Correction and Retractions

CORRECTION

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The authors note that Fig. 5 appeared incorrectly. The corrected figure and its legend appear below.

![Corrected figure](https://www.pnas.org/doi/10.1073/pnas.1400058111)
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The authors note, “Recently it has come to our attention that Fig. 3D contained inappropriate data arrangements and manipulations. To avoid further confusion to the related scientific community, we hereby retract this report. We sincerely apologize for any inconvenience from this outcome. All of the authors agreed with the retraction.”

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The authors wish to note, “Recently it has come to our attention that inappropriate data arrangements and manipulations were made in Figs. 1, 2C, S4A, S4B, and 5. To avoid further confusion to the related scientific community, we hereby retract this report. We sincerely apologize for any inconvenience from this outcome. All of the authors agree with the retraction.”

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Distinct function of 2 chromatin remodeling complexes that share a common subunit, Williams syndrome transcription factor (WSTF)

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A number of nuclear complexes modify chromatin structure and operate as functional units. However, the in vivo role of each component within the complexes is not known. ATP-dependent chromatin remodeling complexes form several types of protein complexes, which reorganize chromatin structure cooperatively with histone modifiers. Williams syndrome transcription factor (WSTF) was biochemically identified as a major subunit, along with 2 distinct complexes: WINAC, a SWI/SNF-type complex, and WICH, an ISWI-type complex. Here, WSTF+/− mice were generated to investigate its function in chromatin remodeling in vivo. Loss of WSTF expression resulted in neonatal lethality, and all WSTF+/− neonates and ~10% of WSTF+/− neonates suffered cardiovascular abnormalities resembling those found in autosomal-dominant Williams syndrome patients. Developmental analysis of WSTF+/− embryos revealed that Gja5 gene regulation is aberrant from E9.5, conceivably because of inappropriate chromatin reorganization around the promoter regions where essential cardiac transcription factors are recruited. In vitro analysis in WSTF+/− mouse embryonic fibroblast (MEF) cells also showed impaired transactivation functions of cardiac transcription activators on the Gja5 promoter, but the effects were reversed by overexpression of WINAC components. Likewise in WSTF−/− MEF cells, recruitment of Snf2h, an ISWI ATPase, to PCNA and cell survival after DNA damage were both defective, but were ameliorated by overexpression of WICH components. Thus, the present study provides evidence that WSTF is shared and is a functionally indispensable subunit of the WICH complex for DNA repair and the WINAC complex for transcriptional control.

WICH | WINAC | heart development | SWI/SNF | ISWI

Chromatin structure is reorganized through chromatin remodeling and epigenetic modifications in the process of nuclear rearrangement. Two major classes of chromatin-modifying complexes that support nuclear events on chromosomes have been well characterized (1). One class is a histone-modifying complex (2, 3), and the other class is an ATP-dependent chromatin-remodeling complex (4). This complex uses ATP hydrolysis to rearrange nucleosomal arrays in a noncovalent manner to facilitate, or prevent, access of nuclear factors to nucleosomal DNA. These ATP-dependent chromatin-remodeling complexes have been classified into 4 subfamilies, the SWI/SNF-type complex, the ISWI-type complex, the INO80 complex, and the NuRD-type complex. Each complex contains a major catalytic component that possesses DNA-dependent ATPase activity, such as Brg-1/Brm (SWI/SNF-type complex) or Snf2h (ISWI-type complex) (5, 6). Selection of catalytic ATPase subunits, combined with other complex components, defines the role of these complexes in various nuclear events including transcription, DNA replication, or DNA repair (7). Genetic analyses have shown that core components of the chromatin remodeling complexes are indispensable for embryonic development whereas coregulatory subunits appear to support the spatiotemporal function of the complexes (8, 9). BAF60c was recently identified as a heart-specific subunit of the SWI/SNF-type complex (10).

We reported that Williams syndrome transcription factor (WSTF) (11) is a subunit of WINAC, which is a subclass of the SWI/SNF-type ATP-dependent chromatin remodeling complexes (12). WSTF is crucial for gene regulation by the vitamin D receptor (VDR) and is expressed during embryogenesis (12, 13). WSTF is also reported to assemble with Snf2h to form WICH, an ISWI-type chromatin complex (14). Co-localizing with replication foci, WICH is considered to support DNA replication (15). WSTF was initially found as 1 of several genes (including Cyn2, Limk1, Elastin, Bcl7b, and Fzd) deleted in patients with autosomal-dominant Williams syndrome, a disease that displays a wide spectrum of developmental defects, including cardiovascular abnormalities (11, 16). In the present study, to address the physiological significance of WSTF as a component of chromatin remodeling complexes, we ablated WSTF expression in mice. All WSTF−/− mice and 10% of WSTF+/− mice exhibited overt cardiovascular abnormalities similar to those observed in Williams syndrome patients. Detailed analysis of the mutant mice and cells revealed that the function of both WINAC and WICH complexes was impaired in the absence of WSTF. Our findings suggest that WSTF is shared and is a functionally indispensable subunit of 2 distinct chromatin remodeling complexes; WICH for DNA repair and WINAC for transcriptional control.


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The physiological impacts of WSTF were addressed by elastin expression in mice. Williams syndrome patients thus appears attributed, at least in part, to a malfunction of WSTF in a VDR-mediated gene cascade (12, 19).

To address a possible unique role of WSTF in WINAC during embryogenesis, gene expression of BAF60c and BAF180, specific components of other SWI/SNF-type complex subclasses responsible for heart development (20, 21), was examined by in situ hybridization at E9.5. No exclusive expression patterns for WSTF, BAF60c, or BAF180 were found in the trabeculae of heart ventricles. In the pharyngeal arch 1 (Pa1), expression of WSTF and BAF180, but not BAF60c, was detected in E9.5 embryos. Conversely, ubiquitous expression of Snf2h was observed (Fig. 1F).

**Results and Discussion**

**WSTF Is Essential for Life and Exhibits a Specific Expression Pattern in Embryos.** The physiological impacts of WSTF were addressed by gene disruption in mice (17) (Fig. S1). WSTF-deficient mice were born with Mendelian frequency but died within a few days. They had smaller body sizes but were of normal appearance (Fig. 1A and B). Expression of the WSTF gene was not detected in WSTF<sup>−/−</sup> pups as expected, but transcript levels of several other candidate Williams syndrome genes (Cyclin2, Limk1, Elastin, Bcl7b, and Fzd9) were not detected (Fig. 1C). The reported role of WSTF in gene regulation (9, 11) was then tested by expression analysis of the key enzymes regulating vitamin D biosynthesis and catabolism, which are the 25(OH)D<sub>3</sub> 1α-hydroxylase (1α(OH)ase) and 25(OH)2D<sub>3</sub> 24-hydroxylase (24(OH)ase) genes (18). Activation of the VDR by binding of 1α(OH)D<sub>3</sub> served as a transcriptional repressor of 1α(OH)ase, but was a transcriptional activator of 24(OH)ase (12, 13, 18). Although upregulated expression of 1α(OH)ase was observed, no clear induction of 24(OH)ase was detected despite an excess of 1α,25(OH)D<sub>3</sub> observed in WSTF<sup>−/−</sup> embryos (Fig. 1D). Supporting these observations, serum calcium levels were elevated in WSTF<sup>−/−</sup> pups (Fig. 1E). Infantile hypercalcaemia found in Williams syndrome patients thus appears attributed, at least in part, to a malfunction of WSTF in a VDR-mediated gene cascade (12, 19).

From the phenotypic observations of elastin-deficient mice, elastin was considered to be responsible for the vascular deformities, for instance, supraavalvar aortic stenosis (SVAS), in Williams syndrome patients (11, 16). However, elastin gene expression was not detected, even in wild-type WSTF<sup>+/−</sup> embryos at E9.5 (Fig. 2F). Considering also that no overt cardiac defects were obvious in elastin<sup>−/−</sup> embryos at E9.5 (Fig. 2A), lack of WSTF expression appeared to be responsible for the cardiac defects seen in this hereditary disease.
Dysregulation of Cardiac Transcription Activators in Developing Hearts. As severe defects were observed in WSTF−/− E9.5 embryonic hearts, we presumed that the function of cardiac transcription factors in E9.5 embryos was impaired due to dysregulation of WSTF-containing chromatin remodeling complexes. To test this idea, a cardiac conduction system-related gene Gja5, which encodes connexin40 (Cx40) (22, 23), their upstream transcriptional regulators, Nkx2.5, Tbx5, and Gata4 (22, 24, 25), and the cardiac trabeculation marker, Irx3 (26), were analyzed by RT-PCR assay in E9.5 embryonic hearts (Fig. 2G). Although WSTF ablation appeared unlikely to affect the expression of cardiac transcription factors, a significant reduction in the expression of Gja5(Cx40) and Irx3 was found (Fig. 2G and H). Such reduced expression of Gja5(Cx40) and Irx3 genes was seen even in WSTF+/− hearts (Fig. 2G). WISH analysis of E9.5 embryos confirmed the altered gene expression patterns (Fig. 2H). However, cardiac muscle markers, Mlc2a and Mlc2v (27), were expressed normally in WSTF−/− hearts and thus appeared to be independent of WSTF-mediated gene regulation. Such dysregulated expression of cardiac development markers was further studied by in situ hybridization of E9.5 hearts (Fig. S2). These findings imply that WSTF, presumably as a WINAC subunit, is crucial for the normal gene cascades in the developing heart and that WINAC is required for normal function of cardiac transcriptional regulators.

WINAC Components Support the Function of Cardiac Transcription Factors. WISH analysis of developing hearts suggested that the major transcriptional activators responsible for cardiac development require WSTF, presumably as a WINAC component, for their transcriptional regulation. The association of endogenous WSTF with endogenous Brg1 or Snf2h in MEF cells was verified by coimmunoprecipitation (Fig. 3A). Although Brg1 was coimmunoprecipitated with all of the tested BAF components, BAF 180 was not seen in WSTF immunoprecipitates in MEF cells or in cardiogenic-P19.CL6 cells (RIKEN) (Fig. 3D). Physical association of WSTF with endogenous Brg1 or Snf2h in MEF cells was verified by coimmunoprecipitation (Fig. 3A). The fourth aortic arch artery of WSTF mutant mice at E10.5 was hypoplastic. Aortic arch arteries were visualized mutant mice were evident at E9.5. Sagittal sections of the embryo of each genotype are shown. Atrial chamber (a), left ventricle (lv), and right ventricle (rv) are shown. (C) Aortic arch defects of WSTF mutant mice. Stereomicroscopic images are shown. Aorta (ao), pulmonary artery (pa), and right ventricle are indicated by la, lv, ra, and rv, respectively. Arrow indicates atrial septal defect (ASD); arrow head shows ventricular septal defect (VSD). (D) The fourth aortic arch artery of WSTF mutant mice at E10.5 was hypoplastic. Aortic arch arteries were visualized by ink injection. 3, 4, and 6 indicate the number of aortic arch arteries. (E) The frequency of cardiovascular abnormalities of WSTF mutant hearts is shown. Coarctation of the aorta (CoA) and double-outlet right ventricle (DORV) are displayed. (F and G) Cardiac gene expression patterns at E9.5 analyzed by RT-PCR. Expression of elastin was not detected at E9.5 (F). Down-regulated expression of Gja5(Cx40), with normal expression of its activators, in WSTF mutant mice (G).

Fig. 2. Cardiac defects and aortic arch disorganization in WSTF+/+ and WSTF−/− embryos. (A) WSTF mutant (WSTF+/− and WSTF−/−) mice exhibited severe heart defects. Coronal sections of typical dilation of both ventricles of WSTF mutant mice and elastin−/− mice at P0 are shown. Left atrium, left ventricle, right atrium, and right ventricle are indicated by la, lv, ra, and rv, respectively. Arrow indicates atrial septal defect (ASD); arrow head shows ventricular septal defect (VSD). (B) Heart defects in WSTF mutant mice were evident at E9.5. Sagittal sections of the embryo of each genotype are shown. Atrial chamber (a), left ventricle (lv), and right ventricle (rv) are shown. (C) Aortic arch defects of WSTF mutant mice. Stereomicroscopic images are shown. Aorta (ao), pulmonary artery (pa), and coartation of the aorta (arrow) are indicated. (D) The fourth aortic arch artery of WSTF mutant mice at E10.5 was hypoplastic. Aortic arch arteries were visualized by ink injection. 3, 4, and 6 indicate the number of aortic arch arteries. (E) The frequency of cardiovascular abnormalities of WSTF mutant hearts is shown. Coarctation of the aorta (CoA) and double-outlet right ventricle (DORV) are displayed. (F and G) Cardiac gene expression patterns at E9.5 analyzed by RT-PCR. Expression of elastin was not detected at E9.5 (F). Down-regulated expression of Gja5(Cx40), with normal expression of its activators, in WSTF mutant mice (G). (H) Altered gene expression in WSTF mutant embryos at E9.5 by WISH analysis. Left ventricle (lv) and atrium (a) are shown. Expression of most cardiac genes, Nkx2.5, Tbx5, Gata4, Mlc2a, and Mlc2v, is normal in WSTF+/+ and WSTF−/− hearts at E9.5. [Scale bars, 500 μm in A, 100 μm in B, 800 μm in C, 300 μm in D, and 1 mm in H.]

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To test the physiological impact of the WINAC complex on the function of cardiac transcription factors, regulation of Gja5 (Cx40) expression by WINAC components in cardiogenic P19 cells was tested. Gja5 (Cx40) expression was attenuated by siRNA of the tested WINAC components, but not by the non-WINAC component BAF180 (Fig. 3C). Consistently, recruitment of WINAC components and activators was observed by using a ChIP assay (12) with the Gja5 (Cx40) gene promoter in embryos at E9.5. In vivo ChIP assays were performed as described in Material and Methods. For the Re-IP assay, the immunoprecipitates (IP) and their supernatants (sup.) were sequentially applied for the following ChIP analysis (13). (E) WINAC components were recruited to the Gja5 (Cx40) promoter exclusively in E18.5 hearts, but not in livers. ChIP assays were performed with the indicated tissues in both wild-type mice (WSTF+/+) and WSTF homozygous mice (WSTF−/−). The Right image shows a qPCR analysis using the same chromatin samples from E18.5 hearts shown in the Left image using specific primers for qPCR.

**WICH Components Are Essential for the DNA Repair Process but Not for DNA Replication.** In previous reports, both WICH and WINAC were shown to support DNA replication in vitro (12, 15). Therefore, using WSTF−/− MEF cells, we asked whether WSTF mediated DNA replication. From flow cytometric analyses of the distribution of DNA content (12) (Fig. 4D), cell cycle regulation appeared intact in the absence of WSTF. WSTF and Snf2h are anchored to Pcna (15), a major protein for controlling DNA repair after damage (28). Thus, we determined whether WICH was involved in DNA repair by assessing cell survival after treatment with methyl methanesulfonate (MMS), an inducer of DNA damage (29). Recovery from DNA double-strand breaks appeared lower in WSTF−/− MEF cells compared with wild-type cells. Furthermore, reduced survival could be normalized when both WSTF and Snf2h were over-expressed (Fig. 4B). After MMS-induced DNA damage in WSTF−/− MEF cells, recruitment of Snf2h to Pcna was not detected by coimmunoprecipitation (Fig. 4D). Consistently, chromatin-bound Snf2h was not seen in MMS-treated WSTF−/− MEF cells by immunofluorescence analysis (Fig. 4E). Together, these findings imply that certain phenotypic abnormalities in WSTF−/− mice, and presumably shared with Williams syndrome patients, may be caused by an impaired DNA repair process owing to the dysfunction of WICH (Fig. 4F).

The present findings suggest that WSTF is a chromatin remodeler that is essential for physiological functions of certain sequence-specific transcriptional regulators other than VDR (12, 13) (Fig. 4F). Although WSTF was indispensable for proper in vitro function of transcriptional activators specific for cardiovascular development like other BAF components (12, 30), WSTF−/− embryos did not exhibit the same abnormalities seen in the embryos deficient in either BAF components (10, 21) or...
cardiac activators (22, 24, 25). This clearly suggests a unique role for WINAC among the SWI/SNF-type chromatin remodeling complex subclasses in cardiovascular development. Considering that WINAC shares most of its components with other complex subclasses of the SWI/SNF-type (12), it is likely that a particular combination of common components with a subclass-specific subunit enables each complex to carry out its specific function in gene regulation. The detailed phenotypic analysis of E9.5 hearts showed similar but distinct abnormalities found in Baf60c knocked-down mice (see Fig. S2) (10). Thus, we presume that Baf60c cooperatively works with WSTF on common target gene promoters presumably by forming a single protein complex (see Fig. 3A and C) at a specific developmental stage in cardiac development.

WSTF is also a WICH component (14, 15) so certain phenotypes of WSTF−/− mice may be attributed to dysfunction of WICH. Although both WICH and WINAC were reported to affect DNA replication (15), no overt defects in the cell cycle or growth were found in WSTF−/− MEF cells, as was seen in Xenopus eggs (31). Thus, WSTF appears dispensable for DNA replication in developing mice. Conversely, it is likely that WICH is indispensable for repair of DNA damage because restored expression of WSTF, together with the WICH component Snf2h, ameliorated impaired survival after DNA damage in WSTF−/− MEF cells. In conclusion, the WSTF subunit appears to serve as a chromatin remodeler and is a component of 2 functionally distinct complexes.

Materials and Methods

Whole-Mount In Situ Hybridization. Whole-mount section in situ hybridization by using digoxigenin-labeled probes was performed as described in ref. 12. Embryos were fixed with 4% paraformaldehyde, stored in 10% methanol, and 10−H9262 paraffin sections were cut. In situ hybridization was performed by using digoxigenin-labeled probes generated by in vitro transcription (Roche) and standard procedures. The following mouse cDNAs were used as templates for riboprobe synthesis: Gja5 (Cx40), Irx3, Gata4, WSTF, BAF60c, BAF180, Snf2h, Nkx2.5, Tbx5, Mlc2a, and Mlc2v. Light microscopy was performed at room temperature by using a microscope (AX10; Zeiss) fitted with an Axio Cam CCD camera (Zeiss). Images of embryos were taken with a stereomicroscope (Leica MZ 16FA) under a Planapo 1.0× objective equipped with an Axio Cam CCD camera (Zeiss). Images were processed by using Adobe Photoshop.

Ink Injection. At E10.5, India ink was injected into the left ventricle of the embryo’s heart. Embryos were fixed in Carnoy’s fixative, dehydrated through a series of graded ethanol/PBS solutions to 100% ethanol, and cleared for several hours in 2 volumes of benzyl benzoate and 1 volume of benzyl alcohol.

MEF Preparation. Primary murine embryonic fibroblasts (MEFs) were isolated and cultured as described in ref. 13. For the luciferase assay, MEFs were transfected with the indicated plasmids by using Lipofectamine plus reagents (Invitrogen).
ChIP Assay. Preparation of soluble chromatin for PCR amplification was performed according to the protocol provided by Upstate Biotechnology. To test in vivo binding of each transcription factor to the mouse Cx40 promoter, Culti- and liver tissue from C57BL/6J mice was used (22). One gram of tissue was homogenized in 50 mL PBS and treated with 1% formaldehyde for 15 min at 37°C. Primer pairs were as follows: Gja5 (Cx40) promoter: 5′-TTTCCTGCTGGCTTTAGAGGGA-3′ and 5′-TCTGAGCCTGTATGGTTCCTCGG-3′, and for quantitative RT-PCR (qPCR) 5′-CTTTCTGGACGTGGAGGAA-3′ and 5′-GAAGTTATCTTGTGCCTCGGGGTG-3′ for GAPDH. The primers were designed to yield a product of approximately 300 bp. Transfected cells were treated with medium containing 0.02% MMS for 1 h, washed with PBS, and maintained for 4 days in fresh medium. Surviving cells were then counted. All values are means ± SD from 6 independent experiments. P values (lanes 9–11) were calculated by Student’s t test (n = 6).

Immunofluorescence. MEF cells from WSTF−/− and wild-type cells were treated with 0.02% MMS for 30 min, washed with PBS, and maintained for 30 min in fresh medium. They were then treated with a hypotonic lysis solution containing 10 mM Tris-HCl pH7.4, 2.5 mM MgCl2, 1 mM PMSF, and 0.5% Nonidet P-40 for 8 min on ice, as described in ref. 32. The following immunofluorescence procedure was performed by using standard methods as described in ref. 33. Primary antibodies used were Polyclonal PAb for GFP (Santa Cruz Biotechnology), Cx40, Cx30, and Cx50 (Abcam), GABA (Abcam), and Xenopus sGFP (Abcam). Secondary antibodies were Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 594 goat anti-mouse IgG and Alexa Fluor 488 goat anti-rabbit IgG. All fluorescence images were captured at room temperature by using a 63×/1.4 oil-immersion objective on a confocal microscope (LSM510; Zeiss) with LSM 510 software (Zeiss). Images were processed by using Adobe Photoshop.

Cell Survival Assay. All experimental procedures were conducted as described in our previous report, with some modifications (29). MEF cells from WSTF−/− and wild-type mice were plated on 60-mm dishes at 40% confluency. The expression vectors, or Smartpool RNAi (Dharmacon), were transfected with Lipofectamine (Invitrogen). After 24 h, transfected cells were treated with medium containing 0.02% MMS for 1 h, washed with PBS, and maintained for 4 days in fresh medium. Surviving cells were then counted. All values are means ± SD from 6 independent experiments. P values (lanes 9–11) were calculated by Student’s t test (n = 6).