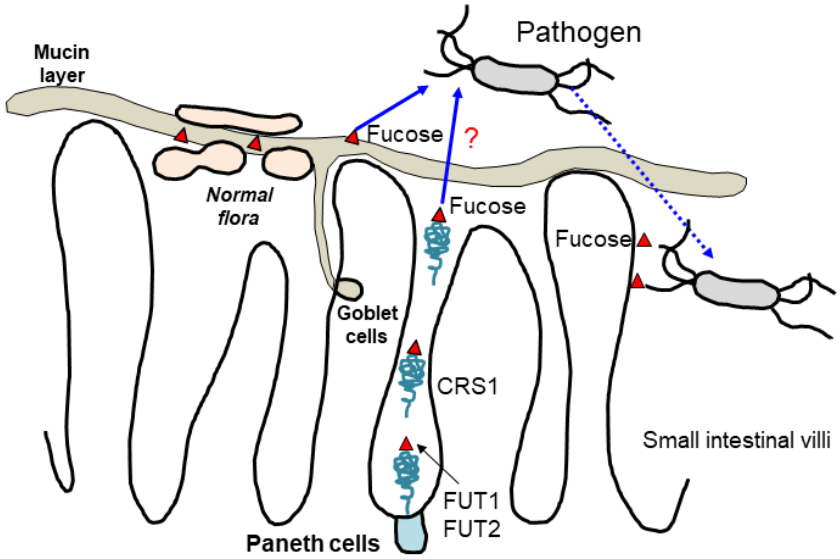
News Release

## Title

**Identification of α1,2-fucose modification, important modification in intestinal immunity, on CRS1, an antimicrobial protein.**

## Key Points

- In the small intestine, glycosylation with α1,2-fucose has been implicated in the symbiosis with intestinal bacteria and the elimination of pathogens.
- However, it remains largely unknown which proteins are modified with α1,2-fucose in the small intestine.
- We found that α1,2-fucosylation that predominantly occurs on the O-glycans of CRS1, an antimicrobial protein secreted from Paneth cells.
- Our results suggest that α1,2-fucosylation on O-linked glycans of CRS1 is likely to regulate the antimicrobial activity of CRS1 and maintain the intestinal environment.




## Summary

A research group led by Dr. Hiroki Hashiguchi (first author), a medical staff in the Department of Gastroenterology, Nagoya University Hospital, Prof. Mitsuhiro Fujishiro, Department of Gastroenterology, Nagoya University Graduate School of Medicine, and Prof. Tetsuya Okajima (corresponding author), Department of Molecular Biochemistry, Nagoya University Graduate School of Medicine, has revealed an antimicrobial protein glycosylated with α1,2-fucose using mass spectrometry analysis of mouse small intestine.

The modification of galactose with α1,2-fucose is involved in symbiosis with intestinal bacteria and elimination of pathogenic bacteria. It is postulated that mucin secreted from goblet cells is involved in defending an organism against infections, but the detailed molecular mechanisms are yet to be elucidated. It

was previously reported that Paneth cells of the small intestine were positive for UEA-1 lectin staining. However, glycoproteins in Paneth cells carrying α1,2-fucose have not yet been identified. Glycoproteomic analysis of ileal lysates identified 348 O-linked and 1,165 N-linked glycopeptides. In particular, cryptdin-related sequence 1 (CRS1) expressed in Paneth cells was found to be α1,2-fucosylated. Unlike other antimicrobial α-defensin proteins, CRS1 contains unique Thr residues, which are modified with O-glycans, with 3HexNAc2Hex1Fuc1NeuAc being the main glycoform. Identification of α1,2-fucose on the O-glycans of CRS1 expressed in Paneth cells will pave the way for a mechanistic understanding of α1,2-fucose-dependent symbiosis with intestinal bacteria and elimination of pathogenic bacteria in the intestine.

The research was published in the electronic version of the journal ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, published on line by ELSEVIER INC in 27 Oct, 2020.

## Research Background

The interaction between glycans and glycan recognition molecules is closely related in developmental control of organisms and is involved in differentiation, proliferation, adhesion, gene expression and signal transduction. These findings will be applicable to various fields such as diagnosis and treatment of diseases related to abnormalities of glycans.

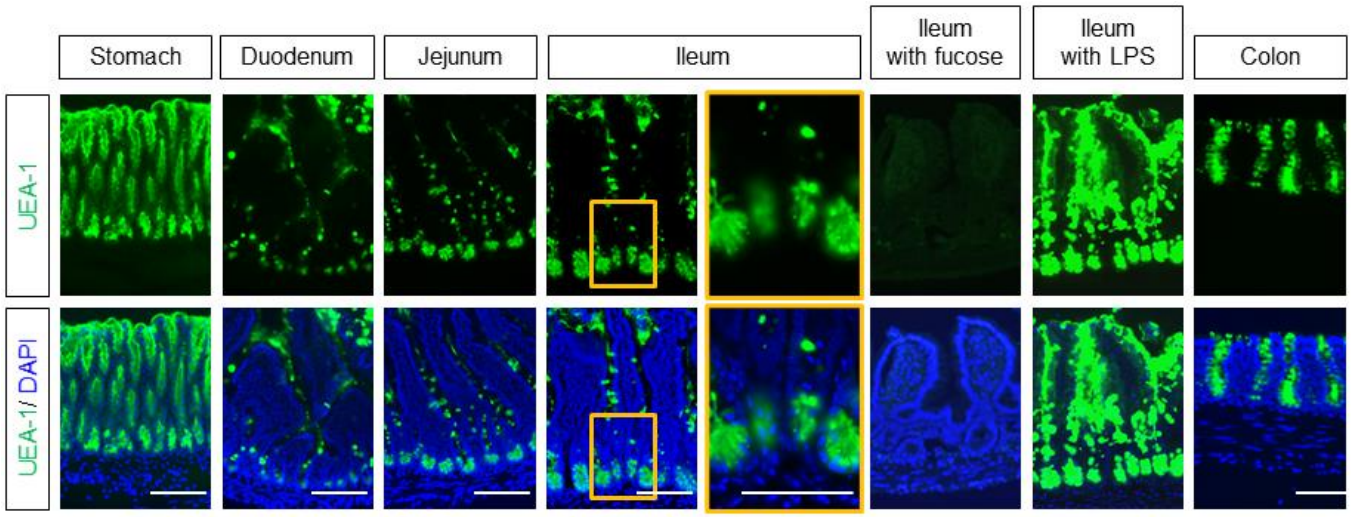
Modification of N-acetyl-lactosamine by α1,2-fucose has been implicated in the symbiosis with intestinal bacteria and the elimination of pathogens. It has been speculated that mucin secreted by goblet cells is involved in the defense against infection, but the detailed molecular mechanism has not yet been elucidated. Although the small intestine is in constant contact with a wide variety of symbiotic flora and pathogenic microorganisms, the function of α1,2-fucose, which is involved in the maintenance of intestinal homeostasis, remains largely unknown, and glycoproteins modified with α1,2-fucose have rarely been reported. In this study, we analyzed the endogenous glycoproteome by integrated glycoproteomic analysis to identify a novel α1,2-fucosylated glycoprotein associated with intestinal homeostasis.

## Research Results

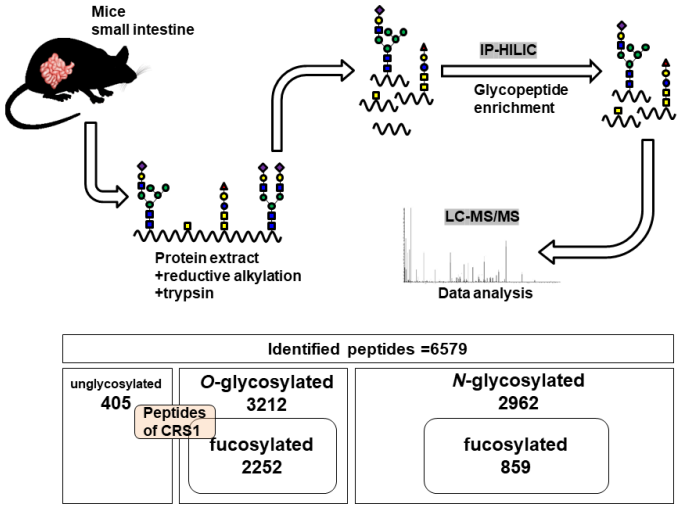
Fluorescence staining using UEA-1 lectin, which binds to α1,2-fucose, detected intracellular granules in the Paneth cells of the small intestine (Figure 1). This staining was observed regardless of previous exposure to LPS. Lectin blots revealed 15 kDa glycoproteins that were α1,2-fucosylated in the small intestine.

Mass spectrometry analysis with IP-HILIC identified 3,212 O-linked and 2,962 N-linked glycopeptides in the small intestine (Figure 2). Among them, we found a 15 kDa cryptdin-related sequence 1 (CRS1), which is structurally similar to cryptdin, an antimicrobial protein abundantly expressed in Paneth cells, which is highly expressed and modified with α1,2-fucose. MS analysis detected CPVCPTCPQCPK and TAITTQAPNTQHK peptides bearing α1,2-fucose on O-glycans (Figure 3).

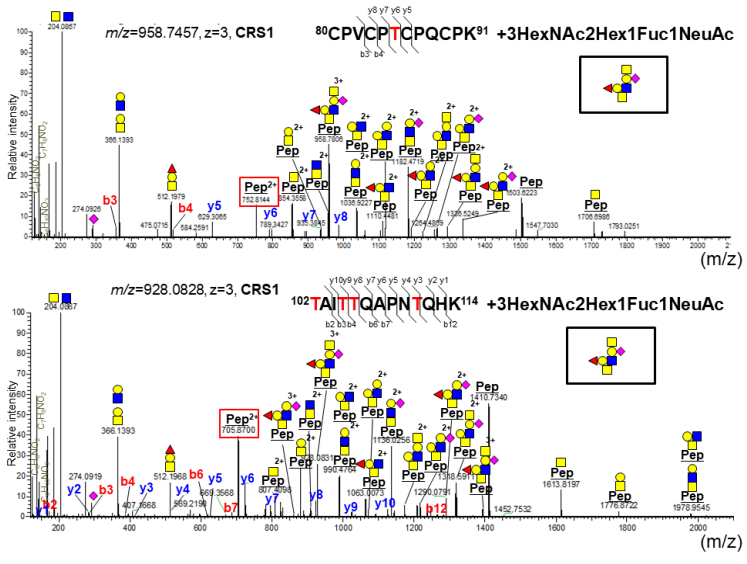
## Figure 1




## Figure 2




## Figure 3



## Research Summary and Future Perspective

The results of this study add a potential new player in intestinal immunity regulated by α1,2-fucose. This discovery of α1,2-fucosylated CRS1 is the first example in α-defensin family proteins that is glycosylated with O-linked glycans or α1,2-fucose. If glycosylation modifies the antimicrobial activity of CRS1, it would invoke the new concept of "antimicrobial glycoproteins". Recent studies have shown that pathogenic *Salmonella* bacteria bind to α1,2-fucose and are important for colony formation in the intestinal tract. Presumably, the α1,2-fucosylation may direct CRS1 to the microorganisms to enhance antimicrobial activity. This study will facilitate the understanding α1,2-fucose-dependent symbiosis and the defense mechanism for pathogenic bacteria.

## Publication

**ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS**, published on line 27 Oct, 2020.

Glycoproteomic analysis of fucose-containing proteins in small intestine identified cryptdin-related sequence 1 as O-glycosylated proteins modified with α1,2-fucose

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