The Fission Yeast Inhibitor of Growth (ING) Protein Png1p Functions in Response to DNA Damage*

Received for publication, January 7, 2010, and in revised form, March 11, 2010 Published, JBC Papers in Press, March 18, 2010, DOI 10.1074/jbc.M110.101832

Jian-Qiang Chen,1 Yang Li,1 Xian Pan, Bing-Kun Lei, Cheng Chang, Zheng-Xun Liu, and Hong Lu2

From the State Key Laboratory of Genetic Engineering, School of Life Sciences, and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China

In budding yeast and human cells, ING (inhibitor of growth) tumor suppressor proteins play important roles in response to DNA damage by modulating chromatin structure through collaborating with histone acetyltransferase or histone deacetylase complexes. However, the biological functions of ING family proteins in fission yeast are poorly defined. Here, we report that Png1p, a fission yeast ING homolog protein, is required for cell growth under normal and DNA-damaged conditions. Png1p was further confirmed to regulate histone H4 acetylation through collaboration with the MYST family histone acetyltransferase 1 (Mst1). Additionally, both fission yeast PGN1 and MST1 can functionally complement their budding yeast correspondent homologs YNG2 and ESAI, respectively. These results suggest that ING proteins in fission yeast might also conserve function, similar to ING proteins in budding yeast and human cells. We also showed that decreased acetylation in Δpng1 cells resulted in genome-wide down-regulation of 756 open reading frames, including the central DNA repair gene RAD22. Overexpression of RAD22 partially rescued the png1 mutant phenotype under both normal and DNA-damaged conditions. Furthermore, decreased expression of RAD22 in Δpng1 cells was confirmed to be caused by decreased H4 acetylation at its promoter. Altogether, these results indicate that Png1p is required for histone H4 acetylation and functions upstream of RAD22 in the DNA damage response pathway.

The ING family members are well known candidate tumor growth suppressors (1). They are associated with certain areas of oncogenesis and cellular growth, such as cell cycle regulation, senescence, DNA damage repair, and apoptosis (2). Reduced mRNA expression, allelic loss, or somatic mutation of ING proteins were reported in many types of human cancers, including breast cancer, gastric cancer, melanoma, glioma, esophageal squamous cell carcinoma, and head and neck squamous cell carcinoma (2). However, the underlying mechanisms of ING proteins are still poorly understood.

The DNA damage response process is very important for all living things to defeat the continuous threat of DNA damage caused by endogenous and exogenous factors and to maintain genome integrity (3). Chromosomal DNA in the eukaryotic nucleus is packaged into a very compact structure, and a critical step in DNA repair is to ensure that the DNA repair machinery can access the DNA. ING proteins always act as co-factors of histone acetyltransferases (HATs)3 or histone deacetylases (HDACs) to acetylate or deacetylate the N-terminal tail of histone proteins (4, 5). This process can promote or inhibit access of DNA repair or gene transcription machinery to the DNA and is involved in DNA repair, gene transcription, and genome integrity (6, 7). Thus far, five human members (INGs1–5) and three budding yeast members (Yng1p, Yng2p, and Pho23p) have been characterized, and they all function with HATs or HDACs (8). Among these members, human ING3 is a stable component of the NuA4 HAT complex and functions in the DNA damage response pathway through regulation of histone H4/H2A acetylation or deacetylation (7, 9). The three budding yeast ING family proteins, Yng1p, Yng2p, and Pho23p, are subunits of the NuA3 HAT, NuA4 HAT, and Sin3/Rpd3 HDAC complexes, respectively. They regulate H3 acetylation, H4 acetylation, and histone deacetylation, respectively (10–12). Each of these ING family proteins contain an N-terminal protein–protein interaction region, a nuclear localization signal, and a C-terminal plant homeodomain finger (1, 13). The most conserved motif, plant homeodomain, always functions as a histone code-signaling domain that recognizes the trimethylated lysine 4 residue of histone H3 (H3K4me3) to sense upstream signals (14–17). However, the downstream pathway of histone code transduction by ING proteins still needs to be investigated.

Fission yeast is an ideal system to study the molecular mechanisms of the DNA damage response. However, the biological functions of fission yeast ING family proteins Png1p and Png2p are poorly defined. In 2007, Grewal’s laboratory (18) found that Png2p functions as a component of the Clr6 HDAC complex, and cells with deletion of PNG2 were insensitive to DNA damage, suggesting that Png2p is likely not involved in the DNA damage response. In previous bioinformatics reports, the other ING family protein Png1p in fission yeast was closely related to Yng2p, an ING family protein in budding yeast involved in the

* This work was supported by grants from the National Program on Key Basic Research Project (973 Program, 2009CB825601), the National Natural Science Foundation of China (30771145 and 30671175), the Specialized Research Fund for the Doctoral Program of High Education (SRDFP 20060246017), and the Innovation Fund of Fudan University.

1 Both authors contributed equally to this work.

2 To whom correspondence may be addressed: The State Key Laboratory of Genetic Engineering, School of Life Sciences, and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China. Tel. and Fax: 86-21-65642505; E-mail: honglu0211@yahoo.com.

3 The abbreviations used are: HAT, histone acetyltransferase; MST1, MYST family histone acetyltransferase 1; MMS, methylmethane sulfonate; CPT, camptothecin; HDAC, histone deacetylase; NuA4, nucleosome acetyltransferase of H4; HCS1, 3-hydroxy-3-methylglutaryl-CoA synthase; GFP, green fluorescent protein; WT, wild type; ChIP, chromatin immunoprecipitation.
response to DNA damage (1, 19). These reports prompted us to address whether Png1p conserves function in the DNA damage response similar to Yng2p and to determine the downstream pathway. In the present study, we found that Png1p is required for cell growth under normal and DNA-damaged conditions. The Png1p protein is required for histone H4 acetylation in vivo. Decreased acetylation in png1 mutants resulted in decreased expression of 756 open reading frames related to chromatin remodeling, the cell cycle, and DNA repair, including the central DNA repair gene RAD22. Furthermore, downregulation of Rad22 was attributable to decreased H4 acetylation. Overexpression of RAD22 in Δpng1 mutants partially rescued the defective phenotype under normal and DNA-damaged conditions. Our results indicate that fission yeast Png1p is required for H4 acetylation and functions upstream of Rad22 in the DNA damage response pathway.

EXPERIMENTAL PROCEDURES

Yeast Strains and Gene Knock-out—The yeast strains used in this study are listed in Table 1. Budding yeast strains were cultured in YPD medium at 30 °C, and fission yeast strains were cultured in YES or EMM medium at 32 °C. PNG1 and PNG2 were knocked out from fission yeast according to homologous recombination (20). The knocked out png1 or png2 yeast strains were renamed Δpng1 or Δpng2.

DNA Damage Assay—The fission yeast cells, grown to A600 1.0 in liquid medium, were 10-fold serially diluted and spotted onto YES or EMM plates containing 0.005% (w/v) methyl methanesulfonate (MMS) or 1 μM camptothecin (CPT) and then incubated a 32 °C for 3–4 days. For the barker’s yeast, the cells were grown at 30 °C and spotted onto a YPD plate containing 0.01% (w/v) methyl methanesulfonate or 10 μg/ml CPT and then incubated at 30 °C for 3–4 days. The plates were photographed with a scanner.

Analysis of Cell Cycle by Flow Cytometry—Approximately 10² yeast cells at logarithmic phase were harvested and fixed in 70% (v/v) cold ethanol at 4 °C for 1 h. The fixed cells were resuspended in 50 mM Tris–HCl (pH 7.5) containing 0.1 mg/ml RNase A, incubated at 37 °C for 2 h, and then stained with 4 μg/ml propidium iodide at room temperature for 15 min. Approximately 1–2 × 10⁴ were measured by a FACScalibur flow cytometer (Becton Dickinson) and analyzed by Cell Quest software (21).

Preparation of Histone and Analysis of Histone Acetylation—Histone was prepared by sulfuric acid extraction as previously described (22). Acetylation of histone H4 at different sites was analyzed by Western blotting using the following antibodies: anti-acetyl histone H4K5 (07-327; Upstate Biotechnology), anti-acetyl histone H4K8 (07-328; Upstate Biotechnology), and anti-acetyl histone H4K12 (07-323; Upstate Biotechnology). Anti-histone H4 (07-108; Upstate Biotechnology) was used as a control.

Co-immunoprecipitation Assay—Δpng1 cells were co-transformed with plR2−41U-Png1-His₆ and pREP1−3×FLAG-Mst1 and cultured in the EMM medium without uracil and leucine overnight. 100 A600 cells were harvested and washed twice with cold phosphate-buffered saline, treated with Zymolyase (Sigma) for 30 min, and then lysed in TPER lysis buffer (Pierce) (23). After analyzing the expression of Png1-His₆ and 3×FLAG-Mst1 in co-transformed cells by Western blotting using anti-His or anti-FLAG antibody, the anti-FLAG M2 affinity gel was used to immunoprecipitate FLAG-fused protein, and anti-His was used in Western blotting (24).

Subcellular Protein Localization—Png1 and Mst1, fused separately with GFP and Cherry at the C terminus, were cloned into pJR2−41U and pREP1 vectors, respectively, and then transformed individually or co-transformed into fission yeast CHP428. After incubation of transformed cells at 32 °C overnight, the cells were washed with phosphate-buffered saline, stained with 1 μg/ml 4',6'-diamidino-2-phenylindole to visualize nuclei, and then observed and captured using a Zeiss Axioplan equipped with a chilled video charge-coupled device camera (C4742–95; Hamamatsu Photonics, Bridgewater, NJ) connected to a computer using kinetic image AQM software (Kinetic Imaging, Nottingham, UK). The images were further processed using Adobe Photoshop (version 7.0).

DNA Microarray Analysis—cDNAs were prepared according to a previous report (25). Gene expression in fission yeast wild type (WT) and Δpng1 was analyzed by the Shanghai Genetech Company (Shanghai, China) using the Yeast Genome 2.0 array (900553; Affymetrix). Gene names for each open reading frame and their functions were obtained from the Schizosaccharomyces pombe genome data base.

Chromatin Immunoprecipitation—ChIP analysis experiments were performed as previously described (22). Briefly, sol-
uble chromatin fractions, prepared from fission yeast strains WT, ΔPNG1, or WT/Rep1-FLAG-Mst1, were incubated separately overnight with 20 μl of protein A/G-Sepharose and 10 μl of one of the following antibodies: anti-aceH4K5, anti-aceH4K8, anti-aceH4K12, or anti-FLAG. WT/Rep1-FLAG was used as a negative control. DNA recovered from the immunoprecipitated fractions was amplified using PCR with the following primers: Rad22 (5′-AAGACGGCCATTTACAC-3′ and 5′-TCTGTTCTTTTAGCTTC-3′), CDC22 (5′-CGGACTATTAGCGGAACTTTG-3′ and 5′-CTCTTGTTCTTAGCTTGT-3′), and HCS1 (5′-CCATTTATCCAGAATTG-3′ and 5′-CTCTGCTCTTAGCTTGT-3′). The promoter of 3-hydroxy-3-methylglutaryl-CoA synthase (HCS1) was amplified and used as a control.

Real Time Quantitative PCR—The experiments were performed as previously described (26). Briefly, 4 μg of total RNA was prepared from fission yeast by using TRIzol and reverse transcribed to first strand cDNA, which was used as a template for real time PCR. The PCR amplification reactions were performed using SYBR Premix ExTaq TMII (DRR081C; Takara) on an ABI Prism 5700 sequence detection system (Applied Biosystems) with an initial step at 95 °C for 3 min, followed by 40 cycles at 95 °C for 5 s and 60 °C for 20 s. C_T was determined automatically by the instrument. All of the samples were analyzed in triplicate. HCS1 was used to normalize the mRNA expression level.

RESULTS

Png1p Functions in Response to DNA Damage—ING proteins have been reported to be involved in the DNA damage response (19). To determine whether the homologs of ING family proteins in fission yeast have similar functions, the effect of PNG1 or PNG2 deletion on cell growth was first analyzed using the cell growth curve and serially dilution assay. As shown in Fig. 1 (A and B), deletion of PNG1 slowed cell growth, whereas deletion of PNG2 did not. In the flow cytometry assay, the haploid WT cells showed only one 2C DNA peak, whereas the cells of the ΔPNG1 and ΔPNG2 strains showed two DNA peaks of 2C and 4C (Fig. 1C). The 4C DNA peak is well known to indicate cell cycle arrest with failure to complete cytokinesis (27, 28). These results show that fission yeast cells without the ING family gene PNG1 or PNG2 yield cell cycle arrest after DNA synthesis.
Furthermore, the sensitivity of \( \Delta \text{png1} \) or \( \Delta \text{png2} \) mutants to the DNA damage agents was determined. The DNA damage reagents used in our experiments are MMS, which can alkylate primarily guanine bases and induce single- or double-strand breaks, and CPT, which can directly inhibit topoisomerase I activity and induce intra-S phase double-stranded breaks (29). As shown in Fig. 1B, the \( \Delta \text{png1} \) mutants were supersensitive to both 1 \( \mu \text{M} \) CPT and 0.005\% (w/v) MMS, whereas the growth of \( \Delta \text{png2} \) mutants was the same as that of WT under identical DNA-damaged conditions. In the flow cytometry assay, after treatment of logarithm phase cells by 0.005\% (w/v) MMS or 1 \( \mu \text{M} \) CPT for 2 h, \( \Delta \text{png1} \) cells were arrested in intra-S phase because the cells with DNA content between 2C and 4C were increased compared with WT cells (Fig. 1C). These results demonstrate that Png1p is not only required for cell growth but is also involved in the DNA damage response pathway. DNA repair may be one downstream pathway affected by Png1.

**Png1p Is Required for Histone H4 Acetylation**—Png1p shares the highest amino acid sequence identity with *Saccharomyces cerevisiae* Yng2p (1), which is reported to be a subunit of the NuA4 complex and functions in the intra-S phase DNA damage response through regulation of H4 acetylation (19). Png1p also has a very close relationship with ScYng2p in the phylogenetic tree assay (1). To investigate whether Png1p also shares similar biological functions with ScYng2, a functional complementation test was performed. As shown in Fig. 2A, SpPNG1 rescued the growth defect of yng2 mutants in budding yeast under both normal and DNA-damaged conditions. ScYNG2 also functionally complemented *png1* mutants in fission yeast (Fig. 2B).

The functional complement between *SpPNG1* and *ScYNG2* prompted us to address whether Png1p is involved in histone acetylation. As shown in Fig. 2C, the presence of 50 \( \mu \text{g/ml} \) trichostatin A, an inhibitor of deacetylation (30), partially complemented the growth defect caused by deletion of Png1 either in normal or CPT- or MMS-induced DNA damage conditions. These results suggest that Png1p is involved in histone acetylation. Furthermore, the effect of Png1p deletion on the acetylation level of histone H4 was analyzed by Western blot. In the \( \Delta \text{png1} \) mutant, the acetylated levels of H4 at K5, K8, or K12 were lower than WT cells (Fig. 2D). However, acetylation of H4 in \( \Delta \text{png2} \) mutants was not detectably different from WT. These results indicate that Png1p plays an important role in the acetylation of histone H4* in vivo.*

**Png1p Is Associated with Histone Acetyltransferase Mst1p in Fission Yeast**—The homolog proteins of histone acetyltransferase (i.e. Mst1p in fission yeast, Esa1p in budding yeast, and Tip60 [KAT5] in human yeast) share 56.56\% amino acid sequence identity (31, 32). These three acetyltransferases also share the conserved MYST histone acetyltransferase structure features (31, 32). To understand whether Mst1p has biological functions similar to Esa1p during DNA damage, a functional complementation test was performed in an *S. cerevisiae eso1-1851* mutant, which is temperature-sensitive and specifically hypersensitive to CPT and MMS, even at a permissive temperature (33). As shown in Fig. 3A, the eso1-1851 mutant grew well at 30 °C but not at 37 °C. However, it grew poorly at 30 °C in the presence of CPT or MMS in the medium. After overexpression of *MST1* in the eso1-1851 mutant, cells grew well at both 30 and 37 °C, even in the presence of the DNA damage reagents CPT and MMS in the medium (Fig. 3A). These results indicate that *SpMST1* shares similar biological functions with *ESA1* in the DNA damage response pathway.

Considering that ING family proteins are always associated with HATs, we next identified the physical and physiological relevance between Png1p and Mst1p in fission yeast. In the co-immunoprecipitation assay, the fission yeast cells overexpressing FLAG-tagged Mst1p and His6-tagged Png1p were

![Figure 2](image_url)
Png1p Functions in Response to DNA Damage

Lyased. The anti-FLAG M2 affinity gel was then added to the cell lysates to immunoprecipitate FLAG-Mst1p. As shown in Fig. 3B, Png1p-His6 was detected in the precipitate by Western blotting using anti-His6 as primary antibody. In the fluorescence localization analysis, Mst1p-RFP protein was exclusively localized in the nucleus, which is consistent with previous reports (31, 32), whereas Png1p-GFP protein was localized primarily in the nucleus (Fig. 3C). When Png1p-GFP and Mst1p-RFP were co-expressed in fission yeast, these two proteins were co-localized predominantly in the nucleus (Fig. 3C). Altogether, Png1p is associated with MYST family histone acetyltransferase Mst1p in fission yeast in biochemical and biological functions.

Png1p Is Required for Expression of Cell Cycle-related Genes—Previous studies demonstrated that hyper- or hypo-acetylation of histone H4 could change the expression levels of specific genes (34). To identify the disparity of expressed genes between Δpng1 and WT genome-wide, DNA microarray was performed. As shown in Table 2, 756 open reading frames were decreased 2-fold or more in Δpng1 cells. Remarkably, some of these down-regulated genes are related to the cell cycle, chromatin remodeling, and DNA repair. Among these down-regulated genes, real time PCR assay further confirmed the expression levels of those in which we were most interested. Consistent with the microarray analysis results, the relative mRNA expression level of the tested genes, including ENGI, AGNI, ASF1, SPT3, SNFI, CDC22, CDC20, and RAD22, were markedly down-regulated (Fig. 4). Accordingly, the decreased acetylation of histone H4 caused by png1 deletion down-regulated the expression of cell cycle- and DNA damage response-related genes.

![Image](50x431 to 407x633)

**FIGURE 3.** Png1p is associated with the fission yeast histone acetyltransferase Mst1p. A, complementation test between ScMST1 and ScESA1 in budding yeast. The budding yeast wild type (QY204), esa1-1851 mutant (SC1002), and esa1-1851/pHR2-ScMST1 (SC1003) cells were spotted separately on the YPD and YPD-containing CPT or MMS. B, co-immunoprecipitation analysis of association between Png1p and Mst1p. Fission yeast sp1010 was co-transformed with Png1p-6His and 3FLAG-Mst1 or Png1p-6His and 3FLAG. Anti-FLAG M2 affinity gel was used for immunoprecipitation of FLAG-Mst1p and anti-His6 antibody for Western blotting. C, co-localization of Png1p-GFP and Mst1p-RFP. Fission yeast sp1010s was transformed with Png1p-GFP and Mst1p-RFP individually or co-transformed with both. 4',6-Diamidino-2-phenylindole (DAPI) was used to stain the nucleus. Scale bar, 10 μm.

**TABLE 2**

Open reading frames down-regulated in Δpng1 mutants

The DNA microarray analysis demonstrated that 756 open reading frames were down-regulated at least 2-fold in Δpng1 cells compared with wild type cells. The important open reading frames are listed in this table. Fold change \(= \frac{|−1|}{2}\) (Signal Log Ratio).

| Signal log ratio | GeneDB name | Gene name | Function |
|------------------|-------------|-----------|----------|
| −4.7             | SPAC821.09  | ENGI      | Endo-1,3-β-glucanase |
| −3.6             | SPAC14A.09  | AGNI      | Endo-1,3-α-glucosidase |
| −3.2             | SPAC823.06  | TAF2      | TFID complex subunit |
| −2.6             | SPAC17H9.19c| CTD2(SEV1)| Cell division cycle protein |
| −2.6             | SPBC828.18  | CDT1      | Cell division cycle protein |
| −2.3             | SPAC12G12.14c| PFS2     | Sister chromatin segregation |
| −2.1             | SPAC3H5.06c | POL1      | DNA polymerase α subunit |
| −2.1             | SPAC27A10.02| CDR2      | DNA polymerase α subunit |
| −2.0             | SPBC20F10.10| PHO85     | GIN4 family protein kinase |
| −1.9             | SPAC1F7.05  | CDC22     | Cyclin |
| −1.9             | SPAC20H2.13c| CDC20(POL2)| Cyclin |
| −1.8             | SPCC645.01  | SIG1      | CDK inhibitor |
| −1.6             | SPAC20G8.05c| CDC15     | Cyclin |
| −1.5             | SPAPB2B4.03 | CIG2      | Mitotic B-type cyclin |
| −1.4             | SPAC29A10.15| CDC18     | Origin recognition complex |
| −1.3             | SPAC14C8.07c| CDC18     | MCM loader |
| −2.2             | SPAC25C4.02 | CRN1      | Actin binding protein |
| −2.5             | SPCC534.03  | CIA1(ASF1)| Histone chaperone |
| −2.3             | SPCC61.02   | SWD3      | M3-K4 methylation |
| −1.9             | SPAC2F7.08c | SNF5      | SWI/SNF complex subunit |
| −1.8             | SPBC428.08c | CLN4     | H3 histone transferase |
| −1.5             | SPAC25H1.02 | JMJ1     | jmjC domain protein |
| −1.5             | SPAC30D11.10| RAD22    | Double-strand break repair |
| −1.4             | SPAC3C7.03c | RHP55     | DNA repair protein |
| −1.4             | SPAC6B12.02c| MUS7     | DNA repair protein |
| −2.1             | SPAC9C5.02c | HOS1     | GTpase Ryh1 |

**Origin recognition complex**

**Double-strand break repair**

**DNA repair protein**

**DNA damage related genes**
Png1p Functions in Response to DNA Damage

These down-regulated genes may underlie the cell cycle delay and the DNA damage sensitivity of Δpng1 cells.

**Png1p Functions in DNA Damage Response through the Rad22 Pathway**—We found that decreased histone H4 acetylation caused by png1 deletion decreased the expression of serial genes, including the central DNA repair gene RAD22 (Table 2 and Fig. 4). We then sought to determine whether Rad22 is involved in the same signal pathway with PNG1 in fission yeast. If so, overexpression of RAD22 in Δpng1 mutants could rescue the phenotype defect. The coding region of RAD22 was cloned to the fission yeast expression plasmid pJR2–41U and transformed to Δpng1. As shown in Fig. 5A, overexpression of RAD22 in Δpng1 partially rescued the growth defect of the Δpng1 mutant in the presence of CPT or MMS in the medium.

In the ChIP assay, H4 acetylation at K5, K8, and K12 at the RAD22 promoter region in Δpng1 was further found to be decreased compared with WT (Fig. 5B). Acetylated H4 at the CDC22 promoter region, which was markedly down-regulated in Δpng1, was also decreased, whereas that of the internal control HCS1 did not change (Fig. 5B). In contrast, acetyltransferase Mst1, which is associated with Png1 in fission yeast, was also found to be recruited at the RAD22 promoter (Fig. 5C). These results suggest that the lower expression of RAD22 in Δpng1 cells was attributable to decreased H4 acetylation at its promoter, indicating that Png1p might facilitate Mst1 to acetylate H4 at the Rad22 promoter to regulate Rad22 expression. This conclusion was further confirmed by real time PCR, which indicated overexpression of Mst1 in WT cells and Δpng1 cells. As shown in Fig. 5D, overexpression of Mst1 increased the expression of RAD22 in fission yeast WT cells and rescued the down-regulation of RAD22 in Δpng1 cells. Thus, Png1p may directly regulate Rad22 expression. This conclusion was further confirmed by real time PCR, which indicated overexpression of Mst1 in WT cells and Δpng1 cells. As shown in Fig. 5D, overexpression of Mst1 increased the expression of RAD22 in fission yeast WT cells and rescued the down-regulation of RAD22 in Δpng1 cells. Thus, Png1p may directly regulate Rad22 expression.

**DISCUSSION**

ING family members are conserved proteins in different types of eukaryotic cells (1, 8). In the present study, we found that the phenotype of the fission yeast Δpng1 was similar to that of the budding yeast Δyn2, but their molecular mechanisms were not completely the same. In the phenotype study, we found that Png1p is essential for cell growth under normal and DNA-damaged conditions (Fig. 1), which parallels Yng2p
Png1p Functions in Response to DNA Damage

in budding yeast (19). The molecular mechanism study showed that Png1p collaborates with the histone acetyltransferase Mst1p/KAT5 and is essential for global histone H4 acetylation in vitro and in vivo, which is similar to the functions of Yng2p in budding yeast (19, 35). Additionally, PNG1 and YNG2 are functional homologs in the DNA damage response pathway, reflected by the results of the complementation assay (Fig. 2, A and B). However, we also found differences between the molecular mechanisms of Png1p and Yng2p. Contrary to a previous report showing that Yng2p localizes in the nucleus (35), we found that Png1p did not function exclusively in the nucleus (Fig. 2B). The different localizations of Png1 and Yng2 suggest differences between their physiological functions. Png1p might function not only in the nucleus but also in the cytoplasm. Furthermore, the analysis of genome-wide gene expression using a DNA microarray showed that 756 open reading frames are down-regulated at least 2-fold in Δpng1 mutants (Table 2). These down-regulated genes contain the cell division-related genes ENS1 and AGNI1 (36, 37), cell cycle-related genes CDC22 and CDC20 (38, 39), chromosome remodeling-related genes ASFI (40) and SNFS1 (41), and DNA damage repair-related gene RAD22 (Table 2 and Fig. 4). These results are opposite to the report of Choy et al. (35), which did not find gene expression changes between budding yeast WT and Δyng2 mutant.

The DNA recombination protein Rad22 is well known to play an important role in promoting S phase completion and acts as the central protein in repair of DNA double-strand breaks (42). Rad22 can directly bind to the end of linear DNA and is essential for the formation of the DNA repair complex (42, 43). Although the functions of Rad22 have been identified in budding yeast and mammalian cells, but the complex of Mst1p in fission yeast has not. The actin-related protein Alp5 is a component of the Mst1p HAT complex (45). In the present study, associations between Png1p and Mst1p in vitro and in vivo suggest that Png1p is also a component of the Mst1p complex (Fig. 3, B and C). Identifying the Mst1p-formed HAT complex that is responsible for histone acetylation, similar to the NuA4 complex in budding yeast and humans, will be interesting. Further functional research regarding Mst1p and its complex will provide deeper insights into how this conserved protein affects chromatin remodeling and subsequent gene expression.

In summary, we used Schizosaccharomyces pombe to investigate the role of Png1, one member of the ING family. Png1p is required for cell growth under normal and DNA-damaged conditions. Png1 was shown to be associated with acetyltransferase Mst1 by co-immunoprecipitation and fluorescence localization and to be a functional ortholog of S. cerevisiae Yng2p (a component of the NuA4 HAT complex) by complementation testing. Deletion of Png1 led to reduced acetylation of histone H4 and down-regulation of 756 genes containing Rad22, which are critical for double-strand break repair. Overexpression of Rad22 partially suppressed the growth defects and DNA damage sensitivity caused by Δpng1 deletion. ChIP assays further demonstrated that H4 acetylation at the Rad22 promoter is lacking or drastically reduced in Δpng1 cells, and Mst1 is localized to the Rad22 promoter. These results suggest that Png1p facilitates Mst1p to acetylate H4 at the Rad22 promoter and regulates the expression of Rad22. In summary, Png1p is involved in H4 acetylation and acts upstream of Rad22.

Acknowledgements—We thank Prof. Janet Leatherwood (Stony Brook University) for kindly providing the CHP428 fission yeast WT strain and the fission yeast expression vectors pR2-411L and pREPI. We also thank Prof. Stephen J. Kron (University of Chicago) for kindly providing the Scyng2 mutant and Prof. Jacques Cote (Laval University) for kindly providing the QY203 and QY204 strains. We thank Prof. Michael F. Christman (Boston University) for kindly providing the esa1-1851 strain.

REFERENCES

1. He, G. H., Helbing, C. C., Wagner, M. J., Sensen, C. W., and Riabowol, K. (2005) Mol. Biol. Evol. 22, 104–116
2. Jones, P. A., and Baylin, S. B. (2007) Cell 128, 683–692
3. Zharkov, D. O. (2008) Cell. Mol. Life Sci. 65, 1544–1565
4. Jenuwein, T., and Allis, C. D. (2001) Science 293, 1074–1080
5. Saksouk, N., Avvakumov, N., and Côté, J. (2008) Cell. Mol. Life Sci. 65, 1013–1018
6. Struhl, K. (1998) Genes Dev. 12, 599–606
7. Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, H., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Cell 102, 463–473
8. Shi, X., and Gozani, O. (2005) J. Cell. Biochem. 96, 1127–1136
9. Doyon, Y., Cayrou, C., Ullah, M., Landry, A. J., Côté, V., Selleck, W., Lane, W. S., Tan, S., Yang, X. J., and Côté, J. (2006) Mol. Cell 21, 51–64
10. Nourani, A., Doyon, Y., Uley, R. T., Allard, S., Lane, W. S., and Côté, J. (2001) Mol. Cell. Biol. 21, 7629–7640
11. Nourani, A., Howe, L., Pray-Grant, M. G., Workman, J. L., Grant, P. A., and Côté, J. (2003) J. Biol. Chem. 278, 19171–19175
12. Howe, L., Kusch, T., Muster, N., Chaterji, R., Yates, J. R., 3rd, and Workman, J. L. (2002) Mol. Cell. Biol. 22, 5047–5053
13. Russell, M., Berardi, P., Gong, W., and Riabowol, K. (2006) Exp. Cell Res. 312, 951–961
14. Wysocka, J., Swigut, T., Xiao, H., Milne, T. A., Kwon, S. Y., Landry, J.,
Kauer, M., Tackett, A. J., Chait, B. T., Badenhorst, P., Wu, C., and Allis, C. D. (2006) Nature 442, 86–90
15. Li, H., Ilin, S., Wang, W., Duncan, E. M., Wysocka, J., Allis, C. D., and Patel, D. I. (2006) Nature 442, 91–95
16. Shi, X., Hong, T., Walter, K. L., Ewalt, M., Michishita, E., Hung, T., Carney, D., Peña, P., Lan, F., Kaadige, M. R., Lacoste, N., Cayrou, C., Davrazou, F., Saha, A., Cairns, B. R., Ayer, D. E., Kutateladze, T. G., Shi, Y., Côté, J., Chua, K. F., and Gozani, O. (2006) Nature 442, 96–99
17. Peña, P. V., Davrazou, F., Shi, X., Walter, K. L., Verkhusha, V. V., Gozani, O., Zhao, R., and Kutateladze, T. G. (2006) Nature 442, 100–103
18. Nicolas, E., Yamada, T., Cam, H. P., Fitzgerald, P. C., Kobayashi, R., and Grewal, S. I. (2007) Nat. Struct. Mol. Biol. 14, 372–380
19. Choy, J. S., and Kron, S. J. (2002) Mol. Cell. Biol. 22, 8215–8225
20. Gregan, J., Rabitsch, P. K., Rumpf, C., Novatchkova, M., Schleiffer, A., and Nasmyth, K. (2006) Nat. Protoc. 1, 2457–2464
21. Kumar, S., and Huberman, J. A. (2004) J. Biol. Chem. 279, 43574–43580
22. Ostrin, M. L., Belenguer, P., Leroy, D., Hoffmann, I., and Ducournou, B. (1995) Biochimie 77, 279–287
23. Chlebowicz, E., and Jachymczyk, W. J. (1979) Mol. Gen. Genet. 167, 279–286
24. Verdin, E., Dequiedt, F., and Kasler, H. G. (2003) Trends Genet. 19, 286–293
25. Go´mez, E. B., Nugent, R. L., Laria, S., and Forsburg, S. L. (2008) Genetics 179, 757–771
26. Go´mez, E. B., Espinosa, J. M., and Forsburg, S. L. (2005) Mol. Cell. Biol. 25, 8887–8903
27. Bird, A. W., Yu, D. Y., Pray-Grant, M. G., Qiu, Q., Harmon, K. E., Megee, P. C., Grant, P. A., Smith, M. M., and Christman, M. F. (2002) Nature 419, 411–415
28. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Cell 84, 843–851
29. Choy, J. S., Tobe, B. T., Huh, J. H., and Kron, S. J. (2001) J. Biol. Chem. 276, 43653–43662
30. Martin-Cuadrado, A. B., Dueñas, E., Sipiczki, M., Vázquezde Aldana, C. R., and del Rey, F. (2003) J. Cell Biol. 166, 1689–1698
31. Dekker, N., Speijer, D., Grün, C. H., van den Berg, M., de Haan, A., and Hochstenbach, F. (2004) Mol. Biol. Cell 15, 3903–3914
32. D’Urso, G., and Nurse, P. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 12491–12496
33. Nasmyth, K., and Nurse, P. (1981) Mol. Gen. Genet. 182, 119–124
34. Ostrin, M. L., Osman, F., Dixon, J., and Whitby, M. C. (2004) Nucleic Acids Res. 32, 5570–5581
35. Minoda, A., Saitoh, S., Takahashi, K., and Toda, T. (2005) Mol. Biol. Cell 16, 316–327