Cloning of the Promoter Regions of Mouse TGF-β Receptor Genes by Inverse PCR with Highly Overlapped Primers

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Abstract

In order to isolate promoters of mouse TGF-β receptor genes, we used inverse PCR with highly overlapped primers corresponding to the 5' sequence of the receptor cDNAs. Nested primer sets only covered a 30- to 40-base region of the sequences. Hinfl-digested and self-ligated mouse genomic DNA was used as a PCR template. Only one band for each receptor was seen after PCR. The amplified DNA fragments could direct luciferase production when the luciferase coding sequence was ligated after the fragments. The sequence of the fragment which corresponded to the type II receptor showed partial homology with the promoter region of the human TGF-β type II receptor. Thus, the inverse PCR with highly overlapped primers could be an easy way to isolate the promoter regions of many genes.

Key words: inverse PCR, TGF-β receptor, promoter, cDNA, 5'-end

In order to understand the regulation of gene expression, it is necessary to isolate the promoter of a gene. This step usually required an elaborate cloning step. We hoped to simplify this step by using the inverse polymerase chain reaction (PCR) (1, 2). The development of long PCR (3, 4) allowed us to take this approach, because long PCR can amplify long DNA fragments with much greater accuracy than was possible with previous techniques (3, 4).

For the target of the promoter cloning by inverse PCR, we chose mouse type I and type II transforming growth factor-β (TGF-β) receptor genes. TGF-β has a strong growth-inhibitory activity to various types of cells and plays important roles in development and differentiation. The TGF-β signal is transduced by type I and type II TGF-β receptors (TGF-β RI and TGF-β RII) (5). Because they are the major components for transducing the TGF-β signal, it is important to understand the regulation of the expression of these genes. The cDNA sequences were known for these important genes (6, 7, 8, 9). Furthermore, the promoter of the human TGF-β type II receptor gene was known (10). Thus, they could be good target genes.

In the design of the primers for inverse PCR amplification of the promoter of mouse TGF-β RI and TGF-β RII, we considered following points: 1) Nested primers should be used to increase the specificity of the PCR amplification; 2) primer sets should come from very short sequences of cDNA because the exon-intron structure of mouse TGF-β RI and TGF-β RII were not known and because exon 1 of some genes was short; and 3) the primer length should be more than 20 bp in order to allow the specific amplification of the promoters. Therefore, we used highly overlapped primers as shown in Fig. 1. The primers came from only 30- to 40-bp stretches near or at the 5' end of the known cDNA sequence of TGF-β RI and TGF-β RII (7, 8). The highly overlapped primers were also used for the chromosomal assignment of cDNAs by PCR (12).

To make template DNA, mouse genomic DNA was digested with Hinfl, blunt-ended by T4 polymerase, and self-ligated by T4 DNA ligase (13). Using about 1 mg of template DNA, we performed the first PCR amplification with the outer primers [primers 1 and 2 for TGF-β RI and primers 5 and 6 for TGF-β RII (see Fig. 1)]. After 25 cycles of PCR at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min in the volume of 100 µl, the PCR products were phenol-extracted, ethanol-precipitated and subjected to further PCR amplification using inner primers [primers 3 and 4 for TGF-β RI and primers 7 and 8 for TGF-β RII (see Fig. 1)] for 25 cycles under the same temperature conditions. For this PCR steps, Gene Amp XL PCR Kit (Perkin-Elmer Cetus) was used.

Only one band was seen for each amplification (data not shown). These bands were cloned into the luciferase assay plasmid pGL-2-basic (Promega) and their
Figure 1. Primers for inverse PCR amplification of mouse TGF-β receptor type I and type II. A: primers for type I receptor. B: primers for type II receptor. Primers were aligned with the cDNA sequence which is shown at the center. Nucleotides were numbered according to Tomoda et al. (7) for A and Suzuki et al. (8) for B. * indicates the 5' end of the primers.

Figure 2. Promoter activities of PCR amplified products. PCR amplified bands, a 1000-bp band for TGF-β RI (TGF-β RI) and a 700-bp band for TGF-β RII (TGF-β RII) (data not shown), were cloned into pGL-2-basic (Promega) in an orientation-defined manner, transfected into CV1 cells (11) and the promoter activities were compared with SV40 early promoter (pGL-SV). Lysates were made using cell culture lysis buffer (Promega) and the luciferase activity was measured using luciferase assay substrate (Promega) according to the manufacturer’s instructions.

Figure 3. Partial sequence of cloned band. A: The sequence of putative mouse TGF-β type I receptor promoter. B: The sequence of putative mouse TGF-β type II receptor promoter. The sequence which matched with cDNA are underlined. The sequences which matched with primers are boxed. C: Alignment of putative mouse type II promoter sequence (bottom) with human promoter sequence (top) (10). The 1st base of human cDNA (9) is underlined and is taken as nucleotide number 1. Alignment was performed with FASTA (15).

promoter activity was tested by the luciferase assay. As shown in Fig. 2, both bands showed significant promoter activity when transfected into cultured cells. Thus, we think they may be promoters of TGF-β RI and TGF-β RII.

We determined the partial sequence of the both ends of
these putative promoters (Fig. 3 A, B) and we compared the sequence of the putative mouse TGF-β RII promoter with that of the human promoter (Fig. 3 C). The results indicate that the 3' portion of the clone matched with the corresponding cDNA sequence. Furthermore, the sequence of mouse TGF-β RII matched well at least up to −300 bp of the 5' portion of the cDNA.

From these results, at least, the promoter of the type II receptor seems to be the correct one. Thus, the inverse PCR with highly overlapped primers is an effective way to clone the promoter region of the gene which only cDNA sequence were known. There is also a kit (PromoterFinder, CLONTECH laboratories, Inc.) for isolation of promoter region by PCR. This kit is based on a improved adaptor ligation PCR method (14). Our inverse PCR-based method has better specificity because both the 5' and 3' primers were specific for the cDNA sequence, while only the 3' primer is specific in case of adaptor ligation PCR. In contrast, one disadvantage of our inverse PCR method is that the amplified PCR product contains the downstream sequence as well as the upstream sequence.

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