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Solo or in Concert: SUMOylation in Pathogenic Fungi

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SUMOylation plays a pivotal role in DNA replication and repair, transcriptional stability, and stress response. Although SUMOylation is a conserved post-translational modification (PTM) in eukaryotes, the number, type, and function of SUMOylation-associated components vary among mammals, plants, and fungi. SUMOylation shares overlapping features with ubiquitination, another well-known PTM. However, comparative studies on the interplay between these two PTMs are largely limited to yeast among fungal species. Recently, the role of SUMOylation in pathogenicity and its potential for crosstalk with ubiquitination have gained attention in fungal pathogens. In this review, we summarize recent findings on the distinct components of SUMOylation across organisms and describe its critical functions in fungal pathogens. Furthermore, we propose new research directions for SUMOylation in fungal pathogens, both independently and in coordination with other PTMs. This review aims to illuminate the potential for advancing PTM crosstalk research in fungal systems.

Keywords **:** pathogenic fungi, post-translational modifications (PTMs), PTMs crosstalk, small ubiquitin-like modifier (SUMO), ubiquitin

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Post-translational modifications (PTMs) induce the generation of diverse proteins with various functions by adding polypeptides or functional groups to proteins (Leach and Brown, 2012). In eukaryotes, PTMs regulate multiple processes such as apoptosis, transcriptional regulation, DNA repair, cell cycle, and protein interactions (Enserink, 2015; He et al., 2017; Oh et al., 2012; Zhao, 2007). Among these PTMs, ubiquitination is the most vigorously studied, primarily for its role in targeting proteins for degradation through the ubiquitin-proteasome system (Dielen et al., 2010). In addition, proteins similar in structure to ubiquitin have been identified and are termed ubiquitin-like modifiers (UBLs) (Kerscher et al., 2006).

The small ubiquitin-like modifier (SUMO) is one of the UBLs that has garnered significant attention in recent years. As suggested by its full name, SUMOylation shares many hallmarks with ubiquitination. During SUMOylation, SUMO forms an isopeptide bond with lysine (K) residues on substrate proteins through a sequential process involving E1-activating enzymes (E1), E2-conjugating enzymes (E2) and E3 ligases (E3) (Gill, 2004). SUMO is initially synthesized as an inactive precursor. After the exposure of its C-terminal di-glycine residues by a SUMO hydrolase, SUMO is activated in an ATP-dependent manner by binding to a cysteine (C) residue on E1. SUMO is serially transferred to C residues on E2 and to the substrates, either with or without the mediation of E3. SUMO is subsequently removed from modified substrates by SUMO isopeptidase and recycled in this reversible pathway (Fig. 1) (Enserink, 2015; Gill, 2004; Morrell and Sadanandom, 2019).

Unlike ubiquitination, SUMOylation utilizes a heterodi-

Table 1. SUMOylation-associated components in *Homo sapiens*, *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, and *Magnaporthe oryzae*

Class of components	*H. sapiens*	*A. thaliana*	*S. cerevisiae*	*M. oryzae*
SUMO	SUMO1[a]	SUM1[d]	Smt3[e]	MoSmt3[f]
	SUMO2[a]	SUM2[d]		
	SUMO3[a]	SUM3[d]		
	SUMO4[b]	SUM4[d]		
	SUMO5[c]	SUM5[d]		
		SUM6[d]		
		SUM7[d]		
		SUM8[d]		
E1	Sae1[g]	Sae1a[h]	Aos1[i]	MoAos1[f]
	Sae2[g]	Sae1b[h]	Uba2[i]	MoUba2[f]
		Sae2[h]		
E2	Ubc9/Ube2I[j]	Sce1[k]	Ubc9[l]	MoUbc9[f]
E3	Pias1[m]	Siz1[s]	Siz1[u]	MoSiz1[y]
	Piasxα[m]	Mms21/Hpy2[t]	Siz2/Nfi1[v]	MoSiz2[f]
	Piasxβ[m]		Mms21[w]	MoMms21[f]
	Pias3[m]		Cst9/Zip3[x]	
	Piasy[m]			
	Kap1[n]			
	Cbx4/Pc2[o]			
	Mms21[p]			
	Ranbp2[q]			
	Hdac4[r]			
E4	Znf451[z]	Pial1[aa]		
		Pial2[aa]		
Protease	Senp1[bb]	Ulp1a/Els1[ee]	Ulp1/Pli1[mm]	MoUlp1[y]
	Senp2[bb]	Ulp1b/Els2[ee]	Ulp2[nn]	MoUlp2[y]
	Senp3[bb]	Ulp1c/Ots2[ff]	Wss1[oo]	MoWss1[y]
	Senp5[bb]	Ulp1d/Ots1[gg]		
	Senp6[bb]	Esd4[hh]		
	Senp7[bb]	Asp1/Spf1[ii]		
	Desi1[cc]	Spf2[ii]		
	Desi2[cc]	Desi1[jj]		
	Uspl1[dd]	Desi2A[jj]		
		Desi2B[jj]		
		Desi3A[jj]		
		Desi3B[jj]		
		Desi3C[jj]		
		Desi4A[jj]		
		Desi4B[jj]		
		Fug1[kk]		
		Ubp6[ll]		
		Ubp7[ll]		

[a]Saitoh and Hinchey (2000). [b]Wang and She (2008). [c]Liang et al. (2016). [d]Kurepa et al. (2003). [e]Seufert et al. (1995). [f]Lim et al. (2018). [g]Gong et al. (1999). [h]Castaño-Miquel et al. (2013). [i]Johnson et al. (1997). [j]Bernier-Villamor et al. (2002). [k]Saracco et al. (2007). [l]Johnson and Blobel (1997). [m]Schmidt and Müller (2002). [n]Liang et al. (2011). [o]Kagey et al. (2003). [p]Potts and Yu (2005). [q]Tatham et al. (2005). [r]Zhao et al. (2005). [s]Catala et al. (2007). [t]Ishida et al. (2012). [u]Takahashi et al. (2001). [v]Johnson and Gupta (2001). [w]Zhao and Blobel (2005). [x]Cheng et al. (2006). [y]Liu et al. (2018). [z]Eisenhardt et al. (2015). [aa]Tomanov et al. (2014). [bb]Mendes et al. (2016). [cc]Shin et al. (2012). [dd]Schulz et al. (2012). [ee]Hermkes et al. (2011). [ff]Colby et al. (2006). [gg]Cheng et al. (2006). [hh]Murtas et al. (2003). [ii]Liu et al. (2017). [jj]Orosa et al. (2018). [kk]Lois (2010). [ll]Morrell and Sadanandom (2019). [mm]Li and Hochstrasser (1999). [nn]Li and Hochstrasser (2000). [oo]Balakirev et al. (2015).

mer as E1, and E3 is not essential for modification and target selection but rather enhances modification efficiency when non-covalently bound to substrates (Gill, 2004; Morrell and Sadanandom, 2019). The number of SUMOylation components is smaller than in ubiquitination, with two E1s, fewer than 40 E2s, and fewer than 600 E3s (Salas-Lloret and González-Prieto, 2022) (Table 1). While SUMOylation has a well-defined consensus binding motif (hydrophobic amino acids-K-x-D/E, minimal K-x-D/E, and inverted D/E-x-K), ubiquitination lacks a specific ubiquitin binding motif for lysine selection (Gill, 2004; Trulsson and Vertegaal, 2022). Interestingly, even the human cyclin-dependent kinase inhibitor p16, which has no K residue, undergoes ubiquitin conjugation at its N-terminus, leading to its degradation (Kelsall, 2022). In ubiquitination, the E4 elongase and mono-, multi-, and poly-ubiquitination play distinct roles, with specific K residues in ubiquitin (K6, K11, K27, K29, K33, K48, and K63) forming polyubiquitin chains. Mono-ubiquitination is vital for histone regulation, while poly-ubiquitination is responsible for proteasomal degradation and endocytosis (Kocaturk and Gozuacik, 2018; Ronai, 2016). SUMO E4 elongases, such as PIAL1, PIAL2, and ZNF451, have been reported in *Arabidopsis thaliana* and *Homo sapiens* and are known to enhance SUMO chain formation (Eisenhardt et al., 2015; Hoppe, 2005; Li and Ye, 2008; Matic et al., 2008; Tomanov et al., 2014). However,

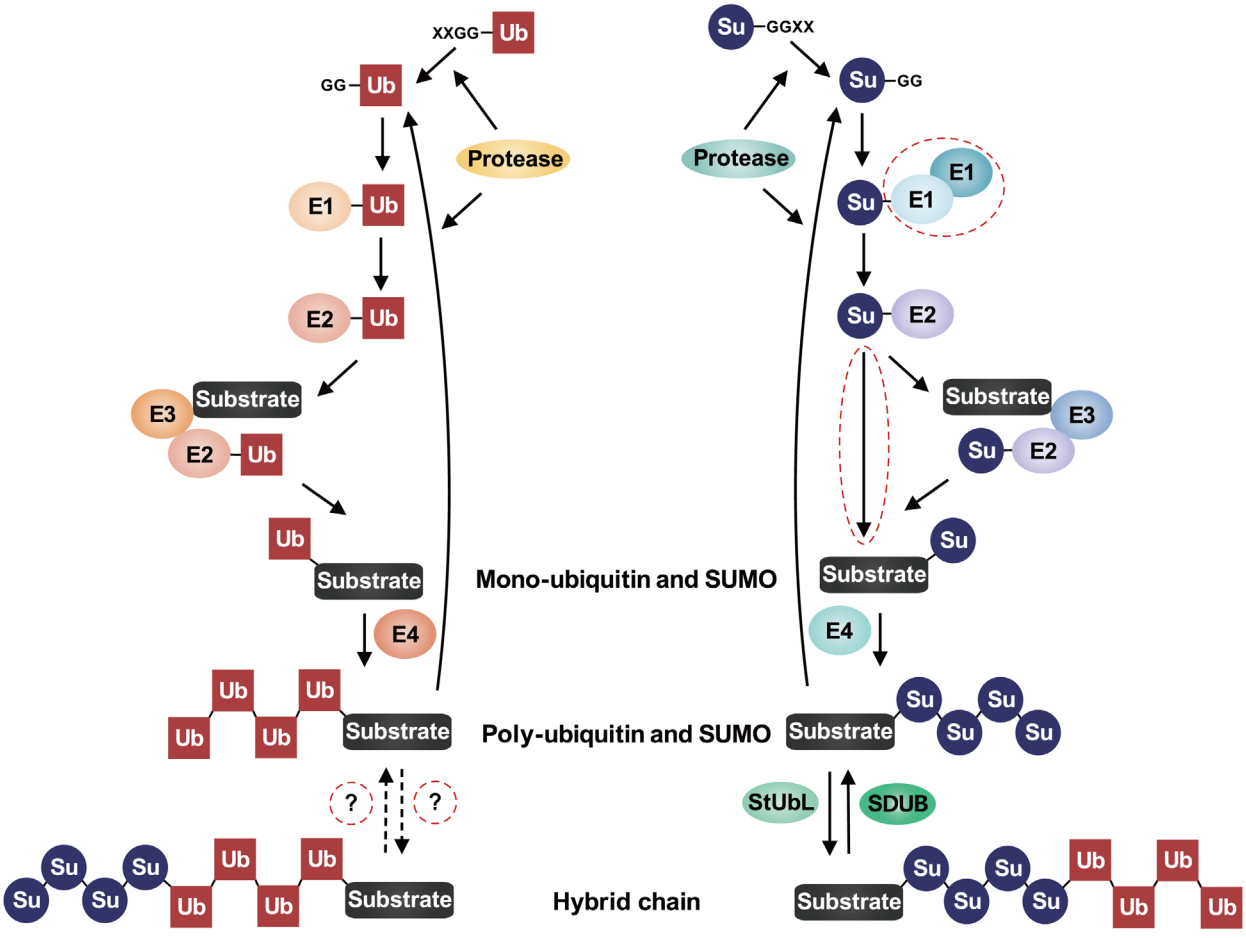

Fig. 1. Reversible ubiquitination and SUMOylation pathways and linkage types of ubiquitin and SUMO on substrates. Schematic illustration of the sequential ubiquitination and SUMOylation pathways. The enzymes E1, E2, and E3 are essential for the modification of substrates by ubiquitin, while the E4 enzyme is only involved in poly-chain formation on the substrates. Proteases have dual hydrolase and isopeptidase functions. Most SUMOylation processes are similar to the ubiquitination pathway, but, unlike ubiquitination, E1 has activity as a heterodimer and E3 is not crucial for SUMOylation. Moreover, the SUMO-ubiquitin hybrid chain is well understood, but ubiquitin-SUMO hybrid chain formation has not been clearly elucidated. Red dotted circles indicate differences between SUMOylation and Ubiquitination pathways. Ub, ubiquitin; Su, SUMO; E1, E1-activating enzymes; E2, E2-conjugating enzyme; E3, E3 ligase; E4, E4 elongase; STUbL, SUMO-targeted ubiquitin ligase; SDUB, SUMO deubiquitinase.

the specific functions of mono- and poly-SUMOylation remain to be clearly elucidated (Fig. 1).

Ubiquitin and SUMO were first discovered in 1975 and 1995, respectively, and have since been extensively studied in model organisms such as human, mice, *A. thaliana*, and yeast (Celen and Sahin, 2020; Goldstein et al., 1975; Meluh and Koshland, 1995). However, compared to ubiquitination, SUMOylation remains less understood across many species, including fungal pathogens. Due to the above-described differences, ubiquitination and SUMOylation have often been studied as separate pathways (Gill, 2004). Recently, studies have begun exploring potential interactions between SUMOylation and ubiquitination (Lamoliatte et al., 2017; Lim and Lee, 2020; Parker and Ulrich, 2012). In this review, we summarize the current understanding of SUMOylation's role in mammalian and plant fungal pathogens and provide future perspectives on the crosstalk between ubiquitination and SUMOylation in fungi.

Differences on SUMOylation Components among Mammals, Plants, and Fungi

The roles and components of SUMOylation in mammals and plants are well-documented, regarding their involvement in abiotic stress responses and immune responses during infection by pathogenic fungi (Augustine et al., 2016; Benlloch and Lois, 2018; Colignon et al., 2017; Enserink, 2015; Morrell and Sadanandom, 2019; Verma et al., 2018; Yang et al., 2017). In fungi, the functional roles of these SUMOylation components have been vigorously studied in *Saccharomyces cerevisiae* (Esteras et al., 2017). However, the SUMOylation pathway in pathogenic fungi remains less understood. Compared to mammals and plants, fungi generally possess fewer SUMOylation-associated components (Table 1).

E1-Activating Enzyme, SUMO, E3 Ligase, and E4 Elongase

In most species, the E1 enzyme gains activity through the formation of a heterodimer with two proteins (Sae1/Aos1-Sae2/Uba2), except in certain plant species (Enserink, 2015; Gong et al., 1999; Lim et al., 2018). In plants such as *A. thaliana* and some *Brassicaceae*, two types of E1 heterodimers (Sae1a-Sae2 or Sae1b-Sae2) exist due to a duplication of Sae1 (Castaño-Miquel et al., 2013). These two Sae1 isoforms, Sae1a and Sae1b, are not fully redundant. Sae1a has higher SUMO conjugation efficiency and stronger thioester binding to Sae2-SUMO than Sae1b (Castaño-Miquel et al., 2013).

In *H. sapiens*, five SUMO isoforms are present. SUMO1 is associated with mono-SUMOylation and terminates poly-chain formation, while SUMO2, SUMO3, SUMO4, and SUMO5 are involved in poly-SUMOylation (Liang et al., 2016; Saitoh and Hinchey, 2000; Wang and She, 2008). *A. thaliana* has eight SUMO isoforms. SUM1 and SUM2 participate in poly-SUMOylation, whereas SUM3 lacks the motif required for SUMO chain formation. In addition, SUM5 is expressed, though its functions are still undefined, while SUM4, SUM6, SUM7, and SUM8 are not expressed and lack the di-glycine residues essential for SUMOylation (Budhiraja et al., 2009; Colby et al., 2006; Kurepa et al., 2003; Roy and Sadanadom, 2021). In contrast, fungi have only a single SUMO protein (Lim et al., 2018). It is speculated that the diversity of SUMO isoforms in higher organisms emerged to handle the functional specialization required for both mono- and poly-SUMOylation, allowing PTM to target a broader range of proteins.

All organisms possess at least one E3 ligase that contains the characteristic SP-RING enzymatic motif (Jmii and Cappadocia, 2021). However, due to their role in the specific recognition of target substrates, E3 ligases exhibit much lower interspecies sequence similarity compared to SUMO, E1-activating enzymes (E1), and E2-conjugating enzyme (E2) (Wang et al., 2022). While *Candida glabrata* and *H. sapiens* show high sequence similarity for SUMO, E1s, and E2, their E3 ligase similarity was significantly lower (Gupta et al., 2020). Furthermore, although homologs of the *S. cerevisiae* E3 ligase MMS21 are predicted to exist in the rice blast fungus, *Magnaporthe oryzae* and *A. thaliana*, they are absent in *Oryza sativa* (Lim et al., 2018).

A new type of SUMOylation-associated component, E4 elongase, has been identified in humans and plants. E4 elongase facilitates the formation of poly-SUMO chains on substrate proteins (Eisenhardt et al., 2015; Tomanov et al., 2014). In humans, ZNF451 has dual functions with both E3 ligase and E4 elongase activities, requiring a zinc-finger domain and SUMO-interacting motifs (SIMs) for its function (Eisenhardt et al., 2015). In *A. thaliana*, the SP-RING domain, which includes a zinc-finger MIZ-type (Zf-MIZ) domain (IPR004181), along with SIM1, is essential for the E4 elongase activities of PIAL1 and PIAL2. Siz1, an E3 ligase in *A. thaliana*, also contains a Zf-MIZ domain and has overlapping functions with PIAL1 and PIAL2. However, while PIAL1 and PIAL2 are involved in SUMO chain extension, Siz1 exclusively facilitates SUMO conjugation (Tomanov et al., 2014). In fungi, E4 elongase is absent, instead, the E2-conjugating enzyme Ubc9 contributes to SUMO chain formation through non-covalent interactions (Klug et al., 2013). However, the precise mechanism of

poly-SUMO chain assembly is still unclear in fungi. In humans, SENP6 and SENP7 play specific roles in editing SUMO2/3 chains (Nayak and Müller, 2014). To better understand the function of poly-SUMOylation, ongoing proteomic research in humans are identifying targets of chain-specific isopeptidase SENP6. SENP6-targeted substrates participate in DNA repair, cell cycle progression, and chromosome segregation (Keiten-Schmitz et al., 2019; Liebelt et al., 2019; Wagner et al., 2019). In addition, SENP7 is associated with chromatin remodeling and dynamics (Garvin et al., 2013). Unlike ubiquitination, the functions according to different chain types on lysine residues have not been studied in detail. However, poly-SUMOylation is believed to play a role in DNA-related processes (Liebelt et al., 2019).

Roles of SUMOylation in Mammalian Fungal Pathogens

The fungi *C. albcians*, *C. glabrata*, and *Aspergillus nidulans* are the most medically significant pathogens affecting mammals. *Candida* species and *A. nidulans* represent yeast and filamentous forms of opportunistic fungal pathogens, respectively, causing mucosal candidemia and pulmonary aspergillosis in immune deficiency patients (Grosset et al., 2016; Paulussen et al., 2017). In *Candida albicans*, SUMOylation components including a single SUMO (Smt3), two E1-activating enzymes (Aos1 and Uba2), one E2-conjugating enzyme (Ubc9), and one E3 ligase (Mms21) are crucial for cell differentiation and responses to DNA damage and cell wall integrity stress, but they are not essential for viability (Islam et al., 2019; Leach et al., 2011). In addition, the E3 ligase Wos1 regulates CO_2-induced switching from white to purple cell forms (Yan et al., 2015). In *C. glabrata*, one E3 ligase (Siz2) and one protease (Ulp2) are required for growth, DNA damage and oxidative stress tolerance, repression of pseudohyphal structure formation, and SUMOylation. In particular, Ulp2 plays a critical role in fungal virulence (Gujjula et al., 2016). In *A. nidulans*, SUMOylation components, including one SUMO (SumO), two E1-activating enzymes (AosA and UbaB), one E2-conjugating enzyme (UbcN), one E3 ligase (SizA), and two SUMO proteases (UlpA and UlpB), are important for growth, asexual and sexual reproduction, and responses to DNA damage, but they are not essential for survival (Harting et al., 2013; Wong et al., 2008).

SUMOylation substrates as well as their roles have been identified in mammalian fungal pathogens (Horio et al., 2019; Islam et al., 2019; Leach et al., 2011). A large fraction of SUMOylation substrates are associated with stress response, cell cycle progression, cytoskeleton organization, polarized growth, endocytosis, and nuclear segregation in *C. albicans* (Islam et al., 2019; Leach et al., 2011). Similarly, in *A. nidulans,* most SUMOylated substrates are involved in transcriptional regulation, RNA maturation, DNA replication and repair, and cell cycle progression (Horio et al., 2019). Furthermore, some SUMOylation components (such as E1-activating enzymes, E2-conjugating enzymes, and E3 ligases) and ubiquitination components (such as proteasome subunits and E1-activating enzymes) have also been identified as SUMO-modified (Harting et al., 2013; Horio et al., 2019).

Roles of SUMOylation in Plant Fungal Pathogens

The functions of SUMOylation components and the profiling of SUMOylated proteins have been investigated relatively recently in plant pathogenic fungi. *Magnaporthe oryzae* is one of the most critical plant pathogens that causes severe blast disease in rice and wheat (Dean et al., 2012; Malaker et al., 2016). In *M. oryzae*, deletion mutants of one SUMO (MoSmt3), two E1 enzymes (MoAos1 and MoUba2), one E2 enzyme (MoUbc9), one E3 enzyme (MoSiz1) exhibit pleiotropic phenotypes, including defects in conidiation, septum formation, tolerance to stress, pathogenicity, and SUMOylation (Lim et al., 2018; Liu et al., 2018). In addition, SUMOylation of four septins is essential for fungal virulence and proper septins arrangement in appressoria. Furthermore, SUMOylation is critical for the localization of both apoplastic and cytoplasmic effectors in *M. oryzae*, as well as the localization and stability of the nuclear effector, MoHTR1 (Lim and Lee, 2020; Lim et al., 2024; Liu et al., 2018). In *Aspergillus flavus*, SUMO is important for growth, cell separation, stress sensitivity, asexual and sexual reproduction, aflatoxin production, and pathogenicity, but it is not essential for viability (Nie et al., 2016). In the watermelon Fusarium wilt fungus, *Fusarium oxysporum* f. sp. *niveum*, deletion mutants of FonUBA2 (E1 enzyme) and FonSIZ1 (E3 enzyme) are lethal, whereas deletion mutants of FonSMT3 (SUMO), FonAOS1 (E1 enzyme), FonUBC9 (E2 enzyme), and FonMM21 (E3 enzyme) are viable. These SUMOylation components are also pleiotropic and involved in growth, asexual reproduction, stress sensitivity, and apoptosis (Azizullah et al., 2023). In *F. graminearum*, a causal agent of Fusarium head blight, SUMO (FgSMT3), E1 enzymes (FgAOS1 and FgUBA2), E2 enzyme (FgUBC9), proteases (FgULP1 and FgULP2) are particularly important for pathogenicity and DNA damage response (DDR). SUMOylation of FgSR, a transcription factor, is responsible for DDR regulation,

nuclear localization, and phosphorylation by kinases under DNA damage stress (Jian et al., 2022). *Botrytis cinerea,* which infects various fruits, also relies on SUMOylation components, including SUMO (BcSmt3), E1 enzymes (BcAos1 and BcUba2), E2 enzyme (BcUbc9), and protease (BcUlp1), for mycelial growth and pathogenicity. SUMOylated BcSsb, a heat shock protein, stabilizes microtubules and enhances cold tolerance. SUMOylated BcRad18, an E3 ubiquitin ligase, modulates ubiquitination on proliferating cell nuclear antigen (PCNA) (sliding clamp protein) and the response to oxidative DNA damage (Shao et al., 2023). The soil-borne fungus *Verticillium dahliae* deploys the protease VdUlpB to facilitate virulence by deconjugating SUMO from enolase (VdEno). SUMOylated VdEno is important for the transcription of the effector VdSCP8 (Wu et al., 2023).

The SUMOylation substrates of *M. oryzae* mostly involved in ribosome biogenesis, nutrient utilization, and stress responses. In addition, a significant number of previously identified pathogenesis-associated proteins, including Hsp70 family members, components of the UPR machinery, and reactive oxygen species-scavenging enzymes, are also present in identified SUMOylated substrates (Liu et al., 2018).

Crosstalk between SUMOylation and Ubiquitination in Fungi

Various PTMs, including phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation, interact with each other (Duan and Walther, 2015). Ubiquitin itself can undergo phosphorylation, acetylation, and SUMOylation (Kliza and Husnjak, 2020). In addition, quinary interactions among phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation in histone proteins are critical for chromatin condensation (Cheung et al., 2000; Krogan et al., 2003; Zhang and Reinberg, 2001). Here, we mainly focus on the crosstalk between SUMOylation and ubiquitination. Numerous studies in both humans and plants have demonstrated overlapping roles between these two PTMs (Cuijpers et al., 2017; Lamoliatte et al., 2017; McManus et al., 2017; Praefcke et al., 2012; Ulrich, 2008). Overexpression or loss of function in SUMOylation- and ubiquitination-associated components often exhibits overlapping effects, except in the case of protein degradation, which is a specific role of ubiquitination (Celen and Sahin, 2020; Garvin and Morris, 2017; Park et al., 2011; Zhang et al., 2015; Zhou and Zeng, 2017).

In fungi, unlike in humans and plants, interactions between the SUMO and ubiquitin pathways are poorly understood. Beyond yeast, crosstalk between SUMOylation and ubiquitination has been primarily explored in the rice blast fungus, *M. oryzae*. In *M. oryzae*, MoFbx15 and MoCue1, ubiquitination-associated components, are involved in the induction of SUMOylation under heat stress and the transcriptional expression of deSUMOylation proteases (Lim and Lee, 2020). In addition, SUMOylated substrates in *M. oryzae* include ubiquitination-associated components such as MoUba1 (E1-activating enzyme) and MoUbr1 (E3 ligase) (Liu et al., 2018).

Further evidence for direct or indirect crosstalk between SUMO and ubiquitin in yeast is detailed below.

SUMO-Targeted Ubiquitin Ligase and SUMO-Ubiquitin Hybrid Chain Specific Receptors

Poly-SUMO chains induce activation of the SUMO-targeted ubiquitin ligase (STUbL) pathway (Mullen and Brill, 2008; Sriramachandran and Dohmen, 2014). The SIMs of STUbLs recognize the poly-SUMO chains on SUMOylated substrates, adding ubiquitin either to the poly-SUMO chain or directly to the substrates (Wilson and Heaton, 2008). STUbL is important for maintaining genome stability by facilitating DNA repair, mitosis, and DNA replication (Nie et al., 2017). Yeast has four STUbLs, including Slx5-Slx8 heterodimer, Uls1/Ris1, and Rad18 (Sriramachandran and Dohmen, 2014). In the Slx5-Slx8 heterodimer, both Slx5 and Slx8 possess RING domains, but only Slx8 has E3 ligase activity (Xie et al., 2007). Slx5-Slx8 recognizes SUMOylated Cse4, a kinetochore protein, inducing proteolysis to prevent Cse4 mislocalization and thus maintain genome stability (Ohkuni et al., 2016). Uls1 has dual functions as a SWI/SNF family ATPase and STUbL. It prevents telomere-telomere fusion by inhibiting the accumulation of poly-SUMOylated Rap1 (Garza and Pillus, 2013). Uls1 physically interacts with SUMOylated Yen1, a nuclease that cleaves Holiday junctions during homologous recombination, and plays an essential role in DNA repair through genetic interactions with another Holiday junction-cleaving nuclease, Mus81 (Bauer et al., 2019). Rad18 is involved in the ubiquitination of SUMOylated PCNA, a cofactor of DNA polymerase. In response to DNA damage, Rad18 controls DNA synthesis and repair processes by forming a complex with ubiquitin E2 to participate in the mono-ubiquitination of PCNA (Geng et al., 2010; Han et al., 2014; Hibbert et al., 2011). However, the Rad18's preference for SUMOylated substrates is not conserved in all species. Human Rad18, the ortholog of yeast Rad18, lacks SIMs and does not act as a STUbL (Parker and Ulrich, 2012).

Not only STUbLs but also the ubiquitin-selective chaperone complex (Cdc48-Ufd1-Npl4) facilitates the degradation of SUMOylated substrates (Nie et al., 2012). Ufd1, a cofactor of the Cdc48 ATPase along with Npl4, has both a ubiquitin-interacting motif (UIM) and a SIM (Nie et al., 2012). These two interaction motifs enable the recognition of SUMO-ubiquitin hybrid chains or SUMO-ubiquitin co-modified substrates, which organize the repair factors (Nie and Boddy, 2016; Sato et al., 2019). Cdc48 and Ufd1 directly interact with the STUbL Slx8, and STUbLs and the ubiquitin-selective chaperone share several SUMOylated substrates (Køhler et al., 2013, 2015). However, further research is needed to clarify how STUbLs and ubiquitin-selective chaperone determine their substrates and specific roles.

While STUbLs add ubiquitin to SUMOylated substrates, SUMO deubiquitinases (SDUBs) remove ubiquitin from substrates that are co-modified with SUMO and ubiquitin (Martín-Rufo et al., 2022). In humans, USP7/HAUSP is recognized as an SDUB and plays a critical role in DNA replication by deubiquitinating co-modified substrates at the replication fork (Smits and Freire, 2016). In yeast, a SUMO-ubiquitin hybrid chain-specific protease, Wss1, exists and plays important roles not only as a SUMO isopeptidase but also in the deubiquitination of SUMO-ubiquitin hybrid chains (Balakirev et al., 2015; Mullen et al., 2010). However, in fungi, the existence and function of SDUBs are not yet fully elucidated.

SUMO and Ubiquitin Modifications on the Same Substrates

PTMs, including SUMOylation, ubiquitination, acetylation, and methylation, commonly occur at K residues of proteins, regulating various biological processes (Li et al., 2018). PCNA, a DNA sliding clamp, is modified by both SUMOylation and ubiquitination, and the K164 residue of PCNA is a major site for these modifications (Papouli et al., 2005). Although the same K residue of PCNA is modified, the role of PCNA varies depending on the type of modifier (Gali et al., 2012; Northam and Trujillo, 2016; Zhang et al., 2011). SUMOylated PCNA inhibits homologous recombination during an unperturbed S-phase and stabilizes the anti-recombinogenic helicase Srs2. Ubiquitinated PCNA, on the other hand, is involved in the function of helicases such as Rad5 and Mph1, as well as fork regression (Gali et al., 2012; Northam and Trujillo, 2016; Zhang et al., 2011). Histone protein H2B is modified on different K residues by two distinct PTMs (Trujillo et al., 2011). SUMOylation occurs at K6-7 and K16-17 residues, with K6-7 residues being more important for SUMOylation levels in yeast. SUMOylated H2B is required for transcriptional repression of regulatory pathway genes such as *Gal1*, *Suc2*, and *Trp3* (Nathan et al., 2006). In contrast, ubiquitination at K123 residue of H2B functions in DNA replication and also inhibits SUMOylation at K6-7 residues of H2B (Chandrasekharan et al., 2009; Hung et al., 2017; Nathan et al., 2006). Protein quality control through the particiation of the proteasome is major function of ubiquitination (Tanaka, 2009). The proteasome, composed of more than 45 subunits, includes subunits with both SUMOylation and ubiquitination sites (Cui et al., 2014). Ubiquitination at K71, K84, K99, and K268 residues of Rpn10, a proteasome subunit, disrupts its UIM and inhibits the recruitment of modified substrates to the proteasome by preventing interactions with other poly-ubiquitinated substrates (Isasa et al., 2010). Rpn10 is also SUMOylated, but it's the function of this modification is not yet understood (Cui et al., 2014).

In the human proteome, numerous substrates undergo conjugation by both SUMO and ubiquitin, with modifications occurring either at distinct sites or at the same site, known as the site of alternative modification (SAM) (Ulman et al., 2021). Similarly, SAM-containing substrates have been identified in the yeast proteome (Ulman et al., 2021). Among these substrates, KSS1 (a mitogen-activated protein kinase) and CPR1 (a cyclophilin) were mutated to selectively abolish either SUMOylation alone or both SUMOylation and ubiquitination by substituting the K residue at the SAM. The localization and abundance of these proteins differ between the non-mutated substrate and substrates with different mutations (Ulman et al., 2021).

Concluding Remarks and Perspectives

The intricate interplay between SUMOylation and ubiquitination, along with other PTMs, is crucial in cellular regulatory networks (Duan and Walther, 2015). Across various organisms, from humans to fungi, these PTMs influence essential processes such as DNA repair and replication, chromatin dynamics, stress response, and protein quality control (Alonso et al., 2015; Su et al., 2020). This review summarized the latest knowledge on how SUMOylation-associated components differ among mammals, plants, and fungi. Furthermore, we highlighted studies of the crosstalk between SUMOylation and ubiquitination in fungi, showing that these two PTMs are not independent pathways but interact in complex ways.

SUMOylation and ubiquitination, in particular, exhibit both cooperative and antagonistic interactions (Chandrasekharan et al., 2009; Hung et al., 2017; Nathan et

al., 2006). In fungi, where the crosstalk between SUMO and ubiquitin pathways is less well understood, emerging studies on plant pathogens such as *M. oryzae* reveal that SUMOylation and ubiquitination play significant roles in pathogenicity, stress tolerance, effector secretion and localization, providing a foundation for further exploration of PTMs in fungal pathogens (Lim and Lee 2020; Lim et al., 2018, 2024; Liu et al., 2018). Despite advancements, the precise mechanisms regulating the selection of substrates and defining the roles of SUMOylation and ubiquitination remain uncharted areas for future investigation. Moreover, the presence of SUMO-ubiquitin hybrid chains, STUbLs, SDUBs, and SAMs indicates that merits in-depth exploration of crosstalk between PTMs.

Given the pivotal roles of SUMOylation in fungal virulence and stress adaptation, targeting this pathway offers a promising avenue for antifungal intervention. This could be achieved through the development of inhibitors that specifically target the components of SUMOylation, which exhibit low sequence similarity between fungal pathogens and their hosts. It might be allowed for disruption of the pathogen's SUMOylation while leaving the host unaffected.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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