Intestinal handling of glide peptides: modulation by probiotics

ELFID
(European Laboratory for the Investigation of Food Induced disease)

and

DISMET
(Dipartment of Traslational Medical Science, University Federico II, Naples, Italy)

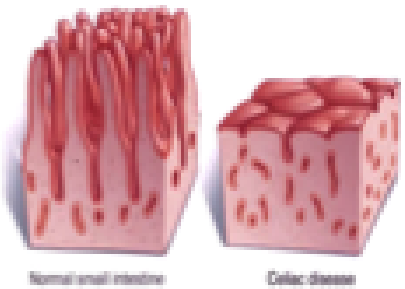
Rome 2019

CELIAC DISEASE

Gliadin :
enviromental factor

HLADQ2/DQ8 : genetic factor

Autoimmune disease

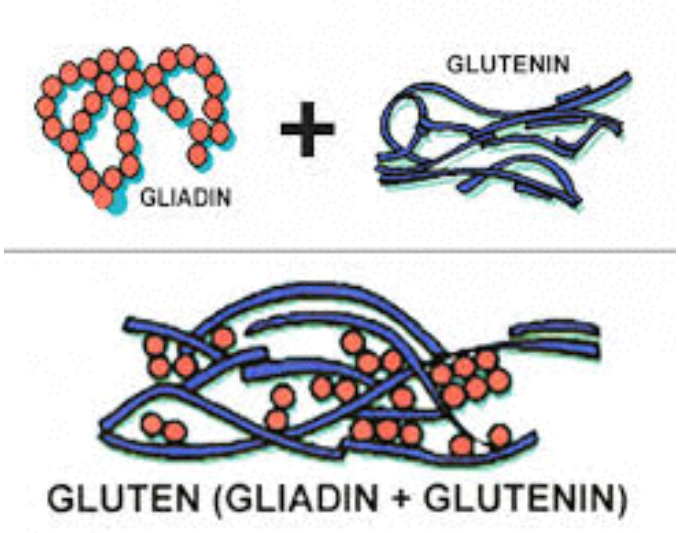


The intestinal mucose of the celiac patient presents it:

- Increase in intraephitelial lymphocytes .
- Hyperplasia of crypts.
- Atrophy of the villi.

Gliadin is a protein component of wheat and it is fundamental to the pathogenesis of celiac disease.

Gliadin is a wheat storage protein



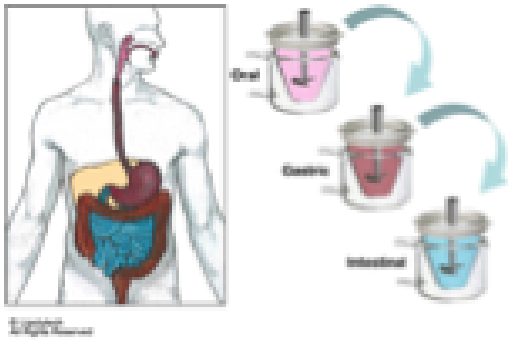

```
         10          20          30          40          50
MVRVPVPQLQ PQNPSQQQPQ EQVPLVQQQQ FPGQQQPFPP QQPYPQPQPF
         60          70          80          90         100
PSQQPYLQLQ PFPQPQLPYP QPQLPYPQPQ LPYPQPQPFR PQQPYPQSQP
        110         120         130         140         150
QYSQPQQPIS QQQQQQQQQQ QQKQQQQQQQ QILQQILQQQ LIPCRDVVLQ
        160         170         180         190         200
QHSIAYGSSQ VLQQSTYQLV QQLCCQQLWQ IPEQSRCQAI HNVVHAIILH
        210         220         230         240         250
QQQQQQQQQQ QQPLSQVSFQ QPQQQYPSGQ GSFQPSQQNP QAQGSVQPQQ
        260         270         280         290
LPQFEEIRNL ALETLPAMCN VYIPPYCTIA PVGIFGTNYR
```

In Vitro Digestion Systems

Some gliadin peptides are resistant to digestive enzymes

In vitro gliadin digestion

Digestion:
Salivation/ mastication
Stomac
pancratic



Chromatography analysis

25 peptides

Chromatography analysis

2 peptides:

Digestion:
brush border enzimes;

These two peptides can be fpund in the stools

(Mamone G. et al J Chromatography B, 2007
(Mamone G. et al Agri. Food Chem, 2015

Dynamic In Vitro Digestion System

This digestion system is dynamic and composed of different parts which can simulate all the phases of digestion.

It simulates the digestion apparatus of a 6 months baby

MIDA system

Mastication

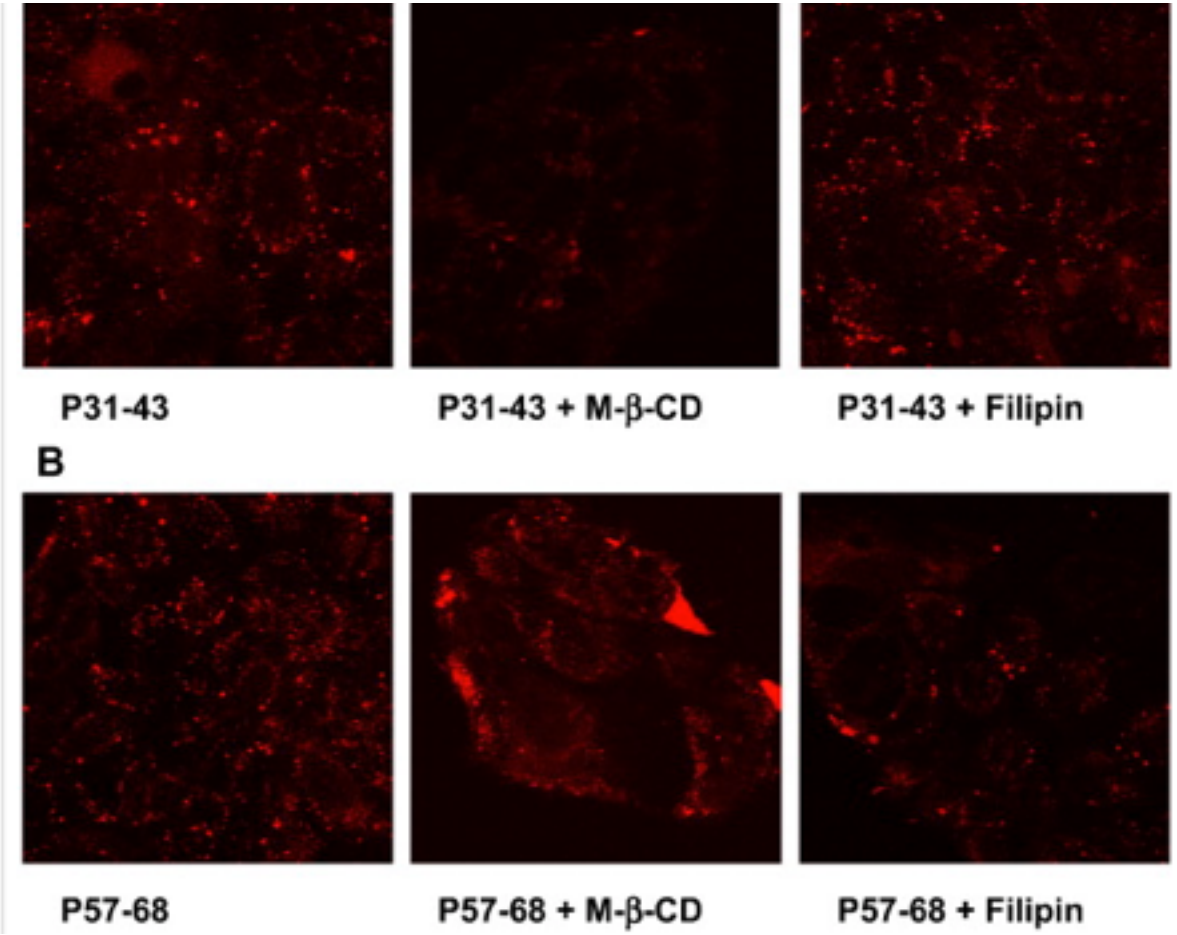
Gastric digestion

Intestinal digestion

Passannanti F et al: In vitro dynamic model simulating the digestive tract of 6-month-old infants. PLoS One. 2017
Gallo M et al: Effect of pH control during rice fermentation in preventing a gliadin P31-43 entrance in epithelial cells. Int J Food Sci Nutr. 2019

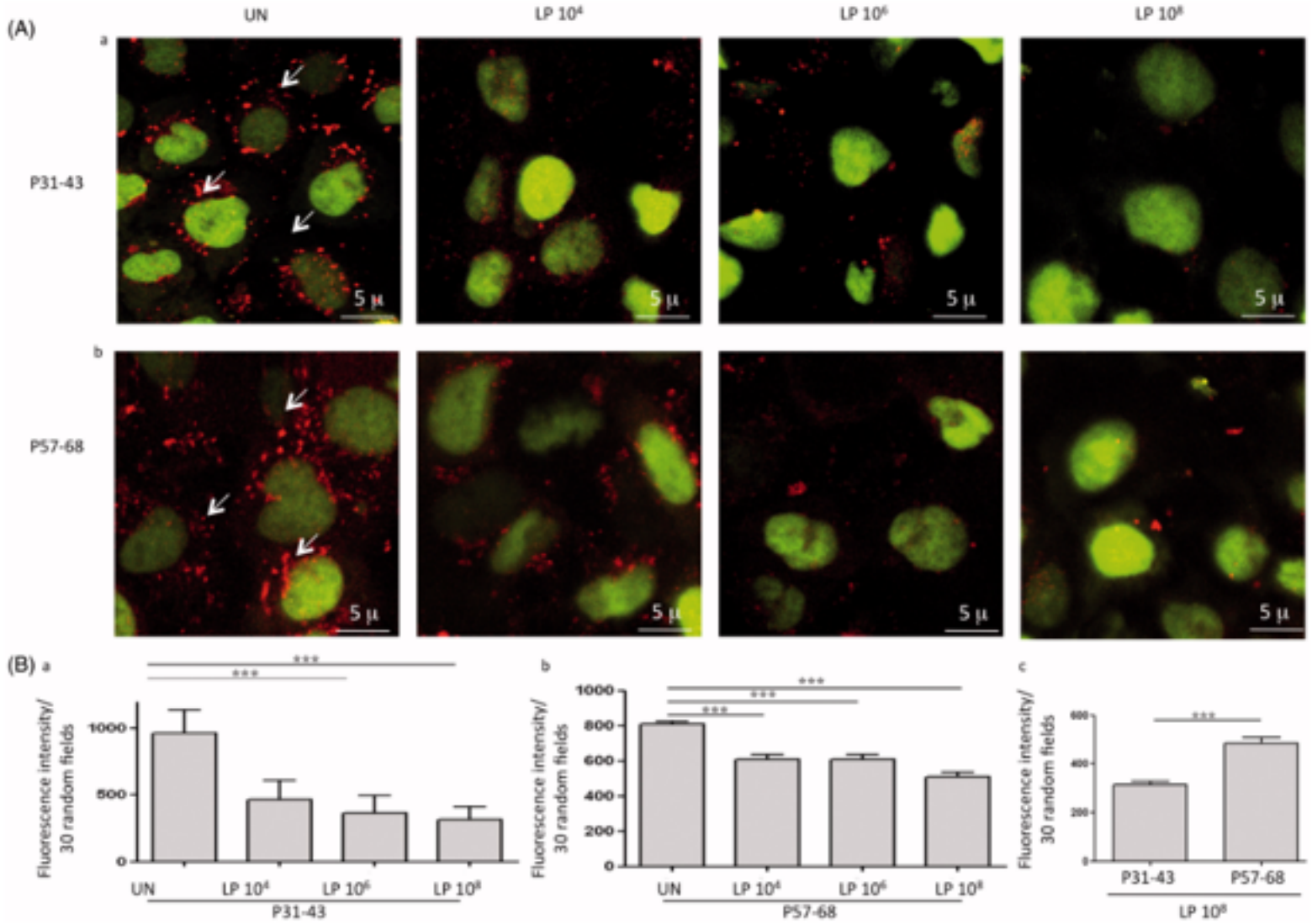
Gliadin peptides entered epithelial cells by endocytosis

Entrance of labelled peptides required:
1)37C Temperature
2)Ca++



Caputo I et al BBA 2010, Zimmermann C PLoS One 2014,

LB CBA L74 can prevent gliadin peptides entrance into CaCo2cells



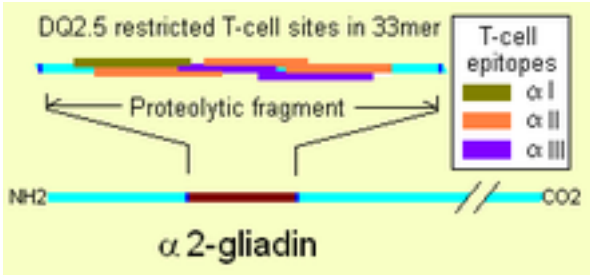
Barone MV et al J of Functional Food 2014 and 2019

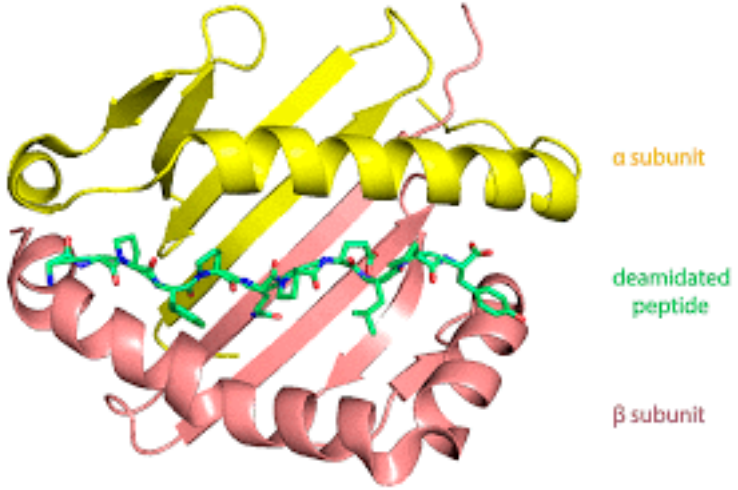
The two indigested peptides have different biological effects

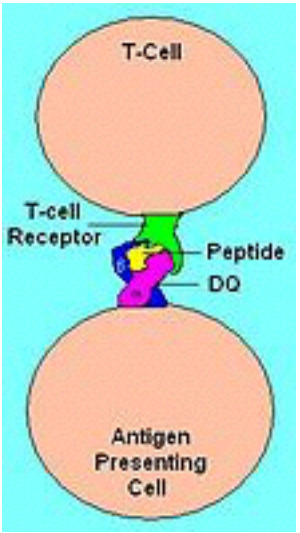
P57-68 is one of the main peptides able to activate the T cell mediated immune response

"T-CELL IMMUNOGENIC" PEPTIDE

P56-68 QLQPFPQPQLPY





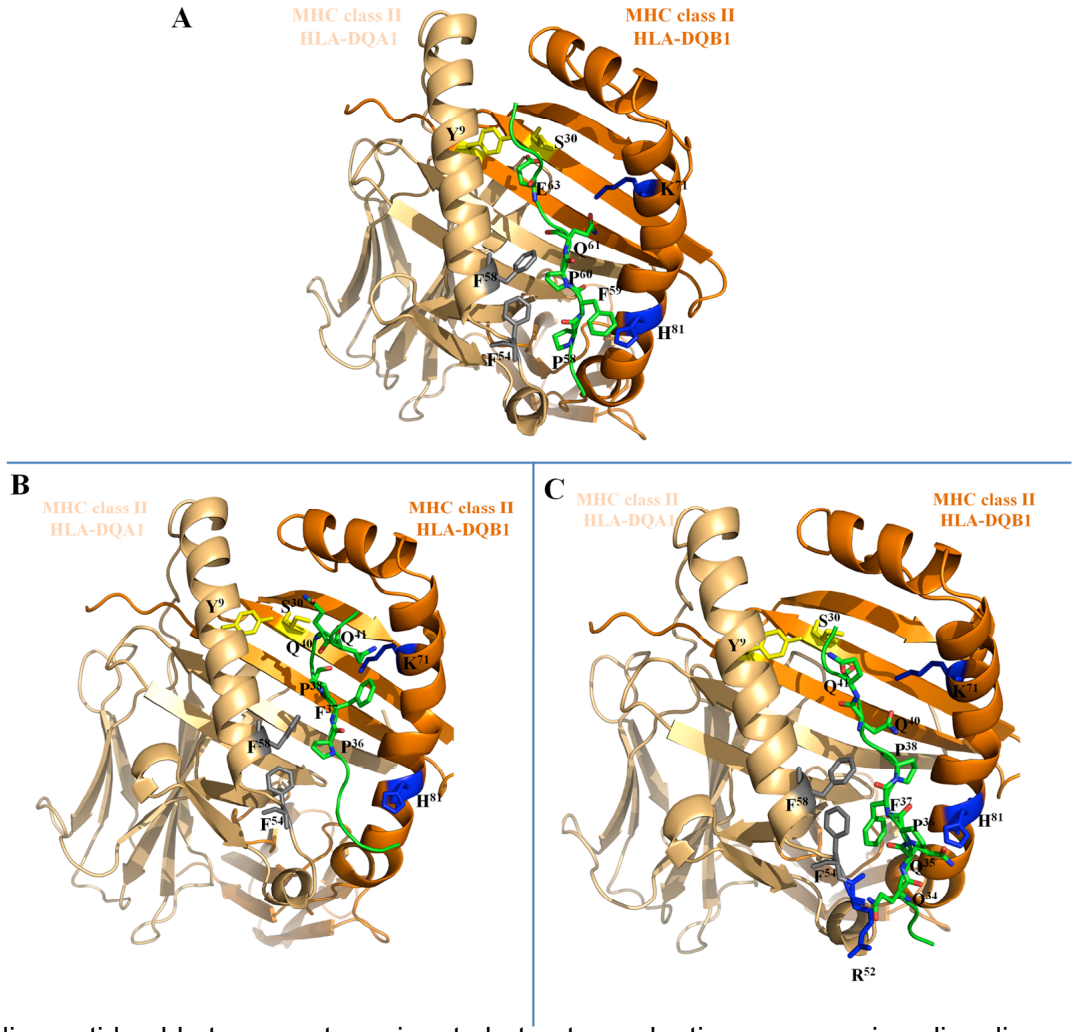


P31-43 is not presented and activates the innate immune response

"TOXIC" PEPTIDE

31-43 LGQQQPFPPQQPY

Agglutination of K538 cells
Apoptosis
Actin rearrangements
Induces morphological changes in CD patients intestine
Not immunogenic for T-cells
Activates innate immunity



Calvanese L et al : Structural insights on P31-43, a gliadin peptide able to promote an innate but not an adaptive response in celiac disease. J Pept Sci. 2019

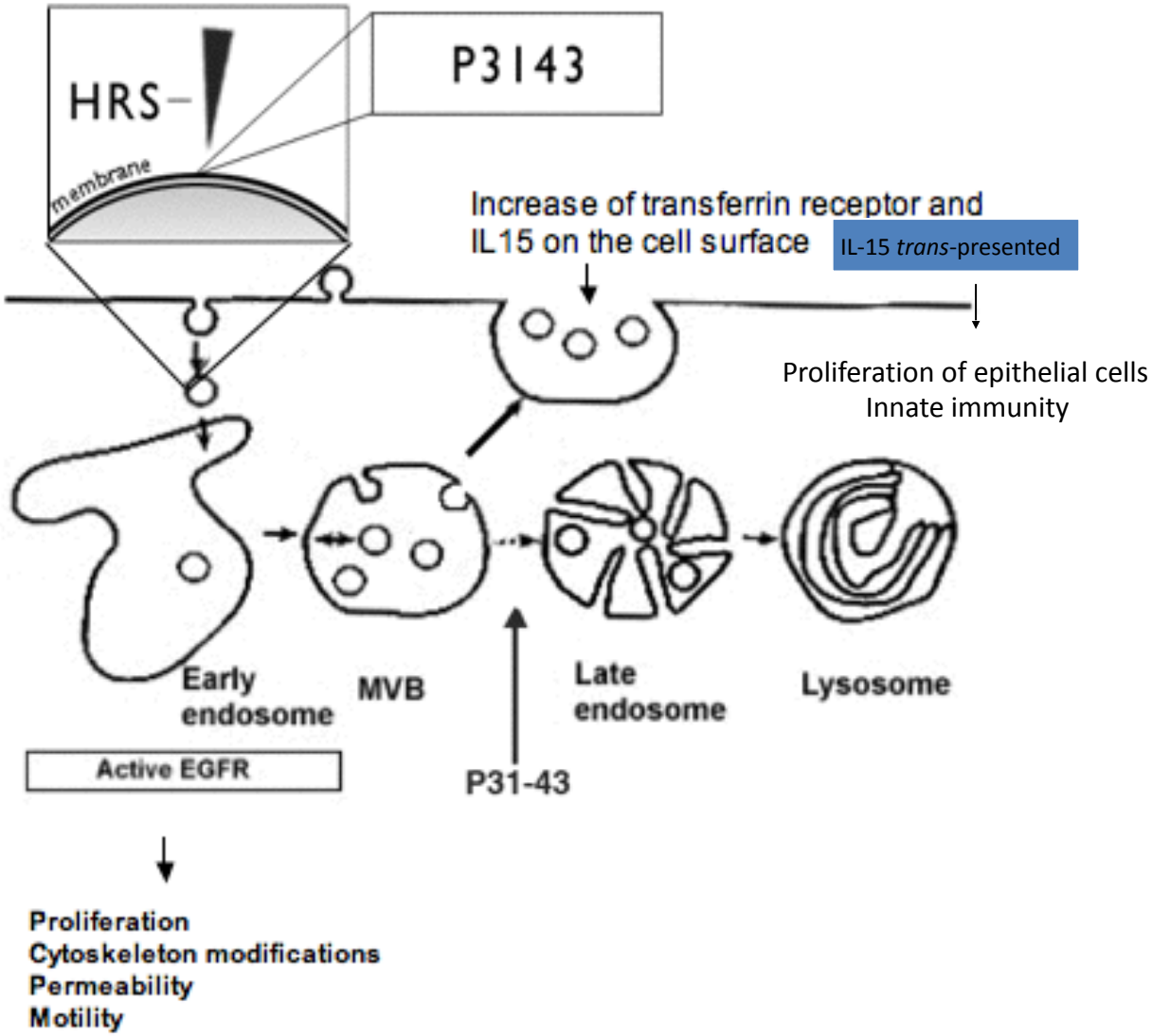
Gliadin peptide P31-43 is similar to HRS

(Hepatocyte growth factor-regulated tyrosine kinase substrate)

Hrs is a key protein for the regulation of endocytic maturation

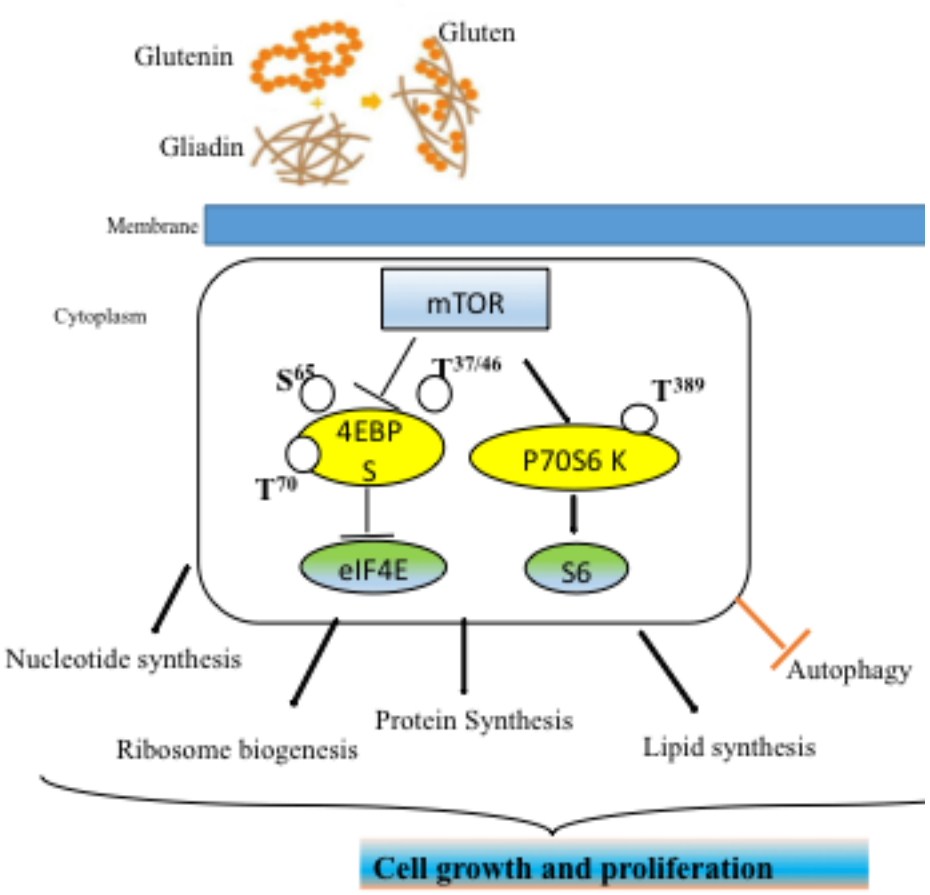
Barone MV et al GUT 2007, PloS One 2010

Gliadin peptides can delay endocytic maturation and increase recycling vesicles

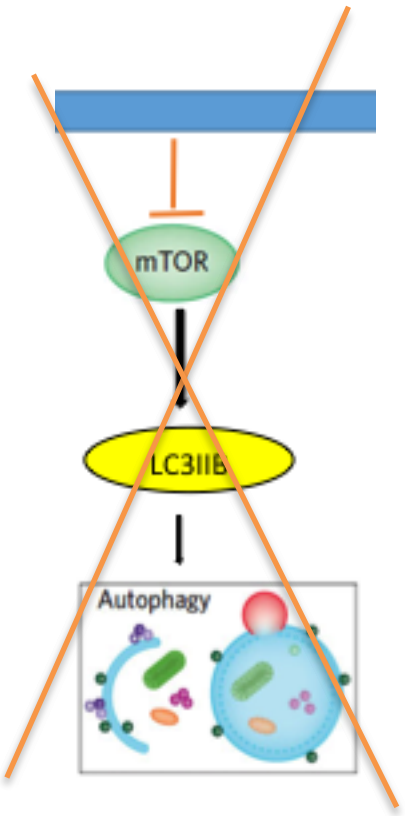


M.V. Barone et al
Plos One 2010 and 2011,
Am J Clin Nutr. 2013
Sci Rep. 2018 ,
Cell Biol Int. 2018
Communications Biology 2019

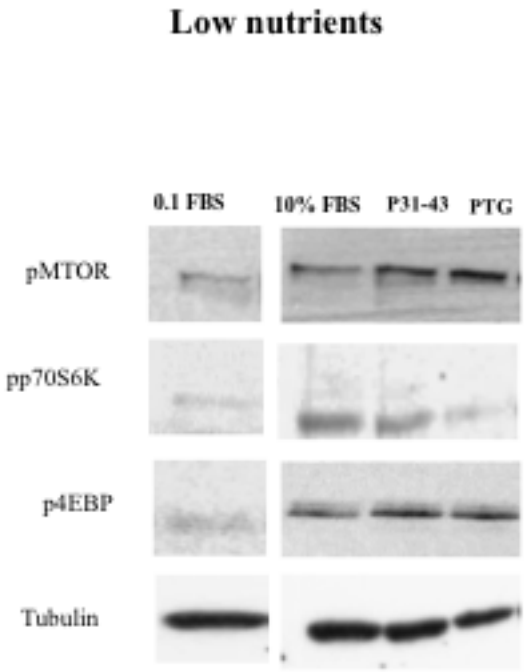
Gliadin peptides can activate the mTOR pathway

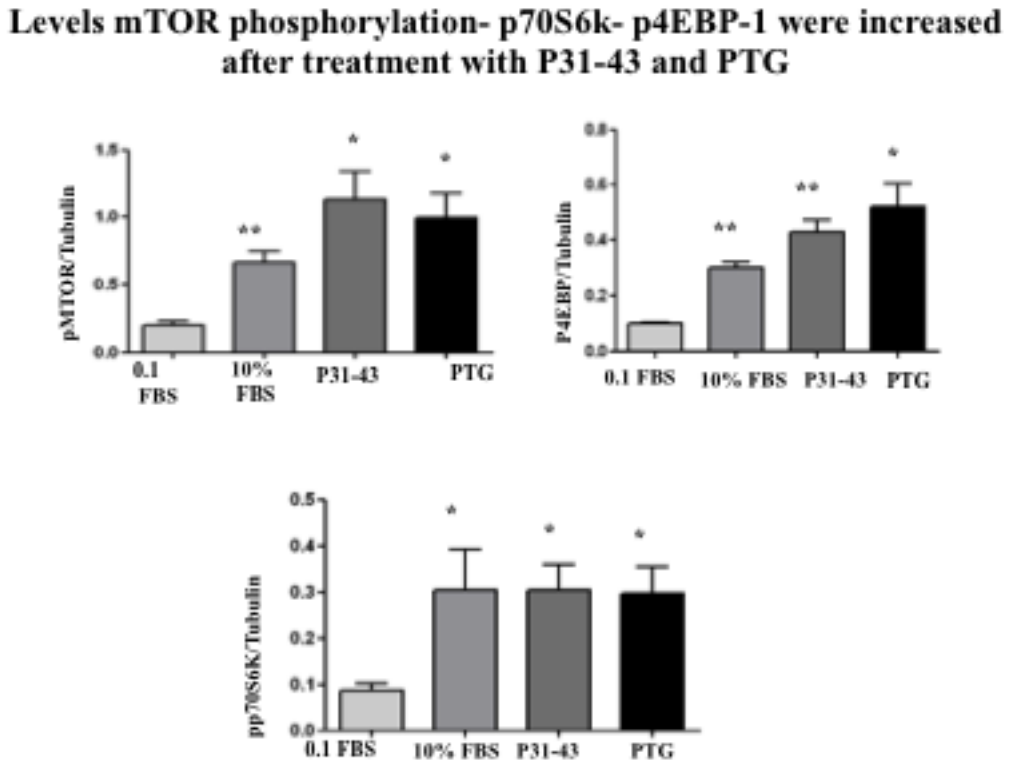


Authophagy

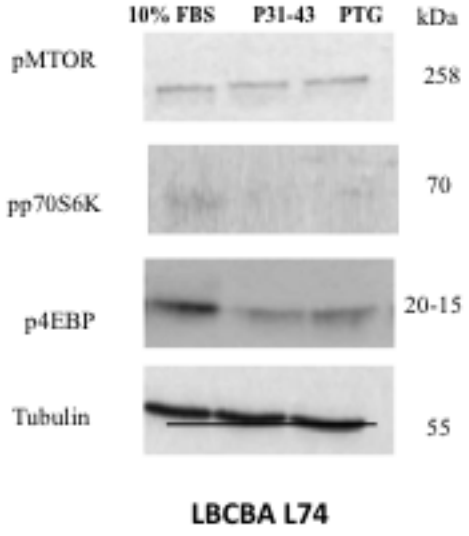


P31-43 can activate the mTOR pathway

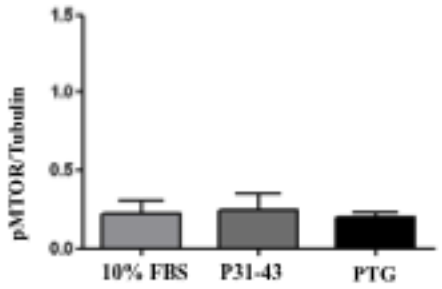


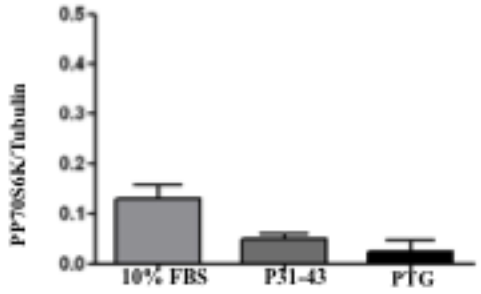


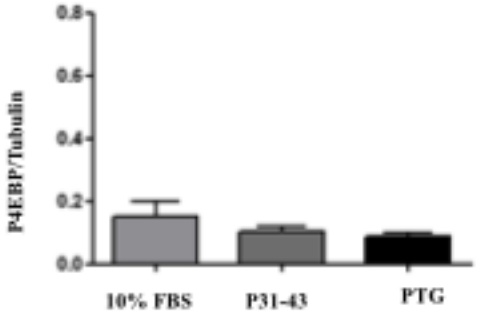
LB CBA L74 can prevent P31-43 effects on mTOR pathway



LBCBA L74

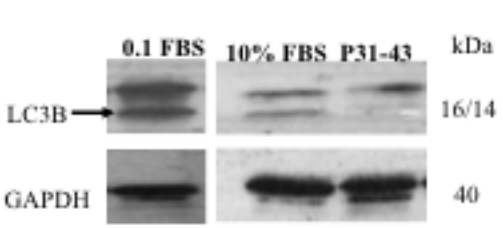


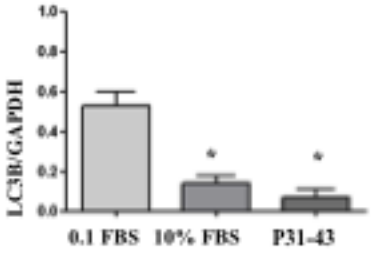


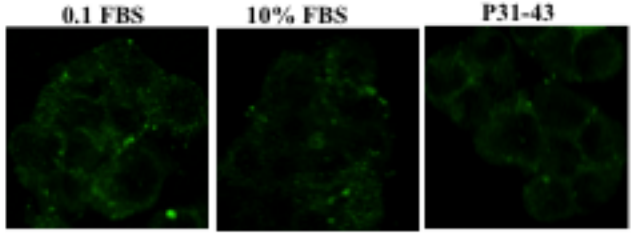


P31-43 decreases LC3 levels and LB CBA L74 can prevent this effect

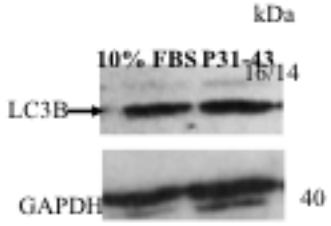
Not treated

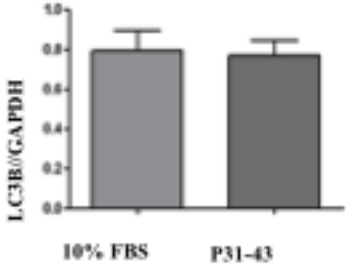


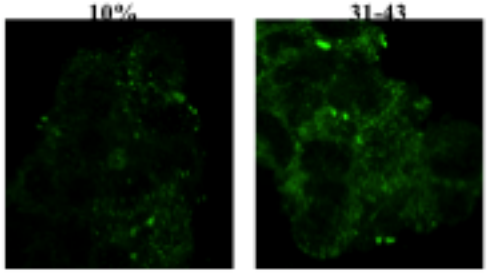




Treated with LB CBA L74



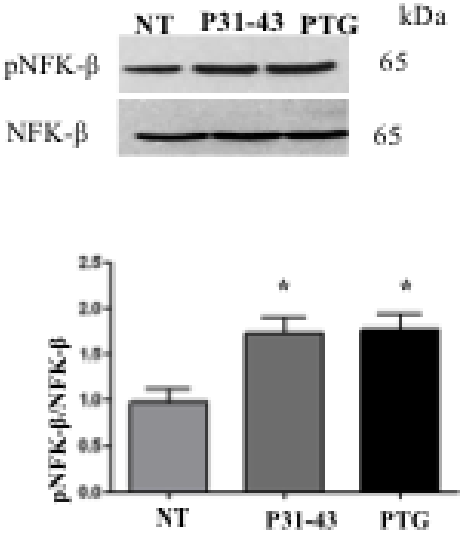




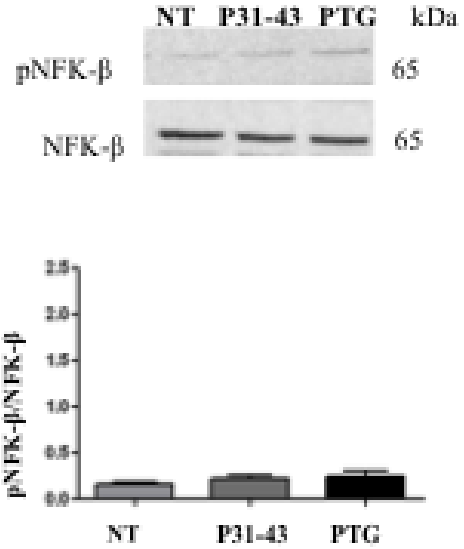
Low nutrients

P31-43 induces marker of inflammation NFkB and LB CBA L74 can prevent this effect

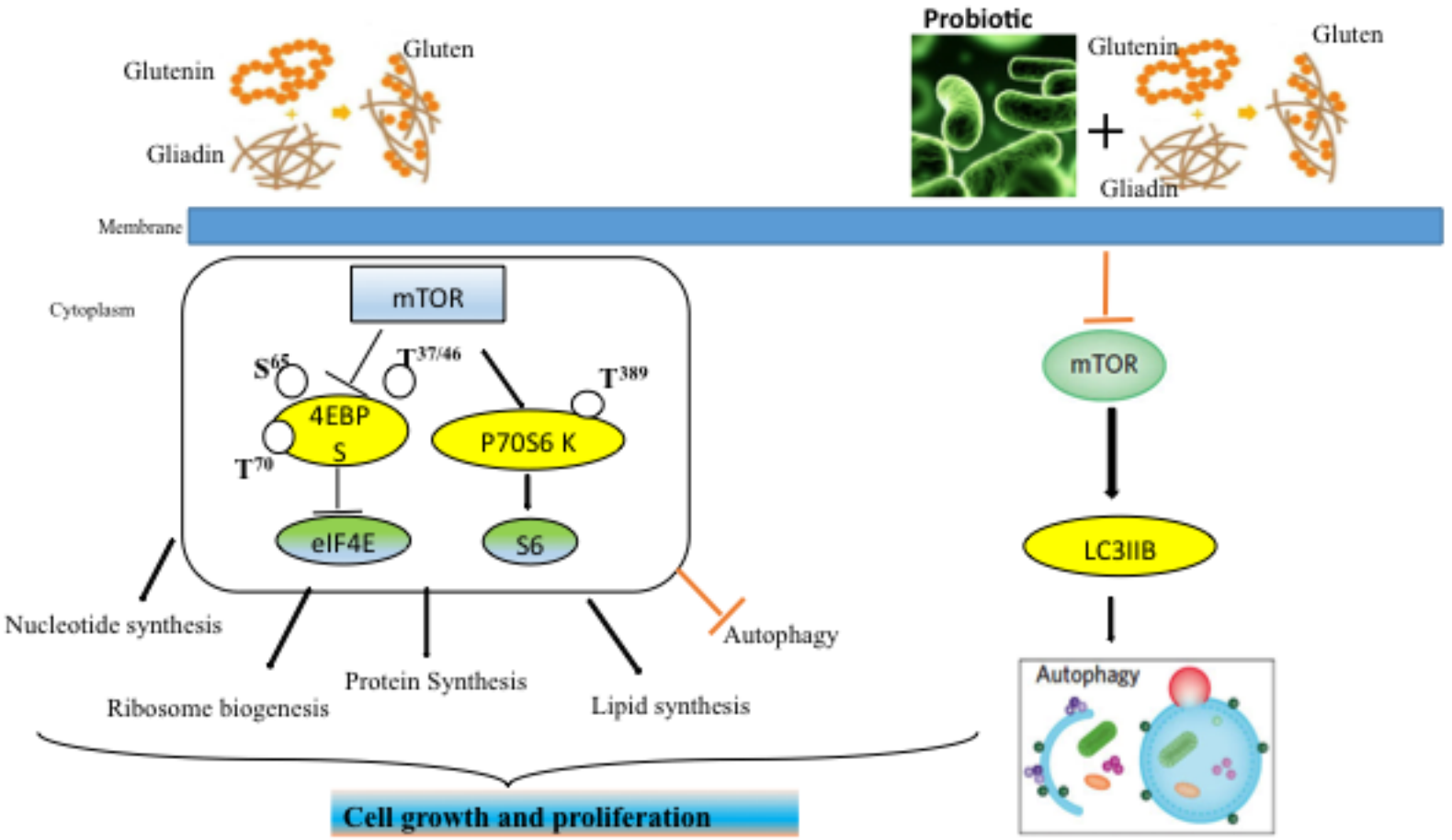
Not treated



Treated with LB CBA L74



LB CBA L74 can prevent P31-43 activity on mTOR pathway



WILEY CELLULAR MICROBIOLOGY

This Article | For Authors | Learn More | Submit

Cell Microbiol. 2019 Aug; 21(8): e13035.
Published online 2019 May 20. doi: 10.1111/cmi.13035

PMCID: PMC6618323
PMID: 31042331

Celiac disease-associated Neisseria flavescens decreases mitochondrial respiration in CaCo-2 epithelial cells: Impact of Lactobacillus paracasei CBA L74 on bacterial-induced cellular imbalance

Giuseppe Labruna,[1] Merlin Nanayakkara,[2] Chiara Pagliuca,[3] Marcella Nunziato,[3,4] Laura Iaffaldano,[4] Valeria D'Argenio,[3,4,5] Roberta Colicchio,[3] Andrea L. Budelli,[6] Roberto Nigro,[7] Paola Salvatore,[3] Maria Vittoria Barone,[✉2] and Lucia Sacchetti[✉4,5]

Conclusions

Lactobacilli can prevent many effects of gliadin peptides in vitro

Future plans

- Find the element/s that are effective in the lactobacilli supernatant
- test the effects of the Lactobacilli on a Celiac Cellular Model

Acknolegments

ELFID

Merlin Nanayakkara

Giuliana Lania

Mariantonia Maglio

Renata Auricchio

Riccardo Troncone

Salvatore Auricchio

CEINGE

Lucia Sacchetti

Valeria D'Argenio

Chemical Engineering
University of Naples Federico II
Roberto Nigro

Francesca Passannanti

Department of Pharmacy,
University of Naples Federico II

Gabriella D'Auria

Giancarlo Morelli

Lucia Falcigno

TiGEM

Antonietta de Matteis

IBP-CNR

Alberto Luini

Riccardo Rizzo

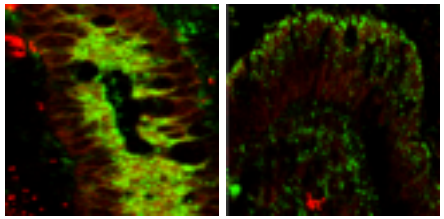
Cellular models of intestine

Intestinal biopsies in colture

Techniques
Biochemistry
Molecular biology
Immunohystochemistry
Immunofluorescence

Limits
No propagation
Small amount
No engineering

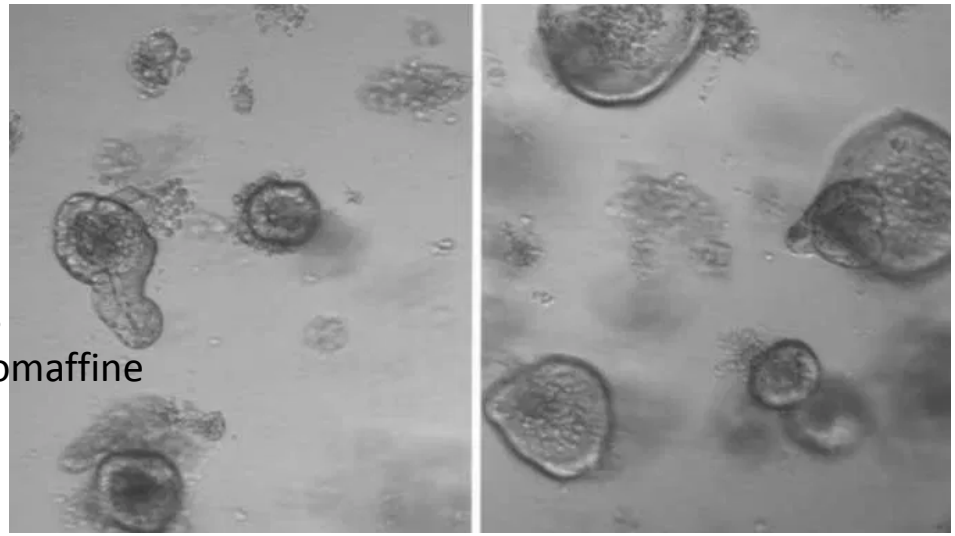
GCD CD



Crypts Villi

Intestine from staminal cells

Intestinal Organoids in 3D colture



Cells:
Epithelial
Mucipars
Enterocromaffine
Paneth

Techniques

Biochemistry
Molecular biology
Immunohystochemistry
Immunofluorescence

They can be propagated indefinitely

They can be engineered