Volume 258, number 2, 301–304 FEB 07900 December 1989

A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor

Shin-ichi Tominaga

Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105, Japan

Received 19 October 1989

A cDNA clone, which represents a species of mRNA that is expressed in growth-stimulated BALB/c-3T3 cells but not in resting cells, was found to encode a protein remarkably similar in sequence to the members of the immunoglobulin superfamily, especially to the extracellular portion of the mouse interleukin 1 receptor The immunoglobulin superfamily is believed to be involved in cell adhesion and cell-to-cell interaction The evidence that a member of this family is induced in the course of the initiation of cell proliferation is intriguing

Cell cycle, Cell growth, G_0/G_1 transition, Immunoglobulin superfamily, Interleukin 1 receptor

1. INTRODUCTION

The signal transduction for the initiation of the cell cycle, especially the early responses, has been investigated intensively [1–6]. On the other hand, the late response that is related to the G_0/G_1 transition has not been studied very extensively [7]. To elucidate the mechanisms of the G_0/G_1 transition, I have been studying genes which are activated at 10 h after serum stimulation of resting BALB/c-3T3 cells. In the preceding paper I reported the isolation of a battery of growth-specific cDNAs from BALB/c-3T3 cells [8]. One of them encoded the β-subunit of integrin which plays a significant role in cell-to-cell and cell-to-matrix interactions [8,9]. Here I report about another cDNA clone (tentatively named ST2) which encodes a protein highly similar in sequence to the members of the immunoglobulin superfamily, especially to the extracellular portion of the mouse IL1-R.

2. MATERIALS AND METHODS

BALB/c-3T3 cells (clone A31) were provided by Dr C Stiles (Harvard Medical School) Poly(A) RNAs from various tissues of CD-1 (10-week-old) mice were purchased from Clontech Laboratories, Inc Exonuclease III, Mung bean nuclease and pUC 19 plasmid were from Takara Shuzo A multiprime DNA labeling system was from Amersham [α-^{32}P]dCTP (specific activity, about 3000 Ci/mmol) was from New England Nuclear

The total cytoplasmic RNAs and poly(A) RNAs were prepared from BALB/c-3T3 cells as described [8] Northern blotting was performed using glyoxal, and dimethylsulfoxide [10] Probes for Northern hybridization were labeled with [α-^{32}P]dCTP by the multiprime labeling method [11] Hybridization was carried out at 42°C and the filter (Zeta probe, Bio Rad) was washed at 50°C in 0 1 × SSC, 0.1% SDS solution [10]. For nucleotide sequence analysis, cDNA was subcloned into the pUC 19 plasmid. Using exonuclease III and Mung bean nuclease, plasmids containing inserts of various lengths were obtained [12], thereafter the nucleotide sequence was determined by the chain termination DNA sequencing method [13,14] Local homology search in translated protein sequence was performed using the IDEAS (Integrated Database and Extended Analysis System for Nucleic Acids and Proteins) program [15–17]

3. RESULTS AND DISCUSSION

As reported previously [8], 7 clones screened from 6.5×10^5 plaques of the λ gt10 cDNA library showed remarkable differences in intensity of Northern hybridization when poly(A) RNAs from resting, growth-stimulated, or continuously growing cells were compared. One of them had been determined to be the β-subunit of integrin. Subsequently, another clone (clone 87) was examined by Northern hybridization (fig.1). The appearance of mRNA was almost all or none between the resting (Q) and growth-stimulated (S) state (fig.1a). And moderate appearance of the mRNA in the continuously growing (G) state suggested that this gene is probably expressed in the G_1 phase. The mRNA appeared as early as 2 h but the peak in the distribution of this mRNA among the total cellular RNAs was 10 h after serum addition (fig.1b), just before the initiation of DNA synthesis [18,19]. Corresponding mRNA in various tissues of mouse was below detectable level even after intensified exposure (fig.2). This also indicates that the expression is not constitutive but growth specific.

Correspondence address. S Tominaga, Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105, Japan

Abbreviations. IL1-R, interleukin 1 receptor, poly(A), polyadenylic acid, dCTP, deoxycytidine 5′-triphosphate

The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no Y07519

Published by Elsevier Science Publishers B.V (Biomedical Division)
00145793/89/$3 50

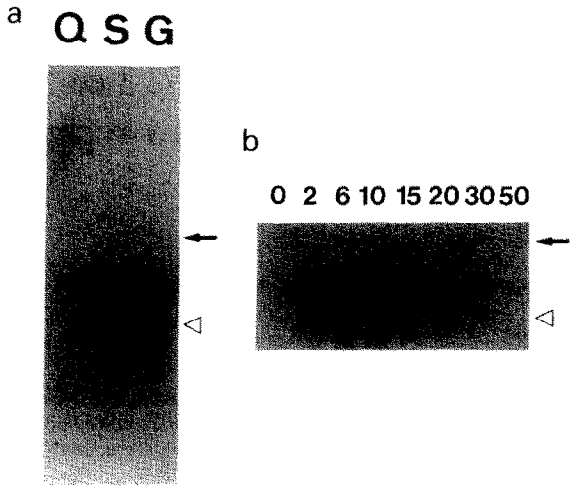

Fig.1. Growth specific appearance of ST2 mRNA . (a) Total cellular RNAs were extracted from quiescent BALB/c-3T3 cells (Q), cells at 10 h after serum stimulation (S), or continuously growing cells (G) as described [8]. RNAs (5 μg) were loaded on a 1.1% agarose gel for Northern blotting. Complementary DNA fragment of clone 87 was excised from the λ gt10 phage and labeled with [α-^{32}P]dCTP. Hybridization was carried out as described in section 2. The arrow and the open arrowhead correspond to the positions of 28S and 18S ribosomal RNA, respectively. (b) Total cellular RNAs were extracted at the indicated hours after serum stimulation. Northern hybridization was performed as in (a).

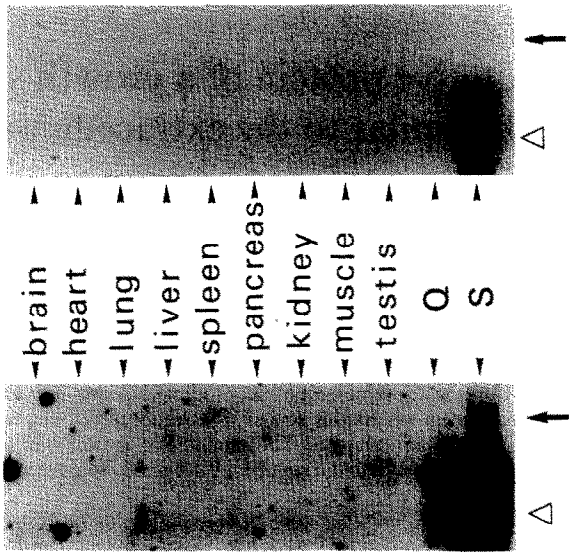

Fig.2. Absence of ST2 mRNA in various tissues of mouse. Poly(A) RNAs (1 μg) from various tissues of CD-1 mouse and quiescent (Q) or serum-stimulated (S) BALB/c-3T3 cells were loaded on an agarose gel and processed for Northern hybridization as in fig.1. The blot was exposed overnight with (lower panel) or without (upper panel) intensifying screen. The arrow and the open arrowhead correspond to the positions of 28S and 18S ribosomal RNA, respectively.

Next, using a cDNA fragment of clone 87, the original cDNA library was rescreened and clone ST2 containing a 2.7 kb insert was obtained. Since the size of this mRNA was about 2.7 kb in Northern blot, ST2 cDNA was nearly full length. The entire nucleotide sequence was determined and shown in fig.3. This cDNA had an open reading frame of 337 amino acids. There were three candidates for initiating methionine residues at positions 1, 7 and 19. According to Kozak's rule, the second ATG codon seems to be the best candidate for initiation site [20]. However, it is difficult to exclude the possibility that the upstream ATG codon is also functional. For simplicity, the most upstream methionine residue was assumed as the translation initiation site. There were nine potential N-linked glycosylation sites. Computer searches of the March 1989 edition of GenBank revealed that this putative ST2 protein had significant similarity to the protein related to the immunoglobulin superfamily, including mouse neural cell adhesion molecule (percent match of 22.7%), mouse basement membrane proteoglycan (19.0%), HLA-6-2 (20.8%), constant region of secreted form of chicken IgM heavy chain (16.5%). The highest similarity was found to be with mouse IL1-R (percent match of 25.1%). Detailed comparison between the ST2 protein and IL1-R revealed that ST2 protein was similar to the extracellular portion of the IL1-R (hatched area in fig.4A) and that it did not have a corresponding transmembrane (dotted area in fig.4A) or cytoplasmic domain [21]. Three loops could be constructed as in the case of the IL1-R and the amino acids 2 residues upstream of cysteine at the N-termini of immunoglobulin-like domains (arrows in fig.4B) were valine, isoleucine, and isoleucine, all of which matched with the consensus amino acid residues [22]. Also, amino acids 2 residues upstream of cysteine at the C-termini of immunoglobulin-like domains (arrowheads in fig.4B) were all tyrosine, which again matched with the consensus residue. In conclusion, this putative protein of growth-specific cDNA is also a member of the immunoglobulin superfamily.

The immunoglobulin superfamily is one of the key groups not only in immunity but also in the mediation of cell surface recognition to control the behavior of cells in various tissues [22]. The unique properties of ST2 protein among the members of the immunoglobulin superfamily are lack of transmembrane domain and its growth-specific expression. It is intriguing to speculate that the protein is possibly secreted as a signal molecule and has something to do with the growth signal transduction. It is also possible, in this respect, that ST2 protein is one of the members of the growth-specific secreted proteins which had been reported earlier [23].

Further investigation of the native product using antibodies to synthetic polypeptides are in progress to understand the localization and the function of ST2 protein.

Acknowledgements: I thank Drs Masato Nakafuku and Yoshito Kaziro for helpful discussions, and Dr Reiko Takemura for reading the manuscript. This work was supported in part by a research grant from the Japanese Ministry of Education, Science and Culture.

```
                                                                    GCAGAAATGAGA  -183

CGAAGGAGCGCCAAGTAGCCTCACGGCTCTGAGCTTATTCTCTCCAGCCCTTCATCTGGGTATCTACAGTGATTTCTCTTCTGGACCCTAC   -92

CTCAGAGAGCACTTGTCAACCGCCTAGTGAACACACCATTACTATCCTGTGCCATTGCCATAGAGAGACCTCAGCCATCAATCACTAGCAC    -1

ATG ATT GAC AGA CAG AGA ATG GGA CTT TGG GCT TTG GCA ATT CTG ACA CTT CCC ATG TAT TTG ACA GTT    69
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr Leu Pro Met Tyr Leu Thr Val    23

ACG GAG GGC AGT AAA TCG TCC TGG GGT CTG GAA AAT GAG GCT TTA ATT GTG AGA TGC CCC CAA AGA GGA   138
Thr Glu Gly Ser Lys Ser Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly    46

CGC TCG ACT TAT CCT GTG GAA TGG TAT TAC TCA GAT ACA AAT GAA AGT ATT CCT ACT CAA AAA AGA AAT   207
Arg Ser Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro Thr Gln Lys Arg Asn    69

CGG ATC TTT GTC TCA AGA GAT CGT CTG AAG TTT CTA CCA GCC AGA GTG GAA GAC TCT GGG ATT TAT GCT   276
Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala    92

TGT GTT ATC AGA AGC CCC AAC TTG AAT AAG ACT GGA TAC TTG AAT GTC ACC ATA CAT AAA AAG CCG CCA   345
Cys Val Ile Arg Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys Lys Pro Pro   115

AGC TGC AAT ATC CCT GAT TAT TTG ATG TAC TCG ACA GTA CGT GGA TCA GAT AAA AAT TTC AAG ATA ACG   414
Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr   138

TGT CCA ACA ATT GAC CTG TAT AAT TGG ACA GCA CCT GTT CAG TGG TTT AAG AAC TGC AAA GCT CTC CAA   483
Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu Gln   161

GAG CCA AGG TTC AGG GCA CAC AGG TCC TAC TTG TTC ATT GAC AAC GTG ACT CAT GAT GAT GAA GGT GAC   552
Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn Val Thr His Asp Asp Glu Gly Asp   184

TAC ACT TGT CAA TTC ACA CAC GCG GAG AAT GGA ACC AAC TAC ATC GTG ACG GCC ACC AGA TCA TTC ACA   621
Tyr Thr Cys Gln Phe Thr His Ala Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr   207

GTT GAA GAA AAA GGC TTT TCT ATG TTT CCA GTA ATT ACA AAT CCT CCA TAC AAC CAC ACA ATG GAA GTG   690
Val Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr Asn His Thr Met Glu Val   230

GAA ATA GGA AAA CCA GCA AGT ATT GCC TGT TCA GCT TGC TTT GGC AAA GGC TCT CAC TTC TTG GCT GAT   759
Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp   253

GTC CTG TGG CAG ATT AAC AAA ACA GTA GTT GGA AAT TTT GGT GAA GCA AGA ATT CAA GAA GAG GAA GGT   828
Val Leu Trp Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln Glu Glu Glu Gly   276

CGA AAT GAA AGT TCC AGC AAT GAC ATG GAT TGT TTA ACC TCA GTG TTA AGG ATA ACT GGT GTG ACA GAA   897
Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu   299

AAG GAC CTG TCC CTG GAA TAT GAC TGT CTG GCC CTG AAC CTT CAT GGC ATG ATA AGG CAC ACC ATA AGG   966
Lys Asp Leu Ser Leu Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr Ile Arg   322

CTG AGA AGG AAA CAA CCA AGT AAG GAG TGT CCC TCA CAC ATT GCT TGA ATAAATTGGCTGAATCAGCTGTGCACT  1041
Leu Arg Arg Lys Gln Pro Ser Lys Glu Cys Pro Ser His Ile Ala End                               337

GCATCCGTTTTCTCCGAGGACTGTGTGTTGTAGCTTGGTCCCAGGGAATCCATCATGATCAAGGGAATAGTTGGCCTGTTTCATCAAGTGT  1132

TCTTCTCACGTTGAGGAAGCTCCTTAAATCTGGTCTTTCCAGAATGTTTCTGTCTTCCAACAGGAATCTCTGTCATTGTATCCTTCCCCTC  1223

TCTGTGTCCCCTCCTCCTTGTTCTCCCGGCAGTCCTCCCCATCTCCTCACCTCCCTTAATGTGTTCTTGACCCCCTTCTCTCTTTTCCTTC  1314

TCTCTGAGCTCCTTCTCACCCAATAGTGGCTTTTGCAGTCATCCTTTGTACCGACTACAAGGGACATTGGTATTGGTAGTGGGTTCAGAGC  1405

AGTAATAACTCTGCTGTGTCTCTTTGTATAACCTTGTCATGGAAAACAACTTACAAACTTTCATTCTGAGCAGTTATTAATTCCCTTGCTT  1496

GGTCCTTGGGTTGACAGGTGCAGCCATCATGATAGATAGATGACCAACCTGATCCGATTTTAAAAGAGTAAACATCTTTTTTACCCTTATC  1587

ACTCTCTTATGATACTGACCACTGCCTTACTGGCAATACAACTAATATGAAAACATTTTTAATTTCTTTCAAATATCAAGAGGGCATGGGA  1678

GGGAGAGAGACACTAACTCTAAGATCATAGCAATATGTGGGGCATTTATTTGGATGAATATATTGATTAAAAGGGTAGGGTGGAGGTACCT  1769

ATTAGATTCAGTCATGCTGTGTCTCTGCCTGAAGTGGTATTTGGGATTTTTGTTGATTCTGTTTGTCTTCTTTTGTTTGTTTTTACTATAG  1860

AAACTATTCTGCCCTTGTACTCCTAGAGTCACCTGTCTTTGCCTCCAGTTACTGGGACTAAAGCTATGTGTCACCTTACTGAGCCAGGGTG  1951

TTTCTTGTTTTGGTTTTGATTTTAGAGCCTCTGGCTTGTAACATTTTTATAAAACAGAATTTTGATTCCTAGGTGGCCAGAGTTGTGACTC  2042

ATAGAGGGATTTTTGTGCTGTTGTGATCAGTGAGGTCTTGGGGATCTGCCCCTGATAATGGTGTTACTCCGGGTGACTGTGGACCACAGCA  2133

CTGTGTTCCCAGATGGTGGTGGTCACTGCACATTCTGCAGGAAAAGAGAATCCAAACCCCTATTCTCACCCAGTTTGACCTTGATTCCACA  2224

ATGCCTTCCTCTGTAACAGGATCTTTTGTCTAGATTTCTGAGTGTACTTTAGTTCACGTTTGTATTAGAATTATATTTTTTAATCAGTAAT  2315

TTTGTATTTGTTTTGTTTGTGTGTGATTTCTTTGTTTTCCAGTTTATTTTTAATTCACTTGTTGCTATTCAAATCAATGTGTTCATACTGT  2406

TTGAACAACACAGCGTATTAAATAAAATTCGTGTCTATTGTTCTTG                                               2452
```

Fig 3 Complementary DNA sequence and deduced amino acid sequence of ST2 The nucleotide sequence of ST2 cDNA was determined as described in section 2 The putative signal peptide cleavage site is designated by an arrow [24]. The poly(A) signal is underlined The potential sites for N-linked glycosylation are shown by arrowheads The cysteine residues involved in the formation of immunoglobulin-like domain are boxed

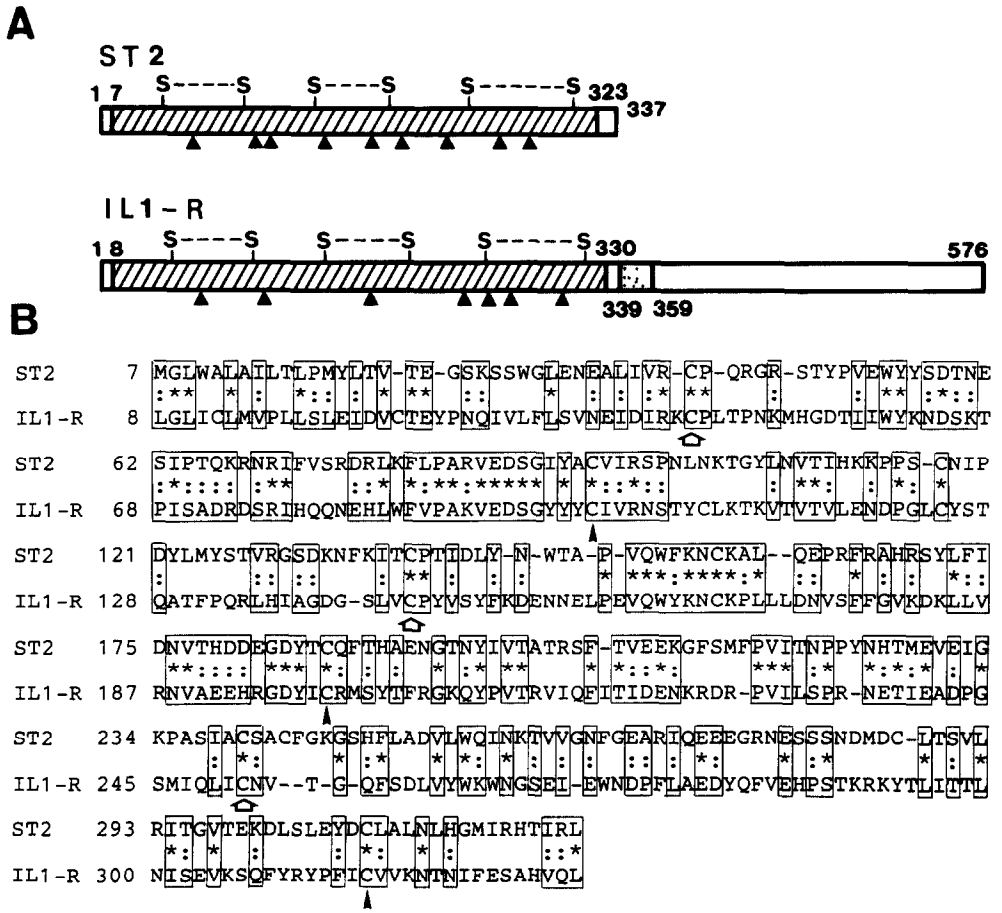

Fig 4 Comparison between the ST2 protein and IL1-R (A) The regions of high similarity are shown by the hatched area Triangles are the potential sites for N-linked glycosylation Disulfide bridges involved in the formation of immunoglobulin-like domains are shown by the broken lines The dotted area of IL1-R corresponds to the transmembrane domain. Numbers in the figure represent amino acid residue numbers (B) Boxed areas are the portions of high similarity * represents the identical amino acid residues represents similar amino acid residues according to Dayhoff's mutation data [25]. The N-terminus and C-terminus of each immunoglobulin-like domain are indicated by the arrow and arrowhead, respectively

REFERENCES

[1] Linzer, D I H and Nathans, D (1983) Proc. Natl Acad Sci USA 80, 4271-4275
[2] Linzer, D I H and Nathans, D. (1984) Proc Natl Acad Sci USA 81, 4255-4259
[3] Hirschhorn, R R., Aller, P., Yuan, Z -A., Gibson, C W and Baserga, R (1984) Proc. Natl Acad. Sci USA 81, 6004-6008.
[4] Lau, L F and Nathans, D (1985) EMBO J 4, 3145-3151
[5] Lau, L.F. and Nathans, D (1987) Proc Natl Acad Sci USA 84, 1182-1186
[6] Chavrier, P , Zerial, M , Lemaire, P , Almendral, J , Bravo, R and Charnay, P (1988) EMBO J. 7, 29-35
[7] Tominaga, S. (1987) FEBS Lett. 226, 53-57
[8] Tominaga, S. (1988) FEBS Lett 238, 315-319
[9] Hynes, R O (1987) Cell 48, 549-555
[10] Maniatis, T , Fitsch, E F and Sambrook, J (1982) in Molecular Cloning A Laboratory Manual Cold Spring Harbor Laboratory Press, New York
[11] Feinberg, A P and Vogelstein, B (1983) Anal Biochem 132, 6-13.
[12] Henikoff, S (1984) Gene 28, 351-359
[13] Sanger, F , Nicklen, S and Coulson, A R (1977) Proc Natl Acad Sci USA 74, 5463-5467
[14] Tabor, S and Richardson, C C (1987) Proc Natl Acad Sci USA 84, 4767-4771
[15] Kanehisa, M. (1982) Nucleic Acids Res 10, 183-196
[16] Needleman, S B and Wunsch, C D (1970) J Mol Biol 48, 443-453
[17] Wilbur, W J and Lipman, D J (1983) Proc Natl Acad Sci USA 80, 726-730
[18] Sokawa, Y , Watanabe, Y , Watanabe, Y and Kawade, Y (1977) Nature 268, 236-238
[19] Balkwill, F and Taylor-Papadimitriou, J (1978) Nature 274, 798-800
[20] Kozak, M (1987) Nucleic Acids Res. 15, 8125-8148
[21] Sims, J E , March, C J , Cosman, D , Widmer, M.B., MacDonald, H R , McMahan, C.J , Grubin, C E., Wignall, J.M , Jackson, J L , Call, S M , Friend, D , Alpert, A R , Gillis, S , Urdal, D and Dower, S K (1988) Science 241, 585-589
[22] Williams, A F and Barclay, A N (1988) Annu Rev Immunol 6, 381-405
[23] Tominaga, S and Lengyel, P (1985) J Biol Chem 260, 1975-1978
[24] Von Heijne, G (1986) Nucleic Acids Res 14, 4683-4690
[25] Dayhoff, M.O., Schwartz, R.M and Orcutt, B C (1979) in Atlas of Protein Sequence and Structure, vol 5, suppl. 3 (Dayhoff, M.O ed), pp 345-352, National Biomedical Research Foundation, Washington, DC