Factor H-related proteins determine complement activating surfaces.

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Summary

The complement factor H-related proteins (FHRs) are strongly associated with different diseases involving complement dysregulation, which suggests a major role for these proteins regulating complement activation. Because the FHRs are evolutionarily and structurally related with the complement inhibitor factor H (FH), the initial assumption was that the FHRs are also negative complement regulators. Despite weak complement inhibiting activities were originally reported for these molecules, recent developments indicate that the FHRs may rather enhance complement activation and are providing novel insights into their role in health and disease. FHRs are now envisioned as a complex set of surface recognition molecules that, by competing with FH, provide improved discrimination of the self and non-self/foreign surfaces where complement activation should appropriately take place.

Introduction

The complement system is an essential part of the innate immunity, with decisive roles in the protection against infections, but also participating in the modulation of inflammation, the disposal of immune complexes, cellular waste and apoptotic/necrotic cells, or in the activation and regulation of innate and adaptive immune cells [1]. Preserving the physiological balance between complement activation and inhibition is absolutely required to maintain homeostasis by targeting appropriate sites for activation but also avoiding at the same time bystander damage to host tissues. This is ensured by proper target recognition mechanisms that initiate and amplify complement activation only when it is necessary, by the built-in specificity and limited activity of the enzymatic complexes (C3/C5 convertases) of the system, and by an array of receptors and regulatory molecules that control the activation cascade at different steps (BOX 1). Imbalance between activation and inhibition due to excessive activation or improper regulation has pathological consequences [2-5]. Here we will review recent developments that provide novel insights into how complement is regulated on self and non-self biological surfaces and the implications that this understanding have in health and disease.

The FH/FHR protein family

Six genes positioned in tandem arrangement encode the members of the FH/FHR protein family. This family includes FH and FHL-1, both derived from *CFH* via alternative splicing, and the FHR-1, -2, -3, -4 and -5 proteins coded by the *CFHR1-5* genes. FHR-1 has two allelic variants FHR-1*A and FHR-1*B, and FHR-4 two isoforms FHR-4A and FHR-4B (Figure 1). All these proteins are composed of repetitive units of ~60 aa named short consensus repeats (SCRs) (GLOSSARY), arranged in a continuous fashion. The *CFHR1-5* genes originated from the *CFH* gene by tandem duplication events [6] (Figure 1A). The C-terminal region (SCRs 18-20) and SCRs 6-7 of FH, harbouring the major surface recognition sites of FH, are

retained with different degrees of conservation in all FHRs, explaining their capacity to interact with most of the FH ligands (Table 1) [7-9]. However, none of the FHRs contain regions homologous to FH SCRs 1-4, which questions conservation of the FH complement regulatory activities in the FHRs (Figure 1B, 1C).

A remarkable characteristic of FHR-1, FHR-2 and FHR-5 is that the two N-terminal SCRs, which are almost identical in these FHRs (Figure 1C), include a dimerization domain [10]. This determines that these FHRs, in contrast to FH, always circulate in plasma as dimers or tetramers (Figure 1D) [10, 11]. This structural organization has major functional implications. By assembling into homo- and hetero-oligomeric complexes, FHR-1, FHR-2 and FHR-5 increase their avidity for their ligands (C3b, iC3b, C3dg and carbohydrates). In addition, because the FHR composition of the different oligomers influences the carbohydrate binding characteristics for each of these FHR complexes, a combinatorial repertoire of different FHRs likely provide finer identification of opsonised surfaces with different carbohydrate compositions and different densities and rates of deposition of C3 fragments.

FHRs and Disease

Genetic variations in the FHRs are associated with different diseases, including C3 Glomerulopathies (C3G), atypical Hemolytic Uremic Syndrome (aHUS), IgA Nephropathy (IgAN), Systemic Lupus Erythematosus (SLE) and Age-related Macular Degeneration (AMD) [12-24]. Among them, variations involving *CFHR1-5* genomic rearrangements (Table 2) are most remarkable and informative. These rearrangements are not unusual because the region contains large genomic duplications (Figure 1A), which makes it highly prone to genomic rearrangements through gene conversion and non-allelic homologous recombination [6].

Rearrangements resulting in the generation of hybrid genes between *CFH* and *CFHR1* or *CFHR3* strongly associate with aHUS, a rare kidney disease characterized by impaired

regulation of complement activation on endothelial surfaces leading to thrombotic microangiopathy. Notably, FH proteins in which the last C-terminal SCR20 has been replaced by the C-terminal SCR5 of FHR-1 or by the whole FHR-3, namely FH::FHR-1 and FH::FHR-3 hybrids, are unable to regulate complement properly on endothelial surfaces [25-28]. Similarly, the FHR-1::FH hybrid protein in which the C-terminal SCR5 of FHR-1 has been replaced by SCR20 of FH also impairs complement regulation by competing binding of FH itself to the endothelium [29]. As a whole, data generated from the study of the *CFH*::*CFHR1/CFHR3* hybrid genes [25-27, 29-31] illustrate the critical role that the C-terminal region of FH plays in the protection of host surfaces from complement damage and that this region in FH cannot be replaced by the C-terminal region of FHR-1 or FHR-3; FH and FHR-1/FHR-3 have different binding specificities. Notably, the observation that the FHR-1::FH hybrid protein impairs complement regulation also indicates that FHR-1 lacks appropriate complement regulatory activity to substitute FH [29].

A second type of rearrangement in the *CFH/CFHR1-5* region is the deletion of the *CFHR3* and *CFHR1* genes. This is a common polymorphism in humans with allelic frequencies ranging from 0 to 0.55 in different populations [32], which originated from a single non-allelic homologous recombination event involving a duplicated region downstream of *CFH* and *CFHR1* [33]. Remarkably, the *CFHR3-CFHR1* deletion is strongly associated with lower risk of AMD [17] and IgAN [16], two prevalent conditions affecting the retina and the kidney, respectively. In contrast, the *CFHR3-CFHR1* deletion is a risk factor for SLE and autoimmune aHUS [24, 32, 34].

Finally, there is a set of genomic rearrangements resulting in the duplication of the dimerization domain of FHR-1, FHR-2 and FHR-5, which are specifically associated with C3G, a heterogeneous group of rare glomerulopathies leading to renal failure characterized by massive deposition of C3 derivatives along the glomerular basement membrane (GBM) [11, 12, 14, 15, 35, 36]. This duplication of the dimerization domain causes abnormal

multimerization of FHR-1, FHR-2 and FHR-5, which increases avidity for their ligands and enhances competition with FH [10, 11]. It is postulated that these gain-of-function mutant FHR-1, FHR-2 and FHR-5 proteins are pathogenic because they over-compete FH binding to host surfaces and impair complement regulation.

As described, the association of the *CFH/CFHRs* rearrangements with disease suggests that FHRs originated to modulate complement activation by competing the binding of FH to surfaces and that this competition can be beneficial or detrimental depending on the surfaces/circumstances.

The evolving understanding of the role of the FHR proteins

Because of their homology with FH, complement regulatory roles were originally postulated for the FHRs [37]. The initial studies, however, could not demonstrate FH-like activity for these proteins [38]. Subsequent studies showed weak cofactor activities of FHR-3, FHR-4 and FHR-5 [39, 40] and synergistic activity with FH, namely, enhancement of the cofactor activity of FH in the case of FHR-3 and FHR-4 [39]. Notably, though, high and now known to be non-physiological concentrations of the FHR-3 and FHR-4 proteins were required for these activities. FHR-5 was also reported to inhibit the alternative pathway (AP) C3 convertase [40] and more recently strong cofactor activity was shown for FHR-3 [41], although this again required a concentration above the likely (yet precisely undefined) plasma concentration of FHR-3 [42]. One study reported a complement inhibiting function for FHR-2 [43] and another showed that FHR-1, instead of having FH-like regulatory activity, inhibits the lytic pathway [44], although others demonstrated that FHR-1 could not significantly influence and inhibit complement-mediated lysis of sheep erythrocytes [29, 45].

The ability of recombinant FHR-1 and FHR-5 proteins or native FHR-1, FHR-2 and FHR-5 oligomers to regulate C3b and the AP has now been more extensively analysed using hemolytic assays and surface plasmon resonance. These analyses demonstrated that these

FHRs bind to C3b, iC3b, and C3dg, but provided no evidence of cofactor activity for the FI-mediated proteolysis of C3b or AP C3 convertase decay accelerating activities [10, 11]. These studies also failed to detect any significant interaction of FHR-1 with C5 [10], indicating that FHR-1, FHR-2 and FHR-5 have no intrinsic C3 or C5 regulatory activity at physiological concentrations. On the other hand, these experiments showed that the FHR-1, -2 and -5 proteins, through their ability to compete with FH for binding to C3b, actually prevent FH-mediated complement regulation. This interference with the FH regulatory activities is apparently facilitated by their oligomerization, which increases avidity for their ligands, and is significantly enhanced by the rare C3G-associated mutations that results in abnormal multimerization of the FHR-1, FHR-2 and FHR-5 proteins [10, 11]. Importantly, it has been reported that FHR-3 and FHR-4 are also able to compete for FH ligands and therefore have the potential to interfere with FH regulation [41, 46].

In addition to playing important roles in controlling FH activities on self, FH and some of the FHRs have also been found to interact with several microbes and microbial proteins. However, only few studies have addressed the functional consequences of the interaction of the FHR proteins with pathogens. These studies showed that none of the bound FHRs (FHR-1, FHR-2 and FHR-5) conferred protection from complement activation and deposition of C3b and C5b-9, whereas binding of FH could reduce or completely block C3b and C5b-9 deposition and lysis [47]. This suggests that FH but not the FHRs protect the microbes from opsonophagocytosis and/or complement-mediated damage when bound on the microbial surface. Strikingly, the FH domains that are well conserved among the FHRs are those that mediate the binding of FH to most known FH-binding pathogens and microbial proteins (Figure 2), raising the possibility that the FHRs have evolved as decoys to reduce the amount of FH bound by the pathogens and, consequently, potentiate their elimination mediated by the host complement system.

Very recent experiments with the major human pathogen *Neisseria meningitidis* provide strong support to these ideas [46]. *N. meningitidis* recruits FH via fHbp, a surface lipoprotein that mimics host carbohydrates and binds FH with high affinity [48]. In their report, Caesar et al. elegantly show that FHR-3 binds fHbp with similar affinities that FH and that FHR-3 competes with FH on the bacterial surface, influencing *N. meningitidis* survival in serum sensitivity assays. These findings may explain the earlier observation that individuals carrying a particular extended *CFH-CFHR3-CFHR1* haplotype present significant protection to *N. meningitdtis* infections [49]. Notably, this haplotype (*H3* haplotype), which is also an important risk factor for aHUS, determines significantly lower levels of FH and includes polymorphisms in potential *CFHR3* regulatory regions that may also affect FHR-3 expression in plasma (Bernabeu-Herrero et al., Submitted).

Some FHRs seem to have acquired functions that enhance complement activation on the surface of pathogens. For example, FHR-4 binds preferentially the native, pentameric form of CRP (pCRP) and can thus allow complement activation [50, 51], whereas FH binds mainly the modified, monomeric form of CRP (mCRP) at lower concentrations [51-53]. Similarly, both FHR-4 and FH bind to C3b, but only FH promotes inactivation of C3b efficiently; FHR-4 rather enhances complement activation by allowing AP convertase formation on FHR-4-bound C3b [54]. This mechanism may result in enhanced opsonization of dying cells or some pathogens in the presence of FHR-4 [55, 56].

Taken together, all of these recent data suggest that the FHRs modulate complement activation by competing with FH for binding to its ligands. In contrast to the binding of FH to surfaces, which prevents further C3b generation and deposition (negative regulation), the binding of the FHRs enables C3b amplification to proceed unhindered. This competition with FH would be influenced by the concentration and composition of the FHRs relative to FH at the site of complement activation, the density and relative deposition of C3b, iC3b and C3dg, the carbohydrate and polyanion composition and the presence of additional ligands on the

complement-activating surface. Understanding all these factors will help to understand the association of the FHRs with disease.

Competition between FH/FHL-1 and FHRs on host and altered host surfaces or molecules.

The main function of FH/FHL-1 is to control the AP amplification loop and to prevent tissue damage by accidental complement activation on self-surfaces. Basically, the role of FH is to maintain the density of C3b molecules on host surfaces below a critical threshold, because if this threshold is exceeded, C3b amplification runs off without control and tissue damage occurs. A first consideration regarding the postulated competitor role of the FHRs is what prevents them from inhibiting the function of FH protecting the normal host cell surfaces and causing disease. A likely answer to this question is that despite the very high conservation of the FH surface recognition domains in some of the FHRs (Figure 1), competition on normal host tissues is very limited. This affirmation is supported by observations in aHUS patients indicating that exchanging the C-terminal regions between FH and FHR-1 has identical pathogenic consequences to that of the disease-associated FH mutations that disrupt the C-terminal functionalities (Figure 3A). Therefore, despite there are only two amino acid differences (S1191L, V1197A) between the C-terminal regions of FH and FHR-1, these differences are sufficient to alter sialic acid recognition [57], conferring distinct surface binding specificity to FH and FHR-1 and eliminating the risk of an undesirable competition between them for host tissues. The possibility that the FH/FHR competition affects mainly a specific subset of surfaces, which does not include normal host surfaces, may help to understand the association of the FHRs with pathology. Our proposal is, therefore, that the FHRs originated through evolution to prevent binding of FH to certain pathogens (Figure 3B) and that they also compete binding of FH to altered host surfaces (Figure 3C), perhaps because these altered host surfaces include molecules that resemble

those present on the surface of pathogens. This proposal assumes that there must be complement activation and C3 deposition on these surfaces (spontaneous, following a trigger, or both) that would be accelerated in the presence of these FHR proteins. The presence of the activated C3 fragments iC3b and C3dg, which are better ligands for some FHRs than for FH [10, 11], may be crucial to sustain a competition between FH and the FHRs at these surfaces. Additional poorly characterized "injury-associated" changes in host surfaces may favour this competition [58].

In this setting, the relative amounts of FH and FHRs, established by their levels of expression or activity, appear critical to modulate complement regulation and to determine susceptibility to complement-mediated injury. Plasma levels of FH vary widely (116–562 μg/ml) in humans as the result of the combined effect of genetic and environmental factors. Notably, 63% of the variation in plasma levels of FH is determined genetically by polymorphisms likely linked to the *CFH* gene in 1q32 [59]. Strong linkage dysequilibrium limits the genetic variability in that genetic region to a few *CFH-CFHR3-CFHR1* extended haplotypes [2, 17]. Interestingly, two of these haplotypes that have been strongly associated with various diseases determine significantly different plasma levels of FH and FHRs. Haplotype H3 determines significantly lower levels of FH in plasma and may also affect FHR-3 levels in plasma (Bernabeu-Herrero et al., submitted). This could imply an increased competition between FH and FHR-3 and may explain the association of this haplotype with protection to meningococcal disease [59]. Haplotype H4 carries the *CFHR3-CFHR1* deletion and also determines increased levels of FH [60], a combination that, enhancing FH regulation on surfaces, could explain the association of this haplotype with protection against AMD and IgAN [2, 17].

Despite these genetically determined population variations, FH serum levels are not actively regulated individually [59], which may not be the case for FHRs. One report suggests that the concentrations of FHRs increase upon infection [61] and there is evidence that FHRs

levels may also change in other situations (our own data) [62, 63]. Changes in FHRs levels may explain the episodic nature of some diseases associated with complement dysregulation in which relapses often associate with infection (i.e., C3G, IgAN). The possibility that FHR levels may be influenced by physiological and pathological conditions warrant further studies since it would be predicted that a local increase in the concentration of the FHRs would, through enhanced FH competition, enable (rapid) enhancement of complement activation.

The FH/FHRs balance may also be altered in situations in which the FHRs are mutated and acquire gain-of-function activities. Hybrid and mutant FHR-1, FHR-2 or FHR-5 proteins containing a duplicated dimerization domain are good examples of this situation [10, 11, 14]. Similarly, the association with C3G of novel *CFHR3::CFHR1* and *CFHR5::CFHR2* hybrid genes may be explained by an altered FH/FHR balance [64]. These hybrid genes express the hybrid protein on top of the FHRs produced by the normal copies of *CFHR1* and *CFHR3*, which result in increased levels of these FHRs to compete FH.

The identification of the specific surfaces that sustain the FH/FHR competition in these pathologies is, however, challenging. The GBM and the Bruch's membrane (BM) are good candidate surfaces for competition between FH/FHL-1 and FHRs. It has been shown that FH, FHL-1, FHR-1 and FHR-5 bind to extracellular matrix components (ECM), the major constituent of the GBM and the BM, and that FHR-5 inhibits the surface-associated cofactor activity of FH by competing its binding to the ECM [65-67]. This competition may increase by the presence of microbial ligands or other physiological or pathological substances deposited together with C3 activated fragments along the GBM or the BM.

In addition, there are additional molecules on apoptotic cells, retinal deposits and other structures that can sustain this FH/FHRs competition. One of such ligands is malondialdehyde (MDA) epitopes that originate from peroxidation of membrane lipids due to oxidative stress [68]. It is thought that MDA epitopes are responsible for the recruitment of FH to the surface of apoptotic cells, where FH neutralizes their proinflammatory properties and halts

complement activation [69]. In AMD, a common eye condition among elderly people this situation may be particularly relevant as in the retina dying cells are continuously generated and need to be efficiently removed. FH binds MDA epitopes through SCRs 6-7 and SCRs 19-20, which may explain the association of both, the 402His polymorphism in SCR7 with increased risk and the *CFHR3-CFHR1* deletion with strong protection to AMD [17, 70-73]. Accordingly, the FH risk variant 402His was shown to present decreased binding to MDA compared to the 402Tyr variant and evidence was provided suggesting that FHR-1 could compete the binding of FH to MDA [69].

IgA nephropathy (IgAN) is a common form of primary glomerulonephritis characterized by galactose-deficient IgA1 (Gd-IgA1)-containing immune complexes that deposit in the glomerular mesangium producing progressive kidney disease [74]. IgAN is also strongly associated with the *CFHR3-CFHR1* deletion [16]. Similarly to AMD, the reported protection to IgAN conferred by the lack of FHR-3 and FHR-1 may also relate to the generation of altered host surfaces. Pathogenesis of IgAN is currently modelled as a sequence of multiple events, one of them being the generation of antiglycan antibodies that recognize GalNAc-containing epitopes on Gd-IgA1 [75]. It is possible that Gd-IgA1-containing immune complexes deposited in the mesangium may resemble pathogen surfaces where competition between FH and FHRs may occur. We speculate that this competition would be further enhanced by the deposition of the C3 activated fragments generated by activation of the lectin pathway [76]. The advantage of individuals carrying the *CFHR3-CFHR1* deletion would be to have less FHRs to interfere with the regulation by FH of the complement activation induced by these (Gd-IgA1)-antiglycan immune complexes in the kidney.

Complement also plays an important role in SLE, a severe autoimmune disease characterized by the presence of autoantibodies that result in tissue injury of multiple organs [24]. Here, in contrast to AMD and IgAN, the *CFHR3-CFHR1* deletion represents an important predisposition factor, suggesting that in this case decreased FH/FHR competition is

deleterious. Two possibilities have been proposed to explain this association; one is based on the capacity of FHR-1 to inhibit C5 convertase activity and MAC formation, a role of FHR-1 that is currently controversial. The second explanation relates to the possibility that carriers of the *CFHR3-CFHR1* deletion generate autoantibodies against FH, similarly to the situation in aHUS where these antibodies are associated with the homozygous deficiency of FHR-1 [77]. The contrasting phenotypic association of SLE with the *CFHR3-CFHR1* deletion, opposing the protective effect observed for IgAN and AMD, may also suggest that some AP activation could be beneficial in SLE. For example, the *CFHR3-CFHR1* deletion may result in less opsonization and opsonophagocytic removal of autoantigens due to increased complement regulation by FH on apoptotic cells. In that setting, enhanced levels of autoantigens may promote production of pathogenic autoantibodies recognizing these targets.

Conclusions

As a whole, there is little convincing evidence for the existence of physiologically-relevant complement-inhibitory activities of the FHRs. On the other hand, recent data and critical evaluation of previous studies suggest a FH antagonistic function for the FHRs, termed complement de-regulation. Future studies will define the ligands and surface specificity of the FHRs in more detail and also the conditions under which competition with FH occurs. This knowledge will advance our understanding of the pathogenic mechanisms of several diseases associated with complement dysregulation. We do not exclude that FHRs may have functions independent of the activity of FH, such as the enhancement of complement activation as showed for FHR-4 and even some complement inhibitory activities. Currently, however, strong in vitro evidence and data from different diseases suggest a major role of the FHRs as competitive inhibitors of FH for fine-tuning discrimination of complement activating surfaces.

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Conflicts of interest

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BOX. Complement activation and its regulation by FH

Pathogens, altered host cells or molecules and certain host ligands released/exposed during infections and inflammatory processes, such as pentraxins, molecules of the extracellular matrix and DNA, can activate complement. The classical complement pathway is activated by immunoglobulins, pentraxins or by the direct binding of C1q to such ligands/surfaces. The lectin pathway is initiated via target-bound mannose-binding lectin or ficolins. The C3b fragment generated from C3 through these activation routes feeds into the alternative pathway (AP), amplifying the classical and lectin pathway-mediated activation. The tick-over mechanism [78] ensures a low-rate constant activation of C3 in plasma through the AP. Properdin may also act as an AP initiator [79]. Cleavage of C3 into C3b results in the covalent binding of C3b to the activating surface. Incorporation of additional C3b molecules to the surface-bound AP C3-convertases generates the C5-convertases with the capacity to bind and cleave C5, leading to the initiation of the lytic pathway and the generation of terminal complement complexes (termed membrane attack complex (MAC) when integrated into target cell membranes) (Figure). Regulation of complement activation is a necessary and complex process involving several soluble and membrane-associated proteins (Depicted in red in the figure). Among them factor H (FH), the major regulator of the AP, is the prototypical member. FH is a relatively abundant plasma protein that is essential to maintain complement homeostasis and to restrict the action of complement to activating surfaces. FH binds to C3b, accelerates the decay of the AP C3-convertase (C3bBb) and acts as a cofactor for the factor I (FI)-mediated proteolytic inactivation of C3b. FH regulates complement both in fluid phase and on cellular surfaces. However, while FH binds and inactivates promptly C3b in fluid phase, the inactivation of surface-bound C3b by FH is dependent on the chemical composition of the surface to which C3b is bound. In the presence of polyanions like sialic acids, glycosaminoglycans or sulphated polysaccharides (heparins), the affinity of FH for surface-bound C3b increases as a consequence of the simultaneous recognition of both

polyanionic molecules and bound C3b by the same FH molecule [57, 67, 80, 81]. Abbreviations for other complement proteins depicted in the figure are C4b-binding protein (C4bp), membrane cofactor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and complement receptor type 1 (CR1; CD35). These proteins restrict complement activation on self-tissues by either catalyzing proteolytic inactivation of C3b/C4b by FI (MCP, CR1, C4bp) or accelerating convertase dissociation (DAF, CR1, C4bp).

Glossary Box

Age-related macular degeneration: a major cause of blindness among the elderly, with multiple predisposing factors including complement gene variants, and characterized by the accumulation of waste material along the Bruch's membrane in the retina.

Atypical hemolytic uremic syndrome: a form of thrombotic microangiopathy characterized by hemolytic anemia, low platelet count and acute renal failure, with predisposing complement gene variants or autoantibodies to factor H.

C3 glomerulopathy: various forms of glomerulonephritis characterized by involvement of the alternative complement pathway and C3 deposition in the glomeruli.

Cofactor activity: the ability of factor H and some other complement regulators to assist the serine protease factor I in the enzymatic degradation of the central complement fragment C3b.

Complement de-regulation: the ability of the factor H-related proteins to competitively inhibit the complement regulator factor H.

Decay accelerating activity: the capacity of factor H and a few other complement regulators (decay accelerating factor, complement receptor type 1) to facilitate the disassembly of the C3 convertase enzyme (C3bBb).

Haplotype: A set of DNA variations (i. e., mutations, single nucleotide polymorphisms, copy number variations) found on the same chromosome and that tend to be inherited together.

IgA nephropathy: a form of glomerulonephritis characterized by IgA-containing immune complexes.

MAC: membrane attack complex, a pore formed by complement proteins C5b, C6, C7, C8 and C9 in the cell membrane of target cells.

MDA epitopes: malondialdehyde epitopes that originate from peroxidation of membrane lipids due to oxidative stress and that modify primary amino groups in proteins and lipids .

SCR domain: short consensus repeat or Sushi domain, also known as complement control protein (CCP) domain, a domain characteristic to many complement regulatory and other proteins.

SLE: systemic lupus erythematosus, a systemic autoimmune disease characterized by anti-DNA and other autoantibodies.

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Table 1. Known host ligands of the human FHR proteins

Protein	Ligand	Associated function	Reference
FHR-1	C3b, C3d	Competition with FH (no cofactor / decay accelerating activity)	[10, 11, 38, 44]
	Heparin	Unknown	[44]
	PTX3	Unknown	[67]
	C5	Terminal pathway inhibition (Questioned by others)	[10, 44, 82]
	Lipoproteins (HDL)	Unknown	[83]
	CR3	Cellular adhesion	[55]
FHR-2	C3b, C3d	Inhibition of C3 convertase (no cofactor and decay accelerating activity). Terminal pathway inhibition	[43]
	Heparin	Unknown	[43]
	Lipoproteins (HDL)	Unknown	[83]
FHR-3	C3b, C3d	Enhancement of FH cofactor activity	[39]
	C3b	Cofactor activity	[41]
	Heparin	Unknown	[39]
FHR-4A **FHR-4B**	C3b, C3d	Enhancement of FH cofactor activity	[39, 54]
	C3b	Enhancement of AP activation	[54]
	CRP	Enhancement of CP activation	[50]
	Lipoproteins (Chylomicron, LDL, VLDL)	Unknown	[84]
FHR-5	C3b	Inhibition of C3 convertase; weak cofactor activity	[40]
		Competition with FH	[10]
	Heparin	Unknown	[40]
	CRP	Unknown	[40]
	Lipoproteins (HDL)	Unknown	[40]

408 **HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein**

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410 **TABLE 2.** CFHRs gene rearrangements associated with disease.

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Disease		Genetic findings	Outcome	Significance	Risk/ Protection	Prevalence	References
aHUS		CFH::CFHR1 hybrid genes	Substitution of the C-terminal SCR of FH for that in FHR-1.	Loss of complement regulation at cell surfaces.	R	Several unrelated cases described	[28]
		CFHR1::CFH hybrid genes	Substitution of the C-terminal SCR of FHR1 for that in FH. Two C-term SCRs for those of FH	Loss of complement regulation at cell surfaces.	R	Few unrelated cases described	[29, 85]
		CFH::CFHR3 hybrid gene	Substitution of the last C-terminal SCR20 of FH for the whole FHR-3.	Loss of complement regulation at cell surfaces	R	Very rare	[86]
		DelCFHR3-CFHR1 DelCFHR1-CFHR4	Loss of FHR-3 and FHR-1. Loss of FHR-1 and FHR-4.	Associated with FH autoantibodies impairing cell surface regulation.	R	Common	[12, 87, 88]
C3G	**DDD**	DupCFHR1	Mutant FHR-1 with SCR123412345	Abnormal oligomerization. Increased competition with FH.	R	Very rare	[11]
		CFHR2::CFHR5 hybrid gene	Hybrid protein containing SCR1-2 of FHR-2 followed by the whole FHR-5 molecule.	Abnormal oligomerization. Increased competition with FH.	R	Very rare	[14]
	C3-GN	CFHR3::CFHR1 hybrid gene	Hybrid protein containing SCR1-2 of FHR-3 followed by the whole FHR-1 molecule.	Increased levels of FHR-1 Increased competition with FH?	R	Very rare	[19]
		CFHR5::CFHR2 hybrid gene	Hybrid protein containing SCR1-2 of FHR-5 followed by the whole FHR-2 molecule.	Increased levels of FHR-2 Increased competition with FH?	R	Very rare	[64]
		DupCFHR5	Mutant FHR-5 with SCR12123456789	Abnormal oligomerization. Increased competition with FH.	R	Several related cases described, 1 unrelated	[10, 15, 89]
AMD		DelCFHR3-CFHR1	Loss of FHR-3 and FHR-1.	No competition with FH?	P	Common	[17]
IgAN		DelCFHR3-CFHR1	Loss of FHR-3 and FHR-1.	No competition with FH?	P	Common	[16]
SLE		DelCFHR3-CFHR1	Loss of FHR-3 and FHR-1.	No competition with FH?	R	Common	[24]

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627 **Figure legends**

628 **Figure 1. The FH/FHRs family of proteins**

629 A) Genomic organization of the *CFH* and *CFHR1-5* genes. Arrows represent the genes with
630 their names. The coloured boxes underneath indicate the sequence repeats. The vertical
631 lines indicate the position of the exons of the *CFH* and *CFHRs* genes.
632 B) Structural organization of the FH and FHR proteins. Short consensus repeats (SCRs) are
633 represented by ovals and are numbered from the N-terminal end. Homologous SCRs are
634 aligned. Colours illustrate SCRs presenting almost complete identity of amino acid
635 sequences.
636 C) Percentages of amino acid similarities are provided for SCRs 1 and 2 between FHR-1,
637 FHR-2 and FHR-5 and for SCRs 6 and 7 in FH and SCRs 1 and 2 in FHR-3. The diagram
638 also includes alignments to illustrate the similarities between the C-terminal regions of
639 FH and the two FHR-1 alleles, as well as between FHR-3 and the two FHR-4 isoforms.
640 D) FHR-1, FHR-2 and FHR-5 complexes for which there is experimental evidence. Models
641 are drawn based on structural data demonstrating that the first two amino terminal SCRs
642 of these proteins form dimers in a head to tail orientation [10]. For simplicity, tetramers
643 are also depicted showing interactions through these SCRs.
644

645 **Figure 2. Main binding sites within FH for host and bacterial ligands.**

646 Figure depicts a diagram of the 20 SCR domains of FH. SCRs 1-4 are responsible for the
647 cofactor and decay accelerating activities (yellow), and SCRs 7 and 19-20 contain major
648 recognition sites for host and bacterial ligands (blue). The main host ligands are listed and
649 their binding sites in FH indicated by red horizontal lines. Selected microbial ligands are
650 similarly shown at the bottom part of the figure.
651

Figure 3. Competition between FH and FHRs on self, altered-self and pathogen surfaces.

The figure depicts potential scenarios of FH/FHRs competition. Host cells, available data suggest that on normal endothelial cells, there is no competition between FH and FHR-1 and FHR-3. In aHUS, it has been established that the pathogenic mechanism is a defect in the protection of endothelial cells from complement damage. The prototypical genetic defects are C-terminal FH mutations and hybrid proteins involving FH, FHR1 and FHR3. The pathogenicity of the latter demonstrate both that FHR-1 and FHR-3 cannot replace the complement regulatory activities of FH and that they do not compete binding of FH to the aHUS relevant surfaces. Certain pathogens, like *Neisseria* express FH binding proteins in their surface that contribute to survival of the pathogen. Different FH/FHRs ratios could explain differences in susceptibility of the host to *Neisseria* infections. Alteration of the FH/FHRs ratio and modification of host surfaces by genetic or environmental factors, potentially contribute to sustain a competition between FH and the FHRs and result in pathology. Singular and altered host surfaces are terms used here in a wide and overlapping sense to refer, for example, to extracellular matrix and other cell surface components modified by aging, microbial and chemical agents, or by deposition of immune complexes (including those containing galactose deficient-IgA), or even to iC3b, C3dg opsonised surfaces. We like to suggest that on these singular and altered host surfaces an unbalanced FH/FHRs ratio causes complement dysregulation.

Fig. 1.

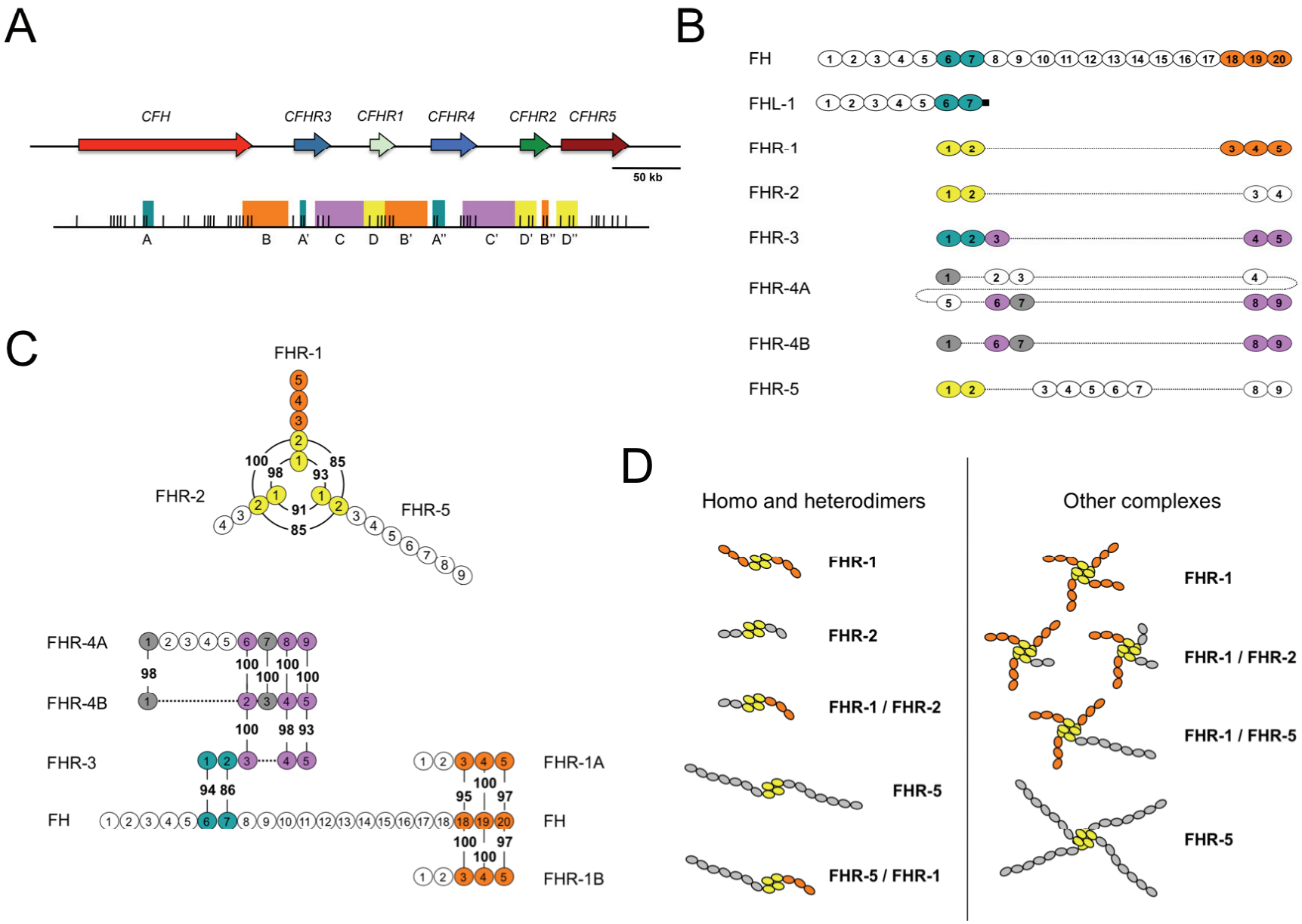

A
CFH
CFHR3
CFHR1
CFHR4
CFHR2
CFHR5
50 kb
A
B
A'
C
D
B'
A''
C'
D'
B''
D''
B
FH
FHL-1
FHR-1
FHR-2
FHR-3
FHR-4A
FHR-4B
FHR-5
C
FHR-1
FHR-2
FHR-5
100
98
93
85
91
85
FHR-4A
FHR-4B
FHR-3
FH
FHR-1A
FHR-1B
98
100
100
100
100
98 93
94 86
95
97
100
97
100
D
Homo and heterodimers
Other complexes
FHR-1
FHR-2
FHR-1 / FHR-2
FHR-5
FHR-5 / FHR-1
FHR-1
FHR-1 / FHR-2
FHR-1 / FHR-5
FHR-5

Fig. 2.

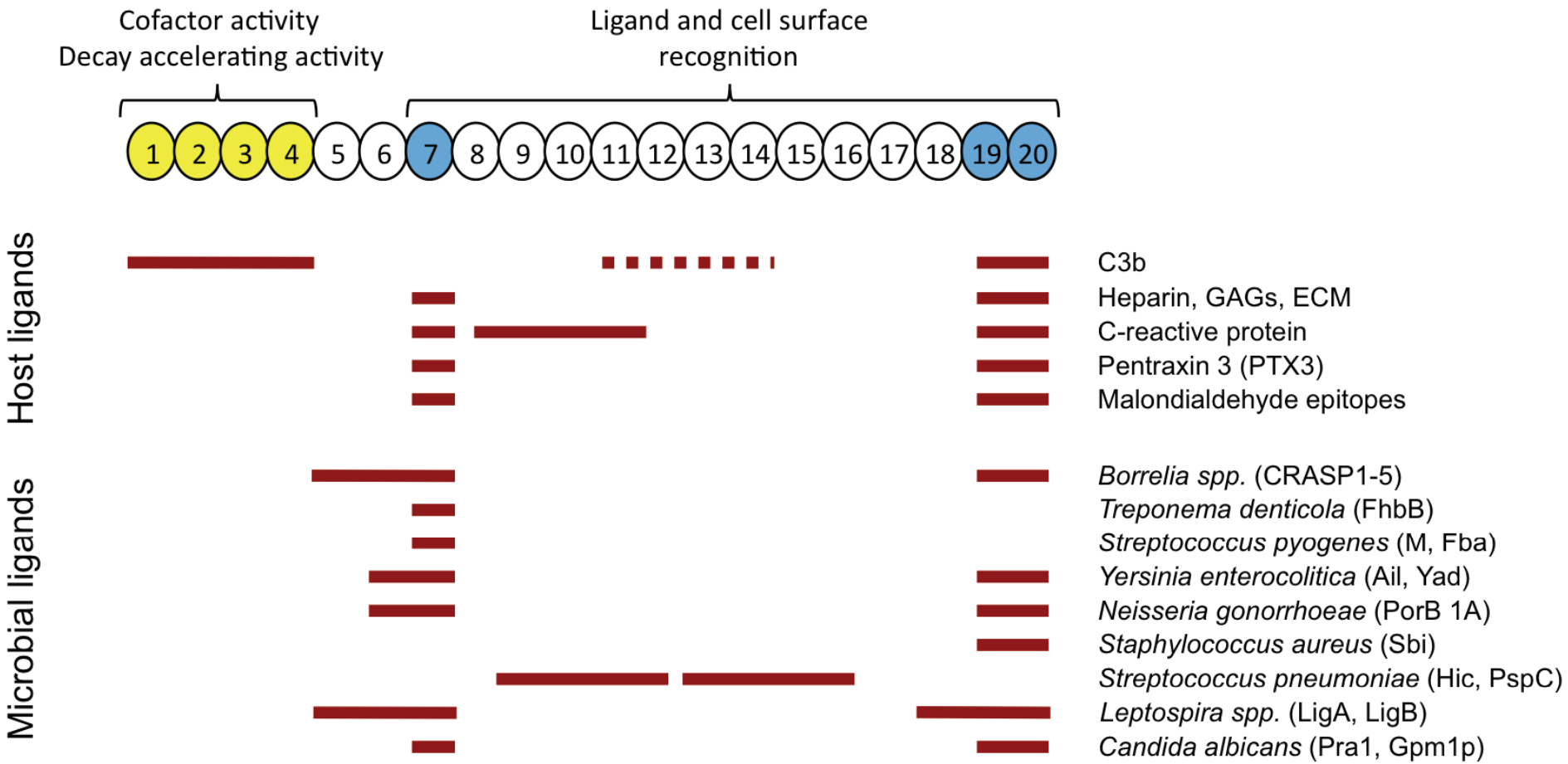
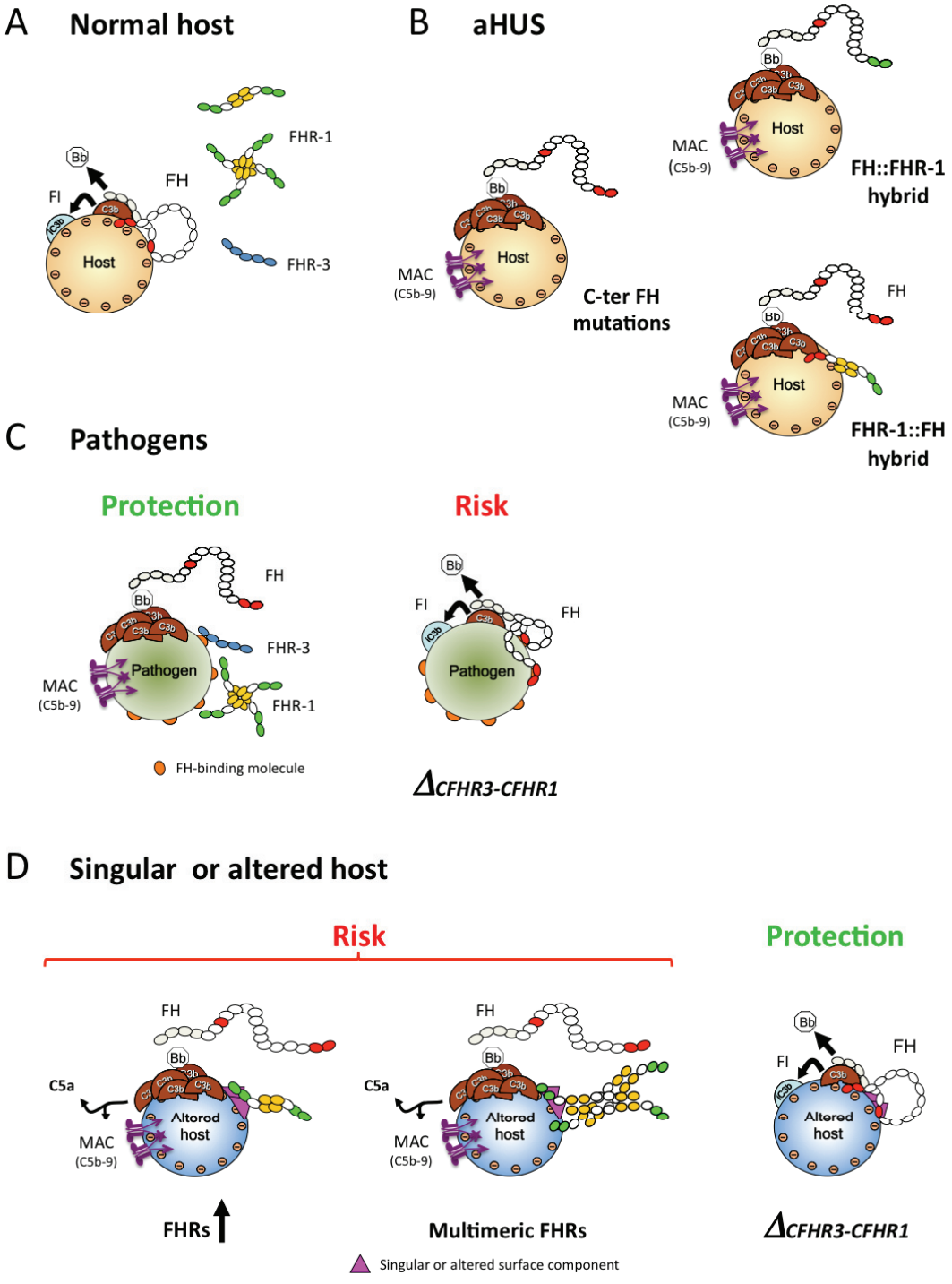
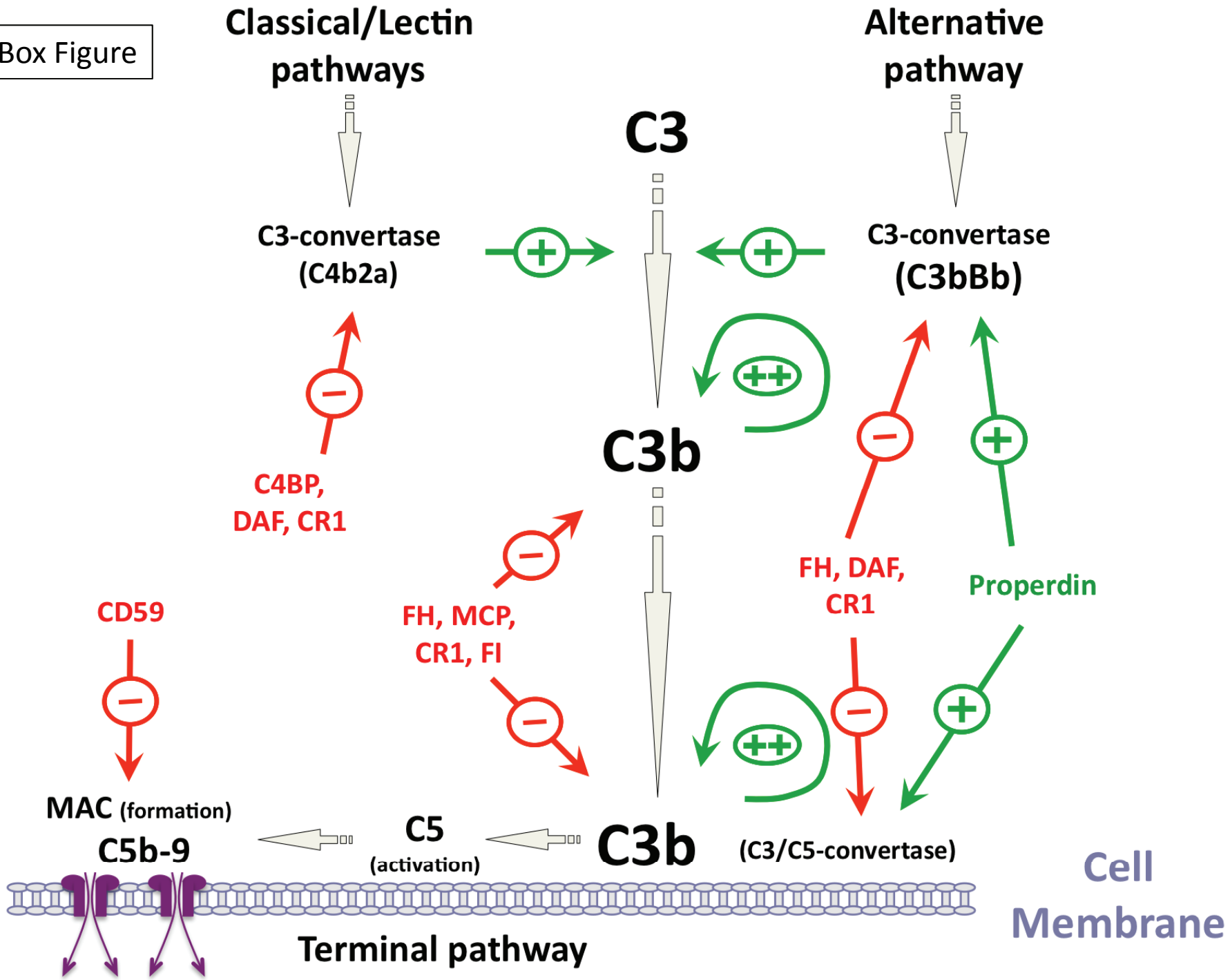

Cofactor activity
Decay accelerating activity
Ligand and cell surface
recognition
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Host ligands
Microbial ligands
C3b
Heparin, GAGs, ECM
C-reactive protein
Pentraxin 3 (PTX3)
Malondialdehyde epitopes
Borrelia spp. (CRASP1-5)
Treponema denticola (FhbB)
Streptococcus pyogenes (M, Fba)
Yersinia enterocolitica (Ail, Yad)
Neisseria gonorrhoeae (PorB 1A)
Staphylococcus aureus (Sbi)
Streptococcus pneumoniae (Hic, PspC)
Leptospira spp. (LigA, LigB)
Candida albicans (Pra1, Gpm1p)

Fig. 3.


A Normal host
FHR-1
FH
FI
Bb
C3b
Host
FHR-3
B aHUS
Bb
C3b
Host
MAC
(C5b-9)
C-ter FH
mutations
Bb
C3b
Host
MAC
(C5b-9)
FH::FHR-1
hybrid
FH
Bb
C3b
Host
MAC
(C5b-9)
FHR-1::FH
hybrid
C Pathogens
Protection
Risk
FH
Bb
C3b
FHR-3
Pathogen
MAC
(C5b-9)
FHR-1
FH-binding molecule
Bb
FI
FH
C3b
Pathogen
ΔCFHR3-CFHR1
D Singular or altered host
Risk
Protection
FH
Bb
C5a
C3b
Altered
host
MAC
(C5b-9)
FHRs
FH
Bb
C5a
C3b
Altered
host
MAC
(C5b-9)
Multimeric FHRs
Bb
FI
FH
C3b
Altered
host
ΔCFHR3-CFHR1
Singular or altered surface component

Box Figure


Classical/Lectin pathways
Alternative pathway
C3
C3-convertase (C4b2a)
C3-convertase (C3bBb)
C3b
C4BP, DAF, CR1
FH, MCP, CR1, FI
FH, DAF, CR1
Properdin
CD59
MAC (formation)
C5b-9
C5 (activation)
C3b
(C3/C5-convertase)
Terminal pathway
Cell Membrane