IMMUNOLOGY

STING condensates on ER limit IFN response

STING is a key player in the IFN response to cytosolic DNA, and its multimerization is commonly associated with activation of the pathway. A new study now shows that STING forms 'puzzle'-like condensates to limit the IFN response and constrain antiviral immune activation.

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Formation of higher-order assemblies including liquid and gel condensates has recently emerged as a major mechanism of signal transduction in innate immunity[1]. Indeed, activation of pathways through multimerization of sensor and adaptor proteins is known for Toll-like receptor signalling[2], inflammasome activation[3], retinoic acid-inducible gene I (RIG-I)[4] and cyclic GMP–AMP synthase (cGAS)[5] nucleic acid detection, as well as many others. However, the question as to whether and how a multimerization principle may also be involved to inhibit immune pathways has not been fully addressed. In this issue of *Nature Cell Biology*, Yu et al. report that excess 2′,3′-cyclic GMP–AMP (2′,3′-cGAMP) induces STING to phase separate on the endoplasmic reticulum (ER) in a puzzle-like structure, thereby preventing STING overactivation[6].

STING is a major mediator of interferon (IFN) activation in response to cytosolic DNA, a powerful danger-associated signal emerging from bacterial or viral infection or pathological processes within the host cell[7]. The upstream sensor cGAS recognises cytosolic DNA and synthesises a messenger molecule, 2′,3′-cGAMP, which binds and activates STING to trigger its association with TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), thus leading to IFN production[7]. Dysregulation of this complex pathway is associated with autoinflammatory diseases and cancer, making STING a desirable target for drug discovery[8,9].

Recent studies have unraveled the underlying mechanism of cGAS–STING activation at the biological and structural levels. For example, an inactive STING dimer normally residing on the ER was shown to translocate to Golgi and Golgi-derived vesicles upon activation, where it formed tetramers and higher-order oligomers[10] (Fig. 1). Such multimerization is required for the downstream cascade because it allows STING-associated TBK1 to interact with neighbouring TBK1 and IRF3 molecules to phosphorylate and activate them[11]. Interestingly, the upstream sensor cGAS also requires oligomerization for its activity and has been shown to form phase-separated droplets upon DNA binding[12]. However, much less is known about the regulatory mechanisms of the pathway. Can these STING multimers disassemble? Or will the pathway components be degraded? Yu et al. provide a surprisingly different answer that involves phase-separation-mediated sequestration.

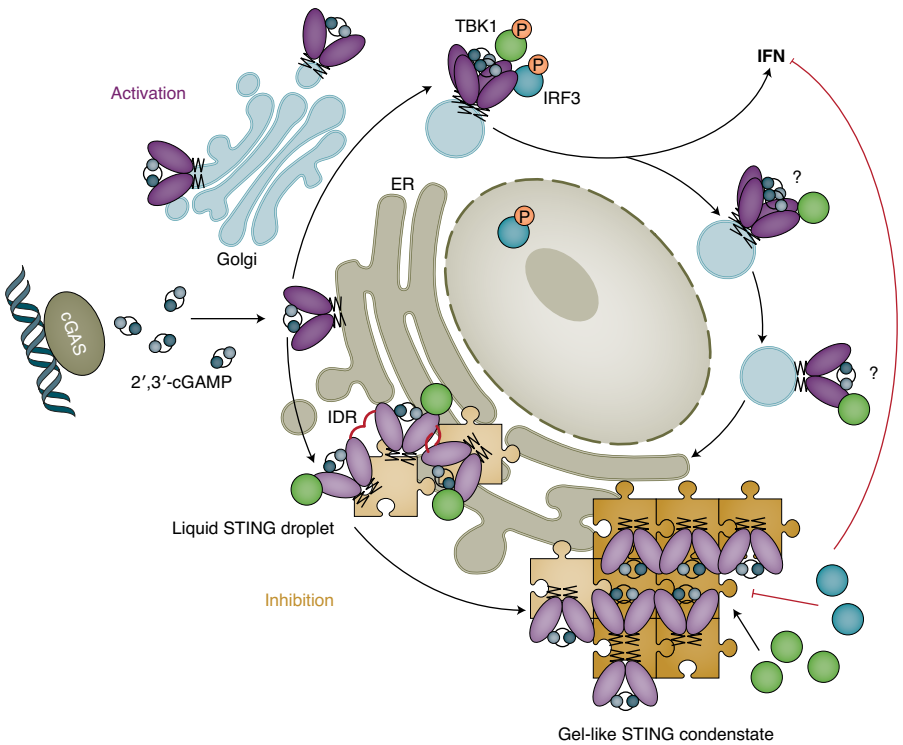

Fig. 1 | A proposed model of STING activation (clockwise) and inhibition (counterclockwise). Clockwise: DNA binding by cGAS (grey) results in 2′,3′-cGAMP production and STING activation. 2′,3′-cGAMP bound STING translocates from the ER to Golgi and further to Golgi vesicles to engage with TBK1 and IRF3, which results in their phosphorylation (P) and activation. Phosphorylated IRF3 translocates into the nucleus to induce IFN production and anti-viral response. Golgi vesicles with active STING can be further fused with STING condensate structures for inactivation, the mechanism of which is yet to be discovered (question marks). Counterclockwise**:** Excess 2′,3′-cGAMP induces STING phase separation on the ER, mediated partly by the intrinsically disordered region at STING C terminus (IDR, red). STING liquid droplets are further converted into biocondensates with gel-like properties, in which STING and TBK1 are unphosphorylated and incapsulated between ER membrane fragments, making STING inaccessible for IRF3 binding and downstream signalling. These puzzle-like STING condensates act like a 2′,3′-cGAMP and TBK1 sponge to limit IFN overactivation at the later stages of the antiviral response.

Yu and colleagues started their discovery by observing that STING activation by the DNA virus Vaccinia (VACV) or by 2′,3′-cGAMP led to the formation of not only smaller submicron-sized STING condensates, which are commonly associated with Golgi-translocated STING[13],

but also larger micron-sized STING entities at the ER. These larger STING condensates were especially prevalent with excess 2′,3′-cGAMP, and fluorescence recovery after photobleaching indicated a liquid to gel transition at the later time points after stimulation of the cGAS–STING pathway. In line with the formation of biocondensates, sequence analysis revealed that the STING C terminus comprises a conserved intrinsically disordered region (IDR), which is a common feature among proteins with phase-separation propensities. Indeed, mutations in this region abolished the formation of STING condensates in living cells and droplets in vitro in response to 2′,3′-cGAMP.

STING condensates formed on the ER appeared to be different from phase-separated droplets of soluble proteins. Super-resolution microscopy indicated the presence of a three-dimensional mesh-like pattern within STING condensates. This pattern emerged from the highly organised ER membrane structures folded together in a form of a puzzle, as revealed by transmission electron microscopy (TEM). The puzzle was closely connected to the membranes of both lamellae and granulated ER in response to the binding of 2′,3′-cGAMP by STING (Fig. 1). Unexpectedly, STING molecules residing on these membrane structures are incapsulated between the puzzle 'pieces' and inaccessible to the cytosol. By contrast, the smaller STING condensates translocated from the ER to the Golgi appeared to be simpler, whereby STING was presumably accessible from the surface to engage proteins in the cytosol.

So how and why does STING form such a puzzle structure? STING condensates on the ER appear to be formed solely as a response to high 2′,3′-cGAMP level, likely acting as a cGAMP sponge to prevent overactivation of the pathway while allowing lower levels of 2′,3′-cGAMP to still activate the IFN response. Such a sponge-based mechanism has also been shown for the helicase DDX3X, which is important for activating the NLRP3 inflammasome but can be sequestered within stress granules to reprogram the cellular response from NLRP3-induced cell death to survival[14]. STING condensates on the ER are not dependent on the downstream components TBK1 and IRF3 or on complete activation of the STING pathway. Indeed, STING translocation to Golgi, its palmitoylation or phosphorylation, or recruitment of IRF3 are all dispensable for the puzzle formation. The notion of a cGAMP sponge is supported by the observation that in vitro reconstituted wild-type STING condensates, but not STING soluble IDR mutants, were able to inhibit IFN production in cell lysates. In addition, while not being involved in the puzzle formation, TBK1, but not IRF3, was strongly enriched in these STING condensates, suggesting that they also act as a TBK1 sponge to disrupt an active STING–TBK1–IRF3 complex. Further analysis revealed that both STING and TBK1 are not phosphorylated within the puzzle-like structures, which excludes a possibility for STING to induce IFN response.

Interestingly, Yu et al. observed a decreased puzzle formation from constitutively active STING variants, which have been previously hypothesised to favour a rotation in their cytosolic domains to undergo 2′,3′-cGAMP-independent multimerization and activation[6,10]. Thus, it may be assumed that the STING condensates at the ER accommodate a conformation different than that of the IFN-activating STING tetramers and higher-order oligomers as previously reported[10], and it can be inferred that IFN-inducing state of STING is incompatible with the puzzle-like structure. Because small Golgi-derived vesicles presumably containing active STING multimers are fused with the ER STING condensates, it remains unclear how this active state of STING is reverted within the puzzle. It is possible that STING multimerization may simultaneously utilise two competing mechanisms: a side-by-side stacking of active STING dimers on Golgi vesicles and an IDR-driven multivalent interaction in the STING puzzle on the ER. If a prion-like conformational change is at work here, we hypothesise that upon vesicle fusion, the ER STING condensates may convert a stacking interaction between STING dimers to an inactive IDR-driven interaction, thus locking STING within the puzzle and shifting the equilibrium towards inactivation.

Given that maintenance of ER membranes relies strongly on microtubules, it is not surprising that the authors found that pharmacological disruption of microtubule structures prevented the ER from forming the puzzle-like STING condensates with a concomitant increase in IFN response. This relationship of STING signalling with the cytoskeleton is opposite to what has been observed for the NLRP3 inflammasome, in which microtubule disruption inhibits the transport of inflammasome components, thus impairing instead of enhancing its activation[15].

This discovery raises a number of important scientific questions. For example, how does STING trigger such extensive ER membrane reorganisation? Is 2′,3′-cGAMP bound to every STING dimer within the puzzle or does it only initiate such STING multimerization? Does the puzzle inhibit STING-induced autophagy and NF-κB signalling? Can STING condensates on ER dissolve to enable a second round of STING activation? And how general is this principle of inhibition in other innate immune pathways? Further research would be needed to address these and many more questions. For now, a novel STING phase separation mechanism offers a new target for development of anti-inflammatory and anticancer treatments. ❐

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Competing interests

H.W. is a co-founder of Ventus Therapeutics. L.A. declares no competing interests.