Toll-like receptors — taking an evolutionary approach

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Abstract | The Toll receptor was initially identified in *Drosophila melanogaster* for its role in embryonic development. Subsequently, *D. melanogaster* Toll and mammalian Toll-like receptors (TLRs) have been recognized as key regulators of immune responses. After ten years of intense research on TLRs and the recent accumulation of genomic and functional data in diverse organisms, we review the distribution and functions of TLRs in the animal kingdom. We provide an evolutionary perspective on TLRs, which sheds light on their origin at the dawn of animal evolution and suggests that different TLRs might have been co-opted independently during animal evolution to mediate analogous immune functions.

Toll-like receptors (TLRs) are type-I transmembrane proteins with extracellular leucine-rich repeat (LRR) motifs and an intracellular Toll/interleukin-1 receptor (TIR) domain. Members of the TLR family contribute both to cell–cell interactions and to signalling, linking extracellular signals to specific gene-expression programmes. Toll, the founding member of the TLR family, was initially implicated in the establishment of dorsoventral polarity in the early *Drosophila melanogaster* embryo[1]. Genetic analysis of *Drosophila* Toll and another *Drosophila* TLR, Toll2 (also called 18 wheeler (18w)), revealed an additional role in embryogenesis and post-embryonic development[2,3]. Functional studies in vertebrates have not uncovered a role for TLRs in development. Mammalian TLRs have essential roles in the direct recognition of infectious agents, initiating signalling through nuclear factor-kappa B (NF-κB), leading to the initiation of both innate and adaptive immune responses[4,5]. Similarly, *Drosophila* Toll also contributes to NF-κB-mediated host immune defences and is essential for resisting infections[6]; although, in contrast to mammals, *Drosophila* Toll does not directly recognize microorganisms but is activated by its endogenous ligand, Spätzle. Such observations, and the recent accumulation of genomic and functional data in diverse organisms, are challenging the view that the insect and vertebrate innate immune systems share a common ancestry.

Here, we review our knowledge of TLR distribution and function in the animal kingdom. After describing TLR structure in terms of domain organization, we report the distribution and diversification of TLR genes among the animal kingdom and outline their functions in model organisms. This survey confirms the ancient origin of TLR genes but reveals major differences in the way TLRs function among species. Finally, we discuss what this tells us about the ancestral TLR function, their evolution and the emergence of TLR-mediated immunity.

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Molecular signatures of TLRs

TLR ectodomain. The main part of the TLR ectodomain is composed of LRR motifs. This ancient domain has been identified in many proteins in viruses, archaea, bacteria, plants, fungi and animals. It is defined by a 22 to 29 amino-acid repeat with characteristically spaced hydrophobic residues[7]. LRR motifs provide a versatile structural framework for the formation of protein–protein interactions[8]. However, TLR ectodomains also interact with lipids, carbohydrates and nucleic acids. The crystal structure of the extracellular region of human TLR3 reveals that the LRR motifs form a horseshoe-shaped solenoid that is directly involved in ligand interaction[9,10] (FIG. 1). This direct interaction has recently been reported for other TLR family members, including *Drosophila* Toll[11], human and mouse TLR1, TLR2, TLR4, TLR5 (REFS 12–14), and murine TLR9 (REFS 15,16). Interestingly, in addition to TLRs, other proteins with LRR motifs — such as NACHT-LRR (NLR) in vertebrates[17,18] or NBS-LRR in plants[19] — have been implicated in the activation of host antimicrobial defences. In contrast to TLRs, these proteins are cytosolic but their LRR motifs are, like TLRs, generally associated with a signalling domain involved in protein–protein interaction such as a caspase recruitment domain (CARD), a TIR domain or a pyrin N-terminal homology domain(PYD)[20].

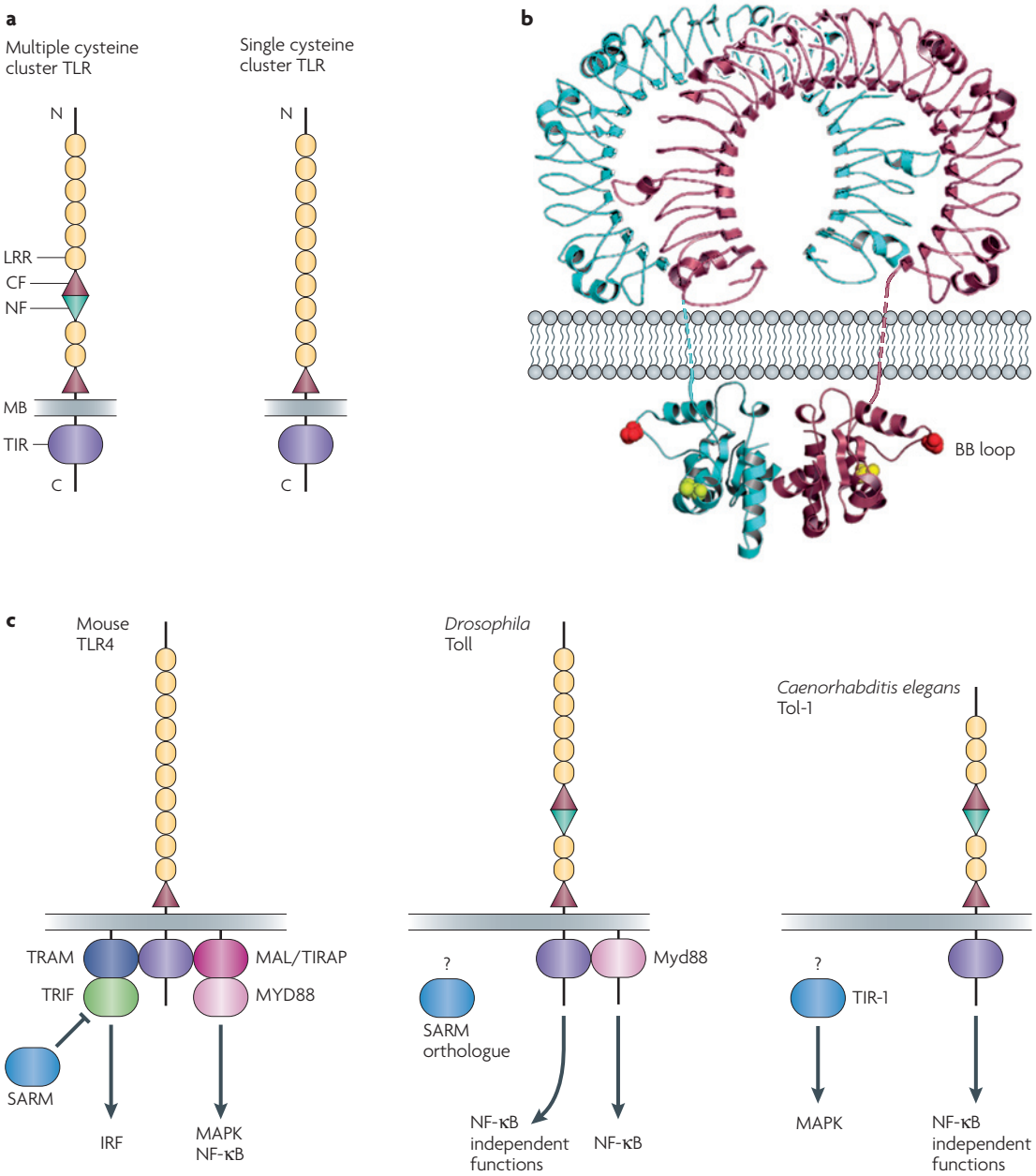

Figure 1 | **Toll-like receptors and downstream signalling pathways. a** | Molecular signatures of Toll-like receptors (TLRs). TLRs share a prototypical organization of N-terminal (N) extracellular leucine-rich repeat (LRR) motifs, a C-terminal (C) intracellular Toll/interleukin-1 receptor (TIR) domain spaced by a single transmembrane-spanning domain. Based on the variation of their ectodomains, two types of TLRs exist: the multiple cysteine cluster TLR (mccTLR) and the single cysteine cluster TLR (sccTLR). Both types present a cysteine cluster on the C-terminal end of LRRs (CF motif) that is juxtaposed to the plasma membrane (MB), whereas only mccTLRs have two or more CF motifs and another cysteine cluster on the N-terminal side of the proximal LRRs (NF motif)[35]. **b** | Schematic organization of TLRs based on the structure of the human TLR3 ectodomain and human TLR2 TIR domain: TLRs are dimerized, the ectodomain forms a horseshoe-shaped solenoid and the intracellular domain is compact and globular. The BB loop site of the TIR domain is essential for TIR–TIR homotypic interactions between TLRs and most intracellular signalling adaptors. **c** | TLR signalling does not exclusively rely on cytoplasmic TIR adaptors. Mouse TLR4 triggers the activation of interferon response factor (IRF) transcription factors through the adaptors TRAM and TRIF and induces the mitogen activated protein kinase (MAPK) cascade and nuclear factor-kappa B (NF-κB) signalling through the adaptors MAL (also known as TIRAP) and MYD88. The adaptor SARM is a negative regulator of TRIF[129]. *Drosophila* Toll controls the NF-κB factors Dorsal or Dif through intracellular signalling through the adaptor Myd88. Toll also mediates NF-κB independent functions such as cell adhesion. The function of the *Drosophila* SARM orthologue remains unknown. *Caenorhabditis elegans* Tol-1 functions independently of TIR adaptors. However, a *C. elegans* TIR adaptor exists. Tir-1, the orthologue of *Drosophila* and human SARM, regulates MAPK signalling independently of Tol-1 (REFS 75–77). Note that NF-κB molecules are absent from the *C. elegans* genome. Part **b** modified, with permission, from REF. 78 © (2006) Annual Reviews.

TLR cytoplasmic domain. The intracellular part of TLRs contains a TIR domain, which also has an ancient evolutionary origin. It has been identified in proteins from plants and most metazoans, and is found in a few bacterial and viral species. The presence of a TIR domain in bacterial and viral proteins might be a recent acquisition by horizontal transfer, serving a 'decoy' function to weaken TIR-dependent host defences (as proposed for the TIR-containing proteins found with the vaccinia virus[21]). The intracellular domain of TLRs has been associated with the signalling cascade leading to the nuclear translocation of the transcription factor NF-κB[22,23]. In both *Drosophila* and mice, the Toll (TLR in mice)–NF-κB pathway involves the recruitment of a TIR-containing adaptor such as Myd88, leading to the activation of the kinase Pelle (IRAK in mice) and subsequent phosphorylation and degradation of Cactus (I-κB in mice) — an inhibitor of NF-κB, which induces the rapid nuclear translocation of NF-κB transcription factors.

The compact and globular TIR domain[24] is associated with several immune-related molecules other than TLRs in both animals and plants. In vertebrates, a TIR intracellular domain is also found in interleukin-1 and interleukin-18 receptors (IL1R and IL18R). These receptors are key mediators of inflammation and engage the NF-κB signalling cascade in a manner that is similar to TLRs. However, their extracellular regions contain immunoglobulin-like domains instead of LRRs. As these two cytokine receptors are restricted to deuterostomes, the IL1R and IL18R families probably diverged from TLRs at the dawn of deuterostome evolution[25]. However, recent genomic analysis in cnidarians has revealed the existence of molecules with similar domain signatures to vertebrate IL1Rs but with highly diverged TIR domains, suggesting a separate evolutionary origin for these cnidarian and vertebrate molecules[26].

Plants also express many TIR- and LRR-containing proteins, the so-called R proteins, many of which are involved in disease resistance[19]. These proteins are distinct from TLRs in three ways: their TIR domain has only low sequence similarity to that of TLRs; they lack a transmembrane domain — that is, the LRR motifs are intracytoplasmic — and they control different downstream signalling cascades. The recurrent use of similar modules such as TIR and LRR in both plant and animal proteins that are linked to host defence is intriguing and points to an old link between these protein folds and disease-resistance mechanisms[27].

Origin and evolution of TLR genes

Recent genomic data from diverse organisms suggest that TLR genes are absent from non-animal phyla but are present in most eumetazoans, with the probable exception of platyhelminthes (TABLE 1). Based on the new animal phylogeny that splits protostomes into two major lineages — ecdysozoans (including nematodes and arthropods) and lophotrochozoans (including molluscs, annelids and platyhelminthes)[28] — we can infer that TLRs might have been lost in specific phyla such as Platyhelminth (FIG. 2). This loss might be due to the particular evolutionary history of the flatworm lineage, which has resulted in dramatic developmental and physiological simplifications.

Origin of TLRs. The phylum Cnidaria provides crucial insights into the early evolution of animals because it is the likely sister group of the superphylum Bilateria (FIG. 2). A TLR gene is present in the genome of the starlet sea anemone, *Nematostella vectensis*[29] (a basal cnidarian), but not in the genomes of other cnidarians, such as Hydra (*Hydra magnipapillata*) or the coral species *Acropora millepora* (the data were taken from ESTs)[26,30]. However, TIR-containing receptors with short extracellular domains that are devoid of LRR motifs are present in the Hydra and *A. millepora* genomes. Sequence comparison provides further evidence that these TIR-domain sequences cluster with TIR domains of other animal TLRs, rather than with intracellular TIR-domain adaptors, suggesting that they are TLR-related molecules[26,31]. Similarly, no true TLR genes have been found in the demosponge *Suberites domuncula* (of the Porifera phylum, a sister group of Cnidaria and Bilateria) but a TLR-related gene was identified[32,33].

Together, these data point to an origin of TLRs in the eumetazoan ancestor more than 600 millions years ago (mya) — before the separation of bilaterians and cnidarians. The TLR-related molecules that are found in more divergent cnidarian species and in sponges suggest that TLR-related genes emerged in the common ancestor of all animal phyla more than 700 mya (FIG. 2). The existence of these molecules that lack extracellular LRR motifs could indicate that TLR initially evolved by the association of a cytoplasmic TIR domain-containing molecule with a transmembrane domain, later followed by the independent acquisition of extracellular LRRs[34]. Alternatively, the TLR-related molecules of cnidarians and sponges might associate with other transmembrane proteins that contain LRR motifs.

Diversification of TLRs. A sequence analysis of TLR ectodomains indicates the existence of two major structural types[35]. Single cysteine cluster TLRs (sccTLRs) are characterized by the presence of a single cysteine cluster on the C-terminal end of LRRs (a CF motif), which is juxtaposed with the plasma membrane (FIG. 1a). Most TLRs found in deuterostomes have this domain organization, and one insect TLR, Toll9, also belongs to this type (FIG. 2). Conversely, multiple cysteine cluster TLRs (mccTLRs) are characterized by an ectodomain with two or more CF motifs and another cysteine cluster on the N-terminal side of the LRRs (NF motif) (FIG. 1a). They are systematically found in protostomes, but have also been recently identified in the invertebrate deuterostome *Strongylocentrotus purpuratus* (a sea urchin of the Echinodermata phylum) and in *N. vectensis* (FIG. 2), suggesting that mccTLRs reflect the ancestral domain structure of TLRs that were already present in the eumetazoan ancestor (FIG. 2).

Phylogenetic analysis reveals that TLR genes from different protostomian and deuterostomian phyla fall into separate clusters, showing that they share a

Metazoans
Heterotrophic multicellular organisms (that is, animals).

Deuterostomes
Animal taxon including all animal species in which the blastopore forms the anus.

Eumetazoans
The clade comprising all major animal groups except sponges (that is, cnidarians to vertebrates).

Protostomes
Animal taxon including all animal species in which the blastopore forms the mouth.

Bilaterians
Animals with bilateral symmetry.

common ancestor but evolved independently by gene duplication[36–39], suggesting a functional divergence between protostomian and deuterostomian TLRs.

Multiple functions of insect Toll

Drosophila *Toll in development*. Most of our knowledge about the functions of insect TLRs comes from *D. melanogaster*. The genome of this Dipteran contains nine distinct Toll genes, three of which have been studied genetically. The first *Toll* alleles were identified in large genetic screens that uncovered maternally expressed genes controlling the determination of the dorsoventral axis of the embryo[40]. Female flies that lack Toll activity produce dorsalized embryos, whereas those carrying a dominant gain-of-function *Toll* allele produce ventralized embryos[1] (FIG. 3a). The molecular characterization of other dorsoventral patterning genes has defined the components of a signalling cascade named the Toll pathway[41]. During oogenesis, a molecular cue that is localized on the ventral part of ovarian follicular cells initiates a proteolytic cascade in the perivitelline space outside the fertilized embryo, resulting in the ventral processing of Spätzle in a graded manner. The cleaved form of Spätzle then acts as a ligand for the Toll receptor. Localized activation of the Toll receptor leads to the stimulation of an intracellular pathway involving

Table 1 | **Toll-like receptor (TLR) genes in representative species with a sequenced genome**

Phylum	Subphylum	Class	Order	Common name	Scientific name	Number of TLRs (pseudogene) [TLR-like]*	Ref.
Chordata	Vertebrata	Mammals	Primates	Human	*Homo sapiens*	10 (1); TLR1–10 (TLR11)	39
Chordata	Vertebrata	Mammals	Rodentia	Mouse	*Mus musculus*	12 (1); TLR1–13 (TLR10)	39
Chordata	Vertebrata	Mammals	Rodentia	Rat	*Rattus norvegicus*	10; TLR1–7, 9, 10, 13	39
Chordata	Vertebrata	Mammals	Carnivora	Dog	*Canis familiaris*	10 (2); TLR1–10 (TLR11–12)	39
Chordata	Vertebrata	Mammals	Carnivora	Cat	*Felis catus*	9; TLR1–9	39
Chordata	Vertebrata	Mammals	Artiodactyla	Cow	*Bos taurus*	10; TLR1–10	39
Chordata	Vertebrata	Mammals	Marsupial	Opossum	*Monodelphis domesticus*	11;TLR1–12	39
Chordata	Vertebrata	Aves	Galliformes	Chicken	*Gallus gallus*	13 [4]; TLR1a, b, c, 2a, b, 3, 4, 7a, b, 8, 15, 21	39
Chordata	Vertebrata	Amphibian	Anura	*Xenopus*	*Xenopus tropicalis*	19; TLR1a, b, c, 2–5, 7–9, 11, 13, 14a, b, c, d, 16, 21, 22	39
Chordata	Vertebrata	Actinopterygii	Cypriniformes	Zebrafish	*Danio rerio*	17; TLR1–3, 4a, b, 5a, b, 7, 8a, b, 9, 18, 20a, b, 22	39
Chordata	Vertebrata	Actinopterygii	Cypriniformes	Japanese puffer fish	*Takifugu rubripes*	12 [1]; TLR1–3, 5, 7–9, 14, 21–23 [TLR5S]	39
Chordata	Vertebrata	Actinopterygii	Cypriniformes	Green spotted puffer fish	*Tetraodon nigroviridis*	10; TLR1a, b, 2, 3, 5, 8, 9, 21–23	39
Chordata	Urochordata	Ascidiacea	Phlebobranchia	Solitary tunica	*Ciona savignyi*	7–19	39
Chordata	Urochordata	Ascidiacea	Phlebobranchia	Solitary tunica	*Ciona intestinalis*	3	102
Chordata	Cephalochordata	–	–	Amphioxus	*Branchiostoma floridae*	42	100
Echinodermata	Eleutherozoa	Echinoidea	Echinoida	Purple sea urchin	*Strongylocentrotus purpuratus*	222	98
Arthropoda	Hexapoda	Insecta	Hymenoptera	Honey bee	*Apis mellifera*	5; Toll1, 2, 6, 8, 10	67
Arthropoda	Hexapoda	Insecta	Coleoptera	Flour beetle	*Tribolium castaneum*	9; Toll1–4, 6–10	70
Arthropoda	Hexapoda	Insecta	Lepidoptera	Silk worm	*Bombyx mori*	11 [2]; Toll2a, b, 3–11	68
Arthropoda	Hexapoda	Insecta	Diptera	Fruit fly	*Drosophila melanogaster*	9; Toll1–9	65
Arthropoda	Hexapoda	Insecta	Diptera	Mosquito	*Anopheles gambiae*	10; Toll1A, B, 5A, B, 6–11	66
Arthropoda	Hexapoda	Insecta	Diptera	Mosquito	*Aedes aegyti*	12; Toll1A, B, 4, 5A, B, 6–8, 9A, B, 10, 11	69
Nematoda	–	Secernentea	Rhabditidae	Round worm	*Caenorhabditis elegans*	1; Tol-1	73
Cnidaria	–	Anthozoa	Actiniaria	Starlet sea anemone	*Nematostella vectensis*	1	30

*Vertebrate TLR numbering is based on the order of their discovery in humans and mice spanning the range from TLR1 to 13. Fish numbering has started with TLR18 to allow room for some further mammalian consecutive numbering. Vertebrate TLRs with the same number are generally orthologous. Invertebrate and vertebrate TLR nomenclature does not correspond.

the adaptors Tube and DmMyD88 and the kinase Pelle, leading to the phosphorylation and degradation of Cactus. Cactus physically interacts with the NF-κB-family transcription factor Dorsal and retains it in the cytoplasm. Degradation of Cactus allows Dorsal to enter the nucleus where it regulates the expression of several genes that are involved in the dorsoventral regionalization[41]. The role of the Toll pathway in early dorsoventral patterning might be a recent acquisition because it seems to be specific to holometabolous insects, and the mechanisms that are involved in axis induction during oogenesis among insects evolve rapidly[42].

Toll also has important zygotic functions later in development. Lack of Toll activity causes lethality, and individuals that survive show a tubby-like phenotype[2,43]. The origin of these phenotypes is not yet known. However, a lack of Tube and Pelle — but not of Spätzle — led to similar phenotypes, indicating that this effect is mediated through the intracellular Toll pathway and does not involve the canonical Toll ligand Spätzle.

Toll has also been identified as a direct regulator of organogenesis. Loss of zygotic Toll induces muscle pattern defects[44]. *spätzle*, *tube* and *pelle* mutant embryos

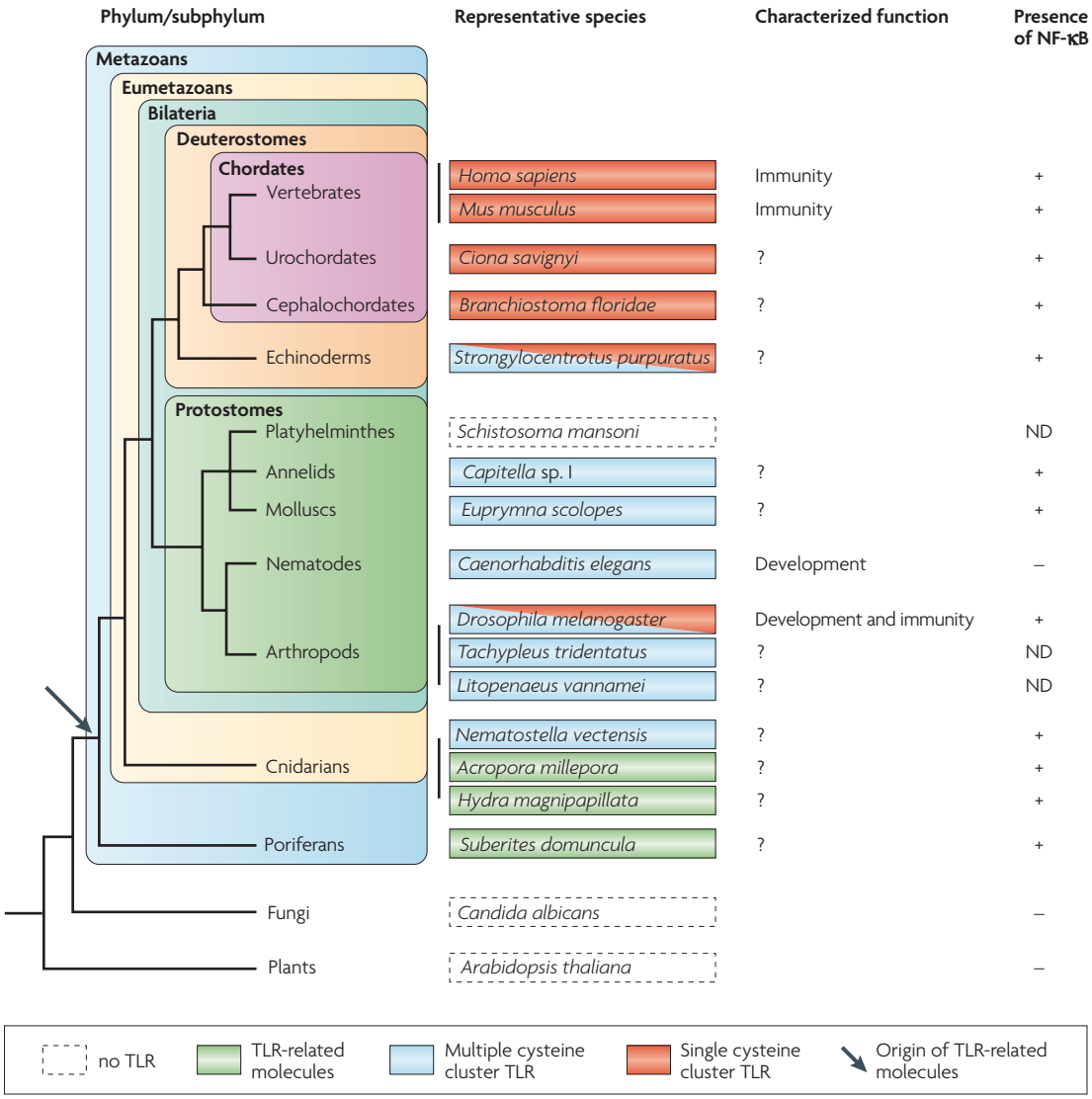

Figure 2 | **Origins, distribution and functions of Toll-like receptors in the animal kingdom.** A simplified phylogenic tree depicting the general relationship of the major metazoan phyla and subphyla[28,130]. This highlights the origin of Toll-like receptors (TLRs), their distribution in the animal kingdom, their molecular type, their characterized function and the presence of nuclear factor-kappa B (NF-κB) in the species. The black arrow points to the possible origin of TLR-related genes in a lineage that is ancestral to all metazoans. TLRs are present in most eumetazoans from cnidarians to vertebrates, although they seem to be absent from platyhelminthes. TLRs are not found in non-animal phyla. Functional studies have been performed in only five species (humans, mice, *Caenorhabditis elegans*, *Drosophila melanogaster* and *Aedes aegyptis* (not shown)) and reveal important immune and/or developmental functions of TLRs. Fungi and plants are shown as out-groups of the metazoans. This figure is not intended to represent all known species in which TLRs have been identified. ?, unknown; ND, not detected.

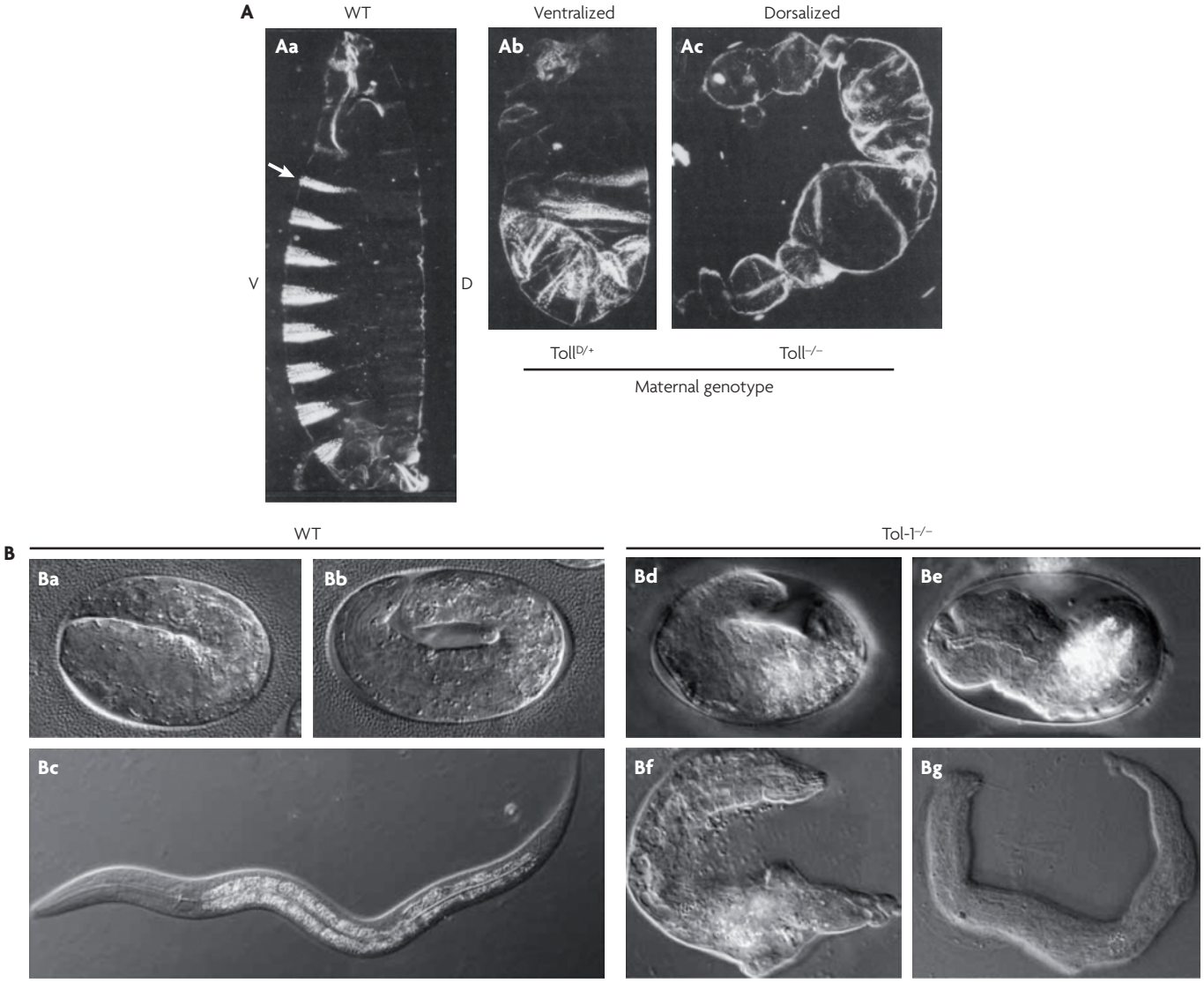

Figure 3 | **Toll-like receptor functions in development. A** |*Drosophila* Toll is required for the maternal determination of the dorsoventral axis of the embryo. Dark-field photography of the cuticle of a first instar larva produced by wild-type (WT) *Drosophila* females shows a normal dorsoventral pattern (**Aa**). By contrast, females that are heterozygous for a dominant *Toll* gain-of-function mutation produce ventralized embryos (**Ab**), whereas females that are homozygous for a recessive *Toll* loss-of-function mutation produce dorsalized embryos (**Ac**). Note the characteristic difference in the presence of the thick short bristles (arrow) arranged in segmental bands in the ventral cuticle (**Aa**). They are observed throughout the ventralized cuticle (**Ab**) but are absent from the dorsalized larvae (**Ac**). **B** | The essential role of Tol-1 in *Caenorhabditis elegans* development is illustrated by embryonic (**Ba**, **Bb**, **Bd**, **Be**) and larval lethality (**Bc**, **Bf**, **Bg**) of *Tol-1*-null mutant worms. Shown are *Tol-1* mutant worms arrested in their embryonic development (**Bd**, **Be**) compared with wild-type embryos (**Ba** and **Bb**). Larvae eventually emerge but are small and deformed (**Bf** and **Bg**) compared with wild-type (**Bc**). D, dorsal; V, ventral. Pictures for part **A** reproduced, with permission, from REF. 1 © (1985) Elsevier Ltd. Pictures for part **B** reproduced, with permission, from REF. 73 © (2001) Elsevier Ltd.

present similar defects, suggesting that the Toll signalling cascade — including its extracellular ligand — controls muscle development[45]. Motor-neuron defects are also observed in Toll mutant embryos[44,46]. Therefore, the dynamic expression of Toll in musculature regulates synaptic initiation of motor neurons and contributes to the local cues controlling the development of neuronal networks[46]. Toll is also essential during the secondary phase of heart formation for the correct alignment and migration of cardioblasts[47]. Although the precise molecular mechanisms underlying these different processes are still unclear, all of them require cell–cell communication. This suggests that one aspect of Toll function in development is to promote cell–cell interaction and adhesion. Proteins with LRRs are often implicated in cell adhesion, and

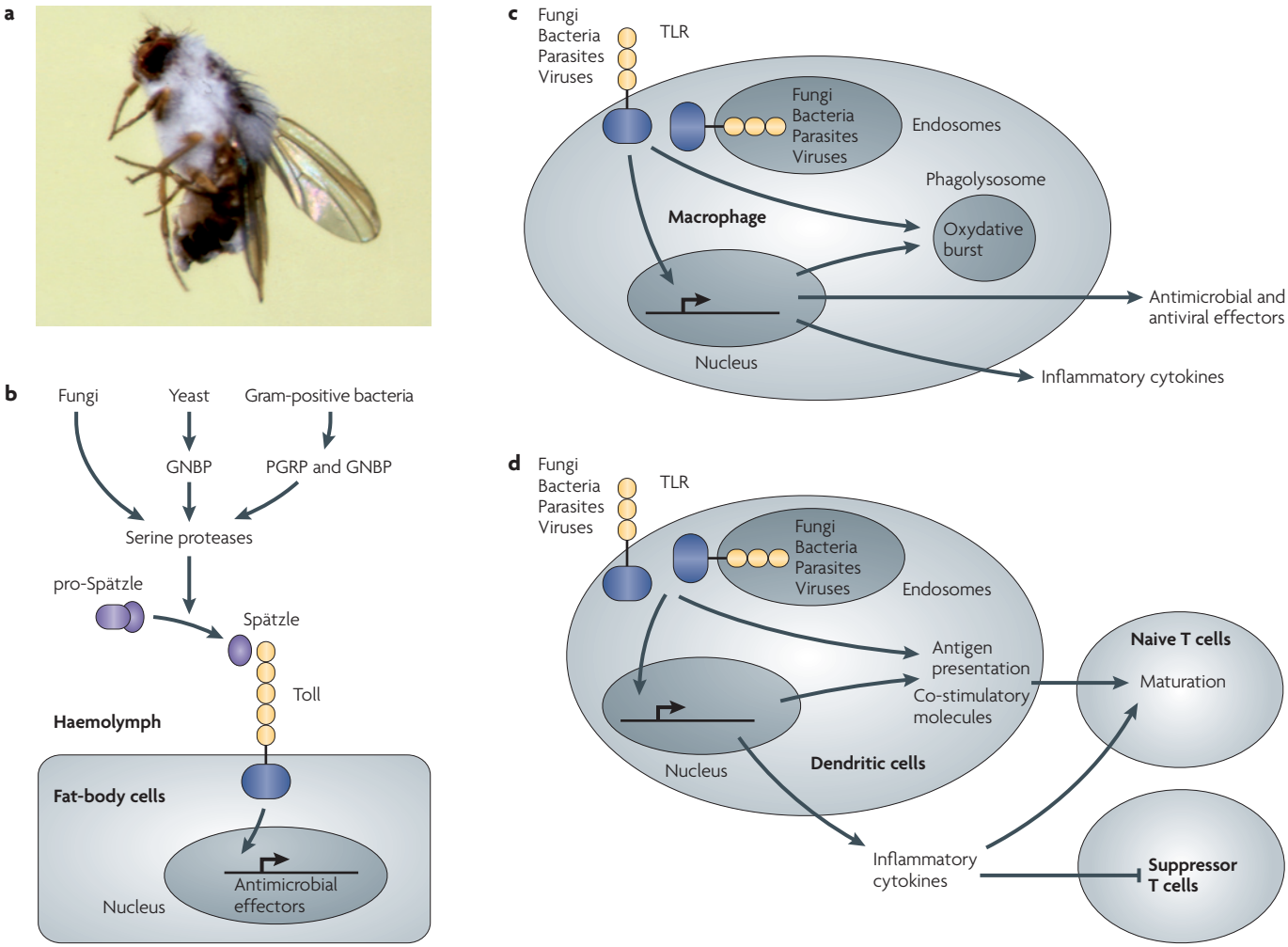

Figure 4 | **Toll-like receptor functions in immunity. a** | A picture of a *Toll* mutant *Drosophila* that has succumbed to an infection by the entomopathogen fungi *Neurospora crassa* (picture by B. L. and P. Tzou). Note the growing fungal hyphae on the dead fly cuticle. Wild-type flies normally resist this type of infection. **b** | *Drosophila* Toll activates antimicrobial responses to fungal, yeast or Gram-positive bacterial infection. The Toll pathway functions in the fat body, a major immune responsive tissue, and triggers the expression of a battery of target genes, including genes encoding antimicrobial peptides. Fungi, yeast and bacteria are sensed in the haemolymph by secreted peptidoglycan recognition proteins (PGRPs) and β-glucan recognition proteins (GNBPs). Following microbial recognition, serine-protease cascades lead to the maturation of Spätzle, the Toll ligand. **c** | Vertebrate Toll-like receptors (TLRs) are essential for innate immune defence. TLRs are expressed on macrophages and directly recognize products from various types of microorganisms, including fungi, bacteria, parasites or viruses. TLR signalling initiates acute inflammatory responses through numerous means: induction of enhanced phagocytosis; oxidative burst; antimicrobial and antiviral factors; pro-inflammatory cytokines that lead to direct killing of the microorganisms; and the recruitment of other immune effectors. **d** | Vertebrate TLRs also contribute to the activation of adaptive immune responses. TLR signalling in dendritic cells allows their maturation to become efficient antigen-presenting cells through the induction of co-stimulatory molecules, the upregulation of major histocompatibility complex molecules and the secretion of pro-inflammatory cytokines. Mature dendritic cells activate naive T cells and modulate suppressor T cells.

overexpression of Toll in cultured *Drosophila* cells promotes their aggregation[48]. A role for Toll in cell adhesion is further reinforced by its complex spatial and temporal expression pattern, which correlates with regions of invaginating cells[2].

Drosophila *Toll in immunity.* Toll was the first TLR member to be linked with immunity. Mutations affecting both intracellular Toll-signalling-pathway components and Spätzle dramatically reduce survival after some fungal and Gram-positive bacterial infections[6,49,50] (FIG. 4a). This stems from the central role of Toll signalling in the expression of a battery of immune genes by the fat body, including antimicrobial peptide genes[6,51] (FIG. 4b). The Toll signalling cascade controlling the antimicrobial response differs from the pathway that is involved in dorsoventral patterning at two levels: by the serine proteases acting upstream of Spätzle

Fat body
The functional equivalent, in insects, of the mammalian liver.

Table 2 | **Loss-of-function analysis of Toll-like receptor genes**

Gene	Species	Major loss-of-function phenotypes	Ref.
Toll	*Drosophila melanogaster*	Dorsalization of the embryo	1
		Defects in motor-neuron number	44
		Improper muscle patterning	44
		Improper motor-neuron synaptogenesis	46
		Incomplete dorsal-vessel formation (embryonic)	47
		Reduced number of circulating cells	54
		Defective antimicrobial-gene regulation (adult)	6
		Defective larval development	2
		Defective pupal development	43
Toll2 (*18 wheeler*)	*Drosophila melanogaster*	Defective morphogenesis	3
		Defective epithelial morphogenesis	57
Toll8 (Tollo)	*Drosophila melanogaster*	Loss of neural-specific glycosylation	59
Toll5A	*Aedes aegypti*	Susceptibility to fungal infection (adult)	72
Tol-1	*Caenorhabditis elegans*	Embryonic lethality	73
		Pathogen-avoidance defects (adult)	73
Tlr1	*Mus musculus*	Defective triacyl lipopeptide response	112
Tlr2	*Mus musculus*	Defective lipopeptide response	110
Tlr3	*Mus musculus*	Defective dsRNA response	116
Tlr4	*Mus musculus*	Defective lipopolysaccharide response	107
Tlr5	*Mus musculus*	Defective bacterial flagellin response	113
Tlr6	*Mus musculus*	Defective diacyl lipopeptide response	111
Tlr7	*Mus musculus*	Defective ssRNA response	121
Tlr9	*Mus musculus*	Defective bacterial-DNA response	122
		D efective viral-DNA response	123
Tlr11	*Mus musculus*	Susceptibility to uropathogenic bacteria	114
		Defective response to a profilin-like protein from *Toxoplasma gondii*	115
TLR3	*Homo sapiens*	Herpes simplex encephalitis	92

and by the use of a different NF-κB protein Dif in the adult fat body, rather than Dorsal, during oogenesis[52]. Microbial infections are sensed in the haemolymph by secreted peptidoglycan recognition proteins (PGRPs) and β-glucan recognition proteins (GNBPs), which, following binding to microbial compounds, trigger the activation of Spätzle through distinct and complex proteolytic cascades[53] (FIG. 4b). The Toll pathway has also been implicated in other aspects of the *Drosophila* immune response, such as the regulation of haemocyte proliferation and density[54,55]. Thus, *Drosophila* Toll is a text-book example of a multifunctional molecule that can use different upstream or downstream partners in different contexts.

***Other* Drosophila *Toll*.** Similar to Toll, *Drosophila* Toll2 and Toll5 to Toll9 have dynamic embryonic expression patterns, which suggest a role in development[56]. Such a role has been demonstrated genetically for *Toll2* and *Toll8* (also known as *Tollo*) (TABLE 2). Mutation in *Toll2* causes death during larval development and early adulthood. Forced expression of *Toll2* promotes the rapid and robust aggregation of cells in culture, suggesting that it can function as a cell-adhesion molecule and that it can facilitate cell movements[3]. Accordingly, *Toll2* mutant embryos present salivary gland invagination defects similar to the embryos that lack components of the Rho pathway[57] and *Toll2* mutant ovarian follicular cells show delayed migrations[58]. Therefore, *Toll2* has an adhesive and a signalling role in epithelia that are engaged in cell migration that does not involve the canonical Toll cascade but possibly the Rho–GTPase pathway. Finally, it has been reported that the loss of Toll8 function abolishes specific glycosylation patterns in the embryonic nervous system[59,60].

Thus, *Drosophila* Toll-like proteins are known to have a range of important roles in development, whereas their role in the control of immune responses is currently limited to Toll. Two other *Drosophila* TLRs, Toll5 (also known as Tehao) and Toll9, have been linked to an immune function[61–64] but additional *in vivo* experiments are needed to clarify this.

Haemolymph
Insect blood.

Box 1 | **Mouse TLR ligands**

Twelve Toll-like receptors (TLRs) have been identified in the mouse genome and each TLR seems to recognize distinct molecules that are derived from various types of microorganism. TLRs can be classified into several groups based on the types of ligand they recognize. TLR1, 2, 4 and 6 recognize lipids. TLR4, together with its extracellular components such as MD-2 and CD14, associate with lipopolysaccharide (LPS) from Gram-negative bacteria[107–109]. TLR2 forms heterodimers with TLR1, with TLR6 and with non-TLR molecules such as CD36 to differentiate between a wide variety of ligands including peptidoglycans, mycoplasma lipopeptides, fungal zymosan, and lipopeptides and lipoproteins from Gram-positive bacteria[110–112]. TLR5 and TLR11 recognize protein ligands. TLR5 is abundantly expressed in intestinal dendritic cells, where it senses bacterial flagellin[113]. TLR11 recognizes currently unknown components of uropathogenic bacteria and a profilin-like molecule of the protozoan parasite *Toxoplasma gondii*[114,115]. The third class of TLR includes TLR3, 7, 8 and 9, which are localized in endosomes where they detect nucleic acids that are derived from viruses and bacteria. TLR3 was shown to sense double-stranded RNA (dsRNA), which is produced by many viruses during replication[116]. TLR7 recognizes synthetic imidazoquinoline-like molecules, guanosine analogues such as loxoribine, small interfering RNA and single-stranded RNA (ssRNA) derived from various viruses[117–119]. An immune function of TLR8 remains unknown in mice but human TLR8 can sense synthetic imidazoquinoline-like molecules and ssRNA, like mouse TLR7 (REFS 120,121). TLR9 recognizes CpG DNA motifs that are present in bacterial and viral genomes as well as non-nucleic acids such as haemozoin from the malaria parasite[122–124].

Other insect TLRs. So far, the analysis of sequenced genomes from the orders Lepidoptera, Diptera, Coleoptera and Hymenoptera has revealed that insects have between 5 and 12 TLRs[65–70] (*Apis mellifera* and *Aedes aegyptis,* respectively) (TABLE 1). Despite this diversity, high sequence similarities suggest that insect TLRs are not fast evolving but instead evolve by gene duplication. Insect TLRs fall into 3 families: the Toll1 group, consisting of Toll1/5 and 3/4 subfamilies; the Toll2 group, including Toll2/7, 6, 8 and 10/11 subfamilies; and the Toll9 group[38]. Toll9 is clearly distinct from other insect TLRs as this is the only sccTLR, and its expression pattern in *Drosophila* seems restricted to the haematopoietic system during development and the digestive tract at the adult stage[56,71]. The variable numbers of Toll1/5 and Toll9 subfamily members found in Diptera reflect specific expansions that occurred after the split between *Drosophila* and mosquitoes 250 mya.

The immune function of *Drosophila* Toll family members is conserved in other Diptera (TABLE 2). RNAi knockdown of the mosquito *A. aegyptis* Toll5A and its putative ligand Spz1C results in increased susceptibility to infection by the entomopathogenic fungus *Beauveria bassiana*, albeit to a lesser extent than RNAi knockdown of the mosquito Dorsal homologue, Rel1 (REF. 72). In addition, both *A. aegyptis* Toll1A and Toll5B are induced following fungal infection and their expression is dependent on Rel1 (REF. 72). This, together with the specific expansion of Toll1/5 and Toll9 subfamily members in Dipterans might reflect the consequence of diversifying selective pressure imposed by pathogens[69].

The *Caenorhabditis elegans* TLR

Only one TLR gene, *tol-1*, has been identified in *Caenorhabditis elegans* and *Caenorhabditis brigssae*[73]. TOL-1 is an mccTLR but is molecularly distant from arthropod TLRs. TOL-1 seems to have a major developmental function (strong loss of function leads to a high proportion of embryonic lethality) and no essential role in the control of immune responses[73] (FIG. 3b; TABLE 2). However, its molecular function remains elusive. Surprisingly, hypomorphic *tol-1* mutants with a small deletion of the TIR domain are healthy and fertile but exhibit a weak larval lethality[73]. This suggests that the TIR domain is largely dispensable for the embryonic function and that the protein might act at the level of the cell surface, where it might contribute to correct cell–cell adhesion. In addition, the hypomorphic *tol-1* mutants show defects in prototypical avoidance behaviour to pathogenic bacteria, although other chemosensory behaviours seem normal. However, recently it has been reported that *Salmonella enterica* can invade the pharynx of such hypomorphic *tol-1* mutants[74]. Pujol *et al.* reported a reduced lifespan of such mutant worms and a restricted adult expression pattern of *tol-1* in neurons[73]. This correlates well with TOL-1 function in a neuronal sensory pathway. However, additional experiments are needed to clarify how *tol-1* loss of function might account for the observed increased susceptibility to *S. enterica*. The absence of a major immune function of TOL-1 correlates with the fact that NF-κB factors are absent from the *C. elegans* genome. However, TIR-1, a TIR-containing adaptor similar to human and *Drosophila* SARM, has been characterized and functions independently of TOL-1 in the control of MAPK signalling[75–77] (FIG. 1c).

Vertebrate TLRs: the immune sentinels

Functional and molecular studies have revealed that mammalian TLRs play an essential part in the recognition of infectious agents, and act as sentinels and regulators of host defence mechanisms.

Mouse TLRs. Mice have twelve TLRs (TABLE 1). TLR mutant mice are viable and healthy but show increased susceptibility to a wide range of microorganisms[4,78]. In contrast to *Drosophila* Toll, vertebrate TLRs directly recognize products from various types of microorganisms, including viruses, bacteria, fungi and parasites. TLRs can be classified into several groups based on the types of ligand they recognize (BOX 1; TABLE 2). Signalling events downstream of vertebrate TLRs are similar but more diverse than in the *Drosophila* Toll–NF-κB pathway. In mammals, five TIR-containing adaptors — MyD88, TIRAP (also known as MAL), TRIF (also known as TICAM1), TRAM (also known as TICAM2) and SARM — mediate or modulate intracellular TLR signalling[79]. Based on the combination of adaptors used, mammalian TLRs activate several intracellular cascades leading to nuclear translocation of NF-κB. However, recent studies indicate that TLRs can also signal independently of NF-κB, through transcription factors belonging to the interferon response factors family (IRF3, 5 and 7) or signalling cascades activated by mitogen activating protein kinase (MAPK)[79] (FIG. 1c).

Avoidance behaviour
C. elegans worms that are fed on bacterial lawn in experimental conditions have the capacity to discriminate between bacterial species and avoid pathogenic bacteria such as *Serratia marcescens*, while being attracted by non-pathogenic species such as *Escherichia coli*.

TLR signalling initiates acute inflammatory responses by the induction of antimicrobial genes, inflammatory cytokines and chemokines in various cell types — especially those of myeloid origin and also paneth cells of the gut epithelium[80] (FIG. 4c). Subsequent events, such as the recruitment of neutrophils and activation of macrophages, lead to direct killing of the microorganisms[81]. TLRs also contribute significantly to the activation of adaptive immune responses, which are vertebrate specific[82,83]. TLR signalling causes dendritic cells to become efficient antigen-presenting cells by the induction of co-stimulatory molecules, the upregulation of major histocompatibility complex molecules and the secretion of cytokines and chemokines (FIG. 4d). This maturation occurs in peripheral tissues or secondary lymphoid organs and leads to the activation of T cells and B cells, the main cellular effectors of adaptive immune responses. TLRs are also expressed in certain subsets of T and B cells and can modulate the activity of these cells directly[83,84]. Overall, TLR activation enables the potent induction of immune responses, a function that is analogous to the role of Toll in insect immunity. However, in *Drosophila*, Toll directly regulates the expression of a large array of antimicrobial molecules by the fat body, whereas vertebrate TLRs control a complex cytokine network.

Most insect TLR functions seem to be developmental. To the best of our knowledge, a similar function for vertebrate TLRs has not been identified. Nevertheless, recent reports show that TLRs are expressed in mouse neurons and neuronal progenitors and might modulate neurite outgrowth in a manner similar to *Drosophila* Toll in motor-neuron synaptogenesis[44,46,85,86] and neuronal-progenitor differentiation and/or self-renewal[87]. Although preliminary, these results pave the way for studies of non-immune vertebrate TLR function.

Human TLRs. Ten TLRs containing polymorphisms associated with several infectious or inflammatory diseases have been identified in humans[88,89]. Patients with a null mutation in *IRAK4*, which encodes an essential intracellular mediator of TLR signalling, develop recurrent invasive pneumococcal infections but are otherwise healthy[90]. Similarly, patients with altered *UNC93B* function affecting TLR3, 7, 8 and 9 signalling or *TLR3* loss-of-function frequently develop herpes simplex virus 1 (HSV-1) encephalitis but have no other obvious immune defects[91,92]. The narrow spectrum of infections in these patients is surprising given the role of TLRs in mice in defence against a wide range of microorganisms. Although there is probably redundancy between human TLRs for protective immunity to most microorganisms, they seem to be non-redundant for protective immunity to particular infections. Intrinsic differences between the ecosystems of mice and humans analysed in these studies (experimental versus natural), and differences in TLR-independent responses might account for the observed discrepancies[93].

Phylogeny of vertebrate TLRs. Analysis of other vertebrate genomes ranging from primates to jawed fish has revealed a minimal number of ten genes encoding sccTLRs (TABLE 1), which fall into six major families: TLR1, 3, 4, 5, 7 and 11. Most vertebrates have at least one gene from each family[39]. There are occasional exceptions: *Tetraodon nigroviridis* and *Takifugu rubripes* lack TLR4, which correlates with the known resistance of these fish to endotoxin shock[94,95]. Chickens lack TLR9, the function of which might have been substituted with the avian-specific TLR15 or other TLR-related genes (TABLE 1). No genome sequence is available for jawless vertebrates but recently two TLR14-like sccTLRs have been identified in the lamprey (*Lampreta japonica*), suggesting that TLRs are also part of the immune recognition arsenal of jawless vertebrates[96].

The phylogeny of each major vertebrate TLR family recapitulates the phylogeny of vertebrate species, and sequence analyses show that all vertebrate TLRs evolve at about the same slow rate, suggesting strong selection for maintenance of function[39]. This high conservation relates to the fact that microorganisms cannot easily mutate their structural motifs, which are recognized by TLRs. Apart from humans and mice, no functional data are available for other vertebrate TLRs. However, the observation that zebrafish embryos treated with *Myd88* morpholinos are susceptible to bacterial infections supports a conserved immune function of TLRs from fish to humans[97].

Expansion of TLRs in invertebrate deuterostomes

The draft genome sequences of representative invertebrate deuterostomes provide the opportunity to compare their gene repertoire with that of vertebrates (FIG 2; TABLE 1). This is particularly interesting for immune-related genes because the immune response has experienced a drastic change during chordate evolution, ultimately leading to the emergence of adaptive immunity early in the vertebrate lineage (~500 mya).

The genome sequence of the sea urchin *S. purpuratus* reveals an enormous expansion of three classes of innate immune recognition proteins, including TLRs, NLRs and scavenger receptors[98]. There have been 222 TLR genes identified and these can be separated into two broad categories based on the comparison of their TIR domain sequences[99]. A greatly expanded multigene family consists of 211 genes encoding sccTLRs and a more limited group of 11 divergent genes includes 3 mccTLRs, 3 divergent sccTLRs and 5 atypical TLRs with a short extracellular domain. These sea-urchin-specific TLRs seem to have been duplicated and diversified recently and sequence diversity is greatest in the ectodomain, which could be consistent with an associated diversification of recognition specificity[98].

In the absence of any functional data, it has been proposed that sea urchin TLRs could be a component of the host defence system because their expression pattern is reminiscent of immune genes rather than developmental genes[99]. Indeed, a wide range of sea urchin TLRs are expressed in circulating coelomocytes, whereas their expression seems to be low or absent in embryos[99]. Interestingly, 26 genes encoding TIR adaptor proteins have been identified, suggesting that a modest expansion has also taken place in TLR adaptor signalling

Paneth cells
Specialized epithelial cells of the small intestine, which provide host defence against microorganisms.

Endotoxin shock
A medical condition that is caused by decreased tissue perfusion and oxygen delivery as a result of lipopolysaccharide contamination of the blood stream.

Morpholinos
A synthetic molecule used to modify gene expression.

Coelomocytes
Circulating cells that are present in the body cavity (coelome) of sea urchins and other invertebrates.

Box 2 | **Lophotrochozoan TLRs and the quest for the TLR ancestral function**

Lophotrochozoans comprise annelids, molluscs and flatworms. They represent the sister group of ecdysozoans (that is, arthropods and nematodes) and, therefore, studies on TLRs in this group might shed light on the ancestral function of TLRs in the bilaterian ancestor. Multiple cysteine cluster TLRs (mccTLRs) have been identified in cephalopod molluscs, including the Hawaiian squid (*Euprymna scolopes*)[125] and in a divergent marine bivalve, the Zhikong scallop (*Chlamys farreri*)[126]. TLRs are also present in the annelid phylum as several mccTLRs have been identified in genomic traces of the polychaete annelid *Capitella* sp. I (M. Vervoort and G. Balavoine, personal communication). However, a TLR gene has yet to be found in platyhelminthes even though significant genomic information is available for the flatworms *Schistosoma japonicum*, *Schistosoma mansoni* and *Schmidtea mediterranea*[31]. This provides evidence that TLR genes are likely to exist throughout the molluscs and annelids phyla and might have been secondarily lost in a lineage leading to platyhelminthes (FIG. 2). Given the molecular divergence of lophotrocozoan TLRs, it is evident that they have evolved independently from arthropod and nematode TLRs[125]. However, contrary to nematodes, nuclear factor-kappa B (NF-κB) factors have been identified in molluscs[125] and annelids[127,128] (G. Balavoine, personal communication), suggesting that lophotrochozoan TLRs might have retained the ability to control NF-κB signalling (FIG. 2). Nevertheless, the existence of a TLR–NF-κB pathway in these species remains purely speculative and the biological importance of lophotrochozoan TLRs remains to be studied.

proteins. Nevertheless, NF-κB signal transduction components are not expanded in the *S. purpuratus* genome[98]. Therefore, it is probable that the engagement of TLR proteins leads to the activation of NF-κB factors in sea urchin coelomocytes. It has been proposed that, in the absence of an adaptive immune system in this species, the specificity of the immune response could be provided by the spatiotemporal regulation of the TLR repertoire[99]. A causal explanation for the versatility of the sea urchin TLR system might stem from its complex life history, intricate water vascular system, large body size (compared with other invertebrates) and long lifespan (more than 30 years). An expanded immune receptor repertoire might also have a pivotal role in the surveillance of the endosymbiotic microbial communities that these animals harbour[99,100].

Multiple TLR-gene expansion and diversification has also occurred in invertebrate chordates: 42 TLR genes have been identified in the amphioxus (*Branchiostoma floridae)* genome, a cephalochordate, one of the three subphylum of chordates[100]. As with the sea urchin, the need for such an expanded TLR repertoire in the amphioxus genome might stem from its water filtering activity as a suspension feeder animal that is buried in sand. However, two other filter-feeding invertebrate deuterostomes, the solitary ascidians *Ciona savignyi* and *Ciona intestinalis* show no expansion of TLRs (having between 3 and 7 TLR genes each). These species belong to the other invertebrate subphylum of chordates, the urochordates, which is the sister group of vertebrates[101]. However, a striking expansion of genes encoding putative proteins of the complement system and genes encoding the prelude to adaptive immunity with allorecognition and self-incompatibility reactions have been reported in these species[102,103].

Why have certain invertebrate deuterostomes vastly expanded their TLR genes? One possibility is the requirement of a higher diversity of immune recognition capacities at an early stage of deuterostome evolution. Long-term coexistence between animals and microorganisms might have favoured the evolution of such large arsenals of specific microbial recognition molecules, which might have become obsolete or even detrimental in lineages where primitive adaptive immune systems emerged. Studying TLR functions in such organisms could refine our understanding of the ancestral innate immune system of deuterostomes.

Evolutionary perspective on TLR function

Functional information on TLRs is limited to a small number of model organisms (TABLE 2). Still, the range of known functions, from host immune responses in insects and vertebrates to development and cell adhesion in insects and nematodes, make any inference about the function of TLRs in the bilaterian ancestor (immunity, development or cell adhesion) and the origins of immune and developmental functions as they are known today highly speculative.

However, phylogenetic studies point to an ancient origin of TLR genes at the dawn of animal evolution about 700 mya. With the exception of nematodes, which have lost many pathways, the presence of TLR genes in genomes ranging from humans to cnidarians always correlates with the presence of NF-κB transactivators (FIG. 2). This, together with the well-established similarities between the NF-κB signalling pathways controlled by *Drosophila* and mammalian TLRs, suggests an ancient link between TLR and NF-κB, which might date from the origin of TLR function. Nevertheless, it is important to note that in *Drosophila* and *C. elegans* TLRs also contribute to cell adhesion during development, independently of NF-κB activation. This facet of TLR activity has received little attention so far and further work is required. Presently, it is unclear when the developmental role of TLRs appeared but studies on lophotrocozoan and cnidarian TLRs might help to shed light on this issue and on the function of TLRs in the eumetazoan ancestor (BOX 2).

Convergent evolution of TLR-mediated immunity?

The findings that TLRs are implicated in the immune response in mammals and that Toll participates in the host defence of *Drosophila* has led to the proposition that TLR-mediated innate immune responses are ancient, originating in the common ancestor of bilaterian animals. However, the recent accumulation of genomic, phylogenetic and functional data on TLRs in diverse organisms instead suggests that some TLRs have been independently co-opted for mediating innate immunity functions in insects and mammals[36–38,104].

First, sequence comparison of TLR genes from different phyla reveals that TLR families evolved independently and that no relationships of orthology can be drawn. In particular, mammalian TLRs and *Drosophila* Toll do not form a clade as expected in the case of the continuity hypothesis, but rather they fall into two distinct clusters. This shows that they share a common ancestor but evolved independently by gene duplication after the split between protostomes and deuterostomes[36–38].

Adaptive immune system
The long-lasting host defence response to infection, which is acquired during the life of the host.

Chordates
The phylum of animals that is defined by the presence of a notochord.

Complement system
A complex system of proteins that interact in a proteolytic cascade, leading to pathogen clearance in the serum.

Innate immune response
The first line of defence against invading organisms, which is inherited.

Clade
A taxonomic group of organisms comprising a single common ancestor and all the descendants of that ancestor.

Convergent evolution
The process whereby organisms that are not closely related (not monophyletic) independently evolve similar traits as a result of having to adapt to similar environments or ecological niches.

Second, significant functional differences exist between *Drosophila* and mammalian TLR-mediated immunity. The use of a cytokine intermediate, Spätzle, as a ligand for *Drosophila* Toll seems fundamentally different from the direct sensing of microorganisms by vertebrate TLRs. There are also major differences in signalling downstream of TLR: the TAK1–TAB–IKK signalling module is an essential part of vertebrate TLR signalling upstream of I-κB (an inhibitor protein of NF-κB) but does not seem to function in the *Drosophila* Toll pathway; instead it is involved in a distinct pathway controlling NF-κB — the Imd pathway[52]. Finally, the role of *Drosophila* Toll in the control of the systemic antimicrobial response is probably a recent adaptation in holometabolous insects because, with the exception of hemipterans, such an antimicrobial response is generally poorly developed in hemimetabolous insects in comparison to cellular reactions or other humoral reactions involving lectins, lysozymes and phenoloxidase[105,106].

One evolutionary scenario that agrees with these observations is that the bilaterian ancestor harboured mccTLRs (of currently unknown function), which would have been co-opted for immunity before the bilaterian lineages diverged. Subsequent independent evolution of these lineages would have led to the actual divergence of TLR structures and functions. However, an alternative interpretation of the similarities and differences between Toll-mediated humoral immunity in *Drosophila* and TLR-mediated immunity in vertebrates is convergent evolution. TLR-mediated immunity would have been independently co-opted in several lineages to mediate immune functions: once, early in the deuterostome lineage, and later, in the insect lineage. This is in line with many evo–devo observations of high malleability in pathway utilization among species for analogous function. Ecological factors are likely to have had a particularly important role in the diversification of the immune system, given the diverse pressure of pathogens. Nevertheless, TLR function in the innate immune response in both *Drosophila* and mammals is probably not entirely coincidental, and raises the question of why evolution has retained a limited number of analogous regulatory modules in separate evolutionary lineages. It could be that the intrinsic properties of signalling modules are particularly well-suited to a specific function[27]. This assumes that, despite a common denomination, signalling pathways might not be equivalent but instead are more or less appropriate to mediate particular tasks. The recurrent implication of the JAK–STAT, TLR–NF-κB and MAPK pathways in the immune responses of species belonging to various phyla might arise from their capacity to rapidly modulate transcription of target genes in response to an external stress — a characteristic that is essential for efficient and robust immune responses.

Concluding remarks

TLRs have multiple functions in addition to immunity, ranging from developmental signalling to cell adhesion. However, we currently lack the functional information on TLRs in several important lineages, such as lophotrochozoans or cnidarians, that is required to draw a robust evolutionary scenario of the emergence of TLR-mediated immunity and the ancestral function of TLRs. Therefore, one important challenge for the future will be to study the function of TLRs in these lineages. In addition, analysing the function of TLRs in invertebrate deuterostomes will clarify when TLRs emerged as direct sensors of microorganisms and might refine our understanding of the ancestral innate immune system of deuterostomes.

1. Anderson, K. V., Jurgens, G. & Nusslein-Volhard, C. Establishment of dorsal–ventral polarity in the *Drosophila* embryo: genetic studies on the role of the *Toll* gene product. *Cell* **42**, 779–789 (1985). **This paper describes the initial characterization of *Drosophila Toll* alleles.**
2. Gerttula, S., Jin, Y. S. & Anderson, K. V. Zygotic expression and activity of the *Drosophila Toll* gene, a gene required maternally for embryonic dorsal–ventral pattern formation. *Genetics* **119**, 123–133 (1988).
3. Eldon, E. *et al.* The *Drosophila* 18 wheeler is required for morphogenesis and has striking similarities to Toll. *Development* **120**, 885–899 (1994).
4. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124**, 783–801 (2006).
5. Pasare, C. & Medzhitov, R. Toll-like receptors: linking innate and adaptive immunity. *Adv. Exp. Med. Biol.* **560**, 11–18 (2005).
6. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. & Hoffmann, J. The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983 (1996). **This paper describes the first implication of a TLR in host defence.**
7. Buchanan, S. G. & Gay, N. J. Structural and functional diversity in the leucine-rich repeat family of proteins. *Prog. Biophys. Mol. Biol.* **65**, 1–44 (1996).
8. Kobe, B. & Kajava, A. V. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* **11**, 725–732 (2001).
9. Bell, J. K. *et al.* The molecular structure of the Toll-like receptor 3 ligand-binding domain. *Proc. Natl Acad. Sci. USA* **102**, 10976–10980 (2005).
10. Choe, J., Kelker, M. S. & *Wilson*, I. A. Crystal structure of human Toll-like receptor 3 (TLR3) ectodomain. *Science* **309**, 581–585 (2005).
11. Weber, A. N. *et al.* Binding of the *Drosophila* cytokine Spätzle to Toll is direct and establishes signaling. *Nature Immunol.* **4**, 794–800 (2003).
12. Mizel, S. B., West, A. P. & Hantgan, R. R. Identification of a sequence in human Toll-like receptor 5 required for the binding of Gram-negative flagellin. *J. Biol. Chem.* **278**, 23624–23629 (2003).
13. Andersen-Nissen, E., Smith, K. D., Bonneau, R., Strong, R. K. & Aderem, A. A conserved surface on Toll-like receptor 5 recognizes bacterial flagellin. *J. Exp. Med.* **204**, 393–403 (2007).
14. Jin, M. S. *et al.* Crystal structure of the TLR1–TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* **130**, 1071–1082 (2007).
15. Cornelie, S. *et al.* Direct evidence that Toll-like receptor 9 (TLR9) functionally binds plasmid DNA by specific cytosine–phosphate–guanine motif recognition. *J. Biol. Chem.* **279**, 15124–15129 (2004).
16. Rutz, M. *et al.* Toll-like receptor 9 binds single-stranded CpG–DNA in a sequence- and pH-dependent manner. *Eur. J. Immunol.* **34**, 2541–2550 (2004).
17. Kufer, T. A., Fritz, J. H. & Philpott, D. J. NACHT-LRR proteins (NLRs) in bacterial infection and immunity. *Trends Microbiol.* **13**, 381–388 (2005).
18. Martinon, F. & Tschopp, J. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* **26**, 447–454 (2005).
19. DeYoung, B. J. & Innes, R. W. Plant NBS-LRR proteins in pathogen sensing and host defense. *Nature Immunol.* **7**, 1243–1249 (2006).
20. Werts, C., Girardin, S. E. & Philpott, D. J. TIR, CARD and PYRIN: three domains for an antimicrobial triad. *Cell Death Differ.* **13**, 798–815 (2006).
21. Bowie, A. *et al.* A46R and A52R from vaccinia virus are antagonists of host IL-1 and Toll-like receptor signaling. *Proc. Natl Acad. Sci. USA* **97**, 10162–10167 (2000).
22. Belvin, M. P. & Anderson, K. V. A conserved signaling pathway: the *Drosophila* Toll–Dorsal pathway. *Annu. Rev. Cell Dev. Biol.* **12**, 393–416 (1996).
23. Medzhitov, R., Preston-Hurlburt, P. & Janeway, C. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397 (1997). **This paper shows the first link between TLR- and NF-κB-mediated immune responses in vertebrates.**
24. Xu, Y. *et al.* Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* **408**, 111–115 (2000).
25. Boraschi, D. & Tagliabue, A. The interleukin-1 receptor family. *Vitam. Horm.* **74**, 229–254 (2006).
26. Miller, D. J. *et al.* The innate immune repertoire in cnidaria — ancestral complexity and stochastic gene loss. *Genome Biol.* **8**, R59 (2007).
27. Ausubel, F. M. Are innate immune signaling pathways in plants and animals conserved? *Nature Immunol.* **6**, 973–979 (2005).
28. Philippe, H., Lartillot, N. & Brinkmann, H. Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. *Mol. Biol. Evol.* **22**, 1246–1253 (2005).
29. Putnam, N. H. *et al.* Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86–94 (2007).
30. Sullivan, J. C., Kalaitzidis, D., Gilmore, T. D. & Finnerty, J. R. Rel homology domain-containing transcription factors in the cnidarian *Nematostella vectensis*. *Dev. Genes Evol.* **217**, 63–72 (2007).

This paper describes the initial identification of TLR and NF-κB genes in a cnidarian.
31. Zheng, L., Zhang, L., Lin, H., McIntosh, M. T. & Malacrida, A. R. Toll-like receptors in invertebrate innate immunity. *Invertebrate Survival Journal* **2**, 105–113 (2005).
32. Wiens, M. *et al.* Innate immune defense of the sponge *Suberites domuncula* against bacteria involves a MyD88-dependent signaling pathway. Induction of a perforin-like molecule. *J. Biol. Chem.* **280**, 27949–27959 (2005).
33. Wiens, M. *et al.* Toll-like receptors are part of the innate immune defense system of sponges (demospongiae: Porifera). *Mol. Biol. Evol.* **24**, 792–804 (2007).
34. Beutler, B. Innate immunity: an overview. *Mol. Immunol.* **40**, 845–859 (2004).
35. Imler, J. L. & Zheng, L. Biology of Toll receptors: lessons from insects and mammals. *J. Leukoc. Biol.* **75**, 18–26 (2004).
36. Luo, C. & Zheng, L. Independent evolution of *Toll* and related genes in insects and mammals. *Immunogenetics* **51**, 92–98 (2000).
37. Friedman, R. & Hughes, A. L. Molecular evolution of the NF-κB signaling system. *Immunogenetics* **53**, 964–974 (2002).
38. Kanzok, S. M. *et al.* Origin of Toll-like receptor-mediated innate immunity. *J. Mol. Evol.* **58**, 442–448 (2004).
39. Roach, J. C. *et al.* The evolution of vertebrate Toll-like receptors. *Proc. Natl Acad. Sci. USA* **102**, 9577–9582 (2005).
References 35 to 39 provide phylogenetic data supporting the independent evolution of TLRs in different phyla.
40. Anderson, K. V. & Nüsslein-Volhard, C. in *Pattern Formation: A Primer in Developmental Biology* (eds Malacinsky, G. M. & Bryant, S.) 269–289 (MacMillian Publishers Ltd, New York, 1984).
41. Moussian, B. & Roth, S. Dorsoventral axis formation in the *Drosophila* embryo — shaping and transducing a morphogen gradient. *Curr. Biol.* **15**, R887–R899 (2005).
42. Roth, S. The origin of dorsoventral polarity in *Drosophila*. *Phil. Trans. R. Soc. Lond.* **358**, 1317–1329 (2003).
43. Letsou, A., Alexander, S., Orth, K. & Wasserman, S. A. Genetic and molecular characterization of *tube*, a *Drosophila* gene maternally required for embryonic dorso-ventral polarity. *Proc. Natl Acad. Sci. USA* **88**, 810–814 (1991).
44. Halfon, M. S., Hashimoto, C. & Keshishian, H. The *Drosophila Toll* gene functions zygotically and is necessary for proper motoneuron and muscle development. *Dev. Biol.* **169**, 151–167 (1995).
45. Halfon, M. S. & Keshishian, H. The Toll pathway is required in the epidermis for muscle development in the *Drosophila* embryo. *Dev. Biol.* **199**, 164–174 (1998).
46. Rose, D. *et al.* Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in *Drosophila*. *Development* **124**, 1561–1571 (1997).
47. Wang, J. *et al.* Expression, regulation, and requirement of the Toll transmembrane protein during dorsal vessel formation in *Drosophila melanogaster*. *Mol. Cell Biol.* **25**, 4200–4210 (2005).
48. Keith, J. & Gay, N. The *Drosophila* membrane receptor Toll can function to promote cellular adhesion. *EMBO J.* **9**, 4299–4306 (1990).
49. Tauszig-Delamasure, S., Bilak, H., Capovilla, M., Hoffmann, J. A. & Imler, J. L. *Drosophila* MyD88 is required for the response to fungal and Gram-positive bacterial infections. *Nature Immunol.* **3**, 91–97 (2002).
50. Rutschmann, S., Kilinc, A. & Ferrandon, D. Cutting edge: the Toll pathway is required for resistance to Gram-positive bacterial infections in *Drosophila*. *J. Immunol.* **168**, 1542–1546 (2002).
51. De Gregorio, E., Spellman, P. T., Tzou, P., Rubin, G. M. & Lemaitre, B. The Toll and Imd pathways are the major regulators of the immune response in *Drosophila*. *EMBO J.* **21**, 2568–2579 (2002).
52. Lemaitre, B. & Hoffmann, J. The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* **25**, 697–743 (2007).
53. Ferrandon, D., Imler, J. L. & Hoffmann, J. A. Sensing infection in *Drosophila*: Toll and beyond. *Semin. Immunol.* **16**, 43–53 (2004).
54. Qiu, P., Pan, P. C. & Govind, S. A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis. *Development* **125**, 1909–1920 (1998).
55. Sorrentino, R. P., Carton, Y. & Govind, S. Cellular immune response to parasite infection in the *Drosophila* lymph gland is developmentally regulated. *Dev. Biol.* **243**, 65–80 (2002).
56. Kambris, Z., Hoffmann, J. A., Imler, J. L. & Capovilla, M. Tissue and stage-specific expression of the Tolls in *Drosophila* embryos. *Gene Expr. Patterns* **2**, 311–317 (2002).
57. Kolesnikov, T. & Beckendorf, S. K. 18 wheeler regulates apical constriction of salivary gland cells via the Rho–GTPase-signaling pathway. *Dev. Biol.* **307**, 53–61 (2007).
58. Kleve, C. D., Siler, D. A., Syed, S. K. & Eldon, E. D. Expression of 18 wheeler in the follicle cell epithelium affects cell migration and egg morphology in *Drosophila*. *Dev. Dyn.* **235**, 1953–1961 (2006).
59. Seppo, A., Matani, P., Sharrow, M. & Tiemeyer, M. Induction of neuron-specific glycosylation by Tollo/Toll8, a *Drosophila* Toll-like receptor expressed in non-neural cells. *Development* **130**, 1439–1448 (2003).
60. Aoki, K. *et al.* Dynamic developmental elaboration of N-linked glycan complexity in the *Drosophila melanogaster* embryo. *J. Biol. Chem.* **282**, 9127–9142 (2007).
61. Tauszig, S., Jouanguy, E., Hoffmann, J. A. & Imler, J. L. Toll-related receptors and the control of antimicrobial peptide expression in *Drosophila*. *Proc. Natl Acad. Sci. USA* **97**, 10520–10525 (2000).
62. Lazzaro, B. P., Sackton, T. B. & Clark, A. G. Genetic variation in *Drosophila melanogaster* resistance to infection: a comparison across bacteria. *Genetics* **174**, 1539–1554 (2006).
63. Luo, C., Shen, B., Manley, J. L. & Zheng, L. Tehao functions in the Toll pathway in *Drosophila melanogaster*: possible roles in development and innate immunity. *Insect Mol. Biol.* **10**, 457–464 (2001).
64. Ooi, J. Y., Yagi, Y., Hu, X. & Ip, Y. T. The *Drosophila* Toll9 activates a constitutive antimicrobial defense. *EMBO Rep.* **3**, 82–87 (2002).
65. Rubin, G. M. *et al.* Comparative genomics of the eukaryotes. *Science* **287**, 2204–2215 (2000).
66. Christophides, G. K. *et al.* Immunity-related genes and gene families in *Anopheles gambiae*. *Science* **298**, 159–165 (2002).
67. Evans, J. D. *et al.* Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* **15**, 645–656 (2006).
68. Cheng, T. C. *et al.* Identification and analysis of Toll-related genes in the domesticated silkworm, *Bombyx mori*. *Dev. Comp. Immunol.* (2007).
69. Waterhouse, R. M. *et al.* Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science* **316**, 1738–1743 (2007).
70. Zou, Z. *et al.* Comparative genomic analysis of the *Tribolium* immune system. *Genome Biol.* **8**, R177 (2007).
71. Chintapalli, V. R., Wang, J. & Dow, J. A. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. *Nature Genet.* **39**, 715–720 (2007).
72. Shin, S. W., Bian, G. & Raikhel, A. S. A Toll receptor and a cytokine, Toll5A and Spz1C, are involved in Toll antifungal immune signaling in the mosquito *Aedes aegypti*. *J. Biol. Chem.* **281**, 39388–39395 (2006).
This paper shows a similar role for TLRs in the antifungal immune response in Diptera.
73. Pujol, N. *et al.* A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 809–821 (2001).
This paper presents a functional analysis of the unique *C. elegans* TLR.
74. Tenor, J. L. & Aballay, A. A conserved Toll-like receptor is required for *Caenorhabditis elegans* innate immunity. *EMBO Rep.* **9**, 103–109 (2008).
75. Chuang, C. F. & Bargmann, C. I. A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev.* **19**, 270–281 (2005).
76. Couillault, C. *et al.* TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nature Immunol.* **5**, 488–494 (2004).
77. Liberati, N. T. *et al.* Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *Proc. Natl Acad. Sci. USA* **101**, 6593–6598 (2004).
78. Beutler, B. *et al.* Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. *Annu. Rev. Immunol.* **24**, 353–389 (2006).
79. O'Neill, L. A. & Bowie, A. G. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nature Rev. Immunol.* **7**, 353–364 (2007).
80. Kawai, T. & Akira, S. TLR signaling. *Semin. Immunol.* **19**, 24–32 (2007).
81. Takeda, K. & Akira, S. Toll-like receptors in innate immunity. *Int. Immunol.* **17**, 1–14 (2005).
82. Pasare, C. & Medzhitov, R. Toll-like receptors: linking innate and adaptive immunity. *Microbes Infect.* **6**, 1382–1387 (2004).
83. Iwasaki, A. & Medzhitov, R. Toll-like receptor control of the adaptive immune responses. *Nature Immunol.* **5**, 987–995 (2004).
84. Sutmuller, R. P., Morgan, M. E., Netea, M. G., Grauer, O. & Adema, G. J. Toll-like receptors on regulatory T cells: expanding immune regulation. *Trends Immunol.* **27**, 387–393 (2006).
85. Ma, Y. *et al.* Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. *J. Cell Biol.* **175**, 209–215 (2006).
86. Cameron, J. S. *et al.* Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals. *J. Neurosci.* **27**, 13033–13041 (2007).
87. Rolls, A. *et al.* Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biol.* **9**, 1081–1088 (2007).
References 85 to 87 report the involvement of mouse TLRs in neurogenesis.
88. Cook, D. N., Pisetsky, D. S. & Schwartz, D. A. Toll-like receptors in the pathogenesis of human disease. *Nature Immunol.* **5**, 975–979 (2004).
89. Bochud, P. Y., Bochud, M., Telenti, A. & Calandra, T. Innate immunogenetics: a tool for exploring new frontiers of host defence. *Lancet Infect. Dis.* **7**, 531–542 (2007).
90. Picard, C. *et al.* Pyogenic bacterial infections in humans with IRAK4 deficiency. *Science* **299**, 2076–2079 (2003).
91. Casrouge, A. *et al.* Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* **314**, 308–312 (2006).
92. Zhang, S.-Y. *et al.* TLR3 Deficiency in patients with herpes simplex encephalitis. *Science* **317**, 1522 (2007).
93. Ku, C. L. *et al.* Inherited disorders of human Toll-like receptor signaling: immunological implications. *Immunol. Rev.* **203**, 10–20 (2005).
94. Berczi, I., Bertok, L. & Bereznai, T. Comparative studies on the toxicity of *Escherichia coli* lipopolysaccharide endotoxin in various animal species. *Can. J. Microbiol.* **12**, 1070–1071 (1966).
95. Iliev, D. B., Roach, J. C., Mackenzie, S., Planas, J. V. & Goetz, F. W. Endotoxin recognition: in fish or not in fish? *FEBS Lett.* **579**, 6519–6528 (2005).
96. Ishii, A. *et al.* Lamprey TLRs with properties distinct from those of the variable lymphocyte receptors. *J. Immunol.* **178**, 397–406 (2007).
97. van der Sar, A. M. *et al.* MyD88 innate immune function in a zebrafish embryo infection model. *Infect. Immun.* **74**, 2436–2441 (2006).
98. Rast, J. P., Smith, L. C., Loza-Coll., M., Hibino, T. & Litman, G. W. Genomic insights into the immune system of the sea urchin. *Science* **314**, 952–956 (2006).
99. Hibino, T. *et al.* The immune gene repertoire encoded in the purple sea urchin genome. *Dev. Biol.* **300**, 349–365 (2006).
References 98 and 99 describe and discuss the striking amplification of TLR genes in the sea urchin genome.
100. Pancer, Z. & Cooper, M. D. The evolution of adaptive immunity. *Annu. Rev. Immunol.* **24**, 497–518 (2006).
101. Bourlat, S. J. *et al.* Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. *Nature* **444**, 85–88 (2006).
102. Azumi, K. *et al.* Genomic analysis of immunity in a urochordate and the emergence of the vertebrate immune system: 'waiting for Godot'. *Immunogenetics* **55**, 570–581 (2003).
103. Khalturin, K., Panzer, Z., Cooper, M. D. & Bosch, T. C. Recognition strategies in the innate immune system of ancestral chordates. *Mol. Immunol.* **41**, 1077–1087 (2004).
104. Hughes, A. L. Protein phylogenies provide evidence of a radical discontinuity between arthropod and vertebrate immune systems. *Immunogenetics* **47**, 283–296 (1998).
105. Boman, H. G. & Hultmark, D. Cell-free immunity in insects. *Annu. Rev. Microbiol.* **41**, 103–126 (1987).
106. Hultmark, D. Insect lysozymes. *EXS* **75**, 87–102 (1996).

107. Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088 (1998).
108. Hoshino, K. *et al.* Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J. Immunol.* **162**, 3749–3752 (1999).
109. Qureshi, S. T. *et al.* Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (TLR4). *J. Exp. Med.* **189**, 615–625 (1999).
References 107 to 109 are the first reports of the essential role of TLRs in vertebrate immunity.
110. Takeuchi, O. *et al.* Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a Toll-like receptor 2- and MyD88-dependent signaling pathway. *J. Immunol.* **164**, 554–557 (2000).
111. Takeuchi, O. *et al.* Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int. Immunol.* **13**, 933–940 (2001).
112. Takeuchi, O. *et al.* Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J. Immunol.* **169**, 10–14 (2002).
113. Hayashi, F. *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**, 1099–1103. (2001).
114. Zhang, D. *et al.* A Toll-like receptor that prevents infection by uropathogenic bacteria. *Science* **303**, 1522–1526 (2004).
115. Yarovinsky, F. *et al.* TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* **308**, 1626–1629 (2005).
116. Alexopoulou, L., Holt, A. C., Medzhitov, R. & Flavell, R. A. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* **413**, 732–738. (2001).
117. Hemmi, H. *et al.* Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nature Immunol.* **3**,196–200 (2002).
118. Heil, F. *et al.* The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur. J. Immunol.* **33**, 2987–2997 (2003).
119. Hornung, V. *et al.* Sequence-specific potent induction of IFN-α by short interfering RNA in plasmacytoid dendritic cells through TLR7. *Nature Med.* **11**, 263–270 (2005).
120. Jurk, M. *et al.* Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nature Immunol.* **3**, 499 (2002).
121. Heil, F. *et al.* Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. *Science* **303**, 1526–1529 (2004).
122. Hemmi, H. *et al.* A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745 (2000).
123. Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. Toll-like receptor 9-mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. *J. Exp. Med.* **198**, 513–520 (2003).
124. Coban, C. *et al.* Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J. Exp. Med.* **201**, 19–25 (2005).
125. Goodson, M. S. *et al.* Identifying components of the NF-κB pathway in the beneficial *Euprymna scolopes–Vibrio fischeri* light organ symbiosis. *Appl. Environ. Microbiol.* **71**, 6934–6946 (2005).
126. Qiu, L., Song, L., Xu, W., Ni, D. & Yu, Y. Molecular cloning and expression of a Toll receptor gene homologue from Zhikong Scallop, *Chlamys farreri*. *Fish Shellfish Immunol.* **22**, 451–466 (2007).
127. Matsuo, K., Yoshida, H. & Shimizu, T. Differential expression of caudal and dorsal genes in the teloblast lineages of the oligochaete annelid *Tubifex tubifex*. *Dev. Genes Evol.* **215**, 238–247 (2005).
128. Goldstein, B., Leviten, M. W. & Weisblat, D. A. Dorsal and snail homologs in leech development. *Dev. Genes Evol.* **211**, 329–337 (2001).
129. Carty, M. *et al.* The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nature Immunol.* **7**, 1074–1081 (2006).
130. Adoutte, A. *et al.* The new animal phylogeny: reliability and implications. *Proc. Natl Acad. Sci. USA* **97**, 4453–4456 (2000).

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
tol-1
UniProtKB: http://ca.expasy.org/sprot
18w | Cactus | Dorsal | Myd88 | Pelle | Spätzle | Toll | Tube

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