

Regulation of the Human FcεRI α-Chain Distal Promoter¹

Masanari Hasegawa,*[†] Chiharu Nishiyama,^{2*} Makoto Nishiyama,[‡] Yushiro Akizawa,*[§] Kyoko Takahashi,[¶] Tomonobu Ito,* Susumu Furukawa,[†] Chisei Ra,[¶] Ko Okumura,* and Hideoki Ogawa*

The α-chain of the high affinity receptor for IgE (FcεRI) is essential for cell surface expression of FcεRI and binding of the IgE Ab. The human α-chain gene possesses two promoters: the proximal promoter, which is highly conserved with that of rodent; and the distal promoter, the structure and role of which are largely unknown. Transcriptional regulation of the α-chain distal promoter was investigated in this study. Transient reporter assay revealed critical region for transcription activity located within -27/-17. EMSA identified Elf-1, YY1, and PU.1 as transcription factors binding to this region. In contrast to the proximal promoter, which was *trans*-activated by YY1 and PU.1, these transcription factors exhibited repressive function on this promoter. Addition of IL-4 caused a marked increase in transcription from the distal promoter and subsequently increased the intracellular production of the α-chain. These results indicate that IL-4-dependent up-regulation of the human α-chain was due to enhancement of distal promoter activity and suggests that the two promoters have different regulatory mechanisms for α-chain expression. *The Journal of Immunology*, 2003, 170: 3732–3738.

The high affinity receptor for IgE, FcεRI, is expressed on the surface of effector cells, such as mast cells and basophils. Cross-linking of IgE Abs bound with FcεRI by multivalent Ags induces activation of these cells and results in secretion of allergic mediators as well as induction of cytokine gene transcription. Therefore, FcεRI plays a central role in the induction and maintenance of the allergic responses.

Among the three subunits (α-, β-, and γ-chains) forming FcεRI, the α-chain is the specific component of FcεRI that directly binds IgE. Involvement of the α-chain in the FcεRI-mediated allergic reaction was definitively demonstrated by the absence of allergic reactions in α-chain-deficient mice (1).

Expression of FcεRI was up-regulated by IL-4 in human mast cells, eosinophils, or monocytes (2–7). In those studies, IL-4 stimulation increased the amount of α-chain mRNA and its product. Because expression of the other two subunits is not affected by IL-4 stimulation, it is believed that α-chain expression determines the cell type-specific expression of FcεRI in response to IL-4 stimulation. Therefore, elucidation of the mechanisms for the FcεRI α-chain expression may provide important information on the prevention of allergic diseases. We analyzed a promoter just upstream of the exon, which was previously identified as the first exon of the α-chain gene, and found that this promoter was up-regulated by PU.1 and YY1 cooperating with GATA-1 and down-regulated by

Elf-1 (8). However, this promoter, which we tentatively termed the proximal promoter in this report, was not affected by IL-4 stimulation (C. Nishiyama, unpublished observation).

We recently identified two novel exons at 18.4 and 12.6 kb upstream of the first exon of human FcεRI α-chain gene. However, the biological function of this region has not yet been elucidated (9). We herein report that a promoter, tentatively designated the distal promoter, which is located upstream of these newly identified exons of human α-chain gene, is up-regulated by IL-4 stimulation.

Materials and Methods

Cell cultures

KU812 and HML/SE cells were cultured in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 10% heat-inactivated FCS (Biological Industries, Haemek, Israel), 100 U/ml penicillin, 100 μg/ml streptomycin, 10⁻⁴ M 2-ME, and 10 μM MEM nonessential amino acids solution. For cultivation of HML/SE cells, 5 ng/ml human rGM-CSF (Genzyme, Minneapolis, MN) was added to the medium (10). HMC-1 cells were cultured in IMEM (Sigma-Aldrich) supplemented with 10% heat-inactivated FCS, penicillin, and streptomycin. THP1 cells were cultured in RPMI 1640 containing 10% heat-inactivated FCS, 10 mM HEPES (pH7.4), penicillin, and streptomycin. RT-PCR and 5'-RACE were performed using mRNA prepared from KU812, HML/SE, and HMC-1 as the template. For transient reporter assay and EMSA, KU812 was used. THP1 was used for IL-4 stimulation experiments.

RT-PCR and 5'-RACE

Total RNA was prepared from each cell using TRIzol reagent (Invitrogen, Leek, The Netherlands). RT-PCR was performed using superscript II and oligo(dT) primer (Invitrogen) for reverse transcription, the Advantage 2 polymerase mix (Clontech Laboratories, Palo Alto, CA) was used for PCR, and the 5'-RACE kit (Invitrogen) was used for 5'-RACE analysis. The following oligonucleotides were used as primers (see Fig. 1): Ex. 1A (5'-GAATTCCTCCATGCTACTAAGAG-3'); Ex. 2A (5'-TCTCCAGCATCCTCCACCTGTCTAC-3'); Ex. 3 (5'-GTGACTCTTATATGCAATGGAACAATT-3'); and Rev. (5'-GTGTCCACAGCAACAGAATCACC-3') for RT-PCR; and human α-GSP1 (5'-CCTTATAATAGATCACCTTG TACACATCC-3'), human α-GSP2 (5'-CCTCCAACCATGGCACCT GAGGAAGAGGG-3'), human α-GSP3 (5'-CCACCTCAGCAGAGGC CTGAAGGAGCAGCC-3'), and human α-GSP4 (5'-CCCATGCTCGGT GGTAGACAGGTGGAGGAT-3') for 5'-RACE. To amplify mRNA for β-actin, PCR was performed using the synthetic oligonucleotides, 5'-GCGCTCGTCGTCGACAACGG-3' and 5'-CATCGGAACCGCTCATT

*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo, Japan; [†]Department of Pediatrics, Yamaguchi University School of Medicine, Ube-shi, Yamaguchi, Japan; [‡]Biotechnology Research Center, University of Tokyo, Tokyo, Japan; [§]Pharmacobioregulation Research Laboratory, Hanno Research Center, Taiho Pharmaceutical Co., Ltd., Saitama, Japan; [¶]Department of Molecular Cell Immunology and Allergy, Advanced Medical Research, Nihon University School of Medicine, Tokyo, Japan

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² Address correspondence and reprint requests to Dr. Chiharu Nishiyama, Atopy (Allergy) Research Center, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo, 113-8421, Japan. E-mail address: chinishi@med.juntendo.ac.jp

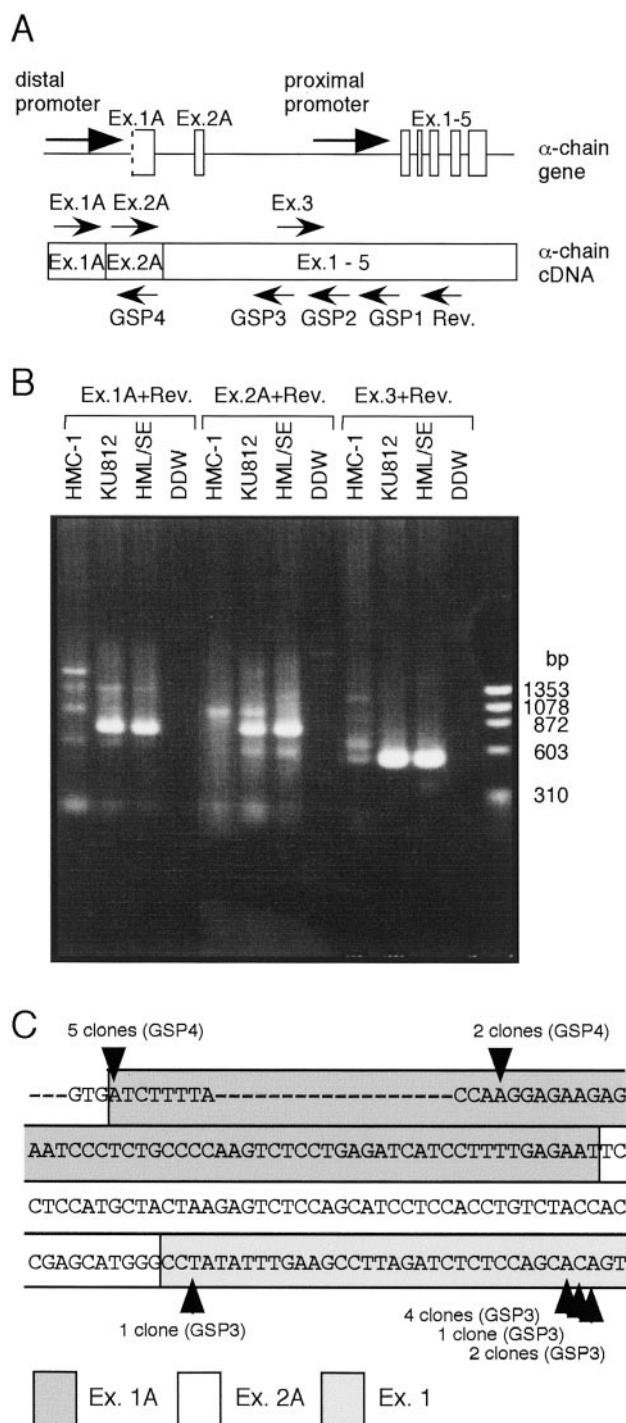


FIGURE 1. The distal promoter of human FcεRI α-chain gene is functional in KU812 and HML/SE. **A**, Structures of human FcεRI α-chain genomic DNA and cDNA. Both promoters of the α-chain and primers used in this study are indicated by arrows on the structure of genomic DNA and cDNA, respectively. **B**, Agarose gel electrophoresis of RT-PCR products containing exons (Ex.) 1A and 2A. **C**, 5'-End of α-chain mRNA determined by 5'-RACE analysis.

GCC-3'. Each PCR product was subcloned into pCR2.1 using a TOPO TA cloning kit (Invitrogen), and nucleotide sequences were determined using an ABI PRISM377 DNA sequencer (Applied Biosystems, Foster City, CA).

Plasmid construction

Several reporter plasmids were constructed as follows. A Quick change site-directed mutagenesis kit (Stratagene, La Jolla, CA) was used to intro-

duce a *Hind*III restriction site at +7/+12 of exon 1A of human α-chain gene on pGV-B2-α1EB3.8 (9), which carried part of intron 1A, exon 1A, and an upstream region of 1 kb containing the distal promoter, in reverse order. *Hind*III digestion followed by self-ligation of the resulting plasmid resulted in generation of a reporter plasmid carrying the distal promoter (−1033/+7) at the 5'-site of the luciferase gene. Other plasmids containing a variety of 5'-truncation or nucleotide replacement in the distal promoter were constructed similarly to procedures in previous reports (8, 9, 11) by site-directed mutagenesis and/or restriction endonuclease digestion. Plasmids, pCR-PU.1 (8), pCR-Elf-1/type 1 (8, 12), pCR-YY1 (8, 13), and pCR-Oct-1 (26), were used to produce PU.1, Elf-1, YY1, and Oct-1, respectively.

Transfection and luciferase assay

Transient reporter assay was performed as described previously (8, 9, 11). Harvested KU812 cells were suspended in culture medium supplemented with 10% FCS. Cells ($5\text{--}10 \times 10^5$ in 0.5 ml) were cotransfected with 5 μg of test reporter construct and 25 ng of pRL-CMV (Promega, Madison, WI) by electroporation using Bio-Rad Gene Pulsar II (Bio-Rad, Hercules, CA) set at 320 V and 950 μF and were cultured for 24 h (Figs. 3 and 4). For coexpression analysis, 3 μg of reporter plasmid and a total of 9 μg of expression plasmids were introduced into cells (Fig. 6). The luminescence of each cell lysate was measured by a MicroLumat Plus (Berthold, Postfach, Germany), as described previously (8, 9, 11).

EMSA

EMSA probes were prepared by annealing FITC-labeled synthetic oligonucleotides (5'-CAATAGGAAGGAGAAGGAAGCACTTTCCAG-3') with oligonucleotides having the complementary sequence. A nuclear extract of KU812 cells was prepared as described previously (8, 9, 11). EMSA was performed in the same way as described previously (8, 9, 11, 14, 15). Anti-Elf-1, anti-PU.1, and anti-YY1 Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). For competition experiments, nonlabeled competitor oligonucleotides were added to the reaction mixture. Band shift on polyacrylamide gel was subjected to fluorescence detection using a FluorImager 595 (Molecular Dynamics, Sunnyvale, CA).

IL-4 stimulation

Monocytes were enriched from human peripheral blood by a Percoll-based density gradient centrifugation technique. Enriched cells and THP1 were incubated with 200 and 25 U/ml of human rIL-4, respectively, and cultivated for an additional 4–24 h, as described in previous reports (7, 16).

Flow cytometric analysis

For detection of cell surface expression of the FcεRI α-chain, THP1 cells (5×10^6) were washed with PBS and incubated with FITC-conjugated anti-human FcεRI α-chain Ab (17), or FITC-conjugated mouse IgG2b (isotype control; BD PharMingen, San Diego, CA) for 1 h at 4°C in PBS containing 2% FCS. For detection of intracellular expression of the FcεRI α-chain, cells were permeabilized by treatment with cold 70% ethanol for 5 min at 4°C in PBS containing 2% BSA and 2% FCS and were then incubated with FITC-conjugated anti-human FcεRI α-chain Ab or FITC-conjugated mouse IgG2b. Finally, cells were washed with PBS twice, and α-chain expression was analyzed by FACSCalibur (BD Biosciences, Mountain View, CA).

Results

Transcriptional initiation site of the human α-chain distal promoter

We initially screened for cell lines in which the distal promoter was functional. For this purpose, we performed RT-PCR analysis with total RNA prepared from the human basophil-like cell line KU812, mast cell line HMC-1, and megakaryocyte cell line HML/SE. Major bands of the expected sizes, 776 bp, 753 bp, and 521 bp, were detected by primer set Ex.1A/Rev., Ex.2A/Rev., and Ex.3/Rev., respectively, on agarose gel electrophoresis, when total RNA prepared from KU812 and HML/SE was used as the template for PCR, whereas no corresponding fragments were produced by PCR using cDNA from HMC-1 as the template and Ex.1A or Ex.2A as the forward primer (Fig. 1B). This result indicated that KU812 and HML/SE cells were suitable for analysis of the distal promoter.

We recently found that the 5'-terminal 9 bp of the longest cDNA reported by Shimizu et al. (18) corresponds to a part of exon 1A (9) and suggested that exon 1A possesses further 5'-terminal extension. To determine the actual 5'-terminal end (the transcription initiation site) of exon 1A, we performed 5'-RACE analysis with α-chain mRNA from KU812. When 5'-RACE was conducted using GSP1, 2, and 3 as the primers for reverse transcription, first, and second PCR, respectively, all clones started within exon 1 but did not contain any parts of exons 1A and 2A (Fig. 1C). This suggested that the transcript from the proximal promoter was major and that from the distal promoter was minor under the conditions used. We then used GSP4 instead of GSP3 as the primer for second PCR to amplify the α-chain mRNA containing exons 1A and 2A (Fig. 1C) and determined the major and minor transcriptional initiation sites to be ~330 and 60 bp upstream of the 5'-end of the previously reported longest cDNA (9, 18). Similar results were obtained using mRNA from HML/SE cells (data not shown). The nucleotide sequences of exon 1A and its 5'-flanking region are shown in Fig. 2 (EMBL accession number AB059236).

Transcriptional regulatory region of the distal promoter

For identification of *cis*-acting elements of the promoter, several constructs carrying the 5'-flanking regions of various lengths (−1030 to −27; the major initiation site is designated as +1) were generated, and introduced into KU812 cells (Fig. 3). We found that even the shortest region (−27/+7) gave luciferase activity comparable with that of longer regions, suggesting that the region contained essential elements for activation of the distal promoter.

For fine mapping of the enhancer elements in the minimum promoter region (−27/+7), we then constructed several plasmids, each of which contained 6-bp nucleotide replacements between −27 and +2 of the minimum distal promoter (Fig. 4). Nucleotide substitutions between −27 and −17 and between −4 and +2 resulted in a marked decrease in promoter activity, whereas substitutions between −16 and −5 did not show any apparent effects.

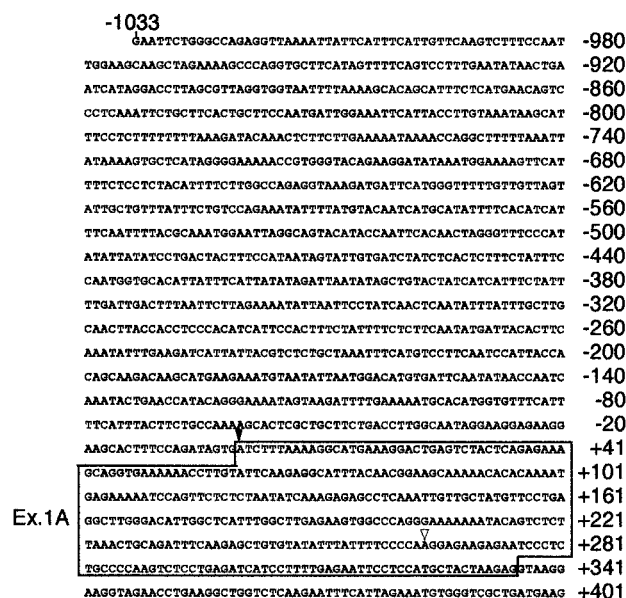


FIGURE 2. Nucleotide sequence of the 5'-flanking region of the distal promoter of human FcεRI α-chain gene (EMBL accession number AB059236). Transcriptional initiation site determined by 5'-RACE in Fig. 1 is indicated as +1. ▼, Major transcriptional initiation site; ▽, minor initiation site.

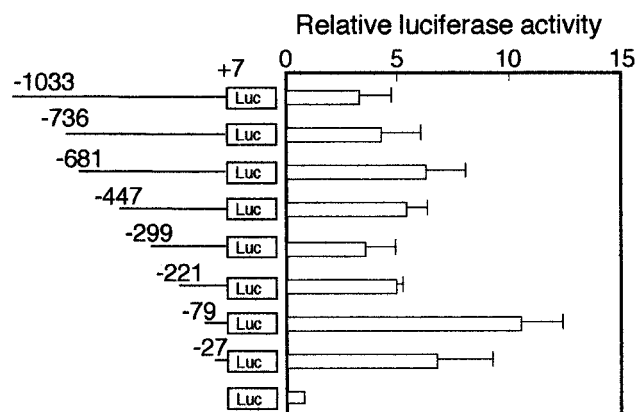


FIGURE 3. Determination of transcriptional regulatory region of the distal promoter. 5'-Deleted constructs of the distal promoter were introduced into KU812 cells by electroporation. The relative luciferase (Luc) activity is given relative to that of pGL3-Basic (promoterless reporter plasmid), and the results are expressed as the mean $\pm$ SE of more than three independent experiments performed in duplicate for each sample.

Identification of transcription factors binding to −27/−17

Transient reporter assay revealed that −27/−17 and −4/+2 were essential for promoter activity. We performed EMSA to identify the transcription factors binding to −27/−17 but not to −4/+2, because the decrease in luciferase activity after nucleotide substitution at −4/+2 may have been due to effects on the initiator sequence, Inr. As shown in Fig. 5A, lane 2, five shift bands (shown by arrows) were observed in the assay using −36/−7 as a probe. The band shown with an asterisk disappeared in the presence of competitor 2, 3, or 4 as well as competitor 1, suggesting that the protein responsible for the band shift bound portions other than −32/−17, i.e., −36/−33 and/or −16/+7, in the probe DNA. Two shifted bands, indicated by the letters "E" and "P," were assigned to be the bands for Elf-1- and PU.1-DNA complexes, respectively, because addition of anti-Elf-1 or anti-PU.1 Ab resulted in disappearance of each band (lanes 4 and 3). Both bands substantially

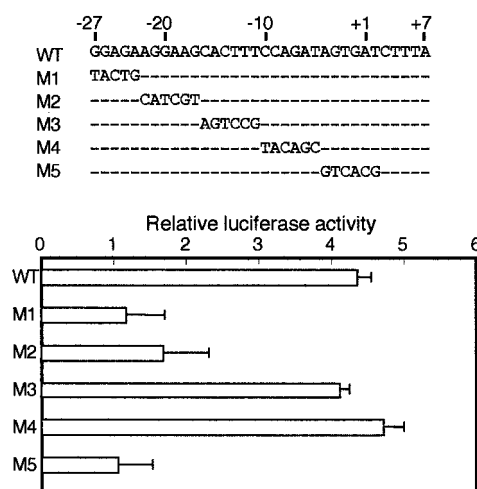


FIGURE 4. Determination of *cis*-enhancing elements in the minimum promoter by site-directed mutagenesis. Mutations were introduced into −27/+2 of the α-chain distal promoter plasmid carrying the minimum promoter (−27/+7). The various mutant plasmids were introduced into KU812 cells. Nucleotides that differed from the original are shown and lines represent unchanged nucleotides in mutant plasmids. Results are expressed as the mean $\pm$ SE of more than three independent experiments performed in duplicate for each sample. WT, Wild type.

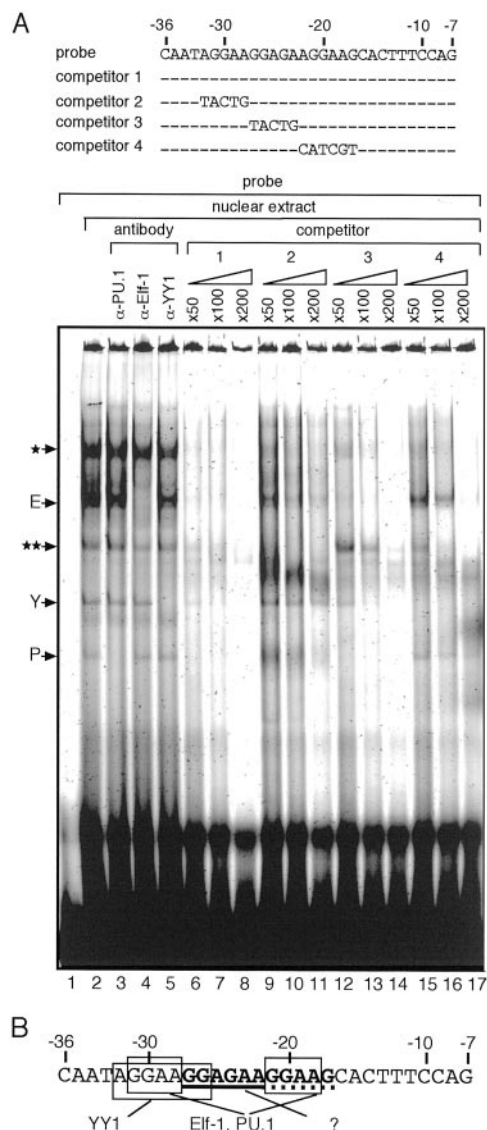


FIGURE 5. Nuclear proteins binding the critical elements in the distal promoter. *A*, EMSA profile. Lane 1, probe only; lanes 2–17, probe with nuclear extracts prepared from KU812 cells. Additives are: lane 1, none; lane 2, none; lane 3, anti-PU.1 (P); lane 4, anti-Elf-1 (E); lane 5, anti-YY1 (Y); lanes 6–8, competitor 1; lanes 9–11, competitor 2; lanes 12–14, competitor 3; lanes 15–17, competitor 4. *B*, Structure of the α -chain distal promoter. Putative binding core sequences for transcription factors, PU.1, Elf-1, and YY1 are shown. A putative binding region for an unidentified transcription factor is underlined. Nucleotides critical for activation of the distal promoter are in bold.

lost intensity by addition of competitor 3 as well as self competitor but were detected even when a 100-fold excess of competitor 2 or 4 was added, suggesting that two regions, $-32/-28$ and $-22/-17$, containing the motifs for Elf-1 and PU.1 were recognized by the transcription factors. Similarly, YY1 was identified to form a complex with probe DNA (shown with letter “Y”) by recognizing mainly $-32/-28$. Nuclear protein(s) causing a band shift shown with double asterisks was suggested to bind region $-27/-23$ of the probe but was not identified in the present study.

Transcription factors Elf-1, PU.1, and YY1 repressed activity of the distal promoter

According to the results of transient reporter assay and EMSA, the structure of the distal promoter was determined as shown in Fig.

5B, where three transcription factors, Elf-1, PU.1, and YY1, recognize limited and overlapping portions of the α -chain promoter. To investigate the roles of these transcription factors on the distal promoter, coexpression analysis was performed. Interestingly, all the transcription factors served to down-regulate the distal promoter (Fig. 6A). To examine the individual dose dependency of PU.1, Elf-1, or YY1, we performed coexpression analysis using 1.5–4.5 μ g of each expression plasmid. In this range, dose-dependent overproduction (10-fold more) of each transcription factor was confirmed by Western blotting analysis (data not shown). However, as shown in Fig. 6B, a dose-dependent inhibitory effect was observed in the range of 0–3.0 μ g of expression plasmids, and no further decrease in luciferase activity derived from cells transfected with 4.5 μ g of each expression plasmid was observed. In contrast, when Oct-1, which is a transcription factor unrelated to this promoter, was exogenously produced, no inhibitory effect of overproduced protein was observed. These results suggest that

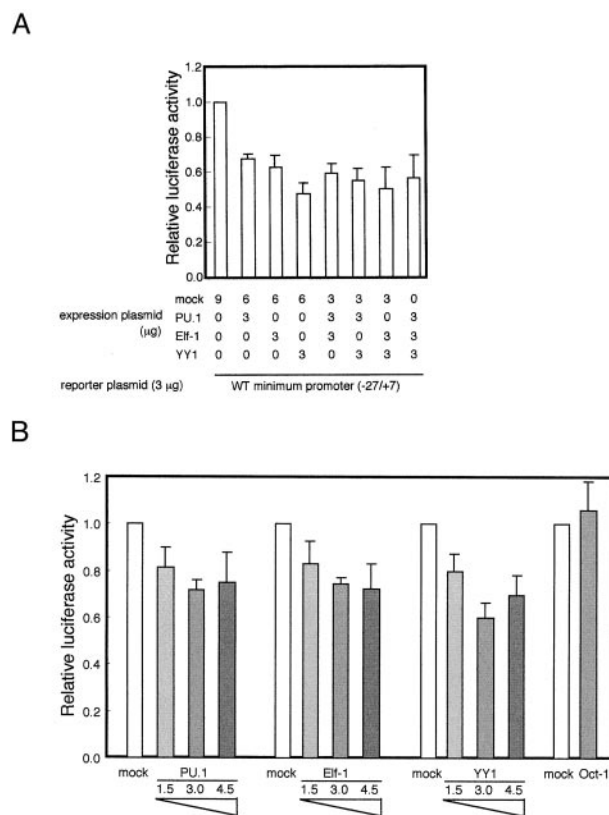


FIGURE 6. Cotransfection analysis. *A*, Effect of exogenously produced transcription factors for the minimum promoter. KU812 cells were transfected with 3 μ g of the promoter-reporter plasmid (wild-type (WT) minimum promoter ($-27/+7$)) and 3 μ g of each of the expression plasmids. The total amount of plasmids used for transfection was adjusted to 12 μ g (3 μ g of reporter plasmid and totally 9 μ g of expression plasmids) by addition of the empty plasmid pCR3.1-self. The relative luciferase activities were expressed as the ratio of the activity in cells carrying the promoter construct to that of cells carrying the empty plasmids. Average values obtained from three independent experiments are shown. *B*, Dose dependency of inhibitory effects. KU812 cells were transfected with 3 μ g of reporter plasmid (wild-type minimum promoter; $-27/+7$) and 1.5–4.5 μ g of each expression plasmid. The relative luciferase activities derived from cells transfected with 3 μ g of pCR3.1-self and pCR-Oct-1 are shown as mock and Oct-1, respectively, because no effects were observed on these expression plasmids in the range of 1.5–4.5 μ g. Overproduction (>10 -fold) of exogenously produced transcription factors was confirmed by Western blotting analysis.

PU.1 and YY1 as well as Elf-1 repress the distal promoter, in contrast to the proximal promoter, which is positively regulated by PU.1 and YY1 (8).

Activation of distal promoter by IL-4 stimulation

We analyzed the effects of IL-4 stimulation on promoter activity of the human α -chain gene, because Fc ϵ RI α -chain mRNA and/or its translated product increase in response to IL-4 in human cells (2–7). Levels of both α -chain mRNA and its product remained unchanged in the cell lines KU812 and HML/SE in response to IL-4 stimulation. This observation concurs with a previous report in which KU812 showed no response to IL-4 stimulation (19). Therefore, THP1, which is intrinsically α -chain negative but expresses α -chain mRNA and Fc ϵ RI in response to IL-4 (16), was used for IL-4 stimulation experiments. We performed RT-PCR for α -chain

mRNA from THP1 treated with IL-4 for 4 h, because α -chain mRNA in THP1 was reported to be most abundant 4–5 h after IL-4 stimulation in a previous report (16). As shown in Fig. 7A (top), Fc ϵ RI α -chain mRNA accumulated in response to IL-4. In addition, when RT-PCR to detect the portion corresponding to exons 1A and 2A was performed, the portion (exons 1A/2A) accumulated in an IL-4-dependent manner (Fig. 7A, center). Similar increases in mRNA transcribed from the distal promoter was also observed when enriched monocytes were prepared from human peripheral blood by a Percoll-based density gradient centrifugation technique and stimulated with human IL-4 (Fig. 7A). According to FACS analysis, basophils were also included in this enriched monocyte fraction as a minor population (< 5%). Basophils express the Fc ϵ RI α -chain even in the absence of IL-4 stimulation, and thus might be a source of α -chain mRNA detected by PCR using common primers without IL-4 stimulation (Fig. 7A, top, lane 3). Nonetheless, the α -chain transcript from the distal promoter was increased by IL-4 stimulation in primary cells, as well as in the THP1 cell line. To elucidate whether the transcript from the distal promoter was actually translated to the α -chain protein, we then analyzed the expression level of the α -chain protein. In our experiment, α -chain cell surface expression in THP1 was not affected by IL-4 stimulation (Fig. 7B). In contrast, the intracellular levels of Fc ϵ RI α -chain protein were apparently increased by IL-4-stimulation and peaked at 6–7 h after stimulation. Elevated production of the α -chain decreased to nearly basal levels within 24 h after IL-4 stimulation (Fig. 7B).

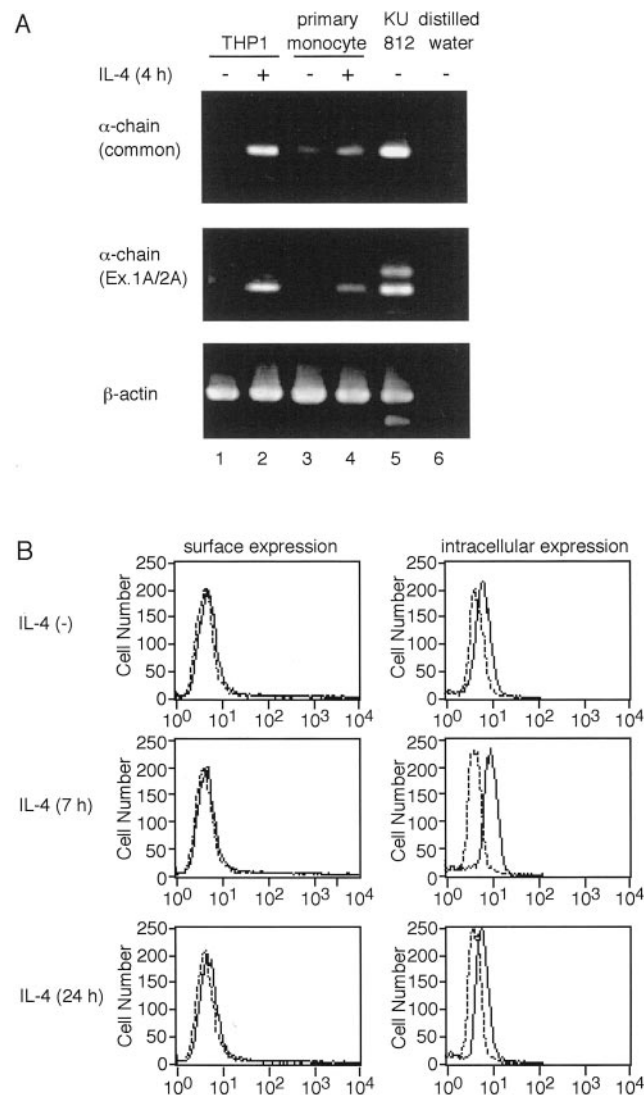


FIGURE 7. Effect of IL-4 stimulation on α -chain expression level. **A**, RT-PCR analysis of the α -chain mRNA. Total RNA from THP1 and enriched peripheral blood monocyte cells was prepared after the treatment with or without IL-4 for 4 h. Lanes 1 and 2, THP1; lanes 3 and 4, peripheral blood monocytes; lane 5, KU812 (positive control); lane 6, without RNA (negative control). **B**, Surface and intracellular expression of the α -chain in IL-4-stimulated THP1. The α -chain expression of the cells (—) was analyzed in correlation to the respective isotype control (.....). Typical results of a single experiment are shown. Similar results were observed in three other independent experiments.

Discussion

Expression of the Fc ϵ RI α -chain is known to be up-regulated by IL-4 in human cells (2–7). In contrast, IL-4-dependent up-regulation of Fc ϵ RI α -chain expression was not observed in mice (20). The nucleotide sequence of the proximal promoter was highly conserved between the human and mouse α -chain promoters, suggesting that similar regulation mechanisms operate for proximal promoters in both organisms. However, the proximal promoter did not respond to IL-4 stimulation in human cells (C. Nishiyama, unpublished observation). We recently identified two additional exons upstream of the exon that is known as the first exon of the α -chain gene (9). This also revealed that two promoters separated by ~19 kb functioned in expression of the gene.

In this study, we observed that IL-4 stimulation of THP1 and enriched peripheral blood monocytes caused marked increases in

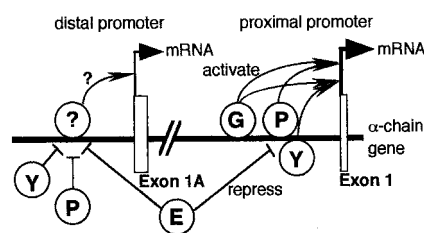


FIGURE 8. Schematic drawing of the human Fc ϵ RI α -chain distal and proximal promoters. E, Elf-1; G, GATA-1; P, PU.1; Y, YY1. *cis*-Enhancing elements in the proximal promoter are highly conserved between human and rodent, whereas the distal promoter has not yet been identified in rodent. Transcription of α -chain gene depends on the proximal promoter under normal conditions, whereas IL-4 stimulates transcription from the distal promoter. An erythroid-specific transcription factor GATA-1 trans-activates the proximal promoter cooperatively with a myeloid- and lymphoid-specific transcription factor, PU.1, or a ubiquitous transcription factor, YY1. Repression of the proximal promoter by Elf-1 and that of the distal promoter by PU.1, Elf-1, and YY1 is probably due to competition for DNA binding between transcription activators.

α -chain mRNA transcribed from the distal promoter. To date, the presence of exon 1A, exon 2A, and the distal promoter was not indicated in the mouse gene. Therefore, we may postulate that the distal promoter is responsible for human-specific up-regulation of IL-4-induced α -chain expression.

In this report, we determined the transcriptional initiation sites of the distal promoter of the α -chain gene and found that the promoter was negatively regulated by Elf-1, PU.1, and YY1. Taking into consideration our recent findings that the proximal promoter is positively regulated by PU.1 and YY1 (8), these transcription factors appear to possess opposing functions in controlling the two different promoters for the α -chain gene (Fig. 8). The presence of another nonidentified nuclear protein that recognizes a region $-27/-23$ was detected, although it has not yet been identified. This nuclear protein may activate the distal promoter competitively with Elf-1, PU.1, and YY1, which negatively regulate the promoter. As shown in Fig. 6A, the presence of one transcription factor is as inhibitory as all three. We presently believe that binding of one transcription factor is sufficient to prevent the nonidentified activator protein from binding to the core sequence, GGAGAA, due to the close proximity of the target sequences of each transcription factor, and this may be the reason for the absence of additive effects of the down-regulating transcription factors. Addition of competitor 2 (Fig. 5A, lanes 9–11) led to the formation of a complex causing a new band shift between the bands indicated by double asterisks and the letter “Y.” The protein causing the new band may be involved in regulation of the distal promoter, although we do not deal with the protein in the present study.

We identified $-27/+7$ as the minimum promoter by performing reporter assays for various 5'-truncated promoters in this study (Fig. 2). The highest luciferase activity was observed in the construct carrying $-79/+7$. This result may suggest that other cis-regulatory elements are present in the $-221/-28$ region; one may be the suppressing region ($-221/-80$), and the other may be the activating region ($-79/-28$). Further detailed analysis will be required to investigate this possibility.

The IL-4 signal is transduced to target genes via the transcription factor STAT6. Determination of the genomic sequence for the α -chain gene revealed two STAT6 recognition sequences, at $-874/-865$ in the distal promoter and just downstream of exon 1A ($+373/+382$). This suggests that the distal promoter is up-regulated by IL-4 stimulation through these putative STAT6 motifs. Further analysis will be required to elucidate the activation mechanisms of the distal promoter via these transcription factors.

In this study, we detected no apparent increase in cell surface expression of the α -chain by IL-4 stimulation, although intracellular levels of the α -chain protein were significantly increased. This seems contradictory to previous observations that IL-4 stimulates cell surface expression of Fc ϵ RI (7, 16). However, an IL-4-dependent increase in α -chain mRNA and α -chain protein in the intracellular space was shown in various studies (2, 21, 22), whereas an IL-4-dependent increase in the α -chain protein as Fc ϵ RI on cell surface was observed only in limited cells, such as primary cultured mast cells (3–6). The β and γ subunits are components of Fc ϵ RI and are involved in cell surface expression of Fc ϵ RI (21, 23). These subunits are also components of other different receptors (24, 25), and because expression of the β and γ subunits was not stimulated by IL-4 (3, 4), IL-4-induced overexpression of the α subunit does not necessarily result in increased cell surface expression of Fc ϵ RI. Therefore, we may attribute this apparent discrepancy in IL-4-dependent Fc ϵ RI expression on the

cell surface to shortage of the other subunits (β - and γ -chains) in cells that produce both subunits in small amounts, such as monocytes, eosinophils, and Langerhans cells (21). Nonetheless, the present findings indicate that IL-4 stimulation increases transcription of the α -chain via the distal promoter, which assures high level production of the protein. Increases in α -chain mRNA and/or protein were also observed in allergic patients (2, 4, 7, 21, 22). Therefore, it will be interesting in the near future to analyze the relationship between the increase of α -chain expression and distal promoter activity in allergic patients.

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