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Structural Analysis of *Helicobacter pylori* VacA Reveals Insights into Oligomerization

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Helicobacter pylori is a Gram-negative bacterium that persistently colonizes the stomachs of >50% of the human population, with a prevalence as high as 90% in developing nations [1, 2]. *H. pylori* infection causes gastritis and can lead to the development of peptic ulcer disease and gastric cancer in a subset of infected individuals [3, 4]. Gastric cancer is the third leading cause of cancer-related deaths worldwide and the World Health Organization has classified *H. pylori* as a type 1 carcinogen [5].

An important *H. pylori* virulence factor implicated in these diseases is the pore-forming toxin vacuolating cytotoxin A (VacA) [6, 7]. VacA is named for its ability to induce vacuolation in cultured eukaryotic cells [8, 9]. VacA has been reported to cause multiple cellular effects in addition to cellular vacuolation, including membrane permeabilization, mitochondrial dysfunction, cell death, autophagy, T cell inhibition, and other immunomodulatory effects [10].

VacA is secreted from *H. pylori* as an 88 kDa monomer (p88), which shares very little sequence similarity to any characterized proteins from other bacterial species. p88 binds to the surface of gastric epithelial cells, oligomerizes, and forms anion-selective membrane channels [10]. p88 is comprised of two domains, an N-terminal p33 domain and a C-terminal p55 domain [11]. The p33 domain contains a hydrophobic region required for formation of the channel and regions within both the p33 and p55 domains mediate VacA oligomerization and binding to host cells [10]. Although most of the cellular effects of VacA are dependent on oligomerization, the underlying mechanism for how VacA oligomerizes is not understood.

To investigate the structural basis of VacA oligomerization, we analyzed VacA oligomers and VacA p88 monomers by single particle cryo-electron microscopy (cryo-EM) (Figure 1). Examining the oligomer particles by 2D classification with RELION revealed VacA oligomerizes into hexamers, heptamers, dodecamers, and tetradecamers [12]. We generated 3D reconstructions using RELION and cisTEM of VacA as a hexamer, heptamer, and dodecamer [12, 13]. Our highest resolution structure was of a VacA hexamer. Additionally, we generated a 3D reconstruction of a VacA p88 monomer using cryoSPARC [14]. Comparison of these structures enabled us to identify regions within the VacA p33 and p55 domains involved in oligomerization.

This study provides important insights into how VacA oligomerizes. Since the molecular

mechanisms by which VacA elicits its variety of cellular responses are not fully elucidated, these structural studies will be used as springboard to understand VacA function within the context of cells [15].

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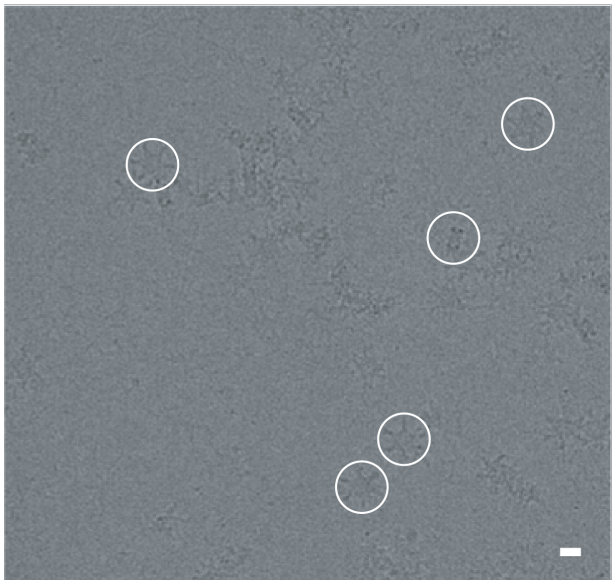

Figure 1. Cryo-EM analysis of VacA s1m1 oligomers. (A) Representative cryo-EM micrograph of VacA oligomer particles in vitreous ice. Some particles are highlighted with white circles. Scale bar = 20 nm.

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