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MICROBIAL REGULATION OF PPARγ: NUCLEAR RECEPTOR NETWORKS IMPORTANT FOR COLONIC HOMEOSTASIS

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“Probably my worst quality is that I get very passionate about what I think is right.”
Hillary Rodham Clinton

ABSTRACT

One of the central issues surrounding the physiology of good health is to uncover and understand the molecular networks responsible for its maintenance. The alimentary tract in particular, demands a properly sustained homeostatic control since it is constantly challenged by potentially harmful agents along with innocuous nutrients, with the burden of basic metabolic functions in its ward. The colon is thus a digestive and absorptive organ with life sustaining authority, and central to its great protective nature is its need to sense and use bacterial products. Our microflora is an organ in itself, representing a greatly efficient and stable bioreactor where dietary constituents are degraded for our benefit. An understanding of how the intestinal barrier senses and responds to bacterial products is critical to gaining insights into the pathogenesis of disease as well as the impact of bacterial metabolism on energy balance of the host.

My work summarized in this thesis, highlights that development of the gastrointestinal tract is subject to regulation by colonizing microorganisms, and explores to what extent the microflora can tune its functions. These processes require crosstalk between commensal bacteria and host cells by way of signaling through for example Toll-like receptor pathways, activating transcription factors such as nuclear receptors. By comparing mRNA expression profiles of nuclear receptors and Toll-like receptors, I have identified a subset of these to be conditionally regulated by the gut microbiota. It would seem that the gut flora affects receptors intimately connected to innate immunity and metabolic control.

Apart from transcriptional communication, bacteria can also converse through post-translational effects. By altering phosphorylation status of the nuclear receptor PPARγ much like a specific ligand would, commensal bacteria are able to skew cell fate into maturation and anti-inflammation, thus affecting overall homeostasis. This implies that the manner in which the crosstalk is carried out may be through secreted molecules which affect host cells. Except for the clues presented in this thesis, there still remains a paucity of information regarding bacterial influence on host physiology, and even less information on how this influence is mediated. The search for mediators for fine tuning of body homeostasis has recognized probiotics as potentially beneficial. In my work I have shown that nuclear receptors such as the PPARs, can be regulated by microflora both in expression and function. One of their target genes, fasting induced adipose factor (FIAF), has a potentially interesting role in obesity because of its actions as a lipoprotein lipase inhibitor. Certain probiotic strains are able to upregulate FIAF, possibly through PPARs, with the implication of regulatory effects on body fat storage.

Although the purported health benefits attributed to bacteria are numerous, the precise molecular mechanisms governing the cross-talk within the intestinal ecosystem remain to be discovered. Research now points toward host–microbe interactions being essential to several conditioning aspects of normal physiology. The work presented here adds to our understanding of the molecular basis for the complex and dynamic interactions between the microbiota and its host, and further underscores the huge potential in manipulation of the microbiota as a tool for sustained health.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

I. Lundin A, Bok CM, **Aronsson L***, Björkholm B, Gustafsson J-Å, Pott S, Arulampalam V, Hibberd M, Rafter J, Pettersson S.
Gut flora, Toll-Like-Receptors and Nuclear Receptors: a tripartite communication that tunes innate immunity in large intestine
Cell Microbiol (2008) 10(5):1093-1103

II. Are A, **Aronsson L***, Wang S, Greicius G, Lee YK, Gustafsson J-Å, Pettersson S, Arulampalam V.
Enterococcus faecalis from newborn babies regulate endogenous PPARγ activity and Interleukin-10 levels in colonic epithelial cells
Proc Natl Acad Sci U S A (2008) 105(6):1943-8

III. **Aronsson L**, Huang Y, Parini P, Gustafsson J-Å, Pettersson S, Arulampalam V, Rafter J.
Lactobacilli Targets Fat Storage by Activating Fasting Induced Adipose Factor (FIAF)
PLoS Biology, submitted

* shared first authorship

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LIST OF ABBREVIATIONS

ADRP	Adipophilin, Adipose differentiation-related protein
AF	Activation function
CCL2/MCP-1	Chemokine ligand 2/Monocyte chemoattractant protein-1
CD	Crohn's disease
DBD	DNA binding domain
DSS	Dextran sodium sulphate
FIAF	Fasting induced adipose factor
GF	Germ free
HDL	High density lipoprotein
IBD	Inflammatory bowel disease
IL	Interleukin
LBD	Ligand binding domain
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
NBS/LRR	Nucleotide binding site/Leucine-rich repeat protein
NEC	Necrotizing enterocolitis
NF-κB	Nuclear factor-kappa B
NOD	Nucleotide binding oligomerization domain
NR	Nuclear receptor
PAMP	Pathogen associated molecular pattern
PI-3K	Phosphatidylinositol 3-OH kinase
PPAR	Peroxisome proliferator activated receptor
PPRE	PPAR response element
PRR	Pattern recognition receptor
RXR	Retinoid X receptor
siRNA	Small interfering RNA
SPF	Specific pathogen free
SUMO	Small ubiquitin related modifier
TLDA	TaqMan Low-Density Array
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TZD	Thiazolidinedione
UC	Ulcerative colitis
VLDL	Very low density lipoprotein

1. INTRODUCTION

The amount and type of proteins present in a cell largely determines its actions and properties. Despite the theoretical potential in externally controlling proteins and their signaling pathways, many of these are still too inadequately understood to be considered targets for drug design. The complexity of the protein networks involved in any given disorder would demand multiple target points in both time and space, which today is poorly comprehended. Fine tuning of gene regulation is crucial for all biological systems since they by nature demand a dynamic ability of adaptation. Knowledge of how to appropriately tweak these signals may solve a lot of our problems with unwanted side effects that always come with modern medicine.

The gastrointestinal tract is especially a hot spot for external influence and subsequent response. Here, cells serve as both a barrier and an absorptive surface which demands intricate signaling control. In addition to this, a constant discourse between the present microflora and the host is maintained to ensure proper symbiosis. This dialogue is of particular importance for the understanding of homeostasis in the gastrointestinal tract.

1.1 Environment and Homeostasis

Westernization of developing countries has been, correctly or not, deemed the culprit for the steady increase in diseases such as allergy, colonic cancer and inflammatory bowel diseases. Migrants from countries of low prevalence are even tending to take on the occurrence of their adopted country [3,4]. Which elements of the culture of underdeveloped countries that might represent a protective factor against various diseases common in “western” parts of the world is becoming a hot area for research.

Of all the vital organs in the body, the one that suffers the most abuse from modern dietary habits is the colon. Inflammatory bowel disease is one of the conditions on steady rise in Western Europe and North America [5]. Clearly, access to experienced medical care is likely to influence the variability between countries. However, changes in diagnostics cannot be the sole basis for the seen variation, given the fairly stable rates of disease in most areas. Environmental factors associated with bowel diseases include diet, smoking, microbial situation, drugs, stress, and socioeconomic status [6]. The strategy of identifying modifiable causes for disease has made some progress. Life-style variables along with genetic information thus have the potential to clarify causation of a variety of diseases.

When comparing cultures, diet is often the most apparent feature that differs. Semi-manufactured foods are a widespread way of time-saving by simplifying everyday life. Storage, shelf life, and taste can, by modern knowledge about compounds, be adjusted according to demand which introduces food supplements not there naturally. There is growing concern regarding the effects of food additives and their contribution to various diseases. It has been suggested that added inorganic, non-nutrient microparticles can act in concert with additional individual factors to influence intestinal permeability important for epithelial cell integrity and healing [7]. Increased hygiene associated with improved socioeconomical conditions is also believed to have global effects on the developing infantile immune system. The hygiene hypothesis imparts the message of caution against the westernized attitude toward microbes, stating that it leaves the immune system more naïve than it would be in underdeveloped countries [8,9]. This could in turn result in an increased susceptibility to inflammatory diseases.

1.2 Intestinal Biology

Function and disease of the gastrointestinal tract is a complex subject that demands both fundamental and specialized research in order to understand all its facets.

The intestinal ecosystem is composed of three closely interacting components: host cells, nutrients and microflora. The main function of the gastrointestinal tract is to provide the body with nutrients and energy for satisfactory metabolism. Uptake is however not as simple as absorption, but is closely linked to risk of invasion. Along with absorption, the intestine needs to selectively hamper uptake of harmful agents as well as microorganisms in the lumen. The barrier responsible for all this consists of a single layer of epithelial cells (Figure 1), the integrity of which is maintained even though it is shed every other day. Surface enterocytes are interconnected with tight junctions and overlain with mucus, and their preferred energy source is a short chain fatty acid called butyrate produced by the intestinal flora [10].

1.2.1 Small Intestine

The epithelium of the small intestine consists of many different cell types with specialized functions. The enterocytes are the absorptive cells which actively take up nutrients. The epithelium also has scattered mucus secreting cells, called goblet cells, which accumulate as you go further on down the intestine. Both enterocytes and goblet cells have a lifespan of 5-6 days. At the base of the protruding villi you find Paneth cells which release anti-bacterial factors, and also phagocytize some bacteria and protozoa. Paneth cells play a role in depressing the flora in the small intestine and have a lifespan

of about 4 weeks. Enteroendocrine cells, which are also present in the stomach, often occupy the lower part of the villi where they secrete products that stimulate secretion from the pancreas, and gall bladder contraction such as cholecystokinin. The base also holds undifferentiated stem cells that give rise to all other cell types in the epithelium. The complete mucosa, together with the epithelium of both the small intestine and colon, also includes the lamina propria where capillaries and lymph vessels along with lymphoid tissue are accommodated.

1.2.2 Colon

The classical functions of the large intestine are electrolyte absorption and storage of excrement. The colon has no villi as compared to the small intestine and produces no digestive enzymes. The mucosa consists of numerous straight, tubular crypts responsible for extraction of fluids, electrolytes and vitamins. The glands and the surface are lined with simple columnar epithelium whose cell types are as described for the small intestine. However, Paneth cells are usually absent in the adult colon and enteroendocrine cells are rare, while absorptive cells and goblet cells are abundant. Goblet cells are more prevalent in the crypts than along the surface, and their number increases distally toward the rectum. The mucus they generate facilitates the passage of

Figure 1.

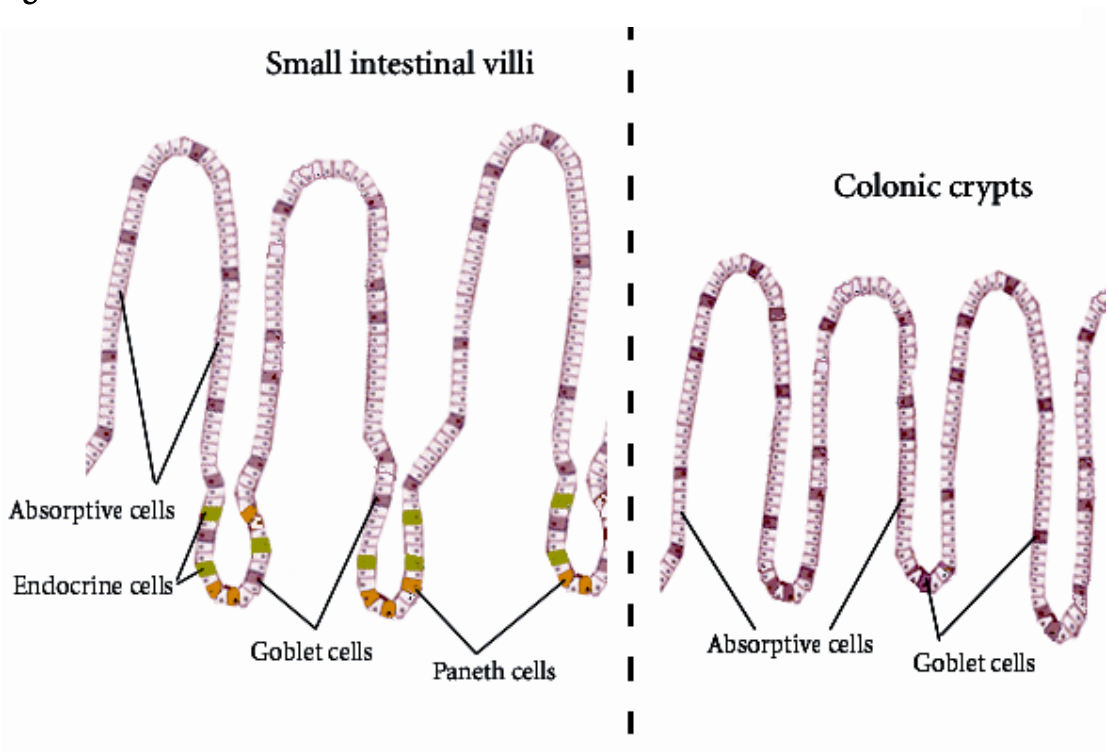

Figure 1. Schematic over small and large intestinal cell types and general wall structure of epithelium.

the increasingly solid colonic contents, and covers bacteria and particulate matter. As in the small intestine, undifferentiated cells are found at the base of the crypts.

1.3 Intestinal Disease

To protect the body, the intestine can mount an inflammatory reaction to deal with a potentially harmful invasion [11]. Enterocytes detect danger signals in the lumen by secreting defensins, chemokines, and cytokines alerting innate and adaptive immune systems [12,13]. The epithelium may therefore be viewed as the primary sensor of infection in the gut. Many pathogens have developed strategies to breach the epithelial barrier, but in the case of commensals, their crossing is largely restricted to sampling of luminal content through microfold cells or directly by dendritic cells. These dendritic cells then travel to mesenteric lymph nodes where they activate the adaptive immune system to secrete IgA antibodies which coat the luminal microbes to prevent them from violating the gut lining [14].

When the intestine interprets these signals incorrectly or overreacts to them inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and Ulcerative colitis (UC), but also milder food allergies such a celiac disease ensue. Causes for these types of ailments include immunological overreactivity where nature and nurture cooperate with several unknown triggering factors. Chronic inflammation in itself is a risk factor for developing cancers such as colorectal carcinoma [15].

Acid, bile, and pancreatic secretions hinder most bacterial colonization of the stomach and proximal small intestine. After these more bacterially sparse gastrointestinal parts, the density of bacteria increases dramatically, ending in colon where 60% of the fecal matter is of bacterial origin [16]. Since this is the intestinal niche that contains the most bacteria and discarded food agents, the colon is in particular need of ways to distinguish between adverse and innocuous signals from the luminal content. Many animal models have been instrumental in determining causative factors in IBD. Three genetically engineered models, T cell receptor α [17], Interleukin (IL) -2 [18] and IL-10 knockout mice [19] were independently found in 1993 to develop spontaneous colitis. Many transgenic and knockout models have since been added to this group. The IL-10 knockout mouse has educated us on the implications of bacterial presence, as they no longer develop disease under germ free conditions [20]. Other predisposed animals, such as dextran sodium sulphate (DSS) induced colitis subjects, no longer develop colorectal cancer when bacterial factors are excluded [21]. Other studies have gone deeper into the mechanisms of these inflammatory signals and have found a link between nuclear receptors and the type of response generated by infection [22]. It is however not as easy as blaming inflammatory mediators as recently shown by targeting

signaling by the transcription factor NF-κB [23]. Here they conclude that disruption of NF-κB activation causes an IBD-like phenotype, indicating that this pathway is crucial for maintenance of immune homeostasis in the gastrointestinal tract.

1.4 Intestinal Microbiota

A prominent feature of multicellular organisms and their microbiota is their well established co-evolved symbiotic relationship. Adult humans are actually composed of more prokaryotic than eukaryotic cells making the microflora a formidable force to be reckoned with [24]. Indigenous microbial communities are often referred to as "commensals", the implication of which is that these microbes have no discernable effect on the fitness of their host, which could however be a mere reflection of our lack of knowledge about their specific contributions. This ecosystem is by and large still considered a black box since the majority of the bacteria cannot be cultured.

During maturation of the host, the developing intestine can be affected by intestinal flora with a subsequent change in interaction with the nutrient environment. The microbiota participates a great deal in providing metabolic traits that we ourselves have not fully mastered. These traits include the ability to break down plant polysaccharides, promote conversion of conjugated bile acids, synthesize certain vitamins [25-27], and assist in absorption of calcium, magnesium and iron. If colonic bacteria are normal, vitamins B-1, B-2, B-12 and K are produced by them, and all with the possible exception of B-12 are absorbed and used by the body.

Germ free animals have provided a crucial experimental system to study in particular the epigenetic effects of microorganisms on gastrointestinal development. Studies in germ free mice have shown that bacteria regulate such diverse host responses as glycoconjugation, production of antimicrobial proteins, and intestinal angiogenesis [28]. An experimental germ free existence is very much compatible with life if certain vitamins are supplemented. The animals are, however, more susceptible to infection and display alteration in vascularity, digestive enzyme activity, muscle wall thickness, cytokine and immunoglobulin production [29]. One interesting finding is that chronic inflammation is abrogated if the animals in question are maintained germ free, supporting the notion of the fine balancing act that is mutualism [30].

Acquisition of our microbial nation begins at birth, before which the intestine is sterile [31]. In the initial colonization, the maternal flora is the most predominant followed by environmental microorganisms. The first colonizing bacteria are *Escherichia coli* and *Enterococcus* sp., quickly followed by the obligate anaerobes, represented by *Bifidobacterium* sp., together with *Bacteroides* sp. [32]. Depending on diet, the flora

then continues to evolve over the first 24 months of life before it is comparable to adulthood conditions even though further colonization is an ongoing process. The most common genera include the anaerobic *Bifidobacterium*, *Clostridium*, *Bacteroides*, and *Eubacterium*, and the aerobic *Escherichia*, *Enterococcus*, *Streptococcus*, and *Klebsiella* are also found.

Postnatal colonization of our intestine induces changes in gut epithelial homeostasis such as increases in cell proliferation, maturation including major shifts in surface expressed glycans [33-35]. The new flora educates our immune system, so we become tolerant of a wide variety of microbial immunodeterminants. When normal flora is lost, in the context of infection or antibiotic treatment, beneficial stimulation on GI mucosal development may be impaired along with innate and adaptive immune responses. It has been reported that this education may reduce allergic responses to food or environmental antigens [36]. Apart from effects on the immune system, indigenous microbes shape the development of the intestine's elaborate microvasculature [37]. When comparing germ free mice to conventionally raised mice, the complexity of the submucosal capillary network appears quite primitive. These findings illustrate that certain postnatal developments in mammals are consequences of host-flora co-evolution. Since the microbial population in the premature infant affects maturation and optimal function of the intestinal innate and adaptive immune system, a pathogenic role has been suggested in several diseases such as necrotizing enterocolitis (NEC), chronic lung diseases, and hematopoietic abnormalities. The small intestine of immature fetuses responds excessively to external inflammatory stimuli compared to the small intestine from infants and children [38]. With this in mind, the altered course of colonization as a result of premature birth or even breast-fed versus formula-fed infants should be considered very important. Human milk is an important factor in the initiation, development and, composition of microbiota in the healthy neonatal gut. Prematurely born babies have fewer episodes of late-onset sepsis, NEC, and diarrhea, and need less antibiotic therapy when fed their own mother's milk rather than formula [39].

The bacterial diversity in the gut is currently considered to consist of eight phyla, with members of the Gram-negative Bacteroidetes, and Gram-positive Firmicutes representing 60-80% of the total faecal community [40]. Alterations in gut microflora during intestinal disorders have been given some attention with interesting results. A reduction in Firmicute complexity has been seen in Crohn's disease patients compared to healthy controls [41]. Here they show that the main group affected is a major contributor to the pool of the aforementioned epithelial energy source, butyrate, in the gut.

1.5 Probiotics

In recent years people have begun to understand the benefits of a well composed intestinal flora and pro- and prebiotics are readily discussed. Probiotics are living microbial food ingredients beneficial to health beyond basic nutrition. Prebiotics in turn, are non-digestible to the host and modify the composition of the intestinal flora in a health-promoting fashion. The most common and researched species belong to genera Lactobacillus, Bifidobacterium and Saccharomyces, particularly *L. casei*, *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, *B. longum*, *B. breve*, *B. bifidum*, *S. cerevisiae boulardii* [42]. The mechanisms of action for probiotics differ between strains, but also depending on the surrounding flora and disease setting. With fermented milk products, the effects are known to depend on the strain used, whereas the metabolites produced by the fermentation process may also exert immunomodulatory activity, which further complicates the research. However, there are a number of common mechanisms evident for a variety of probiotics [43]. One such mechanism is prevention of colonization by pathogenic bacteria by competing for mucosal niches. Another mechanism of action is to promote anti-inflammatory mediators which could have a significant effect on the gut overreacting to external stimuli.

Probiotics have been suggested to be beneficial as part of IBD treatments possibly due to their immunomodulatory effects. Disease profiles for both DSS induced colitis and IL-10-/- mice have been alleviated by administration of Lactobacillic strains [44,45]. Clinically, a mixture of probiotics have been used to prolong remission in UC patients with promising results [46-49].

The rational for using prebiotics is to elevate numbers of beneficial endogenous bacterial strains. Inulin and oligosaccharides are commonly used to stimulate Lactobacilli and Bifidobacteria and/or decrease pathogen colonization. Another option to consider is a combination of pro- and prebiotics, so called synbiotics, where the prebiotic is present to boost the escorted probiotic.

1.6 Pattern Recognition Receptors

Microbial products are generally sensed through pattern recognition receptors (PRRs) and often activate signaling pathways that converge on NF-κB. There are two major PRR systems where nucleotide binding oligomerization domain 1 and 2 (NOD1/2) function intracellularly, while members of the Toll-like receptor (TLR) family reside on cellular membranes [50,51]. These receptors are used as a first line of defense against foreign challenge, whereas acquired immunity depends largely on production of an antigen-specific defense system providing specificity and memory.

Cytoplasmic PRRs consists of many different types of related molecules, out of which the NODs represents a group of well studied and yet enigmatic bacterial sensors. NOD2 is a general sensor for most bacteria, whilst NOD1 recognizes peptides from Gram negative bacteria. The sensing capabilities of NOD2 has been implicated in susceptibility for chronic inflammatory disorders such as Crohn's disease [52,53], thus relating bacterial components and development of disease.

All TLRs are type I intregral membrane glycoprotein receptors that recognize highly conserved microbial motifs called pathogen associated molecular patterns (PAMPs), from both pathogens and commensals. Working as homo- or heterdimers with other PRRs, they recognize a plethora of ligands such as lipopolysaccharide (LPS), bacterial flagellin, or CpG DNA, enabling the innate immune system to recognize nonself, and activate both innate and adaptive immune responses [54-56]. TLRs 1, 2, 4, 5 and 6 are located mainly on the cell surface and primarily recognize bacterial components, while TLRs 3, 7, 8 and 9 are located in endocytic compartments where they mainly sense viral products. Signals mediated through TLRs by commensals are essential for intestinal barrier function and repair [57].

The epithelium and resident immune cells are dependent upon commensals to provide molecules such as LPS, which interact with the TLRs. The resulting signaling enhances the ability of the epithelial cells to withstand injury while also priming the surface for repair responses [58]. Given the continuous presence of bacteria the signaling of the mucosal PRRs must be under strict control to avoid aberrant stimulation. Signals of both conciliation and danger need to be managed. Apart from differential expression levels, control is achieved by both negative feedback mechanisms and cross-interference with cytokine signaling pathways such as that of IL-1 [50,59]. Prolonged exposure to bacterial products induces tolerance in the form of hyporesponsiveness by for example down-regulating the surface expression of TLRs [60]. However, down-regulation is not always the answer since signaling through some TLRs is actually needed to stop inappropriate epithelial behavior. TLR-4 dependent signaling has been shown to inhibit allergic hyperreactivity to food allergens [61], implicating it in an important balancing act between extremes in inflammatory cytokine production. Whether TLRs are the primary cause for intestinally originating inflammations or not, they represent an important area of research for further understanding the balancing act of bacterial co-existence.

1.7 Nuclear Receptors

The mammalian nuclear receptors (NRs) are a family of transcription factors of approximately 50 members, including receptors for steroid hormones, thyroid hormone, vitamin D, and retinoic acid as well a group of orphan receptors [62,63]. NRs are implicated in nearly every aspect of vertebrate development and adult physiology, and alterations in their structure, function and expression, as well as altered environmental stimuli can give rise to pathogenic conditions such as obesity, insulin resistance, type 2 diabetes, and atherosclerosis [64]. Figure 2 highlights NRs important in the gastrointestinal tract, capitalizing on their general function.

Figure 2.

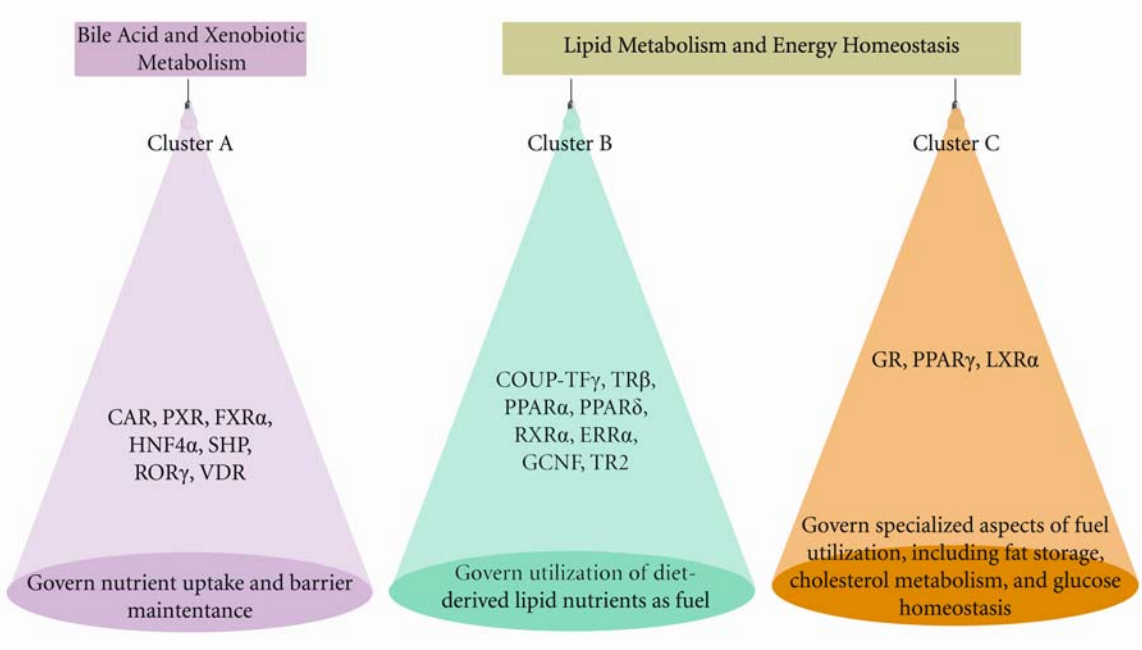

Figure 2. Nuclear receptors important in gastrointestinally relevant tissues clustered according to general function [2].

Nuclear receptors bind to the promoter of a target gene, where they interact with the basal transcription machinery, recruit co-activators, and maintain a pre-initiation complex in order to drive transcription. Nuclear receptors can be subdivided into classes based on their ligand- and DNA-binding properties [65,66]. The steroid and thyroid hormone receptors make up the first and more classical group of receptors, which is also the most extensively characterized class. In absence of ligand these are sequestered in complexes with heat shock proteins and/or co-repressors and thus kept transcriptionally inactive. The second group is comprised of so called adopted orphan receptors where former orphans have been conferred a ligand and physiological role. These are able to bind to their cognate DNA sites even in absence of ligand and often form heterodimers with other member of this class. The third subclass consists of

orphan receptors, which have not yet been linked to a specific ligand, or function in a ligand-independent manner.

Nearly all members the NR family contain two activation domains separated by a DNA binding domain (Figure 3), thus conveying both ligand-independent and ligand-dependent activation functions, AF-1 and AF-2 respectively [67-69]. The DNA binding domain (DBD) is often highly conserved, mediating sequence specific

Figure 3.

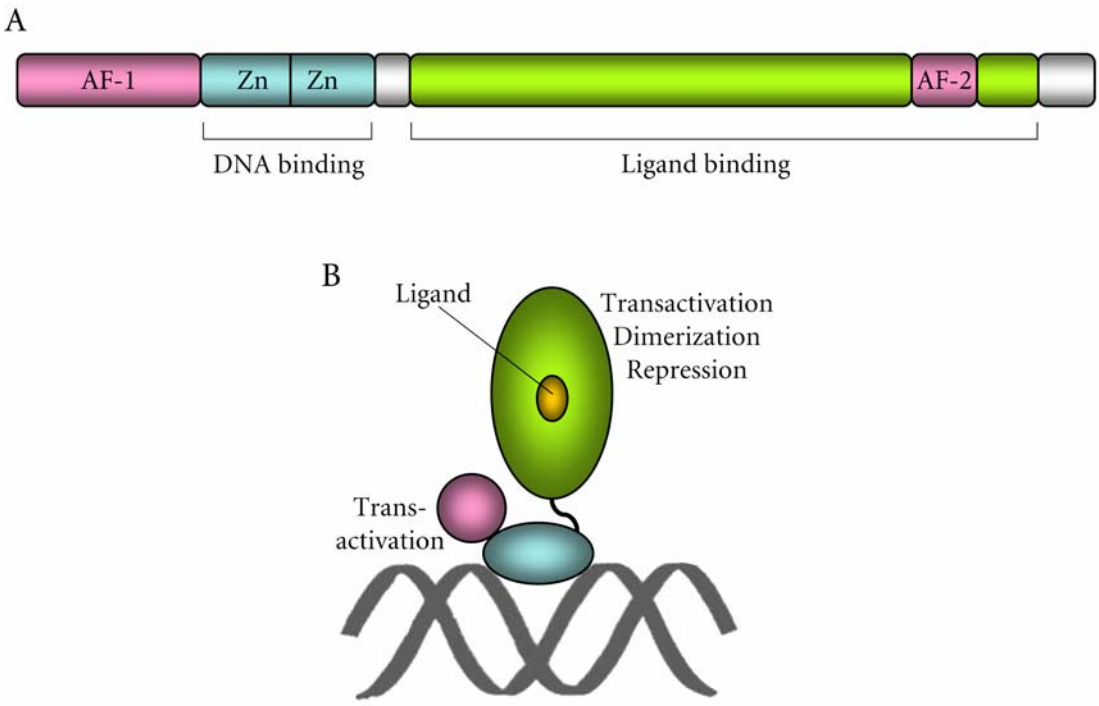

Figure 3. Domains of nuclear receptors. A) Nuclear receptor domain structure is comprised of an amino-terminal activation domain (AF-1) followed by a central zinc finger based DNA binding domain and a carboxy-terminal ligand binding domain including a second activation domain (AF-2). B) When in tertiary structure the domains of nuclear receptors gain functional characteristics such as transactivation and/or repression functions along with ability to bind ligands and other proteins.

recognition of target genes. The DBD mediates specificity of mono-, homo- or heterodimeric NRs by binding to response elements in enhancer or promoter regions of targets. Most of these response elements contain two or more closely spaced core recognition motifs, each coming in contact with a DBD. However specific the binding might seem, there is a surprisingly high level of promiscuity between different receptors. Even though many receptors share the same response element selectivity is achieved from relative orientation as well as spacing of the element. If the optimal orientation and spacing is not present a receptor may still bind but then instead acts as a repressor on that spot [70].

The amino terminus (AF-1) is poorly conserved among different NRs and conveys ligand independent action, while the C-terminal domain determines specific ligand binding properties of each receptor and overlaps the second activation function (AF-2). Hormone recognition in the ligand binding domain (LBD) ensures selectivity of a physiological response. In addition to binding ligand, the LBD integrates transcriptional activation or repression with a role in dimerization through leucine-rich sequences which form coil-coil interactions [71]. Presence of ligands represents one of the most important determinants of activity. Working as signaling components of both external and endogenous origin, ligands are as diverse as the LBDs recognizing them. Whether the receptor in question requires ligands to be very small or allows for larger and more complex molecules, they commonly share a non-polar character matching the interior of most ligand binding pockets.

Nuclear receptors do not only activate target genes but are equally proficient in repressing expression [72]. Passive repression refers to competition either for DNA binding or for dimerization partners which results in steric hindrance. Inactive heterodimer formation has been documented. Active silencing on the other hand is when an unliganded receptor either affects transcriptional initiation or recruits an array of factors with the function of creating an environment incompatible with pre-initiation complex assembly – called transrepression. Ligand dependent repression is poorly understood but seems to stem from inhibition of other signal dependent transcription factors, an activity referred to as ligand dependent transrepression.

1.7.1 Nuclear Receptor Co-regulators

Transcriptional regulation by NRs is a flowing multistep process, in which different factors have temporally and spatially distinct functions at promoters. NR co-regulators can roughly be divided into co-activators and co-repressors. Co-activators can be defined as molecules directly recruited by NRs to enhance gene expression. Co-activators can be subdivided into primary and secondary molecules, where the secondary are a group of activators that do not directly contact the NRs but rather contribute by being a constituent of multisubunit co-activator complexes. Co-repressors act in an opposite manner primarily through interaction with unliganded NRs.

Table 1. Nuclear receptor co-regulators

Cofactor	Comments	Ref.
CBP	Interacts with and co-activates multiple activators including NRs	[73,74]
HMG-1	Specific for steroid receptors	[75]
NCoR	Interacts with and co-represses unliganded TRα, RARα and COUP-TF1, RevErb and DAX-1	[76-79]
NSD-1	Interacts with both unliganded and liganded NR LBD	[80]
p300	Functionally similar to CBP, interacts with and co-activates NRs	[81]
PBP/TRIP2	Binds to RARα, RXR and TRβ1 and co-activates PPARγ, originally isolated as a TR-binding protein	[82,83]
PGC-1	Interacts with PPARγ ligand-independently, induced at low temperatures	[84]
RIP-140	Interacts with and co-activates ER, co-repressor for TR2	[85,86]
SRA	Functions as RNA transcript, co-activates AF-1 of steroid receptors	[87]
SMRT	Interacts with and co-represses unliganded TR and RAR, similar to NCoR	[88,89]
SRC-1	Interacts with and co-activates NRs, contacts basal transcription factors	[90,91]
TIF-1α	Interacts with and co-activates RXR and RAR AF-2, interacts with factors related to chromatin modifying proteins	[92,93]
TRAP/DRIP	Interacts with liganded TR and VDR	[94,95]
TSC-2/Tuberin	Interacts with RXR, co-activates PPARγ and VDR	[96]

The structural shift that takes place upon ligand binding enables attachment of co-activator proteins to the LXXLL interaction motif in NRs, by recognition through a so called NR box [65]. The sequence divergence around individual NR boxes might determine their binding affinity for the AF-2 ligand-induced hydrophobic groove of NRs. The recruitment of co-activator complexes allows for modification of chromatin structure and facilitates assembly of the general transcriptional machinery at the promoter. Co-regulators are often organized into preformed complexes, which facilitates assembly into multiple configurations and make them readily available to competing pools of activators and promoters. Relative expression of co-activators and –repressors is also an important determinant of an appropriately graded response to ligand.

1.7.2 Peroxisome Proliferator Activated Receptors

The peroxisome is a ubiquitous organelle that participates in fatty acid metabolism and rids the cell of toxic peroxide by using it as an oxidizing agent. Peroxisomes are very dynamic and can replicate upon stimuli. The peroxisome proliferator activated receptor (PPAR) family of nuclear receptors, consisting of three members (PPARα/δ/γ), were cloned in the early 1990s [97], starting with recognizing PPARα as

the protein binding agents responsible for peroxisome proliferation [98]. Despite their name however, neither PPARδ nor PPARγ responds to peroxisome proliferators very well.

PPARs control many different target genes involved in both lipid and glucose homeostasis, which have identified the PPARs as master regulators of lipid and carbohydrate metabolism [99]. By forming heterodimers with the retinoid X receptor (RXR), PPARα/δ/γ regulates transcription of many genes in a ligand-dependent manner. The typical response element for PPARs is a direct repeat of the core sequence AGGTCA separated by one or two nucleotides.

In contrast to other NRs, the PPARs are quite promiscuous in their binding of ligand and accommodate quite differently structured ligands, while still keeping a fair amount of specificity between the three by ligand interacting residues inside the cavities [100]. PPAR-RXR complexes can be activated by ligand for either receptor, with simultaneous binding being the most efficient. PPARα binds unsaturated fatty acids, albeit with a lower affinity than PPARγ, along with arachidonic acid metabolites and synthetic fibrates. PPARδ binds both saturated and unsaturated fatty acids as well as the prostanoid prostacyclin. Examples of natural ligands for PPARγ are unsaturated fatty acids, arachidonic acid, and a prostanoid called 15-deoxy-δ-12,14-prostagladin J2. Synthetic ligands include members of the thiazolidinedione (TZD) family of anti-diabetic compounds [101].

PPARα is highly expressed in hepatocytes, cadiomyocytes, skeletal muscle, and enterocytes, where its activation favors fatty acid catabolism. PPARδ is ubiquitously expressed, often at a higher level than the other two PPARs. Functionally, PPARδ is a bit of an enigma, but knock-out mice indicate action in lipid metabolism, skin, and small intestine [102], transgenic mice have also shown PPARδ to have a considerable fat burning capacity [103]. PPARγ is found to be highly expressed in white and brown adipose tissue, immune cells, colonic mucosa, and placenta.

1.7.3 Roles of PPARγ in Physiology

PPARγ can be found as two separate isoforms (γ1 and γ2), where the longer one has 28 additional amino acids resulting in a lengthening of its AF-1 domain, and is virtually only expressed in adipocytes while the rest of the PPARγ sites of expression are in the form of PPARγ1. PPARγ is well known as one of the master genes driving differentiation programs during adipogensis [104,105]. It not only drives maturation but is also a pivotal coordinator of fat uptake and storage by regulating genes such at fatty acid binding proteins and lipoprotein lipase.

PPARγ plays a vital role in stimulating release of fatty acids from triglycerides, facilitating intracellular fatty acid transport, and fatty acid esterification [106]. Because of its large and quite open LBD, PPARγ is often viewed as a generic sensor of fatty acid fluxes, without a particular optimal ligand.

Type 2 diabetes is considered a disorder of fatty acid metabolism and adipokine production [107]. Obesity related insulin resistance involves release of mediators such as free fatty acids, tumor necrosis factor (TNF) α, or the adipokine resistin from adipocytes, all of which impair insulin action on skeletal muscle. An obese state will impair the normal adipokine mediated crosstalk between adipose tissue and other organs, and unless excess triglycerides are properly taken care of by adipocytes, lipid deposition will occur in liver and muscle, impairing their normal function which can lead to insulin resistance [108]. A role for PPARγ in type 2 diabetes has been inferred by the efficacy of TZD ligands in ameliorating insulin resistance [109,110]. TZDs lower hyperglycemia, hyperinsulinemia, and hypertriglyceridemia as well as increase high density lipoproteins (HDL) by enhancing tissue sensitivity to insulin. This can, however, result in long term adverse effects, especially in combination with high caloric intake, in the form of weight gain. The sites of action for the TZDs have been studied with the help of mice lacking white adipose tissue, and there it has been shown that insulin and glucose levels cannot be reduced in absence of this tissue [111]. However, the high triglyceride levels could still be affected by TZDs even without presence of white fat, indicating additional tissue targets of these anti-diabetic drugs.

PPARγ ligands inhibit production of an array of different inflammatory cytokines depending on cell type either by transcription factor competition or direct transcription. In monocytes they inhibit proinflammatory molecules such as TNFα, IL-1β and IL-6 [112], while in macrophages affecting inducible nitric oxide synthase, matrix metallopeptidase 9, and scavenger receptor 1 [113]. In induced colitis studies one has also reported that PPARγ action has beneficial effects on disease progression [114]. Furthermore, the anti-inflammatory properties of PPARγ have been linked to its anti-atherosclerotic effects where both PPARγ and RXR ligands have beneficial outcomes on the disease. Although activation of PPARγ induces the fatty acid transporter CD36, recent work shows that it also causes lipid efflux by increasing levels of ATP binding cassette protein A1 in concert with LXRα [115].

PPARγ also has anti-proliferative actions which have been implemented in different types of malignancy. Impediment of proliferation due to PPARγ seems to be true for

liposarcomas [116], but also for colon cancer where PPARγ activation results in a more mature, less malignant phenotype [117].

2. AIMS OF THESIS

The objective of this thesis was to characterize the colonic expression of nuclear receptors in general and functional properties of PPARγ in colonocytes in particular, all with the ultimate goal of elucidating important players in the regulation of local gut homeostasis. The importance of intestinal flora in achieving a balanced biological setting through NRs was also to be addressed.

Specific aims for each paper:

I. Through the use of germ free mice address whether NRs can be regulated by bacteria in colonocytes, and if these differences vary depending on exposure to commensals or pathogens.

II. Investigate how ligand induced phosphorylation of PPARγ1 regulates its transcriptional activity and address possible pathways of host-microbe interactions involved in protein modification.

III. Assess the PPAR target gene Fasting-induced adipose factor (FIAF) as a function of probiotic regulated weight control.

3. METHODOLOGICAL HIGHLIGHTS

3.1 Germ Free Mice

We have had the opportunity to utilize germ free mice in our investigations of host-microbe interactions. The concept of the germ free animal is attributed to Pasteur even though he hypothesized that a bacteria-free existence would not be compatible with life. Today the rearing of germ free animals is a standardized antiseptic procedure with specialized equipment. Germ free animals are obtained by germicide treated eggs, in the case of birds, and by cesarean section, in the case of mammals. Cesareans are performed on pregnant females at term, and the intact amniotic sac is passed through an antiseptic into a clean isolator. The pups are subsequently delivered, resuscitated and placed with a germ free foster mother that has a newly delivered litter of her own. The drawbacks of this approach include the precarious decision of timing regarding the Caesarian section as well as the acceptance by the foster mother. Because of our bacteria laden environment, it is fairly easy to contaminate germ free animals. Meticulous protocols and experimental controls are required to make sure contamination is not a factor influencing phenotypes. Germ free animals also need dietary supplements, such as vitamins B and K, as these will be lost without bacterial presence.

Depending on the animal facility, availability of different animals and strains provides the researcher with tools to determine the effect of a single, or a combination, of bacteria on whole animal physiology. Germ free animals colonized purposefully with a known microflora is called gnotobiotic animals, meaning "known life". The germfree system provides unique experimental advantages for studies in which microbial flora might have a modifying influence on the health of the host.

3.2 Expression Analysis

The TaqMan Low-Density Array (TLDA; Applied Biosystems) is a micro fluidic card designed to simultaneously run between 12 and 384 real time PCR reactions with a single sample loading (Figure 4). Regular quantitative or semi-quantitative, as with the usage of SYBR Green, real time PCR is accurate enough but does not really translate efficiently to large expression profiles, such as validation of microarray data. The TLDA card is a high throughput approach that will

Figure 4

Figure 4. Loading of TLDA card [1].

generate large expressional profiles with high reproducibility.

The arrays, where the assays have been pre-loaded in the wells, can be custom made by choosing from the inventoried gene list or ordered as pre-composed cards with different specializations. We have employed these cards for the generation of the large data sets while, the smaller ones and subsequent validation have been performed using regular SYBR Green based qPCR.

4. RESULTS AND DISCUSSION

This thesis is based upon three perspectives of microbe-host interactions with special emphasis on nuclear receptors such as the PPARs. The first paper deals with the impact that normal flora has on NR expression in the gut, implying a broad microbe-host cross-talk potentially necessary for proper colonic development. The second paper explores bacterial points for regulation of NRs, and identifies phosphorylation of PPARγ as a way of altering its transcriptional potential. By affecting expression and/or activity of nuclear receptors in the colon one might envisage bacterial content to be a player in regulation of gastrointestinally related organs as well as metabolically important tissues such as muscles and fat. The third and last paper is based on this extended view of bacterial signals. Here it is shown that presence of probiotic bacteria can influence expression of metabolically active factors, potentially through PPARs, and thus affect lipid homeostasis.

4.1 Paper I: Gut Flora, Toll-Like Receptors and Nuclear Receptors: A Tripartite Communication that Tunes Innate Immunity in Large Intestine

Considering that the amount of bacteria present in our body exceeds our own human cells in number, it is difficult to resolve whether the bacteria are subordinated to our existence or vice versa. The intestine is however known for its exclusivity even though the sheer numbers of species can seem daunting. Despite differences in early colonizers depending on environment, the adult intestine is, as mentioned before, really only dominated by two divisions of bacteria: the Bacteroidetes and the Firmicutes. It would seem that the intestine has strict membership clauses even though it is bombarded by new applicants every day. It is conceivable that this is a part of a survival strategy brought about by co-evolution, where some functions of life can be outsourced to the ever present bacteria for the price of providing provisions and accommodation. With this in mind one can also imagine that it is not only metabolic functions that have been subcontracted but also certain base stages of development. As seen in germ free animals, the biological system is quite capable of coping with life without bacteria, even though aspects of tissue maturation have not been fulfilled [118]. Except for small clues, there still remains a paucity of information on bacterial influence on host physiology, and even less information on how this influence is mediated.

In this paper, we have monitored the effects of bacterial presence on the nuclear receptor family of transcription factors. The diversity of NRs links this group of receptors to virtually all aspects of life, and bacterial manipulation of these would help explain how flora affects host properties.

Especially five of the 49 tested NRs show an inherent difference in expression pattern between germ free and specific pathogen free (SPF) control mice. Intestinal microbiota seems to elevate GCNF and Nur77, while reducing the expression of CAR, LXRα and RORγ, the implications of which are yet to be determined. Functional repercussions in diverse facets of physiology, such as metabolic, immunological and maturation processes can be inferred by alterations of these particular NRs. In view of developmental consequences, GCNF in particular conveys interesting signals to the gastrointestinal tract. GCNF is highly influential during embryogenesis and has been shown to be a powerful agent of differentiation in germ and neuronal cells [119]. Elevated differentiation seems to fit well with the intestinal alterations inherent to colonization, since the germ free gut is less mature. Being a transcriptional repressor, GCNF also has a noteworthy role in inhibiting the action of other NRs such as ERRα, which would squelch inflammatory signaling pathways [120,121]. We performed a small comparative study on 12 CD, 14 UC patients and 11 healthy controls where these five NRs were investigated. Only GCNF showed a significant differential expression pattern, as it was downregulated in both UC and CD patients compared to controls (Figure 5). This could be a reflection of the chronic inflammatory state where the anti-

Figure 5

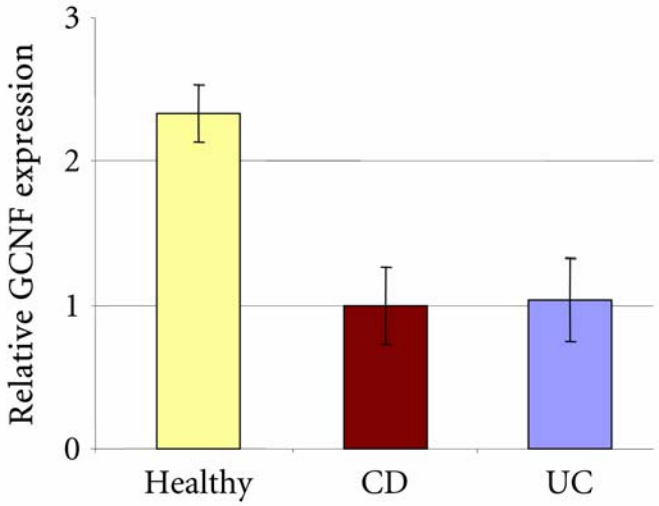

Figure 5. GCNF shows altered expression in IBD. Out of the five bacterially regulated NRs, GCNF levels are significantly lower in human Crohn's disease (CD) and Ulcerative colitis (UC) patients compared to healthy controls.

inflammatory properties of GCNF are subdued, or it might represent a targeted drop in differentiation to quench the need for a constant flow of new cells to replace the ones lost to the detrimental inflammation.

We go on to show that the naïve germ free gut is not practiced in the art of inflammation and thus responds very differently to pathogenic infection compared to conventional animals. Since two of our original five NRs, Nur77 and RORγ, have clear ties to immune cells, it is tempting to speculate on the topic of their importance in gut "immunoeducation". Nur77 has been attributed pro-apoptotic signaling in colon cancer cells, but is mostly mentioned in relation to T cell receptor signaling, where it is responsible for selection and elimination of autoreactive populations [122,123]. RORγ activation on the other hand, results in inhibition of T cell receptor induced proliferation and cell death by affecting IL-2 and Fas ligand expression [124]. Based on this information these two NRs could act in concert to educate immune cells, but also play an important role in the colonic mucosa. The elevation of Nur77 could be explained by the need for controlled cell death in a properly functioning mucosal layer since it has a higher rate of cell proliferation and cell number than epithelial linings which have not encountered bacteria. The decrease in RORγ could allow for proper expansion and function of immune cells through epithelial secreted factors.

CAR and LXRα are primarily found in liver and are responsible for metabolic processes such as elimination of xenobiotics and cholesterol metabolism. We show that LXRα is downregulated in the presence of bacteria. A possible link between bacterial signals and LXRα has been made prior to our study, where its downstream targets are shown to be negatively affected by TLR-3 and TLR-4 signaling [125]. It has also been shown that LXR activation represses cholesterol absorption [126], which would suggest that a metabolic switch has taken place in the SPF mice, where cholesterol absorption is increased due to low levels of LXRα.

The main organ of expression for CAR is the liver, but since it acts as a xenosensor to protect from exogenous insults, its presence in the intestine is reasonable. Given the large amount of new metabolites present in a colonized intestine it might seem odd that CAR is in fact downregulated in this setting compared to germ free. We also found a possible link between TLR-2 signaling and CAR expression which would imply that this NR is under bacterial regulation. This can be interpreted as a placating property of the healthy commensal setting on xenosensing to ensure low overreactivity. This represents yet another route of developmental stimuli orchestrated by the resident flora.

Our study mainly focuses on expressional differences while the posttranslational and/or stimulatory differences are unfortunately not within the scope of this paper. There are however some interesting connections between NRs and TLRs which involve general signaling pathways. One such pathway is the phosphatidylinositol 3-OH kinase (PI-3K) which can be manipulated to lessen LPS stimulated inflammatory targets [127,128]. PI-

3K is a part of signaling pathways often shared and affected by NRs. This pathway is also underscored in Paper II of this thesis where its action has a hand in the regulation of NR transcriptional activity, exemplified by PPARγ.

4.2 Paper II: Enterococcus Faecalis from Newborn Babies Regulate Endogenous PPARγ Activity and Interleukin-10 Levels in Colonic Epithelial Cells

Our microflora is an organ in itself, representing a greatly efficient and stable bioreactor. Functional redundancy between bacteria keeps the stability constant, if not disturbed by highly pathogenic species or antibiotics. Proper colonization early in life is crucial for further development and aids in laying the ground work for future stability. Bacterial legacies are hard to pin down, both due to redundancy between species, and also because of the intricate network of the host signaling pathways. Understanding these networks will help us grasp the microbial bestowals shaping our physiology.

The human intestine is the equivalent of a chemostat where exogenous complex carbohydrates and endogenous glycoproteins together create a rich culture medium where fermentation to produce short chain fatty acids takes place. This fermentation is a very important source of calories and carbon in other animals [129] and probably figured prominently in human nutrition when we were less well fed. Through the production of these short chain fatty acids, resident bacteria can positively influence not only metabolic effects, but also intestinal epithelial cell differentiation and proliferation [130]. We often focus on NRs as the mediators for these effects, as they readily bind fatty acid derived ligands. Mechanistically, these processes are still poorly understood, and to truly know these pathways one needs to delve into the complex nature of intracellular signaling.

Here we focus on the phosphorylation aspect of signaling, and try to establish this as a potential transcriptional modulation pathway for bacterial influence. Phosphorylation of NRs and other transcription factors is a well known mode of altering activity. Post-translational modifications are very important in regulation of NRs, each with their own implications. In the field of PPARγ there is a clear schism of whether phosphorylation connotes positive or negative effects of transcriptional activity [131-134]. Differences in results might be explained, while speculatively, by divergent methodology, cell type, or genetic predisposition. This chapter is long from closed and we have tried to further our knowledge by including specific ligand work together with bacterial data, to prove our stand-point of highly active phospho-PPARγ. We show that a ligand dependent phosphorylation of PPARγ correlates to increased activity in the

form of target gene expression. We show this relationship in a colonic epithelial cell line, but have obtained similar results from a macrophage lineage as well. This suggests that the seen effect is not cell type specific but could rather be a general mechanism of activation (Figure 6).

Figure 6

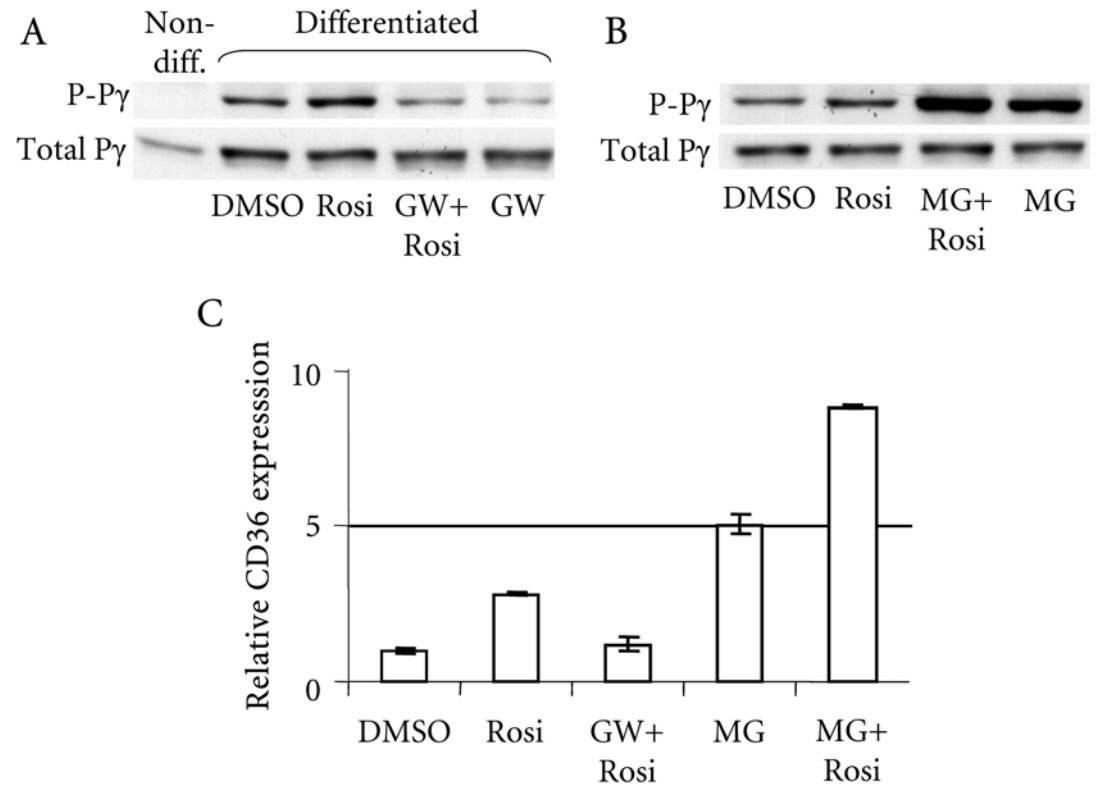

Figure 6. Role of PPARγ phosphorylation in macrophages. A) Western blot showing effects of Rosiglitazone (Rosi) and GW9662 (GW) on phospho-PPARγ1 (P-Pγ1). B) Accumulation of phospho-PPARγ1 in presence of the proteasomal inhibitor MG-132 (MG). C) Real time PCR of CD36 under the influence of Rosiglitazone, GW9662, and MG-132.

We go on to demonstrate that bacterial presence, in the form of *E. faecalis*, has the same effect on PPARγ function as ligand. The *Enterococci* strains we are using are early colonizers isolated from infants, and could potentially be important in gut development knowing the importance of early interplay as stated previously.

The reported inconsistencies on phosphorylation effects could be a consequence of differences in addressing proteasomal degradation. Phosphorylation is intimately coupled to ubiquitination and degradation, and could thus signify a mere means to an end where the protein is decommissioned, as seen for other NRs [135,136]. In accordance with this premise we show that this modification is coupled to proteasomal degradation and increases in amount when this pathway has been obstructed. We propose a model where phosphorylation instigates increased transcriptional activity,

which in turn is kept under strict control by degradation systems as not to disturb the cellular balance. We also allude to a possible signaling pathway for this particular posttranslational modification. It would seem that the PI-3K pathway is a crucial part of this phosphorylation event as inhibition results in ablation of receptor activation in response to bacteria.

These interconnected networks set the stage for controlled manipulation of the colonic mucosa by commensal bacteria such as *E. faecalis*, where differentiation and anti-inflammatory expression programs, such as IL-10, may be initiated. Here we also show the targets ADRP and FIAF which are linked to fatty acid transport and metabolism. Both can be conceived as part of a developmental program important early in life when a metabolic switch is made upon birth.

Probiotic bacteria have been shown to attenuate TNFα mediated induction of NF-κB activation by inhibiting the proteasome [137]. The bacteria are thus able to regulate epithelial cell responses to both increase cytoprotection and decrease inflammation. In said paper the focus is on proteasomal influences on the NF-κB pathway, while it is highly likely that it would affect a number of other signaling cascades as well. That these probiotic influences could be mediated through PPARγ and its phosphorylation is a very appealing concept. Anti-inflammatory consequences due to phospho-PPARγ, such as upregulation of IL-10 expression, would be a natural synthesis between our data and theirs.

A continuation of bacterial activation of PPARγ can be seen in Paper III where also probiotics are featured.

4.3 Paper III: Lactobacilli Targets Fat Storage by Activating Fasting Induced Adipose Factor (FIAF)

Microbial ability to degrade dietary constituents is well established, while impact of bacterial metabolism on energy balance of the host is granted less focus. Given the diversity of responses that can be mounted by the gut epithelium, alteration of microflora through probiotics could represent a possible route of total body manipulation. Combined efforts have however established that ingested probiotic strains generally do not persist as members of the normal microbiota beyond the dosing period [138,139]. Although permanent microbiota alteration may not be possible, association of probiotics to host cells or their release of relevant factors might be sufficient to trigger important signaling cascades.

The metabolic syndrome (also known as Syndrome X) is a constellation of metabolic abnormalities that includes glucose intolerance, insulin resistance, abdominal adiposity,

dyslipidemia, and hypertension and is often linked to cardiovascular disease and type 2 diabetes. The metabolic syndrome is often initiated by an excess of weight which is coupled to low grade inflammation. Insulin resistance and cardiovascular disease seem to share mechanistic traits in the form of macrophage derived cell types and their actions on adipose tissue and atherosclerotic plaques. Diet induced obesity leads to recruitment of macrophages to white adipose tissue and an increase in inflammatory mediators [140]. The importance of inflammation in atherosclerosis is highly respected, and the role of macrophages in lipid rich plaques has been given a lot of attention. The precursors of foam cells are attracted to plaques by the same chemokine (CCL2/MCP-1) that attracts macrophages to adipose tissue during obesity [141,142]. Interestingly, the receptor for this CCL2 is downregulated by PPARγ when activated by oxidized LDL [143]. Signaling circuitry involved in such complex diseases is naturally quite intricate and does not involve any one pathway or transcription factor. The PPARs are however a favorite group of mediators tied to metabolic control and have been known to reverse many aspects of the metabolic syndrome. One pathway intimately associated with type 2 diabetes is insulin and the body's pathogenic resistance to it. PPARγ ligands are, as stated before, known for their anti-diabetic properties and reversal of insulin resistance. The promising effects of PPAR ligands do however not tell the whole story since they in practice have mixed effects on metabolic endpoints.

The angiopoietin-like protein 4 (ANGPTL4), also known as hepatic fibrinogen/angiopoietin-related protein (HFARP), PPARγ angiopoietin-related gene (PGAR), or fasting induced adipose factor (FIAF) is a circulating plasma protein upregulated during fasting by PPAR agonists, involved in regulating glucose homeostasis and lipid metabolism through its inhibition of lipoprotein lipase (LPL) [144,145]. LPL hydrolyzes circulating very low density lipoproteins (VLDL) and chylomicrons, generating fatty acids for energy through beta oxidation or alternatively for storage and presumed future use. This means that an inhibition of LPL would result in decreased uptake of fat into storage facilities such as adipocytes.

It has been shown that FIAF is a player in the microbially related propensity for obesity, as normal gut flora is suggested to downregulate its expression [146,147]. Mice devoid of FIAF, display a reduced expression of peroxisomal proliferator activated receptor co-activator (PGC-1α) and enzymes involved in fatty acid oxidation. The GF state is believed to exhibit higher levels of FIAF and an accompanied lean phenotype even when exposed to Western type diets.

We show that the probiotic *Lactobacillus* F19, in contrast to previously published whole flora, induces FIAF expression in colonic cells. It seems to do so through a secreted lipophilic factor possibly affecting PPAR gene transcription programs. Systemically, F19

colonization results in elevated levels of circulating FIAF, the effect of which can be seen on lipoprotein content in serum as a consequence of LPL inhibition. Storage of fat is thus circumvented by less uptake of lipids to adipocytes. Homeostatic changes such as these do of course have their own set of consequences. Increased levels of the lower density lipoproteins are associated with disorders of the metabolic syndrome and could lead to pro-atherogenic lipid oxidation. On the other hand, studies have shown that LPL derived from macrophages is pro-atherogenic in the arterial wall [148], in which case a drop brought about by FIAF would be highly favorable. This stresses the importance of molecular origin and cell type specific actions.

PPARγ activation modulates gene expression leading to decreases in circulating glucose and triglycerides. Because of this, its synthetic ligands are used to treat type 2 diabetes where they reverse insulin resistance. The undesirable consequence of this is an increase in fat mass through promotion of triglyceride storage into adipose tissue, as well as generation of new adipocytes. Fibrates, sensed by PPARα, are used as pharmacological tools for reducing triglyceride levels and increasing the concentration of HDL cholesterol [149]. Both PPARα and PPARγ have also been known to directly induce expression of LPL [150,151]. This of course introduces the concept of direct and indirect effects brought about by NR activation (Figure 7). The direct effect of PPAR ligands allowed to act systemically will be quite different from their indirect effects where factors such as FIAF are the ones allocated to act on homeostatic parameters.

Figure 7

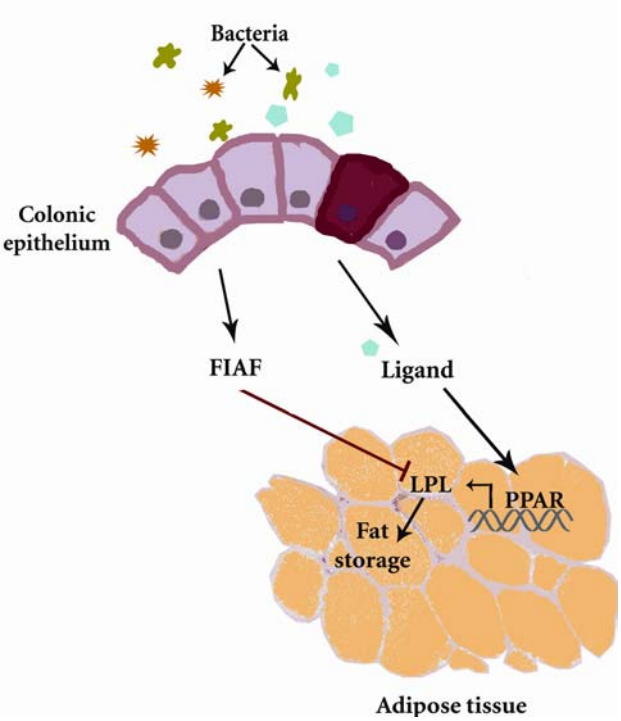

Figure 7. Simple representation of PPAR activation. Direct effects brought about by ligand recognition is here represented by the target gene LPL in adipose tissue. A secondary, or indirect, effect of PPAR activation in colonic epithelium is expression of FIAF, which subsequently can inhibit LPL action.

These potentially health promoting actions of F19 encourage further studies into it being used as a pro-active complement during weight loss. In addition to basic life style interventions, multi-strain probiotic combinations could be used to ensure successful weight loss, and it is therefore necessary to intensely investigate strain selection, dosing, and colonization issues. Mechanisms by which this Lactobacillus is able to accomplish this feat have not yet been elucidated. Since a link to PPARs have been shown one might envisage both ligand dependent and independent pathways. Whether the effect we are seeing is on account of a secreted factor acting as ligand or, as mentioned in Paper II, probiotic effect on proteasomal function tying directly to PPAR action as a downstream mediator, is not known but worthy of further investigation.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

One might view both PRRs and NRs as complementary parts of a bacterial sensing strategy. PRRs are responsible for recognition of their structural and physical properties such as components of cell wall and DNA, while NRs sense bacterial products for example in the form of secreted or conjugated molecules. Sensing bacterial presence is both a protective and developmental endeavor where pathogenesis is balanced against symbiosis. How this balance is achieved is of utmost interest to a vast array of disease settings.

Revealing NR mechanisms of conduct has widespread implications for our understanding of biological regulation. As future biomarkers, it might be possible to match NRs with different diseases based on the expression patterns of the receptor and its target gene profile in relevant cell types. Combinatorial therapy using NR agonists to work in synergy might represent an alternative, and potentially complementary, strategy to achieve desirable health effects while minimizing the adverse outcomes of overstimulating only one signaling pathway. Using chronic inflammatory diseases as an example, it is possible that anti-inflammatory actions of glucocorticoids could be achieved at lower doses with fewer side effects by a simultaneous administration of for example PPARγ ligand. Transrepression constitutes one mechanistic basis for the anti-inflammatory properties of PPARs. Ligand stimulation of PPARγ was shown to trigger its association with the co-repressor NCoR, thereby preventing dissociation of NCoR from the iNOS promoter and subsequent gene action [158]. The post-translational modification responsible for this PPARγ action was SUMOylation (conjugation of a small ubiquitin related modifier), as this alteration targeted the receptor for interaction with the co-repressor.

Taking inflammatory bowel disease into account, regulatory systems of host-microbe interactions are yet another important field of research as it impinges on the origin of disease. Here signaling pathways, as the ones discussed in this thesis, are at the hub of regulation and could represent tangible points of modulation. Degradation and activation coupled phosphorylation discussed for PPARγ in Paper II, is only one possible route of bacterial influence, but offers intellectually stimulating ramifications. The involvement of ubiquitin-protein conjugation enzymes for efficient transcription factor activation raises the intriguing possibility that clearance of factors from promoters enables sequential interfacing of activated NRs with different co-regulator complexes. While this thought adds to the expressional complexity of any given system,

it also offers an explanation to divergent results depending on cell type and transcriptional machinery. Further mapping of these differences will be instrumental for future composite knowledge.

The benefits of TZDs are attributed to direct effects on lipid metabolism in adipose tissue and to secondary effects on lipid and glucose metabolism in liver and skeletal muscle. PPAR involvement in atherosclerosis and insulin resistance is inferred by their lipid sensing roles central to starvation, feeding and inflammatory control. Macrophage infiltration is a prominent feature of these diseases, and both pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages are present in fatty streaks and adipose tissue. Activation of PPARγ is critically involved in macrophage polarization to the M2 phenotype in both adipose tissue and atherosclerotic lesions and improves insulin sensitivity. Conversely, selective deletion of PPARγ in macrophages increases insulin resistance and exacerbates atherosclerosis [115,159]. Together with our observations on PPARγ action in macrophages, these data provide evidence that tissue macrophages and their PPARγ is a potential therapeutic target for the treatment of both the metabolic syndrome and its related diseases.

Modern environment versus ancient genotype is postulated to expose inherent genetic imprints of "thriftiness" regarding syndromes such as cardiovascular disease and obesity [160]. Thrifty genes would be the ones that favor insulin resistance and fat storage during times of famine. This can be coupled to the symbiotic relationship developed with surrounding bacteria where their fermentation products provides us with about 10% of our daily caloric intake [161]. Our microbiota is well equipped to degrade and ferment luminal constituents, an ability which has been given some attention regarding health and disease. The relative proportion of Bacteroidetes and Firmicutes studied in obese patients, indicates that the presence of Bacteroidetes species is inversely correlated with the obese state [162]. Theoretically this would imply that Firmicutes, which generate more energy in the form of butyrate, get a larger playground and can thus produce more calories readily available to the host. It could also entail signaling brought about by an increased luminal content of butyrate. Butyrate is known for its effects on histone acetylation and could thus be influential in altering gene expression and function of epithelial cells [163]. Whether the short chain fatty acids are involved in the effects we see in both mice and cell lines is yet to be determined but they could represent at least a part of the explanation.

Inter-molecular relationships at the helm of homeostatic regulation show us the importance of a balanced co-existence. Similarly, symbiotic union between host and microbe dictates our wellbeing, confirming that happiness comes from within.

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7. REFERENCES

1. Http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_040127.pdf
2. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, et al. (2006) Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 126: 789-799.
3. Harding S, Rosato M (1999) Cancer incidence among first generation Scottish, Irish, West Indian and South Asian migrants living in England and Wales. Ethn Health 4: 83-92.
4. Montgomery SM, Morris DL, Pounder RE, Wakefield AJ (1999) Asian ethnic origin and the risk of inflammatory bowel disease. Eur J Gastroenterol Hepatol 11: 543-546.
5. Farrokhyar F, Swarbrick ET, Irvine EJ (2001) A critical review of epidemiological studies in inflammatory bowel disease. Scand J Gastroenterol 36: 2-15.
6. Danese S, Sans M, Fiocchi C (2004) Inflammatory bowel disease: the role of environmental factors. Autoimmun Rev 3: 394-400.
7. Lomer MC, Harvey RS, Evans SM, Thompson RP, Powell JJ (2001) Efficacy and tolerability of a low microparticle diet in a double blind, randomized, pilot study in Crohn's disease. Eur J Gastroenterol Hepatol 13: 101-106.
8. Adlerberth I, Lindberg E, Aberg N, Hesselmar B, Saalman R, et al. (2006) Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr Res 59: 96-101.
9. Gwee KA (2005) Irritable bowel syndrome in developing countries--a disorder of civilization or colonization? Neurogastroenterol Motil 17: 317-324.
10. Roediger WE (1980) Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21: 793-798.
11. Shibahara T, Wilcox JN, Couse T, Madara JL (2001) Characterization of epithelial chemoattractants for human intestinal intraepithelial lymphocytes. Gastroenterology 120: 60-70.
12. Stadnyk AW (2002) Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. Can J Gastroenterol 16: 241-246.
13. Takahashi A, Wada A, Ogushi K, Maeda K, Kawahara T, et al. (2001) Production of beta-defensin-2 by human colonic epithelial cells induced by Salmonella enteritidis flagella filament structural protein. FEBS Lett 508: 484-488.
14. Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303: 1662-1665.
15. Bernstein CN, Blanchard JF, Kliewer E, Wajda A (2001) Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer 91: 854-862.
16. Stephen AM, Cummings JH (1980) The microbial contribution to human faecal mass. J Med Microbiol 13: 45-56.
17. Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, et al. (1993) Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell 75: 274-282.
18. Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, et al. (1993) Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell 75: 253-261.
19. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75: 263-274.
20. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, et al. (1998) Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 66: 5224-5231.
21. Narushima S, Itoh K, Mitsuoka T, Nakayama H, Itoh T, et al. (1998) Effect of mouse intestinal bacteria on incidence of colorectal tumors induced by 1,2-dimethylhydrazine injection in gnotobiotic transgenic mice harboring human prototype c-Ha-ras genes. Exp Anim 47: 111-117.
22. Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, et al. (2005) Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell 122: 707-721.
23. Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, et al. (2007) Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 446: 557-561.
24. Hooper LV, Midtvedt T, Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr 22: 283-307.
25. Hill MJ (1997) Intestinal flora and endogenous vitamin synthesis. Eur J Cancer Prev 6 Suppl 1: S43-45.

26. Hylemon PB, Harder J (1998) Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems. FEMS Microbiol Rev 22: 475-488.
27. Salyers AA, West SE, Vercellotti JR, Wilkins TD (1977) Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. Appl Environ Microbiol 34: 529-533.
28. Hooper LV (2004) Bacterial contributions to mammalian gut development. Trends Microbiol 12: 129-134.
29. Smith K, McCoy KD, Macpherson AJ (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 19: 59-69.
30. Seksik P, Sokol H, Lepage P, Vasquez N, Manichanh C, et al. (2006) Review article: the role of bacteria in onset and perpetuation of inflammatory bowel disease. Aliment Pharmacol Ther 24 Suppl 3: 11-18.
31. Favier CF, Vaughan EE, De Vos WM, Akkermans AD (2002) Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol 68: 219-226.
32. Bourlioux P, Koletzko B, Guarner F, Braesco V (2003) The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine," held in Paris, June 14, 2002. Am J Clin Nutr 78: 675-683.
33. Rawls JF, Samuel BS, Gordon JI (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proc Natl Acad Sci U S A 101: 4596-4601.
34. Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI (1999) A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc Natl Acad Sci U S A 96: 9833-9838.
35. Bates JM, Mittge E, Kuhlman J, Baden KN, Cheesman SE, et al. (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev Biol 297: 374-386.
36. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, et al. (2002) Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 347: 869-877.
37. Stappenbeck TS, Hooper LV, Gordon JI (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci U S A 99: 15451-15455.
38. Nanthakumar NN, Fusunyan RD, Sanderson I, Walker WA (2000) Inflammation in the developing human intestine: A possible pathophysiologic contribution to necrotizing enterocolitis. Proc Natl Acad Sci U S A 97: 6043-6048.
39. Hylander MA, Strobino DM, Dhanireddy R (1998) Human milk feedings and infection among very low birth weight infants. Pediatrics 102: E38.
40. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. (2005) Diversity of the human intestinal microbial flora. Science 308: 1635-1638.
41. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, et al. (2006) Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55: 205-211.
42. Rastall RA, Gibson GR, Gill HS, Guarner F, Klaenhammer TR, et al. (2005) Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. FEMS Microbiol Ecol 52: 145-152.
43. Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361: 512-519.
44. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN (1999) Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 116: 1107-1114.
45. Matsumoto S, Hara T, Hori T, Mitsuyama K, Nagaoka M, et al. (2005) Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. Clin Exp Immunol 140: 417-426.
46. Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, et al. (2005) VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 100: 1539-1546.
47. Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, et al. (2005) Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 54: 242-249.
48. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT (1999) Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 354: 635-639.
49. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, et al. (1999) Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 13: 1103-1108.

50. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2: 675-680.
51. Harton JA, Linhoff MW, Zhang J, Ting JP (2002) Cutting edge: CATERPILLER: a large family of mammalian genes containing CARD, pyrin, nucleotide-binding, and leucine-rich repeat domains. J Immunol 169: 4088-4093.
52. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, et al. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411: 599-603.
53. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411: 603-606.
54. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, et al. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410: 1099-1103.
55. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, et al. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408: 740-745.
56. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282: 2085-2088.
57. Fukata M, Abreu MT (2007) TLR4 signalling in the intestine in health and disease. Biochem Soc Trans 35: 1473-1478.
58. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118: 229-241.
59. O'Neill LA (2003) SIGIRR puts the brakes on Toll-like receptors. Nat Immunol 4: 823-824.
60. Otte JM, Cario E, Podolsky DK (2004) Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 126: 1054-1070.
61. Bashir ME, Louie S, Shi HN, Nagler-Anderson C (2004) Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol 172: 6978-6987.
62. Evans RM (1988) The steroid and thyroid hormone receptor superfamily. Science 240: 889-895.
63. Robinson-Rechavi M, Carpentier AS, Duffraisse M, Laudet V (2001) How many nuclear hormone receptors are there in the human genome? Trends Genet 17: 554-556.
64. Francis GA, Fayard E, Picard F, Auwerx J (2003) Nuclear receptors and the control of metabolism. Annu Rev Physiol 65: 261-311.
65. McKenna NJ, Lanz RB, O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20: 321-344.
66. Tsai MJ, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63: 451-486.
67. Giguere V (1999) Orphan nuclear receptors: from gene to function. Endocr Rev 20: 689-725.
68. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, et al. (1995) The nuclear receptor superfamily: the second decade. Cell 83: 835-839.
69. Warnmark A, Treuter E, Wright AP, Gustafsson JA (2003) Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Mol Endocrinol 17: 1901-1909.
70. Naar AM, Boutin JM, Lipkin SM, Yu VC, Holloway JM, et al. (1991) The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. Cell 65: 1267-1279.
71. Nagy L, Schwabe JW (2004) Mechanism of the nuclear receptor molecular switch. Trends Biochem Sci 29: 317-324.
72. Pascual G, Glass CK (2006) Nuclear receptors versus inflammation: mechanisms of transrepression. Trends Endocrinol Metab 17: 321-327.
73. Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, et al. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85: 403-414.
74. McKenna NJ, Nawaz Z, Tsai SY, Tsai MJ, O'Malley BW (1998) Distinct steady-state nuclear receptor coregulator complexes exist in vivo. Proc Natl Acad Sci U S A 95: 11697-11702.
75. Boonyaratanakornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, et al. (1998) High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. Mol Cell Biol 18: 4471-4487.
76. Crawford PA, Dorn C, Sadovsky Y, Milbrandt J (1998) Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. Mol Cell Biol 18: 2949-2956.
77. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, et al. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377: 397-404.

78. Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, et al. (1997) The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11: 693-705.
79. Shibata H, Nawaz Z, Tsai SY, O'Malley BW, Tsai MJ (1997) Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Mol Endocrinol 11: 714-724.
80. Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, et al. (1998) Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. Embo J 17: 3398-3412.
81. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, et al. (1996) Role of CBP/P300 in nuclear receptor signalling. Nature 383: 99-103.
82. Yuan CX, Ito M, Fondell JD, Fu ZY, Roeder RG (1998) The TRAP220 component of a thyroid hormone receptor- associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. Proc Natl Acad Sci U S A 95: 7939-7944.
83. Zhu Y, Qi C, Jain S, Rao MS, Reddy JK (1997) Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. J Biol Chem 272: 25500-25506.
84. Puigserver P, Wu Z, Park CW, Graves R, Wright M, et al. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92: 829-839.
85. Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, et al. (1995) Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. Embo J 14: 3741-3751.
86. Lee CH, Chinpaisal C, Wei LN (1998) Cloning and characterization of mouse RIP140, a corepressor for nuclear orphan receptor TR2. Mol Cell Biol 18: 6745-6755.
87. Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, et al. (1999) A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell 97: 17-27.
88. Chen JD, Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377: 454-457.
89. Sande S, Privalsky ML (1996) Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. Mol Endocrinol 10: 813-825.
90. Onate SA, Tsai SY, Tsai MJ, O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270: 1354-1357.
91. Smith CL, Onate SA, Tsai MJ, O'Malley BW (1996) CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. Proc Natl Acad Sci U S A 93: 8884-8888.
92. Fraser RA, Heard DJ, Adam S, Lavigne AC, Le Douarin B, et al. (1998) The putative cofactor TIF1alpha is a protein kinase that is hyperphosphorylated upon interaction with liganded nuclear receptors. J Biol Chem 273: 16199-16204.
93. Le Douarin B, Nielsen AL, Garnier JM, Ichinose H, Jeanmougin F, et al. (1996) A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors. Embo J 15: 6701-6715.
94. Fondell JD, Ge H, Roeder RG (1996) Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc Natl Acad Sci U S A 93: 8329-8333.
95. Rachez C, Suldan Z, Ward J, Chang CP, Burakov D, et al. (1998) A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell-free system. Genes Dev 12: 1787-1800.
96. Henry KW, Yuan X, Koszewski NJ, Onda H, Kwiatkowski DJ, et al. (1998) Tuberous sclerosis gene 2 product modulates transcription mediated by steroid hormone receptor family members. J Biol Chem 273: 20535-20539.
97. Issemann I, Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347: 645-650.
98. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, et al. (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68: 879-887.
99. Wahli W, Braissant O, Desvergne B (1995) Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more. Chem Biol 2: 261-266.
100. Zoete V, Grosdidier A, Michielin O (2007) Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators. Biochim Biophys Acta 1771: 915-925.
101. Balakumar P, Rose M, Singh M (2007) PPAR ligands: are they potential agents for cardiovascular disorders? Pharmacology 80: 1-10.

102. Peters JM, Lee SS, Li W, Ward JM, Gavrilova O, et al. (2000) Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta). Mol Cell Biol 20: 5119-5128.
103. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, et al. (2003) Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 113: 159-170.
104. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA (1994) Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology 135: 798-800.
105. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79: 1147-1156.
106. Rosen ED, Spiegelman BM (2001) PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem 276: 37731-37734.
107. Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.
108. Unger RH, Orci L (2000) Lipotoxic diseases of nonadipose tissues in obesity. Int J Obes Relat Metab Disord 24 Suppl 4: S28-32.
109. Iwamoto Y, Kuzuya T, Matsuda A, Awata T, Kumakura S, et al. (1991) Effect of new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14: 1083-1086.
110. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, et al. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270: 12953-12956.
111. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, et al. (2000) Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. J Clin Invest 106: 1221-1228.
112. Jiang C, Ting AT, Seed B (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 391: 82-86.
113. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 391: 79-82.
114. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, et al. (2001) Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 193: 827-838.
115. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, et al. (2001) A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 7: 161-171.
116. Tontonoz P, Singer S, Forman BM, Sarraf P, Fletcher JA, et al. (1997) Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. Proc Natl Acad Sci U S A 94: 237-241.
117. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, et al. (1998) Differentiation and reversal of malignant changes in colon cancer through PPARgamma. Nat Med 4: 1046-1052.
118. Gordon HA (1960) The germ-free animal. Its use in the study of "physiologic" effects of the normal microbial flora on the animal host. Am J Dig Dis 5: 841-867.
119. Zechel C (2005) The germ cell nuclear factor (GCNF). Mol Reprod Dev 72: 550-556.
120. Sonoda J, Laganiere J, Mehl IR, Barish GD, Chong LW, et al. (2007) Nuclear receptor ERR alpha and coactivator PGC-1 beta are effectors of IFN-gamma-induced host defense. Genes Dev 21: 1909-1920.
121. Yan Z, Jetten AM (2000) Characterization of the repressor function of the nuclear orphan receptor retinoid receptor-related testis-associated receptor/germ cell nuclear factor. J Biol Chem 275: 35077-35085.
122. Cho SD, Yoon K, Chintharlapalli S, Abdelrahim M, Lei P, et al. (2007) Nur77 agonists induce proapoptotic genes and responses in colon cancer cells through nuclear receptor-dependent and nuclear receptor-independent pathways. Cancer Res 67: 674-683.
123. Sohn SJ, Thompson J, Winoto A (2007) Apoptosis during negative selection of autoreactive thymocytes. Curr Opin Immunol 19: 510-515.
124. He YW, Deftos ML, Ojala EW, Bevan MJ (1998) RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. Immunity 9: 797-806.
125. Castrillo A, Joseph SB, Vaidya SA, Haberland M, Fogelman AM, et al. (2003) Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. Mol Cell 12: 805-816.

126. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, et al. (2000) Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289: 1524-1529.
127. Guha M, Mackman N (2002) The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 277: 32124-32132.
128. Martin M, Rehani K, Jope RS, Michalek SM (2005) Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat Immunol 6: 777-784.
129. McBee RH (1970) Metabolic contributions of the cecal flora. Am J Clin Nutr 23: 1514-1518.
130. Sakata T, Yajima T (1984) Influence of short chain fatty acids on the epithelial cell division of digestive tract. Q J Exp Physiol 69: 639-648.
131. Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK (1997) Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J Biol Chem 272: 5128-5132.
132. Akahoshi T, Namai R, Murakami Y, Watanabe M, Matsui T, et al. (2003) Rapid induction of peroxisome proliferator-activated receptor gamma expression in human monocytes by monosodium urate monohydrate crystals. Arthritis Rheum 48: 231-239.
133. Compe E, Drane P, Laurent C, Diderich K, Braun C, et al. (2005) Dysregulation of the peroxisome proliferator-activated receptor target genes by XPD mutations. Mol Cell Biol 25: 6065-6076.
134. Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, et al. (1996) Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. J Biol Chem 271: 31771-31774.
135. Dace A, Zhao L, Park KS, Furuno T, Takamura N, et al. (2000) Hormone binding induces rapid proteasome-mediated degradation of thyroid hormone receptors. Proc Natl Acad Sci U S A 97: 8985-8990.
136. Lonard DM, Nawaz Z, Smith CL, O'Malley BW (2000) The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol Cell 5: 939-948.
137. Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, et al. (2004) Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 127: 1474-1487.
138. Klingberg TD, Budde BB (2006) The survival and persistence in the human gastrointestinal tract of five potential probiotic lactobacilli consumed as freeze-dried cultures or as probiotic sausage. Int J Food Microbiol 109: 157-159.
139. Tannock GW (2002) The bifidobacterial and Lactobacillus microflora of humans. Clin Rev Allergy Immunol 22: 231-253.
140. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, et al. (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112: 1796-1808.
141. Boring L, Gosling J, Cleary M, Charo IF (1998) Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394: 894-897.
142. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, et al. (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest 116: 115-124.
143. Han KH, Chang MK, Boullier A, Green SR, Li A, et al. (2000) Oxidized LDL reduces monocyte CCR2 expression through pathways involving peroxisome proliferator-activated receptor gamma. J Clin Invest 106: 793-802.
144. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, et al. (2005) Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc Natl Acad Sci U S A 102: 6086-6091.
145. Yoshida K, Shimizugawa T, Ono M, Furukawa H (2002) Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. J Lipid Res 43: 1770-1772.
146. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101: 15718-15723.
147. Backhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 104: 979-984.
148. Ichikawa T, Liang J, Kitajima S, Koike T, Wang X, et al. (2005) Macrophage-derived lipoprotein lipase increases aortic atherosclerosis in cholesterol-fed Tg rabbits. Atherosclerosis 179: 87-95.
149. Heller F, Harvengt C (1983) Effects of clofibrate, bezafibrate, fenofibrate and probucol on plasma lipolytic enzymes in normolipaemic subjects. Eur J Clin Pharmacol 25: 57-63.
150. Auwerx J, Schoonjans K, Fruchart JC, Staels B (1996) Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects. J Atheroscler Thromb 3: 81-89.

151. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, et al. (1996) PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. Embo J 15: 5336-5348.
152. Diaz ML, Watkins BA, Li Y, Anderson RA, Campbell WW (2008) Chromium picolinate and conjugated linoleic acid do not synergistically influence diet- and exercise-induced changes in body composition and health indexes in overweight women. J Nutr Biochem 19: 61-68.
153. Laso N, Brugue E, Vidal J, Ros E, Arnaiz JA, et al. (2007) Effects of milk supplementation with conjugated linoleic acid (isomers cis-9, trans-11 and trans-10, cis-12) on body composition and metabolic syndrome components. Br J Nutr 98: 860-867.
154. Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, Leesnitzer LA, Belury MA (1999) Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha. J Lipid Res 40: 1426-1433.
155. Yu Y, Correll PH, Vanden Heuvel JP (2002) Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. Biochim Biophys Acta 1581: 89-99.
156. Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, et al. (2004) Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. Am J Clin Nutr 79: 1118-1125.
157. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, et al. (2002) Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation 106: 1925-1929.
158. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, et al. (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 437: 759-763.
159. Babaev VR, Yancey PG, Ryzhov SV, Kon V, Breyer MD, et al. (2005) Conditional knockout of macrophage PPARgamma increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol 25: 1647-1653.
160. Grun F, Blumberg B (2006) Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 147: S50-55.
161. Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70: 567-590.
162. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444: 1022-1023.
163. Sanderson IR (2004) Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. J Nutr 134: 2450S-2454S.