Probiotic Approach for Mitigation of Stress Adverse Effects

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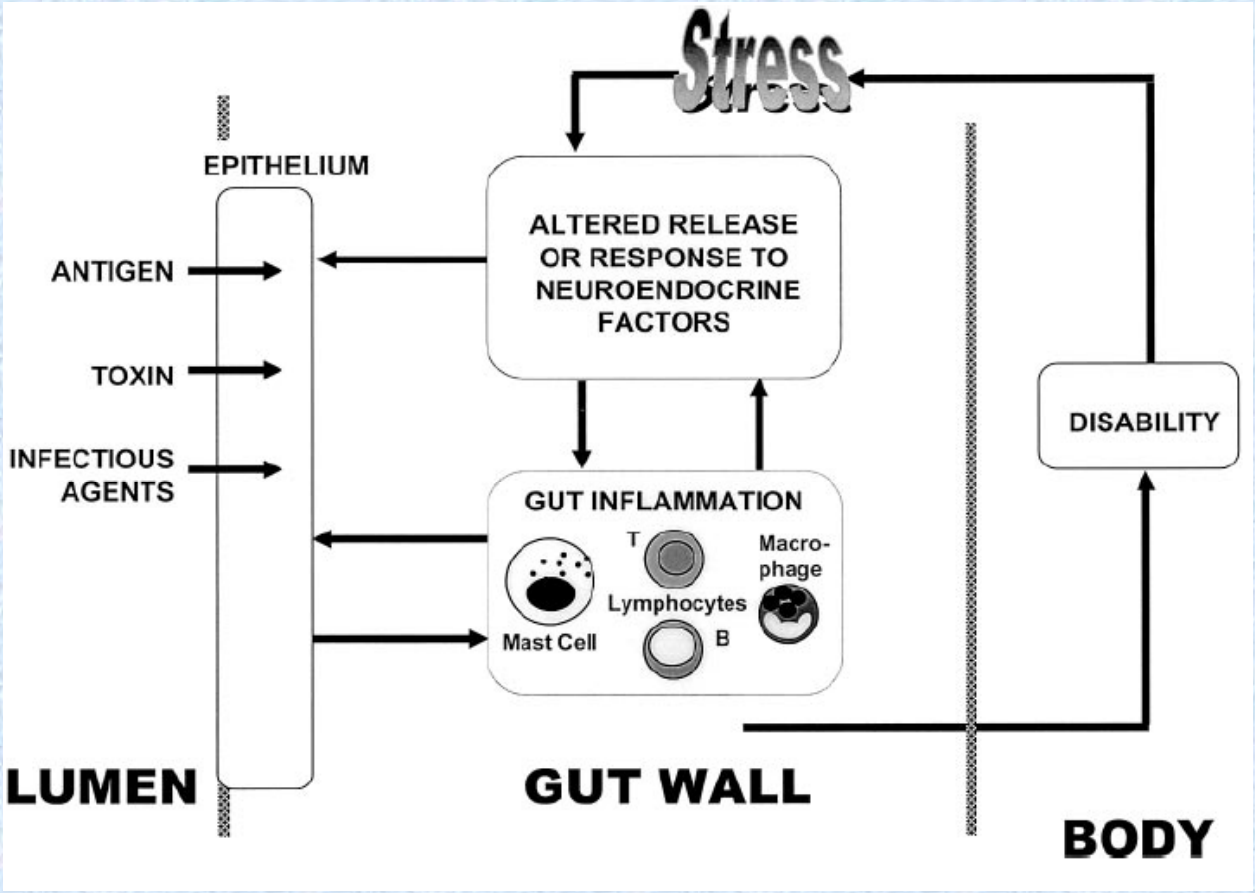
“Stress is any threat to the homeostasis of an organism”

Selye H., Nature, 1936

Conditions Associated With Changes in Gut Microflora

Condition	References
Thermoregulation	Kent et al., 1992
Neuroendocrine control	Kent et al. 1992
Sleep	Kent et al. 1992
Social behavior	Bercik, et al., 2011; Li, 2009
Cognition	Kent et al. 1992
Gut neuro-motor function	Verdu et al., 2009
Muscular activity	Verdu et al, 2006
Memory	Li, 2009
Anxiety	Bercik, et al., 2010, 2011a

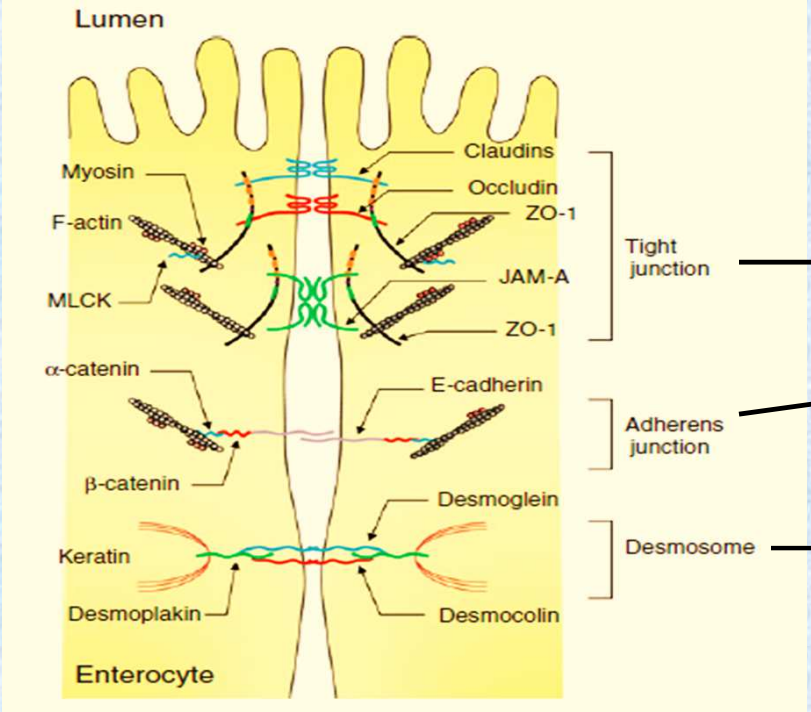
Impact of Stress on Intestinal Barrier Function

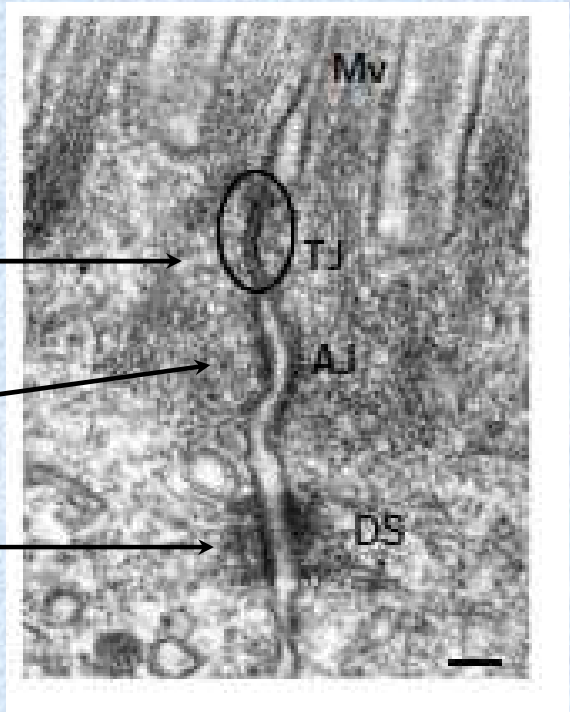


- **Stress, via signals from the central nervous system, leads to the altered release of and response to neuroendocrine factors (acetylcholine, neurotensin) in the intestinal mucosa.**
- **Such factors may act on the epithelium, inducing barrier dysfunction and the uptake of proinflammatory material from the gut lumen.**
- **The resultant inflammation causes disability and increases stress, which further amplifies the defect.**

Soderholm, Perdue, 2001

Intestinal barrier function - the ability to control uptake across the mucosa and to protect the gut from harmful substances present in the lumen. The intercellular junctions of intestinal epithelial cells are sealed by different protein complexes, including tight junctions (TJs), adherens junctions (AJs), and desmosomes. The TJs, multiple protein complexes, locate at the apical ends of the lateral membranes of intestinal epithelial cells and they act as a primary barrier to the diffusion of solutes through the intercellular space.





Tight Junction Integral Proteins

Claudin

- a family of ≥24 members
- the main structural components of intramembrane strands
- determine ion selectivity of paracellular pathway

Occludin

- regulates paracellular diffusion of small hydrophilic molecules
- has been linked to the formation of the intramembrane diffusion barrier
- regulates the transepithelial migration of neutrophils

Junctional adhesion molecule (JAMs)

- JAM is involved in formation and assembly of TJs in intestinal epithelial cells

The intestinal TJ barrier is dynamically regulated by physiological and pathophysiological factors:

- **microorganisms (probiotics and pathogens)**
- **cytokines**
- **food factors**

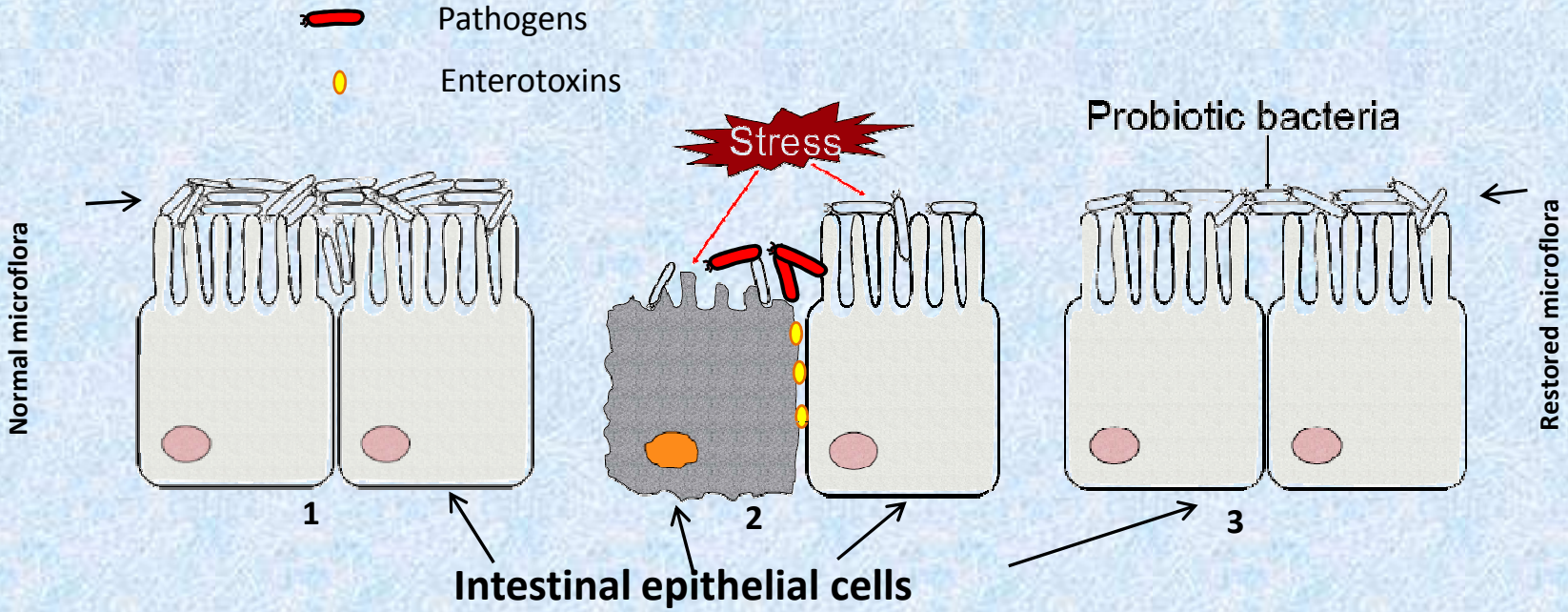
Downregulation of Intestinal Tight Junction by Pathogens

- *Vibrio cholerae*
- Enteropathogenic *E. coli*
- *Clostridium perfringens*

Upregulation of Tight Junction Proteins by Probiotics

- *Streptococcus thermophilus*
- *Lactobacillus acidophilus*
- *Escherichia coli* Nissle 1917
- *Saccharomyces boulardii*

Effect of Probiotic Bacteria on Stress-Inhibited Intestine



Healthy intestine (1):

- physical barrier to hinder invasion of pathogens
- immune system development
- activation of immune and inflammatory response

Stress effects (2):

- depression of mucosal barrier function
- immune system depression
- reduction of the bacteria of the normal microflora

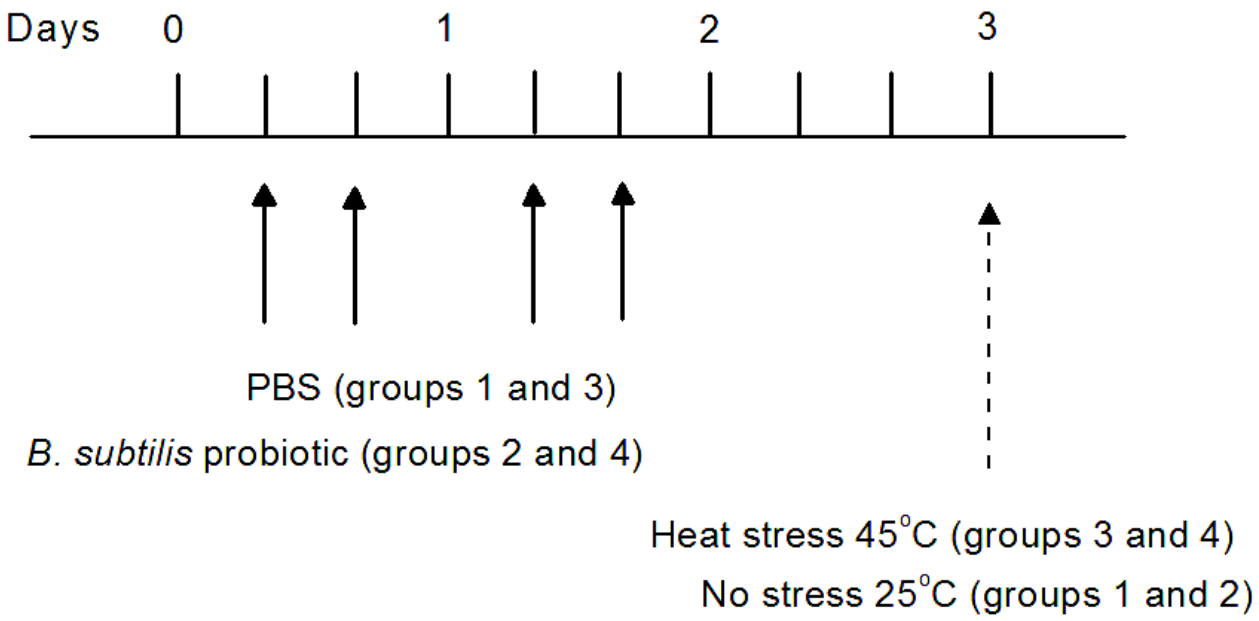
Effect of probiotic (3):

- restoration of normal microflora and mucosal barrier function
- activation of immune system

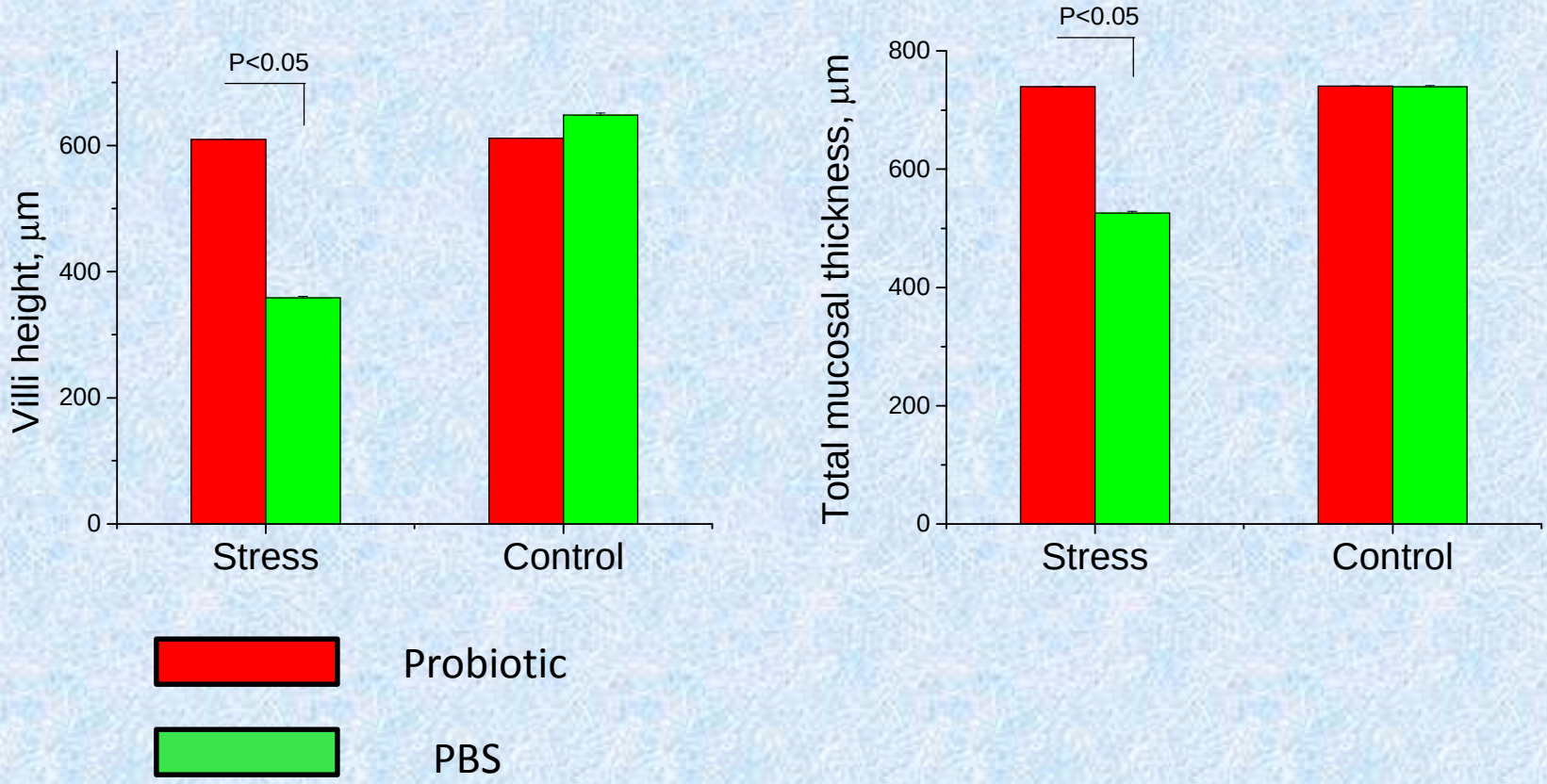
Types of Stressors

- Physical
- Psychological
- Chemical

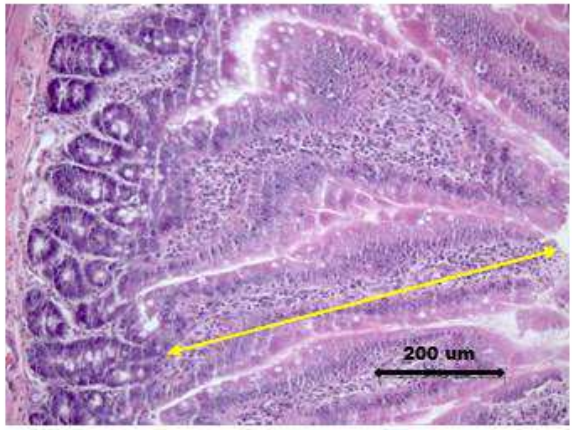
Experimental Design



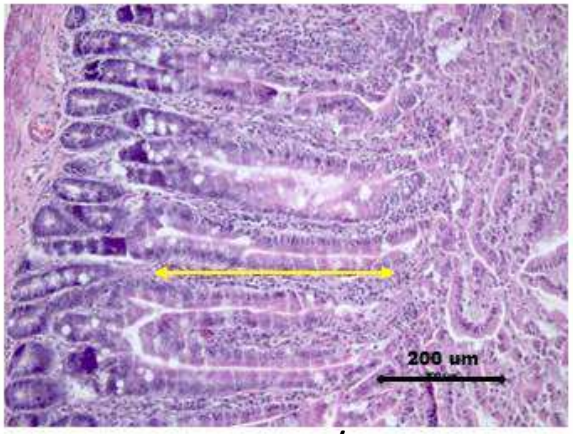
Protective Effect of *Bacillus subtilis* Probiotic on Gut Epithelial Cells



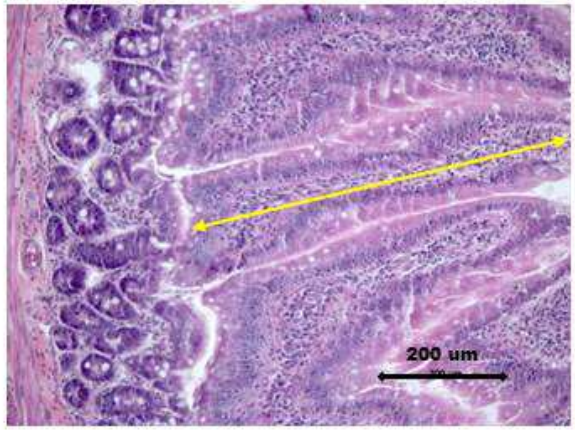
Histological images of intestinal mucosa



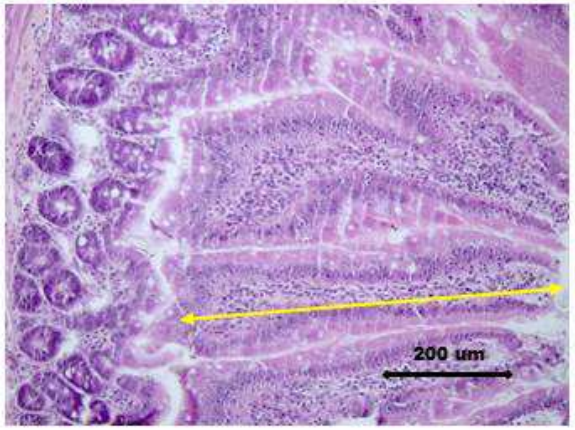
PBS/25°C



PBS/45°C

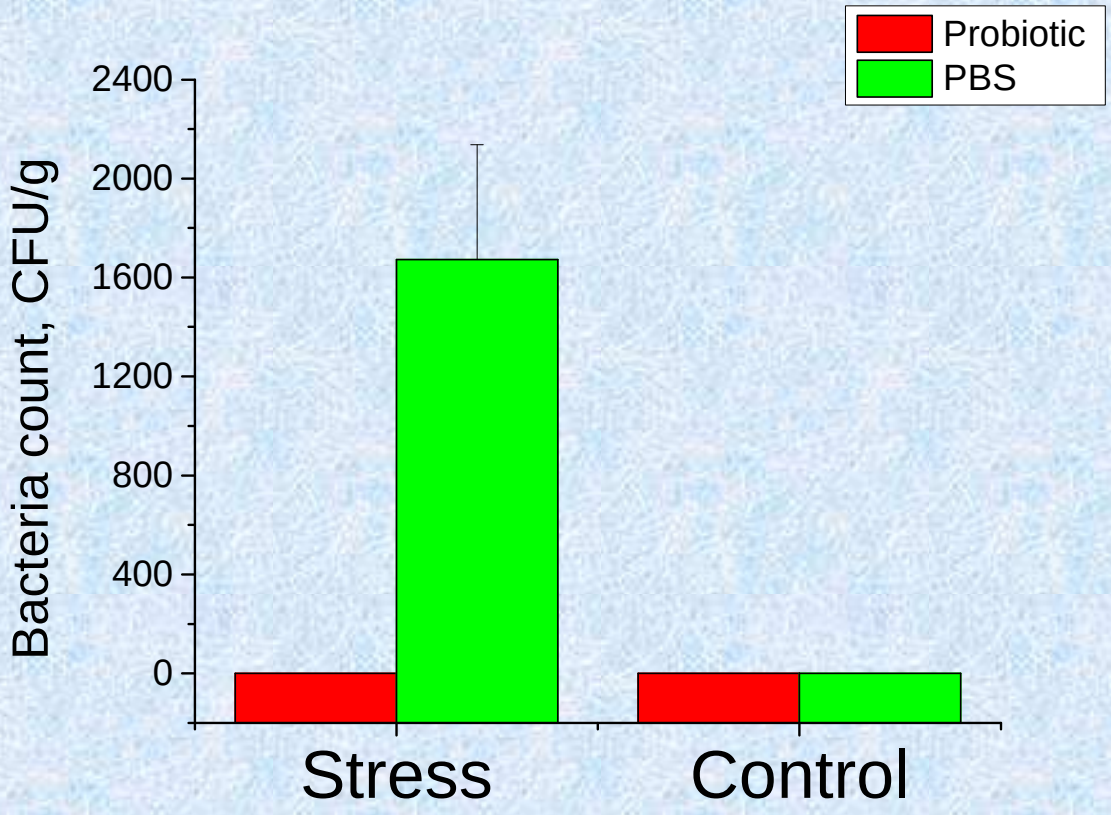


Probiotic/25°C

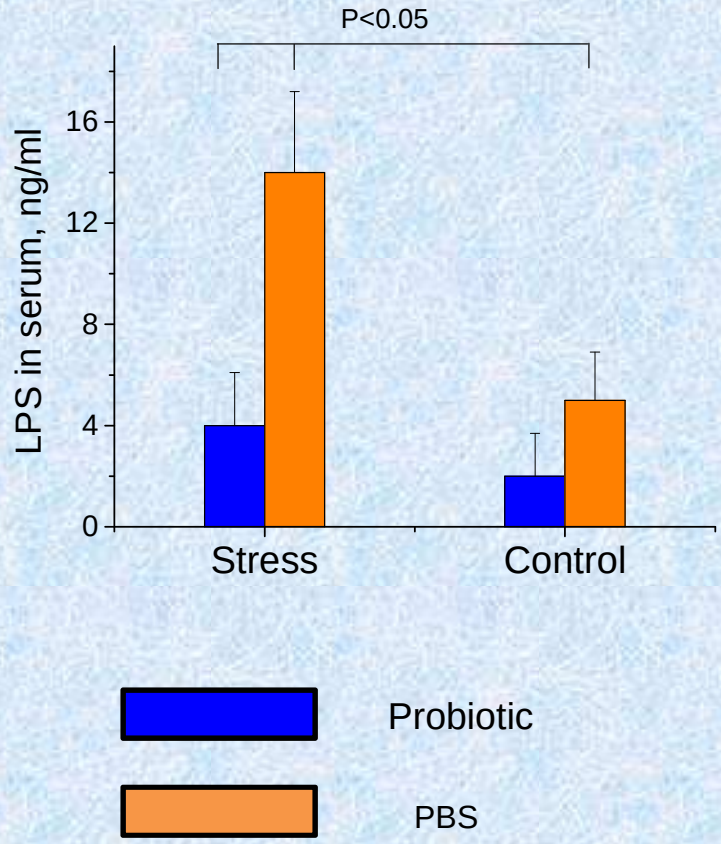


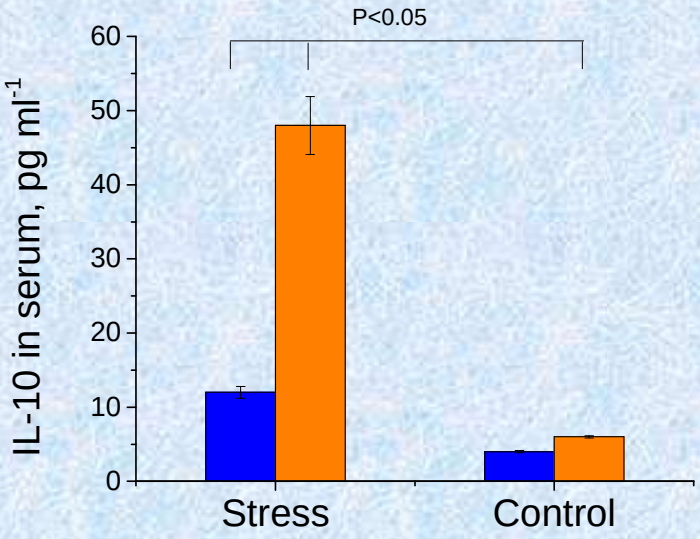
Probiotic/45°C

Prevention of Bacterial Translocation by Probiotic

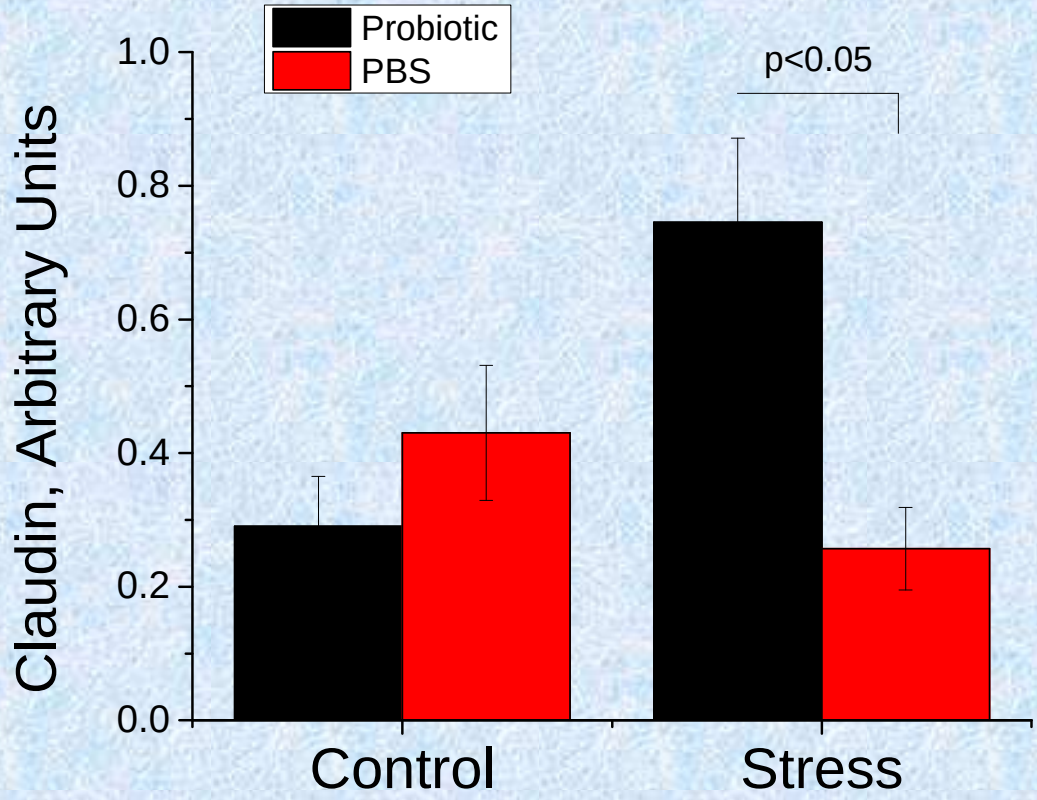


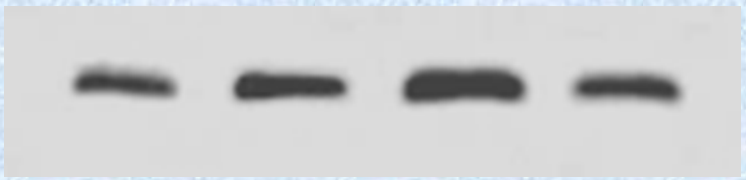
Protective Effect of Probiotic





Beneficial Effect of *Bacillus* Probiotic on Intestinal Tight Junction

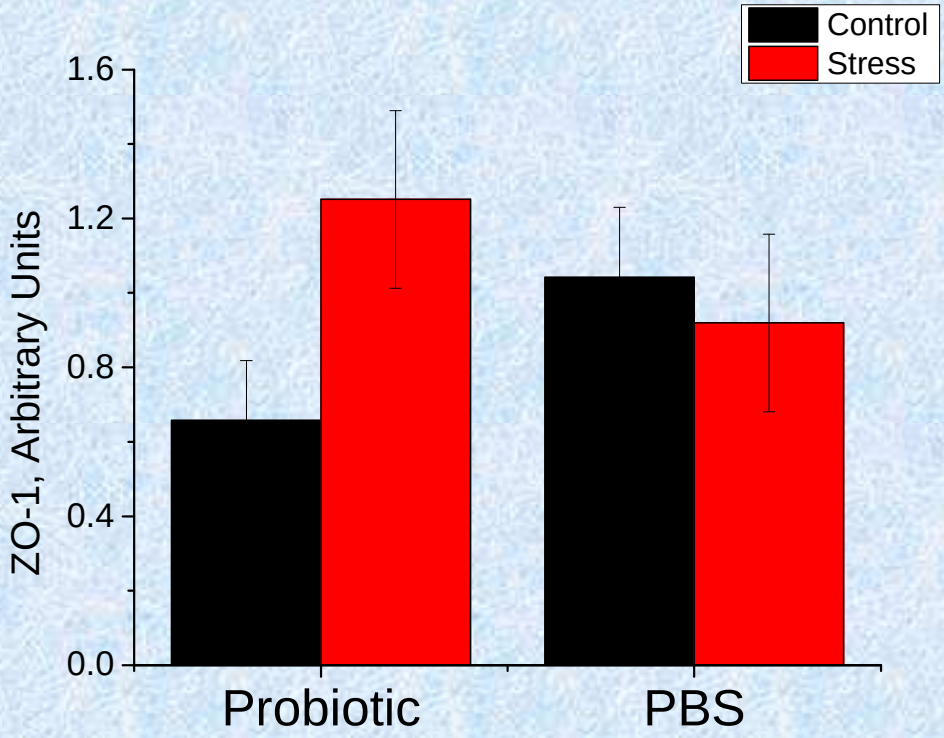


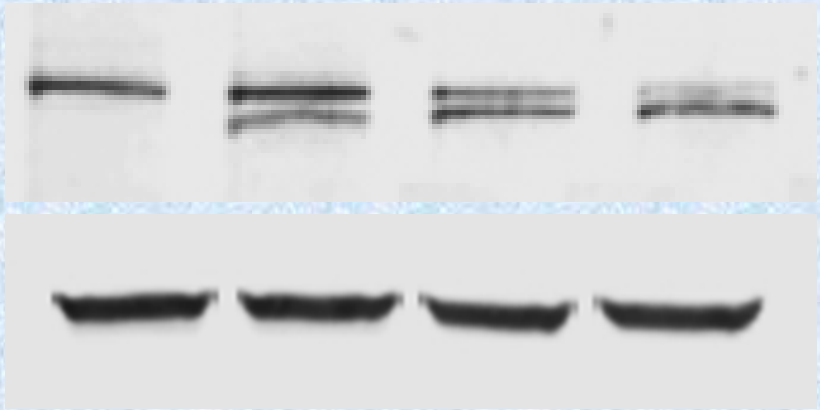


Claudin

Actin

Beneficial Effect of *Bacillus* Probiotic on Intestinal Tight Junction

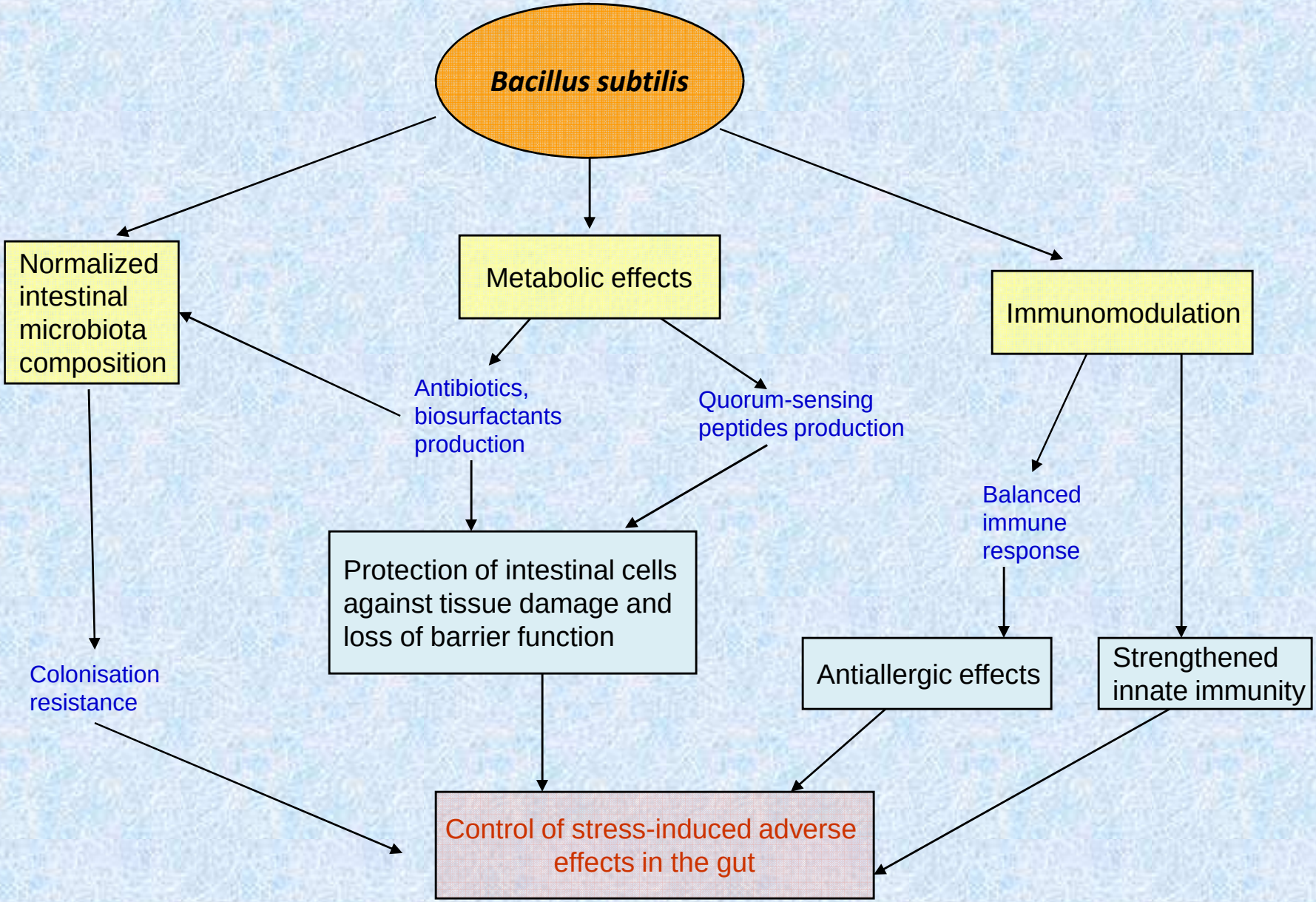




ZO-1

Actin

Mechanism of Action



Conclusion

- ***Bacillus subtilis* probiotic prevents heat stress-related complications:**
  - changes in morphology of intestinal cells
  - translocation of bacteria into lymph nodes and liver
  - elevation of LPS level in serum
  - changes of serum cytokines composition
  - changes of TJ proteins composition
- **Upregulation of TJ proteins with probiotic in rats exposed to heat stress is one of the mechanisms of animal protection against stress-related adverse effects.**

Probiotics:

“Live microorganisms which when administered in adequate amounts confer a health benefit on the host”

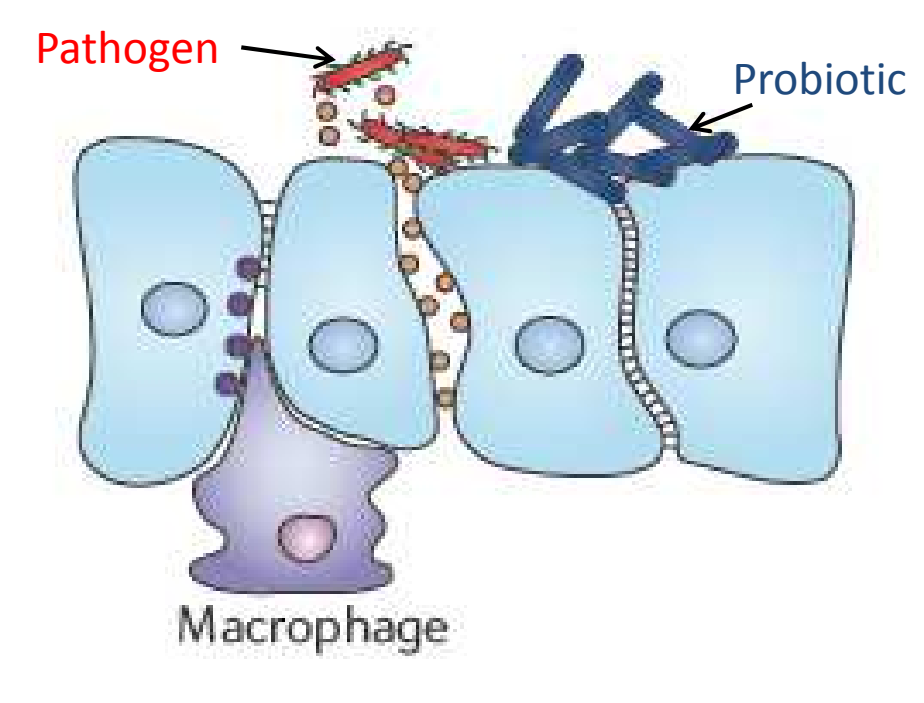
Joint FAO/WHO Expert Consultation, October 2001

http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf

Probiotic Microorganisms

- *Bifidobacterium breve*
- *B.bifidum*
- *B. adolescentis*
- *B.infantis*
- *B.lactis*
- *B.longum*
- *B. thermophilum*
- *Lactobacillus acidophilus*
- *L.delbrueckii* subs*.bulgaricus*
- *L.casei*
- *L.johnsonii*
- *L. reuteri*
- *L. crispatus*
- *L. fermentum*
- *L. Gasseri*
- *L. brevis*
- *L.plantarum*
- *L. ramnosus*
- *L. salivarius*
- *Lactococcus lactis*
- *Enterococcus faecium*
- *Streptococcus salivarius*
- *Pediococcus acidilactici*
- *Bacillus cereus*
- *B. clausii*
- *B.coagulans*
- *B. subtilis*
- *B. licheniformis*
- *Escherishia coli*
- *Propionibacterium shermanii*
- *Saccharomyces cerevisiae*
- *S. boulardii*

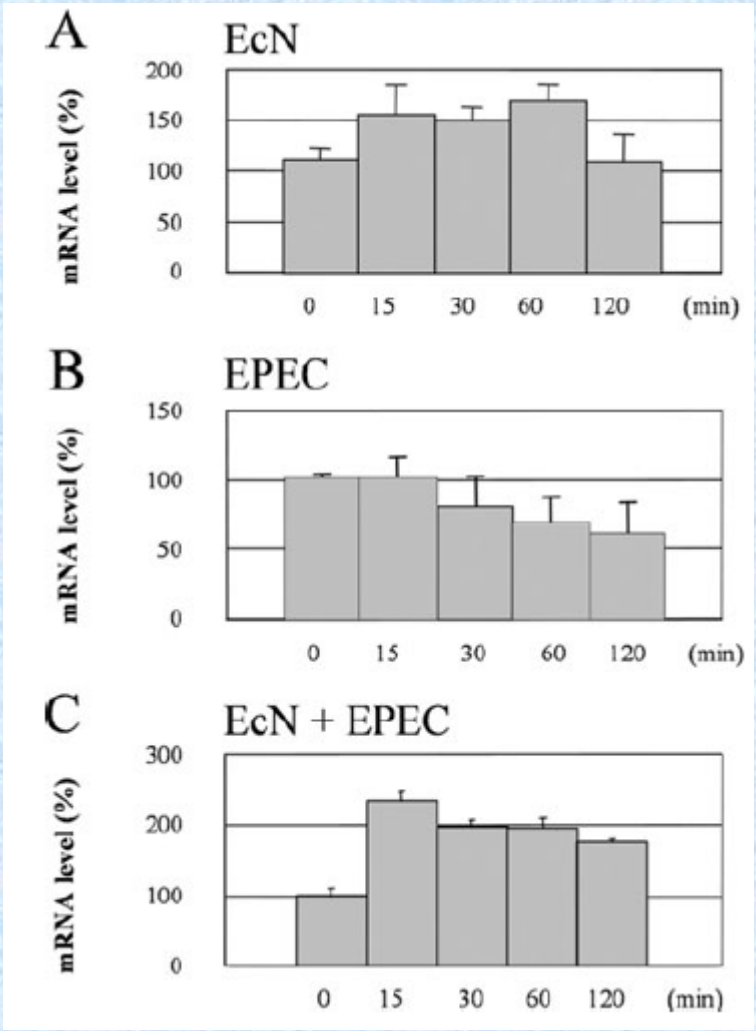
Modulation of tight junctions



Upregulation of tight junction proteins (**occludin, claudin, and junctional adhesion protein)** might help to limit the damage that is caused to epithelia by inflammatory processes or pathogens.

The probiotic-coated surface retains an intact junction.

Beneficial Effect of Probiotic on Intestinal Tight Junction

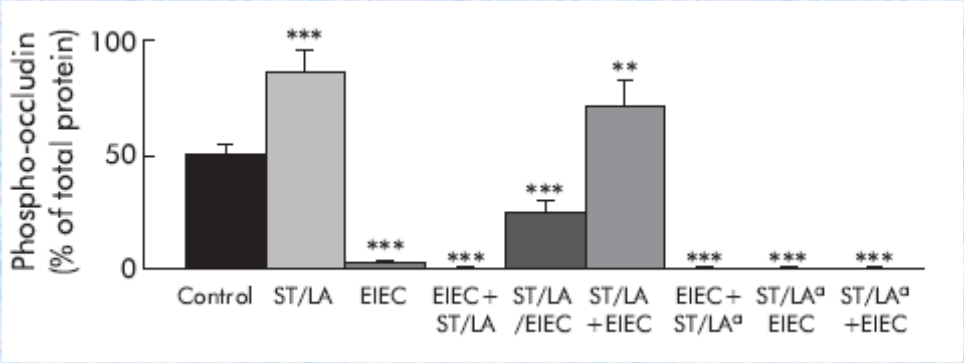


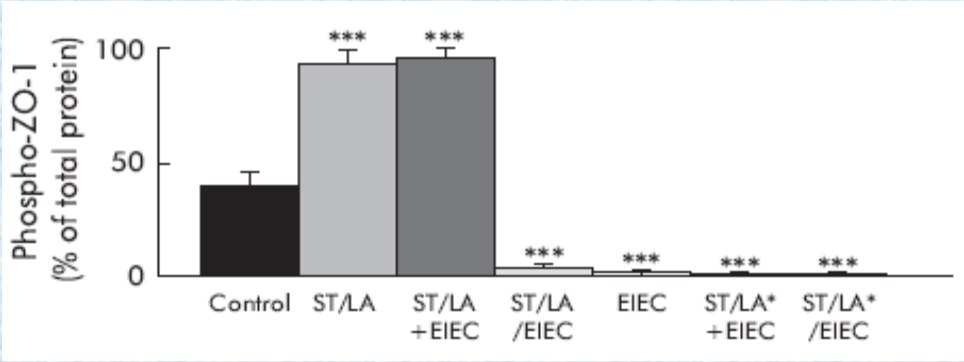
Effect of probiotic bacteria *Escherichia coli* Nissle 1917 on changes in ZO-2 mRNA of T84 epithelial cell after infection with enteropathogenic *E.coli* (EPEC)

Zyrek，2006

ZO-2

Beneficial Effect of Probiotic on Intestinal Tight Junction



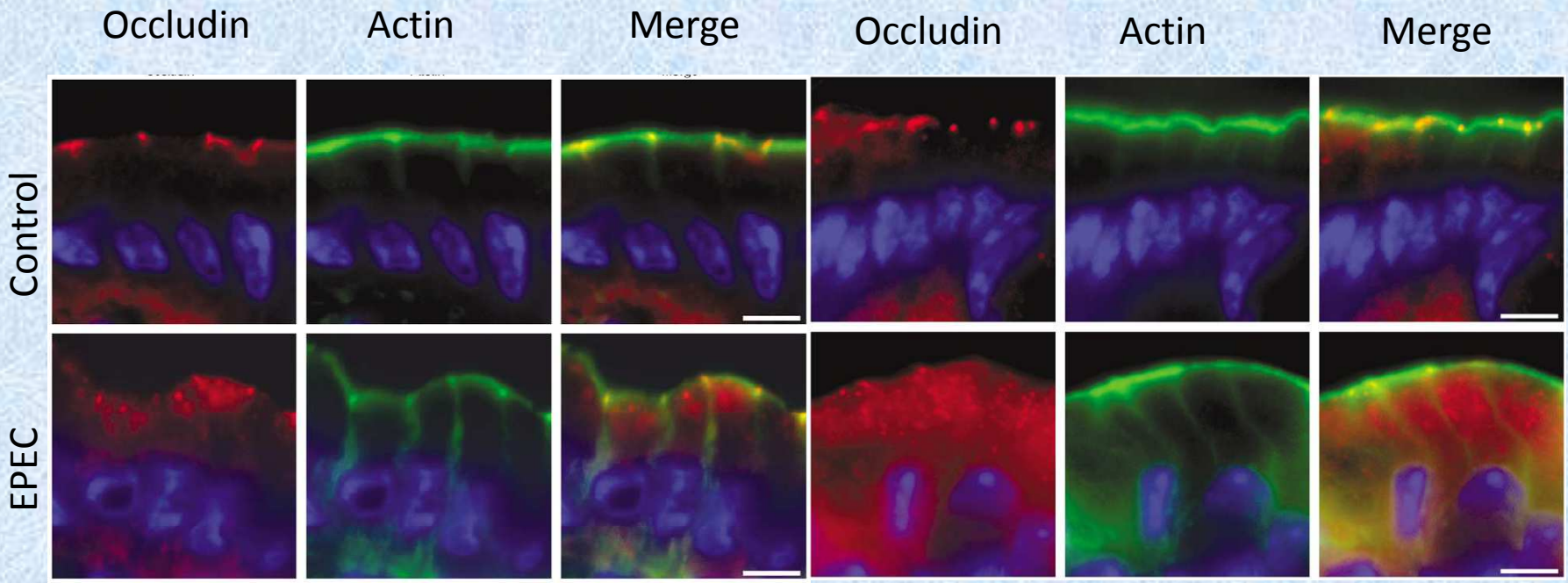


ST/LA - *Streptococcus thermophilus* and *Lactobacillus acidophilus*

EIEC - enteroinvasive *Escherichia coli*

Resta-Lenert, 2003

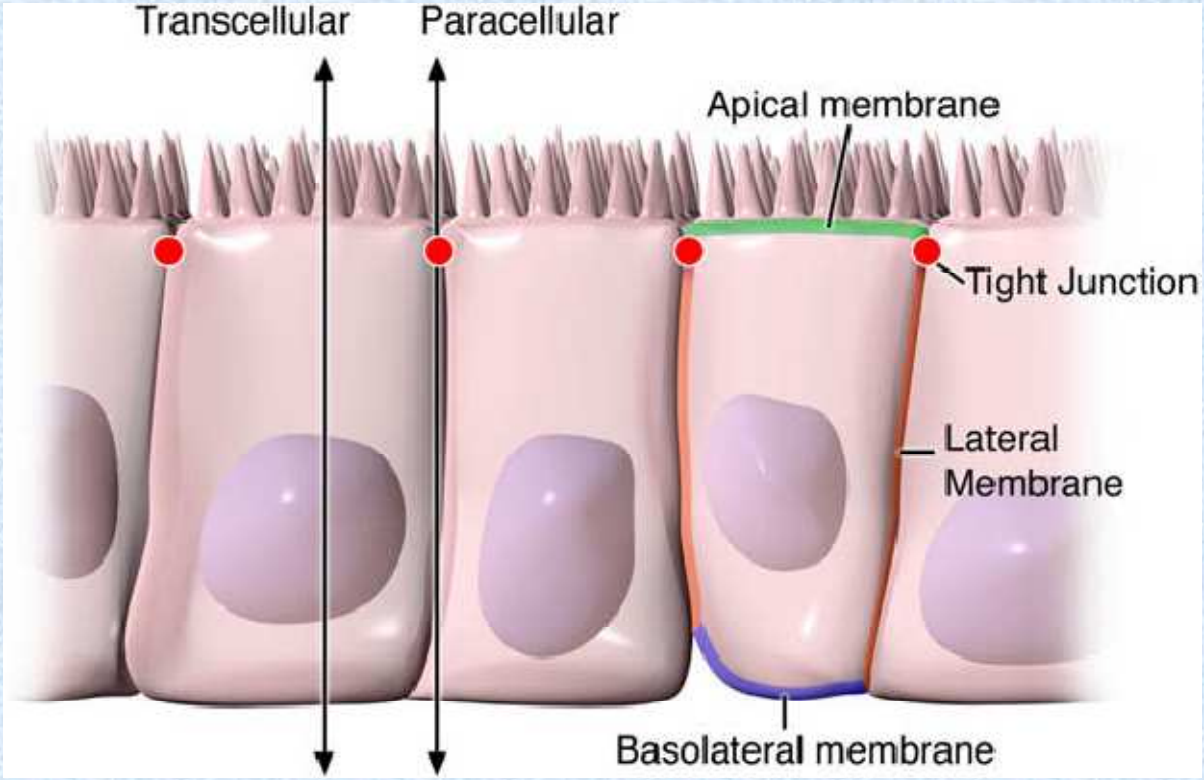
Occludin distribution after infection with enteropathogenic *E. coli* (EPEC)



a b

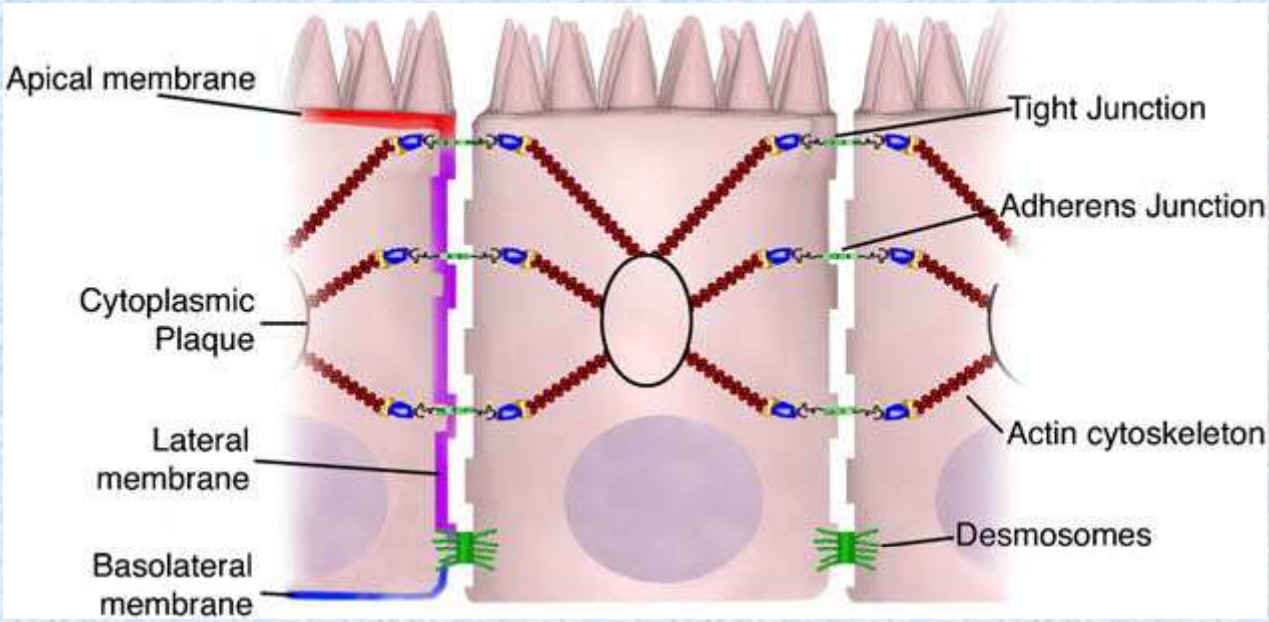
Ileal (a) and colonic (b) epithelium

Shifflett, 2005



Pathways of epithelial permeability. Transcellular permeability is associated with solute or water movement through intestinal epithelial cells. Paracellular permeability is associated with movement in the intercellular space between epithelial cells and is regulated by TJs localized at the junction of the apical-lateral membranes.

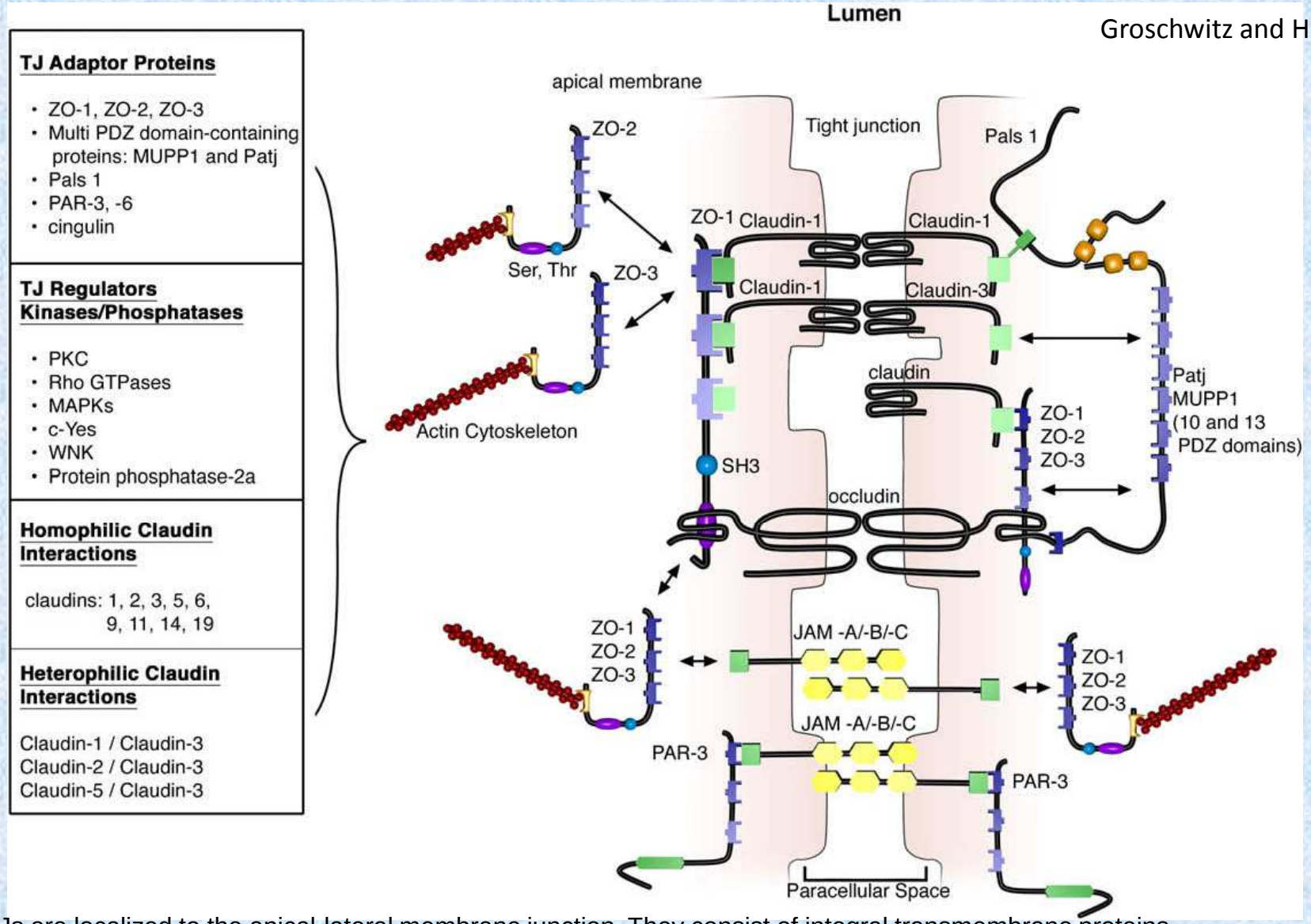
Groschwitz and Hogan, 2009



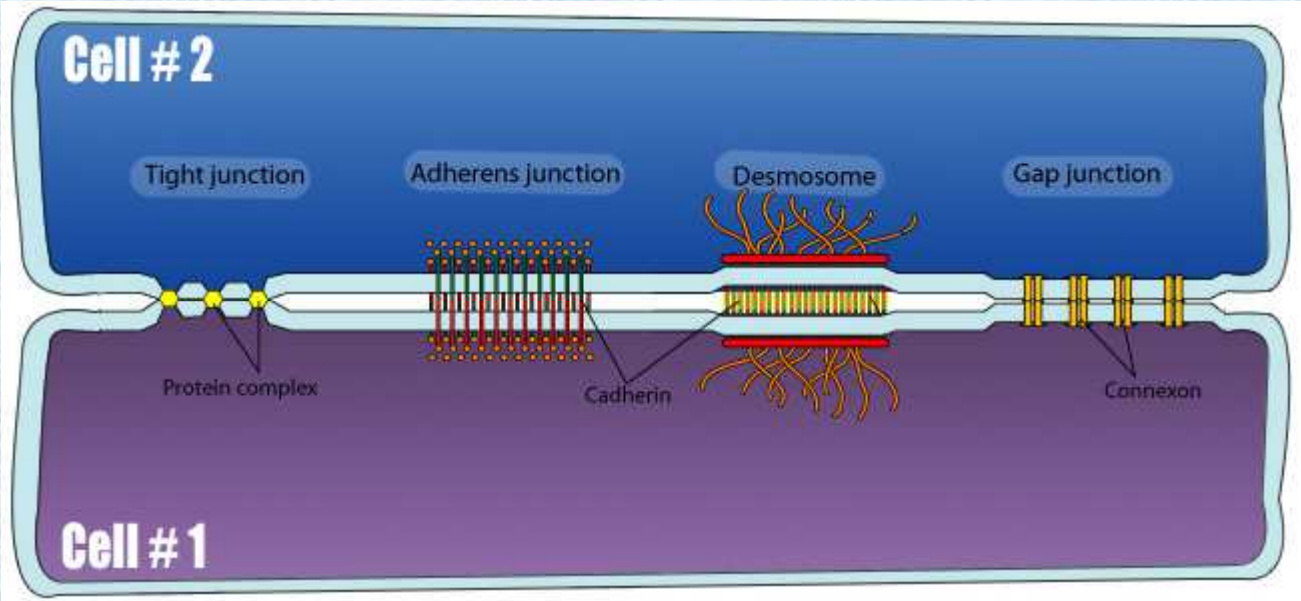
Overview of intestinal epithelial junctional complexes. The intestinal epithelium consists of a single layer of polarized epithelial cells. Adjacent cells are connected by 3 main junctional complexes: desmosomes, AJs, and TJs. Desmosomes are localized dense plaques that are connected to keratin filaments. AJs and TJs both consist of transcellular proteins connected intracellularly through adaptor proteins to the actin cytoskeleton. The collection of proteins in the junctional complexes forms cytoplasmic plaques.

Groschwitz and Hogan, 2009

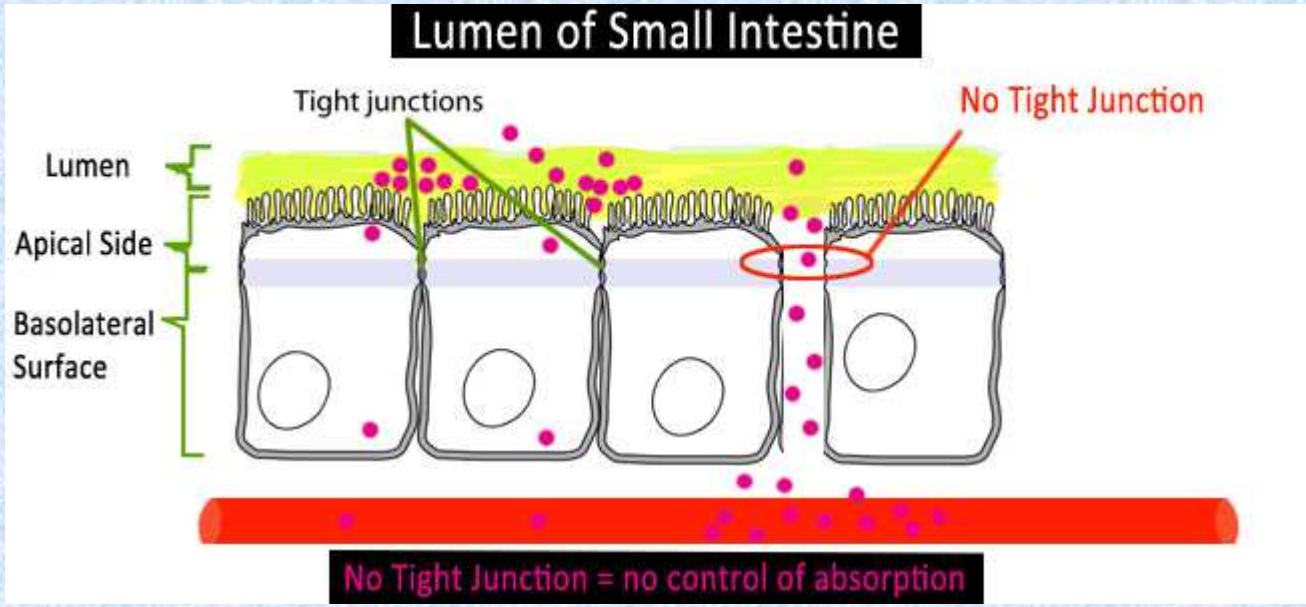
Groschwitz and Hogan, 2009



TJs are localized to the apical-lateral membrane junction. They consist of integral transmembrane proteins (occludin, claudins, and JAMs) that interact in the paracellular space with proteins on adjacent cells. Interactions can be homophilic (eg, claudin-1/claudin-1) or heterophilic (eg, claudin-1/claudin-3). The intracellular domains of transmembrane proteins interact with PDZ domain–containing adaptor proteins that mechanically link the TJ complex to the actin cytoskeleton. TJ proteins are regulated by means of phosphorylation by kinases, phosphatases, and other signaling molecules



http://www.dbriers.com/tutorials/2012/12/junctions-between-cells-simplified/



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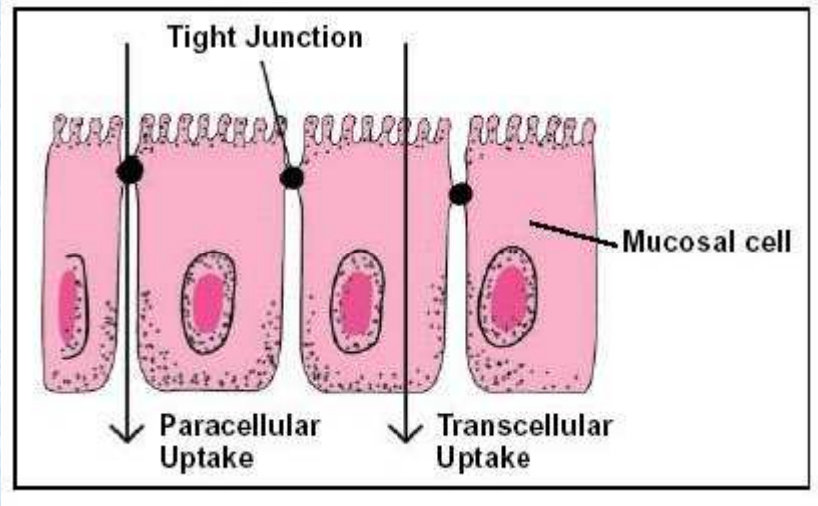
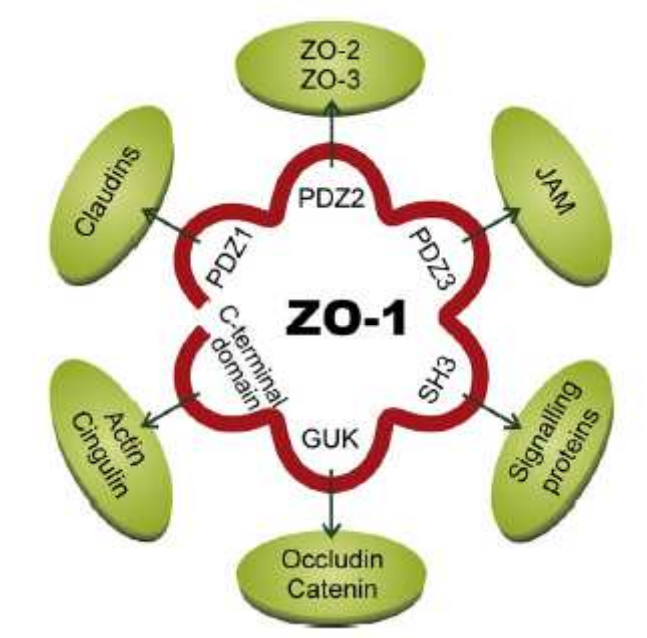
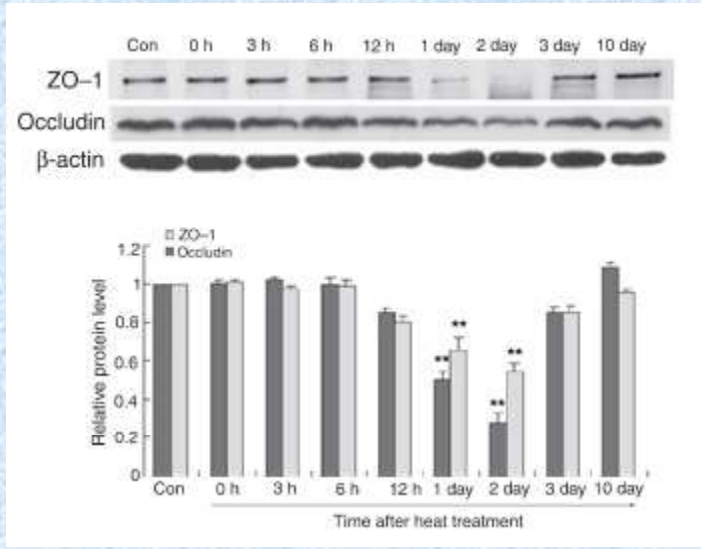


http://allnaturaladvantage.com.au/how_gastrointestinal_health_affe.htm



Schematic diagram of interactions of ZO-1 (zonula occludens-1)with transmembrane, cytosolic and cytoskeletal proteins. JAM, junctional adhesion molecule; PDZ, Post synaptic density 95, Disc large and ZO-1 domain; SH3, Src homology domain; GUK, guanylate kinase domain.

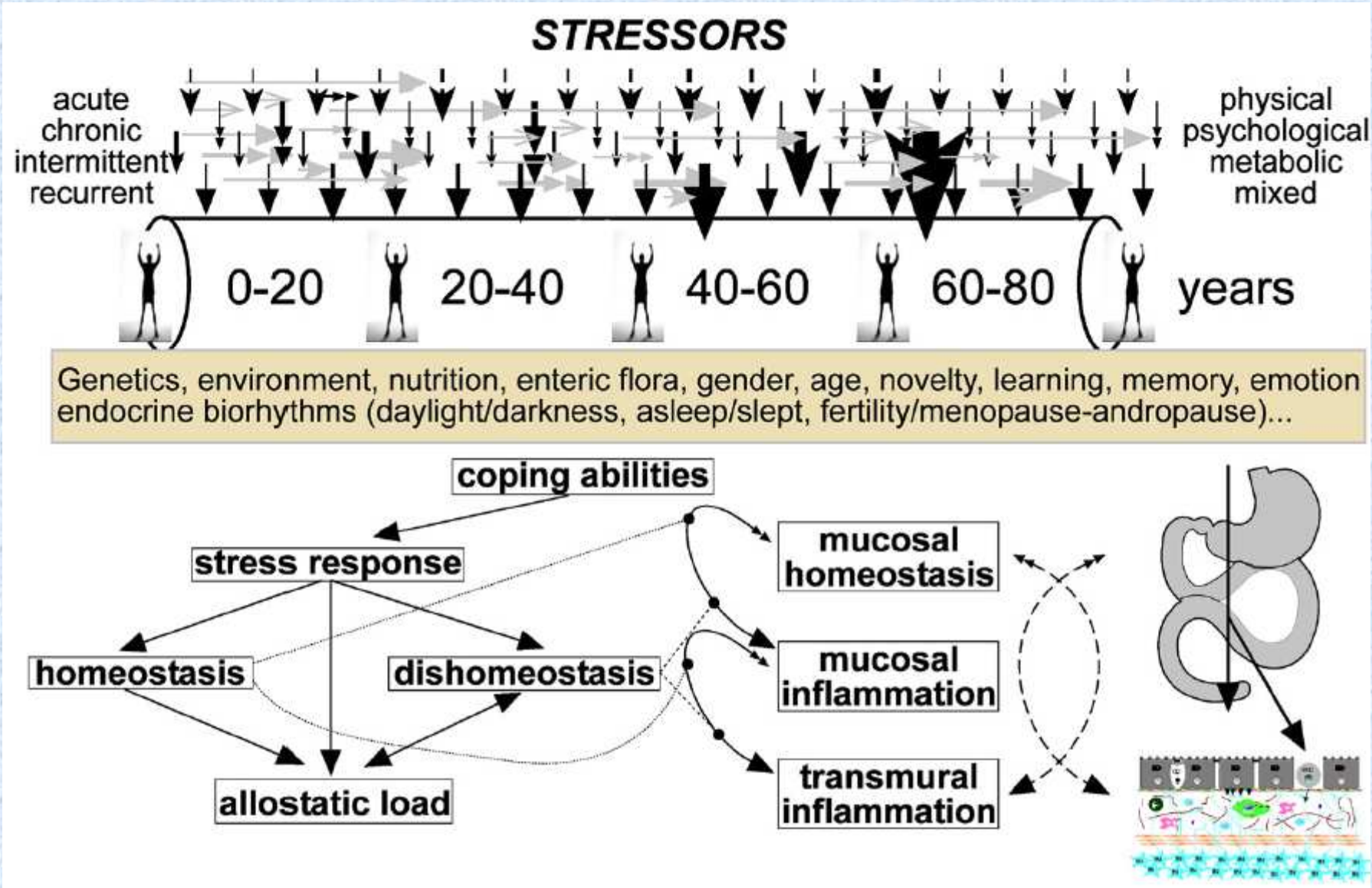
Kosinska, 2013


Con
0 h
3 h
6 h
12 h
1 day
2 day
3 day
10 day
ZO–1
Occludin
β-actin
ZO–1
Occludin
Relative protein level
1.2
1
0.8
0.6
0.4
0.2
0
Con
0 h
3 h
6 h
12 h
1 day
2 day
3 day
10 day
Time after heat treatment

Cai, 2010

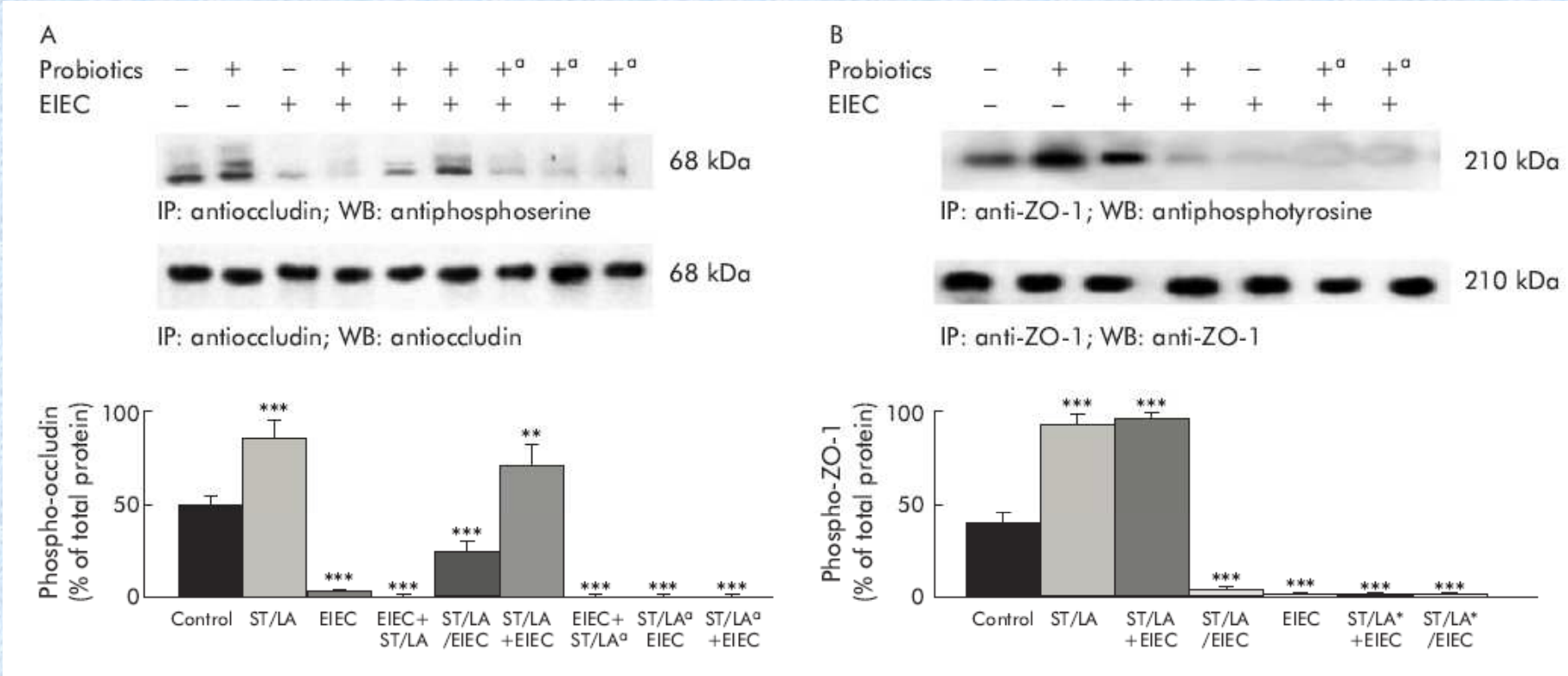
Acknowledgement

- Dr. Vitaly Vodyanoy
- Dr. Benson Akingbemi
- Mrs. Ludmila Globa
- Mr. Oleg Pustovyy


STRESSORS
acute
chronic
intermittent
recurrent
physical
psychological
metabolic
mixed
0-20
20-40
40-60
60-80
years
Genetics, environment, nutrition, enteric flora, gender, age, novelty, learning, memory, emotion
endocrine biorhythms (daylight/darkness, asleep/slept, fertility/menopause-andropause)...
coping abilities
stress response
homeostasis
dishomeostasis
allostatic load
mucosal homeostasis
mucosal inflammation
transmural inflammation

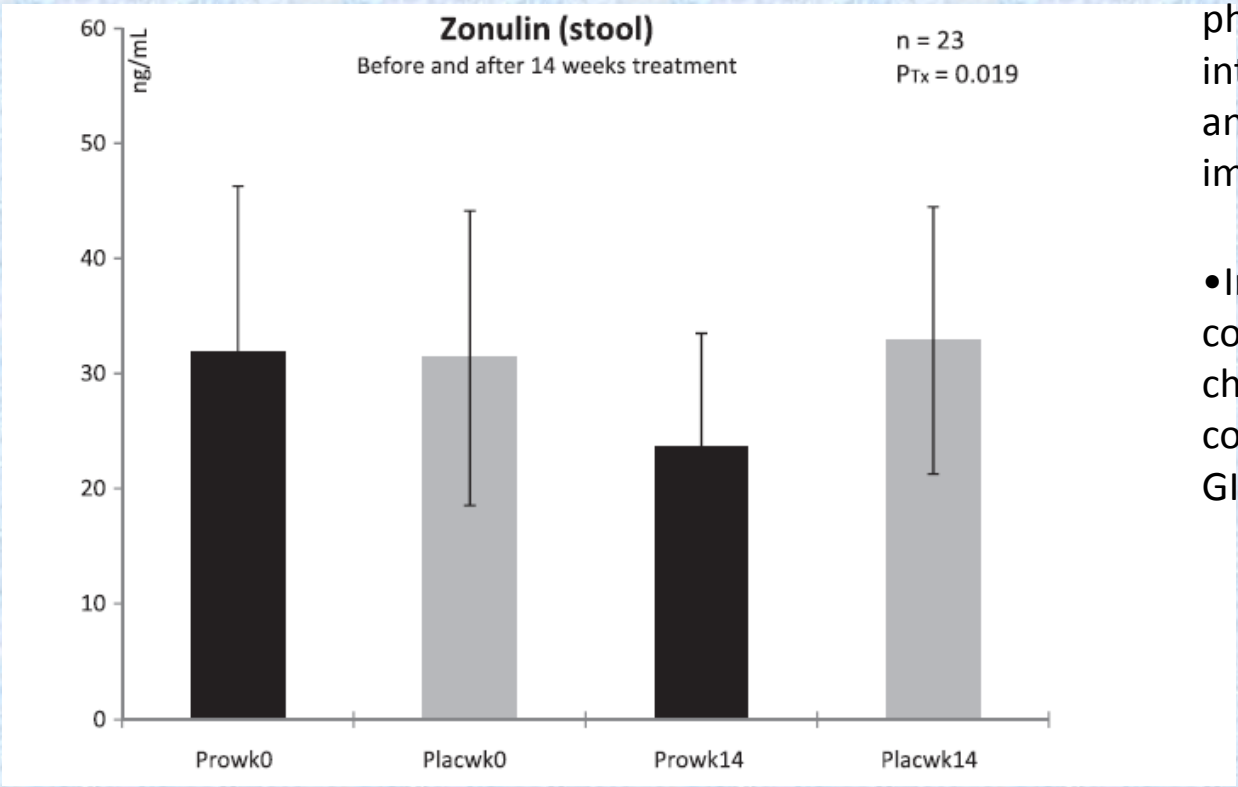
Santos, 2008

Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC)



- Infection with EIEC alters phosphorylation of the tight junction proteins occludin and ZO-1.
- *Streptococcus thermophilus* and *Lactobacillus acidophilus* (ST/LA) living and antibiotic killed (a) were tested

Resta-Lenert, 2003

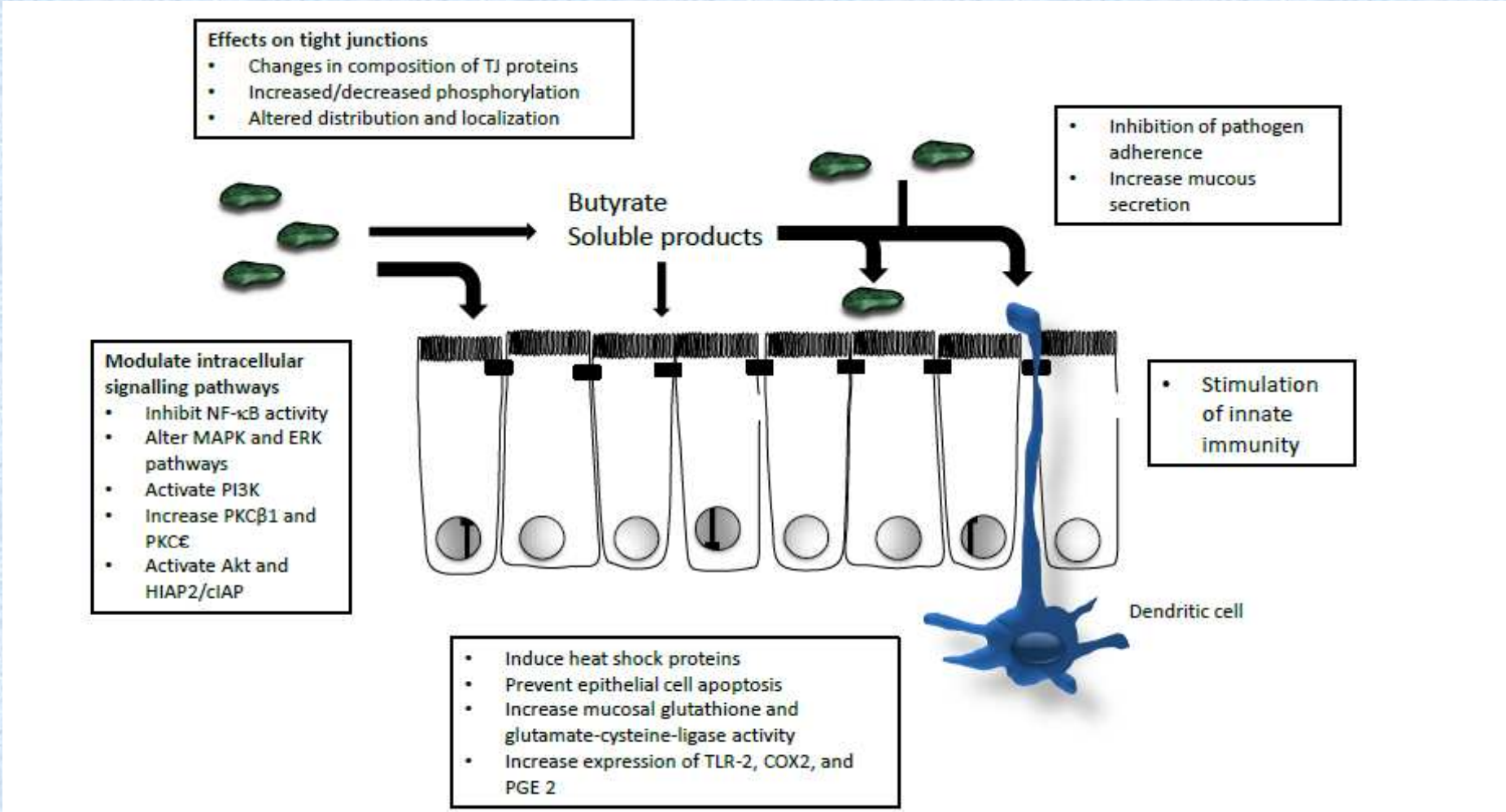


- Zonulin is regarded as a phyiological modulator of intercellular tight junctions and a surrogate marker of impaired gut barrier.

- Increased zonulin concentrations are related to changes in tight junction competency and increased GI permeability

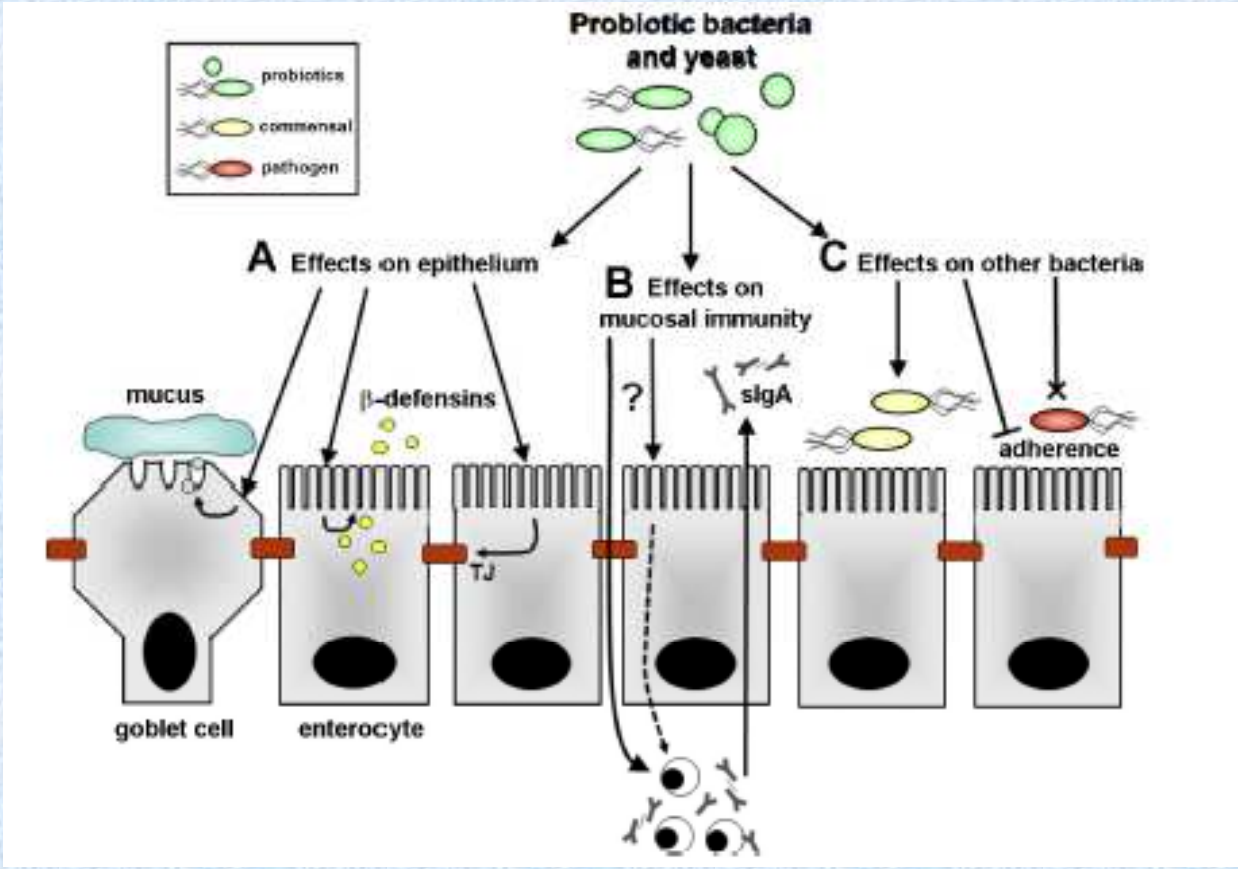
Stool concentrations of zonulin in trained men before and after 14 weeks of treatment. Pro with probiotics supplemented group, Plac placebo group, Tx treatment, wk week; n = 11 (probiotic supplementation), n = 12 (placebo). Values are means ± SD. There was a
signficant difference between groups after 14 wk of treatment: PTx < 0.05.

Lamprecht, 2012

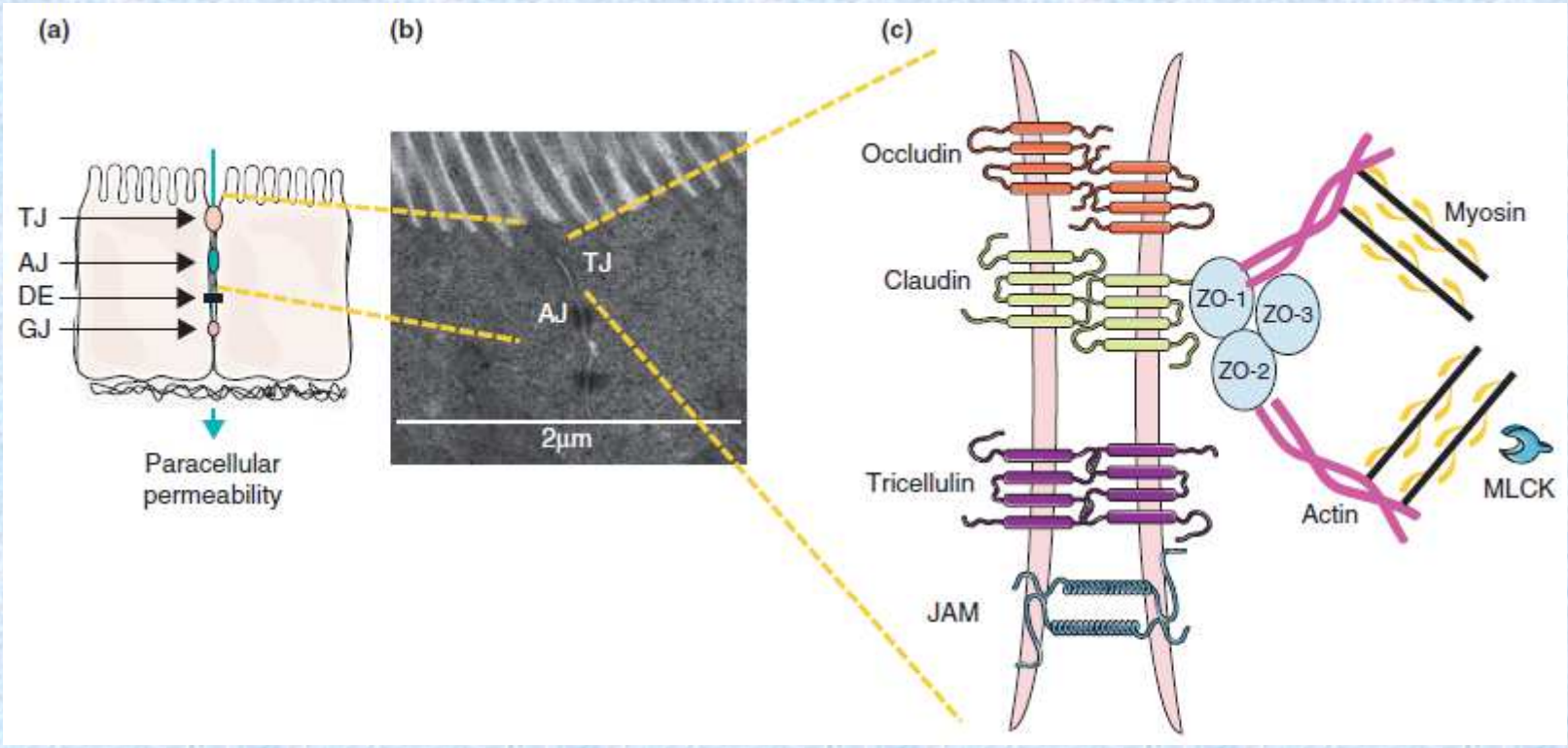


An overview of mechanisms involved in probiotic-induced enhancement of epithelial barrier function. These include direct modulation of epithelial cell signaling pathways and tight junctions, as well as effects on microbial ecology and innate and adaptive immune function

Madsen, 2012


Probiotic bacteria
and yeast
probiotics
commensal
pathogen
A Effects on epithelium
B Effects on
mucosal immunity
C Effects on other bacteria
mucus
β-defensins
?
sIgA
adherence
TJ
goblet cell
enterocyte

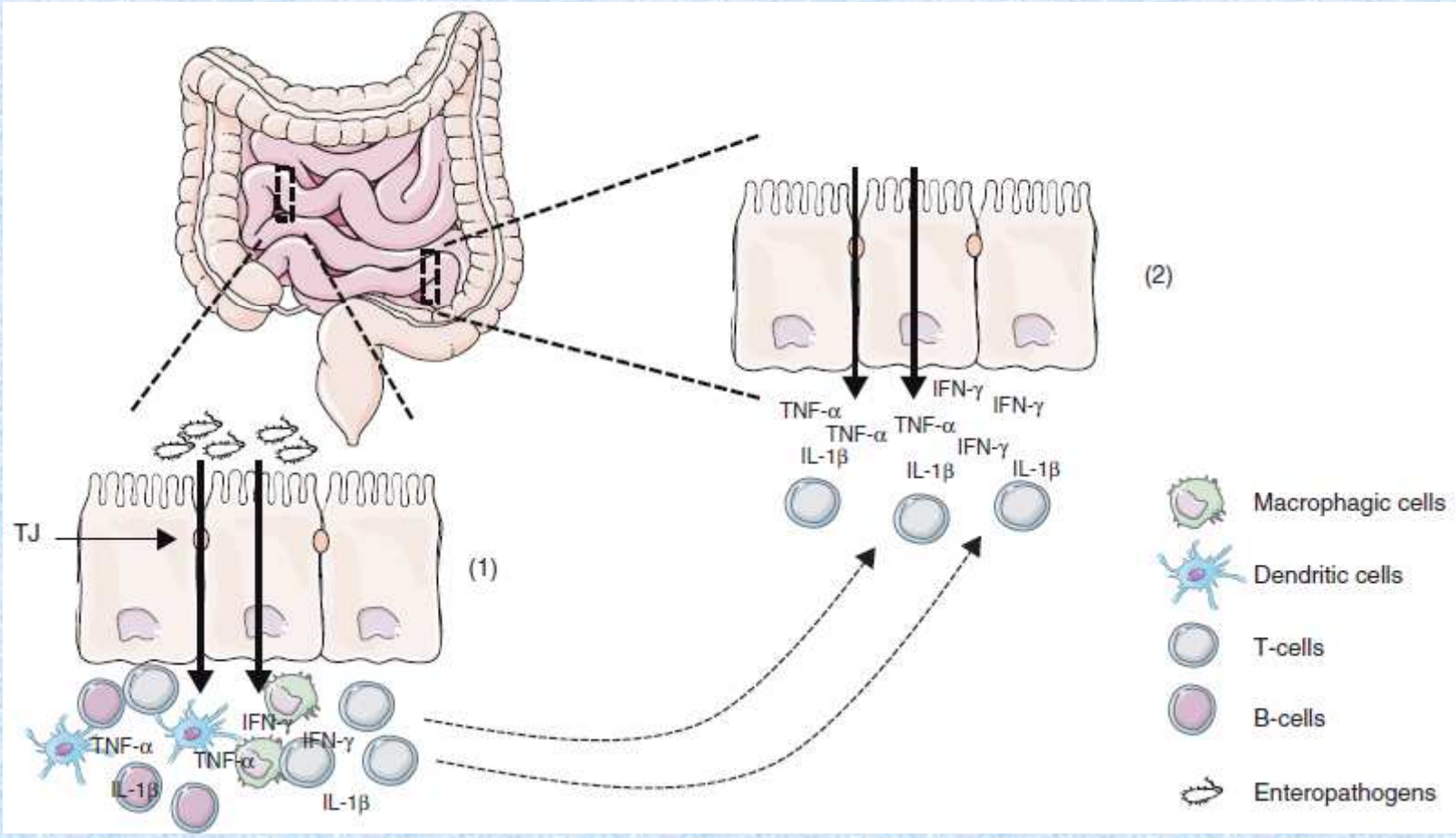
Ohland,2010



Mechanisms of paracellular permeability. **(a and b)** Epithelial cells are joined to each other by junctional complexes consisting of tight junctions (TJ), adherens junctions (AJ), desmosomes (DE), and gap junctions (GJ). **(c)** Four transmembrane families of proteins (occludin, claudins, junctional adhesion molecule (JAM) and tricellulin) contribute to TJ formation. The extracellular domains of the transmembrane proteins form a selective barrier through homophilic and heterophilic interactions with the adjacent cells. The intracellular domains of these transmembrane proteins interact with cytosolic scaffolding proteins like zonula occludens (ZO) proteins, which in turn anchor the transmembrane proteins to the perijunctional actinomyosin ring. The interaction of TJ proteins with the actin cytoskeleton is vital to the maintenance of TJ structure and function. The interaction of the TJ with the acinomyosin ring permits the cytoskeletal regulation of TJ barrier integrity. The circumferential contraction of the actinomyosin ring is regulated by the myosin light chain kinase (MLCK) which phosphorylates the myosin light chain.

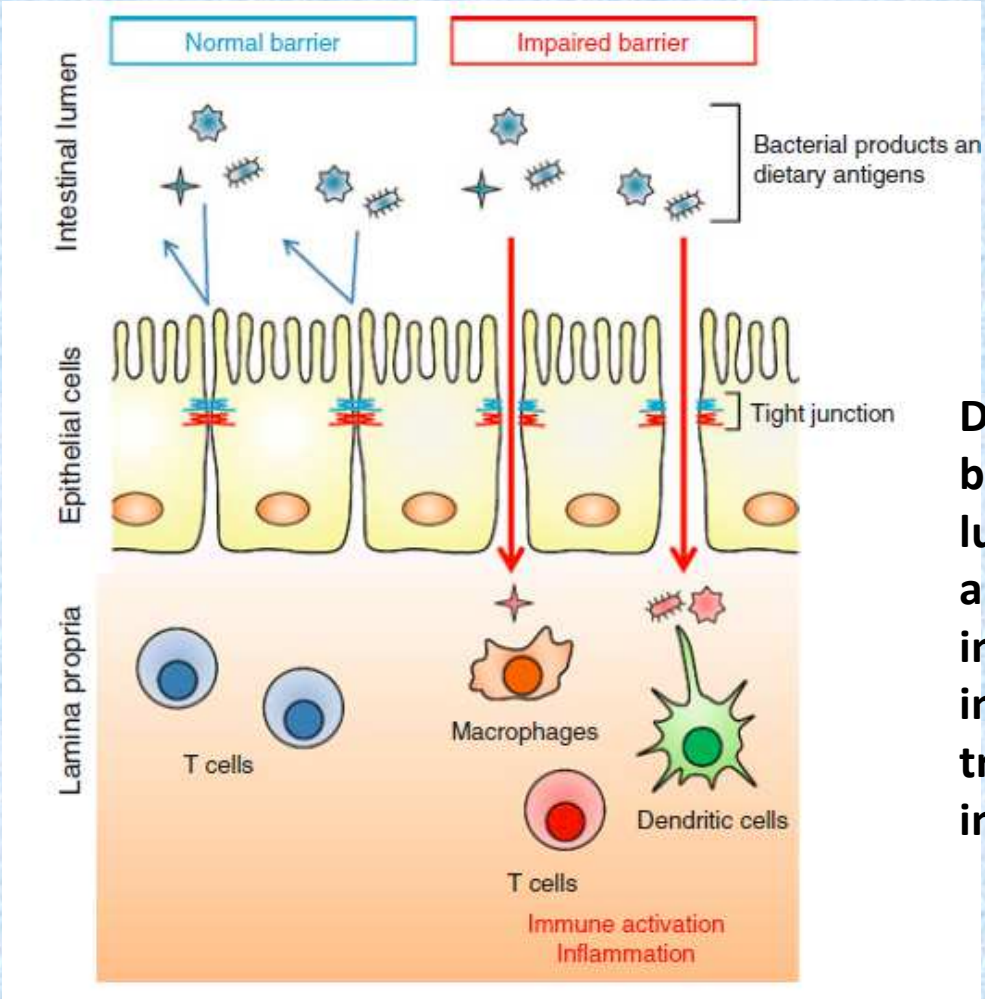
Barreau, 2014

Putative mechanisms by which enteric bacteria could disrupt the epithelial barrier function. The enteropathogenic bacteria are able to adhere at the surface of the enterocytes and M-cells. **(1)** When pathogenic bacteria encounter favorable conditions for their adherence and proliferation, they induce a local inflammation. At this place, paracellular and transcellular permeabilities are increased in relation with the recruitment and expansion of immune cells producing inflammatory cytokines like IL-1β, TNF-α and/or IFN-γ. As it has been extensively described these cytokines are able to alter the structure of the TJ by inducing the expression and activity of the MLCK (IL-1β, TNF-α and IFN-γ) and/or triggering the endocytosis (TNF-α and IFN-γ) of TJ proteins. **(2)** The recirculation of the activated immune cells contributes to propagate the barrier defect at distance of the infected area.



Barreau, 2014

Gastrointestinal selective permeable barrier is achieved by intercellular tight junction (TJ) structures



Disruption of the intestinal TJ barrier, followed by permeation of luminal noxious molecules, induces a perturbation of the mucosal immune system and inflammation, and can act as a trigger for the development of intestinal and systemic diseases.

Suzuki, 2013

Tight Junction Proteins

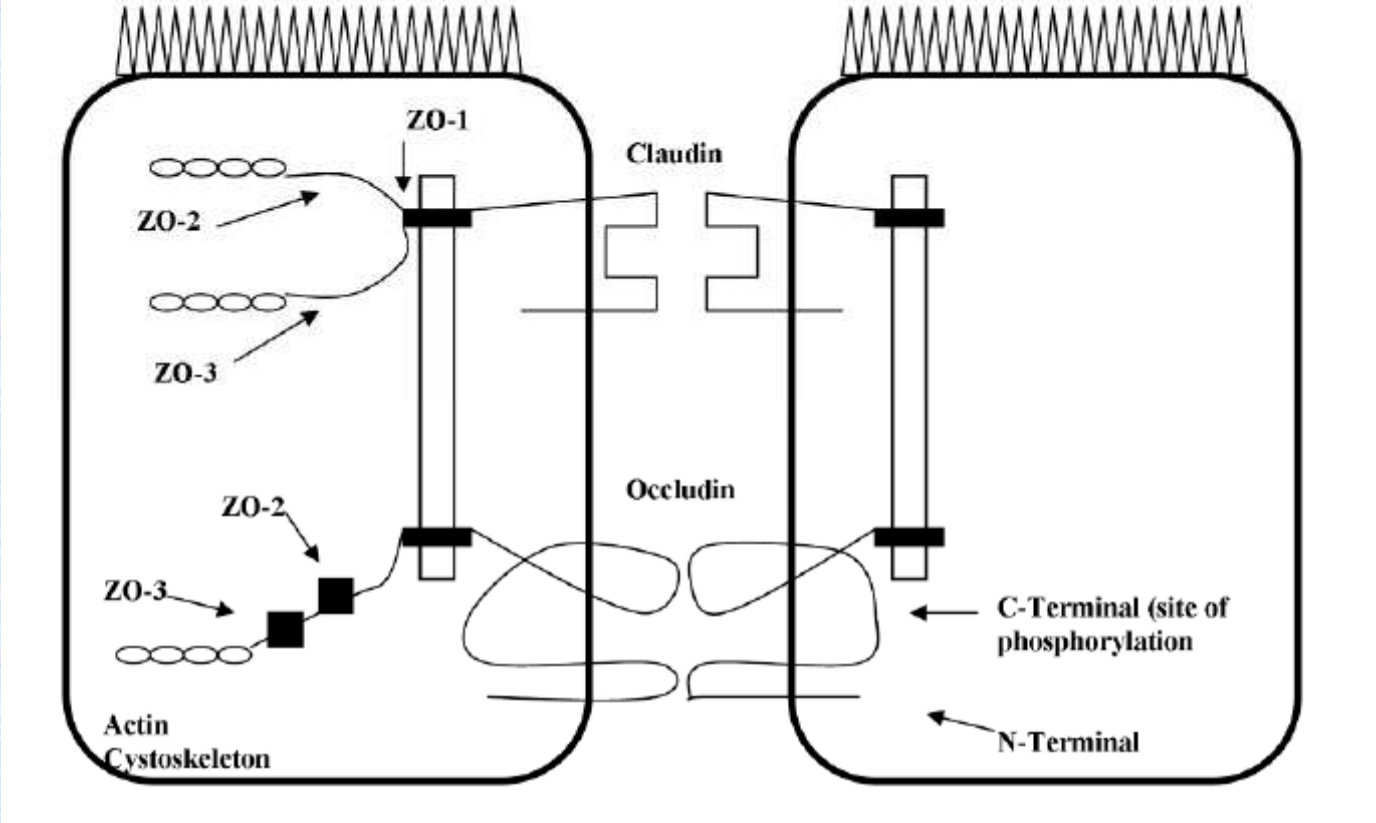


Figure 2 The tight junction barrier is composed of tetraspanning membrane proteins claudins and occludin, and the regulatory proteins ZO-1, ZO-2 and ZO-3.

Zuhl, 2014

Structure disruption/protection

gene expression alteration

Intestinal TJ regulation by

CYTOKINES

Intestinal TJ regulation by cytokines

The roles of cytokines in intestinal TJ regulation under pathophysiological conditions have been well investigated using cell cultures and animal models.

The cytokine mediated dysfunction of the TJ barrier, resulting in immune activation and tissue inflammation, is thought to be important in the initiation and/or development of several intestinal and systemic diseases . In contrast, some growth factors play roles in the protection and maintenance of TJ integrity.

Cytokines which increase intestinal TJ permeability

Cytokines	Cell lines	Mechanism
IFN-γ	T84	Myosin II-dependent vacuolarization, internalization of JAM-A, occludin, claudin-1 and claudin-4 (Bruewer M, *et al* 2003; Bruewer M, *et al.*,2005)
TNF-α	Caco-2	ZO-1↓ [Ma TY, et al., 2004] MLCK ↑, pMLC ↑ [Ma TY, et al.2005; Ye D, et al. 2006]
	HT29/B6	Claudin-2 ↑ (Mankertz J, et al. 2009)
TNF-α /IFN-γ	Caco-2	MLCK ↑, pMLC ↑ (Wang F, et al. 2005; Wang F, et al.2006)
LIGHT /IFN-γ	Caco-2	MLCK ↑, pMLC ↑, Caveolar endocytosis (occludin, ZO-1 and claudin-1) (Schwarz BT, 2007)

Cytokines which increase intestinal TJ permeability

Cytokines	Cell lines	Mechanism
IL-1β	Caco-2	Occludin ↓ (Al-Sadi RM, Ma TY, 2007)
	Caco-2	MLCK ↑, pMLC ↑ (Al-Sadi R,2008)
IL-4	T84	Claudin-2 ↑ (Wisner DM,2008)
IL-6	Caco-2, T84	Claudin-2 ↑ (Kusugami K,1995)
IL-13	T84	Claudin-2 ↑ (Weber CR,2010)
	HT29/B6	Claudin-2 ↑ (Prasad S,2005)
	Caco-2	Potentiate oxidant (Rao R,1999)

Cytokines which decrease intestinal TJ permeability

Cytokines	Cell lines	Mechanism
IL-10	T84	Decrease Neutralize IFN-c （Madsen KL, 1997）
IL-17	T84	Claudin-1 ↑, Claudin-2 ↑ (Kinugasa T, et al., 2000)
TGF-α antibody	Caco-2	Neutralize hydrogen peroxide (Forsyth CB,2007)
TGF-β	T84	Claudin-1 ↑ (Howe KL, et al, 2005)
	HT29 / B6	Claudin-4 ↑ (Hering NA,et al.,2011)
	T84	Neutralize EHEC, restoration of occludin, claudin-2 and ZO-1 expression (Howe KL, et al,2005)

Cytokines which decrease intestinal TJ permeability

Cytokines	Cell lines	Mechanism
TGF-β	T84	Neutralize IFN-γ
	T84	Neutralize cryptosporidium parvum (Roche JK, et al.,2000)
EGF	Caco-2	Neutralize hydrogen peroxide, restoration of occludin and ZO-1 distribution (Basuroy S, et al, 2006)
	Caco-2	Neutralize hydrogen peroxide, restoration of actin cytoskeleton assembly (Banan A, et al, 2001; Banan A, et al., 2004]
	Caco-2	Neutralize ethanol, restoration of microtubule assembly and oxidation/nitration of tubulin (Banan A, et al, 2007)
	Caco-2	Neutralize acetaldehyde, restoration of occludin and ZO-1 distribution (Suzuki T, et al., 2008; Samak G, et al. 2011)

- Intestinal TJ regulation by

food factors

Nutrients and food factors **decrease** and restore intestinal TJ permeability

Amino acid	Cell	Mechanism [b]
Gln	Caco-2	claudin-1← →
Gln	Caco-2	Neutralize acetaldehyde, restoration of occludin and ZO-1 distribution
Trp	Caco-2	Unknown

Nutrients and food factors **decrease** and restore intestinal TJ permeability

Fatty acid	Cell	Mechanism [b]
EPA, DHA, arachidonic acid, γ -LA, di-homo- γ -LA	T84	Unknown
EPA, DHA, arachidonic acid, di-homo- γ -LA	T84	Neutralize IL-4
Acetic acid	Caco-2，T84	Unknown
Propionic acid	Caco-2，T84	Unknown
Butyric acid	Caco-2	Promotion of occludin and ZO-1 assembly in Ca-induced TJ reassembly

Nutrients and food factors **decrease** and restore intestinal TJ permeability

Vitamin	Cell	Mechanism [b]
Vitamin A	Caco2	Neutralize Clostridium difficile toxin A (Maciel AA,2007)
Vitamin D	SW480, not determined permeability	ZO1 ↑, claudin-1 ↑, claudin-2 ↑, E-cadherin ↑ (Kong J,et al,2008)
	Caco-2	Neutralize DSS (Kong J,et al,2008)

Nutrients and food factors **decrease** and restore intestinal TJ permeability

Polyphenol	Cell	Mechanism [b]
Genistein	Caco2	Neutralize hydrogen peroxide, occludin ← →, ZO-1 ← → (Rao RK,2002)
	Caco2	Neutralize acetaldehyde, occludin ← →, ZO-1 ← → (Atkinson KJ,2001)
Curcumin	Caco-2	Neutralize TNF-a (Ye D,2006)
	Caco-2	Neutralize IL-1b (Al-Sadi RM,2007)
EGCG	T84	Neutralize IFN-c (Watson JL,2004)
Quercetin	Caco-2	Claudin-4 ↑, ZO-2 ← →, claudin-1 ← →, occludin ← → (Suzuki T, Hara H,2009)
Kaempferol	Caco-2	ZO-2 ↑, claudin-4 ↑ occluidn ← →, claudin-1 ← →, claudin-3 ← →(Suzuki T,et al 2011)
Myricetin	Caco-2	Unknown (Suzuki T, Hara H,2009)