A Yeast Two-Hybrid Screen Identifies Histone H2A.Z as a Transcription Factor ZNF24 Interactor

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ABSTRACT

ZNF24 is a pleiotropic factor that has a role in transcription regulation, hematopoiesis, brain development, and cancers, but the molecular mechanisms underlying its functions are not clearly understood. In this study, histone variant H2A.Z has been identified in yeast-two-hybrid assays with ZNF24 as bait. GST pull-down, co-immunoprecipitation and co-localization assays confirm the interaction between ZNF24 and H2A.Z. H2A.Z has been implicated in many diverse biological processes. High expression of H2A.Z is ubiquitously detected in the progression of breast cancer, and is significantly associated with lymph node metastasis and patient survival. Thus, our results provide important information for the molecular mechanisms of ZNF24 functions and suggest that ZNF24 may be is implicated in transcriptional regulation of genes associated with oncogenesis by interaction with H2A.Z. J. Cell. Biochem. 113: 3411–3418, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: ZNF24; H2A.Z; YEAST-TWO-HYBRID; INTERACTION; TRANSCRIPTIONAL REGULATION; TUMORIGENESIS

The zinc finger protein 24 (ZNF24, also known as ZNF/Zfp191 [Shi et al., 1998; Han et al., 1999]) belongs to the SCAN domain subfamily of Krüppel-like zinc finger transcription factors [Sander et al., 2003]. This gene is initially named as RSG-A (for retinoic acid suppressed gene-A) because its mRNA can be amplified by homologous RT-PCR only in retinoic acid-untreated but not in retinoic acid-treated acute promyelocytic leukemia NB4 cells [Han et al., 1999]. ZNF24 contains four continuous typical C_2H_2 zinc fingers in its C-terminus, and one SCAN domain in its N-terminus [Rousseau-Merck et al., 1991; Han et al., 1999]. Whereas Krüppel-like zinc fingers bind to DNA-specific sequences and are widely represented in all species [Klug and Schwabe, 1995], the SCAN domain participates in protein–protein interactions and has to date only been found in vertebrates [Schumacher et al., 2000; Nam et al., 2004] with a remarked absence in birds. ZNF24 shows 94% identity to its mouse homolog Zfp191, which is the most highly conserved among the human-mouse SCAN family member orthologs pairs [Edelstein and Collins, 2005]. The SCAN domain of ZNF24 displays a suppressive effect on the transcription in CHO and NIH3T3 cells [Han et al., 1999]. The ZNF24 gene is located on chromosome 18q12.1 [Rousseau-Merck et al., 1991; Shi et al., 1998; Han et al., 1999], a region frequently deleted in a number of

human cancers [Vogelstein et al., 1988; Kern et al., 1989; Papadimitrakopoulou et al., 1998; Richard et al., 2000].

ZNF24 is involved in negative regulation of VEGF and PDGFRB and may represent a novel repressor of VEGF and PDGFRB transcription [Harper et al., 2007; Li et al., 2010]. We have recently shown that ZNF24 is a pleiotropic factor that has a role in hematopoiesis, brain development and cancers [Li et al., 2009], but the molecular mechanisms underlying its functions are not clearly understood. Our previous study, gene targeting provided the first evidence that ZNF24 has important functions, because the null mutation caused early embryonic lethality in mice [Li et al., 2006]. Recent studies suggest ZNF24 is necessary to maintain neural cells in a cycling progenitor status by preventing them from leaving the cell cycle and being committed into a differentiation pathway [Khalfallah et al., 2009]. In addition, ZNF24 is required for the myelinating function of differentiated oligodendrocytes [Howng et al., 2010].

As identification of proteins that interact with ZNF24 may provide important clues to understanding its regulation and function, we performed a protein–protein interaction screen using ZNF24 as bait. By doing so, we identified the histone H2A.Z, as a novel ZNF24 interacting protein. Based on our data and those

reported by others, we propose that this newly identified protein–protein interaction might be involved in transcriptional regulation of genes associated with oncogenesis.

MATERIALS AND METHODS

PLASMID CONSTRUCTION

DNA encoding amino acids 1–250 of human ZNF24 was amplified by PCR using appropriate primers, and the product was cloned into the *Eco*RI/*Sal*I sites of pGBKT7 (Clontech) vector. The resulting construct, pGBKT7-ZNF24 (1–250), was used as the bait plasmid in yeast two-hybrid experiments described throughout this report. DNA encoding amino acids 1–250 or full-length of human ZNF24 was amplified by PCR and was cloned into the *Xho*I/*Sac*II sites of pEGFP-N1 (Clontech), the *Sal*I/*Bgl*II sites of pCMV-HA (Clontech) and the *Bam*HI/*Xho*I sites of PGEX-4T-2 (GE Healthcare Life Sciences) yielding pEGFP-ZNF24-1-250, pEGFP-ZNF24, pCMV-HA-ZNF24, and pGEX-ZNF24, respectively. Full-length H2A.Z was amplified by PCR and was cloned into the *Hind*III/*Sac*II sites of pDsRed-Monomer-N1 (Clontech) and the *Xho*I/*Hind*III sites of pcDNA3.1-myc-His B (Invitrogen), yielding H2A.Z-dsRed and Myc-H2A.Z fusion proteins, respectively. The sequences of all constructs were confirmed by DNA sequencing.

YEAST TWO–HYBRID ASSAYS

The yeast strain AH109 was cotransformed with the Hela cDNA library (cloned into the GAL4 activation domain of the vector pACT2 (BD Clontech)) and the pGBKT7-ZNF24 (1–250) clone. Colonies that grew successfully on the selective media SD/-Ade/-His/-Leu/-Trp, were screened further for reporter gene β-galactosidase expression by the colony lift filter assay. Plasmid DNA was isolated from positive clones and transformed into *Escherichia coli* competent cells to enable isolation and sequencing. Plasmid DNA was sequenced and clones identified by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/).

CO–IMMUNOPRECIPITATIONS

Approximately 2×10^5 human embryonic kidney (HEK293) cells (obtained from American Type Culture Collection) were transiently transfected with 2 μg of the each relevant plasmid using LipofectAMINE 2000 (Invitrogen), according to the instructions of the manufacturer. After 48 h, cells were harvested and lysed in 50 mmol/L Tris–HCl (pH 8), 150 mmol/L NaCl, and 1% Triton X-100 supplemented with complete protease inhibitor cocktail (Roche Diagnostics). Lysates were sonicated and cell debris was removed by centrifugation. Supernatants were incubated with 1 μg mouse monoclonal anti-myc or anti-HA or anti-ZNF24 antibody or mouse normal IgG (Santa Cruz Biotechnology) overnight at 4°C, followed by the addition of 20 μl Protein A/G PLUS-Agarose (Santa Cruz Biotechnology). The beads were pelleted by centrifugation; the supernatant was removed, and the pelleted beads were washed five times with 1 ml of phosphate buffered saline (PBS). Proteins were eluted with 40 μl of 1× SDS protein loading buffer [0.0625 mol/L Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, and 5% β-mercaptoethanol] for 5 min at 100°C. Inputs and co-immunoprecipitates were subjected to SDS–PAGE and transferred to a nitrocellulose membrane (Hybond-C, Amersham Biosciences).

WESTERN BLOTTING

For analyzing the immunoprecipitates, Western blots carrying inputs and immunoprecipitated samples were first incubated with primary antibody (rat anti-HA or mouse anti-myc or rabbit H2A.Z) followed by appropriate HRP-conjugated antibodies. Proteins were visualized by enhanced chemiluminescence kit (Amersham Biosciences).

GST PULL–DOWN ASSAY

BL21-Gold (DE3) bacteria (Stratagene) were transformed with GST constructs, and single colonies were grown in a lysogenic broth (LB) starter culture overnight. Two hundred milliliters of LB were inoculated with 5 ml starter culture at 37°C with shaking for approximately 2 h until absorbance at 600 nm reached between 0.6 and 0.8. Culture was induced with 0.25 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and grown at 30°C for 4 h. Cells were pelleted by centrifugation at 3,000*g* for 15 min. Cells were resuspended in lysis buffer [50 mM Tris pH 8.0, 150 NaCl, 0.5% (v/v) NP-40, 1× Complete EDTA-free protease inhibitor complex (Roche)]. Resuspension was incubated with lysozyme for 0.5 h, then sonicated to homogenize lysate. Lysate was centrifuged at 15,000*g* for 30 min. Supernatant was collected and stored at −80°C until use. Cleared lysates were thawed and protein concentration was determined with BCA assay (Pierce), according to manufacturer's instructions. Glutathione-sepharose beads (Amersham-Pharmacia) were washed and resuspended in lysis buffer, to make a 50%-bead slurry. Two hundred microliters of bead slurry was incubated with 2 μg bacterial lysate for 1 h at 4°C. Beads were washed with lysis buffer. H2A.Z-Myc transfected HEK 293 cells were harvested 48 h after transfection in lysis buffer. Homogenates were cleared by centrifugation, as described in immunoprecipitation procedure. Cleared homogenates were precleared with unbound 100 μl glutathione-sepharose bead slurry for 1 h at 4°C. Extracts were then incubated with 100 μl of GST protein-bound glutathione beads for 2 h at 4°C. Beads were then washed with lysis buffer. Samples were boiled, and separated by polyacrylamide gel electrophoresis for analysis with Coomassie stain or immunoblotting.

FLUORESCENCE MICROSCOPY

HEK293 cells and human breast adenocarcinoma cells (MCF-7; obtained from American Type Culture Collection) were seeded into 24-well plates containing sterilized glass cover slips and transiently co-transfected with 250 ng vector DNA (125 ng pEGFP-ZNF24 or pEGFP-ZNF24-1-250 and 125 ng pDsRed-H2A.Z) and 2 μl Lipofectamin™ 2000 transfection reagent (Invitrogen). At 48 h post-transfection the cells mounted on coverslips were fixed with 4% (w/v) formaldehyde in PBS for 10 min at room temperature, then permeabilized with 0.5% Triton X-100 in PBS for 10 min. Images were captured using confocal laser scanning microscopy (Leica SP5). For immunofluorescence staining, the cells were fixed with 4% (w/v) formaldehyde in PBS for 10 min at room temperature, then permeabilized with 0.5% Triton X-100 in PBS for 10 min and blocked with 0.2% Triton X-100 and 5% horse serum in PBS for 1 h.

Cells were washed in PBS, subsequently incubated for 1 h with anti-ZNF24 mouse monoclonal antibody (Santa cruz Biotechnology) and anti-H2A.Z rabbit antibody (Cell signaling), washed with PBS, and incubated with donkey anti-mouse Alexa Fluor 488 antibody and donkey anti-rabbit Alexa Fluor 546 antibody (Molecular Probes, Invitrogen) for 40 min. Cells were washed with PBS, and nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI).Images were captured using Nikon Diaphot 200 inverted fluorescence microscope.

RESULTS

IDENTIFICATION OF THE HISTONE H2A.Z BINDING PROTEIN

To search for novel ZNF24 interactors, the Matchmaker Two-Hybrid System 3 was used to screen the Hela cDNA libraries fused to the GAL4 activation domain (Fig. 1B). Transfection of the ZNF24-bait alone in yeast cells did not yield any growth on histidine-deficient plates, as expected. Co-transfection of the bait with the Hela cDNA interaction library resulted in 54 colonies that grew on fully selective medium. Of these, 36 were selected for further analysis. Sequencing revealed that two of these yeast colonies contained pACT2 plasmids encoding the full-length H2A.Z. Co-transfection of these pAD-H2A.Z plasmids with standard control plasmids encoding lamin-B and p53 turned out to be negative. However, yeast cells co-transfected with the ZNF24 bait and the pACT2-H2A.Z plasmids were able to grow on fully selective medium and turned blue in a β-galactosidase assay (Fig. 1C), thereby validating the yeast two-hybrid interactions observed.

ZNF24 INTERACTS WITH H2A.Z IN VITRO

To investigate whether the ZNF24 protein interacts directly with H2A.Z, glutathione *S*-transferase (GST) pull-down analyses were performed, using GST-ZNF24 fusion proteins produced in *E. coli*.

Full-length Myc-tagged H2A.Z (H2A.Z-Myc) proteins were obtained by transfecting HEK293 cells, leading to the generation of a SDS–PAGE protein band of expected size, 14 kDa (Fig. 2A; Input). After performing GST pull-down assays using the GST-ZNF24 fusion protein and GST alone, we observed that the H2A.Z protein co-precipitated with the GST-ZNF24 fusion protein (Fig. 2A), but not with the GST protein, thus confirming a direct interaction between ZNF24 and H2A.Z.

ZNF24 INTERACTS WITH H2A.Z IN VIVO

In order to assess the ZNF24-H2A.Z interaction in mammalian cells, we transiently transfected HEK293 cells with HA-ZNF24 and/or H2A.Z-Myc constructs. Subsequently, immunoprecipitations were performed on single and double transfectants, using anti-HA and anti-Myc antibodies to precipitate the respective proteins (Fig. 2B,C). The resulting immunoprecipitation products were analyzed using western blotting in conjunction with either anti-HA (Fig. 2B) or anti-Myc (Fig. 2C) antibodies. Ten percent of total lysates were loaded as controls (Fig. 2B,C; Input). In single ZNF24 and double ZNF24/H2A.Z transfectants, ZNF24 was readily detected as a 43 kDa protein (including the HA-tag; Fig. 2B) which was not present in single H2A.Z transfectants. Conversely, in single H2A.Z and double ZNF24/H2A.Z transfectants, H2A.Z could readily be detected as a 14 kDa protein (including the Myc-tag; Fig. 2C). The variations observed in band intensities are due to variations in the respective transfection efficiencies. In order to exclude cell-type specific effects, these immunoprecipitations were replicated in Hela cells in the same fashion as outlined above. Again, we observed co-immunoprecipitation of exogenous ZNF24 and H2A.Z (data not shown).

In order to assess whether the ZNF24-H2A.Z interaction is present at physiologically relevant levels, we performed co-immunoprecipitation with an anti-ZNF24 antibody or mouse normal IgG using
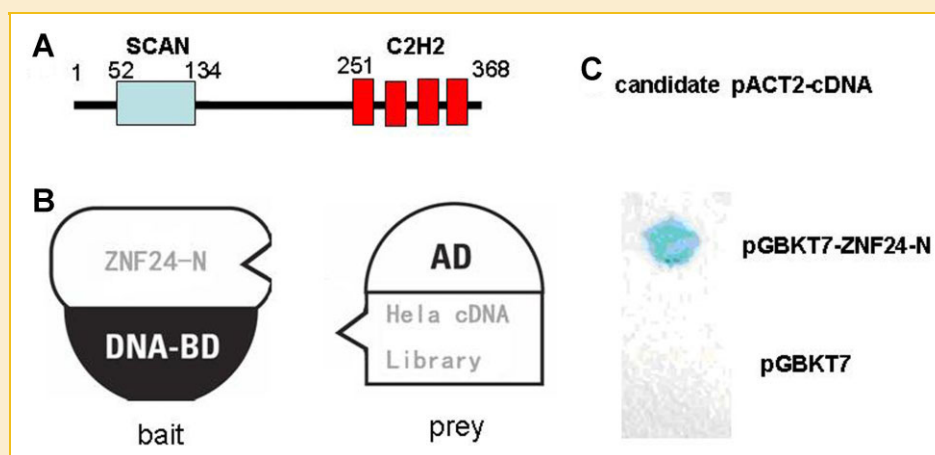

Fig. 1. Screening of interaction partners of ZNF24 in a yeast two-hybrid assay. A: Schematic diagram illustrating the structure of ZNF24 with its SCAN domain N-terminus, zinc finger C-terminus. B: Components of the yeast two-hybrid assay: bait (ZNF24-N(1–250aa) fused to GAL4-DNA binding domain) and prey (Hela cDNA library fused to GAL4 activation domain. N = N-terminus, C = C-terminus, GAL4 BD = GAL4-DNA binding domain, GAL4 AD = GAL4 activation domain. C: Results from co-transformation of the Yeast strain AH 109 with the prey-candidate (H2A.Z) and either pGBKT7-ZNF24 (bait) or pGBKT7 (control plasmid). Transformed colonies were spotted and grown on nutritionally selective plates (–Ade, –Leu, –His, –Trp) and examined by β-galactosidase activity. Blue colonies indicate reporter gene expression in AH109. When AH109 is cotransformed with the candidate plasmid pACT2-cDNA and the insert-less pGBKT7 absent colony growth proved for the correctness of the candidate. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]
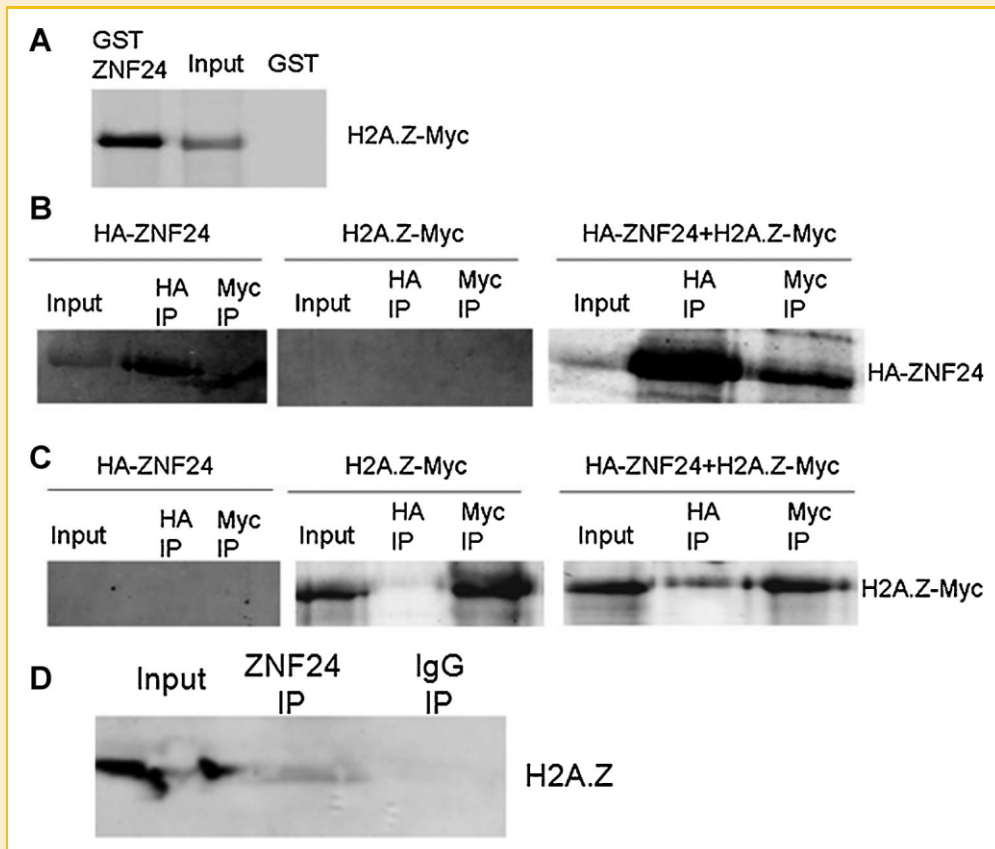
Fig. 2. ZNF24 interacts with H2A.Z. A: HEK293 cells expressing H2A.Z–Myc protein were lysed and probed for interaction with either GST–ZNF24 or GST alone. Specific interaction between ZNF24 and H2A.Z was detected by western blot analysis using an anti–Myc antibody. B, C: HEK293 cells were transiently transfected with HA–ZNF24, H2A.Z–Myc or both as indicated on top. Immunoprecipitations were performed with anti–HA and anti–Myc antibodies as indicated. Western blot analyses were performed with (B) an anti–HA antibody, and (C) an anti–Myc–antibody. The positions of the immunoreactive HA–ZNF24 and H2A.Z–Myc proteins are marked on the right. D: Immunoprecipitations were performed with an anti–ZNF24 antibody or normal IgG using normal HEK293 cells, followed by immunoblotting with an anti–H2A.Z antibody.

normal HEK293 cells, followed by immunoblotting with an anti-H2A.Z antibody. Figure 2D showed that after immunoprecipitation with the ZNF24-specific antibody, H2A.Z was detected, but not with mouse normal IgG. Taken together, these combined immunoprecipitation analyses confirm the presence of an in vivo physical interaction between these two proteins in mammalian cells.

ZNF24 AND H2A.Z CO-LOCALIZE WITHIN MAMMALIAN CELLS

To assess whether the observed interaction between ZNF24 and H2A.Z is operative in mammalian cells, we determined the sub-cellular localization of both proteins in HEK293 and MCF-7 cells. HEK293 and MCF-7 cells were transiently co-transfected with ZNF24-GFP and H2A.Z-dsRed expression constructs. Subsequently, the transfected cells were assayed for the sub-cellular localization of the respective proteins. Double ZNF24 and H2A.Z transfectants showed a (near) perfect nuclear co-localization (Fig. 3A,B). Moreover, immunofluorescence staining also shows that ZNF24 and H2A.Z proteins have a (near) perfect nuclear co-localization in HEK293 (Fig. 3C), suggesting that the interaction of ZNF24 with H2A.Z is present at physiologically relevant levels.

In order to assess whether the ZNF24-1-250 which lacks zinc finger region (251-368aa) and H2A.Z interaction is present in living cells, HEK293 cells were transiently transfected with expression plasmids encoding ZNF24-1-250-EGFP and H2A.Z-dsRed, either each alone or both together. HEK293 cells transfected with GFP or red fluorescent protein (RFP) alone displayed diffuse fluorescence (data not shown). As shown in Figure 4A, cells transfected with GFP-tagged ZNF24-1-250 indicated a whole-cell distribution. Cells transfected with H2A.Z-dsRed indicated a predominantly nuclear localization (Fig. 4B). When cells co-expressed GFP-tagged ZNF24-1-250 and dsRed-tagged H2A.Z (Fig. 4C), the subcellular localization of H2A.Z remained primarily in the nucleus; interestingly, a small quantity of cells showed nuclear localization of ZNF24-1-250-EGFP. Fluorescence images analysis showed that ZNF24-1-250 and H2A.Z co-localized in the nucleus, suggesting that the N-terminus of ZNF24 from amino acids 1 to 250 was sufficient for H2A.Z binding.

DISCUSSION

Zinc finger proteins represent the largest family of transcription factors, because of their large representation within all species, their implication in a wide range of functions, and their association with
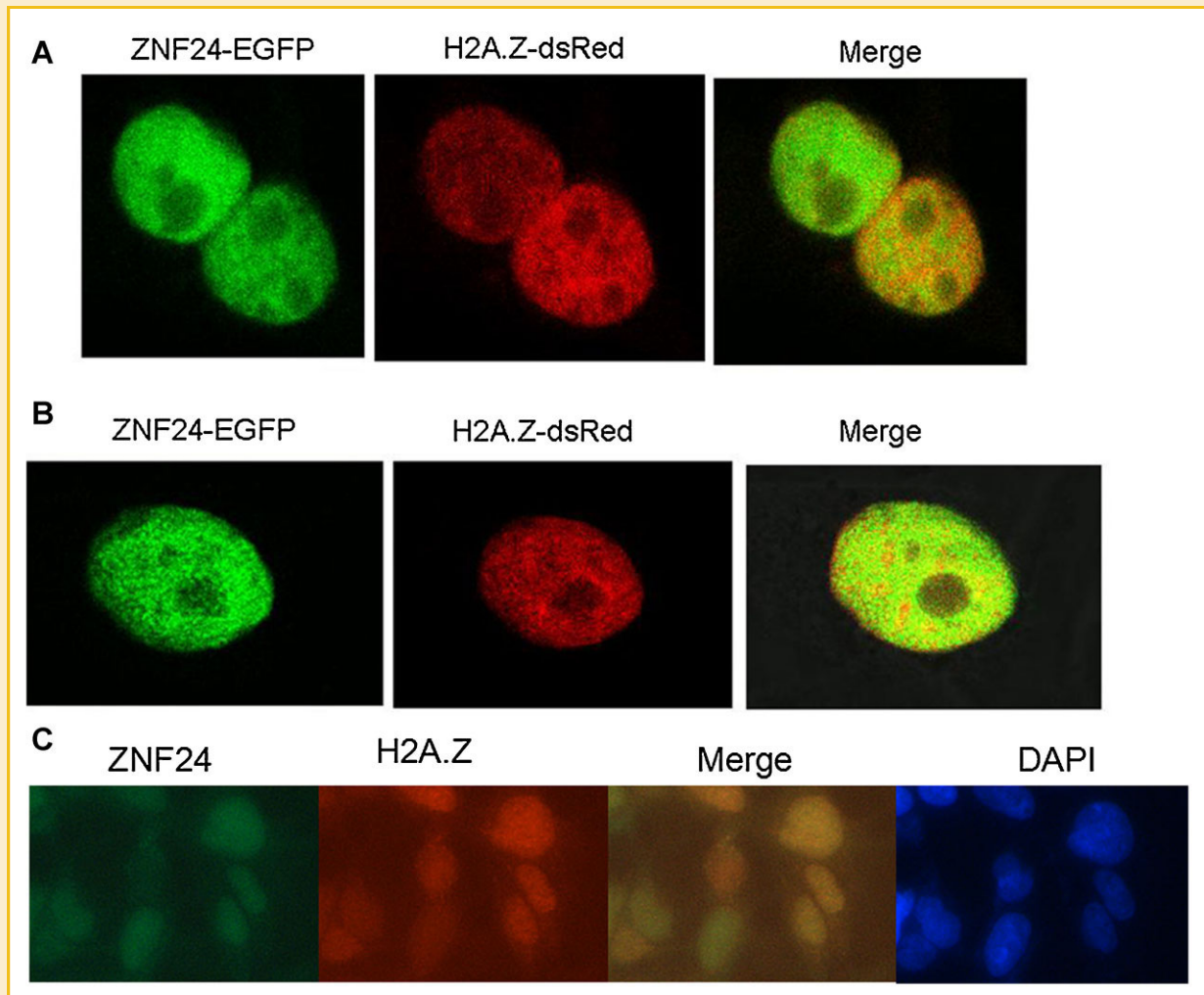
Fig. 3. Sub-cellular (co-)localization of ZNF24, H2A.Z in HEK293 and MCF-7 cells. A: HEK293 cells were transiently co-transfected with ZNF24-EGFP (green) and H2A.Z-dsRed (red) expression constructs. B: MCF-7 cells were transiently co-transfected with ZNF24-EGFP (green) and H2A.Z-dsRed (red) expression constructs. Images were captured using confocal laser scanning microscopy. C: HEK293 cells were stained with anti-ZNF24 antibody (green), anti-H2A.Z antibody (red) and DAPI staining of the nuclei (blue). The overlay of the different signals (Merge) reveals a partial colocalization of the respective proteins. Images were captured using fluorescent microscopy. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

other functional domains, such as homeodomains or KRAB or SCAN domains. The SCAN domain [Williams et al., 1995] (an acronym for the proteins in which it was first found—SRE-ZBP, CTfin-51, AW-1, Number 18 cDNA—and also known as LER, for Leucine Rich Repeat) is a highly conserved 84-residue motif that appears to be specific to vertebrates [Letunic et al., 2004] and may play a role in the assembly and function of the SCAN zinc finger transcription factors by mediating homo- and hetero-oligomerization [Sander et al., 2000; Williams et al., 1999]. ZNF24 has been isolated from a screening for Krüppel-like zinc finger proteins expressed in hematopoietic cells [Han et al., 1999]. This protein also contains a SCAN domain and is able to act as a repressor of transcription [Han et al., 1999], but its function in hematopoietic cells has not been clarified. In support of a function for ZNF24 in transcription, we found that ZNF24-EGFP localized to the nucleus of cultured HEK293 and MCF-7 cells (Fig. 3). Our previous study, gene targeting provided the first evidence that ZNF24 has important functions, because the null mutation caused early embryonic lethality in mice [Li et al., 2006]. Our further analysis reveals that ZNF24 is a pleiotropic factor that has a role in hematopoiesis, brain development and cancers [Li et al., 2009]. More recently, studies show that ZNF24 is necessary to maintain neural cells as cycling progenitors [Khalfallah et al., 2009] and the myelinating function of differentiated oligodendrocytes [Howng et al., 2010], but the molecular mechanisms underlying its functions are not clearly understood.

In the present work, using a yeast two-hybrid interaction trap we identified the H2A.Z as a novel ZNF24 interacting protein. The conclusion that H2A.Z is a ZNF24-interacting protein was supported by results from several different experimental approaches. First, the in vitro GST pull-down assays of GST-ZNF24 expressed in bacteria and H2A.Z-Myc produced in HEK293 cells showed the interaction between the full-length ZNF24 and H2A.Z (Fig. 2A). Second, co-immunoprecipitation of ZNF24 and H2A.Z co-expressed in HEK293 and Hela cells confirmed their interaction (Fig. 2B,C). Third, confocal
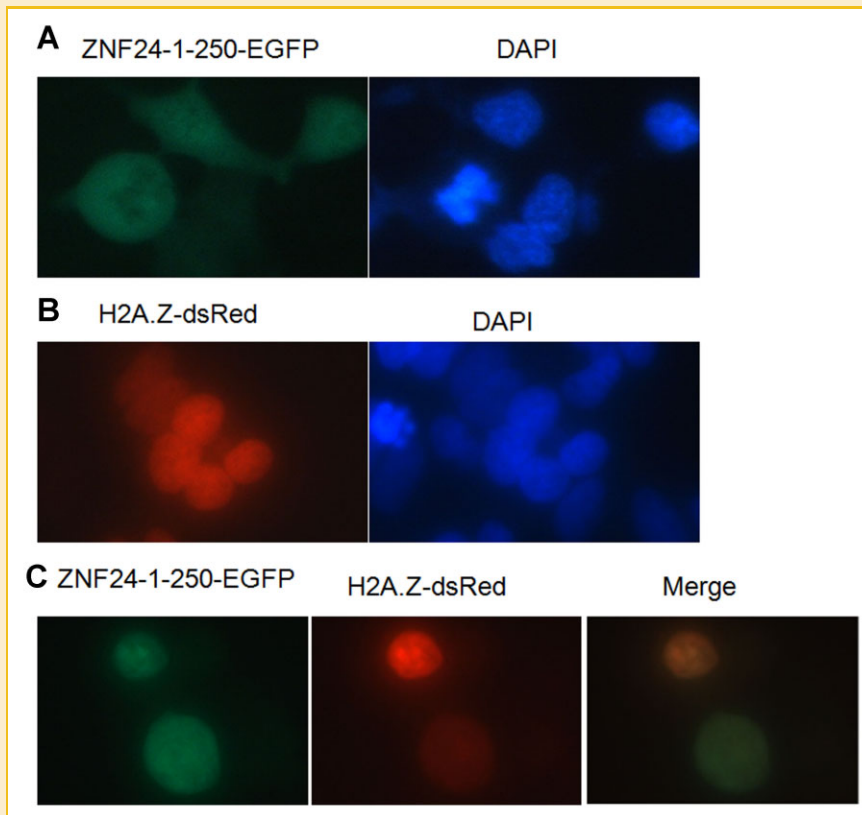
Fig. 4. Common subcellular localization between ZNF24-1-250 and H2A.Z protein was identified. A: HEK293 cells were transiently transfected with ZNF24-1-250-EGFP (green) or (B) H2A.Z-dsRed (red) expression constructs. C: HEK293 cells were transiently co-transfected with ZNF24-1-250-EGFP and H2A.Z-dsRed expression constructs. Images were captured using fluorescent microscopy. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

analysis showed that ZNF24 and H2A.Z co-localized in the nucleus of cultured HEK293 cells (Fig. 3). Although we have not determined the precise boundaries of the H2A.Z binding site within ZNF24 protein, our data suggested that the N-terminus of ZNF24 was sufficient for H2A.Z binding.

H2A.Z is a highly conserved histone variant. It is found in organisms as diverse as the malaria-causing protozoan parasite *Plasmodium falciparum*, the yeast *Saccharomyces cerevisiae*, and *Homo sapiens*, with sequence conservation of ~90% [Iouzalen et al., 1996], which suggests unique and important functions for H2A.Z. Although not essential to yeast cells, the loss of H2A.Z is lethal in others species, such as *Tetrahymena thermophila* [Liu et al., 1996], *Xenopus laevis* [Iouzalen et al., 1996; Ridgway et al., 2004], *Drosophila melanogaster* [Clarkson et al., 1999] and mice [Faast et al., 2001]. In mammalian cells, H2A.Z is important for chromosome segregation, centromeric functions and transcriptional regulation [Rangasamy et al., 2003, 2004; Gevry et al., 2007, 2009; Hardy et al., 2009]. Genome-wide studies have provided strong evidence of the non-random pattern of H2A.Z distribution. In euchromatic regions, a sharp peak of H2A.Z is observed at the 5' end of numerous genes, as well as in enhancers and in insulators. H2A.Z nucleosomes are observed in regions flanking the transcriptional start site, with a region that is devoid of nucleosomes in between, thus forming a nucleosome-free region (NFR) over the start site [Guillemette et al., 2005; Zhang et al., 2005; Albert et al., 2007; Barski et al., 2007]. The enrichment of H2A.Z at promoter sequence often affects local histone modification patterns and chromatin conformation, which in turn influences transcriptional output. In yeast cells, the presence of H2A.Z at gene promoters is generally inversely correlated with transcription levels [Guillemette et al., 2005; Zhang et al., 2005]. In contrast to the situation in yeast, the presence of H2A.Z at promoters is positively correlated with transcription in human cells and in Drosophila [Barski et al., 2007; Mavrich et al., 2008]. Recent analysis of genome-wide mapping of nucleosome positions in human genome reveals that H2A.Z is enriched at transcriptional regulatory elements of several important genes including BRCA1 [Barski et al., 2007; Schones et al., 2008]. Interestingly, a inverse correlation between the levels of ZNF24 and BRCA1 is observed by ZNF24 overexpression and knockdown in HEK293 cells [Li et al., 2009]. Taken together, these findings suggest that the interaction of ZNF24 and H2A.Z may be involved in transcriptional regulation. The immediate future goal is to understand functions of ZNF24 in transcription when it interacts with H2A.Z.

H2A.Z has been implicated in many diverse biological processes, such as gene activation, chromosome segregation, heterochromatin silencing, and progression through the cell cycle [Zlatanova and Thakar, 2008]. Studies in cancer patients have reported significant

overexpression of H2A.Z in several major types of malignancies, especially at the metastatic stage [Dunican et al., 2002; Rhodes et al., 2004; Zucchi et al., 2004; Hua et al., 2008]. *ZNF24* was frequently down-regulated in ovarian malignant tumors, colon carcinomas and breast carcinoma compared with respective adjacent normal tissues [Zhang. et al., 2003; Harper et al., 2007]. Taken together, these findings suggest that both H2A.Z and ZNF24 are likely involved in oncogenesis.

In summary, H2A.Z has been identified as a novel ZNF24 interacting protein. This novel protein–protein interaction might be involved in transcriptional regulation of genes associated with oncogenesis.

ACKNOWLEDGMENTS

REFERENCES

Albert I, Mavrich TN, Tomsho LP, Qi J, Zanton SJ, Schuster SC, Pugh BF. 2007. Translational and rotational settings of H2A.Z. nucleosomes across the *Saccharomyces cerevisiae* genome. Nature 446:572–576.

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007. High-resolution profiling of histone methylations in the human genome. Cell 129:823–837.

Clarkson MJ, Wells JR, Gibson F, Saint R, Tremethick DJ. 1999. Regions of variant histone His2AvD required for Drosophila development. Nature 399:694–697.

Dunican DS, McWilliam P, Tighe O, Parle-McDermott A, Croke DT. 2002. Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. Oncogene 21:3253–3257.

Edelstein LC, Collins T. 2005. The SCAN domain family of zinc finger transcription factors. Gene 359:1–17.

Faast R, Thonglairoam V, Schulz TC, Beall J, Wells JR, Taylor H, Matthaei K, Rathjen PD, Tremethick DJ, Lyons I. 2001. Histone variant H2A.Z. is required for early mammalian development. Curr Biol 11:1183–1187.

Gevry N, Chan HM, Laflamme L, Livingston DM, Gaudreau L. 2007. p21 transcription is regulated by differential localization of histone H2A. Z Genes Dev 21:1869–1881.

Gevry N, Hardy S, Jacques PE, Laflamme L, Svotelis A, Robert F, Gaudreau L. 2009. Histone H2A.Z. is essential for estrogen receptor signaling. Genes Dev 23:1522–1533.

Guillemette B, Bataille AR, Gevry N, Adam M, Blanchette M, Robert F, Gaudreau L. 2005. Variant histone H2A.Z. is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol 3:e384.

Han ZG, Zhang QH, Ye M, Kan LX, Gu BW, He KL, Shi SL, Zhou J, Fu G, Mao M, Chen SJ, Yu L, Chen Z. 1999. Molecular cloning of six novel Kruppel-like zinc finger genes from hematopoietic cells and identification of a novel transregulatory domain KRNB. J Biol Chem 274:35741–35748.

Hardy S, Jacques PE, Gevry N, Forest A, Fortin ME, Laflamme L, Gaudreau L, Robert F. 2009. The euchromatic and heterochromatic landscapes are shaped by antagonizing effects of transcription on H2A.Z. deposition. PLoS Genet 5:e1000687.

Harper J, Yan L, Loureiro RM, Wu I, Fang J, D'Amore PA, Moses MA. 2007. Repression of vascular endothelial growth factor expression by the zinc finger transcription factor ZNF24. Cancer Res 67:8736–8741.

Howng SY, Avila RL, Emery B, Traka M, Lin W, Watkins T, Cook S, Bronson R, Davisson M, Barres BA, Popko B. 2010. ZFP191 is required by oligodendrocytes for CNS myelination. Genes Dev 24:301–311.

Hua S, Kallen CB, Dhar R, Baquero MT, Mason CE, Russell BA, Shah PK, Liu J, Khramtsov A, Tretiakova MS, Krausz TN, Olopade OI, Rimm DL, White KP. 2008. Genomic analysis of estrogen cascade reveals histone variant H2A.Z. associated with breast cancer progression. Mol Syst Biol 4:188.

Iouzalen N, Moreau J, Mechali M. 1996. H2A.ZI, a new variant histone expressed during Xenopus early development exhibits several distinct features from the core histone H2A. Nucleic Acids Res 24:3947–3952.

Kern SE, Fearon ER, Tersmette KW, Enterline JP, Leppert M, Nakamura Y, White R, Vogelstein B, Hamilton SR. 1989. Clinical and pathological associations with allelic loss in colorectal carcinoma [corrected]. JAMA 261:3099–3103.

Khalfallah O, Ravassard P, Lagache CS, Fligny C, Serre A, Bayard E, Faucon-Biguet N, Mallet J, Meloni R, Nardelli J. 2009. Zinc finger protein 191 (ZNF191/Zfp191) is necessary to maintain neural cells as cycling progenitors. Stem Cells 27:1643–1653.

Klug A, Schwabe JW. 1995. Protein motifs 5. Zinc fingers. FASEB J 9:597–604.

Letunic I, Copley RR, Schmidt S, Ciccarelli FD, Doerks T, Schultz J, Ponting CP, Bork P. 2004. SMART 4.0: Towards genomic data integration. Nucleic Acids Res 32:D142–D144.

Li J, Chen X, Yang H, Wang S, Guo B, Yu L, Wang Z, Fu J. 2006. The zinc finger transcription factor 191 is required for early embryonic development and cell proliferation. Exp Cell Res 312:3990–3998.

Li J, Chen X, Gong X, Liu Y, Feng H, Qiu L, Hu Z, Zhang J. 2009. A transcript profiling approach reveals the zinc finger transcription factor ZNF191 is a pleiotropic factor. BMC Genomics 10:241.

Li J, Chen X, Liu Y, Ding L, Qiu L, Hu Z, Zhang J. 2010. The transcriptional repression of platelet-derived growth factor receptor-beta by the zinc finger transcription factor ZNF24. Biochem Biophys Res Commun 397:318–322.

Liu X, Li B, Gorovsky MA. 1996. Essential and nonessential histone H2A variants in Tetrahymena thermophila. Mol Cell Biol 16:4305–4311.

Mavrich TN, Jiang C, Ioshikhes IP, Li X, Venters BJ, Zanton SJ, Tomsho LP, Qi J, Glaser RL, Schuster SC, Gilmour DS, Albert I, Pugh BF. 2008. Nucleosome organization in the Drosophila genome. Nature 453:358–362.

Nam K, Honer C, Schumacher C. 2004. Structural components of SCAN-domain dimerizations. Proteins 56:685–692.

Papadimitrakopoulou VA, Oh Y, El-Naggar A, Izzo J, Clayman G, Mao L. 1998. Presence of multiple incontiguous deleted regions at the long arm of chromosome 18 in head and neck cancer. Clin Cancer Res 4:539–544.

Rangasamy D, Berven L, Ridgway P, Tremethick DJ. 2003. Pericentric heterochromatin becomes enriched with H2A.Z. during early mammalian development. EMBO J 22:1599–1607.

Rangasamy D, Greaves I, Tremethick DJ. 2004. RNA interference demonstrates a novel role for H2A.Z. in chromosome segregation. Nat Struct Mol Biol 11:650–655.

Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A, Chinnaiyan AM. 2004. Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. Proc Natl Acad Sci USA 101:9309–9314.

Richard F, Pacyna-Gengelbach M, Schluns K, Fleige B, Winzer KJ, Szymas J, Dietel M, Petersen I, Schwendel A. 2000. Patterns of chromosomal imbalances in invasive breast cancer. Int J Cancer 89:305–310.

Ridgway P, Brown KD, Rangasamy D, Svensson U, Tremethick DJ. 2004. Unique residues on the H2A.Z. containing nucleosome surface are important for *Xenopus laevis* development. J Biol Chem 279:43815–43820.

Rousseau-Merck MF, Huebner K, Berger R, Thiesen HJ. 1991. Chromosomal localization of two human zinc finger protein genes, ZNF24 (KOX17) and

ZNF29 (KOX26), to 18q12 and 17p13-p12, respectively. Genomics 9:154–161.

Sander TL, Haas AL, Peterson MJ, Morris JF. 2000. Identification of a novel SCAN box-related protein that interacts with MZF1B. The leucine-rich SCAN box mediates hetero- and homoprotein associations. J Biol Chem 275: 12857–12867.

Sander TL, Stringer KF, Maki JL, Szauter P, Stone JR, Collins T. 2003. The SCAN domain defines a large family of zinc finger transcription factors. Gene 310:29–38.

Schones DE, Cui K, Cuddapah S, Roh TY, Barski A, Wang Z, Wei G, Zhao K. 2008. Dynamic regulation of nucleosome positioning in the human genome. Cell 132:887–898.

Schumacher C, Wang H, Honer C, Ding W, Koehn J, Lawrence Q, Coulis CM, Wang LL, Ballinger D, Bowen BR, Wagner S. 2000. The SCAN domain mediates selective oligomerization. J Biol Chem 275:17173–17179.

Shi SL, Liu MM, Yu L, Chen SJ, Zheng QP, Wu GJ, Chen Z, Zhao SY. 1998. Assignment of a novel zinc finger gene ZNF191 to human chromosome 18Q12.1 by human/rodent somatic cell hybrid panel and fluorescent in situ hybridization. Shi Yan Sheng Wu Xue Bao 31:21–27.

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. 1988. Genetic alterations during colorectal-tumor development. N Engl J Med 319:525–532.

Williams AJ, Khachigian LM, Shows T, Collins T. 1995. Isolation and characterization of a novel zinc-finger protein with transcription repressor activity. J Biol Chem 270:22143–22152.

Williams AJ, Blacklow SC, Collins T. 1999. The zinc finger-associated SCAN box is a conserved oligomerization domain. Mol Cell Biol 19:8526–8535.

Zhang H, Roberts DN, Cairns BR. 2005. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell 123:219–231.

Zhang H, Cai Y, Lu L. 2003. Expression of ZNF191 mRNA in ovarian malignant tumors. Acad J Sec Mil Med Univ 24:1381–1382.

Zlatanova J, Thakar A. 2008. H2A.Z: View from the top. Structure 16: 166–179.

Zucchi I, Mento E, Kuznetsov VA, Scotti M, Valsecchi V, Simionati B, Vicinanza E, Valle G, Pilotti S, Reinbold R, Vezzoni P, Albertini A, Dulbecco R. 2004. Gene expression profiles of epithelial cells microscopically isolated from a breast-invasive ductal carcinoma and a nodal metastasis. Proc Natl Acad Sci USA 101:18147–18152.