Beyond sensing DNA: A role for cGAS in the detection of extracellular cyclic di-nucleotides

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Running title: cGAS detects extracellular CDNs

Abstract

Cyclic GMP-AMP synthase (cGAS) is best known as an innate immune receptor that detects pathogen DNA in the cytosol. In this issue of EMBO Reports, Kaufmann and colleagues [1] report that cGAS has an additional role in innate immunity: It can also bind cyclic di-nucleotides (CDNs), signalling molecules produced by bacteria. The authors show that when extracellular CDNs are taken up by endocytosis, they bind to cGAS, causing it to form a complex with the CDN receptor STING (STimulator of INterferon Genes), thereby enhancing its activation. As cGAS is dispensable for the detection of intracellular CDNs, this work exemplifies how the localisation of pathogen-associated molecular patterns (PAMPs) influences innate immune signalling.

Many bacteria produce CDNs, such as cyclic di-AMP or cyclic di-GMP, as signalling molecules. These bacterial messengers can also be detected by the innate immune system, and thus act as PAMPs during infection. When CDNs gain access to the cytosol of human or mouse cells, e.g. during infection with intracellular bacteria, they are detected by STimulator of INterferon Genes (STING) which triggers the expression of type I interferons. CDN binding to STING promotes its translocation from the endoplasmic reticulum (ER) to ER-Golgi intermediate compartments (ERGICs) where STING activates the TANK binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3) [2], which finally results in the transcription of interferon-β and other cytokines and chemokines (Fig. 1). STING is not only a receptor for CDNs, but also an adaptor in the signalling pathway following the detection of cytosolic DNA, as the cytosolic DNA receptor cyclic GMP-AMP synthase (cGAS) produces a mammalian cyclic di-nucleotide, cyclic GMP-AMP (cGAMP), that also activates STING [2].

In this issue of EMBO Reports, Kaufmann and colleagues report that cGAS has an additional function in innate immunity, in that it can promote the activation of STING by extracellular bacterial CDNs [1]. The authors show that

human and murine macrophages can take up CDNs by clathrin-dependent endocytosis, and respond to these PAMPs by producing interferon-β. The response to extracellular CDNs required 10- to 100-fold higher CDN concentrations than those used for direct delivery into the cytosol by digitonin-mediated membrane permeabilisation, which may explain why a response to extracellular CDNs has not been observed previously. Using mouse bone marrow-derived macrophages (mBMDMs) or human THP-1 monocytes lacking cGAS, the authors demonstrate that cGAS specifically enhances the response to extracellular CDNs, but is dispensable for the detection of intracellular CDNs delivered by membrane permeabilisation. STING, on the other hand, is essential for the recognition of CDNs in both scenarios. The authors confirm that the role of cGAS in enhancing the interferon response to extracellular CDNs was not due to trace amounts of DNA or other non-specific contaminants in the synthetic CDN preparations, as the response was abolished by the addition of snake venom phosphodiesterase, which specifically degrades CDNs.

Kaufmann and colleagues [1] provide evidence that cGAS can bind CDNs directly, as determined using pull-down experiments with immobilised CDNs and *in vitro* binding assays. Notably, the binding affinity of cGAS for different CDNs correlated with the extent of interferon-β expression elicited. For instance, in human cells, cyclic di-AMP binds cGAS and stimulates interferon production more potently than cyclic di-GMP, suggesting that cGAS may be able to provide a further layer of specificity to CDN recognition. The authors find that CDN binding by cGAS induces cGAS dimerisation *in vitro*, and promotes an interaction between cGAS and STING and their co-localisation in a signalling complex in CDN-treated cells (Fig. 1).

The role of cGAS in sensing endocytosed CDNs differs from its role in the recognition of cytosolic DNA. First, despite an involvement of additional co-sensors and co-factors in DNA sensing, cGAS has been shown to be essential for the response to cytosolic DNA in many different systems, while its role in the detection of extracellular CDNs seems to be to enhance, rather than cause, the activation of STING. Second, cGAS seems to promote CDN-induced STING activation through association with STING in a signalling complex, rather than through the production of cGAMP, which then diffuses through the cytosol and binds STING during DNA sensing (Fig. 1). The detection of extracellular CDNs by cGAS probably does not involve cGAMP production as intermediate [1]. This is shown in reconstitution experiments where STING-deficient RAW264.7 cells are supplemented with wild type STING or with the R231A STING mutant, which can respond to cGAMP, but fails to bind bacterial CDNs such as cyclic di-GMP [3]. The authors observe that R231A STING cannot rescue the response to extracellular cyclic di-GMP, while the cGAMP-dependent response to transfected DNA is sustained. This suggests that the binding of phagocytosed CDNs by cGAS instead results in a different mode of STING activation *via* interaction in a signalling complex and the promotion of CDN binding by STING [1]. While the authors find that residues in the active site of cGAS are important for the detection of extracellular CDNs, it is conceivable that this is because those residues might also mediate CDN binding. Thus, the function of cGAS might be more similar to that of the CDN and DNA co-sensor DDX41, which also binds DNA and cyclic di-nucleotides and promotes interferon induction by forming a complex

with STING [4]. The question remains whether a cGAS-STING interaction could also play a role in DNA sensing, possibly aiding STING activation by making the transfer of cGAMP from cGAS to STING more efficient. Identifying which amino acid residues in cGAS and STING are important in mediating the interaction of the two proteins will be key to assess the relative contributions of cGAS catalytic activity and STING binding in the activation of STING under different circumstances.

Since the discovery of cGAS as cytosolic DNA sensor only a few years ago, its mechanism of action has been investigated in great detail. Novel functions of cGAS in different subcellular locations, including in the nucleus, have recently been discovered [5,6], and, as further demonstrated by Kaufmann and colleagues [1], cGAS function is not always restricted to DNA sensing and cGAMP production. Indeed, the way in which STING is activated by cGAS or other regulators appears to have consequences for its mode of signalling. The authors observe that after detection of extracellular CDNs, STING does not translocate to the ERGIC, but instead moves to perinuclear foci that depend on dynein-mediated transport for their formation (Fig. 1). This has parallels with other recent findings suggesting that different modes of STING activation exist, which influence its subcellular location and downstream signalling [7]. While the detection of extracellular CDNs causes the activation of TBK1 and IRF3, similar to conventional STING activation during DNA sensing, it is possible that the differential localisation of STING in dynein-dependent foci could make it susceptible to additional modes of regulation, or could induce alternative downstream effects, in addition to the interferon response. As extracellular CDNs act as a sign for bacterial, rather than viral, infection, it remains to be examined whether this mode of STING signalling is beneficial to the host during bacterial infection *in vivo*. Indeed, two other recently discovered CDN receptors, the oxidoreductase RECON and the ER membrane protein ERAdP promote the activation of the transcription factor NF-κB rather than IRF3, which results in a more pronounced anti-bacterial, rather than anti-viral response [8,9]. As STING activation can also promote NF-κB activation as well as autophagy, which has anti-bacterial effects, the downstream consequences of STING activation after the detection of extracellular CDNs remains to be examined in more detail.

A further outstanding question concerns how extracellular CDNs gain access to the cytosol after endocytosis, as CDNs are unable to pass through intact membranes, and would require either non-specific membrane leakage, pore formation or an active transport process. Similar questions arise from the observation that dendritic cells can use phagocytosis to take up extracellular DNA released from tumour cells, which also causes a cytosolic cGAS- and STING-dependent interferon response [10]. It is possible that cGAS guards the cytosolic face of the endolysosomal membrane for release of DNA or CDNs, in order to detect locally elevated concentrations of phagocytosed PAMPs.

Kaufmann and colleagues have now demonstrated the existence of a novel cGAS-dependent mode of STING activation [1]. Further studies will tell whether local extracellular CDN concentrations would be sufficient to elicit such a response during infection. It is conceivable that this could be relevant for the immune system's interaction with commensal bacteria, for instance at barrier locations such as the gut or the skin. Overall, this study provides a

further example for the multi-faceted regulation of STING signalling, beyond the core cGAS-cGAMP-STING axis, and demonstrates how STING can integrate the input from upstream regulators to orchestrate the resulting innate immune response.

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Conflict of interest statement

The author declares that she has no conflict of interest.

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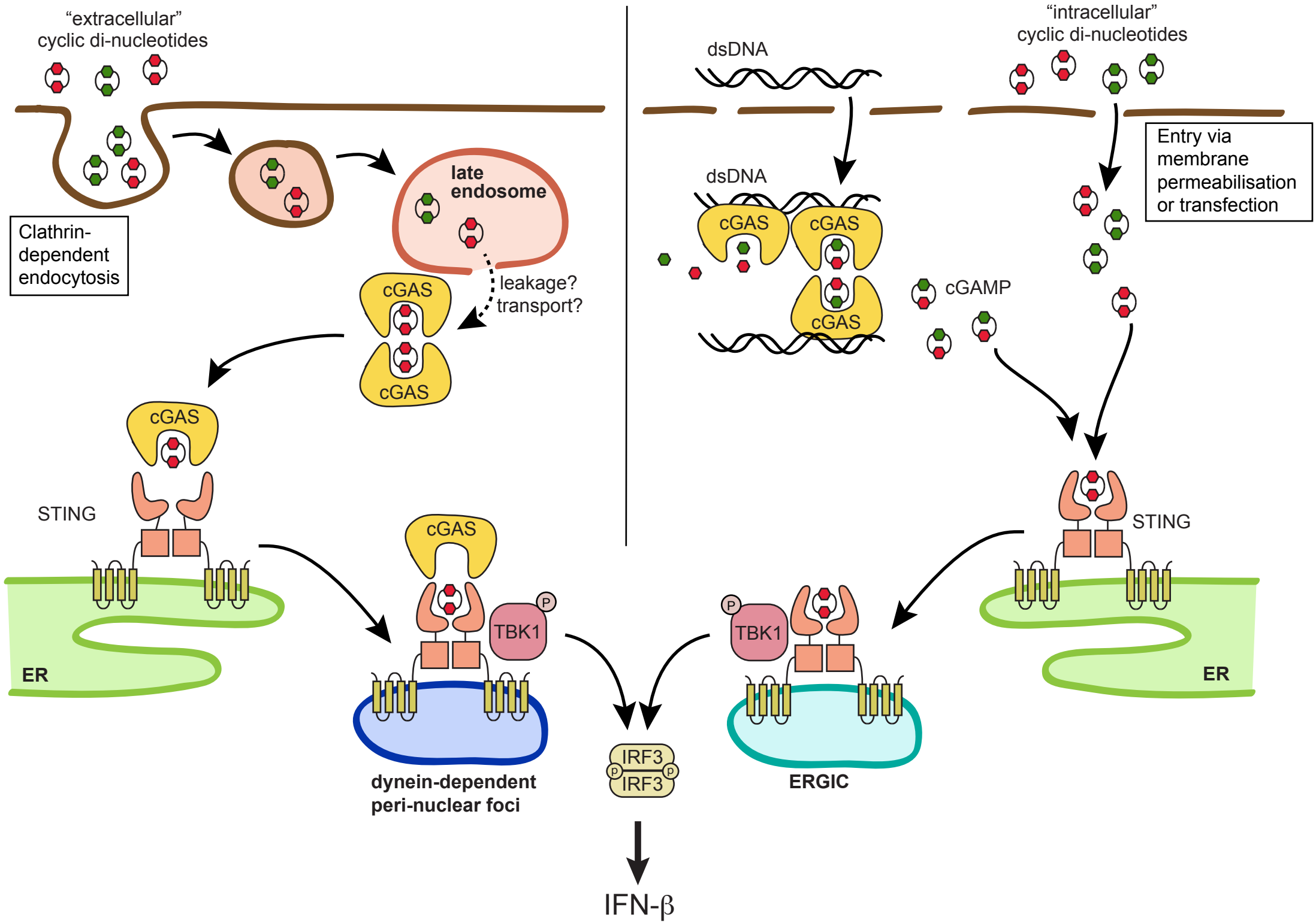
Figure Legend

Fig. 1: The innate immune response to extracellular and intracellular cyclic di-nucleotides.

Extracellular cyclic di-nucleotides (CDNs) are taken up by clathrin-mediated endocytosis (left). After export or leakage from late endosomes, CDNs can bind cyclic GMP-AMP synthase (cGAS), which then dimerises and forms a complex with the CDN receptor STimulator of INterferon Genes (STING). STING and cGAS co-localise at dunein-dependent perinuclear foci in a signalling complex also containing TANK-binding kinase (TBK1) which then activates Interferon Regulatory Factor 3 (IRF3), leading to the transcription of interferon-β (IFN-β). In contrast, when CDNs are delivered directly into the cytosol by membrane permeabilisation or transfection ("intracellular" CDNs, right), they can bind to STING in a cGAS-independent manner, causing STING translocation from the endoplasmic reticulum (ER) to the ER-Golgi-Intermediary Compartment (ERGIC) where TBK1 activation takes place. This is similar to the response to cytosolic DNA, where cGAS produces the mammalian cyclic di-nucleotide cGAMP (cyclic GMP-AMP), which also binds and activates STING.


"extracellular"
cyclic di-nucleotides
Clathrin-
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