The Murine Interleukin-3 Receptor α Subunit Gene: Chromosomal Localization, Genomic Structure, and Promoter Function

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The interleukin-3 receptor (IL-3R) is composed of α and β subunits, members of the class I cytokine receptor family. Here we describe isolation and characterization of the chromosomal gene for the mouse IL-3R α subunit (mIL-3Rα). Whereas the human IL-3Rα gene is tightly linked with the granulocyte-macrophage colony-stimulating factor receptor α subunit (GM-CSFRα) gene in the pseudoautosomal region of the X and Y chromosomes, the mIL-3Rα gene (Il3ra) is located in the proximal region of mouse chromosome 14, separated from the mouse GM-CSFRα gene, which is on chromosome 19. The mIL-3Rα gene spans about 10 kb and is divided into 12 exons. All the exon-intron boundaries possess the splicing junction consensus sequences (5’GT-AG3’), and the whole genomic structure is similar to those of the previously reported class I cytokine receptor genes. There are two major transcription initiation sites that are located at 215 and 188 nucleotides upstream of the initiator codon. The promoter region is GC-rich and contains potential binding sites for GATA, Ets, c-myb, Sp1, Ap-2, and G-C boxes, but not a typical TATA or CAAT sequence. A fusion gene containing 0.8 kb of the 5’ noncoding sequence linked to the firefly luciferase gene directed the transcription in mouse mast cells but not in fibroblasts or T cells, suggesting that this promoter functions in a cell type-specific manner. Further sequential deletion of the 5’ region suggests two potential regulatory regions for transcription of the mIL-3Rα gene.

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MURINE IL-3 RECEPTOR α SUBUNIT GENE

MATERIALS AND METHODS

Cell culture. A mouse mast cell line, PT18\(^{8}\) was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 \(\mu\)mol/L 2-mercaptoethanol (2-ME), and 50 U/mL mGM-CSF. A mouse fibroblastic cell line, NIH3T3, was cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 5% FCS. An IL-2-dependent T cell line, CTLL2,\(^{2}\) was maintained in RPMI 1640 medium containing 10% FCS, 50 \(\mu\)mol/L 2-ME, and 100 U/mL mL-2.

Isolation and subcloning of genomic clones. A Balb/c spleen genomic library in Charon 4A (provided by Dr. Mark Davis, Stanford University, Stanford, CA) and an adult Balb/c liver genomic library in a SP6/T7 (Clontech, Palo Alto, CA) were screened with the 1.3-kb fragment encoding the entire IL-3Ra. Furthermore, a C129 mouse genomic library in ZEMBL was screened with a 0.6-kb Nco I-Sma I fragment of cDNA. The probes were labeled with \(^{32}\)PdCTP by the T7 Quick Prime kit (Pharmacia, Alameda, CA) to a specific activity of greater than 4 \(\times\) 10\(^{6}\) cpm/\(\mu\)g. Plaques were transferred to a nitrocellulose filter and hybridized for 18 hours at 42°C in hybridization solution (\(\times\) SSPE, 5% formamide, \(\times\) Denhardt solution, 100 \(\mu\)g/mL yeast tRNA, 0.2% sodium dodecyl sulfate [SDS]). Positive plaques were purified, and phage DNA was prepared by a standard plate lysate procedure. The mIL-3Ra gene was subcloned into pUC18 for further characterization and sequencing.

Characterization of cloned DNA fragment and DNA sequencing. The subcloned DNA fragments were mapped by restriction enzyme analyses, and the exons were localized by Southern hybridization using\(^{m}\)-3\(\alpha\)-cDNA as a probe. Supercircular plasmid DNA was alkali-denatured and sequenced by the dyeoxy chain termination method using Sequenase (United States Biochemical Corp, Cleveland, OH) for all the intron-exon junctions and short introns. The sizes of introns were determined by polymerase chain reactions (GeneAmp PCR reagent kit; Perkin Elmer Cetus, Norwalk, CT) using several oligonucleotide primers corresponding to mIL-3\(\alpha\) cDNA. The reaction mixture including 1 ng template DNA and 100 pmol/L each primer was incubated in a thermal cycler (Perkin Elmer Cetus) for 30 cycles (denatured for 1 minute at 94°C, annealed for 2 minutes at 50°C, elongated for 3 minutes at 72°C). Products were analyzed by electrophoresis on a 1.6% agarose gel in the presence of ethidium bromide.

Southern hybridization analysis of mouse genomic DNA. Genomic DNA (10 \(\mu\)g) from liver of adult Balb/c (Clontech) and C129 SV mice were digested to completion by the restriction endonucleases (EcoRI, BamHI, and HindIII). After electrophoresis on a 1% agarose gel, the DNA was treated with 0.25 \(\mu\)mol/L HCl, alkali-denatured (0.5 mol/L NaOH, 1.5 mol/L NaCl), neutralized (0.5 mol/L Tris HCl, ph 7.5; 1.5 mol/L NaCl), transferred to a Hybond-N nylon membrane (Amersham, Arlington Heights, IL) using 20\(\times\) SSPE, crosslinked by UV transilluminator (Stratagene, La Jolla, CA), and then hybridized with the \(^{32}\)P-labeled 1.3-kb mL-3Ra cDNA.

SMAC: rapid amplification cDNA ends (SMAC: RACE) assay. PolyA\(^{+}\) RNA was prepared from PT18 cells by the Fast Track mRNA Isolation kit (Invitrogen Corp, San Diego, CA). The mL-3Ra cDNA was reverse transcribed from 10 \(\mu\)g of polyA\(^{+}\) RNA together with an 18-mer oligonucleotide primer complementary to a sequence of the third exon (ST104; 5’GCCTACAGTTGATGACGGCCG3’). The cDNA purified by agarose gel electrophoresis was then ligated with AmpliFINDER anchor according to the manufacturer’s protocol (Clontech). The cDNA including 5’ end was amplified by polymerase chain reaction (PCR) as follows. One tenth of the ligiation product was amplified using a 38-mer anchor primer and an antisense 18-mer primer (ST-125; 5’GAGTGAGAAGGAGGGAGGC3’), under the following condition: 35 cycles of 94°C for 1 minute, 57°C for 1 minute, 72°C for 1 minute, and eight more cycles of the same enzyme. The first amplified product was then diluted one tenth and further amplified using another nested antisense primer (ST-143; 5’GCCCTGAGGCGCTGGGGGCGG3’) under the same condition except for annealing temperature at 55°C. The final amplified product was treated with T4 DNA polymerase to create blunt ends. After heat inactivation of polymerase, the DNA was cleaved with EcoRI, as the anchor sequence includes an EcoRI site. The resultant DNA fragments were purified by agarose gel electrophoresis and spin column (MicroSpin S-200; Pharmacia) and then subcloned into the EcoRI-Sma I sites of pUC18. The cloned DNAs were sequenced to identify the site(s) of 5’-cDNA ends.

Interspecific backcross mapping. Interspecific backcross progeny were generated by mating (C57BL/6J \(\times\) Mus spretus)F1 females and C57BL/6J males as described.\(^{11}\) A total of 205 \(N\) mice were used to map the Il3ra locus (see Results for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed as described.\(^{11}\) All blots were prepared with Zetabind nylon membrane (AMF-Cuno, Meriden, CT). The probe, a 1.3-kb Xho I fragment of mL-3Ra cDNA (SUT-1), was labeled with \(^{32}\)PdCTP using a random primed labeling kit (Boehringer Mannheim, Indianapolis, IN); washing was performed to a final stringency of 1.0X SSCP, 0.1% SDS, 65°C. A major fragment of 7.6 kb was detected in Bgl II-digested C57BL/6J DNA, and a major fragment of 11.0 kb was detected in Bgl II-digested M spretus DNA. The presence or absence of the 11.0-kb M spretus-specific Bgl II fragment was followed in backcross mice.

A description of the probes and restriction fragment length polymorphisms (RFLPs) for the loci linked to Il3ra, including retinoic acid receptor beta (Rarb, formally referred to as Hap), plasmagin activator urokinase (Plau), and SP-A pulmonary surfactant protein (Sftp1), has been reported previously.\(^{14,15}\) Recombination distances were calculated as described\(^{16}\) using the computer program Spretus Madness. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Transient expression assays. For the analysis of promoter activity, the fragment containing the 5’ noncoding region of the mL-3Ra gene was fused to the firefly luciferase gene. The luciferase expression vector pGL2- Basic Vector (Promega Corp, Madison, WI) was used. Cells (5 \(\times\) 10\(^4\) PT18 cells, 5 \(\times\) 10\(^4\) CTLL-2 cells, 10\(^6\) NIH3T3 cells) were transfected with supercoiled plasmid DNA (40 \(\mu\)g for PT18, 40 \(\mu\)g for CTLL-2, 30 \(\mu\)g for NIH3T3) by electroporation. At 18 hours after transfection, the luciferase activity was assayed using the luciferase assay kit (Promega Corp, Madison, WI). The SRa promoter (hybrid promoter containing SV40 and HIV-LTR)-luciferase fusion gene was used as a positive control.

RESULTS

Chromosome mapping. The mouse chromosomal location of the IL-3Ra gene (Il3ra) was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J \(\times\) Mus spretus)F1 \(\times\) C57BL/6J] mice. This interspecific backcross meiotic mapping panel has been typed for over 1,500 loci that are well distributed among all the autosomes as well as the X chromosome.\(^{12}\) This indicates that, on average, markers are spaced about 1 centiMorgan apart over the genome. To place a new marker, an RFLP is established by Southern blot analysis of the two parental strains in the cross, and the backcross mapping panel is then typed for the informative RFLP. The segregation patterns of the new marker are then compared with known
segregation patterns of all other markers. Gene order is determined by minimizing the number of double or multiple crossovers required to explain the new probe distribution.

C57BL/6J and *M. spreitus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative RFLPs using a mouse IL-3Ra cDNA probe. An 11.0-kb *M. spreitus Bgl Il RFLP* (see Materials and Methods) was used to follow the segregation of the *Il3ra* locus in backcross mice. The mapping results indicated that *Il3ra* is located in the proximal region of mouse chromosome 14 linked to *Rarb, Plau,* and *Sftp1*. Although 161 mice were analyzed for every marker and are shown in the segregation analysis (Fig 1), up to 185 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinants to the total number of mice analyzed for each pair of loci and the most likely gene order are as follows: centromere-*Rarb-0/174-*Il3ra-2/185-*Plau-19/183-*Sftp1*. The recombination frequencies (expressed as genetic distances in centiMorgans [cM] ± the standard error) are *(Rarb, Il3ra)-1.1 ± 0.8—Plau—10.4 ± 2.3—Sftp1*. No recombinants were detected between *Rarb* and *Il3ra* in 174 animals typed in common, suggesting that the two loci are within 1.7 cM of each other (upper 95% confidence limit).

We have compared our interspecific map of chromosome 14 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations. **Il3ra** mapped in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus (data not shown).

**Isolation of chromosomal gene for the mIL-3Ra gene.** Of 5 x 10^6 independent phage plaques of the Balb/c sperm library screened with the 1.3-kb cDNA (SUT-1) fragment of mIL-3Ra as a probe, one positive clone (Asut-1) was obtained. Using the same probe, three positive clones (Asut-3, -4, and -6) were obtained from 2 x 10^6 plaques of the Balb/c liver library. These four clones were plaque-purified, and the A DNA were analyzed by restriction enzyme mapping and Southern hybridization. Three clones (Asut-1, -3, and -4) were overlapped, covering the 3' region of the gene, while one clone (Asut-6) contained the 5' portion (Fig 2). These four clones covered most of the cDNA sequence of mIL-3Ra, except for the short fragment located in the middle of cDNA including the exon V. To isolate the DNA for this missing portion, 10^6 plaques of the C129 mouse genomic library were screened with the 0.6-kb SmaI-Nco fragment of the mIL-3Ra gene. Only one positive clone (Asut-7) was isolated, and it contained the sequence that was missing in the other clones (Fig 2).

To confirm that the cloned DNA fragments represented the mIL-3Ra gene locus, mouse genomic DNA from the liver of Balb/c and C129 mice was digested with EcoRI, BamHI, or HindIII and analyzed by Southern blotting using the mIL-3Ra cDNA as a probe. The EcoRI or HindIII cleavage generated a single fragment of approximately 18 kb and approximately 13 kb, respectively, while BamHI cleavage produced two fragments of 7.5 kb and 4 kb (data not shown). The 10-kb cloned chromosomal gene of mIL-3Ra, which...
The clones used for the analysis are shown by bars. There is a single BamHI site present in exon 4, but no HindIII or EcoRI site within about 10 kb of mIL-3Ra gene from exons 1 to 12. Clones hst3 and hst6 are derived from the Balb/c, and hst7 is derived from C129. The dashed lines represent λ arms.

Spans 12 exons, has no recognition site for EcoRI or HindIII and has a single site for BamHI (Fig 2), whereas an EcoRI site, a HindIII site, and two BamHI sites are located nearby in the surrounding regions, which indicates that one copy of the mIL-3Ra gene is present per genome and is covered by the cloned DNA fragments.

Structure of the mIL-3Ra gene. The overall structure of the mIL-3Ra gene was determined by Southern hybridization, PCRs, and DNA sequencing: PCR and restriction enzyme analyses were performed to determine intron length using the cloned λ DNAs and the subcloned plasmid DNAs. All exon-intron borders and several smaller introns were sequenced entirely. As shown in the schematic diagram of the gene and restriction map (Fig 2), the estimated length of the gene from the transcription start site to the polyadenylation site is approximately 10 kb. The gene contains 11 introns, and all the sequences of intron-exon junctions conform with the consensus sequences of eukaryotic splice junctions (Table 1).

Exon I encodes 5′-untranslated sequence, and exon II encodes more 5′-untranslated sequence and the signal peptide. The extracellular domain is encoded by seven exons (exons III to IX), the transmembrane domain by a single exon (exon X), and the cytoplasmic domain by two exons (exons XI and XII). Exon XII also contains the 3′-untranslated sequences.

Identification of the transcriptional start site(s). To identify the functional promoter region that regulates mIL-3Ra gene expression, we attempted to map the 5′-end of the transcript by S1 nuclease protection assay and primer extension. However, the results were rather ambiguous, probably due to the presence of the G/C rich sequences in the 5′ region. Therefore, we mapped the transcriptional start site(s) using the 5′ RACE assay. First, the 5′-ends of the mRNA were reverse transcribed from polyA+ RNA of PT18 cells. The cDNA was amplified and subcloned into pUC18. Plasmid DNA from 13 individual clones was isolated, and the cloned DNA fragments were sequenced. Six clones had the same 5′-end at 215 nucleotides, and seven clones had the 5′-end at 188 nucleotides upstream from the initiation codon (Fig 3), suggesting that the transcription starts at these two positions.

Sequences of the 5′ flanking regions. Although there are no conventional TATAAA and CCAAT sequences in the 5′ flanking region of the mIL-3Ra gene (Fig 3), the potential promoter region carries several noteworthy features. First, the overall G + C content (65%) is unusually high. There are several GC boxes, Sp1 binding sites (G/TG/AGGC/CG/TG/AG/G/T), AP-2 binding sites (CCCC/NG/C/CC/G/C), and a G-stretch of a consensus sequence (CS) for erythrocyte-specific binding protein GCP1. Second, an A + T stretch of 21-mer is present and includes a CS (C/TGA/GTTCA/GC/TTTC/TG/CTN) for interferon CS binding protein ICSBP. Third, two known recognition sequences are present tandemly: GATA (A/TGATAA/G) and Ets (G/CAG/CG/AG/CG/TG/GA/CT), two Myb sequences (T/CACAAG/GC/TG), and a 21-bp potential palindromic sequence (GAG/GCCCCGCGGCG/CC/AAGG). Moreover, there is a 21-bp potential palindromic sequence (GAG/GCCCCGCGGCGGCG/CC/AAGG) just upstream of the GATA-Ets sequences. An NFkappaB site (GGA/GAA/GAT/GT/CCCCC/TG) is also found at about −350 bp.

The promoter region contains several repetitive sequences, including some CS for known transcription factors: three GC boxes, three γ-interferon responsive elements (CA/TGG/C/GA/AG/GA/CT/TG/GA/CT).
for the luciferase constructs in Fig 4 are also indicated.

TAAGT/C, five AP-2 binding sites, and eight GCF CS around -570, and a sequence of 5'GCGCCGTGGGCG3' is present at around +80 and -610. In addition, the sequence (T/G)(A/T)GGCAG, which is similar to the NF-IL6 motif, is located in the 5'-flanking region (Figs 2, 3, and 4). This motif contains one of the transcription start sites mapped (T/G)(A/T)GGCAG, which is similar to the NF-IL6 motif. The apparent palindromic structure. NF-IL6-like sequences (see Results) are marked by asterisks. The mRNA is expressed in various hematopoietic cell lines, including mast cells, myeloid progenitors, pro-B cells, and transfected into the mouse mast cell line PT18, the T-cell line CTLL-2, and the fibroblastic cell line NIH3T3. As a control, human IL-3Ra gene. The transcriptional start sites determined by S'RACE assay. The resultant plasmid, Pst-luc, was transfected into these cell lines. Luciferase activity in the transfected cell lysates was measured 18 hours after transfection. As shown in Fig 4, luciferase activity was clearly detected in PT18 but not in CTLL-2 or NIH3T3 cells transfected with the Pst-luc plasmid. The plasmid with the Pst I-Stu I fragment in an opposite direction (shown as a Pst-luc-) generated a significantly low level of luciferase activity, suggesting an orientation dependency of the promoter. These results also suggest that the 0.8-kb fragment is able to direct transcription in a cell type-specific manner.

To locate the promoter function within the 0.8-kb Pst I-Stu I fragment, the 0.4-kb Pvu II-Stu I and the 0.17-kb Eag I-Stu I fragments were also fused to the reporter gene, and the resultant plasmids, Pvu-luc and E-luc (Fig 4), were used to evaluate the promoter function. Pvu-luc directed production of luciferase in PT18 cells, but not in CTLL or NIH3T3 cells. The luciferase expressed in PT18 by Pvu-luc was about half of that produced by Pst-luc. In contrast, luciferase produced by E-luc in PT18 was about the same level as that produced by Pst-luc (Fig 4). These results suggest that multiple DNA elements may be required for the transcription of the IL-3Ra gene in hematopoietic cells.

**DISCUSSION**

The genes for IL-3, IL-5, and GM-CSF are tightly linked on human chromosome 5 and mouse chromosome 11. In contrast, while the human IL-3Ra (IL3RA) and GM-CSF-Rα (CSF2RA) genes are also tightly linked on the pseudautosomal region of the human X and Y chromosomes, the IL-5Ra gene (IL5R) is on 3p24-3p26. All the mouse α subunit genes are located on different chromosomes: mIL-3Ra (IL3RA), mGM-CSF-Rα (CSF2RA), and mIL-5Ra (IL5R) are on mouse chromosomes 14, 19, and 6, respectively. Interestingly, the proximal region of mouse chromosome 14, where IL3Ra and Rarb genes reside, shares regions of homology with human chromosome 3p24 and human RARB has been placed on 3p24. Although the human IL3RA gene is located on pseudautosomal regions of X and Y chromosomes, the human IL5-R gene resides on 3p24. While this is an interesting finding, it is unknown whether this is due to a result of recombination between these loci or is a simple coincidence.

The mIL-3Ra gene spans about 10 kb and is divided into two exons. As shown in Fig 5, the overall structure of the gene is similar to that of previously reported cytokine receptors, including human and mouse erythropoietin receptors (h/m Epor), human IL-2 receptor β subunit (hIL-2Rβ), mIL-4R, h/m IL-7R, human G-CSF receptor (hG-CSFR), and the mouse β subunits, AIC2A and AIC2B. In general, the gene encoding the class I cytokine receptor motif is divided into four exons, except for hIL-2Rβ and mIL-3Ra (Fig 5). In IL-2Rβ, one of the two exons encoding the conserved cysteine residues is split into two exons. In contrast, there is a small exon of 30 nucleotides in front of the exon for the WSXWS motif in the mIL-3Ra gene. It would be interesting to see whether a unique small exon is also present in the GM-CSF-Rα and IL-5Ra genes.

When linked to the promoterless firefly luciferase gene, the 0.8-kb Pst I-Stu I fragment, including a transcription initiation site and a 14-bp 5'-untranslated region, directed

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Fig 2. Nucleotide sequence of the 5'-flanking region of the mouse IL-3Ra gene. The transcriptional start sites determined by 5' RACE are indicated by arrowheads. The numbering (bp) in sequence is based on the longer 5'-cDNA end as +1. A sequence of the previously isolated cDNA is marked with pluses. The potential binding sites for various transcription factors are shown. An arrow indicates the apparent palindromic structure. NF-IL6-like sequences (see Results) are marked by asterisks. The Pst I, Pvu II, Eag I, and Stu I sites used for the luciferase constructs in Fig 4 are also indicated.
the transcription in mouse mast cells but not in either mouse fibroblasts nor T cells in an orientation-dependent manner (Fig 4). In addition, another IL-3-dependent hematopoietic cell line, OTT-1, also expressed luciferase after transfection of Pst-luc at a level comparable to PT18 (data not shown). These results indicate that the Pst I-Stu I fragment contains the promoter that functions in a hematopoietic cell-specific manner. In addition, there are at least two regions of positive regulatory elements within 0.8 kb, as the 0.4-kb Pvu II-Stu I fragment produced less luciferase than the Pst I-Stu I fragment (Fig 4). Although there is no typical TATA and CCAAT sequence, TAATAA and GCAAT are present at -200 and -140, respectively. Instead, like many housekeeping genes, the most proximal region to the start site is GC-rich (GC content is 70%). Although there are many potential recognition sites for transcription factors as described above (Fig 3), the role of these elements for cell type-specific expression is unknown. Identification of the cis elements for cell type-specific transcription is important to understand the regulation of the IL-3Rα.

Because IL-3 interacts with very early multipotential hematopoietic progenitors, the IL-3 receptor must be present on these cells. In fact, the early human hematopoietic progenitors with a hematopoietic stem cell marker CD34+ express IL-3Rα and IL-3Rβ subunits. The β subunit RNA is not present in mouse embryonic stem (ES) cells, and it appears on day 7 of in vitro development after initiation of embryoid body culture. Likewise, β RNA is not detectable in blastocysts but is induced by in vitro culture of blastocysts. Timing of the appearance of the β subunit RNA is consistent with the onset of hematopoiesis. In contrast, the IL-3Rα transcript is detectable in ES cells as well as blastocysts. However, the IL-3Rα expression is clearly tissue-specific and is restricted mainly to myeloid lineages. Thus, transcription of the IL-3Rα and IL-3Rβ subunit genes appears to be differentially regulated during development. Consistent with the differential regulation, there is no significant similarity of the 5' upstream sequences between the mIL-3Rα and the mIL-3Rβ genes, although the GATA motif, frequently found in hematopoietic specific genes, is present in both genes.

Another interesting feature of the 5' upstream sequence of the mIL-3Rα gene is a high frequency of CpG dinucleotide. Whereas CpG dinucleotides are usually found one fifth as

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**Fig 4.** Promoter activity of the 5' flanking region of the mIL3Rα gene. The 0.8-kb Pst I-Stu I, 0.4-kb Pvu II-Stu I, or 0.15-kb Eag I-Stu I fragment containing the 5' flanking region of the IL-3Rα gene was fused to the luciferase gene as described in Results. Pst-luc+ and Pst-luc- indicate the correct and reverse orientation of the fragments, respectively. Plasmid DNA was transfected into NIH3T3 (solid bars), CTLL-2 (shaded bars), or PT18 cells (open bars) and the luciferase activity in cell extracts was determined at 18 hours after transfection. The results are shown as relative luciferase activity; ie, luciferase activity obtained with SRα-luc (raw value of luciferase activity/100 μg cell extract protein) is regarded as 1.0. Values represent the average of three to four independent experiments with standard deviation.
frequently as GpC in mammalian DNA (CpG:GpC ratio is 0.2)\(^\text{41}\), the CpG:GpC ratio in the region between -1 to -200 of the IL-3Ra gene is as high as 1.0. Interestingly, like IL-3Ra, the mouse pim-1 gene, one of the hematopoietic specific genes, also contains a high GC content and has a high CpG:GpC ratio (1.0) in the corresponding region.\(^\text{42}\) In contrast, the CpG:GpC ratio of the S subunit genes between -1 and -200 is 0.27.\(^\text{8}\) As DNA methylation often inhibits gene expression and CpG is known to be a common target of DNA methylation,\(^\text{33}\) high frequency of CpG in the 5'-flanking region may indicate gene inactivation by methylation. If methylation occurs in a cell type-specific manner during differentiation, it may explain why ES cells express IL-3Ra RNA but the differentiated nonmyeloid cells do not.\(^\text{5,22}\) However, exogenously introduced unmethylated plasmid DNAs containing the mIL-3Ra-luciferase fusion gene did not express luciferase in NIH3T3 cells. Thus, cell type-specific methylation, if it is present, does not solely explain the cell type-specific function of the mIL-3Ra promoter. Presumably, cell type-specific factors are required for regulated expression of mIL-3Ra, either positively or negatively. Identification of such transcription factors is critical to understanding the mechanism of IL-3 receptor expression.

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