A complex of atypical PKC and Par6 is a common regulator for cell polarity-related processes, which is an essential clue to evolutionary conserved cell polarity regulation. Here, we determined the crystal structure of the complex of PKC\(\alpha\) and Par6 PB1 domains to a resolution of 1.5 Å. Both PB1 domains adopt a ubiquitin fold. PKC\(\alpha\) PB1 presents an OPR, PC, and AID (OPCA) motif, 28 amino acid residues with acidic and hydrophobic residues, which interacts with the conserved lysine residue of Par6 PB1 in a front and back manner. On the interface, several salt bridges are formed including the conserved acidic residues on the OPCA motif of PKC\(\alpha\) PB1 and the conserved lysine residue on the Par6 PB1. Structural comparison of the PKC\(\alpha\) and Par6 PB1 complex with the p40phox and p67phox PB1 domain complex, subunits of neutrophil NADPH oxidase, reveals that the specific interaction is achieved by tilting the interface so that the insertion or extension in the sequence is engaged in the specificity determinant. The PB1 domain develops the interaction surface on the ubiquitin fold to increase the versatility of molecular interaction.

Cell polarity is a fundamental property related to many cellular processes. Recently, a complex of atypical protein kinase C (aPKC)\(^1\) and Par6 (aPKC-Par6) has received much attention as a common cell polarity regulator conserved from nematodes to mammals. Par6 was initially identified as one of the products of par genes (par-1 to par-6) encoding essential components of cell division in the early embryo found by lethal genetic screening of Caenorhabditis elegans (1). In addition to Par proteins, a defect in Pkc3 (a homologue of aPKC in C. elegans) also disrupts embryonic polarity (2). aPKC-Par6 is a conserved signaling complex from C. elegans to Homo sapiens (3) and is now regarded as a common regulator for asymmetric cell division (4, 5), directed cell migration (6), axon specification (7), and tight junction (TJ) formation in epithelial cells (8) in various species. Thus, aPKC-Par6 is an essential clue to evolutionary conserved cell polarity regulation (9).

aPKC-Par6 is characteristic ly located at a specific region of cells, such as the anterior cortex of the nematode embryo (1, 2), apical cortex of fly neuroblasts (10, 11), leading edge of migrating cells (6), and TJs in epithelial cells (8), implying that the complex formation is required for aPKC specific localization. The regulation of cellular localization as well as kinase activity of aPKC is essential for phosphorylation of its intrinsic target. Thus, the scaffold proteins that bind to PKCs play a crucial role in the control of the cellular localization of PKCs (12, 13). In particular, TJ formation is a prototype of cell polarity events. TJs encircle cells at the most apical region of the lateral membrane and function as both paracellular and intramembrane diffusion barriers. Moreover TJ formation triggers cell proliferation and differentiation (14, 15). aPKC-Par6 is localized to the TJs and together with Par3 is involved in TJ assembly. Although recruitment of the ternary complex to the TJs and regulation of TJ formation are still elusive, phosphorylation by aPKC seems to be a clue to signal transduction because the kinase activity deficient aPKC abrogates TJ formation (16). Notably, Par6 can regulate aPKC kinase activity in a Cdc42-dependent manner (17).

The complex formation of aPKC and the scaffold protein Par6 is established through the interaction between PB1 domains existing at the N terminus of both proteins. In TJ formation, the disruption of PB1-PB1 interaction in aPKC-Par6 causes mislocalization of aPKC or Par6 (18). Therefore, PB1-PB1 interaction between aPKC and Par6 is related to their localization and has an indispensable role in cell polarity-related processes.

Until now, the PB1 domain has been found in more than 200 signaling proteins including p40phox, p67phox, cytosolic factors of NADPH oxidase, Bem1p, Cdc24p, MEK5 and p62ZIP on the SMART data base (19). Recent studies have demonstrated that PB1 domain functions as a protein binding module through PB1-mediated heterodimerization or homo-oligomerization (20–23). The PB1 domain is further classified into two types, type I and type II PB1 domains. The PB1-mediated complex is formed through the interaction between type I and type II PB1 domains. Type I PB1 adopts the OPCA motif as a binding site, a remarkable consensus sequence in the PB1 domain with conserved acidic and hydrophobic residues (12, 24, 25). On the other hand, type II PB1 requires a conserved lysine residue in \(\beta1\) (Fig. 1). Binding assays for PB1-PB1 interaction suggest that the electrostatic interaction between conserved acidic residues on the OPCA motif in type I-PB1 and a conserved basic residue in type II PB1 is responsible for PB1-PB1 interaction.
Despite its electrostatic nature, the PB1-PB1 interaction is highly specific and exclusive (20). Interestingly, aPKC binds to several proteins such as Par6, ZIP/p62, and MEK5 through its PB1 domain (18, 26) and mediates distinct signals in a binding action mode are yet to be elucidated. Furthermore, it provides insights into the general and specific properties of the PB1-PB1 interaction.

Here, we determined the crystal structure of the PB1 domain complex of PKCδ and Par6α. Although the electrostatic interaction was assumed to be responsible for complex formation, the structure reveals salt bridge formation in the PB1-PB1 complex. Furthermore, it provides insights into the general and specific properties of PB1-PB1 interaction. We also identified the role of PB1-PB1 interaction in TJ formation in aPKC-Par6.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Complementary DNA fragments encoding various lengths of human Par6α and PKCδ were prepared as described previously (18, 29). Mutations leading to the indicated amino acid substitutions were introduced by PCR-mediated site-directed mutagenesis, and the mutated fragments were cloned into the indicated vectors. All the constructs were sequenced to confirm their identities.

**Sample Preparation for Crystallization**—The PKCδ-(16–99) gene was cloned in pGEX-6P-1 (Amersham Biosciences), and the Par6α(14–95) gene was cloned in pET HP (Novagen), converting the thrombin protease recognition site into the PreScission protease recognition site. Both constructs were co-expressed in E. coli strain BL21 (DE3). Because PKCδ-(16–99) and Par6α(14–95) form a complex in E. coli cells, the complex is acquired via purification from bacterial lysate by affinity chromatography using glutathione-Sepharose 4B resin (Amersham Biosciences). After proteins were treated with PreScission protease (Amersham Biosciences), purification using anion exchange chromatography and gel filtration chromatography were performed. On the process of purification, PKCδ (16–99) and Par6α(14–95) retained a stable complex. Finally, the complex of PKCδ PB1 and Par6α PB1 was concentrated up to 12–15 mg/ml in a buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, and 5 mM dithiothreitol, and this solution was used for crystallization.

Selenomethionine (SeMet)-substituted protein was expressed in E. coli strain B834 (DE3) with LeMaster medium (30). Purification was performed in the same manner as native protein preparation, and SeMet substitution for Met was confirmed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. After concentration to 12–15 mg/ml, SeMet derivatives were used as a crystallization sample.

**Crystallization**—Crystallization screening was performed using a sitting drop vapor diffusion method at 293 K. 100 μl of volume of reservoir was applied to wells on crystal clear strips no indent (Hampton Research). Each drop consisted of 0.5 μl of protein solution and an equal amount of reservoir solution. Approximately 12–15 mg/ml of the complex of PKCδ PB1 and Par6α PB1 in 50 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM dithiothreitol was used for crystallization. A single crystal was obtained in 3.6 M sodium formate and 0.1 mM Tris (pH 8.0) at 293 K by using a microseeding technique.

**Data Collection and Structure Determination**—Prior to x-ray diffraction experiments, crystals were soaked in a cryoprotectant solution (3.6 M sodium formate, 0.1 mM Tris (pH 8.0), 20% glycerol) for several seconds and were immediately flash-cooled to 100 K in a nitrogen gas stream. Native diffraction data were collected on an MAR CCD detector using synchrotron radiation at the BL41XU beam line, SPring-8. The camera to crystal distance was set to 150 mm, and a total of 180 images were collected at 1° oscillation angle per image. The diffraction data were processed using the HKL2000 program (31). The crystal belongs to space group P2₁2₁2₁ with unit cell parameters a = 94.150 Å, b = 38.363 Å, c = 45.178 Å and a cell volume of 164.261 Å³. The molecular mass of a complex of PKCδ PB1 and Par6α PB1 was ~19,427 Da, thus, the Matthews coefficient (Vₘ) was 2.1 Å³/Da and the solvent content was 42%, assuming one molecule exist in an asymmetric unit. Multiwavelength anomalous dispersion data sets were collected using a single crystal of SeMet-substituted protein at three wavelengths at the BL41XU beam line, SPring-8 as well as native data sets. The x-ray diffraction statistics are summarized in Table I.

Determination of the selenium site in SeMet-substituted proteins, phasing, and refinement were performed by crystallography and NMR system software (32). Modeling was performed by Turbo-Frodo (33). Finally, refinement was performed up to 1.5 Å, and the statistics are summarized in Table I. The coordinates and structure factor of a PB1 domain complex of PKCδ and Par6α have been deposited in the Protein Data Bank (accession code 1WMH). The molecular surface model was generated by the GRASP program (34). All figures of the structural model except the model of electrostatic surface potential were created by Pymol software.

**Yeast Two-hybrid Experiments**—Yeast HF7c cells containing a HIS3 reporter gene were co-transformed with a pair of pGBT9 (Clontech) encoding the wild-type or mutant PKCδ and pGADGH (Clontech) encoding the wild-type or mutant Par6α as described previously (35). Following the selection for "Try" and "Leu" phenotype, the transfer-

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**FIG. 1. Structure-based sequential alignment of the PB1 domain.** The conserved hydrophobic residues are highlighted in red, and hydrophilic residues are highlighted in blue. Secondary structure components of type I, type I and type II, or type II PB1 are derived from the tertiary structure of Cdc24p, PKCδ, or Par6α, respectively. The OPCA motif is enclosed in green. The two conserved acidic regions of type I, A1 and A2, are boxed, and the two conserved basic region of type II, B1 and B2/B2’, are highlighted in orange and boxed. Residues involved in the interaction between PKCδ PB1 and Par6α are mapped on the sequence. Asterisks represent residues with side chains involved in an intermolecular salt bridge and/or hydrogen bond. Asterisks represent residues involved in an intermolecular hydrophobic interaction. Filled circles represent residues with the main chain involved in an intermolecular hydrogen bond.
Complex Structure of Cell Polarity Regulator

### RESULTS AND DISCUSSION

**Overall Structure of the PB1 Complex of PKCα and Par6α—** The crystal structure of the PB1 complex of PKCα and Par6α has been determined to a resolution of 1.5 Å by a multiwavelength anomalous dispersion technique using a SeMet-substituted protein as described under "Experimental Procedures." The crystal belongs to the space group P2₁ 2₁ 2, and one complex exists per asymmetric unit. Of a total of 175 residues, 5 N-terminal residues of PKCα PB1 and 4 N-terminal residues of Par6α PB1 that are derived from cloning artifacts and Cys99 of PKCα PB1, a C-terminal residue of the construct, are disordered. In Par6α PB1, Pro87 adopts a cis conformation. A summary of the collected data and the structural statistics is given in Table I.

The overall structure of the complex is shown in Fig. 2A. Both PB1 domains comprise two α helices and a mixed β-sheet which consists of five β strands that belong to the ubiquitin superfamily. The OPCA motif region of PKCα (Leu⁵⁶-Leu⁸₃) has a βββ fold, and the β turn is categorized as a type I + G1 β-bulge, characteristic of type I PB1 domains (23). The PB1 domains of PKCα and Par6α are remarkably similar as shown in Fig. 2B (root mean square deviation = 0.86 Å for backbone atoms). Because the solution structure of PKCα (free state) was determined by NMR (28), the free and the complex states of PKCα PB1 can be compared (Fig. 2C). Both of the overall structures are well superimposed (root mean square deviation = 0.96 Å for backbone atoms). We performed superimposition between free and complex state structures, using not only all over structure but also more limited region such as OPCA motif. As the result, free and complex state structures are uniformly superimposed, and notable conformational exchange is not observed except for the loop region that is flexible in free structure.

**Interaction Surface of the PKCα and Par6α PB1 Complex—** Despite the overall structural similarity between the PKCα and Par6α PB1 domains, they formed an asymmetric heterodimer using a different surface from each protein (Fig. 2A). The interaction surface of PKCα PB1 is the OPCA motif corresponding to β3, β4, and α2, whereas that of Par6α PB1 is composed of β1, β2, the C-terminal region of α1, and the N-terminal region of β5. Thus, PKCα serves as a type I PB1 domain and Par6α serves as a type II PB1 domain, respectively, as was suggested by the previous mutational analyses (18, 27). In PKCα PB1, the OPCA motif is responsible for the interaction with Par6α PB1, but no other regions are involved in the interaction.

The interaction surface of PKCα PB1 presents two acidic regions, A1 (Asp⁵⁹, Glu⁶₅, and Asp⁶⁷) and A2 (Glu⁶⁷), whereas that of Par6α is comprised of two basic regions, B1 (Lys¹⁹, Arg²⁸) and B2 (Arg²⁷) (Fig. 3, A and B). In PKCα PB1, Arg²⁷ and Arg²⁸, two consecutive basic residues on β2, direct their side chains to the opposite side so that Par6α PB1 presents two distinct basic regions, B1 and B2’, at both sides of β2 (Fig. 3A, right). The complementarity of the surface potential between A1-B1 and A2-B2 supports the notion that electrostatic interaction plays a major role in PB1 heterodimer formation. In addition to these interactions, Arg⁴² (PKCα)-Glu¹⁷ (Par6α) and a hydrophobic interaction, including Leu⁷⁰ of PKCα and Phe²⁹,
Leu$^{45}$, and Val$^{49}$ of Par6α, also contribute to the intermolecular interaction (Fig. 3A).

Description of the Interface in the PKC-Par6α PB1 Domain Complex—A detailed inspection of the interface gives the characteristic interaction mode in the PKC-Par6α complex (Fig. 3, A and B). In PKC PB1, A1 is located on the β turn in the OPCA motif formed by the conserved acidic residues, Asp$^{63}$, Glu$^{65}$, and Asp$^{67}$. As the β turn adopts a type I + G1 β bulge, these acidic residues orient to the same side to form an acidic surface. B1 of Par6α consists of Lys$^{19}$ in β1, Arg$^{28}$ in β2, and Arg$^{89}$ in β5. The complementarily charged side chains of PKC and Par6α form salt bridges as shown in Fig. 2B. Notably, Lys$^{19}$ of Par6α PB1 forms salt bridges with all of the three conserved acidic residues. The interaction mediated by Lys$^{19}$ is consistent with its strict conservation in type II PB1. Arg$^{28}$ and Arg$^{89}$ of Par6α interact with Glu$^{65}$ and Asp$^{67}$ of PKC, which fix these carboxyl groups to assist salt bridge formation with Lys$^{19}$ as well as to compensate for charges on the salt bridge network. Thus, A1-B1 is a major interaction site in the complex formation (Fig. 3B). In the complex of p40phox-p67phox PB1 domains, the corresponding acidic residues on the OPCA motif in p40phox (Asp$^{269}$, Glu$^{291}$, and Asp$^{293}$) interact with the conserved lysine residue in p67phox (Lys$^{325}$) in a manner similar to that in the PKC-Par6α complex (27). The importance of this interaction is evident considering that the type I PB1 domain contains an arginine residue instead of a lysine residue. To form tight salt bridges with the three acidic residues, the lysine residue is essential in this position (Fig. 1).

In addition to the A1-B1 interaction, two other interactions, including A2-B2′ and Arg$^{82}$-Glu$^{17}$, are to be noted (Fig. 3B). A2 is located on α2 of PKC PB1 to interact with B2′ of Par6α PB1. Glu$^{76}$ located on α2 of PKC PB1 forms a salt bridge with Arg$^{27}$ on β2 of Par6α PB1, which also forms a hydrogen bond with Ser$^{72}$ of PKC. Glu$^{17}$ of Par6α and Arg$^{82}$ of PKC are located close to each other to form a salt bridge. Furthermore, intramolecular salt bridge formation between Glu$^{79}$ and Arg$^{82}$ of PKC and between Glu$^{17}$ and Arg$^{28}$ of Par6α is observed (Fig. 3B). Besides these salt bridges, there are hydrophobic interactions involving Leu$^{75}$ on α2 of PKC and Leu$^{45}$ and Val$^{49}$ on β3 and Phe$^{29}$ on β2 of Par6α (Fig. 3A).

In the interface, not only intermolecular salt bridges but also a number of intramolecular salt bridges and hydrogen bonds are formed. These interactions are observed throughout the interface and form a network (Fig. 3C). Although the intramolecular salt bridges do not directly contribute to the binding, the salt bridge network may affect their side chain coordination to assist in the formation of a stable complex. There are a relatively small number of hydrophobic interactions and water-mediated hydrogen bonds in the interface. The surface area of the PKC-Par6α complex is −1141 Å$^2$, which is appreciably smaller than those of other protein complexes (37). Tight interaction between PB1 domains is mainly caused by the salt bridge and hydrogen bond formations that amounted to 10 in the complex and much less to hydrophobic interactions. Therefore, the interface of the PKC-Par6α PB1 complex is very compact but sufficient to form a stable protein-protein complex.

Mutational Analyses of the Residues Responsible for Complex Formation—The mutational analyses using the yeast two-hybrid method were performed to confirm the molecular interaction. The conserved acidic residue (Asp$^{67}$) in PKC PB1 and the conserved lysine residue in Par6α (Lys$^{19}$) were mutated to alanine, which completely abrogated the binding with each counterpart. The K20A mutation in PKC PB1 and the D63A mutation in Par6α showed similar binding affinity to the wild type (Fig. 4). These results are consistent with the previous mutational experiments using a pull-down binding assay (18, 27) and confirm that PKC PB1 serves as type I and Par6α PB1 as type II (Fig. 1). Furthermore, we designed several mutants based on the present structure of the PKC-Par6α PB1 complex. First, we made mutations at residues R28A, R89A, and R28A/R89A in Par6α to investigate the contribution of these residues to the A1-B1 interaction. Although R28A and R89A slightly diminished the interaction, the R28A/R89A double mutant completely impaired the interaction with PKC PB1, consistent with the present structure (Fig. 3, B and C). Then we further investigated the two interaction sites, A2-B2′ (Ser$^{72}$/
Glu76-Arg27) and Arg82-Glu17. Although E76A or R82A mutations in PKCβ/β9259 had a slight effect on binding to Par6α/β9251, the E76A/R82A double mutant completely abolished binding to Par6α/β9251. Thus, the Glu76 and Arg82 of PKCβ/β9259 were confirmed to be involved in the complex formation. The mutational studies by the yeast two-hybrid method (Fig. 4) support the interaction mode revealed herein in the structure of the PKCβ/β9259-Par6α/β9251 PB1 domains (Fig. 3).

General Properties of PB1-PB1 Interaction—The PB1 domains were classified into two types, the type I and the type II PB1 domains. Type I uses the OPCA motif, whereas type II uses the conserved lysine residue located on the opposite surface to the OPCA motif for heterodimerization. There is another type of PB1 domain, classified as type I and type II, which contains both the OPCA motif and the conserved lysine residue (23). For example, αPKC PB1 binds to Par6 PB1 as a type I PB1 domain and to MEK5 PB1 as a type II PB1 domain. Now, we aligned the PB1 domains on the structural basis, taking the previous studies into consideration (Fig. 1) (21, 23, 27, 28). We show the interfaces of both type I and type II PB1 domains in an open book style (Fig. 5). The interface in the type I PB1 domain is strictly located on the OPCA motif. Two acidic regions, A1 and A2, are presented on the OPCA motif of the type I PB1 domains that interact with the type II PB1 domains in an electrostatic manner. In contrast to the type I PB1 domain, the general properties of the type II PB1 domain are less understood because of low sequence homology except for the strictly conserved lysine residue involved in B1 that interacts with A1.

Our present study reveals the generality of the PB1-PB1 interaction especially in the type II PB1 domain. In the PB1 complex of PKCβ/β9259 and Par6α/β9251, A1 of PKCβ/β9259 interacts with B1, including Lys19, Arg28, and Arg89 of Par6α/β9251. Notably, as the Arg89 of Par6α is positioned in β5 as well as Lys19, the conserved lysine residue in β5 interacts with A1. Arg89 corresponds to a highly conserved basic residue in β5 of the type II PB1 domain (Fig. 1). Thus, the conserved basic residue in β5 of the type II PB1 domain is suggested to generally interact with A1 of the type I PB1 domain.

In contrast to the A1-B1 interaction, the A2-mediated interaction is quite complicated. In the structure of the p40phox-p67phox PB1 heterodimer, Lys382 in α1 of p67phox PB1 interacts...
with A2 of p40\textsuperscript{phox} PB1 (27). This basic residue in a1 (referred to basic region 2, B2) is also conserved in Bem1p PB1 (corresponding to Arg\textsuperscript{27}), and its contribution to the formation of the PB1 domain complex is supported by the tertiary structure and mutational experiments (21, 23). However, in other type II PB1 domains, the residue involved in B2 is not conserved on the sequential alignment (Fig. 1). Accordingly, the A2-B2 interaction appears to be specific for p40\textsuperscript{phox}-p67\textsuperscript{phox} and Cdc24p-Bem1p PB1. However, surprisingly, Arg\textsuperscript{27}, a basic residue in B2 of Par6\textalpha, interacts with Glu\textsuperscript{76} in A2 of PKC\textalpha. Arg\textsuperscript{27} presents its side chain to a similar position as Lys\textsuperscript{322} in p67\textsuperscript{phox} PB1 in the tertiary structure regardless of their different position in the sequence (Fig. 1). So Arg\textsuperscript{27} of Par6\textalpha and Lys\textsuperscript{322} of p40\textsuperscript{phox} give similar electrostatic potential at an equivalent position and form a conserved basic surface (Fig. 5). This basic residue in B2 (basic region 2', B2') is consistently conserved in type II PB1 domains except for p67\textsuperscript{phox} and Bem1p. Thus, the A2-B2/B2' interaction is considered to be conserved as a general property of the PB1-B1 interaction.

Specificity Determinants for PKC\textalpha-Par6\textalpha and p40\textsuperscript{phox}, p67\textsuperscript{phox} Complex Formation.—Although the formation of salt bridges between A1-B1 and A2-B2/B2' has been identified as a general property of PB1-PB1 interaction, the PB1-PB1 interaction is known to be highly specific and exclusive. As a unique feature of the p40\textsuperscript{phox}-p67\textsuperscript{phox} PB1 heterodimer, the C-terminal non-conserved region of p40\textsuperscript{phox} PB1 was shown by mutational analyses to be responsible for stable complex formation with p67\textsuperscript{phox} PB1 (25). Therefore, in addition to the typical A1-B1 and A2-B2/B2' interactions, the C-terminal extension of p40\textsuperscript{phox} located outside of the canonical PB1 domain boundary provides a specific interface essential for the formation of the p40\textsuperscript{phox}-p67\textsuperscript{phox} PB1 complex. However, the PB1 domain of Cdc24p exists at the C terminus of the protein, and therefore involvement of the C-terminal extension outside of the canonical PB1 domain is excluded from the complex formation with Par6\textalpha PB1.
In the complex of p40\textsuperscript{phox}-p67\textsuperscript{phox} PB1 domains, the C-terminal extension of p40\textsuperscript{phox} PB1 is engaged in the interaction with the H9252\textsuperscript{1}/H9252\textsuperscript{2} loop, and the C-terminal region of p67\textsuperscript{phox} is mainly through hydrophobic interaction and hydrogen bond formation (27). However, in contrast to the complex of PKC\textsuperscript{Par6}\textsuperscript{PB1} domains, p40\textsuperscript{phox} PB1 only slightly interacts with p67\textsuperscript{phox} because of the upward tilt (Fig. 6B). Thus, the change in the tilt angle between the two PB1 complexes is sufficient to endow specificity in the complex of the PB1 domains. Such specific interaction caused by the tilt in the interface was shown in the complex of Ras binding domain and Ras or Rap1A. Ras or Rap1A interacts with a specific effector such as Raf, RalGDS, or phosphatidylinositol 3-kinase \(\gamma\) by adjusting the relative orientation of each domain (38). Beside the case of Ras or Rap1A-Ras binding domain interaction, ubiquitin-mediated interactions with Npl4 zinc finger (39) and ubiquitin E2 variant domain (40) is a good example. Ubiquitin uses a hydrophobic patch on a \(\beta\) sheet as a common interface with them, whereas ubiquitin confers a specific interface with each domain by tilting its orientation. The specific recognition in domain-domain interaction achieved by tilting the orientation may be a general strategy developed in the process of evolution.

**Role of the PB1-PB1 Interaction between PKC\textsubscript{a} and Par6\textsubscript{a} in Tight Junction Formation—**MDCK cells normally form TJs when aPKC (wt) is overexpressed, whereas TJ assembly in MDCK cells is disturbed when a kinase-deficient mutant of aPKC is overexpressed (16). Thus, the kinase activity of aPKC is known to be important for epithelial polarity. However, the importance of the PB1-PB1 interaction between aPKC and Par6 in TJ assembly is still unclear. Accordingly, we investigated the role of PB1-PB1 interaction in the control of TJ assembly. In accordance with previous reports, MDCK cells normally formed TJs when PKC\textsubscript{a} (wt) was overexpressed (Fig.
The crystal structure of the PB1 heterodimer of PKC\(\alpha\) and Par6 demonstrated that the crystal structure of the PB1 heterodimer of PKC\(\alpha\) and Par6 revealed that the salt bridge formation between the PB1 domains is involved in the regulation of epithelial cell polarity. We evaluated the effect of Par6 mutants that do not bind with aPKC to clarify the role of the Par6-PB1 complex in TJ assembly. When Par6 (wt) was overexpressed in MDCK cells, TJ assembly was inhibited, which is consistent with the results of the previous study (Fig. 7) (41). However, when Par6 K19A and R27A/R28A mutants that do not interact with aPKC were overexpressed in MDCK cells, TJ assembly was little inhibited (Fig. 7). Thus, the role of Par6 in the TJ assembly is to interact with aPKC via the PB1 domain. These results strongly suggest that the PB1-PB1 interaction between aPKC and Par6 plays an important role in the regulation of epithelial cell polarity.

CONCLUSION

The crystal structure of the PB1 heterodimer of PKC\(\alpha\) and Par6 revealed that the salt bridge formation between the conserved acidic residues in the OPCA motif of PKC\(\alpha\) and the conserved lysine residue in Par6 is mainly responsible for complex formation. Interestingly, the ammonium group of the conserved lysine residue is tightly held with the three conserved acidic residues in the OPCA motif of PKC\(\alpha\) PB1. Such salt bridge formation is also observed in the p40\(^{phox}\)-p67\(^{phox}\) PB1 complex and is regarded as characteristic in PB1 heterodimer formation. In addition, the formation of another salt bridge (A2-B2) was observed. Although the electrostatic surface potential is quite similar in the Par6 and p67\(^{phox}\)-PB1 domains, the residue responsible for the formation of basic surface is not aligned in the sequence but makes up the equivalent basic surface in the tertiary structure. Thus, the A1-B1 and A2-B2 interactions are regarded as general properties in the PB1-PB1 interaction.

The PB1-PB1 interaction is known to be highly specific and exclusive. The comparison of the PB1 complexes of PKC\(\alpha\)-Par6 and p40\(^{phox}\)-p67\(^{phox}\) revealed differences in the relative orientation between PB1 domains. The C-terminal extension outside of the PB1 domain boundary of p40\(^{phox}\) PB1 is involved in the interaction with p67\(^{phox}\) PB1, whereas a2 in aPKC PB1 extensively interacts with Par6. Therefore, not only diversity in amino acid sequence but also the relative orientation of the PB1 domains endow specificity in PB1-PB1 interaction.

Although the PB1 domain adopts a ubiquitin fold, it develops insertions and extensions in the sequence as interaction sites. For example, the PB1 domain develops a C-terminal extension as a specific interaction site with the p67\(^{phox}\) PB1 domain. The above consideration leads to the hypothesis that the ubiquitin scaffold presents several surfaces as interaction sites for cognate partners, thus expanding the versatility of the protein interaction modes.
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