MOLECULAR AND CELLULAR BIOLOGY, Jan. 1988, p. 511–513
0270-7306/88/010511-03$02.00/0

Vol. 8, No. 1

Transfection of an Immunoglobulin κ Gene into Mature Human B Lymphocytes

LÊ THI BICH-THUY[1,2]* AND CARY QUEEN[1]†

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892,[1] and Institut National de la Santé et de la Recherche Médicale U 80, Hôpital E. Herriot, 69374 Lyon Cédex 08, France[2]

Received 26 June 1987/Accepted 13 October 1987

We show in this report that the transcription induced by interleukin-2 or pokeweed mitogens of the κ MOPC 41 immunoglobulin light-chain gene transfected into primary human or murine B lymphocytes initiates from a previously unobserved start site about 26 base pairs upstream of the start site used in myeloma cell lines.

The regulation of immunoglobulin genes has been intensively studied by transfecting the genes into myeloma cell lines and measuring their expression (for reviews, see references 11 and 13). To further understand the relationships among B-cell development, lymphokines, and immunoglobulin gene expression, we transfected cloned genes into primary (i.e., normal, nontransformed) lymphoid cells. We report here that the MOPC 41 κ light-chain gene can be introduced into human or murine primary B lymphocytes, where its expression can be induced by lymphokines or polyclonal B-cell activators. Surprisingly, in all cases, the transcription start site is about 26 base pairs upstream of the site used in myeloma cells.

The plasmid pLT40K (Fig. 1A) and derivatives used for the transfections contain part of the κ gene from the mouse myeloma MOPC 41 (1, 8, 14). The derivative plasmid pLX40K contains the polyomavirus replicon, which allows the plasmid to replicate in mouse cells, thus increasing the amount of RNA synthesized by increasing the amount of DNA template (6, 12). Similarly, the plasmid pLB40K contains most of the genome of human BK virus, allowing it to replicate in at least some human cells (9).

Human B cells extensively purified from peripheral blood (3) were transfected with 10 μg of pLB40K DNA or mock transfected without DNA by the calcium-phosphate method (5). Recombinant interleukin-2 (IL-2) was used as an activator, because we have shown that this lymphokine induced both growth and differentiation of human B cells in the absence of T cells (2). RNA extracted from transfected cells was assayed by the S1 nuclease method (Fig. 2c and d) with a probe specific for transcripts from the κ promoter region (Fig. 1B). RNA from S194 mouse myeloma cells transfected with pLX40K was assayed in parallel with the B-cell RNA (Fig. 2a). RNA from the B cells transfected with pLB40K protected a fragment of the probe about 106 base pairs in length, which was not protected by RNA from the mock-transfected cells, indicating that the κ gene was transcribed from a start site at about position −26 (Fig. 3). To more precisely locate this start site, the S1-assayed RNA was run on a sequencing gel in parallel with the sequenced probe. The protected band spanned the 3 bases ATA from position −24 to position −26. The position of the start site was the same when B cells from 10 different individuals were used (data not shown). In contrast, the κ gene transfected into the S194 cells was transcribed from a start site at position +1 (Fig. 3), as seen in all previous experiments with this gene in MPC 11 cells (1, 8, 12), and from the endogenous gene in the original MOPC 41 cells (1).

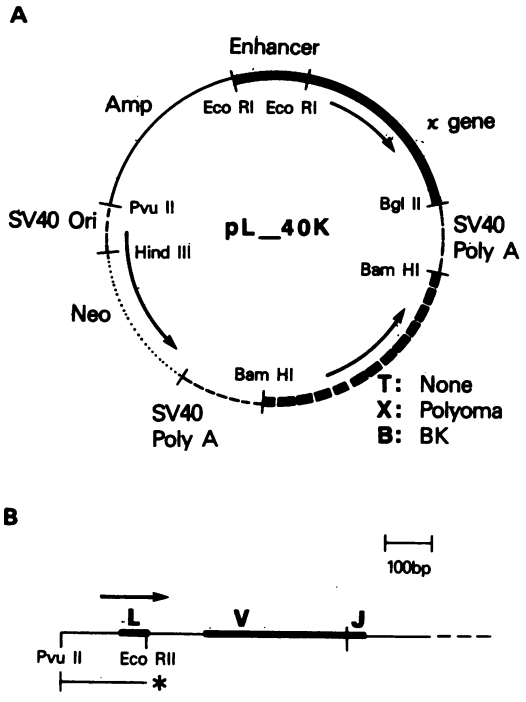

FIG. 1. (A) Diagram of the plasmids pLT40K, pLX40K, and pLB40K (not drawn to scale). The structure of pLX40K has been described previously (8). In pLT40K, the polyoma region has been deleted, and in pLB40K, it has been replaced by the *Eco*RI-*Bam*HI fragment of BK virus (9) by means of a *Bam*HI linker. Arrows show the direction of transcription from the κ, simian virus 40 (SV40) early, and polyoma or BK early promoters. (B) Strategy for S1 nuclease assay. The κ-specific probe is shown underneath the gene; *, ^{32}P-labeled end. The direction of κ transcription is shown by an arrow. L, Leader segment; V, variable segment; J, joining segment; Ori, origin.

* Corresponding author.
† Present address: Protein Design Labs, Palo Alto, CA 94304.

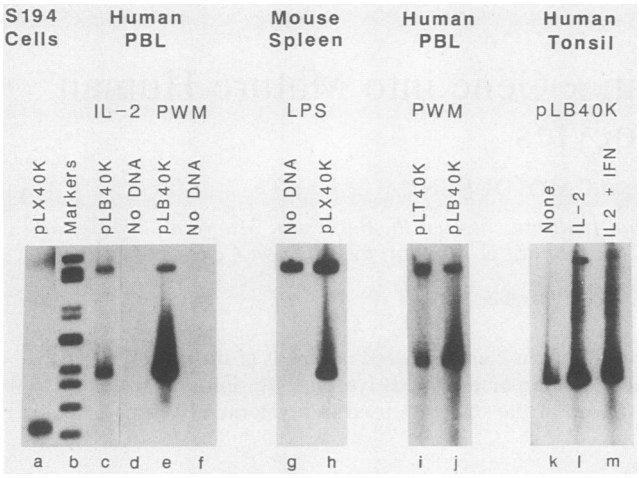

FIG. 2. S1 nuclease assay of RNA from transfected cells. Cells, activators, and plasmids used for transfection are as indicated. RNA was extracted and S1 assays were performed as described previously (8). The upper band in each lane ran at the position of undigested probe. Lanes a through h, i through j, and k through m were from three separate gels. The markers were from a *Hpa*2 digest of pSV2neo and had sizes (from top to bottom) of 190, 181, 171, 147 (running as a doublet), 131 and 127 (running together), 110, 103, 90, and 77.

It has been observed that gamma interferon (IFN-γ) added to IL-2 synergistically stimulates expression of the endogenous light-chain genes in human B cells (4). Therefore, we transfected B cells from human tonsils without any activator, with IL-2 alone, or with IL-2 and recombinant human IFN-γ. Transcription was stimulated strongly by IL-2 and an additional two- to threefold by IFN-γ (Fig. 2k through m), an effect reproducible in five other experiments. Transcription initiated at the same start site in the presence and in the absence of IFN-γ.

The above experiments were performed by using highly purified B cells. To assess the role of T cells on the transcription start site, in two experiments we stimulated transfected B cells with IL-2 in the presence of 10% autologous T cells. The amount of RNA detected was decreased, an effect in keeping with our previous finding that T cells inhibited B-cell differentiation induced by IL-2 when present at a T- to B-cell ratio of 1:9 (2). However, transcription initiated at the same start site as in the absence of T cells (data not shown). In a series of experiments, the purified peripheral B cells were transfected, mixed with 10% of their number of T cells from the same sample of blood, and then stimulated with the T-cell-dependent polyclonal B-cell activator pokeweed mitogen. The κ gene was then transcribed with much higher efficiency than when B cells were stimulated with IL-2 in the absence of T cells, but the same start site was still used (Fig. 2c and e).

To determine the effect of the BK replicon on transcription, pLT40K was transfected in parallel with pLB40K (Fig. 2i and j). The plasmid without BK transcribed less RNA than the plasmid with BK did but from the same start site (at position −26). To be certain that the unexpected transcription start site in primary B cells was not due to the transfection of a mouse κ gene into human cells, the plasmid pLX40K was also transfected into fresh mouse spleen cells activated with lipopolysaccharide (Fig. 2g and h). The start site was the same as in the primary human B cells and differed from that of the mouse myeloma cells. This result was also obtained when the mouse spleen cells were stimulated with pokeweed mitogen or human IL-2. When the plasmid pLT40K without the polyoma replicon was transfected into the mouse primary splenic cells, the κ gene was transcribed with a lower efficiency than when pLX40K was used for transfection, but again transcription started at position −26 as in primary human B lymphocytes, with no band corresponding to the myeloma start site detectable (data not shown).

We have shown here that an immunoglobulin gene can be readily transfected and expressed in normal peripheral and functional cells of the immune system, namely mature circulating B cells. Interestingly, for this gene, the transcription start site after transfection into primary B cells differs from the start site in myeloma cell lines. However, as there are no ATG codons between the two start sites (Fig. 3), transcripts initiating at either site should code for the same light-chain protein. It is very unlikely that the protected band in these S1 experiments (Fig. 2c through m) represents a splice site for upstream transcripts rather than a transcription start site, because splicing after the nearest instance of the strongly conserved splice acceptor sequence AG (10), at position −18, would create a band 6 to 8 base pairs shorter than the observed band (Fig. 3).

Since transcription of immunoglobulin genes results from their interaction with multiple protein factors (15), it seems possible that primary B cells possess the factors needed to activate the MOPC 41 κ gene promoter for transcription but may be missing a factor necessary to specify the start site. In the absence of this factor, transcription initiates about 35 base pairs after the TATA-like sequence CATA (position −61 to −66) rather than after the TATA sequence at position −27 used in myeloma cells. However, the presence of T cells, which are currently considered to be the source of lymphokines required for B-cell growth and differentiation, did not supply or induce the putative missing factor. The same κ gene transfected into a transformed B-cell line and a transformed pre-B-cell line initiates transcription at the start site used in the myeloma cells (11). Whether the transformation process used in establishing these lines may have selected cells constitutively expressing any proteins needed to direct initiation at the myeloma start site is currently under investigation.

We thank Hoffmann-La Roche Inc. and Genentech Corp. for their supply of recombinant lymphokines. The secretarial assistance of B. Miller is greatly appreciated.

L.T.B.-T. was partially supported by an exchange program between the EORTC and the National Cancer Institute.

FIG. 3. Sequence of the MOPC 41 κ promoter region (14). Transcription in myeloma cells initiates at position +1. *, Most likely transcription start site in transfected B cells, on the basis of the size of the protected band (Fig. 2). The TATA box is in boldface, and a conserved octanucleotide (7) is underlined.

LITERATURE CITED

1. **Bergman, Y., D. Rice, R. Grosschedl, and D. Baltimore.** 1984. Two regulatory elements for immunoglobulin κ light chain gene expression. Proc. Natl. Acad. Sci. USA **81**:7041–7045.
2. **Bich-Thuy, L. T., and A. S. Fauci.** 1985. Direct effect of interleukin-2 on the differentiation of human B cells which have not been preactivated in vitro. Eur. J. Immunol. **15**:1075–1079.
3. **Bich-Thuy, L. T., and A. S. Fauci.** 1986. Recombinant interleukin-2 and gamma interferon act synergistically on distinct steps of in vitro terminal human B cell maturation. J. Clin. Invest. **77**:1173–1179.
4. **Bich-Thuy, L. T., C. Queen, and A. S. Fauci.** 1986. Interferon gamma induces light chain synthesis in interleukin-2 stimulated human B cells. Eur. J. Immunol. **16**:547–550.
5. **Chu, G., and P. Sharp.** 1981. SV40 DNA transfection of cells in suspension: analysis of the efficiency of transcription and translation of T antigen. Gene **13**:197–202.
6. **Deans, R., K. A. Denis, A. Taylor, and R. Wall.** 1984. Expression of an immunoglobulin heavy chain gene transfected into lymphocytes. Proc. Natl. Acad. Sci. USA **81**:1292–1296.
7. **Falkner, F. G., and H. G. Zachau.** 1984. Correct transcription of an immunoglobulin κ gene requires an upstream fragment containing conserved sequence element. Nature (London) **310**:71–74.
8. **Foster, J., J. Stafford, and C. Queen.** 1985. An immunoglobulin promoter displays cell-type specificity independently of the enhancer. Nature (London) **315**:423–425.
9. **Milanesi, G., G. Barbanti-Brodano, M. Negrini, D. Lee, A. Corallini, A. Caputo, M. P. Grossi, and R. P. Ricciardi.** 1984. BK virus-plasmid expression vector that persists episomally in human cells and shuttles into *Escherichia coli*. Mol. Cell. Biol. **4**:1551–1560.
10. **Mount, S. M.** 1982. A catalogue of splice sequences. Nucleic Acids Res. **10**:459–470.
11. **Queen, C.** 1984. Regulation of immunoglobulin transcription. Oxford Surv. Eukaryotic Genes **1**:169–191.
12. **Queen, C., and D. Baltimore.** 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell **33**:741–748.
13. **Queen, C., J. Foster, C. Stauber, and J. Stafford.** 1986. Cell-type specific regulation of a κ immunoglobulin gene by promoter and enhancer elements. Immunol. Rev. **89**:49–68.
14. **Seidman, J. G., E. E. Max, and P. Leder.** 1979. An immunoglobulin gene is formed by site-specific recombination without further somatic mutations. Nature (London) **280**:370–375.
15. **Sen, R., and D. Baltimore.** 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell **46**:705–716.