University of Crete

Department of Biology

MSc Program in Molecular Biology and Biomedicine

Master Thesis

The role of SATB1 in long-range chromosomal interactions in murine CD4+ T cells

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I hereby declare that this thesis is my own work. Where other sources of information have been used, they have been acknowledged

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Abstract

Upon antigen encounter, naive $CD4^+$ T cells can differentiate into two major and functionally distinct subpopulations; T helper 1 (Th1) and T helper 2 (Th2). Several epigenetic phenomena regulate the differentiation of naive CD4 cells into the Th1 and Th2 cell lineages. Special AT-rich binding protein 1 (SATB1) plays a crucial role in the establishment and promotion of the epigenetic changes. SATB1 organizes chromatin architecture by tethering distant genomic loci into its network facilitating their coordinated regulation.

In thymocyte nuclei SATB1 forms a cage as it is excluded from heterochromatin regions. Here, we provide evidence for the *in vivo* binding of murine SATB1 in gene loci involved in $CD4^+$ cell lineage commitment. These cytokine gene loci interchromosomally interact in the nucleus of naive CD4 cells and this interaction poises chromatin for transcription. Moreover, by performing rapid amplification of cDNA ends (RACE) experiments, we have identified the different SATB1 transcripts which are expressed in peripheral $CD4^+$ cells and we show their distinct expression, in terms of their mRNA levels, during differentiation. Rabbit polyclonal antisera against a unique SATB1 transcript carrying an extra exon, revealed its protein expression in different $CD4^+$ cell populations.

Our findings provide insight into the role of SATB1 in transcriptional regulation during $CD4^+$ T cell differentiation and support the hypothesis for its crucial role in the establishment of epigenetic mechanisms that regulate the $CD4^+$ T cell fate**.**

Introduction

T cell development and the maturation of effector T cells is a crucial process in acquired immunity. It begins early in embryogenesis and it occurs in the propitious microenvironment of the thymus. After several stages of selection, T cells mature and they are ready to forsake the thymus. During this developmental process the vast majority of T cells die and only 3% of them survive and migrate to peripheral lymphoid organs.

One class of effector T cells is CD4+ T cells, which exert a prominent role in the orchestration of the immune responses. Naive CD4+ T cells, upon activation of T cell Receptor (TCR) can differentiate into one of the following categories of effector cells; **Th1, Th2, Th17 and Treg** depending on the signals they receive during the differentiation process. The above mentioned cell fates, are distinct in their functions, cytokine production and the transcription factors involved in the determination of cell fate. In this assignment we dealt only with Th1 and Th2 fates.

Th1 cells produce IFN-γ as their signature cytokine as well as TNF-β and IL-2 and are responsible of cell-mediated immune responses.**[1]** Th1 cells are also involved in autoimmune responses. On the other side, Th2 cells produce Interleukin 4 (IL4), IL5 and IL13 and are responsible for the immune responses against extracellular parasites, by boosting antibody production from B cells.

Th1, Th2 polarization depends not only in cytokine signaling but on transcription factors that govern the process as well. Each T helper lineage is characterized by the presence of specific transcription factors which act as master regulators who coordinate the expression of cytokine genes.

Members of the STAT family, exert an important role in the signaling cascade during the T helper differentiation. Signaling through the Notch receptor also contributes to the cell fate determination.

T-bet transcription factor is the “so called” master regulator of Th1 cell fate and is up-regulated during Th1 differentiation. Activated STAT-1, enhances the expression of T-bet, which in turn activates the IFNγ expression. STAT-4 elevates activation at the IFNγ locus. [2]. One of the genes encoded by T-bet is Runx3, which in collaboration with T-bet binds to IFNγ promoter and several other enhancers as well. [3] The sequence of those events, generate a positive feedback loop for expression of genes involved in the commitment towards Th1 lineage. In addition T-bet blocks the expression of GATA-3 in Th1 cells.

Cells lacking T-bet, suffer severe defects in Th1 differentiation and they are more likely to develop asthma-like diseases. [4]

In the presence of IL4 naive cells are directed to follow Th2 lineage commitment. GATA-3 is the master regulator transcription factor of the Th2 differentiation. It was the first transcription factor identified to be necessary for the transcription of all Th2 cytokine genes. [5] Activation of STAT-6 as a response to IL-4 stimuli results the induction of GATA-3, which subsequently binds to the promoter of IL-5 and IL-13 genes and is responsible for the reorganization of the IL-4 locus. [6] GATA-3 also constraints potential signaling to Th1 differentiation, by inhibiting the expression of STAT-4.[3]

The following image summarizes the major events of the T helper differentiation.

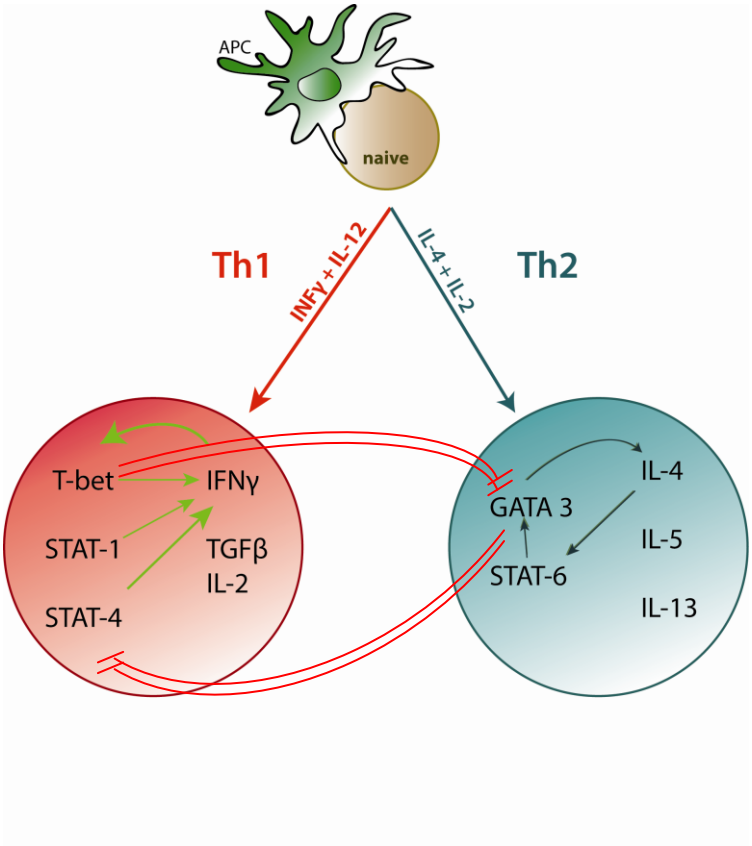

Figure 1. The major events during T helper cell differentiation. T-bet and GATA-3 are the master regulators of Th1 and Th2 commitment respectively.After stimulation of their T cell receptor (TCR), naive cells follow one four possible fates depending on the stiuli they receive. In the presence of IL-12 the *IFNg* gene is upregulated through STAT-4. T-bet also promotes the expression of IFNg and at the same time blocks the expression of Gata3 to inhibit signalling to Th2 differentiation. On the other hand, exogenous addition of IL-2 uppon TCR activation of naive cells promotes the Th2 differentiation pathway. GATA-3 and IL-4 are expressed in low levels. Signalling via the IL4 receptor further enhances the expression of those two genes. Direct inibition of *Stat4* gene via GATA-3 prevents differentiation towards Th1 commitmnet. Curved arrows indicate the feedback loops during cytokine signalling.

As mentioned above, Notch signalling appart from its significant role in the development of the Central Nervous System, is also involved in the T cell development. Direct evidence link different Notch ligands with the commitment to each one of the T helper lineages. Another perspective is that Notch sigalling does not influence the lineage commitment , but as an alternative, orchestrates the expansion of differentiated effector cells. [7]

Appart from the signalling pathways, chromatin structure is an important player of the transition towards Th1, Th2 lineages and undergoes drastic changes within this developmental context.

For istance, chromatin in naive cells is largely condensed. Under this circumstance, DNA is not accessible to transcription factors and this is the major cause for keeping naive cells in a stem state.[8]

Appart from their small size due to chromatin condensity, quiesence is another key feature of naive cells. Upon stimulation, chromatin goes through rapid decondension which makes it accessible for tanscription, which now exits the quiesence state and starts to proliferate.

Condensation of chromatin itself, is not the only determinant of trancriptional activity or repress. Inter and intachromosomal interactions and a series of regulatory elements such as; *enhancers, Locus control Regions {LCR}, Conserved non Coding Sequences {CNS}, Hypersensitive sites* combined with epigenetic changes, have been reported to regulate the transcriptional status in each stage of T helper cell differentiation.

Among the epigenetic changes, the differential methylation, acetylation status of different cell types is included. In the cytokine promoters for example, histones are hypo-methylated in naive cells. A condition that rapidly changes upon stimulation.

Epigenetic changes on Th2 locus, is a representative example of such changes during cell differentiation. Th2 locus encompasses the cytokine genes involved in T helper 2 differentiation; Interleukin-4 (IL-4), IL-5, IL-13, in which -*Rad50*- a gene encoding for a DNA repair protein is interjected. At the 3' end of *Rad50* a Locus Control Region (LCR) has been identified. LCR seems to control the expression of the Th2 locus genes. Under Th2 differentiation conditions, specific sites on the entire Th2 locus and especially inside the LCR become sensitive to DNaseI treatment. This observation opposes to the status of naive or Th1 cells, where these sites are not identified. [4] Furthermore, Spilianakis & Flavell reported the interaction of all the cytokine gene promoters of the Th2 locus in effector T cells and highlighted the role of LCR in this phenomenon. [9]

Moreover, the spatial organization of chromatin inside the nucleus is not incidentally arranged. During the differentiation procudure, radical changes in gene expression take place, comprising sets of genes that are activated and other sets of genes that are repressed. Large chromosomal loops, contributes to the co-regulation of those groups. [10]

Long range chromosomal interactions have been reported in Th1 cells, at theIFNγ locus and are mediated through cohesin.[11] Extensive research on chromosomal interactions in Th1 cells brought into light that the monoallelic interaction of the IFNγ gene, with the Interferon gamma receptor 1, is mediated by CTCF. As the DNA between IFNγ and IFNγR1 genes is looped out, the two genes are placed nearby in the 3D organization of the nucleus. [12]

Similarly to Th1, chromosomal interactions have been reported in Th2 cells where chromatin at the Th2 cytokine locus is forced to form a unique structure, comprising of many loops between distal and proximal regulatory elements. Interestingly the frequency which LCR participates in these intrachromosomal interactions is particularly high. Cai & Shigematsu reported that intrachromosomal interactions are mediated through SATB1. In non activated CD4 T cells, some basal level of chromosomal interaction is detected, which is basiclly based on two distal SATB1 binding sites, spotted on the opposed edges of the 120kb Th2 locus. Once, cells are stimulated to follow Th2 commitment, the expression level of SATB1 is elevated together with the frequency of intrachromosomal interactions inside the Th2 locus. [14]

SATB1, binds on specific sequences on DNA, the so called Matrix Attachment Regions (MARs). SATB1 selectively recognizes and binds on AT rich sequences. These sequences due to the less hydrogen bonds connecting the two helices, have unwinding properties and are also known as Base Unpaired Regions (BURs). Mutations that eliminate the unwinding properties of those sequences, have an impact on the ability of SATB1 to bind on BURs. [13] It has previously been reported that genomic sequences that contain BURs contribute to tissue specific expression of genes. [15] Uppon binding on DNA, SATB1 organizes chromatin architecture by tethering distant genomic loci into its network facilitating their coordinated regulation. In the nucleus of thymocytes SATB1 has a unique ***cage-like*** pattern distribution. This unique pattern is maintained in early stages of Th2 differentiation, but abolishes at terminal Th2 differentiation where SATB1 becomes diffused inside the nucleus.

SATB1 -/- mice have a remarkably small size and die three weeks post natal, because they fail to develop a functional immune system. T cell development is blocked at the Double Positive stage (CD4+ CD8+). Dysregulation of gene expression is also observed in the molecular level. [23] Those observations underline the significant role of SATB1 to regulate gene expression during development.

The significant role of SATB1 in the Th2 lineage commitment, has been recently reported by Natani et al. Researchers, described the interaction between SATB1 and β-catenin as a crucial step for the regulation of Th2 cytokines expression uppon Wnt signalling. [22]

Satb1 gene, is located on mouse chromosome 17. Five transcripts of the murine Satb1 have been so far identified. Four of them give rise to the exact same protein and they only differ at the 5' Untranslated Region. Whereas the fifth transcript carries an extra exon and leads to the production of a protein which is 32 amino-acids longer.

The following image shows the different Satb1 mRNA trancripts.

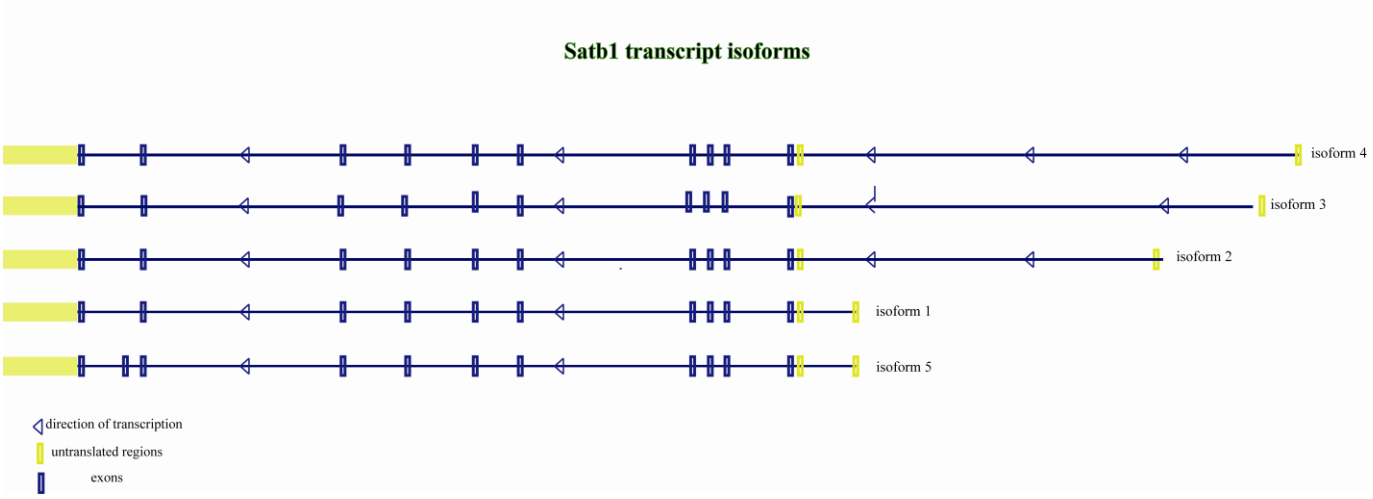

Figure 2: Schematic representation of Satb1 transcripts as visualized in ECR browser.

As the role of SATB1 in the archtectural organization of the chromatin is under extensive research, we wanted to assess its role in the organization of the nucleous in murine Thymocytes and naive CD4+ T cells. Moreover, we wanted to evaluated the potential role of the different *Satb1* transcripts, in the developmental context naive CD4+ differentiation into Th1 or Th2 lineages.

Experimental Procedures

Animals

C57BL/6 mice were used for thymi spleen and peripheral lymph nodes isolation

Isolation on naïve CD4+ T cells

Spleens and peripheral lympm nodes were collected from 4-6 week old mice. MACS LS Columns and L3T4 microbeads (miltenyi Biotech) were used for positive selection of naïve CD4+ T cells, according to manufacturers instructions.

Differentiation of CD4+ T cells

After positive selection of CD4+ T cells, 3000 cells were seeded in a 12-well plate, and cultured in Click's Medium (Sigma-Aldrich) supplemented with 10%FBS, 00,5mM β-mercaptoethanol, 100µg/mL Pen/Strep, 2mM Glutamine and 25mM Hepes pH 7.2. Mouse anti-CD3 and anti-CD28 (R&D Systems) were used for TCR stimulation. For Th1 conditions, cels were treated with 20 units/ml IL-2, 10µg/ml anti IL-4 and 10µg/ml IL-12. (R&D Systems). For Th2 conditions celles were treated with 20 units/ml IL-2, 10µg/ml anti-IFNγ and 1000 units/ml IL-4. (R&D Systems)

RNA isolation cDNA synthesis and qPCR.

Total RNA from naive, Th1 and Th2 cells at the time points of 24h 48h 72, 72+R was isolated using TRIzol reagent (Invitrogen). cDNA synthesis was carried out using M-Mulv reverse trancriptase (New England Biolabs) and using oligo dt primer. The qPCR for target genes were performed using a DNA engine – Opticon 2 real time cycler (MJ Reserch).

Primers for the detection of Satb1 transcripts.

Isoform 1: FW: 5' TCAAGAATCCCGGCTGCAAAGG 3' REV: 5' AATCTTGGCGGGTGGACCCTTC 3', Isoform 2: FW 5' ACTTCGCACTGTCACCCCATCA 3' REV: 5' AATCTTGGCGGGTGGACCCTTC 3', Isoform 5 (long) FW: 5' TTCTTACCAAGCCTGCTGACC 3' , REV: 5' TGGTCTTCTGCCGGTTTTCC 3'

The following primer pair was used for the detection of the total *Satb1* mRNA: FW: 5' CAGTATTTGCACGCGTGGCTTT 3' REV: 5' AGGCTGCGTTCAAGCTCCTTTC 3'

Chromatin Immunoprecipitation

$50x10^6$ thymocytes or naive CD4+ T cells were used for chromatin preparation. Cells were crosslinked for 10 minutes at room temperature in Formaldehyde buffer (11% Formaldehyde, 100mM NaCl, 1mM EDTA, 0.5mM EGTA, 50mM Hepes pH: 8.0) in a final formaldehyde concentration 1,1%. Glycine in a final concentration of 0.125M was added to the solution and cells were incubated in the presence of Glycine for 5 minutes at room temperature and 5 minutes on ice. Subsequently cells were centrifuged at 1400rpm for 5 minutes at 4°C and were lysed in 500μl Lysis Buffer (4% SDS, 50mM Tris pH: 8.0, 100mM NaCl, 1mM EDTA, 0.5mM EGTA) and then subjected to a 5-8 M Urea gradient ultracentrifugation at 30000rpm for 16hours (15°C). Cross-linked chromatin was collected and washed several times with TE Buffer and subsequently sonicated in 800μl Sonication Buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl pH: 7.5) using an ultrasonic homogenizer (LABSONIC ®). 20μg of SCS were incubated with 5μg anti-SATB1 or anti-IgG (Santa Cruz Biotechnology) overnight at 4°C with rotation. The next day 25μl of pre-cleared magnetic beads (novex-life technologies) were added to each sample and incubated 1hour at 4°C with rotation. Complexes on beads were successively washed with Buffer A (20mM Tris-HCl pH 8.1, 150mM NaCl, 1mM EDTA, 1% Triton-X 100, 0.1% SDS, 0.1% Na-Deoxycholate, 0.5mM PMSF, 1x protase inhibitors) Buffer B ((20mM Tris-HCl pH 8.1, 500mM NaCl, 1mM EDTA, 1% Triton-X 100, 0.1% SDS, 0.1% Na-Deoxycholate, 0.5mM PMSF, 1x protase inhibitors) and Buffer C (20mM Tris-HCl pH 8.0, 250mmLiCl, 1mM EDTA, 0.5% NP-40, 0.5% Na-Deoxycholate, 0.5mM PMSF, 1x protase inhibitors) respectively. Precipitated chromatin was resuspended in 100μl TE + 1% SDS. Samples were incubated at 55°C for 3 hours in the presence of Proteinase K and then at 65°C overnight to reverse crosslink chromatin. The next day samples were treated for 30 minutes with 100μg RNAse A (Qiagen) at 37° C, before they subjected to phenol-chloroform extraction and ethanol precipitation with glycogen (30μg).

Primers used for the detection of SATB1 binding sites:

On **Th2 locus**: SBS-1: FW: 5' CTAGCACATAACAACAGGGACT 3' , REV: 5' CTGGGTAACAGGTGTCAATTAG 3' , SBS-2: FW: 5' ACCCATGTATCAAGTTTGGCAC 3' , REV: 5' TCAAGCCATCTTGCTGACTAGT 3' ,

SBS-3: FW: 5' GATCCTCAAAGTCCCTAACTCA 3' , REV: 5' AGAACCACTGAGGTAGCGCTTG 3' , SBS-4: FW: 5' CTCCAGGCTTACTGTTTCAGCA 3' REV: 5' GCTGCCTCTGTACAATCATTCC 3' , SBS-5: FW: 5' CCTCTCATGTTCTGTCTCCCCT 3' , REV: 5' ATTAACTGCTGAGAAGAGAAGAGG 3'

On **TNFa locus**: SBS-6: FW: 5' GCTGTGATGAATCAGGGACAGA 3' , REV: 5' GAAAAGAAGCTGCAGCTGAAAG 3' , SBS-7: FW: 5' CACAACTCACACACACACACAC 3' REV: 5' AGATCTGAGTCAAAGTGAAGGA 3'

On **IFNγ locus**: SBS-8: 5' CCTTCAAACCCTGAAATACCT 3' , REV: 5' TGGGTTTGTAACACAGTAAGC 3' , SBS-9: FW: 5' AGGCCAGAAACATTTCAAGATT 3' , REV: 5' GCAAATCATAAACTCTTTCCG 3'

Immunofluorescence staining

$3x10^5$ cells were seeded in Poly-L- Lysine coated coverslips and fixed for 10 minutes at room temperature, in freshly prepared 2% Para-Formaldehyde in 1x PBS and then washed 3 times with 1x PBS (5 minutes per wash). Cell membranes were permeabilized on ice using 0.5% Triton X-100 pH 7-7.2. Samples were washed 3 times with 1x PBS at room temperature before blocking with 1% BSA –Fraction V (Gibco). We incubated cells with primary antibody, anti-SATB1 or anti-IgG overnight at 4°C. (Rabbit polyclonal anti-SATB1 against GKGESRGVFLPSLLTPAPWPHAA peptide, specific for the long SATB1 protein isoform). The next day cells were incubated with secondary antibody in a dark humid chamber for 1 hour at room temperature. Cells were again washed 3 times with 1x PBS before they were counterstained for 90 seconds with Topro3 (1:8000) and finally coverslips were mounted on a slide with DAPI (Invitrogen).

Images were collected using an Observer Z1 Apotome microscope (ZEISS). Optical sections of 0.3µm were taken using a 100x lens. Collected images were processed using Volocity 6.2.1 Software (Perkin Elmer).

Results and Discussion

Differential expression pattern of Satb1 transcripts.

As, transcription from alternative promoters has been associated with differential signaling stimulus, or tissue specific gene expression, [16] we examined the expression profile of *Satb1* transcript isoforms during T helper cell differentiation and after restimulation of Th1, Th2 cells. We were able to detect the expression of only two of the *Satb1* transcripts.

When we activated naive CD4+ cells and differentiated them into Th1 and Th2 lineages we noticed a differential expression pattern. Isoform 1 is expressed in a higher level in naive cells comparing to Isoform 2 and its expression gradually increases during Th1 differentiation, until it reaches the maximum level of expression after 3 days of differentiation followed by re-stimulation of the TCR, while it is expressed in a basal level during Th2 differentiation.

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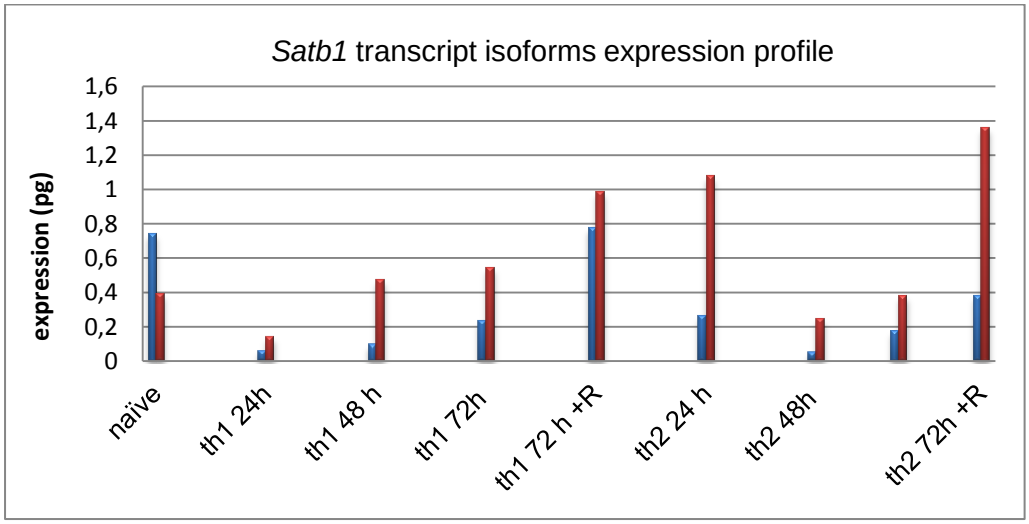
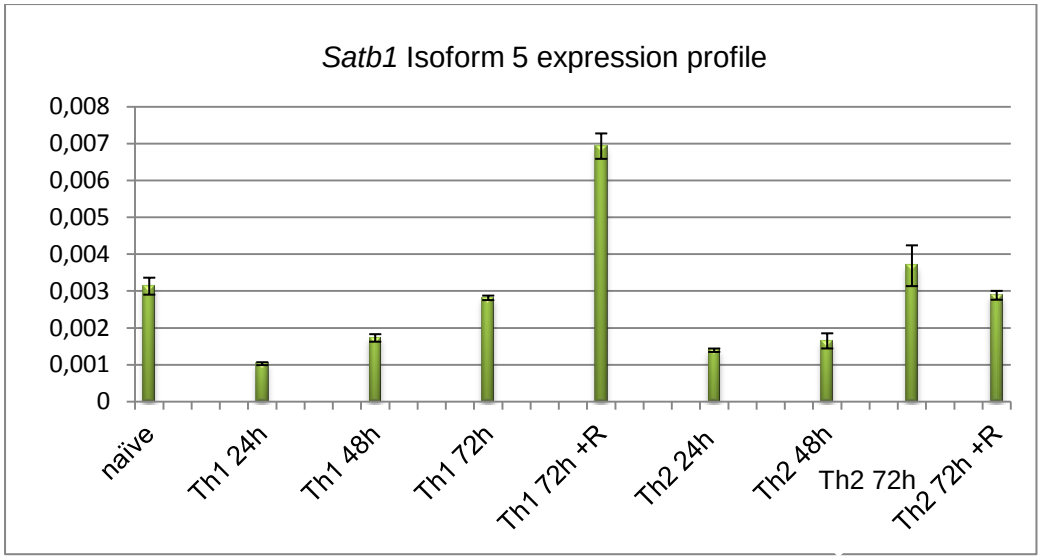


Figure 3: (a) Expression profile of the different *Satb1* transcripts at the time points of 24, 48, 72, 72+R hours after differentiation. Naive CD4+ T cells were cultured under Th1 or Th2 differentiation conditions for the time points indicated. Total mRNA was collected from each time point and the expression level of each transcript was detected by qPCR, targeting unique sequence of each transcript at the 5'. (b) same as in (a) - Expression profile of the Satb1 transcript, carrying the extra exon at the same time points.



When we tested the expression profile of the Satb1 transcript carrying the extra exon, we noticed that it follows the expression pattern of Isoform 1, raising the possibility that these two transcript isoforms are transcribed from the same promoter.

SATB1 long protein isoform contributes to ‘cage-like’ network formation

We next wanted to investigate the nuclear localization of the long SATB1 [E9PVB7] protein isoform (never reviewed before). A rabbit polyclonal antibody against GKGESRGVFLPSLLTPAPWPHAA peptide, specific for the long SATB1 protein isoform was used for immunofluorescence studies.

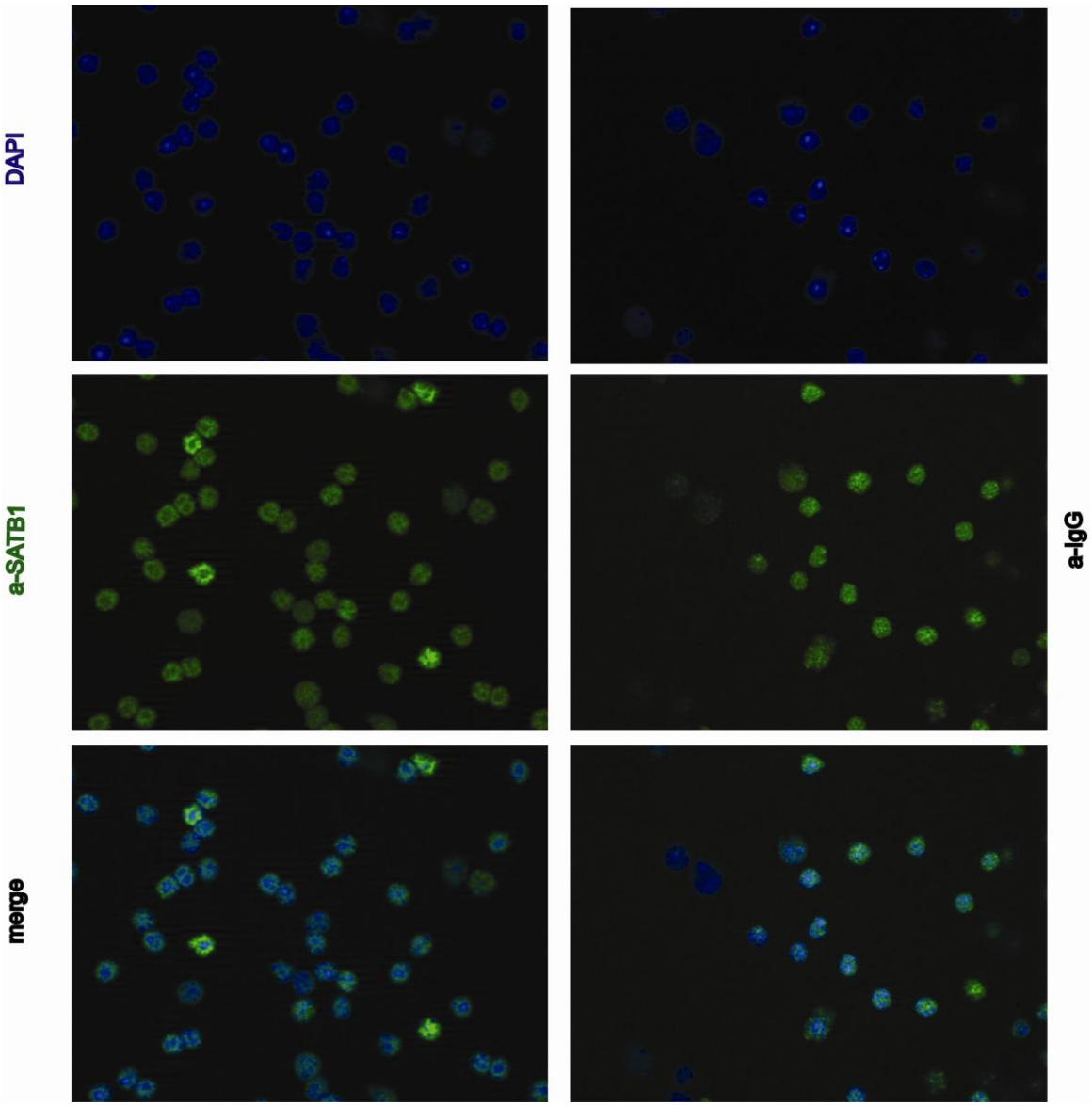


Figure 4: Immunoflorescence staining of murine thymocytes against E9PVB7 SATB1 variant.

Immunostaining with anti-SATB1 in thymocytes nuclei revealed that E9PVB7 variant is also abundantly expressed in thymocytes, is excluded from heterochromatin and contributes to the formation of the cage-like structure. (Fig 4). When we tested the expression of E9PVB7 in Raw 264.7 cell line we did not detect any signal as expected. (data not shown)

The role of SATB1 in long range chromosomal interactions

We next wanted to evaluate the role of SATB1 in the establishment of long range chromosomal interactions between genomic loci involved in T helper cell differentiation. Previous ImmunoDNA FISH experiments from our group, revealed that Th2, IFNγ and TNFa loci separately interact with SATB1 in a great percentage. For instance, 81% of Th2 loci tested in ImmunoDNA FISH were found to interact with SATB1. This observation, together with the fact that these loci have previously been reported reported to interchromosomally interact [10], suggest the possibility that these interactions are mediated directly or indirectly through SATB1. Because SATB1 binds to Matrix Attechment Regions on DNA, we used Biocomputing approaches, to predict MAR sites on Th2, IFNγ and TNFa loci. We then performed Chromatin Immunoprecipitation experiments followed by quantitative PCR for the predicted MARs to confirm SATB1 binding.

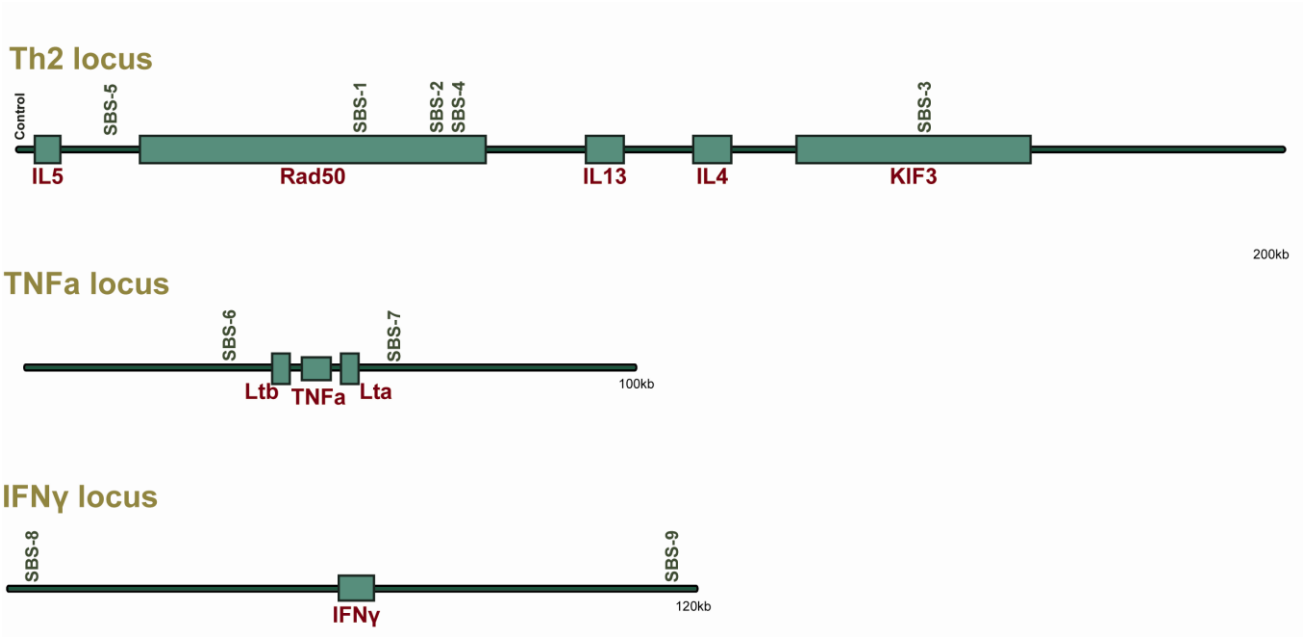


Figure 5: Schematic representation of SATB1 binding sites confirmed by ChIP experiments on Th2, TNFa and IFNγ locus respectively.

Indeed we were able to confirm the *in vivo* SATB1 binding on 9 sites. Five of them are located on Th2 locus. We also identified two binding sites on TNFa and IFNγ locus (Fig. 5). SATB1 binding site 4 (SBS-4) corresponds to Rad50 Hypersensitive site 6. RHS6 is a 151 bp sequence showing great conservation among species and is located inside the Th2 LCR region (at the 3' end of the *Rad50* gene). As previously described, LCR controls the cytokine expression during T helper differentiation not only by regulating chromatin folding[14] but also acting as a benchmark for epigenetic modifications. [17]. Using mouse models deficient for RHS6, Flavell and colleagues highlighted *in vivo* the role of RHS6 in Th2 cytokine expression, as mice in which RHS6 was truncated were facing a vigorous inability to produce the Th2 cytokines. [18]

Confirmation of SATB1 binding on RHS6 provides the first evidence that this binding promotes the active state of chromatin, as it goes in line with a peak in acetylation status of the same region in Th2 cells.

SATB1 binds *in vivo* on loci involved in T helper cell differentiation

It has been reported that SATB1 binds on Th2 locus and forces chromatin for looping formation, facilitating coordinated gene regulation. Here we provide evidence for the *in vivo* binding of SATB1 in Th2 locus. SATB1 binds to numerous sites on Th2 locus. We noticed a strong binding of SATB1 on SBS-4 (RHS6). (Fig. 6) We have also confirmed SATB1 binding on RHS6 in a DNA binding affinity assay, coupled with Mass Spectrometry. (Data not shown)

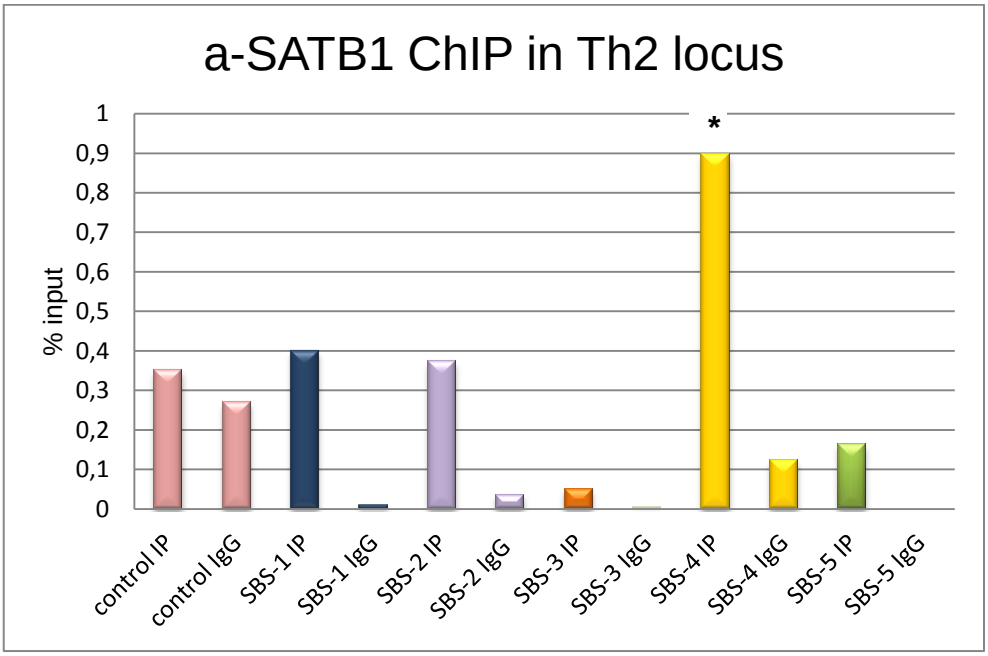


Figure 6: Chromatin Immunoprecipitation in thymocytes nuclei followed by qPCR targeting the in silico predicted MAR sites on Th2 locus for the confirmation of SATB1 binding.

Data Analysis showed that SATB1 is phosphorylated at Serine 638 when binds to DNA. Mass Spectrometric analysis revealed two more potential phosphorylation sites at serine 634 ande Serine 635. We next asked whether these phosphorylation sites can affect the nuclear localization of SATB1. We generated constructs where the *Satb1* gene carrying a point mutation at position 638 S638A, S634A, S635A respectively was in frame with a GFP tag. We overexpressed the mutated SATB1 in HEK293 cells and monitored its subcellular localization. Apart from the observation that mutated SATB1 tends to form speckles inside the nucleus, we did not detect any significant defects in the ability of mutated SATB1 to enter the nucleus. (data not shown).

These findings not only confirm SATB1 binding on RHS6 but also highlight the importance of this specific region of the LCR as well as the role of SATB1 in the establishment of the epigenetic phenomena, which occur in the thymocytes nuclei. SBS-1 has also been reported by Cai et al, as a strong binding site for SATB1. [14] As we have previously found RHS6 to interact with IFNγ locus (Spilianakis CG unpublished data) we queried for MARs/SBS on IFNγ locus. Indeed we were able to identify two binding sites for SATB1 on the two opposite ends of the 120 kb IFNγ locus. (Fig. 5 and Fig. 7a)

a

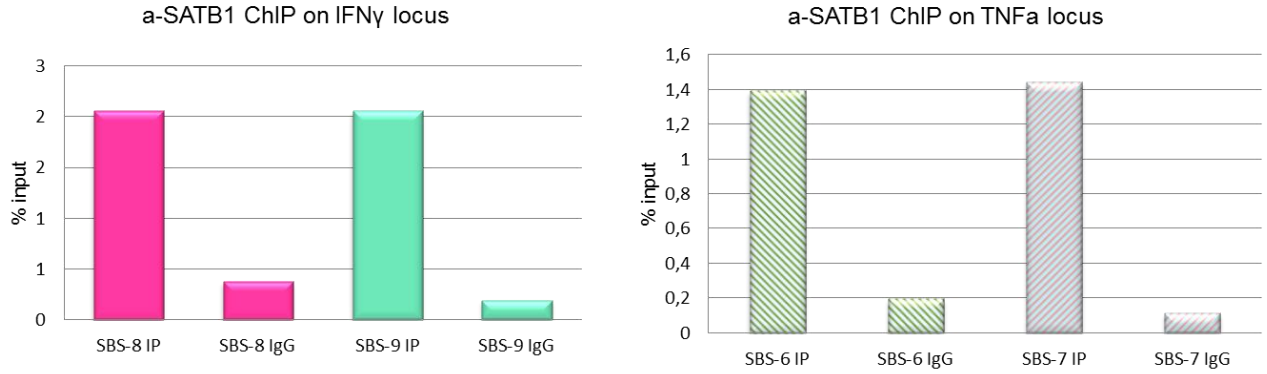


Figure 7: Chromatin Immunoprecipitation in thymocytes nuclei followed by qPCR targeting the in silico predicted MAR sites on (a) IFNγ locus and (b) on TNFa locus for the confirmation of SATB1 binding.

We observed a strong binding of SATB1 on SBS-8, SBS-9. This comes in agreement with the fact that these two sites were predicted in silico as potential MARs and consequently as possible SATB1 binding sites with a high score, as long as with the fact that IFNγ locus on mouse chromosome 10 is in contact with the RHS6 of the Th2 locus on mouse chromosome 11 in the nucleus of naive cells. We also detect a strong binding of SATB1 in the promoter region of Lta and Ltb on the 100 kb TNFa locus.

Having obtained a basic knowledge on the SATB1 binding profile on thymocytes, we next wanted to investigate the same question in naive cells. Preliminary data do not show any strong binding of SATB1 on the MAR sites tested in thymocytes. In fact, we observed a signal close to background levels in all the SBS tested. (data not shown). Partially this could be explained by the lower expression of SATB1 in naive cells in contrast to thymocytes.

Our data provide evidence for the differential expression of the 5 *Satb1* transcripts in terms of their alternative promoters. Taking into account that Satb1 overexpression in non-aggressive breast cancer cell lines results the transition into aggressive phenotypes, [21] it is likely that this discrepancy in Satb1 transcripts expression, might be correlated with the initiation of tumorigenesis. Following to the above, further Immunohistochemistry studies will provide more evidence on whether SATB1 protein variants have unique patterns in their expression in different tissues.

By combing computational and biochemical methods we were able to identify 9 SBS on Th2, IFNγ and TNFa locus. Those findings are in line with the theory that SATB1 plays a role of great importance during the T helper cell differentiation, in terms of 3D chromatin structure. SATB1 binds frequently on Th2, IFNγ and TNFa locus respectively. It coordinates the looping formation of DNA, bringing distal regulatory elements in close proximity. It is likely that SATB1 orchestrates chromatin in T cells in a similar way it organizes the spatial architecture of MHC I cluster genes for synchronized regulation. [20] As we confirmed SATB1 binding on previously reported interchromosomal interacting loci in thymocytes nuclei, our data also support the hypothesis that interchromosomal interactions observed in naive cells are formed in early developmental stage of T cell differentiation persist to the stage of naive CD4+ cells and weaken in favor of intrachromosomal interactions upon Th1 or Th2 commitment.

SATB1 is predominantly expressed in thymocytes, forming a cage-like pattern, is expressed in lower levels in naive cells and is again rapidly induced upon Th2 differentiation. Correspondingly, in a similar model, recent studies reported a slightly lower expression of SATB1 in Hematopoietic Stem Cells (HSC) which maintains their quiescence and keeps them in a non-differentiated state. Upon differentiation of HSC towards lymphoid lineages, SATB1 is rapidly induced. [19] A possible model based on the above observation, could be that SATB1 possibly through an interplay with yet uncharacterized factors, acts as a rheostat controlling the initiation process of Th2 differentiation.

Referances

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