Toll-like receptors (TLR) cascades

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Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 79

This document contains 8 pathways (see Table of Contents)
Toll-like receptors (TLR) cascades

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Toll-like receptors (TLRs) are a group of highly conserved pathogen recognition receptors which initiate and regulate the immune response by controlling cytokine and chemokines expression.

Mammalian and avian lineages diverged from the common ancestor approximately 300 millions years ago. Although most of the genes encoding proteins of the chicken TLR cascade molecules have not been cloned and characterized directly, analyses of the chicken genome sequence has defined ten TLRs [Lynn et al. 2003, Temperley ND et al. 2008]. The avian TLR repertoire consists of single orthologs of mammalian TLRs 3, 4, 5 and 7 and distinct new chicken genes TLR15 and TLR21. The TLR2 subfamily is represented by tandemly duplicated avian TLR2 and TLR1 genes and consists of two isoforms of each gene - TLR2 type1 and 2, TLR1 type1 and 2. No functional orthologs of mammalian TLR8, TLR9 and TLR10 have been detected in the chicken genome. However, chicken heterophils and spleen cells are responsive to the broad range of mammalian TLR antagonists including ligands that stimulate mammalian TLR7/8 and TLR9 [Schwarz et al. 2007, He et al. 2006, Kogut et al. 2007].

TLR signaling pathways are highly conserved among vertebrates and the chicken proteins involved in the TLR signaling cascade show moderate to high identity with their human counterparts [Yilmaz et al. 2005, Temperley et al. 2008, Cormican et al. 2009]. Thus, a homology-based strategy was used to reconstruct most parts of the chicken TLR pathways in this Reactome module.

All TLRs share a similar structure consisting of N-terminus ectodomain with several leucine-rich regions (LRR), one or two trans-membrane domains and an intracellular C-terminus Toll/Interleukin-1 receptor domain (TIR).

Activation of TLR pathways occurs upon recognition and interaction with conserved motifs expressed by invading microbes, also known as pathogen-associated molecular patterns (PAMPs). Each TLR recognizes specific PAMPs.

Upon PAMP binding TLRs form heterodimers (TLR2 subfamily) or homodimers (all other TLRs). Activ-
ated TLRs recruit one or several TIR adaptor proteins myeloid differentiation primary response gene 88 (MyD88), TIR domain containing adaptor protein (TIRAP or MAL), TIR domain-containing adapter protein inducing IFN-beta (TRIF or TIKAM-1), and TRIF related adaptor molecule (TRAM). The fifth known adaptor SARM binds TIR as a negative competitor to TRIF.

All TLRs except TLR3 can initiate downstream signaling through MyD88 adaptor protein. In the MyD88-dependent pathway, once the adaptor is bound to TLR, it leads to recruitment of IL1 receptor associated kinase family IRAK, followed by activation of tumour necrosis factor receptor-associated factor 6 (TRAF6). TRAF6 is an ubiquitin E3 ligase, which in turn induces TGF-beta activating kinase 1 (TAK1) auto-phosphorylation. Once activated, TAK1 can ultimately mediate the induction of the transcription factor NF-kB or the mitogen-activated protein kinases (MAPK), such as JNK, p38 and ERK. This results in the translocation of the activated NF-kB and MAPKs to the nucleus and the initiation of appropriate gene transcription leading to the production of many proinflammatory cytokines and antimicrobial peptides.

In contrast to other TLRs, TLR3 functions only through the MyD88-independent signaling cascade, recruiting TRIF, which in turn leads to the interferon regulatory factor 3 or 7 (IRF3/7) activation. Activated IRF3 or 7 mediates innate anti-viral responses through interferon-beta expression.

Mammalian TLR4 can ultimately utilize both MyD88-dependent (controlled by the MyD88-TIRAP pair of adaptors) and MyD88-independent (controlled by TRAM-TRIF adaptor proteins) signaling pathways, in contrast, chicken TLR4 signaling is mediated by MyD88-TIRAP exclusively.

**Literature references**


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In chicken, TLR1 and 2 genes are individually duplicated to encode four different proteins of the TLR2 subfamily:

- chTLR1 type 1 (TLR1-1, or TLR1LA, or TLR16)
- chTLR1 type 2 (TLR1-2 or TLR1LB)
- chTLR2 type 1(TLR2A or TLR2-1)
- chTLR2 type 2 (TLR2B or TLR2-2)

The TLR1 and TLR2 gene duplication events were estimated to have taken place about 147 and 65 million years ago respectively (Temperley ND et al. 2008).

Analyses of chicken genome revealed that all chTLR2 and chTLR1 isoforms are located on Chromosome 4 and are encoded by one exon, like human TLR2 and TLR1. The phylogenetic comparative analysis also showed that chTLR2 isoforms are two distinct genes (not splicing products of the same gene) and TLR2A and B are both orthologs of the single TLR2 of mammals (Temperley ND et al 2008, Cormican et al 2009). Although chicken TLR1 type 1 and 2 genes were reported by Yilmaz et al. (2004) to be highly similar to human TLR1 and TLR6 genes, further analysis revealed that chicken TLR1 and human TLR1/6/10 are not orthologs (Temperley ND et al 2008).

The functional profile of chicken TLR2 subfamily proteins is similar to human; members of TLR2 subfamily respond to lipoproteins through heterodimer formation.

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Toll-like receptor 3 (TLR3) as was shown for mammals is expressed on myeloid dendritic cells, respiratory epithelium, and macrophages, and appears to play a central role in mediating both the antiviral and inflammatory responses of the innate immunity in combating viral infections.

Chicken TLR3 protein shows 61% identity to human TLR3.

The chicken ortholog of mammalian TLR3 specifically recognizes poly I:C, a dsRNA analogue, like its mammalian counterpart. In the chicken as in mammals, poly I:C rapidly induces type 1 IFN.

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Toll-like Receptor 4 is an evolutionarily conserved microbe associated molecular pattern receptor well known for its sensitivity to bacterial lipopolysaccharides (LPS). LPS is a major cell wall component in all Gram-negative bacteria and a potent activator of the innate immune response in mammals.

Chicken TLR4 encodes a 843-amino-acid protein that shows 46% identity to human TLR4. Like its human homologue chicken TLR4 contains a leucine-rich repeat extracellular domain, a short transmembrane domain typical of type I transmembrane proteins, and a Toll-interleukin-1R signaling domain. (Lebeque et al. 2003). Chicken TLR4 was shown to sense LPS of diverse Gram-negative bacteria (Kogut et al. 2005, Leveque et al. 2003). The major difference between mammalian and chicken TLR signaling is the lack of MyD88-independent IFN-beta production in chicken after TLR4 activation (Keestra et al. 2008, Zoete et al. 2009). Unlike its human counterpart activated chicken TLR4 exclusively induces the MyD88/TIRAP-dependent, but not the TRAM/TRIF mediated signaling pathway. At present, inspection of the chicken genome indicated no orthologs for intracellular human adaptor TRAM (Keestra et al. 2008), which acts as a bridge to recruit TRIF to activated TLR4.

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[https://reactome.org](https://reactome.org)
In mammals, the interaction of flagellin with TLR5 induces a signaling cascade that utilizes MyD88 adaptor, IRAK family members, and downstream signaling mediators such as MAP kinases and NF-kB that regulate pro-inflammatory genes [Smith KD et al. 2003].

The chicken tlr5 gene was cloned from the chicken NCSU macrophage cell line; it encodes a protein of 861 amino acids. The cloned chTLR5 showed 69% and 68% overall amino acid similarity to human and murine TLR5, respectively (Keestra AM et al., 2008). Chicken TLR5 mRNA is expressed in a broad range of tissues, immune cell subsets and chicken cell lines. The strongest signal was detected in colon, spleen, kidney, lung, heart, and testes (Iqbal M et al., 2005).

Comparisons of gene structure, genomic location, amino acid composition, and patterns of leucine-rich regions (LRR) consistent with the suggestion that chTLR5 is an orthologue of mammalian TLR5. Like its mammalian counterpart chTLR5 was reported to respond to bacterial flagellin (Iqbal M et al., 2005 and Keestra AM et al., 2008).

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In mammals, TLR7 and TLR8 belong to TLR9 subfamily and senses RNA in endosomal compartments, initiating the MyD88 signaling cascade.

Although functional assays showed that chicken cells respond to known mammalian TLR7 and TLR9 ligands in vitro and in ovo [Philbin et al 2005, Jenkins KA et al 2009], bioinformatics analysis of the chicken genome have identified a disrupted ortholog of TLR8 and no direct ortholog of TLR9. Only TLR7 gene was found intact [Philbin et al 2005, Temperley ND et al 2008]. The chicken TLR7 (chTLR7) amino acid sequence shows 62% identity to its human counterpart. chTLR7 mRNA was detected in immune-related tissues - spleen, caecal tonsil [Iqbal et al 2005, Phiblin et al 2005]. Chicken leukocytes, heterophils, monocytes and the chicken macrophage cell line HD11 have been also shown to express chTLR7 [Philbin et al 2005].

**Literature references**


Bioinformatics analysis of the chicken genome revealed a pathogen recognition receptor - TLR15, whose ortholog is absent in mammals [Higgs R et al 2006]. TLR15 orthologs were also found in other avian genomes - turkey, duck and zebra finch [Boyd AC et al 2012].

Chicken tlr15 gene was mapped on chromosome 3 and encodes a protein that like other TLRs consists of a cytoplasmic Toll/IL-1 receptor (TIR) region, a transmembrane domain, and an extracellular domain with multiple leucine-rich regions (LRR) [Higgs et al 2006]. However, phylogenetic analysis revealed that TLR15 does not belong to any of the TLR groups identified to date [Temperley ND et al 2008; Boyd AC et al 2012].

TLR15 mRNA was detected in a broad range of chicken tissues: spleen, thymus, ileum, colon, bursa, bone marrow, liver and cecum [Boyd AC et al 2012; Higgs R et al 2006].

Modeling of the TLR15 TIR domain revealed the presence of a conserved BB-loop structure including a conserved proline residue, known to be required for MyD88-dependent signaling in mammalian TLR family members [deZoete MR et al 2011]. This Reactome project describes TLR15-mediated induction of MyD88 pathway in chicken, however the mechanism of TLR15-mediated responses remains unclear.

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Chickens express TLR21 which is absent in human; chTLR21 has orthologs in fish and frog genomes and shows similarity with murine TLR13. Cloning and expression analysis revealed that the TLR21 gene encodes a protein of 972 amino acids residues. The protein was predicted to contain an extracellular domain containing 27 leucine rich regions (LRR), a single trans membrane region and cytoplasmic TIR domain, thus resembling the protein architecture of TLR family members.

It has been reported that despite the low similarity in amino acid sequence, chTLR21 senses DNA and acts as a functional homologue of mammalian TLR9 (Keestra et al. 2010). Confocal microscopy located chTLR21 in the endoplasmic reticulum. Besides, inhibition of the chTLR21-mediated response by bafilomycin A (Brownlie R et al 2009) or chloroquine (Keestra AM et al 2010) suggested that endosomal maturation is required for the receptor activation, as is the case for mammalian TLR9.

A direct structural orthologue to mammalian TLR9 is absent in the chicken genome.

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