Published in final edited form as:

Aging Cell. 2009 June ; 8(3): 288–295. doi:10.1111/j.1474-9726.2009.00476.x.

JNK signaling in insulin-producing cells is required for adaptive responses to stress in *Drosophila*

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Summary

Adaptation to environmental challenges is critical for survival of an organism. Repression of Insulin/IGF Signaling (IIS) by stress-responsive Jun-N-terminal Kinase (JNK) signaling is emerging as a conserved mechanism that allows reallocating resources from anabolic to repair processes under stress conditions. JNK activation in Insulin producing cells (IPCs) is sufficient to repress Insulin and Insulin-like peptide (ILP) expression in rats and flies, but the significance of this interaction for adaptive responses to stress is unclear. Here we show that JNK activity in IPCs of flies is required for oxidative stress-induced repression of the *Drosophila* ILP2. We find that this repression is required for growth adaptation to heat stress as well as adult oxidative stress tolerance, and that induction of stress response genes in the periphery is in part dependent on IPC-specific JNK activity. Endocrine control of IIS by JNK in IPCs is thus critical for systemic adaptation to stress.

Keywords

JNK; Insulin; stress response; *Drosophila*; metabolism; growth

Introduction

Metabolic adaptation to environmental challenges is imperative for the long-term homeostasis and reproductive success of metazoans. Ectotherms, which are particularly vulnerable to environmental changes, have evolved particularly strong adaptive responses (termed diapause, (Tatar & Yin 2001; Denlinger 2002)), but metabolic adaptation to stress is also conserved in higher organisms, including vertebrates (Buteau *et al.* 2007; Russell & Kahn 2007; Schumacher *et al.* 2008).

The molecular regulation of metabolic adaptation is only starting to be understood. Studies in *C.elegans* have established the evolutionarily conserved Insulin/IGF Signaling (IIS) pathway as a central regulator of metabolic adaptation, controlling the transition into a larval diapause, the dauer state (Kimura *et al.* 1997; Burnell *et al.* 2005; Kenyon 2005). The same signaling pathway controls stress tolerance and metabolic homeostasis in higher organisms, influencing lifespan (Tatar & Yin 2001; Denlinger 2002; Bishop & Guarente 2007a; Russell & Kahn 2007; Sim & Denlinger 2008; Taguchi & White 2008), but the mechanism(s) by which IIS activity is modulated in response to stress remain to be established.

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Recent work in flies, worms and mice suggest that stress signaling pathways play an important role in this regulation, increasing lifespan and stress tolerance by repressing IIS activity (Oh *et al.* 2005; Wang *et al.* 2005) (Bishop & Guarente 2007b; Schumacher *et al.* 2008). Interestingly, a similar antagonism between stress signaling and IIS also seems to be central to the etiology of type II diabetes, suggesting that repression of IIS activity is an important acute response to stress, but has deleterious effects on metabolic homeostasis under chronic conditions (Hotamisligil 2006). In particular, the stress-responsive Jun-N-terminal Kinase (JNK) pathway has been shown to regulate IIS activity at multiple levels, influencing lifespan and stress tolerance in lower organisms and promoting insulin resistance in mouse models for diabetes (Hirosumi *et al.* 2002; Kaneto *et al.* 2002; Kajimoto & Kaneto 2004; Oh *et al.* 2005; Wang *et al.* 2005; Karpac & Jasper in press). JNK can repress IIS signal transduction by phosphorylating the Insulin receptor substrate, as well as by activating the IIS-regulated transcription factor Foxo, but can also interfere with Insulin-like peptide (ILP) expression in Insulin Producing Cells (IPCs) (Hirosumi *et al.* 2002; Kaneto *et al.* 2002; Essers *et al.* 2004; Kajimoto & Kaneto 2004; Oh *et al.* 2005; Wang *et al.* 2005).

The regulation of Insulin-like peptide expression suggests a central role for IPC-specific JNK signaling in coordinating metabolic adaptation systemically, i.e. throughout the whole organism. Insulin-like peptides are produced by pancreatic beta cells in mammals, and by neurosecretory cells in *Drosophila* and *C.elegans* (IPCs in *Drosophila* and ASI and ASJ neurons in worms (Brogiolo *et al.* 2001; Ikeya *et al.* 2002; Li *et al.* 2003). The central role of these neurosecretory cells in metabolic adaptation and lifespan has been established in worms and flies. Ablation of ASI neurons promotes dauer formation (Bargmann & Horvitz 1991), while expression of the Insulin-like peptide daf28 in these neurons is downregulated in response to stress (Li *et al.* 2003). Furthermore, ablation of IPCs results in flies with diabetic phenotypes, but extended lifespan and increased stress tolerance (Rulifson *et al.* 2002; Wessells *et al.* 2004; Broughton *et al.* 2005), and repressing ILP expression in IPCs by over-expression of either the JNK Kinase Hemipterous (Hep), or a dominant-negative version of p53 results in increased lifespan (Wang *et al.* 2005; Bauer *et al.* 2007).

While these studies confirm that forced repression of Insulin-like peptide expression is sufficient to impair IIS activity and increase stress tolerance and longevity, it remains unclear whether this repression is part of an acute adaptive response to environmental stress. Here we show that IPC-specific JNK activity is required for ILP repression in response to oxidative stress in flies, mediating stress-induced growth repression during development and promoting adult stress tolerance. IPC-specific JNK signaling is thus central to systemic adaptive responses to acute environmental challenges.

Results and Discussion

JNK represses dILP2 expression in response to oxidative stress

The increased stress tolerance and extended lifespan of flies in which IPCs are ablated, as well as the repression of dILP2 transcription by JNK signaling in IPCs, suggests that repression of dILPs plays an important role in coordinating stress responses systemically. To test this hypothesis, we analyzed dILP transcript levels in response to treatment with the oxidative stress-inducing compound Paraquat. Supporting a potential role for ILP repression in metabolic adaptation to stress, we observed significant down-regulation of the genes encoding dILP2 and dILP5, while dILP3 expression was not affected (Figure 1). Importantly, stress-induced repression of dILP2 transcription was not observed in flies in which JNK signaling was specifically inhibited in IPCs, by expression of a dominant-negative form of the *Drosophila* JNK Basket (Bsk^{DN}) (Weber *et al.* 2000). Interestingly, dILP5 repression was unaffected in these flies, indicating that JNK signaling is specifically

required for the transcriptional control of dILP2 in response to environmental stress (Figure 1). While little is known about the specific functions of different dILPs in the regulation of IIS activity, various studies have demonstrated specific regulation of the encoding genes in response to nutritional cues or to specific signaling events (Ikeya *et al.* 2002; Hwangbo *et al.* 2004; Wang *et al.* 2005; Gershman *et al.* 2007). Further studies are needed to establish whether this specific regulation of individual dILPs has physiological consequences *in vivo*, and to assess how individual dILPs would elicit specific responses through the sole Insulin Receptor (InR) encoded in the fly genome.

Growth adaptation is regulated by IPC-specific JNK activity

DILP2 has been described as a major regulator of growth in flies, and can alone rescue the growth defects of flies with ablated IPCs (Ikeya *et al.* 2002; Rulifson *et al.* 2002), suggesting that its repression by JNK signaling in IPCs significantly impacts growth and physiology. To test this, we assessed the requirement for IPC-specific JNK activity in heat-induced growth repression, an adaptive strategy conserving energy resources under adverse conditions. To assess whether IPC-specific JNK activity is required for this response, we compared adult wing sizes of flies reared at 25 °C with wing sizes of flies that were shifted to 29°C early in development. Wild-type animals (OreR) exhibit significantly reduced body weight as well as wing size when shifted to 29°C, caused by a decrease in cell numbers and a slight reduction in cell size (Figure 2 A-E). This phenotype is reminiscent of the consequences of IIS repression, or Foxo activation, which impacts primarily cell proliferation, but also influences cell growth (Junger *et al.* 2003; Puig *et al.* 2003). This reduction is less pronounced in mutants for JNKK/Hep (Figure 2 A-E; *hep*¹ is a P-element insertion into the *hep* locus, resulting in viable hemizygous adult males with deficient JNK activity, (Glise *et al.* 1995; Wang *et al.* 2003)). Strikingly, flies expressing Bsk^{DN} or a double-stranded RNA targeting *bsk* (Bsk^{RNAi}; this line efficiently represses *bsk* transcription; Figure S2) specifically in IPCs (using the IPC specific driver, *dilp2G4* (Rulifson *et al.* 2002)) also exhibited significantly reduced growth repression compared to wild-type controls, demonstrating that IPC-specific JNK is required for growth adaptation (Figure 2 F, G; see also Figure S1). As seen for conditions in which JNK activity is increased in flies (Wang *et al.* 2003), inhibiting JNK signaling in IPCs does not affect developmental timing, indicating that the observed effects are primarily mediated through a control of cell proliferation (Figure S1C). Importantly, growth repression was also reduced in flies constitutively expressing dILP2 in IPCs, confirming the role of dILP2 repression in this response (Figure 2 F, G). Further highlighting the importance of dILP2 repression downstream of JNK signaling in this adaptive response, we found that co-expressing a double-stranded RNA targeting *dILP2* (*dILP2*^{RNAi}; this lines efficiently reduces dILP2 transcription; Figure S2) was sufficient to rescue the growth repression in animals expressing Bsk^{DN} in IPCs.

IPC-specific JNK activity regulates systemic stress tolerance

Since IIS regulates both growth during development, as well as stress tolerance in the adult, we asked whether IPC-specific JNK signaling would also influence stress tolerance. We tested the sensitivity of flies expressing Bsk^{DN} or Bsk^{RNAi} in IPCs to Paraquat in conditions identical to the ones used in the experiments described in Figure 1 (Figure 3A). Supporting a critical function for IPC-specific JNK activity in systemic stress tolerance, these flies were significantly more sensitive to Paraquat compared to sibling controls. To control for genetic background effects, and to test whether acute rather than chronic repression of JNK activity would be sufficient to induce the observed stress sensitivity, we generated an RU486-inducible IPC-specific Gal4 driver line (using a 1500bp fragment of the *dILP2* promoter to drive Gal4 fused to a progesterone binding domain (Osterwalder *et al.* 2001). This driver specifically and efficiently drives expression of UAS-linked transgenes in IPCs in response

to RU486; Figure 3C and data not shown). Expression of Bsk^{DN} or Bsk^{RNAi} using this driver increased stress sensitivity of adult flies in a RU486-dependent manner, further supporting the notion that IPC-specific JNK activity is required for adaptive responses and thus increased tolerance to environmental stress (Figure 3B). Importantly, fecundity was not affected in animals expressing Bsk^{DN} in IPCs, indicating that JNK-mediated repression of *dILP2* transcription influences cytoprotective mechanisms directly, and not by compromising reproduction. This view is supported by the fact that fecundity is also not affected in long-lived JNK gain-of-function flies (Wang *et al.* 2003). Interestingly, inhibiting JNK activity in IPCs does not lead to shortened lifespan under optimal culture conditions (not shown), indicating that JNK activity is not normally required in IPCs, but becomes critical under stress conditions. This observation further suggests that the basal activity of JNK detected in IPCs using a JNK lacZ reporter line (*puc^{E69}*, (Wang *et al.* 2005)) is likely a consequence of acute stress responses that result in accumulation of beta-Galactosidase activity in IPCs throughout the lifetime of the animal, and does not demonstrate a requirement for chronic basal JNK activity in these cells.

To confirm that the observed stress sensitivity of flies with IPC-specific inhibition of JNK was caused by a lack of *dILP2* repression (as shown in Figure 1), we assessed whether repressing *dILP2* transcription constitutively was sufficient to rescue the stress sensitivity of flies with impaired IPC-specific JNK signaling activity (Figure 3D). Indeed, stress tolerance of flies expressing both Bsk^{DN} and *dILP2^{RNAi}* was similar to the stress tolerance observed in wild-type control flies, while over-expression of *dILP2* resulted in significant increase of oxidative stress sensitivity (Figure 3E). These results further underline the importance of JNK-mediated repression of *dILP2* for systemic stress tolerance.

IPC-specific JNK activity is required for peripheral stress responses

Our results suggest that activation of JNK signaling in IPCs increases stress tolerance by inhibiting IIS activity in the periphery. IIS repression results in increased nuclear translocation of the transcription factor Foxo, which promotes the expression of stress response genes and growth regulators (Junger *et al.* 2003; Puig *et al.* 2003; Wang *et al.* 2005; Vihervaara & Puig 2008). To test whether this transcriptional stress response in the periphery was dependent on IPC-specific JNK activity and *dILP2* repression, we analyzed the induction of the Foxo target and stress response genes *thor*, *dLip4* and *gstD1* in the abdomen and thorax of stressed flies (Figure 4A). Supporting a requirement for IPC-specific JNK activation for systemic transcriptional stress responses, we found that induction of *thor*, *dLip4* and *gstD1* was significantly impaired in flies expressing Bsk^{DN} in the IPCs. We further tested whether nucleo-cytoplasmic distribution of Foxo in abdominal fatbodies would be affected by stress. Indeed, we found that Paraquat exposure resulted in increased nuclear localization of Foxo in abdominal fatbody cells, and, strikingly, that this effect is strongly reduced in flies over-expressing *dILP2* in IPCs (Figure 4B). The repression of *dILP2* in response to oxidative stress is thus critical for increased nuclear localization and activity of Foxo in peripheral tissues.

Interestingly, however, while induction of Foxo target genes was significantly reduced, it was not completely abolished in flies expressing Bsk^{DN} in IPCs, suggesting that IPC-specific JNK activity is important for a sustained and efficient systemic stress response, while local responses to ROS are still functional when JNK is inhibited in IPCs (Figure 4C). Cell autonomous activation of Foxo by JNK signaling has been described in mice and flies (Essers *et al.* 2004; Oh *et al.* 2005; Wang *et al.* 2005; Luo *et al.* 2007; Nielsen *et al.* 2008), and it likely plays an important role in cytoprotection and/or apoptosis in response to local insults. Our results suggest that the repression of *dILP2* transcription by IPC-specific activity of JNK adds a critical regulatory dimension that allows coordinating cellular stress responses systemically (Figure 4C). Recent work in mice suggests that such a systemic

regulation of IIS activity might be an evolutionarily conserved response to damaging stimuli (Niedernhofer *et al.* 2006; van der Pluijm *et al.* 2007).

Interestingly, while we find that repression of *dILP2* transcription is a critical component of the adaptive systemic response to stress, a recent study reports that constitutively repressing *dILP2* transcription is not sufficient to increase stress tolerance of wild-type flies, an observation that is consistent with our own findings (Broughton *et al.* 2008, and compare wild-type and *dilp2>dILP2^{RNAi}* in Figure 3D). The lack of increased stress tolerance when *dILP2* is repressed constitutively is due to compensatory up-regulation of *dILP3* and *dILP5* expression in IPCs (Broughton *et al.* 2008, and Karpac, data not shown). Based on our results reported here, it is evident that such a compensatory induction of *dILP3* and *dILP5* does not occur immediately after stress exposure and that repression of *dILP2* thus promotes stress tolerance under these acute conditions. Accordingly, when repression of *dILP2* transcription is impaired, as in IPC-specific JNK loss-of-function conditions, reduced stress tolerance is observed.

Our analysis focuses primarily on oxidative stress as a well-established insult that promotes loss of homeostasis in aging organisms, as well as on growth responses to elevated temperature during development. It remains unclear whether the observed endocrine mechanisms govern adaptive responses to other challenges, such as inflammation or DNA damage. Interestingly, repression of the IIS/GH signaling axis has been described in mice with elevated DNA damage (Schumacher *et al.* 2008), but the mechanisms mediating this repression have not yet been uncovered. Based on the evolutionary conservation of JNK signaling and of the repression of Insulin production by JNK in rat islet cells (Kaneto *et al.* 2002), it can be expected that similar endocrine mechanisms might govern the metabolic adaptation to stress in vertebrates.

An interesting question that arises from our observations is whether the adaptive repression of *dILP2* expression by stress-induced JNK activation can result in lifespan extension, and whether low level exposure to oxidative stress should therefore extend lifespan. Indeed, we have shown before that lifespan can be extended when JNK is moderately activated in IPCs (Wang *et al.*, 2005), and various studies have shown that moderate exposure to stress can have beneficial effects on the organism's lifespan (an effect termed hormesis, see for example (Hercus *et al.* 2003)). The regulation discussed here implies a mechanism for such hormetic effects of oxidative stress.

Experimental Procedures

Drosophila strains and fly handling

Fly strains: OreR, from the Bloomington *Drosophila* stock center; *hep¹/FM6*, gift from S. Noselli; UASBsk^{RNAi} and UASDilp2^{RNAi}, from Vienna *Drosophila* Research Center; UASHep^{act} and UASBsk^{DN}, gifts from M. Mlodzik; UASDilp2, gift from E. Hafen; and *dilp2Gal4*, gift from E.J. Rulifson.

dilp2GS: The *dilp2* promoter (1500 bp amplified with 5'-TTC CGT GCG GCC GCG GCC ATG GCG ATG GCG ATG A-3' and 5'-TTC CGT GCG GCC GCC TTT ACG ATC AAA TGG ATT A-3') was cloned into the NotI site of pP{UAS-Geneswitch} (Osterwalder *et al.* 2001). P element-mediated transformation was performed by Genetic Services, Inc.

For paraquat treatments, flies (4-6d old, reared on 12 hr light:dark cycle) were starved for 6 hr and then transferred to vials containing filter paper soaked in 5% sucrose solution with or without 25 mM paraquat (Methyl Viologen, Sigma). Survival was assessed after 24-28hr. Driver and UAS lines were kept in *w¹¹¹⁸* genetic background.

RU486-induction was performed by feeding flies with 500 µg/ml RU486 in 5% sucrose on filter paper for 1.5 hr. Filter paper was then exchanged for filter paper soaked with 500 µg/ml RU486 and 25 mM paraquat in 5% sucrose. Survival was assessed after 28 hr. Compared genotypes were always treated in parallel.

To assess stress-induced growth response, 30 females and 10 males for each cross were placed in bottles and allowed to mate for 3 days. Next, an equal amount of females from each cross were placed into two vials, and allowed to lay eggs for 16hr at 25°C. The flies were removed from the vials after 16hr, and each vial was kept at 25°C for an additional 24hr. After 24hr, one vial from each cross was transferred to 29°C, the other left at 25°C. Wings (10 per genotype) were collected from male flies (5-7d) and wing area, cell size, and cell number were quantified using Adobe Photoshop.

qRT-PCR analysis

Expression of genes was measured using qRT-PCR. Male, whole flies (0-3d) were processed in Trizol (Invitrogen) to generate total RNA. In experiments using paraquat, flies were processed 16 hrs after starting treatment, ensuring that flies had fed on the provided sucrose/Paraquat solution and allowing time for transcriptional responses to fully engage. One µg of RNA was reverse transcribed using the SuperScript II reverse transcriptase (Invitrogen). Primer sequences are listed in supplementary material. Standard qRT-PCR reaction using SYBR Green was performed on a Bio-Rad iCYCLER iQ. Data is presented as expression relative to *actin5C*.

Immunohistochemistry

Flies were dissected in fixative (4% Paraformaldehyde in PBS), and fixation was continued for 15 min at room temperature (RT). After washing in PBS/0.1 % Triton, tissues were blocked in BBTr (PBS/0.1% Triton/0.1% BSA) for 1 hr at RT. Primary antibody incubations were performed in BBTr overnight at 4°C (using rabbit anti-Foxo N-term (Puig *et al.* 2003), at 1:200 dilution). The tissue was then washed in PBTr (2 × 10' at RT), and incubated for 2hrs at RT with TRITC-labeled secondary antibody and DAPI. Tissues were then mounted in mounting medium and inspected by confocal microscopy (Leica SP5 confocal microscope).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute on Aging (RO1 AG028127 to HJ) and the National Institute of Diabetes and Digestive and Kidney diseases (F32 DK083862 to JK).

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Figure 1. Reduction of *dilp* transcription after paraquat treatment is dependent on JNK activity in IPCs

dilp2 and *dilp5*, but not *dilp3*, transcript levels are significantly reduced in control flies (*dilp2G4>+*) 16hrs after paraquat treatment. The repression of *dilp2*, but not *dilp5*, after paraquat treatment can be inhibited by impairing JNK activity in IPCs (*dilp2G4>Bsk^{DN}*). Data from qRT-PCR is presented as relative expression normalized to *actin5C*. Adult heads were used in the experiments, and each bar represents the average of at least three independent experiments $\pm$ SD. P values are from Student's Ttest.

Figure 2. JNK is required in IPC's for stress-induced growth repression

A-E, Growth defects induced by shifting animals to higher temperature (25°C to 29°C) during development are reduced in *hep¹* mutant males as compared to OreR controls. **A,** Wing images showing changes in wing size after shifting temperatures from 25°C to 29°C in OreR controls and *hep¹* mutants. **B,** Histograms showing a reduction of wing area after shifting temperatures in *hep¹* mutants as compared to OreR controls. Each bar represents the average of at least 10 wings, measured using Adobe Photoshop. **C,** Histogram showing the change in wing area between flies reared at 25°C and flies shifted to 29°C. OreR (wild-type) controls show significantly larger changes than *hep¹* hemizygotes. Each bar represents the average of 8-10 ratios $\pm$ SD, wing area was measured using Adobe Photoshop. **D,** Histograms showing a significant reduction in the number of cells per wing in wild-type, but not *hep¹* hemizygous flies, and no significant change in wing cell size in either genotype after the temperature shift. Each bar represents the average of cells counted in 15 defined fields (cells per wing) or 15 cells (cell size) $\pm$ SD, all measurements done in Adobe Photoshop. **E,** Histogram showing a significant reduction in the weight of wild-type, but not *hep¹* hemizygous males after temperature shift. **F-G,** Impairing JNK activity in IPC's specifically can inhibit stress-induced growth repression. **F,** Wing images showing changes in wing size after shifting temperatures from 25°C to 29°C. **G,** Histogram showing reduced changes in wing area between flies reared at 25°C and flies reared at 29°C after inhibiting JNK activity in IPC's (*dilp2G4>Bsk^{DN}* and *dilp2G4>Bskⁱ* (*BskRNAi*)) or over-expressing Dilp2 (*dilp2G4>UAS Dilp2*) as compared to controls (*dilp2G4>+*). Over-expressing Dilp2^{RNAi} in a *dilp2G4>Bsk^{DN}* background (*dilp2G4>Dilp2^{RNAi}, Bsk^{DN}*) can rescue the change in wing area. Each bar represents the average of 8-10 ratios $\pm$ SD (only 5 ratios were used to determine *dilp2G4>UAS-Dilp2* average); wing area was measured using Adobe Photoshop. See also Figure S1 for controls.

Figure 3. JNK signaling in IPCs is required for stress tolerance in adults

A, Repression of JNK activity in IPCs ($dilp2G4>Bsk^{DN}$ and $dilp2G4>Bsk^i$ (BskRNAi)) results in decreased survival 28 hrs after paraquat (25mM) treatment as compared to sibling controls ($UAS-Bsk^{DN}/+$ and $UAS-Bsk^i$). To ensure identical genetic backgrounds, all lines were kept in the w^{1118} background. Outcrossed w^{1118} ; $dilp2Gal4/+$ virgins were crossed to the individual UAS lines, and sibling progeny was compared. Each bar represents the average of at least 5 cohorts (~20 male flies per cohort) $\pm$ SD from one experiment, multiple independent experiments were performed with similar results. Total populations are as follows; $+/+$ (139), $dilp2G4>+$ (136), $Bsk^{DN}/+$ (114), $dilp2G4>Bsk^{DN}$ (153), $Bsk^i/+$ (116), $dilp2G4>Bsk^i$ (155). 20 male flies were treated with sucrose alone as a survival control. Decreased survival was also found in females expressing Bsk^{DN} or Bsk^i in IPCs (not shown). **B**, Reduced stress tolerance in flies expressing Bsk^{DN} in IPCs using an RU486-inducible system ($dilp2GS$). Histograms compare survival (28 hrs after 25mM paraquat) of genetically identical siblings treated with or without RU486. Each bar represents the average of at least 4 cohorts (~20 male flies per cohort) $\pm$ SD from one experiment, multiple experiments were performed. Total populations are as follows; $dilp2GS>+$ (- RU486) (81), $dilp2GS>+$ (+ RU486; 200 μ M) (80), $dilp2GS>Bsk^{DN}$ (- RU486) (133), $dilp2GS>Bsk^{DN}$ (+ RU486; 200 μ M) (135), $dilp2GS>Bsk^i$ (- RU486) (144), $dilp2GS>Bsk^i$ (+ RU486; 200 μ M) (156). **C**, Characterization of the $dilp2GS$ driver. Induction of GFP in larval brains ($dilp2GS>UAS-nlsGFP$) in response to feeding RU486 (200 μ M). Similar induction was observed in adult brains (not shown). No GFP was observed in other parts of the larval or adult body (not shown). **D**, The decreased survival in $dilp2G4>Bsk^{DN}$ flies can be rescued by over-expressing $Dilp2^{RNAi}$ ($dilp2G4>Dilp2^i; Bsk^{DN}$). Survival was measured after 24 hrs treatment with 25 mM paraquat. Each bar represents the average of at least 4 cohorts (~20 male flies per cohort) $\pm$ SD from one experiment, multiple independent experiments were performed. Total populations are as follows; $dilp2G4/+$ (75), $dilp2G4>Bsk^{DN}$ (71), $dilp2G4>Dilp2^{RNAi}; Bsk^{DN}$ (81), $dilp2G4>Dilp2^{RNAi}$ (65). **E**, Constitutive expression of

Dilp2 results in increased stress sensitivity. Population sizes: dilp2G4/+ (117), dilp2G4>Dilp2 (138).

Figure 4. JNK signaling in IPCs is required for transcriptional stress response in the periphery
A, Stress response and Foxo target genes *gstD1*, *thor* and *dLip4* are significantly upregulated 16hrs after 25 mM paraquat treatment (*dilp2G4>+*). Inhibiting JNK activity in IPCs (*dilp2G4> Bsk^{DN}*) represses this induction. Whole bodies were used in the experiments, and each bar represents the average of at least three experiments $\pm$ SD.

B, Foxo translocates to the nucleus of adult fatbody cells in response to Paraquat exposure in wild-type flies, but not in flies constitutively expressing *Dilp2*. 5 day-old flies were exposed to 25mM Paraquat for 16hrs, and immunostained using antibodies against *Drosophila* Foxo. Confocal sections are shown here: Foxo in red (left panels) or white (right panels, only Foxo channel is shown); DAPI in blue.

C, Model for the systemic control of stress responses by JNK signaling activity in IPCs. Repression of *dilp2* transcription by JNK signaling in IPCs is required for proper adaptive responses to environmental challenges, decreasing peripheral IIS activity and promoting expression of genes that influence growth and stress tolerance. IIS can also be repressed directly by JNK in peripheral tissues (Wang et al., 2005), suggesting that local and systemic regulation of IIS by JNK is coordinated to optimize the organism's stress response.