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Molecular Immunology 41 (2004) 103–111
Molecular Immunology
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Review

# Primitive complement system—recognition and activation

Teizo Fujita[a,*], Yuichi Endo[a], Masaru Nonaka[b]

[a] Department of Biochemistry, Fukushima Medical University, Fukushima 960-1295, and CREST, Japan Science and Technology Agency, Japan
[b] Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

**Abstract**

The complement system, composed of more than 30 serum and cell surface components, is collaborating in recognition and elimination of pathogens as a part of both the innate and acquired immune systems. The two collagenous lectins, mannose-binding lectin (MBL) and ficolins, are one of the pattern recognition molecules acting in innate immunity and upon recognition of the pathogens, they trigger the activation of the lectin complement pathway through attached serine proteases (MASPs). A similar lectin-base complement system, consisting of the lectin-protease complex and C3, is present in ascidians, our closest invertebrate relatives and functions in an opsonic manner. On the other hand, ongoing genome projects in both vertebrates and invertebrates revealed that most domains used by mammalian complement components are found in both protostomes and deuterostomes. However, the unique combinations of them as found in mammalian complement components are present only in deuterostomes, indicating the deuterostome origin of the complement system. Unexpectedly, the complement system of an invertebrate deuterostome, ascidian, shows a similar level of complexity as that of mammals, suggesting that expansion of complement genes by gene duplications occurred independently both in the ascidian and vertebrate lineages. Although most characteristic domain structures of the mammalian complement components are found in ascidians, detailed evolutionary analysis casts doubt on their mutual reactivity in several points. Thus, another integrative step seems to have been required to establish the modern complement system of higher vertebrates.
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*Keywords:* Complement; Lectin pathway; Innate immunity; Evolution

## 1. Introduction

Immunity to infection is mediated by two general systems, acquired (or adaptive) and innate (or natural). The innate immune system is an evolutionarily ancient form and crucial for the first line of defense before the acquired immune system comes into play (Hoffmann et al., 1999). Innate immunity was formerly thought to be a nonspecific immune response characterized by phagocytosis. However, innate immunity has considerable specificity and is capable of discriminating between pathogens and self as proposed in the concept of pattern recognition molecules of host. These molecules recognize conserved pathogen-associated molecular patterns (PAMPs) shared by large group of microorganisms, thereby successfully defending invertebrates and vertbrates against infection (Medzhitov and Janeway, 2000).

The complement system is a highly sophisticated biological reaction system, playing a major role in body defense as a part of both the innate and adaptive immune systems (Walport, 2001a,b). Complement was first described in the 1890s as a heat-labale protein in serum that 'complemented' heat-stable antibodies in the killing of bacteria. Fifty years later it was proposed that complement could be activated by bacterial surfaces through an antibody-independent pathway, the alternative pathway that was not easily acceptable at that time. Recently, the third pathway, the lectin pathway, was found (Fujita, 2002; Matsushita and Fujita, 1996). Now, the complement system consists of three activation pathways, classical, alternative and lectin pathways, which merge at the proteolytic activation step of C3, the central component of the complement system. C3 is equipped with a unique intramolecular thioester bond which is exposed to the molecular surface upon activation and forms a covalent bond with invading microorganisms (Law et al., 1980). This covalent tagging of foreign molecules by C3 is considered to be one of the most important function of the complement system, and once C3 tag is added, which enhances the phagocytosis of pathogens through C3 receptors on phagocytes, and activates the complement late components, C5–C9, leading to cytolytic complex (membrane attack complex (MAC)) formation. The classical pathway is activated by antibody-antigen complexes and is a major effector of antibody-mediated immunity, and the other two, the lectin and alternative pathways function in innate immune defense. The lectin pathway involves carbohydrate recognition by mannose-binding lectin

* Corresponding author. Fax: +81-24-548-6760.
*E-mail address:* tfujita@fmu.ac.jp (T. Fujita).

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doi:10.1016/j.molimm.2004.03.026

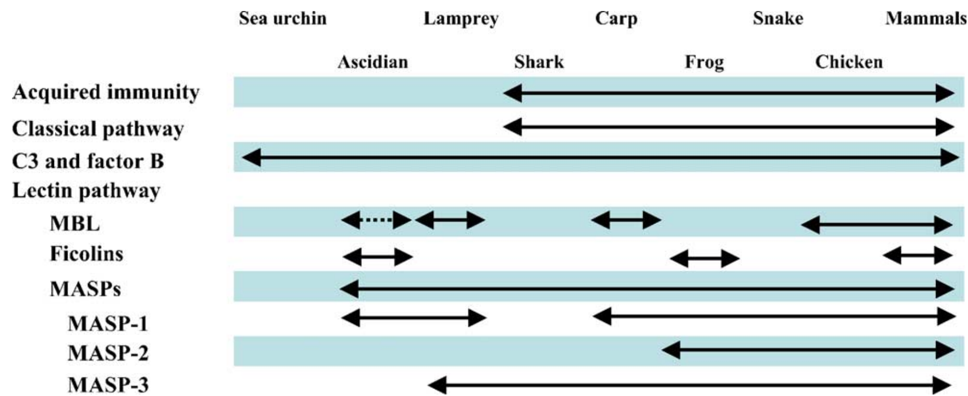


Fig. 1. The complement system from an evolutionary perspective. Acquired immunity was established at an early stage in the evolution of the jawed vertebrates, illustrated with a shark in this figure. Evolutionary studies have revealed that cartilaginous fish (shark) and higher vertebrates possess a well-developed complement system with all three activation pathways, although not all components of each pathway have been identified. C3, the central component of the complement system, and C2/fB-like sequence have been identified in a marine invertebrate, sea urchin. In ascidian several pivotal molecules such as GBL homologous to MBL, ficolins, MASPs, C3 and C3 receptor have been identified. The development of each type of MASP is also shown.

(MBL) and ficolins, that are typical pattern recognition molecules (Holmskov et al., 2003; Matsushita and Fujita, 2001, 2002), and the subsequent activation of associated unique enzymes, MBL-associated serine proteases (MASPs) (Matsushita et al., 1998; Schwaeble et al., 2002). The alternative pathway is initiated by the covalent binding of a small amount of C3 to hydroxyl or amine groups on cell surface molecules of microorganisms and does not involve specific recognition molecules. This pathway also functions to amplify C3 activation (amplification loop) (Walport, 2001a,b).

Most components of the classical pathway have their structural and functional counterparts in the alternative or lectin pathways, suggesting that gene duplications played an important role in establishing these three pathways (Nonaka et al., 1998). Evolutionary analysis of the complement system is expected to elucidate when and how these gene duplications and modification occurred to establish the modern complement system as found in mammals. Accumulating evidence indicates that adaptive immunity was established at an early stage of the jawed vertebrate evolution, and it is proposed that the two rounds of tetraploidization postulated at the early stage of vertebrate evolution have played some role in this process (Kasahara et al., 1997). The complement system has a more ancient origin, and all major invertebrate deuterostome groups so far studied, sea urchin, ascidians and amphioxus, as well as jawless vertebrates such as lamprey and hagfish have this system (Fujita, 2002; Nonaka, 2001). Since these animals are believed to have diverged from the jawed vertebrate lineage prior to the two rounds of tetraploidization, their complement systems are expected to be simpler than those of higher vertebrates. These duplications could be an epoch-making event, which enabled generation of the classical pathway, considered to be the most modern pathway contrary to its name, from the alternative and lectin pathways (Nonaka et al., 1998). Thus, the modern complement system seems to have been established by the emergence of jawed vertebrates, and accumulating evidence indicates that the complement system of bony and cartilaginous fish has a basically the same set of components as the mammalian complement system (Nonaka, 2001). The development of the complement system is illustrated in Fig. 1.

In addition, one of the outstanding advances in recent complement research is the discovery of the lectin pathway. In the lectin pathway, MBL and ficolin act as the recognition molecules and activate complement in association with MASPs, a C1r/C1s-like serine protease that is capable to cleave the complement components C4, C2 and C3. Recent biochemical identification of several components of the lectin pathway from solitary ascidian, *Halocynthia roretzi*, revealed that the primitive complement system is one of the most highly organized innate immune system in invertebrate. In addition, ongoing genome projects, especially, genome analysis of an ascidian, *Ciona intestinalis* revealed possible architecture of the ascidian complement system.

In this review we summarized recognition molecules and associated serine proteases in complement activation briefly. For evolution of ficolins, MASPs, C3, and C2/Bf which are also important for understanding the molecular evolution of the complement system, we refer the reader to other reviews (Matsushita et al., 1998, 2001; Matsushita and Fujita, 2001, 2002; Nonaka and Miyazawa, 2002; Nonaka and Yoshizaki, 2004a,b; Fujita et al., 2004). In addition, we introduce the detailed architecture of the primitive complement system as revealed by the functional and biochemical data and by the draft genome sequence of *C. intestinalis* (Dehal et al., 2002).

## 2. Recognition molecules and associated serine proteases in complement activation

Activation of the mammalian classical and lectin complement pathways is initiated by C1q and MBL or ficolin,

respectively (Fujita, 2002). All these initiation molecules have an N-terminal collagen structure and a C-terminal globular domain: the C1q domain, the C-type lectin domain and the fibrinogen domain, respectively. C1q has an unusual modular structure consisting of six globular heads each connected by strand to a central fibril-like region, composed of collagen-like triple-helical structure (Kishore and Reid, 2000). The overall structure of C1q is similar to those of two types of lectins, MBL and ficolins, recognizing moiety of the lectin pathway (Holmskov et al., 2003). The classical pathway activation is triggered by binding of the recognition subcomponent C1q to the antibody that, in turn, is translated into activation of the serine protease C1r and C1s (Arlaud et al., 2002). Likewise, binding of the lectin pathway recognition molecules (i.e. MBL or ficolins) to microbial carbohydrates activates the serine protease MASPs–MASP-1 (Matsushita and Fujita, 1992; Sato et al., 1994; Takada et al., 1993), MASP-2 (Thiel et al., 1997) and MASP-3 (Dahl et al., 2001)—specific for the lectin pathway (Thiel et al., 2000).

### 2.1. MASPs

Among three MASPs, MASP-2 is the enzyme component that—like C1s in the classical pathway—cleaves the complement components C4 and C2 to form the C3 convertase C4b2a, common for both the lectin and the classical pathway activation route (Thiel et al., 2000). On the other hands, alternatively, MASP-1 is capable of cleaving C3 directly (Dahl et al., 2001; Matsushita and Fujita, 1995; Rossi et al., 2001), resulting in activation of the alternative pathway (Matsushita and Fujita, 1995), although the C3-cleaving activity of human MASP-1 was questioned, based on recombinant proteins (Ambrus et al., 2003). The function of MASP-3 is not yet clear. In addition, a nonprotease, small MBL-associated proteins (sMAP or MAP19; truncated form of MASP-2) (Stover et al., 1999; Takahashi et al., 1999) was associated with MBL and ficolins.

As described above, MASPs, C1r and C1s are proteolytic enzymes responsible for the activation of the mammalian complement system through the lectin or classical pathway. They share a unique domain structure that is not found in any other proteases. In humans, at least four protein forms are known; MASP-1, MASP-2, MASP-3, and sMAP. They are derived from two MASP genes, MASP-1 and MASP-3 from the MASP-1/3 gene and MASP-2 and sMAP from the MASP-2 gene. The MASP-1/3 gene has dual serine protease-encoding regions, one without intron and the other with introns, and differential usage of these serine protease-encoding regions generates MASP-3 and MASP-1, respectively. They share the same N-terminal sequence termed the heavy chain. sMAP is produced form of the MASP-2 gene, by differential usage of the poly(A) addition sites, and lacks its C-terminus including the serine protease domain. Recently, we reported the origin of MASP-1 and MASP-3 (Endo et al., 2003), and also several review articles (Fujita, 2002; Nonaka and Miyazawa, 2002; Nonaka and Yoshizaki, 2004b; Fujita et al., 2004).

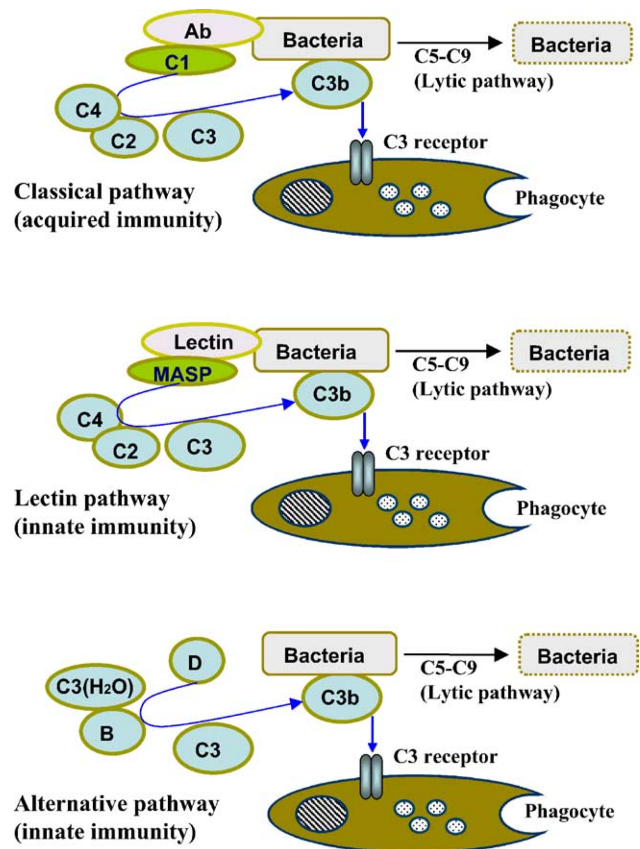


Fig. 2. Activation of the classical, lectin, and alternative pathways. The classical pathway is initiated by the binding of the C1 complex to antibodies bound to antigen on the surface of bacteria. The C1 complex consists of C1q and two molecules of C1r and C1s. The binding of the recognition subcomponent C1q to the Fc portion of immunoglobulins which results in autoactivation of the serine protease C1r. C1r then cleaves and activates C1s, the enzyme that translates the activation of the C1 complex into complement activation through the cleavage of C4 and C2 to form a C4bC2a enzyme complex. C4bC2a acts as a C3 convertase and cleaves C3 resulting in products that bind to and result in the destruction of invading bacteria by formation of lytic pathway, C5–C9 (MAC). The lectin pathway is initiated by binding of either of MBL or ficolin, associated with MASP-1, -2 -3 and sMAP to an array of carbohydrate groups on the surface of a bacterial cell. As with C1s, MASP-2 is responsible for the C4 and C2 activation, leading to generation of the same C3 convertase as the classical pathway. The alternative pathway is initiated by the low grade activation of C3 by hydrolyzed C3 (C3 ($H_2O$)) and activated factor B (Bb). The activated C3b binds factor B (B) which is cleaved into Bb by factor D (D) to form the alternative pathway C3 convertase, C3bBb. Once C3b is attached to the surface, the amplification loop consisting of the alternative pathway components is activated and the C3 convertase enzymes cleave many molecules of C3 to C3b, which bind covalently around the site of complement activation.

### 2.2. Ficolins

Ficolins are a group of proteins containing both a collagen-like and fibrinogen-like domain and found in varying tissues. Recent characterization has shown that ficolins

present in serum are lectins with a common binding specificity for *N*-acetylglucosamine (GlcNAc) (Matsushita and Fujita, 2002). The fibrinogen-like domain is responsible for the carbohydrate binding. MBL is also a serum collagenous lectin specific for GlcNAc and mannose whose domain organization is similar to that of ficolins except that MBL has a carbohydrate-recognition domain instead of a fibrinogen-like domain, as mentioned above. Investigations of two types of human serum ficolins, L-ficolin and H-ficolin (Hakata antigen), revealed that they are associated with MASPs and sMAP, and activate the lectin pathway (Matsushita et al., 2001, 2002). Recently, we reported that L-ficolin binds to lipoteichoic acid (LTA), a cell component found in all gram-positive bacteria and activate the lectin pathway (Lynch et al., 2004). These findings indicate that, serum ficolins act as one of the pattern recognition molecules and thus play an important role in innate immunity. Also, the precise function, structure and phylogeny of ficolins have been reviewed (Matsushita et al., 2001; Matsushita and Fujita, 2001, 2002; Fujita et al., 2004)

### 2.3. MBL

MBL is a C-type lectin that plays a crucial role in the first line of host defense (Drickamer et al., 1986; Ezekowitz et al., 1988; Kawasaki et al., 1978; Turner, 1996). The importance of this molecule is underlined by a number of clinical studies linking MBL deficiency with increased susceptibility to a variety of infectious diseases (Jack et al., 2001; Neth et al., 2000; Summerfield et al., 1995; Super et al., 1989). MBL belongs to the collectin family of proteins that consist of collagen-like domain and carbohydrate recognition domain (CRD) (Holmskov et al., 1994). Through its CRD, MBL binds carbohydrates with 3- and 4-hydroxyl groups in the pyranose ring in the presence of $Ca^{2+}$ through the five conserved residues (Glu184, Asn186, Glu191, Asn205, and Asp206) in the MBL CRD (Drickamer, 1992; Weis et al., 1992). Prominent ligands for MBL are thus D-mannose and GlcNAc, whereas carbohydrates that do not fit this steric requirement—D-galactose and sialic acid, which usually decorate the mammalian glycoprotein—have undetectable affinity for MBL. This steric selectivity of MBL, along with differences in the spatial organization of the ligands, allows for the specific recognition of carbohydrates on pathogenic microorganisms including bacteria, fungi, parasitic protozoans and viruses, and avoids recognition of self (Holmskov et al., 2003).

As mentioned above, sequence analysis of CRDs in comparison with monosaccharide specificity revealed that Glu185 and Asn187 (EPN type) are highly conserved in CRDs that bind mannose/glucose. Galactose-binding CRDs have Gln185 and Asp187 (QPD type) at these critical positions (Weis et al., 1992), and site-directed mutagenesis has shown that mannose-specificity can be changed to galactose specificity by replacing Glu185 and Asn187 (EPN type) with Gln185 and Asp187 (QPD type) (Drickamer, 1992). In addition to mammalian and chicken MBL, several lectins in bonny fish were characterized. The deduced primary structure of these lectins in carp, zebrafish and goldenfish indicates selectivity for galactose, having QPD type (Vitved et al., 2000). Recently, another carp MBL with a specificity for mannose (EPN type) was also purified (Nakao et al., 2003). Recently, we purified and cloned MBL-like lectin from a urochordate, the solitary ascidian *H. roretzi* (Sekine et al., 2001). The purified lectin binds specifically to glucose but not to mannose or GlcNAc and it was designated glucose-binding lectin (GBL). Sequence analysis of GBL reveals that the C-terminal half of the ascidian lectin contains a CRD which is homologous to C-type lectin (EPN type), but a collagen-like domain was replaced by the other sequence which has an α-helix structure similar to the configuration of Gly-*X*-*Y* repeats. The above results raise the possibility that GBL has evolved early as a prototype of MBL. During evolution GBL may have acquired the broad binding specificity for carbohydrates and the collagen structure characteristic of MBL. To prove this hypothesis, we also purified the lectin associated with MASP in lamprey, one of the most primitive vertebrates. The deduced amino acid sequence shows that this lectin has a collagenous region and a typical EPN-type CRD. Therefore, in conjunction with the phylogenetic analysis, it seems likely that the lamprey lectin is an orthlogue of the mammalian MBL (manuscript in preparation).

Interestingly, nine collectin-like genes are found in *C. intestinalis* genome which encode proteins composed of the collagen and lectin-like domains. They are transcriptionally active as shown by the presence of ESTs. Some of these genes are duplicated in tandem whereas some EST clones suggest the possibility of alternative splicing. Unlike other modular complement components, however, the domain structure of these molecules is relatively simple, and it is difficult to clarify molecular orthology based solely on domain structure. Thus, the phylogenetic analysis was performed to test orthology between human collectin and the corresponding ascidian molecules (data not shown). The C-type lectin domains of the *C. intestinalis* genes with the collagen and C-type lectin domains formed a monophyletic cluster outside of the vertebrate collectin cluster. These two clusters formed a clade supported by a high bootstrap value, and all C-type lectin domains of non-collectin type genes came outside of this clade. These results indicate that the *C. intestinalis* genes directly derived from a common ancestor of vertebrate collectin genes including MBL, suggesting that some of them could play a homologous role to vertebrate MBL. As mentioned above, mannose recognition by MBL requires the tripeptide sequence of Glu-Pro-Asn (EPN type), which is conserved among mammalian and bird MBL (Drickamer, 1992). In bony fish, the sequence is altered into Gln-Pro-Asp (QPD type), which prefers galactose for binding (Vitved et al., 2000). Among the nine MBL-like sequences found in *C. intestinalis*, three had EPN type sequence, thus they are expected to have a binding specificity to mannose. The

others had either Glu-Pro-Thr or Glu-Pro-Ser, which may be responsible for different sugar binding preference on pathogen surfaces. Thus, *C. intestinalis* seems to have many collectin-like molecules that differ in their carbohydrate recognition domain, suggesting that the duplication of these genes expanded variation of the innate immune system to compensate for the absence of the adaptive immune system.

## 3. The primitive complement system of invertebrates and jawless vertebrates

From the structural viewpoint, evolution of the complement system is divided into three stages, preceding, primitive and modern. At the preceding stage, from the emergence of metazoa to the emergence of deuterostome, most domain structures used by the vertebrate complement components has been created, but unique combination of them as found in the vertebrate complement components has not been established yet. Thus, no complement proper function is expected at this stage. At the primitive stage, from the emergence of deuterostome to the emergence of jawed vertebrate, most complement–specific domain combinations have been established, suggesting that basic function of individual complement component has been also established at this stage. Thus each component could function as discussed in detail in this section. At the modern stage, after the emergence of jawed vertebrate, acquisition of new components by gene duplication and following modification has made it possible to integrate complement components into a more sophisticated system (Nonaka and Yoshizaki, 2004a,b).

Sea squirts, ascidians occupy a pivotal intermediary position between invertebrates and vertebrates. Studies on the ascidian complement system have been performed by using mainly two species, *H. roretzi* and *C. intestinalis*, and it is the best-studied complement system among non-vertebrate animals. Due to its big body size, *H. roretzi* is suitable for biochemical analysis, and several proteins such as C3 (Nonaka et al., 1999), two lectins (GBL, and ficolins) (Kenjo et al., 2001; Sekine et al., 2001), and two MASPs (Sekine et al., 2001) have been isolated. In addition, cDNA clones for C3 (Nonaka et al., 1999), GBL (Sekine et al., 2001), ficolin (Kenjo et al., 2001), C2/Bf (Nonaka and Miyazawa, 2002), MASPs (Ji et al., 1997), and CR3 α (Miyazawa et al., 2001), and CR3 β (Miyazawa and Nonaka, 2004) have been identified, and the presence of the opsonic complement system has been demonstrated by the functional analysis (Miyazawa et al., 2001; Nonaka et al., 1999; Sekine et al., 2001). However, it is difficult to get whole view of the complement system of a certain animal species by the approach to identify components one by one. Recent assembly of the draft genome sequence of *C. intestinalis* (Dehal et al., 2002) made it possible to perform a genome-wide search for the possible complement component genes *in silico*. In the following, we will discuss architecture of the ascidian complement system revealed by biochemical approach and by genomic approach.

### 3.1. Biochemical view of the primitive complement system in ascidian

The alternative pathway has been regarded as the original complement pathway, because it does not require the participation of the adaptive immune system. However, the accumulated evidence indicates that complement originates as a lectin-based opsonized system. As described above, MBL-like 36 kDa lectin, GBL, was purified as a major protein from the ascidian body fluid. Sequence analysis of GBL cDNA revealed that the C-terminal half of the ascidian lectin contains a carbohydrate recognition domain (CRD) that is homologous to C-type lectin, but a collagen-like domain was replaced by the other sequence. Although the structure and binding specificity of GBL is different from mammalian MBL, GBL associated with two ascidian MASPs, and GBL-MASPs complex activates ascidian C3 as like human MBL-MASP-1 complex (Sekine et al., 2001). In addition, we have isolated ascidian ficolins as GlcNAc-binding lectins that have characteristic features of mammalian ficolins (Kenjo et al., 2001). Although it is presently unknown whether these ficolins associated with MASPs and activate complement, these observations indicate that ficolins probably, as well as GBL, act as the recognition molecules of the primitive ascidian complement system in a similar manner to the mammalian lectin pathway. Although C3 and C2/Bf were identified in sea urchin and ascidian, the sophisticated recognition mechanism of the alternative pathway to recognize a broad spectrum of pathogens seems to have developed more recently. This assumption was supported by findings that no opsonization was observed in the absence of GBL in ascidian as described below. However, the possibility of a simple role of C2/Bf-like protein, as an amplifier of C3 deposition cannot be excluded completely. In any case function of C2/Bf is still to be clarified (Fig. 1).

C3 was identified as the main opsonic factor in ascidian plasma (Nonaka et al., 1999), and a C3 receptor was also identified on ascidian haemocytes as the homologue of mammalian complement receptor type 3 or 4 (CR3 or CR4) (Miyazawa et al., 2001; Miyazawa and Nonaka, 2004). As reported previously, this opsonic activity is derived from C3 (Nonaka et al., 1999). Interestingly, antibodies that are specific for GBL, C3 (Sekine et al., 2001) and C3 receptor (Miyazawa et al., 2001) completely inhibited the phagocytosis of yeast in ascidians, which indicates that complement-mediated phagocytosis is a central part of the physiological function of this primitive complement system. Furthermore, yeast treated with purified GBL-MASPs complex and C3 enhanced the phagocytosis by haemocytes (Sekine et al., 2001). These observations strongly suggest that GBL-MASP complex, C3, and its receptor may have developed as the minimal ancestral components of the

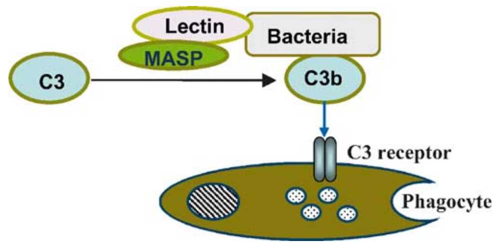


Fig. 3. Functional model of an ancient lectin-based complement system. The lectin-protease (MASP) complex, C3 and C3 receptor are probably the minimal ancestral components of the primordial complement system which functioned in an opsonic manner and appeared in the ascidian lineage. The complement system of lamprey (the most primitive vertebrate) lacks the classical and lytic pathway and so lamprey appear to have a similar complement system to ascidians. Therefore, the complement system developed dramatically at an early stage of vertebrate evolution into a sophisticated, multifunctional system as shown in Fig. 2.

primordial complement system in the ascidian lineage as shown in Fig. 3 (Fujita, 2002).

### 3.2. Recognition molecules of complement in lamprey

The classical and lytic pathways of the complement system seem to have emerged at the cartilaginous fish stage, coincident with the emergence of adaptive immunity (Nonaka and Miyazawa, 2002). The complement system of lamprey, the most primitive vertebrate, also lacks the classical and lytic pathway, suggesting that lamprey has a similar complement system to the ascidian. Recently we purified two lectins from lamprey serum using GlcNAc-agarose: one was eluted with mannose, and the other with GlcNAc (manuscripts in preparation). According to cDNA cloning, the former was identified as lamprey MBL as described above, and surprisingly, the latter is homologue of C1q. Interestingly, both lectins were associated with MASP-A, a serine protease of the MASP family and MASP-A exhibits a proteolytic activity against lamprey C3. The deduced amino acid sequence of lamprey C1q cDNA revealed that it consists of a collagen-like domain and antibody recognition domain, gC1q domain found in a variety of proteins including mammalian C1q. A phylogenetic tree of the gC1q domains of proteins shows that lamprey C1q and mammalian C1q form a cluster. These observations strongly suggest that C1q may have emerged as a lectin and functioned as an initial recognition molecule of the complement system before the establishment of acquired immunity such as immunoglobulins in the cartilaginous fish. As mentioned below, the two *C. intestinalis* genes, genewise 377.28.1 and grail 77.41.1, with the collagen and C1q domains showed a close relationship to human CQT1 (C1q and tumor necrosis factor related proteins) and CQT3, respectively, belonging to the C1q/TNF superfamily (Shapiro and Scherer, 1998). Human CQT1 gene was originally identified as a protein interacting with the V2 vasopressin receptor (V2R), and called G-protein-coupled receptor interacting protein, GIP (Innamorati et al., 2002). CQT1 has the C1q domain and collagen repeats for its cytosolic region, in addition to the transmembrane and extracellular domains. The globular C1q domain of CQT1 was found to interact with V2R, but its function is not yet clear. Human CQT3 is a homologue of mouse CORS26, which was identified from TGF-beta 1 treated embryonic fibroblast cell line and thought to have an important role in skeletal development (Maeda et al., 2001). In this case, therefore, it is not likely that these two *C. intestinalis* genes play a role similar to vertebrate C1q.

Although the molecular composition of the lectin pathway in cartilaginous and bony fish has not been fully clarified, the C1r and C1s components of C1 are clearly derived from the MASP lineage and C1q is closely related to MBL or ficolins with the substitution of antibody recognition domains for the CRDs or fibrinogen-like domain. From an evolutionary point of view, the primitive lectin pathway in innate immunity appears to have developed into the more sophisticated, multifunctional complement system of the classical pathway though gene duplication, to serve as an effector system of acquired immunity. A strong link between the innate immune systems of invertebrates and acquired immunity in vertebrates is therefore established.

### 3.3. Genomic view of the *C. intestinalis* complement system

Recent publication of the draft genome of *C. intestinalis* revealed a genome-wide and detailed architecture of the primitive complement system which details were described in recent reviews by Nonaka (Nonaka and Yoshizaki, 2004a,b). Briefly, the α2M/C3/C4/C5 family is different from other complement components in two points: one is that they show no clear domain structures, and another is that this family genes are also found in protostome genomes such as *Drosophila melanogaster* and *Caenorhabditis elegans*. The *C. intestinalis* genome contains two C3-like genes and two α2M-like genes (Azumi et al., 2003). And the three *C. intestinalis* Bf/C2 genes, termed Bf-1, Bf-2 and Bf-3, are all linked together within ∼50 kbp of the genomic region. As mentioned above, there are nine collectin-like, nine ficolin-like and two C1q like genes, and most of them are transcriptionally active as shown by the presence of ESTs. In addition, 11 gene models with the MAC/perforin domain were found from the *C. intestinalis* genome, and nine of them have a unique domain structure similar to that of the human complement late components. Except for one, all *C. intestinalis* C6-like genes have corresponding EST sequences.

Based solely on the derived domain structure of the possible *C. intestinalis* complement components, and extrapolating structure-function relationship of these domains in mammals, the postulated ascidian complement system is schematically shown in Fig. 4. The ascidian complement system is probably composed of three functional units. The first unit contains C3 and Bf, and is considered to be components of the vertebrate alternative pathway. This unit is considered to have an opsonic activity through C3-tagging,

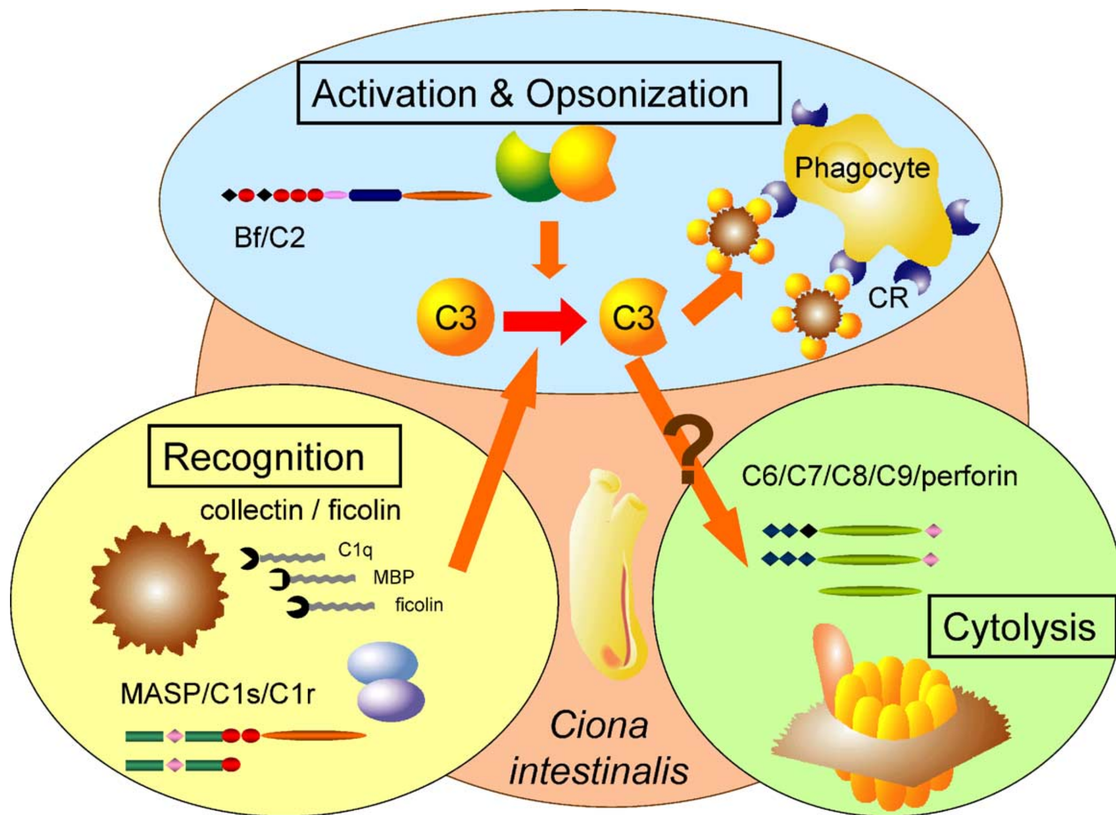


Fig. 4. Genomic view of the *C. intestinalis* complement system. The possible complement components of *C. intestinalis* are deduced from the draft genome sequence. Based on structural comparison with the vertebrate counterparts, these possible complement components are classified into three functional units. C3 and Bf are considered to form an activation system like a vertebrate alternative pathway, which leads to C3 deposition on non-self surface enhancing phagocytosis. Collagenous lectin molecules and MASP type serine proteases most probably associated with them form the second unit. Proteolytic target of them is proposed to be C3 as mentioned in Fig. 3. The third unit is formed by the possible cytolytic molecules. The activation mechanism of these molecules is still to be clarified.

although the function of Bf is still to be clarified. The second unit contains the possible recognition molecules for microorganisms, composed of the collagen and lectin-like domains, and serine proteases most probably associated with the recognition molecules. The substrates for these serine proteases are proposed to be C3 as mentioned above. The third unit contains C6-like molecules with the MACP domain, and their obvious function is cytolysis. However, activation mechanism of these molecules is still to be clarified. In the modern complement system, components belonging these three units are functionally linked, thereby constituting a single reaction system. In the ascidian components some domains or structures required for these interactions are missing, suggesting that these three units are not completely functionally linked. Especially, a functional linkage between C3 and the possible ascidian lytic components is still to be examined. The unique combination of several domains assign these components to the C6–C9 family, and their cytolytic function is strongly suggested by the presence of the MAC/perforin domain. However, it is still open if they are functionally linked to the ascidian C3 or not, since they lack the several C-terminal domains of human C6 or C7. Thus, it is proposed that these units are established independently to play an independent role, and are functionally combined later during the vertebrate evolution.

## 4. Conclusion

The identification and functional characterization of the lectin-based activation mechanisms of the complement system have provided new insights into the role of complement in innate immunity, which enables molecular patterns that specifically characterize microorganism to be detected. In addition, possible complement genes, established by molecular phylogenetic analyses, are present in all extant deuterostomes so far analyzed. The draft genome sequence of one ascidian species indicated that most modular complement genes show a similar or even higher level of expansion compared to the mammalian counterparts. The activation mechanisms and function of the ascidian complement system is expected to reveal the evolutionary process of this interesting biological reaction system. Also, an ancient lectin-based complement system in ascidian reveals that the primitive complement system is one of the most highly organized innate immune system in invertebrates.

## Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research (13143204) from the Ministry of Education, Science, Sports and Culture of Japan.

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