Complete nucleotide sequence of the gene for human interleukin 1 alpha

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ABSTRACT

The chromosomal gene for human interleukin 1 alpha (IL-1 α) was isolated from a human genomic DNA library by using as a probe cloned human IL-1 α cDNA. Complete nucleotide sequence of about 12 kilobase pairs (kbp) long was determined and the structure and organization of this gene were elucidated. This gene contains seven exons and six introns. The first exon encodes the 5'-untranslated region. Most of the prepeptide portion of the precursor polypeptide is encoded by the next three exons, and the mature form of IL-1 α is encoded by the remaining three exons. The last exon also encodes the intronless 3'-untranslated region. The fourth intron as well as both 5'- and 3'-flanking regions contains the sequence of an Alu family member. In the middle part of the last intron, a 46-bp sequence with a unique structure is repeated five times in a head to tail manner. These repeats are flanked by the regions containing alternative purine and pyrimidine tracts. In the 5'-flanking region, immediately upstream of the putative TATA box, the 16-bp sequence highly homologous to the binding site of the adenovirus 2 major late promoter transcription factor is identified. The nucleotide sequence reported here showed only one nucleotide substitution in the 3'-untranslated exon in comparison with the nucleotide sequence of the cDNA from HL-60, a promyelocytic leukemia cell line, previously cloned and sequenced in our laboratories.

INTRODUCTION

Interleukin 1 (IL-1) is a cytokine having many biological functions which is originally defined as the product of macrophages (1). Recent studies have indicated that some other cells such as fibroblasts, keratinocytes, astrocytes, mesangial cells, corneal cells, large granular lymphocytes and Epstein-Barr virus (EBV)-transformed B lymphocytes, also produce IL-1 or closely related substance(s) (see refs 2-4 for review, and refs 5 and 6). The various biological functions of IL-1 have been reported, including augmentation of mitogen induced proliferation of thymocytes (1), induction of fever (7), stimulation of fibroblast proliferation (8), induction of acute phase protein (9), cytotoxic effect on certain tumor cell lines (10, 11), enhancement of B-lymphocyte proliferation (12, 13) and

maturation (13, 14), and corticotropin-releasing factor activity (15). This wide variety of functions of IL-1 reflects its broad spectrum of the target cell types. Thus IL-1 may play an important role in immune and inflammatory responses as well as neuroendocrine one. More recent works on protein sequencing of purified IL-1 from the culture supernatant of peripheral blood monocytes or monocyte-like cell lines and nucleotide sequencing of cloned IL-1 cDNA in several laboratories directly demonstrated the presence of two structurally distinct forms of IL-1 (IL-1 α and IL-1 β corresponding to acidic IL-1 of pI 5 and neutral IL-1 of pI 7 respectively) (16-20). The cloning, characterization and expression of cDNAs have enabled us not only to produce large quantity of the polypeptides, but also to isolate corresponding genomic clones.

In the present investigation, we have isolated and characterized the chromosomal gene for human IL-1 α . We report here the complete nucleotide sequence and the structural organization of the gene for human IL-1 α .

MATERIALS AND METHODS

Materials

Restriction endonucleases, E. coli DNA polymerase I, T4 polynucleotide kinase and T4 DNA ligase were purchased from Takara Shuzo Co., LTD., Avian myeloblastosis virus reverse transcriptase from Life Sciences, nuclease Bal31 from Bethesda Research Laboratories Inc. and DNase I from Sigma. The plasmid pUC18 was purchased from Pharmacia, M13 sequencing kit from Takara Shuzo Co., LTD., and [α -³²P]dCTP and [γ -³²P]ATP from Amersham International or New England Nuclear.

Cloning and sequencing of human IL-1 α gene

A human genomic λ Charon 4A library constructed by Lawn et al. (21) was screened by plaque hybridization according to the procedure of Benton and Davis (22). The hybridization probe used was the BalI-HincII fragment of 847 bp containing entire protein coding region except for the first five nucleotides from ATG initiation codon, which was excised from clone pHL4 (18) and was labeled with ³²P by nick-translation (23). Hybridization positive phage clones were purified by successive plaque hybridization. The purified phage was then amplified and the recombinant phage DNA was isolated. The DNA insert was digested in combination with EcoRI and HindIII and the digest was separated by electrophoresis on a low melting agarose gel. The DNA fragments were eluted individually from the gel and purified by phenol extraction and ethanol precipitation. Aliquots of the resulting DNA

fragments were then systematically shortened by treatment with nuclease *Bal31* as described (24) and subcloned into *HincII* site of plasmid pUC18 followed by transformation (25) into *E. coli* HB101 cells. The nucleotide sequences of pUC18 subclones containing sequentially shortened DNA inserts were determined from both ends by the dideoxynucleotide chain termination method (26) using universal primers or synthetic 15 to 18-mer primers complementary to the sequence of the human IL-1 α gene. Oligodeoxyribonucleotide primers used for nucleotide sequencing and a primer extension experiment were synthesized by the phosphotriester method (27), using a DNA synthesizer (Biosearch Sam I) according to the procedure recommended by the manufacturer.

Southern blot hybridization

Southern blot hybridization (28) was conducted using human placental DNA prepared by the method of Blin and Stafford (29). The DNA (7.5 μ g) was digested with several restriction endonucleases, electrophoresed on a 0.7 % agarose gel, transferred to a nitrocellulose filter and hybridized at 65 °C or 55 °C with the same probe as that used for cloning of the human IL-1 α gene.

Primer extension

Poly(A)RNA was prepared as described previously (18). The synthetic oligodeoxyribonucleotides used were 5'-TCTGGAACCTTGGCCATCTTGACTT-3' (corresponding to positions -8 to 17 of the human IL-1 α cDNA sequence, see ref. 18) and 5'-TCAGGGACTTGGCCATCTTGATTT-3' (corresponding to positions -8 to 17 of the rabbit IL-1 α cDNA sequence, see ref. 18). Primer extension was performed essentially as described (30). The oligodeoxyribonucleotide was end-labeled with [γ -³²P]ATP and T4 polynucleotide kinase. The end-labeled oligodeoxyribonucleotide (500,000 cpm) was annealed to 10 μ g of poly(A)RNA and extended at 42 °C for 1 hr by 20 units of reverse transcriptase. The products were analyzed by the electrophoresis on an 8 M urea-6 % polyacrylamide gel.

RESULTS AND DISCUSSION

A human genomic DNA library kindly provided by Dr T. Maniatis (21) was screened for phages containing human IL-1 α gene sequence by plaque hybridization with human IL-1 α cDNA previously cloned in our laboratories (18). Out of about 6x10⁵ phage plaques, six hybridization positive clones were identified. Recombinant phage DNAs were isolated individually and characterized by restriction endonuclease mapping. All of the phage clones tested seemed to carry an overlapping DNA insert sharing several restriction

Figure 1. Cloning of overlapping clones containing human IL-1 α gene sequences. λ Charon 4A phage clones containing human IL-1 α gene sequences were isolated as described in Materials and Methods. The gene for human IL-1 α and its flanking regions are schematically displayed in the top line, where exons (1 to 7) are represented by closed boxes. The extent and overlap of the DNA sequences carried by isolated phage clones are shown below.

fragments common in size (Fig. 1).

Southern blot hybridization analysis (28) of human placental cellular DNA with human IL-1 α cDNA represented hybridization positive bands of about 16, 5.0, 2.6 and 1.3 kilobase pairs (kbp) in EcoRI digestion and about 7.4 and 4.2 kbp in HindIII digestion (Fig. 2, lane 1 and lane 3). No additional hybridization positive band was detected even in low stringent hybridization condition (data not shown). Two clones, out of six hybridization positive clones, λ HL#4 and λ HL#6, contained all HindIII fragments of the same sizes as the hybridization positive bands. We selected λ HL#4 for further characterization, because this clone contained a longer DNA insert extending

Figure 2. Autoradiography of blot hybridization analysis of human placental DNA. Samples of human DNA (7.5 μ g) were digested with EcoRI (lane 1), HindIII (lane 3) and EcoRI plus HindIII (lane 2), electrophoresed on a 0.7 % agarose gel, transferred to a nitrocellulose filter and hybridized at 65 $^{\circ}$ C with the 847-bp BalI-HincII fragment of a human IL-1 α cDNA clone (18). The size markers were λ DNA (49) digested with HindIII, the lengths of which are indicated in kb.

Figure 3. Structure of the human IL-1 α gene and sequencing strategy. The top line indicates the human IL-1 α mRNA, for reference. The open and shaded boxes represent the prepeptide region (amino acid positions 1 to 112) and the mature IL-1 α coding region (amino acid positions 113 to 271), respectively. The restriction map of the human IL-1 α gene is shown below the mRNA. The locations of the exons are indicated by closed boxes. The slash mark located at right end of the map indicates the EcoRI site generated from the linker sequence used for cloning into λ Charon 4A. Each DNA fragment obtained by double digestion with EcoRI and HindIII was systematically shortened by nuclease Bal31 and subjected to nucleotide sequencing from both ends by the dideoxynucleotide chain termination method (26) after subcloning of fragments into pUC18. Arrows indicate the direction and extent of the sequencing. The vertical lines at the end of arrows indicate specific restriction sites where the nucleotide sequencing started, and the open circles at the end of arrows indicate the positions of specific sequencing primers used.

in both the 5' and 3' direction than clone λ HL#6. The DNA insert from λ HL#4 was digested in combination with EcoRI and HindIII and separated by electrophoresis on a low melting agarose gel. The restriction patterns observed were all compatible with the data from genomic southern blotting (Fig. 2). The digested DNA fragments were eluted from the gel and then digested with nuclease Bal31 to give systematically shortened DNA fragments.

The nucleotide sequence of the 3'-terminal two third of the DNA insert from clone λ HL#4 was determined according to the strategy as indicated in Fig. 3.

The complete nucleotide sequence of the human IL-1 α gene is shown in Fig. 4. The nucleotide sequence reveals the structural organization of the gene. This gene contains 10,206 bp, including seven exons (exon 1-7) and six introns (intron 1-6). The first intron interrupts the 5'-untranslated region 8 nucleotides upstream from the ATG initiation codon. The next three introns interrupt the prepeptide portion of the precursor polypeptide. The fourth intron interrupts the C-terminal region of the prepeptide 6 amino acids upstream from the N-terminus of the mature IL-1 α polypeptide. The last two

[illegible]

Figure 4. Nucleotide sequence of the human IL-1 α gene. Nucleotide residues are numbered in the 5' to 3' direction beginning with the putative transcription initiation site. The sequences of exons are boxed. The putative TATA box, Alu repetitive sequences and alternative purine and pyrimidine tracts are underlined. Direct repeats are shown by horizontal arrows, and inverted repeats are shown by arrows with broken lines.

Figure 5. Determination of the 5' end of IL-1 α mRNA by primer extension. The end-labeled primers (see Materials and Methods) were annealed to poly(A)RNA from induced HL-60 cells (lane 2), uninduced HL-60 cells (lane 3), induced rabbit alveolar macrophages (lane 4) or uninduced rabbit alveolar macrophages (lane 5), and extended at 42 °C for 1 hr by reverse transcriptase. The products were analyzed by electrophoresis on an 8 M urea-6 % polyacrylamide gel. End-labeled PstI-HinfI fragments of pBR322 DNA were used as the size markers (lane 1).

introns interrupt the coding sequence for the mature IL-1 α polypeptide. All the intron and exon junctions in the human IL-1 α gene are similar to those of other genes in agreement with the established GT/AG rule (31). There are 3' splice signals homologous to the proposed consensus sequence (32, 33) in all the intron sequences 21-56 bp upstream from 3' splice sites. The nucleotide sequences of the exons in the human IL-1 α gene are wholly identical with the reported cDNA sequence (18) from HL-60, a promyelocytic leukemia cell line, except for one nucleotide difference at position 9,403 in the 3'-untranslated region (T for C).

March et al. (19) have also reported the nucleotide sequence of human IL-1 α cDNA prepared from peripheral blood monocyte mRNA. A major discrepancy between the two human IL-1 α cDNA sequences is a nucleotide difference observed in the protein coding region which causes a difference in deduced amino acid at the second position of the mature IL-1 α sequence (Ala for Ser at amino acid position 114). Additional nucleotide sequencing of other genomic clones in this paper showed that three clones (λ HL#1, λ HL#2 and λ HL#4) out of six clones carried the same nucleotide sequence as reported here, while the other three clones carried the same nucleotide sequence as reported by March et al. Therefore, we concluded that the difference may result from polymorphism (34, 35). The expression of our cloned cDNA sequence corresponding to mature IL-1 α demonstrated that recombinant human IL-1 α produced is fully active in respect to LAF activity in com-

parison with natural IL-1 (T. Fukui, manuscript in preparation).

The transcription initiation site has been determined by the primer extension experiments. The synthetic 25-mer primer complementary to the human IL-1 α mRNA (positions -8 to 17, see ref. 18) was extended by reverse transcriptase and the transcripts were analyzed by the electrophoresis on a urea-polyacrylamide gel. A single cDNA band corresponding to about 76 nucleotides in length was identified only on the mRNA from induced HL-60 cells, but no detectable band was observed on the mRNA from uninduced cells (Fig. 5, lane 2 and lane 3). The same result was obtained when the 25-mer primer specific for the rabbit IL-1 α mRNA (the positions corresponding to the human primer) and the rabbit IL-1 α mRNA from induced and uninduced alveolar macrophages were used (Fig. 5, lane 4 and lane 5). From these data and the fact that many of the eukaryotic mRNA start with an A residue (see ref. 36 for review), we tentatively assigned the transcription initiation site of the human IL-1 α gene to the A residue at position 1. The possibility that the human IL-1 α mRNA starts with the A residue at position 2 can not be excluded. In this case, the transcription initiation site corresponds to the 5' end of the cDNA sequence previously reported (18), demonstrating that our cDNA clone (pHL4) has a complete copy of the mRNA.

The typical TATA box (see ref. 36 for review) and the typical CAAT box (37) were not found in the region immediately upstream from the transcription initiation site, but a TATA-like sequence, TACAAAA, was located 31 bp upstream from the transcription initiation site. The additional potential TATA boxes were also found in the further upstream region (positions -932 to -926 and -239 to -234). It is possible that these TATA sequences also provide promoter functions to give multiple transcription initiation sites as reported (30).

The sequence of 16 bp homologous to the 17-bp sequence of the adenovirus 2 major late promoter transcription factor (MLTF) binding site (38) was identified immediately upstream of the putative TATA box. As aligned in Fig. 6, the two sequences are highly conserved. Moreover, they are present at quite the same position in the 5'-flanking region of the genes. This 16-bp sequence of the human IL-1 α gene having perfect tandem duplication exhibits imperfect but significant dyad symmetric structure similar to that of the adenovirus 17-bp element. It has been suggested that part or all of the 12-bp dyad sequence in the adenovirus 17-bp element might be important for recognition and binding of MLTF (39). The significance of the homology reported here is not yet clear. This 16-bp sequence may play a

Figure 6. Homology between the 5'-flanking region of the human IL-1 α gene and the adenovirus 2 major late promoter. The positions from the transcription initiation site are represented by negative numbers. The hyphen (-) in IL-1 α sequence indicates a gap. The direct repeats are shown by arrows, and the palindromic sequences are denoted by arrows with broken line. The sequence of adenovirus 2 major late promoter (Ad2 MLP) was taken from ref. 38.

role in gene expression as a cis-acting transcription activating element.

The fourth intron contains a member of the Alu repetitive family (see ref. 40 for review) in the reverse orientation as indicated in Fig. 4, which is bounded by short repeated sequences (11 bp long). In addition, Alu family members are also identified in both the 5'- and 3'-flanking region of the gene. The inverse and complementary structure of the first monomer sequence of an Alu family member is located over 1,000 bp upstream from the transcription initiation site. The incomplete Alu repetitive sequence lacking 5' one third is located immediately downstream from the end of the gene in which 3' one third is missed from the determined nucleotide sequence, and is followed directly by the sequences of the EcoRI linker and the lambda short arm.

The sequences of alternative purine and pyrimidine, poly(dG-dT) or poly(dC-dA), were found in introns 5 and 6 (underlined in Fig. 4). These sequences are considered to be potential regions to adopt the Z-DNA conformation (see ref. 41 for review). One of them having a (AC)₁₉(CA)(TG)(CA)₄ sequence is located near the end of intron 5, 71 bp upstream from the splice junction. The others, a (AC)₇A(AC)A sequence and a (TG)₃TA(TG)₆TA(TG)₇T sequence are both present in intron 6 at the indicated positions respectively.

In the middle part of intron 6, we found a region containing the fifth tandem repeat of a 46-bp sequence. These repeated sequences are perfectly conserved among them except for a single nucleotide difference in repeat 1 (A for G at position 7,488) and a 4-nucleotide deletion in repeat 5 (between positions 7,690 and 7,691). Each repeat contains several short sequences homologous to the known functional elements, (I) a GGCGG sequence found in the 5'-terminal portion; the binding site of a transcription factor SP1 (see ref. 42 for review), (II) a GTGGTTG sequence or a GTGGTTTT sequence found in the middle portion; an imperfect copy of the core sequence of the viral

enhancer elements (GTGG^{AAA}_{TTT}G) which is also found in the immunoglobulin tissue-specific enhancer (43, 44), (III) a AGGACA sequence found in the 3'-terminal portion; an inverse and complementary sequence of the ligand binding glucocorticoid receptor binding site (TGTPyCT) (45). Three copies of the core sequence of the viral enhancer elements are also found near the 46-bp repeat region (positions 7,433 to 7,440, 7,735 to 7,742 and 8,012 to 8,019). In this context, it is interesting to note that some EBV-transformed B lymphocyte lines also produce IL-1-like factor with acidic pI (46), and that murine recombinant IL-1 α possesses corticotropin-releasing factor activity (15). In addition, inhibition of IL-1 production by glucocorticoids has previously been reported (47). Note also that the 46-bp repeats is flanked both upstream and downstream by the sequences containing alternative purine and pyrimidine tract(s) as described above, implying its functional significance for gene expression and regulation as proposed in ribosomal DNA (48).

Isolation and characterization of the gene for human IL-1 α reported here should provide a clue to elucidation of the molecular mechanism of the IL-1 regulation. Expression study by DNA mediated gene transfer may allow us to identify the regulatory element responsible for LPS induction or tissue-specific expression of the human IL-1 α gene.

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REFERENCES

1. Gery, I., Gershon, R. I. and Waksman, B. H. (1972) *J. Exp. Med.* 136, 128-142.
2. Oppenheim, J. J. and Gery, I. (1982) *Immunol. Today* 3, 113-119.
3. Mizel, S. B. (1982) in *Biological Response in Cancer*, Mihich, E. Eds., pp. 89-119, Plenum, New York.
4. Dinarello, C. A. (1984) *Reviews of Infectious Diseases* 6, 51-95.
5. Scala, G., Allavena, P., Djeu, J. Y., Kasahara, T., Ortaldo, J. R., Herberman, R. B. and Oppenheim, J. J. (1984) *Nature* 309, 56-59.
6. Matsushima, K., Kuang, Y.-D., Tosato, G., Hopkins, S. and Oppenheim, J. J. (1985) *Cell. Immunol.* 94, 406-417.
7. Murphy, P. A., Simon, P. L. and Willoughby, W. F. (1980) *J. Immunol.* 124, 2498-2501.
8. Schmidt, J. A., Mizel, S. B., Cohen, D. and Geen, I. (1982) *J. Immunol.* 128, 2177-2182.
9. Sipe, J. D., Vogel, S. N., Ryan, J. L., McAdam, K. P. W. J. and Rosenstreich, D. L. (1979) *J. Exp. Med.* 150, 597-606.
10. Onozaki, K., Matsushima, K., Aggarwal, B. B. and Oppenheim, J. J. (1985) *J. Immunol.* 135, 3962-3967.
11. Lovett, D., Kozan, B., Hadam, M., Resch, K. and Gems, D. (1986) *J. Immunol.* 136, 340-347.

12. Howard, M., Mizel, S. B., Lachman, L., Ansel, J., Johnson, B. and Paul, W. E. (1983) *J. Exp. Med.* 157, 1529-1543.
13. Pike, B. L. and Nossal, G. J. V. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 8153-8157.
14. Lipsky, P. E., Thompson, P. A., Rosenwasser, L. J. and Dinarello, C. A. (1983) *J. Immunol.* 130, 2708-2714.
15. Woloski, B. M. R. N. J., Smith, E. M., Meyer III, W. J., Fuller, G. M. and Blalock, J. E. (1985) *Science* 230, 1035-1037.
16. Lomedico, P. T., Gubler, U., Hellmann, C. P., Dukovich, M., Giri, J. G., Pan, Y.-C. E., Collier, K., Semionow, R., Chua, A. O. and Mizel, S. B. (1984) *Nature* 312, 458-462.
17. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M. and Dinarello, C. A. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 7907-7911.
18. Furutani, Y., Notake, M., Yamayoshi, M., Yamagishi, J., Nomura, H., Ohue, M., Furuta, R., Fukui, T., Yamada, M. and Nakamura, S. (1985) *Nucleic Acids Res.* 13, 5869-5882.
19. March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P. and Cosman, D. (1985) *Nature* 315, 641-647.
20. Van Damme, J., De Ley, M., Opdenakker, G., Billiau, A. and De Somer, P. (1985) *Nature* 314, 266-268.
21. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. and Maniatis, T. (1978) *Cell* 15, 1157-1174.
22. Benton, W. D. and Davis, R. W. (1977) *Science* 196, 180-182.
23. Rigby, P. W. J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* 113, 237-251.
24. Yoshitake, S., Schach, B. G., Foster, D. C., Davie, E. W. and Kurachi, K. (1985) *Biochemistry* 24, 3736-3750.
25. Cohen, S. N., Chang, A. C. Y. and Hsu, L. (1972) *Proc. Natl. Acad. Sci. U. S. A.* 69, 2110-2114.
26. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 5463-5467.
27. Efimov, V. A., Buryakova, A. A., Reverdatto, S. V., Chakhmakheva, O. G. and Ovchinnikov, Y. A. (1983) *Nucleic Acid Res.* 11, 8369-8387.
28. Southern, E. M. (1975) *J. Mol. Biol.* 98, 503-517.
29. Blin, N. and Stafford, D. W. (1976) *Nucleic Acids Res.* 3, 2303-2308.
30. Leonard, W. J., Depper, J. M., Kanehisa, M., Krönke, M., Pfeffer, N. J., Svetlik, P. B., Sullivan, M. and Greene, W. C. (1985) *Science* 230, 633-639.
31. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. and Chambon, P. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 4853-4857.
32. Ruskin, B., Krainer, A. R., Maniatis, T. and Green, M. R. (1984) *Cell* 38, 317-331.
33. Keller, E. B. and Noon W. A. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 7417-7420.
34. Jeffreys, A. J. (1979) *Cell* 18, 1-10.
35. Slightom, J. L., Blechi, A. E. and Smithies, O. (1980) *Cell* 21, 627-638.
36. Breathnach, R. and Chambon, P. (1981) *Annu. Rev. Biochem.* 50, 349-383.
37. Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1980) *Nucleic Acids Res.* 8, 127-142.
38. Carthew, R. W., Chodosh, L. A. and Sharp, P. A. (1985) *Cell* 43, 439-448.
39. Yu, Y.-T. and Manley, J. L. (1984) *Nucleic Acids Res.* 12, 9309-9321.
40. Schmid, C. W. and Jelinek, W. R. (1982) *Science* 216, 1065-1070.
41. Rich, A., Nordheim, A. and Wang, A. H.-J. (1984) *Ann. Rev. Biochem.* 53, 791-846.

42. Kadonaga, J. T., Jones, K. A. and Tjian, R. (1986) Trends Biochem. Sci. 11, 20-23.
43. Weiher, H., König, M. and Gruss, P. (1983) Science 219, 626-631.
44. Gillies, S. D., Morrison, S. L., Oi, V. T. and Tonegawa, S. (1983) Cell 33, 717-728.
45. Karin, M., Haslinger, A., Holtgreve, H., Richards, R. I., Krauter, P., Westphal, H. M. and Beato, M. (1984) Nature 308, 513-519.
46. Matsushima, K., Tosato, G., Benjamin, D. and Oppenheim, J. J. (1985) Cell. Immunol. 94, 418-426.
47. Snyder, D. S. and Unanue, E. R. (1982) J. Immunol. 129, 1803-1805.
48. Thomas, J. R., Bolla, R. I., Rumbyrt, J. S. and Schlessinger, D. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7595-7598.
49. Sanger, F., Coulson, R., Hong, G. F., Hill, D. F. and Petersen, G. B. (1982) J. Mol. Biol. 162, 729-773.