Minireview

Adding injury to insult: pathogen detection and responses

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Abstract

Genomic approaches to the study of the expression of plant genes induced in response to disease and attack are now showing that there is an intimate association between pathogen perception and general stress detection.

Considerable space in the genomes of most higher organisms is devoted to encoding the machinery necessary for perceiving pathogens and signaling from this perception to trigger defense gene expression. Plants are no exception, since they are subject not only to attack by microbial pathogens but also by myriad invertebrates and vertebrates. They must also be able to deal with environmentally imposed stresses such as drought and physical injury. The question is: what proportion of the genome encodes products involved only in pathogen defense signaling, and what proportion is involved in less specific survival mechanisms that also protect against physical injury? Genomic approaches are rapidly shedding light on this issue and already offer part of the answer, yielding a new understanding of the strategies employed by attacking organisms. An element of the story comes from a new study [1] of genes rapidly activated or repressed when a transgenic plant cell suspension culture perceives a component of a fungal pathogen. Additional evidence comes from a number of studies of plant gene expression responses to a number of different pathogens and predators.

Durrant *et al*. [1] used transgenic tobacco cells expressing the tomato *Cf-9* resistance gene, which enables the cells to respond to the Avr9 peptide elicitor produced by specific races of the fungal pathogen *Cladosporium fulvum*. On perception of the elicitor, defense responses are rapidly activated. Along with other well-established cell-suspension culture systems [2], *Cf-9*-tobacco cell cultures have already proven to be a useful tool for pharmacological studies of early events in signal transduction triggered by pathogen-derived elicitors [3]. The novelty of the study by Durrant *et al*. [1] is, in part, due to the fact that the authors isolated rapidly regulated cDNAs rather than downstream defense genes, thus giving new information on signal transduction in response to elicitation.

The Avr9 elicitor peptide (in the intercellular fluid in which it is produced) can be added to a cell suspension where it is quickly perceived by the cells. Elicitors trigger a variety of rapid responses in cell cultures, including superoxide production [2]. In the experiments of Durrant *et al*. [1], superoxide production was inhibited specifically to reduce the complexity of the induced responses. Elicitor (or control intercellular fluid lacking an elicitor) was added to the cell suspension and, after 30 minutes, cells were frozen and mRNA was converted into cDNA for amplified fragment length polymorphism (AFLP) analysis [4]. These cDNAs were restricted with MseI and ApoI. Adapters were then ligated onto the ends of the cDNAs and the resultant molecules were selectively amplified with primers complementary to the MseI and ApoI adapters but containing two extra bases. These primers are only extended if the fully complementary sequence is present in the cDNA, thus reducing the number of PCR products and facilitating visualisation of bands [4]. This strategy resulted in a theoretical coverage of about 76% of expressed genes. A total of 30,000 polymorphisms were analysed, yielding 290 derived from differentially expressed genes. Re-amplification

and sequencing revealed that some of the fragments were homologous to genes encoding known signaling proteins, such as protein kinases, transcription factors, 13-lipoxygenase and so on. Then, 13 full length 'ACRE' (for *Avr9/Cf9* rapidly elicited) cDNAs were cloned using these fragments. Some of the cDNAs encoded signaling proteins with strong homology to known gene products, such as a truncated form of the N-resistance protein (involved in perception of viral attack), ethylene response-element binding proteins (EREBPs), and a calcium-binding protein, and several appeared to encode pioneer proteins. Among these latter sequences, at least five were predicted to encode polypeptides of 97 to 156 residues. These are interesting candidate defense-related genes. The cDNAs were used as probes to study Avr9 regulated gene expression in intact leaves. It is here that the story gets more interesting. ACRE gene expression was, as expected, strongly induced by infiltrating Avr9 into leaves. But control infiltrations lacking Avr9 also induced transient expression of the ACRE genes. It remains to be seen whether the transcription of other genes not encountered in these experiments is activated exclusively by pathogens and whether some of the genes in the study encode proteins regulated by post-translational protein modification and which might respond selectively to different inputs. In either case, the tobacco plants in this experiment use similar signaling machinery to respond to a fungal elicitor and to a physical stress (infiltration). It is this observation which merits attention.

The idea has emerged recently that a common signaling machinery is used to cope with different forms of biological attack in plants. There appear to be strong parallels in the ways plants respond to attacks by insects and pathogens. Elicitors, for example, are not confined to microbial pathogens and the rigorous characterisation of elicitors in insect saliva [5] immediately invited comparison with elicitor display by pathogens [6]. This was followed by the discovery of a single plant gene, *Mi-1*, capable of confering resistance both to a nematode and to an aphid, organisms from two different phyla [7,8]. Confronted by very different attacking organisms, common perception mechanisms were employed by the plant [9]. It should be noted that, at the level of outcome (defense), plants can respond very differently to various attackers, for example some insects and microbial pathogens, in part due to signal pathway cross-talk [10]. The signal pathways activated during attack can interact with one another in an antagonistic manner, with the result that an insect attack might make a plant more susceptible to a microbial pathogen [10].

The work of Durrant *et al.* [1] now suggests that the signaling machinery responds in a very similar way upon perception of a microbial elicitor and to the physical stress of infiltrating fluid into leaves. This report is not an isolated example, and a new study of plant-insect interaction extends the idea of shared signaling in response to biological and physical stress. This work [11] employed silverleaf whiteflies, which are phloem-feeding insects. Differential display revealed two genes in squash plants induced by silverleaf whitefly. Interestingly, the expression of the two genes was not strongly induced by infliction of a crushing wound or by bacterial infection, but was strongly upregulated by water-deficit. Together, the results suggest that attacking organisms can be sensed by the plant, which uses shared signaling functions to respond to biological input from the attacker as well as any physical stress its ingress might engender. The results will lead to a renewed interest in the 'physical' component of attack in plants.

A free-living organism that attacks a plant would be perceived both via elicitor recognition and by its physical effects on host tissues. This means that a 'perfect pathogen' - lacking elicitors for a given host - might still encounter the result of activating the physical-stress signaling machinery. Well-adapted attackers must therefore minimize the display of elicitors and also avoid injuring their host since both types of input will surely lead to better detection and stronger defense, as illustrated in Figure 1. Work from my own group [12] describes an example of an attack strategy that may be tailored to do just this and to reduce physical injury. In this case, cDNA microarray analysis revealed that responses to a crushing mechanical wound caused the powerful activation of many genes, including those encoding water-stress-related proteins, in the model plant *Arabidopsis*. When feeding on *Arabidopsis* leaves, a pierid caterpillar appeared to use a deliberate strategy to minimize activating physical-stress-related gene expression. This was achieved by cutting tissues with sharp mouth parts and removing the maximum mass of tissue while reducing the cut edge of the leaf to a minimum. In fact, this may be why this insect often cuts almost perfect semicircular and circular holes in leaf tissues - to minimize the physical component (cut leaf edge) of injury. Insects also have strategies to reduce their chances of being perceived via elicitor production. Some insects rapidly consume tissue from cut leaf edges, thus re-ingesting much of the elicitor they produce [13]. Thus attackers - at least some insects - use the strategy best adapted to reducing elicitor display and keeping physical injury to a minimum. In parallel, there has been recent progress in understanding the nature and regulation of signal pathways for cold stress and dehydration in plants [14]. Several transcription factors important in these responses (for example, bZIP proteins and EREBPs) have close relatives involved in plant defense and development. At least one of the ACRE sequences described by Durrant *et al.* [1] encodes an EREBP. The ACRE genes are thus excellent candidates with which to further investigate cross-talk between defense and environmental stress signal networks.

In conclusion, there are probably simple reasons why the plant uses shared signaling machinery to cope with pathogenesis and physical environmental stress. The latter can be

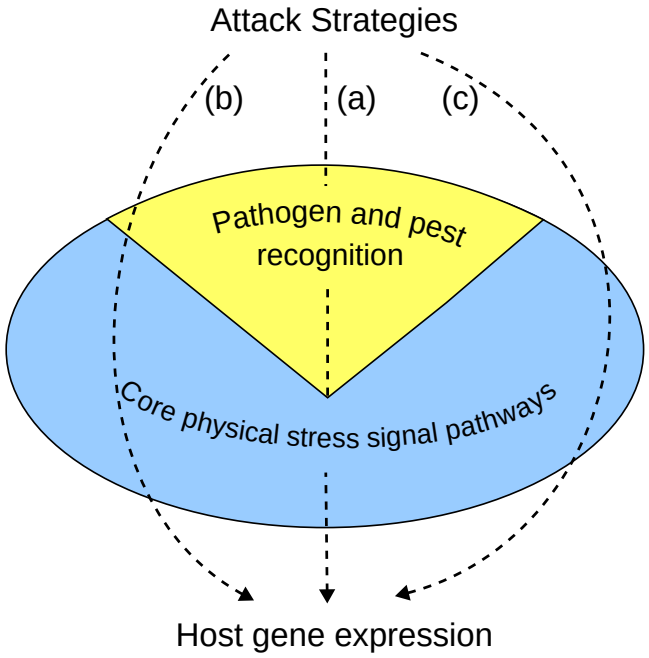

Figure 1
Adding injury to insult. The signaling machinery for pathogen and pest recognition may be intimately associated with core signaling elements that respond to physical stress. Organisms that attack plants inevitably modify host gene expression through being recognized by molecular determinants (elicitors) and through physical damage to cells and tissues **(a)**. Attackers which succeed in modifying their elicitors to either partially **(b)** or completely **(c)** escape detection still activate host gene expression through interaction with physical stress pathways. One strategy to ensure successful attack, which might be used by some insects, would minimize both insult and injury.

a consequence of attack, and it seems that being attacked by an insect or a pathogen is like adding insult to injury. A common set of defense-related signaling genes is differentially expressed in response to different stresses, but their degree of activation or inactivation depends on the nature of the attacker. Thus, at least two sets of variables will play important roles in the host response: first, the exact nature of the physical injury inflicted by the attacker; and second, the nature of the elicitors displayed to the host. Attackers may try to minimise both inputs. While research on the biological component of attack proceeds at a fast pace, new insights on physical aspects of attack are just emerging. These new considerations raise the exciting possibility that among the many genes discovered by researchers interested in environmental stress there are defense genes and defense signaling genes. There is at least one example of such a gene, osmotin [15], but the stage is set for the discovery of many more. Recognition of the fact that some or many defense genes can be induced by physical stresses will allow us to investigate defenses from other perspectives: what can be learned about the wound response from plant-pathogen interactions? Finally, given the conservation of strategies of innate defense in eukaryotes [16] it will be interesting to re-examine the effects of wounding and other physical stresses on defense gene expression in many types of organism.

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