phosphate synthase are present in virtually all cells. The genes were delivered by an adenoviral vector. Infection of the cells with increasing quantities of adenovirus correlated with increased biosynthesis of trehalose. Although the genetically modified cells were dried to a level at which free water was no longer detectable, they retained viability for five days. The level of viability might be improved further by altering the storage conditions; oxygen, for example, is well known to attack dry membranes, producing free radicals and free fatty acids that are extremely damaging. Surprisingly, some enzymes are active when water content is low, which might be another source of damage[8]. Panek and colleagues[9] have shown that a trehalose transporter is required in yeasts to transport trehalose out of the cell during drying. As trehalose is required on both sides of the membrane to stabilize it[4], this is a way of meeting that requirement. The gene for this transporter has now been cloned[10], so there is no obvious reason why it could not be incorporated into the cassette already in use for trehalose synthesis by Guo et al., a treatment that might well extend the period of viability.

It is important to note that the work from these two groups of investigators has its roots in basic research on anhydrobiotic organisms, work that initially did not appear to have any practical applications in biotechnology. Nature will most likely provide many more useful lessons as we continue to extend our knowledge of the mechanisms for preserving mamalian cells.

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Taming the uncultured

Nicholas J. Talbot and Lisbeth Hamer

Powdery mildew fungi cause some of the most severe and persistent diseases of crops, particularly of European and North American barley and wheat[1]. Finding effective control strategies represents an important challenge to the agricultural biotechnology industry, and one with high incentives: the powdery mildew fungicide market is worth more than 380 million dollars per year[2].

As an obligate parasite, *Erysiphe graminis* (the causal agent of barley powdery mildew) only grows on and in living hosts, which creates something of a nightmare for molecular geneticists who cannot culture it on artificial media. For this reason, the first successful transformation of *E. graminis*, reported by Chaure and colleagues[3] in this issue, is an important breakthrough. DNA-mediated transformation—long since taken for granted by those studying model fungal organisms such as *Saccharomyces cerevisiae*, *Aspergillus nidulans*, or *Neurospora crassa*—represents the first step toward functional genetic analysis in *E. graminis*.

The introduction and stable expression of foreign DNA in the organism constitutes an impressive technical feat. The authors

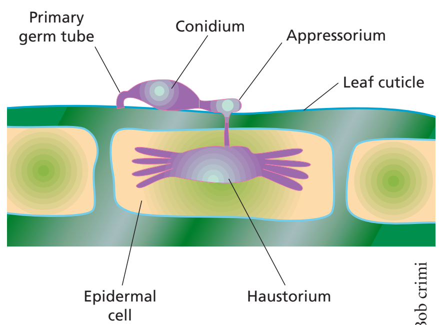

Figure 1. Diagram showing infection of barley by the fungal pathogen *Erysiphe graminis* f. sp. *hordei*, an obligate parasite that infects barley leaves using a specialized cell called an appressorium which penetrates the leaf cuticle. The fungus is an extremely efficient parasite of the living plant, and produces a specialized feeding structure, the haustorium, that allows it to subsist in leaf epidermal cells.

first cloned the β-tubulin genes from *E. graminis* f. sp. *hordei* and used site-directed mutagenesis to create an allele conferring resistance to the benzimidazole fungicide, benomyl. This allele was introduced by biolistic transformation while the fungus grew within a barley plant. The plant was then transferred to media containing the fungicide, killing the original recipient fungal strain, and leaving the plant unaffected. Thereafter, plant disease symptoms were produced subsequently only if the fungal strain was transformed with the selectable marker gene. The authors used the method to express the bialaphos resistance gene in barley lines as well as the β-glucuronidase reporter gene, demonstrating the benefits of transformation for studying gene expression in *E. graminis* f. sp. *hordei*.

Many aspects of the fungal life cycle and life style could be investigated using this new technology. Particularly exciting is the prospect of identifying genes and products that confer host resistance or susceptibility, and establishing the molecular basis of obligate fungal parasitism. The infection process starts when vegetative spores (conidia) of the fungus land on the barley leaf surface. There, in the apparent absence of free water, the spores germinate, sending out a short primary germ tube that secures the spore to the leaf. A second germ tube emanates from the tip of the spore to become an infection cell, the appressorium (see Figure 1). The fungus uses this appressorium to penetrate the leaf cuticle (probably using a combination of enzymes and physical force to dissolve the waxy cell wall layer and enter the epidermal cells). Once inside the epidermal cell, the fungus produces an haustorium, a specialized feeding structure that allows the fungus to derive nutrients from the living plant and subsist in the epidermal cell. The fungus colonizes adjacent cells and erupts from the leaf surface to spread conidia, causing severe and persistent disease outbreaks.

How the fungus is able to perceive signals for cellular development from the leaf in the absence of water is unknown, as is the genetic basis of appressorium formation. The exchange of signals between host and parasite following infection is another mystery. It is clear, however, that the signal transduction pathways required for plant infection differ between this fungus and its

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more easily studied relatives, rice blast and corn smut fungi[4,5].

The elucidation of the genes involved in cultivar-specific resistance to *E. graminis* f. sp. *hordei* could be enormously beneficial. Certain types of plant disease resistance are mediated by plant gene products involved in the recognition of pathogens. These so-called "gene-for-gene interactions" can protect plants from pathogens expressing particular virulence genes. Barley powdery mildew shows a gene-for-gene interaction

Figure 2. A powdery mildew outbreak on barley. The white powdery lesions are sites of intense spore production by *E. graminis* f. sp. *hordei* which further spread the disease. Powdery mildew is one of the most serious diseases of temperate cereals and represents the most important target for development of resistant cultivars and novel fungicides. Photo by H.P. Jensen. Courtesy of H. Østergaard, RISØ National Laboratory, Denmark.

with barley, and resistant barley cultivars carry race-specific dominant resistance genes[6]. These genes encode products that recognize fungal strains expressing corresponding avirulence genes, enabling the plant to mount an effective defense from invasion by the fungus. Genetic analysis of avirulence genes is possible in *E. graminis* f. sp. *hordei*, and positional cloning strategies are being used to identify and characterize avirulence loci[7,8]. Current transformation technology allows easy testing of putative avirulence genes. This can be accomplished by producing transformants carrying a putative avirulence gene, maintaining them on a susceptible barley host, and then testing for the presence and function of the avirulence gene after transfer to a host carrying the corresponding resistance gene. Lack of disease symptoms would confirm the presence of the appropriate avirulence gene.

In fungi that can be cultured, the most typical form of gene function analysis is construction of a null allele of a nonessential gene. However, this is not an option in an obligate pathogen, because the targeting of a gene essential for either pathogenicity or survival would result in an inviable mutant. Thus development of a transient and controllable means of attenuating gene expression is the next challenge. One possibility would be to express an inducible antisense construct for a target gene, an approach that is essentially untested in filamentous fungi[9,10]. However, this would require the development of a highly inducible promoter, or a heterologous promoter that works in *E. graminis*. Alternatively, an extra copy of the putative pathogenicity gene in the sense orientation could be expressed for gene silencing by cosuppression, the phenomenon by which additional copies of a gene turn off expression of the endogenous gene.

Genome-level analysis of the barley powdery mildew fungus is being carried out in the public and private sectors and promises to be enormously valuable for identifying the key components of obligate parasitism. Analyses of large collections of expressed sequence tags have revealed genes that are not only conserved among related pathogen species, but also appear distinct from the most similar genes in yeast. Comparative genomics approaches have also made it possible to introduce heterologous genes to study their capacity to substitute for presumed orthologs in a recipient organism.

Development of this efficient transformation system is a milestone for the study and control of *E. graminis*, and the timing couldn't be better. This technique will make it possible to exploit the growing wealth of genetic information from genome sequencing projects and provide a unique opportunity to carry out functional genomics on an obligate parasite. Introducing genes into the barley mildew genome to allow artificial propagation of the fungus is an especially exciting prospect, as it would allow study of how different genes contribute to the obligate life style and pathogenicity of this fascinating, but destructive, organism.

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