The Molecular Basis of dsDNA Sensing Through the cGAS-STING Pathway

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A conserved PLPLRT/SD motif of STING mediates the recruitment and activation of TBK1

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Nucleic acids from bacteria or viruses induce potent immune responses in infected cells[1–4]. The detection of pathogen-derived nucleic acids is a central strategy by which the host senses infection and initiates protective immune responses[5,6]. Cyclic GMP-AMP synthase (cGAS) is a double-stranded DNA sensor[7,8]. It catalyses the synthesis of cyclic GMP-AMP (cGAMP)[9–12], which stimulates the induction of type I interferons through the STING–TBK1–IRF-3 signalling axis[13–15]. STING oligomerizes after binding of cGAMP, leading to the recruitment and activation of the TBK1 kinase[8,16]. The IRF-3 transcription factor is then recruited to the signalling complex and activated by TBK1[8,17–20]. Phosphorylated IRF-3 translocates to the nucleus and initiates the expression of type I interferons[21]. However, the precise mechanisms that govern activation of STING by cGAMP and subsequent activation of TBK1 by STING remain unclear. Here we show that a conserved PLPLRT/SD motif within the C-terminal tail of STING mediates the recruitment and activation of TBK1. Crystal structures of TBK1 bound to STING reveal that the PLPLRT/SD motif binds to the dimer interface of TBK1. Cell-based studies confirm that the direct interaction between TBK1 and STING is essential for induction of IFNβ after cGAMP stimulation. Moreover, we show that full-length STING oligomerizes after it binds cGAMP, and highlight this as an essential step in the activation of STING-mediated signalling. These findings provide a structural basis for the development of STING agonists and antagonists for the treatment of cancer and autoimmune disorders.

To test whether STING binds TBK1 directly, we expressed several biotin-labelled STING truncation mutants (Extended Data Fig. 2a, b) and conducted TBK1 binding studies by surface plasmon resonance (SPR) analysis (Extended Data Table 1). We observed that unphosphorylated STING binds TBK1 directly with relatively low affinity (Extended Data Fig. 2c, d). The binding affinity does not change notably in the presence of cGAMP (Extended Data Fig. 2e, f). However, after phosphorylation, STING binds to TBK1 at almost 20 times higher affinity (Fig. 1e, Extended Data Fig. 2g). The binding affinity does not change notably in the absence of cGAMP (Extended Data Fig. 2h, i). In addition, phosphorylation of TBK1 does not affect its binding affinity with STING (Extended Data Fig. 2j). To map the TBK1-binding site, we conducted binding studies with several truncated forms of STING (Extended Data Fig. 2a, b). We observed that truncation of the C-terminal 9 or 37 residues of STING abolish TBK1 binding (Fig. 1e, f, Extended Data Fig. 2g, k). By contrast, peptides containing the C-terminal 37 or 14 residues of STING are fully capable of binding TBK1 (Fig. 1f, Extended Data Fig. 2k, l).

To determine the exact STING residues that contribute to binding of TBK1, we generated several STING mutants (Fig. 2a, Extended Data Fig. 3a) and conducted binding studies (Fig. 2a, Extended Data Fig. 3b–l). These studies showed that the Leu374Ala mutation disrupts binding of TBK1 (Fig. 2a, Extended Data Fig. 3f). However, this mutation does not affect binding of IRF-3 (Extended Data Fig. 3m). In addition, the Arg375Ala and Phe378Ala mutations reduce the binding affinity

cGAMP binding induces the oligomerization of full-length hSTING