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Evolutionary Immunology to Explore Original Antiviral Strategies

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26 **ABSTRACT**

27

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

43

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

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

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

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

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
88 less conserved and evolve rapidly, as do the effector molecules regulated by these
89 pathways (see for example ref.¹⁵⁻¹⁷ and Khimovitch & Bosch in this issue). Hence,
90 innate immunity pathways exhibit an information bottleneck, akin to the bottleneck in
91 gene expression corresponding to the establishment of the axis of the embryo during
92 development. Although the evo-immuno concept does not entail an ontogeny aspect,
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.

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

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

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

139

140 **STING signaling participates in insect antiviral immunity**

141 In the course of our work on induced-antiviral responses in *D. melanogaster*^{35–}
142 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
144 whole³⁰, participated in the control of infection by two picorna-like viruses, *Drosophila*
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
150 illustrates 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
153 and showed that dSTING was acting upstream of IKK β and Relish to regulate
154 expression of genes induced by viral infections³⁰ (**Fig. 3**). Similar results were
155 obtained in the silkworm *Bombyx mori*⁴⁰. Altogether, our findings pointed to the
156 existence of a new pathway activated by viruses and controlling expression of
157 *STING-regulated genes* to curb viral infection.

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

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-

168 dependent manner. Of note, 2'3'-cGAMP was a more potent agonist of dSTING than
169 3'3'-connected cyclic dinucleotides of bacterial origin, suggesting that an enzyme
170 producing 2'3'-cGAMP was present in insects³¹. This discovery led to an analysis of
171 the transcriptome of 2'3'-cGAMP -injected flies, which revealed more than 400 genes
172 stimulated at least 1.5-fold 6, 12 or 24h post injection. It is worth mentioning here that
173 2'3'-cGAMP-induced genes include components of the small interfering RNA (*Dicer-*
174 *2*, *Argonaute 2*) and autophagy (*Ref(2)P*, encoding the homologue of p62) pathways,
175 pointing to interactions between the STING pathway and other antiviral
176 mechanisms⁴⁷⁻⁵². Strikingly, co-injection of 2'3'-cGAMP with viruses reduced viral
177 replication and improved the survival of wild-type flies, but not *dSTING* or *Relish*
178 mutant flies. This effect was observed on 5 different viruses belonging to different
179 families (*Dicistroviridae*, *Nodaviridae*, *Alphaviridae*, *Rhabdoviridae*, *Nudiviridae*),
180 indicating that 2'3'-cGAMP triggers broad antiviral immunity in *D. melanogaster*³¹.
181 Altogether, our results revealed that 2'3'-cGAMP triggers a dSTING/NF- κ B-
182 dependent antiviral transcriptional response.

183

184 **cGLRs, an emerging family of pattern recognition receptors**

185 Two cGAS-like receptors, cGRL1 and cGRL2, acting upstream of STING in *D.*
186 *melanogaster* have recently been identified^{33,53}. Although they synthesize cyclic
187 dinucleotides, like cGAS, their characterization revealed intriguing differences with
188 the mammalian enzyme (**Fig. 3**)⁵⁴. For one, the activity of cGRL1 *in vitro* or in
189 transfected mammalian cells depends on the presence of double stranded (ds)RNA,
190 rather than DNA. The *in vitro* activity of recombinant cGRL2 from the species *D.*
191 *bipunctinata* and *D. pseudoananassae* is also enhanced most strongly in the presence
192 of dsRNA³². Another notable difference with cGAS is that *Drosophila* cGLRs produce

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

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

218 indicates that Poxins are widely found in insect poxviruses and are present in some
219 bat and rodent poxviruses but are missing in many other vertebrate poxviruses. This
220 suggests that Poxin was acquired by horizontal transfer from insect viruses, possibly
221 favoured by the insectivorous nature of bats and rodents⁶². Overall, these findings
222 illustrate the relevance of taking a broad look at the repertoire of antiviral defences
223 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**

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

246 Induction of antiviral gene expression can be achieved by analyzing the
247 transcriptome of cells or animals after viral infection. However, it is difficult in these
248 studies to distinguish between the stress reaction triggered by cell lysis or tissue
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
252 the infection. Finally, viruses are notorious for hijacking cellular functions and
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
255 with molecules mimicking the molecular patterns sensed by PRRs to trigger antiviral
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
258 meet PRRs residing in the cytosol or the endosomes⁶⁵. In this context, the ability to
259 activate STING signalling by injection of cyclic dinucleotides provides a powerful
260 shortcut to visualize the modifications of the transcriptome associated with induction
261 of antiviral immunity, in *Drosophila* and also other invertebrates³¹. The emerging
262 diversity of cyclic dinucleotide signals produced by cGLRs represents a caveat but
263 should not be limiting. Indeed, although cGLRs from invertebrates can produce
264 several cyclic dinucleotides, 2'3'-cGAMP is produced by cGLRs from (i) several
265 insects (e.g., *Drosophila* flies^{32,33,53}, the beetle *Tribolium castaneum*³³, the thrip
266 *Frankliniella occidentalis* and the flea *Ctenocephalides felis*⁵⁵); (ii) the sea anemone
267 *Nematostella vectensis*^{50,66}; (iii) the coral *Pocillopora damicornis*⁵⁵ and (iv) the fresh-

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

274

275 **CDN injection provides access to a repertoire of antiviral genes**

276 Injection of 2'3'-cGAMP or other cyclic dinucleotides has now been reported to
277 activate an extensive transcriptional program not only in *Drosophila*, but also the sea
278 anemone *N. vectensis*, the coral *S. pistillata*, the Eastern oyster *Crassostrea virginica*
279 and even the marine choanoflagellate *Monosiga brevicollis*, a free living unicellular
280 and colonial eukaryote that is the closest living relative of metazoans^{31,55,68,69}.
281 Strikingly, comparison of the genes differentially expressed in response to CDN
282 injection in these organisms reveals a common pool of immune genes – including
283 transcription factors (e.g., NF- κ B, IRF), PRRs (e.g., cGLRs; RIG-I-like receptors;
284 Nucleotide binding domain, Leucine-rich repeats containing Receptors (NLRs)) or
285 effectors (e.g., viperin, OAS, RNaseL) –, pointing to an ancestral set of immunity
286 factors that have been conserved over more than 500 million years of evolution. A
287 number of these genes (e.g., viperin, Argonaute or cGLRs and STING themselves,
288 as mentioned above) have been inherited from the arsenal of antiphage defences in
289 prokaryotes⁷⁰. The function of the mammalian orthologues of several of these factors
290 has already been characterized⁷¹. However, besides this evolutionarily ancient and
291 conserved program, many non-conserved genes are also induced in all species.
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
295 the IFN family of cytokines in vertebrates, or the fly-specific gene *pastrel*, which
296 encodes a potent restriction factor for picorna-like viruses and whose expression is
297 induced by 2'3'-cGAMP in all ten *Drosophila* species analysed^{72,73} (Hédelin, Thiébaud
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
300 of antibacterial genes, in addition to antiviral genes, which may represent an early
301 innovation in the animal lineage of multicellular eukaryotes to connect the pathway to
302 infectious agents beyond viruses^{68,69}.

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
305 identified a conserved core of 62 IFN-stimulated genes (ISG), thus highlighting the
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
308 attests to the constant expansion of ISGs in vertebrates, most likely driven by the
309 constant arms race between host and viruses². Of note, many ISGs are also induced
310 directly upon sensing viral infection by PRRs and predate the evolution of interferons,
311 such that they have homologs in invertebrates and even in procaryotes^{70,75,76}. The
312 possibility to investigate the “STINGome” of invertebrates using the cyclic
313 dinucleotide injection assay now provide the opportunity to investigate evolution of
314 antiviral responses in these animals as well, which offer access to a much broader
315 biodiversity. In particular, with the tools now at hand, insects represent powerful
316 models to identify new restriction factors against families of viruses with broad host
317 tropisms, such as *Picornaviridae*, *Poxviridae*, *Flaviviridae*, *Togaviridae*.

318

319 **Concluding remarks**

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

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

343 and evolution-guided pipelines⁸⁴ to select non conserved candidate genes with
344 interesting features, represents a powerful strategy for discovery. Mechanistic studies
345 on the molecular function of these non-conserved genes may lead to the discovery of
346 potentially unique strategies of antiviral defence, revealing discrete weak spots in the
347 targeted viruses. These could be exploited to develop innovative antiviral therapies.
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
351 conserved can be as important as what is conserved, as superbly illustrated by the
352 evo-devo field.

353

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365

366

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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.

653

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

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

659

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

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

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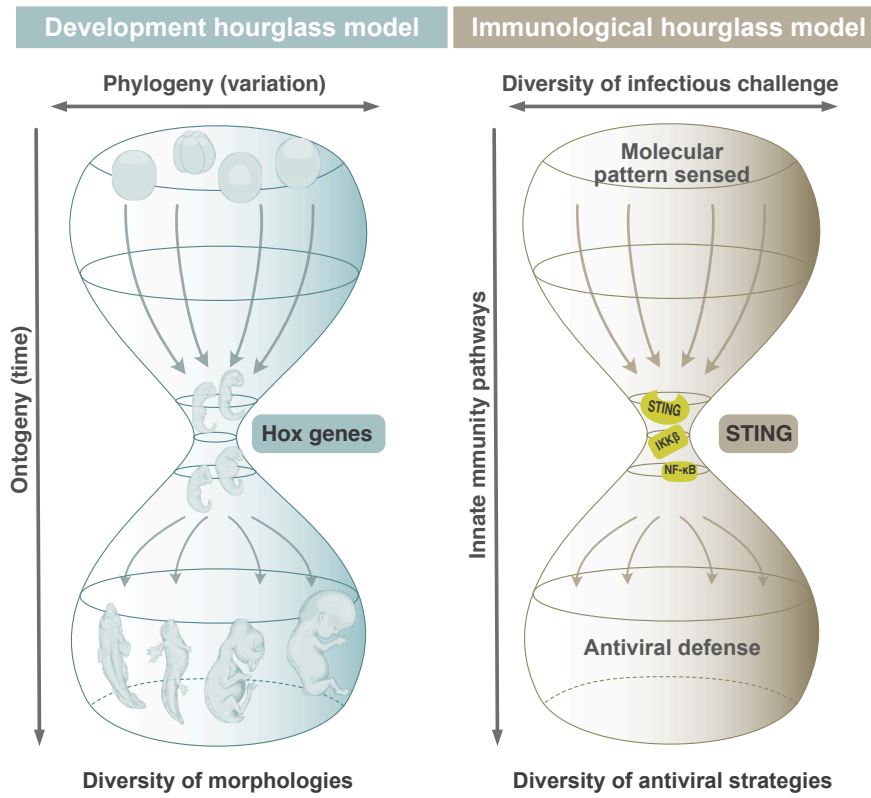


Figure 1: Parallel between evo-devo and evo-immuno. The STING pathway can be used to probe original antiviral strategies, much like homeobox genes were instrumental to decipher evolution of morphology in animals.

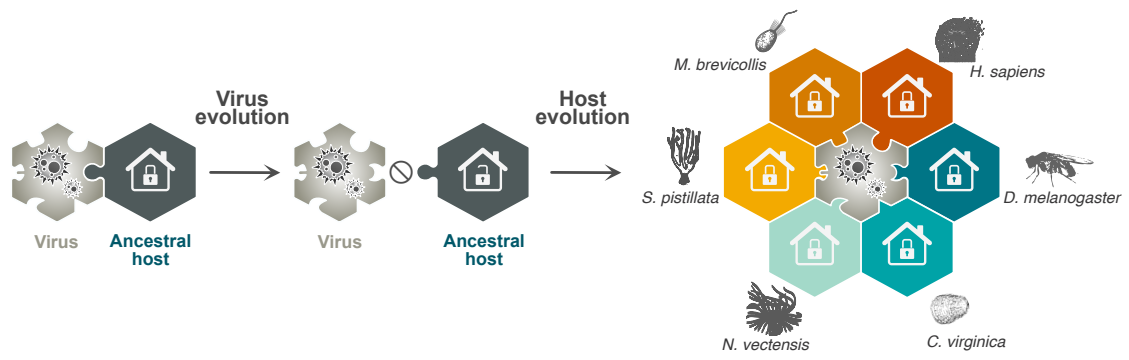


Figure 2: Evolutionary arms race between hosts and viruses. When confronted to rapidly evolving viruses that escape host restriction factors, hosts adapt by modifying the restriction factor (deep blue) or by finding another target in the virus. As a result, each animal may exhibit lineage-specific restriction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

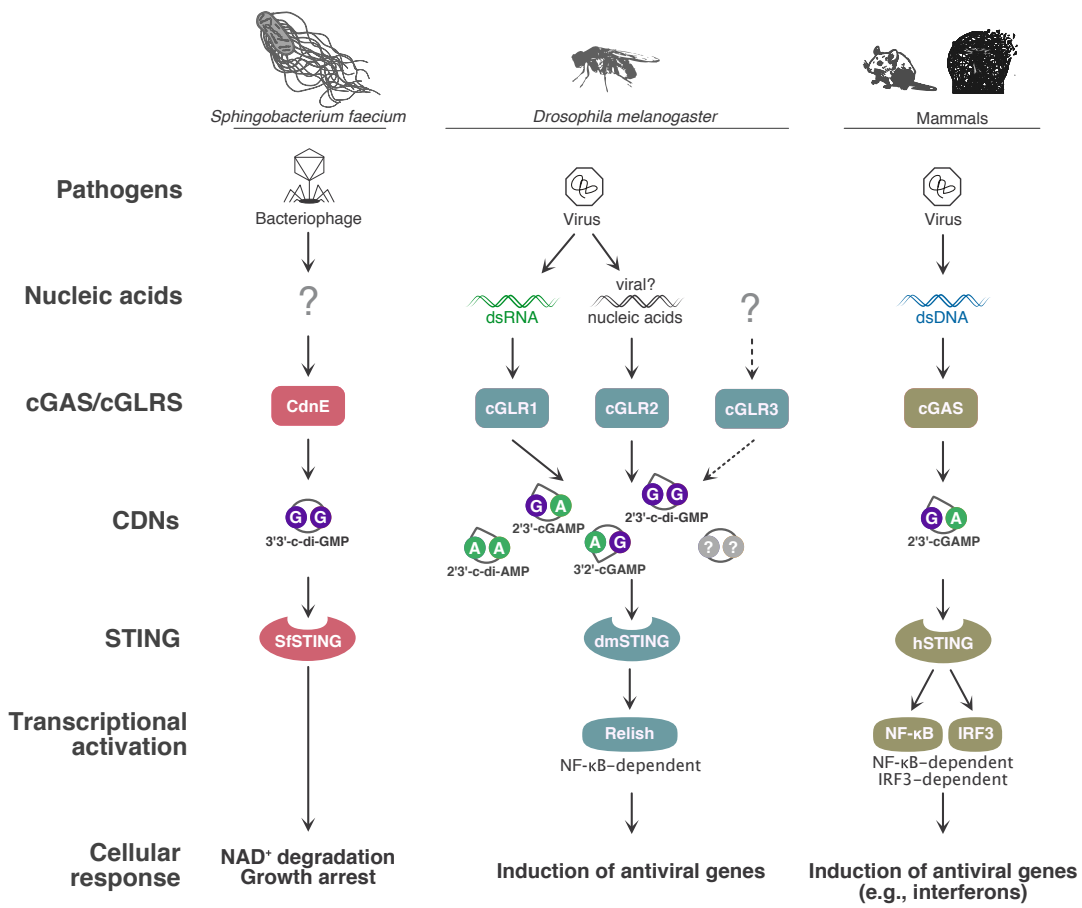


Figure 3: Evolutionary conservation of the cGLR-CDN-STING cassette. Enzymes of the CD-NTase family (cGAS, cGLRs, CdnE), the cyclic oligonucleotides they produce and STING related molecules participate in the control of phage or viral infections in bacteria and animals, from invertebrates to mammals. 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).