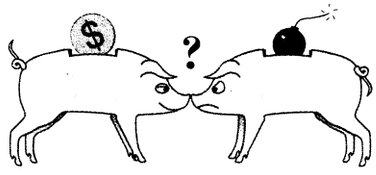
we would need to investigate whether PERV caused disease and whether it could be transmitted to other individuals. To address the risks of infection, the U.S. Food and Drug Administration (FDA) established an Advisory Panel on Xenotransplantation. And in 1997, the British government, still reeling from the evidence that bovine spongiform encephalopathy ("mad cow disease") had spread to humans, moved quickly to set up the UK Xenotransplantation Interim Regulatory Authority (UKXIRA).

With the latest reports (*4, 8*) on the lack of evidence for PERV infection in vivo, the keenest advocates of xenotransplantation may mutter that the concern over the risk of PERV infection unnecessarily delayed progress in the field. The Novartis/CDC teams, however, conclude that only cautious progress in closely monitored, prospective clinical trials will help in "assessing the safety and efficacy of using porcine cells, tissues, or organs therapeutically in humans" (*4*). Both the FDA and UKXIRA take this attitude and appear ready to approve, in principle, small-scale human trials of porcine cellular therapy.



Whereas the endogenous retroviruses in our house guests (cats and mice) have not naturally been transmitted to humans, we have known for more than 20 years that human tumor xenografts grown in immunosuppressed animals sometimes become infected (*15*). PERV, however, does not proliferate as readily in human cells as human-tropic feline and murine endogenous retroviruses (*5, 6*). But the possibility remains that, say, one among 1000 xenograft recipients may become infected by PERV or by a virus resulting from recombination between PERV and human retroviral sequences.

The concern, then, will be the potential for onward transmission from the rare, infected transplant recipient to his or her contacts. Lest we dismiss this notion as ridiculous, we should bear in mind that HIV-1 began as a zoonosis, probably from chimpanzees, and that the worldwide pandemic of the major HIV-1 subgroups may be attributable to a single cross-species event (*16*). Neither can we be sure that AIDS did not have an iatrogenic (medically caused) origin, if chimpanzee kidneys were used in Africa to propagate certain batches of poliovirus vaccine (*17*).

Although the public may demand evidence of no risk, retrospective epidemiological surveys can at best provide no evidence of risk, which is a rather different matter. We should heed the Hippocratic precautionary principle—"at least do no harm." Yet no new medical procedure can be deemed entirely safe, so we need to balance risk with benefit, for the patient and for the human population.

For the individual transplant recipient, the real promise seems to be greater than uncertain peril. Indeed, one of the potential advantages of xenotransplantation over allotransplantation (person-to-person grafts) is that pathogen-free pigs might pose a lesser threat of infection than a graft from an unknown human donor. After all, many cases of life-threatening infections have been transmitted by human transplantation and transfusion: HIV, hepatitis B and C viruses, various herpesviruses, tuberculosis, and Creutzfeldt-Jakob disease.

For the community at large, the risk-benefit equation is much more difficult to quantify. It took more than 20 years for HIV-1 to spread out of Africa, and it is only after 55 years of individual benefit from antibiotics that we are facing the public health threat of multidrug-resistant microbes. The ethical and technical problems of maintaining vigilance over xenotransplantation should not be underestimated.

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PERSPECTIVES: PLANT BIOLOGY

Taking Transgenic Plants with a Pinch of Salt

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About one-third of the world's irrigated land is unsuitable for growing crops because of contamination with high levels of salt. Currently more arable land is lost through salinity than is gained through the clearing of valuable forests. Most trees and crop plants are highly sensitive to salty conditions, experiencing a water deficit because of osmotic stress and biochemical perturbations due to the influx of sodium ions (Na^+). Varieties of a single plant species, such as barley or tomato, exhibit a high degree of variation in salt tolerance (*1*). This suggests that only a few mutations in several key transporter or regulatory proteins could confer salt tolerance on salt-sensitive plants. For example, the differing ability of two species of *Plantago* to withstand salty soil is due to the presence of the sodium/proton (Na^+/H^+) antiport protein in salt-tolerant *P. maritima* and its absence in salt-sensitive *P. media* (*2*).

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Twenty years after Epstein and his colleagues proposed genetically engineering salt-resistant crops (*1*), Apse *et al.* now report on page 1256 that they have engineered salt-tolerant *Arabidopsis* by overexpressing a single endogenous gene (*AtNHX1*) encoding a Na^+/H^+ antiport protein (*3*). With this strategy, it should be possible to engineer a whole spectrum of salt-tolerant crop plants, enabling them to be irrigated with seawater or water of marginal quality. Public acceptance of genetically engineered salt-tolerant plants (which could enable crops to thrive in salty environments, thus saving further deforestation in the quest for more arable land) is likely to be greater than for plants engineered to be pesticide- or herbicide-resistant.

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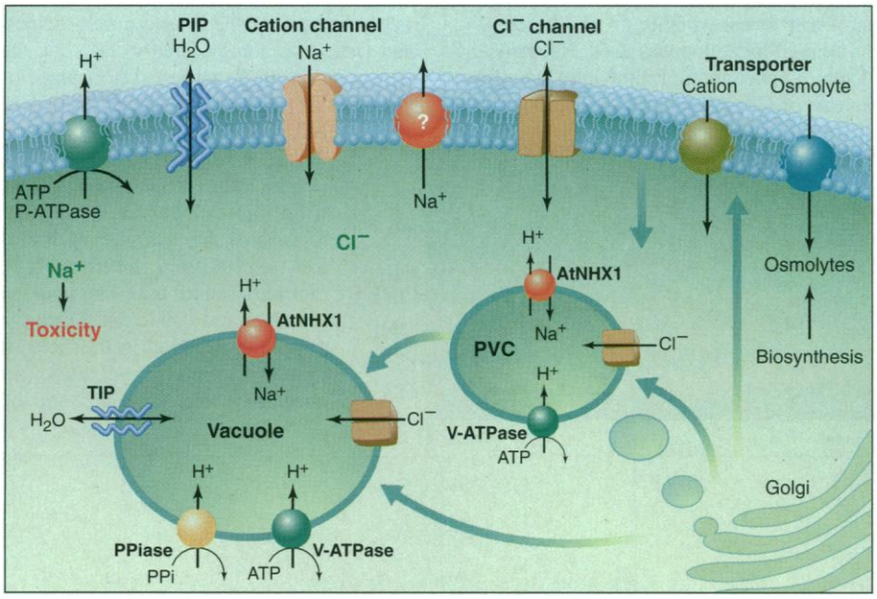
SOURCE: C. PATIENCE

Plants combat a saline environment in two principal ways: either by excluding Na^+ at the plasma membrane or by sequestering them in the large intracellular vacuole (in both circumstances there is also accumulation of compatible solutes that restore the correct osmolarity to the intracellular milieu). Compatible solutes, such as proline or sugar alcohols, are essential for maintaining salt tolerance—a reduced proline content makes plants salt-sensitive, whereas an increase in sugar alcohol levels improves salt tolerance (*4*). Sodium ions flow through the Na^+/H^+ antiport in the large vacuole membrane—down an electrochemical proton gradient generated by two vacuolar proton pumps—and accumulate inside the large intracellular vacuole (see the figure).



Salt of the earth. Ion transport proteins involved in salt tolerance in the plant *Arabidopsis*. The sodium/proton antiport (AtNHX1) is the principal protein responsible for sequestering Na^+ in the large vacuole of the plant cell. *Arabidopsis* plants genetically engineered to overexpress AtNHX1 are salt-tolerant. AtNHX1 and other ion transport proteins are thought to be localized within vacuolar and prevacuolar (PVC) membranes. (P-ATPase, P-type proton ATPase; V-ATPase, vacuolar proton pump; PPiase, pyrophosphatase; TIP, tonoplast intrinsic protein; PIP, plasma membrane intrinsic protein.)

Apse *et al.*, together with Gaxiola and colleagues (*5*), were able to identify and characterize the Na^+/H^+ antiport gene in *Arabidopsis* because of its similarity to bacterial, fungal, and mammalian homologs. They overexpressed AtNHX1 in *Arabidopsis* and by cell fractionation showed that the antiport protein was expressed primarily in the membrane of the large intracellular vacuole. Engineered *Arabidopsis* expressed greater levels of AtNHX1 and showed increased vacuolar uptake of Na^+ compared with wild-type plants. Transgenic plants were also significantly more salt-tolerant, thriving in soil irrigated with 200 mM NaCl.

But is the mechanism of salt tolerance in the transgenic *Arabidopsis* as straightforward as it seems? The increase in AtNHX1 mRNA and protein was relatively small yet translated into a huge increase in vacuolar Na^+ uptake and marked salt tolerance. Recent studies on a simpler but extremely salt-tolerant eukaryote, the unicellular yeast *Saccharomyces*, have shed more light on this paradox. Yeast both sequester Na^+ in intracellular compartments through the activity of a Na^+/H^+ antiport (homologous to AtNHX1) and extrude Na^+ across the plasma membrane by means of plasma membrane cation pumps and a cation/proton antiport (*6*). Interestingly, the intracellular Na^+/H^+ antiport (as well as chloride channels that sequester chloride ions in intracellular compartments) is located primarily in the membranes of prevacuolar compartments and not in the vacuole membrane itself (*6*), suggesting that salt might be transferred into the large vacuole through a pathway of vesicles (see the figure).

Plants living in a saline environment must contend not only with the toxicity of Na^+ ions but also with water loss caused by osmotic stress. It has been suggested that the vacuolar salt gradients in salt-tolerant transgenic *Arabidopsis* should help to drive water into plant cells, resulting in plants that are not only salt-resistant but that use water more efficiently. If this is the case, then chloride counterions would have to be taken up into the cell and large vacuole by chloride channels in the plasma and vacuolar membranes (see the figure).

Ubiquitous and continuous overexpression of AtNHX1 in transgenic *Arabidopsis* results in the salt-resistant phenotype. In wild-type *Arabidopsis*, AtNHX1 may be expressed in the membrane surrounding the prevacuole (analogous to the situation in yeast). Engineering plants to overexpress AtNHX1 could lead to missorting of the Na^+/H^+ antiport from the prevacuolar membrane into the large vacuole membrane and plasma membrane. If, through mistrafficking, AtNHX1 ended up in the vacuole membrane, then Na^+ would be sequestered in the large vacuole, as Apse *et al.* observed. Alternatively, if missorted to the plasma membrane, AtNHX1 would be able to exclude Na^+ ions from the cell interior by transporting them across the plasma membrane. Finally, if AtNHX1 is overexpressed in the prevacuolar membrane, the resulting change in ion homeostasis might affect the sorting of other proteins, which in turn could result in salt tolerance (*7*). The ability to sequester Na^+ may have been lost during evolution as plants colonized inland areas away from the sea. The lack of efficient Na^+ uptake into the vacuole of salt-sensitive plants may be explained if high AtNHX1 activity has unwanted side effects. (These may become apparent when transgenic plants are tested in the field). Combining AtNHX1 overexpression with strategies to engineer, for example, the accumulation of compatible solutes may improve salt tolerance in plants still further.

Salinity has been an important historical factor in the decline of ancient agrarian societies, and more recently has resulted in a decline in crop production in large areas of the Indian subcontinent. As in the case of transgenic plants tolerant to aluminum or acid soil (*8*), yields should rise by several percent worldwide if crops can be engineered to thrive in saline environments that are currently unsuitable for agriculture.

Salt-tolerant crops will be particularly important for developing countries. Free licenses to CGIAR (Consultative Group on International Agricultural Research) institutes allowing them to incorporate salt-tolerant traits into different germplasms will be a prerequisite for poor countries to develop locally adapted salt-resistant crops. Increasing yields from marginal soils by growing transgenically adapted crops is one step toward solving the problem of feeding the world's rapidly growing population.

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