Visualization of agonistic and inhibitory DNA recognition by Toll-like receptor 9

Our bodies often face dangers from bacterial and viral invasions. Pathogenic microorganisms (bacteria, viruses, fungi, and parasites) threaten us continuously but we are equipped with powerful defense mechanisms. The innate immune system is the first line of defense against pathogenic microorganisms by detecting certain kinds of microbial products as a sign of danger and activating the downstream immune response. The Toll-like receptors (TLRs) are among the best-known receptors in the innate immune system and recognize 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]. TLRs have received significant attention due to their critical roles in the innate immune system. TLRs are type I transmembrane receptors consisting of an extracellular leucine-rich repeat (LRR) domain, a transmembrane domain, and an intracellular Toll IL-receptor (TIR) domain. The LRR and TIR domains are responsible for ligand recognition and signaling, respectively. To date, 10 TLRs (TLR1 to TLR10) have been identified in humans and each TLR recognizes a distinct type of molecule commonly found in microorganisms that cause disease (Fig. 1). TLR9 recognizes CpG-DNA, a DNA sequence with a 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 the potential to be a target for vaccine adjuvants 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 viewpoint, remains unknown.

A research group at the Graduate School of Pharmaceutical Sciences of the University of Tokyo used brilliant synchrotron X-rays at SPring-8 beamline **BL41XU** and KEK to determine the crystal structures of the LRR domain of TLR9 in three forms: unliganded, inhibitory DNA-bound, and CpG-DNA-bound forms [5].

In the ring-shaped monomer structure of TLR9, its N- and C-termini directly interact (Fig. 2(a)). In the crystal structures, the unliganded and inhibitory DNA-bound forms of TLR9 are monomeric (Figs. 2(a) and 2(b)). Upon ligand binding, TLR9 and the CpG-DNA complex form a 2:2 complex with the CpG-DNA wedged between the two TLR9 protomers in an extended conformation (Fig. 2(c)). The dimerization of TLR9 brings the two C-termini in close proximity and induces the association of the intracellular TIR domain, leading to the activation (Fig. 3), while the unliganded and inhibitory DNA-bound forms of TLR9 are unable to associate the TIR domain.

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, it acts as a molecular glue to bridge the two TLR9 molecules and induce the activated form. The bases of the CpG motif are accommodated in the groove and engage in multiple specific interactions with TLR9, which define the specificity of the TLR9 toward the CpG dinucleotide. 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 to form stem-loop structures with three or four intramolecular base pairs. The high affinity of the inhibitory DNA is achieved mainly through the recognition of the backbone of the stem-loop

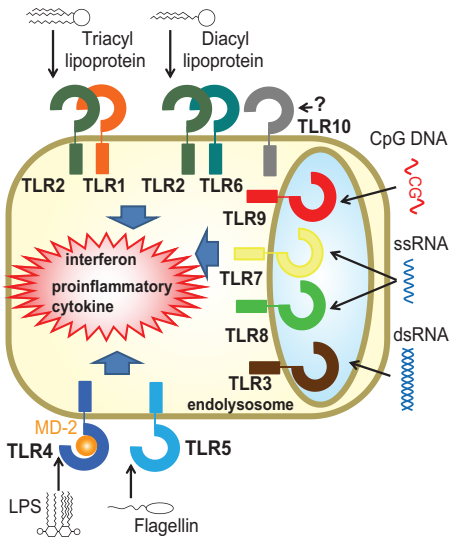

Fig. 1. Schematic illustration of human TLR signaling. TLR3 and TLR7–9 localize at the endosome, where they sense microbial and host-derived nucleic acids, whereas TLR5 and heterodimers of TLR2 and TLR1 or TLR6 are expressed at the cell surface. TLR4 localizes at both the plasma membrane and the endosome. TLR signaling is initiated by ligand-induced dimerization of receptors. TLR signaling induces proinflammatory cytokines and type I interferons.

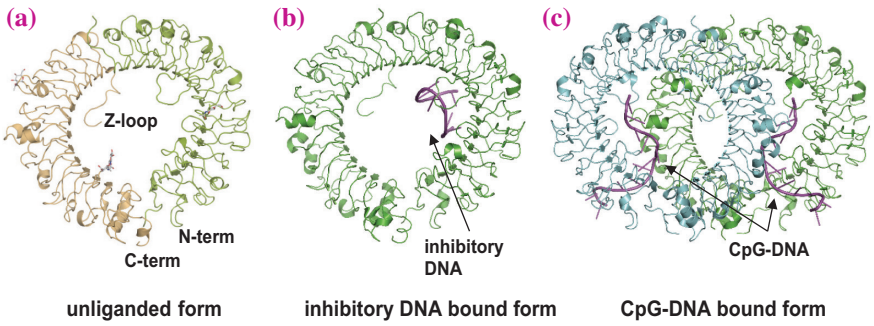

Fig. 2. Structures of TLR9 in the unliganded **(a)**, inhibitory DNA-bound **(b)**, and CpG-DNA-bound **(c)** forms. Bound DNA is shown in sticks in purple. The N- and C-terminal halves of TLR9 are shown in green and brown, respectively, in (a). The two protomers in the dimer are shown in green and cyan in (c).

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 agonistic CpG-DNA and inhibitory DNA recognition and the activation mechanism of TLR9, which also open a new avenue for developing novel therapeutic agents targeting TLR9.

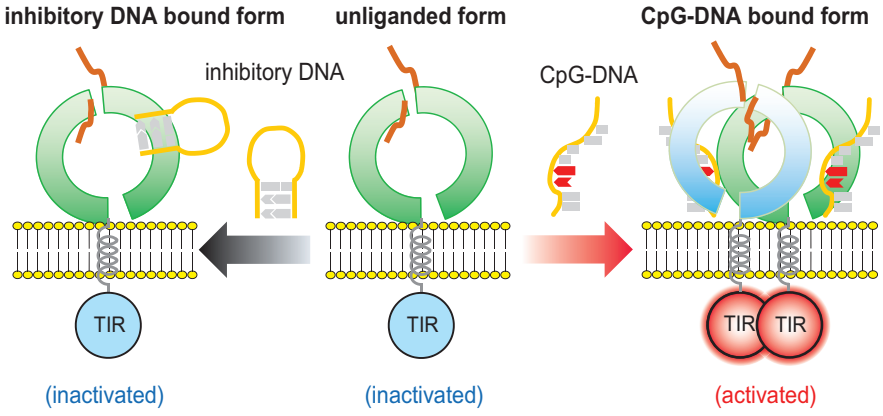

Fig. 3. Schematic regulation mechanism of TLR9 by CpG-DNA and inhibitory DNA. The unliganded, inactivated form of TLR9 (middle panel) transforms into the activated form (right panel) upon the binding of CpG-DNA. Upon the binding of inhibitory DNA, TLR9 remains inactivated (left panel).

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