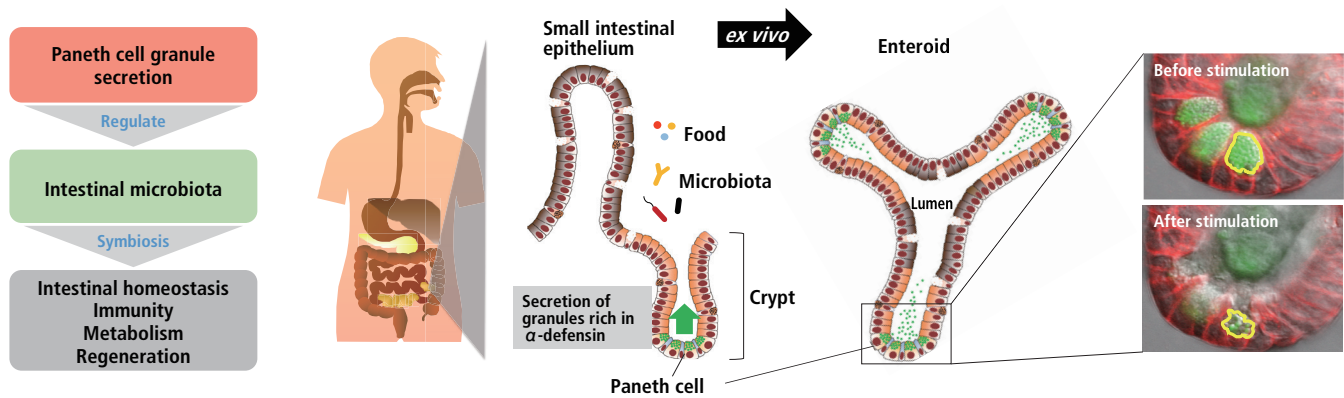


Live Imaging of Paneth Cell Secretory Responses in Innate Immunity by Using Three-Dimensional Culture of Small Intestinal Epithelial Cells

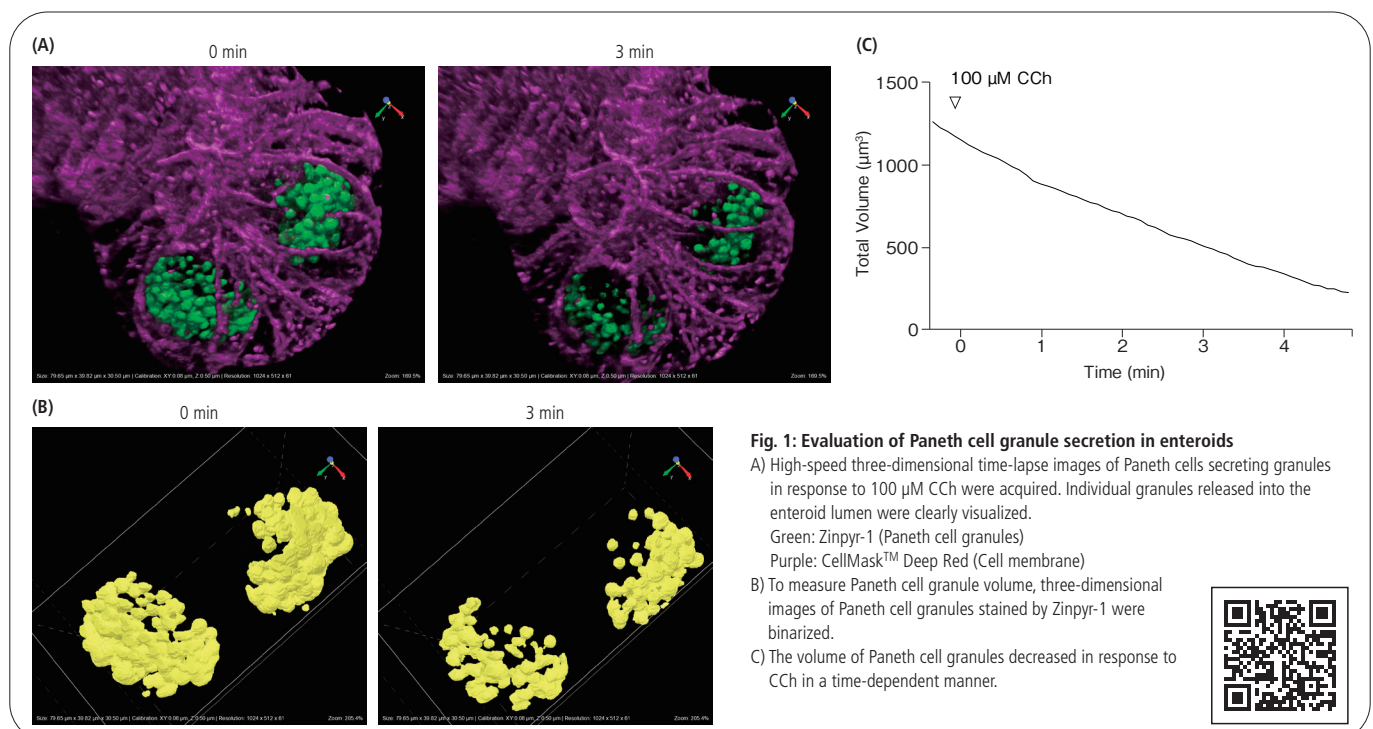
Paneth cells, a lineage of small intestinal epithelial cells, secrete granules rich in antimicrobial peptides, α -defensins, in response to cholinergic agents and bacteria, and regulate the intestinal microbiota by killing enteric pathogens, while less killing commensal bacteria. In this Application Note, we introduce examples that clarify the mechanisms of α -defensin secretion by visualization and quantification of Paneth cell granule secretory responses *ex vivo* using enteroid, a three-dimensional culture system of small intestinal epithelial cells.



Three-dimensional time-lapse analysis of Paneth cell granule secretion

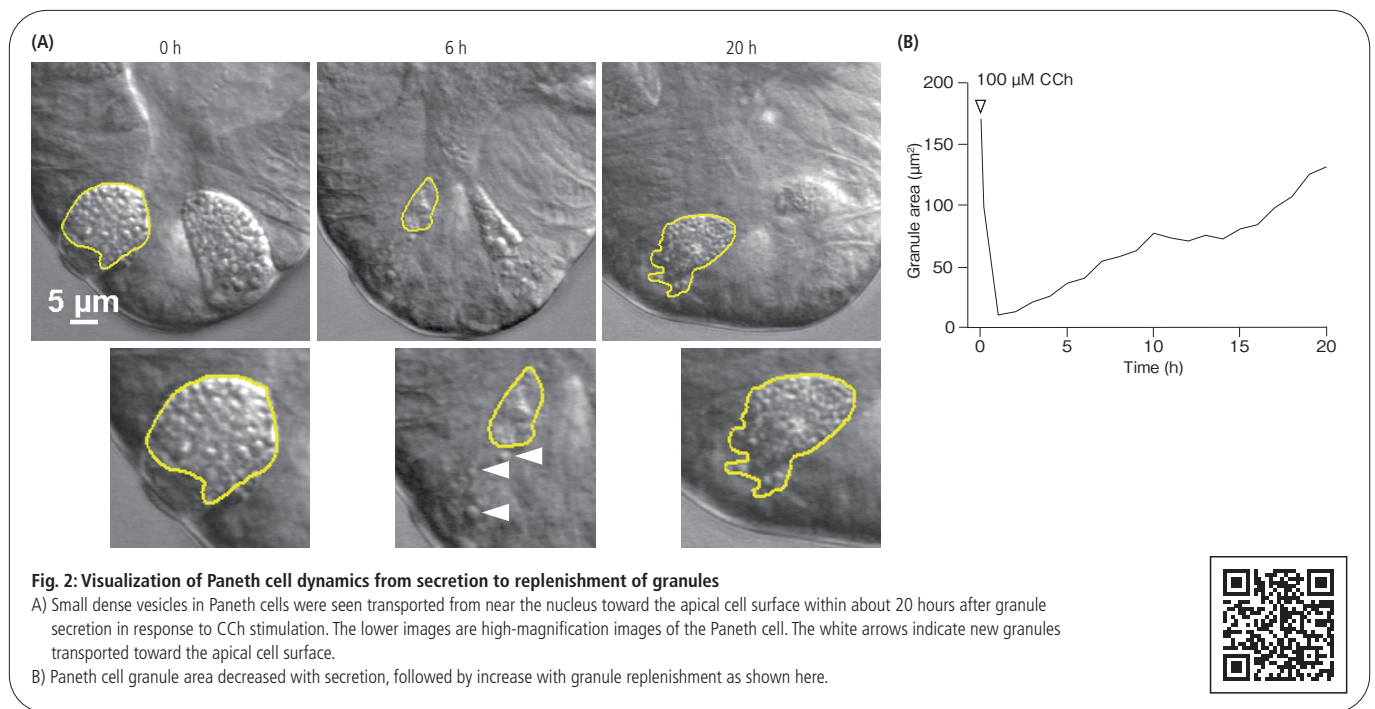
Carbachol (CCh), a cholinergic agent, was added to enteroids, and Paneth cell immune responses were observed.

Paneth cell granule secretion toward the lumen of the enteroids was evaluated by visualization and quantification using high-speed 3D time-lapse imaging with a high-speed resonant scanner and a piezo Z stage (Fig. 1).



Long-term tracking of Paneth cell granule replenishment after secretory responses

Paneth cell granule replenishment happening within 20 hours after granule secretion was observed by long-term time-lapse imaging with differential-interference contrast (Fig. 2 (A)). In addition, the process of the secretion and the replenishment of Paneth cell granules in innate enteric immunity was evaluated quantitatively by measuring the granule area (Fig. 2 (B)).



Summary

In this study, we evaluated Paneth cell dynamics by visualization and quantification for the first time, from granule secretion in response to cholinergic stimulation until replenishment of newly generated granules toward the apical side by vesicular trafficking within 20 hours, and clarified dynamics of Paneth cells in innate enteric immunity. In future, we would like to reveal underlying molecular mechanisms of Paneth cell secretory responses by three-dimensional calcium imaging *ex vivo* and *in vivo*.

Acknowledgement

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Reference

Yokoi Y, Nakamura K, Yoneda T, Kikuchi M, Sugimoto R, Shimizu Y, Ayabe T. *Scientific Reports* 9, 2710 (2019).

Product Information

A1R HD25 Confocal Microscope

This resonant scanner, which enables high-speed acquisition of up to 720 frames per second, has low phototoxicity toward living cells and is capable of high-speed 3D imaging in combination with a piezo Z stage.

NIS-Elements imaging software provides total support from image acquisition to image analysis.



Piezo Z stage



CFI Apochromat LWD Lambda S 20XC WI

This lens provides a long working distance as well as a high numerical aperture. It has chromatic aberration corrected over a broad wavelength range from visible to IR. Nano Crystal Coat is applied to increase transmittance. It is capable of capturing bright, sharp images into deep areas in thick living samples such as 3D culture systems and tissue sections.

