PLANT IMMUNITY

# Deceiving the chaperone

Heat-shock protein 90 (HSP90) chaperones play an essential role in plant defence by assisting the folding of client proteins needed for immunity. A newly identified bacterial effector promotes disease by mimicking a HSP90 client, functioning as a minimal kinase that phosphorylates and inactivates the chaperone.

## Huan Chen, Fengquan Liu and Zheng Qing Fu

In nature, plants constantly interact with a wide variety of microorganisms. Among them, many bacterial, viral, fungal, oomycete and nematode pathogens seek every opportunity to invade and steal nutrients from their host plants[1]. A deeper understanding of how plant pathogens cause diseases is needed for developing novel and effective control strategies. Given that plants have developed a robust defence system, scientists show great interest in investigating how plant pathogens circumvent the host innate immune system to cause diseases[2,3]. A recent study published in *Cell* provides a perfect example of a unique virulence strategy used by a plant pathogen[4]. Lopez et al. report that the bacterial type III effector HopBF1 suppresses plant defence by phosphorylating and inactivating the molecular chaperone heat-shock protein 90 (HSP90) to promote disease development.

Plant Gram-negative bacterial pathogens rely on effectors that are injected into plant cells by the type III secretion system to cause diseases. One of these type III effectors, HopBF1, is conserved in animal and plant bacterial pathogens, including *Pseudomonas syringae*[4]. HopBF1 is also found in plant symbionts and free-living bacteria. Analysis of its sequence indicates that HopBF1 shows similarity to a protein kinase[4]. To better understand its exact function, Lopez et al. solved the crystal structure of HopBF1 from the opportunistic human pathogen *Ewingella Americana*. This HopBF1 adopts a minimal kinase-like fold and can be superimposed onto the tertiary structure of protein kinase A. In fact, *E. Americana* HopBF1 is an atypical kinase because canonical kinases are rich in α-helices in the C-lobe and have a GHI helical subdomain involved in substrate recognition, while *E. Americana* HopBF1 has more β-strands than α-helices in its C-lobe and does not have the GHI helical subdomain.

In order to find out whether this putative kinase activity plays a role in bacterial virulence, the authors then focused on the HopBF1 from the model plant bacterial pathogen *P. syringae*[4]. When the wild-type effector was transiently expressed in *Nicotiana benthamiana* or *Nicotiana tabacum* plants by agroinfiltration or delivered into plants by the *P. syringae* type III secretion system, the authors observed robust necrosis and tissue collapse. In contrast, these phenotypes were not observed when a catalytically inactive kinase was used. These data indicate that HopBF1 contributes significantly to disease development, and this role is dependent on its kinase activity.

It is crucial to identify the host targets of HopBF1, so the authors performed in vitro phosphorylation assays. In the presence of HopBF1, a single protein from a yeast extract with molecular weight of 80–90 kDa was labelled. A co-immunoprecipitation assay revealed that only one protein forms a complex with HopBF1 kinase in yeast. Mass spectrometry identified this protein as the yeast homologue of HSP90. In vitro kinase assays confirmed that HopBF1 indeed phosphorylates yeast, wheat and human HSP90. More importantly, HopBF1 phosphorylates HSP90 during infection.

HSP90 is a molecular chaperone, which assists the proper folding of its client proteins (Fig. 1). It is phosphorylated by HopBF1 on serine 99. As a conserved residue in HSP90, serine 99 supports nucleotide binding through its interaction with the β-phosphate of ATP. As expected, phosphorylation of HSP90 by HopBF1 inactivates its ATPase activity[4].

Unlike HSP70, which is a general chaperone, HSP90 prefers proteins involved in signalling transduction pathways, including protein kinases, as its clients[4]. HSP90 is an indispensable component in animal and plant immunity[5]. In mammals, HSP90 binds the co-chaperone SGT1 and this complex is required for the nucleotide-binding leucine-rich repeat (NB-LRR) immune sensors nucleotide-binding oligomerization domains 1/2 (NOD1/2) and NLRP3-activated immune responses[6]. When two human HSP90 clients — the oncogenic tyrosine kinase v-Src and NLRP3 — were co-expressed with wild-type *E. Americana* HopBF1, the levels of v-Src and NLRP3 were significantly reduced, supporting the idea that HopBF1 inactivates HSP90's chaperone function through phosphorylation[4]. In plants, HSP90 and its co-chaperones SGT1 and RAR1 play key roles in effector-triggered
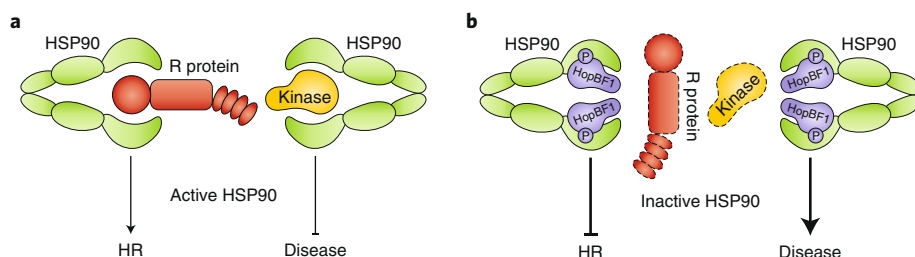


**Fig. 1 | A schematic model on the disruption of HSP90's function in plant defence by the bacterial type III effector HopBF1. a**, HSP90 functions as a molecular chaperone, which facilitates the proper folding of its clients, including NB-LRR resistance (R) proteins and kinases. NB-LRR R proteins promote effector–trigger immunity by turning on hypersensitive response (HR), while kinases confer plant defence responses. **b**, The plant bacterial pathogen *P. syringae* delivers the type III effector HopBF1 into plant cells. As a minimal and atypical kinase, HopBF1 appears to mimic a HSP90 client. HopBF1 inactivates HSP90 through phosphorylating serine 99 on the lid segment of the chaperone HSP90. Without an active HSP90, NB-LRR R proteins and kinases become unstable. In the presence of HopBF1, NB-LRR R proteins cannot function properly to induce HR and kinases cannot activate plant defence. Consequently, *P. syringae* causes severe disease on its host plants.

immunity[7,8]. Currently available data suggest that NB-LRR resistance proteins, such as RPM1, are clients of HSP90 (refs. [4,7]). To investigate the impact of HopBF1 on resistance protein-mediated hypersensitive response (HR) or cell death, Lopez et al. transiently expressed wild-type HopBF1 or kinase-inactive HopBF1 mutants with an autoactive mutant of RPM1 (D505V), which elicit HR in the absence of a pathogen, in *Nicotiana benthamiana* plants. When RPM1 (D505V) was co-expressed with wild-type HopBF1 but not the kinase-inactive D154A and D169A mutants, cell death was delayed and reduced, suggesting that HopBF1 blocks RPM1 (D505V) activation through phosphorylation of HSP90.

Several kinases, including cyclin-dependent kinase 4, haematopoietic cell kinase and Janus kinase 1, have been shown to be clients of HSP90 (ref. [9]). HopBF1, as a minimal and atypical kinase, appears to mimic a client in order to disable HSP90. HopBF1 undermines plant defence, probably through reducing the levels of NB-LRR resistance proteins and

kinases, as it is well known that inhibition of HSP90's ATPase activity leads to the degradation of its client proteins (Fig. 1)[4,10]. Previously, it was reported that another *P. syringae* type III effector, avirulence protein B (AvrB), interacts with HSP90 through HSP90's co-chaperone RAR1 (ref. [11]). Both AvrB and HSP90 promote MPK4 activation, and HSP90 is required for AvrB-induced susceptibility. HSP90 plays a crucial role in animal and plant immunity[5]. In mammals, HSP90 has been shown to interact with the type III effector NleH1 from enterohaemorrhagic *Escherichia coli*[12]. Therefore, animal and plant bacterial pathogens have probably independently evolved strategies to disrupt the functions of HSP90, highlighting the importance of targeting chaperones during the development of animal and plant bacterial diseases. ❑

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**Competing interests**
The authors declare no competing interests.