Mitogen-activated protein kinase (MAPK) or extracellular signal-regulated kinase are ubiquitous kinases conserved from fungi to mammals. Their activity is regulated by phosphorylation on both threonine and tyrosine, and they play a crucial role in the regulation of proliferation and differentiation. We report here the cloning of the murine p44 MAP kinase (extracellular signal-regulated kinase 1) gene, the determination of its intron/exon boundaries, and the characterization of its promoter. The gene spans approximately eight kilobases (kb) and can be divided into nine exons and eight introns, each coding region exon containing from one to three of the highly conserved protein kinase domains. Primer extension analysis reveals the existence of two major start sites of transcription located at −183 and −186 base pairs (bp) as well as four discrete start sites for transcription located at −178, −192, −273, and −292 bp of the initiation of translation. However, the start site region lacks TATA-like sequences but does contain initiator-like sequences proximal to the major start sites obtained by primer extension. 1 kb of the promoter region has been sequenced. It contains three putative TATA boxes far upstream of the main start sites region, one AP-1 box, one AP-2 box, one Malt box, one GAGA box, one half serum-responsive element, and putative binding sites for Sp1 (five), GC-rich binding factor (five), CTF-NF1 (one), Myb (one), p53 (two), Ets-1 (one), NF-1-L6 (two), MyoD (two), Zeste (one), and hepatocyte nuclear factor-5 (one). To determine the sites critical for the function of the p44 MAPK promoter, we constructed a series of chimeric genes containing variable regions of the 5'-flanking sequence of p44 MAPK gene and the coding region for luciferase. Activity of the promoter, measured by its capacity to direct expression of a luciferase reporter gene, is strong, being comparable with the activity of the Rous sarcoma virus promoter. Progressive deletions of the −1 kb (−1200/−78) promoter region allowed us to define a minimal region of 186 bp (−284/−78) that has maximal promoter activity. Within this context, deletion of the AP-2 binding site reduces by 30–40% the activity of the promoter. Further deletion of this minimal promoter that removes the major start sites (−167/−78) surprisingly preserves promoter activity. This result implicates a major role of this region that contains the Sp1 sites. Finally, removal of the major start sites of transcription as well as the Sp1 sites reveals additional promoter activity at the upstream transcription minor start sites (−240/−167), an activity that is enhanced by the upstream cis-acting elements. In summary, our findings reveal a complex pattern of transcriptional regulation of the mouse p44 MAPK promoter.

Mitogen-activated protein kinases (MAPKs) or extracellular signal-regulated kinases were first described as two proteins of 42 and 44 kDa that were phosphorylated on both tyrosine and threonine residues following stimulation of 3T3-L1 adipocytes with insulin (1, 2). These same phosphoproteins had been visualized previously by twodimensional gel electrophoresis (3–5). MAPKs are ubiquitously expressed, being found in all cell systems studied including yeast, worms, flies, frogs, plants, and mammals (6). They are activated by a wide variety of extracellular signals, and their activation requires the phosphorylation of the highly conserved TEY motif present in almost all described MAPKs. An increasing body of data in particular in yeast suggests that MAPKs belong to a multigene family. In yeast, each reported isoform has been implicated in a different signaling pathway, leading to mating, cell wall synthesis, or regulation of osmotic pressure. Studies with the mammalian homologues of yeast MAPK suggest that they play equivalent roles in different processes, including proliferation, differentiation, and response to environmental stress (7–11).

As far as the p42 and p44 MAPK are concerned, two approaches have demonstrated their role in controlling fibroblast cell growth. First, we showed that overexpression of either a dominant-negative p44 MAPK mutant or an antisense construct prevented growth factor-induced cell cycle entry. Second, we (12) and others (13, 14) demonstrated that expression of a constitutively active form of a MAPK activator (MEK1) led to the constitutive activation of p42 and p44 MAPK, an action sufficient to promote cell cycle entry and oncogenicity in fibroblasts. So, at this stage, both MAPK isoforms that are coordinately regulated and capable of phosphorylating identical substrates in vitro appear to be redundant. Alternatively they might serve different functions as a consequence of alternative spliced isoforms that display distinct subcellular localization as recently reported (15). To resolve this issue we isolate genomic MAPK clones in order to study their regulation and to subsequently disrupt each corresponding mouse gene. Here we de-
scribe the detailed structure of the murine p44 MAPK (extracellular signal-regulated kinase 1) gene. We have also investigated its promoter to identify cis elements important to drive transcription by a deletion analysis.

EXPERIMENTAL PROCEDURES

Materials—Restriction and DNA modifying enzymes were obtained from New England Biolabs (Ozyme, France) or from Eurogentec (Liege, Belgium). [α-32P]dCTP, [α-35S]dATP, and [γ-32P]dATP were from Amersham Corp. or ICN. Synthetic oligonucleotides were from Eurogentec. The genomic DNA library was made with SV129 D3 embryonic stem cell DNA and constructed in a GEM.2 vector. This library was kindly provided by P. Chambron, Strasbourg, France.

Genomic Clones—The genomic library was screened with a 0.73-kb KpnI/KpnI fragment from the hamster p44 MAPK cDNA (16). Six λ phage plaques were isolated from the library with the first screening. Among these different clones two clones (clones 14 and 15) hybridized to the greatest extent to the probe at high stringency and were subcloned for a further analysis. Clone 15 was found to correspond to p44 MAPK, and clone 14 corresponded to another isoform of p44 MAPK with approximately 80% homology at the nucleotide level. Two SacI fragments of 4.3 and 4.8 kb adjacent in clone 15 were subcloned into Bluescript KS. The partial sequence of these subclones was obtained using Universal M13, T7, T5, and K5 primers as well as oligonucleotide primers designed according to the sequence of the probe hybridizing to the genomic library. The 4.3- and 4.8-kb fragments were then subcloned into Bluescript E. coli (see Table I) and characterised by DNA sequencing.

DNA Sequence Analysis—Sequencing was performed by the double-stranded dideoxy chain termination technique using the Pharmacia kit. Restriction analysis and determination of overlapping sequence were done using the Mac Vector program for Macintosh (IBI, New Haven, CT).

Primer Extension Analysis—Three oligonucleotides, ERS 2 corresponding to bases 20–1, and GP9 corresponding to bases 208–189 were hybridized to the probe at high stringency and were subcloned for a further analysis. Clone 15 was found to correspond to p44 MAPK, and clone 14 corresponded to another isoform of p44 MAPK with approximately 80% homology at the nucleotide level. Two SacI fragments of 4.3 and 4.8 kb adjacent in clone 15 were subcloned into Bluescript KS. The partial sequence of these subclones was obtained using Universal M13, T7, T5, and K5 primers as well as oligonucleotide primers designed according to the sequence of the probe hybridizing to the genomic library. The 4.3- and 4.8-kb fragments were then subcloned into Bluescript E. coli (see Table I) and characterised by DNA sequencing.

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ently implicated in the specificity of substrate recognition of the MAP kinase family plus 5.4% of the 3'-untranslated region; and exon 9 encodes the remaining 3'-untranslated region.

Identification of the Transcription Start Site—The 5' end of the mRNA was determined by primer extension using different 20-base oligonucleotides (see "Experimental Procedures") derived from the sequence of the mouse p44 MAPK. ERS 2 and GP9 are specific for p44 MAPK, while ERS 5 can hybridize to p42 MAPK as well as p44 MAPK. The ERS 5 oligo was used to determine the exact position of the start sites because the extended fragments obtained with GP9 or ERS 2 were too long for their size to be determined accurately on classical sequencing gels. The experiment performed with ERS 5 reveals the existence of two major start sites (2183 and 2186) as well as four minor initiation sites at positions 2178, 2192, 2273, and 2292 bp of the ATG (A of ATG 511) (Fig. 2). Because of the high proportion of GC content upstream of and including the ERS 5 primer, it was difficult to obtain reliable sequence within this region, and therefore an unrelated template and primer were used to calibrate the primer extension analysis. When the primer extension analysis was performed with the GP9 oligonucleotide, we detected a major band of 44 bp, indicating the existence of another possible transcript shorter than the classical p44 MAPK transcript (data not shown). Interestingly, the sequence shown in Fig. 2 also reveals the presence of one splice donor (CGGGCAGG at −138, TGGTGAGG at +92, and GGCGAC at +100) consensus sites within the promoter, the 5'-untranslated sequence, and the beginning of the coding region. Fig. 2 only shows the positions of the start sites of transcription in the absence of alternative splicing.

Sequence of the 5'-Regulatory Region—To obtain the sequence of the ATG 5'-flanking region, we subcloned a 2-kb SacII/SacII fragment from the original 4.3-kb SacI/SacI fragment. This SacII/SacI fragment was digested with BstEII, AccI, NheI, and BglII in order to obtain smaller fragments. The sequence obtained is shown in Fig. 3. It was searched for reported consensus sequences that are recognized by DNA-binding proteins. The sequence reveals three putative TATA boxes (TATAAAA at −846; GATACATA at −638; CATAGAGA at −384), one hepatocyte nuclear factor-5 site (TATTTGT at −1321) (21), two p53 sites (GGGCTTGCTT at −1193 and GGGCTAGCCT at −369) (22), two MyoD sites (CACCTG at −952 and CACCTG at −723), one Zeste site (TGAGCC at −947) (24), two sites for NF-IL6 (TGAAGGAAT at −754 and TGTGGCAAT at −680) (25), one-half serum-responsive element site (GATGTCC at −743) (26), one GAGA box (AGAGAGAGAG at −574) (24), one Malt box (GGATGGG at −491) (27), one site for Ets-1 (GAGGATGT at −413) (25), one Myb site (TAACTG at −304) (28), one AP-2 site in reverse orientation (GGCCTGGGG at −259) (29), one AP-1 site in reverse orientation (AGACTAA at −232) (30), one CTF-NF1
site (ACCTAGTTGCCAA at -222) (31), five Sp1 sites (GGCGGG at -112, -97, -91, -46, and -35) (32), and five GC-rich binding factor (GCF) binding sites ((G/C/T)(G/C)CG(C/T)G) overlapping the Sp1 sites (33). All bind to direct production of a luciferase reporter enzyme in transient transfection assays in CCL 39 lung fibroblasts. Positive and negative controls included RSV luciferase, which contains the transcription start sites of transcription are shown by arrows. The double-stranded DNA fragment described above, was obtained with the RP oligonucleotide used to sequence an internal PstI site of the p44 MAPK gene subcloned in the PTZ vector (Bio-Rad) (see “Experimental Procedures”).

Identification of the Promoter Region—The promoter activity of a 1128-bp BgII/SalI (BH) fragment (see “Experimental Procedures” and Fig. 3) was analyzed by measuring its ability to direct transcription of a luciferase reporter enzyme in transient transfection assays in CCL 39 lung fibroblasts. Positive and negative controls included RSV luciferase, which contains the RSV promoter, PxP1 (EV), which has no promoter and a splice donor (see “Experimental Procedures”). The promoter activity of the p44 MAPK gene is driven by the few remaining sequences containing the TATA box; AP-2; AP-1; Myb; p53; Ets-1; GAGA box; Malt box; NF-IL6; CTF-NF1; Sp1; GCF; serum-responsive element (SRE); MyoD; Zeste; hepatocyte nuclear factor 5 (HNF-5); splice donor or splice acceptor sequences, oligonucleotides responsive element (H alf SRE); MyoD; Zeste; hepatocyte nuclear factor 5 (HNF-5)), splice donor or splice acceptor sequences, oligonucleotides were used in primer extension analysis (ERS 2, ERS 5, GP9), and major restriction sites are underlined. The restriction enzyme sites are BglII, PstI, Nhel Styl, Bsh111, and Sad1. The positions of the start sites of transcription are shown by R (178, 183, 186, 272, and 293). The lowercase letters represent the position of the intron. In the case of Sp1 or GCF the number of each site in the underlined sequence is given.

+AP-2 construct. A deeper deletion to the StyI site deleting the AP-1 and CTF-NF1 site decreased by 66% the activity of the +AP-2 construct. Therefore, these results indicate that AP-2, AP-1, and CTF-NF1 potential binding sites participate in the strength of the promoter.

Transcription Can Be Initiated from All of the Start Sites Determined by Primer Extension—An intriguing observation is that deletion of the major start sites of transcription (BSH construct) decrease but did not abolish transcription, suggesting intervention of initiator-like sequence (34) within the −167/−78 region or specific initiation of transcription in fibroblasts. The quite strong promoter activity (50% of maximum) is probably driven by the few remaining sequences containing the three Sp1 binding sites. However, these Sp1 sites are dispensable since their removal (+AP-2/Bs or +AP-2/Bs constructs) preserves promoter activity (12 or 8% of maximal). On the other hand, a 3′ deletion to the PstI proximal site (NP con-
struct) that deletes Sp1 sites as well as the major transcription start sites still possesses 5–10% of promoter activity. The addition to this construct of the upstream cis-acting elements (P3' construct) enhanced 10-fold this activity. This result shows that transcription can be initiated from the discrete start sites (−273, −292) and that there exist positive cis-active elements in the −939/−367 region. Indeed deletion of these discrete start sites (BN construct) abolished promoter activity.

**DISCUSSION**

MAPK belongs to a multigene family, and previous reports have shown that expression of dominant negative mutants or antisense constructs of p44 MAPK were able to inhibit fibroblast proliferation (7). Because of their potential importance in growth control (7, 35) and differentiation (36–38) the genes for human p44 MAPK (extracellular signal-regulated kinase 1), p42 MAPK (extracellular signal-regulated kinase 2), and p63 MAPK (extracellular signal-regulated kinase 3) have been mapped (39). However, it has not been possible to attribute specific biological roles to the individual isoforms even if some data describe differential activation of p42 MAPK versus p44 MAPK in platelets (40). As a first step in such an analysis, we have isolated and partially characterized several different mouse MAPK genomic clones and characterized the gene for mouse p44 MAPK (extracellular signal-regulated kinase 1) and a portion of its 5'-flanking regulatory region in detail.

The p44 MAPK (extracellular signal-regulated kinase 1) gene spans approximately 8-kb and is divided into nine exons. An interesting aspect of the gene's structure is that one or more of the domains highly conserved among protein kinases are contained within individual exons. This is the first example of such a distribution, and it is strikingly different from what is observed in related kinases, such as mammalian cdc2 (41) which is divided into four exons only, without precise division of the protein kinase subdomains among them. This unusual subdivision could result from the evolution of an ancestral gene that has progressively acquired specific characteristics. The first 7 exons encode the protein kinase domains. An additional exon, exon 8, encodes the carboxyl terminus of MAPK. The C-terminal domain it encodes can be considered to be specific for p44 MAPK because it is one of the most divergent domains among the MAPK related kinases, the other variable domain being subdomain X. The ninth exon directly encodes 95% of the 3'-untranslated region of p44 MAPK mRNA.

We also describe the presence of two predominant start sites of transcription located at −183 and −186 bp upstream from the ATG (A = 1). However, overexposure of the gels allowed us to see additional discrete start sites. The presence of multiple sites of transcription initiation is open to interpretation. First, the oligonucleotides used in primer extension analysis could have hybridized to mRNA not yet described. This possibility has to be considered because during the screening of the genomic library five other clones, each apparently encoding a different gene, were shown to hybridize to the p44 MAPK probe at high stringency. The phenomenon could also be explained by the absence of a real consensus sequence for a TATA box. For SV 40 and histone H2A genes, removal or mutation of the

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**Fig. 4.** Measurement of p44 MAP kinase promoter activity by transient expression of chimeric luciferase reporter gene-promoter constructs in CCL39 lung fibroblasts. Activities from different p44 MAPK promoter constructs measured in cells stimulated with 10% fetal calf serum were compared and plotted (percentage of the luciferase activity of the BH construct considered as 100%). The names of the constructs and the numerotation relative to the initiation of translation (+1) are given (see also Fig. 3 and "Experimental Procedures"). These data are representative of five independent transient transfection experiments.
The p44 MAP Kinase Gene

The p44 MAP kinase gene is expressed in many tissues, including the brain, muscle, and liver, but its regulation is still not fully understood. The promoter region of the p44 MAP kinase gene contains several cis-acting elements, including a TATA box, a GC box, and an AP-1 site, which are implicated in the regulation of transcription. The TATA box is essential for the initiation of transcription, while the GC box and AP-1 site are involved in the activation of transcription by various transcription factors.

The GC box contains binding sites for several transcription factors, including Sp1, which is a ubiquitous transcription factor that binds to GC-rich sequences. The AP-1 site contains binding sites for AP-1 and AP-2 transcription factors, which are activated by various cellular signaling pathways, including extracellular signal-regulated kinase (ERK) and c-jun

The presence of these transcription factors in the promoter region suggests that the p44 MAP kinase gene is regulated by a variety of signaling pathways. The regulation of the p44 MAP kinase gene is also influenced by the tissues in which it is expressed. For example, in the liver, the p44 MAP kinase gene is regulated by transcription factors such as c-jun and AP-1, while in the muscle, the p44 MAP kinase gene is regulated by transcription factors such as Sp1 and AP-2.

In summary, the regulation of the p44 MAP kinase gene is a complex process that involves the interaction of various transcription factors and signaling pathways. A better understanding of the regulation of the p44 MAP kinase gene will provide insights into the function of this important kinase in various physiological and pathological processes.
the inactivation of the gene by homologous recombination in embryonic mouse stem cells. Parallel studies with the p42 MAPK-gene will be necessary to determine whether each MAP kinase serves specific function or is totally redundant and can entirely substitute for each other.

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REFERENCES

1. Ray, L. B., and Sturgill, T. W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1502–1506.
2. Ray, L. B., and Sturgill, T. W. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3753–3757.
3. Cooper, J. A., Sefton, B. M., and Hunter, T. (1988) Mol. Cell. Biol. 4, 30–37.
4. Kohn, M., and Pouyssegur, J. (1986) Biochem. J. 238, 451–457.
5. Vila, J., and Weber, M. J. (1988) Cell Physiol. 135, 285–292.
6. L'Allemain, G. (1994) Prog. Growth Factor Res. 5, 291–334.
7. Page's, G., Lenormand, P., L'Allemain, G., Chambard, J. C., Meloche, S., and Pouyssegur, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8319–8323.
8. Deirjard, B., Hilsi, M., Wu, I.-H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) Cell 76, 1025–1037.
9. Galcheva-Gavgova, Z., Deirjard, B., Wu, I.-H., and Davis, R. J. (1994) Science 265, 806–808.
10. Kyrki, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160.
11. Rouse, J., Cohen, P., Trigori, M., Morange, M., Alonso-Lamazares, A., Zamanillo, D., Hunt, T., and Nebreda, A. R. (1994) Cell 78, 1027–1037.
12. Brunet, A., Pagès, G., and Pouyssegur, J. (1994) Oncogene 9, 3379–3387.
13. Cowley, S., Paterson, H., Kemp, P., and Marshall, C. J. (1994) Cell 77, 841–852.
14. Mains, S. J., Maren, W. T., Hermann, A. S., Candia, J. M., Rong, S., Fukusawa, K., and Van Woude, G. F. (1994) Science 265, 966–970.
15. Boulton, T. G., Nyc, N. H., Robins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Parayotatob, N., Cobb, M. H., and Rison, G. D. (1991) Cell 65, 663–675.
16. Melton, D. W., McEwan, C., McClelland, R. A., and Reid, A. M. (1986) Cell 44, 319–328.
17. Darnell, W. S., Sazer, S., Tjian, R., and Schinke, R. T. (1986) Nature 321, 246–248.
18. McGrogr, M., Simonsen, C. C., Smouse, D. T., Farnham, P. J., and Schinke, R. J. (1985) J. Biol. Chem. 260, 2307–2314.
19. Sazer, S., and Schinke, R. T. (1986) J. Biol. Chem. 261, 4685–4690.
20. Yang, X., Yudorov, D., and Deneris, E. S. (1995) J. Biol. Chem. 270, 8514–8520.
21. Pecorino, L. T., Darrow, A. L., and Strickland, S. (1991) Mol. Cell. Biol. 11, 3139–3147.
22. Watt, R., Nishikawa, K., Sorrentino, J., ar-Rushdi, A., Croce, C. M., and Rogel, V. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6307–6311.
23. Ishii, S., Xu, Y., Stratton, R. H., Roe, B. A., Merlino, G. T., and Pastan, I. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4920–4924.
24. Ishii, S., Merlino, G. T., and Pastan, I. (1985) Science 230, 1378–1381.
25. Johnson, A. C., Ishii, S., Inno, Y., Pastan, I., and Merlino, G. T. (1988) J. Biol. Chem. 263, 5693–5699.
26. Hudson, G. L., Thompson, K. L., Xu, J., and Gill, G. N. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7336–7340.
27. Saffer, J. D., Jackson, S. P., and Annarella, M. B. (1991) Mol. Cell. Biol. 11, 2189–2199.
28. Hagen, C., Mulher, S., Beato, M., and Suske, G. (1992) Nucleic Acids Res. 20, 5519–5525.
29. Jackson, S. P., Mac Donald, J. J., Lees-Miller, S., and Tjian, R. (1990) Cell 63, 155–165.