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RESEARCH HIGHLIGHT

Building unique bonds to fight misplaced DNA

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A cyclic dinucleotide comprised of GMP and AMP was previously shown to be a key intermediate during activation of innate immune responses to cytosolic DNA. A report by Patel and Tuschl groups published in *Cell* reveals the structure of the enzyme involved in the synthesis of this second messenger and identifies this cyclic dinucleotide as a unique compound in metazoan cell signaling.

For more than 100 years it has been known that DNA stimulates immune responses [1]. Hence, when DNA reaches the cytoplasmic compartment in a cell, no matter originating from an infectious agent like viruses or from the damaged nucleus or mitochondria, it is recognized as a sign of danger. DNA can provoke severe consequences as it can be seen from aberrant recognition of lost DNA in autoimmune conditions such as systemic lupus erythematous and Sjogren's syndrome. To perceive such a dreadful insult, several DNA-sensing proteins are present in mammalian cells. Some of these DNA sensors activate a cytoplasmic protein called stimulator of interferon (IFN) genes (STING). STING then turns on a series of protein kinases, culminating in the production of type I IFNs and other cytokines that participate in host immune responses [2]. Gaining details about the structures and the mechanisms associated with such cellular responses has been a matter of great interest in the immunology field and may bear relevance for both infectious and autoimmune conditions.

It was recently demonstrated that STING activation by DNA is mediated by a cyclic dinucleotide comprised of GMP and AMP, called cGAMP. Hence, upon infection with DNA viruses or delivery of DNA into the cytoplasm of some immune cells, cGAMP levels build up, and the dinucleotide binds directly to STING, leading to type I IFN production through activation of IRF3 via TBK1 [3]. Therefore, cGAMP acts as a second messenger during DNA-triggered innate immune response. It was also shown that cGAMP synthesis relies on the activity of the enzyme cyclic GMP-AMP synthase (cGAS), which belongs to the nucleotidyltransferase family [4]. cGAS, therefore, acts as a cytoplasmic DNA sensor that generates the second messenger cGAMP, essential for activating STING-mediated type I IFN production.

Cyclic dinucleotides are well-known bacterial intracellular signal transducers, and cyclic di-GMP (c-di-GMP) has been acknowledged as a universal bacterial second messenger [5]. The structural and biochemical analysis of the bacterial enzymes responsible for the synthesis of this second messenger suggested that c-di-GMP is formed from two molecules of GTP via a two-step reaction that generates a 3′-5′-phosphodiester linkage between the two GMP nucleotides [6]. Taking the bacterial synthesis as a model and based on the fact that chemically synthesized cGAMP with the 3′-5′-phosphodiester linkage stimulates STING-dependent type I IFN production in mammalian cells [3], one would assume that cGAS-derived cGAMP likely contains the same phosphodiester linkage. However, in an outstanding paper published by *Cell*, Gao *et al*. [7] challenged this view. Combining structural, chemical, biochemical and biological techniques, they definitely establish that cGAMP contains a 2′-5′ linkage, position this second messenger as the first 2′-5′ linkage-containing metazoan second messenger ever described, and distinguish it from the bacterial cyclic dinucleotides. The previous study had concluded that the form of cGAMP generated in mammalian cells was a 3′-5′-phosphodiester nucleotide. In this study, however, Gao *et al*. identify cGAMP as actually cyclic [G(2′,5′)pA(3′,5′)p] cGAMP. This form is unique to metazoans. The bacterial form is therefore subtly different and is less potent as an activator of STING [3].

As a first approach for understanding the mechanisms involved in cGAMP synthesis after DNA recognition, the authors compared the structure of the crystalized cGAS in its free state with the structure of the enzyme complexed with double-stranded DNA (dsDNA). dsDNA interaction with the enzyme led to pronounced conformational changes on the protein, allowing cGAS to adopt a catalytically competent conformation, a feature considered to be essential for a cytosolic DNA sensor. Comparison of the structures of the dsDNA-bound cGAS complexed with GTP, or with GMP + ATP or with GTP + ATP suggested that one of the phosphodiester linkages in the dinucleotide produced by the reaction was of the 2′-5′ nature, in contrast to the previously assumed 3′-5′ conformation. This unexpected result was supported by biochemical analysis and confirmed after comparison of the purified cGAS-derived product with chemically synthesized dinucleotide standards.

The authors have also provided evidence suggesting that cyclization occurs

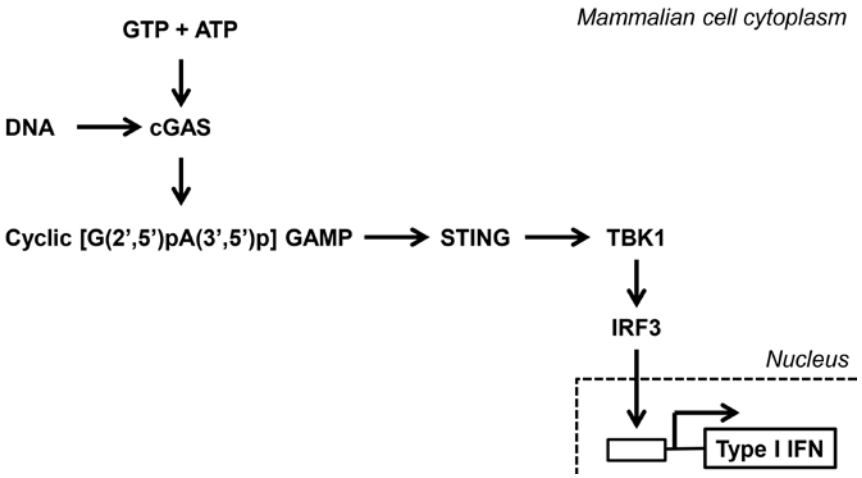

Figure 1 The study by Gao *et al*. shows that, upon DNA recognition in cytoplasm, cGAS suffers a conformational shift that allows it to convert GTP and ATP nucleotides into the cyclic compound cGAMP, containing the [G(2′,5′)pA(3′,5′)p] linkages. The 2′-5′ linkage is a unique feature of metazoan cyclic dinucleotides, as bacterial ones described so far present exclusively 3′-5′ phosphodiester bonds. cGAMP subsequently binds to STING, leading to TBK1-mediated IRF3 activation and robust type I IFN production.

in a stepwise manner and showed that a pair of divalent cations is necessary for phosphodiester bond formation. Finally, the use of functional mutants of the dsDNA-binding site or of the catalytic pocket of cGAS reinforced the conclusions gained from the structural analysis, confirming the importance of complex formation between cGAS and dsDNA and of the nucleotide-interacting residues in the catalytic pocket for activity of cGAS and consequent STING-mediated type I IFN production.

The great amount of data presented by Gao *et al*. provide detailed information regarding the synthesis of cGAMP by cGAS, and valuable knowledge for understanding the control of cellular responses to cytosolic DNA. Although 2′-5′ bonds were previously shown to occur in mammalian biochemical reactions during the polymerization of ATP into linear oligoadenilate by the dsRNA sensor oligoadenylate synthetase 1 (OAS1) [8], this is the first documented case of such a linkage in dinucleotides. This kind of phosphodiester bond is uncommon, and few nucleases are reported to be able to hydrolyze 2′-5′ linkages [9]. This might promote a greater stability of the second messenger in cells and consequently enable effective and, maybe, long-lasting signal transduction. This unique structure establishes cGAMP as a founding member of a potentially broader class of metazoan second messengers. Importantly, the fact that the second messenger and the enzymes involved in dinucleotide synthesis in bacterial systems present some structural distinctions from the ones found in metazoan cells points to features that may be explored for selectively targeting the prokaryotic or the metazoan pathway. In addition, although it was not clearly demonstrated that cGAS and cGAMP directly impact in autoimmune responses, the structural and biochemical information provided by Gao *et al*. may bear relevance for the development of small molecule inhibitors with therapeutic potentials in such conditions.

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References

1 O'Neill LA. *Science* 2013; **339**:763-764.
2 Paludan SR, Bowie AG. *Immunity* 2013; **38**:870-880.
3 Wu J, Sun L, Chen X, *et al*. *Science* 2013; **339**:826-830.
4 Sun L, Wu J, Du F, *et al*. *Science* 2013; **339**:786-791.
5 Romling U, Galperin MY, Gomelsky M. *Microbiol Mol Biol Rev* 2013; **77**:1-52.
6 Krasteva PV, Giglio KM, Sondermann H. *Protein Sci* 2012; **21**:929-948.
7 Gao P, Ascano M, Wu Y, *et al*. *Cell* 2013; **153**:1094-1107.
8 Donovan J, Dufner M, Korennykh A. *Proc Natl Acad Sci USA* 2013; **110**:1652-1657.
9 Kubota K, Nakahara K, Ohtsuka T, *et al*. *J Biol Chem* 2004; **279**:37832-37841.