ZNF143 Mediates Basal and Tissue-specific Expression of Human Transaldolase*

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Transaldolase regulates redox-dependent apoptosis through controlling NADPH and ribose 5-phosphate production via the pentose phosphate pathway. The minimal promoter sufficient to drive chloramphenicol acetyltransferase reporter gene activity was mapped to nucleotides −49 to −1 relative to the transcription start site of the human transaldolase gene. DNase I footprinting with nuclear extracts of transaldolase-expressing cell lines unveiled protection of nucleotides −29 to −16. Electrophoretic mobility shift assays identified a single dominant DNA-protein complex that was abolished by consensus sequence for transcription factor ZNF143/76 or mutation of the ZNF76/143 motif within the transaldolase promoter. Mutation of an AP-2α recognition sequence, partially overlapping the ZNF143 motif, increased TAL-H promoter activity in HeLa cells, without significant impact on HepG2 cells, which do not express AP-2α. Cooperativity of ZNF143 with AP-2α was supported by supershift analysis of HeLa cells where AP-2 may act as cell type-specific repressor of TAL promoter activity. However, overexpression of full-length ZNF143, ZNF76, or dominant-negative DNA-binding domain of ZNF143 enhanced, maintained, or abolished transaldolase promoter activity, respectively, in HepG2 and HeLa cells, suggesting that ZNF143 initiates transcription from the transaldolase core promoter. ZNF143 overexpression also increased transaldolase enzyme activity. ZNF143 and transaldolase expression correlated in 21 different human tissues and were coordinately up-regulated 14- and 34-fold, respectively, in lactating mammary glands compared with nonlactating ones. Chromatin immunoprecipitation studies confirm that ZNF143/73 associates with the transaldolase promoter in vivo. Thus, ZNF143 plays a key role in basal and tissue-specific expression of transaldolase and regulation of the metabolic network controlling cell survival and differentiation.

Metabolism of glucose through the pentose phosphate pathway (PPP) fulfills two unique functions: formation of ribose 5-phosphate for the synthesis of nucleotides, RNA and DNA; and generation of NADPH as a reducing equivalent for biosynthetic reactions. Medical importance of PPP was first appreciated when deficiencies of certain enzymes of the pathway, most often deficiency of glucose-6-phosphate dehydrogenase (G6PD), were found to be associated with hemolytic anemia (1). PPP is important in host defense mechanisms in all tissues against oxidative stress (2), in embryogenesis/morphogenesis (3), neu- rulation (4), myelination (5), inflammation (6–9), lymphocyte activation (10, 11), phagocytosis (7, 9), cardiac arrhythmias (12), radiation resistance of malignant tumors (13), and toxicity of high glucose concentrations in diabetes mellitus (14–16). Many of these processes are associated with apoptosis, a funda- mental form of programmed cell death (17). Apoptosis is indispensable for normal development and maintenance of homeostasis within multicellular organisms (18). Reactive oxygen species (ROS) have long been considered as toxic by-products of aerobic existence, but evidence is now accumulating that controlled levels of ROS modulate cellular function and are necessary for signal transduction pathways, including those mediating programmed cell death (19). A normal reducing atmosphere, required for cellular integrity is provided by reduced GSH that protects cells from damage by ROS (2). Regeneration of GSH from its oxidized form, GSSG, is dependent on NADPH produced by the PPP (2).

Understanding how the PPP is regulated has been compli- cated by the fact that the pathway is composed of two separate phases, oxidative and nonoxidative. Reactions in the oxidative phase are irreversible, whereas all reactions of the nonoxida- tive phase are fully reversible. Nevertheless, the two phases are functionally connected. The nonoxidative phase can convert ribose 5-phosphate into glucose 6-phosphate for the oxidative phase, and thus, indirectly, it can also contribute to generation of NADPH (20). The rate-limiting enzymes for the two phases are different. The oxidative phase is primarily dependent on G6PD (21). Although the control of the nonoxidative branch is less well established, transaldolase (TAL) has been proposed as its rate-limiting enzyme (21, 22).

TAL catalyzes the transfer of a 3-carbon fragment, corre- sponding to dihydroxyacetone, to α- glyceraldehyde 3-phosphate, α-erythrose 4-phosphate, and a variety of other acceptor aldehydes, including nonphosphorylated trioses and tetroses. Enzymatic activity of TAL is regulated in a tissue-specific (21–28) and developmentally specific manner (4, 29). In the brain, TAL is expressed selectively in oligodendrocytes at high levels (28). This is particularly interesting because myelin sheaths are formed by oligodendrocytes, and lesions in the reactive oxygen species; TAL, transaldolase; np, nucleotide positions; snRNA, small nuclear RNA; CAT, chloramphenicol acetyltransferase; H-TAL, human TAL; M-TAL, murine TAL; Staf, selencysteine tRNA gene transcription activating factor; GPx, glutathione peroxidase.

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The abbreviations used are: PPP, pentose phosphate pathway; NE, nuclear extracts; Abs, antibodies; PMSF, phenylmethylsulfonyl fluoride; ChIP, chromatin immunoprecipitation; EMSAs, electrophoretic mobility shift assays; G6PD, glucose-6-phosphate dehydrogenase; ROS, reactive oxygen species; TAL, transaldolase; np, nucleotide positions; snRNA, small nuclear RNA; CAT, chloramphenicol acetyltransferase; H-TAL, human TAL; M-TAL, murine TAL; Staf, selencysteine tRNA gene transcription activating factor; GPx, glutathione peroxidase.
most common demyelinating disease of the central nervous system, multiple sclerosis, are characterized by a progressive loss of oligodendrocytes and demyelination (30). High levels of TAL expression in oligodendrocytes may be related to synthesis of lipids as major constituents of myelin and exquisite susceptibility of these cells to damage by reactive oxygen species, nitric oxide, and tumor necrosis factor α released by activated macrophages and astrocytes (31).

Data from this laboratory have provided evidence that TAL can regulate susceptibility to apoptosis through control of the balance between the two branches of the PPP and its overall output as measured by NADPH and GSH production (32). Overexpression of TAL in Jurkat and H9 human T cell lines results in a decrease in G6PD and 6-phosphogluconate dehydrogenase activities and NADPH and GSH levels and renders the cell highly susceptible to apoptosis induced by serum deprivation, H2O2, nitric oxide, tumor necrosis factor α, Fas antibody, or infection by human immunodeficiency virus, type 1 (32–34). In addition, reduced levels of TAL results in increased G6PD and 6-phosphogluconate dehydrogenase activities and increased GSH levels with inhibition of apoptosis. Disruption of the mitochondrial transmembrane potential (Δψm), the point of no return in the effector phase of programmed cell death (35, 36), is subject to regulation by an oxidation-reduction equilibrium of ROS, pyridine nucleotides (NAD/NADH and NADP/ NADPH), and GSH levels (37). The extent of Fas-induced mitochondrial ROI production, changes in Δψm, caspase activation, and cell death are regulated by TAL expression levels (34). The impact of TAL on apoptotic signaling (32) may be related to an overwhelming influence of TAL-catalyzed dihydroxyacetone transfer reactions on the balance between the two branches of PPP (32) that controls intracellular NADPH levels and neutralization of ROS (38). TAL-dependent changes in NADP/NADPH levels may regulate expression of G6PD through oxidative stress-response elements located in the G6PD promoter (39). Therefore, regulation of TAL expression may play a pivotal role in tissue- and cell-type-specific metabolic signaling (19, 38).

TAL-H is a single copy gene located on the short arm of human chromosome 11 at p15.4–15.5 (40). The eight exons encoding a 337-amino acid protein of 37.6 kDa (41) are contained within a 17.5-kb genomic locus (TALDO1) (42). In the present study, we localized the core promoter of TAL-H to nucleotide positions (np) −49 to −1 that harbors a DNA element that is recognized and functionally activated by transcription factor ZNF143, a human homolog of Xenopus selenocysteine tRNA gene transcription activating factor (Staf) (43, 44). This is a seven-zinc finger transcription factor capable of enhancing transcription of mRNA, tRNA, snRNA, and snRNA-type genes via RNA polymerase II or III (43–47). ZNF143 is encoded by a single copy genomic locus within a 10-megabase distance from the TAL-H locus at band p15.4 of human chromosome 11 (48). Site-directed mutagenesis of the ZNF143/TALDO1 motif abolished transcriptional activity of the core TAL-H promoter. Expression of TAL-H correlated with that of ZNF143 in 21 different human tissues and both were up-regulated in lactating mouse mammary glands as compared with nonlactating controls. Overexpression of ZNF143 enhanced TAL-H promoter activity, and a dominant-negative form of ZNF143 blocked transcription from the TAL-H promoter, indicating that ZNF143 may play a key role in regulating tissue-specific expression of TAL-H.

MATERIALS AND METHODS

Cell Culture—HepG2 human hepatoma cells (ATCC, Manassas, VA) were cultured in minimum essential medium with Earle's salts, 10% fetal bovine serum, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM t-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. HeLa human cervix carcinoma cells (from ATCC) and M03.13 human oligodendroglialoma cells (kindly provided by Dr. Neil Cashman, Montreal Neurological Institute, Montreal, Canada) were grown in Dulbecco's modified Eagle's medium with 2 mM t-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml amphotericin B. HOG human oligodendroglialoma cells (University of Chicago, Chicago) were grown in Dulbecco's modified Eagle's medium with 2 mM t-glutamine, 0.1 mM nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml amphotericin B. Jurkat human T cell leukemia cells (49) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM t-glutamine, and antibiotics. All cell lines were maintained in a humidified atmosphere with 5% CO2 at 37 °C. Cell culture products were purchased from Cellogro (Mediatech Inc., Herndon, VA).

Plasmids and Oligonucleotides—Mutagenesis of TAL-H promoter constructs were generated by PCR using the QuickChange Site-directed Mutagenesis kit as suggested by the manufacturer (Stratagene, La Jolla, CA). 25 ng of template was incubated with 125 ng of sense and antisense primers, dNTPs, and subjected to 15 PCR cycles with Fps turbo DNA polymerase with denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 68 °C for 10 min. DpnI was used to digest the parental supercoiled double-stranded methylated DNA for 1 h at 37 °C. Transformations were performed in Escherichia coli XL-1 Blue cells using DpnI-treated DNA. Plasmids were prepared using Qiagen Plasmid Maxi Kit Columns (Qiagen, Valencia, CA). Introduced alterations into the TAL-H promoter constructs were confirmed by DNA sequencing. AP-2 and Sp1 consensus oligonucleotides were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). TARE-6 oligonucleotide, derived from full-length TARE-6 (42), was used as a nonspecific competitor in electrophoretic mobility shift assays (EMSAs). ZNF143/76 (50) and SOX5 (51) consensus sequences were generated based on published reports. All oligonucleotides used in EMSAs and for generating TAL reporter-chloramphenicol acetyl transferase (CAT) reporter gene constructs were synthesized by Ransom Hill Bioscience (Ramona, CA).

Transient Transfections and Reporter Gene Assays—HepG2 cells were transfected with 1.6 μg of pbCAT3-based (52) TAL-H promoter reporter constructs at 80% confluency in 9.5-cm2 wells using 20 μl of PLUS reagent and 4.8 μl of LipofectAMINE reagent (Invitrogen). 80% confluent HeLa cells in 3.8-cm2 wells were transfected with 0.8 μg of pbCAT3-based TAL-H promoter vectors using 10 μl of PLUS reagent and 2.4 μl of LipofectAMINE reagent. Each cell line was cotransfected with pSVβ-gal (β-galactosidase reporter gene driven by the Rous sarcoma virus promoter) (53) using 1.6 μg for HepG2 and 0.8 μg for HeLa, respectively, in order to normalize transfection efficiency. In each transfection, the promoterless pBLCAT3 vector was used as a negative control. For overexpression of recombinant transcription factors ZNF76 and ZNF143, the corresponding expression vectors or pcAGGS empty vector (54) were cotransfected with the TAL-H promoter plasmid and internal control pB8β-gal using 1 μg of each plasmid, 20 μl of PLUS reagent, and 8 μl of LipofectAMINE reagent. After 4 h of exposure to the DNA-LipofectAMINE complex in serum- and antibiotic-free media, the DNA complex was removed, and cells were further cultured for 36 h in complete growth media. Cells were harvested in 150 μl of 250 mM Tris, pH 7.8, and solubilized by 3 rounds of freezing and thawing. For β-galactosidase assay, 30 μl of lysate was incubated with 270 μl of reaction mixture (1 mM MgCl2, 50 mM β-mercaptoethanol, 3 mM o-nitrophenyl-β-N-galactopyranoside, and 0.1 mM NaF, pH 7.5) at 37 °C and terminated with the addition of 500 μl of 1 M Na2CO3 once the reaction turned yellow. Absorbance values were measured at 420 nm and employed to adjust the quantity of cell lysates to be used in the CAT assay for normalization of transfection efficiencies. Prior to CAT assay, lysates were heated to 65 °C for 10 min to deacetylate acetylases. CAT assays were performed at 37 °C in a 50-μl reaction mixture with normalized volumes of cell lysates in 250 mM Tris, pH 7.8, 0.4 mM acetyl coenzyme A, and 0.025 μCi of [14C]choloramphenicol. Acetylated chloramphenicol was extracted with ethyl acetate and dried in a vacuum centrifuge. Pellets were resuspended in ethyl acetate, spotted on a silica gel TLC plate (Analtech, Newark, DE), and resolved by an equilibrated thin layer chromatography tank containing chloroform/methanol until the solvent front approached the top of the TLC plate. A 445 SI PhosphorImager with ImageQuant software (Amerham Biosciences) was used to determine the ratio of acetylated to unacyl- lated [14C]chloramphenicol. All assays were conducted within the range of linearity of CAT activities with respect to incubation time, based on
the β-galactosidase assay. Each transfection experiment was repeated at least four times.

Preparation of Nuclear Extracts—Nuclear extracts (NE) were prepared according to a protocol described previously (55) at 4 °C. Briefly, cells in log phase were harvested, washed with cold phosphate-buffered saline, and resuspended in 1× packed cell volume of ice-cold hypotonic buffer A (10 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol, and 10 mM leupeptin). Following a 15-min incubation on ice, the suspension was rapidly flushed eight times through a 25-gauge syringe to lyse cells. Homogenates were centrifuged at 16,000 × g, and pelleted nuclei were resuspended in 2/3 original packed cell volume of ice-cold buffer C (20 mM HEPES, pH 7.9, 20% glycerol, 550 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 1 mM dithiothreitol) for 15 min. Following a 10-min incubation on ice, the supernatant containing nuclear proteins was aliquoted and stored at −80 °C. Protein concentrations were determined by the Bradford method using the Bio-Rad Protein Assay (Bio-Rad).

DNase I Footprinting—DNase I footprinting was carried out with modifications of an established protocol (56, 57). Briefly, a double-stranded gel-purified TAL-H promoter DNA fragment (np 153 to +52) from a BamHI-TaqI digestion of clone 1994 (Fig. 3) was end-labeled by incorporating 3000 Ccpmm [α-32P]dCTP (ICN Biomedicals, Aurora, OH) into the 3'-end of the sense strand using Sequenase version 2.0 DNA polymerase (U. S. Biochemical Corp.) and purified through a Sephadex G-25 column (Shelton Scientific, Shelton, CT). DNA footprinting reactions were conducted in 50 µl of EMSA binding buffer (see below) without bovine serum albumin incubating 0.035 pmol (65,000–80,000 cpm) of TAL-H promoter probe with 40 µg of NE for 10 min at room temperature. Then the reaction was supplemented with 50 µl of 5 mM CaCl₂, 10 mM MgCl₂, and allowed to equilibrate for an additional minute at room temperature. Subsequently, the mixture was treated with 1.5 units of DNase I (Promega, Madison, WI) for 1 min at room temperature and terminated with the addition of 90 µl of 30 mM EDTA. Nuclear proteins were digested with 25 µg of proteinase K for 10 min at room temperature. After phenol/chloroform extraction, the aqueous phase was ethanol-precipitated overnight with 30 µg of GlyoBlue (Ambion, Inc., Austin, TX). The DNA pellet was resuspended in 5 µl of 1:2 diluted loading dye (U. S. Biochemical Corp. Stop Solution: 95% formamide, 0.5% bromophenol blue, 0.05% xylene cyanol FF), denatured at 95 °C for 5 min, and separated on a 6% polyacrylamide, 8 µm sequencing gel run at 65 watts in 1× Tris borate/EDTA (TBE) buffer. The gel was fixed in 10% methanol, 10% acetic acid for 30 min, dried under vacuum at 37 °C for 1 h, and subjected to autoradiography at −80 °C with an intensifying screen. Control reaction consisted of 0.035 pmol of probe incubated with 0.75 units of DNase I in the absence of nuclear extract. Identification of the location of the protected regions within the TAL-H promoter was determined by alignment with a 32P-labeled sequencing ladder run alongside the footprinting reactions. The map of TAL-H promoter oligonucleotides (Fig. 4) were generated by heating primer pairs to 94 °C for 1 min and slowly cooling to room temperature. These double-stranded oligonucleotides were 5'-labeled sequencing ladder run alongside the footprinting reactions. These double-stranded oligonucleotides were 5'-labeled sequencing ladder run alongside the footprinting reactions.

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Statistical Analysis—Statistical analyses were performed with Prism version 3.0 for Windows (GraphPad Software, San Diego). CAT activities of different constructs were compared with Student’s t test. Correlation of ZNF143, ZNF76, and TAL expression in human postmortem tissue was analyzed by linear regression. Data were expressed as the means ± S.E. of individual experiments. Changes were considered significant at a p value < 0.05.
and seven human TAL cDNA clones as well as primer extension studies, the transcription start site was mapped 48 bases upstream from the ATG codon of our original cDNA clone (GenBank™ accession number L19437). As described previously, the TAL-H locus is transcribed into a single 1.3-kb mRNA (42).

Functional Mapping of the TAL-H Minimal Promoter to np -49 to -1—In order to delineate the promoter of the TAL-H gene, a 2.7-kb genomic DNA fragment, flanked by 5′ PstI and 3′ TaqI sites with the latter located at nucleotide position +52 with respect to the transcriptional start site, was cloned into the pBLCAT3 reporter plasmid (52) immediately upstream of the chloramphenicol acetyltransferase (CAT) reporter gene. A 955-base-long TaqI fragment, nucleotides -903 to +52 contained in construct 1357 (Fig. 1), was sequenced previously (GenBank™ accession number AF058912) along with the 17,479-nucleotide-long genomic locus harboring all eight coding exons of TAL-H (GenBank™ accession number AF058913). 5′ deletions of this upstream TAL-H genomic DNA were created, and the resulting constructs were tested for promoter activity by transient transfection of HepG2 and HeLa cell lines (Fig. 1), which endogenously express TAL-H. The β-galactosidase reporter gene driven by the Rous sarcoma virus promoter, pRSVβ-gal (53), was cotransfected with each promoter construct in order to normalize transcription efficiency. The promoterless pBLCAT3 vector was used as a negative control in each transfection. Promoter strength was computed relative to the construct containing nucleotides -153 to +52 (1994 =

![Fig. 1. Deletion mapping of TAL-H promoter activity within a 2.7-kb DNA upstream element based on CAT reporter gene assays.](Image)

Left panel, physical maps of pBLCAT3-based constructs containing upstream DNA segments serially deleted from the 5′-end. Nucleotide positions of promoter DNA sequences are designated relative to the transcription start site of TAL-H (+1, represented by an arrow). BamHI (B), PstI (P), and TaqI (T) restriction sites within the TAL-H promoter sequence are shown as landmarks. Shaded boxes indicate the promoter sequence retained in each construct, whereas deleted portions (Δ) are denoted by flanking nucleotide residues. Maps of constructs 1994, 6863, 6886, 6881, and 6996 are magnified for visual clarity. pBLCAT3-based TAL-H promoter-CAT reporter constructs, with corresponding names shown on the left-hand side, were transiently transfected into HepG2 and HeLa cells, using pBLCAT3 and pRSVCAT as negative and positive controls, respectively, in all experiments. Each plasmid was cotransfected together with the pRSVβ-gal expression vector to standardize for transfection efficiency. A representative transfection of HepG2 cells is shown in the bottom inset. Right panel, transcriptional activities relative to construct 1994 composed of nucleotides -153 to +52 (100%, open bar) are displayed as bar charts. Data represent mean ± S.E. of at least 18 experiments. Significant differences in CAT activity relative to construct 1994, defined as p < 0.05 (*) or p < 0.001 (**), are shown for each promoter segment.
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100%; Fig. 1). 5′ serial deletions from −2.7 kb to −154 did not eliminate promoter activity in either cell line, implying that the basal promoter was contained within a 205-bp segment spanning bases −153 to +52. Decreased promoter activities were observed in constructs 2075 and 1357, suggesting the presence of negative cis-acting regulatory sequences farther upstream from the TAL-H core promoter. Removal of nucleotides −143 to −50 (6881) from this 205-bp 5′ promoter had no impact on transcriptional activation in either cell line. Although deletion of bases −49 to −1 (6896) completely abrogated CAT activity in HepG2 cells, significant transcriptional activity was still retained at 35% of base line in HeLa cells (p < 0.0001).

Reduction of the promoter element to np −49 to −1 (6886) was sufficient to drive basal transcription in both HepG2 and HeLa cells, albeit at lower levels than the intact 205-bp promoter element. In the antisense orientation, none of the TAL-H DNA fragments initiated CAT activity (data not shown). Taken together, these observations indicate that a 49-nucleotide region immediately upstream from the transcriptional start site contains the core sequence required for basal TAL-H transcription. Furthermore, because nucleotides +1 to +52 only yielded promoter activity in HeLa but not HepG2 cells, it is possible that a DNA element(s) within this region may mediate tissue-specific regulation of TAL-H gene expression. Transfections conducted in Jurkat human T cells paralleled the results observed in HeLa cells (data not shown).

**DNase I Footprinting of the cis-Acting DNA Element of the TAL-H Promoter**—To delineate precisely the cis-acting DNA element(s) mediating basal transcription of TAL-H, DNase I footprinting was employed using NE from HeLa, HOG, MO3.13, Jurkat, and HepG2 cell lines. A 3′-end radiolabeled DNA fragment harboring nucleotides −153 to +52 was assessed for the presence of putative transcription factor-binding sites. Two footprinted regions, −29 to −16 and −102 to −89, were unveiled in all cell lines tested (Fig. 2). Protection of nucleotides −29 to −16 was consistent with CAT assay data, thus mapping a functional core promoter within residues −49 to −1 of the TAL-H gene. Whereas protection by HeLa, HOG, and HepG2 NE was confined to np −29 to −16, MO3.13 and Jurkat NE also protected nucleotides −15 to −14 (Fig. 2). Jurkat cells displayed the most variability, with additional footprints at bases −1 to +3, −12 to −6, −51 to −35, and −64 to −55. Similar protection patterns were generated by all NEs with the 205-bp probe labeled at the opposite end (data not shown). Although the protected nucleotides −102 to −89 were not necessary for basal promoter activity (Fig. 1), this region could be involved in modulating TAL-H gene expression. DNase I footprinting also revealed protection at nucleotides +12 to +45 (not shown), which may play a role in tissue- or cell type-specific promoter activity in HeLa cells (Fig. 1).

**Cell Type-specific Recognition of ZNF143/76 and AP-2-binding Motifs of the TAL-H Core Promoter**—Mapping potential transcription factor-binding sites within the protected region of np −29 to −16 using the TRANSFAC data base (60) revealed the presence of binding motifs for transcription factors AP-2, Sp1, SOX5 (SRy-related HMG box 5), and Staf (selenocysteine tRNA gene transcription activating factors; Zinc Finger 143 (ZNF143) and/or 76 (ZNF76); Fig. 3). Binding of transcription factor(s) to this core promoter region was assessed by EMSAs, using an oligonucleotide probe spanning np −41 to −4 (TAL-H_P12, Fig. 4). A single dominant shifted DNA-protein complex was detected with all NE (Fig. 5, A, B, D, and E, and Fig. 6, A–C). By using HepG2 cell NE, formation of this sequence-specific complex was completely abrogated with 200-fold molar excess of cold TAL-H_P12 probe, although it remained unaltered in the presence of excess cold nonspecific competitor sequence (derived from TARE-6 (42)) (Fig. 5A). A 200-fold molar excess of cold double-stranded oligonucleotides containing Sp1, SOX5, or AP-2 consensus sequences did not significantly affect formation of the shifted complex with the TAL-H probe TAL-H_P12 (Fig. 5, A, B, D, and E). By contrast, a 200-fold excess of cold ZNF143/76 consensus sequence (Fig. 4) (50) completely abolished the complex formed by the TAL-H_P12 probe using HepG2 NE (Fig. 5, A and B, and Fig. 6, A and C). The ZNF143/76 consensus sequence also profoundly, but not entirely, quenched the shifted complex formed between the TAL-H_P12 probe and HeLa NE (−5% residual activity; Figs. 5, D and E, and 6B). This residual complex was eliminated by a combination of excess cold ZNF143/76 and AP-2 consensus sequences (Figs. 5E and 6B). Combination of excess cold AP-2 and Sp1 or ZNF143/76 and Sp1 consensus sequences did not affect the complex shifted by the TAL-H_P12 (Fig. 5, B and E). In turn, complexes formed between ZNF143/76 consensus sequence and HepG2 (Fig. 5C) or HeLa NE (Fig. 5P) was completely eliminated by TAL-H_P12, further supporting a critical role of ZNF143/76 in recognition of the TAL-H promoter.

Involvement of the ZNF143/76 motif was further examined by mutagenesis of the TAL-H promoter sequence (TAL-H_ZNFmut, Fig. 4) at residues critical for transcription factor bind-
ing (50, 61). A 200-fold molar excess of TAL-H/ZNFmut, containing a substitution of five nucleotides within the ZNF143/76 motif, failed to eliminate the shifted complex formed by TAL-H_P12 (Figs. 5, A and D, and 6A) or ZNF consensus (Fig. 5, C and F) with HepG2 and HeLa NE, thus suggesting the following: 1) involvement of the ZNF143/76 motif in binding to TAL-H_P12, and 2) efficacy of the introduced mutations. Mutation of the SOX motif within TAL-H_P12 probe (Figs. 5, A and D) or ZNF consensus (Fig. 5, C and F) probes, arguing against involvement of SOX. In parallel, the ZNF143/76 consensus motif markedly inhibited formation of complexes with the TAL-H_P12 (Fig. 5, A, B, D, and E) or ZNF consensus (Fig. 5, C and F) probes using HepG2 or HeLa NE, indicating a highly specific and dominant role for the ZNF143/76 motif within the TAL-H promoter (Fig. 5).

The profound but incomplete blocking by ZNF143/76 consensus of the shifted complex produced by HeLa NE and TAL-H_P12 probe led us to further investigate cooperativity with other transcription factors. Excess AP-2 consensus sequence alone only marginally diminished the shifted complex formed with HeLa NE (Fig. 6B). Combination of ZNF143/76 and AP-2 consensus sequences as cold competitors fully abrogated the shifted complex formed in the presence of HeLa NE and TAL-H_P12 probe (Figs. 5E and 6B). Combination of Sp1 with the ZNF143/76 consensus sequence was a less effective competitor than the combination of AP-2 and ZNF143/76 consensus sequences (Figs. 5E and 6B).

To characterize further the DNA-protein complex generated with np 41 to 4 of the TAL-H promoter, polyclonal antibodies to transcription factors AP-2α, Sp1, and anti-peptide antibodies to the N or C terminus of ZNF143 and ZNF76 (termed ZNF N-terminal or ZNF C-terminal, kindly provided by P. Carbon (58)) were utilized in supershift assays. Both the N and C terminus-specific ZNF143/76 antibodies markedly retarded migration of the DNA-protein complex formed with the TAL-H_P12 probe and HepG2 (Fig. 6, A and C) or HeLa NE (Fig. 6B), respectively. As a negative control, a polyclonal antibody directed against the TAL-H protein failed to alter the migratory pattern of the DNA-protein complex (Fig. 6). Antibody to AP-2α reduced formation of the TAL-H_P12-HeLa NE complex and further modified migration of the TAL-H_P12 DNA-protein complex supershifted with a ZNF143/76-specific antibody (Fig. 6B). These results demonstrated that the ZNF143/76 motif within np 41 to 4 of the TAL-H promoter is recognized by the transcription factors ZNF143 and/or ZNF76 in both HepG2 and HeLa cells, alone or in combination with AP-2α (only in HeLa), respectively.

Site-directed Mutagenesis of the ZNF143/76 Motif Abrogates TAL-H Promoter Activity—The ability of a transcription factor to interact with a DNA element in vitro may not confer promoter activity in vivo. Therefore, to assess a functional role for ZNF143 and/or ZNF76 in mediating transcription of TAL-H, the ZNF143/76 motif was mutated through substitution of five nucleotides at np 18 to 15 and np 13 within all TAL-H promoter CAT reporter gene constructs. These mutations were identical to those within the TAL-H/ZNFmut oligonucleotide (Fig. 4), which was rendered incapable of competing for NE proteins bound to the wild-type TAL-H np 41 to 4 probe (Figs. 5, A and D, and 6A) or ZNF consensus in EMSAs (Figs. 5, C and F, and 6A). Certain critical residues previously found crucial for the binding affinity of ZNF143/76 (45, 50, 62) were not mutated in the TAL-H promoter, because doing so would have also resulted in mutation of the overlapping SOX5 and AP-2 motifs. To ensure that this mutation was potent at completely eliminating binding of ZNF143/76 to the TAL-H promoter, EMSA analysis was conducted with the TAL-H/ZNF-
Fmut probe. Surprisingly, two specific complexes were formed, albeit of weak intensity, that exhibited both a faster and slower migratory pattern compared with the ZNF143/76 DNA-protein complex generated with the wild-type probe (Fig. 6). We realize that a new recognition motif, of rather low affinity, may have been created. Data base analysis of putative proteins that may recognize this mutant promoter include AP-2, myeloid zinc finger 1 (MZF1), and Ikaros-2 (Ik-2) (60). However, the TAL-H ZNFmut complex did not contain ZNF143/76 because of the following: 1) excess ZNF143/76 consensus sequence was incapable of competing out the shifted bands, and 2) ZNF143/76-specific antibodies did not alter the migration of these DNA-protein complexes (Fig. 6A). This indicated that the ZNF143/76 motif had been destroyed and thus could be effectively used in reporter assays. As shown in Fig. 7A, such substitutions in the ZNF143/76 recognition motif abolished CAT activity in both HepG2 and HeLa cells, providing functional evidence for involvement of ZNF143/76 in initiation of TAL-H transcription. Background activity of the promoterless pBLCAT3 vector was somewhat higher in HeLa as compared with HepG2 cells (Figs. 1 and 7A), which may account for the apparent residual CAT activity observed in HeLa cells in accordance with earlier data (52). Furthermore, site-directed mutagenesis of critical AP-2 residues enhanced TAL promoter activity in HeLa cells by 20 ± 7.3% (construct 7110; p = 0.021), 28 ± 9.8% (construct 7158; p = 0.027), and 42 ± 12.3% (construct 7150; p = 0.011), respectively (Fig. 7B). Mutagenesis of AP-2 sites did not, however, affect promoter activity in HepG2 cells. This was consistent with a lack of expression by AP-2 in HepG2 cells (63, 64). Substitutions of residues involved in both AP-2 and ZNF143/76 motifs in constructs 7116, 7133, and 7141 reduced CAT activities in both HeLa and HepG2 cells. These mutagenesis studies suggested that basal transcription from the core TAL-H promoter is primarily mediated by ZNF143/76, whereas AP-2 may act as a repressor in HeLa cells.

**Regulation of TAL-H Promoter Activity by Transfection of ZNF143 Expression Vectors**—To delineate involvement of ZNF143 versus ZNF76 in activation of the TAL-H promoter, HepG2 cells were cotransfected with TAL-H promoter reporter constructs and pCAGGS-based expression vectors coding for full-length human ZNF143 or ZNF76 protein or the DNA-binding domain of ZNF143 that lacks the transcriptional activation domain and acts in a dominant-negative fashion (54) (Fig. 8). Promoter activity was normalized to the CAT activity exhibited by the individual TAL-H promoter constructs co-transfected with the pCAGGS mammalian expression vector.
Fig. 5. EMSAs of in vitro binding by HepG2 and HeLa NEs to the TAL-H core promoter (np -41 to -4). Binding of 10 μg of HepG2 (A–C) or HeLa NE (D–F) to 20 fmol of 32P-labeled TAL-H_P12 probe (A, B, D, and E) or ZNF consensus probe (C and F) was assessed in EMSAs. + indicates presence of NE, whereas minus indicates control reactions without NE. Competitions were carried out with 200× molar excess of the indicated cold competitor. Sequences of the oligonucleotides employed are shown in Fig. 4.
alone (65). As shown in Fig. 8, transcription from all TAL-H promoter constructs was up-regulated by ZNF143, whereas unaffected by ZNF76 overexpression. As an example, CAT activity from the minimal TAL-H promoter consisting of np−49 to −1 (6886) was enhanced 3.2 ± 0.8-fold (p = 0.0081) by full-length ZNF143, whereas ZNF76 produced no significant alteration. With respect to HepG2 cells transfected with pCAGGS alone, overexpression of ZNF143 also increased TAL enzyme activity by 27.7 ± 0.7% (p < 0.01). Acting in a dominant-negative fashion, the ZNF143 DNA-binding domain elim-
uated transcriptional activity of all TAL-H promoter constructs. Base-line TAL enzyme activities were not affected by overexpression of dominant-negative ZNF143, which was attributed to a >4-day-long half-life of endogenously produced TAL. Similar results were obtained with HeLa cells (data not shown).

**Fig. 7. A**, effect of site-directed mutagenesis of the ZNF143/76 recognition site on TAL-H promoter activity. Mutation of five residues corresponding to those in TAL-H_ZNFmut (Fig. 4) were introduced into each TAL-promoter-CAT reporter plasmid. Left panel, shaded boxes indicate the promoter region driving CAT activity of each construct; $M$ represents the location of the 5-bp mutation within the ZNF143/76 recognition motif of the TAL-H promoter. Right panel shows CAT activities of promoter constructs normalized to plasmid 1994 (100%). Data represent mean ± S.E. of at least 12 independent experiments. Effect of each mutation was compared with the corresponding wild-type construct depicted by matching shaded bars. B, effect of site-directed mutagenesis of AP-2-binding residues (constructs 7110, 7150, and 7158) or AP-2- and ZNF143/76-binding residues (constructs 7116, 7133, and 7141) on CAT activity of TAL promoter constructs in HeLa and HepG2 cells. The AP-2 and ZNF143/76 recognition motifs within np −49 to −1 of the TAL-H promoter are boxed. Capital letters show wild-type nucleotides and boldface lowercase letters indicate mutated residues. CAT activities relative to construct 6886 (100%, open bar) are displayed in the bar chart in the right panel. Data represent mean ± S.E. of at least four independent experiments. Significant differences compared with wild-type sequence are defined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (***)

**Tissue-specific Expression of ZNF143 and TAL**—According to previous studies, enzymatic activity of TAL is regulated in a
FIG. 8. Effect of recombinant ZNF143, ZNF76, and the dominant-negative DNA-binding domain of ZNF143 on TAL-promoter activity. HepG2 cells were cotransfected with the indicated TAL-H promoter reporter constructs (2075, 1357, 2020, 1994, and 6886) in combination with pCAGGS-based expression vectors encoding full-length human ZNF143, ZNF76, or the DNA-binding domain of ZNF143 or the pCAGGS backbone vector alone. Left panel shows map of constructs with shaded promoter regions. CAT assays were performed based on β-galactosidase activity of cotransfected pRSV/gal. CAT activities were normalized to controls yielded from coexpression with pCAGGS (100%, open bars). Data represent mean ± S.E. of at least four independent experiments. Statistical significance, $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (***) , is indicated relative to CAT activities with pCAGGS.
tissue-specific (21–28) and developmentally specific manner (4, 29, 66). Additionally, ZNF76 (67) and ZNF143 are expressed in different tissues at variable levels (43, 61). To examine the relationship between ZNF143/76 and TAL-H expression, their relative abundance was assessed by Western blot analysis of protein lysates from 21 different human postmortem tissues (Fig. 9). Relative expression of each gene product was assessed with respect to the mean of all tissues examined with setting expression levels in the ovary at 100%. Linear regression analysis revealed a striking correlation between ZNF143 and TAL levels \( (p = 0.0199) \). High ZNF143 and TAL levels were observed in the adult ovary, adult kidney, neonatal lung, adult pancreas, and adult liver, whereas their expression was relatively low in the skeletal muscle, heart, stomach, small intestine, and colon from an adult patient. With respect to ZNF143, TAL levels were low in adrenal glands suggesting involvement of additional transcriptional and post-translational factors in controlling TAL expression. ZNF76 and TAL levels did not correlate. Neither of the ZNF isoforms nor TAL correlated with \( \beta \)-actin or G6PD levels (not shown). Similar results were obtained from analysis of tissues from three different human donors.

Increased activity of PPP enzymes has been associated previously with an NADPH requirement for reductive syntheses, such as fatty acid biosynthesis in the lactating mammary tissue (2, 23, 68, 69). Elevated expression (70) and binding activity of mStaf, the mouse homolog of human ZNF143, was noted in lactating mammary tissue of the mouse (61, 70). As shown in Fig. 10, mStaf/ZNF143, TAL, and G6PD expression were increased 14.4-, 34.4-, and 13-fold, respectively, in lactating mammary tissues as compared with nonlactating ones, further supporting this tissue-specific correlation between TAL and ZNF143 expression.

**ZNF143/76 Associates with the Human and Mouse Transaldolase Promoter in Vivo**—Localization of ZNF143/76 to the human TAL (TAL-H) promoter in vivo was investigated with the ChIP assay. Formaldehyde cross-linked chromatin with DNA sheared to 200–1000 bp in length was prepared from HeLa cells and incubated with rabbit antibody directed to the C terminus of ZNF143/76, termed ZNF C-terminal, or control antibody directed to TAL-H. DNA was extracted from immunoprecipitated protein-DNA complexes and analyzed by PCR. As shown in Fig. 11A, a 194-bp TAL-H promoter fragment containing the ZNF143/76 motif was detected in chromatin selectively precipitated by ZNF C-terminal antibody. By con-

**Fig. 9.** Comparative analysis of tissue-specific expression of human ZNF143, ZNF76, and TAL. 20 \( \mu g \) of total protein lysates were loaded per lane. N, neonatal; A, adult human post-mortem tissue. Expression levels of ZNF143 and ZNF76 were assessed with a polyclonal antibody to their common C terminus (ZNF C-terminal) using enhanced chemiluminescence detection. Subsequently, the same blot was developed with antibodies to TAL-H and \( \beta \)-actin by detection with 4-chloronaphthol. Values indicate relative expression levels with respect to those in the ovary set at 100%. Since \( \beta \)-actin is also expressed in a tissue-specific manner, it was not used as a basis for comparison. \( p \) values indicate correlation with expression of TAL-H.
ZNF143 Regulates Transaldolase Expression

TAL, a rate-limiting enzyme of the PPP, has a profound impact on the balance between the two branches of the pathway and its ultimate output of NADPH and GSH (32). These findings are in agreement with a dominant role of TAL within the metabolic network that controls intracellular NADPH levels and neutralization of ROS (38). Levels of TAL expression serve as a critical determinant of tissue and cell type-specific sensitivity to apoptosis triggered by serum deprivation, hydrogen peroxide, nitric oxide, tumor necrosis factor α, anti-Fas monoclonal antibody, or infection by human immunodeficiency virus, type 1 (32–34). Therefore, regulation of TAL expression may play a pivotal role in tissue- and cell type-specific functioning of the PPP.

The present study localized the core promoter of TAL-H to np –49 to –1 which is recognized and functionally activated by transcription factor ZNF143, the human homolog of Xenopus Staf (43, 44), a seven-zinc finger transcription factor capable of enhancing basal transcription of mRNA, tRNA^Sec, snRNA, and snRNA-type genes via RNA polymerase II or III (43–47). Unlike most transcription factor-binding motifs, ZNF143 recognizes a relatively long and restrictive consensus sequence of 21 bp, 5′-NNY(AY)CCC/G(A/G)N(A/C)AT(G/C)(A/C)YYRCRN (46, 50). The minimal TAL-H promoter has a high (78%) GC content (Fig. 3). Unlike most G/C-rich TATA- and Inr-less genes where transcriptional initiation is under the control of Sp1 (71–76), basal expression of TAL-H is mediated through ZNF143. DNase I footprinting and EMSA analyses mapped the ZNF143-binding motif to nucleotides –26 to –6 of the TAL-H promoter. Formation of a DNA-protein complex between the TAL-H promoter and HepG2 NE was completely abolished with a ZNF143/76 consensus sequence or a mutation of the ZNF143 motif within the TAL-H promoter. Polyclonal antibodies to ZNF143 specifically recognized and supershifted this DNA-protein complex formed by the TAL-H promoter. Point mutations of the ZNF143 motif within the TAL-H promoter completely extinguished CAT activity in HeLa and HepG2 cells. In CAT assays, transcriptional activation of the TAL-H promoter was enhanced by overexpression of full-length recombinant ZNF143. In contrast, TAL promoter activity was completely abolished by cotransfection of a dominant-negative ZNF143 producing construct.

Our study provides evidence that ZNF143 is capable of interacting with additional factors, such as AP-2α, in mediating transcription from the TAL-H promoter in HeLa cells. Mutations of the AP-2α recognition sequence increased TAL-H promoter activity in HeLa cells without significant impact in HepG2 cells, which do not express AP-2α (63, 64). Cooperativity of ZNF143 with AP-2α was further supported by EMSA and supershift analysis (Fig. 6). This was conceivable because both ZNF143 (77) and AP-2α are capable of interacting with other transcription factors (73, 78–81). Whereas excess ZNF143/76 consensus oligonucleotide completely abolished the TAL-H promoter DNA-protein complex with HepG2 NE, formation of the TAL-H promoter DNA-protein complex with HeLa NE was drastically, but incompletely, reduced. Only in the presence of both excess ZNF143/76 consensus and AP-2α consensus competitor DNA was the complex completely abolished. Furthermore, antibody to AP-2α only affected mobility of the TAL-H promoter DNA-protein complex in the presence of antibody to ZNF143/76. This indicated that the ZNF143 Ab must first alter the conformation of the complex, thereby exposing hidden AP-2α epitopes that can subsequently be recognized by AP-2α Ab. These results suggested that AP-2α may have primarily complexed with ZNF143 and did not directly bind to the TAL-H promoter DNA. Our data are indicative of a protein-protein interaction.

**DISCUSSION**

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*Fig. 10. Western blot analysis of ZNF143, TAL, G6PD, and β-actin in lactating and post-lactating mouse mammary tissues. In each lane, 20 μg of total protein lysates from four lactating (samples 1, 3, 5, and 7) and three post-lactating tissues (samples 2, 4, and 6) was separated on a 12% SDS-polyacrylamide gel and immunoblotted with a rabbit polyclonal antibody to the C terminus of ZNF143 (ZNF C-terminal antibody). Values below each lane indicate relative abundance of ZNF143, TAL, and G6PD with respect to β-actin using automated densitometry. p values reflect differences between lactating and nonlactating tissues as compared with Student’s t test.

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\( ^2 \) A. Perl and K. Banki, unpublished data.
interaction in which the binding of ZNF143 may tether AP-2α to the TAL-H promoter and thereby modulate transcription of the TAL-H gene in HeLa cells.

Although we have clearly demonstrated that ZNF143 can act alone or in combination with AP-2α to initiate TAL-H expression, the DNase I protection pattern (–29 to –16), does not completely overlap with the identified ZNF143 recognition sequence at –26 to –6. This discordance may be related to the versatility of the zinc fingers of ZNF143 in binding to DNA (50). Zinc finger 1 preferentially interacts with a GCG triplet located at the 3′-end of the ZNF143 recognition motif (50, 58), which is also present within the TAL-H promoter (–25 to –23 in Fig. 3). Binding of zinc fingers 1 and 2 of ZNF143 to the antisense TAL-H promoter increases 4 kb upstream of the first ATG codon, does not directly bind to the TAL-H promoter. Site-directed mutagenesis of AP-2α-binding motifs up-regulated TAL-H promoter activity in HeLa cells. HepG2 and HeLa NE suggest that AP-2α does not directly bind to the TAL-H promoter. Site-directed mutagenesis of AP-2α-binding motifs up-regulated TAL-H promoter activity in HeLa cells but not in HepG2 cells (Fig. 1B). Thus, AP-2α may modulate ZNF143-mediated transcription in HeLa cells by acting as a
ZNF143 Regulates Transaldolase Expression

Enzymatic activity of TAL is regulated in a tissue-specific (21–28) and developmentally specific manner (4, 29). TAL is highly active in the thymus (22), intestinal mucosa (22), adrenal gland (23), kidney (22), and mammary tissue of lactating and pregnant mice (23). Conversely, TAL activity is particularly low in the brain (23, 25), liver (23), heart (22, 24, 25), and skeletal muscle (22, 25, 84, 85). In the brain, TAL expression is high in myelin-producing oligodendrocytes, compared with neurons and astrocytes (28). During development of the nervous system, TAL activity levels peak at the time of active myelination (86) possibly related to a greater demand of NADPH for lipid biosynthesis (5, 87). Northern blot analysis of human tissues revealed high levels of ZNF143 mRNA in the ovary, testis, and prostate (43). In the mouse, ZNF143 is highly expressed in the lung (61), ovary (43, 61), and thymus (61). Low ZNF143 mRNA levels were found in human leukocytes, colon, thymus, and spleen (43) as well as in mouse brain, liver, and kidney (61). To our knowledge, there have not been any studies on the relative expression of TAL among various tissues. Western blot analysis of 21 different freshly processed human postmortem tissues revealed a striking correlation between ZNF143 and TAL levels (p = 0.0199). The highest ZNF143 and TAL levels were observed in the ovary, kidney, and liver. ZNF76 and TAL levels did not correlate. Neither ZNF isoforms nor TAL correlated with actin or G6PD levels. Overexpression of ZNF143 enhanced TAL-H promoter activity, and a dominant-negative form of ZNF143 blocked transcription from the TAL-H promoter (Fig. 8), indicating that ZNF143 may play a key role in regulating tissue-specific expression of TAL.

ZNF143 is required for transcriptional activation of selenocysteine transfer RNA tRNA Sec, which mediates incorporation of selenocysteine into selenoproteins such as glutathione peroxidase (GPx) (88, 89) and type 1 thyroxine 5'-deiodinase (90). The mouse homologs of ZNF143 (m-Staf) (61), tRNASec, and GPx are up-regulated during lactogenesis in the mouse mammary gland (70). Most interesting, expression of the ZNF143-activated cytosolic chaperonin containing t-complex polypeptide 1, Ccta, is particularly elevated in FM3A mammary carcinoma cells (91). Lac-tation has long been associated with increased G6PD activity and NADPH requirement for lipid biosynthesis (92, 93). Indeed, ZNF143, TAL, as well as G6PD expression were up-regulated with respect to total protein or β-actin content in lactating mouse mammary glands. No ZNF143/76 consensus sequence was noted in the G6PD promoter (GenBankTM accession number NM_000402) (94), further supporting the notion that expression of TAL, but not G6PD, is selectively regulated by ZNF143. ChIP analyses show that ZNF143/73 associates with the TAL promoter in vivo and ZNF143/76 occupancy of the TAL-M promoter correlates with TAL expression in lactating versus nonlactating mammary tissues. With respect to total DNA input, detection of TAL-M promoter PCR product was increased from chromatin of lactating mammary tissue, exhibiting increased ZNF143/76 and TAL expression.

It is remarkable that activity of ZNF143-regulated enzymes, GPx, neuronal nitric-oxide synthase, and TAL are connected through a requirement for GSH (GPx) and NADPH (neuronal nitric-oxide synthase) or provision of NADPH via the PPP (TAL). Because maintenance of glutathione in a reduced state is dependent on NADPH provided by the PPP, it is conceivable that ZNF143-mediated up-regulation of TAL expression may represent an arm of coordinated metabolic changes underlying mammary gland proliferation. Increased G6PD activity is likely to be mediated through its promoter responsive to oxidative stress (59, 95) and, it is associated with increased metabolic activity of rapidly proliferating cells. By contrast, expression or enzymatic activity of TAL is not altered by H2O2 or Fas stimulation (92, 40). Therefore, TAL expression may be a critical determinant of tissue-specific sensitivity to oxidative stress and other cell death and differentiation signals.

ZNF143 also enhances transcription of the human interferon regulatory factor-3 gene via binding to an upstream consensus sequence at −181–−163 (96), which lies outside the core promoter (97). IRF-3, which is induced in response to viral infection, increases paramyxovirus-induced apoptosis (98). ZNF143 is necessary for direct or indirect (via tRNA Sec) synthesis of certain pro- and anti-oxidant enzymes including neuronal nitric-oxide synthase exon 1c (99), GPx, IRF-3, and now TAL-H, all of which can regulate susceptibility to apoptosis signals (32–34). Among these genes, TAL alone appears to be dependent on ZNF143 for both basal and tissue-specific expression. Of note, both TAL-H (40) and ZNF143 have been mapped within a <10-megabase segment of the short arm of human chromosome 11 at band p15.4 (48) and a syntenic region of mouse chromosome 7, further underlying an important genetic and functional relationship between these two genes. Thus, ZNF143 and TAL may play key roles in controlling the metabolic network that regulates cell survival and differentiation.

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