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ATP-dependent chromatin remodeling in T cells

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Abstract

One of the best studied systems for mammalian chromatin remodeling is transcriptional regulation during T cell development. The variety of these studies have led to important findings in T cell gene regulation and cell fate determination. Importantly, these findings have also advanced our knowledge of the function of remodeling enzymes in mammalian gene regulation. In this review, first we briefly present biochemical/cell-free analysis of 3 types of ATP dependent remodeling enzymes (SWI/SNF, Mi2, and ISWI), to construct an intellectual framework to understand how these enzymes might be working. Second, we compare and contrast the function of these enzymes, during early (thymic) and late (peripheral) T cell development. Finally, we examine some of the gaps in our present understanding.

Three classes of remodeling enzymes

Chromatin remodeling enzymes fall into three broad categories. One group is ATP-dependent remodeling enzymes, the focus of this review. Another group of remodeling enzymes covalently modify histone proteins, including methyltransferases, demethylases, acetyltransferases and deacetylases. A third group of enzymes covalently modify DNA, which is subject to methylation and hydroxymethylation. These remodeling systems are often studied independently; however, they are known to work together, and can even be found in the same complex. Chromatin remodeling is thought to be important in many nuclear processes, including transcription, DNA replication and DNA repair; this review will focus on the role of ATP-dependent remodeling enzymes in gene regulation in T cells.

Classification of ATP-dependent remodeling enzymes

ATP-dependent remodeling enzymes contain SNF2-like ATPase subunits. SWI/SNF, Mi2, ISWI, and other ATP-dependent remodeling enzymes are classified into subfamilies (Fig 1) based upon homology of the ATPase subunit (Flaus et al., 2006; Saha et al., 2006). These ATPases couple the hydrolysis of ATP to changes in chromatin structure. While all of the details of remodeling enzyme mechanism have not yet been determined, translocation of these molecular motors along the DNA substrate appears to be important, while these helicase-like ATPases do not appear to have helicase activity (Clapier and Cairns, 2009; Pazin and Kadonaga, 1997). Biochemical activities include chromatin assembly, nucleosome displacement, nucleosome translocation (sliding), histone replacement, and nucleosome unfolding. The intrinsic biochemical function of remodeling ATPases are similar in very simple assays, resulting in increasing local accessibility, though they appear to differ in more complicated assays (Aalfs et al., 2001; Boyer et al., 2000; Fan et al., 2005;

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Competing financial interests

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Ishii et al., 2009). Remodeling enzymes are thought to be recruited to specific sites through interactions with transcription factors, histone modifications and non-coding RNAs, and are generally thought to lack DNA-binding specificity (Biddie and Hager, 2009; Clapier and Cairns, 2009; Ho and Crabtree, 2010; Tarakhovsky, 2010; Thompson, 2009; Wysocka et al., 2006). Differential recruitment of remodeling enzymes may impart a measure of specificity to remodeling enzyme function. Generally ATPase remodeling enzymes are multisubunit enzymes, and it is often the case that several versions of complex exist with different accessory subunits and the same ATPase (Ho and Crabtree, 2010; Kasten et al., 2011; Lusser and Kadonaga, 2003) (Fig 1).

SWI/SNF subfamily

Mammalian SWI/SNF complexes contain one copy of either the BRG1 or Brm ATPase, and approximately 10 additional accessory subunits to form complexes that are generally over a megadalton in size (Fig 1). BRG1 is required for embryonic development (Bultman et al., 2000), while the paralog Brm is dispensable (Reyes et al., 1998). SWI/SNF ATPases have been demonstrated to displace, unfold and slide nucleosomes in cell free systems (Lorch et al., 2006; Schnitzler et al., 1998; Whitehouse et al., 1999). BRG1 complexes can be recruited by transcription factors and other remodeling enzymes (Hassan et al., 2001; Neely et al., 1999; Yudkovsky et al., 1999). Several versions of SWI/SNF complex have been described, based on subunit composition. The BAF and PBAF complexes (Fig 1) have been found in several cell types in both mammals (Huang et al., 2008; Kaeser et al., 2008; Kasten et al., 2011; Lemon et al., 2001; Nie et al., 2000; Yan et al., 2005) and flies (Carrera et al., 2008; Mohrmann et al., 2004). Additional SWI/SNF complexes have been identified that appear to be specific to ES cells and neurons (Ho and Crabtree, 2010). BRG1 is the one of the few remodeling ATPases that has been analyzed genome-wide for binding in mammalian cells; this was first performed in ES cells (Ho et al., 2009) and subsequently in several T helper lymphocyte subsets (De et al., 2011) and the HeLa cell line (Euskirchen et al., 2011). CHD7 has also been analyzed in ES cells, revealing modest genome-wide overlap with BRG1 binding regions and a direct physical interaction between CHD7 and PBAF (Bajpai et al., 2010; Schnetz et al., 2010).

ISWI subfamily

Mammalian ISWI complexes contain one copy of the SNF2H or SNF2L ATPase. SNF2H and the ISWI binding partner BPTF are essential for embryonic development (Landry et al., 2008; Stopka and Skoultschi, 2003). ISWI complexes are generally dimeric; however, the larger number of binding partners, including ACF1/BAZ1A, WSTF/BAZ1B, TIP5/BAZ2A, CECR2, RSF1 and BPTF (Fig 1), leads to many forms of ISWI complexes (Dirscherl and Krebs, 2004; Yadon and Tsukiyama, 2011). ISWI binding partners alter the intrinsic enzymatic activity of ISWI complexes (He et al., 2008). ISWI binding partners can also recruit complexes to targets by binding modified histones (Clapier et al., 2001; Wysocka et al., 2006). BPTF binds SNF2L (Barak et al., 2003). BPTF can apparently be targeted by recognizing modified histones (Corona et al., 2002; Ruthenburg et al., 2011; Wysocka et al., 2006). In cell free systems ISWI enzymes can assemble chromatin, slide nucleosomes, and space nucleosomes (Ito et al., 1997; Langst et al., 1999). These activities may be responsible for the ability of ISWI to activate transcription in cell-free systems (Ito et al., 1997; LeRoy et al., 1998; Mizuguchi et al., 1997).

Mi2 subfamily

Mammalian NuRD complexes contain one copy of a chromodomain helicase DNA-binding (CHD) subfamily ATPase, either Mi-2 α /CHD3, Mi-2 β /CHD4 (Denslow and Wade, 2007; Ramirez and Hagman, 2009; Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998) or the

brain-specific paralog CHD5 (Potts et al., Pazin, in press). NuRD complexes also contain a histone deacetylase, generally HDAC1 or 2, thus combining two different types of chromatin remodeling enzyme in the same complex (Fig 1). NuRD complexes are approximately megadalton in size, and other subunits include metastasis-associated proteins (MTA1/2/3), methyl CpG binding domain proteins (MBD)2/3, histone-binding proteins/retinoblastoma-binding proteins (RBBP4/7). Due in part to its direct association with a histone deacetylase, NuRD has largely been viewed as a negative regulator of transcription. Mi2 can alter chromatin structure to increase nuclease accessibility in cell free systems (Boyer et al., 2000; Xue et al., 1998; Zhang et al., 1998) and can also “erase” open chromatin marks created by another remodeling enzyme in a reaction that is not well understood (Ishii et al., 2009).

ATP-Dependent Chromatin Remodeling During T cell Differentiation

One important measure of chromatin structure is the detection of DNase hypersensitive sites (DHS). Historically, this method has proved useful to identify functional elements (eg. enhancers, silencers and promoters) involved in gene regulation (Gross and Garrard, 1988; Wu et al., 1979). More recently, histone modifications have been mapped for the same purpose. T cells are a system where DHS and histone modification mapping have been successfully applied by many groups to identify regulatory elements. Early DHS mapping in T cells identified a T cell specific locus control region (LCR) in the CD2 locus, and changes in accessibility in cytokine loci which are activated rapidly in response to T cell signaling (Cockerill et al., 1993; Festenstein et al., 1996; Greaves et al., 1989; Siebenlist et al., 1986; Wotton et al., 1989). In T cells, chromatin structure can be dependent on cell fate, cell activation, or both. For example, during thymic development immature T cells differentiate into either T helper cells (expressing the coreceptor CD4) or cytotoxic T cells (expressing the CD8 coreceptor). Analysis of the chromatin structure of the CD4 gene allowed for the identification of a T cell-specific enhancer and a silencer (Kioussis and Ellmeier, 2002). Mature T cells in the lymphoid periphery can further differentiate into effector lineages, along with changes in chromatin structure (Ansel et al., 2003; Wilson et al., 2009). There has been a great deal of interest in studying the chromatin structure changes in the lineage-restricted genes, to determine the mechanism of activation of these genes. While studies of T cell chromatin structure have provided important insights into gene regulation, the enzymes that catalyze these chromatin structure changes in T cells have been less well studied.

Early T cell development in the thymus

Thymocyte development results in mature T cells expressing a functional T cell receptor (TCR) together with an appropriate coreceptor depending on the TCR specificity; CD4 for MHC class II restricted T helper cells and CD8 for MHC class I restricted cytotoxic T cells (Starr et al., 2003). The earliest thymocytes are deficient for both CD4 and CD8 expression and are referred to double negative (DN) cells (Fig 2). Based on unique cell surface marker expression the DN stage can be further subdivided into stages DN1-4. The DN stages involve active cell proliferation and expansion, as well as TCR rearrangement and expression. After progression through the DN stages, thymocytes upregulate expression of both CD4 and CD8 coreceptors and are termed double positive (DP) cells. Although not actively proliferating, DP cells comprise the vast majority of the cells in the thymus and undergo a selection process to positively select for cells that can recognize self-MHC but also eliminate those that are overtly self-reactive. The positively selected cells downregulate one of the two coreceptors to become single positive (SP) cells, either CD4-expressing T helper cells or CD8-expressing cytotoxic T cells (Fig 2). The molecular mechanisms involved in the developmentally regulated expression of CD4 and CD8 during thymic differentiation have been studied intensely for years (Bosselut, 2004). A stage-specific

transcription silencer in the CD4 locus serves to repress CD4 expression during both the early DN stage as well as in mature CD8 expressing cytotoxic T cells (Sawada et al., 1994; Siu et al., 1994). Additionally, a number of positively acting enhancer elements have been characterized in both the CD4 and CD8 loci (Adlam and Siu, 2003; Ellmeier et al., 1998; Ellmeier et al., 2002; Kioussis and Ellmeier, 2002).

BRG1, SWI/SNF and Thymic Development

Although BRG1 complexes are thought to bind DNA nonspecifically, they are likely recruited to specific sites through interactions with transcription factors, histone modifications and perhaps other mechanisms (Biddie and Hager, 2009; Clapier and Cairns, 2009; Hassan et al., 2001; Ho and Crabtree, 2010; Neely et al., 1999; Tarakhovsky, 2010; Wysocka et al., 2006; Yudkovsky et al., 1999). A role for BRG1 in the regulation of primary and secondary response genes has been shown for macrophages, cells of the innate immune system (Ramirez-Carrozzi et al., 2006). Here we will discuss how BRG1 participates in gene regulation in T lymphocytes, cells from the adaptive immune response.

A role for chromatin remodeling in the appropriate development of T cells in the thymus was first suggested in several mouse models deficient for the BRG-containing SWI/SNF complex (Chi et al., 2002; Gebuhr et al., 2003). Homozygous germline deletion of BRG ATPase results early embryonic lethality; however mice heterozygous for BRG1 exhibit delayed activation of CD8 expression in DP cells (Fig 2), suggesting a positive role for BRG1 CD8 transcription (Chi et al., 2002). Additionally, thymic specific expression of a transgene expressing a dominant negative version of a SWI/SNF component, BAF57, also disrupted the appropriate activation of CD8 expression during thymic development (Chi et al., 2002). In addition to the loss of CD8 expression, the BAF57 mutants also revealed a defect in the silencing of CD4, as CD4 was derepressed in DN cells. BAF complexes appeared to negatively regulate CD4 expression through the activation of the intronic CD4 silencer and ChIP experiments suggested that BRG1 affected silencer activity through a direct interaction (Wan et al., 2009). An analysis of the chromatin structure of the CD4 silencer in BAF57 DN thymocytes indicated that BAF complexes are required for CD4 silencer accessibility and facilitated the recruitment of the CD4 silencer factor, Runx 1 (Wan et al., 2009).

Compared to the BRG^{+/-} mice described above, mice with a conditional deletion of BRG1 specifically in T cells displayed a profound thymic development block, also suggesting a role for BRG1 in thymocyte survival and proliferation (Chi et al., 2003; Gebuhr et al., 2003). Mice deficient for BRG in the thymus displayed severely reduced numbers of thymocytes (40–100 fold reduction) and a nearly complete block in the transition from DN4-DP cells. BRG1-deletion appeared to induce apoptosis at the DN stage, as introduction of a Bcl-xL transgene rescued the cell loss (Chi et al., 2003). However, despite Bcl-xL overexpression, BRG-deficient thymocytes still failed to develop beyond the DN4 stage due to a cell cycle block, due in part to a defect in activation of CD8 expression. The phenotype of the BRG-deleted thymocytes closely resembled that of mice deficient for the Wnt effector, Tcf1 (van de Wetering et al., 2002). Indeed, gene expression changes in BRG-1-deficient thymocytes overlapped with the Wnt- pathway as several Wnt targets, including c-myc, were reduced in Brg-deficient thymocytes (Chi et al., 2003). These results suggest that BRG1 acts downstream of the Wnt pathway and plays a role in Wnt-target gene transcription in the thymus.

An ATPase-independent role for BRG1 in T cell development was explored with an elegant genetic model using mice with a specific point mutation in the BRG1 ATPase generated alongside littermates with a complete BRG1 gene deletion (Jani et al., 2008). Most of the

BRG1-dependent events described above (CD8 activation, CD4 depression at the DN3 stage and proliferation/apoptosis defects) above could be attributed to a loss of the ATP-dependent remodeling activity. However, this strategy revealed an additional positive and ATPase-independent role in the promotion of CD4 expression specifically during the transition from DN4 to DP. ChIP experiments provided evidence for direct BRG1 binding at activating regulatory elements in the CD4 locus, including the CD4 enhancer and LCR. Interestingly, the chromatin structure at these elements was not altered in cells expressing the ATPase-deficient BRG1 mutant, consistent with a remodeling-independent role for BRG1 in the activation of CD4 expression during this later stage of thymocyte development. BRG1 could be interacting with elements in the CD4 locus and recruiting coactivators, although this model has not been formally tested. These results illustrate that though these remodeling enzymes have an obvious biochemical activity, one should not assume all functions are dependent on ATPase activity.

In addition to regulating gene expression during T cell development, the chromatin remodeling activity of BRG1 has also been found to regulate thymic T cell receptor (TCR) gene rearrangement (Osipovich et al., 2007). During early thymocyte differentiation, each precursor T cell selects individual V, D and J gene segments from the T cell antigen receptor locus and joins them to create a functional exon coding for unique antigen specificity. This process requires providing accessibility to and recruitment of the VDJ recombinase, RAG1 and RAG2, to specific sites in the TCR locus. Enhancers and promoters in the TCR locus serve as accessibility control elements (ACE) and are required for efficient recombination. BRG1 is actively recruited to the endogenous TCR β locus through the ACE's. Functional targeting of BRG1 to a TCR β minilocus lacking a promoter ACE element overcame the requirement of a promoter for effective rearrangement. These results suggest that a critical function of ACE is to recruit BRG1 chromatin remodeling activity (Fig 2), allowing for remodeling of neighboring nucleosomes and subsequent accessibility to the recombinase.

Mi-2, NuRD and Thymic Development

Like BRG1, Mi-2/NuRD chromatin remodeling activity is involved in the developmental regulation of CD4 gene expression in the thymus (Williams et al., 2004). Mi-2 β /CHD4, the most abundant of the Mi-2 subunits, was conditionally deleted in thymus. Similar to conditional BRG1 knockout, conditional Mi-2 β knockout disrupts the successful transition of thymocytes from DN to DP (Fig 2), resulting in a decrease in thymic cellularity and loss of the functional proliferative responses of mature T cells. However, unlike BRG1, Mi-2 appears to positively regulate CD4 expression specifically at the later DP stage when the thymocytes are undergoing positive selection. Mi-2 β appeared to play a direct role in activating CD4 expression through interaction with the CD4 enhancer and facilitating recruitment of the E box binding protein HEB and histone acetyltransferase p300, resulting in hyperacetylation of the enhancer region. This finding was somewhat surprising since, as discussed above, HDAC-containing NuRD complexes are largely viewed as repressive in their function. However, in this gene context, Mi-2 was specifically required for the recruitment of an activating acetyltransferase activity instead. These results suggest that Mi-2 could be functioning outside of the conventional NuRD complex or conversely the combinatorial recruitment of associating enzymatic activities is determining whether the functional output is activating or repressive. Regardless, in the context of CD4 gene expression, Mi-2 β is an activator of gene expression.

Using protein interaction screens, the lymphoid lineage determining transcription factor, Ikaros, was identified as interacting with Mi-2 β in thymocytes (Kim et al., 1999; Sridharan and Smale, 2007). Ikaros is an important regulator of lymphocyte development and homeostasis and can behave either as an activator or repressor of transcription

(Merkenschlager, 2010). A genetic approach generating single and double mutants of Ikaros and Mi-2 β was used in order to determine the functional consequences of the Ikaros/Mi-2 β interaction in vivo (Naito et al., 2007). DN thymocytes from Ikaros-deficient mice failed to repress CD4 expression, suggesting that Ikaros plays a repressive role in CD4 expression while Mi-2 functions as an activator of CD4 expression as described above. Mice deficient for both Ikaros and Mi-2 β exhibited enhanced CD4 expression, suggesting the Ikaros mutation was epistatic. These studies suggest that Ikaros is required for the repression of CD4 during the early DN stage while Mi-2 β relieves this repression at the DP stage, allowing for appropriate CD4 expression. Biochemical evidence suggests that the functional antagonism between Ikaros and Mi-2 β occurs at the CD4 silencer element. Unopposed binding of Ikaros to the silencer during the DN stage leads to activation of the silencer element and repression of CD4 while recruitment of Mi-2 β to the silencer at the DP stage results in silencer inactivation and CD4 expression. Taken together, these studies suggest that Mi-2 β activates CD4 expression during T cell development both through activation of enhancer and promoter elements as well as antagonism of the silencer element.

NURF, ISWI and Thymic Development

Recently a T cell-specific deletion of BPTF was described, revealing a role for NURF in thymic development (Landry et al., 2011). BPTF-deficient mice display a block in development after the DP stage, fail to robustly produce CD4 or CD8 SP T cells and lack a thymic medulla, the thymic region populated by positively selected mature thymocytes (Fig 2). However, the overall rates of cell proliferation, apoptosis and CD4/CD8 expression of thymocytes from BPTF-deficient mice appear normal. Therefore, while SWI/SNF and NuRD appear to be important for thymocyte proliferation, cellularity and CD4/CD8 expression, NURF is essential for later stages of T cell development, subsequent to thymocyte expansion. NURF target genes identified in thymocytes are activated downstream of T cell receptor stimulation: Egr1, Egr2, Ikaros, IL-2, and IL7ra. Additionally, BPTF was required at the Egr1 and IL2 locus to maintain chromatin structure at the earliest stages of thymocyte development. At Egr1 these changes in chromatin structure correlate with reduced accessibility for the activation-inducible transcription factor, Srf. These results suggest that the BPTF-containing NURF complex plays a significant role in transmitting activating cellular signals to facilitate changes in chromatin structure and gene transcription

Chromatin Remodeling and Peripheral T Helper Cells: T helper Differentiation

The CD4 SP, or T helper (Th), cells that emigrate from the thymus are competent to respond to peptide antigens presented by class II MHC on antigen presenting cells. Depending on the context of this initial stimulation, Th cells can further differentiate into a variety of effector cell fates including (but not limited to) Th1, Th2, Th17, Treg and Th9 (reviewed in Murphy and Reiner, 2002; Wan and Flavell, 2009). Each of these effector subsets are marked by unique cytokine expression profiles, the expression of which is induced in an acute manner in response to antigen stimulation. There is also increasing interest in the ability of these cell types to change gene expression profiles, or even lineage, after differentiation (O'Shea and Paul, 2010). Lineage specific transcription factors are distinctively expressed within the each subset and are required for subset-specific cytokine expression (Zhu et al., 2010). Although a number of different stimuli can influence Th cell fate decisions, the surrounding cytokine milieu during the initial antigen stimulation is the most influential in directing effector cell fate. For example, Th1 cells express high levels of the hallmark cytokine IFN γ and develop in response to stimulation with antigen in the presence of IFN γ and IL-12. Th2 cells are marked by high levels of IL-4, IL-5, IL-13 and are induced after antigen stimulation in the presence of IL-4. Different Th subsets are required for clearance of different pathogens. For

example, Th1 cells help combat intracellular bacteria while Th2 cells are important for eradicating parasitic infections. Inappropriate Th cytokine expression is associated with pathologies, for example, an exaggerated Th1 or Th17 response is associated with autoimmunity while abundant Th2 cytokines are associated with allergy and asthma.

Since Th cytokine signature expression and mis-expression is tightly coupled with clinical conditions, a number of groups have aimed to dissect the molecular processes of Th differentiation and cytokine expression (Ansel et al., 2006). Initial work focused on the importance of activation-inducible transcription factors involved in binding cytokine promoters and inducing cytokine expression after T cell stimulation, including NFATs, NF- κ B and AP-1. Remarkably, the activation-specific NFATs bind Th1 gene in Th1 cells and Th2 genes in Th2 cells, perhaps because the underlying chromatin structure (Agarwal et al., 2000). Additionally, mice deficient for several signal transducer and activator of transcription (STATs), the transcription factors induced following cytokine receptor stimulation, revealed a prominent role for these transcription factors in the generation of different Th subsets; Th2 development is largely dependent on IL-4-induced STAT6 while Th1 development requires STAT1 and STAT4 in response to IFN γ and IL-12, respectively (Kaplan et al., 1996; Wurster et al., 2000).

A role for chromatin accessibility changes in Th-specific cytokine expression was demonstrated by DHS mapping of cytokine loci from different Th subsets (Ansel et al., 2006; Wilson et al., 2009). The Th2 cytokine cluster, containing the Th2 cytokines IL-4, IL-5 and IL-13 in a 250 kb locus, has been intensively studied at the level of chromatin (Agarwal and Rao, 1998). These studies revealed a dramatic increase in the number and intensity of DHS formation in the loci of the signature cytokines in Th2 cells compared to undifferentiated Th precursors (Thp) or Th1 cells. These HS sites were both proximal to the genes (ie promoters) and 20–100 kilobases away from the promoters. Many of the distal DHS were subsequently determined both genetically and biochemically to be enhancer and silencer elements important to Th2 cytokine expression (Ansel et al., 2006; Wilson et al., 2009). Although changes in nuclease accessibility across cytokine loci in response to differentiation signals has been well documented, very little is known with regards to the specific enzymes and remodeling proteins responsible.

BRG1 and Th2 Differentiation

BRG1 was tested as a candidate remodeling enzyme in Th2 differentiation using siRNA-mediated depletion (Wurster and Pazin, 2008). BRG1 is required *during* Th2 differentiation for efficient Th2 differentiation and cytokine expression. BRG1 expression is also required *after* the completion of Th2 differentiation for IL-4 and IL-13 expression, indicating that BRG1 activity is important in acute cytokine expression as well as differentiation. The action of BRG1 on Th2 cytokine expression appeared to be direct as ChIP studies identified Th2-specific and developmentally regulated BRG1 recruitment to known regulatory elements throughout the locus, including the Th2 Locus Control Region (LCR). One particular DHS within the LCR, RHS7, binds BRG1 in a highly Th2-specific and activation-inducible fashion. BRG1 recruitment to RHS7 is dependent on both the cytokine-induced transcription factor, STAT6, and an activation-induced transcription factor NFAT (Fig 3). BRG1 is required for the formation of the DHS at RHS7, suggesting a direct role for BRG1 in the chromatin remodeling at this regulatory element. Taken together, these results suggest that BRG1 chromatin remodeling integrates mitogenic and lineage directed signals to facilitate lineage specific gene expression.

We compared the role of BAF and PBAF versions of the SWI/SNF complex in Th2 cells (Wurster and Pazin, 2008). We found that proteins that are specific for the BAF complex

(Brm and BAF250a/Arid1a) bind the LCR/RHS7 region of the Th2 cytokine cluster. We also found that depletion of BAF250 using siRNA in Th2 cells reduced IL-4 expression, similar to depletion of BRG1 (Wurster and Pazin, 2008). Depletion of BAF250 or BRG1 decreased the maximum expression in stimulated, differentiated cells. By contrast, depletion of a PBAF-specific protein (BAF180) did not alter expression of IL-4 in Th2 cells (Wurster and Pazin, unpublished). We have detected PBAF targets in Th2 cells, where depletion of BAF180 appears to repress some target genes and activate others (Wurster, Z. Wang and Pazin, unpublished).

BRG1 and Th1 Differentiation

In addition to regulating chromatin structure changes and gene expression of the Th2 locus, BRG1 has functionally been linked to Th1-specific gene expression as well (Letimier et al., 2007; Placek et al., 2009; Zhang and Boothby, 2006). BRG1 was shown to be recruited to the promoter of the Th1 cytokine, IFN γ , in a Th1-specific fashion and this binding correlated with Th1-specific nucleosome remodeling at that region (Zhang and Boothby, 2006). Knockdown of BRG1 during Th1 development *in vitro* resulted in reduced IFN γ production suggesting that BRG1 is an activator of IFN γ transcription. Similar to what was observed at the Th2 locus, BRG1 recruitment and activity was dependent on signaling that activates the both the activation-inducible NFAT and cytokine-inducible STAT pathways. This theme continues in an analysis of another Th1-specific locus, the IL-12R β 2 gene (Letimier et al., 2007). BRG1 and STAT4 were recruited in a coordinated fashion to regulatory elements within the IL-12R β 2 gene and both were required for optimal IL-12R β 2 expression following T cell stimulation. Once again, BRG1 functionally coordinates activation-induced signals with lineage-specific signals to facilitate lineage specific transcription.

In addition to its direct role in nucleosome remodeling, BRG1 also regulates Th1 –specific gene expression through interactions with other chromatin modifying enzymes. The H3K27-methyl histone modification suppresses the transcription of lineage-specific genes in progenitor cells and this mark is actively removed as cells differentiate resulting in changes in gene expression profiles. A recent report identified an interaction between BRG1, the H3K27 demethylases, Jmjd3 and UTX and T-bet, the Th1 lineage specific transcription factor (Miller et al., 2010). This interaction was necessary for the chromatin remodeling observed at the IFN γ promoter in Th1 cells. This report highlights the importance of interactions between ATP-dependent and covalent histone chromatin remodeling enzymatic activities.

Genome-wide analysis of BRG1 binding during T helper differentiation

The studies described above examined BRG1 at a few loci in two Th fates, and they suggest that BRG1 might play a broader role. This work was expanded to additional cell fates and genes by determining the dynamics BRG1 recruitment in a variety of Th subsets and activation states using ChIP-Seq technology (De et al., 2011). There are approximately 10,000 to 20,000 binding regions in each Th cell fate, and they are discrete binding regions with an average length of about 150 bp (De et al., 2011); a similar number of binding regions were found in ES cells, but the average reported length was much larger, 6.2 kb (Ho et al., 2009). Globally, BRG binding is positively associated with gene expression in naïve, Th1, Th2 and Th17 cells; thus, a major function for BRG1 is to facilitate gene activation. At genes with a lineage-specific gene expression pattern, BRG1 binding was also enriched in a lineage-specific manner, demonstrating the role of BRG1 in multiple cell fates. For example, at the locus encoding the transcription factor c-maf, an important regulator of Th2 cytokine expression, the number and intensity of BRG1 binding sites is greater in Th2 cells than Th1

cells (Figure 4A). BRG1 binding frequently colocalized with STAT6 and STAT5 binding in Th2 cells and STAT4 binding in Th1 cells, suggesting the previously mentioned examples of STAT recruitment of BRG1 may be general (De et al., 2011). An attractive hypothesis is that BRG1 might also be important for T helper plasticity, the ability of T helper cells to change gene expression pattern or even fate after differentiation. BRG1 binding correlates with expression of fate-specific miRNA genes in T helper cells, suggesting they might use similar mechanisms for regulation as protein-coding genes.

BRG1 binding is frequently found at distal and promoter elements; both modes of binding are globally correlated with gene expression, demonstrating that both modes of binding are functional. The GATA3 locus, encoding a transcription factor required for Th2 differentiation, was used as a test case for distal functional enhancers, as prior studies suggested unidentified elements at least 150 kb from the promoter were required for T cell expression. A number of distal BRG1 binding regions are present in the GATA3 locus, including Th2-specific sites more than 700kb from the promoter (De et al., 2011). These sites were associated with Th2-specific DHS, and functional analysis indicates they possess BRG1-dependent and chromatin-dependent enhancer-like activity (De et al., 2011). A recent independent study found one of these distal elements (+284k in mouse) was functional in transgenic mouse assays in a variety of T cells and also NK cells, and confirmed that as yet unidentified enhancers explain Th2 expression of GATA3 (Hosoya-Ohmura et al., 2011). These results suggest BRG1 binding could be a useful marker for active enhancers.

BRG1 and T cell activation

The above studies clearly indicate a role for chromatin remodeling during peripheral T helper differentiation. However, this does not imply chromatin remodeling is important during T cell activation; indeed, chromatin structure could be static following differentiation to provide an epigenetic memory. This appears to be the case for the ISWI remodeling enzyme SNF2H, which is not strongly regulated by T cell activation (Precht et al., 2010). Similarly, histone modifications have been reported to be relatively stable during activation in T cells (Barski et al., 2009; Lim et al., 2009). By contrast, analysis of BRG1 binding to the Th2 cytokine genes revealed that BRG1 binding was induced during differentiation, then reduced at some of these sites in resting, differentiated cells, and increased again when differentiated cells were restimulated (Wurster and Pazin, 2008). BRG1 binding to the GM-CSF promoter is also regulated by T cell activation, though opposing effects have been reported (Brettingham-Moore et al., 2008; Holloway et al., 2003; Wurster et al., 2011). We found that depletion of a BAF-specific protein (BAF250a) decreased IL-3 activation, similar to depletion of BRG1, while depletion of a PBAF-specific protein (BAF180) had no effect on IL-3 expression (Wurster et al., 2011). Genome-wide analysis of BRG1 binding revealed extensive activation-dependent changes (De et al., 2011). At activation-inducible genes, BRG1 binding was increased by T cell activation, while at activation-silenced genes, BRG1 binding was decreased by T cell activation (De et al., 2011). BRG1 binding is activation inducible at immediate early genes, such as EGR1-3, NFAT1, 2, FOS, JUN and Myc (Figure 4B). At the Myc locus, we have found inducible BRG1 binding at a recently identified enhancer (De et al., 2011). BRG1 binding is also induced by activation at lineage-specific genes in Th1 cells (IFN γ , Tbx21/T-bet, EOMES) and Th2 cells (IL4, IL5, IL13, IL-24, GATA3, c-MAF, NFIL3/E4BP4). Thus, BRG1 appeared to integrate lineage and activation signals.

SNF2H and T cell cytokine expression

The SNF2H ATPase is a member of the ISWI subfamily of remodeling enzymes. In contrast to BRG1 and Mi-2, much less is known about the role of these remodeling enzymes in T cell

function. Mammalian ISWI complexes are frequently dimeric, consisting of either the SNF2H or SNF2L ATPase combined with one of a variety of cofactors, including ACF1 or BPTF (Corona and Tamkun, 2004; Lusser and Kadonaga, 2003). SNF2H regulates cytokine expression at the level of transcription, as found in studies using a T cell line and siRNA-mediated depletion (Precht et al., 2010). By contrast to BRG1, SNF2H primarily functions as a repressor of cytokine genes, including IL-2, IL-5, IL-13 and IL-17. However, SNF2H appears to behave as an activator of IL-3 expression in the same cells under the same conditions, suggesting that SNF2H can have opposing effects on cytokine expression. SNF2H and its binding partner, ACF1, directly bind proximal and distal elements in the cytokine target genes in primary T helper cells and the T cell line, suggesting that regulation of the cytokine genes is a direct effect of SNF2H on the cytokine genes. Depletion of SNF2H alters nuclease accessibility of SNF2H binding sites; the change in accessibility correlates with gene activity (ie, decreased accessibility and decreased transcription at the IL-3 locus, and increased accessibility and increased transcription at the IL-2 locus). SNF2H binding is largely insensitive to stimulation of cells, in both primary T helper cells and the cell line. By contrast, as reviewed above, BRG1 binding is highly responsive to cell activation. For example, a distal enhancer in the IL-3 locus termed CNSa is bound by both BRG1 and SNF2H in T cells; BRG1 recruitment is strongly inducible (Wurster et al., 2011) and SNF2H much less so (Precht et al., 2010). Both remodeling enzymes appear to directly regulate the chromatin structure and accessibility of this enhancer, and the linked gene. These results suggest a potential cooperative relationship between BRG1 and SNF2H complexes where SNF2H may maintain the chromatin structure at a specific element in resting T cells while BRG1 could be responsible for accessibility changes induced by cellular activation.

NuRD and Peripheral T cell Function

Although studies on the function of NuRD in T cells has been largely limited to thymocytes, several lines of evidence suggest that NuRD is also important in mature T cell function in the lymphoid periphery as well. In Mi-2 β -deficient mice, relatively few T cells successfully exit the thymus and accumulate in the lymphoid periphery and those that do have profound proliferative defects in response to TCR stimulation (Williams et al., 2004). Although this proliferation defect could be due to an aberration in the development of the Mi-2 β -deficient T cells, this finding does suggest that NuRD is involved in peripheral T cell expansion. A separate study targeted a different member of NuRD the complex, Mta2. Mta2 is a member of the Mta protein family and Mta2 and 3 have been shown to form complexes with NuRD components (Denslow and Wade, 2007). Mice deficient for Mta2 exhibited a partial embryonic lethality but the surviving animals developed a lupus-like autoimmune syndrome suggestive of a hyperactivated immune system (Lu et al., 2008). Mta2-deficient T cells were also hyperproliferative in response to T cell stimulated and exhibited enhanced cytokine induction. In contrast to the depressed proliferative responses observed with Mi-2 β -deficient T cells, these results suggest that Mta2-containing NuRD complexes are important negative regulators of peripheral T cell activity. Future experiments will be required to determine if the presence of different Mta family members in NuRD complexes have distinct functional outcomes in T cells.

Summary

While chromatin has been a useful tool for studying T cell fate and gene expression, T cells have returned the favor and provided valuable insights into the role of chromatin remodeling in gene expression. In turn, this increased understanding has also heightened our awareness of current gaps in our knowledge. We conclude this review by examining some of these questions, considering possible explanations, and suggesting future lines of experiments.

Perhaps the biggest question, from which the others follow, is the easiest to state: *Why are there so many remodeling enzymes?* There are different subfamilies of ATPases, often there are multiple paralogs in the same subfamily, and different accessory proteins make different complexes from the same ATPase. Perhaps the simplest explanation is the expression patterns of these components could be different, as observed for BRG1 and its paralog Brm, or the brain-specific expression of CHD5. However, many remodeling ATPases and accessory proteins are broadly expressed, suggesting other models must account for much of the variety.

Do remodeling enzymes have different intrinsic activity?

Cell-free studies reveal remodeling enzymes can have different intrinsic enzymatic activities; an important caveat is too few of these studies are side-by-side comparisons. When two remodeling enzymes have opposing effects at the same locus (eg. CD4 silencer), the simplest explanation is they are doing different things. Some examples where different remodeling enzymes are recruited to the same locus in cell-free (Ishii et al., 2009) and cell based systems (Gao et al., 2009; Precht et al., 2010; Ramirez-Carrozzi et al., 2006) illustrate this point. ATP-dependent remodeling has been found to alter histone modifications, a simple illustration of remodeling enzymes having different roles at the same locus (DiRenzo et al., 2000; Letimier et al., 2007; Ramirez-Carrozzi et al., 2006; Wurster and Pazin, 2008). Whether functional antagonism, cooperation or neutrality is the more common mode for remodeling enzyme interaction remains to be determined.

How are remodeling enzymes targeted to the genes they regulate?

We have reviewed evidence of transcription-factor-mediated recruitment here (eg., BRG1 in T helper cells). There is also evidence, from other systems, of recruitment by RNA, histone modifications, and even DNA structure. It is unclear to what extent remodeling enzyme binding patterns overlap; the answer to this question awaits further genome-wide analysis of remodeling ATPases and accessory subunits. Already, there is evidence for differences in remodeling enzyme binding; this suggests the hypothesis that perhaps remodeling enzymes sometimes perform the same reaction, but are recruited to different sites.

How can one remodeling enzyme activate and repress gene activity?

A remodeling enzyme can have opposing effects on gene expression, in the same cell (eg. SNF2H). Perhaps part of the explanation is remodeling enzymes exert local effects on chromatin structure; thus, a remodeling enzyme that opens chromatin structure could activate one gene if it were targeted to an enhancer, while repressing another gene if recruited to a silencer. An alternative explanation is that different versions of a remodeling complex might have different intrinsic enzyme activity, such that one version could open chromatin while another version could close chromatin.

How are remodeling enzymes regulated?

To date very few studies have been performed on regulation of remodeling enzymes. Remodeling enzyme binding might not strictly correlate with function, and not all studies examine the effects of remodeling enzyme binding on chromatin structure. SWI/SNF and ISWI complexes can be regulated by phosphorylation and ligand binding (Lai et al., 2009; Shen et al., 2003; Sif et al., 1998). The great variety of accessory proteins and even ATPases might exist, in part, to permit remodeling to respond to different signaling pathways. Given the pervasive nature of protein regulation by phosphorylation, it would seem surprising if there is not much to be learned in this area. Genome-wide comparison of remodeling enzyme binding and DHS could clarify these questions, especially if coupled with perturbation analysis (John et al., 2008).

Are remodeling enzyme binding sites useful markers for enhancers?

H3K4me1 and p300 have been identified as a powerful marker for enhancers in a number of studies (Ernst and Kellis, 2010; Ghisletti et al., 2010; Heintzman et al., 2009; Visel et al., 2009). However, more recently it has been suggested that H3K4me1 may not strongly respond to the activation state of cells, while H3K27ac and p300 might better capture this feature (Creighton et al., 2010; Ernst and Kellis, 2010; Ghisletti et al., 2010; Kim et al., 2010; Rada-Iglesias et al., 2011). We have found BRG1 to be a useful enhancer marker; we have validated only a small number of BRG1 sites, but we also show that BRG1 binds to many known regulatory elements in T cells (De et al., 2011). The remodeling ATPase CHD7 has also been proposed to be enriched at enhancers (Bajpai et al., 2010; Schnetz et al., 2010). It is not known at this time what marks (eg. H3K4me1, H3K27ac, p300, BRG1, CHD7, small RNA transcripts) have the best specificity and sensitivity for enhancer detection, and it remains possible that different marks identify different classes of enhancers.

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Figure 1.

Subunit composition of selected remodeling enzymes. Alternatively used subunits are labeled in the same bubble and separated by commas. SWI/SNF complexes; BAF-specific subunits are shown in red in the upper drawing, PBAF-specific components in blue in the lower drawing. ISWI complexes; two of many possible complexes are shown. CHD5 was found in the NURD family in PLoS ONE 6(9): e24515. doi:10.1371/journal.pone.0024515.

Figure 2.

T cell development and ATP-dependent remodeling enzymes. A) Stages of thymic development progress from left to right. Remodeling enzymes activate (arrowheads) or inhibit (T stops) the events shown; proliferation (Pro, CD4 and CD8 expression, and TCR β rearrangement. B) schematic of CD4 gene functional elements, and action of remodeling enzymes.

Figure 3.

Th2 cytokine LCR integrates signals. IL-4, a lineage signal, activates the transcription factor STAT6, and T cell receptor stimulation, a T cell activation signal, activates the transcription factor NFAT. Together, these factors recruit BRG1 to the LCR/RHS7 site; all three factors contribute to cytokine gene activation (Wurster and Pazin, 2008).

Figure 4.

BRG1 and transcription factor binding in Th cells. A) c-Maf locus. Data from stimulated Th1 and Th2 cells in red; STAT5B (black), STAT6 (red), and H3K4me3 (black) are below; Input (control) is in black. c-Maf is transcribed right to left. B) c-Myc locus. BRG1 binding in resting Th2 cells (blue) and stimulated Th2 cells (red) and input chromatin (black). Vertical arrow beneath BRG1 Th2S binding track indicates region homologous to enhancer C identified in (Sotelo et al., 2010). c-Myc is transcribed left to right. Scale (y axis) for all tracks in both graphs is identical to allow direct comparison (minimum tag frequency of 0, maximum tag frequency of 1.14×10^{-5}). BRG1 and input data from (De et al., 2011), STAT5 data from (Liao et al., 2008), STAT6 data from (Wei et al., 2010), H3K4me3 data from (Wei et al., 2009); genomic coordinates are for MM9 assembly.