Hypothesis: Bacteria Control Host Appetites

Vic Norris,a Franck Molina,b Andrew T. Gewirtzc

Theoretical Biology Unit, EA3829, Faculty of Science, University of Rouen, Mont Saint Aignan, Francea; Sysdiag UMR 3145-CNRS/BIO-RAD, Montpellier, Franceb; Center for Inflammation, Immunity & Infection, Department of Biology, Georgia State University, Atlanta, Georgia, USAc

To help investigate the relationship between inflammatory and other diseases and the composition of the gut microbiota, we propose that a positive-feedback loop exists between the preferences of the host for a particular dietary regimen, the composition of the gut microbiota that depends on this regimen, and the preferences of the host as influenced by the gut microbiota. We cite evidence in support of this hypothesis and make testable predictions.

Few people underestimate the danger of bacteria. Shigellosis alone causes 1.1 million deaths a year. Disease caused by certain species of the bacteria in the gut is the leading cause of death in the world. What may be underestimated, however, is the full extent to which they can affect us. Bacteria both recognize and synthesize neuroendocrine hormones, and this has led to the hypothesis that microbes within the gut comprise a community that forms a microbial organ interfacing with the mammalian nervous system that innervates the gastrointestinal (GI) tract, the enteric nervous system.

The enteric nervous system is embedded in the lining of the gastrointestinal system and consists of around five hundred million neurons, including afferent neurons (which carry nerve impulses from receptors or sense organs toward the central nervous system [CNS]), efferent neurons (which carry impulses the other way), and interneurons (which connect other neurons). The enteric nervous system contains support cells which resemble the astroglia in the brain as well as a diffusion barrier around the capillaries surrounding ganglia which resembles the blood-brain barrier of cerebral blood vessels. The enteric nervous system can operate autonomously and has been likened to a second brain. This system regulates gastric acid secretion, peristalsis/motility, fluid flow across the lining epithelium, local blood flow, and nutrient handling; it also interacts with the endocrine and immune systems of the gut. Normally, the communication of the enteric nervous system with the central nervous system involves the parasympathetic nervous system (via, for example, the vagus nerve and the sympathetic nervous system (via, for example, the prevertebral ganglia). The enteric nervous system uses over 30 neurotransmitters, most of which are the same as those used by the central nervous system, including acetylcholine, dopamine, and serotonin. A wide range of such neurohormones, neurotransmitters, and their receptors—including corticotropin, somatostatin, and γ-aminobutyric acid (GABA)—are homologous to those found in microorganisms.

The brain can influence gut microbiota via changes in gastrointestinal motility, secretion, and permeability and via signaling molecules released into the gut lumen from cells in the lamina propria such as neurons, immune cells, and enterochromaffin cells (which regulate communication between the gut lumen and the nervous system); indeed, enterochromaffin cell signaling to neuronal circuits via vagal, afferent innervation is believed to have an important role in pain and immune-response modulation, control of background emotions, and other homeostatic functions.

HYPOTHESIS
The hypothesis is based on two sets of findings. First, as is well attested, human behavior helps control which species of bacteria are present in the gut; second—and more speculatively—bacteria influence human behavior. In the hypothesis, there is a mutual reinforcement between the behavior of the human host and the bacterial population within that host.

Dependence of bacterial composition on human behavior.
The composition of the population of bacteria in the gut is selected to a large extent by the nutrients consumed by the host and by the stresses to which the host is subjected.

Bacteria influence host appetite. The bacteria in the gut possess several mechanisms for influencing the physiology of their...
hosts and, in particular, can influence the preferences of their hosts for certain nutrients.

Positive feedback between gut microbiota and appetite. A system of selection exists based on the positive-feedback relationship between the particular nutrients consumed by the host and the bacterial composition in the gut such that this system leads to stable attractors of bacterial composition and host behavior.

EVIDENCE

There are three lines of evidence for the hypothesis. First, the composition of bacteria in the gut depends on the behavior of the host and, in particular, on what food is eaten and on whether the host is under stress. Second, bacteria in the gut have many established mechanisms for influencing their hosts as in the case of susceptibility to a variety of diseases; such influence can extend to emotional disturbances and eating disorders. Third, eating changes both pleasure and the sensation of satiety. Both experiences involve processes that may be modulated by bacteria.

Dependence of bacterial composition on human behavior.

The quantity, frequency, and nature of the food ingested by the hosts and the hormones they release during stresses have major effects on the bacterial composition in the gut.

(i) Nutrients determine the gut microbiota. The composition of the population of bacteria in the gut is selected to a large extent by the nutrients consumed by the host. If food intake determines the gut microbiota, fasting and feasting are the most extreme dietary lifestyles, should be expected to produce major changes in the microbiota. Using the Burmese python as a model system, it was indeed found that fasting was associated with increased abundances in the large intestine of the genera Bacteroides, Rikenella, Synergistes, and Akkermansia and with reduced overall diversity, while, after feeding, Firmicutes, including the taxa Clostridium, Lactobacillus, and Peptostreptococcaceae, gradually outnumbered the fasting-dominant Bacteroidetes and increased overall “species”-level diversity (6). In humans, Bacteroides species proliferate in response to fructans, a class of fructose-based dietary polysaccharides. This was attributed to a fructose-binding, hybrid two-component signaling sensor that controls the fructan utilization locus; this locus is different in different Bacteroides species and results in different fructans being useable by the bacteria (7).

Comparison of the fecal microbiota of African children with a high-fiber, low-fat diet to the fecal microbiota of European children with a modern diet revealed that the former had more Bacteroidetes and fewer Firmicutes bacteria and a particular abundance of bacteria of the genera Prevotella and Xylanibacter (8); this led to the conclusion that gut microbiota coevolved with the polysaccharide-rich diet of the children from Burkina Faso to allow them to maximize energy intake from fibers and to protect them from inflammation and colonic disease. It should, however, be noted that giving a low-fat, high-carbohydrate diet for 10 days to a group of subjects who started with the Bacteroides enterotype did not result in switching to the Prevotella enterotype (9).

(ii) Stress. Psychological stress increases the circulating levels of the catecholamine hormones norepinephrine and epinephrine, which play a central role in the “flight or fight” response in higher animals. These hormones affect bacterial growth by scavenging iron or by inducing the production of autoinducers, and in a study of 43 oral bacterial species, catecholamines elicited the greatest response from the periodontal pathogens most closely associated with gum disease (10). Overgrowth by species such as Escherichia coli is responsible for gut-derived sepsis following surgery (11). Significantly, norepinephrine, epinephrine, and dopamine increased the growth of commensal E. coli in vitro by over 4 orders of magnitude (12). Mice exposed to a social stressor have increased levels of circulating cytokines and an innate immune system that is primed for enhanced reactivity. Stressor exposure significantly changed the community structure of the microbiota in the cecum by decreasing the proportion of Bacteroides and increasing the proportion of bacteria in the genus Clostridium. The stressor also increased circulating levels of interleukin-6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1), which were correlated with changes to the proportions of Coprococcus, Pseudobutyribivibrio, and Dorea (13).

Influence of the bacteria in the gut on their hosts.

The molecules that bacteria use to exert control over host behavior may have originated in several different ways; for example, such host-control compounds may have originated (and may still function) as signals between bacteria in biofilms, or as the fermentation products of indigestible foods, or as cometabolites, or as the products of bacterial lysis. Indeed, it has been suggested that quorum sensing might also be a “language” by which bacteria and host cells communicate (14). The evolution of host-control compounds was probably guided by molecular complementarity which selects from the diversity of biological molecules those that are able to stabilize and interact functionally with each other (15); hence, candidate host-control compounds produced by bacteria include the macromolecules or fragments of macromolecules (16) that bind to metabolites or that bind to the receptors of those metabolites or even the receptors themselves (such as the bacterial adrenergic receptors [17]). One possible example of molecular complementarity is the major cell wall breakdown product muramyl dipeptide, or “adjuvant peptide,” which not only stimulates an inflammatory response via the NOD proteins (18) but also mimics serotonin to cause drowsiness (19, 20). Drowsiness may also be caused by the administration of another bacterial product, flagelin, which acts via a Toll-like receptor, TLR5 (A. Gewirtz, unpublished data). The abundance of a wide variety of other molecules in the gut has also been attributed to the action of the microbiota. These molecules, some of which may help bacteria control the behavior of their hosts, include short-chain fatty acids, branched-chain fatty acids, γ-aminobutyric acid (GABA), biotin, vitamin K, p-hydroxyphenylacetic acid, m-hydroxyphenylacetic acid, hydroxy-cinnamic acid, phenylvaleric acid, p-aminobenzoic acid, indoxyl sulfate, indoleacetic acid, indoleacetaldehyde, 6-hydroxymelatonin sulfate, putrescine, spermidine, spermine, taurine, cadaverine, tryptophan, tyrosine, and peptides (21–25).

There are several examples in which the ensemble of the gut microbiota, rather than an individual species, has major effects on the health of a host. Sometimes, transplanting “diseased”—but “pathogen”-free—microbiota to a healthy but susceptible host also transfers the disease. Moreover, some of the molecular mechanisms responsible for this control by bacteria of their hosts are known. They include signaling pathways such as those involving c-Fos, Toll-like receptors (TLR), NF-κB, mitogen-activated protein kinase (MAPK), Jun N-terminal protein kinase (JNK), and CyclinD, while signals include flagellin, lipopolysaccharide (LPS), and trimethylamine N-oxide (TMAO). These different mechanisms reveal the capacity of bacteria in the gut to influence human health—or the behavior associated with health. The effects of
these mechanisms range from metabolic syndrome and even to thyroid disease, cardiovascular disease, cancer, and anxious behavior.

(i) Mood disorders and illnesses. The hypothalamus-pituitary-adrenal response is involved in the neurobiology of mood disorders and illnesses that include anxiety disorder, bipolar disorder, depression, posttraumatic stress disorder, borderline personality disorder, attention deficit–hyperactivity disorder (ADHD), major depressive disorder, burnout, chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, and alcoholism. The normalization of this response, as well as increased angiogenesis, is correlated with increased numbers of Bacillus infantis (26). As mentioned above, a role for the microbiota in stressor-induced increases in levels of circulating cytokines in normal mice was shown in antibiotic-treated mice in which the stressor did not increase IL-6 and MCP-1 levels (13). In a remarkable experiment, challenging mice with live Campylobacter jejuni led to a reduction in their exploratory behavior—consistent with anxiety—and an activation of brain regions implicated in anxiety behavior, including the lateral septum, paraventricular, and dorsomedial hypothalamic nuclei, basolateral and central nuclei of the amygdala, and the bed nucleus of the stria terminalis and periaqueductal gray (5); this activation was correlated with c-Fos expression in the bed nucleus of the stria terminalis. The higher motor activity and reduced anxiety of germfree mice, compared with pathogen-free mice with a normal gut microbiota, are associated with altered expression of genes involved in second messenger pathways and synaptic long-term potentiation in brain regions implicated in motor control and anxious behavior (27). Moreover, germfree mice exposed to gut microbiota early in life resemble mice with a normal microbiota and have reduced expression of postsynaptic density protein 95 (PSD-95) and synaptophysin in the striatum (27). To explain how the treatment of Helicobacter pylori infection helps those suffering from Parkinson’s disease, it has been proposed, with some experimental support, that this is because the L-DOPA supplements given to these patients are actually used by H. pylori (28). This would be consistent with yet another connection between neurotransmitters and bacteria. Finally, Desulfovibrio species are more common in autistic subjects than in controls (29). The controversial idea is that these anaerobic bacilli may actually be selected during the treatment of common childhood infections because of their resistance to antimicrobial agents such as cephalosporins.

(ii) IBD. Murine models of inflammatory bowel disease (IBD) require “normal” microbiota for disease development, suggesting that human IBD is mediated by an aberrant immune response to gut microbiota. The mechanism involves TLRs on the surface of the host cells which bind to bacterial constituents such as flagellin, lipoprotein, lipopolysaccharide, and DNA and then activate pro-inflammatory gene expression via NF-κB and interferon regulatory factor (IRF). Macrophages respond to lipopolysaccharide, while epithelial cells respond to flagellin. Both bacterial constituents lead to increases in the level of serum cytokines, in the former case, tumor necrosis factor alpha (TNF-α), and in the latter, IL-8. In the absence of TLR5 in particular, a variety of inflammatory gut conditions can result, including colitis and metabolic diseases. Improvements in immunocompetence and tolerance are correlated with increased numbers of (i) Lactobacillus spp. via the tolerization of dendritic cells and (ii) Bacteroides fragilis via cellular immunity, lymphoid organogenesis, and mucosal immunity (26).

Immunocompetence tolerance, angiogenesis, and lipid metabolism are also affected by the presence of Bacteroides thetaiotaomicron (26). A major gut commensal, B. fragilis, uses polysaccharide A to signal via TLR2 on Foxp3(+) regulatory T cells to allow niche-specific colonization (30); this has led to the proposal that the immune system can discriminate between pathogens and the microbiota through recognition of bacterial molecules to allow symbiosis (30).

(iii) Obesity and metabolic syndrome. Studies of mice have underpinned the idea that the gut microbiota affect the use of the energy provided by the diet and that this can lead to obesity (31). Subsequent studies of human twins revealed that obesity is associated with phylum-level changes in the microbiota, reduced bacterial diversity, and altered representation of bacterial genes and metabolic pathways (32). Such connections between obesity and the microbiome are motivating intriguing albeit still anecdotal experiments (33). Obesity is closely linked to metabolic syndrome, which is a set of metabolic abnormalities associated with insulin resistance. In addition to obesity, these abnormalities include hyperglycemia, hyperlipidemia, and hypertension, and those affected often progress to type 2 diabetes. TLRs are again involved. Mice genetically deficient in TLR5 exhibit hyperphagia and the metabolic abnormalities associated with metabolic syndrome (34). Significantly, the microbiota from these TLR5 knockout mice are sufficient to transfer metabolic syndrome to wild-type (WT) germfree mice (34). It has been argued that the loss of Toll-like receptor 5 function results in a failure to manage microbiota, increased activation of hematopoietic TLR4, changes in gene expression that affect both nutrient acquisition and the production of antimicrobials and pro- and anti-inflammatory cytokines, and, finally, metabolic syndrome or chronic inflammation in the gut. As part of this, an inflammatory explanation for insulin resistance has been advanced based on the following sequence of events: increased caloric consumption (leading to obesity [32]), nutrient excess, endoplasmic reticulum stress, microbiota-mediated inflammatory signaling (via NF-κB, MAPK, and JNK), and, finally, desensitization of insulin receptor signaling, which loops back to increase caloric consumption. Another Toll-like receptor, TLR2, is also implicated in insulin resistance. TLR2 mutant mice, raised under normal rather than germfree conditions, have gut microbiota with a 3-fold increase in Firmicutes and a slight increase in Bacteroidetes similar to those found in obese mice (and humans) and develop symptoms characteristic of metabolic syndrome, including subclinical inflammation, insulin resistance, glucose intolerance, and obesity as well as increased LPS absorption; significantly, these symptoms responded to antibiotic treatment (35). In turn, transplantation of these microbiota to wild-type mice led to symptoms of metabolic syndrome. In this case, the TLR4 and JNK pathway was again activated but the IκB kinase (IKKβ)–IκB–NF-κB pathway was not.

(iv) Thyroid disease. Lipopolysaccharide (LPS), a glycolipid present in the cell wall of Gram-negative bacteria, is mainly recognized by the TLR4/MD2/Cluster of differentiation 14 complex (CD14). In rodents, LPS acts directly on thyroid cells via TLR4 to upregulate thyroglobulin gene expression. LPS increases thyroid-stimulating hormone (TSH)-induced iodide uptake as well as synthesis of the sodium iodide symporter, the first step in thyroid hormonogenesis. The fact that thyroid cells are able to recognize and respond to LPS supports the idea of a role of the endotoxin as a potential modiﬁer of thyroid function (36).
(v) **Cardiovascular disease.** Heart weight was significantly reduced in germfree mice compared with mice that had received a gut microbiota transplant from conventionally raised donors (37). This myocardial-mass phenotype was reversed in the germfree mice by a ketogenic diet (37). Cardiovascular disease is associated with choline, TMAO, and betaine, which are derived from the dietary lipid phosphatidylcholine (38). Using germfree mice, the gut microbiota were shown to be important in TMAO production, increased macrophage cholesterol accumulation, and foam cell formation; moreover, suppression of intestinal microflora in atherosclerosis-prone mice inhibited dietary-choline-enhanced atherosclerosis (38).

(vi) **Cancer.** Media conditioned by the growth of *Bacillus polymyxa* inhibited the growth of human colon cancer cells in vitro and of tumors of such cells in mouse xenograft experiments (39); these effects were associated with a reduction in the mRNA and protein levels of ErbB2 and ErbB3 as well as the levels of E2F-1, which regulates CyclinD1 production, and of CyclinD1 itself, which is required for ErbB-dependent transformation. These results have led to *B. polymyxa* administration being proposed as a preventative treatment for bowel cancer (39). Recently, inflammation was shown to increase the frequency of colorectal cancer in mice by favoring the proliferation of an *E. coli* strain that produces a DNA-damaging toxin, colibactin (40).

**Pleasure and food.** The choice of one sort of food over another is often determined by the pleasure given by the different foodstuffs available, while the amount consumed can be determined by feeling “full.” There is some evidence that bacteria may be able to modulate human reward systems based on dopaminergic activity and to modulate feelings of satiety based on the presence of peptide YY (PYY).

(i) **Dopamine.** The pleasure that results from eating is largely mediated by dopaminergic activity in the mesolimbic system, which has been implicated in reinforcement and motivation, and the rewarding stimuli include chemicals that directly promote dopaminergic neurotransmission (41). Bacteria can produce chemicals that affect this reward system. A common soil bacterium, *Streptomyces venezuelae*, produced a metabolite that, by disrupting the ubiquitin-proteasome system, destroyed dopamine neurons in *Caenorhabditis elegans* (42); this neurodegeneration was exacerbated by the presence of dopamine. Recently, a role for *H. pylori in Parkinsonism* has been suggested, following studies that show *H. pylori*-infected mice are more likely to have lower levels of dopamine in the area of the brain controlling movements (43); it is believed that this has a chemical basis insofar as the effect occurred even when the bacteria were dead.

(ii) **Peptide YY.** There is an obvious possible relationship between the amount of food consumed and the nature of the bacteria that proliferate. The rules of the game are clearly different when competition between the microbiota (and the host) is for a very limited quantity of nutrients versus when it is for an almost unlimited quantity. One factor affecting the amount of food consumed is the sensation of feeling full. PYY is a pancreatic polypeptide produced by the endocrine L cells in the distal small intestine and colon. The main circulating form of PYY reduces food intake and prolongs intermeal intervals in several animal models (for references, see reference 44). The concentrations of PYY and glucagon-like peptide 1 in the plasma after eating, as well as the impression of satiety, were increased in healthy humans by supplementing their diet with prebiotics such as glucosyl-(fructosyl)$_n$-fructose and (fructosyl)$_n$-fructose (45). Significantly, these indigestible carbohydrates promote the growth of certain species of bacteria, including bifidobacteria.

**POSITIVE-FEEDBACK POSSIBILITIES**

The molecules produced by bacteria may act on the host central nervous system in a variety of ways. For example, the bacterial signaling molecule indole affects the tight junctions in epithelial cells (46) and is structurally similar to melatonin, which helps regulate the sleep-wake cycle by causing drowsiness and lowering body temperature; tyrosine and tryptophan, which can cross the blood-brain barrier (47), are converted into dopamine and serotonin in the brain, with dopamine inducing pleasure and alertness and serotonin inducing peacefulness and sleep (48); fatty acids may affect signaling via the lipid rafts present in host cell membranes (49). This signaling could, via dopamine- and peptide YY-influenced feelings, condition in Pavlovian fashion the choices made by the hosts between feasting and fasting, between high- and low-fat intake, and between high- and low-carbohydrate diets. Such choices themselves would then help determine the composition of the gut.

Positive feedback could also operate in the case of catecholamine-mediated stress in which the microbiota are implicated; for example, microbial activation of the stria terminalis can result in anxious behavior, as discussed above (5). Similar stressed behavior may lead to dysfunctional eating habits and altered transit times which might then affect the production of autoinducers and thereby help maintain the very microbiota that contribute to the stress.

What might happen in the absence of positive feedback? Conceivably, a Red Queen situation might occur in which the host immune system responds to the composition of the microbiota, which then changes composition in response, thus causing the immune system to change again, *ad infinitum*. Such immune-mediated alteration of the microbiota may constitute a much greater perturbance than that resulting from normal feedback between microbiota and host. The temporal sequence of compositions of the gut microbiota might then resemble what happens in a chaotic system in which small differences in initial conditions lead to widely diverging outcomes and long-term prediction is usually impossible (50); that said, such a prediction might be envisaged using modeling approaches designed to study the behavior of systems with many parameters (51). It is tempting to draw a parallel between the idea of a Red Queen situation in the microbiota-immune relationship and the suggestion that AIDS results from a prolonged immune overactivation induced by HIV during the course of chronic infection, ending in “exhaustion” and the eventual collapse of the immune system (52).

**PREDICTIONS**

**Food preferences, bacterial populations, and diseases of couples.** We predict that people living together and eating the same food (as in the case of many married couples) should suffer similar ailments due to similar bacterial populations in their guts. It might be argued that pregnant women are known to develop hyperphagia and cravings for particular foods, although their diet may be similar to that of their partners (44, 53). What is not known is whether these hormonal changes lead to changes in the composition of their gut microbiota that precede the changes in food preferences (54, 55). It should be noted that the risk for a disease...
Depends on the location and the associated gut microbiome rather than genetic differences between the populations (54), which are likely to be related to the combination of major metabolic processes in mammals being under symbiotic control (55) and different food preferences being characteristic of human populations in different parts of the world.

**Microbial endocrinology.** The areas in the brain known to be involved in pleasure should be stimulated by injection or ingestion of some of the components generated by bacteria such as indole, polyamines, short-chain fatty acids, tryptophan, etc. (see above). Such stimulation might be tested using functional magnetic resonance imaging which images the changes in blood flow related to neural activity via the paramagnetic properties of oxygenated and deoxygenated hemoglobin. Alternatively, labeled radioligand (an antagonist of dopamine for binding to dopamine receptors in the striatum) might be used in conjunction with positron emission tomography scanning (56, 57).

**Behavioral experiments.** Mice could be reared separately on two different diets with associated food preferences. Fecal transplants could be performed so that some of the mice on diet A receive the gut microbiota from those on diet B and vice versa. The mice would then be given a choice of diets A and B to see whether food preferences are altered.

**Compositional stability experiments.** In the hypothesis in which the composition of the gut microbiota changes constantly and results in disease, the prediction is that health would be improved by any stabilization of this composition. Such stabilization might be achieved by regularly feeding animals a large inoculum which the composition of the gut microbiota changes constantly. Health benefits should accrue from such stabilization despite wide variations in the composition of the inoculum (that is, the bacterial species used may not matter much).

**Discussion**

The gut is a complex ecosystem in which different species of bacteria must compete and collaborate with one another and with the cells of their host in order to survive and multiply. The food eaten by the host is an important factor in the continuous selection that confronts these bacteria, and the nature of this food is often determined by the preferences of the host. If a bacterial species, or group of bacterial species, could manipulate host preferences, it should be fitter than those that have not. The first issue, then, is whether bacteria are capable of having acquired such a capacity. It seems reasonable to suppose that bacteria have had both the time—millions of years—and the formidable adaptive machinery needed to control their hosts.

The second issue is whether there is any evidence supporting the hypothesis. It has long been clear that the gut microbiota respond both to the nutrients consumed by their hosts and to the state of their hosts as signaled by various hormones. It is now becoming clear that the gut microbiota may play a role in diseases other than those usually associated with the gut—and that this entails bacteria influencing host signaling pathways. These diseases include thyroid disease, cancer, and metabolic syndrome. There are, therefore, several ways in which bacteria can manipulate their hosts. In addition, a few pioneering studies implicated the gut microbiota in mood disorders and suggested that bacteria have the capacity to manipulate our feelings via, for example, actions exerted on dopamine (42, 43) and peptides involved in appetite such as glucagon-like peptide 1 and peptide YY (45). In deed, it was found that *C. jejuni* could induce anxiety-like behavior in mice and it was proposed that this involved the vagal pathway (5). The ensemble of these results is therefore consistent with the idea that bacteria could have evolved the capacity to affect our behavior and, in particular, our appetite.

The third issue is whether the gut microbiota really do use this capacity to influence our choice of food. We propose here a number of experiments that may help address this issue. These experiments include epidemiological studies and experiments correlating the presence of particular bacterial metabolites with images of the activity of regions of the brain associated with appetite and pleasure.

**Acknowledgments**

We thank Bob Root-Bernstein and the reviewers for helpful comments.

**References**


