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Interaction between the immunoglobulin heavy chain 3′ regulatory region and the IgH transcription unit during B cell differentiation

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ABSTRACT

The immunoglobulin heavy (*Igh*) chain locus is subject to precisely regulated processes, such as variable region gene formation through recombination of variable (V_H), diversity (D_H), and joining (J_H) segments, class switching and somatic hypermutation. The 3′ regulatory region (3′ RR) is a key regulator of the *Igh* locus, and, as revealed by deletions in mouse plasma cell lines and mice, is required for IgH expression as well as class switching. One of the mechanisms by which the 3′ RR regulates its targets is through long-range physical interactions. Such interactions between elements of the 3′ RR and a target site in the IgH transcription unit have been detected in plasma cells, and in resting and switching B cells, where they have been associated with IgH expression and class switching, respectively. Here, we report that lentiviral shRNA knockdown of transcription factors, CTCF, Oct-2, or OBF-1/OCA-B, had no discernible defects in loop formation or H chain expression in plasma cells. J_H–3′ RR interactions in pre-B cell lines were specifically associated with IgH expression. J_H–3′ RR interactions were not detected in either Pax5-deficient or RAG-deficient pro-B cells, but were apparent in an Abelson-derived pro-B cell line. These observations imply that the 3′ RR has different loop interactions with target *Igh* sequences at different stages of B cell development and *Igh* regulation.

1. Introduction

During B cell maturation and activation, immunoglobulin heavy chain (IgH) genes are subject to DNA rearrangements required for VDJ joining and class switch recombination (CSR), and to V_H gene somatic hypermutation (SHM) (reviewed in Dudley et al., 2005). These processes are tightly regulated by cis elements of the *Igh* locus, including V region promoters, I/switch region promoters, the intronic enhancer (Eμ) and the hs sites of the 3′ regulatory region (3′ RR), several of which are associated with the four known enhancers (hs3a, hs1.2, hs3b and hs4) (Cogne and Birshtein, 2004). Additional 3′ RR hs sites contain binding sites for Pax5 and CTCF and display their accompanying insulator activity (hs5-7 and "38") (Fig. 1A) (Chatterjee et al., 2011; Garrett et al., 2005). Functionally, the 3′ RR is important for immunoglobulin heavy chain gene expression in plasma cells and for class switching in activated B cells, as revealed by mouse knockout models (Pinaud et al., 2001; Vincent-Fabert et al., 2010). A contribution of the 3′ RR to SHM was revealed by a reduction in SHM resulting from a deletion of the entire 3′ RR enhancer region from a BAC transgenic mouse (Dunnick et al., 2009).

Chromosome conformation capture (3C) studies have identified loops that juxtapose 3′ RR enhancers with target *Igh* sequences, such as J_H sequences associated with the IgH transcription unit. In switching cells, additional interactions of the 3′ RR with I/switch region promoters have been identified (Wuerffel et al., 2007; Yan et al., 2011). That J_H–3′ RR interactions occur in splenic B cells but not in splenic T cells indicates that these interactions are B cell-specific. Loss of H chain expression in a plasma cell line (Ju et al., 2007) or defects in CSR (Wuerffel et al., 2007), each resulting from deletions of 3′ RR enhancers, showed concomitant defects in loop formation. Hence, loop interactions involving the 3′ RR are implicated in these roles for expression and recombination. Additional 3′ RR loop interactions have been identified, one through DICE elements at the V_H promoter, which is also associated with Igh expression in a plasma cell line (Ren et al., 2011), and a second with CTCF sites upstream of DFL16.1 in pro-B cells that is associated with *Igh* locus contraction (Degner et al., 2011) and predicted to impact on VDJ joining.

The transcription factor CTCF has been shown to contribute to loop formation in various loci. Initially identified as a regulator of the chicken c-myc promoter (Lobanenkov et al., 1990), CTCF was subsequently found to be involved in insulation of enhancers from promoters, potentially regulating boundaries between active and silent chromatin (rev. in Phillips and Corces, 2009). CTCF sites identified in the imprinted control region (ICR) regulate H19/Igf2 imprinting through loop-formation as measured by chromosome

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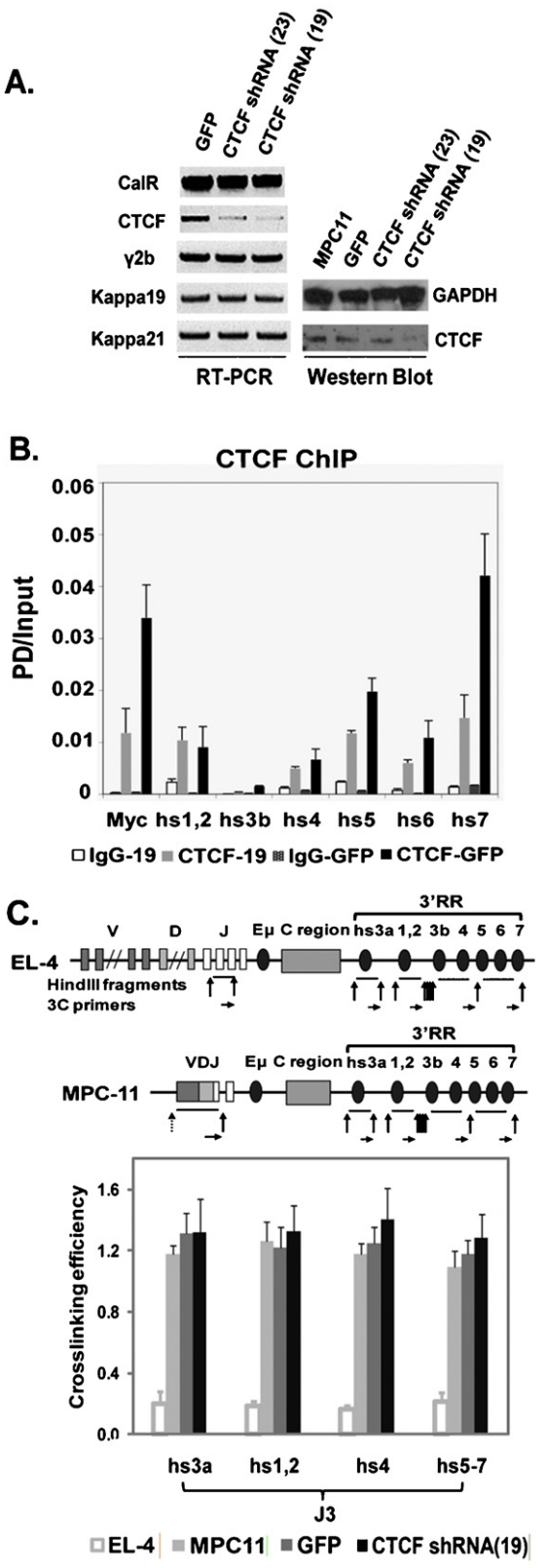

Fig. 1. Effect of CTCF on Ig expression and 3C interactions in MPC11 plasma cells. (A) CTCF mRNA (RT-PCR) and protein levels (Western blot) in CTCF shRNA treated MPC11 plasma cells, with GFP- infected MPC11 cell lines as a control. MPC11 H (γ2b) and L(kappa 19 and 21) chain expression were unchanged. Calreticulin (CalR) housekeeping gene and GAPDH were used for normalization of mRNA and protein levels, respectively. (B) Effect of CTCF KD on ChIP analysis of CTCF binding to 3′ RR enhancers and insulator region. CTCF binding, as measured by PD (pulldown)/input, was reduced by lentiviral shRNA, as shown in the gray bars. Note that the predominant CTCF binding sites are in hs5-7 (Chatterjee et al., 2011). The 5′ end of the c-myc gene was used as a positive control. Levels of binding with non-CTCF specific IgG are also shown. (C) Effect of CTCF KD on 3C interactions involving J_H3 and 3′ RR. Schematic maps of immunoglobulin heavy chain (IgH) loci in EL-4 T cells and MPC11 plasma cells used for 3C analysis. Vertical arrows indicate HindIII cleavage sites. Horizontal arrows indicate the primers for 3C assay. 3C interactions between J3 and 3′ RR elements were analyzed in EL-4 T cells, MPC11 cells, and MPC11 cells infected with GFP or CTCF shRNA 19. No changes were observed with CTCF KD.

conformation capture (3C) experiments (Kurukuti et al., 2006), and similarly, contribute to MHC locus regulation (Majumder and Boss, 2010). CTCF also plays a role in looping in the β-globin locus, but apparently without a critical role in gene expression (Splinter et al., 2006). Recently, CTCF has been shown to affect *Igh* locus contraction apparently through an impact on loop interactions (Degner et al., 2011).

Octamer binding motifs are found in many *Igh* regulatory elements, including V gene promoters, Eμ and the 3′ RR. Together with the co-factor OBF-1, Oct-1 or Oct-2 binds to octamer motifs in B cells and exerts its function. Oct-1 is ubiquitously expressed while Oct-2 is expressed only in B cells and neuronal cells. Although B cell development in fetal liver is not affected in Oct-2 knockout mice, these mice die soon after birth for unclear reasons (Corcoran et al., 1993). OBF-1 (also known as OCA-B and Bob-1) expression is B cell-specific, and OBF-1 knockout mice show reduced levels of IgG isotypes and no germinal center formation (Schubart et al., 1996). Oct-2 and OBF-1 have been shown to regulate 3′ RR activity in B cells (Tang and Sharp, 1999). In human t(14;18) lymphoma cells, the bcl2 gene is involved in a translocation with the *Igh* locus, and there is an interaction between the translocated *bcl2* gene and the 3′ RR. The contribution of Oct-2 to the *bcl2*-3′ RR interaction was revealed by siRNA knockdown of Oct-2 (Duan et al., 2007). Recent studies have shown a role for OBF-1/OCA-B in interactions between hs4 and DICE sequences downstream of the V_H promoter (Ren et al., 2011).

In this report, we find that loop interactions involving the 3′ RR and J_H target sequences in a plasma cell line, although critical for *Igh* expression, do not rely on CTCF, Oct-2 or OBF-1. J_H–3′ RR loop interactions detected on the single VDJ rearranged and expressed allele in a pre-B cell line imply their association with *Igh* gene expression. The analysis of J_H–3′ RR interactions in pro-B cell sources gives insight into the ontogeny of 3′ RR interactions with the IgH transcription unit during early stages of B cell development. Collectively, these studies monitor the engagement of the 3′ RR with the IgH transcriptional unit during B cell development and suggest that the 3′ RR participates in a variety of functional loops.

2. Materials and methods

2.1. B cell lines

Murine EL-4 T cells (ATCC #TIB-39) were used as a control for cell type specificity. The mouse pro-B cell line 63-12 (Shinkai et al., 1992), pre-B cell lines 3-1 and 70Z/3 (Nelson et al., 1983; Paige et al., 1978) were maintained in RPMI-1640 medium with 10% fetal calf serum; plasma cell line MPC11 (Laskov and Scharff, 1970) was cultured in DMEM medium with 10–15% fetal calf serum. All cells were grown at 37 °C in an atmosphere of 5% CO_2. 63-12 cells reconstituted with RAG-2 protein were a generous gift from Dr. Kathryn Calame (Columbia University) (Angelin-Duclos and Calame, 1998). Pro-B cells derived from a Pax5$^{-/-}$ mouse and transduced with a Pax5 expression construct coupled with estrogen receptor (ER) (Pax5-ER (B1.1)) (Nutt et al., 1998) were a generous gift from Dr. Meinrad Busslinger (Research Institute of Molecular Pathology, Vienna, Austria), and were received from Dr. Paolo Norio (Montefiore Medical Center, Bronx, NY). Pax5-ER cells were maintained