

Keeping the Death Protein in Check

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<https://doi.org/10.1016/j.immuni.2019.06.011>

Gasdermin D is an executioner of inflammatory cell death. In this issue, Liu et al. report the crystal structures of full-length human and mouse gasdermin D, which contributes to our understanding of gasdermin D autoinhibition and activation and will inform the future development of therapeutics targeting gasdermin D.

Gasdermin D (GSDMD) mediates an inflammatory programmed cell death known as pyroptosis. Pyroptosis starts when immune cells recognize pathogens and danger signals, resulting in activation of inflammasomes that dimerize and process inflammatory caspases such as caspase-1 and caspase-11. An active caspase then cleaves IL-1 family pro-inflammatory cytokines and GSDMD. Cleaved GSDMD damages membranes and induces cytokine release and pyroptotic cell death (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015). The cytokines will attract other immune cells and induce inflammation at the site of infection. Excessive pyroptosis has been associated with diseases such as sepsis and chronic inflammation; the latter is linked to heart diseases, autoimmunity, and Alzheimer's disease. In this issue, Liu et al. (2019) report the full-length crystal structures of murine and human GSDMD in the autoinhibited state. These structures shed light on the regulatory mechanism of GSDMD and inform the future development of therapeutics targeting GSDMD.

In human, there are six gasdermins (GSDMs): GSDMA, GSDMB, GSDMC, GSDMD, GSDME (also known as DFNA5), and DFNB59 (also known as pejavakin). Mice have 10 GSDMs: three GSDMAs (GSDMA1-3), four GSDMCs (GSDMC1-4), GSDMD, GSDME, and DFNB59. All GSDMs except DFNB59 consist of two domains, the N-terminal domain (NTD) and the C-terminal domain (CTD). It has recently been discovered that the NTD of GSDMD binds to acidic phospholipids, forms pore on the cell membrane, and induces pyroptosis (Aglietti et al., 2016; Chen et al., 2016; Ding et al., 2016; Liu et al., 2016; Sborgi et al., 2016). Tremendous progress has been made on mouse GSDMA3. The struc-

tures of full-length GSDMA3 in the autoinhibited state and GSDMA3 NTD in the pore state have been obtained, revealing the mechanism of autoinhibition, lipid binding, oligomerization, and membrane insertion (Ding et al., 2016; Ruan et al., 2018). However, although it is expected that GSDMD exhibits a similar mechanism of autoinhibition and activation as GSDMA3, there was no full-length GSDMD structure reported until now. A substantial problem in obtaining the structure of GSDMD is its instability. The truncation of internal flexible loop regions in GSDMD proves to be crucial for allowing the crystallization and structure determination of human and mouse GSDMD, which were achieved at 3.5 Å and 3.3 Å resolutions, respectively.

Liu and colleagues' work offers the first structures of full-length human and mouse GSDMD (Figure 1). The NTD of human and mouse GSDMD overlays very well with mouse GSDMA3, except that the α 4 helix and the β 4 strand are missing, likely due to flexibility (Ding et al., 2016). The CTD of full-length GSDMD does not show noticeable changes with previous reported GSDMD CTD structures (PDB: 6AO3, PDB: 6AO4, and PDB: 5WQT), informing that the CTD domain does not undergo conformational changes for inhibiting the NTD. These findings suggest that GSDMs are structurally conserved and likely share the same activation mechanism. The cellular functions of GSDMs other than GSDMD and GSDME are largely unknown. These questions are intriguing puzzles to be solved and will potentially give us new insights into programmed cell death.

In contrast to the high similarity between individual domains, the relative position between NTD and CTD is different between GSDMD and GSDMA3. A shift of 20 Å is observed in NTD if the CTDs of GSDMD and GSDMA3 are superimposed.

Despite the relative shift between NTD and CTD, the extensive binding interface between NTD and CTD is mediated by hydrophobic interactions between the β 1- β 2 loop from NTD and the CTD pocket in all GSDMD and GSDMA3 structures. Several hydrophobic residues are identified to be crucial for such interaction, and mutations of these residues disrupt the autoinhibition of mouse GSDMD. These findings emphasize that the autoinhibition mediated by the NTD β 1- β 2 loop and the CTD pocket is common across GSDMs.

Although the structures of GSDMD are in the autoinhibited state, the authors were able to model the active state of GSDMD NTD using homology modeling based on the cryoelectron microscopy (cryo-EM) structure of GSDMA3 pore (Ruan et al., 2018). The authors then identified positively charged residues in α 1 helix and β 1- β 2 loop and showed that mutations of these residues compromise liposome leakage and pyroptosis mediated by GSDMD.

Similar to lipid binding, the oligomerization interfaces of GSDMD can also be identified through modeling. Based on the three oligomerization interfaces of GSDMA3, three interfaces have been identified, and mutations of residues on these interfaces abolish the oligomerization of GSDMD NTD, as well as pyroptosis. It is worth noting that the crystallization construct of GSDMD does not form pores due to its truncations at oligomerization interfaces. The GSDMA3 pore structure describes a pore consist of 26–28 GSDMA3 monomers with an inner diameter of ~18 nm (Ruan et al., 2018). Although the trans-interactions between monomers can be modeled, the size of the GSDMD pore cannot be accurately inferred. It would be interesting to know if the size and oligomerization state of the GSDMD



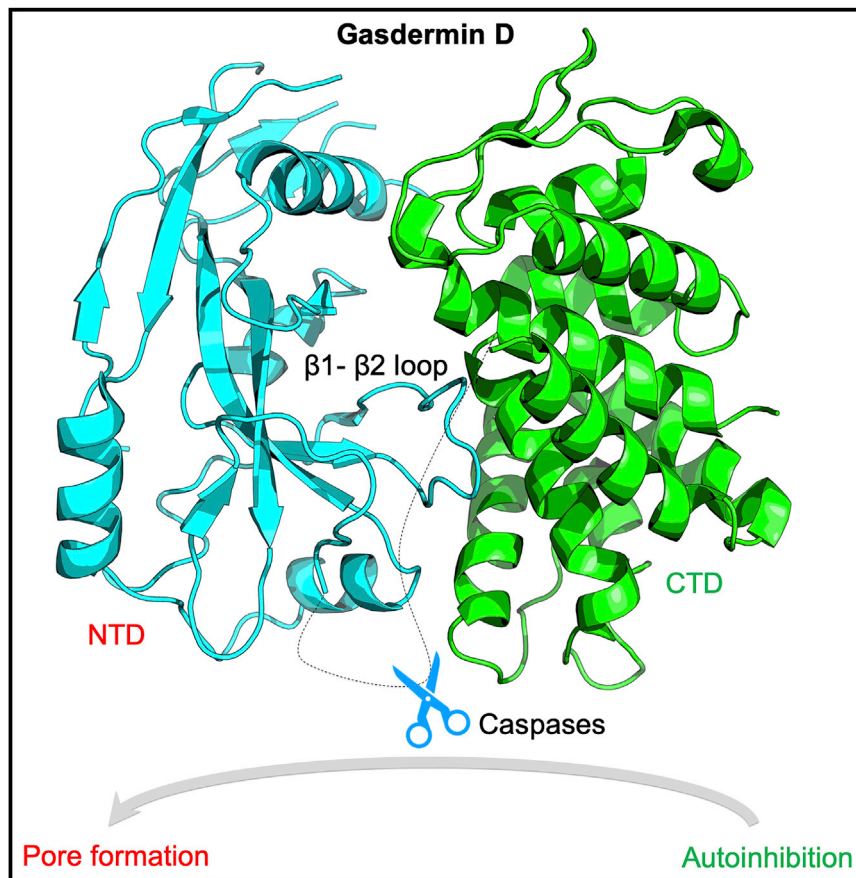


Figure 1. Structure of Full-Length Mouse GSDMD

Liu et al. report the structures of full-length human and mouse GSDMD in the autoinhibited state. The NTD of GSDMD is colored in cyan, and the CTD is in green. Interactions between NTD and CTD are mediated largely by hydrophobic contacts between the NTD $\beta 1$ - $\beta 2$ loop and the CTD pocket. Upon cleavage of the linker between NTD and CTD, the NTD is activated and forms pores on cell membrane.

NTD pore are different from GSDMA3 pore and if the different sizes of the pores underline different cellular functions.

Activation of GSDMs is a complicated process that involves cleavage of the linker between NTD and CTD, lipid binding of NTD, oligomerization of NTD, and membrane insertion and pore formation through conformational changes in NTD. Disruption of this process will result in the loss of function of GSDMs. However, it is currently under debate whether these processes happen at the same time or follow sequential steps. To fully uncover the molecular mechanism for the activation of GSDMs, structural snapshots of GSDMs in intermediate states are required.

The crystal structures of full-length GSDMD in the autoinhibited state is the first structure of a full-length GSDM with known activators, which may enable further functional and structural studies of

the recognition and cleavage mechanism of GSDMD by inflammatory caspases. It will be interesting to address how the cleavage of the linker between NTD and CTD in an autoinhibited state and leads to the pore-forming activity of NTD. Detailed computational analysis and structure simulations are needed to answer this question.

GSDMD has been associated with many inflammatory diseases and is a promising therapeutic target. Liu et al. provide us with wonderful structures of GSDMD as the basis for therapeutic design. Based on the insights from GSDMA3 and GSDMD structures, the GSDMD-mediated pyroptosis can be suppressed in various ways, such as stabilizing the autoinhibited state, disrupting lipid binding, inhibiting oligomerization, or abolishing membrane insertion of

GSDMD. It would be exciting to see future development of GSDMD-targeting drugs with high potency.

ACKNOWLEDGMENTS

The work was supported by National Institutes of Health (1R01 AI139914 to H.W.).

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