The nuclear protein NIPP1 (nuclear inhibitor of protein Ser/Thr phosphatase-1) interacts with the splicing factors SAP155 and CDC5L and is involved in a late step of spliceosome assembly. In addition, NIPP1 is an interactor of protein phosphatase-1 and a COOH-terminal NIPP1 fragment displays an RNase E like endoribonuclease activity. A yeast two-hybrid screening resulted in the identification of the Polycomb group protein EED (embryonic ectoderm development), an established transcriptional repressor, as a novel NIPP1 interactor. NIPP1 only interacted with full-length EED, whereas two EED interaction domains were mapped to the central and COOH-terminal thirds of NIPP1. The NIPP1-EED interaction was potentiated by the binding of (d/G)-rich nucleic acids to the central domain of NIPP1. Nucleic acids also decreased the potency of NIPP1 as an inhibitor of PP1, but they did not prevent the formation of a ternary NIPP1/EED-PP1 complex. EED had no effect on the function of NIPP1 as a splicing factor or as an endoribonuclease. However, similar to EED, NIPP1 acted as a transcriptional repressor of targeted genes and this NIPP1 effect was mediated by the EED interaction domain. Also, the histone deacetylase 2 was present in a complex with NIPP1. Our data are in accordance with a role for NIPP1 as a DNA-targeting protein for EED and associated chromatin-modifying enzymes.

NIPP1 (39 kDa, 351 residues) is a ubiquitously expressed nuclear protein that was originally discovered as a potent and specific inhibitor of protein Ser/Thr phosphatase-1 (PP1), hence its name nuclear inhibitor of PP1 (1). NIPP1 contains binding sites for PP1 in its central and COOH-terminal domains (2, 3)

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tions as a methyltransferase and methylates nucleosomal histone H3 on Lys-9 and Lys-27, which facilitates the binding of the PRC1 complex (19–22). Recruitment of PRC1 would then prevent the access of nucleosome-remodeling factors, such as SWI/SNF, leading to the formation of a repressive chromatin state. A further complexity in the understanding of PcG-mediated transcriptional repression arises from observations that the PcG complexes also contain non-PcG proteins and that the composition of the PcG complexes is cell-type and differentiation-dependent (23). For example, the PRC2 complex has been reported to contain the histone deacetylases HDAC1 and HDAC2, which may contribute to the transcriptional repression by the PRC2 complex (24, 25). However, the association of HDACs with the PRC2 complex has not been a universal finding (19, 20). Another component of the PRC2 complex is the transcription factor Yin Yang 1 (YY1), which has been suggested to mediate the binding of the PRC2 complex to specific DNA sequences, the so-called Polycomb response elements (26).

As a component of the PRC2 complex, EED is essential for a proper anterior-posterior patterning of the primitive streak in early gastrulation (27–29) and for the stable maintenance of random and imprinted X chromosome inactivation in the mouse (17, 18). However, EED is also expressed in a variety of adult tissues (30–32) and functions in a cell-type and differentiation-dependent manner. For example, EED represses proliferation of myeloid and B-cell precursors in adult bone marrow (33) but promotes the differentiation of early intrathymic T-cell precursors. Intriguingly, EED also suppresses the development of thymic lymphomas induced by the carcinogen N-methyl-N-nitrosourea.

To identify protein interactors that mediate the splicingosomal function of the COOH-terminal domain of NIPP1 and/or modulate its endoribonuclease activity, we have performed a yeast two-hybrid screening with NIPP1-(225–351) as bait. This study led to the identification of the PRC1 protein EED as a novel interactor of NIPP1. We did not find any evidence for a role of EED in the splicingosomal and endoribonuclease functions of NIPP1. However, like EED, NIPP1 functioned as a potent transcriptional repressor. The latter NIPP1 effect depended on binding sites for EED but did not require functional interaction sites for either splicing factors or PP1.

**EXPERIMENTAL PROCEDURES**

**Materials**—poly(A), poly(U), poly(C), and poly(G) were obtained from Sigma, and the 20-mer poly(dC) and poly(dG) were purchased from Sigma. Microcystin-Sepharose was purchased from Upstate Biotechnology. PP1 was purified from rabbit skeletal muscle (34). Recombinant inhibitor-2 from rabbit skeletal muscle was prepared as described previously (35). A synthetic peptide comprising the COOH terminus of NIPP1 (341PGKKPTPSLLI351) coupled to keyhole limpet hemocyanin was used to generate a rabbit polyclonal antibody. The NIPP1 antibodies were affinity-purified on glutathione-agarose beads (Sigma) that has been preblocked with TBS plus bovine serum albumin (1 mg/ml). 0.5% Triton X-100, and 1 mM dithiothreitol. After washing with TBS plus 0.1% Nonidet P-40, the beads were incubated with either COS-1 cell lysates or polyhistidine-tagged EED (mutants) for 1 h at 4 °C. Subsequently, the beads were sedimented (30 s at 1000 × g), washed three times with 500 μl of TBS plus 0.1% Nonidet P-40, and subsequently probed for the presence of His-EED. The cells were lysed in buffer A containing 50 mM Tris at pH 7.5, 0.3 mM NaCl, 0.3% Triton X-100, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonfonyl fluoride, 0.5 mM benzamidine, 5 μM leupeptin, 1 mM orthovanadate, and 20 mM NaF.

For immunoprecipitations, mouse embryo (7.5 dpc, including decidua) extracts, cell lysates, or a mixture of purified GST-NIPP1 and His-EED (plus or minus PP1 and nucleic acids as indicated) was incubated for 1–4 h at 4 °C with either NIPP1 or EED antibodies coupled to protein A-BSA (AffiL) beads. After three washes with 500 μl of TBS plus 0.1% Nonidet P-40, the beads were screened for the presence of NIPP1 and EED by Western analysis and for PP1 by the assay of phosphatase activity.

**Cell Culture**—COS-1 cells were grown in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum. FuGene 6 (Roche Applied Science) was used for transfection with mammalian expression vectors encoding GST or GST-NIPP1. 48 h after transfection, the cells were washed twice with ice-cold phosphate-buffered saline and lysed in buffer A plus 0.1% Nonidet P-40 and 10 mM butyrate. After lysis of the cells, histidine bromide (50 μg/ml) was added to disrupt protein-DNA interactions, and after an incubation for 30 min at 0 °C, the lysates were cleared by centrifugation (10 min at 16,000 × g) and the supernatants were used for GST pull-down assays.

**Phosphorylase Phosphatase Assays**—The tryptic-revealed protein phosphatase activities with different proteins were measured as described previously (1) using 32P-labeled phosphorylase a as substrate. One unit of phosphatase released 1 nmol of phosphate/min at 30 °C. The inhibitory potency of NIPP1 and NIPP1-(1–329) was assayed in the presence of 0.4 μM PP1.C.

**Transcriptional Repression Assays**—We used the assay described by Thié et al. (36, 37). For these experiments, EED, NIPP1, and the control vectors were transfected in the mammalian expression system SV40 pm in-frame with the GAL4 DNA-binding domain (residues 1–147). COS-1 cells and 293 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. For repression assays, 0.06 × 10^5 cells were seeded in each well of a 24-well plate. After 1 day, the cells were transfected with (i) 50 ng of the luciferase reporter plasmid (ii) 5 GAL4-binding sites (UAS) either upstream of the SV40 promoter (plasmid pUA3S–SV40-Luc) or downstream of the polyadenylation signal (plasmid pSV40-Luc-UAS), (iii) 100 ng of GAL4 (fusion) encoding plasmid, and (iii) 20 ng of the β-galactosidase encoding plasmid. Two days after transfection, the cells were harvested in passive...
lysis buffer (Promega). The luciferase activities were measured in a Luminoskan Ascent luminometer (Labsystems) using a luciferase assay kit (Promega). The β-galactosidase activities were measured with 2-nitrophenyl-β-D-galactopyranoside as substrate. The luciferase activities were normalized for transfection efficiency using the β-galactosidase activities. The data are represented as means ± S.E. of percentages of the luciferase activity that were measured with GAL4 (n = 4–9 separate transfections).

RESULTS

Yeast Two-hybrid Analysis of the NIPP1-EED Interaction—A yeast two-hybrid screening of a HeLa cell library with the COOH-terminal third of NIPP1 (residues 225–351) as bait resulted in the identification of the transcriptional repressor EED as an interacting protein (Fig. 1B). A deletion of the RNA-binding domain of NIPP1 as in NIPP1-(225–329) improved the binding to EED considerably, but a further deletion as in NIPP1-(225–310) abolished the interaction, indicating that residues 310–329 are essential for the binding of EED (Fig. 1B). Accordingly, NIPP1-(280–329) was also found to interact with EED in the two-hybrid assay. We have not been able to explore the interaction between EED and full-length NIPP1 in two-hybrid assays since the latter already induced the expression of the reporter gene in the absence of EED.

The central domain of NIPP1 also contains two binding sites for PP1. One of these represents a polybasic stretch (residues 193–197) that is important for the inhibition of PP1 (2). However, mutation of this polybasic stretch as in NIPP1-(143–224)-K193A,R194A,K195A,R196A,K197A did not hamper the interaction with EED in two-hybrid assays (data not shown). A second PP1-binding site in the central domain of NIPP1 is represented by the 200RVTF203 sequence (2). This sequence is a variant of the so-called RVXF motif that is present in most interactors of PP1 (38). The RVXF motif of NIPP1 is flanked by two serines (Ser-199 and Ser-204), and phosphorylation of these serines or their replacement by aspartic acid impedes the binding of PP1 (2). We found that NIPP1-(143–224)-S199D,S204D interacted normally with EED in a two-hybrid assay (data not shown). Collectively, the above data indicate that EED and PP1 have distinct binding sites in the central domain of NIPP1.

The two-hybrid screening with NIPP1-(225–351) as bait yielded six prey clones that all encoded full-length EED (441 residues). There is considerable evidence that murine EED is a
larger polypeptide (535 residues), and this was explained by the use of an upstream Val-encoding GTG as translation initiation codon. However, the corresponding Val triplet in the human EED gene is a GTA, which is never used as a translation initiation codon. We and others (26, 31) have therefore used the first ATG as the putative translation initiation codon, yielding a protein of 441 residues, which is similar to the size of Drosophila EED (425 residues) better known as ESC. Based on the Protein Sequence Analysis program (bmerc-www.bu.edu/psa/), EED largely consists of seven WD40 domains (Fig. 1A).

WD40 domains are protein-interaction domains that often occur tandemly in proteins. WD40 repeats are known to fold into four-strand β-blades that, similar to the pieces of a pie, form a circular structure known as a β-propeller (39). EED is predicted to form a seven-bladed β-propeller. We found that the deletion of the first one or the last three WD40 domains abolished the ability of EED to interact with the central and carboxyl-terminal domains of NIPP1 in two-hybrid assays (Fig. 1B). Likewise, EED-(1–156), EED-(157–289), and EED-(290–441) did not interact with the NIPP1 fragments. Interestingly, two point mutants, i.e. I193N and L196P, did not interact with NIPP1 either. These two residues are predicted to reside in the third WD40 domain (Fig. 1B), and the same mutations of the corresponding residues in mouse EED are embryologically lethal (L196P) or hypomorphic (I193N) (27). The latter two EED mutants are also inactive in transcriptional repressor assays (see also Fig. 8) (30) and do not interact with EZH2 (40).

Co-precipitation of NIPP1 and EED—The NIPP1-EED interaction that was detected by yeast two-hybrid analysis (Fig. 1) was confirmed by co-immunoprecipitation of both components from the yeast extracts using antibodies against either NIPP1 or HA-tagged EED (Fig. 2A). In the control condition, no primary antibody was added. The immunoprecipitates were examined for the presence of NIPP1 and EED by Western blot analysis. B, NIPP1 or EED was immunoprecipitated from a mouse embryo (7.5 dpc) extract, and co-precipitation of EED or NIPP1 was evaluated by Western analysis with EED and NIPP1 digoxigenin-labeled antibodies, respectively. C, a lysate of COS-1 cells was incubated with either GST or GST-NIPP1 coupled to glutathione-agarose. Co-sedimented EED was detected by Western analysis. D, similar amounts of purified recombinant His-tagged EED, His-EED I193N, or His-EED L196P were incubated with GST-NIPP1 bound to glutathione-agarose beads. The washed beads were screened for the presence of EED using an anti-His antibody and for GST-NIPP1 using a anti-NIPP1 antibody.

![Fig. 2. Co-immunoprecipitation and GST pull-down experiments confirm the NIPP1-EED interaction.](image-url)
with NIPP1 in yeast two-hybrid assays (Fig. 1B), could not be pulled down with GST-NIPP1 (Fig. 2D).

The NIPP1-EED and NIPP1-PP1 Interactions Are Modulated by Nucleic Acids—We have subsequently explored whether previously identified interactors of NIPP1 interfere with the binding of EED. Cdk2-phosphorylated SAP155-(223–322) or CDC5L-(272–606), which bind to the FHA domain of NIPP1 (9, 10) did not affect the binding of EED to GST-NIPP1 in pull-down experiments (data not shown). The COOH-terminal 22 residues of NIPP1 comprise an RNA-binding domain that preferentially interacts with A/U-rich sequences (11). We found that the NIPP1-EED interaction as determined by pull-down assays with GST-NIPP1 is enormously increased by the addition of single-stranded RNA poly(U), poly(A), poly(C), and in particular by poly(G) (Fig. 3A). The NIPP1-EED interaction was similarly increased by single-stranded poly(dC), poly(dG), and by double-stranded poly(dC/dG) (Fig. 3B). The stimulatory effects of nucleic acids on the NIPP1-EED interaction were so large that the interaction of both components in the absence of nucleic acids was sometimes difficult to see with the same exposure time of the blots (Figs. 3B and 6A).

The stimulatory effect of nucleic acids on the NIPP1-EED interaction was surprising since two-hybrid assays had indicated that the RNA-binding domain of NIPP1 hampered the interaction with EED (Fig. 1). The effects of poly(G) and poly(dC/dG) were also unexpected because the COOH terminus of NIPP1 does not have an affinity for these polynucleotides (41), suggesting that their effects on the NIPP1-EED interaction involves a different part of NIPP1 and/or is mediated by EED. Pull-down experiments with GST-NIPP1-(1–329), GST-NIPP1-(143–224) indeed revealed that the stimulatory effects of poly(G) on the binding of EED to NIPP1-EED only involved the central domain of NIPP1 (Fig. 4A). We have subsequently set up competition experiments with overlapping synthetic peptides covering a large part of the central domain of NIPP1. NIPP1-(181–200) and NIPP1-(191–210) were able to compete for the poly(G)-induced binding of the central domain of NIPP1 to EED (Fig. 4B). However, NIPP1-(190–200), which covers the residues that are common to these two peptides, did not show any competition (data not shown). Thus, the flanking residues also appear to be involved in the increased binding of NIPP1 to EED that is detected in the presence of nucleic acids.

The above data indicated that nucleic acids interact with a region of NIPP1 (residues 181–210) that also mediates inhibi-
tion of PP1 (see above). This raised the possibility that nucleic acids also control the inhibitory potency of NIPP1. We indeed found that poly(G) decreased the inhibitory potency of full-length NIPP1 considerably (Fig. 5A). Poly(A), poly(U), and poly(C), which are much less potent stimulators of the NIPP1-EED interaction (Fig. 3A), also had little to no effect on the inhibitory potency of NIPP1 (Fig. 5A). Poly(dC/dG) was as efficient as poly(G) in decreasing the inhibitory potency of NIPP1 (data not shown). In Fig. 5B, it is shown that poly(G) also decreased the inhibitory potency of NIPP1-(1–329), showing again that the effects of poly(G) are not mediated by the RNA-binding domain in the COOH terminus.

A Complex of NIPP1, EED, PP1, and HDAC2—The previous data suggested that NIPP1 has distinct binding sites for PP1 and EED and that the NIPP1-EED and NIPP1-PP1 interactions are oppositely affected by nucleic acids. However, this does not necessarily imply that nucleic acids disrupt the NIPP1-PP1 interaction, in particular, because NIPP1 also contains PP1-binding sites outside its central domain (3). Therefore, we have explored whether NIPP1, EED, and PP1 can form a ternary complex in the presence of nucleic acids. In Fig. 6A, it is shown that an in vitro reconstituted NIPP1-EED complex formed in the presence of poly(G) and immunoprecipitated with either EED antibodies (left panel) or NIPP1 antibodies (right panel) co-precipitated with exogenous PP1 as determined by the assay of trypsin-revealed phosphorylase phosphatase activity. The amount of PP1 that was immunoprecipitated with NIPP1 and with the NIPP1-EED complex was the same, indicating that the binding of EED does not affect the recruitment of PP1. We have also found that a protein phosphatase activity is immunoprecipitated from 293 cell lysates using antibodies against either EED or NIPP1 (Fig. 6B). These protein phosphatase activities stemmed from PP1 since the activity was blocked by inhibitor-2, a specific inhibitor of PP1. Thus, using both purified components (Fig. 6A) as well as cell lysates (Fig. 6B), PP1 could be shown to be part of a complex that also contains NIPP1 and EED.

Microcystin is a cyclic heptapeptide that binds with high affinity to PP1 (and related phosphatases) and can be used for affinity purification of PP1 holoenzymes. We found that PP1 from mouse embryo extracts was indeed bound by microcystin-Sepharose (Fig. 7A). NIPP1 and EED were also retained on microcystin-Sepharose, providing additional evidence for the existence of an EED-NIPP1-PP1 complex. Intriguingly, HDAC2, an established ligand of EED (14–16), was also retained by microcystin-Sepharose (Fig. 7A). HDAC2, PP1, and EED also co-precipitated with GST-NIPP1 that was expressed in COS-1 cells (Fig. 7B), confirming that these components are part of a single complex. In contrast, no immunodetectable amounts of YY1 or EZH2 could be precipitated from COS-1 cell lysates with GST-NIPP1 (data not shown), two other established ligands of EED (14–16).

Functional Analysis of the EED-NIPP1 Interaction—We originally used NIPP1-(225–351) as bait for a two-hybrid screening to identify proteins that control the splicing function or endoribonuclease activity of NIPP1 (see Introduction). However, pu-
rified EED (up to 2.2 μM) did not affect the endoribonuclease activity of NIPP1-(225–351) and did not endow full-length NIPP1 with an endoribonuclease activity (data not shown). We have previously shown that residues 225–329 of NIPP1, which contains an EED interaction site (Fig. 1B), are required for a late step of spliceosome assembly (8). However, the addition of 2 μM EED, EED I193N, or EED L196P to HeLa cell nuclear extracts did not affect the splicing of α2-globin pre-mRNA fragment (data not shown). Also, while NIPP1 was found to co-precipitate with spliceosomes (8), the immunoprecipitation of exogenous EED from splicing extracts did not result in the co-precipitation of spliceosomal components (data not shown).

The above data did not provide any evidence for an involvement of EED in the splicing and endoribonuclease functions of NIPP1. This prompted us to consider the opposite view, namely that NIPP1 functions in cellular processes that are also affected by EED. It is generally accepted that EED causes transcriptional inhibition of targeted promoters (16). To study the transcriptional effects of full-length EED, NIPP1, and some mutants, these proteins were fused in-frame to the GAL4 DNA-binding domain (amino acids 1–147) in the mammalian expression vector pM1. The vector encoding only the GAL4 DNA-binding domain was used as negative control. As positive controls, we expressed fusions of the GAL4 DNA-binding domain and either the transcriptional repression domain of NK10 (residues 1–112) (37, 42) or the transcriptional co-repressor NAB1 (36). The plasmids were transiently co-expressed in COS-1 cells with a reporter luciferase gene plasmid. In the reporter plasmid, five tandem GAL4-binding sites (UAS) were located either upstream of the minimal SV40 promoter (Fig. 8A) or downstream of the polyadenylation signal (data not shown). Compared with the GAL4 DNA-binding domain, the expression of GAL4-EED or GAL4-NIPP1 resulted in a 5-fold reduction of reporter activity with either type of reporter plasmid (Fig. 8B). Thus, NIPP1 and EED function as transcriptional repressors both from proximal and distal positions with respect to the promoter. The transcriptional repression by EED and NIPP1 was similar in magnitude to that observed with NK10 and NAB1. On the other hand, EED L196P, which has no affinity for NIPP1, did not show any transcriptional repression.
and even consistently showed a transcriptional activation of approximately 30–40%, in accordance with a previous report (30). Deletion of the COOH-terminal binding sites for RNA and PP1 (GAL4-NIPP1-(1–324)) mutation of the PP1 binding RVXF motif in the central domain (GAL4-NIPP1 V201A,F203A) or its flanking residues (GAL4-NIPP1 S199D,S204D) or mutation of the FHA domain (NIPP1 S68A,R69A,V70A,H71A) did not affect the transcriptional repressor activity of NIPP1. GAL4-NIPP1-(1–324) V201A,F203A also showed the same repressor activity as wild-type NIPP1 (data not shown). However, the expression of GAL4-NIPP1-(1–142), which does not bind to EED (Fig. 1B), had only a minor (<25%) effect on the transcription of the reporter gene. Thus, NIPP1 acts as a transcriptional repressor in a cellular context. This NIPP1 function is independent of its ability to interact with PP1 or ligands of the FHA domain but requires the EED-interacting domain.

**DISCUSSION**

**Mechanism of Transcriptional Repression by NIPP1**—The original aim of this study was to identify proteins that modulate the endoribonuclease or splicing activities of NIPP1. Using a yeast two-hybrid screening with NIPP1-(225–306) as bait, we identified EED as a novel interactor of NIPP1 (Fig. 1) and the interaction was subsequently confirmed by co-immunoprecipitation analysis and GST pull-down assays (Figs. 2 and 6). EED did not appear to interfere with the endoribonuclease or splicing functions of NIPP1 (fragments), but unexpectedly, NIPP1 was found to display a transcriptional repressor activity similar to that of EED. It is tempting to speculate that the transcriptional repression activity of NIPP1 is mediated by EED-associated chromatin-modifying enzymes. In accordance with this view, we found that the transcriptional effect of NIPP1 required the EED interaction domain but was independent of the previously identified interactors, PP1, SAP155, and CDC5L (Fig. 8). Also, we found that HDAC2, which has been suggested to mediate at least in part the transcriptional repression by EED (16), is a component of a complex that also contains NIPP1, EED, and PP1 (Fig. 7). Furthermore, we have identified NIPP1 as a DNA-binding protein (Figs. 3 and 4) and NIPP1 therefore qualifies as a candidate mediator of the binding of EED and associated (chromatin-modifying) proteins to specific DNA sequences. This function would be similar to that proposed for the transcription factor YY1 (26). In this respect, it is important to note that the NIPP1-associated pool of EED did not contain immunodetectable amounts of YY1 (data not shown), suggesting that another protein, e.g., NIPP1, mediates the targeting of this EED complex and its associated proteins to DNA.

**The Role of NIPP1/EED-associated PP1**—We have been able to reconstitute a ternary complex of purified NIPP1, EED, and PP1, and such a complex could also be identified in cell lysates (Fig. 6). Similarly, PP1 was identified as a component of another chromatin-remodeling complex, which also contains the PP1 interactor GADD34 and the SNF5 protein (43). GADD34 is a stress-induced protein that facilitates cell-cycle arrest, whereas SNF5 is a component of a SWI/SNF chromatin remodeling complex that acts by repositioning nucleosomes. PP1 was also recently identified as an antagonistic interactor of the trithorax protein, a component of a complex of proteins that acts oppositely to the PcG proteins and is required for the maintenance of normal expression of homeotic genes (44). Finally, PP1 was identified as a component of a complex that also includes HDAC1 and promotes the dephosphorylation and inactivation of the transcription factor CREB (45). Thus, PP1 emerges from these studies as a novel regulator of transcription.

The NIPP1-PP1 complex did not show any protein phosphatase activity, but the mere addition of (d)G-rich nucleic acids already resulted in an activation of the holoenzyme (Fig. 5). Thus, in addition to the phosphorylation of NIPP1 by various protein kinases (see Introduction), the binding of (d)G-rich nucleic acids to NIPP1 represents yet another means to modulate the activity of associated PP1. The de-inhibition of NIPP1-associated PP1 by DNA can be accounted for by the binding of DNA to the central domain of NIPP1 (Fig. 4), resulting in the (partial) dissociation of PP1 from this domain. Since PP1 also has an interaction site in the COOH terminus of NIPP1 (3), the binding of DNA does not cause the disruption of the NIPP1-PP1 complex (Fig. 6A). It is important to note that NIPP1 appears to have two nucleic acid-binding sites, one for single-stranded A/U-rich sequences in the COOH terminus (11) and another one for (d)G-rich single/double-stranded nucleic acids in the central domain (Fig. 4). We have previously shown that the binding of AU-rich RNA decreases the inhibition of PP1 by the COOH-terminal domain of NIPP1 and, moreover, potentiates the phosphorylation of Tyr-335 by the protein tyrosine kinase Lyn (3). Our two-hybrid analysis suggested that the COOH-terminal RNA-binding domain of NIPP1 is inhibitory to the binding of EED (Fig. 1). In conclusion, our data suggest that the interaction among NIPP1, EED, and PP1 is complexly modulated by nucleic acids in a sequence-dependent manner.

What could be the function of PP1 in the NIPP1-EED-PP1 complex? The transcriptional repression by NIPP1 is not dependent on functional PP1-binding sites (Fig. 8), but this does of course not rule out the possibility that PP1 modulates the transcriptional repression by NIPP1. PP1 appears to be a component of complexes that also contain HDACs (Fig. 7) (45), and the dephosphorylation and deacetylation of histone H3 by these enzymes are expected to result in transcriptional repression (45). Although these data suggest that PP1 promotes transcriptional repression, it cannot be excluded that PP1 (also) functions in the reversal of transcriptional repression, e.g., by promoting the disassembly of the repression complex. Such a function would be similar to that recently proposed for the pool of PP1 that is associated with NIPP1 in the splicesomes, namely to promote spliceosome disassembly and/or the shutting of splicing factors to storage sites (8). Potential substrates of EED-associated PP1 are EED itself, EED-associated HDACs, or histone H3. All of the three proteins are established phosphoproteins, and HDACs and histone H3 have been identified as physiological substrates of PP1 (46–48).

In conclusion, we have described here an interaction between NIPP1, an established splicing factor and PP1 regulator, and EED, a protein implicated in gene silencing and transcriptional repression. The binding of EED is mediated by two binding sites in the central and carboxyl-terminal thirds of NIPP1 and is potentiated by the binding of (d)G-rich nucleic acids to the central domain of NIPP1. We also demonstrate that NIPP1 itself acts as a transcriptional repressor in transient expression experiments, and this effect does not require functional interaction sites for splicing factors and PP1 but is mediated by the EED-interacting domain. We hypothesize that NIPP1 causes transcriptional repression by the recruitment to specific DNA sequences of an EED-containing chromatin-modifying complex that includes HDAC2.

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The Protein Phosphatase-1 (PP1) Regulator, Nuclear Inhibitor of PP1 (NIPP1), Interacts with the Polycomb Group Protein, Embryonic Ectoderm Development (EED), and Functions as a Transcriptional Repressor
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