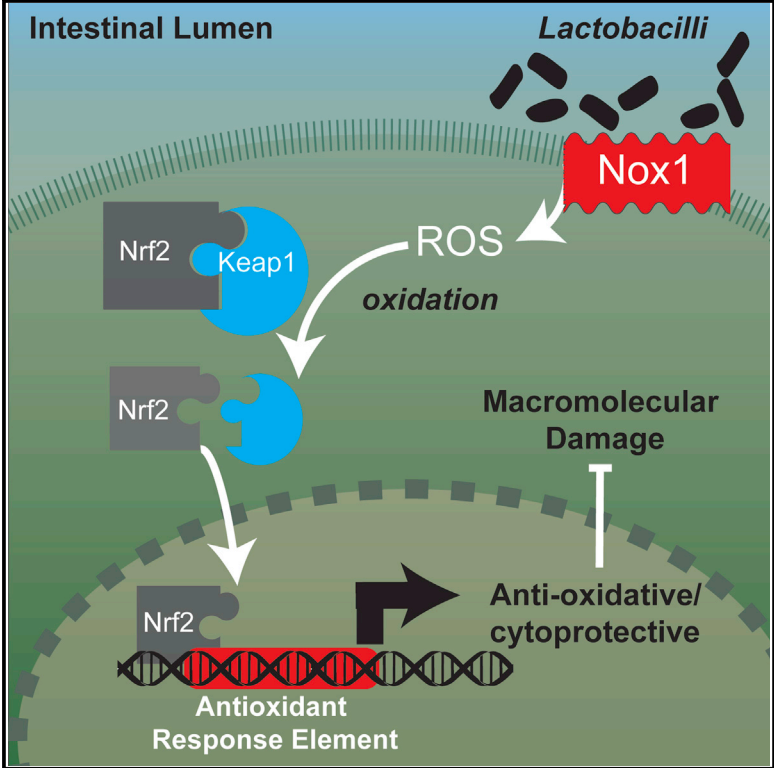


Lactobacilli Modulate Epithelial Cytoprotection through the Nrf2 Pathway

Graphical Abstract



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In Brief

Jones et al. report that the commensal gut bacterial taxa lactobacilli are able to mediate beneficial cytoprotective effects in the gut of both flies and mice. These highly conserved events are mediated by ROS-dependent activation of the Nrf2 xenobiotic pathway and conserved effector genes.

Highlights

- Commensal lactobacilli activate the xenobiotic responsive Nrf2 pathway
- Nrf2 pathway activation upregulates cytoprotective genes in both *Drosophila* and mice
- Nrf2 target genes protect against environmental stresses
- Lactobacilli transduce cytoprotective effects via Nrf2 pathway activation



Lactobacilli Modulate Epithelial Cytoprotection through the Nrf2 Pathway

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SUMMARY

An optimal gut microbiota influences many beneficial processes in the metazoan host. However, the molecular mechanisms that mediate and function in symbiont-induced host responses have not yet been fully characterized. Here, we report that cellular ROS enzymatically generated in response to contact with lactobacilli in both mice and *Drosophila* has salutary effects against exogenous insults to the intestinal epithelium via the activation of Nrf2 responsive cytoprotective genes. These data show that the xenobiotic-inducible Nrf2 pathway participates as a signaling conduit between the prokaryotic symbiont and the eukaryotic host. Indeed, our data imply that the capacity of lactobacilli to induce redox signaling in epithelial cells is a highly conserved homeostatic adaptation to impel cellular conditioning to exogenous biotic stimuli. These data also highlight the role the microbiota plays in eukaryotic cytoprotective pathways and may have significant implications in the characterization of a eubiotic microbiota.

INTRODUCTION

The prokaryotic microbiota of virtually all metazoans participate in many symbiotic functions, including regulation of cellular growth and survival (Neish and Jones, 2014). However, the host intrinsic mechanisms that mediate non-immune symbiont-induced effects are largely unknown. A well-studied and evolutionarily highly conserved system for transducing exogenous stimuli into eukaryotic transcriptional responses is the Nrf2 pathway. Nrf2 activation upregulates a regulon of genes including those involved in xenobiotic and reactive oxygen species (ROS) detoxification, as well as pro-restitutive function. This pathway has attracted considerable attention because small molecule inducers of Nrf2 have cytoprotective effects against oxidant and electrophilic environmental stressors (Kobayashi et al., 2009). Our research group has recently reported that lactobacilli, which are constituents of the microbiota in many meta-

zoans and well-known probiotic agents, are highly adapted to induce the generation of physiological levels of ROS in cells of the intestinal epithelium and consequent induction of cell proliferation (Jones et al., 2013).

Gut bacteria stimulate reactive oxygen species (ROS) production in epithelial cells by an enzymatic mechanism analogous to the pathogen-induced respiratory burst in phagocytes (Alam et al., 2014; Jones et al., 2013). Cellular ROS are generated by the catalytic action of NADPH oxidases. The first and exemplary member of this family of enzymes, Nox2, was characterized in phagocytes and is well known to function in neutrophil microbicidal ROS generation in response to pathogens. Thereafter, isoforms of Nox2 were identified in non-phagocytic tissues, including Nox1, which is highly expressed in colonic enterocytes of both flies and mice (Lambeth and Neish, 2014). Interestingly, enzymatically generated ROS in the epithelia is stimulated not only by potential pathogens, but also by symbiotic bacteria, especially members of the lactobacilli taxon, and promotes cell proliferation and migration (Wentworth et al., 2010, 2011), accelerates restitution post injury (Swanson et al., 2011), and modifies epithelial NF- κ B signaling (Kumar et al., 2007).

The Nrf2 system is a well-characterized ubiquitin-dependent signaling pathway that responds to oxidative stress and electrophilic xenobiotics and is recognized as a major regulator of cytoprotective responses against such environmental cellular stresses (Jones et al., 2012). Components of the Nrf2 signaling are functionally conserved even in lower metazoans such as *C. elegans* and are fully developed in *Drosophila* and mammals. Disruption of Nrf2 signaling results in increased sensitivity to oxidative stress in flies (Sykietis and Bohmann, 2008) and increased sensitivity to radiological insult in mice (McDonald et al., 2010). The Nrf2 pathway has been implicated in proliferative control of gut stem cells (Hochmuth et al., 2011). At low levels of ROS, Nrf2 is bound to its cytoplasmic inhibitor Keap1, which suppresses the activity of Nrf2 by targeting it for constitutive polyubiquitination by a Cullin3-based E3 ligase complex and consequent proteasomal degradation. Keap1 contains redox-sensitive cysteine residues that under oxidative stresses conditions react and alter the functional conformation of Keap1, thereby abolishing the inactivation of Nrf2. Thereafter, Nrf2 translocates to the nucleus, binds to Antioxidant Response Element (ARE) sequences, and initiates the transcription of a

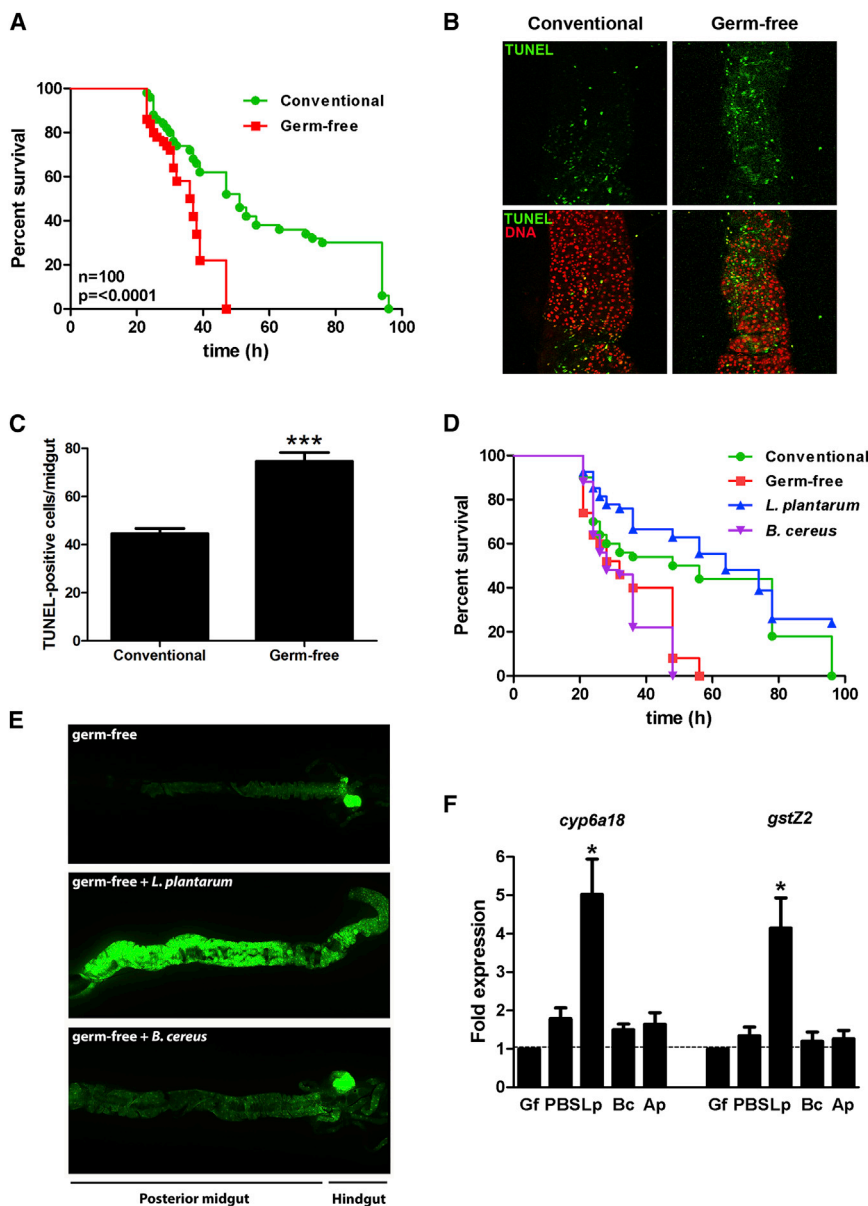


Figure 1. *Lactobacillus plantarum* Induces Cytoprotection against Oxidative Insult and Potentiates Upregulation of CncC Pathway Responsive Genes in *Drosophila*

(A) Relative survival of 5-day-old germ-free and conventionally raised adult *Drosophila* in response to Paraquat challenge. log-rank test $p < 0.001$, $n = 100$.

(B) TUNEL analysis of posterior midgut tissues dissected from *Drosophila* described in (A) at 24 hr following Paraquat challenge. Note the high concentration of TUNEL-positive cells in the posterior 3 (P3) section of the midgut.

(C) Numeration of TUNEL-positive cells per midgut examined in (B). *** $p < 0.001$, $n = 10$.

(D) Relative survival of germ-free adult *Drosophila* gnotobiotically monocolonized with *L. plantarum* or *B. cereus* in response to Paraquat challenge. Note increased survival of flies monocolonized with *L. plantarum* (log-rank test for g-f versus g-f + *L. plantarum*, $p < 0.0001$, $n = 100$). Note also significantly increased survival of germ-free fed *L. plantarum* compared to *B. cereus*-fed flies (log-rank for g-f + *L. plantarum* versus g-f + *B. cereus*, $p < 0.0001$, $n = 100$).

(E) Detection of ARE-dependent GFP expression in the midgut of germ-free $P_{gstD11-gfp}$ third-instar larvae following ingestion of *L. plantarum*. Images represent a mosaic of three 20 \times confocal images of the larval midgut taken at the same confocal settings.

(F) Real-time qPCR analysis for the detection of *cyp6a18*, and *gstZ2* transcript enrichment in the midgut of germ-free third-instar larvae at 4 hr following ingestion of either *L. plantarum* (Lp), *B. cereus* (Bc), *A. piechaudii* (Ap). * $p < 0.05$, $n = 30$. Error bars indicate SEM.

battery of antioxidant enzymes and detoxifying proteins, known as the “Phase 2 detoxification response.” We hypothesized that symbiont-induced ROS may activate epithelial Nrf2 pathway signaling, and thereby mechanistically mediate the beneficial influences of a eubiotic microbiota.

RESULTS

Lactobacillus plantarum* Induces Cytoprotection against Oxidative Insult and Potentiates Upregulation of CncC Pathway Responsive Genes in *Drosophila

We first implicated a role for the indigenous microbiota in cytoprotection against oxidative injury in the intestine by showing that adult germ-free *Drosophila*, compared to conventionally

raised controls, are significantly more sensitive to Paraquat-induced toxicity (Figure 1A) and exhibit elevated numbers of apoptotic cells in the highly proliferative posterior 3 (P3) section of the midgut (Figures 1B and 1C). To identify whether, or which, specific bacterial genera contribute to cytoprotection, we studied germ-free *Drosophila* gnotobiotically colonized with monocultures of bacterial species that we previously isolated from the luminal content of adult *Drosophila* (Jones et al., 2013) and examined the extent to which individual members of the microbiota influenced cytoprotection. Midguts were dissected and comparable levels of bacterial ingestion confirmed by plate count. Of those tested, only flies gnotobiotically colonized with pure *Lactobacillus plantarum* cultures significantly protected *Drosophila* from oxidative injury (Figure 1D) and (Figure S1). To demonstrate the beneficial hormetic effect of pre-exposure of germ-free adult *Drosophila* to lower levels of ROS, we show that lower doses of paraquat, with 5 mM for 24 hr being optimal, can protect against ensuing severe insults triggered by 25 mM paraquat feeding (Figure S2). Because we also previous showed that only

L. plantarum possessed the capacity to induce ROS generation in *Drosophila* enterocytes following colonization (Jones et al., 2013), these data suggested the possibility of redox signaling in commensal-mediated cytoprotection. To examine this notion, we used a GFP reporter fly bearing an antioxidant response element (ARE)-dependent promoter (*gstD1*-GFP) that responds to Nrf2 (known as CncC in *Drosophila*) (Sykiotis and Bohmann, 2008). Third-instar larva were utilized as their feeding behavior allows rapid colonization and synchronous induction of signaling events. We observed that colonization of either germ-free larvae or adult *Drosophila* harboring a CncC-responsive reporter was activated following *L. plantarum*, but not *B. cereus* ingestion (Figures 1E and S2). To assess gene expression associated with *L. plantarum*-induced cytoprotection, we employed microarray analysis on dissected larval midgut tissue following *L. plantarum* ingestion by germ-free *Drosophila*. Gene ontology analysis identified highly represented transcripts in *L. plantarum*-fed larva (compared to germ free) (Table S1), many of which were found to be located downstream of a promoter region harboring a putative antioxidant response element (ARE) homologous to the 10-bp core element that lies at the center of the 20-bp sequence ARE consensus sequence (Nioi et al., 2003). Many of these genes have a known cytoprotective function, including glutathione S-transferase zeta 1 *GstZ1*, *GstZ2*, and *GstD10*, as well as a number of Cytochrome P450, E-class, group I enzymes, including *Cyp4p1*, *Cyp4ac1*, and *Cyp4ac3*. Consistently, upregulation of Cytochrome P450 family of genes was detected in midgut sections P2, P3, and P4 in other recent investigations (Marianes and Spradling, 2013). Real-time qPCR analysis corroborated the detection of the increased representation of transcripts for the aforementioned proteins in midgut tissues of larva-fed *L. plantarum*, but not in the midgut regions of larvae colonized with other, non-ROS inducing bacteria (Figure 1F). These data indicate that the transcriptional response is specific to *L. plantarum* ingestion, and, based on transcriptional profile analysis, that the mechanism of *L. plantarum*-induced cytoprotection may be as a result of the activation of genes under the regulatory control of an ARE promoter sequence.

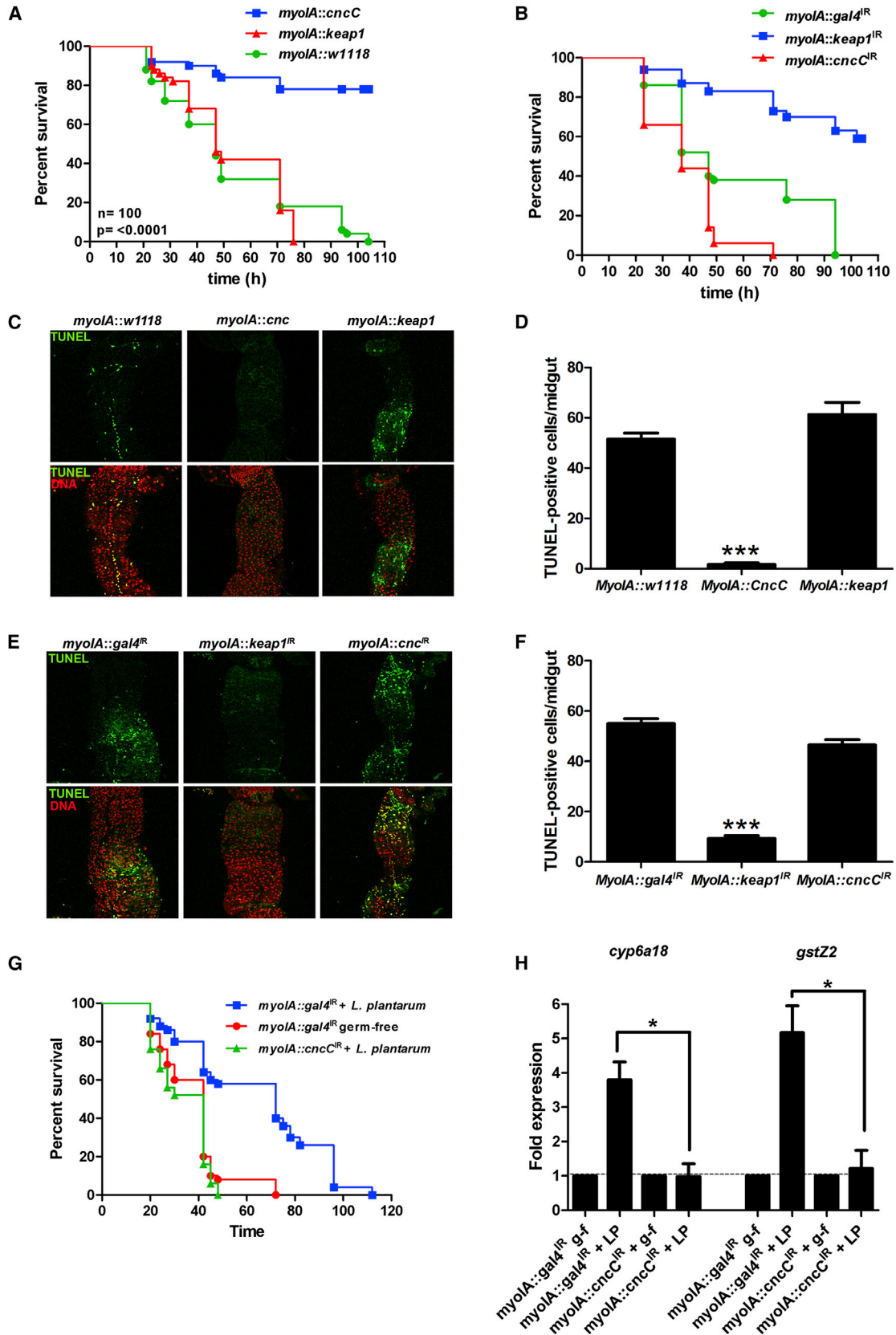
CncC Pathway Signaling Mediates *Lactobacillus plantarum*-Induced Cytoprotection against Oxidative Insult in *Drosophila*

To mechanistically substantiate that CncC signaling is mediating *L. plantarum*-induced cytoprotection, we employed the bipartite GAL4/UAS system to modulate CncC pathway activity by constitutively driving the expression of Keap1 or CncC, or by using RNAi constructs against CncC as previously described (Sykiotis and Bohmann, 2008), under the enterocyte-specific *myoIA*-GAL4 driver fly. Depletion of transcript levels in the midgut of these genotypes were confirmed by qPCR (Figure S3). Constitutive enterocyte-specific expression of CncC (Figure 2A), or depletion of Keap1 levels (Figure 2B), both of which result in Nrf2 pathway activation, significantly enhanced survival of adult *Drosophila* in response to Paraquat-induced toxicity, correlating with the extent of apoptotic cells observed in segments P3 and P4 of the midgut (Marianes and Spradling, 2013) (Figures 2C and 2D). Conversely, constitutive activation of Keap1 (Fig-

ure 2A), or depletion of CncC transcripts (UAS-*cncC*-RNAi) (Figure 2B) (both of which inhibit pathway activation) in the midgut rendered flies significantly more susceptible to Paraquat-induced toxicity, again with correlative apoptotic damage to the midgut (Figures 2E and 2F). The cytoprotective function of CncC in enterocytes was also detected in germ-free adult *Drosophila* (Figure S3). Importantly, depletion of CncC, while having no effect on bacterial numbers (see Experimental Procedures), essentially abolished *L. plantarum*-induced cytoprotection against Paraquat to levels observed for germ-free adult *Drosophila* (Figure 2G). Furthermore, enterocyte-specific depletion of CncC-levels significantly reduced *L. plantarum*-induced activation of *cyp6a18* and *gstZ2* in the adult *Drosophila* midgut (Figure 2H) together indicating that *L. plantarum*-induced protection against oxidative insult was dependent on CncC pathway signaling.

Colonization of the Murine Intestine with *Lactobacillus rhamnosus* GG Induces Nrf2-Dependent Cytoprotection

Nrf2 signaling is highly conserved across metazoans. To determine whether lactobacilli-induced and Nrf2-pathway-mediated cytoprotection occurs in mammals, we recapitulated our investigations in the murine model. Transcriptional profiling and gene ontology analysis identified highly represented transcripts in the colonic tissues of germ-free mice orally gavaged with *L. rhamnosus* GG, a mammalian adapted lactobacillus, when compared to transcripts in the colonic tissues of germ-free mice orally gavaged with equivalent inoculum of *E. coli*, which in our previous studies could not induce detectable generation of ROS in intestinal epithelial cells (Jones et al., 2013). Interestingly, *L. rhamnosus* GG-induced transcripts cluster distinct from transcripts induced by *E. coli* or vehicle (Figure S4). Indeed, comparison of *L. rhamnosus* GG-fed to *E. coli*-fed tissues identified a set of enriched transcripts from the Cytochrome P450 family of proteins, which have conserved upstream ARE promoter sequences, consistent with our observations in the analogous *Drosophila* experiments (Table S2). Specifically, increased transcript representation of *Cyp2c65*, *Cyp2c55* and *Cyp4b1* was detected, and confirmed in these samples by qPCR (Figure 3A). Importantly, qPCR analysis also confirmed that transcripts for these three Cytochrome P450 genes were not enriched in colonic tissues of *E. coli*-fed mice, further supporting our observation that of the bacteria tested, the upregulation of these genes are specifically induced in response to lactobacilli (Figure 3A). Previous reports have described the cytoprotective effects of *L. rhamnosus* GG in mice against radiological insult (Ciorba et al., 2012). We corroborate these data by confirming that mice fed *L. rhamnosus* GG have significantly enhanced survival, are protected against weight loss, and have fewer apoptotic cells at within colonic crypts following irradiation (Figures 3B–3E). Strikingly, each of the measured cytoprotective influences of *L. rhamnosus* GG against irradiation were completely abolished in *Nrf2*^{-/-} mice (Figures 3B–3E). Note also that we did not detect any changes in the response to irradiation insult between untreated wild-type and *Nrf2*^{-/-} mice. Together, these data show that Nrf2 is required for the optimal cytoprotective influences of *L. rhamnosus* GG in the intestine in conventionally raised mice.



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Nox1 Is Required for Optimal Lactobacilli-Induced Cytoprotection

We previously showed that Nox1 in mice and the orthologous dNox in *Drosophila* are required for lactobacilli-induced ROS generation in intestinal enterocytes (Jones et al., 2013). To show that Nox1 generated ROS function in lactobacilli-induced cytoprotection, we employed the same bipartite GAL4/UAS system as above to expressed RNAi constructs against dNox under the enterocyte-specific *myoIA*-GAL4 driver fly. Depletion of transcript levels in the midgut of these genotypes were confirmed in Jones et al. (2013). Constitutive depletion of *dnox* transcripts (UAS-*dnox*-RNAi), but not *dduox*, while again having no effect on bacterial numbers (see Experimental Procedures), significantly reduced *L. plantarum*-induced cytoprotection against Paraquat (Figure 4A) with correlative apoptotic damage to the midgut (Figures 4B and 4C). These data corroborate our previous observations in the fly that symbiotic lactobacilli-induced ROS generation is mediated via the enzymatic activity of dNox, and not by dDuox, which by contrast is reported to function in the *Drosophila* anti-microbial response to pathogens (Ha et al., 2005). To show that Nox1 is also required for *L. rhamnosus* GG-induced cytoprotection against irradiation in mice, we used the intestinal epithelial cell-specific Nox1-deficient knockout (B6.No^{x1}^{ΔIEC}) (Leoni et al., 2013). Consistent with our previous data, *L. rhamnosus* GG-induced enhanced survival and reduced loss of body weight were significantly abrogated in the B6.No^{x1}^{ΔIEC} compared to wild-type littermates (Figures 4D–4G). In summary, these data are compelling evidence showing that enterocyte-expressed ROS generation catalyzed by Nox1 is required for lactobacilli-potentiated cytoprotection.

DISCUSSION

In this study, we identified the Nrf2 pathway as a distinct system for the non-immune perception and response to specific members of the microbiota, namely, lactobacilli. In addition, we show that this ROS-sensitive signal transduction system mediates cytoprotection, consistent with our previous observations of lactobacilli-induced enzymatic generation of ROS in the gut of mammals and *Drosophila* (Jones et al., 2013). Members of the diverse Lactobacillus taxon have long been exploited by hu-

mans in the production of fermented dairy products and are commonly employed as candidate probiotic agents. They are characteristic early colonists of the neonatal mammalian gut and are fructose fermenters present in decaying fruit that form the major energy source of *Drosophila*. Lactobacilli-specific symbiotic functions include cytoprotection in the mouse (Ciorba et al., 2012) and effects in *Drosophila* ranging from mate selection (Sharon et al., 2010) to metabolic regulation (Storelli et al., 2011). These effects likely involve the mucus binding adhesive properties of these bacteria (Ardita et al., 2014). Thus, members of the genus lactobacilli may have evolved symbiotic relationships where microbial-induced generation of ROS functions as a transducer of bacterial signals into host gene regulatory events that potentiate multiple effects in disparate biological systems. We describe a molecular mechanism by which lactobacilli can elicit their beneficial influences on host gut tissues, wherein ROS generated by Nox following bacterial contact activates downstream cytoprotective signaling. These observations were particularly striking in germ-free *Drosophila* mono-associated with *L. plantarum*. Importantly, this influence of lactobacilli was also observed in conventionally raised mice indicating that animals do not necessarily have to be germ free to benefit from lactobacilli-induced cytoprotection.

One intriguing observation is that flies depleted of *dnox* transcripts and monoassociated with *L. plantarum* have a similar sensitivity as germ-free flies over the first half of the experimental time assayed, and thereafter exhibit a degree of resistance to oxidative stress over the second half of the assay (Figure 4A). This infers that lactobacilli-Nox-Nrf2 signaling may not be the only evolved mechanism that lactobacilli exploited to elicit cytoprotective influences on the host. Indeed, other pathways reported to be activated in response to symbiotic bacteria include the TOR pathway in *Drosophila* (Storelli et al., 2011). Consistent with our study, Storelli et al. (2011) concluded that mono-ingestion of *L. plantarum* is sufficient to recapitulate the effects of the normal microbiota. In addition, lactobacilli were reported to protect the murine intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner (Ciorba et al., 2012). Going forward, it will be interesting to determine the extent to which both TOR signaling, and TLR-MAPK signaling synergistically interacts with ROS generated by Nox in cytoprotection. Lactobacilli's impact on host physiology has been found

Figure 2. CncC Pathway Signaling Mediates Lactobacillus plantarum-Induced Cytoprotection against Oxidative Insult in Drosophila

- (A) Relative survival in response to Paraquat challenge of conventionally raised adult *Drosophila* either constitutively expressing *cncC* (UAS-*CncC*) or constitutively expressing *keap1* under the enterocyte-specific *myoIA*-GAL4 driver (log-rank test for *myoIA*-GAL4 *w1118* versus *myoIA*-GAL4 UAS-*cncC*, $p < 0.0001$, $n = 100$).
- (B) Survival in response to Paraquat challenge of 5-day-old conventionally raised adult *Drosophila* where the levels of *cncC* (UAS-*cncC*^{IR}) or *Keap1* (UAS-*keap1*^{IR}) are diminished (log-rank test for *myoIA*-GAL4 *w1118* versus *myoIA*-GAL4 UAS-*cncC*^{IR}, $p < 0.0001$, $n = 100$).
- (C) TUNEL analysis of posterior midgut dissected from adult *Drosophila* listed in (A) and exposed to Paraquat challenge for 48 hr.
- (D) Numeration of TUNEL-positive cells per midgut examined in (C). *** $p < 0.001$, $n = 10$.
- (E) TUNEL analysis of posterior midgut tissues dissected from adult *Drosophila* described in (C), following 48 hr exposure to Paraquat challenge.
- (F) Numeration of TUNEL-positive cells per midgut examined in (E). *** $p < 0.001$, $n = 10$.
- (G) Survival in response to Paraquat challenge of germ-free adult *Drosophila* monocolonized with *L. plantarum* where the levels of *CncC* (UAS-*cncC*^{IR}) are diminished under the enterocyte-specific *myoIA*-GAL4 driver (log-rank for *myoIA*-GAL4;UAS-*gal4*^{IR} + *L. plantarum* versus *myoIA*-GAL4;UAS-*cncC*^{IR} + *L. plantarum*, $p < 0.0001$, $n = 100$).
- (H) Real-time qPCR analysis for the detection of *cyp6a18* and *gstZ2*; transcript enrichment in the midgut of adult *Drosophila* treated as described in (G). * $p < 0.05$, $n = 30$.

Error bars indicate SEM.

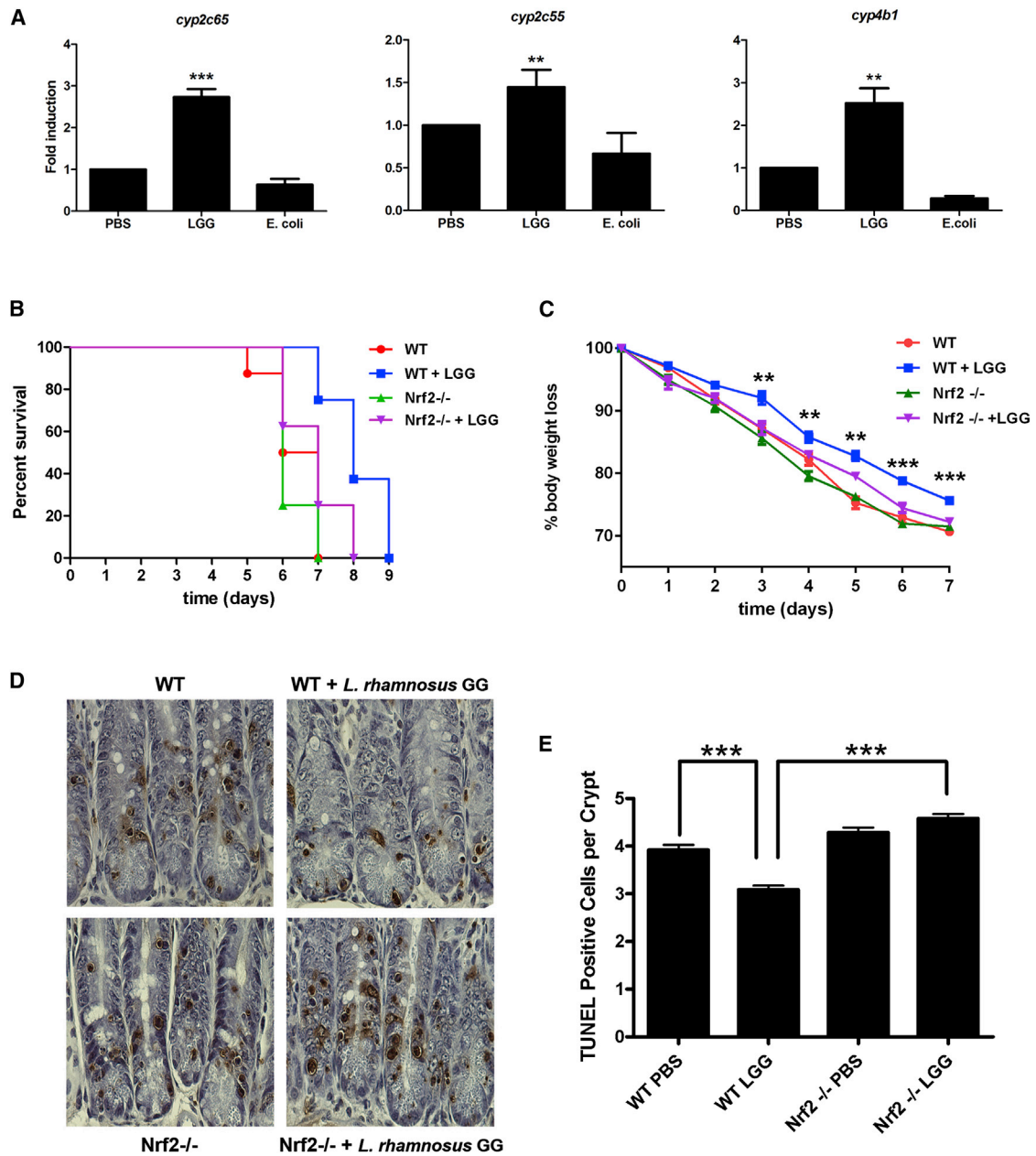


Figure 3. Colonization of the Murine Intestine with *Lactobacillus rhamnosus* GG Induces Nrf2-Dependent Cytoprotection

(A) qPCR analysis to measure the abundance of mRNA transcripts expressed from *cyp2c65*, *cyp2c55*, and *cyp4b1* in colonic tissues of wild-type B6 germ-free mice fed either 2×10^9 cfu total *L. rhamnosus* GG, or *E. coli* by oral gavage for 4 hr.

(B) Survival of 7-week-old Nrf2^{-/-} or wild-type littermates fed *L. rhamnosus* GG (LGG) following 12 Gy irradiation insult. Note significantly increased survival of irradiated wild-type (WT) mice fed LGG in response to irradiation (log-rank test for WT versus WT+LGG, $p = 0.0013$, $n = 8$). Also note significantly decreased survival rate of Nrf2^{-/-} mice fed LGG compared to WT mice fed LGG (log-rank test for WT+LGG versus Nrf2^{-/-} +LGG, $p = 0.0142$, $n = 8$) and no significant increase in survival of Nrf2^{-/-} mice fed LGG compared to unfed WT (log-rank test for WT versus Nrf2^{-/-} +LGG, $p = 0.2150$, $n = 8$).

(C) Percentage of body weight loss of mice described in (B). Statistical analysis represents comparison of WT+LGG versus Nrf2^{-/-} +LGG on each respective day. * $p < 0.05$, ** $p < 0.01$, $n = 8$.

(D) Detection of TUNEL-positive cells within colonic tissues harvested from mice described in (A) and (B).

(E) Quantification of TUNEL-positive cells in (C). *** $p < 0.001$, $n = 5$.

Error bars indicate SEM.

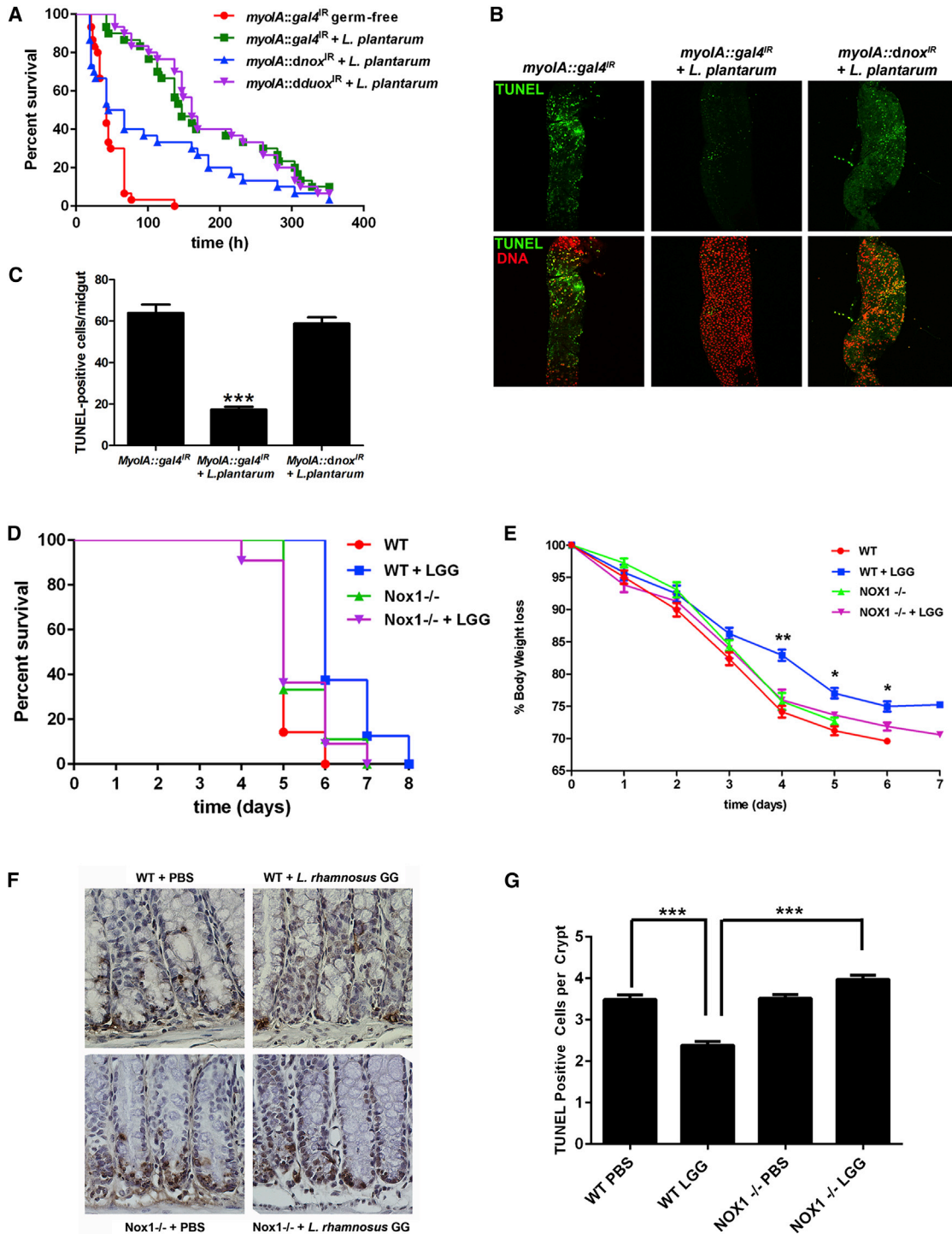


Figure 4. Nox1 Is Required for Optimal Lactobacilli-Induced Cytoprotection

(A) Survival in response to Paraquat challenge of 5-day-old conventionally raised adult *Drosophila* where the levels of *dnox* (UAS-*dnox*^{IR}) are diminished, under the enterocyte-specific *myoIA*-GAL4 driver (log-rank test for *myoIA*-GAL4; UAS-*gal4*^{IR} + *L. plantarum* versus *myoIA*-GAL4; UAS-*dnox*^{IR} + *L. plantarum*, $p = 0.0185$, $n = 100$).

(B) TUNEL analysis of posterior midgut tissues dissected from adult *Drosophila* listed in (A) and exposed to Paraquat challenge for 48 hr.

(C) Numeration of TUNEL-positive cells per midgut examined in (B). *** $p < 0.001$, $n = 10$.

(D) Survival of 7-week-old intestinal epithelial cell-specific Nox1-deficient (B6.Nox1^{ΔIEC}) and wild-type littermates fed *L. rhamnosus* GG (LGG) following 12 Gy irradiation insult. Note significantly increased survival of irradiated WT mice fed LGG in response to irradiation (log-rank test for WT versus WT+LGG, $p = 0.0012$,

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to be strain specific (Storelli et al., 2011). In our experiments, we utilized a single *L. plantarum* strain isolated from the intestine of *Drosophila* from our fly stock. Whether the influence of *L. plantarum* in *Drosophila* (or the influence of *L. rhamnosus* GG in mice) is strain and species specific, or a more general phenomenon of lactobacilli is a subject of intense focus within our research group.

Taken together, these observations are consistent with the concept of “hormesis,” wherein low, near threshold levels of a stressor is protective against more intense or prolonged stimuli, which is explicitly demonstrated in Figure 1E. This concept has been implicated in ROS-induced, Nrf2-mediated protection against irradiation injury (in the bone marrow) (Kim et al., 2014) and cytotoxic mucosal injury (in the airway) (Paul et al., 2014). The microbiota, despite having a beneficial relationship with the host, nevertheless is an extrinsic influence, and ROS stimulated by microbes is a potent signaling stressor. Thus, hormesis, as a response to xenobiotic and environmental stimuli, evidently extends to the acquisition of and adaption to exogenous microbiota, illustrating a mechanism of co-evolution and symbiosis between host cells and microbes.

EXPERIMENTAL PROCEDURES

Drosophila Paraquat Resistance Assays

Whole-animal cytoprotection in *Drosophila* was measured in response to Methyl viologen dichloride (Paraquat)-induced oxidative stress. Groups of ten per vial of 5-day-old adult *Drosophila* of assayed genotypes were starved for 3 hr and then fed a solution of 5% sucrose containing a semi-lethal dose of Paraquat (25 mM). Survivors were scored for up to 5 days, or until 100% lethality. Percentage of surviving flies were recorded and compared by log-rank Mantel-Cox test. From each genotype and gender, triplicate assays of initial surviving 100 flies were scored. Gut tissue cytoprotection in response to (Paraquat)-induced oxidative stress was analyzed following dissection of the fly midgut, fixing the tissue in 4% paraformaldehyde, followed by TUNEL assay analysis using In Situ Cell Death Detection Kit (Roche).

See Supplemental Information for other experimental procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.07.042>.

AUTHOR CONTRIBUTIONS

R.M.J. and A.S.N. conceived and designed the experiments. R.M.J., L.L., A.R.R., and E.S.K. performed experiments on the *Drosophila* animal model. R.M.J., C.D., and C.S.A. performed experiments using the Nrf2-null murine model, and T.M.D. performed experiments using the Nox1-null murine model. R.M.J., C.D., C.D.S., E.S.K., and A.A.W. analyzed the data. R.M.J. and A.S.N. wrote the manuscript.

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n = 8). Also note significantly decreased survival rate of B6.No^{x1}^{ΔIEC} mice fed LGG compared to WT mice fed LGG (log-rank test for WT+LGG versus B6.No^{x1}^{ΔIEC} + LGG, p = 0.0118, n = 8). Finally, note no significantly increased survival rates of irradiated WT unfed mice and B6.No^{x1}^{ΔIEC} mice fed LGG compared (log-rank test for WT versus B6.No^{x1}^{ΔIEC} + LGG, p = 0.4465, n = 8).

(E) Percentage of body weight loss of mice described in (D). Statistical analysis represents comparison of WT+LGG versus B6.No^{x1}^{ΔIEC}+LGG on each day, *p < 0.05, **p < 0.01, n = 8.

(F) Detection of TUNEL-positive cells within colonic tissues harvested from mice described in (C) and (D).

(G) Quantification of TUNEL-positive cells in (E) ***p < 0.001, n = 5.

Error bars indicate SEM.

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