

Review

# The Multifunctions of WD40 Proteins in Genome Integrity and Cell Cycle Progression

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## Abstract

Eukaryotic genome encodes numerous WD40 repeat proteins, which generally function as platforms of protein-protein interactions and are involved in numerous biological process, such as signal transduction, gene transcriptional regulation, protein modifications, cytoskeleton assembly, vesicular trafficking, DNA damage and repair, cell death and cell cycle progression. Among these diverse functions, genome integrity maintenance and cell cycle progression are extremely important as deregulation of them is clinically linked to uncontrolled proliferative diseases such as cancer. Thus, we mainly summarize and discuss the recent understanding of WD40 proteins and their molecular mechanisms linked to genome stability and cell cycle progression in this review, thereby demonstrating their pervasiveness and importance in cellular networks.

Key words: WD40 protein, DNA damage, cell death, cell cycle

## 1. Introduction: Overviews of WD40 proteins and their functions

WD40 repeats proteins are abundant in eukaryotes, but rarely present in prokaryotes [1]. Human genome encodes 349 predicted WD40 repeat-containing proteins [2], and plant genomes harbor more than 200 putative WD40 proteins [1, 3, 4]. Generally, WD40 proteins are characterized by the presence of repeating units with 44–60 variable residues that ended with tryptophan (W) and aspartate (D) dipeptides [5]. The basic function of WD40 proteins is to serve as rigid platforms for protein-protein and protein-DNA interactions [6]. During the past decades, multiple WD40 protein complexes have been identified, leading to a better understanding of WD40 domain functions involved in many biological processes such as signal transduction, gene expression regulation, protein ubiquitination and histone methylation, genome stability and cell cycle control [1, 6].

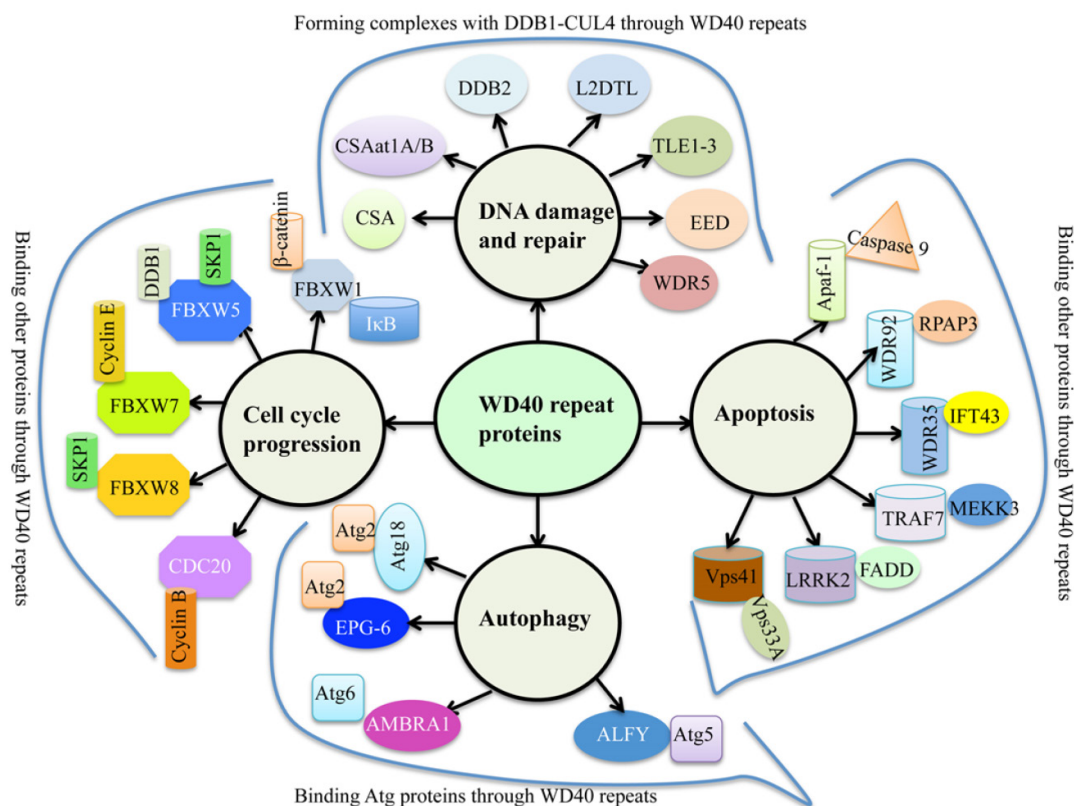
Interestingly, a variety of proteins contain conserved “WDxR” motif(s) in their WD40 repeat regions, such as CSA (Cockayne syndrome A) [7], CSAat1A/B (CSA-like proteins in *Arabidopsis* 1A and 1B) [8], DDB2 (Damaged DNA binding protein 2) [9], L2DTL (*Drosophila* lethal(2) denticleless protein) [10], TLE1-3 (Transducin-like enhancer of split 1 to 3) [11], EED (Embryonic ectoderm development protein) [11], and WDR5 (WD repeat-containing protein 5) [11] (Figure 1). These proteins commonly form complexes with DDB1 (damaged DNA binding protein 1) through the “WDxR” motif in response to DNA damage and repair (Figure 1). In addition, numerous WD40 proteins also have been identified to interact with other proteins in response to apoptosis, autophagy and cell cycle progression (Figure 1). These WD40 proteins mainly include Apaf-1 (Activation of apoptotic protease activating factor 1) [12], WDR35 [13], WDR92 [14], TRAF7 (Tumor necrosis factor receptor-associated factor) [15], LRRK2 (Leucine-rich repeat

kinase 2) [16], Vps41 (Vacuolar protein sorting 41) [17], Atg18 (Autophagy-related protein 18) [18], EPG-6 (Ectopic PGL granules 6) [19], AMBRA1 (Autophagy/beclin-1 regulator 1) [20], ALFY (autophagy-linked FYVE protein) [21], FBXW1 (F-box/WD repeat-containing protein 1) [22], FBXW5 [23], FBXW7 [24], FBXW8 [25], and CDC20 (Cell division cycle protein 20) [26].

Some of these WD40 proteins involved in genome stability and cell cycle progression have been determined their structures (Table 1). Generally, the WD40 domain exhibits a  $\beta$ -propeller architecture, which typically contains seven blades and each of them is composed of four anti-parallel  $\beta$ -sheets [1]. WD40 domain recognizes and binds to other proteins mainly through the smaller top surface, but also through the bottom surface and sides in some cases [1]. Clinically, inherited or acquired defects of WD40 proteins always lead to severe health problems and are genetically associated with a variety of diseases such as Cockayne syndrome [27], multiple cancer types [28], Lissencephaly [29] and Parkinson's disease [30].

## 2. WD40 proteins, DNA damage and repair

The genotoxic chemicals, ionizing radiation (IR) and ultraviolet (UV) radiation can cause DNA damage [31, 32]. Organisms have evolved complex and highly conserved systems to rapidly and efficiently repair damaged DNA. Commonly, DNA double-strand breaks (DSBs) are repaired through three distinct pathways, including non-homologous end-joining (NHEJ), alternative NHEJ and homologous recombination (HR) [31, 33]. UV-induced DNA lesions and other DNA bulks are removed from duplex DNA by nucleotide excision repair (NER) [31, 34]. Small and non-helix-distorting base lesions that affect only one DNA strand are repaired by base-excision repair (BER) [31]. Erroneous insertion, deletion and misincorporation of DNA bases are recognized and repaired by mismatch repair (MMR) [31]. DNA crosslinks, including intrastrand and inter-strand crosslink, are repaired by Fanconi anemia (FA) pathway [31].

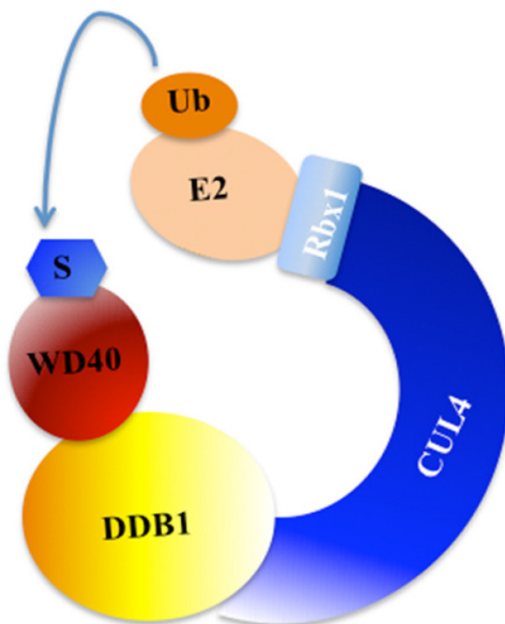


**Figure 1. The involvement of WD40 proteins in genome stability and cell cycle progression.** Multiple WD40 proteins interact with DDB1, Atg proteins, Cyclin proteins and other proteins through their WD40 repeats in response to DNA damage and repair, autophagy, cell cycle progression and/or apoptosis. CSA, Cockayne syndrome A; CSAAt1A/B, CSA-like proteins in *Arabidopsis* 1A and 1B; DDB2, Damaged DNA binding protein 2; L2DTL, *Drosophila* lethal(2) denticleless protein; TLE1-3, Transducin-like enhancer of split 1 to 3; EED, Embryonic ectoderm development protein; WDR5, WD40 repeat-containing protein 5; Apaf-1, Activation of apoptotic protease activating factor 1; WDR35/92, WD40 repeat-containing protein 35/92; TRAF7, Tumor necrosis factor receptor-associated factor; LRRK2, Leucine-rich repeat kinase 2; Vps41, Vacuolar protein sorting 41; Atg18, Autophagy-related protein 18; EPG-6, Ectopic PGL granules 6; AMBRA1, autophagy/beclin-1 regulator 1; ALFY, autophagy-linked FYVE protein; FBXW1/5/7/8, F-box/WD repeat-containing protein 1, 5, 7 and 8; CDC20, Cell division cycle protein 20.

**Table 1:** The bind partners, binding domain/motifs, biological functions and available structures of WD40 proteins

WD40 protein	Binding partner	Binding domain and consensus motif	Biological functions	Available structure	References
CSA	DDB1, RNA polymerase II TFIIF	WD40 domain, VKLWDVRR	TCR DNA repair	Yes	[47]
CSAat1A/1B	DDB1A	WD40 domain, DGAIRFWDIR	TCR DNA repair	No	[8]
DDB2	DDB1, E2F1	WD40 domain, VKIWDLR	GGR DNA repair	Yes	[47]
WDR5	DDB1, Histone H3	WD40 domain, R-X-K <sub>dimethylation</sub>	Chromatin regulator	Yes	[11]
L2DTL (CDT2)	DDB1, CDT1	WD40 domain, IKVWDLRK	DNA damage, cell cycle control	Yes	[10]
EED	DDB1	WD40 domain, LRLWNIQTD	Chromatin regulator	Yes	[121]
WDR18	TopBP1	WD40 domain, Unknown motif	DNA damage checkpoint	No	[56]
TLE1-3	DDB1	WD40 domain, VRSWDLREGR	Transcription regulator	Yes	[122]
Apaf-1	Caspase 9	WD40 domain, Unknown motif	Apoptosis	Yes	[123]
WDR92	RPAP3	WD40 domain, Unknown motif	Apoptosis	Yes	[1]
WDR35	IFT43, BCL6	WD40 domain, Unknown motif	Apoptosis	Predicted structure	[124]
TRAF7	MEKK3	WD40 domain, Unknown motif	Apoptosis	No	[15]
Vps41	VPS33A, VPS16, 18	WD40 domain, Unknown motif	Apoptosis	No	[17]
VprBP	DDB1	WD40 domain, VLWDVRSQAQ	Apoptosis, cell cycle control	Yes	[125]
LRRK2	FADD	WD40 domain, Unknown motif	Apoptosis	Yes	[76]
RACK1	p63	WD40 domain, Unknown motif	Apoptosis	Yes	[78]
Atg18	Atg2	WD40 domain, IYQFRRGTYA	Autophagy	Yes	[126]
EPG-6	Atg2	WD40 domain, Unknown motif	Autophagy	No	[19]
AMBRA1	Atg6, PIK3C3	WD40 domain, Unknown motif	Autophagy	No	[20]
ALFY	Atg5, GABARAP	WD40 domain, DGFIFV	Autophagy	Yes	[21]
LYST	LAST1, LAST2	Unknown domain and motif	Autophagy	No	[101]
LRBA	WDR23	Unknown domain and motif	Autophagy	No	[102]
WDR26	DDB1	Unknown domain and motif	Cell death	No	[103]
MDV1	Dnm1	WD40 domain, Unknown motif	Cell death	No	[104]
FBXW1	$\beta$ -catenin, I $\kappa$ B	WD40 domain, D-pS-G- $\Phi$ -X-pS	Cell cycle control	Yes	[127]
FBXW5	SKP1, DDB1	WD40 domain, IWDRHY	DNA damage and cell cycle control	No	[128]
FBXW7	Cyclin E, Sic1p, Gcn4p	WD40 domain, L-I/L/P-pT-P	Cell cycle control	Yes	[129]
FBXW8	SKP1	WD40 domain, Unknown motif	Cell proliferation	No	[25]
CDC20	Cyclin B	WD40 domain, Unknown motif	Cell cycle control	Yes	[112]

Post-translational modifications, such as phosphorylation, ubiquitination and sumoylation, play crucial roles in coordinating cellular response to DNA damage [31]. Previous studies have indicated that ubiquitination regulates and coordinates pathways involved in DNA damage recognition, signaling and repair [31, 35]. The eukaryotic ubiquitin-proteasome system (UPS) involves three enzymes: the E1 Ub-activating enzyme, E2 Ub-conjugating enzyme, and E3 Ub-protein ligase [36]. In mammals, cullin family proteins associate with RING proteins, adaptor proteins and substrate recognition receptors, forming the largest family of E3 ubiquitin ligases, namely, Cullin-RING ubiquitin ligases (CRLs) [37, 38]. Among these CRLs, CRL4 has been widely reported to function in the regulation of DNA damage and repair processes [37, 38]. CRL4 E3 ligase is consist of cullin 4A or 4B (CUL4A or CUL4B), RING-box protein 1 (RBX1, also known as ROC1), adaptor protein DDB1, and substrate recognition receptor DCAFs (DDB1 and CUL4-associated factors, also referred to as WD40 proteins) [37, 38]. Interestingly, the specificity of CRL4 E3 ligases is conferred by these WD40-containing substrate receptors [37, 38]. A variety of WD40 proteins, such as CSA, CSAat1A/1B, DDB2, WDR5, TLE1-3, L2DTL and EED, have been identified to interact with CUL4A/B-DDB1 complex in response to DNA damage (Figure 2) [7, 39].



**Figure 2. A model for the assembly of the CUL4-DDB1-WD40 ubiquitin ligase complex.** CUL4 serves as a rigid scaffold, and directly contacts with DDB1 with its N-terminal domain. CUL4 can also recruit Rbx1 and the E2 ubiquitin-conjugating enzyme via its C-terminal domain. The DDB1 further binds a variety of WD40 proteins to form E3 ligases, recognizing different substrates in response to different cellular processes [9]. S, substrate. Ub, ubiquitination.

Cockayne syndrome (CS) is a genetic recessive disorder characterized by growth retardation, impairment of nervous system development, hypersensitivity to sunlight and premature aging [40, 41]. CS can be resulted from mutations in either CSA (also known as ERCC8) or CSB gene (also known as ERCC6) [42]. Previous studies have demonstrated that CSA and CSB act in a transcription-coupled repair (TCR) pathway [43]. CSA is a subunit of CRL4 E3 ligase complex and CSB is a substrate of this E3 ligase [43]. After DNA damage caused by UV irradiation, CSB is ubiquitinated by the CRL4<sup>CSA</sup> E3 ligase and degraded by the proteasome at a late stage of the repair process, and that CSB degradation affects the recovery of RNA synthesis after TCR [43]. Interestingly, CSA contains two WDxR motifs in WD40 repeats and the second WDxR motif plays critical role for its interaction with DDB1 [7]. Further studies have revealed that CSA<sup>R217A</sup> mutation abolishes its binding to DDB1 *in vitro*, but not CSA<sup>R218A</sup> mutation [7]. Additionally, CSA<sup>A205P</sup> mutation, which is also located in the second WDxR motif, is identified to cause CS and block DDB1 binding [7]. In *Arabidopsis*, two CSA-like proteins, namely, CSAat1A and CSAat1B can form an E3 ligase with CUL4 and DDB1A to regulate substrates in response to DNA damage through TCR [8]. Both CSAat1A and 1B contain a WDxR motif in the WD40 domain, and single mutations at positions Asp-212, Trp-218, Asp-219, or Arg-221 in CSAat1A and 1B reduce their ability to interact with DDB1A [8].

The CUL4-DDB1 complex specifically binds to DDB2 to form the UV-damaged DNA-binding protein complex (the UV-DDB complex) [44], which further recognizes UV-induced DNA damage and recruits proteins of the NER pathway to initiate DNA repair [44, 45]. The UV-DDB complex preferentially binds to cyclobutane pyrimidine dimers (CPD), 6-4 photo-products (6-4 PP), apurinic sites and short mismatches [46]. Biochemically, the CRL4<sup>DDB2</sup> E3 ligase serve functions in global genome repair (GGR) after UV irradiation [47]. Similarly, DDB2 also contains a WDxR motif in the WD40 domain, which is critically important for DDB1-DDB2 interaction [45]. The R273H mutation within the WDxR motif is deficient for DDB1 binding and is causally associated with human xeroderma pigmentosum (XP-E) [45]. In human cells, the CRL4<sup>DDB2</sup> E3 ligase can ubiquitinate histone H3, H4, and XP-C (xeroderma pigmentosum group C) protein to regulate their activities [48].

The immunoprecipitation and mass spectrometry assays have identified a variety of other WD40 proteins associated with CUL4-DDB1 complex, such as WDR5, TLE1-3 (TLEs), L2DTL and EED [49]. WDR5 is a core subunit of the human MLL



(mixed-lineage leukaemia) and SET1 (hCOMPASS) histone H3 Lys4 (H3K4) methyltransferase complex [50]. TLEs are transcriptional corepressors that bind to a number of transcription factors, thereby inhibiting NF-kappa-B-regulated gene expression, and inhibiting transcriptional activation mediated by FOXA2 (forkhead box protein A2) [51], by CTNNB1 (catenin beta-1) and TCF (transcription factor) family members in Wnt signaling [52, 53]. The crystal structures of WD40-repeat domains in WDR5 and TLE1 indicate that the Asp and Arg/Lys in the WDxR/K motif are exposed on the bottom surface of the barrel-shaped WD40 propeller folds, and are thus available for interaction with CUL4-DDB1 [49]. L2DTL also requires the WD40-repeat domain for DDB1 binding and it is responsible for CDT1 proteolysis in response to DNA damage [49]. The CRL4<sup>L2DTL</sup> E3 ligase can promote the ubiquitination of proteins in S phase and after DNA damage [49]. Numerous substrates of CRL4<sup>L2DTL</sup> E3 ligase have been identified, such as CDT1, the CDK inhibitor, the histone methyltransferase Set8, the his-

tone acetyltransferases GCN5, the checkpoint kinase CHK1 and the TOB anti-proliferative protein [54]. EED can strongly interact with CUL4A-DDB1 and participate in regulating the methylation of H3 histone at K9 and K27 [49]. However, EED protein sequence does not contain a recognizable WDxR/K motif, it has a WNIQ motif in the N-terminus of the WD40-repeat domain instead [49]. Deletion of any of the five putative WD40 repeats in EED abolishes its ability to mediate H3K27 methylation [55].

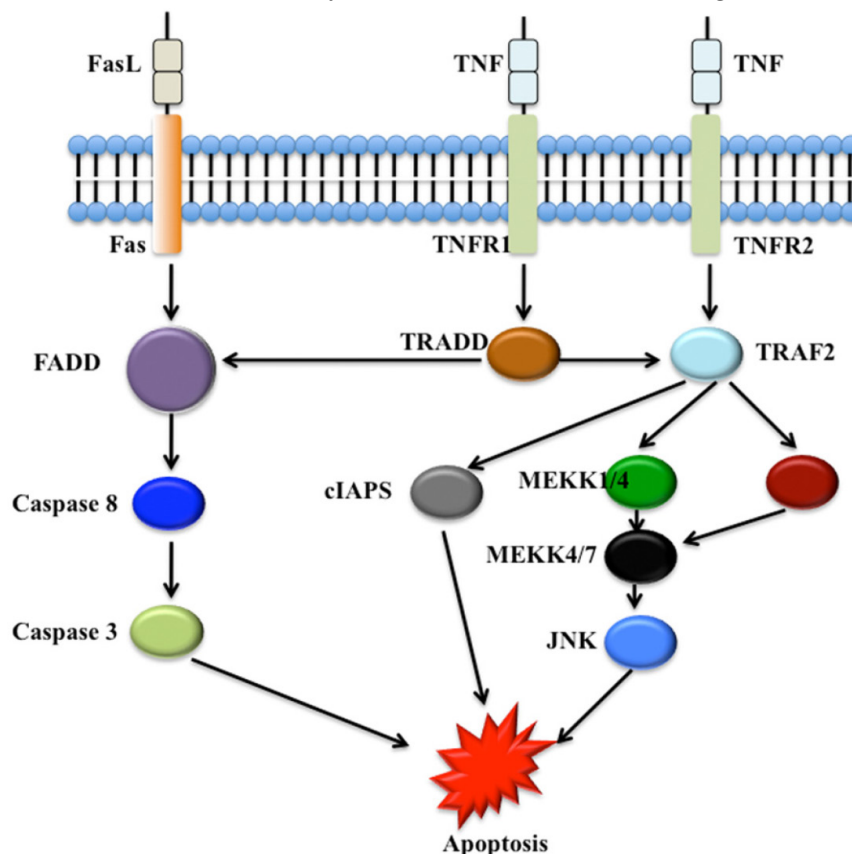
Additionally, the recent studies indicate that a WD40-repeat protein WDR18 can collaborate with DNA topoisomerase 2-binding protein 1 (TopBP1) to facilitate DNA damage checkpoint signaling [56]. WDR18 associates with the C-terminus of TopBP1 *in vitro* and *in vivo*, and the association between WDR18 and TopBP1 is required for AT70-induced Chk1 phosphorylation [56]. WDR18 facilitates ATR-dependent Chk1 phosphorylation via interacting with both C-terminus of TopBP1 and Chk1, promoting DNA damage checkpoint signaling [56].

### 3. WD40 proteins and cell death

Numerous WD40 proteins have been revealed to involve cell death, in particular apoptosis and autophagy processes. In mammals, two main apoptotic initiation mechanisms have been suggested: the tumor necrosis factor (TNF) induced pathway and the Fas-Fas ligand-mediated pathway (Figure 3) [57]. Autophagy process involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes [60, 61]. Three different forms of autophagy are commonly described: macroautophagy, microautophagy and chaperone-mediated autophagy [62].

#### 3.1 WD40 proteins and apoptosis

The intracellular caspases are required for the deliberate disassembly of cells into apoptotic bodies during apoptosis [63, 64]. Twelve caspases have been identified in human and they are present as inactive pro-enzymes that are activated by proteolytic cleavage [65]. Caspases 3, 8, and 9 are func-



**Figure 3. The tumor necrosis factor (TNF) induced apoptosis pathway and the Fas-Fas ligand-mediated apoptosis pathway.** The Fas, tumor necrosis factor receptor type 1 and 2 (TNFR1 and TNFR2) localize in the cell membrane. Numerous adaptor proteins and signal transducing molecules are involved in signal transduction initiated by the binding of the Fas ligand (FasL) to Fas and TNF to TNFR1 and TNFR2, respectively [58, 59]. cIAPs, cellular inhibitors of apoptosis; FADD, Fas-associated death domain protein; JNK, c-Jun N-terminal kinase; MEKK, Mitogen-activated protein kinase kinase; TRADD, TNF receptor-associated death domain protein; TRAF2, TNF receptor-associated factor 2.

tion critically in apoptosis pathways [64]. Caspase3 can interact with both caspase8 and caspase9, and it appears to amplify caspase8 and caspase9 initiation signals into full-fledged commitment to disassembly [63]. Caspase8 and caspase9 activate caspase3 by proteolytic cleavage and caspase3 then cleaves vital cellular proteins or other caspases [66]. Caspase8 is responsible for initiating disassembly in response to extracellular apoptosis-inducing ligands [67, 68, 69]. Caspase9 activates disassembly in response to induction of stress signaling pathways including stress-activated protein kinase/c-Jun NH(2)-terminal kinase (SAPK/JNK) [70], which trigger the release of cytochrome *c* from mitochondria and activation of Apaf-1 [71]. Apaf-1 is a WD40 protein, and it is activated in the presence of dATP and cytochrome *c* binding, resulting in the activation of caspase9 [12]. The WD40 domain of Apaf-1 is located in the C-terminal region and plays critical role for its self-association and pro-caspase9 activation [72]. Interestingly, the WD40 repeats can interact with its N-terminal CED-4 homologous region [72]. Mutational analysis reveals that expression of the WD40 repeat region (residues 468-1194) inhibits Apaf-1 (residues 1-559) self-association and the maturation of pro-caspase9 [72]. The homologues of Apaf-1, including CED-4 in *C. elegans* and Dark in *Drosophila*, also can activate the caspases to initiate apoptosis [67, 73]. Interestingly, these proteins appear to have different regulation and biological roles [67]. Of them, Apaf-1 is inhibited by its WD40 repeats until it is activated by cytochrome *c*, derived from damaged mitochondria [67]. CED-4 is restrained by the homologue of B-cell lymphoma 2 (Bcl-2), namely, CED-9 [67]. The activation of Dark does not seem to involve cytochrome *c*, even though it also contains WD40 repeats [67].

WDR92 (also known as Monad) is a WD40 repeat protein and is widely expressed in human tissues [14]. Overexpression of *WDR92* in HEK293 cells promotes apoptosis and caspase3 activation induced by TNF- $\alpha$  and cycloheximide, indicating that *WDR92* may function as a novel modulator of apoptosis pathway [14]. *WDR35* (also known as Naofen) is a novel WD40-repeat protein [13]. Overexpression of *WDR35* in HEK293 cells spontaneously induces caspase3 activation and apoptosis, and exhibits extremely high susceptibility to TNF- $\alpha$ -induced apoptosis, suggesting that *WDR35* may function as a novel modulator activating caspase3 and promote TNF- $\alpha$ -stimulated apoptosis [13]. However, there is no available information about the function of WD40 repeats in these two proteins.

The TRAF protein family members associate

with, and mediate the signal transduction from members of TNF receptor superfamily [15]. Among these TRAF proteins, only TRAF7 contains seven WD40 repeats at its C-terminus [15]. In addition, TRAF7 also contains another important domain: RING finger [15]. The biochemical analysis indicates that its WD40 repeats specifically interacts with MEKK3 (MAP3K3) and induces MEKK3-mediated AP1 and CHOP activation [15]. The RING finger domain is required for its apoptotic activity as its deletion significantly reduces the ability to induce cell death [15].

*Vps41* contains a WD40 domain, a CLH domain and a RING-finger motif mediating membrane association [17, 74]. *Vps41* binds to caspase8 through its WD40 region and RING-finger motif [17]. Overexpression of *Vps41* promotes Fas-induced apoptosis in A549 human lung adenocarcinoma cells [17].

The human immunodeficiency virus type 1 (HIV-1) Vpr-binding protein VprBP contains WD40 repeats [75]. VprBP has been found to mediate Vpr-induced G2 cell cycle arrest, and bind stoichiometrically with DDB1 through its WD40 domain to form a complex with CUL4A [75]. Conditional deletion of the *VprBP* gene in mouse embryonic fibroblasts results in severely defective progression through S phase and subsequent apoptosis [75].

LRRK2 also contains WD40 repeats [30], and mutations in LRRK2 cause autosomal-dominant Parkinson's disease (PD) [30]. The WD40 repeats are required for LRRK2 neurotoxicity and deletion of WD40 repeats completely blocks the neurotoxicity associated with LRRK2 PD mutations [76]. LRRK2 is also involved in cell death processes as it interacts with FADD (Fas-associated protein with dead domain), which is implicated in the activation of apoptosis [77]. Some studies have further indicated that FADD/caspase8 contributes to the apoptosis induced by LRRK2 [77].

The receptor for activated C kinase 1 (RACK1) is a member of WD40 proteins [78]. In response to stress, RACK1 is sequestered into stress granules and inhibits apoptosis by suppressing stress-responsive MAPK pathways [79]. The p53 family members such as p63 and p73 can induce cell cycle arrest and promote apoptosis through a transactivation (TA) domain [80].  $\Delta$ Np63 isotypes lacking the TA-domain promote cell proliferation and tumorigenesis *in vitro* and *in vivo* [80]. Interestingly, RACK1 physically associates with the p63 $\alpha$  C-terminal domain through its WD40 domain, regulating apoptosis [80].

### 3.2 WD40 proteins and autophagy

Autophagy is a self-degradative process in-

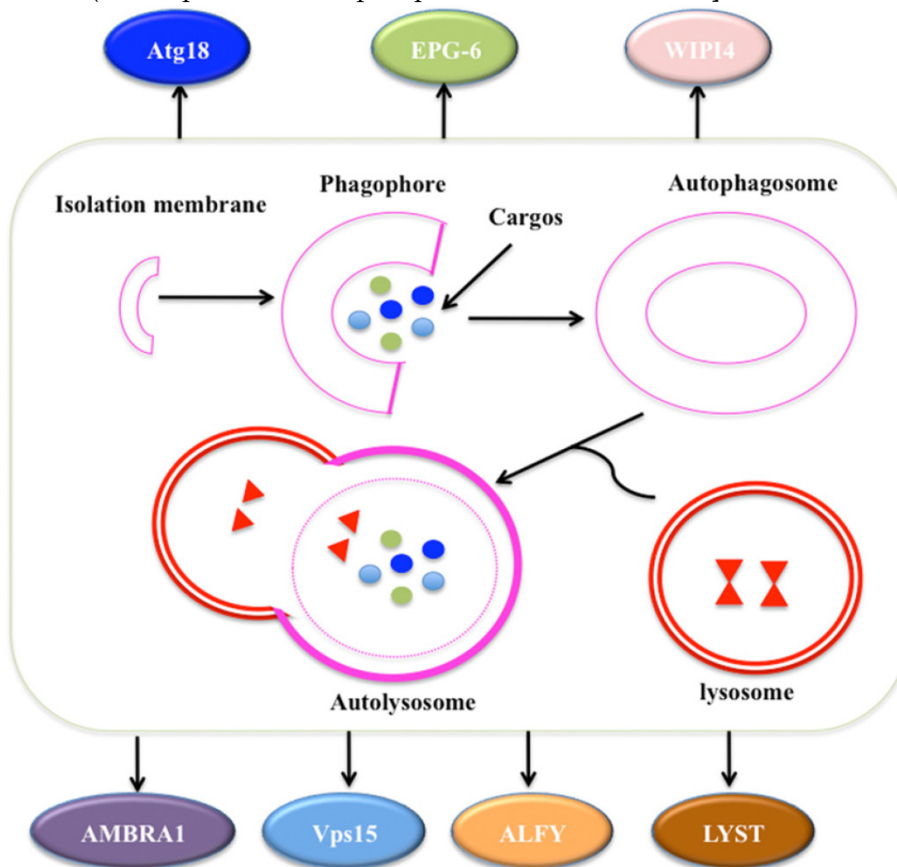
involved in many aspects: (1) autophagy is initiated by the ULK1 (unc-51 like autophagy activating kinase 1) complex, which consists of ULK1, Atg13, Atg17 and receives stress signals from mTOR (mammalian target of rapamycin) complex 1 [81, 82]; (2) autophagosome formation is controlled by Beclin-1/Vps34 at membranes in response to stress signaling pathways [83, 84]; (3) Atg5-Atg12 conjugates and interacts with Atg16L, thereby polymerising at the autophagosome [83, 84]; (4) the LC3 (Microtubule-associated protein 1A/1B-light chain 3) system facilitates the transport and maturation of the autophagosome [85]; and (5) autophagosome fuses its external membrane with lysosomes to degrade its cargos (Figure 4) [86].

Atg family proteins are required for autophagosome formation [88]. Among these Atg proteins, Atg18 protein contains a WD40 domain with 7 beta-propellers [89]. Phosphatidylinositol 3-phosphate (PtdIns(3)P), generated by the Vps34 PI(3)K complex, can recruit Atg18 to the preautophagosomal structure (PAS) in yeast and the mammalian Atg18 homologs, WIPI1 (WD repeat domain, phosphoinositide inter-

acting 1) and WIPI2, to early autophagic structures [19, 90, 91, 92]. The WD40 domain in Atg18 contains a conserved FRRG motif, which binds to phosphoinositides and promotes Atg18 recruitment to the PAS [18]. In the absence of the FRRG motif, the inability of Atg18 interacting with Atg2 impairs its association with the PAS, causing an autophagy block [18]. The homolog of Atg18 in *C. elegans*, namely, EPG-6, is also a WD40 repeat protein with PtdIns(3)P-binding activity [19]. EPG-6 directly interacts with Atg2 through the sixth WD40 repeat, regulating progression of omegasomes to autophagosomes, and their loss of function causes accumulation of enlarged early autophagic structures [19]. The mammalian EPG-6 homolog, namely, WIPI4, is also essential for progression of omegasomes to autophagosomes in starvation-induced autophagy [19].

AMBRA1 is a WD40 protein that regulates autophagy and development of the nervous system [20]. AMBRA1 can interact with Atg6 and PIK3C3 (phosphatidylinositol 3-kinase, catalytic subunit type 3) [93, 94]. The decreased expression of AMBRA1 leads to

autophagy impairment and associates with increased cell death [20]. The functional inactivation of AMBRA1 in mouse causes lethality in utero, leading to severe neural tube defects associated with autophagy impairment, accumulation of ubiquitinated proteins, and excessive apoptosis [20, 95]. The core Vps34/PI3K complex consists of Atg6, Vps15 and Vps34 [96]. This complex is critical for recruiting autophagy-related proteins to the preautophagosomal structure and for other vesicular trafficking processes [97]. Vps15 kinase contains WD40 repeats, and is required for both stress-induced and developmentally programmed autophagy in various tissues in *Drosophila melanogaster* [96]. The X-ray crystal structure of the Vps15 WD40 domain reveals a seven-bladed propeller resembling that of typical G $\beta$  subunits [98]. The WD40 domain is sufficient to bind Gpa1 (Guanine nucleotide-binding



**Figure 4. The process of autophagy.** The process of macroautophagy consists of three main stages: initiation of the formation of the isolation membrane/phagophore; elongation and completion of the mature autophagosome; and fusion of the autophagosome with the lysosome to form an autolysosome [87]. A variety of WD40 proteins have been identified to involve this process, such as Atg18, EPG-6, WIPI4, AMBRA1, Vps15, ALFY and LYST.



protein alpha-1 subunit) and Atg14 [98].

A variety of autophagy proteins contain an LC3-interacting region (LIR), which is required for their interaction with Atg8 homolog proteins [97]. ALFY (also known as WDFY3) binds selectively to LC3C and the GABARAPs (gamma-aminobutyric acid receptor-associated protein) through a LIR in its WD40 domain [97]. The ALFY-GABARAP interaction is required for the clearance of certain p62-bound aggregates by autophagy [97].

Most BEACH (named after 'Beige and Chediak-Higashi') domain-containing proteins (BDCPs) harbor WD40 repeats [99]. Studies indicate that mutations in individual BDCPs result in severe human diseases [99]. The human lysosomal trafficking regulator (LYST) is the most studied BDCP protein in human, which can affect lysosome size and quantity, but not trafficking or degradation through autophagy or endocytosis [100]. However, the homolog of LYST in *Drosophila* exhibits a critical role in the maturation of phagosomes and autophagosomes [101]. LRBA (Lipopolysaccharide-responsive and beige-like anchor protein) is also a WD40 repeat-containing BDCP protein. Mutations of LRBA cause an immune deficiency characterized by defects in B cell activation and autophagy and by susceptibility to apoptosis [102].

### 3.3 WD40 proteins and other types of cell death

Some other WD40 proteins are also involved in cell death, such as WDR26 and MDV1 (mitochondrial division, also known as Net2). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) can induce cell death and significantly up-regulate the expression of WDR26 [103], and WDR26 overexpression in turn strongly suppressed H<sub>2</sub>O<sub>2</sub>-induced cell death [103]. MDV1 is a multi-domain protein containing three major regions: an N-terminal unknown structure, a central coiled-coil (C-C) domain, and a C-terminal WD40 domain [104]. Studies indicate that the MDV1 interacts with Dnm1 (dynamin-related GTPase) through WD40 domain, and interact with Fis1 (Mitochondria fission 1 protein) via N-terminal region [104]. The MDV1-Dnm1-Fis1 complex has been demonstrated to regulate programmed cell death in the mitochondria in budding yeast *Saccharomyces cerevisiae* [104].

## 4. WD40 proteins and cell cycle control

Coordinated progression through the cell cycle is critical for eukaryotic cells [105]. Eukaryotic cell cycle progression is mainly controlled by a family of protein kinases known as cyclin-dependent kinases (CDKs), which consist of an activating cyclin subunit and a catalytic subunit CDK [39, 106]. The CDKs ac-

tivities are controlled by the availability of their cyclin partners and the expression of specific CDK inhibitors (CDKIs) [103]. Interestingly, WD40 proteins have emerged as crucial regulators of cell cycle progression via interacting with other proteins.

Cell cycle progression is tightly controlled by UPS, as cyclin expression levels are executed via UPS and CDKIs are targeted for degradation by the UPS-mediated ubiquitination [107]. Two ubiquitin ligases, namely, SKP1-CUL1-F-box-protein (SCF) complex and anaphase-promoting complex/cyclosome (APC/C), specifically function in the ubiquitination of many of these CDKs and CDKIs [107]. SCF can control the transition of G1/S, and two key targets of SCF, cyclin E and the CDKI p27<sup>Kip1</sup>, are controlled by CUL4-DDB1 [49, 108]. In addition, several F-box proteins with WD40 repeats, such as FBXW1 (also known as  $\beta$ -TRCP), FBXW5, FBXW7 and FBXW8, can bind DDB1 through WD40 repeats *in vitro* in response to DNA damage, repair and cell cycle progression [22]. FBXW1 plays important roles in regulating cell cycle checkpoints [109]. In response to genotoxic stress, it contributes to turn off CDK1 activity by mediating the degradation of CDC25A in collaboration with Chk1 [109, 110], thereby preventing cell cycle progression before the completion of DNA repair [109, 110]. FBXW5 is a cell cycle-regulated protein with expression levels peaking at the G1/S transition [111]. FBXW5 levels are controlled by the anaphase-promoting (APC/C) complex, which targets FBXW5 for degradation during mitosis and G1, thereby helping to reset the centrosome duplication machinery [111]. FBXW7 protein is a well-established tumor suppressor and responsible for substrate recognition in an SCF ubiquitin ligase complex [24, 28]. The WD40 repeats in FBXW7 is essential for binding substrates such as cyclin E, Myc (myelocytomatosis oncogene), JUN (jun proto-oncogene) and Notch [24]. FBXW8 plays an essential role in cancer cell proliferation through proteolysis of cyclin D1, but it remains unclear whether FBXW8 is necessary for cell cycle progression in normal cells [25]. The CRL4A<sup>L2DTL</sup> E3 ligase also interacts with and targets the tumor suppressor protein p53, mediating cell cycle arrest or apoptosis in response to genotoxic stress [112].

CDC20 is a WD40 protein, which is an essential cell-cycle regulator required for the completion of mitosis in organisms from yeast to mammals [26]. In mitosis, CDC20 binds to and activates the ubiquitin ligase activity of APC/C and enables the ubiquitination and degradation of securin and cyclin B [20]. APC/C<sup>CDC20</sup> contributes to the proteolysis of securin, triggering chromosomal separation at anaphase [107]. The structure of WD40 domain in CDC20 indicates



that it folds into a seven-bladed  $\beta$ -propeller and has an ideal architecture for multiple protein-protein interactions [113]. Thus, CDC20 appears to bridge the interactions between APC/C and its substrates through WD40 repeats [113].

In budding yeast *Saccharomyces cerevisiae*, Sic1 encodes a cyclin-dependent kinase inhibitor that regulates the cell cycle at the G1 to S transition by inhibiting the activity of the CDC28 [114]. The phosphorylated Sic1 binds to a WD40 protein, namely, CDC4 (also known as FBXW7 in mammals), which is the substrate recognition subunit of the SCF<sup>CDC4</sup> E3 ligase [115]. Versions of Sic1 that lack multiple CDK phosphorylation sites fail to bind CDC4, are stabilized *in vivo*, and cause cell cycle arrest [116, 117]. Sic1 binds to the WD40 domain of CDC4 when a nominal set of six of the nine CDK consensus sites is phosphorylated, terming CDC4 phosphodegron (CPDs) [118]. The human CDC4 ortholog FBXW7 shares similar phosphodegron to that of CDC4, with the exception of an additional phosphorylated residue at the P + 4 position [119, 120].

## 5. Conclusion

Genome integrity and cell cycle progression are tightly regulated by a variety of pathways that mainly involve DNA repair and recombination, apoptosis, autophagy and cell cycle checkpoints. Numerous WD40 proteins participate in these processes through the following mechanisms: (1) function as substrate receptors of CRL4 E3 ligases to target different substrates selectively [1]; (2) utilization of insertion motifs and inter-blade binding grooves of WD40 domains in ligand binding [1]; and (3) recognition of post-translational modification marks [1]. Considering the critical roles of WD40 proteins in genome stability and cell cycle progression, it is clear that there is much more work to be investigated about the E3 ligase substrates and WD40 interaction proteins specifically responding to DNA damage and repair, apoptosis, autophagy and cell cycle progression in the future.

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## Compliance with Ethics Guidelines

This article does not contain any studies with human or animal as subjects performed by the author.

## Competing Interests

The authors have declared that no competing interest exists.

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