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Journal of Cellular Biochemistry 114:2569–2576 (2013)

Journal of Cellular Biochemistry

Breakpoint Regions of *ETO* Gene Involved in (8; 21) Leukemic Translocations Are Enriched in Acetylated Histone H3

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ABSTRACT

One of the most frequent chromosomal translocation found in patients with acute myeloid leukemia (AML) is the t(8;21). This translocation involves the *RUNX1* and *ETO* genes. The breakpoints regions for t(8;21) are located at intron 5 and intron 1 of the *RUNX1* and *ETO* gene respectively. To date, no homologous sequences have been found in these regions to explain their recombination. The breakpoint regions of *RUNX1* gene are characterized by the presence of DNasaI hypersensitive sites and topoisomerase II cleavage sites, but no information exists about complementary regions of *ETO* gene. Here, we report analysis of chromatin structure of *ETO* breakpoint regions. Chromatin immunoprecipitation (ChIP) were performed with antibodies specific to acetylated histone H3, H4, and total histone H1. Nucleosomal distribution at the *ETO* locus was evaluated by determining total levels of histone H3. Our data show that in myeloid cells, the breakpoint regions at the *ETO* gene are enriched in hyperacetylated histone H3 compared to a control region of similar size where no translocations have been described. Moreover, acetylated H4 associates with both the whole *ETO* breakpoint regions as well as the control intron. Interestingly, we observed no H1 association either at the breakpoint regions or the control region of the *ETO* gene. Our data indicate that a common chromatin structure enriched in acetylated histones is present in breakpoint regions involved in formation of (8;21) leukemic translocation. J. Cell. Biochem. 114: 2569–2576, 2013.

KEY WORDS: ETO GENE; CHROMOSOMAL TRANSLOCATION; CHROMATIN ORGANIZATION; HISTONE ACETYLATION

Acute myeloid leukemia (AML) is a heterogeneous bone marrow malignancy and patients with the cytogenetic abnormality of (8;21) translocation represent majority of the cases. This translocation involves the *RUNX1* (*AML1*) and *ETO* (*MTG8*) genes located on chromosome 21 and 8, respectively. *RUNX1* gene encodes a transcription factor essential for definitive hematopoiesis. Homozygous mice null for *Runx1* gene dies midgestation due to complete failure of hematopoiesis [1;2]. *ETO* gene, on the other hand, encodes a protein of as yet unknown function; which associates with N-CoR/Sin3a/HDAC complexes in vivo and acts as a corepressor for the promyelocytic zinc finger protein. Moreover, ETO protein is associated with nuclear matrix at sites also occupied by histone deacetylase enzymes and mSin3a. These data suggest that ETO protein functions as transcriptional corepressor [Davis et al., 2003].

Conflicts of interest: None.

Author's contributions: MS participates in the conception and design of the study, carried out molecular studies and the analysis and interpretation of data. SN contributes to the analysis and interpretation of data. AJ and SEG conceived of the study, and participated in its design and coordination and wrote the manuscript. All authors read and approved the final manuscript.

Grant sponsor: Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT); Grant numbers: AT24091060, 1100670.

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Manuscript Received: 21 May 2013; Manuscript Accepted: 23 May 2013

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 6 June 2013

DOI 10.1002/jcb.24605 • © 2013 Wiley Periodicals, Inc.

The breakpoints regions for (8;21) translocation are located in intron 5 of *RUNX1* gene and intron 1 of *ETO* gene [4]. Interestingly, no homologous sequences are found between the breakpoint regions of the *RUNX1* and *ETO* genes. However, these breakpoint regions co-localize with chromatin structural elements like topoisomerase II cleavage sites and DNAse I hypersensitive sites [Zhang et al., 2002] suggesting that chromatin organization has a role in the formation of leukemia-associated chromosomal translocations.

The basic structure of chromatin is the nucleosome, which consists of an octamer of four core histones (H2A, H2B, H3, and H4) around which 147 base pairs of DNA are wrapped. A fifth histone, H1, binds to this core particle and facilitates the formation of higher-order chromatin structure [Luger et al., 1997, 2012]. Through various mechanisms, the organized chromatin structure is made accessible for readout by the complex machinery involved in gene transcription, DNA replication and DNA repair [Liu et al., 2012; Chiruvella et al., 2013; Patel and Wang, 2013; Serrano et al., 2013]. Among them we find post-translational modifications of nucleosomal core histones, which include acetylation, methylation, and phosphorylation. These modifications regulate the access to DNA and thus influence all the processes ranging from DNA replication to gene transcription. The best-characterized modification corresponds to histone acetylation, mainly in histones H3 and H4. In general, histone acetylation is related with chromatin decondensation and DNA accessibility. In fact, histone acetylation has been associated with presence of DNAse I hypersensitive and topoisomerase cutting sites [Marchion et al., 2005; Catalano et al., 2006; Fang et al., 2009; Kim and Kim, 2013].

Previous work from our lab have shown that pattern of histone acetylation at the *RUNX1* intron 5 is very different compared to a control intron of the same gene in which no translocation has been found [Stuardo et al., 2009]. This pattern was characterized by several regions with high-levels of histone H3 and H4 acetylation, while in the control intron we identified very few regions enriched in H3 acetylation. Furthermore, the breakpoint regions of the *RUNX1* gene were devoid of histone H1 [Stuardo et al., 2009]. These results suggest that histone acetylation and H1 absence may be common theme in breakpoint regions involved in formation of chromosomal translocations. In this report, we analyzed histone acetylation and H1 presence in the breakpoint regions and a similar length control intron of *ETO* gene. Our results indicate that acetylation of histone H3 is a common denominator in breakpoint regions of both *RUNX1* and *ETO* genes.

MATERIALS AND METHODS

CELL CULTURES

The hematopoietic cell line HL-60; which express RUNX1 protein and have no reported abnormalities in the *RUNX1* locus, was cultured in RPMI media supplemented with 10% fetal bovine serum. Cell cultures were routinely maintained in T-75 flasks and incubated in humidified chamber at 37°C with 5% carbon dioxide (CO_2). Cells were maintained at $\leq 1 \times 10^6$ cells/ml and seeded at 1×10^5 cells/ml after each passage.

CHROMATIN IMMUNOPRECIPITATION ASSAYS

Chromatin immunoprecipitation (ChIP) assay was performed as described elsewhere [Soutoglou and Talianidis, 2002] with some modifications. Cells were treated with 1% (v/v) formaldehyde at 37°C for 10 min. Crosslinking reaction was stopped by adding glycine to a final concentration of 0.125 M. Cells were then washed with ice cold PBS, and collected by centrifugation at 165*g* for 5 min at 4°C. The cell pellet was resuspended in lysis buffer (25 mM HEPES pH 7.8, 1.5 mM MgCl2, 10 mM KCl, 0.1% (v/v) NP-40, 1X Cømplete, 25 mM MG-132) and incubated on ice for 10 min. Following dounce homogenization (20 strokes, pestle A), the nuclei were collected by centrifugation at 750*g* for 5 min, resuspended in sonication buffer (50 mM HEPES pH 7.9, 140 mM NaCl, 1 mM EDTA, 1%(v/v) Triton X-100, 0.1% (v/v) Na-deoxycholate, 0.1% (v/v) SDS, 1XCømplete, 25 mM MG32) and sonicated on ice to a DNA size of 200–800 bp. The samples were centrifuged at 16,000*g* for 15 min and precleared with A/G plus-agarose beads precoated with 2 mg/ml sonicated salmon sperm DNA, and 1 mg/ml BSA.

Precleared chromatin (30 A260 units) was immunoprecipitated with 5 mg of antibody and the immunocomplexes were collected by binding to A/G plus-agarose beads. The beads were washed twice with each of the following buffers: sonication buffer, sonication buffer containing 500 mM NaCl, LiCl buffer (20 mM Tris pH 8, 1 mM EDTA, 250 mM LiCl, 0.5%(v/v) NP-40, 0.5%(v/v) Na-deoxycholate), and 10 mM Tris pH 8. The immunocomplexes were eluted in 50 mM Tris pH 8, 1 mM EDTA and 1%(v/v) SDS at 65°C for 15 min, adjusted to 200 mM NaCl, and incubated overnight at 65°C to reverse the crosslinking. The anti-acetylated H3 (catalog number 06–599), and anti-acetylated H4 (catalog number 06–866) antibodies used for the immunoprecipitations were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). The anti-acetylated histone H3 antibody recognizes acetylated lysine 9 and 14 while the anti-acetylated histone H4 antibody recognizes acetylated lysine 5, 8, 12, and 16. The anti-H1 antibody (SC 10806) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

DNA PURIFICATION AND ANALYSIS

After reversing the crosslink, the immunoprecipitated chromatin was treated with RNase One (1 U/ml) and Proteinase K (20 mg/ml) at 37°C for 4 h. DNA was then purified by phenol: chloroform (1:1 v/v), and chloroform:isoamyl alcohol (24:1 v/v) extraction followed by ethanol precipitation. DNA pellet was washed with 70% ethanol and resuspended in 10 mM Tris pH 8. One-tenth of the immunoprecipitated DNA and input DNA were analyzed by real-time PCR (Light cycler, Roche Applied Science) using primers designed every 1 kilobase as indicated in Figure 1 for intron 1 and control region of *ETO* gene. The sequences and size of primers and PCR products are described in Tables I and II, respectively.

RESULTS

CHROMATIN OF ETO BREAKPOINT REGIONS ARE COMPOSED OF HIGHLY ACETYLATED HISTONE H3

The hyperacetylation of histone H3 and H4 is associated with an open and relaxed chromatin structure. We have previously reported an enrichment of acetylated histone H3 and H4 in the breakpoint regions of the *RUNX1* gene involved in t(8;21) compared to similar size region not involved in translocations [Stuardo et al., 2009]. To determine if histone acetylation represent a common structural factor among breakpoint regions of the (8;21) translocation partner genes, we

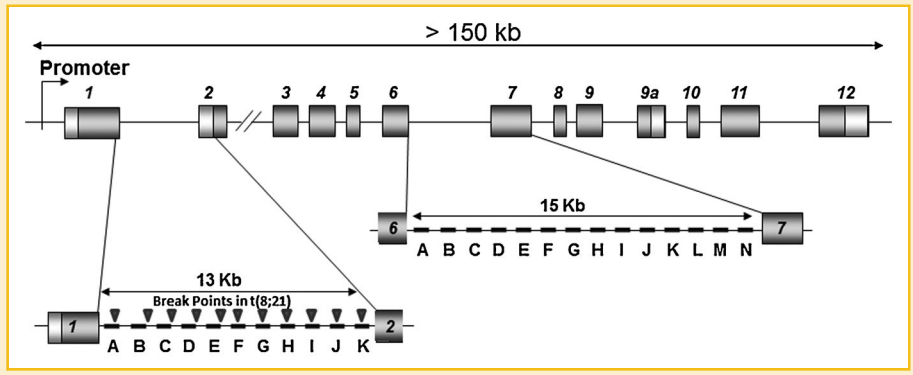

Fig. 1. Breakpoints involved in formation of (8;21) translocation are spread throughout intron 1 of the ETO gene. Top panel shows a diagrammatic representation of the exon–intron distribution in the ETO locus. Boxes in light gray represent untranslated regions of indicated exons. Bottom panel correspond to a magnification of intron 1 and intron 6 control region respectively. Arrowheads indicate breakpoints within intron 1 that are reported for (8;21) translocations. No chromosomal translocations have been reported for the 15 kb intron 6. Regions tested for histone modifications and nucleosomal distribution by ChIP analysis are shown as black blocks (A–K for intron 1 and A–N for intron 6).

analyzed *ETO* chromatin organization of intron 1 (Fig. 1). Acetylation pattern of H3 and H4 were determined by chromatin immunoprecipitation and analyzed by quantitative real time PCR with oligos specific to intron 1 of the *ETO* gene (Table I). Our results reveal that intron 1 of *ETO* gene has a significant enrichment of acetylated histone H3 and H4 (Fig. 2). Interestingly, while acetylated histone H4 is present throughout the whole *ETO* breakpoint regions, acetylated histone H3 is enriched only in discrete areas of *ETO* gene. To out rule random modifications of histones in the nucleosomes covering large 13 kb regions, we compared histone hyperacetylation pattern with a region of the *ETO* gene in which no breaks have been found (Fig. 1). For this, intron 6 of the *ETO* gene was analyzed by ChIP using antibodies against acetylated histones H3 and H4 (Fig. 3, Table II). Surprisingly, only two regions were found enriched in acetylated histone H3 in intron 6, suggesting differential chromatin remodeling of *ETO* regions involved in leukemic translocation. We confirmed that lack of H3 acetylation is not due to experimental error or a particular lack of histone association to this DNA region as a robust acetylation of H4 was noted in intron 6 of the *ETO* gene (Fig. 3).

Taken together these results demonstrate different chromatin organization of *ETO* gene regions involved in (8;21) translocation.

REGIONS OF *ETO* GENE INVOLVED IN LEUKEMIC TRANSLOCATION ARE DEVOID OF NUCLEOSOMES

To better understand if the difference in acetylation patterns noted at breakpoint regions of *ETO* gene is related to nucleosomal

TABLE I. Sequences of Primers Sense (S) and Antisense (AS) Designed for Chromatin Immunoprecipitation Analysis of Intron 1 of *ETO* Gene

Intron 1	D	Sequence 5′-3′	Product size (pb)
A	S	AGAGGGGGAGCGAAATACTC	230
	AS	GATTAAGGCTTCCCCAAAGG	
B	S	GAATTCGTGGCTGCAGTGAG	230
	AS	CCAATGGATTCCAGGTGTT	
C	S	CAGCATGCCAGTTGTTTTCA	257
	AS	TGTGTGTGCAAAGTCACCAA	
D	S	TTGCGGAAGTTGATATGCAG	250
	AS	AACCCTCTTCCACACAATGC	
E	S	AGCTGGCATCATCGTGATCTAT	233
	AS	CCTGCCAAGAGTTTGTTGGT	
F	S	CACCACCCACAATTGCATTA	239
	AS	GCCTTCTGTAGACATGCAGCTT	
G	S	AGGGAAAGTTCATTGGAGCA	278
	AS	GCATTTCCCATAAAACAATTCC	
H	S	AGGTGAAGGCTAGGGGTTGT	271
	AS	GGCTGTCCAAGAGAGCCTAA	
I	S	CGTTGGGTGATCATTGAGTG	259
	AS	CAGAGCAGATGGGGTAGGAA	
J	S	GAATGTTTGAGCTTGGTGCTT	225
	AS	CCCAAGAAGGGCTGCTTTAT	
K	S	CCTGGCATTGTTGTGTGTTT	215
	AS	ATACCCAGCCAGGTTCAATG	

Sequences are presented in 5′–3′ orientation and the length of the amplified product is indicated.

TABLE II. Sequences of Primers Sense (S) and Antisense (AS) Designed for Intron 6 of *ETO* Gene (Control Region)

Intron 6	D	Secuence 5′-3′	Product size (pb)
A	S	TGATGGTTTGGAAGCTTGTCT	254
	AS	GGGTTTAATGGAGAAGTGGTTTT	
B	S	TGTTCCCCTTGACATAGAACC	269
	AS	ATTGTTGAGCCACCTCTTGG	
C	S	TGTTCCCCTTGACATAGAACC	288
	AS	TCAGACCATATGCACCAACAA	
D	S	ATTGTTGAGCCACCTCTTGG	214
	AS	TCCATTCTGCAGTCTCAAAGAA	
E	S	TATAGGCATGAGCCACCACA	268
	AS	GGGCCTTAGTTTGTGCAAGA	
F	S	AGACGGGGTTTCACCATATT	220
	AS	AGCAGCAAGAAATCAGGCTA	
G	S	GCCACAATTTGAAGCCATCT	237
	AS	TGGTCCCTCAACCTTCCATA	
H	S	ACGCTGGGGAATTTGTAATG	216
	AS	CGTGCAGTTTGTTAAGATTGC	
I	S	CGCTTCTGCCATGTAGATTT	219
	AS	CGAGACTCCATCTCAAGGAA	
J	S	ACACTGAAAAGGGCAATGAA	202
	AS	TGAAGGATCTCTCTGGTGCT	
K	S	CCCTAGCCTGCCTTTTAGGA	230
	AS	GGTCAAACCACCCCAACTTA	
L	S	TTCCAGCTGATTTATGCTGA	278
	AS	CTAATGCTGCTCCCTTCAAA	
M	S	GTCACACGGCTGAGTCCTAA	215
	AS	ACCATCCTTTATGGCCCTTT	
N	S	CAAGCGTACCTGTTGAGCAC	256
	AS	CCCTAAGACCGACTGTATTTGA	

Sequences are presented in 5′–3′ orientation and the length of the amplified product is indicated.

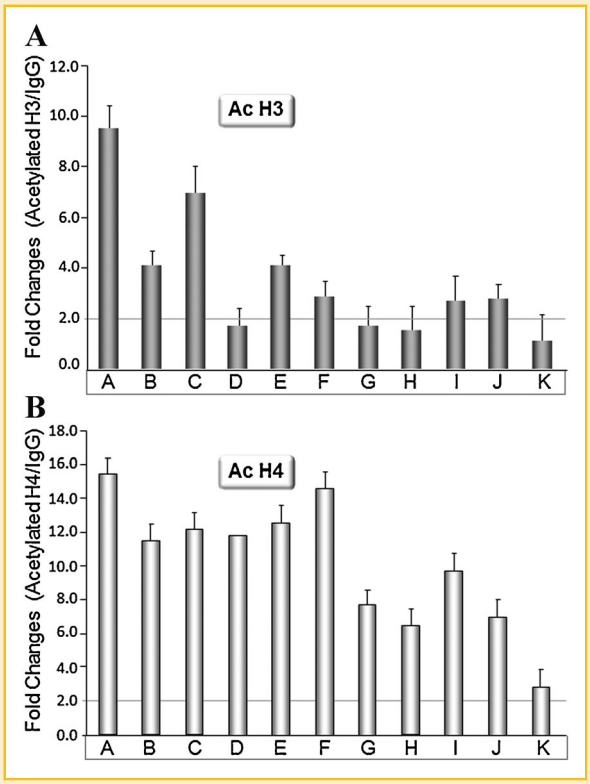

Fig. 2. Chromatin of the breakpoint regions in *ETO* gene is enriched in acetylated histones H3 and H4. ChIP assays were performed in HL-60 promyeloid cells with either anti-acetylated H3 (A) or anti-acetylated H4 (B) antibodies. Immunoprecipitated DNA was quantified as percent with respect to input and plotted as fold over non-specific IgG for each region analyzed. Pooled data from five independent experiments with standard error are shown. Values above the indicated lines represent statistically significant acetylated histone association. AcH3: Acetylated histone H3; AcH4: Acetylated histone H4.

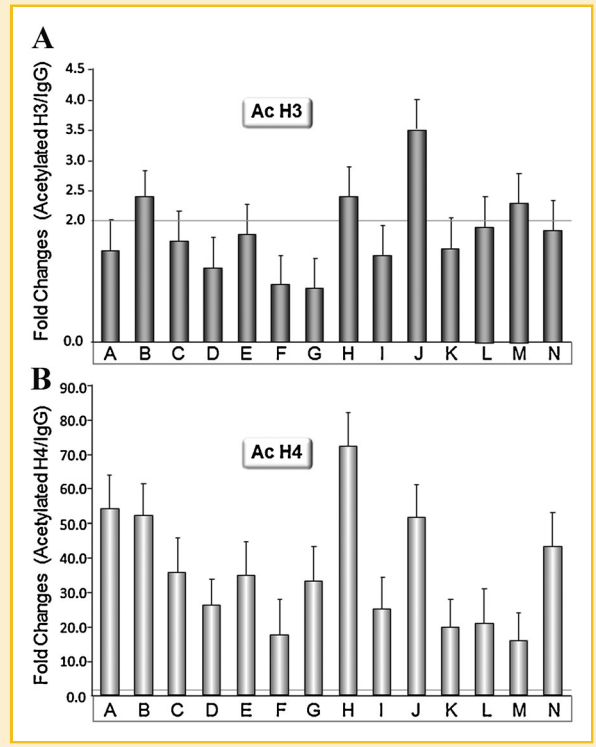

Fig. 3. Intron 6 of the ETO gene is preferentially enriched in acetylated histone H4. ChIP assays were performed in HL-60 promyeloid cells with either anti-acetylated H3 (A) or anti-acetylated H4 (B) antibodies. Immunoprecipitated DNA was quantified as percent with respect to input and plotted as fold over non-specific IgG for each region analyzed. Pooled data for five independent experiments with standard error are shown. Values above the indicated lines represent statistically significant acetylated histone association. AcH3: Acetylated histone H3; AcH4: Acetylated histone H4.

content and density, we analyzed the nucleosome distribution at the breakpoint and control regions of the *ETO* gene. To this end, we performed ChIP assays using an anti H3 C-terminal domain antibody.

Our results show a significant difference in nucleosome occupancy of the *ETO* breakpoint regions. Total of eleven different regions that encompass intron 1 were mostly devoid of nucleosome presence (Fig. 4A). Low-levels of ChIP were consistently noted with C-terminal H3 antibody. In sharp contrast, the similar size control region (intron 6) exhibited high levels of ChIP with C-terminal H3 antibody indicating high density of nucleosome association (Fig. 4B).

The differential nucleosomal pattern exhibited by introns 1 and 6 prompted us to re-analyze the association of acetylated histones in breakpoint region and control intron of ETO gene. To this end, we determined the relationship between nucleosome density and histone H3 or H4 acetylation. These ratios were calculated as acetylated histone over histone H3 content (Fig. 5).

Our findings confirm that *ETO* breakpoint regions are highly enriched in both acetylated histones H3 and H4 while the control intron is only associated with acetylated histone H4. Interestingly, when corrected by nucleosome occupancy, the pattern of acetylated histone H3 associated with *ETO* breakpoint regions are maintained with the exception of region B (compare Figs. 2 and 5). In contrast, the two region (H and J) that where found enriched in acetylated histone H3 in the intron 6 of the *ETO* gene when corrected by nucleosomal occupancy do no exhibit enrichment in this modified histone (compare Figs. 3 and 5).

Taken together these results demonstrate that breakpoint regions of *ETO* gene exhibit a substantially different chromatin organization than a control region of similar size that does not undergo leukemic translocations.

NUCLEOSOME DENSITY AT THE BRC OF THE ETO GENE IS NOT LINKED WITH PRESENCE OF HISTONE H1

Histone H1 is related with higher order chromatin structure and compaction. Specifically this histone protein facilitates inter-nucleosomes interactions and formation of higher order chromatin structures, thus preventing accessibility to the DNA [Bednar et al., 1998; Happel and Doenecke, 2009]. Due to its role in DNA compaction, we investigated the presence of histone H1 in control and breakpoint regions of the *ETO* gene. Our results show a lack of

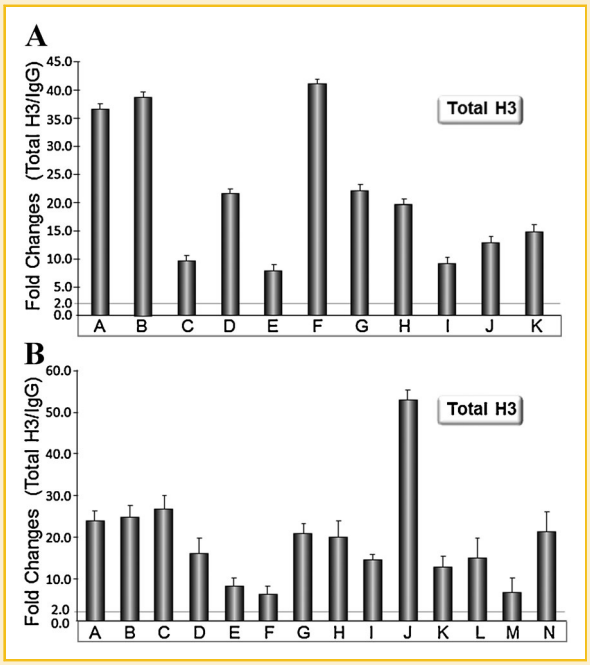

Fig. 4. Breakpoint regions of *ETO* gene involved in (8;21) translocation have low density of nucleosome. Pro-myeloid cells were used for chromatin immune precipitation assays. Antibody specific for C-terminal domain of histone H3 was used. Immuno precipitated DNA was quantified for presence of intron 1 (A) and intron 6 (B) regions of the ETO gene. ChIP DNA is presented as percent of input and plotted as fold over non-specific IgG of indicated regions. Pooled data for five independent experiments with standard error are shown. Values above the indicated lines represent statistically significant acetylated histone association.

histone H1 association with *ETO* breakpoint region. However, similar absence of histone H1 was also observed in the control regions of the *ETO* gene (Fig. 6).

Taken together, these results suggest that depletion of histone H1 is not a structural factor exclusive of chromosomal breakpoint regions, at least for genes involved in (8;21) translocation.

DISCUSSION

Chromosomal translocations are formed by wrong joining of double strand DNA breaks most likely by the activity of the non-homologous end joining DNA repair system. It has become clear that dynamic changes in chromatin structure play a key role in regulating genome functions, including DNA replication, DNA repair and gene transcription [Liu et al., 2012; Chiruvella et al., 2013; Patel and Wang, 2013; Serrano et al., 2013]. Genomic regions with highly compact chromatin are enriched in nucleosomes. DNA in such regions is inaccessible to most, if not all proteins. In contrast, dynamic chromatin modifications, such as histone acetylation, or a net loss of nucleosomes from gene-specific regulatory regions, increases chromatin accessibility and binding of different protein complexes to target DNA. Therefore, both chromatin structure and biochemical modifications of histone proteins have emerged as important mechanisms for the regulation of all the processes in which DNA participates.

In this work, we have analyzed histone acetylation in intron 1 of ETO gene, which is involved in (8;21) translocation, and compared it with a control region of similar size, where no breaks or translocations has been described (intron 6 of the ETO gene). Our result show that pattern of both acetylated histone H3 and H4 and in the breakpoint regions of the ETO gene is very different from the one present in intron 6 region of the same gene. This pattern is characterized by predominant acetylation of histone H3 in specific areas of intron 1 of ETO gene. Interestingly, these results are similar to the acetylation pattern observed in the breakpoint regions of *RUNX1*, the *ETO* partner gene involved in (8;21) translocation. In fact, all breaks within *RUNX1* gene are clustered in intron 5, which exhibits high enrichment of H3 acetylation compared to a control region [Stuardo et al., 2009]. We also found low association of histone H1 with intron 1 of the *ETO* gene. However, this structural feature is also noted in intron 6 control region, suggesting that low presence of histone H1 is related with transcriptional status of the gene.

Interestingly, analysis of the nucleosomal organization at the *ETO* breakpoint regions and control regions reveals that nucleosome distribution is vastly different. The control intron 6 exhibits a higher nucleosomal density than breakpoint regions of the *ETO* gene (Fig. 4). It is important to note that when acetylation status is normalized with actual nucleosomal content, we find near absence of acetylated histone H3 in the control intron 6 regions compared to breakpoint regions of the ETO gene. In contrast, we found acetylated histone H4 present in both intron 1 and intron 6. However, breakpoint regions exhibit from 2- to 12fold higher acetylated histone H4 association than the control region (intron 6).

Histone acetylation has been related to chromatin accessibility; mainly in regions of DNA with chromatin structural elements like, DNase I hypersensitive sites and topoisomerase II cleavage sites. These accessible regions are found in transcriptionally active chromatin, such as promoters of actively transcribed genes and active enhancer modules [Yaragatti et al., 2008; Sakabe and Nobrega, 2010; Beaulieu et al., 2011]. Interestingly, the genomic location of DNA double strand breaks (DSBs) generated during developmentally programmed events is directly stimulated by post-translational modifications of histones. The clearest example of this phenomena is the formation of DSBs by the RAG1–RAG2 endonucleases during V(D)J recombination in lymphocytes [Schatz and Ji, 2011]. Several studies have shown that the basic mechanism that promotes V(D)J recombination involves stepwise epigenetic modifications that results in permissive chromatin structure for transcription and gene rearrangement [Maes et al., 2001; Shih et al., 2012; Bevington and Boyes, 2013]. In fact, in regions involved in V(D)J recombination, nucleosome remodeling supports the formation of a DNaseI hypersensitive domain associated with acetylated histones H3 and H4 [Maes et al., 2006; Schatz and Ji, 2011], which is determinant for intiation of V(D)J recombination.

Chromatin organization also plays a critical role in DNA repair processes. Multiple recent reports have highlighted a context specific repair response, wherein the cell senses and evaluates the chromatin environment in which the DNA lesion lies and adjusts

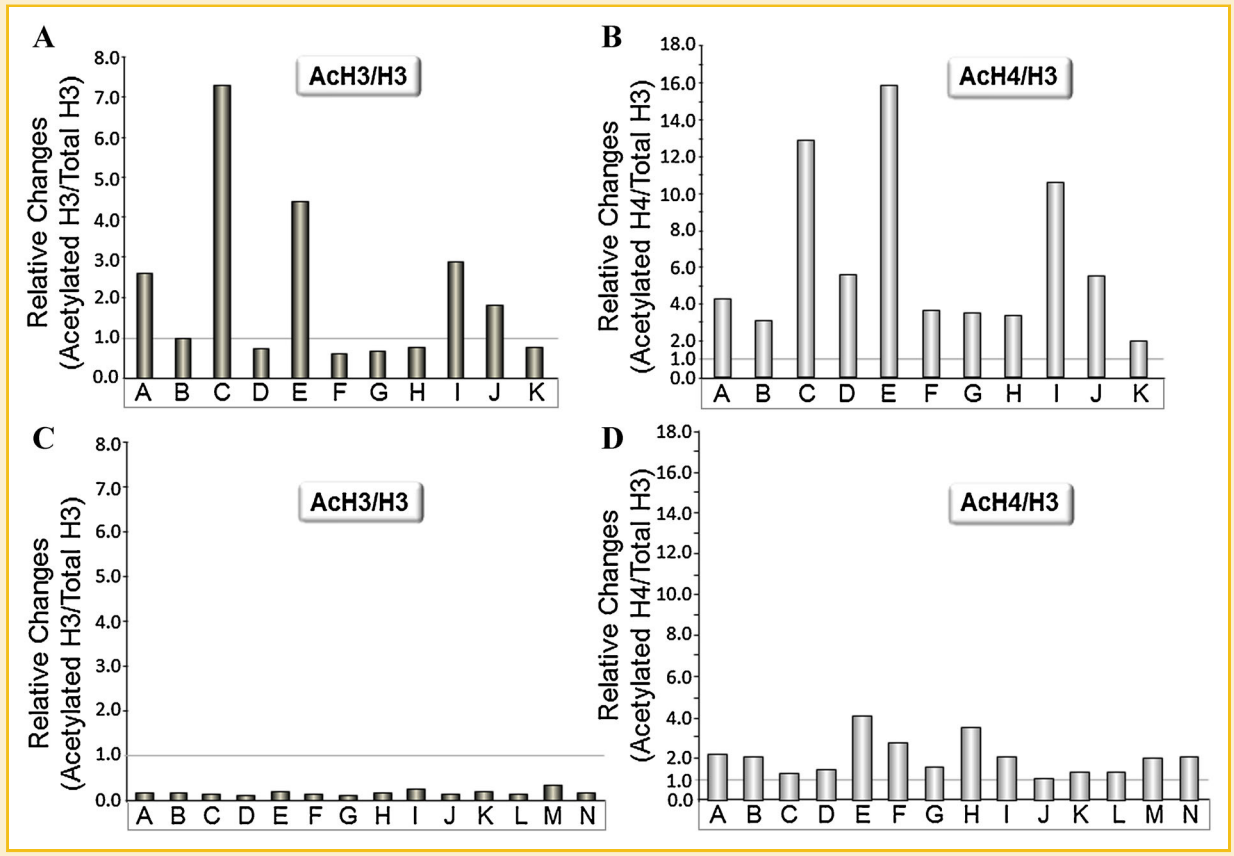

Fig. 5. Breakpoint regions in intron 1 of the *ETO* gene are enriched in nucleosomes with acetylated histone H3. Immunoprecipitated DNA using either anti-Acetylated H3 (A and B) or anti-acetylated H4 (B and D) antibodies were normalized with respective values from total histone H3 content. Results are shown as relative changes in acetylated H3 or acetylated H4 for intron 1 (A and B) and intron 6 (C and D) of the ETO gene.

the molecular response accordingly [Ayoub et al., 2008; Goodarzi et al., 2008]. All DNA repair necessitates rapid recognition of the lesion and coordinated mechanisms for recruitment of the repair machinery in the context of the chromatin template. Histone acetylation is a key modulator of chromatin accessibility. Studies in early eukaryotes showed that mutation in N-terminal lysines of histone H4 that block their acetylation also abolishes repair of both DSB and UV-induced damage [Bird et al., 2002]. Similarly, blockage of acetylation in N-terminal lysine in histone H3 make cells more sensitive to DNA damage. The GCN5 enzyme is recruited to DNA double strand breaks and catalyzes acetylation of N-tail lysines of histone H3. The GCN5 mutant cells exhibit decrease viability following induction of a single DNA double strand break [Tamburini and Tyler, 2005]. In higher eukaryotes and in mammals, DNA damage is also known to induce H3 acetylation [Vidanes et al., 2005; Yang et al., 2013]. Therefore, chromatin changes are an integral part of the DNA damage response and involve assembly of large multiprotein complexes.

The leukemic (8:21) translocation is considered a product of an erroneous repair process. In mammals the DSB are repaired by homologous recombination (HR) or non-homologs end-joining (NHEJ). Histone acetylation plays a critical role in marking the lesion, facilitating accessibility and interaction of recombinase enzymes with the target DNA [Gontijo et al., 2003]. Thus DNA regions associated with highly acetylated histones and/or chromatin remodelers are preferentially repaired. However, the relationship between acetylation of histones and repair seems to be more complex than a simple model in which more acetylation results in better accessibility and therefore more efficient DSB repair. Histone acetylation also determines which pathway will be utilized for DSB repair. For example, enhanced acetylation due to p300 and CBP activity enhances NHEJ by prompting recruitment of KU proteins [Ogiwara et al., 2011].

Our results demonstrate that chromatin organization at the breakpoint regions of both partner genes in (8;21) translocation, *RUNX1* and *ETO* genes, exhibit a common structural feature, characterized by increased histone H3 acetylation. This particular chromatin organization maybe relevant either in formation of the DNA breaks and/or during the NHEJ recombination process that gives rise to the (8;21) chromosomal translocation. Taken together our results suggest that chromatin organization at the breakpoint regions of the ETO gene may have a functional linkage with the increased susceptibility of this region to undergo chromosomal translocations.

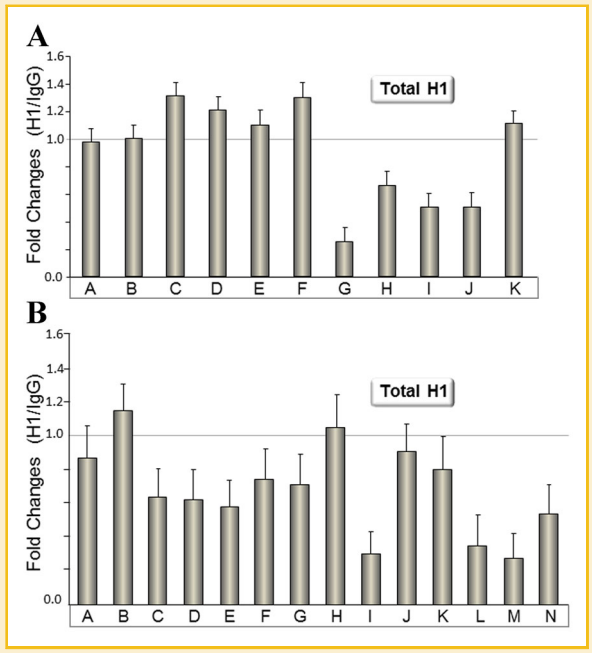

Fig. 6. Chromatin of ETO gene lacks histone H1. ChIP assays were performed in pro-myeloid cells with anti-H1 antibody that recognize all isoforms of histone H1. Real time PCR were perform for intron 1 (A) and intron6 (B) of the *ETO* gene. Immunoprecipitated DNA was quantified as percent with respect to input and plotted as fold over non-specific IgG for various regions as indicated. Pooled data for five independent experiments with standard error are shown. Values above the indicated lines represent statistically significant acetylated histone association.

ACKNOWLEDGMENTS

This work has been supported by Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) Grant AT24091060 awarded to MS and Grant 1100670 awarded to SEG.

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