Molecular and Genetic Characterization of the *Drosophila melanogaster* 87E Actin Gene Region

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ABSTRACT

A combined molecular and genetic analysis of the 87E actin gene (*Act87E*) in *Drosophila melanogaster* was undertaken. A clone of *Act87E* was isolated and characterized. The *Act87E* transcription unit is 1.57 kb and includes a 556-base intervening sequence in the 5′ leader of the gene. The protein-coding region is contiguous and encodes a protein that is >93% identical to the other Drosophila actins. By *in situ* hybridization with a series of deficiencies that break in 87E, *Act87E* was localized to a region encompassing one to three faint, polytene chromosome bands. The region between the deficiency endpoints that flank the actin gene was isolated and measures approximately 24–30 kb. The closest proximal deficiency endpoint lies 8–10 kb 5′ to the actin gene; the closest distal deficiency endpoint lies 16–20 kb 3′ to the actin gene. A single, recessive lethal complementation group lies between the deficiency endpoints that flank the actin gene. An EMS mutagenesis screen produced four additional members of this recessive lethal complementation group. Molecular analysis of the members of this complementation group indicated that two of the newly induced mutations have deletions of approximately 1 kb in a transcribed region 4–5 kb 3′ (distal) to the actin gene. This result suggests that the recessive lethal complementation group represents a gene separate from and distal to the actin gene. The mutagenesis screen failed to identify additional recessive lethal complementation groups in the actin gene-containing region. The implications of the failure to identify recessive lethal mutations in the actin gene are discussed in reference to studies of other conserved multigene families and other muscle protein mutations.

ACTIN is a major contractile protein found in all eukaryotic cells, accounting for 1–2% of the total cellular protein. As the major component of thin filaments, actin is one of the primary proteins responsible for muscle contraction. In addition, actin is critical to many cellular processes, including cell motility, cytokinesis and morphogenesis (for reviews, see POLLARD and WEIHING 1974; KORN 1978). In all eukaryotes studied, with the exception of yeast, different forms of actin are encoded by highly conserved multigene families whose members are expressed differentially, exhibiting temporal and/or positional specificity. The evolution of these multigene families has allowed diversification of actin expression and/or function.

The *Drosophila melanogaster* actin gene family consists of six highly conserved genes, dispersed throughout the genome (TOBIN *et al.* 1980; FYRBERG *et al.* 1980), that exhibit both developmental stage- and tissue-specific expression. The expression patterns of the individual genes have suggested differing functional roles for the various actins. In particular, the 5C and 42A actin genes (*Act5C* and *Act42A*) appear to encode cytoplasmic actins; the 79B gene (*Act79B*), muscle actins in adult leg and thorax; the 57B and 87E genes (*Act57B* and *Act87E*), intersegmental muscle actins in larvae and adults; and the 88F gene (*Act88F*), a flight-muscle-specific actin (ZULAUF *et al.* 1981; SANCHEZ *et al.* 1983; FYRBERG *et al.* 1983). Mutants in the *Act88F* gene exhibit a range of flight defects, but are otherwise normal, supporting the conclusion that the 88F actin functions specifically in flight muscles (KARLIK, COUTU and FYRBERG 1984; MAHAFFEY *et al.* 1985; HIROMI and HOTTA 1985).

Since no mutations have yet been identified in the other five Drosophila actin genes, their exact roles and the extent of their functional overlap is still uncertain. To address questions concerning the specific function and expression of the 87E actin gene, we undertook a molecular and genetic analysis of the 87E actin gene and its flanking regions. We chose the *Act87E* gene because there were already many recessive lethal and readily visible mutations in 87E, as a result of extensive genetic analysis of the region surrounding the *rosy* locus (HILLIKER *et al.* 1980; 1981). We examined the existing lethal mutations in this region and generated a large collection of new ones in an attempt to identify mutants in the 87E actin gene. In the course of these studies we also obtained new molecular information about the 87E actin gene.

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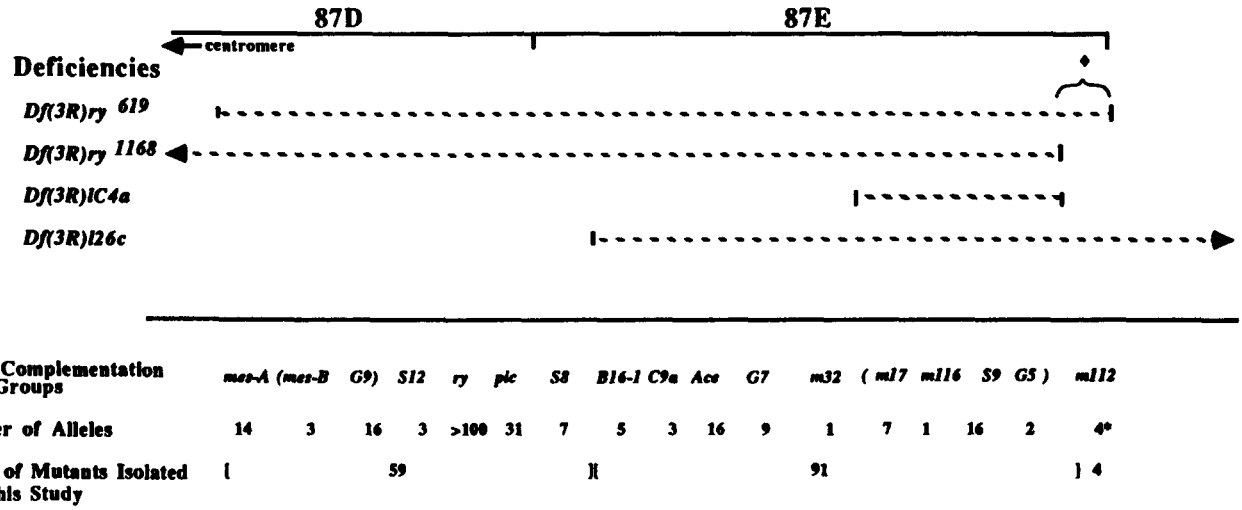

FIGURE 1.—Summary of the relevant deficiencies and complementation groups of the polytene chromosome region 87D7-9 to E12-F1. The dashed horizontal lines represent the chromosomal regions that are missing in the deficiencies. The positions of the known complementation groups with the number of alleles in each complementation group are noted at the bottom of the figure. The centromere is to the left. The *Act87E* gene maps to the most distal region of 87E by *in situ* hybridization (indicated by ♦). The number of mutants isolated in this study is indicated at the bottom of the figure. The brackets indicate the position of these newly isolated mutants with respect to the *Df(3R)l26c*, *Df(3R)ry*^{1168}, and *Df(3R)ry*^{619} endpoints. [Adapted from HILLIKER *et al.* (1980), Figure 1.]

MATERIALS AND METHODS

Drosophila strains: Previously isolated recessive lethals and deficiency strains used for *in situ* hybridization, deletion mapping, and mutant screens are described in HILLIKER *et al.* (1980, 1981). The breakpoints for the various deficiencies used in this study are as follows: *Df(3R)ry*^{619}—87D7-9; 87E12-F1, *Df(3R)ry*^{1168}—87B15-C1; 87E9-12, *Df(3R)lC4a*—87E5-7; 87E11-F1, and *Df(3R)l26C*—87E1-2; 87F11-12 (HILLIKER *et al.* 1981). Additional genetic markers used are described in LINDSLEY and GRELL (1968).

Screens for recessive lethal mutations uncovered by *Df(3R)ry*^{619}: Prior to mutagenesis, and iso-3 *kar*^{2}*ry*$^{+11}$/*kar*^{2}*ry*$^{+11}$ stock was constructed by standard crosses. *kar*^{2}*ry*$^{+11}$/*kar*^{2}*ry*$^{+11}$ males were treated with ethyl methane sulfonate (EMS) (LEWIS and BACHER 1968) and then mated with *TM6, Ubx/Tp(3)MKRS, M(3)S34 kar ry*^{2} *Sb* females at 25°. Treated males were removed after 4–5 days. Single F_1 males carrying a mutagenized *kar*^{2}*ry*$^{+11}$ third chromosome heterozygous with the *MKRS* balancer were mated to *Df(3R)ry*^{619}/*MKRS* females. The *kar, Sb* flies from F_2 vials that lacked non-*Sb* progeny (*kar*^{2}*ry*$^{+11}$/*Df(3R)ry*^{619}) were saved as potential recessive lethals uncovered by *Df(3R)ry*^{619}. Balanced stocks heterozygous for the newly induced recessive lethal and *MKRS* were established by standard crosses and were classified by their complementation pattern with *Df(3R)l26c/MKRS*, *Df(3R)ry*^{1168}/*MKRS*, and *Df(3R)ry*^{619}/*MKRS* (Figure 1).

Complementation tests: For complementation tests between two recessive lethal mutants *m1* and *m2*, the progeny of matings between *m1/MKRS* and *m2/MKRS* were counted. If 5% or less of the offspring were *Sb*$^{+}$ then the mutations were considered to be non-complementing. The only exception to this is in tests with the allele *l(3)ml12*. *l(3)ml12* is a leaky mutation because *l(3)ml12/Df* flies were partially viable—1.3% of the total offspring of matings between *l(3)ml12/MKRS* and *Df(3R)ry*^{619} were *Sb*$^{+}$. For this reason, mutations were considered to be noncomplementing with *l(3)ml12* if less than 8% of the total offspring were *Sb*$^{+}$. All matings for complementation tests were performed at 21–25°.

Isolation of the *Act87E* Gene: An Oregon R genomic plasmid library was screened with an *Act88F* gene (K1a) probe (TOBIN *et al.* 1980) according to a modification of the GRUNSTEIN and HOGNESS (1975) procedure described by CRAIG, MCCARTHY and WADSWORTH (1979). Of the five clones that hybridized to K1a, one clone (pK60) labeled 87E upon *in situ* hybridization to salivary gland polytene chromosomes. Using a nonprotein-coding region of pK60 as a probe, S. TOBIN isolated (and generously provided us with) Canton S clones of the *Act87E* gene and flanking regions (λ8, λ12, λ24) from a wild-type phage library (MANIATIS *et al.* 1978).

Primer extensions and S1 nuclease experiments: RNA isolation and primer extension experiments were performed as described in CRAIG, INGOLIA and MANSEAU (1983). Primer extensions utilized hybrids of larval RNA and an end-labeled 92 bp *Hin*fI fragment of pK60 that is located 62 bases into the protein-coding region.

Hybridizations for the S1 nuclease experiments were performed as described in the primer extension protocol using larval poly(A)$^{+}$ RNA and an end-labeled 770 bp *Bgl*II fragment of pK60 that crosses the 5′ end of the protein-coding region. S1 nuclease digestions of the RNA-DNA hybrids were conducted following the protocol of BERK and SHARP (1977). The digestion products were analyzed on a 6% polyacrylamide-urea gel.

DNA sequencing: The DNA sequence of the *Act87E* gene and of the ends of the cDNA clone inserts was determined by the dideoxy chain termination sequencing method (SANGER, NICKLEN and COULSON 1977) using an Amersham dideoxy sequencing kit. The Canton S isolate of the *Act87E* gene was sequenced from the pBR322

subclone of λ24 that contains the actin gene (p24-RI-2). All bases were sequenced on both strands and all cloning sites were sequenced across. Manipulation and analysis of the sequence data was performed using the Sequence Analysis Software Package of the University of Wisconsin Genetics Computer Group.

Screening phage libraries: Phage libraries were screened using the modification of the BENTON and DAVIS 1977) *in situ* hybridization technique described in MANIATIS *et al.* (1978). cDNA clones were obtained by screening a pupal library (POOLE *et al.* 1985) with an *Act87E* gene-specific, single-stranded, RNA probe from the 3′ end of the gene (pK60 LS). cDNA clones that also contain the 5′ end of the gene were identified by screening phage that hybridized to the 3′ end probe with an end-labeled 15 base synthetic oligonucleotide corresponding to a sequence in the 5′ untranslated leader of the gene (5′-CTTGGCTACTGGCTG-3′).

Southern blot analysis of mutant fly DNA: Genomic DNA was isolated from adult flies by a procedure of R. LIFTON (cited and described in BENDER, SPIERER and HOGNESS 1983). Typically, 3–4.5 μg DNA were digested with restriction endonuclease, size fractionated by electrophoresis through a 1% agarose gel, and transferred to nitrocellulose (SOUTHERN 1975).

Filter hybridizations: Nitrocellulose filters for Southern blots or screening phage libraries were prehybridized for at least 3 hr in 50% formamide, 5× SSC, 5% dextran sulfate, 5× Denhardt's solution, 10 mM Tris, pH 7.5, 1 mM EDTA, 0.1% sarkosyl, 0.1 mg/ml denatured calf thymus DNA. The filters were incubated with radiolabeled probe for at least 15 hr in the above solution with 10% dextran sulfate. If DNA probes were used, incubations were at 37–42°; if RNA probes were used, incubations were at 45–50°. The filters were washed as in MANIATIS, FRITSCH and SAMBROOK (1982).

Identification of the genomic region that encodes the first exon: To identify the genomic region that encodes the first exon, a single-stranded probe was made following standard protocols from an M13mp18 *Eco*RI subclone of a full length cDNA clone of the *Act87E* gene that was oriented with the 5′ end of the gene next to the M13mp18 priming region. The DNA was then digested with *Bgl*II, denatured, and electrophoresed on a 6% polyacrylamide-urea gel. After autoradiography, the *Bgl*II fragment corresponding to the 5′ end of the gene was excised from the gel and eluted by shaking overnight at 37° in 0.5 M ammonium acetate, 1 mM EDTA, pH 8.0. Hybridization of this probe with a Southern blot of digests of a pK60 *Bam*HI-*Hin*dIII subclone that is 5′ to the protein coding region identified the region that encodes the first exon of the actin gene.

Strategy for the chromosomal walk: A "chromosomal walk" (BENDER, SPIERER and HOGNESS 1983) was undertaken to the $Df(3R)ry^{619}$ endpoint on the distal side of the actin gene and to the closest deficiency endpoint on the proximal side of the actin gene (either the distal breakpoint of *Df(3R)lC4a* or $Df(3R)ry^{1168}$). The distal step in the chromosomal walk utilized nick-translated p12-R1-5 to screen a Canton S Drosophila library (MANIATIS *et al.* 1978). The $Df(3R)ry^{619}$ breakpoint was localized by *in situ* hybridization to a region 16–20 kb distal to the 3′ end of the actin gene. In hybridizations of $Df(3R)ry^{619}$/*MKRS* chromosomes with λ12 or p12-R1-5 (see Figure 8) grains are observed only over the *MKRS* chromosome (MANSEAU 1986), while the probe λD311 (see Figure 8) labels both the $Df(3R)ry^{619}$ and *MKRS* chromosomes (MANSEAU 1986). This indicates that λ12 and p12-R2-5 contain DNA sequences that are mainly within the deleted region of $Df(3R)ry^{619}$ and λD311 contains DNA sequences that are beyond the deleted region of $Df(3R)ry^{619}$.

The $Df(3R)ry^{1168}$ endpoint is the closest breakpoint on the proximal side of the actin gene and has been localized by Southern blot analysis to a region 8–10 kb 5′ to the gene. A Southern blot of *Eco*RI-digested genomic DNA probed with nick-translated p24-R1,H3-16 (see Figure 8) has a novel fragment resulting from a deficiency breakpoint within the region of the probe (MANSEAU 1986). This novel fragment is not detected with a subclone of the most proximal sequences of p24-R1,H3-16, confirming the presence of a deficiency in this region (MANSEAU 1986). The *Df(3R)lC4a* chromosome is not deficient in this region as *in situ* hybridization with p24-R1,H3-16 as a probe labels both third chromosomes of *Df(3R)lC4a/MKRS* (data not shown).

RESULTS

Isolation and structural analysis of *Act87E* Gene Clones: The *Act87E* gene was isolated to analyze the gene structurally and to provide the basis for identifying mutants. An *Act87E* gene clone (pK60) was identified by *in situ* hybridization to polytene chromosomes (BONNER and PARDUE 1976) from among clones that hybridized to an 88F actin protein-coding region probe (Figure 2). The restriction map of the *Act87E* gene is shown in Figures 8 and 9. Because of the high degree of homology among actins, comparison of the *Act87E* gene map with restriction maps of other Drosophila actin gene clones (TOBIN *et al.* 1980; FYRBERG *et al.* 1981) and hybridization of protein-coding region probes from the *Act88F* gene to restriction enzyme digests of the 87E clone allowed us to identify the approximate position of the actin protein-coding region and the direction of transcription.

The actin gene is located in 87E9–12: In their analysis of the *ry* locus [located at 87D(11–13)], HILLIKER *et al.* (1980, 1981) identified 11 complementation groups in the 87E region, all but four of which are separable by deletion endpoints. Each complementation group contains from 1 to 16 alleles, adding up to a total of 74 lethals in the entire region. As a first step in determining whether any of these complementation groups result from alterations in the actin gene, it was necessary to define the location of the actin gene with respect to the deficiency endpoints that divide the 87E region. Any complementation groups mapping cytologically to the region containing the actin gene would be candidates for actin mutants.

In situ hybridization (BONNER and PARDUE 1976) of ^{3}H-cRNA from the *Act87E* clone pK60 to polytene chromosomes of the deletion strains that divide the 87E region localized the actin gene relative to the endpoints of these deletions. There was no apparent hybridization to the $Df(3R)ry^{619}$ chromosome, but there was to the *Df(3R)lC4a* and $Df(3R)ry^{1168}$ chro-

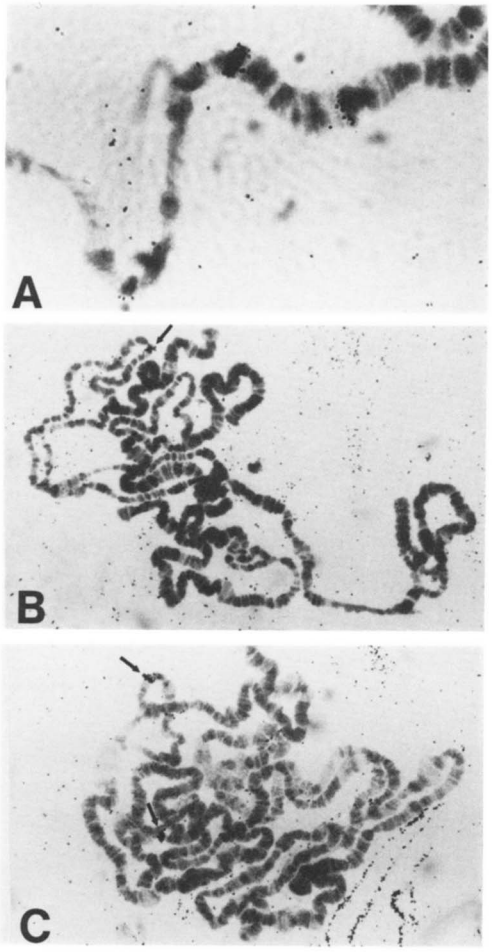

FIGURE 2.—*In situ* hybridization of ^{3}H-labeled cRNA synthesized from the clone pK60 A, High magnification view of the grains at the 87E region. B and C, *In situ* hybridization to polytene chromosomes of deletion stocks. Because the deletion chromosome is heterozygous with the *MKRS* balancer chromosome, asynapsis and separation of the third chromosome homologues occurs. Hybridization to the *Act87E* locus on both third chromosomes indicates that the deletion does not remove the actin gene, while hybridization at only one of the loci indicates that the deletion does remove the actin gene. B, $Df(3R)ry^{619}/MKRS$; C, $Df(3R)ry^{1168}/MKRS$. The pattern of labeling seen with $Df(3R)lC4a$ is the same as that seen in C. Hybridizations included 300,000 cpm/slide. Exposure times were 5–10 days.

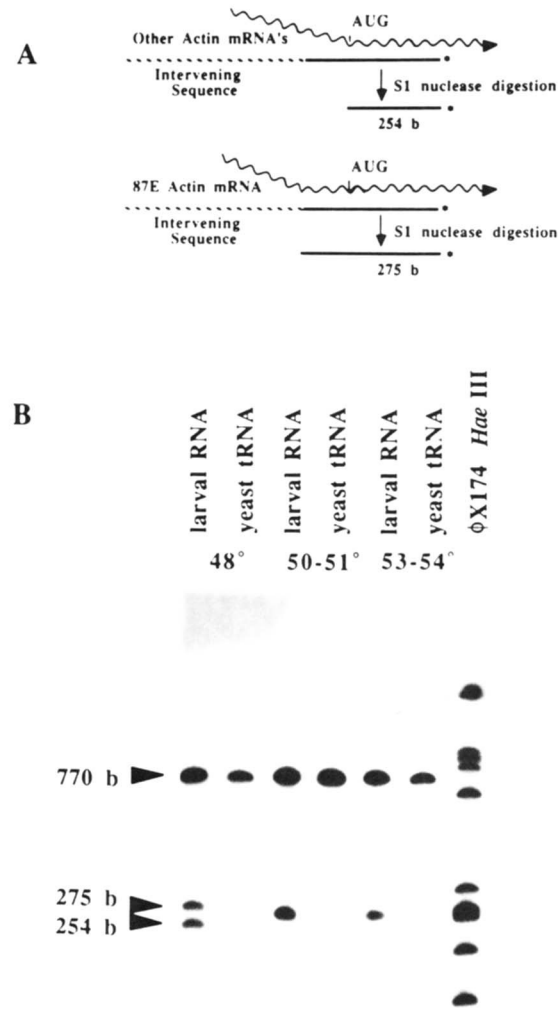

FIGURE 3.—S1 nuclease experiments. A, Diagrammatic representation of proposed hybrids and S1 nuclease digestion products. The probe used is the 770 b pK60 *Bgl*II fragment that includes the 5′ end of the protein-coding region. The end-labeled probe (indicated by the straight line, dashed line indicates the proposed intervening sequence) is annealed with larval RNA (wavy line) and then digested with S1 nuclease. The result of digestion of hybrids between the probe and mRNA from the other (non-87E) Drosophila actin genes is shown at the top of A. The result of digestion of hybrids between the probe and mRNA from the *Act87E* gene is shown at the bottom of A; B, S1 nuclease digestions of hybrids of the end-labeled 770 b pK60 *Bgl*II fragment hybridized with larval poly(A)$^+$ RNA. The temperatures indicated are the hybridization temperatures. The largest molecular weight band is the original DNA probe, while the next two lower molecular weight bands result from S1 cleavage. The lower molecular weight band (254 b), not present at higher hybridization stringenecies, corresponds to an S1-protected region extending to the 5′ end of the protein-coding region. This band presumably results from hybrids between the protein-coding region of the probe and RNA's transcribed from the other Drosophila actin genes. The larger band (275) corresponds to a hybrid that includes approximately 21 bases 5′ to the protein-coding region, as well as the protein-coding region. This band is the only one present at higher hybridization stringency, suggesting that this hybrid results from RNA transcribed from the *Act87E* gene. 20 μg of yeast tRNA was used in control experiments. The size marker is ϕX174 DNA cut with *Hae*III.

mosomes (Figure 2). Thus, *Act87E* was localized to a region distal to the right endpoints of $Df(3R)ry^{1168}$ (87E9-12) and $Df(3R)lC4a$ (87E11-F1) and proximal to the right endpoint of $Df(3R)ry^{619}$ (87E12-F1). This is a very small chromosomal region (87E9–12) comprising, at the most, three faint polytene chromosome bands.

Position of the actin gene with respect to the $Df(3R)ry^{619}$, $Df(3R)ry^{1168}$, and $Df(3R)lC4a$ breakpoints: To determine more precisely the location of the entire actin gene between the distal breakpoints of $Df(3R)ry^{1168}$ and $Df(3R)lC4a$ and the distal breakpoint of $Df(3R)ry^{619}$, the 5′ and 3′ ends of the actin gene were localized and the position of the actin gene

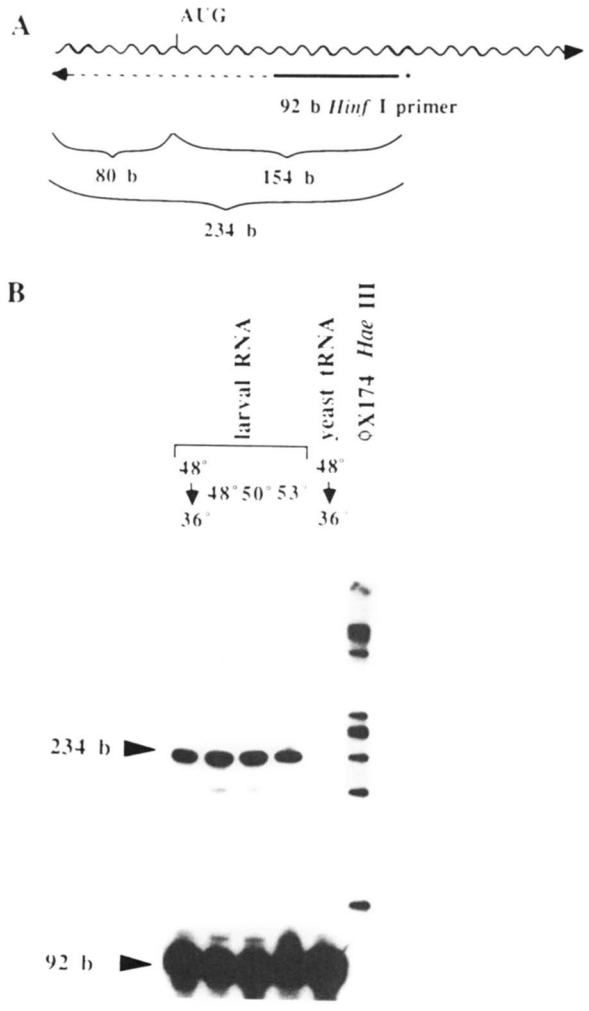

FIGURE 4.—cDNA extension experiment, A, Diagrammatic representation of the cDNA extension experiment. The primer is a 92 b *Hin*fI fragment that is located 62 bp 3′ to the 5′ end of the protein-coding region. The end-labeled primer (solid line) is annealed with larval RNA (wavy line). Reverse transcriptase extends the primer (dashed line) using the hybridized RNA as a template. B, cDNA extensions of hybrids between a 92 b *Hin*fI fragment in the protein-coding region of the actin gene and 200 μg of larval RNA. The 234 b extended primer indicates the presence of an 80 b transcribed leader. The temperatures indicated are the hybridization temperatures. 48° → 36° indicates that the hybridization was cooled slowly from 48° to 36°. 20 μg of yeast tRNA was used in control experiments. The size marker is ϕX174 DNA cut with *Hae*III.

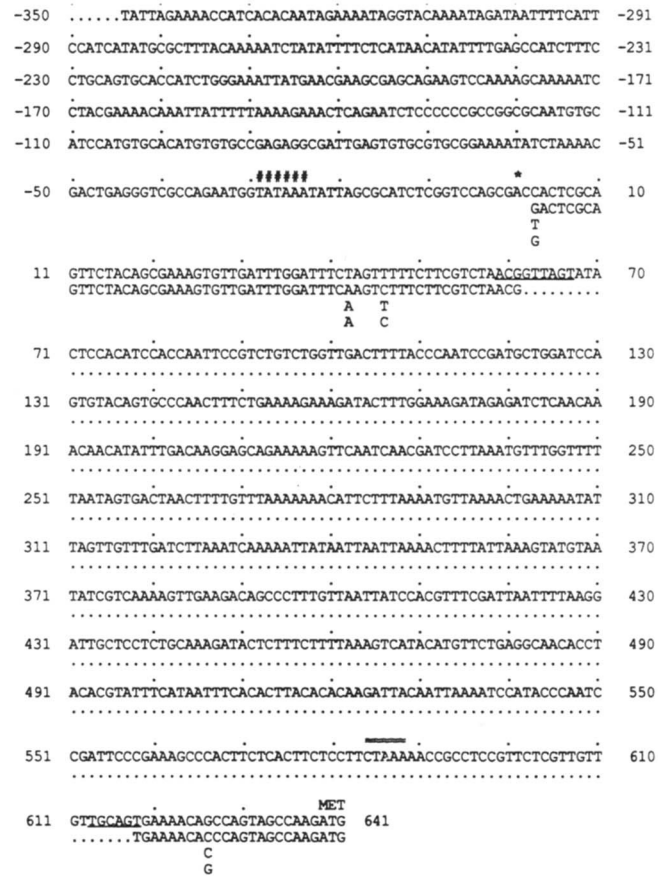

FIGURE 5.—Comparison of the DNA sequence of genomic and cDNA clones in the region 5′ to the protein-coding region. The top sequence is from p24-R1-2, a Canton S genomic clone, while the second sequence is from an Oregon R cDNA clone. Positions where the three cDNA clones sequenced differ from each other or from the genomic sequence are indicated in the third and fourth rows of a numbered DNA segment. The proposed transcription initiation site (*) is numbered as +1. The 556 bp intervening sequence begins after +61 and ends at +617. Regions similar to 5′ and 3′ intervening splice junction consensus sequences are underlined. A 3′ splice signal is indicated by ≈≈≈. A sequence resembling a TATA box is marked by #.

transcription unit with respect to the deficiency endpoints was established (as described in the following four sections).

Localization of the 5′ end of the actin transcript: The results of S1 nuclease and primer extension experiments suggested the presence of an intervening sequence in the 5′ transcribed, non-translated leader region. S1 nuclease experiments indicated that there are approximately 21 bases contiguous with the 5′ end of the protein-coding region present in the *Act87E* mRNA (Figure 3). Primer extension of hybrids between a fragment from the *Act87E* protein-coding region and larval RNA indicate the presence of a transcribed leader of approximately 80 bases (Figure 4). This result, together with the data from the S1 nuclease experiments, strongly suggests the presence of an intervening sequence that ends approximately 21 bases 5′ to the initiating AUG and predicts that approximately 59 bases are transcribed 5′ to the start of the intervening sequence. DNA sequence comparison of full length cDNA clones with the genomic clone in the region 5′ to the protein-coding region established the presence of an intervening sequence 21 bases 5′ to the beginning of the protein-coding region (Figure 5).

Figure 5 shows the genomic DNA sequence of the region that encodes the 5′ end of the mRNA aligned with the sequence of the cDNA clones. The intervening sequence is 556 bp long. Sequences corresponding to the consensus sequence for 5′ intervening

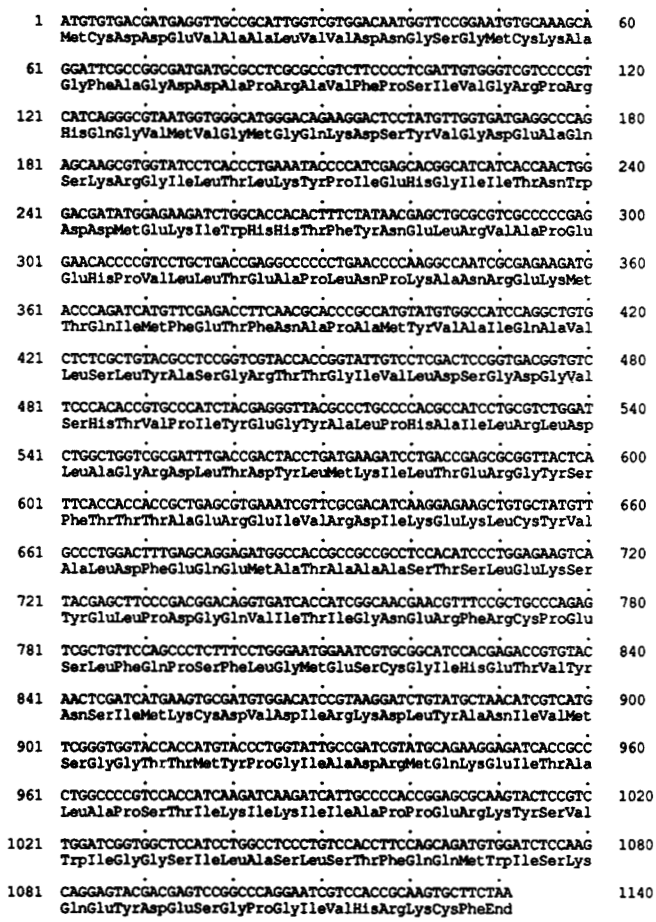

FIGURE 6.—The DNA sequence and predicted amino acid sequence of the protein-coding region of the *Act87E* gene.

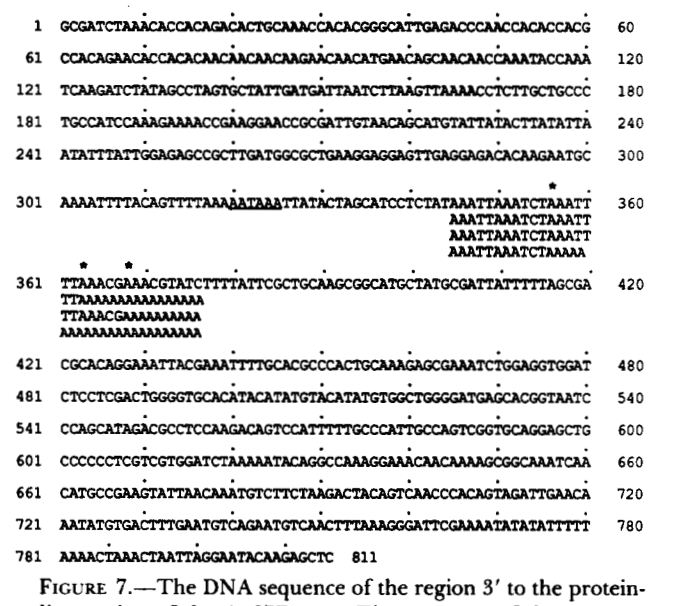

FIGURE 7.—The DNA sequence of the region 3′ to the protein-coding region of the *Act87E* gene. The sequence of the genomic clone p12-R1–2 is shown. The sequences of the 3′ ends of three cDNA clones are shown aligned with the genomic sequence. The base immediately past the stop codon is labeled +1. A polyadenylation signal is underlined. Polyadenylation sites, identified in cDNA clones, are indicated by *. Poly(A) runs found at the ends of the cDNA clones are denoted by small A's.

sequence splice junctions [*Act87E*: ACG/GTTAGT; consensus: $^{C}_{A}$AG/GT$^{A}_{G}$AGT (MOUNT 1982)], 3′ intervening sequence splice junctions [*Act87E*: TGCAG/T; consensus: $(^{T}_{C})_{n}$N$^{C}_{T}$AG/G (MOUNT 1982)], and 3′ intervening sequence splice signals [*Act87E*: CTAAA; consensus: CTAAT (KELLER and NOON 1984)] are present. All three cDNA clones end 59 bp upstream from the splice junction. The sequence at this position (ACTCGCA) does not correspond well with the Drosophila CAP-consensus sequence [ATCA$^{G}_{T}$T$^{C}_{T}$ (HULTMARK, KLEMENZ and GEHRING 1986)], but two bases upstream from this site there is a reasonable match with the CAP-consensus (ACCACTC). We propose the upstream site as the transcription initiation site. The size of the predicted transcribed leader is 79 bases long. This corresponds well with the S1 nuclease and primer extension results. A sequence resembling a TATA box (GOLDBERG 1979) begins 23 bases upstream from the proposed transcription initiation site.

DNA sequence of the actin 87E protein-coding region: The DNA sequence of the protein-coding region of the *Act87E* gene is shown in Figure 6. Comparison with published actin sequences indicates that the protein-coding region is continuous. The protein-coding regions of the Drosophila actin genes are, as expected, highly conserved, with the majority of the DNA sequence differences between the genes lying in the third base codon position. The *Act79B* and *Act88F* genes, which have been completely sequenced (SANCHEZ *et al.* 1983), are 87% (different at 145 bp out of 1131 bp) and 88% (different at 132 bp out of 1131 bp) identical to the *Act87E* gene at the DNA level. The remaining genes are slightly less similar to the *Act87E* gene: the *Act5C* gene is 85% identical (different at 47 bp out of 326 bp); the *Act42A* gene is 83% identical (different at 161 bp out of 926 bp); and the *Act57B* gene is 83% identical (different at 28 bp out of 161 bp) (sequence data for the 5C, 42A and 57B genes is from FYRBERG *et al.* (1981).

The Drosophila actins are greater than 90% identical at the amino acid level. The 79B and 88F actins are both 96% identical to the 87E actin, differing at 14 of 376 amino acid positions from the 87E actin (SANCHEZ *et al.* 1983). The 57B actin differs at only 3 positions from the 87E actin (FYRBERG *et al.* 1981; E. A. FYRBERG, personal communication). The 5C actin, which differs at 3 out of 108 amino acids, is 97% identical to the 87E actin, and the 42A actin, which differs at 13 out of 198 amino acids, is 93% identical to the 87E actin (FYRBERG *et al.* 1981).

Localization of the 3′ end of the actin transcript: Comparison of the DNA sequence of the actin cDNA clones with that of the genomic clone in the region 3′ to the protein-coding region indicates that the

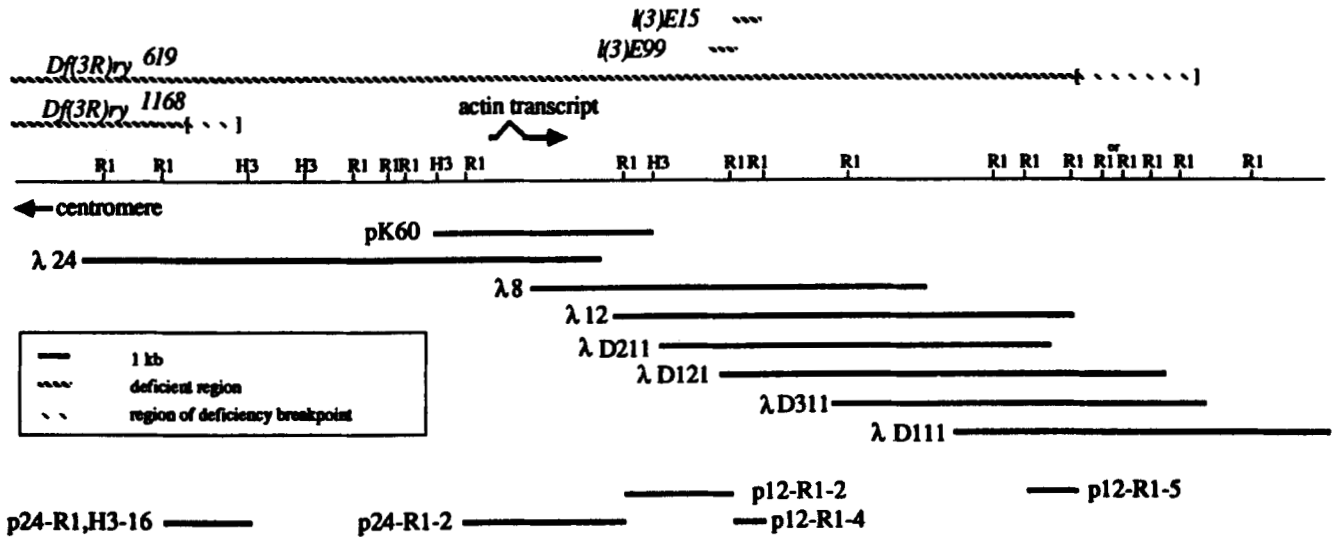

FIGURE 8.—Restriction map of the 87E actin gene flanking regions. The deficient regions of *Df(3R)ry*^{619} and *Df(3R)ry*^{1168} are indicated by dotted lines at the top of the figure, with the regions that must contain the deficiency breakpoints indicated in brackets. The restriction map of the region is in the center of the figure. The position of the actin transcript is indicated above the map; the arrow indicates the direction of transcription. The centromere is to the left. Clones of the region are indicated at the bottom of the figure. λ24, λ8, λ12, λD211, λD121, λD311, and λD111 are lambda clones from a wild-type Canton S Drosophila library (MANIATIS *et al.* 1978) that contain the indicated regions. p24-R1-1 and p24-R1-2 are pBR322 subclones that contain the indicated *Eco*RI fragments from λ24. p24-R1,H3-16 is a pBR322 subclone that contains the indicated *Eco*RI, *Hin*dIII fragment of λ24. p24-R1,Nru is a subclone of p24-R1,H3-16 that was made by digestion of p24-R1,H3-16 with *Nru*I and *Pvu*II, followed by religation. p12-R1-2 and p12-R1-4 are pBR322 subclones of the indicated *Eco*RI fragments of λ12. The p12-R1-2 *Bam*HI, *Bgl*II isolated fragment is the indicated *Bam*HI, *Bgl*II isolated fragment from p12-R1-2. The "or" at the right side of the restriction map indicates that the *Eco*RI site could be at either of the marked R1 positions. R1 = *Eco*RI, H3 = *Hin*dIII.

mRNA and the genomic sequence are colinear. A polyadenylation signal [*Act87E*: AATAAA) identical to the consensus sequence (AATAAA) (PROUDFOOT and BROWNLEE 1976)] is present 321 bp past the stop codon (Figure 7). Three different polyadenylation sites are evident in the five cDNA clones sequenced in this region. The poly(A) tails begin 31, 38 or 43 bases past the polyadenylation signal, as is typical of eukaryotic genes, resulting in a 3′ transcribed, non-translated region of 357, 363 or 368 bases.

The predicted size of the *Act87E* mRNA, obtained by summing the sizes of the sequenced flanking regions and the protein-coding region, is 1.57 kb [poly(A) tail not included]. The observed *Act87E* transcript is approximately 1.7 kb (FYRBERG *et al.* 1983; data not shown). In addition, a faint, large molecular weight band (approximately 4 kb) of unknown origin is sometimes seen on Northern blots using *Act87E* probes (FYRBERG *et al.* 1983; data not shown).

Isolation of the region between the distal endpoints of *Df(3R)ry*^{1168} and *Df(3R)ry*^{619}: To identify potential actin mutants among the existing lethal complementation groups in the region, the location of the actin gene transcription unit with respect to the flanking deficiency breakpoints was determined. A "chromosomal walk" (BENDER, SPIERER and HOGNESS 1983) was undertaken to the *Df(3R)ry*^{619} endpoint on the distal side of the actin gene and to the closest deficiency endpoint on the proximal side of the actin gene (either *Df(3R)lC4a* or *Df(3R)ry*^{1168}). The 43-kb walk is diagrammatically represented in Figure 8. The *Df(3R)ry*^{619} breakpoint is located 16–20 kb distal to the 3′ end of the actin gene. The *Df(3R)ry*^{1168} endpoint is the closest breakpoint on the proximal side of the actin gene and is located 8–10 kb 5′ to the gene (see MATERIALS AND METHODS). Because the *Df(3R)ry*^{1168} breakpoint is 8–10 kb 5′ to the beginning of the *Act87E* transcript, it seems very unlikely that this deficiency affects actin gene expression. Thus, complementation groups located in the region between the *Df(3R)ry*^{1168} and *Df(3R)ry*^{619} breakpoints are strong candidates for containing *Act87E* mutations.

Complementation pattern of preexisting recessive lethal mutations in the same genetic region as the actin gene: The region between the distal endpoints of *Df(3R)ry*^{1168} and *Df(3R)ry*^{619} was previously found to contain one recessive lethal complementation group, *l(3)m112*, defined by seven alleles (see Figure 1) (HILLIKER *et al.* 1980, 1981). These mutations were chosen for further analysis.

Complementation tests with these seven mutants were performed and indicated a somewhat different complementation pattern from that previously published (HILLIKER *et al.* 1981). In the present analysis, only *l(3)m112*, *l(3)B16-2*, *l(3)B17-1* and *l(3)H21* appeared to be members of a simple complementation group that maps to the same small region as the actin gene (Table 1). Although previously included in the

TABLE 1

Complementation tests of putative *l(3)m112* alleles

m1 / *MKRS*	*Df(3R)ry*^{619} / *MKRS*		*Df(3R)ry*^{1168} / *MKRS*		*Df(3R)lC4a* / *MKRS*		*Df(3R)l26c* / *MKRS*		*l(3)m112* / *MRS*		*l93)B9-2* / *MKRS*		*l(3)B17-1* / *MKRS*		*l(3)B16-2* / *MKRS*		*l(3)H21* / *MKRS*		*l(3)H24* / *MKRS*	
m2 / *MKRS*	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$	Sb	Sb$^+$
l(3)m112 / *MRS*	442	6	316	127	455	211	85	0												
l(3)B9-2 / *MKRS*	210	0	240	0	122	0	52	0	203	84										
l(3)B17-1 / *MKRS*	117	0	108	42	51	22	44	0	320	2	206	0								
l(3)B16-2 / *MKRS*	182	0	141	48	143	62	78	0	366	1	357	158	324	0						
l(3)H21 / *MKRS*	253	0	159	80	72	26	56	0	410	20*	177	62	223	0	351	0				
l(3)H24 / *MKRS*	455	113	436	69	154	31	8	13	257	66	258	97	220	114	250	99	ND			
l(3)H25 / *MKRS*	381	76	371	19	165	44	29	9	166	17	ND		ND		ND		ND		124	24

Data shown are the result of crosses between *m1/MKRS* and *m2/MKRS*. *MKRS* carries *Sb*. ND, not determined. * Partial complementation between *l(3)m112* and *l(3)H21*.

TABLE 2

Complementation tests of newly isolated *l(3)m112* alleles

m1 / *MKRS*	*Df(3R)ry*^{619} / *MKRS*		*Df(3R)ry*^{1168} / *MKRS*		*Df(3R)lC4a* / *MKRS*		*Df(3R)l26c* / *MKRS*		*l(3)m112* / *MRS*		*l(3)B9-2* / *MKRS*		*l(3)B17-1* / *MKRS*		*l(3)B16-2* / *MKRS*		*l(3)H21* / *MKRS*		*l(3)E15* / *MKRS*		*l(3)E99* / *MKRS*		*l(3)E118* / *MKRS*	
m2 / *MKRS*	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$	*Sb*	*Sb*$^+$
l(3)E15 / *MKRS*	219	0	206	62	79	43	224	0	77	3*	72	27	118	0	115	0	107	0						
l(3)E99 / *MKRS*	153	0	80	29	65	33	104	0	202	13*	54	22	87	0	105	0	107	0	106	0				
l(3)E118 / *MKRS*	93	0	34	13	106	42	52	0	139	11*	38	9	131	0	159	0	110	0	131	0	167	0		
l(3)E203 / *MKRS*	94	0	103	29	78	40	163	0	116	0	35	20	57	0	126	0	97	0	96	0	72	0	112	0

Data shown are the result of crosses between *m1/MKRS* and *m2/MKRS*. *MKRS* carries *Sb*. * Partial complementation.

l(3)m112 group (HILLIKER *et al.* 1981), the mutation *l(3)B9-2* does not appear to be a member of this complementation group because it is lethal in combination with *Df(3R)ry*^{1168} and *Df(3R)lC4a* and shows high viability (40%) when heterozygous with the allele *l(3)m112*. Although *l(3)B9-2* is lethal in combination with *l(3)B17-1*, a member of the *l(3)m112* group, subsequent experiments showed that this was the result of a shared lethal elsewhere on the third chromosome (MANSEAU 1986). Additional crosses revealed that *l(3)B9-2* is instead a member of the *l(3)m17* complementation group (MANSEAU 1986). In addition, *l(3)H24* and *l(3)H25* were previously placed in the *l(3)m112* complementation group because surviving *l(3)H24/l(3)m112* and *l(3)H25/l(3)m112* heterozygotes had thin bristles and irregular ommatidia (HILLIKER *et al.* 1981). However, this phenotype appears to be as strong or stronger when the mutations are heterozygous with *Df(3R)ry*^{1168} or *Df(3R)lC4a*, indicating that the mutations causing the mutant phenotype are probably not in the region between the most distal endpoints of *Df(3R)ry*^{1168} and *Df(3R)ry*^{619}. Thus, in the actin gene region a single complementation group, the *l(3)m112* complementation group, containing four alleles has been identified.

Isolation and genetic analysis of additional mutations in the actin gene region: Southern blot anal-

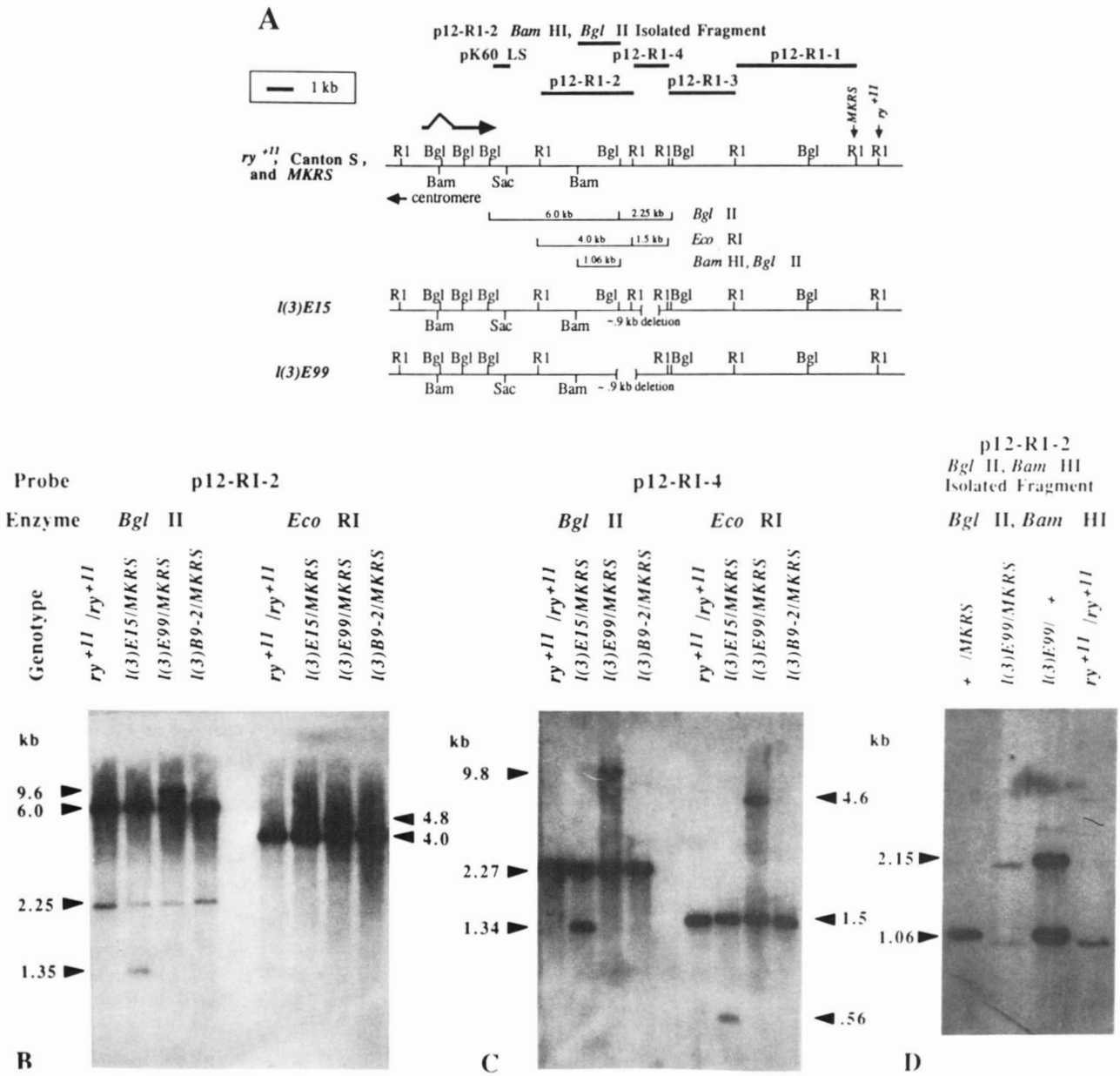

FIGURE 9.—Southern blot analysis of the *l(3)E15* and *l(3)E99* mutations. A, Diagram of the probes used and results of the Southern blot analysis. The probes used are indicated at the top of the figure. p12-R1-1, p12-R1-2, p12-R1-3, and p12-R1-4 are all pBR322 subclones of the indicated *Eco*RI fragments. pK60 LS is a pSP65 subclone of the indicated *Bgl*II-*Sac*I fragment. The restriction map of the ry^{+11}, *MKRS*, and Canton S chromosomes is shown in the middle of A. The arrow above the map represents the actin transcript. The centromere is to the left. The *Eco*RI sites at the right of the region differ in the ry^{+11} and *MKRS* chromosomes. The sizes of the wild-type *Bgl*II, *Eco*RI and *Bam*HI-*Bgl*II fragments that are important to the Southern blot analysis are indicated below the restriction map. The restriction maps of the *l(3)E15* and *l(3)E99* mutant chromosomes in this region are shown at the bottom of the figure. R1 = *Eco*RI, Bgl = *Bgl*II, Bam = *Bam*Hi, Sac = *Sac*I; B and C, Southern blots of genomic DNA from the indicated stocks digested with *Bgl*II or *Eco*RI and hybridized with nick-translated p12-RI-2 in B and p12-R1-4 in C. ry^{+11} is the parental chromosome of *l(3)E15/MKRS* and *l(3)E99/MKRS*. *l(3)B9-2/MKRS* does not contain any observable changes in these regions and is included to control for the *MKRS* balancer chromosome in the *l(3)E15/MKRS* and *l(3)E99/MKRS* lanes.; D, Southern blot of genomic DNA from the indicated stocks digested with *Bgl*II and *Bam*HI and hybridized with the nick-translated *Bgl*II-*Bam*HI fragment from p12-R1-2. + indicates a wild-type chromosome from Canton S. Please see text for interpretation of these blots.

ysis of the existing mutants in the *l(3)m112* complementation group failed to reveal any molecular changes in the *Act87E* gene. Because we would expect only a small proportion of the mutations within the *l(3)m112* group to have a lesion readily detectable by Southern analysis, even if they did reside within the actin gene, we generated additional mutant alleles to increase the likelihood of producing a molecularly detectable alteration. In addition, because the 87E actin gene could correspond to a complementation group not previously identified by HILLIKER *et al.* (1980, 1981), we screened for new mutations in the relevant region that were outside the *l(3)m112* group. The 87E actin is thought to be a major actin in larval

muscles essential for movement (FYRBERG *et al.* 1983), therefore it seemed reasonable to expect that some lesions in this actin gene would produce a lethal phenotype. Consequently, mutagenized chromosomes were screened for mutations with either recessive lethal or obvious morphological phenotypes. From 16,000 EMS-treated chromosomes, 154 new mutants uncovered by $Df(3R)ry^{619}$ were isolated. Four of these *l(3)E15*, *l(3)E99*, *l(3)E118*, and *l(3)E203*) are not uncovered by $Df(3R)ry^{1168}$ (Table 2) and thus localize to the same cytological region as the actin gene.

Complementation tests indicated that the four newly induced recessive lethal mutations are all members of the *l(3)m112* complementation group (Table 2) and that no new complementation groups were identified.

Identification of the molecular lesion in two members of the *l(3)m112* complementation group: The four newly isolated mutants, like the previous members of the *l(3)m112* complementation group, did not have any lesions within the actin gene that were detectable by Southern blot analysis. However, aberrations were found in two of the mutants a few kb 3′ (distal) to the actin gene. The EMS-induced *l(3)E15* allele appears to have a 0.9-kb deletion ~5 kb distal to the actin gene. The neighboring probes p12-R1-2 and p12-R1-4 both hybridized to a novel fragment in Southern blots of *Bgl*II-cut *l(3)E15/MKRS* DNA (Figure 9). In addition, p12-R1-4 probes detected a novel fragment in *Eco*RI-cut *l(3)E15/MKRS* DNA (Figure 9). Additional Southern blot analysis with flanking region probes indicated that the lesion is within the *Eco*RI fragment of p12-R1-4. Since both the novel *Bgl*II fragment and the novel *Eco*RI fragment seen in *l(3)E15/MKRS* are approximately 0.9 kb smaller than the parental ry^{+11} chromosome fragments, the *l(3)E15* mutation is most likely a 0.9 kb deletion.

A similar analysis indicated that the EMS-induced *l(3)E99* chromosome has a deletion of approximately 0.9 kb beginning 3.5–4.5 kb distal (3′) to the actin gene. Southern blots indicated that both the *Bgl*II and *Eco*RI sites of p12-R1-2 are missing in *l(3)E99*, suggesting that the *l(3)E99* mutation is a deletion. (pK60LS, p12-R1-2, and p12-R1-4 probes hybridized to a novel, larger fragment of *Bgl*II-cut *l(3)E99/MKRS* DNA and p12-R1-2 and p12-R1-4 detected a novel, larger fragment of *Eco*RI-digested mutant DNA (Figure 9).) The proximal end of the deletion lies between the *Bam*HI and *Bgl*II sites of p12-R1-2, as *Bam*HI, *Bgl*II-cut DNA hybridized with an isolated fragment from the region detected a new fragment (Figure 9). The size of the deletion is approximately 0.9 kb, based on comparison of the fragment sizes in *Eco*RI-cut and *Bgl*II, *Bam*HI-cut mutant and wild-type DNAs.

The region that contains the identified *l(3)m112* complementation group lesions is transcribed: In an attempt to determine whether the region containing the *l(3)E15* and *l(3)E99* alterations is transcribed, a late pupal cDNA library (POOLE *et al.* 1985) was screened using p12-R1-2 as a probe. Six cDNA clones, containing inserts ranging from 0.8 to 4.3 kb, were isolated, at least three of which hybridize to the *Eco*RI fragments of λ12 that are subcloned into p12-R1-2 and p12-R1-4. None of these cDNA clones hybridize to pK60 L2, a plasmid containing the bulk of the actin protein-coding region.

The lesions detected in the *l(3)E15* and *l(3)E99* mutants are 4–5 kb distal to the actin gene in a region that is transcribed. This suggests that the *l(3)m112* complementation group is not in the actin gene, but in a gene distal (3′) to the actin gene.

DISCUSSION

A single lethal complementation group, *l(3)m112*, exists within the region that contains the 87E actin gene, which is defined cytologically as one to three faint polytene chromosome bands and molecularly as 24–31 kb of DNA. Our analysis indicates that this complementation group comprises mutations in a transcribed region 4–5 kb distal to the actin gene. Therefore, no complementation group containing actin mutants has been identified in this study.

Evidence that the *l(3)m112* complementation group is not in the *Act87E* gene: The major evidence indicating that the *l(3)m112* complementation group is not in the *Act87E* gene is that two of the eight *l(3)m112* alleles, *l(3)E15* and *l(3)E99*, have identifiable lesions in the same small region 4–5 kb distal to the actin gene. The existence of lesions in the same small region of DNA in two independently-isolated mutants argues strongly that these alterations are responsible for the mutant phenotype. The isolation of cDNA clones using probes from the region that is altered in the mutants indicates that a transcribed gene lies in this area. In addition, a wild-type *Act87E* gene, introduced via *P*-element-mediated transformation, fails to rescue the recessive lethal phenotype of the *l(3)m112* complementation group mutants (MANSEAU 1986). However, there are a number of possible reasons (besides the mutations not being in the actin gene) why the transduced *Act87E* gene might not have rescued the phenotype of the mutants, including improper expression of the introduced gene.

Possible explanations for the failure to identify *Act87E* mutants: Four possible explanations for the failure to identify *Act87E* mutations will be discussed: (1) that mutant screens in 87E might not have been extensive enough; (2) that *Act87E* mutations might be uncovered by $Df(3R)ry^{1168}$ and thus, have been

discarded in this study; (3) that *Act87E* mutations might have a phenotype other than recessive lethality; and (4) that other Drosophila actins might substitute for the loss of the 87E actin.

Mutant screens might not have been extensive enough: It seems unlikely that the *Act87E* gene can be mutated to a readily visible or recessive lethal phenotype because the region surrounding the actin gene is thought to be saturated or nearly saturated for mutations with these phenotypes (HILLIKER *et al.* 1980, 1981). In screens for mutations in the *ry* region, HILLIKER *et al.* (1980, 1981) identified 74 mutations in the 87E1,2 to E12,F1 region which fall into eleven complementation groups. In our screens, an additional 95 mutations in this region have been obtained, but no new complementation groups have been identified in the small region that contains the actin gene—between the distal $Df(3R)ry^{1168}$ and $Df(3R)ry^{619}$ endpoints. Because of the unequal mutability of loci, it is not possible to estimate the number of loci in the region that remain unmutated (LEFEVRE and WATKINS 1986). Moreover, two of the complementation groups identified by HILLIKER *et al.* (1980, 1981) contain only one mutation, so the possibility of additional unmutated loci cannot be ruled out. However, since our study has more than doubled the number of mutations in this region, it seems unlikely that numerous loci remain that can be mutated to readily visible or recessive lethal phenotypes.

Mutations in *Act87E* might be uncovered by $Df(3R)ry^{1168}$: One possibility for the failure to identify mutations in the *Act87E* gene is that $Df(3R)ry^{1168}$ might disrupt expression of the *Act87E* gene. If this were true, then recessive lethal *Act87E* gene mutations would be among those discarded in the present screens. However, it seems unlikely that this deficiency drastically reduces actin gene expression, since $Df(3R)ry^{1168}$ is broken 8–10 kb proximal to the 5′ end of the actin gene transcript. This is probably outside of the regulatory region of the *Act87E* gene. Transformation experiments with the *Act88F* gene have demonstrated that only only 1 kb of 5′ flanking sequences is required to direct normal expression of the *Act88F* gene (HIROMI *et al.* 1986). The possibility exists, however, that through some type of position effect, the deficiency could be exerting a negative effect on *Act87E* transcription.

Mutations in *Act87E* might result in a phenotype other than recessive lethality: It is possible that mutations in the *Act87E* gene produce a phenotype missed in these studies. Because the 87E actin is thought to function in larval and adult intersegmental muscles, we originally believed that *Act87E* mutations would result in recessive lethality. If, however, the 87E actin functions only in a small subset of these muscles not required for survival or if loss of the 87E actin can be partially compensated for by the other actins (see the next section), then *Act87E* mutations might result in slow growth, in uncoordinated or slightly paralyzed larvae, or in uncoordinated adults.

Dominant *Act87E* mutations would also have been missed. A number of muscle genes are known to be haplo-insufficient for flight (MAHAFFEY *et al.* 1985; HIROMI and HOTTA, 1985; OKAMOTO *et al.* 1986; BERNSTEIN *et al.* 1983; KARLIK and FYRBERG 1985). However, since flies heterozygous for deficiencies of the *Act87E* gene ($Df(3R)ry^{619}$ and $Df(3R)l26c$) have no detectable phenotype, mutations that simply reduce or eliminate expression of the *Act87E* gene should not result in dominant phenotypes. There are dominant flightless mutations in the flight-muscle-specific actin gen (*Act88F*) that produce stable, but functionally altered actin (KARLIK, COUTU and FYRBERG 1984; HIROMI and HOTTA 1985; HIROMI *et al.* 1986; OKAMOTO *et al.* 1986). Similar mutations of the *Act87E* gene might result in such dominant phenotypes as lethality, uncoordinated or slightly paralyzed larvae, or uncoordinated adults. Because there is no way to specifically target a particular cytogenetic region in screens for dominant mutations, we did not screen for dominant *Act87E* phenotypes.

Other Drosophila actins might substitute for loss of the 87E actin: 1. *Intergenic complementation in other multigene families.* An important question in the study of a highly-conserved multigene family, such as the Drosophila actin gene family, is whether the product of one gene could functionally substitute for the product of another member of the gene family. Such an occurrence could make it impossible to identify recessive mutations in the gene family using a simple screen like that used in the experiments described here. Precedence for this type of intergenic complementation exists. The histone H2A (*HTA*) (KOLODRUBETZ, RYKOWSKI and GRUNSTEIN 1982), histone H2B (*HTB*) (RYKOWSKI *et al.* 1981), ribosomal protein 51 (*RP51*) (ABOVICH and ROSBASH 1984), *ras* (TATCHELL *et al.* 1984), *Hsp70* (CRAIG and JACOBSON 1984, 1985) and α-tubulin (SCHATZ, SOLOMON and BOTSTEIN 1986) gene families of yeast all contain members that can substitute for one another. Another example of genes that can functionally substitute for one another occurs in hereditary persistence of fetal hemoglobin (HPFH). In HPFH, synthesis of fetal hemoglobin persists into adulthood compensating for hereditary defects in adult hemoglobin chain genes that would otherwise result in thalessemia (reviewed in WEATHERALL and CLEGG 1979; COLLINS and WEISSMAN 1984).

The most relevant example of genes that can substitute for one another is the actin gene family of *Caenorhabditis elegans*. There are four actin genes in the nematode, three of which are clustered. Five dominant, gain of function mutations that affect motility and muscle structure map to the same region

as the actin gene cluster (WATERSTON, HIRSH and LANE 1984). Four phenotypically wild-type revertants of these dominant mutations have been found that have major disruptions in at least one of the actin genes of the cluster. Since these animals are phenotypically wild type, the actin genes of *C. elegans* must have overlapping functions.

2. *Intergenic complementation in the Drosophila actin gene family.* Compensation for loss of the 87E actin by the other actins could explain our failure to isolate mutants in the *Act87E* gene. As discussed below, our current knowledge of the patterns of expression and structural similarities of the Drosophila actin genes is consistent with the possibility that other actins may substitute for the 87E actin. Because there is no technique for directed gene disruption in Drosophila, it is currently impossible to test directly whether the other Drosophila actins can substitute for loss of the 87E actin.

Our present understanding of the expression of the Drosophila actins suggests that the other actins are present in a temporal and spatial pattern that might allow for compensation for the loss of the 87E actin. In the developmental stages and adult tissues that have been studied, the 87E actin is never present without at least one of the other actins (FYRBERG *et al.* 1983). Transcripts from the *Act87E* gene are present in RNA isolated from adult abdomens, from abdominal walls, from adult heads, and from adult legs. The *Act57B* transcript is present in similar amounts to the *Act87E* transcript in adult abdomens, abdominal walls, and adult heads. Transcripts from the *Act79B* gene are present in greater amounts than the *Act87E* transcript in RNA isolated from adult legs (FYRBERG *et al.* 1983). A more detailed study of the expression of the actin genes would help determine whether the actins are expressed in the appropriate tissue at levels abundant enough to substitute for one another. Even if other actin genes are not *normally* expressed in a pattern similar to that of *Act87E*, the expression of the other actins may be altered when 87E actin is absent. Such compensatory alterations in expression pattern have been observed in strains containing mutations in certain members of the Hsp70 gene family of yeast (WERNER-WASHBURNE, STONE and CRAIG 1987).

Whether any other actins are functionally equivalent to the 87E actin is unknown. The 57B actin is the most similar to the 87E actin, differing at only 3 amino acids (FYRBERG *et al.* 1981; E. A. FYRBERG, personal communication). Since the 57B and 87E actins are nearly identical and are expressed in the same temporal and positional patterns, the 57B actin may well compensate for loss of the 87E actin in the tissues in which they are both normally expressed: the larval and adult intersegmental muscles. The *Act79B* gene, which is expressed in legs in greater abundance than the *Act87E* gene (FYRBERG *et al.* 1983), encodes an actin that is 96% identical to the 87E actin; thus, the 79B actin may compensate for loss of the 87E actin in legs.

However, the functional significance of the observed amino acid differences in the actin isoforms is unknown. By substitution of portions of the *Act88F* gene with analogous portions of other Drosophila actin genes, it has been shown that the other actins are at least partially equivalent functionally to the 88F-encoded flight-muscle-specific actin and all can partially substitute for actin 88F and direct the formation of flight muscle myofibrils in *Act88F* mutants, although one of the fusion actins is somewhat better at rescuing flight than the others (E. A. FYRBERG, personal communication). Whether this observed difference in flight ability results from differences in the amount of actin produced from the various inserts or from subtle functional differences in the chaemeric actins is currently unknown (E. A. FYRBERG, personal communication). These experiments are possible because of the existence of a null allele for the *Act88F* gene.

In conclusion, it seems reasonable that *Act87E* mutants were not identified in this study either because the 87E actin functions in muscles not essential for viability or because one or more of the other actins are able to substitute for the loss of the 87E actin. Additional knowledge about the tissue expression of this gene family will help determine whether the first possibility is tenable, but directly testing whether other actins compensate for loss of the 87E actin is currently not possible. In light of the difficulties that we have encountered in seeking recessive *Act87E* mutants, an alternative genetic approach is desirable. By analogy with the dominant flightless mutants in the flight-muscle specific *Act88F* gene (HIROMI *et al.* 1986; OKAMOTO *et al.* 1986), it should be possible to engineer changes in the *Act87E* gene that when introduced into wild-type flies via *P*-element-mediated transformation result in a dominant mutant phenotype. Analysis of these *Act87E* dominant mutants would help define the function of the *Act87E* gene.

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