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Regulation of Chromatin Structure During Thymic T Cell Development

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Abstract Development is the process whereby a multipotent cell gives rise, through series of divisions, to progeny with successively restricted potentials. During T cell development, the process begins with a multipotent hematopoietic stem cell (HSC) in the bone marrow, moves to the thymus where early T cells or thymocytes pass through signal-initiated developmental checkpoints, and ends in the periphery where mature T cells reside. At each step along this developmental pathway, T lymphocyte progenitors must be able to turn genes on and off, creating a specialized program of gene expression, to allow further development. How is gene expression coordinated? This review will summarize what has been learned about the function of chromatin structure in generating a ''blueprint'' of gene expression during T cell development. This will include discussion of mechanisms of chromatin remodeling, histone modification, and heritable gene silencing. In many cases, these processes are carried out by multi-protein complexes whose components are largely ubiquitously expressed. The spatial and temporal specificity of these complexes is contributed by sequence specific DNA binding factors, some of which are cell type restricted in their expression. This review will summarize research underway to identify these key genetic ''targeters.'' Taken together, the research reviewed here provides a glimpse into the importance of regulation of chromatin structure in T cell development and the ''players'' involved. J. Cell. Biochem. 95: 466–477, 2005.

Key words: chromatin remodeling; histone acetylation; gene silencing; T cell development

INTRODUCTION

A cell's identity is defined by the genes it expresses. The hematopoietic system consists, in part, of cells of the lymphocyte, erythroid, granulocyte, and monocyte lineages, each of which is derived from a single pluripotent cell, the hematopoietic stem cell (HSC). Each step in development, as cells progress from the HSC to the progenitor to the fully differentiated cell, consists of a narrowing of potential. In molecular terms this means that genes are becoming programmed to be either "on" or "off." Programming is accomplished by the array of transcription factors expressed in that cell and, perhaps more importantly, the epigenetically heritable chromatin structure that acts as a blueprint for that cell's program of gene expression.

The first step in unraveling the mystery of cell type specific gene expression was the identification of lineage specific transcription factors. It was believed that these factors worked primarily through direct recruitment of the basal transcriptional machinery to the gene promoter, thereby activating transcription [Ptashne and Gann, 1997]. Recently, however, some proteins identified as canonical transcription factors have been found to be components of large multi-protein chromatin remodeling complexes.

DNA does not exist freely in the nucleus of a eukaryotic cell. It is wrapped around an octamer core of highly basic histone proteins to form individual units called nucleosomes. Nucleosomes are fundamental units of a higher level structure called chromatin, responsible for the compacting of chromosomal DNA. The spacing of nucleosomes as well as the nature of

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histone:DNA contacts is controlled by chromatin remodeling complexes. Therefore, these complexes are centrally important in determining whether a gene within a specific region of DNA is accessible to transcription factors ("on") or not ("off").

Chromatin structure is gene specific and is based on nucleosomal location and structure. Chromatin remodeling complexes regulate nucleosomal location by promoting "sliding" to uncover or cover genes. They also regulate nucleosomal structure through association with enzymes that post-translationally modify histones. Modifications include methylation, acetylation, phosphorylation, and ubiquitination. Incorporation of sequence-specific DNA binding proteins into chromatin remodeling complexes is thought to confer specificity of remodeling to specific genetic loci. This bestows cells of a specific lineage their unique gene expression "fingerprint." In addition to their importance in conferring cell identity, chromatin remodeling complexes impart chromatin flexibility to the cell, allowing a cell to change gene expression in a heritable manner in response to external stimuli.

Chromatin remodeling complexes were first identified in yeast and are remarkably similar to those later identified in humans, highlighting their biological significance. ATP-dependent chromatin remodeling complexes contain enzymatic activities that result in mobilization of nucleosomes using energy provided by ATP and/or that result in the post-translational modification of histone proteins, which can have either an "activating" or a "repressive" effect on chromatin structure. There are three major families of ATP-dependent chromatin remodeling complexes—the SWI/SNF, ISWI, and Mi-2 families—each of which is made up of multiple family members (reviewed by Vignali et al. [2000]). Whereas SWI/SNF and ISWI complexes are largely thought of as activating complexes, the Mi-2 complexes have been regarded as repressive complexes.

SWI/SNF complexes consist of 9–11 proteins/complex (reviewed by Workman and Kingston [1998]). Their purpose is to alter the histone–DNA contacts, thereby enhancing access of DNA binding proteins to nucleosomal DNA. They do this in an ATP-dependent manner. The enzymatic component of SWI/SNF complexes is either Brahma (Brm) or Brahma related gene 1 (Brg1). Both Brm and Brg1 possess ATPase/helicase activity [Muchardt and Yaniv, 1993; Randazzo et al., 1994]. Other proteins in these complexes include BAFs or Brg1-associated factors, which increase the efficiency of nucleosomal disruption by Brm or Brg1.

The second group of ATP-dependent remodeling complexes is the ISWI family, so called because they contain the ISWI protein as the ATPase activity [Tsukiyama et al., 1995]. The ISWI chromatin remodeling complexes consist of fewer proteins than their SWI/SNF counterparts. They are also considered "activating" complexes since they can facilitate transcription from chromatin templates using ex vivo nucleosomal remodeling assays. Mechanistically, they catalyze the movement of nucleosomes along DNA to "open up" regions of chromatin.

Finally, the Mi-2/nucleosome remodeling and deacetylase (NuRD) family consists of "repressive" chromatin remodeling complexes [Tong et al., 1998]. These complexes are unique in that they contain two enzymatic activities—an ATPase activity, supplied by Mi-2, and histone deacetylase activity, supplied by HDAC1 and HDAC2. Histone deacetylases catalyze deacetylation of histones, resulting in a more condensed nucleosomal structure and are, therefore, linked to gene silencing. NuRD complexes, like SWI/SNF complexes, are large complexes. They consist of up to 10 protein subunits, including proteins believed to bind methylated DNA (MBD3) and modulate deacetylase activity (MTA2) [Zhang et al., 1999].

TOOLS FOR THE STUDY OF T CELL DEVELOPMENT

The study of how chromatin remodeling is controlled during development and the role of the ATP-dependent chromatin remodeling complexes in this complex process is still in its infancy. Since lymphocyte development can be manipulated ex vivo and the lymphoid system is not required for survival in mice (at least not in the specific pathogen free environment of most barrier facilities), it has been utilized as a model system to study these, as well as many other, developmental events. Fetal thymic organ culture (FTOC) and reaggregate thymic organ culture (RTOC) are culture systems almost ubiquitously used by T cell developmental biologists. In both cases, T cell progenitors are allowed to mature in the context of their

natural microenvironment of the thymus. Thymic stroma as well as bone marrow-derived antigen presenting cells that reside in the thymus provide many of the signals required during T cell development, either by secretion of growth and survival factors (i.e., interleukin-7) or direct cell to cell interaction via molecules on their surface (i.e., MHC:peptide complexes). These signals drive changes in gene expression that lead to differentiation, proliferation, apoptosis, and/or survival of the developing T cell. A new system has recently been developed by Juan Carlos Zúñiga-Pflücker's laboratory in which T cell development can be even more easily visualized and manipulated. In this system, T cell progenitors are co-cultured with a bone marrow-derived stromal cell line that has been stably transfected with a ligand, Delta-like ligand 1, for the Notch receptor that is expressed on T cells [Schmitt and Zuniga-Pflucker, 2002]. T cell development can be induced from fetal liver stem cells as well as progenitors from adult thymus allowing the study of events from the very beginning to the end of the developmental process [Schmitt and Zuniga-Pflucker, 2002; Ciofani et al., 2004]. The potential of this system is just starting to be realized.

Another important tool in the study of chromatin remodeling in development in general and T cell development in particular is the generation of tissue-specific knockout mice. This system allows the abrogation of expression of a gene, such as one encoding an essential component of a chromatin remodeling complex, in a tissue-specific manner. Restricted disruption prevents the pleiotropic effects or lethality that can arise using a conventional knockout strategy.

Taken together, these experimental systems are beginning to prove to be important in unraveling the complicated mechanisms behind regulation of T cell development.

SWI/SNF COMPLEXES IN T CELL DEVELOPMENT

Tissue-restricted knockout mice have been elegantly used by two groups to investigate the importance of the Brg1 containing SWI/SNF complex in T cell development. This strategy is essential since targeted mutation of Brg1 results in early embryonic lethality in the mouse [Bultman et al., 2000; Klochendler-Yeivin et al., 2000; Roberts et al., 2000; Kim et al., 2001]. Both groups inactivated Brg1 specifically in the T cell lineage using the Cre-loxP system. Cre recombinase was placed under the control of the lck proximal promoter ensuring that it would be expressed in thymocytes, thereby leading to Brg1 inactivation during T cell development. Their conclusions were very similar, demonstrating that Brg1 plays a role at very specific stages of T cell development [Chi et al., 2003; Gebuhr et al., 2003].

First, Brg1 is integral in regulating expression of the T cell co-receptor genes, *CD4* and *CD8*. Activation or repression of co-receptor molecules has been correlated with cell fate choices in T cell development. Early thymic progenitors express neither CD4 nor CD8, and therefore, have been designated as double negative (DN) thymocytes. Signals received by DN thymocytes lead to up-regulation of both CD4 and CD8, resulting in development of CD4+CD8+, or double positive (DP), thymocytes. In turn, signals received by the DP thymocyte result in the subsequent down-regulation of either CD4 or CD8, leading to the development of mature helper (CD4+CD8−) or cytotoxic (CD4−CD8+) T cells that can exit the to the periphery. Inactivation of Brg1 impairs both CD4 silencing and CD8 activation, resulting in a thymus that contains no cells that are phenotypically DP or CD4−CD8+.

Second, Brg1 deficient thymuses are highly hypocellular due to the combination of a developmental block at the DN to DP transition and decreased thymocyte viability. The developmental block is hypothesized to be the result of an inability of Brg1 null thymocytes to respond to developmental signals initiated through pre-T cell receptor (TCR) [Chi et al., 2003], signaling that is essential for the DN to DP stage transition. Interestingly, there is not a complete block in T cell development since Brg1 null T cells are found in the periphery, albeit in greatly reduced numbers [Gebuhr et al., 2003]. However, Brg1 null T cells are unable to proliferate in response to activation stimuli, a phenotype likely to be due to their defective ability to respond by changes in gene expression to activation-induced signaling pathways.

These studies clearly implicate Brg1 and, by extension, SWI/SNF complexes in regulation of thymocytes' responses to developmental cues and of mature T cells' responses to activation-induced signaling pathways that lead to their proliferation. Brg1 null T cells that do develop

could be the result of some level of redundancy, perhaps provided by SWI/SNF complexes that do not contain Brg1, which permits partial rescue of the developmental phenotype. In order to stringently test the role of SWI/SNF complexes in T cell development, T cell specific inactivation of a common subunit of all SWI/SNF complexes (i.e., SNF5/INI1) must be performed.

If SWI/SNF complexes are important for regulating CD4 and CD8 expression, how are they targeted to these specific genetic loci? One group suggests that a sequence specific DNA binding factor, Runx1, may play this role [Taniuchi et al., 2002]. Runx proteins are important regulators of multiple developmental processes from *Drosophila* to mouse (and, most probably, man). By using the Cre-loxP system to inactivate Runx1 in thymocytes, it was shown that Runx1 is required both for activation of CD8 and repression of CD4 during T cell development [Taniuchi et al., 2002]. This is a very similar phenotype to that observed in the thymocyte specific Brg1 inactivation experiments described above. Therefore, it was hypothesized that Runx1 may be required for recruitment of the SWI/SNF complex to the CD4 and CD8 loci. However, Runx1 has not yet been identified as associating with a SWI/SNF complex, the first step in confirming this hypothesis. Another group suggests that the sequence-specific DNA binding factor Ikaros, which has been shown to interact with chromatin remodeling complexes [Kim et al., 1999], is important in this role for the *CD8a* gene [Harker et al., 2002].

Interestingly, these studies suggest both an "activating" and "repressive" role for SWI/SNF complexes in regulation of the *CD8* and *CD4* genes, respectively, providing evidence that these complexes may perform both roles during T cell development. How they accomplish this is still an unexplored frontier.

Mi-2β IN T CELL DEVELOPMENT

The role of Mi-2β, the ATPase component of the NuRD complex, in T cell development has also been studied through the use of a T cell specific knockout [Williams et al., 2004]. Using the Cre-loxP system, expression of Mi-2β was abrogated in T cells. Cre recombinase was expressed from the lck proximal promoter as in the studies discussed above. Mi-2β is the most abundantly expressed of the Mi-2 homologues (the other being Mi-2α) and is an essential component of the NuRD complex. The most striking result of this study was that Mi-2β was found to be involved in positive regulation of CD4 during T cell development. It does this through its interaction with the histone acetyltransferase (HAT) p300 and the sequence specific DNA binding protein, HEB. This interaction results in increased histone H3 acetylation, a state linked with gene activation, in a CD4 regulatory region previously shown to be important for CD4 expression [Sawada and Littman, 1991]. Significantly, it is known that HEB plays a direct role in promoting CD4 expression [Sawada and Littman, 1991], and the study described here suggests that HEB may do so through recruitment of p300 and Mi-2β to the *CD4* gene enhancer.

Significantly, this study describes, for the first time, an association of Mi-2β with a HAT. It also suggests that Mi-2β, a critical component of the repressive NuRD complex, may be involved in activation of genes, possibly through its association with another, as yet unidentified, complex. The basic idea of Mi-2 possessing both transcriptional repressing and activating functions is not entirely unique to this study. In a previous study performed by another group, evidence was provided demonstrating that Mi-2β has both transcriptional repressing and activating domains [Shimono et al., 2003]. These studies were performed by transient transfection of reporter constructs and Mi-2β fusion proteins into the human embryonic kidney cell line, 293. The activating potential of Mi-2β was linked to its ability to interact with the SWI/SNF complex ATPase, Brg1. Taken together, these studies suggest that the paradigm of SWI/SNF as activating complexes and Mi-2/NuRD as repressive complexes may be too simplistic.

DO CHROMATIN REMODELERS ONLY REGULATE CD4 AND CD8 EXPRESSION?

Although the studies described above have provided insight into a role for SWI/SNF and Mi-2β complexes during T cell development, the results beg the question, "Is regulation of *CD4* and *CD8* gene expression all there is?" Much effort has been expended towards defining the regulators of *CD4* and *CD8* due to the importance of these genes in T cell development

and function. However, it is undisputed that they are but two of many genes whose expression must be modified in response to external signals, leading to the development of mature T cells. The future holds many challenges in defining the role of chromatin remodeling complexes in initiating and imprinting programs of gene expression at each stage of T cell development. Perhaps some insight into the crucial role of these complexes in cell identity can be gained by what has been defined as their role in development of two other lineages of blood cells, B cells and erythroid cells.

In a recent study, it was demonstrated that the Mi-2/NuRD complex regulates cell fate during B cell development [Fujita et al., 2004]. It accomplishes this feat, at least in part, through its interaction with the sequence specific DNA binding protein, BCL6. BCL-6 is necessary for preventing development of B cells into terminally differentiated, antibody-secreting plasma cells through its active repression of the plasma cell differentiation program [Reljic et al., 2000; Fujita et al., 2004]. Dramatically, exogenous expression of BCL-6 in a plasma cell line leads to reprogramming of cell fate toward that of a non-antibody secreting B lymphocyte through repression of many plasma cell specific genes (e.g., *Blimp-1*, *XBP1*) [Fujita et al., 2004]. It has now been shown that this repression is mediated through the interaction of BCL-6 with the Mi-2/NuRD complex [Fujita et al., 2004], demonstrating the importance of incorporation of sequence specific DNA binding factors into chromatin remodeling complexes for their targeting to a combination of genetic loci to determine cell fate.

Target genes of chromatin remodeling complexes that define the erythroid cell lineage have also been identified. Globin gene expression is unique to erythroid lineage cells. The globin genes that are expressed differ during fetal versus adult life. The mechanism behind how fetal globin gene expression turns off and adult globin gene expression turns on was largely a mystery until the identification of the PYR complex, an erythroid developmental stage specific SWI/SNF-related chromatin remodeling complex [O'Neill et al., 2000]. This complex co-purifies with components of both the SWI/SNF and NuRD complexes as well as the sequence specific DNA binding factor, Ikaros [O'Neill et al., 2000]. The PYR complex has been shown to be essential for fetal to adult globin gene switching which occurs shortly after birth [O'Neill et al., 2000]. It is hypothesized that it facilitates opening of the chromatin surrounding the adult globin locus to permit binding of transcriptional activators. Ikaros may function as a targeter for the PYR complex. This supposition is supported by analyses of genetically engineered Ikaros null mice. Ikaros null mice lack the PYR complex, suggesting that Ikaros is essential for its formation [Lopez et al., 2002]. In addition, they display a delay in fetal to adult globin switching and anemia. Therefore, the chromatin remodeling PYR complex appears to be essential for normal red blood cell development.

Sequence specific DNA binding proteins that target remodeling complexes for regulation of programs of gene expression, such as BCL-6 for the B cell lineage and Ikaros for the erythroid lineage, have not yet been identified for the T cell lineage. Interestingly, Ikaros null mice display defects in T cell development [Wang et al., 1996; Winandy et al., 1999; Urban and Winandy, 2004], suggesting that Ikaros may also target T cell specific chromatin remodeling complexes to developmentally important genes during T cell development. Defects in the Ikaros null T cell compartment include an inability of thymocytes to proliferate normally and increased development of CD4+CD8− T cells [Wang et al., 1996; Urban and Winandy, 2004]. It has been hypothesized that T cell developmental signaling pathways modulate Ikaros activity, and that this modulation is important for normal transition through developmental checkpoints [Winandy et al., 1999; Urban and Winandy, 2004]. In support of a role for Ikaros in targeting chromatin remodeling for regulation of a T cell specific program of gene expression, it has been shown that re-introduction of Ikaros into an Ikaros null T cell line initiates expression of a number of T cell specific genes, including *CD4*, *CD8*, and *TCR*, while concomitantly increasing widespread levels of histone H3 acetylation [Kathrein et al.]. In addition, Ikaros is a component of both SWI/SNF and NuRD complexes in T cells [Kim et al., 1999]. If Ikaros does play the role of targeter for chromatin remodelers in thymocytes, it is likely that it does so with the help of a T cell specific factor, since it is expressed in all lineages of blood cells. Therefore Ikaros, by itself, cannot impart T cell specificity.

CHROMATIN STRUCTURE IN SILENCING OF THE DEOXYNUCLEOTIDYL TERMINAL TRANSFERASE (Dntt) LOCUS

Chromatin remodeling, as defined by shifting of DNA relative to the histone core proteins, is almost always associated with other changes in chromatin structure, most notably post-translational modifications of histone proteins. Insight into the role of histone modifications in expression of a developmentally regulated gene during T cell development can be gleaned by studies on regulation of the deoxynucleotidyl terminal transferase (*Dntt*) gene. Deoxynucleotidyl terminal transferase (Dntt or TdT) is a DNA polymerase responsible for generating diversity in the T cell repertoire through insertion of random nucleotides at regions of joining during *TCR* gene rearrangement (the process of rearrangement will be discussed in more detail in "Role of the Chromatin Environment in Rearrangement of Antigen Receptor Genes"). Expression of Dntt is restricted to windows in development when receptor genes are rearranging. Evidence suggests that regulation of Dntt is primarily at the level of gene silencing through modulation of chromatin structure [Su et al., 2004]. A transformed thymocyte cell line,VL3-3M2, was an important tool in these studies. VL3-3M2 can undergo molecular events upon stimulation that are similar to those observed during thymic T cell development. Using this system, as well as primary murine thymocytes, it was demonstrated that silencing of Dntt is accomplished by histone deacetylation and differential histone methylation of the core histone H3. More specifically, silencing is accompanied by deacetylation of histone H3 (H3-Lys9), loss of methylation at H3-Lys4, and acquisition of methylation at H3-Lys9. These modifications are nucleated at the promoter and, from there, spread throughout the locus. They are correlated with reduced restriction enzyme accessibility of the Dntt promoter region, suggesting a "closed down" chromatin structure. Interestingly, methylation at H3-Lys9 provides a binding site for heterochromatin protein 1 (HP1), a structural component of silent chromatin [Bannister et al., 2001; Lachner et al., 2001]. Therefore, this modification may facilitate heterochromatin formation within the locus.

How are chromatin remodeling and histone modification activities targeted to Dntt in a temporal and cell specific fashion? Although this is still an open question, studies have implicated the sequence specific DNA binding protein, Ikaros, in this role [Trinh et al., 2001]. Ikaros binds an element in the Dntt promoter that is required for down-regulation of expression of Dntt. The Ikaros binding site overlaps nucleotides shown to be important for activation of the gene. This activating element binds Elf-1, a sequence specific DNA binding protein that is a member of the Ets family of transcription factors. It has been hypothesized that whereas Elf-1 is necessary for activation of the *Dntt* gene, Ikaros is required for its silencing. This may be accomplished through mutually exclusive binding of these factors at different T cell developmental stages. However, the questions still remain "Is Ikaros required for the modifications observed in histone H3 that have been linked to silencing?" and "Is this silencing linked to Ikaros' ability to target the NuRD complex with its accompanying HDAC activity?" The genetically engineered Ikaros null mice and the VL3-3M2 cell line in combination with small interfering RNA (siRNA) technology may be the perfect tools to answer these questions.

ROLE OF THE CHROMATIN ENVIRONMENT IN REARRANGEMENT OF ANTIGEN RECEPTOR GENES

Rearrangement of *TCR* genes, which include the α, β, γ, and δ loci, are regulated in a stage-specific manner during T cell development in the thymus. For example, during early development of T cells of the αβ lineage, the DN to DP transition represents a major checkpoint known as β-selection. Passage through β-selection is dependent upon expression of a productively rearranged *TCRβ* gene. *TCRβ* is assembled by a process known as V(D)J recombination from separate V, D, and J gene segments. Once thymocytes are β-selected, they must productively rearrange the *TCRα* gene in order to express a TCRαβ on their surface. *TCRα* gene rearrangement proceeds by a similar mechanism to that of *TCRβ*, and results in the development of mature T cells after transition through further developmental checkpoints. Rearrangement of these genes always occurs in the sequence of *TCRβ* followed by *TCRα*, even though they utilize the same recombination machinery. This is accomplished through

regulation of chromatin structure at these genetic loci.

The "accessibility hypothesis" of antigen receptor rearrangement was first put forth approximately 20 years ago. This hypothesis states that antigen receptor genes typically reside in a chromatin structure that is refractory to recognition by the recombination machinery. When appropriate signals are received by the cell, chromatin structure becomes accessible and rearrangement occurs in an ordered fashion. It was not until recently, however, that insight has been gained into the molecular mechanism by which this occurs. Changes in histone H3 acetylation and recruitment of a SWI/SNF remodeling complex have been implicated as playing important roles in priming antigen receptor genes for rearrangement.

A series of studies from different groups have shown that hyperacetylation of histone H3 is tightly linked to recombination [Kwon et al., 2000; Mathieu et al., 2000; McBlane and Boyes, 2000; McMurry and Krangel, 2000]. In one of the first studies, it was shown that thymocytes demonstrate a shift to hyperacetylated histone H3 in regions of *TCRβ* at the DN stage, the stage at which rearrangement of this gene occurs [McMurry and Krangel, 2000]. In a similar fashion, increased acetylation of histone H3 was identified in regions of *TCRα* at the DP stage, the stage at which rearrangement of this locus occurs. The shift in histone H3 from a hypoacetylated to a hyperacetylated state within the *TCRα* locus was dependent upon a functional transcriptional enhancer, designated Eα. Strengthening the link between histone H3 acetylation and rearrangement, Eα also must be intact for *TCRα* gene rearrangement to occur [Sleckman et al., 1997]. This same observation has been made for *TCRδ*, a gene that encodes a chain of TCRγδ, the expression of which identifies cells of the γδ T cell lineage. In this case, it has been demonstrated that a three base pair mutation which prevents the binding of a sequence specific DNA binding factor, CBF/PEBP2, to a TCRδ enhancer (Eδ) prevents H3 hyperacetylation of a *TCRδ* mini-locus transgene [Hernandez-Munain and Krangel, 2002]. Interestingly, it has been demonstrated that CBF/PEBP2 can interact with HATs [Kitabayashi et al., 1998], suggesting that its ability to recruit HAT activity to Eδ nucleates the hyperacetylation that is required for TCRδ rearrangement.

Hyperacetylation alone is insufficient to generate the "open" *TCR* gene conformation necessary for stable binding of the recombination machinery. Higher order chromatin structure must also be altered. It is hypothesized that this is accomplished by a SWI/SNF complex. Using an in vitro nucleosome remodeling assay, it was shown that introduction of SWI/SNF activity to nucleosomal structures containing recombination substrates greatly increased the accessibility of DNA to the recombination machinery [Kwon et al., 2000].

Taken together, these studies suggest that histone acetylation and SWI/SNF remodeling together may prepare antigen receptor gene loci for rearrangement. However, this is just the beginning of the story. Mechanisms of stage-specific remodeling during T cell development have yet to be uncovered, although they almost certainly will include stage-specific DNA binding factors that, like CBF/PEBP2, are required for rearrangement. These factors, expressed only in the correct window of development, would recruit the histone modifying and chromatin remodeling activities to the appropriate TCR loci. Identifying these factors and unraveling how their activity is controlled are two of the next important scientific questions.

MAINTENANCE OF SILENT CHROMATIN DURING T CELL DEVELOPMENT

Since chromatin structure defines the genes that are expressed in a given cell, and the genes that are expressed impart cell identity, it follows that maintenance of chromatin structure is crucial in maintenance of cell identity. Members of the Polycomb-group (*PcG*) gene family have been shown to play a fundamental role in this process through their roles in the heritable maintenance of gene silencing over successive generations during development (reviewed by Raaphorst et al. [2001]).

PcG proteins are components of large (2–5 MD) complexes that can bind DNA [Franke et al., 1992]. However, the core components of PcG complexes cannot directly bind DNA. It is hypothesized the PcG complexes are targeted to genetic loci through their interaction with sequence specific DNA binding proteins. Proteins that have been found associated with PcG complexes include transcriptional activators, transcriptional repressors, and HDACs, which together could contribute

to the specificity and function of these complexes. Indeed, evidence suggests that one PcG complex, designated the EED–EZH complex, controls gene repression through the recruitment of a HDAC followed by local histone deacetylation [Sewalt et al., 1998; van Lohuizen et al., 1998]. Another PcG complex, designated the polycomb repressive complex 1 (PRC1), has been shown to negatively regulate chromatin accessibility promoted by the SWI–SNF complex [Shao et al., 1999].

PcG complexes are critical for normal T cell development. Bmi-1 is a PcG family protein that is a component of the PRC1 complex. Bmi-1 knockout mice show progressive loss of mature T cells due to a severe proliferative defect [van der Lugt et al., 1994]. Similar results were obtained when other *PcG* genes (i.e., *Mel-18*, *M33*, *Rae28*, and *Eed*) were mutated in mice (reviewed by Raaphorst et al. [2001]). This phenotype is the result of a partial arrest in development at the β-selection checkpoint, a stage at which proliferation is initiated during T cell development. However, the genes affected by PcG deficiency that result in this phenotype have not yet been definitively identified. It has been hypothesized that deregulation of the cell cycle dependent inhibitor genes *p16INK4a* and *p19ARF* may contribute to this phenotype [Jacobs et al., 1999], although neither have shown to be direct targets for polycomb complex action. The inability of thymocytes to proliferate may suggest that PcG complexes are important in the maintenance of chromatin structural changes that occur as a result of developmental signals. Therefore, in the absence of this activity, cells cannot respond normally to proliferative signals, which is manifested as their inability to proliferate. However, it is likely that absence of PcG complexes may also result in the inability to maintain repression of genes that must be silenced in order for lymphocyte development to occur, a phenotype that is more difficult to explore.

CENTROMERIC HETEROCHROMATIN—A RESERVOIR FOR INACTIVE GENES?

Silent genes lie in restrictive chromatin environments. The "closed" chromatin conformation may be isolated to individual genetic loci. However, another mechanism of silencing involves recruitment of genes to larger regions of highly condensed chromatin, called heterochromatin, that are located in centromeric and pericentromeric regions of each chromosome. It has been proposed that these heterochromatic regions might be reservoirs for genes that are heritably silenced during development. The cells used to define this hypothesized mechanism of gene silencing were B and T lymphocytes [Brown et al., 1997, 1999; Klug et al., 1998], suggesting that this method of regulating chromatin structure may play an important role during lymphocyte development.

How are specific genes recruited to centromeric and pericentromeric regions of heterochromatin during development? To explain this mechanism, sequence specific DNA binding factors must again be invoked. Ikaros is a potential candidate for a targeting factor during B and T lymphocyte development. Ikaros has been localized to pericentromeric heterochromatin in an overlapping pattern with that of silent genes using an ingenious approach called immuno-FISH [Brown et al., 1997, 1999]. Using immuno-FISH, heterochromatin was identified within interphase nuclei through hybridization with a FITC-labeled probe for γ-satellite sequences, repetitive elements found within mouse heterochromatin. Within these same cells, Ikaros was detected by an anti-Ikaros antibody, followed by a Texas-Red-conjugated secondary antibody. Regions of overlapping fluorescence were revealed using indirect immunofluorescence and confocal microscopy. Using these techniques, it was revealed that Ikaros co-localizes with γ-satellite DNA and, therefore, pericentromeric heterochromatin. Immuno-FISH was also utilized to demonstrate that, within the heterochromatic foci, Ikaros co-localizes with heritably silent genes and not with actively transcribed genes [Brown et al., 1997]. In cells committed to the B lymphocyte lineage, Ikaros associates with genes encoding the T cell developmental markers, CD4 and CD8α. It does not associate with the highly expressed B lymphocyte gene encoding CD19. Localization of Ikaros to pericentromeric heterochromatin is dependent on Ikaros' ability to bind DNA, and Ikaros binds directly to γ-satellite DNA sequences [Cobb et al., 2000]. Taken together, these data provide evidence that Ikaros may bind DNA sequences in genes that must be silenced, initiating their recruitment to centromeric regions, where they then assemble into heterochromatin.

The use of centromeric heterochromatin as a reservoir for heritably silent genes has also been proposed for T lymphocytes. During T lymphocyte development, genes that are expressed early in development, but must be heritably silenced at later stages, are repositioned into pericentromeric heterochromatin. This was shown for genes encoding proteins required for gene rearrangement and generation of TCR diversity, the Rag proteins (Rag1 and Rag2, components of the recombination machinery) and Dntt. In primary DP thymocytes, these genes are at first active to allow rearrangement of TCRα, but must be silenced once successful rearrangement has occurred. Pericentromeric repositioning of these genes within the nucleus was demonstrated to accompany this pattern of heritable silencing [Brown et al., 1999].

CONCLUSION

During development, cells "instruct" their DNA to differentially express or silence genes, creating a program of gene expression that will define that cell. This instruction is delivered, in part, by (1) chromatin remodeling complexes, (2) enzymes that catalyze changes in histone structure through post-translational modifications, and (3) sequence specific DNA binding factors that target chromatin remodeling and enzymatic activities to specific genetic loci and, in addition, recruit genes to heterochromatic regions resulting in their heritable silencing (Table I). Although the study of regulation of chromatin structure during T cell development is still in its early stages, many significant observations have been made.

Defining the role of individual chromatin remodeling complexes in T cell development has been hampered by the redundancy within families. Nevertheless, roles for SWI/SNF and Mi-2/NuRD family complexes have been uncovered. These include regulation of expression of T cell specific genes, of thymocyte expansion and viability, and of accessibility of *TCR* gene loci for rearrangement. The role of the ISWI family of chromatin remodelers in T cell development has been much less studied. A recent study suggests that, like SWI/SNF complexes, ISWI complexes possess the capacity to remodel antigen receptor gene loci to allow access by the recombination machinery [Patenge et al., 2004]. However, almost certainly, this family of complexes has other roles waiting to be discovered.

The hunt for target genes regulated by chromatin structure during T cell development has, to date, primarily identified three genes: *CD4*, *CD8α*, and *Dntt*. Although these are certainly important developmentally regulated genes, they are most likely but a peek into the vast array of genes whose expression patterns are under the control of epigenetic regulation. Studies on regulation of these three genes have provided insight into the importance of sequence specific DNA binding factors in targeting chromatin structural changes to genetic loci. Interestingly, some of these factors have dual roles. For example, Runx1 is required both for activation of CD8 and repression of CD4 during T cell development. Understanding how Runx1 functions to differentially regulate expression of key target genes is as yet unexplored. Another sequence specific DNA binding factor, Ikaros, has been shown to interact with both SWI/SNF and Mi-2/NuRD complexes in primary T cells. This suggests that it is important for function of both, but no insight has yet been gained as to how Ikaros may differentially target these complexes. One possibility is that Ikaros targets genes based on its ability to

TABLE I. Summary of Chromatin Remodeling Activities That Regulate T Cell Development

Complex/activity	Function	Known role(s) during early T cell development	References
SWI/SNF	Chromatin remodeling	CD4 silencing, CD8 activation, thymocyte viability and proliferation, *TCR* gene accessibility	Kwon et al. [2000]; Chi et al. [2003]; Gebuhr et al. [2003]
Mi-2β	Chromatin remodeling	CD4 activation, T cell proliferation	Williams et al. [2004]
ISWI	Chromatin remodeling	*TCR* gene accessibility	Patenge et al. [2004]
Histone acetyltransferase (HAT)	HAT	CD4 activation, *TCR* gene accessibility	McMurry and Krangel [2000]; Williams et al. [2004]
HDAC	Histone deacetyltransferase	Gene inaccessibility, Dntt silencing	Su et al. [2004]
Polycomb	Heritable gene silencing	Thymocyte proliferation	van der Lugt et al. [1994]
Nuclear repositioning	Heritable gene silencing	Not known	Brown et al. [1997]

A compiled table of chromatin remodeling activities that function during T cell development and their roles.

interact with a sequence specific DNA binding "partner." Ikaros' incorporation into the SWI/SNF or Mi-2/NuRD complex may affect its ability to interact with one partner versus another. For example, it may be that Ikaros must be phosphorylated in order to interact with the Mi-2/NuRD complex, but that this modification is not required for interaction with SWI/SNF. Moreover, phosphorylation could control Ikaros' ability to interact with cell type specific DNA binding proteins. The mechanism of this type of "fine-tuning" for differential recruitment of chromatin remodeling complexes is certainly a ripe field for future research.

Interestingly, the majority of insight into the role of chromatin structure in T cell development has come in the field of gene silencing and maintenance of a repressive chromatin environment. Could it be that the majority of epigenetic regulation is controlled at this level? Certainly it is a fact that mammalian cells express large multi-protein complexes, such as those containing the PcG proteins, whose only job appears to be maintenance of the silent gene state.

In conclusion, the study of regulation of chromatin structure during lymphocyte development has just begun. Insights gained into how chromatin structure is modeled and remodeled during lymphocyte development, an easily manipulated system, will almost certainly provide insight into how this highly regulated process occurs in other cell types.

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