Crystal Structure of Inhibitor of Growth 4 (ING4) Dimerization Domain Reveals Functional Organization of ING Family of Chromatin-binding Proteins

The protein ING4 binds to histone H3 trimethylated at Lys-4 (H3K4me3) through its C-terminal plant homeodomain, thus recruiting the HBO1 histone acetyltransferase complex to target promoters. The structure of the plant homeodomain finger bound to an H3K4me3 peptide has been described, as well as the disorder and flexibility in the ING4 central region. We report the crystal structure of the ING4 N-terminal domain, which shows an antiparallel coiled-coil homodimer with each protomer folded into a helix-loop-helix structure. This arrangement suggests that ING4 can bind simultaneously two histone tails on the same or different nucleosomes. Dimerization has a direct impact on ING4 tumor suppressor activity because monomeric mutants lose the ability to induce apoptosis after genotoxic stress. Homology modeling based on the ING4 structure suggests that other ING dimers may also exist.

Chromatin structure is highly dynamic and largely regulated by post-translational covalent modifications at the N-terminal histone tails, which are modified by enzymatic complexes with domains that recognize one or more histone modifications (1). The ING (inhibitor of growth) family of tumor suppressors consists of five homologous proteins (2) that regulate the transcriptional state of chromatin by recruiting remodeling complexes to sites where histone H3 is trimethylated at Lys-4. This modification is recognized by their C-terminal plant homeodomain (PHD)3 fingers (3). ING1 and ING2 form part of the histone deacetylase complexes, whereas ING3, ING4, and ING5 form part of the histone acetyltransferase complexes (4). ING4 recruits the histone acetyltransferase complex HBO1 to ING4 target promoters and facilitates histone H3 acetylation (5).

The PHD structure of ING4 bound to histone H3 trimethylated at Lys-4 (H3K4me3) (6, 7) shows a specific recognition based on interactions that are conserved in the ING protein family (8–10), but little is known about the full-length structure of any ING protein. Low-resolution data show that ING4 forms dimers through its N-terminal domain and that the two PHDs of the dimer bind to H3K4me3 peptides independently and with the same affinity (11). This is a consequence of the central ~85-residue-long region (the linker, which contains the nuclear localization sequence) being disordered and flexible. However, these data could not establish the relative orientation of the two protomers in the dimer, and the functional implication of ING4 oligomerization inside living cells is not known.

**EXPERIMENTAL PROCEDURES**

**Cloning and Mutagenesis**—The clones used for the production of full-length ING4 (UniProt code Q9UNL4, isoform 1) and N-terminal domain 1–105 in bacterial or human cells have been described previously (11, 12). The mutant versions were generated with the QuikChange site-directed mutagenesis kit (Stratagene) and confirmed by DNA sequencing. The templates were a synthetic gene with codons optimized for expression in *Escherichia coli* and the cDNA corresponding to the *Homo sapiens* ING4 protein cloned in the retroviral vector pLPC.

**Protein Expression and Purification**—Proteins were produced in *E. coli* BL21(DE3) cells transformed with the plasmid pET11d(+) containing the ORF coding for the different ING4

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* This work was supported by Spanish Ministerio de Ciencia e Innovación Grants SAF2009-09031 (to I.P.), BFU2011-23815 and CSD2006-00023 (to G.M.), and CTQ2011-28680 (to F.J.B.).

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3 The abbreviation used is: PHD, plant homeodomain.
constructs as described previously (11, 12). Protein identity was confirmed by mass spectrometry for every sample, and it was found that all of the proteins lacked the initial methionine. Protein concentration was determined by absorbance at 280 nm using the extinction coefficients calculated from the amino acid composition of each protein. The sample purity was checked on Coomassie Blue-stained SDS-polyacrylamide gel, and its polydispersity was evaluated using dynamic light scattering. The level of incorporation of selenomethionine was confirmed by mass spectrometry to be high enough for multiwavelength anomalous dispersion phasing.

Crystallographic Data Collection, Structure Solution—Crystallization and diffraction data collection and analysis were performed as described (12). Diffraction data were indexed, integrated, and scaled using the XDS program suite (13). A 2.27 Å resolution structure of the selenomethionyl-labeled protein was solved by the single-wavelength anomalous dispersion phasing method. The data were collected at the selenium K-edge, and SHELX (14) was used to locate the selenium sites, and SHARP (14) was used to refine those initial positions. Initial phases were subjected to solvent flattening as implemented in SOLOMON (15). The major part of the structural model was automatically built using the Autobuild routine as implemented in PHENIX (16). The remaining part of the structure was completed by iterative cycles of manual building in Coot (17) and refinement with PHENIX (16).

Molecular Modeling and Analysis—Molecular modeling and free energy calculations were done with FoldX 3.0 (18) using the corresponding plug-in in YASARA (19). Helical wheels were drawn with Drawcoil. Figures were prepared using PyMOL (DeLano Scientific LLC).

Circular Dichroism Spectroscopy—The CD spectra were collected on a Jasco J-810 spectropolarimeter equipped with thermoelectric temperature control. Measurements were made at 25 °C on protein samples at a concentration of 25 μM (on a monomeric basis) in 20 mM phosphate (pH 6.5), 200 mM NaCl, and 1 mM DTT using a quartz cell with a 2-mm path length. Protein unfolding was induced by increasing the temperature at a rate of 1 °C/min from 5 to 100 °C, and the ellipticity at 222 nm was recorded at intervals of 1 °C.

Size Exclusion Chromatography—Multiangle Light Scattering—Static light scattering experiments were performed at room temperature using a Superdex 200 10/300 GL gel filtration column (GE Healthcare) in-line with a DAWN® HELEOS™ light scattering detector (Wyatt). The column was equilibrated with running buffer (0.02 M Tris (pH 8) and 0.3 M NaCl, 0.1 μM-filtered), and the system was calibrated using BSA (Sigma). Samples of 100 μL of protein at concentrations in the range of 0.3–1.5 g/liter were analyzed. Data acquisition and analysis were carried out using the ASTRA software. On the basis of numerous measurements on BSA samples at 1 g/liter under the same or similar conditions, we estimated that the experimental error in the molar mass was ~5%.

Cell Cultures and Co-immunoprecipitation Assays—Human cell lines HT1080 (fibrosarcoma) and 293T (embryonic kidney epithelium) and immortalized mouse fibroblasts (NIH3T3) were cultured in DMEM (Invitrogen) containing 10% FCS and penicillin/streptomycin at 37 °C and 5% CO₂. Transient transfection of 293T cells was performed using the calcium phosphate method as described previously (20, 21). Retroviral infection of HT1080 and NIH3T3 cells was performed essentially as described (21). Cell lysate preparation, electrophoresis, and Western blot analysis were done as described (22). Immunoprecipitation was done essentially as described (23) on 293T cell lysates transiently transfected with vectors expressing HA and AU5-tagged ING4 constructs. The antibodies used were anti-ING4 (1:500 dilution; ab3714, Abcam), anti-HA (1:500 dilution; AC-15, Sigma) as a loading control.

DNA Damage-induced Apoptosis Assays—Infected HT1080 and NIH3T3 cells (1.5 × 10⁵) were plated and treated with doxorubicin over 24 h. After collection and staining with trypan blue, the viable cells were counted. Two independent experiments were performed, each one with four different plates for every doxorubicin concentration and for every construct. The total number of cells counted in each plate was in the range of 50–170. The statistical significance of the differences was evaluated by Student’s t test using the program PRISM (GraphPad Software).

Anchorage-independent Growth—HT1080 cells were resuspended in a prewarmed solution of 0.3% agarose in complete medium and plated in 60-mm plates with a bottom layer of 0.5% agarose. Colonies with a diameter of >0.2 mm were visually

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**TABLE 1**

| Data collection, phasing, and refinement statistics | Selenium single-wavelength anomalous dispersion |
|---------------------------------------------------|----------------------------------------------|
| Data collection                                    |                                              |
| Wavelength (Å)                                    | 0.979                                       |
| Crystal-to-detector distance (mm)                  | 368.0                                       |
| Resolution range (Å)                              | 47.09–2.27 (2.27–2.40)                     |
| Space group                                       | C222                                         |
| Unit cell parameters                              | a = 128.45, b = 186.6, c = 62.22 Å; α = β = γ = 90.0° |
| Multiplicity                                      | 6.8 (3.3)                                   |
| Mean I/σI                                        | 11.1 (2.1)                                  |
| Completeness (%)                                  | 96.8 (96.8)                                 |
| R_merge (%)                                       | 9.3 (35.7)                                  |
| R_max (%)                                         | 11.7 (24.1)                                 |
| R_fom (%)                                         | 5.7 (28.0)                                  |

**Phasing statistics**

| No. of Se atoms found | 25/30  |
|-----------------------|--------|
| FOM (SHARP)           | 0.3    |
| R_merge (SHARP)       | 0.77   |
| Phasing power         | 1.2    |
| Density modification FOM (SOLOMON) | 0.825 |

**Refinement statistics (|F| > 0)**

| R_work (%) | 20.9 |
| R_free (%) | 25.1 |
| No. of atoms | 4913 |
| No. of amino acids | 589 |
| No. of waters | 203  |
| r.m.s.d. from ideal bond length (Å) | 0.004 |
| r.m.s.d. from ideal bond angle | 0.746° |

**Ramachandran statistics**

| Residues in favored regions | 544 (98.03%) |
| Residues in allowed regions | 8 (1.39%)    |
| Residues in disallowed regions | 10 (1.67%)   |

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* R_merge = Σ|F|/|F| (hkl - |F| (hkl))/|F| (hkl), where the summation is over all symmetry-equivalent reflections, excluding reflections observed only once.
* FOM, figure of merit; r.m.s.d., root mean square deviation.
* Calculated with 10% of reflections. Data collection values in parentheses are relative to the high-resolution shell.
counted after 15 days of growth. Two independent experiments were performed, each one with three different plates for every construct. For every plate, 10 randomly selected fields were counted in a non-blind mode. The total number of colonies counted in each plate was in the range of 60–100. A t test was used to evaluate the statistical significance of the differences.

**RESULTS**

**ING4 Forms Antiparallel Coiled-coil Dimer** —N-terminal fragment 2–118 of ING4 was shown by NMR to contain a structured domain (11), but it did not crystallize. Three shorter folded fragments were identified by limited proteolysis experiments coupled to mass spectrometry and N-terminal sequencing. These fragments differed at their C-terminal ends, and one of them (residues 2–105) yielded crystals that, after optimization, were suitable for high-resolution data collection (12). The crystal structure was solved at 2.27-Å resolution and refined to an Rwork of 20.9% and Rfree of 25.1%. A summary of the crystallographic data statistics is shown in Table 1.

The asymmetric unit contained six monomers forming three dimers (supplemental Fig. S1), with some local differences and/or missing electron density at the chain ends, loops, and in some solvent-exposed residues, probably due to local high flexibility as indicated by the high B-factors in these regions. The dimer in the asymmetric unit formed by chains A and C (as named in Protein Data Bank code 4AFL) displays 96% of the polypeptide chain (residues 3–104 in chain A and residues 4–105 in chain C) and is henceforth discussed as the representative structure of the N-terminal domain of ING4.

The domain is folded into an antiparallel coiled coil (Fig. 1A). Therefore, full-length ING4 has an elongated shape, with the PHD pointing to opposite directions. This arrangement implies that the PHD fingers, involved in the recognition of the histone H3 tail, are able to sample a large space around the dimerization domain due to the flexible nuclear localization sequence region.

Each of the two protomers forms a helix-loop-helix structure with two long helices (Ile-13–Met-50 and Ser-58–Asp-103, named α2 and α3, respectively) plus a short α1 helix (Met-5–Asp-12). The dimer arises from an antiparallel left-handed coiled-coil assembly of the four long helices of the two protomers, with helical cross angles of ~10°. The coiled-coil assembly is stabilized by the characteristic clustering of hydrophobic residues in its buried core and by interhelical polar interactions at the more solvent-exposed regions (Fig. 2A). There is a general correspondence between the pattern of heptad repeats ((abcdefg)ₙ), the local positions in the coiled coil, and the chemical nature of the amino acid. The largely buried positions a, d, and
g are occupied mainly by hydrophobic residues, with leucine and isoleucine residues frequently appearing at position g and forming the zipper structure. Polar residues are common at the partially solvent-exposed positions b and e, and charged residues are most frequent in the predominantly exposed positions c and f. The main exceptions to this pattern are two charged residues (Lys-39 and Asp-32) at position a in helix α2, which form two buried intermolecular salt bridges (Fig. 1B). The alignment of the N-terminal sequences of the five ING proteins (Fig. 2B) shows a conservation pattern that, in light of the ING4 structure, arises largely from structural constraints.

The mutation M50V has been detected in ING4 transcripts of colorectal carcinoma cells (24). This mutation eliminates two of the four intermolecular interactions between the Met-50 sulfur atom and the Phe-19 aromatic ring (25, 26) and reduces the buried hydrophobic area in the pocket where Met-50 sits (Fig. 1B). Therefore, the M50V mutation is expected to destabilize the formation of the dimer. Experimental measurements on the N-terminal domain show that this mutant is slightly less stable than the wild type, but it still forms a dimeric coiled coil, with no monomers detected at room temperature (Fig. 3A). The CD spectrum presents a more negative minimum at 222 nm than at 208 nm, typical of coiled-coil structures (27), and is the same as the spectrum of the wild type (11). The midpoint denaturation temperature is 51.2 ± 0.5 °C, which is 4 °C smaller than that of the wild type under the same experimental conditions (Fig. 3A). These results suggest that, if this mutation is related to the cancer phenotype of the colorectal...
cancer cells in which the mutated transcripts were detected, it is not through its effect on the dimeric coiled-coil structure of the N-terminal domain.

Monomeric ING4 Mutants Do Not Associate inside Cells but Still Interact with HBO1—To investigate the impact of the dimerization of ING4 on its binding to HBO1, we designed three mutations that disrupt six (three per monomer) intermolecular interactions: the buried salt bridges Asp-32/Lys-39 located in the central part of the coiled coil, the salt bridges Lys-66/Asp-101 at the tips of the molecule, and the hydrogen bonds between the side chains of Gln-69 and Asp-94 (Fig. 1). The mutation of Lys-66, Lys-39, and Gln-69 to Asp introduces electrostatic repulsive forces, which are expected to strongly destabilize the dimer but not the monomer.

Single, double, and triple ING4 mutants were made both on the full-length protein and on the N-terminal domain. Light scattering measurements at room temperature revealed that the triple K39D/K66D/Q69D mutant was predominantly monomeric (Fig. 3C), independent of the concentration used (0.24 and 1 g/liter). The single K39D and Q69D mutants still formed stable dimers, and the double K39D/Q69D mutant displayed an intermediate behavior (data not shown). CD measurements on the N-terminal domains showed that the mutations disrupted the dimer but preserved the helical structure of the monomers (supplemental Fig. S2). The changes in the ratio of the ellipticity at 222 and 208 nm with temperature indicate that dimer disruption is linked to coiled-coil destabilization, but at low temperatures, a small population of coiled-coil structures, possibly dimeric, may still exist (supplemental Fig. S3). For this reason, a deletion mutant lacking the first helix (ING4/H92512–56) was used as an additional control for non-dimerizing ING4.

Human embryonic kidney epithelial 293T cells were cotransfected with vectors expressing ING4 or its monomeric mutants with the HA and AU5 tags. Immunoprecipitation against one of the tags would yield only co-precipitated ING4 protein containing the other tag if they associate. Co-precipitation occurred with wild-type ING4, but not with the mutants, which were unable to self-associate inside the cells (Fig. 4A). Immunoprecipitated ING4 proteins probed with an antibody specific for HBO1 (Fig. 4B) show that all ING4 variants could bind to HBO1, independently of their ability to form dimers or not. This result is consistent with the previous finding that an ING4 splice variant lacking the first helix (ING4Δ1–56) was used as an additional control for non-dimerizing ING4.

Dimerization Is Essential for ING4-mediated Apoptosis Induction and for Anchorage-independent Cell Growth Inhibition—Overexpression of ING4 in tumor cells mediates apoptosis induction in response to DNA damage (29), as other members of the ING family similarly do (30). To investigate the relevance of ING4 dimerization with regard to apoptosis, we used retroviral infection to produce stable cell lines expressing ING4 or the monomeric mutants in human fibrosarcoma HT1080 cells. These tumor cells are a suitable model for the functional analysis of ING4 because the level of endogenous ING4 protein is very low (5). Infected HT1080 cells were treated for 24 h with doxorubicin prior to measurement of cell
viability. ING4 expression almost doubled the number of dead cells, whereas the expression of the monomeric mutants did not significantly increase the number of dead cells (Fig. 5A). The expression of the ING4 variant proteins in the HT1080 cells was confirmed by Western blotting (Fig. 5B). The differential effect on cell death in response to DNA damage was also evaluated in mouse NIH3T3 fibroblasts, a well characterized system previously used to study cell proliferation and apoptosis mediated by ING proteins (21, 31). NIH3T3 cells infected with the same ING4 variants yielded results similar to those of HT1080 cells (supplemental Fig. S4A). That cell death was at least partially due to apoptosis was confirmed by the observation of poly(ADP-ribose) polymerase fragmentation (supplemental Fig. S4B). Poly(ADP-ribose) polymerase is a DNA nick sensor used as an apoptosis marker (32).

ING4 was identified as a tumor suppressor in a screen for genes that suppress loss of contact inhibition in human cancer cells as measured by anchorage-independent cell growth in soft agar (24). We show that ING4 expression reduced the number of HT1080 colonies in soft agar by almost 50%, whereas the expression of the triple mutant had a much smaller effect, and the effect of the deletion mutant was statistically non-significant compared with the vector (Fig. 5C).

Are Other ING Proteins Also Dimeric?—The long α2 helix contains the so-called leucine zipper-like region present in the family of ING sequences (33). To evaluate the possibility that the other ING proteins also form homodimers, we modeled them on the ING4 structure and inspected their intermolecular interactions (supplemental Fig. S5). Because the overall distribution of hydrophobic and polar residues is conserved and matches, to a large extent, the heptad pattern of coiled coils, the modeled structures of all of the homodimers are very similar. However, the buried salt bridge Asp-32/Lys-39, which stabilizes the ING4 dimer, cannot be formed by ING1, ING2, or ING3 because the residues corresponding to Lys-39 are hydrophobic in those proteins (Fig. 2B). The buried charge of Asp-32 would destabilize the dimer unless conformational changes expose it to the solvent. Salt bridges and/or hydrogen bonds between residues at positions 94/69 and 101/66 (ING4 numbering) are possible, however, in all of the putative homodimers except in ING3, where residue 66 is an alanine. A quantitative estimation of the potential for dimerization was obtained by computation of the free energy of interaction of the two protomers for each ING protein model. The calculated free energies are not precise estimations of the stability of the dimers, but their relative values using ING4 as a reference provide a criterion to rank them by their relative stability (18). Nevertheless, this theoretical prediction based on the ING4 structure will need experimental validation. The relative free energies predict that ING5 may form a dimer as stable as ING4, whereas other homodimers are predicted to be much less stable (supplemental Fig. S5). The possibility of heterodimer formation has been evaluated in the same way, with the prediction that ING4/ING5 heterodimers may be as stable as the corresponding homodimers (supplemental Fig. S6).
DISCUSSION

Experimentally determined protein coiled-coil structures consist of two to six helices, with a single reported case of seven helices in a designed variant of the transcription factor GCN4 (34). The crystal structure of the ING4 N-terminal domain resembles the structure of the RNA-binding protein Rop (repressor of primer), the paradigm of four-helix antiparallel coiled coils (35), but with longer helices and loops and an additional two-turn-long α1 helix. The residues of this short helix interact with residues not within the dimer where they belong but with one of the other two homodimers that exist in the asymmetric unit (for example, a cation-π interaction between the aromatic ring of Tyr-6 and the guanidinium group of Arg-29 in a different dimer) or with a dimer located in an adjacent asymmetric unit (such as the ionic pair between the carboxyl of Glu-8 and the guanidinium of Arg-98 of another dimer). This observation suggests that helix α1 does not form an integral part of the dimeric coiled-coil structure and may be a dynamic local structure stabilized in the crystal lattice.

The antiparallel arrangement of the dimerization domain of ING4 has important implications for ING4 targeting to chromatin sites. The full-length ING4 protein is an elongated molecule with two PHD fingers tethered by flexible regions to the 65-Å-long dimerization domain and pointing to opposite directions. Each PHD has access to the space in a sphere centered at the tip of the coiled coil with a radius of ∼250 Å (the length of the 85 residues of the central region in the fully extended conformation, 3 Å/residue), excluding the volume occupied by the ING4 protein itself. Because of its elongated shape and the large conformational space accessible to the PHD fingers, the ING4 dimer may be able to bind simultaneously two histone H3 tails on the same or different nucleosomes.

The structure of a tetranucleosome shows a possible association of the nucleosomes within the chromatin fiber and their relative orientation (36). The flexible tails of histone H3 are not visible, but an estimation of the separation between the N-terminal tails can be obtained from the distance between the His-38 residues (the first ones visible in the electron density). The distance between the histone H3 tails in the same nucleosome is 73 Å, and that between two tails in two consecutive nucleosomes is in the range of 79–112 Å. Thus, a single ING4 dimer could bind the two histone H3 tails of the same or adjacent nucleosomes (Fig. 6). In the nuclei, with an estimated DNA concentration of 100 g/liter (37), ING4 could bind nucleosomes far away in the chromatin fiber but close in space, acting at the

FIGURE 5. Dimerization of ING4 is essential for apoptosis induction and is important for anchorage-independent growth. A, percentages of nonviable HT1080 cells expressing ING4 constructs after 24 h of treatment with different concentrations of doxorubicin. B, Western blot analysis of the expression levels of the different ING4 variants in the infected HT1080 cells. The asterisk indicates an unknown protein. C, average number of HT1080 cell colonies per field expressing the different ING4 constructs after 15 days of plating and growth in soft agar. In A and C, the represented data are the mean ± S.D. of two independent experiments; see “Experimental Procedures” for a detailed description of each experiment. The statistical significance of the differences in each independent experiment is indicated: ***, p < 0.0001; **, p < 0.003; *, p < 0.05.

FIGURE 6. ING4 dimers can bind two histone H3 tails on same or different nucleosomes. Models are shown of the ING4 dimer engaging the histone H3 N-terminal tails of the same nucleosome (left) or of two different nucleosomes (right) through its PHD fingers. The flexible nuclear localization sequence regions of ING4 and the histone H3 tails are represented as curvy lines. The single nucleosome structure (48) is represented with the DNA as an orange rod (backbone) and blue-green sticks (bases) and the histone proteins as ribbons of different colors: H3 in blue, H4 in red, H2A in magenta, and H2B in yellow. For the sake of clarity, the structure of the tetranucleosome (36) is represented with the histone octamers as white surfaces (except for the histone H3 N-terminal ends, which are in blue) and the PHD fingers as colored surfaces. The Protein Data Bank codes for the nucleosome and tetranucleosome structures used in the figure are 1AOI and 1ZBB, respectively.
same time on the transcriptional regulation of distant DNA sequences. It is also possible that the ING4 dimer binds only one nucleosome through one PHD, leaving the other free or available for recruiting other binding partners. The only other protein reported to bind to the ING4 PHD is the hypoxia-inducible factor prolyl hydroxylase-2, an association linked to repression of hypoxia-inducible factor-α under hypoxic conditions (38). These binding modes are not mutually exclusive because different ING4 pools could be involved in distinct association modes. ING4 binds histone H3K4me3 with an affinity 2 orders of magnitude larger than for the non-methylated tail. Thus, this histone modification would favor one structural assembly over another depending on the methylation state of the histone H3 tails. ING4 binding to two nucleosomes could increase the compactness of the regions with the H3K4me3 mark, sequestering them from other complexes. As part of the HBO1 complex, ING4 could recruit it to one or two adjacent nucleosomes, modulating its histone acetyltransferase activity.

ING4 is expressed in many human tissues but is down-regulated in several types of cancer cells (39). In HT1080 cells, ING4 has been shown to facilitate apoptosis in response to genotoxic stress and to inhibit anchorage-independent cell growth in a manner dependent on its ability to interact with H3K4me3 (5). We have shown here that dimerization is also necessary for both of these functions (Fig. 5). This result is consistent with the leucine zipper-like region being required for ING2-mediated apoptosis after UV irradiation (40). A full account of the link between dimerization of ING4 and its tumor-suppressing activity is not possible with the available data. The bivalent binding may be necessary for (or may enhance) the transcription of certain apoptosis-promoting genes. This effect is not directly related to the HBO1 complex because the monomeric ING4 mutants can associate with HBO1 inside the cells. However, dimerization of ING4 increases its local concentration at the target chromatin sites. Dimerization could also be relevant for the binding of ING4 to the transcription factors p53, NF-κB, and hypoxia-inducible factor-α (41, 42); for the levels of ING4 in the cytoplasm; and/or for its binding to cytoplasmic partners (43, 44). The tumor suppressor p53 partially localizes to the cytoplasm also, where it has a positive effect on apoptosis binding Bcl-2, Bcl-x, and Bak (45).

Structural models of the other ING proteins suggest that they may also form homodimers, albeit with different stabilities (supplemental Fig. S5). ING5 is predicted to form a dimer as stable as ING4 and even a stable heterodimer with ING4 (supplemental Fig. S6). Although these predictions are consistent with the high sequence identity between the dimerization domains of these two ING proteins (75%), they need experimental validation. Interestingly, in HeLa cells, both proteins form part of HBO1 complexes and are required for chromatin modification, although they play different roles in DNA replication (4). Heterodimerization could have a regulatory role, analogous to what has been observed in transcription factors (46). Other putative ING heterodimers are predicted to be much less stable (supplemental Fig. S6).

In summary, the molecular basis of ING4 dimerization and its relevance for apoptosis induction after DNA damage and cell growth have been unveiled. The dimerization and putative binding to two H3K4me3 marks on the same or different nucleosomes may be common to other ING dimers.

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