

STRUCTURAL GENE FOR HUMAN MEMBRANE COFACTOR  
PROTEIN (MCP) OF COMPLEMENT MAPS TO WITHIN  
100 kb OF THE 3' END OF THE C3b/C4b RECEPTOR GENE

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Membrane cofactor protein (MCP) of complement is a C3b/C4b binding glyco-  
protein (1) that possesses cofactor activity for the Factor I-mediated proteolysis of  
C3b and C4b (2). MCP has a wide tissue distribution (1, 3-5), and its physiologic  
role may be to protect autologous tissue from complement-mediated damage (6-7).

Our laboratory has recently cloned and sequenced a full-length MCP cDNA (8).  
Sequence analysis demonstrated that MCP contains at its NH<sub>2</sub> terminus four copies  
of the 60 amino acid cysteine rich short consensus repeats (SCRs) which are also  
present in the other members of the C3b/C4b binding gene family of regulatory  
and receptor proteins (reviewed in references 9 and 10). Using in situ hybridization,  
the structural gene for MCP was localized to human chromosome 1, bands 1q31-41  
(8). Structural genes for C4 binding protein (C4bp), Factor H, the C3b/C4b receptor  
or complement receptor type one (CR1), C3d/EBV receptor or complement receptor  
type two (CR2), and decay-accelerating factor (DAF) have been localized to the same  
region of human chromosome 1 (11-14). Two recent reports have shown that genes  
encoding CR1, CR2, DAF, and C4bp are aligned on a 750-800 kb DNA segment  
within this regulator of complement activation (RCA) gene cluster (15, 16). The order  
for these genes within this cluster was shown to be CR1-CR2-DAF-C4bp (15, 16).

In the present report we demonstrate that the gene for MCP is on the same 1,250-  
kb Not I fragment that also contains CR1, CR2, DAF, and C4bp and maps within  
100 kb of the 3' end of the CR1 gene.

### Materials and Methods

*Preparation of DNA.* High molecular weight genomic DNA was prepared from a human  
promyelocytic cell line (HL-60) cells according to the method of Kenwrick et al. (17). Briefly,  
freshly grown mammalian cells were harvested, pelleted, and washed two times with PBS  
(0.15 M NaCl, 1.92 mM NaH<sub>2</sub>PO<sub>4</sub>, 8.7 mM K<sub>2</sub>HPO<sub>2</sub>, pH 7.4). The cells, suspended in PBS  
at 2 × 10<sup>7</sup> cells/ml, were mixed with an equal volume of 1% low gel agarose (Bethesda Re-  
search Laboratories, Gaithersburg, MD). The mixture was cooled to 50°C and immediately  
poured to a depth of 2 mm in an ice cooled sterile Petri dish. When set, the agarose was  
cut into blocks of 2 × 5 × 10 mm. These blocks were then incubated in ESP (0.5 M EDTA,

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pH 9.0, 1% sodium lauroylsarcosine, 1 mg/ml proteinase K [Boehringer Mannheim Biochemicals, Indianapolis, IN]) for 2 d at 50°C with gentle shaking. The samples were stored in ESP at 4°C.

*Restriction Enzyme Digestion and Analysis by Pulsed Field Gel Electrophoresis (PFGE).* Before digestion, the agarose plugs containing ~5 µg of DNA were washed twice with 1 ml of 1 mM PMSF in TE (10 mM Tris-HCl [pH 7.4], 0.1 mM EDTA) by slow rotation at room temperature for 90 min. This was followed by three washes in 1 ml TE at room temperature.

Restriction enzyme digestions were carried out according to the supplier's recommendations (New England Biolabs, Beverly, MA). 100 U of restriction endonucleases were used in a final volume of 0.5 ml. Samples were digested for 8 h at the recommended temperature. Reactions requiring more than one enzyme were conducted in two stages, using the enzyme with activity in the lower salt concentration or temperature first.

After the enzymatic digestion DNA blocks were washed for 2 h at 50°C in ES followed by 2 h of treatment with proteinase K (1 mg/ml in ES). The restriction fragments were separated either by orthogonal field alternation gel electrophoresis (OFAGE) (18) or by field inversion gel electrophoresis (FIGE) (19).

For OFAGE, a 1.5% agarose gel (10.5 × 10.5 × 0.4 cm) in 0.5 × TBE (1 × TBE = 90 mM Tris base, 90 mM boric acid, 1 mM EDTA) was used. Electrophoresis was performed for 22 h at 14°C using a switch interval of 15, 35, or 50 s with an applied voltage of 300 V.

For FIGE, a 23 cm long, 15 cm wide, and 0.5 cm thick gel containing 1% agarose was cast and run in 0.5 × TBE. To create switching-interval ramps, a PPI-100 microcomputer (MJ Research, Inc., Cambridge, MA) was used. A switching-interval ramp in which the forward migration interval varied linearly from 10 s at  $t = 0$  h to 60 s at  $t = 12$  h with a constant reverse interval of 5 s was used for separations between 260 and 700 kb. To achieve separations above 700 and up to 1,500 kb, the forward migration interval was varied linearly from 9 s at  $t = 0$  h to 60 s at  $t = 12$  h and a ratio of 3:1 between forward and reverse intervals was used (19). Electrophoresis was performed for 24 h at 260 V at 14°C. Intact yeast chromosomes (*Saccharomyces cerevisiae*, strain AB972) prepared in agarose blocks (20) and tandemly annealed λDNA concatemers with a monomer length of 48.5 kb were used as molecular weight markers (18).

*Transfer and Hybridization.* After ethidium bromide staining, the gels were treated with 0.25 M HCl twice for 15 min according to Maniatis et al. (21). DNA was then transferred to nitrocellulose according to the method of Smith and Summers (22). DNA probes were labeled with <sup>32</sup>P by the random oligonucleotide priming method, as described by Feinberg and Vogelstein (23), to a specific activity of at least  $2 \times 10^8$  cpm/µg. Hybridization and high stringency washing (0.2 × SSC, 0.1% SDS, 65°C) were carried out following standard procedures (21). Autoradiography was done for 1–5 d using Kodak RX-Omat films at –80°C. Filters were stripped of a probe by washing in 0.4 M NaOH at 45°C for 30 min followed by washing in 0.2 M Tris HCl (pH 7.5), 0.1 × SSC, 1% SDS at 45°C for 30 min.

*Probes.* cDNA probes for CR1 (24, 25), DAF (26), MCP (8) and leukocyte common antigen or T-200 (27) have been described. The CR1 probe is 980 bp in length, extending from nucleotide 422 to nucleotide 1402, covering the area from 3rd to the 7th SCR. The DAF probe is a full-length 1.9 kb cDNA probe whose sequence is described by Medof et al. (26). The MCP probe is also a full-length 1.5-kb cDNA probe whose sequence is described by Lublin et al. (8). The probe used for human leukocyte common antigen (LCA) is 1.8 kb in length and is described in reference 27. CR2 probe is 1.45-kb Eco RI fragment of a CR2 cDNA clone (Holers, V. M., unpublished data).

## Results and Discussion

Probes for CR1, DAF, and MCP hybridized to a common fragment of ~1,250 kb of Not I-digested DNA (Fig. 1 a). Two recent reports have shown that the genes for CR1, CR2, DAF, and C4bp are present on a common Not I fragment of ~1,250 kb (15, 16). Our results confirm these observations and place the MCP gene on this same fragment. Digestion of DNA with Mlu I gave two fragments of 800 and 550

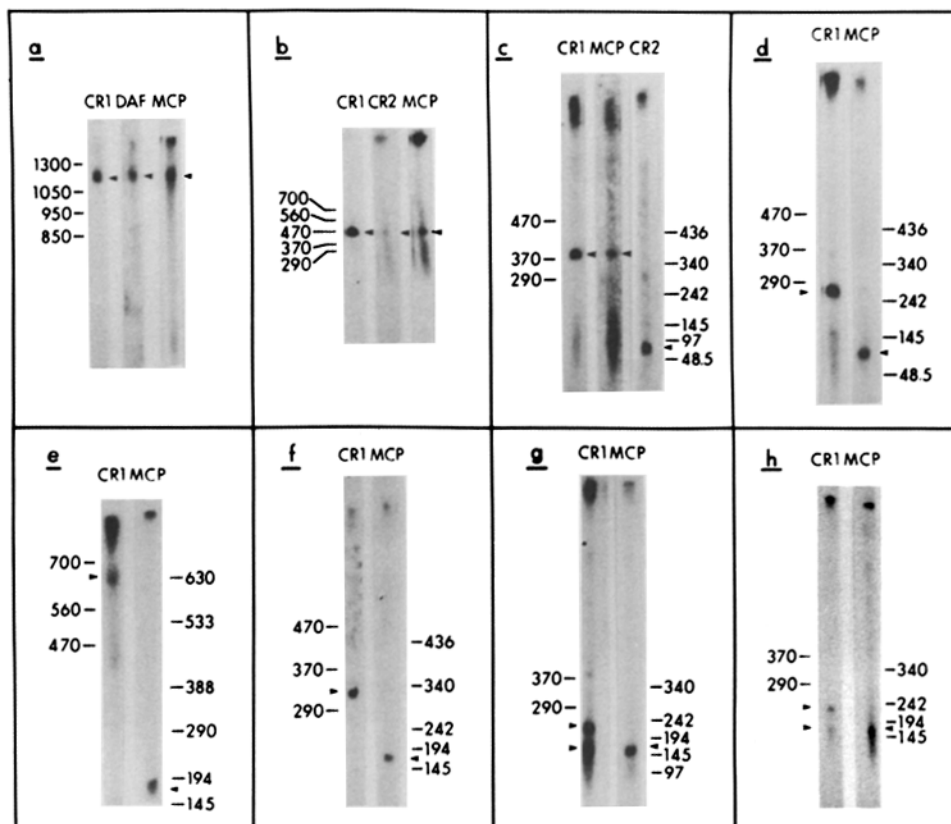


FIGURE 1. Southern blot analyses of genomic DNA digests separated by PFGE. Yeast chromosomes and, in some cases,  $\lambda$  phage concatemers were employed as size markers. Hybridization at the top of the gel in some of these blots may represent undigested DNA. (a) Fragments generated by Not I. Southern blot was sequentially hybridized with CR1, DAF, and MCP probes. CR1, DAF and MCP probes identify a common fragment of 1,250 kb. (b) Fragments generated by Not I and Mlu I. Autoradiograms were obtained from a single filter hybridized sequentially with the probes shown. A 470-kb fragment hybridizes to all three probes. (c) Fragments generated by Sfi I. This blot was sequentially hybridized with CR1, MCP and CR2 probes. CR1 and MCP probes recognize a 380 fragment. CR2 identifies a fragment of 70 kb. The apparent hybridization between 50 and 150 kb in the MCP lane may represent partially degraded DNA. (d) Fragments generated by Sfi I and Sal I. CR1 probe hybridizes to a 280-kb fragment while MCP hybridizes to a 100-kb piece. (e) Fragments generated by Sal I. Sequential hybridization with CR1 and MCP probes. CR1 probe identifies a restriction fragment of 630 kb while MCP probe hybridizes to a 180-kb fragment. (f) Fragments generated with Mlu I and Sal I. CR1 hybridizes to a 320-kb fragment and MCP to a 180-kb fragment. (g) Fragments generated by Sac II. MCP hybridizes to a 160-kb fragment while the CR1 probe recognizes the 240- and 160-kb fragments. (h) Fragments generated by Sac II and Sal I. Sequential hybridization employing a MCP probe that hybridizes to a 160-kb piece followed by the CR1 probe that identifies 240- and 160-kb fragments.

kb. Probes for CR1, CR2, and MCP hybridized to a 550-kb piece and DAF probe hybridized to a 800-kb piece (not shown). When the DNA was digested with both Not I and Mlu I, the DAF probe hybridized to a 780-kb fragment (not shown), while

the probes for CR1, CR2, and MCP hybridized to a 470-kb fragment (Fig. 1 *b*). These data place the Not I site within the Mlu I fragment. Earlier reports of Rey-Campos et al. (15) and Carroll et al. (16) have shown that the CR1 and CR2 genes are located on a 450-kb Not I/Mlu I fragment. Thus, our results place the MCP gene closer to CR1 and CR2 than DAF and C4bp genes.

To localize the gene encoding MCP relative to those for CR1 and CR2, DNA was digested with Sfi I, an enzyme that has previously been shown to separate CR1 and CR2 (15). MCP and CR1 probes hybridized with the 380-kb Sfi I fragment while the CR2 probe hybridized to the 70-kb fragment (Fig. 1 *c*). Digestion with Sfi I/Sal I further cleaved the 380-kb Sfi I fragment into 280- and 100-kb pieces. The CR1 probe recognized the 280-kb fragment, while the MCP probe identified the 100-kb piece (Fig. 1 *d*). Since probes for CR1 and MCP hybridized to the same Sfi I restriction fragment and since others have shown that CR1 is oriented 3' to 5' with CR2 next to the 5' end (16), these results indicate that the MCP gene is located either on the 3' or 5' end of the CR1 gene.

To further define the position of the MCP gene relative to CR1, DNA was digested with Sal I and hybridized to CR1 and MCP probes. The CR1 probe hybridized to a 630-kb fragment, while the MCP probe recognized a 180-kb fragment (Fig. 1 *e*). A combined digestion of DNA with Sal I and Mlu I decreased the size of the DNA fragment hybridizing to CR1 from 630 to 320 kb, while the MCP probe still hybridized to the 180-kb restriction fragment (Fig. 1 *f*). CR1, CR2, DAF, and C4bp probes were previously shown to hybridize to a common Sal I restriction fragment of ~630 kb (15, 16). In our experiments the CR1 probe but not the MCP probe hybridized to this 630-kb piece. Since MCP and CR1 probes hybridized with different Sal I fragments, we conclude that the MCP gene must be at the 3' end of the CR1 gene. Taken together these data indicate that order of the genes in the RCA gene cluster is as follows: MCP-CR1-CR2-DAF-C4bp (Fig. 2).

Further analysis using Sac II-digested DNA is shown in Fig. 1 *g*. The MCP probe hybridized to a 160-kb piece, while the CR1 probe hybridized with two pieces of 240 and 160 kb. Using both Sac II/Sal I, the fragments that hybridized to the MCP and CR1 probes were unchanged (Fig. 1 *h*). Thus these results place the MCP gene within ~100 kb of the 3' end of the CR1 gene.

The gene encoding human leukocyte-common antigen (LCA) is also located on the long arm of chromosome 1 at 1q32 (27). However, the leukocyte common antigen probe did not hybridize to the 1,250-kb Not I fragment which in the same set of experiments was recognized by MCP, CR1, and DAF probes. This observation indicates that the gene encoding for T-200 is not present on the same piece of DNA as those for MCP, CR1, CR2, DAF, and C4bp.

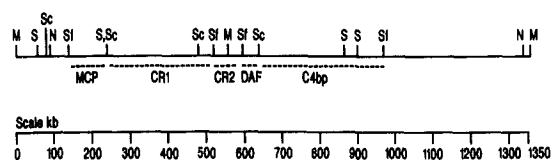


FIGURE 2. Diagram of the RCA locus on human chromosome 1. Abbreviations used for restriction endonucleases are as follows: M, Mlu I; S, Sal I; Sc, Sac II; N, Not I; SF, Sfi I. Restriction sites for C4bp have been taken from reference 15.

### Summary

The structural gene for membrane cofactor protein (MCP), a widely distributed C3b/C4b binding regulatory glycoprotein of the complement system, has been mapped to the same locus as the structural genes for CR1, CR2, DAF, and C4bp. The order of the genes within an ~800-kb DNA fragment on the long arm of chromosome 1 is MCP-CR1-CR2-DAF-C4bp. Further, the MCP gene maps to within 100 kb of 3' end of the CR1 gene.

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