Molecular Recognition in Dimerization between PB1 Domains*

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The PB1 (Phox and Bem 1) domain is a recently identified module that mediates formation of a heterodimeric complex with other PB1 domain, e.g., the complexes between the phagocyte oxidase activators p67\textsuperscript{phox} and p40\textsuperscript{phox} and between the yeast polarity proteins Bem1p and Cdc24p. These PB1 domains harbor either a conserved lysine residue on one side or an acidic OPCA (OPR/PC/AID) motif around the other side; the lysine of p67\textsuperscript{phox} or Bem1p likely binds to the OPCA of p40\textsuperscript{phox} or Cdc24p, respectively, via electrostatic interactions. To further understand molecular recognition by PB1 domains, here we investigate the interactions mediated by proteins presenting both the lysine and OPCA on a single PB1 domain, namely Par6, atypical protein kinase C (aPKC), and ZIP. Par6 and aPKC form a complex via the interaction of the Par6 lysine with aPKC-OPCA but not via that between the aPKC lysine and Par6-OPCA, thereby localizing to the tight junction of epithelial cells. aPKC also uses its OPCA to interact with ZIP, another protein that has a PB1 domain presenting both the lysine and OPCA, whereas aPKC binds via the conserved lysine to MEK5 in the same manner as ZIP interacts with MEK5. In addition, ZIP can form a homotypic complex via the conserved electrostatic interactions. Thus the PB1 domain appears to be a protein module that fully exploits its two mutually interacting elements in molecular recognition to expand its repertoire of protein-protein interactions.

The cytoplasmic proteins that control the signaling pathways and regulatory systems in eukaryotic cells are commonly constructed of modular domains that mediate molecular interactions or have an enzymatic function (1). Interaction domains bind specifically to one another or to phospholipids, nucleic acids, or small molecule second messengers. Several protein interaction domains recognize specific sequences exposed on their partner proteins to mediate a modular protein-protein interaction: the SH2 domain binds to specific phosphorylserine motifs (2); the SH3 domain makes contact with a proline-rich region (3); and the PDZ domain interacts with the C terminus of target proteins in a sequence-specific manner, or with another PDZ domain to form a heterodimer (4). By using combinations of these modules, protein networks have presumably been developed for fine regulation of various cellular activities.

Among modules that mediate protein-protein interactions is the PB1 (Phox and Bem 1) domain, which has been recently identified (5). This domain, comprising about 80 amino acid residues (Fig. 1A), is designated as such because it was originally found in the phagocyte oxidase activator p67\textsuperscript{phox} and the yeast polarity protein Bem1p (5). According to a recent computer search (6), the PB1 domain is known to occur in nearly 200 proteins in yeasts, plants, and animals. Structural analysis by NMR has revealed that the Bem1p PB1 domain adopts a ubiquitin-like β-grasp fold and presents a conserved lysine residue on the first β-strand (7). The PB1 domains of p67\textsuperscript{phox} and Bem1p interact, via the lysine, with p40\textsuperscript{phox} and Cdc24p, respectively, by recognizing the PC (Phox and Cdc) motif in these target proteins (8). The PC motif (8), also known as OPR (9) or AID (10), is an -20-amino acid stretch composed of conserved acidic and hydrophobic residues (Fig. 1A) and is presently designated as the OPCA (OPR/PC/AID) motif (11). Current sequence and structural analyses show that the OPCA motif are all embedded within PB1 domain homologues (11). Thus, the PB1 domains of p40\textsuperscript{phox} and Cdc24p contain an OPCA motif, and those of p67\textsuperscript{phox} and Bem1p do not (Fig. 1A). The conserved lysine found in p67\textsuperscript{phox} and Bem1p, however, is replaced by arginine in p40\textsuperscript{phox} and Cdc24p (Fig. 1A).

Interactions between PB1 domains appear to participate in a variety of biological events. The NADPH oxidase in mammalian phagocytes is dormant in resting cells but becomes activated during phagocytosis to produce superoxide, a precursor of microbicidal oxidants (12–14). The activation of the oxidase requires translocation of p67\textsuperscript{phox}, p40\textsuperscript{phox}, p47\textsuperscript{phox}, and the small GTPase Rac from the cytoplasm to the membrane, where they assemble to activate the catalytic core of the oxidase, i.e. membrane-integrated cytochrome B\textsubscript{552} (12–14). In this process, p40\textsuperscript{phox} functions via the PB1-PB1 interaction with p67\textsuperscript{phox} (Fig. 1B) to greatly enhance the membrane recruitment of p67\textsuperscript{phox} and p47\textsuperscript{phox} (15). The PB1-mediated interaction between Bem1p and Cdc24p (Fig. 1B) plays a crucial role in the polarity establishment of Saccharomyces cerevisiae, which is essential for both budding and mating (5, 19). The Bem1p and Cdc24p homologues of the fission yeast, scd2 and scd1, respectively...
Cell polarization is also of fundamental importance in the organization of animal cells (18–20). The polarity protein Par6 and atypical protein kinase C (aPKC), each harboring the PB1 domain in the N terminus (Fig. 1A), form a heterodimer via a head-to-head interaction (21–25), which is likely involved in the establishment of cellular asymmetry during epithelial morphogenesis, asymmetric cell division, and directed cell migration (18–20). The Par6–aPKC complex not only serves as a target of the small GTPase Cdc42, which is known to be central to polarity in a variety of cells (18–20), but also functions by interacting directly with a variety of proteins such as another polarity protein Par3 (21–28), glycogen synthase kinase-3β (29), and the cytoskeletal protein Lgl (30–32). In mammalian epithelial cells, the Par6-aPKC-Par3 complex localizes to the tight junction and is considered to be involved in the establishment and maintenance of apico-basolateral polarity (18–20).

In contrast to the conventional PB1 domains, Par6-PB1 and aPKC-PB1 possess both the conserved lysine and OPCA motif (Fig. 1A). Here we show that Par6 interacts with aPKC via binding of the conserved lysine of Par6-PB1 to the OPCA motif of aPKC-PB1. The interaction by itself is required for localization of both proteins to the tight junction, which likely plays a crucial role in polarity establishment in epithelial cells. Based on experiments using various PB1 domains, we describe molecular mechanism underlying target recognition by the interaction module.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Complementary DNA fragments encoding various lengths of Par6, Par6β, Par6γ, PKCα, PKCi, and ZIP were prepared as described previously (5, 24, 28). We also amplified the DNA fragment that encodes MEK5 (amino acid residues 1–186) by reverse transcriptase-PCR using mRNA from human neuroblastoma SH-SY5Y cells. The DNA fragments were ligated to the indicated expression vectors. 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 of the constructs were sequenced to confirm their identities.

**Two-hybrid Experiments**—Various combinations between pGBT and pGADGH plasmids were co-transformed into competent yeast Y190 cells containing HIS3 and lacZ reporter genes, as described previously (33, 34). Following selection for the Trp+ and Leu+ phenotype, the transformants were tested for their ability to grow on plates lacking histidine supplemented with 25 µg 3-aminoacrylate to suppress background growth, according to the manufacturer’s recommendation (Clontech).

An in Vitro Pull-down Binding Assay Using Purified Proteins—For expression in Escherichia coli, cDNA fragments were ligated to the following vectors: pGEX-2T (Amersham Biosciences) for glutathione S-transferase (GST) fusion protein; pMALC2 (New England Biolabs) for maltose-binding protein (MBP) fusion protein; or pProEX-HTb (Invitrogen) for His-tagged protein. GST-, MBP-, or His-tagged proteins were purified by glutathione-Sepharose 4B (Amersham Biosciences), amyllose resin (New England Biolabs), or His-bind resin (Novagen), respectively, according to the manufacturers’ protocols. Pull-down binding assays were performed as described previously (24, 34, 35). Briefly, a pair of GST and MBP fusion proteins or a pair of GST fusion and MBP fusion proteins of Par6 and His-tagged proteins were mixed in 500 µl of phosphate-buffered saline (PBS) (137 mM NaCl, 2.68 mM KCl, 8.1 mM Na2HPO4, and 1.47 mM KH2PO4, pH 7.4) containing 10 mM dithiothreitol and incubated for 30 min at 4°C. A slurry of glutathione-Sepharose 4B or amyllose resin was subsequently added, followed by further incubation for 60 min at 4°C. After washing two times with PBS containing 0.5% Triton X-100 and 10 mM dithiothreitol, proteins were eluted from glutathione-Sepharose 4B with 10 mM glutathione or from amyllose resin with 10 mM maltose. The eluates were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue (CBB).

In Vivo Interaction of Par6 with aPKC—CO-IP assays were transfected with Lipofectamine (Invitrogen) with the expression vector pEF-BOS (36), which encodes the indicated protein, and cultured for 40 h in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (24, 28). The cells were lysed with 1 ml of a lysis buffer (138 mM NaCl, 2 mM EDTA, 10% glycerol, and 20 mM Tris, pH 8.0) containing 1% Triton X-100. The lysate was precipitated with a monoclonal antibody against Myc (9E10; Roche Applied Sciences), FLAG (M2, Sigma), or HA (16B12, Covance) in the presence of protein G-Sepharose (Amersham Biosciences). After washing three times with the lysis buffer, the precipitate was applied to SDS-PAGE, and transferred to a polyvinylidene difluoride membrane (Millipore). The membrane was probed with anti-Myc polyclonal antibodies (Santa Cruz Biotechnology), anti-FLAG (M2) monoclonal antibody, or anti-aPKC rabbit polyclonal antibodies (Santa Cruz Biotechnology). The blots were developed using ECL-plus (Amersham Biosciences) to visualize the antibodies.

**RESULTS**

Alignment of the Amino Acid Sequences of PB1 Domains—The sequences of PB1 domains from various proteins are aligned in Fig. 1A on the basis of the three-dimensional structure of this module of Bem1p (7). The originally identified PB1 domains, e.g., those of p67phox and Bem1p, lack an OPIC motif but harbor the conserved lysine residue on the first β-strand; Lys-355 of p67phox and Lys-482 of Bem1p are crucial for the interaction with their target proteins (5, 15). The lysine is replaced by arginine in a group of PB1 domains that possess the OPIC motif, such as those of Cdc42p, p40phox, and MEK5 (Fig. 1A).

On the other hand, the conserved lysine and OPIC motif are both present on single PB1 domains of aPKC, Par6, and ZIP (Fig. 1A). It is known that aPKC-PB1 directly interacts with the N-terminal regions of Par6 (24) and ZIP (5), each containing a PB1 domain (Fig. 1A). The roles of the lysine and OPIC motif presented on the same PB1 domain, however, have remained elusive.

**The PB1 Domain of Par6 Is Responsible for the Interaction with the PB1 Domain of aPKC**—The polarity protein Par6...
FIG. 1. The PB1 domains. A, the amino acid sequence alignment of PB1 domains. Secondary structures based on those of Bem1p are presented at the top, and a tentative consensus sequence is shown at the bottom where # symbols represent hydrophobic residues. The amino acids in the OPCA motif are shaded. B, domain structures of PB1 domain-containing proteins. TPRs, tetratricopeptide repeats; PX, phox homology domain; CH, calponin homology domain; DH, Dbl homology domain; PH, pleckstrin homology domain; ZZ, ZZ zinc finger domain; UBA, ubiquitin-associated domain.
FIG. 2. Role for the PB1 domain and the conserved lysine of Par6. A, pinpointing the Par6 domain, which interacts with the PB1 domain of aPKC. Yeast Y190 cells were co-transformed with a pair of pGBT9 encoding PKC/α and pGAD encoding Par6α, which were variously deleted from either the N or C terminus. Following the selection for the Trp⁻ and Leu⁻ phenotype, its histidine-dependent (right) and -independent (left) growth of the transformant was tested as described under “Experimental Procedures.” B, GST-Par6α-PB1 (14–95) was incubated with MBP-PKC/α-PB1, MBP-PKC/β-PB1, or MBP alone and pulled down with glutathione-Sepharose. The precipitated proteins were subjected to SDS-PAGE followed by staining with CBB. C, yeast Y190 cells were co-transformed with a pair of pGBT9 encoding PKC/α and pGAD encoding the PB1 domain of the wild-type Par6 (wt), the one with the K19A substitution, or the one with the D63A substitution. Following the selection for the Trp⁻ and Leu⁻ phenotype, the histidine-dependent (right) and -independent (left) growth of the transformant was tested as described under “Experimental Procedures.” D, GST-Par6α-PB1 (wt) or GST-Par6α-PB1 (K19A) was incubated with MBP-PKC/α-PB1, MBP-PKC/β-PB1, or MBP alone, and pulled down with amylose resin. The precipitated proteins were subjected to SDS-PAGE followed by staining with CBB. Positions for marker proteins are indicated in kDa. E, COS-7 cells were transfected with pEF-BOS-Myc-Par6α (wt) or pEF-BOS-Myc-Par6α (K19A). Lysates of the transfected cells were analyzed by immunoprecipitation (IP) with the anti-Myc monoclonal antibody followed by immunoblot (Blot) with the anti-aPKC (upper panels) or anti-Myc (lower panels) polyclonal antibodies. For details, see “Experimental Procedures.” These experiments were repeated more than three times with similar results.
harbors a PDZ domain and a CRIB-like motif (Fig. 2A); the former participates in interaction with Par3 (23), and the latter serves as a target for the small GTPases Cdc42 and Rac in the GTP-bound state (21–25). It is also known that the N-terminal region of Par6α (amino acid residues 1–115) interacts directly with aPKC (24). Here we pinpointed the aPKC-interacting region using a series of deletion mutants of Par6α in the yeast two-hybrid system. As shown in Fig. 2A, the fragment comprising amino acid residues 14–95 of Par6α was essential and sufficient for the binding to PKCα/λ; further deletion from either end completely abolished the two-hybrid interaction. When PKCζ was used instead of PKCα/λ, essentially the same interactions were observed (data not shown). The minimally required region (residues 14–95) corresponds precisely to the PB1 domain predicted from the alignment of the module from various proteins (Fig. 1A). Furthermore, in an in vitro pull-down assay using purified proteins, GST-Par6α(14–95) was precipitated efficiently with MBP-PKCα/λ-PB1 or MBP-PKCζ-PB1 but not with MBP alone (Fig. 2B). Thus the PB1 domain of Par6 seems to bind directly to the same module of aPKC.

The Conserved Lysine in the Par6 PB1 Domain Is Crucial for the Interaction between Par6 and aPKC—As described above, the PB1 domain of Par6 harbors both the conserved lysine and OPCA motif, the latter of which includes the stretch DXXGD (X, any amino acid) (Fig. 1A). We first tested the role of the lysine, Lys-19 of Par6α, in the interaction with the PB1 domain of aPKC. The K19A substitution led to an impaired interaction with PKCα/λ (Fig. 2C) or with PKCζ (data not shown), as estimated in the yeast two-hybrid system. The role of Lys-19 was verified by an in vitro binding assay using purified PB1 domains and by an immunoprecipitation assay using mammalian cells expressing full-length proteins. As shown in Fig. 2D, the GST-fused PB1 domain of Par6α carrying the K19A substitution, GST-Par6α-PB1(K19A), was incapable of binding to MBP-PKCα/λ-PB1 or MBP-PKCζ-PB1 under the conditions where the wild-type Par6α-PB1 fully bound to aPKC. When full-length Par6α was expressed as a Myc-tagged protein in COS-7 cells, aPKC co-immunoprecipitated with the wild-type Par6α(wt) but not with the mutant protein Par6α(K19A) (Fig. 2E). The K19A substitution does not appear to disturb the protein integrity because the mutant protein fully interacted with other targets, including the small GTPases Cdc42 and Rac, and Par3 (data not shown).

On the other hand, even when Asp-63, an invariant residue among the OPCA motif (Fig. 1A), was replaced by alanine, Par6α fully bound to aPKC (Fig. 2, C and E). In addition, the substitution of alanine for other conserved residues in the motif, such as Asp-67, Leu-69, and Ser-76, did not affect the interaction with aPKC (data not shown). Taken together, the binding of Par6 to aPKC is mediated via the conserved Lys-19 but not via the conserved lysine, to interact with Par6. Taken together, the interaction between Par6 and aPKC is probably mediated via binding of the conserved lysine of Par6-PB1 to the OPCA motif of aPKC-PB1. On the other hand, neither Par6-OPCA nor the lysine of aPKC is involved.

We next tested the interaction of aPKC with ZIP (a protein linking the ζ isofrom of protein kinase C to RIP and/or potassium channels) (39–41), also known as p62 (42). The binding is mediated via their PB1 domains (Ref. 5; see Fig. 1B), and ZIP-PB1 also possesses both the conserved lysine and OPCA motif (Fig. 1A). To know the manner of this PB1-PB1 interaction, we tested the role for the lysine and OPCA of these PB1 domains in the two-hybrid system. As shown in Fig. 5A, ZIP was capable of binding to PKCα/λ(K20A) but not to PKCα/λ(D63A) (Fig. 5A). On the other hand, PKCα/λ-PB1 failed to bind to a mutant ZIP carrying the K7A substitution, under the conditions where it fully interacted with a mutant ZIP-PB1 carrying the D71A substitution in the OPCA motif. These findings in-
dicate that aPKC-PB1 recognizes ZIP-PB1 in a manner similar to its recognition of Par6-PB1; the OPCA motif of aPKC interacts with the conserved lysine in PB1 domains of target proteins.

It has been shown that aPKC-PB1 also interacts with MEK5 (43), a mitogen-activated protein kinase kinase that activates ERK5/BMK1 (44–46). In this PB1-PB1 interaction, the conserved lysine of aPKC is supposed to be involved instead of the OPCA motif, because MEK5 contains a canonical OPCA motif but lacks the conserved lysine (Fig. 1A). As expected, PKC/\(\lambda\)(K20A) failed to bind to MEK5, whereas PKC/\(\lambda\)(D63A) interacted with MEK5 (Fig. 5B). Thus aPKC-PB1 likely recognizes MEK5 in a manner different from its recognition of Par6 and ZIP; the recognition requires the conserved lysine, but not the OPCA motif, in aPKC-PB1.

ZIP Can Bind to MEK5 and Form a Homotypic Complex via PB1-PB1 Interaction—During the course of experiments using various combinations of PB1 domains, we found two novel
PB1-PB1 interactions: ZIP made contact with MEK5 (Fig. 5C); and ZIP formed a homotypic complex (Fig. 5D), although such self-association was not observed in aPKC-PB1 or Par6-PB1 (data not shown). In the interaction between ZIP and MEK5, the conserved lysine in ZIP-PB1 played an essential role, whereas the OPCA motif in ZIP-PB1 was dispensable (Fig. 5C). Hence it is likely that ZIP-PB1 recognizes MEK5-PB1 in a manner similar to its recognition of aPKC-PB1 (Fig. 5B), and MEK5-PB1 interacts with ZIP-PB1 as with aPKC-PB1 (Fig. 5A). Although ZIP-PB1 harbors the two mutually interacting elements, ZIP uses solely the conserved lysine in the interaction with MEK5 as well as aPKC.

Finally we investigated the PB1-mediated self-association of ZIP. Although neither the K7A nor the D71K substitution affected the interaction (Fig. 5D), a doubly substituted mutant, ZIP(K7A/D71K), was incapable of interacting with the wild-type protein (Fig. 5D). The interaction thus seems to require either the conserved Lys-7 or the OPCA motif in ZIP-PB1. Even in the homotypic interaction, the lysine in a PB1 domain appears to bind to the OPCA motif in the other PB1: ZIP(K7A) or ZIP(D71K) failed to self-associate, whereas ZIP(K7A) and ZIP(D71K) could interact with each other (Fig. 5D). In addition to the conserved lysine, therefore, the OPCA motif in ZIP-PB1 is also of functional importance.

DISCUSSION

Here we describe molecular recognition in interactions between PB1 domains. The PB1 domain of about 80 amino acid residues adopts a ubiquitin-like β-grasp fold, which consists of two α-helices and a single β-sheet (7). Heterodimerization of PB1 domains is presumably mediated via two mutually interacting elements, i.e. a conserved lysine on the first strand and an OPCA motif (for details, see below). The PB1 domains found initially, such as p67phox-PB1 and Bem1p-PB1, harbor the lysine but lack the OPCA motif (Fig. 6A), whereas the PB1 domains of their targets (p40phox-PB1 and Cdc24p-PB1, respec-
determinant in the recognition of OPCA motifs. The two aspara-
gly) contain the OPCA motif but not the lysine (Fig. 6A). On
the other hand, the two elements occur simultaneously in a
single PB1 domain of various proteins such as Par6, aPKC, and
ZIP (Fig. 1A).

The cell polarity protein Par6 is known to associate tightly
with aPKC (18–20). In the present study, we clearly show that
the association is mediated via binding of the conserved lysine
of Par6-PB1 to the OPCA motif of aPKC-PB1; neither the
lysine of aPKC nor the Par6 OPCA motif participates in the
PB1-PB1 interaction (Figs. 2 and 3; see also Fig. 6B). We also
demonstrate that the interaction by itself, but not the kinase
activity of aPKC, is required for the localization of both pro-
teins to the tight junctions in mammalian epithelial cells (Fig.
4). In mammals, aPKC plays an essential role in the formation
of the epithelia-specific cell-cell adhesion structure (18–20).
For this function, it seems important that aPKC correctly lo-
calizes to a junctional complex such as the tight junctions.
This may be supported by the observation that assembly of the
tight junctions is inhibited by ectopic expression of an isolated PB1
domain of aPKC, which probably serves as a dominant negative
fragment, but is not affected by the mutant aPKC-PB1, which
is incapable of binding to Par6 (38). The correct localization of
aPKC, which requires the PB1-PB1 interaction with Par6, may
thus be implicated in epithelial tight junction assembly.

In contrast to the association of aPKC with Par6, the con-
served lysine of aPKC-PB1, instead of the OPCA motif, partici-
pates in the interaction with MEK5, which contains the OPCA
motif but not the lysine (Fig. 5). Thus the two target-interact-
ng elements of aPKC-PB1 are both functional (Fig. 6B). Sim-
ilarly, ZIP-PB1 uses the conserved lysine or the OPCA motif to
recognize distinct targets; ZIP interacts via the lysine with
aPKC and MEK5, whereas the homotypic interaction of ZIP-
PB1 requires the OPCA motif as well (Fig. 6C).

Among the PB1 domains that can interact with OPCA mo-
tifs, the lysine of the first β-strand is the sole invariant residue.
The substitution of alanine for the lysine abrogates the PB1-
PB1 interaction without exception (Refs. 5 and 15, and the
present study), indicating that this residue serves as a core
determinant in the recognition of OPCA motifs. The two aspar-
tate residues in the conserved DXXGD stretch of the OPCA
motif play a common role in PB1-PB1 interactions; amino acid
substitutions for either aspartate lead to an almost complete
loss of all the PB1-PB1 interactions tested thus far in various
studies (5, 7, 8, 15) including the present one. Hence it is likely
that the aspartates interact directly with the invariant lysine of
the partner PB1 domain, probably via electrostatic
interactions.

Judging from the three-dimensional structure of Bem1p-PB1
(7) and Cdc24p-PB1 (47), the invariant lysine is supposed to be
located on a surface distinct from that of an acidic OPCA motif.
Thus it is highly probable that the PB1 domains in the het-
erodimeric complex are asymmetrically aligned, which complex
is similar to the heterodimer formed from the CAD domains of
the caspase-activated DNase CAD and its inhibitor ICAD (48).
The CAD domain consists of one α-helix and five β-strands
forming a single sheet, the overall structure of which is cate-
gorized in the ubiquitin superfold. In the heterodimeric com-
plex, the two CAD domains are not aligned symmetrically,
using the same side for the binding interface, but rather side by
side; positive charges on the strand β2 at one end of the β-sheets
of CAD are paired with negative charges around the opposite
end of the β-sheet of ICAD (48).

It is presently unknown how a PB1 domain selects its specific
partner. The PB1 domain of Bem1p recognizes the OPCA motif
of Cdc24p (5) but not that of p40phox (5) or aPKC (data not
shown). In the recognition of the OPCA motif of Cdc24p,
Bem1p-PB1 uses not only the invariant lysine (Lys-482) but
also Ala-491 on the N terminus of the second
β-strand (see Fig. 1A), albeit to a lesser extent; the A491N or
R510A substitution in Bem1p-PB1 results in a slightly decreased interaction with Cdc24p (47). In
Par6-PB1 and ZIP-PB1, both of which bind to aPKC (Figs. 3
and 5) but not to Cdc24p (data not shown), the alanine residue
is substituted with arginine (Arg-28 of Par6; Arg-22 of ZIP),
whereas the arginine on the α-helix is replaced by a hydropho-
bic residue (Val-49 of Par6; Leu-52 of ZIP). It is thus possible
that these amino acids serve as a determinant of specificity in
the recognition of OPCA motifs.

[Image: Molecular recognition by PB1 domains. K, the conserved lysine on the first β-strand of PB1 domain; PC, the OPCA motif presented on PB1 domains.]
Unconserved residues in the OPCA motif may also function to determine the specificity. For instance, an aspartate residue on the second α-helix of p40phox and Cdc24p (Asp-302 and Asp-833, respectively; see Fig. 1A) participaties in interaction with their partners; the D302A substitution in p40phox leads to an impaired interaction with p67phox (15), and Cdc24p carrying the D383A substitution interacts only weakly with Bem1p (47). This residue, however, is not conserved among OPCA motifs (Fig. 1A), suggesting its role in specific recognition of PB1 partners. In this context, it should be noted that the residue is glutamate in the OPCA motifs of aPKC, ZIP, and MEK5 (Fig. 1A), all of which can be recognized by ZIP-PB1 (Fig. 5). The extra-OPCA region is also supposed to be involved in specificity determination. The truncation of the five C-terminal residues of p40phox (amino acids 335–339) results in a defective interaction with the PB1 domain of p67phox (8). This fragment is located on the C-terminal extension outside of the ubiquitin-like fold of amino acids 236–329 (Fig. 1A). Further studies are required, including determination of the tertiary structure of PB1 heterodimers, to understand mechanism whereby PB1 domains recognize their specific partner.

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