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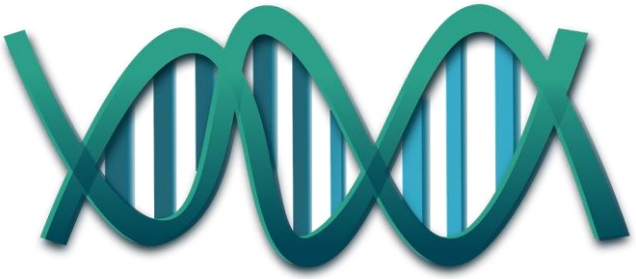
Innate Immune Gene Expression in Fish



Submitted by

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Innate immune gene expression in fish

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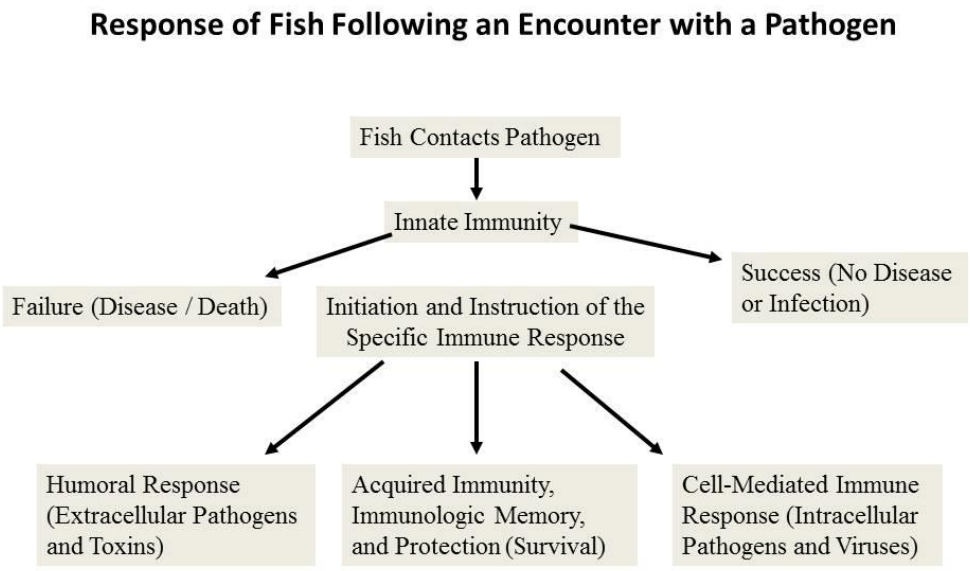
Terminologies

- **Immunology:** Study of the immune system and its responses to invading pathogenic organisms.
- **Immune system** includes the molecules, cells, tissues and organs that are associated with immunity in the host defence mechanisms.
- **Immune response** is the combined reaction of these cells and molecules to invading pathogenic organism.
- **Resistance**: any type of barrier within the host that allows it to resist the pathogen
- **Immunity** is referred as the state of acquired or innate resistance or protection from a pathogenic microorganism or its products or from the effect of toxic substances.
- **Innate or natural immunity**: attributed to inherited ability to produce antibodies without stimulation by antigens
- **Acquired immunity**: host is stimulated by contact with antigens
- **Passive immunity**: acquired through the use of antibodies from other animals (vaccination)

1. Immune system

Either the innate or acquired variety requires the interaction of specific molecules, cells and tissues for the generation of an immune response. The cells of the immune system consist of lymphocytes, specialized cells that capture and display microbial antigens, and effector cells that eliminate pathogens. Non-specific immunity is a fundamental defence mechanism in fish and it also plays a key role in activating acquired immune response. Microbial pathogen in the environment are blocked by physical barriers like skin, mucus etc from being enter into the host. If the pathogens overcome this physical barrier and invade the host the innate immune mechanism is activated and destroys the pathogen. If the pathogen survives the innate immunity the adaptive immunity is activated and combats the pathogen. The adaptive immunity also helps the immune system during the secondary invasion of the pathogen by keeping the memory of the same pathogen.

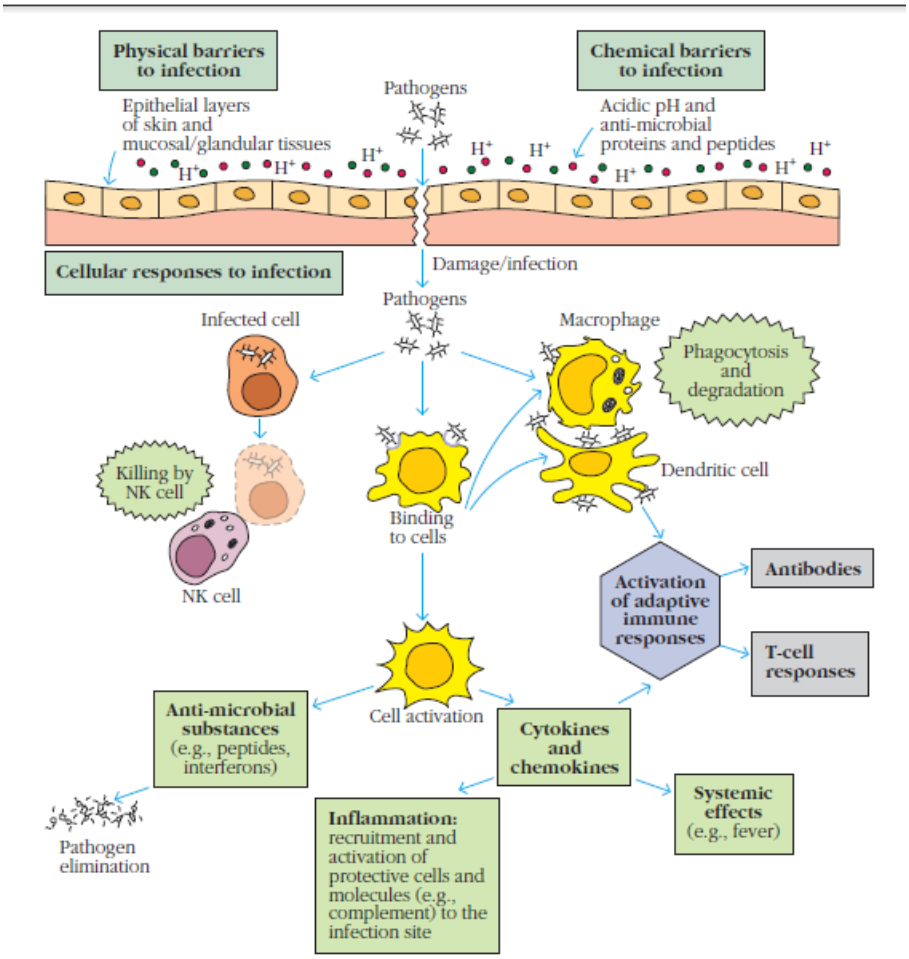


2. Innate immunity

The innate immunity system is the first-line of defence against pathogen infections. Innate immunity serves a dual role: controlling and destroying pathogens and additionally, triggering comprehensive immune responses needed for development of proper adaptive immune responses. Innate immunity is broadly divided into two a sensing arm and an effector arm. Each arm of innate immunity is further divided into cellular and humoral components.

Key elements of innate immunity include the physical and chemical barriers that prevent infection, provided by the epithelial cell layers of the skin, mucosal tissues (e.g., gastrointestinal, respiratory, and urogenital tracts), and glandular tissues (e.g., salivary, lacrimal, and mammary glands). Once pathogens enter the body, such as through a breach in an epithelial layer, they are confronted by an array of cells with cell surface and intracellular receptors that recognize pathogen components and trigger a variety of cellular responses. Pathogen recognition by these receptors activates some cells to phagocytose and degrade the pathogen, and many cells are activated through their receptors to produce a variety of antimicrobial substances that kill pathogens, as well as cytokine and chemokine proteins that recruit cells, molecules, and fluid to the site of infection, leading to swelling and other symptoms collectively known as inflammation. The innate natural killer (NK) cells

recognize and kill some virus-infected cells. Cytokines and chemokines can cause systemic effects that help to eliminate an infection, and also contribute—along with dendritic cells that carry and present pathogens to lymphocytes—to the activation of adaptive immune responses.



3. The sensing arm of innate immunity

Recognition of microbial pathogens is an essential element for the initiation of innate immune responses such as inflammation and is mediated by germline-encoded pattern-recognition receptors (PRRs) that recognize molecular structures that are broadly shared by pathogens, known as pathogen-associated molecular patterns (PAMPs) (Janeway, 1989). Upon PAMP recognition, PRRs initiate a serious of signalling programs that execute the first line of host defensive responses necessary for killing infectious microbes. In addition, PRR signalling simultaneously induces maturation of dendritic cells (DCs), which is responsible for alerting induction of the second line of host defense, so-called adaptive immunity.

The sensing and effector arms of the innate immune system in fish have cellular and humoral

	Sensing	**Effector**
Humoral	C3b, pentraxins, mannose binding lectin,	Cytokines including interferons, antimicrobial peptides, lysozymes, BPI, complement (sp. late components), acute phase reactants
Cellular	TLRs, NOD-like receptor, RIG-I like receptors, C-type lectin	Antimicrobial peptides, protease, lipase, cell adhesion molecules, reactive oxygen species like O_2- and H_2O_2 and nitrogen intermediates

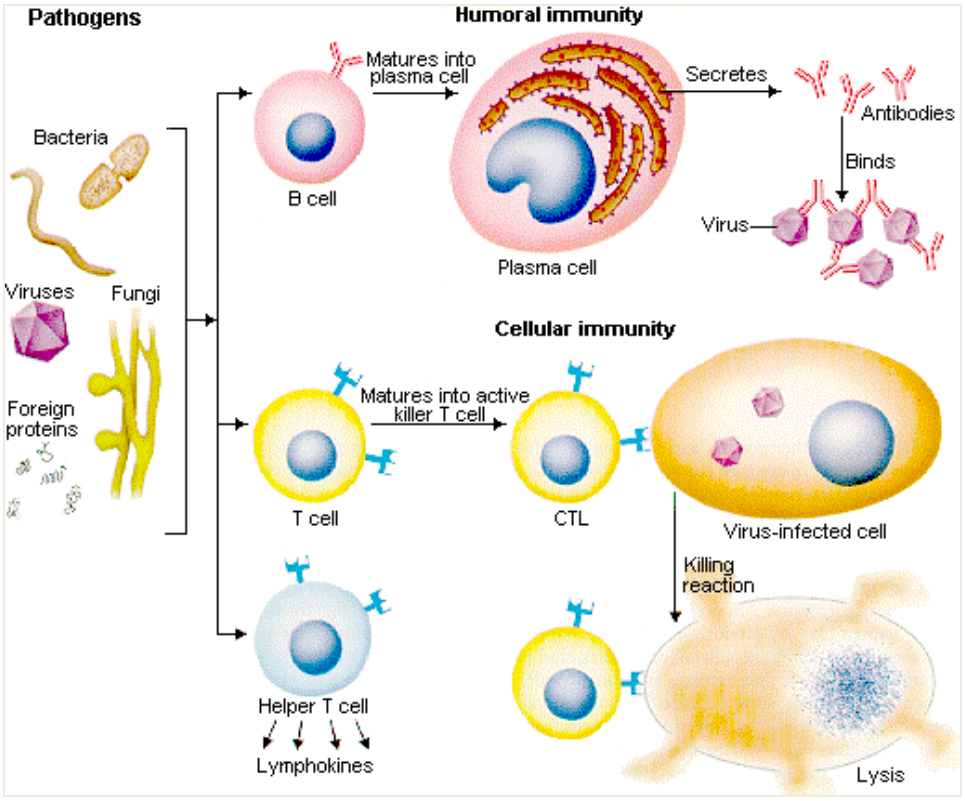
3. Humoral and cell mediated immunity

- **The humoral response** (or antibody-mediated response) involves B cells that recognize antigens or pathogens that are circulating in the lymph or blood ("humor" is a medieval term for body fluid). The response follows this chain of events:

1. Antigens bind to B cells.
2. Interleukins or helper T cells costimulate B cells. In most cases, both an antigen and a costimulator are required to activate a B cell and initiate B cell proliferation.
3. B cells proliferate and produce plasma cells. The plasma cells bear antibodies with the identical antigen specificity as the antigen receptors of the activated B cells. The antibodies are released and circulate through the body, binding to antigens.
4. B cells produce memory cells. Memory cells provide future immunity.

- **The cell-mediated response** involves mostly T cells and responds to any cell that displays aberrant MHC markers, including cells invaded by pathogens, tumor cells, or transplanted cells. The following chain of events describes this immune response:

1. Self cells or APCs displaying foreign antigens bind to T cells.
2. Interleukins (secreted by APCs or helper T cells) costimulate activation of T cells.
3. If MHC-I and endogenous antigens are displayed on the plasma membrane, T cells proliferate, producing cytotoxic T cells. Cytotoxic T cells destroy cells displaying the antigens.
4. If MHC-II and exogenous antigens are displayed on the plasma membrane, T cells proliferate, producing helper T cells. Helper T cells release interleukins (and other cytokines), which stimulate B cells to produce antibodies that bind to the antigens and stimulate nonspecific agents (NK and macrophages) to destroy the antigens.

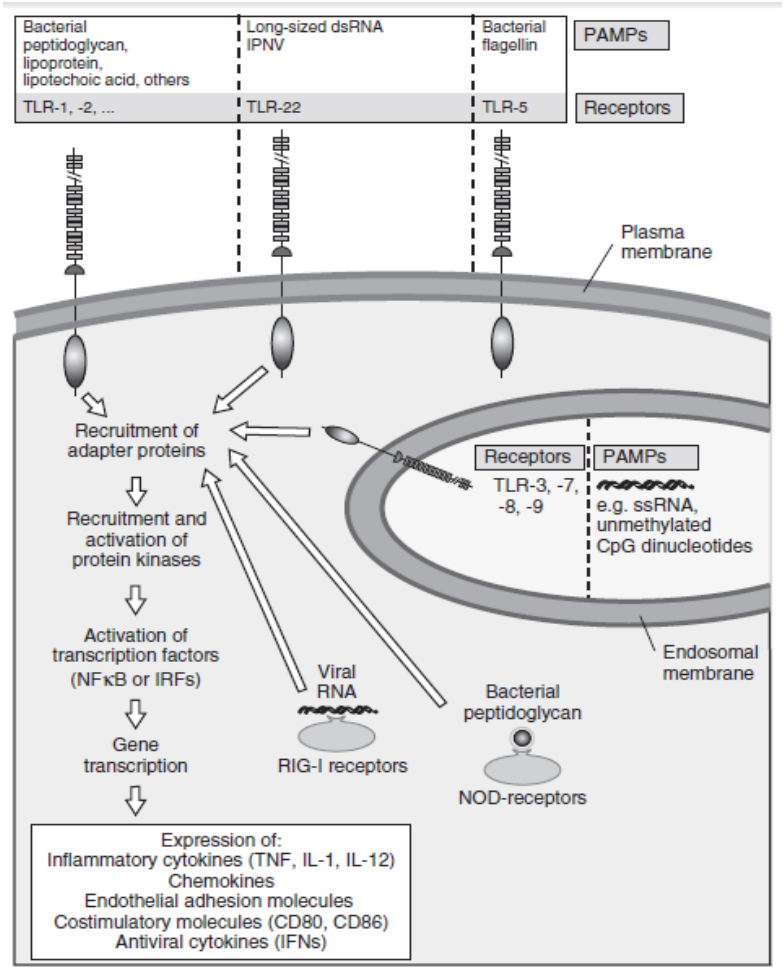


5. Innate immunity relevant genes and signalling

5.1. Pattern recognition receptors

The recognition of microbial pathogens mediated by pattern recognition receptors (PRRs) is critical to the initiation of innate immune responses. PRRs sense the conserved molecular structure of a pathogen, known as pathogen-associated molecular patterns (PAMPs), and induce subsequent host immunity through multiple signalling pathways that contribute to the eradication of the pathogen (Janeway and Medzhitov, 2002). To date, several classes of PRRs, such as Toll-like receptors (TLRs), RIG-I like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs) have been characterized from many species, including humans, rodents, birds, and teleost fishes.

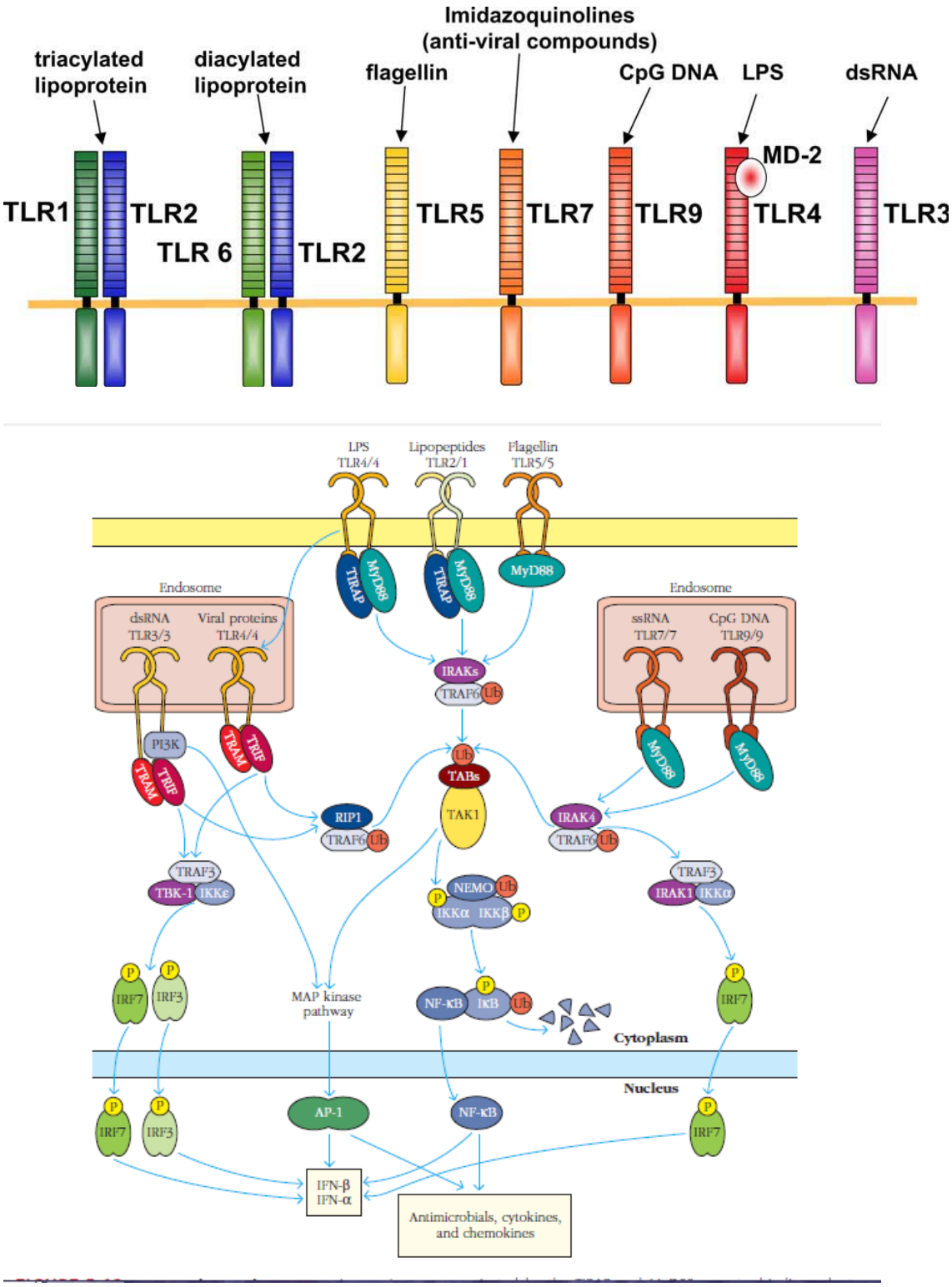
Specification and functions of pathogen recognition receptor



Toll-like receptors (TLRs) in fish

Among the best-studied PRRs are the toll-like receptors (TLRs), of which 17 types have been described in different fish species so far (Palti, 2011). In humans, two major subtypes of TLRs are identified based on their cellular localization; TLR1, 2, 4, 5, 6 and 10 (type I) are found on the cell surface and recognize microbial lipids and sugars derived from different bacteria and fungi, while TLR3, 7, 8 and 9 (type II) reside within the endosomal compartments and primarily respond to nucleic acids such as double-stranded (ds) or single stranded (ss)-RNA and CpG DNA, derived from viruses and bacteria. Representatives from both of these subtypes are identified in fish (Rebl *et al*., 2010; Palti, 2011). However, the fish TLRs have a much greater diversity compared with mammals, and additionally six non-mammalian TLRs are identified in fish. These include TLR14, which shares sequence and structural similarities with TLR1 and 2 (Oshiumi *et al*., 2003; Jault *et al*., 2004; Meijer *et al*., 2004; Hwang *et al*., 2011; Star *et al*., 2011), and the fish-specific TLR-branch including TLR 19, 20, 21 22 and 23 (Oshiumi *et al*., 2003; Meijer *et al*., 2004). Within the latter group, the one studied in detail is fugu TLR22, which was shown to recognize long-sized dsRNA on the cell surface (Matsuo *et al*., 2008).

Fish TLRs exhibit distinct features; for example, TLR5, which in mammals is a membrane-bound receptor that recognizes the flagellin component of bacterial flagella, is found both as a membrane-bound (TLR5M) and soluble (TLR5s) protein in rainbow trout (Tsujita *et al*., 2004) and other species as well (Oshiumi *et al*., 2003; Hwang *et al*., 2010). The membrane-bound TLR5 signaling in trout in response to flagellin, as measured by NFκB activation, is amplified through interaction with the soluble form (TLR5s) in a positive feedback loop (Tsujita *et al*., 2004). Atlantic cod TLRs are unique due to the absence of several TLRs that recognize bacterial surface antigens (TLR1, TLR2 and TLR5), while the families of TLRs that recognize nucleic acids (TLR7, TLR8, TLR9 and TLR22) are expanded (Star *et al*., 2011). These data point to an increased importance of the recognition of pathogens through the nucleic acid detection system for the cod immune defense.



MyD88-Dependent Signalling Pathways

After associating with a TLR dimer following ligand binding, MyD88 initiates a signalling pathway that activates the NF-_B and MAPK pathways by essentially the same pathway as that activated by IL-1. As shown for plasma membrane TLRs 2/1, 4, and 5 in Figure, MyD88 recruits and activates several IRAK protein kinases, which then bind and activate TRAF6. TRAF6 ubiquitinates NEMO and TAB proteins,

leading to the activation of TAK1, which then phosphorylates the IκB kinase (IKK) complex. Activated IKK then phosphorylates the inhibitory IκB subunit of NF-κB, releasing NF-κB to enter the nucleus and activate gene expression. TAK1 does double duty in this TLR signalling cascade. After separating from the IKK complex, it activates MAPK signalling pathways that result in the activation of transcription factors including Fos and Jun, which make up the AP-1 dimer. In addition to activating NF-κB and MAPK pathways via the MyD88-dependent pathway, the endosomal TLRs 7, 8, and 9, which bind microbial nucleic acids (especially viral RNA and bacterial DNA), also trigger pathways that activate IRFs. As shown in Figure 5-13, when triggered by these TLRs, the MyD88/IRAK4/TRAF6 complex activates a complex containing TRAF3, IRAK1, and IKK1, leading to the phosphorylation, dimerization, activation, and nuclear localization of IRF7. IRF7 induces the transcription of genes for both Type 1 interferons, IFN-α and -β, which have potent antiviral activity. Thus different TLRs may differentially activate distinct transcription factors (NF-κB, certain IRFs, and/or those activated by MAPK pathways), leading to variation in which genes are turned on to help protect us against the invading pathogens.

TRIF-Dependent Signalling Pathways

For the two endosomal TLRs that recruit the TRIF adaptor instead of MyD88—TLR3 and endosomal TLR4—the downstream signalling pathways differ somewhat from those activated by MyD88. TRIF recruits the RIP1 protein kinase that in turn recruits and activates TRAF6, which then initiates the same steps as in the MyD88-dependent pathway. TLR3 also activates PI3K, which enhances MAPK pathway activation. In addition, TRIF activates TRAF3 and a complex of TBK-1 and IKKε, which phosphorylates and activates IRF7 and a different IRF—IRF3. IRF3 and IRF7 dimerize and move into the nucleus and (together with NF-κB and AP-1) induce the transcription of the IFN-α and -β genes. Thus, all of the intracellular TLRs that bind viral PAMPs in an infected cell induce the synthesis activated by the TIRAP and MyD88 receptor-binding adaptors. TRIF-dependent signalling pathways are activated by the TRAM and TRIF receptor-binding adaptors.

PAMP Detection by TLRs and Other PRRs

Species	PAMPs	TLR Usage	PRRs Involved in Recognition
Bacteria, mycobacteria	LPS	TLR4	
	Lipoproteins,	LTA, PGN, lipoarabinomannan TLR2/1, TLR2/6	NOD1, NOD2, NALP3, NALP1
	flagellin	TLR5	IPAF, NAIP5
	DNA	TLR9	AIM2
	RNA	TLR7	NALP3
Viruses	DNA	TLR9	AIM2, DAI, IFI16
	RNA	TLR3, TLR7, TLR8	RIG-I, MDA5, NALP3
	structural protein	TLR2, TLR4	
Fungus	zymosan, b-glucan	TLR2, TLR6	Dectin-1, NALP3
	Mannan	TLR2, TLR4	
	DNA	TLR9	
	RNA	TLR7	
Parasites	tGPI-mutin (Trypanosoma)	TLR2	
	glycoinositolphospholipids (Trypanosoma)	TLR4	
	DNA	TLR9	
	hemozoin (Plasmodium)	TLR9	NALP3
	profilin-like molecule (Toxoplasma gondii)	TLR11	

A number of TLR ligands (agonists) may induce strong innate responses that may be decisive for the outcome of adaptive responses. Teleost fish species may possess close to twice the number of different TLRs compared with mammalian species, and one may think about how diverse the immune response may be with respect to fish TLR-induced signalling. A review article on immune-relevant genes including TLR-like receptors in fish has recently been published by Zhu *et al*. (2013). In general, those TLRs that, after ligand binding, induce the production of IL-12 favour a Th1 response (TLR 3, 4, 5, 7, 8, 9 and 11), and in addition the activation of

these TLRs may induce cross-presentation of antigens facilitating a cytotoxic T-cell response under certain conditions (Manicassamy and Pulendran, 2009). It should be mentioned that ligand binding to TLRs 3 and 4, 7 and 9 may also induce type I IFN responses via interferon regulating factors.

NOD-like and RIG receptor in fish

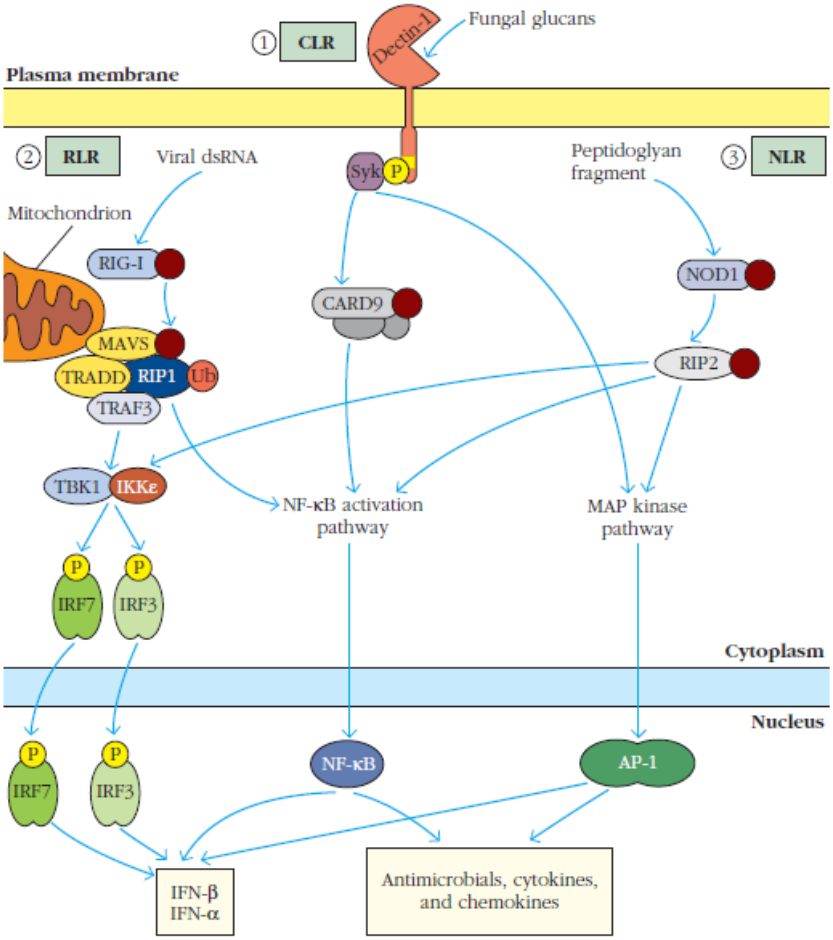
Two additional families of innate receptor have been described in fish that join the TLRs as key pathogen sensors: the NOD-like receptor (NLR) and retinoic acid-inducible gene-1 (RLRs) (Laing *et al*., 2008). Both of them are intracellular cytosolic receptor, mainly involved in bacterial or viral defense, respectively. The NLRs family members are best known for ability to signal NFkB activation (NOD 1 and NOD 2) or secretion of the pro-inflammatory cytokines IL-1b and IL-18 (NALPs) (Chen *et al*., 2009). In teleost, the NLRs family genes have been identified in the zebrafish genome and also form number of various fish species including channel catfish, grass carp, rainbow trout and flounder. The RLRs comprises three families such as RIG-I, MDA-5 and LGP2 that are responsible for detection of cytoplasmic viral RNA (Kumar *et al*., 2011). RIG-I has been cloned in a various fish species including grass carp and channel catfish, LGP2 and MDA5 in rainbow trout and grass carp. Moreover, MDA5 has been derived from rainbow trout, Japanese flounder and grass carp and rainbow trout LGP2, exhibit antiviral functions (Chang *et al*., 2011a), thus demonstrating that fish RLRs, like their mammalian homologs, have roles in host surveillance against viral infections. There are several recent reviews about innate recognition in fish, where a more detailed description about the genomics and biology of piscine PRRs can be found (Zhang and Gui, 2012; Zhu *et al*., 2013).

CLR, NLR, RLR signalling pathway

C-type lectin receptors bind carbohydrates on the surfaces of extracellular pathogens

The second family of cell surface PRRs that activate innate and inflammatory responses is the **C-type lectin receptor (CLR)** family. CLRs are plasma membrane receptors expressed variably on monocytes, macrophages, dendritic cells, neutrophils, B cells, and T-cell subsets. CLRs generally recognize carbohydrate components of fungi, mycobacteria, viruses, parasites, and some allergens (peanut and dust mite proteins). CLRs have a variety of functions. Unlike TLRs, which do not promote phagocytosis, some CLRs function as phagocytic receptors, and all CLRs

trigger signalling pathways that activate transcription factors that induce effector gene expression. Some CLRs trigger signalling events that initially differ from TLR signalling but generally lead to downstream steps similar to the MyD88-dependent TLR pathways that activate the transcription factors NF-κB and AP-1. An example of this is Dectin-1, which binds cell wall β-1,3 glucans on mycobacteria, yeast, and other fungi Dectin-1 contains a cytoplasmic domain with an ITAM similar to those in the signalling chains of B-cell and T-cell antigen receptors. After Dectin-1 binds a ligand, its ITAM is phosphorylated and then recognized by the tyrosine kinase Syk, also involved in the initial stages of B-cell activation. Syk triggers MAPK pathways, leading to the activation of the transcription factor AP-1, and also activates CARD9, one of many signalling components with **Caspase recruitment domains (CARD)**. CARD9 forms a complex with additional signalling components, leading to the activation of IKK and the nuclear translocation of NF-κB as described above for TLR. NF-κB and AP-1 cooperate in inducing expression of inflammatory cytokines and IFN-β.



Retinoic acid-inducible gene-i-like receptors bind viral RNA in the cytosol of Infected Cells

The **Retinoic acid-inducible gene-I-like receptors (RLRs)** are soluble PRRs that reside in the cytosol of many cell types, where they play critical roles as sensors of viral infection. The three known RLRs (RIG-I, MDA5, and LGP2) are CARD-containing RNA helicases that recognize viral RNAs, usually from RNA viruses such as influenza, measles, and West Nile. These receptors appear to distinguish viral from cellular RNA on the basis of particular structural features not shared by normal cellular RNA, such as double stranded regions of the RNA, virus-specific sequence motifs, and, in the case of RIG-I, a 5' triphosphate modification that arises during viral RNA synthesis and processing. Upon viral RNA binding, RIG-I undergoes a conformational change that leads to binding via CARD-CARD interactions to its downstream adaptor molecule located in mitochondrial membranes, the mitochondria-associated MAVS (*m*itochondrial *a*nti-*v*iral *signalling*) protein. MAVS aggregates and recruits additional proteins, including the adaptor TRADD, TRAF3, and RIP1, which activate NEMO/IKKs that lead to the activation of IKK complexes, which activate IRFs 3 and 7 and the NF-κB pathway, leading to expression of IFNs α and β, other antimicrobials, chemokines, and pro-inflammatory cytokines.

Nod-Like receptors are activated by a variety of PAMPs, DAMPs, and other harmful substances

The final family of PRRs is the NLRs. (NLR is an acronym that stands for both Nod-like *r*eceptors and *n*ucleotide oligomerization domain//eucine-rich repeat-containing *r*eceptors). The NLRs are a large family of cytosolic proteins activated by intracellular PAMPs and substances that alert cells to damage or danger (DAMPs and other harmful substances). They play major roles in activating beneficial innate immune and inflammatory responses. PAMP binding to the LRR regions of NODs initiates signalling by activating NOD binding to the serine/threonine kinase RIP2 through their CARDs. RIP2 then activates IKK, leading to nuclear translocation of NF-κB. RIP2 also activates MAPK pathways, leading to AP-1 activation. The activated NF-κB and AP-1 initiate transcription of inflammatory cytokines—including TNF-α, IL-1, and IL-6—and antimicrobial and other mediators. In addition, RIP2 activates the TRAF3/TBK1/IKKε complex, leading to phosphorylation of IRFs 3 and 7 and to production of IFNs-α and -β.

5.2. Antimicrobial peptides

The integument and integumental secretions of fish, such as the multifunctional skin mucus have been shown to play a significant role in host defence against bacteria and viruses. Antimicrobial polypeptides have been found in the tissues of some teleost species, including halibut and flounder e.g., mucus, liver and gills. The low molecular weight polypeptides have the ability to break down cell wall of both Gram-positive and Gram-negative bacteria. There are two different viewpoints about the anti-bacterial mechanisms of AMPs: (1) the amphiphilic structure of AMPs can selectively bind to the bacterial membrane and form transmembrane channels, which destruct of their membrane integrity and kill incursive bacteria; and (2) AMPs can directly enter the bacterial cell to interact with specific intracellular targets to interfere with bacterial growth and metabolism, thus playing a role in bacterial death.

Fish AMPs are important components of the non-specific immune system. The major groups of AMPs studied in fish include defensins, natural resistance-associated macrophage protein (Nramp), NK-lysin, and hepcidin. Defensins could be classified into three subfamilies (e.g. α-defensins, β-defensins, and θ-defensins) according to the distribution of the disulphide bonds formed by the six conserved cysteine residues. They play important roles both in innate immunity as microbicidal agents and in adaptive immunity as enhancers. Multiple β -defensins have been identified in bony fish. For instance, two β -defensins were identified in common carp (*C. carpio*) (Marel *et al*., 2012), three were cloned in zebrafish (*D. rerio*) (Zou *et al*., 2007a), four were detected in rainbow trout (*O. mykiss*) (Casadei *et al*., 2009; Falco *et al*., 2008), and many others were found in other fish species including olive flounder (*Paralichthys olivaceus*) (Nam *et al*., 2010), medaka (*Oryzias latipes*) (Zhao *et al*., 2009), and orange-spotted grouper (*E. coioides*) (Jin *et al*., 2010). The orange-spotted grouper b-defensin is a newly identified family member with 63 amino acids whose sequence shares very high identity with that of green puffer, medaka, rainbow trout, and zebrafish β -defensin-2 (57.14–87.30%) and very low identity with other β -defensins in fish.

Hepcidin has been found to act as an important iron regulator by binding to ferroportin (a key iron exporter on macrophages) and inducing ferroportin-mediated endocytosis and proteolysis, emerging studies have focused on its regulation of iron metabolism and indirect host defense (Ganz, 2011; Kroot *et al*., 2012; Nicolas *et al*.,

2001; Shi and Camus, 2006). To date, hepcidin homologs have been found in various teleost fish, including marine medaka (*Oryzias melastigma*) (Bo *et al*., 2011), orange-spotted grouper (*E. coioides*) (Zhou *et al*., 2011), ayu (*Plecoglossus altivelis*) (Chen *et al*., 2010b), large yellow croaker (*P. crocea*) (Wang *et al*., 2009a), turbot (*S. maximus*) (Chen *et al*., 2007a), Japan sea bass (*Lateolabrax japonicas*) (Ren *et al*., 2006), and black porgy (*Acanthopagrus schlegelii*) (Yang *et al*., 2007).

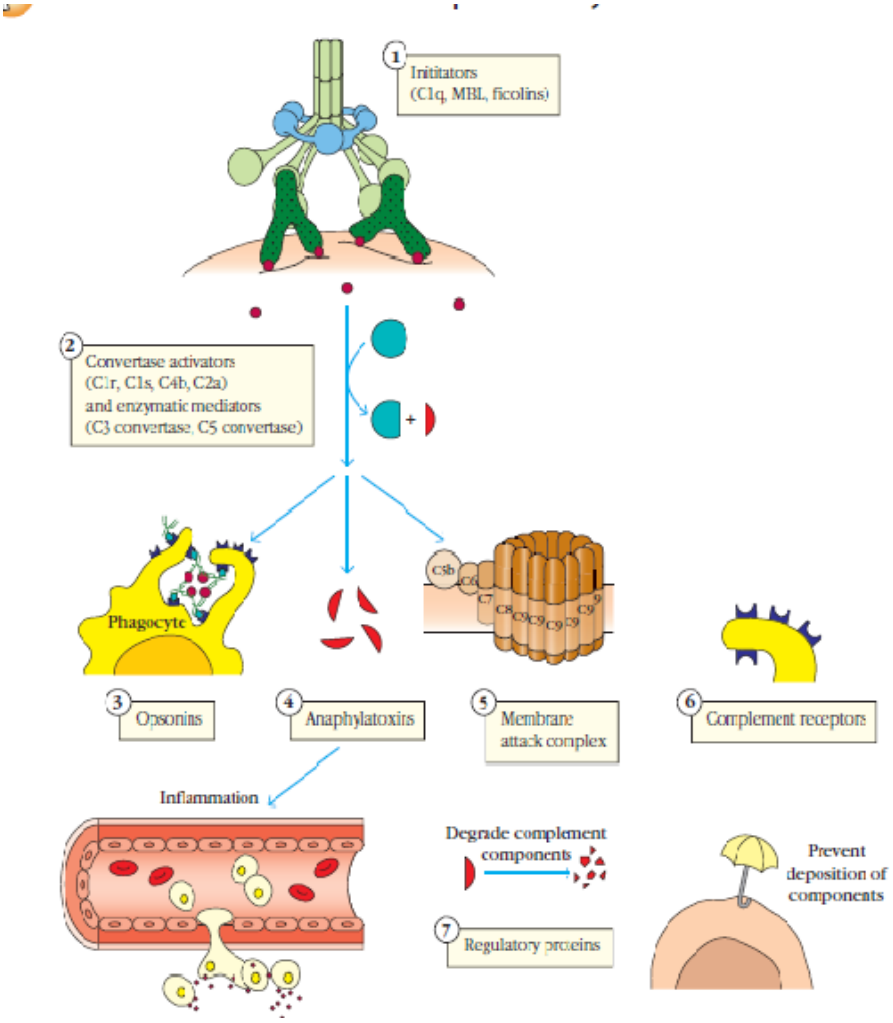
5.3. Complement molecules

There are three different ways to activate the complement on the surface of invading pathogens: the classical pathway, the mannan-binding lectin pathway, and the alternative pathway. Although initiation of each approach depends on different elements, they all produce the same anti-infectious effects: (1) opsonization or the promotion of pathogen phagocytosis by phagocytes with complement receptors due to the large number of activated complement proteins generated and covalently bound with the invading pathogen; (2) recruitment of phagocytic cells to sites of inflammation and promotion of their activation; and (3) formation of membrane-attack complexes to punch holes in the cell membranes of pathogens, thus causing their death. In addition to the major functions of complements in innate immunity, some studies reported that a series of complements could also play essential roles in adaptive immunity, immunologic memory, and even in tissue regeneration and tumor growth.

Several complement components, such as C3 and Bf, have been confirmed to be maternal factors that are transferred from maternal source into eggs in different fish species, including spotted wolfish (Anarhichas minor Olafsen) (Ellingsen *et al*., 2005), rainbow trout (*O. mykiss*) (Løvoll *et al*., 2006), common carp (*C. carpio*) (Huttenhuis *et al*., 2006), grass carp (*Ctenopharyngodon idella*) (Shen *et al*., 2011b), Atlantic salmon (*Salmo salar*) (Løvoll *et al*., 2007), and zebrafish (*D. rerio*) (Wang *et al*., 2008). Although there is little evidence to confirm the existence of the mannan-binding lectin pathway in teleost fish in the past, the homologues of mannose-binding lectin (which is the initiating component capable of activating this pathway) have been characterized in three members of the carp (*Cyprinidae*) family, including zebrafish (*D. rerio*), goldfish (*C. auratus*), and crucial carp (*C. carpio*) (Vitved *et al*., 2000), as well as in turbot (*S. maximus*) (Zhang *et al*., 2010a) and channel catfish (*I. punctatus*) (Zhang *et al*., 2011).

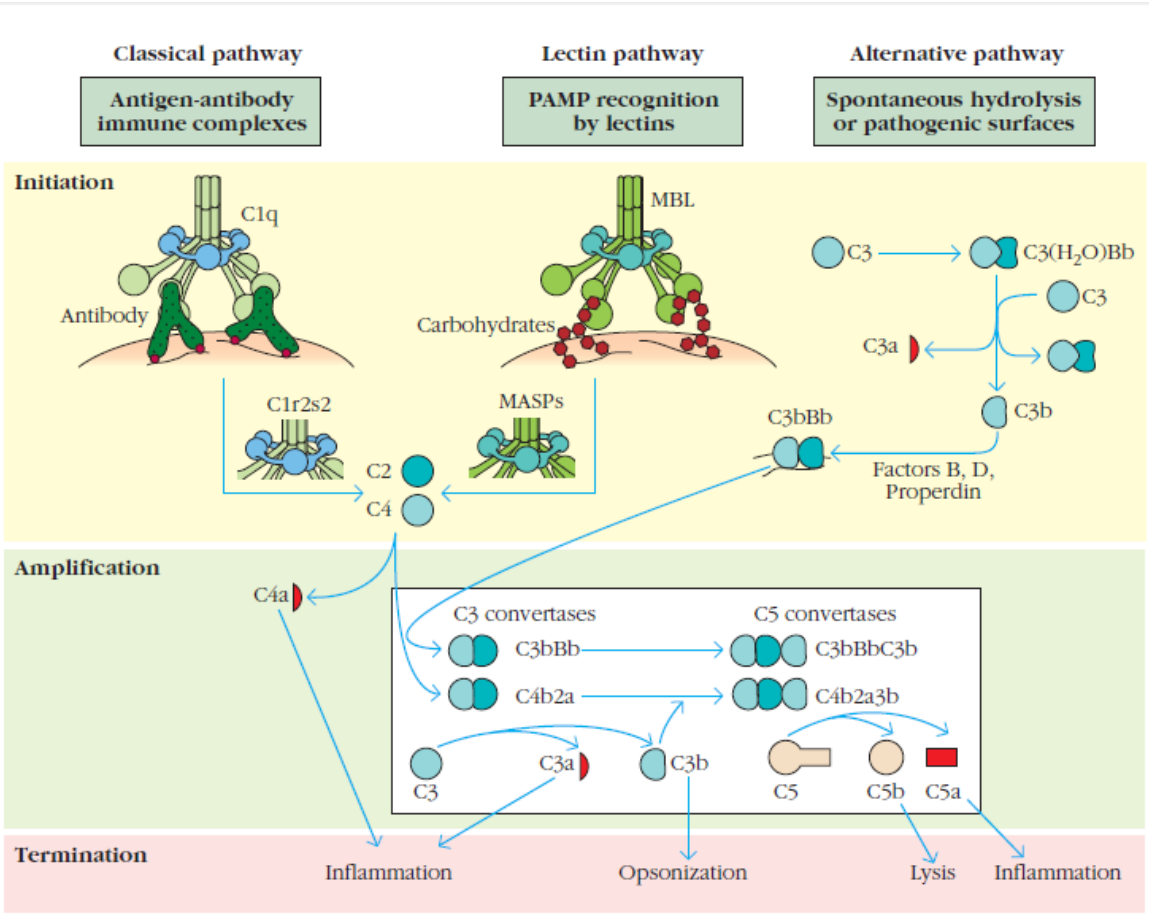
Proteins involved in complement system

(1) The complement pathways are initiated by proteins that bind to pathogens, either directly or via an antibody or other pathogen specific protein. After a conformational change, (2) enzymatic mediators activate other enzymes that generate the central proteins of the complement cascade, the C3 and C5 convertases, which cleave C3 and C5, releasing active components that mediate all functions of complement, including (3) opsonization, (4) inflammation, and (5) the generation of the membrane attack complex (MAC). Effector complement proteins can label an antibody-antigen complex for phagocytosis (opsonins), initiate inflammation (anaphylatoxins), or bind to a pathogen and nucleate the formation of the MAC. Often, these effectors act through (6) complement receptors on phagocytic cells, granulocytes, or erythrocytes. (7) Regulatory proteins limit the effects of complement by promoting their degradation or preventing their binding to host cells.



Proteins involved in complement system

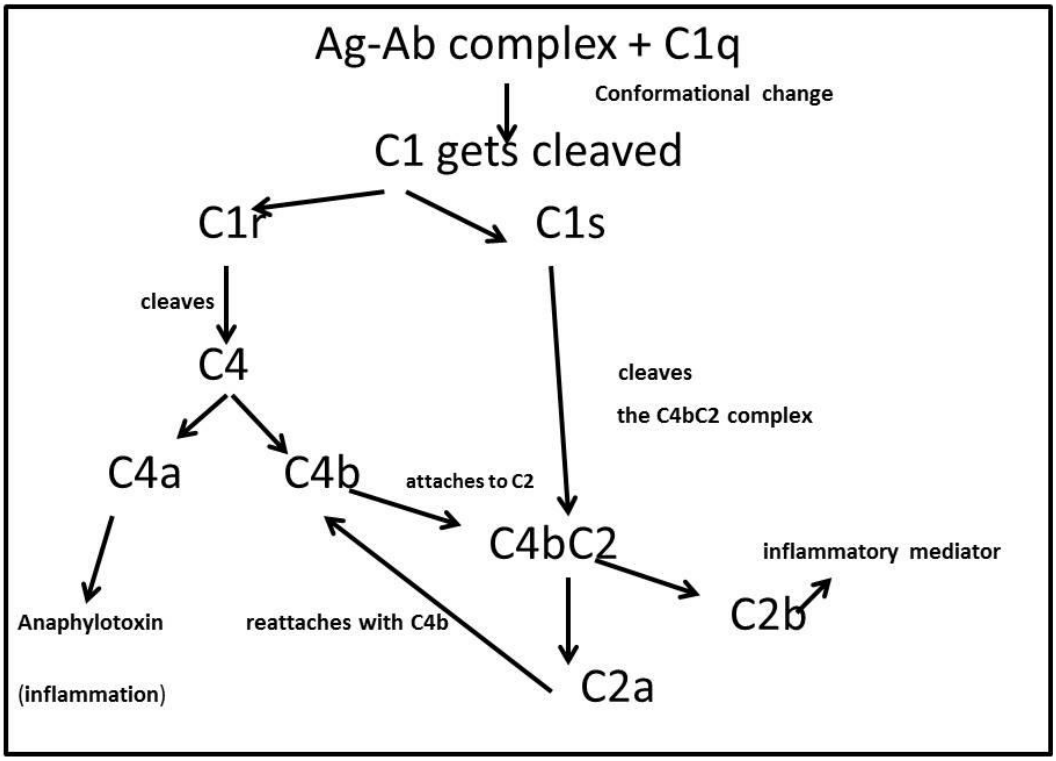
Major pathways of complement activation



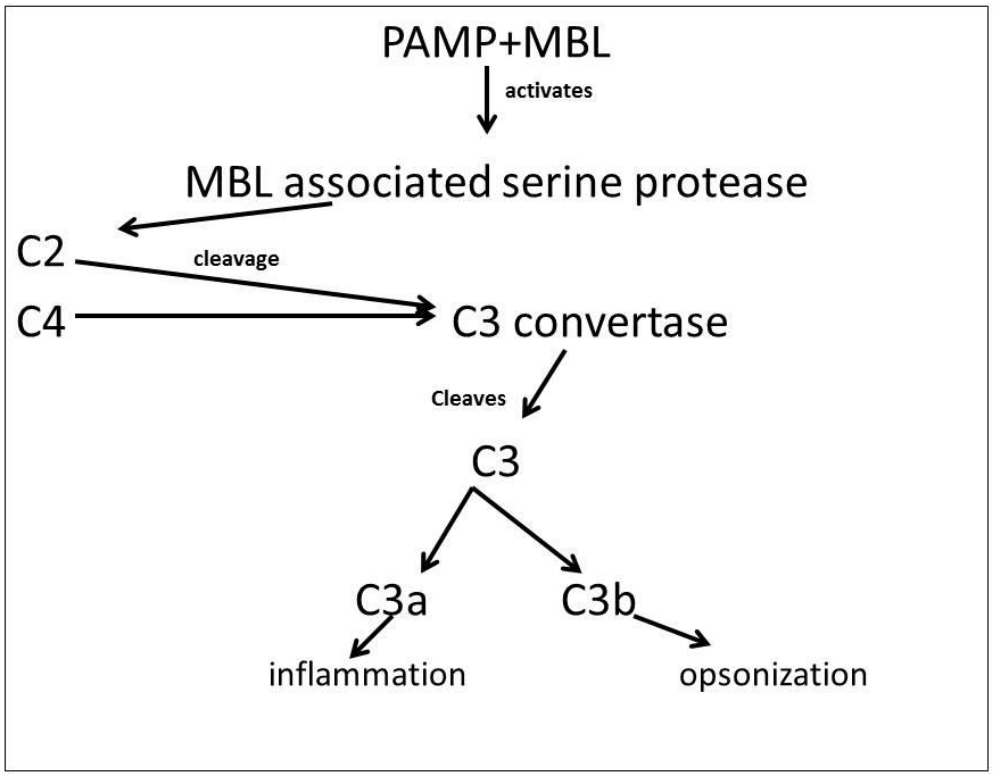
The **classical pathway** is initiated when C1q binds to antigen-antibody complexes. The antigen is shown here in dark red and the initiating antibody in green. The C1r enzymatic component of C1 (shown in blue) is then activated and cleaves C1s, which in turn cleaves C4 to C4a (an anaphylatoxin, bright red) and C4b. C4b attaches to the membrane, and binds C2, which is then cleaved by C1s to form C2a and C2b. (C2b is then acted upon further to become an inflamatory mediator.) C2a remains attached to C4b, forming the classical pathway C3 convertase (C4b2a). In the **lectin pathway**, mannose binding lectin (MBL, green) binds specifically to conserved carbohydrate arrays on pathogens, activating the MBL-associated serine proteases (MASPs, blue). The MASPs cleave C2 and C4 generating the C3 convertase as in the classical pathway. In the **alternative pathway**, C3 undergoes spontaneous hydrolysis to C3(H2O), which binds serum factor B. On binding to C3 (H2O), B is cleaved by serum factor D, and the resultant C3(H2O)Bb complex forms a fluid phase C3 convertase. Some C3b, released after C3 cleavage by this complex, binds to microbial surfaces. There, it binds factor B, which is cleaved by factor D, forming the cell-bound alternative pathway C3

convertase, C3bBb. This complex is stabilized by proper din. The C5 convertases are formed by the addition of a C3b fragment to each of the C3 convertases.

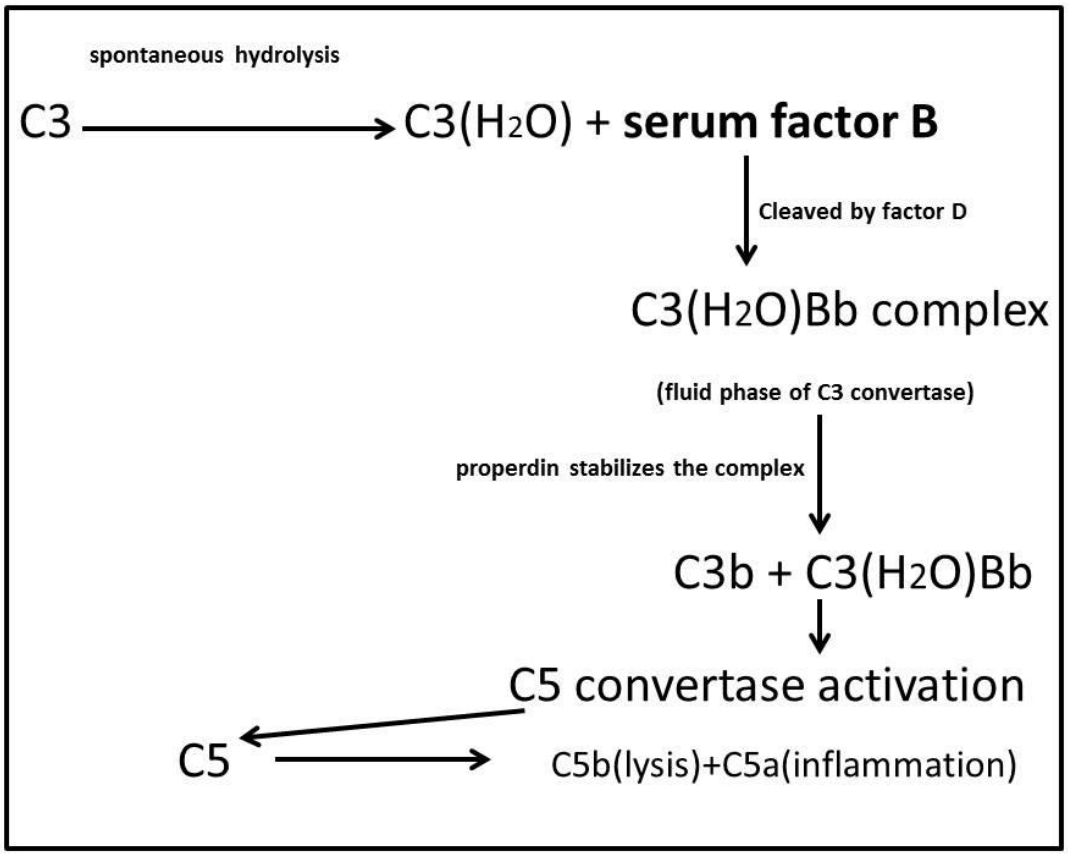
Classical pathway



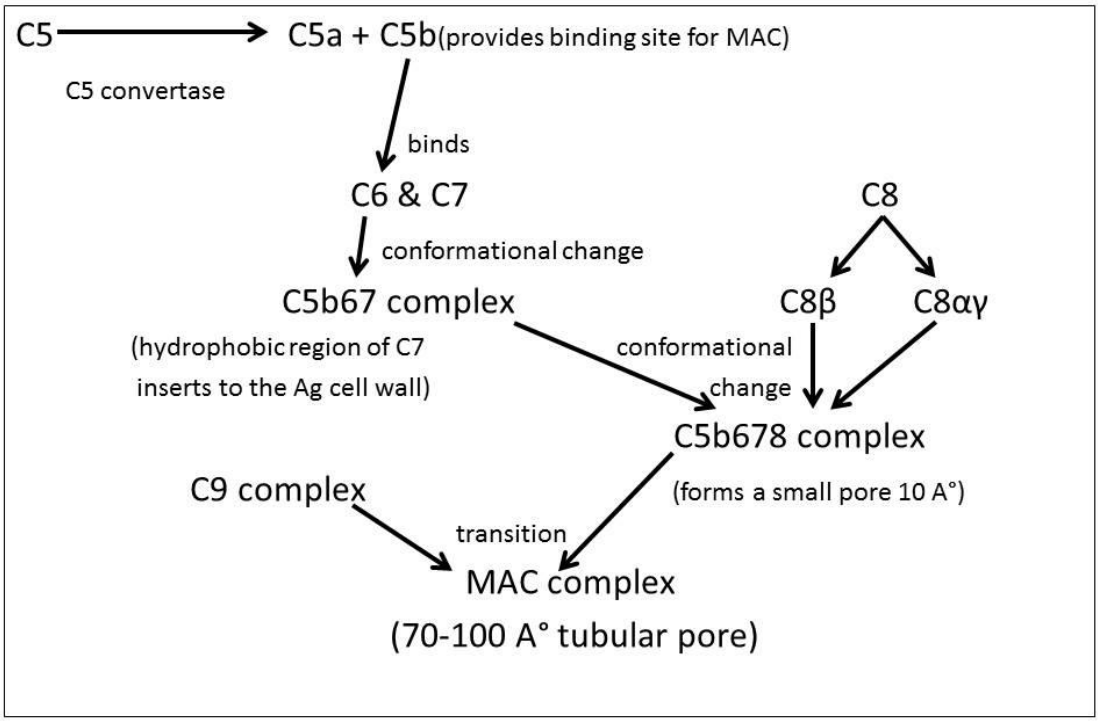
Lectin pathway



Alternative pathway



MAC formation – happens when all three complement pathways converge - C5 convertase production



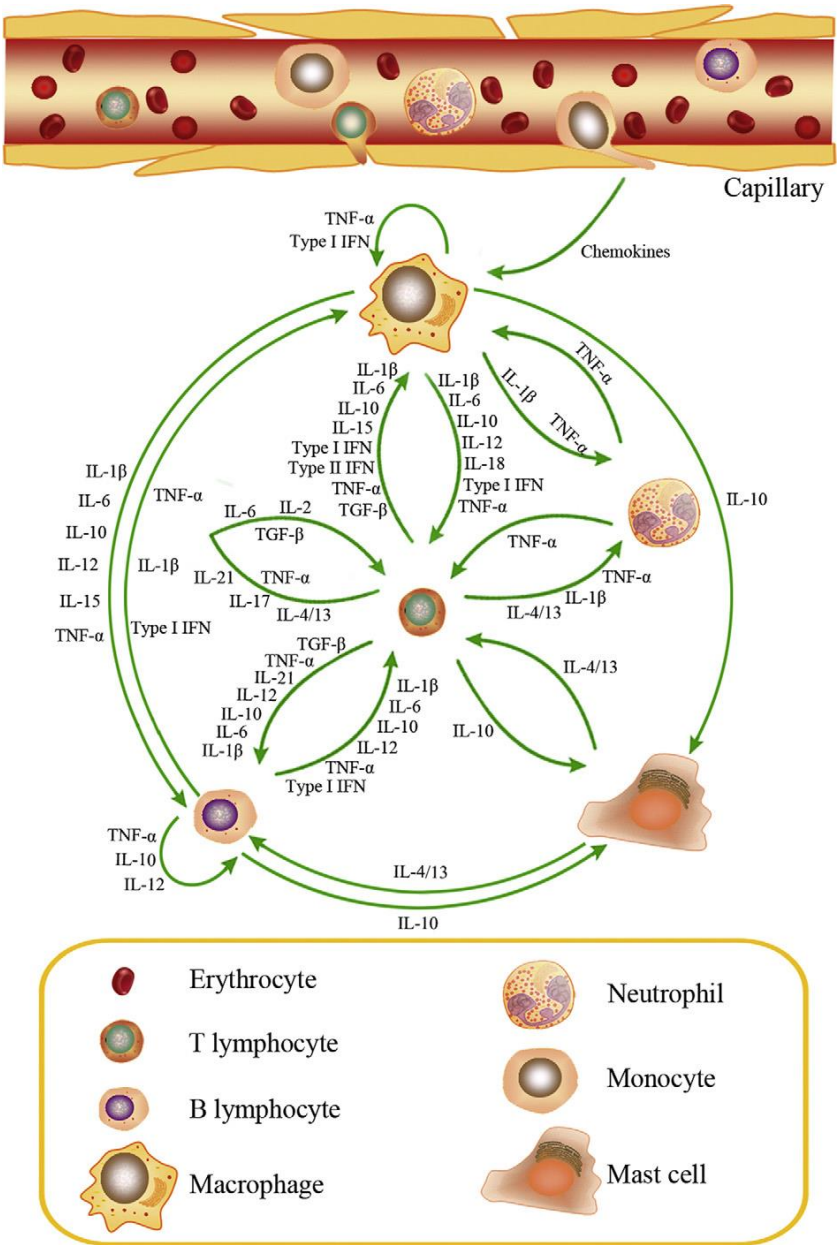
5.4. Lectin family members

Biomembranes contain an amount of carbohydrates mainly in the form of glycoproteins and glycolipids. Lectins are a group of sugar binding proteins that can specifically bind to carbohydrate structures from viruses, bacteria, fungi and animals. Lectins are an enormous superfamily that consists of a great number of members throughout almost all living creatures, including virus, bacteria, fungi, protists, plants, and animals. In animals, the lectins are pivotal components of innate immune response by inducing phagocytosis, activating platelet, initiating complement system, and enhancing the natural killer cell activity. With the emergence of adaptive immunity in vertebrates, the lectins have corresponding functions as regulators of adaptive immune responses by recognizing bacterial or viral components on dendritic cells (DCs), promoting signals that initiate or modulate cytokine responses and inducing lymphocyte maturation, and polarization to the invading pathogens. A total of 15 major lectin families have been identified in animals, among which C-type lectins, galectins, F-type lectins, rhamnose-binding lectins, and intelectins have also been demonstrated to exist in fish species.

C-type lectin is a large family that includes all known collectins and selectins in animals. Its most basic feature is its ability to interact with carbohydrate residues in glycoprotein through the C-type carbohydrate recognition domain (CRD) or C-type lectin domain. Aside from the mannan-binding lectin in the complement system described above, several other members of the C-type lectin family have been discovered in rainbow trout (*O. mykiss*) (Zhang *et al*., 2000), common carp (*C. carpio*) (Savan *et al*., 2004), Japanese eel (*Anguilla japonica*) (Tasumi *et al*., 2002), bighead carp (*Aristichthys nobilis*) (Pan *et al*., 2010), zebrafish (*D. rerio*) (Lin *et al*., 2009a), turbot (*S. maximus*) (Zhang *et al*., 2010a), and grass carp (*C. idellus*) (Liu *et al*., 2011). All these C-type lectins could be significantly induced by bacterial invasion and have shown different functions of agglutination and inhibition with various invading pathogens. For instance, the purified bighead carp C-type lectin possesses anti-*Vibrio harveyi* activity but no antifungal activity, whereas recombinant turbot C-type lectin can agglutinate the Gram-negative fish pathogen *Listonella anguillarum* but cannot function against the gram-positive pathogen *Streptococcus iniae*.

5.5. Cytokines in innate immunity

Lymphoid cells, inflammatory cells and haematopoietic cells are involved in the development of an effective immune response. The complex interaction of these three cells are mediated by a group of protein are collectively called as cytokines.



Cytokines are a family of low molecular weight proteins that are often glycosylated and are secreted by activated immune-related cells upon induction by various pathogens such as parasitic, bacterial, or viral components. They can modulate immune responses through an autocrine or paracrine manner upon binding to their corresponding receptors. Cytokines are derived from macrophages, lymphocytes, granulocytes, DCs, mast cells, and epithelial cells, and can be divided into interferons (IFNs), interleukins (ILs), tumor necrosis factors (TNFs), colony

stimulating factors, and chemokines. In innate immunity, macrophages can secret IL-1, IL-6, IL-12, TNF-a, and chemokines such as IL-8 and MCP-1, all of which are indispensable for macrophage, neutrophil, and lymphocyte recruitment to the infected tissues and their activation as pathogen eliminators. Meanwhile, cytokines released by phagocytes in tissues can also induce acute phase proteins, including mannose-binding lectin (MBL) and C-reactive protein (CRP), and promote migration of DCs. All these cytokines have been found in bony fish, and their functions and signalling are being explored with great progress.

5.5.1. Interferons and signalling factors

Interferons (IFNs) are most important molecules during viral infection and they have ability to interfere with replication of virus. In human and other mammals, IFNs are the first line of defense against virus infections and can be induced through different signalling pathways in response to pathogen infection or pathogen associated molecular pattern stimulation. A large number of IFNs have been identified in various species of vertebrates. They are classified into three groups with different structures and functions (e.g. type I IFNs, type II IFNs, and type III IFNs) and interact with different cell-surface receptors. Type I IFNs consist of about 20 members, including IFN-α, IFN-β, IFN-ω, IFN-κ, IFN-ε, and limitin, while both type II and type III IFNs only have one member called IFN-c and IFN-k, respectively. In recent years, progress has been made in fish IFN research. Since IFN-like activity was first discovered in a permanent cell line from fathead minnow (*Pimephales promelas*) stimulated with infectious pancreatic necrosis virus (IPNV) (Gravell and Malsberger, 1965), similar observations were also described in various fish species induced by different viruses (de Kinkelin and Dorson, 1973; Mathews and Vorndam, 1982; Tamai *et al*., 1993). Careful property analysis in grass carp (*Ctenocpharyngodon idellus*) revealed that virus-induced fish IFN shares similar properties with mammalian type I IFN (Shao *et al*., 1998). Grass carp type I-like IFN can be produced by leucocytes from head kidney, spleen, and peripheral blood (Xiang and Shao, 2000).

IFN genes have been identified from various fish species, including Atlantic salmon (*S. salar*), rainbow trout (*O. mykiss*), fugu (*Takifugu rubripes*), and catfish (*I. punctatus*). Studies suggest that teleost species possess several genes encoding virus-induced IFNs; specifically, 4 genes in zebrafish, 4 in catfish, and 11 in Atlantic salmon. The classification and nomenclature of fish virus-induced IFNs remain

controversial. Several reports classified them as type I IFNs based on putative structural features, whereas others suggested that they are type III IFNs since they are encoded by genes with four introns and their receptor structures are more similar to that of type III IFNs. Fish IFN is further divided them into three subtypes (e.g. IFNα, IFNβ, and IFNY) depending on sequence and expression patterns or named them as IFN1, 2, 3, 4, etc. according to the order in which they were discovered in a certain species. Based on cysteine patterns, virus-induced fish IFN can be divided into two groups: the 2 cysteine containing group (group I) the two groups bind to two distinct receptor complexes. Group I binds to a receptor comprised of CRFB1 and CRFB5, while group II binds to a receptor comprised of CRFB2 and CRFB5. This binding pattern differs from that of mammalian type I IFNs, which all bind to a common ternary receptor complex containing two chains of IFNAR1 and IFNAR2.

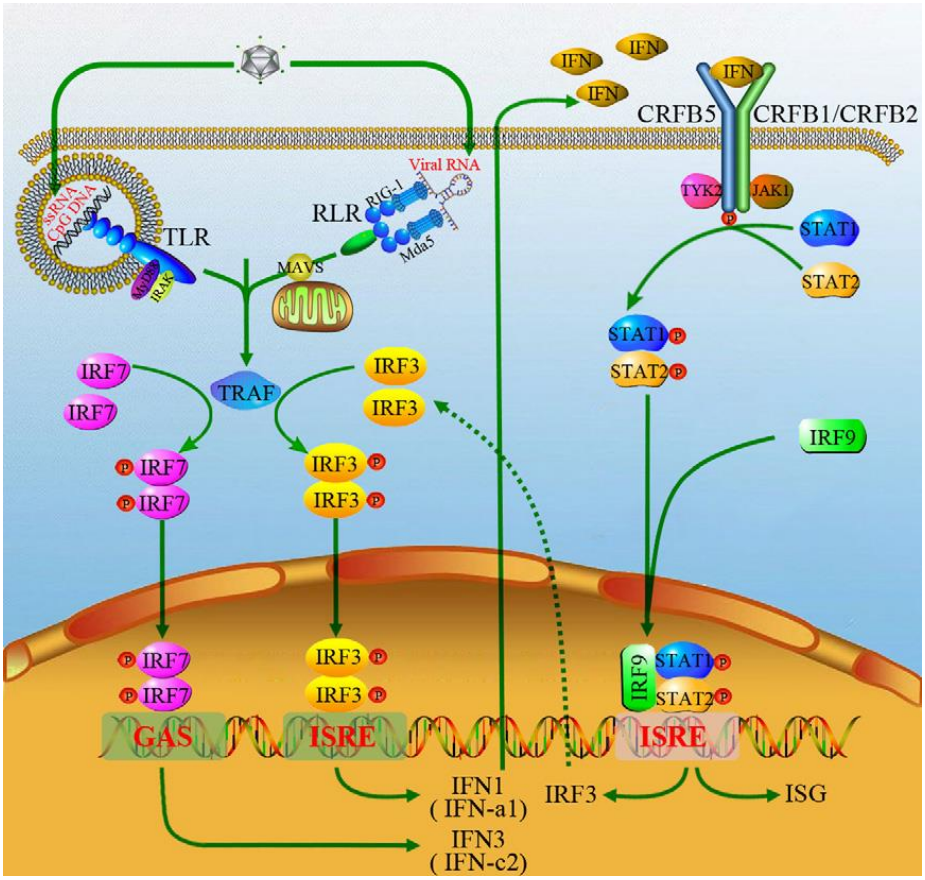
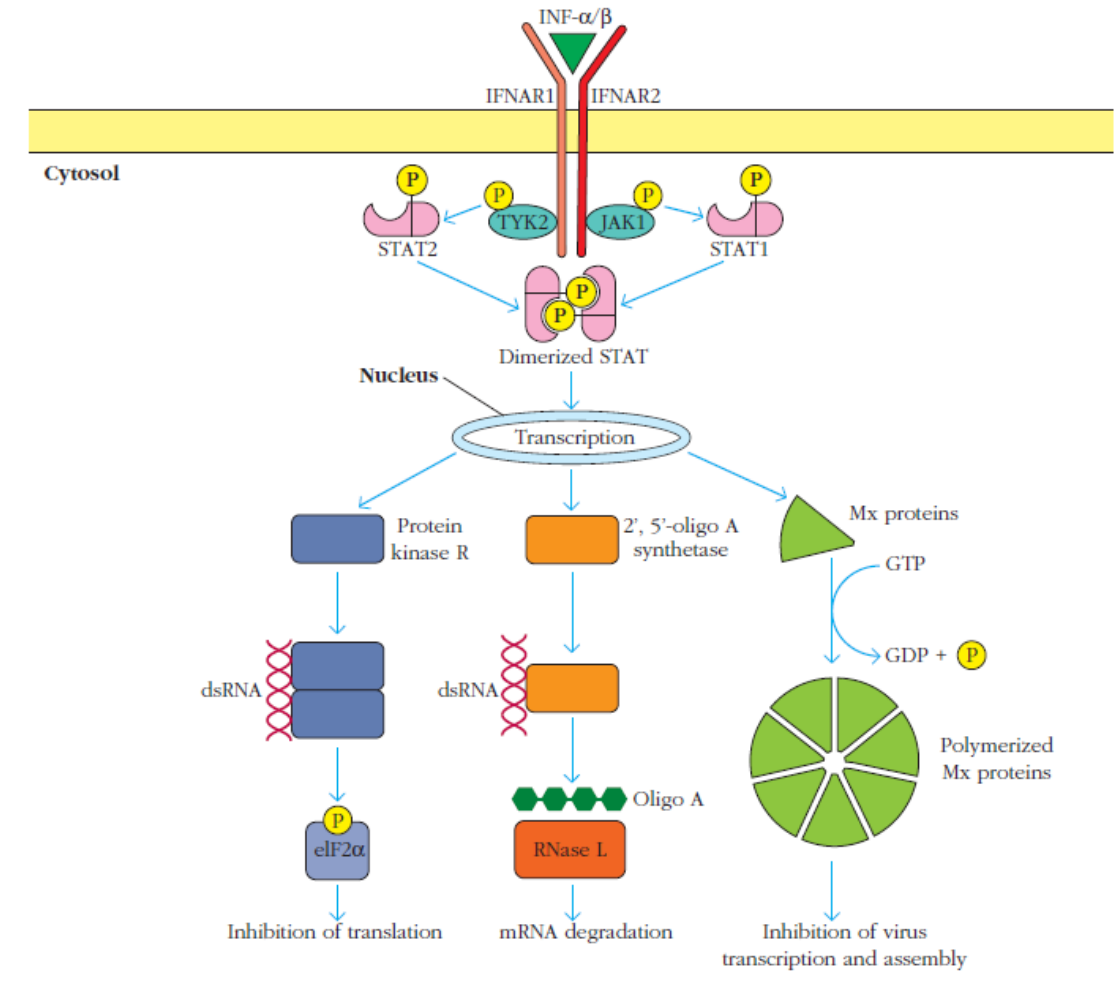


Diagram of the deduced anti-virus signalling of type I IFNs in teleost fish.

Virus-infected cells recognize viral pathogen-associated molecular patterns (PAMP) by pattern recognition receptors (PRR). After TRAF3 or TRAF6 recruitment by PRRs and their associated adaptors, downstream kinases catalyze the phosphorylation of IRF3 or IRF7 respectively. These, in turn, form dimers, translocate into the nucleus, attach to the specific motif in the IFN1 (IFN-a1) or IFN3 (IFN-c2) promoter, and regulate the expression of type I

IFNs in collaboration with AP-1 and NF-κB. The newly produced type I IFNs could bind to two groups of receptors on most host cells in fish, both of which contain a common low affinity CRFB5 chain and a distinct high affinity CRFB1 or CRFB2 chain. After phosphorylation of STAT1 and STAT2 by TYK2 and JAK1 kinases in the cytoplasm, activated transcription factors form a dimer and cross the nuclear membrane associated with IRF9. Following this, the transcription factor complexes bind to interferon stimulated response elements (ISRE) motifs and the interferon-stimulated genes (ISGs) could be finally induced to exert antiviral functions. Fish IRF3 is an ISG distinct from that of tetrapods due to its unique ability for a self-positive feedback loop of the IFN regulatory system in order to trigger cascade amplification during antivirus response in teleost.

Type II IFN is encoded by two genes in fish: IFN-c with similar functions to its homologues in mammals and a teleost specific IFN-c related (IFN-crel) molecule. The IFN-c molecules primarily promote cell-mediated immunity and have been identified in lot of species, including rainbow trout (*O. mykiss*), channel catfish (*I. punctatus*), and Atlantic salmon (*S. salar*) (Martin *et al*., 2007; Robertsen *et al*., 2006; Zou *et al*., 2005).



Fish virus induced genes and IFN type I signalling. Following virus entry in the cell, viral nucleic acids bind different sensors as RLRs and TLRs. Additionally fish possess TLR-22, a unique cell-surface receptor, which binds dsRNA. Activation of these sensors leads to the induction and secretion of IFNs. IFNs have auto- and paracrine activity mediated through interactions with a transmembrane receptor (IFNAR) that activates the JAK/STAT pathway. It then leads to the induction of interferon-stimulated genes (ISGs) such as Mx, ISG15, and vig/viperin.

Similar to signalling pathways downstream of many cytokine receptors, binding of IFN-α or -β activates IFNAR to recruit and activate specific JAKs (JAK1 and Tyk2), which then activate specific STATs (STATs 1 and 2). STAT1/1 and STAT1/2 dimers then induce expression of various genes, including those for the three proteins that block viral replication: protein kinase R (PKR), which inhibits viral (and cellular) protein synthesis by inhibiting the elongation factor eIF2α; 2',5'-oligoadenlyate A synthetase, which activates the ribonuclease RNase L that degrades viral mRNA; and Mx proteins, which inhibit both the transcription of viral genes into mRNAs and the assembly of virus particles.

Interferon-regulatory transcription factors (IRF) are a family of transcription factors essential for modulating the expression of IFN genes and IFN-stimulated genes by binding to characteristic elements in their promoters. A global characterization of IRF family members was performed in fish and a number of other vertebrates. Results showed that all IRF family members ubiquitously exist in all vertebrates. Phylogenetic analysis showed that fish IRF family members can be divided into two clusters: one consisting of IRF-1 and IRF-2, and another cluster of all other remaining IRFs (Shi *et al*., 2010). IRF-1 was the first IRF family member known to activate the IFN-b gene, whereas IRF-2 was described as a multifunctional transcription factor that exhibits both transcriptional activating and repressing activities by antagonizing the effect of IRF-1 (Sato *et al*., 2001).

Among all IRF members, IRF-3 has the greatest structural homology to IRF-7. Both IRF-3 and IRF-7 are early IRFs activated by TLRs and other signalling modes that play a pivotal role in the initial induction of type I IFNs (Ozato *et al*., 2007). IRF-3 is required for the activation of early-phase IFNs, including IFN-b, which in turn amplify the expression of late-phase IFN-α gene by IFN-induced IRF-7 through the STAT1 pathway. Although type I fish IFN genes cannot be classified as IFN-α or IFN-β, zebrafish IFN1 (IFN-α1) and IFN3 (IFN-γ2) were recently found to be regulated by

IRF-3 and IRF7, respectively. This finding suggests that zebrafish IFN1 (IFN- α1) resembles mammalian IFN-β and that IFN3 (IFN- γ2) resembles mammalian IFN-a (Sun *et al*., 2011a). Recently, IRF-3 and IRF-7 were also characterized in crucian carp (*C. auratus*) and crucian carp blastulae embryonic (CAB) cells (Sun *et al*., 2010a; Zhang *et al*., 2003). IRF-7 expression could be significantly elevated when CAB cells were treated with GCHV, UV-inactivated GCHV, or CAB-produced IFN protein.

5.5.2. Interleukin-1 family members and receptors

Interleukin-1 (IL-1) is an important early response pro-inflammatory cytokine that mediates immune regulation in both innate and adaptive immunity. IL-1 could be secreted by monocytes, activated macrophages, granulocytes, endothelial cells, activated T lymphocytes, and many other cell types. There are 10 ligand proteins in the IL-1 gene family, the main members of which include IL-1a, IL-1b, and IL-18. IL-1a and IL-1b share the same receptor on target cells and exert similar biological functions, although IL- 1b shows more potent function in humoral immune response. Over the years, IL-1b genes have been identified in various teleost fish species, including rainbow trout (*O. mykiss*), carp (*C. carpio*), sea bass (*Dicentrarchus labrax*), channel catfish (*I. punctatus*), and yellowfin sea bream (*Acanthopagrus latus*).

In general, only one IL-1b gene seems to exist in fish. However, two IL-1-b-like genes encoding 280-amino acid peptides with high identity (94.3%) with each other have been cloned from catfish. Multiple alignments showed that catfish IL-1b genes shared low identity with that of mammals and higher vertebrates (approximately 20%) and low identity with other identified fish IL-1β genes (31–38%). There are differences in the expression levels of these two IL-1β genes in various tissues. IL-1β gene 1 is significantly expressed in liver, head kidney, spleen, intestine, and muscle, but minimally in stomach, brain, ovary, skin, and trunk kidney. In contrast, IL-1β gene 2 is highly expressed in all tested tissues except the brain. In addition, IL-1β gene 1 could be more significantly induced than IL-1 β gene 2 after bacterial infection.

IL-1 activates target cells by binding to IL-1 receptors on the cell surface and ultimately triggering inflammation to cope with pathogen infection. Two IL-1 receptors (IL-1RI and IL-RII) that display opposite functions in IL-1 signalling exist in mammals.

IL-1RI is the positive receptor participating in all known IL-1 functions, whereas IL-1RII antagonizes IL-1 function as a decoy receptor. Fish also possess both contrary IL-1Rs, indicating that the composition of fish IL-1Rs was conserved in mammals. Both IL-1RI and IL-1RII have been found in various fish species, including salmon (*S. salar*), rainbow trout (*O. mykiss*), pufferfish (*Fugu rubripes*), and zebrafish (*D. rerio*) for IL-1RI (Huising *et al*., 2004); and rainbow trout (*O. mykiss*), gilthead seabream (*Sparus aurata*), and Japanese flounder (*P. olivaceus*) for IL-1RII (Fan *et al*., 2010; Lopez-Castejon *et al*., 2007; Sangrador-Vegas *et al*., 2000).

5.5.3. Tumor necrosis factors

The TNF superfamily plays a key role in inflammation, host defense, autoimmunity, organogenesis, cellular apoptosis, and differentiation. Until recently, only a limited number of TNF superfamily members have been identified in teleosts, and a great number of them are similar to mammalian TNF-a. This factor has been identified from several fish species, including mandarin fish (Siniperca chuatsi) (Xiao *et al*., 2007), zebrafish (D. rerio) (Savan *et al*., 2005c), common carp (C. carpio) (Saeij *et al*., 2003), and turbot (Psetta maxima) (Ordas *et al*., 2007). Several other family members have also been characterized in teleosts, such as TRAIL-like (Chang *et al*., 2006; Gao *et al*., 2008), CsTL (Zhang *et al*., 2008), and BAFF (Ai *et al*., 2011). TNF-b does not seem to exist in fish. Similar to their counterparts in mammals, teleost TNF-a genes consist of four exons and three introns. However, the identity of fish TNF-as are generally lower than that in mammals, and the average size of TNF-as in teleost is a little larger than that of mammals (Goetz *et al*., 2004). Zebrafish TNF-a can mediate cell death and regulate the expression of some essential molecules in relative pathways, suggesting that the function of this molecule was conserved in mammals (Wang *et al*., 2011).

TNF fulfils its functions by interacting with its specific receptors. The TNF receptor (TNFR) family members are classified into three groups: TNF receptor-associated factor (TRAF) binding receptors, death domain (DD)-containing receptors, and decoy receptors (Aggarwal, 2003; Dempsey *et al*., 2003; Locksley *et al*., 2001). Instead of having DDs, TRAF binding receptors (including TNFR2) have motifs that recruit TRAF proteins to exert functions (Chung *et al*., 2002). DD-containing TNFRs, including TNFR1 and FAS, activate caspase cascades through DD-containing signalling adaptors, resulting in caspase activation and cell apoptosis. These adaptors bind to TNFRs and to each other through homo- and hetero-typic

interactions to induce apoptosis. To date, six TRAF molecules have been identified in mammals (Xu *et al*., 2008). They share common structures, such as a single RING finger at the N-terminal (with the exception of TRAF1, which does not have an N-terminal RING finger domain), and several zinc fingers and a TRAF domain at the Cterminal. The TRAF domain is responsible for binding to associate receptors. Among the six TRAF molecules, TRAF2, TRAF5, and TRAF6 are adaptors that connect receptors to downstream kinase cascades. This binding results in activation of NF-jB and activator protein-1(AP-1) (Inoue *et al*., 2000), which in turn leads to apoptosis, inflammation or cell survival. TRAF1 can indirectly associate with TNFR2 with the help of TAFR2 to form a heterodimeric complex. TRAF1 can also interact with TNFR1 and act as a substrate for caspases activated by receptors with DDs. TRAF3 antagonizes the effects of TRAF2 in NF-jB activation (Watts, 2005). TRAF4 is a unique member of this family in terms of both structure and function.

The TRAF gene has been characterized in many vertebrates, little is known about it in fish. The first detailed description of the structure and expression of TRAF in fish is TRAF1 in grass carp (*C. idella*). Compared with TRAF1 in other vertebrates, its identity varies from 44% to 72% in terms of the most important domain and varies from 52% to 58% in reference to the TRAF domain (TD) (Xu *et al*., 2008). In mammals, TRAF1 is selectively expressed in spleen, lung, tonsils, and testis (Rothe *et al*., 1994), but not in kidney, liver, heart, and brain. However, in grass carp, TRAF1 mRNA is widely distributed even in heart, head kidney, thymus, brain, gill, liver, and spleen. TRAF1 may play a negative role in the regulation of TNF signalling in teleost, although the main function of this molecule and its homologs in bony fish remains to be investigated.

5.5.4. Chemokines

Chemokines refer to a group of chemotactic cytokine family members that are released by most infected tissues in the early stages of infection. They are small heparin-binding secreted cytokines recruiting monocytes, neutrophils, and other effector cells from vessels towards the focus of infection. Chemokines play a key role in the movement of immune effector cells to sites of infection and it is becoming increasingly clear that their function is also necessary to translate an innate immune response into an acquired adaptive response. Innate immune stimuli activate toll receptors and set in motion the expression of chemokines from resident tissue macrophages and dendritic cells and, modulate the expression of chemokine

receptors on dendritic cells. These changes in chemokine/chemokine-receptor expression direct the movement of antigen-loaded dendritic cells from the tissue into lymphoid tissue to activate native T and B cells and initiate the adaptive response.

Chemokines could be divided into several families (e.g. CXC, CC, C, and CX3C) according to the organization of cysteines next to the amino terminal. The two most important and deeply studied families of chemokines are the CC chemokines (characterized by two adjacent cysteine residues next to the N-terminal) and the CXC chemokines (characterized by two cysteine residues separated by one amino acid next to the N-terminal). These two main chemokine families and their receptors have also been found in bony fish. Compared with human chromosome 17, which contains the largest group of CC chemokine genes, several catfish CC chemokines share high identities on the same chromosomal stretch in humans, such as SCYA108, SCYA123, SCYA120, SCYA124, and SCYA104. In addition, SCYA101 and SCYA107 have been shown to be specific to fish.

Investigations of fish CC chemokine receptors have accompanied fish CC chemokine discovery. A total of 17 CC chemokine receptor members, together with their gene structure and organization, have been determined in zebrafish (Liu *et al*., 2009). They are homogenous to CCR6, CCR7, CCR8, CCR9, and CCRL1 of mammals. No CCR1, CCR2, CCR3, CCR4, CCR5, and CCR10 homologs could be identified in zebrafish. Expression analyses revealed that majority of these receptors are expressed in fertilized eggs or early embryos, indicating they are of maternal origin. However, differential expression patterns were found in adult fish, suggesting that they have functional diversity. IL-8 is one of the first CXC chemokines to be discovered in fish (Lee *et al*., 2001) and is classified into two groups (Abdelkhalek *et al*., 2009; van der Aa *et al*., 2010). An IL-8 ortholog has been identified in fugu (*T. rubripes*) (Saha *et al*., 2007), black seabream (*A. schlegelii*) (Zhonghua *et al*., 2008), and Half-smooth tongue sole (*Cynoglossus semilaevis*) (Sun *et al*., 2011b)

6. Conclusion

It is generally believed that the innate immune system may have been conserved in all vertebrates and that the development of innate immunity in high vertebrates evolved from simple mechanisms in ancient vertebrates to complex mechanisms in modern vertebrates. However, the presence of far more abundant immune-relevant genes and networks in fish innate immunity than that of mammals

suggests that the evolution of innate immunity might have progressed oppositely. Compared with mammals, the complexity of the fish innate immune system is reflected by several aspects, such as the composition of PAMP recognition system, signaling regulatory factors, immune effector molecules, and their corresponding mechanisms. For instance, compared with 11 TLRs in human, at least 17 TLRs have been found in fish, with 7 being unique non-mammalian TLRs.

Although many immune related molecules such as pattern recognition receptors, interferon, and some cytokines are less conserved from fish to mammals in terms of molecular amount, type, and homology, it is noteworthy that pivotal molecules and transcription factors involved in their signalling pathways show a high degree of conservation. For example, the TIR domain containing adaptor molecules (such as Myd88, TRAM and TIRAP) and their mediated NF-κB signalling pathway in PRR initiated responses, some critical IRFs, the RLR-triggered IFN response mediated by MITA-TBK1-IRF3, the JAK-STAT signalling pathway, and some participants of the inflammatory signalling pathways are highly conserved from fish to mammals.

7. Abbreviations

AD, acidic domain
AMPs, antimicrobial peptides
AP-1, activator protein-1
APC, Antigen presenting cells
b-2m, b-2 microglobulin
BAC, bacterial artificial chromosome
BPI, Bactericidal/permeability-increasing protein
BCR, B cell receptor
Bf, B factor
C1INH, complement component 1 inhibitor
CAB cells, crucian carp blastulae embryonic cells
CARD, caspase activation and recruitment domains
cDC, conventional dendritic cells
CLRs, C-type lectin receptors
CRD, carbohydrate recognition domain
CRD, cysteine-rich domain
CRP, C-reactive protein

C-terminal domain
DBD, DNA-binding domain
DC-SIGN, DC-specific ICAM-3 grabbing nonintegrin
DD, death domain
Df, D factor
DIGIRR, double-Ig-IL-1R related molecule
DreIFN B, zebrafish IFN allele B
dsRNA, double strand RNA
ICE, interleukin-converting enzyme
ICS, IFN-containing supernatant
IFN-crel, IFN-c related
IFNs, interferons
Igs, immunoglobulins
IgSF, immunoglobulin superfamily
Ii, invariant chain
ILs, interleukins
IPNV, infectious pancreatic necrosis virus
IRAK, including IL-1R-associated kinase
IRF, interferon-regulatory transcription factors
ISGs, interferon-stimulated genes
ISREs, IFN-stimulated response elements
KIR, kinase inhibitory region
KLH, keyhole limpet hemocyanin
LECT2, leukocyte cell-derived chemotaxin 2
LGP2, laboratory of genetics and physiology 2
LRR, leucine-rich repeats
MAPK, mitogen-activated protein kinase
MBL, mannose-binding lectin
MDA5, melanoma differentiation-associated gene 5
MHC, major histocompatibility complex
MITA, The mediator of IRF3 activation
NF-κB, nuclear factor-κB
NO, nitric oxide
NOD like receptor, nucleotide-binding oligomerization domain-like receptors
Nramp, natural resistance-associated macrophage protein

PAMPs, pathogen-associated molecular patterns
pDCs, plasmacytoid DCs
PGRPs, peptidoglycan recognition proteins
PHA, phytoagglutinin
PIAS, protein inhibitor of activated STATs
PRRs, pattern-recognition receptors
RAG1, recombination-activating gene 1
RAG2, recombination-activating gene 2
RD, repressor domain
RIG-1, retinoic acid-inducible gene I
RLD, RING-finger-like zinc-binding
RLRs, RIG-I like receptors
RSS, recombination signal sequence
SIGIRR, single-Ig-IL-1R related molecule
SOCS, suppressors of cytokine signaling
ssRNA, single-strand RNA
STAT, signal transducer and activator of transcription protein
TCR, T cell receptors;
TD, thymus dependent
TD, TRAF domain
TGF-b, transforming growth factor-b
THD, TNF homology domain
TIR, Toll-IL-1 receptor
TLRs, Toll-like receptors
TNFR, TNF receptor
TNFs, tumor necrosis factors
TRAF, TNF receptor-associated factor

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