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Structure of Toll-like receptors: the input from solution NMR spectroscopy

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Motivation and Aim: Toll-like receptors (TLRs) are the key players in the innate immune response. TLRs recognize various pathogen-associated molecular patterns and induce the inflammation. They are involved in severe diseases, including cancer, which makes them prospective targets for the drug design. Despite their obvious importance, the structure of TLRs is not well investigated, especially of the transmembrane and intracellular parts. In the present work, we utilized protein engineering, NMR spectroscopy, computer simulations and functional assays to study the spatial structures of TLR transmembrane domains, cytoplasmic juxtamembrane regions and intracellular TIR domains.

Results: All the TLRs include the portion of hydrophobic residues in their cytoplasmic juxtamembrane regions. We solved the structures of four protein constructs, which encompass the transmembrane and juxtamembrane domains of TLR2, TLR3, TLR5 and TLR9 in a membrane mimetic environment. According to our data, the representatives of all the TLR subfamilies except for TLR4 form a short α-helix in cytoplasmic juxtamembrane regions. The bioinformatic analysis reveals that these juxtamembrane domains are more conservative than the transmembrane ones. Functional assays demonstrate that the correct amino acid sequences of juxtamembrane domains are essentially important for the receptor activation. The intracellular TIR domain is another important part of TLR, which is believed to launch the downstream signaling via the interaction with the adapter proteins. Here we solved the solution structure of TLR1 TIR domains and studied its interactions with TLR2. As we found out, TLR1 TIR domains are capable of binding the zinc ions with nanomolar affinity in two different modes. Using mutagenesis, we identified the key cysteine residues responsible for the zinc binding; the structure of zinc-bound TLR1 states were proposed by computer simulations. According to the experiments with living cells, one of the zinc binding sites is functionally relevant, its blocking completely inhibits the activity of full length TLR1/TLR2 receptor.

Conclusion: NMR spectroscopy is a potent technique that can provide the structural data on the organization of single-pass membrane proteins, which is impossible to obtain using the alternative approaches. Application of NMR to the TLR systems allowed identifying several key points, essential for the correct functioning of these receptors:

the proper sequence of cytoplasmic juxtamembrane regions, and zinc binding by the intracellular globular domains. The obtained structures provide an insight on how the active state of full-length TLR could look like and allows putting forward the hypotheses on the mechanics of the receptor activation.

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