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Induction of Abnormal Nuclear Shapes in Two Distinct Modes by Overexpression of Serine/Threonine Protein Phosphatase 5 in HeLa Cells

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Abstract Okadaic acid-sensitve serine/threonine protein phosphatase 5 (PP5) is expressed ubiquitously in various tissues and is considered to participate in many cellular processes. PP5 has a catalytic domain in the C-terminal region and three tetratricopeptide repeat (TPR) motifs in the N-terminal region, which are suspected to function as a protein–protein interaction domain. Physiological roles of PP5 are still largely unknown, although several PP5-binding proteins were reported and a few in vivo functions of PP5 were suggested. In the present study, the effects of expression of the full-length wild-type PP5 fused with EGFP (EGFP-$PP5_{WT}$) and its phosphatase-dead mutant EGFP-$PP5_{H304A}$ were investigated. Transient expression of either EGFP-$PP5_{WT}$ or EGFP-$PP5_{H304A}$ in HeLa cells induced deformed nuclei with a 10-fold frequency compared to that of EGFP. Abnormal-shaped nuclei were also substantially increased by induced moderate expression of PP5 in tet-on HeLa cells. Many HeLa cells expressing EGFP-$PP5_{WT}$ possessed multi-nuclei separated from each other by nuclear membrane, while expression of EGFP-$PP5_{H304A}$ induced deformed nuclei which were multiple-like in shape, but not separated completely and were surrounded by one nuclear membrane. These results suggest that PP5 plays important roles at the M-phase of the cell cycle, especially in separation of chromosomes and formation of nuclear membrane. J. Cell. Biochem. 101: 321–330, 2007.

Key words: PP5; protein phosphatase; abnormal nuclei; deformed nuclei; (E)GFP

Protein phosphatase 5 (PP5) is a member of serine/threonine phosphoprotein phosphatases and is expressed ubiquitously in various tissues. Its cDNA was isolated by the nucleotide sequence similarity of the catalytic domain with that of PP2A and other members of serine/threonine protein phosphatase (PPase) and by a yeast two-hybrid screen to identify proteins interacting with atrial natriuretic peptide (ANP) receptor [Becker et al., 1994; Chen et al., 1994; Chinkers, 1994]. Being distinct from other PPases, PP5 has a unique structural feature and contains three tetratricopeptide repeat (TPR) motifs in the N-terminal region and a catalytic domain in the C-terminal region [for reviews, see Cohen, 1997; Chinkers, 2001]. The TPR motifs are considered to serve as an interface for protein–protein interaction [Lamb et al., 1995]. The TPR domain and C-terminal 11 amino acid residues of PP5 were supposed to be auto-inhibitory domains [Chen and Cohen, 1997; Sinclair et al., 1999; Kang et al., 2001]. The crystal structure of PP5 reported recently proved that the TPR domain blocks the catalytic channel, and the C-terminal residues interact with the TPR domain and stabilize the auto-inhibitory conformation [Yang et al., 2005]. Some unsaturated long-chain fatty acids, including arachidonic acid and long-chain fatty acyl-CoA esters, bind to the N-terminal TPR domain of PP5 and enhance the phosphatase activity *in vitro* [Skinner et al., 1997; Ramsey and Chinkers, 2002]. A recent study showed that arachidonic acid and nocodazole induce the cleavage of the C-terminal of PP5 and stimulate

Abbreviations used: PP5, protein phosphatase 5; TPR, tetratricopeptide repeat; EGFP, enhanced green fluorescence protein; ASK1, apoptosis signal-regulating kinase.

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its enzymatic activity as a consequence [Zeke et al., 2005], but molecular mechanisms of PP5 activation in the living cell are still largely unsolved.

In vivo functions of PP5 have been proposed in several studies through the identification of associating partners, such as heat shock protein 90 (Hsp90), glucocorticoid receptor (GCR), ANP receptor, apoptosis signal kinase 1 (ASK1), ataxia telengiectasia mutated (ATM), and ATM- and Rad3-related (ATR) kinases, and two components of anaphase-promoting complex, CDC16 and CDC27. Taking the biological functions of these interacting proteins into consideration, PP5 is supposed to be involved in a variety of biological signal pathways, including functional modification of molecular chaperons, signal transduction through receptors, mitotic processes, and cell cycle checkpoint [Chen et al., 1996; Ollendorff and Donoghue, 1997; Silverstein et al., 1997]. PP5 is also suspected to be involved in DNA-damage-induced activation of ATM and ATR and oxidative-stress-induced apoptosis signal-regulating kinase 1 (ASK1) inactivation [Morita et al., 2001; Ali et al., 2004; Zhang et al., 2005]. In addition, PP5 associates with microtubule-binding proteins, tau and dynein [Galingniana et al., 2002; Gong et al., 2004], which may indicate the role of PP5 in cytoplasmic molecular transport through the physical interaction with structural proteins, such as tubulin and/or actin. We have recently demonstrated that PP5 localizes at microtubules, and the inhibition of PP5 expression by siRNA in HeLa cells induced abnormal organization of microtubules [Hara et al., our unpublished observation]. Zuo et al. [1998] reported that inhibition of PP5 induces G1 arrest through activation of p53-p21 pathway; however, a detailed molecular mechanism is not clarified yet.

In the present study, we demonstrated an intriguing and novel phenotype caused by the altered expression of the wild-type and mutant PP5. Overexpression of the full-length wild-type PP5 fused with EGFP (EGFP-$PP5_{WT}$) and its phosphatase-dead mutant (EGFP-$PP5_{H304A}$) induced an abnormal nuclear shape in HeLa cells. Furthermore, distinct structural differences were observed between the nuclear shape abnormality induced by wild-type and mutant EGFP-PP5 proteins in terms of the continuity of the nuclear membrane in a cell. For example, overexpression of the former induced multiple nuclei, each of which being demarcated separately by nuclear membrane, but the latter induced clover-like nuclei surrounded by a continuous nuclear membrane, suggesting the presence of two distinct modes of action for PP5 in the induction of abnormal nuclear shapes by its overexpression. A possible and novel role of PP5 is discussed on its involvement in the reconstitution of the nuclear membrane at mitosis.

MATERIALS AND METHODS

Construction of Expression Plasmids with Wild-Type and Phosphatase-Dead Mutants of PP5 and Purification of Those Recombinant PP5 Proteins Expressed in *Escherichia coli*

Prokaryotic expression plasmids for the full-length wild-type rat PP5 fused with glutathione S-transferase (GST) [GST-$PP5_{WT}$] and deletion mutants lacking N-terminal 177 amino acids of PP5 [GST-$PP5C_{WT}$] were constructed as described previously [Fukuda et al., 1996]. An expression plasmid for GST-$PP5C_{H304A}$, harboring an amino acid substitution (His to Ala) at codon 304 in the catalytic domain, was generated by PCR-based site directed mutagenesis, as described previously [Sambrook and Russel, 2001]. GST fusion proteins of PP5 were expressed in *E. coli* and purified, as described previously [Fukuda et al., 1996]. Phosphatase activity of these proteins was determined and the GST-$PP5C_{H304A}$ mutant was later confirmed to be enzymatically inactive.

We also constructed eukaryotic expression vectors for the full-length wild-type rat PP5 fused with EGFP (EGFP-$PP5_{WT}$) and an EGFP-fused deletion mutant of PP5 lacking N-terminal 236 amino acids (EGFP-$PP5C_{WT}$) from the counterpart of each GST-fused proteins. Their phosphatase-dead counterparts, EGFP-$PP5_{H304A}$ and EGFP-$PP5C_{H304A}$, were generated by replacement of the C-terminal catalytic domain from the corresponding counterparts of GST-$PP5_{H304A}$ and GST-$PP5C_{H304A}$.

Cell Culture and Plasmid Transfection

HeLa cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). For transient transfection of plasmids, cells were seeded at 5×10^4 cells/ml

and transfected with various plasmid DNA, including control pEGFP plasmid, using TransIT reagents (Takara Bio, Inc.) according to the manufacturer's instructions.

Phosphatase Assay

Phosphatase assays using ^{32}P-labeled phosphorylase *a* as a substrate were performed as detailed earlier [MacKintosh and Moorhead, 1993; Fukuda et al., 1996]. The final concentrations of the substrate and GST-PP5 were 0.35 mg/ml and 61 μU/ml, respectively. Phosphatase assays using *p*-nitrophenylphosphate as a substrate were also carried out as described earlier [MacKintosh and Moorhead, 1993] with or without arachidonic acid (Sigma) and okadaic acid (Wako Pure Chemical).

For PP5 proteins expressed in cultured cells, EGFP, EGFP-PP5$_{WT}$, and EGFP-PP5$_{H304A}$ were purified from HeLa cells by immunoprecipitation. Cells, transfected with individual PP5 constructs as described above, were harvested and lysed in a single-detergent lysis buffer composed of 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM DTT, 5 mM EDTA, 1% NP-40, 0.1 mM PMSF, and 3 μg/ml leupeptin. The lysate was incubated with anti-GFP antibody (MBL) for 60 min at 4°C and further with protein A sepharose beads for 60 min. The beads were washed extensively by the same buffer three times and PBS twice, and small aliquots of precipitated protein were used for determination of protein content by SDS–PAGE, following silver staining. The remaining beads were divided into three parts and mixed with phosphatase assay buffer. Phosphatase activities of the proteins were measured by the same method as described above.

Establishment of *tet-on* HeLa Cells Expressing PP5 or Phosphatase-Dead Mutants

Expression plasmid pTRE2hyg and HeLa tet-on cells were purchased from Clontech. *Tet-on* HeLa cells were transfected with pTRE2hyg-derivative plasmids, coding either EGFP-PP5$_{WT}$, EGFP-PP5$_{H304A}$, or EGFP. Two days later, the cells were harvested and seeded on 96-well culture plates, and kept propagated for more than 3 weeks. Cell culture was carried out in the presence of G418 (100 ng/ml) and hygromycin B (50 ng/ml). Several clones, which stably express either the wild-type or the phophatase-dead mutant PP5 protein, were isolated. Before conducting further experiments, induction of EGFP-PP5$_{WT}$ and EGFP-PP5$_{H304A}$ proteins were confirmed by the addition of doxycycline (Dox), and cell clones with considerable amounts of PP5 proteins were selected and stored in liquid nitrogen until use.

Immunofluorescence Microscope Observation

After transient transfection of various PP5 constructs, cells were incubated at 37°C for 24 h for further analyses. For *tet-on* HeLa cells, 5×10^4 cells were seeded on culture plates and incubated for another 48 h and subjected to immunocytochemical analysis. For immunofluorescence, cells were fixed with cold methanol for 20 min and incubated in a buffer consisting of 1% BSA-PBS with the first antibody for respective proteins for 60 min, then further incubated with the second antibodies for 60 min at RT or at 4°C. Cells were analyzed under a Zeiss Axiovert 200 fluorescence microscope (Carl Zeiss). Anti-lamin A/C (Chemicon) and B (Oncogene), and Alexa 594 conjugated anti-mouse IgG (Molecular probe) were purchased from each supplier. Counting of cells, possessing deformed nuclei, including di- or multi-nuclei, was carried out by fluorescence images of EGFP or EGFP-PP5 because these proteins localize mainly in cytoplasm and can illustrate the shapes of nuclei. For detailed observation, the images of Hoechst 33342 (Sigma) staining for DNA and immunostaining of lamins were utilized in addition to the EGFP-image.

RESULTS

A Phosphatase-Dead Mutant of PP5 by a Single Amino Acid Substitution

In our previous study and other reports, the N-terminal region containing three TPR motifs, which are suspected to provide a suitable interface for protein–protein interaction [Lamb et al., 1995], inhibits the phosphatase activity of the C-terminal catalytic domain of PP5 [Sinclair et al., 1999; Yang et al., 2005]. The recombinant PP5 protein, GST-PP5C$_{WT}$, containing the rat PP5 from Gly^{178} to Met^{499}, lacking 177 amino acids of the N-terminal, was successfully expressed as a soluble protein in *E. coli* and purified. The purified GST-PP5C$_{WT}$ protein conspicuously showed phosphorylase *a* phosphatase activity (Fig. 1). Amino acid sequence in the catalytic domain of PP5, especially those between cystein at codon 240

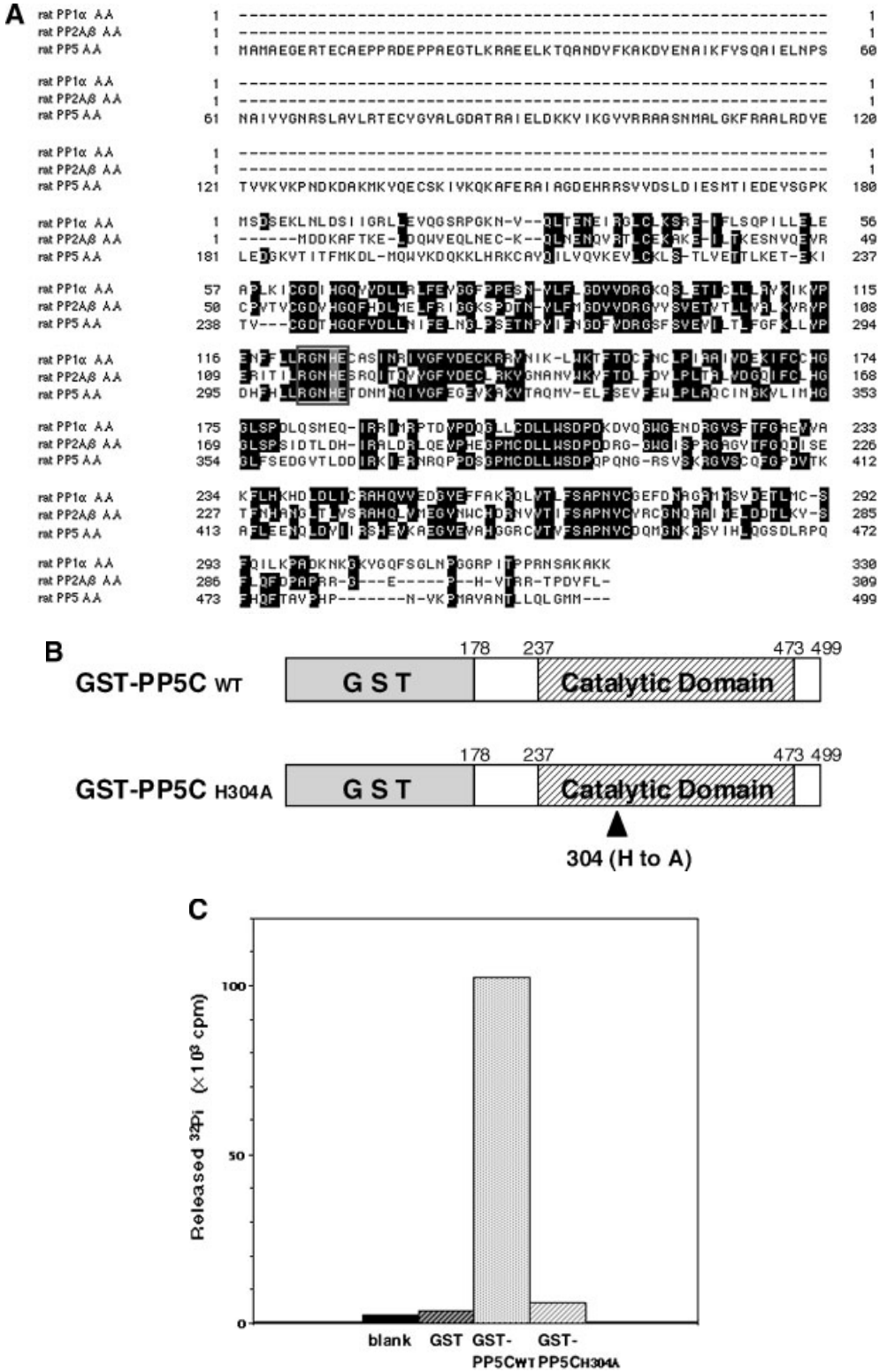


Fig. 1. Generating a phosphatase-dead mutant of PP5. **A**: Conserved amino acids in the catalytic domain between PP5 and other PPases. Alignment of amino acid sequences of rat PP5, rat PP1α, and rat PP2Aβ. Conserved amino acids are indicated by black shadows. **B**: Structure of recombinant GST-PP5C expressed in *E. coli*. GST-PP5C$_{WT}$ is a GST-fused PP5 protein, which has the C-terminal catalytic domain of PP5, lacking the N-terminal 177 amino acids. GST-PP5C$_{H304A}$ is a mutant counterpart of GST-PP5$_{WT}$ with an introduced substitution from histidine to alanine at codon 304. **C**: Phosphatase activities of recombinant GST-PP5C. Phosphatase activities were measured by released ^{32}P using ^{32}P-labeled phospholyrase *a* as a substrate.

and tyrosin at codon 323, is highly conserved among other members of protein phosphatases, such as PP1α and PP2Aβ (Fig. 1A). A single amino acid substitution from histidine to alanine at codon 304 was introduced into GST-$PP5C_{WT}$, aiming to generate a phosphatase-dead mutant of PP5, since amino acid substitution at the corresponding residues of PP1 was reported to lose phosphatase activity [Egloff et al., 1997]. The purified mutant protein, GST-$PP5C_{H304A}$, completely lost phosphorylase *a* phosphatase activity (Fig. 1C). Two other mutant proteins having an amino acid substitution, GST-$PP5C_{D274V}$ and GST-$PP5C_{Y313N}$, lost phosphorylase *a* phosphatase activity as well (data not shown).

Next, we constructed mammalian expression vectors for the full-length rat PP5 fused with EGFP (EGFP-$PP5_{WT}$) and its phosphatase-dead mutant EGFP-$PP5_{H304A}$. Likewise, we also constructed expression vectors for EGFP-$PP5C_{WT}$ and EGFP-$PP5C_{H304A}$, lacking 236 amino acids of the N-terminal part of PP5 (Fig. 2A). After we confirmed that HeLa cells transfected transiently with the plasmids expressed EGFP-$PP5_{WT}$ and EGFP-$PP5_{H304A}$, respectively (data not shown), we attempted to produce tet-on HeLa cells where EGFP-$PP5_{WT}$ or EGFP-$PP5_{H304A}$ was expressed under the control of Dox in the culture medium. Induction of the proteins with Dox was confirmed by immunoprecipitation with anti-GFP antibody (Fig. 2B), followed by immunoblotting with anti-PP5 antibody (data not shown). Immunoprecipitate with anti-GFP antibody from the cells expressing EGFP-$PP5_{WT}$ showed phosphatase activity (Fig. 2C). On the other hand, that from the control cells expressing EGFP alone did not show significant phosphatase activity. The activity of EGFP-$PP5_{WT}$ was remarkably stimulated by arachidonic acid at 160 μM and inhibited by okadaic acid at 100 nM (Fig. 2C). These results indicate that the phosphatase activity monitored in the present study was indeed derived from EGFP-$PP5_{WT}$, not from other phosphatases contaminated in the immunoprecipitate. It was also confirmed that the imunoprecipitate from the cells expressing EGFP-$PP5_{H304A}$ did not show phosphatase activity, even with addition of arachidonic acid (Fig. 2C).

Subcellular Localization of Wild-Type and Phosphatase-Dead Mutant of PP5 in HeLa Cells

When the wild-type PP5 was expressed transiently in HeLa cells as a form of EGFP-$PP5_{WT}$,

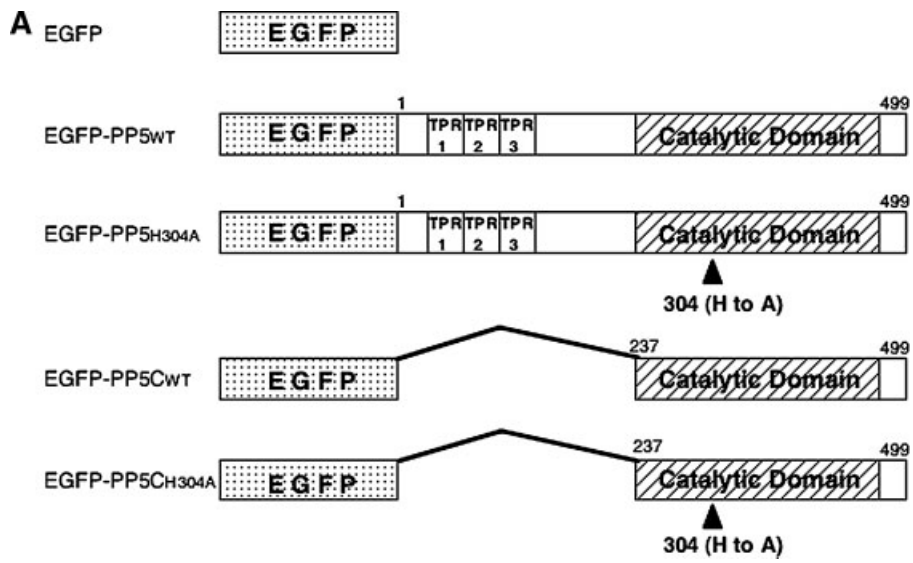
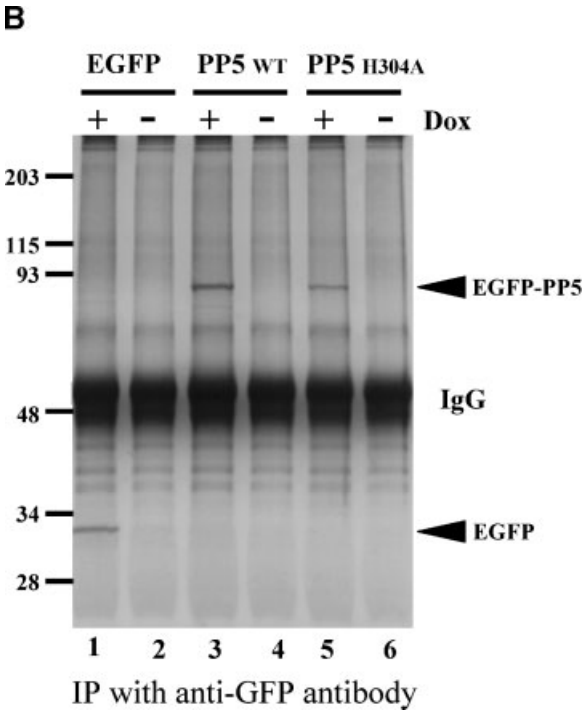


Fig. 2. Establishment of tet-on HeLa cells expressing PP5 or phosphatase-dead mutant. **A**: Structure of various constructs of EGFP-PP5 for eukaryotic expression. **B**: Dox-induced expression of EGFP-PP5 proteins in tet-on HeLa cells. A silver-stained SDS–PAGE gel showing immunoprecipitants with anti-GFP antibody from tet-on Hela cells expressing EGFP (**lane 1**), EGFP-$PP5_{WT}$ (**lane 3**), and EGFP-$PP5_{H304A}$ (**lane 5**) in the presence of Dox. Positions of EGFP-PP5s and EGFP are indicated by arrow-heads. **C**: Phosphatase activities of immunoprecipitants with anti-GFP antibody from each tet-on HeLa cells. Phosphatase activities were measured using *p*-nitrophenylphosphate (PNPP) as a substrate with or without 160 μM of arachidonic acid (AA) and 100 nM of okadaic acid (OA). Open, closed, dotted, and hatched bars indicate (AA+, OA+), (AA+, OA−), (AA−, OA+), and (AA−, OA−), respectively.



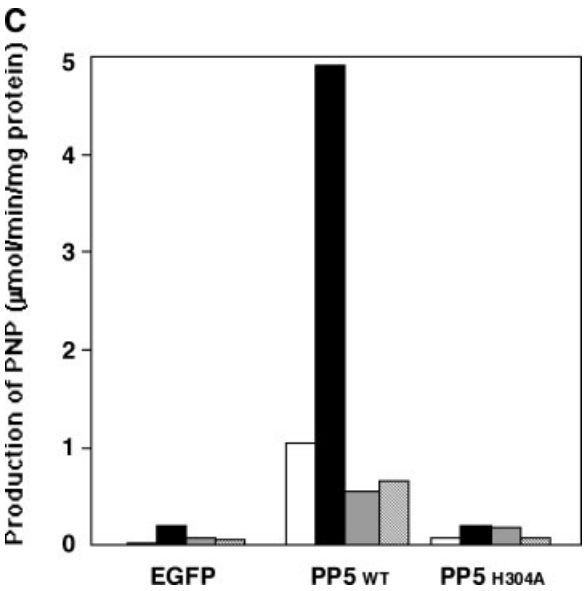


Fig. 2. (*Continued*)

it mainly localized in the cytoplasm and partly at the nucleus (Fig. 3A-c). EGFP-PP5C$_{WT}$, lacking an N-terminal region, showed a diffused subcellular localization pattern and a considerable fraction of the protein localized in the nucleus, compared to its full-length counterpart, EGFP-PP5$_{WT}$ (Fig. 3A-a). This suggests that TPR motifs in the N-terminal region are necessary for precise localization of PP5 to cytoplasm. No obvious difference was observed in the localization of PP5 between the wild-type and mutant proteins, whether TPR existed (Fig. 3A-c,d) or not (Fig. 3A-a,b).

Induction of Abnormal Nuclear Shape by Transient Expression of PP5 and its Derivatives

Notably, multiple nuclei were frequently observed in HeLa cells transiently trasfected with EGFP-PP5$_{WT}$ at an incidence of more than 20% (Fig. 3B-a). Ratios of cells with deformed nuclear shape were counted and are listed in Table I. HeLa cells transfected with EGFP-PP5$_{WT}$ had deformed nuclei with a 10-fold frequency compared with the control of EGFP. Similarly, the deformed nuclei were induced by the mutant EGFP-PP5$_{H304A}$ with a 10-fold higher frequency compared with the control EGFP (Fig. 3B-c, Table I). Frequencies of deformed nuclei induced by transfection with EGFP-PP5C$_{WT}$ and EGFP-PP5C$_{H304A}$ were increased 2.8- and 3.5-fold compared with EGFP, respectively. These results indicate that the catalytic domain of PP5 does not play an essential role in producing the deformed nuclei, but the N-terminal domain, possibly TPR domains, may contribute to the phenotype.

It is also important to note that the pattern of nuclear shape abnormality was apparently different between the wild-type EGFP-PP5$_{WT}$ and the mutant. EGFP-PP5$_{H304A}$ induced deformed nuclei, which appeared as clover-like segmented shapes having a constricted part (Fig. 3B-c). In contrast, overexpression of wild-type PP5 induced multiple nuclei, each of which seemed to be separated individually by nuclear membrane (Fig. 3B-a). The results suggest the presence of two distinct modes of action for PP5 in the induction of abnormal nuclear shape.

Nuclear Shape Abnormality in *tet-on* HeLa Cells of PP5

To confirm the substantial impact of PP5 on the induction of nuclear shape abnormality, we established several tet-on HeLa cell clones with moderate expression levels of PP5. Induction and activity of the proteins were confirmed by conducting immunoprecipitation and immunoblot analysis as described above. We next examined the effects of PP5-overexpression on nuclear shape using *tet-on* HeLa cells. Induction of EGFP-PP5$_{WT}$ by addition of Dox produced deformed nuclei with a twofold frequency

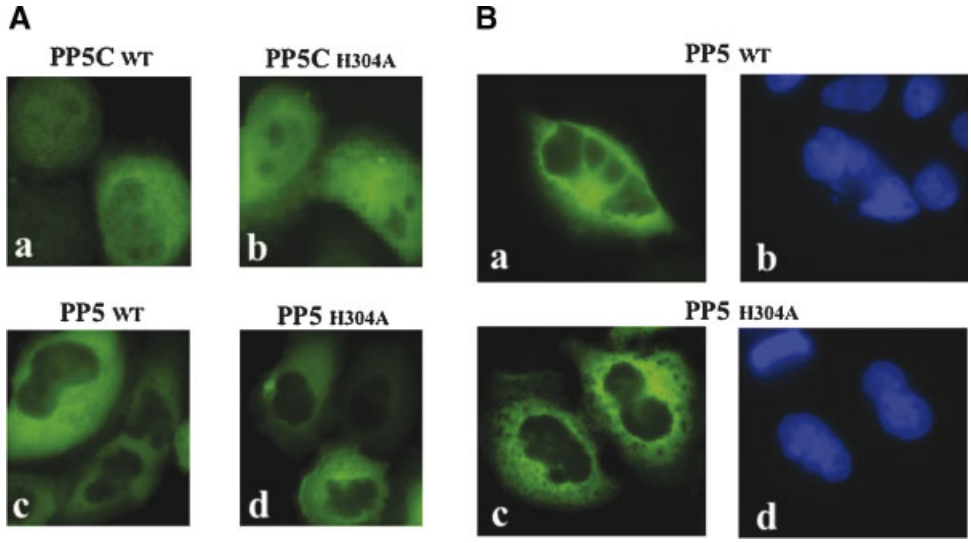


Fig. 3. HeLa cells transfected transiently with EGFP-PP5 variants. **A**: Fluorescence microscope images of EGFP in the cell expressing EGFP-PP5C$_{WT}$ (**a**), EGFP-PP5C$_{H304A}$ (**b**), EGFP-PP5$_{WT}$ (**c**), and EGFP-PP5$_{H304A}$ (**d**), showing subcellular localization of each EGFP-PP5 variants. **B**: Deformed nuclei induced by overexpression of EGFP-PP5 and phosphatase-dead mutants in HeLa cells. Direct fluorescence microscope images of EGFP in the cell expressing EGFP-PP5$_{WT}$ (**a**) and EGFP-PP5$_{H304A}$ (**c**), and Hoechst 33342 staining of the same cells (**b**, **d**).

as EGFP (Table II). The induction of EGFP-PP5$_{H304A}$ also produced deformed nuclei with a similar frequency to that of EGFP-PP5$_{WT}$ (Table II). Differences between the abnormal nuclear structures induced by the wild-type and mutant PP5 in tet-on HeLa cells were similar to that in transient expressing cells (Fig. 4A). In order to distinguish subtle but distinct differences of the abnormal nuclear structures, lamins A/C and B, backing-up proteins of nuclear membrane, were immunostained (Fig. 4B). Interestingly, *tet-on* HeLa cells expressing EGFP-PP5$_{H304A}$ had deformed nuclei delineated with a continuously reconstructed nuclear membrane (Fig. 4B-c). On the other hand, nuclear membranes were discontinuously formed with the expression of EGFP-PP5$_{WT}$, and deformed nuclei were separated and demarcated individually by nuclear membrane (Fig. 4B-a).

TABLE I. Ratios of Cells With Deformed Nuclei (Transient Transfection)

	Counted cells	No. of cells with deformed nuclei (%)
EGFP-PP5$_{WT}$	379	79 (20.8)
EGFP-PP5$_{H304A}$	299	75 (25.1)
EGFP-PP5C$_{WT}$	319	18 (5.6)
EGFP-PP5C$_{H304A}$	393	28 (7.1)
EGFP	408	8 (2.0)

DISCUSSION

In the present study, we found a novel biological function of PP5 on the reconstruction of nuclear membrane, and a clearly different phenotype was observed between the wild-type and phosphatase-dead mutant PP5. The results embrace two distinct modes of action of PP5: abnormality in nuclear shape was caused in a phosphatase activity-independent manner, but in the N-terminal domain-, possibly a TPR-domain-dependent manner. The results from the inducible expression of PP5 derivatives using *tet-on* HeLa system were in good agreement with those obtained from the transient expression experiment, although the frequencies of the deformed nuclei were lower than that of the transient transfection. The low frequency of abnormal nuclei in tet-on HeLa cells may reflect the expression levels of exogeneously introduced PP5-derivatives per cell, those being

TABLE II. Ratios of Cells With Deformed Nuclei (*Tet-on* HeLa)

	Counted cells	No. of cells with deformed nuclei (%)
EGFP-PP5$_{WT}$	400	52 (13.0)
EGFP-PP5$_{H304A}$	403	57 (14.0)
EGFP	400	28 (7.0)

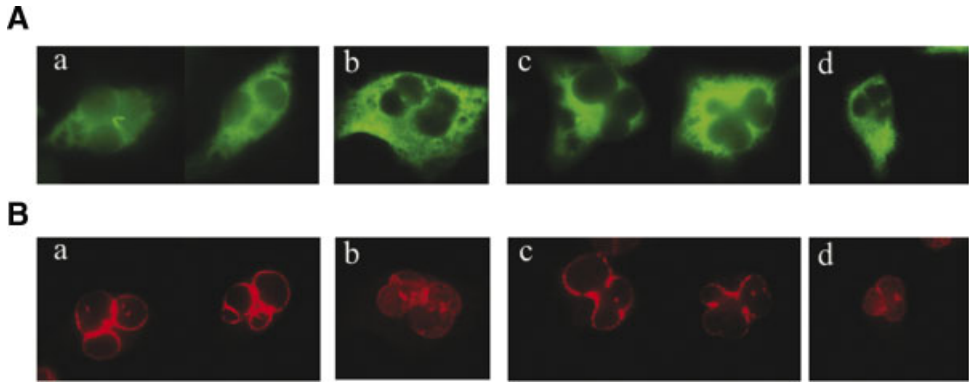


Fig. 4. Differences between the abnormal nuclear structures induced by wild-type and mutant PP5. **A**: Fluorescence microscope images of EGFP in the tet-on HeLa cells expressing EGFP-PP5$_{WT}$ (**a**, **b**) and EGFP-PP5$_{H304A}$ (**c**, **d**). **B**: Immunostaining with anti-lamin B (**a**, **c**) and anti-lamin A/C (**b**, **d**) antibodies of the same cells as in the upper panel A.

much lower in the inducible system compared to the transient expression system.

In the cases of the transient transfection with EGFP-PP5C$_{WT}$ and EGFP-PP5C$_{H304A}$, which are lacking the N-terminal part containing TPR motifs, the frequency of the deformed nuclei was less than one-third of that with their full-length counterparts. This result indicates the N-terminal domain of PP5 may have a substantial impact on the induction of the deformed nuclei. It is conceivable that the N-terminal domains of EGFP-PP5$_{WT}$ and EGFP-PP5$_{H304A}$ sequester some PP5-associating proteins and a consequent loss of their function may lead to the generation of the deformed nuclei. There are several possible mechanisms for the deformed and multiple nuclei induced by overexpression of EGFP-PP5$_{WT}$ and EGFP-PP5$_{H304A}$, in addition to the absorption of PP5-associating proteins by the TPRs in the EGFP-PP5 proteins described above. (1) Acceleration of the PP5-driven processes by overexpression of PP5; (2) abnormal dephosphorylation of PP5's substrates or other proteins by overexpression of PP5; (3) dominant negative effect of phosphatase-dead mutant; (4) dominant negative effect by EGFP-fused proteins inhibiting the real *in vivo* ability of PP5.

Accumulating studies have suggested that PP5 could be involved in G1 arrest, apoptosis and DNA-damage responses through regulating the activities of p53, ASK-1 and ATM in response to various stresses [Zuo et al., 1998; Morita et al., 2001; Ali et al., 2004]. In addition, our current study suggests the possible involvement of PP5 in chromosome partition in mitosis. Particularly, the phosphatase activity of PP5 is suspected to be required for the last step of chromosome separation and nuclear membrane. Molecular mechanisms of these complex processes are largely unknown and await further investigation. Therefore, we only propose several possible mechanisms to form the unusual shaped nuclei by aberrant PP5 activity here. In the exit from mitosis and progression of cytokinesis, dephosphorylation of many different cyclin-B-cdk-1 substrates is required for different events, including cytokinesis, spindle disassembly, and nuclear reformation [Pines, 2006]. Nuclear reassembly requires phosphate activity, and at least for B-type lamins, involves PP1 [Steen and Collas, 2001; Margalit et al., 2005]. It is, therefore, highly possible that PP5 is also involved in nuclear reassembly and other events in the late or post-mitotic stages. PP5 was reported to colocalize with both cytoplasmic dynein and microtubles [Galigniana et al., 2002]. Because nuclear envelope breakdown is dynein- and microtuble-dependent [Beaudouin et al., 2002; Salina et al., 2002], it is possible that a dynein- and microtuble-dependent event is also required for nuclear envelope reformation and PP5 modulates this event. Another possibility is abnormality of APC/C function by aberrant PP5 activity may lead to deformed nuclei because PP5 associates with two components of anaphase-promoting complex (APC), CDC16, and CDC27 [Ollendorff and Donoghue, 1997]. PP5 was recently reported to be an inactivator for Raf [von Kriegsheim et al., 2006], and it was reported by many studies that Raf/MEK/ERK signal pathways induced cell

cycle progression via multiple effecter molecules, including p53, p21, p16, and cyclins [Chang et al., 2003]. Therefore, it is possible that misregulation of downstream genes of the Raf pathway induced by aberrant PP5 activity leads to the deformed nuclei.

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