Minireview

Unraveling regulatory networks in plant defense using microarrays

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Abstract

DNA microarrays are being used to comprehensively examine gene expression networks during the plant defense response that is triggered when a plant encounters a pathogen or an elicitor molecule. In addition to identifying new genes induced during defense, these studies are providing new insights into the complex pathways governing defense gene regulation.

Plants induce an array of defenses in response to pathogen attack [1]. For example, a rapid defense mechanism known as the hypersensitive response is often induced upon recognition of a pathogen-derived molecule [2]. The hypersensitive response results in rapid death of plant cells at the site of infection, which probably serves to prevent pathogen spread. Subsequent to the hypersensitive response, a long-lasting enhanced resistance develops throughout the plant, termed systemic acquired resistance (SAR) [3]. SAR confers resistance not only to secondary challenge by the initial infecting pathogen, but also to a wide range of normally virulent pathogens. SAR is associated with increased levels of salicylic acid (SA), both at the infection site and systemically. SA is necessary and sufficient for SAR: application of SA onto plants can induce SAR, whereas expression of an SA-oxidizing enzyme, salicylate hydroxylase, suppresses SAR. SAR is also correlated with the induced expression of genes in uninfected secondary tissues. These genes include the pathogenesis-related (*PR*) genes, some of whose protein products have antimicrobial activity. A limited set of SAR marker genes has been identified using traditional methods of screening [3,4]. Now, Maleck *et al.* [5] have applied microarray technology to provide a much more comprehensive description of SAR genes from *Arabidopsis thaliana*. Additionally, knowledge of the genomic sequence for *Arabidopsis* has enabled them to identify a common promoter element associated with a particular set of SAR-induced genes.

Maleck *et al.* [5] used an *Arabidopsis* microarray containing 10,000 expressed sequence tags (ESTs). Because of redundancy in the EST set, this represented about 7,000 genes or 25-30% of all *Arabidopsis* genes. This microarray was used to profile gene expression changes under 14 different conditions related to SAR. Most of these conditions corresponded to fully induced SAR generated by either chemical or biological means. For example, plants were treated with benzothiodiazol, a chemical analog of SA, and RNA was extracted 48 hours later. RNA was also collected from uninfected secondary tissue 44 hours after infection of primary leaves with avirulent bacteria. Three *Arabidopsis* mutants (*cim6, 7,* and *11*) that have a constitutive SAR phenotype and one mutant (*npr1/nim1*) that is compromised for SAR were also analyzed. Maleck *et al.* [5] then compared the gene expression profiles between the 14 experiments involving SAR and identified 413 ESTs (about 300 genes) that differ in expression during SAR. The criterion for selecting these ESTs was conservative: ESTs had to exhibit differential expression equal to or greater than 2.5-fold in at least two SAR-relevant samples.

The identification of these SAR genes, many of which are novel, is a significant achievement in itself. This study goes much further, however, by analyzing in detail the expression profiles of the 413 SAR-associated ESTs. Two types of clustering algorithms were used to derive groups of SAR genes with highly similar regulation patterns. The cluster group containing the *PR-1* gene, termed the *PR-1* regulon, was

further analyzed; *PR-1* is a commonly used marker for SAR. The induction of the *Arabidopsis PR-1* gene is dependent on the protein NPR1 (also known as NIM1) [6,7]. NPR1 has been shown to interact with members of the TGA family of basic leucine zipper (bZIP) transcription factors [8-10]. The promoter of the *Arabidopsis PR-1* gene contains a binding site for TGA-bZIP factors (the sequence TGACG) that serves as a positive *cis*-acting element for SA induction [11]. Thus, it was expected that all genes of the *PR-1* regulon would contain a TGA-bZIP binding site in their promoters. Maleck *et al.* [5] inspected the promoters (about 1 kb of upstream sequence) of 26 *PR-1* regulon genes for known *cis* elements. Surprisingly, the minimum TGA-bZIP recognition site (TGACG) is absent from 9 of 26 *PR-1* regulon promoters and its overall occurrence in the promoter set is less than that expected at random. This suggests that TGA-bZIP factors are unlikely to act as common regulators of *PR-1* regulon genes. In contrast, the core binding site for WRKY transcription factors [12], known as a W box (the sequence TTGAC), is found in all 26 promoters. It is present on average 4.3 times per promoter and the boxes are often clustered. The clustering of W boxes has been shown to be important for a strong and rapid transcriptional response [13]. Inspection of the promoter sequences of a random set of genes that are not co-regulated with *PR-1* gives an average occurrence of less than two WRKY factor sites per promoter. These data suggest that WRKY factors are essential for the co-regulation of *PR-1* regulon genes. Although the core binding site for TGA-bZIP and WRKY factors are similar, a distinction can be made between these two sites because no functional W box has been found that has a G after the TTGAC core sequence [12].

A WRKY binding site in the promoter of the *PR-1* gene has previously been shown to be an important regulatory element for response to 2,6-dichloroisonicotinic acid, a chemical analog of SA [11]. In this case, the WRKY site acts as a negative *cis* element. Maleck *et al.* [5] thus propose that *PR-1* regulon genes may be co-repressed by WRKY factors and that during SAR these genes would be de-repressed (Figure 1). This de-repression would probably be mediated through NPR1, because SAR-associated expression of all *PR-1* regulon genes was dependent on NPR1 activity. No direct interaction between NPR1 and a WRKY factor has yet been reported, however. A negative regulator of *PR-1* gene expression, SNI1, has recently been characterized [14]. SNI1 has no obvious DNA-binding domain, but it is localized in the nucleus. It is possible that SNI1 interacts with WRKY factors bound to negatively acting W boxes. During SAR, NPR1 may inactivate SNI1 to de-repress *PR-1* regulon genes. It is also possible that distinct WRKY factors act on individual promoters within the *PR-1* regulon. In some cases, a WRKY factor may act through a de-repression mechanism, as in the case of the *PR-1* gene, whereas in other cases a different WRKY factor may act through a simple activation mechanism. The parsley WRKY1 protein is an example of a WRKY factor acting as a positive regulator of defense gene expression [13]. In either case, the WRKY factor would act together with other types of transcription factors to achieve precise regulation of gene expression during SAR.

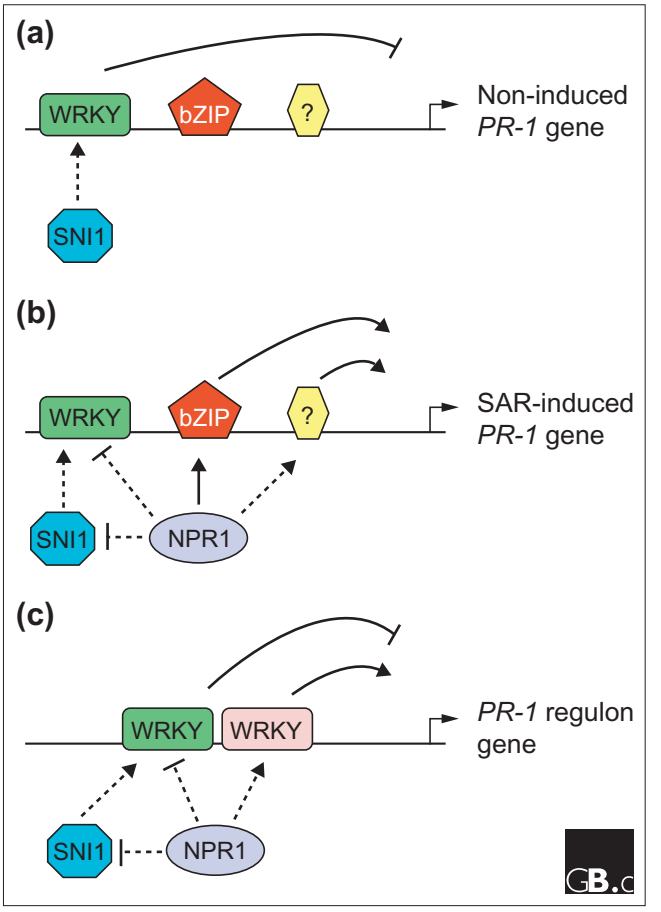

Figure 1
WRKY proteins are key regulators of gene expression during SAR. **(a,b)** The *PR-1* gene is thought to be regulated by three transcription factors: a TGA-bZIP factor and an unknown factor that activate transcription, and a WRKY factor that represses transcription [11]. SNI1 is a negative regulator of *PR-1* gene expression, possibly acting through the WRKY factor. (b) NPR1 regulates *PR-1* gene expression through interactions with the TGA-bZIP factor, and possibly with the other two transcription factors and/or with SNI1. **(c)** Common regulation of genes in the *PR-1* regulon is proposed to occur through WRKY transcription factors acting either positively or negatively and in an NPR1-dependent fashion. Dashed lines indicate putative interactions.

Maleck *et al.* [5] have uncovered an interesting biological insight regarding transcriptional reprogramming during SAR in *Arabidopsis*. This insight was made possible through whole genome analysis using DNA microarrays. Another recent study using *Arabidopsis* DNA microarrays examined gene expression changes of 2,375 genes after inoculation with the fungal pathogen *Alternaria brassicicola* or treatment with the defense-related signaling molecules SA,

methyl jasmonate (MJ), or ethylene [15]. It was found that 705 ESTs on the microarray showed differential expression in one or more of these treatments. The high proportion of ESTs showing changes was due to preferential representation of putative defense-associated ESTs on the microarray. The interesting finding from this study was the level of coordinated gene expression changes between the four treatments. A comparison of the expression profiles from the four treatments showed that 126 genes were induced by multiple treatments. For example, SA and MJ coinduced 55 genes. Furthermore, half the genes induced by ethylene were also induced by MJ and 50 genes induced by the fungus were also induced by SA, MJ, and/or ethylene. Previous studies have indicated that there is coordination between different plant defense pathways, but these studies have only focused on one or a few genes at a time. The extent of the overlap between the pathways can only be fully appreciated with a more global analysis of gene expression, however.

Future microarray experiments will provide further insights into the signaling networks governing plant defense. Microarrays containing the full complement of *Arabidopsis* genes will provide a more complete analysis. Microarrays developed for other plant species, including important crop species, will also provide further information. It is anticipated that the genome-scale information garnered from these experiments will in due course provide avenues for enhancing disease resistance in crop plants.

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