CellPress

Cell Host & Microbe
Perspective

The Evolution of Antiviral Defense Systems

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http://dx.doi.org/10.1016/j.chom.2016.01.006

Self-replicating genetic material presumably provided the architecture necessary for generating the last universal ancestor of all nucleic-acid-based life. As biological complexity increased in the billions of years that followed, the same genetic material also morphed into a wide spectrum of viruses and other parasitic genetic elements. The resulting struggle for existence drove the evolution of host defenses, giving rise to a perpetual arms race. This Perspective summarizes the antiviral mechanisms evident across the tree of life, discussing each in their evolutionary context to postulate how the coevolution of host and pathogen shaped the cellular antiviral defenses we know today.

Introduction

The combination of time and adaptability has bestowed extraordinary diversity into all life. The molecular understanding of cellular processes, particularly with the recent advent of high throughput sequencing and computational biology, has provided us the means to use conservation to infer the evolutionary past. This technique has given us profound insight with regards to the origins of life and has led to significant advances in our understanding of the processes that drive its diversity.

Arguably, one of the most powerful drivers of evolution stems from genetic parasites such as transposable elements and viruses (Koonin and Dolja, 2013). Their capacity to change, duplicate, edit, and/or transfer genetic information is often credited for the diversity of life. Despite the value provided by mobile genetic information, if left unchecked, this process would be catastrophic. It therefore follows that in addition to natural attenuation, the ability to successfully block expansion and spread of genetic pathogens is an essential attribute of host evolutionary survival. From prokaryotes to archaea to eukaryotes, every living species on the planet has evolved elaborate measures to recognize and react to viruses and/or transposable elements in some capacity. These defenses are composed of diverse strategies that often reflect the evolutionary trajectory that shaped each species, with some general themes proving to be timeless.

Despite the mechanistic overlap of some antiviral defenses across both kingdoms and species, there is immense diversity in how they evolved and function (Figure 1). Moreover, specific evolutionary branches demonstrate the emergence of defense systems that are exclusive to a given lineage. Here the various antiviral pathways are summarized in their evolutionary context to highlight both common and unique features of each strategy as a means of postulating how they may have emerged. Although necessarily speculative, the evolutionary trajectory for each specific defense system is based on observed conservation and the principle of evolutionary parsimony. This concept states that the number of assumptions needed to generate a hypothesis should be inversely correlated to its likelihood. When applied to evolutionary biology, maximum parsimony equates to the idea that any biological component that can be found in all three domains of life (bacteria, archaea, and eukaryotes), is likely to have existed in some form in our last universal ancestor (Kolaczkowski and Thornton, 2004; Koonin, 2003). This inference can be further reinforced should the conserved elements in question cluster independently to a phylogenetic analysis that can be superimposed onto the tree of life (Woese et al., 1990). Based on this framework, it is argued that the best predictions for what the first defense against parasitic genetic elements may have looked like demands a comprehensive analyses of genes involved in processing nucleic acid (Anantharaman et al., 2002). While this application of parsimony theory is probabilistic in nature and not always universally agreed upon, it serves as the best means of reconstructing ancient evolutionary events. This review aims to summarize the brilliant work of many groups on the specific origins of distinct evolutionary trajectories to put forth a plausible theory as to how the evolution of viruses may have shaped our cellular defense systems.

The Evolution of Genomic Parasites

Clues to the emergence of different antiviral defenses can be derived both from the evolution of life as well as the virome that it coexists with (Koonin and Dolja, 2013). However, while tracing the lineage of bacteria, archaea, and eukaryotes is generally clear, viruses represent the most abundant and diverse entity on the planet with no evidence that they derive from a single ancestor (Edwards and Rohwer, 2005; Koonin et al., 2006; Krupovic et al., 2011). Rather, virus diversity appears to have emerged as a result of genetic exchanges initially between themselves and then later with their hosts, in addition to any independent de novo emergence generated by self-amplification (Koonin and Dolja, 2014). Such exchanges undoubtedly began in the primordial pool as self-replicating RNAs. Here, selection would have been based exclusively on RNA replication (Koonin and Martin, 2005). Following countless rounds of amplification, peptides and later proteins would add to the complexity of these dynamics, eventually giving rise to polymerases. Generation of an RNA-dependent RNA polymerase (RdRp) would subsequently result in the evolution of an RNA-dependent DNA polymerase and the beginning of a new era of genetic storage. This process also spawned a set of core gene products that would go on to represent the most conserved hallmarks of viruses and the very beginnings of life itself (Koonin et al., 2006).

Once established, horizontal gene transfer events of these hallmark genes would give rise to the viral diversity that exists today. While determining the exact phylogeny of virus evolution

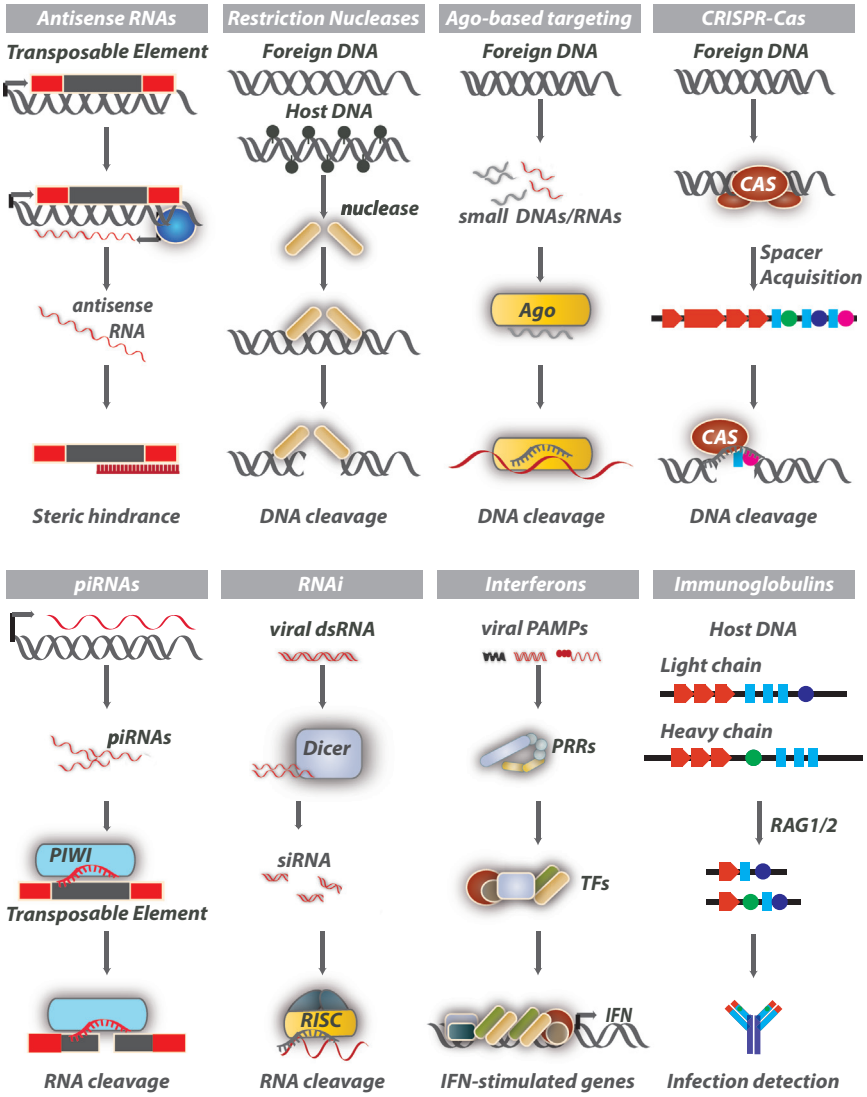

Figure 1. Defense Systems across the Tree of Life

Cartoon models depicting the various defense systems utilized by bacteria, archaea, and eukaryotes to defend against foreign genetic material. Defense systems are denoted in the gray box above each model. Antisense RNAs can be generated by transcribing the opposite strand of a transposable element or some other foreign DNA to produce a high-affinity RNA that can bind the DNA and disrupt function through steric hindrance. Restriction nucleases are proteins that recognize and cut DNA is a sequence-specific manner. The host can be protected from this action by the absence of that sequence or by the modification of self DNA (depicted by gray circles). The Argonaute (Ago)-based defense depicts the bacterial generation of small DNAs or RNAs that can be used to cleave complementary foreign DNA. The CRISPR-Cas (clustered regularly interspersed short palindromic repeats)-Cas (CRISPR-associated) complex depicts the capturing of small DNA fragments from incoming phage and the incorporation of that genetic material into the bacterial or archaeal genome (spacer acquisition). These spacers are then transcribed and used with another Cas member to mediate cleavage of the phage DNA. PIWI-associated RNAs (piRNAs) are small RNAs complementary to transposable elements. piRNAs associate with a PIWI nuclease to mediate RNA cleavage. RNA interference (RNAi) is initiated following the generation/recognition of double stranded RNA (dsRNA) which is cleaved by an RNase III nuclease called Dicer to yield small interfering RNAs (siRNAs). These siRNAs are loaded into the RNA-induced silencing complex (RISC) to mediate RNA cleavage of complementary targets. The interferon (IFN) system depicts the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). PRR engagement results in the activation of transcription factors (TFs) and the subsequent transcriptional induction of IFN and IFN-stimulated antiviral genes. The immunoglobulin system is denoted by light and heavy chain recombination that is mediated by the RAG recombinases generating a diverse range of antigen-specific receptors and antibodies. This enables the detection of diverse pathogens.

and the history of horizontal gene transfer events is unachievable, the inferences that can be made on current data suggest that it was very complex (Koonin et al., 2006). For example, while the first virus-like elements would have presumably been RNA-based, it is DNA viruses that dominate among present day prokaryotes. In fact, with the exception of only a few bacterial families, there is little evidence for RNA viruses in the vast majority of prokaryotes (bacteria or archaea). In stark contrast, DNA viruses in plants are very rare and RNA viruses abound in all eukaryotic lineages (Koonin et al., 2006, 2015).

Given the lack of diverse RNA phage populations, it seems feasible to assume that the first bona fide parasitic genetic element to impact the first unicellular population was DNA based. The lack of RNA-based entities, which would have been bountiful in the primordial pool, suggests that the environment of these early cells was considerably less amenable for RNA replication and spread—perhaps a result of inherent bottlenecks in the horizontal transfer of RNA elements between cells. In any case, expansion of these early self-replicating DNA elements, which would go on to include bacteriophages, drove the evolution of the first prokaryotic defenses (Figure 2).

The DNA viruses of the early prokaryotes provided the evolutionary platform for eukaryotic genetic pathogens. For example, based on structural similarities and limited homology, it would seem that the DNA phage that emerged from self-replicating DNA elements would later adapt to eukaryotic biology in the form of Polintons (Fischer and Suttle, 2011; Krupovic and Koonin, 2015). These transposable elements have been hypothesized to be the ancestor of most eukaryotic DNA viruses (Krupovic and Koonin, 2015). In contrast, RNA-based bacteriophages show very little evidence of being the direct ancestral counterpart for eukaryotic viruses (Koonin et al., 2015). One notable exception to this is an RdRp in a plant virus family that derives from a mitochondrial confined self-replicating RNA of phage ancestry (Koonin et al., 2015). Apart from this event, it would seem that the vast majority of eukaryotic RNA viruses may have also been derived from DNA phages by combining some of their structural components with an RdRp from a

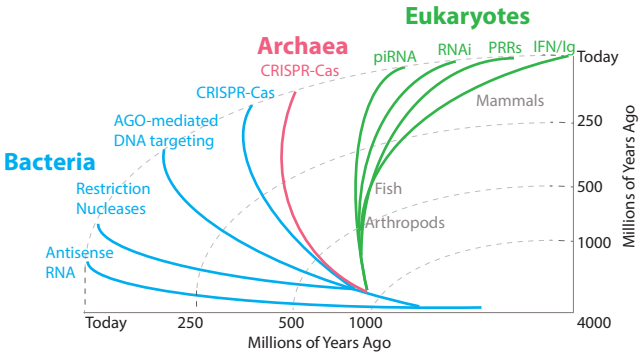

Figure 2. Evolution of Life's Cellular Defense Systems
Graph depicting an evolutionary timeline for the appearances of genetic defense systems as they relate to the phylogenetic tree of the three domains of life (bacteria, blue; archaea, red; and eukaryotes, green). Ago, Argonaute; CRISPR, clustered regularly interspersed short palindromic repeats; Cas, CRISPR-associated genes; piRNAs, piwi-interacting RNA; RNAi, RNA interference; PRR, pattern recognition receptors; IFN/Ig, interferon/immunoglobulins.

self-splicing retroelement (Koonin et al., 2008). This event was responsible for the eventual formation of the picornavirus-like virus lineage (Goldbach and Wellink, 1988; Koonin and Dolja, 1993; Koonin et al., 1991). The rapid amplification and diversification of these early positive-stranded RNA viruses was presumably driven by new niches, provided by the membranous and compartmentalized environment of eukaryotes. It was this environment that allowed the expansion and diversification of the picornavirus-like viruses that would later give rise to the alphavirus-like and flavivirus-like lineages. Flavivirus-like viruses are thought to be the ancestors of the negative-stranded RNA viruses of eukaryotes (Koonin et al., 2015). With the predicted evolutionary transition of DNA viruses to RNA viruses as the foundation, one can envision how present day antiviral defense mechanisms needed to adapt to the changing genetic makeup of the pathogens that would have been driving their selection.

Simple Beginnings—Antisense RNA

There is strong evidence indicating that RNA-based life existed before protein- and DNA-based life, yet how these processes gave rise to the first protocells is still very much unknown (Robertson and Joyce, 2012). Ensuring continuity of this genetic information would have demanded an RdRp activity which would eventually give rise to an RNA-dependent DNA polymerase (RdDp) (Makeyev and Grimes, 2004). The capacity to interconvert RNA to DNA set the stage for the generation of unicellular life and the subsequent emergence of retroelements, small self-replicating DNAs that amplify via RNA intermediates (Xiong and Eickbush, 1990). While the self-amplifying nature of retroelements would have been necessary as a driver for protocell diversity, at some point in this evolutionary trajectory these elements would become problematic. Thus, biological measures to limit the amplification of self-replicating genetic material would have emerged out of necessity and been rapidly selected for. The measures that evoked this control would have constituted the earliest antiviral platform which, given the simple nature of the organism, would have been based on the production of antisense-mediated targeting.

To inhibit mobile DNA elements, the earliest protocell could probably do no better than to limit this activity by transcribing antisense RNA that was, at least in part, complementary to the element itself (Figure 1). Many adaptations of this approach are still evident in bacteria and archaea (Gottesman and Storz, 2011; Thomason and Storz, 2010; Wagner et al., 2002). In general, this form of regulation is mediated by small RNAs of less than 150 nt long, which engage their targets in a manner that, in part, depends on sequence complementarity as well as secondary structures formed during these interactions (Wagner et al., 2002). Known functions of antisense RNAs in prokaryotes include the control of plasmid, transposon, and bacteriophage amplification (Wagner et al., 2002). This early use of non-coding RNA to control pathogens would form a central platform for how all subsequent life modulated both gene regulation and pathogen control.

RNAs, Nucleases, and the Emergence of Multicomponent Systems

While generating antisense RNA may have effectively saved early prokaryotes from transposable elements, the eventual emergence of DNA phages would have demanded more powerful defense systems. While speculative, one could envision this need as the driver for the emergence of sequence-specific endonucleases (Ishikawa et al., 2010; Kobayashi, 2001; Mochizuki et al., 2006). This system may have arisen as a relatively straightforward means of cutting a specific DNA sequence that was absent in the host genome. In time, this same system grew in complexity as the host began modifying its own DNA, thereby protecting it from cutting and allowing for the utility of more promiscuous nucleases that could cut very short palindromes. This two-component defense provided prokaryotes with a potent weapon to fend off foreign DNA.

The emergence of restriction endonucleases and other two-component systems would have imposed significant selective pressure on the phage virome, but no defense is infallible. As such, unicellular organisms would have needed to generate additional strategies to fend off phage infection. To achieve this, it would seem that evolution combined the general strategies of antisense biology and nucleases to combat foreign nucleic acid and merged them together to form a brand new means of defense. The first example of this comes in the form of a family of nucleases called Argonaute proteins (Swarts et al., 2014b). In bacteria, these proteins have been shown to associate with small fragments of complementary foreign DNA or RNA and compose a defense system to combat prokaryotic mobile genetic elements (Makarova et al., 2009; Olovnikov et al., 2013; Swarts et al., 2014a). A second system comes in the form of the CRISPR (clustered regularly interspersed short palindromic repeats)-Cas (CRISPR-associated) complex (Marraffini, 2015). CRISPR-Cas systems can be found in 90% of sequenced archaea and 40% of bacterial genomes (Grissa et al., 2007; van der Oost et al., 2009). This adaptive prokaryotic response functions by incorporating fragments of plasmid or phage DNA into specific genomic loci (Marraffini, 2015). These loci are transcribed and processed into small RNAs that can subsequently be used to guide a Cas nuclease complex to combat the viral threat. This defense not only provides the host with a potent and phage-specific restriction

factor, but it also enables transmission of the defense to its progeny (Koonin and Wolf, 2009).

A third example of combining antisense- and nuclease-based strategies comes in the form of the Piwi-interacting RNA (piRNA) system in eukaryotes. In all of these examples specificity is achieved via a non-coding RNA that enables the targeted elimination of the genetic threat by a nuclease. The piRNA system utilizes genome-encoded, transposon-derived, clustered small RNAs to defend against random integration events during development (Ishizu et al., 2012). piRNAs are ~25–30 nt and, similar to small noncoding RNAs in prokaryotes, can associate with a member of the Piwi/Argonaute family of nucleases. Found in genomic clusters riddled with transposable elements, piRNAs are generated by the thousands in a manner that is still relatively unclear (Iwasaki et al., 2015). The resulting small RNAs, bound to nuclease, function by associating and cleaving transposon-derived RNA. This activity is thought to be confined to germline cells (Gunawardane et al., 2007). Evolutionary conservation of the piRNA pathway suggests that it was present in the last common ancestor of metazoans and may be related to the Ago-based prokaryotic system (Grimson et al., 2008; Iwasaki et al., 2015; Shah and Garrett, 2011). While there does not appear to be any common ancestry between the piRNA and CRISPR systems, these analogous pathways provide an effective and heritable tool against genetic parasites.

Antisense, restriction nucleases, and CRISPR-Cas systems all provide effective protection against DNA viruses but their design is inadequate to deal with the rapidly dividing and mutating nature of RNA agents that thrived in eukaryotic cells (Lauring et al., 2013). Possibly as a result of this dynamic, one can observe extensive modifications and elaborations, many on the prokaryotic Ago-based system, which presumably gave rise to a potent eukaryotic defense called RNA interference (RNAi). Like Ago-based systems in bacteria, RNAi builds on the theme of coupling a small, sequence-specific oligonucleotides to a nuclease (Shabalina and Koonin, 2008; Swarts et al., 2014b). While the biology of RNAi differs from prokaryotes and even within eukaryotes, the general principles of the system are the same.

The RNAi system functions by processing dsRNA or RNA with extensive secondary structure into 19-21nt fragments using an RNase III nuclease called Dicer (Nayak et al., 2013; Szittya and Burgyán, 2013). Production of dsRNA can be derived directly from the pathogen or can be generated and amplified by the host through use of RdRps, although this latter step is not universal. For example, plants and worms utilize their own genome-encoded RdRps to generate and amplify these RNA fragments, an attribute they acquired through evolutionary acquisition of a phage polymerase (Shabalina and Koonin, 2008). However, this genetic function is not present in arthropods, resulting in a system where transport of antiviral small interfering RNAs (siRNAs) are critical and where the strength of the defense diminishes in cells more distal to the site of infection (Saleh et al., 2009; Tomoyasu et al., 2008). Following production, antiviral siRNAs are loaded into an RNA-induced silencing complex (RISC) containing an Ago effector nuclease and used to cleave the target RNA from which it was derived.

The Emergence of Pattern Recognition

Many of the core RNAi elements can be found in each of the five eukaryotic superkingdoms, suggesting this system existed in our last common ancestor (Cerutti and Casas-Mollano, 2006). However, it remains somewhat controversial whether chordates have retained the antiviral functions of RNAi (Cullen et al., 2013; Li et al., 2013; Maillard et al., 2013). Eukaryotes do encode a related pathway in the form of microRNAs (miRNAs), which would have originated from our ancestral antiviral system, but this class of endogenous small RNAs no longer appears to function in an antiviral capacity (Aguado et al., 2015; Bogerd et al., 2014). The idea that microRNAs perform a unique biological function, independent of antiviral activity, is in agreement with studies in *Drosophila* which utilize both systems in parallel (Obbard et al., 2006). In this study, the authors compared the evolution of *Dicer-1* and *Dicer-2* and found only the later gene product, which is the one involved in antiviral immunity, showed signs of selective pressure indicative of immune-related function (Obbard et al., 2006). Given this, it would seem that eukaryotic defenses as a whole diversified—with some superkingdoms maintaining small RNA-mediated defenses whereas others adapted additional or completely novel antiviral strategies. While there is experimental evidence to support that chordates could have used RNAi as their principal antiviral defense (Benitez et al., 2015; Kennedy et al., 2015), it would seem that the use may be limited to very specialized pluripotent cells, perhaps similar to the piRNA system (Cullen et al., 2013; Iwasaki et al., 2015; Li et al., 2013; Maillard et al., 2013).

In place of RNAi, chordates have evolved a stratified defensive system composed of core cellular effector proteins (innate) and a multifaceted and highly specific response (adaptive). This system relies on the recognition of common replication intermediates formed as a result of virus replication—commonly referred to as pathogen-associated molecular patterns or PAMPs. Proteins responsible for recognizing PAMPs are called pattern recognition receptors (PRRs), which evolved from a pre-existing family of proteins called Toll-like receptors (TLRs). TLRs are transmembrane proteins involved in embryogenesis and post-embryonic development, and have roles in both cell-cell interactions and signaling (Leulier and Lemaitre, 2008). TLRs originated some 700 million years ago at the dawn of animal evolution (Putnam et al., 2007). While the ancient Tolls were likely used exclusively in development in nonchordates, their structural organization and capacity to elicit a transcriptional response appears to have led to independent coopting events to transform them into sentinels for foreign material (Leulier and Lemaitre, 2008). This family of proteins expanded and diversified to detect different viral PAMPs expressed both extracellularly and intracellularly. In chordates, PRR engagement induces the direct activation of transcription factors to elicit a response aimed at pathogen inhibition (Janeway and Medzhitov, 2002).

The Emergence of Antiviral Cytokines

The existence of TLRs enabled multicellular organisms to detect the presence of a pathogen and elicit a transcriptional response. While this strategy can be effective in isolation, coupling of the transcriptional response to the generation of a second, secreted (paracrine) signal would protect the organism as a whole more efficiently; indeed, this is the function RdRps serve in large plants (Baulcombe, 1999). While cytokines would have already been in use as biological tools for establishing development gradients, their application in defense would allow distal cells the time to

fortify themselves in anticipation of infection. It is therefore not surprising that one of the earliest examples of an antiviral cytokine is tumor necrosis factor (TNF), whose transcriptional activity can be coupled to TLR engagement (Quistad et al., 2014). TNF, which can induce apoptosis (another strategy utilized in pathogen defense in all three domains of life; Makarova et al., 2012), can be found in most eukaryotic lineages and is thought to represent a 550-million-year functional conservation (Quistad et al., 2014). While TNF is still an active pathogen defense component in many species, the induction of cell death to prevent further spread is a luxury not always afforded to the organism (Iranzo et al., 2015). This is especially true of chordates. As a result, other antiviral cytokines including the interleukins and interferons (IFNs) emerged (Blair and Hedges, 2005).

In chordates, PRR engagement results in the induction of a conserved family of antiviral IFNs (Blair and Hedges, 2005). Similar to the RNAi machinery in plants, IFNs, especially type I IFNs (IFN-I), have expanded due to a series of gene duplication events (Manry et al., 2011). The family of IFN-I genes, which can include as many as sixty functional members, is rapidly induced at the transcription level in response to virus infection (Levy et al., 2011). IFNs act in both an autocrine and a paracrine manner to elicit a second signal transduction event that induces hundreds of host genes that collectively generate a cellular environment that is not conducive for virus propagation (Schneider et al., 2014).

Alongside IFN came the evolutionary emergence of recombinatorial adaptive immunity (Pancer and Cooper, 2006). In this system, specialized cells are dedicated to making pathogen-specific immunoglobulin receptors through DNA rearrangements. Generating the diversity necessary to recognize specific pathogens at a protein level was, similar to PRRs, enabled by TLR-like proteins (Pancer and Cooper, 2006). This early recombinatorial response later grew in complexity through the splicing and joining of three gene fragments to generate immunoglobulins in a process referred to as V(D)J rearrangement. The capacity to dedicate cellular lineages to a given pathogen provided the host with unique means to specifically neutralize and clear the infection. Furthermore, given that V(D)J recombination is irreversible, maintaining these cells provides the host with lifelong memory should the pathogen ever be encountered again.

Upgrades and Incompatibility Issues

Interestingly, the evolutionary loss of RNAi appears to coincide with the appearance of both IFN-I and the recombinatorial adaptive immune system. While it is not possible to determine the evolutionary cause of this transformation, one attractive hypothesis is based on an incompatibility between the small RNA targeting strategy and chordate biology. In this regard there are two attributes of chordates that may have caused the shift from RNAi to IFN-I; the mode of virus spread and the size of chordates.

As large multicellular organisms, chordates would require an RdRp for siRNA amplification and their circulation to distal sites within the organism in a manner akin to plants (Rajeswaran et al., 2014). In fact, the only phylum that relies on an RdRp-independent RNAi defense system is arthropods whose overall size provides protection by transport in the absence of amplification (Saleh et al., 2009). Given the significant difference in size between chordates and arthropods, siRNA transportation in the absence of amplification would be inadequate to offer system-wide protection. In support of this hypothesis, it is interesting to note that experimentally RNAi can replace the IFN-I system in chordates when the need for an RdRp is eliminated (Benitez et al., 2015). Based on the findings of this study, the loss of RNAi may have everything to do with RdRp biology and an inherent incompatibility with the IFN-I system. This is perhaps best supported by the fact that exogenous expression of an RdRp in mammalian somatic cells has now been demonstrated by two independent groups to be sufficient to trigger innate immunity (Painter et al., 2015; Yu et al., 2012).

Moreover, there is additional evidence that RNAi and the IFN-I system may be mutual exclusive. For example long dsRNA can induce functional small interfering RNAs in stem cells in the absence of an IFN-I response whereas the same stimulation in somatic cells produces IFN-I and no RNA processing (Billy et al., 2001; Maillard et al., 2013; Paddison et al., 2002). In addition, virus infection of somatic cells has been found to induce the shutdown of RISC while the restoration of an RNAi response inhibited IFN-I signaling pathways (Girardi et al., 2015; Seo et al., 2013). Together, these data support the notion that the IFN-I system may be fundamentally incompatible with a small RNA-mediated defense pathway. As to why chordates evolved to use the IFN-I system in place of RNAi: it stands to reason that IFN-I was better suited to provide system wide protection to larger organisms. While it could be argued that plants successfully use RNAi, the biology of plants provides an environment that makes this uniquely possible (Heinlein, 2015).That is, circulatory siRNAs in plants is more adaptable to combating infection because viruses spread from cell to cell independent of receptors and cell wall entry (Heinlein, 2015). This biology allows siRNAs to naturally follow the movement of the virus. In contrast, chordate virus infection is initiated by receptor-mediated cell entry, which would then demand a separate pathway for the internalization of siRNAs akin to what is observed in arthropods (Saleh et al., 2009). Together, this theory would suggest IFN was needed to protect chordates as they grew larger and, due to an incompatibility, the RNAi system needed to be eliminated.

Defenses, Counterdefenses, and Adaptive Innovations

Often referred to as the ''Red Queen Hypothesis,'' the biological arms race between host and pathogen represents a never-ending evolutionary struggle to maintain a relatively constant fitness level for both entities (Elde and Malik, 2009). Despite the host's best defenses, RNAi, restriction endonucleases, CRISPR-Cas, or the IFN-I system, viruses adapt to circumvent and/or disable these pathways (García-Sastre and Biron, 2006; Labrie et al., 2010; van der Oost and Brouns, 2015). Loss of functional host defense demands the invention of novel neutralizing strategies that are inevitably overcome ad infinitum. While these viral counter-defenses often represent antagonists to a central component of the defense pathway, others are more exploitative. For example, there is a phage of *Vibrio cholera* that encodes both its own cas genes and gRNAs that enable the virus to target a putative bacterial defense system aimed at interfering with phage replication (Seed et al., 2013). Similarly, cytokine signaling networks and stress response pathways of mammalian

hosts have been found to contribute to viral persistency (Moore and Chang, 1998). These types of interactions between host and pathogen have not only resulted in rapid evolution of intrinisic antiviral defenses, they are also directly responsible for the emergence of adaptive immune responses.

A rapid response to pathogen is important to host survival but the nature of this defense demands that it be non-specific. In contrast, an adaptive response, while requiring more time, can provide life-long customized protection. In an ideal setting, both defenses would be in place and would complement each other (Ago and CRISPR-Cas in bacteria or IFN-I and antibodies in chordates). In this regard, it is interesting to note that the two known forms of adaptive immune responses, the CRISPR-Cas and immunoglobulin-centered systems both derive from genetic pathogens (Koonin et al., 2015; Koonin and Krupovic, 2015). Similar to the coopting of components from transposable elements in the invention of eukaryotic telomerases (Nakamura and Cech, 1998), parasitic genetic elements appear to be responsible for the independent evolution of CRISPR-Cas and immunoglobulin-based systems (Koonin and Krupovic, 2015). Interestingly, despite evolving completely independently, these two adaptive responses appear to have emerged as a result of transposable elements enabling a pre-existing innate immune response with the capacity to undergo recombination (Koonin and Krupovic, 2015). For example, the type II CRISPR-Cas system that has become so popular for genome editing (Travis, 2015) seems to consist almost entirely of transposon-derived genes from a family of elements dubbed casposons (Koonin and Krupovic, 2015; Krupovic et al., 2014). These elements provided the enzymatic activities needed to acquire foreign DNA and integrate them into the genome to provide life-long immunity to the organism. Similarly, the capacity to undergo somatic recombination to form a diverse repertoire of immunoglobulins, a cornerstone of adaptive immunity in all jawed vertebrates, is the product of a recombinase that derived from a different transposable element from the so-called Transib family (Kapitonov and Jurka, 2005; Kapitonov and Koonin, 2015). In an ironic evolutionary twist, it would seem that the systems that provide us with the most protection derived from some of the very agents that made them necessary.

Summary

The need for defensive systems in the ongoing struggle for survival inevitably led to the evolutionary emergence of many different strategies. In fact, it could be argued that these defenses provide one of the common threads that tie all life together. From our modest antisense-based techniques to the more complex adaptive systems, life has evolved under extraordinary pressure from genetic pathogens. In fact, comparing archaeal, bacterial, and eukaryotic gene duplication events shows that host defense systems diverge more rapidly than any other process. While eukaryotes show a much greater propensity for creating new proteins with novel functions, the basic tools they build upon were largely present in their most ancient ancestors (Daugherty and Malik, 2012; Siddle and Quintana-Murci, 2014). Given all that has been learned thus far from tracing the evolution of host and pathogen it would seem likely that future studies will reveal more unknown biological systems that will further clarify the evolution of all antiviral systems.

ACKNOWLEDGMENTS

I am grateful to the members of my lab and colleagues for critical reading of this manuscript. I also sincerely apologize to the many authors whose original work could not be included or cited due to space limitations.

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