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Plant Disease Resistance Genes – 7th International Symposium on Molecular Plant-Microbe Interactions

Meeting Report

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This year's ISMPMI in Edinburgh, Scotland, June 26–July 1, 1994, was marked by reports on the cloning and characterization of disease resistance genes from several plants. In addition to the *Pto* gene conferring resistance of tomato to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* [Martin et al., 1993], the *Cf9* gene encoding resistance to the fungal tomato pathogen *Cladosporium fulvum* was isolated (Jones et al., Norwich, UK). The first gene conferring resistance to a viral pathogen, TMV, was cloned from tobacco, the *N* gene (Baker et al., Albany, USA).

Also from the model plant of modern plant molecular biology, *Arabidopsis thaliana*, the *RPS2* gene for resistance to strains of *P. syringae*, has now been cloned by two groups (Ausubel et al., Boston, USA; Staskawicz et al., Berkeley, USA). It is particularly satisfying however, that among the first plant disease resistance genes isolated and characterized, there is also one from the system studied by Flor, the L^6 gene conferring resistance of flax to flax rust (Ellis et al., Canberra, Australia). Thus, approximately 50 years after the formulation of the gene-for-gene hypothesis [Flor, 1955] and 10 years after the cloning of the first bacterial avirulence gene [Staskawicz et al., 1984] we finally got the first glimpse into the molecular basis of plant disease resistance.

Basically, two generally applicable strategies have been successfully used to isolate plant disease resistance genes: map-based cloning *(Pto, RPS2)* and transposon tagging (*Cf9, N,* L^6). Map-based cloning aims at identifying a gene by its position relative to known markers. Essential requirements for this strategy are the availability of comprehensive genomic libraries in yeast artificial chromosome vectors and of high-density RFLP or RAPD chromosome maps. Isolation of the gene of interest is eventually achieved by cloning of the DNA between closely flanking markers, identification of open reading frames and complementation analyses. Since these requirements are fulfilled in particular for *A. thaliana* and tomato, both plants with small genome sizes, it is not surprising that this strategy led to the isolation of resistance genes from these plant species.

In transposon tagging a gene is mutated by the insertion of a known transposon which is then used as a tag to isolate the flanking DNA regions containing the gene. Transposable elements were first described for maize.

Transposon tagging was therefore used to isolate genes from this species or by transferring maize transposons to other plants. In particular, the maize *Ac* element was used to isolate the *Cf9* gene from tomato, the *N* gene from tobacco and the L^6 gene from flax. In maize, the *Mu* element was used as a tool to get a hold of the *Rp1* region conferring resistance to *Puccinia sorghi* (Bennetzen et al., Purdue, USA) and to isolate the very first cloned resistance gene, *Hm1*, encoding an enzyme that specifically detoxifies the toxin from *Cochliobolus carbonum* [Johal and Briggs, 1992]. The *Rp1* region is an example of a resistance gene cluster: not only are there several closely linked *Rp1* genes interspersed between pseudogene sequences, but in addition other resistance genes [Bennetzen et al., 1990].

What is known about structure and putative function of the resistance gene products? Two structural motifs are present in the *RPS2*, *N* and L^6 proteins: a P-loop domain and a leucine-rich repeats (LRR) domain. P-loops are nucleotide-triphosphate binding regions of many ATP- and GTP-binding proteins. LRRs have been found in a number of extra- and intracellular proteins, including plant receptor-like protein kinases (e.g. RLK5 from *A. thaliana* [Walker, 1993]). The LRR domain is presumed to interact with proteins or other macromolecules, thereby mediating cell to cell, cell to protein, or protein to protein interactions. In addition, the *RPS2* gene product has a leucine zipper-like putative dimerization domain, a membrane-spanning region, an N-terminal cytoplasmic anchoring domain and six possible *N*-glycosylation sites. Interestingly, similarity of the tobacco *N* gene to the *A. thaliana RPS2* gene was reported. The LRRs are not only in a similar position, but also of similar length. Taken together these features point at the *RPS2* gene product as being a typical transmembrane receptor-like protein.

The Cf9 protein is also characterized by LRRs. Its N-terminus has homology to plant receptor-like protein kinases (e.g., RLK5 of *A. thaliana*) and, surprisingly, to polygalacturonase inhibitor proteins (PGIPs) of several plant species directed against fungal polygalacturonases. For example, the *pgip* gene from *Phaseolus vulgaris* (De Lorenzo et al., Rome, Italy) encodes a completely extracellular LRRs-containing protein that is synthesized upon fungal attack. In comparison, the C-terminus of the Cf9 protein seems to be located in the cytoplasma as indicated by putative extra- and intracellular anchoring domains flanking a putative transmembrane domain. No protein kinase or other intracellular signalling domains were identified.

The *Pto* protein was described previously [Martin et al., 1993]. It appears to be an intracellular serine/threonine-specific protein kinase that may be attached to the plasma membrane *via* myristoylation at the N-terminus. The complex *Rp1* locus of maize also contains at least 10 copies of a putative serine/threonine kinase gene of the SNF1 subfamily [Hanks et al., 1988]. In comparison, the L^6 gene has no similarity to other

cloned genes in addition to P-loop and LRR sequences and no obvious membrane-spanning region.

What is the picture emerging for plant disease resistance genes and their role in cell signalling? Cells are enabled to respond to external stimuli by signalling pathways consisting of chains of intercommunicating proteins. However, these pathways must not be imagined as being linear. One such pathway is the Ras pathway which seems to be present in most eukaryotic cells [Egan and Weinberg, 1993]. Protein phosphorylation/ dephosphorylation has turned out to be one of the major mechanisms of signal integration in eukaryotic cells and the Ras pathway is characterized by a cascade of protein kinases which are regulated by multiple signals and which probably also have numerous targets.

Disease resistance-related signalling in plants may work in a similar way. An extracellular signal (e.g. a specific elicitor) is perceived at the cell surface by receptor-like proteins such as RPS2 or Cf9. The perceived signal is then transduced into the cytoplasma. This may be achieved *via* the same transmembrane protein (RPS2) or mediated in a two-element receptor complex. The product of *Cf9* may represent an extracellular receptor element while the *Pto* product is an intracellular effector component. It will be very interesting to see what additional compounds of signalling pathways or rather networks will show up during the coming years. In this respect, not only additional resistance genes such as the *RPS3/RPM1* gene from *A. thaliana* (Dangl et al., Cologne, Germany; Innes et al., Bloomington, USA) or the *Cf2/Cf5* resistance genes from tomato (Jones et al., Norwich, UK) will be informative. The dissection of pathways required for resistance gene function will reveal new classes of signal propagating components. Examples for such genes are the *Nar* and *Nor* genes from barley that are *n*ecessary for *mla* or *mlo*-mediated *r*esistance to powdery mildew (Schulze-Lefert et al., Aachen, Germany). Other genes are presumed to be involved in triggering the hypersensitive response. Mutants in these genes such as the *acd* and *lsd* mutants from *A. thaliana* have been identified [Greenberg et al., 1994; Dietrich et al., 1994]. Also the tomato *Prf* and *Fen* loci [Salmeron et al., 1994] are candidates for encoding additional components of this plant's disease resistance related signalling pathway.

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