Association of Two Nuclear Proteins, Npw38 and NpwBP, via the Interaction between the WW Domain and a Novel Proline-rich Motif Containing Glycine and Arginine*

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We have previously reported a nuclear protein possessing a WW domain, Npw38 (Komuro, A., Saeki, M., and Kato, S. (1999) Nucleic Acids Res. 27, 1957–1965). Here we report a Npw38-binding protein, NpwBP, isolated from HeLa cell nuclear extracts and its characterization using a cloned cDNA. NpwBP contains two proline-rich regions that are capable of binding to the WW domain of Npw38. The binding analysis using an oligopeptide-immobilized membrane revealed that the WW domain of Npw38 preferentially recognizes a short proline-rich sequence, PPGPPP, surrounded by an arginine residue, so we named it a PGR motif. Localization analysis using green fluorescent protein fusion protein and immunostaining showed that Npw38 and NpwBP are colocalized in the same subnuclear region. Coimmunoprecipitation experiments confirmed the association between Npw38 and NpwBP, which were expressed as epitope-tagged forms in COS7 cells. Furthermore, the N-terminal region of NpwBP has binding ability for poly(rG) and G-rich single-stranded DNA. These results suggest that NpwBP is a physiological ligand of Npw38 and that the Npw38-NpwBP complex may function as a component of an mRNA factory in the nucleus.

**WW domains are globular modules composed of 35–40 amino acids in which two tryptophans and one proline are conserved (1–4). WW domains have been revealed to be involved in mediating protein-protein interaction that resulted from a binding between the WW domain and a proline-rich motif in a corresponding ligand. Recent reports described various pairs of proteins mediated by WW domains, which have potential involvement in signaling processes that underlie human disease (5–9). The analysis of the binding regions in these pairs revealed that the WW domains are classified into three classes by the proline-rich motifs of the ligands: a PY motif (10), a PPLP motif (11–13), and a PGM motif (14). Recently, a different class of motif has been found in phosphoproteins to which WW domains of the Pin1 and the ubiquitin ligase NEDD4 bind in a phosphorylation-dependent manner (15).

We have previously reported a novel WW domain-containing protein Npw38 (16). Npw38 is localized in the nucleus and has binding ability for poly(rG). The WW domain of Npw38 possesses eight hydrophobic amino acid residues in a relatively acidic region, while other WW domains have rather hydrophilic and more basic residues. These characteristics may contribute to the ligand binding specificity of Npw38. To characterize a binding motif in a Npw38 ligand, we tried to find a physiological ligand of the WW domain of Npw38 using a yeast two-hybrid screening system. However, we failed to identify the ligand because the WW domain itself caused a basal transcriptional activation (16).

In this report, we describe the isolation of a Npw38-binding protein, NpwBP, from HeLa nuclear extracts using affinity purification and the characterization of NpwBP using its cDNA. NpwBP has been shown to have two proline-rich regions that are capable of interacting with the WW domain of Npw38. The precise binding motif was determined by a binding assay using an oligopeptide-immobilized membrane, demonstrating a novel WW domain-binding motif composed of proline, glycine, and arginine. Localization and immunoprecipitation analyses indicate that Npw38 and NpwBP associate with each other in the nucleus. Furthermore, NpwBP was shown to have nucleotide-binding ability as does Npw38.

EXPERIMENTAL PROCEDURES
cDNA Clones—The Npw38 cDNA has been described in a previous paper (16). The human EST clone encoding NpwBP was purchased from Genome Systems. Both strands of all cDNA clones were sequenced using a primer walking method with a DNA sequencer (Perkin-Elmer ABI, model 377).

Preparation of GST Fusion Proteins—GST-Npw38 fusion proteins containing a single mutation were described in the previous paper (16). The coding regions containing a test sequence of NpwBP (positions 1–192 and 1–352) were amplified by PCR using primers with an EcoRI or a SalI site. This PCR product was digested with restriction enzymes and subcloned into an EcoRI-SalI site of pGEX-5X-1 (Amersham Pharmacia Biotech). GST-NpwBP(190–210) and GST-NpwBP(120–210) were constructed by annealing two oligonucleotides (5′-CGTCCACAGTGGCCCTCCCCCTGGCGCAGCAAACTCCTGTCGACCCCTCCTCCCTCCTCAAG-3′ and 5′-TGGCTCCACACTTCTCCCTTCCTACAG-3′) and 5′-CGTCCACAGTGGCCCTCCCCCTGGCGCAGCAAACTCCTGTCGACCCCTCCTCCCTCCTCAAG-3′ and 5′-TGGCTCCACACTTCTCCCTTCCTACAG-3′, respectively, and ligating them into pGEX-5X-1. The coding region of the FBP21 was amplified by PCR using primers with a SalI and a NotI site and subcloned into a SalI-NotI site of pGEX-6P-1. The constructed vector was introduced into E. coli JM109 and the expression was induced for 3 h by adding isopropyl-1-thio-D-galactopyranoside to a final concentration of 0.2 mM. The proteins were affinity-purified with glutathione-Sepharose.

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The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EMBL Data Bank with accession number AB029309 (for NpwBP cDNA).

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The abbreviations used are: GST, glutathione S-transferase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; HA, hemagglutinin; ss, single-stranded.
rose beads and eluted with reduced glutathione as described in the manufacturer’s instruction manual (Amersham Pharmacia Biotech).

Identification of the Npw38-associated Protein—For the detection of Npw38-binding proteins, GST or GST-Npw38 (500 ng each) was added to HeLa nuclear extracts, and the binding proteins were purified by glutathione-Sepharose beads and eluted with SDS sample buffer. Bound proteins were separated by SDS-PAGE and visualized by silver staining.

For the large scale preparation of nuclear extract, HeLa-S3 cells (1 × 10^8 cells) were harvested in phosphate-buffered saline and transferred into a hypotonic buffer (20 mM HEPES (pH 7.4), 10 mM KCl, 1.5 mM MgCl₂, 1 mg/ml leupeptin, 2 mM pepsin, 10 μg/ml aprotinin) to swell for 10 min. The cells were then lysed by vigorous Dounce homogenization, and nuclei were recovered by centrifugation at 1500 × g. The nuclei were purified by washing twice in a hypotonic buffer containing 0.5% Nonidet P-40. Purified nuclei were extracted with a high salt buffer (20 mM HEPES (pH 7.4), 1.5 mM MgCl₂, 250 mM NaCl, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin). A monoclonal anti-HA 3F10 (rat, Roche Molecular Biochemicals), anti-FLAG M2 (mouse, Eastman Kodak Co.), or control IgG (rat or mouse) was added and the mixture incubated overnight at 4 °C. The immunocomplexes were collected with protein G-agarose beads and washed with immunoprecipitation buffer. Precipitated proteins were separated on SDS-PAGE, transferred onto a PVDF membrane, and analyzed by immunoblotting with either anti-HA and anti-FLAG antibodies.

Antibody Preparation—Anti-NpwBP antisera was prepared in a rabbit using the bacterially expressed GST-NpwBP-(1–192) fusion protein. Anti-GST antibodies were removed from the serum by GST affinity chromatography using an Affi-Gel 10 hydroxysuccinimide-activated column (Amersham Pharmacia Biotech). The anti-NpwBP antibodies were purified using a GST-NpwBP-(1–192) affinity column. The absence of reactivity by the purified anti-NpwBP antibodies against GST was confirmed by Western blot analysis.

Pull-down Assay Using In Vitro Translation Products—The in vitro transcription/translation was carried out in a reaction mixture containing [³⁵S]methionine (Amersham Pharmacia Biotech) using a TnT-coupled reticulocyte lysate system (Promega) according to the manufacturer’s instructions. Four μl of the [³⁵S]-labeled translation product was diluted in 500 μl of immunoprecipitation buffer (20 mM Tris-HCl (pH 7.4), 1% Triton X-100, 0.25% sodium deoxycholate, 250 mM NaCl, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin). A monoclonal anti-HA 3F10 (rat, Roche Molecular Biochemicals), anti-FLAG M2 (mouse, Eastman Kodak Co.), or control IgG (rat or mouse) was added and the mixture incubated overnight at 4 °C. The immunocomplexes were collected with protein G-agarose beads and washed with immunoprecipitation buffer. Precipitated proteins were separated on SDS-PAGE, transferred onto a PVDF membrane, and analyzed by immunoblotting with either anti-HA and anti-FLAG antibodies.

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Colocalization Analysis—HeLa cells transiently expressing the GFP-Npw38 fusion gene (16) were washed with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. The fixed cells were washed with PBS and permeabilized with 0.2% Triton X-100 in PBS. After blocking with 5% milk in PBS, the cells were incubated with the anti-NpwBP antibody for 45 min, washed with PBS, and stained with rhodamine-conjugated secondary antibody for 45 min. GFP and rhodamine images were individually or simultaneously observed on a fluorescence confocal microscope (Nikon, Bio-Rad).

Oligopeptide Binding Assay—GST fusion proteins were ³²P-labeled with protein kinase (17). Briefly, Sepharose beads containing the desired GST fusion protein were washed once with HKM buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 12 mM MgCl₂) and resuspended in 2–3 volumes of HKM buffer containing 1 mM dithiothreitol, 1 μCi/μl of [³²P]ATP (5000 Ci/mmole, Amersham Pharmacia Biotech) and 1 unit/μl of the catalytic subunit of cAMP-dependent protein kinase (Sig-
A kinase reaction was performed at 4 °C for 30 min and terminated by the addition of 1 ml of HKM stop buffer (10 mM sodium phosphate (pH 8), 10 mM sodium pyrophosphate, 10 mM EDTA). The beads were washed five times with PBS and eluted in 8 bed volumes of elution buffer (25 mM glutathione, 50 mM Tris (pH 8), 150 mM NaCl, 0.1% Triton X-100 and 0.75% Sarkosyl). A SPOTs membrane that is immobilized by 12- or 13-nucleotide long oligopeptides derived from the proline-rich sequence of NpwBP and peptides from several WW domain binding sequences was obtained from Genosys Biotechnologies, Inc. The membrane was blocked overnight at room temperature with blocking buffer (Tris-buffered saline (pH 8.0) containing 0.05% Tween 20 and 5% bovine serum albumin). The complexes were separated by electrophoresis through a 5% polyacrylamide gel using 1 TBE buffer (100 mM Tris base, 100 mM boric acid, and 2 mM EDTA (pH 8.0)) at 100 V. The gels were subjected to autoradiography.

To find a putative binding partner of Npw38, an affinity purification approach was used. After GST-Npw38 was incubated with HeLa cell nuclear extracts, binding proteins were purified by glutathione-Sepharose beads and were analyzed by SDS-PAGE. As shown in Fig. 1A, a specific band with a molecular mass of 94 kDa was detected only when the GST-Npw38 fusion protein was used as an affinity probe, whereas several common binding proteins were detected for both GST and GST-Npw38 probes. To identify the 94-kDa protein, a large scale affinity purification was performed. The proteins bound to glutathione beads were analyzed by SDS-PAGE. Lane 1, 25% input; lane 2, GST; lane 3, GST-Npw38-(1–265); lane 4, GST-Npw38-(1–43); lane 5, GST-Npw38-(25–84); lane 6, GST-Npw38-(43–98); lane 7, GST-Npw38-(81–265). C, association of NpwBP with GST-Npw38 point mutants. Lane 1, 20% input; lane 2, GST; lane 3, GST-Npw38. The conserved two Trps and one Pro in the WW domain of Npw38 were substituted with Ala (W52A, Ala lane 4, Ala (W52A, lane 5), and Gly (F78G, lane 6), respectively. D, schematic representation of the various deleted mutants of NpwBP used for the GST pull-down assay and the yeast two-hybrid system. Interaction + indicates that the deletion mutant interacted with Npw38 in the yeast two-hybrid system. E, in vitro translated 35S-Npw38 was incubated with GST-Npw38 fusion proteins. The proteins bound to glutathione beads were analyzed by SDS-PAGE. Lane 1, 25% input; lane 2, GST; lane 3, GST-NpwBP-(1–192); lane 4, GST-NpwBP-(1–352); lane 5, GST-NpwBP-(190–210); lane 6, the basic amino acids (Arg-192, Arg-197, Lys-198) in NpwBP-(190–210) were substituted with Ala residues.

mRNA. A kinase reaction was performed at 4 °C for 30 min and terminated by the addition of 1 ml of HKM stop buffer (10 mM sodium phosphate (pH 8), 10 mM sodium pyrophosphate, 10 mM EDTA). The beads were washed five times with PBS and eluted in 8 bed volumes of elution buffer (25 mM glutathione, 50 mM Tris (pH 8), 150 mM NaCl, 0.1% Triton X-100 and 0.75% Sarkosyl). A SPOTs membrane that is immobilized by 12- or 13-nucleotide long oligopeptides derived from the proline-rich sequence of NpwBP and peptides from several WW domain binding sequences was obtained from Genosys Biotechnologies, Inc. The membrane was blocked overnight at room temperature with blocking buffer (Tris-buffered saline (pH 8.0) containing 0.05% Tween 20 and 5% bovine serum albumin). The complexes were separated by electrophoresis through a 5% polyacrylamide gel using 1 TBE buffer (100 mM Tris base, 100 mM boric acid, and 2 mM EDTA (pH 8.0)) at 100 V. The gels were subjected to autoradiography.

RESULTS

Identification of a Protein Interacting with Npw38—To find a putative binding partner of Npw38, an affinity purification approach was used. After GST-Npw38 was incubated with HeLa cell nuclear extracts, binding proteins were purified by glutathione-Sepharose beads and were analyzed by SDS-PAGE. As shown in Fig. 1A, a specific band with a molecular mass of 94 kDa was detected only when the GST-Npw38 fusion protein was used as an affinity probe, whereas several common binding proteins were detected for both GST and GST-Npw38 probes. To identify the 94-kDa protein, a large scale affinity purification was performed. The proteins bound to GST-Npw38 were separated by SDS-PAGE and blotted onto a PVDF membrane. The N-terminal amino acid sequence of the 94-kDa protein, designated by NpwBP, was determined by a protein sequencer to be GXRSTSSTKS6GKFMNPTDQAKREEA.

A BLAST search based on a GenBank™ data base using this sequence revealed that this amino acid sequence was included in an open reading frame of one EST cDNA clone (GenBank™ accession no. AA206624). Sequencing analysis of this cDNA showed that it contains a cDNA insert of 2654 base pairs capable of encoding a 641-amino acid protein (Fig. 1B) in which the N-terminal sequence is in agreement with that of the purified Npw38. The molecular mass of the Npw38 protein predicted from the cDNA sequence is 70 kDa, which is lower than the 94 kDa of the purified protein. This discrepancy may be explained by the effect of the highly charged region on NpwBP. The molecular mass of the in vitro translated 35S-Npw38 was identical to that of the purified protein (Fig. 2B).

The characteristic feature of the deduced amino acid sequence is the existence of two proline-rich regions (positions 191–209 and 404–538) and two acidic cluster regions (positions 231–302 and 341–361) (Fig. 1C). A PSORT II program predicted the coiled-coil region (position 71–133) and four putative nuclear localization signals (RKEARKREKKNKKQR at 22, RKKK at 318). The C-terminal region (position 218–599) is similar to the mouse homolog of the NpwBP cDNA.
Npw38 and NpwBP Interact through the WW Domain in Vitro—To confirm that NpwBP directly interacts with Npw38, the in vitro translation product of the NpwBP cDNA was incubated with GST-Npw38 and then GST-Npw38 was isolated from the reaction mixture using glutathione beads. As a result, 35S-NpwBP was copurified with GST-Npw38 even under the stringent buffer condition containing detergents (1% Triton X-100, 0.25% sodium deoxycholate), but not with GST alone (Fig. 2B). Next, to determine the NpwBP-binding region in Npw38, we prepared the GST fusions with various deletion mutants of Npw38 (Fig. 2A). As shown in Fig. 2B, the WW domain-containing construct GST-Npw38-(25–84) associated with NpwBP with the same affinity as GST-Npw38, but GST-Npw38-(43–98) did so with lower affinity. The WW domain-deleted constructs such as GST-Npw38-(1–43) or GST-Npw38-(81–265) failed to associate with NpwBP. Furthermore, the point mutants in which one of the conserved two Trps or Pro in the WW domain of Npw38 was replaced with Ala or Gly lost the NpwBP-binding ability (Fig. 2C). All these results confirmed that the association between Npw38 and NpwBP was made through the WW domain.

The WW Domain of Npw38 Interacts with the Proline-rich Regions of NpwBP—To explore the WW domain-binding region in the NpwBP, we performed a GST fusion pull-down assay and a yeast two-hybrid assay. The NpwBP has two proline-rich regions that are candidates for the binding site to the WW domain of Npw38, because known WW domain-binding proteins have a short proline-rich sequence capable of binding to their WW domains. The GST pull-down assay using GST fusions with the deleted NpwBP mutants revealed that the GST-NpwBP-(1–352) and GST-NpwBP-(190–210), both containing the first proline-rich region, associate with NpwBP, but GST-NpwBP-(1–43) and GST-NpwBP-(81–265) failed to associate with NpwBP. Furthermore, the point mutants in which one of the conserved two Trps or Pro in the WW domain of Npw38 was replaced with Ala or Gly lost the NpwBP-binding ability (Fig. 2C). All these results confirmed that the association between Npw38 and NpwBP was made through the WW domain.

TABLE I

| Peptide no. | Sequence | Relative intensity |
|------------|----------|--------------------|
| 22         | PRLLPPGPPPGR | 100 |
| 3          | RKPGGPPGPPPFP | 85 |
| 14         | PGGPPGAPPFL  | 72 |
| 25         | PGPPGPPGPPP  | 65 |
| 30         | PPGPPGPPPGR  | 59 |
| 23         | LGPGLPPGPPP  | 55 |
| 27         | PPGGLPGLPP  | 43 |
| 24         | GPPPGGPPGPP  | 42 |
| 2          | PPGRPPGPPGPP | 42 |
| 29         | PGLPPGPPGPP  | 37 |
| 11         | RPPGPPGTPGPP | 37 |
| 26         | PGPPGPGLPP  | 33 |
| 15         | PGGPPGPPGLP  | 31 |
| 10         | PPLLPPGPPGGL | 24 |
| 31         | PPGPPGPPGLP  | 15 |
| 21         | GPPGLPPGPP  | 15 |
| 9          | FPAPPLPPGPP  | 14 |
| 17         | PLLPPGCMGGL  | 13 |
| 12         | GPPGLPPGPP  | 4 |

Each peptide is derived from the proline-rich regions of NpwBP as shown in Fig. 3A.
interacts with Npw38, we performed a binding analysis using a SPOTs membrane that is immobilized by 10–13-nucleotide long oligopeptides derived from the first and second Pro-rich regions of NpwBP (Fig. 3A) in addition to Pro-rich peptides derived from several WW domain ligands (Fig. 3B). Fig. 3D shows positive signals resulting from the binding of GST-Npw38, and Fig. 3F shows the relative intensity of each spot. Table I shows the sequences arranged in order of the degree of intensity. These results clearly indicate that the top 10 peptides showing strong intensity contain a Pro-rich motif, PPGPPP. Peptides 12, 13, and 28, however, showed a very weak signal despite containing PPGPPP. This discrepancy can be explained by the existence of an Arg residue surrounding a PPGPPP motif. The PPGPPP in peptides showing a high intensity signal are flanked by Arg within three amino acids upstream and/or downstream except for peptides 14 and 27. The importance of Arg on binding ability is implicated by the fact that several peptides containing an Arg residue within three amino acids upstream and/or downstream flank the motif (peptides 9, 10, 11, 15, 17, 21, and 31) become positive despite substitution or deletion of one amino acid residue in the sequence of the PPGPPP motif. Other evidence is obtained from the comparison between the results of peptides 24 and 28. Peptide 24 showed relatively strong binding ability, but peptide 28 in which Arg in the peptide 24 is replaced by Leu did not. The contribution of Arg has been further confirmed by a pull-down assay using a mutated probe. When three basic amino acids (Arg-192, Arg-197, Lys-198) in the first Pro-rich region were substituted with Ala residues, the binding to Npw38 was abolished in the GST pull-down experiments (see Fig. 2E). The strong signals of the peptides 14 and 27 not containing Arg may be explained by the effect of the location of PPGPPP in the probe peptide. These two peptides are flanked by no or only one additional residue upstream of PPGPPP, whereas peptides 12, 13, and 18 showing a very weak signal have more than three residues, suggesting that the upstream region of PPGPPP may suppress binding if this region does not contain Arg.

Finally, we attempt to examine whether Npw38 associates with Pro-rich motifs derived from several WW domain ligands. Npw38 did not bind to any motifs including PGM motifs (UIC, SF1, and SmB/B’), PPXY motifs (WP2 and epithelial sodium channel), and PPLP motifs (Mena) (Fig. 3D), but the faint signals were detected from the PPXY motifs of the epithelial sodium channel β and γ chain and the PPLP motifs of Mena only on long term exposure to the x-ray film (Fig. 3F). All these results indicate that the binding motif of NpwBP to the WW domain of Npw38 is PPGPPP surrounded by Arg. Thus, we named this motif a “PGR motif,” which is the fourth class of a Pro-rich WW domain binding motif (cf. Ref. 18).

**NpwBP Associates with Npw38 in Nuclei—** Previously, we demonstrated that Npw38 is localized in nuclei (16). To confirm the colocalization of Npw38 and NpwBP in nuclei, HeLa cells producing a GFP-Npw38 fusion protein were immunostained with anti-NpwBP antibody. The green fluorescent pattern resulting from GFP-Npw38 (Fig. 4A) is similar to the red fluorescent pattern observed with the anti-NpwBP antibody staining (Fig. 4B). When the two images were merged, the green fluorescence parts became yellow (Fig. 4C), indicating that these proteins were colocalized in the same subnuclear regions.

To test whether NpwBP associates with Npw38 in cells, we performed coimmunoprecipitation experiments. Both HA-tagged Npw38 and FLAG-tagged NpwBP were produced in COS7 cells, and the lysate was then immunoprecipitated with anti-HA (3F10) antibody, anti-FLAG (M2) antibody, or control IgG. Fig. 4, D and E, show that NpwBP and Npw38 were coimmunoprecipitated in any combinations, indicating that NpwBP associates with Npw38 in cells.

**NpwBP Associates with Poly(rG) and G-rich ssDNA—** Previ-
results indicate that NpwpB-(1–192) has affinity preferentially for the G-rich sequences.

**DISCUSSION**

We have purified a nuclear protein associating Npwp38 from HeLa nuclear extracts and have named it NpwpB. The cloning and characterization of the NpwpB cDNA revealed that NpwpB has two proline-rich regions. Two kinds of binding assays using GST fusion proteins and in vitro translated products and using the yeast two-hybrid system showed that NpwpB associates with Npwp38 through interaction between the WW domain of Npwp38 and the two proline-rich regions in NpwpB. The conserved two Trp and one Pro residues have been shown to be essential for this interaction by mutation analysis. Localization analysis showed that NpwpB is colocalized with Npwp38 in the nucleus. Npwp38 and NpwpB expressed in COS7 cells were coimmunoprecipitated. All these results indicate that NpwpB is a physiological ligand of the WW domain of Npwp38.

The binding assay using an oligopeptide-immobilized membrane demonstrated that the sequence motif interacting with the WW domain of Npwp38 is PPGPPP. Npwp38 has five sites containing a PPGPPP motif in which two sites (positions 199–204 and 459–464) are the most likely binding sites based on the results of the oligopeptide binding assay. It should be noted that these two PPGPPP sequences are flanked by Arg within three amino acids. The importance of the flanked Arg was confirmed by oligopeptide binding assay and site-directed mutation analysis. Thus we named it a PGR motif. The contribution of Arg to the interaction may be attributed to an acidic environment of the WW domain of Npwp38 compared with the basic ones of other WW domains. The structural analysis of the complex between the WW domain of YAP and a peptide containing a PPXY motif revealed that the peptide binds to a hydrophobic surface formed by three amino acids, including the second Trp-39, Tyr-28, and Leu-30 (19), which correspond to Trp-75, Tyr-64, and Trp-66 in the WW domain of Npwp38. This hydrophobic region of Npwp38 is surrounded by three acidic amino acids (Asp-58, Asp-68, and Asp-71). These acidic residues may contribute to stabilize the binding of a PGR motif sequence to the hydrophobic pocket through ionic interaction between Arg and Asp.

A protein data base search showed 21 sequences containing PPGPPP, in which the following three sequences have a PPGPPP motif flanked by Arg: (i) human hypothetical protein KIAA0755 (DDBJ/GenBankTM/EBI accession number AB018298), TRGM-LPPGPPLUSGPHG (53–68); (ii) human atrophin-1 (Swiss-Prot accession number P08392), YKTASPPFPPPYGKRA (623–638); (iii) herpes simplex virus trans-acting transcriptional protein ICFP (Swiss-Prot accession number P08392), PGRTPPPPP-LPLEAP (232–249). These sequences may not be a physiological ligand of Npwp38, because Arg in KIAA0755 or Atrophin-1 is not located within three amino acids flanked by PPGPPP, and the trans-acting transcriptional protein ICF4 is flanked not only by upstream Arg but also by downstream Glu. Experiments are needed to confirm this.

Previously, we demonstrated that the WW domain of Npwp38 can activate a basal transcription in yeast and mammalian cells, suggesting that the WW domain may interact with transcriptional machinery. The single mutation among conserved two Trps and one Pro did not affect this transcription activation, whereas the same mutation abolished the interaction between Npwp38 and NpwpB. These results imply that the transcriptional activation by the WW domain of Npwp38 may not result from the interaction via a PGR motif and that the WW domain of Npwp38 could have dual binding abilities for different ligands through which Npwp38 may play dual roles in...
nuclear events. At present, it is not clear whether the interaction of the WW domain with transcriptional machinery is physiologically meaningful.

NpwBP has been shown to have binding ability for poly(rG) and G-rich ssDNA, whereas Npw38 binds preferentially to poly(rG) but not ssDNA as described in the previous paper (16). The G-specific binding to AdMLP has been reported on hTAF68, which is a component of a multienzyme complex composed of the TATA-binding protein and TATA-binding protein-associated factors (20). Like this complex, the Npw38-NpwBP complex may play a role in mediating a link between DNA and RNA in transcriptional or posttranscriptional events. The identification of other proteins interacting with the Npw38-NpwBP complex will help elucidate the physiological role of this complex in these nuclear events.

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