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# How *Xanthomonas* type III effectors manipulate the host plant

Sabine Kay and Ulla Bonas

Pathogenicity of *Xanthomonas* and most other Gram-negative phytopathogenic bacteria depends on a conserved type III secretion (T3S) system which injects more than 25 different effector proteins into the plant cell. Extensive studies in the last years on the molecular mechanisms of type III effector function revealed that effector proteins with enzymatic functions seem to play important roles in the interaction of *Xanthomonas* with its host plants, for example, the SUMO protease XopD. In addition, xanthomonads express a unique class of type III effectors to pursue another strategy. Effectors of the AvrBs3 family, so far only identified in *Xanthomonas* spp. and *Ralstonia solanacearum*, mimic plant transcriptional activators and manipulate the plant transcriptome.

**Address**
Institute of Biology, Department of Genetics, Martin-Luther-University Halle-Wittenberg, Weinbergweg 10, D-06120 Halle (Saale), Germany

Corresponding author: Bonas, Ulla (ulla.bonas@genetik.uni-halle.de)

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## Introduction

Pathogenic bacteria of the genus *Xanthomonas* infect a wide range of host plants and are responsible for important crop plant diseases. Examples include bacterial blight of rice and citrus canker caused by *X. oryzae* pv. (pathovar) *oryzae* and *X. axonopodis* pv. *citri*, respectively. The bacteria enter through natural openings, such as stomata and hydathodes, or wounds, and multiply in the intercellular spaces of the plant tissue and/or the xylem. Protein secretion systems play an important role in the interaction of pathogens with their host. *Xanthomonas* spp. contain genes for all known protein transport systems in Gram-negative bacteria, i.e. the Sec, signal recognition particle, and TAT pathways; type I, type II, type III, and type IV secretion systems of different types, type V autotransporters, two-partner secretion systems, and a type VI secretion system [1,2]. However, for most *Xanthomonas* secretion systems the substrates and their importance for bacterial virulence are unknown.

Here, we focus on substrates of the type III secretion (T3S) system which is highly conserved in plant and animal pathogens and is essential for *Xanthomonas*' pathogenicity [3,4]. This 'molecular syringe' transports Xops (*Xanthomonas* outer proteins) without N-terminal processing across both bacterial membranes [3,4]. Most proteins, termed type III effectors, are translocated directly into the plant cell. The molecular function of most effectors is still unknown; however, there is some conservation across bacteria with T3S systems (Table 1). Some effectors, formerly designated as avirulence proteins, are specifically recognized in resistant plants containing corresponding resistance (*R*) genes. Such recognition triggers plant defense reactions often culminating in the hypersensitive response (HR), a rapid localized cell death, and restricting pathogen ingress [5]. This review covers new insights of the past two years into selected *Xanthomonas* effectors with a focus on their molecular mode of action within the plant cell.

## Type III effectors are important virulence factors

T3S mutants are impaired in growth *in planta* and fail to cause disease symptoms in susceptible plants indicating that effector proteins play an essential role in pathogenicity [6,7]. Although individual *Xanthomonas* strains secrete a battery of 15 and more type III effector proteins [1,8] only a few effectors were shown to be major virulence factors because their deletion leads to a dramatic loss of virulence. For instance, AvrBs2 from the pepper and tomato pathogen *X. campestris* pv. *vesicatoria* strongly contributes to the multiplication of the bacteria *in planta*, symptom development, and epiphytic survival [9]. In contrast, mutations in genes encoding other effectors like AvrXccC and XopXccN from *X. campestris* pv. *campestris* only weakly affect bacterial growth [10,11]. Several recent studies suggest that many effectors of *Pseudomonas* contribute to virulence via suppression of plant defense mechanisms [12]. In contrast, to date there are only a few type III effectors from *Xanthomonas* with a presumed role in defense suppression. One example is XopX from *X. campestris* pv. *vesicatoria* which promotes lesion development and growth of nonhost pathogens in *Nicotiana benthamiana* suggesting that XopX suppresses basal plant defense [13].

## *Xanthomonas* effectors with enzymatic functions

Several effectors from *Pseudomonas syringae* display enzymatic activities and modify host proteins to fulfill their function [12]. Subspecies of *Xanthomonas* possess homologs of some of these proteins suggesting that the



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**Table 1**

**Known type III effectors of *Xanthomonas* and their (putative) function**

| Protein(s) | (Predicted) function | Homologs[a] in | | | | | | | | Reference[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Xac* | *Xcc* | *Xcv* | *Xoc* | *Xoo* | *Ps* | *Rs* | apb | |
| AvrBs1 | Unknown function | – | + | + | – | – | + | – | – | [9] |
| AvrBs2 | Putative glycerophosphoryl-diester phosphodiesterase | + | + | + | + | + | – | – | – | [9] |
| AvrBs3, Avrb6, AvrXa7, PthA, and others | AvrBs3 family, TAL activity | + | – | + | + | + | – | + | – | [9,28••,29••,41] |
| AvrRxo1 | Unknown function | – | – | + | + | – | – | – | – | [47] |
| AvrRxv, AvrBsT, AvrXv4, XopJ | YopJ/AvrRxv-family, putative cysteine proteases (C55 family) or acetyltransferases | – | (+) | + | – | – | + | + | + | [9,16] |
| AvrXccC | AvrB family, unknown function | – | + | – | – | – | + | – | – | [10] |
| AvrXv3 | Unknown function | – | – | + | (+) | – | + | – | – | [48] |
| Ecf XopX | HopAE1 family, unknown function | + | + | + | + | + | + | – | – | [9] |
| HpaA | Unknown function | + | + | + | + | + | – | – | – | [49] |
| XopB | Unknown function | – | – | + | – | – | + | + | – | [9] |
| XopC | Unknown function | – | – | + | – | – | – | (+) | – | [9] |
| XopD | SUMO cysteine protease (C48 family), DNA-binding (HLH motif), EAR motifs | – | + | + | – | – | (+) | – | – | [9,18••] |
| XopE1, XopE2 | HopX family, putative transglutaminases | + | + | + | – | – | + | – | – | [16] |
| XopF1, XopF2 | Unknown function | (+) | (+) | + | + | + | – | – | – | [9] |
| XopN | Unknown function | + | + | + | + | + | + | – | – | [9,11] |
| XopO | Unknown function | – | – | + | + | – | + | – | – | [9] |
| XopP | Unknown function | + | + | + | + | + | – | + | – | [9] |
| XopQ | HopQ1-1 family, putative inosine-uridine nucleoside *N*-ribohydrolase | + | + | + | + | + | + | + | – | [9] |

[a] Homologs of effectors from *X. campestris* pv. *vesicatoria* and, in case of AvrXccC, *X. campestris* pv. *campestris* were identified using BLAST algorithms [50]. (+) indicates partial homology or disrupted homologs. *Xac*, *X. axonopodis* pv. *citri*; *Xcc*, *X. campestris* pv. *campestris*; *Xcv*, *X. campestris* pv. *vesicatoria*; *Xoc*, *X. oryzae* pv. *oryzicola*; *Xoo*, *X. oryzae* pv. *oryzae*; *Ps*, *P. syringae*; *Rs*, *R. solanacearum*; apb, Gram-negative animal–pathogenic bacteria.

[b] *Xanthomonas* effectors are reviewed in [9]. Additional results are cited separately.

effectors have similar activities. For instance, there are putative effectors which show homology to HopAO1 (also known as HopPtoD2) from *P. syringae* that has tyrosine phosphatase activity and suppresses basal defense and the HR [14,15]. XopE1 and XopE2 belong to the HopX (AvrPphE) family of putative transglutaminases [16] with a cysteine-based catalytic triad essential for function [17]. The transglutaminase superfamily comprises proteins with different enzymatic activities like proteases, peptide *N*-glycanases, and DNA repair proteins [16]. However, an enzymatic activity has yet to be shown for the *Xanthomonas* effectors, and their putative virulence effects are unknown.

## The SUMO protease XopD

XopD from *X. campestris* pv. *vesicatoria* has recently been studied in detail. Interestingly, this effector promotes bacterial growth in tomato and delays onset of leaf chlorosis and necrosis in late infection stages of tomato, presumably to sustain the bacterial population in the infected tissue [18••]. The XopD protein has a modular structure and shows different biochemical activities (Figure 1a). The C terminus contains a cysteine protease domain of the C48 superfamily with homology to yeast Ulp1 (ubiquitin-like protease), a small ubiquitin-like modifier (SUMO) protease [19]. XopD isopeptidase activity with a specificity for plant SUMO was demonstrated *in vitro* and *in planta* [19,20•]. Similarly to ubiquitin conjugation, SUMO is covalently linked to eukaryotic proteins but, in contrast to ubiquitination, SUMOylation stabilizes proteins. In plants, SUMOylation and deSUMOylation regulate a number of processes, for example, in abiotic stress responses, pathogen defense, and flower induction [21]. The localization of XopD in subnuclear foci in the plant cell suggests that it probably targets nuclear SUMO-conjugated proteins. Furthermore, XopD contains an N-terminal helix–loop–helix domain for DNA binding and two ERF (ethylene response factor)-associated amphiphilic repression (EAR) motifs [18••]. Recently, XopD was shown to bind to DNA, albeit unspecifically, and to repress transcription of defense- and senescence-associated plant genes [18••] (Figure 1c). The EAR motifs are required for gene repression suggesting a direct effect of XopD on plant transcription by heterodimerization with DNA-bound transcription factors [18••]. Alternatively,



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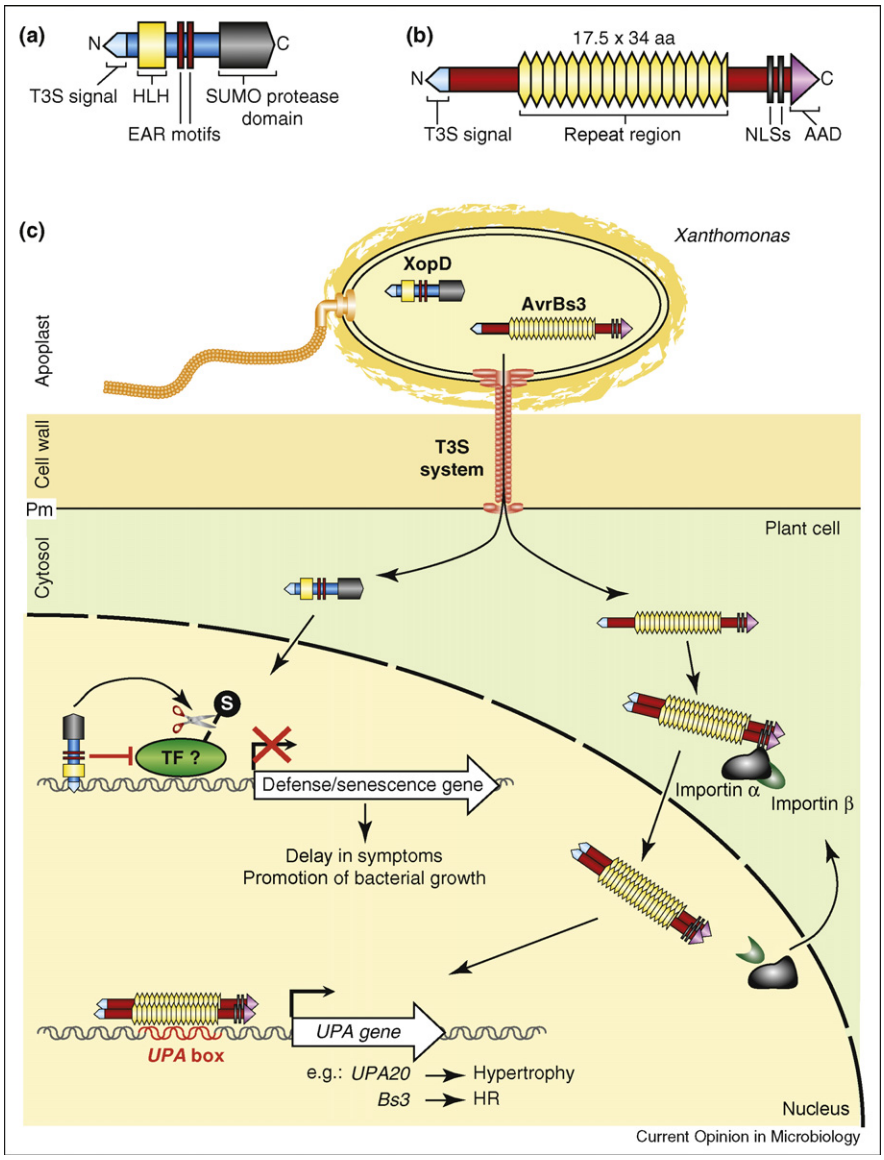
**Figure 1**




Model of the molecular function of the type III effectors XopD and AvrBs3. The protein structures of **(a)** XopD and **(b)** AvrBs3 are shown, and **(c)** the proposed mode of action of the effectors is illustrated. After translocation by the T3S system XopD is transported into the nucleus of the plant cell where it localizes to nuclear foci. Here, it binds to DNA unspecifically via a helix–loop–helix (HLH) domain. Via two EAR motifs, XopD might inhibit yet unidentified plant transcription factors (TFs). Furthermore, XopD possesses SUMO protease activity mediating deSUMOylation of yet unknown target proteins [19]. The XopD activities lead to the suppression of defense- and senescence-associated genes resulting in delayed disease symptoms and increased bacterial multiplication [18••]. AvrBs3 dimerizes in the plant cell cytoplasm and interacts with importin α via its nuclear localization signals (NLSs) [9]. The protein complex is bound by importin β mediating nuclear import. Here, AvrBs3 binds to a specific DNA sequence, the *UPA* box, and activates transcription of more than 10 *UPA* genes [28••,29••]. *UPA20*, one of the induced genes, is the key regulator of plant cell hypertrophy [28••]. In resistant pepper plants, activation of *Bs3* leads to the HR [29••]. AAD, acidic activation domain; Pm, plasma membrane.

XopD might affect chromatin remodeling which was reported for other EAR motif-containing proteins [18••]. Mutant studies revealed that both SUMO protease and transcriptional repressor activities and, to a lesser extent, the DNA binding ability of XopD, contribute to the virulence function of the effector, i.e., promotion of bacterial growth and delay of disease symptoms in tomato [18••].

## Effectors of the YopJ/AvrRxv family

A function as SUMO protease has also been proposed for effectors of the YopJ/AvrRxv family from plant and mammalian pathogens. This family of predicted C55 peptidases comprises four members in *X. campestris* pv. *vesicatoria*, AvrRxv, AvrXv4, AvrBsT, and XopJ [9]. Each protein contains a putative catalytic triad (histidine, glutamate, and cysteine) that is required for *R*-gene-mediated



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recognition ([9]; R Szczesny and U Bonas, unpublished). *In planta* expression of AvrXv4 leads to a reduction in SUMOylated proteins dependent on the catalytic triad [22]. However, a SUMO protease function of AvrXv4 could not be demonstrated [22]. YopJ from human-pathogenic *Yersinia* spp., the best-analyzed protein of the effector family, also decreases the cellular concentration of SUMOylated proteins [23]. On the other hand, a deubiquitinase activity was demonstrated for YopJ *in vivo* and *in vitro*, and components of the host immune response were identified as target proteins [24,25•]. However, the enzymatic function of YopJ/AvrRxv family proteins is a controversial issue since it was also reported that YopJ has acetyltransferase activity [26••]. YopJ-mediated acetylation of critical serine and threonine residues of a mitogen-activated protein kinase which is involved in immune response prevents its phosphorylation and activation [26••]. The idea that YopJ/AvrRxv family members are acetyltransferases is supported by a recent study on AvrBsT [27•]. The *R*-gene-mediated recognition of AvrBsT in the plant requires the catalytic triad suggesting that it depends on the enzymatic function of the effector protein [23,27•]. Cunnac *et al.* identified a specific suppressor of the AvrBsT-caused HR, *SOBER1* (suppressor of AvrBsT-elicited resistance), which encodes a carboxylesterase that deacetylates chromogenic substrates in *in vitro* assays [27•]. This raises the possibility that SOBER1 counteracts AvrBsT by deacetylating its target proteins thereby avoiding detection of the AvrBsT action within resistant plant cells [27•].

## The AvrBs3 family: manipulators of host transcription

The activities of several type III effector proteins ultimately result in a change of plant gene expression. Recently, it has been shown that AvrBs3 from *X. campestris* pv. *vesicatoria* (Figure 1b), type member of a large protein family also known as TAL (transcription activator-like) effectors, acts as a transcription factor and directly induces expression of plant genes [28••,29••]. The AvrBs3 family is the largest and best-analyzed class of type III effectors with TAL activity known so far, although there are sequence-unrelated effector proteins that probably also mimic eukaryotic transcription factors, for example, HsvB and HsvG from *Pantoea agglomerans* [30•]. The AvrBs3 family is restricted to *Xanthomonas* spp. and, with some less conserved relatives, *Ralstonia solanacearum*, and comprises several major virulence factors [9]. For instance, AvrXa7, PthXo1, and other AvrBs3-like proteins from *X. oryzae* pv. *oryzae* strongly support bacterial growth and lesion development in rice [31,32]. Other effectors of this family play an important role in the elicitation of citrus canker by *X. axonopodis* pv. *citri* [33–36]. Avrb6 and other AvrBs3 homologs from the cotton pathogen *X. campestris* pv. *malvacearum* increase the development of water-soaked lesions in leaves [37]. This is associated with an increased release of *Xanthomonas* from the apoplast facilitating distribution of the bacteria [37], an important epidemiological aspect. AvrBs3 from *X. campestris* pv. *vesicatoria* causes a hypertrophy of mesophyll cells that also might support bacterial release to the plant surface in late infection stages [38], and it contributes to bacterial spreading in the field [39].

### Targets of AvrBs3 and presumably of related effectors are plant promoters

The molecular characteristics of AvrBs3 and related proteins include a central repeat region consisting of nearly identical repetitions of usually 34 amino acids which mediate protein dimerization [40] and DNA binding [28••]. Number and order of the repeats, which mainly differ at amino acid positions 12 and 13, determine the specificity of protein activity [9,41]. In addition, the proteins contain nuclear localization signals and an acidic activation domain in the C terminus which are essential for protein function and mediate nuclear import and activation of plant gene expression, respectively [9] (Figure 1b and c). Recent microarray analyses of rice identified a number of target genes of AvrBs3-like proteins from *X. oryzae* pv. *oryzae* [42•,43], for example, *Os8N3* which is induced by PthXo1. As this plant gene is required for bacterial virulence it is thought to be a susceptibility gene [42•]. Os8N3 belongs to the MtN3 family of predicted membrane proteins from animals and plants and in healthy plants has a developmental role in inflorescence development [42•]. However, the biochemical function of Os8N3 is unknown. For AvrBs3, several target genes, termed *UPA* (upregulated by AvrBs3), were isolated from bell pepper using cDNA-AFLP and suppression subtractive hybridization [28••,38]. *UPA20* encodes a transcription factor of the bHLH (basic helix–loop–helix) family which is a key regulator of plant cell hypertrophy elicited by AvrBs3 [28••]. Promoter analyses revealed the presence of a conserved AvrBs3-responsive element, the *UPA* box, in the promoters of *UPA20* and other target genes of AvrBs3 which is directly bound by the effector protein [28••,29••] (Figure 1c). So far, AvrBs3 is the only TAL effector for which a specific binding to a plant promoter has been demonstrated. We believe, however, that AvrBs3 homologs have the same molecular mechanism of action, i.e., hijacking promoters as their plant targets.

### Plant defense strategies against TAL effectors

In response to the molecular mechanism of TAL effector function, plants have evolved a sophisticated recognition strategy using *R* gene promoters as molecular traps. Promoter activation by a certain TAL effector leads to resistance gene induction and subsequent cell death [29••,44]. The analysis of *Bs3* and *Bs3-E*, which recognize AvrBs3 and its derivative AvrBs3Δrep16, respectively, demonstrated that a sequence variation in the promoter, namely a 13 bp deletion in the *UPA* box, and not changes in the coding sequence is responsible for the specific recognition of each effector protein in the corresponding resistant pepper plant [29••].



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In contrast, resistance conferred by the recessive *R* gene *xa13* from rice is not based on induction of the *R* gene promoter but rather on loss of inducibility [45•]. *xa13* is most likely allelic to the disease susceptibility gene *Os8N3* which is induced by the TAL effector PthXo1 described above [42•]. Promoter sequence variations lead to loss of *xa13* inducibility and hence render the rice plant resistant to *X. oryzae* pv. *oryzae* strains which depend on PthXo1 as a major virulence factor [42•,45•]. Interestingly, this resistance can be overcome by another TAL effector, AvrXa7, that does not induce *Os8N3* but probably another susceptibility gene in rice [42•]. In summary, in case of both *Bs3* and *xa13* (*Os8N3*), plant resistance is mediated by the mutation of effector target promoters; however, the outcome is induction of a suicide gene in case of *Bs3* [29••] or lack of gene induction [42•,45•].

Another plant resistance mechanism is based on a subunit of the basal transcription machinery as a polymorphic component of TAL effector recognition in rice. The recessive *R* gene *xa5* from rice encodes the γ subunit of the general transcription factor TFIIA which differs from the product of the susceptible allele (*Xa5*) in only one amino acid (E39V) [46]. TFIIAγ is involved in the recruitment of the basal transcription machinery by eukaryotic transcription factors. Recognition of the corresponding effector Avrxa5 from *X. oryzae* pv. *oryzae*, most likely a member of the AvrBs3 family [31], might be on the basis of a missing interaction of Avrxa5 with the xa5 protein and therefore the inability to promote transcription of susceptibility genes in *xa5*/*xa5* rice lines.

## Conclusion

To promote pathogenicity *Xanthomonas* uses type III effector proteins with novel activities so far not described for any other T3S system-containing pathogen. While effectors like XopD and members of the YopJ/AvrRxv family display enzymatic activities on so far unknown plant proteins, members of the AvrBs3 family act as eukaryotic transcriptional activators and directly modify the host's transcriptome by binding to cognate promoter boxes. Further analysis of type III effector targets will not only provide a deeper insight into virulence of *Xanthomonas* spp. but also shed light on basic plant developmental processes.

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