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WDR26: A Novel Gβ-Like Protein, Suppresses MAPK Signaling Pathway

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Abstract WD40 repeat proteins play important roles in a variety of cellular functions, including cell growth, proliferation, apoptosis, and intracellular signal transduction. Mitogen-activated protein kinases (MAPKs) are evolutionary conserved enzymes in cell signal transduction connecting cell-surface receptors to critical regulatory targets within cells and control cell survival, adaptation, and proliferation. Previous studies revealed that G-protein coupled receptors (GPCRs) play important roles in the signal transduction from extracellular stimuli to MAPKs and the WD40-containing Gβ proteins as well as Gβ-like proteins are involved in the stimulation and regulation of the MAPK signaling pathways. Here we report the identification and characterization of a novel human WD40 repeat protein, WD40 repeat protein 26 (WDR26). The cDNA of WDR26 is 3,729 bp, encoding a Gβ-like protein of 514 amino acids in the cytoplasm. The protein is highly conserved in evolution across different species from yeast, *Drosophila*, mouse, to human. Northern blot analysis indicates that *WDR26* is expressed in most of the examined human tissues, especially at a high level in skeletal muscle. Overexpression of WDR26 in the cell inhibits the transcriptional activities of ETS proteins, ELK-1 and c-fos serum response element (SRE), mediated by MEKK1. These results suggest that WDR26 may act as a negative regulator in MAPK signaling pathway and play an important role in cell signal transduction. J. Cell. Biochem. 93: 579–587, 2004.

Key words: WD40 repeat protein; WD-40; Gβ-like protein; WDR26; MAPK signaling pathway

Members of the WD-40 family of proteins, including the well-known Gβ subunits of heterotrimeric GTP-binding proteins, play a variety of roles in intracellular signaling, RNA processing and degradation, gene expression, vesicular traffic and fusion, cytoskeletal assembly, and the cell cycle as described in earlier reviews [Smith et al., 1999; Yu et al., 2000; Cabrera-Vera et al., 2003]. All proteins within the WD-40 family contain repeating sequences that are separated by approximately 40 amino acids. Each WD-40 repeat consists of two signature sites: a poorly conserved site A containing a glycine and histidine (GH) pair, and a well-conserved site B containing a tryptophan and aspartate (WD) pair. Although each protein contains four to eight WD-40 repeating motifs, the separating distance and the actual sequence of individual repeats are high variable. The functional diversity of WD-40 proteins suggests that WD-40 motifs may not necessarily confer any particular function while it may contributes to the formation of anti-parallel β-strands that stabilize the three-dimmensional β-propellers [Sondek et al., 1996; Smith et al., 1999].

Abbreviations used: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-related kinases; JNK, Jun amino-terminal kinases; GPCRs, G-protein coupled receptors; WDR26, WD40 repeat protein 26; MAPKK, MKK, or MEK, MAPK kinase; MAPKKK, MAPKK kinase; MEKK MEK kinase; EST, expressed sequence tags; WDR, WD40 repeat protein; SRE, c-fos serum response element; SRF, serum response factor; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's sodium.

Ying Zhu, Yuequn Wang, and Xiushan Wu contributed equally to the work.

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Mitogen-activated protein kinases (MAPK) are common components in signal transduction connecting cell-surface receptors to critical regulatory targets within cells and control a wide range of processes from the cell-cycle arrest and mating in yeast to cell proliferation and differentiation in metazoans (reviewed by Feng et al., 1998; Cobb, 1999; Kyriakis and Avruch, 2001). The MAPK cascades are regulated through three-tiered cascades composed of a MAPK, MAPK kinase (MAPKK, MKK, or MEK) and a MAPKK kinase or MEK kinase (MAPKKK or MEKK), which work in series and comprise a module (reviewed by English et al., 1999). In mammals there are at least four distinct groups of MAPKs: extracellular signal-related kinases (ERK)-1/2, Jun amino-terminal kinases (JNK1/2/3), p38 proteins (p38 $\alpha/\beta/\gamma$), and ERK5, which are activated by specific MAPKKs and phosphorylate specific cellular targets. As reviewed by Chang and Karin [2001], one of the most explored targets of MAPK signaling modules is transcription factors, such as c-jun, Elk-1 which regulates transcription immediate early gene expression through binding to the serum response element (SRE) [Gille et al., 1995]. For example, most MAPKs phosphorylate ETS transcription factors that are involved in induction of *fos* genes, whose products heterodimerize with Jun proteins to form activation protein 1 (AP-1) complexes [Treisman, 1996]. Presumably each MAPKKK confers responsiveness to distinct stimuli, such as growth factors, hormones, stress, and inflammation.

The multiplicity of both GPCR and MAPK signaling pathways suggests the activities of both G-protein coupled receptors (GPCRs) and MAPKs is tightly regulated, depending on cooperation of a number of activators and suppressors. Although, we have known a lot about the activation process from receptors to MAPKs, the knowledge on the mechanism of negative regulation is far from well developed. Recently, our laboratory identified a human PAK1-interacting protein, hPIP1, which is a negative regulator of p21-activated protein kinase (PAK). Overexpression of hPIP1 can inhibit the Cdc42-stimulated PAK kinase activity as well as PAK-mediated JNK and NF-κB signaling pathways through interaction with the N-terminal regulatory domains of PAK1 [Xia et al., 2001b]. Here we report the identification and characterization of a novel Gβ-like protein, WD40 repeat protein 26 (WDR26). Northern blot analysis demonstrates that this gene is expressed in most of the human tissues, and especially at high levels in fetal brain and skeletal muscle of both fetal and adult stages. This cytoplasmic protein contains five Gβ-like WD40 repeats similar to hPIP1 and have similar sequence to WD40 repeat protein 5 (WDR5), which can be induced by BMP-2 and dramatically accelerate the program of osteoblastic differentiation [Gori et al., 2001]. Transfection of *WDR26* in mammalian cells can significantly suppress the transcriptional activation of MEKK-mediated SRE and ELK-1. These results suggest that this new Gβ-like WD40 repeat protein may act as a repressor in MAPK signaling pathway to mediate cellular functions.

MATERIALS AND METHODS

Cloning the Full-Lenghth cDNA and Bioinformatics Analysis

In order to identify novel Gβ-like proteins in cell signal transduction, we used the consensus sequence of WD40 region as the subject to search human expressed sequence tags (EST) database with BLASTx algorithm (http://www.ncbi.nlm.nih.gov/BLAST). Through combined BLAST search and PCRs analysis as previously described [Zeng et al., 2002], we identified four overlapping ESTs—AW968761, BG825203, BI757309, and BF971220, belonging to the same novel gene. A heart cDNA library was constructed as PCR template with the kits obtained from TAKARA®. 5′-RACE PCR was performed with TAKARA 5′-RACE Core Kit to confirm the 5′ terminus of *WDR26*. Jellyfish 1.4 was used to find the open reading frame (ORF) and the deduced translated product. Then, the coding sequence was cloned from human heart library. All the primers and reaction conditions for these PCRs are listed in Table I and these PCR products were confirmed by sequencing (Biotech®). For mammalian expression, the *WDR26* ORF was subcloned into the BamHI site of pCMV-tag2C (Stratagene®). The full-length sequence of *WDR26* was submitted to GeneBank with an accession number AY304473.

BLASTn (http://www.ncbi.nlm.nih.gov/BLAST) and Pfam 9.0 [Bateman et al., 2002] were used to analyze genomic structure and the protein domain, respectively. The homologues of

TABLE I. Sets of Specific Oligonucleotide Primers and PCR Conditions

Primers	Sequences	Programs	Cycles
E1	5′-GCAAGAGTCAGGATGTCG-3′	94°C for 30 s; 50°C for 30 s; 72°C for 1 min	30
E2	5′-CCAAAGCTCAGAGCAGTC-3′		
E3	5′-GGAGGCACTTCAAGTTCTAC-3′	94°C for 30 s; 50°C for 30 s; 72°C for 1 min	30
E4	5′-TCCATCACTCAAGCACCA-3′		
E5	5′-ACTGGAGGTCAGCGTGGGC-3′	94°C for 30 s; 52°C for 30 s; 72°C for 1 min	30
E6	5′-CGGGCTTCAGAAATGGTT-3′		
RT1	5′-TCAGCGGCGTCAAT-3′	30°C for 10 min; 50°C for 60 min; 80°C for 2 min	1
S1	5′-CGGAATTTGGTAGCAGAAGG-3′	94°C for 4 min; 56°C for 1 min; 72°C for 1 min	30
A1	5′-CATGGAAGGAGACTGGGATAAG-3′		
S2	5′-CAGTCTGGTTGAGCCCTAA-3′	94°C for 1 min; 58°C for 1 min; 72°C for 1 min	30
A2	5′-GGCACTTCAAGTTCTACGC-3′		
Os	5′-CAGGATCCCCGCCTCCTCTTCCT-3′	94°C for 30 s; 55°C for 30 s; 72°C for 2 min	30
Oa	5′-GGATCCCAACTATCCATGCTACTGCAT-3′		

E1 to E6 are for expressed sequence tags (ESTs) analysis (E1 at the 3 terminus of AW968761 and E2 at 5 terminus of BG825203, E3 at the 3 terminus of BG825203 and E4 at the 5 terminus of BI757309, E5 at the 3 terminus of BI757309, and E6 at the 5 terminus of BF971220). RT1, S1, A1, S2, and A2 are for 5′-RACE PCR. Os and Oa are for cloning the ORF of WD40 repeat protein 26 (WDR26) from heart cDNA library. The template of all the PCR are human heart cDNA library.

WDR26 were found with BLASTp (http://www.ncbi.nih.nlm.gov), and the most similar hits of them were used to perform sequence alignment with ClustalW 1.8 [Jeanmougin et al., 1998] and phylogenetic tree analysis with MegAlign program (DNAstar, Inc., Madison, WI).

Cell Culture, Transient Transfection, Immunocytochemistry, and Fluorescence Imaging

COS-7 was maintained in Dulbecco's modified Eagle's sodium (DMEM), supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2. Cells were transfected with pCMV-WDR26 with LipofectAMINE (Invitrogen, Carlsbad, CA) according to the method described before [Xia et al., 2001a]. Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde for 15 min at room temperature (RT), then blocked with 0.2% bovine serum albumin (BSA) for 30 min, followed by incubation with monoclonal antibody against Flag (M2 monoclonal, Sigma-Aldrich, St. Louis, MO) for 1 h at RT, wash with PBS (pH 7.4), and incubated with FITC-conjugated phalloidin anti-mouse IgG (Molecular Probe, Inc., Eugene, OR). Actin filaments were labeled by rhodamine-conjugated phalloidin (Sigma), and nuclear were stained with DAPI. Fluorescent images of cells were captured on a cooled charge-coupled device camera mounted on an Olympus inverted research microscope using Ultraview imaging software (Olympus®, Inc., Melville, NY) [Guo et al., 2003].

Transient Expression Reporter Gene Assays

COS-7 cells were transfected with LipofectAMINE (Invitrogen) according to the method described before [Xia et al., 2001a]. The reporter constructs for SRE-Luc was obtained from Stratagene and reporter constructs for ELK-1-Luc were kindly provided from Dr. K.L. Guan at the University of Michigan. Luciferase activity assays were performed according to the protocols of Stratagene. Each experiment was performed in triplicates and each assay was repeated at least three times. The mean of the data from three individual transfected wells are presented.

Northern Analysis of *WDR26* Expression in Human Fetal and Adult Tissues

To study the expression pattern of *WDR26* in different human tissues at fetal and adult stages, a RNA filter of purified adult human mRNAs of multiple tissues (Clontech, Inc., Palo Alto, CA) and a RNA filter of total RNA of fetal (gestation 17-week) multiple tissues were hybridized with specific ^{32}P-labeled cDNA as described previously [Luo et al., 2002]. The therapeutically aborted fetuses (gestation 17 week) were obtained under the approval of Health Center of Changsha Hospital of Women and Children, China, with the consent of the patients and the regulation of university policy. The RNA filter was prepared as described in previous studies [Luo et al., 2002].

RESULTS

Molecular Cloning and Domain Structure of WDR 26

During the search for novel Gβ-like proteins in cell signaling and development, we identified a novel gene through scanning the human EST database with WD40 consensus sequence. Combined the results of the overlapping EST

analysis, Northern blotting and 5′-ACE the 3,729 bp full-length cDNA of the novel gene was confirmed, which was named *WDR26* as approved by HUGO nomenclature committee. The nucleotide sequence data reported here is available in GenBank with the accession number AY304473.

Alignment between the cDNA sequence and human genome indicates that *WDR26* is identical to the genomic sequence of PAC RP11-397G23 on chromosome 1q42.13, spanning approximately 46 kb in reverse manner on the genome and organized into 14 exons. All exon–intron junctions contain the gt/ag consensus splice site (the data not shown here). The translation start codon ATG is in the second exon and the TAA stop codon in the 14th exon. The complete cDNA of *WDR26* consists of an ORF of 1,545 bp from 199 to 1,743, a 198 bp 5′-untranslated terminus and a long 3′-untranslated terminus of 2,029 bp with a consensus polyadenylation signal (AATAAA) (Fig. 1A). The deduced WDR26 protein is 514 amino acids and its calculated relative molecular mass is 58,603 Da (~59 kDa) (Fig. 1A). This protein contains a WD40 region (199 amino acid to 494 amino acid), including five WD40 repeats in tandem arrays (Fig. 1B).

WDR26 Belongs to a Novel Protein Family Conserved During Evolution

The amino acid sequence of WDR26 is highly identical to its homologues in other species, whose functions are unknown. Sequence alignment of these proteins demonstrates that it is one of the most conserved proteins during evolution (Fig. 2A). The sequence identity between human and mouse homologues is even higher than that of Gβ-subunit family of proteins. We tried to analyze the evolutionary relationship between the 5-WD40-repeat Gβ-like proteins and Gβ proteins with phylogenetic tree analysis (Fig. 2B). The data shows that despite their similar domain structure, these Gβ-like proteins such as hPIP1 and WDR26 belong to new subfamilies of proteins differing from the Gβ-subfamily of proteins. Furthermore, WDR26 and its homologues make up a new conserved subfamily not known before (Fig. 2B).

WDR 26 Is Widely Expressed at Embryo and Adult Stages

To characterize the transcript of *WDR26* with respect to its size and expression distribution, adult and fetal multiple tissue northern blot were performed using *WDR26* cDNA as the probe. A 3.7 kb transcript of *WDR26* was detected in most adult tissues with the highest expression detected in skeletal muscle and heart (Fig. 3A). In human fetal tissues, *WDR26* was detected mostly in skeletal muscle and brain with low level of expression in liver, lung, and heart (Fig. 3B). The results indicate that *WDR26* is expressed in most of human tissues and the expression level is much higher in skeletal muscle than in other tissues both during early developmental stages and in adult tissues. Furthermore, we noticed that the expression of *WDR26* is similar to *hPIP1*, both of which are strongly expressed in skeletal muscle.

WDR26 Protein Is a Cytoplasmic Protein Suppressing SRE- and ELK-1-Mediated Transcriptional Activation

To examine the subcellular location of WDR26, the pCMV-WDR26 was transfected into COS-7 cells, and 48 h after the transfection, the cells were visualized with epifluorence microscope after labeled with FITC rhodamine for actin and DAPI for nuclei. As shown in Figure 4A, WDR26 protein distributes evenly in cytoplasm when overexpressed in the cells (Fig. 4A, arrow). The combined image shows that WDR26 protein with actin and nucleus in the cell (Fig. 4D). Although we consider WDR26 as a cytoplasmic protein, we could not rule out the possibility that the protein also exists in the nucleus as shown in the fluorescence staining of the protein in the cell.

Recent studies have revealed the importance of multiple intracellular signaling pathways mediated by activation of GPCRs, through which GPCRs transduce extracellular signals into nucleus to regulate the activity of various transcript factors, such as c-fos serum response element (SRE), in cellular processes. Although WDR26 shares WD-40 repeat domains with Gβ-subunits, the potential role of WDR26 is not clear. As a first step in our understanding of WDR26 in cell signal transduction, we examined whether WDR26 is directly or indirectly involved in the regulation of transcription factors, especially in the MAPK pathway. As an important nuclear effector of MAPK signaling pathway, the c-fos SRE forms a ternary complex together with serum response factor (SRF) and ETS proteins. To examine the effect

A

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1    GCG GCC GCC GCA TCC TCA GCC ACC GTC GCA GCT GCC TCC GCC ACC ACC GCC GCC TCC TCT TCC TTG GCC ACC CCA GAA CTG GGC AGC AGC
91    CTC AAG AAG AAG AAG CGG CTC TCC CAG TCA GAT GAG GAT GTC ATT AGG CTA ATA GGA CAG CAC TTG AAT GGC TTA GGG CTC AAC CAG ACT
181   GTT GAT CTC CTC ATG CAA GAG TCA GGA TGT CGT TTA GAA CAT CCT TCT GCT ACC AAA TTC CGA AAT CAT GTC ATG GAA GGA GAC TGG GAT
1                    M  Q  E  S  G  C  R  L  E  H  P  S  A  T  K  F  R  N  H  V  M  E  G  D  W  D
271   AAG GCA GAA AAT GAC CTG AAT GAA CTA AAG CCT TTA GTG CAT TCT CCT CAT GCT ATT GTG GTA AGA GGC GCA CTT GAA ATC TCT CAA ACG
27    K  A  E  N  D  L  N  E  L  K  P  L  V  H  S  P  H  A  I  V  V  R  G  A  L  E  I  S  Q  T
361   TTG TTG GGA ATA ATT GTG AGG ATG AAG TTT TTG CTG CTG CAG CAG AAG TAC CTA GAA TAC CTG GAG GAT GGC AAG GTC CTG GAG GCA CTT
57    L  L  G  I  I  V  R  M  K  F  L  L  L  Q  Q  K  Y  L  E  Y  L  E  D  G  K  V  L  E  A  L
451   CAA GTT CTA CGC TGT GAA TTG ACG CCG CTG AAA TAC AAT ACA GAG CGC ATT CAT GTT CTT AGT GGG TAT CTG ATG TGT AGC CAT GCA GAA
87    Q  V  L  R  C  E  L  T  P  L  K  Y  N  T  E  R  I  H  V  L  S  G  Y  L  M  C  S  H  A  E
541   GAC CTA CGT GCA AAA GCA GAA TGG GAA GGC AAA GGG ACA GCT TCC CGA TCT AAA CTA TTG GAT AAA CTT CAG ACC TAT TTA CCA CCA TCA
117   D  L  R  A  K  A  E  W  E  G  K  G  T  A  S  R  S  K  L  L  D  K  L  Q  T  Y  L  P  P  S
631   GTG ATG CTT CCC CCA CGG CGT TTA CAG ACT CTC CTG CGG CAG GCG GTG GAA CTA CAA AGG GAT CGG TGC CTA TAT CAC AAT ACC AAA CTT
147   V  M  L  P  P  R  R  L  Q  T  L  L  R  Q  A  V  E  L  Q  R  D  R  C  L  Y  H  N  T  K  L
721   GAT AAT AAT CTA GAT TCT GTG TCT CTG CTT ATA GAC CAT GTT TGT AGT AGG AGG CAG TTC CCA TGT TAT ACG CAG CAG ATA CTT ACG GAG
177   D  N  N  L  D  S  V  S  L  L  I  D  H  V  C  S  R  R  Q  F  P  C  Y  T  Q  Q  I  L  T  E
811   CAT TGT AAT GAA GTG TGG TTC TGT AAA TTC TCT AAT GAT GGC ACT AAA CTA GCA ACA GGA TCA AAA GAT ACA ACA GTT ATC ATA TGG CAA
207   H  C  N  E  V  W  F  C  K  F  S  N  D  G  T  K  L  A  T  G  S  K  D  T  T  V  I  I  W  Q
901   GTT GAT CCG GAT ACA CAC CTG CTA AAA CTG CTT AAA ACA TTA GAA GGA CAT GCT TAT GGC GTT TCT TAT ATT GCA TGG AGT CCA GAT GAC
237   V  D  P  D  T  H  L  L  K  L  L  K  T  L  E  G  H  A  Y  G  V  S  Y  I  A  W  S  P  D  D
991   AAC TAT CTT GTT GCT TGT GGC CCA GAT GAC TGC TCT GAG CTT TGG CTT TGG AAT GTA CAA ACA GGA GAA CTA AGG ACA AAA ATG AGC CAG
267   N  Y  L  V  A  C  G  P  D  D  C  S  E  L  W  L  W  N  V  Q  T  G  E  L  R  T  K  M  S  Q
1081  TCT CAT GAA GAC AGT TTG ACA AGT GTG GCT TGG AAT CCA GAT GGG AAG CGC TTT GTG ACT GGA GGT CAG CGT GGG CAG TTC TAT CAG TGT
297   S  H  E  D  S  L  T  S  V  A  W  N  P  D  G  K  R  F  V  T  G  G  Q  R  G  Q  F  Y  Q  C
1171  GAC TTA GAT GGT AAT CTC CTT GAC TCC TGG GAA GGG GTA AGA GTG CAA TGC CTT TGG TGC TTG AGT GAT GGA AAG ACT GTT CTG GCA TCA
327   D  L  D  G  N  L  L  D  S  W  E  G  V  R  V  Q  C  L  W  C  L  S  D  G  K  T  V  L  A  S
1261  GAT ACA CAC CAG CGA ATT CGG GGC TAT AAC TTC GAG GAC CTT ACA GAT AGG AAC ATA GTA CAA GAA GAT CAT CCT ATT ATG TCT TTT ACT
357   D  T  H  Q  R  I  R  G  Y  N  F  E  D  L  T  D  R  N  I  V  Q  E  D  H  P  I  M  S  F  T
1351  ATT TCA AAA AAT GGC CGA TTA GCT TTG TTA AAT GTA GCA ACT CAG GGA GTT CAT TTA TGG GAC TTG CAA GAC AGA GTT TTA GTA AGA AAG
387   I  S  K  N  G  R  L  A  L  L  N  V  A  T  Q  G  V  H  L  W  D  L  Q  D  R  V  L  V  R  K
1441  TAT CAA GGT GTT ACA CAA GGG TTT TAT ACA ATT CAT TCA TGT TTT GGA GGC CAT AAT GAA GAC TTC ATC GCT AGT GGC AGT GAA GAT CAC
417   Y  Q  G  V  T  Q  G  F  Y  T  I  H  S  C  F  G  G  H  N  E  D  F  I  A  S  G  S  E  D  H
1531  AAG GTT TAC ATC TGG CAC AAA CGT AGT GAA CTG CCA ATT GCG GAG CTG ACA GGG CAC ACA CGT ACA GTA AAC TGT GTG AGC TGG AAC CCA
447   K  V  Y  I  W  H  K  R  S  E  L  P  I  A  E  L  T  G  H  T  R  T  V  N  C  V  S  W  N  P
1621  CAG ATT CCA TCC ATG ATG GCC AGC GCC TCA GAT GAT GGC ACT GTT AGA ATA TGG GGA CCA GCA CCT TTT ATA GAC CAC CAG AAT ATT GAA
477   Q  I  P  S  M  M  A  S  A  S  D  D  G  T  V  R  I  W  G  P  A  P  F  I  D  H  Q  N  I  E
1711  GAG GAA TGC AGT AGC ATG GAT AGT TGA TGG TGA ATT TGG AGC AGA CGA CTT CTG TTT AAC TTA AAA TTA GTC GTA TTT TAA TGG CTT GGG
507   E  E  C  S  S  M  D  S  *
1801  ATT TGG TGC AAA CAA ACA TGA TTG ATA GCT GGA CAG ACA TGC TCG TCA TGA AAA AAG AAC CAT TTC TGA AGC CCG ATT GGG GCC AAA CAT
1891  TTA CAC CTT GCT TCA TAG TAA CCA GTT GAG ATG AAG CAC GTC GTT AGA ACG TTG TTG GAC ACC ATG TTG AAT TAT TCC CCC ATC GGT TGT
1981  GAA GAA CTG TGC TAC ATT CAG GCT TAC CCA TTG AAC TCA GTA TAT ATA TTT TTT TCC TTC CTG TCT TTT GTC TGG CAG GAT ACC ATT CTT
2071  GTT GCT CTT CTG TGT AAT GAA GTT TAA ATG CTT GTT TGG AAA ACT TTA TTT AAC AGT TTA GAA GGC TTG ATA GAA AGA GTG CAT TAG TCT
2161  GAA GAG TAT ACA TTG GAT AGG AAA GAA TTT CCT TCT TTT GTT TCT CCA AAT CTT TCC GCC TTA TTT AGC TTG AGA TCT TTG CAG CTT GGT
2251  TCA TGG ATT CTA GCC TTG CCC GTT GCG CAG TAT ATA CTG ATC CAG ATG ATA AAC CAG TGA ACT ATG TCA AAA GCA CTC TCA ATA TTA CAT
2341  TTG ACA AAA AGT TTT GTA CTT TTC ACA TAG CTT GTT GCC CCG TAA AAG GGT TAA CAG CAC AAT TTT TTA AAA ATA AAT TAA GAA GTA TTT
2431  ATA GGA TTA AAG TGA CTT CAT TTG TAT ACA TTT GGA ATC TAA ACC AGC TTA AAA ACA GTT TCC TCA ATG ACT TAG ATA CAC AGT ATA ACT
2521  GAT GCT CTT CTG GAA TAC CAC ATG AGA CAT GGT CAG AAA CAG TGC TTG GAA GGA CAT TAC ACA AGA AAT TCA GAG TAA TGC TTT GAA GAT
2611  TTC CCC CCT TTT GTT TTA TTC CTG AAG GAA CAT CAG TAC CCG ATC TTG AAG AAA TTC AAG ATT CAA AAA GAA TTT TAA ATA CAC CAA CAT
2701  GAG ACA TCA GTA GTC AGT TGG TTT TCA GTA AAG CTT GTT CCA AGT TGT TCT CAA CTT AGG AAG TAA TTT TGG TGT GAT CTA GCA AAA GAG
2791  TAG GAA TCA GCG ATA CAA CCA CTT TGG AAG TTT ATA GTA TAA TTG AAA TTA TTA GAA GAA TTC AGC AGG TTA CAG ACA TAC TTA AAC TGG
2881  GAT TAA AAC CTC ATA GTC ATT TTT CTT AAT TGC CCT TAA TAT TTT GAC ATA TAG GGA TAC ATA AAT TTA AAG AAT ATT TTT TCT CAG TTT
2971  TTT CAG ATA TTG CCA TAC TGA ACC TCA TTC TAA ACT GGT GCT GTG GAT AGT CTT TCC CTC TCC CCT CCT GTT TTA GTT TAA GGA AAG GTT
3061  TCC TTC ATG GAA AAG CAC TTT TAA TTT TGA GAA TTT CAC ATT TAA AAT AAG CCA AAA GCC TGA TGT TCA CAT TGT GCT TTT AAA CTC CGT
3151  AAC AGC TGA ATA CAA CAT AAC ATA GTC TCC CTT GAA ATT CCC TCC CTT TTT CAG TAG AGG ATA AAT AGG GTG ACT CCA GCT GTT GGA ATG
3241  AGA ATG GGG ATA CCC CCA AAA TAA GAT TCT GGT GAA GAA AAA GTC TTG TAC TAA GTT CCC CTT TAG GTG TAG CCA AGT CTA TAA TAT AAT
3331  TAT GAT CAT GAA TAG ACT CCA GAA TCC ACA AAG AGA AGA GGG CTT GGA TGT CAA AAA TTC TTC CAT TGT GGG GTA GGT GGT CGG GGA TGG
3421  GAG AAA GGG AAA AGT CAG GGA AAG GGG AGA AGA AAG AAA AAC ACA TTT GGC CTT AAA ATA GCA TGC ATT CTC CCA GAA GCC CAT AGT AAA
3511  GAA AAT GAC AGA TCG TGG TCA GCA GAC TCT TAT CAA ATT GGT ATG TAA AAG ATT TCT TCA AGC TCC ATT TTG CAG AGA CCA CCA CTT AGA
3601  GAA TCA AAA AAT TCC TGT TTT GAT ACT TCT AAG TTA AAA TAT ATG TTA CAG TTT ATC TGG TAC TTC ATT TTT CTT AAC TAA AAT TAC TTT
3691  TTA CTT TAA GCT TGA ATA AAA ATC TTC ATT GGT AAC TGT
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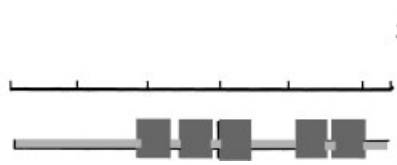

Fig. 1. **A**: Nucleotide sequence and deduced protein sequence of the human WD40 repeat protein 26 (*WDR26*) gene. *WDR26* encodes a polypeptide of 514 amino acids. The initiation ATG and termination TAA codons are boxed and shaded in gray. Amino acids are identified by their one-letter code. The five WD40 repeats are underlined. Both nucleotides and amino acids are numbered at the left side of each line, respectively. The putative polyadenylation signal sequence AATAAA is underlined. **B**: The domain structure of WDR26. The boxes indicate the location of five WD40 repeats with WD-repeat 1 (aa 200–237), WD-repeat 2 (aa 246–283), WD-repeat 3 (291–328), WD-repeat 4 (413–453), and WD-repeat 5 (458–496).

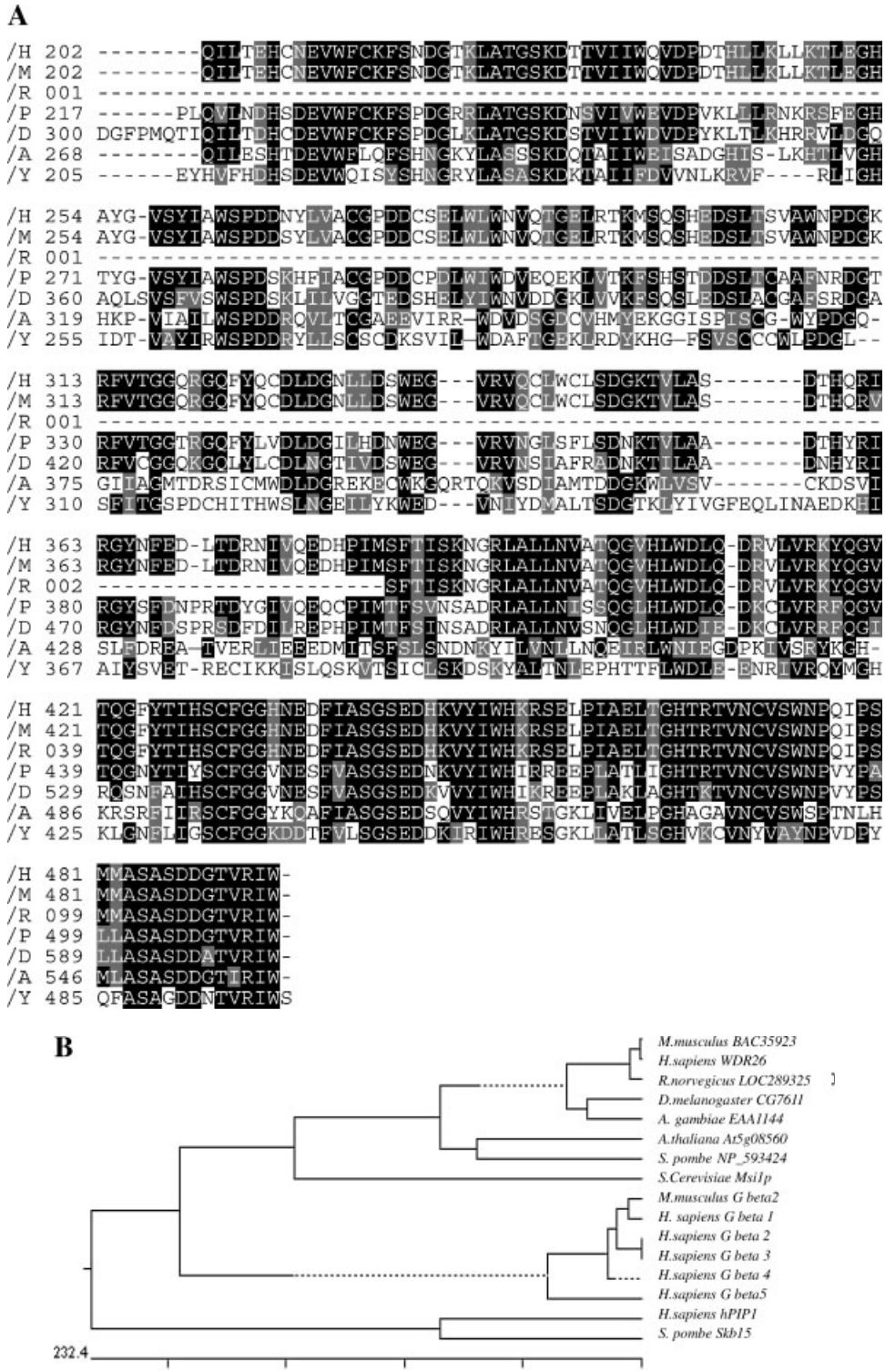

Fig. 2. WDR26 is conserved during evolution. **A**: Comparison of the amino acid sequences of WD40 region among WDR26 and its homologues: H, human WDR26; M, *Mus musculus* BAC35923; R, *Rattus* LOC289325; D, *Drosophila melanogaster* CG7611-PA, A, *Arabidopsis thaliana* NP_196473; P, *Anopheles gambiae* EAA11448.1; Y, *Schizosaccharomyces pombe* trp-asp repeat protein T38653. Identical residues fitting the WD40 repeat consensus have been boxed and are shaded in dark. Human WDR26 protein is 99% identical to *Mus musculus* BAC35923, 56% to *Drosophila melanogaster* CG7611-PA, 38% to *Arabidopsis thaliana* NP_196473, 61% to *Anopheles gambiae* EAA11448.1, 32% to *Schizosaccharomyces pombe* trp-asp repeat protein T38653. **B**: Un-rooted phylogenetic tree analysis of WD40 repeat proteins shows that WDR26 belong to a new subfamily differing from Gβ proteins and the PAK inhibitor hPIP1. WDR26 and its homologues from other species shown in (A), five human Gβ proteins, and several Gβ-like proteins were used in the unrooted phylogenetic tree analysis.

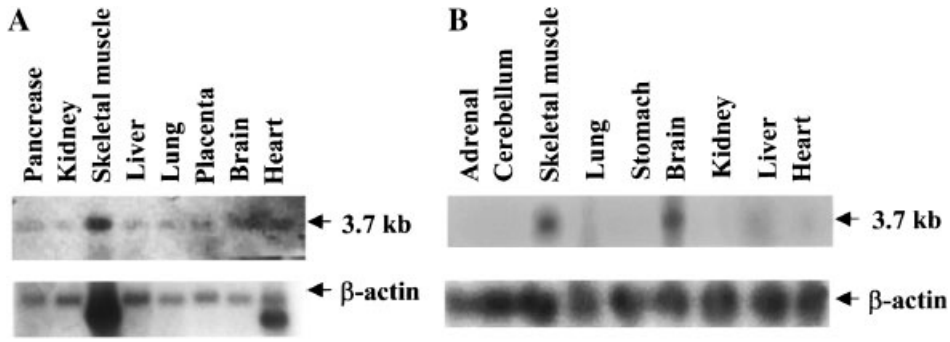

Fig. 3. Expression of *WDR26* in human adult (**A**) and 17-week-fetal tissues (**B**) analyzed using Northern blot. The RNA filters were hybridized with a ^{32}P random-labeled cDNA probe, which contains coding sequence of WDR26. The same filters were also hybridized with β-actin to normalize for loading differences. A band at ~3.7 kb was detected.

of WDR26 in this pathway, we performed reporter gene assays to measure the modulation of SRE and ELK-1 by WDR26 in the cell. COS-7 cells were co-transfected with the expression plasmids pCMV-WDR26, pFC-MEKK, pSRE-Luc, and pFA2-Elk-Luc, which encodes for luciferase controlled by SRE and Elk-1 respectively. As shown in Figure 5A, expression of WDR26 significantly reduced the endogenous SRE-luciferase activity by ~60%. Furthermore, co-expression of WDR26 with MEKK1 strongly inhibited MEKK1-stimulated SRE-luciferase activity, suggesting a potential role of this protein in MEKK-mediated signal transduction. We then tested the effect of WDR26 on the transcriptional activity of Elk-1, a member of the ternary complex. As observed in the SRE-luciferase assays, we found that expression of WDR26 strongly inhibited the endogenous transcriptional activity of Elk-1 and the MEKK-mediated Elk-1 transcriptional activity (Fig. 5B). Taken together, our results suggest that WDR26 is a new WD40 repeat protein that potentially participates in the MAPK signaling pathways in the cell.

DISCUSSION

WD-40 repeat proteins play important roles in a variety of cellular functions from cell proliferation, cell apoptosis, to different cell signal transduction pathways. MAPKs are important signal transducing enzymes that are involved in many facets of cellular regulation; therefore, their activity should be tightly regulated. In this report, we described the identification of a novel Gβ-like protein, WDR26. The protein contains five WD-40 repeats and is highly conserved across different species and organisms. Expression of *WDR26* was found in most of the tissues tested with the highest in human adult skeletal muscle and heart, in fetal skeletal muscle and brain. Overexpression of WDR26 in the cell negatively regulates MAPK signaling pathway by significantly inhibiting the activities of SRE and ELK-1, which are the targets of ERK, JNK and p38 [Treisman, 1996].

Structural study on WD40 repeat proteins revealed that the propeller ring structure constructed with WD40 repeats is conserved among this superfamily, suggesting that WDR26 may have a similar ternary structure like Gβ-subfamily of proteins. Therefore, it is interesting to demonstrate the opposite roles of Gβ-like protein, WDR26, and the Gβ proteins in MAPK pathways. Besides WDR26, there are more Gβ-like proteins that act as regulatory factors in signaling pathway, such as hPIP1 and its homologue in yeast skb15, the negative regulators in Cdc42/Rac-PAK medicated JNK pathway in mammalian [Xia et al., 2001b] and yeast cells [Kim et al., 2001]. All these Gβ-like proteins share a WD region consisting five or six WD-40 repeats, which is different from the seven WD-40 repeats of Gβ subunits. The phylogenetic tree analysis also demonstrates that Gβ-like proteins, including WDR26 and its homologues, belong to a new WD40 repeat protein superfamily differing from Gβ proteins (Fig. 3B). Together, these data suggest that the new subfamily of Gβ-like proteins may cooperate with Gβ proteins in the regulation of cell signaling pathways.

MAPK pathways are involved in multiple cellular living processes through phosphorylating their specific endpoint targets, such as ELK-

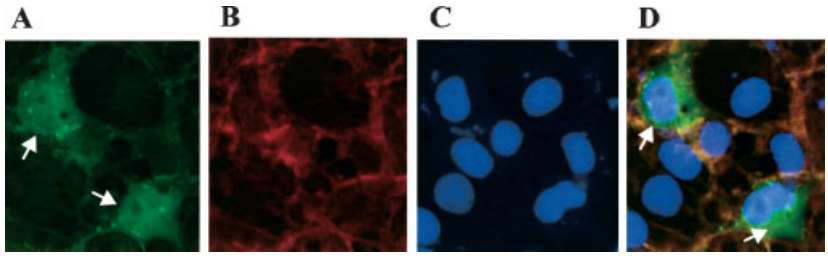

Fig. 4. WDR26 is a cytoplasmic protein. **A**: Cells expressing Flag-tagged WDR26 were stained with monoclonal antibody against Flag epitope of WDR26 and FITC-conjugated phalloidin. **B**: Cells in (A) stained with rhodamine-conjugated phalloidin. **C**: The nucleus of cells in (A) stained with DAPI. **D**: The combined image of (A–C). [Color figure can be viewed in the online issue, which is available at www.interscience. wiley.com.]

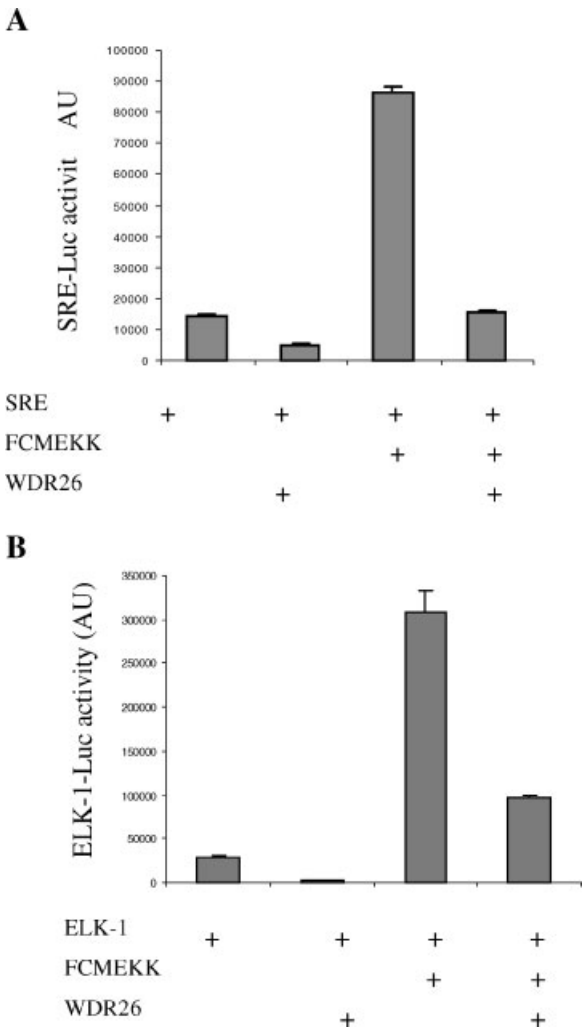

Fig. 5. Overexpression of WDR26 suppresses transcriptional activities of SER and Elk1 in cos-7 cells. **A**: Co-transfection of pCMV-WDR26 and pSRE-Luc suppresses the endogenous and MEKK-mediated c-fos serum response element (SRE) activation in the reporter assay. **B**: Inhibition of endogenous and MEKK-mediated Elk-1 transcriptional activity by expression of WDR26. COS-7 cells transfected with individual reporter plasmid and the corresponding plasmids shown in the figure. The data are the mean of three repeats in a single transfection experiment. Each transfection experiment was performed at least three times.

1 and SRE, which compose a ternary complex together with SRF to induce expression of c-fos and other early growth response genes that control the transition from quiescence to proliferation [Gille et al., 1995; Graf et al., 1997]. *WDR26* is a conserved gene during evolution with high sequence identity among homologues from various species, suggesting a potential role in regulating some essential cellular processes, such as cell growth and proliferation, through the MAPK signaling pathway. The exact function of WDR26 in the cell is not clear and is under active investigation.

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