Translational mini-review series on complement factor H: Structural and functional correlations for factor H

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Translational Mini-Review Series on Complement Factor H: Structural and functional correlations for factor H

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Summary

The 155-kDa glycoprotein, complement factor H (CFH), is a regulator of complement activation that is abundant in human plasma. Three-dimensional structures of over half the 20 complement control protein (CCP) modules in CFH have been solved in the context of single-, double- and triple-module segments. Proven binding sites for C3b occupy the N and C termini of this elongated molecule and may be brought together by a bend in CFH mediated by its central CCP modules. The C-terminal CCP 20 is key to the ability of the molecule to adhere to polyanionic markers on self-surfaces where CFH acts to regulate amplification of the alternative pathway of complement. The surface patch on CCP 20 that binds to model glycosaminoglycans has been mapped using nuclear magnetic resonance (NMR), as has a second glycosaminoglycan-binding patch on CCP 7. These patches include many of the residue positions at which sequence variations have been linked to three complement-mediated disorders: dense deposit disease, age-related macular degeneration and atypical haemolytic uraemic syndrome. In one plausible model, CCP 20 anchors CFH to self-surfaces via a C3b/polyanion composite binding site, CCP 7 acts as a 'proof-reader' to help discriminate self- from non-self patterns of sulphation, and CCPs 1–4 disrupt C3/C5 convertase formation and stability.

Keywords: age-related macular degeneration, atypical haemolytic uraemic syndrome, complement, glycosaminoglycans, NMR

Introduction

Factor H is a 155-kDa soluble glycoprotein regulator of the complement system. It is abundant in plasma and can associate with host cell membranes and other self-surfaces via recognition of polyanions such as glycosaminoglycans (GAGs) and sialic acid [1]. Through intervention at the level of the alternative-pathway C3 and C5 convertase enzymes it modulates both fluid-phase and surface-associated complement amplification. Factor H works in several ways [2]: it competes with factor B for binding to C3b, thus impeding formation of alternative-pathway C3 convertases (C3bBb); when bimolecular convertase complexes do succeed in assembling, CFH accelerates their subsequent dissociation (decay); CFH also accelerates decay of the alternative-pathway C5 convertase ($C3b_2Bb$); and CFH is a co-factor for factor I-mediated proteolytic cleavage of C3b to iC3b. As well as having binding sites for C3b, polyanions, and factor I, CFH reportedly binds to C-reactive protein (CRP) [3], fibromodulin [4] and adrenomedullin [5]. Finally, it is bound by proteins borne on microorganisms that surface-sequester CFH to evade complement [6].

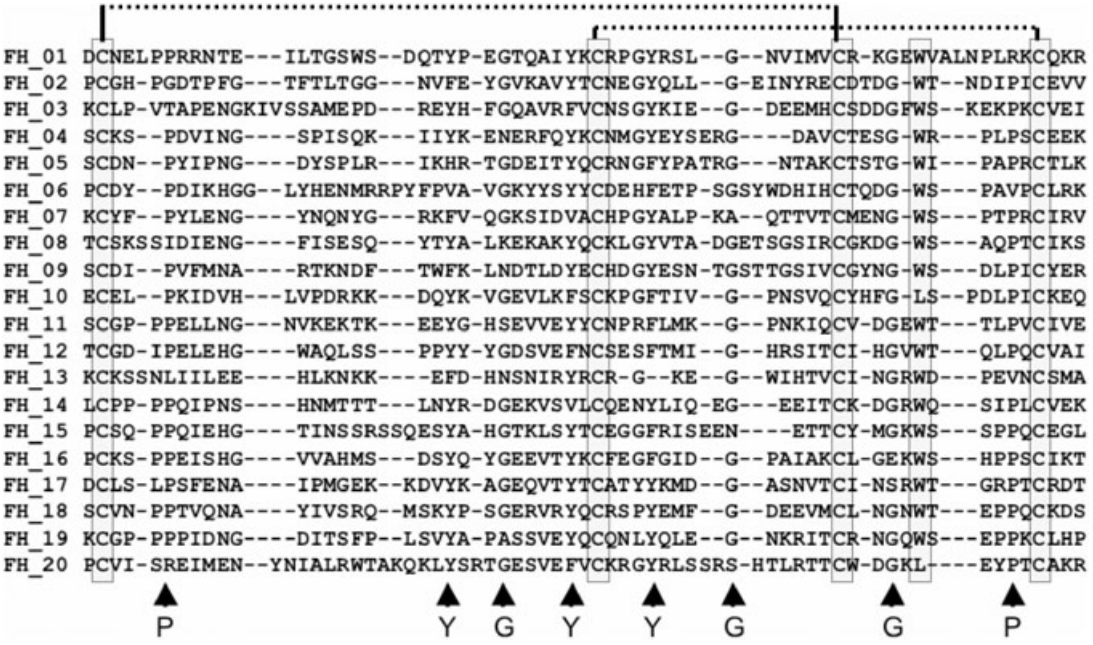

Fig. 1. Multiple sequence alignment of 20 short consensus repeats in sequence of complement factor H (CFH). One-letter codes used throughout; invariant Cys residues and almost invariant Trp residue are highlighted. Arrows indicate other well-conserved residues. Each short consensus repeat (SCR) probably folds into a complement control protein (CCP) module; horizontal lines indicate disulphides within CCP module.

The structure of factor H

The 1213 amino acid residues of mature CFH (155 kDa) [7,8] consist of 20 short consensus repeats (SCRs), each of ~60 residues [9]. A multiple alignment of the 20 SCRs (Fig. 1) highlights four invariant Cys residues and a near-invariant Trp residue between Cys(III) and (IV). This consensus sequence also occurs multiple times in other members of the regulators of complement activation (RCA) family [10], including C4b-binding protein (C4 BP), complement receptor type 1 (CD35), decay accelerating factor (CD55), membrane co-factor protein (CD46) and a set of CFH-related proteins. Within CFH, 'linkers' of between three and eight residues lie between Cys(IV) (last residue) of one SCR and Cys(I) (first residue) of the next SCR. Each of the 20 SCRs (plus one or two residues within the linkers at either end) is presumed to fold into a distinct three-dimensional (3D) structure termed the complement control protein module (CCP) [11], stabilized by Cys(I)–Cys(III), Cys(II)–Cys(IV) disulphide linkages.

Structures rich in β-sheet were predicted originally for SCRs/CCPs based on sequence analysis [12,13]. While circular dichroism spectra of CFH were uninterpretable (due to contributions from the 40 putative disulphide bonds) [14], Fourier-transformed infrared spectroscopy [15] confirmed extensive β-sheet in CFH and a paucity of α-helices. Infrared spectroscopy also demonstrated rapid exchange of backbone amide protons with solvent, indicative of an elongated structure for CFH in which the multiple CCPs do not, in general, contribute to a common globular arrangement. This inference of an extended structure concurs with electron microscopy, small-angle X-ray scattering and analytical ultracentrifugation studies of C4 BP, CFH and other RCAs [16–19]. Currently, a 'folded-back' model for CFH is favoured, bringing together its N- and C-terminal regions [20].

There are 3D structures currently available for 11 of the 20 CFH CCPs that have been expressed recombinantly as single, double or triple modules [21–28] (and unpublished data); and reliable homology-based models have been produced for several others [29]. Each CCP has an ovoid structure (Fig. 2), of dimensions approximately 40 Å by 15 Å by 10 Å.

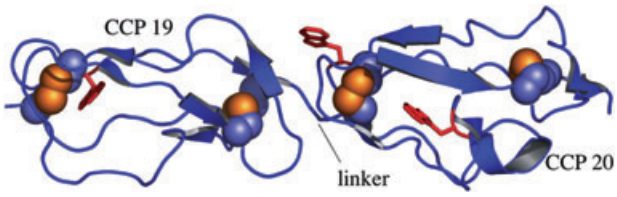

Fig. 2. Cartoon showing three-dimensional structure of the complement control protein (CCP) module pair, complement factor H (CFH) 19–20. Cys side-chains (green and orange) drawn [*PyMol* (Warren L. DeLano, 'The PyMOL Molecular Graphics System', DeLano Scientific LLC, San Carlos, CA, USA. http://www.pymol.org)] in space-fill representation; the three Trp side-chains (red) present are drawn in stick representation. Module 19 is typical of CCP modules; CCP 20 (lacking the consensus Trp) is less elongated than CCP 19.

Five extended stretches of residues (that often form β-strands and small antiparallel β-sheets) run back or forth in a direction that is approximately parallel with the long axis of the CCP. Thus the module's N and C termini occupy opposite poles consistent with a 'head-to-tail' arrangement of adjacent modules. Indeed, 3D structures of the module pair consisting of CCPs 15 and 16 (i.e. CFH 15–16) [24], and of the triple-module CFH 6–8 [28], are elongated with small intermodular buried surface areas. Bulges or loops, corresponding to insertions and areas of low sequence conservation, project laterally from the body of the module potentially contributing to binding specificity. Because stabilizing interactions between neighbouring modules are limited, intermodular flexibility is possible. The degree of overall flexibility of CFH is unknown, as are the extents of any conformational changes upon interaction with binding partners.

Binding sites of factor H for C3b

The N-linked glycans of CFH are dispensable for complement regulation [30], although whether they modulate interactions electrostatically between CFH and surface-borne polyanions requires further investigation. Early mapping of functional sites to specific CFH CCP modules focused on a 38-kDa tryptic N-terminal fragment, with fluid-phase C3b-binding and co-factor activity [31,32], corresponding to CCPs 1–5 plus part of CCP 6. A 42-kDa CFH splice variant (CFH-like 1) containing CCPs 1–7 is similarly able to regulate fluid-phase complement [33]. To pinpoint key functional modules, fluid-phase co-factor activity was measured for (non-purified) module-deletion and truncation mutants of the 38-kDa fragment expressed recombinantly and secreted from Chinese hamster ovary cells [34] (see Fig. 3). The results imply that the four N-terminal CCPs are required for full co-factor activity in the fluid phase, although CFH 1–3 (and to a lesser extent CFH 2–4) retained residual activity. A subsequent study [35] (involving constructs prepared in a baculovirus expression vector) largely reinforced these findings: while the CFH 1–4 construct displayed full fluid-phase co-factor activity the triple-module constructs CFH 1–3, CFH 2–4, CFH 1–2, 4 and CFH 1, 3–4 lacked it, as did the four-module construct CFH 1, 6–7, 4 (where CCPs 6 and 7 replace CCPs 2 and 3), and CFH 1–4 with non-native linker lengths. Subsequently, CCPs 1–4 were also shown to be required for the decay accelerating activity of this molecule, although full-length CFH is apparently ~100-fold more potent than CFH 1–4 or CFH-like 1 in this respect. In summary [43], the N-terminal four CCPs of CFH are necessary and sufficient to engage with C3b and C3 convertase in the fluid phase and thereby regulate amplification of the cascade via the alternative pathway. Interestingly, patients with an amino acid residue deletion in CCP 4 developed dense deposit disease [44], a renal pathology also seen in factor H-deficient humans, pigs and mice [45].

Further C3b-binding sites were identified using immunoaffinity-purified module-deletion CFH mutants from a baculovirus expression vector [41]. Constructs lacked (Δ) modules: 2; 5; 1–5; 6–10; 11–15; 16–20; 1–10; or 11–20. All these deletion mutants exhibited C3b-co-factor activity except CFHΔ2, CFHΔ1–5 and CFHΔ1–10. Crucially, CFHΔ1–5 (and CFHΔ2) none the less retained some binding affinity for cell-surface (sheep erythrocyte)-bound C3b (csbC3b), demonstrating that CFH CCPs other than modules 1–4 bind C3b. Deletions of CCPs 16–20 decimated affinity for csbC3b, thus implicating a C-terminal region of CFH as a second C3b-binding site. A third C3b-binding site was suggested because CFHΔ6–10 exhibited a decreased affinity for csbC3b, similar to that of CFHΔ1–5.

An antibody (131X) specific for CCPs 8–15 weakened interactions of full-length CFH with csbC3b [46], suggesting that a third C3b-binding region lies in these CCPs. Subsequently Jokiranta *et al.* [36] investigated binding of purified CFH constructs, cloned in a baculovirus system, to C3b, and its fragments C3c and C3d, attached to Biacore 'CM5'-chips. In these surface plasmon resonance (SPR) experiments both CFH 1–6 and CFH 19–20 associated with immobilized C3b, confirming the presence of independent C3b-binding sites near both the N and C termini of CFH. While CFH 8–20 bound immobilized C3b, CFH 8–11 and CFH 15–18 did not [36]. Hence this study could not confirm directly the existence of a third site, in the central segment of CFH, able to bind C3b independently. On the other hand, no CFH 12–14 construct was tested, thus it remains possible that one or more of these three CCPs contribute to a putative third C3b-recognition region. According to the same study [36] (Fig. 3) CFH 1–6 bound to immobilized C3b but not to immobilized C3c or C3d (C3c and C3d are non-overlapping proteolytic cleavage fragments of C3b; C3d corresponds to the thioester domain of C3b), while CFH 19–20 recognized C3d in addition to C3b but not C3c [and the C-terminal C3b(C3d)-binding site was mapped subsequently to CCP 20] [40]. Intriguingly, CFH 8–20 bound to both C3c and C3d, suggesting [36] that the inferred, third, C3b-binding site is specific for C3c. A three-module, CCPs 10–12, C3b(C3c)-binding site would explain most of these results, i.e. interference by the131X antibody, the loss of C3b-affinity by CFH Δ6–10 (but not the full activity displayed by CFH Δ11–15) and the results obtained with CFH 8–11, CFH 15–18 and CFH 8–20; this hypothesis requires testing with the appropriate constructs. An alternative explanation consistent with the evidence is that measurable affinity for C3c requires simultaneous engagement of two subsites, one within CCP 10 and the other within modules 16–18. In summary, two distinct binding sites for C3b lie at the N (CCPs 1–4) and C termini (CCP 20) of CFH; the latter is also able to bind C3d. Intervening modules participate in the binding process, but evidence for a distinct, third, C3b (C3c)-binding site remains circumstantial.

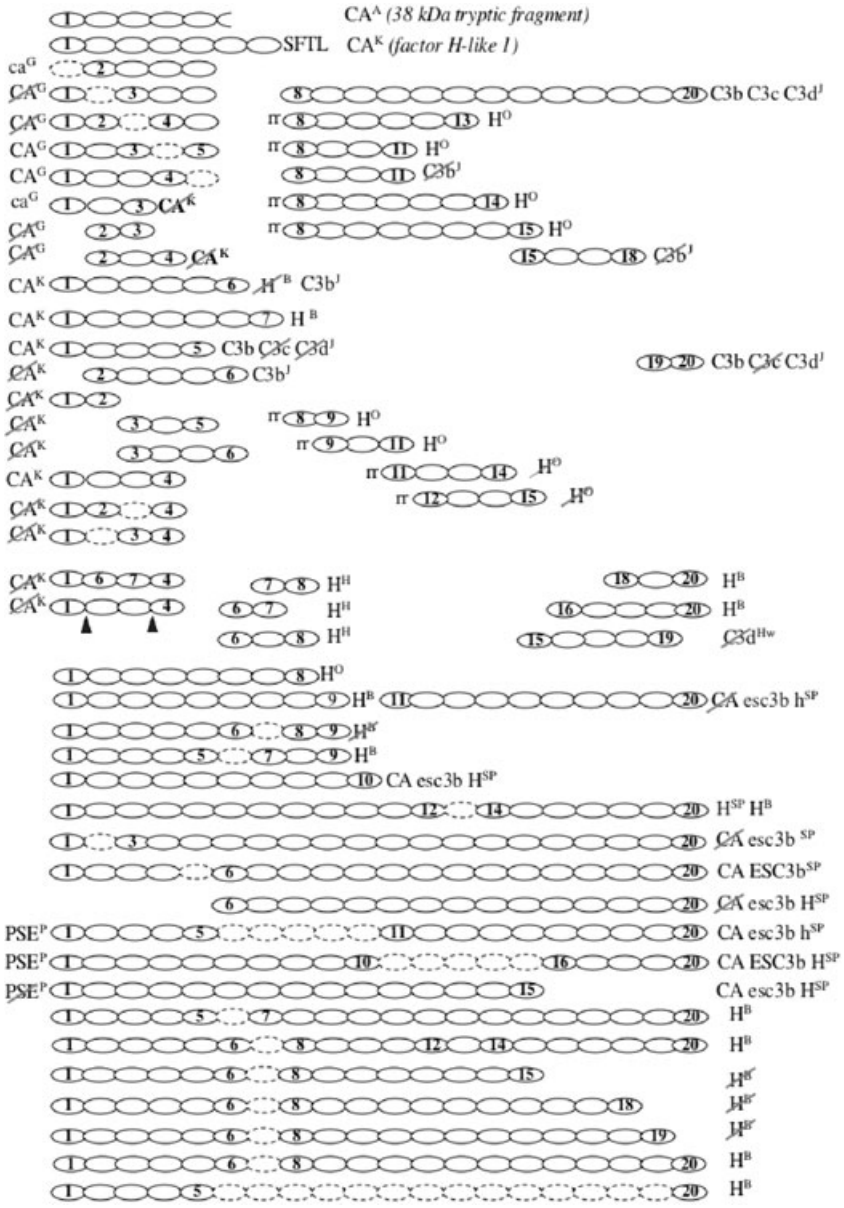

Fig. 3. Summary of module-deletion/truncations of complement factor H (CFH). Complement control protein (CCP) modules are shown as ovals within a cartoon-type representation of each deletion/truncation mutant. The black triangles indicate non-native linker lengths in one mutant. 'CA' written alongside indicates it has co-factor activity; similarly 'H', 'C3b', 'C3c', etc. indicate the mutant protein has binding affinity for heparin-affinity resin, C3b, C3c, etc. (ESC3b signifies sheep erythrocyte-bound C3b, as opposed to fluid-phase or chemically immobilized C3b); 'PSE': protects sheep erythrocytes from complement-mediated lysis. A strike-through means a particular activity was investigated but found not to be measurable; lower-case letters signify substantial reduction in measured activity; SFTL, the four residues specific to the C-terminus of CFH-like 1; 'rr', non-native N-terminal sequence containing two Arg residues. Superscripts refer to cited works as follows: A, Alsenz *et al.* [31,32]; G, Gordon *et al.* [34]; K, Kuhn [35]; J, Jokiranta *et al.* [36]; O, Ormsby *et al.* [37]; B, Blackmore *et al.* [38,39]; H, Herbert *et al.* [27]; HW, Hellwage *et al.* [40]; SP, Sharma and Pangburn [41]; and P, Pangburn [42].

Binding sites of factor H for polyanions

Factor H binds to non-complement–activating surfaces through interactions with polyanions. This is fundamental to its ability to regulate complement on surfaces [1]. In early studies, CFH CCP 13 (which is highly basic) and CCP 14 were implicated using a photoaffinity-tagging heparin analogue [47]. However, experiments on CFHΔ13 and CFHΔ11–15 [41] indicated that deletion of CCP 13 from CFH results in only very slightly reduced ability to bind a heparin-agarose column (and negligible loss of binding to C3b-coated sheep erythrocytes). On the other hand, CFHΔ6–10 showed significantly weaker heparin affinity [41] implying a stronger GAG/sialic acid-binding site exists in the 6–10 region. A prominent role for CCP 7 in GAG binding was confirmed subsequently because CFH 1–6, one of a series of constructs generated in CHO cells [38] (Fig. 3), barely bound heparin while CFH 1–7 was a good heparin-binder. Furthermore CFH 1–6, 8–9 (i.e. a module 7-deletion of CFH 1–9) lost all affinity for heparin [38]. Latterly, constructs of CFH 6–8 were shown to bind GAGs and GAG analogues [27,48].

Interestingly, CFHΔ7 (and CFHΔ7Δ13) bound heparin almost equally as well as CFH, so module 7 is not the only one that binds polyanions [38]. That CFHΔ6–10, unlike CFHΔ7 or CFHΔ7Δ13, eluted from a heparin-affinity column at relatively low salt [41], suggests that modules 6, 8, 9 or 10 also participate in heparin binding. Some of these modules could contribute to the same heparin-binding site as CCP 7 or they could form a distinct, third site. A more recent study of constructs CFH 8–9, CFH 9–11 and CFH 11–14 seems to support the notion of a third site centred on

module 9; CCPs 8–9 bind most strongly, followed by CCPs 9–11, while CCPs 11–14 did not bind heparin [37]. It is worth noting that an artificial sequence containing two arginine residues (EFTWPSRPSRIGT) was apparently included at the N terminus (part of the cloning procedure) of CFH 8–9 and CFH 9–11; in combination with a native lysine residue prior to the Cys(I) of CCPs 8 or 9, this introduces a potential heparin-interacting artefact. Indeed, in a previous study CFH 1–6, 8–9 (i.e. a construct containing both CCPs 8 and 9 but lacking CCP 7) had been shown not to bind heparin [39].

In fact, the evidence suggests CCP 20 is the primary heparin-binding determinant in CFHΔ7. The CFHΔ7Δ20 construct eluted from a heparin-affinity column at low salt while CFH 18–20 bound relatively tightly [39]. Moreover, non-heparin-binding CFH 1–5 was converted to heparin-binding CFH 1–5, 20 by inclusion of CCP 20 in the construct [39]. Human CFH from an individual with a mutation (CFH-E1172Stop) resulting in a lack of module 20 bound weakly to a heparin-affinity column [49]. Highly purified, structurally characterized CFH 19–20 [25] bound well to a heparin-agarose column. In summary, while GAG-binding sites in module 7 (with contributions from CCPs 6 and 8) and module 20 (with possible contributions from CCP 19) are well established, current evidence for involvement of either CCPs 9 or 13 is inconclusive.

C terminus of factor H recognizes C3b in the context of the self-surface

The importance of the C-terminal heparin-binding site for self *versus* non-self discrimination was shown by experiments on CFHΔ6–10, CFHΔ11–15 and CFH 1–15. Of these three constructs, only CFH 1–15 could not protect sheep erythrocytes against lysis by human complement (Fig. 3) [42]. In a dramatic illustration of the role played by CCPs 19 and 20, Ferreira *et al.* [50] showed that purified, *Pichia pastoris*-produced, CFH 19–20 competitively inhibited the action of CFH on cell surfaces. This double-module construct overcame the protective effects of full-length CFH and thereby promoted aggressive complement-mediated lysis of sheep erythrocytes. Further support for a dominant role of the CFH C terminus is provided by the ability of monoclonal CCP 20-specific antibodies [51] to block interactions of CFH with endothelial cells. Thus this C-terminal polyanion- and C3b-binding site is critical for the ability of CFH to recognize and protect host cells bearing sialic acids and GAGs.

Besides disrupting the protection of normally non-activating surfaces by full-length CFH, CFH 19–20 completely abolishes CFH binding to immobilized C3b (but not to fluid-phase C3b) [50], despite the presence of the C3b-binding site in CCPs 1–4. Moreover [51], CCP 20-specific antibodies blocked CFH binding, in an enzyme-linked immunosorbent assay (ELISA), to C3b and C3d as well as to heparin (and to endothelial cells, as mentioned above). In order to reconcile these intriguing results with the multiple C3b- and GAG-binding sites identified by module-deletions and CFH truncations, two models were proposed: (i) the C terminus is unique among C3b-binding sites of CFH in having a high affinity for cell surface-bound (csb) C3b (as opposed to fluid-phase C3b or C3b immobilized artificially on a chip or microtitre plate). The other C3b-binding site (in CFH 1–4) has only poor affinity for C3b after the activated C3 fragment has become attached to a surface; this CFH 1–4 site requires initial anchoring of CFH via the C terminus before it can engage, to a significant extent, with its binding site on csbC3b. Thus CFH 19–20 competes with the sole csbC3b-binding site in full-length CFH for binding to csbC3b. Because CFH 19–20 has no complement regulatory region associated with it, the csbC3b to which it is bound is not destroyed by factor I. Similarly, if the csbC3b-binding site of CFH is blocked by an antibody then CFH will not be able to bind to csbC3b. In the fluid phase, binding of CFH 19–20 to C3b is functionally irrelevant as the CFH N-terminal modules bind well elsewhere on fluid-phase C3b and this latter interaction is sufficient for co-factor activity; hence – in agreement with experimental evidence – CFH 19, 20 does not inhibit fluid-phase co-factor activity. The C terminus probably recognizes a composite site consisting of both GAGs and C3b; note that the C-terminal C3b-binding site is the only one that also binds GAGs. (ii) According to an alternative, or supplementary, model, the other CFH C3b-binding site (in CCPs 1–4) is cryptic, only becoming available following occupation of the C-terminal site by C3b. The C-terminal site binds initially to the thioester domain of C3b inducing a conformational change within CFH. For example, the binding site in CCPs 1–4 could be occluded initially by interactions with other CCPs in a compact conformation of CFH. This notion of proximity between N- and C-terminal modules tallies with SPR experiments showing that CFH 1–7 binds full-length CFH on a Biacore chip [51] and with low-resolution structural studies discussed earlier. A problem with this notion of a cryptic site is that it predicts that fluid-phase C3b, in the presence of full-length CFH and an excess of CFH 19–20, will not be cleaved by factor I; this is because the CFH 19–20 site on C3b would not be available for binding by CFH. In fact, CFH 19–20 does not inhibit the co-factor activity of CFH in the fluid phase. It therefore remains necessary to invoke structural or accessibility differences between fluid-phase and csbC3b, as in model (i).

Mutations in CFH linked to atypical haemolytic uraemic syndrome

Further support for a key role of the CFH C terminus in complement regulation *in vivo* comes from studies of atypical haemolytic uraemic syndrome (aHUS) [52] (described by Rodriguez de Cordoba and Goicoechea de Jorge in this issue). The majority of aHUS-linked CFH mutations occur

towards the C terminus, with CCP 20 being a hotspot [53]. Strikingly, a mouse model of aHUS was generated in CFH-knock-out mice (that develop a different renal pathology, dense deposit disease [45]) by transgenic expression of CFH 1–15 [54] (for further details see review by Pickering and Cook in this issue). It was hypothesized that a predisposition to aHUS is linked directly to an inability of mutant forms of CFH either to bind properly to C3b(C3d) or to recognize polyanionic markers on non-activating surfaces (or a diminishment of both these roles). This hypothesis has been tested by mutagenesis and structural studies.

Table 1 lists laboratory-generated and naturally occurring CFH mutants containing sequence changes within CCPs 19 and 20, all of which have been tested for function. Some were expressed recombinantly within the contexts of partial versions of CFH such as CFH 19–20 or CFH 8–20, hence there is variation in the extent to which other binding sites within the protein can contribute to the functional outcome. Some changes coincide with aHUS-linked mutations while others were designed to identify residues participating in functional sites.

All mutated proteins for which C3b(C3d)-binding data are reported exhibit decreased affinity; K1186A, which is not aHUS-linked, is the exception. Most mutants display loss or reduction in affinity for heparin-affinity resin and for human umbilical vein endothelial cells (exceptions are R1182A, K1186A, S1191L and V1197A). Thus all the aHUS-linked mutants tested for function exhibited a deficiency in binding to either C3b(C3d) or GAGs, and in several cases to both.

Pathophysiological insights based solely on the data in Table 1 are limited for several reasons. (i) No distinction was made between perturbation of a specific binding site and widespread structural disruption of the module. (ii) Contradictory results were obtained, e.g. W1183L in the context of CFH 19–20 dimerized [as judged by mobility on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)] and the dimer binds tightly to a heparin-affinity column; yet W1183L in the context of CFH 8–20 binds more weakly to heparin-affinity resin than the native-sequence 8–20 construct. (iii) Glycosaminoglycans are diverse; their levels and patterns of sulphation, for example, vary between tissue-type and over the course of development and ageing [61]. This raises a critical issue that has repercussions for the organ-limited nature of particular complement-associated diseases: can the ability of CFH to protect host surfaces be attributed to specific GAG-recognition processes [62]? Unfortunately, heparin-affinity chromatography is a crude probe of the capacity to recognize specific surface polyanions. Thus, unfortunately, it is difficult to test directly the hypothesis that mutations in CFH predispose to aHUS if they disrupt regions of CFH involved in recognition of specific GAG-sulphation patterns in the glomerulus (for example).

In an attempt to deal with some of these issues, the 3D structural basis of GAG recognition by CCPs 19 and 20 was investigated using nuclear megnetic resonance (NMR) [25] and crystallography [26]. The two modules are organized in the linear, end-to-end arrangement observed in other structures of CCP-module pairs. A model GAG compound – pure, fully sulphated heparin tetrasaccharide (dp4), enzymatically cleaved from heparin – was titrated into the CFH 19, 20 sample and the NMR frequencies, or chemical shifts, of protein backbone atoms were monitored for perturbations. The presence of bound dp4 induces changes in the magnetic field experienced by nearby nuclei and thus amino acid residues in or near the binding site will resonate with slightly different chemical shifts. A surface patch of CFH 19, 20 residues was thus implicated in binding to dp4. Strikingly, these coincided well with aHUS-linked mutations [25]. For example, R1182, W1183, T1184, E1198 and R1215 (see Fig. 4) showed significant chemical shift perturbations. Thus, these experiments support a disease model in which dysfunctional CFH fails to protect fully the GAG-rich layers of the glomerular basement menbrane from complement activation.

A credible, theoretical docking exercise [25] placed C3d on the reverse face, relative to the GAG-recognition site, of CFH 20 (i.e. on the back-face as viewed in Fig. 4). None the less, in reality, C3d (and C3b) binding might be disrupted by aHUS-linked mutations, as suggested by Jokiranta *et al.* [26]. To investigate this possibility, the location of residues listed in Table 1 was mapped onto the 3D structure (Fig. 4). The role of R1210 is difficult to assess from the data because of the disulphide-forming potential of R1210C, but its location on the back-face (Fig. 4) is consistent with the putative location of the C3d-binding site based on the outcome of the docking studies. On the other hand, five side-chains, forming a band across the front face of CCP 20 (Fig. 4), were also implicated in C3d binding according to mutagenesis studies. Four of these residues are implicated additionally in binding to a heparin-affinity column, and also appear in the list of significantly perturbed chemical shifts obtained from dp4 NMR-titration experiments. One possible explanation for these apparently confusing results is that CFH 19–20 has GAG- and C3d(C3b)-binding sites that are sufficiently close to interfere or co-operate with one another depending on the nature of the ligand tested (e.g. heparin *versus* a physiological GAG and C3b *versus* C3d) and the order of binding events. Thus heparin inhibits binding of C3d to CFH 15–20 while C3d enhances binding of native-sequence CFH 15–20 to heparin [49]. These observations are not inconsistent with the intuitively feasible hypothesis that a tertiary complex containing GAGs, C3b and CFH forms during the process of complement regulation on self-surfaces. A caveat to interpretation of these studies is that electrostatic 'steering interactions' (as probed here by mutating Arg and Lys residues to uncharged residues, or potentially neutralizing their side-chains by addition of a polyanion such as heparin) may represent just one step in the multi-step process whereby C3d interacts with CCPs 19–20. Electrostatic steering [63] could enhance the number of productive encounters

Table 1. Mutations in complement control proteins (CCPs) 19 and 20 of complement factor H (fH).

Mutation	Source of protein	aHUS link?	CA?	Binding to		
				C3b(C3d)	GAG	HUVEC
W1157R	In context of fH 8–20 (Baculovirus) (Jz 2006)	Yes	NR	<< to C3b/C3d in CPA; none by SPR;	Slight reduction in heparin binding	Slightly reduced
E1172-stop	Purified from heterozygous patient (M 2003; J 2005)	Yes	NR	<< to C3b by SPR	Slightly weaker than WT	No
R1182A	In context of fH 19–20 (*Pichia pastoris*) (J 2006)	Resembles R1182S	NR	< to C3d in CPA; by SPR, sl. < C3b, < C3d	Binds to heparin-affinity column	NR
W1183L	Ex heterozygous patient (and ex COS cells) (S-C 2002,4)	Yes	Yes*	< to C3b in CPA	NR	NR
W1183L	In context of fH 8–20 (Baculovirus) (Jz 2006)	Yes	NR	< to C3b and C3d (SPR); < to C3b/C3d in CPA	Binds weakly to heparin column	Weak
W1183L	In context of fH 19–20 (*P. pastoris*); note – dimer on SDS-PAGE (J 2006)	Yes	NR	< to C3d in CPA < to C3d; anomalous result for C3b by SPR	Dimer binds more tightly than native fH 19–20	NR
K1186A	In context of fH 19–20 (*Pichia pastoris*) (J 2006)	No	NR	Full binding to C3b in CPA; SPR NR	Similar heparin affinity to fH 19–20	NR
K1188A	In context of fH 19–20 (*Pichia pastoris*) (J 2006)	No	NR	< to C3d in CPA; < to C3b and C3d by SPR	sl < binding than fH 19–20 to hep.	NR
S1191L	Ex heterozygous patient (H2006)	Yes	*	NR	Full binding to heparin column	NR
S1191L, V1197A	Ex heterozyg. patient (and in fH 18–20 context) (H 2006)	Yes	*	< to C3b and C3d by SPR	Full binding to heparin column	NR
V1197A	Ex heterozyg. and also ex hemizyg. patients (H 2006)	Yes	*	NR	Full binding to heparin column	NR
V1197A	Ex homozygous patient (and ex COS cells) (S-C 2002,4)	Yes	Yes*	< to C3b in CPA	NR	NR
V1197A	In context of fH 8–20 (Baculovirus) (Jz 2006)	Yes	NR	< to C3b/C3d-coated plates, and by SPR	Binds weakly to heparin column	Weak
E1198A	In context of fH 19–20 (*Pichia pastoris*) (J 2006)	Yes	NR	< to C3d in CPA SPR: sl < to C3b, spurious C3d	> to hep. column cf fH 19–20	NR
E1198K	Ex heterozygous patient (V-S 2006)	Yes	*	NR	NR	Weak
R1203E, R1206E, R1210S, K1230S, R1231A	In context of fH 15–20 (*P. pastoris*) (HW 2005)	R1210S Resembles R1210C	NR	Significantly < to C3d and C3b (by SPR)	No binding to heparin column	No
R1203E, R1206E, R1210S	In context of fH 15–20 (*P. pastoris*) (J 2005)	See above	NR	NR	No binding to heparin column	No
R1210C	Ex heterozyg. patient (di-S with other proteins) (& ex COS cells) (S-C 2002, 2004)	Yes	Yes*	< to C3b in CPA	NR	NR
R1210C	Ex heterozyg. patient and in context of fH 8–20 (Baculovirus) (M 2003; Jz 2006)	Yes	NR	< to C3b/C3d-coated plates; < to C3d by SPR	Binds weakly to heparin column	Weak
R1215G	In context of fH 8–20 (Baculovirus) (M 2003)	Yes	NR	Significantly < binding to C3d by SPR	Binds weakly to heparin column	Weak
P1226S	In context of fH 8–20 (Baculovirus) (Jz 2006)	Yes	NR	Not to C3b/C3d-coated plates; none, by SPR	Binds weakly to heparin column	NR
K1230S, R1231A	In context of fH 15–20 (*P. pastoris*) (J 2005)	No	NR	NR	Binds hep. column equal to fH 15–20	Weak

aHUS: atypical haemolytic uraemic syndrome; GAG, glycosaminoglycan; HUVEC, human umbilical vein endothelial cell; NR, not reported; CPA, coated plate assay; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SPR, surface plasmon resonance; sl, slight; hep, heparin; ex, from; <, reduced binding; CA, co-factor activity; *reduced ability to protect sheep erythrocytes from complement-mediated haemolysis. Superscripts refer to cited work as follows: Jz 2006, Jozsci *et al.* [55]; M 2003, Manuelian *et al.* [56]; J 2005, Jokiranta *et al.* 2005 [49]; J 2006, Jokiranta *et al.* 2006 [26]; S-C 2002, 2004, [57,58]; H 2006, Heinen *et al.* [59]; V-S 2006, Vaziri-Sani *et al.* [60].

between a pair of protein molecules by influencing their orientations as they approach one another, but the eventual complex could be stabilized by other interactions involving an entirely different set of side-chains. Another factor that complicates interpretation is the putative oligomerization of CFH that could be important and may occur via the C-terminus [26]. Thus despite much recent progress, in the absence of detailed thermodynamic studies of binding and 3D structures of binary and tertiary complexes, the picture remains obscured.

SNPs in CFH linked to age-related macular degeneration

Patients suffering from dense deposit disease, a rare but serious form of glomerulonephritis, often develop the soft ocular drusen regarded as a hallmark of early age-related macular degeneration [64] (AMD), which is a leading cause of geriatric vision-loss. The recently discovered links between the Y/H 402 polymorphism in CCP 7 of CFH and both dense deposit disease [65] and AMD [66] hints at the physiological importance of this second (after CCP 20) polyanion-binding site in CFH. The at-risk sequence variation is also present in CFH-like 1, where module 7 represents the sole polyanion binding site. This GAG-binding module has been reported additionally to participate in binding sites for CRP [67], fibromodulin [68], DNA [68] and various pathogen-borne proteins [67,69,70].

Structural and chemical shift perturbation studies of CCP 7 – similar to those carried out on CFH 19–20 – revealed that the H/Y 402 side-chain is positioned in order to contribute specificity to a GAG-binding groove [27]. Working with the single module, Y402 CCP 7 bound significantly more strongly than H402 CCP 7 both to a heparin-affinity column and to dp4. This appears to support the case for a causal link between the polymorphism and a mechanism for AMD involving insufficient complement regulation in the ageing choroid. When examined in the context of the triple-module CFH 6–8, however, the two variants bound equally well to some GAGs [27] but differently to others [48], with either variant binding more tightly depending upon the GAG tested. Both Y402 and H402 versions of full-length CFH bound equally well to a series of fully sulphated heparin fragments and (as might have been predicted from the aforementioned results obtained for CFHΔ7) to a heparin-affinity column [27,68,71,72].

In all probability, what these results illustrate is that the CFH–GAG interaction is both dual-site (i.e. involves two physiologically relevant GAG-binding sites on CFH) and tissue-specific. The results obtained with isolated CCP 7 in complex with a chemically defined heparan sulphate analogue, together with the data for interaction of CFH 6–8 with a range of heterogeneous GAGs, indicate strongly that the disease-linked polymorphism is tweaking the GAG-(self)-recognition capabilities of CFH in a subtle fashion not apparent in the test tube with full-length CFH and the 'blunt instrument' of heparin. Such a conclusion is supported by the detailed picture of protein–sugar interactions provided by the crystal structure of CFH 6–8 in complex with sucrose octasulphate [28]. A subtle difference between the two allotypic variants is consistent with the H402 (at-risk) variant of CFH – present in 35% of Western populations – functioning adequately until at least old age. Indeed, the H402 variant is less tightly sequestered than the Y402 variant by the M6 protein of *Streptococcus pyogenes* and might confer an evolutionary advantage in this respect [72].

Consistent with a 'GAG hypothesis' for a causal link with AMD there is also evidence for differential binding of the

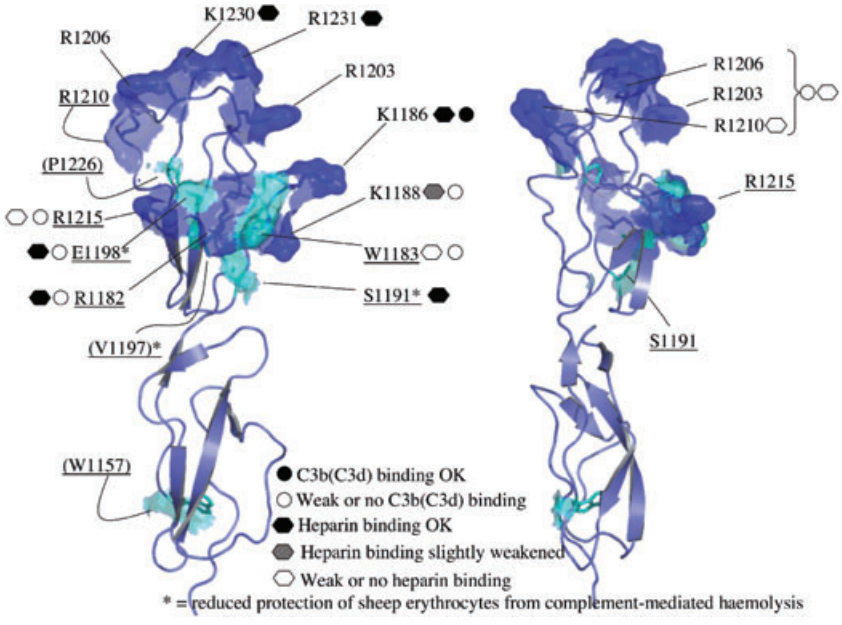

Fig. 4. Amino acid residue positions of mutations listed in Table 1 within the structure of complement factor H (CFH) 19, 20. The structure of CFH 19, 20 is drawn as a cartoon in PyMol (see legend to Fig. 2). Mutated side-chains are labelled (and in parentheses if they are likely to be structurally critical) and drawn and their exposed surfaces are rendered (dark blue if they are positively charged, otherwise cyan). Underlining indicates that mutations in the residue concerned are linked to atypical haemolytic uraemic syndrome. The effect on function – where measured – is indicated by symbols (see key), except for residues likely to be critical for structural integrity.

Y/H 402 variants to cell surfaces. Flow cytometry and confocal laser scanning microscopy revealed a slightly lower binding of the H402 variant to retinal pigment epithelial cells and to endothelial cells, and indeed (in the context of CFH-like 1) the H402 variant has reduced co-factor activity at the cell surface even though there is no difference in fluid-phase co-factor activity between the two variants [73]. In another flow cytometry study the H402 variant exhibited higher binding to necrotic Jurkat T cells, which may reflect a measured difference in DNA-affinity between the variants (with H402 CFH 6–8 binding to DNA more tightly than Y 402 CFH 6–8) [68].

Controversy surrounds the effects of the polymorphism on the affinity of CFH for CRP as measured by SPR or by ELISA. Five reports concur that the H402 allotypic variant (within the context of full-length CFH, CFH-like 1, CFH 5–7 or CFH 6–8) is the weaker CRP binder [27,68,71–73]. Previous work showed that CRP is more abundant in the eyes of AMD patients with the H402 variant of CFH than in those with the Y402 variant [74] and that CRP is present in drusen [68,74]. It has been suggested that CFH recognizes CRP borne on the membranes of apoptotic cells and acts to ensure that apoptotic cell clearance proceeds in a non-inflammatory setting [75]. Overlap has been reported for CRP- and GAG-binding sites on CCP 7 [67], suggesting that the relative affinities for these two ligands might be critical for CFH function at self-surfaces in situations where both ligands are encountered; if the Y/H 402 side-chain contributes to both sites it could be a key player in this respect. So a 'GAG hypothesis' and a 'CRP hypothesis' need not be mutually exclusive. However, a study by Hakobyan *et al.* [76] casts doubt on the relevance of the CFH–CRP interaction. In the hands of these authors, CFH interacts with CRP only under circumstances where the CRP pentamer is disrupted through Ca^{2+} ion-removal, a situation that would never prevail in physiological circumstances.

Conclusions

Taking all these data together, a mechanism for CFH is emerging. The two ends of CFH (modules 1–7 and modules 19–20) contain all the proven discrete binding sites and are also the sites of most disease-linked sequence variations. The modules joined by long linkers towards the centre of CFH (CCPs 12–14) allow CFH to kink so that these two ends are brought into proximity. The C-terminal modules are the only ones with the potential to bind to a composite site consisting of C3b and the polyanions on the self-surface to which C3b is attached, and hence are dominant in distinguishing self-surfaces from non-self (complement-activating) ones. The N-terminal three or four CCPs, like other similar blocks of CCPs in other RCAs (e.g. CD46, CD55, CD35) are able to perform the task of disrupting the surface-associated C3/C5 convertases once the CFH is anchored in place by its C-terminus. To position correctly the N-terminal modules in the GAG-convertase-CFH complex, a further interaction of CFH with GAGs is mediated by the specific recognition capabilities of CCP 7 – indeed, this module could act as a 'proof-reader' to make it more difficult for bacteria to emulate the chemistry at self-surfaces. Further understanding probably depends on more detailed binding studies of chemically pure GAG analogues combined with 3D structural studies of the protein–protein and protein–carbohydrate complexes.

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