

**Winterschool: Role of the GI tract for development of allergy and obesity**

DHI & ØFN, Copenhagen  
09-12-2008

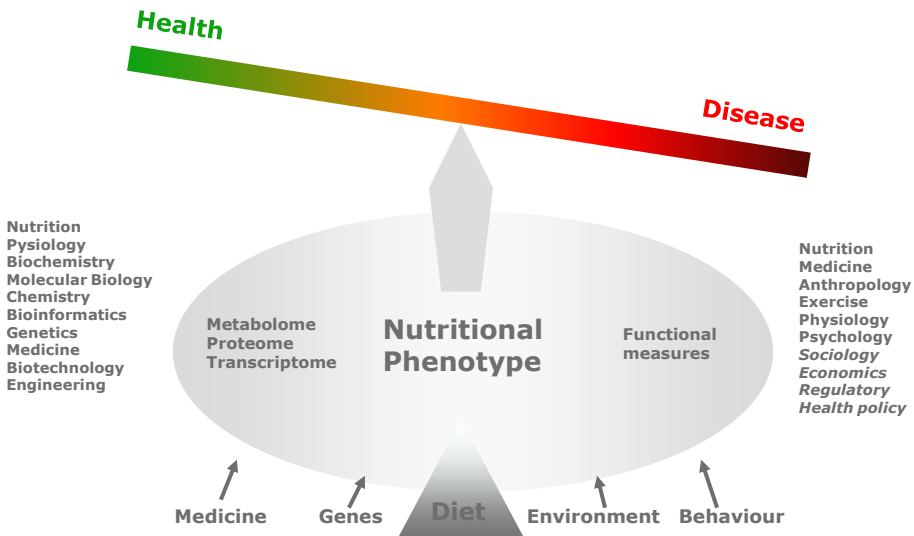
**Interplay between the gut flora and the immune system in the development of chronic inflammation**



LIFE, University of Copenhagen  
ActiFoods ApS  
Aff. Professor, PhD  
Peter Olesen

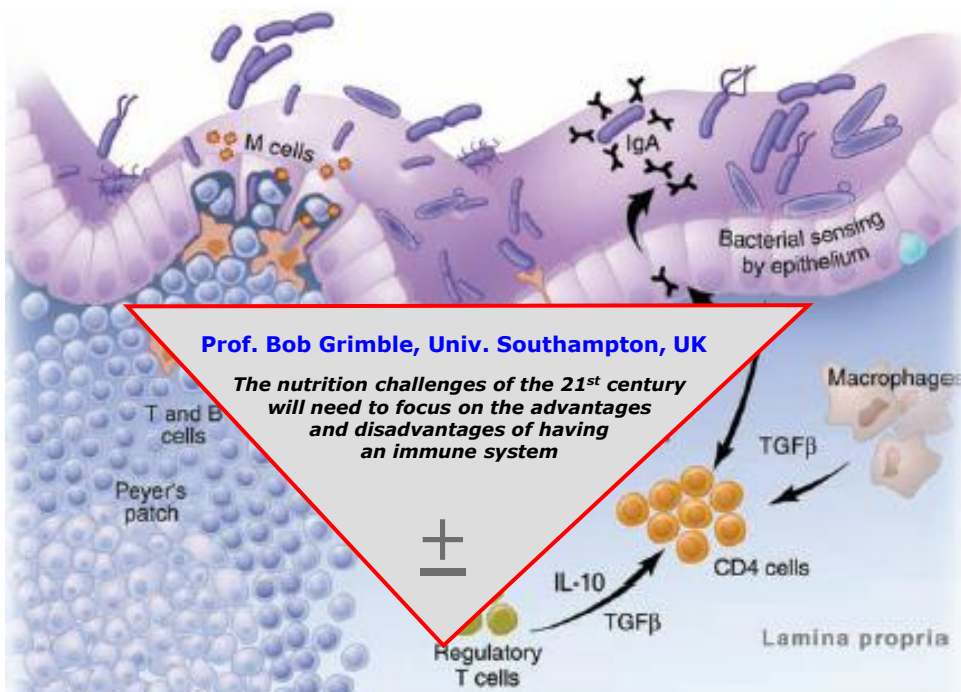


**A complexity and multidisciplinary issue**

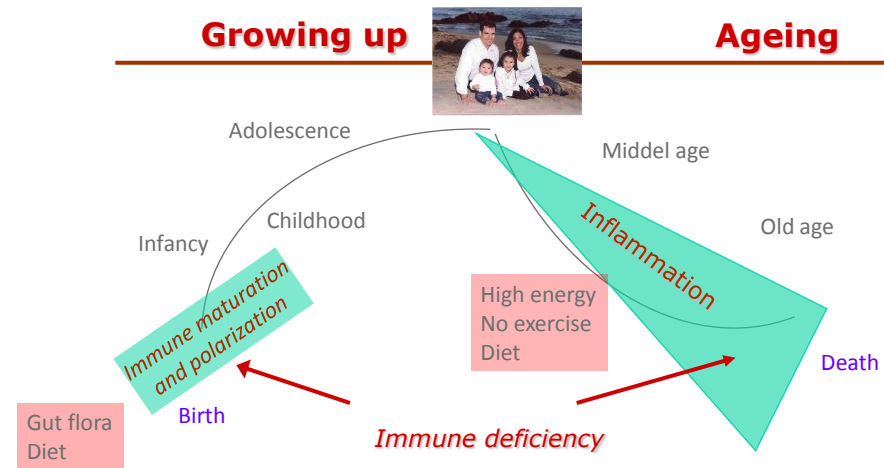


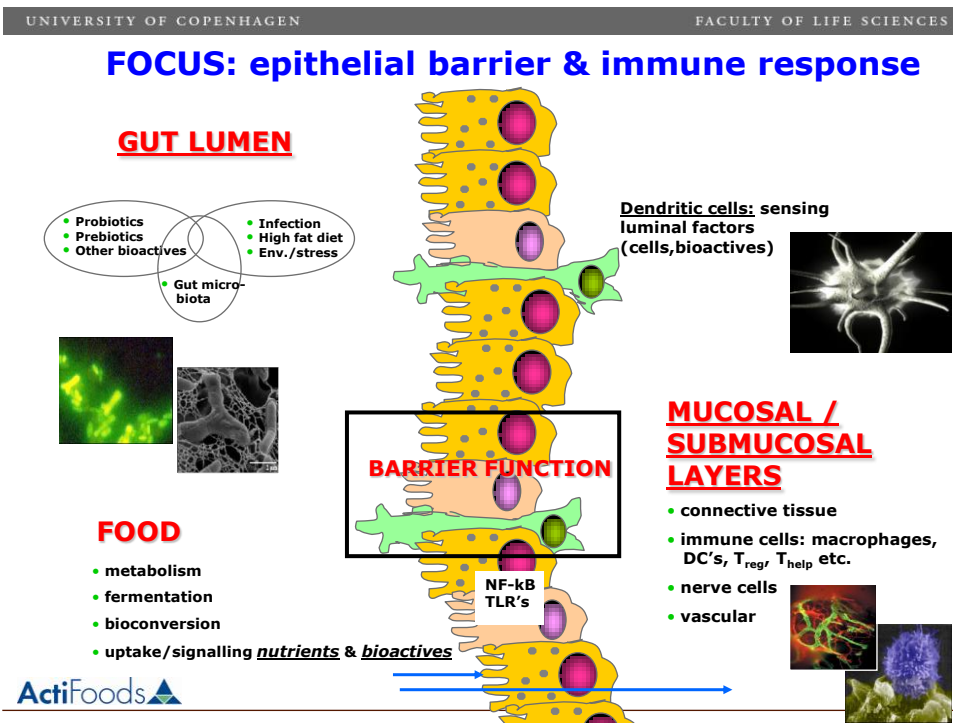
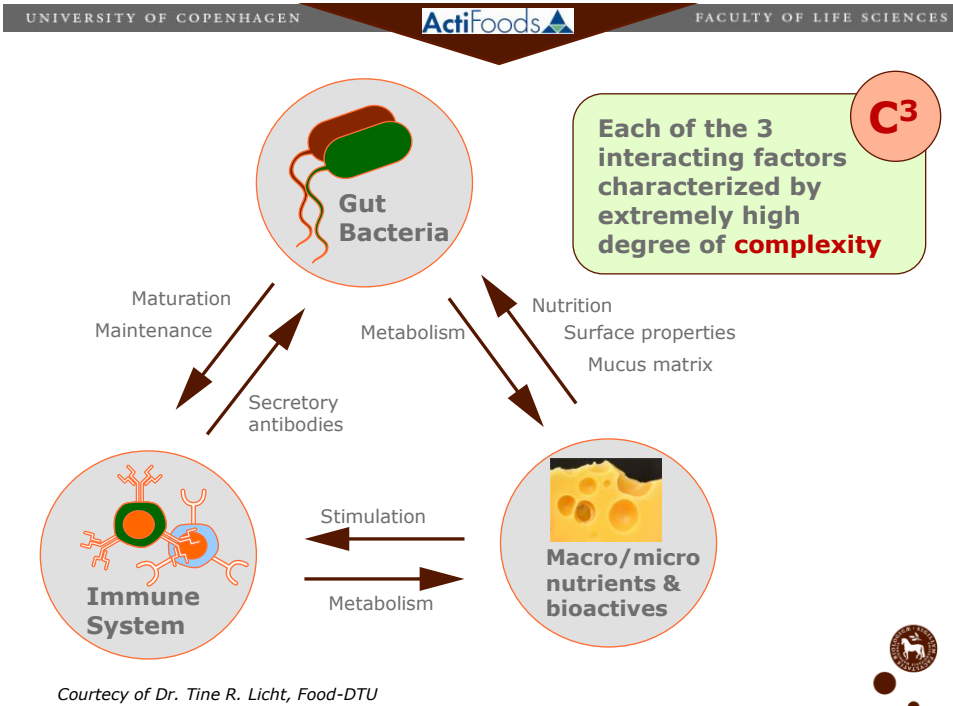
From: Steve Zeisel et al., 2005





## Immune maturation and inflammation





## Modes of action for food bioactives

### Direct action

- as ingested or bioconverted in gut
- ex: milk peptides
- ex: resveratrol
- ex: antioxidative food

### Microbiota

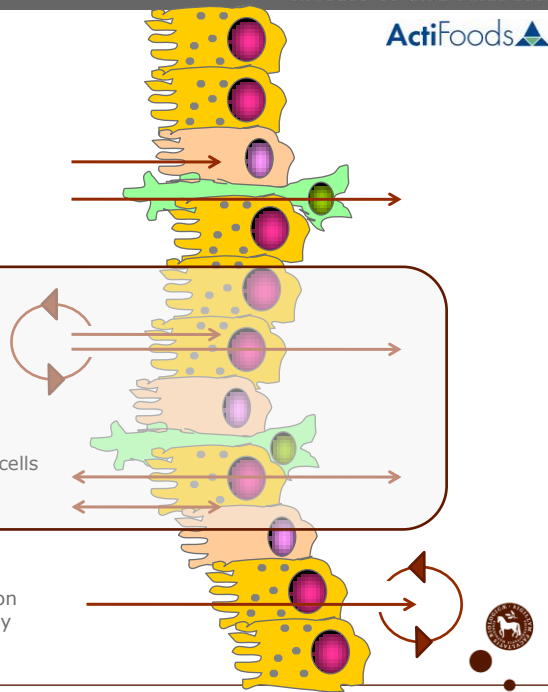
- diversity and dynamics
- ex: chicken flora
- ex: probiotic effects
- ex: prebiotic / synbiotic effects

### Complex communications

- bioactives/microbiota/host immune cells
- ex: microbial symbiosis factor
- ex: epithelial response factor

### Drug interference

- bioactive improving antibiotic function
- ex: flavor compounds and TB therapy

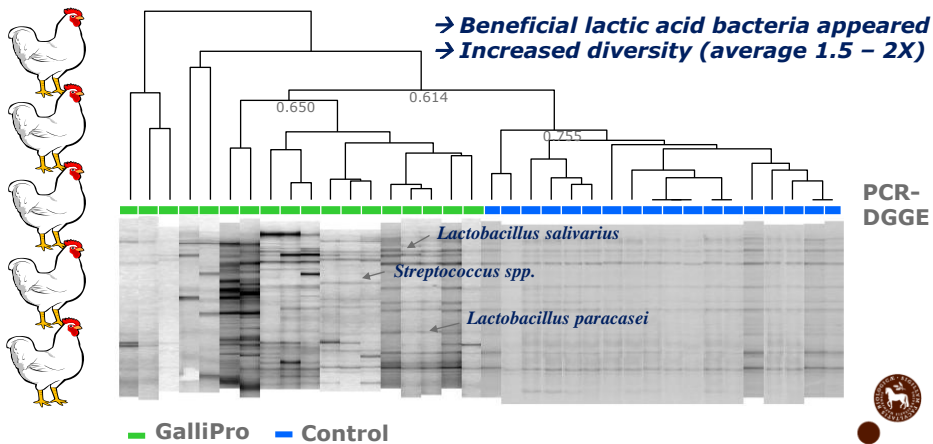


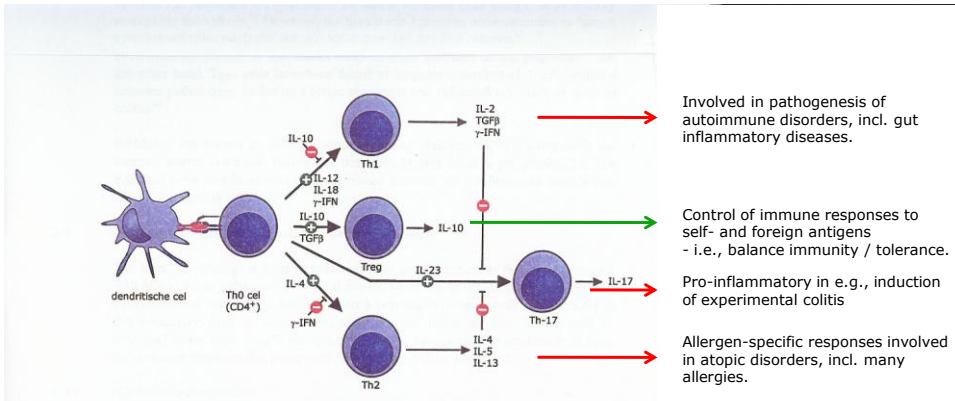
## Feeding GalliPro (*Bacillus subtilis*) to broiler chicken alters the bacterial community in the ileum and enhances growth

N Milora et al. 2008 J Prob Preb Res (in press):

Dice coefficient analysis of the ileal profiles from control birds and birds fed GalliPro resulted in a grouping associated with the dietary treatment

- Beneficial lactic acid bacteria appeared
- Increased diversity (average 1.5 – 2X)

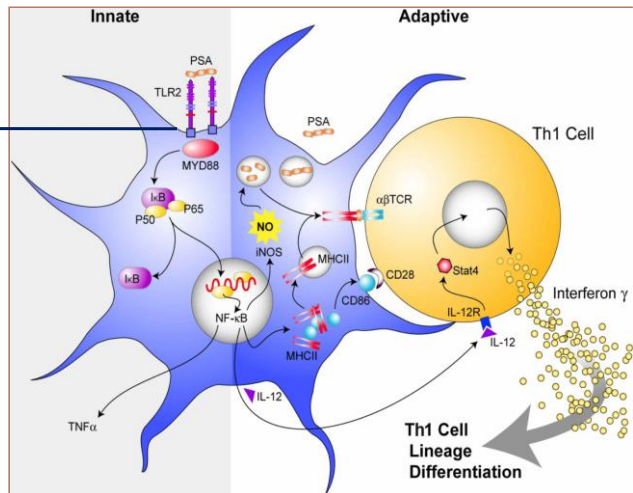




Generic model of the immune system: differentiation of T-cells after activation by maturing dendritic cells (DC's)

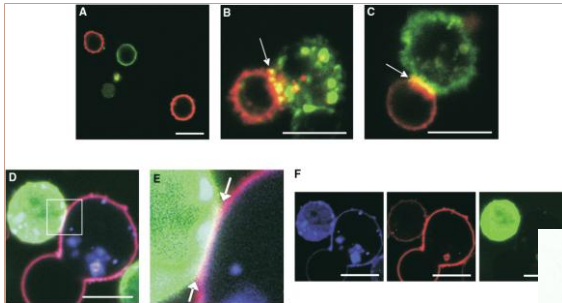
Toll-like receptors (TLR's) are involved in sensing the microbes present and initiation of immunity/tolerance responses.

TLR's recognize highly conserved structural motifs like LPS endotoxin, peptidoglycans, PSA on pathogens as well as commensals



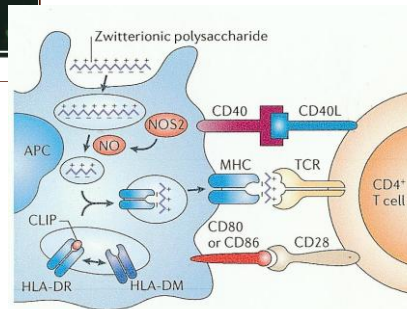
Wang, Q et al. (2003) J.Exp.Med. 203 (13) 2853-63.

### The Immunological Synapse concept



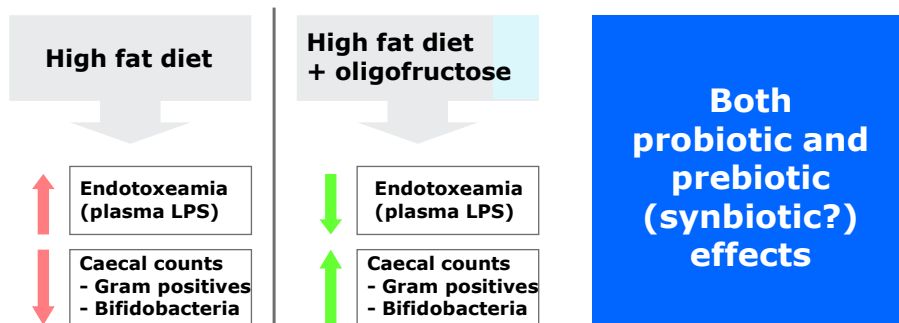
Cobb, BA et al. Cell 117, 677-87 (2004).

- APC's = red cells
- T-cells = green cells
- Synapses = yellow structures (antigen + MHCII)



Mazmanian SK & Kasper DL. Nature Rev. Immunol. 6, 849-58 (2006).

### Increase in Bifidobacteria may help prevent inflammation and development of metabolic syndrome



Selective Increases of Bifidobacteria in Gut Microflora Improve High-Fat-Diet-Induced Diabetes in Mice Through a Mechanism Associated with Endotoxaemia  
Cani PD et al. 2007, Diabetologia, in press

**Modifying the gut microbiota in favour of Bifidobacteria may prevent deleterious effects of high-fat-diet-induced metabolic diseases...**

## Dendritic cells (DC's) are sensing and responding to gut microbes *in vitro*



Courtesy of Dr. Hans van Noort TNO, NL

### Receptors

cytokine receptors  
chemokine receptors  
growth factor receptors  
toll-like receptors, TLR's  
DC-SIGN  
DEC 205  
...



### Molecular signals to the immune system

cytokines  
chemokines  
growth factors  
MHC molecules  
Co-stimulatory and adhesion molecules  
...

DC's are crucial messengers of immune-regulatory signals and central in defining strength and quality of immune responses

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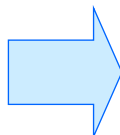
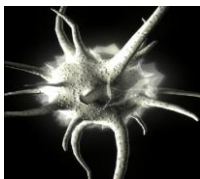
## Human DC's show differential *in vitro* responses to different probiotic bacteria



Courtesy of Dr. Hans van Noort TNO, NL

LPS  
L. acidophilus NCFM  
L. acidophilus La-5  
B. lactis Bb-12  
B. lactis BI-07

- activation of pattern-recognition receptors on DC's incl. TLR's
- powerful induction of DC maturation
- early cytokine & chemokine responses are often similar, indicating common activation of NF-κB
- other cytokine responses are quantitatively very different
- late DC responses diverge and indicate differential induction by bacteria of many growth and differentiation factors



**The *in vitro* response of human DC's to probiotics involve secretion of several immune regulatory and/or health-promoting factors**

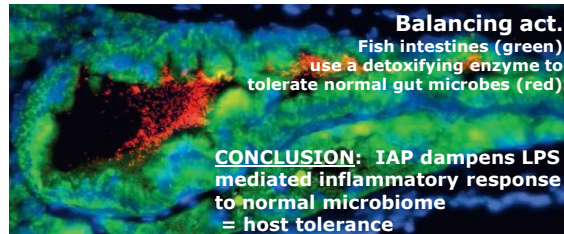
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ActiFoods   **Microbial signals can elicit host responses involved in maintaining a healthy balance** 

### A 'fishy' story about Intestinal Alkaline Phosphatase, IAP

Source:

Guillemin K et al. 2007: *Cell Host & Microbe* (Dec 13, 2007) & commentary *Science* 318: 1853 (Dec 21, 2007).



**FINDINGS:**

- No IAP activity and no neutrophils in germ free fish
- + IAP and neutrophils when microflora or LPS added
- IAP dephosphorylates LPS
- Chemically/genetically abolished IAP activity leads to boost in neutrophils and LPS-induced death

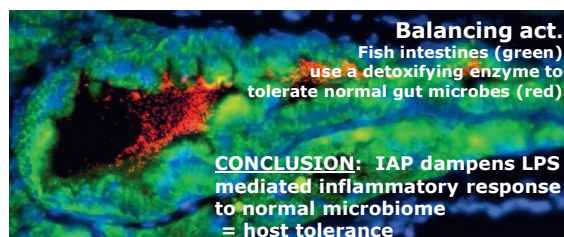
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**QUESTIONS:**

- ❖ May pathogens 'fool' the IAP response by high LPS loads or interference with regulation of the IAP response?
- ❖ May 'unhealthy' microbiota (e.g. diet-induced obesigenic composition) disturb the IAP/LPS mechanism and lead to chronic endotoxaemia, inflammation and MS?
- ❖ May emphasise role of LPS in cross-talk between microflora, immune system and inflammation?



## Microbial signals can elicit host responses involved in maintaining a healthy balance



### Intestinal inflammation in mice can be tamed by bacterial sugars

Source:

Mazmanian K et al. 2008; *Nature* Vol 453/29 May 2008, 620-25.

**A microbial symbiosis factor prevents intestinal inflammatory disease**

Experimental colitis induced by *Helicobacter hepaticus* (a murine commensal) can be abolished by a single microbial molecule (polysaccharide A, PSA) produced by e.g. *Bacteroides fragilis* (a human commensal).

- ❖ PSA seems to suppress pro-inflammatory IL-17 production in intestinal immune cells a.o. *in vitro* cell cultures
- ❖ PSA protection is based upon a functional requirement for IL-10 producing CD4+ T-cells.
- ❖ Germ-free mice have defects in CD4+ T-cell development and human *B fragilis* can correct the deficiency through PSA expression

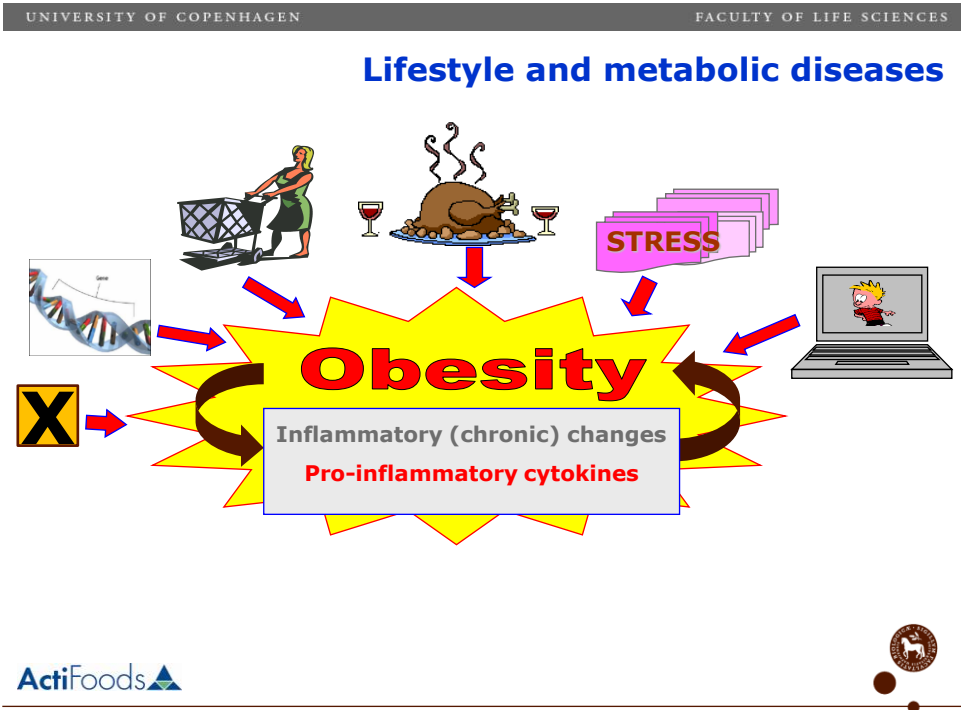
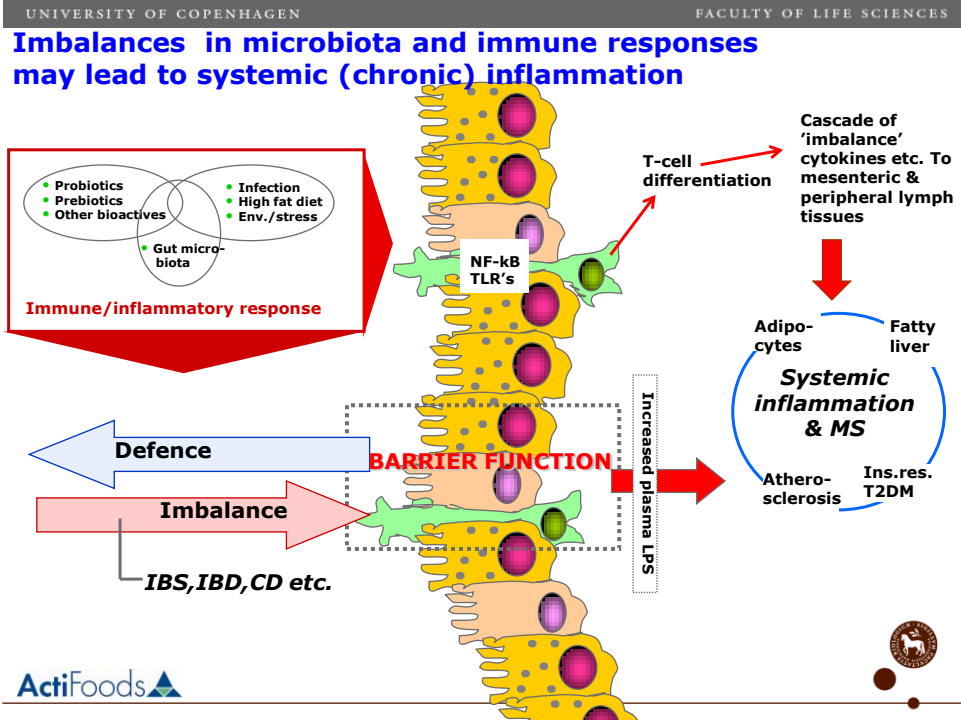
## WORKING HYPOTHESIS



FOOD

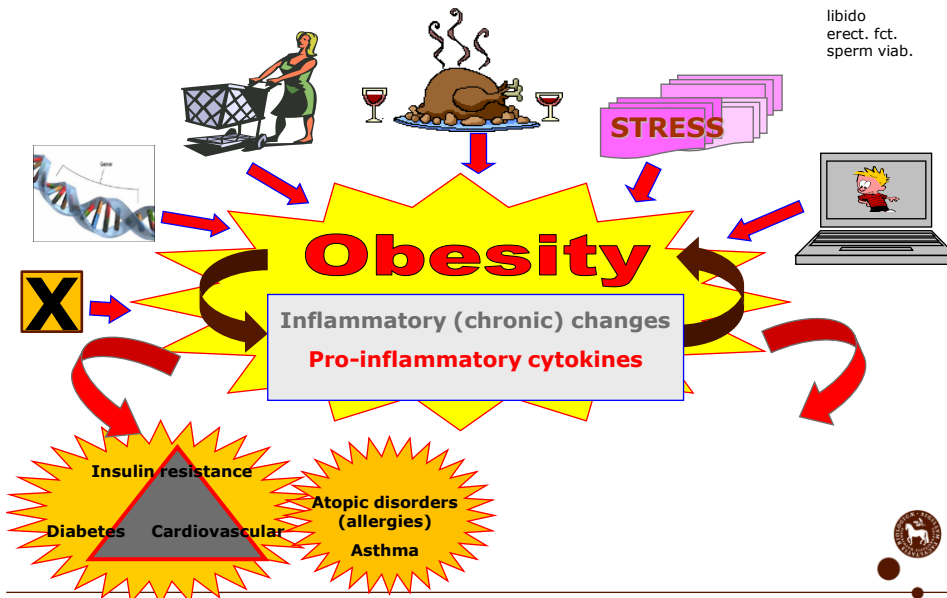
Based upon *in vitro* and animal models, it is anticipated that the microbiota (incl. probiotics a.o. 'intruders') are constantly monitored by the immune system via DC's a.o. antigen presenting cells of the immune system

HEALTH





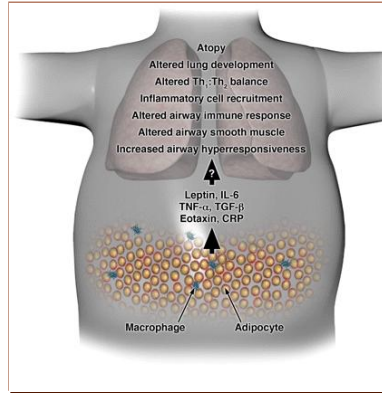
### Lifestyle and metabolic diseases



## Inflammation, IR, obesity and asthma

### Conclusions (from Inter99 study)

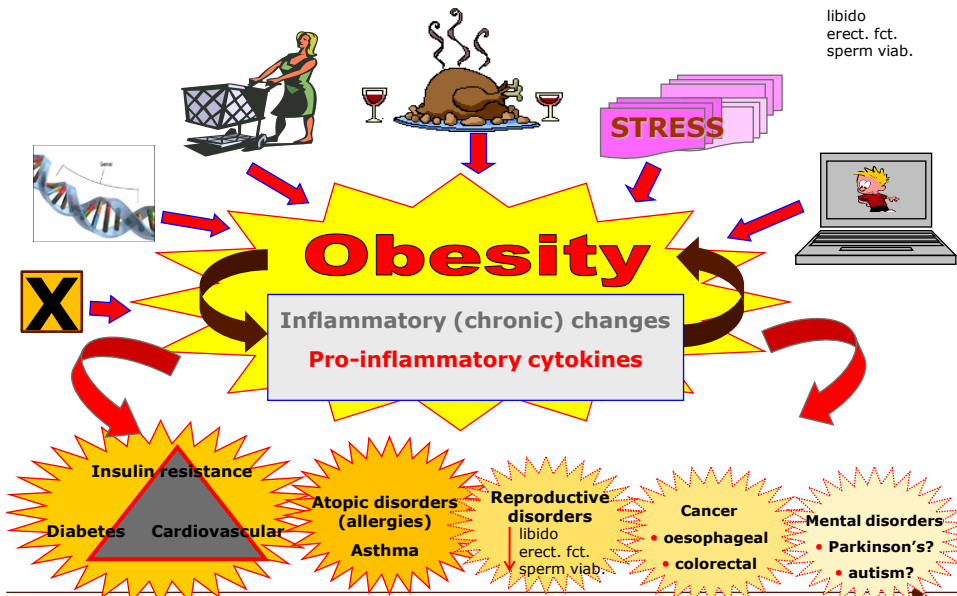
- All considered obesity measures were associated with incident asthma in adults
- Insulin resistance is a risk factor for incident asthma symptoms in adults – the effect is independent of obesity
- Inflammatory pathways involved in insulin resistance may also contribute to the pathogenesis of asthma
- These inflammatory processes may be part of the underlying biological mechanism linking obesity to asthma



Allan Linneberg, MD, PhD  
Lise-Lotte Husemoen, M.Sc., PhD  
Lars-Georg Hersoug, M.Sc.  
Betina H. Thuesen, M.Sc.

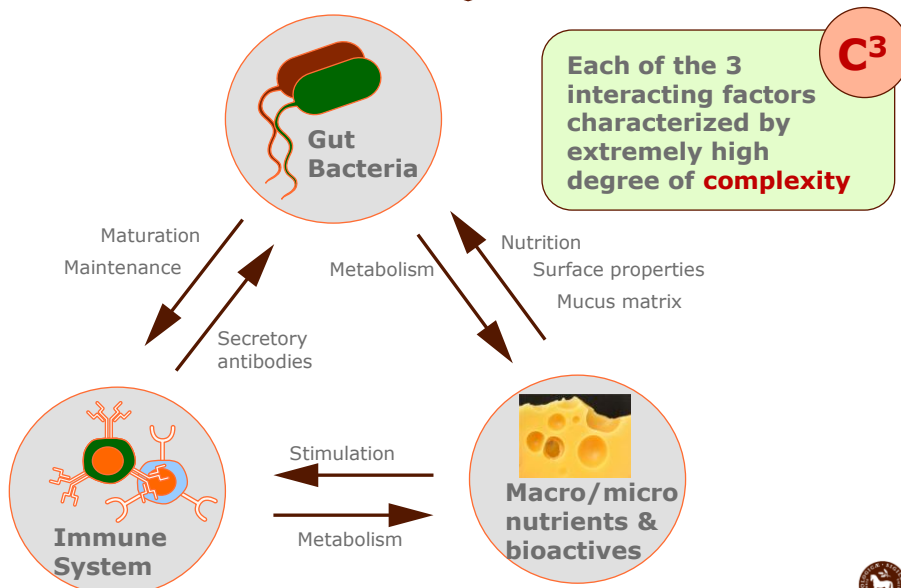
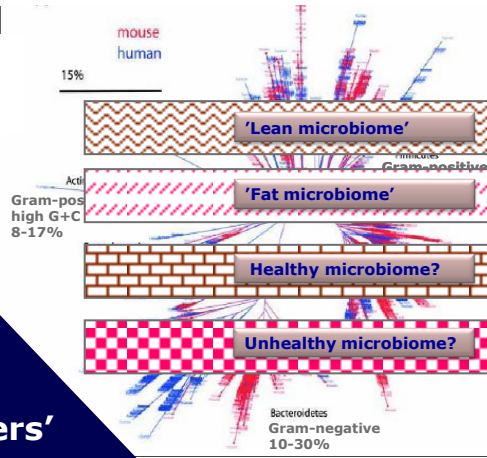
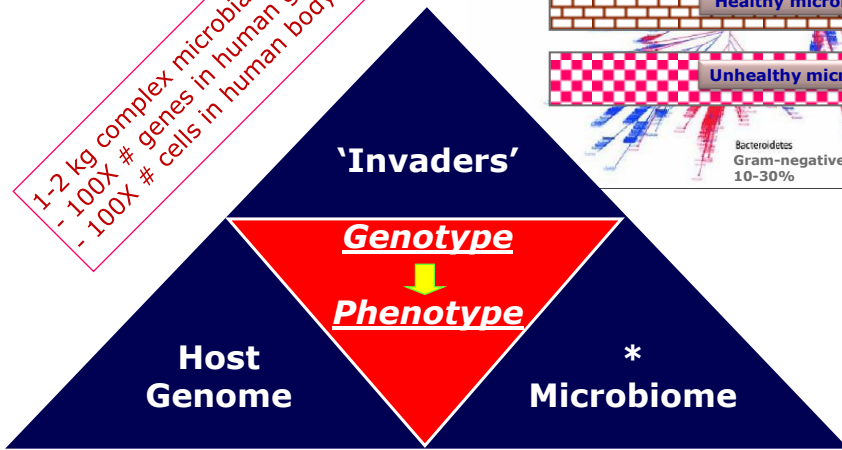


## Lifestyle and metabolic diseases



# Genes, genomes and metagenomes...

1-2 kg complex microbial ecosystem  
 - 100X # genes in human genome  
 - 100X # cells in human body

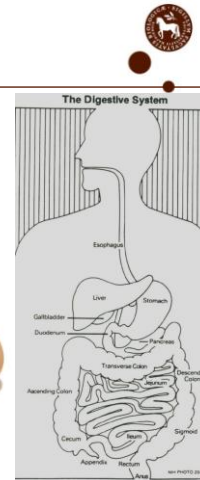
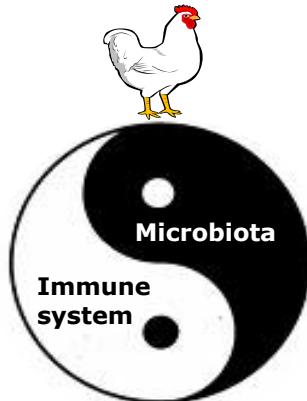
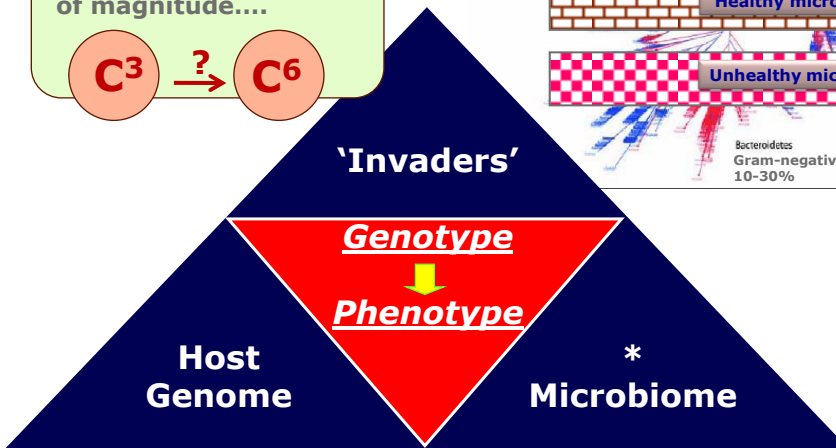
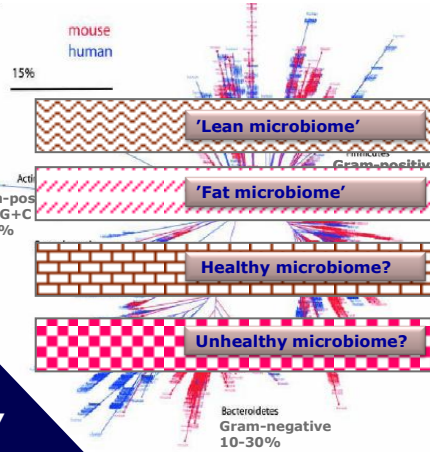
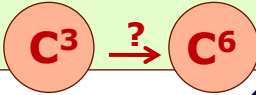


Courtesy of Dr. Tine R. Licht, Food-DTU



# Genes, genomes and metagenomes...

Increases **complexity** with several orders of magnitude....



We only see bits and pieces (e.g., effects of added probiotics and single commensals) but the overall picture is still far away....

*A classical biological conundrum in understanding metabolic homeostasis*

## Clinical efficacy a major shortcome

**3-4 main categories of food bioactives known to have health-promoting (and disease prevention) functionalities, BUT.....**

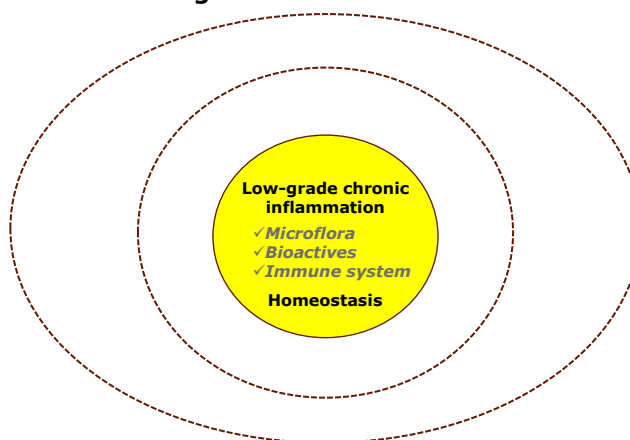
<b><u>Probiotics &amp; Prebiotics</u></b>	<b><u>(Fermented) Milk Peptides</u></b>	<b><u>Resveratrol a.o. Plant Phenolics</u></b>
<ul style="list-style-type: none"> <li>➤ GI stability</li> <li>➤ Inflammatory gut diseases</li> <li>➤ Anti-inflammatory</li> <li>➤ Anti-infective</li> <li>➤ Immune stimulating</li> <li>➤ Anti-allergic</li> <li>➤ Satiating / anti-obesigenic)</li> </ul>	<ul style="list-style-type: none"> <li>➤ Anti-hypertensive</li> <li>➤ Anti-arrhythmic</li> <li>➤ Anti-cholesterolaemic</li> <li>➤ Anti-atherosclerotic</li> <li>➤ Anti-inflammatory</li> <li>➤ Satiating (anti-obesigenic)</li> </ul>	<ul style="list-style-type: none"> <li>➤ Anti-oxidant</li> <li>➤ Anti-inflammatory</li> <li>➤ Anti-diabetic</li> <li>➤ Anti-obesigenic</li> <li>➤ Liver protecting</li> <li>➤ Energy (endurance)</li> <li>➤ Anti-carcinogenic</li> </ul>

**COMMON FEATURES:** small, additive, multiple/multifactorial effects  
**COMMON CHALLENGE:** impressive laboratory and pre-clinical data  
 – BUT human clinical trials often inconclusive

## Documentation: cause – effect - efficacy



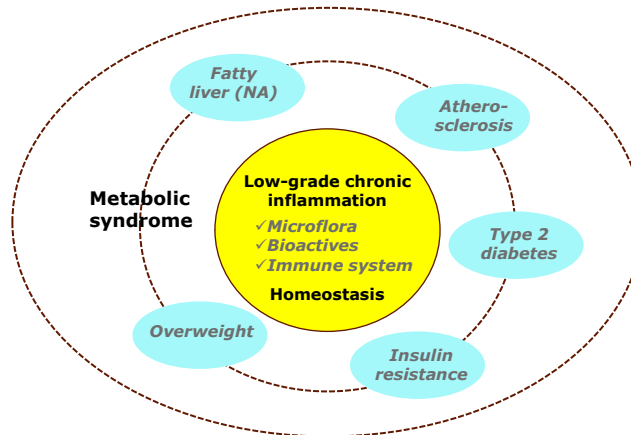
### The biomarker challenge



## Documentation: cause – effect - efficacy



### The biomarker challenge

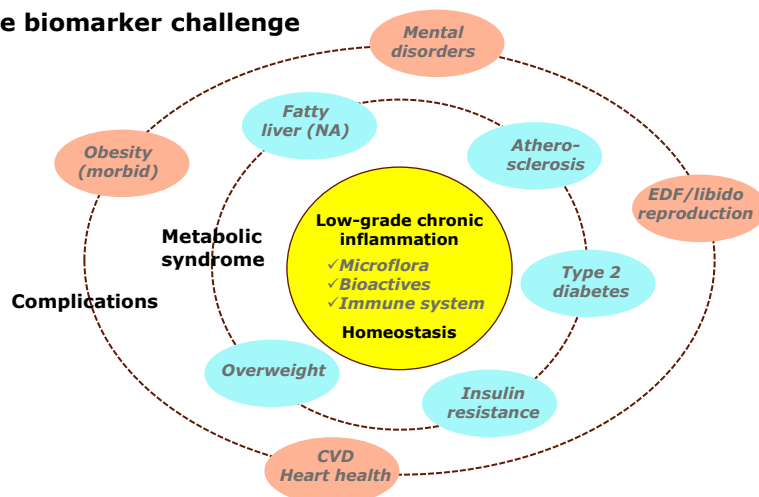


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## Documentation: cause – effect - efficacy



### The biomarker challenge

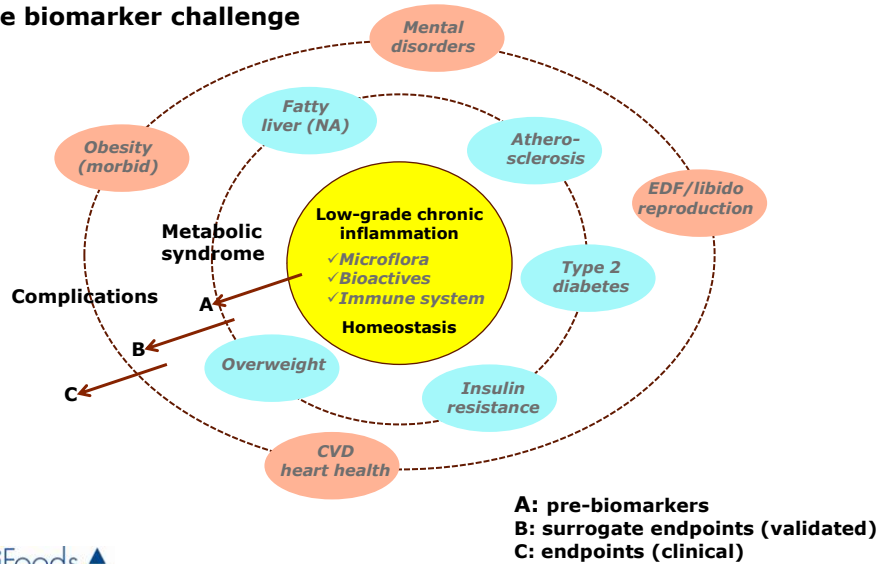


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## Documentation: "The Clinical Dogma"



### The biomarker challenge



## The omics ('holistic') approach

The genome

The transcriptome

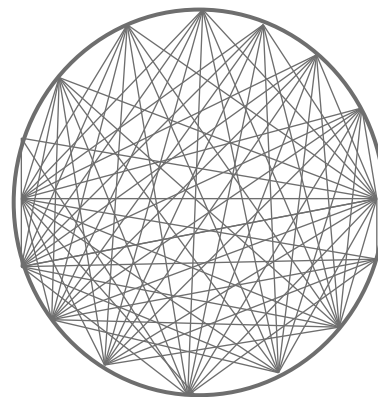
- response to diet
- individuals
- metagenome(s)

The metabolome

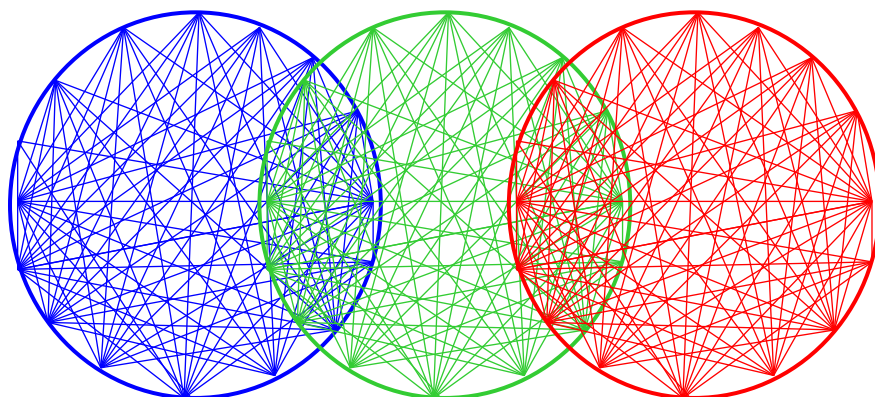
- lipidome, proteome
- 'urinome'

Other 'omes'?

- 
- 



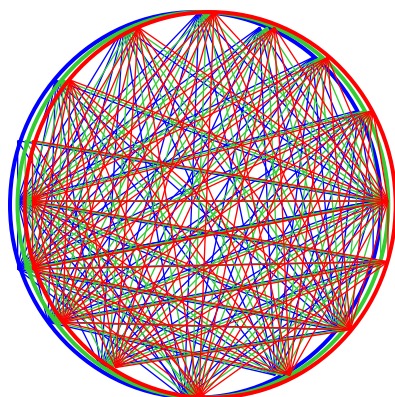
## A (very) complicated pattern profiling



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## A (very) complicated pattern profiling



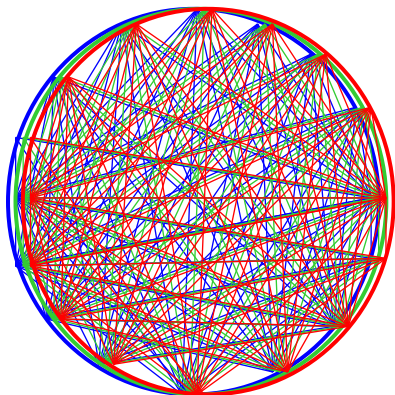
### ***DOABLE?***

Analytical chemistry  
Bioinformatics  
Chemometrics/PCA  
New advanced  
technologies

-



## A (very) complicated pattern profiling



**DOABLE? - YES!**

Analytical chemistry  
 Bioinformatics  
 Chemometrics/PCA  
 New advanced technologies



- Members of the microflora and the gut epithelial/immune cells do it all the time



## NEED: Metagenomic approaches to microbiota dynamics - the way to reduce complexity?

"Interactions between symbiotic or pathogenic microbes and the hosts they colonize are central to both health and disease.

This rapidly advancing field is now bearing the fruits of interdisciplinary efforts by microbiologists, immunologists, cell biologists, geneticists and ecologists"



Cover illustration: A human epithelial cell infected with enteropathogenic *E. coli*. (Courtesy of J.A. Gutman, A. W. Vaid and R. K. Hoyle.)

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## HOST-MICROBE INTERACTIONS

More than a century ago, Robert Koch established that infectious diseases are caused by microbes, a discovery that won him the Nobel Prize in Physiology or Medicine in 1905. At around the same time, Ilya Mechnikov, one of the pioneers of cellular immunology, was the first to recognize that microbes might also have beneficial effects on human health, when he proposed that "lactic-acid bacteria can prolong life."

Since then, a tremendous amount has been discovered about encounters between microbes and the animals they colonize — their hosts. These microbial interactions are as diverse as the organisms involved: they can be accidental or obligatory; they can result in temporary or permanent intimate associations; and they can involve subtle or intense subcellular and cellular responses. But the outcome for the host is simple: health or disease.

In the quest to understand and combat infectious diseases and, more recently, to uncover the basis of non-pathogenic microbial colonization, microbes have been found to produce a multitude of factors that either confer or promote colonization by other means. The activities of these factors are counteracted by the equally diverse responses of the host immune system. This Insight highlights advances in the study of this dynamic interplay between host and microbe, focusing on humans and bacteria. It also provides an overview of the current understanding of the ecology, evolution, immunology, cell biology and genomics of these interactions. We thank the authors and reviewers, who contributed their time, effort and enthusiasm to this collection.

We are pleased to acknowledge the financial support of Pfizer in producing this Insight. As always, Nature carries sole responsibility for all editorial content and peer review.

Claudia Lupp, Senior Editor

### FEATURE

**804 The Human Microbiome Project**  
 P. J. Turnbaugh, R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight & J. L. Gordon

### REVIEWS

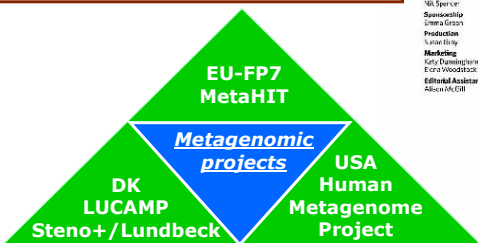
**811 An ecological and evolutionary perspective on human-microbe mutualism and disease**  
 L. DeLencastre, M. McFall-Ngai & D. A. Relman

**819 Recognition of microorganisms and activation of the immune response**  
 R. Medzhitov

**827 Manipulation of host-cell pathways by bacterial pathogens**  
 A. Bhaydar, J. A. Gutman & B. B. Finlay

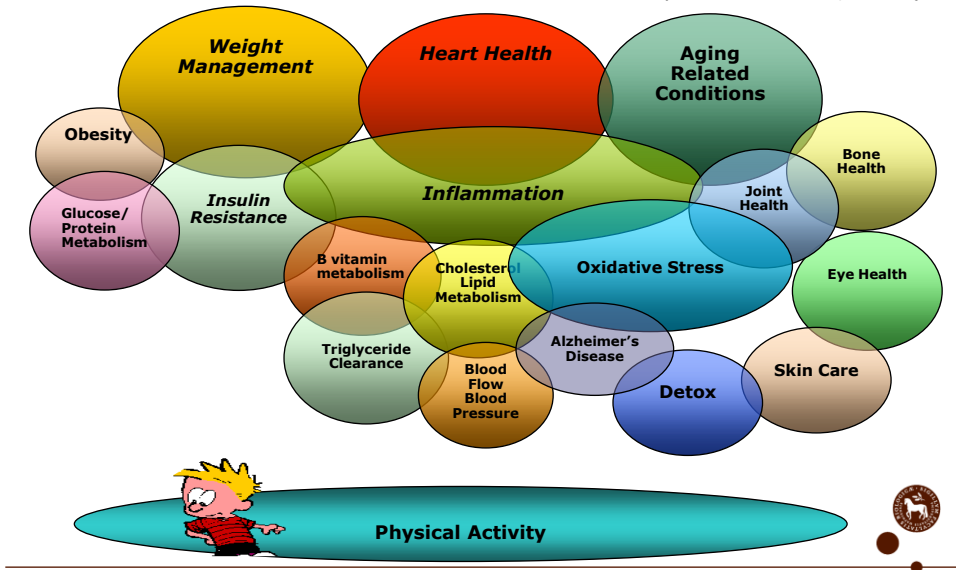
**835 Bacterial pathogenomics**  
 M. J. Fallan & B. W. Wain

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### ...and there is enough for everybody!

(Dr. Keith Grimaldi, Sciona)



### ...and there is enough for everybody!

(Dr. Keith Grimaldi, Sciona)

