Sentinels of disease: Plant resistance genes

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Concepts in Plant Innate Immunity

Successful defense against a pathogen requires perception of its whereabouts. In multicellular organisms this ability is genetic information that is 'hard-wired' into the genome and is called 'innate immunity', drawing its origins from a phylogenetically ancient form of immunity that is common to all Metazoa and Viridiplantae. Recognition of 'non-self' components as opposed to the components of 'self' is accomplished efficiently by the presence of constitutive pattern recognition receptors that like sentinels can then alert the organism to activate cellular defense. The plant cellular defense arsenal includes rapid reactive oxygen species (ROS) bursts, cell-wall strengthening, induction of defense gene expression (defensins) and rapid apoptopic-like cell death at the site of infection. We characterized two molecular components that produce ROS, a prerequisite of some apoptopic pathways. One major component is generated by a plant gp91phox homologue (NADPH oxidase). However, unlike its mammalian counterpart it is constitutively active and can be directly regulated by calcium levels. Another source belongs to the aldehyde oxidase class of enzymes that we show produce ROS production and play a role in stress hormone biosynthesis. How are these pathways activated during the pathogenesis response?

Molecular components of sentinels and their rapid divesification

The agents that plant sentinels detect are called avirulent factors. They are produced by the pathogen and likely play a role in the process of pathogen colonization of the host. A basic molecular motif that makes up innate sentinels common to animals and plants consists of leucine rich repeats (LRR). This element has been shown to participate in many protein-protein interactions. The LRR domain appears in combination with accessory motifs that participate in signal relay. These include in animals and plants: membrane anchor or transmembrane domains, Toll-and Il-1R-like domains (TIR) and nucleotide binding domains (NBD). A plant specific domain that may serve a function homologous to TIR are coiled coil (CC) and kinase domains. In contrast to animal TIR-dependent innate immunity which consists of a limited number of pattern recognition genes, the plant class have undergone major amplification. The phylogenetic tree shows the early origins of TIR motifs (Fig. 1).

We isolated a plant resistance gene whose presence is essential for resistance to the fungal vascular disease caused by Fusarium oxysporum. This gene is made up of a CC, NBD and LRR components. The LRR domain likely acts as the receptor for pathogen generated ligands. What are the biophysical properties of the LRR domain that promoted its choice as the sentinel pattern receptor? The protein structure solved by the crystal structure of the porcine ribonuclease inhibitor (RI) has served as a rough structural platform for conceptualizing what the distantly related plant LRR may look like (Fig. 2). It shows that the solvent exposed beta-sheets region interact with the ribonuclease. The expectation is that the solvent exposed regions should show an apparent higher degree of mutation rate, due to positive selection of residue changes that are responsible for interaction with pathogen factors. Adaptive evolution can be ascertained by comparing the distribution of the ratio (Ka) of nonsynonymous nucleotide

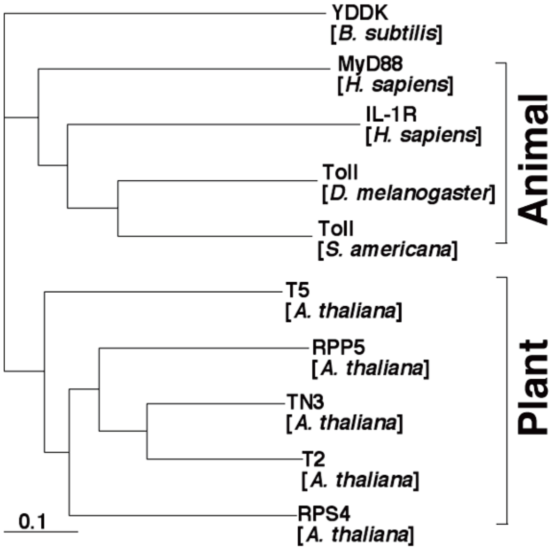

Fig. 1 *Phylogenetic tree of the TIR domain (Toll/Interleukin-1Receptor) collated from representative plant and animal sources. In animals TIR domains appear with LRR. TIR domains in plants appear alone or with NBD and LRR as part of plant resistance genes.*

substitutions to the (Ks) synonymous nucleotide substitution. The remarkable subtlety of this procedure is conceptualized in Fig. 2 with an example that compares Ka/Ks ratio along the NBD-LRR-type resistance gene for the tomato Fusarium oxysporum R-gene, I2. A high ratio is obtained particularly within the ‘solvent exposed’ beta-sheet region. Thus, the evidence is consistent with a role of the LRR domain in coordinating pathogen-ligand binding. How these genes work and transduce signals are major goals of our research into understanding the biological strategy of innate immunity.

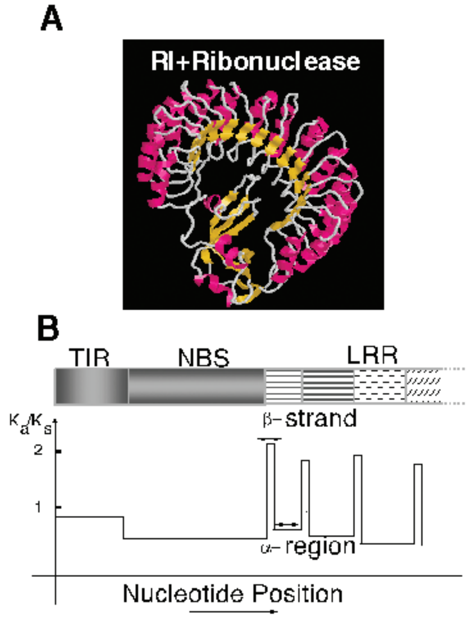

Fig. 2 *The LRR motif of plant resistance genes. A. The Porcine ribonuclease inhibitor and ribonuclease B. Perceived ratio of nonsynonymous/synonymous amino acid substitutions in NBD-LRR haplotypes of I2C-2 vs I2C-5 tomato Fusarium resistance genes.*

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Acknowledgements

This work is supported by the Minerva Foundation, Germany.

R.F. is the head of the Charles W. and Tillie K. Lubin Center for Plant Biotechnology and the Harry and Jeanette Weinberg Center for Plant Molecular Genetics Research. His work is supported by the Raymond Burton Fund for Plant Genome Research.