Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7883–7887, October 1986
Immunology

Identification of the 5′-flanking regulatory region responsible for the difference in transcriptional control between mouse complement *C4* and *Slp* genes

(complement/promoter/nucleotide sequence/chloramphenicol acetyltransferase assay)

MASARU NONAKA*, HIROSHI KIMURA†, YU DAE YEUL*, SHIGERU YOKOYAMA*, KOHZO NAKAYAMA*, AND MORINOBU TAKAHASHI*

*Department of Immunobiology, Cancer Research Institute, Kanazawa University, Kanazawa, 920, Japan; and †Department of Experimental Radiology, Shiga University of Medical Science, Seta, Otsu, Shiga, 520-21, Japan

Communicated by Donald C. Shreffler, July 7, 1986

ABSTRACT **To elucidate the molecular basis underlying the difference in the mode of gene expression between mouse complement *C4* (constitutive) and sex-limited protein (*Slp*) (testosterone-regulated), we compared nucleotide sequences and transcriptional regulatory activities of the 5′-flanking regions of these two genes. Although the two sequences showed a high degree of overall homology (95%) up to 1.9 kilobases (kb) upstream from the transcription initiation site, the *Slp* sequence lacked a 31-nucleotide segment containing ACACCC repeats and a 60-nucleotide segment containing ACAC repeats, which are present, respectively, 1.6 kb and 200 base pairs (bp) upstream from the transcription initiation site of the *C4* gene. When assayed in human hepatoma-derived HepG2 cells, the 1.8-kb 5′-flanking DNA fragment of the *C4* gene demonstrated strong transcriptional activity, whereas the corresponding DNA fragment of the *Slp* gene showed only negligible activity. By progressive deletion experiments, it was shown that the difference in the constitutive transcriptional activity of the *C4* and *Slp* genes was accounted for by the presence or absence of the positive regulatory domain located between 1700 bp and 400 bp upstream of the transcription initiation site.**

The fourth component of complement (C4) plays a pivotal role in activation of the classical pathway as a constituent of the C3 convertase (1). Two *C4* isotypes have been described in humans (*C4A* and *C4B*) and in mice [*C4* and sex-limited protein (*Slp*)], and their structural genes are closely linked to each other and to the *C2* and factor B genes in the major histocompatibility complex (2, 3). Nucleotide sequence analysis of cDNA clones revealed a high degree of sequence homology between isotypes in both species (4–6), suggesting that they were duplicated very recently and/or there is a mechanism maintaining identity. Both human C4A and C4B are active as complement components, although some difference in reactivity has been reported (7). In mouse, in contrast, only C4 shows complement activity; Slp is hemolytically inactive and has no known function (8). Comparison of the complete primary structures of the mouse C4 and Slp proteins deduced from the cDNA sequences revealed a cluster of amino acid differences close to the C1 cleavage site of C4, and the possibility has been suggested that these differences render Slp resistant to C1 and, hence, hemolytically inactive (6, 9).

The mode of gene expression of *C4* isotypes also shows a striking contrast between humans and mice. Whereas both human *C4A* and *C4B* are expressed constitutively, in mice of standard inbred strains, only *C4* is constitutively expressed and *Slp* is testosterone-regulated—that is, *Slp* is present only in male adult mice. In addition to this standard pattern, mouse *C4* and *Slp* show extensive genetic variation in serum levels among inbred and wild mice (10), thus providing an attractive system for the study of eukaryotic gene regulation. For example, mice with $H\text{-}2^k$ haplotype have ≈1/20th as much C4 as other common inbred strains. *Slp* has not been detected in either males or females in certain inbred mice or in almost all wild mice so far studied. The possibility that these mice lack the bulk of the *Slp* gene has been excluded (3, 11, 12). In mouse strains carrying wild-type-derived $H\text{-}2^{w7}$, $H\text{-}2^{w16}$, and $H\text{-}2^{w19}$ haplotypes, *Slp* is constitutively expressed. Mice of these strains have multiple *Slp* genes, and possible involvement of the extra genes in constitutive expression was suggested (12–14). In addition, trans regulation for the female expression of *Slp* genes was reported for mouse strains FM, PL/J, and NZB. In these strains, recessive genes not linked to *H-2* and not linked to each other regulate female expression of *Slp* by a testosterone-independent mechanism. However, the breeding experiment showed that the *Slp* gene of FM strain mice *per se* is a testosterone-regulated gene (15). Recently, C4- and Slp-specific cDNA probes became available for quantification of liver mRNA levels of C4 and Slp by RNA blot analysis. In almost all cases so far studied, differences in serum levels of C4 and Slp are paralleled by differences in the steady-state levels of the respective liver mRNA (11), suggesting that the regulation mechanism for *C4* and *Slp* gene expression is in most cases acting at the transcriptional level. To analyze at the molecular level the nature of the transcriptional control mechanism of mouse *C4* and *Slp* genes, here we compared the nucleotide sequences of the 5′-flanking region of the $C4^{FM}$ (constitutive gene) and Slp^{FM} (testosterone-regulated gene) and the transcriptional control activities of these genes using a chloramphenicol acetyltransferase (CAT) assay system.

MATERIALS AND METHODS

Materials. We obtained [α-^{32}P]dATP, [α-^{32}P]dCTP, [γ-^{32}P]ATP, and [^{14}C]chloramphenicol from the Radiochemical Centre; restriction enzymes, Klenow fragment, alkaline phosphatase, T polynucleotide kinase, S1 nuclease, and *Hin*dIII linker were from Takara Shuzo (Kyoto, Japan) and Boehringer Mannheim; reverse transcriptase was from Bethesda Research Laboratories. pSV0cat and pSV2cat were a gift from B. H. Howard; HepG2 cells were a gift from H. R. Colten.

Isolation and Characterization of Genomic Clones Encompassing the 5′-End Regions of the *C4* and *Slp* Genes. Isolation

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: C1, C2, and C4, first, second, and fourth components of complement; Slp, sex-limited protein; CAT, chloramphenicol acetyltransferase; kb, kilobase(s); bp, base pair(s).

FIG. 1. Nucleotide sequences of the 5′ end and flanking regions of the mouse *C4* and *Slp* genes. The nucleotide sequence of the *C4* gene is presented, with residue numbers on the left. The A of the initiator methionine codon is denoted +1, and residues preceding it are given negative numerals. Nucleotide residues placed below the *C4* sequence are those of the *Slp* sequence that are different from the *C4* sequence. Dashes indicate deletions introduced to maximize homology. Since no homology was detected from −1973 further upstream between the *C4* and *Slp* sequences, all residues of both *C4* and *Slp* sequences are presented. Arrowheads and solid circles around position −60 indicate transcription start sites determined by primer-extension and S1 nuclease mapping analysis, respectively. Boxed sequence preceding the transcription initiation site indicates the possible TATA box. The underlined +2 to +19 represents the nucleotide sequence corresponding to the primer used for primer extension. Arrows and solid lines over the *C4* sequence or under the *Slp* sequence indicate ACACCC repeats and ACAC repeats, respectively. Restriction enzyme sites used for CAT assay are shown above or below the sequences.

of genomic clones containing the *C4* and *Slp* genes from FM strain mouse and characterization of exon–intron structures at the 5′-end regions have been described (16, 17). The transcription initiation sites for the *C4* and *Slp* genes were determined by S1 nuclease mapping (18) and primer-extension analysis (19), using liver mRNAs of FM mouse isolated as described (9). All nucleotide sequences were determined at least twice in both directions following the dideoxy-chain termination method (20).

CAT Assay. Fragments of the 5′-flanking DNA of the *C4* and *Slp* genes were introduced into the *Hin*dIII site of the plasmid pSV0cat using *Hin*dIII linkers. The orientation of the insert was determined by restriction analysis. Plasmid DNA was used for transfection after two cycles of purification by centrifugation in CsCl gradients. HepG2 cells ($1–3 \times 10^6$) (21) propagated in 100-mm plates were transfected with 16 μg of recombinant plasmids by the calcium phosphate/DNA coprecipitation method (22). Glycerol shock was performed at 25% and CAT activities were estimated by the method of Gorman *et al.* (23) with the following modification. The cell extract containing 100 μg of protein was assayed for CAT activity in 180 μl of reaction mixture.

RESULTS

Nuelcotide Sequences of 5′-Flanking Regions of the *C4* and *Slp* Genes. λ phage clones containing the 5′-end regions of the *C4* gene, λC4-2.2, and the *Slp* gene, λSlp-3.22, of FM strain mouse were isolated and characterized as described (16, 17). The nucleotide sequences of the 5′-flanking regions of the *C4* and *Slp* genes were determined up to 2.7 kilobases (kb) (C4) or 2.2 kb (Slp) upstream from the AUG initiation codon, and major parts of these sequences are shown in Fig. 1. In these sequences, we denoted the A of the AUG initiation codon as position +1. Upstream nucleotides in the genomic sequence are given consecutive negative numerals. Although there are some insertion/deletions of nucleotides between the *C4* and *Slp* genes, all the nucleotide positions of the *Slp* gene are also expressed according to the C4 numbering system for the convenience of a comparison. When the nucleotide sequences of the 5′-flanking regions of the *C4* and *Slp* genes were compared, a high degree of homology (95%) was observed up to 1972 base pairs (bp) upstream from the AUG initiation codon (Fig. 1). Further upstream from this point, the *C4* and *Slp* sequences diverge markedly and no homology was recognized between the 700-bp *C4* sequence and the 400-bp *Slp* sequence 5′ to this point (only part of these sequences is shown in Fig. 1). In spite of the overall sequence homology up to −1972 from the AUG initiation codon, two major deletions 31 and 60 nucleotides long were observed in the *Slp* sequence, ≈1.7 kb and ≈300 bp, respectively, upstream from the AUG initiation codon. In both regions, the corresponding segments of the *C4* gene consist of C+A-rich sequences. In the −1.7-kb region, the *C4* sequence has seven ACACCC repeats (Fig. 1, arrows), whereas the *Slp* sequence has only two repeats of this motif because of the deletion and substitutions. In the −300-bp region, the *C4* sequence has ACAC repeats and the *Slp* sequence again lacks the major part of these repeats (Fig. 1, solid lines).

Mapping of 5′ Termini of C4 and Slp mRNA. To determine the initiation point of transcription for mouse C4 and Slp mRNA we used S1 nuclease mapping and primer elongation analysis. The initiation sites for both the *C4* and *Slp* genes had been expected to be present between the *Hap* II site (−119) and the A (+1) of the AUG initiation codon, because only DNA fragments of the *C4* gene downstream of the *Hap* II site hybridized with mouse liver mRNA (16). In the primer elongation experiment, we used as primer a synthetic 18-mer oligonucleotide complementary to the 5′ end of the leader sequence, +2 to +19 (Fig. 1, underlining). Three major initiation sites were detected (Fig. 2*A*, arrows) at positions −55, −56, and −65 (Fig. 1, arrowheads). Since the relative content of C4 mRNA and Slp mRNA in FM liver RNA preparations has been estimated as 6:1 based on the numbers of isolated C4-specific and Slp-specific cDNA clones (9), the major bands shown in Fig. 2*A* are probably attributable to the *C4* gene. However, as the nucleotide sequences of the *C4* and *Slp* genes are completely identical in the region corresponding to the primer, it is likely that the initiation site of the *Slp* gene was also detected by this experiment. At present, we can neither understand the significance of multiple initiation sites detected by primer extension analysis nor determine which initiation sites are attributable to the *C4* and *Slp* genes. However, these results clearly indicate that the main transcription initiation site of the *C4* gene is between −65 and −55. The same conclusion was obtained by S1 nuclease mapping analysis. In S1 nuclease mapping, we used a uniformly labeled probe synthesized by M13 phage containing the *Taq* I (−437)/*Bam*HI (−9) fragment of the *C4* gene sequence. As shown in Fig. 2*B*, a ladder of protected fragments was consistently detected. Some of these multiple bands are probably due to overdigestion and/or underdigestion of the DNA·RNA hybrids by S1 nuclease. From the position of the major bands, it is concluded that the 5′ untranslated region of C4 mRNA consists of 58–66 nucleotides (Fig. 1, solid circles). The major start sites detected by primer elongation and S1 nuclease mapping analysis showed a few nucleotides difference, which appear to be within the error of these methods. Together, these results indicate that the initiation point of the *C4* gene is most likely to be present between −66 and −55. S1 nuclease mapping analysis by using a single-stranded probe synthesized according to the *Slp* sequence showed the same digestion pattern as shown in Fig. 2*B* (data not shown), suggesting that a single nucleotide deletion at position −25 and one nucleotide substitution at position −18 were not recognized by S1 nuclease under the conditions used, and therefore the fragments protected by the Slp mRNA are also included in the ladder shown in Fig. 2*B*. As shown in Fig. 1, the nucleotide sequences of the *C4* and *Slp* genes are highly homologous near the C4 initiation sites. Therefore, on the basis of the results of primer extension, S1 nuclease mapping, and sequence analysis, the initiation site of the *Slp* gene was also located ≈60 bp upstream of the AUG initiation codon. Similar observations have been made by Tosi *et al.* (25), who used mRNA isolated from BALB/c (*H-2*^{d}) mice. The result of a CAT assay in which the DNA fragment containing nucleotides −244 to −9 of the *Slp* gene showed promoter activity (to be described in the following section) also supports this conclusion. Both the *C4* and *Slp* gene lack typical TATA and CAAT boxes. However, a possible variant of a TATA box—GATCAAA—is recognized ≈20 bp 5′ of the transcription initiation sites determined by primer elongation and S1 nuclease mapping analysis (Fig. 1, box).

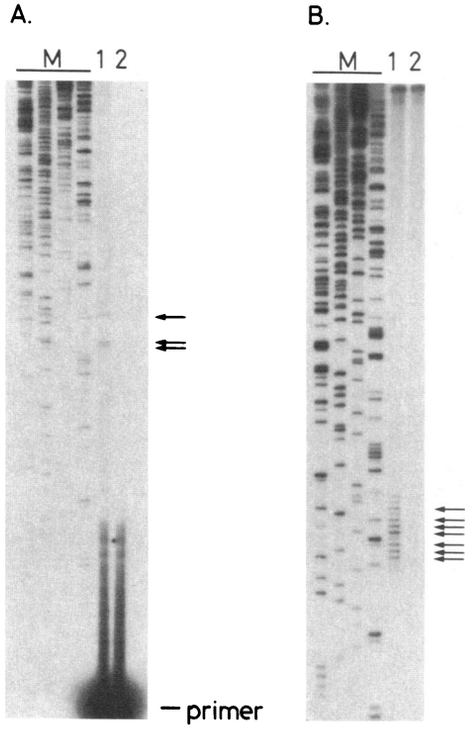

FIG. 2. Determination of the transcription initiation sites of the *C4* and *Slp* genes. (*A*) Primer extension of the C4 and Slp mRNAs. A 5′-end-labeled oligonucleotide primer synthesized according to the nucleotide sequence from +2 to +19 was hybridized with 4 μg of >28S mRNA isolated from FM mouse liver (lane 1) or 4 μg of yeast tRNA (lane 2). After incubation with 80 units of reverse enzyme at 42°C for 30 min, primer-extended products were analyzed in 7 M urea/8% polyacrylamide gel. Arrows indicate positions of the extended products. Lanes M, size markers. (*B*) S1 nuclease mapping of the 5′ portion of the C4 and Slp mRNAs. The *Taq* I (−437)/*Bam*HI (−9) fragment of the *C4* gene was subcloned in M13mp11. Uniformly labeled probes for S1 nuclease mapping were synthesized as described (24). Ten micrograms of poly(A)$^+$ RNA isolated from FM mouse liver (lane 1) or 10 μg of yeast tRNA (lane 2) was hybridized to the single-stranded probe. After digestion with S1 nuclease (3 units/μl) for 40 min at 30°C, resistant products were separated in 7 M urea/8% polyacrylamide gel. Arrows indicate positions of major bands in the autoradiograph. Lanes M, size markers.

Difference in Transcriptional Control Between the *C4* and *Slp* Genes Estimated by CAT Assay. We tested transcriptional regulation activity of the 5′-flanking regions of the *C4* and *Slp* genes by using a CAT assay system (23). CAT assays were performed with human hepatoma-derived HepG2 cells, which have been shown to produce human C4 in culture (21). As positive and negative controls, we transfected HepG2 cells with pSV2cat, which contains the simian virus 40 early promoter region, and with pSV0cat, which lacked the entire simian virus 40 promoter region (23). As expected, HepG2 cells transfected with pSV2cat showed a significant CAT activity, whereas HepG2 cells transfected with pSV0cat did not show any detectable CAT activity. Transcriptional regulation activity of the 5′-flanking DNA segments of the *C4* and *Slp* genes was tested after subcloning into the *Hin*dIII site of pSV0cat, which is located immediately upstream of the CAT structural gene. In all the following CAT experiments, the *Bam*HI (−9) site found between the transcription initiation sites and the AUG initiation codon of both C4 and Slp sequences (Fig. 1) was used as the 3′ end of the inserted DNA fragments. When a 2.9-kb *Bam*HI/*Bam*HI 5′-flanking fragment of the *Slp* gene and 2.0-kb *Hin*dIII/*Bam*HI 5′-flanking fragment of the *C4* gene were inserted in either orientation into pSV0cat, only the 5′-flanking sequence of the *C4* gene in the correct orientation showed a significant activity to direct CAT transcription in HepG2 cells (Fig. 3). To determine the initiation point of the CAT transcript directed by the 5′-flanking sequence of the *C4* gene, we performed S1 nuclease mapping analysis using the same probe as described in the legend for Fig. 2*B*. We found that this CAT transcript was

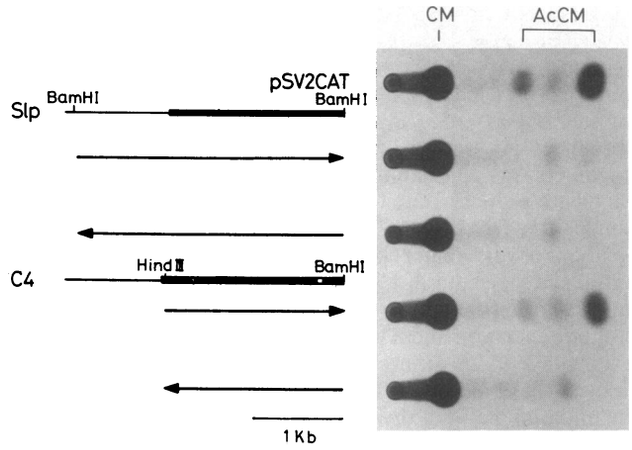

FIG. 3. CAT activities directed by the 5'-flanking DNA fragments of the *C4* and *Slp* genes. (*Left*) Schematic representation of the *C4* and *Slp* genes. Thick lines represent the regions where a high degree of homology is recognized between the *C4* and *Slp* genes. White areas in the thick line of the *C4* gene show the positions of the C+A-rich sequences corresponding to the two major deletions of the *Slp* gene. Arrows indicate ranges and directions of the inserts subcloned into the *Hin*dIII site of pSV0cat. HepG2 cells were transfected with these recombinant plasmids or with pSV2cat (positive control), and CAT activities were evaluated as described (23). (*Right*) Autoradiogram shows the conversion of chloramphenicol (CM) to its acetylated products (AcCM).

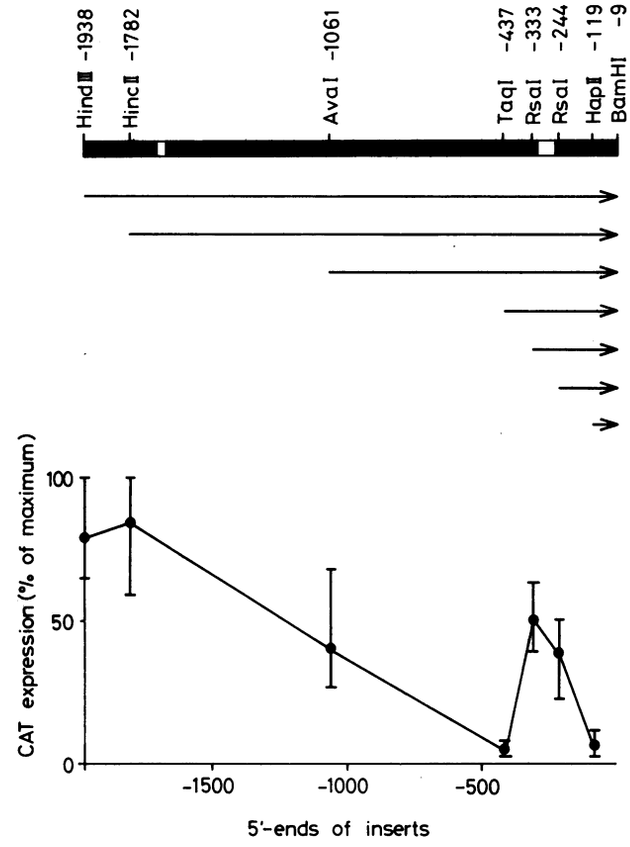

FIG. 4. Effect of successive 5' deletion on the transcriptional regulation activity of the 5'-flanking region of the *C4* gene. (*Upper*) Schematic representation of the 5'-flanking region of the *C4* gene is shown as a bold line. White areas show positions of C+A-rich sequences corresponding to the two major deletions of the *Slp* gene. Arrows indicate range of inserts subcloned into the *Hin*dIII site of pSV0cat. They have common 3' ends (*Bam*HI site, −9) and 5' deletions of various extents. (*Lower*) CAT activities directed by these 5'-deleted inserts are plotted against the 5' ends of the inserts. After visualizing the results of the CAT assay by autoradiography, areas containing chloramphenicol and its acetylated metabolites were cut from the silica plates and measured by scintillation counting. CAT activity was calculated as percentage conversion to acetylated metabolites and then values were converted to percentage of the maximum of each experiment. Closed circles indicate average values from three experiments involving independent clones or independent plasmid preparations; vertical bars indicate range of values.

initiated faithfully from the transcription start site of the *C4* gene (data not shown). To identify the DNA region required for the transcriptional activity, we subcloned 5'-flanking segments of the *C4* gene of various lengths into pSV0cat and assayed for CAT activity. These fragments had a common 3' end, the *Bam*HI (−9) site, and variable 5' ends. As shown in Fig. 4, the *Hin*dIII (−1938)/*Bam*HI (−9) and *Hin*cII (−1782)/*Bam*HI (−9) fragments showed high activities of the same level. A substantial reduction in activity was observed with the *Ava* I (−1061)/*Bam*HI (−9) fragment. A shorter *Taq* I (−437)/*Bam*HI (−9) fragment showed little or no activity. Then, much shorter fragments, the *Rsa* I (−333)/*Bam*HI (−9) and the *Rsa* I (−244)/*Bam*HI (−9) again showed a significant activity, which amounted to 50% the level of maximum activity. The shortest *Hap* II (−119)/*Bam*HI (−9) fragment, however, did not show any detectable activity. These results indicate that the 5'-flanking region of the *C4* gene contains at least three functional domains; (*i*) a putative promoter located between the *Rsa* I (−244) site and the transcription initiation sites; (*ii*) an inhibitory domain present between the *Taq* I (−437) site and the *Rsa* I (−333) site; and (*ii*) a positive regulating domain still further upstream. To identify the domains of the *Slp* gene that are functionally different from those of the *C4* gene, we subcloned three 5'-flanking segments of the *Slp* gene into pSV0cat and assayed for their activity to direct CAT transcription. These three fragments are *Rsa* I (−244)/*Bam*HI (−9), *Hin*cII (−439)/*Bam*HI (−9), and *Hin*cII (−1782)/*Bam*HI (−9), which, respectively, correspond to the promoter, the promoter plus the inhibitory domain, and the promoter plus the inhibitory domain plus the positive regulatory domain of the *C4* gene. As shown in Fig. 5, very similar levels of CAT transcription were directed by the putative promoter domain of C4 and Slp fragments. The DNA fragments of both the *C4* and *Slp* genes containing the putative promoter domain plus the putative inhibitory domain directed only negligible CAT transcription. However, a marked difference in the CAT activity was recognized when the C4 and Slp fragments containing all of the three putative functional domains were compared. Thus, the Slp fragment directed only very negligible CAT transcription, while the corresponding C4 sequence directed quite high CAT activity. It is highly likely that the *Hin*cII (−1782)/*Bam*HI (−9) fragment of the *Slp* gene lacks the positive regulatory function. The absence of the positive regulatory sequence in the 5'-flanking region of the *Slp* gene and in the presence of the functionally intact promoter and the negative regulatory domains probably explains the difference in the activity to direct CAT transcription between the long fragments of 5'-flanking sequences of *C4* and *Slp* genes.

DISCUSSION

When tested by CAT assay, the 5'-flanking regions of the *C4* and *Slp* genes showed a marked difference in the transcriptional regulation activity. The plasmids containing the 1.8-kb 5'-flanking DNA of the *C4* gene showed strong activity to direct CAT transcription in HepG2 cells at a comparable level as pSV2cat. In contrast, the plasmid containing the corresponding 5'-flanking fragment of the *Slp* gene showed a negligible activity indistinguishable from the background level. The extreme inefficiency of the 5'-flanking region of the

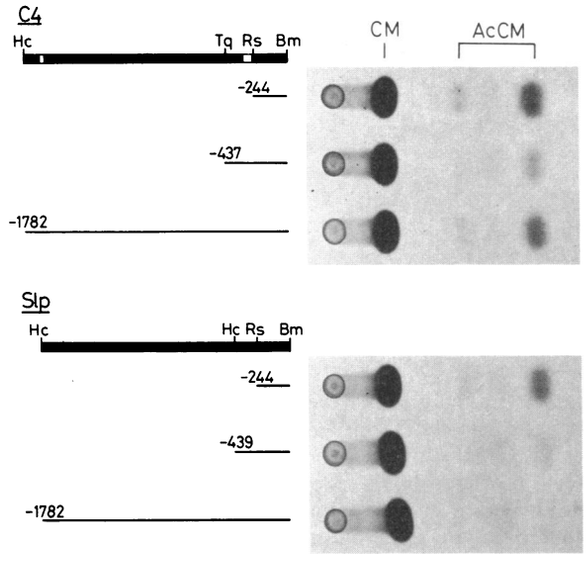

FIG. 5. Expression of CAT activities in HepG2 cells transfected with recombinant plasmids containing various 5'-flanking fragments of the *C4* and *Slp* genes. The 5'-flanking fragments of the *C4* gene containing one (−244 to −9), two (−437 to −9), or three (−1782 to −9) functional domains and the corresponding fragments of the *Slp* gene were subcloned into pSV0cat and assayed for transcriptional control activity. Restriction enzyme sites used were as follows: Hc, *Hin*cII; Tq, *Taq* I; Rs, *Rsa* I; Bm, *Bam*HI. Explanations for the schema of the 5'-flanking regions of the *C4* and *Slp* genes and the autoradiogram are in the legend for Fig. 3. This figure shows the result of one of three independent assays. The other two assays showed very similar results.

Slp^{FM} gene in the transcriptional regulation is at first glance contradictory to the apparent constitutive Slp expression in FM strain mice, but it is compatible with the idea proposed by Brown and Shreffler (15) that the seemingly constitutive expression in FM mice is due to the extra H-2, trans-acting factor. The 5'-deletion experiment of the 5'-flanking region of the *C4* gene showed the presence of at least three functional domains; a putative promoter domain, an inhibitory domain, and a positive regulating domain in this order from proximal to distal. The difference in transcriptional regulation between the *C4* and *Slp* genes is explained by the difference in the most upstream positive regulating regions. The putative promoter region of the *Slp* gene showed the same level of activity as the *C4* gene.

In contrast to a marked difference in the transcriptional regulation, a high degree of nucleotide sequence homology (95%) was observed between the *C4* and *Slp* genes up to 2 kb upstream from the AUG initiation codon. The degree of homology is striking in the sense that it is almost the same degree as the homology observed between the protein-coding regions of these two genes (96%) (5). However, the *Slp* sequence had two major deletions with the length of 31 nucleotides and 60 nucleotides in the 5'-flanking region. The corresponding segments of the *C4* gene were occupied by characteristic C+A-rich sequences, ACACCC repeats (−1685 to −1655 region), and ACAC repeats (−315 to −256 region). ACAC repeating sequences are known to be widespread in a broad range of eukaryotic genomes. For example, mammalian genomes contain 10^4–10^5 copies of this sequence (26). The ACACCC repeating sequences of the *C4* gene closely resemble the simple repeating sequence of the yeast telomere, $(C_{1-3}A)_n$ (27, 28). The biological functions of these C+A-rich sequences in the 5'-flanking region of the *C4* gene are not apparent. However, an intriguing hypothesis is that these C+A-rich regions of the *C4* gene play some regulatory role in transcription, since the ACACCC sequence of the β-globin gene just 5' to the CAAT box has been shown to be important for the transcriptional regulation of this gene (29, 30).

We are grateful to Dr. B. H. Howard for providing pSV0cat and pSV2cat; to Dr. H. R. Colten for providing HepG2 cells; to Drs. K. Yamamoto, A. Shimizu, and S. Natsuume-Sakai for helpful discussions and advice; and Ms. M. Miyagishi and Y. Nakano for technical assistance. This work was supported partly by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

1. Müller-Eberhard, H. J. (1975) *Annu. Rev. Biochem.* **44,** 697–723.
2. Carroll, M. C., Campbell, R. D., Bentley, D. R. & Porter, R. R. (1984) *Nature (London)* **307,** 237–241.
3. Chaplin, D. D., Woods, D. E., Whitehead, A. S., Goldberger, G., Colten, H. R. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6947–6951.
4. Belt, K. T., Carroll, M. C. & Porter, R. R. (1984) *Cell* **36,** 907–914.
5. Nonaka, M., Nakayama, K., Yu, D. Y. & Takahashi, M. (1986) *J. Immunol.* **136,** 2989–2993.
6. Ogata, R. T. & Sepich, D. S. (1985) *J. Immunol.* **135,** 4239–4244.
7. Law, S. K. A., Dodds, A. W. & Porter, R. R. (1984) *EMBO J.* **3,** 1819–1823.
8. Ferreira, A., Nussenzweig, V. & Gigli, I. (1978) *J. Exp. Med.* **148,** 1186–1197.
9. Nonaka, M., Takahashi, M., Natsuume-Sakai, S., Nonaka, M., Tanaka, S., Shimizu, A. & Honjo, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6822–6826.
10. Shreffler, D. C., Atkinson, J. P., Chan, A. C., Karp, D. R., Killion, C. C., Ogata, R. T. & Rosa, P. A. (1984) *Philos. Trans. R. Soc. London Ser. B* **306,** 395–403.
11. Ogata, R. T. & Sepich, D. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4908–4911.
12. Levi-Strauss, M., Tosi, M., Steinmetz, M., Klein, J. & Meo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1746–1750.
13. Levi-Strauss, M., Georgatsou, E., Tosi, M. & Meo, T. (1985) *Immunogenetics* **21,** 397–401.
14. Rosa, P. A., Sepich, D. S., Shreffler, D. C. & Ogata, R. T. (1985) *J. Immunol.* **135,** 627–631.
15. Brown, L. J. & Shreffler, D. C. (1980) *Immunogenetics* **10,** 19–29.
16. Nonaka, M., Nakayama, K., Yu, D. Y. & Takahashi, M. (1985) *J. Biol. Chem.* **260,** 10936–10943.
17. Nonaka, M., Nakayama, K., Yu, D. Y., Shimizu, A. & Takahashi, M. (1985) *Immunol. Rev.* **87,** 81–99.
18. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
19. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306,** 557–561.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
21. Morris, K. M., Aden, D. P., Knowles, B. B. & Colten, H. R. (1982) *J. Clin. Invest.* **70,** 906–913.
22. Graham, F. & van der Eb, A. (1973) *Virology* **52,** 456–457.
23. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
24. Fujita, T., Ohno, S., Yasumitsu, H. & Taniguchi, T. (1985) *Cell* **41,** 489–496.
25. Tosi, M., Levi-Strauss, M., Georgatsou, E., Amor, M. & Meo, T. (1985) *Immunol. Rev.* **87,** 151–183.
26. Hamada, H., Petrino, M. G. & Kakunaga, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6465–6469.
27. Shampay, J., Szostak, J. W. & Blackburn, E. H. (1984) *Nature (London)* **310,** 154–157.
28. Walmsley, R. W., Chan, C. S. M., Tye, B.-K. & Petes, T. D. (1984) *Nature (London)* **310,** 157–160.
29. Dierk, P., van Ooyen, A., Cochran, M. D., Dobkin, C., Reiser, J. & Weissman, C. (1983) *Cell* **32,** 695–706.
30. Treisman, R., Orkin, S. H. & Maniatis, T. (1983) *Nature (London)* **302,** 591–596.