Department of Microbiology, Tumor and Cell Biology

The Microbiome, PPARγ and AhR in the inflammation-metabolism interface within the gastrointestinal tract

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THE MICROBIOME, PPARγ AND AhR IN THE INFLAMMATION-METABOLISM INTERFACE WITHIN THE GASTROINTESTINAL TRACT

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Dentro de nós há uma coisa que não tem nome, essa coisa é o que somos

Inside us there is a thing that does not have a name, this thing is what we are

- José Saramago

ABSTRACT

The mammalian body is a mosaic of different organisms - a holobiont, which contains all the biomolecules and their metabolites encoded in our eukaryotic genome and supplemented by an even larger pool of prokaryotic genes and products. This symbiotic coexistence is presumed to have evolved to necessitate the sharing of biological and biochemical needs important for growth, body physiology, survival and reproduction. In this thesis, the communication between the microbiome and its host has been studied using state-of-the-art high-throughput methodologies, modelled on two known ligand-activated transcription factors, AhR and PPARγ. We show that commensal bacteria and their metabolites, short chain fatty acids (SCFA) can induce the expression of ANGPTL4 and CYP1A1 in gut epithelial cells. These genes are regulated by PPARγ and AhR respectively. ANGPTL4 is known to regulate metabolic processes connected to energy storage and utilisation, whilst CYP1A1 is involved metabolism of toxins and pollutants. These results illustrate how innate and metabolic properties of intestinal cells can be modulated by gut microbial products. This model is further evaluated using the pathogen *Salmonella enterica* serovar Typhimurium. Infection studies in mice show that *Salmonella* inactivates PPARγ and elicits acute colitis and activation of the protein lipocalin 2 (LCN2). LCN2 stabilises the metalloproteinase protein MMP9 which, in turn, further fuels tissue damage and colitis. Interestingly, the use of LCN2 KO mice drastically alleviates colitis. This highlights PPARγ/LCN2/MMP9 as a set of metabolic and immune regulators of the host that *Salmonella* needs to "hijack" in order to pave its way for intestinal colonisation. As PPARγ is a regulator of energy balance and LCN2 is an important metabolic regulatory protein, these studies establish a functional link between pathophysiology, metabolism, immune responses and gut microbes. In the final study, the bidirectional communication between the AhR signalling pathway and gut microbiome is explored. While the bacterial metabolite SCFA can regulate AhR function and expression of its target genes in intestine and liver, the composition of the gut microbiota is altered in AhR KO mice. Furthermore, metabolomic studies of AhR KO mice show that these mice, when under metabolic stress are compromised in their ability to produce ketone bodies. The evidence of metabolic stress is further supported by the observation that young AhR KO mice show growth retardation at a developmental stage that is prone to dynamic fluctuations in microbiota composition. These findings illustrate the link between immunity and metabolic functions through the sharing of biological and biochemical modulators within the holobiont. The re-discovery of the gut microbiome and its apparent influence on body functions represent a paradigm shift. We are just beginning to appreciate the importance of the microbiome as a mediator of health and are starting to understand how microbes contribute to who we are and how we function as one organism.

LIST OF PUBLICATIONS

This thesis is based on three publications:

I. Kundu P, Ling TW, **Korecka A**, Li Y, D'Arienzo R, Bunte RM, Berger T, Arulampalam V, Chambon P, Mak TW, Wahli W, Pettersson S. Absence of Intestinal PPARgamma Aggravates Acute Infectious Colitis in Mice through a Lipocalin-2-dependent Pathway. *Plos Pathogens, 2014:10 (1); e1003887*

II. **Korecka A**, de Wouters T, Cultrone A, Lapaque N, Pettersson S, Doré J, Blottière HM, Arulampalam V. ANGPTL4 expression induced by butyrate and rosiglitazone in human intestinal epithelial cells utilizes independent pathways. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 1 June 2013 Vol. 304 no. G1025-G103*

III. **Korecka A**, D'Arienzo R, Dona A, Tett AJ, Braniste V, Tylor SL, Nepelska M, Reichardt N, Abbaspour A, Nicholson J, Fujii-Kuriyama Y, Rafter J, Blottière H, Narbad A, Holmes E, Pettersson S, Arulampalam V. Cross-talk between AhR, gut microbiota and energy homeostasis. *Manuscript*

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LIST OF ABBREVIATIONS

3MC	3-methylcholanthrene
ACAT1	acetoacetyl-CoA thiolase
ACOX	acetyl-CoA oxidase
AhR	aryl hydrocarbon receptor
ANGPTL-4	angiopoietin-like 4
ARNT	aryl hydrocarbon receptor nuclear translocator
AMP	antimicrobial peptide
bHLH	basic helix-loop-helix
BNF	β-naphtoflavone
CD	Crohn's disease
CD36	cluster of differentiation 36
cfu	colony forming units
CLA	conjugated linoleic acid
CoA	co-enzyme A
CPT	carnitine palmitoyl transferase
CYP	cytochrome
DC	dendritic cell
FABP1	fatty acid binding protein 1
FFA	free fatty acids
FICZ	6-formylindolo[3,2-b]carbazole
GI	gastrointestinal
GPR	G-protein coupled receptor
HAT	histone acetyltransferase
HDAC	histone deacetylase
HSP90	heat shock protein 90
I3C	indole-3-carbinol
IBD	inflammatory bowel disease
IEC	intestinal epithelial cell
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
IID	intestinal inflammatory disorder
IL	interleukin
ILC	innate lymphoid cell
KO	knock-out
LCN2	lipocalin 2
LPS	lipopolisaccharide
MAMP	microbe-associated molecular pattern
NCoA	nuclear receptor co-activator
NCoR	nuclear receptor co-repressor
NFκB	nuclear factor kappa B
NLR	nuclear oligomerisation domain-like receptor

NR	nuclear receptor
PAS	PER-ARNT-SIM
PPAR	peroxisome proliferator-activated receptor
PPRE	PPAR response element
PRR	pattern recognition receptor
REG	regenerating islet-derived protein
RORγt	retinoic acid-related orphan receptor gamma t
SCFA	short chain fatty acid
TCA	tricarboxylic (citric) acid cycle
TCDD	2,3,7,8- tetrachlorodibenzo-*p*-dioxin
TFF	trefoil factor
TMA	trimethylamine
TLR	toll-like receptor
Trp	tryptophan
TSA	trichiostatin A
TZD	thiazolidinedione
UC	ulcerative colitis
WD	western diet
WT	wild-type
XRE	xenobiotic response element

1 INTRODUCTION

The gastrointestinal tract is a complex ecosystem where prokaryotic bacteria meet eukaryotic cells and interaction between them are influenced by nutrition, toxins and drugs. A delicate balance is created between the host defence machinery preparing itself against pathogenic invasions whilst at the same time providing a niche for trillions of commensal bacteria that will support the host in a symbiotic manner. Many receptors and signalling pathways are engaged in this process. Many more remain to be discovered. Two known guardians of intestinal homeostasis are the peroxisome proliferator-activated receptor gamma (PPARγ) and the aryl hydrocarbon receptor (AhR). These two proteins control many of the physiological processes taking place in the gut, including those at the inflammation-metabolism interface. They also respond to bacterial signals allowing for bidirectional exchange of signals and nutrients between the host and its microbiome, which is necessary for the maintenance of health.

1.1 The gastrointestinal tract

The gastrointestinal (GI) tract is a multifunctional organ with a complex structure and diverse physiological roles. Rather than a single organ, it is an ecosystem composed of three main components: host cells, microbiota and environmental cues, including nutrients. The main function of the gastrointestinal tract is to provide energy as well as micro- and macronutrients to the rest of the body. In recent years, however, it became apparent that the GI tract is much more than just a digestive and absorptive organ. The GI tract provides the first line of defence against natural toxins and man-made chemicals. It also contains trillions of bacteria and maintains a balance between tolerogenic and inflammatory signals. This balance is necessary for peaceful coexistence and exchange of signals between the host and the microbiome, which in turn ensures proper regulation of many physiological processes. The GI tract is also an endocrine organ, which produces signalling molecules communicating with the rest of the body, including the brain. All these functions are strongly related to the particular structure and morphology of the GI tract.

1.1.1 Structure and morphology of the gastrointestinal tract

In broad terms, the lower GI tract can be divided into the stomach, small intestine and colon (large intestine). Each of these compartments has a slightly different anatomy, role and physiology (Table 1).

Name	Function	pH	Length (cm)	Diameter (cm)
Stomach	HCl secretion, digestion of macromolecules	2	variable	variable
Small intestine	Main digestion. Absorbtion of monosaccharides, fatty acids, amino acids and water	4-5	600	2,5
Colon	Absorbtion of water, vitamins and bile acids.	7	150	7,5

Table 1. *Function, pH and dimensions of different parts of the human lower GI tract.*

The small intestine is further divided into duodenum, jejunum and ileum. The colon is divided into proximal and distal parts (ascending, transverse and descending colon in humans). From the anatomical point of view, the small and large intestine share a similar structure of a tube covered by many layers of distinct tissues. The main layers surrounding the intestinal lumen are mucosa, submucosa and smooth muscles. The mucosa is composed of a layer of smooth muscles, lamina propria, a single layer of epithelial cells separating the host from the environment and mucus - glycoprotein gel-like structure secreted to the lumen. The structure of the mucosal layer of the small intestine and colon vary significantly. The epithelial cells of the small intestine form villi and crypts. In the colon, the villi are absent. The mucus layer structure is also distinct in the small intestine and the colon (Figure 1) (1).

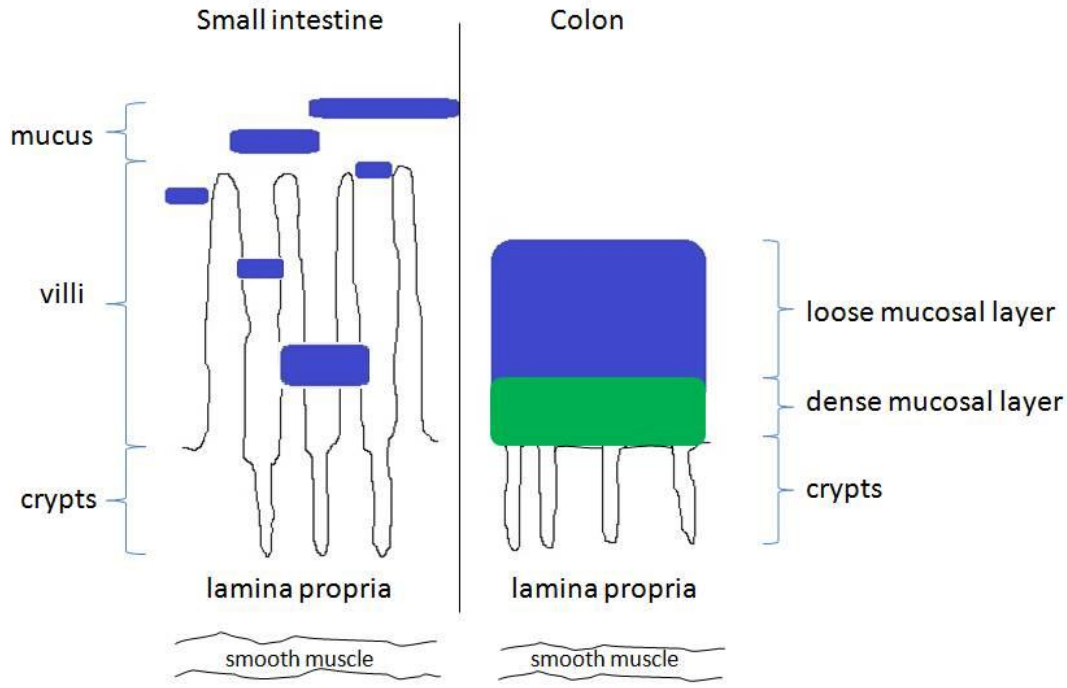

Figure 1. *Structure of the small intestinal and colonic mucosa.*

The epithelial cells are connected with each other by tight junctions to prevent leakage of large molecules in-between the cells into the lamina propria. Epithelial cells are not uniform and several types can be distinguished based on morphology and function: stem cells, enterocytes, enteroendocrine, goblet and Paneth cells. The stem cells are located at the bottom of the crypts and give rise to all the other cell types building the epithelial layer. The enterocytes are the most abundant cell type and function as absorptive cells, responsible for transport of nutrients from the lumen to lamina propria, from where they are further transported to the blood or lymph circulation. Goblet cells are specialised in producing mucins - the glycoproteins building the mucosal barrier. Enterocytes and goblet cells have a life span of 5 to 6 days. Enteroendocrine cells produce signalling molecules that regulate many digestive processes, e.g. release of enzymes from the pancreas. Paneth cells are present in the small intestine but not in the colon. They are situated at the bottom of the crypts, in proximity to

stem cells. Paneth cells are long lived cells, with a life span up to one month. Paneth cells produce antimicrobial peptides, which play an important role in protecting the host from invasion of pathogenic bacteria. In the colon, which lacks the Paneth cells, the antimicrobial peptides are secreted by enterocytes (2,3). Apart from epithelial cells, the first layer of contact between the host and the environment also consists of infiltrating immune cells, called intraepithelial lymphocytes (IEL). One other type of immune cells present in the lamina propria - dendritic cells are thought to produce protrusions between the epithelial cells to reach the lumen and sample the intestinal antigens. The sampling of antigens also occurs directly through a specific cell type called the M-cells. The presence of cells with immune function in the close proximity of bacterial and nutritional antigens assures proper tolerogenic responses within the gut. It also provides a very sensitive system for rapid detection of pathogenic bacteria invading the GI ecosystem.

1.1.2 Gastrointestinal tract as a multifunctional organ

The GI tract has been for many years regarded as a digestive organ. Recently, it has become apparent that the GI tract is much more than just an absorptive surface. Many metabolically active enzymes are expressed in the gut epithelium influencing the energy balance of the whole body. Harmful substances, like chemicals and toxins are also absorbed and/or metabolised in the epithelial cells of the gut. The GI tract is a place of bidirectional exchange of signals and nutrients between the host and the microbiota. One of the tasks of the GI tract is to maintain a balance between tolerance to nutritional and commensal antigens at the same time providing an effective defence system against pathogens. This balance is achieved thanks to a mucus layer and production of antimicrobial peptides by the epithelial cells as well as by the presence of competent immune cells in the lamina propria. Additionally, it has now became apparent that the GI tract communicates (via the vascular lymphatic system, vagus nerve, signalling peptides and others) with the rest of the body and influences many physiological processes.

1.1.2.1 Digestive function of the GI tract

The digestive function of the GI tract is its most well known and most studied function. Digestion of food starts with a breakdown of macromolecules like fat, carbohydrates and proteins into smaller molecules (fatty acids, simple sugars and peptides). This process begins already in the mouth and continues in the stomach and throughout the small intestine. Enzymes, like pepsin, are released from the pancreas and upon activation in the small intestine break down proteins. Bile acids are secreted from the gall bladder and facilitate the breakdown and absorption of fats. Most nutrients are absorbed to the circulation before reaching the colon. In the colon, water, vitamins and minerals are absorbed. It is also in the colon where the residing microbiota ferment proteins and complex carbohydrates, providing additional source of energy for the host in form of short chain fatty acids (SCFA).

1.1.2.2 Xenobiotic metabolism

The main function of the GI tract is to extract energy and minerals from ingested food. However, not everything that reaches the intestine has a nutritional value. Together with food and water we also ingest toxins, chemicals and environmental pollutants, collectively known as xenobiotics (from Greek *xeno* meaning foreign). Xenobiotics (including medicines) introduced orally are a subject of so called first-pass metabolism in the gastrointestinal canal and liver before they reach the rest of the body. The

metabolism of xenobiotics is divided into two phases: I and II. In phase I xenobiotics are modified chemically (most commonly hydroxylated, deaminated, oxidated or dealkylated). These chemical modifications allow for the phase II reactions in which xenobiotics are conjugated with a water-soluble molecules (UDP-glucuronic acid, acetyl-coA, glycin or sulphates to name a few) to be further exported out from the cells to the blood stream or back to the intestinal lumen. Many of the genes coding phase I and phase II enzymes, as well as specific transporters for conjugated xenobiotics are expressed in the epithelial cells of the GI tract. In general, the expression and activity of xenobiotic metabolising enzymes decreases with descending from duodenum towards the ileum (4,5). The most important group of phase I enzymes is the family of cytochrome P450 proteins (CYP450). There are 57 members of CYP450 family in human and 102 in mice (http://drnelson.uthsc.edu/CytochromeP450.html). The CYP450 genes are divided into 18 families and 43 subfamilies. The most important ones from the pharmacological point of view are enzymes belonging to CYP1, CYP2, CYP3 families (6). CYP1 genes are expressed in the epithelial cells of the GI tract of mice and humans and are responsible for metabolism of drugs and steroids, especially oestrogen (7,8). CYP1 proteins are divided into 3 subfamilies: CYP1A1, CYP1A2 and CYP1B1. Metabolism of xenobiotic in the epithelial cells of the GI tract can be protective or detrimental to the host, depending on the type of substance. Additionally, some xenobiotics are metabolised by bacteria resident in the GI tract (9,10).

1.1.2.3 Mucosa: the intestinal gatekeeper

The characteristic anatomy of the gastrointestinal tract with villi and crypts forms an enormous inhabitable surface of around 200m^2 (11). This surface and the lumen of the gut are densely populated by bacteria, fungi and protozoa. Bacteria inhabiting the gut outnumber our own cells 10 times and contain 100 times more genes. We harbour around 1,5 kg of bacteria in our gut, more than 10^{12} cells/cm^3 of colonic content. On the other hand, our "insides" are separated from the lumen and inhabiting microbiota by a single layer of cells, about 10µm thick. To make their task of protecting the host from bacterial invasion even more difficult, the epithelial cells do not remain steady, but are in constant movement from the crypts to the top of the villi, during which time they differentiate. The epithelial lining is exchanged by new cells every week, proving great challenge of keeping the intestinal barrier intact. The bacterial community also fluctuates, responding to changes in diet, antibiotic intake or presence of opportunistic pathogens (12,13). Even though we are in constant alert to spot the potentially dangerous bacteria, we must remember that the majority of bacteria inhabiting our intestines are neutral or beneficial for our health, providing vitamins and nutrients (like SCFA). The intestinal defence system must therefore have a way to distinguish between the "good" and the "bad" bacteria, allowing for the growth of the first, and preventing invasion of the latter. During many years of evolution a system that provides means to maintain the correct balance between the host and the microbiome in the GI tract has developed. This system is composed of many elements: mucosal layer, antimicrobial peptides, pattern recognition receptors and gut associated immune tissues, to name a few.

1.1.2.3.1 The mucus layer

The surface of the GI tract is covered by a layer of mucus, a gel-like structure protecting the thin epithelial lining from the invasion of bacteria. This layer is composed of cross-linked glycoproteins, called mucins where the oligosaccharide part forms up to 80% of the molecule (14). Mucins are produced and secreted by goblet cells. The mucus layer in the small intestine is not organised in any specific way but scattered randomly among the villi (1). In the colon, however, mucus is organised in two distinguishable layers - dense, bacteria free layer closest to the epithelial cells, and loose mucin layer, where the commensal bacteria reside (Figure 1) (15). The thickness of the mucosal layer in the colon reaches up to 150μm (16). It can, however, be diminished by antibiotic treatment, predisposing the host to bacterially-induced colitis (17). Thinner mucosal layer is also observed in patients with inflammatory bowel disease (IBD) (18). The mucosal layer is however not only an important barrier that keeps bacteria at distance from the epithelial lining. It also provides a habitat for mucin degrading bacteria. Glycans forming the mucosal layer represent an important energy source for commensal bacteria, which in turn produce SCFA, providing energy for colonocytes. In this way, part of the energy lost in the process of mucin production is re-gained by the host (1,19,20). Mucus has one more important function. It provides a matrix on which antimicrobial peptides secreted from epithelial cells are concentrated preventing translocation of bacteria to the proximity of epithelial cells (21).

1.1.2.3.2 Antimicrobial peptides

Antimicrobial peptides (AMPs) produced by the epithelial cells are natural antibiotics, with a task to kill or deactivate invading bacteria. In contrast to antibiotics used in clinics, to which bacteria fast develop resistance, very few bacterial species are resistant to AMP indicating how important AMPs are in basic defence against pathogens (3,22). One of the few pathogenic species that did develop resistance to many of the AMPs is *Salmonella enterica* (23). AMPs are evolutionally conserved and can be found in all organisms, from plants to mammals (24). AMPs are found on the skin, in the intestine, respiratory and reproductive tracts. Some of the AMPs are expressed constitutively, i.e. independently from microbial presence. For some AMPs, however, specific bacterial signals are required to induce their expression and secretion to the lumen. Many AMPs are secreted in response to bacterial signals sensed by pattern recognition receptors, present on epithelial cell and cells of the immune system.

There are several groups of AMPs with different functions and structure, corresponding to the diversity of the bacterial communities. The most prevalent group of AMPs is formed by α-defensins, also known as cryptidins in mice. α-defensins are positively charged molecules that bind to bacterial cell membranes which are charged negatively. This causes membrane disruption, being effective against both Gram-positive and Gram-negative bacteria, some fungi, viruses and protozoa. α-defensins are mostly present in the small intestine where they are produced by Paneth cells (25). In the colon, which lacks Paneth cells, β-defensins are produced instead by the enterocytes.

Another group of AMPs are C-type lectins, including regenerating islet-derived protein family (REG). The most important example of a protein belonging to this group is REG3γ in mice and REG3α in humans, both secreted from enterocytes and Paneth cells of the small intestine (26). In the colon, their expression is induced in enterocytes upon infection (27). REG proteins are most efficient in killing

Gram-positive bacteria, because they bind peptidoglycans, one of the components of bacterial cell wall of Gram-positive bacteria (28).

Apart from disrupting the bacterial cell membrane, other AMPs fight the bacteria by different mechanism, for example by interfering with mineral supply. Lipocalin 2 (LCN2) is an example of such an AMP, which when secreted from enterocytes and immune cells sequesters iron-laden siderophores, making iron inaccessible for bacteria (29). This sequestration is believed to dampen growth and invasion of intestinal pathogens.

AMPs can also exert their functions indirectly, by stimulating the immune responses of the host. Defensins have chemo-attractant properties and recruit leukocytes to the site of infection. Deregulation in AMPs production and activity can lead to breakage of self-tolerance and development of autoimmune disorders (30).

1.1.2.3.3 Pattern recognition receptors

Pattern recognition receptors (PRRs) are proteins expressed in epithelial and immune cells with a function of recognising microbial presence, by binding to microbe-associated molecular patterns (MAMPs). MAMPs are generally conserved structures of bacterial cells, like cell wall components (e.g. lipopolisaccharide and peptidoglycan), flagella or microbial DNA and RNA molecules. There are two main families of PRRs - Toll like receptors (TLRs) and nuclear oligomerisation domain-like receptors (NLRs). Once MAMPs are detected a cascade of signalling events is activated inside the host cell leading to the production of defence molecules, like mucins, AMPs, immunoglobulins or cytokines, depending on the type of activated cell (31,32). One of the most important cytokines for the maintenance of immunological balance between inflammation and tolerance in the gut is interleukin-22 (IL-22), which is produced by specific type of cells called innate lymphoid cells (ILC).

1.1.2.3.4 Innate lymphoid cells

Apart from defence system provided by the epithelial cells, the lamina propria of the intestine hosts a variety of immune cells. One of the cell types crucial for the maintenance of intestinal homeostasis are innate lymphoid cells (ILCs) (33). There are three families of ILCs, characterised by activity of specific transcription factors and production of cytokines. Group 3 ILCs expresses Retinoic acid-related Orphan Receptor gamma t (RORγt) and aryl hydrocarbon receptor (AhR) transcription factors and produce IL-22 and IL-17A in response to bacteria (34). IL-22 stimulate production of mucins and AMPs in the IEC which express the IL-22 receptor (35).The cross-talk between ILCs and the intestinal flora is bidirectional. Bacterial signals influence the function of ILCs, and the activity of ILCs changes the composition of the microbiota in the gut (33).

1.1.2.3.5 Other components of the immune system

Epithelial cells and ILCs provide very important defence systems against invasion of potentially harmful bacteria. Many other cell types participate in protection of the host against pathogens. These include adaptive immune cells and innate immune cells, like dendritic cells (DC) or macrophages. One of the most important cell type within the adaptive immune system are B-cells, which produce immunoglobulins (Ig). In the gut, it is IgA, that plays the most important role in host's protection.

IgA is secreted to the lumen where it causes bacterial agglutination and trapping in the mucus (36,37) Another adaptive immune cell type that is important for the intestinal homeostasis are T-cells, especially T helper -17 (Th17 cells) and T regulatory cells (Tregs in the small intestine and Tr1 in the colon). T regulatory cells are crucial for the development of tolerance to commensal bacteria, nutrients and other molecules that can be found in the intestinal lumen. The development of tolerance is directed via production of interleukin 10 by these cells (38).

1.2 The Microbiome

Mucosal surfaces, like skin and respiratory, uro-genital, and gastrointestinal tracts, are constantly in touch with bacteria, viruses, fungi and protozoa. In fact, our bodies contain more prokaryotic than eukaryotic cells. The GI tract, due to its anatomy provides the largest inhabitable space. Bacteria residing in or GI tract are collectively known as the gut microbiota. Microbiome, on the other hand, describes all constituents of microbiota: genes, proteins and metabolites; which collectively influence an array of host's physiological processes. Our microbiome is often referred to as commensal bacteria, giving the impression that the bacteria and host have no influence over each other's physiology. However, this statement might underestimate the potential of the microbiota to influence host condition whilst host genetics can influence the composition and functions of its microbiota. Today we know that our bacterial communities contribute to many physiological processes taking place in the GI tract (39). These functions include breakdown of complex polysaccharides (40), conversion of conjugated bile acids (41) and production of vitamins B-1, B-2, B-12 and K (42). In this way the microbiota provide a set of metabolic traits, which are needed for our lives, but which we do not intrinsically possess.

1.2.1 Development and composition of intestinal microbiota

Foetus developing in the mother's uterus is believed to be free of bacteria. It does, however, remain under the influence of maternal microbiome. Delivery provides the first contact between bacteria-free neonate and bacteria-laden environment. The mode of delivery is important for the establishment of proper intestinal bacterial communities. Vaginally born children are first colonised by the maternal faecal and vaginal flora. Caesarean section delivery leads to disrupted colonisation of the gut in children, where the flora is acquired mostly from the skin of hospital stuff and the hospital environment (43–48). Colonisation of the infant's gut at birth seem to be important for proper energy extraction from milk, which is a new source of nutrients for the newborn. Certain bacterial species, especially Bifidobateria and *Bacteroidetes thetaiotaomicron* were reported to play a major role in the breakdown of milk oligosaccharides, which provides easily available energy source for the infant (49). The colonisation progresses gradually from birth throughout our entire life-span. During early childhood the number of bacteria inhabiting the gut is growing and changing dynamically. Aerobic and facultative anaerobic bacteria, which are the first gut colonizers are slowly replaced by obligate anaerobes. This process can be disrupted by the use of antibiotics, which may lead to permanent changes in the composition of intestinal flora and development of several diseases, including childhood obesity (50–53). Bacterial numbers and composition stabilises within 2-3 years from birth, reaching 10^{12} bacteria in one ml of faecal content. At this stage the infants microbiota resembles that of an adult individual. In general mammalian intestinal bacteria belong to nine phyla: Firmicutes,

Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Fusobacteria, Spirochaetes and TM7 (54). These phyla are represented by numerous bacterial species, of which only a very small proportion is shared by majority of individuals underlining the enormous diversity of mammalian commensal microbial community (55). In 2011 Arumugam and colleagues proposed to divide human microbiota into three functional groups (56). These groups were called enterotypes and the division was based on the functional characteristics of bacteria inhabiting the gut. Enterotype I, for example, is thought to be dominated by Bacteroidetes, which have the ability to ferment complex carbohydrates and synthesise biotin. Enterotype II would be dominated by Prevotella, which degrade mucins and other proteins (56). The presence of these two enterotypes was later confirmed in an independent study (57). Enterotypes seem also to be linked with long term dietary habits, distinguishing between meat eaters and vegetarians. Indeed, it seems that the environmental cues (including diet), rather than genetic factors, are responsible for the huge variation of bacterial composition amongst humans and other mammals (58,59). The concept of enterotypes requires further investigation in order to confirm its universality, but even now provides an interesting way of looking at functional groups and their role in shaping the physiological processes of the host, rather than single at the presence or absence of particular species of bacteria. While looking at the microbiota we must therefore consider not only the different species but also the genes that are carried by the bacteria which will be responsible for their function in the gut. There are 100 more bacterial open reading frames in our intestines than our own genes. The most abundant bacterial genes code for enzymes involved in the digestion of complex sugars and their fermentation to SCFA.

Intestinal compartment	**Bacterial abundance (cfu/ml)**	**Dominant bacteria**
Stomach	$1\text{-}10^2$	*Lactobacillus, Streptococcus, Helicobacter, Peptostreptococcus*
Duodenum	10^1 - 10^3	*Streptococcus, Lactococcus, Staphylococcus*
Jejunum	10^2 - 10^4	*Lactobacillus, Streptococcus, Enterococcus*
Ileum	10^7 - 10^9	Segmented filamentous bacteria, Enterobacteriaceae, *Bacteroides, Clostridium*
Colon	10^{11} - 10^{12}	*Bacteroides, Clostridium*, Lachnospiraceae, Proteobacteria, Actinobacteria, Prevotellaceae, TM7, Fusobacteria, Verrucomicrobium

Table 2. *Spatial distribution and composition of the microbiota along the GI tract* (60,61).

1.2.2 Short chain fatty acids

Short chain fatty acids (SCFA) are bacterial metabolites produced by anaerobic fermentation of dietary complex carbohydrates and proteins. There are three major SCFA produced in the gut: acetic acid, propionic acid and butyric acid. It is not uncommon for these acids to form salts with sodium in the colon, therefore they are often named: sodium acetate, propionate or butyrate respectively (Figure 2). SCFA are produced in millimolar concentrations. In mouse the concentration of SCFA reaches 10mM in the proximal small intestine and 40mM in the colon (62). The most abundantly produced SCFA is acetate, followed by propionate and butyrate (63). In human faeces the concentration of butyrate is

between 10-20mM. In mice the colonic concentration of butyrate vary between 0,5mM to 3,5mM (64). Butyrate is the main energy source for colonocytes (65). Butyrate can also be transported to the liver, where together with propionate and acetate it may become a substrate for lipogenesis (66,67). Acetate is also transported to the peripheral tissues and is detected in the blood stream at micromolar concentrations (68). Apart from being energy source for the host, SCFAs also regulate various cellular processes. SCFAs enter the cell by simple diffusion or by binding to various transport proteins on the cell membrane. Once inside the cells SCFAs work as histone deacetylase (HDAC) inhibitors. HDACs control the acetylation status of histones and many other proteins, including transcription factors and regulators, DNA repair enzymes, chaperon proteins and inflammatory mediators (69,70). Apart from their function as HDAC inhibitors, SCFAs also affect cellular processes before entering the cell. This signalling pathway is mediated mostly by binding of SCFA to G-protein coupled receptors, mostly to GPR41 and GPR43. Activation of these receptors by SCFA leads to secretion of peptide YY which slows down gastrointestinal transit. SCFA signalling via GPR 43 is also crucial in regulation of energy balance and adiposity of the host. Additionally SCFA may signal through GPR41 in other tissues, like adipocytes which leads to increased adipogenesis, decreased lipolysis and inducing leptin secretion (71). GPR43, on the other hand, is expressed on variety of immune cells including neutrophiles, eosinophiles and macrophages. Signalling via GPR43 on these cells is linked to anti-inflammatory properties of SCFAs.

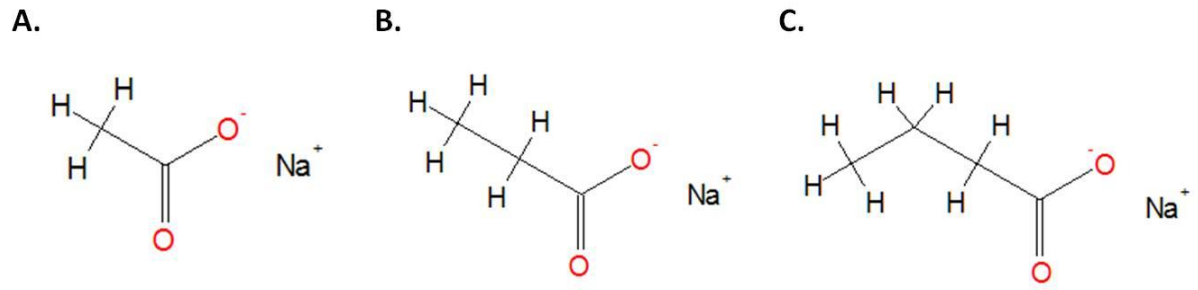

Figure 2. *Schematic structures of sodium acetate (A), sodium propionate (B) and sodium butyrate (C). Generated with BioClipse 2.0.*

1.2.3. Interactions between the microbiome and the host

The host and the microbiome co-evolved for millions of years to minimize the genetic pool and to use each other's genes and enzymatic functions in the most optimal way (72,73). The mammalian microbiome controls digestive function of the GI tract, immune responses (74), energy metabolism (75) and even behaviour (76–78). The presence of commensal microbiota inhibits the growth of pathogens. Microbiota can also metabolize toxins to less or more harmful substances contributing to the intestinal xenobiotic metabolism. They also produce a variety of biologically active substances like SCFAs, conjugated linoleic acid (CLA), phenoles, indoles, or trimethylamine (TMA) (79–81). Microbiota play an important role in preventing or accelerating development of lifestyle diseases like cancer, obesity or cardiovascular diseases (82). Maintenance of peaceful relationship between the microbiome and the host is therefore essential for health.

The best model to study how bacteria influence host's physiology is the germfree (GF) mouse, which is devoid of bacteria. GF mice can be monocolonised with a specific bacterial species to assess the role

of this very species in the regulation of many physiological processes, including energy homeostasis. Whole bacterial community from conventionally raised mice (specific pathogen free, SPF) can also be introduced to GF mice, a process called conventionalisation. Colonisation of mice has profound effects on gene expression in host's cells. Processes in which microbiota play the most visible role are regulation of energy homeostasis, immune system regulation and activation of host defence systems.

1.2.3.1 The microbiome and energy metabolism

The most straight forward way to assess if the microbiome plays a role in energy metabolism, and as a consequence in the development of diet-induced obesity and metabolic disorders, is to compare the outcome of feeding of GF and SPF mice specially composed semi-synthetic diets with increased amount of fat. Several studies with such a design have been conducted with an overall conclusion that GF mice are protected from high-fat diet induced weight gain and insulin resistance (83,84). However, one other study suggests that this resistance is dependent on the composition of the diet and thus more investigation is needed in order to unravel the contribution of the intestinal microbiota to the development of obesity (85). Conventionalisation, i.e. introduction of a "normal" SPF microbiota to a previously GF mouse is another method to study the contribution of bacteria to regulation of metabolic processes and allows for studying the dynamic metabolic changes from the moment of introduction of microbiota to a given point in time (usually up to 14-30 days). Colonisation of GF mice results in increased body weight and adiposity. These effects are mimicked by the introduction only two bacterial species, namely *Bacteroidetes thetaiotaomicron* and *Methanobrevibacter smithii*. These two species have the ability to ferment carbohydrates to SCFAs (86,87). This discovery raised a question if SCFAs contribute to development of obesity in mice and humans. Many studies were conducted but no consensus is reached so far. Some results indicate association of SCFAs with increased body mass, some do not find this correlation, other show the opposite, i.e. that increased SCFA concentrations in the animals' caecum or supplementation of diet with SCFAs results in lower body mass (61). A recent elegant publication on human subjects, trying to solve this conundrum, was performed on monozygotic twins discordant for obesity. In these twin pairs, one individual was obese, and the other one lean. Presence of bacteria producing SCFAs was associated with lean phenotype. Interestingly, the lean and obese phenotypes could be transferred to GF mice together with the "lean" and "obese" microbiome (88).

Intestinal bacteria have also been shown to influence other processes connected with energy and nutrients metabolism and to play a role in the development of several metabolic diseases. Intestinal microbiota and their metabolites were shown, for example, to play a role in cholesterol metabolism and the development of non-alcoholic fatty liver disease (89–91).

1.2.3.2 Bacteria and intestinal inflammatory diseases

Bacteria that live in our intestine play an important role in modulating the balance between immune tolerance and host defence. Many mouse models have been developed to study in details the role of microbiome in the development and pathogenesis of intestinal inflammatory disorders (IIDs). The IIDs comprise both inflammatory bowel disease (IBD) and more acute, pathogen driven inflammatory conditions. IBD is a complex disease comprising at least two subtypes with different pathologies, namely Crohn's disease (CD) and Ulcerative Colitis (UC). The causes of IBD are not well understood

but are believed to be a combination of genetic predispositions, bacterial dysbiosis and environmental factors. Pathogen-driven inflammatory disorders are easier to study in the sense that the cause of the disease in usually an infection with a specific pathogenic bacteria. The most commonly studied mouse models for acute inflammation involve infections with *Citrobacter rodentium* or *Salmonella enterica* subs. *enterica* serovar Typhimurium. Many mice strains are naturally resistant to these pathogens due to so called colonisation resistance. Colonisation resistance is a phenomenon where commensal bacteria residing the in gut provide protection against invasion of opportunistic pathogens. Antibiotic administration is required prior to infection in order to obtain intestinal inflammation resembling diseases observed in humans. The use of antibiotics diminishes the numbers of commensal microbiota and changes the prevalence of certain bacterial groups abrogating the colonisation resistance and making the mice more susceptible to the infection (224).

The presence of commensal bacteria is also necessary for the proper development of mucosal layer, production of certain types of AMPs and proper functioning of the ILCs. De-regulation of these processes may lead to alterations in the composition of intestinal microbiota and development of IBD (17,30,92). This in turn will alter the prevailing microbiome, including soluble metabolites which can transverse the mucosa, and affect epithelial and lamina propria cells. Some of these metabolites can act as activators of host receptors, such as PRRs and transcription factors. Rapid changes necessary for host responses in recognition of a changing microbial environment are best countered by transcription factors, especially ones that are ligand-inducible.

1.3. Ligand-activated transcription factors

In order for the bacteria to have an impact on the host, bacterial signals need to be sensed by an eukaryotic cell. This is usually done by the PRRs, some of which are expressed at the cell surface. However, signals must be transmitted within the cell to cause a specific reaction, for example modulation of host gene expression or activity of enzymatic pathways. One such sensors are ligand-activated transcription factors. These proteins have the ability to respond quickly to environmental signals and induce expression of a given group of target genes. Ligand-activated transcription factors are a large group of structurally unrelated proteins, but all activated by a ligand and able to induce gene expression in response to ligand binding. There are many transcription factors that work in a ligand-dependent manner and respond to environmental cues. This work focuses on two of them, that are especially important for intestinal physiology: Peroxisome proliferator-activated receptor gamma (PPARγ), belonging to the nuclear receptors family and Aryl hydrocarbon receptor (AhR) - a member of basic helix-loop-helix/PER-ARNT-SIM family.

1.3.1 Nuclear receptors

Nuclear receptors (NR) are a group of structurally-conserved ligand-activated transcription factors. In humans 49 members of the NR superfamily have been identified. Almost all NRs contain two activation domains, AF-1 and AF-2 of which one functions in a ligand-dependent manner and the other one in a ligand-independent way. AF-1 and AF-2 are separated by DNA-binding domain, a linking region and ligand-binding domain (Figure 3). These domains acquire a characteristic conformation upon ligand- and DNA-binding which allows for interaction with a set of co-factors (activators or repressors) as well as for homo- or heterodimerisation with other NRs. Depending on the

type of ligand, NRs are divided into three main classes. The first class includes the steroid- and thyroid-hormone receptors like oestrogen and glucocorticoid receptors. The second class consists of so called orphan receptors that share the structure and functionality of a typical NR but for which no naturally occurring ligand has been identified yet. It is also possible that no such ligand exists and that these receptors function in a ligand-independent way. The third class of NRs consists of so called adopted NRs, for which the natural ligands were discovered only recently.

Figure 3. *Schematic representation of NR protein domains.*
Green – activation domains (AF-1 and AF-2), blue – DNA binding domain composed of two zinc-fingers based domains, red – ligand binding domain, gray – variable regions including hinge region linking the DNA-binding domain with ligand-binding domain.

Upon ligand binding NRs bind specific DNA sequences and induce transcription of their respective target genes. By regulating gene expression, NRs control virtually all processes in our bodies, including reproduction, development, immune responses and metabolism. Based on NRs' expression patterns and functions of their target genes, NRs are divided into two main clusters (93). The first cluster contains NRs essential for the proper development of an organism and basic physiological functions. NRs assigned to this cluster are expressed in the reproductive organs controlling sexual maturation and function (oestrogen receptor, testosterone receptor). This cluster also contains NRs expressed in the central nervous system which controls neural development. Other members of this cluster are expressed in the liver and heart, and their function is coupled to circadian clock rhythms controlling for example cardiac function or cholesterol efflux to the liver. The second cluster contains NRs that are expressed mainly in the gastro/enterohepatic axis and key metabolic tissues (muscle, adipocytes). This cluster is divided into three groups with distinct functions:

- bile acid and xenobiotic metabolism with function in nutrient uptake and barrier maintenance
- lipid metabolism and energy homeostasis - use of dietary-derived lipids as fuel
- lipid metabolism and energy homeostasis - specialised aspects of fuel utilisation like glucose homeostasis, lipid storage, cholesterol metabolism

Nuclear receptors do not only induce the expression of target genes, they can also block transcription of certain subsets of genes, a phenomenon known as trans-repression. During infection pro-inflammatory genes are often repressed by ligand-bound NRs. There are many models trying to explain the molecular basis of trans-repression. Two main mechanisms are postulated to explain this phenomenon: (i) activated NR prevents removal of repressive complexes from the promoters of pro-inflammatory genes, or (ii) activated NR competes for transcriptional activators, which cannot be recruited to the promoters of pro-inflammatory genes (94,95).

1.3.2 Nuclear receptors co-regulators

Nuclear receptors do not work alone but rather in combination with a battery of co-regulators. These co-regulators can enhance the function of a NR (co-activator, NCoA) or block it (co-repressor, NCoR). Co-activators play an important role in regulation of NR's function and fine-tuning of the target genes' expression levels. Co-activators recruit transcription machinery to the promoter allowing for chromatin conformation changes by acetylating or methylating histone tails. Co-repressors usually bind the non-liganded form of NR preventing its unspecific activation. Binding of a co-repressor to a liganded NR was also reported and probably plays a role in trans-repression of genes. NCoR have often histone deacetylase (HDAC) activities whereas NCoA functions often as histone acetyltrasferase (HAT) (96).

1.3.3 Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the superfamily of nuclear receptors. PPARs were first discovered and cloned in the early 1990s (97). Three forms or PPARs have been described so far: PPARα, PPARβ (also known as PPARδ) and PPARγ. In order to induce gene expression PPARs must form heterodimers with another NR, called Retinoic X Receptor (RXR). PPARs bind specific DNA sequences called PPAR response elements (PPREs), which are variation of a consensus sequence AGGTCA (98). PPARs' ligand binding pockets are quite big and flexible allowing for attachment of various types of ligands, sometimes with quite different chemical structures including fatty acids, arachidonic acid, prostaglandin derivates and thiazolidinediones (TZD) (99–101).

Each of the three PPARs has a specific tissue and cell type expression pattern as well as a set of target genes that determines its physiological function. PPARα is expressed in the gastrointestinal epithelium, liver, skeletal muscle and heart. It favours utilisation of lipids as energy source in those tissues (102). PPARβ is expressed virtually in all tissues and its exact function in each of them remains unknown. In the skin and intestine PPARβ plays an important role in wound healing, whereas in the skeletal muscle it regulates lipid metabolism. PPARγ has been most extensively studied as an important regulator of adipocyte maturation and lipid storage (103). However, PPARγ is expressed not only in the adipocytes, but also in other cell types.

1.3.4 The functions of PPARγ

PPARγ is activated by a variety of ligands, including unsaturated fatty acids (100), oxidised low density lipoproteins (oxLDL) (104), prostanoids like 15-deoxy-d-12,14-prostaglandin J2 (101) as well as synthetically obtained thiazolidinediones (TZD) (99). PPARγ is expressed as two isoforms: γ1 and γ2 which differ by 28 aminoacids in the AF-1 domain. The longer γ2 version is expressed almost exclusively in adipocytes. The shorter γ1 isofom is expressed by a variety of different cell types including immune system, skeletal muscle, bone building cells and epithelial cells of the gastrointestinal tract (105).

1.3.4.1 PPARγ and metabolism

The most well known and most intensively studied function of PPARγ is its ability to regulate metabolic processes. PPARγ is necessary and sufficient for adipocyte differentiation (106) and regulates the storage or release of fat from the adipose tissue. PPARγ regulates also the production and secretion of hormones from adipose tissue, including lectin and adiponectin as well as other signalling molecules like Angiopoietin-like 4 protein (ANGPTL-4) a.k.a Fasting induced adipose factor (FIAF). PPARγ is also expressed in the brown adipose tissue (BAT), which has a function in maintaining body temperature via thermogenesis (107,108). Activation of PPARγ by TZD improves glucose and insulin tolerance in subjects with obesity and metabolic disorders. This effect might be mediated simply by instructing the adipose tissue to store and retain more fatty acids, which are otherwise toxic to the peripheral tissues. However, obesity and diabetes are not purely metabolic diseases. Many other factors including the aberrant activation of the immune system play a significant role in the development of these pathologies. Interestingly, activation of PPARγ in macrophages was partially responsible for the therapeutic effects of TZDs (109,110).

1.4.4.2 PPARγ and immunity

Although PPARγ has been studied predominantly as a regulator of energy metabolism the evidence for its importance as an immune regulator is growing. PPARγ is expressed in variety of immune cells, out of which the most well studied are macrophages and dendritic cells. Stimulation of macrophages with PPARγ ligands prevents expression of pro-inflammatory genes upon stimulation with MAMPs like LPS (111). These effects are believed to be due to trans-repression of Nuclear Factor κB (NFκB) target genes which may lead to development of so called alternatively activated macrophages (or M2), in which PPARγ is important for the maintenance of the anti-inflammatory properties. The anti-inflammatory properties of M2 macrophages are connected with PPARγ-induced metabolic changes in these cells (112,113). PPARγ activation in DC also induces expression of genes connected to lipid transport and metabolism. Activation of PPARγ enables lipid presentation by DC, which is needed for activation of invariant natural killer cells, which then secrete variety of cytokines (including IL-4 and IL-5).

1.4.4.3 PPARγ in the intestinal epithelial cells

PPARγ is highly expressed in the intestinal epithelial cells (IEC) (114,115). In the colon PPARγ is expressed in both differentiated cells and stem cells. In the small intestine PPARγ's expression is induced at the crypt/villus junction, where the epithelial cells start to differentiate (116–118). Activation of PPARγ in the GI tract can be achieved in many ways. Oral administration of TZD activates the receptor and induces expression of many of the PPARγ target genes, including that of ANGPTL4 (119). Activity of PPARγ can be modulated *in vitro* by bacteria and their metabolites (119–121). Activation of PPARγ in the IEC leads to elevated expression of specific target genes. Those target genes can be functionally divided in 4 major cluster controlling different physiological processes: (i) metabolism (mostly lipid transport and metabolism), (ii) cellular signal transduction, (iii) proliferation (withdrawal from cell cycle and differentiation), and (iv) cellular motility and adhesion (117).

Expression and activity of PPARγ in IEC is important for the maintenance of homeostasis between microbiota and the host. Deletion of PPARγ results in decreased expression of defensins and reduced antimicrobial defences against infections caused by *Escherichia coli* and *Bacteroides fragilis* in the colon (122). PPARγ protects also against experimentally induced colitis in mice (123–126). In humans, patients with IBD have lower expression of intestinal PPARγ then healthy people (127). Additionally several polymorphisms in the PPARγ gene were identified and associated with risk of developing the disease. The most commonly found polymorphism is observed at position 12 in amino acid sequence. The Ala12Ala genotype seems to protect against development of IBD in the European population (128) whereas the Pro12Ala allele was associated with increased risk of developing IBD in several studies (129–131).

1.4.3 Basic helix-loop-helix/PER-ARNT-SIM proteins

Basic helix-loop-helix (bHLH) proteins are transcription factors, as are nuclear receptors. Even though the functional properties of these two groups are similar, they have very different molecular structures. bHLH transcription factors posses two α-helixes linked by a loop which provides flexibility. Similarly to NR bHLH protein usually require dimerisation with other proteins in order to exert its function. The conserved DNA sequence to which bHLH proteins bind is called E-box and has a consensus sequence CANNTG. The PER-ARNT-SIM (PAS) proteins form a subgroup within bHLH transcription factors, making a group called bHLH/PAS proteins. bHLH/PAS have two PAS domains, PASA and PASB, which are however poorly conserved in amino acid levels within the group. The structure of a typical bHLH/PAS protein is shown in Figure 4. The PAS domains provide specificity of action and influence the dimerisation properties of each protein. PASA domain prevents binding with bHLH proteins that do not contain a PAS domain. The presence of PASA also allows the transcription factor to bind non-classical E-box sequences. PASB binds small molecules and provides interaction with other proteins that respond to metabolic or environmental signals (132,133). There are 19 members of bHLH-PAS family with broad range of functions including regulation of developmental processes and response to stress. bHLH/PAS proteins can be divided into two classes. Class I is usually tissue restricted or signal specific. The members of class II are ubiquitously expressed and activated by a variety of stimuli. In order to bind DNA class I bHLH/PAS proteins dimerise with proteins from class II. The examples of class I bHLH/PAS proteins include: single-minded homolog 1 (SIM1) expressed in the neurons, ubiquitously expressed CLOCK (Circadian Locomotor Output Cycles Kaput) and stimuli-responsive hypoxia inducible factor 1 alpha (HIF1α) and aryl hydrocarbon receptor (AhR).

bHLH/PAS proteins also bind a set of co-activators similarly to NRs. In most cases binding of the co-factor enhances the expression of specific target genes. Additionally some co-regulators initially identified as NR co-activators have been shown to have a high sequence similarity to bHLH/PAS proteins and this domain allows them to interact with many proteins including p53 and signal transducer and activator of transcription 6 (STAT6). NCoA1 and 3 were shown to bind to AhR and aryl hydrocarbon nuclear translocator (ARNT) and modulate the activity of these receptors.

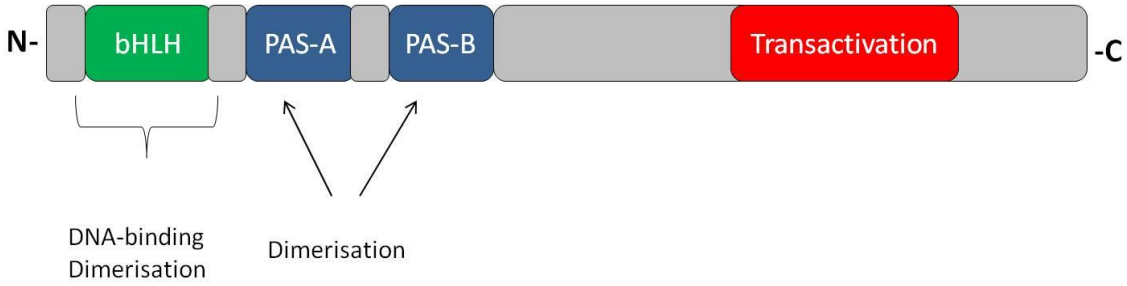

Figure 4. *Schematic representation of bHLH/PAS protein domains.*
Green – bHLH domain responsible for DNA binding and dimerisation with other proteins, blue – PAS domains responsible for dimerisaton, red – transactivation domains, gray – variable regions

1.4.3.1 Aryl hydrocarbon receptor (AhR)

AhR is a ligand activated transcription factor that belongs to the bHLH/PAS family (134–136). The PASB domain of AhR provides interaction with heat shock protein 90 (HSP90). Other two chaperon proteins, namely p23 and AhR interacting protein (AIP) are also bound to AhR in the cytoplasm. AhR-HSP90-p23-AIP complex remains in the cytoplasm and does not possess transcriptional activity. Within this complex AhR is in a conformation that exposes the ligand binding pocket (137). Upon ligand binding the conformation of the AhR changes exposing the DNA-binding domain and translocation of the AhR to the nucleus. In order to bind to DNA AhR needs to form a heterodimer with ARNT (138,139). The DNA binding specificity is provided by PASA domain.

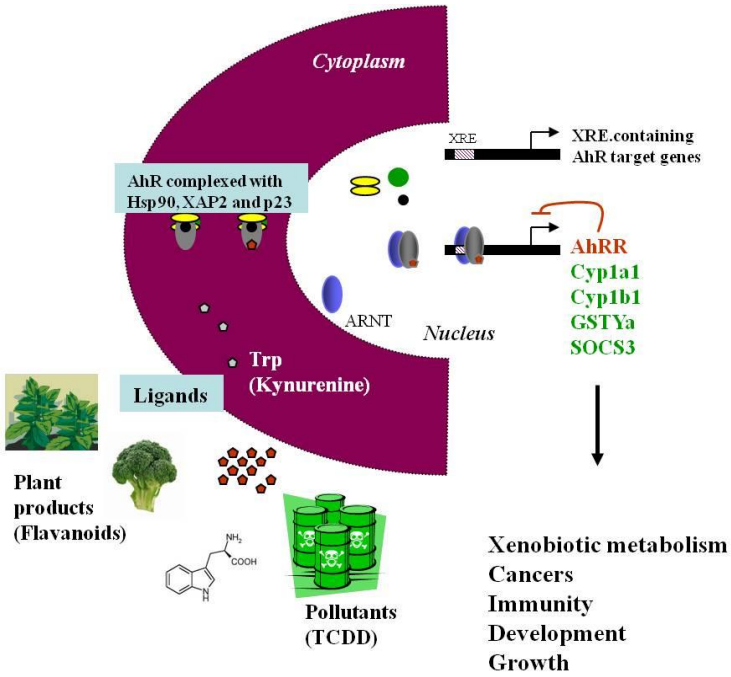

Figure 5*. AhR as a ligand-activated transcription factor*

The consensus sequence TNGCGTG to which AhR-ARNT complex bind is called xenobiotic response element (XRE) or drug responsive element (DRE) (140). The mechanism of action of AhR as a transcription factor is depicted in Figure 5. Deletion of PASA domain of the AhR makes the protein non-functional, whereas deletion of PASB results in constitutively active AhR protein (141).

1.4.3.2 The functions of AhR

AhR is highly conserved evolutionarily and expressed in many cell types (142,143). AhR plays multiple roles in induction of metabolism of xenobiotics and regulation of physiological processes, including immune regulation and energy metabolism. Historically the most studied AhR target genes are Phase I drug metabolizing enzymes CYP450 family members: CYP1A1, CYP1A2 and CYP1B1, inducing the detoxification cascade (144). The prototypic AhR ligands include man-made pollutants, 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD), 3-methylcholanthrene (3MC), benzo(a)pyren and other polycyclic aromatic hydrocarbons (145). Activation of AhR by those ligands leads to several toxic effects including alterations in lipid metabolism, and embryonic development as well as tumorigenesis and immunosuppression (146). In recent years, however, a battery of natural AhR ligands has been discovered, including metabolites or derivates of tryptophan, like FICZ (6-formylindolo[3,2-b]carbazole) or kynurenine as well as other dietary ligands, like indole-3-carbinol (I3C), present in cruciferous vegetables, such as broccoli and cauliflower (147–149). Activation of the AhR by these naturally-derived ligands was shown to have physiological effects, sometimes opposite to the ones caused by the environmental pollutants (150–152). Apart from its function as a transcription factor AhR has also been shown to act as a E3 ubiquitin ligase and may in this way regulate the stability of the oestrogen receptor (153). Deletion of AhR results in a variety of immunological, metabolic and developmental defects. One of the most striking feature of the AhR KO mice is liver deformation caused by a failure to close the *ductus venosus* (a blood vessel shunt in the liver) after birth leading to lifelong impairment of blood flow in the liver. As a consequence, the liver of an AhR KO mouse is roughly half the size of a liver of a WT mouse (154).

1.4.3.3. AhR and immunity

Apart from being a metabolic regulator, AhR also plays an important role in modulating the functions of the immune system and response to bacterial infections (reviewed in (155)). AhR is especially important in the regulation of development and function of dendritic cells and T-cells, including T helper cells (especially Th17) and Treg cells. Treg cells play a crucial role in the distinguishing between self and non-self and development of tolerance. AhR is very important in the induction of expression and stability of the transcription factor FoxP3, which promotes the differentiation of T cells into Tregs. AhR is also important for the development and functioning of Tr1 in the colon. Both Treg and Tr1 produce the anti-inflammatory IL-10, a process which is most probably AhR-dependent. Apart from being a direct regulator of T cell development, the AhR is also involved in generation and activity of DCs. DCs on the other hand influence differentiation of T cells to a variety of subtypes, including Th17, Treg and Tr1 cells. AhR decreases the ability of DC to present antigens and inhibits secretion of T-cell stimulatory cytokines. In this way, the AhR indirectly promotes the development of Treg and Tr1 cells, rather than Th17 cells. AhR increases the expression of IDO (idoleamine 2,3-dioxygenase) in DCs. This results in production of kynurenine which is immunosuppressive.

Kynurenine may in turn activate AhR in T-cells which promotes the development of Treg cells. One another AhR-dependent process is the production of retinoic acid by DCs. Retinoic acid is required to promotes the differentiation of Treg at mucosal surfaces.

1.4.3.4 AhR in the GI tract

AhR is an important payer in maintaining the intestinal homeostasis. It is required for development of intraepithelial lymphocytes (IEL) and innate lymphoid cells producing IL-22 (ILC22). AhR KO mice are more susceptible to chemically and bacterially induced colitis (34,150,205-209). Lymphocytes taken from patients with CD have decreased expression of AhR. In experimental animal models, activation of AhR resulted in amelioration of disease and decreased inflammation. However, these studies had very limited potential for translating into humans because the AhR's ligands used (like TCDDs or benzo(a)pyren) have very high toxicity (156). Only the recent discovery of endogenous or food-derived AhR ligands opened new horizons for treatment of inflammatory conditions by activating AhR. Indol-3-carbinol (I3C) is a component of dietary vegetables that is known to activate AhR *in vivo* and to provide a signal to retain the IEL in the gut, which gives protection against the intestinal inflammation (151). AhR expression in ILC is critical to protect the mice against *Citrobacter rodentium* infections, as mice lacking the AhR are much more susceptible to infection (157). Apart from the role of AhR in modulation of function of IEL and ILC22, AhR blocks the development of Th17 cells and promotes that of Tr1 during experimentally-induced colitis (158). In parallel to nutritionally-derived anti-inflammatory AhR ligands, bacterial signals also ameliorate colitis in mouse models in a potentially AhR-dependent manner. More studies, especially clinical trials with humans are needed to fully appreciate the role of AhR in the function and protection of the gastrointestinal tract.

1.4.3.5 AhR and metabolism

The role of AhR in metabolism has been studied almost exclusively in conjunction with toxicity of man-made pollutants on animal models. Chronic administration of TCDD to mice results in profound changes in expression of genes regulating cholesterol synthesis, *de novo* lipogenesis and glycolisis (159). Acute exposure to TCDD lowers the expression of genes involved in gluconeogenesis in the liver (160) and decreases glucose transport into the adipose tissue (161). Administration of 3MC in C57Bl/6J mice resulted in fat accumulation in the liver and development of liver steatosis in conjunction with increased levels of PPARα mRNA (162). Transgenic mice expressing constitutively active AhR in the liver and intestine develop hepatic steatosis as well (163). On the other hand deletion of the AhR results in lower expression of PPARα and decreased expression of genes, whose protein products are involved in β-oxidation of fatty acids (164). All these studies indicate a strong link between AhR expression/activity and metabolism of fats and glucose, mainly the regulation of gluconeogenesis and β-oxidation of fatty acids.

The only evidence of involvement of the AhR in regulation of metabolic processes in human comes from epidemiological studies. Studies have indicated a correlation between global obesity epidemic world-wide and the increasing exposure to environmental pollutants, which are potential AhR ligands. The presence and concentration of many of these pollutants in human serum is positively correlated

with diabetes and metabolic syndrome (165)**.** A recent study also links circulating serum AhR-ligands with mitochondrial dysfunction (166). Additionally, many of the these substances have oestrogen-like properties and exposure to these leads to disturbed hormonal balance and metabolic problems. This problem is of particular importance because AhR may directly and indirectly interact with oestrogen receptor itself (153).

In summary we can say that some small molecules (of dietary or microbial origin) may function as ligands for the ligand-activated transcription factors. These factors, including PPARγ and AhR would then bind to their respective response elements on the DNA and induce expression of their specific target genes. In this work we have focused on two main groups of target genes, which play a role in modulation of inflammation and metabolism. By regulating transcription of these two functional categories of genes the ligand-activated transcription factors regulate the function of the GI tract, liver and muscle (summarised in Figure 6)

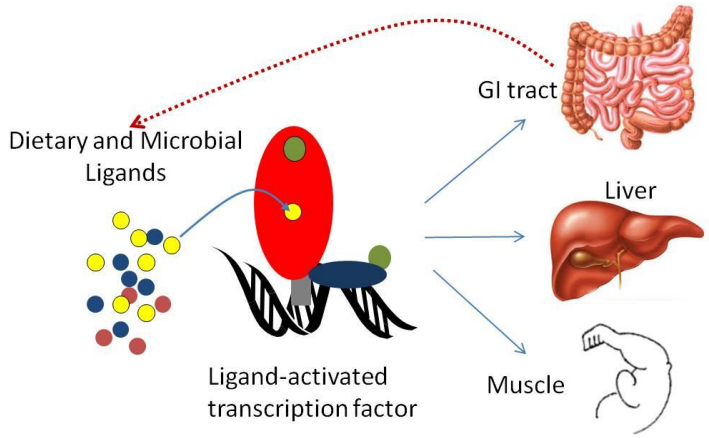

Figure 6. *Small molecules can act as agonist to trigger ligand-activated transcription factors, which execute various functions in organs such as the intestine, liver and muscle.*

1.5 Liver and the control of energy metabolism

Liver is traditionally not included in the concept of the GI tract. However, one should not forget the connection of this organ to the functioning of the GI tract. Liver is directly connected to the small intestine by the hepatic portal circulation. Hepatic portal vein transports nutrients, toxins and signalling molecules absorbed in the intestine directly to the liver, where they can be stored or metabolised before reaching the general circulation. Liver is therefore the first target organ for the intestinal signals and a sensor of intestinal health.

Liver is also one of the main organs responsible for the regulation of the host energy balance and metabolism. During fasting periods lipids are liberated from adipose tissue to the blood stream in form of glycerol and free fatty acids (FFA). FFA are taken up by the liver by various fatty acid transporters, including CD36 (167,168) and fatty acid binding protein 1 (FABP1, a.k.a. L-FABP) (169,170). Once inside the cell, FFA are activated by the Acyl-CoA synthetase which adds a Coezyme A (CoA) molecule to FFA generating Acyl-CoA. The fate of Acyl-CoA depends on its carbon chain length. Very long (>20C) and long (C14-20) acyl-CoA molecules are directed primarily to peroxisomes. However, it was shown that in brown adipose tissue the medium chain fatty acids can also be oxidised

inside peroxisomes (171). The first reaction of β-oxidation (desaturation) inside peroxisomes is catalysed by Acetyl-CoA oxidases (ACOX) that donate electrons directly to molecular oxygen, thereby producing hydrogen peroxide that is later disposed of by catalase. (172). Long, medium and short chain fatty acids are oxidised in the mitochondria. If the mitochondrial β-oxidation pathway is compromised medium and short chain fatty acids may be oxidazied in the endoplasmatic reticulum by enzymes belonging to the Cytochrome P450 family, like CYP4A1. Short and medium chain fatty acids enter the mitochondria by simple diffusion, but long chain fatty acids depend on a transportation system involving carnitine palmitoyl transferases (CPT1 and CPT2) and carnitine-acylcarnitine translocase (CACT a.k.a. Slc20a25) proteins. These proteins convert Acyl-CoA to acylcarnitine and then back to acyl-CoA inside the mitochondrial matrix in order to move the fatty acid molecule through the double mitochondrial membrane. The first step in the β-oxidation pathway inside the mitochondria is catalysed by chain length specific acyl-coA dehydrogenases: very long chain (VLCAD), long chain (LCAD), medium chain (MCAD) or short chain (SCAD) acyl-CoAs dehydrogenases. These Acyl-CoA dehydrogenases remove two carbons from the C-terminus of the acyl-CoA in each reaction and the process is repeated until acyl-CoA is degraded to two carbon atoms Acetyl-CoA molecule (for Acyl-CoA with even numbers of carbons) or a three carbon atoms propionyl CoA (for Acyl-coA with odd number of carbons) (173). Propionyl Co-A is used as a substrate for gluconeogenesis whereas Acetyl-CoA can either enter the citric acid cycle (TCA) to be fully oxidised to CO_2, or serves as substrate for ketone bodies synthesis. There are two main ketone bodies synthesised in the liver: acetoacetate and 3-hydroxybutyrate. Acetoacetate is produced in the liver in HMG-CoA cycle catalysed by acetoacetyl-CoA thiolase (ACAT1), HMG-CoA synthase (HMGCS2) and HMG-CoA lyase (HMGCL). Acetoacetate can then be reversibly transformed to 3-hydroxybutyrate by D-3-hydroxybutyrate dehydrogenase (BHD1). Ketone bodies diffuse into the blood stream and are taken up by peripheral tissues, like the brain and skeletal muscle. In these tissues ketone bodies are converted back to Acetyl-CoA before they can be utilised as energy source and oxidizaed to CO_2. This step catalysed by BHD1 (converting B-hydroxybutyrate to acetocetate), 3-oxoacid CoA-transferase (OXCT1) and ACAT1. Brain tissue cannot oxidise fatty acids present in the blood, it therefore depends in a big part on the energy delivered in a form of ketone bodies during periods of low blood glucose levels caused by food shortage. Skeletal muscles are more flexible in the source of energy they use for proper functioning. Apart from ketone bodies skeletal muscle cells can directly use circulating fatty acids or glucose to gain enrgy. Based on the preference for utilizing glucose or fatty acids muscles are divided into two main types: white (where anaerobic glycolysis predominates) and red where β-oxidation of fatty acids take place. Both types can use ketone bodies as energy source during fasting periods. Lactate is a main product of glycolysis and an increased level of lactate in the plasma is an indicator of ongoing glycolisis.

In this thesis I have perused the question as to whether the intestinal microbiome can either directly or indirectly influence the metabolic processes activated during periods of stress, such as fasting. We know from the work of others that bacteria can regulate adipose tissue, liver and brain function (Figure 7). Some of these effects may be mediated by metabolites potentially linked to the composition of the microbiota. Variations in the microbiome may confer different levels of adaptability to different kinds of metabolic stress.

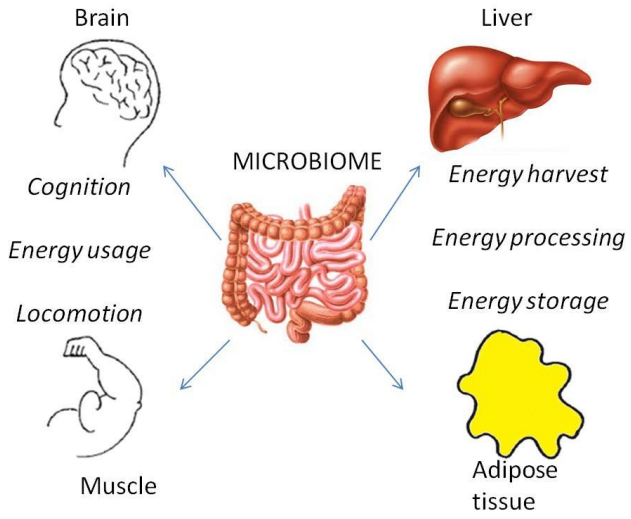

Figure 7. *The microbiome is thought to influence metabolic processes in a variety of organs. Signals from the intestine can impact on both sides of the energy equation: energy harvest and storage in organs such as liver and adipose tissue, balanced against energy usage in e.g. brain and muscle.*

2. AIMS

The objective of this thesis was to investigate the interactions between the host and its intestinal microbiota. First of all, we were interested to see how intestinal bacteria, both commensal and pathogenic influence the expression and activity of two ligand-activated transcription factors PPARγ and AhR. Secondly, we set out to investigate the role of these receptors in sensing the bacterial signals and responding to them by regulation of expression of genes involved in metabolism and/or immune response.

Specific aims for each paper:

I. To evaluate the role for epithelial PPARγ in defence against *Salmonella* Typhimurium infection

II. To investigate if the expression of ANGPTL4 in the intestinal epithelial cells is regulated by bacteria and their metabolites - short chain fatty acids and if this regulation was mediated via activation of PPARγ

III. To study the cross-talk between gut microbiota and AhR and its role in the regulation of energy metabolism. This paper has three specific aims:

a. To study the impact of microbiota and its metabolites on the expression and activity of the AhR in the intestinal epithelial cells.
b. To assess if absence of AhR influences the composition of intestinal microbiota
c. To evaluate the role of the AhR in regulation of energy metabolism, in particular on glucose and fat utilisation during fasting

3. METHODOLOGICAL HIGHLIGHTS

3.1 IEC-specific PPARγ knock-out

In order to investigate the role of PPARγ on development of *Salmonella*-induced colitis we have used a mouse model, with non-functional PPARγ in IEC. These mice were generated by crossing two mouse strains. The first, called PPARγ-flox was a strain where the exon 2 of PPARγ DNA sequence was flanked from the 3' and 5' ends by two LoxP sequences. The second mouse strain was a transgenic mouse that expressed Cre gene under the control of villin promoter. Villin promoter is transcriptionally active in the epithelial cells of the GI tract but not in any immune cell type, which allows for IEC specific expression of Cre. Cre is an endonuclease that recognizes and cuts LoxP DNA sequence generating double strand breaks at both ends of the region flanked by LoxP sequences. Crossing the Villin-Cre mouse to PPARγ-flox mice (F0) gives rise to mice (F1) in which Cre, expressed in IEC, generates double strand breaks at both ends of exon 2 of PPARγ gene. As a result of Cre-mediated DNA recombination the two LoxP sites are linked together omitting exon 2 of the PPARγ gene generating a frame shift, resulting in non-functional PPARγ. However, F1 mice have only one PPARγ allelflanked by the LoxP sites, the second one being a wild type allel, which not be targeted for Cre-mediated recombination. F1 mice are therefore back-crossed to PPARγ-flox mice in order to obtain a mouse with two allels with PPARγ LoxP sites and a Cre transgene (F2), which are than selected for further breeding. F3 provides mice with IEC specific PPARγ deletion (named PPARγ IEC KO or Cre+) and control mice (named PPARγ IEC LoxP or Cre-). The breeding scheme is shown in Figure 8. The presence or absence of Cre transgene as well as the deletion or PPARγ can be confirmed by PCR to allocate mice to KO or WT group.

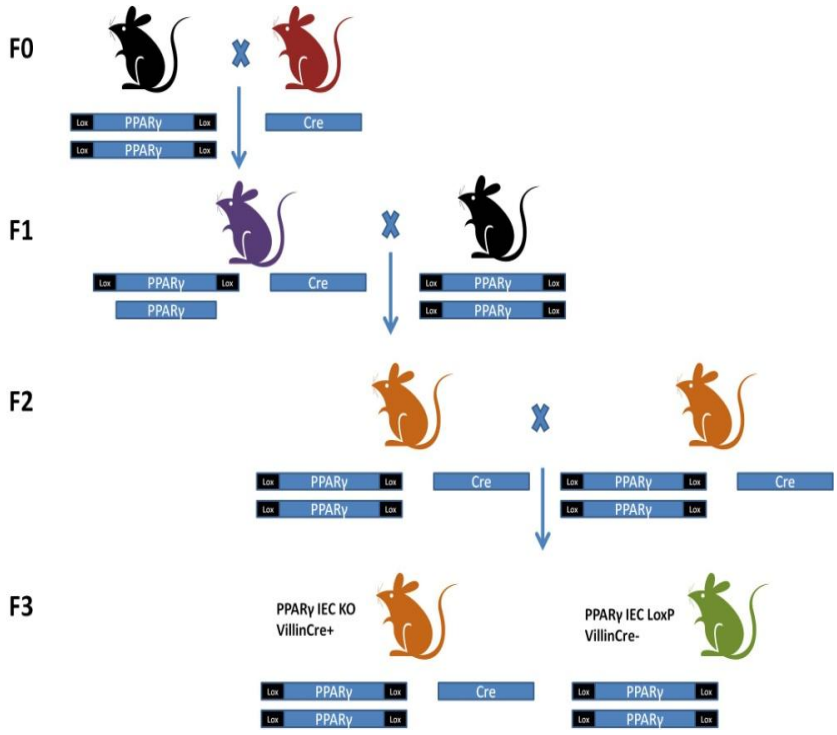

Figure 8. *Breeding scheme for obtaining PPARγ IEC specific knock-out as described in the text.*

3.2 Gnotobiotic mouse models

Gnotobiotic animals are a powerful tool to study the host-bacterial interactions and their physiological importance. A gnotobiotic animal (from Greek *gnotos* = known and *bios* = life) is an animal in the which microbial composition is known. In practice, the gnotobiotic animals can be completely devoid of bacteria - GF, containing only a single bacterial species - monocolonised or have several different species whose identity is known. GF, mono- and bicolonised mice have been used to assess the role of the microbiota in many processes including immune system development and resistance to infections, development of the gastrointestinal tract, energy metabolism with special attention to obesity and metabolic disorders, and recently signalling between the gut and the brain as well as behaviour.

Maintenance of gnotobiotic animals requires special precautions and specific conditions, so that the animal cannot be colonised by unwanted microorganisms. In order to do this, gnotobiotic animals, mostly mice and rats, are kept in specially designed sterile isolators where all the incoming air is sterile filtered. Cages, bedding, and other housing materials as well as water and food must be autoclaved before given to the animals.

Rearing a new strain of mice into GF conditions is based on a principle that pups in the uterus remain sterile and do not have direct contact with microorganisms and is done by transferring the pups while still within in the uterine sac to a GF foster mother. Even though the pups reared in this way and raised inside an isolator are considered GF one must remember that their mother was not, which means that they remained under microbial influence during *in utero* development. At least one or two generations of mice born in strictly GF conditions inside the isolators must pass before starting any experiments.

Most GF strains are viable and healthy, if certain vitamins, e.g. vitamin K and B vitamins are supplemented with food. The most characteristic feature differentiating a conventionally raised mouse from a GF mouse is that GF mice have enlarged caecum, most probably containing the undigested fibres and carbohydrates. Most mouse strains breed normally under GF conditions. They do however have many developmental irregularities. Many of these are directly connected to the development and functioning of the GI tract, including altered vascularisation and muscle wall thickness (174). Expression of many of the NR and xenobiotic metabolizing enzyme is also disrupted in GF mice (175,176). Experiments performed on GF mice show that bacteria play a major role in the development of the hosts defence systems, including structure of mucus, production of AMPs by enterocytes, cytokines and immunoglobulins, which is diminished in mice lacking microbiota (15,177). All these features make GF mice more susceptible to infections with pathogens. However, GF mice seem to be resistant to certain inheritable chronic inflammatory diseases, underlining the importance of microbiota in development of immune system and responses (178).

3.3 Metabolomics

Metabolomics, together with genomics, transcriptomics and proteomics form the holistic way to look at an organism and all the biochemical processes that take place in it. The "omics" research exploded at the end of the last century due to huge technological advances allowing for processing hundreds, thousands or even millions samples in a very short time. Genomics aims to obtain a full DNA sequence of a given organism, transcriptomics studies the pool of genes transcribed to RNA,

proteomics the pool of mRNA translated into proteins. Metabolomics is the study of the metabolome. The metabolome is a collection of metabolites, especially small molecules that are present in a cell, tissue or other biological sample (usually blood or urine) under particular circumstances and are products of enzymatic reactions ongoing in the body. Metabolomics, therefore, allows getting a direct insight into which chemical and/or enzymatic processes are active at the given moment in the organism providing a functional readout of cellular state. Another advantage of metabolomics is that many biofluids like urine or blood can be analysed without any pre-treatment. For solid tissues, pre-treatment usually confers separation of organic and aqueous phases prior to screening and analyses of these fractions separately. The most commonly used method in metabolomic screening in nuclear magnetic resonance (NMR). NMR consists of placing the sample of interest in a magnetic field, where the atoms, when excited by the field, produce a characteristic signal depending on the mass and structure of the chemical molecule. ^{1}H-NMR methods are based on recording signals produced by atoms of hydrogens. These will generate spectra where hydrogen atoms in each molecule generate a specific signal. One molecule might therefore emit several signals, depending on the placement and bonding of hydrogen. The emitted spectra are then compared with standard spectra for each metabolite to generate the metabolite profile of a given sample. There are two main approaches for analysis of samples in metabolomic studies: targeted and un-targeted screenings. In targeted metabolomics only a small spectrum of metabolites of interest is recorded and analysed. This approach is used mainly for screening for drug bi-products, or indicators of certain physiological processes, in which the metabolites of interest are known in advance. In un-targeted metabolomics the aim is to obtain the broadest spectra of metabolites and identify as many metabolites as possible. The spectra obtained in this way are usually used to compare the metabolic signatures between groups of individuals, for example healthy and diseased people, patients taking an active drug or a placebo. Such comparison allows for discovery of biomarkers for diseases. It also indicates which metabolic process are important for a particular pathophysiological state, which might later be used in a drug-discovery process.

4. RESULTS AND DISCUSSION

In this thesis I set out to get an insight into possible bidirectional interactions between the microbiome, composed of both commensal and potentially pathogenic bacteria, and the host. In order to study these complex interactions we have selected short chain fatty acids, as a model of a bacterially-derived product with known beneficial effects on host's health. From the host's side we have focused on two ligand-activated transcription factors known to be important regulators of metabolic and immune processes - PPARγ and AhR.

4.1 Paper I: Absence of intestinal PPARγ aggravates acute infectious colitis in mice through a lipocalin-2-dependent pathway

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a Gram-negative, food-borne, pathogenic bacterium causing intestinal inflammation in humans. *S.* Typhimurium is also a model organism for studying bacterial virulence factors and invasion mechanisms. Animal models have been developed in order to study the pathology of *Salmonella*-driven colitis. However, mice infected with *S.* Typhimurium tend to develop systemic typhiod fever syndromes rather than intestinal inflammation. This phenomenon is most probably caused by colonisation resistance, the protective effects of intestinal flora preventing the out-growth and invasion of the pathogen. Pre-treating mice with an antibiotic streptomycin prior to *S.* Typhimurium infection is needed for the development of colitis that resembles human salmonellosis. Administration of the antibiotic leads to marked reduction of normal intestinal microbiota (up to 90%), which allows for the out-growth of *Salmonella* and development of a full-blown colitis within 24 hours from infection. For this reason *S.* Typhimurium infection is also used as a model to study the role of commensal bacteria in providing the host protection against the pathogenic invasion.

In this paper we have investigated the role of PPARγ expressed in the IEC in the protection against *Salmonella* invasion and regulation of *Salmonella*-driven colitis. We showed that PPARγ protects mice against *S.* Typhimurium-induced colitis and tissue damage. During *S.* Typhimurium infection PPARγ expression levels drop in the IEC which results in increased expression of pro-inflammatory cytokines and anti-microbial peptides, amongst them LCN2. LCN2 is at least partially responsible of inducing the colonic tissue damage upon *S.* Typhimurium infection.

4.1.1 PPARγ expression in the intestinal epithelial cells protects mice against colitis caused by *Salmonella* Typhimurium infection

Expression and activity of PPARγ in the IEC seems to play an important role in the development of defence mechanisms against bacterial invasion and development of intestinal inflammation. PPARγ plays an important role in modulating the anti-inflammatory signals during infections. Deletion of PPARγ results in increased susceptibility to bacterial infections and chemically induced colitis (122–126). On the other hand expression of PPARγ was shown to be lower in patients with IBD in one study (127). However, the role of PPARγ in *S.* Typhimurium-triggered colitis remained unknown. We first set out to investigate if infection of mice with this pathogen has any influence on the expression of PPARγ. Indeed the expression of PPARγ was reduced in the caecal and colonic epithelium of mice infected with *S.* Typhimurium. To make sure that the reduction of PPARγ expression is a direct effect

of *Salmonella* executed in the IEC we used an *in vitro* system, where colonic epithelial cell line was co-cultured with *S.* Typhimurium. The mRNA levels of PPARγ in *Salmonella*-exposed cells was lower than in mock-treated cells, indicating that the presence of immune cells in not necessary for the decrease in expression levels of PPARγ in IECs. There are reports suggesting interactions between pathogenic bacteria and PPARγ. *Helicobacter pylori* and *Mycobacterium tuberculosis* were shown to up-regulate the levels and activity of PPARγ in order to induce anti-inflammatory signals and prevent the release of cytokines and anti-microbial peptides (179,180). In the case of *Salmonella* many reports show that this pathogen needs the full-blown inflammation within the gut in order to have a survival advantage compared to normal commensal microbiota. *S.* Typhimurium, by down-regulating PPARγ expression induces anti-microbial responses, to which *S.* Typhimurium is resistant, which change the microbial composition and provide conditions for it to grow.

Further on, we investigated if the downregulation of PPARγ in IEC is important in the pathogenesis of *Salmonella*-induced colitis. In order to do that we used the IEC-specific PPARγ knock-out mice. The Cre+ mice, which do not express PPARγ in the IECs were more susceptible to *Salmonella* infection. Cre+ mice had smaller caecum and shorter colons than Cre- mice. The activity of pro-inflammatory transcription factors, NFκB and AP-1 as well as the expression of pro-inflammatory cytokines were all higher in the infected Cre+ mice than in their Cre- siblings. The observation of increased pro-inflammatory signals in the absence of PPARγ is probably due to trans-repression mechanisms described for PPARγ- NFκB interaction upon pro-inflammatory stimulation (111). Other mechanisms might also play a role in upregulation of pro-inflammatory signals in the PPARγ IEC KO. PPARγ regulates expression of trefoil factor 3 (TFF3), which is necessary for epithelial restitution and mucosal integrity promoting mucins cross-linking (181,182). It is therefore possible that PPARγ IEC KO has disrupted mucosal layer, which on the other hand disrupts intestinal homeostasis and predisposes the mice to *S.* Typhimurium - induced colitis. It seems therefore that intestinal epithelial PPARγ provides a level of protection against the full-blown inflammation in the colon. This observation is in accordance with other reports, showing that PPARγ expression and activity ameliorates chemically induced colitis (125,126,181).

4.1.2 PPARγ regulates LCN2

Deletion of PPARγ in IEC resulted in upregulation of expression of IL-17 and IL-22 in response to *S.* Typhimurium infection. These two cytokines are necessary for the production of AMPs by IEC. Antimicrobial peptides are important defence molecules that limit the invasion of intestinal pathogens and their expression is induced upon pathogenic infection. Indeed the expression of two AMPs: Reg3γ and LCN2 was induced upon *S.* Typhimurium infection, and their expression was much higher in the epithelial cells of in the PPARγ IEC KO. In order to investigate if the deletion of PPARγ had a direct effect on the expression of LCN2, or rather it was just due to increased intestinal inflammation we have used our in-vitro model. Knock-down of PPARγ by siRNA in HT-29 resulted in increased expression of LCN2, even without co-culturing of the cells with *Salmonella*. The level of LCN2 was increased in HT-29 following co-culturing the cells with *Salmonella*, and the effect was stronger in cells in which PPARγ expression was lowered by siRNA. The finding that LCN2 levels increase in response to *Salmonella* was surprising because it is resistant to LCN2 effects. The resistance of Salmonella to LCN2 is due to the fact that Salmonella uses another iron-chelating system, called

salmochelin, which does not bind to LCN2. Instead of reducing the growth of *Salmonella*, LCN2 is likely blocking the iron transport systems of commensal bacteria, causing a growth advantage for *Salmonella* (183–185). If that is the case then increased levels of LCN2 would favour the growth of *Salmonella* in the gut of PPARγ IEC KO mice. However, in our study we did not observe any differences in the numbers of *S.* Typhimurium cells between Cre+ and Cre- mice. What we observed was increased tissue damage in the PPARγ KO mice upon *Salmonella* infection. It therefore occurred to us that LCN2 might somehow contribute to the intestinal damage, which we investigated in the last part of the paper.

4.1.3 LCN2 activity causes intestinal damage upon *Salmonella* infection

LCN2 is a multi-functional protein, secreted to the intestinal lumen where it functions as an AMP sequestering iron and hindering bacterial growth, in this way preventing invasions of pathogens. LCN2 is also able to bind and stabilize matrix metalloproteinase 9 (MMP-9) in the intestinal lamina propria (186,187). MMPs play an important role in the remodelling of intestinal tissue by regulating the extracellular matrix turnover. MMP-2 and MMP-9 are associated with colitis. Expression of MMP-2 and MMP-9 is higher in patients with IBD. MMP-2 and MMP-9 have opposite functions: MMP-9 mediates tissue injury during colitis, whereas MMP2 maintains the gut barrier and protects against tissue damage. MMP-9 expression is low in normal colonic mucosa and is induced during colitis caused by administration of DSS or during *S.* Typhimurium infection. *S.* Typhimurium was shown to stimulate secretion and activation of MMP9 from macrophage via its surface protease PgtE (188). MMP9 KO mice are protected against colitis and tissue damage (189,190). Additionally activation of PPARγ by TZD results in lowered secretion of MMP9 from LPS-activated macrophages (191). We have observed an increased protein levels and activity of MMP9 in Cre+ mice infected with *S.* Typhimurium, whereas the level and activity of MMP-2 did not differ between Cre+ and Cre- mice. Additionally, we detected the LCN2-proMMP9 complex in protein extracts from the colon of mice infected with *S.* Typhimurium. This complex was present in larger amounts in the samples isolated from Cre+ mice in comparison to Cre- mice.

To further investigate if the increased levels of LCN2-MMP9 complex had a deteriorating impact on colonic tissue integrity we used a LCN2 KO model. LCN2 KO mice were protected against S. Typhimurium-induced colitis and exhibited reduced colon shortening and tissue damage. Additionally, LCN2 KO mice in comparison to wild type mice had slightly higher levels of proMMP-9 protein, but not its activity upon infection with *S.* Typhimurium. Secreted MMP-9 was also detected in *S.* Typhimurium treated LCN2 KO mice, but to a much lesser extent that in PPARγ IEC KO mice. We observed similar dowregulation of PPARγ expression following *S.* Typhimurium infection in LCN2 KO and wild type mice. There was also no difference in the levels of pro-inflammatory cytokines or Rag3 expression. These observations indicate that LCN2 expression plays a major role in mediating S. Typhimurium-induced tissue damage but does not influence the levels of PPARγ or pro-inflammatory signals. We speculate that blocking LCN2 would result in amelioration of colitis due to decreased tissue damage. This might be an important therapeutic strategy to reduce severity of intestinal inflammation because LCN2 seem not to affect the beneficial activity of over-all beneficiary MMP2. Blocking of LCN2 might therefore lower the stability and activity of MMP9 without influencing the function of MMP2.

4.1.4 Downregulation of PPARγ and the subsequent increase in LCN2 expression upon *Salmonella*-infection is TLR4-independent.

In Paper I we show that *S.* Typhimurium down-regulates the expression of PPARγ in the IEC. We also show that deletion of PPARγ results in increased levels of LCN2, which in turn causes an increase activity of MMP-9 resulting in damage of the colonic tissue. The mechanisms by which *S.* Typhimurium exerts these effects remain unknown. Salmonella is a Gram-negative expressing various MAMPs on its cell surface, including LPS. LPS binds to and activates TLR4, which in turn initiates the signalling cascade activating NFκB transcription factor and induces expression of pro-inflammatory cytokines. A previous report has shown that the PPARγ protein levels dropped upon exposure of macrophages to LPS in TLR4 dependent manner (192). We have therefore investigated if the effects of Salmonella on PPARγ expression could be attributed to activation of TLR4. We have used TLR4-deficient mice to test that hypothesis. Upon infection with *Salmonella* we observed a similar reduction in the PPARγ mRNA levels in TLR4 KO mice and in wild type mice. *Salmonella*-induced downregulation of expression of PPARγ appears therefore to the independent of TLR4 signalling. Additionally, we compared the effects of S. Typhimurium and LPS on the expression of LCN2 and PPARγ in an *in vitro* model. LCN2 was increased and PPARγ expression decreased after co-incubation of cells with *Salmonella*. LPS had no effect on expression of LCN2 or PPARγ, it did however induce expression of IL-8, proving that the cells were responsive to LPS (Figure 9)

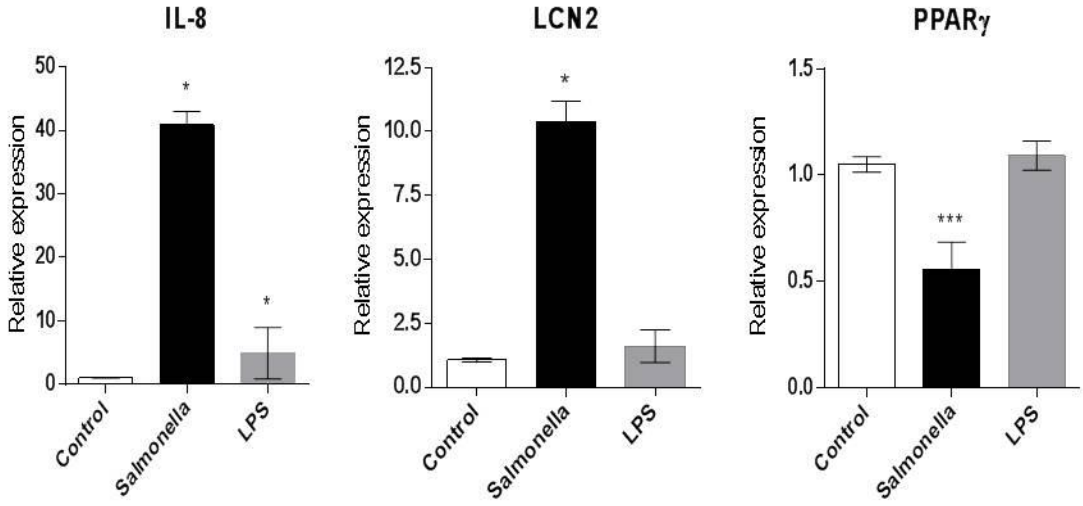

Figure 9. *Expression of IL-8, LCN2 and PPARγ in HT-29 cells co-cultured with Salmonella Typhimurium or LPS. Cells were exposed to treatments for 6 hours, after which they were washed with RPMI-medium and cultured in no-treatment containing medium for 18 hrs. Expression level of genes was analysed by qPCR and normalised to β-actin. Statistics: Students-t-test *, p<0,05; ***, p<0,001, against the control*

Other TLRs might be involved in the recognition of *Salmonella* and mediating its effect on PPARγ expression. Some other MAMPs present on the cell surface of *S.* Typhimurium include amyloids (curli fibrils), which binds to TLR2 and flagella, recognised by TLR5 (193–195). It is therefore possible that these TLRs are also responsible for initiating the signal transduction pathways leading to downregulation of PPARγ expression in the IEC. This question remains un-answered in Paper I.

It would however be interesting to investigate which, if any, TLRs mediate the down regulation of PPARγ in IEC in response to *S*. Typhimurium infection, as it might provide a therapeutic window of inducing PPARγ-dependent protective functions in the gut.

4.2 Paper II and Paper III: SCFA induce expression of metabolic genes in the intestinal epithelial cells *in vitro* and *in vivo*

In Papers II and III we focused on the role of commensal bacteria and one of their metabolites, short chain fatty acids (SCFA) on shaping the metabolic processes within the GI tract. We used an *in vivo* model of GF mice, which received sodium butyrate in oral gavage or were colonised with one commensal bacterial strain *Clostridium tyrobutyricum*, a butyrate producer. We also used *in vitro* models to study in more details the mechanism of action of SCFA, in particular if they can regulate the expression or activity or PPARγ and AhR, and in which way.

4.2.1 Butyrate induces the expression of ANGPLT4 and CYP1A1 in colonic epithelial cells

We first investigated the effects of stimulation of colonic cell lines with SCFAs on the expression of ANGPTL4, a well established PPARγ target gene (119,121) and CYP1A1, an AhR target gene (144). We screened various epithelial cell lines for induction of these genes after treatment with a PPARγ ligand - Rosiglitazone, and an AhR ligand - β-naphtoflavone (BNF). We selected HT-29 colonic carcinoma cell line for further experiments as in this cell line the induction of expression of ANGPTL4 and CYP1A1 expression was the highest. We did not observe any cross-reactivity, i.e. treatment with Rosiglitazone did not induce expression of CYP1A1, nor did BNF influence the expression of ANGPLT4. Having established the inducibility of ANGPTL4 and CYP1A1 in HT-29 cells, we stimulated the cells with three SCFAs: acetate, propionate and butyrate to investigate if they could induce their expression. Butyrate was the strongest inducer of expression of ANGPTL4 and CYP1A1, however, with different kinetics. The expression of ANGPTL4 was induced 6 hrs after treatment, whereas CYP1A1 expression was induced the strongest after 24 hrs. The GI tract provides a complex environment where bacteria and their metabolites influence the regulation of the host's physiological processes in the presence of other stimuli, both nutritional and toxic. The PPARγ and AhR ligands are ingested with food and it is very probable the intestinal response to those dietary ligands is influenced by the bacteria and their metabolites. We set out to investigate what is the effect of a specific PPARγ or AhR ligand in combination with SCFA on the expression of ANGPTL4 and CYP1A1. When HT-29 cells were treated simultaneously with Rosiglitazone or BNF and butyrate a much higher induction of expression of ANGPTL4 and CYP1A1 was seen (Figure 10).

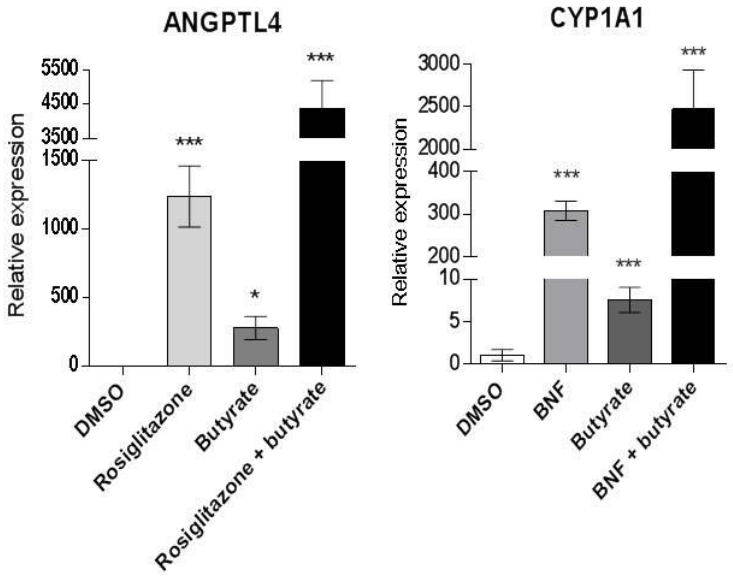

Figure 10. *Expression of ANGPTL4 and CYP1A1 in HT-29 treated with DMSO (0,1%), sodium butyrate (2mM), Rosiglitazone (5μM) BNF (10μM) and a combination of Rosiglitazone or BNF with butyrate. Cells were treated for 24 hrs prior to extraction of mRNA. Expression was analysed by qPCR and normalised to B-actin. Statistics: Students-t-test ***, p<0,001, against DMSO treated control*

4.2.1.1 Butyrate induces ANGPTL4 expression in PPARγ-independent manner.

In has been suggested that butyrate activates PPARγ and in this way induces the expression of PPARγ target genes (62,196). Other bacterial metabolites were also reported to activate PPARγ and ANGPTL4 (119–121,197). We wanted to investigate if the effect of butyrate on ANGPTL4 expression could be attributed to the activation of PPARγ. We tried to answer this question by blocking the expression or activity of PPARγ by siRNA and a chemical inhibitor. Knocking down of PPARγ or blocking its activity did not influence butyrate-induced ANGPTL4 expression. These results suggest that the activation of expression of ANGPTL4 by butyrate is PPARγ-independent, which is in stark contrast with a recent publication by Alex and colleagues (62), in which the authors show that butyrate activates PPARγ in colonic epithelial cells and this activation is responsible for induction of ANGPTL4 expression. Many factors can be responsible for this discrepancy, including different cell type and culture media used. The culture media composition might be of big importance for the outcome of butyrate treatment for two reasons. First of all the transcriptional outcome of butyrate treatment is dependent on metabolic status of the cells, which varies depending on glucose concentration in culture medium (64). It is also possible that linoleic and linolic acids present in the DMEM/F12 medium routinely used for cultivation of T84 cells act as PPARγ ligands. This would mean that the activation status of PPARγ is different in HT-29 and T84 cells, which may influence the outcome of butyrate treatment. Whatever the reason behind the discrepancies, it remains an interesting phenomenon worth investigating in more detail because it might help to understand physiological functions of butyrate in the gut and its role in protection against colorectal cacinoma. The observation that ANGPTL4 expression in HT-29 cells is independent of PPARγ led us to investigate other possible mechanisms of action of SCFA. ANGPTL4 promoter analysis revealed one Sp1 binding site located in the promoter fragment of 0,5 kbp upstream from the transcription initiation site. This promoter fragment was also responding to butyrate treatment, again in a PPARγ-independent manner. It was also responsive to treatment with Trichiostatin A (TSA), a potent histone deacetylase inhibitor, that is structurally unrelated to butyrate. Interestingly, TSA was shown previously to be able to activate gene

transcription by modulating the acetylation status of Sp1 (198). It is therefore possible that butyrate influences the acetylation status of not only histones but also transcription factors and possibly co-activators and in this way exert their function as transcriptional regulators.

4.2.1.2 Butyrate induces CYP1A1 expression in an AhR-dependent manner

In Paper II we have established that expression of ANGPTL4 induced by butyrate in HT-29 is PPARγ-independent. In Paper III, we investigated whether butyrate-induced expression of CYP1A1 in those cells is dependent on AhR. We could show that knock-down of AhR by using siRNA decreases butyrate induced CYP1A1 expression. Additionally, blocking of AhR activity by a chemical inhibitor also abrogated butyrate effect on CYP1A1 expression. CYP1A1 expression was induced by butyrate only after longer stimulation (longer than 8 hours) we therefore wanted to check if butyrate directly stimulates the AhR or if the butyrate mediated effect was dependent on synthesis of other proteins. In order to answer this question we blocked protein translation by treating the cells by cycloheximide prior to butyrate stimulation. Butyrate-induced CYP1A1 expression does not require *de novo* protein synthesis because the effects of butyrate were not blocked by cycloheximide (Figure 11).

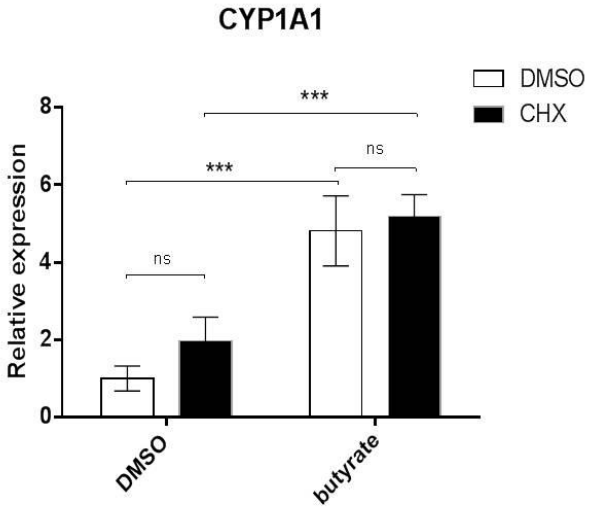

Figure 11. *Expression of CYP1A1 in HT-29 following 24 hrs stimulation with sodium butyrate after 1 hour pre-treatment with cycloheximide (CHX, 10μM) or vehicle (DMSO, 0,1%). Expression was analysed by qPCR and normalised to β-actin. Statistics,, Two Way ANNOVA, ***, p<0,001, ns, p>0,05*

In order to induce transcription of its target genes AhR needs to translocate from the cytoplasm to the nucleus, where it forms a heterodimer with ARNT which in turn allow for DNA binding at specific XRE. We have therefore investigated if treatment of HT-29 cells increases the nuclear pool of AhR. Indeed, after addition of butyrate the levels of nuclear AhR do increase (Figure 12).

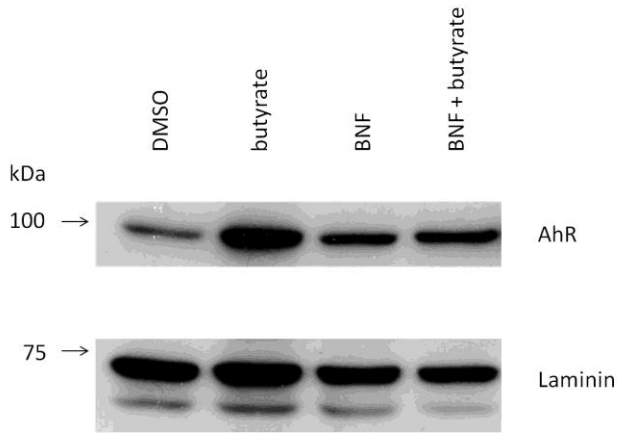

***Figure 12**. Nuclear extracts from HT-29 treated with DMSO (0,1%), sodium butyrate (2mM), BNF (10µM) and a combination of BNF and butyrate. Cells were treated for 24 hrs prior to extraction of nuclear proteins. The extraction was carried using NEPER kit (Thermo Scientific). 30µg of protein was analysed using laminin as a loading control. Antiboides were from EnzoLife Sciences (AhR) and Cell Signaling (Laminin A/C).*

To our knowledge there are no reports suggesting that SCFAs might work as AhR ligands and such an interaction is rather improbably due to the fact that AhR ligands share planar structure, which is not the case for butyrate. There are however reports showing that the cell culture media (including RPMI) contain AhR ligands. These ligands come from light-driven degradation of tryptophan and include amongst others FICZ (199). The presence of AhR ligands in HT-29 cell culture medium may allow for basal AhR activity. We speculate that the addition of butyrate changes acetylation status of AhR or its co-factors, allowing for binding of a co-activators, removing co-repressors, or facilitating nuclear translocation. This interactions would result in increased expression of CYP1A1 that is AhR dependent. It is also possible that butyrate influences the chromatin structure by modifying the acteylation status of histones allowing for binding of AhR itself or of other proteins building the transcriptional machinery. The mechanism by which butyrate drives the AhR-dependent expression of CYP1A1 remains undiscovered and requires further detailed analysis.

4.2.2 Oral administration of butyrate in GF mice induces the expression of ANGPTL4 and CYP1A1

We have shown that butyrate induces expression of ANGPTL4 and CYP1A1 in HT-29 colonic carcinoma cell line. In order to confirm our results in physiological settings we gave butyrate solution intragastrically to GF mice. Gavage with butyrate stimulated the expression of ANGPTL4 and CYP1A1 in the epithelial cells of the distal small intestine, confirming the stimulatory effect of butyrate on the expression of ANGPTL4 and CYP1A1 in the GI tract. Administration of butyrate is effective in GF mice, which do not have any bacterially-produced SCFAs. However, the high concentrations of SCFAs in the colon of a mouse harbouring normal microbiome would most probably mask any effect of butyrate administered by gavaging. Additionally, butyrate gavage did not induce any changes in gene expression in the colon, most probably due to reduced transfer through the ceacum or earlier absorption by epithelial cells. Although significant amounts of SCFAs are found in the small intestine, it is the colon where the concentration of butyrate is the highest. We have therefore chosen to use a butyrate producing bacterial strain to achieve a more physiological setting and to see if SCFA producers were able to mimick the effect of exogenously supplemented SCFA.

4.2.3 *Clostridium tyrobutyricum* induces the expression of ANGPTL4 and CYP1A1 in intestinal epithelium

Having established that butyrate induces the expression of ANGPTL4 and CYP1A1 expression in intestinal epithelial cells *in vitro* and *in vivo*, we asked ourselves if similar effect could be achieved by directly using a butyrate producing bacteria. We have chosen a butyrate producer *Clostridium tyrobutyricum*, a strain previously shown to prevent colitis in mice (200,201). *C. tyrobutyricum* is a part of normal commensal flora in mice and it is also present in cheese, therefore is a feasible bacteria to use in intervention studies in the future. Another reason for choosing this particular species was the fact that it is known to produce mostly butyrate and not the other SCFA: propionate and acetate, which was important since we have used butyrate in our *in vitro* studies as the most potent of the three SCFAs tested. We have tested the effects of *C. tyrobutyricum* on the expression of ANGPTL4 and CYP1A1 both *in vitro* and *in vivo*. We first monocolonised GF mice with *C. tyrobutyricum* in order to establish if the effects mediated by oral administration of butyrate are mimicked by the presence of a butyrate producing bacteria. Expression of ANGPTL4 and CYP1A1 was higher in the colon and distal small intestinal epithelial cells of monocolonised mice than in GF mice. We have also compared the levels of CYP1A1 and ANGPTL4 in *C. tyrobutyricum* monocolonised mice and specific pathogen free mice, harbouring normal microbiota. We found that the levels of CYP1A1 and ANGPTL4 expression was comparable between colonic epithelial cells of *C. tyrobutyricum* and SPF. However, in the small intestine the SPF mice had lower expression levels of ANGPTL4 and CYP1A1 than *C. tyrobutyricum* monocolonised mice or even the GF mice in case of ANGPTL4. Lower levels of ANGPTL4 expression in the small intestine of SPF mice as compared to germ free mice was previously reported (86,202). The reason behind the differential regulation of ANGPTL4 and CYP1A1 expression by the entire microbiome in SPF mice and *C. tyrobutyricum* is not know. One explanation might be, that the flora inhabiting the small intestine is more diverse and have other functions, apart from producing SCFA, which results in overall lower expression of CYP1A1 and ANGPTL4.

In order to be able to distinguish the effects caused by direct contact between bacteria and the eukaryotic cells and the effects mediated indirectly by bacterial metabolites we used *in vitro* system based on HT-29 cells. We stimulated HT-29 cells with live and heat-inactivated *C. tyrobutyricum* as well as with different bacterial culture media. From these experiments we concluded that it is indeed the soluble bacterial metabolites and not membrane-bound components of bacteria that play a major role in induction of ANGPTL4 and CYP1A1 expression. One cannot, however, formally exclude the presence of metabolites other than SCFAs in this soluble fraction.

4.3 Paper III: Cross-talk between AhR, gut microbiota and energy homeostasis

Given that SCFA and intestinal microbiota influence CYP1A1 expression in an AhR dependent manner we sought to investigate in more detail the cross-talk between the AhR and the microbiome. AhR is an important regulator of intestinal homeostasis and by regulating the activity of ILC22 and development of T-cells, AhR influences the production of antimicrobial peptides. AhR activity could therefore regulate the composition and function of the intestinal of microbiota. We used the AhR KO mice, in which AhR gene was deleted to test this hypothesis.

4.3.1 Deletion of the AhR changes the composition of small intestinal microbiota

Profound changes in the composition of intestinal microbiota have been reported for mice lacking TLR5 and NLRP6 (203,204). In both cases the disbalance in microbial ecology results in the development of colitis that could be transmitted to WT mice that received the microbiota from the KO mice. We first sought to investigate if deletion of AhR compromises the microbial *status quo* in the intestine. We collected the content of the distal part of the small intestine, the colon as well as faecal material. To eliminate external influences, we compared only co-housed littermates of AhR heterozygous crosses. Thus both AhR KO and AhR WT mice had equal chances to acquire the same bacterial flora. There were no differences between AhR KO and WT mice when faecal or colonic microbiota were compared. However, the comparison of prevalence of bacterial phyla and classes inhabiting the small intestine revealed significant differences between mice expressing the AhR and AhR-deficient mice. The prevalence of Actinobacteria, Tenericutes and Bacteroidetes was lower and the prevalence of Firmicutes was higher in the intestine of AhR KO mice. We also observed significant difference in prevalence of two classes of bacteria belonging to the Firmicutes phyla. The Bacilli class was more abundant and the Clostridia class less so in the intestine of the AhR KO mice. Bacteria that belong to the phylum Bacteroidetes as well as the ones that belong to the class Clostridia within the Firmicutes phyla are the main contributors to the production of SCFA in the GI tract. We hypothesise that the AhR KO mice have decreased fermentation ability, and thus lower levels of SCFA in the GI tract. Although lower levels of SCFA in the intestine of AhR KO is speculative it does however remain an interesting possibility because SCFA have anti-inflammatory effects and AhR KO mice are more prone to chemically and bacterially induced colitis (34,150,205–209). The fact that there was no difference in the composition of faecal and colonic microbiota between the AhR WT and AhR KO mice, but we did observe major shifts in the proportions of small intestinal bacteria is of great importance. The majority of studies aiming to characterize the differences between various genetic or environmental conditions is usually based on comparisons of faecal material because it is easily available. However, the knowledge gained by sequencing bacterial DNA from faeces might not be as informative as previously though. There might be changes in the populations of bacteria inhabiting the small intestines which are not detectable when comparing the faecal microbiome. Our results are in accordance with a report aiming to characterize the "active" microbiome at various sites along the GI tract, that found that there are important differences between the fecal and intestinal bacterial composition (54). The shift in the composition of intestinal microbiota favoring the prevalence of anaerobic fermenting bacteria (including Clostridia) occurs mainly during the weaning period. Interestingly, the AhR KO mice have altered weight gain during that phase of development. It is therefore an interesting question is if the changes in the composition of microbiota observed in the AhR KO have a causative effect on the growth retardation of these mice or if they may account for higher susceptibility of these mice to intestinal inflammation. It would be of great interest to perform microbiome transfer experiments to confirm the role of bacterial imbalance in the intestine of the AhR KO in susceptibility of these mice to colitis. Apart from higher susceptibility for intestinal inflammation the changes in bacterial composition are also reported to cause obesity and metabolic disorders in mice (88,210). In the final part of Paper III we investigated if AhR can play an important role in regulating energy balance.

4.3.2 AhR is an important regulator of energy homeostasis

Several reports indicated a role of AhR in the regulation of energy balance and metabolism. Many of these studies were performed in conjunction with the investigation of toxicity of man-made environmental pollutants in animal models. Chronic administration of TCDD in mice, for instance results in profound changes in expression of genes regulating cholesterol synthesis, *de novo* lipogenesis and glycolisis. (159). Acute exposure to TCDD lowers the expression of genes involved in gluconeogenesis in the liver (160) and decreases glucose transport to the adipose tissue (161). Administration of 3MC in C57Bl/6J mice resulted in fat accumulation in the liver and development of liver steatosis in conjunction with increased levels of PPARα mRNA in the liver, probably due to increased expression of fatty acid translocase (FAT a.k.a. CD36) (162). On the other hand AhR KO mice have lower expression of PPARα and genes coding for enzymes involved in β-oxidation of fatty acids (Paper III and (164)). Altogether these animal models indicate a strong link between the expression and activity of the AhR and metabolism of fat and glucose, mainly β-oxidation of fatty acids and gluconeogenesis. Gluconeogenesis and β-oxidation of fatty acids are two metabolic processes that are active mostly during caloric restriction or fasting, when the easily available reserve of glucose and glycogen are exhausted and there is a need to retrieve energy from fat deposits. There were however no reports to our knowledge that investigated the role of the AhR in the regulation of metabolic processes occurring during fasting in mice. In order to study in detail the role of AhR in the regulation of fasting-associated metabolic processes we embarked on global scanning of metabolites present in the blood, liver and muscle tissue of AhR KO and AhR WT mice after 12 hours of food removal. This unsupervised approach allowed us to dig deep into the possible molecular mechanisms and discover several pathways affected in the KO mice during fasting. Here we report for the first time that AhR is an important factor regulating oxidation of fats in the liver and production of ketone bodies. Metabolic NMR profiling also revealed higher levels of lactate, pyruvate and alanine in the plasma of AhR -/- mice suggesting increased rate of glycolysis in peripheral tissues.

Presence and concentration of many of environmental pollutants that are ligands for the AhR, in human serum is positively correlated with diabetes and metabolic syndrome (165)**.** One of the hallmarks of metabolic syndrome is elevated fasting glucose levels as a consequence of glucose intolerance in peripheral tissues, up-regulated gluconeogenesis even in the presence of glucose in the blood and disrupted mitochondrial oxidation of fatty acids. Mitochondrial dysfunction has been recently associated with increased levels of AhR-ligands in human blood serum in an independent study (166). We therefore fed the AhR KO mice semi-synthetic chow in which the proportion of calories coming from fat is about 40%. This chow is called western diet (WD) as it is supposed to mimic the dietary habits of westernised, developed countries (including Europe and United States of America). Such diet is associated with development of obesity and metabolic diseases in humans and in mice (211,212). Feeding adult AhR KO and WT mice western diet for 11 weeks resulted in increased body weight gain as compared to normal chow (R36) fed mice. We did not however observe any differences between the AhR KO and AhR WT mice. To assess glucose intolerance, we performed an oral glucose tolerance test. We observed that AhR KO mice had lower fasting glucose levels and improved glucose tolerance than AhR WT mice. The absence of AhR signalling might be beneficial

during dietary challenge, down-regulating gluconeogenesis in the liver and switching the metabolism from fats to glucose which result in the lower fasting glucose levels of AhR KO mice.

Most of the studies trying to answer the role of AhR in regulation of physiological processes is done using male mice. However many of the environmental pollutants linked with obesity and metabolic problems have oestrogen-like properties and exposure to such chemicals leads to disturbed hormonal balance. Additionally AhR may directly and indirectly interact with oestrogen receptor itself (153), giving yet another twist to the role of oestrogen-like chemicals in development of metabolic syndrome. We therefore used females AhR KO and AhR WT mice to study the development of diet-induced obesity and glucose intolerance. We found that AhR female mice were protected against western-diet induced weight gain and glucose intolerance (Figure 13).

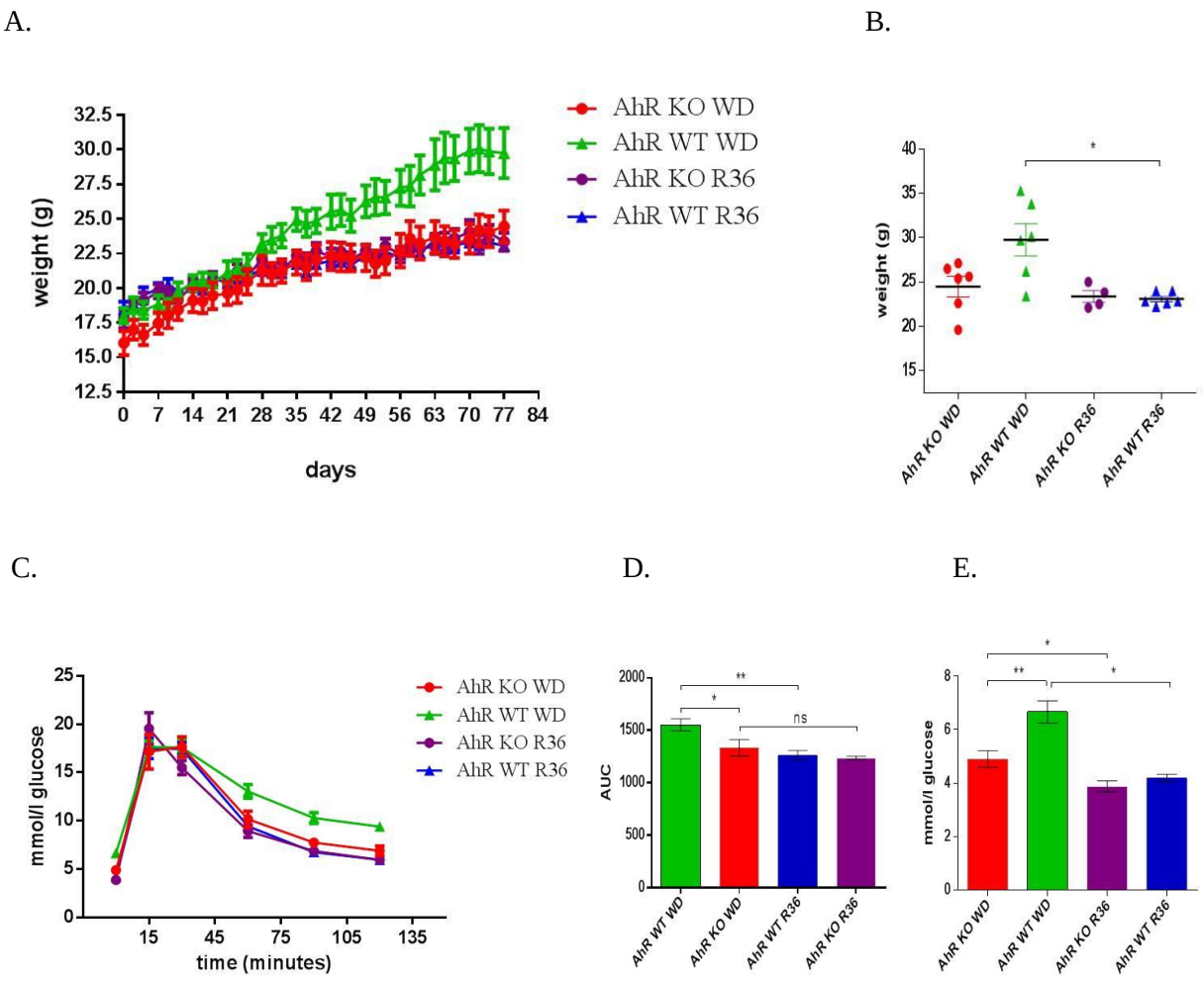

Figure 13. Metabolic parameters of female AhR KO and AhR WT mice fed Western Diet.
A*. Weight changes.* ***B****. weight after 11 weeks of feeding. Statistics T-test *, p<0,05; **, p<0,01; ***, p<0,001. Bars and error bars show means +/- SEM.* ***C-E****. Oral glucose tolerance test. Changes of glucose concentration in blood after oral administration of glucose (2g/kg) (C), Area under curve (D) and fasting glucose levels (E). Statistics One-Way ANNOVA: *, p<0,05; **, p<0,01; Bars and error bars show means +/- SEM.*

Previous studies have shown that WT C57/Bl6 female mice do appear to be significantly less susceptible to the metabolic lesions brought about by fat-rich diets (213). In that study both males and females gain weight, but the female are able to handle the consequences of higher visceral fat better than the males.

Given that the AhR KO males undergo changes in the microbiota and exhibit metabolic variables leading to inefficient energy utilisation, one could speculate that similar conditions in the female AhR KO mice under WD allows the shunting of excess energy from storage to usage, hence having no appreciable weight gain. This is at present speculative, and would be of interest to explore in terms of underlying molecular mechanisms and implications for gender-based therapies.

5. CONCLUDING REMARKS AND PERSPECTIVES

When I first registered as a PhD student in October 2008, the field of human commensal microbiology had just became a very hot topic. Virtually every day new data and new knowledge was made available to the world. At this time I could describe the components of my project plan as "The Good, the Bad and the Ugly" making a reference to Clint Eastwood's famous Western movie. The Good was the PPARγ, protecting us from pathogens and preventing too strong immunological responses. The Bad was the AhR, at this time mostly known for mediating toxic effects of dioxins and other environmental pollutants having immunosupressive and carcinogenic effects. And the Ugly, were the intestinal microbiota, due to their obscure function and unknown properties. The composition of the intestinal microbiota was first characterised in the late 1970s, it was however not before the 16S rRNA typing techniques conquered the field that we got the full insights into which bacteria inhabit our intestines. The two big collaborative projects: The NIH Human Microbiome Project (http://commonfund.nih.gov/hmp/index) and the European Meta-HIT Consortium (http://www.metahit.eu) were the main contributors to the knowledge in this field. These two large groups of scientists sequenced and characterised the vast majority of bacterial DNA that could be extracted from human faeces. The majority of results of their work and the work of many other groups on intestinal bacterial composition were published between year 2005 and 2011 (reviewed in (61)). It is therefore only recently that we have gained the knowledge about who is there. At the moment huge efforts are undertaken to understand what they are doing there. We are just at the beginning of a journey to understand the role, mechanisms and importance of our microbiome in regulating the host's physiological processes and shaping the balance between health and disease. Much has also be learnt about how our bodies adjust to changes in the microbiome and how our physiology shapes the composition and functionality of the microbiome. This work is a modest attempt to unravel some of the mysteries and conundrums behind this bidirectional interaction.

In Paper I we focused on the interaction of a pathogenic bacteria with the host and the consequences it has for the progression of intestinal inflammation. We could show that infection with *S*. Typhimurium decreases the levels of PPARγ in the IEC and that deletion of PPARγ results in increased inflammatory response and tissue damage. This might be mediated by several possible mechanisms. One of them is the lack of NFκB transrepression in the absence of PPARγ. It was previously shown that PPARγ is able to influence nuclear shuttling of NFκB, resulting in lowered expression of pro-inflammatory genes. In that study the anti-inflammatory effects of PPARγ were induced by a commensal bacteria *Bacteroidetes thetaiotaomicron* (214), via a yet unknown mechanism. *B.thetaiotaomicron* belongs to the Bacteroidetes phylum and is an anaerobic bacteria that produces SCFAs acetate and propionate as products of fermentation (87). Other fermenting bacteria that were shown to ameliorate intestinal inflammation are *Clostridium butyricum* and *Clostridium tyrobutyricum*, belonging to the Firmicutes phylum (200,201). It is therefore possible that the changes in bacterial composition induced by streptomycin treatment contribute to the development of colitis upon *S*. Typhimurium infection. Indeed streptomycin was shown to decrease the amount of SCFA and some SCFA producers (Firmicutes) in the mouse intestine (215). Clostridia, including *C. butyricum* and *C. tyrobutyrium*, exert their effect via TLR2 *in vitro* and *in vivo* (201,216). Additionally, gavaging of mice with butyrate induces the expression of TLR2 in the IEC (Figure 14). In paper I we

showed that the downregulation of PPARγ in response to *S.* Typhimurium infection is TLR4 independent, however other TLRs, like TLR2 play an important role in mediating this effect. It is probable due to the fact that one of the *S.* Typhimurium MAMPs - curli fibrils is recognized by TLR2. This recognition is needed for tightening the epithelial barrier which in turn prevents invasion of S. Typhimurium to the lamina propria (193). We speculate that decreases in SCFA and SCFA producers in the intestine caused by streptomycin treatment lowers the TLR2 dependent signaling, which loosens the epithelial barrier predisposing mice to colitis. It remains an open question if deletion of PPARγ in the IEC influences the composition of microbiome, for example by lowering the amounts of fermenting microbiota (similarly to the effect observed in the AhR KO mice in Paper III), which would be yet another way to predispose these mice to colitis. This effect could be achieved for example by decreased expression of TFF3 regulating the mucosal integrity, by changing the immune landscape in the gut or by yet unidentified mechanisms.

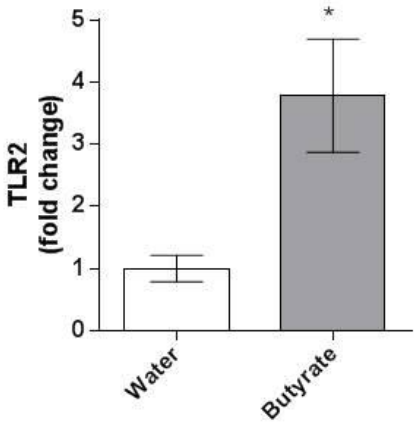

***Figure 14**. Expression of TLR2 in IEC of GF mice gavaed with water or sodium butyrate (1g/kg) 24 and 6 hours prior to sacrifice. Gene expression was analysed by qPCR and normalised to B-actin. Statistics: Students-t-test *, p<0,05,*

Another interesting observation connecting the intestinal microbial composition with colitis is that a mixture of Clostridia species was able to ameliorate intestinal colitis as well by activating the expression of IL-10 in Treg cells (201). AhR was shown to regulate IL-10 production in Treg cells and in Paper III we show that *C. tyrobutyricum* was able to transcriptionally activate AhR. It is therefore possible that streptomycin induced changes in the intestinal microbiome lead to downregulation of AhR-mediated signaling, providing yet another explanation as how the colonisation resistance works. We and others could show the dependence of PPARα expression on the presence of AhR (Paper III and (164)), but the AhR and PPARγ have not been robustly linked at this point in time, to our knowledge. However, these receptors share the co-activators and might be indirectly linked via bacterial interactions.

PPARγ, AhR, ANGPTL4 and LCN2 are all multi-functional proteins occupying the inflammation-metabolism junction. PPARγ protects against intestinal colitis, while at the same time regulates the expression of metabolic enzymes and storage of fat. This laboratory has also shown that PPARγ is amenable to post-translational regulation by bacteria (119,121,214). AhR is an important regulator of response to fasting, and appears to govern PPARα which is fundamental for energy extraction from lipids. AhR at the same time influences the composition of the microbiome in the gut and mediates immune responses to intestinal pathogens and environmental pollutants. ANGPTL4, although mostly studied as an adipokine involved in usage of fatty acids was also shown to prevent formation of foam

cells and in this way to protect against fatty-acid induced inflammation (217). LCN2 is an antimicrobial peptide keeping intestinal bacteria at safe distance from the IECs in the intestine. During infection it can also mediate remodeling of the intestinal wall by forming complexes with MMP9. Interestingly, LCN2 was postulated to be an acute phase response protein secreted from the liver in response to infection and a biomarker for renal injury and ongoing inflammation (218). Some studies have even linked LCN2 to the development of metabolic syndrome in humans, further pointing to the link between systemic inflammation and metabolic diseases (219). We now know from our own work and from the work of others that PPARγ, AhR as well as ANGPTL4 and LCN2 can be directly or indirectly regulated by the intestinal microbiome. Additionally nutritional ligands have been identified for PPARγ and AhR. Crucially, it has also been shown that composition of bacteria in the GI tract is dependent on diet (12,57,220,221). The rapidity of diet induced changes of the microbiota requires efficient sensory receptors and genetic effectors. Ligand-activated transcription factors are a unique group that can work simultaneously as receptors and effectors and respond to nutritional cues.

Paper II and Paper III deal directly with microbiome derived cues that can impact on host metabolic properties. SCFA was chosen as a model microbial molecule as it represents the symbiotic nature of the microbiota and the diet. SCFA production is firmly dependent on bacterial fermentation of dietary fibres. Beyond its importance in intestinal immunity and colonocytes survival, SCFAs act as a metabolic mediator, in this case we believe through ANGPTL4 (Paper II). We and others have shown that gut microbiota driven ANGPTL4 has ameliorating effects on fat storage (86,121). More recent studies indicate that ANGPTL4 is crucial for reducing intestinal lipase activity (222). This prevents intestinal lipid overload, in an ANGPTL4-mediated manner, as previously shown in cardiomyocytes and macrophages (217). Hence, it tempting to speculate that that the diet-microbiota interplay that produces a steady stream of SCFAs has a crucial role in downstream lipid processing in the host.

More subtle effects of the diet-microbiota marriage on metabolism are seen in Paper III, where a bidirectional relationship between AhR and microbiome is proposed. The absence of a functional AhR changes the composition of the microbiota but crucially only in the small intestine. Whether the microbiota population is sculptured by epithelial or immune factors under the direction of AhR remains to be elucidated. What is of interest is exactly how far the microbiome changes can double back and impact of the metabolic properties seen in the AhR KO mice, e.g. compromised lipid metabolism in the liver. These insights are useful when considering different approaches to understand and treat chronic immune and metabolic disorders.

The microbiome, e.g. SCFAs influences expression of AhR target genes in the intestine. This microbiome induced expression of AhR may indicate a mechanism to increase sensitivity towards AhR ligands. In the course of mammalian development, governance of AhR activity by SCFA can occur primarily after weaning, when there is sufficient dietary complex carbohydrate content. Milk oligosaccharides, together with maternal microbiota may have similar effects before weaning. The importance of SCFA-driven CYP1A1 expression in the intestine is indeed significant, as intestinal CYP1A1 reduces the burden of processing xenobiotics in the liver (223). In the early stages of development, the clearance of food-derived xenobiotics without impinging much on liver activity is crucial to ensure proper liver function for the purpose of energy harvest. Tellingly TLR2, a PRR which

we show to be inducible by SCFA in the intestine, is essential for of intestinal CYP1A1 activity (223). TLR2, like AhR and PPARγ also occupies the interface of immunity and metabolism and is open to regulation by the microbiome.

The dialogues between immunity and metabolism is a prerequisite for growth, reproduction and survival, the common currency of which is energy availability. It is essential to maintain a cordial intercourse, as any disruption will result in a cascade of inflammatory and metabolic diseases. This will inadvertently divert energy usage from maintenance and growth to protection and defense. Complex organisms as ourselves have reduced the burden of maintaining this crosstalk, by "out sourcing" certain functions in maintaining this peaceful relations to the microbiota. The microbiota in turn has recruited an array of host responders to rapidly and effectively manage the energy needs (survival) of both the host and the microbial community.

Multiple roles and functions of PPARγ, AhR, ANGPTL4, TLR2 and LCN2 have made them great candidates to study the inflammation-metabolism interface within the gut epithelium. Much, however, remains unknown and we are only now beginning to appreciate how these and other proteins are interconnected and influence each other's function in response to nutritional or bacterial cues which then together shape the balance between health and disease. The interaction between the microbiome, PPARγ and AhR require much more attention and investigation before we will be able to translate the knowledge into methods for disease prevention or treatment.

To conclude my work presented in this PhD thesis, I would like to quote the Nobel Prize Laureate in literature, José Saramago. Although he most probably used it in a metaphorical and psychological sense, I think it perfectly describes the biology of human kind and our dependence on so many other organisms, whose roles are barely understood.

Saberemos cada vez menos o que é um ser humano.

Each time, we know less of what a human being is.

- José Saramago

6. ACKNOWLEDGEMENTS

Five and a half years have passed since I registered as a PhD student at the Department of Microbiology, Tumor and Cell Biology and more than six since I first met Sven and Gesan. Many people have joined and left the group during these years and all of them in some way contributed to my development as a scientist and as a person. Many other people not connected to science in any way helped me through hard times and enjoyed the good ones together with me. I would like to thank all of you for sharing a part of your lives with me and for helping me overcoming difficulties and finally arrive to the date of my PhD defence.

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7. REFERENCES

1. Johansson ME V, Larsson JMH, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 4659–65.
2. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol. Nature Publishing Group; 2014 Feb 25;14(3):141–53.
3. Müller C a, Autenrieth IB, Peschel a. Innate defenses of the intestinal epithelial barrier. Cell Mol Life Sci. 2005 Jun;62(12):1297–307.
4. Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, et al. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. J Pharmacol Exp Ther. 1997 Dec;283(3):1552–62.
5. Strassburg CP, Kneip S, Topp J, Obermayer-Straub P, Barut a, Tukey RH, et al. Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. J Biol Chem. 2000 Nov 17;275(46):36164–71.
6. Anzenbacher P, Anzenbacherová E. Cytochromes P450 and metabolism of xenobiotics. C Cell Mol Life Sci. 2001;58:737–47.
7. Zhang Q, Dunbar D, Ostrowska A, Zeisloft S, Yang J, Kaminsky LS, et al. Characterization of human small intestinal cytochromes P-450. Drug Metab Dispos. 1999;27(7):804–9.
8. Zhang Q, Dunbar D, Kaminsky LS. Characterization of mouse small intestinal cytochrome P450 expression. Drug Metab Dispos. 2003;31(11):1346–51.
9. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. Nat Rev Microbiol. 2005 May;3(5):431–8.
10. Kang MJ, Kim HG, Kim JS, Oh DG, Um YJ, Seo CS, et al. The effect of gut microbiota on drug metabolism. Expert Opin Drug Metab Toxicol. 2013 Oct;9(10):1295–308.
11. Neish AS. The gut microflora and intestinal epithelial cells: a continuing dialogue. Microbes Infect. 2002 Mar;4(3):309–17.
12. Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. Nat Immunol. Nature Publishing Group; 2011 Jan;12(1):5–9.
13. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol. Nature Publishing Group; 2011 Apr;9(4):233–43.
14. Lievin-Le Moal V, Servin AL. The Front Line of Enteric Host Defense against Unwelcome Intrusion of Harmful Microorganisms : Mucins , Antimicrobial Peptides , and Microbiota. Clin Microbiol Rev. 2006;19(2):315–37.
15. Johansson ME V, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A. 2008 Sep 30;105(39):15064–9.
16. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr Gastroenterol Rep. 2010 Oct;12(5):319–30.
17. Wlodarska M, Willing B, Keeney KM, Menendez a, Bergstrom KS, Gill N, et al. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. Infect Immun. 2011 Apr; 79(4):1536–45.
18. Fyderek K. Mucosal bacterial microflora and mucus layer thickness in adolescents with inflammatory bowel disease. World J Gastroenterol. 2009;15(42):5287.
19. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. J Appl Microbiol. 2007 May;102(5):1197–208.
20. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science. 2005 Mar 25; 307(5717):1955–9.
21. Meyer-Hoffert U, Hornef MW, Henriques-Normark B, Axelsson L-G, Midtvedt T, Pütsep K, et al. Secreted enteric antimicrobial activity localises to the mucus surface layer. Gut. 2008 Jun; 57(6):764–71.
22. Gallo RL, Hooper L V. Epithelial antimicrobial defence of the skin and intestine. Nat Rev Immunol. Nature Publishing Group; 2012 Jul;12(7):503–16.
23. Hornef MW, Wick MJ, Rhen M, Normark S. Bacterial strategies for overcoming host. 2002;3(11).

24. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(January):389–95.
25. Ouellette AJ. Paneth cell α-defensins in enteric innate immunity. Cell Mol Life Sci. 2011 Jul; 68(13):2215–29.
26. Christa L, Carnot F, Simon M-T, Levavasseur F, Stinnakre M-G, Lasserre C, et al. HIP / PAP is an adhesive protein expressed in normal Paneth , and pancreatic cells. Am J Clin Nutr. 1996;271(6):G993–G1002.
27. Ogawa H, Fukushima K, Naito H, Funayama Y, Unno M, Takahashi K, et al. Increased expression of HIP/PAP and regenerating gene III in human inflammatory bowel disease and a murine bacterial reconstitution model. Inflamm Bowel Dis. 2003 May;9(3):162–70.
28. Lehotzky RE, Partch CL, Mukherjee S, Cash HL, Goldman WE, Gardner KH, et al. Molecular basis for peptidoglycan recognition by a bactericidal lectin. Proc Natl Acad Sci U. 2010 May 27; 107(17):7722–7.
29. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004;432 (16 Dec):917–21.
30. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang Y-H, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007 Oct 4; 449(7162):564–9.
31. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. Elsevier Inc.; 2010 Mar 19;140(6):805–20.
32. Carvalho F a, Aitken JD, Vijay-Kumar M, Gewirtz AT. Toll-like receptor-gut microbiota interactions: perturb at your own risk! Annu Rev Physiol. 2012 Jan;74:177–98.
33. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity. Elsevier Inc.; 2012 Oct 19;37(4):601–10.
34. Qiu J, Heller JJ, Guo X, Chen ZE, Fish K, Fu Y-X, et al. The Aryl Hydrocarbon Receptor regulates Gut Immunity through Modulation of Innate Lymphoid Cells. Immunity. 2012;36(1):92–104.
35. Sonnenberg GF, Monticelli L a, Elloso MM, Fouser L a, Artis D. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. Immunity. Elsevier Inc.; 2011 Jan 28;34(1):122–34.
36. Mestecky J, Russell MW. Specific antibody activity, glycan heterogeneity and polyreactivity contribute to the protective activity of S-IgA at mucosal surfaces. Immunol Lett. 2009 Jun 4;124(2):57–62.
37. Peterson D a, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe. 2007 Nov 15;2(5):328–39.
38. Foussat A, Cottrez F, Brun V, Fournier N, Breittmayer J-P, Groux H. A comparative study between T regulatory type 1 and CD4+CD25+ T cells in the control of inflammation. J Immunol. 2003 Nov 15;171(10):5018–26.
39. Hooper L V, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr. 2002 Jan;22:283–307.
40. Salyers AA, West SE, Vercellotti JR, Wilkins TD. Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the Fermentation of Mucins and Plant Polysaccharides by Anaerobic Bacteria from the Human Colon. appl Environ Microbiol. 1977;34(5):529–33.
41. Hylemon PB, Harder J. Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems. FEMS Microbiol Rev. 1998;22(5):475–88.
42. Hill MJ. Intestinal flora and endogenous vitamin synthesis. Eur J Cancer Prev. 1997;6 Suppl 1:S43–S45.
43. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):11971–5.
44. Biasucci G, Rubini M, Riboni S, Morelli L, Bessi E, Retetangos C. Mode of delivery affects the bacterial community in the newborn gut. Early Hum Dev. Elsevier Ltd; 2010 Jul;86 Suppl 1(1):13–5.

45. Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of Delivery – Effects on Gut Microbiota and Humoral Immunity. Neonatology. 2008;93(4):236–40.
46. Pandey PK, Verma P, Kumar H, Bavdekar A, Patole MS, Shouche YS. Comparative analysis of fecal microflora of healthy full-term Indian infants born with different methods of delivery (vaginal vs cesarean): Acinetobacter sp. prevalence in vaginally born infants. J Biosci. 2012 Oct 18;37(S1):989–98.
47. Palmer C, Bik EM, DiGiulio DB, Relman D a, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol. 2007 Jul;5(7):e177.
48. Grönlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. Journal of pediatric gastroenterology and nutrition. 1999. p. 19–25
49. Falony G, Calmeyn T, Leroy F, De Vuyst L. Coculture fermentations of Bifidobacterium species and Bacteroides thetaiotaomicron reveal a mechanistic insight into the prebiotic effect of inulin-type fructans. Appl Environ Microbiol. 2009 Apr;75(8):2312–9.
50. Antonopoulos D a, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect Immun. 2009 Jun;77(6):2367–75.
51. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an Antibiotic on the human gut microbiota, as revealed by deeo 16S rRNA sequencing. PLoS Biol. 2008 Nov;6(11):e280.
52. Ubeda C, Pamer EG. Antibiotics, microbiota, and immune defense. Trends Immunol. Elsevier Ltd; 2012 Sep;33(9):459–66.
53. Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ. Infant antibiotic exposures and early-life body mass. Int J Obes (Lond). Nature Publishing Group; 2013 Jan;37(1):16–23.
54. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005 Jun 10;308(5728):1635–8.
55. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010 Mar 4;464(7285):59–65.
56. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011 May 12;473(7346):174–80.
57. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh S a, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011 Oct 7;334(6052):105–8.
58. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457(7228):480–4.
59. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of Mammals and Their Gut Microbes. Science (80-). 2008;320(20 June):1647–52.
60. Brown EM, Sadarangani M, Finlay BB. The role of the immune system in governing host-microbe interactions in the intestine. Nat Immunol. 2013 Jul;14(7):660–7.
61. Korecka A, Arulampalam V. The gut microbiome: scourge, sentinel or spectator? J Oral Microbiol. 2012 Jan;4:1–14.
62. Alex S, Lange K, Amolo T, Grinstead JS, Haakonsson AK, Szalowska E, et al. Short-chain fatty acids stimulate angiopoietin-like 4 synthesis in human colon adenocarcinoma cells by activating peroxisome proliferator-activated receptor γ. Mol Cell Biol. 2013 Apr;33(7):1303–16.
63. Cook SI, Sellin JH. Review article: short chain fatty acids in health and disease. Aliment Pharmacol Ther. 1998 Jun;12(6):499–507.
64. Donohoe DR, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. Mol Cell. Elsevier Inc.; 2012 Nov 30;48(4):612–26.
65. Roediger WE. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut. 1980 Sep;21(9):793–8.
66. Wolever TM, Spadafore P, Eshuis H. Interaction between colonic acetate and propionate in humans. Am J Clin Nutr. 1991;53:681–7.
67. Scheppach W. Effects of short chain fatty acids on gut morphology and function. Gut. 1994 Jan;35(1 Suppl):S35–8.

68. Pomare EW, Branch WJ, Cummings JH. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. J Clin Invest. 1985 May;75(5):1448–54.
69. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006 Sep;5(9):769–84.
70. Marks PA, Xu W-S. Histone Deacetylase Inhibitors: Potential in Cancer Therapy. J Cell Biochem. 2009;107(4):600–8.
71. Ichimura A, Hirasawa A, Hara T, Tsujimoto G. Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. Prostaglandins Other Lipid Mediat. 2009 Sep;89(3-4):82–8.
72. Bäckhed F, Ley RE, Sonnenburg JL, Peterson D a, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005 Mar 25;307(5717):1915–20.
73. Ley RE, Lozupone C a, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol. 2008 Oct;6(10):776–88.
74. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut Microbiota in Health and Disease. Physiol rev. 2010;90:859–904.
75. Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 2011 Jan;62:361–80.
76. Bravo JA, Forsythe P, Chew M V, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011;108(38):16050–5.
77. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011 Feb 15;108(7):3047–52.
78. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011 Aug;141(2):599–609, 609.e1–3.
79. al-Waiz M, Mikov M, Mitchell SC, Smith RL. The exogenous origin of trimethylamine in the mouse. Metabolism. 1992 Feb;41(2):135–6.
80. Smith E, Macfarlane G. Formation of Phenolic and Indolic Compounds by Anaerobic Bacteria in the Human Large Intestine. Microb Ecol. 1997 Apr;33(3):180–8.
81. Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C, Carbo A, Shaykhutdinov R, et al. Probiotic bacteria produce conjugated linoleic acid locally in the gut that targets macrophage PPAR γ to suppress colitis. PLoS One. 2012 Jan;7(2):e31238.
82. Wall R, Ross RP, Ryan C a, Hussey S, Murphy B, Fitzgerald GF, et al. Role of gut microbiota in early infant development. Clin Med Pediatr. 2009 Jan;3(0):45–54.
83. Rabot S, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J. 2010 Dec;24(12):4948–59.
84. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A. 2007 Jan 16;104(3):979–84.
85. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M. Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr. 2010 Sep;104(6):919–29.
86. Bäckhed F, Ding H, Wang T, Hooper L V, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004 Nov 2;101(44):15718–23.
87. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A. 2008 Oct 28;105(43):16767–72.
88. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science (80-). 2013 Sep 6;341(6150):1241214.
89. Dumas M-E, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A. 2006 Aug 15;103(33):12511–6.

90. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 2013 Dec;62(12):1787–94.
91. Gérard P, Lepercq P, Leclerc M, Gavini F, Raibaud P, Juste C. Bacteroides sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. Appl Environ Microbiol. 2007 Sep;73(18):5742–9.
92. Kimura K, Kanai T, Hayashi A, Mikami Y, Sujino T, Mizuno S, et al. Dysregulated balance of retinoid-related orphan receptor γt-dependent innate lymphoid cells is involved in the pathogenesis of chronic DSS-induced colitis. Biochem Biophys Res Commun. Elsevier Inc.; 2012 Nov 2;427(4):694–700.
93. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell. 2006 Aug 25;126(4):789–99.
94. Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. Trends Endocrinol Metab. 2006 Oct;17(8):321–7.
95. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, et al. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature. 2005 Sep 29;437(7059):759–63.
96. Perissi V, Rosenfeld MG. Controlling nuclear receptors: the circular logic of cofactor cycles. Nat Rev Mol Cell Biol. 2005 Jul;6(7):542–54.
97. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature. 1990;347(6294):645–50.
98. Palmer CN, Hsu MH, Griffin HJ, Johnson EF. Novel sequence determinants in peroxisome proliferator signaling. The Journal of biological chemistry. 1995. p. 16114–21.
99. Zoete V, Grosdidier A, Michielin O. Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators. Biochim Biophys Acta. 2007 Aug;1771(8):915–25.
100. Kliewer S a, Sundseth SS, Jones S a, Brown PJ, Wisely GB, Koble CS, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci U S A. 1997 Apr 29;94(9):4318–23.
101. Kliewer S a, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell. 1995 Dec 1;83(5):813–9.
102. Contreras A V., Torres N, Tovar AR. PPAR- as a Key Nutritional and Environmental Sensor for Metabolic Adaptation. Adv Nutr An Int Rev J. 2013 Jul 15;4(4):439–52.
103. Rosen ED, Spiegelman BM. PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem. 2001 Oct 12;276(41):37731–4.
104. Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM. Oxidized LDL Regulates Macrophage Gene Expression through Ligand Activation of PPARg. Cell. 1998;93:229–40.
105. Heikkinen S, Auwerx J, Argmann CA. PPARγ in human and mouse physiology. Biochim Biophys Acta. 2007;1771(8):999–1013.
106. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell. 1994 Dec 30;79(7):1147–56.
107. Park KW, Halperin DS, Tontonoz P. Before they were fat: adipocyte progenitors. Cell Metab. Elsevier Inc.; 2008 Dec;8(6):454–7.
108. Cannon B, Nedergaard J. Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). Int J Obes (Lond). Nature Publishing Group; 2010 Oct;34 Suppl 1(S1):S7–16.
109. Hevener AL, Olefsky JM, Reichart D, Nguyen MTA, Bandyopadyhay G, Leung H, et al. Macrophage PPAR γ is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J Clin Invest. 2007;117(6):1658–69.
110. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature. 2007 Jun 28;447(7148):1116–20.

111. Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. Proc Natl Acad Sci U S A. 2003 May 27;100(11):6712–7.
112. Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. Nat Rev Immunol. Nature Publishing Group; 2010 May;10(5):365–76.
113. Chawla A. Control of macrophage activation and function by PPARs. Circ Res. 2010 May 28;106(10):1559–69.
114. Mukherjee R, Jow L, Croston GE, Paterniti Jr JR. Identification, Characterization, and Tissue Distribution of Human Peroxisome Proliferator-activated Receptor (PPAR) Isoforms PPARgamma 2 versus PPARgamma 1 and Activation with Retinoid X Receptor Agonists and Antagonists. J Biol Chem. 1997 Mar 21;272(12):8071–6.
115. Fajas L. The Organization, Promoter Analysis, and Expression of the Human PPARgamma Gene. J Biol Chem. 1997 Jul 25;272(30):18779–89.
116. Drori S, Girnun GD, Tou L, Szwaya JD, Mueller E, Xia K, et al. Hic-5 regulates an epithelial program mediated by PPARgamma. Genes Dev. 2005 Feb 1;19(3):362–75.
117. Chen L, Bush CR, Necela BM, Su W, Yanagisawa M, Anastasiadis PZ, et al. RS5444, a novel PPARgamma agonist, regulates aspects of the differentiated phenotype in nontransformed intestinal epithelial cells. Mol Cell Endocrinol. 2006 Jun 7;251(1-2):17–32.
118. Su W, Bush CR, Necela BM, Calcagno SR, Murray NR, Fields AP, et al. Differential expression , distribution , and function of PPAR- g in the proximal and distal colon. Physiol genomics. 2007;30:342–53.
119. Are A, Aronsson L, Wang S, Greicius G, Lee YK, Gustafsson J-A, et al. Enterococcus faecalis from newborn babies regulate endogenous PPARgamma activity and IL-10 levels in colonic epithelial cells. Proc Natl Acad Sci U S A. 2008 Feb 12;105(6):1943–8.
120. Voltan S, Martines D, Elli M, Brun P, Longo S, Porzionato A, et al. Lactobacillus crispatus M247-derived H2O2 acts as a signal transducing molecule activating peroxisome proliferator activated receptor-gamma in the intestinal mucosa. Gastroenterology. 2008 Oct;135(4):1216–27.
121. Aronsson L, Huang Y, Parini P, Korach-André M, Håkansson J, Gustafsson J-Å, et al. Decreased fat storage by Lactobacillus paracasei is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). PLoS One. 2010 Jan;5(9).
122. Peyrin-Biroulet L, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, et al. Peroxisome proliferator-activated receptor gamma activation is required for maintenance of innate antimicrobial immunity in the colon. Proc Natl Acad Sci U S A. 2010 May 11;107(19):8772–7.
123. Annese V, Rogai F, Settesoldi A, Bagnoli S. PPARγ in Inflammatory Bowel Disease. PPAR Res. 2012 Jan;2012:620839.
124. Ramakers JD, Verstege MI, Thuijls G, Te Velde A a, Mensink RP, Plat J. The PPARgamma agonist rosiglitazone impairs colonic inflammation in mice with experimental colitis. J Clin Immunol. 2007 May;27(3):275–83.
125. Mohapatra SK, Guri AJ, Climent M, Vives C, Carbo A, Horne WT, et al. Immunoregulatory actions of epithelial cell PPAR gamma at the colonic mucosa of mice with experimental inflammatory bowel disease. PLoS One. 2010 Jan;5(4):e10215.
126. Adachi M, Kurotani R, Morimura K, Shah Y, Sanford M, Madison BB, et al. Peroxisome proliferator activated receptor gamma in colonic epithelial cells protects against experimental inflammatory bowel disease. Gut. 2006 Aug;55(8):1104–13.
127. Yamamoto-Furusho JK, Peñaloza-Coronel A, Sánchez-Muñoz F, Barreto-Zuñiga R, Dominguez-Lopez A. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression is downregulated in patients with active ulcerative colitis. Inflamm Bowel Dis. 2011 Feb;17(2):680–1.
128. Zhang Z-F, Yang N, Zhao G, Zhu L, Wang L-X. Association between the Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma 2 and inflammatory bowel disease: a meta-analysis. PLoS One. 2012 Jan;7(1):e30551.

129. Andersen V, Christensen J, Ernst A, Jacobsen B a, Tjønneland A, Krarup HB, et al. Polymorphisms in NF-κB, PXR, LXR, PPARγ and risk of inflammatory bowel disease. World J Gastroenterol. 2011 Jan 14;17(2):197–206.
130. Poliska S, Penyige A, Lakatos PL, Papp M, Palatka K, Lakatos L, et al. Association of peroxisome proliferator-activated receptor gamma polymorphisms with inflammatory bowel disease in a Hungarian cohort. Inflamm Bowel Dis. 2012 Mar;18(3):472–9.
131. Aoyagi Y, Nagata S, Kudo T, Fujii T, Wada M, Chiba Y, et al. Peroxisome proliferator-activated receptor γ 2 mutation may cause a subset of ulcerative colitis. Pediatr Int. 2010 Oct;52(5):729–34.
132. Pongratz I, Antonsson C, Whitelaw ML. Role of the PAS Domain in Regulation of Dimerization and DNA Binding Specificity of the Dioxin Receptor. Mol Cell Biol. 1998;18(7):4079–88.
133. Taylor BL, Zhulin IB. PAS Domains : Internal Sensors of Oxygen , PAS Domains : Internal Sensors of Oxygen, Redox Potential, and Light. Microbiol Mol Biol Rev. 1999;63(2):479–506.
134. Ema M, Sogawa K, Watanabe N, Chujoh Y, Matsushita N, Gotoh O, et al. cDNA cloning and structure of mouse putative Ah receptor. Biochem Biophys Res Commun. 1992;184(1):246–53.
135. Burbach KM, Poland A, Bradfield C a. Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. Proc Natl Acad Sci U S A. 1992 Sep 1;89:8185–9.
136. Swanson HI, Bradfield CA. The AH-receptor - genetics, structure and function. Pharmacogenetics. 1993;3:213–30.
137. Furness SGB, Lees MJ, Whitelaw ML. The dioxin (aryl hydrocarbon) receptor as a model for adaptive responses of bHLH/PAS transcription factors. FEBS Lett. 2007 Jul 31;581(19):3616–25.
138. Lees MJ, Whitelaw ML. Multiple Roles of Ligand in Transforming the Dioxin Receptor to an Active Basic Helix-Loop-Helix / PAS Transcription Factor Complex with the Nuclear Protein Arnt. Mol Cell Biol. 1999;19(8):5811.
139. Pollenz RS, Sattler CA, Poland A. The Aryl Hydrocarbon Receptor and Aryl Hydrocarbon Receptor Nuclear Translocator Protein Show Distinct Subcellular Localizations in Hepa 1 ci c7 Cells by Immunfluorescence Microscopy. Mol Pharmacol. 1993;45:428–38.
140. Hines RN, Mathis JM, Jacob CS. Identification of multiple regulatory elements on the human cytochrome P450IA1 gene. Carcinogenesis. 1988 Sep;9(9):1599–605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3409463
141. McGuire J, Okamoto K, Whitelaw ML, Tanaka H, Poellinger L. Definition of a dioxin receptor mutant that is a constitutive activator of transcription: delineation of overlapping repression and ligand binding functions within the PAS domain. J Biol Chem. 2001 Nov 9;276(45):41841–9.
142. Kewley RJ, Whitelaw ML, Chapman-Smith A. The mammalian basic helix–loop–helix/PAS family of transcriptional regulators. Int J Biochem Cell Biol. 2004 Feb;36(2):189–204.
143. Frericks M, Meissner M, Esser C. Microarray analysis of the AHR system: tissue-specific flexibility in signal and target genes. Toxicol Appl Pharmacol. 2007 May 1;220(3):320–32.
144. Rowlands JC, Gustafsson J-A. Aryl Hydrocarbon Receptor-Mediated Signal Transduction. Crit Rev Toxicol. 1997;27(2):109–34.
145. Denison MS, Soshilov A a, He G, DeGroot DE, Zhao B. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. Toxicol Sci. 2011 Nov;124(1):1–22.
146. Gonzalez FJ, Fernandez-salguero P. The Aryl Hydrocarbon Receptor: Studies Using the AhR-null Mice. Drug Metab Dispos. 1998;26(12):1194–8.
147. Rannug U, Rannug a, Sjöberg U, Li H, Westerholm R, Bergman J. Structure elucidation of two tryptophan-derived, high affinity Ah receptor ligands. Chem Biol. 1995 Dec;2(12):841–5.
148. Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol. 2003 Jan;43:309–34.
149. Opitz C a, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature. Nature Publishing Group; 2011 Oct 13;478(7368):197–203.

150. Kiss E a, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science. 2011 Dec 16;334(6062):1561–5.
151. Li Y, Innocentin S, Withers DR, Roberts N a, Gallagher AR, Grigorieva EF, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell. Elsevier Inc.; 2011 Oct 28;147(3):629–40.
152. Hao N, Whitelaw ML. The emerging roles of AhR in physiology and immunity. Biochem Pharmacol. Elsevier Inc.; 2013 Sep 1;86(5):561–70.
153. Ohtake F, Baba A, Takada I, Okada M, Iwasaki K, Miki H, et al. Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. Nature. 2007 Mar 29;446(7135):562–6.
154. Harstad EB, Guite CA, Thomae TL, Bradfield CA. Liver Deformation in Ahr -Null Mice: Evidence for Aberrant Hepatic Perfusion In Early Development. Mol Pharmacol. 2006;69(5):1534–41.
155. Quintana FJ, Sherr DH. Aryl hydrocarbon receptor control of adaptive immunity. Pharmacol Rev. 2013 Jan;65(4):1148–61.
156. Hankinson O. The Aryl Hydrocarbon receptor complex. Annu Rev pharmacolToxicol. 1995;35:307–40.
157. Lee JS, Cella M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, et al. AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. Nat Immunol. 2012 Feb;13(2):144–51.
158. Monteleone I, MacDonald TT, Pallone F, Monteleone G. The aryl hydrocarbon receptor in inflammatory bowel disease: linking the environment to disease pathogenesis. Curr Opin Gastroenterol. 2012 Jul;28(4):310–3.
159. Sato S, Shirakawa H, Tomita S, Ohsaki Y, Haketa K, Tooi O, et al. Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver. Toxicol Appl Pharmacol. 2008 May 15;229(1):10–9.
160. Weber LWD, Lebofsky M, Stahl BU, Gorski JR, Muzi G, Rozman K. Reduced activities of key enzymes of gluconeogenesis as possible cause of acute toxicity of (TCDD) in rats. Toxicology. 1991;66(2):133–44.
161. Liu PC, Matsumura F. Differential Eftects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the “Adipose-Type” and "Brain-Type” Glucose Transporters in Mice. Mol Pharmacol. 1995;47(1):65–73.
162. Kawano Y, Nishiumi S, Tanaka S, Nobutani K, Miki A, Yano Y, et al. Activation of the aryl hydrocarbon receptor induces hepatic steatosis via the upregulation of fatty acid transport. Arch Biochem Biophys. Elsevier Inc.; 2010 Dec 15;504(2):221–7.
163. Lee JH, Wada T, Febbraio M, He J, Matsubara T, Jae M, et al. A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis. Gastroenterology. 2010;139(2):653–63.
164. Wang C, Xu C-X, Krager SL, Bottum KM, Liao D-F, Tischkau S a. Aryl hydrocarbon receptor deficiency enhances insulin sensitivity and reduces PPAR-α pathway activity in mice. Environ Health Perspect. 2011 Dec;119(12):1739–44.
165. Neel B a, Sargis RM. The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. Diabetes. 2011 Jul;60(7):1838–48.
166. Park W-H, Jun DW, Kim JT, Jeong JH, Park H, Chang Y-S, et al. Novel cell-based assay reveals associations of circulating serum AhR-ligands with metabolic syndrome and mitochondrial dysfunction. BioFactors. 2013;39(4):494–504.
167. Koonen DPY, Jacobs L, Febbraio M, Young ME, Soltys CM. Increased Hepatic CD36 Expression Contributes to Dyslipidemia Associated With Diet-Induced Obesity. Diabetes. 2007;56(December):2863–71.
168. Zhou J, Febbraio M, Wada T, Zhai Y, Kuruba R, He J, et al. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. Gastroenterology. 2008 Feb;134(2):556–67.
169. Binas B, Erol E. FABPs as determinants of myocellular and hepatic fuel metabolism. Mol Cell Biochem. 2007 May;299(1-2):75–84.

170. Thumser a E, Storch J. Liver and intestinal fatty acid-binding proteins obtain fatty acids from phospholipid membranes by different mechanisms. J Lipid Res. 2000 Apr;41(4):647–56.
171. Alexson SE, Cannon B. A direct comparison between peroxisomal and mitochondrial preferences for fatty-acyl beta-oxidation predicts channelling of medium-chain and very-long-chain unsaturated fatty acids to peroxisomes. Biochim Biophys Acta. 1984;796(1):1–10.
172. Reddy JK, Mannaerts GP. Peroxisomal lipid metabolism. Annu Rev Nutr. 1994 Jan;14:343–70.
173. Eaton S, Bartlett K, Pourfarzam M. Mammalian mitochondrial beta-oxidation. Biochem J. 1996 Dec 1;320:345–57.
174. Hooper L V. Bacterial contributions to mammalian gut development. Trends Microbiol. 2004 Mar;12(3):129–34.
175. Björkholm B, Bok CM, Lundin A, Rafter J, Hibberd ML, Pettersson S. Intestinal microbiota regulate xenobiotic metabolism in the liver. PLoS One. 2009 Jan;4(9):e6958.
176. Lundin A, Bok CM, Aronsson L, Björkholm B, Gustafsson J-A, Pott S, et al. Gut flora, Toll-like receptors and nuclear receptors: a tripartite communication that tunes innate immunity in large intestine. Cell Microbiol. 2008 May;10(5):1093–103.
177. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol. 2007 Apr;19(2):59–69. 2
178. Seksik P, Sokol H, Lepage P, Vasquez N, Manichanh C, Mangin I, et al. Review article: the role of bacteria in onset and perpetuation of inflammatory bowel disease. Aliment Pharmacol Ther. 2006 Oct;24 Suppl 3(June):11–8.
179. Mahajan S, Dkhar HK, Chandra V, Dave S, Nanduri R, Janmeja AK, et al. Mycobacterium tuberculosis modulates macrophage lipid-sensing nuclear receptors PPARγ and TR4 for survival. J Immunol. American Association of Immunologists; 2012;188(11):5593–603.
180. Konturek PC, Kania J, Kukharsky V, Raithel M, Ocker M, Rembiasz K, et al. Implication of peroxisome proliferator-activated receptor gamma and proinflammatory cytokines in gastric carcinogenesis: link to Helicobacter pylori-infection. J Pharmacol Sci. 2004;96(2):134–43.
181. Borniquel S, Jadert C, Lundberg JO. Dietary Conjugated Linoleic Acid Activates PPAR g and the Intestinal Trefoil Factor in SW480 Cells and Mice with Dextran Sulfate. J Nutr. 2012;142:2135–40.
182. Mashimo H, Wu DC, Podolsky DK, Fishman MC. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science. 1996 Oct 11;274(5285):262–5.
183. Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio S-P, Paixao T a, et al. Lipocalin-2 resistance confers an advantage to Salmonella enterica serotype Typhimurium for growth and survival in the inflamed intestine. Cell Host Microbe. 2009 May 8;5(5):476–86.
184. Raffatellu M, Baumler AJ. Salmonella's iron armor for battling the host and its microbiota. Gut Microbes. 2010;1(1):70–2.
185. Fischbach M a, Lin H, Zhou L, Yu Y, Abergel RJ, Liu DR, et al. The pathogen-associated iroA gene cluster mediates bacterial evasion of lipocalin 2. Proc Natl Acad Sci U S A. 2006 Oct 31;103(44):16502–7.
186. Kjeldsens L, Johnsen H, Sengelbv H. Isolation and Primary Structure of NGAL , a Novel Protein Associated. J Biol Chem. 1993;268(14):10425–32.
187. Triebel S, Bläser J, Reinke H, Tschesche H. A 25 kDa alpha 2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. FEBS Lett. 1992 Dec 21;314(3):386–8.
188. Ramu P, Lobo LA, Kukkonen M, Bjur E, Suomalainen M, Raukola H, et al. Activation of pro-matrix metalloproteinase-9 and degradation of gelatin by the surface protease PgtE of Salmonella enterica serovar Typhimurium. Int J Med Microbiol. 2008 Apr;298(3-4):263–78.
189. Castaneda FE, Walia B, Vijay-Kumar M, Patel NR, Roser S, Kolachala VL, et al. Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central role of epithelial-derived MMP. Gastroenterology. 2005 Dec;129(6):1991–2008.
190. Garg P, Vijay-kumar M, Wang L, Gewirtz AT, Merlin D, Sitaraman S V. Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis. Am J Physiol Gastrointest Liver Physiol. 2009;30322(296):175–84.

191. Shu H, Wong B, Zhou G, Li Y, Berger J, Woods JW, et al. Activation of PPARalpha or gamma reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. Biochem Biophys Res Commun. 2000 Jan 7;267(1):345–9.
192. Necela BM, Su W, Thompson EA. Toll-like receptor 4 mediates cross-talk between peroxisome proliferator-activated receptor gamma and nuclear factor-kappaB in macrophages. Immunology. 2008 Nov;125(3):344–58.
193. Oppong GO, Rapsinski GJ, Newman TN, Nishimori JH, Biesecker SG, Tükel Ç. Epithelial cells augment barrier function via activation of the Toll-like receptor 2/phosphatidylinositol 3-kinase pathway upon recognition of Salmonella enterica serovar Typhimurium curli fibrils in the gut. Infect Immun. 2013 Feb;81(2):478–86.
194. Hayashi F, Smith KD, Ozinsky a, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001 Apr 26;410(6832):1099–103.
195. Gewirtz a T, Navas T a, Lyons S, Godowski PJ, Madara JL. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol. 2001 Aug 15;167(4):1882–5.
196. Kinoshita M, Suzuki Y, Saito Y. Butyrate reduces colonic paracellular permeability by enhancing PPAR c activation. 2002;293:827–31.
197. Grootaert C, Van de Wiele T, Van Roosbroeck I, Possemiers S, Vercoutter-Edouart A-S, Verstraete W, et al. Bacterial monocultures, propionate, butyrate and H2O2 modulate the expression, secretion and structure of the fasting-induced adipose factor in gut epithelial cell lines. Environ Microbiol. 2011 Jul;13(7):1778–89.
198. Huang W, Zhao S, Ammanamanchi S, Brattain M, Venkatasubbarao K, Freeman JW. Trichostatin A induces transforming growth factor beta type II receptor promoter activity and acetylation of Sp1 by recruitment of PCAF/p300 to a Sp1.NF-Y complex. J Biol Chem. 2005 Mar 18;280(11):10047–54.
199. Oberg M, Bergander L, Håkansson H, Rannug U, Rannug A. Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. Toxicol Sci. 2005 Jun;85(2):935–43.
200. Hudcovic T, Kolinska J, Klepetar J, Stepankova R, Rezanka T, Srutkova D, et al. Protective effect of Clostridium tyrobutyricum in acute dextran sodium sulphate-induced colitis: differential regulation of tumour necrosis factor-α and interleukin-18 in BALB/c and severe combined immunodeficiency mice. Clin Exp Immunol. 2012 Feb;167(2):356–65.
201. Hayashi A, Sato T, Kamada N, Mikami Y, Matsuoka K, Hisamatsu T, et al. A single strain of Clostridium butyricum induces intestinal IL-10-producing macrophages to suppress acute experimental colitis in mice. Cell Host Microbe. Elsevier; 2013 Jun 12;13(6):711–22.
202. Larsson E, Tremaroli V, Lee YS, Koren O, Nookaew I, Fricker A, et al. Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. Gut. 2012 Aug;61(8):1124–31.
203. Vijay-kumar M, Sanders CJ, Taylor RT, Kumar A, Aitken JD, Sitaraman S V, et al. Deletion of TLR5 results in spontaneous colitis in mice. J clin Inv. 2007;117(12):3909–21.
204. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss C a, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. Elsevier Inc.; 2011 May 27;145(5):745–57.
205. Furumatsu K, Nishiumi S, Kawano Y, Ooi M, Yoshie T, Shiomi Y, et al. A role of the aryl hydrocarbon receptor in attenuation of colitis. Dig Dis Sci. 2011 Sep;56(9):2532–44.
206. Monteleone I, Rizzo A, Sarra M, Sica G, Sileri P, Biancone L, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. Gastroenterology. Elsevier Inc.; 2011 Jul;141(1):237–48, 248.e1.
207. Qiu J, Guo X, Chen Z-ME, He L, Sonnenberg GF, Artis D, et al. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. Immunity. Elsevier Inc.; 2013 Aug 22;39(2):386–99.
208. Singh NP, Singh UP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Activation of aryl hydrocarbon receptor (AhR) leads to reciprocal epigenetic regulation of FoxP3 and IL-17 expression and amelioration of experimental colitis. PLoS One. 2011 Jan;6(8):e23522.

209. Takamura T, Harama D, Matsuoka S, Shimokawa N, Nakamura Y, Okumura K, et al. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. Immunol Cell Biol. Nature Publishing Group; 2010 Aug;88(6):685–9.
210. Vijay-Kumar M, Aitken JD, Carvalho F a, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010 Apr 9;328(5975):228–31.
211. West DB, Boozer CN, Moody DL, Atkinson RL. Dietary obesity in nine inbred mouse strains. Am J Physiol. 1992;262(6 Pt 2):R1025–R1032.
212. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes. 1988;37(9):1163–7.
213. Pettersson US, Waldén TB, Carlsson P-O, Jansson L, Phillipson M. Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLoS One. 2012 Jan;7(9):e46057.
214. Kelly D, Campbell JI, King TP, Grant G, Jansson E a, Coutts AGP, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004 Jan;5(1):104–12.
215. Garner CD, Antonopoulos D a, Wagner B, Duhamel GE, Keresztes I, Ross D a, et al. Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a Salmonella enterica serovar typhimurium murine model of infection. Infect Immun. 2009 Jul;77(7):2691–702.
216. Gao Q, Qi L, Wu T, Wang J. Clostridium butyricum activates TLR2-mediated MyD88-independent signaling pathway in HT-29 cells. Mol Cell Biochem. 2012 Feb;361(1-2):31–7.
217. Lichtenstein L, Mattijssen F, de Wit NJ, Georgiadi A, Hooiveld GJ, van der Meer R, et al. Angptl4 protects against severe proinflammatory effects of saturated fat by inhibiting fatty acid uptake into mesenteric lymph node macrophages. Cell Metab. 2010 Dec 1;12(6):580–92.
218. Sultan S, Pascucci M, Ahmad S, Malik IA, Bianchi A, Ramadori P, et al. LIPOCALIN-2 is a major acute-phase protein in a rat and mouse model of sterile abscess. Shock. 2012 Feb;37(2):191–6.
219. Jang Y, Lee JH, Wang Y, Sweeney G. Emerging clinical and experimental evidence for the role of lipocalin-2 in metabolic syndrome. Clin Exp Pharmacol Physiol. 2012 Feb;39(2):194–9.
220. Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, et al. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. Gut. 2010 Dec;59(12):1635–42.
221. David L a, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. Nature Publishing Group; 2014 Jan 23;505(7484):559–63. 7
222. Mattijssen F, Alex S, Swarts HJ, Groen AK, van Schothorst EM, Kersten S. Angptl4 serves as an endogenous inhibitor of intestinal lipid digestion. Mol Metab. Elsevier; 2014 Apr;3(2):135–44.
223. Do KN, Fink LN, Jensen TE, Gautier L, Parlesak A. TLR2 controls intestinal carcinogen detoxication by CYP1A1. PLoS One. 2012 Jan;7(3):e32309.
224. Ferreira RB, Gill N, Willing BP, Antunes LC, Russell SL, Croxen MA, Finlay BB. The Intestinal Microbiota Plays a Role in Salmonella-Induced Colitis Independent of Pathogen Colonization.Plos One 2011 6(5): e20338