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The systematic identification and mRNA expression profiles post viral or bacterial challenge of complement system in grass carp *Ctenopharyngodon idella*

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ABSTRACT.

Complement system is an immemorial and pivotal element in innate immunity, protecting individuals from invading pathogens. Due to the emergence of whole genomes and functional researches, systematic identifications of complement system are feasible in many non-model species. In the present study, BLAST analysis was employed to systematically identify and characterize complement system in grass carp (*Ctenopharyngodon idella*). The results showed that *C. idella* complement system consists of 64 members, including the complement system pattern recognition, proteases, complement components, receptors and regulators. In which, most genes were well conserved with those in higher vertebrates over the course of evolution. Phylogenetic and syntenic analyses revealed their homologous relationships with other species. mRNA expression analyses of complement system related genes indicated that many members are sustainably expressed in multiple tissues before and after grass carp reovirus (GCRV) or *Aeromonas hydrophila* infection, which provide *in vivo* evidence for the response patterns of complement system after viral or bacterial infection. Meanwhile, this study also explored the evolution of complement system from ancestral protists to mammals and then investigated the changes in gene diversification during the evolution. These results will serve the comparative studies on the complement system in evolution and further functional investigations in *C. idella*.

1. Introduction

Complement system is a central component of innate immunity and has been regarded as an important defender against pathogens, which is now known as an essential humoral system of innate immunity as well as a link between innate and adaptive immune responses [1,2]. Complement system plays multiple roles in immune responses, such as immune cell activation, chemotaxis, opsonization and lysis of antigens. Both the lack of certain complement components as well as an excessive complement activation are reasons for serious illnesses, demonstrating the requirement for a delicate balance of complement activation and regulation for health [3].

According to the evolutionary history of complement system, which is widely present in both invertebrates and vertebrates [4]. For example, complement C3 gene has been reported in sea sponge (*Amphimedon queenslandica*) genome [5]. Similarly, a C3-like gene has also been identified in sea anemone (*Nematostella vectensis*) and coral (*Swiftia excreta*), respectively [6]. Complement system comprises more than 35 distinct soluble blood plasma proteins and cell membrane proteins in higher vertebrates, forming three convergent pathways of activation: classical pathway, lectin pathway and alternative pathway [7]. A major function of the soluble complement proteins is to destroy invading pathogens [8,9]. Meanwhile, the cell membrane proteins of complement system are divided into complement receptors and complement regulators [10]. Complement receptors on phagocytic cells promote binding and engulfment of pathogens coated with complement opsonins, whereas complement regulators protect healthy tissues from accidental damage by these soluble complement proteins [11]. Upon binding of complement proteins or protein fragments that are generated during complement activation, these receptors and regulators transduce various signals into cells bearing them [12,13]. Finally, complement membrane attack complex (C5b-9) binds to cell membranes, independent of any receptor, and also activates multiple signaling pathways. The receptor-dependent and -independent signals transduced by

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complement components are of great consequence to health and disease [7,14].

Complement system have been well studied in higher vertebrates. Meanwhile, a large number of studies on complement components have also been conducted in teleost fishes. For instance, complement C3, C5, C6, C7, C8 and C9 have been reported in zebrafish (*Danio rerio*) [6] and rainbow trout (*Oncorhynchus mykiss*) [15]. Besides, complement C1q was also identified in mollusk, such as Zhikong scallop (*Chlamys farreri*) [16] and razor clam (*Sinonovacula constricta*) [17,18]. Furthermore, studies on complement regulators have also been conducted in teleost fishes, for example, CFH, CFHL, CD46 and C1-INH have been reported in *D. rerio* [6] and channel catfish (*Ictalurus punctatus*) [19]. A CD46-like complement regulatory membrane protein was cloned and characterized in common carp (*Cyprinus carpio*) [20]. And the expression profiles of some complement system related genes have also been characterized in teleost fishes, for example, C1-INH was found to be significantly upregulated at early stages after bacterial infection with *Edwardsiella ictaluri* in channel catfish [21]. And in grass carp (*Ctenopharyngodon idella*), Bf/C2 in interference cells down-regulated the transcript levels and induced NF-κB signaling after *Aeromonas hydrophila* challenge [22]. Upon challenge with *A. hydrophila*, C6 expression was significantly upregulated in muscle, trunk kidney, head kidney, hepatopancreas, spleen, heart and intestine, whereas it was down-regulated in brain and skin [23]. C7 expression was significantly upregulated in head kidney and spleen tissues, which was suppressed in trunk kidney, hepatopancreas, skin, heart and intestine tissues following *A. hydrophila* infection [24]. These findings indicate the vital role of complement system related genes in immunity in *C. idella* as well as other teleost fishes.

C. idella is one of the important freshwater economic fish species in China. However, viral and bacterial diseases have caused high mortality in both wild and cultured fish, and had a severe impact on *C. idella* aquaculture. A better understanding of the innate immune response in *C. idella* can facilitate the development of effective methods for disease control. Up to now, some important proteins and gene families involved in innate immune mechanisms have been identified in *C. idella*, which suggests that many components in innate immunity, including complement system, and signaling pathways known from mammals are highly conserved. This study mainly focuses on the evolution and expression of complement system in *C. idella*. Meanwhile, we will discuss the research progresses and future perspectives in complement system.

2. Materials and methods

2.1. Databases mining and gene identification

Complement system related genes were identified by blasting against *C. idella* whole genome [25] and transcriptome [26] based on a query set of available sequences from fishes to mammals. BLASTN similarity searches were conducted against the whole genome and transcriptome databases to identify all complement system related sequences with an e-value $< 10^{-5}$. To confirm these candidate sequences, BLASTN was conducted against the NCBI non-redundant database with an e-value $< 10^{-10}$. After that, these novel sequences were deposited in the GenBank database and their accession number being listed in Table 1.

2.2. Sequence and motif analyses

Firstly, the coding sequences of genes were predicted using ORF (opening reading frames) finder (https://www.ncbi.nlm.nih.gov/orffinder/), and then translated into hypothetical proteins and further validated by BLASTP against NCBI non-redundant protein databases. Subsequently, protein sequences of complement system were analyzed for the presence of signal peptides using the program Signal P 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Isoelectric point (PI) and molecular weight (MW) of complement system were analyzed by Compute PI/Mw (http://web.expasy.org/compute_pi/). Beyond, chromosome positions of complement system related genes were also analyzed by blasting against *C. idella* whole genome (http://www.ncgr.ac.cn/grasscarp/).

2.3. Phylogenetic, evolutionary and syntenic analyses

To analyze the phylogenetic relationship of *C. idella* complement system, phylogenetic analyses were performed using neighbor-joining method and maximum likelihood method within the MEGA6 program, with the Poisson model for amino acid substitution and pairwise deletion for gaps treatment. The degree of confidence for each branch point was determined by bootstrap analysis (10,000 times) and recorded, some bootstrap values were not shown. For the nomenclatures of *C. idella* complement system related genes, we named them after *D. rerio* and human (*Homo sapiens*), since orthologs to mammalian complement system could be established for many fish complement system. Meanwhile, concise evolutionary analysis of complement system from ancestral protists to mammals was conducted.

Analysis of conserved syntenies was conducted to provide additional evidence for the identification of *C. idella* complement system related genes. In most cases, the comparison was made between the genes in *C. idella* and *D. rerio*. The flanking genes of complement system related genes were predicted from the *C. idella* genomic scaffolds and validated by running BLASTP against NCBI non-redundant database. The genomic pattern of complement system related genes as well as their neighboring genes in *D. rerio* were obtained from NCBI database. For the nomenclatures of *C. idella* complement system related genes, we named them after *D. rerio* (http://zfin.org/), since orthologs to mammalian complement system could not be established for some fish complement system related genes. When the orthologous relationship was obscure, especially for those tandem duplicated complement copies originated from one single gene, we named them based on their genomic locations on the chromosome.

2.4. mRNA expression analyses

The expression profiles of complement system related genes in response to grass carp reovirus (GCRV) (*C. idella* infected with GCRV and showing typical symptoms of disease) and *A. hydrophila* (AH10; Aquatic Pathogen Collection Center of Ministry of Agriculture, China) infections were determined based on the RNA-Seq datasets. All experiments with fish were conducted in accordance with the guidelines on the care and use of animals for scientific purposes.

Briefly, for viral challenge, *C. idella* weighing about 30 g were chosen for infection study. *C. idella* were immersed in 8% NaCl for 2 min, and then experiment groups were bathed with the virus working solution for 30 min. Finally, all *C. idella* were moved into aerated water at 28 °C containing 100 U/mL of streptomycin and penicillin. Spleen, gill, hepatopancreas and intestine tissues were harvested from 15 individuals before GCRV infection and used as the control profiles. And four tissues were harvested at 2, 24, 48, 72, 96 and 120 h after GCRV infection, respectively. In the duplicate experiments, four tissues of 10 individuals were obtained and pooled respectively at the same time points as previously. Subsequently, total RNA of same tissues at the same time points ($n = 25$) were pooled together for RNA-Seq library construction and sequencing with the Illumina HiSeq™ 2000 platform [27]. For bacterial challenge, 12 months old *C. idella* weighing about 70 g were chosen for infection study. Animals were randomly separated into four tanks (40 individuals/tank). Each fish in the two tanks was given 100 μL intraperitoneal injections of *A. hydrophila* that was suspended in PBS at a dose of 2.7×10^{7} CFU/mL. Control fish in the other two tanks was injected with the same volume of PBS. Three animals were randomly selected from each control tank at 0 h and from the experimental tank at 4, 8, 12, 24, 48 and 72 h after *A. hydrophila* infection, respectively. Then, equal amounts of RNA extracted from the

Table 1
Features of complement system in *C. idella*.

Function	Type	Subtype	Amino acid	Chr	PI	MW	Length of signal peptide	Accession No.
Pattern recognition	CRP	CRP	225	19	5.94	25028.61	18	MG599717*
		CRP-like	170	19	5.65	19463.10	–	MG599718*
	PTX3	PTX3a	448	7	5.20	49491.83	22	MG599719*
		PTX3b	412	22	5.91	45760.90	22	MG599720*
	C1q	C1q-A	245	3	8.77	25806.36	21	MG599677*
		C1q-B	243	3	5.14	25625.16	26	KC708227
		C1q-C	244	3	8.75	26162.55	23	JQ358795
	MBL	MBL	250	22	5.48	26527.11	21	KM275626
	Collectin	Collectin 10	271	12	8.90	29466.83	25	MG599721*
		Collectin 11	271	23	5.44	29167.42	25	MG599722*
		Collectin 12	739	22	5.26	79987.33	–	MG599723*
	FP	FP	442	21	6.73	48901.70	20	MG599683*
	Ficolin	Ficolin-like	–	21	–	–	–	MG599711*
Proteases	C1r	C1r	1155	12	5.29	128506.23	–	MG599703*
	C1s	C1s-like	–	12	–	–	–	MG599704*
	MASP	MASP2	685	6	6.13	76592.57	17	MG599707*
		MASP3	847	15	5.13	94512.74	–	MG637442*
	B/C2	B/C2-A1	731	3	7.84	81600.71	21	MG599710*
		B/C2-A3	752	3	5.64	84464.30	21	JF747038
		B/C2—B	837	–	6.56	94393.46	22	JF747039
	FI	FI	531	6	6.51	59487.32	–	HM776035
	FD	FD	250	5	6.30	27063.48	20	KF672346
Complement components	C3	C3.1	1643	8	6.03	183551.77	21	AY374472
		C3.2	1948	8	7.64	219499.95	21	MG599688*
		C3.3	1509	8	6.27	167941.70	21	MG599687*
		C3.4	1546	8	7.84	172081.24	21	MG599689*
		C3.5	1360	8	6.27	151913.56	22	MG599690*
		C3.6	1750	2	7.99	198727.45	18	MG599691*
		C3.7	> 1573	24	–	–	–	MG599692*
		C3.8	> 581	–	–	–	–	MG599693*
		C3-like	> 385	–	–	–	–	MG599694*
	C4	C4A	1695	15	6.53	190651.37	17	MG599708*
		C4B	1715	12	6.51	192706.85	21	MG599709*
	C5	C5	1687	17	6.74	190771.86	19	MG599678*
	C6	C6	907	3	6.09	102633.05	22	HQ416903
	C7	C7a	842	21	6.27	94187.59	23	MG637443*
		C7b	821	3	6.98	92082.02	18	JN710442
	C8	C8α	477	22	6.00	53671.52	–	MG599682*
		C8β	567	22	7.06	63561.14	29	MG599679*
		C8γ	209	19	9.20	24246.26	23	MG599699*
	C9	C9	650	17	5.58	71044.74	19	AY391781
Receptors	C1qR	C1qR	551	23	6.40	62006.81	16	MG599695*
		C1qR-like	339	23	8.22	37701.49	–	MG599706*
	gC1qR	gC1qR	–	–	–	–	–	MG599712*
	Calreticulin/cC1qR	Calreticulin	–	21	–	–	–	MG599713*
		Calreticulin 3a	–	24	–	–	–	MG599714*
		Calreticulin 3b	–	5	–	–	–	MG599715*
		Calreticulin-like	1210	24	4.76	135323.83	–	MG599716*
	CR3	CD11-like	1157	–	5.42	127846.74	–	MG637444*
		CD18-like	773	16	6.16	84794.66	21	MG637445*
	C3aR	C3aR	339	24	6.74	38669.25	–	MG599686*
	C5aR	C5aR	358	7	8.12	40682.69	–	MG599680*
Regulators	C1—INH	C1—INH	601	21	5.06	67951.95	17	MG599685*
	FH	FH	868	24	6.29	97893.23	22	MG599698*
		FHL1	> 250	24	–	–	–	MG599701*
		FHL2	493	24	6.48	55163.95	22	MG599702*
		FHL3	444	24	5.79	50463.36	22	MG599700*
	CD59	CD59	118	1	8.28	12907.05	21	MG599684*
	MCP-like	MCP-like	338	6	7.40	37081.45	24	MG637446*
	C4BP-like	C4BP-like	> 598	–	–	–	–	MG637447*
	DAF-like	DAF-like	–	18	–	–	–	MG599705*
	Clusterin	Clusterin	462	14	5.45	53138.40	19	MG599681*
	Vitronectin	Vitronectin a	541	15	7.53	63880.13	15	MG599696*
		Vitronectin b	452	3	8.48	51839.87	16	MG599697*

Note: PI, MW and Chr represent isoelectric point, molecular weight and chromosome, respectively. "-" indicates that the features of complement system are unknown or not exist in *C. idella*. * indicates the sequence was deposited in GenBank by ourselves. Full names of complement system related genes were listed in Table S5.

spleen samples at the same time points (n = 6) were pooled together for RNA-Seq library construction and sequencing with the Illumina NextSeq™ 500 platform [28]. Finally, their accession numbers were **SRA099702** [27] and **SRP060308** [28], including 28 RNA-Seq libraries in viral infection experiment and 7 RNA-Seq libraries in bacterial infection experiment.

To preliminarily investigate the mRNA expression profiles of complement system related genes after viral and/or bacterial infection, BLASTN analysis was employed to detect their expressions in these tissues at seven time points based on the RNA-Seq datasets. In the present study, mRNA sequences were searched by BLASTN program, mapped reads with an e-value $< 10^{-10}$ were annotated and counted with RPKM (reads per kilobase per million mapped reads) values. Complement system related genes were bidirectionally clustered according to their expression profiles by pheatmap software (R package).

3. Results

3.1. Identification of complement system related genes

Due to the conservatism of complement system during the process of evolution, they were often identified by automated gene predictions and detailed analyses. Hence, we performed *de novo* complement system predictions on the draft genome of *C. idella* and merged our gene predictions with any available gene information characterized in the literatures. In the present study, a total of 64 complement system members were identified in *C. idella*, thereinto, 11 complement system related genes have been reported in *C. idella*. The GenBank accession numbers and characteristics are shown in Table 1, including their transcript sizes, coding sequences, isoelectric points, molecular weight patterns and chromosomal locations.

3.2. Origin and evolution of complement system in different species

To construct the evolutionary history of complement system as well as to provide a catalog of complement system in important model species, we sought to identify complement system members within different species based on the various databases (NCBI and Ensemble), including *H. sapiens*, mouse (*Mus musculus*), chicken (*Gallus gallus*), zebra finch (*Taeniopygia guttata*), anole (*Anolis carolinensis*), crocodile (*Alligator mississippiensis*), frog (*Xenopus tropicalis*), *D. rerio*, elephant fish (*Callorhinchus milii*), sea squirt (*Ciona intestinalis*), sea urchin (*Sea purpuratus*) and fruit fly (*Bactrocera tryoni*) (Fig. 1 and Table S1). Interestingly, complement system related genes were found in various species with the exception of ancestral protists.

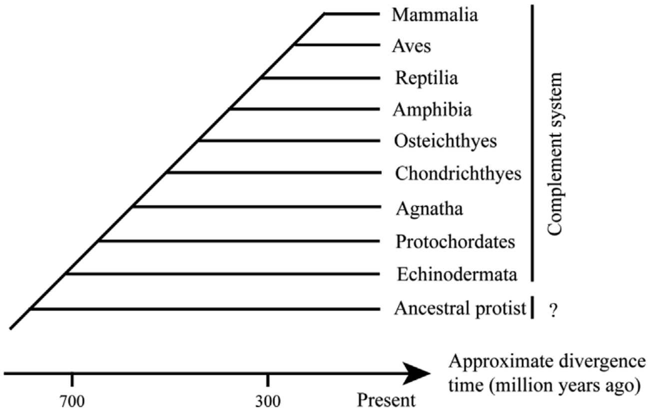

Fig. 1. Phylogenetic relationship of complement system among animals. Phylogeny shows the dates across multiple taxonomic species. "?" indicates that complement system related genes are not found in these species up to now.

3.3. Phylogenetic and syntenic analyses of complement system

Phylogenetic analyses were conducted to annotate the *C. idella* complement system related genes using the amino acid sequences of other species (Fig. 2 and S1). As shown by phylogenetic trees, most of *C. idella* complement system members were clustered into clades with their respective counterparts in *D. rerio* (Fig. S1). In the 64 *C. idella* complement system members, some of them are highly related duplicates, which included 9 genes for C3 (Table 1). Meanwhile, phylogenetic relationships provided strong support for the identities of most *C. idella* complement system related genes. According to phylogenetic analyses, nomenclatures of some ambiguous complement system related genes were established in *C. idella* (Fig. 2). Hence they were renamed according to phylogenetic or syntenic analyses, and their correct names are included in Table 1.

Though phylogenetic relationships provided strong support for the identities of most *C. idella* complement system related genes, the syntenic analyses were necessary to provide additional evidence for annotations and identifications of complement system related genes. On the one hand, the syntenic analyses provided strong evidence for the identification of *C. idella* C3 gene whose phylogenetic relationship were somewhat obscure. As shown in Fig. 3 and Table S2, the syntenic block containing CRP, PTX, C1, Collectin, FP, MASP, FD, C3, C4, C5, C6, C7, C8, C9, C1−INH, FH and Vitronectin b were well conserved between *C. idella* and *D. rerio.* On the other hand, the synteny results also supported the annotations of remaining genes and their duplicates, especially for those clustered genes. For example, 5 members of C3 were apparently derived from tandem duplications (Fig. 3).

3.4. Molecular characterizations and chromosomal location analyses of complement system

A number of tools were employed to systematically analyze the molecular characterizations and distributions of complement system related genes in *C. idella*. For complement system, most of which were from both genome and transcriptome datasets (Table 1). Meanwhile, signal peptides were not found in 23 complement system members and their PIs are relative low (Table 1). Overall, 58 of 64 identified complement members are distributed on 18 different chromosomes (chromosomes 1, 2, 3, 5, 6, 7, 8, 12, 14, 15, 16, 17, 18, 19, 21, 22, 23 and 24), but the chromosome locations of other 6 genes have not yet been mapped (Table 1). There are two large complement system clusters that located on chromosomes 3 and 24 in *C. idella* genome, containing 8 genes, respectively. Other genes are present in mini-clusters or as single genes on 16 different chromosomes (Table 1). The detail information of chromosome location about these genes were listed in Table S3.

3.5. mRNA expressions of complement system in tissues after viral or bacterial challenge

To get insights into the potential involvement of complement system after viral and bacterial infection, their expression profiles were determined based on the RNA-Seq datasets. We performed a BLASTN profiling across 64 complement members constituting the recognition of reads in *C. idella*. Regarding the tissue-specific expression, RNA-Seq experiment indicated that some complement system related genes may respond to GCRV infection in spleen, gill, hepatopancreas and intestine (Fig. 4A, B, C and D). Spleen, hepatopancreas and intestine tissues suggested that many genes have strong immune responses against viral infection, while some complement immune responses in gill were generally much weaker than other three tissues. Comparing the relative immune intensities among the various genes, we recorded the strong immune responses for 16 genes in spleen, 8 genes in gill, 19 genes in hepatopancreas, 13 genes in intestine (Table S4). While other complement system related genes were found to be expressed at comparatively low levels in these tissues (Fig. 4A, B, C and D).

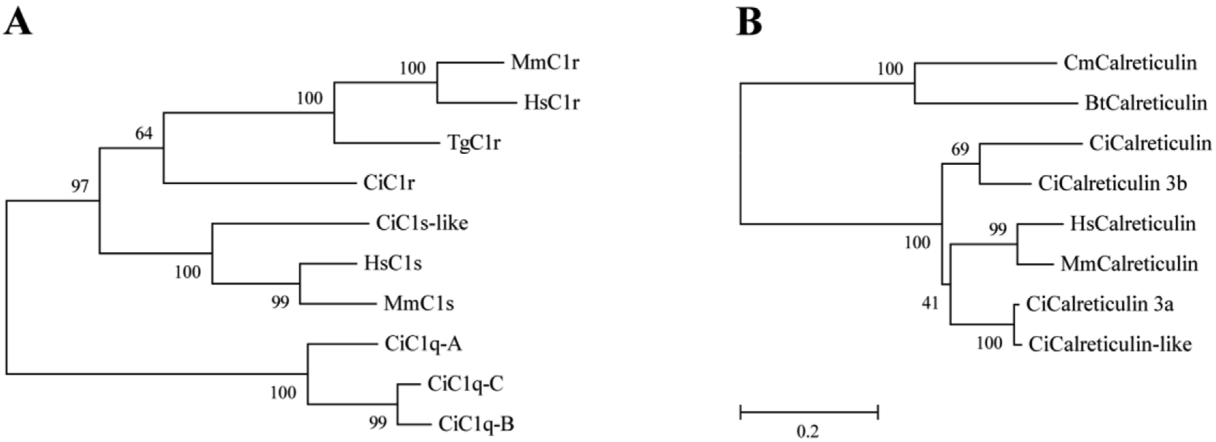

Fig. 2. Phylogenetic relationship annotate nomenclatures of ambiguous complement system members in *C. idella* with Maximum likelihood dendrogram method. Accession numbers of these genes are available in Table S1.

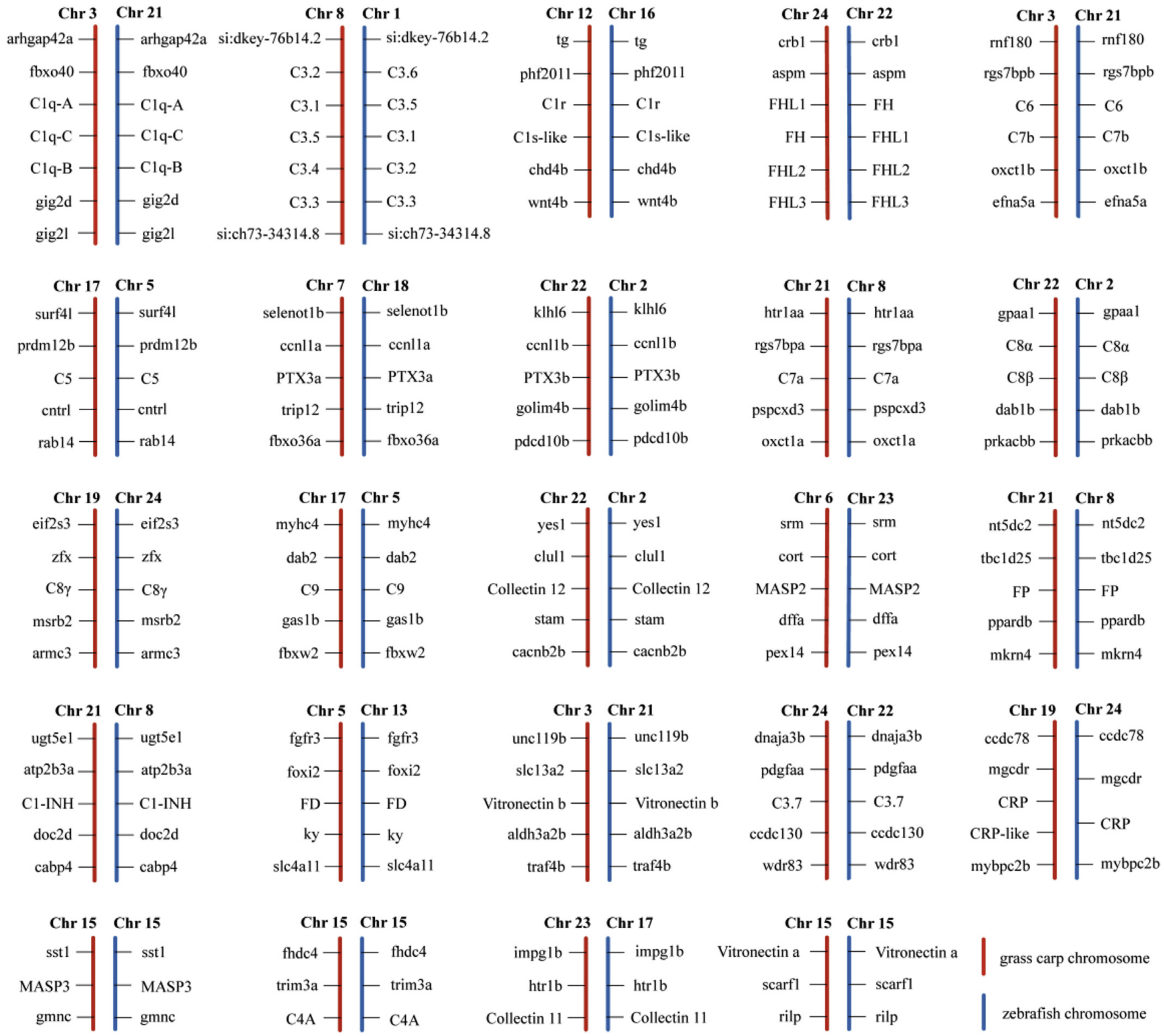

Fig. 3. Syntenic analyses of complement system related genes between *C. idella* and *D. rerio*. These syntenies were generated with the information obtained from the NCBI and ZFIN.

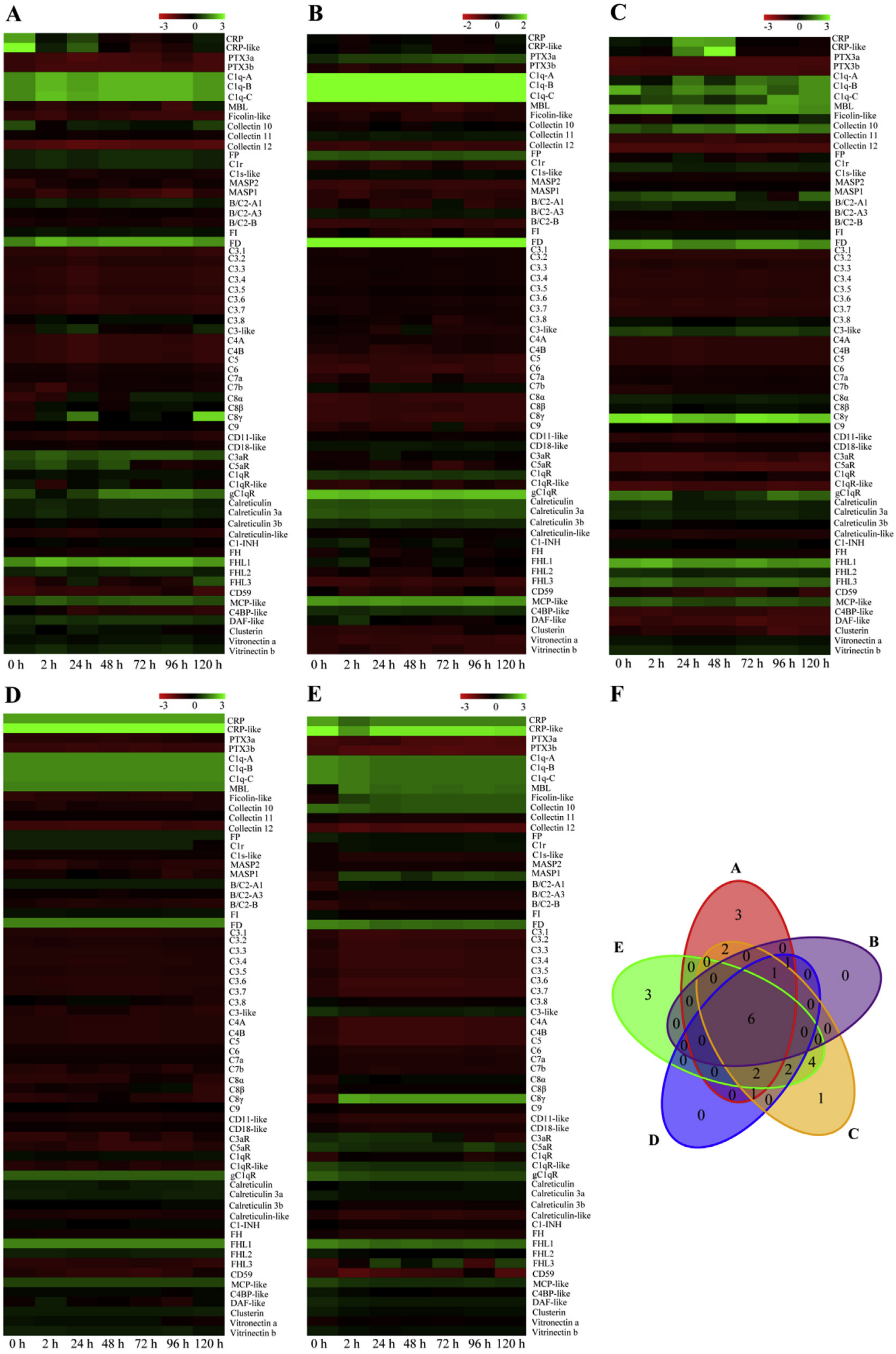

Fig. 4. mRNA expression changes of complement system related genes upon GCRV or *A. hydrophila* infection. (A), (B), (C) and (D) represent changes in expression of complement system related genes after GCRV infection in spleen, gill, hepatopancreas and intestine tissues, respectively. (E) stands for changes in expression of complement system related genes after *A. hydrophila* infection in spleen tissue. (F) Venn diagram describes overlaps among complement system related genes with high expression level in A, B, C, D and E.

Meanwhile, RNA-Seq experiment suggested that some complement system related genes may respond to *A. hydrophila* infection in spleen and there are different from basal level (Fig. 4E). Comparing the relative immune intensities among various genes, we recorded the strong immune responses for 17 genes in spleen after *A. hydrophila* infection, while other members may have weak immune responses in

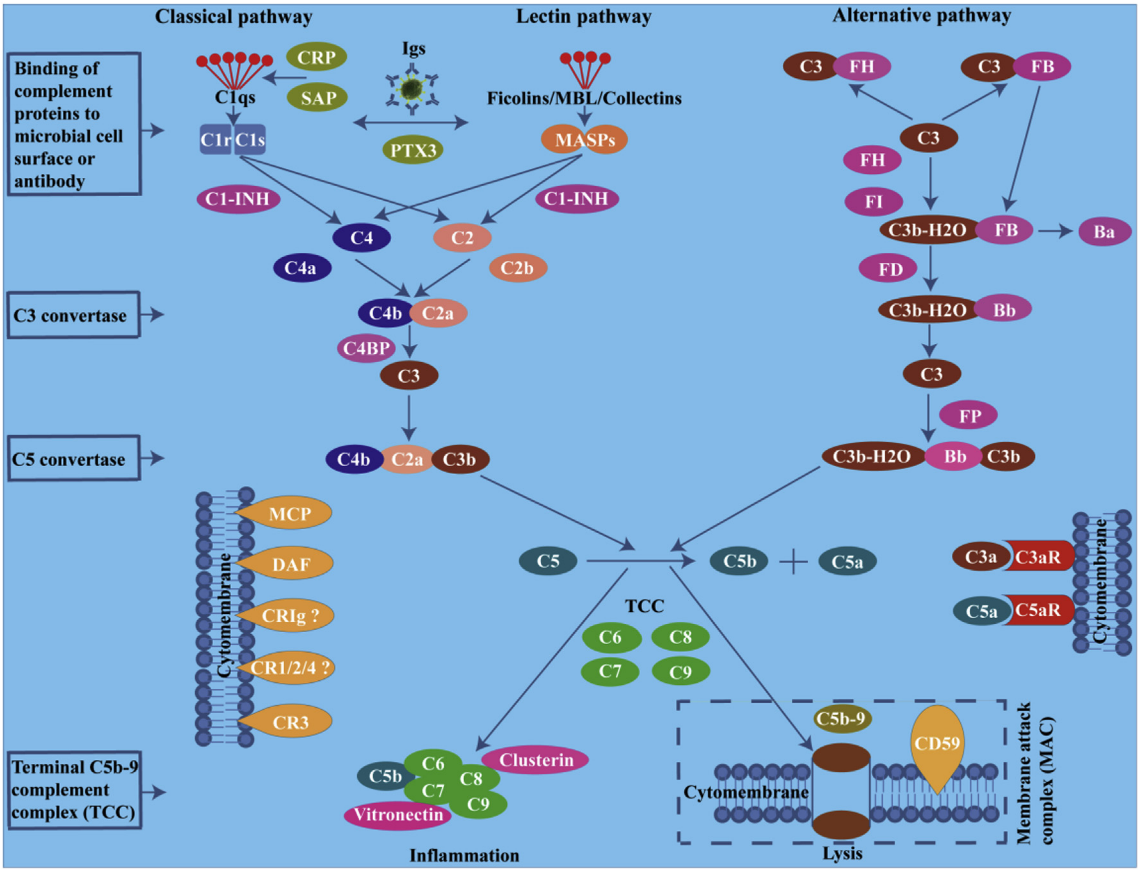

Fig. 5. Suppositional activation and regulation of complement system in teleost fishes. The complement system can be activated through three pathways, all converging to the cleavage of C3 to generate C3a and C3b. The classical pathway is typically activated by pentraxins (CRP, SAP and PTX3) that can activate C1q. The lectin pathway is activated through recognition of carbohydrates by MBL, ficolins and collectins. The alternative pathway is activated by foreign or damaged own cells, facilitated by the continuous spontaneous hydrolysis of C3. The alternative pathway also has an important function for providing an amplification loop to enhance C3 activation. Then, activation of C3 leads to formation of a C5 convertase, cleaving C5 into C5a and C5b. The anaphylatoxins C3a and C5a bind to their receptors C3aR and C5aR, leading to downstream production of inflammatory mediators. Finally, C5b initiates the formation of terminal complement complex (C5b-9), which either forms the membrane attack complex if inserted into a membrane that can lead to lysis of bacteria and cells, or results in inflammation in soluble manners. The complement system is tightly regulated by soluble inhibitors (C1−INH, FH, FI, C4BP, Vn and Cl), keeping the continuous low-grade activation in the fluid phase. Host cell membranes are equipped with a number of inhibitors to protect them against attack by complement, including MCP, CR1, DAF, controlling C3 and C4 activation, and CD59 protecting against final assembly of the C5b-9 complex. "?" indicates that complement system related genes are not found in teleost fishes but existed in higher vertebrates. Detailed functions of complement system related genes were listed in Table S5.

spleen tissue (Fig. 4E). In all the high expression members, spleen and hepatopancreas after GCRV challenge have 3 and 1 specific genes, respectively. Spleen after *A. hydrophila* infection contains 3 specific genes (Fig. 4F and Table S4).

4. Discussion

Complement system is a major humoral system of innate immunity and protecting individuals from invading pathogens, which could be activated in teleost fishes through all the three pathways as those in mammals [29,30]. In recent studies, the homolog-hunting identification of complement system in model animals has been completed, but systematic analyses were vacant in many important species [4]. Consistently, our global searching for complement system from *C. idella* genome and transcriptome datasets, together with the published data, has revealed *C. idella* possesses most of orthologs in mammalian complement system so far. In this study, we systematically identified 64 complement system related molecules in *C. idella*. Establishing one-to-one orthologous relationships between teleost and mammalian complement systems will be successful due to their well conserved evolution. Further functional experiments in various species should clarify the conserved and novel roles in this gene family.

The recent availability of complement system datasets from a number of species has allowed new insights into the evolution and diversity of this important gene family [31]. However, type and number of complement system related genes have a big difference in various taxonomic species. Diversity of complement system appears to vary among teleost fishes in many cases, may be greater than those in higher vertebrates. Expansion of complement system likely occurred through whole genome duplication and tandem duplication [32]. For example, CR1, CR2 and CR4 exist in *H. sapiens* and *M. musculus* genomes, but they are not found in *C. idella* and *D. rerio* (Table S1). Although we cannot discount the possible role of gene deletion in all non-mammalian species, the comparative genomic analysis of multiple species suggests that a number of these genes are lineage-specific in mammals [33,34]. In teleost fishes, SAP also exist in tongue sole (*Cynoglossus semilaevis*) and ayu (*Plecoglossus altivelis*) according to the NCBI datasets, but not found in *C. idella* and *D. rerio*. Even in cyprinid fishes, there are many differences in terms of the number and variety of complement system related genes. Interestingly, ficolin-like molecular has also been identified in *C. idella*, while it has not been reported in many teleost fishes, hence the functions of ficolin-like gene need to further study. Many complement system members have well conserved evolution between *C. idella* and *D. rerio* (Fig. S1), while some subgroups have different numbers among them, which may result from the tandem duplication in their specific evolutionary environment. Meanwhile, by systematically identifying complement system across multiple taxonomic species, we can further infer the origin and model the evolutionary history of complement system.

The replicators of ancestral genes show functional redundancy and one of the pair will be degenerated to a pseudogene or will be lost entirely from the genome, while the other copy maintains its original function and is constrained by purifying selection [35]. Meanwhile, this effect also promotes the retention of duplicated copies when a large amount of gene product is required [36]. For a multigene family descended from a common ancestor, birth-and-death evolution and concerted evolution models are proposed [37]. According to the birth-and-death model, some duplicates are maintained for a long time, whereas others are inactivated or deleted from the genome by neutral drift or purifying selection [37]. Complement system may have also evolved in a similar fashion, since some lineage-specific functional genes and pseudogenes are found in various species. Meanwhile, some genes have more than one copy that with relatively high similarity in their genomes, hence it is difficult to distinguish these genes by amplification analysis.

Complement components are mainly produced in liver as acute phase reactants in mammals, but they are also synthesized in extrahepatic sites, suggesting that complement components might mediate distinct fine-tuning roles in the context of tissue homeostasis and immune surveillance [6]. Compared with the ever-growing amount of the data at the mRNA level, there is limited number of the functional data on the physiological role of complement system in teleost fishes. While expression patterns and conclusive identifications of complement system related genes, especially those with isotypic diversity, are still necessary and important. Current study trends in the complement system in teleost fishes appear to be shifting to analysis and elucidation of function and mechanism, modulation of innate and adaptive immune response as well as unconventional role in complement triggered pathways. For example, transcriptome analysis of RAG1 knock-out *D. rerio*, which has deficient adaptive immunity, shows elevated expression levels of some complement components (such as MASP2 and C3) in intestine [38], contrasting to the RAG1-deficient *M. musculus*, which suffers a lethal symptom of the severe combined immunodeficiency [39]. Complement system plays crucial roles in teleost fishes, while current expression evidence is insufficient to conclusively connect complement genomic environs with inflammatory or homeostatic function.

This study has systematically identified the members of complement system in *C. idella* (Table 1). Combining the previous literatures [40–42], we summarized the schematic illustration of complement system in teleost fishes according to that in higher vertebrates (Fig. 5). Firstly, the classical pathway is activated by the binding of a plasma protein called C1 to antibodies bound to the surface of a microbe or other structure, the alternative pathway is triggered by direct recognition of certain microbial surface structure and the lectin pathway is initiated by MBL or FCN. Subsequently, these three pathways merge at a common amplification step involving the formation of unstable protease complexes, named C3-convertases (C3bBb in the alternative pathway and C4b2a in the classical/lectin pathways). Finally, the terminal stage that initiates the assembly of C5, C6, C7, C8 and C9, forms a membrane attack complex which leads to complement-mediated lysis, or induces inflammation in soluble manner (Fig. 5). Activated complement system plays multiple immune roles including elimination of invading pathogens, promotion of inflammatory response as well as clearance of apoptotic cell and necrotic cell debris, in addition to modulation of adaptive immune responses [12].

In order to understand the complement system and their functions in the organisms, it is better to study them from an evolutionary perspective. Whereas the functions of complement system are more probably linked to resistance to infectious agents as well as specific functions related to their expression sites. Complement system participates in the development of immune system, inflammatory responses as well as innate and acquired immune responses. The studies on complement system in the last few years have led to important advances in our understanding of immune responses. However, function studies are still in infancy and required to uncover the unconventional roles in complement pathways.

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Appendix A. Supplementary data

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