Crystal Structure of Innate Immune Toll-Like Receptor 9 Recognizing Bacterial CpG DNA

Toll-like receptor 9 (TLR9) recognizes microbial DNA containing CpG motifs and activates innate immune responses. We determined the crystal structures of three TLR9 forms: unliganded, bound to agonistic CpG-DNA, and bound to inhibitory DNA (iDNA). Agonistic CpG-DNA–bound TLR9 formed a symmetric TLR9/CpG-DNA complex with 2:2 stoichiometry, whereas iDNA-bound TLR9 was a monomer. CpG-DNA was recognized by both protomers in the dimer, specifically by the N-terminal fragment from one protomer and the C-terminal fragment from the other. The iDNA, which forms a stem-loop structure suitable for binding by intramolecular base pairing, binds to the concave surface of TLR9.

Our bodies often face dangers from bacterial and viral invasions. The immune system is a defense against the infections, which eliminates pathogenic microorganisms. The innate immune system provides the first line of defense by detecting certain kinds of microbial products as a sign of danger and activating the downstream immune response. The toll-like receptor (TLR) is one of the best-known receptors in the innate immune system that recognizes a wide variety of microbial products. Dr. Beutler and Dr. Hoffmann won the Nobel Prize in Physiology or Medicine 2011 for their discovery concerning the activation of innate immunity by TLRs [1, 2]. TLR is a type I transmembrane receptor consisting of extracellular leucine-rich repeat (LRR) domain, transmembrane domain, and intracellular Toll/IL-1 receptor (TIR) domain. The LRR and TIR domains are responsible for the ligand recognition and signaling, respectively. In humans, ten members of TLRs (TLR1 to TLR10) are identified and each TLR recognizes different ligand. TLR9 recognizes CpG DNA, a DNA sequence with Cytosine-phosphate-Guanine dinucleotide (CpG) motif that is specific to bacterial and viral DNA [3, 4]. The activation of TLR9 by CpG DNA leads to the release of interferon and inflammatory cytokines. TLR9 thereby has a potential to be a target for the vaccine adjuvant or therapeutic agents for viral infections and allergy diseases. Although TLR9 has been studied extensively since its discovery in 2000, the way it functions, especially from a structural point of view, remains unknown.

We determined the crystal structures of the LRR domain of TLR9 in three forms: unliganded, inhibitory DNA bound, and CpG DNA bound forms [5].

The ring-shaped monomer structure of TLR9, where its N- and C-termini directly interact (Fig. 1A) is similar to that of TLR8 [6], the other member of TLRs that recognizes single stranded RNA, however, the ligand

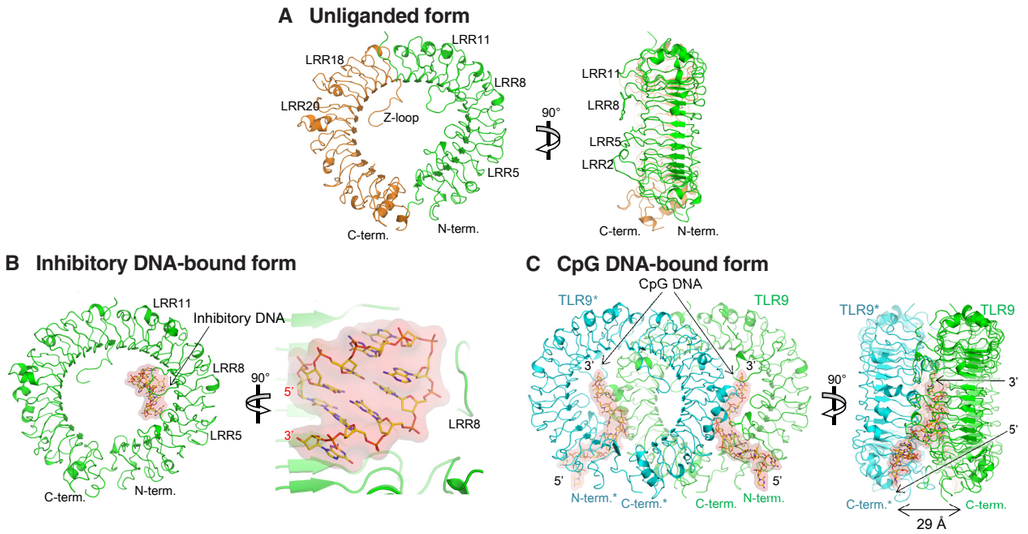

Figure 1: Structures of TLR9 in the unliganded (A), inhibitory DNA bound (B), and CpG DNA bound (C) forms. Bound DNAs are shown in sticks with semi-transparent surface representations in magenta. The N- and C-terminal halves of TLR9 are shown in green and brown, respectively in (A). The two protomers in the dimer are shown in green and cyan in (C).

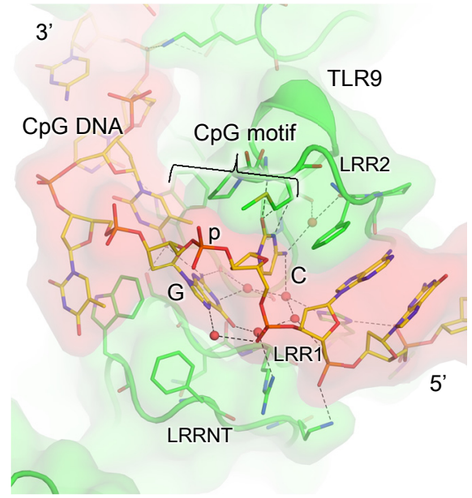

Figure 2: CpG motif binding mode of TLR9. CpG DNA and residues of TLR9 interacting with DNA are shown in sticks with semi-transparent surface representations in magenta (CpG DNA) and green (TLR9). Hydrogen-bonds are indicated with dashed lines.

binding modes are strikingly different between the two. In the crystal structures, the unliganded and inhibitory DNA bound forms of TLR9 are monomeric (Fig. 1A and 1B) while the agonistic CpG DNA bound form of TLR9 is dimeric (Fig. 1C), thus representing inactivated and activated forms, respectively. The dimerization of TLR9 brings the two C-termini in close proximity and would induce the association of the intracellular TIR domain, leading to the activation (Fig. 1C).

The agonistic CpG DNA in an extended conformation is recognized by both protomers in the dimer (Fig. 1C). The CpG DNA binds to the groove formed at the lateral face of the ring structure near the N-terminus in one protomer and simultaneously to the lateral face of the C-terminal side of the other protomer, thus act as a molecular glue to bridge the two TLR9 molecules and induce the activated form. The core hexamer regions of CpG DNA, including the CpG motif, are mainly recognized by TLR9 (Fig. 2). The bases of the CpG motif are accommodated in the groove and engage in multiple specific interactions with TLR9, which defines the specificity of the TLR9 toward CpG dinucleotide (Fig. 2). In addition, the flanking regions of the CpG motif further strengthen the interaction between TLR9 and CpG DNA.

The inhibitory DNA binds to the concave surface of TLR9 forming stem-loop structures with intramolecular three or four base-pairs (Fig. 1B). The high affinity of the inhibitory DNA is achieved mainly through the recognition of the backbone of the stem-loop structures. Since the binding site for the inhibitory DNA partially overlaps with that for agonistic CpG DNA, inhibitory DNA competes for the binding site with agonistic CpG DNA and thereby inhibits the TLR9 activation.

These structural analyses enable us to understand the detailed CpG DNA and inhibitory DNA recognition and activation mechanism of TLR9 and also open a new avenue for developing the novel therapeutic agents targeting TLR9.

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BEAMLINES

BL-17A and AR-NE3A

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