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The periodontal pathogen *Porphyromonas gingivalis* exits from gingival epithelial cells via recycling pathways

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Introduction

Periodontitis is one of the most common infectious diseases in humans, which leads to eventual tooth exfoliation¹⁾. *Porphyromonas gingivalis* is a Gram-negative black pigmented anaerobe and reported to be strongly associated with severe periodontal diseases²⁾. In the subgingival area, gingival epithelial cells are colonized by a diverse and abundant microbiota and prevent intrusion by periodontal bacteria. However, *P. gingivalis* can enter and persist in gingival epithelial cells³⁾. In this study, by monitoring two regulators of recycling endosomes, we were able to illustrate that intracellular *P. gingivalis* can exit from gingival epithelial cells via recycling pathways.

Endocytic compartments co-localized with intracellular *P. gingivalis*

Endosomes are membrane-bounded compartments in eukaryotic cells. The recycling endosome is organelle that is specialized for transporting several molecules back to the plasma membrane. To trace the pathways from entry to exit of intracellular *P. gingivalis*, we first examined the intracellular localization of *P. gingivalis* in immortalized human gingival epithelial cells (IHGECs). After internalization, *P. gingivalis* was localized early endosomes positive for FYVE domain of EEA1 (an early endosome marker; **Figure 1A**). We

observed that some bacteria were transported to recycling endosomes positive for Rab11A (recycling endosome markers; **Figure 1B**) and others were transported to lytic compartments (lysosomes/autolysosomes) with time. In addition, we observed that RalA (a mediator of GTP-dependent exocytosis; **Figure 1C**)

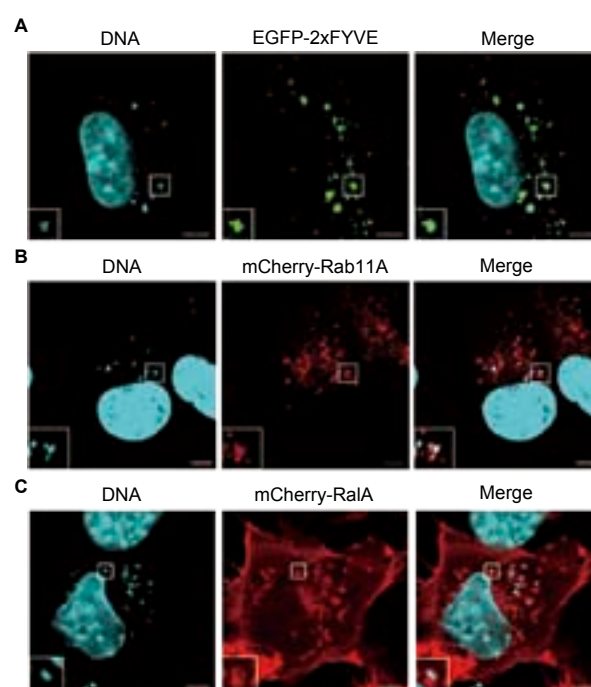


Fig. 1. Co-localization of intracellular *P. gingivalis* with endocytic compartments.

IHGECs expressing EGFP-2xFYVE (A; green), mCherry-Rab11A (B; red), or mCherry-RalA (C; red) were infected with *P. gingivalis* (blue). IHGECs were fixed, stained with DAPI, and analyzed using confocal microscopy. Bars, 5 μ m.

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also co-localized with intracellular *P. gingivalis*.

Involvement of Rab11A and RaLA in *P. gingivalis* exit

To investigate the functions of Rab11A and RaLA, we next introduced siRNAs for Rab11A and RaLA into gingival epithelial cells. We observed that knockdown of Rab11A and RaLA significantly decreased *P. gingivalis* exit and increased the intracellular viable bacteria (Figure 2A and 2B). RaLA was shown to

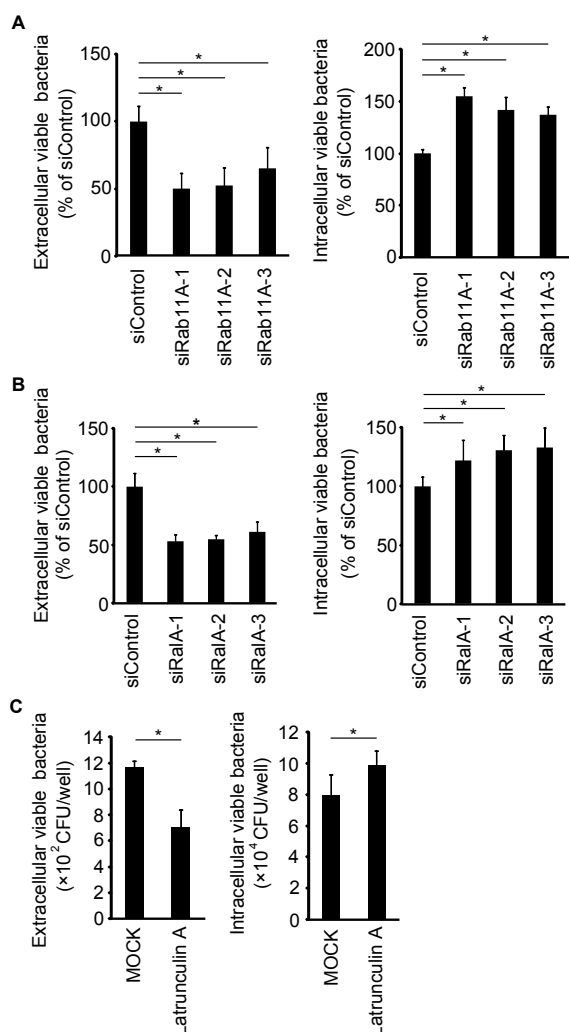


Fig. 2. Exit of intracellular *P. gingivalis* to extracellular milieu.

Effects of siRab11A (A), siRaLA (B), or latrunculin A (C) on intracellular or extracellular *P. gingivalis* organisms were determined at 6 h after infection with colony formation assay. *p<0.05, n=3, Dunnett test (A and B), two-tailed t test (C).

affect the actin-cytoskeletal rearrangements⁴⁾. We hypothesized, therefore, that actin polymerization would be involved in *P. gingivalis* exit. To test this possibility, we performed inhibitor assays with latrunculin A (an inhibitor for actin polymerization). We observed that latrunculin A significantly decreased *P. gingivalis* exit and increased the viable bacteria in IHGECs (Figure 2C).

Conclusion

In this study, we showed that *P. gingivalis* can exit from gingival epithelial cells via recycling pathways, at least regulated by Rab11A, RaLA and actin-cytoskeletal rearrangements (Figure 3). An unexpected finding was that intracellular *P. gingivalis* were sorted to the different organelles within the same cells, namely recycling endosomes and lytic compartments (lysosomes/autolysosomes). We still do not know what factors of bacteria and host cells can affect transportation into different destinations. Nevertheless, the finding is an important step for understanding the etiology of periodontitis.

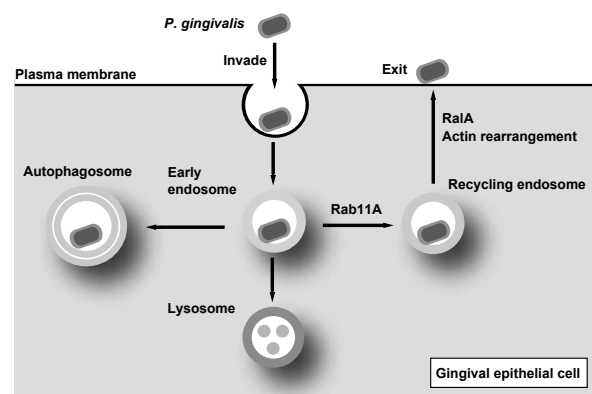


Fig. 3. Proposed model of *P. gingivalis* trafficking in human gingival epithelial cells.

P. gingivalis are initially localized within early endosomes after entry. Some bacteria are routed to lysosomes/autophagosomes, and other bacteria are able to escape from endosomes to recycling pathways, which may enable further penetration of host tissues in a transcellular manner.

Acknowledgements

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References

- 1) Deas, D. E. and Mealey, B. L. (2010): Response of chronic and aggressive periodontitis to treatment. *Periodontol 2000*, **53**, 154–166.
- 2) Amano, A. (2007): Distribution of epithelial barrier and impairment of cellular function by *Porphyromonas gingivalis*. *Front Biosci*, **12**, 3965–3974.
- 3) Lamont, R. J., Chan, A., Belton, C. M., Kzutsu, K. T., Vasei, D. and Weinberg, A. (1995): *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infect Immun*, **63**, 3878–3885.
- 4) Sugihara, K., Asano, S., Tanaka, K., Iwamatsu, A., Okawa, K. and Ohta, Y. (2002): The exocyst complex binds the small GTPase RalA to mediate filopodia formation. *Nat Cell Biol*, **4**, 73–78.
- 5) Takeuchi, H., Furuta, N., Morisaki, I. and Amano, A. (2011): Exit of intracellular *Porphyromonas gingivalis* from gingival epithelial cells is mediated by endocytic recycling pathway. *Cell Microbiol*, **13**, 677–691.