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# C4b-Binding Protein, a Regulatory Protein of Complement

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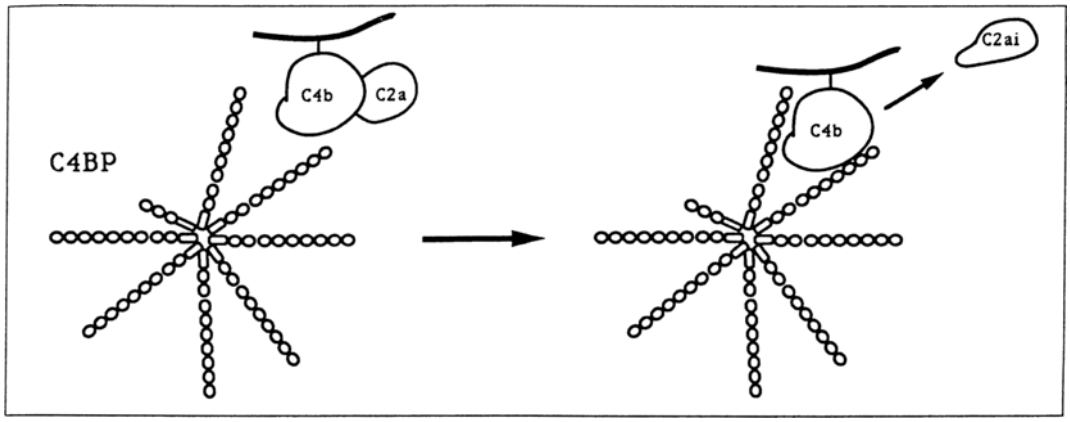
## Introduction

C4b-binding protein (C4BP) has been studied for over a decade, primarily because of its function in regulating the classical pathway of complement. Biochemical characterization during this time period revealed an interesting multimeric structure. In the past few years there has been a renewed interest in C4BP because sequence analysis revealed that it is a member of a growing superfamily of proteins (both complement and noncomplement) that are composed, at least in part, of a short consensus repeat (SCR) unit (60–75 amino acids in length). Equally interesting is the localization of C4BP to the regulator of complement activation (RCA) locus on chromosome 1, along with all the complement-regulatory components composed of SCRs. This, of course, has evolutionary implications and a number of schemes for the evolutionary pathways for some of these molecules have been put forth; however, this is beyond the scope of this review. The purpose of this review is to provide an overview of C4BP structural and functional features and to cover new information on the molecular biology, biosynthesis and regulation of C4BP expression.

## Regulation of Complement Activation by C4BP

C4BP is a key regulatory molecule of the classical pathway of complement. That C4BP regulates the classical pathway was elegantly demonstrated by Gigli et al. [1] who showed a dose-dependent acceleration of decay of the C3 convertase (C4b2a) and inhibition of the formation of the convertase in the presence of C4BP. Mechanistically, C4BP accelerates the decay of the C3 convertase by dissociating C2a from the complex, as shown in figure 1a. This was demonstrated by the release of $^{125}$I-C2a from the C4b2a complex in the presence of C4BP. In contrast, studies in which the C3 convertase was stabilized either by the use of $C2^{oxy}$, a decay-resistant form of C2, or an anti-C3 convertase antibody demonstrated that C4BP was unable to accelerate decay of these convertases [2]. The latter C3 convertase is schematically represented in figure 1b. It is not clear whether C4BP competes for the same binding site as C2a or for nearby binding sites on the C4b molecule. There is currently no evidence to suggest binding sites of C4BP for C2a. The importance of C4BP in classical pathway regula-

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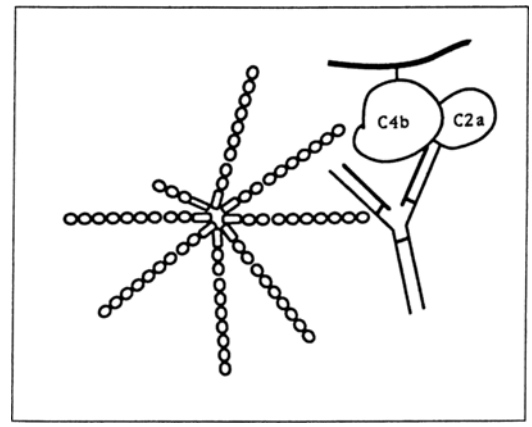


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**Fig. 1. a** Schematic representation of C4BP interacting with the classical pathway C3 convertase. C4BP binds to C4b in the classical pathway C3 convertase, shown covalently bound to an activating surface. This interaction causes the release of C2a, which contains the active site of the convertase, rendering the convertase inactive. **b** Stabilization of the C3 convertase by an auto-anti-C3 convertase antibody. Studies by Daha and Van Es [2] provided experimental evidence for the role of C4BP in C3 convertase destabilization. The binding of an auto-anti-C3 convertase antibody markedly increased the half-life of the convertase in the presence of C4BP. It is unclear whether the antibody sterically inhibits C4BP interaction or actually binds to the C4BP binding site.

tion is seen in studies in which treatment of serum with anti-C4BP antibody leads to rapid consumption of C3. Reconstitution of treated serum with purified C4BP inhibits C3 consumption [1].

In addition to accelerating the decay of the C3 convertase, C4BP serves as a cofactor in the complement protein I-mediated cleavage of C4b to C4c and C4d which prevents the formation or reconstitution of the C3 convertase [1, 3–6]. Cleavage of C4b by I is dependent on the presence of C4BP [1, 5] and can occur when C4b is cell-bound or in the fluid phase [1, 3–6]. The cleavage of C4b occurs in the α-chain and leads initially to the formation of iC4b, a functionally inactive form of C4b. The second cleavage releases the disulfide-linked C4c fragment from C4d [4–6].

## Structure, Molecular Biology and Genetics of C4BP

In all species in which C4BP has been identified and characterized (human, mouse and guinea pig), it is a multimeric glycoprotein with a molecular weight of 550,000–

1,000,000 as determined by chromatographic methods [7–9]. Purified protein from humans and guinea pigs appears as a single or double band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions with a molecular weight of 550,000 [7, 9], while murine C4BP has an apparent molecular weight of 60,000–80,000 under nonreducing conditions [8]. Under reducing conditions, C4BP from all 3 species appears as a single band with an apparent molecular weight of 70,000–80,000 [7, 9, 10]. C4BP is composed of 7 identical subunits (α-chains; see fig. 2) which are disulfide-linked near the carboxy terminus in humans [7, 11–14] and guinea pigs [9] but noncovalently associated in mice [8, 10]. The disulfide linkage, at least in human C4BP, is probably mediated by cysteines 498 or 510 which are not part of the repeating units that compose most of the molecule [14]. This is supported by studies in which limited chymotrypsin digestion (presumably at the Tyr 395 or Trp 425 residues) produced a disulfide-linked core fragment of 160,000 (composed of carboxy-terminal 25,000-dalton polypeptide fragments) and an amino-terminal fragment of 48,000, demonstrating that the linkage occurs near the carboxy terminus [12, 15, 16]. The additional carboxy-terminal cysteines (numbers 498 and 510) found in human C4BP are not present in murine C4BP [17].

In human C4BP, a 45,000 molecular weight subunit, initally seen in electron-microscopic studies by Dahlback et al. [18], has now been shown to be a covalently attached subunit and is termed the β-chain. No analogous subunit has been identified in any other species. The β-chain binds to the carboxy-terminal region of the C4BP complex to 2 of the α-chains [19] (fig. 2). The stoichiometry of β-chain to α-chain by gel filtration studies suggests 1 β-chain per C4BP molecule. The β-chain is chymotrypsin-sensitive and treatment of the C4BP complex with chymotrypsin abrogates the binding of protein S, the primary ligand of the β-chain, to C4BP [19]. Other forms of human C4BP have been reported [20] in which the molecule has 6 α-chains and 1 β-chain or 7 α-chains and no β-chains. The relative percentage that these forms of C4BP contribute to total serum levels is unknown.

The derived amino acid sequence of the human and mouse C4BP α-chain has been determined from the sequence of cloned DNA [14, 17, 21]. The homology between the species at the nucleotide level ranges from 50 to 67%, depending on the region, and from 51 to 61% at the amino acid level, depending on the use of identical or chemically similar residues [17]. The molecular weight of the C4BP α-chain calculated from the amino acid sequence is 61,500 for the human [14] and 45,281 for the mouse [17]. The human molecule has 136 more residues than the mouse. Four potential N-linked carbohydrate sites have been identified in the human molecule, 3 of which are glycosylated [14], and it has been calculated that carbohydrate composes 10–16% of the molecular mass of human C4BP [22]. In contrast, there are 7 potential N-linked carbohydrate sites on murine C4BP [17]. It is not known how many of these sites are glycosylated, but it is clear that carbohydrate contributes substantially (30–48%) to the molecular mass of murine C4BP since its apparent molecular weight as determined by SDS-PAGE ranges from 60,000 to 80,000 [8, 10].

The β-chain has also been sequenced [23] and contains 235 amino acids giving a calculated molecular weight of 26,300. The β-

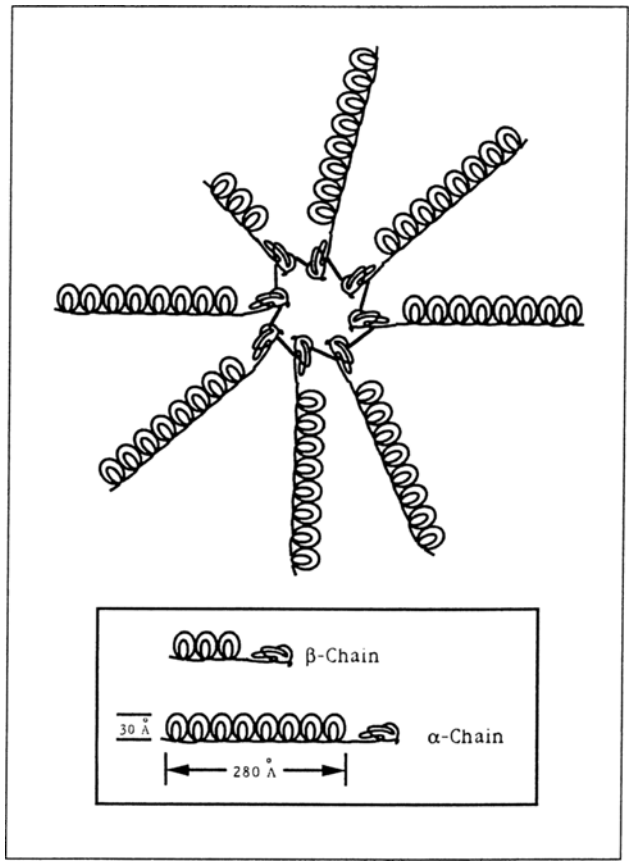


**Fig. 2.** Schematic representation of human C4BP. Human C4BP contains 7 disulfide-linked α-chains and 1 β-chain. The linkage of the β-chain is shown as a dotted line since the specific subunit(s) it is disulfide-linked to is not known. The double-looped structures represent individual SCR units, while the globular region at the end of both chain types represents a nonrepeat portion of the polypeptide and is the site of linkage between the chains. **Inset:** Each α-chain contains 8 SCRs and a single nonrepeat region at the 3′ end of the chain, while the β-chain contains 3 SCRs and a nonrepeat region. Previous electron-microscopic studies by Dahlback et al. [18] have demonstrated that each α-chain is approximately 30 Å thick and the SCR portion of the molecule is approximately 280 Å in length.

chain has 5 potential N-linked glycosylation sites. Thus, based on the observed molecular weight of 45,000 on SDS-PAGE, the β-chain is approximately 58% carbohydrate. Treatment of the β-chain with endo-F glycosidase decreases the molecular weight to 29,000, demonstrating tht the β-chain contains complex carbohydrate side chains as well [23].

Other interesting features of the primary structure of human and mouse C4BP include two potential in-frame translational start signals which would yield leader sequences of 13 or 32 amino acids for human [21] and 13 or 56 residues for mouse [7] C4BP. It is not known which translational start site is preferentially utilized. Both molecules contain contiguous regions of internal homology each approximately 60 amino acids in length. These SCRs (fig. 3) are composed of a framework of conserved residues including 4 cysteines, 2 prolines, 3 glycines, 2 phenylalanine/tyrosines and 1 tryptophan for each unit [for review, see ref. 25–27]. The human C4BP α-chain has 8 SCRs in tandem followed by a nonrepeat region of 58 residues, while the murine C4BP has 6 SCRs in tandem followed by a nonrepeat region of 55 residues. Sequence comparison suggests that

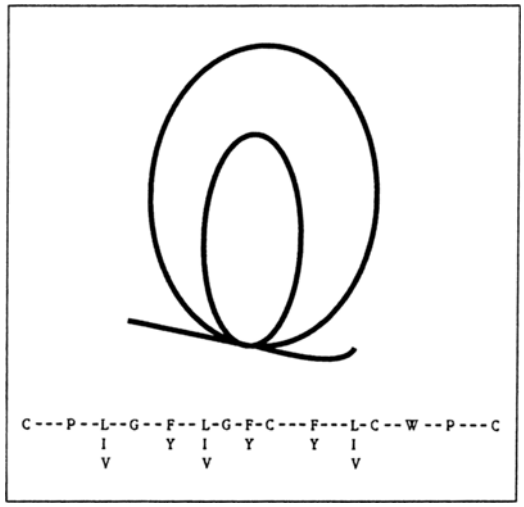


**Fig. 3.** Schematic representation of the SCR structure. The double-looped structure of a representative SCR is shown in the upper part of the figure. Studies by Janatova et al. [24] have shown that the first and third and second and fourth cysteine residues in each SCR are disulfide-linked, giving rise to the depicted structure. Shown below the figure is the consensus of the conserved framework residues found in human C4BP SCRs. In addition to the basic conserved residues, human C4BP has several conserved Tyr/Phe and hydrophobic residues. The positions of the residues are not proportionally gapped and are from Chung et al. [14].

the regions corresponding to SCRs 5 and 6 in human C4BP are missing in mouse C4BP. The β-chain contains 3 SCRs followed by a 60-amino-acid nonrepeat region which has sequence homology (~25%, identical residues) with the C-terminal nonrepeat region in the α-chain [23].

The SCRs are present in 11 other complement proteins and range in number from 2 SCRs (in C1r, C1s, C6 and C7) to 37 SCRs (CR1) per protein (table 1). Those molecules serving as regulatory components of the complement system [C4BP, H, CR1, CR2, membrane cofactor protein (MCP) and decay-accelerating factor (DAF)] are composed almost entirely (or entirely in the case of H) of SCRs. The remaining molecules (B, C2, C1s, C1r and I) are all serine proteases involved in activation of complement and have only 1 or a few SCRs as one of several protein domains. All of the SCR-containing molecules(with the exception of C6 and C7) bind or interact with C3 or C4 or their proteolytic fragments.

A large number of noncomplement proteins also contain SCR units (table 1). These proteins are diverse in function and range from components of the coagulation cascade to cell surface receptors. The function of the SCRs in these molecules is unclear since none of these proteins interact with C3b or C4b or other fragments of these molecules. However, it has been suggested that the SCRs in a secretory protein of vaccinia virus may interact with C3b to escape neutralization by complement [31]. It is possible that SCR units in these proteins serve as carriers of binding sites for ligands other than C3- and C4-derived fragments; however, this remains to be demonstrated.

Structurally, C4BP has a spider-like appearance as seen by electron microscopy [18]; however, more recent analysis using synchroton X-ray scattering and hydrodynamic techniques suggests that the 7 arms of C4BP are close together with an average arm axis of 5–10° [22]. Such a structure would bind only a small number of C4b molecules (2–4) relative to the number of binding sites available (7, see section Binding Specificity), similarly to what has been demonstrated by Ziccardi et al. [32]. These studies also suggest that the base or core of the molecule (the carboxy-terminal region) comprises 23% of the molecular volume. Secondary structure

**Table 1.** Complement and noncomplement proteins containing SCRs

| Protein | Number of repeats | Protein | Number of repeats |
|---|---|---|---|
| Factor B | 3 | $\beta_2$-Glycoprotein | 5 |
| C1r | 2 | IL-2 receptor | 2 |
| C1s | 2 | Factor XIII | 10 |
| C2 | 3 | Haptoglobin | 2 |
| C6 | 2 | Vaccinia virus (SP-35) | 4 |
| C7 | 2 | Cartilage glycoprotein | |
| Factor H (human/mouse) | 20 | (rat/bovine/chicken) | 1 |
| CR1 | 23/30/37 | Lymph node | 2 |
| CR2 | 15/16 | ELAM-1 | 6 |
| DAF | 4 | Thyroid peroxidase | 1 |
| MCP | 4 | Factor C-horseshoe crab | 1 |
| C4BP α-chain | | GMP-140 | 9 |
| Human | 8 | Vesican | 1 |
| Mouse | 6 | | |
| C4BP β-chain | 3 | | |

Data are compiled from references 17, 25 and 27–30.

predictions indicate that the SCRs are composed mainly of β-sheets, β-turns and random coils [17]. Klickstein et al. [33] have suggested that, in each SCR, the first and third and second and fourth cysteines are disulfide-linked, giving rise to a double-looped structure, and that linked in tandem yield an elongated structure (i.e. the arms seen in electron-microscopic studies and as schematically diagrammed in figure 2). The elongated structures generated by tandem SCRs are due, in part, to the short distance between the repeats (only a few amino acids) and also to the amino- and carboxy-terminal location of the cysteine residues involved in loop formation. Limited proteolytic digestion and amino-acid-sequencing studies by Janatova et al. [24] have substantiated the disulfide linkages suggested by Klickstein et al [33]. In this same study, the double-loop structure of SCRs was compared to that of part of a kringle domain found in prothrombin fragment 1. Kringle domains are well-studied structural modules found in the regulatory regions of coagulation and fibrinolytic proenzymes. This comparison suggests that SCRs may have a triple-loop structure similar to that found in kringle domains. All these findings await confirmation by X-ray crystallographic studies. The nonrepeat region of the molecule is most likely α-helical in structure.

*Allelic Variants*

Two allelic variants of murine C4BP have been identified [34] with a pH range of 6.5–7.0 for the a type and 6.3–6.6 for the b type. These are the only 2 alleles seen in over 25 strains of mice. Crosses between strains bearing distinct alleles resulted in progeny exhibiting both alleles; thus, the gene is inherited in an autosomal codominant man-

ner. This same study, employing backcross analysis, suggested that the C4BP gene is linked to the H-2 complex between H2-D and C3-1 [34]. Recent data, however, demonstrate that the murine C4BP gene is located on chromosome 1 [35, 36] (see below). In humans there are 3 C4BP allelic variants termed C4BP*1, C4BP*2 and C4BP*3 with gene frequencies of 0.986, 0.010 and 0.004, respectively [37]. The pIs of these alleles are 6.65, 6.60 and 6.75, respectively. The human variants can probably be accounted for at the protein level since protein and cDNA sequencing studies have identified a threonine or glutamine at residue 44, a tyrosine or histidine at residue 309 and a tyrosine or alanine at residue 344 [13, 14].

*Gene Structure and Chromosome Location*

The organization of both the human and mouse C4BP gene has been examined [35, 38, 39]. The structure of the human gene has recently been determined and found to contain 12 exons and be over 40 kilobases (kb) in length [39]. All the SCRs are encoded by separate exons with the exception of the second repeat which is encoded by 2 exons 8 kb apart. The 5′ untranslated region and the leader sequence are encoded by 2 exons, separated by a 9-kb intron, while the 3′ nonrepeat region is encoded by a single exon. Interestingly, pulsed-field electrophoresis has demonstrated a partial duplication of the C4BP gene which maps to the same region on chromosome 1 as the gene itself (see below).

The structure of the murine gene has only been partially determined [35]. Seven exons have been identified so far in the mouse gene, extending from the second repeat through the nonrepeat region of the molecule. Based on the human gene structure, there will likely by 3 or 4 additional exons in the mouse gene. The second SCR in the mouse gene is also encoded by 2 exons giving rise to a 'split repeat'. This feature has been seen in the genes of several SCR-containing proteins such as H [40], CR1 [41], CR2 [42], MCP [43] and DAF [44]. The 3′ end of the murine gene displays sequence homology with the SV40 class II (late) RNA sequence element CAYUG described by Benoist et al. [45]. These elements are found in tandem (twice) and immediately 3′ to the polyadenylation site [35]. This sequence motif if thought to be involved in determining specificity of precursor RNA cleavage and has been found in a number of genes [46]. The murine gene, estimated to be 20–30 kb, is similar in length to the human gene [35, 38]. With message sizes of 2.5 and 1.8 kb for the human and the mouse, respectively, it is clear that the genes are composed predominantly of introns.

The C4BP α-chain gene is located on chromosome 1 in humans and mice in what is termed the RCA locus. This locus is shown diagrammatically in figure 4. In humans, the genes for several of the complement-regulatory proteins are clustered in a 900-kb region with the gene order MCP-CR1-CR2-DAF-C4BP. The H gene is located approximately 7 centimorgans (cM) centromeric to this cluster [47–49]. In mice the RCA locus is much less tightly clustered, with C4BP the most centromerically positioned gene, 9 cM from mouse H and 45 cM from the mouse homologues of CR1 and CR2, Mcry and Mcr2 [35, 36]. It has been suggested that the mouse RCA region may have undergone a translocation or inversion during its development because of the breakpoint between C4BP and H and the murine CR1 and CR2

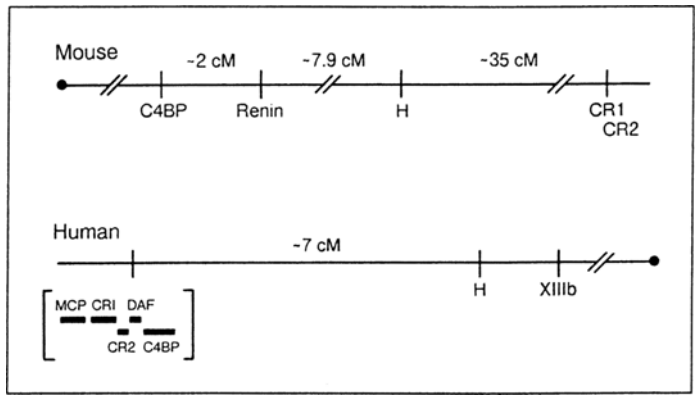


**Fig. 4.** Map of the RCA region of mouse and humans on chromosome 1. In the murine RCA region, the C4BP gene is located approximately 10 cM centrometic to the factor H gene and 45 cM centromeric of the murine equivalent of CR1 and CR2. The human RCA region is significantly more clustered than the murine region. The unit cM correspond to approximately 1,000,00 bases. The figure is reproduced from Barnum et al. [35] with permission.

homologues [36]. There are several other genes found in the conserved linkage groups of both mouse and human chromosome 1, including the renin and T200 genes [50–53]. Recent studies by Dahlback et al. [in preparation] have localized the β-chain gene to chromosome 1, band 1q32, using in situ hybridization techniques. In addition, Pardo-Manuel et al. [54] have, using pulsed-field electrophoresis, determined that the α-chain and β-chain genes are closely linked.

### Biosynthesis and Regulation

The biosynthesis and regulation of C4BP are probably the least studied and understood aspects of C4BP biology. It is likely that the liver is the primary site of biosynthesis and that hepatocytes produce most if not all of serum C4BP. The serum concentration of human C4BP is 200 μg/ml [55]. Until recently, the only demonstration of C4BP production by the liver was the isolation of cDNA clones from liver libraries and the presence of message on Northern blots of poly(A)$^+$ RNA from whole liver [14, 17]. De novo synthesis of C4BP by HepG2 cells, a human hepablastoma cell line, has recently been demonstrated [56, 57]. In addition, human synovial fibroblasts appear to be a biosynthetic site for C4BP; however, more studies are needed [J. Kidd and S. Barnum, unpubl. findings]. Northern blot analysis of total RNA from HepG2 demonstrated a message size of 2.5 kb [57], comparable to that seen in human liver [14]. No other cell or tissue type has been shown to transcribe a C4BP message or synthesize and secrete the protein.

There are limited data describing the regulation of C4BP expression; however, there is evidence that C4BP is an acute-phase reactant [56, 58, 59]. The serum levels of C4BP increase up to 286% in response to surgical trauma [59] or pneumonia [56]. In addition, C4BP synthesis by HepG2 cells increased up to 190% in response to treatment with interleukin-6 with (IL-6) [56, 57], further suggesting that C4BP is an acute-phase reactant. Serum C4BP levels are elevated in systemic lupus erythematosus (SLE; 186% higher than in healthy controls); however, the levels are unrelated to disease activity or drug therapy [59]. The slight increase in C4BP levels in SLE patients versus the acute-phase re-

sponse suggests several possibilities. One is that C4BP is differentially regulated in these two states. Alternatively, the modest increase in both C4BP and C-reactive protein, a well-described acute-phase reactant, in SLE may suggest a reduced ability of these individuals to respond to a cytokine-mediated signal (perhaps through IL-6 and/or IL-1) or reduced production of these cytokines in these individuals. Although the adult serum concentration of C4BP is 200 μg/ml using pooled serum [55], fetal and newborn levels are only 5 and 20%, respectively, of adult levels [60–62]. C4BP is hormonally regulated with levels increasing slightly during pregnancy [61–63] and in women on oral contraceptives [58, 61, 64]. In humans there is no apparent difference in serum C4BP levels between the sexes. In mice the levels are androgen-regulated with male mice having 2.5 times the levels of females [8]. No significant differences in serum C4BP levels were seen in mice of different H-2 haplotypes [8].

### Binding Specificity

The binding specificity of C4BP is highest for C4b (see below), a characteristic which has been utilized for affinity purification of the molecule [9], although several purification schemes have been employed [7, 8, 10, 11]. The binding of C4b to C4BP in humans has been extensively characterized. At physiological ionic strength, C4BP displays 4 binding sites for C4b, each with an association constant of $1.2 \times 10^7\ M^{-1}$ in the fluid phase [32]. The reported association constant of $4.6 \times 10^8\ M^{-1}$ for C4BP bound to cell-bound C4b is significantly higher (40-fold) and is most likely due to the multivalent interaction of the molecule [6]. Each binding site is independent based on a calculated Hill coefficient of 1.1. At reduced ionic strength (half-physiological strength), 2 additional binding sites have been detected. It is thought that each binding site is identical and that the number of C4b molecules bound does not exceed 4, probably due to steric hindrance [32].

In addition to C4b, C4BP displays affinity for C4($H_2O$) (native C4 in which the thiolester bond has been hydrolyzed), C4c and C4b but not native C4 or C4d. The affinity of C4BP for C3b is approximately 100-fold less than for C4b; however, the binding may have some functional relevance since C3b can be cleaved to C3bi in the presence of C4BP and I [32, 65]. There is no evidence that C4BP can serve as a cofactor for the second cleavage of C3b to C3d and C3c [66, 67]. However, on molar basis, C4BP is 17–20 times less efficient than H in serving as a cofactor for cleavage [68, 69] and does not serve as a decay accelerator for the alternative pathway C3 convertase C3bBb [69]. It has been reported that factor H also serves as a cofactor in the I-mediated cleavage of C4b [70], but it is significantly less able to inhibit the classical pathway C3 convertase than the alternative pathway C3 convertase (200-fold less on a molar basis) [69]. This 'cross-pathway regulation' thus may be of significance only during high levels of complement activation, especially since the affinity of H for C3b is considerably higher than that of C4BP for C3b ($4.4 \times 10^6$ vs. $10^5\ M^{-1}$) [71]. It is interesting to note that C4BP is as efficient as CR1, on a molar basis, in inhibiting the classical pathway C3 convertase, but 4-fold more efficient than DAF [69].

The efficiency of classical pathway regulation by C4BP with respect to its serum con-

centration and the amount of C4b generated during complement activation has been previously noted [32], but is relevant to this discussion. Since the serum concentration and number of binding sites per molecule of C4BP are greater than the dissociation constant of C4BP-C4b interactions ($0.8 \times 10^{-7} M$), any C4b spontaneously generated will be bound and inactivated. In addition, since the concentration of C4BP will, under normal conditions, always exceed the C4b concentration, it is likely that only 1 molecule of C4b will be bound to a molecule of C4BP. Thus, the multivalent nature of C4BP may be more important during infection or the acute phase when complement activation may be markedly elevated and especially at localized sites of inflammation where the concentration of C4BP may be limited by physical factors. It is clear that C4BP tightly regulates the classical pathway of complement and under appropriate conditions may augment the regulation of the alternative pathway as well.

*Localization of Binding Sites for Complement and Noncomplement Ligands*

The binding sites through which C4BP mediates its role in complement regulation have not been unambiguously localized, although some progress has been made in defining the regions involved on the subunits. Early studies utilized limited proteolytic cleavage of C4BP with chymotrypsin [16, 72] or trypsin [73] to determine the ultrastructure of the molecule and isolate functional fragments involved in the binding and cofactor activity of the molecule. From the results of Nagasawa et al. [72] it was suggested that the cofactor activity resides in a 48-kilodalton amino-terminal fragment of each subunit. These results were extended and confirmed by Chung and Reid [17] who subjected C4BP to limited proteolysis with chymotrypsin, trypsin or pepsin and then examined the resulting fragments for their ability to bind C4b and also to serve as a cofactor in the I-mediated cleavage of C4b to C4c and C4d. These studies tentatively identified 2 unique regions in each C4BP subunit: a carboxy-terminal A region (residues 332–395), which spans portions of SCRs 6 and 7, and a more amino-terminal B site (residues 177–322) spanning primarily SCRs 3 and 4. The A region is suggested to be important in cofactor activity, while the B region is implicated in binding activity. This latter point was confirmed in studies by Fujita et al. [74].

Studies using a monoclonal antibody [74], with specificity for the 48-kilodalton amino-terminal chymotryptic fragment, identified this region as essential for the binding of C4b and for mediating the functional roles of C4BP. This antibody appears specific for the binding site of C4BP since it is able to completely inhibit C4b binding, while another antibody to this fragment is noninhibitory. Although taken together these data suggest that the binding and cofactor activities of C4BP are separate, it is difficult to envision the cofactor activity of the molecule without binding to C4b. A more precise analysis of the regions mediating C4BP activity will require deletion and site-specific mutagenesis as has been initiated for CR1 [75] and CR2 [76].

Aside from interacting with complement ligands, C4BP also has a binding site for the vitamin-K-dependent protein S, a regulatory component of the coagulation system [55, 77]. This binding is mediated through the β-chain [19, 20]. A similar function or subunit

has not been identified for mouse C4BP. Treatment of the subunit with chymotrypsin abolishes its binding to protein S and, once protein S is bound, the subunit is protected from proteolytic digestion [19]. The subunit is a functionally important structural feature of C4BP since approximately 50–60% of plasma C4BP is complexed with protein S ($K_D = 0.7 \times 10^{-7} M$) [55, 77]. The presence of protein S has no effect on the functional activities of C4BP in regulation of complement [78]; however, the ability of protein S to augment factor Va degradation by protein Ca is lost in the presence of C4BP [79, 80]. Interestingly, protein S is also afforded protection from proteolysis when complexed with C4BP, and it has been suggested that binding to C4BP serves to protect protein S during inflammatory responses [81].

## Conclusion

Despite the wealth of structural and functional information on C4BP, it is clear that several aspects of C4BP biology and regulation under normal conditions and in the acute-phase response remain unresolved. Studies to identify which interleukin and cytokines regulate C4BP expression (both α- and β-chains) are underway in our laboratory. In addition, upstream cis-acting regulatory sequences need to be characterized to attempt to correlate structural regulatory elements with expression. C4BP is a unique macromolecular protein and the mechanisms involved in controlling intracellular assembly also will be of interest. Finally, the modest increase of C4BP in SLE suggests dysregulation of altered cytokine responses in these individuals. Further studies in this and other autoimmune diseases should aid in understanding our present findings and may help in understanding C4BP gene regulation.

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