Published in final edited form as:

Curr Med Chem. 2012 ; 19(10): 1519–1529.

Reactive Oxygen Production Induced by the Gut Microbiota: Pharmacotherapeutic Implications

R.M. Jones, J.W. Mercante, and A.S. Neish*

Emory University School of Medicine, Atlanta, GA, USA

Abstract

The resident prokaryotic microbiota of the mammalian intestine influences diverse homeostatic functions, including regulation of cellular growth, maintenance of barrier function, and modulation of immune responses. However, it is unknown how commensal prokaryotic organisms mechanistically influence eukaryotic signaling networks. Recent data has demonstrated that gut epithelia contacted by enteric commensal bacteria rapidly generate reactive oxygen species (ROS). While the induced generation of ROS *via* stimulation of formyl peptide receptors is a cardinal feature of the cellular response of phagocytes to pathogenic or commensal bacteria, evidence is accumulating that ROS are also similarly elicited in other cell types, including intestinal epithelia, in response to microbial signals. Additionally, ROS have been shown to serve as critical second messengers in multiple signal transduction pathways stimulated by proinflammatory cytokines and growth factors. This physiologically-generated ROS is known to participate in cellular signaling *via* the rapid and transient oxidative inactivation of a defined class of sensor proteins bearing oxidant-sensitive thiol groups. These proteins include tyrosine phosphatases that serve as regulators of MAP kinase pathways, cytoskeletal dynamics, as well as components involved in control of ubiquitination-mediated NF- κ B activation. Consistently, microbial-elicited ROS has been shown to mediate increased cellular proliferation and motility and to modulate innate immune signaling. These results demonstrate how enteric microbiota influence regulatory networks of the mammalian intestinal epithelia. We hypothesize that many of the known effects of the normal microbiota on intestinal physiology, and potential beneficial effects of candidate probiotic bacteria, may be at least partially mediated by this ROS-dependent mechanism.

Keywords

Formyl peptide receptors; Inflammatory bowel disease; Nox enzymes; Probiotics; *Lactobacillus*; Wound healing; Hormesis; Crohn's disease

EUKARYOTIC-PROKARYOTIC INTERACTIONS AND INTESTINAL EPITHELIAL HOMEOSTASIS

Mutually beneficial, or symbiotic host-microbe interactions have coevolved over millennia in many animals, with the human luminal microbiota representing an example of significant medical importance [1]. The vast majority of these microbes, represented by ~500 genera of bacteria, are broadly grouped into two taxonomic divisions: the Bacteroidetes and Firmicutes. An accurate accounting of the microbiota is not practical by conventional microbiological techniques; however, recent high-throughput sequencing and molecular taxonomic methodologies have greatly increased our understanding of the population composition, dynamics, and ecology of the gut microbiota [2] (and reviewed in [3–7]).

The mammalian gut is sterile in utero and is colonized immediately both during and after birth, rapidly developing a diverse and stable community, though marked variations in microbial composition between individuals is typical [8]. Members of the microbiota may exist in a planktonic state, free-living in the luminal stream, or they may be partially adherent to the gut mucosa. Total numbers vary greatly from $\sim 10^{11-12}$ cells/gram of intestinal luminal content within the ascending colon, to $\sim 10^{7-8}$ in the distal ileum, and $\sim 10^{2-3}$ in proximal ileum and jejunum. Most members of the microbiota are autochthonous, meaning indigenous and stable, though allochthonous, or transient members are not uncommon (certainly most enteric pathogens fall into this category). Each of these variables could influence the manner and degree of any bacterial-host crosstalk.

The gastrointestinal mucosa comprises the gut epithelia and the immune, vascular and structural support in the lamina propria that together function as a dynamic barrier between the luminal contents and the underlying systemic compartment. Impressively, the mucosa performs vital fluid and nutrient absorptive functions in the presence of the microbiota and their products. Ultimately, the microbiota is separated from the host interior by only a single layer of epithelial cells (or epithelial-derived, e.g. mucus layer). Epithelial cells, by definition, act as interfaces between the host and the environment, and are equipped with apical surface specializations (e.g., microvilli, intercellular junctions) to permit physiological function (e.g., mucus production, vectorial ion secretion) while contacting the luminal contents. Additionally, the intestinal epithelium actively and continually renews itself in a process requiring proliferation of progenitor stem cells, and migration and regulated shedding at the luminal surface (a process that occurs in 5–7 days in humans), while maintaining proper barrier function.

Intestinal bacteria thrive in a stable, nutrient rich environment while providing beneficial functions to the host including energy salvage of otherwise indigestible complex carbohydrates, vitamin and micronutrient syntheses, stimulation of immune development, and competitive exclusion of pathogenic microorganisms [3, 9]. Interestingly, studies with germ-free mice have revealed that the microbiota is not functionally insulated from the mucosa, but rather gut bacteria fundamentally influence epithelial barrier function, metabolism, proliferation and survival [3, 10–13]. For example, the small intestinal villi of the germ-free gut are elongated, while crypts are atrophic, and exhibit slower turnover of epithelial cells [14] along with defective angiogenesis [15]. Germ-free mice monocolonized

with a single gut symbiont species (*Bacteriodes thetaiotaomicon*) exhibit robust host transcriptional response, indicating that host recognition of the microbiota occurs [13]. Thus, there exists a dynamic and largely symbiotic relationship between the host and its commensal flora. However, under some conditions, a “dysbiotic” microbial population may be sufficient to provoke intestinal inflammation, such as that seen in inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn’s disease (CD) [16]. In addition, there is increasing interest in quantitative and/or qualitative abnormalities of the flora that may be associated with other systemic immune, allergic, metabolic and infectious disorders [17, 18]. Consequently, there is a concerted effort to understand the potential therapeutic benefits of supplementing the normal flora with exogenous viable bacteria. This approach, termed “probiotics”, has been reported to dampen inflammation, improve barrier function and increase reparative responses *in vitro* and has shown promise as therapy in several inflammatory and developmental disorders of the intestinal tract [19, 20]. Thus, a growing body of compelling evidence suggests that the gut flora beneficially affects intestinal homeostasis and, by extension, systemic organismal health. However, little is known of how the host perceives non-pathogenic bacteria, or how the microbiota mechanistically influences gut biology. Herein, we describe a fundamental, highly conserved response of host epithelial cells to bacteria that likely forms a component of the host-microbiota interaction.

INTESTINAL PERCEPTION OF THE MICROBIOTA

The gut must respond to bacterial pathogens; and, by extension, the gut must also respond to and manage the commensal microbiota [21, 22]. The now well-studied Toll-like receptors (TLRs) and related Nod proteins, both designated “pattern recognition receptors” (PRRs), recognize and bind to conserved structural motifs present on the surface of a wide range of microbes, termed “microbe associated molecular patterns” (MAMPs) [22]. Pattern recognition receptor initiated signaling is generally considered pro-inflammatory; however, the microbiota exerts positive influences on normal homeostatic maintenance and reparative responses through basal, low-level Pattern recognition receptor activation [23, 24].

An understudied form of pattern recognition receptor is the formylated peptide receptors (FPR). While not typically considered pattern recognition receptors in the same biochemical class as leucine-rich repeat-bearing TLRs or Nods, the FPRs are clearly, by definition, pattern recognition receptors that recognize and respond to bacterial products. Classically, the FPRs are seven membrane pass, G-protein-linked surface receptors expressed on neutrophils and macrophages, where they perceive bacterial cell wall products and stimulate phagocyte functions [25]. Human FPRs consist of three structurally-related receptors that have recently been renamed as FPR1, FPR2 and FPR3 (the respective previous nomenclature was FPR, FPRL-1 and FPRL-2) [26]. An unusual yet important feature of FPR family members is their marked ligand diversity and overlapping ligand recognition properties. FPRs respond to bacterial components such as translation products tagged with a characteristic bacterial specific N-formyl group; the classic example of which is N-formyl methionyl-leucyl-phenylalanine (fMLF). FPR1 has been characterized as the high-affinity receptor for fMLF with an ED₅₀ in the nanomolar range, while FPR2 is the low affinity receptor that responds to fMLF in the micromolar range. Additionally, endogenous agonists

(e.g., AnxA1, mitochondrial formyl peptide, LXA4 and SAA) also stimulate FPRs and transduce pleiotropic biological responses [26]. Interestingly, FPR2 was initially identified as a receptor for the lipid LXA4 [27, 28], a host endogenous compound with anti-inflammatory (and potential therapeutic) functions. FPR1-null mice (i.e., mFPR^{-/-} or mFpr1) have increased susceptibility to bacterial infection supporting a role of this receptor in host defense [29], while mitochondrial formyl peptides can activate systemic shock in an FPR1-dependant manner, suggesting a role in non-infectious tissue injury [30].

FPRs are subject to regulation by phosphorylation and glycosylation in an agonist, concentration, and time-dependent manner; thus, downstream FPR-dependent signaling events vary greatly with the specific ligand, dose, duration of stimulus and receptor bound [31, 32]. For example, FPR1 has 22 potential serine/threonine phosphorylation sites on its cytoplasmic face which likely generate unique signaling complexes that differentially modulate cellular signaling pathways and outcomes. Studies of FPR function in phagocytes have revealed that activated FPR receptors undergo a conformation change that allows binding with pertussis toxin-sensitive Gi proteins which in turn triggers exchange of GDP for GTP in the α subunit and dissociation of the β and γ subunits [33, 34]. Subsequent signaling involves both phosphatidylinositol 3-kinase (PI3K) and MAPK pathways, calcium release, and small GTPase activation which eventuate in: 1) changes in actin dynamics and initiation of chemotaxis; 2) transcriptional upregulation of inflammatory effectors and cytokines; and 3) the activation of NADPH oxidase enzymes and ROS generation (e.g., the respiratory burst) [35–37]. It is this latter function, recently described in the intestinal epithelium, which has provided the impetus toward investigating the role of ROS in host-commensal interactions, beyond the traditionally accepted host-pathogen relationship. This concept was further advanced when immunohistochemical staining experiments detected FPRs located on the apical surface of the intestinal epithelia, prompting interest that this, and related epithelial receptors, may mediate physiological responses in the gut [38]. Subsequent studies have demonstrated that, in epithelial cells, the MAPK ERK pathway is activated by formylated peptides in a FPR-dependant manner and that the process depends of FPR dependant ROS generation in the epithelia [39]. These data suggest FPRs can be viewed as key pattern recognition receptors that control biological responses of cells to both endogenous and exogenous (i.e., microbial) ligands and they provide a currently well-understood link between perception of bacteria and the generation of ROS.

PHYSIOLOGICAL GENERATION OF REACTIVE OXYGEN SPECIES

Reactive oxygen species are short lived, highly electrophilic molecules that result from the incomplete reduction of molecular oxygen, and include radical forms (superoxide, O₂⁻) and non-radical peroxide forms (H₂O₂). Superoxide is largely membrane impermeant while H₂O₂ is considerably more stable, can cross cell membranes and travel the equivalent of several cell distances. At high levels they are considered potentially microbiocidal, necessary for the killing of engulfed organisms within phagocytic cells. In non-phagocytic cells ROS are commonly conceptualized as inadvertent and deleterious by-products of aerobic respiration within the mitochondria. ROS, especially superoxide, are highly reactive and are capable of inflicting macromolecular damage on vital cellular components such as membrane lipids and nucleic acids. Thus, cells have necessarily developed biochemical

machinery to manage the presence of intracellular ROS. Enzymatic scavengers include superoxide dismutase (SOD) that serves to convert the superoxide radical into H_2O_2 , and numerous catalases that further convert H_2O_2 to H_2O . Additionally, the cell can increase levels of cysteine-rich proteins and peptides such as glutathione, the thioredoxins, peroxiredoxins and others that function as “redox sinks”. These proteins contain numerous cysteine residues in a basal reduced thiol anionic form that are rapidly oxidized by ROS, quenching further biochemical reactivity. These compounds can be transcriptionally regulated by the action of redox-sensitive signaling pathways such as the Keap1/Nrf2/ARE regulatory mechanism (discussed below) providing a feedback mechanism for management of redox stresses.

As mentioned, the reactive nature of superoxide has been harnessed for beneficial effect in phagocytic cells. “Professional” phagocytes include short lived neutrophils (with a half-life of 6 hours upon exiting the bone marrow) and macrophages (multifunctional immune regulator and effector cells that can live the life of the organism) and are capable of detecting invading bacterial pathogens *via* surface FPRs. Upon initial perception of formyl peptides, the phagocytes undergo cytoskeletal rearrangements that allow extension of cytoplasmic processes (pseudopodia) that induce directed migration (chemotaxis), and when a critical concentration is reached, engulf the offending bacteria. Next, FPRs, *via* small GTPase proteins, set in motion the “oxidative burst”, the large scale physiological generation of superoxide within the phagocytic vacuole containing the bacterium. In this case, the generation of ROS is deliberate, and the product of specialized and dedicated enzymatic machinery. Classically, the oxidative (or respiratory) burst is mediated by a membrane-bound NADPH-dependant multi component enzyme complex. The phagocyte NADPH oxidase, Nox2 (formerly gp120phox), is a basally inactive multi-subunit complex comprised of a membrane-bound dimer of p22phox and gp91phox [40]. Given the toxicity of high levels of superoxide, understandably, this process is tightly regulated by G- protein mediated activation. The *in vivo* role of this enzyme in host defences is vividly illustrated by the fact that the genetic absence of Nox2 function results in chronic granulomatous disease (CGD), a condition where phagocytes fail to produce ROS and patients are predisposed to recurrent pyogenic infections. Invertebrate phagocytes stimulated by microbial products (e.g., formylated peptides) generate ROS in the same manner as mammalian neutrophils, and plants also induce ROS generation in response to bacterial pathogens and symbionts [40, 41], highlighting the conserved and fundamental nature of bacterially-elicited ROS.

The classical phagocyte Nox2/gp120phox is the founding member of the NADPH oxidase family or “Nox’es”. This family is represented in many non-phagocytic cell types, with Nox1 and Dual oxidase 2 (Duox2) strongly expressed in intestinal epithelia [40, 42, 43]. In general, the non-phagocytic NADPH oxidases exhibit similar, but not identical organization to the phagocyte enzyme. All subunits of Nox1 are membrane bound and solely dependent on Rac-GTPase-mediated events for activity, while Duox enzymes are calcium induced. Nox-dependant generation of ROS, critical for modulating numerous signal transduction pathways, has been observed after receptor activation by various hormones, cytokines and growth factors [40, 43]. Orthologs of the Nox/NAPDH family mediate ROS generation throughout multicellular life [44–47], including *Drosophila*, where ROS is necessary for

commensal-induced gut epithelial homeostasis [48] and in plants, where ROS levels were shown to control the transition from proliferation to differentiation in the root [49]. Interestingly, in the case of the fly, ROS generation occurs in the epithelia, and is necessary for control of the luminal flora. Here, Duox was shown to be responsible for infection-induced ROS generation, and for limiting the onset of microbial proliferation in the gut of non-pathogenic, commensal microbes [48, 50]. This latter observation suggests a conserved role for epithelial ROS generation (as opposed to strictly phagocytic) in gut homeostasis and microbial control. Other roles for epithelial ROS have been suggested. Nox1 activation has also been shown to be important for migration of colon epithelial cells in culture [51], strongly suggesting direct effects on epithelial proliferation/migration. In summary, Nox-dependent ROS generation, while critical for phagocyte-mediated defense, may play an important role for bacterial-induced ROS mediating critical intestinal epithelial processes.

A recent key discovery was the demonstration that several species of human commensal gut bacteria induce rapid, “deliberate” generation of physiological levels of ROS within human epithelial cells (Fig. (1)) [52]. Furthermore, epithelial cells co-cultured with certain bacteria rapidly show increased oxidation of soluble redox sinks, such as glutathione and thioredoxin, and exhibit an increase in redox-stimulated transcriptional activation, reflecting a cellular reaction to increased ROS. Interestingly, different strains of commensal bacteria can elicit markedly different ROS levels from contacted cells. *Lactobacilli* are especially potent inducers of ROS generation in cultured cells and *in vivo*, though all bacteria tested have some ability to alter the intracellular redox environment. This is not surprising given that phagocytes can induce a respiratory burst regardless of whether they encounter nominal pathogens or stray commensals. High ROS-stimulating bacteria, such as *Lactobacilli*, may possess specific membrane components or even secreted factors that activate cellular ROS production. For instance, Yan [53] reported soluble factors of *Lactobacilli* that mediated beneficial effects in *in vivo* inflammatory models. Alternatively, high ROS-stimulating bacteria may simply possess enhanced adhesion or the ability to penetrate mucous layers wherein they gain additional proximal access to cellular receptors (e.g., TLRs and FPRs). FPRs, especially, are promising candidates for this function as they are expressed on apical surfaces and are known to directly stimulate ROS production in phagocytes and epithelial cells. This process, however, may not be as simply explained because bacteria, unlike individual peptides and cytokines, are multifaceted biological stimuli and would be expected to interact with a complex range of cellular receptors and influence diverse processes.

ROS SIGNALING AND REACTIVE CYSTEINES

Besides being unavoidable by-products of metabolic processes, or functional antimicrobial molecules, evidence is emerging that certain ROS species, such as H₂O₂, possess essential signaling roles. The specificity of biological responses to altered levels of ROS within these signaling events can be modulated by the specific ROS molecule, the intensity/duration of the production, the subcellular sites of generation and the developmental stage of the cell [43, 47]. As short lived molecules, ROS can have very small functional radii, which contributes to their selective action. Indeed, certain receptors physically interact with a ROS-generating Nox enzyme, presumably to limit ROS-mediated influences to the immediate vicinity of effector proteins [54].

Cellular redox signaling is mediated by “sensor” proteins, generally regulatory enzymes, whose activity can be variably modulated by ROS. These redox-sensitive proteins are modified by reversible H₂O₂-mediated oxidation of their active site cysteines, thus allowing for graded perception of intracellular H₂O₂ concentrations and control of critical steps in signal transduction pathways. In most redox-responsive proteins, cysteine residues are protonated at physiological pH (Cys-SH) ($pK_a \sim 8.5$), whereas so-called low pK_a cysteines exist as thiolate anions (Cys-S[−]) and are more readily oxidized by H₂O₂ as a result of vicinal charged amino acids (Fig. (2)) [55]. These redox-sensitive thiolates are present under physiological conditions in a limited but increasingly recognized subset of enzymes [55–59]. Specific examples of such oxidant-sensitive proteins include class I protein tyrosine phosphatases, such as lipid phosphatase (PTEN) and MAP kinase phosphatases (MAPK-P or DUSPs); class II protein tyrosine phosphatases, such as low-molecular-weight protein tyrosine phosphatases (LMW-PTPase); and regulatory enzymes of ubiquitin and ubiquitin-like proteins such as SUMO and Nedd8 [56, 57, 60, 61], (Fig. 1). Highlighting the intimate interplay between redox-regulation and innate immunity is the discovery that reduction of disulphide-bridges within human β -defensin 1 is necessary for the molecule’s potent antimicrobial activity against a pathogenic fungus and anaerobic Gram-positive bacteria [62]. Other oxidant sensors are employed in the control of overall cellular redox balance, discussed next.

Keap1/Nrf2/ARE Signaling

The Keap1/Nrf2/ARE signaling module is an evolutionarily conserved pathway through which eukaryotic cells sense and rapidly respond to electrophilic and oxidative stresses, thus maintaining intracellular redox homeostasis. While originally discovered in a screen for proteins that bind the β -globin gene cluster enhancer element [63], Nrf2 (NF-E2-Related Factor 2) and its antagonist Keap1 (Kelch-like ECH-Associated Protein 1) were quickly recognized as central to the host response to xenobiotics (Fig. (3)) [64–67]. In addition to humans, components of the pathway have been functionally described in most widely used metazoan model systems including *C. elegans* [68], *D. melanogaster* [69], zebrafish [70], and mouse [65]. Nrf2 is a member of the CNC (cap-n-collar) family of basic leucine zipper (b-Zip) transcription factors [71], and is post-translationally regulated through the action of its specific inhibitor, Keap1 [67, 70, 72]. Under basal reducing conditions Keap1 binds to Nrf2, in a manner similar to the well-studied NF- κ B/I- κ B/ β -catenin pathway, promoting Nrf2 cytoplasmic degradation through a cullin-dependent E3 ubiquitin ligase [73–77]. Cellular pro-oxidant or electrophilic stress causes oxidation of redox-sensitive cysteines within Keap1 [78, 79] (and possibly in Nrf2 itself [80, 81]) leading to a conformational change in Keap1, inhibition of proteosomal degradation and release of Nrf2 [76, 77]. Liberated Nrf2 then translocates to the nucleus, dimerizes with a small Maf protein [65, 82] and binds to a well-defined antioxidant response element (ARE) sequence (5-RTGACnnnGC-3) [83] thereby activating expression of an extensive gene network (Fig. (3)) [64, 84].

Fundamentally, Keap1/Nrf2/ARE is a cytoprotective pathway [85] that responds to both physiologically generated, endogenous ROS, and xenobiotic stresses. While a comprehensive review of direct-binding Nrf2 targets is beyond the scope of this review, it is

generally accepted that Nrf2-regulated genes fall into at least 2 categories, the antioxidant enzymes, which include superoxide dismutase (SOD), glutathione peroxidase (GPX) and thioredoxin (TXN), and the detoxification enzymes, including those designated “phase II”, such as glutathione S-transferase (GST), heme oxygenase-1 (HMOX1) and multidrug resistance-associated proteins (MRP’s) (for a review see [86–89]). Mice and flies null in Nrf2, or biochemically unable to activate the pathway, fail to upregulate the antioxidant or detoxification effector genes and are hypersensitive to a variety of exogenous insults including UV radiation [90–94], sepsis [95, 96] and inhaled xenobiotic oxidants and toxins [97–106].

Over the past decade, a number of studies have described the Nrf2-inducible and basal regulon, which is currently between ~160 and 670 genes, although estimates vary widely [107–110]. These studies revealed that in addition to overt electrophile and oxidant detoxification, Nrf2 also directly or indirectly regulates concomitant cellular processes including cell cycle progression/proliferation, protein trafficking, proteolysis and ubiquitination, chaperone-dependent stress responses, cell growth and apoptosis, and inflammation and immunity [107, 108, 111–114]. During injury, a high level of ROS is generated by inflammatory responses, secondary to the large respiratory burst from professional phagocytes, which further contributes to tissue injury associated with acute inflammation. Thus, it is likely that ROS in inflamed tissue requires the Nrf2 pathway to elevate antioxidant defences, promote cellular repair and re-establish physiologically proper redox balance (for a review see [115]). And with the recognition of ROS as a modulating factor in cellular proliferation, Nrf2-dependent effects are being examined in closer detail. Experiments in dermal models suggest Nrf2-mediated cytoprotective responses may serve to protect stem cell populations and thus protect normal and restitutive cellular proliferation [90]. Nrf2-modulated ROS may indeed represent an evolutionarily conserved pro-proliferative/differentiation circuit [116, 117]. For example, induced ROS is a key elicitor of hematopoietic differentiation in *Drosophila* [118], while it also stimulates root development in plants [46]. Nrf2 pathway control of cell proliferation and differentiation is further demonstrated in Keap1 null mice, where constitutive Nrf2 activation results in a proliferative phenotype in squamous epithelia [119]. In addition, Nrf2 activation has been shown to upregulate the Notch differentiation pathway [120].

Keap1/Nrf2/ARE has been most heavily studied in the context of the skin, respiratory tract and nervous system, but it is clear that this pathway is also critical for intestinal health and maintenance. In the gut, Nrf2 null mice are hypersensitive to DSS colitis [121, 122] and traumatic injury [123, 124]. And work from our research group (unpublished data) as well as others [125] confirm that gut-directed over-expression of the *Drosophila* nrf2 homologue, cncC, or keap1RNAi protects flies from oxidant-induced mortality. Interestingly, we have previously described the modulation of epithelial restitution and inflammatory signaling *via* commensal-bacteria-induced cellular ROS generation [52, 126–128]. Therefore, the relationship between bacterial-dependent ROS generation and Nrf2 may be of considerable potential importance given that all metazoans live in intimate contact with a native microbial population. Preliminary results from our research group suggest that *Drosophila* colonized with human commensal bacteria rapidly generate a pulse of ROS in the intestine which is

followed, both spatially and temporally, by Nrf2-dependent gene expression. Importantly, the Nrf2/Keap1/ARE signaling pathway may represent a novel mechanism by which the host perceives and responds to microbial stimuli and therefore may play a major role in regulating gut physiology and host-microbial crosstalk through bacteria-induced ROS generation.

MICROBIAL EFFECTS ON INFLAMMATORY SIGNALING

Microbially-elicited ROS may affect other pathways besides those dedicated to ROS detoxification, suggesting ROS-mediated signaling may have evolved to regulate numerous epithelial processes. A variety of reports have described commensals, many employed as probiotics that suppress eukaryotic inflammatory signaling pathways, such as NF- κ B, and block inflammatory effector functions (Fig. (1)) [53, 129–132]. For example, the gut symbiont *Bacteroides thetaiotaomicron* has been shown to inhibit NF- κ B by regulating nuclear translocation of the p65 subunit [133]. Several laboratories have demonstrated that intestinal bacteria are able to influence inflammation, and likely other cellular regulatory processes, by manipulating the ubiquitin-proteasome pathway [134–137]. Ubiquitination is a covalent modification increasingly recognized to play a regulatory role in a wide spectrum of biochemical events, generally by targeting modified proteins for controlled degradation *via* the proteasome organelle. The NF- κ B pathway employs this process through ubiquitination of its inhibitory component, I κ B, thus controlling signal transduction and subsequent immune activation [138]. Indeed, there are numerous examples of pathogens that utilize preformed effector proteins to influence I κ B ubiquitination and thus innate immunity [139–141].

Commensals interacting with the epithelia *in vitro* and *in vivo* are capable of blocking I κ B ubiquitination and thus NF- κ B activation by interfering with the I κ B ubiquitin ligase, SCF β^{TrCP} (Skp1, Cdc53/Cullin, E box receptor) [134, 142, 143]. This enzymatic complex is activated by a second covalent modification, neddylation, on the cullin-1 (Cul-1) regulatory subunit. Neddylation is the covalent modification of the SCF ubiquitin ligase by the ubiquitin-like protein Nedd8, representing a central regulatory event in cellular processes that are controlled by protein degradation, including NF- κ B and β -catenin. This biochemical event occurs by an enzymatic cascade analogous to the ubiquitination reaction, specifically catalyzed by a Nedd8 ligase called Ubc12. Interaction of commensal bacteria with epithelia *in vitro* and *in vivo* results in the rapid and reversible loss of the Nedd8 modification, accounting for the loss of overall SCF ubiquitin ligase function and consequent blockade of NF- κ B activation [142]. The neddylation reaction has been shown to be negatively regulated by oxidative signaling, resulting in transient inactivation of the Nedd8 ligase, Ubc12 (Fig. (1)) [52]. This mechanism for controlling protein activity is reminiscent of the transient oxidative modification of related enzymes in the ubiquitin-like SUMOylation process which also modulates regulatory protein function.

Thus, ROS generation can influence the equilibrium between neddylated and un-neddylated Cul-1 in cells of the gut in contact with the normal flora and thus modulate the activity of the SCF ligases and the pathways these enzymes control. Dynamic changes in ROS production may be involved in suppression of NF- κ B; an effect that may account for the

usual inflammatory tolerance of the intestinal mucosa to native commensals. However, excessive or prolonged ROS generation may result in deleterious suppression of NF- κ B-mediated survival factors and contribute to tissue injury. Finally, E3-SCF ^{β TrCP} is required for the regulation of the β -catenin, Snail, Twist and Hedgehog pathways [144], suggesting additional targets whereby microbially-mediated modulation of SCF E3s could influence many aspects of mucosal homeostasis.

Of note is a recent report describing how pathogenic bacteria require ROS during pathogenesis. ROS generated during *Salmonella typhimurium*-induced inflammation was shown to react with thiosulfate in the intestinal lumen to form tetrathionate, which was identified as a novel respiratory electron acceptor that confers a growth advantage for *S. typhimurium* over the competing gut microbiota [145].

MICROBIAL EFFECTS ON EPITHELIAL CELL MOTILITY

As mentioned, germ-free mice show defective epithelial proliferation and wound healing, suggesting commensal enteric bacteria are able to stimulate epithelial cell migration during development and post-injury. How commensal bacteria affect this process is unclear. Epithelial cell migration depends on coordinated rearrangement of the actin cytoskeleton with spatial and temporal changes in adhesion of the protruding membrane edge to the extracellular matrix at specialized signaling nidus points called focal adhesions (FA). Focal adhesion assembly is regulated by focal adhesion kinase (FAK), a 125 kDa protein that is maintained in an inactive, dephosphorylated form by the constitutive action of the redox-sensitive tyrosine phosphatases LMW-PTPase and SHP-2 [146]. Endogenous physiological stimuli, such as growth factors and integrin engagement with the epithelial basement membrane, induce local ROS production *via* Nox1 activation which results in rapid oxidative inactivation of these PTPase's, consequent focal adhesion kinase phosphorylation and initiation of cellular motility (Fig. (1)) [147]. Similarly, interaction(s) of intestinal epithelia with natural commensal bacterial strains (and formylated peptides) is associated with rapid accumulation of ROS, especially at the leading edge of the migrating cell monolayer. ROS generation results in reversible oxidation of redox-sensitive low pKa cysteines in LMW-PTPase and SHP-2, upregulation of FAK phosphorylation and an increase in the number of FA at the migrating edge of the monolayer, followed by enhanced cell adhesion and velocity of epithelial migration. Functionally, commensal bacteria mediate enhanced wound closure in an *in vitro* model of injury and resolution of dextran sodium sulfate-induced mucosal damage in a mouse model. Thus, ROS production associated with commensal-epithelial contact can stimulate epithelial motility and likely contribute to wound restitution. This data suggests another means by which the microbiota mediates homeostatic effects on the gut lining and a mechanism for certain beneficial effects of probiotics.

MICROBIAL EFFECTS ON EPITHELIAL GROWTH AND DIFFERENTIATION

Germ free mice also exhibit reduced migration of epithelial cells along the crypt-villus axis, suggesting a role for the microbiota in modulating the dynamics of epithelial growth and development. The balance between cellular proliferation and differentiation is a key aspect

of development in multicellular organisms. Physiological levels of ROS generated by Nox or Duox enzymes have been shown to play essential roles in maintaining this balance. An increasing number of manuscripts report these responses in a variety of metazoans. For example, ROS were shown to prime *Drosophila* haematopoietic progenitors for differentiation [118]. It was shown that multipotent haematopoietic progenitors, which are similar to the mammalian myeloid progenitors, display increased levels of ROS under *in vivo* physiological conditions. Dampening ROS levels in these cells dysregulated their differentiation into mature blood cells, whereas increasing ROS in haematopoietic progenitors induced differentiation into all three mature blood cell types found in *Drosophila* through a signaling pathway that involves JNK and FoxO activation as well as Polycomb downregulation [118]. Additionally, modulation of the cellular redox state by the Keap1/Nrf2/ARE signaling module (discussed above) was shown to control intestinal stem cell proliferation in the *Drosophila* midgut in response to oxidative stress, and was shown to function in maintaining intestinal homeostasis in aging flies [148]. In plants, transcriptional regulation of ROS levels was shown to control the transition from proliferation to differentiation in the root by a mechanism that is dependent on the transcription factor UPBEAT1 (UPB1) [49]. UPB1 directly regulates the expression of a set of peroxidases that modulate the balance of ROS between the zones of cell proliferation and elongation where differentiation occurs. Disruption of UPB1 activity alters this ROS balance, leading to a delay in the onset of cellular differentiation. Furthermore, modulation of either ROS balance or peroxidase activity through chemical reagents also affects the onset of differentiation [49]. Growth/differentiation control by a similar mechanism has also been demonstrated in animals; Nox modulation of Wnt and Notch1 signaling can influence the fate of proliferative progenitor cells in the murine colon [149]. Nox1 was shown to be a pivotal determinant of cell proliferation and fate by a mechanism that also integrates the Wnt/ β -catenin and Notch1 signaling pathways. Nox1-deficient mice exhibit increased conversion of undifferentiated progenitors into goblet cells, with a concurrent reduction in the number enterocytes, due to the concerted repression of PI3K/AKT/Wnt/ β -catenin and Notch1 signaling. The report concluded that Nox1 controls the balance between goblet and absorptive cell types in the colon [149]. Together, these examples demonstrate trans-kingdom conservation of function in maintaining a balance between cellular proliferation and differentiation.

However, the intrinsic source of physiological levels of ROS, or the extrinsic stimulus that induces Nox and Duox enzymes to generate ROS is yet to be fully described. As stated previously, our research group recently demonstrated that contact of commensal bacteria with cultured epithelial cells induces the rapid generation of ROS in these cells [128]. Furthermore, we identified that the bacterial product fMLF induces the FPR-dependent generation of ROS in cultured epithelial cells (Fig. (1)) [150]. Using the *Drosophila* midgut model, we have also been able to show that stem cells exist in an area of low ROS levels, and these progenitor cells migrate to areas of higher ROS where differentiation occurs (unpublished data). Furthermore, in mammals, Paneth cells are located at the base of the intestinal crypts and proximal to the stem cell niche in the small intestine. Paneth cells secrete large quantities of antimicrobial proteins to protect the adjacent stem cells from bacterial contact. We thus speculate that as progenitor cells emerge from the stem cell niche/Paneth cell region, and migrate up the villus, they come into contact with bacteria and/or

their products. Here, bacterial-induced generation of ROS plausibly facilitates differentiation and thus represents a mechanism by which microbes influence epithelial cell growth and differentiation. This speculation is currently the focus of intense investigations within our research group.

FUTURE PERSPECTIVES

A series of manuscripts have shown that epithelia exhibit increased ROS generation in response to commensal bacteria in a manner analogous to the events induced in phagocytic cells, suggesting a deep functional conservation. Indeed, recent data in invertebrates suggest that ROS generation for signaling and microbicidal functions in the gut epithelia may represent the primordial ancestral response to bacteria [48]; additional studies in lower metazoans and plants also indicate ROS as a fundamental regulatory (and defensive) molecule. In the mammalian gut, epithelial ROS generated in response to bacteria serves a signaling role (as in many non-epithelial cells), and likely there are numerous ROS-sensitive enzymes that could be influenced by changes in cellular redox status. As has been discussed, reversible oxidative inactivation of a wide range of regulatory enzymes is an increasingly recognized mechanism of signal transduction [47, 60]. Current proteomic approaches that exploit reactive cysteines to label individual peptides may be employed as a high throughput system to screen for microbial-specific, oxidant-sensitive regulatory proteins [151]. Alternatively (but not contradictorily), questions of bacterial-elicited ROS stimulating an epithelial antimicrobial response (as occurs in phagocytes and the *Drosophila* gut), especially in limited locations such as the intestinal crypt, are still to be resolved. Functional analyses of microbial and ROS-dependent outcomes on multiple pathways *in vivo* will be challenging future work.

The source of ROS is an intriguing topic. Clearly the Nox enzymes, especially Nox1 and Duox2 are prime candidates given their pattern of tissue expression in the gut, but other sources such as mitochondrial respiratory chain enzymes, lipoxygenases and others could contribute to redox changes in the cell. The initial inducers and/or triggers for ROS generation are also open questions. FPRs are attractive candidates for receptor-stimulated ROS production, given that many of the same mechanisms that mediate FPR signaling in professional phagocytes are conserved in epithelial cells. Toll-like receptors and Nod proteins have been shown to elicit ROS signaling upon ligand binding. Additionally, it is unclear whether certain commensals, which clearly generate ROS by their own enzymatic machinery, influence eukaryotic signaling by exogenous ROS; conversely, some bacteria could achieve this result by producing anti-oxidants. The secondary effects of ROS production from phagocytic cells under non-inflammatory, physiological conditions or during active inflammation are entirely unknown.

Modulation of ROS-mediated signaling may occur during rapid quantitative changes in microbial populations or qualitative changes in the composition in the gut, during abrupt dietary changes, antibiotic administration, probiotic use, or the developmental acquisition of the normal microbiota during the neonatal period. Additionally, the total prokaryotic population can vary widely along the length of the digestive tract, ranging in number from $\sim 10^4$ cfu/ml in the small bowel, to $\sim 10^{12}$ cfu/ml in the cecum and ascending colon, and

markedly less in the descending colon [152]. Different anatomical regions of the gut may thus experience different levels of ROS. Of note, there is an inverse correlation between bacterial numbers in the colon and the pathological distribution of inflammatory ulcerative colitis, with the disease characteristically most predominant in the rectum and descending colon, which are regions with the lowest bacterial numbers. The observation that different taxa of bacteria exhibit markedly different ROS-inducing potential supports the idea that qualitative changes in community composition can affect host biology. It is possible that the relative abundance of particular gut taxa, with dissimilar ROS-stimulatory capacities could have functional consequences at the organism level. Understanding this relationship between microbes and cellular ROS could allow for defining parameters of “eubiotic” or “dysbiotic” flora associated with health or inflammatory disease, and may be relevant to the development and optimization of probiotics. Long term biochemical accommodation to tonic bacterial presence, as in the colon, may also affect different aspects of redox biology.

We further speculate that the microbiota may upregulate an ROS and Nrf2-dependent adaptive stress response. This concept, termed “hormesis”, is loosely defined as a low level or sub-lethal stress that stimulates endogenous defence mechanisms, such that a system is more resistant to the stress upon second exposure [153]. The hormetic potential of dietary phytochemicals, as well as numerous stressors, is well documented; and the relationship between Nrf2 activation and numerous hormetics is also well established (for a review see [89]). However, while this protective phenomenon has been described in bacteria for a range of stressors including acid [154], heat shock [155] and antibiotics [156], microbes themselves have never been considered hormetics. Overall, an appropriate regulation of cell oxidative balance is emerging as an important regulator of cellular survival, growth and organismal homeostasis.

CONCLUSIONS

In conclusion, cellular generation of ROS through microbe-epithelial contact is a conserved process with many known, expected, and plausible consequences. Therefore, this mechanism is an attractive general and non-selective means by which a complex microbial community could influence a wide range of host signaling and homeostatic processes [143]. It is hoped that a more complete understanding of this mechanism may advance our appreciation of the natural, commensal microbiota and allow for their exploitation as potential probiotics.

ACKNOWLEDGEMENTS

RMJ is supported by NIH Grant K01DK081481. JWM is supported funding from the NIH/NIGMS IRACDA program grant 5K12GM000680, and ASN is supported, in part, by National Institutes of Health Grant R01DK071604.

REFERENCES

1. Neish AS. Microbes in gastrointestinal health and disease. *Gastroenterology*. 2009; 136(1):65–80. [PubMed: 19026645]
2. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J,

- Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Jian M, Zhou Y, Li Y, Zhang X, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010; 464(7285):59–65. [PubMed: 20203603]
3. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. *Science*. 2001; 292(5519):1115–1118. [PubMed: 11352068]
 4. Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, Henrissat B, Coutinho PM, Minx P, Latreille P, Cordum H, Van Brunt A, Kim K, Fulton RS, Fulton LA, Clifton SW, Wilson RK, Knight RD, Gordon JI. Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol*. 2007; 5(7):e156. [PubMed: 17579514]
 5. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*. 2007; 449(7164):811–818. [PubMed: 17943117]
 6. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. *Science*. 2006; 312(5778):1355–1359. [PubMed: 16741115]
 7. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Hostbacterial mutualism in the human intestine. *Science*. 2005; 307(5717):1915–1920. [PubMed: 15790844]
 8. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science*. 2005; 308(5728):1635–1638. [PubMed: 15831718]
 9. Marchesi J, Shanahan F. The normal intestinal microbiota. *Curr. Opin. Infect. Dis*. 2007; 20(5):508–513. [PubMed: 17762785]
 10. Ismail AS, Hooper LV. Epithelial cells and their neighbors. IV. Bacterial contributions to intestinal epithelial barrier integrity. *Am. J. Physiol. Gastrointest. Liver Physiol*. 2005; 289(5):G779–G784. [PubMed: 16227525]
 11. Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology*. 2001; 121(3):580–591. [PubMed: 11522742]
 12. Smith K, McCoy KD, MacPherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol*. 2007; 19(2):59–69. [PubMed: 17118672]
 13. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001; 291(5505):881–884. [PubMed: 11157169]
 14. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc. Natl. Acad. Sci. USA*. 2005; 102(1):99–104. [PubMed: 15615857]
 15. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes *via* Paneth cells. *Proc. Natl. Acad. Sci. USA*. 2002; 99(24):15451–15455. [PubMed: 12432102]
 16. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008; 134(2):577–594. [PubMed: 18242222]
 17. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat. Rev. Immunol*. 2001; 1(1):69–75. [PubMed: 11905816]
 18. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol*. 2004; 12(12):562–568. [PubMed: 15539116]
 19. Park J, Floch MH. Prebiotics, probiotics, and dietary fiber in gastrointestinal disease. *Gastroenterol. Clin. North Am*. 2007; 36(1):47–63. [PubMed: 17472874]
 20. Hord NG. Eukaryotic-microbiota crosstalk: potential mechanisms for health benefits of prebiotics and probiotics. *Annu. Rev. Nutr*. 2008; 28:215–231. [PubMed: 18489258]
 21. Sansonetti PJ. The innate signaling of dangers and the dangers of innate signaling. *Nat. Immunol*. 2006; 7(12):1237–1242. [PubMed: 17110939]

22. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe*. 2008; 3(6):352–363. [PubMed: 18541212]
23. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118(2):229–241. [PubMed: 15260992]
24. Lee J, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, Lee HK, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat. Cell Biol.* 2006; 8(12): 1327–1336. [PubMed: 17128265]
25. Migeotte I, Communi D, Parmentier M. Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev.* 2006; 17(6):501–519. [PubMed: 17084101]
26. Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN, Murphy PM. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* 2009; 61(2):119–161. [PubMed: 19498085]
27. Parkinson JF. Lipoxin and synthetic lipoxin analogs: an overview of anti-inflammatory functions and new concepts in immunomodulation. *Inflamm. Allergy Drug Targets*. 2006; 5(2):91–106. [PubMed: 16613568]
28. Serhan CN, Chiang N. Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. *Scientific World Journal*. 2002; 2:169–204. [PubMed: 12806051]
29. Gao JL, Lee EJ, Murphy PM. Impaired antibacterial host defense in mice lacking the N-formylpeptide receptor. *J. Exp. Med.* 1999; 189(4):657–662. [PubMed: 9989980]
30. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010; 464(7285):104–107. [PubMed: 20203610]
31. Ali H, Richardson RM, Tomhave ED, Didsbury JR, Snyderman R. Differences in phosphorylation of formylpeptide and C5a chemoattractant receptors correlate with differences in desensitization. *J. Biol. Chem.* 1993; 268(32):24247–24254. [PubMed: 8226971]
32. Tardif M, Mery L, Bouchon L, Boulay F. Agonist-dependent phosphorylation of N-formylpeptide and activation peptide from the fifth component of C (C5a) chemoattractant receptors in differentiated HL60 cells. *J. Immunol.* 1993; 150(8 Pt 1):3534–3545. [PubMed: 8468487]
33. Bokoch GM, Gilman AG. Inhibition of receptor-mediated release of arachidonic acid by pertussis toxin. *Cell*. 1984; 39:301–308. [PubMed: 6094010]
34. Lad PM, Olson CV, Smiley PA. Association of the N-formyl-Met-Leu-Phe receptor in human neutrophils with a GTP-binding protein sensitive to pertussis toxin. *Proc. Natl. Acad. Sci. USA*. 1985; 82(3):869–873. [PubMed: 2983319]
35. Jesaitis AJ, Naemura JR, Sklar LA, Cochrane CG, Painter RG. Rapid modulation of N-formyl chemotactic peptide receptors on the surface of human granulocytes: formation of high-affinity ligand-receptor complexes in transient association with cytoskeleton. *J. Cell Biol.* 1984; 98(4): 1378–1387. [PubMed: 6325470]
36. Sklar LA, Hyslop PA, Oades ZG, Omann GM, Jesaitis AJ, Painter RG, Cochrane CG. Signal transduction and ligand-receptor dynamics in the human neutrophil. Transient responses and occupancy-response relations at the formyl peptide receptor. *J. Biol. Chem.* 1985; 260(21):11461–11467. [PubMed: 2995337]
37. Jesaitis AJ, Tolley JO, Painter RG, Sklar LA, Cochrane CG. Membrane-cytoskeleton interactions and the regulation of chemotactic peptide-induced activation of human granulocytes: the effects of dihydrocytochalasin B. *J. Cell. Biochem.* 1985; 27(3):241–253. [PubMed: 3838753]
38. Babbitt BA, Jesaitis AJ, Ivanov AI, Kelly D, Laukoetter M, Nava P, Parkos CA, Nusrat A. Formyl peptide receptor-1 activation enhances intestinal epithelial cell restitution through phosphatidylinositol 3-kinase-dependent activation of Rac1 and Cdc42. *J. Immunol.* 2007; 179(12):8112–8121. [PubMed: 18056353]

39. Wentworth CC, Jones RM, Kwon YM, Nusrat A, Neish AS. Commensal-epithelial signaling mediated *via* formyl peptide receptors. *Am. J. Pathol.* 2010; 177(6):2782–2790. [PubMed: 21037077]
40. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* 2004; 4(3): 181–189. [PubMed: 15039755]
41. Schneeweiss H, Renwanz L. Analysis of the attraction of haemocytes from *Mytilus edulis* by molecules of bacterial origin. *Dev. Comp. Immunol.* 1993; 17(5):377–387. [PubMed: 8270090]
42. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* 2007; 87(1):245–313. [PubMed: 17237347]
43. Ogier-Denis E, Mkaddem SB, Vandewalle A. NOX enzymes and Toll-like receptor signaling. *Semin. Immunopathol.* 2008; 30:291–300. [PubMed: 18493762]
44. Kotchoni SO, Gachomo EW. The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. *J. Biosci.* 2006; 31(3):389–404. [PubMed: 17006022]
45. Pauly N, Pucciariello C, Mandon K, Innocenti G, Jamet A, Baudouin E, Herouart D, Frendo P, Puppo A. Reactive oxygen and nitrogen species and glutathione: key players in the legume-*Rhizobium* symbiosis. *J. Exp. Bot.* 2006; 57(8):1769–1776. [PubMed: 16698817]
46. Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B. Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. *Plant Cell.* 2006; 18(4):1052–1066. [PubMed: 16517760]
47. Terada LS. Specificity in reactive oxidant signaling: think globally, act locally. *J. Cell. Biol.* 2006; 174(5):615–623. [PubMed: 16923830]
48. Ha E-M, Oh C-T, Bae YS, Lee W-J. A direct role for dual oxidase in *Drosophila* gut community. *Science.* 2005; 310(5749):847–850. [PubMed: 16272120]
49. Tsukagoshi H, Busch W, Benfey PN. Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell.* 2010; 143(4):606–616. [PubMed: 21074051]
50. Ha EM, Oh CT, Ryu JH, Bae YS, Kang SW, Jang IH, Brey PT, Lee WJ. An antioxidant system required for host protection against gut infection in *Drosophila*. *Dev. Cell.* 2005; 8(1):125–132.
51. Sadok A, Bourgarel-Rey V, Gattacceca F, Penel C, Lehmann M, Kovacic H. Nox1-dependent superoxide production controls colon adenocarcinoma cell migration. *Biochim. Biophys. Acta.* 2008; 1783(1):23–33. [PubMed: 18023288]
52. Kumar A, Wu H, Collier-Hyams LS, Hansen JM, Li T, Yamoah K, Pan ZQ, Jones DP, Neish AS. Commensal bacteria modulate cullin-dependent signaling *via* generation of reactive oxide species. *EMBO J.* 2007; 26(21):4457–4466. [PubMed: 17914462]
53. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology.* 2007; 132(2):562–575. [PubMed: 17258729]
54. Karrasch T, Kim JS, Muhlbauer M, Magness ST, Jobin C. Gnotobiotic IL-10^{-/-};NF-κB(EGFP) mice reveal the critical role of TLR/NF-κB signaling in commensal bacteria-induced colitis. *J. Immunol.* 2007; 178(10):6522–6532. [PubMed: 17475882]
55. Rhee SG, Kang SW, Jeong W, Chang TS, Yang KS, Woo HA. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr. Opin. Cell. Biol.* 2005; 17(2):183–189. [PubMed: 15780595]
56. Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell.* 2005; 120(5):649–661. [PubMed: 15766528]
57. Heneberg P, Draber P. Regulation of Cys-based protein tyrosine phosphatases *via* reactive oxygen and nitrogen species in mast cells and basophils. *Curr. Med. Chem.* 2005; 12(16):1859–1871. [PubMed: 16101506]
58. Barford D. The role of cysteine residues as redox-sensitive regulatory switches. *Curr. Opin. Struct. Biol.* 2004; 14(6):679–686. [PubMed: 15582391]
59. Husain A, Jeelani G, Sato D, Nozaki T. Global analysis of gene expression in response to L-Cysteine deprivation in the anaerobic protozoan parasite *Entamoeba histolytica*. *BMC Genomics.* 2011; 12:275. [PubMed: 21627801]

60. Chiarugi P, Buricchi F. Protein tyrosine phosphorylation and reversible oxidation: two cross-talking posttranslation modifications. *Antioxid. Redox Signal.* 2007; 9(1):1–24. [PubMed: 17115885]
61. Heneberg P, Draberova L, Bambouskova M, Pompach P, Draber P. Down-regulation of protein-tyrosine phosphatases activates an immune receptor in the absence of its translocation into lipid rafts. *J. Biol. Chem.* 2010; 285(17):12787–12802. [PubMed: 20157115]
62. Schroeder BO, Wu Z, Nuding S, Groscurth S, Marciniowski M, Beisner J, Buchner J, Schaller M, Stange EF, Wehkamp J. Reduction of disulphide bonds unmasks potent antimicrobial activity of human β -defensin 1. *Nature.* 2011; 469(7330):419–423. [PubMed: 21248850]
63. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc. Natl. Acad. Sci. USA.* 1994; 91(21):9926–9930. [PubMed: 7937919]
64. Venugopal R, Jaiswal AA. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase 1 gene. *Proc. Natl. Acad. Sci. USA.* 1996; 93(25):14960–14965. [PubMed: 8962164]
65. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* 1997; 236(2):313–322. [PubMed: 9240432]
66. Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene.* 1998; 17(24):3145–3156. [PubMed: 9872330]
67. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.* 1999; 13(1):76–86. [PubMed: 9887101]
68. An JH, Blackwell TK. SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes Dev.* 2003; 17(15):1882–1893. [PubMed: 12869585]
69. Sykietis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev. Cell.* 2008; 14(1):76–85. [PubMed: 18194654]
70. Kobayashi M, Itoh K, Suzuki T, Osanai H, Nishikawa K, Katoh Y, Takagi Y, Yamamoto M. Identification of the interactive interface and phylogenetic conservation of the Nrf2-Keap1 system. *Genes Cells.* 2002; 7(8):807–820. [PubMed: 12167159]
71. Motohashi H, O'Connor T, Katsuoka F, Engel JD, Yamamoto M. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. *Gene.* 2002; 294(1–2):1–12. [PubMed: 12234662]
72. Zipper LM, Mulcahy RT. The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. *J. Biol. Chem.* 2002; 277(39):36544–36552. [PubMed: 12145307]
73. Sekhar KR, Soltaninassab SR, Borrelli MJ, Xu Z-Q, Meredith MJ, Domann FE, Freeman ML. Inhibition of the 26S proteasome induces expression of GLCLC, the catalytic subunit for gamma-glutamylcysteine synthetase. *Biochem. Biophys. Res. Commun.* 2000; 270(1):311–317. [PubMed: 10733945]
74. Sekhar KR, Yan XX, Freeman ML. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. *Oncogene.* 2002; 21(44):6829–6834. [PubMed: 12360409]
75. McMahon M, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J. Biol. Chem.* 2003; 278(24):21592–21600. [PubMed: 12682069]
76. Kobayashi A, Kang M-I, Okawa H, Ohtsui M, Zenke Y, Chiba T, Igarashi K, Yamamoto M. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell. Biol.* 2004; 24(16):7130–7139. [PubMed: 15282312]
77. Zhang DD, Lo S-C, Cross JV, Templeton DJ, Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell. Biol.* 2004; 24(24):10941–10953. [PubMed: 15572695]

78. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. USA.* 2002; 99(18):11908–11913. [PubMed: 12193649]
79. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol. Cell. Biol.* 2003; 23(22):8137–8151. [PubMed: 14585973]
80. Li W, Yu S-W, Kong A-NT. Nrf2 possesses a redox-sensitive nuclear exporting signal in the Neh5 transactivation domain. *J. Biol. Chem.* 2006; 281(37):27251–27263. [PubMed: 16790425]
81. He X, Ma Q. NRF2 cysteine residues are critical for oxidant/electrophile-sensing, Kelch-like ECH-associated protein-1-dependent ubiquitination-proteasomal degradation, and transcription activation. *Mol. Pharmacol.* 2009; 76(6):1265–1278. [PubMed: 19786557]
82. Marini MG, Chan K, Casula L, Kan YW, Cao A, Moi P. hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. *J. Biol. Chem.* 1997; 272(26):16490–16497. [PubMed: 9195958]
83. Wasserman WW, Fahl WE. Functional antioxidant responsive elements. *Proc. Natl. Acad. Sci. USA.* 1997; 94(10):5361–5366. [PubMed: 9144242]
84. Nguyen T, Huang HC, Pickett CB. Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK. *J. Biol. Chem.* 2000; 275(20):15466–15473. [PubMed: 10747902]
85. Chen XL, Kunsch C. Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. *Curr. Pharm. Des.* 2004; 10(8):879–891. [PubMed: 15032691]
86. Singh S, Vrishni S, Singh BK, Rahman I, Kakkar P. Nrf2-ARE stress response mechanism: a control point in oxidative stress-mediated dysfunctions and chronic inflammatory diseases. *Free Radic. Res.* 2010; 44(11):1267–1288. [PubMed: 20815789]
87. Baird L, Dinkova-Kostova AT. The cytoprotective role of the Keap1-Nrf2 pathway. *Arch. Toxicol.* 2011; 85(4):241–272. [PubMed: 21365312]
88. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses *via* the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* 2007; 47:89–116. [PubMed: 16968214]
89. Birringer M. Hormetics: dietary triggers of an adaptive stress response. *Pharm. Res.* 2011; 28:2680–2694. [PubMed: 21818712]
90. Schäfer M, Dütsch S, auf dem Keller U, Navid F, Schwarz A, Johnson DA, Johnson JA, Werner S. Nrf2 establishes a glutathione-mediated gradient of UVB cytoprotection in the epidermis. *Genes Dev.* 2010; 24(10):1045–1058. [PubMed: 20478997]
91. Hirota A, Kawachi Y, Itoh K, Nakamura Y, Xu X, Banno T, Takahashi T, Yamamoto M, Otsuka F. Ultraviolet A irradiation induces NF-E2-related factor 2 activation in dermal fibroblasts: protective role in UVA-induced apoptosis. *J. Investig. Dermatol.* 2005; 124(4):825–832. [PubMed: 15816842]
92. Kawachi Y, Xu X, Taguchi S, Sakurai H, Nakamura Y, Ishii Y, Fujisawa Y, Furuta J, Takahashi T, Itoh K, Yamamoto M, Yamazaki F, Otsuka F. Attenuation of UVB-induced sunburn reaction and oxidative DNA damage with no alterations in UVB-induced skin carcinogenesis in Nrf2 gene-deficient mice. *J. Invest. Dermatol.* 2008; 128(7):1773–1779. [PubMed: 18200051]
93. Marrot L, Jones C, Perez P, Meunier J-R. The significance of Nrf2 pathway in (photo)-oxidative stress response in melanocytes and keratinocytes of the human epidermis. *Pigment Cell Melanoma Res.* 2008; 21(1):79–88. [PubMed: 18353146]
94. Hirota A, Kawachi Y, Yamamoto M, Koga T, Hamada K, Otsuka F. Acceleration of UVB-induced photoageing in nrf2 gene-deficient mice. *Exp. Dermatol.* 2011; 20(8):664–668.
95. Kong X, Thimmulappa R, Kombairaju P, Biswal S. NADPH oxidase-dependent reactive oxygen species mediate amplified TLR4 signaling and sepsis-induced mortality in Nrf2-deficient mice. *J. Immunol.* 2010; 185(1):569–577. [PubMed: 20511556]

96. Kong X, Thimmulappa R, Craciun F, Harvey C, Singh A, Kombairaju P, Reddy SP, Remick D, Biswal S. Enhancing Nrf2 pathway by disruption of Keap1 in myeloid leukocytes protects against sepsis. *Am. J. Respir. Crit. Care Med.* 2011; 184:928–938. [PubMed: 21799073]
97. Sykietis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci. Signal.* 2010; 3(112):re3. [PubMed: 20215646]
98. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tudor RM, Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J. Clin. Invest.* 2004; 114(9):1248–1259. [PubMed: 15520857]
99. Knörr-Wittmann C, Hengstermann A, Gebel S, Alam J, Müller T. Characterization of Nrf2 activation and heme oxygenase-1 expression in NIH3T3 cells exposed to aqueous extracts of cigarette smoke. *Free Radic. Biol. Med.* 2005; 39(11):1438–1448. [PubMed: 16274879]
100. Iizuka T, Ishii Y, Itoh K, Kiwamoto T, Kimura T, Matsuno Y, Morishima Y, Hegab AE, Homma S, Nomura A, Sakamoto T, Shimura M, Yoshida A, Yamamoto M, Sekizawa K. Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. *Genes Cells.* 2005; 10(12): 1113–1125. [PubMed: 16324149]
101. Singh A, Rangasamy T, Thimmulappa RK, Lee H, Osburn WO, Brigelius-Flohe R, Kensler TW, Yamamoto M, Biswal S. Glutathione peroxidase 2, the major cigarette smoke-inducible isoform of GPX in lungs, is regulated by Nrf2. *Am. J. Respir. Cell Mol. Biol.* 2006; 35(6):639–650. [PubMed: 16794261]
102. Adair-Kirk TL, Atkinson JJ, Griffin GL, Watson MA, Kelley DG, DeMello D, Senior RM, Betsuyaku T. Distal airways in mice exposed to cigarette smoke: Nrf2-regulated genes are increased in Clara cells. *Am. J. Respir. Cell Mol. Biol.* 2008; 39(4):400–411. [PubMed: 18441282]
103. Singh A, Ling G, Suhasini AN, Zhang P, Yamamoto M, Navas-Acien A, Cosgrove G, Tudor RM, Kensler TW, Watson WH, Biswal S. Nrf2-dependent sulfiredoxin-1 expression protects against cigarette smoke-induced oxidative stress in lungs. *Free Radic. Biol. Med.* 2009; 46(3):376–386. [PubMed: 19027064]
104. Malhotra D, Thimmulappa R, Vij N, Navas-Acien A, Sussan T, Merali S, Zhang L, Kelsen SG, Myers A, Wise R, Tudor R, Biswal S. Heightened endoplasmic reticulum stress in the lungs of patients with chronic obstructive pulmonary disease: the role of Nrf2-regulated proteasomal activity. *Am. J. Respir. Crit. Care Med.* 2009; 180(12):1196–1207. [PubMed: 19797762]
105. Gebel S, Diehl S, Pype J, Friedrichs B, Weiler H, Schüller J, Xu H, Taguchi K, Yamamoto M, Müller T. The transcriptome of Nrf2^{-/-} mice provides evidence for impaired cell cycle progression in the development of cigarette smoke-induced emphysematous changes. *Toxicol. Sci.* 2010; 115(1):238–252. [PubMed: 20133372]
106. Lee SE, Jeong SI, Kim G-D, Yang H, Park C-S, Jin Y-H, Park YS. Upregulation of heme oxygenase-1 as an adaptive mechanism for protection against crotonaldehyde in human umbilical vein endothelial cells. *Toxicol. Lett.* 2011; 201(3):240–248. [PubMed: 21238556]
107. Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW, Biswal S. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res.* 2010; 38(17):5718–5734. [PubMed: 20460467]
108. Shen G, Xu C, Hu R, Jain M, Nair S, Lin W, Yang C, Chan J, Kong AN. Comparison of (–)-epigallocatechin-3-gallate elicited liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2^{-/-} mice. *Pharm. Res.* 2005; 22(11):1805–1820. [PubMed: 16132347]
109. MacLeod AK, McMahon M, Plummer SM, Higgins LG, Penning TM, Igarashi K, Hayes JD. Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. *Carcinogenesis.* 2009; 30(9):1571–1580. [PubMed: 19608619]
110. Wu KC, Cui JY, Klaassen CD. Beneficial role of Nrf2 in regulating NADPH generation and consumption. *Toxicol. Sci.* 2011; 123:590–600. [PubMed: 21775727]

111. Kwak M-K, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, Kensler TW. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J. Biol. Chem.* 2003; 278(10):8135–8145. [PubMed: 12506115]
112. Cho H-Y, Reddy SP, Kleeberger SR. Nrf2 defends the lung from oxidative stress. *Antioxid. Redox Signal.* 2006; 8(1–2):76–87. [PubMed: 16487040]
113. Hu R, Xu C, Shen G, Jain MR, Khor TO, Gopalkrishnan A, Lin W, Reddy B, Chan JY, Kong A-NT. Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray. *Life Sci.* 2006; 79(20):1944–1955. [PubMed: 16828809]
114. Barve A, Khor TO, Nair S, Lin W, Yu S, Jain MR, Chan JY, Kong A-N. Pharmacogenomic profile of soy isoflavone concentrate in the prostate of Nrf2 deficient and wild-type mice. *J. Pharm. Sci.* 2008; 97(10):4528–4545. [PubMed: 18236473]
115. Menshikova E, Tkachev V, Zenkov N. Redox-dependent signaling system Nrf2/ARE in inflammation. *Mol. Biol.* 2010; 44(3):343–357.
116. Hernández-García D, Wood CD, Castro-Obregón S, Covarrubias L. Reactive oxygen species: A radical role in development? *Free Radic. Biol. Med.* 2010; 49(2):130–143. [PubMed: 20353819]
117. Covarrubias L, Hernandez-Garcia D, Schnabel D, Salas-Vidal E, Castro-Obregon S. Function of reactive oxygen species during animal development: passive or active? *Dev Biol.* 2008; 320:1–11. [PubMed: 18555213]
118. Owusu-Ansah E, Banerjee U. Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature.* 2009; 461(7263):537–541. [PubMed: 19727075]
119. Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, Imakado S, Kotsuji T, Otsuka F, Roop DR, Harada T, Engel JD, Yamamoto M. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat. Genet.* 2003; 35(3):238–245. [PubMed: 14517554]
120. Wakabayashi N, Shin S, Slocum SL, Agoston ES, Wakabayashi J, Kwak M-K, Misra V, Biswal S, Yamamoto M, Kensler TW. Regulation of notch1 signaling by nrf2: implications for tissue regeneration. *Sci. Signal.* 2010; 3(130):ra52. [PubMed: 20628156]
121. Osburn WO, Karim B, Dolan PM, Liu G, Yamamoto M, Huso DL, Kensler TW. Increased colonic inflammatory injury and formation of aberrant crypt foci in Nrf2-deficient mice upon dextran sulfate treatment. *Int. J. Cancer.* 2007; 121(9):1883–1891. [PubMed: 17631644]
122. Khor TO, Huang M-T, Kwon KH, Chan JY, Reddy BS, Kong A-N. Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. *Cancer Res.* 2006; 66(24):11580–11584. [PubMed: 17178849]
123. Jin W, Wang H, Ji Y, Hu Q, Yan W, Chen G, Yin H. Increased intestinal inflammatory response and gut barrier dysfunction in Nrf2-deficient mice after traumatic brain injury. *Cytokine.* 2008; 44(1):135–140. [PubMed: 18722136]
124. Jin W, Wang H-D, Hu Z-g, Yan W, Chen G, Yin H-X. Transcription factor Nrf2 plays a pivotal role in protection against traumatic brain injury-induced acute intestinal mucosal injury in mice. *J. Surg. Res.* 2009; 157(2):251–260. [PubMed: 19394962]
125. Misra JR, Horner MA, Lam G, Thummel CS. Transcriptional regulation of xenobiotic detoxification in *Drosophila*. *Genes Dev.* 2011; 25(17):1796–1806. [PubMed: 21896655]
126. Lin PW, Myers LES, Ray L, Song S-C, Nasr TR, Berardinelli AJ, Kundu K, Murthy N, Hansen JM, Neish AS. *Lactobacillus rhamnosus* blocks inflammatory signaling *in vivo* via reactive oxygen species generation. *Free Radic. Biol. Med.* 2009; 47(8):1205–1211. [PubMed: 19660542]
127. Kumar A, Wu H, Collier-Hyams LS, Kwon Y-M, Hanson JM, Neish AS. The bacterial fermentation product butyrate influences epithelial signaling *via* reactive oxygen species-mediated changes in cullin-1 neddylation. *J. Immunol.* 2009; 182(1):538–546. [PubMed: 19109186]
128. Swanson PA, Kumar A, Samarin S, Vijay-Kumar M, Kundu K, Murthy N, Hansen J, Nusrat A, Neish AS. Enteric commensal bacteria potentiate epithelial restitution *via* reactive oxygen species-mediated inactivation of focal adhesion kinase phosphatases. *Proc. Natl. Acad. Sci. USA.* 2011; 108(21):8803–8808. [PubMed: 21555563]

129. Menard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut*. 2004; 53(6):821–828. [PubMed: 15138208]
130. Pena JA, Versalovic J. *Lactobacillus rhamnosus* GG decreases TNF-alpha production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. *Cell Microbiol*. 2003; 5(4):277–285. [PubMed: 12675685]
131. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology*. 1999; 116(5):1107–1114. [PubMed: 10220502]
132. Ghosh S, Dai C, Brown K, Rajendiran E, Makarenko S, Baker J, Ma C, Halder S, Montero M, Ionescu VA, Klegeris A, Vallance BA, Gibson DL. Colonic microbiota alters host susceptibility to infectious colitis by modulating inflammation, redox status, and ion transporter gene expression. *Am. J. Physiol. Gastrointest. Liver Physiol*. 2011; 301(1):G39–G49. [PubMed: 21454446]
133. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat. Immunol*. 2004; 5(1):104–112. [PubMed: 14691478]
134. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. Prokaryotic regulation of epithelial responses by inhibition of I κ B- α ubiquitination. *Science*. 2000; 289(5484):1560–1563. [PubMed: 10968793]
135. Tien M-T, Girardin SE, Regnault B, Le Bourhis L, Dillies M-A, Coppee J-Y, Bourdet-Sicard R, Sansonetti PJ, Pedron T. Anti-inflammatory effect of *Lactobacillus casei* on *Shigella*-infected human intestinal epithelial cells. *J. Immunol*. 2006; 176(2):1228–1237. [PubMed: 16394013]
136. Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB. Probiotics inhibit nuclear factor- κ B and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. *Gastroenterology*. 2004; 127(5):1474–1487. [PubMed: 15521016]
137. Iyer C, Kusters A, Sethi G, Kunnumakkara AB, Aggarwal BB, Versalovic J. Probiotic *Lactobacillus reuteri* promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF- κ B and MAPK signalling. *Cell. Microbiol*. 2008; 10:1442–1452. [PubMed: 18331465]
138. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- κ B activity. *Annu. Rev. Immunol*. 2000; 18:621–663. [PubMed: 10837071]
139. Kim DW, Lenzen G, Page AL, Legrain P, Sansonetti PJ, Parsot C. The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc. Natl. Acad. Sci. USA*. 2005; 102(39):14046–14051. [PubMed: 16162672]
140. Angot A, Vergunst A, Genin S, Peeters N. Exploitation of eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III and type IV secretion systems. *PLoS Pathog*. 2007; 3(1):e3. [PubMed: 17257058]
141. Rytönen A, Holden DW. Bacterial interference of ubiquitination and deubiquitination. *Cell Host Microbe*. 2007; 1(1):13–22. [PubMed: 18005678]
142. Collier-Hyams LS, Sloane V, Batten BC, Neish AS. Cutting edge: bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. *J. Immunol*. 2005; 175(7):4194–4198. [PubMed: 16177058]
143. Lee WJ. Bacterial-modulated signaling pathways in gut homeostasis. *Sci. Signal*. 2008; 1(21):pe24. [PubMed: 18506033]
144. Zhou BP, Hung MC. Wnt, hedgehog and snail: sister pathways that control by GSK-3 β and β -Trcp in the regulation of metastasis. *Cell Cycle*. 2005; 4(6):772–776. [PubMed: 15917668]
145. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsois RM, Roth JR, Baumlér AJ. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature*. 2010; 467(7314):426–429. [PubMed: 20864996]
146. Neishi Y, Akasaka T, Tsukiji M, Kume T, Wada N, Watanabe N, Kawamoto T, Kaji S, Yoshida K. Reduced coronary flow reserve in patients with congestive heart failure assessed by

- transthoracic Doppler echocardiography. J. Am. Soc. Echocardiogr. 2005; 18(1):15–19. [PubMed: 15637483]
147. Chiarugi P, Pani G, Giannoni E, Taddei L, Colavitti R, Raugei G, Symons M, Borrello S, Galeotti T, Ramponi G. Reactive oxygen species as essential mediators of cell adhesion: the oxidative inhibition of a FAK tyrosine phosphatase is required for cell adhesion. J. Cell. Biol. 2003; 161(5):933–944. [PubMed: 12796479]
148. Hochmuth CE, Biteau B, Bohmann D, Jasper H. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in *Drosophila*. Cell Stem Cell. 2011; 8(2):188–199. [PubMed: 21295275]
149. Coant N, Ben Mkaddem S, Pedruzzi E, Guichard C, Treton X, Ducroc R, Freund JN, Cazals-Hatem D, Bouhnik Y, Woerther PL, Skurnik D, Grodet A, Fay M, Biard D, Lesuffleur T, Deffert C, Moreau R, Groyer A, Krause KH, Daniel F, Ogier-Denis E. NADPH oxidase 1 modulates WNT and NOTCH1 signaling to control the fate of proliferative progenitor cells in the colon. Mol. Cell. Biol. 30(11):2636–2650. [PubMed: 20351171]
150. Wentworth CC, Alam A, Jones RM, Nusrat A, Neish AS. Enteric commensal bacteria induce extracellular signal-regulated kinase pathway signaling *via* formyl peptide receptor-dependent redox modulation of dual specific phosphatase 3. J. Biol. Chem. 2011; 286:38448–38455. [PubMed: 21921027]
151. Sethuraman M, McComb ME, Huang H, Huang S, Heibeck T, Costello CE, Cohen RA. Isotope-coded affinity tag (ICAT) approach to redox proteomics: identification and quantitation of oxidant-sensitive cysteine thiols in complex protein mixtures. J. Proteome Res. 2004; 3(6):1228–1233. [PubMed: 15595732]
152. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006; 7(7):688–693. [PubMed: 16819463]
153. Mark PM. Hormesis defined. Ageing Res. Rev. 2008; 7(1):1–7. [PubMed: 18162444]
154. Foster JW, Hall HK. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. 1990; 172(2):771–778. [PubMed: 2404956]
155. Periago PM, van Schaik W, Abee T, Wouters JA. Identification of proteins involved in the heat stress response of *Bacillus cereus* ATCC 14579. Appl. Environ. Microbiol. 2002; 68(7):3486–3495. [PubMed: 12089032]
156. Daikos GL, Jackson GG, Lolans VT, Livermore DM. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. J. Infect. Dis. 1990; 162(2):414–420. [PubMed: 2115555]

Fig. (1). Cellular signaling pathways regulated by microbial-elicited ROS generation

Commensal microbiota and/or their products within the intestinal lumen influence the activity of fundamental cellular processes through the regulation of cellular redox status. For example, luminal bacteria produce and shed small formylated peptides which are perceived *via* formyl peptide receptors localized to the apical surface of gut epithelia. These and likely other receptors activate NADPH oxidases that transduce microbial signals *via* highly localized ROS production, affecting the oxidation status, and thus activity of redox sensor regulatory proteins (in red) such as DUSP3, LWM-PTPase and the Nedd8 ligase, Ubc12. As discussed in the text, downstream basic cellular processes including proliferation, motility and inflammation can thus be modulated by changes in microbial-dependent cellular redox potential.

Fig. (2). Oxidation sensitive cysteines

At low pKa conditions, cysteine exists in the thiolate form. Cellular generation of ROS (H₂O₂) results in the reversible oxidation of the thiol group to sulfenic acid. Generation of higher levels of H₂O₂ result in reactions that lead to the formation of irreversible cysteine oxidation states, including sulfenic acid (-SO₂H), or sulfonic acid (-SO₃H) (not shown). Cysteines avoid irreversible oxidation by the formation of disulfide bonds. This is achieved by either mixed disulfide formation with glutathione (S-glutathionylation), or by disulfide bond formation with another cysteine.

Fig. (3). Activation of the Keap1/Nrf2/ARE signaling module through microbe-induced ROS generation

Under normal, basal reducing conditions, Keap1 physically associates with Nrf2, leading to constitutive ubiquitin-dependent proteolysis and absence of Nrf2 transcriptional activity. Establishment of a pro-oxidative intracellular environment, such as during the application of an oxidative chemical or through microbe-mediated ROS signaling, results in a cysteine-dependent Keap1 conformational change, Nrf2 release, stabilization and cytoplasmic accumulation. Nrf2 then translocates to the nucleus where, together with a small DNA binding Maf protein, it transcriptionally activates the expression of a large Nrf2 regulon that includes cytoprotective factors such as superoxide dismutase and glutathione S-transferase. Nrf2-dependent gene products then act to quench the initial offending oxidative stress and also protect against subsequent insults.