

Review

Mitotic inactivation of the cGAS–MITA/STING pathways

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The cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS)–mediator of interferon response factor 3 activation/stimulator of interferon genes (MITA/STING) axis has emerged as a major pathway, which senses microbial or mislocated cellular DNA in the cytosol to trigger innate immune responses. cGAS senses cytosolic DNA without a preference of self- or nonself-DNA. How the cGAS–MITA/STING axis is inactivated upon nuclear envelope breakdown (NEBD) at mitotic entry in vertebrate cells to avoid self-DNA sensing remains unclear until very recently. In this review, we summarize the recent advances on how cGAS responds to chromosomes upon NEBD and the mechanisms involved in the inactivation of the cGAS–MITA/STING pathways in mitosis.

Keywords: cGAS, MITA, STING, mitosis, innate immune response, DNA

Introduction

The innate immune response is the first line of host defense, which is initiated by sensing of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). It has been shown that sensing of exogenous DNA in the cytosol by the host cell activates an interferon response factor 3 (IRF3)-dependent innate immune response (Stetson and Medzhitov, 2006). Microbial DNA represents one class of PAMPs, which is sensed by host cells during infection (Ablasser and Chen, 2019; Hu and Shu, 2020). Sensing and elimination of foreign DNA while insulating cellular self-DNA from host defense are conserved functions for most organisms. Compartmentalization and regulation of DNA turnover can prevent innate sensing of self-DNA in most cases, as self-DNA is sequestered from the cytosol by nuclear envelope or other organelle membranes, and mislocated self-DNA is quickly degraded by cytosolic deoxyribo-nucleases (DNases) (Ablasser and Chen, 2019). However, the nuclear envelope disassembles at mitotic entry in vertebrate cells and the chromatin is exposed to cytosol in mitosis, which

raises a question on how mitotic cells restrain innate immune responses to self-DNA.

The cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) has emerged as a major cytosolic DNA sensor, which recognizes cytosolic DNA and utilizes GTP and ATP as substrates to synthesize the second messenger cGAMP (Sun et al., 2013). cGAMP binds to the endoplasmic reticulum (ER)-located adaptor protein MITA [mediator of IRF3 activation, also called stimulator of interferon genes (STING)], leading to activation of downstream transcription factors and transcriptional induction of type I interferon (IFN) and inflammatory genes (Ishikawa and Barber, 2008; Zhong et al., 2008; Luo and Shu, 2018; Hu and Shu, 2020). cGAS recognizes double-strand DNA (dsDNA) in a sequence-independent manner and is unable to distinguish self- or non-self-DNA (Civril et al., 2013; Hu and Shu, 2018). Activation of cGAS by aberrant and/or mislocated self-DNA, such as mitochondrial DNA (mtDNA) or genomic DNA, is associated with the pathogenesis of various autoimmune diseases (Gluck et al., 2017; Yang et al., 2017; Hu and Shu, 2020). Studies over the past decade reveal that the cGAS–MITA/STING axis is tightly regulated in a spatial and temporal manner to ensure efficient innate immune responses to microbial DNA at the early phase of infection and its timely turning off at the late phase (Hu and Shu, 2018, 2020; Luo and Shu, 2018; Hu et al., 2019; Yang and Shu, 2020). However, how cGAS responds to mitotic

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chromosomes upon nuclear envelope breakdown (NEBD) and how the cGAS–MITA/STING axis is regulated during mitosis remained enigmatic until recently. Several recent studies suggest that cGAS translocates to chromosomes upon NEBD and the cGAS–MITA/STING axis is inactivated by multiple mechanisms in mitotic cells (Zierhut et al., 2019; Uhlorn et al., 2020; Zhong et al., 2020; Li et al., 2021a). In this review, we summarize these recent advances and provide a perspective view on how the cGAS–MITA/STING axis is inert for innate immune responses to DNA.

Overview of the cGAS–MITA/STING pathways

cGAS is activated by binding to dsDNA in a length-dependent manner (Luecke et al., 2017). cGAS contacts with the sugar-phosphate backbone of DNA and forms a minimal 2:2 complex (Civril et al., 2013; Andreeva et al., 2017). Upon binding to dsDNA, cGAS undergoes conformational changes and forms a droplet complex with DNA through liquid–liquid separation (Du and Chen, 2018). Activated cGAS utilizes ATP and GTP as substrates to synthesize the second messenger cGAMP, which binds to the ER-located adaptor MITA/STING, and causes a 180° rotation of its cGAMP-binding domain (Shang et al., 2019). This conformational change enables two adjacent MITA/STING dimers to form a tetramer, which serves as a signaling platform to recruit and activate TANK-binding kinase 1 (TBK1). It has been shown that recruitment of TBK1 occurs after translocation of MITA/STING from the ER to Golgi apparatus (Ishikawa and Barber, 2008). A conserved PLPLRT/SD motif within the C-terminal tail of MITA/STING is needed for its binding to the dimer interface of TBK1, leading to recruitment and activation of TBK1 (Zhao et al., 2019). In turn, the TBK1 dimer transphosphorylates the consensus pLxIS motif in the C-terminus of MITA/STING (Liu et al., 2015). Phosphorylated MITA/STING then recruits IRF3 for its phosphorylation by TBK1. The phosphorylated IRF3 dimerizes and translocates into the nucleus, leading to transcriptional induction of type I IFN and other antiviral genes (Figure 1).

cGAS binds to cytosolic dsDNA without a preference of DNA sequence. Except for microbial DNA, self-DNA released into the cytosol under cellular stress conditions also activates cGAS-triggered innate immune responses (Hartlova et al., 2015; West et al., 2015; Gluck et al., 2017; Yang et al., 2017). For examples, both DNA damage and senescence cause enrichment of genomic DNA in the cytosol and induction of type I IFN (Hartlova et al., 2015; Gluck et al., 2017; Yang et al., 2017). Recently, it has been demonstrated that the vaccinia virus-related kinase 2 promotes association of mtDNA with voltage-dependent anion channel 1 (VDAC1) and formation of VDAC1 oligomeric pores upon viral infection or mitochondrial stress, leading to release of mtDNA and activation of the cGAS–MITA/STING axis (He et al., 2021).

Aberrant activation of the cGAS–MITA/STING pathways by self-DNA is associated with multiple autoimmune and autoinflammatory diseases (Hu and Shu, 2020). It has been well

established that the cGAS–MITA/STING pathways are tightly and delicately regulated by cofactors, posttranslational modifications (PTMs), posttranscriptional and other mechanisms to ensure proper activation of innate immune responses against microbial DNA (Hu et al., 2016; Liu et al., 2016, 2018b; Luo et al., 2016; Lian et al., 2018; Wei et al., 2018; Li and Shu, 2020; Hu and Shu, 2020; Sun et al., 2020).

Cellular localization of mitotic cGAS

Although it has been well established that cGAS plays important roles in sensing cytosolic DNA, the subcellular distributions of cGAS are not completely clear. cGAS was first identified as a cytosolic DNA sensor, which predominantly localized in the cytosol in THP1 and L929 cells (Sun et al., 2013). The physical separation between cGAS and self-DNA by the nuclear envelope and mitochondrial membrane is viewed as a crucial regulatory strategy to avoid self-DNA sensing and auto-immune activity. However, following studies have shown additional subcellular localizations for cGAS, including on the plasma membrane or in the nucleus at interphase (Barnett et al., 2019; Volkman et al., 2019). It has been reported that the N-terminus of cGAS is needed for its association with phosphatidylinositol 4,5-bisphosphate, which mediates its plasma membrane localization in human and mouse phagocytes (dendritic cells, monocytes, and neutrophils) (Barnett et al., 2019). The nuclear localization of cGAS is found in long-term hematopoietic stem cells (LT-HSCs), epithelial cells, and certain cancer cells (Xia et al., 2018; Volkman et al., 2019; Zierhut et al., 2019). Nuclear cGAS is tethered tightly by a salt-resistant interaction and requires intact nuclear chromatin (Volkman et al., 2019). These studies suggest that the subcellular distributions of cGAS vary in cell types.

In addition to cell types, the subcellular distributions of cGAS also change in response to cellular stress or physiological processes. It has been demonstrated that constitutive phosphorylation of cGAS at Y215 (cGAS^{Y215}) by BLK facilitates cytosolic retention of cGAS in resting cells, whereas cGAS undergoes dephosphorylation and translocation into the nucleus in response to DNA damage in PC-9 cells (Liu et al., 2018a). Moreover, it has been shown that cGAS is translocated to chromatin in migrating mammalian cells in which the nuclear–cytoplasmic barrier opens at high frequency (Raab et al., 2016).

The cellular distributions of cGAS in mitosis have been recently investigated by live cell imaging. It has been shown that cGAS is promptly translocated from the cytoplasm to chromosomes upon NEBD in several cell types (Zhong et al., 2020). Endogenous cGAS is associated with chromosomes from prophase to telophase in primary murine lung fibroblast and Raw264.7 cells (Zhong et al., 2020). The chromosomal localization of mitotic cGAS has also been observed in other cell types including MEF and HaCaT cells (Mackenzie et al., 2017; Yang et al., 2017; Zierhut et al., 2019; Uhlorn et al., 2020; Zhong et al., 2020). Collectively, these studies suggest

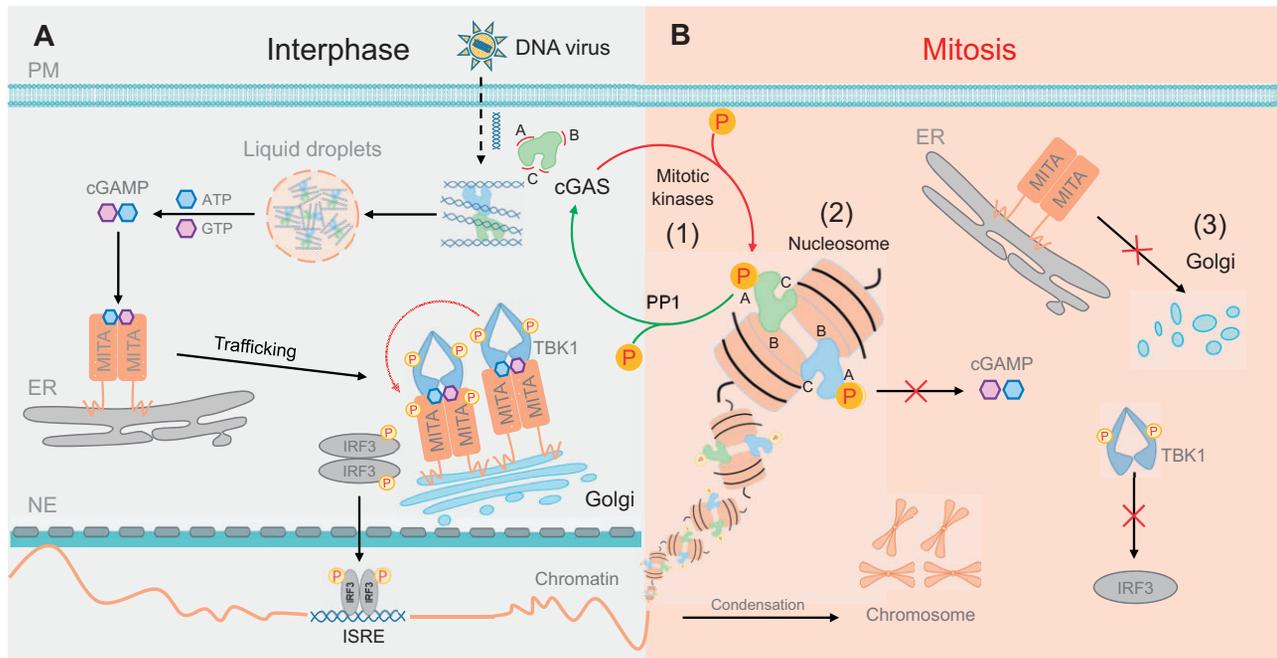


Figure 1 Reorganization and inactivation of cGAS–MITA/STING pathways during mitosis. **(A)** In interphase, cGAS senses cytosolic dsDNA with the DNA binding sites A, B, and C, resulting in the formation of dsDNA–cGAS liquid droplets in which cGAS synthesizes cGAMP with ATP and GTP. cGAMP binds to the ER-located MITA/STING, which then translocates to the Golgi apparatus. In this process, MITA/STING recruits and activates TBK1, which then transphosphorylates the adjacent MITA/STING. Phosphorylated MITA then recruits IRF3 for its phosphorylation by TBK1, leading to activation of IRF3 and eventual induction of antiviral genes. **(B)** In mitosis, (1) cGAS is translocated into the nucleus upon NEBD and phosphorylated by mitotic kinases (e.g. CDK1 and AURKB) at distinct sites, leading to inhibition of its activity; (2) at chromosomes, cGAS binds to the nucleosome core particles with sites B and C, which impairs its interaction with DNA and disrupts its dimerization; (3) mitotic Golgi vesiculation suppresses MITA/STING and IRF3 activation. TBK1 is phosphorylated in mitosis but unable to phosphorylate IRF3, probably due to loss of MITA/STING-mediated regulation.

that the subcellular localizations of cGAS are regulated by cell types, intra/extracellular environmental changes, and cell cycle progression.

Mitotic inhibition of the cGAS–MITA/STING axis

As both compartmentalization and regulation of DNA turnover are not applicable in mitosis, the cGAS–MITA/STING axis must be tightly controlled to avoid self-DNA sensing in mitotic cells. The production of cGAMP by cGAS converts DNA recognition into a chemical signal, which is the essential first step of the DNA sensing pathways (Sun et al., 2013). cGAMP is very stable and potent in inducing innate immune responses (Eglesham et al., 2019). Therefore, interfering the binding of cGAS to DNA or regulating the activity of cGAS to avoid cGAMP production would be a preferred way to avoid self-DNA-triggered innate immunity. Indeed, recent studies suggest that nucleosomal binding and phosphorylation of cGAS are critical mechanisms responsible for its inactivation in mitotic cells (Zierhut et al., 2019; Zhong et al., 2020; Li et al., 2021a). It has been reported that the downstream components of cGAS are also inactivated to prevent innate immune responses in mitotic cells (Uhlorn et al., 2020; Zhong et al., 2020).

Chromatin tethering suppresses cGAS activity

Unlike naked DNA, eukaryotic DNA is packaged into chromatin and undergoes condensation to form chromosomes during cell division. The perichromosomal layer of proteins and hypercompaction of DNA have been proposed to mitigate against cGAS binding and activation (Mackenzie et al., 2017). Indeed, both *in vitro* reconstituted chromatin and chromatin isolated from mouse NIH3T3 cells induce less cGAMP production when incubated with recombinant cGAS (Mackenzie et al., 2017).

Nucleosomes are basic structural units of a chromatin, which are composed of a segment of DNA wrapped around the histone octamer (H2A, H2B, H3, and H4) (Kouzarides, 2007). It has been proposed that histones structurally mark DNA as ‘self’, as many DNA viruses contain either naked, unchromatinized DNA or DNA that is packaged with nonhistone viral core proteins (Uhlorn et al., 2020). Recently, it has been demonstrated that the nucleosome has a higher affinity for cGAS than naked DNA, which suppresses DNA-dependent activation of cGAS (Zierhut et al., 2019). Structural analysis has demonstrated that cGAS associates with the acidic patch of H2A–H2B heterodimer through site B and interacts with DNA from the other symmetrically placed nucleosomes via site C, without the

involvement of site A (Boyer et al., 2020; Cao et al., 2020; Kujirai et al., 2020; Pathare et al., 2020). Thus, the activation of cGAS by nucleosomes can be prevented by lack of DNA bound at sites B and C. In addition, the monomeric cGAS binds to nucleosome core particles with 1:1 and 2:2 stoichiometries, in which nucleosomes can competitively disrupt cGAS dimerization (Cao et al., 2020). Mutations of R236 or R255 of human cGAS, which are critically involved in the interaction between cGAS and H2A–H2B, can rescue dsDNA-induced cGAS activation from the nucleosome-mediated inhibition (Cao et al., 2020). Although less effective, cGAS R236E and R255E mutants can produce cGAMP in mitotic cells, suggesting that the interaction between cGAS and nucleosomes is an important mechanism for its inactivation during mitosis (Li et al., 2021a). These structural and biochemical studies suggest that chromosome-bound cGAS is kept inactive via impairment of its interaction with DNA and dimerization.

Nuclear DNA and histones are modified and their modifications change during mitosis (Wang and Higgins, 2013; Yao et al., 2015). Genomic DNA derived from mitotic or asynchronized cells induces the transcription of *IFNB1* to a similar level, indicating that DNA modifications do not affect cGAS activation (Zhong et al., 2020). Whether histone modifications affect cGAS activation in interphase and mitotic cells is unknown. In addition to histones, chromatin is also consisted of other proteins as well as RNAs and undergoes condensation to form mitotic chromosomes during cell division. Whether these proteins/RNAs and the highly condensed structures of mitotic chromosomes impair the ability of cGAS to sense self-DNA remains unclear.

Barrier-to-autointegration factor (BAF) is a chromatin-binding protein, which plays important roles in nuclear assembly and chromatin organization (Sears and Roux, 2020). Two independent groups have shown that BAF inhibits self-DNA-induced activation of cGAS-triggered pathways (Guey et al., 2020; Ma et al., 2020). It has been shown that BAF dynamically outcompetes with cGAS for DNA binding upon nuclear envelope rupture, which inhibits the formation of DNA–cGAS complexes (Guey et al., 2020). Overexpression of BAF reduces cGAMP synthesis whereas BAF-deficiency enhances cGAMP production catalyzed by two nuclear tethering-defective cGAS mutants (R236A and R255A) (Guey et al., 2020). It has been demonstrated that BAF forms a dense chromatin network at the surface of anaphase chromosomes (Samwer et al., 2017). Thus, BAF may also help to restrict chromatin DNA-induced cGAS activation in anaphase and telophase. Additionally, it has been reported that loss of STAG2, an important component of the cohesin complex that holds sister chromatids together, results in spontaneous genomic DNA damage, recognition of cytoplasmic microchromatin, and activation of the cGAS–MITA/STING pathways (Ding et al., 2018). It is possible that STAG2 and other proteins that maintain the intact structures of chromatin may contribute to inhibition of cGAS-mediated self-DNA sensing by maintaining DNA chromatinization. Furthermore, it has been shown that cia-cGAS (circular RNA antagonist for cGAS)

can bind to nuclear cGAS and inhibits its enzymatic activity in LT-HSCs (Xia et al., 2018). Although cia-cGAS only exists in LT-HSCs, whether other circular RNAs are involved in regulation of the cGAS–MITA/STING pathways during mitosis remains to be investigated. Collectively, the recent studies suggest that chromatin tethering impairs cGAS activation mostly through impairing the interaction of cGAS with DNA and disrupting cGAS dimerization by nucleosomes.

Inactivation of cGAS by phosphorylation during mitosis

Although less effective, nucleosomes, chromatin, and chromosome-derived micronuclei can also activate cGAS in interphase (Mackenzie et al., 2017; Zierhut et al., 2019), suggesting that additional inhibitory mechanisms exist to avoid self-DNA sensing during mitosis. Numerous studies have demonstrated that PTMs, including phosphorylation, ubiquitination, SUMOylation, glutamylation, methylation, and acetylation, play important roles in regulating the activity of cGAS in interphase (Seo et al., 2015; Hu et al., 2016; Xia et al., 2016; Chen and Chen, 2019; Song et al., 2020; Sun et al., 2020; Ma et al., 2021). However, whether these PTMs are involved in regulation of the cGAS–MITA/STING axis during mitosis is unclear until recently. Two recent studies have demonstrated that phosphorylation of cGAS is critical for its inactivation in mitotic cells (Zhong et al., 2020; Li et al., 2021a).

The major mitotic kinase CDK1–cyclin B1 complex is the first one reported to mediate cGAS phosphorylation and inactivation in mitosis. It has been shown that human cGAS^{S305} (corresponding to murine cGAS^{S291}), a residue highly conserved among mammals, is minimally phosphorylated in interphase but highly phosphorylated in mitotic cells in human HT1080, mouse L929, and Raw264.7 cells (Zhong et al., 2020). *In vitro* experiments indicate that purified CDK1–cyclin B complex can phosphorylate cGAS^{S305}, while the CDK1 inhibitor RO-3306 abolishes this phosphorylation (Zhong et al., 2020). Mutation of cGAS^{S305} to aspartic acid (D), which mimics phosphorylation of cGAS, abolishes its ability to activate the IFN- β promoter. Overexpression of CDK1 and cyclin B1 abolishes cGAS-mediated activation of the IFN- β promoter, while CDK1 inhibition increases the production of cGAMP in mitotic cells (Zhong et al., 2020). These findings suggest that CDK1–cyclin B1 kinase complex is responsible for cGAS phosphorylation and inactivation in mitosis. In addition, it has been shown that the phosphatase PP1 dephosphorylates cGAS^{S305} upon mitotic exit, which conditions cGAS for activation by DNA in interphase (Zhong et al., 2020).

Recently, another study shows that phosphorylation of several serine (S13, S37, S64, S116, and S143) and threonine (T69 and T91) residues in the N-terminus of cGAS is increased during mitosis in HeLa cells (Li et al., 2021a). Replacing all 20 N-terminal serine and threonine residues of cGAS with acidic residues (cGAS^{20DE}) to mimic phosphorylation impairs the ability of cGAS to form liquid droplets with DNA and catalyze

cGAMP synthesis (Li et al., 2021a), while cGAS^{20A} (in which all serine and threonine residues are replaced with alanine to prevent phosphorylation) produces more cGAMP than its wild-type counterpart. In addition, cGAS^{20A} restores activation of cGAS R236E or cGAS R255E mutation in mitotic cells, suggesting that chromatin tethering and phosphorylation of cGAS N-terminus are important mechanisms for suppression of cGAS activation during mitosis (Li et al., 2021a). The study further shows that the kinase AURKB contributes to phosphorylation of cGAS at S13 and S64. It is also demonstrated that the N-terminus of cGAS is critical for sensing chromatin DNA but not mtDNA (Li et al., 2021a). However, the N-terminus of cGAS is highly variable among species. The lack of conservation of the N-terminus of cGAS raises a question on how different species adapt the same strategy to avoid self-DNA sensing during evolution. In addition, mutation of 20 serine/threonine residues at the N-terminus of cGAS may dramatically disrupt its structure, which may hinder interpretation of the results. Moreover, AURKB inhibition only reduces the phosphorylation of mitotic cGAS at S13 and S64 (Li et al., 2021a), which opens a question on how the other serine/threonine residues of the N-terminus of cGAS are phosphorylated during mitosis.

In contrast to the results showing that cGAS^{S305} (murine cGAS^{S291}) is minimally phosphorylated in interphase but dramatically phosphorylated in mitotic human HT1080, mouse L929, and Raw246.7 cells (Zhong et al., 2020), Li et al. (2021a) found that cGAS^{S305} was already phosphorylated in interphase and slightly increased in mitosis in HeLa cells. Previous studies have demonstrated that while cGAS is mostly localized in the cytoplasm in most cells, it is predominantly localized in the nuclei of interphase HeLa cells (Volkman et al., 2019; Zierhut et al., 2019). It has been shown that except in mitotic cells, low level of cyclin B (but not cyclin A)–Cdk1 activity exists in interphase cells (Tachibana et al., 2008). Therefore, it is possible that nuclear cGAS is phosphorylated by CDK1–cyclin B complex at S305 to inhibit self-DNA sensing in HeLa cells. In addition, it has been reported that CDK1 and AURKB sequentially phosphorylate Sororin, in which AURKB phosphorylates Sororin only if CDK1 phosphorylates Sororin first (Nishiyama et al., 2013; Borton et al., 2016). Whether CDK1 and AURKB function in sequential to control cGAS phosphorylation and inactivation during mitosis needs further investigation.

Other mechanisms of mitotic inhibition of the cGAS–MITA/STING pathways

Since cGAMP is very stable and potent in inducing innate immune responses (Eaglesham et al., 2019), additional mechanisms may contribute to suppression of the cGAS–MITA/STING axis in mitotic cells. It has been shown that nuclear cGAS is enriched on centromeric satellite DNA and to a lesser extent on LINE elements in HeLa and U2OS cells (Gentili et al., 2019). It would be interesting to investigate whether DNA configuration and local chromatin proteins are involved in regulating cGAS

activity in mitotic cells. Several DNA-binding proteins, including ZCCHC3, G3BP1, PQBP1, and PCBP1, have been shown to be associated with and help cGAS to bind to dsDNA (Yoh et al., 2015; Lian et al., 2018; Liu et al., 2019; Liao et al., 2021). It would be interesting to investigate whether these proteins regulate cGAS activity in a cell cycle-dependent manner.

A recent study shows that cGAMP-induced phosphorylation of MITA/STING and IRF3 is inhibited in mitotic cells, suggesting that the downstream signaling pathway of cGAMP is also inactivated in mitosis (Zhong et al., 2020). It has been shown that fragmentation and dispersal of the pericentriolar Golgi complex are required for mitotic entry of mammalian cells (Sutterlin et al., 2002). Recent studies show that Golgi vesiculation impairs MITA/STING activation in response to either foreign DNA or exogenous cGAMP stimulation (Uhlorn et al., 2020), pointing to the possibility that loss of Golgi integrity contributes to inactivation of MITA/STING-mediated innate immune responses in mitosis.

In addition to innate immune responses, TBK1 also plays important roles in cell division and other signaling pathways (Liu et al., 2015; Pillai et al., 2015). It has been shown that phosphorylation of TBK1^{S172} is increased during mitosis (Pillai et al., 2015), which raises a question on why phosphorylated TBK1 does not induce IRF3 phosphorylation in mitosis and other cellular processes. In this context, it has been demonstrated that adaptor proteins, e.g. virus-induced signaling adaptor, MITA/STING, and TIR-domain-containing adapter-inducing interferon- β , specify TBK-induced IRF3 activation (Liu et al., 2015). Therefore, loss of this specification by the adaptor proteins may contribute to the inability of activated TBK1 to phosphorylate IRF3. Transcription in mitosis is generally shut down, though low-level mitotic transcription may occur (Prescott and Bender, 1962; Palozola et al., 2017). It has been shown that IRF3 phosphorylation slowly accumulates during a mitotic arrest without inducing the transcription of *IFNB1* and IFN-stimulated genes (Zierhut et al., 2019). How the transcriptional activity of phosphorylated IRF3 is suppressed in the mitotic-arrested cells needs further investigation.

Concluding remarks and perspectives

The cGAS–MITA/STING pathways are essential to innate immune responses against cytosolic DNA derived from pathogens, including DNA viruses, retroviruses, and bacteria, as well as mislocated mtDNA and nuclear DNA. As the DNA sensor cGAS is unable to distinguish self (cellular) from nonself (pathogenic) DNA, the cGAS–MITA/STING axis must be tightly controlled to avoid self-DNA-triggered innate immunity, particularly upon NEBD in mitosis. Recent studies have revealed that the cGAS–MITA/STING axis is inactivated in mitotic cells via multiple inhibitory mechanisms, including impairment of cGAS–DNA interaction and cGAS dimerization in nucleosomes, inactivation of cGAS by mitotic kinase-mediated phosphorylation, and restrain of MITA/STING activation by Golgi vesiculation (Figure 1).

Although these findings provide important explanations on how mitotic cells restrain the cGAS–MITA/STING pathways to avoid self-DNA sensing, some questions remain unresolved. For example, plasma membrane-bound and cytosolic-located cGAS is translocated to chromosomes during mitosis. The physiological significance of the translocation and the potential functions of the chromosomal cGAS are unknown. Multiple mechanisms including cofactors for cGAS and MITA/STING have been reported to regulate the cGAS–MITA/STING axis in interphase cells (Zhou et al., 2014; Liu et al., 2016, 2019; Lian et al., 2018; Xia et al., 2019; Li et al., 2021b). Whether and how these mechanisms are regulated to modulate the cGAS–MITA/STING axis in mitotic cells remain enigmatic. Since the cGAS–MITA/STING axis is inactivated in mitotic cells, whether mitotic cells are more vulnerable to DNA viruses and how mitotic cells defend DNA pathogens are open questions. A more comprehensive understanding of cell cycle-dependent regulation of the cGAS–MITA/STING axis may help to understand the complicated mechanisms of the innate immune system to distinguish nonself from self and to exploit these mechanisms for the development of therapeutics for autoimmune and immunodeficient diseases and cancers.

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