Positional cloning for pathogen resistance


Mutant alleles of the Mlo gene of barley confer broad-spectrum resistance to powdery mildew (Erysiphe graminis f. sp. hordei). By employing a positional cloning approach, Büsches et al. have isolated the Mlo gene. The sequence analysis revealed that, unlike many other pathogen-resistance genes, the putative 60 kDa protein lacks leucine-rich repeats and nucleotide binding sites. Instead, there is evidence of six hydrophobic stretches that are predicted to anchor the protein within a membrane.

By crossing lines containing the different mutant alleles (which are resistant to powdery mildew), it was possible to identify individuals in a selfed F1 population that were susceptible to the fungus. Apparently intragenic recombination events between the separated mutation sites in mlo had given rise to the wild-type Mlo sequence. This was confirmed by sequencing. Thus, restoration of the Mlo wild-type gene coincided with susceptibility to the pathogen.

The authors also discussed possible functions of Mlo, and proposed two models: the first was that Mlo protein controls leaf cell death – a spontaneous lesion phenotype occurs in seedlings carrying different mlo alleles even in the absence of the pathogen; the second suggests that Mlo downregulates multiple defence-related functions, because mlo plants have elevated resistance to pathogens before lesions occur.

Calmodulin and the oxidative burst


In an imaginative attempt to delineate the signalling pathway following environmental and fungal challenge, these authors have transformed tobacco containing a dominant mutation in the calcium-regulated protein, calmodulin. This calmodulin mutation was chosen because a lys115 to Arg substitution prevents it from being methylated post-translationally. Methylation has the effect of attenuating calmodulin activity, and in the mutant this leads to hyperactivation of NAD kinase in vitro. Analysis of transgenic plants revealed that this was also the case, with a fourfold increase in NADPH concentrations in transformed plants relative to the controls. These experiments also revealed that there was an accompanying rise in hydrogen peroxide levels in the transgenic plants, suggesting a correlation between calcium signalling and the production of active oxygen species.

Cell cultures generated from transgenic lines were then used to assess what triggers the oxidative burst and changes in nictotinamide coenzyme fluxes. Mechanical stimulation, cellulosed treatment and addition of pathogens or fungal elicitor all had the effect of accelerating the release of activated oxygen species. Further analysis revealed that the NADPH concentrations also rose in response to cellulosed treatment, and that the activation of NAD kinase was dependent on both calcium and calmodulin. The possibility that the activation of NAD kinase provides NADPH required for the oxidative burst was tested using an inhibitor of O2- producing NADPH oxidases. The inhibitor had the effect of promoting the accumulation of NADPH, consistent with a defence response lacking calcium, calmodulin, NAD kinase, NADPH and the generation of active oxygen species.

Novel plastid promoters


Many plastid genes containing prokaryotic -10 and -35-like consensus type (CT) sequences are expressed in a light-and development-dependent manner. Here, Kapoor et al. show that the presence of a novel nonconsensus promoter (designated type NC-II) is sufficient for the low-level constitutive expression of specific 'housekeeping' genes.

Analysis of the atpB/E operon in tobacco revealed four transcription initiation sites, but only three attributable to CT promoters. With leaves, the absence of light for 5 d reduced the CT promoter-derived transcripts to approximately 10% of their original levels, but had little effect on the ~290 atpB/E NC-II-type promoter-derived transcript. In fact, when nongreen cell types were examined, the latter transcript was the only atpB/E transcript detected.

Levels of various CT promoter-derived transcripts declined significantly when seedlings were exposed to tigitoxin (a selective inhibitor of chloroplast RNA polymerase) or cycloheximide (an inhibitor of cytoplasmic protein synthesis). In comparison, the levels of certain transcripts showed a similar sensitivity to cycloheximide, but increased markedly after a 6 d exposure to tigitoxin, suggesting the existence of two transcription systems that differ in their sensitivity to tigitoxin.

The authors have detected regions of homology between the ~290 atpB/E NC-II-type promoter and two well-characterized nonconsensus promoters. These genes, rnm16 and rps12, encode fundamental elements of the plastid genetic machinery and are just the type of genes that might require constitutive expression throughout development.

This month's literature focus articles were contributed by Hugh Blackburn, Daniel Holloway and Robert Schuurink. If you would like to contribute a brief synopsis of a recent research paper then please contact the Editor (plants@elsevier.co.uk).