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Emerging Roles of Post Translational Modifications in Plant Pathology

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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. 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).

PTMs are reversible or irreversible chemical modifications of a polypeptide chain that occur after DNA has been transcribed into RNA and translated into protein. Such modifications encompass the events of phosphorylation (addition of a phosphoryl group), glycosylation (addition of glycosyl donor), acetylation (introduction of an acetyl functional group) methylation (addition of methyl group), ubiquitination (attachment of a ubiquitin protein) and others.

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.

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.

POST TRANSLATIONAL MODIFICATIONS

PTMs are chemical modifications of a polypeptide chain that occur after DNA has been transcribed into RNA and translated into protein .The protein may be modified during the translation (Co-translational modifications) or After the translation (Post translational modifications).These chemical modifications of a polypeptide chain after its biosynthesis extends the range of amino acid structures and properties, and consequently, diversifies structures and functions of proteins. Although DNA typically encodes 20 primary amino acids, proteins contain more than 140 different

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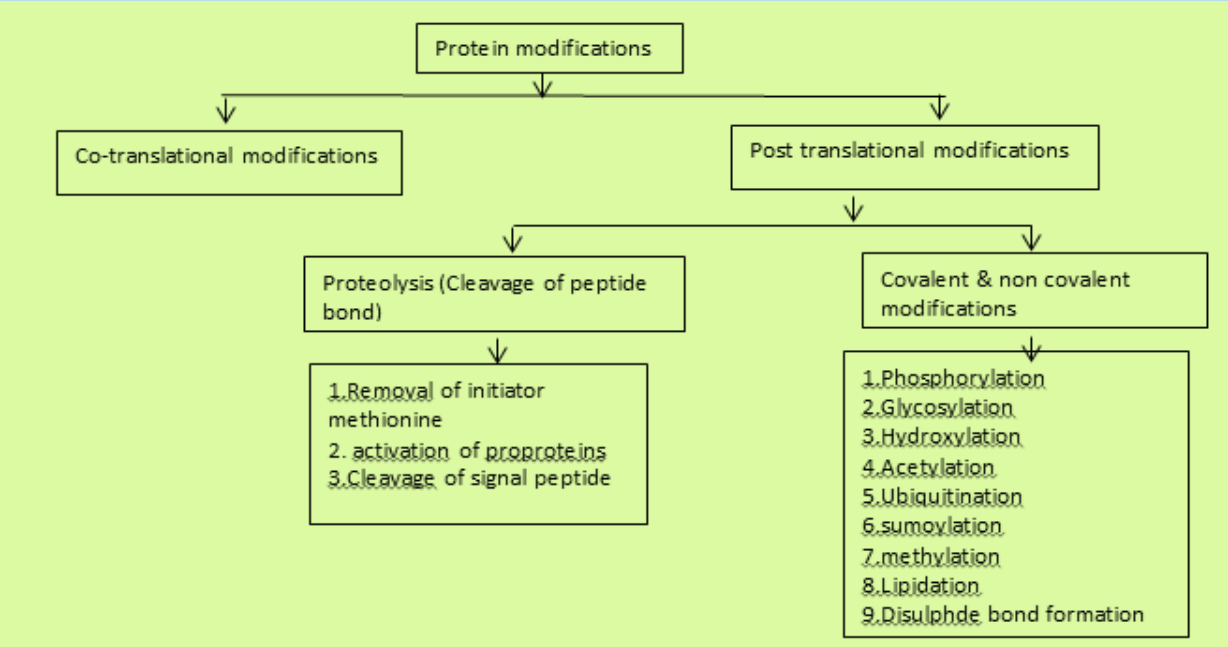
residues, because of various PTMs.

These chemical alterations range from the enzymatic cleavage of peptide bonds to the covalent additions of particular chemical groups, lipids, carbohydrates, or even entire proteins to amino acid side chains.Among them Covalent modifications play important roles in both palnt pathogenic fungi and bacteria. (Retanal *et al*., 2021),

recent years as key players in fungal and bacterial plant disease.(Liu *et al*., 2021)

Mitogen Activated Protein Kinase Cascades:

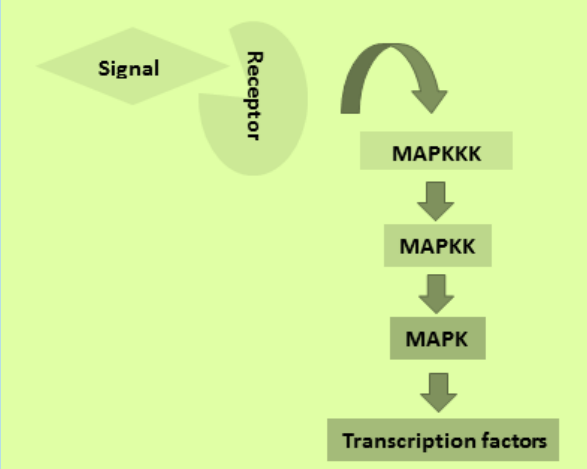
MAPK cascades are three-tiered PK modules that are present in all eukaryotic organisms and function in succession to transmit a variety of cellular signals. The MAP kinase kinase kinase (MAPKKK)



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

phosphorylates the MAP kinase kinase (MAPKK), which in turn activates the MAPK by dual phosphorylation of a pair of conserved threonine and tyrosine residues. MAPK cascades govern a variety of cellular responses, ranging from proliferation and differentiation to stress adaptation and programmed cell death.



Most fungal pathogens contain three MAPKs that are orthologs of the *S. cerevisiae* Fus3/Kss1, Slt2, and Hog1 MAPKs, and function in separate signaling cascades to regulate infection-related morphogenesis, cell wall remodeling, and high osmolarity stress response, respectively.Although all three MAPK pathways contribute to virulence on plants, each of them has distinct and sometimes even opposite functions during the infection process. There is also evidence for cross talk between different fungal MAPK cascades and between MAPK and other key signaling pathways, such as the cAMP-PKA and the target of rapamycin (TOR) pathway.

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. 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.(*Fujita and Kinoshita , *2012)*

***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. 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 Proline).

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

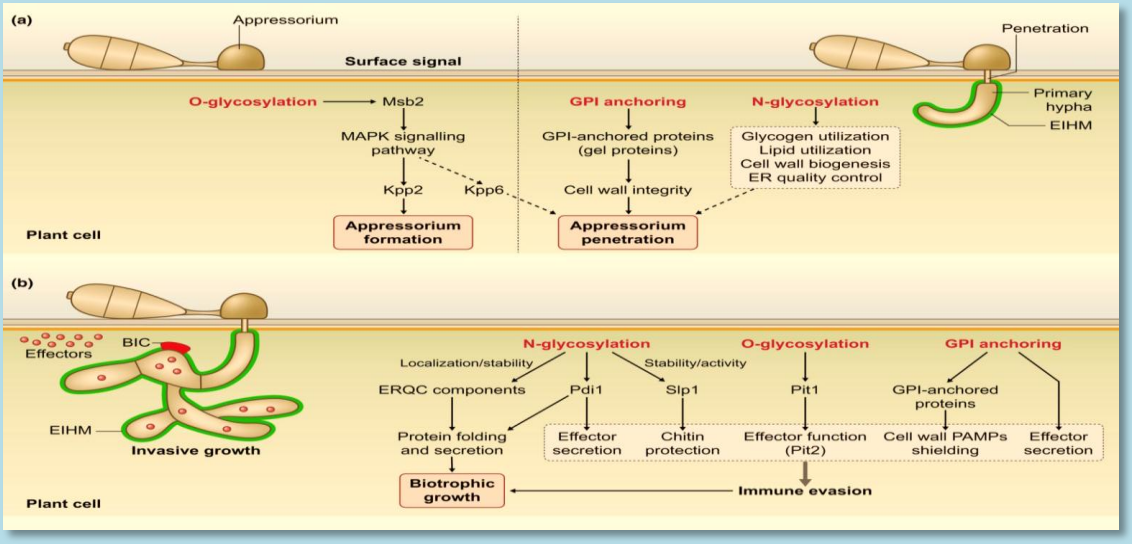
***O*-glycosylation**

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. Initial mannose addition is mediated by protein

mannose transferases (PMTs) in the ER, followed by modification and maturation in the Golgi. Fungal PMTs comprise three widely conserved subfamilies: PMT1, PMT2, and PMT4. PMT deletion usually causes defects in cell wall composition by affecting the amounts of β-glucans, chitin, and glycoproteins found in the cell wall. In *U. maydis*, individual deletion of eight predicted *O*-mannosyl transferase genes showed that only *pmt4* was essential for virulence and played roles in appressorium formation and plant cuticle penetration. 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. 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.

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. *Fusarium graminearum* deletion mutants of the GPI pathway gene *GPI7* formed aberrantly shaped macroconidia and had significantly reduced virulence. 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. In *M. oryzae*, disruption of *GPI7* caused significant defects in appressorial cell wall

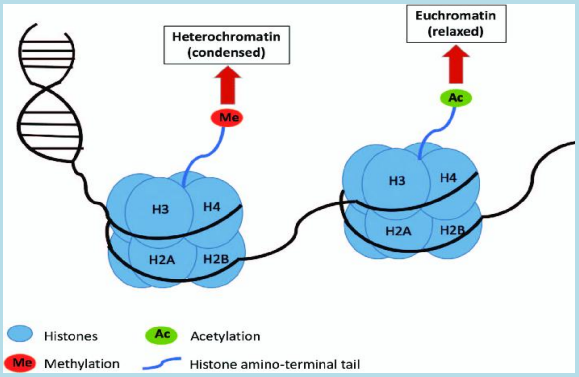


Potential mechanisms of glycosylation during fungal infection process

architecture, which is essential for penetration and invasive growth in the host cells. 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 plant host innate immunity system.(Liu *et al*., 2020)

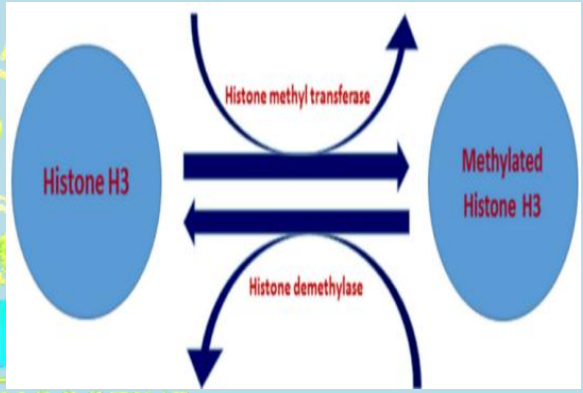
HISTONE MODIFICATIONS:

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. Methylation and acetylation of histones are the most well-studied modifications in plant pathogens. (Rajan *et al*., 2020).



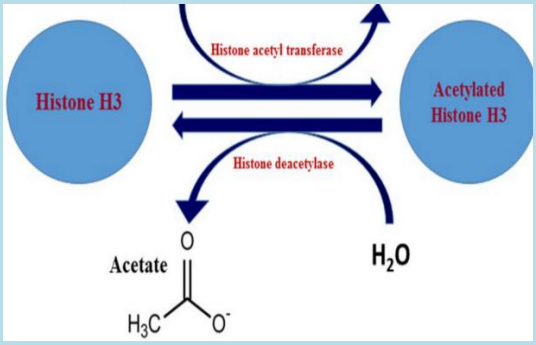
Methylation:

The attachment of methyl groups to histone proteins occurs predominantly at specific lysine or arginine residues on histones H3 and H4 The loosening or restriction of the chromatin structure due to histone methylation or demethylation results in transcriptional repression (heterochromatin) or activation (euchromatin). It is mediated by histone methyltransferases (HMTs), and histone demethylation is carried out by histone demethylases (HDMs).



Acetylation:

Lysine acetylation of residues on histone 3 and histone 4 is generally related to gene activation and regulates diverse processes in plant-pathogenic fungi .HATs catalyze the transfer of an acetyl group from acetyl-CoA molecules to the lysine ε-amino groups on the N-terminal tails of histones. Histone deacetylases (HDACs) reverse histone acetylation thereby work as repressors of gene expression

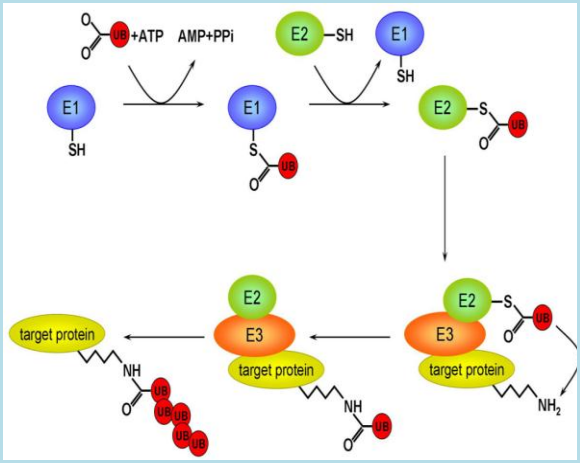


UBIQUITIN AND UBIQUITIN-LIKE MODIFICATIONS

Ubiquitination:

Ubiquitin is a 76-amino acid 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.

Ubiquitination contributes to the regulation of numerous cellular processes, including cell cycle progression, gene transcription, DNA repair, and signal transduction.(Hershko *et al*., 1998)

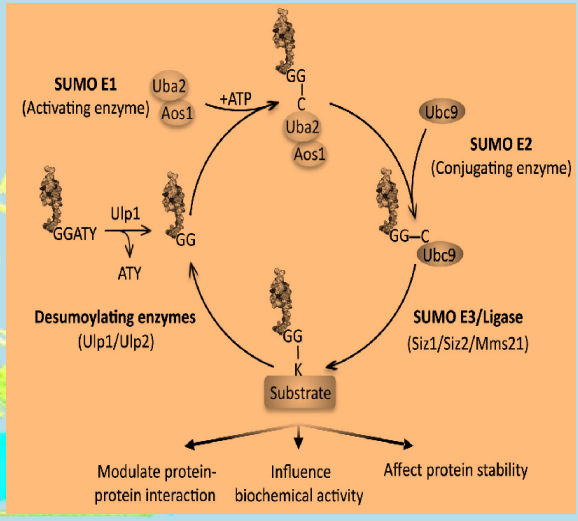


Sumoylation:

Attachment of the small ubiquitin-like modifier (SUMO)(approx. 100 amino acids) to target proteins (mainly lysine residue) by covalent bond.

Functions:

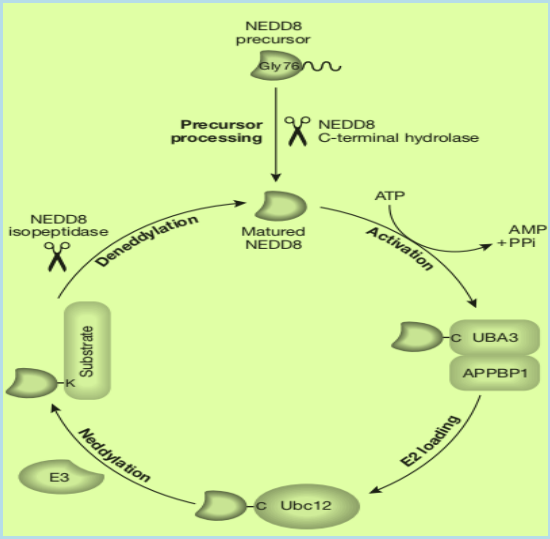
- Plays key roles in colony growth, conidia formation and virulence to the host Secretion of effector proteins
- Regulate proper localization of septins essential for appressorial actin ring formation*(M.oryzae*).



(Liu *et al*., 2018)

Neddylation:

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



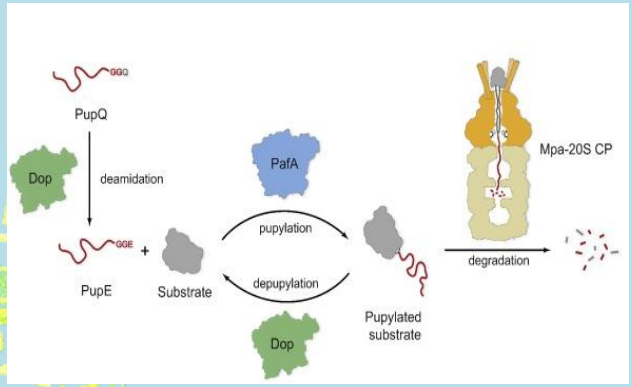
Rabut and Peter (2008)

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. 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

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*. 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. Pupylation has not been studied in plant pathogens, but Pup ligase genes are annotated in the genomes of plantinhabiting *Rhodococcus*, *Streptomyces*, *Leifsonia*, and *Frankia* strains



Muller and Weber-Ban , 2019)

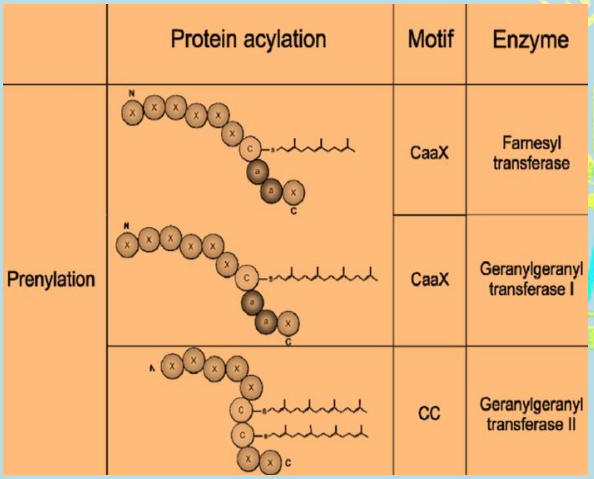
LIPID MODIFICATIONS

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.(Spinelli *et al*., 2018)

Prenylation

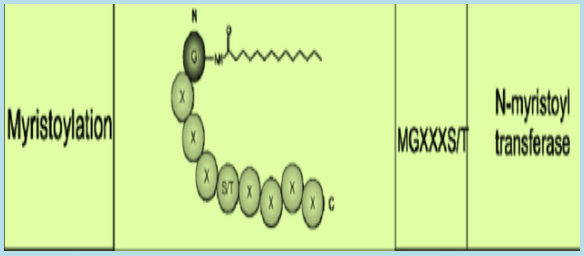
Prenylation is the irreversible addition of 15- or 20-carbon terpenoids to a target cysteine in a C-terminal CAAX motif. In fungi, prenylation most frequently modifies

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. 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. Ram1 is also a critical virulence and mating factor in sugarcane smut fungus *Sporisorium scitamineum*.



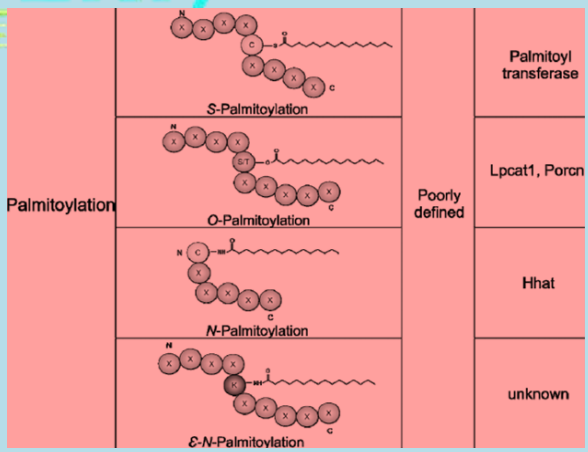
Myristoylation

N-Myristoylation is the addition of the 14-carbon fatty acid myristate to an exposed N-terminal glycine. The process is essential for membrane targeting and viability in human model fungi such as *Aspergillus fumigatus* and *Cryptococcus neoformans*, 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 (Sbacillithiolation), or mycothiol in Actinomycetes (*S*-mycothiolation). 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 .(Vu Van *et al.* 2015).

CONCLUSION

- ✓ Phosphorylation-mediated MAPK, cAMP-PKA, and two-component signaling cascades play key roles in pathogen infection.
- ✓ 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.
- ✓ Histone methylation and acetylation coordinate infection through activating or repressing genome-wide gene expression.
- ✓ Ubiquitin and ubiquitin-like modifications regulate protein stability or localization in plant-pathogenic fungi.
- ✓ Lipid modifications are important for the localization of plasma membrane target proteins.
- ✓ Functional genetics research, genome-wide reverse genetics strategies, PTM proteomics approaches, and bioinformatics site prediction can be used to study PTMs in plant-pathogenic pathogens.
- ✓ Enzymes mediating PTMs provide promising new fungicide targets for disease control.

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