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# A Minor Transactivation Effect of GATA-3 on its Target Sites in the Extrachromosomal Status

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**Abstract** Transcription factor GATA-3 is the critical transcription factor for Th2 cell differentiation. In spite of its importance in Th2 cell differentiation, the molecular mechanism for its action in Th2 differentiation is poorly understood. Previous studies have suggested that GATA-3 may be involved in the chromatin remodeling in the Th2 cytokine locus. To determine whether GATA-3 exerts its effect on its target sites in the extrachromosomal status, cell transfection assay was performed. In this assay, 800 bp IL4 promoter-luciferase constructs linked with GATA-3 target sites were transfected into the M12 B cell line, D10 mouse Th2 cell lines, and human T lymphoma Jurkat cell lines with or without the GATA-3 expression vector. The GATA-3 effects on its target sites were minimal in the extrachromosomal status, supporting the previous propositions that GATA-3 functions at the chromatin level by remodeling chromatin structure.

**Keywords:** GATA-3, Th2, transient transfection, extrachromosomal status, chromatin remodeling, M12, D10, Jurkat

Gene expression is regulated by complex interactions of transcription factors and *cis*-acting regulatory elements [26]. The molecular mechanisms of how transcription factors enhance the transcription rate of RNA polymerase are not clearly defined. Recent evidences suggest that these factors may initiate or facilitate the structural changes of chromatin by inducing histone modification or DNA demethylation [22].

GATA-3 has been implicated in many steps in the fate decision processes [20]. GATA-3 is critically involved in the differentiation of common lymphoid precursor cells to T cell lineage, in the differentiation of naive CD4 T cells to Th2 lineage [15]. Recently, it was reported that GATA-3 is also involved in the differentiation of endothelial cells in the breast [10]. Among these processes, differentiation to Th2 cells from naive CD4 T cells was intensively studied because of the fundamental interest in the fate-decision process and clinical importance of T helper cells in human diseases [1–4, 7, 9, 13, 23]. In addition, *cis* regulatory elements that coordinately regulate the genes for Th2 cells have been relatively well studied [13], giving advantage for the study of the interaction between transcription factors and *cis* regulatory elements during cell differentiation.

GATA-3 has been shown to be the critical transcription factor for Th2 cell differentiation [15, 16, 28]. GATA-3 is selectively expressed in Th2 cells, and inhibition of GATA-3 expression by antisense DNA caused a reduction of Th2 cytokines, IL4, IL5, and IL13 [28]. The role of GATA-3 in chromatin remodeling was suggested by transfer of a retroviral GATA-3 expression vector into Th1 cells [14, 17, 18]. Transgenic expression of GATA-3 caused a Th2-like phenotype, even in Th1 stimulated cells [28]. In this case, GATA-3 induced DNase I hypersensitive sites in the Th2 cytokine locus, which contains *il4-il13-rad50-il5* genes, characteristic for Th2 cells [14, 17, 18]. Whether GATA-3 is directly or indirectly involved in chromatin remodeling in the Th2 cytokine locus is not known, but this result suggested that it is critical in the process. Numerous consensus GATA-3 binding sites are present in the Th2 cytokine locus, among which functional GATA-3 target sites were searched by transfection and transgenic approaches [11, 21]. These studies found that several GATA-3 target sites are present around the *il4*, *il13*, and *il5* genes [11] and in a recently found regulatory sequence, the Th2 locus control region (LCR) [12].

In this study, to gain an insight into how GATA-3 works in its target sites, I examined whether GATA-3 functions as a transactivator on the IL4 promoter in the extrachromosomal status. For this purpose, I used a transient transfection assay using reporter constructs that contain GATA-3 target sites. This study demonstrates that GATA-3 does not perform a transactivation function when its target binding sequences are in the extrachromosomal status.

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## MATERIALS AND METHODS

### Plasmid Construction

The construction of reporter constructs has been described previously [11]. Briefly, the pGL3-IL4P construct was made by inserting the 800 bp promoter region from −741 to +60 of the transcription start site into the BglII site of the pGL3-basic luciferase vector (Promega). The SV40 late poly(A) signal was replaced with the human growth hormone (hGH) poly(A) signal. The pGL3-IL-4P-IE construct was made by inserting a 679 bp BglII fragment from the second intron of the *IL4* gene from the pIL4 plasmid into the BamHI site of pGL3-IL4P. The pGL3-HSS-IL4P construct was made by inserting a 2,738 bp fragment from −1,0463 to −7,725 bp upstream of the IL4 translation start site into an NheI site of the pGL3-IL4P construct. The sequences of all the PCR products were confirmed by sequencing.

### Cell Culture and Transient Transfection Assay

The B cell lymphoma M12, human T cell lymphoma Jurkat cells, and mouse Th2 cell line D10 were cultured in RPMI 1640 plus 5% fetal bovine serum with antibiotics. D10 cells were stimulated every 2 weeks with C3H/He antigen-presenting cells and chicken conalbumin (Sigma) (100 μg/ml). Exponentially growing cells ($1\times10^7$) were transfected with 20 μg of reporter constructs by electroporation with a Bio-Rad gene pulser (280 V, 960 μF). Cells were rested for 16 h and then stimulated with 50 ng/ml PMA+ 1 μM ionomycin for 4 h. Cell extracts were made, and the luciferase activity was measured with the Dual-luciferase Reporter Assay System (Promega). The relative luciferase unit was calculated by dividing the firefly luciferase value with that of cotransfected renilar luciferase for normalizing transfection efficiency.

## RESULTS

### GATA-3 has Marginal Effects on Transactivation of the IL4 Promoter

To investigate the functional role of GATA-3 on the transactivation through its target sites, I used a reporter construct (pGL3-IL4P-luciferase, abbreviated name IL4P) containing an 800 bp IL4 promoter and luciferase [11]. To look at the effects of GATA-3 on transactivation of IL4 promoter activity, I transfected this construct into M12, a B cell line that does not express endogenous GATA-3. To stimulate the M12 cells, I treated with PMA+ionomycin, which are used for activating PKC and calcineurin, respectively, and therefore mimicking activation through a T cell receptor or B cell receptor. Stimulation of M12 cells with PMA+ ionomycin did not enhance IL4 promoter activity in M12 cells (Fig. 1). Introduction of GATA-3 enhanced IL4 promoter activity about 2.5-fold (Fig. 1). I compared this enhancing activity of GATA-3 with that of c-Maf, which was shown to be a strong transactivator in the IL4 promoter [5]. c-Maf enhanced IL4 promoter activity about 20-fold without PMA+ionomycin stimulation, and about 40-fold with PMA+ionomycin stimulation (Fig. 1). The weak transactivation activity of GATA-3 on its target sites are not due to insufficient amount of induction of GATA-3 in the transfected cells, since immunoblot analysis showed that GATA-3 is highly overexpressed in the cells (data not shown). Therefore, the enhancing activity of GATA-3 on the IL4 promoter is much weaker than that of c-Maf.

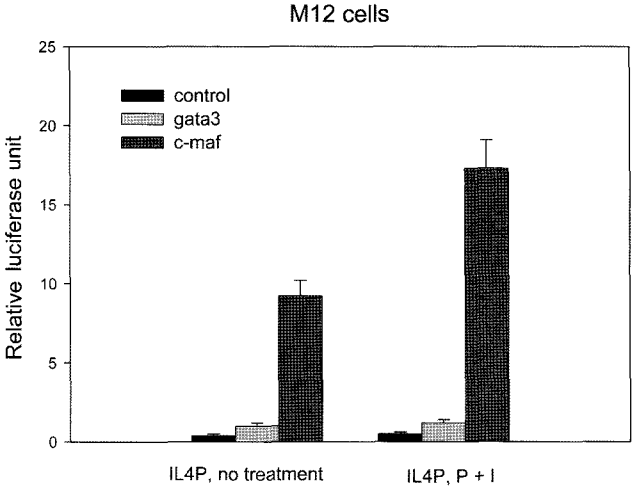


**Fig. 1.** IL4P construct was transfected into M12 (B cell lymphoma cell line) by electroporation.
GATA-3 expression vector or c-Maf expression vector were cotransfected with the IL4P construct. Cells were rested for 16 h and then cells were either nonstimulated or stimulated with 50 ng/ml PMA+1 μM ionomycin for 4 h. Cell extracts were made, and the luciferase activity was measured and transfection efficiency was normalized by dual luciferase. Data represent mean±standard deviation from a triplicate.

### GATA-3 has no Transactivation Effect on its Target Sites in the Extrachromosomal Status

To investigate the role of GATA-3 on its target site, I used two constructs, named pGL3-IL4P-luciferase-IE (IL4P-IE) and pGL3-HSS-IL4P-luciferase (HSS-IL4P) [11]. These constructs contain IE or HSS, which were shown to be GATA-3 responsive elements in the previous study done by a transgenic approach [11] and were shown to bind to GATA-3 and other GATA family members by *in vitro* electrophoresis mobility shift assay (EMSA) [6, 27]. I transfected these constructs into M12 cells with or without GATA-3 (Fig. 2). GATA-3 had no effect on the transactivation of IL4 promoter activity when the IE elements were linked to the IL4 promoter (Fig. 2). Introduction of GATA-3 with HSS-IL4P-luciferase did not enhance IL4 promoter activity (Fig. 2). Rather, IL4 promoter activity in the cells transfected with HSS-IL4P was reduced compared with

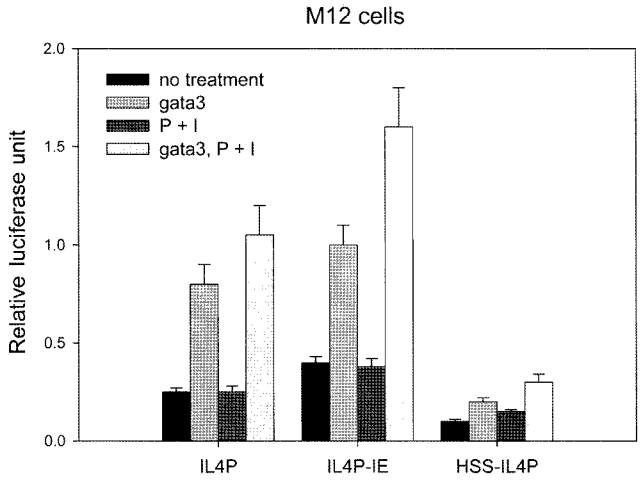


**Fig. 2.** IL4P, IL4P-IE, and HSS-IL4P constructs were transfected into M12 (B cell lymphoma cell line) by electroporation. Experiments were done as the same way as in Fig. 1. Data represent mean±standard deviation from a triplicate.

that in the cells transfected with IL4P (Fig. 2). Taken together, these results suggest that GATA-3 has no effect on the transactivation on the IL4 promoter through its target sites in the extrachromosomal status, at least in M12 cells. Since previous studies indicated that GATA-3 can transactivate IL4 promoter activity in the chromatin context, these results suggest that the chromatin context is crucial in the function of GATA-3.

**Presence of the Necessary Factors for *il4* Gene Expression is not Sufficient for the Enhancement of IL4 Promoter Activity in the Extrachromosomal Status**

One possibility for the lack of GATA-3 activity in the previous experimental setting is that it may result from the lack of other necessary transcription factors for *il4* gene expression, since M12 cells are B cells, and thus they may lack the necessary factors for optimal *il4* gene expression. Therefore, I tested this possibility by performing the same transfection experiment on the D10 mouse Th2 cell line and Jurkat human T cell lymphoma cell line, both of which were shown to express IL4 upon TCR stimulation. PMA+ ionomycin treatment after transfection of the IL4 promoter-luciferase construct into D10 cells increased IL4 promoter activity about 9-fold (Fig. 3A). This result is in stark contrast with that from M12 cells, since PMA+ionomycin had no effect on the IL4 promoter activity (Fig. 2). This result confirms that D10 cells have all the necessary transcription factors for IL4 expression. I examined whether regions containing GATA-3 target sites can enhance the IL4 promoter activity in the environment where all the necessary factors for IL4 gene expression are provided including GATA-3. For this purpose, I introduced IL4P-IE and HSS-IL4P into D10 cells and measured IL4 promoter activity after PMA+ ionomycin stimulation. Addition of IE or HSS regions to

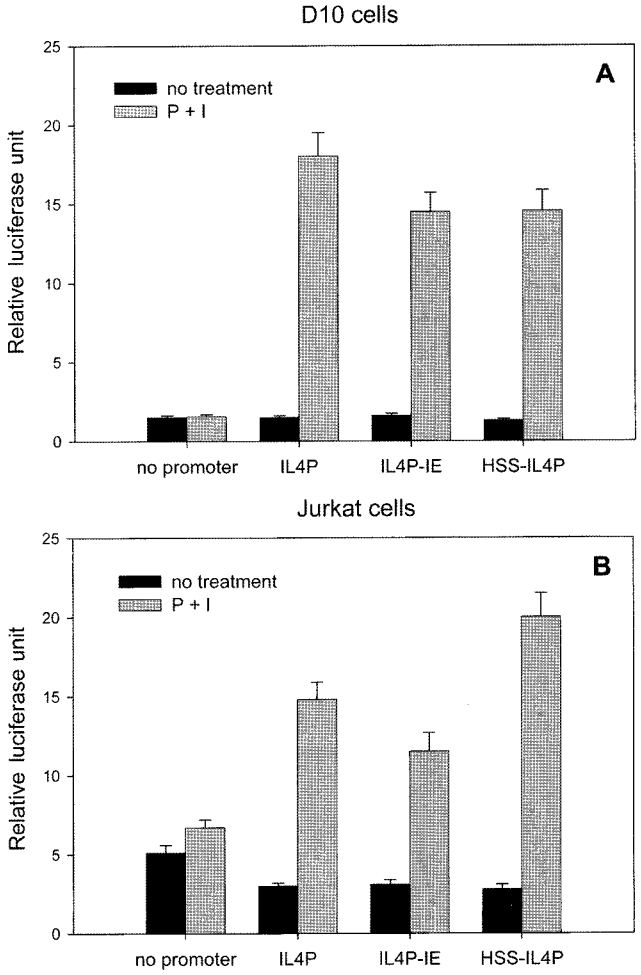


**Fig. 3.** IL4P, IL4P-IE, and HSS-IL4P constructs were transfected into (**A**) D10 (Th2 cell line), and (**B**) Jurkat (human T lymphoma cell line) by electroporation. Experiments were done in the same way as in Fig. 1. Data represent mean±standard deviation from a triplicate.

IL4P-luciferase did not enhance IL4 promoter activity, even in D10 cells (Fig. 3), suggesting that the lack of transactivation activity shown in M12 cells is not from the lack of necessary factors for IL4 gene expression. Similar results were obtained in Jurkat human T cells, which express IL4 upon T cell receptor stimulation (Fig. 3B). These results further support that even in the presence of an optimal amount of transcription factors for IL4 gene expression, the native chromatin context is important in transactivation of the *il4* gene by GATA-3.

## DISCUSSION

In this study, I investigated the role of GATA-3 in transactivation of the IL4 promoter in the extrachromosomal status. This study suggests that GATA-3 does not play a

major role in a direct transactivation of the IL4 promoter, and that GATA-3 does not function in the extrachromosomal status. These results support the current proposition that GATA-3 is involved in chromatin remodeling in the Th2 cytokine locus.

The transactivation activity of GATA-3 was marginal in the IL4 promoter, and it was much smaller than that of c-Maf (Fig. 1), suggesting that this is not the major role of GATA-3 in the Th2 differentiation. It is probable that c-Maf plays a major role in the IL4 promoter and that GATA-3 may play a role in the global Th2 cytokine locus. This is supported by the previous report that deletion of c-Maf abrogates IL4 expression but not other Th2 cytokines, IL13 and IL5 [8]. In contrast, conditional deletion of GATA-3 suppressed all Th2 cytokine expression, suggesting a more global effect of GATA-3 [19, 29].

It was shown previously that the transactivation activity of GATA-3 may require the cooperation of regions containing the GATA-3 target sites and the IL4 promoter region [11]. Only in this context does GATA-3 function fully as a transactivator. In this study, even in this combinatorial context, GATA-3 could not exert its function. This result corroborates the proposition that GATA-3 only functions in the chromatin context. Therefore, the native chromatin context as well as cooperation between the enhancer and promoter seems to be a crucial determinant for GATA-3 action.

The transcriptional activity in the linkage of HSS to IL4P-luciferase was reduced compared with control, IL4P-luciferase, in M12 cells (Fig. 2), but this phenomenon was not observed in D10 or Jurkat cells (Figs. 3A and 3B). Hence, this inhibitory effect is specific for M12 cells. The reason for this reduction is not clear, but it may result from the binding of HSS to inhibitory factors that are present only in B cells, although other explanations are also possible.

Recent studies have suggested that the Th2 cytokine locus rearranges during Th2 cell differentiation by forming chromosomal interactions [24, 25]. Since GATA-3 is the critical transcription factor for Th2 differentiation, GATA-3 may play a role in this process. Therefore, it will be interesting to investigate the role of GATA-3 in the process.

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