Review Article

Role of Nuclear Matrix Associated Region (MAR) Binding Proteins in the Regulation of T Helper Cell Differentiation

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Nuclear matrix is the major hub of nuclear functions that includes transcription, post transcriptional splicing, DNA repair etc. and also majorly involved in the differentiation of T cell programmes. Though plenty of reports illustrate about the role of lineage specific transcription factors involved in the process of T cell differentiation, not much is known about the active participation of nuclear matrix associated proteins in the modulation of chromatin architecture necessary for the T cell differentiation. This review comprehensively explore the necessary role of nuclear matrix associated proteins in the modulation of chromatin architecture relative to gene positioning, necessary to facilitate the T cell differentiation pattern.

Key Words: T Helper Cell Differentiation; SMAR1; T-Bet; GATA-3; HDAC1; IL-17

1. Introduction

(a) Immune Response

Human body has one of the most complex and intricate mechanism of self-defense for resisting the invasion of unwanted environmental agents, causing malfunctioning of the system (Beck and Gail, 1996). Our body has to recognize self and the non-self and react against exogenous agents. Once the antigen is recognized as outsider, it is specifically marked as an opponent, which is targeted and destroyed by the immune responses initiating an armageddon. There can be two fates of this war. One, the immune system wins and the invading agents are destroyed and removed. Or, the exogenous pathogen wins and the body will be succumbing to it and perish (Metchnikoff, 1905).

Appropriate activation of immune system is indispensable for eliciting proper immune response against antigens. There are two lines of defense in the body. The first line of defense is the innate immune response, which makes the physical barrier of the body with the outside world, including the skin, mucus secretions, tears, as well as some of the cells acting non-specifically (still under investigation) to an antigen like macrophages or complements (Janesway *et al*., 2001). The next set of immune cells is highly specific to the type and nature of antigens and is capable of mounting huge immune response specifically targeted against the intruder. This line of defense, called as adaptive immune response requires the recognition and presentation of antigens by the innate immune cells for its action. Thus, adaptive immune response is activated only when the antigen is marked. Once it is marked, the sentinels of the adaptive immune response unleash a catastrophic program cascade killing all the antigens (Alberts *et al*., 2002).

2. Adaptive Immune Response

Adaptive immune response consists of two cell types mainly, T cells and B cells. T cells actuate the cell mediated immune response and B cells raise the antibody mediated humoral immunity (Alberts *et al*., 2002). There are various subsets of T cells for the

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distribution of labour. CD4$^+$ and CD8$^+$ T cells are endowed with specific functions. CD8$^+$ T cells, when activated by APCs, convert into cytotoxic T lymphocytes killing the pathogens by making the pores on its surface through the secretion of cytotoxic perforins and granzymes. CD4$^+$ T cells on the other hand are diverse in their functionalities. They are also called T helper cells as they can help in the activation of adaptive immune response by stimulating the class switch recombination in B cells (Pancer and Cooper, 2006).

3. T Cell Differentiation

There are various subtypes of CD4$^+$ T cells having functional specifications. Th1 cells are safe guarding cells against the intracellular pathogens like *Mycobacteria* and *Leishmania* infections secreting more of IFNγ. By the secretion of this specific cytokine, it can cause direct cell killing (Szabo *et al*., 2002). Another set of T cells secreting IL4 is important for the defense against the extra-cellular bacterial and helminthes infection, by inducing the antibody generation in B cells against these antigens. These cells are called Th2 cells (Ouyang *et al*., 2000). Recent advancements in the field of immune responses paved the way for the identification of many novel T cell subsets including Th17 cells-secreting IL17, which raise localized inflammation, regulatory T cells mediating the suppression of immune responses, Th9 cells, Th22 cells etc (Zhu *et al*., 2010). All these various sets of T cells are generated from the differentiation of a common lineage progenitor cells called naïve T cells. The differentiation process of naïve T cells into various lineages is a complicated phenomenon involving the interplay of diverse lineage specific transcription factors. These factors orchestrate the activation of gene subsets related to a particular committed T cells while shutting down the transcription of all the other genes (Kanno *et al*., 2012). For example, in the case of Th2 cells, initial signaling from IL4 induces a STAT6 mediated activation of GATA-3. GATA-3 can establish Th2 cells by directly binding and activating the Th2 locus mediating the chromatin remodelling of the region including the genes of IL4, IL13 and IL5, at the same time restrict other lineages by

inhibiting IFNγ, RORc, FoxP3 etc (Wei *et al*., 2011). Thus, GATA-3 is necessary and sufficient for the development and maintenance of Th2 cells.

3.1 Orchestration of T Cell Differentiation

T cell differentiation has direct influence on the immune response of the body. Any defect in these differentiation processes ultimately lead to many physiological manifestations including susceptibility to various pathogens or aberrant auto-immune responses. Thus, T cell differentiation is made stringent through the homeostasis and programmed control of various lineage specific transcription factors and cytokines (Fig. 1). Recent research in T cell biology unraveled the existence of these 'master regulators' for each lineage pathway of T cells (Zhou *et al*., 2009). Intracellular pathogens or viruses triggers the secretion of IFNγ from the T cells with the induction of T-bet (T-box protein expressed in T cells) encoded by *Tbx21* gene (Szabo *et al*., 2000). T-bet expression is critical for the formation of a functional Th1 cells and activation of IFNγ gene. It maintains the Th1 cells by blocking the expression of gene subsets belong to other T cell lineage including GATA-3, RORgt etc. (Zhu *et al*., 2010). Alternatively, deficiency of T-bet expression is correlated with defective Th1 response and disease manifestations. T-bet deficiency in T cells caused susceptibility to intracellular pathogens including *Mycobacteria* and *Leishmania* infections (Hsieh *et al*., 1993; Szabo *et al*., 2000). Reports also suggest compromised immunity to cancer cells in T-bet knock-out mice. Such mice also generated spontaneous air way hypersensitivity and allergy against external allergens with enhanced Th2 response and IgE production (Finotto *et al*., 2002). Recent studies also outline a negative regulation of T-bet on Th17 pathway through RORgt (Lazarevic *et al*., 2011). Thus, T-bet is an indispensable lineage specific master regulator of Th1 cells (Lazarevic and Glimcher, 2011). Some T cells are specialized to recognize the extra cellular infections mainly mediated by bacterial, fungal or other parasitic infections. These cells are also derived from a common progenitor naïve CD4$^+$ T cells. When these cells are encountering the antigen presenting cells

(APCs) primed with extra cellular pathogens, they start secreting IL4 specific to Th2 cells. When researchers looked into the transcriptional factors important for this lineage differentiation, they observed GATA-3, specifically induced in these cells (Ouyang *et al*., 2000). Further research work in this field has been yielding enormous information about the functional relevance of GATA-3 and its accessory proteins important for the establishment and maintenance of Th2 cells. GATA-3 is necessary and sufficient for Th2 cells (Zheng and Flavell, 1997). It can establish Th2 cell lineage by directly binding and activating IL4 locus including IL4, IL13 and IL5 (Lee *et al.*, 2000). Binding of GATA-3 mediates chromatin remodeling and epigenetic modifications around the region, which are either activating or repressing. The same GATA-3, which is causing activation of IL4 locus, can trigger a repressive epigenetic condensation of chromatin at the IFNg locus (Zhu *et al*., 2006). Recent genomic study revealed multiple sites of GATA-3 binding with both activating and repressive roles. Deletion of GATA-3 in T cells

resulted in the upregulation of T-bet, IL12rb, RoRc etc. which are important for the differentiation of other lineages (Wei *et al*., 2011). Thus, GATA-3 can possibly control the differentiation of T cells towards Th2 pathway by promoting necessary chromatin changes and repressing Th1 and Th17 lineage gene subsets.

4. Chromatin Dynamics During T Cell Differentiation

The subnuclear localization of various cytokine loci is not random in T cells and specific loci reside in distinct locations (Cremer and Cremer, 2001). As a naïve T cells is differentiating, changes in transcriptional activity are coupled with alterations in subnuclear localization of loci. During Th2 cell differentiation, Th2 cytokines present in IL4 locus are located in close spatial proximity, and forms an initial chromatin core configuration (Tanaka *et al*., 2011). These kinds of interactions were also noticed at the *Ifng* locus during Th1 differentiation suggesting
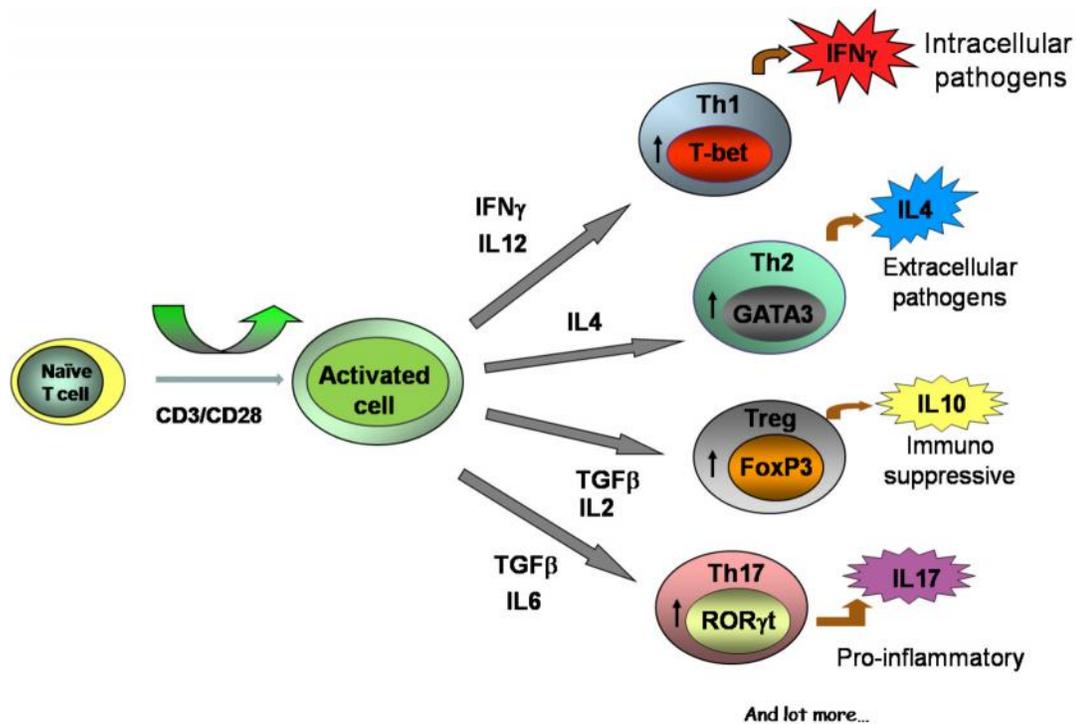

Fig. 1: T cell differentiation. Naïve T cells, when activated with CD3 and CD28 in the presence of various polarizing cytokines yield differentiated T cells of particular lineage. Each effector T cells has specific functions in the activation of immune response against the external antigens. Lineage specific transcription factors orchestrate the gene expression of the signature lineage, while repressing all the other gene subsets

this as a mechanism of gene activation within particular locus (Collins *et al*., 2012). Apart from the intrachromosomal interactions, a higher level of inter-chromosomal interactions between specific gene loci is reported during T cell differentiation (Fig. 2). In naïve T cells, a strong and specific interchromosomal interaction was noticed between IFNg gene and three distinct regions of the Th2 cytokine locus, covering the RHS6, Rad50 and IL5 promoter. Upon differentiation, these inter-chromosomal interactions were lost and localized into separate domains of the nucleus. Thus, when the cell is naïve, it has the potential to form either Th1 or Th2 cells through the activation of specific gene loci and the inter-chromosomal interaction will facilitate the activation of the gene locus. Under Th1 differentiation condition, the interchromosomal interaction between *Ifng* locus and Th2 locus is lost and IFNg locus is pulled to the transcriptionally active domain forming an intra-chromosomal interaction and gene activation.

At this time, Th2 locus will be kept silent by localization to a repressive subnuclear compartment. On the other hand, in Th2 differentiation condition, the loss of interchromosomal interaction will facilitate the localization of Th2 locus to the activation domain and IFNglocus was kept silent. Thus, during the differentiation of naïve T cells to effector cells, there is a switch of inter- to intrachromosomal interactions (Spilianakis *et al*., 2005).

4.1 T Cell Specific Gene Loci

Gene loci contain one or more genes, intergenic regions, conserved non-coding sequences etc. all playing critical role in the regulation of genes (Cremer and Cremer, 2001). A dynamic interplay of interchromosomal and intra-chromosomal interactions may also be possible for actuating the proper regulation of the loci (Isogai and Tjian, 2003). In T cell differentiation, many gene loci are very well
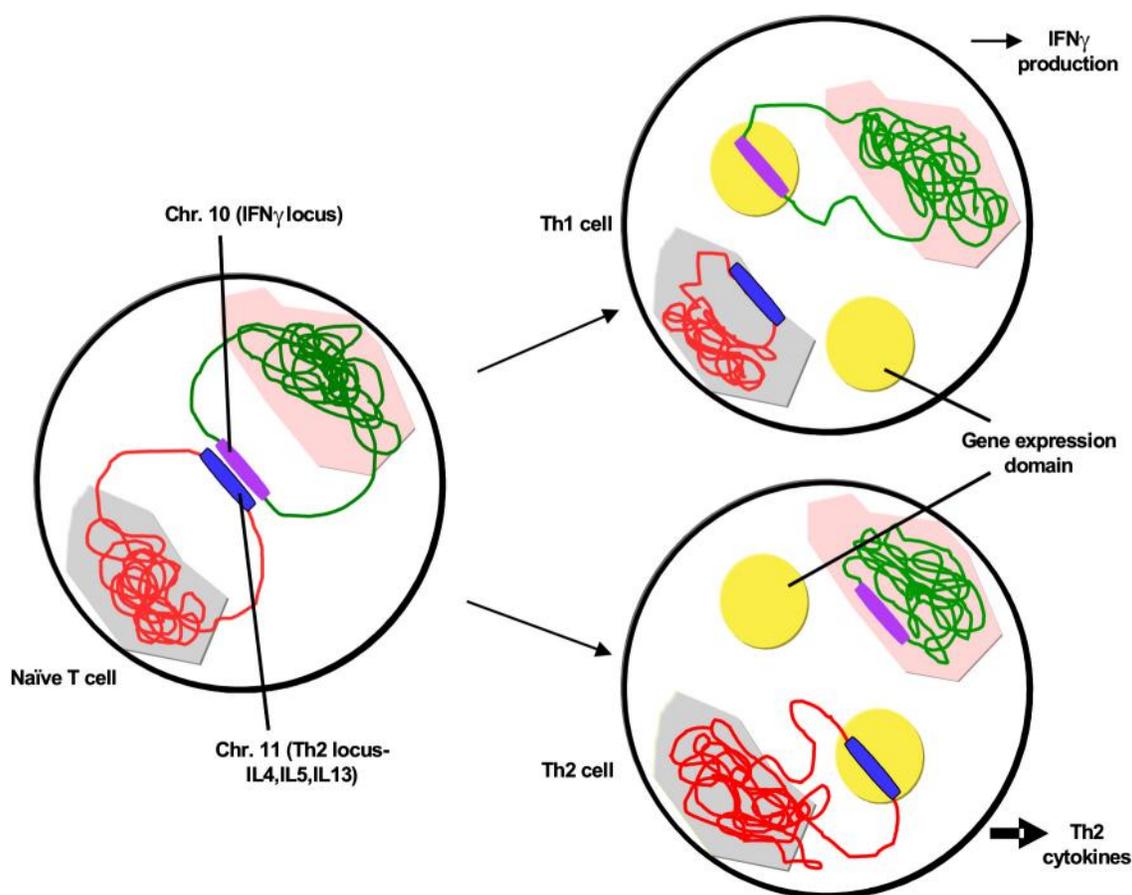

Fig. 2: Chromatin changes during Th1-Th2 cell differentiation. Interchromosomal interactions between IFNγ locus and Th2 cytokine locus in naïve T cells are removed to form intrachromosomal interactions at the active gene expression domain of the nucleus

established like IFNγ locus, Th2 cytokine locus containing IL4, IL5 and IL13 genes and IL17 locus containing IL17A and IL17F. Regulation of these loci is important for the proper control of expression of genes within the loci. Recent studies unraveled the role of many *cis* elements in the regulation of these loci. Conserved non coding sequences and enhancers affect the transcription of loci and thus play critical role in the overall regulation of cytokine gene expression and T cell differentiation (Kanno *et al*., 2012). There are some loci yet to be elucidated or poorly understood which are involved in T cell differentiation.

4.1.1 IFNγ Locus

IFNγ is one of the early cytokine investigated, related to T cell function (Milstone and Wakesman, 1970). It is a dimerized soluble cytokine and is the only member of type II class of interferon (Gray and Goeddel, 1982) IFNγ expression is important for both innate and adaptive immunity against intracellular bacterial infections, viral infestations as well as tumor control. Deregulated IFNγ production is correlated with many inflammatory and autoimmune diseases (Schoenborn and Wilson, 2007). In T cells, Th1 cytokine signals trigger multiple factors to bind on to 140-180kb region of single gene IFNγ locus leading to its activation. Mapping of *cis* elements in the IFNγ locus were initiated with identification of DNase I hypersensitive sites within 8.6kb fragment of human *IFNG* gene (Soutto *et al*., 2002). Later research pointed out the importance of many other regulatory elements is essential for the proper control of the locus. Many of the regulatory DNA elements in the locus are conserved across the species, enabling

database to detect homologous DNA within the IFNγ locus. Nine CNSs, which contribute to the locus control, have been reported where the regulatory proteins and barrier proteins can bind and regulate the locus (Fig. 3). Conventional DNase I hypersensitivity mapping and chromatin immunoprecipitation analysis of histone modifications lead to the initial identification of CNS1 and CNS2. Novel high throughput technologies including massive parallel sequencing coupled with ChIP assay allowed more comprehensive mapping of distal and proximal elements and its associated epigenetic modifications regulate the recruitment of critical trans-acting factors. CNS sites have been discovered recently regulating the locus control. CNS-70 and CNS+66, where CTCF and Rad 21 bind and marks the boundaries of the locus (Bream *et al*., 2004). CNS-54, the region where GATA-3 is bound and repress the IFNg locus under Th2 condition, CNS-34, -22, -6, +17-19, +30, +40. +46, +54 etc, where T-bet, Runx3, STAT-4 and NFkB proteins bind and activate the loci. Under naïve condition, IFNγ locus was kept silent by the binding of mSin3A chromatin remodelling complex at CNS-22 (Balasubramani *et al*., 2010). A recent report suggests CNS-30, CNS-4 and CNS+20 are crucial at distinct development stages of Th1 differentiation, whereas CNS-16 has inhibitory functions with drastic changes in the epigenetics of the region (Collins *et al*., 2012).

4.1.2 Th2 Locus

Th2 locus, containing IL4, IL5, IL13, Rad50 and Kif3a genes has been extensively studied as a model system of chromatin dynamics and gene expression
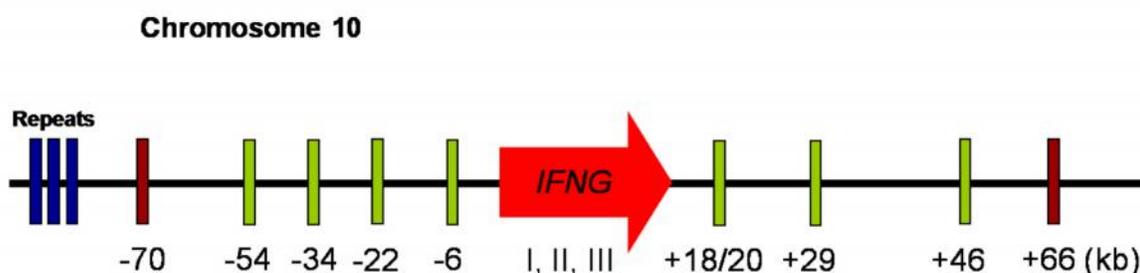

Fig. 3: IFNγ locus. IFNγ locus of the mice is present in chromosome 10. Nine CNS sites have been described stretching from -70kb to +66kb of the locus

in T helper lineage commitment (Loots *et al*., 2000). The genes present in this locus is clustered in a 120kb region on chromosome 11 in mouse and 160kb region on chromosome 5 in human (Fig. 4). Various regulatory elements including CNSs and hypersensitivity sites have been described to have significant role in regulating the locus (Wilson *et al*., 2009). Two CNS sites were described between IL4 and IL13 and downstream of 3' UTR of the IL4 gene respectively, whose deletions cause aberrant activation of the locus (Ansel *et al*., 2006). In mouse IL4 locus, IL4 transcription is regulated by elements that map to conserved sequence (HSS1 and HSS2), HS0 lies in the 5' region of IL4. HS1 site is present in the promoter region of IL4, HS2 and HS3 in second exon; and HS5a and HS5 located at 3' of IL4 (Agarwal and Rao *et al*., 1998, Agarwal *et al*., 2000, Lee *et al*., 2002). There are other regulatory regions also explained, mainly HS sites (Rad 50 HS4 (RHS4), RHS5, RHS6 and RHS7), which are located between the exon 22 and exon 25 of Rad 50 gene. IL13 gene also have some regulatory elements like HS1- also called as conserved GATA-3 response elements (CGRE) upstream of the proximal promoter HS2. Report suggests that GATA-3 activates the IL4 locus by directly binding to the HS2 sites near CGRE site and within the exons of IL4 gene. This HS2, present near the CGRE site and the exon of IL4 functions as enhancer of this locus. Ablation of HS2 showed substantial impairment in eliciting Th2 response with

drastic reduction in IL4 and asthmatic response (Tanaka *et al*., 2011). Th2 LCR is essential for proper Th2 response *in vivo*. Deletion of LCR caused loss of activating epigenetic modifications of the Th2 locus and marked reduction in the airway hyper-responsiveness against allergic antigens (Koh *et al*., 2010). Though the epigenetic modifications of the loci is well studied under various T cell lineages, the factors which dictates and functions these modifications or deciding the chromatin changes are still not understood completely.

4.1.3 IL17 Locus

IL17 locus contains IL17A and IL17F genes which are separated by ~43.9kb in mice and are transcribed in opposite directions. There are eight CNS sites which are very well described in this locus stretching around 120kb (Fig. 5). CNS1 and CNS2 are present upstream of IL17A, CNS3-CNS6 is present in the intergenic region and CNS7 and 8 are present upstream of IL17F gene (Akimzhanov *et al*., 2007). Many of these CNS sites are co-incided with the binding of STAT3. CNS2 was found to be most crucial *cis* element for the activation of the locus. CNS2 physically interact with IL17A and F promoter and targeted deletion of CNS2 resulted in impaired RORγt binding and activation of genes. CNS2 thus functions as an important enhancer for IL17 locus by recruitment of histone modifying enzymes like p300 and JmjC as well as transcription factors at the
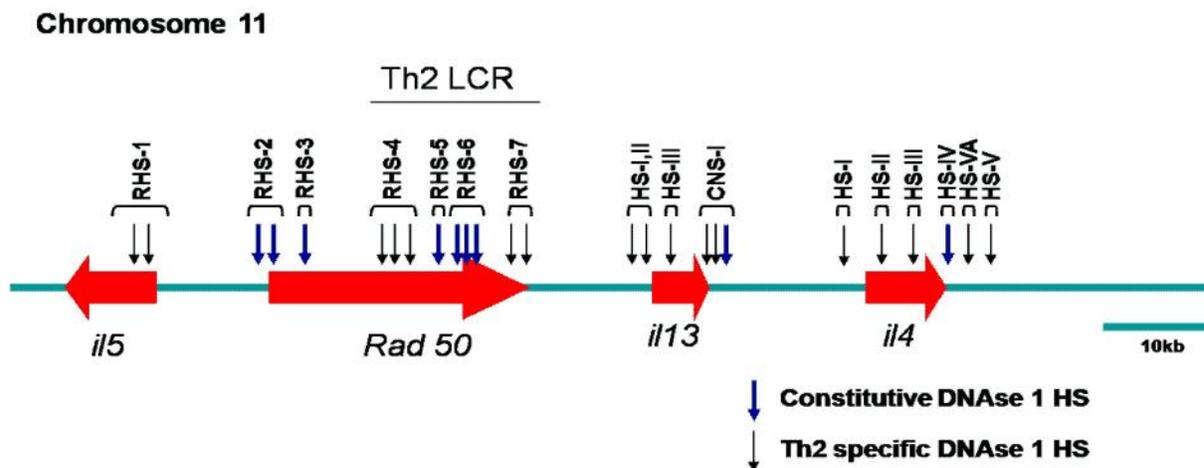

Fig. 4: Th2 Locus. It is present in chromosome 11 and has four genes including IL5, Rad50, IL13 and IL4. Many conserved elements are present through out the locus which play crucial role in the regulation of gene expression

promoters of both IL17A and IL17F. Deletion of CNS2 caused impairment in RORγt dependent IL17 expression and the mice having the deletion in T cells were resistant to EAE (Wang *et al*., 2012). Differential binding of distinct STATs to these CNS sites can streamline the regulation of the locus. STAT3 and STAT5 were found to be binding to various regions other than the promoters present in the locus. Maximum occupancy of STATs was observed to be in the intergenic region where CNS3-CNS6 is clustered. The interplay of STAT3 and STAT5 binding can drive the activation or repression of this locus respectively. Binding of chromatin remodelor NCoR2 was also noticed in the intergenic region along with the binding of STAT5 suggesting the role of chromatin remodelling of the locus depending upon the signal stimuli (Yang *et al*., 2011).

4.1.4 Tbx 21 Locus (T-bet Locus)

Tbx21 locus is a monogenic locus stretching ~50kb region on chromosome 11 in mouse. T-bet, the gene coded by Tbx21 is indispensable for the activation of IFNγ locus and Th1 lineage establishment. Not much research have been carried out in these locus apart from the binding of some factors like Notch 1 signaling (Minter *et al*., 2005), Ikaros (Thomas *et al*., 2010), Chd4 (Hosokawa *et al*., 2013), etc.

4.1.5 Other Loci

Other gene loci including FoxP3 locus (Zheng *et al*., 2010), IL2 locus (Kumar *et al*., 2005), etc are also explained, but not much work has been done to elucidate its relevance and identifications of *cis* elements with respect to the locus control or its effects on T cell differentiation. It is also interesting to note that IL12Rb-IL23R, STAT3- STAT5a, etc. are all present within their own loci and needs more investigation to understand the regulation of these loci during T cell differentiation.

5. Nuclear Matrix and Chromatin Factors Involved in T Cell Differentiation

At the chromatin level, T cell differentiation is associated with dramatic changes in the nuclear architecture. Numerous structural organizations were observed in the interphase chromatin, necessary for the transcriptional processes to be effective and regulated. Nuclear matrix has been identified as the site of major nuclear functions including transcription, post transcriptional splicing, DNA repair etc. (Fig. 6). Nuclear matrix, through AT rich MAR elements tethers the chromatin to it. The group of proteins which do this tethering function is called S/MAR binding proteins (Bode *et al*., 2000) Scaffold/ matrix proteins are nuclear proteins which facilitate nuclear stability by binding to scaffold/matrix attachment region (S/MAR). These are the sequences in the DNA where the nuclear matrix attaches and constitute anchor points of the DNA for the chromatin scaffold proteins, serving the structural domains of the chromatin (Heng *et al*., 2004). These dynamic and complex organizations of the chromatin mediated by S/MAR and the associated proteins regulate the gene transcription by modulating the chromatin architecture (Tetko *et al*., 2006).

5.1 S/MAR Proteins and Its Function

The novel concept of transcriptional regulation encompasses the compartmentalization of nucleus (Carmo-Fonseca, 2002). Disagreement with the older view of transcription where the sigma factor searches the entire chromosome looking for possible target
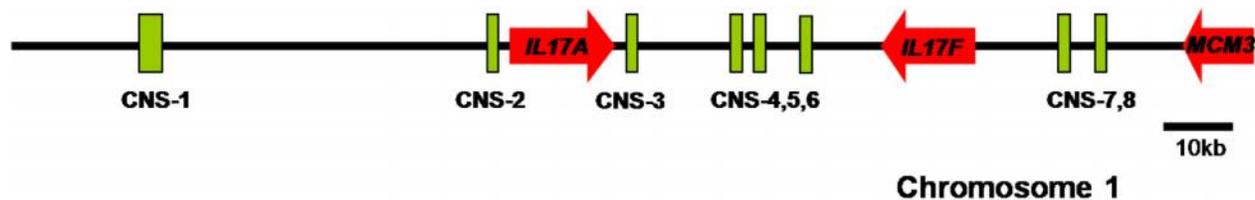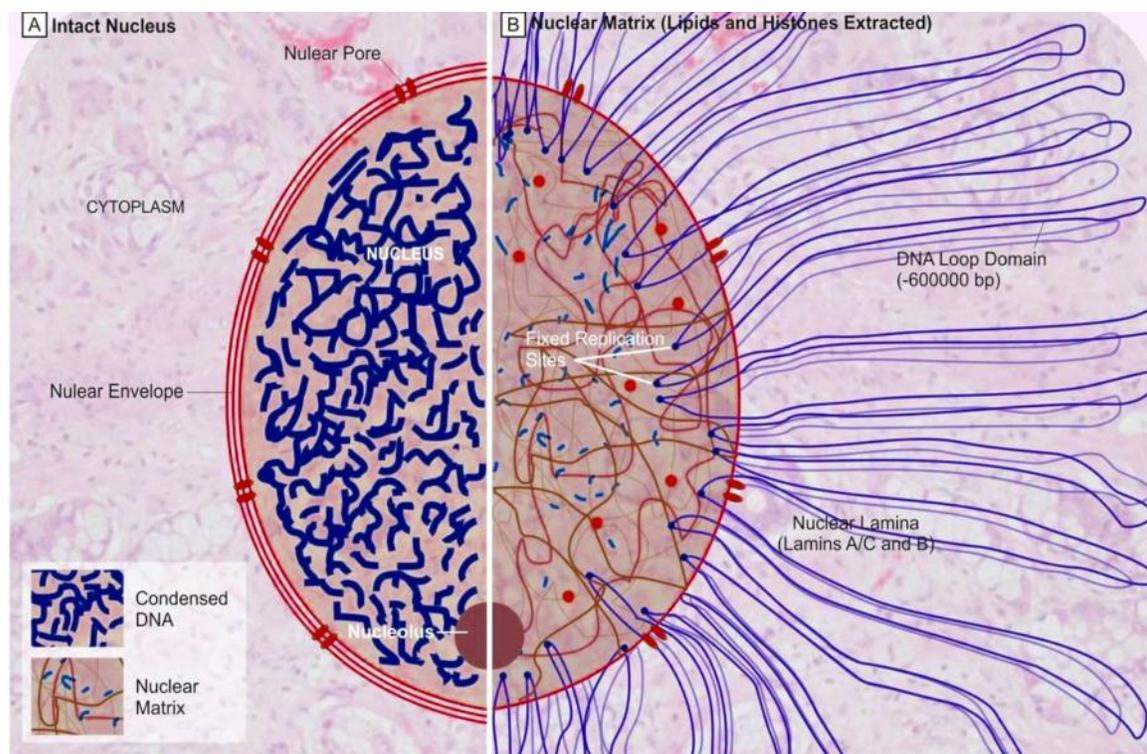

Fig. 5: IL17 Locus. IL17 locus has two genes, inversely oriented (IL17A and IL17F). Eight CNS sites have been well elucidated. CNS-2 is found to be critical for the activation of this locus

Fig. 6: Nuclear matrix. In an interphase nucleus, chromatin is present in relatively condensed state (A). When the histone proteins and the lipids are removed from the nuclear, the remaining fibrous mesh of proteins are called nuclear matrix. Nuclear matrix render the structural and architectural stability to the nucleus and is the major site for the chromatin functions including transcription, splicing, DNA repair, etc.

genes, research shows dynamic nature of chromatin changes (Kumari *et al*., 2012) and epigenetic modifications associated with external stimuli (Jaenisch and Bird, 2003). A particular signal activates specific signaling cascade which ultimately transmits its signals to the nucleus by activated signaling molecules. After reaching the nucleus, these signaling molecules have to activate a set of genes necessary for the functional phenotypic response against those stimuli. Chromatin functions as the receiver and quarterback of external cellular signals in the nucleus (Johnson and Dent, 2013). Recent research in chromatin biology suggests the possible 'nuclear microenvironment' formation within the nucleus which can be either facilitating the gene transcription or silencing (Zaidi *et al*., 2010). This in turn put forward a possible characterization of functional transcriptome complex and its interaction with the chromatin and histone readers, together facilitating the complex process of gene transcription

(Fig. 7) (Musselman *et al*., 2012). As with the new model, the molecular machinery needed for transcription, including the RNA Polymerase and transcription factor complexes are clustered near the activation foci within the nucleus where the chromatin remodelling complexes interplay with chromatin through the code reader-writer complex and marks the neighbouring nucleosomes transcriptionally active (Isogai and Tjian, 2003; Lelli *et al*., 2012).

6.3 Transcriptional Regulation and S/MAR Proteins

The concept of transcriptional regulation and the dynamic interplay of chromatin factors during the transcription are gaining importance day by day and, so is the relevance of nuclear matrix and associated proteins in mediating the transcription (Zaidi *et al*., 2005). The genes to be actively transcribed after receiving the external stimuli are spatially oriented near the activation foci with the help of S/MAR and
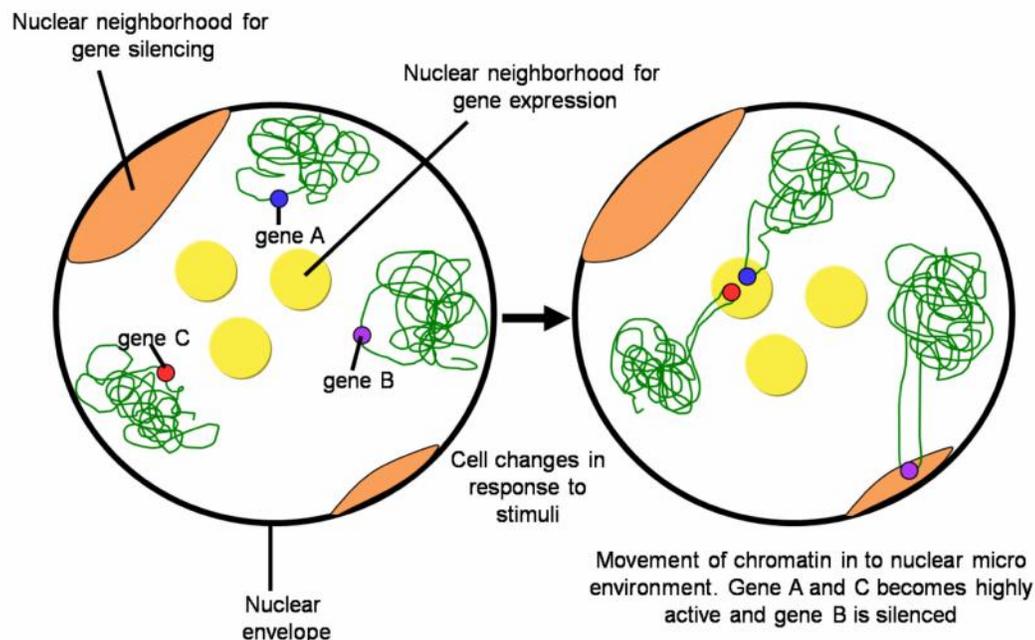
Fig. 7: **Chromatin dynamics of transcriptional regulation. The subnuclear compartmentalization of chromatin in the activation or silencing domain dictates the gene activation or repression and this is mainly occurred through the spatio-temporal orientation of gene loci**

S/MAR binding proteins (Mirkovitch *et al*., 1984; Bode *et al*., 2000) (Fig. 8). Similar to this mechanism, the gene subsets which should be silenced will be spatially moved to the gene silencing compartment of the nucleus where the repressive epigenetic marks keep it transcriptionally inactive (Wu *et al*., 2006, Reddy *et al*., 2008). Even though S/MAR binding proteins share similar sequences for binding to DNA, they show multifaceted functions within the nucleus (Mirkovitch *et al*., 1984).

Many S/MAR binding proteins are well elucidated. SATB1/L2a-P1 is a S/MAR binding protein predominantly expressed in the thymus and in brain. SATB1 can function both as transcriptional repressor as well as activator through its interaction with PDZ domain (Kumar *et al*., 2007). Nucleolin, another member of S/MARBPs is expressed in erythroleukemia cell line K562 necessary for the stabilization of glycosaminoglycans (Dickinson and Shigemastu, 1995). SAF-B functions by competitively binding with RNA PolII (Naylor *et al*., 1998). NFmNR/CDP/Cux protein is also a S/MAR protein shown to interact with SATB1, HDAC1,

SMAR1, etc. and has important role in many organogenesis (Wang *et al*., 1999). Some group of S/MARBPs can function as transcriptional repressor by mediating the chromatin condensation and repressive epigenetic modifications. SP100 functions by repressing Bright and causes the chromosome condensation (Zong *et al*., 2000). Ku is a subunit of PARP protein and functions as an important mediator of DNA damage, especially in the non-homologues double strand break repair (Cheng *et al*., 2011). SMAR1 protein, also known as BANP in human is another member of S/MARBPs represses transcription by the recruitment of HDAC1, mSin3A complex at the target genes (Rampalli *et al*., 2011). DNA-PK is also an important member of this family as it can directly activate other DNA damage response proteins in response to DNA damage (Malewicz *et al*., 2011). In contrast to the transcriptional repressors, many MARBPs functions as activators like SAF-A, and Bright which recruits SWI or p300 and affects the chromatin remodelling and epigenetic modifications (Chattopadhyay and Pavithra, 2007). Broadly, the MARBPs also includes ubiquitous multifunctional proteins including High mobility
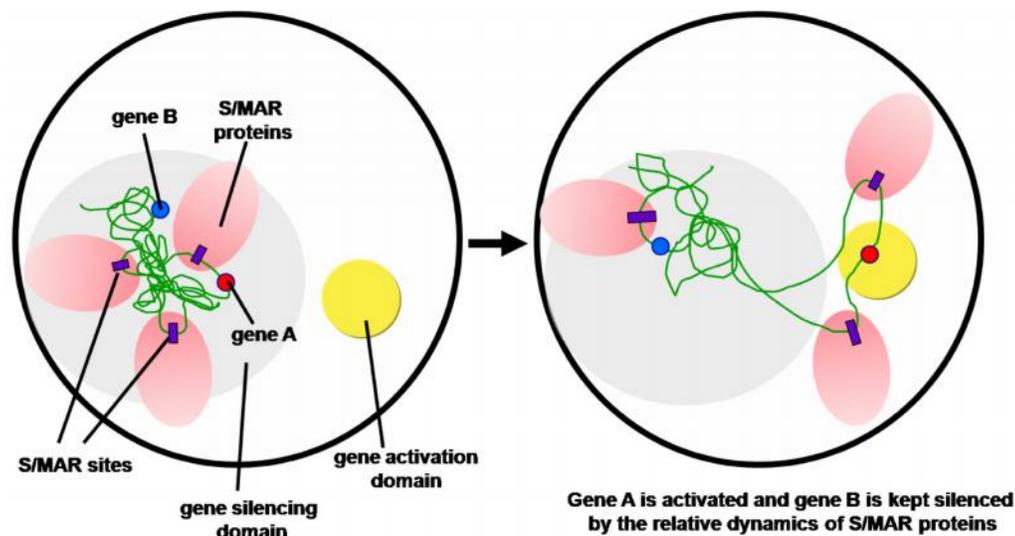
Fig. 8: S/MAR protein mediated chromatin changes MAR proteins can bind to the chromatin through the AT rich S/MAR elements. In response to the external stimuli, S/MAR proteins can modulate the chromatin orchestration of genes causing the activation of one gene, while repressing other

group proteins (HMGs), transcription factors like Y-box, H-box, and CAAT binding proteins etc. Many novel members of MARBPs are being investigated by the modern high throughput technologies including tandem Mass spectrometry.

6.4 Involvement of S/MAR Proteins in T Cell Differentiation

Recent research trend in transcription biology exemplified more on translational research of finding novel targets of transcriptional control. S/MARs and associated proteins thus became an interesting area of research for understanding the molecular mechanism of transcriptional orchestration as well as to unravel novel targets of transcriptional modulation of genes especially in the immune responses. YY1, SATB1, CTCF, Bright, Gfi-1 etc have been investigated for its functional importance in regulating the transcription of T cells by chromatin changes.

6.4.1 Ying Yang 1 (YY1)

YY1 is a Zinc finger S/MARBP, which functions as transcriptional repressor or activator depending upon it's binding to the enhancer or regulatory elements (Gordon *et al.*, 2006). When bound to a particular

cis element, YY1 can function by recruiting histone H4-specific methyl transferase and cause epigenetic change to the region (Rezai-Zadeh *et al.*, 2003). Though much work on YY1 was done in prostrate and other cancer (Seligson *et al.*, 2005), most interesting function of YY1 is elucidated in B cell development in particular reference to V(D)J recombination. YY1 deficiency in mouse B cells caused a defect in somatic gene rearrangement in the immunoglobulin heavy chain (IgH) locus prevents the progenitor B- to precursor-B-cell transition. YY1 binds to the intronic enhancer element of IgH (Em) and mediate long range chromatin interactions between various V, D and J genes causing the gene rearrangement and immunoglobulin class switching (Zaprazna and Atchison, 2012). Utilizing the hypersensitivity regions present on the IgH, YY1 can crosstalk with Em and other insulator proteins like CTCF and induce the locus contractions (Guo *et al.*, 2011). Thus, YY1 works by inducing the histone modifications and alterations in chromatin structures that may facilitate IgH locus contractions and recombination.

6.4.3 Special AT Rich Sequence Binding Protein 1 (SATB1)

SATB1 is a global gene organizer and transcription

factor important for integrating chromatin architecture with gene regulation (Cai *et al*., 2003, 2006). Through interaction with HDAC1, NURD, SMARCA5, CHD4, BAZ1A, SMAR1, etc. it affects the organization of chromatin landscapes, loops and its dynamic architecture in response to the external physiological stimuli. SATB1 expression is induced in higher grades of cancer and it reprograms gene expression to induce breast tumor growth and augments the tumor metastasis. SATB1 directly upregulate metastatic-associated genes while downreulating tumor-suppressor genes in cancer cells by modulating the epigenetic modifications (Shukla *et al*., 2013). Since the expression of STAB1 is high in thymocytes, most of the *in vivo* function of SATB1 is correlated with T cell development and differentiation. Deficiency of SATB1 results in temporal and spatial mis-expression of numerous genes and arrested T cell development (Alvarez *et al*., 2000). Reports also suggest that SATB1 is important for the regulation of MHC I (Kumar *et al*., 2007) and IL2 locus (Kumar *et al*., 2005). During Th2 cell differentiation, expression of SATB1 is enhanced and it forms a transcriptionally permissive chromatin structure at various cytokine loci (Cai *et al*., 2006). Numerous loops of chromatin is folded on to SATB1 which serves as the basement for the recruitment of transcription factors like GATA-3, STAT6 and c-Maf, Brg1 and RNApolII forms a complex. In differentiating Th2 cells, SATB1 recruits activation signaling proteins like ß-catenin and p300 acetyl transferase on GATA-3 promoter, thus favouring the Th2 differentiation both directly and through GATA-3 (Notani *et al*., 2010). Recent report suggests that repression of SATB1 is critical for the phenotype and function of regulatory T cells. T_{reg} lineage transcription factor, FoxP3 can directly bind and repress the SATB1 locus through the synergistic effect of FoxP3 controlled induction of microRNAs. Defective control of SATB1 by FoxP3 renders loss of suppressive function and induction of effector T cell cytokines (Beyer *et al*., 2011). Thus, SATB1 plays pivotal role in the modulation of global chromatin remodelling for maintaining T cell differentiation.

6.4.4 B Cell Regulator of Immunoglobulin Heavy Chain Transcription (Bright)

Bright is a B-lymphocyte specific protein important for the immunoglobulin gene transcription in antigen activated B cell (Rajaiya *et al*., 2006). It binds to DNA through S/MAR region and interacts with other nuclear matrix proteins Sp100 and LYSp100B in the PML nuclear bodies. The competitive interaction of Bright and Sp100 protein at the Em enhancer of IgH locus dictates the transcription of rearranged immunoglobulin genes. Bright affects chromatin configuration and nuclear sublocalization of IgH locus and gene activation (Kim and Tucker, 2006).

6.4.5 Growth Factor Independent 1 (Gfi-1)

(Gfi-1) is a transcriptional repressor specific to Th2 cell lineage (Zweidler-Mckay *et al*., 1996, Zhu *et al*., 2002). It can be induced by IL4 signaling and inhibit the differentiation of naïve T cells to either Th1 (Placek *et al*., 2009), Th17 or T_{regs} (Chalmin *et al*., 2012). Ablation of Gfi-1 in Th2 cells causes the upregulation of T-bet, RORc, IL23r and CD103with concomitant trimethylation of histone 3 lysin 4 of the loci. Binding of Gfi-1 recruits lysine-specific demethylase 1 to the target regions. Gfi-1 also mediates positive epigenetic changes in the IL4 locus as its deficiency caused severe impairment in the IL4 locus activation. Furthermore, in Gfi-1 deficient Th2 cells, IFNγ locus showed striking activation and enhanced ubiquitinylation of GATA-3 (Zhu *et al*., 2002). Thus Gfi-1 is stabilizing the GATA-3 under Th2 cell differentiation.

6.4.6 Switch/Sucrose Non-Fermentable (SWI/SNF)

SWI/SNF was the first ATP-dependent chromatin-remodelling complex to be described. This protein family includes large chromatin remodelling complexes that move or ejects nucleosomes, providing proper nucleosome positioning and density at genes and other loci (Tang *et al*., 2010). SWI/SNF complex of proteins are involved in diverse functions inside the cell like elongation, double strand break repair, nucleotide excision repair, signaling, proliferation, differentiation, splicing, tumor

suppression, development, etc. (Euskirchen *et al.*, 2011). BRG-1, a subunit of SWI/SNF complex is important for the regulation of HIV-1 LTR and transcription (Henderson *et al.*, 2004). SWI/SNF complex is very much essential in T cell development. BRG-1 deficiency blocks the DN4-DP transition through the disruption of Wnt signaling and pre-TCR pathway (Wurster and Pazin, 2008). Deletion of BRG-1 complex also blocks the B cell transition from pre-pro-B to early pro-B cell due to failures in the expression of B lineage-specific genes such as Ebf1 and IL7ra (Choi *et al.*, 2012). T cell activation induces SWI/SNF complex and it rapidly interacts with chromatin and regulate gene expression in T cells. SWI/SNF complex is also known to induce the expression of AP1 by direct recruitment to its promoter (Jeong *et al.*, 2010). T cell receptor signaling induces histone modifications and recruitment of BRG-1 to the IL12Rβ2 regulatory regions and associated with reduced gene transcription of the locus. BRG1 can also bind to a distal site of GAT-3 locus and activate the region in a STAT6 dependent manner. Thus, BRG1 is required for Th2 differentiation and Th2 cytokine transcription (De *et al.*, 2011). Mice with constitutive expression of SWI/SNF complex in T cells were susceptible to EAE diseases (Jeong *et al.*, 2010). This finding unravels the possible role of SWI/SNF chromatin remodelling complexes in Treg/Th17 axis of cell differentiation.

6.4.7 CCAAT Displacement Protein (CDP/Cux)

CDP/Cuxis an atypical homeodomain protein, functions as transcription repressor of lymphoid and myeloid genes like IgH, TCRβ and γ chains and CD8 (Fei *et al.*, 2007). Deletion of cux/CDP caused reduced thymic cellularity and enhanced apoptosis, with drastic reduction of CD4$^+$ CD8$^+$ thymocytes (Sinclair *et al.*, 2001). B cell lymphopoiesis was also perturbed with block in pro-pre-B to pre-BII stage (Wang *et al.*, 1999). These mice also demonstrated myeloid hyperplasia with enhanced TNF levels. CDP/cux can interact with a histone lysine methyltransferase, G9a through *cut* sequence and co localizes in the nucleus mediating the epigenetic changes of the target gene loci (Nishio *et al.*, 2004).

6.4.8 High Mobility Group (HMG) Proteins

HMG is a group nuclear protein that helps in transcription, replication, recombination, DNA repair etc (Reeves, 2010). A member of HMG protein, hLEF/TCF-1α is a lymphoid cell-specific HMG protein upregulated by Notch, that activates the distal enhancer of the gene encoding the α-subunit of the T-cell receptor and have critical roles in normal thymocyte development (Staal and Clevers, 2000). TCF deficient mice show developmental block at the DN to DP transition, but spontaneously generated thymic lymphoma/leukemia at later stages (Yu *et al.*, 2012). Overexpression of TCF1 in the bone marrow progenitor cells skews the development of T cell lineages in the absence of its inducing Notch1 signaling. These TCF1 induced cells also showed enhanced expression of T cell specific transcription factors like GATA-3, Bcl11b, components of T cell receptor etc. suggesting TCF1 induces the T cell lineage downstream of Notch1 signaling. During T cell differentiation, TCF1 was shown to activate the expression of GATA-3 and promote IL4 producing Th2 cells independent of STAT6 signaling. TCF1 simultaneously blocks the Th1 fate by negatively regulating IFNγ expression independently of β catenin (Yu *et al.*, 2010). TCF1 maintains the Th2 cells also by downregulating the Th17 differentiation. It regulates IL17 expression by binding to the regulatory regions of the IL17 gene locus and TCF1 deficient mice were hyper responsive to EAE (Yu *et al.*, 2011).

6.4.9 Methyl CpG Binding Protein 2 (MeCP2)/ ARBP

MeCP2 is an important family of nuclear matrix protein by the presence of methyl CpG binding domain (Amir *et al.*, 1999). They can repress the transcription of genes from methylated promoters and recruits DNA methyl transferase DNMT1 (Kimura and Shiota, 2003). MeCP2 is a ubiquitous protein particularly expressed in high levels in neuron of the post natal brain suggesting a crucial role in brain development. Mutations in MECP2 gene cause a progressive neurologic development disorder called Rett syndrome, which is one of the most common

cause of mental retardation in females. MeCP2 may act as a transcriptional repressor and activator context dependent manner, but its mode of action is by binding to the methylated DNA. Once bound, MeCP2 will condense the chromatin structure and form complexes with HDACs and blocks transcription. It has also been shown as a transcription activator by the recruitment of transcription factor CREB1 (Gadalla *et al*., 2011). Genetic polymorphism of MECP2 gene is related to the lupus T cell formation and adversely affects lymphocyte growth (Koelsch *et al*., 2013).

6.4.10 DNA Dependent Protein Kinase (DNAPK) and Ku Proteins

DNA PK is a serine/threonine protein kinase enzyme encoded by PRKDC gene and is belonged to the phosphatidylinositol 3-kinase-related kinase protein family (Ma *et al*., 2013). DNA PK is the second component of autoimmune antigen Ku protein. The catalytic subunit of DNA PK (DNAPKcs) is inactive and necessitates Ku proteins for its recruitment to the DNA ends as well as for its activation. DNAPKcs is required for the non-homologous end joining (NHEJ) pathway of double strand DNA repair and also in V(D) J recombination (Oksenych *et al*., 2013). Knockout mice of DNA PKcs have severe combined immunodeficiency due to defect in V(D)J recombination and behaves similarly as RAG mutant mice. Thus, DNA PKs have crucial role in T cell development. Related with DNA PK, Ku proteins (Ku 70/80 heterodimer) are also indispensable for the development of T cells by mediating the NHEJ of DNA ends during the gene recombination. The N-terminal region of the DNA-Pkcs is required for the activation of double strand DNA break repair (Davis *et al*., 2013). Recent discovery suggests that, the pathogens communicate with the DNA of the host through its DNA dependent protein kinases to activate several signaling pathways (Watson *et al*., 2012). These findings are opening up the greater role of DNA associated protein kinases in the generation of immune responses.

6.4.11 Poly (ADP-ribose) Polymerase (PARP)

PARP is a family of S/MAR proteins involved in number of cellular processes like DNA repair and programmed cell death. The main role of PARP proteins is to detect single-strand DNA breaks (SSB) and activate the proteins involved in SSB repair. PARP detects the SSB and binds to the DNA, gets activated and starts synthesizing poly (ADP-ribose) chain (PAR) which signals other SSB proteins like DNA ligase III, DNA polymerase beta and scaffolding proteins X-ray cross complementing gene (XRCC1) (Gibson and Kraus, 2012). Recent discovery shows that tumor cells, which are defective of homologous recombination repair pathways, are sensitive to inhibitors of PARP and this has been used to target cancer cell by novel identification of PARP inhibitors for cancer therapy (Javle and Curtin, 2011). YY1 regulate the expression of PARP protein, which in turn can regulate another S/MAR protein CTCF post translationally. Thus, there may be possibility of a feed-back loop for the regulation of nuclear matrix proteins. Inhibition of PARP1 protein causes perturbed DNA repair of SSB and have enhanced apoptosis with accumulation of p53 (Doetsch *et al*., 2012). PARP1 deficiency cause T-cell lymphomagenesis in p53 deleted mice indicating that PARP1 and p53 act concomitantlyto suppress tumorigenesis by maintaining the genomic integrity (Beneke and Moroy, 2001). PARP1 also regulates gene expression of many immune cells like dendritic cells, T cells and macrophages (Aldinucci *et al*., 2007). PARP1 deficiency reduces the secretion of anti-inflammatory cytokines and thus resistant to the induction of septic shock and autoimmune diseases (Pieper *et al*., 1999). PARP1 deficiency also caused increased FoxP3 regulatory T cell formation (Nasta *et al*., 2010). Inhibition of PARP-1 prevents the airway inflammation and eosinophilic recruitment by modulating Th2 cytokine IL5 (Oumouna *et al*., 2006). Thus, PARP-1 may be an essential Th2 factor for the generation of Th2 response in asthma disease progression.

6.4.12 Scaffold Attachment Factor B (SAF-A/B)

(SAF-B) is a protein that has specificity for S/MAR. SAF-B protein is thought to serve as a molecular base to assemble a transcriptosome complex in the vicinity of actively transcribed genes (Hong *et al*., 2012).

Being a scaffold binding protein, SAF-B can couple transcription and pre-mRNA splicing to the S/MAR elements (Nayler *et al*., 1998). Though some research work reveals its role in the regulation of heat shock proteins, not much work has been carried out in understanding the molecular mechanism of SAF protein function in relation to T cell differentiation.

6.4.13 Nucleolin

Nucleolin is a eukaryotic necleolar phosphoprotein involved in the synthesis and maturation of ribosomes. Being a member of S/MAR binding protein, it has multiple functions including viral interactions at the cellular membrane, processing and transport of the ribosomal RNA to the nucleolus. It also functions as histone chaperone and a chromatin remodeler (Mongelard and Bouvet, 2007). Nucleolin was shown to be anti-apoptotic through the stabilization of Bcl2 mRNA. Addition of recombinant nucleolin to extracts of normal B cells showed reduced bcl2 mRNA decay and overexpression is correlated with chronic lymphocytic leukemia (Otake *et al*., 2007). In T cell activation, Nucleolin was found to be an important shuttle protein between the cytoplasmic tail of CD3e (Gil *et al*., 2001) and by stabilizing the IL2 mRNA through a JNK mediated mechanism (Chen *et al*., 2000) suggesting its critical role in T cell activation.

6.4. 14 Heterochromatin Protein 1 (HP1)

HP1belongs to group of conserved adaptor proteins having multifaceted functions inside the nucleus. HP1 proteins are one of the fundamental units of heterochromatin packaging enriched in centromeres and telomeres. HP1 can form strong complexes with HP1-interacting histone methyltransferase (HMTase) SUV39H1/Clr4 (Bannister *et al*., 2001) through chromo domain and mediate the repressive/ condensation epigenetic mark, methylation of histone H3 at Lys 9 (K9). HP1 can also facilitate the gene silencing by its interaction with DNA methyltransferase 1 (DNMT1) (Smallwood *et al*., 2007). Recent finding show that HP1 is involved in telomere capping and its metabolism in mammals (Canudas *et al*., 2011). Another studyexplored the role the SUV39H1-H3K9me3-HP1 silencing

pathway in the control of Th2 lineage stability. SUV39H1-H3K9me3-HP1 alpha pathway helps in silencing the Th1 locus and maintains the Th2 cells and inhibition by small chemical compounds or loss of SUV39H1 drives T cell responses towards Th1 responses associated withreduced lung pathology in response to allergens (Allan *et al*., 2012).

6.4.15 Scaffold/Matrix Attachment Region Binding Protein 1 (SMAR1)

SMAR1 is a MAR binding protein identified in mouse thymocytes, where it play important role in the regulation of V(D)J gene recombination by binding to a proximal MAR region to the Eb enhancer (Chattopadhyay *et al*., 2000). SMAR1 transgenic mice showed aberrant expression of peripheral Vbs in the T cell repertoire (Kaul-Ghanekar *et al*., 2005). SMAR1 functions as chromatin modifying protein by binding to other chromatin remodelor proteins like mSin3A, HDAC1 etc (Rampalli *et al*., 2005). It plays an important role in major stress responses including DNA damage. Many research into the functionality of SMAR1 revealed that it functions as a tumor supressor and an anti-inflammatory molecule (Singh *et al*., 2007). SMAR1 transcriptionaly regulates IκB and thus control the NFκB pathway (Singh *et al*., 2009). SMAR1 can modulate the chromatin architecture by orchestrating intrachromosomal interactions (Sinha *et al*., 2010) and chromatin looping (Sreenath *et al*., 2010). A wider view of SMAR1 function reveals its role in mRNA splicing, glucose metabolism and in many signaling cascades (β catenin) (*unpublished*).

Conclusion

Many nuclear matrix associated proteins are involved in T cell differentiation and growing information unravels the molecular interactions by which these proteins mediate various differentiation programmes. Thus, this group of proteins may function as novel factors streamlining the chromatin changes relative to gene positioning necessary for T cell differentiation. The functional importance of these proteins at the chromatin level may possibly dictate the physiological fate of T cell function during many disease conditions. Aberrant expressions of many of

nuclear matrix associated proteins were reported in cancerous and other disease conditions. Novel research findings also suggest the importance of cell intrinsic chromatin modulation specific to inflammatory or anti-inflammatory response of T cell with respect to the expression of transcription factors and nuclear matrix associated proteins. Emerging research of T cell plasticity and the co-existence of T cell lineage transcription factors in various infections may be better explained by the involvement of nuclear matrix associated proteins, which works at the chromatin level and have substantial role in mediating epigenetic changes. Thus, further investigations are necessary in identifying novel members of this family of proteins which may help in immunomodulatory therapeutic interventions.

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