Synergy between bacterial infection and genetic predisposition in intestinal dysplasia

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Accumulating evidence suggests that hyperproliferating intestinal stem cells (SCs) and progenitors drive cancer initiation, maintenance, and metastasis. In addition, chronic inflammation and infection have been increasingly recognized for their roles in cancer. Nevertheless, the mechanisms by which bacterial infections can initiate SC-mediated tumorigenesis remain elusive. Using a *Drosophila* model of gut pathogenesis, we show that intestinal infection with *Pseudomonas aeruginosa*, a human opportunistic bacterial pathogen, activates the c-Jun N-terminal kinase (JNK) pathway, a hallmark of the host stress response. This, in turn, causes apoptosis of enterocytes, the largest class of differentiated intestinal cells, and promotes a dramatic proliferation of SCs and progenitors that serves as a homeostatic compensatory mechanism to replenish the apoptotic enterocytes. However, we find that this homeostatic mechanism can lead to massive over-proliferation of intestinal cells when infection occurs in animals with a latent oncogenic form of the Ras1 oncogene. The affected intestines develop excess layers of cells with altered apicobasal polarity reminiscent of dysplasia, suggesting that infection can directly synergize with the genetic background in predisposed individuals to initiate SC-mediated tumorigenesis. Our results provide a framework for the study of intestinal bacterial infections and their effects on undifferentiated and mature enteric epithelial cells in the initial stages of intestinal cancer. Assessment of progenitor cell responses to pathogenic intestinal bacteria could provide a measure of predisposition for apoptotic enterocyte-assisted intestinal dysplasias in humans.

cancer | cell polarity | cytokines | tumor | virulence factors

The intestine is one of the fastest-renewing tissues of metazoa and its rapid cell turnover is a necessary response to enterocyte apoptosis or exfoliation caused by the passage, digestion, and absorption of food and various xenobiotics (1). Concurrently, the intestine must tolerate an extensive load of microbiota and is susceptible to various types of acute and chronic infection that can elicit intestinal inflammation. Not surprisingly, alterations in human microbiota, as well as bacterial infections, mainly due to *Helicobacter* species, have been inextricably linked to gastrointestinal disease and cancer (2–4). Yet while bacterial infection has been associated with blood cell infiltration and the induction of immune responses, its role in carcinogenesis has not been demonstrated conclusively (3–5). In addition, while the roles of the Notch and K-Ras signaling pathways in human colorectal cancer have been unequivocally demonstrated (6, 7), the contribution of these pathways in stem cells (SCs) and progenitors in intestinal tumor initiation remains elusive.

The evidence that hyperproliferating intestinal SCs and progenitors drive cancer initiation, maintenance, and metastasis (8–10), and that chronic inflammation has a role in cancer (4, 5, 11), indicates that under some conditions, hyperproliferation diverts from homeostasis to tumorigenesis. Among the various oncogenes, K-Ras is a commonly identified factor that drives gastrointestinal tumors (6). We hypothesized that cytologically undetectable activation of K-Ras and other oncogenes or reduction in expression of tumor suppressors might lead to perturbations in intestinal progenitors during infection. We therefore sought to model tumor initiation upon infection in the genetically-tractable model organism *Drosophila melanogaster*, a species that shares striking similarities with mammals in terms of overall physiology, cell biology, and signal transduction pathway components (12).

We analyzed the intestinal cell changes that accompany infection with *P. aeruginosa*, a prevalent human opportunistic pathogen (13), especially in immunocompromised and neutropenic cancer patients undergoing radiation, chemotherapy or bone marrow transplant (14, 15) in *D. melanogaster*. We found that virulent *P. aeruginosa* induces apoptosis, c-Jun N-terminal kinase (JNK) pathway activation and the proliferation of intestinal SCs and progenitors upon infection. Importantly, infection of flies bearing a latent oncogenic form of the Ras1 protooncogene, produces a profound increase in SCs and progenitors, epithelial multilayering and changes in the apicobasal polarity marker Armadillo.

Results and Discussion

***P. aeruginosa* Infection Increases the Number of *esg*-Positive SCs or Progenitors** In this study, flies were infected with *P. aeruginosa* strains via feeding and the highly virulent PA14 strain (16) killed almost all flies within 20 days, while the avirulent CF5 strain (16) failed to establish fatal infections (Fig. S1). Subsequent analysis of the expression of the SC and progenitor marker gene *escargot* (*esg*) using either the *esg-lacZ* or *esg-GAL4 UAS-GFP* reporter (17) revealed that intestinal infection of flies with the highly-virulent *P. aeruginosa* strain PA14 caused a striking increase in *esg*-positive (*esg*+) cells following infection, predominantly in the posterior part of the midgut (Fig. 1 *A* and *B* and Fig. S2 *A* and *C*). In contrast, the avirulent *P. aeruginosa* strain CF5 did not promote such a prominent effect (Fig. 1*C*). PA14 infection resulted in tissue hyperplasia, manifested by a significant increase in the width of the posterior midgut compared to that of uninfected flies (Fig. S3*A*). Interestingly, the phenotype of hyperplasia (Fig. S3*A*) and the concomitant increase in the number of *esg*+ cells (Fig. S4 *A* and *C*) was reversible upon bacteria clearance, suggesting that the presence of virulent bacteria is a prerequisite for this phenotype.

In control experiments, flies infected with heat-killed PA14 or subjected to PA14 clearance postinfection were not killed (no mortality over the course of 20 days) and did not have an altered SC compartment (Fig. S4). In addition, fly feeding on media containing purified pyocyanin, a secreted virulence factor of *P.*

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The authors declare no conflict of interest.

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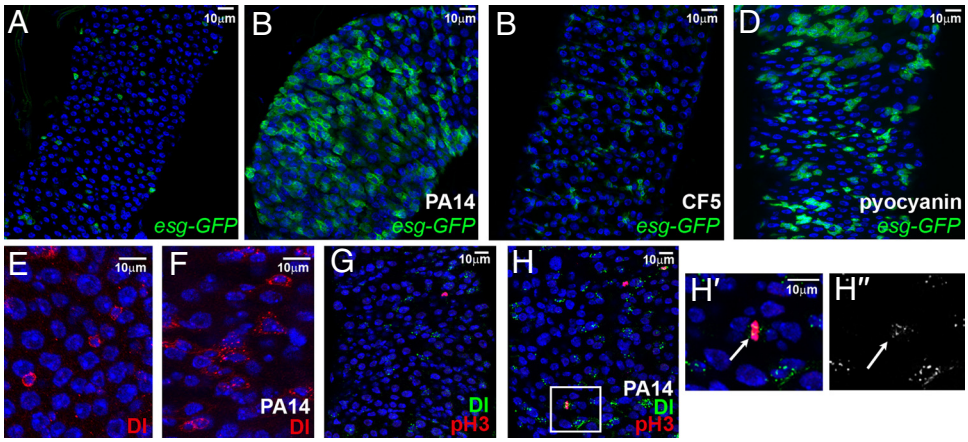

Fig. 1. *P. aeruginosa* infection induces intestinal progenitor expansion. (*A–D*) Posterior midgut cells of uninfected (*A*), strain PA14 infected (*B*), strain CF5 infected (*C*) and pyocyanin-fed (*D*) flies. SCs and progenitors of *esg-GAL4 UAS-srcGFP* flies fed for 5 days are shown in green. (*E* and *F*) Posterior midgut SCs marked with α-Delta antibody (red) of uninfected (*E*) and PA14-fed (*F*) flies. (*G*, *H*) Posterior midgut SCs marked with α-Delta (green) and phospho-histone-H3 (pH3; red) antibody, of uninfected (*G*) and PA14-fed (*H*) flies. (*H′* and *H″*) Magnification of rectangular region in (*H*), showing colocalization of pH3 (*H′*; arrow) and Delta (*H′* and *H″*; arrow) staining. Nuclei in all panels are marked with DAPI (blue).

aeruginosa that is not produced by the CF5 strain, as assessed by comparing the absorbance at $OD_{520\ nm}$ of the fully pyocyanin producer strain PA14 to that of CF5, increased the percentage of *esg*+ cells ($P = 0.03$, $n = 5$ intestines) (Fig. 1*D*). This finding indicates that pyocyanin secretion by virulent *P. aeruginosa* is one of the factors that stimulate proliferation of SCs and progenitors during infection.

Hyperplasia in *P. aeruginosa* Infected Fly-Gut Is Associated Predominantly with SC Divisions To distinguish whether the observed increase in *esg*+ cells was due to proliferation of SCs or progenitors, or to some other mechanism, such as dedifferentiation, we looked at two indicators to determine if SCs divide during infection: the SC restricted expression of Delta (Dl) (18) and the presence of phosphorylated histone-H3 (pH3), a marker for mitosis. We found a 2.5-fold increase in Delta-positive (Dl+) cells upon infection ($P = 0.007$, $n = 6$ intestines, >1,000 cells) (Fig. 1 *E* and *F*), indicating that the increase occurs predominantly in SCs. Likewise, we found a 3-fold increase in mitosis ($P = 0.0005$, $n = 6$ intestines, >1,000 cells) (Fig. 1 *G* and *H*), indicating that the effect of infection is not due to dedifferentiation of cells, but rather to the expansion of cells. Importantly, as in uninfected (18), the pH3 positive (pH3+) cells of infected flies were restricted to the SCs (Fig. 1 *H′* and *H″*), as marked by Delta, indicating that the infection caused an overproliferation of SCs. It is worth noting that transient-amplifying cells that arise from SCs, and that are necessary for the amplification of the mammalian intestine progenitor population, have not been previously described in *Drosophila*. Nevertheless, they might arise from *Drosophila* SCs upon infection and the increase in

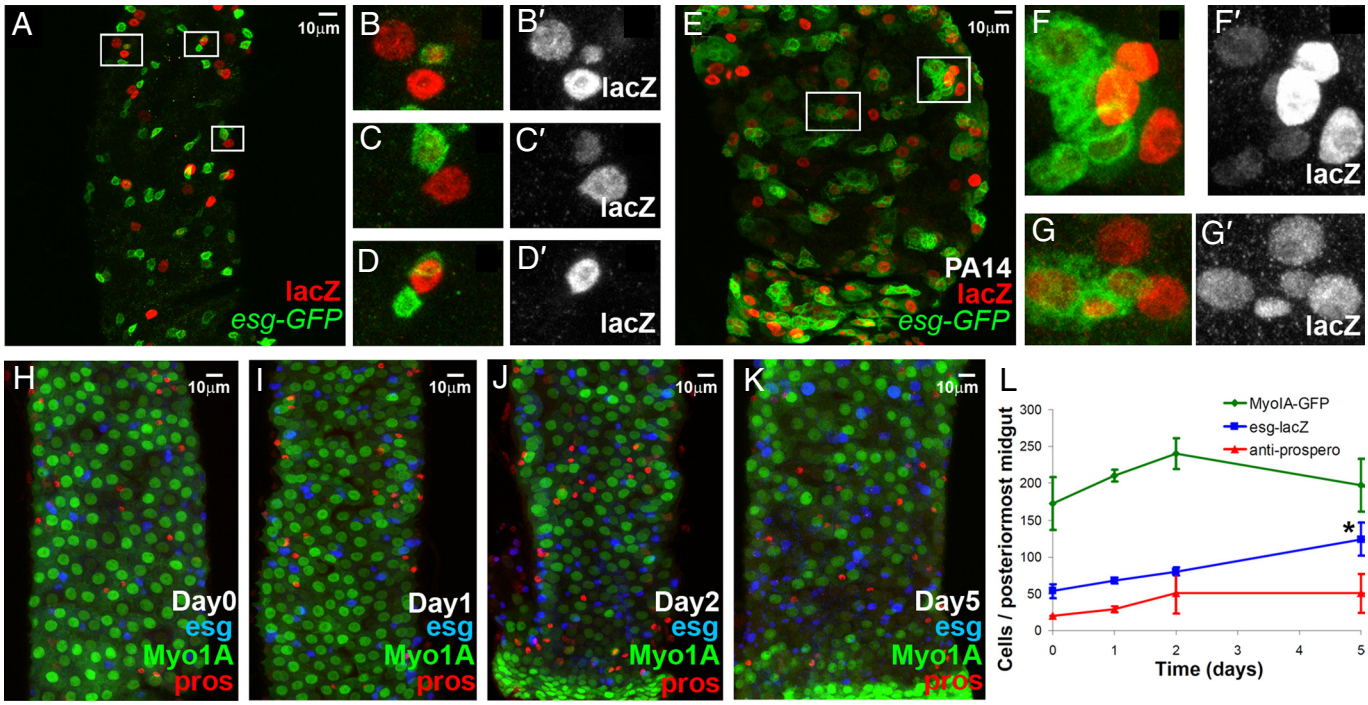

Fig. 2. Mature midgut cells are produced and cellular homeostasis is sustained during infection. (*A–K*) Lineage tracing of midgut clones induced by *esg* expressing cells in the absence (*A–D*) or presence (*E–G*) of PA14 infection. Green represents GFP driven by *esg-GAL4* and red represents nuclear *lacZ*, the lineage-tracing marker. *esg*+ cells produce mature enterocytes in both cases (*B–D*, *F*, and *G*), although in the case of PA14 infection, the clone size increases (*E–G*). (*H–L*) Time-course of PA14 infection. *Myo1A-Gal4 UAS-GFP* flies were fed on PA14-containing food for 0 (*H*), 1 (*I*), 2 (*J*), and 5 days (*K*) and the different cell types [Myo1A+, mature enterocytes (green), esg-lacZ+, SCs and progenitors (blue) and prospero+, enteroendocrine cells (red)] comprising their posterior-most midguts were counted. (*L*) Only *esg*+ cell numbers changed significantly during the course of infection (≈2.5-fold increase at day 5, *, $P = 0.05$; $n = 3$ intestines).

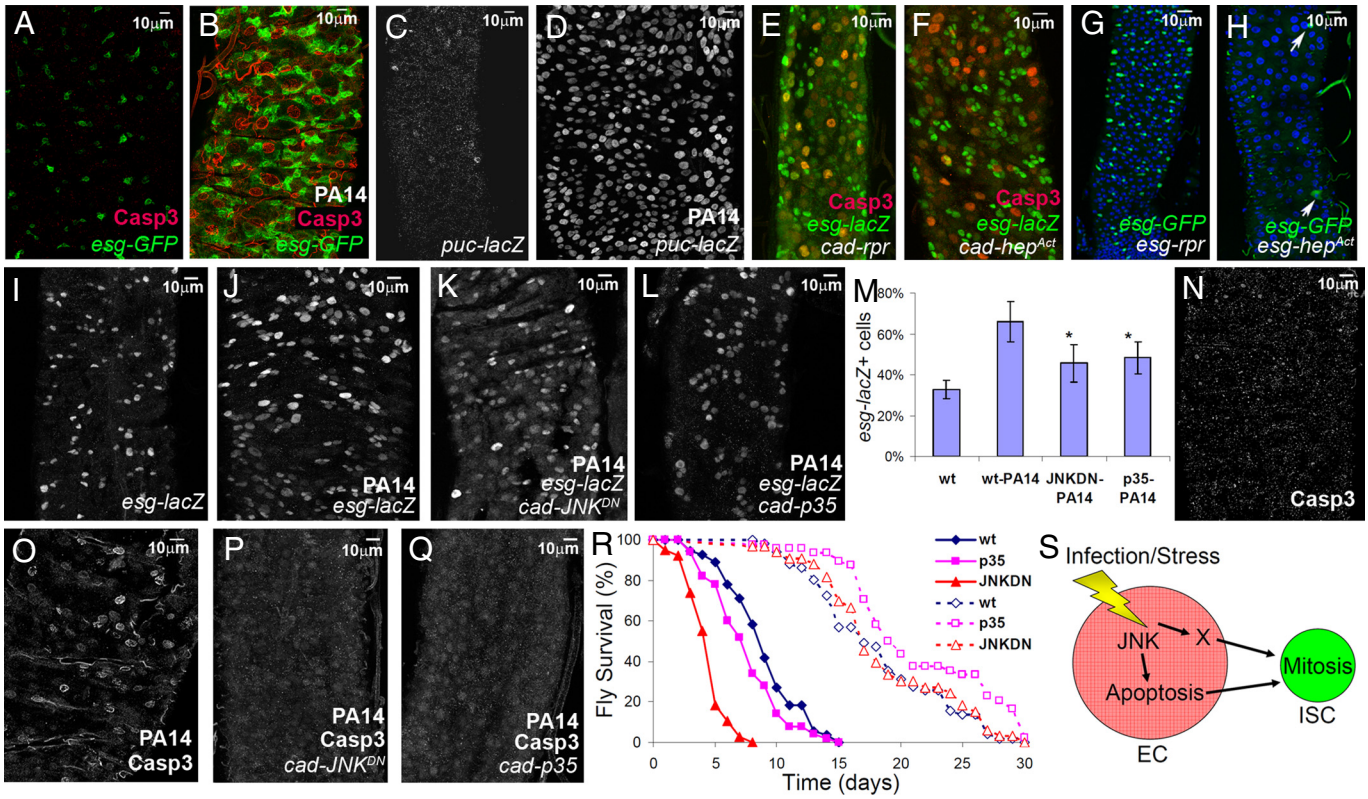

Fig. 3. *P. aeruginosa* infection triggers proliferation of SCs and progenitors via JNK-mediated apoptosis of enterocytes. (*A* and *B*) Uninfected (*A*) and PA14-fed (*B*) midgut enterocytes of *esg-GAL4 UAS-srcGFP* flies show substantial activation of caspase-3 (red) with concomitant accumulation of GFP (*esg*+ cells) in their neighboring SCs and progenitors. (*C*, *D*) JNK pathway activation in uninfected (*C*) and PA14-fed (*D*) midgut cells of *puc-lacZ* flies. (*E* and *F*) Overexpression of either the apoptotic gene *reaper* (*E*) or the activated form of the JNK activating kinase Hep^{Act} (*F*) in the all posterior midgut activates caspase-3 (red) in enterocytes neighboring *esg*+ cells. (*G* and *H*) Expression of *reaper* (*G*) or Hep^{Act} (*H*) in the progenitor population does not increase their numbers (arrows in *H*). (*I*–*R*) JNK pathway inhibition via overexpression of JNK^{DN} or inhibition of cell death by p35 overexpression in all posterior midgut cells exhibited a reduced number of *esg*+ cells ($P < 0.05$, $n = 5$ intestines, >1,000 cells; *I*–*M*), absence of strong apoptotic caspase-3 staining (*N*–*Q*) and increased mortality (solid lines in *R*, $P < 0.001$, $n = 40$) following infection compared to the isogenic control flies. Longevity of these flies (dashed lines in *R*) is not restricted. (*S*) Model illustrating the JNK-apoptosis cassette and a putative factor X acting in parallel, or sequentially, to induce SC mitosis upon enterocyte (EC) infection or stress.

mitosis of Dl+ cells upon infection may reflect the divisions of such transient-amplifying cells rather than SCs *per se*.

Infection Does Not Inhibit Intestinal Cell Differentiation Rate To assess if infection affects other aspects of gut homeostasis, such as the differentiation of SCs into mature cells, we used the flip-out lineage tracing technique (19) to mark *lacZ* clones of cells arising from *esg*+ cells. Fig. 2 shows that clones with differentiated cells were present in both noninfected and infected flies. At day 5 post-PA14 infection, 64 ± 18% of the clones contained mature cells, as evidenced by the lack of esg expression (*esg*−) and the big nucleus size. Similarly, in wild-type (wt) animals, 67 ± 11% of clones contained mature (*esg*−) cells, indicating that infection did not alter the frequency at which SCs gave rise to differentiated cells ($n = 96$ clones) (Fig. 2 *E*–*G*). Thus, the infection appeared to alter SC and progenitor numbers, without restricting subsequent differentiation steps. To assess if the production of differentiated cells during PA14 infection results in mature cell homeostasis, we performed a time-course analysis measuring the numbers of intestinal cells expressing *myosin1A*, *prospero* and *esg*, which correspond to the mature enterocytes, the enteroendocrine cells, and the undifferentiated cells, respectively. We observed no overlap in the expression of these markers under any condition tested ($n = >10$ intestines), indicating that, these markers can be used to label distinct cell populations in infected flies. We did not find a significant increase in the numbers of mature cells, although the numbers of *esg*+ cells gradually increased approximately 2.5-fold in the course of infection (Fig. 2 *H*–*L*). Thus, it can be deduced that infection increases progenitor population leading to the production of mature cells, establishing cellular homeostasis.

Infection Induces Apoptosis of Mature Cells The observation that there was an increase in progenitor population without a continuous increase in enterocytes and enteroendocrine cells, also suggests that the mature cells may have been dying at a faster rate due to the infection. SCs may multiply to compensate for the loss of dying cells. This possibility is consistent with the observation in mammals that proliferating SCs preserve the integrity of the gut (1). To assess this, we used antibodies against the activated cleaved form of caspase-3, which marks apoptotic cells. We also used acridine orange, another indicator of apoptosis, and observed increased staining in patches of cells in infected flies ($n = 5$ intestines). Importantly, *esg*+ cells did not show significant overlap with apoptotic cells (Fig. 3 *B*, *E*, and *F*), indicating that mature cells are more prone to apoptosis.

Infection Induces JNK Pathway Activation Since apoptosis and inflammation are often induced via JNK pathway activation in flies and mammals (20), we monitored the intestines of uninfected and PA14-fed flies for the expression of a reporter of JNK pathway activation, *puc-lacZ*. Only PA14-infected flies expressed this reporter in the majority of the posterior midgut cells (Fig. 3 *C* and *D*). Moreover, *puc-lacZ* positive cells showed no

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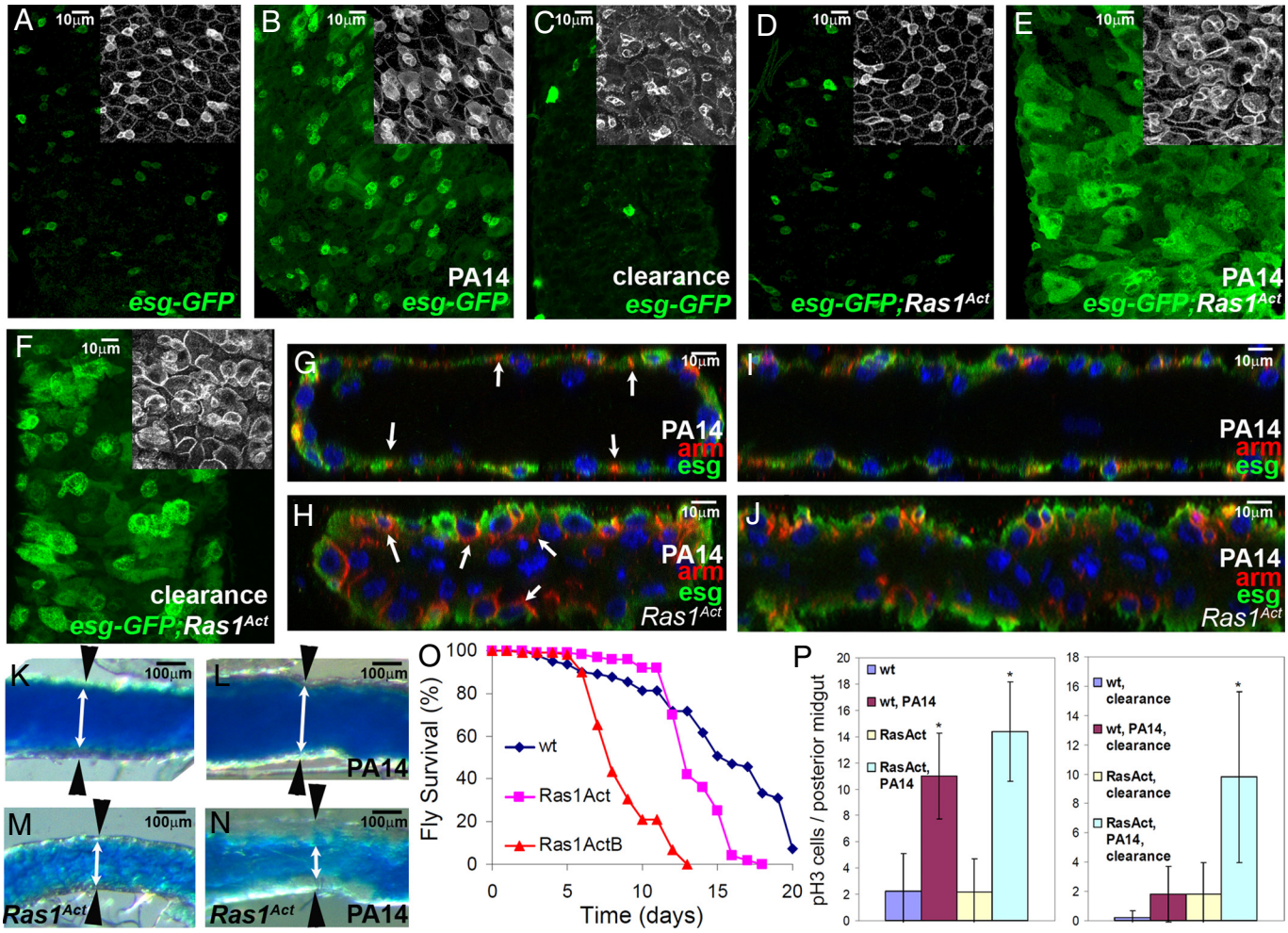

Fig. 4. *Ras1^{Act}* oncogene synergizes with infection to induce SC-mediated intestinal dysplasia. (*A–F*) Control *tub-GAL80^{ts} esg-GAL4 UAS-GFP* flies (*A–C*) alone or in combination with *UAS-Ras1^{Act}* (*D–F*) that are either uninfected (*A* and *D*), PA14-fed (*B* and *E*), or cleared from infection (*C* and *F*). Midgut *esg*+ cells are shown in green (*A–F*) and Arm staining in white in insets. (*G–J*) Cross-sections (*G* and *H*) and sagittal sections (*I* and *J*) of PA14-infected wild-type (wt) (*G* and *I*) and *Ras1^{Act}* (*H* and *J*) midguts indicating the difference in epithelial structure. DAPI marks the nuclei and red Arm staining. Arm is localized in lateral cell junctions, but in *Ras1^{Act}* there is mislocalization of Arm in the apical domain (arrows in *G* and *H*). (*K–N*) Posterior midguts of wt (*K* and *L*) and *Ras1^{Act}* (*M* and *N*) flies fed on sucrose (*K* and *M*) or PA14 (*L* and *N*) for 5 days, followed by ingestion of blue dye cornmeal to highlight the gut lumen. White double arrows and black arrowheads delineate the borders of the intestinal epithelium. (*O*) Control flies survive longer compared to flies expressing two different insertions of *Ras1^{Act}* ($P < 0.001$; $n = 40$). (*P*) pH3+ cells per posterior midgut are significantly and similarly increased during infection in both control and *Ras1^{Act}* flies, but differ following clearance (*, $P < 0.05$; $n = 7$).

overlap with *esg*+ cells ($n = 7$ intestines). These findings indicate that apoptosis and induction of the JNK pathway are specifically activated in mature cells, while undifferentiated *esg*+ cells are resistant to apoptosis and JNK pathway activation.

Enterocyte Apoptosis Induces SC or Progenitor Proliferation in the Absence of Infection. Given that infection causes death of enterocytes, we examined whether this was sufficient to stimulate increased proliferation of SCs and progenitors. Overexpression of the apoptotic gene *reaper* (*rpr*) (Fig. 3*E*) or the activated form of the JNK kinase, *Hep^{Act}* (Fig. 3*F*), in the posterior midgut cells induced enterocyte apoptosis and the concomitant appearance of supernumerary *esg*+ cells. Control experiments driving *reaper* or *Hep^{Act}* expression specifically in *esg*+ cells revealed that the % of these cells did not increase (Fig. 3 *G* and *H*), demonstrating that apoptosis and JNK activation in mature cells, rather than in SCs and progenitors can lead to SC or progenitor multiplication.

Inhibition of Apoptosis During Infection Inhibits Proliferation. To address whether the death of enterocytes during infection is the driving signal for *esg*+ cell expansion, we expressed the baculovirus protein p35, a universal apoptosis inhibitor, in all cells of the posterior midgut. As a result, the increase in the SC and progenitor population due to infection was inhibited (Fig. 3 *G*, *L*, and *M*). In addition, when a dominant-negative form of JNK (JNK^{DN}) was expressed in all cells of the posterior midgut, the percentage of *esg*+ cells and the strong activated caspase-3 staining were both reduced upon infection (Fig. 3 *G*, *K*, and *M–Q*) compared with wt controls (Fig. *3 B* and *D*). Importantly, these isogenic p35 or JNK^{DN} expressing flies died faster after being infected (Fig. 3*R*) ($P < 0.001$), indicating that increased intestinal regeneration during infection is important for host homeostasis. *hep^{1}* flies, which are also defective in JNK activation, showed 50 and 100% mortality post infection at approximately 5 and 9 days, respectively, which is similar to that of JNK^{DN} flies and significantly faster than wt Oregon R flies (Fig. 3*R*). These results collectively show that induction of apoptosis in the mature enterocytes is both necessary and sufficient to induce supernumerary SCs or progenitors, and that at least one consequence of the JNK pathway in the midgut is to induce

mature cell apoptosis, which ultimately contributes to preserve intestinal homeostasis (Fig. 3*S*).

Synergism between Infection and Oncogenic Predisposition in Intestinal Pathology. To assess the synergism between infection and oncogenic predisposition, we expressed a latent oncogenic form of the *Drosophila* ortholog of mammalian K-Ras ($Ras1^{Act}$) (21) in the progenitor compartment. Expression of $Ras1^{Act}$ under semipermissive conditions (see *Materials and Methods*) is cytologically innocuous because the gut diameter and the number and shape of *esg*+ cells appear normal (Fig. 4*D* and Fig. S3*B*). However upon infection, a number of intestinal dysplastic phenotypes became apparent. First, essentially all cells in the posterior midgut were *esg*+, with heightened Armadillo (Arm) staining (Fig. 4*E*, *Inset*), a property of SCs and progenitors of wt uninfected flies (Fig. 4*A*, *Inset*) (22). Second, supernumerary *esg*+ cells that were present predominantly in the posterior midgut upon infection of wt infected flies, were present in both the anterior and posterior midgut of genetically predisposed flies (Fig. S2 *B* and *D*). Third, there was a higher number of Dl+ cells in the intestine (57 ± 4% vs. 30 ± 3% for the wt control; $P = 0.0002$, $n = 4$), indicative of more SCs. Fourth, Arm staining, which was restricted to the lateral region of the wt midgut cells (Fig. 4 *G* and *I*), was found both laterally and apically in Ras^{Act} expressing flies (Fig. 4 *H* and *J*), indicating perturbation of apicobasal cell polarity. Fifth, the epithelial monolayer of Ras^{Act} flies developed additional layers with more apical cells that lack detectable Arm staining, many of which had nuclei of irregular shape (Fig. 4 *G–J* and Fig. S5). Sixth, infected Ras^{Act}-expressing flies had a reduced midgut luminal space, increased defecation rate upon infection and succumbed faster to the infection (Fig. 4 *K–O* and Fig. S6).

Interestingly, the synergy between the genetic background and infection in causing perturbations of the progenitor cell population is not limited to the $Ras1^{Act}$ oncogene. We assessed the effect of expression in the *esg*+ cells of a dominant negative form of Notch ($Notch^{DN}$), a pathway relevant to intestinal SC control and cancer in mammals (23, 24). It has previously been shown that reduction of Notch expression autonomously increases SC numbers and inhibits differentiation, thus resulting in supernumerary SCs in *Drosophila* (17, 18). Under semipermissive $Notch^{DN}$ expression conditions, no such effect on *esg*+ cells was apparent, but following infection, clusters of *esg*+ cells covering most of the surface of the posterior midgut, rendered these flies hypersusceptible to infection (Fig. S7). Similarly, weak RNAi knockdown of the tumor suppressor gene *discs large* (dlg^{RNAi}) (25) was found to have no effect in the absence of infection, but in the presence of infection it resulted in extensive expansion of *esg*+ cells, covering most of the surface of the gut (Fig. S8).

Importantly, $Ras1^{Act}$ and dlg^{RNAi} flies, but not the wt controls, retained a significant overabundance of *esg*+ cells 5 days following bacteria clearance (Fig. 4 *C* and *F* and Fig. S8), while dlg^{RNAi} flies also retained a significant increase in gut diameter (Fig. S3*C*). In addition, 5 days following bacterial clearance, $Ras1^{Act}$ (Fig. 4*P*) and dlg^{RNAi} flies, but not the wt controls, had increased numbers of proliferating (pH3+) cells (>5-fold increase vs. controls). Although assessment of the phenotype at time points further beyond clearance is not feasible, due to the effect of aging in the SC compartment (26), these data demonstrated that genetically predisposed SCs and progenitors exhibit prolonged pathology.

Since our earlier results demonstrated that JNK activation is necessary and sufficient for full induction of SC or progenitor growth upon infection, we also tested whether JNK signaling can synergize with $Ras1^{Act}$ and dlg^{RNAi} in the absence of infection to cause an increase in the progenitor population. To weakly increase JNK activation to a level below the threshold that would trigger an alteration in the progenitor population, we used a genetic background containing only one copy of the JNK pathway inhibitor, *puckered* (*puc*). Expression of $Ras1^{Act}$ and dlg^{RNAi} (under semipermissive conditions) did not produce significant increase in the *esg*+ cell population (Fig. 5 *A*, *C*, and *E*). However, in the puc^{E69}/+ genetic background, $Ras1^{Act}$ and dlg^{RNAi} expression resulted in supernumerary *esg*+ cells (Fig. 5 *D* and *E*), indicating that alterations in these genes can synergize to increase the progenitor cell population.

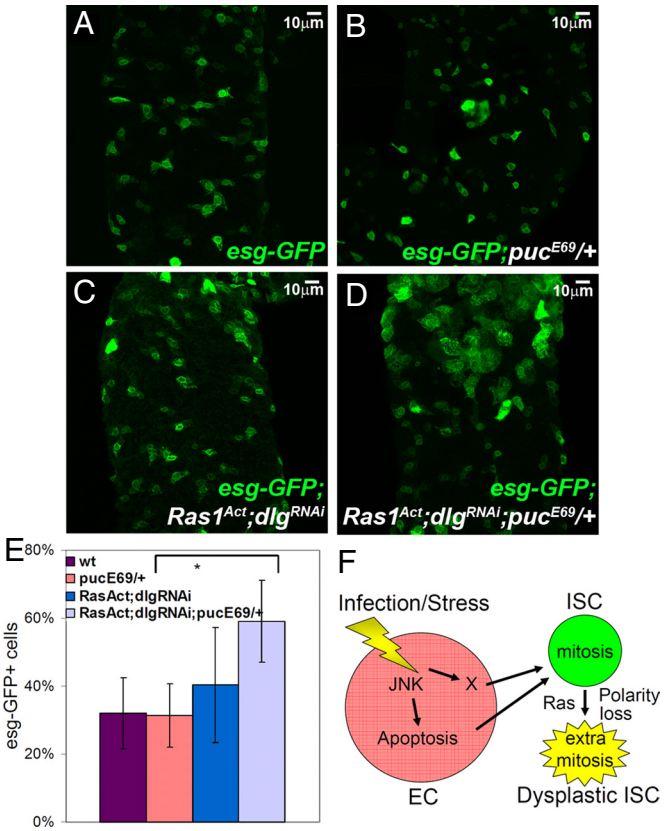

Fig. 5. Synergism between JNK activation and $Ras1^{Act}$ expression and *dlg* downregulation. Posterior midgut SCs and progenitors marked with GFP (green) from flies of the genotype *tub-GAL80^{ts} esg-GAL4 UAS-GFP* without (*A*) or with JNK activation due to the puc^{E69} mutation (*B*) or with $Ras1^{Act}$ and dlg^{RNAi} expression (*C*) or with $Ras1^{Act}$ and dlg^{RNAi} expression in the puc^{E69} background (*D*). (*E*) $Ras1^{Act}$ and dlg^{RNAi} only increased the percent of *esg*+ cells in puc^{E69} flies ($P < 0.05$, $n = 5$ intestines). (*F*) Model of the diversion of homeostasis (as described in Fig. S3) toward dysplasia due to Ras and polarity gene alterations causing intestinal SC predisposition.

Conclusions

The findings presented here support a model (Fig. 3*S*), according to which JNK driven apoptosis is partly responsible for the compensatory proliferation of SCs and progenitors. It is likely that a stress or growth signaling pathway acts in parallel or sequentially to JNK to induce SC and progenitor proliferation. Indeed, recent findings suggest that the JAK/STAT pathway may be a JNK and apoptosis-independent mediator of this response (27, 28). Nonetheless, the signals that trigger these pathways in enterocytes, and those that signal intestinal SC to proliferate in the presence of microbes remain to be discovered.

These signals appear important to preserve intestinal homeostasis by replenishing dying cells and can be subverted toward a dysplasia-like phenotype in genetically predisposed animals (Fig. 5*F*). It is possible that in addition to the Upd cytokines (27, 28) other growth factors secreted from injured enterocytes increase intestinal SC fate proliferation rate. During

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chronic exposure to microbes, such factors might promote irreversible alterations in cell polarity and tissue architecture. Cell aging, infection by *Erwinia carotovora*, *Serratia marcescens*, and *Pseudomonas entomophila*, and exposure to xenobiotics, have recently been shown to induce supernumerary intestinal progenitor cells (26, 27, 29–31). Therefore, it will be important to systematically analyze whether specific environmental factors and intestinal microbiota can also contribute to dysplasia-like phenotypes, as observed in our synergy experiments.

In conclusion, while JNK signaling and SC or progenitor proliferation appear to be beneficial for the host upon infection, an imbalance in the regenerative process can be detrimental for genetically predisposed hosts. This potential risk is especially relevant in cancer patients undergoing chemotherapy, as such individuals may have a genetic predisposition for cancer interacting with an immunocompromised state, making them particularly susceptible to both intestinal infections and cancer recurrence. Hence, it may be beneficial to identify and target bacterial factors that promote SC proliferation. Once such factors are known, cancer screening methods based on the responsiveness of SCs to infection may be developed. In this respect, the ability of bacterial factors to overstimulate the regenerative process, could be interpreted as an index of an individual's risk of developing cancer.

Materials and Methods

Fly Strains *cad-Gal4^{md509}*, *UAS-srcGFP^{M7E}*, *esg-lacZ$^{B7-2-22}$*, *UAS-p35^{BH2}*, *UAS-bsk^{DN}*, and *UAS-Hep^{Act}* were obtained from the Bloomington Stock Center; *Myo1A-GAL4 UAS-GFP* (27); *esg-GAL4* (NP5130, Drosophila Genomics Resource Center); *puc-lacZ^{E69}* (M. Arias, 1993); *tub-GAL80^{ts} esg-GAL4 UAS-GFP*, *UAS-Notch^{DN}* (17); *UAS-Ras1^{Act}* (21); *hep^{1}* (M. Miura) and *UAS-dlg ^{RNAi}* (25).

Transgene Overexpression *esg-GAL4 UAS-srcGFP* flies were reared on normal fly food at 25 °C. For temporal overexpression of transgenes, the *tub-GAL80^{ts} esg-GAL4 UAS-GFP* (progenitor cell-specific) and *tub-GAL80^{ts};cad-GAL4 UAS-GFP esg-lacZ* (posterior midgut-specific) flies were reared on normal fly food at the restrictive temperature (18 °C), where the Gal80 repressor is active and Gal4 expression is suppressed, then transferred onto infection media and incubated at the semipermissive (25 °C) or fully-permissive (29 °C) temperature, respectively. For flip-out clones *tub-GAL80^{ts} esg-GAL4 UAS-GFP* females were crossed with *UAS-FLP;act-FRTstopFRT-lacZ* (19) males at 18 °C and progeny were transferred to infection media at 25 °C for 5 days.

Antibodies Primary antibodies included mouse anti-Arm (1:50; Developmental Studies Hybridoma Bank), mouse anti-β-gal (1:400; Promega), rabbit anti-β-gal (1:10,000; Cappel), rabbit anti-caspase-3 (1:200; Cell Signaling Technologies), rabbit anti-phospho-histone-H3 (1:2,000; Millipore), mouse anti-Delta (Dl) (1:100; DSHB), and guinea pig anti-Dl (1:5,000; a gift from M. Muskavitch). Secondary antibodies conjugated to Alexa Fluor-488, 555, and 647 were diluted to 1:1,000 (Molecular Probes). Standard immunohistochemical methods were used (17, 22). Images were collected on a Leica TCS SP2-AOBS confocal microscope. Additional information can be found in *SI Text*.

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