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The 8th and 9th tandem spectrin-like repeats of utrophin cooperatively form a functional unit to interact with polarity-regulating kinase PAR-1b

Kazunari Yamashita [a], Atsushi Suzuki [a,*], Yoshinori Satoh [a], Mariko Ide [a], Yoshiko Amano [a], Maki Masuda-Hirata [a], Yukiko K. Hayashi [c], Keisuke Hamada [b], Kazuhiro Ogata [b], Shigeo Ohno [a,*]

[a] Department of Molecular Biology, Yokohama City University Graduate School of Medical Science, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan
[b] Department of Biochemistry, Yokohama City University Graduate School of Medical Science, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan
[c] Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8502, Japan

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ABSTRACT

Utrophin is a widely expressed paralogue of dystrophin, the protein responsible for Duchenne muscular dystrophy. Utrophin is a large spectrin-like protein whose C-terminal domain mediates anchorage to a laminin receptor, dystroglycan (DG). The rod domain, composed of 22 spectrin-like repeats, connects the N-terminal actin-binding domain and the C-terminal DG binding domain, and thus mediates molecular linkage between intracellular F-actin and extracellular basement membrane. Previously, we demonstrated that a cell polarity-regulating kinase, PAR-1b, interacts with the utrophin–DG complex, and positively regulates the interaction between utrophin and DG. In this study, we demonstrate that the 8th and 9th spectrin-like repeats (R8 and R9) of utrophin cooperatively form a PAR-1b-interacting domain, and that Ser1258 within R9 is specifically phosphorylated by PAR-1b. Substitution of Ser1258 to alanine reduces the interaction between utrophin and DG, suggesting that the Ser1258 phosphorylation contributes to the stabilization of the utrophin–DG complex. Interestingly, PAR-1b also binds and phosphorylates R8–9 of dystrophin, and colocalizes with dystrophin at the skeletal muscle membrane. These results reveal a novel function of the rod domain of utrophin beyond that of a passive structural linker connecting the N- and C-terminal domain.

Introduction

Utrophin is a widely expressed protein with high sequence similarity to dystrophin, the causative gene product of Duchenne muscular dystrophy (DMD) [1]. Both proteins are large cytoskeletal proteins belonging to the spectrin superfamily which are characterized by an N-terminal actin binding region composed of tandem calponin homology (CH) domains and a rod domain composed of spectrin-like repeats (22 repeats in utrophin and 24 repeats in dystrophin). The rod domain of both utrophin and dystrophin is followed by a unique, evolutionarily conserved C-terminal domain, which anchors to the plasma membrane through association with dystroglycan (DG), a membrane glycoprotein. The dystrophin–DG complex was originally identified in skeletal muscle cells [2], and intensive studies, focusing on DMD, have increased our understanding of the molecular characteristics and functions of this complex [3]. In particular, the finding that the extracellular component of DG, α-DG, associates with laminin, a major component of the basement membrane (BM), led to the idea that the dystrophin–DG complex protect skeletal muscle cells by providing a mechanical link between intracellular F-actin and the extracellular BM [4]. On the other hand, DG is expressed in many developing and adult non-muscle tissues that are not subjected to severe mechanical stresses, and in these tissues DG predominantly associates with utrophin in place of dystrophin [5]. Although the molecular nature and function of this non-muscle utrophin–DG complex remains to be clarified, accumulating evidence has revealed, at least in some epithelial cells, that DG plays indispensable roles in BM assembly [6,7], and that utrophin is required for the stable membrane localization of DG [8]. Given the indispensable roles of the BM for epithelial polarity, these results suggest an essential role of the utrophin–DG complex for epithelial polarity.

Previously, we demonstrated that the utrophin–DG complex and PAR-1b, one of the mammalian paralogues of the cell polarity-regulating kinase PAR-1 [9–11], endogenously interact in cultured Madin Darby canine kidney (MDCK) epithelial cells and colocalize at the basolateral membrane [8]. PAR-1b kinase activity is required for the basolateral localization of the utrophin–DG complex. Since the utrophin–DG complex was required for assembly of

Abbreviations: DG, dystroglycan; CD, circular dichroism; WT, wild-type; SBP, streptavidin binding protein; Tm, melting temperature.

* Corresponding authors. Fax: +81 45 785 4140.
E-mail addresses: abell@med.yokohama-cu.ac.jp (A. Suzuki), ohnos@med.yokohama-cu.ac.jp (S. Ohno).

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extracellular laminin, these results revealed an unexpected inside-out pathway in which an intracellular polarity protein regulates an extracellular polarity cue, laminin assembly, by controlling the utrophin–DG complex. Biochemically, PAR-1b was required for stable interaction between utrophin and β-DG, the membrane spanning subunit of DG. However, the underlying molecular mechanisms of the PAR-1-dependent regulation of this laminin receptor complex remain unclear. In this study, we dissected the molecular interaction between PAR-1b and the utrophin–DG complex and found that the 8th and 9th spectrin-like repeats (R8 and R9) of utrophin cooperatively form a major PAR-1b-binding site in the complex. PAR-1b specifically phosphorylated S1258 within R9, whose substitution to alanine resulted in reduced interaction between utrophin and DG.

Materials and methods

Expression vectors. Human PAR-1b cDNA (Accession No.: NM_017490) was subcloned with 5′ HA-tag sequences into an Epstein–Barr virus (EBV)-based expression vector, pEB6-CAG6 [11]. PAR-1b K82M was used as a kinase-negative mutant. To construct an expression vector for HA-SBP-PAR-1b, we generated an HA-SBP tandem tag sequence by PCR using pNTAP (Stratagene, La Jolla, California, USA) as a template, and subcloned it into the multicloning site of pCAG-GS [12] to produce pCAG-GS-HA-SBP. PAR-1b or PAR-1b K82M cDNA was subcloned into pCAG-GS-HA-SBP. Mouse utrophin cDNA was a kind gift from Rybakova [13]. Dystrophin cDNA fragments were amplified from a human cDNA library (Clontech, Mountain View, California, USA), and validated by sequencing. Appropriate cDNA fragments of utrophin and dystrophin were subcloned into pCAG-GS with 5′ a V5-tag sequence or into pGEX-6P-1 (GE Healthcare, Buckinghamshire, UK). All point mutants of utrophin were generated by PCR-based site-directed mutagenesis, and verified by sequencing.

Antibodies. Rabbit anti-PAR-1b, anti-utrophin (UT-2) and anti-β-DG antibodies have been described previously [11,14]. Other antibodies were purchased as follows: mouse anti-dystrophin antibody (Novocastra Lab, Newcastle upon Tyne, UK); mouse anti-syntrophin antibody (ABR-Affinity BioReagents, Golden, Colorado, USA); rat anti-HA antibody (Roche, Basel, Swiss); and mouse anti-V5 antibody (Invitrogen, Carlsbad, California, USA).

Cell culture and transfection. MDCK II and HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 μg/ml streptomycin. Plasmid transfection into HEK293T cells was performed using Lipofectamine LTX (Invitrogen) according to the manufacturer's instructions.

Immunoprecipitation, GST pull down and in vitro binding assay. Immunoprecipitations were preformed as described previously [8]. Buffer A (50 mM Tris–HCl at pH 8.0, 150 mM sodium chloride, 1 mM EDTA, 1% Triton X-100, 0.5 mM phenyl methylsulphonyl fluoride, protease inhibitor cocktail (Sigma, St. Louis, Missouri, USA), phosphatase inhibitor cocktail (Sigma)) was used as a lysis buffer. To immunoprecipitate HA-tagged proteins, anti-HA antibody-conjugated Sepharose beads (Roche) were used. For GST pull down assay, HEK293T cells expressing HA-PAR-1b were lysed with Buffer A and soluble fractions were incubated with appropriate GST-fusion proteins (1 μM) for 2 h at 4 °C. The resultant protein complexes were purified using glutathione–Sepharose beads, and were analyzed by western blotting or Coomassie Brilliant Blue (CBB) staining. For affinity-purification of HA-SBP-PAR-1b, streptavidin–Sepharose beads (GE Healthcare) were used according to the manufacturer's instructions, and bound proteins were eluted with a buffer containing 2 mM biotin. For the *in vitro* binding assay,

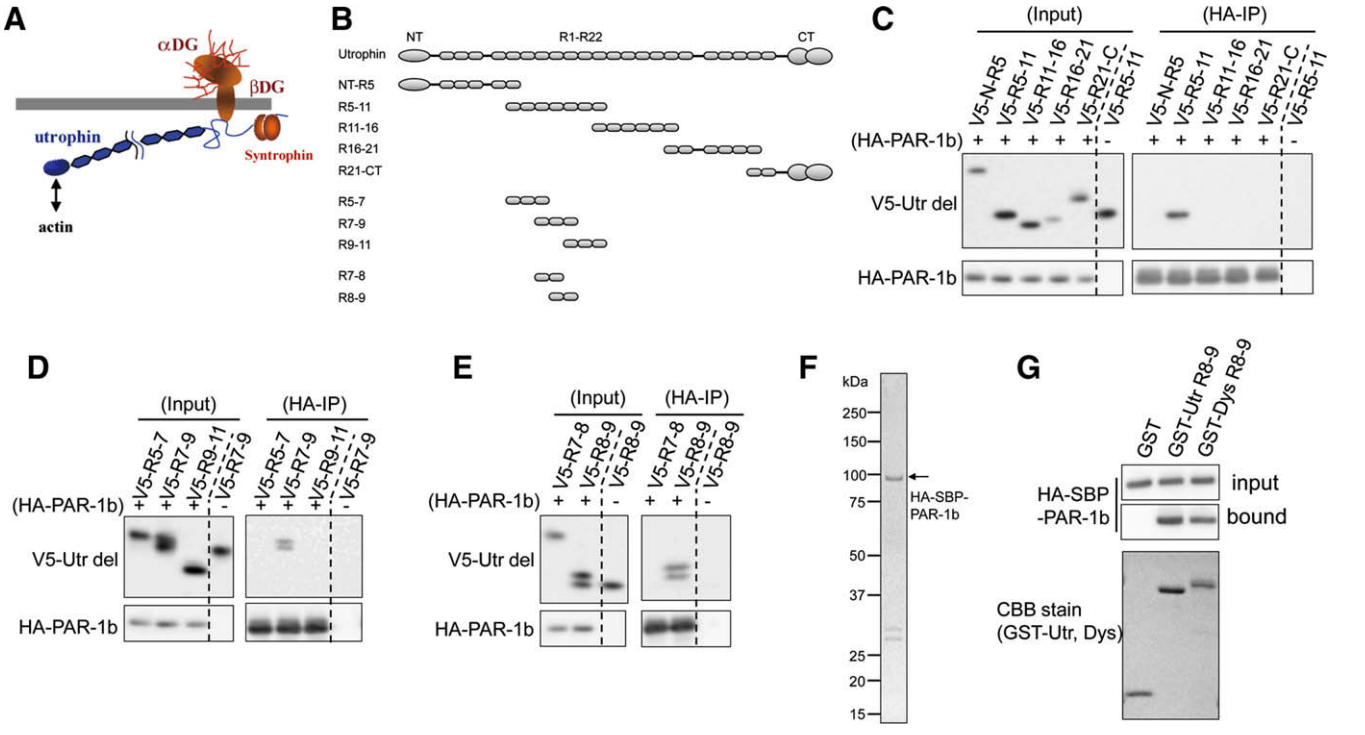

Fig. 1. PAR-1b directly binds to spectrin-like repeats 8–9 of utrophin. (A) Schematic illustration of the utrophin–DG complex. (B) Schematic representation of V5-tagged deletion mutants of utrophin. (C) HEK293T cells ectopically expressing HA-PAR-1b and the indicated V5-utrophin deletion mutants were subjected to immunoprecipitation with anti-HA antibody. Only V5-R5–11 was coimmunoprecipitated with HA-PAR-1b. (D) The shorter deletion mutants of R5–11 were subjected to a similar IP assay. Only V5-R7–9 was coimmunoprecipitated with HA-PAR-1b. (E) V5-R8–9 was specifically coimmunoprecipitated with HA-PAR-1b. Note that mobility shifts of V5-R7–9 (D) and V5-R8–9 (E) were observed in a PAR-1b-dependent manner. (F) CBB staining data of purified HA-SBP-PAR-1b. (G) *In vitro* binding assay using purified proteins. GST-fused R8–9 of utrophin or dystrophin was mixed with purified HA-SBP-PAR-1b, and was pulled down with glutathione beads.

purified HA-SBP-PAR-1b (50 nM) was incubated with GST-fusion proteins in buffer A.

Kinase assay, Immunofluorescence staining of human skeletal muscle and Circular dichroism measurement are described in "Supplementary material and methods".

Results

PAR-1b directly binds to spectrin-like repeats 8–9 within the utrophin rod domain

Previously, we demonstrated that PAR-1b interacts with the utrophin–DG complex which contains multiple components, such as syntrophin and dystrobrevin [5] (Fig. 1A). Here, we found that inclusion of 1% Triton X-100 in cell lysis buffer instead of 1% digitonin specifically eliminated β-DG from anti-PAR-1b immunoprecipitate (Supplementary Fig. 1A). Furthermore, RNAi depletion of utrophin significantly impaired coimmunoprecipitation of β-DG and syntrophin with endogenous PAR-1b (Supplementary Fig. 1B). These results suggest that utrophin is a predominant mediator of the interaction between the DG complex and PAR-1b. To confirm the interaction between PAR-1b and utrophin, we subdivided the utrophin molecule based on the predicted boundary of the spectrin-like repeats in the rod domain [1], and examined which fragments coimmunoprecipitated with PAR-1b (Fig. 1B). When these N-terminal V5-tagged utrophin fragments and HA-PAR-1b were over-expressed in HEK293T cells, only R5–11 was coimmunoprecipitated with HA-PAR-1b (Fig. 1C). Subsequent analysis revealed that PAR-1b interacts with R7–9, but not R5–7 nor R9–11 (Fig. 1D). Finally, R8–9 but not R7–8 was demonstrated to interact with PAR-1b (Fig. 1E). We confirmed the interaction is mediated by direct binding of PAR-1b to utrophin R8–9 (Fig. 1G) by using GST-R8–9 purified from *Escherichia coli* and PAR-1b purified from HEK293T cells as a HA-SBP (streptavidin binding protein)-tagged protein (Fig. 1F).

Utrophin R8–9 cooperatively form a direct binding site for PAR-1b

To further confine the PAR-1b-binding region of utrophin, we prepared GST-fusion proteins containing various regions of R8–9 (Fig. 2A). Consistent with the above results, GST-R8–9 but not GST pulled down HA-PAR-1b from crude extracts of HEK293T cells. However, neither single repeats, R8 or R9, nor two kinds of constructs containing the junction region of R8 and 9 (R8–9 mid1 and mid2) pulled down HA-PAR-1b (Fig. 2B). Furthermore, deletion of the N- or C-terminal region of R8–9, corresponding to a single α-helix of the triple helical bundles, also disrupted the interaction with PAR-1b (Fig. 2C). These results indicate that the whole region spanning R8–9 is required for the interaction with PAR-1b. Consistently, deletion of R9 from full-length utrophin abolished the interaction between V5-utrophin and endogenous PAR-1b in MDCK cells (Fig. 2D). Taken together, we conclude that R8–9 of utrophin is necessary and sufficient for PAR-1b binding.

The spectrin-like repeat is an approximately 110 amino acid triple α-helical bundle motif found in various proteins, including

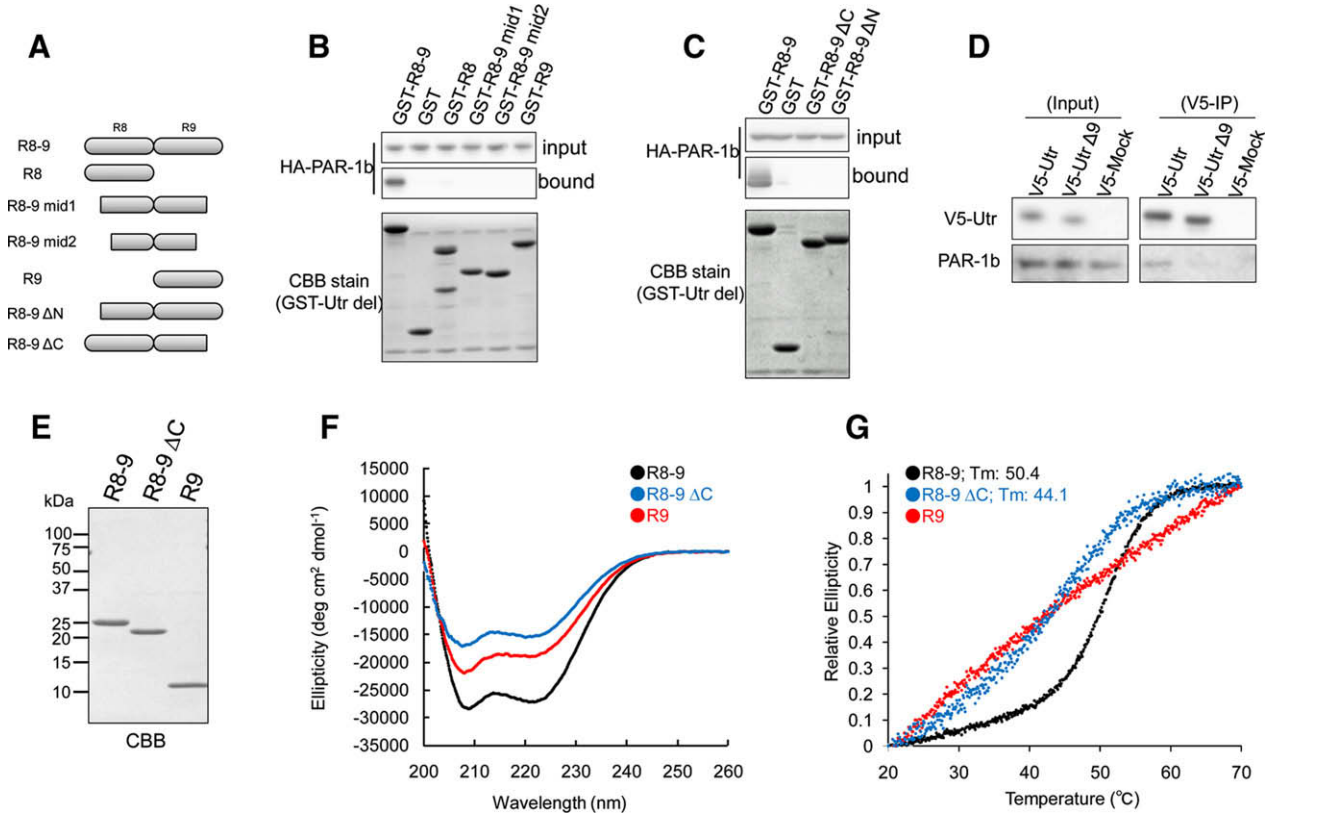

Fig. 2. The entire R8 and R9 region is necessary and sufficient for interaction with PAR-1b. (A) Schematic representation of GST-fused truncated mutants of utrophin spanning various regions of spectrin-like repeats 8–9. (B, C) Pull down assays using the GST-fused protein shown in (A) and HA-PAR-1b expressed in HEK293T cells. Note that only a GST-fusion protein containing the whole spectrin-like repeat 8–9 region (GST-R8–9) pulled down HA-PAR-1b. (D) V5-tagged full-length utrophin and its deletion mutant lacking spectrin-like repeat 9 (Utr Δ9) were expressed in HEK293T cells and subjected to immunoprecipitation using anti-V5 antibody. Endogenous PAR-1b was coimmunoprecipitated with full-length utrophin but not with Utr Δ9. (E) SDS–PAGE analysis of purified R8–9, R8–9 ΔC and R9 used in the CD spectra analysis. (F) CD spectra of recombinant R8–9, its deletion mutant, R8–9 ΔC and R9 obtained at 20 °C. (G) Normalized changes in ellipticity of R8–9 (black), R8–9 ΔC (blue) and R9 (red) at 222 nm with temperature are shown.

spectrin, α-actinin, dystrophin, and utrophin. An early model of the spectrin repeats proposed a beads-on-a-string model with flexibility points between each junction. However, X-ray crystallography of tandem repeats of α-spectrin or α-actinin revealed that tandem spectrin-like repeats form one large rigid rod sharing a long α-helix as the third helix of one motif and as the first helix of the following [15]. It is now suggested that adjacent spectrin-like repeats show differential cooperative stability of folding, with some repeats being independent of their neighbors, and others linked [16,17]. The present results on the broad PAR-1b-binding region of utrophin may, therefore, indicate that utrophin R8–9 corresponds to tandem repeats that have a strong structural cooperativity. To examine this, we compared CD spectra of recombinant R8–9, its deletion mutant R8–9 ΔC and R9 (Fig. 2E). R8–9 showed the typical features of proteins with predominant α-helix folding, as expected (Fig. 2F). α-Helical content derived from the molar ellipticity value at 222 nm is 75.4% [18]. When R8–9 was subjected to heat-induced unfolding, it was denatured in a single step process with a melting temperature of 50.4 ± 0.3 °C (Fig. 2G). On the other hand, R8–9 ΔC, which lacks the third α-helix of R9, showed significantly reduced α-helix content (42.3%) and a lowered melting temperature (Tm) (44.1 ± 0.2 °C) with a broader temperature range for unfolding transition (Fig. 2F and G). R9 also exhibited a lower α-helix content (52.5%), and, strikingly, showed gradual unfolding without a clear cooperative transition. These results indicate that complete sequences spanning R8–9 are required for stable formation of tandem spectrin-like repeats, and are consistent with the idea that utrophin R8–9 cooperatively forms the structural unit required for PAR-1b binding.

PAR-1b phosphorylates Ser1258 of utrophin, and phosphorylation contributes to the stabilization of the utrophin–DG interaction

The immunoprecipitation experiments in Fig. 1 revealed that R7–9 and R8–9 not only bound PAR-1b but also exhibited mobility shifts in a PAR-1b-dependent manner (Fig. 1D and E). Since the mobility shift of R8–9 was abolished by alkaline phosphatase treatment (data not shown), these shifts represent PAR-1b-dependent phosphorylation of these fragments. To determine PAR-1b-dependent phosphorylation target residues, we generated a series of point mutants of V5-R8–9 in which single serine or threonine residues were substituted to alanine (Fig. 3B), and examined which mutations affected the PAR-1b-dependent mobility shift in HEK293T cells. As shown in Fig. 3A, alanine substitution of Ser1258 completely abolished the PAR-1b-dependent mobility shift, while substitution of other residues did not (Supplementary Fig. 2), suggesting that Ser1258 is one of the targets of PAR-1b-dependent phosphorylation of utrophin R8–9. To examine whether PAR-1b directly phosphorylates Ser1258, we next performed an *in vitro* kinase assay using purified HA-SBP-PAR-1b (Fig. 1F). When wild-type GST-R8–9 was used as a substrate, almost equal ^{32}P incorporations were detected in two discrete bands around the molecular weight of GST-R8–9 (Fig. 3C). The lower band (arrow) corresponded to GST-R8–9 itself, which is strongly stained by Coomassie Brilliant Blue (CBB), whereas the upper band (arrowhead) is located slightly higher than the major GST-R8–9 band. Since alanine substitution of Ser1258 eliminated the upper radioactive band, these results indicate that PAR-1b directly phosphorylates Ser1258 as well as other serine/threonine residues,

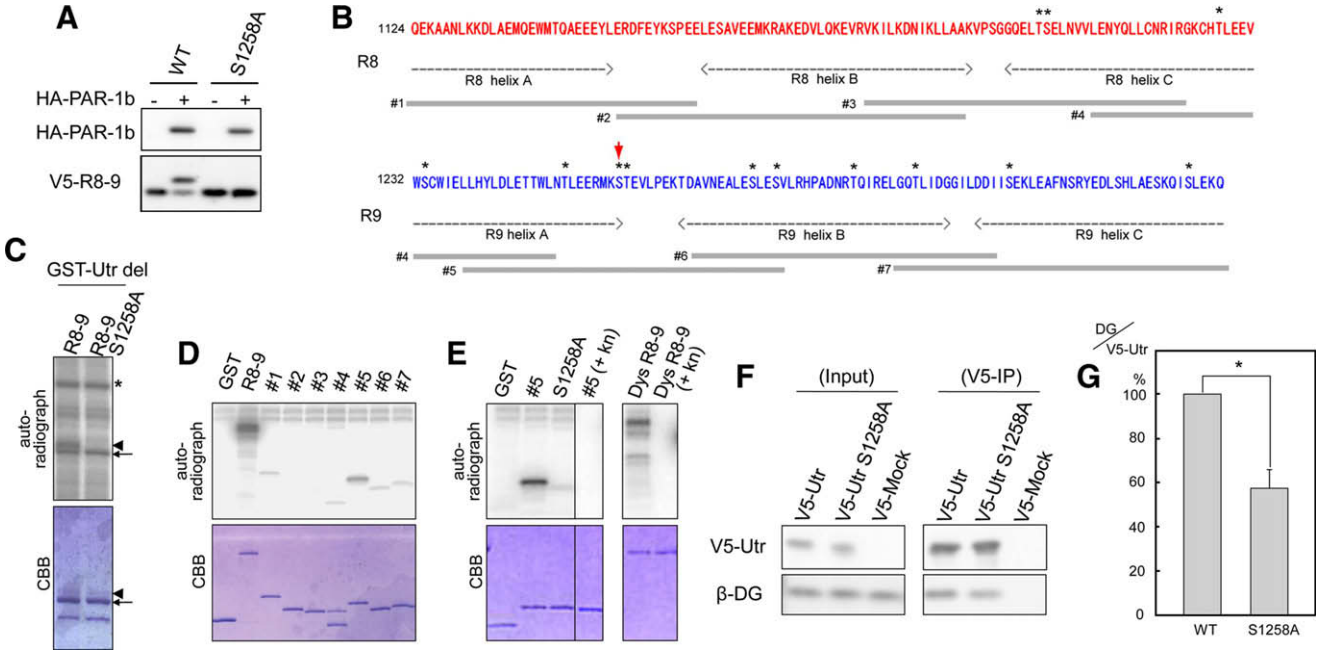

Fig. 3. PAR-1b phosphorylates Ser1258 of utrophin, and suppression of the phosphorylation interfered with the interaction between utrophin and DG. (A) Western blot analysis of HEK293T cell lysates expressing the WT or S1258A mutant of V5-R8–9 with or without HA-PAR-1b. (B) Amino acid sequence of mouse utrophin R8–9 (GenBank Accession No.: NP_035812). Bidirectional arrows indicate the position of predicted α-helices. Gray bars indicate the position of deletion fragments of R8–9 (#1–#7) used in (D). Asterisks indicate the residues mutated in (Supplementary Fig. 2), and a red arrow indicates Ser1258. (C) GST-R8–9 wild-type (WT) and S1258A mutant were subjected to an *in vitro* kinase assay using HA-SBP-PAR-1b. Autoradiographic (upper) and CBB staining (lower) gel images are shown. Asterisk indicates auto-phosphorylation of PAR-1b. Note that wild-type R8–9 revealed two distinct radioactive bands (arrowhead and arrow), whereas the S1258 mutant only showed the lower band. (D) GST-R8–9 and various deletion fragments of GST-R8–9 (#1–#7) indicated in (B) were subjected to an *in vitro* kinase assay using HA-SBP-PAR-1b. Note that GST-R8–9 and its deletion fragment #5 were strongly phosphorylated by PAR-1b. (E) Alanine substitution of Ser1258 (S1258A) abolished phosphorylation of fragment #5 by PAR-1b. In lanes 5 and 6, GST-R8–9 of dystrophin was used as a substrate instead of utrophin. In lanes 4 and 6, the kinase-negative mutant of HA-SBP-PAR-1b (kn) was used instead of wild-type PAR-1b. (F) V5-tagged full-length utrophin and its S1258A mutant were expressed in HEK293T cells, and coimmunoprecipitation of endogenous β-DG with each V5-utrophin was examined. Representative data of western blot analysis are shown. (G) The amounts of coimmunoprecipitated β-DG were normalized with the amount of corresponding V5-utrophin. Statistical analysis of the data from three independent experiments revealed that only 57.3 ± 8.5% of β-DG was coimmunoprecipitated with V5-utrophin S1258A compared to wild-type utrophin. Asterisk denotes significant difference ($^*P < 0.05$) by Student's *t*-test. SD is shown as an error bar. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

phosphorylation of which do not induce mobility shift. Subdivision of R8–9 into shorter fragments (#1–#7) greatly attenuated the phosphorylation efficiencies, probably due to disruption of the direct interaction with PAR-1b, but revealed that ^{32}P incorporation predominantly occurred in fragment #5 containing Ser1258 (Fig. 3D). Mutation S1258A completely abolished the radioactive signal in fragment #5 (Fig. 3E). Weak incorporation of ^{32}P (about 20% of fragment #5) was observed in fragments #1, #4, #6 and #7 (Fig. 3D), suggesting that phosphorylation of the serine/threonine residues within these fragments collectively represent the lower band in Fig. 3C. Taken together, these results demonstrate that PAR-1b directly and predominantly phosphorylates Ser1258 of utrophin R8–9, which is located in the linker region between the first and second α-helix of R9 (Fig. 4B and Supplementary Fig. 3). This is consistent with a recent study indicating that Ser1258 or Thr1259 of utrophin is intrinsically phosphorylated *in vivo* [19].

We previously demonstrated that PAR-1b is required for the stable interaction between utrophin and β-DG [8]. Therefore, we next examined whether alanine substitution of Ser1258 affected the utrophin–β-DG interaction. For this purpose, we expressed wild-type or the S1258A mutant of V5-tagged, full-length utrophin in HEK293T cells and then performed immunoprecipitation using an anti-V5 antibody. As shown in Fig. 3F and G, the amounts of endogenous β-DG coimmunoprecipitated with V5-utrophin were reduced by the S1258A mutation. These results suggest a possibility that phosphorylation of utrophin Ser1258 by PAR-1b plays a role in stabilizing the utrophin–dystroglycan complex.

PAR-1b also binds to the spectrin repeats 8–9 of dystrophin, and colocalizes with dystrophin at the sarcolemma in human skeletal muscle

Finally, we examined whether PAR-1b interacts with dystrophin in a similar manner to that determined for utrophin. Consistent with the results for utrophin, dystrophin R8–9, but not R7–8 nor R9–10, was coimmunoprecipitated with HA-PAR-1b (Fig. 4A). Furthermore, PAR-1b directly binds and phosphorylates dystrophin R8–9 (Fig. 1G and E). Since spectrin-like repeats 8–9 represent a tandem repeat showing significant homology between utrophin and dystrophin (Supplementary Fig. 4), these results indicate that the functional relationship with PAR-1b may be conserved between utrophin and dystrophin. Importantly, PAR-1b colocalized with dystrophin at the sarcolemma (Fig. 4B). These results suggest a possibility that PAR-1b also affects the dystrophin–DG complex in a similar manner to that for the utrophin–DG complex.

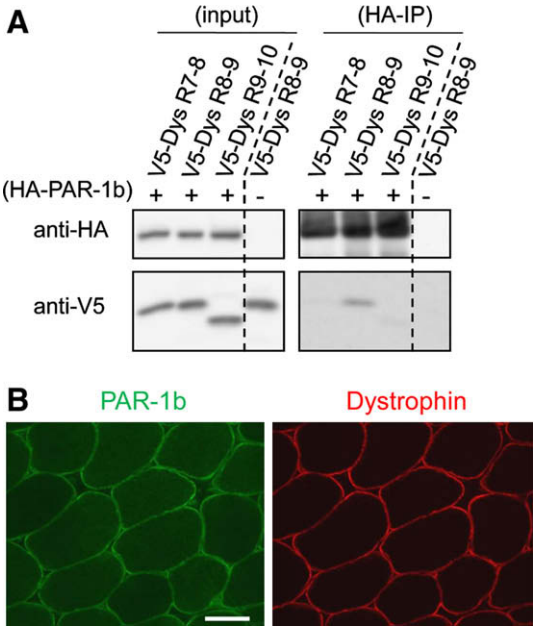

Fig. 4. PAR-1b also interacts with R8–9 of dystrophin, and colocalizes with dystrophin in skeletal muscle. (A) IP using anti-HA antibody from HEK293T cells co-transfected with HA-PAR-1b and the indicated deletion mutants of V5-dystrophin. Again, R8–9 of dystrophin was only coimmunoprecipitated with HA-PAR-1b. (B) Immunofluorescence staining of a cross section of human skeletal muscle using anti-PAR-1b (green) and anti-dystrophin (red). PAR-1b and dystrophin colocalize at the sarcolemma. Bar, 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Discussion

Spectrin-like repeats of utrophin and dystrophin are frequently regarded as passive structural elements [20], some of which can be deleted without significant effects on protein function. This view is based on in-frame deletions found in the dystrophin rod domain that frequently result in milder dystrophic phenotypes, termed Becker muscular dystrophy (BMD) [21]. This is also consistent with the transgenic expression of mini-dystrophin or mini-utrophin genes, which lack numerous spectrin-like repeats, that resulted in the improvement of many dystrophic features of mice lacking dystrophin (mdx mice) [22,23]. However, accumulating evidence has implicated specific roles of the spectrin-like repeats for normal dystrophin and utrophin function beyond a simple structural spacer linking the functional N- and C-terminal domains. For example, full-length utrophin was reported to exert more complete recovery of phenotypes [23,24]. Four spectrin-like repeats from α-actinin-2 could not functionally replace the same number of dystrophin repeats in mini-dystrophin [25]. Finally, single missense mutations, albeit rare, in a spectrin-like repeat of dystrophin can cause severe DMD phenotypes [26]. In this study, we reinforced this idea by revealing that PAR-1b specifically interacts with R8–9 of utrophin, and affects the utrophin–DG interaction by phosphorylating S1258 within R9.

We previously demonstrated that PAR-1b is required for the stable interaction between utrophin and β-DG [8]. The present study provides, at least, one of the mechanisms underlying this regulation by showing that alanine substitution of Ser1258 mildly reduces the utrophin–DG interaction. Interestingly, the first 10 consecutive spectrin-like repeats were shown to be required for the complete actin binding activity of utrophin [13]. Furthermore, a recent study revealed that dystrophin spectrin-like repeats, R7–9, specifically interact with the phospholipids containing phosphatidylserine [27]. Therefore, PAR-1b-dependent phosphorylation of Ser1258 might affect the binding ability of utrophin to other cellular components, such as actin or phospholipids, and thereby indirectly stabilizes the utrophin–DG interaction. Of course, it should be also noted that the S1258A mutation did not completely abolish utrophin–DG binding, as previously observed in PAR-1b knockdown cells [8]. This may indicate the presence of additional mechanisms by which PAR-1b regulates the utrophin–DG complex. Although PAR-1b did not phosphorylate the N- and C-terminal domains of utrophin *in vitro* (Hirata and Suzuki, unpublished data), we cannot exclude the possibility that PAR-1b phosphorylates other sites in utrophin or other components of the utrophin–DG complex. Further studies are required to completely understand the mechanism of PAR-1b-dependent regulation of the utrophin–DG complex.

Here, we demonstrate that utrophin R8 and R9 form a structurally and functionally cooperative unit required for PAR-1b binding. This is not only the first identification of cooperative spectrin-like repeats in utrophin, but is also the first example demonstrating how structural cooperativity of tandem spectrin-repeats is coupled

with their function. We also demonstrate that PAR-1b interacts with dystrophin and phosphorylates dystrophin R8–9 containing a threonine at the corresponding position of utrophin S1258. Taken together, these results suggest a possibility that R8–9 of dystrophin also represents a structurally and functionally important repeat unit. Considering that PAR-1b colocalizes with dystrophin at the skeletal muscle membrane, PAR-1b might also regulate the dystrophin–DG complex by associating with these spectrin-like repeats.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.144.

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