The plant apoplast during plant–pathogen interactions is an ancient battleground that holds an intriguing range of attacking enzymes and counteracting inhibitors. Examples are pathogen xylanases and polygalacturonases that are inhibited by plant proteins like TAXI, XIP, and PGIP; and plant glucanases and proteases, which are targeted by pathogen proteins such as GIP1, EP1, EPIC2B, and AVR2. These seven well-characterized inhibitors have different modes of action and many probably evolved from inactive enzymes themselves. Detailed studies of the structures, sequence variation, and mutated proteins uncovered molecular struggles between these enzymes and their inhibitors, resulting in positive selection for variant residues at the contact surface, where single residues determine the outcome of the interaction.

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Introduction
Extracellular plant–pathogen interactions probably existed long before the evolution of pathogen effector translocation systems and plant resistance (R) genes. The molecular basis of these interactions is mostly undiscovered but some have been investigated in detail and reveal intriguing mechanisms. Here we will highlight major recent findings of extracellular enzyme–inhibitor interactions at the plant–pathogen interface.

Although extracellular plant–pathogen interactions are complex, they can be simplified by assuming that they evolved in several stages (Figure 1). First, microorganisms became pathogens by attacking plants using cell-wall-degrading enzymes and other hydrolases (Figure 1A). In response to this attack, plants secrete inhibitors that suppress these hydrolases (Figure 1B). Initially, these inhibitors were probably constitutively produced, but upon evolution of pathogen recognition systems the production and secretion of these proteins became inducible, becoming part of the arsenal of pathogenesis-related (PR) proteins. Besides suppression of pathogen attack, counter attack mechanisms also evolved in plants through the induced secretion of hydrolytic enzymes (Figure 1C). Examples are the well-studied PR proteins including endo-β-1,3-glucanases (PR-2), chitinases (PR-3), and proteases (PR-7) [1]. Pathogens, in turn, responded to this counter attack by producing inhibitors that suppress these enzymes (Figure 1D). The fifth and latest stage was a sophisticated refinement of the pathogen recognition system by the evolution of R genes that recognize the manipulation of plant targets by pathogens, inducing a severe defense response that includes cell death (Figure 1E). Aspects of this simplified model are consistent with the ‘zigzag’ model for the plant immune system, which explains the suppression of basal defense responses by pathogen effector proteins, followed by the evolution of efficient effector recognition by R proteins [2].

Antagonistic interactions between organisms at the molecular level result in enzymes that evade inhibition, and inhibitors that adapt to these new enzymes. These ‘molecular struggles’ result in positive selection for variation of residues at the interaction surface between enzymes and inhibitors. Selection on these proteins can result in a replacement of outdated versions, causing an ‘arms race’. Alternatively, different isoforms of enzymes and inhibitors are maintained in the population through balancing selection, also compared to ‘trench warfare’. In any case, positive selection for variant residues (here called ‘diversifying selection’) leaves imprints in the gene sequences by the overrepresentation of codon changes that cause significant variation in amino acids.

To illustrate these molecular struggles, we review the structures, sequence variation, and mutagenesis studies of the seven best-characterized enzyme–inhibitor interactions (Figure 1, right). These are cell-wall-degrading enzymes and proteases and their inhibitors, identified in different plant–pathogen interactions (Table 1). Each of these interactions has its own molecular peculiarities and we highlight them in the same order as in Figure 1. To classify the enzymes and inhibitors, we consistently use the classification of glycoside hydrolases (GHs) and proteases of the CAZy and Merops databases, respectively (www.cazy.org and merops.sanger.ac.uk/).
Plant inhibitors targeting pathogen enzymes

Cell-wall-degrading enzymes are important components of the pathogen offensive in the plant apoplast. *Endo*-β-1,4-xylanases (classes GH10 and GH11) degrade xylan, a predominant hemicellulose in many monocotyledon species; whereas polygalacturonases (PGs) (class GH28) hydrolyze homogalacturonan, the major component of pectin [3**]. Microbial xylanases are frequently used in the food industry and their inhibition by wheat grain proteins led to the discovery of xylanase inhibitor proteins of three structural classes: *Triticum aestivum* xylanase inhibitor (TAXI), xylanase inhibitor protein (XIP), and thaumatin-like xylanase inhibitor (TLXI). Recent data indicate that these inhibitors also play a role in plant defense. Polygalacturonase-inhibiting proteins (PGIPs) were discovered earlier and have been extensively studied in various plant–pathogen interactions. All these inhibitors are secreted and localize in the cell wall by their polysaccharide-binding affinity [4,5]. Progress in the recent years revealed interesting structural details on inhibitor specificity, as discussed below.

Wheat inhibitor TAXI targets pathogen GH11 xylanases

A role for TAXI and XIP in plant–pathogen interactions was suggested by the observation that these inhibitors do not act on endogenous plant derived xylanases, but are effective only on microbial xylanases [3**,6**]. Both TAXI and XIP are encoded by gene families which are differently regulated by various forms of stress, such as wounding or infection with the wheat pathogens *Fusarium graminearum* and *Erysiphe graminis* [7,8*,9]. TAXI can only inhibit GH11 xylanases, which are β-jelly roll proteins that fold like a hand with the catalytic glutamine residues in the ‘palm’, covered by a ‘thumb’ [10]. TAXI-I

<table>
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<th>Enzyme</th>
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<tr>
<td>XylA/B, XynBc1 xylanases</td>
<td><em>Fusarium graminearum, Botrytis cinerea</em></td>
<td>TAXI-I (pepsin-like)</td>
<td>Wheat</td>
<td>[11]*</td>
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<td>XynBc1 xylanase</td>
<td><em>B. cinerea</em></td>
<td>XIP-I (chitinase-like)</td>
<td>Wheat</td>
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<td><em>Fm</em>PG1, <em>Bc</em>PG1 polygalacturonases</td>
<td><em>F. moniliforme, B. cinerea</em></td>
<td>PGIP (LRR protein)</td>
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<td>[30]**</td>
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<td><em>Pe</em>GlaseA/B endoglucanase</td>
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<td>GIP1 (trypsin-like)</td>
<td><em>P. sojae</em></td>
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<td>P69B/PR7</td>
<td>Tomato</td>
<td>EP11.10 (Kazal-like)</td>
<td><em>P. infestans</em></td>
<td>[39*,40]*</td>
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<td>PIP1</td>
<td>Tomato</td>
<td>EPIC1,2B (cystatin-like)</td>
<td><em>P. infestans</em></td>
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<td>RCR3</td>
<td>Tomato</td>
<td>AVR2 (small, Cys-rich)</td>
<td><em>Cladosporium fulvum</em></td>
<td>[42**,48**]</td>
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inhibits XylA and XylB, two GH11 xylanases from *F. graminearum* [11*]. *XynBc1*, a GH11 xylanase cloned from *Botrytis cinerea*, was inhibited by TAXI-I but not by TAXI-II, suggesting that these inhibitors can be specific and have perhaps coevolved with their targets [12*]. However, these interactions are just the tip of the iceberg, as *F. graminearum* has over 30 different xylanase genes that are induced during infection [13]. The crystal structure of TAXI has a striking structural homology with pepsin-like aspartic proteases but it lacks the required catalytic triad, suggesting that this inhibitor evolved from a pepsin-like aspartic protease ancestor [14]. The crystal structure of the complex of TAXI-I inhibiting the *Aspergillus niger* xylanase-I (ANXI) revealed substrate-mimicking contacts in the substrate-docking region of the xylanase [14] (Figure 2a). The TAXI–GH11 interactions probably hold a wealth of information on molecular struggles, to be revealed by determining positions for diversifying selection and their importance for the interaction.

**TAXI-like inhibitors target pathogen GH12 glucanases**

In contrast to the inhibitory activity of TAXI toward GH11 xylanases, TAXI family members NEC4, XEGIP (XEG-inhibiting protein), and EDGP target GH12 xyloligosaccharide-specific endoglucanases (XEG). GH12 enzymes are structurally related to GH11 enzymes, but degrade a different cell wall component, called xyloglucan, a major cell wall component of dicots and nongraminaceous monocots [3*,6*]. NEC4 is an abundant protein in the nectar of ornamental tobacco plants and probably protects the nectocoeum from fungal attack [15**]. Both NEC4 and XEGIP (from tomato) inhibit the *endo-β-1,4-glucanase XEG from A. aculeatus* but are unable to inhibit GH11 or GH10 xylanases [15**,16]. The carrot EDGP (extracellular dermal glycoprotein) is the ortholog of tomato XEGIP and accumulates upon wounding [17]. However, a role for XEGIP, EDGP, and NEC4 in plant defense remains to be demonstrated.

**Wheat inhibitor XIP-I targets fungal GH11 and GH10 xylanases**

*XIP-I* from wheat is also transcriptionally induced during wounding and infection with *E. graminis* but not with *F. graminearum* [8*]. XIP-I-like proteins are abundant secreted proteins in maize cell cultures upon treatment with chitosan elicitors [18]. Intriguingly, XIP-I shares sequence homology with class III chitinases of the GH18 family, of which PR-8 is also a member, but it lacks the catalytic residues required for hydrolytic activities [19]. The discovery of another XIP-family member in rice indicates that members of an entire subfamily of presumed chitinases are actually xylanase-inhibiting proteins [20*]. In contrast to TAXI, XIP-I inhibits both GH10 and GH11 xylanases. The crystal structures of XIP-I in complex with *A. nidulans* (GH10) and *Penicillium funiculosum* (GH11) xylanases revealed a striking simultaneous binding of the inhibitor to both target enzymes using two independent enzyme-binding sites (Figure 2b) [10]. GH10 xylanase inhibition is caused by substrate-mimicking contacts in the S2 substrate-binding pocket of the xylanase. GH11 xylanase inhibition is mediated by a loop that sticks in the active site, where an arginine residue directly contacts the catalytic residues of the xylanase (Figure 2c) [10]. XIP-I is an efficient inhibitor of *Botrytis* XynBc1 GH11 xylanase [12*] but it cannot inhibit XylA and XyL GH11 xylanases of *F. graminearum* [11*]. Mutagenesis revealed that the absence of inhibition was because of amino acid adaptations in the ‘thumb’ structural region [21**]. For example, a V151T mutation in XylA restores inhibition by XIP-I by the formation of one additional hydrogen bond with XIP-I [21**]. Notably, amino acid variations in this ‘thumb’ region are common to GH11 xylanases of plant pathogens, suggesting that
adaptations in this region are a frequent strategy to prevent XIP-I inhibition [21**].

Plant PGIPs target pathogen GH28 polygalacturonases

PG–PGIPs complexes are the best characterized enzyme–inhibitor interactions at the plant–pathogen interface. PGs are important virulence factors for various fungi and bacteria, and a partial contribution of PGIPs to plant defense has been shown genetically using overexpression and gene silencing [22,23]. For example, B. cinerea BePG1 is effectively inhibited by bean PcPGIP2 and PcPGIP2, and overexpression of these PGIPs in tobacco and Arabidopsis increases resistance to Botrytis infections [24]. Apart from directly suppressing PG activity, PGIPs are also thought to contribute to pathogen perception by preventing the degradation of oligogalacturonan elicitors that are released during infection [25]. PGIPs are widely distributed in the plant kingdom and are encoded by small gene families that are regulated by different pathways, probably minimizing pathogen interference in PGIP expression [22,26].

PGIPs predominantly consist of leucine-rich repeats (LRRs), which fold with a concave inner surface presenting a cluster of negatively charged residues that are probably involved in interactions with PGs [27]. Extracellular LRRs are also found in receptor-like kinases and it is possible that PGIPs evolved from these LRR proteins by truncation and subsequent specialization. The crystal structure of F. moniliforme FmPG1 showed that this protein has two additional loops that form a lid over the active site, causing the substrate-binding cleft to become narrower [28]. This lid is absent in other PGs, such as B. cinerea BePG1 [29**], and may have evolved to prevent binding of substrate-mimicking inhibitors.

Detailed analysis of the PGs of Fusarium and bean PcPGIPs revealed an intricate molecular struggle. Docking studies of FmPG1 with the crystal structure of PcPGIP2 indicate that PcPGIP2 blocks the active site of FmPG1 by binding the lid of FmPG1 (Figure 2d) [30**]. FmPG1 is inhibited by PcPGIP2 but not by PcPGIP1, even though these proteins only differ in eight residues [31]. With a lysine-to-glutamine mutation at position 224, PcPGIP1 acquired the ability to interact with FmPG1 [31]. Importantly, this position is among the seven sites under diversifying selection that were found around the negatively charged pocket implicated in PG binding (blue and red in Figure 2e) [32,33*]. Sequencing PGs from different Fusarium spp. uncovered diversifying selection at two positions [34**], both located in the lid (red and orange in Figure 2e). Some of the Fusarium PG isoforms are insensitive to inhibition by bean PGIPs and site-directed mutagenesis showed that this is mostly because of a repelling lysine residue at position 121 in the lid [34**]. Interestingly, in the docking model this residue 121 of FmPG1 would be in direct contact with PcPGIP2 residue 224, illustrating the importance of single residues in enzyme–inhibitor interactions, and explaining the diversifying selection at both positions (Figure 2f).

Diversifying selection has also been found in PGIP genes in rice, PG genes of B. cinerea and a large cluster of 17 PG genes of Phytophthora cinnamoni, indicating that these molecular struggles are common to PG–PGIP interactions [35,36*,37]. However, predicting interactions between PG and PGIPs is not straightforward since PGIPs appear to bind PGs in different orientations [30**]. PcPGIP2 is a competitive inhibitor of FmPG1 but it inhibits BePG1 in a noncompetitively mixed mode, allowing the substrate to bind with reduced affinity and reduced kinetics of hydrolysis [29**]. Docking studies of PcPGIP2 with a model of BePG1 indicates that PcPGIP2 binds BePG1 in a different orientation, allowing the substrate to interact (Figure 2f) [29**]. This model indicates that a single PGIP can be versatile in binding and inhibiting their targets in different orientations. The mode of action is very different from inhibitors with substrate-mimicking contacts and probably allows PGIPs to quickly adapt and diversify their spectrum of inhibition.

Pathogen inhibitors targeting plant enzymes

Pathogens secrete a broad range of putative enzyme inhibitors to suppress counteracting plant hydrolases. For example, P. sojae secretes glucanase inhibitor protein-1 (GIP1), which targets specific soybean endo-β-1,3-glucanases [38]. The related tomato/potato pathogen P. infestans secretes Kazal-like inhibitors EPI1 and EPI10 (extracellular protease inhibitor) that target the tomato PR-7 Ser protease P69B; and a cystatin-like EPIC2B protein that targets the tomato papain-like Cys protease PIP1 (Phytophthora-inhibited protease-1) [39*,40*,41*]. A similar tomato papain-like protease is targeted by AVR2 (avirulence protein 2) of the tomato fungal pathogen Cladosporium fulvum [42**]. This RCR3 (required for Cladosporium resistance-3) protease is also required for functioning the tomato resistance gene Cf-2 [43]. The molecular details of each of these enzyme–inhibitor interactions are discussed below.

Phytophthora GIP1 targets soybean GH17 glucanases

The P. sojae glucanase inhibitor GIP1 is expressed during pathogen infection and inhibits soybean Endo-β-1,3 glucanase-A (EGaseA) (GH17 family), but not its PR-2-like relative EGaseB [38,44]. EGaseA is constitutively produced in soybean and releases elicitors, whereas EGaseB only accumulates during pathogen infection and is not thought to contribute to elicitor release [38,44]. GIP1 shares sequence similarity with chymotrypsin Ser proteases (family S1, clan SA) but lacks the catalytic triad, suggesting that it evolved from a secreted protease that lost its proteolytic activity and specialized on glucanase inhibition [38]. The inhibition of defense-related endo-β-
1,3-glucanases by GIP1 probably puts evolutionary pressure on the EGases to counter adapt and evade inhibition; sequencing of EGases from wild relatives of soybean indeed showed that particularly EGaseA is under strong diversifying selection [45**]. Homology modeling and docking studies of GIP1 and EGaseA showed that these variant residues form a ‘ring of fire’ around the active site of EGaseA (Figure 2g) [45**]. Exciting new data on the analysis of GIP1 sequences from Phytophthora spp. reveal that GIP1 is also under diversifying selection and that rapidly evolving and positively selected sites in GIP1 are in close proximity to those in EGases, indicating an ongoing arms race between these protein families [46**].

**Phytophthora** EPIs target tomato S8 subtilisin-like protease P69B
The tomato PR-7 protein P69B is a subtilisin-like Ser protease (clan SB, family S8) that is inhibited by two different Kazal-like protease inhibitors of *P. infestans* called EPIC1 and EPIC2, which are both produced during infection [39*,40*,41*]. EPIC1 and EPIC2 inhibitors are divergent in amino acid sequence and contain two and three Kazal domains, respectively. A unique aspect of these Kazal domains is that they carry an Asp at the position that determines specificity for the protease and that some of these Kazal domains lack the third disulphide bridge [39*,40*,41*]. Purification of EPI-binding proteins from tomato apoplastic fluids showed that P69B is the main target of both EPIC1 and EPIC2 [39*,40*,41*]. This suggests that EPIC1 and EPIC2 play similar roles and that the inhibition of P69B is an important infection strategy of this pathogen [40*].

**Phytophthora** EPIC1 targets tomato C1A papain-like proteases
*Phytophthora infestans* also produces four cystatin-like inhibitors called EPIC1–4, of which EPIC1 and EPIC2 are fast evolving and only expressed upon infection [41*]. Pull-down assays with extracellular proteins from tomato revealed that EPIC2B interacts with PIP1 [41*]. PIP1 is a secreted papain-like Cys protease that is upregulated during defense responses and can therefore be considered a PR protein. Protease activity profiling using biotinylated activity-based probes was used to show that EPIC2B also targets PIP1 for the inhibition in apoplastic fluids [41*]. EPIC2B also inhibits other secreted Cys proteases like RCR3, described below (S Kamoun, unpublished data), and the C14/TDI-65 protease (R van der Hoorn *et al.*, unpublished data).

**Cladosporium** AVR2 targets tomato C1A papain-like proteases
PIP1 is closely related to RCR3. Like PIP1, RCR3 is upregulated and secreted during pathogen infection and can be considered as a PR protein [43]. RCR3, however, is also required for the function of resistance gene *Cf-2*, which confers recognition of the fungal pathogen *C. fulvum* carrying the *Avr2* avirulence gene [43]. The AVR2 protein is a small Cys-rich basic protein that is secreted during infection and is not homologous to any known protein [47]. Protease activity profiling of RCR3 in the presence and absence of AVR2 revealed that AVR2 is an inhibitor of RCR3 [42**]. The RCR3–AVR2 complex, but not RCR3 inhibition itself, activates Cf-2-dependent defense responses, possibly by binding to the extracellu-
lar LRRs of Cf-2 [42**]. The details of this perception mechanism, however, remain to be resolved. Sequence analysis of PIP1 and RCR3 from wild relatives of tomato revealed that both enzymes are under strong diversifying selection at the protease surface surrounding the active site [48**]. Protease activity profiling has shown that PIP1 dominates the induced proteolytic activity in the tomato apoplast during defense and that AVR2 is an effective inhibitor of PIP1, suggesting that PIP1 is the real virulence target of AVR2, and that RCR3 acts as a decoy to inhibit of PIP1, suggesting that PIP1 is the real virulence target of AVR2, and that RCR3 acts as a decoy to trap the fungus into a recognition event [48**]. Interestingly, a single naturally occurring variant residue in RCR3 close to its active site prevents inhibition by AVR2, confirming that variant residues influence interactions with pathogen inhibitors. The molecular and structural basis of this inhibition and the mechanism of perception are exciting topics to resolve.

Conclusions and prospects

Studies of extracellular enzyme–inhibitor interactions at the plant–pathogen interface have provided a number of surprising discoveries regarding the evolution and structural details of these interactions:

- The molecular struggle for inhibitor adaptation and enzyme counteradaptation generates positive selection for variation at enzyme–inhibitor interaction surfaces. Single variant amino acids at these surfaces determine the outcome of the interactions. Good examples of these struggles are the XIP-I–XylA, FmPG1–PePGIP2, and GIP1–EGaseA interactions.
- Besides positive selection, selective pressure also gives rise to large gene families for both enzymes and inhibitors. The family members are usually differentially expressed and have different specificities. Both these measures contribute to the robustness of the systems but may make it difficult to study contributions by individual genes.
- Many inhibitors have evolved from enzymes. The stable structures of these enzymes in the apoplast apparently provided a platform for protein–protein interactions that converted an enzyme into an inhibitor by losing the active site and optimizing an interaction surface. Examples are a former pepsin-like protease (TAXI) and a former chitinase (XIP-I) that inhibit xylanases; and a former trypsin-like protease (GIP1) that inhibits endoglucanases. These examples also illustrate that genome annotations should be interpreted with care.
- Inhibitors bind enzymes in different ways, each providing a different kind of selection pressure. XIP-I, for example, is a substrate-mimicking inhibitor that puts selective pressure on the enzyme to make adaptations in the ‘thumb’ that acts as a lid over the active site (Figure 3A and C). Further extension of such a lid may even go beyond the adaptation possibilities of substrate-mimicking inhibitors (Figure 3B). An example of this might be the extended lid of FmPG1.

In the case of FmPG1, however, the lid became the interaction surface of Pe/PGIP2, putting further selective pressure on the lid to counter adapt (Figure 3D). However, the flexible nature of the PGIP interaction surface even allows it to bind different enzymes in different ways, putting selection pressure on the entire surface of the enzymes.

Future research to find additional enzyme–inhibitor interactions at the plant–pathogen interface requires interdisciplinary approaches of genomics, structural biology, and advanced proteomics. Genomic approaches will contribute by identifying candidate inhibitors and enzymes and determining the sites of positive selection. Structural biology is essential to understand the mode of inhibition, the evolutionary origin of inhibitors and the selective pressures on the interaction surface. Advanced proteomics includes not only sensitive pull-down assays to identify interactors but also activity-based protein profiling to monitor the activities of individual enzymes in complex proteomes [49]. The latter is the focus of the Plant Chemetics Laboratory.

The potential of finding additional enzyme–inhibitor interactions at the plant–pathogen interface is tremendous. Given the clear contribution of enzymes in defense and virulence, it seems probable that the attacked organisms produce inhibitors. Examples of these secreted enzymes from both plants and fungi are lipases [50*,51], chitinases [52], and proteases [53,54]. Besides diversification at interaction surfaces, substrate adaptation and inhibitor inactivation may be additional layers of manipulation in the molecular battlefield of extracellular plant–pathogen interactions.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of the review, have been highlighted as:

- of special interest
- of outstanding interest


This is a comprehensive review about PR-proteins, suggesting that many PR-proteins play roles in other biological processes, rather than only defense.


This is an excellent overview of plant inhibitors targeting pathogen cell-wall-degrading enzymes, summarizing the wealth of available structural data.


This is an excellent review focused on microbial xylanases and their inhibition and perception by plants.


8. Igawa T, Tokai T, Kudo T, Yamaguchi I, Kimura M: A wheat xylanase inhibitor XIP-I, but not TAXI-I, is significantly induced by biotic and abiotic signals that trigger plant defense. Biosci Biotechnol Biochem 2005, 69:1059-1063. XIP-I and TAXI-I xylanases inhibitors were found in food research. This work demonstrates that the corresponding genes are induced during infection of wheat with plant pathogens, suggesting that they play a role by inhibiting pathogen xylanases.


11. Belliën T, van Campenhout S, van Ackerm M, Volckaert G: Cloning and characterization of two endoxylanases from the cereal phytopathogen Fusarium graminearum and their inhibition profile against endoxylanase inhibitors from wheat. Biochem Biophys Res Commun 2005, 327:407-414. Along with Ref. [12*], this paper presents key evidence that XIP and TAXI are inhibitors of xylanases of plant pathogens. These inhibitors were found in food research and so far it was only anticipated that they might target pathogen xylanases.


This study describes the discovery of another XIP-like xylanase inhibitor, in this case from rice, which suggests that an entire subclass of presumed GH18 chitinases actually consists of xylanase inhibitors.


This is an excellent demonstration that single amino acids in the ‘thumb’ of Fusarium XyA and XyB xylanases prevent inactivation by XIP-I. Variation in the ‘thumb’ is common to many xylanases from pathogens, suggesting that variation in this region is a common way to prevent inhibition.


This study is an excellent overview of the current status of the best-characterized enzyme-inhibitor interaction at the plant pathogen interface, pointing out that the structure of these proteins explains their versatility in inhibiting different PGs. It also compares the binding modes of different PGIP–PG interactions using docking models.
This study is an excellent combination of genomics and biochemistry like inhibitors that inhibit the P69B subtilase of tomato.

This paper reveals positive selection for diverse residues at sites in PGs of pathogens.

This is an excellent new data, showing that diversifying selection not only affects interactions of fungal PGs and their inhibitors.

These are excellent new data, showing that diversifying selection not only occurs at glucanases but also in the corresponding GIP1 inhibitors. A structural model is presented indicating direct contacts between diversifying residues of both enzyme and inhibitor.

This is a key evidence supporting the guard hypothesis by showing that the Cf-2 resistance gene confers recognition of host protease RCR3 upon inhibition by pathogen protein AVR2. A role for RCR3 as a defense protein is anticipated, but remains to be demonstrated.

This is the first report that tomato defense proteases PIP1 and RCR3 are targets of the tomato pathogenesis-related Cf-2 mediated resistance by producing truncated AVR2 elicitor proteins.

This is an excellent work about secreted proteins in cultured A. brassicicola cells under stress conditions. One of the secreted proteins is a lipase that affects interactions of fungal PGs and their plant inhibitors. These are excellent new data, showing that diversifying selection not only occurs at glucanases but also in the corresponding GIP1 inhibitors. A structural model is presented indicating direct contacts between diversifying residues of both enzyme and inhibitor.

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