

<https://doi.org/10.7124/bc.000B44>

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COMPARATIVE ACTIVITY OF ALTERNATIVE NUCLEIC ACID STRUCTURAL VARIANTS FOR CGAS ACTIVATION *IN VITRO*

Aim. The cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA sensor that initiates innate immune signaling by recognizing double-stranded DNA and synthesizing 2'3'-cGAMP. Although the effects of DNA length and sequence on cGAS activation are well characterized, the influence of nucleic acid structure and chemical modifications remains poorly understood, limiting the rational design of synthetic nucleic acids with controlled immunostimulatory properties [1]. This study aimed to compare the ability of canonical and structurally modified nucleic acids, including ssDNA, dsDNA, RNA, mismatch-containing DNA, and LNA-based XNA, to activate cGAS *in vitro*. **Methods.** Human and mouse cGAS catalytic domains (hcGAS_{cat} and mcGAS_{cat}) were expressed in *Escherichia coli* Rosetta (DE3) and purified as previously described [2]. Double-stranded nucleic acid variants were prepared by annealing complementary oligonucleotides. cGAS (125 nM) was incubated with nucleic acid samples (600 nM) in reaction buffer containing 20 mM Tris-HCl (pH 7.5), 25 mM NaCl, 10 mM MgCl₂, 1 μM ZnSO₄, 1 μM DTT, 1 mM ATP, and 1 mM GTP at 25 °C for 4 h. Reactions were terminated with acetonitrile, and 2'3'-cGAMP production was quantified by HPLC using an ACQUITY UPLC BEH Amide column and an external calibration curve. **Results.** Both hcGAS_{cat} and mcGAS_{cat} produced the highest amounts of 2'3'-cGAMP in the presence of the positive control ISD70. Among the tested variants, dsDNA (45 bp) significantly activated both enzymes, whereas ssDNA and RNA induced little or no activity. Bulge DNA, with mismatches introduced every 5 or 10 bp, activated both human and mouse cGAS catalytic domains to levels comparable to those induced by canonical dsDNA. Similarly, incorporation of LNA residues every 10 bp did not significantly affect cGAS activity. Overall, hcGAS_{cat} exhibited a broader activation profile than mcGAS_{cat}, responding similarly to 17 and 45 bp dsDNA variants. In contrast, mcGAS_{cat} displayed greater selectivity for longer dsDNA fragments. **Conclusions.** These findings indicate that moderate structural distortions and periodic LNA substitutions are well tolerated by both cGAS orthologs. Moreover, the broader responsiveness of hcGAS_{cat} compared with mcGAS_{cat} suggests species-specific differences in the structural requirements for cGAS activation.

Keywords: cGAS, DNA sensing, alternative nucleic acid structure, 2'3'-cGAMP.

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Received: 06.07.2026; Accepted: 08.09.2026; Published: 29.09.2026

Citation: Olkhovska A.I., Vietrov R.M., Semeniuk D.O., Senchenko N.V., Khadyrov S.L., Novosolov S.O., Velbovets A.R., Hubar O.M. (2026) Comparative activity of alternative nucleic acid structural variants for cGAS activation *in vitro*. *Biopolymers & Cell*, Special issue(42), 11. <https://doi.org/10.7124/bc.000B44>

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