, and a mechanism for dealing with other exceptions would be consistent with this approach.

Second, shifts in environmental contexts may be as important as genetic modifications in determining whether the ecological relationships of an engineered organism will be unique relative to those of a parental form. It is not certain before the fact, for example, that beneficial inhabitants of soil ecosystems will not be adversely affected by some property from a leaf-dwelling organism (for example, toxin production) when this property is engineered into soil bacteria. Although such a toxin may certainly not be "new" and certainly may be "natural," its relocation to a new environmental setting could produce unintended negative results on susceptible organisms exposed for the first time. Some provision is needed in the regulatory scheme to ensure that consideration is given to the specific nature of the receiving community and the species in it in assessing risks.

Finally, transfers of regulatory genes and gene deletions are excluded from the definition of "new," which relegates products with such modifications to low levels of review. But the absence of a protein or the amplification of its production could have profound ecological effects in many instances that are neither difficult to imagine nor highly unrealistic, for example, in modifying important biogeochemical processes. In short, the use of a strictly genetic definition to determine whether a particular product should be treated as high or low risk may underestimate ecologically relevant and important factors. Again, flexibility in implementing the policy is needed to take account of exceptions and shift particular cases between review levels when justified.

At this time, all engineered organisms for environmental release should receive at least a minimal level of review to allow screening of the kinds of ecologically relevant exceptions mentioned here. It is too early to create categories of organisms that are completely exempt from review. Although the process of conducting risk assessments for engineered organisms is currently far from routine, as experience is gained, the process will become both more accurate and more efficient. At the moment, only a few new products are entering the regulatory mill. We have the opportunity to compile the knowledge needed to narrow the concerns and streamline the review process before many products need to be regulated. Credible regulatory oversight is essential to ensure public acceptance of biotechnology's products. Evaluations of both the genetic and ecological properties of engineered organisms will foster confidence in their safety and effectiveness.

Ecologists who have voiced their reservations about biotechnology's environmental products have done so for reasons of professional integrity and because of their concern for the environment. We are not Luddites or alarmists, but merely skeptics who wish to consider what the hidden costs of this promising new technology might be.

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tions of the NIH RAC. The root of the new wave of concern is the assumption that large amounts of engineered bacteria deliberately introduced (*5*) to the environment are much more dangerous than small amounts accidentally released from the laboratory. This tacit proposition has seemed self-evident, by extrapolation from toxic chemicals. But the problems are very different. With bacteria it is not the harmful effects of the released material itself, but its capacity to multiply and hence possibly to spread in the environment, that causes concern; the obverse side to this difference from chemicals is that bacteria also have the capacity to die out rapidly. In any concrete case, then, the crucial question is whether the strain will spread or will die out.

We are thus dealing with a problem in natural selection, where success of a novel strain does not depend on its introduction in large numbers. A new gene arises in evolution in a single individual and then, if successful, spreads in the progeny; a single infected person can initiate an epidemic; and a single pair of rabbits started the rabbit plague in Australia. The same will be true of a novel bacterial recombinant created in the laboratory, if it has an evolutionary advantage. To have this advantage and to succeed in nature, however, an organism must not only be able to grow on the nutrients available in the environment, it must also be better adapted than its natural competitors. If it is, then even a small amount escaping from the laboratory or a greenhouse could start the process of spread. Alternatively, if it grows more slowly than its competitors, by even an infinitesimal amount, the release of tons of the organism (whether deliberate or accidental) will have only a temporary and local effect.

The importance of selection is illustrated by an extraordinarily rapid evolutionary shift, taking place within our lifetimes: increase in the prevalence of drug resistance in bacteria, because of the selection pressure exerted by the antibiotics that humans have introduced into the environment. Similarly, the distribution of soil bacteria will change in response to changes in environmental selection pressures (such as nutrients, moisture, *p*H, host plants), and not, except transiently, as a result of the introduction of genetic novelty. The dense and heterogeneous microbial population of the soil (often well over 10^6 organisms per gram) has an enormous