dTrf2 Is Required for Transcriptional and Developmental Responses to Ecdysone During *Drosophila* Metamorphosis

Arash Bashirullah,[†‡] Geanette Lam,[†] Viravuth P. Yin,[°] and Carl S. Thummel[*]

The TATA box-binding protein (TBP) related factor 2 (TRF2) has been well characterized at a biochemical level and in cultured cells. Relatively little, however, is known about how TRF2 functions in specific biological pathways during development. Here, we show that *Drosophila* TRF2 (dTRF2) plays an essential role in responses to the steroid hormone ecdysone during the onset of metamorphosis. Hypomorphic *dTrf2* mutations lead to developmental arrest during prepupal and early pupal stages with defects in major ecdysone-triggered biological responses, including puparium formation, anterior spiracle eversion, gas bubble translocation, adult head eversion, and larval salivary gland cell death. The transcription of key ecdysone-regulated target genes is delayed and reduced in *dTrf2* mutants. *dTrf2* appears to be required for the proper timing and levels of ecdysone-regulated gene expression required for entry into metamorphosis.

Key words: transcription; steroid hormone; metamorphosis; ecdysone

Accepted 30 August 2007

INTRODUCTION

TFIID is a key component of the transcription preinitiation complex, directing RNA polymerase II to core promoter elements and positioning the transcriptional machinery for subsequent gene expression. The TATA box-binding protein (TBP) acts as a scaffold for TFIID formation, mediating multiple protein–protein interactions between TBP-associated factors (TAFs) and recognizing canonical TATA elements within a subset of core promoters (Smale and Kadonaga, 2003). Biochemical studies have shown that alternate TBP-related factors (TRFs) can mediate the formation of distinct TFIID complexes. Thus, for example, TRF1 can recognize an alternate core promoter, and a TRF1:BRF complex is required for RNA polymerase III transcription of tRNA genes (Holmes and Tjian, 2000; Takada et al., 2000). A third member of the TBP family, TRF2, has been discovered in multiple animal species (Rabenstein et al., 1999). Like TBP and TRF1, TRF2 can interact directly with TFIIA and TFIIB, suggesting that it is part of the core transcriptional machinery and may act to target RNA polymerase II to a specific set of promoters (Rabenstein et al., 1999; Teichmann et al., 1999; Chong et al., 2005). *Trf2* is essential for early embryonic development in *C. elegans*, *Xenopus*, and zebrafish (Dantonel et al., 2000; Kaltenbach et al., 2000; Veenstra et al., 2000; Bartfai et al., 2004). More restricted functions for TRF2 have been observed in mouse mutants, which show defects in spermatogenesis (Martianov et al., 2001; Zhang et al., 2001). In cultured cells, TRF2 interacts with the DREF DNA replication factor and directly regulates genes involved in DNA replication and cell proliferation (Hochheimer et al., 2002). A *Drosoph-*

Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah
Grant sponsor: the Howard Hughes Medical Institute; Grant sponsor: NIH.
[†]Drs. Bashirullah and Lam contributed equally to this work.
[‡]Dr. Bashirullah's present address is School of Pharmacy, Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI 53705-2222.
[°]Dr. Yin's present address is Department of Cell Biology, Box 3709, Duke University Medical Center, Durham, NC 27710.
[*]Correspondence to: Carl S. Thummel, Department of Human Genetics, University of Utah School of Medicine, 15 North 2030 East Room 2100, Salt Lake City, UT 84112-5330. E-mail: carl.thummel@genetics.utah.edu.

DOI 10.1002/dvdy.21350
Published online 17 October 2007 in Wiley InterScience (www.interscience.wiley.com).

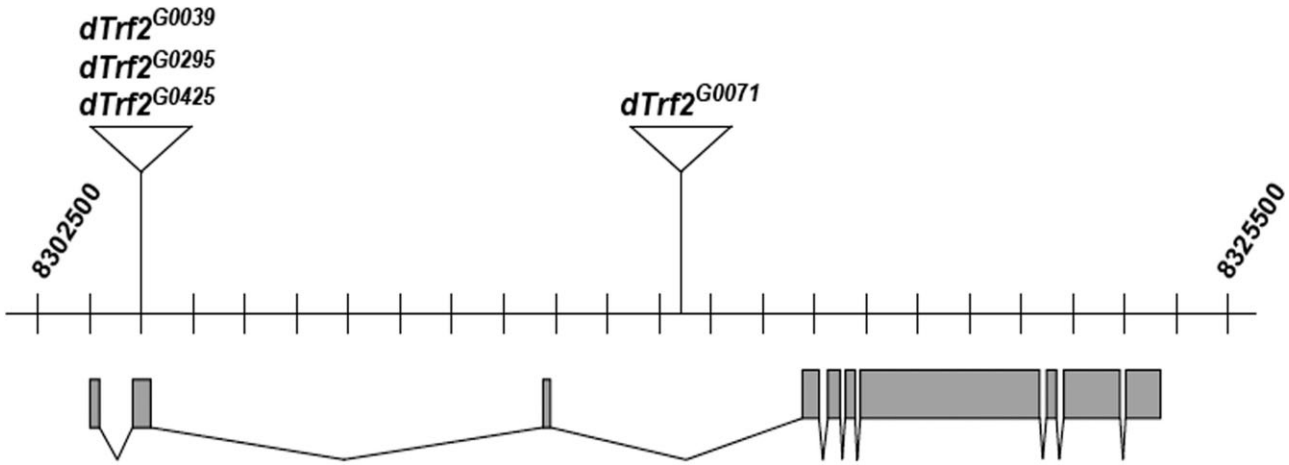

Fig. 1. Map of the *dTrf2* locus. A schematic representation of the 20.7-kb *dTrf2* transcribed region is depicted, extending from genomic sequence reference number 8,303,520 to 8,324,181. Each tick on the map represents 1 kb of genomic sequence. The *dTrf2* gene structure is shown below, from 5′ end (left) to 3′ end (right), with boxes as exons and lines representing introns. The transcript structure is derived from the cDNAs described by Kapytova et al. (2006; GenBank DQ162845) and Rabenstein et al. (1999; GenBank AF136569). The locations of the P-element insertions discussed in the text are marked above the map.

ila ortholog of TRF2, dTRF2, has also been identified (Rabenstein et al., 1999). *dTrf2* encodes two protein products, 75 kDa and 175 kDa, that appear to have redundant functions and that are found in high molecular weight protein complexes, as expected for a TFIID subunit (Kopytova et al., 2006). Weak *dTrf2* mutations are associated with anterior spiracle eversion defects in pupae (Shima et al., 2007) as well as a range of developmental defects in mutant adults, including ectopic bristles, rough eyes, and wing defects (Kopytova et al., 2006).

Here, we describe roles for *dTrf2* in responses to the steroid hormone ecdysone during metamorphosis. Pulses of ecdysone act as critical temporal signals that direct each of the major developmental transitions in the *Drosophila* life cycle, including molting and metamorphosis (Riddiford, 1993). A high titer pulse of ecdysone at the end of the third larval instar acts through the EcR/USP nuclear receptor heterodimer to trigger puparium formation, initiating metamorphosis and the prepupal stage of development (Riddiford et al., 2000). A second ecdysone pulse approximately 10 hr after pupariation triggers adult head eversion and marks pupation, the prepupal-to-pupal transition. Progenitors of specific adult structures respond to each of these ecdysone pulses by undergoing morphogenesis and differentiation, replacing obsolete larval tissues that undergo massive ecdysone-triggered programmed cell death. Detailed functional studies have shown that ecdysone exerts its effects through multiple primary-response transcription factors. These include the zinc finger proteins encoded by the *Broad-Complex* (*BR-C*), the E74 ETS-domain proteins, and E75A nuclear receptor (Burtis et al., 1990; Segraves and Hogness, 1990; DiBello et al., 1991). These transcription factors, in turn, regulate batteries of downstream secondary-response effector genes that direct the appropriate stage- and tissue-specific biological responses to the hormone (Thummel, 1996; Henrich, 2005).

The original description of *dTrf2* reported multiple binding sites for this factor in the giant larval salivary gland polytene chromosomes, including the ecdysone-inducible puffs that correspond to *BR-C*, *E74*, and *E75A* (Rabenstein et al., 1999). This observation raised the interesting possibility that this alternate TBP may play a role in directing transcriptional responses to ecdysone during the onset of metamorphosis. We show here that weak *dTrf2* mutations result in lethality during metamorphosis, with most animals dying during pupation. These mutants display defects in puparium formation, adult head eversion, and salivary gland cell death—characteristic biological responses to ecdysone. Analysis of key ecdysone-regulated target genes shows that many of the transcription factor-encoding genes are delayed and submaximally induced in *dTrf2* mutants. These studies indicate that *dTrf2* plays an essential role in mediating responses to the steroid ecdysone during the early stages of *Drosophila* metamorphosis.

RESULTS AND DISCUSSION

Isolation of *dTrf2* Mutant Alleles

By screening a collection of P-element–induced lethal mutations (Peter et al., 2002), we identified several complementation groups that display lethality late during development along with persistent larval salivary glands (A.B. and C.S.T., unpublished results). Of these, one complementation group of four alleles mapped to the *dTrf2* locus (Fig. 1). Three of these alleles, $dTrf2^{G0039}$, $dTrf2^{G0295}$, and $dTrf2^{G0425}$, map to the second exon of *dTrf2*, as determined from available cDNA sequences (Rabenstein et al., 1999; Kopytova et al., 2006). The fourth allele, $dTrf2^{G0071}$, maps further downstream, approximately halfway through the *dTrf2* locus (Fig. 1). Subsequent studies revealed a total of nine P-element insertions located within an ~500-bp region near the insertion site for $dTrf2^{G0039}$, $dTrf2^{G0295}$, and $dTrf2^{G0425}$, defining this as a hot-

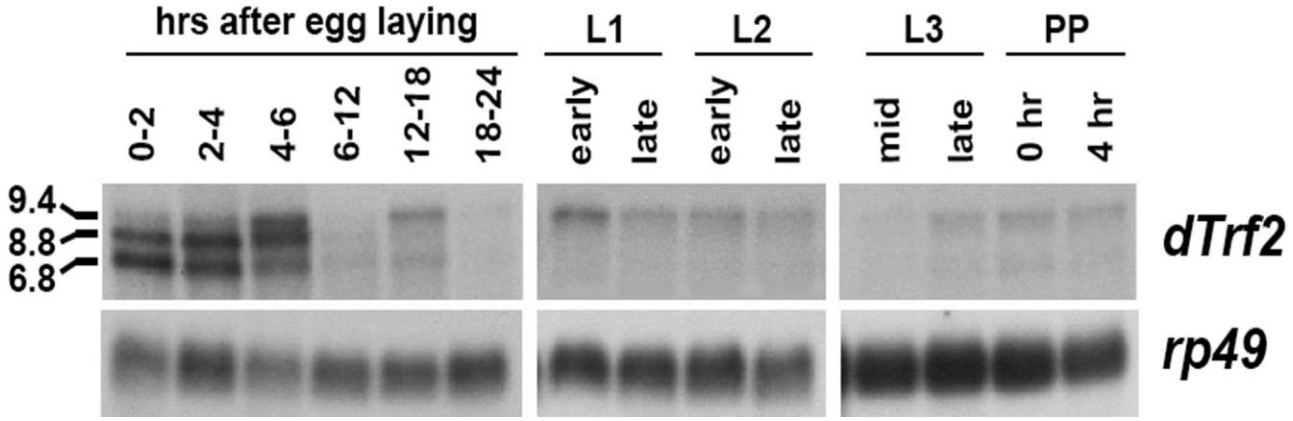

Fig. 2. Temporal pattern of *dTrf2* transcription. Total RNA isolated from staged w^{1118} control animals was analyzed by Northern blot hybridization to detect *dTrf2* mRNA. Hybridization to detect *rp49* was used as a control for loading and transfer. Embryos were staged in hours after egg laying, as depicted. First instar (L1) and second instar (L2) larvae were staged as either early (first half of instar) or late (second half of instar). Third instar larvae (L3) were staged as either blue gut mid-third instar larvae (mid) or clear gut late third instar larvae (late) (Andres and Thummel, 1994). Prepupae (PP) were staged in hours after puparium formation. The approximate sizes of the three *dTrf2* mRNAs are shown on the left, in kilobases (kb).

spot for P integration. These alleles, together with $dTrf2^{G0071}$, all displayed similar lethal phenotypes during the onset of metamorphosis. The $dTrf2^{G0071}$ mutation appeared to have fewer mutant animals that survive to puparium formation, suggesting that it might represent a stronger *dTrf2* loss-of-function allele.

dTrf2 Is Required During the Early Stages of Metamorphosis

RNA isolated from staged embryos, larvae, and prepupae was analyzed by Northern blot hybridization to determine the temporal profile of *dTrf2* expression (Fig. 2). *dTrf2* is expressed at low levels throughout these stages, with a peak in abundance in early embryos. There are three apparent size classes of *dTrf2* mRNA, approximately 9.4, 8.8, and 6.8 kb in length. The 8.8 kb and 6.8 kb *dTrf2* mRNAs are deposited maternally and drop to low levels by mid-embryogenesis. The 9.4-kb zygotic mRNA is up-regulated at 4–6 hr after egg laying and remains the most abundant isoform throughout larval and early prepupal stages. There is no apparent increase in *dTrf2* mRNA levels at puparium formation, suggesting that this gene is not transcriptionally regulated by ecdysone.

Two *dTrf2* mutant alleles were selected to characterize *dTrf2* function. One of these, $dTrf2^{G0071}$, results in only a few animals that survive to puparium formation while the other allele, $dTrf2^{G0039}$, has a higher proportion of mutants that survive to the early stages of metamorphosis. These observations were confirmed by lethal phase analysis. No embryonic lethality was observed in either mutant stock, with 98% of $dTrf2^{G0039}$ mutant embryos (n = 189) and 97% of $dTrf2^{G0071}$ mutant embryos (n = 184) hatching into first instar larvae. A distinct difference between these alleles, however, was observed in the number of mutants that survived larval stages. Of 60 $dTrf2^{G0039}$ mutant larvae, 55 survived to the third instar and 51 pupariated (85%), with 13 of these mutants arresting development as prepupae (22% of the mutant larvae) and 38 dying as pupae (63% of the mutant larvae). In contrast, almost all $dTrf2^{G0071}$ mutant larvae died as first instars, with only one animal surviving to the third instar (1% of the mutant larvae; n = 85). Thus, although both of these alleles are hypomorphic, $dTrf2^{G0071}$ appears to be a stronger loss-of-function mutation than $dTrf2^{G0039}$.

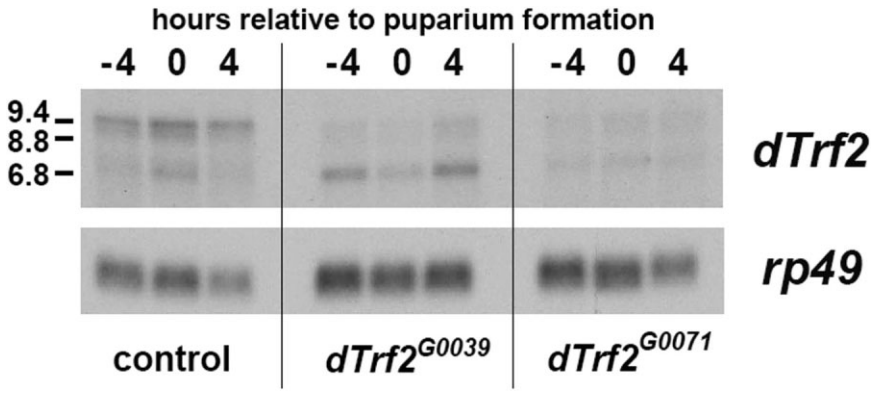

Fig. 3. Effects of *dTrf2* mutations on *dTrf2* transcript levels. Total RNA isolated from w^{1118} controls, $dTrf2^{G0039}$ mutants, or $dTrf2^{G0071}$ mutants, was analyzed by Northern blot hybridization to detect *dTrf2* mRNA. Hybridization to detect *rp49* was used as a control for loading and transfer. Animals were staged as either clear gut late third instar larvae (−4 hr), newly formed prepupae (0 hr), or 4-hr prepupae (Andres and Thummel, 1994).

This conclusion is consistent with Northern blot analysis of RNA isolated from $dTrf2^{G0039}$ and $dTrf2^{G0071}$ mutants, using animals staged as late third instar larvae, newly formed prepupae, or 4-hr prepupae (Fig. 3). The 9.4-kb mRNA is reduced in $dTrf2^{G0039}$ mutants, with an increase in the 6.8-kb mRNA. Significantly lower levels of all *dTrf2* mRNAs, however, are evident in $dTrf2^{G0071}$ mutants, consis-

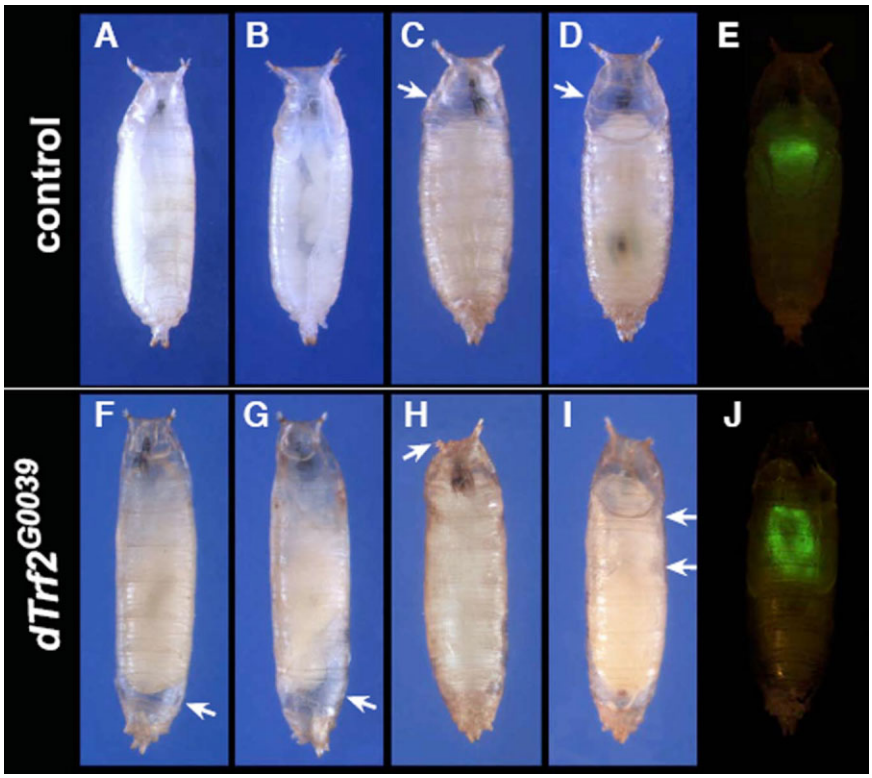

Fig. 4. *dTrf2* mutant phenotypes at the onset of metamorphosis. **A–J:** Control (w^{1118}) prepupae (A,B) and pupae (C–E) are depicted, from either a ventral (A,C,E) or dorsal (B,D) perspective, along with $dTrf2^{G0039}$ mutant prepupae (F,G) and pupae (H–J), from a ventral (F,H,J) or dorsal (G,I) perspective. E,J: Salivary glands were detected using a salivary gland-specific GAL4 driver in combination with a UAS–green fluorescent protein reporter (Ward et al., 2003). C,D,F,G: Gas, trapped at the posterior end of the $dTrf2^{G0039}$ prepupa (arrows, F,G), fails to translocate to the anterior end as seen in controls (arrows, C,D). H: Spiracle eversion defects can be seen in mutant pupae (arrow), compared with controls. The arrows in panel I show the demarcations between the head, thorax, and abdomen.

tent with this representing a stronger loss-of-function mutation. Although the location of the $dTrf2^{G0071}$ P-element in the middle of the gene is consistent with its effects on all *dTrf2* mRNA isoforms (Fig. 1), the reason for the apparent decrease in the 9.4-kb RNA and increase in the 6.8-kb RNA in $dTrf2^{G0039}$ mutants requires further characterization of the *dTrf2* locus. Taken together, these results suggest that the 6.8-kb *dTrf2* mRNA is required for survival during larval stages, and thus its reduced expression in $dTrf2^{G0071}$ mutants accounts for the high degree of larval lethality associated with this allele. In contrast, presence of the 9.4 kb mRNA, which is reduced in both $dTrf2^{G0039}$ and $dTrf2^{G0071}$ mutants, is correlated with survival through the early stages of metamorphosis.

dTrf2 Mutants Display Defects in Major Ecdysone-Triggered Biological Responses

To determine what roles *dTrf2* might play during the onset of metamorphosis, $dTrf2^{G0039}$ and $dTrf2^{G0071}$ mutants were selected as prepupae and followed through their development. Of 88 $dTrf2^{G0039}$ mutant prepupae, 63 (72%) progressed to pupal stages, with 61 displaying defects in head eversion (69%) and dying as early pupae, while two mutants displayed some aspects of adult development before arresting (bristles on the thorax or eye pigmentation). Interestingly, despite its more severe effect on survival during larval development, $dTrf2^{G0071}$ displayed a similar proportion of mutants that failed to progress beyond the prepupal stage. Of 20 $dTrf2^{G0071}$ mutant prepupae, 15 (75%) progressed to pupal stages, with 13 displaying defects in head eversion (65%) and dying as early pupae, while two escapers arrested development at later pupal stages. Thus, both $dTrf2^{G0039}$ and $dTrf2^{G0071}$ have similar effects on survival through the early stages of metamorphosis, with most lethality occurring during early pupal stages.

Representative *dTrf2* mutant phenotypes are depicted in Figure 4. Whereas wild-type prepupae (Fig. 4A,B) and pupae (Fig. 4C,D) have a characteristic barrel shape, $dTrf2^{G0039}$ mutant prepupae (Fig. 4F,G) and pupae (Fig. 4H,I) are elongated and malformed. $dTrf2^{G0039}$ mutants also display defects in gas bubble translocation. A gas bubble normally appears in the center of the prepupa and translocates to the posterior end of the animal (Chadfield and Sparrow, 1985). Rhythmic contractions of the abdominal muscles, accompanied by a swaying motion of the prepupa, then move the gas bubble along the sides of the animal toward the anterior end, driving larval–pupal apolysis and creating a space at the anterior end of the puparium for subsequent adult head eversion. While this space is apparent at the anterior end of wild-type animals (Fig. 4C,D, arrows), it is reduced in $dTrf2^{G0039}$ mutants (Fig. 4F–I), with gas aberrantly accumulating at the posterior end of the puparium (Fig. 4F,G, arrows). As mentioned above, some $dTrf2^{G0039}$ mutants progress to pupal stages and display a distinct head, thorax, and abdomen (Fig. 4I, arrows). These mutants are, however, malformed, and often display incomplete adult head eversion. In addition, as reported previously (Shima et al., 2007), $dTrf2^{G0039}$ mutants display defects in anterior spiracle eversion (Fig. 4H, arrow). Finally, by using a salivary gland-specific GAL4 driver in combination with a GAL4-responsive green fluorescent protein (GFP) reporter transgene, we could follow the fate of larval salivary glands in living wild-type and $dTrf2^{G0039}$ mutant pupae (Ward et al., 2003). Whereas only residual GFP is evident in control pupae at ~20 hr after puparium formation (Fig. 4E), consistent with the normal destruction of the salivary glands at ~14 hr after puparium formation (Jiang et al., 1997), $dTrf2^{G0039}$ mu-

tants have persistent larval salivary glands (Fig. 4J). Dissection of *dTrf2* mutant prepupae also revealed a defect in larval midgut cell death, which normally occurs in early prepupae (data not shown). $dTrf2^{G0071}$ mutant prepupae and pupae display a similar range of phenotypes (data not shown). Taken together, these defects—an elongated and misshapen puparium, improper anterior spiracle eversion, gas bubble translocation defects, adult head eversion defects, and a block in salivary gland and midgut cell death—all involve major ecdysone-regulated biological responses associated with entry into metamorphosis. These observations define *dTrf2* as essential for proper developmental responses to the steroid hormone ecdysone.

dTrf2 Mutants Display Defects in Ecdysone-Regulated Gene Expression at the Onset of Metamorphosis

The widespread effects of *dTrf2* mutations at the onset of metamorphosis raised the possibility that these defects might be accompanied by changes in the expression of key ecdysone-regulated target genes. To test this hypothesis, RNA was isolated from both control and $dTrf2^{G0039}$ mutants staged as either mid-third instar larvae (−18 hr), late third instar larvae (−4 hr), newly formed prepupae (0 hr), or at 2-hr intervals thereafter until 18 hr after puparium formation. These RNA samples were analyzed by Northern blot hybridization to detect the expression of *EcR* as well as five ecdysone-regulated transcription factor-encoding genes: *BR-C*, *E74*, *E75A*, *E93*, and β*FTZ-F*1 (Fig. 5). *dTrf2* mRNA shows no significant changes in abundance as control animals enter metamorphosis, indicating that this gene is not transcriptionally regulated by ecdysone. Only the 6.8-kb *dTrf2* mRNA is clearly detectable in $dTrf2^{G0039}$ mutants, consistent with the hypomorphic nature of this allele (Figs. 3, 5). Despite this effect on *dTrf2* activity, *EcR*, *BR-C*, *E74A*, *E75A*, *E93*, and β*FTZ-F1* are expressed in $dTrf2^{G0039}$ mutants, although they are reduced in abundance compared with controls, and delayed by several hours (Fig. 5). No major defects, however, are observed in the expression of these key ecdysone-regulated genes. The apparent absence of *EcR*, *BR-C*, and *E74B* mRNA in −18 hr third instar larvae is difficult to

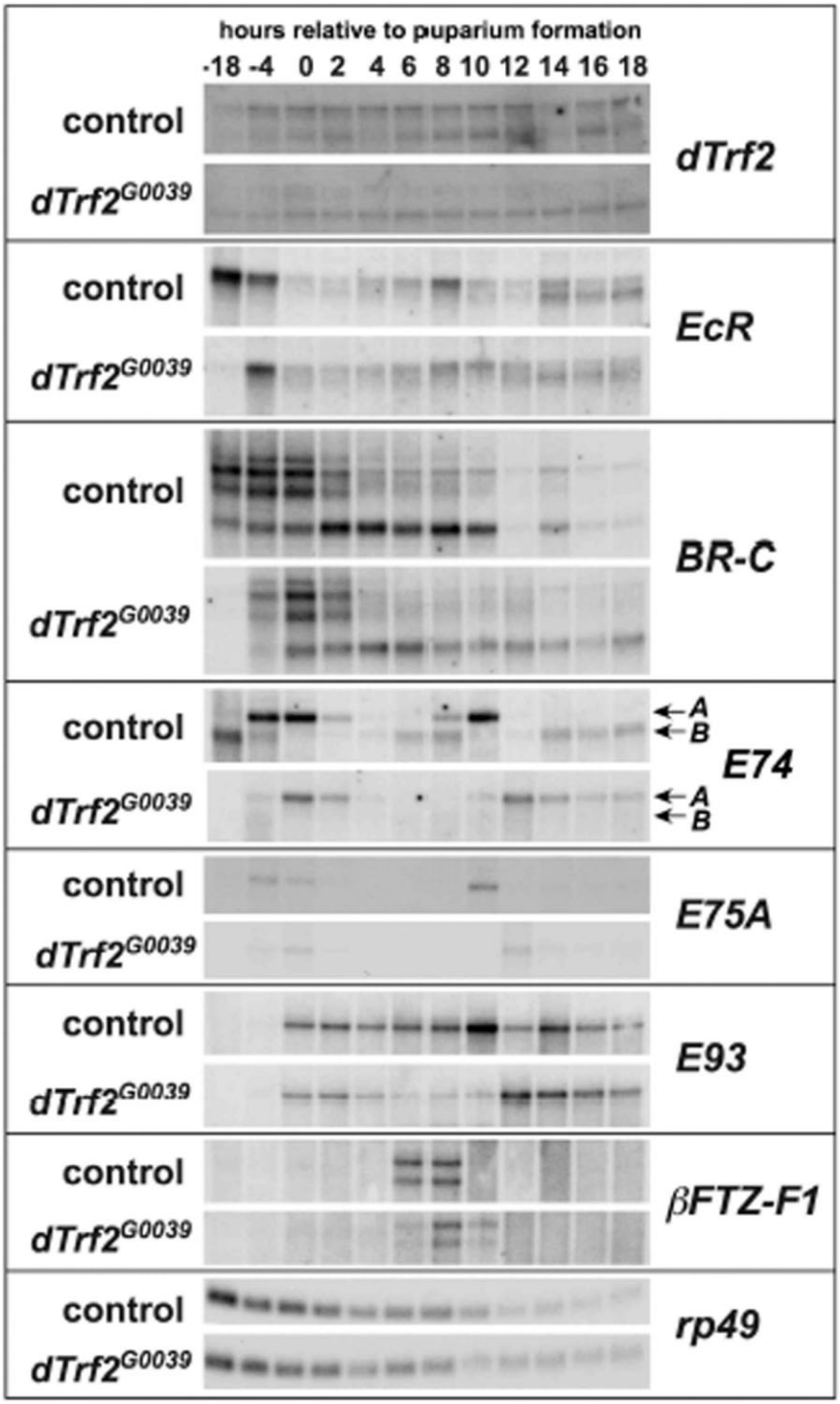

Fig. 5. Delayed and reduced expression of ecdysone-regulated genes in $dTrf2^{G0039}$ mutants. Total RNA from staged control (w^{1118}) and $dTrf2^{G0039}$ mutant third instar larvae, prepupae, or early pupae was analyzed by Northern blot hybridization to detect *dTrf2*, *EcR*, *BR-C*, *E74*, *E75A*, *E93*, and β*FTZ-F1* transcription. Hybridization to detect *rp49* was used as a control for loading and transfer. Numbers at the top indicate hours relative to puparium formation. **A,B:** The two *E74* isoforms (Burtis et al., 1990). Mutant and control blots were treated together to allow direct comparison. The 12-hr control RNA sample is lower due to partial RNA degradation.

interpret because both control and mutant animals can be asynchronous at this stage. Of the transcripts surveyed, only *E74B* displayed a significant change in expression level in $dTrf2^{G0039}$ mutants, with reduced mRNA levels in mutant mid-prepupae (Fig. 5, 6–8 hr) and early pupae (Fig. 5, 14–18 hr). *E74B* mRNA can, however, be detected in $dTrf2^{G0039}$ (Fig. 5) and $dTrf2^{G0071}$ mutants (data not shown), indicating that its reduction alone is not sufficient to explain the lethal phenotypes associated with a loss of *dTrf2* function. *BR-C* and *E74* transcription is reduced and delayed in $dTrf2^{G0071}$ mutants in a manner similar to that seen in $dTrf2^{G0039}$ mutants (data not shown), consistent with the essentially identical effects of the $dTrf2^{G0039}$ and $dTrf2^{G0071}$ mutant alleles on progression through early metamorphosis. Thus, the overall effect of these *dTrf2* alleles appears to be a general reduction in the fidelity of ecdysone-regulated transcription, culminating in lethal defects during the onset of metamorphosis.

dTrf2 Is Required for Proper Genetic and Biological Responses to Ecdysone at the Onset of Metamorphosis

The characterization of *dTrf2* mutant alleles presented here expands our understanding of TRF2 function in *Drosophila*. Our phenotypic analysis indicates that there are two temporally distinct zygotic requirements for *dTrf2* function during development. There is an initial zygotic requirement for *dTrf2* in late embryos or early first instar larvae that is essential for progression beyond the first instar. *dTrf2* function is next required during the early stages of metamorphosis, when it plays a central role in the major ecdysone-triggered biological responses that drive the larval-to-pupal transition (Fig. 4). It is possible that there is also an essential requirement for *dTrf2* during embryogenesis that is masked by maternally deposited wild-type gene product. A test of this hypothesis would require the future characterization of mutants derived from female germline clones.

The lethal defects observed in *dTrf2* mutant prepupae and pupae are accompanied by reduced and delayed expression of key ecdysone-regulated transcription factor-encoding genes (Fig. 5). Although it remains possible that a complete loss of *dTrf2* function would result in more dramatic effects on gene expression at the onset of metamorphosis, our studies indicate that the essential role for *dTrf2* at this stage is not manifested by major overall changes in ecdysone-regulated gene expression, as has been seen for other mutants with similar lethal phenotypes (e.g., Fletcher et al., 1995; Broadus et al., 1999). Rather, *dTrf2* appears to be required to maintain the fidelity of ecdysone-regulated transcription, ensuring the proper levels and timing of gene induction. It is interesting to note that TRF2 protein is bound to the ecdysone-inducible puffs that correspond to *BR-C* (2B5), *E74* (74EF), and *E75A* (75B) in the giant larval salivary gland polytene chromosomes, as well as many other loci (Rabenstein et al., 1999; Kopytova et al., 2006). This finding indicates that dTRF2 may be targeted to these ecdysone-regulated promoters and, thus, directly regulate their activity. In addition, subunits of the NURF chromatin remodeling complex are associated with dTRF2 (Hochheimer et al., 2002), and NURF can bind directly to EcR and enhance the phenotypes associated with a dominant-negative *EcR* mutation (Badenhorst et al., 2005). These observations raise the possibility that *dTrf2* may exert its effects on ecdysone responses, at least in part, through the NURF complex. Further biochemical and genetic characterization of *dTrf2* should provide a molecular basis for understanding its essential stage-specific functions during development.

EXPERIMENTAL PROCEDURES

Stocks and Developmental Staging

The *dTrf2* mutant stocks are available from the Bloomington stock center: $dTrf2^{G0039}$ (stock #11546), $dTrf2^{G0295}$ (stock #12236), $dTrf2^{G0425}$ (stock #12254), and $dTrf2^{G0071}$ (stock #11739). Embryos were collected in 2- to 6-hr intervals on molasses/agar plates supplemented with yeast paste, and allowed to age as necessary. Third instar larvae were staged by maintaining them on standard cornmeal agar food supplemented with 0.05% bromophenol blue, and selecting animals after the cessation of feeding based on their degree of dye clearance in the gut (Andres and Thummel, 1994). Mutant animals were identified by the use of GFP-marked balancer chromosomes.

Northern Blot Hybridizations

RNA was isolated from staged animals using Tripure (Roche). Equal amounts of total RNA were fractionated on 1% formaldehyde gels and transferred to nylon membranes for Northern blot hybridization. Probes were prepared as described (Andres et al., 1993). The probe to detect *dTrf2* mRNA was generated by PCR using the following primers: *Trf2*-5: 5′ TCTGATAGACGCGATCATTCG 3′ and *Trf2*-3A 5′ ACCAGTGCAGGTAATCCTCC 3′.

ACKNOWLEDGMENT

We thank the Bloomington Stock Center for stocks used in this study.

REFERENCES

Andres AJ, Thummel CS. 1994. Methods for quantitative analysis of transcription in larvae and prepupae. In: Goldstein LSB, Fyrberg EA, editors. Drosophila melanogaster: practical uses in cell and molecular biology. New York: Academic Press. p 565–573.

Andres AJ, Fletcher JC, Karim FD, Thummel CS. 1993. Molecular analysis of the initiation of insect metamorphosis: a comparative study of *Drosophila* ecdysteroid-regulated transcription. Dev Biol 160:388–404.

Badenhorst P, Xiao H, Cherbas L, Kwon SY, Voas M, Rebay I, Cherbas P, Wu C. 2005. The Drosophila nucleosome remodeling factor NURF is required for Ecdysteroid signaling and metamorphosis. Genes Dev 19:2540–2545.

Bartfai R, Balduf C, Hilton T, Rathmann Y, Hadzhiev Y, Tora L, Orban L, Muller F. 2004. TBP2, a vertebrate-specific member of the TBP family, is required in embryonic development of zebrafish. Curr Biol 14:593–598.

Broadus J, McCabe JR, Endrizzi B, Thummel CS, Woodard CT. 1999. The *Dro-*

sophila βFTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. Mol Cell 3:143–149.

Burtis KC, Thummel CS, Jones CW, Karim FD, Hogness DS. 1990. The *Drosophila* 74EF early puff contains *E74*, a complex ecdysone-inducible gene that encodes two *ets*-related proteins. Cell 61:85–99.

Chadfield CG, Sparrow JC. 1985. Pupation in *Drosophila melanogaster* and the effect of the *lethalcryptocephal* mutation. Dev Genet 5:103–114.

Chong JA, Moran MM, Teichmann M, Kaczmarek JS, Roeder R, Clapham DE. 2005. TATA-binding protein (TBP)-like factor (TLF) is a functional regulator of transcription: reciprocal regulation of the neurofibromatosis type 1 and c-fos genes by TLF/TRF2 and TBP. Mol Cell Biol 25:2632–2643.

Dantonel JC, Quintin S, Lakatos L, Labouesse M, Tora L. 2000. TBP-like factor is required for embryonic RNA polymerase II transcription in *C. elegans*. Mol Cell 6:715–722.

DiBello PR, Withers DA, Bayer CA, Fristrom JW, Guild GM. 1991. The *Drosophila Broad-Complex* encodes a family of related proteins containing zinc fingers. Genetics 129:385–397.

Fletcher JC, Burtis KB, Hogness DS, Thummel CS. 1995. The *Drosophila E74* gene is required for metamorphosis and plays a role in the polytene chromosome puffing response to ecdysone. Development 121:1455–1465.

Henrich VC. 2005. The ecdysteroid receptor. In: Gilbert LI, Iatrou K, Gill SS, editors. Comprehensive molecular insect science. Oxford: Elsevier. p 243–286.

Hochheimer A, Zhou S, Zheng S, Holmes MC, Tjian R. 2002. TRF2 associates with DREF and directs promoter-selective gene expression in *Drosophila*. Nature 420:439–445.

Holmes MC, Tjian R. 2000. Promoter-selective properties of the TBP-related factor TRF1. Science 288:867–870.

Jiang C, Baehrecke EH, Thummel CS. 1997. Steroid regulated programmed cell death during Drosophila metamorphosis. Development 124:4673–4683.

Kaltenbach L, Horner MA, Rothman JH, Mango SE. 2000. The TBP-like factor CeTLF is required to activate RNA polymerase II transcription during *C. elegans* embryogenesis. Mol Cell 6:705–713.

Kopytova DV, Krasnov AN, Kopantceva MR, Nabirochkina EN, Nikolenko JV, Maksimenko O, Kurshakova MM, Lebedeva LA, Yerokhin MM, Simonova OB, Korochkin LI, Tora L, Georgiev PG, Georgieva SG. 2006. Two isoforms of Drosophila TRF2 are involved in embryonic development, premeiotic chromatin condensation, and proper differentiation of germ cells of both sexes. Mol Cell Biol 26:7492–7505.

Martianov I, Fimia GM, Dierich A, Parvinen M, Sassone-Corsi P, Davidson I. 2001. Late arrest of spermiogenesis and germ cell apoptosis in mice lacking the TBP-like TLF/TRF2 gene. Mol Cell 7:509–515.

Peter A, Schottler P, Werner M, Beinert N, Dowe G, Burkert P, Mourkioti F, Dentzer L, He Y, Deak P, Benos PV, Gatt MK, Murphy L, Harris D, Barrell B, Ferraz C, Vidal S, Brun C, Demaille J, Cadieu E, Dreano S, Gloux S, Lelaure V, Mottier S, Galibert F, Borkova D, Minana B, Kafatos FC, Bolshakov S, Siden-Kiamos I, Papagiannakis G, Spanos L, Louis C, Madueno E, de Pablos B, Modolell J, Bucheton A, Callister D, Campbell L, Henderson NS, McMillan PJ, Salles C, Tait E, Valenti P, Saunders RD, Billaud A, Pachter L, Klapper R, Janning W, Glover DM, Ashburner M, Bellen HJ, Jackle H, Schafer U. 2002. Mapping and identification of essential gene functions on the X chromosome of *Drosophila*. EMBO Rep 3:34–38.

Rabenstein MD, Zhou S, Lis JT, Tjian R. 1999. TATA box-binding protein (TBP)-related factor 2 (TRF2), a third member of the TBP family. Proc Natl Acad Sci U S A 96:4791–4796.

Riddiford LM. 1993. Hormones and *Drosophila* development. In: Bate M, Martinez-Arias A, editors. The development of *Drosophila melanogaster*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. p 899–939.

Riddiford LM, Cherbas P, Truman JW. 2000. Ecdysone receptors and their biological actions. Vitam Horm 60:1–73.

Segraves WA, Hogness DS. 1990. The *E75* ecdysone-inducible gene responsible for the 75B early puff in *Drosophila* encodes two new members of the steroid receptor superfamily. Genes Dev 4:204–219.

Shima S, Aigaki T, Nojima T, Yamamoto D. 2007. Identification of trf2 mutants of Drosophila with defects in anterior spiracle eversion. Arch Insect Biochem Physiol 64:157–163.

Smale ST, Kadonaga JT. 2003. The RNA polymerase II core promoter. Annu Rev Biochem 72:449–479.

Takada S, Lis JT, Zhou S, Tjian R. 2000. A TRF1:BRF complex directs *Drosophila* RNA polymerase III transcription. Cell 101:459–469.

Teichmann M, Wang Z, Martinez E, Tjernberg A, Zhang D, Vollmer F, Chait BT, Roeder RG. 1999. Human TATA-binding protein-related factor-2 (hTRF2) stably associates with hTFIIA in HeLa cells. Proc Natl Acad Sci U S A 96:13720–13725.

Thummel CS. 1996. Flies on steroids - *Drosophila* metamorphosis and the mechanisms of steroid hormone action. Trends Genet 12:306–310.

Veenstra GJ, Weeks DL, Wolffe AP. 2000. Distinct roles for TBP and TBP-like factor in early embryonic gene transcription in *Xenopus*. Science 290:2312–2315.

Ward RE, Reid P, Bashirullah A, D'Avino PP, Thummel CS. 2003. GFP in living animals reveals dynamic developmental responses to ecdysone during Drosophila metamorphosis. Dev Biol 256:389–402.

Zhang D, Penttila TL, Morris PL, Teichmann M, Roeder RG. 2001. Spermiogenesis deficiency in mice lacking the Trf2 gene. Science 292:1153–1155.