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Pregnancy associated increase in mRNA for soluble D-factor/LIF receptor in mouse liver**

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We examined the distribution of mRNAs for differentiation-stimulating factor (D-factor)/leukemia inhibitory factor (LIF) receptor in various mouse tissues by Northern blotting. A mouse cDNA fragment encoding the D-factor receptor was prepared by the RT-PCR method using human cDNA sequences as primers. The smallest mRNA (3 kb) was present in the liver, but not detectable in other tissues examined. Larger mRNAs (5 and 10 kb) were present in the placenta and the M1 cells, and also detectable in the liver, kidney, heart, lung, brain and embryos. Expression of 3 kb mRNA in the liver increased during pregnancy, being 20 times the initial level on day 15. D-factor receptor cDNAs were isolated from a cDNA library prepared from the liver of a pregnant mouse. Most of the cDNA clones encoded a soluble receptor. A cDNA probe specific for the cellular receptor did not hybridize with 3 kb mRNA in the liver. These results suggest that 3 kb mRNA encodes a soluble D-factor receptor and that the liver is the primary site of synthesis of this soluble receptor.

D-factor; Leukemia inhibitory factor; Soluble receptor; mRNA; Mouse liver; Pregnancy

1. INTRODUCTION

D-factor/LIF was purified and cloned as a cytokine inducing differentiation of mouse myeloid leukemic M1-T22 cells into macrophages [1–3]. It was also found to inhibit the proliferation of freshly isolated leukemic blast cells from acute myeloblastic leukemia patients [4]. D-Factor has a wide variety of biological activities besides actions on normal and leukemic hematopoietic cells [5,6]. It regulates the differentiation of embryonic stem cells, neural cells, hepatocytes, adipocytes, osteoblasts and kidney epithelial cells and the proliferation of myoblasts, primordial germ cells and aortic endothelial cells. Although its physiological roles have not yet been confirmed, D-factor has been suggested to play an important role in development of the mouse. It inhibits the differentiation of embryonic stem cells and maintains them in a pluripotent state [7,8]. Developmental expression of the D-factor gene has been detected in preimplantation blastocysts and in extra-embryonic tissues and placenta [9]. Stewart et al. [10] developed mice lacking the D-factor gene and showed that blastocyst implantation depends on expression of D-factor in the uterine endometrial glands. On the other hand, overexpression of D-factor cDNA in chimeric embryos inhibited gasturation [11]. We found that a D-factor-binding protein is present in mouse serum and that it increases transiently during pregnancy. It inhibits the biological activity of D-factor by blocking its binding to receptors on the target cells [12]. Therefore, this D-factor-binding protein may regulate the action of D-factor during embryonic development of mice. It may block the systemic effect of D-factor on various tissues of the mother. cDNA for the human D-factor receptor and cDNA for the mouse soluble receptor were isolated, but nucleotide sequences of the mouse soluble receptor and the cellular receptor have not yet been reported [5,13]. Layton et al. [14] purified the D-factor-binding protein from mouse serum, analyzed its amino acid sequence and showed that it is a soluble D-factor receptor. In this study, we examined site of synthesis of mRNA for the soluble D-factor receptor in mice.

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**The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the following accession number D17444.

Abbreviations: D-factor, differentiation-stimulating factor; LIF, leukemia inhibitory factor; RT-PCR, reverse transcription-polymerase chain reaction.

2. MATERIALS AND METHODS

2.1. *Isolation of RNA*

Total RNAs were isolated from mouse tissues or mouse myeloid leukemic M1-T22 cells (5×10^6) by a single-step isolation method [15]. Pregnant and nonpregnant ICR mice, 12 weeks old, were purchased from CLEA Japan, Tokyo. The day of appearance of a vaginal plug was designated as day 0.

2.2. *cDNA probe*

Based on the published cDNA sequence of the human D-factor receptor [13], a sense primer (residues 2126–2160) and an antisense primer (residues 2729–2748) were synthesized. By use of cDNA prepared from poly(A)$^+$ mRNA of mouse M1-T22 cells, the 568 bases

cDNA between the two primers was amplified by PCR [16] (30 cycles consisting of 1 min at 95°C (denaturation), 1 min at 45°C (annealing) and 1 min at 63°C (extension)). The amplified product was subcloned into a pCR 1000 vector (Invitrogen). The subcloned insert was sequenced with a the Sequenase version 2.0 kit (United States Biochemical). A high degree of homology (83% nucleotide sequence identity) was found between this cDNA fragment and human cDNA encoding the extracellular domain and the transmembrane domain of the D-factor receptor. The first 184 nucleotides of the cDNA are identical with those of mouse cDNA encoding the soluble receptor isolated in this work. Downstream from residue 185 there is a sequence specific to the cellular receptor including a *Sac*I restriction site. The *Sac*I fragment (about 300 bp) was used to detect mRNA and cDNA for the cellular receptor.

2.3. *Northern blot analysis*

Total RNAs (30 μg/lane) were separated on 0.8% agarose-formaldehyde gel and transferred to nitrocellulose membranes (Schleicher and Schuell). The membranes were prehybridized in 5 × SSC (standard saline citrate) containing 50 mM sodium phosphate, 1 × Denhardt's solution, 50% formamide and 250 μg/ml of sonicated-denaturated herring sperm DNA at 42°C for 3 h and then hybridized with a ^{32}P-labeled fragment of mouse D-factor receptor cDNA or β-actin probe (Oncogene Science) in the same solution at 42°C for 16 h. The membranes were washed twice with 2 × SSC at room temperature and then with 0.1 × SSC containing 0.1% SDS at 65°C for 30 min. Autoradiography was performed using a bioimage analyzer, Fujix BAS 2000.

2.4. *cDNA cloning*

Poly(A)$^+$ RNA was prepared from the liver of a mouse on day 13 of pregnancy. A cDNA library (7.2×10^5 independent clones) was constructed using a cDNA Synthesis System, cDNA Cloning System λgt10 (Amersham) and Super Script RT (Gibco BRL). The cDNA library was screened by plaque hybridization using a 0.6 kb cDNA fragment as a probe. Many positive clones were detected and 7 were analyzed by subcloning into PUC 18 or pGEM-7Zf (Promega). Double-stranded plasmid DNA was sequenced by the dideoxynucleotide chain termination method. cDNA clones encoding the cellular receptor were isolated from the same λgt10 plates as used previously. The membranes that hybridized with 0.6 kb cDNA were washed with 0.1 × SSC containing 0.1% SDS at 90°C for 20 min, and then hybridized with the 0.3 kb cDNA probe as described.

3. RESULTS AND DISCUSSION

3.1. *Pregnancy associated increase in mRNA for D-factor receptor in the liver and placenta*

Using cDNA fragment encoding the extracellular domain and the transmembrane domain of the mouse D-factor receptor, we examined the distribution of the mRNA in various mouse tissues. mRNA (3 kb) was detected in the liver of nonpregnant mice. This sized mRNA was abundant in the liver from day 13 of pregnancy (Fig. 1). The placenta and mouse myeloid leukemic M1 cells contained 5 kb and 10 kb mRNAs. On longer exposure of autoradiographs, 5 kb and 10 kb mRNAs were detected in the liver, brain, heart, kidney, lung and embryos (data not shown). These results are consistent with previous reports that radiolabeled D-factor binds to cells from various tissues [15,16].

Next, we examined the change of expression of mRNA for the D-factor receptor during pregnancy. Fig. 2 shows Northern blot analyses of RNAs from pregnant mouse livers. The levels of mRNA were quantitated with a bioimage analyzer (Fig. 3). Expression of the 3 kb mRNA in liver increased during pregnancy to 20 times the initial level (mean of 6 experiments) on days 13–15, and then decreased. We have reported that a single class of D-factor-binding protein (K_d = 10 nM) is present in mouse serum and that it increases in the late stage of pregnancy [12]. This binding protein was shown to be a soluble form of the cellular receptor for D-factor by Layton et al. [14]. The pregnancy-related change in D-factor receptor mRNA is parallel with change in level

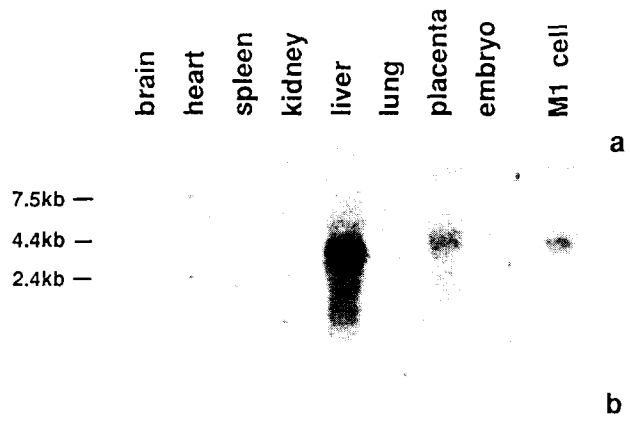


Fig. 1. Northern blot analysis of D-factor receptor mRNA. Total RNAs (30 μg/lane) from various tissues of a mouse on day 13 of pregnancy and M1-T22 cells were electrophoresed in 0.8% agarose-formaldehyde gel and then hybridized sequentially with a ^{32}P-labeled 0.6 kb fragment of mouse D-factor receptor cDNA (a) and β-actin (b) probes.

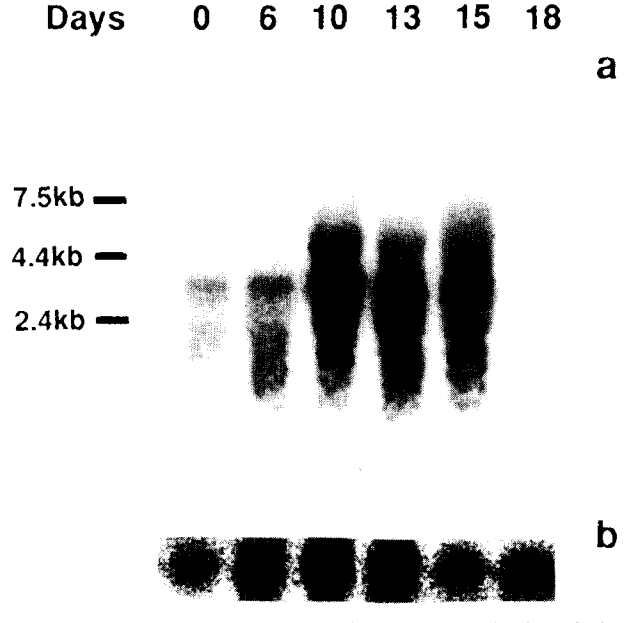


Fig. 2. Expression of mRNA for D-factor receptor in liver during pregnancy. Total RNAs (30 μg/lane) isolated from the liver of mice on the indicated days of pregnancy were analyzed using 0.6 kb D-factor receptor cDNA (a) and β-actin (b) probes.

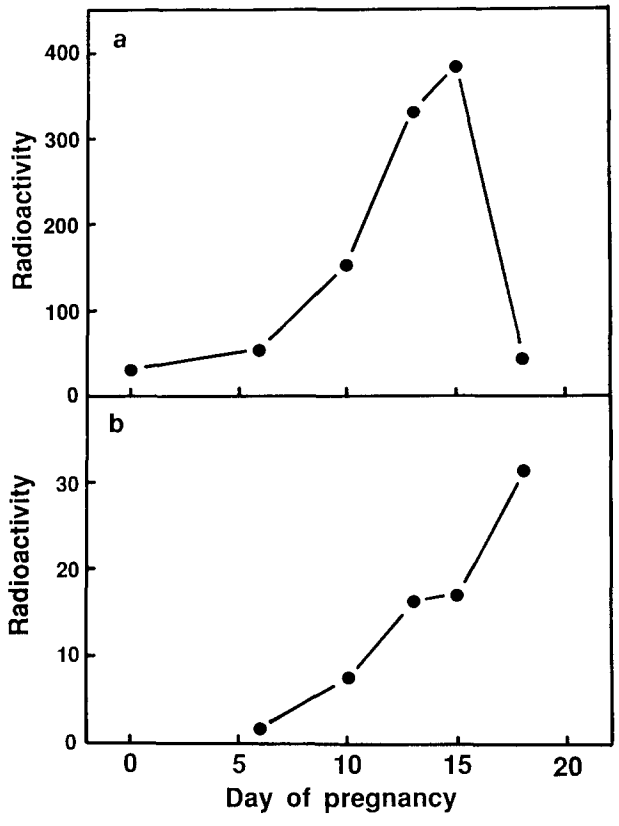


Fig. 3. Expression of mRNA for D-factor receptor in the liver (a) and placenta (b) during pregnancy. Total RNA from the liver or placenta of mice on the indicated days of pregnancy were subjected to Northern blot analysis. Relative radioactivities were measured with a bioimage analyzer.

of the soluble receptor protein in mouse serum [12]. In the placenta, in contrast to the liver, 5 kb mRNA continued to increase during pregnancy (Fig. 3b).

3.2. *Cloning of cDNA encoding soluble D-factor receptor*

We prepared a cDNA library from the liver of a pregnant mouse and detected many positive clones (about 40 clones/2 × 10^4 clones) with a 0.6 kb cDNA probe (Fig. 4A). All 7 clones examined were cDNA clones for the soluble receptor, because they had a stop codon before the transmembrane domain. The nucleotide sequence and predicted amino acid sequence of the longest clone (2551 bases) is shown in Fig. 4B. The amino acid sequence is identical to that reported by Gearling et al. [13]. The nucleotide sequence which is specific to the cellular receptor was found by comparing the sequence of cDNA for the soluble receptor with that of the 0.6 kb cDNA fragment prepared by the RT-PCR method. cDNA clones for the cellular receptor were identified by rehybridization with cDNA probe for the cellular receptor of the same membrane used previously. The number of cDNA clones for the cellular receptor was about 80 times less than that for the soluble receptor in the cDNA library. We analyzed the cDNAs of 8 clones. Although full-length of cDNA has not yet been obtained, the cDNA for the cellular receptor was more than 5 kb. When RNAs were analyzed using the cDNA probe specific to the cellular receptor, bands of 5 kb and 10 kb mRNA were clearly seen but the 3 kb band was faint. These results suggest that 3 kb mRNA encodes the soluble receptor and that 5 kb and 10 kb mRNAs correspond to the cellular receptor. These RNA species may represent alternately spliced transcripts, because all three mRNAs were detected with the cDNA probe derived from the noncoding 3′ portion of the soluble receptor (data not shown). Although expression of 3 kb mRNA in various tissues other than the liver was not clear, we obtained cDNA clones for the soluble receptor from a cDNA library prepared from myeloid leukemic M1 cells. This result suggests that production of the soluble receptor is not

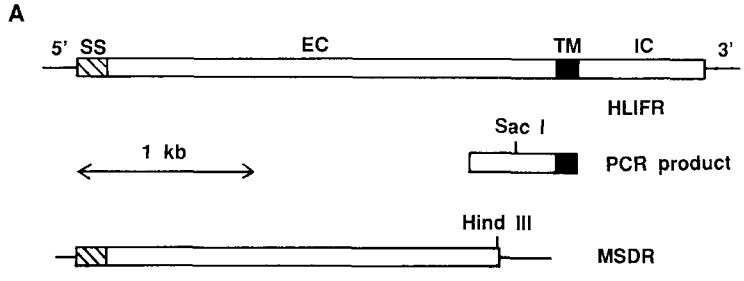
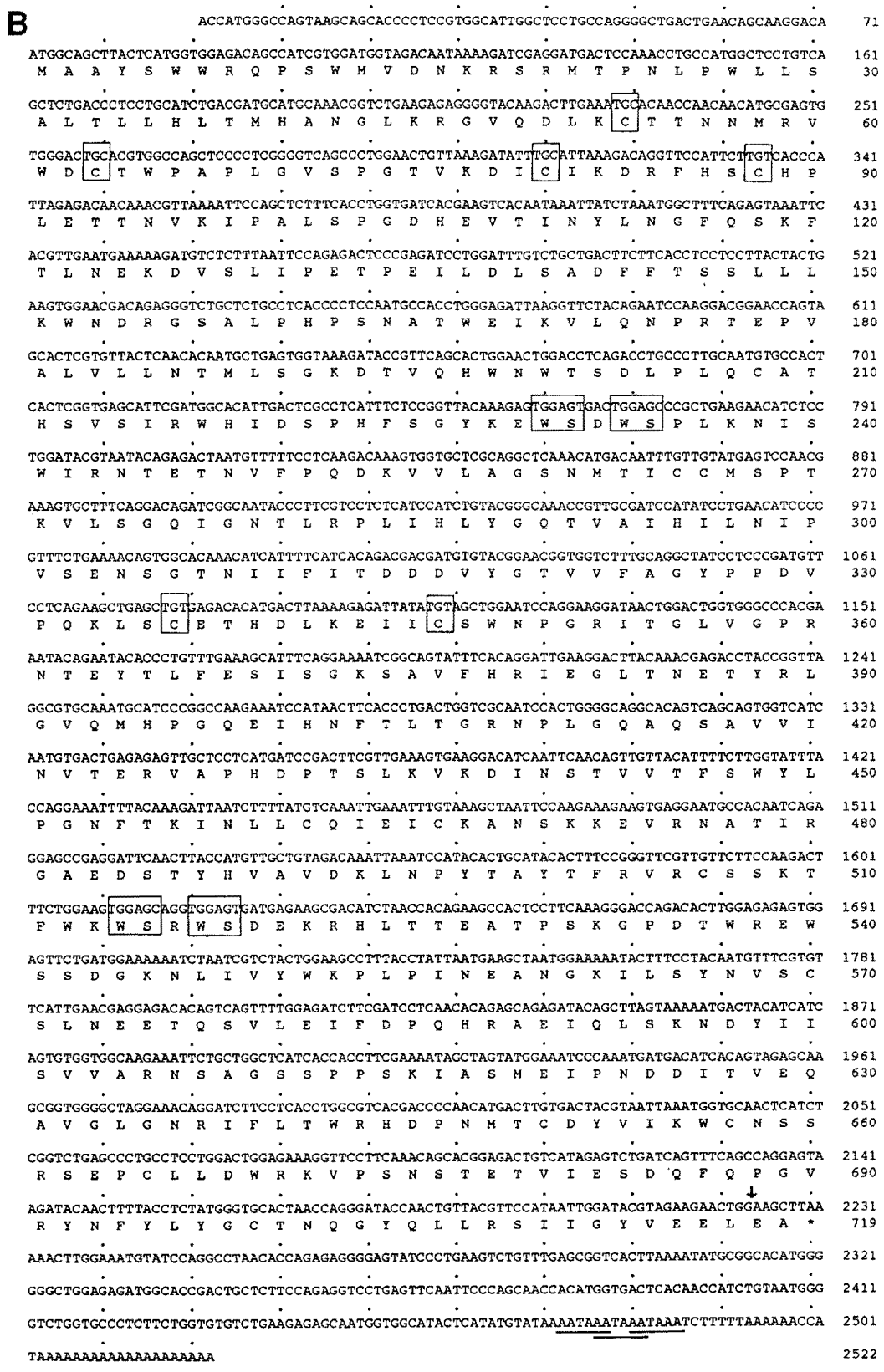


Fig. 4. D-factor receptor cDNA. (A) Schematic representation of human D-factor/LIF receptor cDNA (HLIFR; [13]), 0.6 kb mouse cDNA (PCR product), and mouse cDNA for soluble D-factor receptor (MSDR). The coding region is boxed. SS, signal sequence; EC, extracellular region; TM, transmembrane domain; IC, intracellular region. (B) Nucleotide and deduced amino acid sequences of the mouse cDNA for soluble D-factor receptor. The conserved cysteine residues and the WS motif commonly seen in the cytokine receptor family are boxed. Presumed mRNA processing signals (AATAAA) are underlined.


B
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M A A Y S W W R Q P S W M V D N K R S R M T P N L P W L L S 30
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A L T L L H L T M H A N G L K R G V Q D L K C T T N N M R V 60
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W D C T W P A P L G V S P G T V K D I C I K D R F H S C H P 90
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T L N E K D V S L I P E T P E I L D L S A D F F T S S L L L 150
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R S E P C L L D W R K V P S N S T E T V I E S D Q F Q P G V 690
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TAAAAAAAAAAAAAAAAAAAA 2522

Fig. 4B.

restricted to the liver. However, expression of mRNA for the soluble receptor is most abundant in liver, indicating that the liver is the primary source of the circulating soluble receptor for D-factor.

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REFERENCES

[1] Tomida, M., Yamamoto-Yamaguchi, Y. and Hozumi, M. (1984) J. Biol. Chem. 259, 10978–10982.
[2] Gearing, D.P., Gough, N.M., King, J.A., Hilton, D.J., Nicola, N.A., Simpson, R.J., Nice, E.C., Kelso, A. and Metcalf, D. (1987) EMBO J. 6, 3995–4002.
[3] Lowe, D.G., Nunes, W., Bombara, M., McCabe, S., Ranges, G.E., Henzel, W., Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M. and Goeddel, D.V. (1989) DNA 8, 351–359.
[4] Chen, G., Tomida, M., Hozumi, M., and Nara, N. (1992) Jpn. J. Cancer Res. 83, 1341–1346.
[5] Gearing, G.P. (1993) Adv. Immunol. 53, 31–58.
[6] Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M., Holmes, W., Lowe, D.G. and Goeddel, D.V. (1990) FEBS Lett. 268, 261–264.
[7] Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A. and Gough, N.M. (1988) Nature 336, 684–687.
[8] Smith, A.G., Heath, J.K., Donaldson, D.D., Wong, G.G., Moreau, J., Stahl, M. and Rogers, D. (1988) Nature 336, 688–690.
[9] Conquet, F. and Brulet, P. (1990) Mol. Cell Biol. 10, 3801–3805.
[10] Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992) Nature 359, 76–79.
[11] Conquet, F., Peyrieras, N., Tiret, L. and Brulet, P. (1992) Proc. Natl. Acad. Sci. USA 89, 8195–8199.
[12] Yamaguchi-Yamamoto, Y., Tomida, M. and Hozumi, M. (1993) Leukemia Res. 17, 515–522.
[13] Gearing, D.P., Thut, C.J., VandenBos, T., Gimpel, S.D., Delaney, P.B., King, J., Price, V., Cosman, D. and Beckmann, M.P. (1991) EMBO J. 10, 2839–2848.
[14] Layton, M.J., Cross, B.A., Metcalf, D., Ward, L.D., Simpson, R.J. and Nicola, N.A. (1992) Proc. Natl. Acad. Sci. USA 89, 8616–8620.
[15] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[16] Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A. and Arnheim, N. (1985) Science 230, 1350–1354.
[17] Hilton, D. and Nicola, N.A. (1992) J. Biol. Chem. 267, 10238–10247.
[18] Godard, A., Heymann, D., Raher, S., Anegon, I., Peyrat, M-A., Le Mauff, B., Mouray, E., Gregoire, M., Virdee, K., Soulillou, J-P., Moreau, J-F. and Jacques, Y. (1992) J. Biol. Chem. 267, 3214–3222.