nature genetics

p38 pathway targets SWI-SNF chromatin-remodeling complex to muscle-specific loci

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During skeletal myogenesis, genomic reprogramming toward terminal differentiation is achieved by recruiting chromatin-modifying enzymes to muscle-specific loci[1,2]. The relative contribution of extracellular signaling cascades in targeting these enzymes to individual genes is unknown. Here we show that the differentiation-activated p38 pathway[3–5] targets the SWI-SNF chromatin-remodeling complex to myogenic loci. Upon differentiation, p38 kinases were recruited to the chromatin of muscle-regulatory elements. Blockade of p38α/β repressed the transcription of muscle genes by preventing recruitment of the SWI-SNF complex at these elements without affecting chromatin binding of muscle-regulatory factors and acetyltransferases. The SWI-SNF subunit BAF60 could be phosphorylated by p38α-β *in vitro*, and forced activation of p38α/β in myoblasts by expression of a constitutively active MKK6 (refs. 5–7) promoted unscheduled SWI-SNF recruitment to the myogenin promoter. Conversely, inactivation of SWI-SNF enzymatic subunits abrogated MKK6-dependent induction of muscle gene expression. These results identify an unexpected function of differentiation-activated p38 in converting external cues into chromatin modifications at discrete loci, by selectively targeting SWI-SNF to muscle-regulatory elements.

Pharmacological blockade of p38α/β kinases by SB203580 inhibits the myogenic program[3–5] by repressing the transcription of early (myogenin; *Myog*) and late (muscle-creatine kinase; *Ckm*) muscle genes in myoblasts induced to differentiate (**Fig. 1a**). We monitored by chromatin immunoprecipitation (ChIP) the effect of p38 inhibition on the assembly of the myogenic transcriptosome and on chromatin modifications at the *Myog* promoter and the *Ckm* enhancer during myoblast differentiation. The muscle-regulatory factors (MRFs) MyoD and MEF2C bound the *Myog* promoter within the first hours of incubation in differentiation medium (**Supplementary Fig. 1** online) and throughout the differentiation process, regardless of the presence of SB203580 (**Fig. 1b**). At the *Ckm* enhancer, MRF binding was delayed (data not shown) and not influenced by SB203580 (**Fig. 1b**). p38α was selectively recruited to chromatin occupied by MRFs during muscle differentiation (**Fig. 1b**), as MRFs and p38 were not detected at these sequences in undifferentiated myoblasts (**Fig. 1b**) or in fibroblasts cultured in differentiation medium (data not shown). MRFs and p38 were also not found on the coding regions of *Ckm* (**Supplementary Fig. 2** online) or on the *Igh* enhancer (**Fig. 1b**), which contains E-boxes occupied by E-proteins only in B cells[8]. An interaction between Mef2A-C and p38 was suggested by previous studies[4,5,9,10], and MyoD could bind p38 *in vitro* (**Supplementary Fig. 2** online), suggesting that MRFs might mediate p38 recruitment to muscle-regulatory elements.

We next investigated the effect of p38 inhibition on chromatin modifications produced by MRF-recruited enzymes, including p38, histone acetyltransferases (p300, PCAF)[1,2,11,12] and the chromatin-remodeling SWI-SNF complex[13]. In undifferentiated myoblasts, histones H4 and H3 within the regulatory elements of *Myog* and *Ckm* were hypoacetylated and hypophosphorylated (**Fig. 1b**). We detected hyperacetylation of histones and phosphoacetylation of histone H3 at the *Myog* promoter and the *Ckm* enhancer after incubation in differentiation medium in cells treated with SB203580 and in untreated cells, which at times correlated with MRF binding (**Fig. 1b** and **Supplementary Fig. 1** online). In contrast, treatment with SB203580 eliminated the increased phosphoacetylation of histone H3 at the *Jun* promoter caused by TNFα in myoblasts (**Supplementary Fig. 3** online). These results suggest that p38 is involved in phosphoacetylation of histone H3 at promoters activated by inflammatory cytokines[14], but not muscle-specific promoters.

Histone hyperacetylation reflects the activity of acetyltransferases recruited by MRFs. Previous reports showed that p300-CBP and PCAF directly acetylate MyoD[15,16]. We evaluated the acetylation status of MyoD at the regulatory elements of *Myog* and *Ckm* by using antibodies to acetylated MyoD[17]. Acetylated MyoD was detectable at the *Myog* promoter after a few hours in differentiation medium (**Fig. 1b** and **Supplementary Fig. 1** online) and at the *Ckm* enhancer after 18–24 h in differentiation medium (**Fig. 1b**). The distinct kinetics of chromatin binding of acetylated MyoD at these

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Published online 20 June 2004; doi:10.1038/ng1378

regulatory regions correlated with the presence of PCAF (**Fig. 2a**), which acetylates MyoD at residues recognized by this antibody[18]. Exposure to SB203580 affected neither the amount of chromatin-bound acetylated MyoD (**Fig. 1b**) nor the acetyltransferase activity copurified by antibodies to MyoD from nuclear extracts of myoblasts induced to differentiate (**Fig. 1c**). As local hyperacetylation is not influenced by p38α/β, we considered that a further step in transcription activation, chromatin remodeling, could be the functional target of the p38 pathway. MyoD-associated chromatin remodeling activity has previously been described[19]. Changes in chromatin structure at the endogenous *Myog* and *Ckm* loci underlies the transcription of these genes during differentiation and can be monitored by restriction endonuclease access to specific sites[13,19]. We observed two peaks of endonuclease accessibility at the *Ban*I site of the *Myog* promoter, after 6 h and 24 h in differentiation medium (**Fig. 1d**), whereas the *Ckm* enhancer was accessible to endonucleases only after 18 h in differentiation medium (**Fig. 1e**). Chromatin remodeling at both loci was precluded by SB203580 (**Fig. 1d**,**e**), suggesting that p38α/β stimulates muscle-gene transcription by promoting recruitment or activation of the SWI-SNF chromatin-remodeling complex.

We therefore monitored the effect of SB203580 on the recruitment of acetyltransferases and the enzymatic subunits of the SWI-SNF complex (BRG1 and BRM) at the *Myog* promoter and *Ckm* enhancer in differentiating myoblasts. p300 and PCAF were found on the *Myog* promoter a few hours after incubation in differentiation medium (**Fig. 2a**), and p300 was detected at the *Ckm* enhancer during the first 18 h in differentiation medium (**Fig. 2a**). PCAF bound the *Ckm* enhancer at later stages of differentiation instead (**Fig. 2a**), coincident with the late binding of acetylated MyoD and the late onset of *Ckm* expression (**Fig. 1a**,**b**). BRG1 was reproducibly detected at *Myog* and *Ckm* regulatory elements after incubation in differentiation medium using three different antibodies to BRG1 (**Fig. 2a** and **Supplementary Fig. 4** online). We also detected BRM at the *Myog* promoter but found a much smaller amount at the *Ckm* enhancer (**Fig. 2a**), suggesting that distinct complexes, with potential redundant functions, might be recruited at muscle-regulatory sequences, possibly by different MRFs[18].

Inhibition of p38α/β did not affect the recruitment of p300 and PCAF (**Fig. 2a**) but prevented the engagement of BRG1 and BRM at the *Myog* promoter and the *Ckm* enhancer (**Fig. 2a**), supporting the conclusion that chromatin remodeling is selectively influenced by p38α/β. Recruitment of SWI-SNF complex has been linked to the engagement of the hyperphosphorylated, active fraction of RNA polymerase II, a crucial step in transcription initiation[20]. Consistent with this notion, the inhibition of BRG1-BRM recruitment by SB203580 correlated with reduced levels of RNA polymerase II phosphorylated at Ser5 at the *Myog* promoter and *Ckm* enhancer (**Fig. 2a**). In contrast, RNA polymerase II phosphorylated at Ser2 was detected only in the coding region of transcribed *Ckm* (**Supplementary Fig. 2** online).

Immunoprecipitation of nuclear extracts with antibodies to MyoD uncovered an association between endogenous MyoD, p300, PCAF and BRG1 in differentiating myoblasts (**Fig. 2b**), but only the MyoD-BRG1 interaction was disrupted by SB203580 (**Fig. 2b**). SB203580 did not decrease the cellular levels of BRG1 or other SWI-SNF components (**Fig. 2c**). MyoD-Mef2 interactions have been previously described[21]. As antibodies to MyoD could coprecipitate Mef2 and vice versa, and antibodies to Mef2 also coprecipitated BRG1 (data not shown), the SWI-SNF complex might interact with MyoD, Mef2 or both.

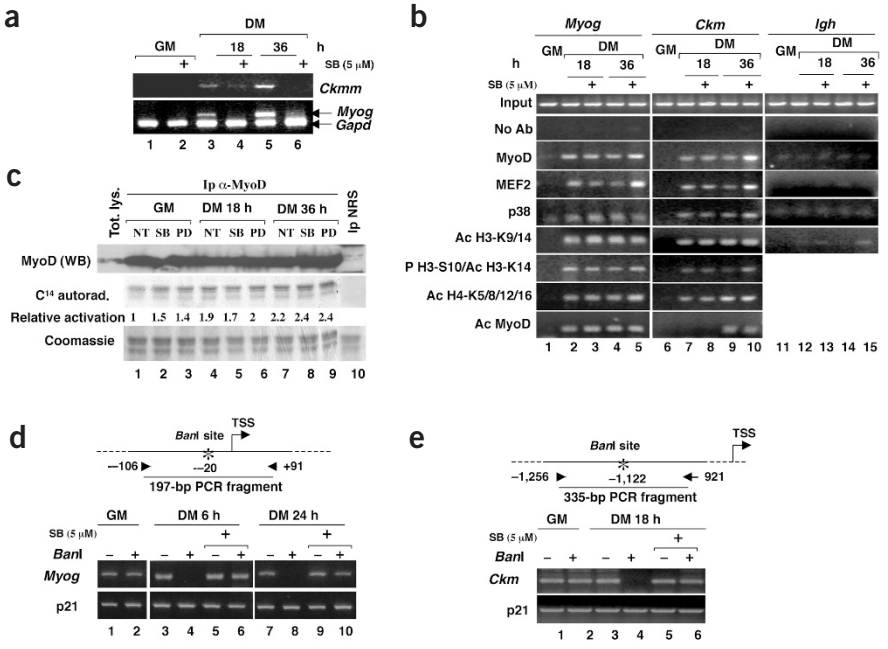

Figure 1 Blockade of p38 by SB203580 inhibits the expression of muscle genes by selectively preventing chromatin remodeling at their regulatory regions. (**a**) Expression of *Myog*, *Ckm* and *Gapd* RNA was monitored by RT-PCR with RNA from C2C12 undifferentiated myoblasts cultured in growth medium (GM) or induced to differentiate by incubation in differentiation medium (DM) for the indicated times, exposed (+) or not exposed to SB203580 (SB). (**b**) ChIP analysis of E-box–containing regions in the *Myog* promoter and the *Ckm* and *Igh* enhancers was done in undifferentiated (GM) or differentiated (DM) C2C12 myoblasts, in the presence (+) or absence of SB203580 (SB), with the indicated antibodies. (**c**) MyoD-associated acetyltransferase activity was monitored after MyoD immunoprecipitation from nuclear extracts of undifferentiated (GM) or differentiated (DM) C2C12 myoblasts, in the absence (NT) or presence (SB) of SB203580 or the MEK1 inhibitor PD. Levels of immunoprecipitated MyoD were detected by western blot (WB, top panel), and the associated acetyltransferase activity was evaluated by C^{14} incorporation (middle panel) of free histones (stained with Coomassie, bottom panel) after *in vitro* acetylation assay. Relative activation quantifies the histone incorporation of C^{14} acetylated coenzyme A relative to the C^{14} incorporation detected in untreated undifferentiated myoblasts (lane 1) and is normalized to the levels of immunoprecipitated MyoD. (**d**,**e**) Restriction endonuclease accessibility was done on nuclei isolated from C2C12 myoblasts either undifferentiated (GM) or differentiated by incubation in differentiation medium (DM) for the indicated times, with or without SB203580 (SB). Nuclei were digested with *Ban*I and then subjected to PCR to amplify a 197-bp fragment from –106 to +91 relative to the transcription start site (TSS) for the *Myog* promoter (**d**) or a fragment from –1,256 to –1,122 relative to the transcription start site (TSS) for the *Ckm* enhancer (**e**). As a control, a p21 promoter fragment devoid of *Ban*I sites was used. Absence or reduction of PCR signal indicates *Ban*I accessibility and reflects chromatin remodeling.

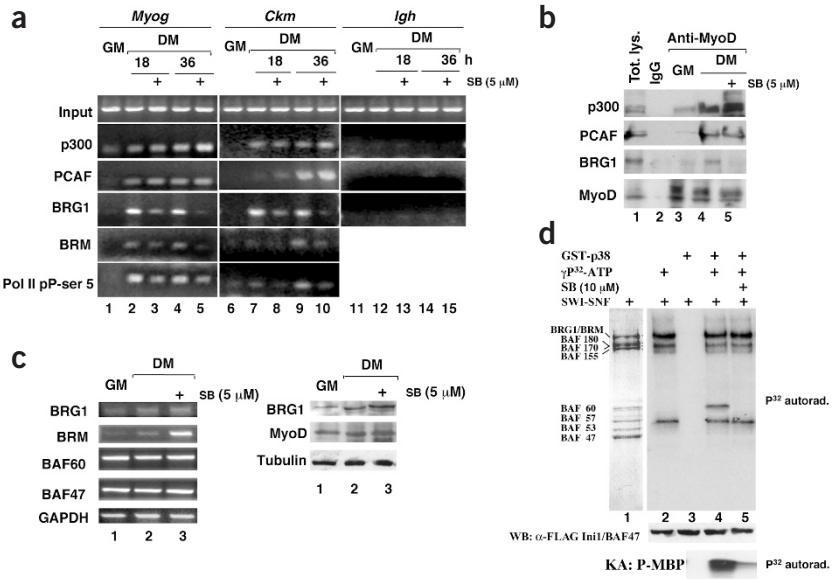

Figure 2 Blockade of p38 by SB203580 selectively prevents SWI-SNF recruitment at regulatory regions of muscle genes. (**a**) ChIP analysis of E-box–containing regions in the *Myog* promoter and the *Ckm* and *Igh* enhancers was done in C2C12 myoblasts undifferentiated (GM) or differentiated (DM) for the indicated times, in the presence (+) or absence of SB203580 (SB), by using the described antibodies. Densitometric analysis showed that binding of BRG1 to chromatin was progressively reduced in cells treated with SB203580 at the *Myog* promoter (by 70% at 18 h and 90% at 36 h) and at the *Ckm* enhancer (by 55% at 18 h and 90% at 36 h). (**b**) Immunoprecipitation of MyoD was done from nuclear extracts of undifferentiated (GM) or differentiated (DM) C2C12 myoblasts in the presence (+) or absence of SB203580 (SB). Levels of precipitated MyoD and coprecipitated p300, PCAF and BRG1 were detected by western blotting. (**c**) Expression levels of BRG1, BRM, BAF60c, BAF47 and GAPDH RNA were monitored by RT-PCR (left panel) and protein levels of BRG1, MyoD and tubulin were monitored by western blotting (right panel) from C2C12 myoblasts cultured in growth medium (GM) or in differentiation medium (DM), with (+) or without SB203580 (SB). (**d**) A kinase assay was carried out by incubating the mammalian SWI-SNF complex with P^{32}-ATP in the presence or absence of recombinant p38 kinases α/β and SB203580 (SB). A fraction of each reaction was used for western blotting (WB) with antibodies to FLAG-tagged Ini-BAF47 to normalize the levels of the complex used for the reaction. A kinase reaction (KA) with recombinant p38 kinases α/β and their known substrate myelin basic protein (MBP) was used as positive control.

To begin elucidating the mechanism that mediates p38-dependent recruitment of SWI-SNF complex to myogenic loci, we carried out an *in vitro* kinase assay with purified mammalian SWI-SNF complex incubated with recombinant p38α/β kinases. Incorporation of P^{32} by some SWI-SNF subunits was apparent in the absence of p38 kinases, but phosphorylation of BAF60 was induced only after incubation with p38 kinases and was eliminated by the inclusion of SB203580 (**Fig. 2d**). Although the functional relevance of this phosphorylation has yet to be established, it is notable that BAF60a–BAF60c isoforms have been implicated as the surface for interactions between SWI-SNF and transcriptional activators[22,23].

We next used a C2C12 subclone stably expressing the p38 activator MKK6EE under the negative control of a tetracycline-responsive element to evaluate whether the ability of MKK6EE to override the antidifferentiation cues in myoblasts[5,6] relies on forced recruitment of SWI-SNF at myogenic loci. Expression of MKK6EE by

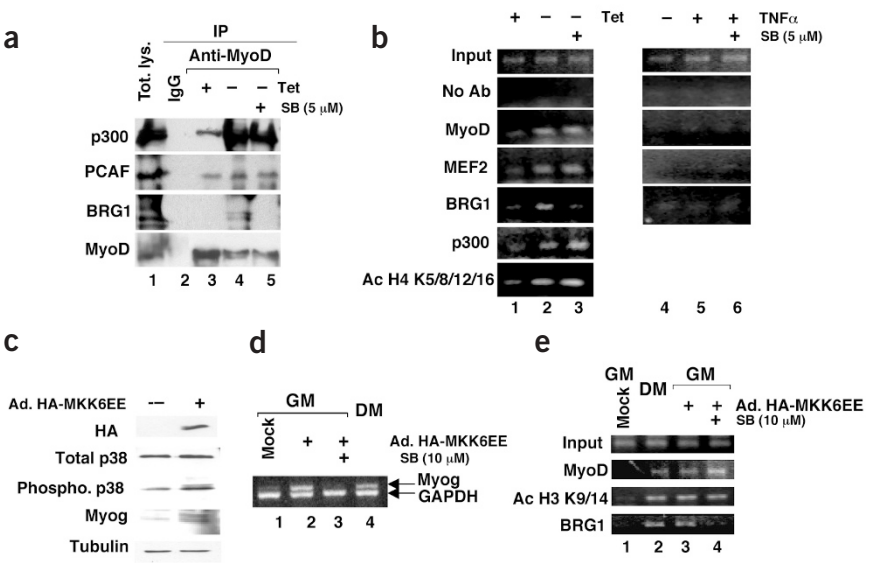

Figure 3 Forced activation of p38 in myoblasts by ectopic expression of MKK6EE induces myogenin expression by promoting BRG1 recruitment on the *Myog* promoter. (**a**) Immunoprecipitation of MyoD was done from nuclear extracts of C2-MKK6EE myoblasts cultured in growth medium (GM), with (+) or without (–) tetracycline (Tet) and SB203580 (SB). Levels of precipitated MyoD and coprecipitated PCAF, p300 and BRG1 were detected by western blotting. (**b**) ChIP analysis of E-box–containing regions in the *Myog* promoter was done in C2-MKK6EE myoblasts cultured in growth medium (GM), with (+) or without (–) tetracycline (Tet) and SB203580 (SB, left panel) or in the parental C2C12 cell line, exposed to TNFα (10 ng ml^{-1}) in the presence or absence of SB203580 (right panel), using the indicated antibodies. (**c**–**e**) C2C12 myoblasts were either infected with adenovirus expressing MKK6EE (+) or mock-infected (–) and then incubated in growth medium (GM). DM, differentiation medium. (**c**) Western-blot analysis was done from whole-cell extracts of MKK6EE- or mock-infected C2C12 cells to determine levels of MKK6EE (HA-tagged), total and phosphorylated p38, myogenin and tubulin. (**d**) RT-PCR analysis of myogenin RNA from MKK6EE- or mock-infected C2C12 cells, in the presence (+) or absence of SB203580 (SB). (**e**) ChIP analysis of E-box–containing regions in the *Myog* promoter was done in MKK6EE- or mock-infected C2C12 cells, with (+) or without SB203580 (SB).

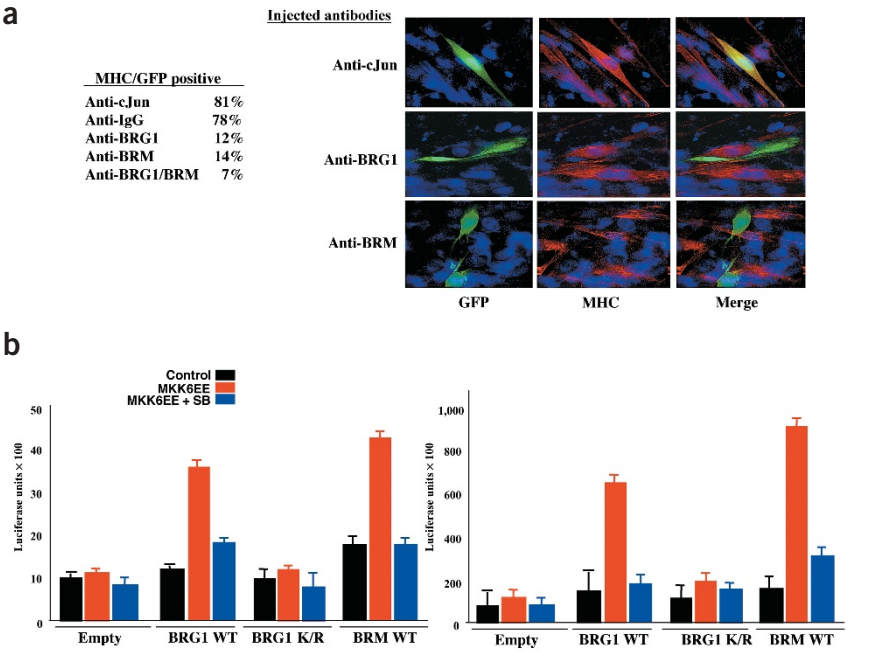

Figure 4 Inhibition or absence of SWI-SNF enzymatic activity abrogates MKK6EE-induced activation of muscle gene transcription and skeletal myogenesis. (**a**) C2-MKK6EE myoblasts cultured in growth medium in the presence of tetracycline were microinjected with a mixture of GFP cDNA and antibodies against BRG1, BRM, cJun and IgG. After microinjection, cells were incubated in growth medium without tetracycline for 36 h and fixed for immunofluorescence studies. Productively injected cells were visualized by GFP expression (green) and differentiation was visualized by staining with antibody to MHC (red). Nuclei were counterstained with DAPI. The differentiation of injected cells was visualized by merging the red and green images, resulting in yellow staining. The number of cells with yellow staining (MHC-GFP double positive) and the number of cells with green staining (at least 50 in each experiment) were counted in two distinct experiments to measure the effect of antibody injection on myoblast differentiation. (**b**) SW13 polyclones with a chromatin-integrated *Gal4-luc* were transfected with wild-type Gal4-MyoD (left) or Gal4-MyoD-N (right; amino acids 1–53 of MyoD) with or without MKK6EE, wild-type (WT) BRG1, enzymatic deficient BRG1 K798R (K/R) and wild-type (WT) BRM, and then cultured for 48 h in growth medium in the presence or absence of SB203580 (SB). Cells were then collected and luciferase activity was measured.

tetracycline removal in myoblasts led to the formation of a complex containing MyoD, p300, PCAF and BRG1 (**Fig. 3a**); SB203580 prevented BRG1 association, but not MyoD, p300 or PCAF interactions (**Fig. 3a**). MyoD, MEF2C, p300 and BRG1 occupied the *Myog* promoter after expression of MKK6EE, leading to histone hyperacetylation (**Fig. 3b**). Inhibition of p38α/β selectively prevented BRG1 recruitment (**Fig. 3b**). These results indicate that MKK6EE can promote the assembly of the myogenic transcriptosome on the *Myog* promoter. Only SWI-SNF recruitment seemed to be directed by p38α/β, as it was abrogated by SB203580. This suggests that other MKK6-activated, SB203580-resistant kinases might promote the recruitment of MRFs and acetyltransferases. Activation of p38 also occurs in response to inflammatory cytokines[9], but p38 activation by TNFα does not promote any step of the transcriptosome assembly on the *Myog* promoter, including MRFs and BRG1 recruitment (**Fig. 3b**).

Acute expression of hemagglutinin (HA)-tagged MKK6EE in myoblasts was also achieved by adenoviral infection. Expression of HA-MKK6EE was detected only in infected myoblasts and correlated with p38 activation and *Myog* expression (**Fig. 3c**). SB203580 inhibited MKK6EE-induced *Myog* transcription (**Fig. 3d**). BRG1, MyoD and hyperacetylated histones were detected at the *Myog* promoter in myoblasts infected with MKK6EE but not in mock-infected myoblasts (**Fig. 3e**). Only BRG1 recruitment was inhibited by blockade of p38α/β (**Fig. 3e**). To establish a causal relationship between MKK6EE-p38 and SWI-SNF-dependent chromatin remodeling at the muscle loci, we inactivated the enzymatic subunits of the SWI-SNF complex by microinjection of neutralizing antibodies in C2C12 cells stably expressing MKK6EE under the control of a tetracycline-responsive element. Antibodies against BRG1 or against BRM prevented MKK6EE-induced expression of muscle genes, such as myosin heavy chain (MHC; **Fig. 4a**).

We further investigated the requirement for SWI-SNF-dependent chromatin remodeling in MKK6EE-p38-dependent activation of MyoD-mediated transcription by monitoring the ability of Gal4-MyoD to activate a chromatin-integrated Gal4-responsive reporter (*Gal4-luc*) in BRG1-BRM-deficient SW13 cells[24]. Gal4-MyoD alone was unable to activate transcription, and MKK6EE did not augment *Gal4-luc* activity in these cells (**Fig. 4b**). Reintroduction of BRG1 and BRM, but not of the enzymatically defective BRG1 point mutant K798R (ref. 25), restored the ability of MKK6EE to activate *Gal4-luc* (**Fig. 4b**), further showing the importance of the catalytic SWI-SNF subunits in enabling MKK6EE to promote MyoD-dependent transcription. SB203580 abrogated MKK6EE-dependent activation of Gal4-MyoD in the presence of BRG1 or BRM (**Fig. 4b**), indicating that p38α/β is essential in promoting SWI-SNF-mediated activation of MyoD-dependent transcription. We made similar observations when we fused the N-terminal acidic transactivation domain of MyoD to Gal4 (Gal4-MyoD-N). As this mutant does not bind Mef2 (ref. 21) and induces transcription autonomously, activation of MyoD-dependent transcription by the MKK6EE-p38-SWI-SNF pathway does not seem to rely on p38-mediated phosphorylation of Mef2A–Mef2C (refs. 4,5,9,10).

Notably, Gal4-MyoD-N does not contain any of the domains previously reported to mediate activation of genes in repressive chromatin[19]. But SWI-SNF can be targeted to promoters by interactions with the acidic transactivation domain[26] or other regions[27] of sequence-specific transcriptional activators, suggesting that interactions of SWI-SNF with transcription factors are multifaceted. Local histone hyperacetylation was proposed to stabilize SWI-SNF binding to the chromatin in the absence of transcription factors[28]. The uncoupling of histone hyperacetylation and chromatin remodeling on muscle-regulatory regions, after blockade of p38, indicates that additional events, such as the activation of signaling cascades, are required to target the SWI-SNF complex to particular loci in response to specific cues.

Our results extend the function of p38 kinases beyond their ability to activate gene expression by direct phosphorylation of transcription factors and establish a link between the differentiation-activated p38 pathway and the recruitment of SWI-SNF complexes to transcriptionally active loci during skeletal myogenesis.

METHODS

Cell cultures, transfections and luciferase assay. We cultured C2C12 myoblasts and SW13 cells in Dulbecco's modified Eagle medium supplemented with 15% fetal bovine serum (growth medium). Differentiation was induced by incubation in Dulbecco's modified Eagle medium supplemented with 2% horse serum and insulin-transferrin (differentiation medium). We generated SW13 polyclones containing integrated *Gal4-luc* by transfecting cells with the *Gal4-luc* reporter and pcDNA3-*neo*^{r} and selecting G418-resistant clones. We combined antibiotic-resistant colonies to yield a polyclonal population of reporter cells. C2-MKK6EE cells are described in ref. 5.

We purchased SB203580 (final concentration 5 M), PD98059 (final concentration 25 M) and TNFα (final concentration 10 ng ml^{-1}) from Calbiochem. We added SB203580 and PD98059 to cell cultures when placed in differentiation medium and added fresh compounds every 18 h. Transfections were done with the Lipofectamine 2000 reagent. We determined luciferase activity with a Promega Luciferase kit and normalized it either to activity for β-galactosidase or using the Dual-Luciferase reporter assay system (Promega).

Adenoviral infections. We excised a cDNA encoding MKK6EE from pcDNA3.1-MKK6EE-HA with *Hin*dIII and *Xba*I and ligated it into pAdShuttle-CMV digested with *Hin*dIII and *Xba*I to create pShuttle-MKK6EE. We created replication-deficient adenovirus expressing MKK6EE from the CMV promoter by homologous recombination of pShuttle-MKK6EE with pAdEasy in *Escherichia coli* BJ5183, generating pAdMKK6EE. The virus was multiplied by transfection of 293 packaging cells. We infected C2C12 cells with pAdMKK6EE for 2 h in serum-free medium and then placed them in growth medium for an additional 18 h, with or without SB203580 (10 M).

RT-PCR. We extracted RNA from C2C12 cells with Trizol Reagent (Invitrogen). We carried out RT-PCR using SuperScript One-Step RT-PCR with platinum *Taq* (Invitrogen). We mixed 400 ng of total RNA with 10 M of specific primers. The RT-PCR reaction was 50 °C for 40 min, followed by 94 °C for 2 min; 25–30 cycles of 94 °C for 15 s, 50–55 °C for 30 s and 72 °C for 40 s; and a final step of 72 °C for 5 min. Primer sequences are available on request.

Kinase assay. We incubated purified recombinant p38α/β with FLAG-tagged Ini1 SWI-SNF complex purified from Hela cells[29], in the presence or absence of ATP and SB203580. We carried out the reaction as described previously[5,6] and detected phosphorylation by P^{32} autoradiography.

Immunoprecipitation studies, acetyltransferase assay and western blotting. We precleared nuclear cell extracts with an A+G sepharose mix for 30 min at 4 °C and immunoprecipitated them with agarose-conjugated antibodies to MyoD (M-318 Ac, Santa Cruz and RGB7; ref. 12). We washed the immunoprecipitates extensively with lysis buffer, resuspended them in Laemmli buffer, separated them on polyacrylamide gels and transferred them to nitrocellulose membranes. We visualized precipitated proteins by ECL (NEN Life Sciences) with antibodies to p300 (N15, Santa Cruz), PCAF (H-369, Santa Cruz), MyoD (M-318, Santa Cruz) and BRG1 (monoclonal G-7, Santa Cruz). We carried out an *in vitro* acetylation assay after immunoprecipitation with antibodies to MyoD as described[15]. We carried out western blotting of whole-cell lysates using antibodies against HA (F7 from Santa Cruz), total p38 and phosphorylated p38 (Cell Signaling), and myogenin (FD5 hybridoma).

ChIP. We carried out a ChIP assay according to instructions from Upstate Biotechnology. We used antibodies to MyoD (M-318 Ac, Santa Cruz), Mef2c, p38α (refs. 5,6), acetylated H4, acetylated H3 and H3 phosphorylated at Ser10 and acetylated at Lys14 (Upstate Biotechnology), acetylated MyoD (ref. 17), p300 (N-15 Ac, Santa Cruz), PCAF (H-369, Santa Cruz), BRG1 (ref. 13), BRG1 (polyclonal N-15 and H-88, Santa Cruz), BRM (polyclonal N19 and C20, Santa Cruz) and RNA polymerase II phosphorylated at Ser5 (H14, Covance). We carried out PCR analysis of 'input' DNA of different samples with specific primers. PCR conditions were 95 °C for 2 min, followed by 25–30 cycles at 95 °C for 30 s, 55–60 °C for 30 s and 72 °C for 30 s. Primer sequences are available on request.

Endonuclease assay. We extracted genomic DNA from C2C12 cells by resuspending cell pellets in RSB buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 5 mM $MgCl_2$, 0.1% Nonidet P-40 and 5 mM butyrate, supplemented with protease and phosphatase inhibitors) and incubating for 20 min on ice. We homogenized nuclei with a 27-gauge syringe, centrifuged them at 2,000 r.p.m. for 5 min at 4 °C, washed them in RSB buffer, centrifuged them again at 2,000 r.p.m. for 5 min and resuspended them in 50 l of RSB buffer. We digested one to five nuclei in 400 l of 1 NEB4 (New England Biolabs) with 100 U of *Ban*I enzyme (for 1 h at 37 °C). Reactions were stopped with proteinase K and 2% SDS for 2 h at 45 °C. We extracted DNA with phenol-chloroform twice, precipitated it in ethanol and resuspended it in water. For PCR, we resuspended an aliquot of each DNA sample in PCR SuperMix (Invitrogen) with specific primers. PCR conditions were 94 °C for 2 min, followed by 25–30 cycles of 94 °C, 58 °C for 30 s, 72 °C for 30 s and 72 C for 7 min. Primer sequences are available on request.

Microinjection studies and immunofluorescence. We microinjected C2-MKK6EE cells with a mixture of GFP cDNA (final concentration of 0.3 g l^{-1}) and antibodies against BRG1 (H88 rabbit, N15 goat or G7 mouse, each at a final concentration of 125 g ml^{-1}) or BRM (N19 goat or C20 goat, each at a final concentration of 125 g ml^{-1}) alone or in combination, and cJun (H79 rabbit, final concentration of 125 g ml^{-1}) or IgG as controls. We placed cells in growth medium without tetracycline 4 h after microinjection. After 36 h, cells were fixed and processed for immunofluorescence studies, using either mouse monoclonal or rabbit polyclonal antibodies to MHC, depending on the species of the injected antibodies, to avoid cross-reaction.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank J. Feramisco for continuous support to the project and for providing the microinjection facility; M. Montminy for support to P.L.P. and C.S.; C. Buckmaster for technical assistance in microinjection; J. Feramisco, V. Sartorelli, J.Y.J. Wang and members of the laboratory of P.L.P. for discussions and critical reading of the manuscript; V. Sartorelli for antibodies to acetylated MyoD; E. Knudsen for BRM, BRG1 and BRG1 (K798R) enzymatic-deficient mutant cDNA; J. Han for antibodies to MEF2c; T. Sudo for antibodies to p38α; and R. Evans for SW13 cells. This project was partially supported by MDA (Research Developmental Grant to P.L.P.) and by Compagnia San Paolo di Torino to Dulbecco Telethon Institute. C.S. was supported by a Telethon fellowship and Sbarro Health Research Organization. S.V.F. is supported by a Marie Curie fellowship.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 8 March; accepted 4 May 2004
Published online at http://www.nature.com/naturegenetics/

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