

Review Articles

Research Progress on the Fish Complement C3 Gene

Rumeng Jin¹, Hu Xia^{1,2a}, Pinhong Yang¹, Juanjuan Lu³, Fuyan Chen², Yunsheng Zhang¹, Liangguo Liu¹, Zhongyuan Chen¹, Hao Zeng¹, Wei Zhou¹, Huimin Liu¹, Shan hong Liu¹

¹ Hunan University of Arts and Science, ² Guangxi Zhuang Autonomous Region Academy of Fisheries Science, ³ Hunan Applied and Technology University

Keywords: complement C3, immune response, complement activation pathways

<https://doi.org/10.46989/001c.73361>

Israeli Journal of Aquaculture - Bamidgah

Vol. 75, Issue 1, 2023

Complement (C) is a serum protein with immunity-related functions and enzymatic activities. It can induce inflammatory responses and antibody formation following activation by substances such as pathogens. Moreover, it is a crucial molecule in the congenital immune defense system. Complement manifests earlier than immunoglobulin in the evolutionary process of fish's immune system. Therefore, the complement system of fish is vital for congenital and acquired immunities. A complement system is a polymolecule system comprising more than 30 soluble proteins, membrane-bound proteins, and complement receptors. It can be activated through three pathways for immunoregulation, the clearance of the immune complex, etc. The liver primarily produces the fish complement C3, which is a significant component of the complement system of fishes. C3 is the hub of three activation pathways. Research works concerning the structure, generation process, expression, and functions of C3 in fishes are reviewed in this study.

INTRODUCTION

Aquaculture is developing quickly in response to the annual escalation of market needs. The expansion of environment-friendly aquaculture has also emerged as a new trend in the industry. The increase in breeding density facilitates rapid pollution and, consequently, toxic substances in the water bodies, making it challenging to ensure the quality of aquatic products. Therefore, studying fish's immune systems and responses is necessary. The complement system of fish is an essential component of their immune systems. Investigating the biological functions of the complement system helps better understand the immune mechanism of fishes, thus enabling the prevention and control of diseases in them more effectively.

The complement system can be divided into a complete inherent component, a complete regulating component, and a complete receptor. C1~C9 belong to the complete inherent components. C3 is the complement component with the highest serum content, connecting specific and non-specific immunities.¹ C3, C4, and C5 are homologous and can produce anaphylatoxin following hydrolysis. All the C-terminal segments contain the structural domain of C345C (complement C3/4/5 C terminals), which facilitates combining C5b with C6 and C7 to create a membrane attack complex.² C3 and C9 are crucial in complement activation, pathogen elimination, etc. Zhang Yanjie et al. demon-

strated that the complement factor promotes phagocytosis, morphogenesis, and immune defense of fishes.³ C3 is the component with the highest content in the complement system and exhibits strong immune responses in various immune tissues.

1. STRUCTURE OF THE C3 GENE

C3 is the molecule that occurs first in the evolutionary complement system. The system occurrence can be dated to the stage of coelenterate.⁴ Since the discovery of Ritg in 1912, C3 has been the most critical component of the complement system. Moreover, it plays an essential role in the classical pathway (CP), lectin pathway (LP), and alternative pathway (AP). It is the intersection of different pathways.⁵ Several forms of C3 can be discovered in almost all Osteichthyes except *Gadus morhua*, *Hippoglossus hippoglossus*, and *Anarhichas minor Olafsen*, even though it is only encoded by one gene.⁶

C3 comprises α chain and β chain. It belongs to the Thioester-containing protein (TEP) superfamily. Such molecules contain a thioester site where the highly conservative $\alpha 2$ macroglobulin (MG) combines with the protease region. The cleavage site between the α and β chains is four alkaline amino acids. They are connected according to disulfide and noncovalent bonds.⁷ A complete C3 molecu-

a Corresponding author. Tel.: 19936716111, fax: +86 0736-7186016, e-mail: xiahu@webmail.hzau.edu.cn

lar is equipped with no bioactivity.⁸ In many known C3 sequences, there is the conservative β - α processing sequence RXXR (most fishes are RRKR or RKRR. The typical sequence in mammals is RRRR. R is arginine, and K is lysine). The thioester domain (TED) of the α chain contains the thiolipid bond motif comprising five amino acids (GCGEQ, where G represents glycine, C represents hemimiosine, E represents glutamic acid, and Q represents glutamine). A unique intra-chain thioester bond between Cys and Gln hides in molecules. This site is important for the immune function of C3.⁹

2. GENERATION PROCESS OF C3

Complements are proteins with enzymatic activity. They are present in serum and interstitial fluid in humans and vertebrates. Complements are synthesized in several histocytes. Hepatocytes constitute a significant source of most plasma complement proteins, especially C3. According to the individual development and extrahepatic synthetic spectra of *O. mykiss*, there is an extensive distribution of multiple complement components.⁹ The comparative study of *H. hippoglossus* and *G. morhua* revealed that the mRNA and proteins of the C3 gene had been detected in several tissues following incubation.¹⁰ Complement is synthesized for a small content in other sites, such as endothelial cells, lymphocytes, monocytes/macrophages and neurogliocytes, and so on.¹¹ Among them, monocytes/macrophages can produce all the complement components, whereas other cells or tissues can produce some complement components.¹¹

The upstream recognition molecules of the serum complement (C1q), mannose-binding lectin (MBL), and ficolin complement participate in the classical pathway (CP) and lectin pathway to finally activate C3. C3a and C3b molecules assume their roles after C3 is split into C3a and C3b. Sulfur lipid bonds in C3b allow C3 molecules to connect with receptor molecules on the cell surface by forming ester groups or amido bonds. Generally, the sulfur lipid bonds in C3 are hidden in the hydrophobic region and can only be exposed after C3 is split into C3b segments by the C3 invertease.¹² After C3 is divided into C3b, the sulfur lipid bonds in C3b are exposed and participate in the trans-acylation between the nucleophilic group on the cell surface and complex carbohydrate or immune complex, thus strengthening the humoral immune response of antigen.¹³ Furthermore, C3b can bind with the H factor. Under the participation of CD46 and CR1, C3B is also split into iC3b and C3f by complement factor I (CFI). C3b is finally divided into C3c, C3dg, and C3d.¹⁴

C3 is the most crucial component in the complement system. It is the activation and effect center. Three activation pathways of the complement system aggregate at C3 and then produce the corresponding effect. C3 is also related to the initiation of activation of AP, which also belongs to a spontaneous activation, a "tickover" mechanism called C3.⁸ C3, accounting for about 1% of total complements, triggers "tickover" every hour. C3 with structural changes is known as C3(H₂O), which can combine with fac-

tor B. Factor B can be split by the serum protease factor D, which is activated because of conformation changes, thereby causing a series of effects to trigger the splitting activation of C3.

3. EXPRESSION OF C3

Previous studies primarily concentrated on the expressions of C3 in humans, but there are few studies on the C3 expression in fishes and other species. Because C3 molecules are present at the hub of complements and are the core molecules of complement activation pathways, they have been widely researched in recent years. The C3 gene in several species, including protein expression, gene and protein sequence analysis, structure and functional analysis, and so on, have been examined and identified over time.¹⁴

Because the tissues in invertebrates have not been differentiated yet in the early stage, the complement components can only be produced in the body cavity equivalent to invertebrates or the original cavity of the blood vessels of vertebrates.¹⁵ Bilateral animals have three germ layers, and complement components hide in the cavity enclosed by the middle germ layer. The two-layer structure of coelenterate has only one coelenteron wrapped by the endoderm, and the C3 gene of coelenterate has expressions of the antenna, pharynx, and mesenteric endoderm in the three tissues of endoderm of adults.¹⁶ In mammals, the C3 gene is primarily expressed in the liver and in other tissues, such as the central nervous system, gastrointestinal tract, reproduction, and lymph.¹⁷

In fishes such as *Pseudosciaena crocea* and *Labeo rohita*, the liver is viewed as the principal organ for the synthesis of C3.^{16,18} Except for a high expression in the liver, the extrahepatic expression of C3 has been reported in many species. For example, C3 has expressions in the gills, skin, skeletal muscles, and heart of *O. mykiss*, *Epinephelus coioides*, carp, *Dicentrarchus labrax*, sea bass, and *Atlantic salmon*, among others. However, expression levels differed.¹⁹⁻²² After incubation, the expression distributions of C3 in 5-99d *H. hippoglossus* were detected using an immunohistochemical technique. This approach revealed the C3 expression in the yolk sac, muscle, liver, brain, cartilage, eyes, heart, stomach, intestines, esophagus, throat, and kidney in different stages.²³ The expression distributions of C3 in Atlantic cod aged from 1d to 57d were detected using the same approach. The results demonstrated that C3 existed in the yolk sac at 1d and in the liver, brain, kidney, and muscle at 2d. Moreover, the C3 protein was expressed in other organs in different development stages, such as the eyes, notochord, stomach, intestines, spleen, heart, and gills.²³ Although the size of C3 in different species and expression levels in different tissues and organs differ, it has stable functions because some acting sites in the conservative functional areas are highly conservative. Due to the various functions of each protein, its spatial and temporal expressions might vary to different extents. With system evolution, the complement components are mainly produced by the liver of vertebrates or hepatopancreas and hemocytes of invertebrates.

4. FUNCTIONS OF C3

4.1 BIOACTIVITY OF C3

(1) Cytotoxicity, sterilization, and dissolution effect: when the membrane attack complex (MAC) is generated, tubular structures are formed on the target cell surface to create pores and cause the breakage of target cells. MAC is the immune measure for the body to resist microbial infection (e.g., bacteria). The excessive activation of MAC may trigger the body's autoimmunity and cause damage to local tissues.²⁴ (2) Opsonisation: C3 in Osteichthyes can mediate and eliminate pathogens through target marking and change the conformation of phagocytes and pathogen cell surface molecules. (3) Removal of immune conjugates: fragment crystallizable (Fc) is affected by absorption between C3b and antibody, thus promoting the cleavage reaction of the immune complex (IC). (4) Inflammation mediating effect: The C3a anaphylatoxin of *O. mykiss* can combine with head-kidney cells, thus causing inflammatory reactions, such as respiratory bursts.²⁵ The C3 molecules of mammals are split into C3b and then combined with the antigen-antibody complex and transfer complex into the liver for removal via blood circulation.²⁶ Moreover, C3d is the smallest fragment of the complement molecule that cannot be split by protease. As a new adjuvant, C3d is integrated with target protein to prepare a vaccine, which enjoys promising application prospects. It shares a covalent linkage with antigens, facilitating cell activation and antibody affinity.

4.2 COMPLEMENT C3-RELATED DISEASES

C3 can be activated by the human immunodeficiency virus (HIV) in the early infection stage. By differently targeting the HIV infection, C3 activation can only produce some MAC to kill and dissolve the virus. It emphasizes opsonization to make HIV enter into cells or infect other cells out of cells.²⁷ In rats with complement factor H deficiency, the de-

position of C3 in the basal membrane of capillary loops requires the participation of i C3b metabolites in the complement regulatory protein.²⁸ Therefore, the deficiency of C3 can hinder the metabolism of i C3b and the deposition of C3 in the basal membrane of glomeruli, thus avoiding dense deposit disease (DDD) of glomeruli.²⁸

5. CONCLUSIONS AND PROSPECTS

To summarise, C3 is a core component of the complement system and develops a critical role. Therefore, profound investigations on the evolution and function of C3 are vital to studies on the evolution and functions of the whole complement system. Recent research works on C3 emphasize elaborating the action mechanism of the immune function. Are there more non-complement functions of C3 and any other essential tasks in the complement system of fishes except for the above four functions? What is the role of C3 in the complement system under bacterial, virus, and parasitic infections? These scientific problems must be further explored in the future.

ACKNOWLEDGMENTS

This work was supported by the Hunan Natural Science Foundation (Grant No. 2022JJ30033), Hunan Provincial Education Department key project (Grant No. 22A0488,18A519), Guangxi Natural Science Foundation (Grant No. 2022JJA130338), National Natural Science Foundation of China (Grant No. 32172965 and No. 31972835), Project of Changde Science and Technology Bureau (2020S096).

The authors declare no conflict of interest.

Submitted: February 09, 2023 CST. Accepted: March 09, 2023 CST. Published: March 16, 2023 CST.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Li XT, Ke XL, Lu MX, Liu ZG, Wang M, Pang JC. Cloning and tissues expression analysis of complement C3 of tilapia. *Biotechnology*. 2016;3:205-212. doi:[10.16519/j.cnki.1004-311x.2016.03.0037](https://doi.org/10.16519/j.cnki.1004-311x.2016.03.0037)
2. Lv LG. *Preliminary Verification of C5/C5a/C5aR Signaling Pathway and Some Related Factors of Grass Carp*. Master's thesis, Hunan Agricultural University; 2020. <https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CMFD202201&filename=1021727843.nh>
3. Zhang Y, Chen J, Yao F, Ji D, Li H, Zhang S. Expression and functional analysis of properdin in zebrafish *Danio rerio*. *Developmental & Comparative Immunology*. 2013;40(2):123-131. doi:[10.1016/j.dci.2013.02.001](https://doi.org/10.1016/j.dci.2013.02.001)
4. Yu YG, Zhang C, Xia XL, Liang YH. Evolution of the complement system. *Life science*. 2012;(4):362-367. doi:[10.13376/j.cbbs/2012.04.014](https://doi.org/10.13376/j.cbbs/2012.04.014)
5. Boshra H, Li J, Sunyer JO. Recent advances on the complement system of teleost fish. *Fish & Shellfish Immunology*. 2006;20(2):239-262. doi:[10.1016/j.fsi.2005.04.004](https://doi.org/10.1016/j.fsi.2005.04.004)
6. Wang ZP, Zhang SP, Wang GF. Advances in the composition, specificity and function of complement system in fishes. *Acta Hydrobiologica Sinica*. 2008;5:760-769. doi:[10.3724/SP.J.0000.2008.50760](https://doi.org/10.3724/SP.J.0000.2008.50760)
7. Sfyroera G, Ricklin D, Reis ES, et al. Rare loss-of-function mutation in complement component C3 provides insight into molecular and pathophysiological determinants of complement activity. *Journal of Immunology*. 2015;194(7):3305-3316. doi:[10.4049/jimmunol.1402781](https://doi.org/10.4049/jimmunol.1402781). PMID:25712219
8. Bavia L, Santiesteban-Lores LE, Carneiro MC, Prodocimo MM. Advances in the complement system of a teleost fish, *Oreochromis niloticus*. *Fish & Shellfish Immunology*. 2022;123:61-74. doi:[10.1016/j.fsi.2022.02.013](https://doi.org/10.1016/j.fsi.2022.02.013)
9. Løvøll M, Kilvik T, Boshra H, Bøgwald J, Sunyer JO, Dalmo RA. Maternal transfer of complement components C3-1, C3-3, C3-4, C4, C5, C7, Bf, and Df to offspring in rainbow trout (*Oncorhynchus mykiss*). *Immunogenetics*. 2006;58(2-3):168-179. doi:[10.1007/s00251-006-0096-3](https://doi.org/10.1007/s00251-006-0096-3)
10. Lange S, Bambir SH, Dodds AW, et al. Complement component C3 transcription in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Fish & Shellfish Immunology*. 2006;20(3):285-294. doi:[10.1016/j.fsi.2005.05.004](https://doi.org/10.1016/j.fsi.2005.05.004)
11. Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. *Immunology Today*. 1994;15(5):218-X. doi:[10.1016/0167-5699\(94\)90247-x](https://doi.org/10.1016/0167-5699(94)90247-x)
12. Law SKA, Dodds AW. The internal thioester and the covalent binding properties of the complement proteins C3 and C4. *Protein Science*. 2008;6(2):263-274. doi:[10.1002/pro.5560060201](https://doi.org/10.1002/pro.5560060201). PMID:9041627
13. Sahu A, Pangburn MK. Investigation of mechanism-based inhibitors of complement targeting the activated thioester of human C3. *Biochemical Pharmacology*. 1996;51(6):797-804. doi:[10.1016/0006-2952\(95\)02398-4](https://doi.org/10.1016/0006-2952(95)02398-4)
14. Nishida N, Walz T, Springer TA. Structural transitions of complement component C3 and its activation products. *Proc Natl Acad Sci USA*. 2006;103(52):19737-19742. doi:[10.1073/pnas.0609791104](https://doi.org/10.1073/pnas.0609791104). PMID:17172439
15. Clow LA, Raftos DA, Gross PS, Smith LC. The sea urchin complement homologue, SpC3, functions as an opsonin. *Journal of Experimental Biology*. 2004;207(12):2147-2155. doi:[10.1242/jeb.01001](https://doi.org/10.1242/jeb.01001)
16. Wang H, Qi P, Guo B, et al. Molecular characterization and expression analysis of a complement component C3 in large yellow croaker (*Larimichthys crocea*). *Fish & Shellfish Immunology*. 2015;42(2):272-279. doi:[10.1016/j.fsi.2014.11.006](https://doi.org/10.1016/j.fsi.2014.11.006)
17. Gasque P, Dean YD, McGreal EP, VanBeek J, Morgan BP. Complement components of the innate immune system in health and disease in the CNS. *Immunopharmacology*. 2000;49(1-2):171-186. doi:[10.1016/s0162-3109\(00\)80302-1](https://doi.org/10.1016/s0162-3109(00)80302-1)
18. Pushpa K, Gireesh-Babu P, Rajendran KV, Purushothaman CS, Dasgupta S, Makesh M. Molecular cloning, sequencing and tissue-level expression of complement C3 of *Labeo rohita* (Hamilton, 1822). *Fish & Shellfish Immunology*. 2014;40(1):319-330. doi:[10.1016/j.fsi.2014.07.008](https://doi.org/10.1016/j.fsi.2014.07.008)

19. Qi ZH, Liu YF, Wang WN, et al. Molecular characterization and functional analysis of a complement C3 molecule in the orange-spotted grouper (*Epinephelus coioides*). *Fish & Shellfish Immunology*. 2011;31(6):1284-1290. doi:[10.1016/j.fsi.2011.09.018](https://doi.org/10.1016/j.fsi.2011.09.018)
20. Sigh J, Lindenstrom T, Buchmann K. The parasitic ciliate *Ichthyophthirius multifiliis* induces expression of immune relevant genes in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Diseases*. 2004;27(7):409-417. doi:[10.1111/j.1365-2761.2004.00558.x](https://doi.org/10.1111/j.1365-2761.2004.00558.x)
21. Saeij JPJ, Vries BJ de, Wiegertjes GF. The immune response of carp to *Trypanoplasma borreli*: kinetics of immune gene expression and polyclonal lymphocyte activation. *Developmental & Comparative Immunology*. 2003;27(10):859-874. doi:[10.1016/S0145-305X\(03\)00083-1](https://doi.org/10.1016/S0145-305X(03)00083-1)
22. Mauri I, Roher N, MacKenzie S, et al. Molecular cloning and characterization of European seabass (*Dicentrarchus labrax*) and Gilthead seabream (*Sparus aurata*) complement component C3. *Fish & Shellfish Immunology*. 2011;30(6):1310-1322. doi:[10.1016/j.fsi.2011.03.013](https://doi.org/10.1016/j.fsi.2011.03.013)
23. Lange S, Bamir S, Dodds AW, Magnadóttir B. The ontogeny of complement component C3 in Atlantic cod (*Gadus morhua* L.)—an immunohistochemical study. *Fish & Shellfish Immunology*. 2004;16(3):359-367. doi:[10.1016/j.fsi.2003.06.001](https://doi.org/10.1016/j.fsi.2003.06.001)
24. Aleshin AE, Schraufstatter IU, Stec B, Bankston LA, Liddington RC, DiScipio RG. Structure of complement C6 suggests a mechanism for initiation and unidirectional, sequential assembly of membrane attack complex (MAC). *Journal of Biological Chemistry*. 2012;287(13):10210-10222. doi:[10.1074/jbc.m111.327809](https://doi.org/10.1074/jbc.m111.327809). PMID:22267737
25. Kania PW, Sorensen RR, Koch C, et al. Evolutionary conservation of mannan-binding lectin (MBL) in bony fish: Identification, characterization and expression analysis of three bona fide collectin homologues of MBL in the rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*. 2010;29(6):910-920. doi:[10.1016/j.fsi.2010.07.020](https://doi.org/10.1016/j.fsi.2010.07.020)
26. Mihlan M, Blom AM, Kupreishvili K, et al. Monomeric C-reactive protein modulates classic complement activation on necrotic cells. *FASEB J*. 2011;25(12):4198-4210. doi:[10.1096/fj.11-186460](https://doi.org/10.1096/fj.11-186460)
27. Webster TR, Udeagu CCN, Shephard CW. Partner services complement routine testing for early HIV diagnosis among older adults. *Am J Public Health*. 2012;102(12):e6-e6. doi:[10.2105/ajph.2012.301053](https://doi.org/10.2105/ajph.2012.301053). PMID:23078470
28. Thurman JM. Factor friction: protective and pathogenic roles for complement factors in immune complex glomerulonephritis. *Kidney International*. 2012;82(9):945-947. doi:[10.1038/ki.2012.259](https://doi.org/10.1038/ki.2012.259)