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The SNF2H Chromatin Remodeling Enzyme Has Opposing Effects on Cytokine Gene Expression

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Abstract

Cytokine gene expression is a key control point in the function of the immune system. Cytokine gene regulation is linked to changes in chromatin structure; however, little is known about the remodeling enzymes mediating these changes. Here we investigated the role of the ATP-dependent chromatin remodeling enzyme SNF2H in mouse T cells; to date, SNF2H has not been investigated in T cells. We found that SNF2H repressed expression of IL-2 and other cytokines in activated cells. By contrast, SNF2H activated expression of IL-3. The ISWI components SNF2H and ACF1 bound to the tested loci, suggesting the regulation was direct. SNF2H decreased accessibility at some binding sites within the IL2 locus, and increased accessibility within some IL3 binding sites. The changes in gene expression positively correlated with accessibility changes, suggesting a simple model that accessibility enables transcription. We also found that loss of the ISWI ATPase SNF2H reduced binding to target genes and protein expression of ACF1, a binding partner for SNF2H, suggesting complex formation stabilized ACF1. Together, these findings reveal a direct role for SNF2H in both repression and activation of cytokine genes.

Keywords

Gene Regulation; T cell regulation; Chromatin Remodeling; SNF2H; ISWI; BRG1; Cytokine Transcription

1. Introduction

Cytokine genes play pivotal roles in determining and executing the function of the immune system, so their regulation is of central importance. Much has been learned of the signaling pathways regulating these genes. A good deal is also known about the transcription factors that regulate cytokines. Activation specific factors, such as the NFAT and rel families, appear to function in activated T cells to directly regulate cytokine transcription. NFAT factors work on different genes in different T helper lineages (Avni et al., 2002; Wurster and Pazin, 2008). Lineage specific factors, such as GATA3 and T-bet, primarily function in particular T helper subsets (Wilson et al., 2009). STAT transcription factors are coupled to cytokine receptors to allow rapid response to the extracellular cytokine milieu. Together, these factors determine cytokine expression through combinatorial control.

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Changes in cytokine gene expression frequently correlate with changes in chromatin structure. Changes in chromatin structure have been detected by nuclease accessibility, primarily DNase I hypersensitivity (DHS). Changes in histone modifications have also been detected. The IL3/GMCSF locus is an early example of successfully using DHS mapping to identify regulatory regions (Cockerill, 2004). In the IL2 locus, there are also reports of chromatin structure changes linked to gene regulation (Attema et al., 2002; Garrity et al., 1994; Yui et al., 2001). Perhaps the best-studied example in lymphocytes is the IL4/IL13/IL5 locus, referred to here as the Th2 locus, approximately 700 kb away from the murine IL3/GMCSF locus. As naïve murine CD4 T cells adopt the T helper 1 (Th1) or T helper 2 (Th2) fates, chromatin structure changes are detected by DNase I hypersensitivity (DHS) over nearly 150 kb, including a central shared locus control region (LCR) (Ansel et al., 2006; Lee et al., 2006). More recently, chromatin structure changes have been found in the IL17A/IL17F locus in the context of Th17 lineage commitment (Akimzhanov et al., 2007; Zhang et al., 2008). DHS mapping has been a general tool to characterize these loci, and in several instances has been used to identify cis-acting regulatory elements.

Though chromatin structure changes in cytokine genes are well documented, little is known about how those changes occur. Unless catalyzed by chromatin remodeling enzymes, changes in chromatin structure are slow. ATP-dependent chromatin remodeling enzymes can rapidly reposition, assemble and disassemble nucleosomes, while other classes of remodeling enzymes covalently modify histone proteins or DNA (Lusser and Kadonaga, 2003; Saha et al., 2006). ATP-dependent remodeling enzymes are often multi-protein complexes, classified by their ATPase subunit into subfamilies such as ISWI, SWI/SNF and Mi2 (Lusser and Kadonaga, 2003). Remodeling ATPases are generally broadly expressed, and primary CD4 T cells express several remodeling ATPases (Wurster and Pazin, 2008).

SWI/SNF complexes are regulators of T cell development (Chi, 2004). SWI/SNF complexes contain 10-15 proteins, including the ATPases BRG1 and Brm. SWI/SNF plays an important role in activating T helper 2 (Th2) gene expression (Wurster and Pazin, 2008) and T helper 1 (Th1) gene expression (Letimier et al., 2007; Zhang and Boothby, 2006). BRG1 is dynamically recruited to the Th2 locus by transcription factors, during differentiation and again during activation (Wurster and Pazin, 2008). Thus, SWI/SNF components activate gene expression to regulate T cell development, differentiation and function.

Mi2 remodeling ATPases, found in NuRD complexes containing histone deacetylases, are also important in T cells. Mi2 β /CHD4 has been found to be important for CD4 expression and T cell development (Williams et al., 2004). Mi2 β can form stable complexes with the transcription factor Ikaros, and Mi2 β and Ikaros appear to antagonize each other (Naito et al., 2007). In macrophages, SWI/SNF and Mi2 β have opposing effects on gene regulation (Ramirez-Carrozzi et al., 2006). Thus the Mi2 remodeling family represses gene expression in the immune system.

Little is known about the role of the ISWI subfamily of remodeling ATPases in T cells, by contrast to the examples above. ISWI complexes are frequently dimeric complexes containing the SNF2H or SNF2L ATPase and one of several accessory proteins (Corona and Tamkun, 2004; Lusser and Kadonaga, 2003). We previously found that SNF2H mRNA is abundant in primary T cells (Wurster and Pazin, 2008). SNF2H is an essential gene with an embryonic lethal phenotype in mice (Stopka and Skoultchi, 2003). ACF1, a SNF2H binding partner, has been implicated in DNA replication and chromatin assembly (Collins et al., 2002; Fyodorov et al., 2004). The ACF1 subunit has been found to alter ISWI activity (Fyodorov et al., 2004; He et al., 2008; He et al., 2006). Here, we test the role of SNF2H in cytokine gene expression. We find SNF2H plays a direct role in transcription of cytokine genes. SNF2H and ACF1 bind to cytokine genes in cells. SNF2H protein is apparently required for ACF1 stability. SNF2H

can increase or decrease gene expression, depending on the cytokine. This activity correlates with changes in SNF2H-mediated chromatin accessibility.

2. Materials and methods

2.1 Cells

Murine EL4 T cell line was obtained from ATCC, grown in RPMI/10% FBS/L Glutamine, penicillin and Streptomycin.

Primary CD4 T cells were prepared from mice and differentiated in culture as described (Wurster and Pazin, 2008). Animal approval was from the NIA ACUC, protocol ASP-365-MJP-Mi, and all experiments conform to the relevant regulatory standards.

2.2 Knockdown

EL4 cells were transduced at high efficiency using nucleofection solution V and program T-14, according to the manufacturer's instructions. Typically, 65-85% of the cells become GFP+ in parallel transfections under these conditions (data not shown). Plasmid encoding SNF2H shRNA was cotransfected with a plasmid encoding a truncated version of the low-affinity NGF receptor (Miltenyi). Transduced cells were enriched 1 day after transfection using magnetic bead purification for NGFr+ cells (Miltenyi), fed on the second day, and analyzed on the third day. SNF2H shRNA plasmid (pBS/U6-606) was constructed by annealing GAGGAGGATGAAGAGCTATCTCGAGATAGCTCTTCATCCTCCTCTTTTG (MP 606) and AATTCAAAAAGAGGAGGATGAAGAGCTATCTCGAGATAGCTCTTCATCCTCCTC (MP 607), followed by ligation into pBS/U6 (Sui et al., 2002). To date, we have not been able to silence SNF2H expression in primary T cells. SNF2H knockdown did not significantly alter proliferation or apoptosis (Fig. S1). SNF2H knockdown did not obviously alter cell recovery or cell morphology, relative to control shRNA transduction.

2.3 mRNA quantitation

Total RNA was purified using RNeasy columns, including DNase treatment on columns (Qiagen). cDNA was made using iScript (BioRad) according to the manufacturer's instructions. The mRNA levels of chromatin remodeling factors, cytokines and transcription factors were determined by real time PCR using SYBR green (Qiagen) on an ABI 7500. Expression levels were normalized to TBP or β -actin as indicated. Oligo sequences are in the supplementary tables. We also confirmed that IL7ra and GADD45 are repressed by SNF2H while FKBP3 and LEF1 were activated, but did not study these genes further.

2.4 Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation was performed using methods similar to those described previously (Wurster and Pazin 2008); details are available on request. Approximately 20 million cells (enough for 5 immunoprecipitations using different antibodies) were crosslinked with 1% formaldehyde and quenched with glycine. Nuclei were prepared with buffer containing 1% Triton X-100, treated with micrococcal nuclease, sonicated using a Bioruptor, and adjusted to 0.1% SDS, 1% Triton X-100 and 150 mM NaCl at 5 ml. Chromatin was precleared with protein A Sepharose (Millipore) and ChIP was performed with the following antibodies: SNF2H (Bethyl A301-017A), ACF1 (Bethyl A301-318A), BRG1 (Wang et al., 1996) H3K9,14ac (Millipore 06-599), H3K27me3 (Abcam 6002) or control (rabbit IgG, Santa Cruz sc-2027). SNF2H ChIP using Santa Cruz SC 13054 (Lot A0605) gave similar results, but with lower signal. Bound chromatin was collected with protein A, washed, eluted with sodium bicarbonate/SDS and crosslinks were reversed, followed by protease treatment. Chromatin was

quantified by real-time PCR (Q-PCR) using an Applied Biosystems 7500 Fast with Sybr Green detection (Qiagen). Immunoprecipitated chromatin was first normalized to input DNA (% input), then normalized to SNF2H ChIP at a control locus. Oligo sequences are in the supplementary tables. We cannot tell whether the lower ACF1 signal relative to SNF2H reflects antibody quality, occupancy, or both. Nfm, Th2 IL13 -11k, and IL17 con are loci we have empirically determined have little or no BRG1 binding.

2.5 DNase I Hypersensitivity Analysis

DNase I Hypersensitivity analysis was performed using EL4 cells, as described previously for primary cells (Wurster and Pazin, 2008). Briefly, nuclei were released by hypotonic lysis in the presence of 0.5% NP40 and digested with the indicated amounts of DNase I (Worthington) for three minutes at room temperature. The samples were then treated with proteinase K, RNase A and the DNA was recovered after phenol/chloroform extraction and ethanol precipitation. Stimulated cells were enriched for viable cells prior to nuclear isolation by ficoll-hypaque separation. DNase I accessibility was assessed by real-time PCR of DNA samples (Wurster and Pazin, 2008). Briefly, DNase-treated DNA was subjected to real-time PCR using primers to indicated regulatory elements in the IL2 and IL3/GMCSF loci. PCR reactions were performed using QuantiTect SYBR green PCR kit (Qiagen) per manufacturer's instructions on an ABI 7500 Fast. The DNase I sensitivity is indicated by % DNA remaining compared to undigested sample and DNA content is normalized to a known DNase I resistant locus (Nfm exon 1); values greater than 100% reflect normalization error. Oligo sequences are in the supplementary tables.

3. Results

3.1 Knockdown of SNF2H protein in EL4 cells

The T cell lymphoma EL4 is a well-studied cell line known to upregulate the mRNA for a number of cytokines upon activation of the cells with pharmacologic agents. We chose to use EL4 cells as a model system to study the role of chromatin remodeling in cytokine gene activation. First, we profiled mRNA encoding remodeling enzyme proteins. We found many were present (Fig 1A), similar to our findings with primary murine CD4⁺ naïve, Th1, and Th2 cells (Wurster and Pazin, 2008). SNF2H, an ATPase from the ISWI family, was found at high levels, while there was little or no mRNA encoding the other ISWI ATPase family member SNF2L (Fig. 1A). Among the SNF2H binding partners, ACF1/BAZ1A mRNA was present at high levels, while BPTF/NURF301, WSTF/BAZ1B and BAZ2A/TIP5 were present at lower levels. There were no large changes in the RNA for these remodeling factors upon stimulation of the cells.

We hypothesized SNF2H might be a regulator of cytokine genes. Little is known about the role of ISWI remodeling enzymes in mammalian gene expression, or T cell gene expression. We developed conditions to deplete EL4 cells of SNF2H protein using nucleofection and a plasmid encoding shRNA targeted to SNF2H. SNF2H protein expression was strongly decreased, while HSP90 protein, a loading control, was unaffected (Fig. 1B). SNF2H knockdown did not obviously alter proliferation, apoptosis, cell recovery or cell morphology, relative to control shRNA transduction (Fig. S1 and data not shown).

3.2 SNF2H Regulates Cytokine mRNA during activation

Regulation of gene expression in T cells is frequently accompanied by changes in chromatin structure. It is thought that chromatin remodeling enzymes alter the accessibility of the DNA contained in chromatin to either augment or reduce gene expression. We hypothesized SNF2H might regulate one or more T cell cytokines, based on previous studies demonstrating the role of SWI/SNF in regulating cytokine loci after T cell stimulation (Letimier et al., 2007; Wurster

and Pazin, 2008; Zhang and Boothby, 2006). We began to test this hypothesis by examining the steady-state mRNA of a number of candidate T cell cytokines. After SNF2H depletion or treatment with a control shRNA, cells were stimulated with PMA and Ionomycin for various times. SNF2H mRNA was strongly decreased by the SNF2H knockdown (mean 6 fold), consistent with the observed protein depletion. By contrast β -actin (a housekeeping gene) and BRG1 (a remodeling ATPase from the SWI/SNF family) were not affected by the SNF2H knockdown, confirming the specificity of the shRNA reagent (data not shown).

We found that mRNA for IL-2 in stimulated cells increased following SNF2H knockdown (Fig. 2A). This suggested SNF2H might function to repress IL-2 expression. There was no change in the timing of IL-2 expression; rather, the maximum amount of IL-2 mRNA was increased. SNF2H depletion also increased expression of IL-17A, IL-5 and IL-13 mRNA (Fig. S2). Analysis of unspliced (nascent) IL-2 and IL-5 RNA revealed increased RNA after SNF2H knockdown, suggesting the SNF2H-mediated regulation was at the level of transcription (Fig. 2C). Together, these findings suggested that SNF2H repressed transcription of IL-2 and other cytokines.

By contrast, examination of the cytokine IL-3 revealed a different mode of action for SNF2H. SNF2H depletion resulted in decreased expression of IL-3 mRNA in stimulated cells (Fig. 2B). This suggested that SNF2H was activating expression of IL-3, rather than acting as a repressor. The maximum amount of IL-3 mRNA was changed by SNF2H depletion, while there was no change in the timing of mRNA accumulation. Thus, SNF2H appeared to have opposing effects on cytokine expression. SNF2H appeared to repress IL-2, IL-5, IL17A and IL-13 mRNA; by contrast, SNF2H appeared to activate IL-3 expression at the same time in the same cells.

3.3 SNF2H Binds Target Loci, suggesting regulation is direct

We hypothesized that SNF2H might be regulating cytokine gene expression directly, by binding the cytokine loci and regulating their chromatin structure. Alternatively, SNF2H might be regulating the expression of intermediary genes that in turn directly regulated the cytokines. We focused these studies on IL-2, which was repressed by SNF2H, and IL3, which was activated by SNF2H, to determine whether there were differences in SNF2H function at repressed and activated loci. We began to test this hypothesis by asking whether SNF2H was present at the IL2 gene using chromatin immunoprecipitation (ChIP). The IL2 gene was previously found to have DNase I HS at -8, -7, -4.5, and -2.5k relative to the promoter (Yui et al., 2001) as well as promoter bound factors (Attema et al., 2002; Garrity et al., 1994). We investigated SNF2H binding in intact cells at these regions as well as conserved, non-coding sequences (CNS; Frazer et al., 2004) at -47k, -26k, -1.8, -1.1, and +1.5k. We found SNF2H binding at these known or putative regulatory regions in the IL2 gene (Fig. 3A), relative to ChIP with control IgG. SNF2H binding was found throughout the locus, not limited to the promoter. There was a small increase in the extent of binding comparing resting and stimulated cells, while there was little if any change in the pattern of binding. We asked whether the SNF2H partner ACF1 was recruited to these sites, as SNF2H generally functions in dimeric complexes, and we had found ACF1 mRNA at high levels in these cells. We found ACF1 was recruited to some of the same sites as SNF2H, consistent with SNF2H binding (Fig. 3A). These findings suggest with a direct role for SNF2H in regulating IL-2 expression.

We next examined SNF2H binding to the IL3 locus. We tested the IL3 promoter, the nearby GM-CSF promoter, an enhancer reported to regulate IL3 expression (Cockerill, 2004; referred to as IL3 enh here, -4.8k upstream of IL3), an enhancer reported to regulate GMCSF expression (Cockerill, 2004; referred to as GM-CSF Enh here, -1.8k upstream up GM-CSF), and CNSa, (a conserved non-coding sequence in this locus, +34.7k downstream of GM-CSF). We found that SNF2H bound all of these regions (Fig. 3B), not limited to the promoters. ACF1 was present at some of these sites, relative to control IgG ChIP. We also found SNF2H and ACF1

binding sites throughout the IL17 and Th2 (IL4, IL13, IL5) loci (Fig. 4). SNF2H occupancy was reduced following SNF2H depletion, confirming the specificity of the SNF2H ChIP signal (Fig. 5).

Remodeling enzymes frequently contain subunits that can bind to modified histones; thus, we tested the hypothesis that histone modifications might target SNF2H to binding sites. However, we found little if any correlation between SNF2H binding and H3K9,14 acetylation or H3K27 trimethylation (Fig. 6). Moreover, we did not discern distinct histone modification signatures for the SNF2H repressed genes relative to the SNF2H activated gene. The amount of acetylation and methylation was low relative to primary Th2 cells (Wurster and Pazin, 2008) and data not shown. SNF2H and ACF1 binding to these loci was more uniform than BRG1 binding; BRG1 occupancy was more strongly dependent on the locus and cell state (Figure S3, S3). The SNF2H binding pattern at the Th2 locus was quite different than what we observed for another remodeling ATPase, BRG1 (Fig S4 and Wurster and Pazin, 2008). The distinct binding patterns for these remodeling ATPases suggested they were recruited by different signals.

The observed binding of SNF2H to cytokine genes in EL4 cells suggested that SNF2H might bind these genes in primary T cells as well. We generated Th1 effector cells in culture from mice, and measured SNF2H binding. Stimulated Th1 cell produce IL-2, IL-3, and GM-CSF, as well as IFN- γ and other cytokines. As in EL4 cells, we found SNF2H and ACF1 present at the IL2 and IL3/GMCSF loci (Fig. 7). Stimulation did not strongly alter SNF2H binding in Th1 cells. By contrast, BRG1 binding was more strongly dependent on the locus examined and the activation state of the cells. Thus, SNF2H and ACF1 were bound to these loci in primary T cells.

It was unexpected to us that SNF2H binding was relatively uniform across these cytokine loci. We considered that SNF2H might 1) constitutively bind target loci, or 2) bind specific targets regulated during development, or 3) bind specific targets regulated during differentiation. We compared resting and activated Th1 cells to naïve Th cells and two regions of brain. We found SNF2H binding to cytokine loci was reduced in brain relative to Th1 cells, while binding to a neuronal locus (Nfm) was increased in brain, consistent with the model that SNF2H binding is regulated during development (Figure 8). We found that SNF2H binding to cytokine loci was increased in Th1 cells relative to naïve Th cells, consistent with the model that SNF2H binding was regulated during differentiation (Figure 8).

3.4 SNF2H Regulates Cytokine Gene Chromatin Structure

We hypothesized that SNF2H might directly regulate cytokine genes by binding them and modifying their chromatin structure. To test this, we asked whether a general measure of chromatin structure, DNase I hypersensitivity, was altered following SNF2H depletion. At the IL-2 promoter and -1.8k regions, we found chromatin was *more* accessible following SNF2H knockdown (Fig. 9A). By contrast, we found that at the IL-3 promoter and CNSa, the chromatin was *less* accessible following SNF2H knockdown (Fig. 9B). SNF2H binding at these sites was reduced following SNF2H depletion, consistent with a direct effect (Figure 5). These results are consistent with the simple model that chromatin accessibility facilitates transcription, and a closed conformation inhibits activity. These results are also consistent with a direct role for SNF2H in regulating IL-2 and IL-3 transcription. SNF2H-dependent chromatin structure was also found in the Th2 cluster (data not shown).

3.5 ACF1, A SNF2H Binding Partner, Is Destabilized By Loss Of SNF2H

We observed that depletion of SNF2H protein reduced ACF1 binding to target loci (Fig. 5). SNF2H is known to form complexes with partners such as ACF1. If ACF1 were the targeting subunit of the complex, ACF1 occupancy could have been unaffected by SNF2H depletion.

Some mutations in components of remodeling enzyme complexes have been found to destabilize the remaining subunits (Chen and Archer, 2005). We hypothesized that loss of ACF1 binding was either because SNF2H was no longer present to recruit ACF1 protein to target loci, or the complex was destabilized. We compared expression of ACF1 protein after SNF2H shRNA or control shRNA treatment. We found that ACF1 protein was depleted when SNF2H protein was depleted using SNF2H shRNA (Fig. 10A). By contrast, ACF1 mRNA was unaffected (Fig. 10B). Thus, some components of ISWI complexes are sensitive to the relative amounts of ISWI ATPase subunits.

4. Discussion

We found that SNF2H repressed expression of cytokine mRNA (IL-2, IL-17A, IL-5, IL-13) in activated cells. By contrast, SNF2H activated expression of IL-3 mRNA. SNF2H bound to the tested loci in both the EL4 cell line and primary T cells, suggesting the regulation was direct. SNF2H decreased accessibility at some binding sites within the IL2 locus, and increased accessibility within some IL3 binding sites. We also found that loss of the ISWI ATPase SNF2H reduced protein expression of ACF1, a binding partner for SNF2H.

The major role for SNF2H on cytokine expression in our study was repression. This is in contrast to our previous work identifying BRG1 as an activator of expression of Th2 genes (Wurster and Pazin, 2008). SNF2H is an ATPase in the ISWI subfamily, while BRG1 is in the SWI/SNF subfamily. SWI/SNF family members have been more frequently linked to gene activation, though repression has also been found. ISWI family members are more frequently linked to repression, though there is also evidence for a role in gene activation. We found little if any change in SNF2H binding upon stimulation of cells, by contrast, we previously found BRG1 binding to target loci could change rapidly upon stimulation (Wurster and Pazin, 2008). We found that SNF2H binding was regulated during development and T helper differentiation. Thus, the SNF2H-mediated chromatin accessibility correlated with gene expression, while the SNF2H binding pattern did not. Consistent with these binding kinetics, SNF2H exerts effects on both the basal and activated chromatin structure of target genes. We found SNF2H binding at distal regions as well as promoter regions, similar to our previous findings with BRG1.

Unexpectedly, we found that SNF2H has opposing effects on cytokine gene expression; SNF2H repressed most of the tested genes, but activated one. These changes in activity correlated with accessibility, suggesting a simple model that accessibility enables transcription. We do not know why SNF2H has opposite effects on the chromatin structure of these loci. Examination of the extent of histone acetylation or histone methylation did not reveal a simple classification of the activation and repression targets.

One hypothesis for the opposing effects of SNF2H is that the SNF2H partners could be different at these loci. SNF2H is known to bind several proteins, including ACF1, BPTF, WSTF, BAZ2A, BAZ2B. Perhaps the identity of the binding partner is different at loci where SNF2H is a coactivator, leading to a different remodeling result. We do detect more ACF1 at CNSa than IL-2 -1.8k and promoter. We were unable to reliably measure BPTF at any of the tested loci. Another hypothesis is that the relative amounts of other remodeling enzymes determines the outcome. CNSa has more BRG1 binding than do the IL2 loci. We have not measured NuRD complexes containing Mi2/CHD3/CHD4 ATPases in this study.

These findings reveal that SNF2H, a chromatin remodeling ATPase not previously examined in lymphocytes, can regulate cytokine gene expression in a direct manner. SNF2H is essential for early development in mouse (Stopka and Skoultschi, 2003), while the fly homolog is required for early development and cell viability (Deuring et al., 2000). In budding yeast, flies, frogs

and mice, ISWI proteins can repress gene expression (Deuring et al., 2000; Dirscherl et al., 2005; Fazio et al., 2001; Fyodorov et al., 2004; Landry et al., 2008; Sherriff et al., 2007). However, ISWI proteins have also been linked to gene activation in several species and cell-free biochemical systems (Badenhorst et al., 2002; Barak et al., 2003; Ito et al., 1997; Landry et al., 2008; LeRoy et al., 1998; Mizuguchi et al., 1997). Moreover, an ISWI binding protein has been reported to be recruited by histone H3K4 trimethylation, a mark frequently found at active promoters (Wysocka et al., 2006). Analysis of other untested remodeling ATPases such as CHD7, which is linked to immunodeficiency (Gennery et al., 2008), will help us to understand the chromatin remodeling landscape and how it determines gene expression programs in lymphocytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Akimzhanov AM, Yang XO, Dong C. Chromatin remodeling of interleukin-17 (IL-17)-IL-17F cytokine gene locus during inflammatory helper T cell differentiation. *J Biol Chem* 2007;282:5969–72. [PubMed: 17218320]
- Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 2006;24:607–56. [PubMed: 16551261]
- Attema JL, Reeves R, Murray V, Levichkin I, Temple MD, Tremethick DJ, Shannon MF. The human IL-2 gene promoter can assemble a positioned nucleosome that becomes remodeled upon T cell activation. *J Immunol* 2002;169:2466–76. [PubMed: 12193716]
- Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002;3:643–51. [PubMed: 12055628]
- Badenhorst P, Voas M, Rebay I, Wu C. Biological functions of the ISWI chromatin remodeling complex NURF. *Genes Dev* 2002;16:3186–98. [PubMed: 12502740]
- Barak O, Lazzaro MA, Lane WS, Speicher DW, Picketts DJ, Shiekhhattar R. Isolation of human NURF: a regulator of Engrailed gene expression. *Embo J* 2003;22:6089–6100. [PubMed: 14609955]
- Chen J, Archer TK. Regulating SWI/SNF subunit levels via protein-protein interactions and proteasomal degradation: BAF155 and BAF170 limit expression of BAF57. *Mol Cell Biol* 2005;25:9016–27. [PubMed: 16199878]
- Chi T. A BAF-centred view of the immune system. *Nat Rev Immunol* 2004;4:965–77. [PubMed: 15573131]
- Cockerill PN. Mechanisms of transcriptional regulation of the human IL-3/GM-CSF locus by inducible tissue-specific promoters and enhancers. *Crit Rev Immunol* 2004;24:385–408. [PubMed: 15777160]
- Collins N, Poot RA, Kukimoto I, Garcia-Jimenez C, Deltre G, Varga-Weisz PD. An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. *Nat Genet* 2002;32:627–32. [PubMed: 12434153]
- Corona DF, Tamkun JW. Multiple roles for ISWI in transcription, chromosome organization and DNA replication. *Biochim Biophys Acta* 2004;1677:113–9. [PubMed: 15020052]
- Deuring R, Fanti L, Armstrong JA, Sarte M, Papoulas O, Prestel M, Daubresse G, Verardo M, Moseley SL, Berloco M, Tsukiyama T, Wu C, Pimpinelli S, Tamkun JW. The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Mol Cell* 2000;5:355–65. [PubMed: 10882076]

- Dirscherl SS, Henry JJ, Krebs JE. Neural and eye-specific defects associated with loss of the Imitation Switch (ISWI) chromatin remodeler in *Xenopus laevis*. *Mech Dev* 2005;122:1157–70. [PubMed: 16169710]
- Fazio TG, Kooperberg C, Goldmark JP, Neal C, Basom R, Delrow J, Tsukiyama T. Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol* 2001;21:6450–60. [PubMed: 11533234]
- Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. VISTA: computational tools for comparative genomics. *Nucleic Acids Res* 2004;32:W273–9. [PubMed: 15215394]
- Fyodorov DV, Blower MD, Karpen GH, Kadonaga JT. Acf1 confers unique activities to ACF/CHRAC and promotes the formation rather than disruption of chromatin in vivo. *Genes Dev* 2004;18:170–83. [PubMed: 14752009]
- Garrity PA, Chen D, Rothenberg EV, Wold BJ. Interleukin-2 transcription is regulated in vivo at the level of coordinated binding of both constitutive and regulated factors. *Mol Cell Biol* 1994;14:2159–69. [PubMed: 8114746]
- Gennery AR, Slatter MA, Rice J, Hoefsloot LH, Barge D, McLean-Tooke A, Montgomery T, Goodship JA, Burt AD, Flood TJ, Abinun M, Cant AJ, Johnson D. Mutations in CHD7 in patients with CHARGE syndrome cause T-B + natural killer cell + severe combined immune deficiency and may cause Omenn-like syndrome. *Clin Exp Immunol* 2008;153:75–80. [PubMed: 18505430]
- He X, Fan HY, Garlick JD, Kingston RE. Diverse regulation of SNF2h chromatin remodeling by noncatalytic subunits. *Biochemistry* 2008;47:7025–33. [PubMed: 18553938]
- He X, Fan HY, Narlikar GJ, Kingston RE. Human ACF1 alters the remodeling strategy of SNF2h. *J Biol Chem* 2006;281:28636–47. [PubMed: 16877760]
- Ito T, Bulger M, Pazin MJ, Kobayashi R, Kadonaga JT. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* 1997;90:145–55. [PubMed: 9230310]
- Landry J, Sharov AA, Piao Y, Sharova LV, Xiao H, Southon E, Matta J, Tessarollo L, Zhang YE, Ko MS, Kuehn MR, Yamaguchi TP, Wu C. Essential role of chromatin remodeling protein bptf in early mouse embryos and embryonic stem cells. *PLoS Genet* 2008;4:e1000241. [PubMed: 18974875]
- Lee GR, Kim ST, Spilianakis CG, Fields PE, Flavell RA. T helper cell differentiation: regulation by cis elements and epigenetics. *Immunity* 2006;24:369–79. [PubMed: 16618596]
- LeRoy G, Orphanides G, Lane WS, Reinberg D. Requirement of RSF and FACT for transcription of chromatin templates in vitro. *Science* 1998;282:1900–4. [PubMed: 9836642]
- Letimier FA, Passini N, Gasparian S, Bianchi E, Rogge L. Chromatin remodeling by the SWI/SNF-like BAF complex and STAT4 activation synergistically induce IL-12Rbeta2 expression during human Th1 cell differentiation. *Embo J* 2007;26:1292–302. [PubMed: 17304212]
- Lusser A, Kadonaga JT. Chromatin remodeling by ATP-dependent molecular machines. *Bioessays* 2003;25:1192–200. [PubMed: 14635254]
- Mizuguchi G, Tsukiyama T, Wisniewski J, Wu C. Role of nucleosome remodeling factor NURF in transcriptional activation of chromatin. *Mol Cell* 1997;1:141–50. [PubMed: 9659911]
- Naito T, Gomez-Del Arco P, Williams CJ, Georgopoulos K. Antagonistic interactions between Ikaros and the chromatin remodeler Mi-2beta determine silencer activity and Cd4 gene expression. *Immunity* 2007;27:723–34. [PubMed: 17980631]
- Ramirez-Carrozzi VR, Nazarian AA, Li CC, Gore SL, Sridharan R, Imbalzano AN, Smale ST. Selective and antagonistic functions of SWI/SNF and Mi-2beta nucleosome remodeling complexes during an inflammatory response. *Genes Dev* 2006;20:282–96. [PubMed: 16452502]
- Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: the industrial revolution of DNA around histones. *Nat Rev Mol Cell Biol* 2006;7:437–47. [PubMed: 16723979]
- Sheriff JA, Kent NA, Mellor J. The Isw2 chromatin-remodeling ATPase cooperates with the Fkh2 transcription factor to repress transcription of the B-type cyclin gene CLB2. *Mol Cell Biol* 2007;27:2848–60. [PubMed: 17283050]
- Stopka T, Skoultschi AI. The ISWI ATPase Snf2h is required for early mouse development. *Proc Natl Acad Sci U S A* 2003;100:14097–102. [PubMed: 14617767]
- Sui G, Soohoo C, Affar el B, Gay F, Shi Y, Forrester WC. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. *Proc Natl Acad Sci U S A* 2002;99:5515–20. [PubMed: 11960009]

- Wang W, Cote J, Xue Y, Zhou S, Khavari PA, Biggar SR, Muchardt C, Kalpana GV, Goff SP, Yaniv M, Workman JL, Crabtree GR. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *Embo J* 1996;15:5370–82. [PubMed: 8895581]
- Williams CJ, Naito T, Arco PG, Seavitt JR, Cashman SM, De Souza B, Qi X, Keables P, Von Andrian UH, Georgopoulos K. The chromatin remodeler Mi-2beta is required for CD4 expression and T cell development. *Immunity* 2004;20:719–33. [PubMed: 15189737]
- Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol* 2009;9:91–105. [PubMed: 19151746]
- Wurster AL, Pazin MJ. BRG1-mediated chromatin remodeling regulates differentiation and gene expression of T helper cells. *Mol Cell Biol* 2008;28:7274–85. [PubMed: 18852284]
- Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, Wu C, Allis CD. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 2006;442:86–90. [PubMed: 16728976]
- Yui MA, Hernandez-Hoyos G, Rothenberg EV. A new regulatory region of the IL-2 locus that confers position-independent transgene expression. *J Immunol* 2001;166:1730–9. [PubMed: 11160218]
- Zhang F, Boothby M. T helper type 1-specific Brg1 recruitment and remodeling of nucleosomes positioned at the IFN-gamma promoter are Stat4 dependent. *J Exp Med* 2006;203:1493–505. [PubMed: 16717115]
- Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. *Nat Immunol* 2008;9:1297–306. [PubMed: 18849990]

Figure 1. Efficient silencing of SNF2H protein expression

A) Several remodeling ATPases are expressed in EL4 cells. mRNA was harvested from EL4 cells, resting or stimulated (5 hr PMA and ionomycin) as indicated. cDNA was synthesized and analyzed by Q-RT-PCR for the indicated genes; reported values are normalized to a housekeeping gene (TBP). B) Cells were transduced in duplicate with SNF2H shRNA plasmid or pBS-U6 vector (as indicated) using nucleofection, transduced cells were enriched (as described in methods), whole cell lysates were made, and 15 ug clarified lysate were loaded per lane, probed with SNF2H antibody and reprobed with HSP90 antibody (loading control), as indicated.

Figure 2. SNF2H regulates production of cytokine mRNA

EL4 cells were transduced with SNF2H shRNA (filled squares and solid line) or control (open diamonds and dashed line), stimulated for the indicated times with PMA and ionomycin, mRNA was harvested, cDNA was synthesized, and analyzed by Q-RT-PCR. mRNA was normalized to TBP. A) Duplicate independent experiments for IL-2. B) Duplicate independent experiments for IL-3. C) Measurement of transcription by detection of unspliced (nascent) transcripts.

Figure 3. SNF2H and ACF1 binding at the IL2 and IL3/GMCSF loci

Resting and stimulated EL4 cells were crosslinked with formaldehyde and analyzed by ChIP using the indicated antibodies. ChIP signal (% input) was normalized to 100 for SNF2H binding from stimulated cells at the GMCSF promoter. Control IgG IP and ACF1 IP are also shown for each primer pair. Loci are shown in scale drawings below data; genes are indicated by boxes with arrows indicating direction of transcription. The line above the x-axis indicates the average + standard deviation for all of the control IP samples. Averages and standard deviations from three experiments (two for ACF1) are shown. A) IL-2 locus. B) IL-3/GM-CSF locus.

Figure 4. SNF2H and ACF1 binding at the IL17 and Th2 cytokine loci

Resting EL4 cells were crosslinked with formaldehyde, and analyzed by ChIP. ChIP signal is expressed normalized to SNF2H+ at GMCSFpro (IL17) or HSV (Th2); averages and standard deviations are shown. Loci are shown in scale drawings below data; genes are indicated by boxes with arrows indicating direction of transcription. A) SNF2H and ACF1 binding at IL17 locus. B) SNF2H and ACF1 binding at Th2 locus.

Figure 5. SNF2H depletion reduces binding of SNF2H and ACF1

Resting and EL4 cells transduced with SNF2H shRNA (SNF2H KD) or control shRNA were crosslinked with formaldehyde and analyzed by ChIP using the indicated antibodies. ChIP signal (% input) was normalized to 100 for SNF2H binding from stimulated cells at the GMCSF promoter. ACF1 ChIP is also shown for each primer pair. Loci are shown in scale drawings below data; genes are indicated by boxes with arrows indicating direction of transcription. The line above the x-axis indicates the average + standard deviation for all of the control ChIP samples. Averages and standard deviations from three experiments (two for ACF1) are shown. A) IL-2 locus. B) IL-3/GM-CSF locus.

Figure 6. H3K9,14 acetylation and H3K27 trimethylation of cytokine loci

Resting EL4 cells were crosslinked with formaldehyde, and analyzed by ChIP. ChIP signal is expressed as % input, and control IgG IP is shown for each primer pair. Loci are shown in scale drawings below data; genes are indicated by arrows along DNA. Similar results were obtained with stimulated cells (data not shown). A) H3K9,14 acetylation at IL2, IL3/GMCSF locus. B) H3K27 trimethylation at IL2, IL3/GMCSF locus.

Figure 7. SNF2H binds cytokine loci in primary Th1 cells

Naïve CD4⁺ cells were harvested from mice, and Th1 effector cells were generated in culture. Resting and stimulated cells were crosslinked with formaldehyde, and analyzed by ChIP using the indicated antibodies. ChIP signal (% input) was normalized to 100 for SNF2H binding from stimulated cells at the GMCSF promoter. Control IgG IP, ACF1 IP and BRG1 IP are also shown for each primer pair. Loci are shown in scale drawings below data; genes are indicated by boxes with arrows indicating direction of transcription. The line above the x-axis indicates the average + standard deviation for all of the control IP samples. A) IL-2 locus. B) IL-3/GM-CSF locus.

Figure 8. SNF2H binding is regulated during development and Th differentiation

Naïve CD4⁺ T cells were harvested from mice, resting and stimulated Th1 effector cells were generated in culture from naïve CD4⁺ T cells, frontal cortex and cerebellum were dissected from mouse brain. Cells/tissue were crosslinked with formaldehyde, and analyzed by ChIP using the indicated antibodies. ChIP signal is presented as averages of % input, error bars are standard deviation. SNF2H IP and BRG1 IP and Control IgG are shown for each primer pair. Gpro is GMCSF pro, GEnh is GMCSF Enhancer, 3 pro is IL3 pro, and 3Enh is IL3 enhancer as before. All 6 panels have identical scaling to facilitate comparison. A) SNF2H binding. B) BRG1 binding. C) Control IgG ChIP

Figure 9. SNF2H programs chromatin structure of cytokine loci

EL4 cells were transduced with SNF2H shRNA (filled symbols and solid line) or control (open symbols and dashed line). Resting cells (triangles) and stimulated cells (squares; 4 hr PMA + ionomycin) were compared. Nuclei were isolated, treated with the indicated amounts of DNase, DNA was purified, and quantified by Q-PCR. Each sample was normalized to a control locus (Nfm). A) IL-2 locus. B) IL-3/GM-CSF locus.

Figure 10. Loss of SNF2H reduces the amount of ACF1 protein

EL4 cells were transduced with SNF2H shRNA or control. A) Whole cell lysates were analyzed by SDS-PAGE, proteins were transferred to membranes, and probed with ACF1 antibodies; the same filter was reprobed for HSP90. B) mRNA was harvested, cDNA was synthesized, and analyzed by Q-RT-PCR. mRNA was normalized to TBP.