IMMUNOLOGY

Sensing the Dark Side of DNA

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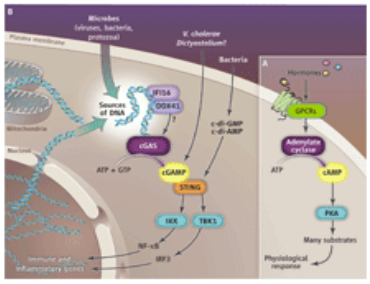
To immunologists, DNA has always had a dark side. Long before it was shown to be the genetic material, it was known to stimulate immune responses (*1*). When DNA is in the wrong place, it is a sign of danger. The danger can be in the form of infection where microbial DNA is sensed, or cellular damage that leaks DNA into the cytoplasm from the nucleus or mitochondria. In the latter scenario, DNA can cause havoc, provoking autoimmune conditions such as systemic lupus erythematosus. On pages 826 and 786 of this issue, Wu *et al.* (*2*) and Sun *et al.* (*3*), show that an enzyme called cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) detects cytoplasmic DNA and triggers a signaling system never before observed in metazoans, to galvanize host defense, inflammatory, and autoimmune responses.

There are several candidate DNA sensors in mammalian cells [including DNA-dependent activator of interferon regulatory factors (DAI), DEAD box polypeptide 41 (DDX41), and interferon inducible protein 16 (IFI16)] (*4*), all of which activate a cytoplasmic protein called stimulator of interferon genes (STING). STING then turns on two protein kinases called IκB kinase (IKK) and TANK binding kinase 1 (TBK1), which in turn, respectively, activate the transcription factors nuclear factor κB (NF-κB) and interferon regulatory factor 3 (IRF3). Both signaling cascades lead to the production of type I interferons and other cytokines that participate in host immune responses. However, the precise mechanism of STING activation has not been clear, nor the relative importance of the possible DNA sensors.

Wu *et al.* and Sun *et al.* searched for a cytosolic sensor of DNA through an in vitro assay based on two mammalian cell lines—one that was screened for a sensor(s), and one that acted as a reporter cell line to detect the sensor(s). Factors in the cytoplasm of the screened cells gained access to the cytoplasm of reporter cells that were permeabilized. Activation of the transcription factor IRF3 in the reporter cells served as the readout for activation of the STING pathway. In this assay, exposure of the screened cells to multiple types of DNA resulted in cytoplasm that could activate the STING pathway in the reporter cells. Through biochemical purification, a factor that activates STING was identified as the cyclic dinucleotide cGAMP. This is intriguing because two other bacterial molecules, cyclic diadenylate monophosphate (c-di-AMP) and cyclic diguanylate monophosphate (c-di-GMP), also bind to STING and induce the production of type I interferons (*5*, *6*). It is also interesting that cGAMP acts as a signaling molecule in the bacterium *Vibrio cholerae* (to control motility) (*7*). Wu *et al.* report that treating the screened cell line with chemically synthesized cGAMP at concentrations as low as 10 nM stimulated the production of interferon-β—an effect much more potent than that of c-di-GMP or c-di-AMP. Exposure of the screened cells to herpes simplex virus I or vaccinia virus also caused an increase in cytoplasmic cGAMP concentration. Wu *et al.* also show that STING binds to cGAMP directly. Whereas c-di-GMP produced by bacteria [and by the protozoan *Dictyostelium* (*8*)] acts as a pathogen-associated molecular pattern (PAMP) molecule that activates STING, cGAMP could be described as a danger-associated molecular pattern (DAMP), although the term "second messenger" is more biochemically correct given that it resembles the well-known second messenger signaling molecule cyclic adenosine monophosphate (cAMP).



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DNA sensor.

(**A**) A well-characterized signaling pathway involves adenylate cyclase, which is activated by many hormones via G protein–coupled receptors (GPCRs) at the cell surface. Adenylate cyclase produces the second messenger molecule cAMP, which activates protein kinase A (PKA) and many cellular processes. (**B**) DNA from diverse microbes is sensed in the cytosol of infected cells as a danger signal. The cyclase cGAS binds this DNA, becomes catalytically active, and generates cGAMP as a second messenger. cGAMP binds to STING, which activates two signaling pathways that increase the expression of immune and inflammatory genes, thereby promoting host defense. The same process is likely to sense host DNA that leaks out of mitochondria or the nucleus in damaged cells, acting as a danger signal. Certain microbes make c-di-GMP or c-di-AMP, which activate STING; other microbes (and protozoa) can also synthesize cGAMP. How other DNA sensors fit into this process is unclear.

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To identify the enzyme generating cGAMP, Wu *et al.* and Sun *et al.* carried out three independent routes of purification of cytoplasm, each consisting of four steps of chromatography. Many proteins copurified with cGAS activity, but only three copurified in all three routes. One of the three is a member of the nucleotidyltransferase family, which includes adenylate cyclase, the enzyme that generates cAMP. This is especially interesting because cGAS would be predicted to be a cyclase on the basis of its amino acid sequence. The expression of endogenous cGAS was high in the screened cell line of the assay and in macrophages (immune cells that are critical for innate immunity) but very low in a cell line that does not contain an endogenous STING pathway. Among the many experiments carried out in both studies, the ectopic expression of cGAS and STING in the latter cell line fully restored responsiveness to DNA—an effect several orders of magnitude greater than that achieved by the ectopic expression of other DNA sensors such as DAI, IFI16, and DDX41. In vitro and in cells, DNA interacted directly with cGAS.

Wu *et al.* and Sun *et al.* provide compelling new insights into how DNA is sensed in the cytoplasm of mammalian cells. DNA binds to the enzyme cGAS, which catalyzes the production of the second messenger molecule cGAMP. This molecule in turn binds to STING, which triggers two different signaling cascades that launch the expression of host defense and inflammatory proteins (see the figure). Moreover, some bacteria appear to bypass cGAS by producing dicyclic nucleotides that bind to STING directly. The discovery of cGAS means that any microbe with DNA that stimulates gene expression by the transcription factors NF-κB and IRF3 will also signal via a cyclic dinucleotide, this time made by the host cell via cGAS. The pathway is also likely to be important for the sensing of self DNA, which can lead to autoimmunity.

What role does cGAS play relative to the other DNA sensors? This is not yet clear, and it is possible that cell type specificity will be found. Because cGAS has catalytic activity, it is possible that a small-molecule inhibitor could have therapeutic potential for autoimmune diseases. Whether that would leave the patient vulnerable to infection would need to be evaluated.

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