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The structural basis of specific protease–inhibitor interactions at the plant–pathogen interface

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Antagonistic host–pathogen interactions offer intriguing insights into coevolutionary processes at the molecular level. Studies on secreted immune proteases from the model plant tomato and their interactions with different unrelated pathogen-derived inhibitors revealed that the inhibitors exhibit a remarkable selectivity towards different host proteases, and that the host proteases accumulate variant residues at the interaction surfaces that interfere with inhibitor binding. Here, we summarize and discuss the recent findings and use structural models to identify the molecular features underpinning protease selectivity. The observed basic principles translate to other examples of secreted immune hydrolases and their putative inhibitors.

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Introduction

In the recent years increasing evidence for important roles of enzyme–inhibitor interactions at the plant–pathogen interface has been uncovered (reviewed e.g. in [1–3]). These molecular interactions in the extracellular space (apoplast) appear to determine the outcome of interactions with many plant pathogens, ranging from bacteria to fungi and from oomycetes to nematodes. In many cases, plants secrete hydrolytic enzymes during pathogen attack as part of their innate immune response and pathogens counteract these host enzymes by secreting corresponding inhibitors interfering with the harmful enzymatic function. Accordingly, plants lacking the enzymes are hypersusceptible for pathogens [4••,5••,6••], whereas pathogens lacking the inhibitors have reduced virulence [7,8].

Some studies revealed a remarkable selectivity in these enzyme–inhibitor interactions. This selectivity is thought to be the consequence of strong reciprocal adaptation as observed in host–pathogen coevolution. However, the molecular mechanisms underpinning these selective interactions are not yet fully understood. Here, we discuss the putative molecular basis of selective inhibition using knowledge of the structures of the enzymes. We will initially focus on secreted papain-like cysteine proteases of the model plant tomato and their inhibition by various pathogen-derived inhibitors, and later discuss the implications for other enzyme–inhibitor interactions at the plant–pathogen interface.

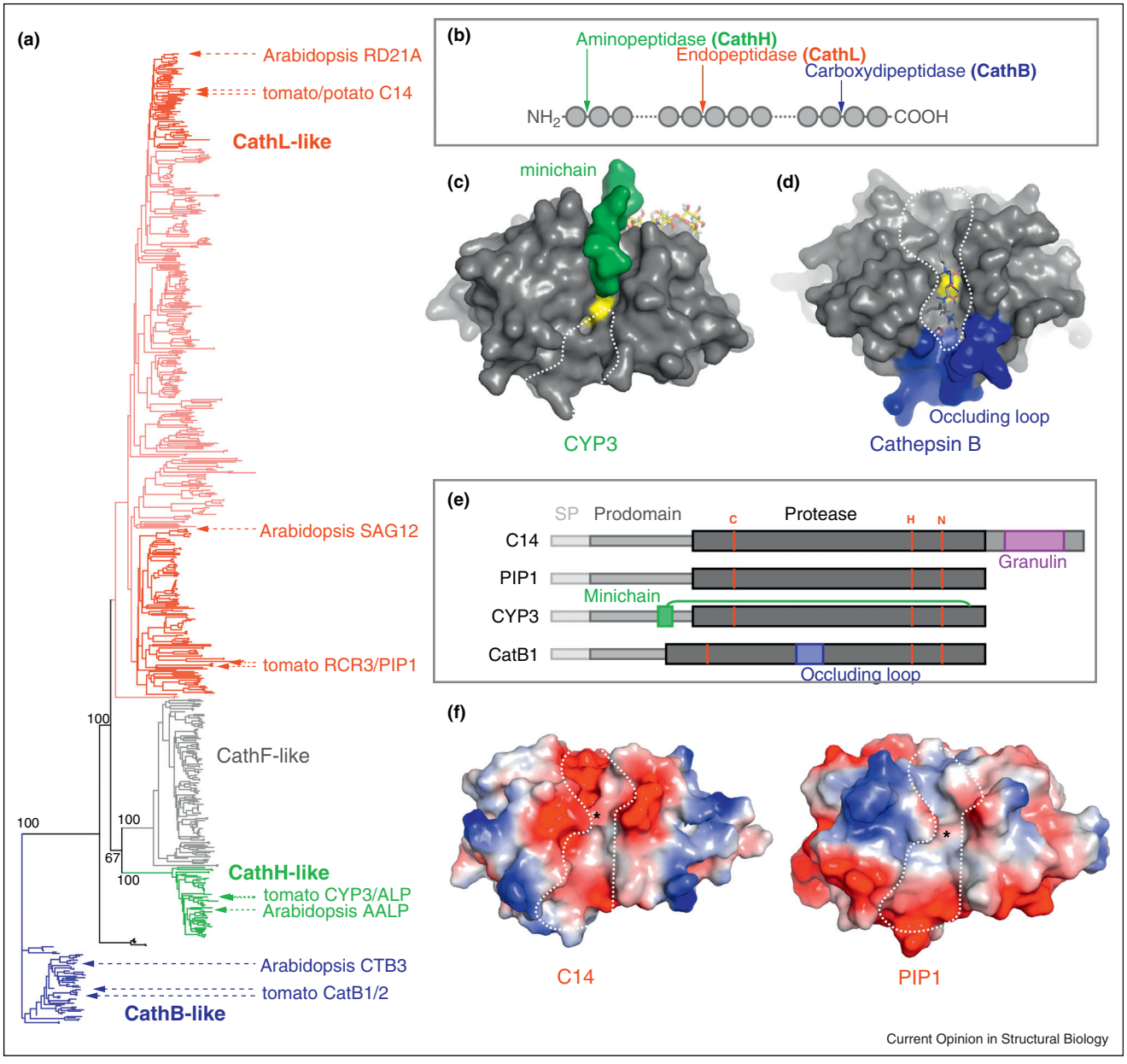
Secretion of different papain-like proteases during immune responses

The extracellular space in plants (apoplast) contains many hydrolytic enzymes (lipases, glycosidases, and proteases) of which many accumulate specifically during immune responses (e.g. [9,10••,11]). Extracellular pathogens (most fungi, oomycetes, bacteria, and even nematodes and insects) are exposed to these hydrolytic enzymes, which will likely have an impact on their survival in the apoplast. Studies on the model plant tomato uncovered seven papain-like cysteine proteases (PLCPs) amongst the hydrolytic enzymes in the apoplast [10••]. PLCPs are also called cathepsins [12]. These proteins are produced as preproproteases that are activated from their precursors upon secretion and accumulate in the apoplast as mature 25–30 kDa proteins. A few PLCPs carry an additional C-terminal granulin domain, increasing the molecular weight to 40 kDa. The mature protease domain of PLCPs consists of two lobes that define a substrate binding groove with a catalytic triad (Cys-His-Asn) in the centre.

Plant PLCPs can be subdivided into nine subfamilies [13]. Three of these families are phylogenetically distinct and share features with their human counterparts: cathepsins B, F, and H. The other six subfamilies are all cathepsin L-like proteases and are distinguished based on the presence of molecular features such as a double cysteine in the active site (subfamily 6, SAG12-like) or a C-terminal retrieval signal for the endoplasmatic reticulum (subfamily 3, CEP1-like) [13].

The seven PLCPs detected in the tomato apoplast represent subfamilies 1 (C14), 6 (RCR3/PIP1), 8 (ALP/CYP3), and 9 (CathB1/CathB2) [10••]. Interestingly, recent analysis of PLCPs in the apoplast of maize resulted in a similar set of PLCPs, with representatives from subfamilies 1 (CP1), 3 (XCP2), 8 (CP2), and 9 (CatB) [14]. Thus, the composition of apoplastic PLCPs is remarkably diverse, containing representatives of several different subfamilies (Figure 1a). Moreover, the fact that

Figure 1



Fundamental differences between plant papain-like proteases explain global sensitivity for pathogen-derived protease inhibitors. **(a)** Phylogenetic classification of plant papain-like cysteine proteases into four major classes, named after their human counterparts. The discussed plant proteases are indicated by arrows. Phylogenetic tree adapted from [13]. **(b)** Functional differences on substrate cleavage by CathH (an aminopeptidase), CathL (an endopeptidase) and CathB (a carboxydipeptidase). **(c)** Structural model of tomato CYP3 (based on 8pch, $Z = -1.666$, [25]), showing the minichain (green) occupying one side of the substrate binding groove. The remaining substrate binding groove is indicated with a dashed line and the catalytic Cys in yellow. **(d)** Structure of *Schistosoma mansoni* Cathepsin B (3qsd, [40]) showing the occluding loop (blue), and the Ca074 inhibitor (sticks). The remaining substrate binding groove is indicated with a dashed line and the catalytic Cys in yellow. **(e)** Protein domains and functional features of the tomato C14, PIP1, CYP3 and CathB proteases. Red, catalytic residues (Cys, His, Asn). The granulin domain, the minichain and the occluding loop are indicated in purple, green and blue, respectively. **(f)** Structural models of tomato C14 (based on 3p5u, $Z = -0.987$, [29]), and PIP1 (based on 1s4vB, $Z = -1.447$, [28]), presented with surface charge (red positive, blue negative). Please note that the regions around the substrate binding groove (dashed line), where also inhibitors bind, are remarkably different between PIP1 and C14. The catalytic Cys is indicated with an asterisk. All structural models have been generated by Swiss Model [41–43] and coloured and rendered in PyMOL (The PyMOL Molecular Graphics System, Schrödinger, LLC).