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SWI/SNF Chromatin Remodeling Complex Is Obligatory for BMP2-Induced, Runx2-Dependent Skeletal Gene Expression That Controls Osteoblast Differentiation

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Abstract Development of bone tissue requires maturation of osteoblasts from mesenchymal precursors. BMP2, a member of the TGFβ superfamily, and the Runx2 (AML3/Cbfa1) transcription factor, a downstream BMP2 effector, are regulatory signals required for osteoblast differentiation. While Runx2 responsive osteogenic gene expression has been functionally linked to alterations in chromatin structure, the factors that govern this chromatin remodeling remain to be identified. Here, we address the role of the SWI/SNF chromatin remodeling enzymes in BMP2-induced, Runx2-dependent development of the osteoblast phenotype. For these studies, we have examined calvarial cells from wild-type (WT) mice and mice that are homozygous for the Runx2 null allele, as well as the C2C12 model of BMP2-induced osteogenesis. By the analysis of microarray data, we find that several components of the SWI/SNF complex are regulated during BMP2-mediated osteoblast differentiation. Brg1 is an essential DNA dependent ATPase subunit of the SWI/SNF complex. Thus, functional studies were carried out using a fibroblast cell line that conditionally expresses a mutant Brg1 protein, which exerts a dominant negative effect on SWI/SNF function. Our findings demonstrate that SWI/SNF is required for BMP2-induced expression of alkaline phosphatase (APase), an early marker reflecting Runx2 control of osteoblast differentiation. In addition, Brg1 is expressed in cells within the developing skeleton of the mouse embryo as well as in osteoblasts ex vivo. Taken together these results support the concept that BMP2-mediated osteogenesis requires Runx2, and demonstrates that initiation of BMP2-induced, Runx2-dependent skeletal gene expression requires SWI/SNF chromatin remodeling complexes. J. Cell. Biochem. 94: 720–730, 2005. © 2004 Wiley-Liss, Inc.

Key words: Cbfa1; Runx2; AML3; null mouse; alkaline phosphatase; SWI/SNF; chromatin; osteoblast differentiation; bone formation

Bone morphogenetic proteins (BMP) are key regulators of bone formation. BMP2 induction of the osteogenic phenotype is observed in several non-osseous mesenchymal cells that include pluripotent C3H10T1/2 cells as well as NIH3T3 fibroblasts, and pre-myogenic C2C12 cells [Ahrens et al., 1993; Wang et al., 1993; Katagiri et al., 1994; Si et al., 1999]. BMP2 signals are directed to the nucleus through Smad heterodimers that converge with Runx2, a transcription factor required for osteoblast differentiation, to regulate the expression of osteogenic genes [Kobayashi et al., 2000; Lee et al., 2000; Zaidi et al., 2002; Franceschi and Xiao, 2003; Ito and Miyazono, 2003; Selvamurugan et al., 2004a]. The mechanisms by which BMP2 signaling and Runx2 are integrated at gene promoters for development of the osteoblast phenotype are minimally understood. Runx2 activation of the bone related *osteocalcin* (OC) gene has been functionally linked to alterations in chromatin structure [Javed et al., 1999]. Although Runx proteins alone lack the ability to remodel chromatin, these transcriptional regulators interact with several factors that have chromatin remodeling activity [Gutierrez et al., 2000; Javed et al., 2000; Paredes et al., 2002; Lian and Stein, 2003]. Together these results suggest that the

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recruitment of chromatin remodeling factors may be coupled to BMP2 signaling and Runx2-mediated gene expression for the commitment of progenitor cells into the osteogenic lineage.

Chromatin remodeling has emerged as a fundamental parameter for control of various physiological events, including steroid hormone and stress response, as well as cellular differentiation [Muchardt and Yaniv, 1993; Chiba et al., 1994; Kowenz-Leutz and Leutz, 1999; de la Serna et al., 2000; de la Serna et al., 2001; Pedersen et al., 2001]. Several factors alter chromatin structure by perturbing nucleosome stability or position in an ATP-dependent manner (e.g., SWI/SNF complexes) or by covalently modifying histones (e.g., histone acetyltransferase) [Workman and Kingston, 1998]. A key step in chromatin-regulated control of transcription is the ordered recruitment of chromatin remodeling factors to gene promoters via interactions with sequence-specific transcription factors [Agalioti et al., 2000; Hassan et al., 2001; Soutoglou and Talianidis, 2002]. Thus, a model has emerged for the combined contribution of tissue specific transcription factors and chromatin remodeling complexes to control cellular differentiation.

Conserved from yeast to humans, SWI/SNF complexes alter chromatin structure in an ATP-dependent manner and provide a critical function for the regulation of gene expression [Workman and Kingston, 1998; Sif, 2004]. These multisubunit complexes are distinguished by their essential DNA dependent ATPase subunit, which in higher eukaryotes is either the Brg or Brm protein [Wang et al., 1996; Sif et al., 2001]. Recent studies using in vitro cell differentiation models have shown that SWI/SNF complexes support transcriptional control of myogenic, adipocytic, and myeloid differentiation [Kowenz-Leutz and Leutz, 1999; de la Serna et al., 2001; Pederson, 2001].

Here, we have addressed the requirement of SWI/SNF activity for induction of the osteoblast phenotype. We first demonstrate that alkaline phosphatase (APase) is a marker for BMP2-induced Runx2-dependent osteoblast differentiation. By analysis of microarray profiles of BMP2-induced osteoblast differentiation, we find that expression of several SWI/SNF subunits is altered. The expression of Brg1, an essential component of the SWI/SNF complex, was identified in developing skeletal structures of the mouse embryo and in ex vivo osteoblast cultures. Functional studies were then carried out to establish the requirement of SWI/SNF for initiation of osteoblast differentiation. Our findings demonstrate that SWI/SNF activity is required for initiating the program of gene expression obligatory for development of the osteoblast phenotype.

MATERIALS AND METHODS

Cell Isolation and Culture Conditions

Primary mouse calvarial cells (17.5 dpc) were isolated from wild-type (WT) and Runx2 null mice, as described in [Pratap et al., 2003], and maintained in α-MEM with 10% FBS and 2 mM L-Glutamine. B22 cells were previously generated, which have an inducible Flag-tagged BRG1 transgene containing a mutation in the ATP binding site (Fig. 5b, [Khavari et al., 1993; de la Serna et al., 2001]). Tet-VP16 cells were generated from NIH-3T3 cells and contain a stably integrated Tet-tA transgene that encodes the Tet-VP16 regulator. B22 cells were generated from Tet-VP16 cells and contain both the Tet-tA transgene (Tet-off) and the Flag-tagged BRG1 mutant transgene (Fig. 5a). Mutant Brg1 has been previously shown in this cell line to associate with endogenous components of the SWI/SNF complex, but is non-functional (Fig. 5d), [de la Serna et al., 2000]. Expression of the mutant transgene is repressed in cells grown in the presence of 2 μg/ml tetracycline; whereas growth in the absence of tetracycline induces expression of the mutant protein as shown by Western blot (Fig. 5c). Both B22 and the Tet-VP16 cells were maintained in DMEM + 10% CS + 4 mM L-glutamine.

Primary rat osteoblasts isolated from fetal calvarial tissue (20 dpc) were cultured under osteogenic culture conditions essentially as described [Owen et al., 1990].

Assessment of Alkaline Phosphatase Activity

For phenotypic rescue experiments Runx2 deficient calvarial cells [Komori et al., 1997] were transduced with an adenovirus vector encoding human Runx2 protein under the control of a CMV promoter or the corresponding empty vector (a kind gift from John Robinson, Wyeth Research, Collegeville, PA). Briefly, viral particles were administered at 50 MOI in α-MEM with 1% FBS, incubated for 1 h at

37°C. After infection, free virus was aspirated, and cells were washed twice in serum-free MEM. Cells were then fed with fresh medium containing 10% FBS and, where indicated, 100 ng/ml BMP2 (a kind gift from Dr. John Wozney, Wyeth Research, Cambridge, MA). Media was changed every second day, with fresh BMP2 where indicated and cultured for 1 week. Cells were then fixed in 2% paraformaldehyde and stained for APase activity, detected by colorimetric reaction using a 0.1M Tris-maleate buffer (pH8.4) containing 0.05% Naphthol AS-MX Phosphate disodium salt, 2.8% NN′-dimethyl formamide, and 0.1% Fast Red salt (Sigma Chemical Co.; St. Louis, MO). Staining was carried out at 37°C for 10 min [Burstone, 1962]. In control experiments cells were transduced with adenoviral particles with empty vector (data not shown).

B22 and Tet-VP16 cells were grown on collagen type-I coated plates (14 ng/mm^2) with media changes every second day. Where indicated 2 μg/ml of Tetracycline was added to suppress transgene expression. After 4 days in culture, cells reached confluence and, where indicated, cells were grown in media containing 200 ng/ml rh-BMP2. After 7 and 14 days in culture, cells were fixed in 2% paraformaldehyde and stained for APase activity as described above.

Real-Time PCR Analysis

B22 and Tet-VP16 control cells were grown to confluence as described above and transduced with 200 ng/ml rh-BMP2. Total RNA was isolated from cells at the indicated time points using Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was purified using the DNA-Free RNA kit (Zymo Research Corporation, Orange CA). cDNA was generated from purified RNA using a reverse transcription reaction with Oligo-dT primers (Invitrogen Corporation, Carlsbad, CA). cDNA was then subjected to real-time PCR reaction using TaqMan chemistry (Applied Biosystems, Inc., Foster City, CA). Primers and probes for rodent GAPDH were purchased from Applied Biosystems, Inc. and mouse APase primers and probes were as follows: (Forward: 5′-CTGCAGGATCGGAACGTCAA-3′), (Reverse: 3′-CTCTTCCCACCATCTGGGC-5′), FAM-MGB probe (5′-CAATTAACATCGACGCTGC-3′).

Amplicon quantities were determined relative to a standard curve generated from a serial dilution of pooled cDNA from all samples. APase quantities were normalized to GAPDH.

Western Blot Analysis

Total protein was isolated from primary rat calvarial osteoblasts at the indicated time in culture. Briefly, cells were lysed on the plate by adding SDS–lysis buffer (2% SDS, 10 mM dithiothreitol, 10% glycerol, 2M urea, 1.0 mM phenylmethylsulfonyl fluoride, 10 mM Tris-HCl, pH 6.8, 0.002% bromphenol blue, complete 1× complete protease inhibitor mixture, Roche Molecular Biochemicals, Indianapolis, IN). Proteins were resolved by SDS–PAGE, transferred to a Immobilon-P PVDF transfer membrane (Millipore Corporation, Bedford MA). Blots were probed with either rabbit polyclonal antibodies to Brg1 (1:2,000; [H-70] Santa Cruz Biotechnology) or mouse monoclonal antibody to INI1 (BAF47) (1:100; B33720 Transduction Laboratories, Lexington, KY). Appropriate HRP conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA. ECL Western immunoblot detection reagent was used to visualize proteins (Amersham Biosciences, Piscataway, NJ).

In Situ Immunofluorescence Microscopy

Mouse embryos at 18.5 dpc were fixed in paraformaldehyde, embedded in paraffin, and heads were serial sectioned at 8 μm for immunolabeling using standard procedures. Slides of serial sections were stained by H&E and by indirect immunofluorescence. Antibodies for indirect immunofluorescence included rabbit anti-Brg1 (1:200; [H-70]), rabbit anti-Runx2 (1:200; [M-70], Santa Cruz Biotechnology, Santa Cruz, CA), and donkey anti-rabbit Alexa 488 (1:800, Molecular Probes, Eugene, OR). DNA was visualized by DAPI (4′, 6-diamidino-2-phenylindole) staining.

Primary osteoblasts isolated from mouse calvaria (17.5 dpc) were grown on gelatin-coated coverslips and processed for in situ immunofluorescence. In brief, cells were rinsed twice with PBS and fixed in 3.7% formaldehyde in PBS for 10 min on ice. After rinsing once with PBS, the cells were permeabilized in 0.1% Triton X-100 in PBS, and rinsed twice with PBSA (0.5% bovine serum albumin in PBS) followed by antibody staining. Cells were double-labeled for Runx2 (green) and Brg1 (red). Affinity purified Brg1 rabbit polyclonal

antibodies (1:200; [de la Serna et al., 2000]), Runx2 monoclonal antibody (a generous gift from Ito Y., Institute of Molecular Cell Biology, Singapore) [Zhang et al., 2000] and anti-rabbit Alexa 568 and anti-mouse Alexa 488 (1:800, Molecular Probes, Eugene, OR) were used. To determine the degree of Runx2 and Brg1 colocalization image cross-correlation was performed essentially as described in [Gupta et al., 2003]. Immunostaining of both cells and tissue sections was recorded using an epifluorescence Zeiss Axioplan 2 (Zeiss, Inc., Thorwood, NY) microscope attached to a CCD camera. Cell images were deconvoluted using Metamorph Imaging Software (Universal Imaging Corp., Downingtown, PA).

RESULTS

Alkaline Phosphatase Expression Reflects BMP2-Induced and Runx2-Dependent Initiation of Osteoblast Differentiation

Runx2 null mice are characterized by a complete absence of bone formation due to a defect in osteoblast differentiation [Komori et al., 1997]. Previous studies have shown that Runx2 co-operates with BMP activated receptor Smads to induce osteoblast differentiation [Kobayashi et al., 2000; Lee et al., 2000; Zaidi et al., 2002; Franceschi and Xiao, 2003; Ito and Miyazono, 2003; Selvamurugan et al., 2004b]. We used Runx2 null cells to determine the extent to which BMP2 signaling promotes osteoblast differentiation in the presence or absence of Runx2, as reflected by APase activity. Primary cultures of cells isolated from the calvaria of Runx2 null and WT mouse embryos were treated with BMP2 and/or exogenously expressed Runx2 for 7 days and monitored daily for APase activity. APase activity was detected in untreated WT calvarial cultures when cells reached confluence (Fig. 1) and was stimulated in cells grown in the presence of BMP2 alone. While exogenous Runx2 expression (by adenovirus infection) alone had a minimal effect on APase activity in WT culture, the presence of both BMP2 and exogenous Runx2 resulted in a synergistic induction of APase activity. In contrast, APase activity remained nearly undetectable in Runx2 null cells, as well as in null cells grown in the presence of either BMP2 or exogenously expressed Runx2. Notably, BMP2 treatment in combination with exogenous Runx2 expression in Runx2 null cells resulted in a synergistic induction of APase activity (Fig. 1). This finding directly demonstrates that BMP2 induction of the osteoblast phenotype requires Runx2.

BMP2-Induced Osteoblast Differentiation Is Associated With the Temporal Expression of SWI/SNF Chromatin Remodeling Subunits

Chromatin remodeling is required to support developmental activation and suppression of genes for phenotype development. Previous studies have shown that SWI/SNF chromatin

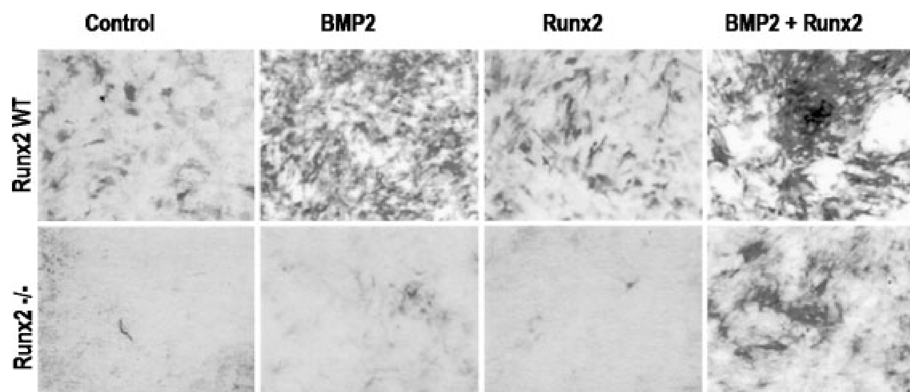
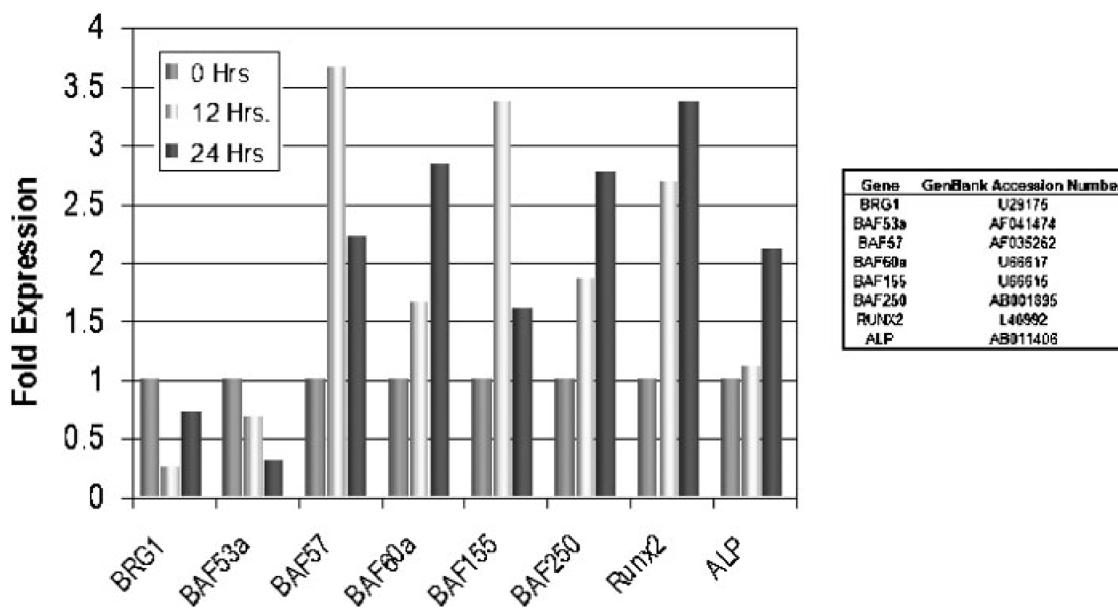


Fig. 1. BMP2 signaling and Runx2 synergistically promote osteoblast differentiation. The dependence of Runx2 expression for BMP2-induced alkaline phosphatase (APase) activity was assessed using mouse calvarial cells (17.5 dpc) from wild-type (WT) or Runx2 null mice. Minimal APase activity was detected in WT cells grown for 7 days in the absence of BMP2 and no expression was observed in Runx2 null cells grown under the same conditions. Induction of APase activity is observed in WT cells treated with BMP2 (100 ng/ml) or exogenous Runx2 delivered by adenoviral vector. In contrast no induction was observed in Runx2 null cells for either treatment alone. Notably, the combination of BMP2 (100 ng/ml) with exogenous Runx2 expression induced APase activity in both WT and Runx2 null cells.

remodeling activity is essential for myeloid, adipocyte, and muscle cell differentiation [Kowenz-Leutz and Leutz, 1999; de la Serna et al., 2001; Pederson, 2001; Salma et al., 2004]. To gain insight into the involvement of the SWI/SNF complex in osteogenic differentiation, we analyzed microarray gene expression data from BMP2-induced osteoblast differentiation of premyogenic C2C12 cells. Induction of the osteoblast phenotype in this system is reflected by the upregulation of both Runx2, within 2 h, and APase expression, by 16 h [Balint et al., 2003]. We find that in response to BMP2 treatment (300 ng/ml) there are temporal alterations in expression of Brg1, BAF53a, BAF57, BAF60a, BAF155, and BAF250 at 12 and 24 h after BMP2 treatment (Fig. 2). These observations provided a basis for exploration of the hypothesis that the chromatin remodeling activity of SWI/SNF is functionally linked to BMP2-induced osteoblast differentiation.

SWI/SNF Components Are Expressed in the Developing Skeleton In Vivo and in Osteoblasts Ex Vivo

We examined the involvement of SWI/SNF complexes during osteogenic differentiation initially by assessing Brg1 expression in developing skeletal structures in vivo. Mouse embryos isolated at 18.5 days-post-coitum (dpc) were examined by immunofluorescence histochemistry for the expression of Brg1 and Runx2 proteins. As shown in Figure 3, both Brg1 and Runx2 proteins are detectable in the nuclei of cells in the developing exoccipital bone. This pattern of Brg1 expression is consistent throughout skeletal components, all of which were also positive for the key osteogenic regulatory protein, Runx2. These observations are consistent with a requirement for SWI/SNF chromatin remodeling activity to support Runx dependent skeletal gene expression. We further find by Western blot and in situ immunofluorescence analyses that Brg1 and Ini1, essential components of the SWI/SNF complex, are expressed in osteoblasts ex vivo (Fig. 4). While the level of Ini1 protein remains constitutive, Brg1 reproducibly exhibits two forms with differing electrophoretic migration at the onset of cellular multilayering and maturation, but, one form at early and late stages of differentiation. Taken together our results suggest a functional relationship between the expression of components of the SWI/SNF chromatin remodeling complex and osteoblast differentiation.



Gene	GenBank Accession Number
BRG1	U29175
BAF53a	AF041474
BAF57	AF035262
BAF60a	U66617
BAF155	U66615
BAF250	AB001895
RUNX2	L40992
ALP	AB011406

Fig. 2. Gene microarray analysis of BMP2-induced osteoblast differentiation reveals temporal alterations in the expression of components of the SWI/SNF complex. Gene microarray expression data from a timecourse, in which pre-myogenic C2C12 cells were induced to differentiation toward the osteogenic lineage by BMP2 treatment [Balint et al., 2003] were analyzed to assess changes in mRNA accumulation for subunits of the SWI/SNF chromatin remodeling complex. Temporal alterations were observed in the expression of each of the SWI/SNF genes that is represented in the microarray data set. For comparison the osteogenic induction of Runx2 as well as APase gene expression is shown. Data were normalized to the initial time point.

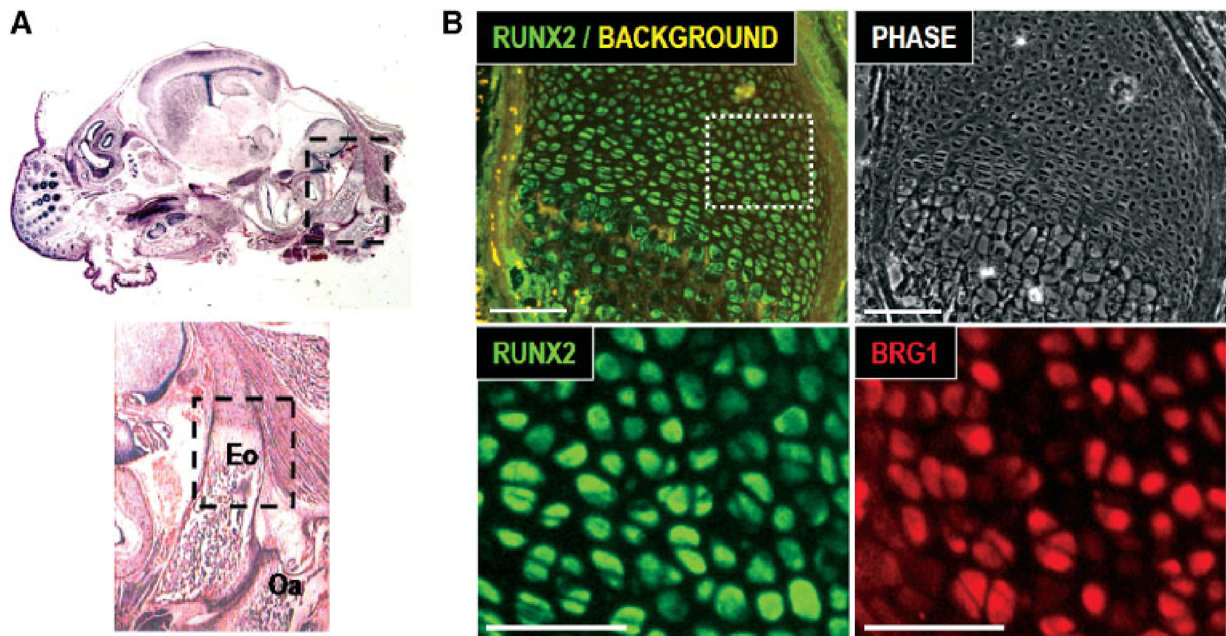


Fig. 3. SWI/SNF factors are expressed in developing skeletal structures. Mouse embryos at 18.5 dpc were fixed in paraformaldehyde, embedded in paraffin, and heads were serial sectioned at 8 μm. Slides of serial sections were stained by H&E dye (**A**) and by indirect immunofluorescence (**B**). Labeled skeletal structures are as follows: exoccipital (Eo) and occipital arch (Oa). Indirect immunofluorescence was performed on adjacent serial sections for Runx2 and Brg1. The Runx2-background image shows the growth plate region of the exoccipital bone. This image was generated by overlaying the Runx2 image with an image that reflects sample auto-fluorescence; in this image background signal is red, autofluorescence signal is yellow, and specific Runx2 signal is green. Staining for Runx2 (green, **lower left panel**) is from the white inset in the Runx2-background image; and staining for Brg1 (red, **lower right panel**) is from an adjacent serial section. (White bars: **upper panel**, 100 μm; **lower panel**, 50 μm).

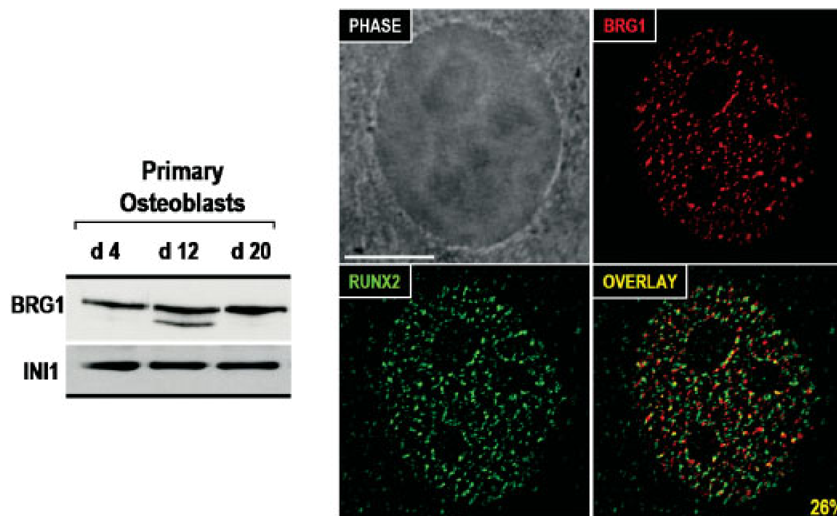


Fig. 4. SWI/SNF factors are expressed in primary osteoblasts. Normal rat diploid osteoblasts were cultured for the indicated time (days). Total protein was isolated and Western blot analysis was performed using antibodies to Brg1 and INI1 (**left panel**). Primary osteoblasts isolated from mouse calvaria (17.5 dpc) were grown on gelatin-coated coverslips and processed for in situ immunofluorescence. Cells were double-labeled for Runx2 (green) and Brg1 (red). As shown both Runx2 and Brg1 are organized in punctate subnuclear foci, and subset of which colocalize (26%), as determined by image cross-correlation analysis. White bar is 10 μm.

SWI/SNF Complex Is Required for BMP2-Induced Alkaline Phosphatase Gene Expression

To determine directly whether there is a role for SWI/SNF-mediated chromatin remodeling in BMP2-induced osteoblast differentiation, we utilized the B22 cell line that contains a stably integrated tetracycline inducible transgene encoding a flag-tagged Brg1 protein with a point-mutation in the ATP binding domain (Fig. 5A,B). This mutant Brg1 associates with components of the SWI/SNF complex, forming catalytically non-functional complexes [de la Serna et al., 2000; de la Serna et al., 2001] (Fig. 5C). Mutant Brg1 protein expression reaches maximal levels within 3 days (Fig. 5D) after removal of tetracycline. As a control we used the B22 parental cell line, Tet-VP16, which expresses the tetracycline responsive repressor protein. B22 cells and the parental Tet-VP16 cell lines express equivalent levels of endogenous Runx2 protein (data not shown).

We examined BMP2-induced osteoblast differentiation in the B22 cells in the absence or presence of the mutant Brg1 protein, ± tetracycline, respectively. We find that BMP2 treatment results in an induction of APase activity in cells expressing endogenous WT Brg1 protein (Fig. 6A). APase expression was detectable as early as 4 days following BMP2 treatment (data not shown) and increased progressively over time in culture. By day 14, robust expression of APase was evident in multilayered tissue-like nodules. Strikingly, the presence of the dominant negative Brg completely blocked the induction of APase by BMP2. APase remained undetectable even after 14 days of chronic BMP2 treatment in the presence of the mutant Brg1 protein. By using Tet-VP16 cells, we confirmed that induction of the tetracycline responsive transactivator alone did not inhibit APase activity (Fig. 6B). We further confirmed by quantitative RT-PCR that the inhibitory effects of the mutant Brg1 protein on APase activity occurred at the level of gene expression (mRNA) (Fig. 6C). These findings indicate that inhibition of SWI/SNF function does not simply delay induction of the osteoblast phenotype but abrogates completion of the BMP2 signaling pathway. Thus SWI/SNF chromatin remodeling activity is essential for induction of the osteogenic lineage.

DISCUSSION

We have combined molecular, biochemical, cellular, and in vivo approaches to demonstrate that SWI/SNF chromatin remodeling activity is obligatory for skeletal gene expression that supports osteoblast differentiation. Our findings in Runx2 null cells show that BMP2-mediated induction of the osteoblast phenotype requires Runx2. However, BMP2 and Runx2 protein are not sufficient to promote osteoblast

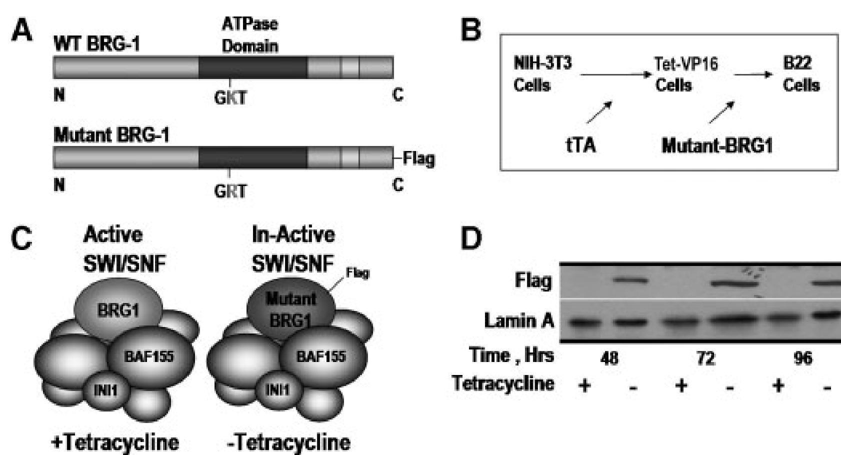


Fig. 5. Tetracycline inducible (Tet-Off) dominant negative SWI/SNF stable cell line. **A**: B22 cells have an inducible flag-tagged BRG1 transgene containing a mutation in the ATP binding site (K to R). **B**: Tet-VP16 cells were generated from NIH-3T3 cells and contain a stably integrated Tet-tA transgene, which encodes the Tet-VP16 regulator. B22 cells were generated from Tet-VP16 cells and contain both the Tet-tA transgene and the flag-tagged BRG1 mutant transgene. **C**: Mutant Brg1 has been previously shown in this cell line to associate with components of the SWI/SNF complex, but is non-functional. **D**: Cells grown in the presence of 2 ug/ml Tetracycline repress the mutant transgene; whereas removal of Tetracycline induces expression of the mutant protein as shown by Western blot. Maximum levels of the mutant Brg1 protein are observed within 3 days.

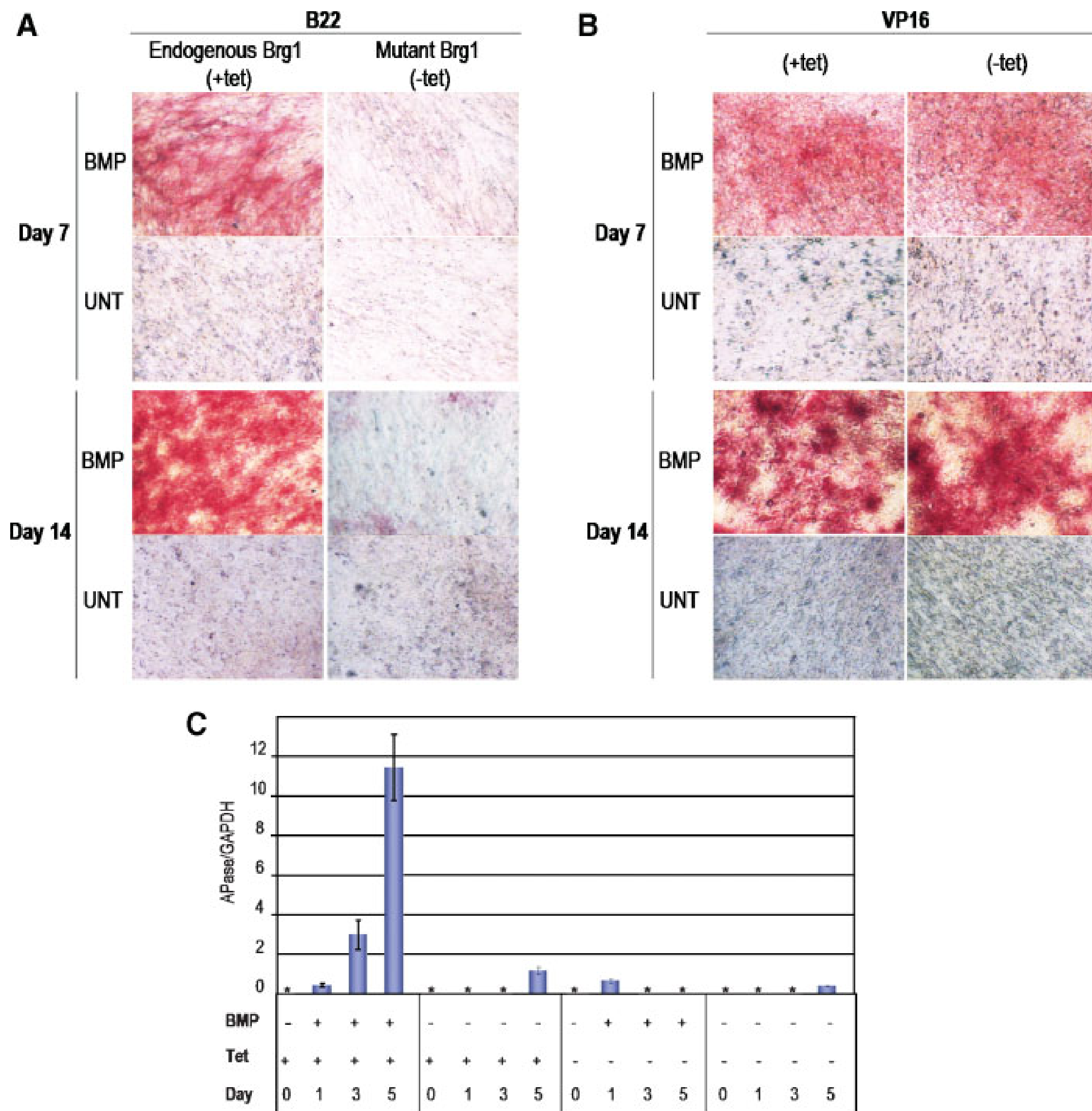


Fig. 6. SWI/SNF complex is required for BMP2-induced APase gene expression. **A**, **B**: B22 and tet-VP16 cells were growth on collagen type-I coated plates with media changes every second day. Where indicated 2 ug/ml of Tetracycline was added to suppress transgene expression. After 4 days in culture, cells reached confluence and, where indicated, were grown in media containing 200 ng/ml rh-BMP2. After 7 and 14 days in culture, cells were fixed in paraformaldehyde and stained for APase activity. B22 cells were isolated from each treatment on day 0, 7, and 14 to confirm by Western blot the expression of the mutant-Brg1 protein (data not shown). **C**: B22 cells were grown and treated as described above. RNA was extracted at the indicated time points and processed for real-time PCR analysis using primers and probes for mouse APase and GAPDH. Amplicon quantities were determined relative to a standard curve generated from a serial dilution of pooled cDNA from all samples (n = 2). APase quantities were normalized to GAPDH and (*) indicates undetectable APase mRNA.

differentiation in the presence of a dominant negative SWI/SNF chromatin remodeling complex.

The concept that chromatin remodeling factors, including SWI/SNF, mediate induction of the osteoblast phenotype is supported by specific modifications in chromatin structure that correlate with basal and vitamin-D enhanced gene expression of the bone-specific OC promoter and that require promoter binding of Runx proteins [Montecino et al., 1996; Javed et al., 1999]. These chromatin alterations include nucleosome displacement and covalent histone modifications [Montecino et al., 1996; Shen et al., 2002; Sierra et al., 2003], and are consistent with the involvement of ATP-dependent

chromatin remodeling as well as histone acetyltransferase activity. Runx proteins are not competent to remodel chromatin [Gutierrez et al., 2002], but interact with coregulatory proteins and chromatin remodeling factors [Javed et al., 1999; Gutierrez et al., 2002]. Thus Runx2 may have a functional role in directing structural alterations in the chromatin organization of skeletal gene promoters to support osteoblast differentiation. Runx2 null mice do not develop a mineralized skeleton and osteoblast differentiation is compromised [Komori et al., 1997]. Together these results predict that the coordination of chromatin alterations is a requirement for the onset of bone formation. Our findings that both Runx2 and SWI/SNF are required for BMP2-mediated induction of osteogenic differentiation strongly support this concept.

The consequences of null mutations in different subunits of the SWI/SNF complex have been examined in vivo. From these studies it is evident that SWI/SNF function is required for embryonic development [Bultman et al., 2000; Klochendler-Yeivin et al., 2000; Roberts et al., 2000; Guidi et al., 2001]. With regard to skeletal development, it is known that 14% of Brg1 null heterozygous mice exhibit exencephaly at embryonic day 16.5–18.5 [Bultman et al., 2000]. This craniofacial defect that is characterized in part by the absence of a calvarium [Ohyama et al., 1997; Bultman et al., 2000], supports a role for Brg1 in skeletal formation. Also, mice that are heterozygous for a BAF155 null allele, a subunit of the SWI/SNF complex, exhibit exencephaly with a similar penetrance as observed for the Brg1 heterozygote mice [Kim et al., 2001]. These genetic observations further indicate a role for the SWI/SNF chromatin remodeling complex in skeletal development. Our demonstration that essential components of the SWI/SNF complex are expressed in developing skeletal structures, as well as throughout osteogenic differentiation of cells isolated from the calvarium, is consistent with such a role.

The rules that govern functional interrelationships between chromatin remodeling and transcriptional control of skeletogenesis remain to be comprehensively established. However, our studies suggest that the SWI/SNF chromatin remodeling enzymes are essential for the initiation of BMP2-induced Runx2-dependent skeletal gene expression that is required for osteoblast differentiation.

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