MK2-Dependent p38b Signalling Protects Drosophila Hindgut Enterocytes against JNK-Induced Apoptosis under Chronic Stress

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Abstract

The integrity of the intestinal epithelium is crucial for the barrier function of the gut. Replenishment of the gut epithelium by intestinal stem cells contributes to gut homeostasis, but how the differentiated enterocytes are protected against stressors is less well understood. Here we use the Drosophila larval hindgut as a model system in which damaged enterocytes are not replaced by stem cell descendants. By performing a thorough genetic analysis, we demonstrate that a signalling complex consisting of p38b and MK2 forms a branch of SAPK signalling that is required in the larval hindgut to prevent stress-dependent damage to the enterocytes. Impaired p38b/MK2 signalling leads to apoptosis of the enterocytes and a subsequent loss of hindgut epithelial integrity, as manifested by the deterioration of the overlying muscle layer. Damaged hindguts show increased JNK activity, and removing upstream activators of JNK suppresses the loss of hindgut homeostasis. Thus, the p38/MK2 complex ensures homeostasis of the hindgut epithelium by counteracting JNK-mediated apoptosis of the enterocytes upon chronic stress.

Introduction

In its function as a protective barrier, the intestinal epithelium is constantly exposed to stressors from the outside [1]. It acts as a mediator between the bacterial flora and the host’s immune system, and the intestinal epithelial cells have to respond to extrinsic and intrinsic factors to ensure their own survival and proper gut homeostasis. Two populations of cells have to orchestrate different aspects of intestinal epithelium survival. While the intestinal stem cells (ISCs) are essential for the proliferative aspects of intestinal homeostasis [2–4], the enterocytes (ECs) form the first line of defence against pathogens and stressors. Signalling cascades that are modulated by external signals and by cellular stress are crucial regulators of intestinal epithelial survival. For example, mice with gut-specific knockout of NEMO spontaneously develop intestinal lesions reminiscent of those in inflammatory bowel diseases (IBDs), indicating an essential role for NFκB signalling in EC survival [5,6]. Recently, ER stress in the ECs has also been found to influence epithelial homeostasis, and mutations in XBPI are sufficient to trigger an IBD-like phenotype [7]. The p38 stress-activated protein kinase pathway has also been implicated in intestinal disorders [8] but its role in intestinal diseases is still controversially discussed [9,10].

The p38 SAPK belongs to the MAPK family and is conserved from yeast to humans. In higher eukaryotes, p38 associates with its major target, the MAPK-activated protein kinase MK2. This complex resides in the nucleus in the resting state. Upon stress, p38 is activated by MKK3/MKK6 and phosphorylates MK2, which results in an exposure of the nuclear export signal of MK2 and a subsequent nuclear export of the complex [11]. Another consequence of the p38/MK2 complex formation is the stabilisation of p38 protein. Interestingly, the kinase activity of MK2 is required neither for the nucleo-cytoplasmic shuttling nor for the p38 protein stabilisation [11–13]. Conversely, MK2 kinase activity is crucial to phosphorylate small heat-shock proteins, transcription factors [e.g., SRF and HSF-1], and TTP [14–16]. The inhibitory phosphorylation of TTP by the p38/MK2 complex has been shown to increase the translation of AU-rich elements (ARE)-containing mRNAs including the mRNA encoding the proinflammatory cytokine TNFα [17]. Furthermore, p38 and MK2 have been shown to act as cytoplasmic checkpoint kinases in parallel to CHK1 [18,19]. Due to the plethora of p38/MK2 functions, targeting the p38 SAPK branch with inhibitors might lead to harmful side effects. Thus, it is important to understand the roles of p38 signalling in a tissue-specific context. The availability of mouse models has helped to decipher some in vivo roles of p38 SAPK signalling [20,21] but the complex nature of the intestinal system has hampered a detailed analysis.

Studies in the model organism Drosophila have provided new insights into intestinal maintenance and how different signalling pathways are employed to ensure proper gut homeostasis. The Drosophila gut consists of the fore-, the mid-, and the hindgut. The larval and adult midgut display a regional specification along the antero-posterior axis and fulfils vital functions such as...
Author Summary

The gut epithelium forms the first barrier against pathogens and stressors in the gut lumen, and a loss of this defence function can result in intestinal diseases. Damage in the gut epithelium triggers the proliferation of intestinal stem cells to replenish the epithelium. However, little is known about how the enterocytes are protecting themselves against stressors. We addressed the function of stress-activated protein kinase (SAPK) signal transduction pathways in the larval gut of *Drosophila*. Our study revealed that a particular module of the p38 SAPK signal cascade is required to protect the larval hindgut epithelium against chronic salt stress. We identified the two kinases, p38b and MK2, as key components of this protective signal. In the absence of p38b or MK2, the stress-inducible JNK cascade is locally upregulated and eventually induces apoptosis. Although the function of the p38b/MK2 module is only required in the enterocytes, the elimination of the affected cells results in atrophy of the overlying muscle layer and subsequent systemic defects in the larvae (e.g., induction of antimicrobial peptides). We hope that our findings will contribute to a better understanding of early (i.e., pre-inflammatory) events in the development of human intestinal diseases.

nutrient absorption [22]. The main part of the embryonic and larval hindgut, the large intestine, is subdivided into a ventral (hv, positive for Delta expression) and a dorsal domain (hd, positive for engrailed expression) [23] that are separated by a single row of boundary cells [24]. The adult hindgut shows a similar yet more complex organisation [25]. The function of the hindgut, however, remains largely unknown. Ultrastructurally, the hd domain is marked by deep infoldings and enriched in elongated mitochondria, resembling the rectal papillae of other insects. Based on these similarities, it has been speculated that the *Drosophila* large intestine plays a role in ion and/or water resorption [23,24].

At least four distinct intestinal epithelial cell types are found in *Drosophila*: intestinal stem cells (ISCs), the hormone-producing enteroendocrine cells (EEs), enterocytes (ECs) and the transient enteroblasts (EBs; progenitors of EEs and ECs). In the adult midgut, ISCs are required for normal gut homeostasis, but in aged and/or stressed individuals the number of midgut ISCs is increased and differentiation is disturbed [26,27]. The orchestrated activation of Hippo, JNK, JAK/STAT, Notch and EGFR signalling within and between the ISCs, the ECs and the visceral muscles is required to coordinate proliferation, differentiation and cellular turnover in the midgut epithelium [26–36]. In contrast to the situation in the midgut, the hindgut stem cells are not required for hindgut homeostasis in the larva and in the adult fly. The hindgut ISCs are rather needed during the shift from larval to adult hindgut, and stress induces proliferation and cell migration in the pylorus region [37]. Thus, especially the adult midgut serves as a good experimental model for the analysis of EC replenishment upon damage, but the mechanisms governing proper stress response in the ECs have remained elusive.

p38 SAPK signalling also plays a role in gut homeostasis in *Drosophila*. In aged adult midguts, an increase in p38b expression has been observed in Delta-positive stem cells, which appears to be partly due to DREF-mediated transcriptional activation [38,39]. Knockdown of p38b in the ISCs prevents age- and stress-induced ISC overproliferation and accumulation of aberrant Delta-positive cells, implying a role for p38b in regulating intestinal regeneration [38]. p38 signalling has been shown to be required for DUOX expression in differentiated ECs and for normal differentiation in the ISCs [38,40]. Consistently, a recent study showed that the larval intestinal epithelium is more susceptible to damage by pathogens in the absence of p38 function [41]. However, the mechanism of p38 action within the ECs remains unclear. In *Drosophila*, p38 signalling can antagonize the closely related JNK SAPK branch [42]. JNK signalling has also been shown to regulate several aspects of intestinal function. It is required in the midgut ECs to induce autophagy and thereby ensure their survival during oxidative stress [43]. In the ISCs, JNK is needed for proper stress response but strong activation of JNK leads to differentiation defects and loss of gut homeostasis [26]. Whether p38 and JNK influence each other in their intestinal function has not been addressed so far.

In this study, we investigate how the *Drosophila* larval hindgut is enabled to maintain homeostasis under stress conditions. Using deletion mutants for *MK2*, *p38a* and *p38b*, we show that *MK2* and *p38b* form a complex that is specifically required to protect the dorsal hindgut ECs against chronic stress. In the absence of this p38b/MK2 complex, JNK is activated in patches of hindgut ECs, resulting in JNK-dependent apoptosis, loss of epithelial organisation, and melanisation of hindgut regions. This melanisation of the ECs does not require the recruitment of hemocytes, indicating an epithelial response that might also precede immune activation in mammalian intestinal diseases. Thus, we identify a specific SAPK signalling module required to maintain hindgut epithelial integrity upon stress.

Results

*Drosophila MK2 Is Dispensable for Normal Development and Survival at Non-Stress Conditions*

The mammalian MAPKAP-K2 is known to be a downstream kinase of the p38 branch of SAPKs [44]. To generate deletion mutants for *Drosophila MK2*, we mobilised a P-element insertion located in the *MK2* locus (Figure 1A). Whereas *A43* is a null allele as judged by the absence of MK2 protein and by the failure of *A43* larval lysates to phosphorylate mammalian small heat shock protein 25 in a kinase assay (Figure S1A), the alleles *A41* and *A12* are likely to represent hypomorphic alleles. *A38* is probably also a null allele although the generation of a truncated protein (initiated from alternative Methionine codons) cannot be excluded (Figure 1A). A precise excision allele of the same P-element (*A14*) was used as control throughout this study. All of the generated alleles show no obvious phenotypic alteration and can be kept as homozygous lines at normal conditions. Since several mutants of the SAPK pathway are sensitive to stresses including high osmolality [45,46], we tested the *MK2* mutants in various stress assays including oxidative stress (paraquat feeding), UV exposure during late embryonic/early larval development, heavy metals (0.5 mM copper sulphate), high osmolality (0.2 M NaCl) and SDS (0.2%) (Figure S1B and data not shown). Interestingly, only salt and SDS feeding resulted in a melanotic phenotype in 35% to 45% of the mutant larvae, characterized by the appearance of a “black dot” (BD) in the posterior part of the body (Figure 1B and Figure S1C–S1E). Closer examination revealed that the BD localised to the posterior hindgut, and the affected hindgut epithelium appeared pathologically altered (Figure 1C). All *MK2* alleles were analysed for the appearance of BDs and survival at three conditions: normal food, weak salt stress (0.1 M NaCl) and strong salt stress (0.2 M NaCl) (Figure 1D). The behaviour of *MK2* hypomorphic larvae indicated that the levels of
MK2 get more important with increasing osmolarity. Introducing a genomic rescue construct completely rescued both the BD phenotype and the lethality of \( D_{43} \) mutants at 0.2 M NaCl (Figure 1D). Thus, MK2 is not essential at normal conditions but is required when larvae are reared on a high sodium chloride diet.

**Drosophila MK2 Is Required to Protect the Hindgut from Stress-Induced Apoptosis**

We speculated that MK2 is specifically required to protect the hindgut epithelium. In sections of the hindgut, dorsal ECs with BDs were apically ruptured (Figure 2Aii). In wild-type hindguts,
ECs of the dorsal domain were not damaged under salt stress conditions (Figure 2Ai and Figure S2C). Similarly, most MK2 mutant hindguts without BDs did not display morphological alterations (Figure S2C). However, a blistering of the apical surface without damaging the apical membrane was occasionally observed (Figure 2Aiii and Figure S2C). Blistering of the apical surface was also observed in MK2 mutant larvae reared on high salt food (0.2 M NaCl with large BDs) in regions away from the BD (Figure S2C). The melanisations occurred at the apical surface within the ECs, probably preceded by the blistering (Figure 2Aiii and Figure S2C). Next, we tested the hindgut tissue for the presence of dying cells. TUNEL staining revealed localised clusters of apoptotic ECs in MK2 mutant hindguts. (Bi) Magnification of apoptotic foci shows the condensed nature of the TUNEL positive nuclei (white arrows). (Biii) No apoptotic cells are present in control hindguts. (C) MK2 mutant larvae reared on high salt food (0.2 M NaCl) show ruptures of the visceral muscles (right panel, white arrow) in comparison to the regular organisation of wild-type larvae (left panel). The location of the BD is marked by an asterisk. (D) The cell-cell junction marker Nrg-GFP indicates morphological defects in ECs in the hd domain. (Di) Wild-type and MK2 mutant larvae reared on 0.1 M NaCl food show normal localisation of Nrg-GFP in the ECs. The hd domain points to the right; the rectum points to the bottom. (Dii) In MK2 mutants with BDs, Nrg-GFP is more diffusely localised at the sites of BDs (white arrow). The BD is discernible in the bright field picture and by its autofluorescence in the far-red channel (red). (Diii) Higher magnification of Nrg-GFP (green) signal in MK2 mutants (reared on 0.2 M NaCl food) with (right) or without BD (left). (E) BDs are always found in the en-gal4-positive hd domain (green) of the hindgut. (F) Rescue of the MK2 mutant phenotype by en-gal4 mediated expression of the indicated cDNA constructs.

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Figure 2. Increased apoptosis and tissue damage in hindguts of MK2 mutants. (A) MK2 mutants show ruptures of ECs when reared on high salt diet. Bright field pictures of sections of hindgut tissue of larvae reared on 0.1 M NaCl food are shown. (Ai) wild-type (D1A), (Aii) MK2 mutant (D43) with BD (white arrow). (Aiii) Higher magnifications of ECs in the dorsal hd domain of MK2 mutants. Blistering of the apical part (upper panel, black asterisk) and melanisation of the apical region (lower panel, white arrow) are frequently observed. The apical surface of the ECs appears to be intact (black arrow). (B) Apoptosis is increased in the hindguts of MK2 mutant larvae reared on high salt conditions. (Bi) TUNEL staining revealed localised apoptosis in MK2 mutant hindguts. (Bii) Magnification of apoptotic foci shows the condensed nature of the TUNEL positive nuclei (white arrows). (Biii) No apoptotic cells are present in control hindguts. (C) MK2 mutant larvae reared on high salt food (0.2 M NaCl) with large BDs show ruptures of the visceral muscles (right panel, white arrow) in comparison to the regular organisation of wild-type larvae (left panel). The location of the BD is marked by an asterisk. (D) The cell-cell junction marker Nrg-GFP indicates morphological defects in ECs in the hd domain. (Di) Wild-type and MK2 mutant larvae reared on 0.1 M NaCl food show normal localisation of Nrg-GFP in the ECs. The hd domain points to the right; the rectum points to the bottom. (Dii) In MK2 mutants with BDs, Nrg-GFP is more diffusely localised at the sites of BDs (white arrow). The BD is discernible in the bright field picture and by its autofluorescence in the far-red channel (red). (Diii) Higher magnification of Nrg-GFP (green) signal in MK2 mutants (reared on 0.2 M NaCl food) with (right) or without BD (left). (E) BDs are always found in the en-gal4-positive hd domain (green) of the hindgut. (F) Rescue of the MK2 mutant phenotype by en-gal4 mediated expression of the indicated cDNA constructs.

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We performed a series of rescue experiments to determine where MK2 function is required. The BD phenotype was rescued by ubiquitous and hindgut-specific but not by midgut-specific expression of MK2 (Figure S2D). Moreover, only wild-type MK2 but not a kinase-dead version of MK2 rescued the BD phenotype. The largest domains of the larval hindgut are the dorsal en-gal4-positive (hd) and the ventral Delta-positive (hv) domain. BDs were only observed in the hd domain (Figure 2E). Consistently, en-gal4 driven expression of a wild-type Drosophila MK2 or a wild-type murine MK2, but not of a kinase-dead Drosophila MK2, rescued the BD phenotype (Figure 2F).

Melanisation in insects can be regarded as a wound healing or defence response and has been shown to be either hemocyte-dependent or hemocyte-independent [47]. We thus attempted to clarify whether BD formation in MK2 mutant larvae was inefficient.
dependent on the recruitment of hemocytes. Staining for the blood cell marker Hemese - either direct (using antibodies) or indirect (using Hi-GAL4 UAS-GFP) - revealed the absence of blood cells at or within the hindgut epithelium in both wild-type and MK2 mutant hindguts (Figure S2A and data not shown). Consistently, the formation of BDs was still observed in hemocyte-ablated MK2 mutants (Figure S2B).

Together, these results show that Drosophila MK2 kinase function is required in the dorsal hindgut compartment to protect larval hindgut ECs from stress-induced apoptosis upon salt stress.

**MK2 Genetically Interacts with p38a and p38b**

Mutations in MEKK1 and p38b but not in MK3/1ic and p38a resulted in a strong BD phenotype even at normal food conditions (Figure 3A). To define the roles of p38a and p38b with respect to MK2, we next tested these kinases genetically for the behaviour at normal conditions and under salt stress (Figure 3E). At unstressed conditions, MK2 was required neither for hindgut homeostasis nor for survival. Similarly, p38a mutants did not display BDs or elevated mortality rates. In contrast, MK2; p38a double mutants developed a weak BD phenotype, indicating that p38a and MK2 act in two parallel stress-signalling pathways. p38b mutants had a decreased survival rate, consistent with published findings [46]. p38b is required for hindgut homeostasis even under normal conditions, because larvae lacking p38b function developed BDs on normal food. Interestingly, MK2; p38b double mutants displayed a slight increase in BDs but no increase in lethality rate compared to p38b single mutants. Thus, p38b and MK2 are likely to function in the same pathway but both kinases may have additional independent functions in the hindgut or in other tissues.

Consistent with a common MK2/p38b pathway, p38b single mutants and MK2; p38b double mutants displayed the same phenotype at both weak (0.1 M NaCl) and strong (0.2 M NaCl) stress conditions. At weak stress conditions, p38a was not crucial for survival and hindgut homeostasis. In contrast, MK2 mutants displayed hindgut defects but no increase in mortality. In agreement with MK2 and p38a acting in two parallel stress-signalling pathways, BD formation was only slightly increased in the MK2; p38a double mutants but the absence of MK2 significantly enhanced the mortality of p38a mutants. This lethality increase of MK2; p38a double mutants was also observed at strong salt stress. At this condition, both MK2 and p38a mutants resulted in increased mortality rates, indicating that both branches of stress signalling are required for survival. Whereas MK2 is specifically required in the hindgut, p38a might be needed in other organs since p38a mutants hardly developed BDs.

Taken together, our genetic data suggest the existence of two major p38 branches in Drosophila that are required to varying extents during normal and different salt stress conditions. The p38a branch is not essential at normal or weak salt stress conditions but required at strong salt stress conditions. In contrast, the p38b branch is required at both normal and salt stress conditions to protect the hindgut. Moreover, the lethality of p38a; p38b double mutants [46] suggests that, besides their specific functions, both p38 kinases engage in a common essential function. MK2 is involved in a sub-branch of the p38b branch and appears to be a key effector of p38b in the hindgut. MK2 becomes more important with increasing stress conditions specifically in this tissue.

**p38b Phosphorylation Is Increased in Melanised Larval Hindguts**

We next checked the activation status of p38 by Western analysis (Figure 3B). Chronic exposure (from L1 to L3) to 0.2 M NaCl did not increase p38 phosphorylation in total and hindgut lysates of wild-type larvae. In contrast, stronger p38 activation was observed in total larval lysates of MK2 mutants under stress conditions. This strong activation was also apparent in the hindgut but only in larvae with BDs. Since we were not able to distinguish the endogenous p38a and p38b, we overexpressed GST-tagged versions of p38 and analysed their activation status in the hindgut (Figure 3C). GST-p38a was strongly activated even under normal conditions, and the activation was slightly increased under stress conditions. In MK2 mutants, GST-p38a was more strongly activated under all conditions and especially in hindguts with BDs. In contrast, GST-p38b was only weakly phosphorylated in wild-type and MK2 mutant hindguts at all conditions, except in hindguts of MK2 mutant larvae with BDs where a boost in GST-p38b phosphorylation was seen. Thus, the increase in endogenous p38 phosphorylation observed in MK2 mutant larval hindguts is probably due to p38b phosphorylation, suggesting that a negative feedback loop operates from MK2 to the upstream signalling components. Alternatively, the lack of a functional stress-protective MK2/p38b function may increase the stress in the ECs and lead to a vicious cycle that boosts p38b activation.

**MK2 Protein Abundance Depends on p38b**

In mammalian cells, MK2 is needed to stabilise p38 protein levels [13], which does not appear to be the case in Drosophila (Figure 3B and 3C). We wondered whether the protein levels of MK2 would depend on the presence of the upstream components. No change in MK2 expression was observed in p38a mutants, and a slight increase was detected in MEKK1 mutants. In sharp contrast, MK2 protein levels were reduced in p38b mutants (Figure 3D). Using a genomic MK2 rescue construct and a genomic MK2-GFP reporter in an MK2 null mutant background, we found that transcription from the MK2 locus was unchanged but protein levels were reduced, probably due to destabilisation of MK2 in the absence of p38b (Figure 3D).

**MK2 Physically Interacts with p38b**

The facts that MK2 and p38b genetically interact and that MK2 protein levels depend on the presence of p38b suggest a close physical interaction of the two kinases. Therefore, we expressed tagged versions of p38a, p38b and MK2 in S2 cells and checked for co-localization of these kinases. In mammalian cells, MK2 is nuclear under normal conditions and translocates to the cytoplasm upon stress [48]. Similarly, in Drosophila S2 cells, GFP-MK2 was mainly found in the nucleus, whereas overexpressed p38a and p38b were largely cytoplasmic (Figure 4A). Co-overexpression of p38b and MK2 (but not of p38a and MK2) resulted in a nuclear-to-cytoplasmic translocation of GFP-tagged MK2 (Figure 4A). Consistently, MK2 was found to bind p38b but not p38a in pull-down experiments (Figure 4B). This interaction as well as the nuclear-to-cytoplasmic shuttling was dependent on a four amino acid motif (DPTD) in p38b resembling the common docking motif (CD domain) that is critical for docking interactions in MAPKs [49]. In p38a, the respective four amino acids are EPSV. Exchanging these motifs revealed that the DPTD motif is necessary and sufficient to dock MK2 to p38 proteins (Figure 4A and 4B).

The expression of GST-p38b completely rescued the BD phenotype of p38b deficient flies, whereas expression of GST-p38a had no influence on the BD phenotype (Figure 4C and Figure S3). Expressing either GST-p38a→b (p38a with docking motif of p38b) or GST-p38b→a (p38b with respective sequences of p38a) resulted in a partial rescue of the BD phenotype. On normal food, both versions rescued partially, indicating that although not
Figure 3. Interactions of MK2 with the p38 SAPK pathway. (A) Mutations in genes encoding p38 SAPK pathway members were tested for a BD phenotype (normal food, 0.1 M NaCl, 0.2 M NaCl). MEKK1 and p38b but not MKK3 or p38a show a BD phenotype similar to MK2 mutants. The wild-type control (D1A) does not display any BDs. (B) The activating pTGpY phosphorylation (PP-p38) of p38 is increased in MK2 mutants under stress, especially in larvae with BDs (lanes marked by asterisks). (C) GST-tagged p38a (G38a) and p38b (G38b) were overexpressed in wild-type or MK2 mutant larvae to distinguish between the activation of the two p38 kinases. The lanes with lysates of MK2 mutant larvae with BDs are marked by asterisks. (D) Stabilisation of MK2 happens at the level of protein stability or translation and not at the level of transcription. In p38b mutant hindguts MK2 protein levels are reduced (upper blot). GFP expressed under the control of the genomic MK2 locus (MK2-GFP, MK2 coding sequence replaced by
essential, binding to MK2 is required for a complete rescue under normal conditions. On 0.1 M and 0.2 M NaCl, GST-p38a resulted in substantial but incomplete rescue, suggesting that other aspects of p38b function not mediated by binding to MK2 are required for a complete rescue. Consistently, a GST-p38b→a protein that is not able to bind MK2 also partially rescued the
p38b phenotype but to a lesser extent than p38a→b (Figure 4C and Figure S3). MK2 protein levels in the hindgut were restored by expressing p38b or p38a→b but not by p38a or p38b→a (Figure 4D). Thus, binding of p38 to MK2 is required to localise and stabilise MK2 and is important for the stress-protective function in the hindgut.

The Catalytic Activity of p38b Is Required to Localise MK2

The catalytic activity of MK2 and MK2 binding to p38b are required to protect the hindgut epithelial cells upon salt stress. To address whether the catalytic activity of p38b is also necessary, we used GST-tagged non-activatable p38bAGF and kinase-dead p38bKR protein mutants. Whereas co-expression of wild-type GST-p38b and GFP-MK2 led to a nuclear export (>70%) of MK2, the localisation of GFP-MK2 was random when GST-p38bAGF or GST-p38bKR were co-expressed (Figure 5A and 5B). Consistently, the BD phenotype of p38b null mutants was not rescued by re-expression of the kinase-dead or of the non-activatable p38b protein version (Figure 5C). Moreover, at high NaCl stress (0.2 M NaCl), the BD phenotype of the kinase-dead or of the non-activatable p38b protein version of MK2 to p38bAGF (DPTD changed to EPSV) do not change the localisation of MK2, but a GST-p38b→b protein mutant can export GFP-MK2 from the nucleus. (B) In pull down experiments, GST-p38b co-precipitates MK2, whereas p38a and the docking mutant p38b→a do not bind MK2. The DPTD motif introduced into p38 (p38a→b) is sufficient for binding to MK2. (C) Whereas overexpression of GST-p38b rescues completely, GST-p38a fails to rescue the BD phenotype of p38b mutants. GST-p38a→b and GST-p38b→a partially rescue the BD phenotype with p38a→b performing better. (D) Docking of MK2 to p38 that harbours a DPTD motif is sufficient to restore wild-type levels of MK2 protein in a p38b mutant background.

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Figure 4. A p38b/MK2 complex is required for hindgut EC stress protection. (A) GFP-MK2 (green) localises to the nucleus. GST-p38a and GST-p38b show a broad, more cytoplasmic distribution (red). GST-p38b co-expression shuttles GFP-MK2 to the cytoplasm in a DPTD-motif dependent manner. GST-p38a or GST-p38b→a (DPTD changed to EPSV) do not change the localisation of MK2, but a GST-p38a→b protein mutant can export GFP-MK2 from the nucleus. (B) In pull down experiments, GST-p38b co-precipitates MK2, whereas p38a and the docking mutant p38b→a do not bind MK2. The DPTD motif introduced into p38 (p38a→b) is sufficient for binding to MK2. (C) Whereas overexpression of GST-p38b rescues completely, GST-p38a fails to rescue the BD phenotype of p38b mutants. GST-p38a→b and GST-p38b→a partially rescue the BD phenotype with p38a→b performing better. (D) Docking of MK2 to p38 that harbours a DPTD motif is sufficient to restore wild-type levels of MK2 protein in a p38b mutant background.

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JNK Signalling Is Induced in MK2 Mutants and Is Linked to Apoptosis

JNK signalling has been implicated in triggering apoptosis [50]. Furthermore, a JNK antagonizing activity of p38 signalling has been observed in developmental processes [42] and at the systemic level during infection [41]. Thus, we tested whether the cell death observed in MK2 mutant larvae reared on high salt correlated with JNK activation. Indeed, elevated levels of phosphorylated JNK were detected in hindgut lysates of MK2 mutant larvae with BDs (Figure 6A). As in vivo readouts for JNK signalling activity, we used reporters for misshapen and puckered [51,52]. An induction of both mis>lacZ and puck>lacZ was observed in MK2 mutant larval hindguts, with highest levels adjacent to the BDs (Figure 6B). To exclude that the induction of JNK signalling is a secondary consequence of wound healing or the melanisation process, we checked for puck-GFP induction in stress-challenged MK2 mutant larvae before BD formation. Interestingly, patches with puck-GFP signal were readily detected upon stress in larvae devoid of BDs, and the area of those patches correlated with the strength of the stress (Figure 6C). We noted that puck-GFP was activated in a graded fashion, with highest activity where ECs undergo apoptosis and a BD will ultimately form (Figure 6D). Strong puck-GFP reporter activity co-localised with TUNEL positive cells close to the BDs (Figure 6E). Removing the JNK upstream components TAK1 and MKK4, respectively, in an MK2 mutant background partially suppressed the BD phenotype, indicating that the hindgut epithelial cells are dying due to JNK-induced apoptosis (Figure 6F). Expressing a dominant-negative version of JNK (BskDN) in the dorsal domain using engrailed-GAL4 resulted in a suppression of the BD phenotype (Figure 6G). Furthermore, expression of BskDN specifically in cells with high JNK activity (using puck-GAL4) substantially suppressed BD formation. In contrast, re-expression of MK2 at this stage reduced the number of larvae with BDs only mildly (Figure 6G). Thus, deregulated JNK

Figure 5. p38b activation and kinase activity are required to protect the hindgut. (A) Co-expression of GST-p38b and MK2 results in cytoplasmic localisation of GFP-MK2. When a kinase-dead (KR) or non-activatable (AGF) version of p38b was expressed, the nuclear export was not efficient. (B) Quantification of GFP-MK2 localisation upon GST-p38b, GST-p38bAGF or GST-p38bKR co-expression. Localisation was assigned to three classes: cytoplasmic (c), equal distribution between nucleus and cytoplasm (e), and nuclear localisation (n). (C) Activation and kinase function are required for proper function of p38b in the hindgut. The rate of BDs of p38b mutants upon re-expression of p38b protein variants by means of Act-GAL4 was assessed.
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activation in the hindgut of MK2 mutants precedes and probably causes cell death and BD formation.

Discussion

Gut homeostasis—under normal and stress conditions—is ensured by complex interactions between the intestinal epithelium, the immune system and the gut flora. Drosophila has been used as a simple model organism to address different aspects of intestinal homeostasis. Replenishment of the gut epithelium by ISCs clearly contributes to epithelial homeostasis but how the differentiated ECs are protected against stressors has remained largely unknown.

Figure 6. JNK activation leads to EC apoptosis in MK2 deficient hindguts. (A) JNK phosphorylation is strongly increased in hindguts of MK2 mutant larvae with BDs reared on 0.2 M NaCl food. Lanes containing lysates of larvae with BDs are marked by asterisks. (B) Reporters of JNK signalling activity (msn-lacZ and puc-lacZ) in wild-type (D1A) and MK2 mutant (D43) larvae reared on 0.2 M NaCl. In MK2 mutants, the JNK activity reporters get induced, especially close to the BDs. (C) Activation of puc-GFP in D43 mutants prior to BD appearance. Whereas no activation of puc-GFP occurs on normal food (Ci), fields of increased puc-GFP appear in the hindgut epithelium on 0.1 M NaCl (Cii) and 0.2 M NaCl (Ciii). The area of the patches and the number of affected ECs correlate with the strength of the stress. (D) puc-GFP induction occurs prior to the melanisation process. Hindgut of an MK2 mutant larva at the initiation of a BD (Di) and of an MK2 mutant larva after BD appearance (Dii), both reared on 0.1 M NaCl food. (E) In D43 mutants on 0.2 M NaCl, highest puc-GFP (green) and TUNEL staining (red) co-localise in the BD border region (white arrows). (F) Removing two copies of TAK1 or of MKK4 partially suppresses the BD phenotype of MK2 mutants. (G) Overexpression of MK2 and of BskDN in the dorsal hindgut (by means of en-GAL4) rescues the BD phenotype of MK2 mutants on 0.1 M NaCl food. Whereas re-expression of MK2 using puc-GAL4 does not rescue the BD phenotype on 0.1 M NaCl food, downregulation of JNK signalling using UAS-bskDN in combination with the puc-GAL4 driver results in a substantial suppression of the BD phenotype.

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critical component in the protection of hindgut ECs against salt stress.

We propose a model that puts a p38b/MK2 complex in the centre of stress-protection of the hindgut ECs (Figure 7). In the absence of this signalling module, cells are undergoing JNK-dependent apoptosis upon stress. The lesion in the EC monolayer results in the damage of the overlying hindgut musculature. This regional loss of the barrier function leads to systemic defects in the larvae (Figure S4), further weakening the larvae and impairing growth under stress conditions. As a consequence, pathogens and toxic substances might enter the body cavity, eventually resulting in the melanisation of pericardial cells and the induction of cecropin in the midgut (Figure S4).

Interestingly, JNK activation in MK2 mutant hindguts precedes the melanisation, and it consistently occurs in patches. Within these areas, some cells acquire highest amounts of JNK activity and eventually undergo apoptosis. The surrounding cells maintain JNK activity, forming a rim around the scar in the tissue. The number of affected ECs remains roughly constant for a given stress. Presently we do not know what determines the patches with high JNK activity within the tissue. Although the dhd domain ECs of the hindgut form a homogeneous epithelium and are facing the same stressor, JNK signalling is only induced in clusters of a certain size but not in surrounding cells. Increased JNK activation was also observed in the p38α deleted intestinal epithelium of a mouse model for IBDs [53]. Furthermore, ulcers occur in similar patchy patterns in IBDs [54]. Thus, the MK2 mutant phenotype may be useful to decipher how a group of cells within a tissue of genetically identical cells transforms into the weakest link in the chain upon stressful conditions.

Several lines of evidence support the notion that the p38b/MK2 signalling complex is key to EC protection against chronic salt stress. (1) p38b and MK2 mutant larvae both develop BDs upon stress conditions. (2) The severity of the p38b; MK2 double mutant phenotype upon stress is similar to the p38b single mutant phenotype, suggesting that they act in the same signalling pathway. (3) p38b but not p38a physically associates with MK2 via its C-terminal CD domain (DPTD motif). (4) The binding of p38b to MK2 stabilises MK2. (5) Upon co-expression, p38b but not p38a redirects MK2 to the cytoplasm. (6) Both the activation and the catalytic activity of p38b are required to efficiently relocalise MK2. (7) The binding of MK2 to p38 and the catalytic activities of both kinases are essential to protect the ECs of the larval hindgut upon salt stress. Taken together, stabilisation, localisation and activation of MK2 by p38b are required for a proper stress response.

Our genetic analysis also revealed that p38 SAPK signalling is contributing to stress protection in different ways in addition to the pivotal role of the p38b/MK2 complex. First, p38b impacts on hindgut homeostasis in an MK2-independent manner. This is apparent from the p38b mutant larvae that, in contrast to MK2 mutant larvae, develop BDs even at normal conditions. Consistently, a p38b protein version that no longer binds MK2 partially rescues the p38b mutant phenotype. Second, p38a is also required for full stress protection. The strong phenotype of MK2; p38a double mutants underscores the importance of the p38a SAPK pathway upon severe salt stress. However, the double mutants do not display an increase in BD formation but rather a decrease in viability. Thus, p38a may be involved in general stress protection that is not specific to the hindgut tissue. Third, a common p38 SAPK branch, encompassing p38a, p38b and potentially also p38c, is essential as the p38a; p38b double mutants die. Since the p38a1 allele affects the coding sequences of p38a and p38c, the p38a1; p38b277 double mutants may even represent p38a; p38b; p38c triple mutants. However, it is unclear to date whether p38c is a pseudogene. Recent studies have suggested an involvement of p38c function in immune gene regulation, early larval survival, and fertility [41,55]. Thus, further studies will be required to clarify whether p38c contributes to p38 signalling subbranches. Finally, the slight increase in BDs seen in MK2; p38b double mutants under normal conditions suggests that MK2 also performs a p38b-independent function in the hindgut. p38b mutants always impact on MK2 signalling since the MK2 protein is not stable and probably not correctly localised if not bound to p38b. Negative feedback regulation acting from MK2 on the activation of p38b further complicates the SAPK signalling network.

What are the upstream components regulating the p38b/MK2 complex? To our surprise, MKK3/Lic does not appear to play a

Figure 7. Protection of hindgut ECs by p38b/MK2. (Ai) Stressors in the gut lumen act on the hindgut ECs and induce p38 and JNK activation. The interaction of p38b with MK2 influences the localisation and protein stability of MK2, ensuring proper stress response by keeping JNK activity low. (Aii) In the absence of a functional p38b/MK2 complex, stress protection of the ECs is reduced and JNK activity is no longer kept in check, resulting in EC cell death and loss of hindgut homeostasis.

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role in the hindgut function of the p38b/MK2 branch. MKK3 but not MKK4 can activate p38 proteins in cell culture [56]. On the other hand, siRNA mediated knockdown of both MKK4 and MKK3 is required to fully block the activation of p38 under certain stresses in S2 cells [57]. Both p38b and MKK3/4 mutants show a strong reduction in p38 activation but no BDs are observed in MKK3/4 mutants. In contrast, mutants for MEKK1, which acts upstream of MEK3, do develop BDs similar to p38b and MK2 mutants. In mammalian cells, it has been shown that p38 can be activated independently of a MAP2 kinase [58–60]. However, no activation of p38 occurred in fibroblasts of MKK3 deficient mice [61]. Since MKK4 is a suppressor of the BD response to Sorbitol or NaCl has been observed [63,64]. Alternatively, p38 might induce a JNK phosphatase. p38 has been shown to impact on JNK activation by inducing DUSP1/MKP-1 in mammalian cells. Sustained JNK activation due to a lack of MKK1/MKK1 in the myeloid lineage had beneficial effects, consistent with the inflammatory nature of BDs. In contrast, p38b deletion in the intestinal epithelium resulted in a lack of gut homeostasis, marked by increased proliferation and by a reduction in goblet cells. Thus, our studies on Drosophila p38 signalling and its role in the larval hindgut provide a basis to specifically address the role of ECs in the maintenance of an intestinal epithelium in the absence of proliferation and immune response.

Materials and Methods

Fly Media and Stock Keeping

1 litre Drosophila media contains 100 g fresh yeast, 55 g cornmeal, 10 g wheat flour, 75 g sugar, and 8 g hacto-agar. For stress medium, Drosophila media were boiled and sodium chloride was added from a 5 M stock solution. 15 ml/l of a stock solution containing 33 g/L nипipag and 66 g/L nипasol in 96% EtOH was added to prevent growth of mould and bacteria. All crosses and experiments were performed at 25°C.

Fly Stocks

GE3296 was remobilised to generate the MK2 deletion mutants. y w; MK2 (genomic rescue), y w; MK2-GFP (genomic GFP reporter); y w; 86b, path1, [GSTM], [X-Sr33a, p38b, p38a > b, p38b > a, p38b5 < y, p38b2 < y, y w; 51D [MK2], y w; 51D [MK2KD], y w; 51D [mouseMK2], y w; nbyn2-GALA4 were generated in this study. For overexpression analyses, the following lines were used: y w; cad-GALA4/CyO y+, y w; pcy-GALA4/TM6B, y w; en-GALA4/CyO y+, y w; NP1-GALA4/CyO y+, y w; da-GALA4 and y w; Act-GALA4. For genetic interactions, the following lines were used: y w; FRT22B D-p38a+/TM6B [66], y w; p38b LoS/CyO y+, y w; lec14 y//Bmn [46], y w; MKK4 y//TM6B, y w; MKK4 y//TM6B [67]; y w; dTAK1+, w; dTAK1+ [63], y w; MKK1 y//TM6B [45]. For hemocyte ablation, y w; He-GALA4, UAS-GFP flies were crossed to y w; UAS-Bax flies [68]. The following reporter lines have been used: my gypsy [69], y w; ece11-LacZ [70]; y w; pucfp-CyO, y w; UAS-EGFP; puc-GALA4/TM6B (gift from K. Basler), and y w; msn-lacZ [92].

Stress Experiments

Females were allowed to lay eggs overnight on apple agar plates. Eggs were collected and 80–120 eggs were transferred to the different food vials. For BD quantification, larvae were analysed before reaching L3 wandering stage. For survival quantification, dead embryos were counted 24 h after seeding to the food and survival to pupae was recorded, respectively.

Plasmid Constructs and Transgenic Animals

For the MK2 genomic rescue construct, the genomic region between CG15771 and CG15770 was PCR-amplified and cloned into pCasper3. For the genomic MK2-GFP reporter, the same region was used but the MK2 coding sequence was replaced by the GFP coding sequence.

For overexpression constructs, the MK2 coding sequence was amplified and cloned into pENTR/D-TOPO (Invitrogen). For the kinase-dead MK2 protein, the mutation leading to the K49A substitution was introduced by PCR mutagenesis. The inserts were shuttled into the destination vector pTGW (http://www.ciwemb.org).
ed1/labs/murphy/Gateway%20vectors.html) for N-terminal GFP tagging. To express untagged MK2, the MK2 coding sequence was cut from the pENTR/D-TOPO and ligated into a pUAST-attB vector.

GST-tagged p38a and p38b overexpression constructs were generated by ligating the GST coding sequence in frame to the p38a or p38b coding sequence, and the resulting fusion sequences were cloned into a pUAST-attB vector. The constructs encoding the p38a or p38b protein mutants were generated by PCR mutagenesis. In the p38a→α and the p38b→α protein mutants, the EPSV motif was changed to DPTD and vice versa. The kinase-dead or non-activatable p38b protein mutants were generated by introducing mutations in the coding sequence that result in the K53R substitution and in the replacement of the TGY motif by two linkers. The anti-D-p38b antibody was generated by Eurogentec by immunizing a rabbit with the peptide H2O-QPKTTLDDYVTSN-COOH, and the final bleed was used 1:500 in WB.

Secondary antibodies: HRP-coupled anti-mouse IgG (Jackson Immunoresearch; 1:10,000; WB), HRP-coupled anti-rabbit IgG (Jackson Immunoresearch; 1:10,000; WB), and Cy3-coupled anti-rabbit IgG (1:500; IHC).

Other Histological Stainings

Larvae were dissected and fixed by standard procedures. After washing with PBS, 500 μl X-gal staining solution was added, and the samples were incubated at 37°C in the dark. The staining progress was observed every 10', and the staining reaction was stopped by two washes with PBT.

Alexa Fluor 594-conjugated phalloidin (Molecular Probes) was used to stain muscles. For apoptosis detection, the TUNEL assay kit ApopTag RED In Situ Detection Kit (Millipore S7165) was used.

Hindgut Sections

Larvae were dissected on ice and hindguts were immediately fixed in 2.5% glutaraldehyde, 1% paraformaldehyde, 1% potassium ferrocyanide, 0.1 M cacodylate buffer for 40'. After washing three times in 0.1 M cacodylate buffer, hindguts were postfixed in 1% osmium tetroxide, 1% potassium ferrocyanide, 0.1 M cacodylate buffer (pH 7.4) for 60'. Hindguts were then dehydrated in an ascending acetone series (30%→50%→70%→90%→100% 3' each and 5' 100%). The samples were incubated overnight in a 1:1 acetone:Spurr solution. After equilibration in Spurr solution for 4 h, samples were embedded in Spurr solution and hardened at 65°C overnight. 2 μm sections were made with a Supercut Reichert-Jung 2050 microtome, and sections were mounted in DPX Mountant for histology (Fluka).

Supporting Information

Figure S1 MK2 null mutants and development of BDs. (A) Larval extracts of wild-type (A1A) larvae but not of MK2 mutants (A43) have in vitro kinase activity towards small heat shock protein 25 (hsp25). Overexpression of wild-type MK2, but not of kinase-dead MK2, boosts hsp25 phosphorylation. Antibodies against Drosophila MK2 do not recognize a band in Western analysis on MK2 mutant total larval lysates. (B) MK2 mutants were tested for the appearance of BDs by feeding different stressors. A rough classification reveals that only high salt and SDS feeding induce BDs in vitro. (C) Representative pictures of MK2 mutant (A43) larvae at indicated time points (white arrows point to BDs in the first two panels). (D) Quantification of BDs of MK2 mutant larvae reared on 0.2 M NaCl food at the time points depicted in (C). (E) The size of the BDs depends on the strength of the stress. MK2 mutant larvae were reared on 0.1 M or 0.2 M NaCl food,
respectively, and BDs were analysed in L3 before wandering stage. White arrows point to BDs in the first two panels.

(TIF)

Figure S2 Hindgut defects and hindgut-specific rescue of MK2 mutants. (A) Hemocytes are observed neither in wild-type (A1A) nor in MK2 mutant (A45) hindguts. No Hemese staining is found at the melanised lesion site (lowest panel) and in MK2 mutant hindguts without BDs (middle panel), even when the visceral musculature (red) is damaged (lowest panel). Staining of blood cells attached to the cuticle (inset and white arrow in the middle panel) demonstrates that the staining protocol worked. (B) Hemocytes are dispensable for BD formation as larvae lacking hemocytes still develop BDs (white arrow). (C) Bright field pictures of hindgut sections of wild-type (A1A) and MK2 mutant (A45) larvae reared on 0.1 M NaCl. In MK2 mutants without BDs, the hindgut structure appears either undamaged (second panel) or displays blistering of ECs in the dorsal hd domain (black asterisk in fourth panel). EC blistering is also observed in MK2 mutants with BDs at a distance of the BD (black asterisk in fourth panel). Panels four and five show sections of the same hindgut ahead of and at the BD lesion site, respectively. White asterisks mark the gut content; black arrows point to undamaged apical membranes; white arrow indicates BD. (D) Various GAL4 lines were used to drive MK2 expression from a wild-type UAS-MK2 cDNA construct in an MK2 mutant background (A45) and the ability to rescue the BD phenotype was scored. Only ubiquitous and hindgut-specific expression of calcitropically active but not of a kinase-dead MK2 rescues the BD phenotype.

(TIF)

Figure S3 p38b BD phenotype rescued by p38 expression. Homozygous p38b mutants were reared on 0.2 M NaCl food. Rescue of the BD phenotype by p38b and p38b was quantified (Figure 4C). Here we show representative examples of larvae that were quantified for their BD appearance.

(TIF)

Figure S4 Systemic effects observed in MK2 mutants. (A) MK2 mutants reared on 0.2 M NaCl food often display a severely ruptured hindgut musculature (white arrows), resulting in a local gut barrier breakdown. The BD can be recognized based on its autofluorescence (yellow). (B) In such strongly affected larvae, the antimicrobial peptide CecA1 is induced in the midgut, indicative of a systemic response. The black bar (labelled with hg) indicates the hindgut; the black arrow marks the BD; the white arrows point to the CecA1-lacZ induction (blue). (C) The systemic disturbance in larvae with large BDs (asterisk) is underscored by the appearance of melanised pericardial cells (arrow).

(TIF)

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Author Contributions

Conceived and designed the experiments: GS EH HS. Performed the experiments: GS. Analyzed the data: GS. Wrote the paper: GS HS.

References

1. Daneman R, Rescigno M (2009) The gut immune barrier and the blood-brain barrier: are they so different? Immunity 31: 722–735.
2. Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, et al. (2009) Crypt stem cells as the cell-of-origin of intestinal cancer. Nature 457: 608–611.
3. Barker N, van Es JH, Jako V, Kasper M, Snippert H, et al. (2008) Very long-term self-renewal of small intestine, colon, and hair follicles from cycling Lgr5+ve stem cells. Cold Spring Harb Symp Quant Biol 73: 351–356.
4. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, et al. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nat. 449: 1003–1007.
5. Nenci A, Becker C, Wallaert A, Gareus R, van Loo G, et al. (2007) Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 446: 557–561.
6. Pasparakis M (2008) IKK/NF-kappaB signaling in intestinal epithelial cells controls immune homeostasis in the gut. Mucosal Immunol 1 Suppl 1: S54–57.
7. Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, et al. (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. J. Biol Chem 283: 14434–14443.
8. Waetzig GH, Seegert D, Rosenstiel P, Nikolaus S, Schreiber S (2002) p38 mitogen-activated protein kinase is activated and linked to TNF-alpha signaling in inflammatory bowel disease. J. Immunol 168: 5342–5351.
9. Malamut G, Cabane C, Dubuquoy L, Malapel M, Derijard B, et al. (2006) No evidence for an involvement of the p38 and JNK mitogen-activated protein kinase pathways in inflammatory bowel disease. J. Biol Chem 281: 14434–14443.
10. Heidenreich O, Neininger A, Schrantz M, Zinck R, Cahill MA, et al. (1999) MAPKAP kinase 2 phosphorylates serum response factor in vitro and in vivo. J. Biol Chem 274: 14141–14149.
11. Brook M, Telen CR, Santalucia T, Mclellan J, Arthur JS, et al. (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Mol. Cell Biol 26: 2408–2418.
12. Reichardt HC, Aslanian AS, Lees JD, Yaffe MB (2007) p53-deficient cells rely on ATM- and ATR-mediated checkpoint signalling through the p38MAPK/ MK2 pathway for survival after DNA damage. Cancer Cell 11: 175–189.
13. Reichardt HC, Hasskamp P, Schmellding I, Morandell S, van Vugt MA, et al. (2010) DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization. Mol. Cell 40: 34–49.
14. Sumara G, Formentini I, Collins S, Sumara I, Windak R, et al. (2009) Regulation of PKD by the MK2 delta in insulin secretion and glucose homeostasis. Cell 136: 235–248.
15. Schwermann J, Rathinam C, Schubert M, Schulmacher S, Noyan F, et al. (2009) MAPKAP kinase MK2 maintains self-renewal capacity of haematopoietic stem cells. EMBO J 28: 1392–1406.
16. Murakami SA, Matsumoto A, Yamaoka I, Tanimura T (1993) Novel tissue units of regional differentiation in the gut epithelium of Drosophila, as revealed by P-Engrailed/Invected repression specifies boundary cells in the Drosophila hindgut. Mech Dev 114: 71–84.
17. Brook M, Telen CR, Santalucia T, Mclellan J, Arthur JS, et al. (2006) Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Mol. Cell Biol 26: 2408–2418.
18. Iwaki DD, Lengyel JA (2002) A Delta-Notch signaling border regulated by Dorsal dependent degradation of the Delta ligand. Development 203: 243–249.
19. Marakou S, Ishii H (2001) Ultrastructure of the hindgut of Drosophila larva, with special reference to the domains identified by specific gene expression patterns. J. Morphol 248: 144–150.
20. Iwaki DD, Lengyel JA (2002) A Delta-Notch signaling border regulated by Engrailed/Invected expression specifies boundary cells in the Drosophila hindgut. Mech Dev 114: 71–84.
21. Schwermann J, Rathinam C, Schubert M, Schulmacher S, Noyan F, et al. (2009) MAPKAP kinase MK2 maintains self-renewal capacity of haematopoietic stem cells. EMBO J 28: 1392–1406.
22. Murakami SA, Matsumoto A, Yamaoka I, Tanimura T (1993) Novel tissue units of regional differentiation in the gut epithelium of Drosophila, as revealed by P-Engrailed/Invected repression specifies boundary cells in the Drosophila hindgut. Mech Dev 114: 71–84.
23. Marakou S, Ishii H (2001) Ultrastructure of the hindgut of Drosophila larva, with special reference to the domains identified by specific gene expression patterns. J. Morphol 248: 144–150.
24. Iwaki DD, Lengyel JA (2002) A Delta-Notch signaling border regulated by Engrailed/Invected expression specifies boundary cells in the Drosophila hindgut. Mech Dev 114: 71–84.
25. Takashima S, Mrkutchyan M, Yousouff-Hartenstein A, Merriam JR, Hartenstein V (2008) The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. Nature 454: 651–655.
26. Biteau B, Hochmuth CE, Jasper H (2008) JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell Stem Cell 3: 442–455.
Stress Protection in the Drosophila Hindgut

27. Choi YJ, Hwang MS, Park JS, Bae SK, Kim YS, et al. (2008) Age-related upregulation of Drosophila caudal gene via NF-kappaB in the adult posterior midgut. Biochim Biophys Acta 1780: 1093–1100.

28. Buchon N, Broderick NA, Kurzinski T, Lemaitre B (2010) Drosophila EGF-R pathway mediates stem cell proliferation and gut remodeling following infection. BMC Biol 8: 152.

29. Biteur B, Jasper H (2011) EGF signaling regulates the proliferation of intestinal stem cells in Drosophila. Development 138: 1045–1055.

30. Staley BK, Irvine KD (2010) Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. Curr Biol 20: 1580–1587.

31. Shaw RL, Kohli-Maier A, Polesello C, Veelken C, Edgar BA, et al. (2010) The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Development 137: 4147–4158.

32. Karpowicz P, Perez-J, Perrimon N (2010) The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development 137: 4135–4145.

33. Cree B, Lee WC, Michelini CA (2010) JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the Drosophila intestinal stem cell lineage. Dev Biol 338: 28–37.

34. Mathur D, Bost A, Driver I, Oltmann B (2010) A transient niche regulates the specification of Drosophila intestinal stem cells. Science 327: 210–213.

35. Jiang H, Patel PH, Kohli-Maier A, Gorenley MO, McEvain DG, et al. (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell 137: 1343–1355.

36. Fee S, Pallevi SK, Huyghe M, Lae M, Janssen KP, et al. (2009) Nerve and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. Proc Natl Acad Sci U S A 106: 6309–6314.

37. Fox DJ, Spradling AC (2009) The Drosophila hindgut lacks constitutively active adult stem cells but proliferates in response to tissue damage. Cell Stem Cell 5: 290–297.

38. Park JS, Kim YS, Yoo MA (2009) The role of p38 MAPK in related modulation of intestinal stem cell proliferation and differentiation in Drosophila. Aging (Albany NY) 1: 637–651.

39. Park JS, Kim YS, Kim JG, Lee SH, Park SY, et al. (2010) Regulation of the Drosophila p38alpha gene by transcription factor DREF in the adult midgut. Biochim Biophys Acta 1799: 510–519.

40. Ha EM, Lee KA, Seo YY, Kim SH, Lim JH, et al. (2009) Coordination of multiple dual oxidase-regulatory pathways in responses to commercial and infectious microbes in drosophila gut. Nat Immunol 10: 949–957.

41. Chen J, Xie C, Tian L, Hong L, Wu N, et al. (2010) Participation of the p38 pathway in Drosophila host defense against pathogenic bacteria and fungi. Proceedings of the National Academy of Sciences of the United States of America 107: 20774–20779.

42. Balakireva M, Rosse C, Langevin J, Chien YC, Ghe M, et al. (2006) The Raf/Exocytosis effector complex counters c-Jun N-terminal kinase-dependent apoptosis in Drosophila melanogaster. Mol Cell Biol 26: 8953–8963.

43. Wu H, Wang MC, Bohmann D (2009) JNK protects Drosophila from oxidative stress by transcriptionally activating autophagy. Mech Dev.

44. Gaestel M (2006) MAPKAP kinase - MKs - two’s company, three’s a crowd. Cell Biol Int 30: 481–495.

45. Staples CJ, Narasimamurthy R, Basler K (2005) A genetic screen targeting the tumor necrosis factor/Eiger signaling pathway: identification of Drosophila TAB2 as a functionally conserved component. Genetics 171: 1683–1694.

46. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is a functionally conserved component of the p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

47. Cheung PC, Campbell DG, Nehreda AR, Cohen P (2005) Feedback control of the protein kinase TAK1 by SAPK2a/p38alpha. The EMBO journal 22: 5793–5803.

48. Geuking P, Narasimamurthy R, Basler K (2005) A genetic screen targeting the tumor necrosis factor/Eiger signaling pathway: identification of Drosophila TAB2 as a functionally conserved component. Genetics 171: 1683–1694.

49. Zhang Y, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.

50. Staples CJ, Owens DM, Maier JV, Cans AC, Keyse SM (2010) Cross-talk between the p38alpha and JNK MAPK pathways mediated by MAP kinase phosphatase-1 determines cellular sensitivity to UV radiation. The Journal of biological chemistry 285: 25928–25930.

51. Craig GR, Finak JL, Yapi Y, Ip YT, Cagan RL (2004) A Drosophila p38 orthologue is required for environmental stress responses. EMBO Rep 5: 1058–1063.

52. Geuking P, Narasimamurthy R, Lemaitre B, Basler K (2005) A non-redundant role for Drosophila Mkk4 and hemipterous/Mkk7? in TAK1-mediated activation of JNK. PLoS ONE 4: e7799. doi:10.1371/journal.pone.0007709.

53. Okuda M, Ankon M, Cribier G, and Mauvais J (2001) A Drosophila MAPKKK, D-MEKK1, mediates stress responses through activation of p38 MAPK. EMBO J 20: 5421–5430.

54. Curi G (2003) Cross-talk between the p38alpha and JNK MAPK pathways mediated by MAP kinase phosphatase-1 determines cellular sensitivity to UV radiation. The Journal of biological chemistry 285: 25928–25930.

55. Orsina M, Bocchini D, Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kellner L, et al. (2003) Mechanism of p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

56. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is a functionally conserved component of the p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

57. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.

58. Craig GR, Finak JL, Yapi Y, Ip YT, Cagan RL (2004) A Drosophila p38 orthologue is required for environmental stress responses. EMBO Rep 5: 1058–1063.

59. Orsina M, Bocchini D, Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kellner L, et al. (2003) Mechanism of p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

60. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.

61. Orsina M, Bocchini D, Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kellner L, et al. (2003) Mechanism of p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

62. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.

63. Orsina M, Bocchini D, Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kellner L, et al. (2003) Mechanism of p38 MAPK kinase activation in vivo. Genes & development 17: 1969–1978.

64. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.

65. Zhuang ZH, Sun L, Kong L, Hu JH, Yu MC, et al. (2006) Drosophila TAB2 is required for the immune activation of JNK and NF-kappaB. Cell Signaling 18: 964–970.