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2	Evolutionary Immunology to Explore Original Antiviral Strategies
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25	Keywords : innate immunity ; STING ; cyclic dinucleotide ; cGAS ; CBASS

26 ABSTRACT

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Over the past 25 years, the field of evolutionary developmental biology (evo-devo) 28 29 has used genomics and genetics to gain insight on the developmental mechanisms underlying the evolution of morphological diversity of animals. Evo-devo exploits the 30 key insight that conserved toolkits of development (e.g., Hox genes) are used in 31 32 animals to produce genetic novelties that provide adaptation to a new environment. Like development, immunity is forged by interactions with the environment, namely 33 34 the microbial world. Yet, when it comes to the study of immune defence mechanisms 35 in invertebrates, interest primarily focuses on evolutionarily conserved molecules also present in humans. Here, focusing on antiviral immunity, we argue that immune 36 37 genes not conserved in humans represent an unexplored resource for the discovery of new antiviral strategies. We review recent findings on the cGAS-STING pathway 38 and explain how cyclic dinucleotides produced by cGAS-like receptors may be used 39 to investigate the portfolio of antiviral genes in a broad range of species. This will set 40 the stage for evo-immuno approaches, exploiting the investment in antiviral defences 41 42 made by metazoans over hundreds million years of evolution.

44 Evolution to understand antiviral immunity: lessons from evo-devo.

As we have been reminded with the recent Covid-19 crisis, viruses represent a 45 46 major threat for our societies. In humans, acute or chronic viral infections are associated with many life-threatening diseases, including cancer. Innate immunity is 47 48 the first line of defence that operates in all animals and, in the case of vertebrates, precedes and orients the establishment of adaptive immunity. Host cells have 49 evolved numerous innate defences against viral infections, but the control of viruses 50 51 is complicated by the high mutation rates of most viral polymerases, which promote rapid virus evolution and adaptation to antiviral mechanisms. As a result, animal 52 genomes have been shaped by continuous challenge from viruses and the repertoire 53 54 of antiviral genes in any species reflects the cumulative effects of million years of evolutionary investment in innate immunity defenses^{1,2}. At a time when we have 55 been reminded that (i) we cannot predict what the next viral human pathogen will be 56 57 and (ii) the risks of future zoonosis outbreaks are higher than ever, increasing the diversity of approaches to understand virus-host interaction should be a priority^{3,4}. 58 Studying these interactions in as many settings as possible will provide insight on a 59 wide range of host restriction factors, opening the way to new applications in 60 biomedicine. 61

The concept of evo-immuno –for evolutionary immunology– is inspired by the spectacular successes of the field of evolutionary developmental biology, or evodevo, in the past 30 years. The evo-devo approach exploited genomics and genetics to gain insight on the developmental mechanisms underlying the evolution of morphological diversity in animals^{5,6}. The crucial discovery that *homeobox (Hox)* genes control antero-posterior patterning in both flies and humans provided the first insight into a genetic toolkit shared for the development of morphologically very

different animals and paved the way for the evo-devo field^{7,8}. A key aspect of the 69 70 genetic theory for the evolution of animal morphology revealed by evo-devo and apparent in the hourglass scheme of Denis Duboule is that a conserved gene toolkit 71 (e.g. Hox genes) responds to novelty and regulates novelty, hence providing 72 adaptation to changing environments (Fig. 1)^{7,8}. Indeed, the ontogenic trajectory is 73 74 often influenced by environmental factors, and innovations in morphogenesis are 75 generally selected if they improve adaptation of the animal to its environment or if they facilitate access to a new niche⁹. Besides embryonic development, the immune 76 system is another facet of animal biology that is forged by interactions with the 77 78 environment (e.g., the microbial world) and could benefit from evolutionary perspectives^{10,11}. Of note, the model organism *Drosophila melanogaster*, with its 79 extensive genetic and molecular resources, played a central role in evo-devo and 80 81 other insects provided spectacular examples of emergence of striking morphological novelties from the ancestral conserved toolkit (e.g., refs¹²⁻¹⁴). 82

83

84 Evo-immuno to delve into the biodiversity of antiviral defence strategies in 85 insects

86 Authors exploring comparative immunology have noted that, while signalling 87 pathways tend to be conserved between species, the receptors sensing infection are less conserved and evolve rapidly, as do the effector molecules regulated by these 88 pathways (see for example ref.^{15–17} and Khimovitch & Bosch in this issue). Hence, 89 innate immunity pathways exhibit an information bottleneck, akin to the bottleneck in 90 gene expression corresponding to the establishment of the axis of the embryo during 91 development. Although the evo-immuno concept does not entail an ontogeny aspect, 92 93 it aims at exploiting central evolutionarily conserved regulatory nodes to gain insight

94 on original solutions to adapt to environmental challenges, following in this regard the 95 path of evo-devo (**Fig. 1**). As argued elsewhere in this issue (see article by Hanson), 96 all microbes have specific weaknesses, which can be exploited by host defense 97 mechanisms and thus drive evolution of immune systems. Evo-immuno aims at 98 identifying evolutionarily exploited weak spots in pathogens, which could point to 99 innovative therapeutic strategies.

We propose to explore the concept of evo-immuno in insects, taking 100 advantage of the Drosophila model, but also of the fantastic biodiversity of this class 101 of animals with more than 1.2 million species known and well-established 102 phylogenetic relationships¹⁸. Insects are present and play vital roles in all terrestrial 103 ecosystems, where they are exposed to a broad range of pathogens, including 104 105 viruses. Indeed, recent virome analysis have revealed that insects represent an impressive reservoir of viruses, including most major viral groups found in animals 106 107 and plants (e.g. poxviruses, flaviviruses, alphaviruses, rhabdoviruses, picorna-like viruses, ...)¹⁹⁻²¹. Furthermore, important human viruses are transmitted by 108 hematophagous insect vectors such as Aedes mosquitoes²². Hence, characterizing 109 110 antiviral immunity in insects may reveal original strategies of antiviral defences. Importantly, evo-immuno aims at understanding how a conserved gene toolkit 111 produces a diversity of non-conserved responses, just as evo-devo allowed to 112 understand how a conserved toolkit can build legs, wings, or fins at locations of the 113 body. Hence, an important aspect of evo-immuno is that the study of non-conserved 114 genes is as interesting as -or even, arguably, more interesting than- evolutionarily 115 conserved ones. This represents an important paradigm shift as, until now, studies 116 on insect immunity received much more support and publicity when they reported 117 evolutionarily conserved mechanisms (e.g., Toll receptors)²³. Yet, investigating non-118

conserved genes holds much promise in the context of antiviral immunity. Restriction 119 120 of a virus by a host factor will put pressure on the virus to adapt, often resulting in the emergence of resistant variants. This will in turn put pressure on the host to either 121 modify the restriction factor to recover interaction with its viral target, or to find 122 another solution to control the virus²⁴. Because this continuous evolutionary arms 123 124 race between two genetic entities antagonizing each other goes on permanently, the repertoire of antiviral factors is evolving rapidly^{2,25}. Central to the concept of evo-125 immuno is the notion that this arms race goes on in parallel in all animals, such that 126 each animal may find its own unique solution to counter a virus (Fig. 2). Thus, 127 128 exploring the genomes of insects for species-specific innovations in antiviral immunity may reveal novel antiviral strategies. 129

Although antiviral immunity in insects has been a focus of attention in the past 130 years, studies have focused on few species (flies, vector mosquitoes, honeybees) 131 132 and specific viruses (e.g., ref.^{26–29}). Broader investigations have been hampered by lack of information on the viruses infecting insects, which could be used to trigger 133 antiviral immunity. The discovery of the important role played by the Stimulator of 134 interferon genes (STING) pathway in the antiviral defence of *D. melanogaster* opens 135 new perspectives³⁰. Indeed, STING-dependent signalling can be activated *in vivo* by 136 injection of cyclic dinucleotides^{31–34}. This provides a powerful mean to trigger antiviral 137 immunity in a range of insects, paving the way for evo-immuno (Fig. 1). 138

139

140 STING signaling participates in insect antiviral immunity

In the course of our work on induced-antiviral responses in *D. melanogaster*^{35–}
 ³⁸, we discovered that two components of the antibacterial immune deficiency (IMD)

143 pathway, the kinase IKK β and the NF- κ B factor Relish, rather than the pathway as a whole³⁰, participated in the control of infection by two picorna-like viruses, Drosophila 144 145 C virus (DCV) and Cricket paralysis virus (CrPV). It is worth reminding here that the 146 characterization of *Diedel*, a gene not conserved in mammals but strongly induced in 147 response to viral infection in *D. melanogaster* and hijacked by members of several 148 families of large insect DNA viruses, attracted our attention to the possible 149 involvement of components of the IMD pathway in antiviral immunity³⁹. This illustrates 150 how investigating non-conserved genes may reveal important 151 evolutionarily conserved facets of immunity. We further identified the orthologue of 152 STING, dSTING, among the genes regulated by IKK β in the context of viral infections and showed that dSTING was acting upstream of IKK^β and Relish to regulate 153 expression of genes induced by viral infections³⁰ (Fig. 3). Similar results were 154 obtained in the silkworm Bombyx mori⁴⁰. Altogether, our findings pointed to the 155 156 existence of a new pathway activated by viruses and controlling expression of STING-regulated genes to curb viral infection. 157

In mammals, STING is a key component of the cytosolic DNA sensing 158 pathway^{41,42}. It activates the transcription factor IRF3 through the kinase TBK1 to 159 induce interferon (IFN) gene expression, but also regulates IKK β and NF- κ B through 160 a less characterized mechanism⁴³⁻⁴⁵. STING acts as a signalling receptor for a 161 second messenger, the cyclic dinucleotide 2'3'-cGAMP, which is produced by the 162 enzyme cGAS upon sensing cytosolic DNA^{41,42} (Fig. 3). STING can also be activated 163 164 by other cyclic dinucleotides directly produced by bacteria (e.g., 3'3'-c-di-AMP, 3'3'-cdi-GMP and 3'3'-cGAMP)⁴⁶. 165

166 In *Drosophila*, we showed that injection of cyclic dinucleotides into flies leads 167 to a dose-dependent induction of STING-regulated genes, in a dSTING- and Relish-

dependent manner. Of note, 2'3'-cGAMP was a more potent agonist of dSTING than 168 169 3'3'-connected cyclic dinucleotides of bacterial origin, suggesting that an enzyme producing 2'3'-cGAMP was present in insects³¹. This discovery led to an analysis of 170 the transcriptome of 2'3'-cGAMP -injected flies, which revealed more than 400 genes 171 stimulated at least 1.5-fold 6, 12 or 24h post injection. It is worth mentioning here that 172 173 2'3'-cGAMP-induced genes include components of the small interfering RNA (Dicer-2, Argonaute 2) and autophagy (Ref(2)P, encoding the homologue of p62) pathways, 174 pointing to interactions between the STING pathway and other antiviral 175 mechanisms^{47–52}. Strikingly, co-injection of 2'3'-cGAMP with viruses reduced viral 176 177 replication and improved the survival of wild-type flies, but not dSTING or Relish mutant flies. This effect was observed on 5 different viruses belonging to different 178 families (Dicistroviridae, Nodaviridae, Alphaviridae, Rhabdoviridae, Nudiviridae), 179 180 indicating that 2'3'-cGAMP triggers broad antiviral immunity in D. melanogaster³¹. Altogether, our results revealed that 2'3'-cGAMP triggers a dSTING/NF-kB-181 dependent antiviral transcriptional response. 182

183

184 cGLRs, an emerging family of pattern recognition receptors

Two cGAS-like receptors, cGRL1 and cGLR2, acting upstream of STING in D. 185 melanogaster have recently been identified^{33,53}. Although they synthesize cyclic 186 dinucleotides, like cGAS, their characterization revealed intriguing differences with 187 the mammalian enzyme (Fig. 3)⁵⁴. For one, the activity of cGLR1 in vitro or in 188 transfected mammalian cells depends on the presence of double stranded (ds)RNA, 189 190 rather than DNA. The in vitro activity of recombinant cGLR2 from the species D. bipectinata and D. pseudoananassae is also enhanced most strongly in the presence 191 of dsRNA³². Another notable difference with cGAS is that *Drosophila* cGLRs produce 192

at least three cyclic dinucleotides in addition to 2'3'-cGAMP, which can all activate
STING signalling *in vivo*, albeit with different efficiencies (2'3'-c-di-GMP>3'2'cGAMP>2'3'-c-di-AMP>2'3'-cGAMP)³².

cGLRs represent an emerging family of pattern recognition receptors (PRRs) 196 197 present in all metazoan phyla and producing an array of cyclic di-purine and purinepyrimidine signals in response to binding DNA, RNA and probably also other 198 signals⁵⁵. The biological significance of this diversity of cyclic dinucleotide products is 199 still unclear but could reflect the existence of alternative receptors for these 200 201 nucleotide signals. For example, the stony coral Stylophora pistillata encodes 42 cGLRs and 7 STING paralogs, which exhibit different affinity and selectivity for cyclic 202 203 dinucleotides: Sp-STING3 preferentially binds 3'3'-linked cyclic dinucleotides, while Sp-STING5 is highly selective for 2'3'-cGAMP⁵⁵. Other receptors for cyclic 204 dinucleotides exist in animals, e.g., RECON (reductase controlling NF-KB) in 205 mice^{56,57}. Interestingly, flies synthesize an unusual cGAMP isomer, 3'2'-cGAMP, 206 which has so far only been identified in Drosophila and the bacteria Asticcacaulis 207 sp.^{33,53,58,59}. This *Drosophila* innovation may have been driven by a family of viral 208 suppressors of cGAS-STING signalling, the Poxins³³. Poxins were initially identified 209 in vaccinia virus, a member of the family Poxviridae, and function as 2'3'-cGAMP-210 specific nucleases⁶⁰. Strikingly, *poxin* homologs can be found in the genomes of 211 several large DNA viruses (baculoviruses, entomopoxviruses) infecting Lepidopteran 212 213 insects, but also in the genome of the moths and butterflies that host these viruses, pointing to a likely insect origin for poxin genes⁶¹. Poxin efficiently cleaves 2'3'-214 cGAMP but fails to cleave 3'2'-cGAMP, suggesting that the isomeric switch in 215 216 phosphodiester linkage specificity in Drosophila may have occurred to evade its action³³. It is worth noting here that genomic analysis of different poxvirus genera 217

indicates that Poxins are widely found in insect poxviruses and are present in some bat and rodent poxviruses but are missing in many other vertebrate poxviruses. This suggests that Poxin was acquired by horizontal transfer from insect viruses, possibly favoured by the insectivorous nature of bats and rodents⁶². Overall, these findings illustrate the relevance of taking a broad look at the repertoire of antiviral defences present in animals, rather than focusing on only a few species.

224

225 The cGLR-CDN-STING cassette in the animal toolkit of antiviral defences

It is now clear that the connection of the STING pathway to the regulation of 226 interferon genes is a late addition to an ancestral pathway, which has been 227 228 associated with the control of viral infections long before the onset of vertebrates. Indeed, the three key components of the pathway – cGAS-like enzymes, the cyclic 229 230 oligonucleotides they produce and STING-related molecules – were all inherited from prokaryotes, where they function in the control of phage infections (reviewed in 231 ref.⁶³). Accordingly, genes encoding cGLRs and STING are present in the genomes 232 of invertebrates, although they were lost in some species, such as worms or, within 233 234 insects, mosquitoes. In addition, an array of distinct cyclic dinucleotide signals controling discrete STING signaling pathways has recently been identified in a set of 235 236 invertebrates⁵⁵. Although the exact biological function of cGLRs in most animals remains unknown, the function of cGAS/DncV-like nucleotidyltransferase (CD-237 NTase) enzymes in the control of highly divergent anti-phage defense signaling 238 239 pathways in bacteria⁶⁴; the conserved function of cGAS and cGLRs in antiviral immunity in flies and mammals⁵⁴; and the fact that most cGLRs can be activated in 240 vitro by nucleic acids^{33,53,55,58} – a molecular pattern characteristic of viral infections – 241 242 altogether strongly suggest that the cGLR/STING signalling axis belongs to an

243 ancestral toolkit associated with the control of viral infections⁶³. Therefore, this 244 pathway can be harnessed to gain insight on the diversity of induced antiviral 245 defences in animals, much like the way *Hox* genes were used for evo-devo (**Fig. 1**).

Induction of antiviral gene expression can be achieved by analyzing the 246 247 transcriptome of cells or animals after viral infection. However, it is difficult in these studies to distinguish between the stress reaction triggered by cell lysis or tissue 248 249 damage and the immune response. The analysis is further complicated by the fact 250 that cell infections are not synchronized when animals are used instead of cell lines, 251 preventing the distinction between immediate early responses and late responses to the infection. Finally, viruses are notorious for hijacking cellular functions and 252 253 suppressing host defence mechanisms. The image provided by these transcriptomic 254 studies can therefore be imprecise. This caveat can be avoided by stimulating cells with molecules mimicking the molecular patterns sensed by PRRs to trigger antiviral 255 256 immunity. However, this is not trivial in the case of viral infections, since the nucleic 257 acid mimics known to trigger antiviral immunity have to be delivered into cells, to meet PRRs residing in the cytosol or the endosomes⁶⁵. In this context, the ability to 258 259 activate STING signalling by injection of cyclic dinucleotides provides a powerful shortcut to visualize the modifications of the transcriptome associated with induction 260 of antiviral immunity, in *Drosophila* and also other invertebrates³¹. The emerging 261 diversity of cyclic dinucleotide signals produced by cGLRs represents a caveat but 262 should not be limiting. Indeed, although cGLRs from invertebrates can produce 263 several cyclic dinucleotides, 2'3'-cGAMP is produced by cGLRs from (i) several 264 insects (e.g., Drosophila flies^{32,33,53}, the beetle Tribolium castaneum³³, the thrip 265 Frankliniella occidentalis and the flea Ctenocephalides felis⁵⁵); (ii) the sea anemone 266 Nematostella vectensis^{50,66}; (iii) the coral Pocillopora damicornis⁵⁵ and (iv) the fresh-267

water polyp *Hydra vulgaris*⁵⁵. Furthermore, as described below, 2'3'-cGAMP can
activate STING signaling in many species, including *Drosophila* flies. If necessary
(e.g., lack of significant response to 2'3'-cGAMP), other cyclic dinucleotides may be
used, as shown recently for the *Drosophila* species *D. serrata* and 2'3'-c-diGMP³².
Indeed, approaches suitable for chemical synthesis of cyclic dinucleotides with any
combination of nucleobases and their purification have been described⁶⁷.

274

275 CDN injection provides access to a repertoire of antiviral genes

Injection of 2'3'-cGAMP or other cyclic dinucleotides has now been reported to 276 activate an extensive transcriptional program not only in Drosophila, but also the sea 277 278 anemone N. vectensis, the coral S. pistillata, the Eastern oyster Crassostrea virginica and even the marine chanoflagellate Monosiga brevicollis, a free living unicellular 279 and colonial eukaryote that is the closest living relative of metazoans^{31,55,68,69}. 280 Strikingly, comparison of the genes differentially expressed in response to CDN 281 injection in these organisms reveals a common pool of immune genes - including 282 transcription factors (e.g., NF-kB, IRF), PRRs (e.g., cGLRs; RIG-I-like receptors; 283 Nucleotide binding domain, Leucine-rich repeats containing Receptors (NLRs)) or 284 effectors (e.g., viperin, OAS, RNaseL) -, pointing to an ancestral set of immunity 285 factors that have been conserved over more than 500 million years of evolution. A 286 number of these genes (e.g., viperin, Argonaute or cGLRs and STING themselves, 287 288 as mentioned above) have been inherited from the arsenal of antiphage defences in prokaryotes⁷⁰. The function of the mammalian orthologues of several of these factors 289 290 has already been characterized⁷¹. However, besides this evolutionarily ancient and conserved program, many non-conserved genes are also induced in all species. 291 292 These genes are evidence of divergent evolution in some organisms to adapt to the

293 threat of viruses, which may have resulted in unique strategies to restrict viral 294 replication (Fig. 2). Examples of these clade-specific innovations include the onset of the IFN family of cytokines in vertebrates, or the fly-specific gene pastrel, which 295 296 encodes a potent restriction factor for picorna-like viruses and whose expression is induced by 2'3'-cGAMP in all ten *Drosophila* species analysed^{72,73} (Hédelin, Thiébaut 297 298 et al, manuscript in preparation). In invertebrates, it appears that, at least in some 299 species (e.g., N. vectensis, M. brevicollis), the STING pathway regulates expression of antibacterial genes, in addition to antiviral genes, which may represent an early 300 innovation in the animal lineage of multicellular eukaryotes to connect the pathway to 301 infectious agents beyond viruses^{68,69}. 302

303 A few years ago, Palmarini, Wilson and colleagues investigated in a pioneer 304 study the "type-I interferomes" of fibroblasts from ten species of vertebrates and identified a conserved core of 62 IFN-stimulated genes (ISG), thus highlighting the 305 306 ancestral functions of the IFN response⁷⁴. Notably, this study also revealed that each 307 animal possessed ISGs unique to their species or their phylogenetic lineage. This attests to the constant expansion of ISGs in vertebrates, most likely driven by the 308 309 constant arms race between host and viruses². Of note, many ISGs are also induced directly upon sensing viral infection by PRRs and predate the evolution of interferons, 310 such that they have homologs in invertebrates and even in procaryotes^{70,75,76}. The 311 possibility to investigate the "STINGome" of invertebrates using the cyclic 312 313 dinucleotide injection assay now provide the opportunity to investigate evolution of antiviral responses in these animals as well, which offer access to a much broader 314 315 biodiversity. In particular, with the tools now at hand, insects represent powerful models to identify new restriction factors against families of viruses with broad host 316 tropisms, such as Picornaviridae, Poxviridae, Flaviviridae, Togaviridae. 317

318

319 Concluding remarks

It is now acknowledged that most animals control viral infection through the 320 321 induction of dedicated gene expression programs. Yet only a fraction of mammalian ISGs have been functionally characterized and recent studies reveal that a 322 323 substantial number of genes induced by viral infection are not conserved between animals⁷⁷. Furthermore, recent insight on the emerging role of the evolutionarily 324 conserved cGLR/STING pathway positions it as a central component of the toolkit 325 used to fight viral infections in animals beyond vertebrates. Induction of STING-326 dependent antiviral immunity upon cyclic dinucleotide injection allows rapid 327 328 identification in different species of large sets of genes regulated in context of viral 329 infections, paving the way for the exploration of the putative antiviral functions of nonconserved genes. It is noteworthy that, although we focus here on viruses, the 330 331 concept of evo-immuno also applies to other infections. Indeed, pioneering studies in innate immunity revealed that, although Toll signalling to NF-kB is conserved 332 between insects and mammals, Toll functions as a cytokine receptor in Drosophila, 333 unlike Toll-like receptors, which function as PRRs^{78–81}. Furthermore, recent studies 334 indicate that the antimicrobial peptides regulated by NF-kB in different Drosophila 335 species are subjected to diversification and specialization driven by differences in the 336 ecologies of the flies⁸². 337

In summary, the breakthrough discovery that broad antiviral immunity can be triggered in *Drosophila* and other invertebrates by injection of CDNs provides a rapid and reliable way to access to the repertoire of antiviral genes^{31,33,55,58,68,69}. The next step will be to exploit these lists of genes to identify novel restriction factors. This will be challenging, but large-scale biochemical analysis aided by structural predictions⁸³

and evolution-guided pipelines⁸⁴ to select non conserved candidate genes with 343 344 interesting features, represents a powerful strategy for discovery. Mechanistic studies on the molecular function of these non-conserved genes may lead to the discovery of 345 potentially unique strategies of antiviral defence, revealing discrete weak spots in the 346 targeted viruses. These could be exploited to develop innovative antiviral therapies. 347 348 As we will continue to be confronted with emerging viruses that ignore phylogeny 349 when they jump into new hosts, it is time to extend the search for antiviral restriction 350 mechanisms beyond humans or mammals and to acknowledge that what is not conserved can be as important as what is conserved, as superbly illustrated by the 351 352 evo-devo field.

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649 Figure legends

650 Fig. 1. Parallel between evo-devo and evo-immuno.

651 The STING pathway can be used to probe original antiviral strategies, much like 652 homeobox genes were used to decipher evolution of morphology traits in animals.

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Fig. 2. Evolutionary arms race between hosts and viruses.

When confronted to rapidly evolving viruses that escape host restriction factors, host adapts either by modifying the restriction factor (deep blue) or by finding another target in the virus. As a result, each animal may exhibit lineage-specific antiviral restriction factors. Modified from ref.²⁴.

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660 Fig. 3. Evolutionary conservation of the cGLR-CDN-STING cassette.

Enzymes of the CD-NTase family (cGAS, cGLRs, CdnE), the cyclic oligonucleotides the produce and STING related molecules participate in the control of phage or viral infections in bacteria and animals, from invertebrates to mammals. The mechanism activating CD-NTase enzymes in prokaryotes in response to phage infections is still unknown. Note that as predicted in the hourglass model shown in Fig. 1, cGLRs and cGAS are not activated by the same type of nucleic acids and regulate overlapping but distinct sets of genes (e.g., no interferons in invertebrates).

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Figure 1: Parallel between evo-devo and evo-immuno. The STING pathway can be used to probe original antiviral strategies, much like homeobox genes were instrumental to decipher evolution of morphology in animals.



Figure 2: Evolutionary arms race between hosts and viruses. When confronted to rapidly evolving viruses that escape host restriction factors, hosts adapt by modifying the restriction factor (deep blue) or by finding another target in the virus. As a result, each animal may exhibit lineage-specific restriction factors. Modified from ref. 18.



Figure 3: Evolutionary conservation of the cGLR-CDN-STING cassette. Enzymes of the CD-NTase family (cGAS, cGLRs, CdnE), the cyclic oligonucleotides they produce and STING related molecules participate in the control of phage or viral infections in bacteria and animals, from invertebrates to mammals. The mechanism activating CD-NTase enzymes in prokaryotes in response to phage infections is still unknown. Note that as predicted in the hourglass model shown in Fig. 1, cGLRs and cGAS are not activated by the same type of nucleic acids and regulate overlapping but distinct sets of genes (e.g., no interferons in invertebrates).