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Brief Communication

Genes for C4b-Binding Protein α- and β-chains (*C4BPA* and *C4BPB*) Are Located on Chromosome 1, Band 1q32, in Humans and on Chromosome 13 in Rats

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Abstract—*C4b-binding protein is involved in the regulation of the complement system. It is a multimeric protein composed of seven identical α-chains and a single copy of a unique β-chain. The latter was identified only recently and its structure determined by cDNA cloning. Both subunits in C4b-binding protein belong to the same superfamily of proteins composed predominantly of tandemly arranged short consensus repeats (SCR) approximately 60 amino acid residues in length. The gene for the human α-chain is known to be located in a gene cluster on chromosome 1, band 1q32, which is called the regulators of complement activation (RCA) gene cluster. We have used cDNA probes for both α- and β-chains of human C4b-binding protein to localize their genes with an in situ hybridization technique. We find the genes for both chains to be located on chromosome 1, band 1q32, in the human. This suggests that the β-chain gene is also a member of the RCA gene cluster and that the α- and β-chain genes are located close to each other. The cDNA probes for the α- and β-chains also were used to screen mouse–rat somatic cell hybrids using Southern blotting to localize their genes in the rat. Both the α- and β-chain genes were shown to be located on chromosome 13 in the rat. These are the second and third genes to be located on rat chromosome 13, and the results suggest that the genes for the α- and the β-chains together with the gene for coagulation factor V represent a conserved chromosomal region in rat and man.*

INTRODUCTION

C4b-binding protein is a regulator of the classical complement pathway (1–4). It functions as a cofactor in the factor I-mediated degradation of C4b and in addition accelerates the decay of the enzyme C2a from the classical pathway C3-convertase (C4b, C2a). Another potentially important feature of C4BP is that it interacts with the vitamin K-dependent protein S (5, 6). Protein S is involved in the regulation of the coagulation system, functioning as a cofactor to activated protein C in the degradation of coagulation factors Va and VIIIa (7–9).

The plasma concentrations of C4BP and protein S are approximately equimolar (0.3 μM) and the two proteins form a 1:1 stoichiometric complex (10, 11). Approximately 50% of protein S in plasma is complexed to C4BP. Only free protein S functions as cofactor to activated protein C (12–14) and the physiological importance of this was demonstrated when patients with

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thromboembolic disease and hereditary deficiency of free protein S were identified (12). These patients may have normal or subnormal levels of total protein S but the equilibrium between free and bound protein S is disturbed. The mechanism for this disequilibrium is not known.

C4BP is a high-molecular-weight protein (Mr 570,000), and the major form in plasma is composed of seven identical 70-kDa α-chains and one unique 45-kDa β-chain (11, 15). The chains are linked by disulfide bridges in their carboxy-terminal ends, and the subunit arrangement gives the molecule an octupuslike structure as demonstrated by electron microscopy (16, 17). Each of the α-chains contains a binding site for C4b, whereas the protein S binding site is located on the β-chain (15, 16, 18).

The complete primary structures of both the α- and the β-chain of C4BP are known (19, 20). The two subunits are homologous, and they have a characteristic structural arrangement based on tandemly arranged repetitive units of 60 amino acid residues. Similar repeat units, denoted short consensus repeats (SCR) have been found in many other proteins several of which have C3b- or C4b-binding properties (reviews in references 4, 21). The typical feature of a SCR includes four cysteines, two prolines, one tryptophane, and several other partially conserved glycine and hydrophobic residues. The cysteines are arranged 1–3 and 2–4, giving each SCR unit a tight domain structure (22). Starting at their amino termini, the α- and β-chains have eight and three SCRs, respectively. In their carboxy termini, both chains have a nonrepeat region of approximately 60 amino acid residues containing two cysteines that are probably involved in the interchain disulfide linkage (19, 20).

The genes for several of the SCR containing complement regulatory proteins (MCP, CR1, CR2, DAF, and factor H) are located on the long arm of chromosome 1 (1q32) (23–29). The C4BP α-chain has been linked physically to the same region, which is called the regulator of complement activation (RCA) gene cluster (23). The C4BP gene in the mouse also has been found on chromosome 1 (30, 31).

Using an in situ hybridization technique and cDNA probes for both α- and β-chains, we now demonstrate that in man the β-chain gene also is located in the RCA gene cluster on the long arm of chromosome 1 (1q32). In addition we have used cell hybrids between mouse and rat to localize the two genes to chromosome 13 in the rat.

MATERIALS AND METHODS

cDNA Probes. The cDNA clones that were used in this study have been described previously (20). Both the α- and β-chain probes were isolated from a λ-gt 11 library. The α-chain cDNA was a partial clone corresponding to the 1.5-kb 3′ part of the message whereas the β-chain probe (886 bp) covered the complete coding sequence. Both cDNA clones were subcloned in pUC 18 and the probes were isolated from the plasmid after EcoRI digestion using standard techniques (32).

Chromosome Preparation and In Situ Hybridization. Lymphocytes from a normal female subject were cultured for four days. During the last 22 h of culture, the cells were treated with BrdU followed by thymidine as described by Zabel et al. (33). Air-dried chromosome preparations were made according to standard procedures, with minor modifications (34). In situ hybridization was performed essentially as described for unique sequence probes by Buckle and Craig (35). The probes were labeled to a final specific activity of approximately 10^8 dpm/μg with [^{3}H]dCTP, [^{3}H]dTTP, and [^{3}H]dATP using a random priming kit (Boehringer). The chromosome preparations were treated with formamide at 65°C for 4 min and the subsequent hybridization was performed at 43°C over night. The slides were washed in

2 × SSC for 1.5 h at room temperature followed by 0.1 × SSC for 1 h at 65°C and finally 0.1 × SSC for 30 min at room temperature. They then were dipped in Ilford K2 liquid emulsion and left to expose for nine days. Subsequently, the slides were developed, treated with Hoechst and UV light and stained in Giemsa. Analysis and grain counts were performed on enlarged prints of photographs of randomly chosen well-spread metaphases.

Somatic Cell Hybrids and Southern Analysis. The derivation and isolation of rat–mouse hybrids comprising the rat gene mapping panel has been described earlier (36, 37). Total DNA was extracted (38) from 21 rat–mouse hybrids and from the rat and mouse parental cells. The DNAs were restricted with EcoRI and electrophoresed with ethidium bromide in 0.75% agarose gels. The DNA fragments were transferred to nylon filters as described by Southern (39). The probes were labeled with the random priming method and [^{32}P]dCTP (40) and hybridized over night to the filters in 0.5 M phosphate buffer with 1% SDS for 3 × 15 min at 65°C and then in 0.2 M phosphate buffer with 0.5% SDS for 15 min at 65°C. The hybridization signals from the filters were registered on Fuji X-ray film after two to five days of exposure.

RESULTS

Mapping of Human α- and β-Chain Genes Using In Situ Hybridization. To localize the gene for the β-chain of C4BP, 35 metaphases were analyzed and 74 silver grains were scored (average 2.1 grains/cells). The distribution of the grains over the human chromosomes is shown in Fig. 1A. Nineteen grains (26%) were found over chromosome 1 and 13 of them (18%) were localized to the band 1q32 (Fig. 2A). Using the α-chain cDNA probe, 32 metaphases were analyzed and a total of 103 grains were scored (average 3.2 grains/cell) (Fig. 1B). Forty-two grains (41%) were found over chromosome 1 and 21 of them (20%) were localized to the band 1q32 (Fig. 2B). These results demonstrate that the α- and the β-chain genes are located close to each other on the long arm of chromosome 1 (band 1q32).

Mapping of Rat α- and β-Chain Genes Using Somatic Cell Hybrids. The chromosomal localization of the rat sequences cross-hybridizing with the β-chain probe was determined with 21 rat–mouse hybrids. In Southern blots, 14 of them displayed a 9.5-kb rat-specific EcoRI fragment that hybridized to the probe (Fig. 3A). The presence or absence of this fragment always coincided with the presence or absence of rat chromosome 13 (Table 1). Discordancy analysis showed that there were at least three discordant hybrids for every other rat chromosome.

The chromosomal localization of the rat sequences cross-hybridizing with the α-chain probe was determined with 17 rat–mouse hybrids. In Southern blots, 12 of them displayed a 6.2-kb rat-specific EcoRI fragment that hybridized to the probe (Fig. 3B). The presence or absence of this fragment again coincided with the presence or absence of rat chromosome 13 (Table 1). Discordancy analysis showed that there were at least four discordant hybrids for every other chromosome except the X chromosome. For the X chromosome, only one discordancy was recorded in the present analysis, but the α-chain of C4BP is known not to be sex-linked in rodents (30, 31).

DISCUSSION

Several of the complement regulatory proteins with C3b- or C4b-binding abilities contain multiple tandemly arranged SCR units (4, 21). Both plasma proteins such as factor H and C4BP and membrane proteins such as MCP, CR1, CR2, and DAF are members of this protein family. The genes for all these proteins are located on chromosome 1 in the regulators of complement activation (RCA) gene cluster (23–29, 41). C4BP is

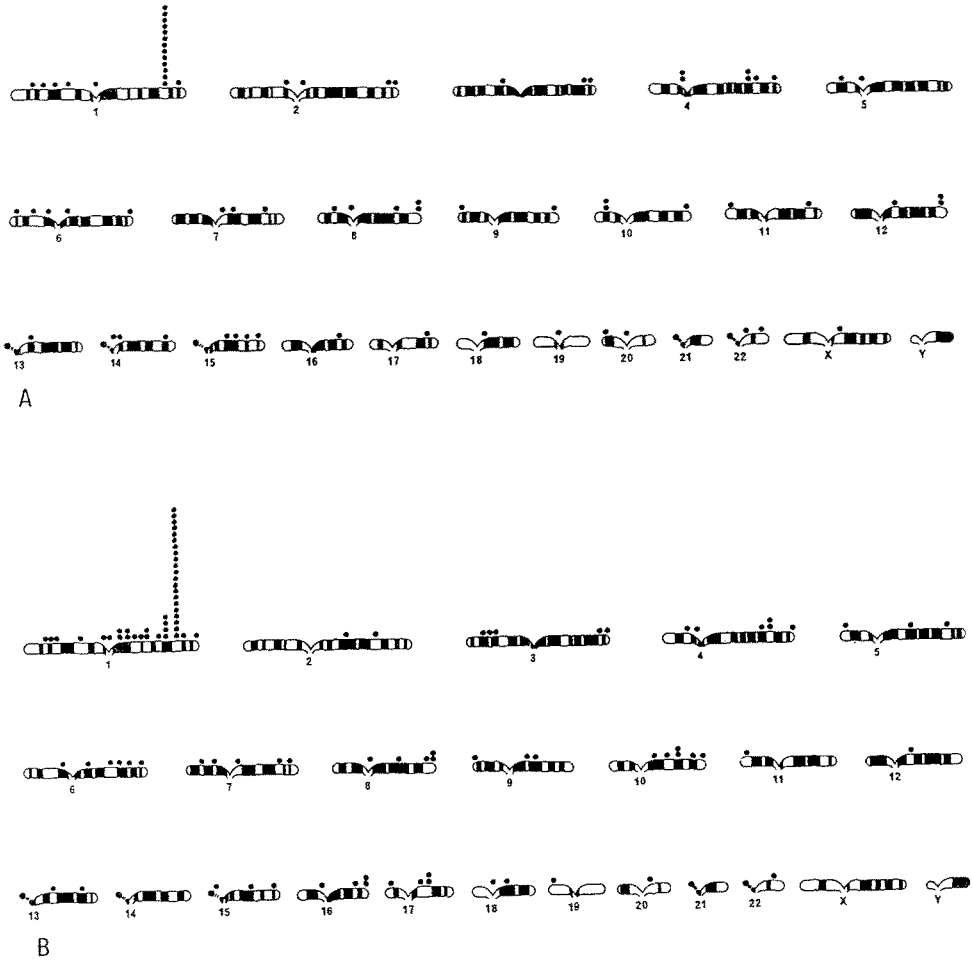

Fig. 1. Localization of the human *C4BPA* and *C4BPB* genes using in situ hybridization. Distribution of grains over the human chromosomes showing specific hybridization at band 1q32 using both the β-chain cDNA probe (A) and the α-chain cDNA probe (B).

unique among these proteins since it contains two different types of subunits, the seven identical α-chains and the single β-chain (15, 20). The ability of C4BP to interact with both a complement protein (C4b interacting with the α-chain) and with a vitamin K-dependent protein (protein S binding to the β-chain) is also a unique feature for C4BP. Both types of chains contain SCRs, and we can now show that in man the genes for both chains are located in the same region on chromosome 1 (band 1q32). Our results confirm the earlier assignment of the α-chain to this chromosomal region (24, 25), whereas the assignment of the β-chain gene is a new mapping. We propose to change the previous gene symbol for the α-chain of the C4b-binding protein from *C4BP* to *C4BPA* and to call the β-chain gene *C4BPB*.

The homologous structural arrangement of the α- and the β-chain with multiple SCRs and a nonrepeat carboxy-terminal region containing two cysteines suggests that the two chains are the results of a gene duplication event. In this respect it is important that both the α- and the β-chain genes are present in

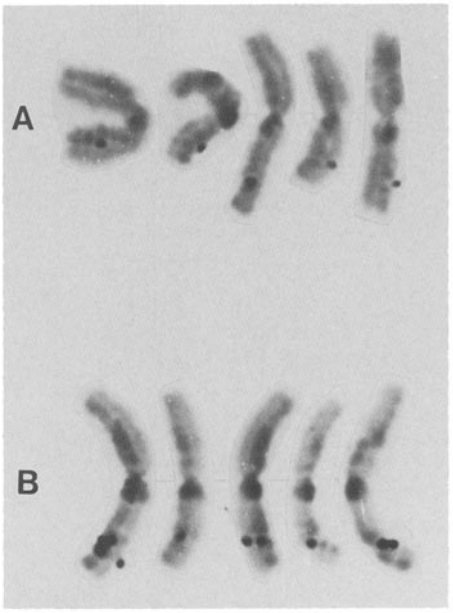

Fig. 2. Visualization of in situ hybridization to chromosome 1. Five instances of human chromosome 1 after hybridization with the β-chain probe (A) or with the α-chain probe (B). Grains are localized to band 1q32.

rodents, suggesting that the gene duplication has occurred in a common ancestor to both rodents and primates. The cDNA cloning of the α-chain of mouse C4BP revealed that the carboxy-terminal portion did not contain the two cysteines that in its human counterpart are thought to be involved in the interchain disulfide bridging (42). In agreement with this, the α-chains in the mouse protein have been demonstrated to be linked by noncovalent bonds. It will be of interest to determine the primary structure of corresponding region in the mouse β-chain to elucidate whether it, like the mouse α-chain, lacks the two cysteines in the carboxy-terminal region. At the protein level, the β-chain has so far only been demonstrated in humans, but it is most likely that the β-chain gene in rodents is transcribed and that it is a constituent component in rodent *C4BP*.

The assignments of the α- and β-chain genes to rat chromosome 13 are the second and third genes to be located on this chromosome. We have previously localized the gene

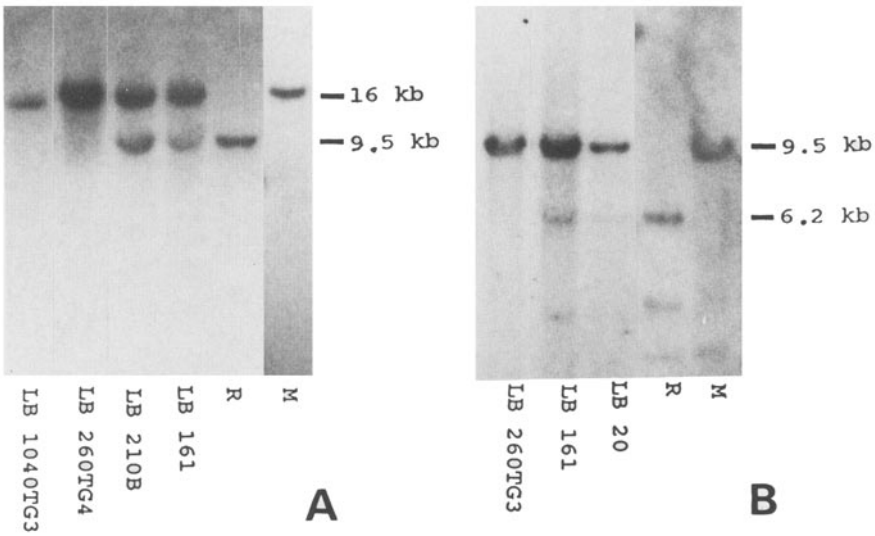

Fig. 3. Southern blot hybridization to filters with EcoRI-digested rat or mouse DNA. (A) The β-chain probe hybridized with 16-kb and 9.5-kb fragments in mouse (lane M) and rat (lane R) parental DNA, respectively. Two hybrids (lane LB 161 and LB 210B) were positive for the rat 9.5-kb fragment and two hybrids (lanes LB 260TG4 and LB 1040TG3) were negative. (B) Hybridization of the α-chain probe against EcoRI-digested mouse (lane M) and rat (lane R) parental DNA yielded major fragments of 9.5 and 6.2 kb, respectively. Two hybrids (lanes LB 20 and LB 161) were positive for the rat fragment and one hybrid (lane LB 260TG3) was negative.

Table 1. Segregation of Rat C4BPB and C4BPA in Rat–Mouse Hybrids[a]

Cell hybrid	Rat chromosome																						C4BPB 45 kDa	C4BPA 70 kDa
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	X	Y		
LB20	−	+	−	−	−	−	+	−	−	−	−	+	+	−	−	+	(+)	+	+	−	+	−	+	+
LB 150-1	−	−	+	+	−	−	+	−	+	−	+	(+)	+	−	−	(+)	(+)	+	(+)	−	+	−	+	ND
LB161	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+
LB210-B	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	+	−	+	+
LB210-C	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−	+	−	+	+
LB210-D	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	(−)	+	−	+	+
LB210-I	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	+	−	+	+
LB251	+	+	−	+	−	(+)	+	−	−	+	−	+	+	−	−	−	+	−	+	−	+	−	+	+
LB260TG3	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−
LB260TG4	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
LB330TG6	−	+	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
LB510	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+	+	+	−	−	+	−	+	ND
LB600	(+)	+	(+)	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	(−)	+	−	+	ND
LB630	−	−	(−)	+	(−)	+	+	−	+	−	+	+	+	(−)	(+)	+	−	+	+	(−)	+	−	+	+
LB780-5	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−
LB780-8	−	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	−	+	−	−	+	−	−	ND
LB780-9	−	+	−	−	−	−	+	−	−	+	+	−	+	−	−	−	−	+	−	−	+	−	+	+
LB810	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	(+)	+	−	+	+
LB860	−	+	+	+	−	−	+	−	+	−	+	+	+	−	+	+	+	+	(−)	(+)	+	−	+	+
LB1040	−	−	+	+	−	+	+	−	−	+	+	+	−	−	+	+	−	+	−	+	+	−	−	ND
LB1040TG3	−	−	−	+	−	+	+	−	−	−	+	−	−	−	+	+	−	+	−	−	−	−	−	−
BS511	−	+	+	+	(−)	+	+	+	−	+	+	−	+	−	+	+	+	−	+	−	+	−	ND	+
Number of discordant lines																								
C4BPB	11	9	9	10	12	12	7	13	9	13	13	7	0	8	10	7	7	5	7	12	3	14		
C4BPA	11	7	8	9	11	9	5	10	10	9	10	8	0	8	8	6	6	4	6	9	1	12		

[a]Symbols: + = chromosome present in more than 55% of the cells, (+) = chromosome present in 25–55% of the cells, (−) = chromosome present in less than 25% of the cells, − = chromosome absent. Hybrids were picked from separate culture dishes and represent independent fusion events with the following exceptions: LB260TG3, LB260TG4, LB330TG6, and LB1040TG3 were clonal isolates from LB260, LB330, and LB1040, respectively, after back-selection in 6-thioguanine. LB210-B, -C, -D and -I were subclones of the primary hybrid LB210 (not included in the analysis), and LB780-5, -8 and -9 were subclones of the primary hybrid LB780 (not included in the analysis).

for coagulation factor V to rat chromosome 13 and to human chromosome 1 (37). Using in situ hybridization, the gene for human coagulation factor V has been localized to 1q21-25 (43). This strongly suggests that the genes for factor V and the α- and β-chains of *C4BP* represent a conserved chromosomal region in rat and man. This homology can be extended to mouse chromosome 1, which carries the *C4BPA* gene (30, 31). The long arm of mouse chromosome 1 also has morphological similarities in its distal end to corresponding region of rat chromosome 13 (44), and it is known to have partial genetic homology to the long arm of human chromosome 1 (45, 46).

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