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Minireview

The WD-40 repeat

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An amino acid sequence motif, called the WD-40 repeat, has been found as a repeat in a large variety of proteins that do not share any obvious functional properties. At present, the function of the repeated motif is not known for any of these proteins. Interestingly, recent experiments in yeast indicate that several proteins containing the WD-40 repeat are genetically associated with members of the TPR-family, a protein family that is characterized by the presence of another repeated motif of unknown function: the tetratricopeptide repeat. It is conceivable that proteins containing the WD-40 repeat interact physically with members of the TPR-family via their respective repeated motifs.

WD-40 repeat; G protein β-subunit; Protein family; TPR-gene family

Many proteins contain repeated motifs that may be considered as building blocks, representing structural elements or functional domains. The deduced amino acid sequence of the β-subunit of heterotrimeric G-proteins revealed that, except for the first 50 N-terminal amino acids, the β-subunit is comprised of 7 segments that show sequence similarity to each other and are arranged in tandem [1]. This repeated motif, referred to as the WD-40 repeat [2], is characterized by the presence of a number of amino acids, conserved not only with respect to the type of side chain, but also in their spacing. A similar motif has been found in several other proteins. Based on the presence of the WD-40 repeat, these proteins may constitute a WD-40 family.

Members of the WD-40 family include: (i) the β-subunit of guanine nucleotide regulatory proteins (G-proteins), a component of the heterotrimeric complex that transduces signals from transmembrane receptors to a variety of second messenger generating effectors [1,2]; (ii) STE4, a functional G-protein β-subunit homologue in yeast (*Saccharomyces cerevisiae*), involved in a signal pathway that controls the response to mating pheromone [3]; (iii) CDC4, a component of the yeast nuclear cytoskeleton, required at the late G_1/S phase boundary of the cell cycle [4,5]; (iv) CDC20, a yeast gene product required for several microtubule-dependent processes at multiple stages in the cell cycle [6–8]; (v) Enhancer of Split, the product of one of the neurogenic genes in *Drosophila* [9]; (vi) 12.3, the product of a gene in the chicken MHC-locus [10]; (vii) PRP4, a stable component of yeast U4/U6 small nuclear ribonucleoprotein particle (snRNP) [11–13]; (viii) PRP17, another protein involved in pre-mRNA splicing in yeast [13]; (ix) AER2/TUP1, a transcriptional repressor in yeast [14–15]; (x) MSI1, a negative regulator of the RAS-cAMP pathway in yeast [16]; (xi) coronin, a component of the actin/myosin complex of the slimemold *Dictyostelium discoideum* [17]; (xii) PWP1, a yeast protein, containing periodic tryptophan residues [18]; (xiii) Clbp, a *Chlamydomonas* protein of unknown function [19]; (xiv) MAK11, the apparently membrane associated product of an essential yeast gene, necessary for the maintenance of killer M1 double-stranded RNA [20]; and (xv) AAC3, the deduced product of a developmentally regulated transcript in *Dictyostelium discoideum* that contains long AAC repeats (reading frame specifies glutamine in this case) [21].

A recent search of Protein Sequence Databases (SWISS, release 18.0 and NBRF/PIR, release 29.0) [22,23] identified only one other protein containing more than one region homologous to the WD-40 repeat, namely, ligninase, a lignin peroxidase, secreted by the fungus *Phanerochaete chrysosporium* [24,25]. It should be noted, however, that the second motif in ligninase displays a rather weak homology to the WD-40 consensus sequence. An alignment of all WD-40 repeats identified and a WD-40 consensus sequence based on this alignment are shown in Fig 1.

The WD-40 repeat can be divided in 2 relatively conserved elements, A and B, spaced by regions variable in both sequence and length (Fig. 1A). The most important features of the repeat are the LxGH in part A and

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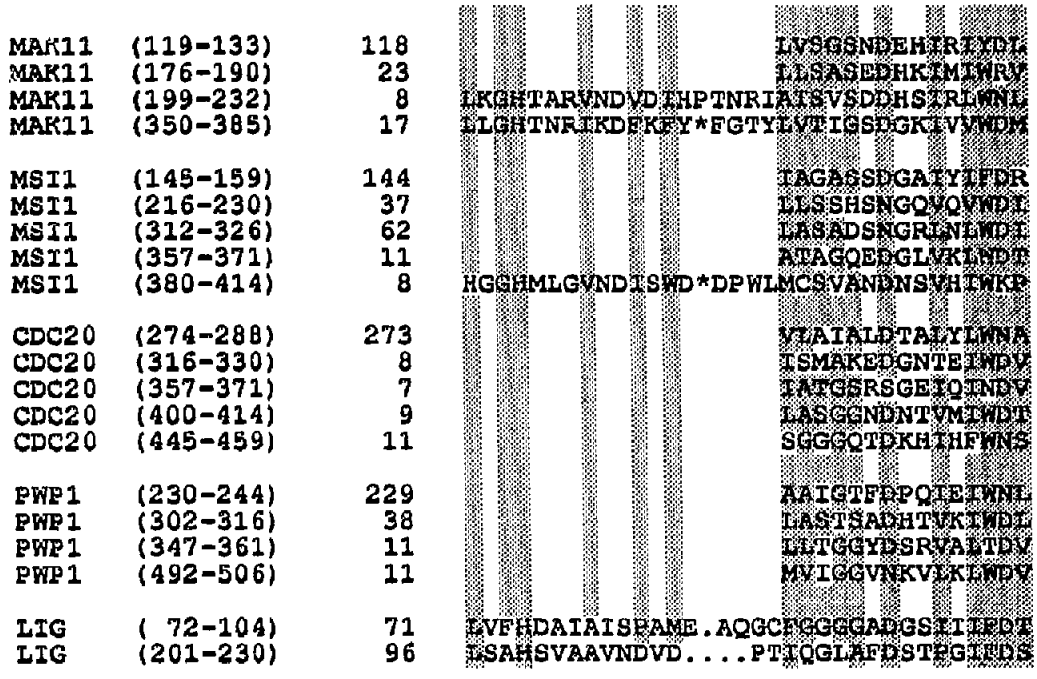
A.

WD-40 repeat consensus		(A)	(B)
	(n1)	LxGHxxxIxxΦxδ	(n2) ΦΦSGGxDxxΦxIWDδ
		F L V	TAA N S C LFN VY

B.

CDC4	(377-409)	376	LRGHMTSVITCLQF.EDNYVITGADDKMIRVYDS
CDC4	(418-450)	8	LSGHDGGVWALKYA.HGGILVSGSTDRTVRVWDI
CDC4	(459-494)	8	FEGHNSTVRCLDIV*NIKYIVTGSRDNTLHVWKL
CDC4	(526-557)	31	LRGHMASVRTVSG..HGNIVVSGSYDNTLIVWDV
CDC4	(566-599)	8	LSGHTDRIYSTIYDHERKRCISASMDTTIRIWDL
CDC4	(628-659)	28	LQGHTALVGLLRL..SDKFLVSAAADGSIRGWDA
Tβ	(51- 84)	50	LRGHLAKIYAMHWGTDSRLLVSASQDGKLIIWDS
Tβ	(112-126)	8	VACGGLDNICSIYNL
Tβ	(139-171)	8	LAGHTGYLSCCRFL.DDNQIVTSSGDTTCALWDI
Tβ	(180-213)	8	FTGHTGDVMSLSLAPDTRLFVSGACDASAKLWDV
Tβ	(222-255)	8	FTGHESDINAICFFPNGNAFATGSDDATCRLFDL
Tβ	(285-299)	10	LLAGYDDFNCNVWDA
Tβ	(308-340)	8	LAGHDNRVSCLGVTDDGMAVATGSWDSFLKLWN
STE4	(88-121)	87	LKGHNNKISDFRWSRDSKRILSASQDGFMLIWDS
STE4	(149-163)	8	VASAGLNNNCTIYRV
STE4	(177-209)	13	PKGHTCYISDIEFT.DNAHILTASGDMTCALWDI
STE4	(243-257)	14	FASCGSDGYTYIWDS
STE4	(285-299)	8	IVAGSDNGAINMYDL
STE4	(364-378)	45	MYSCYTDIGCVVWDV
STE4	(387-420)	8	LEGHGGRVTGVRSSPDGLAVCTGSWDSTMKIWSP
12.3	(11- 45)	10	LKGHNGWVTQIATT*FPDMILSASRDKTIIMWKL
12.3	(59- 92)	13	LRGHSHFVSDVVISSDGQFALSGSWDGTLRLWDL
12.3	(101-134)	8	FVGHTKDVLSVAFSSDNRQIVSGSRDKTIKLWNT
12.3	(165-179)	11	IVSCGWDKLVKVWNL
12.3	(188-221)	8	HIGHTGYLNTVTVSPDGSLCASGGKDGQAMLWDL
12.3	(247-261)	6	WLCAATGPSIKIWDL
12.3	(298-311)	17	LFAGYTDNLVRVWQV
E(spl)	(429-461)	428	TLSHGEVVCAVTIS.NPTKYVVTGGKGCVKVWDI
E(spl)	(494-508)	13	LIVGGEASNLSIWDL
E(spl)	(538-552)	10	CFSCCSDGNIAVWDL
E(spl)	(561-594)	8	FQGHTDGASCIDISPDGSRLWTGGLDNTVRSWDI
E(spl)	(644-676)	49	LHLHESCVLSLRFAACGKWFVSTGKDNLLNAWRT
PRP4	(261-294)	260	LVGHERRISDVKYHPSGKFIGSASHDMTWRLWDA
PRP4	(303-336)	8	QEGHDKGVFSLSFQCDGSLVCSGGHDSLSMLWDI
PRP4	(345-378)	8	LAGHSKPIYTVAWSPNGYQVATGGGDGIINVWDI
PRP4	(410-424)	12	LVSCGYDNLINVYSS
PRP4	(433-465)	8	LAGHTDKIISLDISNNSHFLVSGGWDRSIKLWN
AER2	(458-472)	457	LATGAEDRLIRIWDI
AER2	(481-514)	8	IQGHEQDIYSLDYFPSGDKLVSGSGDRTVRIWDL
AER2	(542-556)	8	IAAGSLDRAVRVWDS
AER2	(572-605)	15	GTGHKDSVYSVVFTRDGQSVVSGSLDRSVKLWNL
AER2	(626-659)	20	YIGHKDFVLSVATTQNDEYILSGSKDRGVLFWDK
AER2	(667-707)	7	IQGHRNSVISVAVA*EYNVFATGSGDCKARIWKY
COR	(75-109)	74	FNGHKSAVLDIAFH*NENLVGSVSEDCNICIWGI
COR	(125-159)	15	LSGHKRKVGTTSFG*ADNVAVTSSGDFLVKTWDV
COR	(168-201)	8	VEGHSDMITSCEWNHNGSQIVTTCKDKKARVFDP
AAC3	(139-153)	138	IASSGSDGTVRVWNE
AAC3	(183-217)	29	LKGHDGSIEKISWS*NNDLLASAGTDKVIKIWDV
AAC3	(314-347)	96	LYGHTASIYCMEFDPTGKYLAAGSADSIVSLWDI



MAK11	(119-133)	118	LVSGSNDEHIRIYDL
MAK11	(176-190)	23	LLSASEDHKIMIWRV
MAK11	(199-232)	8	IKGHTARVNDVDIHPTNRIATSVSDDHSIRLWNL
MAK11	(350-385)	17	LLGHTNRIKDFKFY*FGTYLVTIGSDGKIVVWDM
MSI1	(145-159)	144	IAGASSDGAIYIFDR
MSI1	(216-230)	37	LLSSHSNGQVQVWDI
MSI1	(312-326)	62	LASADSNGRINLWDI
MSI1	(357-371)	11	ATAGQEDGLVKIWDT
MSI1	(380-414)	8	HGGHMLGVNDISWD*DPWLMCSVANDNSVHIWKP
CDC20	(274-288)	273	VIAIALDTALYLWNA
CDC20	(316-330)	8	ISMAKEDGNTEIWDV
CDC20	(357-371)	7	IATGSRSGEIQINDV
CDC20	(400-414)	9	LASGGNDNTVMIWDT
CDC20	(445-459)	11	SGGGQTDKHIHFWNS
PWP1	(230-244)	229	AAIGTFDPQIEIWNL
PWP1	(302-316)	38	LASTSADHTVKIWDL
PWP1	(347-361)	11	LLTGGYDSRVALTDV
PWP1	(492-506)	11	MVIGGVNKVLKLWDV
LIG	(72-104)	71	LVFHDAIAISPAME.AQGCFGGGGADGSIIIFDT
LIG	(201-230)	96	LSAHSVAAVNDVD....PTIQGLAFDSTPGIFDS

Fig. 1. (A) The WD-40 consensus sequence, which represents the consensus for a single repeat, notes those positions at which a single amino acid or group of similar amino acids predominates. n1 and n2 represent stretches of amino acids, variable in both sequence and length, that separate individual members of the WD-40 repeat, and elements A and B within the repeat, respectively. x = any amino acid, ϕ = hydrophobic residues preferred, δ = non-charged amino acid.

(B) Alignment of WD-40 repeats constructed manually. The numbering of the amino acid residues (between parentheses) is according to the references (see text). The number of amino acids constituting n1 is indicated. In the absence of an element recognizable as A, 19 residues (the mean length of the segment otherwise occupied by element A together with n2) have been subtracted. The preferred length for the spacer n1, between individual repeats, is thus around 8 residues. If the length of n2 exceeds 6 amino acids, the extra amino acids are indicated by an asterisk. Some members of the WD40-family are not listed, either because their sequences are not yet available (PRP17), or because they are closely related to members already listed (e.g. the different Gβ-subtypes and Cblp which, upon closer inspection, might be considered as the *Chlamydomonas* homologue of the 12.3-protein (82% similarity).

the [D,N]xxxxx[W,F,Y][D,N] pattern in part B. The connecting regions, n1 and n2, contain many charged residues and prolines. The distances between the individual repeat units (n1 in Fig. 1) vary largely, but n1=8 amino acids seems to be favoured, resulting in a total repeat length of about 42–43 amino acids.

In some motifs, considerable deviation from the consensus sequence is observed: some repeats would not have been recognized as such were it not for the fact that the same protein contains other, better conserved repeats with a proper spacing relative to the more degenerate motif. Obviously, even more divergent versions of the motif may have been missed. On a number of occasions, only element B of the repeat could be identified (e.g. PWP1 and CDC20 contain no part A at all), suggesting that part A may be dispensable. In these cases, no obvious homologies between non-A parts immediately upstream of part B were observed. In the databases, no protein was found that contains a complete WD-40 motif (A+B) in the absence of other sequences homologous to the repeat. This might indicate that multiple copies of the motif (or part of the motif) are required to render it functional.

What could be the function of the WD-40 repeat? Its presence implies some common structural feature(s) for the family members, which may, but need not, imply functional relationships. However, the function(s) of the motif is not known for any of the members of the WD-40 family. The members of the family do not share any obvious functional properties. Furthermore, the subcellular location (if known) of these proteins varies largely: some are found in the nucleus (e.g. CDC4, PRP4 and TUP1), others in the plasma membrane (e.g. Espl, Gβ-subunits, STE4) or as a cytoskeletal component (coronin). None of the members of the family has been crystallized nor has extensive mutational analysis been done.

An intriguing observation concerns the fact that, in yeast, some of the members of the WD-40 family are genetically associated with members of another family of repeat-containing proteins: the tetratricopeptide (TPR) gene family (for a review see [7]). Based on this finding, it has been proposed that members of the WD-40 family work in pairs with members of the TPR-family [7]. Interestingly, TPR-proteins contain multiple repeats of a 34 amino acid sequence motif of unknown function. As with the members of the WD-40 family, members of the TPR-family are involved in many cellu-

lar functions and are found in a wide variety of subcellular locations. In a single case, a direct physical association between members of the two families has been demonstrated: the TPR-protein, Ssn6, when paired with TUP1, a member of the WD-40 family, functions as a general repressor of transcription in yeast [26,27]. Although the evidence is suggestive, it remains to be established whether TUP1 and Ssn6 bind to each other via their respective repeats, but undoubtedly this hypothesis will soon be tested. Clearly, with the relative ease of genetic analysis and the availability of many mutant strains, the yeast *Saccharomyces cerevisiae* provides an attractive system in which to study the function(s) of the WD-40 repeat.

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