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The Ecdysone Regulatory Pathway Controls Wing Morphogenesis and Integrin Expression during *Drosophila* Metamorphosis

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Drosophila **imaginal discs are specified and patterned during embryonic and larval development, resulting in each cell acquiring a specific fate in the adult fly. Morphogenesis and differentiation of imaginal tissues, however, does not occur until metamorphosis, when pulses of the steroid hormone ecdysone direct these complex morphogenetic responses. In this paper, we focus on the role of ecdysone in regulating adult wing development during metamorphosis. We show that mutations in the *EcR* ecdysone receptor gene and *crooked legs* (*crol*), an ecdysone-inducible gene that encodes a family of zinc finger proteins, cause similar defects in wing morphogenesis and cell adhesion, indicating a role for ecdysone in these morphogenetic responses. We also show that *crol* and *EcR* mutations interact with mutations in genes encoding integrin subunits—a family of $\alpha\beta$ heterodimeric cell surface receptors that mediate cell adhesion in many organisms. α-Integrin transcription is regulated by ecdysone in cultured larval organs and some changes in the temporal patterns of integrin expression correlate with the ecdysone titer profile during metamorphosis. Transcription of α- and β-integrin subunits is also altered in *crol* and *EcR* mutants, indicating that integrin expression is dependent upon *crol* and *EcR* function. Finally, we describe a new hypomorphic mutation in *EcR* which indicates that different EcR isoforms can direct the development of adult appendages. This study provides evidence that ecdysone controls wing morphogenesis and cell adhesion by regulating integrin expression during metamorphosis. We also propose that ecdysone modulation of integrin expression might be widely used to control multiple aspects of adult development.** © 2000 Academic Press

Key Words: **metamorphosis; ecdysone; nuclear receptor; transcription; morphogenesis; cell adhesion; integrins.**

INTRODUCTION

Extensive studies over the past decade have focused on the mechanisms by which *Drosophila* imaginal discs are patterned during larval development (Cohen, 1993). In contrast, relatively little is known about the next critical step in disc development—how the determined state is realized by morphogenesis and differentiation of the imaginal tissues during metamorphosis. These complex morphogenetic changes are dependent on pulses of the steroid hormone 20-hydroxyecdysone (referred to here as ecdysone) which coordinate the major developmental transitions during the *Drosophila* life cycle. A high titer ecdysone pulse at the end of larval development triggers puparium formation, initiating the prepupal stage of development. This is followed by another ecdysone pulse, ~10 h after puparium formation, that signals the prepupal–pupal transition. A large surge of ecdysone during pupal development, from 24 to 72 h after puparium formation, controls adult differentiation (Riddiford, 1993). Most larval tissues are destroyed by programmed cell death during prepupal and early pupal stages (Robertson, 1936; Jiang *et al.*, 1997). At the same time, the imaginal discs elongate and evert to the exterior of the animal through a remarkable series of cell shape changes and cell rearrangements, forming rudimentary adult appendages (Fristrom and Fristrom, 1993). A major goal of our studies is to determine how a single hormonal signal can direct these different stage- and tissue-specific biological responses.

Ecdysone exerts its effects on development through a heterodimer of two nuclear receptors, encoded by *EcR* (NR1H1) and *ultraspiracle* (*usp,* NR2B4) (Koelle *et al.*, 1991; Yao *et al.*, 1992; Thomas *et al.*, 1993; Yao *et al.*, 1993). The ecdysone/EcR/USP complex then directly activates cas-

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cades of gene expression (Thummel, 1996; Richards, 1997; Segraves, 1998). *usp* encodes a single protein product, the *Drosophila* homolog of vertebrate RXR (Henrich *et al.*, 1990; Oro *et al.*, 1990; Shea *et al.*, 1990). In contrast, *EcR* encodes three protein isoforms that differ in their N-terminal sequences: EcR-A, EcR-B1, and EcR-B2 (Talbot *et al.*, 1993). Each EcR isoform can heterodimerize with USP to form a functional ecdysone receptor (Koelle, 1992). EcR-A is predominantly expressed in imaginal cells that are destined to form parts of the adult fly while EcR-B1 is predominantly expressed in larval cells that are fated to die. This differential expression pattern has been proposed to dictate, at least in part, the tissue specificity of ecdysone responses (Talbot *et al.*, 1993). Consistent with this hypothesis, leg imaginal discs elongate in *EcR-B* mutants while larval tissues fail to enter programmed cell death (Bender *et al.*, 1997; Schubiger *et al.*, 1998).

In a genetic screen designed to identify genes required for ecdysone-mediated tissue morphogenesis, we identified the *crooked legs* (*crol*) locus (D'Avino and Thummel, 1998). *crol* mutants die during pupal development with defects in leg morphogenesis and adult head eversion. In addition, *crol* mutations specifically affect the transcription of a subset of ecdysone-regulated genes, including *EcR*. The *crol* gene encodes at least three protein isoforms that contain 12–18 clustered C_2H_2 zinc finger motifs, suggesting that *crol* manifests its effects on metamorphosis by directly regulating gene expression. In addition, *crol* transcription is induced by ecdysone during late larval and prepupal development, and *crol* is expressed in a number of ecdysone target tissues including imaginal discs, salivary glands, and the central nervous system (D'Avino and Thummel, 1998). Taken together, these data indicate that *crol* acts as an important regulator of genetic responses to ecdysone during metamorphosis.

In this paper, we investigate the role of ecdysone signaling in wing morphogenesis and cell adhesion during metamorphosis. *crol* and *EcR* mutants display malformed wings, venation defects and partial or total separation of the dorsal and ventral wing epithelia. These phenotypes are similar to those of mutations in integrin subunits, a family of heterodimeric cell surface receptors that mediate cell adhesion in many organisms (Hynes, 1992; Brown, 1993). Three α-integrins have been identified in *Drosophila*: α_{PS1}, α_{PS2}, and α_{PS3}, encoded by the *multiple edematous wings* (*mew*), *inflated* (*if*), and *Volado* (Vol) or *scab* (*scb*) loci, respectively (Brower and Jaffe, 1989; Wilcox *et al.*, 1989; Wehrli *et al.*, 1993; Brown, 1994; Stark *et al.*, 1997; Grotewiel *et al.*, 1998). Like their vertebrate counterparts, α-integrins heterodimerize with a β subunit to form a functional integrin receptor (MacKrell *et al.*, 1988). The *Drosophila* β_{PS} subunit is encoded by the *myospheroid* (*mys*) locus. β_{PS} is expressed over most of the basal cell surface of wing imaginal discs while α_{PS1} is expressed in the presumptive dorsal wing epithelium and α_{PS2} is expressed in the presumptive ventral wing epithelium. This complementary expression pattern appears to be critical for wing morphogenesis, as mutations in any of these integrin subunits results in defects in the apposition of dorsal and ventral wing surfaces, leading to the formation of blisters (Brower and Jaffe, 1989; Wilcox *et al.*, 1989; Zusman *et al.*, 1990, 1993; Wehrli *et al.*, 1993; Brower *et al.*, 1995; Brabant *et al.*, 1996; Bloor and Brown, 1998). Although α_{PS3} may also contribute to the development of adult structures, its only known functions are in short-term memory (Grotewiel *et al.*, 1998) and during embryogenesis (Stark *et al.*, 1997).

Here we show that *EcR* and *crol* mutations interact genetically with *if* and *mys* alleles and that α-integrin transcription is regulated by ecdysone in organ culture. Integrin gene expression is also altered during pupal development in *crol* and *EcR* mutants. These results suggest that *EcR*, together with *crol*, controls wing morphogenesis and cell adhesion by regulating integrin expression during metamorphosis. Since integrins have been shown to mediate a wide range of biological processes, including cell adhesion, muscle attachment, cytoskeleton organization, synaptic plasticity, and gene expression (Hynes, 1992; Brown, 1993; Grotewiel *et al.*, 1998; Martin-Bermudo and Brown, 1999), we propose that the ecdysone regulation of integrin expression is a crucial and general mechanism for controlling tissue morphogenesis during metamorphosis.

MATERIALS AND METHODS

Fly stocks and genetics. Flies were raised on a standard cornmeal/molasses/yeast medium at 18 or 25°C. The *crol* and *EcR* alleles used in this study are listed in Table 1. if^3 and mys^{nj42} are viable hypomorphic X-linked mutations (Brower and Jaffe, 1989; Wilcox *et al.*, 1989). Other stocks are described by Lindsley and Zimm (1992). The $crol^{4418}$, $crol^{6470}$, and $crol^{k08217}$ alleles have been renamed $crol^1$, $crol^2$, and $crol^3$, respectively, according to the temporal order of their isolation. The $crol^2$ and $crol^3$ chromosomes were passed through two rounds of recombination to remove potential flanking mutations. $crol^{1ex15}$ and $crol^{3ex5}$ were generated by mobilization of the *P* elements present in $crol^1$ and $crol^3$, respectively. A *P[Δ2-3]* chromosome (Robertson *et al.*, 1988) was used as source of transposase and the resulting rearrangements were analyzed by Southern blot hybridization. To analyze lethality, $crol^{1ex15}$ homozygous males were crossed to $Df(2L)esc^{10}/CyO$ virgin females and viability was determined in the offspring as the ratio of $crol^{1ex15}/Df(2L)esc^{10}$ to $crol^{1ex15}/CyO$ flies.

The EcR^{k06210} allele was generated by the Berkeley *Drosophila* Genome Project (BDGP) (Spradling *et al.*, 1995) and obtained from the Bloomington stock center. To map the *P* element in this mutant relative to the *EcR* transcription units, its flanking genomic sequences (sequenced by the BDGP, Accession No. AQ025786), and those of the *EcR* cDNAs (Accession Nos. *EcR-A*, S63761; *EcR-B*, M74078) were aligned with the sequence of the 42A8-42A16 genomic region (Accession No. AC007121). In this way, the insertion was mapped to 14,336 bp downstream from *EcR-A* exon 3 and 5826 bp upstream from the *EcR-B* transcription start site (Fig. 3). Lethal phase analysis of EcR^{k06210} was carried out as described previously (D'Avino and Thummel, 1998). To analyze adult phenotypes, *crol* or EcR^{k06210} white prepupae were collected and transferred to a petri dish with wet filter paper. *crol* mutants were incubated at 25°C and EcR^{k06210} mutants were incubated at 18°C until eclosion. Wings were dissected in ethanol and mounted

TABLE 1
EcR and *crol* Alleles Used in This Study

Name	Former designation	Molecular lesion	Classification	Reference
$crol^{1}$	$crol^{4418}$	*PZ* insertion in second intron	Strong hypomorph	D'Avino and Thummel (1998)
$crol^{2}$	$crol^{6470}$	*PZ* insertion in first intron	Strong hypomorph	D'Avino and Thummel (1998)
$crol^{3}$	$crol^{k08217}$	*PlacW* insertion in first intron	Strong hypomorph	D'Avino and Thummel (1998)
$crol^{1ex15}$		2.2-kb insertion. Incomplete deletion of the $crol^{1}$ *P* element	Weak hypomorph	This paper
$crol^{3ex5}$		Excision of the $crol^{3}$ *P* element	Viable	This paper
EcR^{C300Y}		Missense mutation in the DNA binding domain	Strong hypomorph	Bender *et al.* (1997)
EcR^{M554fs}		22-bp deletion within the ligand binding domain	Null allele	Bender *et al.* (1997)
EcR^{k06210}		*PlacW* insertion between *EcR-A* exon3 and *EcR-B1/B2* transcriptional start site	Weak hypomorph, semilethal	This paper
EcR^{214}		Deletion of *EcR-B1/B2* common exon	Null for *EcR-B* functions	Schubiger *et al.* (1998)

in Euparal. Legs were dissected in PBS, cleared by incubation in 45% acetic acid at 65°C for 45 min, and then mounted in CMCP-10 mounting medium (Master's Chemical Company):lactic acid (3:1).

For genetic interaction experiments, either *y w* if^{3} or *w* $mys^{nj42}f$ virgin females were crossed to males heterozygous for a *crol* or *EcR* mutation and a balancer chromosome (*CyO* y^{+}, *SM6b*, or *In(2LR)Gla*). Since the penetrance of wing blisters in if^{3} and mys^{nj42} flies can be influenced by several factors, such as temperature, humidity, and crowding (Brower and Jaffe, 1989), all crosses were performed under identical conditions. Typically, five virgin females were crossed to five males in a 2.5-cm-diameter vial and incubated at 25°C with a 12-h dark/light cycle and 50–60% humidity. Five cohorts were recovered by transferring the parents every 24 h into a fresh vial, after which the parents were discarded. The frequency of wing blisters was determined in the male progeny.

Northern blot hybridization. Animals were staged and collected essentially as described (D'Avino and Thummel, 1998). *EcR* and *crol* mutations were balanced over a *CyO* y^{+} chromosome in a *y w* background. Mutant animals were identified by the *yellow* phenotype of their mouth hooks and denticle belts. For the Northern blots shown in Figs. 7 and 8, *yellow* animals were collected from the following crosses: for $crol^{-}$ mutants − *y w;* $Df(2L)esc^{10}/CyO$ y^{+} virgin females were crossed to *y w*; $crol^{3}/CyO$ y^{+} males; for +/*Df* control animals − *y w;* $Df(2L)esc^{10}/CyO$ y^{+} virgin females were crossed to *y w* males; for EcR^{-} mutants − *y w*; EcR^{M554fs}/CyO y^{22} virgin females were crossed to *y w*; EcR^{k06210}/CyO y^{+} males; for +/*EcR* control animals − *y w*; EcR^{M554fs}/CyO y^{22} virgin females were crossed to *y w* males. Since some *crol* and *EcR* mutant animals die during pupal development, animals were checked before freezing and those showing signs of necrosis or developmental arrest were discarded.

RNA was extracted, fractionated by formaldehyde agarose gel electrophoresis, and transferred onto nylon membranes as described (D'Avino *et al.*, 1995). Twelve to 14 μg of total RNA was loaded per lane. Filters were hybridized, washed, and stripped as described (Karim and Thummel, 1991). To detect *EcR-A*-specific transcripts, a 650-bp genomic DNA fragment encompassing *EcR-A* exons 2 and 3 was PCR amplified from genomic DNA using the following oligonucleotide pair: EcR-A-1 (5′-CTCAGTCGCTAGGAAATGATG-3′) and EcR-A-2 (5′-GGATGCATAGCCGTTGG-3′). Similarly, an 891-bp *EcR-B1* probe was obtained by PCR amplification of the *EcR-B1* exon 2 using the pMK1 cDNA clone as template (Koelle *et al.*, 1991) and the following oligonucleotides: EcR-B3 (5′-GATTGTTTCCC-GCACTAAATG-3′) and EcR-B4 (5′-GCCTACTCCAAGACCTA-3′). PCR conditions have been described previously (D'Avino and Thummel, 1998). A 3-kb *Eco*RI fragment from pMK1 was used as a common region *EcR* probe (Koelle *et al.*, 1991). An 850-bp *Sal*I fragment from the vector pCaSpeR was used as a probe to detect *white* mRNA (Horner and Thummel, 1997). A 1.5-kb *Pst*I fragment from the PS1-41 cDNA clone (a gift from M. Wehrli) was used to detect α_{PS1} mRNA (Wehrli *et al.*, 1993). A 1.55-kb *Bam*HI fragment from the PS2.160 cDNA clone (a gift from N. Brown) was used to detect α_{PS2} mRNA (Brown *et al.*, 1989). A 0.9-kb *Bam*HI fragment from the LM20 cDNA clone (a gift from R. Hynes and K. Stark) was used to detect β_{PS} mRNA (MacKrell *et al.*, 1988). For α_{PS2} and β_{PS}, two transcripts are generated by alternative splicing of small, internally located exons (Brown *et al.*, 1989; Zusman *et al.*, 1993). In both cases, however, the difference in length between these isoforms is too small to be detected by Northern blot hybridization. *Long-* and *Short-*α_{PS3} isoform-specific fragments were obtained by PCR amplification of their 5′ unique sequences, using genomic DNA as template and the following nucleotide pairs: LPS3-1 (5′-CGGGTCGTCGAAGAGTGAAAA-3′) and LPS3-2 (5′-TGGCGGATGACAAGCGTGTA-3′) for *Long-*α_{PS3} and SPS3-1 (5′-GGTGGGGCAAGATCGTGAT-3′) and SPS3-2 (5′-CGTGAA-TCCGAAGTATGACGC-3′) for *Short-*α_{PS3}. All probes were labeled by random priming (Prime-It kit, Stratagene) of gel-purified fragments, with the exception of α_{PS3} probes, which were labeled by asymmetric PCR, as described (Karim and Thummel, 1992).

RESULTS

crol and EcR Mutants Display Similar Defects in Wing Morphogenesis and Cell Adhesion

The three original *crol* alleles are strong hypomorphic mutations that lead to lethality during pupal development

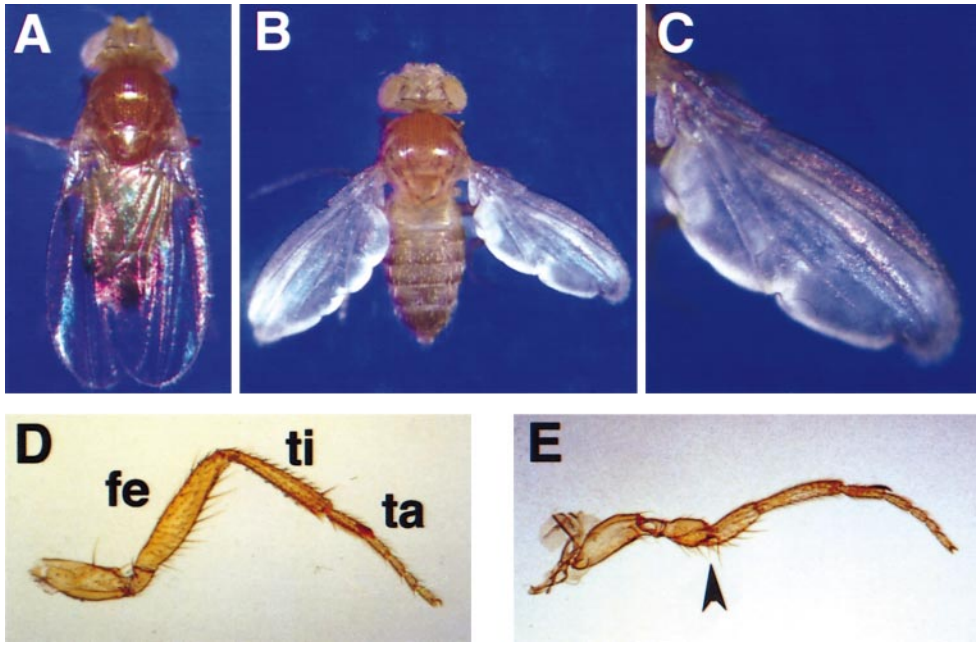

FIG. 1. *crol*^{2}/*crol*^{3} escapers display wing and leg defects. A *y w; crol*^{3ex5} control fly (A) and representative *y w; crol*^{2}/*crol*^{3} escaper (B) are depicted. Both flies are ~3 days old. (C) A higher magnification of the right wing shown in B. First legs dissected from *crol*^{3ex5} (D) and *crol*^{2}/*crol*^{3} (E) adults are depicted below. The femur (fe), tibia (ti), and tarsal segments (ta) of the leg are indicated. The arrowhead marks a severe kink in the femur of the *crol*^{2}/*crol*^{3} mutant leg.

(D'Avino and Thummel, 1998). In some homo- and heteroallelic combinations, however, a few adult escapers can be recovered. For example, 2–3% of *crol*^{2}/*crol*^{3} mutant animals are able to eclose from the pupal case, although these adults have severe morphological defects. Consistent with our earlier study (D'Avino and Thummel, 1998), 71% of these escapers (n = 28) have strongly misshapen legs, often with a severe kink in the femur (Fig. 1E). Unexpectedly, all of these escapers also display wing defects, a phenotype that could not be easily seen in the mutant pharate adults examined in our previous study. Fifty-seven percent of the adult escapers have held out wings with a partial (blister) or complete (balloon) separation of the dorsal and ventral wing surfaces (Figs. 1B and 1C), while the remaining 43% have either malformed or completely unfolded wings (data not shown). The blisters in *crol* mutant wings are generally large and do not appear to have sharp boundaries.

Genetic analysis of a newly isolated viable *crol* allele, *crol*^{1ex15} (see Table 1 and Materials and Methods), provides further evidence of a role for *crol* in wing morphogenesis. This allele is homozygous viable and fertile, but is semilethal in combination with a deficiency for the *crol* locus, *Df(2L)esc*^{10}. Only 44% of *crol*^{1ex15}/*Df(2L)esc*^{10} animals (n = 372) survive to adulthood, leaving many dead pupae with misshapen legs. In addition, 48% of the surviving *crol*^{1ex15}/*Df(2L)esc*^{10} flies (n = 217) have venation defects (Fig. 2C) and 8% have malformed wings (Fig. 2D). The most common venation defects are ectopic vein material originating from the posterior crossvein (pc) and the second longitudinal vein (Fig. 2C, arrowheads). Similar phenotypes can be seen in *crol*^{1ex15} homozygotes (Fig. 2B, arrowheads) as well as *crol*^{1ex15}/*crol*^{3} mutants (data not shown).

Taken together, these phenotypes indicate a role for *crol* in cell adhesion and wing morphogenesis, raising the possibility that ecdysone signaling might play a role in regulating these processes. To test this hypothesis, we analyzed the role of the ecdysone receptor in wing development using the hypomorphic *EcR*^{k06210} allele. This mutation is caused by a *P* element insertion ~14 kb downstream from the *EcR-A* exon 3 and ~6 kb upstream from the *EcR-B* transcriptional start site (Fig. 3A, see also Materials and Methods). At 25°C, 64% of *EcR*^{k06210} homozygous mutants die as pharate adults, while the remaining 36% survive to adulthood (n = 85; Fig. 3B). These adults die after about 1 week at 25°C, but are fertile and can survive for up to 3 weeks at 18°C. Combining the *EcR*^{k06210} allele with the *EcR*^{M554fs} null allele, which inactivates all EcR isoforms (Bender *et al.*, 1997), results in a fully penetrant lethal phenotype (Fig. 3B). Furthermore, *EcR*^{k06210} can only partially complement *EcR*^{214}, a null allele that inactivates both *EcR-B1* and *EcR-B2* functions (Schubiger *et al.*, 1998) (21% of the transheterozygotes survive to adulthood; n = 204). Consistent with the molecular and genetic characterization of *EcR*^{k06210}, Northern blot hybridization revealed that no full-length *EcR-A* mRNA is present in *EcR*^{k06210} mutant

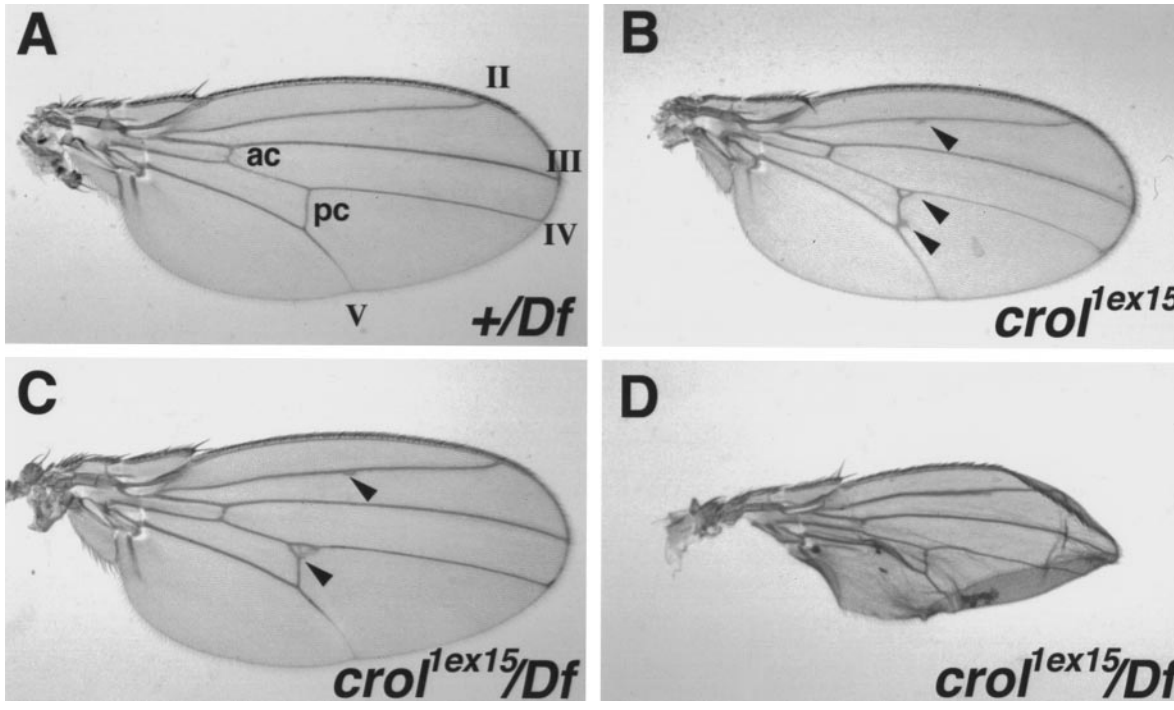

FIG. 2. *crol*^{1ex15} mutants display wing defects. (A) A control wing dissected from a +/*Df(2L)esc*^{10} (+/*Df*) fly appears normal, as do the four longitudinal veins (II–V) and the anterior (ac) and posterior (pc) crossveins. In contrast, *crol*^{1ex15} (B) and *crol*^{1ex15}/*Df(2L)esc*^{10} (*crol*^{1ex15}/*Df*) wings (C) have abnormal venation. Extra vein material (marked by arrowheads) originates from the II longitudinal vein and the posterior crossvein. (D) *crol*^{1ex15}/*Df(2L)esc*^{10} hemizygous flies also display severely malformed wings.

animals, and *EcR-B1* transcription is significantly reduced (Fig. 3C). Hybridization with a probe specific for *EcR-A* mRNA revealed a truncated transcript in *EcR*^{k06210} mutants relative to the full-length mRNA detected in *y w* controls (Fig. 3C). A transcript of the same size as the truncated *EcR-A* mRNA was detected in *EcR*^{k06210} mutants using a probe for the *white* gene, but not using a probe for the *EcR* common region located downstream from the *P* element insertion site (data not shown). Taken together, these data suggest that the truncated transcript in *EcR*^{k06210} mutant animals is the result of splicing the first three *EcR-A* exons with the *white* gene present in the *P* element. Consistent with this hypothesis, the *EcR-A* and *white* transcription units have the same 5′→3′ orientation, and the length of the fusion mRNA corresponds to that of the first three *EcR-A* exons plus the *white* coding region. These three exons encode 197 amino acids at the N-terminus of EcR-A and do not encode either the DNA binding or ligand binding domains of the receptor (Talbot *et al.*, 1993). We conclude that *EcR*^{k06210} is a hypomorphic *EcR* allele that inactivates *EcR-A* function and reduces *EcR-B* activity.

The wings of *EcR*^{k06210} homozygous mutants display cell adhesion and morphogenetic defects similar to those seen in *crol* mutants. At 18°C, 24% of the eclosed adults ($n = 48$) display venation defects (Fig. 4B), 32% have malformed wings (Fig. 4C), and 13% display wing blisters (Figs. 4D, and 4E). The blisters are usually small and centrally located (Fig. 4D), although blistering of the entire wing (a balloon wing) can occasionally be seen (Fig. 4E). The most frequent venation defects include a small, extra vein that originates from the third longitudinal vein, an additional anterior crossvein, and a "delta" thickening at the intersection between the posterior crossvein and the fourth longitudinal vein (Fig. 4B, arrowheads). All of these wing phenotypes, as well as the lethality, can be rescued by excision of the *EcR*^{k06210} *P* element (Fig. 4A), indicating that these defects are due to the *P* element insertion in the *EcR* locus.

Genetic Interactions between crol and EcR Mutations and Integrin Mutations

Mutations in *mew*, *if* and *mys*, which encode the α_{PS1}, α_{PS2}, and β_{PS} integrin subunits, respectively, each cause wing blister phenotypes (Brower and Jaffe, 1989; Wilcox *et al.*, 1989; Wehrli *et al.*, 1993; Zusman *et al.*, 1993; Brower *et al.*, 1995; Bloor and Brown, 1998). Furthermore, a viable hypomorphic *mys* allele, *mys*^{nj42}, displays some venation abnormalities that resemble those observed in *EcR* and *crol* mutant wings (Fig. 4F; see also Wilcox *et al.*, 1989), and *mys*^{nj42}*if*^{3} wings are often highly malformed (Wilcox *et al.*, 1989). To assess whether *EcR*, *crol*, and integrins function in a common pathway that controls wing morphogenesis

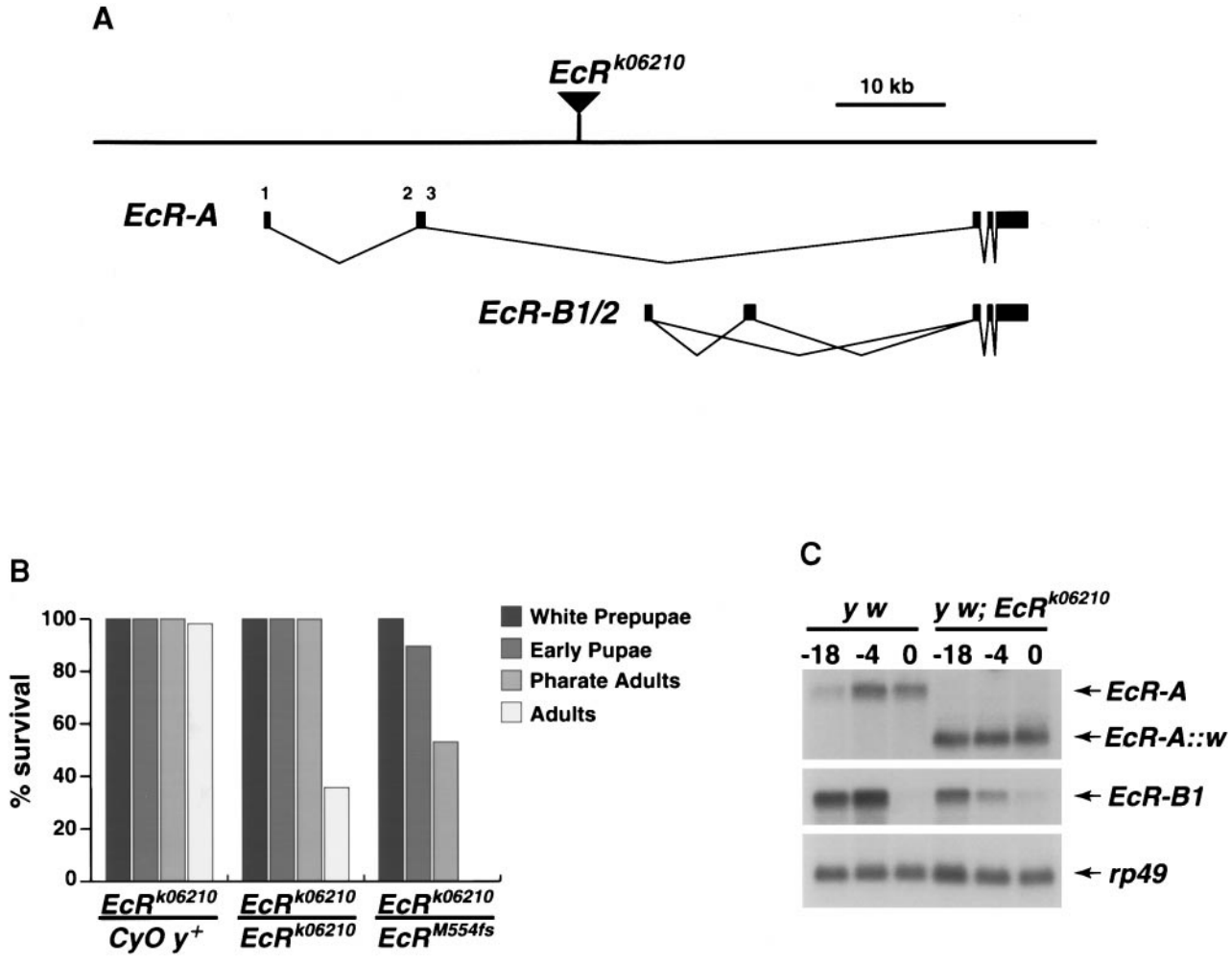

FIG. 3. Genetic and molecular characterization of the EcR^{k06210} mutation. (A) A map showing the genomic region encompassing the *EcR* gene is shown at the top (Talbot *et al.*, 1993), with the location of the *P* element insertion marked. The three *EcR* mRNA isoforms (*A*, *B1*, and *B2*) are depicted at the bottom. (B) Lethal phase analysis. Lethal phases were determined in animals of three genotypes: 1, *y w;* $EcR^{k06210}/CyO\ y^{+}$; 2, *y w;* $EcR^{k06210}/EcR^{k06210}$; and 3, *y w;* $EcR^{k06210}/EcR^{M554fs}$, essentially as described previously (D'Avino and Thummel, 1998). Eighty-five third instar larvae were selected from genotypes 1 and 2 and 96 were selected from genotype 3 and allowed to develop. White prepupae, early pupae (stage P5, after disc evagination), pharate adults, and adult flies were counted from each genotype and used to determine the percent of recovered animals. (C) The *P* element insertion affects both *EcR-A* and *EcR-B* transcription. RNA was extracted from *y w* control animals and from *y w;* EcR^{k06210} homozygous mutants as described (D'Avino *et al.*, 1995). Developmental times are shown in hours relative to puparium formation. Equal amounts of total RNA (~14 μg per lane) were fractionated by formaldehyde agarose gel electrophoresis and analyzed by Northern blot hybridization to detect *EcR-A* and *EcR-B1* transcription. Hybridization to detect *rp49* mRNA (O'Connell and Rosbash, 1984) was used as a control for loading and transfer.

and cell adhesion, we conducted a series of genetic interaction studies. We tested whether *crol* or *EcR* mutations could dominantly enhance the penetrance or expressivity of the wing blister phenotypes associated with two viable hypomorphic integrin mutations, if^{3} and mys^{nj42} (Brower and Jaffe, 1989; Wilcox *et al.*, 1989). These alleles have been used for several genetic interaction studies of cell adhesion (Wilcox, 1990; Prout *et al.*, 1997; Walsh and Brown, 1998).

Under our experimental conditions, flies hemizygous for if^{3} or mys^{nj42} have a low frequency of wing blisters (≤10%, Fig. 5), and the wing layers are completely separated (ballooned) in 1% of mys^{nj42} mutants (black bars, Fig. 5B). The three different chromosomes used to balance *EcR* and *crol* mutations—*CyO* y^{+}, *SM6b*, or *In(2LR)Gla*—did not significantly affect the frequency of blisters in either if^{3} or mys^{nj42} mutants (Fig. 5). Three strong hypomorphic *crol* mutations were used for genetic interaction experiments: $crol^{1}$, $crol^{2}$, and $crol^{3}$ (Table 1;

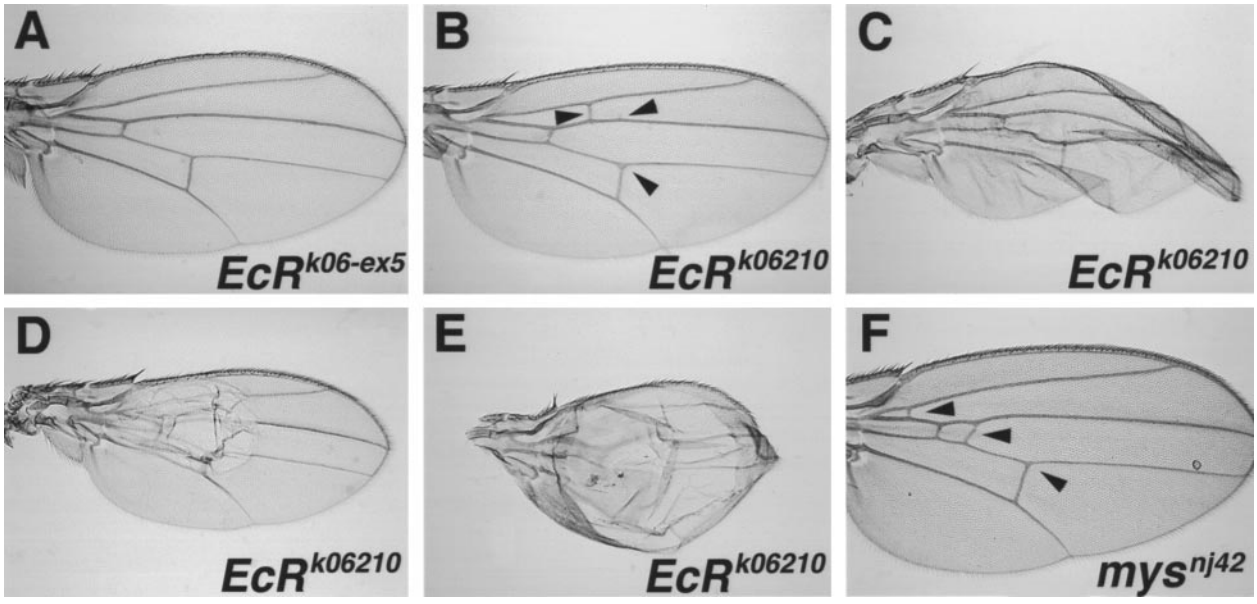

FIG. 4. EcR^{k06210} mutants display wing defects. Shown are wings dissected from an $EcR^{k06\text{-}ex5}$ control fly (A), EcR^{k06210} homozygous mutants (B–E), and a mys^{nj42} homozygous mutant (F). Wings dissected from $EcR^{k06\text{-}ex5}$ flies, a viable line obtained by mobilization of the *P* element in EcR^{k06210}, appear normal (A). The EcR^{k06210} homozygous mutant wings show several abnormalities: (B) venation defects (marked by arrowheads), (C) malformed wing, and (D) small and (E) large blisters. (F) A wing dissected from a homozygous mys^{nj42} fly with arrowheads marking additional anterior crossveins and a "delta" thickening at a vein intersection.

D'Avino and Thummel, 1998). The frequency of blisters in *if* mutants was enhanced three- to fourfold by $crol^1$ and $crol^2$ and approximately sevenfold by $crol^3$ (Fig. 5A). In contrast, no interaction was observed between mys^{nj42} and $crol^1$ or $crol^2$, whereas the frequency of blisters and balloons in mys^{nj42} mutants was increased three- to fourfold by $crol^3$, and the frequency of balloon wings increased approximately sevenfold (Fig. 5B). The increased interactions observed in a $crol^3/+$ genetic background are due largely to the *P* element insertion in the *crol* locus because a fully viable $crol^3$ excision allele, $crol^{3ex5}$, shows a significantly reduced interaction with either the *if* or *mys* mutation (Fig. 5). The $crol^3$ chromosome had also been passed through several rounds of recombination in females, reducing the probability that other mutations might contribute to the observed genetic interactions.

Two *EcR* alleles, EcR^{C300Y} and EcR^{M554fs}, were also tested for genetic interactions with if^3 and mys^{nj42}. These mutations map within the common region of the gene and appear to lack all *EcR* functions (Bender *et al.*, 1997). The frequency of blisters in *if* and *mys* mutants was enhanced approximately five- to ninefold by both *EcR* mutations and the frequency of balloon wings was increased approximately fivefold (Figs. 5A and 5B). Taken together, these results suggest that *crol*, *EcR*, and integrins function in a common pathway during wing morphogenesis.

α-Integrin Transcription Is Regulated by Ecdysone in Organ Culture

Ecdysone exerts its effects on development by triggering genetic regulatory cascades that culminate in stage- and tissue-specific patterns of target gene expression (Thummel, 1996; Richards, 1997; Segraves, 1998). As a first approach to determine whether integrin expression is regulated by ecdysone signaling, we analyzed the transcription of integrin genes in mass-isolated third instar larval organs cultured for various periods of time with ecdysone (Fig. 6). The expression patterns of α_{PS1} and α_{PS2} under these conditions appear almost identical. After ~1 h of culture in the presence of ecdysone, α_{PS1} and α_{PS2} transcript levels decrease rapidly, becoming very low by 8 h after hormone addition (Fig. 6). The α_{PS3} gene consists of two transcription units, *Long*-α_{PS3} (*L*-α_{PS3}) and *Short*-α_{PS3} (*S*-α_{PS3}), that initiate from different start sites (Stark *et al.*, 1997; Grotewiel *et al.*, 1998). Interestingly, *L*-α_{PS3} mRNA accumulates rapidly in response to ecdysone, peaking by 6–8 h after hormone addition, while *S*-α_{PS3} transcription appears unaffected by the hormone (Fig. 6). Similar to *S*-α_{PS3}, β_{PS} mRNA levels remain uniform throughout the time course. Thus, only α-integrin subunits are regulated by ecdysone in cultured larval organs, and they are either induced or repressed in response to the hormone.

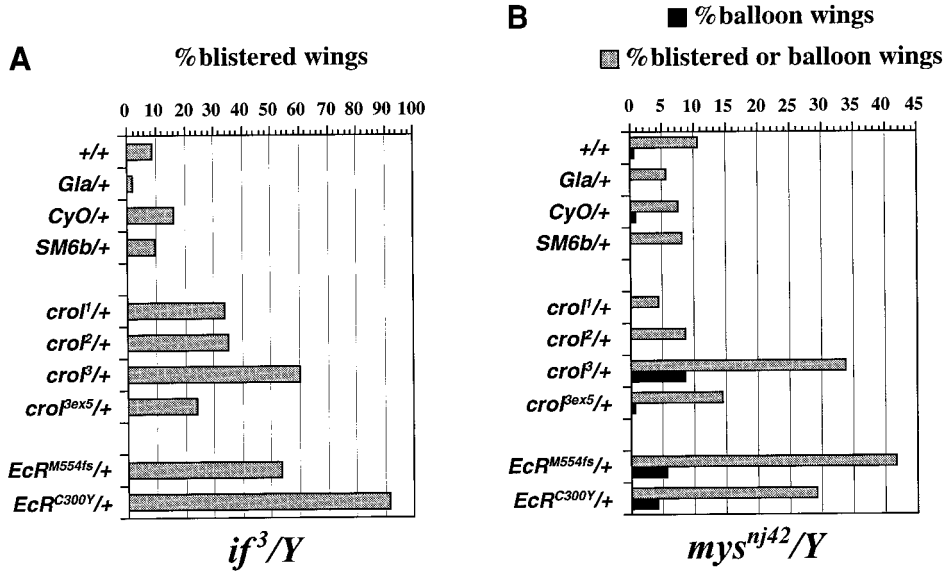

FIG. 5. *crol* and *EcR* interact genetically with *if* and *mys*. A graph displays the frequencies of wing blisters in males hemizygous for if^3 (A) or mys^{nj42} (B) and heterozygous for either a balancer chromosome—*In(2LR)Gla (Gla), CyO* y^+ *(CyO),* or *SM6b*—or for one of the *crol* or *EcR* alleles used in this study (Table 1). Fly crosses and genotypes are described in the text and under Materials and Methods. Each experiment was repeated at least twice and more than 200 flies were scored for each cross. Gray bars indicate the percentage of flies with either blisters or balloon wings, and black bars indicate the percentage of flies with balloon wings; thus the black bars are a subset of the data represented by the gray bars.

crol and EcR Mutations Affect Integrin Transcription during Pupal Development

The observation that α-integrin transcription is regulated by ecdysone in organ culture suggests that the expression of these genes might be modulated by the high titer ecdysone pulses that occur during metamorphosis. In addition, the wing phenotypes of *EcR* and *crol* mutants taken together with the genetic interactions with if^3 and mys^{nj42} raise the possibility that integrin transcription might be dependent on *EcR* and *crol* function. To test these proposals, we analyzed the temporal patterns of α_{PS1}, α_{PS2}, α_{PS3}, and β_{PS} transcription in wild-type animals as well as *crol* and *EcR* mutants. Mid (−18 h) and late (−4 h) third instar larvae were selected, as well as staged prepupae and pupae up to 72 h after puparium formation. This developmental time span encompasses the main ecdysone peaks that control metamorphosis, at −4, 10, and 24–72 h relative to puparium formation (Richards, 1981; Riddiford, 1993; Figs. 7 and 8).

Control animals were selected for analyzing the effects of *crol* and *EcR* mutations on integrin transcription—either $+/Df(2L)esc^{10}$ or $+/EcR^{M554fs}$, respectively. These staged animals allow us to determine the temporal profiles of integrin transcription during wild-type metamorphosis (Figs. 7 and 8). In agreement with the organ culture study, some aspects of α-integrin transcription appear to be regulated by ecdysone. α_{PS1} mRNA increases in abundance in 10-h prepupae and during midpupal development, in paral-

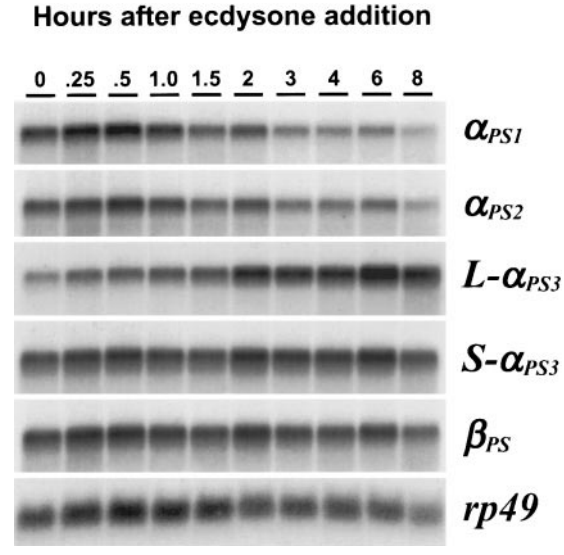

FIG. 6. α-Integrin transcription is regulated by ecdysone in cultured larval organs. Mass isolated third instar larval organs (primarily salivary glands, gut fragments, Malpighian tubules, and imaginal discs) were maintained in culture and treated with 5×10^{-6} M ecdysone for the periods of time indicated. Total RNA was then extracted and analyzed by Northern blot hybridization (~16 μg per lane) to detect integrin transcription. Hybridization to detect *rp49* mRNA (O'Connell and Rosbash, 1984) was used as a control for loading and transfer. This filter has been used previously to study the ecdysone regulation of early gene transcription (Karim and Thummel, 1991; Karim and Thummel, 1992).

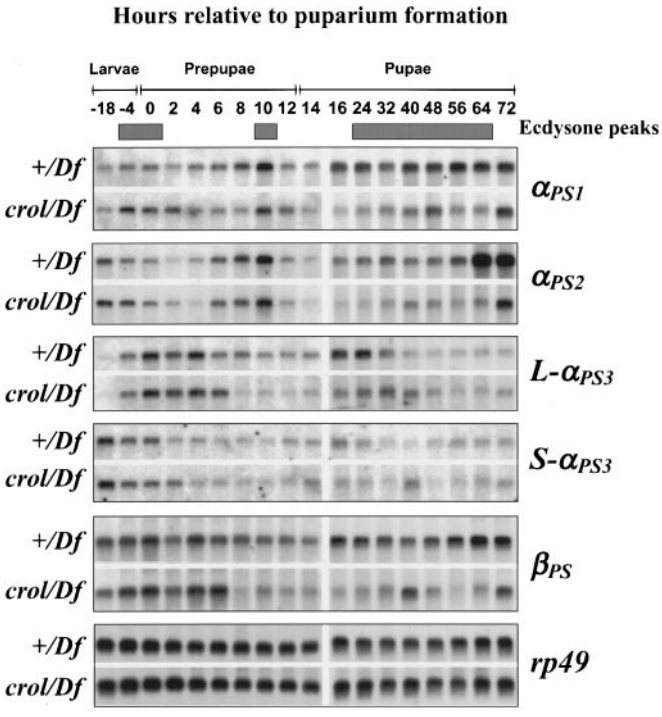

FIG. 7. Integrin transcription is dependent on *crol* function. Equal amounts of total RNA (~12 μg per lane) were isolated from *y w; +/Df(2L)esc*^{10} control animals (*+/Df*) and *y w; crol*^{3}*/Df(2L)esc*^{10} hemizygous mutants (*crol/Df*) at different stages of development and analyzed by Northern blot hybridization. Developmental times are shown on top, in hours relative to puparium formation. The peaks in ecdysone titer are listed below (Richards, 1981). Both control and mutant RNA samples from either the −18- to 14-h time points or the 16- to 72-h time points were run on one gel in order to facilitate direct comparison between the samples. Hybridization to detect *rp49* mRNA (O'Connell and Rosbash, 1984) was used as a control for loading and transfer. *crol* is expressed throughout this time course, with peaks of expression in parallel with each of the three ecdysone pulses (D'Avino and Thummel, 1998; data not shown). This experiment was performed twice with very similar results (data not shown).

lel with the pulses of ecdysone that occur at these stages (Figs. 7 and 8). α_{PS2} mRNA appears to be repressed at puparium formation, similar to the repression observed in third instar larval organs cultured with ecdysone (Fig. 6). In addition, α_{PS2} transcription peaks in late prepupae and shows a modest rise in midpupae. The increase in α_{PS2} mRNA in 6 h prepupae parallels the induction of *EcR* and *E74B*, suggesting that this is an ecdysone-induced response (Karim and Thummel, 1992). *L*-α_{PS3} transcription is significantly induced at puparium formation (Figs. 7 and 8), similar to its induction by ecdysone in cultured larval organs (Fig. 6). In contrast, *S*-α_{PS3} transcription is repressed, rather than induced, at puparium formation and remains at a constant low level throughout metamorphosis (Fig. 7), consistent with the absence of any apparent response to ecdysone in cultured larval organs.

β_{PS} regulation is more difficult to interpret. This gene is unaffected by ecdysone in cultured larval organs (Fig. 6) but displays peaks in mRNA accumulation that correlate with the late larval and pupal pulses of ecdysone (Figs. 7 and 8). In addition, *EcR* mutations interact with *mys*^{nj42}, which encodes the β_{PS} subunit (Fig. 5). Taken together, these observations suggest that β_{PS} transcription is regulated by ecdysone, but that this regulation is not detectable in organs that are cultured from late third instar larvae.

The temporal patterns of integrin transcription during metamorphosis are also dependent on *crol* and *EcR* function (Figs. 7 and 8). α_{PS1}, α_{PS2}, and *L*-α_{PS3} mRNA levels appear normal in *crol* mutant larvae and prepupae, but are reduced during pupal development (Fig. 7). The low levels of *S*-α_{PS3} mRNA seen throughout metamorphosis are unaffected by the *crol* mutation, while β_{PS} mRNA is significantly reduced in *crol* mutant prepupae and pupae (Fig. 7). Interestingly, *EcR* mutants show some similar effects on the patterns of integrin transcription as well as some differences, relative to the *crol* mutants (Fig. 8). α_{PS1} and α_{PS2} are both submaximally transcribed in *EcR* mutant pupae, similar to the

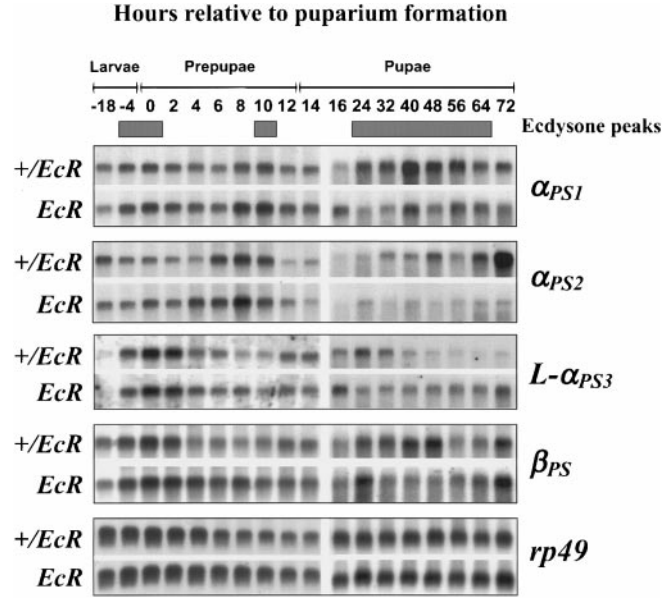

FIG. 8. Integrin transcription is dependent on *EcR* function. Equal amounts of total RNA were isolated from *y w; +/EcR*^{M554fs} control animals (*+/EcR*) and *y w; EcR*^{k06210}*/EcR*^{M554fs} mutants (*EcR*) at different stages of development and analyzed by Northern blot hybridization, essentially as described in the legend to Fig. 7. Developmental times are shown on top, in hours relative to puparium formation. The peaks in ecdysone titer are listed below (Richards, 1981). Hybridization to detect *rp49* mRNA (O'Connell and Rosbash, 1984) was used as a control for loading and transfer. This experiment was performed twice with very similar results (data not shown).

patterns observed in *crol* mutants (Figs. 7 and 8). In contrast, *L*-α_{PS3} mRNA is not significantly reduced in *EcR* mutant pupae, but rather accumulates to higher levels in late pupae (Fig. 8). β_{PS} transcription is reduced in *EcR* mutant midpupae (Fig. 8), reflecting part of the pattern seen in *crol* mutants (Fig. 7), and no effect could be detected on *S*-α_{PS3} transcription in *EcR* mutant animals (data not shown).

DISCUSSION

The remarkable series of cell shape changes associated with the formation of adult appendages during *Drosophila* metamorphosis provides an ideal model system for studying epithelial morphogenesis (Fristrom and Fristrom, 1993; von Kalm *et al.*, 1995). In addition to the effects of ecdysone on gene expression, adult tissue morphogenesis depends on hormonally induced changes in cellular architecture, including the contractile cytoskeleton, adherens junction, and contacts with the extracellular matrix (von Kalm *et al.*, 1995). These morphogenetic changes require alterations in cell adhesion as well as the transmission of signals from the cell surface to the cytoskeleton and nucleus (Waddington, 1941; Fristrom *et al.*, 1993; Brabant *et al.*, 1996). Consistent with this proposal, we provide evidence that ecdysone regulates wing morphogenesis by modulating the expression of the integrin family of cell surface receptors. These results provide a new direction for studying the role of ecdysone in regulating the development of adult appendages during *Drosophila* metamorphosis.

Ecdysone Signaling Controls Wing Morphogenesis and Cell Adhesion

The formation of a flat bilayered wing from a folded imaginal disc monolayer involves four key steps that occur twice during metamorphosis (Waddington, 1941; Fristrom *et al.*, 1993). First, the basal surfaces of the dorsal and ventral wing epithelia rearrange to appose one another. These surfaces then adhere through the formation of basal junctions, followed by expansion of the wing surface through flattening of the epithelial cells. Finally, the dorsal and ventral wing surfaces separate but remain connected by transalar arrays. The timing of these two rounds of wing morphogenesis correlates with the two major rises in ecdysone titer during metamorphosis. The first round of apposition, expansion, adhesion, and separation occurs during mid- and late prepupal stages while the second round occurs from 24 to 60 h after puparium formation, as the ecdysone titer rises dramatically during pupal development (Richards, 1981; Fristrom *et al.*, 1993). In this study, we provide evidence that ecdysone plays a critical role in regulating at least some of these morphogenetic events.

Mutations in *crol* and *EcR* cause defects in wing morphogenesis and cell adhesion, demonstrating a role for ecdysone signaling in both processes. Adult escapers that carry strong hypomorphic *crol* mutations have wing blisters as well as misshapen wings and legs (Fig. 1), and the wings of flies that carry the $crol^{1ex15}$ semilethal allele are often malformed or have abnormal venation (Fig. 2). Similar defects can be seen in adults that carry the hypomorphic EcR^{k06210} mutation (Fig. 4). In addition, a recent study by Tsai *et al.* (1999) identified a high frequency of wing defects in adult escapers that carry the hypomorphic EcR^{A483T} mutation in combination with an *EcR* null allele. It is noteworthy, however, that the additional anterior crossveins associated with *EcR* mutations have never been observed in *crol* mutant wings. Similarly, no leg defects are present in EcR^{k06210} homozygous mutants, but all $EcR^{k06210}/EcR^{M554fs}$ pharate adults have misshapen legs similar to those seen in *crol* mutant pupae (data not shown). Thus, *crol* and *EcR* appear to have overlapping, as well as unique, functions during wing and leg development. In addition, *crol* is required for maximal *EcR* expression during prepupal development and *crol* transcription is induced by ecdysone (D'Avino and Thummel, 1998), indicating that these genes do not function in a linear pathway but rather cross-regulate (Fig. 9). Taken together, these observations indicate that ecdysone signaling and *crol* function are both required for the proper development of adult legs and wings during *Drosophila* metamorphosis.

It is also interesting to note that the wings in some *EcR* and *crol* mutants appear broad (Figs. 2B, 4D, and 4E) resembling wings seen in br^1 mutants of the *Broad-Complex* (*BR-C*) (Kiss *et al.*, 1988). The *BR-C* encodes a family of ecdysone-inducible transcription factors that play a key role in imaginal disc morphogenesis and fusion during the onset of metamorphosis (Kiss *et al.*, 1988; DiBello *et al.*, 1991). The similarity in wing phenotypes between *EcR* and br^1 mutants is consistent with a role for ecdysone in

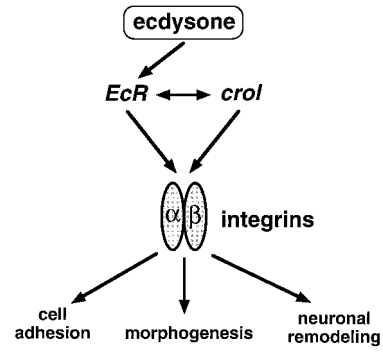

FIG. 9. A model for ecdysone-regulated integrin function during metamorphosis. Ecdysone acts through *EcR* to induce *crol* transcription, and maximal *EcR* expression is dependent on *crol* function (D'Avino and Thummel, 1998), defining a cross-regulatory circuit. Integrin transcription is dependent on both *EcR* and *crol*, positioning them downstream in the genetic cascade. This regulatory pathway may play a crucial role in several biological processes, including leg and wing morphogenesis, cell adhesion, and neuronal remodeling.

inducing the *br* function of the *BR-C* (Emery *et al.,* 1994; Bayer *et al.,* 1996). Similarly, it is possible that the reduced levels of *BR-C* expression in *crol* mutants could, at least in part, be responsible for the broad wing phenotype seen in some *crol* mutants (D'Avino and Thummel, 1998). The *BR-C* could thus mediate at least part of the effects of *EcR* and *crol* on wing morphogenesis.

The EcR^{k06210} Mutation Provides New Insights into EcR Function

The patterns of EcR protein isoform expression led Talbot *et al.* (1993) to propose that different isoforms, or combinations thereof, contribute to the tissue specificity of ecdysone responses during metamorphosis. For example, EcR-A is the predominant isoform expressed in imaginal discs while EcR-B is the predominant isoform expressed in most larval tissues that are fated to die, suggesting that these different isoforms direct the divergent fates of these target tissues. Consistent with this hypothesis, the larval salivary glands and midgut fail to enter programmed cell death in *EcR-B* mutants while the imaginal discs initiate morphogenesis (Bender *et al.,* 1997; Schubiger *et al.,* 1998). Our characterization of *EcR^{k06210}*, however, provides some evidence against this model by showing that EcR-B can direct leg and wing development in the apparent absence of EcR-A.

The *P* element insertion in *EcR^{k06210}* disrupts the *EcR-A* coding region, leading to the synthesis of a truncated mRNA. The level of *EcR-B* mRNA is also reduced in *EcR^{k06210}* mutants (Fig. 3). Based on these observations, we conclude that *EcR^{k06210}* mutants have little or no *EcR-A* function and reduced levels of *EcR-B* activity. Consistent with this proposal, and the model proposed by Talbot *et al.* (1993), most *EcR^{k06210}/EcR^{M554fs}* mutants display severe leg defects and *EcR^{k06210}* homozygotes have wing malformations. Unexpectedly, however, the remaining *EcR^{k06210}* homozygotes survive to adulthood with normal wings and legs (Fig. 3B). How can these appendages develop in the apparent absence of *EcR-A*? One possibility is that low levels of full-length *EcR-A* mRNA are produced in *EcR^{k06210}* mutants, below the level of detection by Northern blot hybridization. Alternatively, we favor the possibility that the residual *EcR-B* activity remaining in *EcR^{k06210}* mutants is sufficient to allow normal disc development in the absence of *EcR-A*. Indeed, EcR-B protein is expressed in imaginal discs, albeit at a lower level than EcR-A, and EcR-B1 is highly expressed during pupal development, when adult tissues differentiate (Talbot *et al.,* 1993). Furthermore, *EcR-B1* mutants can be rescued to adulthood by ectopic expression of either EcR-A or EcR-B, indicating that these isoforms can function in a redundant manner (Bender *et al.,* 1997). We thus propose that EcR-B can function in a redundant manner with EcR-A to direct the development of adult appendages during metamorphosis. A rigorous test of this hypothesis, however, awaits the isolation and characterization of specific *EcR-A* mutations.

Ecdysone Signaling Regulates Integrin Function during Metamorphosis

The wings of *EcR^{k06210}* mutants and *crol* mutants display blisters similar to those seen in animals that carry mutations in the integrin family of cell surface receptors (Figs. 1, 2, and 4). In addition, *EcR^{k06210}* and *crol* mutants display venation defects and *crol^{2}/crol^{3}* escapers have held-out wings, both of which resemble *mys^{nj42}* mutant phenotypes (Figs. 1B, 2, and 4) (Wilcox *et al.,* 1989). These observations prompted us to determine whether integrins might function in the ecdysone regulation of wing morphogenesis. Consistent with this proposal, both *crol* and *EcR* mutations enhance the wing phenotypes of mild hypomorphic *if* and *mys* alleles (Fig. 5). *crol^{1}*, *crol^{2}*, and *crol^{3}* each enhance the blistered wing phenotype in *if^{3}* mutants, while only *crol^{3}* interacts with *mys^{nj42}* (Fig. 5). Unlike *crol^{1}* and *crol^{2}*, which produce truncated mRNAs, *crol^{3}* directs the synthesis of a truncated mRNA that is fused to the *white* gene carried by the *P* element insertion in the *crol* locus (data not shown). This difference in *crol* alleles may, in some as yet unknown way, account for why *crol^{3}* displays stronger interactions with *if^{3}* and *mys^{nj42}* than the other two *crol* mutations.

EcR mutations also interact with integrin mutations, suggesting that ecdysone signaling plays a more general role in integrin function (Fig. 5). *EcR^{M554fs}* shows an approximate fivefold enhancement of the wing phenotype in *if^{3}* and *mys^{nj42}* mutants while *EcR^{C300Y}* displays a much stronger interaction than *EcR^{M554fs}* with *if^{3}*. It is unclear why these *EcR* mutations show allele-specific interactions, although they do affect different *EcR* functions. *EcR^{C300Y}* is a missense mutation in the DNA binding domain while *EcR^{M554fs}* is a small deletion within the ligand binding domain (Bender *et al.,* 1997). Taken together, these genetic interaction studies indicate that *crol*, *EcR*, and integrins function in a common developmental pathway to regulate wing morphogenesis.

Ecdysone Signaling Regulates Integrin Expression during Metamorphosis

Both the ecdysone–receptor complex and *crol* regulate downstream gene expression in the ecdysone-triggered cascades that control metamorphosis (Bender *et al.,* 1997; D'Avino and Thummel, 1998). Given this function, the simplest model for interpreting the genetic interactions of *EcR* and *crol* mutations with integrin mutations would be to position the integrins downstream from *EcR* and *crol* in the ecdysone genetic hierarchy (Fig. 9). Consistent with this model, α_{PS1}, α_{PS2}, and αL-$_{PS3}$ are regulated by ecdysone in cultured larval organs and some changes in their temporal pattern of expression correlate with the ecdysone titer profile during metamorphosis (Figs. 6–8). Most notably, α_{PS2} transcription is repressed by ecdysone and αL-$_{PS3}$ transcription is induced by ecdysone in cultured larval organs, paralleling their responses to the late larval ecdysone pulse at the onset of metamorphosis. In addition, proper α_{PS1}, α_{PS2}, and $L\alpha$-$_{PS3}$ transcription during metamorphosis is dependent on *crol* and *EcR* function (Figs. 7 and 8). Taken together,

these results support the model that α_{PS1}, α_{PS2}, and $L\text{-}\alpha_{PS3}$ integrin expression is regulated by ecdysone during metamorphosis (Fig. 9).

It is interesting to note that the effects of *crol* and *EcR* mutations on integrin transcription are largely restricted to pupal stages during metamorphosis (Figs. 7 and 8). This timing correlates with the stage at which Brabant *et al.* (1996) demonstrated an essential role for integrins in the reapposition of the dorsal and ventral wing surfaces. These authors conclude that integrins function at this stage primarily as adhesion receptors, facilitating the formation of basal extensions and the cell shape changes required for reapposition. Based on their studies, we propose that at least one mechanism by which *crol* and *EcR* exert their effects on wing morphogenesis is to direct normal levels of integrin expression during this critical period of wing development.

Although our Northern blot hybridizations indicate a role for ecdysone in regulating integrin expression in whole animals, they do not address the tissue specificity of this regulation in imaginal discs. This is a difficult issue to address. It takes ~100 leg imaginal discs to provide sufficient RNA for one lane of a Northern blot (R. Ward, personal communication). Efforts to use RT-PCR as a means of quantitating mRNA in isolated tissues have, in our experience, been unable to reproducibly detect modest changes in expression levels, such as those depicted in Figs. 7 and 8. Furthermore, immunohistochemical stains of imaginal discs dissected from late prepupal through midpupal stages are complicated by the impermeable cuticle that is laid down at this time. Nevertheless, defining the ecdysone regulatory cascades in imaginal tissues is a critical step in our understanding of the hormonal regulation of adult development. It seems likely that DNA microarray technology will provide a powerful new method to facilitate our understanding of the genetic regulatory cascades activated by ecdysone in imaginal tissues (White *et al.*, 1999).

A Model for Ecdysone-Mediated Integrin Functions during Metamorphosis

Our findings suggest that altered integrin gene expression in *crol* and *EcR* mutants lead to the defects that we observe in wing morphogenesis and cell adhesion. However, integrins also function in a wide range of other biological pathways during development, including tissue morphogenesis, cytoskeletal reorganization, memory, and gene expression (Hynes, 1992; Brown, 1993; Stark *et al.*, 1997; Bloor and Brown, 1998; Grotewiel *et al.*, 1998; Martin-Bermudo and Brown, 1999). These widespread functions raise the possibility that ecdysone-regulated integrin expression may control multiple events during metamorphosis (Fig. 9). For example, the if^{V2} semilethal allele displays a misshapen leg phenotype that resembles the defective legs seen in *crol* mutants, indicating that α_{PS2} functions may be recruited by the ecdysone pathway to regulate leg morphogenesis (Figs. 1E and 9) (Bloor and Brown, 1998). Furthermore, since α_{PS3} has been proposed to mediate synaptic rearrangements (Grotewiel *et al.*, 1998), its ecdysone-induced expression in late third instar larvae may contribute to the extensive neuronal remodeling that occurs in the central nervous system during metamorphosis (Fig. 9) (Truman, 1996). Further studies of the tissue-specific functions of integrins during metamorphosis will provide a better understanding of how these critical cell surface receptors exert their multiple effects during development.

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