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Functional Anatomy of Complement Factor H

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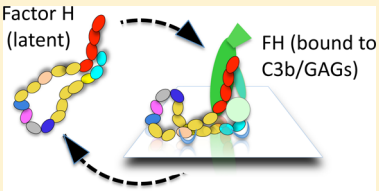
ABSTRACT: Factor H (FH) is a soluble regulator of the proteolytic cascade at the core of the evolutionarily ancient vertebrate complement system. Although FH consists of a single chain of similar protein modules, it has a demanding job description. Its chief role is to prevent complement-mediated injury to healthy host cells and tissues. This entails recognition of molecular patterns on host surfaces combined with control of one of nature's most dangerous examples of a positive-feedback loop. In this way, FH modulates, where and when needed, an amplification process that otherwise exponentially escalates the production of the pro-inflammatory, pro-phagocytic, and pro-cytolytic cleavage products of complement proteins C3 and C5. Mutations and single-nucleotide polymorphisms in the FH gene and autoantibodies against FH predispose individuals to diseases, including age-related macular degeneration, dense-deposit disease, and atypical hemolytic uremic syndrome. Moreover, deletions or variations of genes for FH-related proteins also influence the risk of disease. Numerous pathogens hijack FH and use it for self-defense. As reviewed herein, a molecular understanding of FH function is emerging. While its functional oligomeric status remains uncertain, progress has been achieved in characterizing its three-dimensional architecture and, to a lesser extent, its intermodular flexibility. Models are proposed, based on the reconciliation of older data with a wealth of recent evidence, in which a latent circulating form of FH is activated by its principal target, C3b tethered to a self-surface. Such models suggest hypotheses linking sequence variations to pathophysiology, but improved, more quantitative, functional assays and rigorous data analysis are required to test these ideas.



A network of ~40 plasma and cell-surface proteins, the human "complement system", collaboratively eliminates potentially dangerous cells, debris, and particles from the body. The complement system deploys a proteolytic cascade and a rare example of positive feedback to drive rapid cytocidal and inflammatory responses to episodes of microbial invasion. Remarkably, essentially the same molecular circuitry performs ongoing homeostatic roles not necessarily involving confrontations with infectious hazards.[1] Complement factor H (FH) plays a pivotal role in ensuring the complement system is regulated according to need. Improper functioning of the complement system is linked to infectious, inflammatory, and degenerative diseases.[2] The best-known example is age-related macular degeneration (AMD) associated with inadequate FH functional activity.

Three complement system-activating pathways ("alternative", "classical", and "lectin") converge at the proteolytic activation of the plasma protein, C3 (185 kDa). C3 cleavage entails destabilizing excision of its wedgelike 9 kDa ANA domain, yielding the anaphylatoxin C3a, and rearrangement of the remaining domains comprising C3b.[3,4] The C1r/C1s, Uegf, Bmp1 domain (CUB) and thioester-containing domain (TED) undergo dramatic movements (Figure 1) accompanied by activation and exposure of a previously buried thioester (linking Cys988 to Gln991 within the TED). This unstable group promotes rapid attachment of C3b, via residue 991, to any nearby nucleophile. This is typically solvent water or, importantly, a hydroxyl on a neighboring surface.

Covalent deposition of C3b on a surface has manifold consequences. Nascent binding sites arising from the domain rearrangements accompanying its formation allow C3b to serve as a platform for Mg^{+}-dependent binding of factor B (FB) and subsequent cleavage of FB by factor D (FD), yielding catalytically active C3b-bound Bb (Figure 1). The resultant C3b.Bb complex, labile unless stabilized by properdin, is a C3 convertase; it converts circulating C3 into additional, potentially surface-deposited, C3b in a positive-feedback loop. In this way, C3b can propagate exponentially, potentially creating an opsonic surface coating of C3b molecules that promotes clearance via erythrocyte-borne C3b receptors and phagocytosis. Accretion of a further C3b molecule by C3b.Bb

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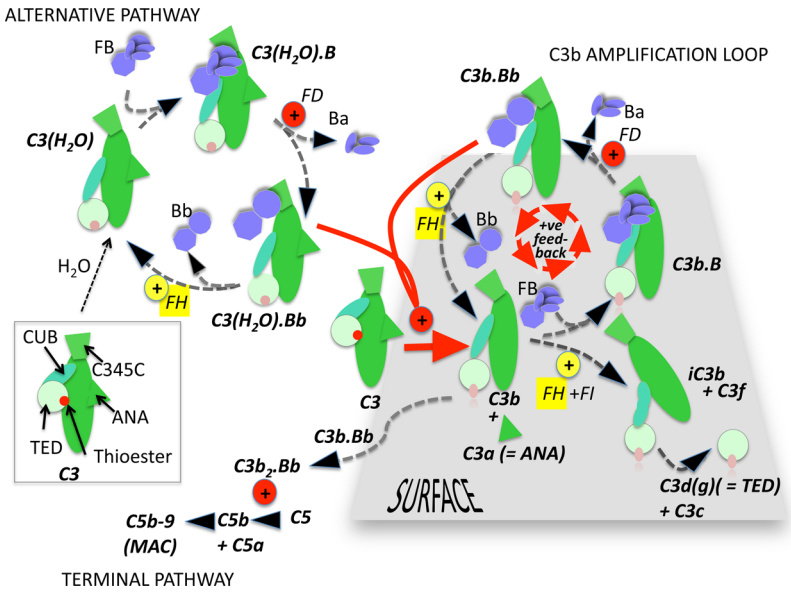

Figure 1. Complement activation. The inset shows the key mobile domains in C3. The left-hand cycle shows the formation of initiating C3 convertase, C3(H_2O).Bb. The right-hand cycle shows the C3b-amplifying loop driven by alternative pathway convertase, C3b.Bb. Both require proteolytic action of factor D (FD) on factor B (FB). The buried thioester in the thioester domain (TED) of C3 can undergo hydrolysis (the red circle turns pink). The reactive thioester exposed in C3b is readily hydrolyzed but can alternatively form an ester linkage (pink oval) with a surface. Factor H (FH) competes with FB for C3b binding, is the cofactor for factor I (FI), and accelerates C3b.Bb decay; it also regulates C3(H_2O).Bb. Consequences of C3b generation include C3a/C5a release, C5 convertase ($C3b_2$.Bb) formation, which triggers membrane attack complex (MAC) assembly, and generation by FI of iC3b and, if other cofactors present, C3dg and C3d that equates to an isolated surface-bound TED.

shifts Bb substrate specificity from C3 to its paralogue, C5. $C3b_2$.Bb splits C5 into the anaphylatoxic C5a and C5b that resembles C3b but lacks a thioester.

Nascent C5b may bind C6 forming a relatively stable complex that nucleates self-assembly (with C7, C8, and $C9_n$), in any local membrane lacking regulator CD59, of a transmembrane pore called the membrane-attack complex (MAC). Thus, uncontrolled C3b deposition causes opsonization, inflammation, and cytolysis. Subsequent proteolysis of C3b prevents further amplification and generates, sequentially, products iC3b, C3dg, and finally (equating to the TED) C3d that remain surface-tethered over the long term and are ligands for receptors promoting noninflammatory clearance and antibody-mediated and cellular immune responses.

Amplification of C3b is spatiotemporally localized because of the high reactivity of its thioester and the tendency of C3b.Bb to rapidly, irreversibly, dissociate. On the other hand, initial C3b deposition via ester formation, while varying in rate depending on the chemical nature of the target,[5] is promiscuous. Moreover, the alternative pathway ensures C3b is ubiquitous;[6] this pathway is initiated by rare, spontaneous, hydrolysis of the buried thioester within C3, generating small quantities of the C3b-like molecule C3(H_2O) (Figure 1). Like C3b, C3(H_2O) is a platform for FB binding and cleavage, yielding the initiating C3b-generating C3 convertase, C3(H_2O).Bb. Potentially then, every surface exposed to blood—healthy, hazardous, or foreign—is susceptible to rapid opsonization with C3b and, via C5b generation, to MAC formation. Activation via classical and lectin pathways is not spontaneous; binding of antibody–antigen complexes or C-reactive protein (CRP) to C1 triggers the classical pathway, while the lectin pathway is launched upon detection, by collectins, of pathogen-associated and damage-associated patterns of oligosaccharides.

FACTOR H INHIBITS C3B AMPLIFICATION ON SELF-SURFACES

Selective prevention of C3b amplification on self-surfaces is vital to avert injury to host tissues. The soluble glycoprotein, FH,[7,8] is a key player in this respect. It circulates in the blood and controls C3b amplification in the fluid phase, i.e., when nascent C3b is hydrolyzed, thus preventing consumption of C3 and FB. Additionally, and importantly, FH is a pattern-recognition protein that will act at any surface, including the extracellular matrix, which carries host-specific molecular signatures.[9] While homologues of FH, including DAF, CR1, and MCP, also play important roles in controlling C3b amplification, they are restricted to cell membranes.[10] On its target surfaces, FH[11] competes effectively (compared to its poor performance on foreign surfaces) with FB for binding to C3b (Figure 1). Once bound by C3b, FH recruits factor I (FI) that is thus activated and cleaves C3b to yield iC3b by destroying the CUB domain and excising the small fragment, C3f[12] (Figure 1). Furthermore, FH accelerates[7,13] the irreversible decay into C3b and Bb of already labile C3b.Bb. It similarly inhibits the initiating C3 convertase, C3(H_2O).Bb. Its potency is underlined by observations that many pathogens as well as some cancer cells avoid activating complement by sequestering FH,[14] and by links between polymorphisms or mutations in the *CFH* gene, or autoantibodies to FH, and disease (as has been reviewed elsewhere[2,15]). Interest in FH has paralleled a growing realization of the potential value of therapeutic intervention in the complement cascade; FH itself, or engineered variants, could be clinically useful.[2,16−21]

Factor H has the remarkable attribute of being effective only when and where control of C3 depletion and C3b amplification is required. How does it work? While much information about its mode of action has been reviewed previously,[22−24] many important questions remain unresolved. It is particularly intriguing that despite functional sophistication, FH has a

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"simple" construction being composed of a single chain of 20 domains or modules all of the same type (Figures 2 and 3).

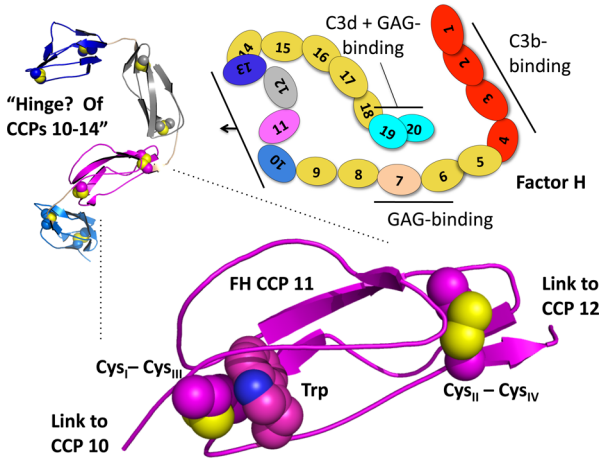

Figure 2. Modular architecture of FH. Numbered ovals represent its 20 CCP modules (CCPs). Binding sites for C3b, polyanions, and other ligands exist in conformationally extended regions toward the ends of FH; central modules (according to NMR/SAXS) adopt a more compact conformation consistent with a bend or hinge. A typical CCP three-dimensional structure (for CCP 11) is shown highlighting secondary structure, invariant Cys residues, and the near-invariant Trp residue. The color coding used here is also used in Figures 3–5.

FACTOR H BELONGS TO A FAMILY OF PROTEINS BUILT FROM CCPS

Human FH, encoded by *CFH* in the "regulators of complement activation" gene cluster, is abundant in plasma (~1–3 μM).[25,26] Produced predominantly in the liver, it is also secreted by endothelial and retinal pigment epithelial[27] cells, among others. Its 1213 amino acid residues comprise 20 consensus repeats of ~60 residues. Each folds into a β-strand-rich domain containing two disulfide bonds (Cys^I–Cys^{III} and Cys^{II}–Cys^{IV}) called a complement-control protein module (CCP)[28] (Figure 2), short consensus repeat, or sushi domain. Eight N-glycosylation sites in FH, on CCPs 9, 12–14, 15 (two), 17, and 18, are occupied predominantly by nonfucosylated diantennary disialylated glycans of ~2.2 kDa, for a total molecular mass of 155 kDa.[29]

Three-dimensional structures have been determined, in the settings of FH truncation fragments, for all FH CCPs except CCPs 9, 14, and 17.[30−41] Each CCP (Figure 2) resembles an ~4 nm × ~2 nm × ~1.5 nm ellipsoid. Its sequence boundaries coincide approximately with the first (Cys^I) and last (Cys^{IV}) cysteine residues. Inclusive of Cys^I and Cys^{IV}, FH CCPs contain between 51 and 62 residues (Figure 3). Spatially, Cys^I and Cys^{IV} of each CCP lie at opposing poles of the ~4 nm axis. Each CCP is connected to its neighbors by linkers (i.e., lying between but not including Cys^{IV} of one CCP and Cys^I of the next) of three to eight residues (see Figure 3). These variable-length linkers allow variation of intermodular angles and flexibility. Low-resolution structural techniques were used to investigate intact FH (discussed below) and several FH fragments,[39,42−45] but no three-dimensional structure of full-length FH is available.

The FH splice variant, FH-like 1, consists of CCPs 1–7 and four additional C-terminal residues. FH-like 1, much less abundant than FH, regulates fluid-phase C3 convertases[46] but is less effective at protecting surfaces. Five FH-related proteins (FHR1–FHR5) (reviewed in ref 47), encoded by separate genes, circulate in the blood, with FHR1 being the most abundant (1–2 μM). FHR1, FHR3, and FHR5 have been

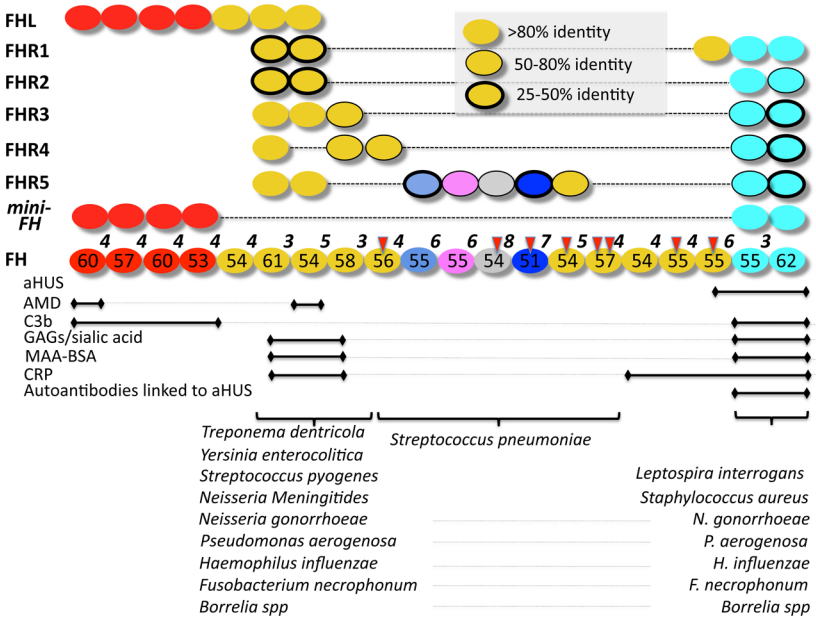

Figure 3. Critical FH regions and similarity with paralogues. The number of residues within each FH CCP (oval) is indicated with intermodular linker lengths written above and N-glycans shown as red triangles. The CCPs of FH-like (FHL) and FH-related proteins (FHRs) are aligned with those of FH to indicate most similar modules, and the percent identity is encoded according to the key (inset). "Mini-FH", an engineered protein based on crystal structures of complexes (see the text and ref 21), is also shown. Ligand-binding sites for C3b, polyanions, malonaldialdehyde acetaldehyde-BSA (MAA-BSA), and C-reactive protein (CRP) are indicated as are binding regions for proteins from various bacteria (adapted from ref 23). The CCPs that contain most SNPs and/or mutations linked to age-related macular degeneration (AMD) and atypical hemolytic uremic syndrome (aHUS), and the binding region for anti-FH autoantibodies related to disease, are shown.

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shown to form tight homodimers and, possibly, heterodimers.[48] Each FHR contains between five and nine CCPs that are similar or identical to various FH CCPs between CCPs 6 and 20 (Figure 3), with N-terminal CCPs (of FHR1, FHR3, and FHR5) forming the dimerization motif. The FHRs do not regulate C3b amplification directly, but FHR1 and FHR5 homodimers were reported to compete with FH for binding to C3b and thereby to modulate the regulatory activity of FH.[48] Other proteins encoded in the same regulators of complement activation gene cluster on chromosome 1q32 also contain multiple CCPs and control C3b, but members of the FH family are unique in that they consist entirely of CCPs; therefore, some FH CCPs participate directly in molecular recognition and surface binding, while others act as spacers, hinges, or tethers.

FACTOR H HAS MULTIPLE BINDING SITES DISTRIBUTED OVER 20 MODULES

With regard to functional regions within FH, most attention has focused recently on sites in CCPs 1−4 and CCPs 19 and 20 that interact with distinct regions of C3b, and two further sites in CCPs 6−8 and CCPs 19 and 20 that interact via molecular markers with self-surfaces.[49] All four sites occur toward the glycan-free N-terminal and sparsely glycosylated C-terminal zones of FH (see Figures 2 and 3), and removal of N-glycans from FH appears not to diminish its functional activity *in vitro*.[50]

CCPs 1−4 Have Decay Accelerating Activity and Cofactor Activity. A recombinant FH fragment consisting of CCPs 1−4 (FH 1−4) binds C3b and acts as a cofactor for FI-catalyzed cleavage of C3b to iC3b. While FI does not bind well to C3b-free FH, C3b-free FH 1−4, or FH-free C3b,[51] its initial rate was a hyperbolic function of the concentration of the FH:C3b (or FH 1−4:C3b) complex (in a fluorescence-based fluid-phase assay) with a K_m of ∼0.2 μM.[51] FH 1−4 also accelerates decay of C3b.Bb[52,53] probably by interacting simultaneously with both C3b and Bb. The acceleration of decay can be measured at low nanomolar FH 1−4 or FH concentrations, consistent with their catalytic roles. Surface plasmon resonance (SPR) measurements show FH 1−4 has a K_D of 5−10 μM for C3b immobilized on a dextran-coated surface.[38,49]

CCPs 19 and 20 Bind to the TED and to Surface Markers. The C-terminal fragment, FH 19−20, has a stronger K_D, of 2−4 μM, for similarly immobilized C3b but, unlike FH 1−4, also binds (with K_D values of 2−4 μM) to iC3b and its cleavage product, C3d (equivalent to the TED of C3b).[49] In the same assay, full-length FH interacts with C3b with a K_D of ∼1 μM,[38,49] consistent with a K_D of ∼0.6 μM inferred from a fluid-phase fluorescence-based assay.[51] Simultaneous occupation of both C3b-binding sites (i.e., CCPs 1−4 and CCPs 19 and 20) within intact FH likely accounts for its tighter binding to immobilized C3b compared to that of either fragment alone, yet greater avidity might be expected were both sites to be fully available for simultaneous binding, be it to the same C3b molecule or to two neighboring C3b molecules bound to a surface. It is conceivable that one or both of the two C3b-binding sites of FH are at least partially occluded in intact FH. Notably, FH itself does not bind well to iC3b[51] despite the presence therein of the intact TED/C3d cleavage product that is the binding site of CCPs 19 and 20. This is discussed further below.

Cooperation of CCPs 6−8 and CCPs 19 and 20. Factor H protected erythrocytes from complement-mediated lysis provided they were sialylated.[54] This and subsequent studies showed that FH recognizes surfaces mainly via glycosaminoglycan (GAG) chains or sialic acid clusters. Recombinant FH truncation fragments, FH 6−8 and FH 19−20, interacted more tightly than any other FH fragments (including FH 8−15) with a heparin affinity resin;[49] heparin is a model for highly sulfated GAGs. FH 6−8 and FH 19−20 bound to soluble heparin with K_D values of 5−10 μM,[33,49,55,56] and bound with diverse affinities to chromatographic resins bearing various GAGs. These sites are also likely responsible for binding to surface-borne sialic acid clusters.[57,58] *In vivo*, FH might utilize these two sites to recognize specific chemical signatures within the glycocalyx.[59−61] Presumably, by employing the two sites simultaneously, full-length FH binds avidly to GAGs,[9,59,62,63] with reported K_D values as low as 30 nM (for heparin, by SPR).[64] CCP 13, like CCPs 7 and 20, carries an electropositive surface patch. Although FH 10−15 and FH 12−13 do not bind appreciably to heparin,[49] CCP 13 could nonetheless augment the affinity of FH for polyanions through nonspecific contacts, consistent with cross-linking studies,[65] or bind an as yet unidentified specific polyanionic marker.

Cooperation between CCPs 1−4 and CCPs 19 and 20 on Self-Surfaces. Affinity measurements for the FH:C3b complex by SPR correspond to the situation on "activating" surfaces (unprotected by FH) because the sensor chip surface is a poor mimic of a self-surface. Indeed, SPR-derived K_D values (of ∼1 μM[38,49]) resemble values obtained for C3b deposited on zymosan and in the fluid phase.[51] On self-surfaces, on the other hand, GAGs/sialic acid and C3b are colocalized; indeed, much surface-deposited C3b is covalently attached via GAG hydroxyl groups. Importantly, FH binds to C3b tethered to sialic acid-bearing (sheep) erythrocytes with a 10-fold enhanced K_D of ∼0.1 μM.[51]

Cooperation between GAG-binding and C3b-binding sites of FH would explain why antibodies recognizing epitopes in CCP 20 blocked binding of FH not just to heparin and endothelial cells but also to C3b.[66] Moreover, recombinant FH 19−20 competed with full-length FH for binding to C3b-decorated erythrocytes, significantly reducing their level of protection from complement-mediated hemolysis afforded by FH.[67] It is unlikely to be coincidental that most pathogen proteins that sequester FH for self-defense purposes bind to one or both GAG/sialic acid-binding sites (reviewed in refs 14 and 68) (Figure 3). Many disease-linked SNPs and mutations occur in these sites, too[2] (Figure 3), a striking example being a cluster of mutations in CCPs 18−20 implicated in atypical hemolytic uremic syndrome, and most have been experimentally determined to modulate binding to polyanions.[36,63,69] When these mutations were reproduced in recombinant FH 19−20, they weakened its ability to block FH function. In a puzzling result, some of these mutations increased, but others decreased, affinities of FH 19−20 for C3d/C3b and/or GAGs,[70] with no clear pattern emerging. These studies cumulatively demonstrate that the various binding sites of FH collaborate, in ways not fully understood, to ensure FH works significantly better on polyanionic self-surfaces than on foreign ones.[11]

Binding by FH to Damaged Cells. Other, putative, binding partners for FH are associated with cellular senescence, stress, or injury. These interactions are less well characterized than those with GAGs or C3b. FH reportedly binds CRP[71] (although this may be an experimental artifact),[72] pentraxin 3,[73]

DNA, histones, annexin II,[74] malondialdehyde acetaldehyde (MAA) adducts of proteins,[75] and oxidized lipids.[76] Clearance of dying cells and cellular debris is assisted by the presence of damage-associated molecular patterns that trigger the classical pathway of complement along with other innate immune responses. Strongly reduced levels of membrane-restricted complement regulators[77] on dying cells imply a reliance on FH to moderate the unwanted pro-inflammatory and cytolytic consequence of unchecked C3b amplification.[78] Factor H in this context promotes formation of iC3b needed for processing and clearance via receptors on macrophages. Thus, direct or indirect recruitment of FH by complement-activating damage-associated molecular patterns may ensure an appropriately nuanced complement response to cell damage. Alternatively, in the case of oxidized lipids, FH might simply block interactions with binding partners.[76]

Advanced MAA adducts (such as 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde derivatives of amino groups on protein molecules) are reliable markers of oxidative stress, so it is of interest that only FH truncation fragments containing CCP 7 or CCP 20 were reported to bind to MAA-modified bovine serum albumin;[75] according to another report, CRP may also bind to these same two regions of FH.[24] These results suggest a bivalent interaction between FH and surfaces bearing oxidation-specific epitopes or CRP similar to that inferred in the case of binding of FH to GAGs or sialic acid-rich surfaces. Of note is the fact that the AMD-linked H402Y variant (in CCP 7) of FH was reported to bind less well than the protective variant to both MAA-modified proteins[75] and oxidized phospholipids and/or low-density lipoproteins.[76] Such markers of oxidative stress are inherently abundant in the macula (a photoreceptor-rich central region of the retina) because of the exposure of photoreceptors to light and the rapid turnover of outer segments, high metabolic activity of cells in the retinal pigment epithelium, and associated high blood flow in the choriocapillaries.

FACTOR H, A BENT-BACK MONOMER, OLIGOMERIZES IN THE PRESENCE OF GAGS

Although eluting anomalously during size-exclusion chromatography, FH was deemed predominantly monomeric[44] based on scattering and analytical ultracentrifugation (AUC) data. Subsequently, FH preparations purified from plasma were found to contain dimers, but with a K_D of ~28 μM for self-association,[79] which is 20-fold higher than actual plasma concentrations. More recent AUC experiments, however, reported the presence of ~5% FH dimer in pure solutions of 0.6−6 μM plasma-derived FH,[80] along with higher-order oligomers comprising 10−20% of the total FH present.

As a string of 20 CCPs (each ~4 nm in length), with diverse net charges, tumbling in solution FH molecules will encounter one another more frequently than would compact globular proteins of the same molecular mass. Some encounters will lead to electrostatically stabilized short-lived self-associations. At any time, there will thus be a range of nonspecific dimers present, perhaps sufficient to account for the ~50% of dimeric molecules in an ~28 μM (i.e., ~17000 molecules per cubic micrometer) solution of purified FH. Therefore, FH at 2 μM in plasma, where it suppresses fluid-phase C3b amplification, does not show a significant tendency to dimerize, unlike FHR1, FHR2, and FHR5. On the other hand, FH could develop a propensity for oligomerization as it ages (during purification and storage, or in circulation) and become modified, oxidized, or proteolytically clipped. It is potentially interesting that FH oligomerization is induced by high concentrations of zinc and other metal ions[81] as well as GAGs (discussed below) and that drusen associated with AMD contain aggregated FH molecules.

In negatively stained electron microscopy images, FH molecules adopted multiple conformations but were predominantly doubled over;[43] AUC and SAXS on FH also indicated an axial ratio that equates to a doubled-over chain of 20 CCPs.[44] This is consistent with intuition because if the molecule is sufficiently flexible for sequentially non-neighboring CCPs in the same chain to make mutual contact, electrostatic interactions are probable given the variations in the pI of CCPs throughout FH. Experimentally, FH 1−7 binds to immobilized FH according to SPR.[66] Indeed, intramolecular (intermodular) interactions might trump intermolecular encounters in a 2 μM solution of FH. High-resolution structural studies show that central CCPs (Figure 2) adopt a compact or hingelike arrangement that encourages interactions between CCPs in N-terminal regions with those in C-terminal regions. The FH molecule became more extended with an increasing salt concentration,[80] consistent with a decrease in compactness accompanying the attrition of electrostatic intermodular interactions.

GAGs and sialic acid, being polyanionic, could likewise influence intramolecular electrostatics and hence the molecular architecture and or oligomerization of FH. Multiple FH molecules can bind one soluble heparin molecule.[82] Moreover, presumably because FH has two GAG-binding sites, large heparin-stabilized oligomers assemble *in vitro*. In another study, soluble dermatan sulfate promoted tetramerization of FH (and self-association of FH 18−20).[83] If four FH molecules binding to one GAG could account for tetramerization, then excess GAGs would push the equilibrium toward a 1:1 FH:GAG complex. However, surprisingly, a tetramerization end point was attained as the GAG:FH ratio was increased,[83] suggesting that some soluble GAGs drive soluble FH self-association toward cooperative tetramer assembly, presumably by exposing cryptic self-association regions of FH. In the same study, soluble GAGs promoted 5-fold FH binding to C3b-decorated zymosan particles (that lack polyanions); moreover, GAG-oligomerized FH displayed increased cofactor and decay accelerating activities on C3b-zymosan.

FACTOR H BINDS TO SURFACES

Because, *in vitro*, FH binds avidly to heparin, sialic acid, etc., FH might be expected to utilize its two GAG/sialic acid-binding sites to bind directly to polyanions on gylcocalyx or extracellular matrix even if they lack deposited C3b (or its fragments), but quantitative data are lacking here. Binding of FH to C3b-free human umbilical vein epithelial cells,[84,85] synovial fibroblasts,[86] and retinal pigment epithelial cells[87] was demonstrated by fluorescence-activated cell sorting or immunofluorescence microscopy.[88] Binding of FH to epithelial cells was inhibited by heparin and various GAGs and terminal sialic acid groups typical of glycoproteins and proteoglycans within the glycocalyx of many cells. Antibodies directed to CCP 20, or mutations in CCPs 19 and 20, inhibited interaction of FH with C3b-free epithelial cells,[69,89] while the role of the GAG interaction site centered on CCP 7[56] in cell binding is less clear.

These data are suggestive but insufficiently quantitative to address a fundamental question: do cells bathed by plasma carry a prophylactic population of FH molecules, or do they recruit significant levels of FH only when they are decorated

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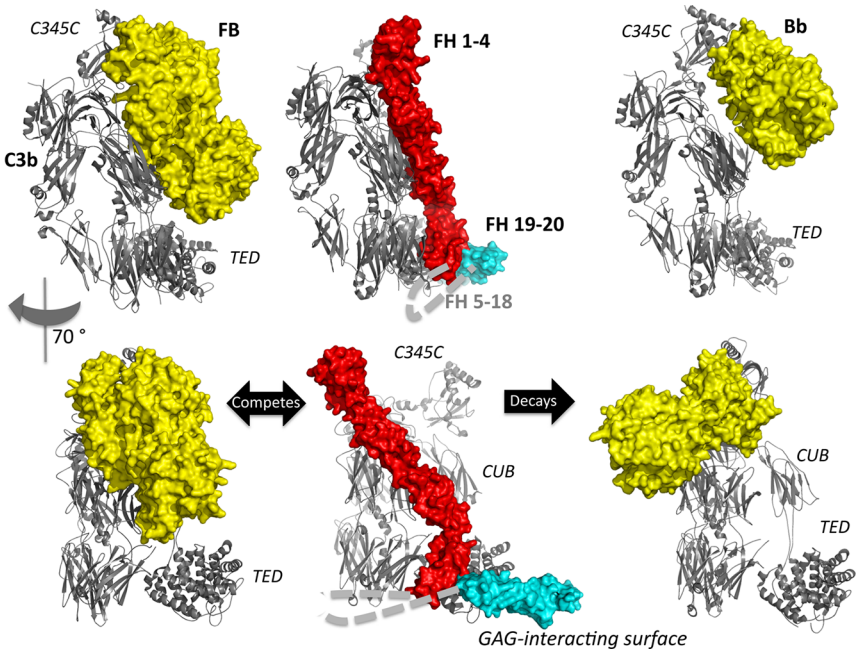

Figure 4. Models of complexes derived from crystal structures. Shown (from left to right) are C3b (gray) in complex with FB (yellow) (PDB entry 2XWJ), FH 1−4 (red) and FH 19−20 (cyan) (modeled on PDB entries 2WII and 3OXU, CCPs 5−18 symbolized by a dotted connector), and Bb (yellow) (extracted from PDB entry 2WIN). TED, CUB, and C345C domains of C3b are labeled where clarity permits. As indicated, FH competes for binding of FB to C3b and accelerates decay of the C3b.Bb complex.

with C3b or its degradation products? In the sole quantitative study, binding of iodinated FH to C3b-free sheep erythrocytes (that have a sialic acid-rich surface and therefore do not activate the human complement) occurred at a low level and without saturation at approximately physiological concentrations of FH, indicating weak binding and a high K_D; in contrast, 4−5 times more FH bound, and exhibited a saturable binding curve, to sheep erythrocytes decorated with ∼30000 C3b molecules.[54]

STRUCTURE OF CCPS 1−4 WITH C3b

The structure of a C3b:FH 1−4 complex [Protein Data Bank (PDB) entry 2WII (Figure 4)][38] provided a treasure trove of information. FH 1−4 buries ∼4500 Å^2 of mutual surface area when it binds C3b; all four CCPs contact C3b over a linear distance of ∼100 Å. The mutual orientations of CCPs 1−3 within the complex resemble those seen in the solution structure of free FH 1−3,[37] with differences in intermodular angles lying only slightly outside the cones of intermodular flexibility inferred for free FH 1−3.[90] Likewise, conformational differences between bound and free C3b were limited, with the C345C domain exhibiting the largest apparent displacement.

Modules 1−4 of FH interact with surface regions created during the transition from C3 (to which FH does not bind) to C3b. Notably, CCP 2 interacts with the new N-terminus of the α-chain created by ANA excision, while CCP 3 bridges the drastically relocated CUB domain (containing two FI-cleavable peptide bonds) and the core of the C3b molecule consisting of MG domains arranged in a "key ring" formation (Figure 4). Module 4 bridges the displaced TED (containing Gln991, the site of surface attachment) and the body of C3b. These interactions explain why FH 1−4 does not bind iC3b wherein the CUB is disrupted. A kink between CCPs 3 and 4 ensures that while CCPs 1−3 head "down" the C3b molecule toward the surface to which it is attached, CCP 4 is almost parallel with the surface presumably projecting CCP 5, etc., away from the complex. This arrangement, confirmed by Forster resonance energy transfer,[91] is consistent with surface-proximal locations for the 16 ensuing CCPs. The structure of the FH:C3b complex explains mutagenesis data[92] and was used, with a crystal structure of FI, to build a plausible model of the FI:FH 1−4:C3b complex.[93] In free FI, the heavy chain inhibits the serine protease domain within the light chain. The model predicts that autoinhibition is relieved by conformational rearrangement of FI following binding, via an exosite, to the FH:C3b complex.

The landmark C3b:FH 1−4 structure, and another of C3b with FB,[94] shows that the FH 1−4-binding site and FB-binding site on C3b overlap, thus accounting for the competition between these two factors.[54] The FH 1−4:C3b structure when compared to a C3b.Bb structure[95] (inferred from a structure of a $C3b_2$.Bb_2 complex stabilized by a bacterial protein) sheds light on decay accelerating activity. In the C3b.Bb structure, only the mobile C345C domain contacts Bb. The C3b.Bb complex decays irreversibly because the Ba portion of factor B (consisting of three CCPs) is obligatory for initial loading of FB onto C3b (Figure 1).[94,96] Hence, there is no scope for FH to inhibit reassociation, and competition between FH and Bb cannot explain the acceleration of decay. The structures of these two complexes along with evidence of flexibility within FH 1−4[90] and mobility of the C345C domain suggest that CCPs 3 and 4 could bind to C3b of the C3b.Bb complex in a manner similar to how they bind C3b alone. The presence of CCPs 1 and 2 might then favor a conformation of the C3b.Bb complex that is inherently unstable, causing the ternary complex to collapse to the binary FH 1−4:C3b complex.

STRUCTURES OF THE COMPLEX OF CCPS 19 AND 20 WITH C3d AND EXTRAPOLATION TO THE COMPLEX OF CCPS 19 AND 20 WITH C3b

Two crystal structures of C3d:FH 19−20 complexes (PDB entries 3OXU[97] and 2XQW[98]) shed further light on the

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C3b:FH complex. Both reports identified the same intermolecular interface (Figure 4), burying ~750 Å^2 and involving mostly CCP 19 with a contribution from the cleft between CCPs 19 and 20, and a region of C3d equating to an exposed region of the TED in C3b. The two CCP modules were mutually tilted by 32°; a near-identical arrangement of CCPs was observed in solution (PDB entry 2BZM)[99] and crystal (PDB entry 2GZI)[34] structures of free FH 19−20 and in a crystal structure of FH 18−20 (PDB entry 3SWO).[40]

These structures indicated that CCPs 19 and 20 bind to a site on the TED of C3b that is adjacent to, but distinct from, the site occupied by CCPs 1−4 (Figure 4), with potential for contacts between CCPs 4 and 19. This conclusion was based on superposition of C3d from the FH 19−20:C3d complex onto the TED of C3b from the FH 1−4:C3b complex, as legitimized by K_D measurements for FH 19−20 mutants confirming that the FH 19−20 residues involved in binding C3d and C3b are identical. A shortcoming of this approach is that it assumes the conformation of C3b is unaltered by the binding of CCPs 19 and 20. Titration of the FH 19−20:C3d complex with a soluble GAG, while monitoring NMR chemical shifts, revealed which FH 19−20 residues were perturbed. This delineated a GAG-binding face on the opposite side of CCP 20 to the one that faces C3d/TED and is consistent with CCPs 19 and 20 binding to a protein-carbohydrate composite consisting of C3b attached via an ester linkage to, or near, surface-borne GAGs[97] (see Figure 4).

In reporting PDB entry 3OXU, one FH 19−20:C3d interface was chosen out of several in the unit cell, based on NMR chemical shift perturbations, mutagenesis data, intermolecular H-bonds, and buried surface area. This interface matched one of two heterotypic interfaces in the unit cell of crystals used to determine the structure of PDB entry 2XQW. The same interface occurred in a third FH 19−20:C3d structure, containing the disease-linked double mutant (S1191L/V1197A) of FH 19−20.[100] The alternative FH 19−20:C3d interfaces could not occur in the context of C3b, unless the TED is very flexibly attached to the rest of C3b. The two studies disagreed about the relevance of these alternative interactions and whether they might allow CCPs 19 and 20 to bridge a C3b and a C3d.

THE STRUCTURE OF CCPS 6−8 SUGGESTS A ROLE FOR TYR402

But what of the 14 intervening CCPs accounting for 70% of FH? In broad confirmation of preceding SAXS studies,[101] a crystal structure of FH 6−8 (PDB entry 2UWN)[35] showed the three CCPs in a predominantly elongated conformation with small intermodular interfaces. Sucrose octasulfate was included in the crystallization solution, and in agreement with NMR chemical shift perturbations, a sucrose octasulfate molecule bound close to the Tyr402 side chain whose substitution with histidine is linked to the risk of AMD. Sucrose octasulfate is a GAG surrogate, and its interaction with Tyr402 suggests substitution of tyrosine with histidine could modulate the ability of FH to recognize self-surface markers. A crystal structure of FH 6−7 determined in complex with a 27 kDa FH-binding protein from *Neisseria meningitidis* (PDB entry 2W80)[102] showed that the bacterial protein binds primarily to CCP 6; its anionic side chains may mimic the sulfates of GAGs.

CCPS 10−15 ADOPT A UNIQUE, COMPACT ARRANGEMENT

Smaller-than-average CCPs linked by longer-than-average intermodular linkers characterize CCPs 10−15 of FH (see Figure 3). The structure of FH 12−13 was investigated (PDB entry 2KMS) as it features both the longest linker (eight residues) and the smallest (51 residues) module (CCP 13) with the highest net positive charge in FH.[39] Unexpectedly, the long linker does not enhance flexibility between modules or allow them to fold against one another in a U shape. Rather, linker residues and those from loops on either side form a hydrophobic minicore holding these CCPs in a rigid V-like structure. Similar findings were made for FH 10−11 (PDB entry 4B2R) and FH 11−12 (PDB entry 4B2S), both with six-residue linkers.[41] Interestingly, preparation of both FH 13−14 and FH 14−15 proved to be difficult. This was ascribed to a requirement of the structural stability of CCP 14 for two or more neighboring CCPs, implying extensive intermodular interactions.[41] These structural observations on module pairs were combined with SAXS studies of FH 10−15 and FH 8−15 to propose a model featuring a compact arrangement, consistent with a hingelike structure toward the middle of FH (see Figure 2). Combining these data with a SAXS-derived rodlike structure of FH 15−19 and the crystal structures of FH 6−8, the FH 1−4:C3b complex, and the FH 19−20:C3d complex produced a model in which FH bends back on itself to allow N- and C-termini to bind simultaneously to adjacent sites on the same C3b molecule (Figure 4) or neighboring C3b molecules on a surface. A tentative theory is that CCP 14 alternates between poorly structured and compact conformations depending on interactions with neighboring CCPs or even an unknown modulator. This would allow articulation of binding sites around a central hinge.

THERAPEUTIC FH

As an efficient alternative pathway modulator that acts in the fluid phase and on self-surfaces but not on foreign ones, FH is a potential anticomplement therapeutic protein. Crucially, if deployed to supplement FH levels in patients, it would not perturb complement-mediated defenses against pathogens. In contrast, a humanized anti-C5 antibody approved for use in paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, like potentially useful anti-FD and anti-FB antibodies,[103,104] compromises MAC formation in cell membranes of both bacterial and human cells. Furthermore, in patients with FH insufficiency, supplementation will both modulate C3b amplification and restore capacity for iC3b production, leading to the promotion of noninflammatory responses to apoptosis or oxidative damage. On the downside, at 155 kDa and with eight glycans and 40 disulfides, FH is challenging to manufacture and administer. Furthermore, its high plasma concentration in healthy controls suggests that doses of hundreds of milligrams might be needed for therapeutic impact unless it can be delivered directly to the site of complement-mediated injury;[105] however, a fine balance is struck between complement activation and regulation, and even a small improvement in FH functionality could "tip the balance" in favor of complement control. A more quantitative understanding of C3b amplification and the role of FH is needed to estimate appropriate doses. Progress in recombinant FH production[20] shows that provision of sufficient quantities of material is feasible.

The structural and functional knowledge described earlier provides a basis for "module engineering" FH for size reduction and efficacy enhancement. Indeed, module engineering approaches to therapeutic complement regulation have worked even when performed without knowledge of the structure (reviewed in refs 103 and 104). For example, fusing three complement regulatory CCPs from CR1 to a peptide-based membrane-anchoring entity created a regulator with potential for protection of transplanted organs. Another innovative approach was to fuse CCPs 1−5 from FH in front of CCPs 1−4 from CR2 (creating "TT30"). These CR2 N-terminal CCPs (like FH CCPs 19 and 20) bind C3d. It was reasoned that this nine-CCP chimeric molecule would be targeted to surfaces decorated with proteolytic fragments of C3b, a legacy of previous cycles of C3b deposition and FI cleavage. Hence, C3d is a marker for self-cells under attack by complement and was used in this instance, successfully, as an anchor for the fusion protein.

In a structural biology-inspired strategy for designing an FH-derived inhibitor, CCPs 5−18 were replaced with a flexible tether.[21] The two C3b-binding regions and the key site for surface interactions in CCPs 19 and 20 were thus retained while the GAG recognition site in CCPs 6−8 (Figure 3) was not. A model of the C3b:FH 1−4:FH 19−20 complex (Figure 4) informed design of the linker between CCPs 4 and 18, which consisted of 12 glycine residues. The resultant six-CCP protein, called "mini-FH", bound to C3b on an SPR chip about as well as FH did (K_D of ∼1 μM). It recognized MAA adducts, and it bound to heparin more tightly than FH (but less well than FH 19−20). Interestingly, it also bound to iC3b and C3d(g) much better than full-length FH. Encouragingly, in a disease model of paroxysmal nocturnal hemoglobinuria, mini-FH was more effective at protecting against complement-mediated hemolysis than FH itself.[21]

■ MODELS FOR THE FH MODE OF ACTION

In summary, the following "duty cycle" of FH is well-established, but the molecular mechanisms underlying each step have not been completely elucidated. FH (typically 1−3 μM in plasma) binds to fluid-phase or surface-deposited C3b. It binds preferentially to C3b deposited on a self-surface. This preference relies upon FH CCPs 19 and 20 recognizing and binding specific molecular markers. FH also binds to C3b in the context of C3b.Bb as well as to C3(H_2O) (that structurally resembles C3b) and C3(H_2O).Bb. CCPs 1−4 of FH occupy a site on C3b that partially overlaps with the FB-binding site. FH thereby competes with FB for binding to C3b. If FH binds to C3b.Bb, it accelerates irreversible decay into the C3b:FH complex (or C3b and FH) and Bb. It also accelerates decay of the C3(H_2O).Bb complex. FI becomes active on binding to the FH:C3b complex, cleaving C3b to iC3b. Finally, FI and FH dissociate from iC3b that remains surface-tethered but may be further degraded to C3d(g). iC3b and C3d(g) are ligands for various cell-surface receptors but do not bind well to FI, FB, or FH.

A better understanding of the molecular basis of its duty cycle would allow the consequences of FH sequence variations to be rationalized and could guide the design of engineered versions of FH with enhanced therapeutic potential. Outlined below (in order of increasing sophistication, and hence speculation) are some tentative models for the molecular basis of FH action that are consistent with varying amounts of the experimental evidence presented in the preceding sections.

The Concentration of FH Is Increased on a Self-Surface. In the simplest model, favorable interactions between FH CCPs 19 and 20 and surface-borne molecular markers attract FH molecules to the self-surface, enriching its local concentration. This increases the encounter frequency between FH and the C3b.Bb complex and, hence, enhances the C3b.Bb complex decay rate. Likewise, encounters between FH and C3b will be more frequent, accounting for an enhancement in the apparent affinity of FH for C3b on a self-surface and providing more effective competition for binding of FB to C3b. The higher population density of C3b:H complexes that results equates to an increase in FI substrate concentration, thus enhancing C3b cleavage rates. Because C3b amplification is a nonlinear process, these differences (between self- and non-self-surfaces) in convertase decay and C3b cleavage rates act as a binary switch between activating and nonactivating scenarios.

A GAG-Induced Oligomeric Anchor for FH? More elaborate models (Figure 5A) must be invoked to explain reports of soluble GAG-induced FH oligomer formation.[83] In one such tentative model, CCPs 19 and 20 mediate formation of an oligomeric structure incorporating surface-bound GAGs. This acts as an anchor, prolonging FH residency times on the surface. In this model, FH CCPs 5−18 form a flexible tether; in "fly-casting mode", they allow CCPs 1−4 to "seek and destroy" C3b molecules within a region around the anchoring point. A putative polyvalent, oligomeric FH is redolent of the six or seven identical chains of C4b-binding protein that performs roles similar to those of FH but in the classical pathway. Each of these chains contains eight CCPs, the N-terminal of which bind C4b and GAGs.[106] The six or seven chains are covalently interlinked at their C-termini, leaving flexible "arms" that can bind avidly to patterns of multiple GAGs and C4b molecules.[107] The oligomeric anchor model of FH allows for modulation of FH by recombinant FH 19−20 as well as FHR1 and FHR5. All of these contain CCPs 19 and 20 (or copies thereof) and could disrupt, via hetero-oligomer formation, assembly of a stable FH homo-oligomer. This might explain why deletions of genes encoding FHR1 (and FHR3) protect against some diseases.[108]

Role of CCPs 6−8. Further elaboration is needed to explain studies that indicate CCPs 6−8 (especially CCP 7) augment the recognition of molecular signatures on self-surfaces by CCPs 19 and 20, potentially providing a combinatorial recognition capability.[11,61] These observations, consistent with a "molecular proofreading" role for CCPs 6−8, are particularly significant because they suggest a molecular basis for the link between the Y402H variant of CCP 7 and the increased risk of developing AMD. It is reasonable to invoke a model in which surface-bound CCPs 6−8 hold CCP 4 of FH proximal to the surface, hence helping to maintain CCPs 1−4 in an optimal pose for productive encounters with surface-attached C3b (Figures 4 and 5A). The job of FH CCPs 9−18, in this tentative model, is to adopt a conformation that favors simultaneous binding, to adjacent surface markers, by both CCPs 7 and 20.

Role for the Interaction of CCPs 19 and 20 with TED and/or C3d. The models invoked above imply all of the affinity of FH for C3b resides within CCPs 1−4. This is at variance with a failure to demonstrate strong binding of FH to sialic acid-rich surfaces lacking C3b.[54] It also fails to explain why (according to SPR) intact FH binds 10-fold better to C3b than the FH 1−4 fragment or why the FH 19−20 fragment binds well to C3b. These models also fail to explain why

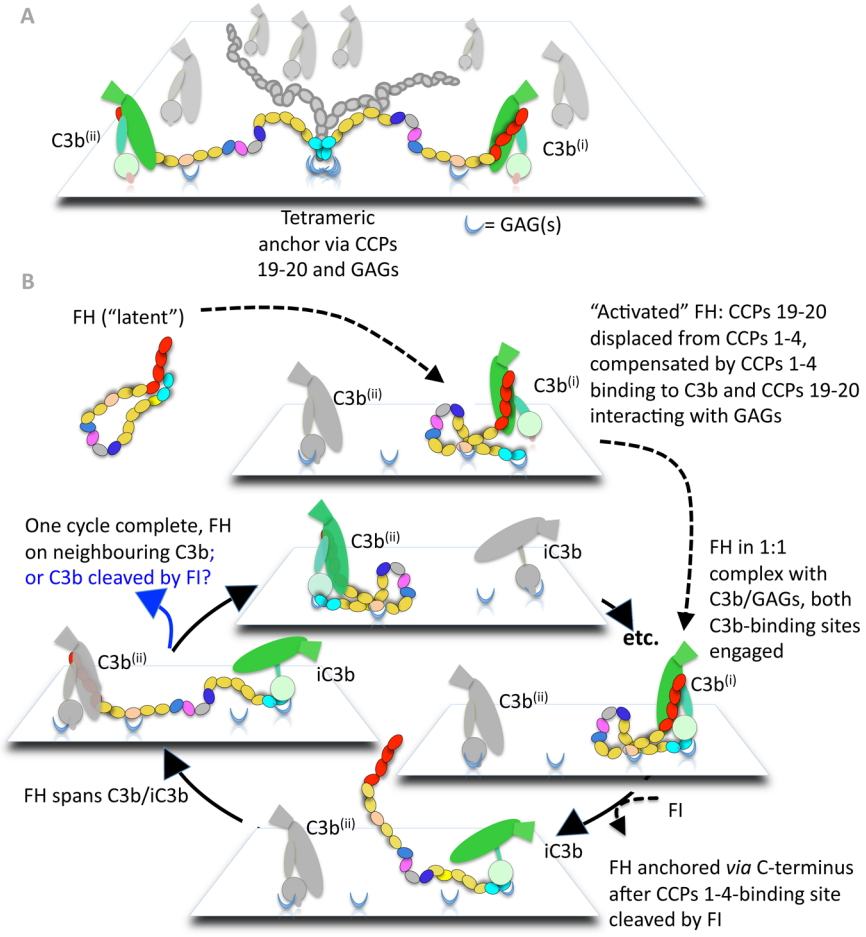

Figure 5. Hypothetical models of the action of FH on nonactivating surfaces. (A) GAG-induced oligomerization model. Four FH molecules (two of which are color-coded as in Figure 2) are anchored [via C-terminal CCPs (cyan) interacting with GAGs] to a self-surface, thereby preventing C3b amplification [through action of N-terminal CCPs (red)] in the environs. (B) Latent/activated model. "Latent" FH is "activated" by interaction with C3b and molecular markers on a self-surface, allowing bivalent interaction with an initial self-surface-tethered $C3b^{(i)}$. Following cleavage of $C3b^{(i)}$, the FH molecule is anchored via its C-terminus to $iC3b^{(i)}$ and surface markers such that N-terminal CCPs could dispatch a neighboring C3b molecule [$C3b^{(ii)}$] or molecules (as in panel A). The C-terminal CCPs could transfer to $C3b^{(ii)}$ before it is cleaved.

antibodies to CCPs 19 and 20 block C3b binding,[109] why FH has a predominantly bent-back structure in solution, and why dimeric FHR1 and FHR5 (that contain copies of CCPs 19 and 20) have been reported to deregulate complement activation by competing with FH for C3b binding.[48] To accommodate these observations, it has been hypothesized that, in the FH:C3b complex, both N-terminal and C-terminal CCPs simultaneously bind, either to the same C3b (i.e., 1:1) as modeled in Figure 4 or to two neighboring C3b molecules on a surface (i.e., 1:2). In these complexes, CCPs 19 and 20 recognize a composite of C3b and an immediately adjacent GAG chain, for example. A 1:1 complex is consistent with structural studies of central segments of FH and a bent-back architecture of FH, but a 1:2 complex might be favored depending on the spatial distribution of C3b targets. A 1:2 mode would additionally allow FH to span a molecule of iC3b or C3d(g) and a C3b molecule (see Figure 5B). If that were the case, these degradation products (and nearby surface markers) could form anchor points for the C-terminus of FH. These, like a putative GAG-induced oligomeric anchor, could be perturbed by FH 19−20, FHR1, or FHR5. Anchors based on iC3b or C3d(g) are feasible because self-surfaces (but not foreign ones) bear C3b degradation products arising from previous actions of surface and membrane-bound regulators. Both TT30 and mini-FH, which have C3d-binding C-terminal regions (and FH CCPs 1−4 as their N-terminal regions), probably work as regulators in this way.

Latent and Activated Conformations of FH. Finally, to account for the lack of affinity of full-length FH for iC3b or C3d(g), despite the presence on these C3b fragments of a binding site for CCPs 19 and 20, a specific train of events for formation of a fully regulatory complex can be invoked. FH is thus (Figure 5B) hypothesized to adopt, preferentially, a low-affinity "latent" conformation, but a higher-affinity "activated" conformation is stabilized by association with its target (C3b on a self-surface). Such a strategy might have evolved under selective pressure from pathogen load because it could make the hijacking of FH for self-defense purposes by bacteria and other pathogens more difficult.

In this model, it is proposed that intramolecular interactions, reinforced by the architecture of its central segment, favor solution conformations of FH in which neither CCPs 1−4 nor CCPs 19 and 20 are fully available to bind C3b; they must first disengage, perhaps from one another. Thus, for complex formation to occur, favorable interactions between C3b and FH must overcompensate for disruption of intramolecular interactions. In the case of C3d binding by FH, it is feasible that the small interface between C3d and CCPs 19 and 20,[97] unlike the 6-fold larger one between C3b and CCPs 1−4, is insufficient to

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compensate for the loss of intramolecular interactions. Even in the case of binding of full-length FH to C3b (where both CCPs 1−4 and CCPs 19 and 20 can bind simultaneously, burying >5200 Å^2), a relatively low-avidity effect occurs for bivalent binding by the full-length protein (giving a K_D of 1 μM for the FH:C3b complex compared to values of 2−10 μM for the two fragments individually). In a key, but speculative, component of this model, it is suggested that surface markers favor the activated conformation of FH because the C-terminus must be exposed for CCPs 19 and 20 to fully engage with surface markers (burying further surface area). This additional interaction pushes the equilibrium toward the ternary (FH:C3b:marker) complex and lowers the K_D, into the 100 nM range. Thus, even at FH plasma levels of only 1.5 μM, a high proportion of C3b:surface marker complexes will engage with FH. According to this tentative model, GAG-induced oligomerization of FH in solution could be a side effect of previously cryptic binding sites becoming available due to "activation" by GAGs, promoting self-association in the absence of the target (C3b).

After C3b cleavage, CCPs 1−4 lose affinity because of binding site disruption (CUB cleavage), while the C-terminus could, in theory, remain temporarily anchored to iC3b; thus, the N-terminus would be liberated to act sequentially on multiple nearby C3b.Bb complexes and C3b molecules, as outlined above. While transient, this hypothetical situation could persist long enough for the dispatch of several surrounding molecules of C3b or the C3b.Bb complex if they lie within range. Depending on the kinetics and flexibility of FH, the C-terminus could release iC3b while the N-terminus is still attached to C3b, whereupon FH binds bivalently and tightly to this second C3b molecule (Figure 5B). As discussed above, the structure of the central segment is consistent with a "sprung hinge" that allows the N-terminal region freedom to search for targets but also energetically favors the 1:1 bivalent interaction with C3b (Figure 4) compared to the iC3b−C3b-spanning alternative conformation (see Figure 5B). Thus, in this most elaborate model, it is suggested that FH remains activated and "walks" on the surface suppressing outbreaks of C3b deposition; its lower likelihood of leaving the surface between duty cycles increases efficacy and could be critical during an episode of heavy bombardment by C3b.

IMPLICATIONS OF MODELS AND FUTURE DIRECTIONS

The latest models invoke a change in the conformational preferences of FH from a latent arrangement of its CCPs in which ligand-binding sites are not fully accessible to an activated, high-affinity for C3b, arrangement. Such a rearrangement would require the substitution of one set of intermodular interactions for another. Available information about the FH architecture suggests that CCPs 1−7 or CCPs 19 and 20 would likely participate in this reorganization, which could therefore be perturbed by some of the many disease-linked sequence variations therein. Likewise, models invoking FH oligomerization imply that some disease-associated sequence variations could have an impact on self-association. FHR1 and FHR5 that themselves form homodimers might compete not only with intermolecular interactions involving the C-terminal CCPs of FH but also with intramolecular ones. A switch in the conformational preferences of FH that increases the level of exposure of a C-terminal C3b-binding site could create a target for autoantibodies to FH that have also been linked to disease. Thus, emerging models of FH action, which entail a subtle interplay between intramolecular and homotypic and heterotypic intermolecular interactions, suggest testable hypotheses linking FH, and FHRs, with risk of disease. Such hypotheses have not been interrogated in previous studies that focused on the use of FH fragments or the measurement of pairwise affinities. For example, the puzzling results obtained for disease-linked FH mutations re-created in recombinant FH 19−20, which had diverse effects on individual affinities of the fragment for GAGs and C3b,[70] perhaps reflect this limitation.

These hypotheses should be tested by mutagenesis in the context of intact FH and rigorous assessment of oligomerization and conformational preferences as well as functional consequences, but the design of insightful, quantitative functional assays that can be interpreted in molecular terms is challenging. At the root of this problem is the spatiotemporal complexity of these highly dynamic surface-tuned phenomena involving complex interactions between multiple participants. In particular, the several roles of FH [pattern recognition, cofactor, catalyst (of decay acceleration), and competitive binder] make it difficult to translate individual pairwise affinity measurements into a holistic understanding.

Computational simulations of the C3b amplification loop could address this issue. These would be founded on quantitative measurement that would in turn require careful fitting of data to equations encapsulating binding, catalysis, thioester half-life and reactivity, and diffusion. A starting point would be simulations of SPR-based experiments involving C3, FB, FD, FI, and FH in which, crucially, real-time measurements may be related to numbers of molecules arriving at or departing from a surface. Such SPR experiments could be complemented by microscopy or fluorescence measurements for identifying surface patterns of deposited molecules. Importantly, SPR chip surfaces can be created with defined molecular properties that mimic those of both complement-activating and nonactivating surfaces, and with varying levels of decoration by C3b, iC3b, or C3d. By coupling this approach with site-directed mutagenesis studies of full-length FH, we could thoroughly test hypothetical mechanisms. Subsequent work with mixtures of FH variants (representing the situation in heterozygous patients), inclusion of FH autoantibodies, properdin (stabilizes the C3b.Bb complex), FHRs, and anticomplement pathogen-produced proteins, would allow deeper insights into the more complex situations that prevail *in vivo*.

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ABBREVIATIONS

AMD, age-related macular degeneration; AUC, analytical ultracentrifugation; CCP, complement control protein module; CRP, C-reactive protein; CUB, C1r/C1s; Uegf, Bmp1 domain; FB, factor B; FH, factor H; FHR, factor H-related protein; FI, factor I; GAG, glycosaminoglycan; MAA, malondialdehyde acetaldehyde; MAC, membrane-attack complex; SAXS,

small-angle X-ray scattering; SPR, surface plasmon resonance; TED, thioester-containing domain.

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