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Crucial role of milk proteins on adhesion of intestinal microbes

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Introduction

Milk

Main source of nutrition and immunological protection for the neonate
Contain glycoproteins (GPs)

Potentially bind bacteria' pili to adhesion site of model intestinal cells

Use of probiotic bacteria

Beneficial for health and intestinal diseases

"Generally Recognized as Safe" (GRAS)

Aim of the study

- ❖ Identification SpaCBA pili role in interaction with intestinal cells and GPs
- ❖ Establishment of interactions and benefits of GPs on bacteria adhesion
- ❖ Improved understanding of the role of milk in the adhesion of bacteria to epithelial intestinal cells

Glycoproteins

What are the different existing interactions ?

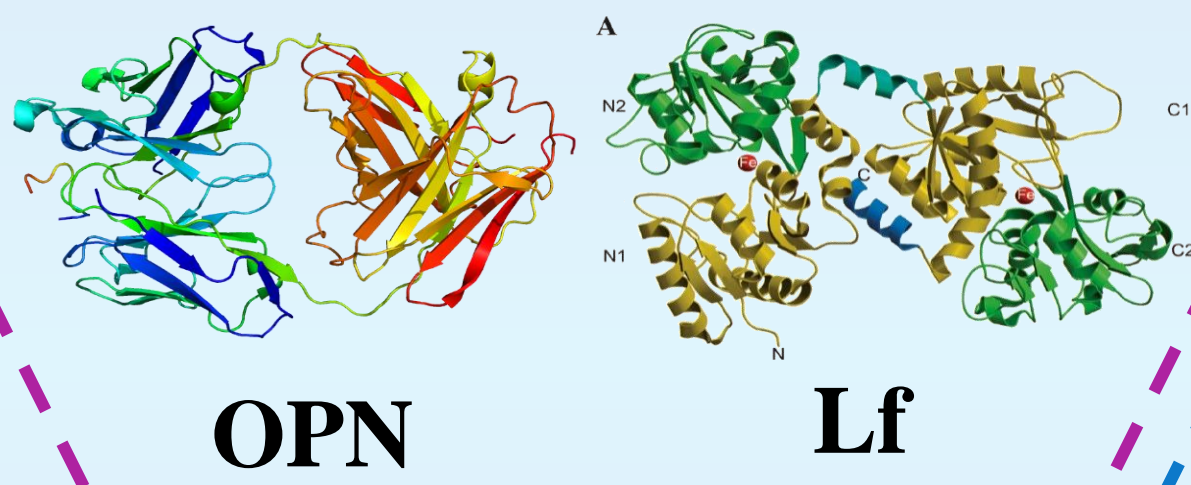
Cell adhesion assay

Use of differentiated cell monolayer obtained after 22 culture days

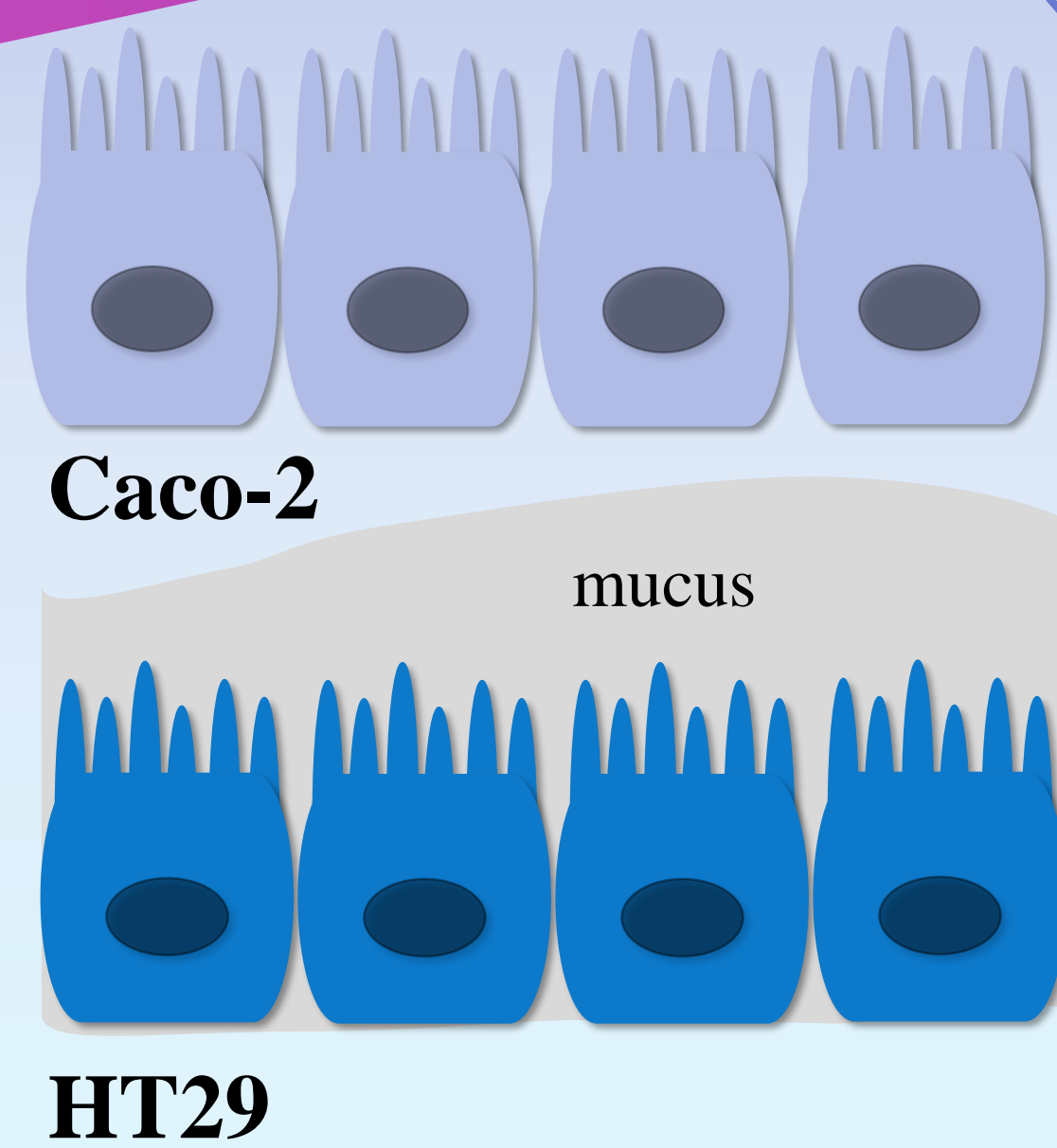
Materials and Methods

Materials used

Milk GPs
Osteopontin
Lactoferrin

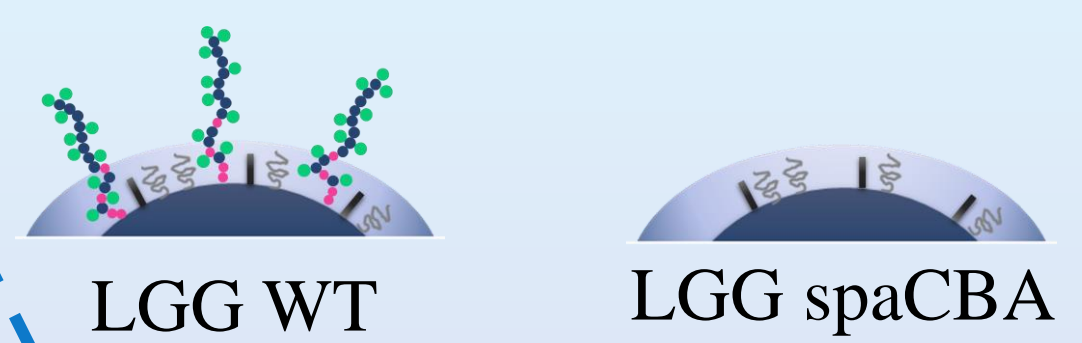


Intestinal epithelial cells
Caco-2 TC7 cells
HT29-CL16E cells (producing mucus)



Mucus layer: potentially impact the bacterial adhesion

Probiotic bacteria
L. rhamnosus GG (LGG) WT
LGG pili depleted (spaCBA)

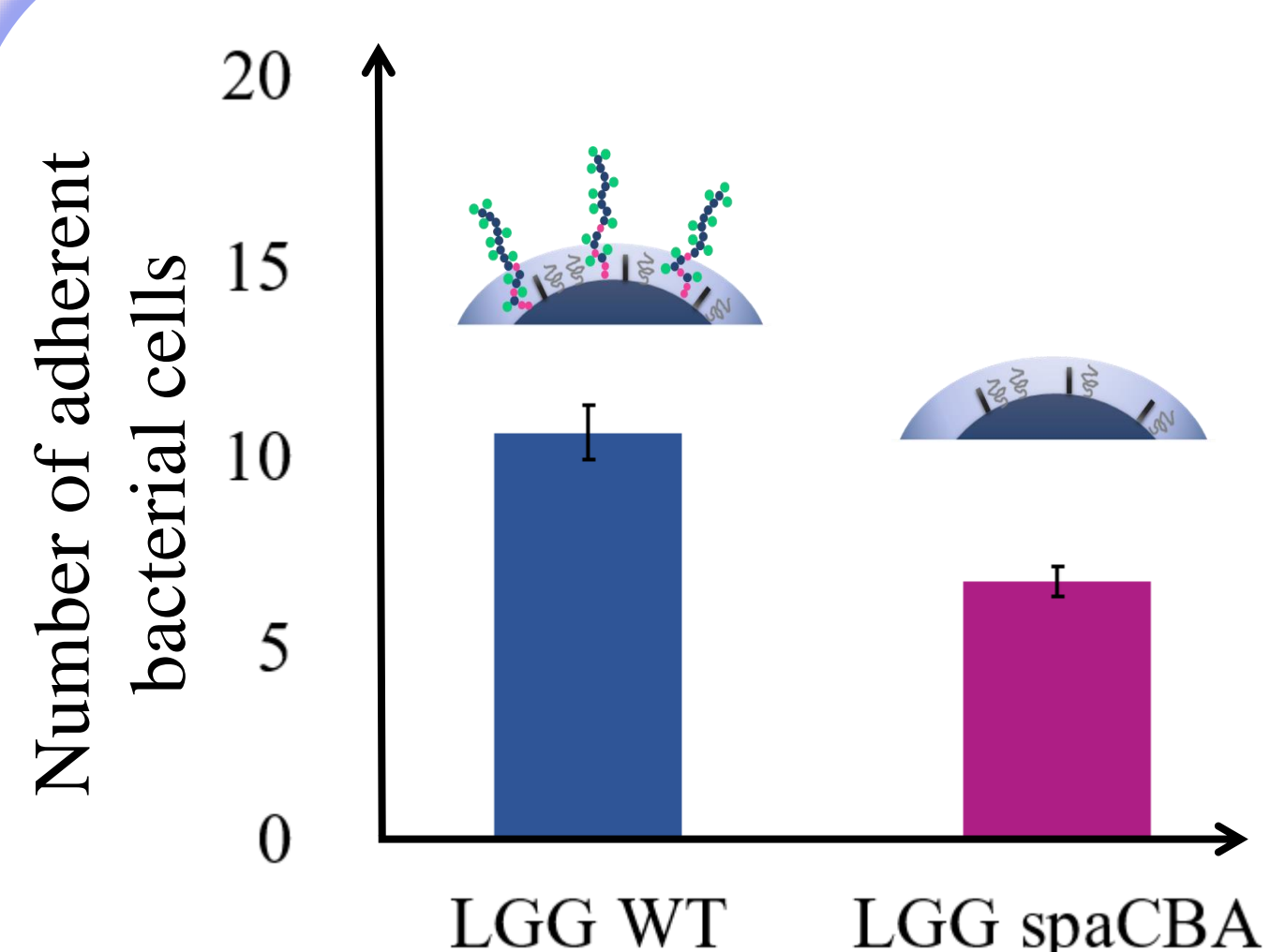


Cell plate with Caco-2 TC7 or HT29-CL16E

- ① Bacterial culture on fresh MRS broth: end of exponential phase (12h)
- ② Epithelial monolayer incubated for 2h with a) Bacteria suspension or b) Bacteria + GPs
- ③ Sequential washing of intestinal cells
- ④ Counting of bacteria attached to epithelial cells

Results and Discussion

Caco-2 TC7 cells



* Percentage increase in cell adhesion compared to results obtained without GPs

Addition of GPs

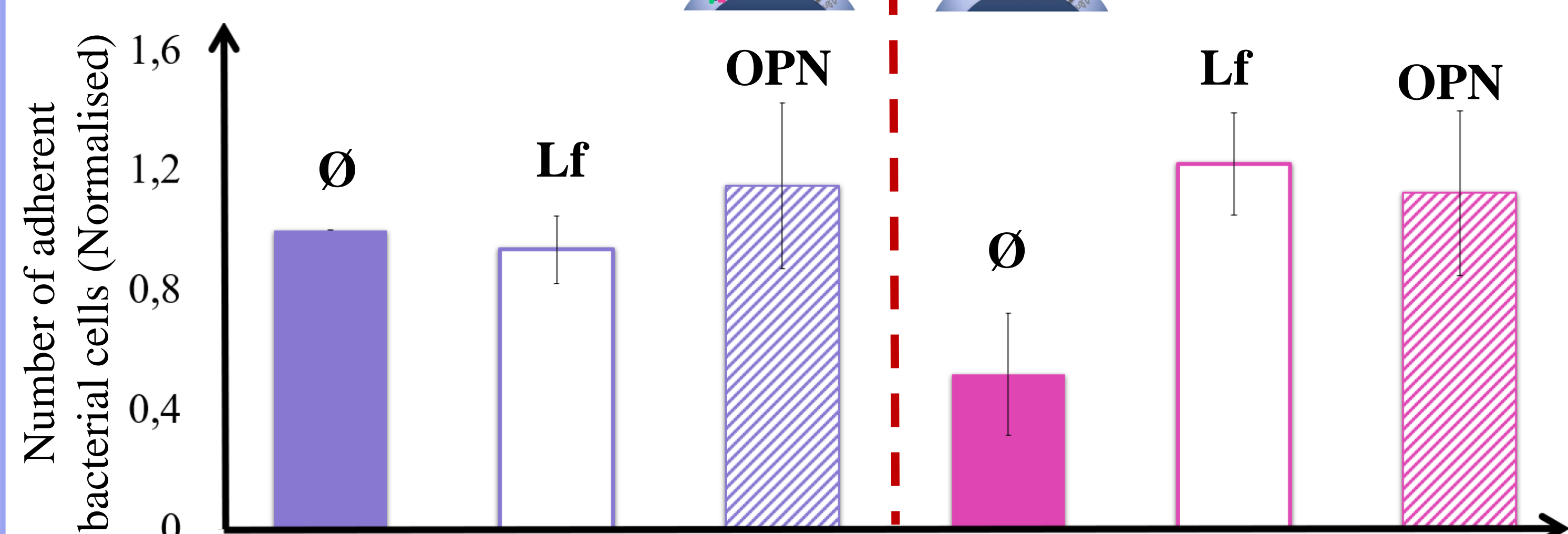
Lf + 23.4 %*
OPN + 51.4 %*

Lf + 14.7 %*
OPN + 70.6 %*

Key role of SpaCBA pilus in adhesion of LGG to Caco-2 cells

- ❖ GPs improve LGG adhesion
- ❖ OPN: major impact on bacterial adhesion

HT29-CL16E cells

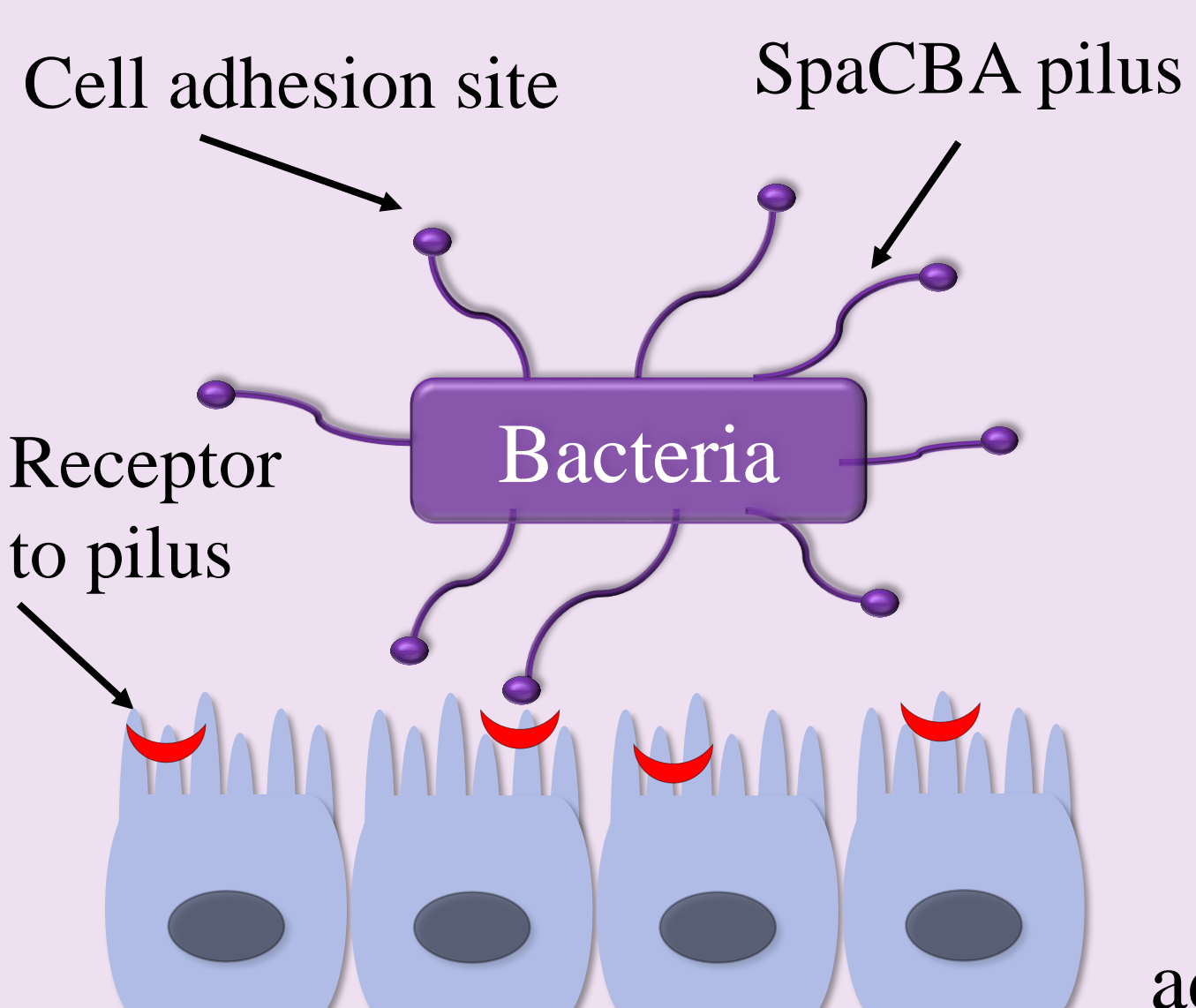


- ❖ Impact of mucus on bacterial adhesion (compared to Caco-2 cells)
- ❖ SpaCBA and WT: almost similar adhesion with GPs
- ❖ OPN: best adhesion results of both cell types in all present cases

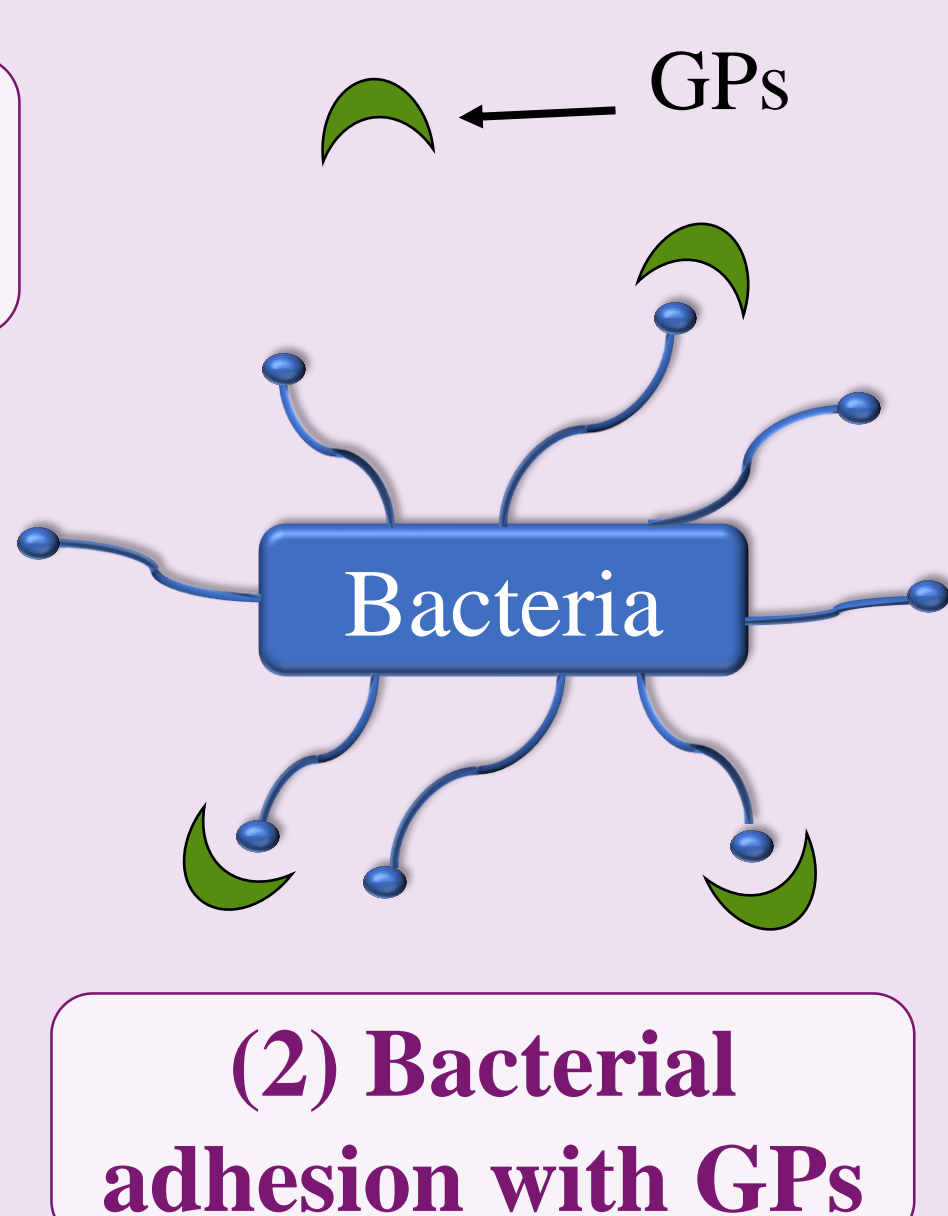
Conclusion / Perspectives

- ❖ Identification of all interactions

(1) Without GPs: bacteria adhesion with epithelial cells



(3) With GPs: increase of bacteria adhesion to epithelial cells



Future works: Identification of molecules engaged in adhesion like Peptins (adhesins, GPs, surface molecules...)

Acknowledgments

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