REVIEW ARTICLE

Microbial endocrinology: the interplay between the microbiota and the endocrine system

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One sentence summary: This review summarizes the links between the host endocrine system and microbiota functions, reporting both effects of the host hormones on bacteria and effects of the microbiota on host hormones influencing behavior, appetite and metabolism, gender and immunity.

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ABSTRACT

The new field of microbiome research studies the microbes within multicellular hosts and the many effects of these microbes on the host's health and well-being. We now know that microbes influence metabolism, immunity and even behavior. Essential questions, which are just starting to be answered, are what are the mechanisms by which these bacteria affect specific host characteristics. One important but understudied mechanism appears to involve hormones. Although the precise pathways of microbiota-hormonal signaling have not yet been deciphered, specific changes in hormone levels correlate with the presence of the gut microbiota. The microbiota produces and secretes hormones, responds to host hormones and regulates expression levels of host hormones. Here, we summarize the links between the endocrine system and the gut microbiota. We categorize these interactions by the different functions of the hormones, including those affecting behavior, sexual attraction, appetite and metabolism, gender and immunity. Future research in this area will reveal additional connections, and elucidate the pathways and consequences of bacterial interactions with the host endocrine system.

Key words: microbiota; microbiome; hormones; germfree; endocrine system; immunity

INTRODUCTION

We are only beginning to understand the pervasive importance of gut microbial communities (microbiota) in health and disease. In the past, bacteria were mostly regarded as either pathogens or irrelevant to host function. However, the growing field of microbiome research—human microbial ecology, studying the communities of bacteria residing within our bodies and the genes they contain—has yielded new perspectives. We now realize that the number of microbial cells we carry can be as much as 10 times greater than the total cell number in the human body, and their genetic information is at least 150-fold greater than that of our human genome. Thus, it is not surprising that microbiota and their hosts interact in numerous complex ways. The gut microbiota in particular plays important roles in host metabolism, immunity and even behavior. Mechanisms by which the microbiota are known to mediate these functions include breaking down dietary components, educating the immune system and degrading toxins (Flint et al., 2012; Elahi et al., 2013; Maurice, Haiser and Turnbaugh 2013).

However, only recently has a critical mechanism of bacterial interaction been revealed: modulation of hormonal secretion. From birth, bacterial colonization of the intestine has a role in the maturation of the immune system (Elahi et al., 2013) and the endocrine system (Clarke et al., 2013). Surprisingly, commensal...
bacteria can produce and secrete hormones. The crosstalk between microbes and hormones can affect host metabolism, immunity and behavior. This interplay is bidirectional, because the microbiota has shown to be both affected by and to affect host hormones, as summarized in Table 1.

Lyte and Ernst were the first to define the field of microbial endocrinology research, after observing that stress-induced neuroendocrine hormones can influence bacterial growth (Lyte and Ernst 1992). Further research of microbial endocrinology discovered hormone receptors in microorganisms and hypothesized that they represent a form of intercellular communication (Lyte 1993). Pathogenic neurotoxins such as neurotoxin 6-hydroxydopamine were shown to alter norepinephrine levels in mice presenting the bidirectional nature of the host–microbe interaction (Lyte and Bailey 1997). A more evolutionary-oriented study showed that many enzymes involved in host hormone metabolism (including epinephrine, norepinephrine, dopamine, serotonin, melatonin, etc.) might have evolved from horizontal gene transfer from bacteria (Iyer et al., 2004).

More clues to the existence of crosstalk between bacteria and the endocrine system came from the discovery of interkingdom signaling, including the hormonal communication between microorganisms and their hosts (Hughes and Sperrandio 2008). This field evolved from the initial observation that bacteria perform quorum sensing (QS), communication based on producing and sensing autoinducer (AI) molecules. These AI molecules are hormone-like elements that regulate functions including coordinated bacterial growth, motility and virulence (Fuqua, Winans and Greenberg 1996). In addition to affecting bacteria, these signals can modulate host cell signal transduction. Some AI molecules have crosstalk with host hormones for activating signaling pathways (Karavolos et al., 2013).

Host hormones also affect bacterial gene expression (Sperrandio et al., 2003), which in turn can have consequences on their hosts. For example, catecholamines enhance bacterial attachment to host tissues, and affect growth and virulence of bacteria (Freestone and Lyte 2008; Hegde, Wood and Jayaraman 2009). In contrast, the human sex hormones estrogen and estradiol decrease bacterial virulence by inhibiting QS (Beury-Crou, et al., 2013).

The effects of host hormones on the microbiota are summarized in Fig. 1.

In this review, we summarize interactions between hormones and the gut microbiota, and propose an important role for the microbiota in hormone regulation. These endocrine effects of bacteria influence a variety of host responses including behavior, metabolism and appetite, and immune responses (Fig. 2). Much of the advances in this field have been made through experiments using germfree (GF) animals (Fig. 3) as well as experiments using probiotics (specific microbes thought to be beneficial to the host) and prebiotics (non-digestible carbohydrates that act as food for probiotics), together with advances in sequencing and bioinformatics platforms.

While this field is in its infancy, future research will likely identify additional strong interconnections between hormones and our microbiome. Microbial endocrinology may also explain how the microbiota affect the host’s gastrointestinal (GI) and psychological health (Lyte 2011). We suggest that hormones are an important mechanism for host-microbial interaction.

**BEHAVIOR**

The gut microbiota influences animal and human behavior in several ways. GF mice have altered cognitive function, memory, stress-response, anxiety and social behavior (Diaz et al., 2011; Neufeld, 2013). The gut microbiota may even influence human emotional states and disease states, such as stress-related irritable bowel syndrome (IBS; reviewed elsewhere in Cryan and O’Mahony 2011), and autism (Sandler et al., 2000; Finegold et al., 2010; Hsiao et al., 2013). These surprising findings show that the microbiota can modulate host behavior, raising the question of how these effects work functionally. The communication between the gut microbiota and the brain has been termed the ‘gut-brain axis’, and is mediated mainly by the long branching vagus nerve. Although the precise pathways of the gut-brain axis have not yet been deciphered, it is thought that the effects are achieved by several different mechanisms mediated by hormones. The effects of the microbiota on host hormone levels may be direct, where the microbiota produce the hormones, or indirect, where microbes may modulate the function of the adrenal cortex (which controls the anxiety and stress responses), or modulate inflammation and immune responses. Two major groups of hormones likely involved in bacterial effects on host behavior are neurohormones, including serotonin and the catecholamines dopamine, epinephrine (adrenaline) and norepinephrine (noradrenaline), and stress hormones, including cortisol, corticosterone, adrenocortosterone and corticotropin. Hormones in both groups can influence physiological changes preparing the body for physical activity (fight-or-flight response), such as increased heart rate and blood pressure, and decreased metabolism (Romero and Butler 2007).

**Neurohormones**

Neurohormones are secreted from neuroendocrine cells in response to a neuronal input. Although they are secreted into the blood for a systemic effect, they can also act as neurotransmitters. Modulation of behavior by the microbiota (such as anxiety in mice) is believed to occur through neurohormone precursors (e.g. serotonin, dopamine) (Lyte 2013). Recently, gut bacteria were shown both to produce and respond to neurohormones such as serotonin, dopamine and norepinephrine (Roshchina 2010). Catecholamines can alter growth, motility, biofilm formation and/or virulence of bacteria (Lyte et al., 2003; Sperrandio et al., 2003; Freestone and Lyte 2008; Karavolos et al., 2008; Hegde, Wood and Jayaraman 2009). These mechanisms are intriguing for researchers studying pathogens because they may influence pathogen susceptibility to host defense responses. For example, in response to host adrenaline, Salmonella downregulates its resistance to antimicrobial peptides and induces key metal transport systems, which affect the oxidative stress balance in the cells (Karavolos et al., 2008). These mechanisms also involve QS: the bacterial QS AI molecule AI-3 and host adrenaline and noradrenaline display crosstalk for activation of the same signaling pathways. These responses most likely depend on bacterial receptor-based sensing and signaling cascades, as they are inhibited by α and β-adrenergic receptor antagonists (Karavolos et al., 2013).

Serotonin, also termed 5-hydroxytryptamine (5-HT), is one of the main neurotransmitters in the brain. However, over 90% of the mammalian host’s serotonin is found in the intestine. Intestinal serotonin secretion is affected by diet, and regulates intestinal movement, mood, appetite, sleep and cognitive functions. This dual role suggests that serotonin may link the intestine (including its microbiota) to host behavior. Brain 5-HT can cross the blood-brain barrier to the blood through the 5-HT transporter, suggesting another link in the gut-brain axis (Nakatani et al., 2008). Serotonin has been implicated in GI...
Table 1. A list of known correlations between hormones and the microbiota.

<table>
<thead>
<tr>
<th>Functional class</th>
<th>Hormone</th>
<th>Model</th>
<th>Finding</th>
<th>Microbial species</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial growth and expressions</strong></td>
<td>Epinephrine, dopamine, dopa</td>
<td>Bacterial growth</td>
<td>Catecholamines induce bacterial growth</td>
<td>E. coli, Yersinia enterocolitica and Pseudomonas aeruginosa</td>
<td>Lyte and Ernst (1992)</td>
</tr>
<tr>
<td></td>
<td>Epinephrine, norepinephrine Epinephrine</td>
<td>QS</td>
<td>QS and host hormone crosstalk</td>
<td>E. coli</td>
<td>Sperandio et al. (2003)</td>
</tr>
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<td></td>
<td>Norepinephrine, epinephrine, dopamine</td>
<td>Bacterial growth</td>
<td>Hormones affect bacterial virulence expression</td>
<td>E. coli, Salmonella enterica and Y. enterocolitica</td>
<td>Freestone and Lyte (2008)</td>
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<td></td>
<td>Norepinephrine</td>
<td>Bacterial growth and characteristics</td>
<td>Enhanced bacterial growth and virulence</td>
<td>Staphylococcus epidermidis Agrobacterium tumefaciens, P. aeruginosa</td>
<td>Lyte et al. (2003)</td>
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<td></td>
<td>Norepinephrine</td>
<td>Biofilm growth</td>
<td>Catecholamines induce biofilm growth</td>
<td>E. coli</td>
<td>Sperandio et al. (2003)</td>
</tr>
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<td></td>
<td>Estriol, estradiol</td>
<td>QS</td>
<td>Hormones decrease bacteria virulence</td>
<td>P. aeruginosa</td>
<td>Hegde et al. (2009)</td>
</tr>
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<td></td>
<td>Norepinephrine</td>
<td>Bacterial production</td>
<td>Gut microbiota produce and respond to norepinephrine</td>
<td>N/A</td>
<td>Roshchina (2010)</td>
</tr>
<tr>
<td></td>
<td>Estrogen, progesterone</td>
<td>Bacterial resistance</td>
<td>Epinephrine decreases bacterial resistance to host antimicrobial peptides</td>
<td>Salmonella</td>
<td>Sperandio et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Tryptophan, 5-HT and 5-hydroxyindoleacetic acid</td>
<td>GF mice</td>
<td>Elevated hipocampal levels in male GF mice</td>
<td>N/A</td>
<td>Clarke et al. (2013)</td>
</tr>
<tr>
<td>Host behavior</td>
<td>Serotonin</td>
<td>Bacterial production of neurotransmitter</td>
<td>Gut microbiota produce serotonin</td>
<td>Streptococcus, Escherichia and Enterococcus spp. Bacillus and Serratia</td>
<td>Roshchina (2010)</td>
</tr>
<tr>
<td></td>
<td>Dopamine</td>
<td>Bacterial production GF mice</td>
<td>Gut microbiota produce and respond to dopamine</td>
<td>N/A</td>
<td>Wikoff et al. (2009)</td>
</tr>
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<td></td>
<td>Serotonin</td>
<td></td>
<td>Low plasma serotonin levels in GF mice</td>
<td></td>
<td>Desbonnet et al. (2008)</td>
</tr>
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<td></td>
<td>Tryptophan, 5-HT</td>
<td>Infected rats</td>
<td>Higher plasma levels in infected rats</td>
<td>B. infantis</td>
<td>Asano et al. (2012)</td>
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<td></td>
<td>Norepinephrine, dopamine GABA</td>
<td>GF mice</td>
<td>Low free lumen catecholamines in GF mice</td>
<td>N/A</td>
<td>Asano et al. (2012)</td>
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<td></td>
<td>GABA</td>
<td>Bacterial production Probiotics in mice</td>
<td>Microbiota produce GABA</td>
<td>Lactobacillus</td>
<td>Bravo et al. (2011)</td>
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<td></td>
<td>Corticosterone</td>
<td>Probiotics in mice</td>
<td>Bacteria alter host GABA receptors</td>
<td>L. rhamnous</td>
<td>Bravo et al. (2011)</td>
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<td></td>
<td>Corticosterone, adrenocorticosterone</td>
<td>Probiotics in mice GF mice</td>
<td>Low levels of corticosterone in treated mice</td>
<td>L. rhamnous</td>
<td>Grenham et al. (2011)</td>
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<tr>
<td>Corticosterone, adrenocorticosterone L-DOPA</td>
<td>Probiotics in rats and humans</td>
<td></td>
<td>Elevated levels in GF mice</td>
<td>N/A</td>
<td>Grenham et al. (2011)</td>
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<tr>
<td>Host mating</td>
<td>cVA and CHs</td>
<td>D. melanogaster</td>
<td>Pheromone levels affected by antibiotics and bacteria addition</td>
<td>L. planatarum</td>
<td>Sharon et al. (2010)</td>
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<td></td>
<td>Volatile fatty acids</td>
<td>Hyena</td>
<td>Species-specific volatiles correlated to bacterial populations in scent glands</td>
<td>N/A</td>
<td>Theis et al. (2013)</td>
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Table 1. (continued.)

<table>
<thead>
<tr>
<th>Functional class</th>
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<tbody>
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<td>Host appetite and metabolisms</td>
<td>Leptin</td>
<td>Antibiotics in rats</td>
<td>Vancomycin leads to decrease in circulating leptin levels</td>
<td>N/A</td>
<td>Lam et al. (2012)</td>
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<tr>
<td></td>
<td>Probiotics in smokers</td>
<td>Reduced serum leptin</td>
<td>Bifidobacterium, Lactobacillus, Clostridium, Bacteroides and Prevotella</td>
<td>L. plantarum</td>
<td>Naruszewicz et al. (2002) Queipo-Ortuno et al. (2013) Queipo-Ortuno et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Male rats</td>
<td>Specific bacteria positively correlated with circulating leptin</td>
<td></td>
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<tr>
<td></td>
<td>Male rats</td>
<td>Specific bacteria negatively correlated with circulating leptin</td>
<td></td>
<td></td>
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</tr>
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<td></td>
<td>Mice (including leptin deficient)</td>
<td>Specific bacteria positively correlated with circulating leptin</td>
<td>Mucispirillum, Lactococcus and an uncultured member of the Lachnospiraceae</td>
<td></td>
<td>Ravussin et al. (2012)</td>
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<td></td>
<td>Probiotics in smokers</td>
<td>Specific bacteria negatively correlated with circulating leptin</td>
<td>Clostridium, Bacteroides and Prevotella</td>
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<td>Queipo-Ortuno et al. (2013) Queipo-Ortuno et al. (2013)</td>
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<td>Male rats</td>
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<td></td>
<td>Probiotics in smokers</td>
<td>Released serum ghrelin</td>
<td>Bifidobacterium, Lactobacillus and B. coccoides – E. rectale group</td>
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<td>Queipo-Ortuno et al. (2013)</td>
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<td></td>
<td>Male rats</td>
<td>Specific bacteria positively correlated with ghrelin</td>
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<td></td>
<td>Male rats</td>
<td>Specific bacteria negatively correlated with ghrelin</td>
<td>Bacteroides and Prevotella spp.</td>
<td></td>
<td>Queipo-Ortuno et al. (2013) Queipo-Ortuno et al. (2013)</td>
</tr>
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<td></td>
<td>Prebiotics (oligofructose) in obese humans</td>
<td>Prebiotics decrease secretion of ghrelin</td>
<td>Promoted growth of Bifidobacterium and Lactobacillus</td>
<td></td>
<td>Parnell and Reimer (2009)</td>
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<td>Insulin</td>
<td>Metagenomics of diabetic women</td>
<td>Negative correlations between insulin levels and bacterial populations</td>
<td>Clostridium spp.</td>
<td></td>
<td>Karlsson et al. (2013) Vrieze et al. (2012)</td>
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<td>Insulin</td>
<td>Bacterial transfer from lean donors to metabolic syndrome patients</td>
<td>Lean human subjects have increased insulin sensitivity due to their microbiome, compared to metabolic syndrome patients</td>
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<td>GLP-1</td>
<td>GF and antibiotic treated mice</td>
<td>Intestinal microbiota lower GLP-1 levels</td>
<td>B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, Streptococcus thermophilus</td>
<td>N/A</td>
<td>Wichmann et al. (2013) Yadav et al. (2013)</td>
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<td>GLP-1</td>
<td>Probiotics in mice</td>
<td>Intestinal microbiota increase GLP-1 levels</td>
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<td>Angptl4</td>
<td>Bariatric surgery in rats</td>
<td>Intestinal microbiota changes correlate with elevated GLP-1 levels</td>
<td>L. paracasei</td>
<td>N/A</td>
<td>Osto et al. (2013)</td>
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<tr>
<td>Alpha-melanocyte-stimulating hormone, neuropeptide Y, agouti-related protein, ghrelin and leptin</td>
<td>GF rats</td>
<td>Bacteria produce somatostatin</td>
<td>Bacillus subtilis</td>
<td>N/A</td>
<td></td>
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<tr>
<td>GIP, GLP-1, insulin</td>
<td>Gastric bypass surgery in diabetic patients</td>
<td>Intestinal microbiota changes correlate with elevated hormone levels</td>
<td></td>
<td>N/A</td>
<td>LaFerrere (2011)</td>
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<td>Angptl4</td>
<td>GF mice colonized with normal gut microbiota</td>
<td>Suppression of Angptl4 expression following colonization</td>
<td></td>
<td>N/A</td>
<td>Backhed (2009)</td>
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</table>
Table 1. (continued.)

<table>
<thead>
<tr>
<th>Functional class</th>
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<th>Model</th>
<th>Finding</th>
<th>Microbial species</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Sex hormones and reproduction</td>
<td>Estrogen</td>
<td>Antibiotics</td>
<td>Antibiotics lead to lower estrogen levels</td>
<td>N/A</td>
<td>Adlercreutz et al. (1984)</td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>Humans</td>
<td>Correlations between urinary estrogen levels and fecal microbiome composition and richness</td>
<td>Clostridia taxa, including non-Clostridiales and three genera in the Ruminococcaceae family</td>
<td>Adlercreutz et al. (1984)</td>
</tr>
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<td>Androgens</td>
<td>Enzymatic and kinetic experiments</td>
<td>Bacteria convert glucocorticoids to androgens</td>
<td>C. scindens</td>
<td>Winter et al. (1984)</td>
<td></td>
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<td>Testosterone</td>
<td>NOD mice</td>
<td>Microbes raise testosterone levels in male NOD mice</td>
<td>N/A</td>
<td>Markle et al. (2013)</td>
<td></td>
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<td>Host growth</td>
<td>Bovine growth hormone</td>
<td>Cows</td>
<td>SCFAs inhibit bovine growth hormone</td>
<td>N/A</td>
<td>Wang et al. (2013)</td>
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<td>Growth hormones</td>
<td>Probiotics in D. melanogaster</td>
<td>Probiotics promote growth hormone signaling</td>
<td>L. plantarum</td>
<td>Storelli et al. (2011)</td>
<td></td>
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<td>Insulin-like peptides</td>
<td>Probiotics in D. melanogaster</td>
<td>Probiotics in mice</td>
<td>Bacterial genes are required for growth factors</td>
<td>A. pomorum</td>
<td>Shin et al. (2011)</td>
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<td>Oxytocin</td>
<td>Probiotics in mice</td>
<td>Upregulation of oxytocin in treated mice</td>
<td>N/A</td>
<td>Poutahidis et al. (2013)</td>
<td></td>
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<tr>
<td>Other</td>
<td>TSH</td>
<td>GF rats</td>
<td>~25% higher TSH levels in GF rats</td>
<td>N/A</td>
<td>Westmann (1996)</td>
</tr>
<tr>
<td></td>
<td>Prolactin</td>
<td>GF rats</td>
<td>~25% higher prolactin levels in GF rats</td>
<td>N/A</td>
<td>Westmann (1996)</td>
</tr>
<tr>
<td></td>
<td>Prolactin</td>
<td>Cow cells</td>
<td>SCFAs inhibit prolactin gene transcription</td>
<td>N/A</td>
<td>Wang et al. (2013)</td>
</tr>
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</table>

Figure 1. Host effects on the microbiota. A variety of host factors (such as diet, exercise, mood, general health state, stress and gender) lead to alterations in hormonal levels, which in turn lead to a variety of effects on the microbiota (including growth, virulence and resistance).
pathologies such as IBS and Crohn’s disease (Manocha and Khan 2012), and these diseases are also associated with differences in the microbiota (Morgan et al., 2012).

To our knowledge, the first direct effect of the microbiome on serotonin was demonstrated in GF mice, which had lower plasma serotonin levels than conventional mice (Wikoff et al., 2009). However, in another study, levels of tryptophan (the precursor of serotonin) and hippocampal concentration of 5-HT and 5-hydroxyindoleacetic acid (its main metabolite) were elevated in male GF mice (Clarke et al., 2013), suggesting that either the effects are facility-specific or tissue-specific. Serotonin can also be produced by Streptococcus, Escherichia and Enterococcus species (Roshchina 2010), and Bifidobacterium infantis modulates 5-HT levels by increasing plasma tryptophan levels (Desbonnet et al., 2008).

Dopamine is also produced by bacteria including Bacillus and Serratia, although little is known about its function in these microorganisms (Roshchina 2010). Levels of free lumen dopamine were significantly lower in GF than conventional mice, and were elevated again upon inoculation with bacteria expressing β-glucuronidase (Asano et al., 2012). These results suggest that there may be correlations between intestinal bacteria and dopamine levels in conditions such as Parkinson’s disease, characterized by insufficient dopamine formation. Because the etiology of Parkinson’s disease is poorly understood, and one of the early symptoms of disease is constipation, it has been hypothesized that bacteria may be involved in its progression or development (Dobbs et al., 2012). Moreover, several rodent models of Parkinson’s disease-like syndrome are established by injection of bacteria such as Nocardia asteroides (Kohbata and Beaman 1991). So far, Helicobacter pylori has been shown to increase the risk for Parkinson’s disease by affecting L-DOPA levels (Pierantozzi et al., 2006), and fecal transplants may have alleviated some neurological symptoms in a Parkinson’s patient.

Figure 2. The effects of the gut microbiota on the host via hormones. Gray arrows and text refer to the effects of the gut microbiota on various hormone levels. Pink arrows and text refer to the effects of these hormonal alterations on host outcomes (e.g. behavior).
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Figure 3. Hormonal alterations reported in GF rodents (Based on Wostmann 1996; Fetissov et al., 2008; Backhed 2009; Wikoff et al., 2009; Grenham et al., 2011; Asano et al., 2012; Clarke et al., 2013; Markle et al., 2013; Wichmann et al., 2013). The arrows refer to the hormone levels in GF compared to conventionally raised rodents.

Hormonal alterations in the germ-free rodent

Figure 3. Hormonal alterations reported in GF rodents (Based on Wostmann 1996; Fetissov et al., 2008; Backhed 2009; Wikoff et al., 2009; Grenham et al., 2011; Asano et al., 2012; Clarke et al., 2013; Markle et al., 2013; Wichmann et al., 2013). The arrows refer to the hormone levels in GF compared to conventionally raised rodents.

Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian CNS, is also produced by the microbiota and may influence host behavior. This is interesting because alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression. GABA production by Lactobacillus has been studied in an attempt to ferment safe GABA on a large scale (Li et al., 2008). Accordingly, administration of Lactobacillus rhamnosus to mice alters the expression of GABA receptors in different CNS regions, decreasing anxiety- and depression-related behavior (Bravo et al., 2011).

Stress hormones

The microbiota may help keep us calm and balanced by altering stress hormone levels. GF mice have elevated plasma levels of the stress hormones corticosterone and adrenocorticotropic hormone (ACTH) in response to mild stress (Sudo et al., 2004; Grenham et al., 2011), increasing behaviors associated with anxiety and stress. ACTH plays an important role in the hypothalamic–pituitary–adrenal axis by further producing corticosteroids. Accordingly, two specific species, L. helveticus and B. longum, reduce levels of the stress hormone cortisol and anxiety-like behavior in both rats and healthy humans (Messaoudi et al., 2011). Furthermore, mice chronically treated with the probiotic L. rhamnosus had lower levels of corticosterone and less depressive behavior in a forced swim test than controls (Bravo et al., 2011).

MATING AND PHEROMONES

Pheromones are hormones that play important roles in sexual recognition, attraction and mating behavior as well as aggression behavior and dominance. Pheromones are also termed ectohormones, chemicals secreted outside of the body of one individual and affect the behavior of others.

In Drosophila melanogaster (D. melanogaster), the most abundant pheromones are cuticular hydrocarbons (CHs), and the most studied pheromone is cis-vaccenyl acetate (cVA). Levels of CHs and cVA are affected by antibiotics, suggesting a role for the microbiota in pheromone regulation (Sharon et al., 2010). In a study characterizing mating preferences between two groups of D. melanogaster fed different diets, assortative mating preferences were eliminated with antibiotics, and depended on a specific gut microbe, L. plantarum (Sharon et al., 2010). Together, these findings suggest a mechanism whereby the microbiota affect host pheromone levels, which in turn affect mating behavior.
Guaiacol (2-methoxyphenol) and related phenolic compounds, which are produced by commensal bacteria involved in vanillate decarboxylation, are another indication of the involvement of the microbiota in pheromone secretion. In response to guaiacol, the desert locust Schistocerca gregaria swarms (Dillon, Vennard and Charnley 2000). Accordingly, GF animals do not release guaiacol or volatile phenols, but monocultures with the commensal species, Pantoea agglomerans, Klebsiella pneumoniae or Enterobacter cloacae lead to pheromone production. Interestingly, this phenomenon is specific to the commensal species, because locusts colonized by a pathogenic bacterial species did not produce guaiacol or other phenolic compounds (Dillon, Vennard and Charnley 2002).

This linkage between commensal bacteria and volatile host social signals may also occur in mammals. Two hyena species harbored different bacterial communities in the scent glands, which correlated with distinct volatile fatty acid profiles of scent secretions. The authors speculate that the symbiotic bacteria make metabolites that provide species-specific odors (Theis et al., 2013).

Additionally, Singh et al. demonstrated in a major histocompatibility complex congenic rat model that discriminative urinary odors that exist in conventional rats do not exist in GF rats. This further supports the idea that bacteria can produce an odor profile that affects host behavior (Singh et al., 1990). Bacterial communities producing specific scents may be linked to host genetic background, as scent-mark communities differed significantly between strains of mice, even when co-housed in a shared environment (Lanyon et al., 2007). Bacteria can also play a role in mate selection: female mice are not attracted to the urine of Salmonella-infected males (Raveh et al., 2014). Because chemosignals and olfactory stimulation also play a role in human behavior, future research will help us understand whether bacterially produced odors affect our own interactions and perhaps evolution.

**SEX HORMONES AND REPRODUCTION**

Examples of bacteria affected by sex hormones have been reported since the 1980s. For instance, Prevotella intermedia takes up estradiol and progesterone, which enhance its growth (Kornman and Loesche 1982). Changes in expression of the estrogen receptor, ER-β, also affect the intestinal microbiota composition (Menon et al., 2013). This interaction goes both ways, as several types of bacteria have also been implicated in steroid secretion or modification (Ridlon et al., 2013). For example, Clostridium scindens converts glucocorticoids to androgens, a group of male steroid hormones (Ridlon et al., 2013). Intestinal bacteria also play a significant role in estrogen metabolism, because use of antibiotics leads to lower estrogen levels (Adlercreutz et al., 1984). Furthermore, strong correlations were found between urinary estrogen levels and fecal microbiome richness, as well as presence of Clostridia, including non-Clostridiales, and three genera within the Ruminococcaceae.

Mittelstrass et al. suggested an interplay between the endocrine system, the gut bacteria and metabolism based on gender-specific differences in fatty acid profiles (Mittelstrass et al., 2011). There are also gender-specific immune changes that may be correlated with the microbiota. The new term ‘microgender’ refers to recent observations that the host microbiome plays a role in the gender bias seen in numerous diseases (Flak, Neves and Blumberg 2013). We discuss this topic further in the immune response section below.

Pregnancy is an interesting period to study the microbiota changes that correlate with the drastic changes in host hormone levels during this time. Normally, hormones such as estrogen, progesterone and leptin increase dramatically with gestational age, while adiponectin and pituitary GH decrease (Newborn and Freemark 2011). The composition of the gut microbiota changes dramatically during pregnancy, specifically in the third trimester, and these changes lead to differences in metabolism (Koren et al., 2012). These microbial changes include a significant increase in Proteobacteria and Actinobacteria as pregnancy progresses. The third trimester is also characterized by elevated low-grade inflammation and insulin desensitization compared to the first trimester. This complex phenotype could be transferred to GF mice, where mice receiving third trimester microbiota gained more weight and had a greater inflammatory response than mice receiving first trimester microbiota (Koren et al., 2012). These changes might be correlated with hormonal changes during gestation.

Dysbiosis of the gut microbiota may also play a role in the development of diseases manifested by hormonal imbalance, such as polycystic ovary syndrome, through their modulation of hormone levels. According to this theory, poor diet leads to changes in gut bacterial communities, creating an increase in gut mucosal permeability, resulting in activation of the immune system. This, in turn, raises serum insulin levels, increases androgen production in the ovaries and interferes with normal follicle development (Tremellen and Pearce 2012). A carbohydrate-poor diet has been proven to alleviate symptoms of the syndrome.

**APPETITE AND METABOLISM**

A classic role of the gut microbiota is in digesting a variety of carbohydrates and fermenting them into short-chain fatty acids (SCFAs). GF mice have different metabolic profiles than conventionally raised mice, including low concentrations of SCFAs, hepatic triacylglycerol and glucose. Interestingly, subtherapeutic doses of antibiotics, which do not eliminate the gut microbial community but rather cause significant changes in its composition, lead to increased levels of SCFAs and to weight gain in mice (Cho et al., 2012). These metabolic effects of the microbiome may further affect regulation of hormone production from cholesterol, peptides or amino acids. For instance SCFAs have been shown to stimulate release of 5-HT and the peptide YY, a hormone released after feeding involved in appetite reduction and decreasing of gut motility (Cherbut et al., 1998; Fukumoto et al., 2003). Additional hormones, mainly neuropeptides that have a role in controlling appetite and regulating metabolism, are likely affected by the gut microbiota. These include alpha-melanocyte-stimulating hormone, neuropeptide Y, agouti-related protein, ghrelin, leptin, insulin and others. Another effect of bacteria on metabolic hormones could be through production of somatostatin, which suppresses the release of the GI and pancreatic hormones (LeRoith et al., 1985).

Several pieces of evidence link the microbiota function to leptin levels. First, use of antibiotics (vancomycin) in rats leads to a dramatic decline (38%) in circulating leptin levels (Lam et al., 2012). Second, the abundance of several bacterial genera (e.g. Mucispirillum, Lactococcus, Bifidobacterium and Lactobacillus) positively correlates with circulating leptin concentrations in mice, while other bacterial genera (e.g. Allobaculum, Clostridium, Bacteroides and Prevotella) negatively correlate with leptin levels. These correlations may stem from bacteria affecting hormone levels, or vice versa. One proposed
mechanism is that diet composition may impact leptin concentrations, which, in turn, may change the microbial community composition through inflammation and/or regulation of mucus production (Ravussin et al., 2012; Queipo-Ortuno et al., 2013). Recently, Rajala et al. demonstrated that leptin might also influence the gut microbiota independently of diet (Rajala et al., 2014). Another model proposes that L. plantarum specifically suppresses leptin by reducing adipocyte cell size in white fat tissue (Takekura, Okubo and Sonyama 2010; Lam et al., 2012). This fits the finding that use of the probiotic L. plantarum in a group of human smokers reduced their serum leptin levels (Naruszewicz et al., 2002). Because leptin is involved in appetite inhibition, metabolism and behavior, deciphering its interconnections with bacteria is of great interest and may help us understand and perhaps control its many effects.

Ghrelin, another important appetite-regulating hormone, is negatively correlated with the abundance of Bifidobacterium, Lactobacillus and E. coliocides–Eubacterium rectale group, and positively correlated with a number of Bacteroides and Prevotella species (Queipo-Ortuno et al., 2013). Intake of oligofructose (a prebiotic that promotes growth of Bifidobacterium and Lactobacillus) decreases secretion of ghrelin in obese humans (Parnell and Reimer 2009).

Insulin, the extremely important metabolic hormone involved in diabetes and metabolic syndrome, may provide another link between the microbiome and hormones. Significant variations in microbiome composition have been observed in diabetes patients compared to healthy controls. Certain bacterial species have been positively or negatively correlated with insulin levels (Qin et al., 2012; Karlsson et al., 2013). Transfer of the intestinal microbiota (including butyrate-producing microbiota) from lean donors to metabolic syndrome patients enhanced insulin sensitivity (Vrieze et al., 2012). The effect is likely mediated by altering immune components. However, additional hormones may also be involved in this process.

One such example is glucagon-like peptide 1 (GLP1), associated with appetite control and insulin secretion. The intestinal microbiota have been recently implicated in lowering levels of the GLP1, and thereby slowing intestinal transit (Wichmann et al., 2013). However, alterations of the microbiome through probiotics (Yadav et al., 2013) or bariatric surgery (Zhang et al., 2009; Liou et al., 2013; Osto et al., 2013) decrease adiposity and increase GLP1 levels in mice. This is primarily attributed to butyrate production by commensal bacteria, which can induce GLP1 production by intestinal L cells (Yadav et al., 2013).

Another example is angiotensin-like protein 4 (Angptl4, also known as fasting-induced adipose factor), a hormone implicated in many metabolic processes including regulation of glucose and insulin sensitivity. Angptl4 is also implicated in lipid metabolism, inhibiting lipoprotein lipase (LPL) and thereby reducing fat storage. Gut microbiota suppress expression of Angptl4, and in a GF mouse study Angptl4 was shown to mediate protection against diet-induced obesity (Adlercreutz et al., 1984). A zebrafish model found a tissue-specific cis-regulatory element that reduced Angptl4 in the intestinal epithelial cells of colonized animals (Camp et al., 2012). Despite the general trend toward repression of Angptl4 by the microbiota, specific bacteria can increase hormone expression. Mice treated with L. paracasei were leaner than controls, had lower circulating lipids and elevated levels of Angptl4 (Aronsson et al., 2010). This probiotic may increase Angptl4 expression through butyrate-mediated mechanisms. First, butyrate is proposed to induce Angptl4 by signaling through peroxisome proliferator-activated receptor γ (PPARγ) (Alex et al., 2013). Butyrate may also interact with the Angptl4 promoter region independently of PPARγ (Korecka et al., 2013) and has also been shown to inhibit proliferation, induce differentiation and repress gene expression by inhibiting histone deacetylase activity (Davie 2003). Hence, it is likely that butyrate plays a role in the microbiota-induced weight maintenance mechanisms that involve hormonal changes.

One interesting mechanism by which the microbiota affects peptide hormones is through autoantibodies. Petissos et al. found that autoantibodies against peptide hormones involved in appetite control (including alpha-melanocyte-stimulating hormone, neuropeptide Y, agouti-related protein, ghrelin and leptin) exist in healthy humans and rats, and affect feeding and anxiety. In GF rats, levels of these autoantibodies are altered, suggesting a novel mechanism by which the microbiome can affect appetite (Petissos et al., 2008). These findings have further implications for the potential role of the microbiota in eating disorders such as anorexia nervosa. In support of this notion, differences in microbial composition have been found between anorexic patients and healthy controls (Armougom et al., 2009).

Finally, new correlations among the microbiota composition, hormonal levels and metabolism come from studies of gastric bypass surgery. Gastric bypass surgery has been shown to alter the intestinal microbiota composition. Following Roux-en-Y gastric bypass (RYGB), in both humans and rodents, the relative abundance of Gammaproteobacteria (Escherichia) and Verrucomicrobia (Akkermansia) increase. While microenvironmental changes such as reduced food intake and reduction of bile acids likely affect this new microbiota composition (Madsbad, Dirksen and Holst 2014), some of the compositional changes are likely due to alterations in the levels of intestinal hormones including elevation of glucose-dependent insulinitropic polypeptide (GIP), GLP1 and insulin following surgery (Laferrere 2011; Osto et al., 2013). These alterations in the gut microbiome further contribute to reduced host weight and adiposity. Accordingly, transfer of the gut microbiota from RYGB-treated mice to non-operated GF mice resulted in weight loss and decreased fat mass in the recipient animals relative to recipients of the microbiota induced by sham surgery (Liou et al., 2013).

**IMMUNE RESPONSE**

A growing amount of evidence has linked both hormones and the microbiome to immune responses under healthy conditions and autoimmune disease (AD). There are many interconnections between them, and the microbiome and hormones may work through shared pathways to affect the immune response. Hormones influence the immune system in many ways. The immune and neuroendocrine systems share a common set of hormones and receptors. Glucocorticoids such as cortisol and interleukin-6 regulate inflammation levels and have effects both on the innate and adaptive immune responses (Francishmont 2004). Additionally, vitamin D affects immune cell responses by enhancing antigen presentation (Bhalla et al., 1989). Moreover, sex hormones affect the immune response in numerous ways (Whitacre 2001; Lasarte et al., 2013; Priyanka et al., 2013; Sankaran-Walters et al., 2013). Many AD are more common in females, perhaps partly due to hormonal differences (Whitacre 2001; Ober, Loisel and Gilad 2008). Mouse models of AD in which hormone levels were altered revealed changes in disease incidence; for example, androgen treatment in a type 1 diabetes (T1D) non-obese diabetic (NOD) mouse model prevents the development of diabetes (Fox 1992).
However, the gut microbiota also plays a role in modulating the immune response, both locally and systemically (Kamada et al., 2013), beyond repressing pathogenic microbes. In the absence of commensal bacteria, GF mice have impaired development of the innate and adaptive immune system (Hill and Artis 2010; Littman and Pamer 2011; Honda and Littman 2012; Hooper, Littman and Macpherson 2012), reduced numbers of IgA-producing plasma cells (Crabbe et al., 1969) and a decreased percentage of CD4+ T cells (Ostman et al., 2006). Additionally, T helper 17 (TH17) cells, which produce pro-inflammatory cytokines, are regulated by gut bacteria and are promoted specifically by segmented filamentous bacteria (SFB) (Tanabe 2013). Finally, AD have also been correlated with alterations of the microbiome (dysbiosis). The most extensively studied example is T1D as presented in Fig. 4 (Brown et al., 2011; Hara et al., 2013).

Thus, both hormones and the gut microbiota play essential roles affecting immunity, and some of their roles may be linked through shared pathways or additive effects. The first to show such a triangular link between the microbiota, hormones and immunity were Markle et al. (2013), who demonstrated in the NOD mouse model that microbiota raise testosterone levels in male hosts, causing protection from T1D. In this mouse model, females are at higher risk of T1D. However, immature females that received microbiome transplants from adult males showed elevated levels of testosterone as well as protection from T1D. In contrast, NOD mice under GF conditions had a similar diabetes risk for both sexes. Furthermore, the researchers found metabolomic changes including levels of glycerophospholipid and sphingolipid metabolites to be different in mice with typically male or typically female microbiota (Markle et al., 2013). This study establishes a direct relationship between the microbiome and hormones and will open new research paths further linking these components. Additional studies of these phenomena, including high-throughput sequencing of the microbiota and gene expression analysis, suggest that hormones and microbiota contribute in an additive manner to T1D protection in NOD mice (Yurkovskiy et al., 2013).

A different example linking the microbiota, hormones and immunity comes from a study in mice, which showed that L. reuteri enhances wound-healing properties in the host through upregulation of the neuropeptide hormone oxytocin, by a vagus nerve-mediated pathway (Poutahidis et al., 2013). The induced hormonal levels lead to higher levels of specific regulatory T cells required for wound healing. Lactobacillus reuteri also supports thick healthy fur in mice, and greater grooming activity, due to a bacteria-triggered interleukin-10-dependent mechanism and higher oxytocin levels, respectively (Levkovich et al., 2013).

**GROWTH AND DEVELOPMENT**

Although no direct connection has been shown to date between the microbiota and growth hormones, the microbiome’s effects on ghrelin and sex hormones may indirectly promote release of growth hormones (Howard et al., 1996). Additionally, SCFAs have been shown to inhibit growth hormones in cows, by affecting gene transcription in a cAMP/PKA/CREB-mediated signaling pathway (Wang et al., 2013). Furthermore, bacteria produce somatostatin, which is a known growth hormone inhibitor (Leroith et al., 1982).

In D. melanogaster, both L. plantarum and Acetobacter pomorum (A. pomorum) were found to be involved in growth promotion. Lactobacillus plantarum promotes larval growth upon nutrient scarcity, by acting genetically upstream of the host nutrient sensing system controlling hormonal growth signaling (Storelli et al., 2011). Accordingly, larval development is prolonged in GF flies, and they exhibit significantly reduced metabolic rates and altered carbohydrate allocations, including elevated glucose levels (Ridley et al., 2012). Moreover, late larval development in GF flies and the metabolic alterations were reversed by adding A. pomorum. Genes in the pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) pathway of A. pomorum are required for expression of Drosophila insulin-like peptides, which act as growth factors and are necessary for normal larval development and metabolism (Shin et al., 2011).

**CONCLUSIONS**

Although the field of microbiome research is new and developing, a significant number of studies already link hormones and the gut microbiota. Hormones regulated by the microbiota span all functional classes and exert broad influences on host behavior, metabolism and appetite, growth, reproduction and immunity. As the understanding of the precise roles of the microbiome deepens, we expect that additional mechanisms will be shown to involve hormones, including novel interactions. Research in the near future will identify both direct and indirect pathways (e.g. via immune system components) by which bacteria affect hormones. Specific classes of bacteria (as well as other
microorganisms including archaea, bacteriophages, eukaryotes and eukaryotic viruses) will likely have regulatory roles controlling host hormone levels. These findings may potentially be used in the future for development of new treatments for hormone-related diseases or disorders, autoimmunity disorders linked to gender or hormonal activity, and even emotional states such as stress. In our vision, we see how behavior could be controlled by the gut microbiota, we see the potential to alter metabolic hormone levels, overcoming depression or stress by swallowing a combination of ‘good bacteria’. While this still sounds like science fiction, these novel approaches may become reality within a few years. However, in order to validate such approaches, the mechanisms, precise bacterial strains and endocrine-microbiome crosstalk must all be thoroughly deciphered.

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