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Recent insights into the evolution of innate viral sensing in animals

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The evolution of viral sensors is likely to be shaped by the constraint imposed through high conservation of viral Pathogen-Associated Molecular Patterns (PAMPs), and by the potential for 'arms race' coevolution with more rapidly evolving viral proteins. Here we review the recent progress made in understanding the evolutionary history of two types of viral sensor, RNA helicases and Toll-like receptors. We find differences both in their rates of evolution, and in the levels of positive selection they experience. We suggest that positive selection has been the primary driver of the rapid evolution of the RNA helicases, while selective constraint has been a stronger influence shaping the slow evolution of the Toll-like receptors.

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Introduction

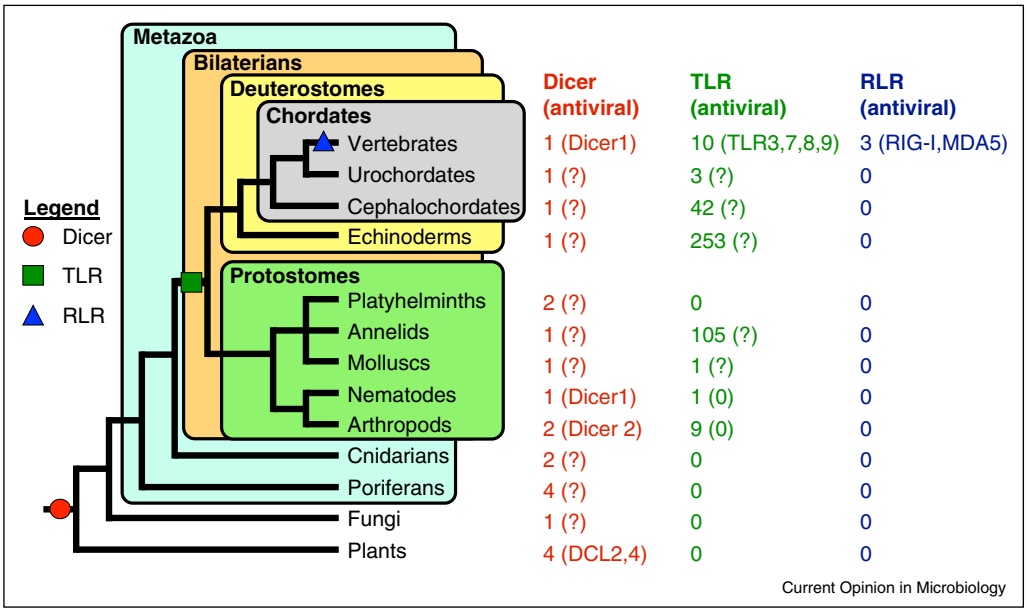
Pathogens reduce host fitness, and thereby exert a strong and ubiquitous selective pressure on hosts that has led to the evolution of a range of immune responses. Immune responses are elicited when sensors detect the presence of pathogens through Pathogen-Associated Molecular Patterns (PAMPs) or through markers of pathogen-associated damage. However, viruses may be uniquely difficult to sense because they use the host's own machinery to replicate, and therefore present fewer exogenous elicitors to immune surveillance mechanisms. Innate antiviral responses are therefore often triggered by conserved signatures of viral nucleic acids, such as dsRNA or CpG dinucleotides, which lead to the activation of multiple downstream immune responses, such as the RNA interference pathway or the vertebrate interferon response.

The conserved nature of these viral PAMPs leads to contrasting predictions regarding the evolution of antiviral genes. On the one hand, sensing these ancient and conserved molecular signatures might be expected to constrain the evolution of viral sensors. On the other hand, viral suppression of the antiviral immune system may lead to rapid evolution of viral sensors, as is seen in some antiviral genes of *Drosophila* [1]. Such rapid evolution may be driven by a host-virus arms race, as viruses escape the host immune response by cleaving or blocking antiviral genes [2]. Mechanisms of viral sensing have recently been reviewed elsewhere [3]; here we summarise the recent progress that has been made in understanding how two important viral sensing mechanisms have evolved, focussing on both phylogenetic history and the ongoing natural selection that shapes antiviral responses of extant populations. We finish by weighing the relative contributions of positive selection and evolutionary constraint during the evolution of viral sensing.

The phylogenetic distribution of viral sensing mechanisms

Although multiple protein families are known to act as viral sensors, many recent evolutionary studies have focussed on the Toll-like receptors (TLRs) and on receptors related to the RNA helicases, such as the Dicers and the RIG-I-like receptors (RLRs). Dicers act as sensors in the RNA interference (RNAi) pathway, binding dsRNA derived from the viral genome, replication intermediates or subgenomic products, and cleaving it into small RNAs that are ultimately used to target the virus or its transcripts for degradation. This is an ancient mechanism that probably arose prior to the most recent eukaryotic common ancestor over 1.5 billion years ago, and has since been conserved in all major eukaryotic lineages, including plants, fungi, ecdysozoa and vertebrates (illustrated in Figure 1) [4]. The helicase domain of the RLRs probably shares a common ancestor with that of Dicer [5], but on sensing viral dsRNA or other PAMPs, RLRs instead activate transcription factors such as nuclear factor-kappa B (NF-κB), and thereby induce the interferon pathway [6]. The RLRs also have a much more recent origin than Dicers, being present only in vertebrates, although homologues to their characteristic CAspase Recruitment Domains (CARDs) and RNA helicase domains are found in more basally branching deuterostomes, such as the tunicate *Ciona intestinalis* and the purple sea urchin

Figure 1



Phylogenetic distribution of viral sensing mechanisms. Gene family sizes are given, with validated antiviral genes in parentheses (0 = no antiviral genes, ? = antiviral function unknown). The three viral sensing mechanisms vary widely in their evolutionary ages: Dicer arose in the early Eukaryotes, whereas TLRs evolved in the early Bilateria, and RLRs first appeared in the vertebrates.

Strongylocentrotus purpuratus [5,7]. At present, direct viral sensing and immune induction functions have only been shown in vertebrates for two of the three RLRs, retinoic acid inducible gene I (RIG-I) [6] and melanoma differentiation associated gene 5 (MDA5) [8]. The third RLR, laboratory of genetics and physiology 2 (LGP2), binds viral RNA but cannot itself induce an immune response, instead triggering interferon production indirectly by signalling to MDA5 [9]. In contrast to the vertebrate-specific RLRs, the antiviral role of Dicer-like genes is much more widespread, being present in plants [10], fungi [11] and animals [12].

The Toll receptors were initially discovered in *Drosophila*, where they are involved in regulating the antibacterial and antifungal immune response [13]. The phylogenetic distribution (Figure 1) of Toll-like receptors (TLRs) suggests that they originated in the early Bilateria, before the divergence of protostomes and deuterostomes. In *Drosophila*, Toll-7 directly binds viruses and activates the autophagy response [14••]. In mammals, four TLRs (TLR3, 7, 8 and 9) play a pivotal role in sensing viral nucleic acids [15–18], subsequently activating the innate and adaptive immune responses through IRF-3, IRF-7 and NF-κB [19]. Other mammalian TLRs recognise different PAMPs, including lipids (TLR1, 2, 4 and 6) [20–22] and proteins (TLR5) [23]. This phylogenetic distribution of antiviral function suggests that TLRs are likely to have evolved a viral sensing role early in animal evolution, before the divergence of the protostomes and deuterostomes.

The evolution of RNA helicases

The most ancient conserved viral sensors are related to RNA helicases present in Archaea and Eukaryotes [5]. Two families of sensing helicases have been the subject of recent evolutionary study: the Dicers [24,25••] and the Rig-I-like receptors (RLRs) [5,7]. Two of the three RLRs (RIG-I and MDA5) each harbour two CARD domains that are integral in triggering the interferon response [6]. Despite this shared function, the two CARD domains appear to have substantially different histories [5], and it has therefore been suggested that the CARDs were gained by RIG-I and MDA5 in two separate events, with the first domain being acquired before the duplication that formed RIG-I and MDA5, and the second domain gained after they diverged [5]. Consistent with this, two CARD domains are found at separate loci in the sea anemone *Nematostella vectensis*, suggesting that the proposed grafting of these CARDs onto RLR may have occurred from these loci after the divergence of the chordates [7]. In contrast to the CARD domains, however, the order of divergence of RIG-I, MDA5 and LGP2 themselves remains unresolved. A neighbour-joining approach suggested that RIG-I diverged in the early deuterostomes, with LGP2 and MDA5 diverging later in the vertebrates