Journal of Cellular Biochemistry 96:1127–1136 (2005)

The Fellowships of the INGs

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Abstract The inhibitor of growth (ING) family of proteins is an evolutionarily conserved family, with members present from yeast to humans. The mammalian ING proteins are candidate tumor suppressor proteins and accordingly can cooperate with p53 to arrest proliferation and induce apoptosis. ING proteins are also reported to function in the promotion of cellular senescence, the regulation of DNA damage responses and the inhibition of angiogenesis. At the molecular level, ING proteins are thought to function as chromatin regulatory molecules, acting as co-factors for distinct histone and factor acetyl-transferase (H/FAT) and deacetylase (HDAC) enzyme complexes. Further, ING proteins interact with a number of additional proteins involved in the regulation of critical nuclear processes, such as gene expression and DNA replication, and also function as nuclear phosphoinositide (PtdInsP) receptors. Despite the increasing number of known molecular interacting partners for ING proteins, the specific biochemical action of mammalian ING proteins and its relationship to tumor suppression remain elusive. In this Prospect, we summarize the present understanding of the binding partners and physiologic roles of ING proteins and propose a general molecular paradigm for how ING proteins might function to prevent cancer. J. Cell. Biochem. 96: 1127–1136, 2005.

Key words: ING1; ING2; PHD finger; phosphoinositide; chromatin modification

ING family members are present throughout eukaryotic proteomes [He et al., 2005]. The best-characterized members include the five human members (INGs1-5) and three *S. cerevisiae* members (Yng1, Yng2, and Pho23.) The founding member of the family, ING1, was identified using an approach designed for discovery of genes whose expression was suppressed in cancer cells [Garkavtsev et al., 1996]. Accordingly, ING1 was subsequently shown to cooperate with p53 to induce apoptosis and cellular senescence, activities consistent with the notion that ING1 is a tumor suppressor [Garkavtsev and Riabowol, 1997; Garkavtsev et al., 1998]. Since the discovery of ING1, studies from multiple groups have implicated ING1 as well as other family members in negative regulation of cell proliferation, promotion of apoptosis and cellular senescence, contact inhibition, DNA damage repair, and inhibition of angiogenesis (for review see [Nouman et al., 2003; Campos et al., 2004; Gong et al., 2005; Kim, 2005] and references therein). In this context, multiple tumors have been found to either (i) harbor mutations within *ING* genes, (ii) have reduced expression of ING proteins, or (iii) have altered ING protein sub-cellular localization. Based on these collective findings, mammalian INGs are now thought to function as type II tumor suppressors (reviewed in [Gong et al., 2005]).

Studies in both yeast and human cells suggest that ING proteins exert their biological functions through their associations with specific molecular partners. For the purpose of this Prospect, we divide these interacting partners of ING proteins into three groups: (i) components of HAT or HDAC complexes, (ii) other proteins involved in nuclear regulatory functions (e.g., p53 and NF-κB), and (iii) the signaling lipids, PtdInsPs. Below we review these interactions and discuss the possibility that ING proteins function by integrating stress-induced PtdInsP signaling to (i) facilitate the assembly and (ii) regulate the sub-nuclear localization of distinct complexes consisting of different combinations of group (i) and (ii) interactors.

Grant sponsor: Burroughs Wellcome Fund and NIH; Grant number: KO8 AG19245.

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Received 28 July 2005; Accepted 29 July 2005

DOI 10.1002/jcb.20625

STRUCTURE AND FUNCTION OF ING PROTEIN DOMAINS

All of the ING proteins share a relatively similar architecture, containing an N-terminal protein–protein interaction region, a centrally located nuclear localization signal (NLS) and a C-terminal plant homeodomain (PHD) finger module. The N-termini of ING proteins mediate the majority of reported protein–protein interactions, with two functionally defined domains, named the PCNA-interacting protein (PIP) and SAP30 interacting domain (SAID) domains [Scott et al., 2001; Kuzmichev et al., 2002]. The PIP domain mediates a direct interaction with PCNA. This interaction is specifically induced upon UV damage and is hypothesized to switch PCNA activity away from DNA replication towards DNA repair [Scott et al., 2001]. Interestingly, in yeast, Yng2 mutants are synthetically lethal with DNA replication mutants, suggesting that regulation of replication-coupled repair might be evolutionarily conserved [Choy and Kron, 2002].

To date, it appears that among ING members, the PIP domain is unique to ING1. In contrast, the SAID domain, which was defined as the region of ING1 that directly interacts with the Sin3a associated protein sin3-associated protein 30 (SAP30), is likely also present on ING2 and PHO23, since both of these proteins directly interact with SAP30 ([Loewith et al., 2001; Kuzmichev et al., 2002]; O. Gozani, unpublished observations). This interaction is thought to bridge ING1, ING2, and Pho23 to SAP30-containing HDAC1/2 complexes.

All of the human ING proteins (with the exception of an alternatively spliced isoform of ING1 (ING1a)) have been shown in overexpression studies to promote the transactivity of the tumor suppressor p53 [Nagashima et al., 2001, 2003; Vieyra et al., 2002b; Shiseki et al., 2003]. It is proposed that ING proteins facilitate p53-dependent transcription through one of several mechanisms, including: (i) opening of chromatin at p53-target promoters by recruitment of ING-containing HAT/HDAC enzymes via ING-p53 interactions, (ii) facilitation of p53 acetylation by ING proteins, likely mediated by ING-p53 interactions and subsequent acetylation by ING-associated HATs, and (iii) inhibition of the p53 deacetylase SIRT1 [Feng et al., 2002; Cheng et al., 2003; Kataoka et al., 2003]. Presently, it remains unclear whether ING-p53 interactions are direct or mediated by co-associated proteins; ING1 has been reported to bind directly to p53 [Leung et al., 2002]. However, we have not detected direct interactions between ING2 and p53 in vitro (O. Gozani, unpublished observations), though the interaction might depend on either ING2 or p53 having undergone a specific post-translational modification in vivo. Regardless, there is clear evidence that ING proteins and p53 are in a common signaling pathway and almost certainly share common protein binding-partners. In this regard, multiple groups have found that ING protein-induced apoptosis requires p53 and that p53-induced apoptosis is potentiated by co-expression of INGs. Further, the ability of ectopically expressed p53 to transactivate a reporter plamsid in *S. cerevisiae* requires Yng2 [Nourani et al., 2001]. Notably, this activity of Yng2 is dependent on the presence of the Yng2 PHD finger, but is not due to a direct interaction between Yng2 and p53 (discussed below) [Nourani et al., 2001].

Recently, ING4 was shown to physically interact with nuclear factor-κB (NF-κB) and repress its transcriptional activity [Garkavtsev et al., 2004]. Interestingly, ING4 truncations augmented NF-κB activity, presumably via dominant-negative effects. This dominant negative activity might indicate sequestration by this domain of ING4 interacting proteins (or ING4 itself) that are normally involved in inhibiting NF-κB [Garkavtsev et al., 2004]. In this regard, reversible acetylation of NF-κB is thought to regulate its transcriptional activation, DNA binding affinity, I-κBα association and subcellular localization (reviewed in [Greene and Chen, 2004]). ING4 has been reported to interact, when overexpressed, with the HAT p300, and is likely to be a component of this HAT or possibly another HAT/HDAC complex [Shiseki et al., 2003]. Thus, ING4 might inhibit NF-κB activity via regulation of acetylation. Further, since p53 and NF-kB are generally thought to have antagonistic functions, potential connections between ING4-p53 interactions and ING4-NF-kβ interactions might exist, though presently such connections have not been explored.

THE PHD FINGER AS A SIGNALING MODULE

The greatest homology among the ING proteins occurs within the highly conserved PHD zinc finger motif [He et al., 2005]. This module is

found throughout eukaryotic proteomes, predominantly on chromatin-associated proteins [Sutherland et al., 2001]. Structurally, the PHD finger belongs to the treble class of zinc-binding domains, containing two zinc ions bound in a cross-braced topology [Pascual et al., 2000]. Zinc-coordination by PHD fingers is achieved via ligation of zinc atoms to alternating pairs of residues from the consensus Cys4-His-Cys3 sequence distribution (zinc one is bound by Cys1, Cys2, His, and Cys6, whereas zinc two is bound by Cys3, Cys4, Cys7, and Cys8) [Pascual et al., 2000; Capili et al., 2001; Kwan et al., 2003; Bottomley et al., 2005]. Beyond the conservation of zinc-coordinating residues, PHD fingers display substantial diversity in their sequences, particularly between Cys6 and Cys7, suggesting that the biological activity of PHD fingers might similarly be diverse [Kwan et al., 2003].

Much evidence argues for PHD fingers mediating important physiologic functions [Aasland et al., 1995]. Mutations within the PHD fingers of numerous proteins are implicated in tumorigenesis, as well as the pathogenesis of immunodeficiency syndromes, autoimmune syndromes, and several other genetic disorders [Gibbons et al., 1997; Pascual et al., 2000; Saugier-Veber et al., 2001; Elkin et al., 2005]. Many of these mutations occur at zinc-coordinating residues, indicating that zinc-ligation and hence integrity of the PHD finger fold is critical for the function of PHD-finger containing proteins. A second class of disease-linked PHD finger mutations do not disrupt zinc-coordination and rather are located between the 6th and 7th zinc-coordinating residues, a segment which, based upon known PHD finger structures and structural modeling, is thought to be at or near the surface of the domain. We and others have postulated that this surface forms a molecular interaction interface and that mutations within this region might disrupt this activity and in so doing manifest the disease phenotype [Gozani et al., 2003; Kwan et al., 2003; Elkin et al., 2005]. Consistent with this idea, we have found that substitution of the basic residues between the 6th and 7th zinc-coordinating residues into alanines disrupt binding of the PHD fingers of ING1, ING2, ATP-dependent chromatin remodeling factor (ACF), and recombination activating gene 2 (*RAG2*) to PtdInsPs. Further, such mutations render ING2 and RAG2 largely inactive [Gozani et al., 2003; Elkin et al., 2005].

Insight into the biologic function of PHD fingers comes in part from studies of the structurally related FYVE and RING finger modules [Pascual et al., 2000; Capili et al., 2001]. The FYVE finger is a well-characterized PtdInsP-binding module, and RING fingers function as components of E3 ubiquitin ligase enzymes (reviewed in [Misra et al., 2001; Stenmark et al., 2002; Fang et al., 2003]). Both of these functions have been reported for PHD fingers from different proteins [Coscoy et al., 2001; Lu et al., 2002; Gozani et al., 2003; Jones and Divecha, 2004; Uchida et al., 2004], though recent analyses argue that putative PHD fingers with E3-ubiquitin ligase activity are more likely to be RING finger variants rather than true PHD fingers [Aravind et al., 2003; Scheel and Hofmann, 2003]. In addition, PHD fingers have also been reported to interact with nucleosomes and to be involved in other protein–protein interactions [Eberharter et al., 2004; Ragvin et al., 2004].

We have found that the ING2 PHD finger, together with a short stretch of basic residues C-terminal to it, preferentially binds to monophosphorylated PtdInsPs, most specifically to the rare PtdInsP species phosphatidylinositol-5-phosphate (PtdIns(5)P) [Gozani et al., 2003]. This interaction appears to facilitate recruitment of ING2 to chromatin, and is required for ING2-dependent activation of p53 during DNA damage responses. The specificity for PtdIns(5)P is intriguing, because levels of PtdIns(5)P undergo dynamic fluctuations within the nucleus during progression of the cell-cycle, suggesting that PtdIns(5)P may be a critical regulator of nuclear signaling events [Clarke et al., 2001]. Consistent with these findings, in response to DNA damage induced by treatment with etoposide, we detect an increase in the levels of chromatin-associated PtdIns(5)P, arguing that this phospholipid might function as a signaling molecule at chromatin during DNA damage responses ([Jones and Divecha, 2004]; M. Ewalt and O. Gozani, unpublished observations). Because ING2 is known to interact with chromatin regulatory complexes, it is possible that the generation of PtdIns(5)P at specific chromatin locations may lead to regulated sub-nuclear localization of ING2 and its associated chromatin-modifying activity (Fig. 1; see below).

This idea is supported by elegant work that investigated the role of the Yng2 PHD finger in modulating histone acetylation and transcription regulation in *S. cerevisiae* [Nourani et al.,

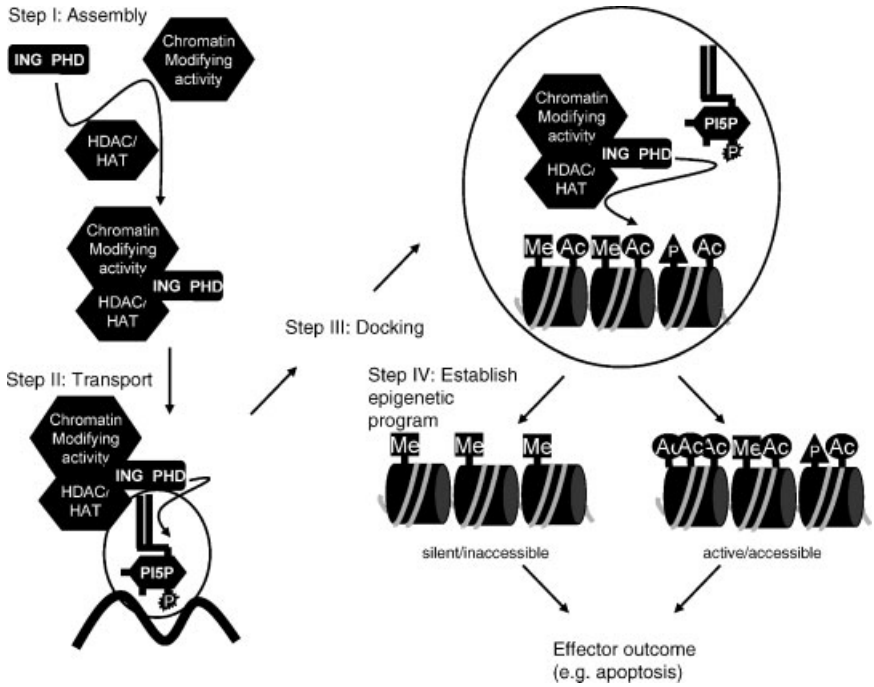

Fig. 1. Schematic representation of potential ING-mediated signaling mechanisms triggered in response to cellular stress such as DNA damage. **Step I**: Assembly; in response to a genotoxic stress, specific ING complexes, consisting of a HAT or HDAC and other associated activities (see text), are assembled. **Step II**: Transport; sub-nuclear localization of ING and associated proteins. DNA damage causes an increase in the levels of a specific PtdInsP species (e.g., PtdIns(5)P), and the PHD finger of an ING protein senses this. ING PHD finger-PtdInsP interactions result in recruitment of ING-associated complexes to the sub-nuclear region containing the highest level of the newly generated PtdInsP. **Step III**: Docking; once ING complexes have been localized to a general sub-nuclear region, finer localization at a distinct genomic locale (e.g., near site of DNA damage) is achieved by docking of the ING complex to specifically modified N-terminal histone tails. **Step IV**: Establish epigenetic program; recruitment of ING associated chromatin-modifying activities to chromatin results in post-translational modification and/or remodeling of nearby nucleosomes and the establishment of a specific epigenetic state. Shown are two examples: (1) recruitment of a HAT leads to increased locale acetylation and increased DNA accessibility, or (2) recruitment of an HDAC leads to decreased locale acetylation and rendering of the DNA inaccessible or silent. In either case, different effector outcome programs, such as apoptosis or cell-cycle arrest, are initiated.

2001]. Yng2 is a stoichiometric component of the NuA4 HAT complex, and the deletion of Yng2 results in a dramatic decrease in cell growth [Choy et al., 2001; Nourani et al., 2001; Choy and Kron, 2002]. This phenotype can be rescued by ectopic expression of Yng2 lacking the PHD finger, indicating that under normal conditions, NuA4 activity does not require the Yng2 PHD finger [Nourani et al., 2001]. However, the PHD finger is needed for efficient activation of specific *NuA4* target genes and p53-dependent transcription of an ectopically introduced p53-responsive gene. The authors of this work demonstrated that the defect was not due to a reduction in the acetyltransferase activity of the NuA4 complex, and reasoned that instead the PHD finger might regulate the subnuclear localization of the NuA4 complex [Nourani et al., 2001]. There is strong biochemical and genetic evidence that in *S. cerevisiae*, PtdInsPs and their metabolites inositol polyphosphates are present and functioning in the nucleus [York et al., 2001]. Thus, it is tempting to speculate that nuclear PtdInsPs, via interactions with the Yng2 PHD finger, might regulate recruitment of NuA4 HAT activity to particular genomic targets in response to specific signals and stimuli. If the PHD finger of Yng2 binds PtdInsPs, because of the power and flexibility of yeast genetics, it will be relatively straightforward to test the functional consequence of Yng2-PtdInsP-interactions. For example, Yng2 mutants unable to bind PtdInsP can be introduced into yng2Δ strains, and the ability of the mutant protein to support Yng2p activities can be tested genetically. Further investigation can test whether temperature sensitive lipid kinase/phosphatase strains phenocopy the specific defects observed with the yng2ΔPHD strains.

INGs AND CHROMATIN REGULATION

In eukaryotes, DNA does not exist in a free state but rather is packaged and compacted by histones and other proteins into the higher order structure of chromatin. Depending on the level of compaction or state of chromatin, the DNA is either accessible or inaccessible to trans-acting factors that carry out fundamental nuclear processes such as gene expression and DNA repair. The basic unit of chromatin is the nucleosome, which contains ~147 base pairs of DNA wound around an octamer of four core histones: H2A, H2B, H3, and H4 [Kornberg and Lorch, 1999]. Nucleosomal-bound DNA is further compacted into secondary and tertiary structures via intrinsic and protein-mediated mechanisms to form chromatin. A key molecular mechanism for regulating chromatin accessibility is achieved via dynamic post-translational modification of the N-terminal tails of histone proteins [Kurdistani and Grunstein, 2003]. Examples of such modifications include acetylation, methylation, phosphorylation, and ubiquitination. The "histone code" hypothesizes that specific patterns of histone modifications are recognized by different DNA and chromatin effector molecules, and these activities in turn initiate downstream pathways, such as gene silencing or apoptosis [Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001]. In this way, molecular structures on chromatin are translated by the histone code into specific functional outcomes. Considerable evidence from the past half decade links ING protein to chromatin regulation and translation of the histone code via physical association with the enzymatic complexes that regulate histone acetylation: the HAT and HDAC complexes [Feng et al., 2002]. Thus, it is thought that through these associations ING proteins execute their biological activity.

CHROMATIN REGULATION: *S. CEREVISIAE* INGs

Loewith et al. [2000] made the first discovery linking ING proteins to histone acetylation, demonstrating that all three yeast INGs are associated with HAT activity. Based on this and several subsequent studies, it is now clear that Yng1 is a stable, stoichiometric component of the NuA3 HAT complex and Yng2 is a stable stoichiometric component of the NuA4 HAT complex [Howe et al., 2002; Doyon and Cote, 2004]. Pho23 is present within the Rpd3 HDAC complex, and the physiologic significance of the originally reported HAT activity is unclear, though it is certainly possible that while Pho23 is predominantly present in the Rpd3 complex, a minor but biologically important fraction associates with a HAT [Loewith et al., 2001].

What role do Yng1 and Yng2 play in their respective HAT complexes? Both proteins appear to facilitate the association and enzymatic activity of their associated HATs with chromatinized substrates. NuA3 acetylates H3, and NuA4 acetylates H4 and to a lesser extent, H2A. The catalytic subunit of NuA3, Sas3p, and Yng1, are both not required for viability [Howe et al., 2002]. However, Sas3p is synthetically lethal with another HAT specific for H3 acetylation, Gcn5p, and gcn5Δyng1Δ strains, while viable, are severely compromised for growth [Howe et al., 2001, 2002]. Similar to the situation with Yng2, the PHD finger of Yng1 is not essential for its activity as expression of Yng1ΔPHD is able to rescue the gcn5Δyng1Δ growth defect [Howe et al., 2002]. Deletion of Yng1 does not affect the integrity of NuA3 protein complex, but rather compromises Sas3 HAT activity toward free histones, an effect that is further evident when nucleosomes are utilized as the substrate [Howe et al., 2002]. Moreover, the ability of NuA3 isolated from yng1Δ strains to interact with nucleosomes are severely decreased, suggesting a role for Yng1p in targeting the NuA3 complex to its chromatin substrates [Howe et al., 2002].

In contrast to Sas3, the catalytic subunit of NuA4, Esa1, is essential for viability in *S. cerevisiae*, and while Yng2 is not strictly essential for viability, Δyng2 strains have markedly decreased growth and are sensitive to DNA damage [Choy et al., 2001; Nourani et al., 2001]. This defect is likely due to decreased NuA4 activity, as purified NuA4 complex from Δyng2 mutant strains, while intact, is low in abundance [Nourani et al., 2001]. Moreover, the complex that is purified, despite having all of the components besides Yng2p, displays weak HAT activity [Nourani et al., 2001].

Esa1 and Yng2 are predominantly found in the ~1.3 MDa NuA4 macromolecular complex. In addition to this large complex, Esa1, Yng2, and a third NuA4 component named Epl1 (homologous to Enhancer of polycomb, E(Pc), a modifier of position effect variegation in *Drosophila*) form a second, smaller (~300 kDa)

NuA4-like complex named Piccolo NuA4 [Boudreault et al., 2003]. This complex is more active than the larger complex and genetic and biochemical evidence indicate that this complex likely functions to maintain global or general acetylation of H4 and H2A [Boudreault et al., 2003]. In contrast, NuA4 is thought to function in a more restricted manner, catalyzing locus-specific acetylation.

Biochemically, Esa1 alone will acetylate free H4 but is unable to acetylate nucleosomal-bound H4. Within the context of the NuA4 complex, Esa1 acetylates free or nucleosome-bound H4 equally well (both activities are reduced in the absence of Yng2), whereas Esa1 from the Piccolo NuA4 complex shows a marked preference for acetylating chromatinized H4 [Boudreault et al., 2003]. Using a recombinant reconstitution system, Boudreault and colleagues demonstrated that Epl1 dramatically augments Esa1 activity on free histones but does not facilitate its activity on chromatin. Yng2 alone has no effect on Esa1 activity or substrate preference, but the addition of both Yng2 and Epl1 to Esa1 together reconstitute Piccolo NuA4 activity on a chromatin substrate. Notably, the PHD finger and the first 66 amino acids of Yng2 are dispensable for Piccolo NuA4 formation and activity [Selleck et al., 2005]. Mechanistically, Yng2 directly binds to nucleosomes and therefore might be facilitating Esa1 activity towards a chromatin substrate by targeting Esa1 to its substrate; however, Yng2 and Epl1 both interact with nucleosomes, a redundancy that argues for additional Yng2 activities.

The involvement of ING proteins in HDAC complexes was also first report by Loewith and colleagues. Deletion of Rpd3, an HDAC enzyme, and Sin3, an evolutionarily conserved co-repressor present in the Rpd3 complex, results in a phenotype of enhanced silencing at rDNA, telomeric, and mating-type loci (reviewed in [Struhl, 1998]). Deletion of the third *S. cerevisiea* ING1 homologue, Pho23, exhibits a similar phenotype [Loewith et al., 2001]. Moreover, Pho23 immunoprecipitates contain HDAC activity, and this activity is absent in rpd3Δ strains. As discussed above, Pho23 associates with the Sin3/Rpd3 HDAC complex via a direct interaction with another subunit of this complex, Sap30. Functionally, Pho23 mutant strains display weakened transcriptional repression of *Rpd3*-target genes (e.g., *PHO5*) and accordingly have reduced Rpd3-dependent HDAC activity [Loewith et al., 2001]. A detailed investigation of this reduced activity has not yet been carried out, however, it is reasonable to postulate that akin to Yng1 and Yng2, Pho23 is likely involved in enabling chromatin to be used as a substrate by Rpd3.

CHROMATIN REGULATION: MAMMALIAN INGs

All five human ING proteins have been reported to associate with a diverse group of HATs and/or HDACs. In certain cases, the reported interactions might be driven by overexpression and not necessarily represent a physiologic association. However, the identification of ING3 as a stable stoichiometric component of the native NuA4-like TIP60 HAT complex and of ING1b and ING2 as substoichiometric components of the native Rpd3-like mSin3a/HDAC1 complex, argue that chromatin regulatory functions by ING proteins are conserved from yeast to mammals [Skowyra et al., 2001; Kuzmichev et al., 2002; Cai et al., 2003; Doyon et al., 2004].

Tip60 is homologous to yeast Esa1, and the multisubunit TIP60 complex, like yeast NuA4, acetylates H4 and H2A. Moreover, Tip60 is also present in a smaller complex that resembles Piccolo NuA4 and consists of Tip60, EPC1 (the mammalian homologue of Epl1) and ING3 [Doyon et al., 2004]. Similar to Esa1, Tip60 alone fails to acetylate a chromatin substrate in vitro, but has potent activity when co-expressed with EPC1 and ING3 [Doyon et al., 2004]. Based on these findings and sequence alignment analyses, it is believed that the mammalian orthologue of Yng2p is ING3. The acetylation activity of Tip60, like Esa1, has been linked to DNA damage responses, including repair and induction of apoptosis [Ikura et al., 2000; Bird et al., 2002; Downs et al., 2004]. The observation that ING3 is found mutated in head and neck cancers suggests a link between ING3-regulation of TIP60 acetylation activity, DNA damage responses and tumorigenesis or tumor progression [Gunduz et al., 2002]. However, the precise mechanism by which ING3 regulates TIP60 DNA damage responses remains to be determined. Of the ING proteins we have tested, we do not detect an interaction between the ING3 PHD finger and PtdInsPs. We postulate that a different signaling molecule, such as inositol polyphosphates, might instead regulate this PHD finger to participate in recruitment of

the TIP60 complex to DNA damaged loci. In this context, inositol polyphosphates are implicated in regulating the recruitment of the ATP-dependent chromatin-remodeling SWI/SNF and INO80 complexes to the PHO5 promoter in response to phosphate limitation [Steger et al., 2003]. PHD fingers have also been shown to bind to nucleosomes [Eberharter et al., 2004; Ragvin et al., 2004], thus it is possible that the PHD finger of ING3 might recognize a histone mark that is specific for DNA damage and thus facilitate docking of the TIP60 complex at or near the area of damage.

In addition to ING3-TIP60 complex interactions, the other four human ING proteins all bind the HATs p300/CBP. Of these interactions, endogenous ING1 and ING2 co-immunoprecipitate CBP and p300, respectively, suggesting that ING1-CBP and ING2-p300 complexes might be present in vivo [Vieyra et al., 2002a; Pedeux et al., 2005]. With respect to ING4-p300/CBP and ING5-p300/CBP interactions, the association was demonstrated via transient overexpression strategies [Shiseki et al., 2003]; whether the endogenous proteins interact physiologically remains to be determined. Evidence indicates, however, that ING4 (and likely ING5) are not functionally redundant with ING1 or ING2, arguing that these two proteins most likely do not physiologically bind p300, and rather might associate with as yet unknown HAT or HDAC complex [Garkavtsev et al., 2004].

Endogenous ING1 IP complexes contain HAT activity towards H3 and H4, and co-precipitate several different HAT enzymes or associated proteins, including p300, CBP, TRRAP, and PCAF [Vieyra et al., 2002a]. These findings highlight a potential divergence in function between the *S. cerevisiae* and mammalian INGs. Specifically, the yeast proteins are thought to interact with a single distinct HAT or HDAC enzyme, whereas the mammalian INGs appear more promiscuous. This is most pronounced for ING1. In addition to the many associations with HATs, ING1 is also reported to associate with two different HDACs: HDAC1 and SIRT1 [Skowyra et al., 2001; Kuzmichev et al., 2002; Kataoka et al., 2003].

Conventional chromatography of the native Sin3a/HDAC1 complex revealed the substoichiometric presence of both ING1 and ING2 as well as components of the Brg1-based SWI/SNF ATP-dependent chromatin-remodeling complex. The authors of this study further demonstrated that ING1 bridges the interaction between the two chromatin-regulatory complexes [Kuzmichev et al., 2002]. ING1 is also implicated in bridging Sin3a/HDAC1 to the DNA methyltransferase DNMT1 complex through interactions with the DNMT1-associated protein DMAP1 [Xin et al., 2004]. The function of ING2 within the Sin3a/HDAC1 complex is not known. It is also unclear whether there are two (or more) distinct Sin3a/HDAC1 complexes, one containing ING1 and a second containing ING2. We postulate that ING1 and ING2, in response to specific stimuli, bridge one of the many different chromatin-modifying enzymes reported to associate with them (e.g., p300, PCAF, SIRT1, etc.) to form distinct subtypes of mSin3a/HDAC 1 complexes. These large macromolecular complexes are further regulated via PtdInsP signaling-mechanisms; to either concentrate them near their genomic target(s) or near additional activities. Such a model might reconcile why ING proteins bind to such a diverse and often seemingly functionally redundant or even contradictory group of proteins.

ING PROTEINS, DNA DAMAGE RESPONSES AND REGULATION OF NUCLEAR SIGNALING

Do the ING proteins function in DNA damage signaling pathways, and if so, is this the basis of their tumor suppressor activity? As discussed above, we speculate that ING proteins function in part to assemble distinct combinations of chromatin-modifying complexes and to regulate the subnuclear localization of these complexes, potentially via PHD finger-PtdInsP interactions. The observations that (i) Yng1 and Yng2 are important for NuA3 and NuA4 binding to chromatin and (ii) the PHD fingers of CBP and ACF directly bind nucleosomes, suggests that a third function for ING proteins might be to participate in the docking of their associated complexes at chromatin through interactions with histones [Howe et al., 2002; Boudreault et al., 2003; Eberharter et al., 2004; Ragvin et al., 2004]. There is a precedent in the literature for domains that have dual functions, binding to both PtdInsPs and peptides (i.e., the PDZ and PTB domains) [Zimmermann et al., 2002; Stolt et al., 2003]. Based on these observations, it is reasonable to hypothesize that PHD fingers of the ING proteins might similarly interact with both PtdInsPs and peptides,

potentially the highly regulated N-terminal tails of histones. In this context, perhaps PHD fingers only recognize histone tails in response to DNA damage, concomitant with the generation of a specifically modified histone mark. Building from this, we postulate that ING proteins are key components of a novel nuclear signaling paradigm operating during the DNA damage response (and possibly in response to other stimuli and stresses) (Fig. 1). The key components of this postulated pathway include: (1) an assembly step, (2) a transport or "zip code" step and (3) a docking or "street address" step. The assembly step consists of bridging of the ING-associated HAT/HDAC with a second activity (e.g., p53 or a chromatin-remodeling complex). In the second step, DNA damage (or other signal-dependent) changes in a specific PtdInsP species level (e.g., PtdIns(5)P for ING2) leads to gross localization of the complex (ING and associated proteins) to within a subnuclear region. In a third step, finer localization of the complex is achieved via recognition of a specifically modified histone tail (e.g., acetylated H4). The docking of the ING associated chromatin-regulatory activities at a genomic locale enriched with this histone modification in turn can result in propagating secondary changes to nearby nucleosomes to effect the establishment of a specific epigenetic state. Taken together, this model provides a testable molecular paradigm for how ING proteins might function to prevent tumorigenesis.

ACKNOWLEDGMENTS

We thank Katrin Chua for critical reading of the manuscript. This work was supported in part by NIH grant to O.G. (KO8 AG19245). O.G. is a recipient of a Burroughs Wellcome Career Development Award in Biomedical Sciences.

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