Complement driven by conformational changes

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Abstract | Complement in mammalian plasma recognizes pathogenic, immunogenic and apoptotic cell surfaces, promotes inflammatory responses and marks particles for cell lysis, phagocytosis and B-cell stimulation. At the heart of the complement system are two large proteins, complement component C3 and protease factor B. These two proteins are pivotal for amplification of the complement response and for labelling of the target particles, steps that are required for effective clearance of the target. Here we review the molecular mechanisms of complement activation, in which proteolysis and complex formation result in large conformational changes that underlie the key offensive step of complement executed by C3 and factor B. Insights into the mechanisms of complement amplification are crucial for understanding host defence and pathogen immune evasion, and for the development of complement-immune therapies.

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doi:10.1038/nri2231
Published online
7 December 2007

The complement system is an important immune surveillance system in the plasma of mammals. This host defence system is comprised of over 30 plasma and cell-surface proteins (reviewed in REF. 1). The complement system enables the host to recognize particles in the form of, for example, invading pathogens and altered host cells. Recognition by components of the complement system initiates a proteolytic cascade producing protein fragments that induce pro-inflammatory responses and mark the particles for clearance by cell lysis or phagocytosis and for stimulation of B cells (FIG. 1). There are three main ways to activate the complement system: through antibodies, C-reactive protein and C1q (the classical pathway), through large multimeric lectins binding to molecular patterns on target surfaces (the lectin pathway) and through a low level, inherent 'tick-over' activation mechanism (the alternative pathway) (BOX 1). These activation pathways converge at a proteolytic amplification step that involves the complement component C3 and the proteases factor B and factor D, the activation of which yields massive labelling — that is, opsonization — of the target surfaces with the large proteolytic fragment of C3 (FIG. 1). The various molecular fragments of C3 evoke the molecular and cellular effector functions: inflammatory responses, lysis by membrane perforation (through the formation of the membrane-attack complex), phagocytosis by macrophages and stimulation of B cells (FIG. 1). Data on the evolution of this elaborate molecular defence system indicate that it most likely developed around C3 and factor B of the central, amplification and opsonization step[2]. In cnidaria (such as anemones and corals) and some protostomes (such as crabs and nematodes), only these two complement proteins are present, indicating that this central part of the complement cascade was established over 1,000 million years ago (reviewed in REF. 2). A more elaborate system evolved as we know it today in higher organisms with multiple recognition mechanisms and effector functions. Modular proteases, such as factor B, and homologues of C3 (C4 and C5) are crucial to the proteolytic cascade in both the initiation and effector pathways. Altogether, the complement proteins form a humoral, molecular defence system that, through the signalling of its various molecular fragments, affects inflammatory and both innate and adaptive immune responses.

Regulation of complement is of critical importance for homeostasis of the organism. On the one hand, complement activity is important for the clearance of foreign pathogenic and altered host cells, whereas on the other hand, this activity must be kept in check to avoid tissue damage. The host expresses several regulators, most of them bound to cell surfaces, that protect against complement activation by downregulating the central proteolytic activity of the amplification and opsonization steps[3]. Deficiencies or mutations in complement proteins may predispose individuals to infectious and immune-related diseases or may lead to excessive complement activation and tissue injury.

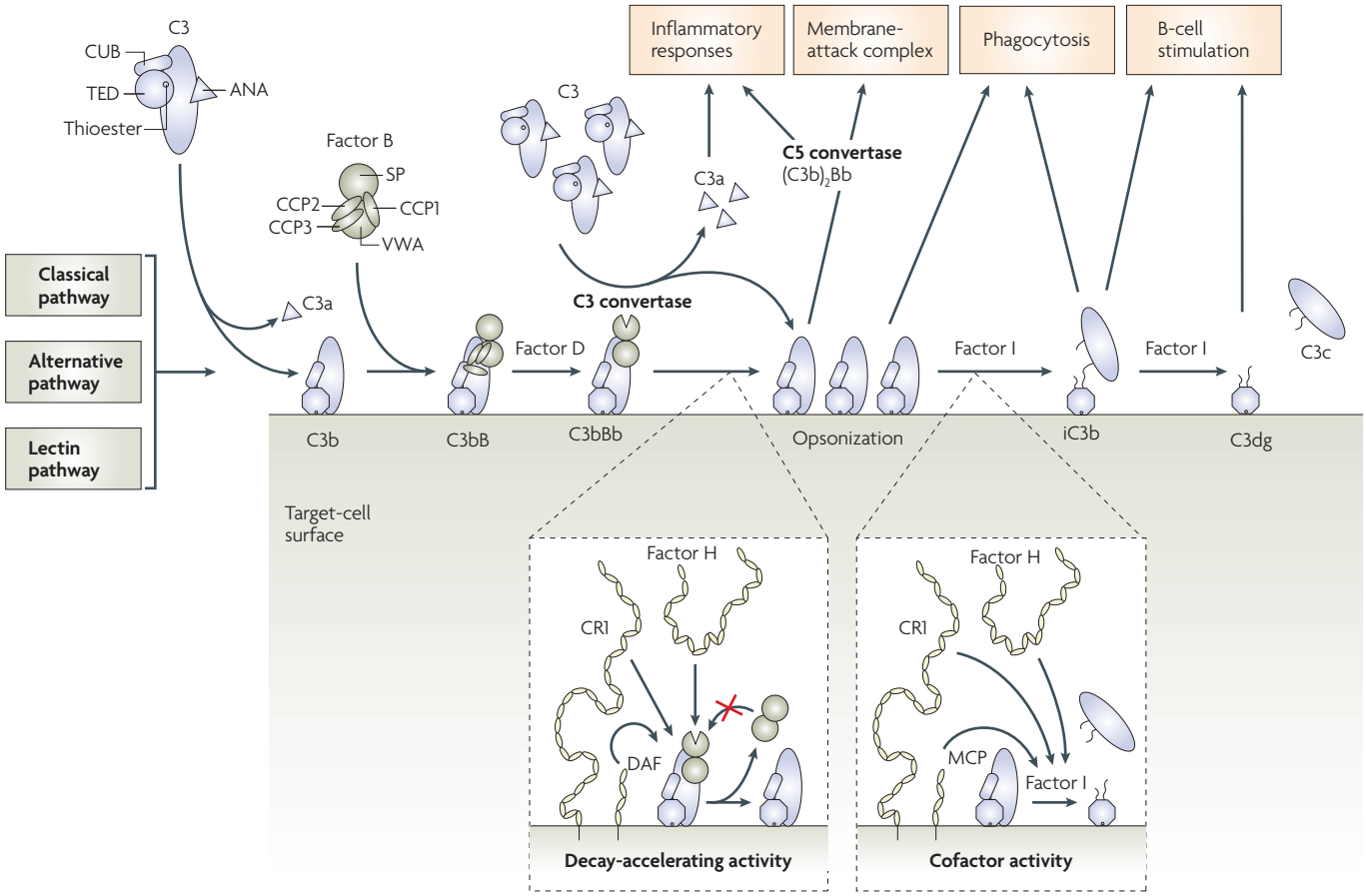


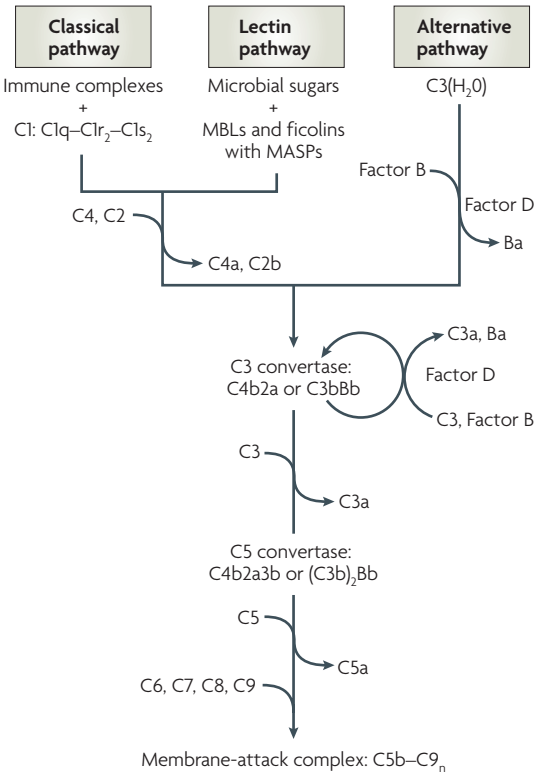
Figure 1 | **Overview of the complement pathways.** The complement response is initiated by one of three initiation pathways and results in the covalent attachment of a small amount of C3b (a proteolytically activated fragment of C3) to target surfaces. This is followed by the central step in the complement response: factor B binds C3b to generate the C3bB complex, which is subsequently activated by the protease factor D to form the active C3 convertase complex C3bBb. The C3 convertase activates many C3 molecules into C3b that covalently couple to reactive surfaces (opsonization), thereby amplifying the complement response. The activation of C3 results in several effector functions, such as inflammatory responses, phagocytosis and B-cell stimulation. Tight regulation of this potentially damaging deposition of C3b is achieved by surface-bound regulators, such as complement receptor 1 (CR1) and decay-accelerating factor (DAF) and soluble factor H, that dissociate Bb from C3b; this is known as decay-accelerating activity. Furthermore, surface-bound CR1 and membrane cofactor protein (MCP) and soluble factor H aid the protease factor I in the processing of C3b; this is called cofactor activity. ANA, anaphylatoxin domain; CCP, complement-control protein; CUB, complement C1r/C1s, UEGF, BMP1; SP, serine protease; TED, thioester-containing domain; VWA, von Willebrand factor A.

Examples of associated pathological conditions are atypical haemolytic uraemic syndrome, systemic lupus erythematosus and age-related macular degeneration (reviewed in REF. 4). Therefore, complement-based immunity depends on a balance between tagging surfaces with activated C3 for clearance and protecting surfaces by preventing tagging.

Many of the biochemical aspects of complement activity have been resolved in the past decades (reviewed in REFS 1,5–10). However, the molecular mechanisms that underlie the central aspects of complement activation have long remained elusive. Recent advances in the determination of the structures of two key proteins, C3 and factor B, and their proteolytic fragments provide a framework to develop mechanistic models. The structural data revealed the complex architecture of C3 (REFS 11,12) and the large conformational changes this molecule undergoes upon activation into fragment C3b, alteration of activity into iC3b and C3dg, and deactivation into C3c by sequential proteolysis[13–15] (FIG. 1). Structures of the pro-enzyme factor B[16] and its proteolytic fragment Bb[17] (as well as its homologue C2a[18,19]) gave insights into the formation and activity of the central C3 convertase, an instable protease complex formed by C3b and Bb (the activated fragments of C3 and factor B, respectively) that is essential for the central amplification and opsonization step.

In this Review, we discuss these recent structural insights and relate them to mechanisms of complement activity, host control and evasion by pathogens. We describe how the intricate structure of C3 markedly rearranges upon proteolytic activation exposing and activating the chemical reactive groups for attachment

Box 1 | **Recognition pathways of complement activation**



Recognition of a pathogenic, immunogenic or apoptotic-cell surface is a first and crucial step in complement activation. Two specific recognition pathways, the classical pathway and the lectin pathway, and a non-specific pathway, the alternative pathway, activate the complement system (see figure). The classical pathway is initiated by the recognition of immune complexes on target surfaces by C1q, which forms part of the large multiprotein complex C1, consisting of C1q, C1r and C1s. The binding event induces conformational changes in C1 that activate the serine proteases C1r and C1s[82]. The lectin pathway is initiated by the recognition of microbial sugars (such as mannose, N-acetylglucosamine or fucose)[6,83]. The sugars are recognized by mannose-binding lectins (MBLs) or ficolins, which are in complex with one of three MBL-associated serine proteases (MASPs; MASP1, MASP2 or MASP3)[8,84,85]; recognition is followed by activation of the MASPs. In both the classical and lectin pathways, the activated C1, MBL–MASP2 or ficolin–MASP2 complexes proteolytically activate complement component C4, which is homologous to C3 (MASP1 and MASP3 have not been shown unambiguously to activate C4 or C2). Similar to C3, activation of C4 by proteolytic cleavage results in release of the anaphylatoxin C4a and the major fragment C4b. C4b can covalently bind to a target surface close to the C1, MBL–MASP or ficolin–MASP complexes. Once attached to the surface, C4b binds the zymogen C2 (which is homologous to factor B) in a magnesium-dependent manner and the nearby C1, MBL–MASP or ficolin–MASP complexes subsequently proteolytically activate C4b-bound C2 into C2b and C2a (which are homologous to Ba and Bb, respectively). Of these only C2a stays attached to C4b. This complex, C4b2a, is called the classical pathway C3 convertase and is able to proteolytically activate C3. Instead of activation by specific recognition, the alternative pathway is spontaneously activated, although at a very low rate[86]. This 'tickover' is achieved by spontaneous hydrolysis of C3 (REF. 32). Hydrolysed C3, termed C3(H_2O), can bind factor B, which is homologous to C2. The bound factor B can be activated proteolytically by the protease factor D into Ba and Bb leaving behind C3(H_2O)Bb, the fluid-phase C3 convertase[5]. This generic process works irrespective of the type of cell surface. Hence, organisms have developed means (by way of soluble and cell-surface bound complement regulators) to avoid complement initiation on their own cells and tissues.

to the target particles. These and subsequent rearrangements induced by further proteolysis effect formation of cryptic binding sites for various proteins, evoking the biological responses in complement activation and regulation, cell lysis, phagocytosis and B-cell stimulation. As we discuss, all of these processes depend on the activity of the C3 convertase that in turn proteolytically activates native C3. Structures of the protease component of the convertase reveal an intricate assembly process, in which a series of conformational changes induced by C3b make the pro-enzyme factor B susceptible to proteolytic activation into Bb. Current data suggest that final C3-convertase activity is only achieved when substrate (C3) binds to the C3bBb protease complex, which would explain the substrate specificity of the C3 convertases. The irreversible dissociation of the C3bBb complex is essential to stop complement activation. Host tissue is protected by complement regulators that dissociate the C3bBb complex or facilitate further proteolysis of C3b. Pathogens have copied these strategies. However, recent examples indicate that pathogens may have developed many more ways of blocking convertase activity.

Complement component C3

C3 is a member of the C3/α_2-macroglobulin protein family of host-defence molecules[20], which are large proteins of 1,400–1,800 amino-acid residues in length[21]. Besides C3 and the protease inhibitor α_2-macroglobulin, this family includes the two homologous complement proteins C4 and C5 (which are 26–30% identical in sequence to C3) and several related molecules in insects and nematodes (known as thioester proteins (TEPs)), which provide multiple pattern-recognition modules that might accommodate for the lack of an adaptive immune system in these animals[20]. A typical feature of these proteins is the presence of a reactive thioester moiety that is required for covalent attachment to molecular or cellular targets (although some members of the family, notably C5, lack this reactive group). The proteins of this family are thought to undergo marked conformational changes upon activation[21,22]. This is clearly the case for C3, as its modification by sequential proteolytic cleavages results in complement amplification and signalling for inflammatory responses, cell lysis, phagocytosis and B-cell stimulation. Crystal structures have been determined of native, intact C3 from humans[11] and cows[12], of the

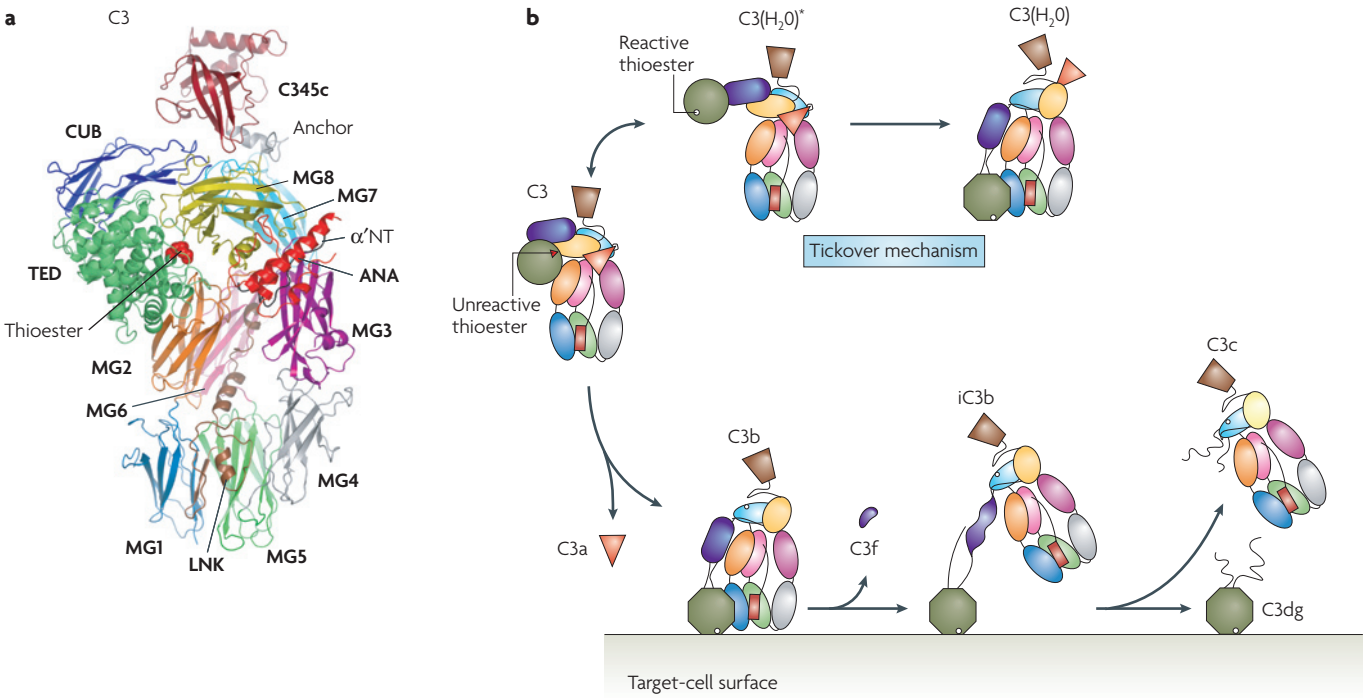


Figure 2 | **Conformational complexity of complement component C3. a** | An X-ray crystal structure of C3, indicating all of its 13 domains (in bold), is shown by ribbon representation. **b** | A schematic representation of the structures of C3 and its activation products show how C3 undergoes large conformational changes upon activation. Low-rate spontaneous hydrolysis of C3 by the so-called tickover mechanism results in the formation of C3(H_2O) through a stable intermediate denoted C3(H_2O)*. Activation of C3 by the C3 convertases results in the formation of C3b, which is similar in structure and properties to C3(H_2O), and the anaphylatoxin C3a. Further processing of C3b results in the formation of iC3b and C3f and finally C3c and C3dg. Elements from this figure are reproduced with permission from *Nature* REF. 11 © (2005) and REF. 13 © (2006) Macmillan Publishers Ltd. α′NT, N-terminus of the α-chain; ANA, anaphylatoxin domain; CUB, complement C1r/C1s, UEGF, BMP1; LNK, linker; MG, macroglobulin; TED, thioester-containing domain.

molecular fragments C3a[23], C3b[13], C3d[24], C3c[11], and of C3b and C3c in complex with complement receptor of the immunoglobulin superfamily (CRIg), which is expressed by Kupffer cells (liver-resident macrophage cells)[14]. In addition, Abdul Ajees *et al.*[25] have also described a structure of C3b. However, this structure has several differences to the two structures of C3b determined by Janssen *et al.*[13] and Wiesmann *et al.*[14], namely an unfolded conformation of the CUB (complement C1r/C1s, UEGF, BMP1) domain and a different position and conformation of the thioester-containing domain (TED) (see below). Because of controversy over the underlying data[26], the structure described by Abdul Ajees *et al.*[25] is not discussed further in this Review. Besides the crystallographic data on C3 and the various fragments, electron-microscopy images provide additional insights into a stable reaction intermediate of C3, an amino-nucleophile-bound form of C3 (which is comparable to hydrolysed C3 or C3(H_2O)) and iC3b[15]. Altogether, these structural data provide a comprehensive view of the various conformational states of C3 and its fragments during complement activation (FIG. 2). Moreover, these data shed light on the thioester activation and surface attachment (that is, conversion from C3 into C3b) and the conformational states of the fragments (C3a, C3b, iC3b and C3dg) that are crucial to the various biological activities of these fragments.

C3 has an intricate domain arrangement with a buried thioester moiety. Human C3 is synthesized as a 1,641 residue long polypeptide precursor. Before secretion, this pro-C3 is cleaved by removal of a tetra-arginine sequence (Arg646–Arg649)[27], which results in mature C3 consisting of a β-chain (residues 1–645) and an α-chain (residues 650–1,641). Together the two chains form 13 domains[11]. The core of the molecule is formed by eight homologous domains, known as macroglobulin (MG) domains. Domains MG1–MG6 form a ring of 1.5 turns, capped by domains MG7 and MG8. Two large inserts (residues 578–745 and 912–1,330) and a C-terminal extension (residues 1,496–1,641) form the other five domains, which include a linker domain, the anaphylatoxin domain, the CUB domain, the TED and the C345c domain (FIG. 2). This domain organization suggests that the C3/α_2-macroglobulin family of host-defence molecules evolved from a core of eight MG domains that may have arisen by gene duplication events[11]. Most recently, Baxter *et al.*[28] determined the structure of the TEP1 isoform TEP1r, which is a distant homologue of C3, from the mosquito *Anopheles gambiae*. Both the similarities and the differences between mammalian C3 and insect TEP1r are striking. TEP1r has the same domains MG1–MG8, the linker domain, the CUB domain and the TED as does mammalian C3. However, the anaphylatoxin domain,

which is functionally important for C3, is missing in TEP1r. The large differences in the domain orientations between mammalian C3 and insect TEP1r are in part related to the absence of anaphylatoxin[28]. However, these differences might also be due to the uncleaved state of the TEP1r molecules and therefore TEP1r may be more related structurally to pro-C3 than mature C3.

The reactive thioester moiety, which is required for covalent attachment to target surfaces, is protected from hydrolysis in native C3. The thioester is formed by the side chains of Cys988 and Gln991, which are part of the TED[29,30]. In the structure of native C3 (REFS 11,12), the thioester is tucked away between the TED and the MG8 domain, this limits the thioester's access to the hydroxyl nucleophiles it can react with and which are for example present in the carbohydrates on the surface of cells. Moreover, the TED–MG8 interface in native C3 prevents the chemical transition of the reactive thioester moiety into a free thiolate and an acyl-imidazole intermediate, which is a far more reactive species[31]. Indeed, it takes hours or days for the thioester in C3 to react with either amino or hydroxyl nucleophiles[5,32–34], whereas the thiolate and acyl-imidazole intermediate has a half-life of less than 100 μs in the presence of hydroxyl nucleophiles[35]. Therefore, the complex structural arrangement of domains in C3 in part serves to maintain the MG8–TED interface, which is crucial in preserving the thioester moiety and hence the native state of C3.

Activation of C3 exposes the thioester for surface attachment. Cleavage of C3 between Arg726 and Ser727 by the C3 convertases removes the anaphylatoxin domain from the N-terminus of the α-chain (α′NT) and yields the small anaphylatoxin C3a and the large fragment C3b. In native C3, the anaphylatoxin domain stabilizes the TED–MG8 interface indirectly by keeping MG8 in its place. Removal of the anaphylatoxin domain causes the MG7 and MG8 domains to swivel, the new α′NT to relocate, and the CUB domain and TED to swing out. These marked conformational changes displace the thioester moiety by 85 Å and expose it completely to the solvent (FIG. 2). Concomitantly, the TED changes its conformation modifying the thioester into the highly reactive thiolate and acyl-imidazole intermediate[11,13,14,24,35]. This intermediate may react with any accessible nucleophile it comes across, which results in C3b becoming covalently bound to molecules and target surfaces close to the site of activation. Reaction with water prevents this damaging attachment from occurring far from the site of activation.

Besides proteolytic activation, C3 can also be activated by spontaneous hydrolysis of the thioester (at a very low rate; half-life ~230 hours). This process is referred to as the tickover mechanism of the alternative pathway. Electron-microscopy data[15] show a stable intermediate of C3, which has reacted with a nucleophile, that has its TED swung away from the MG8 interface (FIG. 2). This intermediate either returns to the native C3 conformation or undergoes a slow (half-life~1 hour) irreversible conformational change to C3(H_2O)[34]. Similar to C3b, C3(H_2O) can bind factor B to form a C3 convertase. However, in the case of C3(H_2O) the anaphylatoxin domain is still attached to the α′NT. The electron-microscopy data indicate that, similar to the α′NT, the bulky anaphylatoxin domain translocates through a narrow hole between the MG2, MG3 and MG6 domains. A comparison of the translocation of the cleaved α-chain versus the uncleaved, bulky anaphylatoxin domain containing α′NT explains the fast conversion of C3 to C3b upon proteolytic activation and the slow, and irreversible, structural transition of C3 to C3(H_2O).

Surface-bound C3b provides a convertase assembly platform. The covalent coupling of C3b to target particles is important to generate local complement amplification. Whereas native C3 interacts with only a few proteins, C3b interacts with many proteins (reviewed in REF. 9). First, C3b binds pro-enzyme factor B and properdin to form the C3 convertase. Second, various regulators (such as factor H, complement receptor 1 (CR1; also known as CD35) or decay-accelerating factor (DAF; also known as CD55)), which consist of extended chains of short complement-control protein (CCP) domains, bind C3b to dissociate the convertase complexes. Third, some of these, and other, regulators (such as factor H, CR1 and membrane cofactor protein (MCP; also known as CD46)) assist protease factor I in the proteolytic degradation of C3b, yielding the fragments iC3b, C3c and C3dg, which no longer support the formation of convertase complexes. Finally, C3b and the subsequent iC3b fragment bind receptors on macrophages to facilitate phagocytosis, whereas fragments iC3b and C3dg bind receptors to stimulate B cells.

C3b provides the binding sites for factor B that are required for C3 convertase formation. The putative binding sites for factor B (α′NT and C345c[36–38]) map to the upper half of the C3b molecule (FIG. 3). This side of the molecule undergoes large rearrangements upon activation of C3 into C3b, which explains why C3 cannot form convertases. Properdin, which enhances convertase formation and stabilizes convertases, binds C3b at the adjacent MG8 domain[39], suggesting that properdin possibly acts by bridging the interactions between C3b and factor B (and those between C3b and Bb)[40]. The binding sites for the regulators factor H and CR1 map to the α′NT, MG6 and TED[36,41–45]. These sites partially overlap with the α′NT site for the binding of factor B, which implies that convertase dissociation (also referred to as 'decay-accelerating activity' of the regulators) may be based in part on steric hindrance with factor B. Besides convertase dissociation, regulators such as factor H, CR1 and MCP facilitate cleavage of the CUB domain of C3b in three places by factor I (which is referred to as the 'cofactor activity' of regulators). The first two cleavages (between Arg1281 and Ser1282 and between Arg1298 and Ser1299) yield iC3b. In contrast to C3b, iC3b does not bind factor B and cannot form convertases. The cleavages in the CUB domain cause it to lose its fold, yielding a flexible linker between the main body of iC3b and the

TED with an average length of 100 Å across approximately 50 residues[15]. As a consequence, the main body of the molecule becomes dislodged and randomly oriented with respect to the surface-bound TED[15]. The third and final cleavage in the CUB domain (between Arg932 and Ser933) severs the link and releases the main body as fragment C3c, leaving C3dg bound to the surface. This proteolytic processing of the CUB domain, however, does not induce further structural rearrangements in the separate fragments, because structures of C3c and C3d are remarkably similar to the equivalent parts in C3b.

These data indicate that formation of convertases probably depends on a proper structure and orientation of the CUB domain. This notion is further supported by the homologous cobra-venom factor that forms fluid-phase convertases and has a CUB domain but not a TED. Therefore, it seems likely that the marked conformational changes upon proteolytic activation of C3 into C3b reorient the α′NT, CUB and C345c regions creating the binding site for factor B to form convertases, and that this binding site crucially depends on a proper structure of the CUB domain.

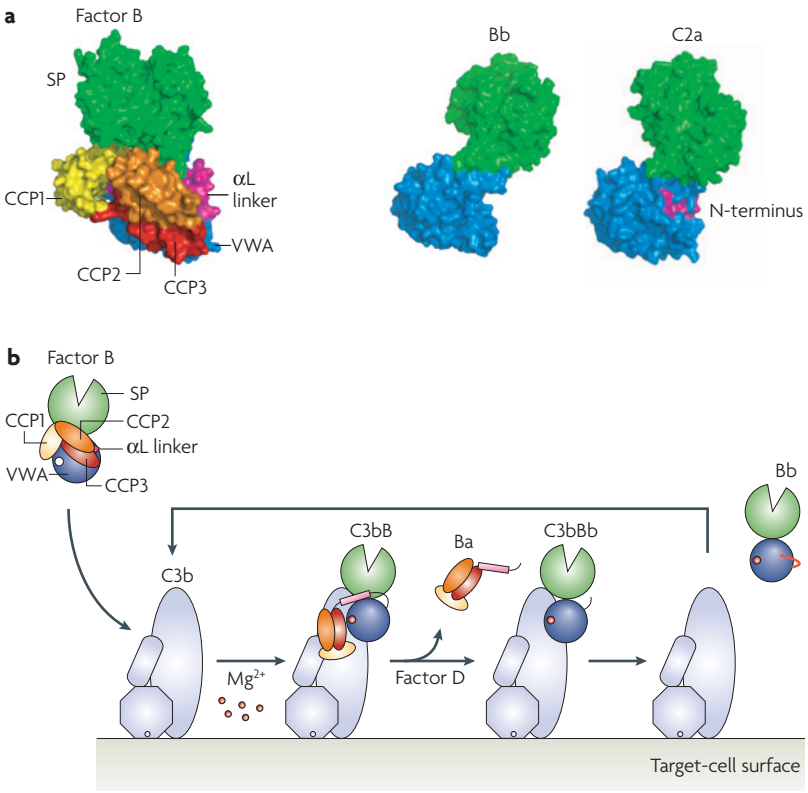


Figure 3 | **Conformational rearrangements of the proteases factor B and C2.** **a** | The structures of pro-enzyme factor B, and fragments Bb and C2a are shown in surface representation. **b** | A schematic representation of C3 convertase formation. Following Mg^{2+}-dependent association to its surface-bound cofactor (C3b), factor B is activated by factor D and the Ba fragment dissociates from the complex. Once dissociated, the N-terminus of Bb and C2a may prevent re-association to their cofactors. Figure modified with permission from *Nature Structural & Molecular Biology* REF. 16 © (2007) Macmillan Publishers Ltd. CCP, complement-control protein; SP, serine protease; VWA, von Willebrand factor A.

C3 convertase converts into a C5 convertase initiating the terminal pathway of complement activation. The action of C3 convertases on target surfaces activates C3, causing high local concentrations of C3b on the target surface, which results in amplification of the complement immune response and formation of the C5 convertase. The C5 convertase is formed from the binding of a C3b molecule to the C3 convertase, which causes the convertase to shift its substrate specificity from C3 to C5 (REF. 46). Cleavage of C5 by the C5 convertase (that is, the $(C3b)_2Bb$ complex) yields C5a, which is one of the most potent mediators of inflammation and chemotaxis, and C5b, which initiates formation of the membrane-attack complex. The membrane-attack complex is the final product of the so-called terminal pathway of complement activation and is formed by C5b, complement proteins C6–C8, and multiple copies of C9 (~16 copies)[47]. On assembly of the membrane-attack complex, C9 inserts into target membranes and forms a large pore-like structure (with a diameter of ~100 Å) that causes cell lysis.

Surface-bound fragments of C3 induce phagocytosis and stimulate B cells. C3b and iC3b both bind CR1 on neutrophils[48] and CRIg on Kupffer cells[49] and this induces phagocytosis of the tagged particle. The crystal structure of the C3b–CRIg complex reveals that CRIg binds C3b at domains MG3–MG6 and at the linker domain; CRIg is only capable of binding C3b and not native C3, owing to the structural reorientations of the MG3 and linker domains[14]. In addition, iC3b induces phagocytosis by binding integrins CR3 (also known as $\alpha_M\beta_2$-integrin or CD11b–CD18) and CR4 (also known as $\alpha_X\beta_2$-integrin or CD11c–CD18) on leukocytes. By contrast, the surface-bound fragment C3dg does not induce phagocytosis. This suggests that the phagocytic activity of complement components depends on those receptors that interact with elements from the main body of iC3b. The situation is different for B-cell stimulation, as both iC3b and C3dg, but not C3b, stimulate B cells. iC3b and C3dg bind to CR2 (also known as CD21) of the co-stimulatory B-cell receptor complex, indicating that interactions with the TED are crucial. This is supported by low resolution structural data that indicate binding of the two N-terminal CCP domains of CR2 to C3dg[50,51]. Clearly, further structural data on C3 fragments in complex with (in some cases large) receptors is needed to understand the binding events. Understanding the molecular mechanism in the subsequent cellular activation presents a formidable challenge to structural biologists. In both cases — that is, phagocytosis and B-cell stimulation — many more molecules on the cell surface and in the cell are involved to establish the biological response.

C3 convertase

C3b bound to the surface of a target particle provides a molecular platform for the formation of C3 convertases. These convertases are highly specific protease complexes that cleave C3 into C3a and C3b generating a local amplification loop (FIG. 1). The complement

pathways contain two homologous C3 convertase complexes: one formed from C3b and pro-enzyme factor B (in the alternative pathway) and one formed from C4b and pro-enzyme C2 (in the classical and lectin pathways). The inactive pro-enzymes factor B and C2 each consist of five domains: three CCP domains followed by a long linker domain that contains the activating scissile bond (that is, the point of cleavage), a von Willebrand factor A (VWA) domain and a C-terminal serine protease (SP) domain. The SP domain has a typical chymotrypsin-like fold and carries the catalytic centre. These proteases, factor B and C2, are activated by a two-step assembly process (FIG. 3): Mg^{2+}-dependent association with their cofactors (C3b and C4b, respectively) and subsequent proteolysis of the protease into a small fragment (consisting of the N-terminal CCP domains and most of the linker domain, denoted Ba and C2b) and a large fragment (formed by the VWA and SP domains, denoted Bb and C2a). The small, N-terminal fragment dissociates from the complex resulting in a final cofactor–protease-fragment complex, either C3bBb or C4bC2a (denoted C4b2a), which are the C3 convertases. These active protease complexes are short-lived (with *in vivo* half-lives of 90 seconds and 60 seconds, respectively[52,53]). Once dissociated, the protease fragments (Bb and C2a) cannot re-associate with their cofactors and the proteolytic activity is lost[54]. Therefore, the assembly process establishes C3 convertase activity, which amplifies the production of C3b, whereas the irreversible dissociation of the convertases ensures the crucial downregulation of complement amplification.

Recent crystal structures of the fragments Bb[17] and C2a[18,19] and of the full-length factor B pro-enzyme[16] revealed marked conformational differences between the fragments and the pro-enzyme. The observed conformational differences allow us to address in several important questions: how are the convertases assembled, what determines the proteolytic activity and the substrate specificity of the C3 convertase complexes, and why do the complexes dissociate irreversibly?

How are C3 convertases assembled? Formation of an active C3 convertase complex requires a two-step assembly process, in which pro-enzyme factor B first binds to C3b in a Mg^{2+}-dependent manner and is, subsequently, cleaved into Bb yielding the active C3bBb protease complex (similarly, for C4b and C2 yielding the C4b2a complex). The recent structural determination of the factor B pro-enzyme[16] and the Bb fragment[17] indicated that this involves intricate conformational changes that convert the locked conformation of the pro-enzyme into a C3b-bound form that can be proteolytically activated. The long linker domain between the CCP and VWA domains of factor B contains the scissile bond and seems to have a pivotal role in this process.

First, the N-terminal part of this linker forms an α-helix (αL) that, surprisingly, is structurally incorporated into the VWA domain at the position of its C-terminal α7 helix. The α7 helix of factor B is homologous to the α7 activation helix found in the integrin inserted (I) domains of integrins. In integrins this helix is, together with the metal-ion-dependent adhesion site (MIDAS), pivotal in bidirectional signalling across the membrane in cell–cell adhesion processes. In outside-in signalling by integrins, ligand binding to the MIDAS on the extracellular I domain causes the α7 helix to move away from the ligand-binding surface inducing large rearrangements of the extracellular domains that convey the signal to the intracellular part of the receptor[55–58]. In turn, signals within the cell that act on the cytoplasmic part of the receptor trigger conformational changes that effect inside-out signalling to activate the integrin[55–58]. In the factor B pro-enzyme, the αL helix blocks the α7 helix from taking its position as observed in Bb, which is analogous to the activated position of I domains in integrins. Second, the C-terminal part of the linker domain forms a long loop containing the Arg234–Lys235 scissile bond. Remarkably, the arginine side chain is bound in between the αL helix and the displaced α7 helix. Because of this binding, this residue is not accessible to the protease factor D making the pro-enzyme resistant to premature proteolysis. Third and finally, factor B and C2 both have a MIDAS for Mg^{2+}-dependent binding of the cofactor C3b[59]. In the pro-enzyme factor B, however, the Mg^{2+}-binding site is distorted and Mg^{2+} binding cannot occur[16,60]. These observations suggest a series of events in the binding of factor B to C3b, which possibly starts with Mg^{2+}-independent binding of the CCP domains to C3b[61,6263,64]. Dislocation of the CCP domains would allow initiation of the MIDAS conformation for Mg^{2+}-dependent binding of C3b. Next, these changes would trigger relocation of the αL and α7 helices (similar to activation of integrin I domains) and consequently expose the scissile bond for proteolytic activation by factor D. Proteolysis by factor D, then finally, removes the Ba fragment and establishes the active C3 convertase (C3bBb) of the alternative pathway.

What determines proteolytic activity and substrate specificity? The catalytic site of the C3 convertases resides in the C-terminal chymotrypsin-like SP domain of factor B and C2. This site is only active in the C3 convertase complex and not in the pro-enzyme nor in the isolated protease fragment (either Bb or C2a). Structures of pro-enzyme factor B and fragment Bb, as well as of C2a, reveal that the catalytic sites are accessible in both the pro-enzyme and the protease fragment[16–19]. Moreover, the catalytic centres, before and after proteolytic activation (factor B versus fragment Bb), are remarkably similar in conformation. By contrast, proteolytic conversion of chymotrypsinogen into chymotrypsin generates a new N-terminus (Ile16) that induces a large conformational change that forms the oxyanion hole required for stabilization of the reaction intermediate[65]. Such proteolytic activation in factor B and C2 takes place in front of the VWA domain and not in the N-terminal region of the SP domain, as in chymotrypsinogen. Comparison of the structures of factor B, fragment Bb and C2a shows that a surface-loop arginine acts in a similar manner as the Ile16 N-terminus in chymotrypsin[16–18,66] (FIG. 4). However, in pro-enzyme factor B and the isolated fragment Bb the conformations of the oxyanion-hole loop are identical. In both proteins, the overall backbone

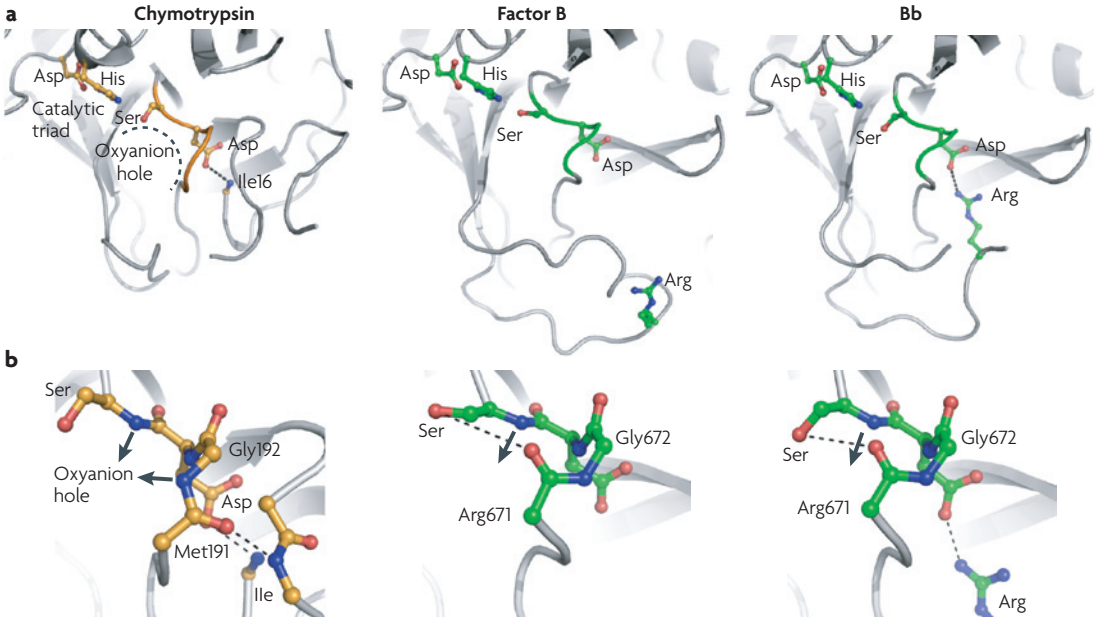


Figure 4 | **The catalytic centre of chymotrypsin and complement factors B and Bb. a** | The catalytic site in serine proteases consists of a catalytic triad (serine, histidine and aspartate) required for nucleophilic attack on the substrate and an oxyanion hole for stabilization of the oxyanion intermediate. The transition of chymotrypsinogen into chymotrypsin generates a new N-terminus (Ile16), which induces conformational rearrangements that result in the formation of an active oxyanion hole next to the catalytic triad. During the activation of factor B into Bb, a surface-loop arginine acts in a similar manner as the N-terminus in chymotrypsin. Nevertheless, in both factor B and Bb, the oxyanion holes are remarkably similar and a full active conformation is not established in fragment Bb. **b** | Two inwards pointing amides (indicated by arrows) form the oxyanion hole in active chymotrypsin, whereas only one of the amides points inwards in Bb. It is possible that the binding of substrate (C3) may induce maturation of the oxyanion hole in Bb, when present in the C3bBb complex. Figure modified with permission from *Nature Structural & Molecular Biology* REF. 16 © (2007) Macmillan Publishers Ltd.

conformation of the oxyanion-hole loop adopts a conformation that is almost similar to the one in active chymotrypsin (that is, irrespective of the arginine side chain making a salt-bridge with an aspartate side chain of the oxyanion-hole loop). Nonetheless, the oxyanion holes in factor B and fragment Bb are in an inactive conformation that is due to a ~180° flip of the Arg671–Gly672 peptide plane[16,17] (FIG. 4). Putatively, this inactive conformation of the oxyanion hole in factor B and fragment Bb is due to a short surface loop, which cannot stabilize the active orientation of the peptide plane, in contrast to the longer surface loop in chymotrypsin. Ponnuraj *et al.*[17] proposed that maturation of the oxyanion hole, which establishes catalytic activity, might be substrate induced, which is analogous to substrate-induced activation of the protease factor D[67,68]. In this case, the convertase cofactor (either C3b or C4b) would provide an exo-site for the substrate C3 promoting substrate-induced activation of the catalytic centre of either Bb or C2a[18]. This model explains the catalytic inactivity of the pro-enzymes (factor B and C2) and of the isolated fragments (Bb and C2a), and explains the activity and substrate specificity of the convertase complexes.

Why do complexes dissociate irreversibly? The irreversible dissociation of the convertase complexes is crucial to the downregulation of the complement response. Once dissociated, Bb and C2a cannot rebind to C3b and C4b, respectively. However, active convertases can be formed by reloading C3b or C4b with pro-enzymes factor B and C2 (FIG. 3). So, either the protease fragments or the cofactors can undergo conformational changes that block re-association. The fact that the core of C3b is very similar to the structure of C3c indicates a structurally stable core of the cofactors. Possibly, the CUB–TED extension takes on an inactive conformation in the free cofactors C3b or C4b that can only be affected by the pro-enzymes and not by the proteolytic fragments Bb or C2a, respectively. Alternatively, the protease fragments can change conformation. One possible clue comes from the structural comparison of full-length C2a and N-terminal-truncated Bb[17,18]. The N-terminal tail of C2a lies in a crevice adjacent to the α7 activation helix. Similar to inhibitors of the integrin I domains[69–71], the N-terminus in C2a dislocates the α7 helix from the activated conformation, as observed in Bb[17], to an intermediate position observed in C2a[18,19]. Putatively, this intermediate position of the α7 helix could prevent association with the cofactor. However, this model, which explains the irreversible dissociation, has not been confirmed experimentally.

Protection against complement activation

Covering target particles with covalently linked C3b molecules — that is, opsonization — is a central step in complement-induced immune responses. The covalent binding of C3b itself to surfaces, however, is not discriminative of host and foreign surfaces (reviewed in REF. 5).

So, both hosts and pathogens have evolved mechanisms to disrupt convertase complexes in protection of their cells. In mammals, complement activity is controlled by various regulators that assist in the discrimination between self and non-self surfaces. Bacteria and viruses have mimicked these proteins and developed additional mechanisms to avoid complement-induced clearance[72,73].

The host discriminates between self and non-self by expressing regulators on the surface of its cells or by expressing soluble regulators that can recognize host cells specifically. These regulators of complement activity affect the convertases in two possible ways: either through decay-accelerating activity that dissociates the convertase complexes and/or through cofactor activity that assists factor I in degrading C3b[74]. The soluble regulator factor H is of particular importance for the protection of cells that lack surface-bound regulators. Factor H discriminates between self and non-self by recognizing glycosaminoglycans on host cells. The regulators of complement activity are all composed of multiple (4–30) connected CCP domains. By contrast, the recently identified complement receptor CRIg, consists of membrane-attached immunoglobulin-like IgV domains[49], which are involved in inhibiting the alternative pathway C3 convertase, C3bBb. Binding of the IgV domain to C3b specifically blocks C3 substrate binding to the convertase, putatively by steric hindrance[14]. This convertase inhibiting activity could be considered a third type of complement regulation in addition to decay-accelerating activity and cofactor activity.

Several pathogens have developed strategies to outmanoeuvre elimination by complement. A large number of bacterial and viral proteins inhibit complement and do so at different stages (reviewed in REFS 75,76). Because C3 is central to the complement system many pathogens have developed mechanisms to control the activation and regulation of C3 and use these interactions to their own advantage. In some instances, pathogens express mimics of complement regulators on their surface or 'hijack' host regulators by binding them to their surfaces. For example, vaccinia virus complement control protein (VCP) is a structural and functional homologue of the regulators of complement activity[77] with both decay-accelerating and cofactor activity, thereby inhibiting complement amplification[78]. HIV virions use opsonized C3b on their surface to interact with and infect complement-regulator-expressing host cells, such as monocytes, macrophages and dendritic cells (reviewed in REF. 73). The tick-borne pathogen *Borrelia burgdorferi* hijacks factor H from the infected host by binding it to *B. burgdorferi* complement regulator-acquiring surface protein (CRASP), and therefore acquires decay-accelerating and cofactor activities that prevent complement amplification on its surface[79]. *Staphylococcus aureus* extracellular fibrinogen-binding protein (Efb) binds to TED in C3 and changes the conformation of C3, disabling its activation into C3b[80]. Another *S. aureus* protein, staphylococcal complement inhibitor (SCIN), stabilizes and inhibits C3 convertases, preventing activation of C3 into C3b[81]. Intriguingly, SCIN inhibits both the human C3bBb and C4b2a complexes, but fails to inhibit mouse or rat complement activation. Further insights into the mechanisms of complement evasion induced by these molecules from pathogens may be particularly advantageous for the development of specific complement therapeutics.

Concluding remarks

The recent structural advances have provided unprecedented insights into the molecular complexity underlying complement activation. In particular, we can begin relating the molecular structures of C3 and its fragments to the diverse molecular and cellular responses they evoke. Structures of the central proteases, factor B and its homologue C2, reveal the intricacy of the assembly process that yield the central, instable convertase complexes that specifically activate C3 molecules. Moreover, with the new structural data on these complex molecules, we can now start addressing many other long-standing questions in this field. What is the structure of the convertase complex, what are the differences among the convertases in alternative and classical or lectin pathways, and how do these complexes alter their substrate specificity from C3 convertases to C5 convertases? How do regulators accelerate convertase dissociation and how do they help factor I in the proteolysis of C3b? How do the various bacterial and viral proteins block C3b generation and deposition? How do C3b, iC3b and C3dg induce phagocytosis and/or B-cell stimulation? These and other important questions, such as the specific recognition and activation mechanisms in the classical and lectin pathways and inflammatory stimulation mechanisms of C3a and C5a, require huge efforts in biochemical and structural studies. In particular, it will require structural determination of many, sometimes large and instable, complexes involving the complement components. These topics present a formidable challenge to structural biologists in the complement field, to which only a small beginning has been made so far.

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Acknowledgements
We thank J. D. Lambris and J. van Strijp for reading the manuscript. This work was financially supported by a 'Pioneer' programme grant to P.G. by the Council of Chemical Sciences of the Netherlands Organization for Scientific Research (NWO-CW).

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
C3 | C4 | C5 | CR1 | DAF | factor B | factor D | factor H | factor I | MCP

FURTHER INFORMATION

Piet Gros's homepage: http://www.crystal.chem.uu.nl/group-gros/

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