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

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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 H4 acetylation through collaboration with the MYST family histone acetylase complexes. However, the biological functions of ING proteins in fission yeast are poorly understood. 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 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 DNA damage response process.

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

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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.

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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, down-regulation 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, respectively.

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% (v/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 mM 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 10⁷ 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 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 × 10⁶ cells were measured by a FACS Calibur 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 pJR2–41U-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 Zymolase (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 database.

Chromatin Immunoprecipitation—ChIP analysis experiments were performed as previously described (22). Briefly, sol-
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A

![Graph showing growth rate of wild type, Δpng1, and Δpng2 strains in YES medium.](A)

B

WT

Δpng1

Δpng2

YES

YES+1μM CPT

YES+0.005% MMS

C

WT

Δpng1

Δpng2

YES

YES+1μM CPT

YES+0.005% MMS

 FIGURE 1. Png1p is required for cell growth under normal and DNA damage-induced conditions. A, the growth rate of wild type (CHP428), Δpng1 (SP1001), and Δpng2 (SP1002) strains in YES medium. A_{600} values at the indicated time were measured. B, serial dilution assay. Wild type, Δpng1, and Δpng2 cells grown to logarithm phase were serially diluted and spotted separately on YES and YES-containing 1 μM CPT or 0.005% (w/v) MMS and incubated at 32 °C for 3–4 days. C, cell cycle analysis by flow cytometry. Wild type, Δpng1, and Δpng2 cells were grown to logarithm phase in the YES and YES-containing 1 μM CPT or 0.005% (w/v) MMS liquid medium at 32 °C and were used for the cell cycle analysis by flow cytometry. All of the experiments were performed in triplicate.

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. 

RESULTS

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VOLUME 285 • NUMBER 21 • MAY 21, 2010

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.

following primers: Rad22 (5’-AAGAGGCATTTTACAC-3’ and 5’-TCCATTTTCATATTATTTAT-3’), CDC22 (5’-CGGACTTATTCTAC-TTGTAAAGTGG-3’), and HCS1 (5’-CTTAATTTCAAACTTTGCTGAGTC-3’ and 5’-TCTTGTAGTTTCAAAAGTGGG-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.

Png1p Is Required for Cell Growth under Normal and DNA Damage-induced Conditions—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 diluted 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,
Furthermore, the sensitivity of Δpng1 or Δpng2 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 Δpng1 mutants were supersensitive to both 1 μM CPT and 0.005% (w/v) MMS, whereas the growth of Δpng2 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 μM CPT for 2 h, Δpng1 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 respond 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 ScYng2 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 μg/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 Png1 deletion on the acetylation level of histone H4 was analyzed by Western blot. In the Δpng1 mutant, the acetylated levels of H4 at K5, K8, or K12 were lower than WT cells (Fig. 2D). However, acetylation of H4 in Δpng2 mutants was not detectably different from WT. These results suggest that Png1p is involved in histone acetylation.

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 esa1-1851 mutant, which is temperature-sensitive and specifically hypersensitive to CPT and MMS, even at a permissive temperature (33). As shown in Fig. 3A, the esa1-1851 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 esa1-1851 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. Png1p is required for histone H4 acetylation.** A, complementation test in budding yeast. Wild type budding yeast cells (QY204), Δpng2 cells (QY203), and Δpng2/pHR2-SpPNG1 cells (SC1001) were serially diluted and spotted separately on YPD or YPD-containing 10 μg/ml CPT or 0.03% (w/v) MMS and then incubated at 30 °C for 3–4 days. B, complementation test in fission yeast. Fission yeast wild type and Δpng1 cells and Δpng1/pREP1-ScYNG2 (SP1004) cells were serially diluted and spotted separately on EMM or EMM-containing 1 μM CPT or 0.005% (w/v) MMS and then incubated at 32 °C for 3–4 days. C, effect of trichostatin A on the growth of Δpng1 cells under normal and DNA-damaged conditions. Fission yeast wild type cells and Δpng1 cells were diluted and spotted separately on YES or YES-containing DNA damage reagents in the presence or absence of trichostatin A and then incubated at 32 °C for 3–4 days. D, analysis of histone H4 acetylation level at K5, K8, and K12. Histone proteins isolated by sulfuric acid extraction from fission yeast wild type cells and Δpng1 cells and Δpng2 cells were separately supplied for Western blotting using anti-acetyl H4K5, anti-acetyl H4K8, and anti-acetyl H4K12 antibodies. Histone H4 was used as a loading control.
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lysed. 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, AGN1, ASF1, SPT3, SNF5, 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.

### TABLE 2

Open reading frames down-regulated in Δpng1 mutants

<table>
<thead>
<tr>
<th>Signal log ratio</th>
<th>GenDB name</th>
<th>Gene name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>−4.7</td>
<td>SPAC821.09</td>
<td>ENGI</td>
<td>Endo-1,3-β-glucanase</td>
</tr>
<tr>
<td>−3.6</td>
<td>SPAC14A.09</td>
<td>AGN1</td>
<td>Endo-1,3-α-glucosidase</td>
</tr>
<tr>
<td>−3.2</td>
<td>SPAC823.06</td>
<td>TAF3</td>
<td>TFIID complex subunit</td>
</tr>
<tr>
<td>−2.6</td>
<td>SPAC17H9.19c</td>
<td>CTD2(SEV1)</td>
<td>Cell division cycle protein</td>
</tr>
<tr>
<td>−2.6</td>
<td>SPBC628.18</td>
<td>CTD1</td>
<td>Cell division cycle protein</td>
</tr>
<tr>
<td>−2.3</td>
<td>SPAC12G12.14c</td>
<td>PBS2</td>
<td>Sister chromatin segregation</td>
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<td>DNA polymerase α subunit</td>
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<td>CDC22</td>
<td>DNA replication</td>
</tr>
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<td>CDC20(POL2)</td>
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</tr>
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<td></td>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>

FIGURE 3. Png1p is associated with the fission yeast histone acetyltransferase Mst1p. A, complementation test between SpMST1 and ScESA1 in budding yeast. The budding yeast wild type (QY204), esa1-1851 mutant (SC1002), and esa1-1851/pha2-SpMST1 (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 SP1005 was 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 SP1005 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.
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 rescued the down-regulation of RAD22 in Δpng1 cells. Thus, Png1p may directly regulate the expression of Rad22, indicating that Rad22 functions downstream of Png1p.

**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 Δyng2, 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, PING1 and YNG2 are functional homolog genes 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 ENGI and AGNI (36, 37), cell cycle–related genes CDC22 and CDC20 (38, 39), chromosome remodeling–related genes ASFI (40) and SNF5 (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 breaks (42). Rad22 can directly bind to the end of linear DNA damage sensitivity of ASF1 and CDC20 (41), and DNA damage repair-related gene SNF5 (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 duplex DNA to protect DNA from exonuclease digestion *in vitro* and is essential for the formation of the DNA repair complex (42, 43). Although the functions of Rad22 have been thoroughly studied, the mechanisms underlying regulation of Rad22 expression are less well known. In the present study, we showed that Δpng1 cells exhibited extreme sensitivity to DNA damage and intra–S phase cell cycle arrest (Fig. 1), similar to rad22 deletion cells (44). Overexpression of Rad22 provides partial suppression of the growth defect and DNA damage sensitivity of png1 deletion (Fig. 5A). We also found that expression of Rad22 is positively correlated to Png1–involved histone H4 acetylation at the Rad22 promoter (Fig. 5, B and D). These results demonstrate that both Png1p and Rad22 are responsible for DNA damage in the same pathway through intra–S phase cell cycle arrest. Png1p facilitated Mst1 to acetylate H4 at the Rad22 promoter and regulated Rad22 expression. Thus, Png1p is involved in H4 acetylation and acts upstream of Rad22. Considering that Rad22 and ING orthologs are conserved from yeast to human (43), upstream and downstream relationships between Png1 and Rad22 in the fission yeast system suggest that this relationship also probably exists in other organisms, especially in human cells and budding yeast. However, this hypothesis needs to be explored further in different eukaryotic cells.

In the present study, we also found that fission yeast Mst1p can rescue budding yeast esa1-1851ts mutants, suggesting that Mst1p may function in histone acetylation, similar to Esa1 in budding yeast. The NuA4 complexes of ScEsa1 and hTip60 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 budding yeast *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. Png1p was shown to be associated with acetyltransferase Mst1p 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 Png1p 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 png1 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 Mst1 to acetylate H4 at the Rad22 promoter and regulates the expression of Rad22. In summary, Png1 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 pR2-41U and pREP1. 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.

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