Divide to conquer: NLRP3 is activated on dispersed trans-Golgi Network.

Kaiwen W. Chen[1]*, Dave Boucher[1]* and Petr Broz[1,2].
*Those authors contributed equally
[1]Department of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland.
[2]Correspondence to petr.broz@unil.ch

NLRP3 is a cytosolic pattern recognition receptor that assembles a multiprotein signaling complex –the NLRP3 inflammasome – upon detecting pathogens or cellular stress. This complex recruits and activates the protease caspase-1, which cleaves interleukin (IL)-1 family proteins, pro-IL-1β and pro-IL-18 to their mature bioactive form; and processes the pore-forming protein Gasdermin D (GSDMD) to induce a form of lytic cell death known as pyroptosis [1]. While many studies show an important role of NLRP3 in host defense, it also plays a deleterious role in chronic inflammatory disorders and gain-of-function mutations in NLRP3 are associated with numerous hereditary inflammatory diseases (e.g. CAPS, neonatal onset multisystem inflammatory disorder) [2]. Therefore, there is an urgent need to better understand the activation mechanisms of NLRP3.

NLRP3 has a unique ability to sense a wide variety of structurally unrelated molecules ranging from whole pathogens, insoluble particulates and endogenous danger signals, indicating that these molecules likely elicit a common stress signal that is sensed by NLRP3. Indeed, potassium efflux, mitochondrial damage, reactive oxygen species, lysosomal rupture and calcium mobilization have all been proposed to be the common event sensed by NLRP3. However, despite more than 10 years of intensive research, the field has yet to reach a consensus opinion. In a recent study published in *Nature,* the group of Zhijian 'James' Chen (refers to Chen *et. al* [3]) report an unexpected finding that dispersion trans golgi network (dTGN) is the early and common stress event that is required for NLRP3 activation in response to diverse agonists.

To investigate the sub-cellular location of NLRP3 assembly, Chen *et. al* established an elegant *in vitro* assay in which they permeabilized the plasma membrane of HEK-293T cells expressing the inflammasome adaptor ASC and caspase-1 with perfringolysin O (PFO), before treating them with fractionated extracts of stimulated NLRP3-positive, but ASC-negative cells. Surprisingly, NLRP3 co-migrated strongly with markers of the TGN, and these fractions elicited robust caspase-1 processing in PFO-permeabilized ASC-Casp1-expressing HEK-293T cells. To investigate this further, the authors examined the changes in morphology and location of subcellular organelles and the NLRP3 protein by microscopy following inflammasome stimulation. Remarkably, all five structurally unrelated NLRP3 agonists: nigericin, ATP, gramicidin, imiquimod and CL097 triggered disassembly of the TGN onto which NLRP3 formed distinct puncta. Disassembled TGN (dTGN)-localized NLRP3 also initiated ASC polymerization, consistent with the idea that TGN disassembly is the common stress event sensed by NLRP3.

To further understand the molecular requirements for NLRP3-dTGN interaction, the authors generated truncated and point mutants of NLRP3 and found that deletion of four consecutive lysine residues between the PYD domain and the NACHT domain of NLRP3 abrogated its recruitment to the dTGN. They further demonstrated that this poly-basic sequence, which is evolutionary highly conserved, mediates binding to phosphatidylinotisol 4-phosphate (PtdIns4P) micro-domain present on the dTGN. This was a surprising finding, since the leucine-rich repeat (LRR) domain of NLRP3 was often believed to mediate ligand sensing [2], much like the LRR of other innate immune sensors such as Toll-like receptor [4]. This is however consistent with a recent report demonstrating that LRR of NLRP3 is not required for ASC oligomerization and caspase-1 processing upon stimulation with classical NLRP3 agonists [4]. Nevertheless, although recruitment of NLRP3 to the dTGN is an essential step for activation it is by itself not sufficient, since induced targeting of NLRP3 to the TGN using the PH domain of OSBP did not yet lead to NLRP3 activation in the absence of NLRP3 agonist.

The discovery that TGN disassembly is a common stress event required for NLRP3 inflammasome assembly has undoubtedly expanded our current knowledge on the activation mechanisms of the this inflammasome, and has important implications to the field. How canonical NLRP3 activators initiate the dispersion of the TGN is unclear and awaits further clarification. NLRP3 activity is tightly regulated by post-translational modifications including phosphorylation and ubiquitination. It is tempting to speculate that such modifications may alter the net charge of NLRP3, or sterically inhibit the interactions between the KKKK motif and PtdIns4P on dTGN. Futhermore, GSDMD and MLKL, two death effectors downstream of the non-canonical inflammasome and necrosome respectively, were shown to disrupt internal membranes including those of the Golgi [5-7]. It will be interesting to investigate whether Gasdermin D or MLKL promote NLRP3 activation by directly inducing dispersion of the TGN.

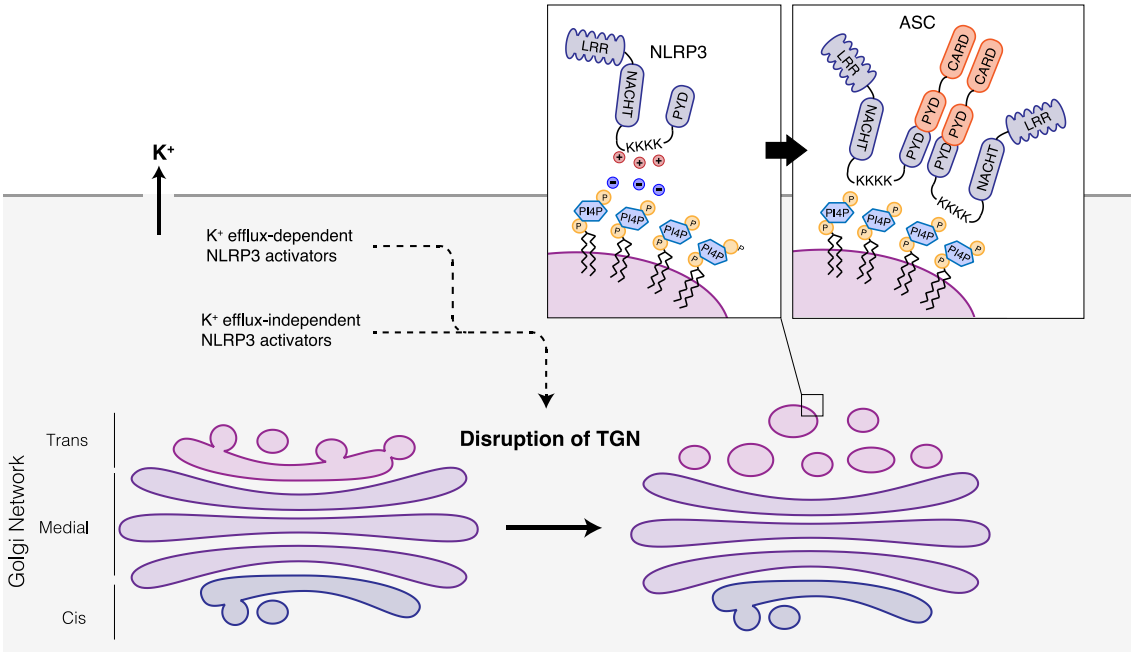

Figure 1: Diverse NLRP3 agonist trigger disassembly of the *trans* Golgi network (TGN) and exposes PI4P microdomains to recruit NLRP3 through its ionic bonding between its polybasic KKKK motif and the negatively charged PtdIns4Ps. NLRP3 aggregates on disassembled TGN and nucleates polymerization of the adaptor protein ASC.

References

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