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A Novel Protein Specifically Interacting with Homer2 Regulates Ubiquitin-Proteasome Systems

Takamasa Ishibashi, Sachie Ogawa, Yasuko Hashiguchi, Yuriko Inoue, Hiroshi Udo, Hiroshi Ohzono, Akihiko Kato, Reiko Minakami and Hiroyuki Sugiyama*

Department of Biology, Graduate School of Science, Kyushu University, Higashi-ku, Fukuoka 812-8581

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Homer family proteins are encoded by three genes, *homer1, 2* and *3*. Most of these proteins are expressed constitutively in nervous systems and accumulated in post-synaptic regions. However, the functional significance of these proteins, especially the significance of the distinction among the proteins encoded by *homer1, 2* and *3*, is still obscure. In the present study, we isolated a cDNA clone encoding a novel protein by two-hybrid system screening using the C-terminal half of Homer2b as the bait. This protein, termed 2B28, has 297 amino acid residues and contains three major domains: a UBA domain, a coiled-coil region, and a UBX domain. When expressed in HEK293T cells, 2B28 showed colocalization with uniquitin and enhanced the expression levels of IκB or Homer1a proteins, which are known to be degraded by proteasomes, indicating that 2B28 is involved in ubiquitin-proteasome functions. 2B28 specifically interacted and colocalized with Homer2 proteins, but not with Homer1 proteins. So far, we have identified no counterpart of 2B28 for Homer1 experimentally or in the protein databases. These results suggest that the specific interaction of 2B28 with Homer2 may play a role in regulation of protein degradation by ubiquitin-proteasome systems and that this function may be specific to Homer2 proteins among Homer family proteins.

Key words: Homer, novel Homer-binding protein, protein degradation, ubiquitin, Ub-proteasome related domains.

Abbreviations: CFP, cyan fluorescent protein; GST, glutathione S-transferase; HA, hemagglutinin; HEK, human embryonic kidney; IκB, I-kappa-B; MBP, maltose binding protein; Ub, ubiquitin.

Homer proteins were originally identified as synaptic plasticity-related proteins in mammalian nervous systems (*1*, *2*) and are now known to be expressed in many other mammalian tissues (*3*). Homer family proteins are transcribed from three separate genes (*homer-1*, *homer-2*, and *homer-3*) and include a short-form protein (Homer1a) and several long-form proteins (such as Homer1b and c, Homer2a and 2b, and Homer3). These proteins are derived from the three genes by alternative splicing. Long form Homer proteins have EVH1 domains in their N-terminals and a coiled-coil region and leucine zipper motifs in their C-terminals, whereas the short-form Homer1a lacks the C-terminal region and consists mainly of the corresponding N-terminal region (*3–5*). The EVH1 domain of Homer proteins bind to their target proteins such as group1 metabotropic glutamate receptors and several cytoskeleton-binding proteins, and the coiled-coil region is required for formation of multimers (*3*, *4*, *6*, *7*). Therefore, Homer proteins are thought to control intracellular signaling pathways by linking receptors and cytoskeleton-binding proteins. However, detailed roles of Homer proteins, especially the specific roles of each isoform of Homer proteins (Homer1, 2 and 3), are still obscure.

Although the N-terminal regions of Homer proteins are highly conserved (over 85% identical), the C-terminal regions are more divergent (approximately 30% identical), and few proteins that interact with this region have been identified. Therefore, to investigate the distinct functions of individual Homer isoforms, we focused on their C-terminal regions and attempted to isolate the binding proteins using yeast two-hybrid screenings. In this study, we identified a novel protein designated as 2B28, which specifically binds to Homer2b. Our results suggest that 2B28 may interact with ubiquitin (Ub) and may be involved in the Ub-proteasome proteolytic pathways.

MATERIALS AND METHODS

Yeast Two-Hybrid Assay—Two-hybrid screening was performed with the Matchemaker LexA two-hybrid system (Clontech) according to the manufacturer's instructions. EGY48 cells, which have a LexA operator upstream of the LEU2 gene, were transformed with a reporter plasmid p8op-lacZ, which contains lacZ located downstream of the LexA operator. The pLexA plasmid containing C-terminal 233 amino acid residues of Homer2b and the candidate plasmid isolated from pB42AD based library (Clontech) were cotransfected into EGY48. Positive clones were detected by β-galactosidase activity in the in vivo plate assay: the positive yeast colonies were selected on X-gal plates lacking urasil, histidine, and tryptophan with blue color developed after 6–36 h. We screened 1×10^6 independent clones and 10 positive clones were found.

*To whom correspondence should be addressed. Tel: +81-92-642-2630, Fax: +81-92-642-2645, E-mail: hsugiscb@mbox.nc.kyushu-u.ac.jp

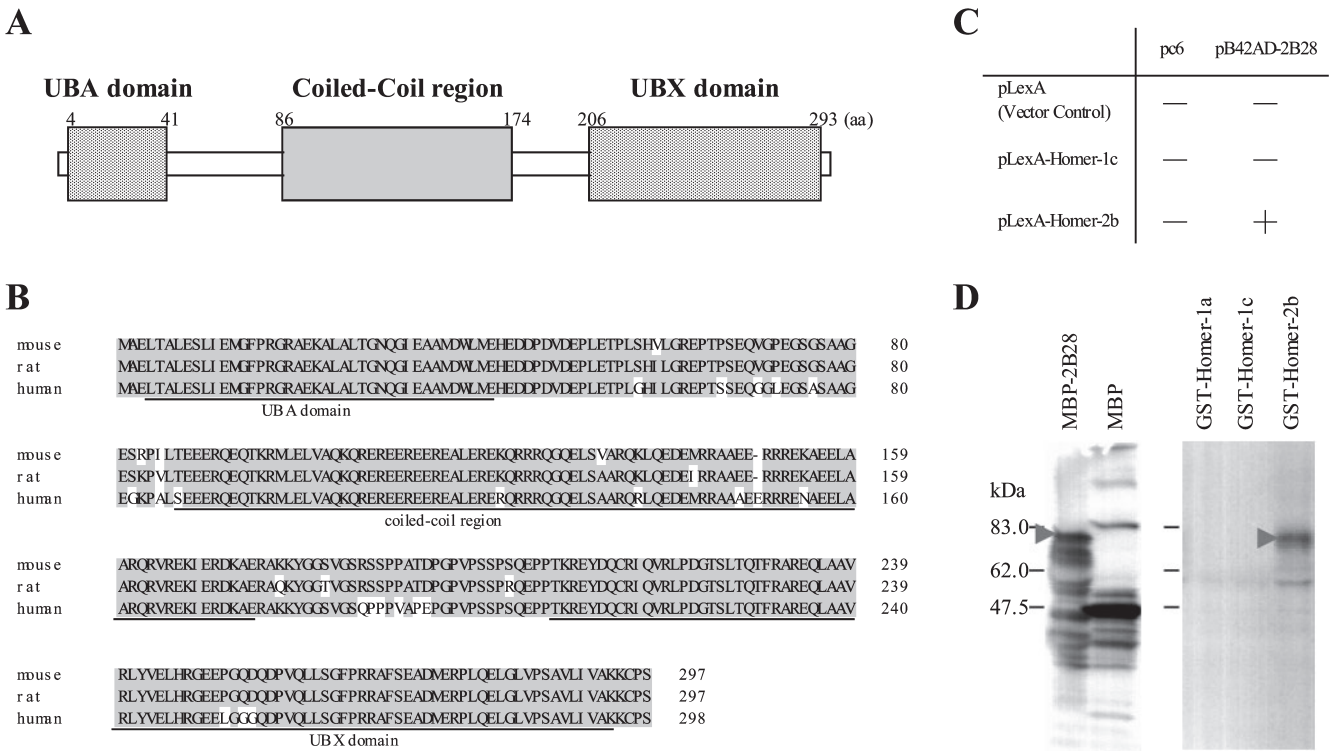

Fig. 1. **(A) Schematic diagram of domain structure of 2B28 protein. (B) Alignment of amino acid sequences of mouse, rat and human 2B28.** Identical amino acids are indicated by shading. The predicted domains are indicated by underlines. **(C) Homer2b interacts specifically with 2B28 in yeast.** Interactions between Homer proteins (in pLexA) and 2B28 (in pB42) were assayed by the LacZ/Leu2 induction in the yeast two-hybrid system. "+" and "–" indicate positive and negative interactions, respectively. (D) Specific *in vitro* binding of 2B28 with Homer2b. Left panel, the lysates prepared from MBP-2B28 expressed in *E. coli* were subjected to Western blotting using anti-MBP antibody. Right panel, the bacterial lysates containing MBP-2B28 were added to GST-Homer proteins and bound proteins were analyzed by Western blotting using anti-MBP antibody. Arrowheads indicate the immunosignals of MBP-2B28 protein.

Cloning of rat 2B28 cDNA and Generating Deletion Mutants—Plaque hybridization was performed with a λZAPII cDNA library constructed from rat hippocampal mRNA (Stratagene). The DNA probes for plaque hybridization were labeled with [α-^{32}P]dCTP using a random-prime labeling kit (Amersham Pharmacia Biotech). The nucleotide sequences of positive clones were determined with a Big-Dye terminator kit (Perkin Elmer) and a Prism 377 automatic sequencer (Perkin Elmer). Three 2B28 deletion mutants were generated by PCR using specific primers.

In Vitro Binding Assay—The coding regions of Homer1a, Homer1c, and Homer2b were subcloned into pGEX 5X-1 (Amersham Pharmacia Biotech) for production of GST fusion proteins. The GST-fused Homer constructions GST-Homer1a, GST-Homer1c, and GST-Homer2b have been described elsewhere (*4*). cDNA fragments encoding rat 2B28 and its deletion mutants were subcloned into pMAL-c2 vector (New England Biolabs) for production of MBP fusion protein.

GST fusion proteins were expressed in *Escherichia coli* XL1-Blue cells and purified with glutathione-Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer's instructions. For MBP fusion proteins, cells were lysed in lysis buffer (PBS containing 1% Triton X-100, 1 mg/ml lysozyme, 1 mM PMSF, 100 μM EDTA, and 5 mM DDT) and centrifuged to remove cell debris. The resin bound to GST fusion protein (1 μg) was added to the bacterial lysate of MBP fusion proteins (40 μl) containing 0.1% BSA. After incubation at 10°C for 1 h, the resin was washed three times with TNN buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% Nonidet-P40, 1% Triton X-100]. The bound proteins were eluted from the resin by boiling in 100 μl of 2× SDS sample buffer. The eluates (20 μl) were analyzed by Western blotting using a rabbit anti-MBP antibody (New England Biolabs). Anti-rabbit antibody conjugated with alkaline phosphatase (Sigma) was used as a secondary antibody. Immunosignals were visualized with Vistra ECF (Amersham Pharmacia Biotech) and a FluorImager (Amersham Pharmacia Biotech).

Mammalian Expression Constructs—The FLAG (DYKDDDDK) -tagged Homer constructs FLAG-Homer1c and FLAG-Homer2b have been described elsewhere (*4*). Myc (EQKLISEEDL)-tagged 2B28 construct was generated by PCR using specific primers and subcloned into a mammalian expression vector pcDNA3 (Invitrogen). FLAG-tagged I-kappa-B (IκB) construct was prepared as described (*8*). Fusion proteins of Homer2 and 2B28 with fluorescent proteins CFP or "Venus" (*9*) were prepared as described (*10*).

Cell Culture—COS-7 and HEK293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4 mM glutamate and 10% (v/v) FBS. Cultures were

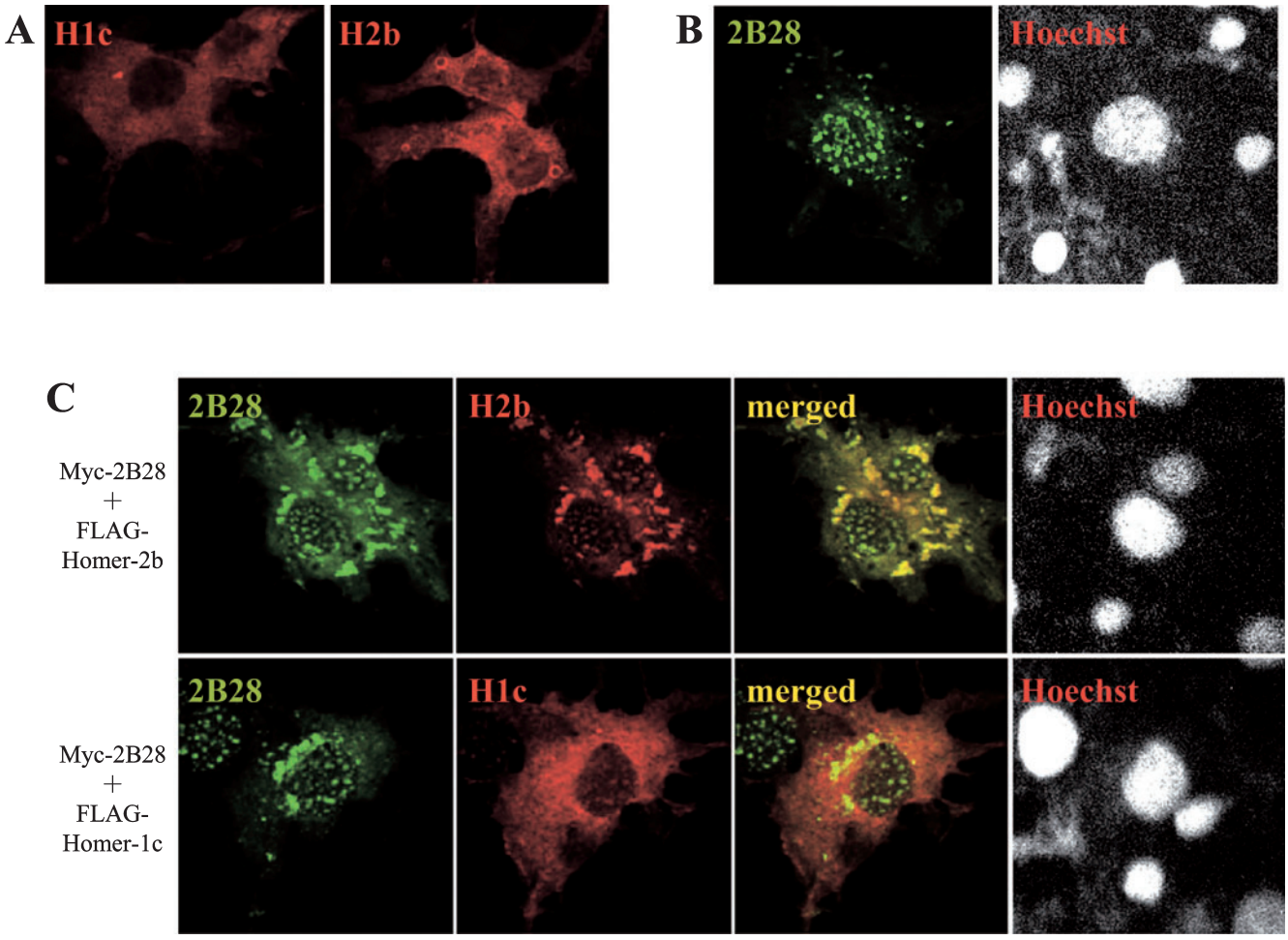

Fig. 2. **2B28 specifically co-localized with Homer2b in mammalian cells.** COS-7 cells were transfected with FLAG-tagged Homer1c or Homer2b alone (A), Myc-tagged 2B28 (B), or both (C). After fixation, cells were stained with anti-FLAG antibody (red, for Homer1c/2b) and anti-Myc antibody (green, for 2B28), and observed by confocal microscopy. Hoechst33258 was used to stain the cell nuclei in panels (B) and (C). Merged are superimposed composite images using two colors (green and red). Scale bars, 10 μm.

maintained at 37°C in a humidified atmosphere containing 5% CO_2. Rat hippocampal neurons were cultured as described (*10*).

Immunocytochemistry—Transfections of COS-7 and HEK293T cells were performed with 20 μg of each plasmid DNA by electroporation (Electro Cell Manipulator 600; BTX) according to manufacturer's instructions. Transfected cells were plated onto glass coverslips. Forty-eight hours after the transfection, cells were rinsed with PBS, fixed with 4% (w/v) paraformaldehyde in PBS for 15 min, and permeabilized with PBS containing 0.2% Triton X-100 for 5 min. After blocking with 8% BSA in PBS for 1 h, cells were incubated with a mixture of mouse anti-FLAG and rabbit anti-Myc antibodies (Sigma) in PBS containing 1% BSA for 1 h at room temperature. They were washed twice for 15 min with PBS and stained with DTAF-conjugated anti-rabbit IgG (Chemicon) and Rhodamine-conjugated anti-mouse IgG (Chemicon) for 1 h, and then the coverslips were rinsed twice for 15 min with PBS. To visualize nuclei, the cells were incubated with H33258 in PBS (1 mg/ml) for 15 min, and mounted with PBS containing 0.5 mg/ml paraphenylenediamine and 80% glycerol. Specimens were examined using a LSM410 laser scanning confocal microscope equipped with Zeiss Axiovert 135M.

Immunoblots for Detecting Transiently Expressed Proteins in HEK293T Cells—HEK293T cells were transfected with DNA constructs by the calcium phosphate methods or lipofection using Lipofectamine2000 (Life Technologies) according to the manufacturer's instructions. After 48–60 h, proteins were extracted in 2× SDS sample buffer. Equal amounts of cell extract were analyzed by Western blotting using anti-FLAG or anti-Myc antibody.

For measuring FLAG-IκB or FLAG-Homer1a expression levels, PVDF membranes were fixed for 45 min with 4% PFA in PBS at 4°C and rinsed three times for 20 min with PBS before blocking.

RESULTS

We isolated a 2B28 clone by yeast two-hybrid screening from a mouse brain cDNA library, using the C-terminal half of rat Homer2 as the bait (Fig. 1C). 2B28 is a novel 297aa protein composed of three domains: a UBA domain, a coiled-coil region, and a UBX domain (Fig. 1, A and B). The domain structure of 2B28, the UBA domain

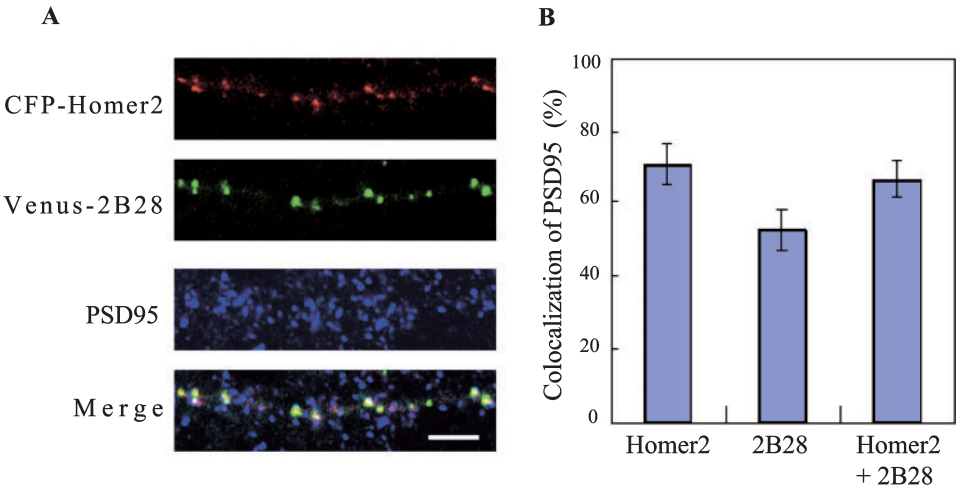

Fig. 3. **Colocalization of 2B28 and Homer2b in cultured hippocampal neurons.** Hippocampal neurons (19d in culture) were injected with DNAs encoding CFP-Homer2 and Venus-2B28, fixed 24 h later, and immunostained with anti-PSD95 and Rhodamine-conjugated secondary antibody. (A) Examples of distribution in a dendrite. Scale bar, 5 μm. (B) Percentages of clusters stained with PSD95 within Homer2 clusters, 2B28 clusters, or clusters harboring both of them (Homer2 + 2B28).

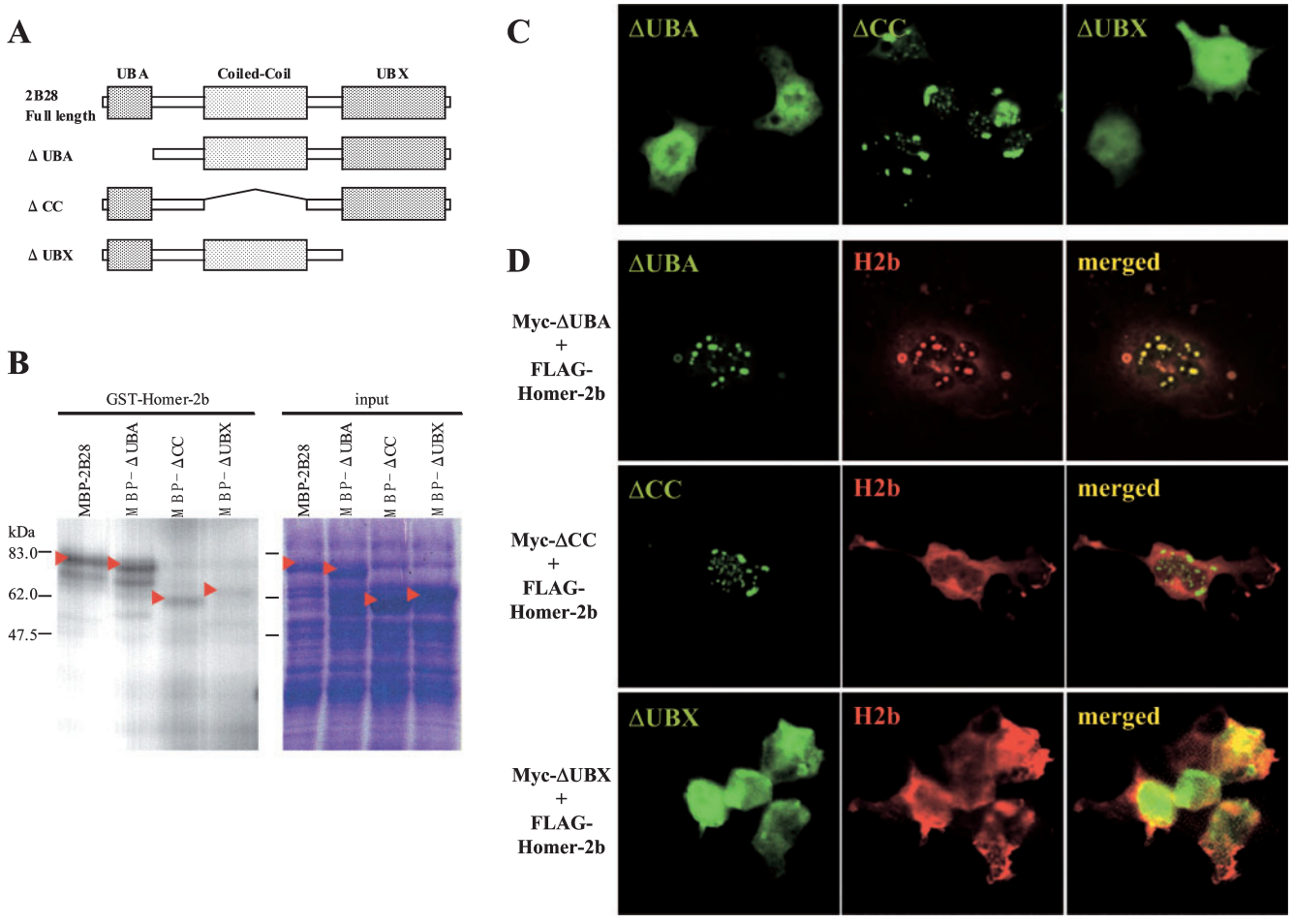

Fig. 4. **(A) Schematic structures of 2B28 deletion mutants. (B) *In vitro* binding of Homer2b with 2B28 deletion mutants.** Left panel, *E. coli* cell lysates of MBP-fused full-length 2B28 or each of the deletion mutants were added to GST-Homer2b Sepharose beads and bound proteins were analyzed by Western blotting using anti-MBP antibody. Right panel, the *E. coli* cell lysates were applied to SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue (CBB). Arrowheads indicate the predicted sizes of individual MBP-fusion proteins. **(C and D) Intracellular localization of 2B28 deletion mutants and coexpressions with Homer2b in mammalian cells.** HEK293T cells were transfected with Myc-2B28 deletion mutants alone (C), or with a mutant and FLAG-Homer2b (D). The cells were stained with anti-Myc antibody (green), and anti-FLAG antibody (red) in (D), after fixation and examined by confocal microscopy. Scale bars, 10 μm.

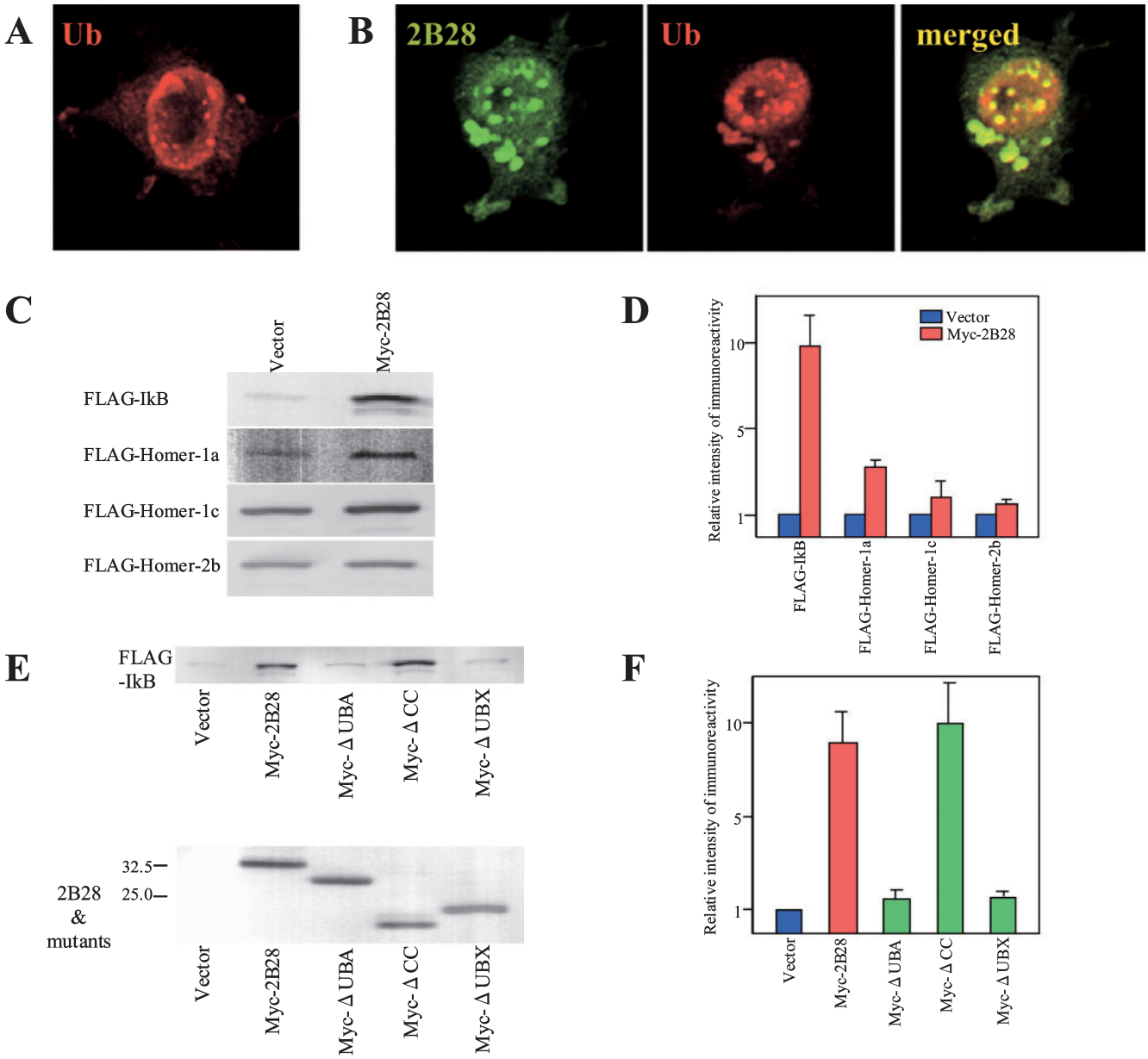

Fig. 5. **(A and B) 2B28 is co-localized with ubiquitin in mammalian cells.** HEK293T cells were transfected with HA-tagged ubiquitin alone (A), or co-transfected with HA-ubiquitin and Myc-2B28 (B). The cells were fixed and stained with anti-HA antibody (red) and anti-Myc antibody (green in B), and observed by confocal microscopy. Scale bars, 10 μm. **(C) Expression of 2B28 increases the stability of Homer1a, which is known to be rapidly degraded by proteasomes.** HEK293T cells were co-transfected with FLAG-tagged proteins and Myc-2B28 (right-hand) or vector alone (left-hand). pcDNA3 with a Myc-tag was used as a vector control. Forty-eight to 60 h after the transfection, these cells were lysed and analyzed by Western blotting using anti-FLAG antibody**. (D) Quantitative analysis of the data depicted in (C).** Relative intensities of immunoreactivity compared to the vector controls are shown. Error bars represent SE. **(E) The UBA and UBX domains of 2B28 are required for the enhancement of the stability of IκB.** HEK293T cells were co-transfected with FLAG-IκB and Myc-tagged 2B28 or deletion mutants, or vector. Forty-eight to 60 h after the transfection, these cells were lysed and analyzed by Western blotting using anti-FLAG antibody (top panel) or anti-Myc antibody (bottom panel)**. (F) Quantitative analysis of the data depicted in (E).** Relative intensities of immunoreactivity compared to the vector control are shown. Error bars represent SE.

in the N-terminal region and the UBX domain in the C-terminal region, implies that 2B28 is related to Ub-proteasome proteolytic system (*11*, *12*). We then obtained a rat homologue of 2B28 by screening a rat brain cDNA library. The homologous sequence was also found for human in GenBank, EMBL, and DDBJ databases. The amino acid sequences of 2B28 were highly conserved in rat, mouse, and human (99.6%, 91.6%, and 91.3% identical between rat and mouse, rat and human, and mouse and human, respectively; Fig. 1B), suggesting that 2B28 may play important roles in mammals. No other isoforms of 2B28 were found in the databases.

To examine whether 2B28 specifically interacts with Homer2, we performed *in vitro* binding assays (Fig. 1D).

GST-fused Homer proteins bound to glutathion-Sepharose resins were suspended in bacterial lysates containing 2B28 fused with MBP. The bound proteins were eluted and subjected to Western blot analyses using anti-MBP antibody. We found that MBP-2B28 bound specifically to GST-Homer2b but not to GST-Homer1c or GST-Homer1a.

To investigate the subcellular localization of 2B28, Myc-tagged 2B28 was expressed in COS7 and HEK293T cells, and immunocytochemical analyses were performed (Fig. 2). When expressed alone, 2B28 displayed a spotty distribution in cytoplasmic and nuclear regions 48 to 72 h after the transfection (Fig. 2B).

FLAG-tagged Homer2 and Myc-2B28 were coexpressed to examine whether Homer2 and 2B28 show colocalization. When expressed alone, Homer2 was distributed in a diffused manner in cytoplasm (Fig. 2A). However, when coexpressed with 2B28, Homer2 showed a spotty distribution and colocalized with 2B28 48 h after transfection (Fig. 2C). In contrast, FLAG-Homer1c showed a diffused distribution even in the presence of 2B28 and was not colocalized (Fig. 2C). These results indicate that 2B28 specifically interacts with Homer2b, but not Homer1c, in mammalian cells, and that 2B28 enhances clustering of Homer2b.

We next examined the distributions of 2B28 and Homer2b in rat hippocampal neurons. Fusion proteins of 2B28 fused with Venus and Homer2 fused with CFP proteins were coexpressed in cultured rat neurons. As shown in Fig. 3, 2B28 and Homer2b were colocalized in dendrites and somata of hippocampal neurons, suggesting the intimate association of endogenous 2B28 and Homer2b in neurons.

To examine which domain of 2B28 is required for the association with Homer2b, we generated three deletion mutants of 2B28, ΔUBA, ΔCC and ΔUBX, lacking UBA, coiled-coil and UBX domains, respectively (Fig. 4A). We first examined the association by in vitro binding assay (Fig. 4B). When MBP-tagged 2B28 or 2B28 mutants were incubated with Homer2b, only the full-length 2B28 and ΔUBA, but not ΔCC and ΔUBX, were able to bind to Homer2b. This result indicates that coiled-coil and UBX domains of 2B28 are required for the interaction with Homer2b.

We further examined the interaction of 2B28 deletion mutants and Homer2b in mammalian cell lines. When the deletion mutants of 2B28 were expressed alone in HEK293T cells, ΔUBA and ΔUBX were found to be dispersed in the cytoplasm, whereas ΔCC produced a spotty distribution as seen in the full-length 2B28 (Fig. 4C), indicating that UBA and UBX domains are required for clustering of 2B28. We next coexpressed 2B28 mutants and Homer2b in HEK293T cells and examined their subcellular localization. Coexpression of ΔUBA and Homer2b produced similar subcellular distribution to that observed for the full-length 2B28 and Homer2b (see Fig. 2C), in which both ΔUBA and Homer2b formed clusters and localized together (Fig. 4D). On the other hand, ΔCC and ΔUBX failed to colocalize with 2B28 and also did not induce Homer2b puncta, indicating that coiled-coil and UBX domains of 2B28 mediate the interaction with Homer2b and the subsequent clustering of Homer2b.

It has been suggested that UBA and UBX domains may associate with Ub (*11*, *12*). To examine the possible implication of 2B28 in Ub systems in mammalian cells, Myc-2B28 and HA-tagged Ub were expressed in HEK293T cells. HA-Ub showed both diffused and spotty distributions when expressed alone (Fig. 5A). In coexpressed cells with 2B28, each dot of HA-Ub was well colocalized with one of Myc-2B28 (Fig. 5B), suggesting that 2B28 interacts with Ub *in vivo*.

The degradation of poly-ubiquitinated proteins by proteasomes is an important cellular process that is regulated by Ub and Ub-related proteins (*14*). We examined whether the overexpression of 2B28 could affect the levels of IκB and Homer1a, known to be regulated by the Ub-proteasome proteolytic systems (*15*, *8*). Western blot analyses showed that the protein amounts of FLAG-tagged IκB and Homer1a were increased dramatically by the coexpression of Myc-2B28 in HEK293T cells (Fig. 5, C and D). In contrast, amounts of Homer1c and Homer2b, which are less influenced by Ub-proteasome systems, remained stable after expression of 2B28 (Fig. 5, C and D). We have also examined three deletion mutants of 2B28 and found that ΔUBA and ΔUBX do not affect the amount of IκB (Fig. 5, E and F). These results suggest that 2B28 may be a component of Ub-proteasome system and may regulate the efficiency of protein degradation.

DISCUSSION

In this study, we identified a novel protein 2B28 as a specific binding partner of Homer2. The *in vitro* binding assay showed that 2B28 specifically binds to Homer2. Immuno- and fluorocytochemical studies also showed the specific colocalization of 2B28 and Homer2 in both mammalian cell lines and rat hippocampal neurons. 2B28 specifically recognized Homer2 but not Homer1. Homer1 and Homer2 are 51% identical overall, but their C-terminal regions including a coiled-coil region and a leucine zipper domain are more divergent (34% identical). This suggests that possible differences in function among Homer isoforms may mainly depend on the properties of binding proteins that specifically recognize the C-terminal regions. We initially investigated whether Homer1 has a similar counterpart corresponding to 2B28. However, two-hybrid screenings using the C-terminal half of Homer1c as a bait failed to detect any Homer1 counterpart (unpublished results). In addition, we did not find any isoforms of 2B28 in GenBank, SwissProt, and PIR databases. Thus, our results suggest that 2B28 may participate in a novel function of Homer family proteins, and that this function may be relevant to Homer2 but not to Homer1c.

The domain organization of 2B28 implied that it is involved in Ub-proteasome proteolytic pathways. 2B28 indeed colocalized with Ub in mammalian cells, indicating that it interacts with Ub. When 2B28 was overexpressed in cells, the stability of IκB and Homer1a, proteins known to be naturally degraded by the proteasomes, increased significantly. We also found that both UBA and UBX domains of 2B28 are required for these stabilizing effects of 2B28. Similar phenomena of protection against protein degradation by proteasomes were observed when a co-factor of proteasomes, which in itself helps proteasomes to degrade substrates, was overexpressed (*16*). Consistent with our results, surplus

amounts of such co-factors, contrary to their original function, can inhibit normal functions of proteasomes in cells. Therefore, it is possible that 2B28 may function as a co-factor of proteasomes, and that the cooperation of Homer2 and 2B28 may bring ubiquitinated proteins to proteasomes. Homer2 may thus be involved in the regulation of proteasome-dependent protein degradation by 2B28.

Recent studies on p47 and VCP provide strong support for the notion that 2B28 may be a co-factor of proteasomes. VCP, also called p97/Cdc48, is an AAA-ATPase which links the ubiquitinated substrates to proteasomes and promotes the degradation of substrates (*17*). p47 has, like 2B28, UBA and UBX domains in its N-terminal and C-terminal regions, respectively. VCP needs to interact with p47 to promote the degradation (*18*). In this interaction, p47 uses the UBX domain as the interface. p47 is thus an adopter protein of VCP, and functions as a co-factor of proteasomes to promote Ub-dependent protein degradation. These properties of p47 are thought to be applicable to the case of 2B28. 2B28 may act as the co-factor of some proteasome-interacting proteins like AAA-ATPases which interconnect proteasomes and ubiquitinated substrates.

McNeill *et al.* (*13*) have recently reported a protein substrate for stress-activated protein kinases, termed SAKS1. This protein turned out to be a human homologue of 2B28. They suggested that the protein may act as a linker of VCP and polyubiquitinated proteins (*13*). VCP is found in nervous systems and implied to be involved in some neurodegenerative disorders (*19*). Thus, it is tempting to speculate that 2B28 might be a co-factor of VCP itself and maintain proteolytic machineries in the nervous system. Although detailed roles and mechanisms of 2B28 are still not clear, our results suggest a specific and distinct role of Homer2 proteins in neuronal functions.

In summary, we have identified a novel protein 2B28 and propose that Homer isoforms may have separate roles, in which Homer2 but not Homer1 may regulate Ub-proteasome system through this protein.

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REFERENCES

1. Brakeman, P.R., Lanahan, A.A., O'brien, R., Roche, K., Barnes, C.A., Huganir, R.L., and Worley, P.F. (1997) Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* **386**, 284–288
2. Kato, A., Ozawa, F., Saitoh, Y., Hirai, K., and Inokuchi, K. (1997) vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. *FEBS Lett.* **412**, 183–189
3. Xiao, B., Tu, J.C., Petralia, R.S., Yuan, J.P., Doan, A., Breder, C.D., Ruggiero, A., Lanahan, A.A., Wenthold, R.J., and Worley, P.F. (1998) Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* **21**, 707–716
4. Kato, A., Ozawa, F., Saitoh, Y., Fukazawa, Y., Sugiyama, H., and Inokuchi, K. (1998) Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. *J. Biol. Chem.* **273**, 23969–23975
5. Sun, J., Tadokoro, S., Imanaka, T., Murakami, S.D., Nakamura, M., Kashiwada, K., Ko, J., Nishida, W., and Sobue, K. (1998) Isolation of PSD-Zip45, a novel Homer/vesl family protein containing leucine zipper motifs, from rat brain. *FEBS Lett.* **437**, 304–308
6. Lim, S., Naisbitt, S., Yoon, J., Hwang, J.I., Suh, P.G., Sheng, M., and Kim, E. (1999) Characterization of the Shank family of synaptic proteins. Multiple genes, alternative splicing, and differential expression in brain and development. *J. Biol. Chem.* **274**, 29510–29518
7. Shiraishi, Y., Mizutani, A., Mikoshiba, K., and Furuichi, T. (2003) Coincidence in dendritic clustering and synaptic targeting of homer proteins and NMDA receptor complex proteins NR2B and PSD95 during development of cultured hippocampal neurons. *Mol. Cell. Neurosci.* **22**, 188–201
8. Hatakeyama, S., Kitagawa, M., Nakayama, K., Shirane, M., Matsumoto, M., Hattori, K., Higashi, H., Nakano, H, Okumura, K., Onoe, K., Good, R.A., and Nakayama K. (1999) Ubiquitin-dependent degradation of IκBα is mediated by a ubiquitin ligase Skp1/Cul 1/F-box protein FWD1. *Proc. Natl Acad. Sci. USA* **96**, 3859–3863
9. Nagai, T., Ibata, K., Park, E.S., Kubota, M., Mikoshiba, K., and Miyawaki, A. (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat. Biotechnol.* **20**, 87–90
10. Inoue, Y., Honkura, N., Kato, A., Ogawa, S., Udo, H., Inokuchi, K., and Sugiyama, H. (2004) Activity-inducible protein Homer1a/Vesl-1S promotes redistribution of postsynaptic protein Homer1c/Vesl-1L in cultured rat hippocampal neurons. *Neurosci. Lett.* **354**, 143–147
11. Buchberger, A. (2002) From UBA to UBX: new words in the ubiquitin vocabulary. *Trends Cell Biol.* **12**, 216–221
12. Hofmann, K. and Bucher, P. (1996) The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem. Sci.* **21**, 172–173
13. McNeill, H., Knebel, A., Arthur, J.S., Cuenda, A., and Cohen, P. (2004) A novel UBA and UBX domain protein that binds polyubiquitin and VCP and is a substrate for SAPKs. *Biochem. J.* **384**, 391–400
14. Doherty, F.J., Dawson, S., and Mayer, R.J. (2002) The ubiquitin-proteasome pathway of intracellular proteolysis. *Essays Biochem.* **38**, 51–63
15. Ageta, H., Kato, A., Hatakeyama, S., Nakayama, K., Isojima, Y., and Sugiyama, H. (2001) Regulation of the level of Vesl-1S/Homer-1a proteins by ubiquitin-proteasome proteolytic systems. *J. Biol. Chem.* **276**, 15893–15897
16. Massey, L.K., Mah, A.L., Ford, D.L., Miller, J., Liang, J., Doong, H., and Monteiro, M.J. (2004) Overexpression of ubiquilin decreases ubiquitination and degradation of presenilin proteins. *J. Alzheimers Dis.* **6**, 79–92
17. Dai, R.M. and Li, C.C. (2001) Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. *Nat. Cell Biol.* **3**, 740–744
18. Hartmann-Petersen, R., Wallace, M., Hofmann, K., Koch, G., Johnsen, A.H., Hendil, K.B., and Gordon, C. (2004) The Ubx2 and Ubx3 cofactors direct Cdc48 activity to proteolytic and nonproteolytic ubiquitin-dependent processes. *Curr. Biol.* **14**, 824–828
19. Hirabayashi, M., Inoue, K., Tanaka, K., Nakadate, K., Ohsawa, Y., Kamei, Y., Popiel, A.H., Sinohara, A., Iwamatsu, A., Kimura, Y., Uchiyama, Y., Hori, S., and Kakizuka, A. (2001) VCP/p97 in abnormal protein aggregates, cytoplasmic vacuoles, and cell death, phenotypes relevant to neurodegeneration. *Cell Death Differ.* **8**, 977–984