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The Carboxyl Terminal Domain of Phosducin Functions as a Transcriptional Activator

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In previous work, we identified a set of phosducin (Phd) isoforms with unknown function including the phosducin (Phd)-like orphan protein 1 (PhLOP1), an amino terminal truncated isoform of the retinal Phd lacking the G$\beta\gamma$ binding domain. To investigate the potential biological function of PhLOP1, PhLOP1 was fused at its amino terminus with the DNA binding domain (BD) of the yeast transcriptional factor, GAL4, and used as bait in a yeast two-hybrid screen. Two potential functional protein partners were identified during the screen: SUG1, a subunit of the 26S proteasome and a putative transcriptional mediator, and CRX, a retina- and pineal-specific transcription factor. Upon localizing the interacting domain of PhLOP1 with one of the new partners, SUG1, we found that a domain of 40 amino acids at the carboxyl terminus of Phd and PhLOP1 had intrinsic transcriptional activation activity in yeast. The transactivation activity was further confirmed in mammalian cells. This region contains an acidic domain that has been shown to be involved in the function of several transcriptional activators. In addition, we showed that Phd is cytoplasmic while PhLOP1 is localized predominantly to the nucleus when fused to an enhanced green fluorescent protein (EGFP) and transiently expressed in transfected cells, suggesting that PhLOP1 may play a distinct functional role in transcriptional regulation independent of the known Phd interaction/regulation of G$\beta\gamma$ transduction. © 2000 Academic Press

Phosducin (Phd) is an acidic phosphoprotein (1, 2) abundantly expressed in retinal photoreceptors and pinealocytes (3–5) but ubiquitously distributed throughout other tissues (6). It has been established that Phd is an important regulator of the retinal phototransduction cascade (1, 7). Phd binds the guanine nucleotide (G)-protein beta/gamma (G$\beta\gamma$) complex, competitively excluding the binding of the Gα and thereby preventing re-association of G protein subunits to form the transduction-competent heterotrimer, G$\alpha\beta\gamma$ (7–10). The efficacy of Phd binding to G$\beta\gamma$ is regulated by protein kinase A (PKA) catalyzed phosphorylation and protein phosphatase 2A (PP2A) catalyzed dephosphorylation on Serine 73 of Phd in a light-dependent manner (8, 9, 11–13). The dephosphorylated form of Phd favors the binding of G$\beta\gamma$, which, in light, prevents receptor-mediated Gα reactivation (7, 8) and blocks interactions between G$\beta\gamma$ and its effectors (14–17). In the dark, Phd is phosphorylated and no longer blocks the reassociation of the G protein subunits, thus more heterotrimeric transducin is available for activation (18).

Phd and its isoforms represent a superfamily of proteins with selected members unable to interact with G$\beta\gamma$. The Phd-like proteins (PhLPs) that bind G$\beta\gamma$, including $PhLP_L$ and $PhLP_S$, share structural and potential functional similarity to Phd (19–21). $PhLP_{L/S}$ has been shown to have widespread tissue distribution (19). Phd and PhLP are encoded by distinct genes, located on chromosome 1q (4) and 9 (22), respectively. Both genes have alternatively spliced variants (19, 23). Of the three Phd isoforms identified by our laboratory from human retina, two failed to bind G$\beta\gamma$ and they were named phosducin-like orphan proteins, PhLOP1 and PhLOP2. PhLOP1 lacks the first 52 N-terminal residues of Phd, but it contains the complete carboxyl (C)-terminus of Phd. PhLOP2 has only a limited amino acid sequence homology to Phd although its nucleotide sequence has significant homology to Phd (23).

To define the potential biological attributes of the PhLOPs, PhLOP1 was used as bait in a yeast two-hybrid screen (24). Two genes encoding proteins that interact with Phd isoforms were identified from a bovine retinal cDNA library: bovine SUG1 (bSUG1, Accession No. AF069053) (24) and bovine CRX (bCRX, Accession No. AF154123) (25). Independent of our

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work, $PhLP_L$ has also been reported to interact with SUG1 (26).

To localize the interacting domain of Phd isoforms with SUG1 and CRX, we created several GAL4 DNA binding domain (BD) fusion constructs with deletion mutants of Phd and PhLOP1. In this study, we demonstrate that the carboxyl terminal 40 amino acids of Phd and PhLOP1 exhibit strong transcriptional activation in both yeast and mammalian cells. Fluorescent microscopy facilitates the study to investigate the subcellular localization of Phd and PhLOP1 fused with an enhanced green fluorescent protein (EGFP), which were transiently expressed in COS-7, PC12 and R28 cells. The functional implications of the transcriptional activation domain of Phd and PhLOP1 are discussed.

MATERIALS AND METHODS

Plasmids construction. The complete coding sequences of human retinal Phd, PhLOP1 and different deletion mutants of PhLOP1 were cloned into the pBD-GAL4 phagemid vector (Stratagene, La Jolla, CA) and the mammalian two-hybrid bait vector, pM (Clontech, Palo Alto, CA) downstream of the GAL4 DNA binding domain (BD) between their *Eco*RI and *Pst*I restriction endonuclease sites as described (24).

The enhanced green fluorescent protein (EGFP) fusion constructs were made with the pEGFP-C2 vector (Clontech). Full-length coding regions of Phd and PhLOP1 were amplified using the following primers with the first two amino acids changed from Met and Glu to Pro and Gly for Phd and from Met and Ser to Pro and Gly for PhLOP1 (the original nucleotide sequences for the first two amino acids are in lowercase and in parentheses) (17): +5′Phd (6–17) *Eco*RI, 5′-CCGAATTC/CCG(atg)/GGA(gaa)/GAA/GCC/AAA/AG-3′; +5′PhLOP1 (6–21) *Eco*RI, 5′-CCGAATTC/CCG-(atg)/GGT(tct)/TCT/CCT/CAG/AG-3′; −3′Phd/PhLOP1 *Pst*I, *Eco*RI, *Bam*HI, 5′GCCGGATCCGAATTCTGCAG/TCA/TTC/AAC/ATC/TTC/TTC-3′ (antisense for both Phd and PhLOP1).

The PCR amplified products were digested with *Eco*RI and ligated to pEGFP-C2 vector through its *Eco*RI site in the multiple cloning sites. The clones that are in the correct orientation were chosen by colony PCR with the above 5′ sense primers and the 3′ vector antisense primer.

All the DNA constructs were completely sequenced from both +5′ and −3′ directions using the ABI PRISM Genetic Analyzer Model 310 (Perkin Elmer, Foster City, CA) to confirm the correct reading frame and the complete nucleotide sequence.

Yeast two-hybrid system. The yeast reporter host strain *Saccharomyces cerevisiae* CG-1945 used for the two-hybrid screen was described previously (24). The yeast cells were grown in YPD (yeast extract/peptone/dextrose) or appropriate selection medium to maintain plasmids. Yeast transformation was done by the lithium acetate method using the YEASTMAKER yeast transformation system (Clontech). Liquid growth assay and quantitative β-galactosidase (β-gal) assay was performed using previously published protocol (24).

Cell culture and transient transfection. COS-7 cells (American Type Tissue Culture, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (DMEM) as described (27). PC12 cells (American Type Tissue Culture) were grown in DMEM supplemented with 10% heat-inactivated horse serum, 5% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin on collagen (Collagen Co., Palo Alto, CA) coated tissue culture dishes. To obtain neuronal phenotype, PC12 cells were primed with 100 ng/ml of nerve growth factor (NGF, Upstate Biotechnology, Lake Placid, NY) for 5 days before plated for transfection. Immortalized rat neuroretinal cells R28 (generously provided by Dr. G. M. Siegel) were maintained as previously published (28–30) in DMEM supplemented with 10% FBS, 2 mM L-glutamine, MEM nonessential amino acids, MEM vitamins and 100 μg/ml gentamicin. All the tissue culture media and supplements except the NGF were obtained from Irvine Scientific (Santa Ana, CA).

Transient transfection was performed using Superfect transfection reagent (Qiagen, Valencia, CA) following the manufacturer's instruction. Briefly, COS-7, R28 or NGF-primed PC12 cells were seeded at 1×10^5 cells/ml into 6-well plates 20 h before transfection. For single plasmid transfection, 2 μg of plasmid DNA was used with 12 μl of Superfect reagent. For cotransfection, 2 μg of GAL4 BD-fusion mammalian expression construct in pM vector and 0.4 μg of pG5CAT reporter construct (Clontech) were used with 12 μl of Superfect reagent.

Chloramphenicol acetyltransferase (CAT) enzyme-linked immunosorbent assay (ELISA). COS-7 cells, cotransfected with a BD fusion construct and the pG5CAT reporter construct, were incubated for 2 days before being harvested for the CAT ELISA assay. Cell extracts were prepared using lysis buffer from the CAT ELISA kit (Boehringer Mannheim, Indianapolis, IN) following the manufacturer's instruction. 0.4 ml of lysis buffer was used for each well. CAT protein was quantitated with 200 μl of cell extracts, following the instruction for the CAT ELISA Kit, with a microtiter plate reader and normalized to total protein in each sample. Data represent the average ± standard error of the mean (SEM) of three independent experiments, each performed in duplicate.

Fluorescent microscopy. COS-7, R28 or PC12 cells transfected with EGFP fusion constructs in 6-well plates were allowed to grow for 24–48 h. Fluorescent microscopy was performed on a Nikon microscope (Nikon Inc., Melville, NY) equipped for epifluorescence. EGFP fluorescence was observed using a standard fluorescein isothiocyanate (FITC) filter, illuminating at 480 nm. Images of cells with EGFP and EGFP-tagged proteins were acquired using a Cooke digital camera with a Sony Interline charge coupled device (CCD) chip. The digitized images were stored on a Macintosh computer, processed and analyzed using Adobe Photoshop.

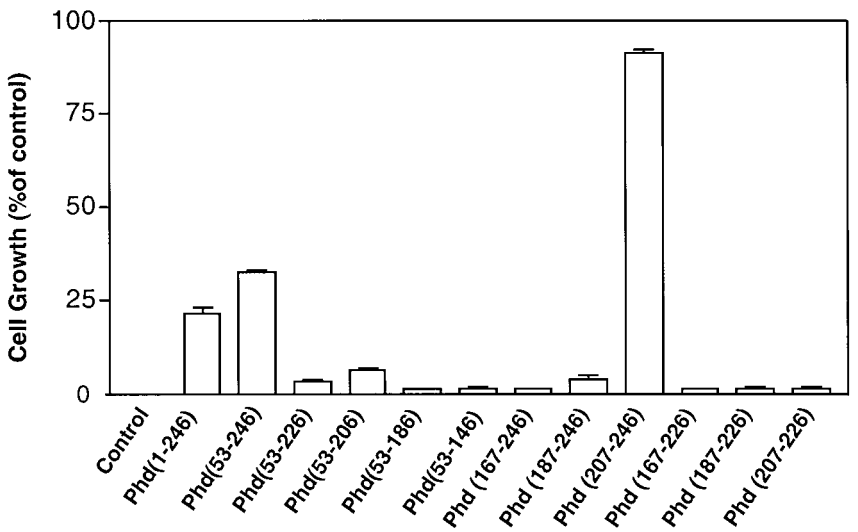
RESULTS

To investigate the biological function of PhLOP1, we did a yeast two-hybrid screen to identify protein-protein interaction partners (24, 25). Two potential functional partners of PhLOP1 were identified from a bovine retina yeast expression cDNA library during a screen with PhLOP1 as bait. One was SUG1, a subunit of the 26S proteasome and a potential transcriptional mediator (24). The other was the cone-rod homeobox (CRX), a recently characterized retina- and pineal-specific transcription factor (31–35). To identify the interacting domain of PhLOP1 with SUG1, we constructed a series of N- and C-terminal deletion mutants of PhLOP1 in the pBD-GAL4 vector (Fig. 1A). These BD fusion constructs were cotransformed to the CG-1945 yeast reporter host strain with either the GAL4 activation domain (AD) vector (pGAD10) or AD-SUG1 (pGAD10-SUG1). The yeast transformants were divided evenly and grown on −Leu-Trp, −Leu-Trp-His (−His) and −Leu-Trp-His + 5 mM 3-aminotriazole (−His + 3-AT) plates at 30°C. Colonies grown on −Leu-Trp plates were pooled and processed for a liquid growth assay and β-galactosidase (β-gal) assay 4 days after cotransformation as described (24). Cell

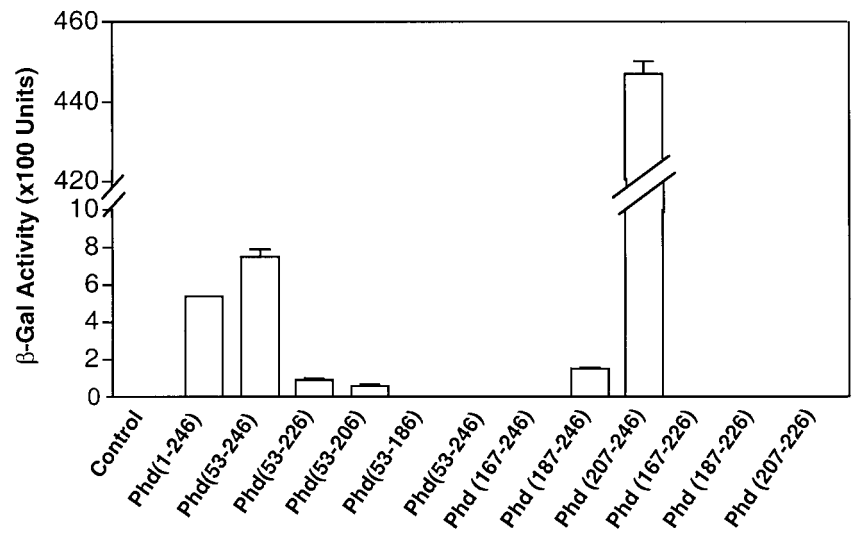
A

		Growth on -His Plates			Growth on -His + 3-AT Plates		
		3 Days	7 Days	9 Days	3 Days	7 Days	9 Days
Control (BD)		-	-	-	-	-	-
Phd (1-246aa)	1–246	+	+++	++++	-	++	+++
PhLOP1 (53-246aa)	53–246	+	+++	++++	-	++	+++
Phd (53-226aa)	53–226	-	++	++++	-	±	++
Phd (53-206aa)	53–206	-	++	++++	-	±	++
Phd (53-186aa)	53–186	-	-	-	-	-	-
Phd (53-146aa)	53–146	-	-	-	-	-	-
Phd (167-246aa)	167–246	-	+	++	-	-	-
Phd (187-246aa)	187–246	-	++	+++	-	-	-
Phd (207-246aa)	207–246	++	++++	++++	-	+++	++++
Phd (167-226aa)	167–226	-	±	+	-	-	-
Phd (187-226aa)	187–226	-	-	-	-	-	-
Phd (207-226aa)	207–226	-	-	-	-	-	-

B



C



growth on either −His or −His + 3-AT plates was recorded 3, 7 and 9 days after cotransformation. As shown in Fig. 1, when AD-SUG1 was cotransformed, full-length Phd, PhLOP1 and several deletion mutants of Phd activated both *HIS3* (growth in −His medium) and *LacZ* (β-gal activity assay) reporter genes. When the AD vector was cotransformed, BD-Phd (207–246) activated the reporter expression to the same extent as when it was cotransformed with AD-SUG1 (data not shown). When further truncated to 20 AA (207–226), no activation was observed. The full-length Phd and PhLOP1 and the other deletion mutants of PhLOP1 did not activate the reporter genes when expressed with the AD vector alone (data not shown). These results indicated that Phd (207–246) could activate transcription on its own when tethered to DNA by a heterologous DNA binding domain.

To further confirm the transactivation activity of Phd (207–246), BD-Phd (207–246) was cotransformed with either the AD vector alone or AD-SUG1 to yeast CG-1945 cells. The strength of the reporter transactivation was measured by the ability of the yeast to survive in 3-AT, a competitor in the histidine pathway (36). BD-Phd (207–246) activated the *HIS*3 gene when expressed with the AD vector alone to the same extent as when it was co-expressed with AD-SUG1 (Fig. 2).

We next sought to investigate if the 40 AA domain at the carboxyl terminus of Phd and PhLOP1 also had transactivation activity in mammalian cells. The full-length Phd, PhLOP1 and truncated PhLOP1 BD-fusion mammalian expression constructs were each cotransfected with the pG5CAT reporter vector, which contains the CAT gene downstream of five consensus GAL4 binding sites and the minimal promoter of the adenovirus E1b gene, into COS-7 cells. The CAT reporter protein was quantitated with the CAT ELISA kit and normalized for total protein. In Fig. 3, Phd (207–246) is shown to activate the CAT reporter expression, while full-length Phd, PhLOP1 and the other truncated PhLOP1 proteins tested did not activate the reporter gene, when tethered to the promoter upstream of the reporter gene by a heterologous DNA binding domain in mammalian cells.

To further explore if the native Phd isoforms have the potential to act as transcriptional activators in the nucleus, we studied the subcellular localization of Phd and PhLOP1 in transiently transfected cells using EGFP as a reporter. Phd and PhLOP1 were fused to the C-terminus of EGFP in the pEGFP-C2 vector and were transiently expressed in three different cell types: non-neuronal cells (COS-7), neuronal cells (NGF-primed PC12) or neuroretinal cells (immortalized rat retinal cells R28), which have been shown to express both photoreceptor and glial cell markers (28, 29). The pEGFP-C2 vector DNA was transfected as a control. Fluorescent microscopy revealed that EGFP-Phd was predominantly localized in the cytoplasm and EGFP-PhLOP1 was predominantly expressed in the nucleus, while the EGFP control was evenly distributed in both cytoplasm and nucleus in each of the three different cell lines tested (Fig. 4).

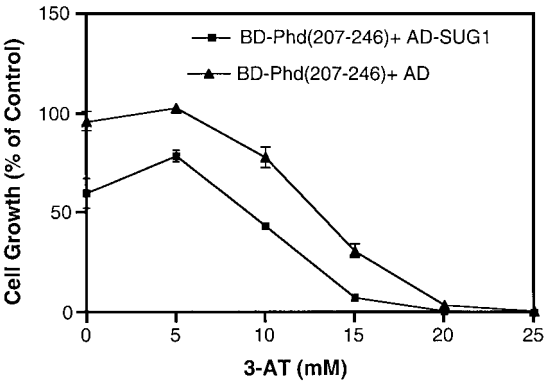


FIG. 2. Transactivation activity of Phd (207–246) in yeast cells. The yeast strain CG-1945 was cotransformed with BD-Phd (207–246) and either AD-bSUG1 or AD yeast expression constructs. The transformants were grown on −Leu-Trp plates at 30°C for 3 days before processed for liquid growth assay. The cells were seeded at 1000 cells/ml in selective medium without histidine but supplemented with indicated concentration of 3-AT. After incubating the cultures for 2 days at 30°C with shaking (250 rpm), growth was measured at OD_{600}. Data is presented as a percentage of the OD_{600} of yeast grown in selective medium supplemented with histidine (−Leu-Trp).

DISCUSSION

The present study shows that the C-terminal 40 AA domain of Phd and PhLOP1 has strong transcriptional activation function in both yeast and mammalian cells,

FIG. 1. Interaction of Phd, PhLOP1, and truncated PhLOP1 with bSUG1. AD-bSUG1 was cotransformed with the indicated BD fusion constructs into yeast CG-1945. The transformants were grown in the presence (−Leu-Trp) or absence (−Leu-Trp-His) of histidine and incubated at 30°C. (A) Schematic alignment representing the BD constructs and their interaction with bSUG1 by estimating the growth on −Leu-Trp-His and on −Leu-Trp-His + 3-AT plates. (−): Small (∅, <1 mm), pale colonies (background growth because of HIS3 gene leaky expression in CG-1945 cells). (+): Small (∅, <1 mm), pink colonies. (++): Medium (∅, 1–2 mm), pink colonies. (+++): Big (∅, 2–3 mm), pink colonies. (++++): Robust (∅, >3 mm), pink colonies. (B) The yeast transformants grown on −Leu-Trp plates were collected after 4 days incubation at 30°C and a pool of colonies was seeded at 1,000 cells/ml into selective medium. After incubating the cultures for 2 days at 30°C with shaking, growth was measured at OD_{600}. Data are presented as a percentage of the OD_{600} of yeast grown in selective medium supplemented with histidine (−Leu-Trp). (C) A pool of colonies was collected from samples grown on −Leu-Trp plates and prepared for β-galactosidase assay. Data represent the mean ± SEM of two independent experiments done in triplicate. The β-galactosidase activity is expressed in standard units multiplied by 1000.

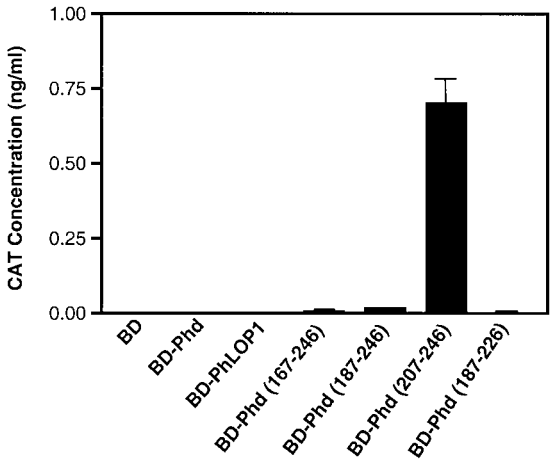


FIG. 3. Transactivation activity of Phd (207–246) in mammalian cells. COS-7 cells were cotransfected with BD-PhLOP1 or BD-truncated PhLOP1 in the mammalian expression vector pM with the pG5CAT reporter vector, which contains five consensus GAL4 binding sites and an adenovirus E1B minimal promoter upstream of the CAT reporter gene. 48 h after transfection, the cells were collected for CAT reporter assay. The results are means ± SEM of three independent experiments performed in duplicate.

whereas the full length Phd and PhLOP1 do not have this activity. These data strongly suggest that a domain of the Phd isoforms is a potential transcriptional regulator. Similar observations have been reported with the transcription factor ATF-2 (37) and the hepatitis C virus nonstructural protein NS5A (38) in which an intrinsic transcriptional activation domain is normally masked by the full length protein. Structural analysis reveals that this short peptide at the C-terminus of Phd isoforms is rich in acidic amino acids, resembling other acidic transcriptional activation domains that function universally in eukaryotes from yeast to human (39–42).

Members of the Phd superfamily have been reported to interact with SUG1, a subunit of the 26S proteasome and a transcriptional regulator, which may target them for degradation by the 26S proteasome or may suggest a role in transcriptional regulation (24, 26). Interestingly, CRX, the other protein-protein interaction partner of Phd isoforms identified from the same yeast two-hybrid screen (25), is a major developmental transcription factor in the eye and the pineal gland (31–33). The identification of this interaction between Phd isoforms and CRX further support our hypothesis that Phd isoforms are potential transcriptional regulators in the retina and pineal.

Both Phd and PhLOP1 are colocalized with CRX to the nucleus of the transfected COS-7 cells and each inhibits the transactivation activity of CRX on an IRBP promoter containing a single CRX binding element (25). Since Phd is predominantly expressed in the cytoplasm of rod photoreceptors and pinealocytes, we sought to determine the subcellular localization of Phd and PhLOP1 in either control COS-7 cells, immortalized rat neuroretinal cells (R28) or NGF-primed neuroblastoma PC12 cells. EGFP fusion of Phd has been shown to have the normal cytoplasmic localization and function in transiently transfected neuronal NG 108-15 hybrid cells (43, 44). Our results confirmed that Phd is localized to the cytoplasm; however, PhLOP1 is

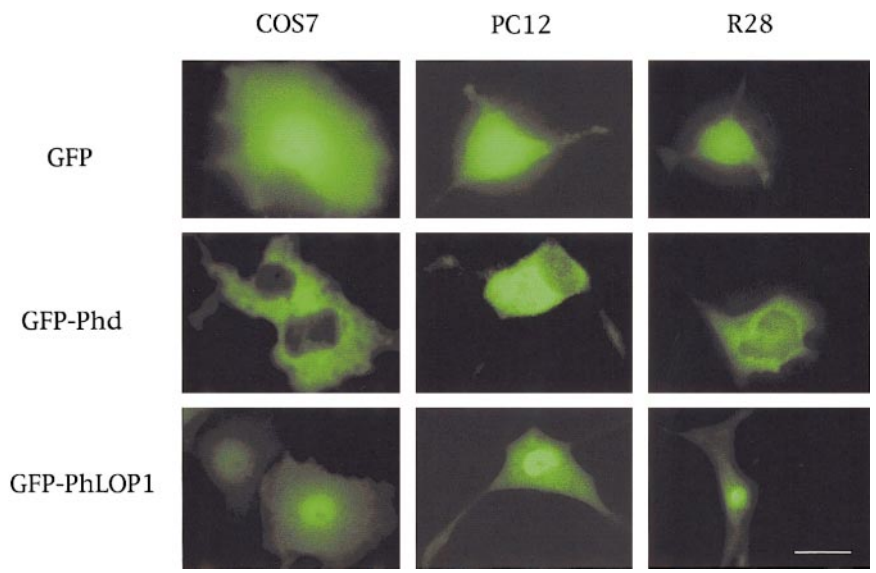


FIG. 4. *In situ* of EGFP-Phd and EGFP-PhLOP1 in transfected COS-7, neuronal PC12, and neuroretinal R28 cells. The coding region of Phd and PhLOP1 were inserted in-frame into the pEGFP-C2 vector, which encodes a red-shifted variant of wild-type GFP (enhanced GFP, EGFP). The EGFP-Phd, EGFP-PhlOP1 fusion constructs, and the EGFP control (pEGFP-C2 vector) were transfected to COS-7, PC12, and R28 cells. Photographs were taken 24–48 h after transfection.

localized to the nucleus in both non-neuronal and neuronal cells. These results are consistent with the previous immunocytochemical study of Phd isoforms with transiently transfected COS-7 cells (25). When cotransfected with CRX, both Phd and PhLOP1 are observed in the nucleus of the cotransfected cells with a Phd C-terminus-specific monoclonal antibody. We have proposed that Phd acts as a transcriptional regulator when its C-terminal peptides are released in the cytoplasm and transferred into the nucleus to exert its transcriptional function (25). The results of this study further support our hypothesis and confirm that the C-terminus of the Phd isoforms is potentially an independent functional unit controlling transcriptional regulation.

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REFERENCES

1. Lee, R. H., Lieberman, B. S., and Lolley, R. N. (1987) *Biochemistry* **26,** 3983–3990.
2. Kuo, C. H., Akiyama, M., and Miki, N. (1989) *Brain Res. Mol. Brain Res.* **6,** 1–10.
3. Lee, R. H., Whelan, J. P., Lolley, R. N., and McGinnis, J. F. (1988) *Exp. Eye Res.* **46,** 829–840.
4. Craft, C. M., Lolley, R. N., Seldin, M. F., and Lee, R. H. (1991) *Genomics* **10,** 400–409.
5. Lolley, R. N., Craft, C. M., and Lee, R. H. (1992) *Neurochem. Res.* **17,** 81–89.
6. Danner, S., and Lohse, M. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10145–10150.
7. Lee, R. H., Ting, T. D., Lieberman, B. S., Tobias, D. E., Lolley, R. N., and Ho, Y. K. (1992) *J. Biol. Chem.* **267,** 25104–12
8. Yoshida, T., Willardson, B. M., Wilkins, J. F., Jensen, G. J., Thornton, B. D., and Bitensky, M. W. (1994) *J. Biol. Chem.* **269,** 24050–24057.
9. Bauer, P. H., Muller, S., Puzicha, M., Pippig, S., Obermaier, B., Helmreich, E. J., and Lohse, M. J. (1992) *Nature* **358,** 73–76.
10. Gaudet, R., Bohm, A., and Sigler, P. B. (1996) Cell **87,** 577–588.
11. Lee, R. H., Brown, B. M., and Lolley, R. N. (1990) *J. Biol. Chem.* **265,** 15860–15866.
12. Chen, F., and Lee, R. H. (1997) *Biochem. Biophys. Res. Commun.* **233,** 370–374.
13. Lee, R. H., Brown, B. M., and Lolley, R. N. (1984) *Biochemistry* **23,** 1972–7
14. Hawes, B. E., Touhara, K., Kurose, H., Lefkowitz, R. J., and Inglese, J. (1994) *J. Biol. Chem.* **269,** 29825–29830.
15. Hekman, M., Bauer, P. H., Sohlemann, P., and Lohse, M. J. (1994) *FEBS Lett.* **343,** 120–124.
16. Muller, S., Straub, A., Schroder, S., Bauer, P. H., and Lohse, M. J. (1996) *J. Biol. Chem.* **271,** 11781–11786.
17. Xu, J., Wu, D., Slepak, V. Z., and Simon, M. I. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2086–2090.
18. Gaudet, R., Savage, J. R., McLaughlin, J. N., Willardson, B. M., and Sigler, P. B. (1999) *Mol. Cell* **3,** 649–660.
19. Miles, M. F., Barhite, S., Sganga, M., and Elliott, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10831–10835
20. Schroder, S., and Lohse, M. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2100–2104.
21. Thibault, C., Sganga, M. W., and Miles, M. F. (1997) *J. Biol. Chem.* **272,** 12253–6
22. Thibault, C., Feng Wang, J., Charnas, R., Mirel, D., Barhite, S., and Miles, M. F. (1999) *Biochim. Biophys. Acta* **1444,** 346–354.
23. Craft, C. M., Xu, J., Slepak, V. Z., Zhan-Poe, X., Zhu, X., Brown, B., and Lolley, R. N. (1998) *Biochemistry* **37,** 15758–15772.
24. Zhu, X., and Craft, C. M. (1998) *Mol. Vis.* **4,** 13.
25. Zhu, X., and Craft, C. M. (2000) Submitted.
26. Barhite, S., Thibault, C., and Miles, M. F. (1998) *Biochim. Biophys. Acta* **1402,** 95–101.
27. Craft, C. M., Murage, J., Brown, B., and Zhan-Poe, X. (1999) *Brain Res. Mol. Brain Res.* **65,** 44–51.
28. Seigel, G. M., Mutchler, A. L., Adamus, G., and Imperato-Kalmar, E. L. (1997) *In Vitro Cell Dev. Biol. Anim.* **33,** 499–502.
29. Seigel, G. M., Mutchler, A. L., and Imperato, E. L. (1996) *Mol. Vis.* **2,** 2.
30. Seigel, G. M. (1996) *In Vitro Cell Dev. Biol. Anim.* **32,** 66–68.
31. Chen, S., Wang, Q. L., Nie, Z., Sun, H., Lennon, G., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Zack, D. J. (1997) *Neuron* **19,** 1017–1030.
32. Li, X., Chen, S., Wang, Q., Zack, D. J., Snyder, S. H., and Borjigin, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1876–1881.
33. Furukawa, T., Morrow, E. M., and Cepko, C. L. (1997) *Cell* **91,** 531–541.
34. Freund, C. L., Gregory-Evans, C. Y., Furukawa, T., Papaioannou, M., Looser, J., Ploder, L., Bellingham, J., Ng, D., Herbrick, J. A., Duncan, A., Scherer, S. W., Tsui, L. C., Loutradis-Anagnostou, A., Jacobson, S. G., Cepko, C. L., Bhattacharya, S. S., and McInnes, R. R. (1997) *Cell* **91,** 543–553.
35. Swain, P. K., Chen, S., Wang, Q. L., Affatigato, L. M., Coats, C. L., Brady, K. D., Fishman, G. A., Jacobson, S. G., Swaroop, A., Stone, E., Sieving, P. A., and Zack, D. J. (1997) *Neuron* **19,** 1329–1336.
36. Goldman, P. S., DeMaggio, A. J., Hoekstra, M. F., and Goodman, R. H. (1997) *Biochem. Biophys. Res. Commun.* **240,** 425–429.
37. Livingstone, C., Patel, G., and Jones, N. (1995) *EMBO J.* **14,** 1785–1797.
38. Tanimoto, A., Ide, Y., Arima, N., Sasaguri, Y., and Padmanabhan, R. (1997) *Biochem. Biophys. Res. Commun.* **236,** 360–364.
39. Ma, J., and Ptashne, M. (1987) *Cell* **48,** 847–853.
40. Hope, I. A., Mahadevan, S., and Struhl, K. (1988) *Nature* **333,** 635–640.
41. Lillie, J. W., and Green, M. R. (1989) *Nature* **341,** 279–280.
42. Cress, W. D., and Triezenberg, S. J. (1991) *Science* **251,** 87–90.
43. Schulz, R., Schulz, K., Wehmeyer, A., and Murphy, J. (1998) *Brain Res.* **790,** 347–356.
44. Wehmeyer, A., and Schulz, R. (1998) *Life Sci.* **62,** L127–L134.