Trends in
Biochemical Sciences

Focus issue: Plant biochemistry

Spotlight

A vigilant gliding bird protects plants

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The plant hormone salicylic acid (SA) receptor NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 (NPR1) plays a critical role for plant defense against biotrophic and hemi-biotrophic pathogens. In a milestone paper, Kumar, Zavaliev, Wu et al. unraveled the structural basis for the assembly of an enhanceosome by NPR1 in activating the expression of plant defense genes.

Just like human beings, plants can be infected by a wide range of pathogens such as bacteria, viruses, nematodes, fungi, and oomycetes. Upon infection by biotrophic and hemi-biotrophic pathogens, a high level of plant defense hormone SA will be induced [1]. SA is not only necessary but also sufficient for plant immunity. Through genetic screens and biochemical studies, NPR1 was identified as a master regulator of SA signaling and a receptor of SA [2,3]. Phenotypically, Arabidopsis npr1 mutants exhibited an elevated susceptibility to pathogen infection, whereas overexpression of Arabidopsis NPR1 in economically important crops provided enhanced resistance to a wide range of pathogens and stresses [2,4].

An important bottleneck in our understanding of NPR1 function in plant immunity was the lack of structural basis underpinning NPR1's functions in promoting plant defense. In a recent landmark paper published in Nature, Kumar, Zavaliev, Wu et al. filled this important knowledge gap [5].

Kumar, Zavaliev, Wu et al. purified full-length Arabidopsis NPR1 protein from insect cells [5]. The reduced form of the purified NPR1 protein predominantly exists as dimers. Single-particle cryo-electron microscopy (cryo-EM) at a resolution of 3.8 Å revealed that the tertiary structure of NPR1 dimers resembles a gliding bird constituted by three domains (Figure 1A) [5].

Next, to obtain the 3D structure of SA-bound NPR1, the authors refolded NPR1 in the presence of SA [5]. The NPR1 full-length cryo-EM structure shows that there is a clear electron density in the putative SA-binding domain (SBD), while the structures of other parts are similar to apo NPR1. Further, Kumar, Zavaliev, Wu et al. modeled SA inducing the folding and docking of SBD onto ANK3/4 via a well-defined hydrophobic interface, which is required for NPR1's function in promoting defense gene transcription; NPR1 interacts with TGA transcription factors in the nucleus to promote the expression of Pathogenesis-Related (PR) genes encoding proteins with antimicrobial activities [6]. To gain insights into the molecular function of NPR1 as a transcriptional coactivator, the tertiary structure of the NPR1-TGA3 protein complex in the presence of TGA binding site cis-element as-1 was determined by single-particle cryo-EM [5]. Although both $TGA3_2$-$NPR1_2$-$TGA3_2$ and $NPR1_2$-$TGA3_2$ configurations were identified, Kumar, Zavaliev, Wu et al. speculated that $TGA3_2$-$NPR1_2$-$TGA3_2$ is the final assembly while $NPR1_2$-$TGA3_2$ represents an assembly intermediate because purified NPR1 proteins exist as dimers (Figure 1B). The connection between NPR1 and TGA3 is mediated by ANK1 in NPR1 and the NPR1-interacting domain (NID) in the C terminus of TGA3. Surprisingly, Kumar, Zavaliev, Wu et al. found palmitic acid in NID, supporting an important role of fatty acid in regulating plant defense [5]. More studies will be required in order to illustrate the exact role of this palmitic acid in the transcriptional regulation of plant defense genes.

Shaped like a gliding bird with two wings, the NPR1 dimer is required for NPR1's function in plant immunity [5]. Dimerization-deficient npr1 (dim) mutant was designed based on a similar strategy that was deployed to disrupt the dimerization of the SPOP BTB domain. Cryo-EM revealed that npr1(dim) adopts a single-wing shape, confirming that npr1(dim) is indeed defective only in dimerization. In stable transgenic Arabidopsis lines, npr1(dim)-GFP failed to activate the expression of plant defense genes. Consequently, these plants were compromised in plant defense.

Kumar, Zavaliev, Wu et al. showed that one of the major functions of SA is to induce the docking of SBD onto ANK3/4 [5]. However, there is no direct contact between SBD and TGA3 [5]. These data suggest that SA-induced SBD-ANK docking might facilitate post-translation modifications of NPR1 and/or recruitment of transcription regulators by NPR1 [5]. For the first time, a C2HC (Cys 150, Cys 155, His 157, and Cys 160) zinc finger motif was identified within the BTB domain of NPR1 [5]. This zinc finger motif plays a key role in mediating the interaction between NPR1 and TGA3.

Because NPR1 regulates the expression of over 2000 genes, it is reasonable to propose that NPR1 not only forms an enhanceosome complex with TGA homodimer (Figure 1C), but also with other transcription factors. Besides TGA transcription factors, NPR1 also interacts with WRKY and TCP transcription factors to regulate the expression of PR genes [7]. It will be interesting to find out whether NPR1-WRKY and NPR1-TCP protein complexes share similar configurations as the $TGA3_2$-$NPR1_2$-$TGA3_2$ protein complex. In addition to transcription factors, NPR1 associates with EDS1, histone acetyltransferases HAC1 and HAC5, and CDK8 to promote the expression of plant defense genes [8–10]. Both EDS1 and NPR1 function as transcriptional coactivators and they bind the same regions in PR1 promoter

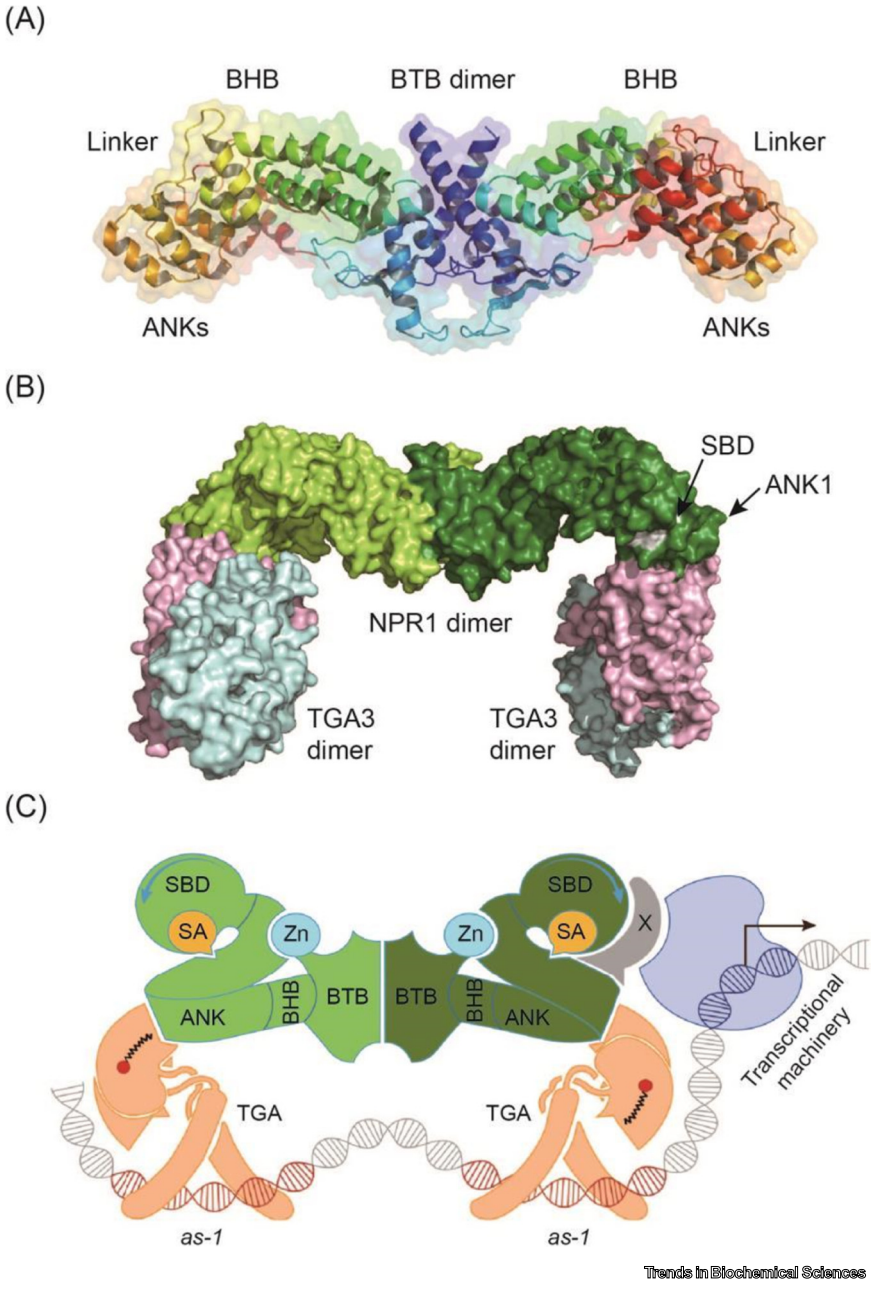

Figure 1. The gliding-bird-shaped NPR1 dimer mediates the enhanceosome assembly to promote plant defense gene expression. (A) Cryo-electron microscopy (cryo-EM) analysis revealed that the tertiary structure of NPR1 homodimer (PDB ID: 7MK2) appears like a gliding bird. The Broad-complex, Tramtrack, and Bric-à-Brac (BTB) dimer region forms the 'body' of a bird, while the two BACK helix bundle (BHB) and ankyrin repeats (ANK) domains are similar to the two 'wings' of the bird. In addition to three apparent ANKs, a fourth noncanonical ANK in NPR1 protein was observed. (B) Cryo-EM density map of the $TGA3_2$-$NPR1_2$-$TGA3_2$ complex (EMDB ID: EMD-25769). The salicylic acid (SA)-binding domain (SBD) with weak electron density is drawn with white color. Due to the limitation of resolution, the authors modeled the structure of SBD using SWISS-MODEL based on the structure of the NPR4 SA-binding core (PBD ID: 6WPG). (C) A schematic model of an enhanceosome in regulating the expression of SA-responsive genes. SA induces SBD-ANK docking, creating a new interface to promote post-translational modifications of NPR1 and/or the recruitment of other transcriptional regulators (X). The assembled enhanceosome will then activate the expression to plant defense genes to suppress pathogen infection. Abbreviation: NPR1, NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1.

to facilitate the expression of PR1 [9]. CDK8, which is a major component in the kinase module of the mediator complex, is engaged in NPR1-dependent plant defense gene expression [10]. Mediator is defined as a large protein complex that is required for transcription by RNA polymerase II. Last but not least, HAC1 and HAC5 can turn tightly packed heterochromatin into lightly packed euchromatin to promote gene transcription [8]. Adding these key players to the NPR1-TGA protein complex will undoubtedly increase our knowledge on how this macromolecule complex coordinates SA and RNA polymerase II to promote plant defense gene expression.

NPR1 was discovered as a key regulator of plant immunity more than two decades ago and has also been shown to regulate plant responses to abiotic stresses [2,4]. Kumar, Zavaliev, Wu et al. solved a long-standing puzzle by showing that gliding-bird-shaped NPR1 dimers form an enhanceosome with TGA3 and other transcription regulators to promote plant defense gene expression [5]. Future studies on the structures of post-translationally modified NPR1, oligomerized NPR1 in the cytosol, and reduced NPR1 in the nucleus, in complex with other transcription regulators, will help us gain important knowledge on the regulations of plant stress responses by NPR1, which could ultimately lead to the development of crops with better resilience [1]. It is imperative for us to meet increasing food demand when we are facing a rising global population and a changing climate.

Declaration of interests

The authors declare no competing interests.

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https://doi.org/10.1016/j.tibs.2022.06.008

Published by Elsevier Ltd.

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