patterns could destabilize benzene and lead to the opening of the ring[8,9].

Qiu and co-workers' computational modelling suggests that, in their reactions, the *bis*(nitrene) intermediates can be formed on the copper atom of the catalyst, and are primed to break apart the benzene ring. They propose that the ring-opening step occurs through fracturing of the C–C bond between the two nitrogen atoms, which is brought about by the ability of these atoms to overload a molecular orbital that is associated with this bond with electrons.

In its current form, Qiu and colleagues' reaction will be challenging to translate to the manufacturing of chemicals. Sodium azide is both explosive and acutely toxic, and the copper catalyst is used in large amounts relative to the quantities of the reactants — which could make manufacturing-scale processes uneconomical, and might produce problematically large amounts of copper waste. The potential applications for synthesis in the laboratory, however, are fascinating. The authors show that their benzene-busting protocol can work on molecules with complicated structures, providing a new approach for chemically manipulating natural products, dyes and pharmaceuticals.

More generally, the development of methods that specifically destroy benzene rings could contribute to the emerging area of molecular editing[10] — the completely selective insertion, deletion or exchange of atoms in organic molecules — and provide new tools for chemical synthesis and the discovery of compounds needed for practical applications. Future methods could build on Qiu and colleagues' findings to find ways of breaking open benzene rings without the need for specific groups to be attached, or to incorporate atoms other than nitrogen into the carbon chain of the product. If a general approach can be found, synthetic chemists might look back and wonder why they did not search for benzene's breaking point sooner.

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Immunology

Fly immune defence finds a surprising way to sting

Cara West & Neal Silverman

Aspects of how immune defence processes evolved remain mysterious. Studies of the fly *Drosophila melanogaster* reveal previously unknown details of a defence pathway with echoes of, but key differences from, a human pathway. **See p.109 & p.114**

The detection of disease-causing agents by the rapidly responding branch of immune defences called the innate immune system relies on several classes of receptor. These detect molecular patterns associated with such agents, or the cellular damage that the agents cause. In particular, the surveillance of RNA and DNA is crucial to limit the replication of infectious organisms, especially intracellular microbes. Indeed, various sensors dedicated to the detection of nucleic acids have been characterized. Slavik *et al.*[1] (page 109) and Holleufer *et al.*[2] (page 114) now describe surprising defence functions for cGAS-like receptors (cGLRs) of the fly *Drosophila melanogaster*, and reveal that this class of receptor is more diverse than was previously anticipated.

Nucleic-acid-sensing receptors typically function by detecting nucleic acids in cellular compartments where these molecules should not reside in a healthy cell[3]. In mammals, the presence of DNA in the cytosol is a signature of infection by a DNA virus. This abnormality is detected by the enzyme cyclic GMP–AMP (cGAMP) synthase, which is also known as cGAS (Fig. 1). Double-stranded DNA binds to cGAS in a manner that is not sequence specific, and this binding triggers cGAS to make a cyclic dinucleotide molecule[4] called 2′3′-cGAMP.

STING, a transmembrane protein located on intracellular membranes, binds to 2′3′-cGAMP and then interacts with the kinase enzyme TBK1, which activates the transcription factors IRF3 and NF-κB (ref. 5). Interestingly, bacterially produced cyclic dinucleotides, such as cyclic-di-GMP, also bind to and activate mammalian STING[6]. Thus, STING serves as an indirect sensor of cytosolic DNA and a direct sensor of some bacteria, including *Listeria monocytogenes*[6].

The version of STING in *D. melanogaster*, dSTING, has been the focus of several studies, which found that it is also activated by cyclic dinucleotides and that it functions in defending against viral and intracellular bacterial infections[7–10]. Unlike mammalian STING, which responds to DNA viruses, dSTING has a crucial role in responses against RNA viruses, including the *Drosophila* C virus and Zika virus[7–10]. dSTING responds[10] to 2′3′-cGAMP, but the enzyme(s) and activation process responsible for synthesizing the activator of dSTING in fly cells was previously unknown. The *Drosophila* genome, and the genomes of insects in general, contains several genes that encode enzymes with a catalytic region called a nucleotidyltransferase domain that is similar to the catalytic domain of cGAS, but none of these enzymes contains the DNA-binding motif that is a component of cGAS.

Slavik *et al.* report that one of these cGAS-like proteins in *D. melanogaster*, called cGLR1, responds not to viral DNA but to double-stranded RNA (Fig. 1), which is a hallmark of viral infection. Interestingly, this recognition process depends on the length of the RNA, a system reminiscent of the RNA-recognition process used in mammalian innate defences[4] by the protein MDA5. Such a length-based mechanism would offer a way to avoid self-recognition of a type of short double-stranded RNA, called a small-interfering RNA, that is made during normal cellular processes.

Surprisingly, the cyclic dinucleotide that cGLR1 produces is 3′2′-cGAMP rather than 2′3′-cGAMP. The phosphate-bond positions of 3′2′-cGAMP are reversed compared with those of 2′3′-cGAMP. The authors show that this reversal arises through a switch in the order in which the cGAMP-forming nucleotides bind to the enzyme. In this system with echoes of the mammalian cGAS–STING pathway, the authors demonstrate that dSTING preferentially binds to 3′2′-cGAMP over 2′3′-cGAMP. This binding activates dSTING, which results in the activation of the NF-κB protein called Relish.

The ability to synthesize 3′2′-cGAMP seems to be unique to insects in the order Diptera, given that the authors found evidence for this biochemical activity in several species of *Drosophila*, but not in other orders of insects

examined, such as the Coleoptera (beetles), which make only 2′3′-cGAMP. This finding suggests that the generation of 3′2′-cGAMP is a comparatively recent adaptation. Genomic analysis shows that the Diptera tend to have an extended number of cGLR-encoding genes compared with the number in other insects.

Slavik *et al.* postulate that the generation of 3′2′-cGAMP is a mechanism to evade poxvirus immune nucleic-acid-cleaving (nuclease) enzymes called poxins[11], which are virus-encoded enzymes that specifically cleave 2′3′-cGAMP. The authors demonstrate that 3′2′-cGAMP is indeed resistant to cleavage by poxins.

The authors' intriguing hypothesis generates more questions. Poxins were originally discovered in DNA viruses (poxviruses), which made sense given the role of cGAS in detecting infection by such viruses. Poxins are also found in some RNA viruses that infect lepidopteran insects, which hints at a possible role for cGAMPs in defences against RNA viruses, at least in insects. Moreover, this begs the question as to what selective pressure — what challenge by a viral poxin (from either a RNA or DNA virus) — was so intense during the evolutionary history of dipterans to select for this new (3′2′-cGAMP) version of cGAMP. And, of course, this also raises the question of whether 3′2′-cGAMP-specific nucleases evolved in viruses to enable effective infection of dipterans.

Holleufer *et al.* took a genetic approach to study cGLR1 and a related protein termed cGLR2. Expression of either protein was sufficient to activate dSTING in adult flies or in cells grown *in vitro*. Flies with cGLR1 mutations behaved similarly to dSTING mutants, with the deficiency resulting in a decrease in the induction of several dSTING-regulated genes and a boost to infection by *Drosophila* C virus (an RNA virus) and Kallithea virus (a DNA virus).

The authors also show that loss of cGLR2 alone does not have a similar outcome, but that cGLR2 has some overlapping functions with cGLR1, given that a double-mutant fly, lacking the genes *cGLR1* and *cGLR2*, is more vulnerable than a single mutant to infection by *Drosophila* C or Kallithea viruses. Moreover, by comparison with a single mutant, the double mutant had elevated levels of virus, and more-severe defects in the induction of dSTING-regulated genes. However, infections by other viruses, including the RNA virus vesicular stomatitis virus and the DNA virus IIV6, were not affected by mutations in cGLRs. Furthermore, these infections did not drive a robust dSTING response — suggesting that a yet-to-be-discovered viral mechanism (or mechanisms) inhibits 3′2′-cGAMP and dSTING.

In human embryonic kidney cells grown *in vitro* by Holleufer and colleagues, cGLR1 required double-stranded RNA to trigger human STING activity (consistent with the results of Slavik and colleagues' biochemical analyses). However, cGLR2 alone (without adding an activator such as double-stranded RNA) was sufficient to drive this STING pathway, perhaps indicating that an activating ligand molecule for cGLR2 already exists in these human cells. Although the *in vitro* biochemical activity of cGLR2 was not determined experimentally using purified protein, in human cells cGLR2 made an almost equal mix of 2′3′- and 3′2′-cGAMP, consistent with the analysis by Slavik and colleagues of other dipteran cGLRs.

These two papers make important contributions to our understanding of the cGAS family as cytosolic immune sensors of nucleic acids, broadening our understanding to include both RNA and DNA ligands for these enzymes, and explaining previous findings linking dSTING to defences against RNA viruses. Furthermore, these results raise intriguing questions about the evolutionary history of the STING component of innate immune defences.

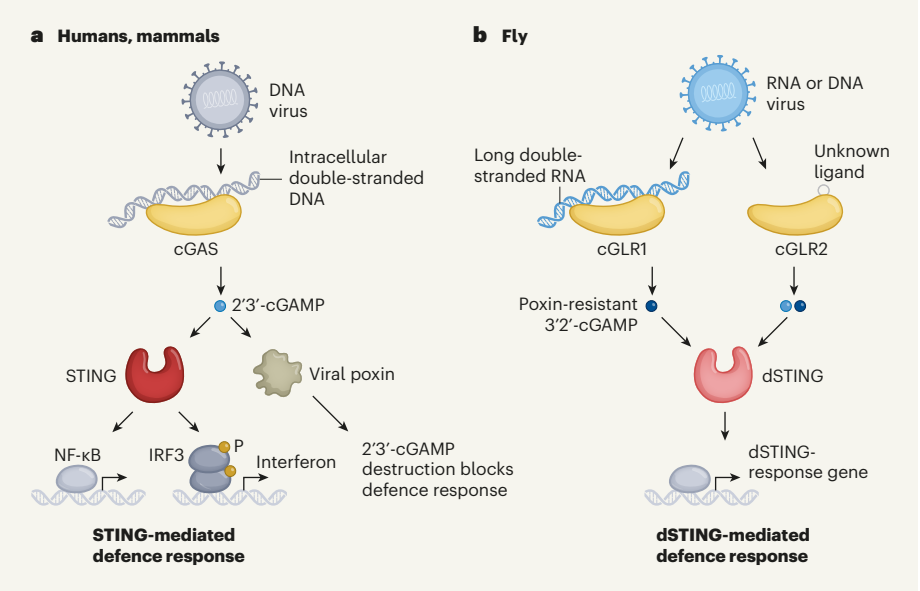

Figure 1 | The evolution of antiviral defence pathways. a, Mammals, including humans, sense signs of viral infection using the cGAS–STING pathway. Double-stranded DNA is not normally present in the cytosol of mammalian cells. If the enzyme cGAS senses it there, cGAS produces the molecule 2′3′-cGAMP. This activates the protein STING to trigger an antiviral defence response in which the transcription factors NF-κB and IRF3 (modified by the addition of a phosphate (P) group), drive expression of molecules such as interferon. Viruses can block this defence by making poxin protein, which targets 2′3′-cGAMP. **b**, Two studies shed light on the cGAS-like receptors (cGLRs) of the fly *Drosophila melanogaster*. cGLR1 responds to long double-stranded RNA produced by RNA viruses, and possibly by DNA viruses (it is assumed, but not determined, that a long double-stranded RNA made by a DNA virus can trigger cGLR1). The binding of cGLR1 to double-stranded RNA triggers the production of 3′2′-cGAMP (which has a slightly different structure to that of 2′3′-cGAMP). Slavik *et al.*[1] report that 3′2′-cGAMP is resistant to poxin-mediated cleavage. When fly dSTING binds to 3′2′-cGAMP, this triggers a defence response driven by NF-κB. Holleufer *et al.*[2] characterized the antiviral role of cGLR2, too, and report that cGLR2 can produce 3′2′-cGAMP as well as 2′3′-cGAMP, although the viral cue sensed by cGLR2 remains unidentified.

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