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Human Genome and Diseases: Review

WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases

D. Li and R. Roberts*

Department of Medicine, Section of Cardiology, 6550 Fannin, MS SM677, Baylor College of Medicine, Houston, Texas 77030 (USA), Fax: +1 713 790 4348, e-mail: rroberts@bcm.tmc.edu

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Abstract. Defined by the presence of four or more repeating units containing a conserved core of approximately 40 amino acids that usually ending with tryptophan-aspartic acid (WD), WD-repeat proteins belong to a large and fast-expanding conservative protein family. As demonstrated by the crystal structure of the G protein β subunit, all WD-repeat proteins are speculated to form a circularized β propeller structure. The importance of these proteins is not only demonstrated by their critical roles in many essential biological functions ranging from signal transduction, transcription regulation, to apoptosis, but is also recognized by their association with several human diseases. Defining the function of a WD-repeat protein is the current challenge. It is, however, paramount to uncover the function of individual WD-repeat proteins, explore the protein interaction mechanism through WD-repeat domains and, ultimately, understand the complex biological processes and organisms themselves.

Key words. WD-repeat; protein interaction; signal transduction; transcription; apoptosis; mutation; Cockayne syndrome; triple-A syndrome.

Introduction

WD-repeats are minimally conserved domains of approximately 40–60 amino acids that are initiated by a glycine-histidine (GH) dipeptide 11 to 24 residues from the N terminus and end with a tryptophan-aspartic acid (WD) dipeptide at the C terminus. Between the GH and WD dipeptides is the conserved, approximately 40-amino-acid core sequence that conforms to the regular expression as given in figure 1 [1]. The repeating unit, first recognized in the β subunit of the GTP-binding protein transducin, has been referred to as the transducin repeat, the GH-WD repeat, or the WD-40 repeat [1–3]. Most WD-repeat proteins contain a cluster of at least 7 or more copies of the WD-repeats with as many as 16 repeats and as few as 4. To date, a large number of WD-repeat proteins have been identified and their associated function defined, while for others, the function remains unknown. Functions identified range from signal transduction to cell cycle control [1, 3]. More WD-repeat proteins are expected to be identified, the majority with an unknown function. The importance of this family of proteins is evident: the sequence has been conserved across all species in eukaryotes; they perform multiple essential functions and, more recently, several human diseases have been recognized due to mutations in WD-repeat proteins. In this review, we will discuss the molecular features, biological functions of WD-repeat proteins, and their association with human disease.

* Corresponding author.

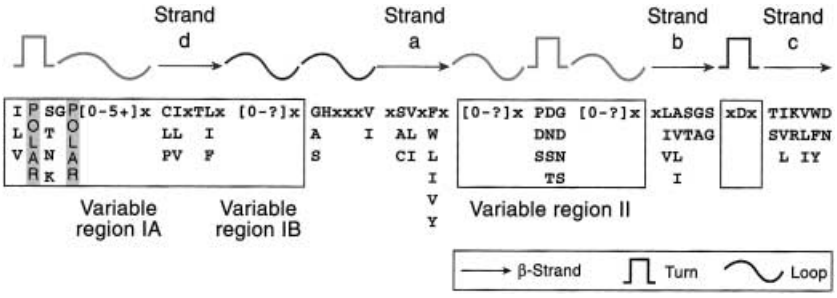

Figure 1.* Structure of one representative blade of the propeller structure of a typical WD-repeat protein. This is different from the WD-repeat unit. In the WD-repeat sequence, there are four strands of a β sheet in the order a, b, c, and d. However, in the propeller blade, there is always one strand from the preceding WD-repeat unit which provides stability for the protein. Thus, each propeller blade begins with a strand from one of the other WD-repeat units designated 'd.' The sequences are also indicated with 'x' indicating that any amino acid can be found in that position; [0-?] indicates the allowed range of the preceding symbol. The variable regions are indicated as I and II with the former divided into IA and IB.

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The WD-repeat family is large and continues to expand

Since it was initially recognized in 1986 [4], the WD-repeat motif has been identified in many proteins. Using a simplified expression derived from the consensus sequence of typical WD repeats, Neer's group identified about 60 true WD-repeat proteins from the protein database at that time (1994). To date, there are at least 123 proteins in the protein database (SWISS-PROT/TrEMBL) and each has at least four WD repeats. A total of 205 WD-repeat proteins have been predicted from the genomes of *Arabidopsis thaliana*, *Caenorhabditis elegans*, and *Saccharonyces cerevisiae*. Interestingly, seven WD-repeat proteins have been identified in prokaryotes like *Synechocystis* in contrast to a previous claim that WD-repeat proteins are only found in eukaryotes (http://bmerc-www.bu.edu/wdrepeat). The recent whole-genome sequence analysis indicates that there are 136 WD-repeat proteins in humans, 98 in *Drosophila melanogaster*, 72 in *C. elegans*, and 56 in *S. cerevisiae* [5].

Probable structure and function of WD-repeat proteins: circularized β-propeller

The WD-repeat proteins vary by two mechanisms. The sequence diversity occurs primarily in the two variable regions within the WD-repeat itself. In addition, the number of structural repeats within a protein also varies. The third variable is not within the WD-repeats themselves but rather in the different multidomains contained in the sequences outside the WD-repeats. The underlying common function of all WD-repeat family members [1] is coordinating multiprotein complex assemblies. These repeating units are believed to serve as a scaffold for protein interactions and these interactions can occur simultaneously with several different proteins. There are several examples of such complexes, including the β subunit of the G protein, the TAFII transcription factor, and the E3 ubiquitin ligase complexes [6]. Structure-function analysis suggests that the repeats act as a very rigid platform or scaffold regardless of the protein involved. The specificity of the proteins is apparently determined by the sequences outside the repeats themselves.

The crystal structure of the classical WD-repeat protein, the G protein β subunit, has been independently determined by two groups [7] and remains the only WD-repeat protein for which the crystal structure is known [8]. It adopts a β propeller fold, a highly symmetrical structure made up of WD-repeats (fig. 2, 3). Each repeat comprises a four-stranded antiparallel β sheet [8, 9]. Thus, each repeat sequence corresponds to a structural repeat of four β strands. However, the repeat structure is not equivalent to each propeller blade; rather, the propeller blade contains the first three strands of the repeat unit and the last strand of the previous propeller blade (fig. 1). Each propeller blade has only four strands, with the initial strand of each propeller blade being the d strand from the previous blade (fig. 1). This sharing of one strand between two blades is believed to stabilize the molecule [3]. This is well illustrated in an example of a bacterial β propeller protein in which a conserved residue in 11 forms a 'tryptophan-docking' structure, which links together the blades in the propeller, thereby stabilizing the overall structure [9]. Murzin [10] proposed that the most stable structure is one of at least seven blades requiring at least seven WD-repeats. There are proteins with four, five and six repeats and how they stabilize remains to be determined. Proteins with only four repeats are postulated to have more residues in the variable region I (fig. 1). The minimum residues in the variable region 1 with just four repeats would be 14 [11]. Normally, this region has at least 20 but can have up to 150 residues [3]. Another possibility is that proteins with just four, five or six repeats dimerize to form at least seven propellers for stability. The propeller

structure occurs in other non WD-repeat proteins and the highest known number of blades in a propeller is eight, in a non WD-repeat protein [12]. Whether proteins with more than eight WD-repeats form smaller or larger propellers is not known.

The conserved sequence and three-dimensional structure of WD-repeat proteins begs the question, why? The WD-repeats themselves do not possess any catalytic activity [3] and do not appear to be a functional domain. The evolutionary pressure to conserve the sequence is apparently to form the propeller structure. The propeller structure provides extensive surface exposure of three surfaces: upper, lower, and the circumference. The surfaces of this structure appear to provide a stable platform for several simultaneous protein, protein interactions. The central core is minimal and not accessible to other proteins. However, the surfaces they form for proteinprotein interaction may be used to define subclasses on the basis of their common binding partners [3].

WD-repeat proteins belong to the β propeller structure. Each of the seven WD-repeats of the G β protein folds into four strands of an antiparallel β sheet (fig. 1) and these sheets are arranged around a central pseudosymmetrical axis into a circular β propeller with the innermost β strand being nearly parallel to the axis of the central tunnel (figs 2, 3). Given the conservation of the WD-repeat, all proteins containing multiple WD repeats likely also form a propeller structure. Such a consistent structure raises the possibility that surface regions of one family can be used to predict the function of new members with similar surface regions [13].

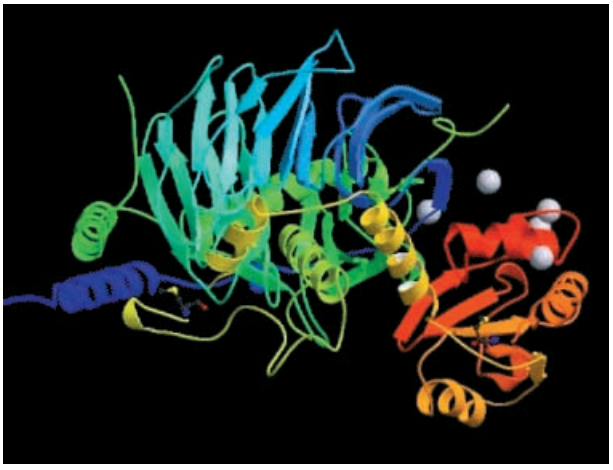

Figure 2. The three-dimensional structure of a β subunit of the G protein [7, 9]. Each of the seven WD-repeats of the β subunit of the G protein fold into four strands of an antiparallel β sheet and those strands form one blade of the β propeller with a total of seven blades making up the β propeller.

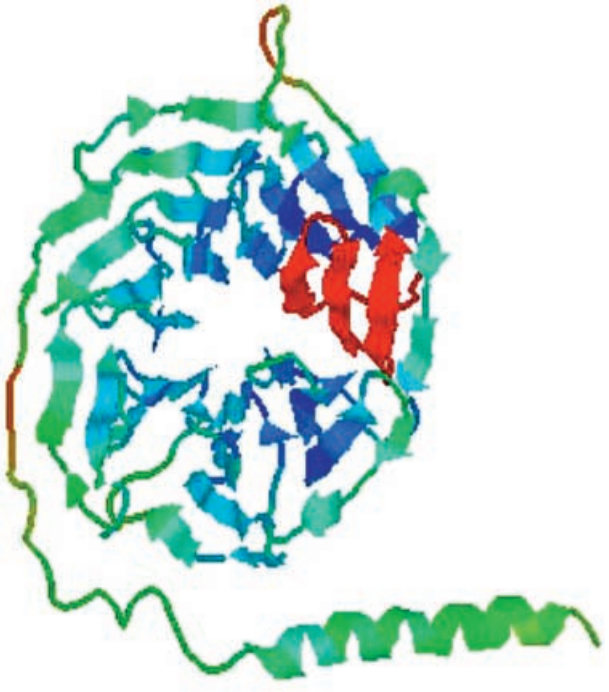

Figure 3. A diagrammatic view of the three-dimensional structure of the G protein. Each blade consists of four strands of a β sheet arranged around a central pseudosymmetrical axis into a circular β propeller with seven blades [13]. The blades are shown in blue. The N terminal and C terminal are shown in red and green, respectively. The sequence repeats and the structural repeats are not identical but overlap to give ring closure.

A conservative protein family with extremely diversified functions

WD-repeat proteins perform a wide range of cellular functions. Most of these proteins seem to be regulatory. A recent study shows that over 30 functional subfamilies can be identified among the current WD-repeat-containing proteins observed in sequenced genomes [14]. These subfamilies can be roughly categorized into signal transduction, RNA synthesis/processing, chromatin assembly, vesicular trafficking, cytoskeletal assembly, cell cycle control, apoptosis, and unknown (table 1).

Signal transduction

All cells, particularly those of a multicellular organism, are constantly being stimulated by a variety of signals to maintain homeostasis. In addition to the classical example of the β subunit of the heterotrimeric G proteins [1, 15], there are many other WD-repeat proteins involved in signal transduction, including RACK1, Stratin, STE4, LIS1, MSI1, PR55, PLAP, RbAp-48.

RACKs (receptors for activated C-kinase) are required for the function of protein kinase C (PKC). The first cDNA encoding a 36-kDa protein was isolated by Ron et al. [16] that fulfilled the criteria for RACKs and was referred to as RACK1. They found that RACK1 is a homolog of the β subunit of G proteins, implicated in mem-

Table 1. Diverse biological functions of WD-repeat proteins.

Biological function	Representatives of WD-repeat proteins involved
Signal transduction	Gβ protein, *RACK1, Stratin, STE4, LIS1, MSI1, PR55, PLAP, RbAp-48*
RNA synthesis and processing	TATA box-binding protein (*TBP*) associated factors (*TAFs*), *TUP1*, cleavage stimulation factor (*CstF*)
Chromatin assembly	chromatin assembly factor-1 (*CAF-1*), *HIR1* and *HIR2* transcriptional repressors of *S. cerevisiae*
Vesicular trafficking	Golgi coat promoter or 'coatomer', most of α and β *COP* proteins, yeast *SEC13*
Cytoskeletal assembly	microtubule-associated protein (*MAP*), intermediate chain of cytoplasmic dynein, actin-related proteins *Arp2* and *Arp3*
Cell cycle regulation	cell division control protein 4 (*CDC4*), *CDC20*, *CDC40*, *p55* (CDC), *Coronin,* the spindle checkpoint protein *Mad2*
Programmed cell death	apoptotic protease activating factor-1 (*Apaf-1*), cell-death proteins, *Dark*, which encodes a *Drosophila* homologue of mammalian Apaf-1 and *C. elegans CED-4*
Function unknown	*WDR1, WDR3, WDR4, WDR6, WDR10*

brane anchorage of β-adrenergic receptor kinases. The data suggested a role for RACK1 in PKC-mediated signaling [17]. In 1997 [18], β'-COPI the coatomer protein, β'-COP was isolated and shown to contain seven repeats of the WD motif and fulfills the criteria previously established for RACKs.

Striatin is a member of the WD-repeat family, the first one known to bind calmodulin (CaM) in the presence of Ca^{2+}. Subcellular fractionation shows that striatin is a membrane-associated protein. Striatin, as demonstrated by Northern blots, in situ hybridization, and immunocytochemistry, is localized to a subset of neurons in the central nervous system, many of which are associated with the motor system. Authors proposed that striatin interacts, through its WD-repeat domain, in a CaM/Ca^{2+}-dependent manner, with one or several members of a surrounding cluster of molecules engaged in a Ca^{2+}-signaling pathway specific to excitatory synapses [19].

RNA synthesis and processing

A key component of the RNA polymerase II transcriptional apparatus, TFIID, is a multiprotein complex containing the TATA box-binding protein (TBP) and several tightly associated factors (TAFs) including hTAFII100, hTAFII18, hTAFII20, hTAFII28, hTAFII55, and hTAFII250 [20]. Interestingly, the majority of TAFs contain multiple WD-repeats. Most of these repeats are located in the carboxyl terminus. Although the functions of most TFIID subunits are unknown, TAFs are not necessary for basal activity but are required to regulate transcription, and so behave as coactivators. The presence of multiple subunits indicates that there is an intricate assembly process whereby TAFs may be responsible for other activities [21, 22]. For example, hTAFII100, which is the human homolog of *Drosophila* TAFII80 and yeast TAFII90, interacts strongly with hTAFII250, hTAFII55, and hTAFII28, less with hTAFII20 and hTAFII18, weakly with TBP, and not at all with DeltaNTAFII135 and hTAFII30. Deletion analysis showed that the C-terminal half of hTAFII100, which contains six WD-repeats, is not required for incorporation into the TFIID complex. These results suggest that hTAFII100 can be divided into two domains, the N-terminal region responsible for interactions within the TFIID complex, and the C-terminal WD-repeat responsible for interactions between hTAFII100 and other factors. An anti-hTAFII100 antibody, raised against a C-terminal epitope, selectively inhibited basal TFIID-dependent in vitro transcription and the specific interaction between hTAFII100 and the 30-kDa subunit of TFIIF (RAP30). Thus, both N termini and WD-repeat-containing C terminals are evidently important in regulating the transcription process [23, 24].

On the other hand, the *Drosophila* 85-kDa TFIID subunit (p85) of transcription initiation factor TFIID interacts directly with both TBP and the 110-kDa subunit (p110) of TFIID, suggesting that p85 may play a role in helping to anchor p110 within the TFIID complex, and TFIID assembly and function may involve a concerted series of subunit interactions. Interestingly, truncated p85 lacking all the WD-40 repeats in its carboxy terminus maintained interactions with both TBP and p110. These observations leave open the possibility for a distinct function for the WD-40 repeats, possibly in transducing signals by interactions with transcriptional regulators and/or other components of the basic transcriptional machinery [25].

Cleavage stimulation factor (CstF), one of the multiple factors required for polyadenylation of mammalian pre-mRNAs, is composed of three distinct subunits of 77, 64, and 50 kDa, and the 64-kDa subunit can be UV-cross-linked to RNA in a polyadenylation signal (AAUAAA)-dependent manner. The 50-kDa subunit of human CstF shares extensive homology with G protein β subunits and has multiple WD-repeats. This WD-repeat protein was suggested to be required for mRNA processing [26].

Chromatin assembly

Chromatin assembly factor-1 (CAF-1) is essential for chromatin assembly in eukaryotes, and comprises three subunits of 150 (p150), 60 (p60), and 48 (p48) kDa. The small subunit of CAF-1 (p48) is a member of a highly conserved subfamily of WD-repeat proteins. There are at least two members in this subfamily in both human (p46 and p48) and yeast (Hat2p, and Msi1p) cells. Human p48 can bind to histone H4 in the absence of CAF-1 p150 and p60. p48, also a known subunit of a histone deacetylase, copurifies with a chromatin assembly complex (CAC), which contains the three subunits of CAF-1 (p150, p60, p48) and H3 and H4. CAC is thought to be a key intermediate in the de novo nucleosome assembly pathway [27, 28].

The small subunit of the chicken CAF-1, chCAF-1p48, consists of 425 amino acid residues, possesses seven WD-repeat motifs, and contains only one amino acid change relative to the human and mouse CAF-1p48s. An immunoprecipitation experiment followed by Western blotting revealed that chCAF-1p48 interacts with chicken histone deacetylases (chHDAC-1 and -2) in vivo. The glutathione S-transferase pulldown affinity assay showed the in vitro interaction of chCAF-1p48 with chHDAC-1, -2, and -3. The Ch p48 subunit binds tightly to two regions of chHDAC-2, located between amino acid residues 82–180 and 245–314, and one C-terminal WD-repeat motif of chCAF-1p48 is required for this interaction. These functions were determined from deletion mutants of the respective regions. Thus, CAF-1p48 is involved in many aspects of DNA-utilizing processes, through alterations in the chromatin structure based on both the acetylation and deacetylation of core histones [29].

Regulation of vesicular trafficking

Regulation of intracellular vesicular trafficking is mediated by specific families of proteins involved in vesicular budding, translocation, and fusion with target membranes. Golgi-derived coated vesicles, termed Golgi coat promoter or 'coatomer,' contain a set of coat proteins of relative molecular mass 160 (α-COP), 110 (β-COP), 98 (γ-COP), and 61 (δ-COP) kDa, and several smaller subunits. Notably, most of the α- and β-COP proteins belong to WD-repeat proteins [30–32]. Several lines of evidence favor the hypothesis that intracellular biosynthetic protein transport in eukaryotes is mediated by non-clathrin-coated vesicles. The vesicles have been isolated and a set of their surface proteins has been characterized as COPs. These COPs exist in the cytosol as a preformed complex, the coatomer, which is known to contain six subunits: four (α-, β-, γ- and δ-COP) with molecular weights between 160 and 58 kDa, and two additional proteins of approximately 36 and 20 kDa, ε- and ξ-COP. The β'-COP subunit occurs in amounts stoichiometric to the established COPs both in the coatomer and in non-clathrin-coated vesicles [33]. The significance of these coatomer proteins was established when disruption of the yeast α-COP gene that contains four WD-40 repeated motifs was shown to be lethal [34].

Cytoskeletal assembly

The major microtubule-associated protein (MAP) of sea urchins and several other echinoderms is a polypeptide of 77 kDa. The echinoderm MAP (EMAP) is abundant in embryonic and differentiated cells, and in mitotic and interphase microtubule arrays. EMAP shows significant sequence similarity with the β subunit of the heterotrimeric G protein, transducin. The homology is mainly in a series of ten WD-repeats. The function of these repeats remains unknown. They are possibly involved in protein-protein interactions, perhaps with the tetratricopeptide-repeat-containing protein family. Alternatively, EMAP may be an important link between signal transduction events and a change in microtubule organization during the cell cycle [35].

The intermediate chain (IC78) of the *Chlamydomonas* outer arm dynein was shown to be located at the base of the solubilized dynein particle and to interact with the α-tubulin in situ, suggesting it binds the outer arm to the doublet microtubule. Sequence comparison indicates that IC78 is homologous to the 69 kDa intermediate chain (IC69) of *Chlamydomonas* outer arm dynein and to the 74 kDa intermediate chain (IC74) of cytoplasmic dynein. The similarity between the chains is primarily in their COOH-terminal halves, that contain six WD-repeats. Using the IC78 cDNA as a probe, Wilkerson et al. [36] screened a group of slow-swimming insertional mutants and identified one which had a large insertion in the IC78 gene, and seven in which the IC78 gene was completely deleted. Electron microscopy of three of these IC78 mutants indicated each is missing the outer arm, suggesting that IC78 is essential for arm assembly or attachment to the outer doublet.

Recent findings indicate that IC78 is likely to provide at least some of the adhesive force that holds the dynein to the doublet microtubule, and support the general hypothesis that the dynein intermediate chains are involved in targeting different dyneins to the specific cell organelles with which they associate. Analysis of the binding activities of various IC78 deletion constructs translated in vitro identified discrete regions of IC78 that affected the binding to microtubules; two of these regions are specifically missing in IC69. Previous studies also showed that IC78 is in direct contact with IC69, and recent studies suggest that the region of IC78 that mediates this interaction contain two of the WD-repeats of IC78. These studies support the hypothesis that WD-repeats are involved in protein-protein interactions within the dynein complex [37, 38].

The Arp2/3 protein complex has been implicated in the control of actin polymerization. The human complex consists of seven subunits, which include the actin-related proteins Arp2 and Arp3, and five others referred to as p41-Arc, p34-Arc, p21-Arc, p20-Arc, and p16-Arc (p complex). Each has homologs in diverse eukaryotes, implying that the structure and function of the complex has been conserved throughout evolution. Human Arp2 and Arp3 are very similar to family members from other species. p41-Arc is a new member of the Sop2 family of WD repeat-containing proteins and may be posttranslationally modified, suggesting its involvement in regulating the activity and/or localization of the complex. Arp3, p34-Arc, and p21-Arc were localized to the lamellipodia of stationary and locomoting fibroblasts, as well to *Listeria monocytogenes* assembled actin tails. They were not detected in cellular bundles of actin filaments. Taken together with the ability of the Arp2/3 complex to induce actin polymerization, these observations suggest that the complex promotes actin assembly in lamellipodia and may participate in lamellipodial protrusion [39].

Cell cycle regulation

The events of late mitosis, from sister-chromatid separation to cytokinesis, are governed by the anaphase-promoting complex (APC), a multisubunit assembly that triggers the ubiquitin-dependent proteloysis of key regulatory proteins. An intricate regulatory network controls APC activity and helps to ensure that late mitotic events are properly timed and coordinated. Many of these regulatory factors are members of the WD-repeat family.

CDC20 is a component of the mammalian cell cycle mechanism. Activation of the APC is required for anaphase initiation and for exit from mitosis. Bancroft et al. [unpublished data] showed that APC was activated during mitosis and G1 by two regulatory factors, CDC20 and HCDH1. These proteins directly bind to the APC and activate its cyclin ubiquitination activity. CDC20 confers a strict destruction-box (D box) dependence on the APC, while HCDH1 shows a much more relaxed specificity for the D box. In HeLa cells, protein levels of CDC20 as well as its binding to APC peak in mitosis and decrease drastically at early G1. Thus, CDC20 is the mitotic activator of APC and directs the degradation of substrates containing the D box.

Initiation of anaphase and exit from mitosis depend on the activation of the APC/cyclosome (APC/C), a multicomponent, ubiquitin-protein ligase. The WD-repeat protein referred to as p55(CDC) (Cdc20) directly binds to and activates APC/C. By using yeast two-hybrid screening, Ohtoshi's group found that cyclin A, a critical cell cycle regulator in the S and G2/M phases, specifically interacts with p55(CDC). Ectopically expressed p55(CDC) and cyclin A form a stable protein complex in mammalian cells. Specifically, the p55(CDC)-cyclin A interaction occurs within the region containing the WD-repeats of p55(CDC) and the region between the D box and the cyclin box of cyclin A. In addition to the physical interaction, p55(CDC) is phosphorylated by cyclin A-associated kinase. These findings suggest that the function of p55(CDC) is mediated or regulated by its complex formation with cyclin A [41].

A mammalian protein, p55Cdc, shows high homology with the cell cycle proteins Cdc20p of *S. cerevisiae* and the product of the *Drosophila* fizzy (fzy) gene, both of which contain WD-repeats and are thought to be required for the metaphase-anaphase transition. The fzy mutants exhibit a metaphase arrest phenotype, which is accompanied by stabilization of cyclins A and B, leading to the hypothesis that fzy function is required for cell-cycle-regulated ubiquitin-mediated proteolysis. p55Cdc expression was initiated at the G1/S transition and steady-state levels of p55Cdc were highest at M and lowest in G1. Immune complexes of p55Cdc obtained at different cell cycle stages showed a variety of proteins with dramatic differences observed in the pattern of associated proteins during the transition from G2 to M. Immunolocalization of p55Cdc demonstrated dynamic changes in p55Cdc localization as the cells transited mitosis. p55Cdc appears to act as a regulatory protein interacting with several other proteins, perhaps via its seven WD-repeats, at multiple points in the cell cycle [42].

Coronin is a ubiquitous actin-binding protein representing a member of proteins portraying a WD-repeat sequence. Coronin has been suggested to participate in multiple, actin-based physiological activities such as cell movement and cell division. Although the slow growth of coronin deletion mutants has been attributed to a defect in the fluid-phase uptake of nutrients, the exact role of coronin in cytoskeletal organization has not been elucidated. Coronin null cells reveal irregularities in organization of actin and myosin II and divide by a process identical to the traction-mediated cytofission reported in myosin II mutants. Overall, this study suggested that coronin is essential for organizing the normal actin cytoskeleton and plays a significant role in cell division [43–45].

Mad2 is a component of the spindle checkpoint, which delays the onset of anaphase until all chromosomes are attached to the spindle. Mad2 formed a complex with Slp1, a WD-repeat protein essential for the onset of anaphase. When the physical interaction between the two proteins was disrupted, the spindle checkpoint was no longer functional [46].

Programmed cell death/apoptosis

Programmed cell death, or apoptosis, is an evolutionarily conserved mechanism of cellular demise that is critical for embryonic development and homeostasis in adult tis-

sues [47–49]. Genetic studies in *C. elegans* have identified two genes, ced-3 and ced-4, that are required for programmed cell death [50]. The protein product of ced-3 was determined to be a cysteine protease [51], and a family of multiple related cysteine proteases (designated caspases) was subsequently identified. These enzymes participate in a cascade triggered in response to proapoptotic signals and culminates in cleavage of a set of proteins, resulting in disassembly of the cell (fig. 4) [49, 52–54].

A human protein homologous to *C. elegans* CED-4, apoptotic protease activating factor-1 (Apaf-1), was identified as a proximal activator of caspase-9 in cell death pathways that trigger mitochondrial damage and cytochrome c release. Notably, the COOH-terminal region of Apaf-1 comprises 12 WD repeats, which are proposed to mediate protein-protein interactions [55]. The mechanism of Apaf-1 action involves the clustering of caspase-9 molecules, thereby facilitating autoprocessing of adjacent zymogens. Apaf-1 can dimerize via the CED-4 homologous and linker domains of the molecule providing a means by which Apaf-1 can promote the clustering of caspase-9 and facilitate its activation. Interestingly, Apaf-1 dimerization was repressed by the C-terminal half of the molecule, which contains multiple WD-repeats, but this repression was overcome in the presence of cytochrome c and dATP. Removal of the WD-repeat region resulted in a constitutively active Apaf-1 that exhibited greater cytotoxicity in transient transfection assays when compared with full-length Apaf-1. Recently, several Apaf-1 isoforms have been identified in tumor cell lines. Functional analysis of all identified Apaf-1 isoforms demonstrated that only those with the additional WD-40 repeat activated procaspase 9 in vitro in response to cytochrome c and dATP, while the NH_2-terminal insert was not required for this activity. Consistent with this result, in vitro binding assays demonstrated that the additional WD-repeat was also required for binding of cytochrome c, subsequent Apaf-1 self-association, binding to procaspase-9, and formation of active Apaf-1 oligomers. These data clearly demonstrated an important regulatory role for the WD-40 repeat region in the apoptotic process [53, 54, 56, 57].

Recently, another cell death protein, Dark, was identified. Like Apaf-1, Dark contains a carboxy-terminal WD-repeat domain necessary for interactions with the mitochondrial protein cytochrome c. Dark selectively associates with the fly apical caspase, Dredd. Dark-induced apotosis is suppressed by caspase-inhibitory peptides and by a dominant-negative mutant Dredd protein, and enhanced by removal of the WD domain. Loss-of-function mutations in Dark attenuate programmed cell deaths during development, causing hyperplasia of the central nervous system, and other abnormalities including ectopic melanotic tumors and defective wings. These findings further establish another WD-repeat protein as an important apoptosis effector in the apoptosis-promoting machinery [58].

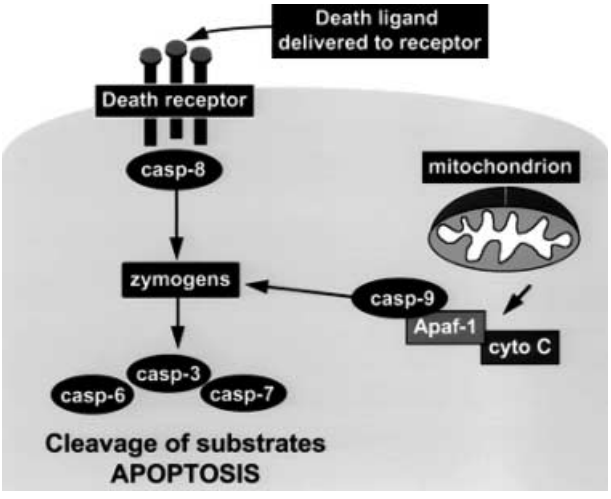

Figure 4. WD-repeat protein (Apaf-1) in the caspase pathway of apoptotic process. Death may be signaled by direct ligand-enforced clustering of receptors at the cell surface, which leads to the activation of the 'initiator' caspase-8 (casp-8). Alternatively, irreparable damage to the genome caused by mutagens, pharmaceuticals that inhibit DNA repair, or ionizing radiation leads to the activation of another initiator, caspase-9 (casp-9). The latter event requires the recruitment of procaspase-9 to proteins such as Apaf-1, which requires the proapoptotic factor cytochrome c (cyto C) to be released from mitochondria. This caspase then directly activates the 'executioner' caspases 3 and 7 (and possibly 6), which are predominantly responsible for the limited proteolysis that characterizes apoptotic dismantling of the cell [52].

Functions to be defined

Fourteen WD-repeat proteins, named WDR1 through WDR14, with no known function are registered with the Human Gene Nomenclature Database. Only five of them (WDR1, WDR3, WDR4, WDR6, WDR10) have been characterized and reported in the literature.

The chick WDR1 gene is expressed at higher levels in the chick basilar papilla after acoustic overstimulation. The 3.3-kb WDR1 cDNA encodes a novel 67-kDa protein containing nine WD-40 repeats, motifs that mediate protein-protein interactions. Sequence database comparisons identified mouse and human cDNAs with high sequence identity to the chick WDR1 cDNA. The mouse Wdr1 and human WDR1 proteins showed 95% sequence identity and 86% identity to the chick WDR1 protein. Northern blot analysis of total RNA from the chick basilar papilla after noise trauma revealed increased levels of a 3.1-kb transcript in the lesioned area. The WDR1 gene has been mapped to human chromosome 4p [59].

WDR3, which encodes a putative 943-amino-acid nuclear protein consisting of ten WD-repeat modules, is widely expressed in hematopoietic cell lines and in non-hematopoietic tissues. Fluorescence in situ hybridization

mapped WDR3 to human chromosome 1p12–p13, a region that is affected by chromosomal rearrangements in a number of hematologic malignancies and solid tumors [60].

To identify candidate genes for Down syndrome phenotypes or disorders that map to human chromosome 21q22.3, trapped exons are being used to isolate full-length transcripts. A full-length cDNA (WDR4) encoding a novel WD-repeat protein and its mouse homolog were isolated. The more highly expressed 1.5-kb transcript was expressed mainly in fetal tissues while the 2.1-kb transcript showed a faint expression in most adult tissues [61].

WDR10 is highly expressed in pituitary and testis. The protein, WDR10p, contains an AF-2 domain as well as seven N-terminal WD-repeats and has been highly conserved through evolution. Chromosomal localization studies placed WDR10 at 3q21, near a locus for the Moebius syndrome, Hailey-Hailey disease, and rhodopsin, which is involved in several forms of retinitis pigmentosa [62].

We previously cloned and characterized a novel human WD-repeat gene, WDR6, which encodes a protein of 1121 amino acids and contains 11 WD-repeat units [1, 63]. The WDR6 gene was mapped to chromosome 15q21 by fluorescence in situ hybridization. Northern analysis demonstrated that WDR6 is ubiquitously expressed in human adult and fetal tissues. WDR6 is not homologous to any previously identified human WD-repeat genes including WDR1 through WDR5. However, it was found to have significant sequence similarity with *A. thaliana* hypothetical protein T7B11.12, a yeast putative elongation factor G, and probable membrane protein YPL183c. All of these have been defined as WD-repeat proteins. Therefore, WDR6 is a novel protein and probably belongs to a highly conserved subfamily of WD-repeat proteins in which T7B11.12 and YPL183c are its distantly related members. The primary structure of WDR6 is unique in that it has 11 WD-repeats clustered into two distinct groups separated by a probable transmembrane domain. Since a group of 4 WD-repeats is able to form a structural unit, typically a β propeller fold [1, 64], WDR6 may form two separate structural units across the plasma membrane. Thus, it may represent a member of a unique and undefined subfamily of WD-repeat proteins [1, 63].

WD-repeat proteins are associated with human diseases

Identification of disease-causing mutations in a gene not only improves our understanding of the disease, but also provides very important information about the function of the gene. This is particularly true for genes of unknown function. At present, there are at least four WD-repeat genes responsible for inherited human diseases.

The lissencephaly-1 gene (LIS1) was the first WD-repeat gene identified as responsible for a human disease. Lissencephaly means 'smooth brain,' i.e., a brain without convolutions or gyri. Deletion of or mutation in the LIS1 gene appears to cause lissencephaly because point mutations have been identified in isolated lissencephaly sequence [65, 66]. The deduced amino acid sequence of LIS1 shows significant homology to β subunits of heterotrimeric G proteins with multiple WD-repeats in their primary structure, suggesting that it may be involved in a signal transduction pathway crucial for cerebral development [67]. LIS1 was later found to be 99% identical with the 45-kDa subunit of bovine platelet-activating factor (PAF) acetylhydrolase, which inactivates PAF by removing the acetyl group at the sn-2 position, indicating that the LIS1 gene product is a human homolog of the 45-kDa subunit of intracellular PAF acetylhydrolase [68].

Cockayne syndrome (CS) is an autosomal recessive disease characterized by abnormal and slow development that becomes evident within the first few years after birth. The characteristics are dwarfism, precociously senile appearance, pigmentary retinal degeneration, optic atrophy, deafness, marble epiphyses in some digits, sensitivity to sunlight, and mental retardation. Disproportionately long limbs with large hands and feet and flexion contractures of joints are usual skeletal features. Remarkably, in striking contrast with other disorders with UV-hypersensitivity, no significant increase in skin cancer is noted. Two clinical types of CS (CSA and CSB) were recognized and, subsequently, two disease genes were identified. The CSB gene encodes a protein with a presumed DNA-unwinding function, i.e., a helicase [69]. In a cell line derived from a patient with type A Cockayne syndrome, Henning et al. [70] found missense mutations, and deletions were identified in a gene encoding a 396-amino-acid protein containing five WD-repeats and the gene was termed CSA. The CSA protein interacts with the CSB protein and with p44, a subunit of the human RNA polymerase II transcription factor IIH. These observations suggest that the products of this interaction are involved in transcription [70].

In the course of constructing a deletion map of the distal portion of the short arm of the X chromosome, a novel gene, transducin β-like (TBL1), was identified. The TBL1 gene shares significant homology with members of the WD-40 repeat-containing protein family containing six WD-repeats in the C-terminal domain. The homology with known β subunits of G proteins and other WD-40 repeat-containing proteins is restricted to the WD-repeat. By genomic analysis, deletion of the TBL1 gene was found to be associated with late-onset sensorineural deafness phenotype [71].

Triple-A syndrome (also known as Allgrove syndrome) is an autosomal recessive disorder characterized by adrenocorticotropin hormone (ACTH)-resistant adrenal insuffi-

ciency, achalasia of the esophageal cardia, and alacrima. Whereas several lines of evidence indicate that triple-A syndrome results from abnormal development of the autonomic nervous system, late-onset progressive neurological symptoms (including cerebellar ataxia, peripheral neuropathy, and mild dementia) suggest that the central nervous system may also be involved in the disease. Using fine mapping and sequencing on a BAC contig encompassing the triple-A minimal region on 12q13, a novel gene, designated AAAS, encoding a protein of 547 amino acids with four WD-repeats in its N terminal was identified and mutations have been found in affected individuals. Most of the mutations result in a truncated protein. In one family, a single splice donor site mutation was detected. The predicted product of the AAAS gene, aladin (alacrima-achalasia-adrenal insufficiency neurologic disorder), belongs to the WD-repeat family of regulatory proteins, indicating a new disease mechanism and novel function of WD-repeat proteins [72, 73].

The challenge of identifying WD-repeat proteins and their function from computational analysis of various gene and protein databases

At present, it is possible but not easy to detect WD-repeats and assign them to a novel protein if four or more such domains are present in the primary structure**.** This can be accomplished by extensive computerized protein sequence homology searches (e.g. BLASTP), protein domain database search (e.g., Pfam, BLOCKS), sequence alignment among homologous proteins across species and distinct WD-repeat domains within the same protein. However, assigning a specific function to a new protein based on sequence analysis alone is still very difficult. The difficulty is mainly with the fact that WD-repeats act as structural platforms for protein-protein interactions and show no functional differentiation or specificity. Based on current understanding, the following factors need to be carefully considered in the effort to define the function of newly cloned WD-repeat proteins.

Analysis of protein sequences outside the WD-repeat clusters

Some WD-repeat proteins such as RACK1 and SEC13 (a 33-kDa WD-repeat protein from *S. cerevesiae* essential for vesicular traffic) are made up of WD-repeats only [74]. The experimental data have established that WD-repeat domains themselves are important for their functions [75, 76]. These motifs may also endow the specific function on the protein. However, such inference is impossible to obtain from sequence analysis of WD-repeats themselves at this stage. In contrast, most WD-repeat proteins have sequence extensions outside the clusters of WD-repeats. These sequences normally offer the information necessary to determine to which subfamily the protein (e.g., G protein, or transcription-associated factors) belongs. Therefore, carefully searching for the homology in all regions is important.

Identification of protein domains in addition to the WD-repeat

Many different functional domains have been identified in WD-repeat proteins. For example, in the primary structures of the WD-repeat protein p57, there are two unique amino acid sequence motifs, a leucine zipper and an actin-binding motif [71]. Other motifs coexisting with the WD-repeat domain include the zinc-binding motif [78], F-box [79], a caveolin-binding motif, a coiled-coil structure, a calmodulin-binding domain [80], the D box, kinase catalytic domain [81], and a KEN box [82]. Specific roles associated with some of these known domains will give clues for the functions of some WD-repeat proteins.

Searching for cellular localization signals

WD-repeat proteins can be found in various subcellular compartments or organelles of the cell. Proteins that assist in chromatin assembly or transcription regulation are found in the nucleus, while proteins involved in Golgi vesicular traffic are localized in the cytoplasm. Cellular localization signals can be identified by using computerized signal search platforms such as PSORT (http://psort.nibb.ac.jp/) and can be tested with in vitro expression or immunohistochemical studies.

Identification of probable structural surface similarity

The only WD-repeat protein whose three-dimensional structure is known is the β subunit of the G protein. It adopts a β propeller fold, a highly symmetrical structure made up of repeats arranged in a small four-stranded antiparallel β sheet [7]. All WD-repeat proteins have been suggested to belong to the same structure class based on molecular modeling [1]. The donut-like β propeller structure contains three potential functional surfaces: the top, the bottom, and the circumference (fig. 2). The most probable common function for the WD-repeat propeller structure is to create a stable platform that can form simultaneous complexes reversibly with other proteins. Thus, the best method to predict the possible binding protein of a WD-repeat protein may be to identify those that share a common surface [1]. One approach is to conduct sequence similarity comparison of WD-repeat proteins while ignoring the non-surface. Unfortunately, this is not a simple task for most biomedical researchers.

Identification of proteins that interact with WD-motifs

WD-repeat proteins perform their function through protein-protein interactions. Needless to say, knowledge of such interactions is critical. Some WD-repeat proteins were isolated through studying protein-proteins interactions utilizing techniques like the yeast two-hybrid screen, coimmunoprecipitation, and pull-down experiments [83, 84]. Others have been able to assign a function based on the protein with which the WD-repeat protein interacts [85, 86].

Identification of WD-repeat protein function through experimentation and association with human diseases

Although not always feasible, experimental studies on animals such as gene knock-outs or finding an inherited disease to be associated with a WD-repeat gene would offer enormous information regarding the function of a particular protein. The Human Genome Project has considerably accelerated our efforts to find genes responsible for human disease. This could be a treasure trove for defining function and cell localization.

Perspective

Recent findings in the analysis of the whole human genome has provided us with a major surprise: the human genome seems to encode far fewer genes (approximately 30,000) than the widely accepted earlier prediction (50,000 to over 140,000) [5, 87, 88]. The number of genes encoded by the human genome is only about twice as big as in the fly (*D. melanogaster*) or worm (*Caenorhabditis elegans*) [89] and does not appear compatible with the complexities of cellular processes we conceive in the human body. The discovery clearly means we must look elsewhere for the mechanism that confers the complexities inherent in molecular processes of human development and physiology. Alternative mRNA splicing, RNA editing, and posttranslation protein modification have been suggested as contributing mechanisms. Another critical contributing source derives from protein-protein interactions in which WD-repeat proteins are central players. This idea is supported by several lines of evidences. First, the fact that WD-repeat proteins are almost exclusively found in eukaryotes that are physiologically or biochemically more complex than prokaryotes: Second, one of the major differences identified between the human genome and other sequenced eukaryotic genomes is that the human genome has the most prominent expansion in proteins involved in intercellular or intracellular signaling pathways in which many WD-repeat proteins are now known to be key mediators. Third, the WD-repeat protein family is among the significant expanded protein families when the human genome is compared with other sequenced eukaryotic genomes.The increase in WD-repeat proteins will conceivably result in much higher complexities than the mere linear increase in number of proteins due to their interacting nature among multiple proteins. The rapidly increasing number of novel WD-repeat proteins without truly homologous proteins in lower species is further indirect evidence that the WD-repeat protein family is probably an important contributor to our inherent complexities. Therefore, it is paramount to uncover the function of individual WD-repeat proteins, explore the protein interaction mechanism through WD-repeat domains, and ultimately understand the roles of WD-repeat proteins in critically important cellular processes like transcription, signaling, and apoptosis.

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