The delayed early serum response gene T1 encodes glycoproteins of the immunoglobulin superfamily with significant sequence similarity to the type 1 interleukin-1 receptor. The T1 gene is transcribed in fibroblasts into an abundant 2.7-kilobase (kb) and a rare 5-kb mRNA in response to proliferation-inducing stimuli. It gives predominantly rise to the longer transcript in the bone marrow of adult mice and in cultured mast cells. Alternative 3' processing is responsible for the two mRNA forms. The short transcript encodes a secreted protein with marked similarity to the extracellular domain of the interleukin-1 receptor, whereas the long mRNA is translated into a protein with an additional putative transmembrane and an intracellular domain. Here we demonstrate that T1 transcription in mast cells and fibroblasts initiates at two different start sites which are 10.5 kb apart. The alternative first exons are both spliced to exon 2 which contains the translation start site. Northern blot analysis and primer extension experiments revealed that promoter usage is strictly cell type-specific. T1 transcription in mast cells is initiated exclusively at the distal promoter, whereas in fibroblasts both the short and the long T1 mRNA start at the proximal promoter. Two GATA-1 elements were identified in the 5'-flanking region of the mast cell-specific distal exon 1.

The T1 gene, also designated ST2 or DER4, was independently isolated as a Ha-ras oncogene-responsive gene (1) and as a gene inducible by growth factors in murine fibroblasts (2, 3). Growth factor-mediated T1 gene induction requires ongoing protein synthesis, thus defining T1 as a delayed early serum responsive gene (3, 4). Upon stimulation of fibroblasts with proliferation-inducing agents such as serum, platelet-derived growth factor, fibroblast growth factor, 12-O-tetradecanoylphorbol-13-acetate, lysophosphatidic acid, or upon oncogene expression T1 is transcribed into an abundant 2.7-kb1 and a rare 5-kb mRNA (4).2 The shorter transcript encodes a primary translation product of 337 amino acids with an NH2-terminal leader peptide sequence (6). The mature small T1 protein is a secreted, heavily N-glycosylated protein (7, 8) of the immunoglobulin superfamily with marked sequence similarity to the carcinoembryonic antigen (6) and the extracellular portion of the interleukin 1 (IL-1) receptors (2). The rare 5-kb T1 mRNA arises by alternative 3' processing of the primary transcript and encodes a 567-amino acid protein. The corresponding cDNA (designated ST2L) was cloned from serum stimulated BALB/c 3T3 cells (9). The sequences of the two T1 proteins are identical at the amino terminus and diverge in nine amino acids in front of the COOH-terminal end of the small protein. The extension of the large T1 protein consists of a putative transmembrane and an intracellular domain with significant homology to the type 1 IL-1 receptor (9).

The in vivo expression patterns of the two T1 transcripts differ drastically. The short mRNA has been found in experimental Ha-ras-induced murine mammary tumors, in the developing mammary gland 3–4 weeks after birth (10) as well as in embryonic skin, retina, and bone (11). Expression of the long transcript is restricted to distinct cells of the hematopoietic organs (embryonic liver, spleen, bone marrow) (11) and to the lung (12) throughout ontogenesis. Despite their similarity to the IL-1 receptor, the T1 proteins neither bind IL-1α nor IL-1β (11), suggesting that the large protein is a novel orphan receptor predominantly expressed in a subset of hematopoietic cells.

Growth factor-induced T1 gene expression in fibroblasts is mediated by AP-1 (4, 13), a homodimeric or heterodimeric protein complex formed by transcription factors of the Jun and Fos families (14). A 12-O-tetradecanoylphorbol-13-acetate-responsive element located within the T1 enhancer at position −3.6 kb is essential for gene induction (13). Furthermore, expression of the immediate early transcription factors c-Fos and FosB resulted in the accumulation of T1 mRNA (4). Likewise, the rat T1 homologue fit-1 was identified as a c-Fos-responsive gene in fibroblasts (15).

Sequence analysis of cDNA clones obtained from T1 mRNA in hematopoietic cells and fibroblasts revealed different transcription initiation sites in these two cell types. Here we demonstrate that the T1 gene is transcribed from two promoters which are 10.5 kb apart. Promoter usage is strictly tissue-specific, the distal and proximal promoters being used exclusively in hematopoietic cells and fibroblasts, respectively.

**EXPERIMENTAL PROCEDURES**

Cell Culture—NFR-2 is a cell clone of NIH 3T3 cells transfected with the Ha-ras (EJ) oncogene (16). NIH 3T3 and NFR-2 cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml), and streptomycin (100 μg/ml). To arrest cell growth, the cells were incubated for 24 h in Dulbecco's modified Eagle's medium containing 0.5% FCS. This medium was replaced with fresh Dulbecco's modified Eagle's medium containing 10% FCS to stimulate entry into the cell cycle.
Anisomycin (Fluka, Biochemica) was added at 200 ng/ml to NFR-2 cells for 6 h. The cell donors 15 and R56 (17) are derivatives of the strictly IL-3-dependent mastocyte cell line PB-3c, which was isolated from mouse bone marrow (18). R56V is an IL-3-dependent, ZIP-v-neomy- cin (19)-infected derivative of R56 and clone 15V-T2 is a ZIP-v- mouse bone marrow (18). R56V is an IL-3-dependent, ZIP-v-mammalian expression system (Promega) to enrich for mRNA. Five or ten micrograms of total RNA and 2.5 μg of poly(A)+ RNA were denatured by glyoxalation, separated on 1% agarose gels (22, 23). The membranes were baked in 25 °C. The membranes were transferred to nylon membranes, and UV cross-linked (UV stratalinker 1800; Stratagene). Hybridization was performed either with DNA fragments labeled with [α-32P]dCTP (3000 Ci/mmol; Amersham Corp.) according to Feinberg and Vogelstein (26, 27) in 5× SSC, 0.1% SDS, 20 mM NaHPO4 (pH 7.0), 5 × Denhardt’s, 1% SDS, 10 μM Tris-HCl (pH 7.5), and 100 μg/ml denatured carrier DNA or with digoxigenin (DIG)-UTP-labeled RNA fragments (26, 28) in 50% formamide, 5 × SSC, 0.1% N-lauroylsarcosine, 0.02% SDS, 20 mM maleic acid, pH 7.5, 30 mM NaCl, and 2% blocking reagent. DIG-labeled RNA was detected using an anti-DIG antibody coupled to alkaline phosphatase followed by the enzymatic reaction with the substrate Lumigen™ PPD (DIG luminescent detection kit; Boehringer Mannheim). The radioactive labeled probes were the following: a 1.15-kb HindIII/BamHI fragment from the plasmid pETH 17 (13) spanning exon 2, a 0.95-kb BamHI/HindIII fragment from the same plasmid, spanning the proximal exon 1, and a 0.6-kb EcoRI fragment from the plasmid pETH, containing the distal exon 1. Southern Blot Analysis—Genomic and plasmid DNA were digested with restriction enzymes, the fragments separated on 1% agarose gels, transferred onto nylon membranes, and the membranes cross-linked. The digoxigenin labeled DNA was further hybridized with DNA fragments labeled with [α-32P]dCTP according to Feinberg and Vogelstein (26, 27) in 5 × SSC, 5 × Denhardt’s solution, 1% SDS, and 100 μg/ml denatured carrier DNA or to the 5′-end-labeled oligonucleotide TG1G (ATTCACCAAGGTCTAGGATCCATTTATT) in 10% dextran sulfate, 5 × SSC, 10 × Denhardt’s, 7% SDS, 20 μg/ml NaCl (pH 7.0), and 100 μg/ml denatured carrier DNA. For end labeling, 50 ng of the oligonucleotide was incubated in 25 μl of 70 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 5 mM dithiothreitol, 0.1 mM spermidine, 0.1 μg of [γ-32P]ATP (5000 Ci/mmol; Amersham Corp.), and 12.5 units T4 polynucleotide kinase (Fermentas) at 37 °C for 45 min. The reaction was stopped by heating at 75 °C for 10 min and unincorporated [γ-32P]ATP removed on a Nick™ column (Pharmacia, Piscataway, N.J.). The following DNA probes were used for hybridization: the 0.6-kb EcoRI fragment from the plasmid pETH containing the distal exon 1 sequence and the 0.85-kb SacI/SphI fragment from the plasmid pETH 17.

Sequence and Primer Extension—The 0.6-kb EcoRI fragment of plasmid pETH was subcloned into pBluescript K' (Stratagene), and the 5′ ends of each DNA was determined by the dideoxynucleoside triphosphate-chain termination reaction (29). For primer extension analysis, 1-2 × 105 pmol of end-labeled oligonucleotides TG1p (GGGGTTGGAGAGGTAAAGCGGTGCGGAGG) or TG2 (GGGTTGGAGAGGTAAAGCGGTGCGGAGG) (purified on 7 μl urea, 13% polyacrylamide gels (30) prior to labeling) were mixed with 5–30 μg of total RNA, ethanol-precipitated, and re-suspended in 30 μl of 50% formamide, 400 mM NaCl, 10 mM PIPES (pH 6.4), and 1 mM EDTA. Annealing was performed by incubation at 80 °C for 3 min followed by slow cooling to room temperature. The nucleic acids were precipitated with isopropanol and primer extension reactions were carried out in 20 μl of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 5 mM dithiothreitol, 1 μg of actinomycin D, 125 μM of each dideoxynucleoside triphosphate, 20 units of RNase Inhibitor (Boehringer Mannheim), and 300 units of RNase H- Moloney murine leukemia virus reverse transcriptase (SuperScript™; Life Technologies, Inc.) at 39 °C for 1 h. For dideoxy chain termination primer extension reactions (29), the conditions were identical to the above with the following changes: the concentration of each of the three dNTPs (not in competition with the ddNTP) was 10 μM, the concentration of the dNTP which was competed for by the corresponding ddNTP was 6 μM, ddATP was 25 μM, ddCTP was 20 μM, ddGTP was 10 μM, and ddTTP was 30 μM. The reactions were stopped by the addition of EDTA to a final concentration of 25 mM, and the RNA was degraded with 5 μg of RNase A at 37 °C for 30 min. After phenol/chloroform extraction of the mixture and subsequent ethanol precipitation the primer extension products were analyzed on 7 μ urea, 9% polyacrylamide sequencing gels (30).

RESULTS

Localization of Mast Cell-specific T1 Gene Sequences—The sequences of cDNA clones derived from mast cells and fibroblasts diverge upstream of the 5′ end of exon 2 (data not shown). The whole genomic T1 DNA has been sequenced up to the Sac I site at position −3.987 (13, 31). No sequence corresponding to the 5′ end of the mast cell-specific T1 cDNA was found within this region. Therefore the T1 transcription start site in mast cells must be located further upstream.

In order to identify this start site, we first determined the genomic organization upstream of the end point of the published sequence (Fig. 1A). DNA of plasmid pETH (Fig. 1A) as well as genomic DNA of NIH 3T3 cells and of primary mast cells were subjected to Southern blot analysis to verify that the organization of the recombinant DNA corresponds to the one of the genomic DNA. The restriction fragment pattern of the different DNAs, obtained by hybridization with the radioactively labeled 0.85-kb SacI/SphI fragment (Fig. 1B), were identical, indicating that no DNA rearrangement had occurred during cloning. This result was confirmed using the 0.6-kb EcoRI fragment as a probe (data not shown).

Next we localized the mast cell-specific sequence by Southern blot hybridization utilizing as a probe the dideoxynucleoside triphosphate chain-termination reaction (Fig. 1B). In order to accurately map the transcription initiation site, we performed primer extension analysis (Fig. 2C). A major and a minor extension product were obtained. Reactions in the presence of dideoxynucleoside triphosphates allowed the exact positioning of the transcription start site. The 5′ ends determined by primer extension and by cDNA cloning coincide precisely.

Cell Type-specific Expression of the Two Alternative First Exons—The T1 gene is transcribed into a major 2.7-kb and a minor 5-kb mRNA in fibroblasts and almost exclusively into the longer 5-kb mRNA in mast cells. Initiation of transcription at the distal and proximal exon 1 could either be cell type-specific or specific for the long and the short transcript as has been suggested for the fit-1 gene, the rat homologue of T1 (12).

Northern blot analyses of RNA extracted from primary and established mast cells, fibroblasts, and adherent primary bone

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3. T. Gaetke, A. K. Werenskiold, and R. Klemzen, unpublished observation.
We therefore analyzed poly(A)-enriched RNA to detect the 5-kb cycloheximide and anisomycin (32). We even observed that this was possible in the presence of the protein synthesis inhibitors in certain cell lines such as NIH 3T3 fibroblasts. T1 induction of proteinsynthesis is a prerequisite for its stimulation. However, serum response gene based on the observation that ongoing transcription in primary adherent bone marrow cells was performed to address this question (Fig. 3). The probes used for hybridization recognized either the T1 mRNA (data not shown). We found particularly high levels of the long 5-kb T1 transcript in fibroblasts (Fig. 3, right panel).

Primer extension experiments were performed to confirm these results at the level of transcription initiation. The primers used in these experiments as well as the expected elongation products are depicted in Fig. 4A. The oligonucleotide TG1, which is complementary to the 5' arm of the 3' transcript, was extended on RNA derived from mast cells and fibroblasts. The two putative TATA boxes for the major and minor transcription start sites are underlined, and putative binding sites for the GATA transcription factors and SP1 are in brackets above the lines and subject to Southern blot hybridization with the oligonucleotide TG1. This probe is complementary to the 5' arm of the distal exon 1 (Fig. 4A). The lengths of the hybridizing restriction fragments are indicated. B, BamHI; H, EcoRI; H, HindIII; S, Sad; Sp, Sphi.

A T1 transcript in fibroblasts (Fig. 3, right panel).

Fig. 3. (A) Genomic organization of the 5' part of the T1 gene. A, different DNA fragments from a λ phage containing approximately 18 kb of the T1 gene upstream of the EcoRI site in the second intron were subcloned (pETRIB, pETH, and pETRI 17) and a restriction map established. Open and filled boxes represent untranslated and coding regions in the first five exons, respectively; the small ellipse marked Enh illustrates the enhancer (13). Restriction sites, the translation start codon, and the extent of sequences subcloned in the plasmids pETRIB, pETH, and pETRI 17 are indicated. The bars represent the probes used for analysis of the genomic Southern blot. d1, distal exon; p1, proximal exon 1. B, Southern blot analysis of cloned and genomic T1 sequences. 0.2 ng of plasmid DNA (pETH) as well as 5 μg of total genomic DNA from NIH 3T3 cells and mast cells were cut with the restriction enzymes indicated above the lines and hybridized to the 0.85-kb SspI probe. M, size marker. B, BamHI; E, EcoRI; H, HindIII; S, Sad; Sp, Sphi.

In certain cell lines such as NIH 3T3 fibroblasts, T1 induction is possible in the presence of the protein synthesis inhibitors cycloheximide and anisomycin (32). We even observed that anisomycin on its own led to a very substantial accumulation of the short 2.7-kb mRNA, whereas the 5-kb transcript accumulated predominantly in mast cells and, to a much lower extent, in primary adherent bone marrow cells.

The T1 gene has previously been identified as a delayed early open reading frame, the exon 2, or the proximal or distal exon 1. As expected, serum-stimulated fibroblasts mainly expressed the short 2.7-kb mRNA, whereas the 5-kb transcript accumulated predominantly in mast cells and, to a much lower extent, in primary adherent bone marrow cells.

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and mast cells (Fig. 4B, lane 3). The extension product indicative for transcriptional initiation at the proximal promoter was obtained with RNA from the two fibroblast cell lines. In contrast, primer extension on mast cell-derived RNA gave rise to the two extension products characteristic for initiation at the distal promoter (Fig. 4B, lane 3). Thus, transcription initiation seems to be strictly cell type-specific. This implies that synthesis of the 5-kb T1 mRNA starts at the distal (Fig. 4B, lane 3) as well as the proximal (Fig. 4B, lane 1) promoter. However, one could argue that the long T1 transcript in fibroblasts is initiated at the distal promoter but that the corresponding extension product could not be detected due to

**Fig. 3. Northern blot analysis of T1 transcripts using probes which discriminate between the proximal and distal promoters and others which do not.** Total RNA from different established mast cell lines (first four lanes, see "Experimental Procedures"), serum-stimulated NIH 3T3 cells, anisomycin-treated NFR-2 cells, primary mast cells, and adherent primary bone marrow cells were analyzed on Northern blots. Four identical blots were prepared with 5 μg of RNA/lane. The filters were independently hybridized to probes corresponding to the T1 whole open reading frame of the 2.7-kb mRNA (T1-ORF), the exon 2, and either one of the alternative exon 1. The positions for the short and long T1 mRNA are indicated. Bottom panel, ethidium bromide-stained 18 S rRNA from one of the gels. Right panel, 2.5 μg of poly(A) mRNA from serum-stimulated NIH 3T3 fibroblasts and anisomycin-treated NFR-2 cells were hybridized to the probe which is specific for the proximal exon 1.

**Fig. 4. Usage of the proximal and distal promoter is cell type-specific.** A, schematic representation of the T1 promoter region. Both the proximal and distal exon 1 are spliced to exon 2. The positions of the oligonucleotides used for Southern blot analysis (TG1d) and for primer extension experiments (TG2, TG1p) as well as the size of the exons (minor transcription start site in parenthesis) are indicated (top). The expected extension products are illustrated (bottom). B, products of primer extension reactions with oligonucleotide TG2 (Fig. 4A) and 18 μg of total RNA isolated from anisomycin-treated NFR-2 cells (lane 1), serum-stimulated NIH 3T3 cells (lane 2), and 15V-T2 mast cells (lane 3) or a mixture of 18 μg of total RNA each from serum-stimulated NIH 3T3 fibroblasts and 15V-T2 mast cells (lane 4). A sequencing reaction of an unrelated DNA fragment was run in parallel on the polyacrylamide gel as a size marker (left panel). The lengths of the extension products are indicated (left margin). C, Northern blot analysis of the same RNAs as used for the primer extension reactions shown in Fig. 4B. Lanes 1–4 correspond to the same lanes in Fig. 4B. The amount of loaded RNA from fibroblasts (lanes 1, 2, and 4) and mast cells (lanes 3 and 4) was 5 μg. The bottom panel depicts part of the ethidium bromide-stained gel around the 18 S rRNA region. D, primer extension reactions with the oligonucleotide TG1p. 10 μg of total RNA extracted from serum-stimulated NIH 3T3 cells (lane 1), serum-starved Swiss 3T3 cells (lane 2), anisomycin-treated NFR-2 cells (lane 3), and primary mast cells (lane 4) were used as templates. The position for the 75-bp extension product, which is characteristic for transcription start at the proximal promoter, is indicated.
its low abundance. To disprove this argument, we performed a primer extension experiment with a mixture of RNA derived from serum-stimulated NIH 3T3 fibroblasts and from mast cells. Northern blot analysis demonstrated that the ratio of the 2.7-kb to the 5-kb T1 transcript in this mixture is approximately the same as in anisomycin-treated NFR-2 cells (Fig. 4C, compare lanes 1 and 4). We obtained extension products indicative of transcription initiation at the proximal and distal promoter (Fig. 4B, lane 4). Even a mixture with a 4-fold reduced amount of mast cell RNA gave rise to easily detectable primer extension products derived from the distal exon 1 (data not shown). Hence, if the long T1 mRNA in NFR-2 cells were initiated at the distal promoter, we should have detected it by primer extension (Fig. 4B, lane 1). Therefore, we conclude that synthesis of the large majority of both T1 transcripts in NFR-2 fibroblasts is started at the proximal exon 1 and that transcript size is independent of the promoter used.

Furthermore, primer extension experiments with the oligonucleotide TG1p, which anneals to the proximal exon 1, yielded a product with RNA from fibroblasts but not from mast cells (Fig. 4D). This confirms that T1 gene transcription in mast cells is exclusively initiated at the distal promoter.

**DISCUSSION**

In this report we have demonstrated that the synthesis of T1 mRNA starts at different promoters in fibroblasts and in mast cells. The two alternative first exons are 10.5 kb apart, and both are spliced to exon 2 where translation starts. Alternative 3’ processing gives rise to a short 2.7-kb and a long 5-kb mRNA that encode a secreted and a putative trans-membrane protein, respectively. In fibroblasts the short transcript predominates, but small amounts of the long mRNA are always observed. Under certain conditions, such as treatment with anisomycin, substantial amounts of the long T1 mRNA are synthesized in fibroblasts, particularly in ras-transformed NIH 3T3 cells. This finding allowed us to demonstrate that all T1 transcripts are initiated at the proximal promoter in fibroblasts, irrespective of their processing mode (Fig. 4, B and C). This is in line with the observation that the two T1 transcripts accumulate in parallel under all inducing conditions in fibroblasts and thus are apparently controlled by the same transcriptional regulatory elements. The large T1 mRNA predominates in mast cells, but substantial amounts of the 2.7-kb T1 transcript could be observed under certain conditions such as treatment with Ca²⁺ ionophores (data not shown). The synthesis of both T1 mRNAs is initiated at the distal promoter in mast cells.

Expression of fit-1, the rat homologous gene of T1, is initiated at two tissue-specific promoters as well, the transcription start sites being 14 kb apart (12). The model has been put forward that transcription initiation is tightly coupled to 3’ processing. Accordingly, whenever transcription starts at the distal promoter, the long mRNA is produced, whereas initiation at the proximal promoter instructs the transcription machinery to terminate in exon 8, giving rise to the short transcript. However, this model is rendered unlikely by our finding that substantial amounts of the long T1 mRNA are observed in fibroblasts under some conditions and that these transcripts are exclusively initiated at the proximal promoter. Rather, we suggest that transcription initiation and 3’ processing (poly(A) selection) are two independent tissue-specific events.

Sequence analysis of the genomic DNA around the mast cell-specific first exon revealed the presence of two GATA elements and one putative SP1 binding site (Fig. 2B). The GATA consensus motif (T/A)GATA(A/G) is the recognition site of the GATA transcription factor family of which six members have so far been described. The patterns of both sequence preferences for DNA binding and expression in tissues as well as cell lines are overlapping, but distinct for these zinc finger proteins. Thus, the GATA transcription factors might exert differential gene regulation by distinct tissue distribution, selective binding to DNA target sequences or by different interactions with other nuclear proteins. Among the GATA factors, expression of GATA-1 (34, 35), GATA-2 and GATA-3 (35) was detected in several mouse and rat mast cell lines but not in mouse 3T3 fibroblasts. These three GATA proteins are reported to regulate the promoter of the carboxypeptidase A gene in mast cells (35). Hence, we consider the possibility that GATA factors direct T1 transcription in mast cells to the distal promoter. It is also striking that two of the four important GATA elements in the murine β-globin promoter (36) are at almost identical relative positions to those in the T1 gene (positions –210 and –75 in the β-globin and positions –210 and –76 in the T1 promoter). A further indication that the GATA elements might be important for mast cell-specific T1 transcription is the finding of a putative SP1 site adjacent to them. Cooperation of SP1 and GATA transcription factors has been observed for several genes (37–39). Promoter studies have been initiated to test whether these GATA elements and the SP1 binding site are indeed instrumental for mast cell-specific T1 gene expression and whether they work in concert with the T1 enhancer. This AP-1 binding regulatory element is centrally located between the two alternative first exons at position –3.6 kb, and it is essential for the induction of the proximal T1 promoter (4, 13). Moreover, Gong et al. have demonstrated that the transcription of the e-globin promoter requires both promoter bound GATA-1 and enhancer bound AP-1NF-E2, whereas GATA-1 acts solely as a mediator of the enhancer effect on transcription (5).

In summary, the T1 gene is transcribed from two promoters which are 10.5 kb apart. Their usage is strictly cell type-specific, as initiation at the distal and the proximal promoter exclusively occurs in mast cells and fibroblasts, respectively. Further characterization of the distal promoter containing the two GATA elements and one SP1 binding site will help to identify the transcription factors that orchestrate tissue-specific 5’ initiation. Differential expression of the putative T1 receptor protein and its soluble form is achieved by two independent tissue-specific events: transcription initiation at two promoters and alternative 3’ processing. T1 is therefore a particularly useful tool for gene regulation studies to reveal the mechanisms responsible for the tissue-specific expression of a transmembrane versus a secreted form of a protein.

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**REFERENCES**

1. Werenskiold, A. K., Hoffmann, S., and Klemenz, R. (1989) Mol. Cell. Biol. 9, 5207–5214
2. Tomina, S. (1989) FEBS Lett. 258, 301–304
3. Lanahan, A., Williams, J. B., Sanders, L. K., and Nathans, D. (1992) Mol. Cell. Biol. 12, 3919–3929
4. Kalousek, M. B., Trub, T., Schuermann, M., and Klemenz, R. (1994) J. Biol. Chem. 269, 6866–6873
5. Gong, Q., and Dean, A. (1993) Mol. Cell. Biol. 13, 911–917
6. Klemenz, R., Hoffmann, S., and Werenskiold, A. K. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5708–5712
7. Takagi, T., Yanagisawa, K., Tsuchimoto, T., Tetsuka, T., Nagata, S., and Tomina, S. (1989) Biochem. Biophys. Acta 1178, 194–200
8. Weireskold, A. K. (1992) Eur. J. Biochem. 204, 1041–1047
9. Werenskiold, A. K. (1992) EMBO J. 11, 1176–1188
10. Rossler, U., Andres, A. C., Reichmann, E., Schnahl, W., and Werenskiold, A. K. (1993) Oncogene 8, 609–617
11. Rossler, U., Thomasen, E., Hultner, L., Baier, S., Danescu, J., and Werenskiold, A. K. (1995) Biochim. Biophys. Acta 1218, 86–97
12. Bergers, G., Reikwester, A., Bruselmann, S., Graninger, P., and Bussinger, M. (1994) EMBO J. 13, 1176–1188
13. Trub, T., Kalousek, M. B., Fröhli, E., and Klemenz, R. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3896–3900
14. Angel, P., and Kärin, M. (1993) Biochim. Biophys. Acta 1072, 129–157
| Reference | Details |
|-----------|---------|
| 15. | Superti-Furga, G., Bergers, G., Picard, D., and Busslinger, M. (1991) Proc. Natl. Acad. Sci. U. S. A. **88**, 5114–5118 |
| 16. | Aoyama, A., Frohli, E., Schafer, R., and Klemenz, R. (1993) Mol. Cell. Biol. **13**, 1824–1835 |
| 17. | Nair, A. P., Hirsch, H. H., and Moroni, C. (1992) Oncogene **7**, 1963-1972 |
| 18. | Ball, P. E., Conroy, M. C., Heusser, C. H., Davis, J. M., and Conscience, J. F. (1983) Differentiation **24**, 74–78 |
| 19. | Schwartz, R. C., Stanton, L. W., Riley, S. C., Marcu, K. B., and Witte, O. N. (1986) Mol. Cell. Biol. **6**, 3221-3231 |
| 20. | Hirsch, H. H., Nair, A. P., and Moroni, C. (1993) J. Exp. Med. **178**, 403-411 |
| 21. | Karasuyama, H., and Melchers, F. (1988) Eur. J. Immunol. **18**, 97-104 |
| 22. | Ishizaka, T., Okudaira, H., Mauser, L. E., and Ishizaka, K. (1976) J. Immunol. **116**, 747-754 |
| 23. | Levi, S. F., Austen, K. F., Gravallese, P. M., and Stevens, R. L. (1986) Proc. Natl. Acad. Sci. U. S. A. **83**, 6485–6488 |
| 24. | Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159 |
| 25. | McMaster, G. K., and Carmichael, G. G. (1977) Proc. Natl. Acad. Sci. U. S. A. **74**, 4835–4838 |
| 26. | Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. **132**, 6–13 |
| 27. | Feinberg, A. P., and Vogelstein, B. (1984) Anal. Biochem. **137**, 266–267 |
| 28. | Hottke, H. J., and Kessler, C. (1990) Nucleic Acids Res. **18**, 5843-5851 |
| 29. | Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. **74**, 5463-5467 |
| 30. | Sanger, F., and Coulson, A. R. (1978) FEBS Lett. **87**, 107-110 |
| 31. | Tominaga, S., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., and Tetsuka, T. (1991) Biochim. Biophys. Acta **1090**, 1–8 |
| 32. | Yanagisawa, K., Tsukamoto, T., Takagi, T., and Tominaga, S. (1992) FEBS Lett. **302**, 51-53 |
| 33. | Aoyama, A., Steiger, R. H., Frohli, E., Schafer, R., von, D. A., Wiestler, O. D., and Klemenz, R. (1993) Int. J. Cancer **55**, 760–764 |
| 34. | Martin, D. I., Zon, L. I., Mutter, G., and Orkin, S. H. (1990) Nature **344**, 444–447 |
| 35. | Zon, L. I., Gurish, M. F., Stevens, R. L., Mather, C., Reynolds, D. S., Austen, K. F., and Orkin, S. H. (1991) J. Biol. Chem. **266**, 22948–22953 |
| 36. | Barnhart, K. M., Kim, C. G., and Sheffery, M. (1989) Mol. Cell. Biol. **9**, 2606-2614 |
| 37. | Leconte, N., Bernard, O., Naert, K., Joulin, V., Larsen, C. J., Romeo, P. H., and Mathieu, M. D. (1994) Oncogene **9**, 2623-2632 |
| 38. | Max-Audit, I., Eleouet, J. F., and Romeo, P. H. (1993) J. Biol. Chem. **268**, 5431-5437 |
| 39. | Merika, M., and Orkin, S. H. (1995) Mol. Cell. Biol. **15**, 2437-2447 |
