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Genomic analysis of NF-κB signaling pathway reveals its complexity in *Crassostrea gigas*

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ABSTRACT

NF-κB signaling pathway is an evolutionarily conserved pathway that plays highly important roles in several developmental, cellular and immune response processes. With the recent release of the draft Pacific oyster (*Crassostra gigas*) genome sequence, we have sought to identify the various components of the NF-κB signaling pathway in these mollusks and investigate their gene structure. We further constructed phylogenetic trees to establish the evolutionary relationship of the oyster proteins with their homologues in vertebrates and invertebrates using BLASTX and neighbor-joining method. We report the presence of two classic NF-κB/Rel homologues in the pacific oyster namely Cgp100 and CgRel, which possess characteristic RHD domain and a consensus nuclear localization signal, similar to mammalian homologues and an additional CgRel-like protein, unique to *C. gigas*. Further, in addition to two classical IκB homologues, CgIκB1 and CgIκB2, we have identified three atypical IκB family members namely CgIκB3, CgIκB4 and CgBCL3 which lack the IκB degradation motif and consist of only one exon that might have arisen by retrotransposition from CgIκB1. Finally, we report the presence of three IKKs and one NEMO genes in oyster genome, named CgIKK1, CgIKK2, CgIKK3 and CgNEMO, respectively. While CgIKK1 and CgIKK3 domain structure is similar to their mammalian homologues, CgIKK2 was found to lack the HLH and NBD domains. Overall, the high conservation of the NF-κB/Rel, IκB and IKK family components in the pacific oyster and their structural similarity to the vertebrate and invertebrate homologues underline the functional importance of this pathway in regulation of critical cellular processes across species.

1. Introduction

The nuclear factor-κB (NF-κB) signaling pathway plays an important and evolutionarily conserved role in immune response, cellular, developmental, and organismal processes in animals. Under several circumstances, NF-κB is activated to enable organisms to respond to environmental stresses, such as cytokines, pathogens, chemicals, and UV irradiation [1,2]. Once activated, NF-κB regulates the expression of target genes to counteract these stresses. The genes regulated by NF-κB include those controlling the response to a variety of stimuli, including bacterial and viral infections and diverse biological functions, such as apoptosis, cell adhesion and proliferation, inflammation, antigen presentation as well as cellular homeostatic mechanisms [2–5]. Moreover, NF-κB family members play additional roles in development. For example, Dorsal, a member of the NF-κB family of transcription factors, controls the establishment of dorsal-ventral polarity in the *Drosophila melanogaster* embryo. Additionally, different NF-κB family members in distinct thymic compartments have important roles during early and late stages of NKT cell development [6–8]. Considering the central role of NF-κB in maintaining cellular homeostasis, it is not surprising that dysregulation of NF-κB can lead to severe consequences, including cancer, autoimmune diseases, and chronic inflammatory disorders [9–11].

In its inactive state, NF-κB exists as a hetero- or homodimer that is sequestered in the cytoplasm by virtue of its association with IκB family of inhibitory proteins. Upon activation of the pathway by an upstream signal, activated IKK phosphorylates IκB, which leads to IκB degradation, allowing NF-κB to translocate to the nucleus, bind DNA, and

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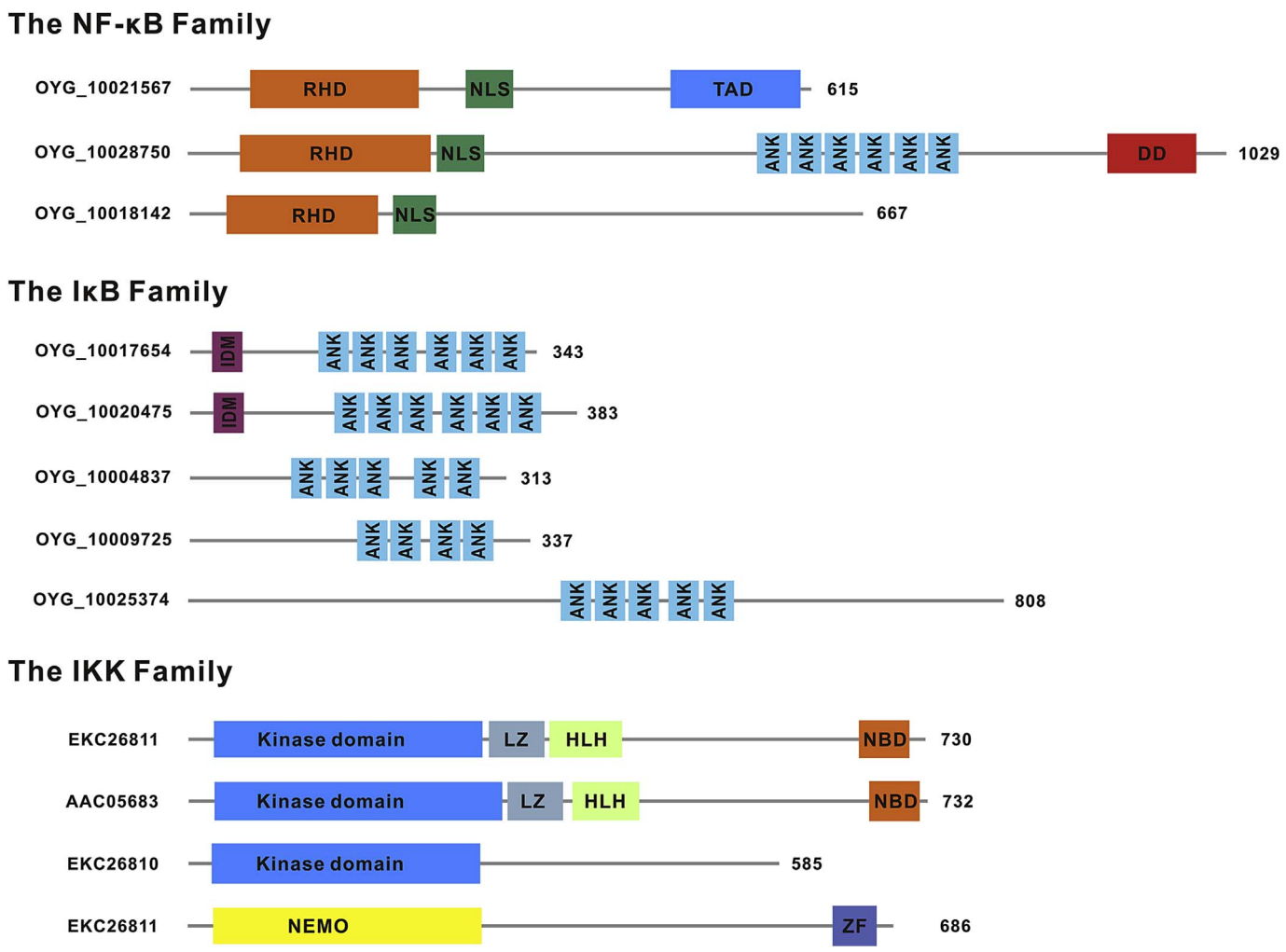

Fig. 1. The domain structure of the NF-κB, IκB and IKK family members in *C. gigas* genome. (A) There are three members of the NF-κB family found in the *C. gigas* genome namely Cgp100, CgRel and CgRel-like proteins. The CgP100 protein has a domain structure similar to mammalian p100/p105 and Relish in *Drosophila*, consisting of an N-terminal RHD, one NLS, six ankyrin repeats (ANKs) and the C-terminus death domain (DD). The TAD domain of Cg-Rel has been shown to have transcriptional activation ability. CgRel-like includes a RHD domain and a NLS constituted of four basic residues "KKRK", implying its basic characteristic as a functional transcription factor. (B) The *C. gigas* genome was found to have 5 IkB family members, similar to mammalian system, namely CgIκB1, CgIκB2, CgIκB3, CgIκB4 and CgBCL3. All the CgIκB proteins have variable number of ankyrin repeats which mediate interaction with NF-κB family members. The last 3 family members however lack the obvious IκB Degradation Motif (IDM). (C) We identified presence of three IKKs and one NEMO genes in oyster genome, named CgIKK1, CgIKK2, CgIKK3 and CgNEMO, respectively. CgIKK1 and CgIKK3 have the same domain organization as its homologues in mammals. Numbers beside each domain structure denotes the number of amino acids in the protein. RHD, N-terminal Rel-homology domain; NLS, nuclear-localization domain; TAD, C-terminal transcriptional activation domain; ANK, ankyrin-repeat motif; DD, region with homolog with death domain; IDM, IκB degradation domain; LZ, putative leucine-zipper domain; HLH, helix-loop-helix domain; NBD, NEMO-binding domain; NEMO, NF-κB essential modifier; ZF, zinc-finger domain.

activate or deactivate specific target gene expression [12,13].

In mammals, there are five NF-κB family members, RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). All the NF-κB family members consist of an N-terminal Rel homology domain (RHD), which mediates DNA binding, homodimerization and heterodimerization [14]. Notably, only RelA, RelB, and c-Rel have a transactivation domain in their C-terminus that is required for gene activation. NF-κB1 and NF-κB2 proteins are generated by processing of the precursor molecules p105 and p100, respectively [5]. IκB proteins IκBα, IκBβ, IκBε, IκBζ, BCL-3, and the precursor proteins p100 as well as p105 are characterized by the presence of multiple ankyrin repeat domains that mediate IκB binding to NF-κB dimers and thereby interfere with the nuclear translocation of the NF-κB dimers. These domains are phosphorylated and degraded in response to certain NF-κB-activating signals [3,12]. Degradation of IκB is a tightly regulated event that is initiated upon phosphorylation of specific residues by activated IKK. The IKK complex consists of three core subunits, the catalytic subunits IKKα and IKKβ (also known as IKK1 and IKK2) and several copies of a regulatory subunit called the NF-κB essential modifier (NEMO, also known as IKKγ) [1,15].

In spite of substantial studies being reported on NF-κB signaling in mammals, relatively less information is available about the evolutionary origins and patterns of this pathway in mollusk. In *Drosophila melanogaster*, NF-κB homologues (Dorsal, Dif, and Relish) are responsible for regulating several biological roles, including humoral immunity and development [16]. Unexpectedly, due to the absence of NF-κB transcription factors in *Caenorhabditis elegans*, the NF-κB signaling pathway was overlooked for several years in organisms more ancient than *Drosophila melanogaster*. However, the recent release of genomic data from various morphologically primitive organisms has revealed that NF-κB is a reasonably ancient signaling pathway [17]. In *Nematostella vectensis*, molecular functions of some members of the Nv—NF—κB pathway have been found to be conserved over the last 600 million years of evolutionary history [13]. In *Carcinoscorpius rotundicauda*, the activation mechanism and transactivation properties of NF-κB and IκB homologues are evolutionarily entrenched [18]. Genome sequencing has identified clear bipartite (RHD-ANK repeat) NF-κB genes in the sponge *Amphimedon queenslandica* (19) and the unicellular