An overview on host proteasomes as a virulence mechanism of plant pathogens

Abstract

Plants' ubiquitin-26S proteasome degradation system (UPS) is involved in the signal transduction of numerous cellular functions, including pathogen-triggered host immunological responses. Pathogens that attack create effectors that are translocated into host cells and disrupt defensive signals in the host in specific ways. Certain bacterial effectors, which are presently best understood in *Pseudomonas syringae*, exploit or depend on the host UPS for their activity, which may not come as a surprise given the extensive role of the host proteasome in plant immunology. Interestingly, certain strains of *P. syringae* express syringolin A, a virulence factor that uses a unique mechanism to irreversibly block the proteasome. This section summarizes the UPS's defense function for plants and how effectors use it. It also covers the biology, taxonomic distribution, and emerging implications for virulence strategies of syringolin A and similar compounds.

Keywords: Ubiquitin-26S proteasome degradation system (UPS), *Pseudomonas syringae*, Programmed cell death (PCD)

Introduction:

"Plants, being sessile creatures subject to varying environmental conditions, have developed various defense mechanisms to fend off abiotic and biotic stressors. These mechanisms include built-in physical barriers, antimicrobial compounds, and the ability to detect and identify pathogen-associated molecular patterns (PAMPs) or effectors specific to a particular pathogen race. The initial line of defense for plants is made up of conserved microbial compounds known as pathogen-associated molecular patterns (PAMPs), which are identified by cell surface pattern-recognition receptors (PRRs)". [21]

Pathogens release effector molecules to counteract the defense responses that pathogens sense and start defense mechanisms. The Zig-zag model of plant microscopic interactions provides a clear picture about host patyhogen interaction. Initial line of protection as PAMP-triggered immunity (PTI) by introducing effector proteins into the host cells, where they alter the host cell's machinery to their advantage, modified plant pathogens are able to evade these PTI reactions. As a result, plants have developed an additional, stronger line of defense that is triggered by intracellular receptors identifying these effectors.

Effector-triggered immunity (ETI) is the term used to describe this second layer. When immunological receptors recognize microbial molecules, hormone signaling and transcriptional reprogramming trigger downstream defense responses. These responses result in the release of antimicrobial chemicals and/or programmed cell death (PCD) to thwart the invasion. Plant-microbe interactions at the molecular level

were studied for years using the zig-zag model, which entails a complicated interplay among various cellular processes.

A high degree of proteome plasticity is needed for the start and maintenance of defense responses, which include both the controlled breakdown and de novo synthesis of regulatory proteins and enzymes. The way that plants respond to both biotic and abiotic stressors is greatly influenced by this proteome flexibility.

Protein degradation can be classified into two categories: non-lysosomal/ubiquitin proteasome mediated protein degradation (Ubiquitin–Proteasome System) and lysosomal protein degradation (Autophagy). Up to 80% of eukaryotic proteins are broken down by the highly conserved Ubiquitin–Proteasome System (UPS). Prior to recycling proteins via the UPS, target proteins must be poly-ubiquitinated. It follows that the discovery of protein breakdown pathways, such as the ubiquitin-protease system, as important regulators of plant immunity and decision-makers in plant-microbe interactions is not surprising.

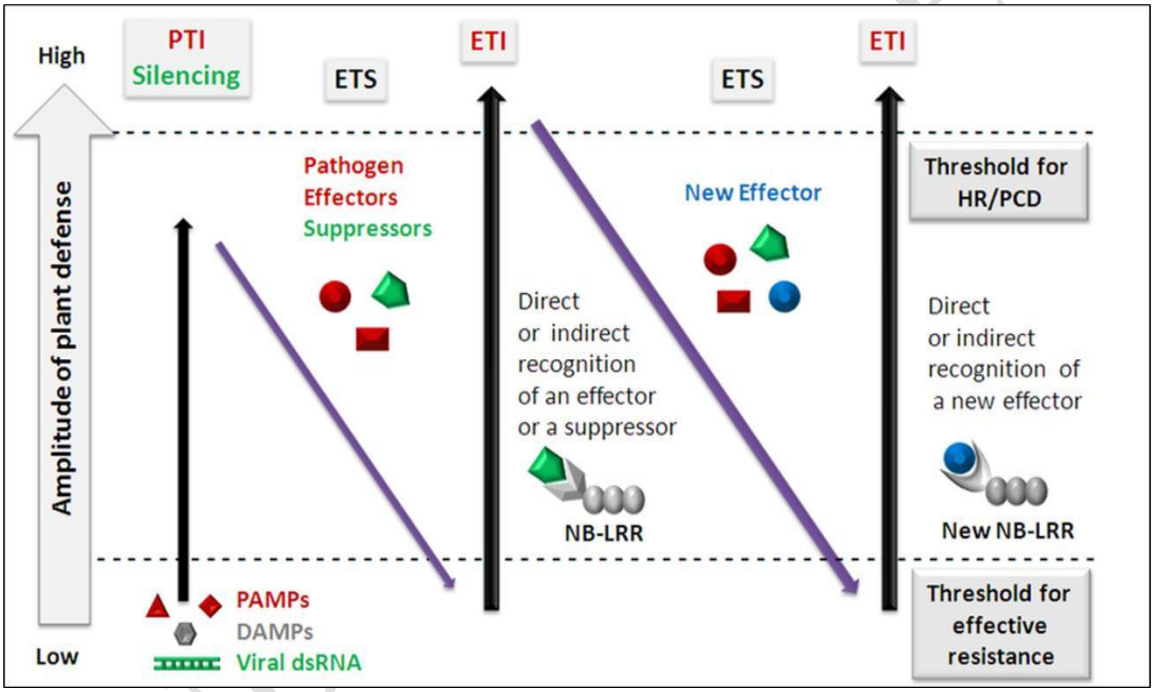

Fig1: Zig-zag model of plant–microbe interactions

Whatis UPS?

The primary non-lysosomal mechanism for breaking down proteins in eukaryotic cells is the UPS (ubiquitin proteasome system) , often referred to as the ubiquitin-proteasome pathway (UPP). One process that occurs after translation and is crucial to the breakdown of proteins is ubiquitination. Targeting misfolded or damaged proteins as well as functioning proteins conveying specific destruction

signals, UPS performs a crucial role in the cell. As a result, UPS serves as a regulatory and quality control system.

Timelineofplantubiquitin-proteasomesystem(UPS)fromitsdiscoverytorecentadvancements:

- ✓ **1975-1980:**EarlyDiscoveries

- Theconceptoftargetedproteindegradationbegantoemergewithstudiesonproteinturnoveri n eukaryoticcells.
- Earlyexperimentsinyeastandmammaliancellslaidthegroundworkforthediscoveryofubiq uitin, asmall regulatory proteininvolved inprotein degradation.

- ✓ **1980:**Ubiquitin,ahighlyconservedproteinfoundinalleukaryotes,wasfirstidentifiedandch aracterized byGideon Goldstein andIrwinRose.

- ✓ **1981:** Aaron Ciechanover and Avram Hershko discovered the role of ubiquitin inproteindegradationandproposedtheATP-dependentubiquitin-proteasome pathway.
- ✓ **1985:**Ciechanoveretal.identifiedtherole ofUPSin cellularprocessesof yeast.
- ✓ **1986:**AlfredGoldbergdemonstratedthepresenceofahigh-molecular-weightproteasecomplexresponsiblefor ATP-dependentproteindegradation,whichlaterbecameknownasthe proteasome.
- ✓ **1990:** Hershko, Ciechanover, & Rose were awarded the Nobel Prize in Chemistry fortheirwork on the UPS.
- ✓ **1996:**PresenceoftheUPSin plantswasconfirmedin*A.thaliana.*
- ✓ **1990-2000:**Identifiedandcharacterized thecomponentsoftheUPSin plants
- ✓ **2004:** Identified the role of UPS in plant immune responses and defense againstpathogens.
- ✓ **2010-present:**Increasedemphasisonunderstandingthemoleculardetailsofinteractionsbetween microbialeffectorsand plantUPScomponents.

Astheprincipalpathwayfordegradingcellularproteins,UPSservestwomainfunctions:

1. **Majorregulatoryfunction:**Itresultsintargeteddegradationofavarietyofshort-livedregulatoryproteins.

2. **Qualitycontrolfunction:**removalofdamagedandfunctionallyincompetentproteins.

ComponentsofUPS:

1. Ubiquitin(Ub):

A 76-amino acid globular peptide called Ub designates proteins for protein breakdown. There are just three residues that differ between yeast, human, and plant species in its highly conserved sequence. Ub exhibits excellent stability due to a large number of intramolecular hydrogen bonds, which are likely to promote recycling over proteolysis during the conjugation/degradation process. Seven lysines are found in ubiquitin (K6, K11, K27, K29, K31, K48, and K63).

2. Ubconjugation cascade:

AttachmentoffreeUbmoietiestoappropriatesubstratesproceedsbyanATPdependent**E1– E2– E3 enzymeconjugation cascade**

E1–ubiquitinactivatingenzyme

E2–ubiquitinconjugatingenzyme

E3–ubiquitinligases

E1 – ubiquitin activating enzyme: The cascade of Ub conjugation is started by the E1 enzymes. One polypeptide, including 1100 residues, is found in plants. It has a nucleotide binding motif that interacts with either AMP Ub intermediates or ATP-bound cysteine to bind active Ub.

E2 – ubiquitin conjugating enzyme: The active cysteine region is surrounded by a distinctive 150 residue catalytic core seen in the E2 enzymes. It seems that distinct E2 enzymes displayed varying preferences of E3 enzymes for interaction based on co-expression and interaction data. Because of their catalytic cysteine residue, E2s can establish a thiol-ester bond with ubiquitin in their structure.

E3 – ubiquitin ligases: To provide substrate selectivity for a wide variety of substrates, the E3s are the most varied proteins in the ubiquitination cascade. A particular E2 interaction domain and a substrate recognition domain are prerequisites shared by all E3s. The composition and method of action of subunits

might serve as broad definitions for subunit classes. The UPS process is made more particular by the E3s, who are in charge of the last protein tagging. Plant genomes have large families of E3 ligases, each of which regulates the ligation of ubiquitin to a single or limited group of substrate proteins, suggesting that a broad range of targets may be identified. Only two E1, 37 anticipated E2, and more over 6% of the predicted Arabidopsis thaliana genome encode UPS proteins.

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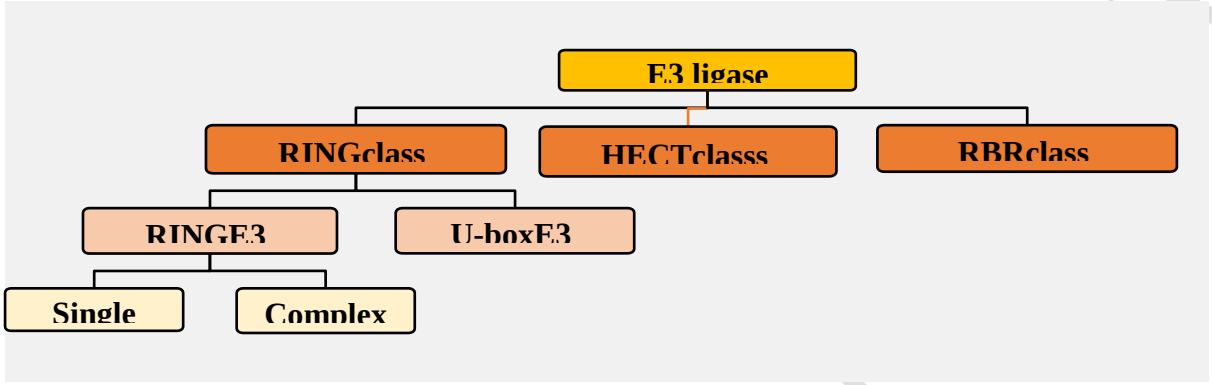

Chart 1: Different types of E3 ubiquitin ligase (Oshima *et al.*, 2023)

Plant E3s are classified into many groups according to the makeup of their subunits and how they function. The two main categories of these families are those that bind to ubiquitin noncovalently [U-box domain and RING (a really fascinating novel gene) E3s] and covalently [HECT (homology to E6-associated protein C-terminus) E3s]. A covalent connection is formed between HECT E3 ligases and ubiquitin prior to the latter's transfer to the target protein. Because they transfer ubiquitin to the substrate through zinc chelation or hydrogen bonds/salt bridges, respectively, U-box and RING E3s are believed to be physically related and functionally similar.

There are two different forms of RING-type E3 ubiquitin ligase: RING-finger proteins as subunits of multiprotein E3 complexes and single subunit E3. The cullin RING ligases (CRL) are one of the most conserved Multi subunit RING E3 families in eukaryotes; among them, the modular SCF group is the largest and most known due to its functions in numerous cellular processes.

. .. Take Arabidopsis thaliana, for instance. Four main components make up SCF complexes: cullin-1 (CUL1), F-box protein, SKP1 (S-phase kinase associated protein)-like protein (ASK1/2), and RBX1 (RING box protein). By interacting with RBX1 at its C-terminal region and SKP1 at its N-terminus, CUL1 functions as a scaffold in the assembly of the various SCF complex subunits. E2-ubiquitin and F-box protein are connected to SKP1 and RBX1, respectively. The ubiquitin transferase activity is mediated by the RBX1–E2 association, while substrate specificity is provided by the SKP1–F-box protein complex.

.Compared to other eukaryotes, plants have a notably larger number of F-box proteins; nevertheless, the cause of this increase is yet unknown. According to Craig et al. (2009), RING/U-box E3s often serve as a molecular adapter for substrates and E2s.

3. 26SProteasome:

The UPS's major component is the 26S proteasome complex. The proteolysis complex that breaks down substrates tagged with ubiquitin is 2 MDa and is dependent on ATP. The 20S core protease (CP) and 19S regulatory particle (RP) are the two subcomplexes that make up the 31 subunits that make up the 26S proteasome. Four stacked rings make up the barrel-shaped structure of the CP, a broad-spectrum protease that is independent of both ATP and ubiquitin (Collins and Goldberg, 2017).

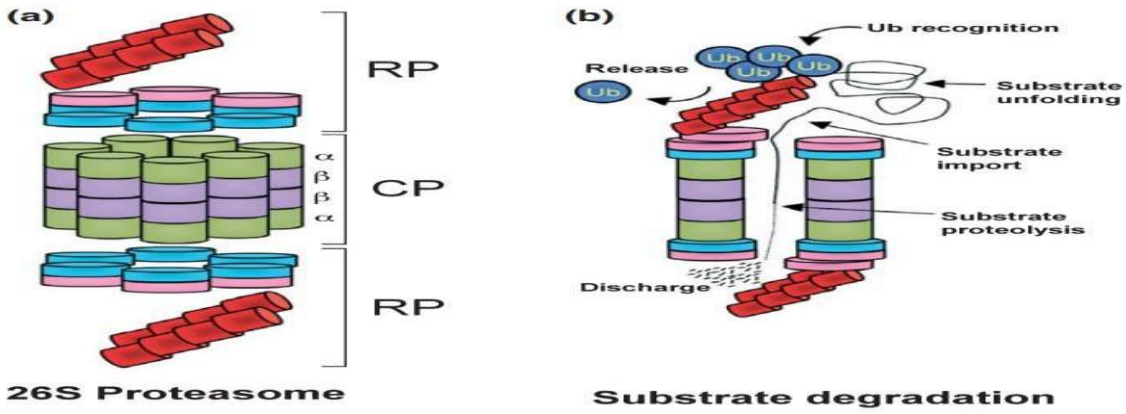

Fig2:Structureof26SProteasome (Oshima *et al*., 2023**)**

4. Deubiquitinatingenzymes(DUBs):

After activation, glutathione and polyamine, two common intracellular nucleophiles, can assault ubiquitin. DUBs work to stop titration by these chemicals in order to prevent loss of active Ub through such pathways. More broadly, it has been suggested that DUB enzymes serve as the last line of defense against destruction, saving proteins that the proteasome has mistakenly been directed towards.

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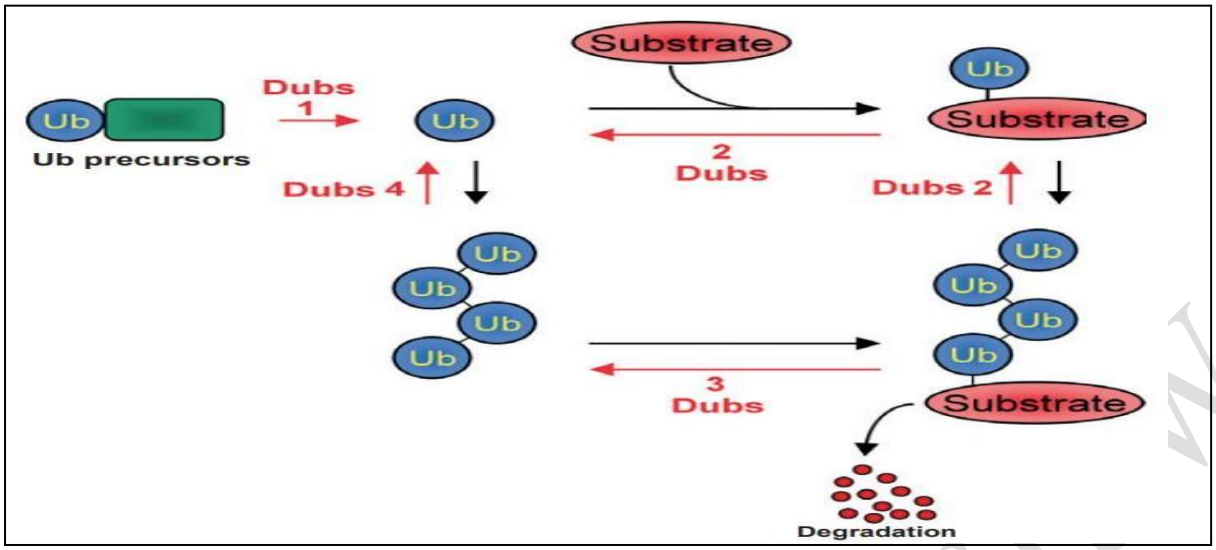

Fig3:Functionsofdeubiquitinatingenzymesinubiquitinsystem (Retanal *et al.*, 2021**)**

Functionsofdeubiquitinatingenzymes(DUBs)intheubiquitin(Ub)system(Fig3).

(1) ubiquitin precursor processing. (2) Ubiquitin conjugates, which are often bound to other proteins within the cell but can also be ligated to numerous tiny nucleophiles like glutathione, can be edited or saved. (3) Recycling of poly-Ub chains or ubiquitin from conjugates of ubiquitin and proteins that are intended for destruction. (4) Unanchored poly-Ub chain disassembly

OverviewofUbiquitinProteasomeSystem:

The ATP-dependent activation of ubiquitin (Ub) by the ubiquitin activating enzyme E1 initiates the ubiquitin–proteasome cascade. A carboxyl group in the terminal glycine of activated Ub forms a thiolester bond with a conserved cysteine in E1. After that, Ub is transferred to the ubiquitin conjugating enzyme (E2) by the creation of an E2-Ub thiolester bond.

The ubiquitin ligase (E3), which is typically formed by an intermediary complex between the target and the E3, is facilitated in transferring the active ubiquitin from the E2 to a lysine residue in the target protein by the E2 itself. The process of initial target ubiquitination begins with the formation of an Ub–protein conjugation connected by an isopeptide bond. Poly-Ub chains are created by ligating additional Ubs. Subsequently, the proteasome complex identifies and targets the proteins that have been tagged with K48 linked poly-Ub chains.

Subsequently, the proteasome complex identifies and targets the proteins that have been tagged with K48 linked poly-Ub chains. A proteasome-associated deubiquitinating activity (DUB) breaks down poly-Ub

chains, releasing free Ub moieties. Substrates localized within proteasomes are subsequently unfolded, imported, and broken down into pieces of peptide.

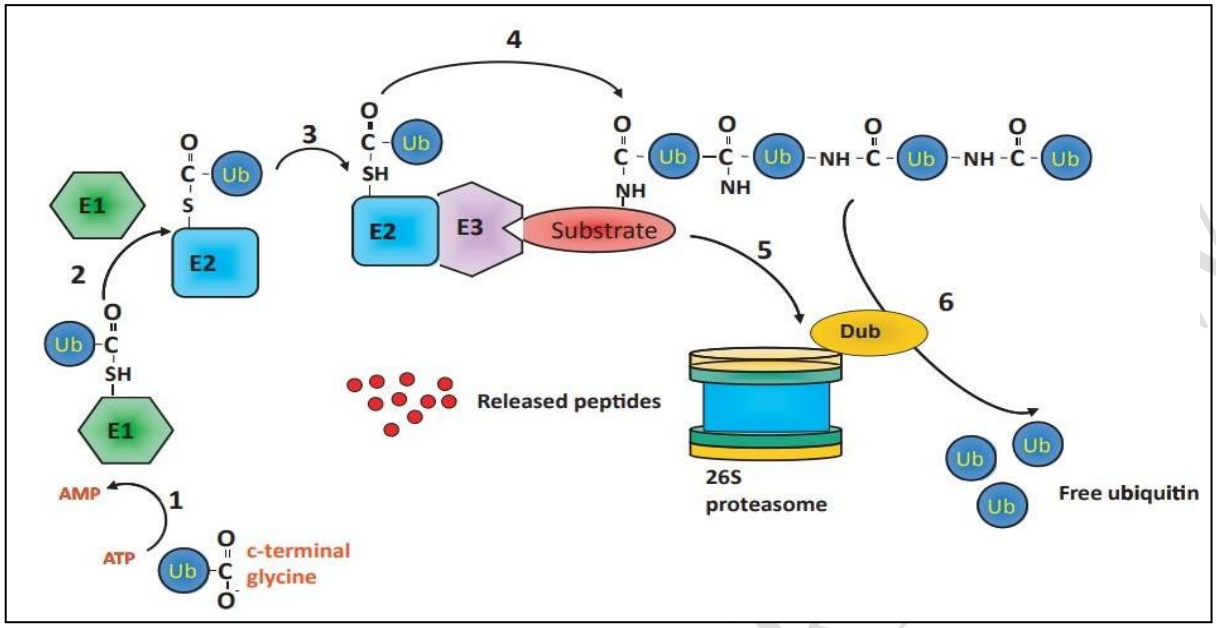

Fig4:Asimplifiedoutline of theubiquitin proteasomesystem (Viana *et al*., 2021**)**

Ubiquitin-mediateddegradation:arecurrentthemeintheplantlifecycle:

Hormone-mediated plant growth and development require UPS.

Maintaining the circadian rhythm is necessary for floral development.

plants' reactions to abiotic stress are coordinated.

Targeting the UPS, plant pathogens demonstrate the UPS's significance for plant immunity

Ubiquitin–proteasomesysteminregulatingallstagesofplantimmunity

The defense responses mediated by PTI and ETI infected plants signify substantial metabolic alterations that must be quickly adjusted to preserve appropriate cell function and biological process regulation. The

26S proteasome's ability to degrade proteins through ubiquitination is one of the most significant methods for regulating PTI and ETI.

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UPSregulatesplantimmunityby:

degradation of important immunological components

1. Downstreamofimmunecomponents

Hormone signaling components associated to defense

2. 26Sproteasomecomplexinimmunity

3. Theholoenzyme anditsregulation

1.UPSdegradeskeyimmunecomponents

Plant immune responses begin with pathogen detection. PAMP perception by cell-surface PRRs and effector recognition by intracellular receptors are the two stages of this recognition process. It has been revealed that immunological receptors of both kinds are targets of UPS-mediated degradation.

Pattern-recognitionreceptorsandassociatedproteins:

"PRRs encode receptor kinases and receptor-like proteins located mostly at the plasmamembrane,wheretheyrecognizeconservedPAMPs.Giventheirimportanceininitiatingplantdefense responses, their controlled turnover is a key regulatory process in which the UPS isheavilyinvolved.Forexample,*Arabidopsis*flagellinsensitive2(FLS2),isthebest-characterizedPRRwhichrecognizesflg22,apeptidederivedfromthebacterialflagellin.Uponflg22 recognition, FLS2 associates with its coreceptor BRI1- associated receptor kinase 1(BAK1)followingmultiplephosphorylationeventsmediatedbytheirrespectivekinasedomains.Afterthisrecognition,FLS2undergoesarapidturnoverthathasbeenassociatedwiththe UPS, as chemical inhibition of proteasomal degradation with MG132 stabilizes FLS2protein levels. Additionally, FLS2 also undergoes ubiquitination by the E3 pair PUB12 and 13andgets degraded byproteasomecomplex"(Furlan*etal*., 2017).

Intracellularimmunereceptors:

"Intracellularimmunereceptorsplaykeyrolesinresistancetoadaptedpathogens.Mostofthemencodenucleotide-bindingleucine-richrepeat(NLR)proteins.NLR-mediatedimmunity usually leads to PCD, also called hypersensitive response (HR) in these cases. Forthis reason, it is vital for the survival of the plant to control the basal activity of these proteinsand avoid autoimmunity. This is mediated by the UPS, as evidenced by multiple reportsidentifying NLR proteins from *Arabidopsis*, *Nicotiana benthamiana*, rice and barley targetedfor proteasomal degradation. Altogether, these reports highlight the importance of the UPS intheregulationofbothPTIandETIresponsesinmultipleplantspeciesagainstawidevariety ofpathogens.Interestingly,theUPScanactasbothapositiveandnegativeregulatorofimmunerespon ses,evidencing thecomplexity andversatility of thesystem". [21]

"ubiquitination-mediated protein degradation via 26S proteasome is one of the most important mechanisms for modulating PTI and ETI. Both PTI- and ETI-mediated defense responses represent significant metabolic changes in the infected plants, which should be modulated rapidly to maintain a proper function and regulation of biological processes in the cell" (Couto and Zipfel, 2016).

Defense-relatedhormonesignallingcomponents:

Plant pathogen perception triggers a vast reprogramming of multiple cellular pathways to coordinate suitable defense responses. Hormone signaling plays a significant role in the transcriptional level of this reprogramming. It's interesting to note that proteasomal degradation is closely related to hormone-induced transcriptional reprogramming in plants.

Salicylic acid (SA) and jasmonic acid (JA), the two primary hormones involved in immunity, are covered under this.

Salicylicacid:

The primary hormone involved in defense reactions against biotrophic infections is SA. The master transcriptional regulator non-expresser of PR genes 1 (NPR1) controls SA-mediated defensive responses. Through the CRL3NPR3/NRP4 receptor complex, SA perception closely regulates the levels of NPR1 protein.

The UPS targets NPR1 for ubiquitination mediated by CRL3NPR3 under baseline circumstances. Early SA buildup inhibits NPR3, which causes NPR1 to stabilize. In response, NPR4 functions as a kind of safety net by activating to cause NPR1 degradation when SA levels go too high. It has been demonstrated

that although primary CRL3 ubiquitination of NPR1 improves its activity, it also serves as a precursor to the ubiquitin chain elongation that is eventually mediated by UBE4 and results in NPR1 destruction. Further complicating the ubiquitin-mediated regulation of NPR1 turnover is the recent identification of two HECT E3s, ubiquitin-protein ligase 3/4 (UPL3 and 4), as regulators of NPR1. A separate mechanism for NPR1 degradation or evolutionary divergence between distinct plant lineages are indicated by the fact that rice OsNPR1 is likewise targeted for degradation by the UPS through the CRL4 complex (Fu et al., 2012) (Fig 5).

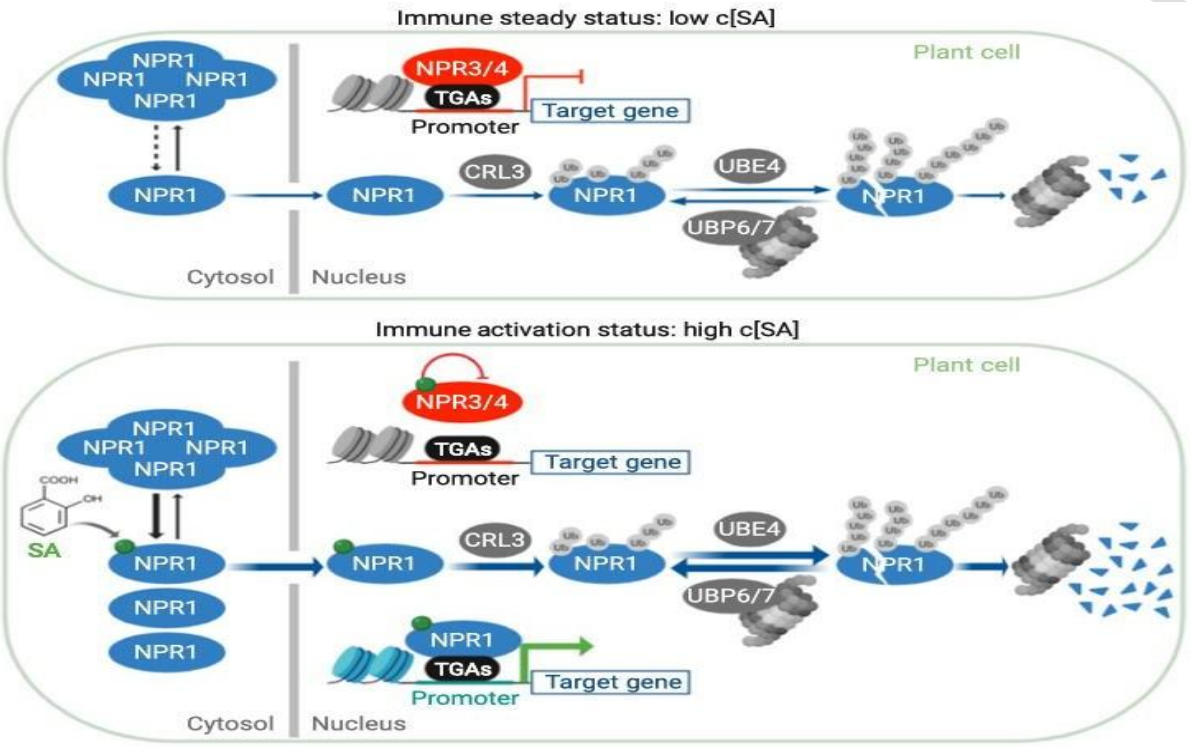

Fig5:SAdependenttranscriptionalreprogrammingagainstpathogensisfine-tunedinthenucleus by the 26Sproteasome (Wang and Zeng., 2022)

Jasmonicacid:

Defense reactions against necrotrophic infections are mediated by JA. The JAZ family of proteins, which primarily mediates transcriptional repression, is essential to JA signaling. According to Mallery et al. (2020), JAZ proteins are transcription factors (TFs) MYC2, 3, and 4's negative regulators. When a pathogen is detected, the SCFCOI1 complex senses JA accumulation in the nucleus. This leads to the ubiquitination/degradation of the JAZ repressor, which in turn derepresses the aforementioned MYCs. Conversely, COI1 is similarly susceptible to proteasomal degradation, indicating distinct functions of the UPS in JA signaling (Fig. 6).

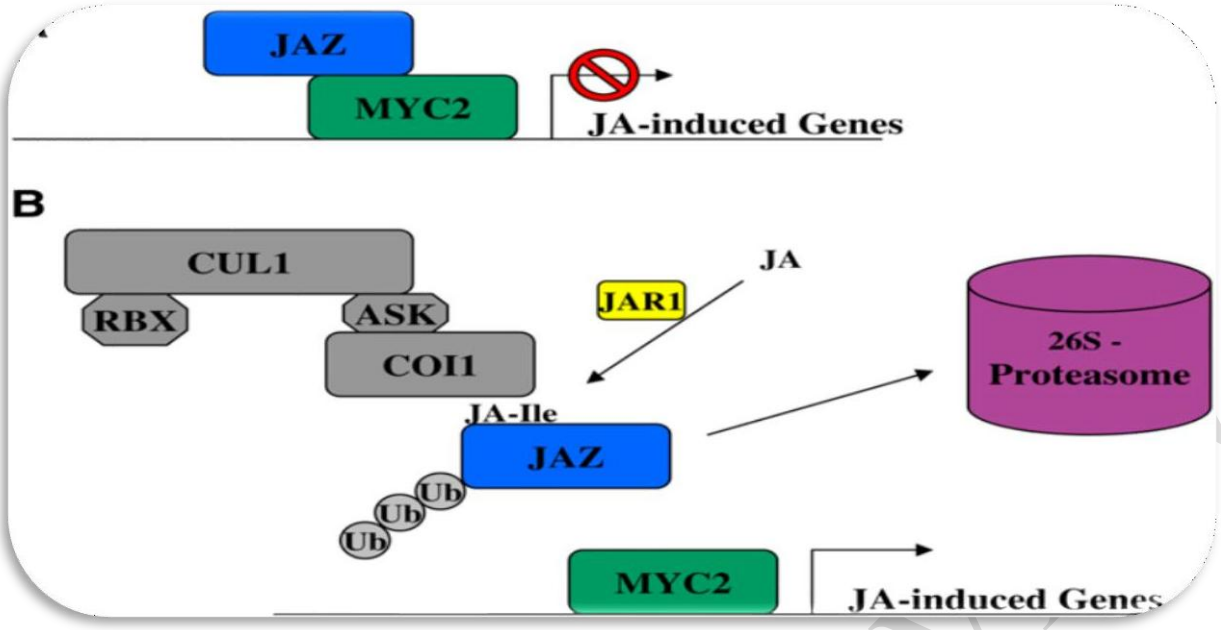

Fig6:JAdependenttranscriptionalreprogrammingagainstpathogensisfine-tunedinthenucleus by the 26Sproteasome (Fu *et al*., 2022**)**

Downstreamimmunecomponents:

Plant immunity is based on an intricate molecular network that combines internal (hormones) and exterior (pathogen detection) information to coordinate the corresponding defense responses. Controlling the quantity and activity of the proteins involved in these processes is crucial for this. Post-translational modifications (PTMs) like phosphorylation, acetylation, and ubiquitination play a major role in this regulation. The UPS uses ubiquitination to keep an eye on the targets' stability.

FunctionsofUbiquitination-RelatedGenesin HostDefense:

RoleofE1in HostDefense:

E1 ubiquitin-activating enzymes are essential to any ubiquitination reaction because they function in the initial phase of ubiquitination. There are two E1 enzymes in Arabidopsis: UBA1 and UBA2. These two enzymes have the ability to bind to ubiquitin and transmit it to enzymes that conjugate ubiquitin to E2. Goritschnig and colleagues discovered a mutation in UBA-1 (the mos5 allele of Uba1) that impacts the resistance provided by the snc1 npr1-1 double mutant. They did this by using a suppressor screen for the snc1 npr1-1 double mutant, which has increased resistance to an oomycete and bacterial disease in Arabidopsis. It's interesting to note that the mos5 single mutant exhibits varied susceptibility to avirulent strains of the pathogen but increased susceptibility to the virulent strain of Pseudomonas syringae pv. maculicola ES4326.

This shows that an E1 enzyme is involved in defense responses and that ubiquitination machinery is necessary for the activation or downstream signaling of certain R proteins. According to Goritznig et al. (2007), UBA1 may be involved in the ubiquitination-mediated activation of positive regulators of the snc-1-mediated resistance pathway or the degradation of regulatory proteins in that process.

RoleofE2inHostDefense:

It is unclear how E2 enzymes directly contribute to plant immunity. Due to the fact that both OsUBC5b and El5 were activated by an elicitor, it was discovered that two rice E2s (OsUBC5a, OsUBC5b) of the Ubc4/5 subfamily function as E2 to catalyze EL5-mediated ubiquitination. This suggests that EL5 and OsUBC5b have roles in the rice defense response through the turnover of protein(s) via the ubiquitin/proteasome system Goritschnig *et al*. (2007).

FunctionofPlantE3Ligasesin HostDefense:

E3 ligases have been the most extensively studied component of the ubiquitinationpathwayin plant host defense.

PlantE3LigasesinRGene-MediatedResistance:

"Although over 70 R genes in plants have been cloned, only a few of their defensepathwaysarerelatedtoubiquitination.Inyeasttwo-hybridscreens,RIN2andRIN3werefoundto interact with RPM1. RIN2 and RIN3 are both RING finger E3 ligases. Although RIN2 andRIN3areE3ligasesandinteractstronglywithRPM1,timeofdisappearanceofRPM1inwild-type Col-0 plants and in rin2rin3 double-mutant plants inoculated with DC3000 (avrRpm1)wasalmostidentical,indicatingthatRIN2andRIN3arenotrequiredfordegradationofRPM1but rather function as positive regulators of RPM1- mediated HR. Because rin2 rin3 plantsshowed a weaker HR than Col-0 but did not alter pathogen growth when inoculated withDC3000, the authors suspect that the RIN2/ RIN3 RING E3 ligases might act on a substratethatregulatesRPM1-dependent HR"(Shirsekar*etal*.2010).

E3ubiquitinligases:

The importance of E3s in regulating plant immunity is evident. Interestingly, some

ofthe E3s involved in immunity also undergo proteasomal degradation. This applies mostly toE3s from the PUB family. PUB22 dimerization and autoubiquitination lead to PUB22'sdegradation in basal conditions and are prevented upon infection through phosphorylation bymitogen-activatedprotein kinase3 (MAPK3).

Signallingandotherimmune-relatedproteins:

One important PTM governing signaling pathways, particularly those connected to defense, is phosphorylation. For example, the essential elements of PTI signaling are MAPK cascades. MAPK kinase 4 and 5 (MKK4 and 5) are ubiquitinated and then degraded by the E3 KEEP ON GOING (KEG). It's interesting to note that KEG appears to be weakened by fungus, confirming its function as the immune system's negative regulator.

Transcriptionfactors:

Several transcription factors (TFs) regulate a significant portion of the plant defensive responses at the transcriptional level. TFs are thus also targets of UPS during immunity modulation. The WRKY family, which is unique to plants, is a well-known family of these TFs. Numerous plant species have been reported to target various members of the WRKY family for proteasomal degradation.

Table1:PlantE3-ligasesinvolvedindefencemechanisms

Protein	**Organism**	**E3 ligasetype**	**Pathways**	**Pathogen**
ACIF1	*N. tabacum*	F-box		TMV
ACRE276	*N. tabacum*	U-box	Cf-genes mediatedHR	TMV
BAH1/NLA	*A. thaliana*	RING	SAsignalling	*P.syringae*
DRF1	*O.sativa*	F-box	ethylenes ignalling	*P.syringae*
RHC1	*O.sativa*	RING		*P.syringae*
PUB22,23,24	*A. thaliana*	U-box	PTI(negative regulation)	*P.syringae*
SPL11	*O.sativa*	U-box		*M.grisea*

Taken together, these data highlight the significance of immune-related protein proteasomal degradation as a regulatory mechanism in plant immunity. This control, which is present throughout the entire process—from perception to action—enables precisely calibrated, potent defensive reactions. This may account for their evolutionary spread throughout the plant lineage, since it ensures an enormous degree of adaptability to respond to shifting environmental conditions. It also demonstrates how susceptible the UPS and its constituent parts are to disruptions by entities capable of controlling these functions, which makes the UPS a prime target for plant diseases.

Microbial manipulation of the UPS is a prime target because of its conservation and significance in optimizing the plant defensive response. As infections only need to target one specific pathway in order to influence and disrupt the entire host system, targeting the proteasome and its constituent parts is a very effective technique to subvert cellular processes.

Perturbationofhostubiquitinsystemsbyplantpathogeneffectorproteins:

Plant pests and pathogens with evolved defense mechanisms weaken host cell defenses by entering host cells with effector proteins. Effector proteins are injected into cells by bacterial pathogens using type III or type IV secretion systems, whereas translocated filamentous pathogen effectors are most likely delivered by haustoria or other specialized structures like the biotrophic interfacial complex (BIC).

Insects and nematodes can also secrete effectors in their saliva. In animal and plant cells, the ubiquitination system has become a specific area of interest for effector protein activity during disease (Banfield, 2015). Effector proteins are used by eukaryotic and prokaryotic pathogens as well as plant pests to interfere with the host's ubiquitin system in order to facilitate colonization (Fig. 7).

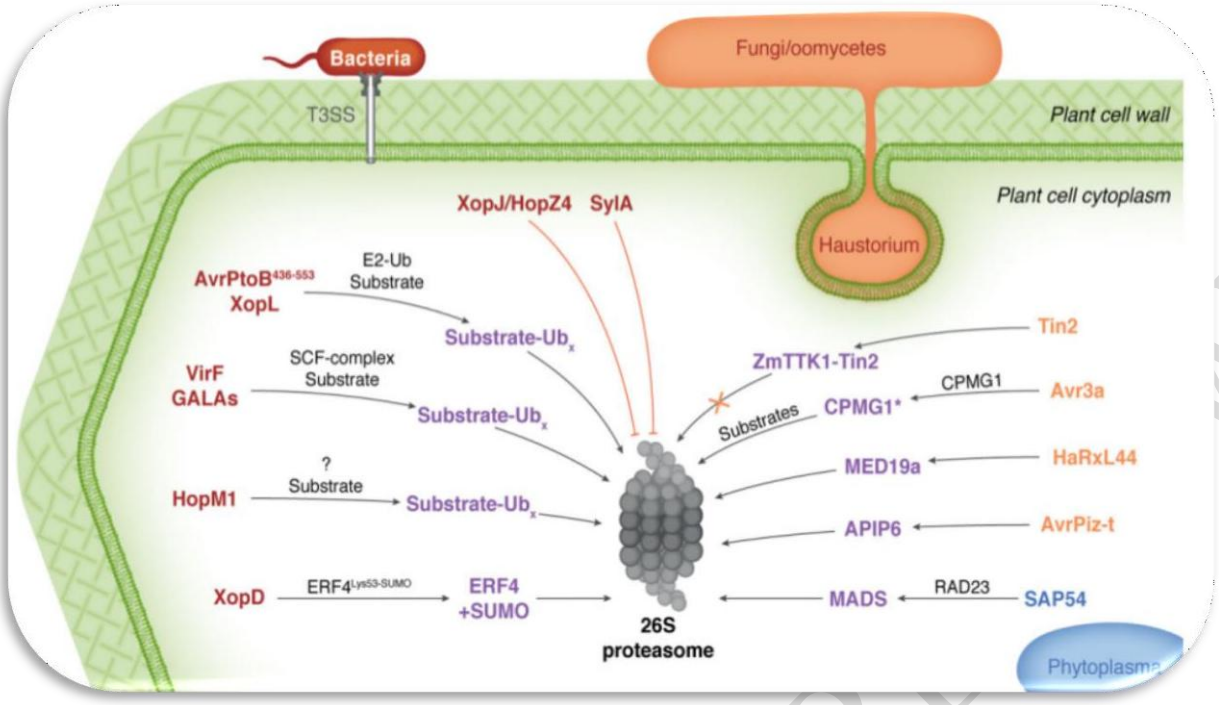

Fig.7:Overviewofplantpathogeneffectorsthatperturbhostubiquitinsystemsandtheiractivities (Denzer *et al*., 2020**)**

Table2:PathogenE3ligasesinvolvedinvirulencemechanisms

Protein	**Organism**	**E3 ligasetype**	**Interactingproteins**
AvrPtoB	*P.syringae*	RING/U-box	Fen,FLS2
CLINK	Fabanecroticyellows virus	F-box	SKP1
GALA1-7	*R.solanacearum*	F-box	ASK1 andASK2
HopM1	*P.syringae*	No knownstructure	AtMINproteins
P0	Beetwesternyellowsvirus, Cucurbit aphid-	F-box	ASK1,ASK2 andAGO1

	borneyellowsvirus		
VirF	*A.tumefaciens*	F-box	VIP1andVirE2

Plantpathogenstargettheubiquitin–proteasomesystemfortheirownbenefit:

Microbial manipulation of the UPS is a prime target because of its conservation and significance in optimizing the plant defensive response. As infections only need to target one specific pathway in order to influence and disrupt the entire host system, targeting the proteasome and its constituent parts is a very effective technique to subvert cellular processes. The pathogen gains an advantage when the host becomes disorganized and is unable to respond to foreign invaders. As a result, viruses evolved sophisticated techniques to target the host UPS both directly and indirectly. Currently, a wide range of plant pathogens, including bacteria, viruses, oomycetes, fungi, and nematodes, employ distinct strategies to alter the UPS for their own advantage.

BacteriahijackinghostUPS:

Plant-pathogenic bacteria have developed a highly conserved type III secretion system (T3SS) to introduce so-called type III effectors (T3Es) into the host cell, circumventing plant defense mechanisms. Targeted to multiple cellular compartments, these T3Es serve as crucial factors that facilitate the course of illness by attenuating diverse plant immunological responses. Based on available data, it appears that T3Es subvert cellular processes in the host system by focusing on more central systems like the UPS (Fig 8).

Early research in the field of effector biology has concentrated on effectors that imitate host eukaryotic proteins. AvrPtoB from P. syringae, which exhibits E3 ligase activity in plants, is one of the most researched effectorproteins. Since the 26S proteasome needs its E3 ligase activity to ubiquitinate and degrade host targets, AvrPtoB is the first effector to hijack the UPS in order to support its virulence role during bacterial infection.

It has been demonstrated that the bacterial effector AvrPtoB interacts with a number of host cell E2 ligases to facilitate the degradation of several PRRs, including CERK1, FLS2, and other PRR-associated proteins like BAK1 and BIK1. This is a prime illustration of how an effector subverts plant defense responses by focusing on the housekeeping recycling mechanism of PRRs in order to hijack and deceive host cellular processes. AvrPtoB has been found to target a wide range of different host targets for

degradation; among them, the SA master regulator NPR1 is one of the most important for plant immunity.The 26S proteasome constitutively degrades NPR1 in the nucleus to ensure a proper SA signaling. This trait is used by the T3E AvrPtoB, which targets NPR1 in a manner dependent on SA. NPR1 is degraded upon association with both proteins; this most likely occurs in the cytoplasm prior to transport into the nucleus. As a result, AvrPtoB has the capacity to reduce PTI and SAR reactions in addition to SA-mediated defense responses. According to Langin et al. (2023), T3E AvrPtoB is not the only bacterial E3 ligase that targets plant immunity by destroying PRRs or master regulators.

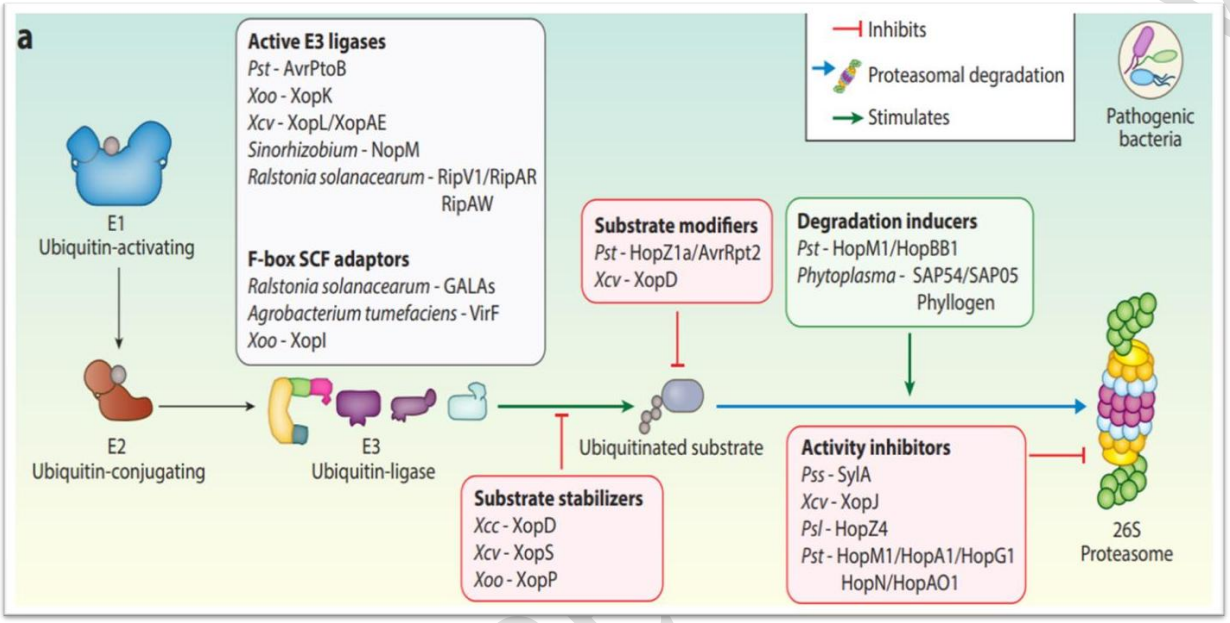

Fig 8: Bacteria use different effector proteins to either degrade or stabilize host targetsthroughdifferent mechanisms (Bonnet *et al.,* 2023**).**

The E3 ligase XopK from Xanthomonas oryzae is another T3E that targets a PRR. Rice OsSERK2 is degraded by the 26S proteasome by interaction with XopK and ubiquitination. Eliminating OsSERK2 makes it possible to effectively inhibit a number of PRR signaling pathways, making it a desirable target for virulence promotion. Some discovered T3Es are not physically similar to known eukaryotic E3 enzymes, despite AvrPtoB's structural homology to RING E3s. A family of bacterial E3s with a unique E3 ligase (NEL) domain is one of them. Leucine-rich repeats (LRRs) are present in the N-terminal domain of the majority of proteins that belong to the NEL family structurally.

The NEL domain, which has a catalytic cysteine residue necessary for the creation of E3-ubiquitin intermediates and E2-ubiquitin complexes, is located in the C-terminal region. Xanthomonas proliferates in plants more readily when autophagic degradation is disrupted by the removal of SH3P2. It's interesting to note that XopL experiences autoubiquitination in plants as well, which could lead to effectorphagy—the autophagic destruction of XopL. This represents yet another instance of the pathogen-plant

evolutionary arms race, wherein XopL may have developed the ability to degrade SH3P2 in order to impede its own removal and maintain its ability to display its virulence function within the host system.

Mechanisms of manipulation of host proteasomes by plant pathogens

- **Mimicking as host E3 ligases**

Xanothomonas campestris pv. *vesicatoria* – XopL -- E2 conjugating enzymes

- **Mimicking as host F-box proteins**

Ralstonia solanacearum -- GALA proteins – SCF complexes

- **Interfering with vesicle trafficking**

Pseudomonas syringae -- HopM1 -- *At*MIN7(vesicle trafficking and the deposition of callose)

- **Inhibition of proteasome activity**

Xanothomonas campestris pv. *vesicatoria* -- XopJ-- RPT6 (subunit of the 19S RP)

- **Promoting transcription factor degradation**

Phytoplasmas -- SAP54 -- RAD23 (MADS-box transcription factors)

- **Stabilizing a U-box protein**

Phytophthora infestans – RXLR type effector AVR3a -- Ubox E3 ligase protein CMPG1

- **Masking of an ubiquitin-proteasome degradation motif**

Ustilago maydis -- Tin2 -- ZmTTK1(stability of the kinase in a 26S proteasome)

- **Suppressing the activity of an E3 ligase**

Magnaporthe oryzae – AvrPiz -- E3 ligase APIP6

TargetingUPSforantimicrobialstrategies:

A new field of study that shows promise for creating innovative treatments for microbial infections is targeting the ubiquitin-proteasome system (UPS) with antibacterial methods. The UPS has been extensively studied in relation to eukaryotic cells, but more recent research indicates that it is also crucial for controlling microbial pathogenesis and host-pathogen interactions. Potential strategies for targeting theUPS in antimicrobial effortsare

1. **InhibitionofPathogenUPSComponents:**

Targeting and identifying particular UPS components in microbial pathogens may interfere with their capacity to overcome host defenses and proliferate inside host cells. This might result in the

creation of antibiotics that selectively block the pathogen's UPS without influencing the host UPS.

2. **ModulationofHostUPStoEnhanceImmuneResponse:**

One tactic to fight infections might be to modulate the UPS to enhance the host immune response. The host's capacity to eradicate infections may be enhanced, for instance, by encouraging the breakdown of proteins that obstruct immunological signaling or by enhancing the presentation of antigens for identification by immune cells.

3. **CombinationTherapieswithExistingAntimicrobials:**

Using UPS-targeting medicines in conjunction with currently available antimicrobial medications may improve treatment outcomes. This strategy may aid in overcoming drug resistance and enhancing antimicrobial treatments' general efficacy.

4. **DevelopmentofProteasomeInhibitors:**

Proteasome inhibitors could be used for antimicrobial purposes; they have been thoroughly researched in the context of cancer treatment. These inhibitors may interfere with the UPS in pathogens as well as host cells, causing the latter to reproduce intracellularly or causing diseased cells to die selectively.

5. **InterferencewithBacterialProteinDegradationSystems:**

Certain bacteria, including ClpXP and ClpAP proteases, have their own mechanisms for breaking down proteins. It is possible to interfere with the UPS-like processes in bacteria or modify these bacterial proteolytic systems, which could prevent the bacteria from surviving and spreading illness.

6. **TargetingUPSinBiofilmFormation:**

Conventional antimicrobial treatments are frequently ineffective against microbial biofilms. One tactic to break up these structures and increase the bacteria' susceptibility to antibiotics would be to target the UPS that is involved in the creation and maintenance of biofilms.

7. **Host-DirectedTherapies(HDTs):**

HDTs work to alter host cell activities in order to create an atmosphere that is detrimental to the survival of microorganisms. As a host-directed antibacterial strategy, manipulating the host UPS to restrict the availability of critical components for pathogen reproduction could be investigated.

8. **ViralInfectionsandUPSModulation:**

Viruses frequently use the host UPS to facilitate their growth and avoid detection by the immune system. Creating antiviral tactics that specifically target UPS components implicated in viral infection may prove to be a viable approach.

Pros:

RegulationofHost-PathogenInteractions: It is possible to change the regulation of proteins involved in host-pathogen interactions by manipulating the plant UPS.

Targeted Protein Degradation: The UPS plays a role in the deliberate breakdown of certain proteins. Plant-microbe interactions may be modulated for desired effects by focusing on key proteins associated with microbial infection or symbiosis.

UnderstandingMolecularMechanisms: Studying the plant UPS in relation to microbial manipulation sheds light on the molecular processes that underlie symbiosis or host defense. This information can help us comprehend plant-microbe interactions on a deeper level.

Biotechnological Applications: The knowledge obtained from modifying the plant UPS may find use in biotechnology. This includes the creation of genetically modified crops that are more resilient to diseases or have a better capacity to build advantageous relationships with microorganisms.

Cons:

1. **UnintendedConsequences:**ManipulatingtheUPSmayhaveunintendedconsequences due to the complex regulatory networks it governs. Changes in the UPScouldaffectothercellularprocesses,leadingtounforeseenoutcomesorcompromisingnormalplant functions.
2. **Specificity Challenges:** The UPS is involved in the degradation of a wide range ofproteins. Achieving specificity in targeting only the desired proteins associated

withmicrobialinteractionscanbechallenging,potentiallyresultinginoff-targeteffects.

3. **EcologicalImpact:**IntroducinggeneticallymodifiedplantswithalteredUPSfunctioninto natural ecosystems may have ecological consequences. Changes in the plant-microbeinteractions could impacttheoverall balanceof theecosystem.
4. **Ethical and Regulatory Concerns:** The use of genetic modification techniques totargettheUPSraisesethicalconsiderationsregardingthereleaseofgeneticallymodified organisms into the environment. There are also regulatory challenges relatedtothesafety andenvironmental impact ofmodifiedplants.
5. **Evolutionary Responses:** Microbes may adapt to changes in the host UPS over time,potentially leading to the development of resistant strains or unintended consequencesinmicrobial communities.

Conclusion:

Host–pathogeninteractionsrepresentanever-endingarmsracebetweenhostorganismsdefending against unwanted invaders and pathogens counteracting the host defence system.“Emergingevidencesuggeststhat theUPSisakeypillaroftheplantimmunesystem.TheUPS governs the turnover of many immune-related components and hence constitutes a vulnerabletarget for pathogens. As such, diverse plant pathogens employ sophisticated strategies tomanipulate the UPS and combat plant immune reactions. Pathogen effectors can induce orblockthedegradationoftargetproteinsinvolvedinimmunereactions.Inthiscontext,effectorscans erveastoolstodissecthowtheUPSisinvolvedinplant–pathogeninteractions” [12].“Pathogenalteration of the host ubiquitination machinery mainly occurs at the step of ubiquitin-substrateligation, whereas activation and conjugation of ubiquitin appears unaffected. This mightindicateatsomelimitationsofpathogensco-optingtheubiquitinationsystem,e.g.requirementsto modify a specific step of the targeted pathway. Interference with host ubiquitination by activationorinhibitioniscommontoplantandanimalpathogensandthereforeitisofgreatinterest to better understand the molecular mechanisms underlying infectious diseases acrosskingdomsalso” [20].“CurrentknowledgeimpliesthattheUPSacts asadouble-edgedsword:ononehand, the UPS is required to maintain efficient plant defence responses; on the other hand,pathogens require its proper function to promote pathogenicity. In addition,

different effectorsfrom the same pathogen can display contrasting functions, which could be partially explainedby their distinct spatiotemporal modes of action. To gain a better understanding, it will becrucial to decipher the fine-tuning of the proteasome and its components on every level: fromtranscriptionandtranslationtotheassemblyoftheproteasomeanditsassociatedcomponents" [21].

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