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Emerging Roles of Posttranslational Modifications in Plant-Pathogenic Fungi and Bacteria

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Abstract

Posttranslational modifications (PTMs) play crucial roles in regulating protein function and thereby control many cellular processes and biological phenotypes in both eukaryotes and prokaryotes. Several recent studies illustrate how plant fungal and bacterial pathogens use these PTMs to facilitate development, stress response, and host infection. In this review, we discuss PTMs that have key roles in the biological and infection processes of plant-pathogenic fungi and bacteria. The emerging roles of PTMs during pathogen–plant interactions are highlighted. We also summarize traditional tools and emerging proteomics approaches for PTM research. These discoveries open new avenues for investigating the fundamental infection mechanisms of plant pathogens and the discovery of novel strategies for plant disease control.

INTRODUCTION

Plant infection depends on complex regulatory networks governing development and infection in the pathogen. The past two decades of work have revealed central roles for signaling pathways mediated by many protein cascades and small-molecule signals in fungal and bacterial pathogens (8, 131). Although the exploration of pathogen regulatory processes has frequently focused on transcript or protein abundance, the activities of many proteins are regulated by posttranslational modifications (PTMs). A PTM can consist of a simple chemical moiety, such as a phosphate, acetyl, methyl, or hydroxyl group, or a more complex modification, such as a nucleotide, sugar, or small polypeptide. PTMs shape the activity state, stability, localization, and interaction partners of proteins. These modifications can activate or deactivate signaling cascades or reshape the surface structures that interface with the plant host. Tight regulatory control depends on a high degree of precision in targeting specific sites with specific modifications.

Historically, much understanding of the PTM mechanism and regulatory significance is based on the model yeast *Saccharomyces cerevisiae*, but studies in recent years have uncovered new contributions of specific PTMs to plant pathogenesis. In this review, we summarize the field of knowledge regarding the identities, mechanisms, and roles of PTMs in fungal and bacterial plant pathogens, including their functions in host–pathogen interactions. We limit our focus to covalent modification of proteins in the pathogen; modifications occurring within the host have been recently discussed elsewhere (6, 38). We highlight several modifications that are the focus of recent research interest (**Table 1**), summarize the approaches used for PTM research, and discuss potential possibilities of developing PTM-based plant disease control strategies.

PHOSPHORYLATION

Phosphorylation is the chemical addition of a phosphoryl group to a protein, catalyzed by a kinase. The reverse reaction, dephosphorylation, is catalyzed by a phosphatase. Mitogen-activated protein kinase (MAPK) and cyclic AMP (cAMP) cascade protein kinase A (PKA) are well-studied paradigms for signaling cascades, but other types of protein kinases (PKs) have emerged in recent years as key players in fungal and bacterial plant disease.

Mitogen-Activated Protein Kinase Cascades

MAPK cascades are three-tiered PK modules that govern a wide variety of cellular responses in all eukaryotic organisms. Orthologs of the *S. cerevisiae* Fus3/Kss1, Slt2, and Hog1 MAPKs exist in most fungal pathogens, among which, Fus3/Kss1-MAPK regulates morphogenesis, Slt2-MAPK regulates cell wall remodeling, and Hog1-MAPK regulates high osmolarity stress response, and all contribute to virulence on plants (**Figure 1**) (68, 131). PMK1-MAPK, the homolog of kss1-MAPK, was discovered in *Magnaporthe oryzae* and is necessary for appressorial development and infection in many important pathogens (68, 145). PMK1 also regulates fungal movement within plant cells and the expression of immune-suppressing effector genes (113). The MAPK Crk1, the homolog of *S. cerevisiae* Ime 2, is widely conserved in plant pathogens and promotes mating in *Ustilago maydis* (44). Slt2-MAPK orchestrates cell wall biosynthesis and actin organization and therefore is important for appressorial penetration in fungi (146). Hog1-MAPK regulates adaptation to hyperosmotic stress, including glycerol accumulation, in fungal pathogens (24).

Protein Kinase A

PKA is the main intracellular target of cAMP and a conserved regulator of fungal cell differentiation. In *U. maydis*, cAMP-PKA controls the transition between budding and sexual development

Table 1 Types of posttranslational modifications (PTMs) in plant-pathogenic fungi or bacteria

PTM types	Important enzymes identified in plant-pathogenic fungi or bacteria	PTM sites	Cellular functions
Phosphorylation			
MAPK	PMK1, MPS1, OSM1	Ser, Thr	Signaling transduction for appressorium formation, cell wall remodeling, osmostress response, mating, etc.
cAMP-dependent protein kinase	PKA, casein kinase 2	Ser, Thr	Signaling transduction for appressorium formation
TOR kinase	AGC kinase Sch9	Ser, Thr	Autophagy processes, nutrient utilization upon starvation
SNF1 kinase	Snf1	Ser, Thr	Carbon nutrient utilization
Histidine kinase	Sln1	His, Asp	Turgor-driven host penetration
Histidine kinase	Bacterial TCS proteins	His, Asp	Sense and respond to environmental stimuli
Glycosylation			
N-glycosylation	ALG family enzymes (Alg2, Alg3), Gnt2	Asn	Nutrient accumulation, storage utilization, cell wall integrity, ERQC system, effector secretion, immune evasion
O-glycosylation (fungi)	PMT1, PMT2, and PMT4 family	Ser, Thr	Nutrient accumulation, cell wall integrity, MAPK signaling pathway, ERQC system, effector secretion, immune evasion
O-glycosylation (bacteria)	*O*-OT-ases	Ser, Thr	Motility, adhesion, biofilm formation, immune response
GPI anchoring	GPI family enzymes (GPI7, GPI8, GPI12)	C-terminal residue (called ω-site) of the substrate protein	Cell wall integrity, effector secretion, immune evasion
Histone modification			
Acetylation	HATs, HDACs, sirtuins	Lys	Gene expression regulation
Methylation	HMTs, Dot1 family of histone KMTs, demethylase LSD1, JmjC domain-harboring enzymes	Lys	Gene expression regulation
Ubiquitin and ubiquitin-like modification			
Ubiquitination	UBI4 (ubiquitin), Rad6 (E2), Skp1-Cul1-F-box-protein (SCF) E3 ligase complex	Lys	Polyubiquitination for protein degradation
Sumoylation	Smt3 (SUMO), Aos1/Uba2 (E1), Ubc9 (E2), Siz1 and Siz2 (E3)	Lys	Protein stability and localization, septin ring formation, effector secretion
Neddylation	Rub1 (NEDD8), Uba3 (E1), Ubc12 (E2), Dcn1 (E3)	Lys	Not known in plant pathogens
Urmylation	Urm1, Uba4 (E1)	Lys	Detoxification of host oxidative stresses
Pupylation	Bacterial carboxylate-amine ligases	Lys	Proteasomal degradation
Lipidation			
Myristoylation	NMT	Cys	Not known in plant pathogens
Prenylation	Ftase, GGTaseI, GGTaseII	Cys	RAS protein localization
Palmitoylation	PATs	Cys	Cell wall integrity

Abbreviations: E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin protein ligase; ERQC, endoplasmic reticulum quality control; Ftase, farnesyl transferase; GGTaseI, geranylgeranyl transferase I; GGTaseII, geranylgeranyl transferase II; GPI, glycolipid phosphatidylinositol; HATs, histone acetyltransferases; HDACs, histone deacetyltransferases; HMTs, histone methyltransferases; JmjC, Jumonji C; KMTs, lysine methyltransferases; MAPK, mitogen-activated protein kinase; *O*-OT-ases, *O*-oligosaccharyltransferases; PATs, protein *S*-acyltransferases; NMT, *N*-myristoyltransferase; TCS, two-component system; TOR, target of rapamycin.

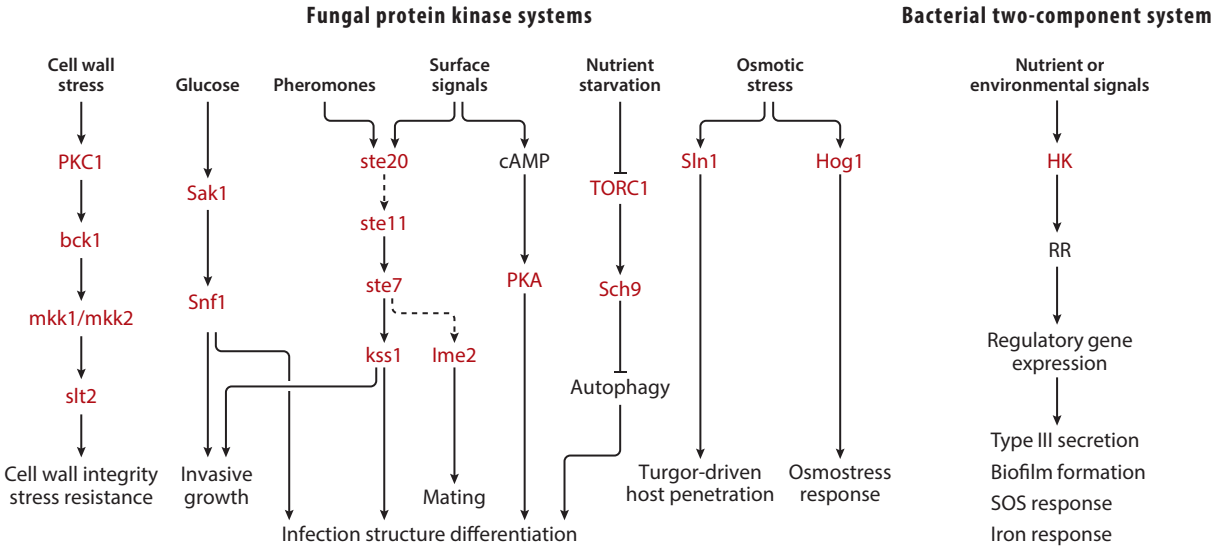

Figure 1

Function of phosphoregulation of protein kinases in plant-pathogenic fungi and bacteria. Different signals are transduced to the appropriate protein kinase modules. Protein kinases in fungi then phosphorylate a downstream substrate for the cellular response, development, or infection process. The Slt2-MAPK cascade (also called Mps1-MAPK cascade) responds to cell wall stress and regulates cell wall integrity. The Kss1-MAPK cascade (also called Pmk1-MAPK cascade) typically regulates infection-related structure differentiation such as appressorium formation. The Hog1-MAPK (also called Osm1-MAPK) controls osmostress response. The histidine kinase Sln1 also responds to osmotic stress but is important for turgor-driven host penetration. The cAMP-PKA signaling pathway is another key regulator of infection-related structure differentiation. Snf1 mediates the response to glucose limitation. Protein kinase Sch9 is involved in the TOR signaling pathway and is also required for appressorium formation. In bacteria, histidine kinase (HK) sensors phosphorylate downstream response regulators (RRs) and subsequently regulate regulatory gene expression for type III secretion, biofilm formation, SOS response, and iron response. Protein kinases are shown in red. Abbreviations: cAMP, cyclic AMP; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; TOR, target of rapamycin.

for infection (45). The cAMP-PKA pathway is essential for infection-related structural differentiation, including appressorium development in *M. oryzae* (**Figure 1**), in many plant-pathogenic fungi (96).

Target of Rapamycin Kinase

Target of rapamycin (TOR) kinase is the central component of a signaling pathway that controls cell growth and proliferation in eukaryotic cells (63). TOR forms the dimeric complex TORC1, which promotes cell growth by stimulating protein synthesis and ribosome biogenesis. Conversely, inhibition of TORC1 triggers autophagy, stress responses, and cell-cycle arrest (63). Through phosphorylation of the kinase Sch9, the TOR signaling pathway is necessary for normal hyphal growth, conidial germination, and osmotic and oxidative stress tolerance as well as mycotoxin synthesis in some pathogens (50, 107). TOR signaling also cooperates with PKA to inhibit autophagy, a process necessary for fungal appressorium formation (**Figure 1**) (93, 94).

SNF1 Kinase

SNF1 kinase mediates the transcriptional response to glucose limitation. Snf1 is required for the normal growth of several plant-pathogenic fungi on complex or host-associated carbon sources

and enhances cell wall–degrading enzyme gene expression and virulence (**Figure 1**) (77, 103, 149). It may also have a role in sexual and asexual development (77, 149).

Histidine Kinases

Histidine kinases (HKs) function at the head of two-component systems (TCSs) used to sense and respond to environmental stimuli and stressors in both prokaryotes and eukaryotes (**Figure 1**) (78). HKs signal through a histidine-containing phosphotransfer protein, which phosphorylates a downstream response regulator protein (78). Some HKs are required for fungicide susceptibility (99, 133). Others regulate conidial development, promote virulence (109, 133), or, in the case of the *M. oryzae* HK Sln1, initiate penetration when the appressorium reaches sufficient pressure (112).

Phytopathogenic bacterial species contain dozens of TCSs that govern bacterial responses to the environment. TCSs may initiate infection- or survival-associated behaviors in response to a variety of stimuli, including quorum sensing signals, osmotic or nutrient stress, light, and phytohormones (9, 138). A few of these systems are essential to pathogenicity: In the model enteric plant pathogen *Erwinia amylovora*, the HrpX/HrpY system activates type III secretion and the RcsCDB phosphorelay regulates production of the external polysaccharide layer (137, 141). The GacS/GacA module is a central virulence regulator in multiple phytopathogens, albeit with context-dependent roles (81, 100).

GLYCOSYLATION

Protein glycosylation is the addition of different polysaccharide cores to specific amino acids containing a special consensus sequence. The polysaccharide cores are synthesized in the endoplasmic reticulum (ER) and transferred to nascent proteins. The glycoproteins undergo maturation in the Golgi apparatus and are then secreted to the plasma membrane–associated cell wall or extracellular region (19, 54). Recent work has uncovered new roles for three types of glycosylation in fungal invasion and infection of plants, primarily in the model systems *U. maydis* and *M. oryzae* (15, 16, 34–36, 88).

N-glycosylation

N-glycosylation is one of the most abundant PTMs in eukaryotes and is necessary for the folding, sorting, stability, and localization of diverse target proteins (54). This PTM is the addition of an oligosaccharide core to the asparagine (N) residue in the sequence Asn-X-Ser/Thr (X is any amino acid except Pro) (54). *N*-glycosylation begins in the ER membrane with glycans assembled on the lipid carrier dolichol pyrophosphate (Dol-PP) by α-glucosidases and transferred to the protein substrate. *N*-glycan-linked proteins are modified to mature *N*-glycosylated proteins by mannosyltransferases in the Golgi apparatus (**Figure 2*a***) (54).

N-glycosylation-deficient mutants of *U. maydis*, *M. oryzae*, and *Mycosphaerella graminicola* have revealed the importance of this modification in fungal plant pathogenesis (15, 16, 97). In *M. graminicola*, the α-mannosyltransferase mediates the switch from yeast-like growth to the hyphal form, whereas in *M. oryzae*, another mannosyltransferase is required for the suppression of host reactive oxygen species (ROS) production (16, 97). In *Fusarium oxysporum*, deletion of the *N*-glycosyltransferase Gnt2 results in differential protein glycosylation patterns, affecting conidium morphology, hyphal fusion rates, and secretion of trafficking vesicles and their protein cargo (92). *N*-glycosylation maintains the stability of the ER quality control (ERQC) system, with implications for infection (**Figure 2*b***). *U. maydis* ERQC glucosidase I and II are required for early

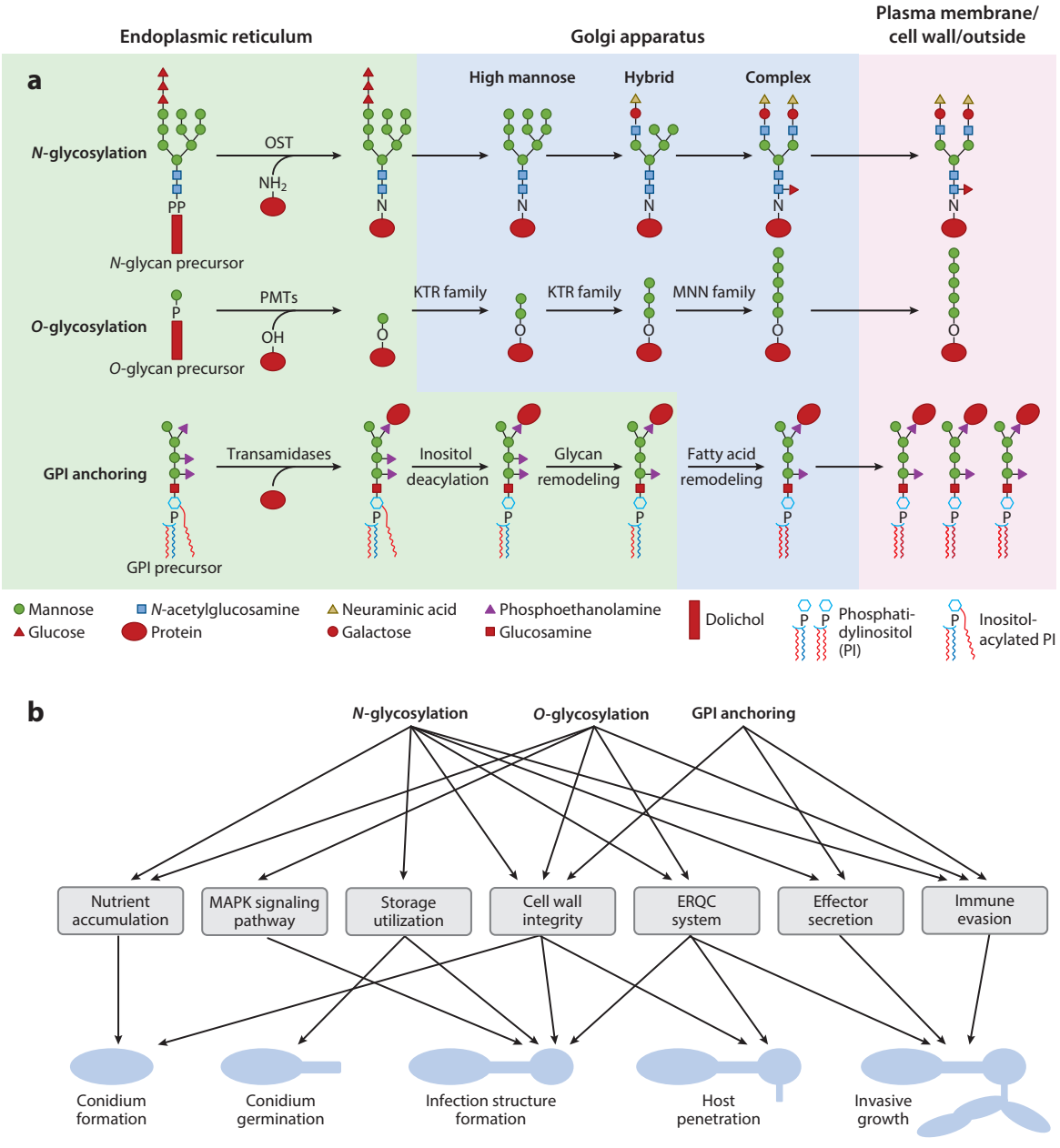

Figure 2

Function of glycosylation in plant-pathogenic fungi. (*a*) Schematic depiction of *N*-glycosylation, *O*-glycosylation, and glycolipid phosphatidylinositol (GPI) anchoring synthesis processes in plant-pathogenic fungi. All three types of glycosylation are catalyzed by corresponding glycan transferases in the endoplasmic reticulum and Golgi apparatus and are eventually secreted into the plasma membrane, cell wall, or outside spaces. (*b*) Biological functions of three types of glycosylation in plant-pathogenic fungi. Each glycosylation type controls several key steps, through different cellular processes, during fungal development or the infection process. Abbreviations: ERQC, endoplasmic reticulum quality control; KTR, Kre-two-related mannosyltransferase; MAPK, mitogen-activated protein kinase; MNN, mannosyltransferase; OST, oligosaccharyltransferase; PMTs, protein *O*-mannosyltransferases.

infection events after host penetration and establishing the initial biotrophic state, respectively (35, 115). ERQC components themselves are also *N*-glycosylated in *M. oryzae*, which affects their ER location and stability (15).

N-glycosylation of effector proteins is a common strategy to help fungal pathogens evade host innate immunity (**Figure 2*b***). The *M. oryzae* chitin-binding virulence effector Slp1 requires *N*-glycosylation at three sites to fully suppress chitin-triggered immunity (16). *N*-glycosylation regulates chitin binding capacity and protein stability of Slp1, suggesting that glycosylation-mediated stabilization is critical to accumulate enough effector to sufficiently lower the free chitin levels (16). Putative *N*-glycosylation sites are found among effector proteins in diverse plant-pathogenic fungi, including *M. oryzae* Bas4, *U. maydis* Pep1 and Pit1, and *Cladosporium fulvum* Ecp6 (21, 25, 26).

O-glycosylation

O-glycosylation, i.e., attachment of sugar to the oxygen of serine or threonine, can incorporate a wider variety of sugars than can *N*-glycosylation. Mannose-based modification, or *O*-mannosylation, is the most common type of *O*-glycosylation in fungi and the best characterized in fungal plant pathogens (91, 115). Initial mannose addition is mediated by protein mannose transferases (PMTs) in the ER, followed by modification and maturation in the Golgi (**Figure 2*a***). Fungal PMTs comprise three widely conserved subfamilies: PMT1, PMT2, and PMT4 (91). PMT deletion usually causes defects in cell wall composition by affecting the amounts of β-glucans, chitin, and glycoproteins found in the cell wall (**Figure 2*b***). In *U. maydis*, individual deletion of eight predicted *O*-mannosyltransferase genes showed that only *pmt4* was essential for virulence and played roles in appressorium formation and plant cuticle penetration (34). This suggests that the virulence roles of *O*-glycosylation are not always linked to cell wall composition. PMT4's effect on early virulence in *U. maydis* is largely attributable to its glycosylation of the signaling mucin Msb2, which regulates a MAPK cascade critical for appressorium formation (**Figure 2*b***) (36). Pmt4 affects virulence independently of Msb2 in later stages of infection, likely through direct glycosylation of the secreted protein Pit1 and putative effector protein Um03749, both of which are required for biotrophic growth (36).

Several other PMTs are critical for cell wall stability, sporulation, and virulence in fungal plant pathogens (46, 52, 53, 104, 147). In *Botrytis cinerea*, PMTs contribute to infection in a variety of hosts, with a primary role in the adhesion and penetration of intact leaves; penetration of grape leaves requires three functional PMTs (46). In *F. oxysporum* f. sp. *cucumerinum*, PMT genes were all required for growth, conidiation, intact cell walls, and virulence, and some PMTs also contributed to thermotolerance and polar growth (147). Deletion of *M. oryzae MoPmt2* and *MoPmt4* resulted in various defects in conidiation, polar growth, and host adhesion as well as penetration and invasion of the host (52, 104).

In bacteria, the flagellum is a common target of *O*-glycosylation. In the model species *Pseudomonas syringae*, flagellin is glycosylated at six sites with a trisaccharide that terminates in a modified viosamine (mVio) (128). Inhibiting glycosylation causes defects in motility, adhesion, and biofilm formation (80, 127), demonstrating importance for both flagellar and regulatory functions. Flagellar glycosylation also determines host specificity in some plant-pathogenic bacteria (57, 129), and recent studies have shown how it affects the host immune response. The multiple immune-triggering epitopes of flagellin are buried in the flagellum and inaccessible to plant receptors (39). During *P. syringae* infection of *Nicotiana benthamiana*, proteases degrade the flagellum to expose these epitopes and trigger immunity, but glycosylation protects the flagella from proteases (7). The *N. benthamiana* glycosidase BGAL1 recognizes mVio and degrades the

glycans, enabling protease function. Modification of flagellar glycosyl groups to evade specialized host glycosidases could be a common pathogen strategy to subvert immunity and expand host range (7). Flagellar glycosylation has been confirmed in species of *Burkholderia*, *Dickeya*, *Pantoea*, *Xanthomonas*, and *Pectobacterium* (60). A genomic analysis in enteric bacteria found that glycosylation islands are horizontally transferred and undergo frequent recombination events (22). Among enteric plant pathogens, flagellar glycosylation islands were found in all published genomes of *Dickeya* and *Lonsdalea* strains but in only a portion of *Pectobacterium*, *Pantoea*, and *Erwinia* genomes. Interestingly, several species in the latter three genera were predicted to have flagellar methylation islands, suggesting that this is an alternate flagellar PTM in plant pathogens (22).

O-oligosaccharyltransferases

O-oligosaccharyltransferases (*O*-OT-ases) are a second group of bacterial glycosyltransferases. *O*-OT-ases modify a variety of bacterial proteins but are most frequently known to modify the type IV pilus, potentially affecting motility or susceptibility to phage (72). $PglL_{Rs}$ is an *O*-OT-ase essential for glycosylation in the wilt pathogen *Ralstonia solanacearum*, and deletion of the corresponding gene compromised biofilm formation and virulence on tomato (29). Proteomic analysis of the deletion mutant identified 20 glycosylation targets, including type IV pilus components, cell division proteins, and several transmembrane proteins of unknown function. Accumulation of pilins and type VI secretion proteins was also reduced (29). Another *O*-OT-ase was identified in the type IV pilin cluster of *Acidovorax avenae* subsp. *citrulli* (117), and pilin glycosylation has also been observed in the citrus pathogen *Xanthomonas citri* (105). A virulence-associated glycoside hydrolase was recently found to affect the abundance of 13 outer membrane proteins in the rice pathogen *Xanthomonas oryzae* (135), suggesting that the impact of glycosylation on pathogenicity may extend well beyond the currently characterized protein targets.

Glycolipid Phosphatidylinositol Anchoring

Glycolipid phosphatidylinositol (GPI) anchoring is the attachment of GPI to a newly synthesized protein to confer membrane association, with the modified protein displayed on the outer cell surface (**Figure 2*a***) (41). *Fusarium graminearum* deletion mutants of the GPI pathway gene *GPI7* formed aberrantly shaped macroconidia and had significantly reduced virulence (110). In the maize pathogen *Colletotrichum graminicola*, RNAi-silenced lines of three GPI pathway genes were all severely defective in cell wall integrity, formed exploding infection cells on the host plant surface, and distorted invasive hyphae (102). In *M. oryzae*, disruption of *GPI7* caused significant defects in appressorial cell wall architecture, which is essential for penetration and invasive growth in the host cells (88). GPI anchoring is a critical process regulating cell wall development and cell wall integrity, likely through modification of cell wall mannoproteins. More interestingly, the GPI-anchored proteins may act as a shield to protect inner cell wall chitin and β-1,3-glucans, therefore helping the fungus to evade recognition of the plant host innate immunity system (**Figure 2*b***) (88).

HISTONE MODIFICATION

Histones form the octameric complex around which DNA winds to form the nucleosome. Histone–DNA interactions are regulated by several types of PTM. Modifications that weaken histone–DNA interactions can cause nucleosomes to loosen to form euchromatin, and PTMs that strengthen these interactions result in tightly packed heterochromatin associated with gene silencing (**Figure 3**). Methylation and acetylation of histones are the most well-studied modifications in plant pathogens (27, 30).

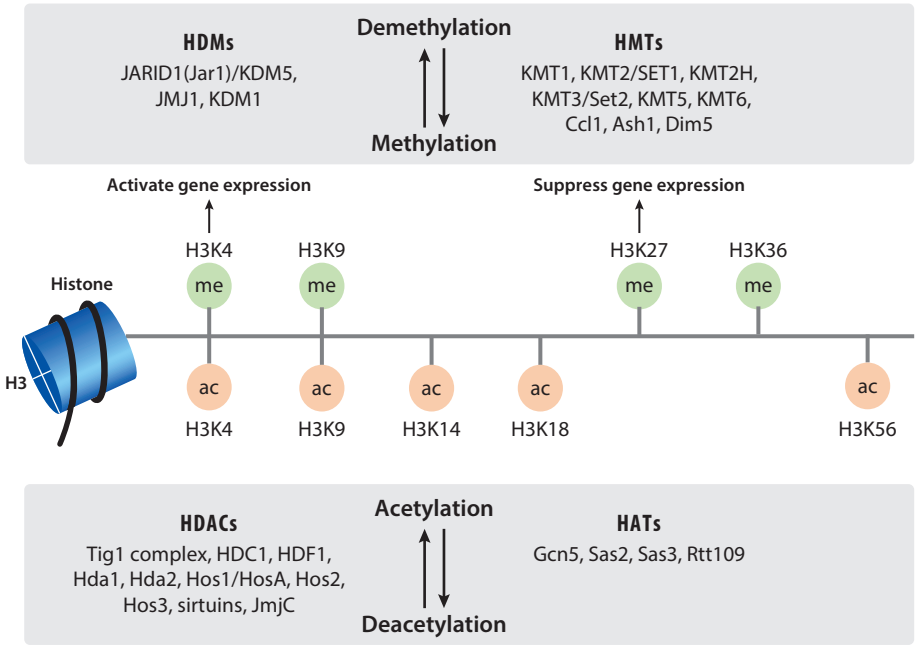

Figure 3

Histone modifications on H3 in plant-pathogenic fungi. Histone methyltransferases (HMTs) and histone demethylases (HDMs) are shown at the top. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are shown at the bottom. Abbreviations: ac, acetylation; me, methylation.

H3K4 Methylation

Methylation of histone 3 lysine 4 (H3K4) is an important epigenetic mark for gene activation (**Figure 3**) (27). Set1 proteins are an important family of H3K4 methyltransferases and function as part of a larger complex called COMPASS (complex proteins associated with Set1p). *M. oryzae MoSET1* encodes a histone methyltransferase that methylates H3K4 at the locus of the cellulase gene *MoCel7C*, activating transcription of the gene in the presence of cellulose (1). *MoSET1* is required for conidiation and appressorium formation, functioning through direct gene activation and indirect gene repression to regulate essential infection processes (70). *Set1* genes also have important roles in many other fungi. Deletion of the *Fusarium verticillioides* gene *FvSET1* led to defects in growth, pathogenicity, synthesis of fumonisin B1 toxin, and environmental stress responses (48). In *Fusarium fujikuroi*, deletion of *Set1* promotes genome-wide transcription and affects the expression of many secondary metabolite synthesis genes as well as the major conidiation-specific transcription factor gene *ABA1* (67). In *F. graminearum*, *FgSet1* is required for the transcription of genes involved in hyphal growth, stress tolerance, virulence, and the synthesis of deoxynivalenol (DON) and aurofusarin toxins (89).

Other proteins in the COMPASS complex are also essential for fungal pathogenesis. In *F. graminearum*, KMT2 is required for normal growth and virulence and altered responses to cell wall stress (89). The COMPASS component Ccl1 is required for the production of secondary metabolites such as gibberellic acid and DON in *F. fujikuroi* and *F. graminearum* (123), suggesting that epigenetic modifications are an important aspect of regulating secondary metabolite productions in fungi. In *Colletotrichum higginsianum*, deletion of the COMPASS component CclA strongly reduced mycelial growth and sporulation but did not impair the formation of appressoria

and biotrophic hyphae (20). However, the appressoria of the *CclA* mutant lacked full penetration capacity. Interestingly, the *CclA* mutant produced an enriched variety of secondary metabolites, including five novel molecules not produced in the wild type (20).

H3K27 Methylation

H3K27 methylation is an epigenetic mark for gene suppression (**Figure 3**), which is catalyzed by KMT6, a part of the polycomb repressive complex 2 (PRC2). In *F. graminearum* and *F. fujikuroi*, H3K27 trimethylation (H3K27me3) covers ~30% of the genes in the genome (18), a majority of which are upregulated in the absence of *FgKMT6*, suggesting that the absence of methylation is sufficient to induce transcription. Deletion of *KMT6* or other PRC2 complex elements results in extreme developmental and metabolic dysfunction in *Fusarium* spp. and *M. oryzae*, including female sterility and constitutive expression of genes related to the biosynthesis of mycotoxins, pigments, and other secondary metabolites (18, 70).

H3K36 Methylation

H3K36 methylation is conferred in *F. fujikuroi* by the methyltransferases Set2 and Ash1, each having unique functions in euchromatin and telomeric heterochromatin, respectively (66). Ash1 is generally thought to be involved in DNA repair processes, although the Ash1-like protein MoKMT2H is important in conidial germination and pathogenesis in *M. oryzae* (10). Set2-mediated H3K36 methylation regulates secondary metabolite biosynthesis, asexual development, and virulence in both *F. fujikuroi* and *F. verticillioides* (49, 66). In *F. verticillioides*, FvSet2-mediated H3K36me3 activates expression of *Fumonisin B1* (*FB1*) and bikaverin biosynthesis genes (49).

H3K9 Methylation

H3K9 methylation is critical for the regulation of chromatin structure and gene transcription. The KHMTase enzyme Dim5 is a lysine histone methyltransferase that regulates H3K9 methylation in eukaryotic cells. In *F. verticillioides*, FvDim5 regulates the H3K9me3 (trimethylation of H3K9) (47), whose deletion mutant was defective in perithecium production, conidiation, and virulence. The mutant also showed increased phosphorylation of the osmotic stress MAPK Hog1 and a corresponding increased tolerance to osmotic stresses (47).

Demethylation

Demethylation is necessary to regulate the histone methylase process. Demethylation of H3K4 is conferred by the demethylase JARID1 (Jar1)/KDM5. In *B. cinerea*, BcJar1 orchestrates global gene expression related to ROS production and response and is critical for infection structure formation but negatively regulates sclerotium production (58). In *M. oryzae*, the histone demethylase MoJMJ1 is also important in vegetative and infectious growth and asexual reproduction (59). In *B. cinerea*, BcKDM1 is an H3K36-specific demethylase required for pathogen penetration as well as responses to light (118).

Acetylation

Acetylation is manipulated by histone acetyltransferases (HATs) and deacetylases (HDACs) with distinct substrates and mechanisms (**Figure 3**) (27). Lysine acetylation of residues on histone 3 and histone 4 is generally related to gene activation and regulates diverse processes in plant-pathogenic fungi (27). The *M. oryzae* HAT MoRtt109, which acetylates H3K56, has an important role in DNA

integrity (74), whereas the HAT MoSAS3 is required for pathogenicity and normal growth (28). *M. oryzae* HATs acetylate nonhistone proteins to regulate pathogenesis-related autophagy (150, 153); Gcn5 acetylates the ubiquitin-like autophagy protein Atg7 in a light-responsive manner (153), whereas MoHat1 acetylates two autophagy-related proteins that are critical for appressorium development and pathogenicity (150). In *F. graminearum*, deletion of four putative HAT genes, including *GCN5*, indicated that all were required for normal growth (71). Two of the HAT genes, *FgSas3* and *FgGcn5*, were also important for sexual and asexual reproduction and were required for wheat and tomato infection and synthesis of DON biosynthesis (71). FgSAS3 acetylates site H3K4, FgGCN5 is essential for H3K9, H3K18, and H3K27 acetylation, and both contribute to H3K14 acetylation (71). GCN5 also has a central role in *F. fujikuroi*, regulating expression of approximately 30% of the genome, including many secondary metabolite genes (111). Finally, in the postharvest pathogen *Aspergillus flavus*, the HAT AflGcnE is an important pathogenicity factor required for colonization and aflatoxin production on maize seeds as well as cell surface hydrophobicity, asexual sporulation, and sclerotia development (75).

Lysine acetylation is also widespread in bacteria, where it regulates many housekeeping and physiological functions such as DNA transcription, replication, and repair (11). Although some bacterial acetylation is mediated by lysine acetyltransferases, the majority occurs independently of enzyme activity (11) and thus cannot be globally inactivated through mutagenesis. A survey in *E. amylovora* found that acetylation levels varied highly with growth conditions and that there were significant differences in the acetylomes of two genetically similar strains with differing virulence levels (144). The 96 acetylated *E. amylovora* proteins identified after affinity enrichment included seven with known virulence roles in addition to many predicted metabolic enzymes. A sensitive liquid chromatography tandem mass spectrometry approach was used to analyze the acetylome in the walnut pathogen *Brenneria nigrifluens*, finding 787 acetylated proteins, including those implicated in motility, nucleoside synthesis, and stress responses (83). The effects of acetylation in plant-pathogenic bacteria are still not well-understood, but it is likely widespread and central to pathogen competence.

Histone Deacetylases

HDACs remove acetyl modifications from histones, usually resulting in the repression of gene expression. In *M. oryzae*, HDAC inhibitor treatment inhibited appressorium formation and decreased fungal pathogenicity (62), as did deletion of components of the Tig1 HDAC complex (23, 76), demonstrating a role for HDACs in transcriptional programming that leads to infectious growth and conidiogenesis. The HDAC homologs *HDC1* and *Hos2* were important genes required for conidiation and mating in the maize pathogens *Cochliobolus carbonum* and *U. maydis*, respectively (2, 31). Deletion of another *U. maydis* HDAC gene, *hda1*, induced expression of several mating locus–regulated genes that are normally expressed only in the dikaryon. The mutant strains can still infect corn but cannot produce teliospores (130). HDACs often have an important role in the production of mycotoxins and other secondary metabolites, as demonstrated by studies on HDF1 in *F. graminearum* (82) and FfHda1 and FfHda2 in *F. fujikuroi* (124).

Sirtuins

Sirtuins are NAD-dependent deacetylases of histones and nonhistone proteins. In plant-pathogenic fungi, sirtuins have an important role in suppressing host innate immunity (37). The *M. oryzae* sirtuin Sir2 works with the histone demethylase MoJmjC to activate expression of superoxide dismutase, which neutralizes the host ROS burst (37). Sir2 itself is regulated by PTM mediated by at least three ubiquitin ligases (79).

UBIQUITIN AND UBIQUITIN-LIKE MODIFICATIONS

Ubiquitin

Ubiquitin is a 76-aa protein attached to target lysine residues through the sequential action of an activating enzyme (E1), a conjugating enzyme (E2), and a ligase (E3). Multiple ubiquitination typically targets proteins for degradation, whereas monoubiquitination can change the location or activity of a protein (56). Deletion of the polyubiquitin encoding gene *UBI4* resulted in defects in growth, sporulation, germination, and appressorium formation as well as a loss of virulence in *M. oryzae* (101). Key ubiquitin components identified in *M. oryzae* include the E2 enzyme Rad6, which works through at least three E3 ligases to ubiquitinate degradation targets, histones, the DNA replication clamp PCNA (119), and members of the Skp1-Cul1-F-box-protein (SCF) E3 ligase complex (106). The essential protein MoSkp1 forms SCF complexes with 17 F-box proteins in *M. oryzae*, of which three are essential for virulence (120), and a fourth F-box protein, MoGrr1, is also critical for conidiogenesis and pathogenicity (51). Grr1 homologs are also key regulators of infection processes in *F. oxysporum* (95). Along with their virulence roles, deletion ubiquitination regulatory genes often cause pleotropic developmental and reproduction defects in systems such as *F. verticillioides* and the chestnut pathogen *Cryphonectria parasitica* in addition to the model pathogens discussed above (14, 51, 106).

Ubiquitin-Like Proteins

Ubiquitin-like proteins (Ubls) are protein modifiers similar to ubiquitin in that they share a β-grasp fold and typically modify lysine (132). These Ubl-mediated modifications, which include sumoylation, neddylation, urmylation, and pupylation, function as important regulators of various cellular processes, including DNA repair, transcription, signal transduction, and cell-cycle control.

Sumoylation

Sumoylation is the attachment of the small ubiquitin-like modifier (SUMO) peptide used to target proteins and is regulated by a cascade of events similar to ubiquitination (43). In *M. oryzae*, a key sumoylation pathway is catalyzed by the subsequently worked enzymes of E1 (activating enzyme; Aos1/Uba2), E2 (conjugation enzyme; Ubc9), and E3 (ligases; Siz1 and Siz2, etc.). Many reported infection-related proteins in this pathogen are sumoylated, including key players in appressorium formation, conidial storage, ROS scavenging and detoxification, and ER effector trafficking and secretion; deletion mutants of the SUMO pathway genes were all significantly reduced in host penetration and invasion (86, 87). Interestingly, SUMO regulates proper localization of the septins, which is essential for appressorial actin ring formation during infection (87).

Neddylation

Neddylation refers to the addition of the NEDD8 polypeptide to lysine residues of a small range of target proteins (108). Neddylation is essential for fungal growth and is closely linked to ubiquitination, regulating the cullin-1 protein of the SCF E3 ligase complex (121). A neddylation protein ortholog exists in plant fungal pathogens such as *M. oryzae*, but the role of neddylation in plant pathogenesis remains largely unknown.

Urmylation

Urmylation is the attachment of the small modifier URM1 (ubiquitin-related modifier 1) to proteins, which was relatively less studied in plant-pathogenic fungi. In *M. oryzae*, URM1 modifies

and activates the thioredoxin Ahp1 during oxidative stress and thus may be part of suppressing the host resistance response (139). URM is necessary for virulence and has other proposed roles in regulating cell wall integrity, vegetative and infectious growth, conidiation, and responses to other stresses (139).

Pupylation

Pupylation is the addition of a ubiquitin-like peptide (Pup) that targets proteins for degradation via the 20S proteosome complex of gram-positive Actinobacteria. The proteosome was originally discovered in the plant symbiont *Frankia alni* (4). In Mycobacteria and Corynebacteria, pupylation contributes to DNA damage and oxidative stress responses, iron homeostasis, and survival in the host as well as some proteosome-independent regulatory roles (98). Pupylation has not been studied in plant pathogens, but Pup ligase genes are annotated in the genomes of plant-inhabiting *Rhodococcus*, *Streptomyces*, *Leifsonia*, and *Frankia* strains (GenBank protein accessions: WP_179273996.1, WP_013004876.1, WP_048678303.1, and WP_041939239.1, respectively).

LIPID MODIFICATIONS

Lipid modification refers to the attachment of diverse fatty acids or sugar–lipid moieties to cysteine, glycine, or serine residues of target proteins (69). In general, lipid modifications function to regulate protein–membrane associations. The GPI anchoring mechanism discussed above in the glycosylation section is one form of lipid modification.

Prenylation

Prenylation is the irreversible addition of 15- or 20-carbon terpenoids to a target cysteine in a C-terminal CAAX motif (152). In fungi, prenylation most frequently modifies members of the small GTPase superfamily that regulate growth and pathogenicity traits as well as lipopeptide pheromones that are important for intercellular communication and mating (55, 125). For example, the *M. oryzae* prenylation enzyme, farnesyltransferase β-subunit Ram1, regulates the membrane localization of two Ras GTPases and contributes to virulence, vegetative and invasive growth, and appressorial and conidial production (55). Ram1 is also a critical virulence and mating factor in sugarcane smut fungus *Sporisorium scitamineum* (125).

N-Myristoylation

N-Myristoylation is the addition of the 14-carbon fatty acid myristate to an exposed N-terminal glycine (33). The process is essential for membrane targeting and viability in human model fungi such as *Aspergillus fumigatus* and *Cryptococcus neoformans* (32, 90), but relevance to plant pathogenesis is not yet known.

Palmitoylation

Palmitoylation is the reversible addition of a fatty acid to the side chain of Cys residues by protein *S*-acetyltransferases (PATs) (12). Functions of palmitoylation in human fungal pathogens have been previously revealed. For example, chitin synthase 3 (CHS3), central signaling protein Ras1, and vacuolar fusion factor Vac8 are palmitoylated by the PATs in the human fungal pathogen *C. neoformans* (114). However, the relevance of palmitoylation to plant pathogenesis has not been studied.

REDOX MODIFICATION

Under stressful conditions, a type of protein modification called redox modifications is usually induced by responding to cellular ROS or reactive nitrogen species signals, which can regulate protein functions to coordinate cellular processes. *S*-Thiolation is the reversible, oxidative stress–induced modification of cysteine residues with a low-molecular-weight thiol redox buffer: glutathione (*S*-glutathionylation) in gram-negative bacteria, bacillithiol in Firmicutes (*S*-bacillithiolation), or mycothiol in Actinomycetes (*S*-mycothiolation) (134). The modification protects cysteines from permanent oxidation and protein damage but can also alter protein function. Although little is known about *S*-thiolation in bacteria, recent studies have revealed that this modification is widespread and plays critical virulence roles in diverse human pathogens (61, 73, 84).

STRATEGIES FOR POSTTRANSLATIONAL MODIFICATION STUDY

Functional Genetics Research

Commonly, functions of different PTMs are characterized by analyzing key genes encoding corresponding modification transferases or hydrolytic enzymes. Through observing phenotypic defects of these genes' mutants, such as defects in vegetative growth, mycelial morphology, conidiation, conidium morphology, germination, appressorium formation, cell wall integrity, stress response, mycotoxin production, sexual reproduction, and virulence, functional roles of specific PTMs can be determined in the pathogenic fungi or bacteria. Through functional genetics research, combining with cellular function determination, gene/protein expression pattern tests, and target identification, the roles of PTMs can be successfully determined.

Genome-Wide Reverse Genetics Strategies

Although some important protein kinases have been characterized, many others remain to be analyzed. In the postgenome era, it is feasible to systematically characterize functions of the predicted kinome of a plant-pathogenic fungus or bacteria. For example, a kinome-wide reverse genetics analysis has been conducted in *F. graminearum* (136). There are 116 genes encoding putative protein kinases identified from the 13,321 predicted genes of *F. graminearum*, 96 of which can be successfully deleted and functionally characterized by observing defects in growth and colony morphology, conidiation and conidium morphology, germination, stress response, DON production, sexual reproduction, and plant infection (136). Combining with a PK–PK and PK–protein interaction network prediction, this strategy can show us a landscape of PK genes' functions in developmental and plant infection processes in the plant-pathogenic pathogens. Similarly, a phosphatome analysis is also performed in *F. graminearum* (151). In a total of 82 genes encoding putative phosphatases, 63 are deleted and characterized for hyphal growth, development, plant infection, and secondary metabolism (151). This strategy can be extended to other pathogenic pathogens to systematically characterize PTM-mediated regulatory mechanisms.

Posttranslational Modification Proteomics Approaches

Many experimental methods have been used to identify potential PTM sites, among which the MS-based proteomic strategy is the most used analytical method for detecting PTM-modified proteins. Gel-based or gel-free approaches, chemical and antibody-based enrichment strategies combined with MS, modern ion separation technologies, and computing algorithms are powerful techniques to characterize the individual parts and provide a global analysis of PTMs (**Figure 4**). These PTM proteomics approaches have been successfully used for identifying sites of

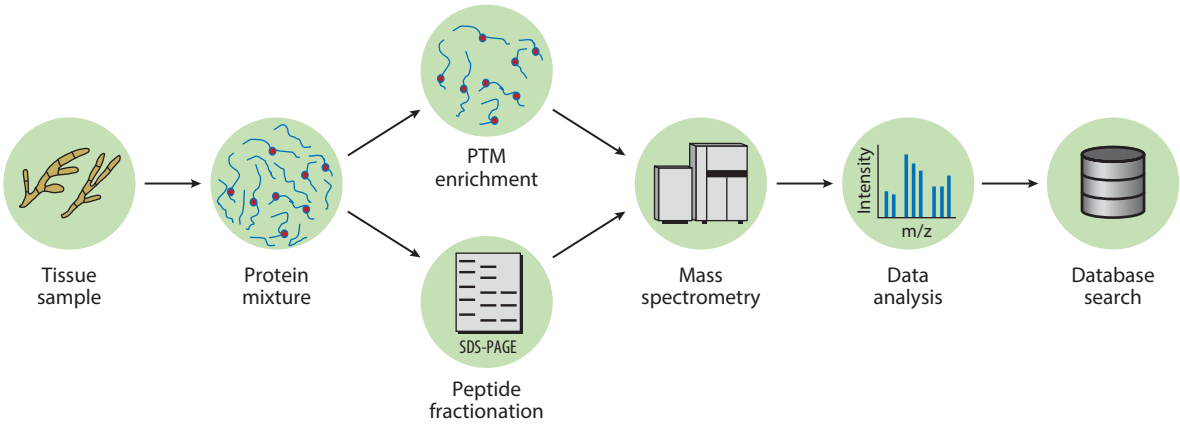

Figure 4

Proteomics analysis to identify posttranslational modifications (PTMs) in plant-pathogenic fungi and bacteria. Fungal or bacterial samples are collected to prepare total proteins, which are enriched by different strategies or separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) gel. The enriched or separated proteins are subjected to mass spectrometry analysis and data analysis with different computing algorithms, after which a database search to determine the target proteins is performed. Abbreviation: m/z, mass-to-charge ratio.

phosphorylation, acetylation, and *N*-glycosylation of different plant-pathogenic pathogens (15, 40, 85, 126, 140), which provides a global view of how PTM proteins regulate the diverse processes of development and pathogenesis.

By using isotopically encoded labels and isobaric tags combined with the spectral counting methodology, quantitative MS has also become a powerful method for studying the regulatory patterns of PTMs. Quantitative analyses of PTM dynamics can be utilized to study regulatory mechanisms under changing cellular conditions or during different plant infection stages. For example, high-throughput *N*-glycosylation proteomics was used to show that *N*-glycosylation is important in coordinating events in vegetative growth, conidia formation, appressoria formation, and invasive growth in *M. oryzae* (15).

ChIP-seq

Chromatin immunoprecipitation (ChIP) combined with high-throughput sequencing (ChIP-Seq) and MS can be used to identify histone modification patterns enriched or deficient in chromatin (3, 142). ChIP-Seq has become a comprehensive tool for investigating various posttranslational histone modifications, including methylation and acetylation. A common approach in ChIP-Seq analysis is to sequence the profiles of an experimental sample and a wild-type sample and compare them to identify differences in the histone modification patterns. These can then be used to uncover the regulatory mechanisms during development and infection of the pathogenic pathogens. Through ChIP-Seq analysis, relationships of specific histone modifications with genome patterns were uncovered in different filamentous fungi, including *F. fujikuroi* (143), *F. graminearum* (18), *M. oryzae* (70), and *Zymoseptoria tritici* (116).

Posttranslational Modification Site Prediction

Because experimental methods are time-consuming and PTM proteomics approaches are high cost, PTM site prediction through bioinformatics has become more valuable. Because the local

sequence of the PTM site is generally conserved in most PTMs, it is appropriate to predict PTM sites through computational methods. Some bioinformatics tools have been developed to predict phosphorylation sites, including NetPhos, Scansite, NetPhosK, GPS, and the bacterial phosphorylation site prediction tool NetPhosBac (**Table 2**). Many other bioinformatics online tools are also developed for the prediction of different PTM sites. For example, ubiquitination sites can be predicted by iUbiq-Lys and UbiNet, lysine acetylation sites can be predicted by BRABSB and PAIL, glycosylation sites can be predicted by NetOGlyc and NetNGlyc, and GPI anchor sites can be predicted by NetGPI and YinOYang (**Table 2**). However, PTM is a complex process related to many biological mechanisms, and local sequence-based PTM site prediction may not be sufficient for the understanding of global function. Other approaches, such as protein–protein interaction (PPI) and protein structure information, can be used to assist the PTM site prediction.

POSTTRANSLATIONAL MODIFICATIONS AND DISEASE CONTROL

As PTMs in the plant-pathogenic fungi and bacteria play critical roles in plant infection and reproduction, various enzymes that mediate PTMs provide promising new agrochemical targets. However, compared with the drug discovery targeting of pathogen PTMs for the treatment of human disease, the applications for plant disease control are still in the early stage. A good example is that a filamentous fungus HK within the HOG pathway is the target of the phenylpyrrole fungicide fludioxonil, which causes uncontrolled hyperactivation of HOG signaling and cell death (42, 64).

Several small molecules inhibit protein kinases, acetylases, and methylases of plant pathogens, which can be loading compounds for the development of specific inhibitors. Marasmic acid, a sesquiterpenoid isolated from the basidiomycete *Marasmus conigenus*, has strong antifungal activity against *M. oryzae* and interferes with the membrane sensor HK MoSln1p of *M. oryzae* (65). In several fungal pathogens, the Hog1 pathway plays a species-specific role in fungal virulence, so this pathway could be a promising target for the development of novel specific fungicides (68). Recently, an in vivo test system for fluorescent-based validation was used to identify the fungicides targeting the HOG pathway in the *MoHOG1*::*GFP* mutant, and this work can provide a tool for screening new fungicidal candidates that interfere with the Hog1p-MAPK (5). HAT and HDAC inhibitors are widely employed in cancer chemotherapy. A specific inhibitor of mammalian histone deacetylase, trichostatin A, also inhibited the appressorium formation of *M. oryzae* and decreased its pathogenesis, and MoHos2 was considered a potential target (62). Phenazine-1-carboxamide, secreted by the biocontrol bacterium *Pseudomonas piscium*, was found to directly inhibit the activity of FgGcn5 in the pathogenic fungus *F. graminearum*, which suggests that the Spt-Ada-Gcn5 acetyltransferase complex could be a valuable target (17).

It has been reported that altering DNA methylation and histone acetylation can affect the synthesis of secondary metabolites of fungi. 5-Azacytidine (5-AC) is a DNA methyltransferase inhibitor and frequently used to elucidate the roles of DNA methylation. 5-AC can dramatically block the aflatoxin production of *A. flavus* and cause concurrent developmental defects (148). In addition, a nonaflatoxigenic mutant of *A. flavus* induced by 5-AC was found to produce decreasing fatty acid–derived volatiles, which are important precursors for aflatoxin biosynthesis (122). After the treatment of an HDAC inhibitor, suberoylanilide hydroxamic acid and two novel compounds, 5-butyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid and 5-(but-9-enyl)-6-oxo-1,6-dihydropyridine-2-carboxylic acid, which are derivatives of fusaric acid, were isolated in the culture medium of *F. oxysporum*, suggesting that chemical epigenetic modifiers could be a feasible strategy to regulate metabolite production (13).

Table 2 Available posttranslational modification (PTM) site prediction tools

PTM type	Tool	URL/web server	Purpose
Phosphorylation	NetPhos	**https://services.healthtech.dtu.dk/service.php?NetPhos-3.1**	Prediction of general phosphorylation status of serine, threonine, and tyrosine phosphorylation sites
	Scansite	**http://scansite.mit.edu**	Prediction of phosphorylation sites on short protein sequence motifs
	NetPhosBac	**https://services.healthtech.dtu.dk/service.php?NetPhosBac-1.0**	Prediction of generic phosphorylation sites in bacterial proteins
	NetPhosK	**http://www.cbs.dtu.dk/services/NetPhosK/**	Prediction of kinase-specific phosphorylation sites
	GPS	**http://gps.biocuckoo.org/**	Prediction of kinase-specific phosphorylation sites
Glycosylation	NetOGlyc	**https://services.healthtech.dtu.dk/service.php?NetOGlyc-4.0**	Prediction of mucin-type GalNac *O*-glycosylation sites
	NetNGlyc	**https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0**	Prediction of *N*-glycosylation sites
	NetGPI	**https://services.healthtech.dtu.dk/service.php?NetGPI-1.1**	Prediction of GPI-anchored proteins
	YinOYang	**http://www.cbs.dtu.dk/services/YinOYang/**	Prediction of *O*-β-GlcNAc attachment sites in eukaryotic protein sequences
Ubiquitination	iUbiq-Lys	**http://www.jci-bioinfo.cn/iUbiq-Lys**	Prediction of lysine ubiquitination sites in proteins
	UbiNet	**http://140.138.144.145/~ubinet/index.php**	Prediction of E3 binding/recognition sites
Sumoylation	GPS-SUMO	**http://sumosp.biocuckoo.org/online.php**	Prediction of sumoylated sites
	SUMOplot	**https://www.abcepta.com/sumoplot**	Prediction of sumoylated sites
Methylation	MASA	**http://masa.mbc.nctu.edu.tw/**	Prediction of the protein methylation site based on the accessible surface area surrounding the modification site
	GPS-MSP	**http://msp.biocuckoo.org/**	Prediction of general or type-specific methylline and methylarginine residues in proteins
Acetylation	NetAcet	**https://services.healthtech.dtu.dk/service.php?NetAcet-1.0**	Prediction of N-terminal acetylation in eukaryotic proteins
	PAIL	**http://bdmpail.biocuckoo.org/prediction.php**	Prediction of Nε-,K-acetylated
Lipidation	TermiNator	**https://bioweb.i2bc.paris-saclay.fr/terminator3/**	Prediction of N-terminal myristoylation and *S*-palmitoylation of proteins
	Myristoylator	**https://web.expasy.org/myristoylator/**	Prediction of N-terminal myristoylation proteins
	NMT	**https://mendel.imp.ac.at/myristate/SUPLpredictor.htm**	Prediction of N-terminal myristoylation proteins
	CSS-Palm	**http://csspalm.biocuckoo.org/online.php**	Prediction of *S*-palmitoylation proteins
	SwissPalm	**http://swisspalm.epfl.ch**	Prediction of *S*-palmitoylation proteins
	SeqPalm	**http://lishuyan.lzu.edu.cn/seqpalm/**	Prediction of *S*-palmitoylation proteins
Pupylation	GPS-PUP	**http://pup.biocuckoo.org/**	Prediction of pupylated substrates

Abbreviations: GPI, glycosylphosphatidylinositol anchoring; GPS, group-based prediction system; GPS-MSP, GPS-methyl-group specific predictor; GPS-PUP, GPS-pupylation; GPS-SUMO, GPS-small ubiquitin-like modifier; NMT, *N*-myristoyltransferase; MASA, methylation site based on the accessible surface area; PAIL, prediction of acetylation on internal lysine.

SUMMARY POINTS

1. Phosphorylation-mediated MAPK, cAMP-PKA, and two-component signaling cascades play key roles in pathogen infection.
2. *N*-glycosylation, *O*-glycosylation, and GPI anchoring regulate appressorium-mediated fungal invasion of plants, and *O*-glycosylation masks bacterial surface proteins from the host interface.
3. Histone methylation and acetylation coordinate infection through activating or repressing genome-wide gene expression.
4. Ubiquitin and ubiquitin-like modifications regulate protein stability or localization in plant-pathogenic fungi.
5. Lipid modifications are important for the localization of plasma membrane target proteins.
6. Functional genetics research, genome-wide reverse genetics strategies, PTM proteomics approaches, ChIP-Seq, and bioinformatics site prediction can be used to study PTMs in plant-pathogenic pathogens.
7. Enzymes mediating PTMs provide promising new fungicide targets for disease control.

FUTURE ISSUES

1. Most current knowledge regarding the roles of PTM in plant pathogenesis is focused on protein phosphorylation. Understanding the important roles of other PTMs, such as ubiquitin-like modifications, histone modifications, glycosylation, lipidation, and others, will require more extensive studies.
2. Interactions between PTMs clearly exist, and therefore regulatory networks mediated by different PTMs will shed new light on the biology and infection of plant-pathogenic pathogens.
3. Specialized PTMs such as *N*-myristoylation, palmitoylation, *S*-thiolation, and *S*-nitrosylation play important roles in model human pathogens, but they are largely unknown in the context of plant disease and warrant further investigation.
4. Emerging novel histone PTMs, including succinylation, crotonylation, butyrylation, benzoylation, and 2-hydroxyisobutyrylation, may have unknown impacts on plant pathogenesis.
5. Developing more effective technologies to identify targets of different PTMs, either through proteomics or bioinformatics, will greatly facilitate the understanding of PTMs in phytopathogen biology and infection. This will provide valuable new targets for fungicide development.
6. Many key enzymes regulating PTMs have been identified, but there are likely many others that need to be uncovered.
7. Inhibition of PTM enzymes may help us to dissect the fascinating biology of PTMs and develop new strategies to control fungal or bacterial diseases.

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