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Report

Retinoids Regulate a Developmental Checkpoint for Tissue Regeneration in *Drosophila*

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Summary

Damage to *Drosophila* imaginal discs elicits a robust regenerative response from the surviving tissue [1–4]. However, as in other organisms, developmental progression and differentiation can restrict the regenerative capacity of *Drosophila* tissues. Experiments in *Drosophila* and other holometabolous insects have demonstrated that either damage to imaginal tissues [5, 6] or transplantation of a damaged imaginal disc [7, 8] delays the onset of metamorphosis. Therefore, in *Drosophila* there appears to be a mechanism that senses tissue damage and extends the larval phase to coordinate tissue regeneration with the overall developmental program of the organism. However, how such a pathway functions remains unknown. Here we demonstrate that a developmental checkpoint extends larval growth after imaginal disc damage by inhibiting the transcription of the gene encoding PTTH, a neuropeptide that promotes the release of the steroid hormone ecdysone. Using a genetic screen, we identify a previously unsuspected role for retinoid biosynthesis in regulating PTTH expression and delaying development in response to tissue damage. Retinoid signaling plays an important but poorly defined role in several vertebrate regeneration models [9–11]. Our findings demonstrate that retinoid biosynthesis in *Drosophila* is important for the maintenance of a condition that is permissive for regenerative growth.

Results and Discussion

A Developmental Checkpoint Is Activated by Imaginal Disc Damage

Damage to *Drosophila* larval tissues produces an extended larval phase [5, 6, 12]. However, it is not known whether this delay in development occurs immediately after damage or later, at a defined developmental checkpoint prior to pupariation. We irradiated third-instar *Drosophila* larvae at 92 hr after egg deposition (AED) with 25 Gy or 40 Gy X-ray irradiation and found that the median length of the larval phase was extended by approximately 23 and 49 hr, respectively (Figure 1A). When larvae were X-irradiated (40 Gy) during either the first (48 hr AED) or second (60 hr AED) instar, they still exhibited delayed pupariation, but the earlier molting transition, from second to third larval instar, proceeded without delay (Figure 1B). Therefore, irradiation of larvae even during the first or second instar delays development only during the third-instar stage, revealing a mechanism that specifically delays entry into pupariation after tissue damage.

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Drosophila larvae achieve a critical size during the third instar, after which neither starvation [13, 14] nor inhibition of tissue growth [15] affects the timing of pupariation. We tested whether, similarly, there is a point in larval development after which tissue damage no longer delays development. Irradiation of larvae at 104 hr AED produces a robust developmental delay, whereas larvae irradiated at 116 hr AED or later pupariate with little delay (Figure 1C). Thus, there is a point late in larval development, between 104 and 116 hr AED, where larvae commit to a timely developmental schedule for pupariation. This is consistent with earlier work that utilized a temperature-sensitive cell-lethal mutation to produce tissue damage [6]. Together, these results demonstrate that a checkpoint-like mechanism operates in the third larval instar to extend the larval phase of development after tissue damage.

Although X-irradiation has the potential to produce widespread tissue damage, very little apoptosis was observed in many larval tissues of irradiated animals including the gut, brain, lymph gland, ring gland, salivary glands, and fat bodies (see Figure S1A available online). In contrast, within 4 hr after irradiation, the larval imaginal discs exhibited elevated caspase activation and cellular apoptosis (Figure S1A). Consistent with earlier observations [12], irradiation of larvae during the first instar had an effect on brain growth (Figures S1B–S1F), but not irradiation after the first larval instar (Figure S1C), which still resulted in delayed pupariation.

Because X-ray-induced apoptosis is restricted predominantly to imaginal discs, we tested whether damage directed primarily to imaginal discs could activate the developmental checkpoint. The *Beadex-GAL4* driver, which expresses primarily in the wing and haltere imaginal discs (Figure S1G), was used to express the proapoptotic gene *reaper* (*Bx* > *rpr*) (Figures S1K and S1L). Because *Beadex-GAL4* is expressed throughout the third instar of larval development, adult *Bx* > *rpr* flies have no wings (Figure 1D, inset) but appear otherwise normal. However, *Bx* > *rpr* larvae exhibit a delay in pupariation that is only slightly shorter than that obtained with 40 Gy X-irradiation (Figure 1D). Although *Beadex-GAL4* is expressed at low levels in the CNS (Figure S1H), it is unlikely that this expression contributes to the developmental delay in *Bx* > *rpr* larvae (Figures S1G–S1R). Indeed, we also observed a similar delay when *reaper* expression was directed to the wing disc with either *Serrate-GAL4* or *rotund-GAL4* (Figures S1Q and S1R). Therefore, damage directed primarily to imaginal discs can elicit a developmental delay.

Persistent expression of *reaper* in the wing disc via the *Bx-GAL4* driver produces continuing damage. Yet pupariation eventually occurs (Figure 1D), indicating that the mechanism that delays development can eventually be overcome, similar to cell-cycle checkpoint adaptation in yeast [16].

Checkpoint-Induced Delay Extends the Period of Regenerative Competence

We examined adult flies developing from larvae irradiated either before the developmental checkpoint (which delays their development) or after the developmental checkpoint (which does not delay development). Larvae irradiated before the developmental checkpoint, at 92 hr AED, produced adults

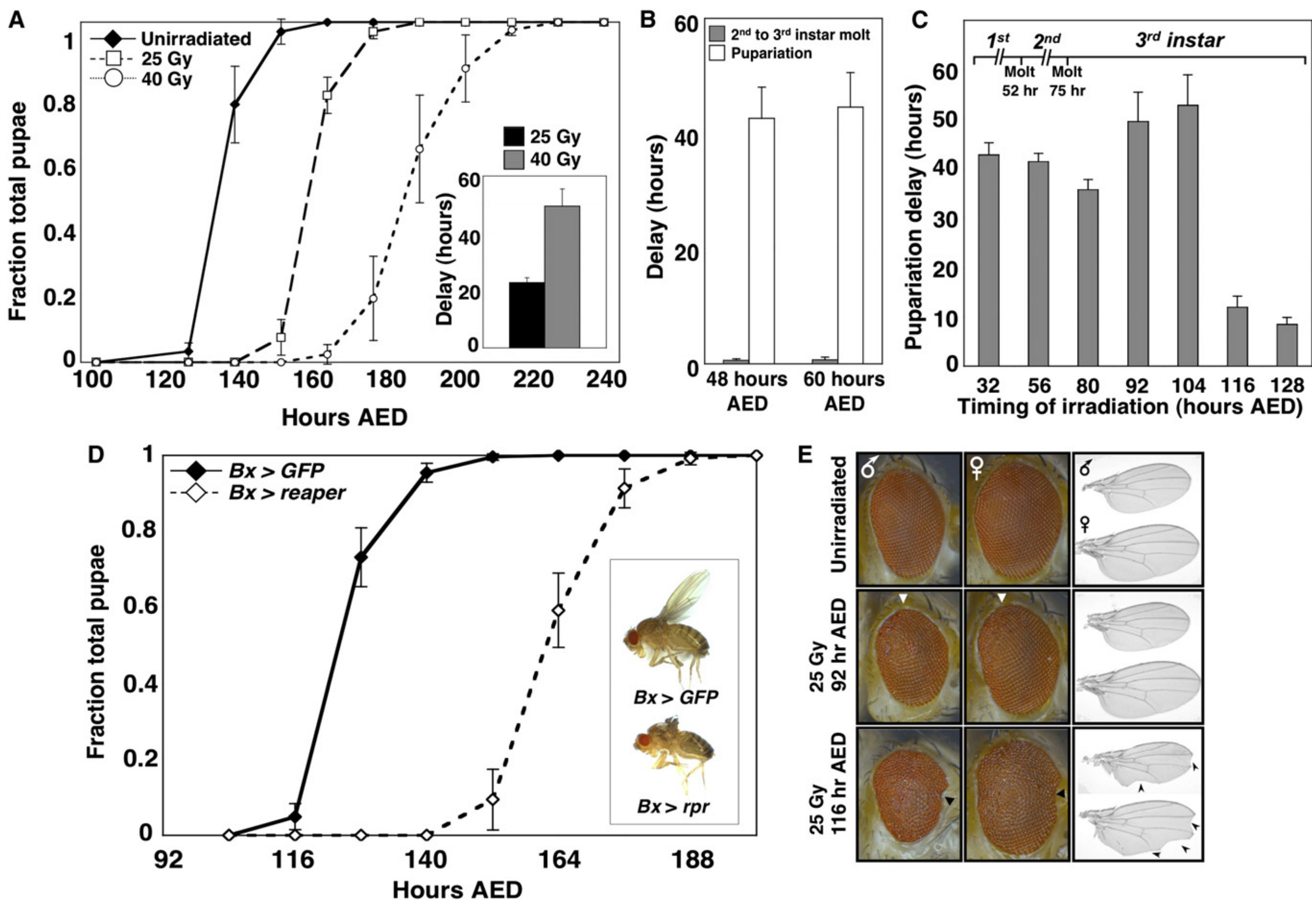

Figure 1. Imaginal Tissue Damage Activates a Developmental Checkpoint during the Third Larval Instar of *Drosophila* Development

(A) Timing of pupariation for unirradiated larvae or larvae X-irradiated at 92 hr after egg deposition (AED) with doses of either 25 Gy or 40 Gy. Developmental timing is represented as both the fraction of total larvae pupariated and in the inset graph as the difference between the median timing to pupariation of irradiated and unirradiated populations of larvae (delay). n = 3 independent populations were assayed for each dosage.

(B) Duration of delay for both second-to-third-instar molting (gray bars) and pupariation (white bars) among larvae irradiated with 40 Gy at either 48 hr AED, during the first larval instar, or at 60 hr AED, during the second larval instar. n = 3 independent unirradiated and irradiated populations were assayed for each data point.

(C) Pupariation delay for larvae irradiated with 40 Gy at progressively later times throughout larval development. n = 3 independent unirradiated and irradiated populations were assayed for each data point.

(D) Timing of pupariation for *Bx > rpr* and *Bx > GFP* larvae. *Bx > rpr* larvae exhibit a substantial delay in pupariation timing. Inset: adult flies expressing either *UAS-GFP* or *UAS-reaper* under the control of *Bx-GAL4*. Eclosed *Bx > rpr* flies have ablated wings but exhibit no other developmental or obvious behavioral abnormalities and are fertile. n = 3 independent populations were assayed for each genotype.

(E) Eyes (shown with posterior to the left) and wings (proximal to the left) from male and female adults irradiated as larvae either before (92 hr AED) or after (116 hr AED) the developmental checkpoint. The boundary of posterior ommatidial disorganization is indicated by white triangles. Anterior notching of eyes is marked indicated by black triangles. Wing notching is indicated by arrowheads. See also Figure S1.

Error bars represent standard error (SE).

that had only occasional tissue disorganization evident in the posterior part of the adult eye (Figure 1D). In contrast, all adults arising from larvae irradiated after the developmental checkpoint, at 116 hr AED or later, exhibited a reduction in tissue size as well as patterning defects in both the eyes and wings (Figure 1E). Thus, checkpoint-induced delay likely facilitates the efficient regeneration of imaginal discs by extending the period of regenerative competence during larval development.

Tissue Damage Inhibits the Endocrine Signals that Both Promote Developmental Progression and Restrict Regenerative Capacity

The primary signal that promotes developmental progression from the larval to the pupal stage is the rapid increase in levels of the steroid hormone ecdysone. Ecdysone is synthesized by an endocrine organ, the larval ring gland, and is converted by larval tissues to the active metabolite 20-hydroxyecdysone (20E; summarized in Figure S2A). In larvae, the level of circulating 20E can be manipulated by feeding 20E [17]. To examine whether irradiation-induced delays could be suppressed by feeding 20E, we irradiated larvae at 92 hr AED. Twelve hours later, irradiated and unirradiated larvae were transferred to food that was supplemented with 0.3, 0.5, or 1.0 mg/ml 20E in ethanol or an equivalent volume of ethanol as a control. Feeding 20E shortened the duration of the delay in pupariation in irradiated larvae (Figure 2A), demonstrating that 20E levels are rate limiting for developmental progression in irradiated animals and that bypassing the regulation of ecdysone synthesis via ectopic feeding of 20E can overcome checkpoint-induced delay.

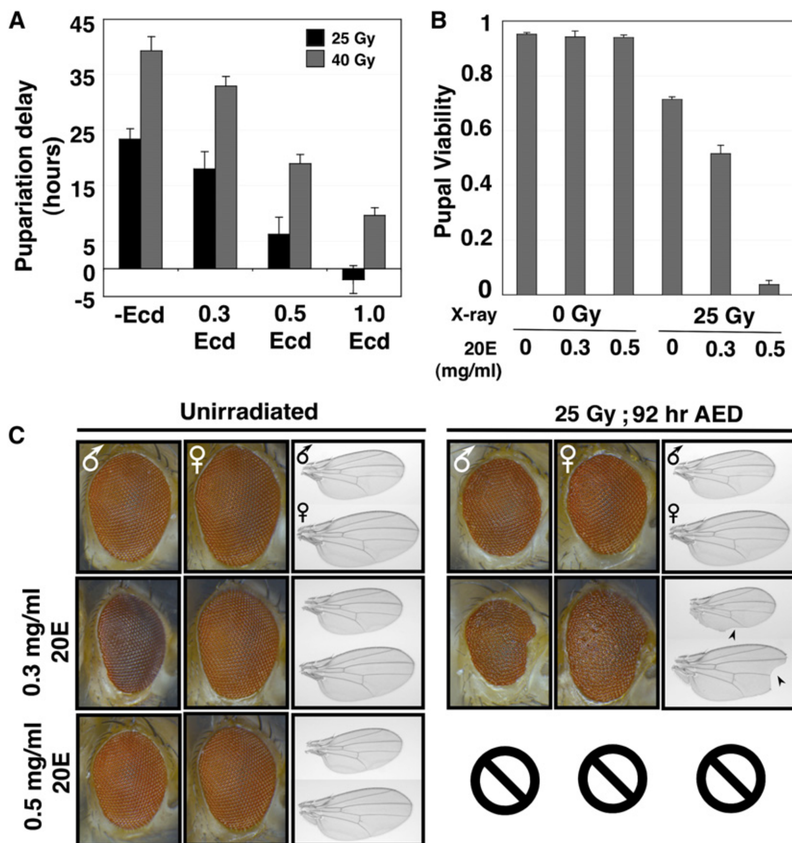

Figure 2. Tissue Regeneration and Pupal Viability in X-Irradiated Larvae Is Compromised by 20E Addition

(A) Developmental delay of larvae exposed to 25 Gy or 40 Gy X-irradiation and fed 0.3, 0.5, or 1.0 mg/ml 20-hydroxyecdysone (20E) dissolved in 100% ethanol, or an equivalent volume of ethanol as a control. n = 3 independent unirradiated and irradiated populations were assayed for each data point.

(B) Pupal viability (as determined by the initiation of adult eclosion from the pupal case) of either unirradiated larvae or larvae irradiated at 92 hr AED that were fed either different concentrations of 20E (0.3 mg/ml and 0.5 mg/ml) in *Drosophila* molasses-cornmeal medium or an equivalent volume of ethanol in molasses-cornmeal medium as a negative control. The viability for n = 3 independent populations was assayed for each condition.

(C) Eyes and wings from male and female adult flies irradiated as larvae at 92 hr AED and then fed 0.3, 0.5, or 1.0 mg/ml 20E or an equivalent volume of ethanol in their normal food. Wing notching is indicated by arrowheads. Two larvae irradiated and fed 0.5 mg/ml 20E eclosed (Figure 2B); however, one adult died before completing eclosion, and the second adult died immediately in the food. Therefore, we were unable to effectively assess the wing and eye phenotypes in these animals.

Error bars represent SE.

To determine whether increasing 20E levels would also restrict the capacity of larvae to regenerate tissues damaged by X-irradiation, we then transferred larvae irradiated at 92 hr AED to food supplemented with 20E. These larvae exhibited a dosage-dependent loss of pupal viability, whereas either irradiation or 20E treatment alone produced little to no decrease in pupal viability (Figure 2B). In addition, 20E-fed larvae exhibited evidence of incomplete repair of irradiation-induced damage in both eyes and wings (Figure 2C).

An early event in the neuroendocrine pathway that regulates ecdysone production at pupariation is an increase in the transcription of the gene encoding the neuropeptide PTTH, in a pair of neurons within the midbrain region of each larval brain lobe (Figure S2A). The presence of these neurons and the appropriate transcriptional regulation of the *ptth* gene are both necessary for the proper timing of the larval-to-pupal transition [18]. The PTTH-expressing neurons innervate the ring gland [18]. PTTH stimulates the ring gland to produce ecdysone, promoting the larval-to-pupal transition [19].

Larvae carrying both a *ptth-GAL4* transgene and a *UAS-GFP* transgene (*ptth* > *GFP*) were examined to determine the levels of GFP expression in the PTTH-expressing neurons of the larval brain [18] (Figure 3A). *ptth* > *GFP* larvae were irradiated at 92 hr AED, and the larval brains were examined 36 hr after irradiation (128 hr AED). In irradiated larvae, there was significantly less GFP fluorescence in the PTTH-expressing neurons than was observed in unirradiated controls (Figure 3B; Figures S2B and S2C). By 60 hr after irradiation (156 hr AED), irradiated larvae had begun wandering, and a restoration of *ptth* expression was observed (Figure S2B). In unirradiated third-instar larvae, *ptth* RNA levels remain low until approximately 12 hr before the median pupariation time, when they increase dramatically. In contrast, larvae exposed to 40 Gy X-irradiation at 92 hr AED exhibited a delay in the upregulation of *ptth* gene expression consistent with their delayed pupariation (Figure 3C). Furthermore, *Bx* > *rpr* larvae exhibited a delayed induction of *ptth* in comparison to *Bx* > *GFP* control larvae, consistent with their delayed developmental timing (Figure 3D), demonstrating that the imaginal tissue-targeted damage is sufficient to delay the upregulation of *ptth* expression prior to pupariation.

To determine whether ectopic *ptth* expression can overcome delays induced by X-irradiation, we examined the effect of constitutive expression of a *UAS-ptth* transgene via either *daughterless-GAL4* or *tubulin-GAL4*, which would express *ptth* throughout the larva, including within the PTTH-expressing neurons. In unirradiated larvae, *da* > *ptth* expression did not effect developmental timing as compared with control *da* > *GFP*-expressing larvae (Figure S2D). In contrast, *tub* > *ptth* larvae pupariated earlier than *tub* > *GFP* larvae (Figure S2E). However, both *da* > *ptth* and *tub* > *ptth* larvae exhibited a significantly reduced delay in pupariation in response to irradiation (Figure 3E), indicating that maintaining low levels of *ptth* RNA is necessary for delaying pupariation upon activation of the developmental checkpoint. Taken together with the delay in *ptth* expression following irradiation, these results indicate that activation of the developmental checkpoint delays pupariation at least in part via the inhibition of *ptth* expression.

Retinoid Activity Regulates Checkpoint-Induced Delay

We screened a collection of stocks with chromosomal deletions that together cover at least 65% of the euchromatic portion of chromosomes 2 and 3 to identify heterozygous deletions that reduce the delay in pupariation after irradiation (Figure S3A). Two deletions, *Df(3R)urd* and *Df(3R)ED5623*, that remove an overlapping genomic region each scored positive in the screen. Among the genes in this region is *ninaB*, which encodes the β-carotene 15,15′-monooxygenase (BCO).

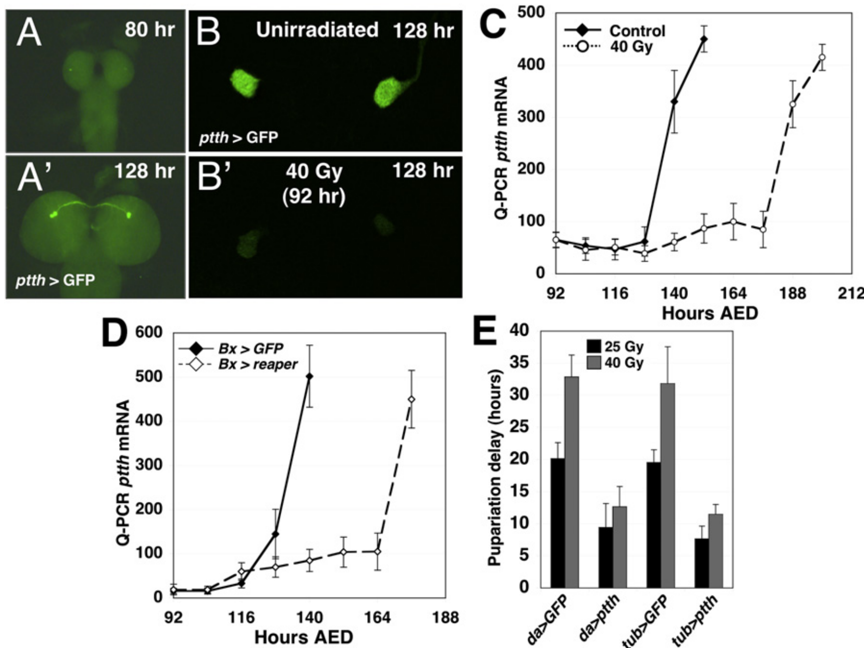

Figure 3. Irradiation-Induced Tissue Damage Inhibits *ptth* Expression and Endocrine Signaling

(A and A′) Larvae carrying *ptth-GAL4* and *UAS-GFP* transgenes can be used to visualize *ptth* gene activity in PTTH-expressing neurons in the larval brain. The previously described [18] upregulation of *ptth* gene activity during developmental progression can be visualized as an increase in GFP fluorescence in the neuron cell bodies (arrowheads) of the PTTH-expressing neurons. (A) *ptth* > *GFP* expression in a larval brain fixed at 80 hr AED is shown; (A′) *ptth* > *GFP* expression at 128 hr AED is shown.

(B and B′) *ptth* > *GFP* expression at 128 hr AED in the brain of an unirradiated larvae (B) or a larva that was X-irradiated at 92 hr AED with 40 Gy (B′).

(C) Measurement of *ptth* transcript levels in unirradiated and X-irradiated (40 Gy) larvae. Larvae were collected every 12 hr after irradiation until the animals had entered the pupal phase of development. *ptth* gene transcript levels were assayed via quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) with probes targeting the *ptth* transcript.

(D) *ptth* transcriptional activity in *Bx* > *rpr* and *Bx* > *GFP* larvae. *Bx* > *rpr* larvae exhibit a delayed upregulation of *ptth* transcript that is consistent with their delayed developmental timing. *ptth* transcript was detected via quantitative RT-PCR.

(E) Ectopic expression of *ptth* attenuates irradiation-induced developmental delays. Larvae carrying either the *daughterless-GAL4* or the *tubulin-GAL4* expression transgenes were crossed to lines carrying either *UAS-GFP* or *UAS-ptth*. In the progeny of these crosses, we assayed the effects of *da* > *ptth* and *tub* > *ptth* expression on the pupariation delay of larvae irradiated at 92 hr AED. Larvae expressing *da* > *GFP* or *tub* > *GFP* were assayed as controls for developmental delay. n = 4 independent unirradiated and irradiated populations were assayed for each data point. See also Figure S2.
Error bars represent SE.

Larvae that are either heterozygous (Table S1) or homozygous (Figure 4A) for mutations that specifically disrupt the coding sequence of *ninaB* gene have a significantly attenuated delay after irradiation at 92 hr. *ninaB* mutations also attenuate delay after irradiation early in larval development (Figure S3B). Because *Drosophila* BCO has been shown to be important for the initial step of metabolism of carotenoids, such as β-carotene, to produce retinoids [20], this finding implicates retinoids in a pathway that links tissue damage to a delay in pupariation.

To date, retinoids have been shown in *Drosophila* to be important only for vision [21] and not in any other aspect of *Drosophila* development; *ninaB* homozygotes are viable and fertile. In contrast, in vertebrates, retinoids regulate development [22] and promote tissue regeneration within individual tissues [10]. Our studies therefore reveal a novel role for retinoid signaling in coordinating tissue regeneration and developmental progression in *Drosophila*.

Mutations disrupting other components of the retinoid biosynthesis pathway (Figure 4B) also reduce the delay in pupariation after irradiation. The scavenger receptor Santa maria, necessary for the transport of carotenoids into cells, acts in concert with BCO for retinoid biosynthesis [23]. Irradiation of homozygous *santa maria*^{1} larvae either in the third instar (Figure 4A; Table S1) or early in larval development (Figure S3B) produces a reduced delay. Cleavage of β-carotene by BCO yields two molecules of retinaldehyde, which can then be converted into vitamin A (retinol). In vertebrates, vitamin A stored within cells can be mobilized through the activity of type III alcohol dehydrogenases, which convert vitamin A to retinaldehyde (retinal). The *Drosophila* protein most similar to mammalian type III alcohol dehydrogenase is encoded by the *Formaldehyde dehydrogenase* (*Fdh*) gene (Figure 4B) [24, 25]. A deletion identified in our screen, *Df(3R)M-kx1*, removes the *Fdh* gene (Table S1). Moreover, we examined two independently derived *P* element insertions within the *Fdh* gene (*Fdh*^{DG29306} and *Fdh*^{BG00983}). When homozygous, each mutation produces a diminished delay in response to irradiation (Figure 4A; Figure S3B; Table S1). In vertebrates, conversion of retinaldehyde into retinoic acid is dependent upon the function of retinaldehyde dehydrogenase. The product of the *Drosophila Aldh* gene, which has been demonstrated to encode an aldehyde dehydrogenase [26], is most similar to the vertebrate retinaldehyde dehydrogenase proteins. Larvae homozygous for a transcriptional suppressor *P* element [27] inserted at the 5′ end of the *Aldh* coding sequence (*Aldh*^{KG02748}) had an attenuated delay after larval irradiation (Figure 4A; Figure S3B; Table S1). Thus, either retinoic acid or a downstream metabolite likely functions in the damage-induced developmental delay.

Both irradiated *Fdh*^{DG29306} and *santa maria*^{1} larvae upregulated *ptth* gene expression earlier than irradiated wild-type larvae (Figure 4C), suggesting that the retinoid biosynthesis pathway plays an important role in the inhibition of *ptth* expression following irradiation. However, the delay in *ptth* upregulation following irradiation in *santa maria*^{1} and *Fdh*^{DG29306} mutants was only partially reduced in comparison to unirradiated control larvae, indicating that other mechanisms also operate to delay pupariation after tissue damage.

To address the possibility that the retinoid biosynthesis genes might be important for the metabolism of a nonretinoid substrate necessary for checkpoint-induced delay, we examined whether the availability of a carotenoid substrate for retinoid biosynthesis affects the checkpoint-induced delay. Flies reared on Sang's defined *Drosophila* medium, which lacks any carotenoid substrates for retinoid biosynthesis [28], have not

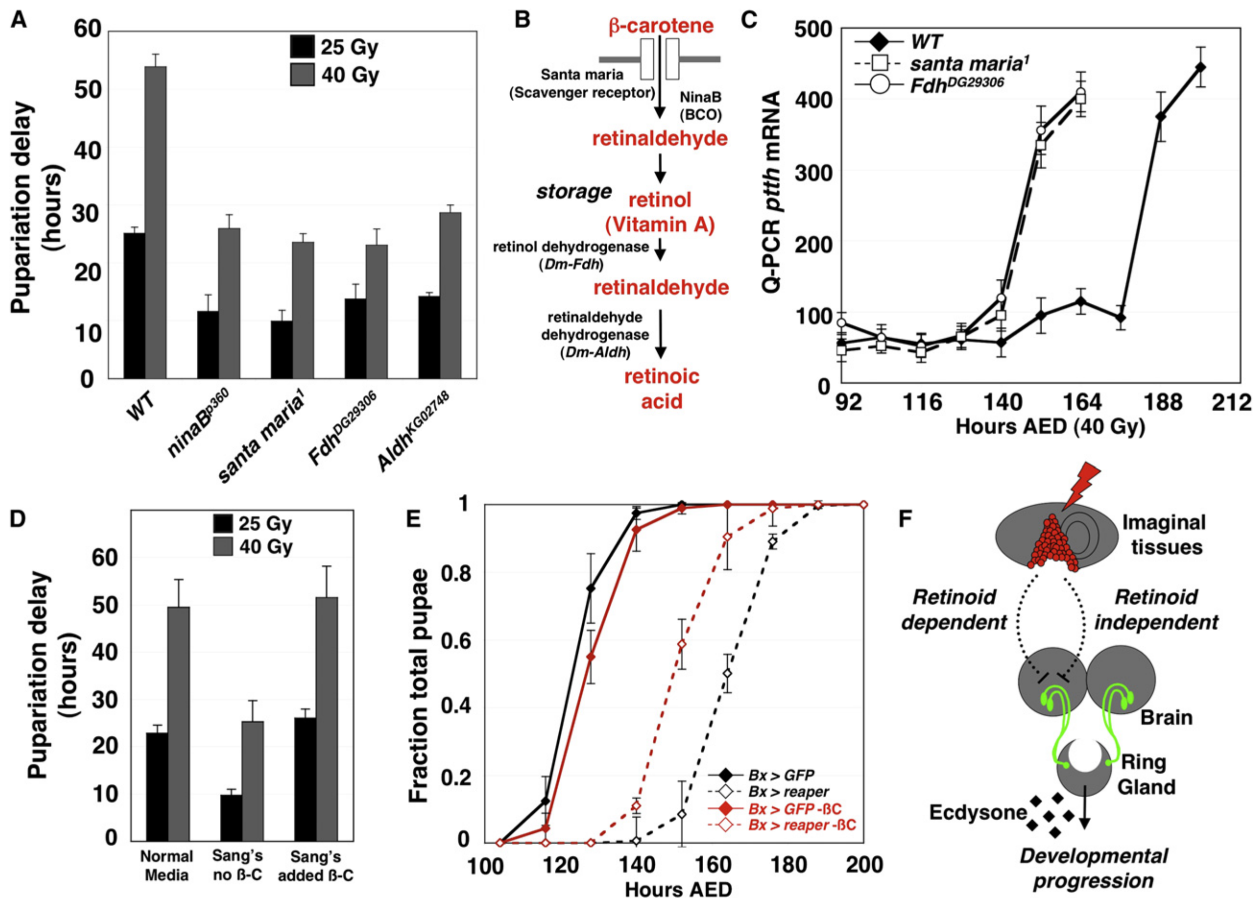

Figure 4. Retinoid Metabolism Regulates Checkpoint-Induced Delay in Irradiated Larvae

(A) Pupariation delay in wild-type larvae or homozygous mutant larvae carrying mutations in genes required for retinoid metabolism. n = 3 independent populations were assayed for all timing experiments with all mutants unless otherwise noted. $p < 0.01$ for each pairwise comparison of wild-type and mutant pupariation delay for all four mutants irradiated at 25 Gy and 40 Gy, as calculated by two-tailed Student's t test.

(B) Schematic illustrating retinoid metabolic pathways and the gene products required for each step of retinoid metabolism. The genes responsible for retinol dehydrogenase and retinaldehyde dehydrogenase activities in *Drosophila* have not been identified. Sequence comparisons suggest that the *Drosophila* gene most similar to vertebrate retinol dehydrogenase is the *Drosophila Formaldehyde dehydrogenase* (*Fdh*) gene; the *Drosophila* gene most similar to vertebrate retinaldehyde dehydrogenase is the *Aldehyde dehydrogenase* (*Aldh*) gene of *Drosophila*.

(C) *ptth* transcript levels in X-irradiated (40 Gy) wild-type, $Fdh^{DG29306}$, and *santa maria*^{1} larvae. *ptth* transcript was detected via quantitative RT-PCR.

(D) Pupariation delay in irradiated wild-type larvae raised on either normal molasses-cornmeal medium or Sang's defined medium either missing (−A) or supplemented with (+A) β-carotene. $p < 0.01$ in a pairwise comparison of delay in normal versus Sang's (−A) medium at both at 25 Gy and 40 Gy . The timing of n = 3 independent populations was assayed for each condition. $p < 0.01$ in a pairwise comparison of delay in Sang's (−A) versus Sang's (+A) medium at both at 25 Gy and 40 Gy; both p values were calculated by two-tailed Student's t test.

(E) Developmental timing of *Bx* > *GFP* and *Bx* > *rpr* larvae raised on carotenoid-deficient Sang's defined medium (red) or Sang's defined medium supplemented with 1.25 g/l β-carotene (black). *Bx* > *rpr* larvae grown on carotenoid-deficient Sang's medium exhibit a shorter larval duration than siblings grown on β-carotene-supplemented medium. The timing of n = 4 independent populations was assayed for each genetic background under each condition.

(F) A model for the developmental checkpoint for tissue repair. Damage to imaginal discs is capable of regulating the expression of the *ptth* gene in the PTTH-expressing neurons via both a retinoid-dependent pathway, defined by the retinoid biosynthesis mutant phenotypes described in this work, and a second, retinoid-independent pathway. See also Figure S3 and Table S1.

Error bars represent SE.

been found to have any developmental abnormalities but exhibit a substantially attenuated delay when compared to controls (Figure 4D). The normal delay could be restored by the addition of β-carotene (Figure 4D), which can be utilized by larvae as a dietary source of retinoids. Similarly, *Bx* > *rpr* larvae exhibited a substantially attenuated delay on carotenoid-deficient medium in comparison to *Bx* > *rpr* larvae raised on carotenoid-supplemented Sang's medium (Figure 4E). Thus, retinoids function to delay pupariation after damage directed primarily to imaginal discs as well. Importantly, in each case depletion of retinoids only partially offsets the delay in pupariation, indicating that retinoid-independent mechanisms also exist. In addition, the relative contribution of the retinoid-mediated mechanism to the developmental delay may vary with specific types of tissue damage and repair (Figure 4F).

Our observations may provide insights into mechanisms that operate in a variety of organisms to delay development in response to tissue damage. For instance, pediatric patients with chronic inflammatory diseases are often observed to experience a delay in the onset of puberty [29–31]. Further analysis of this developmental checkpoint in *Drosophila* may provide a tractable model for understanding how individual tissues communicate the presence of damage to the entire organism.

Supplemental Information

Supplemental Information includes three figures, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2010.01.038.

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References

1. Bryant, P. (1975). Regeneration and duplication in imaginal discs. Ciba Found. Symp. *0*, 71–93.
2. Bryant, P. (1975). Pattern formation in the imaginal wing disc of Drosophila melanogaster: Fate map, regeneration and duplication. J. Exp. Zool. *193*, 49–77.
3. Schubiger, G. (1971). Regeneration, duplication and transdetermination in fragments of the leg disc of Drosophila melanogaster. Dev. Biol. *26*, 277–295.
4. Smith-Bolton, R.K., Worley, M.I., Kanda, H., and Hariharan, I.K. (2009). Regenerative growth in Drosophila imaginal discs is regulated by Wingless and Myc. Dev. Cell *16*, 797–809.
5. Hussey, R., Thompson, W., and Calhoun, E. (1927). The influence of x-rays on the development of drosophila larvae. Science *66*, 65–66.
6. Simpson, P., Berreur, P., and Berreur-Bonnenfant, J. (1980). The initiation of pupariation in Drosophila: Dependence on growth of the imaginal discs. J. Embryol. Exp. Morphol. *57*, 155–165.
7. Dewes, E. (1975). Entwicklungsleistungen Implantierter Ganzer und Halbierter Männlicher Genitalimaginalscheiben von *Ephestia kuehniella* Z. und Entwicklungsdauer der Wirtstiere. Wilhelm Roux Arch. Entwickl. Mech. Org. *178*, 167–183.
8. Rahn, P. (1972). Untersuchungen zur Entwicklung von Ganz-und Teilimplantaten der Flügelimaginalscheibe von *Ephestia kühniella* Z. Wilhelm Roux Arch. Entwickl. Mech. Org. *170*, 48–82.
9. Hind, M., and Maden, M. (2004). Retinoic acid induces alveolar regeneration in the adult mouse lung. Eur. Respir. J. *23*, 20–27.
10. Maden, M., and Hind, M. (2003). Retinoic acid, a regeneration-inducing molecule. Dev. Dyn. *226*, 237–244.
11. Tsonis, P.A., Trombley, M.T., Rowland, T., Chandraratna, R.A., and del Rio-Tsonis, K. (2000). Role of retinoic acid in lens regeneration. Dev. Dyn. *219*, 588–593.
12. Poodry, C., and Woods, D. (1990). Control of the developmental timer for Drosophila pupariation. Rouxs Arch. Dev. Biol. *199*, 219–227.
13. Layalle, S., Arquier, N., and Léopold, P. (2008). The TOR pathway couples nutrition and developmental timing in Drosophila. Dev. Cell *15*, 568–577.
14. Shingleton, A.W., Das, J., Vinicius, L., and Stern, D.L. (2005). The temporal requirements for insulin signaling during development in Drosophila. PLoS Biol. *3*, e289.
15. Stieper, B.C., Kupershtok, M., Driscoll, M.V., and Shingleton, A.W. (2008). Imaginal discs regulate developmental timing in Drosophila melanogaster. Dev. Biol. *321*, 18–26.
16. Sandell, L.L., and Zakian, V.A. (1993). Loss of a yeast telomere: Arrest, recovery, and chromosome loss. Cell *75*, 729–739.
17. Neubueser, D., Warren, J., and Gilbert, L. (2005). Molting defective is required for ecdysone biosynthesis. Dev. Biol. *280*, 362–372.
18. McBrayer, Z., Ono, H., Shimell, M., Parvy, J.-P., Beckstead, R.B., Warren, J.T., Thummel, C.S., Dauphin-Villemant, C., Gilbert, L.I., and O'Connor, M.B. (2007). Prothoracicotropic hormone regulates developmental timing and body size in Drosophila. Dev. Cell *13*, 857–871.
19. Warren, J.T., Yerushalmi, Y., Shimell, M.J., O'Connor, M.B., Restifo, L.L., and Gilbert, L.I. (2006). Discrete pulses of molting hormone, 20-hydroxyecdysone, during late larval development of Drosophila melanogaster: Correlations with changes in gene activity. Dev. Dyn. *235*, 315–326.
20. von Lintig, J., Dreher, A., Kiefer, C., Wernet, M.F., and Vogt, K. (2001). Analysis of the blind Drosophila mutant ninaB identifies the gene encoding the key enzyme for vitamin A formation invivo. Proc. Natl. Acad. Sci. USA *98*, 1130–1135.
21. Montell, C. (1999). Visual transduction in Drosophila. Annu. Rev. Cell Dev. Biol. *15*, 231–268.
22. Duester, G. (2008). Retinoic acid synthesis and signaling during early organogenesis. Cell *134*, 921–931.
23. Wang, T., Jiao, Y., and Montell, C. (2007). Dissection of the pathway required for generation of vitamin A and for Drosophila phototransduction. J. Cell Biol. *177*, 305–316.
24. Ashburner, M. (1998). Speculations on the subject of alcohol dehydrogenase and its properties in Drosophila and other flies. Bioessays *20*, 949–954.
25. Luque, T., Atrian, S., Danielsson, O., Jörnvall, H., and Gonzàlez-Duarte, R. (1994). Structure of the Drosophila melanogaster glutathione-dependent formaldehyde dehydrogenase/octanol dehydrogenase gene (class III alcohol dehydrogenase). Evolutionary pathway of the alcohol dehydrogenase genes. Eur. J. Biochem. *225*, 985–993.
26. Fry, J.D., and Saweikis, M. (2006). Aldehyde dehydrogenase is essential for both adult and larval ethanol resistance in Drosophila melanogaster. Genet. Res. *87*, 87–92.
27. Roseman, R.R., Johnson, E.A., Rodesch, C.K., Bjerke, M., Nagoshi, R.N., and Geyer, P.K. (1995). A P element containing suppressor of hairy-wing binding regions has novel properties for mutagenesis in Drosophila melanogaster. Genetics *141*, 1061–1074.
28. Sang, J. (1956). The quantitative nutritional requirements of Drosophila melanogaster. J. Exp. Biol. *33*, 45–72.
29. Azooz, O.G., Farthing, M.J., Savage, M.O., and Ballinger, A.B. (2001). Delayed puberty and response to testosterone in a rat model of colitis. Am. J. Physiol. Regul. Integr. Comp. Physiol. *281*, R1483–R1491.
30. Ballinger, A.B., Savage, M.O., and Sanderson, I.R. (2003). Delayed puberty associated with inflammatory bowel disease. Pediatr. Res. *53*, 205–210.
31. Simon, D. (2002). Puberty in chronically diseased patients. Horm. Res. *57* (*Suppl 2*), 53–56.