A Deregulated Intestinal Cell Cycle Program Disrupts Tissue Homeostasis without Affecting Longevity in Drosophila*

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**Background:** We know comparatively little about the impact of deregulated intestinal proliferation on an otherwise normal animal.

**Results:** Modified progression through the cell cycle disrupted homeostasis without affecting longevity.

**Conclusion:** Disrupted intestinal homeostasis alone does not shorten the life span of an adult fly.

**Significance:** These questions are of considerable importance, given the relationship between proliferation, dysplasia, aging, and death.

Recent studies illuminate a complex relationship between the control of stem cell division and intestinal tissue organization in the model system Drosophila melanogaster. Host and microbial signals drive intestinal proliferation to maintain an effective epithelial barrier. Although it is widely assumed that proliferation induces dysplasia and shortens the life span of the host, the phenotypic consequences of deregulated intestinal proliferation for an otherwise healthy host remain unexplored. To address this question, we genetically isolated and manipulated the cell cycle programs of adult stem cells and enterocytes. Our studies revealed that cell cycle alterations led to extensive cell death and morphological disruptions. Despite the extensive tissue damage, we did not observe an impact on longevity, suggesting a remarkable degree of plasticity in intestinal function.

Regulated proliferation in a mature intestine provides an essential safeguard against potentially lethal dysplastic conditions. The control of intestinal proliferation is particularly important given the complex and dynamic impacts of the intestinal environment on metazoan behavior, development, immunity, and metabolism (1–5). The model organism Drosophila melanogaster is an ideal system to explore the interplay between host proliferation and the intestinal environment (6–8). The development of the adult Drosophila intestine is well characterized; adult flies have a relatively short life span; the host microbiome consists of a small number of bacterial species that can often be cultured in isolation; and researchers have access to sophisticated genetic tools for manipulation of the intestinal environment.

Substantial experimental evidence suggests that basal intestinal stem cells (ISCs) undergo symmetric or asymmetric divisions to maintain a functional posterior midgut in adult Drosophila (9–11). Inputs from JAK/STAT, insulin, EGF, Wingless, PDGF, and VEGF pathways inform the rate of ISC division (10, 12–30). In the absence of extrinsic challenges, these signals culminate in a division every 1–2 weeks (9, 11). Microbial or chemical damage to epithelial surfaces engages a number of signal transduction pathways that accelerate ISC division (7, 21, 27, 30–40), presumably to ensure preservation of the epithelial barrier. ISC division generates a new stem cell and a transient cell type, the enteroblast, which integrates signals from the Notch/Delta axis to differentiate as secretory enteroendocrine cells or absorptive enterocytes (41–43). The individual cells are easily distinguished by expression of specific markers, with Delta (Dl) expressed in ISCs (42), escargot (esg) restricted to progenitor cells (9), myo1A expressed in enterocytes (33), and prospero expressed in enteroendocrine cells (9). Enterocytes are the most common epithelial cells and undergo several rounds of endoreplication to generate large apical cells with extensive brush borders.

Although the coordinated division and differentiation of progenitor cells maintain a functional epithelial barrier in the intestine, longitudinal studies revealed that progressive rounds of division come at a cost to the host. As flies age, the intestine shows clear signs of dysplasia (25, 31, 44, 45), with a loss of barrier function (46). Bacterial titers increase and lead to elevated signal transduction through the antibacterial immune deficiency pathway and increased production of reactive oxygen species (32, 47–50). The density and composition of the intestinal microbiome shift with age (51), and genetic studies suggest that microbial signatures lead to intestinal dysplasia through the induction of stem cell division (32). Inhibition of the insulin or JNK pathway delays the onset of dysplasia, lowers rates of ISC division, and expands host longevity (44). However, despite correlations between the microbiome and ISC division, the contributions of the microbiome to host longevity are unclear. One study implied a beneficial effect of the microbiome for host life span (52), and another study suggested that the microbiome has no effect on host longevity (47). In addition, there are no data on the phenotypic consequences of direct manipulation of the intestinal cell cycle program.
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The relationship between the intestinal microflora, ISC proliferation, and host longevity remains unclear.

To address this question, we genetically manipulated cell cycle progression in the intestines of otherwise normal flies and examined the phenotypic consequences for the host. We found that modification of the cell cycle in progenitor cells or enterocytes invariably disrupted intestinal homeostasis. Nonetheless, we did not see obvious effects of deregulated proliferation on the longevity of conventionally reared (CR) flies. Instead, we observed that ISC division is required for maintenance of stem cell numbers. Inhibition of cell division diminished the number of stem cells and altered the antimicrobial peptide (AMP) expression profile of the host. Our data suggest an intimate link between proliferation and homeostasis that is independent of fly longevity.

Experimental Procedures

Fly Husbandry—All experiments were performed with virgin male and female flies. w^{1118} flies were used as the wild-type strain. Unless stated otherwise, flies were raised on standard cornmeal medium (Nutri-Fly Bloomington Formulation, Genesee Scientific) at 29 °C. Germ-free (GF) flies were generated by raising adult flies in standard medium supplemented with an antibiotic mixture (100 μg/ml ampicillin, 50 μg/ml vancomycin, 100 μg/ml neomycin, and 100 μg/ml metronidazole dissolved in ethanol) described previously (53). CR control flies were raised on cornmeal medium supplemented with ethanol. Minimal medium was prepared by mixing equal volumes of water and instant Drosophila medium (Carolina Biological Supply Co.). Dyed medium for the Smurf assay was prepared by adding 1% (w/v) erioglaucine disodium salt (Sigma-Aldrich) to fly food medium. The w^{1118}, myo1a[w^{1118}], UASdap, and UASPvrCA flies have been described previously (32, 33, 54, 55). Mitotic clones were generated with flies of the following genotype: y, w, hs-flp, UAS-mCD8:GFPneoFR{T}40A/tubGAL80, neoFR{T}40A). For survival curve analysis, 40–50 flies were raised in vials, with 10 flies/vial, and transferred to fresh vials weekly.

Immunofluorescence—Adults flies were washed with 95% ethanol and dissected in PBS, and guts were fixed for 20 min at room temperature with 4% formaldehyde in PBS. Guts were rinsed with PBS and blocked overnight in PBSTBN (PBS, 0.05% Tween 20, 5% BSA, and 1% normal goat serum) at 4 °C. Guts were stained for 3 h at room temperature in PBSTBN with the appropriate primary antibodies, washed with PBSTBN (PBS, 0.05% Tween 20, and 5% BSA), and stained for 1 h at room temperature in PBSTBN with Hoechst 33258 (1:500; Molecular Probes) and the appropriate secondary antibody (goat anti-mouse Alexa Fluor 647 and 568 and goat anti-rabbit Alexa Fluor 488, Molecular Probes). The guts were washed with PBSTBN and rinsed with PBS prior to visualization. The primary antibodies used in this study were as follows: mouse anti-Delta (1:100; Developmental Studies Hybridoma Bank C594.9B), mouse anti-Armadillo (1:100; Developmental Studies Hybridoma Bank N2 7A1), mouse anti-Propero (1:100; Developmental Studies Hybridoma Bank MRIA), and rabbit anti-GFP (1:1000). For TUNEL assays, dissected guts of virgin female flies were fixed in 4% formaldehyde for 20 min at room temperature, washed with PBS for 30 min at room temperature, and incubated in 100 μl of TUNEL labeling mixture/enzyme solution (in situ cell death detection kit, TMR red, Roche Applied Science) and Hoechst 33258 at room temperature. Gut were washed with PBS and 0.05% Tween 20 for 30 min, washed with PBS overnight, and visualized. Samples were mounted on slides in Fluoromount (Sigma-Aldrich F4680), and the posterior midgut was visualized with a spinning disk confocal microscope (Quorum WaveFX, Quorum Technologies Inc.). Images were collected as a Z-series and processed with Fiji software to generate a single Z-stacked image. Cell numbers were counted using the Harmony software package (PerkinElmer Life Sciences). Electron microscopy was performed at the Faculty of Medicine and Dentistry Core Imaging Cell Imaging Centre of the University of Alberta.

Infection Protocol—Virgin female flies were raised for 10 days at 29 °C. Flies were placed in empty vials for 2 h prior to infection. Pseudomonas entomophila was prepared to an absorbance of 200 in 5% sucrose in PBS. Ten flies/vial were infected with 100 μl of P. entomophila preparations on cotton plugs, and mortality was measured at 8-h intervals.

PCR—The quantitative PCR protocol and primers used in this study have been described previously (56). Bacterial genomic DNA was isolated from flies using the UltraClean microbial DNA isolation kit (MO BIO Laboratories, Inc.) according to the manufacturer’s instructions. Bacterial 16 S DNA was amplified with primers AGAGTTTGATCCTGCGTCA forward) and GGCTACCTTGTTACGACTT (reverse), and the croquemort promoter was amplified with the AATAGCCAAAGGTGGGAAAATAATCCAG and CACCTGCGCAGTTTACAGCTGAG primer pair.

Results

Enterocyte Endoreplication Is Essential for Intestinal Homeostasis—To determine the importance of enterocyte endoreplication for intestinal homeostasis, we used the myo1A[w^{1118}] TARGET system (myo1AGAL4; tubGAL80[w^{1118}], UASGFP) to express the G1-to-S phase transition inhibitor dacapo (dap) (55) in adult enterocytes, and we examined the consequences in the adult posterior midgut. In this system, myo1AGAL4 marks enterocytes as GFP-positive cells (Fig. 1, A and C). Expression of dap in enterocytes greatly reduced the number of GFP-positive cells, and the residual population appeared smaller than their myo1A[w^{1118}] counterpart (Fig. 1, B–E).

Transmission electron microscopy revealed a conventional arrangement of progenitor cells interspersed between larger enterocytes in myo1A[w^{1118}] + midguts (Fig. 1F). In contrast, the nuclei of UASdap/+;myo1A[w^{1118}] + epithelial cells were smaller than those of their control counterparts (Fig. 1G); most cells were heavily vacuolated; and we frequently observed delamination of small irregularly shaped cells, suggesting elevated levels of cell death (Fig. 1G, arrowheads). In agreement with this hypothesis, we detected a substantial level of TUNEL staining in UASdap/+;myo1A[w^{1118}] + midguts (Fig. 1, H–K). Consistent with elevated enterocyte death, we found that inhibition of enterocyte endoreplication led to an increase in the relative proportion of endoendocrine cells in UASdap/+;myo1A[w^{1118}] + midguts, as determined by quantification of Prospero-positive positive.
In short, inhibition of endoreplication disrupts midgut homeostasis with reduced numbers of enterocytes, widespread cell death, and increased proportions of enteroendocrine cells.

Hyperproliferation of ISCs Leads to Intestinal Distension and Cell Death—To examine the impact of deregulated proliferation on midgut organization, we used the esg[ts] TARGET system (esgGAL4; UASGFP, tubGAL80[ts]) to accelerate or block

![Image](https://example.com/image1)

**FIGURE 1.** Endoreplication in adult enterocytes supports midgut homeostasis. A–D, representative immunofluorescence images of the posterior midguts of 20-day-old myo1a[ts]/+ and UASdap−/− myo1a[ts]/+ adults. GFP is shown in green, Prospero and the β-catenin ortholog Armadillo are shown in red, and DNA is shown in blue. Scale bars = 400 μm (A and B) and 25 μm (C and D). E, quantification of GFP-positive cells in the midguts of 20-day-old myo1a[ts]/+ (n = 6) and UASdap+/− myo1a[ts]/+ (n = 6) adults. Statistical evaluations were performed by one-way analysis of variance (ANOVA). *, p < 0.05. F and G, transmission electron microscopy images of 10-day-old control myo1a[ts]/+ and UASdap+/− myo1a[ts]/+ adults, respectively. Enterocytes (EC), progenitor cells (p), and visceral muscle (vm) are labeled. Arrowheads in E indicate irregularly shaped cells in the midgut of UASdap+/− myo1a[ts]/+ adults. Scale bars = 2 μm. H–J, representative immunofluorescence images of TUNEL-positive cells (yellow) in the midguts of 10-day-old UASdap+/− myo1a[ts]/+ adults. DNA is shown in blue. K, quantification of TUNEL-positive cells in the midguts of 10-day-old UASdap+/− myo1a[ts]/+ (n = 5), and UASdap+/− myo1a[ts]/+ (n = 7), and UASdap+ myo1a[ts]/+ (n = 6) adults, respectively. Statistical evaluations were performed by one-way ANOVA. *, p < 0.05; **, p < 0.01. L–N, representative immunofluorescence images of Prospero-positive cells (yellow) in the midguts of 10-day-old UASdap+/−, myo1a[ts]/+, and UASdap+/− myo1a[ts]/+ adults. DNA is shown in blue. O, quantification of Prospero (pros)-positive cells in the midguts of 10-day-old UASdap+/− (n = 4), myo1a[ts]/+ (n = 5), and UASdap+/− myo1a[ts]/+ (n = 5) adults. Statistical evaluations were performed by one-way ANOVA. **, p < 0.01; ***, p < 0.001. Scale bars = 25 μm (H–O and L–N).
the cell cycle of progenitor cells. Consistent with previous reports, we observed GFP expression in small regularly spaced basal progenitor cells in the posterior midgut of newly eclosed esg[ts]/+ adults (Fig. 2A). The organization of GFP deteriorated with age: the number of GFP-positive cells increased, and we observed GFP in epithelial cells (Fig. 2B). These data overlap with several studies that established progressive dysplasia in aging intestines.

We initially used the esg[ts] system to accelerate proliferation of progenitor cells through expression of an active variant of the PDGF and VEGF receptor-related (pvrCA) receptor tyrosine kinase. We previously established that expression of pvrCA in posterior midgut progenitor cells accelerates ISC proliferation and increases the number of DI-positive ISCs (15). esg[ts]-mediated induction of pvrCA greatly disrupted the pattern of GFP expression in adult midguts. Within 1 day of eclosion, we observed a large population of GFP-positive cells that included epithelial cells and resembled 20-day-old control intestines (Fig. 2C). Persistent expression of pvrCA in adult progenitor cells disrupted intestinal morphology with a visible increase in the distribution of GFP-positive cells in the posterior midgut (Fig. 2D). Transmission electron microscopy confirmed that activation of Pvr greatly expanded the number of basal progenitor cells (Fig. 2E), increased the thickness of the midgut, and decreased the volume of the intestinal lumen (Fig. 2, F and G). TUNEL staining showed that hyperproliferation of stem cells also resulted in significant levels of intestinal cell death (Fig. 2, H–K). From these data, we conclude that hyperproliferation in ISCs leads to intestinal distention and an increase in midgut cell death.

Irregularly Shaped Cells Accumulate in the Midguts of Adults with Impaired Stem Cell Division—To examine the effect of impaired stem cell proliferation on the organization of an adult midgut, we determined the number and arrangement of GFP-positive cells in esg[ts]/UASdap flies relative to esg[ts]/+ flies. As expected, we observed a widespread and irregular organization of GFP-positive cells in 20-day-old esg[ts]/+ adults (Fig. 3, A and C). In contrast, we detected very few GFP-positive cells in 20-day-old esg[ts]/UASdap adults raised under identical conditions (Fig. 3, B, D, and E). GFP expression in esg[ts]/UASdap midguts resembled freshly eclosed control adults, with GFP restricted to a limited number of small regularly spaced cells.

Transmission electron microscopy of esg[ts]/UASdap adults revealed dense staining material at various stages of detachment from the epithelium (Fig. 3, F–I, arrowheads). The disrupted integrity of the esg[ts]/UASdap epithelium resembles the damage caused by infection with the enteropathogenic microbe P. entomophila (57) and suggests that prolonged interruption of stem cell proliferation results in accumulation of irregularly shaped epithelial cells. In addition, we noticed that...
the bulk of epithelial cells were continuous with the visceral muscle and that the number of progenitor cells was greatly reduced (Fig. 3, F–I). The few basal cells we found were irregularly shaped and lacked the standard cytological features of wild-type progenitor cells (Fig. 3G, dashed line). Combined, these findings indicate that inhibition of stem cell proliferation results in accumulation of irregularly shaped progenitor and epithelial cells, with a parallel decline in functional progenitor cells.

**Disruptions to Intestinal Proliferation Do Not Impact Drosophila Viability**—In agreement with a previous report (44), we found that expression of a constitutively active version of the Drosophila JNKK ortholog *hemipterous* (*hepCA*) or a dominant inhibitory variant of the Drosophila insulin receptor (*InRDN*) in midgut progenitor cells led to a diminished or an enhanced life span for the adult, respectively (Fig. 4). Building from these findings, we reasoned that the *myo1A*[ts]/UASdap, *esg*[ts]/UASpvrCA, and *esg*[ts]/UASdap fly strains allowed us to genetically isolate the relevance of deregulated proliferation for the overall health of the host. We initially assumed that disrupted proliferation would greatly reduce host longevity. However, we found that the longevities of *myo1A*[ts]/UASdap, *esg*[ts]/UASpvrCA, or *esg*[ts]/UASdap flies raised on conventional medium were indistinguishable from the appropriate control genotypes (Fig. 4). We observed similar effects if we raised flies at the permissive temperature for the first 15 days of adult life and then shifted them to a restrictive temperature (Fig. 4). To determine whether the rich nutritional environment of conventional fly food compensates for partial defects in intestinal function, we repeated the survival assays with flies raised on a minimal nutritional medium. Again, we found that deregulated intestinal proliferation did not reduce the viability of the exper-

**FIGURE 3.** Inhibition of stem cell proliferation greatly reduces the number of progenitor cells in the midguts of adult Drosophila. A–D, representative immunofluorescence images of the midguts of 20-day-old *esg*[ts]/+ and *esg*[ts]/UASdap adults. GFP is shown in green, and DNA is shown in blue. Scale bars = 400 μm (A and B) and 25 μm (C and D). E, quantification of GFP-positive cells in the midguts of 20-day-old *esg*[ts]/+ (n = 6) and *esg*[ts]/UASdap (n = 6) adults. Statistical evaluations were performed by one-way ANOVA. **F–I**, transmission electron microscopy images of 20-day-old *esg*[ts]/UASdap midguts. Visceral muscle (*vm*) and enterocytes (*EC*) are labeled. The dashed line in G indicates the border of an irregularly shaped basal cell, and arrowheads show material detaching from the intestinal epithelium. Scale bars = 2 μm.
Experimental populations relative to the respective controls (Fig. 4). Instead, esg[ts]/UASdap flies appeared to have a moderate advantage over their control counterparts when raised on minimal medium. We then directly challenged the intestines of myo1A[ts]/UASdap, esg[ts]/UASpvrCA, and esg[ts]/UASdap adults with toxic doses of P. entomophila. Here, we also found that impaired or altered intestinal proliferation had no discernible impact on the ability of adult flies to counter a lethal microbial challenge (Fig. 4). Combined, these data suggest that an orderly proliferative program is essential for midgut homeostasis, but that disruptions to this program alone do not negatively affect the viability of the fly under a range of experimental conditions.

ISC Proliferation Supports AMP Expression in Adult Drosophila—As disrupted intestinal cell cycle progression had no obvious effects on Drosophila longevity, we examined connections between the cell cycle and the innate immune response. For these assays, we conducted a longitudinal survey of the expression of the immune deficiency-responsive AMPs attacin (att) and diptericin (dpt) in adult Drosophila of the indicated genotypes at the indicated times after eclosion. Expression levels were standardized to actin in the respective groups. Each graph presents the results of three independent measurements. G, visualization of bacterial 16S ribosomal DNA (16S) isolated from adult Drosophila of the indicated genotypes and ages. A section of the Drosophila croquemort promoter (croPE) was amplified as a control.

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The absence of elevated AMP levels in aging esg[ts]/UASdap flies led us to monitor the bacterial density of esg[ts]/UASdap adults. For these experiments, we examined the total levels of bacterial 16S rDNA in flies of different ages. As expected, we observed an increase in bacterial genomes in esg[ts]/H11001 and UASdap/H11001 flies with age (Fig. 5G). In contrast, we observed a partially reduced density of bacterial genomes in older esg[ts]/UASdap adults, consistent with the reduced induction of AMPs.

Cell Division Maintains Progenitor Cells in the Adult Midgut—Elimination of commensal bacteria from adult esg[ts] flies leads to a decrease in GFP-positive cells, diminished AMP expression, and a reduction in the number of cells positive for the mitotic marker phosphorylated histone H3 (32). The reduced rates of intestinal proliferation and diminished AMP expression in axenic flies are similar to our observations with the midguts of esg[ts]/UASdap adults. These similarities prompted us to further examine the relationship between commensal bacteria and intestinal proliferation. For these experiments, we raised Drosophila larvae under conventional conditions and transferred freshly eclosed adults to a medium enriched with antibiotics. We reasoned that this approach permits developmental inputs from commensal bacteria and allows us to exclusively determine the impact of commensal populations on stem cell proliferation in adult midguts. Consistent with previous reports, we found that 20-day-old esg[ts] flies raised under GF conditions had fewer GFP-positive cells than their CR counterparts (Fig. 6, A and B). Examination of GFP-marked mitotic clones confirmed that the level of progenitor cell mitosis was greatly reduced in GF flies compared with CR flies (Fig. 6, C and D), indicating that commensal bacteria drive the proliferation of stem cells as Drosophila flies age.

We then examined the structure of GF and CR intestines. Newly eclosed CR, newly eclosed GF, and 20-day-old CR flies had similar arrangements of visceral muscle, progenitor, and epithelial cells (Fig. 6, E–G), although the older CR midgut appeared less organized than the younger counterparts. In contrast to esg[ts]/UASdap flies, we did not detect epithelial dam-
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FIGURE 7. Commensal microbes damage barrier integrity in the gut and decrease adult viability. A, percentage of flies with an intact intestinal barrier in CR (dashed line) and GF (solid line) adult Drosophila as a function of time after eclosion. B, viability of CR (dashed line) and GF (solid) adult Drosophila as a function of time after eclosion. For A and B, the y axis indicates that the percentage of flies and curves were compared using a log-rank (Mantel-Cox) test. C, Tukey’s box plot representation of the viabilities of male and female adults of the indicated genotypes raised under CR or GF conditions. Comparisons were performed with Sidak’s multiple comparison test, and values show Sidak’s t value for the respective tests. **, p < 0.01; ****, p < 0.0001. D, 20-day-old CR and GF flies were infected with \( P. \) entomophila at the indicated absorbance. The viability of infected flies was measured at times in hours post-infection. Curves were compared using a log-rank test. For each comparison, GF flies were compared with CR flies infected with the same absorbance of \( P. \) entomophila. *, p < 0.05; ***, p < 0.001.

age in aged GF flies, suggesting that basal levels of proliferation in GF flies replenish damaged epithelial cells. However, similar to \( esg[ts]/UASdap \) flies, we noted a pronounced absence of definable progenitor cells in GF adults (Fig. 6H). The apparent absence of progenitor cells in mitotically quiescent intestines suggests that a basal level of ISC division is required to maintain total stem cell numbers. To test this hypothesis, we directly visualized the ISC marker DI in the midguts of CR and GF adults. We consistently observed a substantial decline in the number of DI-positive cells in GF flies (Fig. 6, K and L) compared with CR flies (Fig. 6, I and J). On average, GF flies had approximately half the number of DI-positive cells as CR flies (Fig. 6M). Combined, our findings suggest that stem cell mitosis is essential for maintenance of total stem cell numbers.

Commensal Bacteria Disrupt Intestinal Barrier Function and Decrease Adult Drosophila Longevity—As previous studies established a link between aging and dysplasia, we examined the relationship between commensal bacterial, host proliferation, and intestinal barrier function in an aging population of flies. For these experiments, we used the recently described Smurf assay (46) to follow loss of intestinal barrier function in adult flies. We found that GF flies took significantly longer to Smurf than CR flies (Fig. 7A), suggesting that raising flies under conventional conditions accelerates the loss of an intestinal barrier. We were somewhat surprised to see that our Smurf data also suggested that raising flies in a GF environment prolonged adult longevity. To exclude contaminating effects of the food dye on our longevity observations, we determined the longevity of CR and GF flies raised on standard fly medium. GF flies had a significantly enhanced longevity relative to CR flies (Fig. 7B), suggesting that commensal microbes gradually diminish the barrier functions of Drosophila intestines and reduce the longevity of the host.

Given the elevated intestinal damage seen in \( esg[ts]/UASdap \) flies compared with GF flies, we then monitored the longevity of GF and CR \( esg[ts]/UASdap \) flies to determine whether stem cell proliferation is a prerequisite for the longevity enhancement observed with flies raised in an axenic environment. We found that adult male or female \( esg[ts]/+ \) or \( UASdap/+ \) flies displayed a moderately increased longevity when raised in a GF environment (Fig. 7C). In contrast, the longevity of male or female \( esg[ts]/UASdap \) flies raised in a CR environment was indistinguishable compared with those raised in a GF environment. These findings imply a novel relationship between commensal bacteria, host stem cell proliferation, and host longevity. The presence of commensal bacteria induces host stem cell proliferation and decreases the longevity of the host. However, a basal level of stem cell proliferation appears to be important for the life span increase observed under axenic culture conditions, as inhibition of proliferation blocks the longevity enhancement associated with a GF environment.

Despite the longevity disadvantages associated with the intestinal microbiota, we found that resident bacteria confer a significant advantage to adult flies challenged with \( P. \) entomophila. We infected 10- and 20-day-old CR and GF female flies with a range of microbial densities. In each case, we noticed that the CR flies had a significant survival advantage over their GF counterparts (Fig. 7D). These findings suggest that the fly microbiota decreases host longevity but provides a significant survival benefit against enteropathogenic infections.

DISCUSSION

The animal intestine establishes a critical interface with the external environment. A healthy intestine maximizes nutritional uptake, coordinates physiological responses to an animal’s habitat, and protects against chemical and microbial challenges. Resident autochthonous and transient allochthonous microbes complicate relationships between an intestine and the external world through the induction, or worsening, of intestinal pathologies that range from chronic inflammation to cancer. Deregulated proliferation is a hallmark of many intestinal pathologies that range from chronic inflammation to cancer. Deregulated proliferation is a hallmark of many intestinal pathologies that range from chronic inflammation to cancer. Deregulated proliferation is a hallmark of many intesti...
proliferation shortened the life span of the fly. However, these studies were performed with modifications to hormonal or stress signaling pathways that influence multiple cellular events. For example, the JNK pathway contributes to events as diverse as cell movement, proliferation, survival, and immunity (59), whereas the insulin response pathway controls cell growth and metabolism in response to nutritional cues (60). In this study, we determined the phenotypic consequences of direct alterations to the intestinal cell cycle program of flies with intact intestinal signal transduction. Our studies include the first reported manipulation of the endocycle program of mature enterocytes.

We found that interruptions to the normal program of ISC division and enterocyte endoreplication disrupted intestinal homeostasis, irrespective of the manipulation. Chronic proliferation of ISCs resulted in substantial intestinal distension and enhanced cell death along the midgut, whereas impaired ISC proliferation led to an accumulation of dead and damaged intestinal cells. Inhibition of endocycles in the mature epithelium resulted in vacuolation throughout the adult midgut. The appearance of vacuoles in enterocytes mirrors a previous report of autophagy in dying enterocytes (61) and suggests that endoreplication is essential for enterocyte viability. Consistent with this hypothesis, we observed elevated cell death and a disproportionately large ratio of enteroendocrine cells in the midguts of adult flies that expressed dap in enterocytes. The elevated numbers of enteroendocrine cells may reflect a loss of total enterocyte numbers. Alternatively, we cannot exclude that impaired endoreplication in enterocytes alters the normal program of enteroblast differentiation. For example, loss of Notch function also leads to a loss of enterocyte fate and an increase in enteroendocrine cells (9). Combined, these data suggest that disruptions to enterocyte endoreplication interrupt Notch activity and lead to a relative increase in enteroendocrine cells.

Our findings establish a clear link between proliferation and tissue homeostasis. Regardless of the manipulation, we consistently observed substantial disruptions to the intestines of flies with modified intestinal cell cycle programs. Contrary to our expectations, we have shown that disrupted intestinal proliferation alone does not substantially affect the life span of an adult fly. Instead, the intestine appears to be a remarkably plastic organ that sustains the host even during prolonged periods of extreme dysplasia. These results uncover an unanticipated degree of functional plasticity in the adult midgut and indicate that a complete block to ISC division alone does not significantly shorten the life span of the fly.

Microscopic examination of adults with impaired stem cell division pointed to a link between intestinal proliferation and the maintenance of ISCs. We found that a prolonged arrest of stem cells in the G1 phase of the cell cycle led to an apparent loss of basally located progenitor cells. This phenotype led us to speculate that a constitutive level of ISC division is essential to maintain ISC numbers. We confirmed this hypothesis with a more detailed examination of flies raised under axenic conditions. In agreement with other studies (32), we found that incubation of adult flies in an axenic environment decreased the rate of midgut mitosis. Similar to observations on the forced inhibition of ISC division, we noted a conspicuous absence of basal progenitor cells in the intestines of adults raised without microflora. In follow-up assays, we established that removal of commensal microbes greatly reduced the number of Dl-positive stem cells in the adult midgut. Combined, these data indicate that commensal microbes provide mitogenic factors that induce stem cell division and that such a constitutive division maintains total stem cell numbers.

We believe that the connections between the intestinal microflora and host proliferation have significant implications for the health of the host. In our hands, flies raised in an axenic environment lived longer than their CR counterparts, with a parallel extension to the duration of intestinal barrier function. The ability of an axenic environment to prolong host longevity requires ISC proliferation, as expression of dap in stem cells appears to revert the beneficial effects of a GF environment. Our results with GF adults differ from those of other groups who reported negative or neutral effects of such culture conditions (47, 52). We suspect that the different results reflect the variability of intestinal microbiomes reported with lab strains of D. melanogaster. We propose that different bacterial strains or strain compositions will have different effects on the overall health of the host. In our case, it appears that the microflora led to intestinal dysplasia and disruptions to the intestinal barrier function, ultimately accelerating host death. In general, our results overlap with a model in which basal levels of ISC proliferation gradually damage and disrupt intestinal function, shortening the life span of the host. Despite the shortened life span associated with the intestinal microflora, we have shown that the Drosophila microflora confers a protective benefit against intestinal challenges with pathogenic microbes.

In summary, our data suggest a complex interplay between proliferation, the microflora, and host longevity. The intestinal microflora induces stem cell proliferation with negative long-term consequences for the host, including dysplasia, diminished barrier function, and shortened longevity. However, microflora-driven proliferation alone does not account for the shorter host life span, as modifications to the intestinal cell division program disrupt homeostasis without any apparent impact on longevity.

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