the enzymes exist that will efficiently and specifically elongate eicosapentaenoic acid to form the docosopentaenoic acid substrate *in vivo*.

Third, the results of Qi *et al.* have so far shown arachidonic acid and eicosapentaenoic acid accumulation only in leaf tissue. It is generally acknowledged that arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid will need to be synthesized in seeds and deposited in their storage oils to realize the maximum production opportunity. To achieve this, the LCPUFAs will need to be efficiently removed from their ultimate phosphatidylcholine substrates and transferred onto triacylglyerols, which will require the presence of acyl exchange enzymes capable of accepting LCPUFAs that are not normally present in plants.

Fourth, LCPUFAs accumulated in seed storage oils will need to be efficiently mobilized during germination to provide energy for plant growth. This will require that the seeds contain lipases capable of cleaving LCPUFAs from triacylglyerols, and that the plant peroxisomes are able to breakdown these complex fatty acids.

Therefore, many scientific challenges remain to be addressed. However, the fact that arachidonic acid and eicosapentaenoic acid synthesis has now been clearly demonstrated in transgenic plants will no doubt provide a significant impetus for further research to resolve these issues.

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The best of both worlds in plant protection

Holger Bohlmann

Fusion proteins combining three different antifungal proteins with an antibody fragment may offer a new weapon against fungal pathogens.

Plants and animals have evolved a range of different weapons to defend against fungal disease. These may be expressed constitutively (*e.g.*, antifungal peptides found in humans, frogs, flies and plants) or in response to a pathogen's presence (*e.g.*, antibodies evolved by humans and other vertebrates). By combining into fusion proteins the potency of the former with the specificity of the latter, Peschen *et al.*[1] have created molecules that can protect transgenic *Arabidopsis thaliana* plants from infection by a pathogenic *Fusarium* sp. Because *Fusarium* spp. are responsible for some of the most costly and devastating grain diseases in the world, this approach may offer an alternative to existing chemical fungicides that are often associated with undesirable environmental consequences.

Holger Bohlmann is at the Institute of Plant Protection at the University of Natural Resources and Applied Life Sciences (BOKU), Peter Jordan-Strasse 82, A-1190 Vienna, Austria.
e-mail: holger.bohlmann@boku.ac.at

To make their fusion proteins, Peschen *et al.* first isolated a single-chain antibody against a protein from the *Fusarium graminearum* cell wall. The DNA encoding this antibody was then fused to the coding sequence of a peptide or protein that had been shown before to have antimicrobial activity. Two different antimicrobial peptides—one from *Raphanus sativus* and one from *Aspergillus giganteus*—and a chitinase from wheat were used. The resulting fusion proteins were produced in *Escherichia coli* and shown to have potent activity against *Fusarium* spp. *in vitro* (see **Fig. 1**). They were also expressed in *A. thaliana*, conferring significant protection against infection by *Fusarium oxysporum*, but not *Sclerotinia sclerotiorum*. The results are interesting for two reasons: first, they should stimulate further research on antifungal fusion proteins; and second, they promise a practical application.

It is known that antimicrobial peptides, which are usually basic and often contain several disulfide bridges, exert their activity by attacking the plasma membrane. The exact mode of action, however, is not known in detail, but seems to involve, at least in some cases, the binding to specific receptors[2]. Antimicrobial peptides have been isolated from a large variety of organisms, and antimicrobial activity has been demonstrated *in vitro* against a variety of fungi (and also bacteria) using the purified peptides. In addition, in fungi, the growing hyphal tip might be especially sensitive to the activity of chitinases that hydrolyze the polymer chitin, a major constituent of the fungal cell wall[3].

Peschen *et al.* demonstrate that the activity of antimicrobial peptides/proteins against *Fusarium* spp. both *in vitro* and in transgenic *A. thaliana* plants can be enhanced by fusing them with an antibody (CWP2) that recognizes a protein in the cell wall of *Fusarium* spp. Do we have an explanation for this effect? The authors themselves speculate that the antibody 'concentrates' the active part of the fusion protein in the cell wall. There, the chitinase would act upon the chitin polymers whereas the peptides would target the plasma membrane. Several different controls support this view. The fusion proteins are not active against *Sclerotinia sclerotiorum* and this fungus is also not recognized by the CWP2 antibody. Using an antibody that does not bind to the fungal cell wall as the fusion partner has no effect. Furthermore, just mixing the cell wall–binding antibody CWP2 with the antifungal peptides/protein is not effective *in vitro*.

The *in vitro* tests were performed with fusion proteins produced in *E. coli*. Although problems with activity because of improper folding in this bacterial expression system might have been anticipated, the fusion proteins appeared efficacious against fungi, as demonstrated by *in planta* results.

What remains to be demonstrated is that this is not a specific effect of the CWP2 antibody. The nature of the CWP2 target is not known yet, but there is reason to believe that the CWP2 antibody might bind a sensitive target in the *Fusarium* cell wall. Expression of the antibody alone significantly enhanced the resistance of the transgenic *A. thaliana* plants against *Fusarium* infection. This is in itself interesting because, to my knowledge, such an effect has not been demonstrated before. But it could also mean that the results obtained for expression of the fusion protein are the additive (or perhaps synergistic) effect of the two partners, the CWP2 anti-

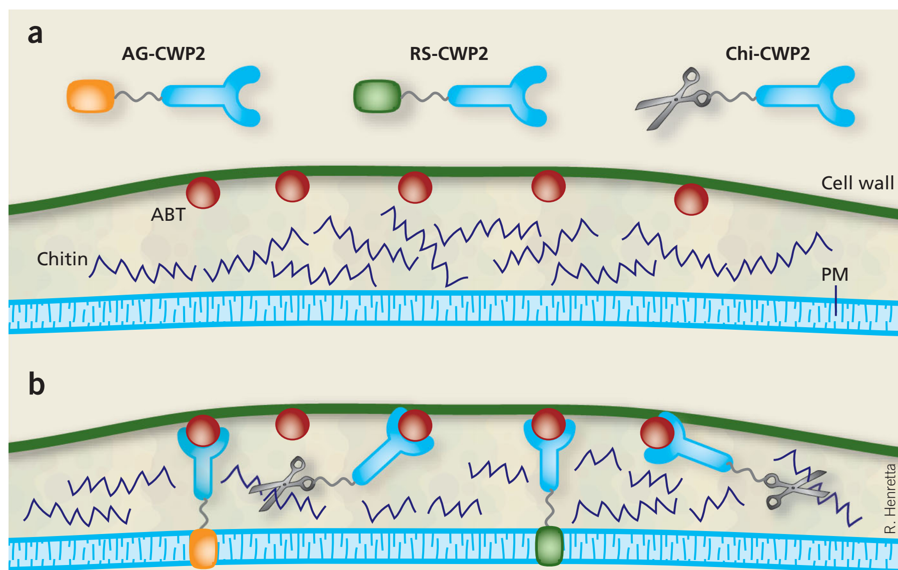

Figure 1 Antifungal fusion proteins. (**a**) Fungal cell wall showing chitin and the protein-binding site for the antibody CWP2. The three structures shown above correspond to three different fusion proteins that gave a strong antifungal effect. CW, cell wall; PM, plasma membrane; ABS, antibody binding site. (**b**) The fusion proteins bind to the cell wall protein (ABS) via their antibody moiety. The antifungal half of the fusion protein then exerts its activity, either on the plasma membrane (AG-CWP2 or RS-CWP2) or by cleaving the chitin polymers (Chi-CWP2).

body and the antimicrobial peptide or the chitinase, respectively.

As has been mentioned above, the *in vitro* tests would be strengthened by further validation in whole plants. The authors should cross the *A. thaliana* lines expressing the different fusion partners to obtain lines that express unfused combinations of the antibody and the antimicrobial peptides/protein. It will be interesting to determine whether these lines have protection levels similar to those of plants expressing a fusion protein. In addition, it will be necessary to use another antibody, binding a different target of the fungal cell wall, as the fusion partner to prove the assumption that the effect seen with the CWP2 antibody is due to targeting the 'effectors' to their battleground. Ideally, this antibody alone would have no effect on the pathogen if expressed in plants.

Antimicrobial peptides and proteins have been attractive candidates for genetic engineering of crop plants for many years. Theoretically, one can express a peptide/protein of interest in plants at a high expression level and then get plants that are resistant to certain pathogens. Although this seems straightforward, in practice results have not been impressive. One reason might be that some antimicrobials are only effective against specific pathogens. Furthermore, to defeat a fungal pathogen, several antimicrobials are often needed to act in concert. Plants produce not only antimicrobial peptides, but also low molecular weight compounds with antimicrobial activity, such as the phytoalexins[4] and the so-called PR (pathogenesis related)-proteins[5], which include glucanases and chitinases, such as the one used in the work by Peschen *et al.* A synergistic effect of thionins and 2S albumins has for instance been demonstrated *in vitro* against filamentous fungi[6].

In this light, the results reported by Peschen *et al.* are very welcome. If it could be demonstrated that the results are broadly applicable, this would have two important implications: first, it would be possible to enhance the activity of antimicrobial peptides so that fungal resistance could be achieved with lower expression levels; second, by choosing the appropriate antibody, it would be possible to target pathogens specifically. Although this may be regarded as a disadvantage in terms of broad-spectrum protection, it may allay concerns about damage to beneficial microbes (and other fauna), which often is brought up in public debates about transgenic plants.

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