Opposite Role of Yeast ING Family Members in p53-dependent Transcriptional Activation*

Received for publication, January 24, 2003, and in revised form, April 2, 2003
Published, JBC Papers in Press, April 2, 2003, DOI 10.1074/jbc.C300036200

Amine Nourani‡‡, LeAnn Howe¶¶, Marilyn G. Pray-Grant¶, Jerry L. Workman**, and Jacques Côté‡§§

From the 3Laval University Cancer Research Center, Hôtel-Dieu de Québec (CHUQ), Quebec City, Quebec G1R 2J6, Canada, the 4Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802-4300, and the 5Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, Virginia 22908

The inhibitor-of-growth (ING) family of proteins was founded by human ING1, a tumor suppressor interacting with p53 in vivo and required for its function in transcription/apoptosis. There are five different ING genes in humans, three of which have been linked to p53 function. In this study, we analyzed the three ING family members present in yeast. We demonstrate that each one is purified as a key component of a specific histone-modifying complex. Pho23 is part of Rpd3/Sin3 histone deacetylase complex, while Yng1 and Yng2 are subunits of the NuA3 and NuA4 histone acetyltransferase complexes, respectively. We also show that the three different ING proteins have opposite roles in transcriptional activation by p53 in vivo. These effects are linked to the presence of each ING in its respective chromatin modifying complex, since mutation of the corresponding catalytic subunit gave similar results. Depletion of Pho23/Rpd3 leads to increased p53-dependent transcription in vivo while depletion of Yng2 abrogates it. Surprisingly, deletion of YNG1 or SAS3 leads to increased transcriptional activation by p53. These data suggest that the NuA3 complex can function in gene-specific repression, an unusual role for a histone acetyltransferase complex. They also demonstrate the key specific role of ING proteins in different chromatin modifying complexes and their opposite functions in p53-dependent transcription.

ING1 (inhibitor of growth) gene family encodes a group of homologous nuclear proteins conserved during evolution and involved in the modulation of various important cellular functions. ING1, the founding member of this family, was initially discovered as candidate tumor suppressor (1). Since then, there were several lines of evidence confirming ING1 as a human tumor suppressor that plays a variety of important roles in the cell (reviewed in (2)). All the family members have significant homology over their entire sequence and share a highly conserved PHD zinc finger domain at their C terminus.

In humans there are five ING genes, named ING1 to ING5 (2). The structure and function of the ING1 gene has been the most thoroughly studied. Overexpression of ING1 hinders cell growth, while an antisense construct activates cell transformation and dramatically increases the replicative life of normal fibroblasts suggesting a role of this protein in the cell senescence. Elevated expression of ING1 and ING4 also promote apoptosis. Expression and mutational analyses in several tumor types show that ING1 is down-regulated in cancers, which is also the case for ING3 (reviewed in Ref. 2).

ING proteins seem to collaborate with the p53 tumor suppressor protein, which is involved in various anti-tumor processes including cell-cycle regulation, apoptosis, and senescence. Interestingly, ING1-mediated growth inhibition requires a functional p53 and results in p53-dependent transcriptional activation of the p21/WAF1 gene, a cyclin-dependent kinase inhibitor (3). In vivo, ING1 and p53 co-immunoprecipitate from cell extracts, suggesting a direct functional interaction (3). In addition, functional interaction was also found between ING2, ING3, and p53 as both enhance the transactivation function of p53 (2).

Although the human ING proteins seem to have important biological functions, the exact mechanism by which they modulate p53 activity and how they affect various cellular processes are not known. Part of the answer comes from studies on the yeast homologs. Indeed, Yng1 and Yng2, two of the three yeast ING proteins, are subunits of the NuA3 and NuA4 histone acetyltransferase (HAT) complexes, respectively (4–6). Pho23, the third yeast ING protein, was shown to interact with the Rpd3/Sin3 histone deacetylase (HDAC) complex (7).

Interestingly, human ING1 is able to suppress yng2 mutation in yeast, suggesting a functional conservation between yeast and human ING proteins (4). The fact that all yeast ING proteins are physically associated to HAT and potentially HDAC complexes suggests that other ING proteins could be linked to such activities. Indeed, ING1 is a stable component of a mSin3/HDAC1 complex and was suggested to interact with various HATs such as PCAF, CBP, and p300 (8, 9). These observations are consistent with a mechanism whereby ING proteins cooperate with p53 by modulating chromatin structure. Interestingly, in addition to the functional conservation between ING1 and Yng2, transcriptional activation of a p21 reporter gene by p53 functions in yeast cells and requires Yng2 (5). This transactivation function is mediated by direct in vivo
interaction between the NuA4 complex and p53, which targets localized histone H4 hyperacetylation (5).

These observations clearly demonstrate that the p53/ING molecular relationship is evolutionary conserved and could explain the biological role of ING proteins. To further understand the mechanism whereby ING proteins modulate different cellular processes, this functional interaction was investigated. Given the conservation between yeast and human INGs, we decided to study the functional relationship between transcription regulation by p53 and all 3 yeast ING paralogs. We show, using tandem affinity purification (TAP) system, that cellular Yng1 and Yng2 are found exclusively in the NuA3 and NuA4 HAT complexes, respectively. We also demonstrate that Pho23 is a stable subunit of the Rpd3/Sin3 HDAC complex. To further elucidate the functional connections between p53 and yINGs, we used a yeast-based system where transcription of a p21/WAF1-HIS3 reporter gene is fully dependent on exogenously expressed p53. Our results demonstrate that yeast ING proteins play different roles in p53-dependent transcription. These roles are directly linked to the type of histone modifying activities they are associated with.

**MATERIALS AND METHODS**

Yeast Strains and Plasmids—The strains expressing Yng1-TAP, Yng2-TAP, and Pho23-TAP were constructed as described previously (10). The strains QY203 and QY204 were described in Ref. 5. To generate pho23 (QY212) and rpd3 (QY213) strains, deletion of PHO23 and RPD3 was done in the strain QY204 by PCR targeting using KanMX4 cassette as selecting marker. The yng1 (YLH505), and sas3 (YJW125) deletion strains were prepared in the FY602 background (Mat a his3Δ1 leu2Δ1 lys2Δ1286 ura3Δ52 trp1Δ63) using HISMX6 as marker. The wild type and PHO23 deleted strains used for conventional purification of Rpd3 complexes were obtained from Regen. The plasmids used for p53-driven transcription assay were described previously (5).

Protein Complex Purifications, Deacetylase Assay, and Northern and Western Blot Analyses—Purification of TAP-tagged proteins was performed as described previously (10). The Rpd3/Sin3 complexes used in the deacetylase assays were purified by successive nickel-agarose, Mono Q, and Superose 6 chromatography as described previously (11). Histone deacetylase activity was measured on NuA4-modified free histones and oligonucleosomes as described previously (12). Northern blots were performed as described previously (5). For Western blots, 5 μl of cell extract (corresponding to 0.5 OD600 of cells) were loaded on SDSPAGE. Monoclonal anti-p53 antibody (pAb 421) was used at 1:1000 concentration.

**RESULTS AND DISCUSSION**

The Yeast ING Proteins Are Found Exclusively in HAT and HDAC Multisubunit Complexes—To confirm the stable association of yeast ING proteins with multisubunit complexes and also to know if each of these proteins belongs to a unique complex, Yng1, Yng2, and Pho23 were TAP-tagged at their chromosomal loci and purified. Following the two-step affinity purification, material was analyzed on silver-stained gel (Fig. 1). We have demonstrated that Yng2 is a stable subunit of the purified NuA4 complex and that it plays primary role in NuA4 complex activity toward chromatin (5).2 Here, with TAP purification from yeast strain expressing physiological levels of Yng2-TAP fusion protein, we observe that Yng2 is clearly associated with NuA4 (Fig. 1A, lane 2). Indeed, based on gel migration, Western and mass spectrometry analysis, all known NuA4 subunits are present in the purified material (data not shown). The three remaining bands (marked by asterisks) are known contaminants that are also obtained from an untagged strain (not shown). We did not observe additional specific bands that might correspond to other Yng2-containing complex. Based on these data, and since Yng2 does not appear more abundant than the other proteins in the purified material, we can conclude that Yng2 is found exclusively associated with NuA4 in the cell.

We have recently found that Yng1 is associated with the NuA3 HAT complex and that it is required for efficient acetylation of chromatin by the complex (6). Importantly, we show here that affinity purification of Yng1-TAP also brings protein bands corresponding to Sas3 and Anc1/Taf30, two previously known subunits of the NuA3 complex (Fig. 1A, lane 1). Again this confirms our previous finding demonstrating Yng1 as a stable component of NuA3 (6). The other bands detected also correspond in size to protein bands previously found in purified NuA3. Since Yng1 is not more abundant than any other band on the gel, this suggests that most if not all Yng1 is associated with NuA3 in the cell.

Pho23 was also linked to chromatin modification. When overexpressed in vivo Pho23 was previously shown to co-immunoprecipitate with Rpd3 and Sap30, two subunits of the Rpd3/Sin3 HDAC complex (7). Deletion of PHO23 was also shown to create phenotypes similar to rpd3 and sin3. Pho23 affinity purification produced several specific protein bands of similar intensity (Fig. 1A, lane 3). Based on gel migration and Western analysis, several of these bands correspond to known components of the Rpd3/Sin3 complex. Since Pho23 is not more abundant than the other bands, this indicates that most if not all Pho23 protein is a stable subunit of the Rpd3/Sin3 complex in the cell. In agreement with this, Pho23 was found associated with Sin3-TAP and Rpd3-TAP (data not shown). In addition, we tested the effect of PHO23 deletion on conventionally purified Rpd3/Sin3 complex deacetylase activity (Fig. 1B). We measured histone deacetylase activity on 3H-labeled chromatin substrate or free histones using Rpd3/Sin3 complexes purified from wild type and pho23 mutant strains. When the assays were normalized for the levels of HDAC activity on free histones, we observed a significant decrease in nucleosomal HDAC activity in the pho23 mutant (Fig. 1B). Our results indicate a crucial role for Pho23 in Rpd3/Sin3 HDAC activity on chromatin substrate, similar to what was found for Yng1 and Yng2 in NuA3 and NuA4 HAT activities (5, 6).2

required for transactivation of a p21-reporter plasmid. In this experiment, we analyzed where p53 is constitutively expressed in cells bearing a p21 reporter gene. As we have shown previously, deletion of strain expression of p53 is required to activate transcription of strain in comparison for this experiment. In the wild type, which were assigned a value of 1 arbitrary unit. Northern blot shows that deletion of transcription levels. C, the expression signals from B were quantified by a phosphorimager. The histogram represents the PHO5/ACT1 (black columns) and p21-HIS3/ACT1 (shaded columns) ratios relative to wild type, which were assigned a value of 1 arbitrary unit.

Yng2 and Pho23 Have Opposite Effect on p53-dependent Transcription in Yeast—Since tumor suppressor p53 is known to function as a transcriptional activator in yeast and human ING proteins have been linked to p53 function, it is interesting to study yeast ING protein requirement for p53-dependent transcription in vivo. We have already shown that Yng2 is required for transcription of a p21/WAF1-HIS3 hybrid reporter gene by p53 and for targeted histone H4 hyperacetylation (5). Since Pho23 is a subunit of the Rpd3/Sin3 HDAC complex, we wanted to know if this factor could also play a role in p53 transcription potential. We used the same yeast system where p53 is constitutively expressed in cells bearing a p21/WAF1-HIS3 reporter plasmid. In this experiment, we analyzed by Northern blot p53-dependent transcription in wild type and pho23-deleted strains (Fig. 2A). We also used an isogenic yng2 strain in comparison for this experiment. In the wild type strain expression of p53 is required to activate transcription of the reporter gene. As we have shown previously, deletion of YNG2 cripples p53-dependent transactivation (Fig. 2A, compare lanes 2 and 4). In contrast, deletion of PHO23 has the opposite effect. Transactivation of p21-HIS3 by p53 is more potent in pho23 cells when compared with wild type (lanes 2 and 6). To elucidate the mechanism by which Pho23 inhibits p53-dependent transcription, we repeated the experiment in an isogenic strain mutated for Rpd3, the catalytic subunit of the HDAC complex containing Pho23. As shown in Fig. 2B, we observe the same increased p53-dependent transactivation of p21-HIS3 in both pho23 and rpd3 strains as compared with wild type strain. Importantly, the activation of the reporter gene by pho23 and rpd3 deletion is equal (Fig. 2C), suggesting that Pho23 role is directly mediated by Rpd3 histone deacetylase activity.

Interestingly, the PHO23 gene was first isolated as a repressor of PHO5 transcription (13). It is also known that Rpd3 has an inhibitory role on PHO5 in rich media (14). These observations are confirmed in our experiment since we observe an increase of PHO5 mRNA levels in both pho23 and rpd3 mutants (Fig. 2, B and C). In contrast to the p21-HIS3 signal, this increase is p53-independent. It is known that p33 mutation affects the histone H3 and H4 acetylation state at the PHO5 promoter, leading to derepression of the gene (14). In the case of p21-HIS3, it is possible that rpd3 and pho23 mutations lead to an increase in H3 and H4 acetylation over the promoter region, provoking an elevated transcriptional activation. Interestingly, we have previously shown that p53 recruits the yeast NuA4 HAT activity and targets histone H4 hyperacetylation to the p21-HIS3 promoter region, a process dependent on the presence of Yng2 (5). Altogether, these observations support a model in which p53 regulates transcription of its target genes in mammalian cells by modifying their chromatin structure through recruitment of ING-containing complexes. Accordingly, it was recently demonstrated that p53 modulates Mdm2 gene transcription by recruiting TRRAP-containing complexes that modify the acetylation status of histone H3 and H4 at that promoter (15). It should be noted that TRRAP is the homologue of yeast Tra1, a subunit of the NuA4 and SAGA HAT complexes (16).

Yng1 Inhibits p53-dependent Transcription—Yng1, the third and last member of yeast ING family, is a subunit of the NuA3 HAT complex. Purified NuA3 complex fails to interact with acidic activators in vitro but can stimulate transcription from pre-assembled chromatin templates (17). Sus3, the catalytic subunit, has not been shown to be involved in transcription of specific genes in vivo. A hint to a role in transcription is provided by the fact that NuA3 interacts with Spt16, a subunit of the yeast CP/FACI complex, supporting a role in transcription elongation (18). Testing Yng1 function in p53-dependent transcription could directly implicate NuA3 in transcription regulation in vivo. In Fig. 3A we analyzed by Northern blot the transcription of p21-HIS3 reporter gene in wild type, yng2, pho23, and yng1 strains, in the presence or the absence of p53. As shown before, Yng2 is required for p21-HIS3 transcription while Pho23 inhibits it, in agreement with the general acetylation/deacetylation model of gene regulation. Surprisingly, deletion of YNG1 results in increased p53-dependent level of p21-HIS3 mRNA when compared with the isogenic wild type strain (compare lanes 8 and 10). This increase in yng1 cells is almost identical to the one observed in pho23 cells, indicating a similar level of inhibition exerted by these two ING proteins on p53-dependent transcription. As shown before, PHO5 transcription requires Yng2/NuA4 and is repressed by Pho23/Rpd3. Importantly, YNG1 deletion has no effect on PHO5 transcription. Therefore, this observation suggests a specific role of Yng1 on p53 function in transcription.

Our results clearly indicate that two of the three yeast ING proteins, Pho23 and Yng1, play negative roles on transcrip-
Opposite Roles of ING Proteins in p53-driven Transcription

Inhibition of p53-dependent Transcription by NuA3 HAT Subunit—It is intriguing that Yng1 specifically inhibits p53-dependent transcription while it is a subunit of the NuA3 HAT complex, a type of activity generally linked to transcription activation. Moreover, Yng1 and Sas3, the catalytic subunit of NuA3, antagonize silencing at mutated HMR loci, suggesting a positive role in transcription (6). Therefore, we asked whether the inhibitory role of Yng1 on p53-dependent transcription is linked to NuA3 HAT activity. To address this question we analyzed the effect of Sas3 deletion on p53 transactivation function. Wild type, yng1, and sas3 strains expressing or not p53 were analyzed for transcription of the p21-HIS3 reporter gene (Fig. 3D). In the presence of equal amounts of p53, deletion of YNG1 or SAS3 results in an increase of p21-HIS3 mRNA compared with wild type. This result indicates a requirement for NuA3 HAT activity in inhibition of p53-dependent transcription. Importantly, it is the first evidence suggesting an inhibitory role of the NuA3 complex in transcription. The question whether this effect is direct remains to be answered although the presence of an ING protein in NuA3 argues for a direct role in p53 function. On the other hand, in contrast to the NuA4 complex, no direct interaction between NuA3 and recombinant p53 could be detected in vitro (6). However, this result must be interpreted with some caution since p53 can be modified in vivo, a process that regulates interactions with partners, including HAT complexes (19). Additionally, we could not detect acetylation of p53 by NuA3 in vitro, which is similar to results found with NuA4 (data not shown). Bulk histone H3 acetylation is controlled in yeast by Gcn5, the catalytic subunit of SAGA complex, and Sas3/NuA3 (20). We have shown that transcription activation of the p21-HIS3 gene by p53 correlates with targeted histone H4 hyperacetylation (5). On the other hand we did not detect any change in the level of histone H3 acetylation over the promoter region. It is possible that histone H3 acetylation by NuA3 on the p21-HIS3 gene is detrimental to p53-dependent transcription. On the other hand the other major H3 HAT in yeast, Gen5, is required for efficient p53-dependent transcription in yeast and interaction between p53 and SAGA was found in vitro.3 One hypothesis is that the role of Gen5 in p53-dependent transcription is through direct acetylation of the p53 protein, as was found for the human homologs, hGcn5 and p/CAF (21). We cannot rule out the possibility that NuA3 modifies directly or indirectly the expression/activity of a p53 co-activator/co-repressor and therefore modulate p21-HIS3 gene expression. While HAT activity involvement in gene repression is unusual, it was recently reported that the Hos2 deacetylase is directly required for gene activation (22). This important observation strongly suggests that histone acetylation and gene activation do not always correlate and supports the possibility of NuA3 being directly involved in gene repression.

We have demonstrated that yeast ING proteins are exclusively found in chromatin modifying complexes. The structural and functional conservation of ING proteins throughout evolution allows us to study the properties and functions of yeast INGs and extend our findings about the mechanisms thereby they affect a number of cellular processes. For example human ING1 is able to complement YNG2 deletion in yeast (4). A number of mammalian INGs cooperate with p53 and are involved in control of its transcriptional activities. We show that in yeast p53 also functionally interacts with the three INGs. While NuA4 HAT component Yng2 plays an essential role, Yng1 and Pho23 hinder p53-dependent transcription. These observations indicate that p53-ING interaction is an important property that could explain how ING proteins regulate important cellular functions such as cell proliferation, senescence, DNA repair, and apoptosis. Our work suggests that ING proteins are key factors in chromatin modification in vivo. Accordingly, human ING1 has been found associated with HDAC and HAT activities (2). In addition to being subunits of chromatin modifying complexes, it seems that INGs play an important role in catalytic function. YNG1 and YNG2 deletions cause important decrease in NuA3 and NuA4 activities toward chromatin without disrupting these complexes (5, 6). Moreover,

---

Opposite Roles of ING Proteins in p53-driven Transcription

recombinant Yng2 is required with Esa1 in a recombinant core HAT complex to acetylate chromatin substrates. 2 The same can now be said about Pho23 for deacetylation of chromatin substrate by the Sin3/Rpd3 complex. Therefore, we suggest that the p53/ING functional link is part of a global function mediated by chromatin-modifying complexes, which is conserved during evolution.

Acknowledgments—We are grateful to N. Lacoste for the Yng2-TAP strain and C. Di Como and B. Séraphin for the FASAY and TAP system plasmids.

REFERENCES
Opposite Role of Yeast ING Family Members in p53-dependent Transcriptional Activation
Amine Nourani, LeAnn Howe, Marilyn G. Pray-Grant, Jerry L. Workman, Patrick A. Grant and Jacques Côté

doi: 10.1074/jbc.C300036200 originally published online April 2, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300036200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 22 references, 15 of which can be accessed free at
http://www.jbc.org/content/278/21/19171.full.html#ref-list-1