PERGAMON

Molecular Immunology 38 (2001) 1193–1199

Molecular Immunology

www.elsevier.com/locate/molimm

Transcriptional regulation of the human high affinity IgE receptor α-chain gene

Kyoko Takahashi [a], Chiharu Nishiyama [b], Chisei Ra [a,*]

[a] *Department of Molecular Cell Immunology and Allergology, Advanced Medical Research Center, Nihon University School of Medicine, 30-1 Oyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan*

[b] *Allergy Research Center, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

Received 28 December 2001; accepted 2 February 2002

Abstract

Transcriptional regulation of the gene encoding human high affinity IgE receptor (FcεRI) α-chain was analyzed. Previously, we reported that GATA-1 and Elf-1 recognition sites were necessary for cell type-specific activation of the α-chain gene promoter. More detailed analysis revealed that other transcription factors bound the regions close to the Elf-1 recognition site and there was a more complex mechanism for the regulation of the promoter activity. On the other hand, during a course of studies to find *cis*-elements over this gene, CAGCTG sequence in the first intron was revealed to serve as an enhancer. A complex composed of USF1 and USF2 activated the human α-chain gene expression via this intronic element. Furthermore, we found two novel exons at 18.4 and 12.6 kb upstream from the reported first exon and discovered an additional distal promoter.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: FcεRI; α-chain; Transcription

1. Introduction

The high affinity receptor for IgE (FcεRI) plays a key role in the IgE-mediated allergic reaction. Crosslinking of FcεRI on mast cells and basophils with antigen-IgE complexes leads to the release of a variety of potent mediators, such as histamine, leukotrienes and prostaglandins. FcεRI is a tetrameric receptor composed of one α, one β and two γ chains. The α-chain is a specific component of FcεRI that binds directly to IgE. Although, the β-chain, also specific for FcεRI, is not always required for the functional expression of FcεRI in human (Miller et al., 1989), amplifies the γ-chain mediated intracellular signals (Lin et al., 1996; Dombrowicz et al., 1998). Recently, it was reported that the β-chain accelerates the cell surface expression of FcεRI by improving the α-chain maturation and receptor trafficking (Donnadieu et al., 2000). On the other hand, despite the cell specific expression of FcεRI on mast cells, basophils, eosinophils, monocytes and Langerhans cells, the γ-chain is also expressed in other kinds of cells to form the receptors for IgG (FcγRI, FcγRIII) and IgA (FcαR) (Ra et al., 1989; Ernst et al., 1993; Pfefferkorn and Yeaman, 1994). Thus, the expression of FcεRI in the limited cells is thought to be regulated by the α- and β-chain expression. Recently, the FcεRI α-chain mRNA was revealed to be upregulated by IL-4 (Toru et al., 1996; Pawankar et al., 1997; Gosset et al., 2001), suggesting the possibility that the inducible expression of FcεRI might be regulated by level of the α-chain transcript.

Therefore, it is crucial to investigate the regulatory mechanism of the α-chain gene expression for elucidating both cell-type specific and inductive regulation of FcεRI expression.

2. Major promoter region

The genomic structure of FcεRI α-chain gene, which is consisted of five exons, was already reported (Pang et al., 1993). Because human FcεRI α-chain gene transgenic mice expressed human α-chain specifically in the same cells as those in human (Fung-Leung et al., 1996; Dombrowicz et al., 1998), it is predicted that the DNA fragment of about 10 kb introduced to the mice contains all the transcriptional regulatory elements of human FcεRI α-chain gene.

Since these two regions were shown to be recognized by GATA-1 and Elf-1, respectively, these transcription

Abbreviations: FcεRI: high affinity IgE receptor; EMSA: electrophoretic mobility shift assay

* Corresponding author. Tel.: +81-3-3972-8111; fax: +81-3-3972-8227.
E-mail address: fcericra@med.nihon-u.ac.jp (C. Ra).

0161-5890/02/$ – see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0161-5890(02)00062-7

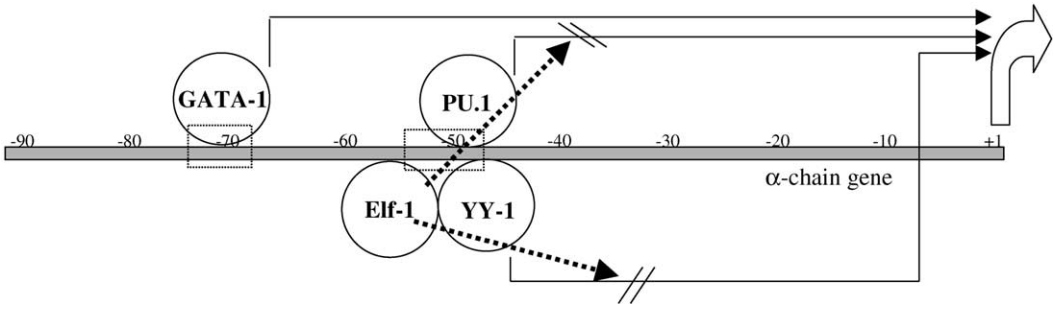

Fig. 1. Schematic drawing of human FcεRI α-chain promoter. Nucleotide numbers are counted from the transcription start site. GATA-1 and Elf-1 recognition sites are drawn with dotted box.

factors were thought to be involved in the upregulation of FcεRI α-chain gene. However, a more complex mechanism for the regulation of the α-chain gene transcription is emerging through further analysis. Overexpression of GATA-1 resulted in the activation of the α-chain promoter, while overexpressed Elf-1 showed negative effect on the promoter (Nishiyama et al., 2000). Recently, we found that other transcription factors, PU.1 and YY-1, bound the sequences partially overlapping with the Elf-1 motif (Fig. 1, Nishiyama et al., 2002). These newly found factors possibly activate the α-chain gene expression by competing with the negative regulator, Elf-1, for the binding sites.

The α-chain gene is thought to be transcribed by using 5′-TATATTT-3′ (−28/−22) as "TATA-box" (Pang et al., 1993), but the base substitutions to alter the TATA sequence did not show apparent negative effects in transient transfection assay (Nishiyama et al., unpublished data). From this result, we speculate that the TATA-like sequence at nt −28/−22 does not work as TATA-box and the promoter of the α-chain is a TATA-less type. Interestingly, both of YY-1 and PU.1 are reported to be involved in the transcriptional initiation from TATA-less promoters (Eichbaum et al., 1994; Feinman et al., 1994; Johansson et al., 1998).

3. Intronic enhancer

Since *cis*-acting elements far from the promoter have been often demonstrated to regulate the gene expression, we screened over 20 kb for *cis*-elements in the region covering the α-chain gene (Takahashi et al., 2001). In a transient transfection assay using a rat mast cell line RBL-2H3, known to express FcεRI constitutively, a genomic fragment containing almost entire part of the first intron increased the promoter activity significantly when inserted upstream of a SV40 promoter followed by a luciferase gene. Similar results were obtained when a murine mast cell line PT18 was employed for the assay.

The first intron contained various motif sequences possibly recognized by transcription factors as shown in Fig. 2A (Takahashi et al., 2001). A series of mutant plasmids which had nucleotide substitutions at these motifs were used for the transient transfection assay in RBL-2H3 cells. Only when three nucleotides in a motif known as E-box were exchanged, the luciferase activity was reduced to <50% of the control (Fig. 2B, Takahashi et al., 2001). This indicated that CAGCTG, one of the CANNTG motifs called E-box, served as an enhancer element in the first intron. We next identified the factors binding to this element by electrophoretic mobility shift assay (EMSA) in which nuclear extract prepared from RBL-2H3 cells was added to a double-stranded DNA probe including the element (Fig. 3, Takahashi et al., 2001). The shifted band indicated with an arrow was disappeared by the addition of a self-competitor A. The competitive effect was not observed when a mutant-type competitor, Amut, which had the substitution of three bases in the CAGCTG motif was used (Fig. 3A and B). These results suggested the existence of nuclear protein(s) that recognized the CAGCTG element.

E-box motif is recognized by the transcription factors belonging to the helix–loop–helix family, such as c-Myc, USF (upstream stimulating factor), MAD, MAX, E47, which function as a homo- or hetero-dimer. When the antibodies against these factors were added to the nuclear extract in EMSA (Fig. 3C), either of anti-USF1 or anti-USF2 antibody eliminated the band (lane 2, 3). USF1 and USF2 were expressed by in vitro transcription/translation and applied for EMSA (Fig. 3D). The oligonucleotide probe complexed with USF1/USF2 heterodimer (lane 1) neither with USF1 nor USF2 homodimer (lane 3, 4) expressed in vitro showed the same mobility as that with the nuclear extract from RBL-2H3 (lane 2). These findings indicated that USF1/USF2 heterodimer bound the CAGCTG element.

Finally, to confirm whether the USF1/USF2 heterodimer actually activates the FcεRI α-chain gene expression, human USF2 antisense expression plasmid was introduced into human basophillic leukemia cell line, KU812 which is reported to express FcεRI α-chain mRNA. RT-PCR analysis revealed that the amount of mRNA for the α-chain was decreased by USF2 antisese plasmid in a dose-dependent manner (Fig. 4, Takahashi et al., 2001), which suggested that USF1/USF2 heterodimer was involved in the FcεRI α-chain

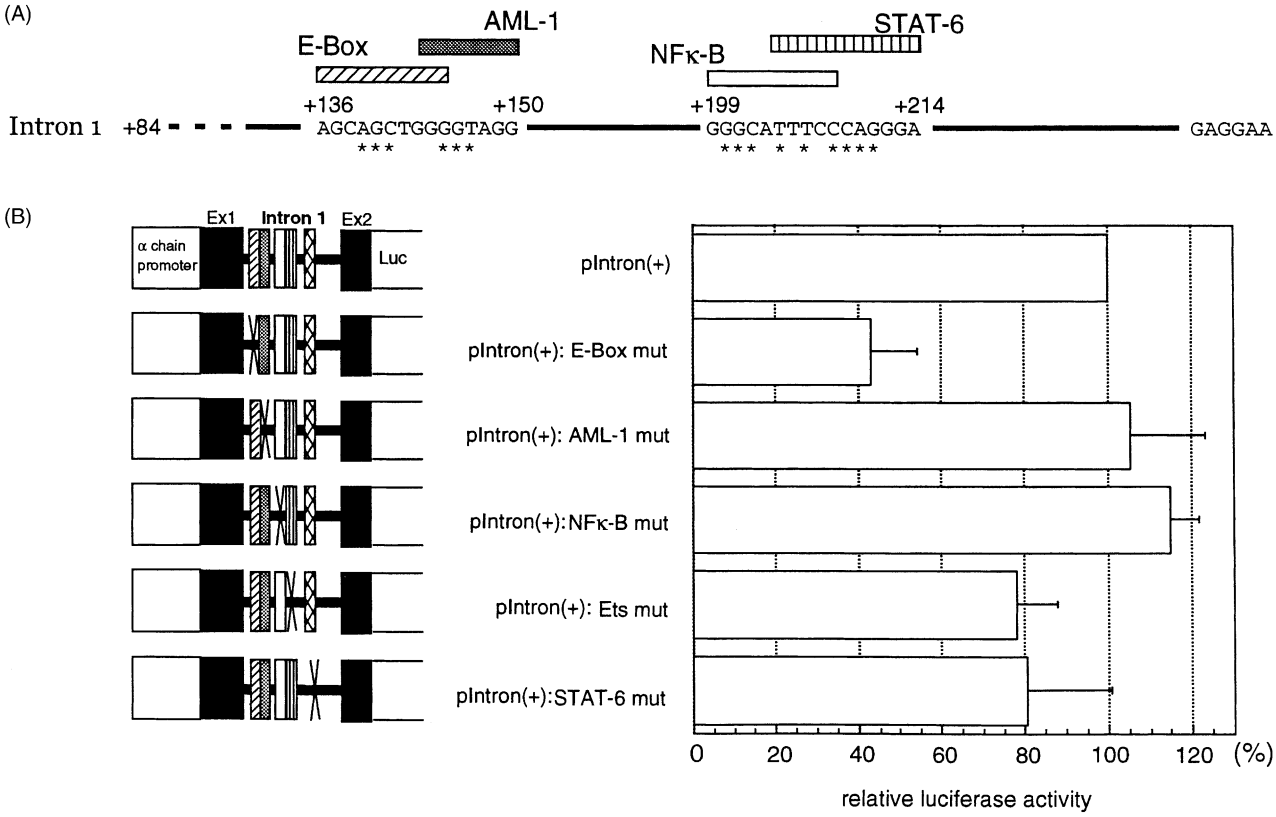

Fig. 2. Determination of enhancer element in the first intron by site-directed mutagenesis (A) Transcription factor binding motifs in the first intron. Replaced nucleotides are marked with asterisks. (B) Nucleotide substitutions were introduced into each motif RBL-2H3 cells were transiently transfected with these mutant plasmids. The results are the average of three independent experiments of double sets.

gene expression even in human mast cells through binding to a putative E-box motif in the first intron.

Although, USF proteins are ubiquitously expressed, they have been reported to be involved in the expression of several tissue-specifically and/or developmentally regulated genes (Navankasattusas et al., 1994; Timchenco et al., 1995; Vallet et al., 1997). It is therefore, possible that USF1/USF2 system might also determine the cell type specific expression of FcεRI α-chain by cooperating with other cell-type specific transcription factors, such as GATA-1 and Elf-1, which were shown to recognize the specific sequences close to the α-chain promoter.

4. Distal promoter

The nucleotide sequence of α-chain cDNA was reported by two independent groups (Shimizu et al., 1988; Kochan et al., 1988). The 5′-end of the cDNA reported by Kochan et al. was about 70 bp longer than that by Shimizu et al. The first exon of FcεRI α-chain gene reported by Pang et al. in 1993 did not include 46 bp of the 5′-end of the cDNA cloned by Kochan et al. Pang et al. attempted to clone a putative exon containing the 46 bp with PCR technique, but failed. We finally found that the 5′-extension of 46 bp was actually present far upstream of the 5′-end of the genomic DNA cloned by Pang et al., forming two exons (Fig. 5, Nishiyama et al., 2001).

A DNA fragment of about 12 kb was amplified by PCR using the primers (primer A and primer B in Fig. 5) which corresponded to the nucleotide sequences of the 5′-end portion of Kochan's cDNA and that of the genomic DNA (about 1.3 kb upstream from exon 1) reported by Pang et al. respectively, indicating that a novel exon occurred about 13 kb upstream of the reported first exon. The PCR product actually contained 37 bp of the 46 bp, but still lacked Kochan's 5′-end portion of 9 bp, which suggested an additional exon was present further upstream. We therefore carried out plaque hybridization employing the DNA fragment of 1.6 kb cloned with a promoter finder kit and that for α-chain cDNA as probes (Fig. 5). Three clones hybridizing with the 1.6 kb probe and two clones hybridizing with the cDNA probe were obtained. Determination of the nucleotide sequence of these clones revealed the structure of human FcεRI α-chain genomic DNA spreading over 20 kb. The exon composed of the remaining 9 bp of Kochan's cDNA was located 18.4 kb upstream from the reported first exon and hereafter we designate the two novel exons as exon 1A and exon 1B.

The expression of α-chain mRNA including exon 1A and 2A in KU812 cells was examined by RT-PCR (Fig. 6,

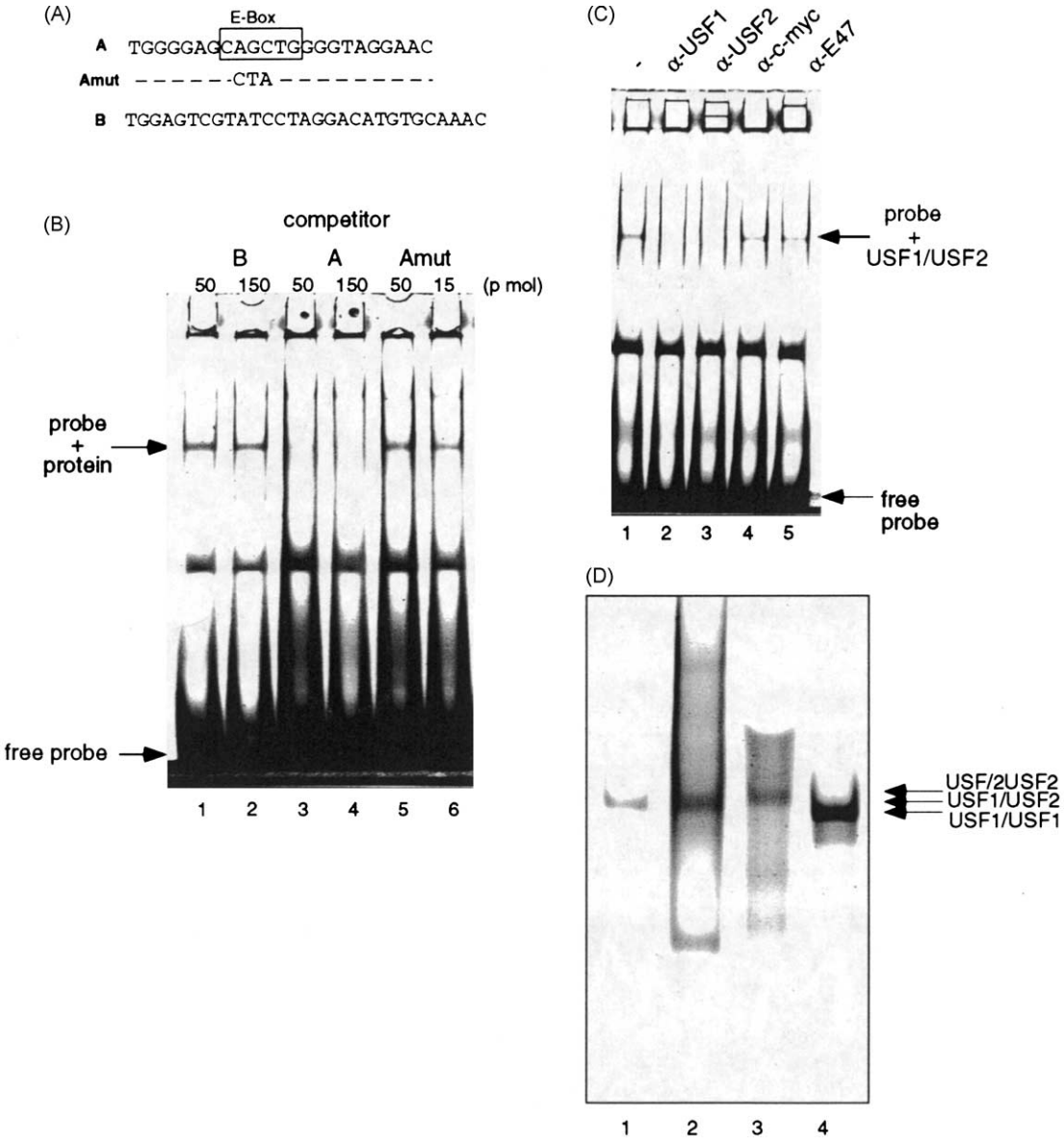

Fig. 3. Identification of nuclear factors binding to the intronic element (A) Nucleotide sequence of the probe and competitors applied in BMSA. A: self competitor; Amut: mutant competitor; B: non-specific competitor. (B) Rhodamine labeled probe A and nuclear extract from RBL-2H3 cells were used for EMSA. Competitive binding assay was performed with unlabeled competitor B, A or Amut. Lanes 1, 2: with competitor B; lanes 3, 4: competitor A; lanes 5, 6: competitor Amut. Lanes 1, 3, 5: 50 pmol of competitors; lanes 2, 4, 6: 150 pmol of competitors. (C) The antibodies against E-box binding proteins were added in EMSA. Lane 1: none; lane 2: anti-USF1; lane 3: anti-USF2; lane 4: anti-c-Myc; lane 5: anti-E47. (D) Lane 1: USF1/USF2; lane 2: nuclear protein from RBL-2H3 cells; lane 3: USF2/USF2; lane 4: USF1/USF1. The proteins in lane 1, 3, 4 were produced by in vitro transcription/translation.

Nishiyama et al., 2001). A product of 521 bp was obtained using a primer set of Ex3-F and Ex5-R which was designed to amplify a DNA fragment including exon 3–5 (lane1). On the other hand, when a different primer Ex1A–F (−11/+9; exon 1A of 9 bp attached with its upstream region of 11 bp at 5′-site) was employed instead of Ex3-F, a product of 776 bp size was obtained (Fig. 6, lane 2), which indicated that the mRNA containing exon 1A and 2A was actually expressed in KU812 cells. Although precise quantitative analysis has not yet been performed, the amount of the product of 776 bp was always less than that of the 521 bp product. It was consistent with the results of 5′-RACE where most of the obtained clones had almost the same 5′-ends as the transcription start site of exon 1 reported by Pang et al. The promoter located upstream of exon 1 seems to serve as a major promoter for the α-chain gene expression.

We next analyzed the transcriptional activity of a putative promoter present at upstream of exon 1A in transient transfection assays of RBL-2H3 and PT18 cells. A DNA fragment containing the 5′-region of 1.36 kb, exon 1A, and 2.4 kb of a 5′-portion of intron 1A (an intron between exon

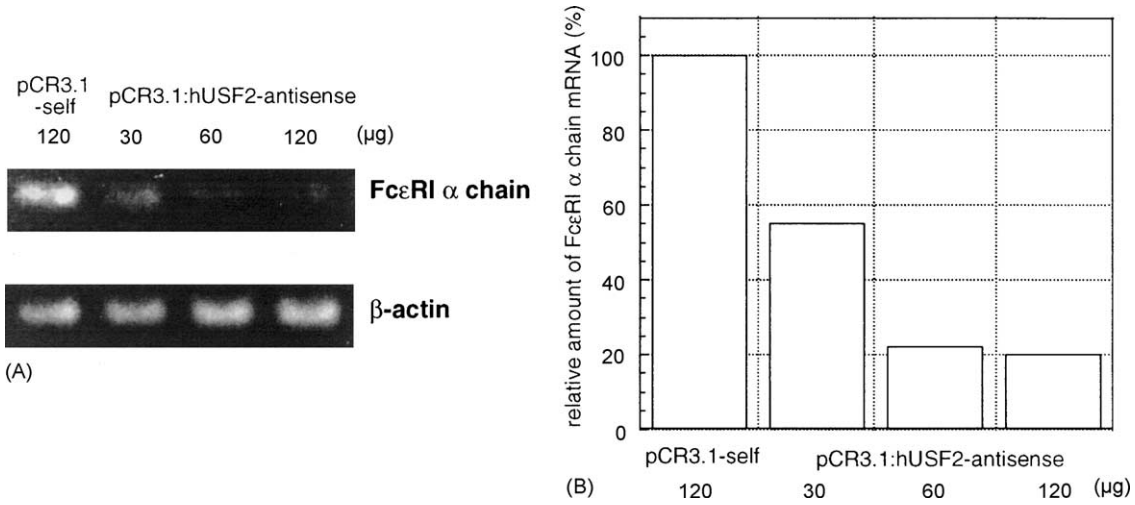

Fig. 4. Reduction of FcεRI α-chain mRNA by the expression of USF2 antisense KU812 cells were transfected with human USF2 antisense expression plasmid. An empty vector, pCR3.1-self was used as a control. After the selection with G418, total RNA was prepared from the cells. The amount of the mRNA for FcεRI α-chain and that for β-actin was quantitated by RT-PCR. (A) Ethidium bromide staining of PCR products in agarose gel. (B) Quantitation of the mRNA levels for FcεRI α-chain. The density of each band for FcεRI α-chain was corrected with that of β-actin. Represented values are relative ratios to the control.

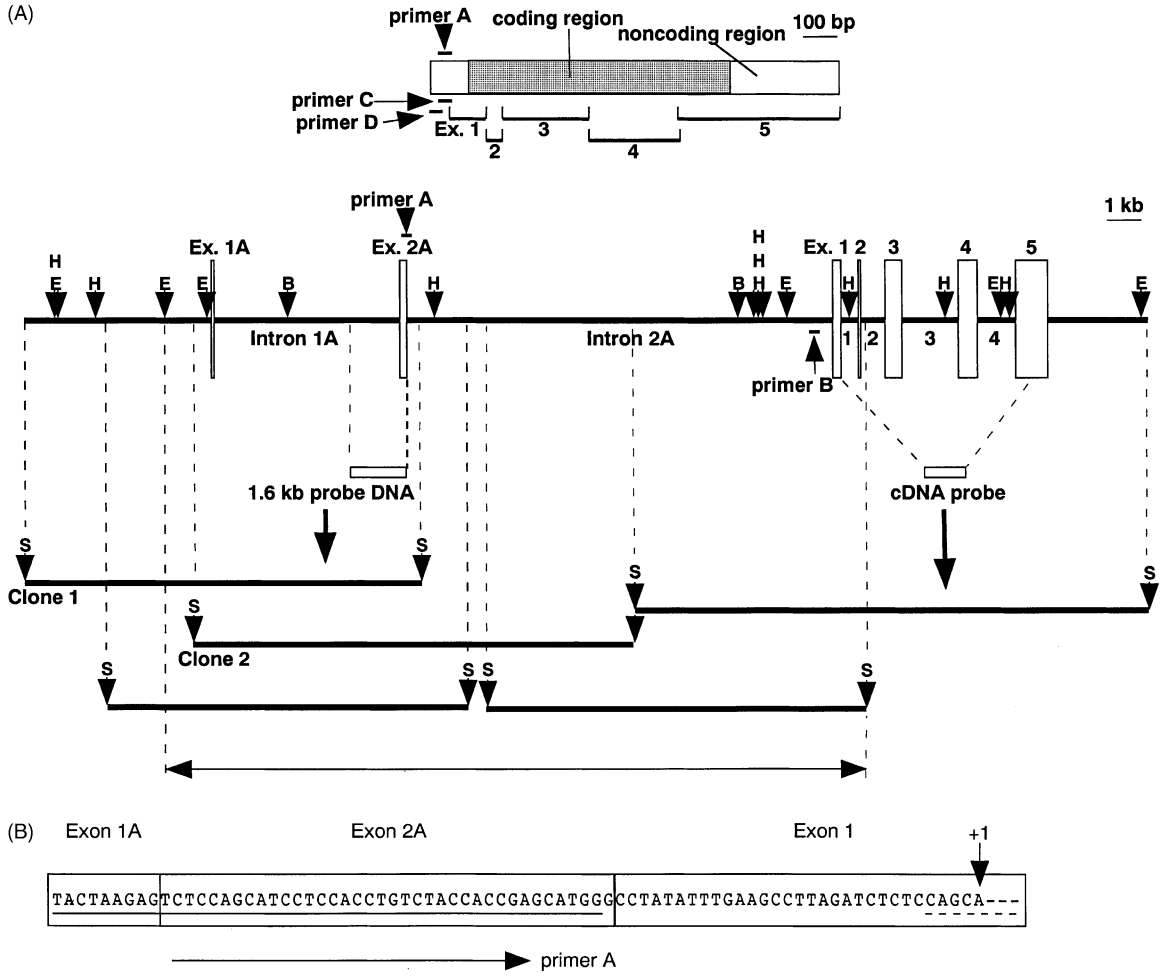

Fig. 5. Structures of human FcεRI α-chain cDNA and genomic DNA (A) Restriction endonuclease recognition sites of *Bam*HI, *Eco*RI, and *Hin*dIII, are shown by B, E, and H, respectively. Region whose nucleotide sequence was determined is indicated by a double-headed arrow. (B) Nucleotide sequence of 5′-end of cDNA encoded by exon 1A, 2A and 1 was boxed. The nucleotide sequence of Kochan's cDNA and that not found in reported genomic DNA was underlined, and cDNA sequence of Shimizu et al. was shown with dotted line. Oligonucleotides used as primer A, C and D are indicated with arrows.

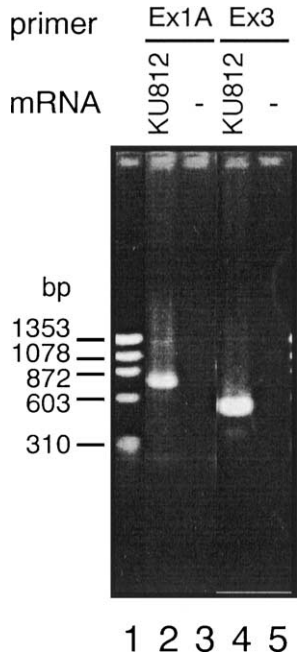

Fig. 6. Agarose gel eleetrophoresis of RT-PCR products containing exon 1A and 2A Lane 1: Molecular Weight marker (ϕX174/*Hae*III digest). 1 μg of total RNA from KU812 cells (lanes 2 and 4) or distilled water (lanes 3 and 5) was used for RT reaction. As the forward primer of PCR, Ex1A–F (lanes 2 and 3) or Ex3-F (lanes 4 and 5) was used.

1A and exon 1B, see Fig. 5) showed the ability to promote transcription when inserted upstream of a luciferase gene. Since, it did not give the transcription activity in an α-chain negative cell line, Jurkat, the promoter was revealed to function only in α-chain positive cells.

Transcription from this promoter was decreased by a partial deletion of intron 1A in the above construct, suggesting the presence of a regulatory element in the intron. Further mapping of the *cis*-acting element(s) by site-directed mutagenesis revealed that the region located at about 360 bp downstream from exon 1A functioned as an enhancer. Consistent with this, EMSA demonstrated the presence of nuclear factors which bound this region in FcεRI α-chain positive cells. One of these factors was identified as YY-1 by EMSA in the presence of anti-YY-1 antibody.

The expression of human FcεRI α-chain gene is known to be upregulated by IL-4 stimulation. However, a typical STAT6 recognition sequence has not been identified around the major promoter (upstream of exon 1), while a STAT6 recognition sequence of 5′-TTCattaGAA-3′ is present just downstream of exon 1A. This suggests the novel promoter might be upregulated by IL-4 stimulation through this possible STAT6 motif. Although, we do not know the physiological role of this promoter at present, it will be interesting to investigate whether this promoter is activated in a specific type or state of cells and whether there are different activation patterns of the two promoters between normal and allergic individuals.

5. Conclusion remarks

This study is the first step for elucidating the regulatory mechanism of FcεRI α-chain gene expression. Taking the fact into consideration that anaphilaxis was actually abolished in the FcεRI α-chain deficient mice (Dombrowicz et al., 1993), the identification of the regulatory elements and binding factors may provide new targets for therapeutic or preventive agents against allergy. For example, the USF2 antisense transfection experiment demonstrated that the inhibition of the binding between *trans*-activator and DNA element would prevent the IgE-mediated allergic reaction by suppressing the expression of FcεRI as a result of decreased expression of the α-chain. More detailed study will reveal the mechanisms of the FcεRI α-chain gene expression in near future.

References

Dombrowicz, D., Flamand, V., Brigman, K.K., Koller, B.H., Kinet, J.P., 1993. Abolish of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α-chain gene. Cell 75, 969–976.

Dombrowicz, D., Lin, S., Flamand, V., Brini, A.T., Koller, B.H., Kinet, J.P., 1998. Allergy-associated FcRβ subunit is a molecular amplifier of IgE- and IgG-mediated in vivo responses. Immunity 8, 517–529.

Donnadieu, E., Jouvin, M.H., Kinet, J.P., 2000. A second amplifier function for the allergy-associated FcεRI-β subunit. Immunity 12, 515–523.

Eichbaum, Q.G., Iyer, R., Raveh, D.P., Mathieu, C., Ezekowitz, R.A.B., 1994. Restriction of interferon γ responsiveness and basal expression of the myeloid human FcγRIb gene is mediated by a functional PU.1 site and a transcription initiator consensus. J. Exp. Med. 179, 1985–1996.

Ernst, L.K., Duchemin, A.M., Anderson, C.L., 1993. Association of the high-affinity receptor for IgG (Fc γ RI) with the γ subunit of the IgE receptor. Proc. Natl. Acad. Sci. U.S.A. 90, 6023–6027.

Feinman, R., Qiu, W.Q., Pearse, R.N., Nikolajczyk, B.S., Sen, R., Sheffery, M., Ravetch, J.V., 1994. PU.1 and an HLH family member contribute to the myeloid-specific transcription of the FcγRIIIA promoter. EMBO J. 13, 3852–3860.

Fung-Leung, W.P., Sousa-Hitzler, J.D., Ishaque, A., Zhou, L., Pang, J., Ngo, K., Panakos, J.A., Chourmouzis, E., Liu, F.T., Lau, C.Y., 1996. Transgenic mice expressing the human high-affinity immunoglobulin (Ig) E receptor α chain respond to human IgE in mast cell degranulation and in allergic reactions. J. Exp. Med. 183, 49–56.

Gosset, P., Lamblin-Degros, C., Tillie-Leblond, I., Charbonnier, A.S., Joseph, M., Wallaert, B., Kochan, J.P., Tonnel, A.B., 2001. Modulation of high-affinity IgE receptor expression in blood monocytes: opposite effect of IL-4 and glucocorticoids. J. Allergy Clin. Immunol. 107, 114–122.

Johansson, E., Hjortsberg, K., Thelander, L., 1998. Two YY-1-binding proximal elements regulate the promoter strength of the TATA-less mouse ribonucleotide reductase R1 gene. J. Biol., Chem. 273, 29816–29821.

Kochan, J., Pettine, L.F., Hakimi, J., Kishi, K., Kinet, J.P., 1988. Isolation of the gene coding for the α subunit of the human high affinity IgE receptor. Nucleic Acids Res. 16, 3584.

Lin, S., Cicala, C., Scharenberg, A.M., Kinet, J.P., 1996. The FcεRI β subunit functions as an amplifier of FcεRI γ-mediated cell activation signals. Cell 85, 985–995.

Miller, L., Blank, U., Metzer, H., Kinet, J.P., 1989. Expression of high-affinity binding of human immunoglobulin E by transfected cells. Science 244, 334–337.

Navankasattusas, S., Sawadogo, M., Van Bilsen, M., Dang, C.V., Chien, K.R., 1994. The basic helix–loop–helix protein upstream stimulating factor regulates the cardiac ventricular myosin light-chain 2 gene via independent *cis*-regulatory elements. Mol. Cell. Biol. 14, 7331–7339.

Nishiyama, C., Takahashi, K., Nishiyama, M., Okumura, K., Ra, C., Ohtake, Y., Yokota, T., 2000. Splice isoforms of transcription factor Elf-1 affecting its regulatory function in transription-molecular cloning of rat Elf-1. Biosci. Biotechnol. Biochem. 34, 2601–2607.

Nishiyama, C., Hasegawa, M., Nishiyama, M., Takahashi, K., Yokota, T., Okumura, K., Ra, C., 2001. Cloning of full-length genomic DNA encoding human FcεRI α-chain and its transcriptional regulation. Biochem. Biophys. Res. Commun. 284, 1056–1064.

Nishiyama, C., Hasegawa, M., Nishiyama, M., Takahashi, K., Akizawa, Y., Yokota, T., Okumura, K., Ra, C., 2002. Regulation of human FcεRI α-chain gene expression by multiple transcription factors. J. Immunol. 168, 4546–4552.

Pang, J., Taylor, G.R., Munroe, D.G., Ishaque, A., Fug-Leung, W.P., Lau, C.Y., Liu, F.T., Zhou, L., 1993. Characterization of the gene for the human high affinity IgE receptor (FcεRI) α-chain. J. Immunol. 151, 6166–6174.

Pawankar, R., Okuda, M., Yssel, H., Okumura, K., Ra, C., 1997. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. J. Clin. Invest. 99, 1492–1499.

Pfefferkorn, L.C., Yeaman, G.R., 1994. Association of IgA-Fc receptors (FcαR) with FcεRIγ2 subunit in U937 cells. J. Immunol. 153, 3228–3236.

Ra, C., Jouvin, M.H., Blank, U., Kinet, J.P., 1989. A macrophage Fc receptor and the mast cell receptor for IgE share an identical subunit. Nature 341, 752–754.

Shimizu, A., Tepler, I., Benfey, P.N., Berenstein, E.H., Siraganian, R.P., Leder, P., 1988. Human and rat mast cell high-affinity immunoglobulin E receptors: characterization of purative α-chain gene products. Proc. Natl. Acad. Sci. U.S.A. 85, 1907–1911.

Takahashi, K., Nishiyama, C., Nishiyama, M., Okumura, K., Ra, C., Ohtake, Y., Yokota, T., 2001. A complex composed of USF1 and USF2 activates the human FcεRI α-chain expression via CAGCTG element in the first intron. Eur. J. Immunol. 31, 590–599.

Timchenco, N., Wilson, D.R., Tayler, L.R., Abdelsayed, S., Wilde, M., Sawadogo, M., Darlington, G.J., 1995. Autoregulation of the human C/EBP α gene by stimulation of upstream stimulatory factor binding. Mol. Cell. Biol. 15, 1192–1203.

Toru, H., Ra, C., Nonoyama, S., Suzuki, K., Yata, J.I., Nakahata, T., 1996. Induction of the high-affinity IgE receptor (FcεRI) on human mast cells by IL-4. Int. Immunol. 8, 1367–1873.

Vallet, V.S., Henrion, A.A., Bucchini, D., Casado, M., Raymondjean, M., Kahn, A., Vaulont, S., 1997. Glucose-dependent liver gene expression in upstream stimulatory factor $2^{-/-}$ mice. J. Biol. Chem. 272, 21944–21949.