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Actin gene mutations in *Drosophila*; heat shock activation in the indirect flight muscles

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We have identified four mutations affecting the actin III isoform in the indirect flight muscles (IFM) of *Drosophila*. One mutation does not produce any protein product, and three direct the synthesis of electrophoretic variants of actin. Complementation tests and recombination mapping indicate that all mutations are alleles of an actin gene at chromosomal band 88F (*act88F* gene). The effect of these mutations is restricted to the IFM. We conclude that the *act88F* gene is expressed only in the IFM to encode actin III, which is its major isoform. In two of the actin mutants, heat shock proteins are constitutively expressed in the IFM. Genetic evidence strongly suggest that this anomaly is primarily caused by the mutations in the *act88F* structural gene.

Key words: Drosophila mutations/actin gene/heat shock activation/indirect flight muscles

Introduction

The genes encoding actins exist as a multiple copy gene family in a number of organisms (reviewed by Firtel, 1981). Many of them are differentially expressed in a tissue- and stage-specific manner. Although the amino acid sequence is highly conserved among different actin genes, the functions they execute in different cell types are manifold. It is, therefore, a useful system to study how subtle changes in primary sequence affect the various actin functions.

In principle, mutations can be used to dissect the complex nature of the protein. Systematic analyses of the mutations of a gene may unravel new functions of the encoded protein. This approach, however, has not been readily applicable to genes essential for basic cellular functions, since such mutations are lethal. Indirect flight muscle (IFM) of *Drosophila* is one system which overcomes the difficulties of such studies. The IFM system is made up of 13 pairs of relatively large muscle fibers in the adult thorax. These muscles are highly differentiated to produce mechanical power during flight (Tregear, 1977). They are dispensable for viability, so that a number of mutations affecting structure and function of the IFM have been obtained by screening flightless mutations (Hotta and Benzer, 1972; Deak, 1977; Koana and Hotta, 1978; Mogami *et al.*, 1981; Mogami and Hotta, 1981; Deak *et al.*, 1982). IFM is the only fibrillar-type muscle in *Drosophila*, and is morphologically distinct from all the other tubular type muscles, such as muscles of the larval body wall, of the adult legs and abdomen. The two muscle types also differ at the biochemical level. Our previous studies showed that a number of proteins exist exclusively in the IFM (Mogami *et al.*, 1982). This suggests the existence of a set of genes which is expressed only in the IFM, and a cellular mechanism to control their expression. IFM can therefore be used to genetically dissect the regulation mechanism of tissue-specific genes.

Recently, Karlik *et al.* (1984a) has reported that one of our dominant flightless mutations (Mogami and Hotta, 1981) carries an amber mutation in the *act88F* actin gene and expresses heat shock proteins (hsp) constitutively. We describe our identification of three additional mutations of the *act88F* gene and show that this gene is expressed only in the IFM. Among the newly found alleles, one shows the same hsp phenotype, suggesting that a certain class, but not all, of the actin mutations can activate *hsp* genes constitutively.

Results

Mutations affecting the actins of IFM

Among the dominant flightless muations we isolated, many showed a reduction of actin isoforms present in the thorax (Mogami and Hotta, 1981). We analyzed protein accumulation and synthesis in the IFM dissected from these mutants, and have identified four mutants as actin mutations (Figure 1). All of these mutations cause reduction in actin III synthesis; in three mutations [KM75, KM88 and KM129; KM75 was previously designated as Ifm(3)7], actin III is entirely absent, whereas in the HH5 mutation, it is reduced to about half the normal amount. The former three mutants also have a reduced amount of actin II in IFM (Figure 1).

In addition, two of the mutants cause synthesis of novel proteins which do not exist in normal muscle. KM75 and KM129 synthesize proteins with a mol. wt. of 42 000 and 38 000, respectively (designated p42 and p38). The HH5 mutation causes an increase in the synthesis of actin I, which is present only in trace amounts in normal IFM.

We identified these novel proteins as actin variants by peptide mapping analysis and hybridization selection experiments. When peptide fingerprints were made by partial proteolytic digestion with *Staphylococcus aureus* V8 protease, maps of the two major actin isoforms in IFM were indistinguishable from each other (Figure 2A). Many of the digestion products of p42 and p38 had the same mobilities as those of normal IFM actins (Figure 2A). These peptide fragments were shown to have identical charge properties, when they were separated by two-dimensional gel electrophoresis (Figure 2B,C). The peptide map of actin I from the HH5 mutant IFM was also similar to that of actin III from normal IFM (Figure 2A). We therefore conclude that the novel proteins have an amino acid sequence similar to those of actins.

Identity of p42 as a variant actin was further confirmed by carrying out hybridization selection experiments with an actin DNA sequence. Poly(A)$^+$ RNA extracted from normal and KM75 adults soon after eclosion was hybrid selected with plasmid DNA containing the coding sequence of a *Drosophila* actin gene (*act5C* gene, Fyrberg *et al.*, 1980), translated *in vitro*, and the products were separated by two-dimensional gel electrophoresis. While only actins were selected from wild-type mRNA with actin DNA, KM75 mRNA selected with the same probe also directed syn-

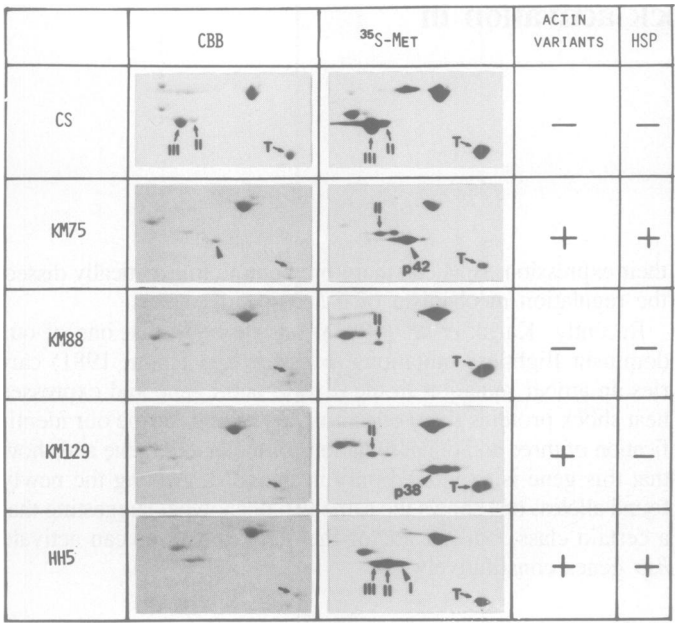

Fig. 1. Two-dimensional electrophoretograms of IFM protein from actin mutants. Indirect flight muscle was dissected from dehydrated flies which had been labeled for 2 h with [^{35}S]methionine. **Lane CBB:** gels stained with Coomassie Brilliant Blue. **Lane ^{35}S-Met:** autoradiogram of the same gel. Only actin regions are shown. III and II indicates actin III and actin II, respectively. Actin variants are indicated by arrowheads. T is tropomyosin.

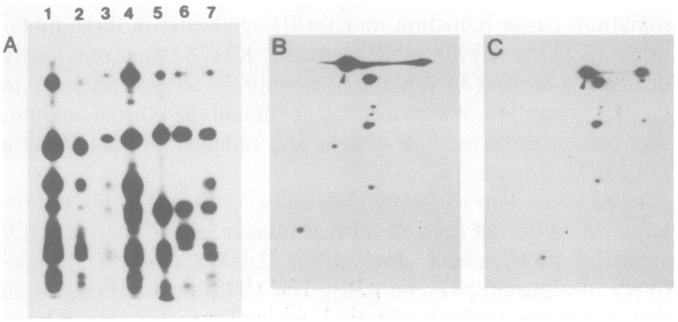

Fig. 2. Peptide maps of actins from normal and mutant muscles. **(A)** One-dimensional peptide map of actins produced by partial proteolytic digestion with *S. aureus* V8 protease. **Lanes 1, 2:** actin II and III from normal tubular muscle (tergodepressor of trochanter). **Lanes 3, 4:** actin II and III from normal IFM. **Lane 5:** p42 from IFM of KM75. **Lane 6:** p38 from IFM of KM129. **Lane 7:** actin I from IFM of HH5. **(B) (C)** Two-dimensional peptide maps of normal IFM actin III **(B)** and p42 from IFM of KM75 **(C)**. Arrowhead indicates undigested protein.

thesis of a protein co-migrating with p42 (Figure 3). Thus the DNA sequence encoding p42 has an extremely high homology with the actin gene. These results indicate that the novel protein synthesized in mutant IFM is an actin variant.

There is an indication that some actin variants are unstable *in vivo*. The variant synthesized in KM75, p42, is stable and is present in large amounts in IFM. On the other hand, the variant actin in KM129 is barely detectable on stained gels (see Figure 1), suggesting that it is quickly degraded after being synthesized.

Two possible mechanisms exist which lead to production of actin variants. One possibility is that the mutations occurred in the structural region of an actin gene. Another is that the variants are produced by abnormal splicing of normal transcripts or by modification of normal translation products. In the former case, the synthesis of actin variants would be dependent on gene

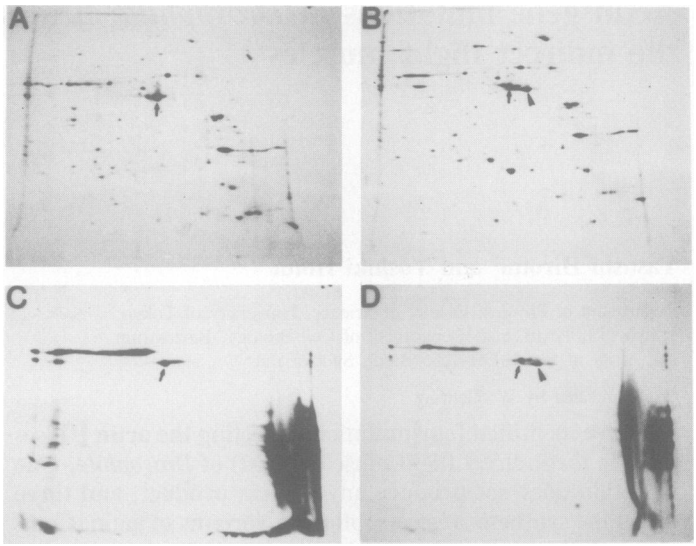

Fig. 3. Hybrid selection of mRNA homologous to actin gene probe. **A, B:** *in vitro* translation product of poly(A)$^+$ RNA from normal **(A)** and KM75 **(B)** adults shortly after eclosion **C, D:** translation product of mRNA from normal **(C)** and KM75 **(D)**, selected by Hd-19 plasmid DNA containing the *Drosophila act5C* gene. Actin is indicated by an arrow, p42 by an arrowhead.

dosage, whereas the latter is most likely recessive.

We examined the proteins synthesized in heterozygotes with respect to the mutant gene. In every case, the actin variants were also synthesized in heterozygotes, and the synthesis of actin III was restored to about half the normal level (Figure 4). The co-dominant synthesis of normal actin III and actin variants indicates that these mutations are structural gene mutations.

All actin mutations we have identified are linked to the third chromosome. Among six *Drosophila* actin genes, three are located on the third chromosome. To determine the allelism of the four mutations, we carried out complementation tests with respect to actin III synthesis (flightlessness cannot be used because it is a dominant character). In all combinations of the mutants which lack actin III (KM75, KM88 and KM129), synthesis of actin III was not restored, indicating lack of complementation (Figure 4). Moreover, in every case the actin variants were synthesized co-dominantly. For example, KM129/KM75 synthesized both p42 and p38, but no actin III. These results indicate that KM75, KM88 and KM129 are alleles of a single actin gene. Since no product has been identified in KM88, the nature of this mutation could be regulatory, rather than structural.

The fourth mutation, HH5, could not be tested for complementation, since apparently normal actin III exists in reduced amounts. We therefore carried out recombination mapping between the four mutations.

Among $\sim 4000-5000$ progeny from mutant *trans*-heterozygote females, no recombinants capable of flying (flight$^+$) were obtained in any combinations tested (Figure 4). This result maps the four mutants within 0.02 map units of one another on the third chromosome. The simplest interpretation of these data is that all mutations are allelic.

The chromosomal locus of KM75 mutation is very close to, if not identical with, band 88F (Mogami and Hotta, 1981), where one actin gene has been localized (Tobin *et al.*, 1980; Fyrberg *et al.*, 1980). We therefore conclude that these four mutations affect either the structural gene or a *cis*-acting regulatory region of the 88F actin gene (*act88F* gene). The four alleles should be called *act88F*^{KM75}, *act88F*^{KM88}, etc., although in this report the

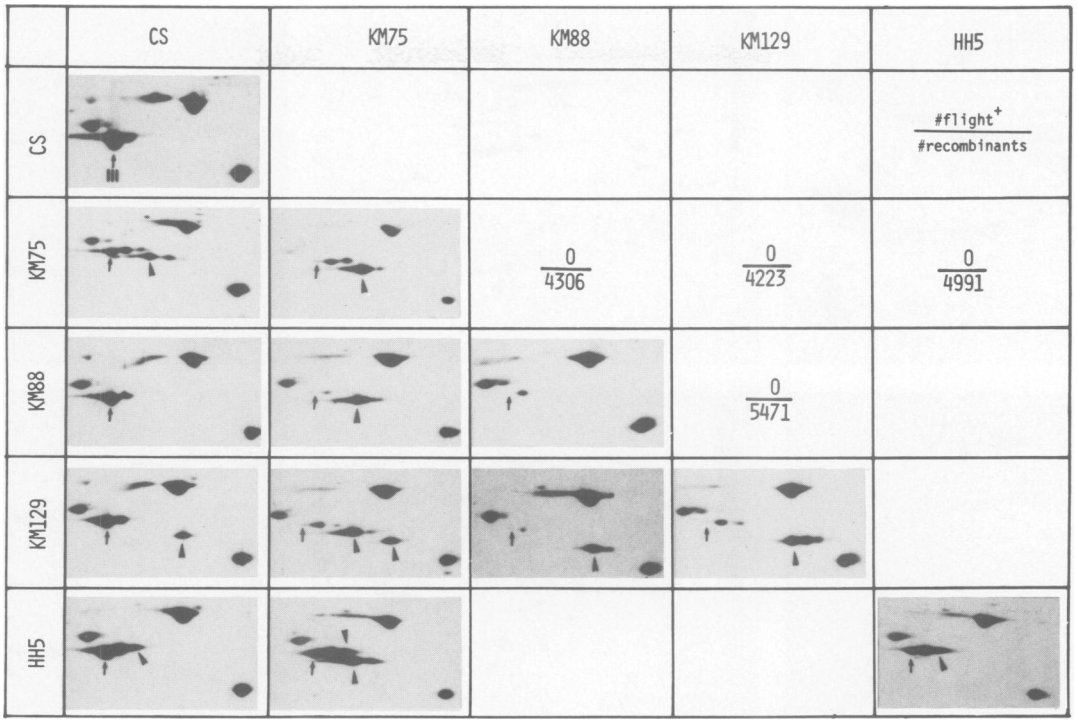

Fig. 4. Complementation test and recombinational mapping of actin gene mutations. Lower left triangle of the matrix shows the results of complementation tests among the actin mutants and the phenotypes of heterozygotes. Parental strains used for construction of heterozygotes are shown above the first column and left of the first row. Homozygous mutants are shown in the diagonal position, and heterozygotes with the normal allele are shown in the first row. Adult flies of each genotype were labeled with [^{35}S]methionine for 2 h just after eclosion, and their IFM proteins were subjected to two-dimensional gel electrophoresis. Actin III is indicated by an arrow, actin variants by arrowheads. Note co-dominant expressions of actin III and actin variants in all combinations, and absence of actin III in the mutant *trans*-heterozygotes. The upper right triangle shows results of recombination mapping between actin gene mutants. Mutant *trans*-heterozygous females (genotype of which can be inferred as above) were crossed with normal males and their progeny was scored for flight ability. The number below the line indicates the number of recombinants. The number of recombinants which can fly (if any) is indicated by the number above the line.

gene name *act88F* will be omitted.

act88F mutations are specific to IFM

The defect in actins caused by these mutations were observed only in the IFM of adult thorax. Actin isoforms in the tubular muscle of the mutants were indistinguishable from normal ones either in the pattern of accumulation, or their peptide maps. Synthesis of the actin variants was not detected in other body parts such as head, abdomen, legs and thorax from which IFMs had been cleanly removed (Figure 5). Neither was any trace of the actin variants detected in tissues from other developmental stages. In addition, we found that peptide maps of tubular muscle actin isoforms differ from those obtained from IFM actins (Figure 2A). The same conclusion was drawn from peptide maps of actins constructed by partial digestion with chymotrypsin (data not shown), suggesting that actin isoforms of the two muscle types differ in primary amino acid sequence, and are encoded by separate genes. These data strongly suggest that the *act88F* gene is expressed only in the IFM.

Some act88F gene mutations synthesize heat shock proteins at low temperature

We have reported that the IFM of KM75 contains a set of unusual proteins which are never observed in normal muscles (Figure 6, Mogami and Hotta, 1981). Isolation of additional dominant flightless mutations led us to the discovery of a new actin mutant HH5 which shows the same abnormality (Figure 6). We identified these proteins as hsps. Co-migration experiments with labeled hsps and unlabeled mutant IFM proteins showed that all major hsps co-focus with the novel proteins in mutant IFM. Pep-

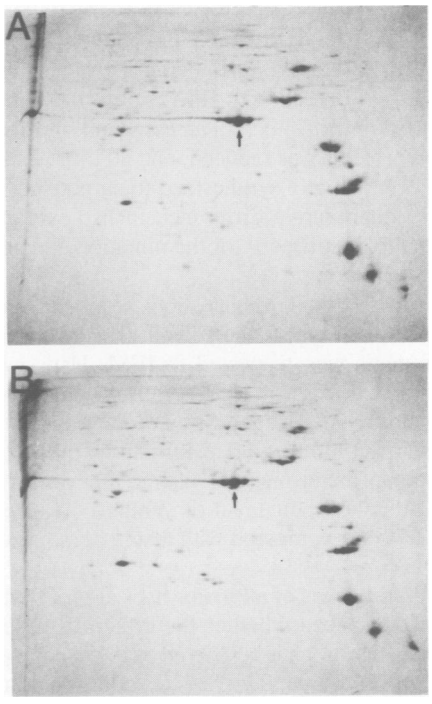

Fig. 5. Proteins synthesized in thorax devoid of IFM. This sample consists mainly of tubular type muscles, thoracic ganglia, parts of digestive tract and cuticle. **(A):** normal, **B:** KM75. No abnormality in either actins (arrowed) or hsps is detectable.

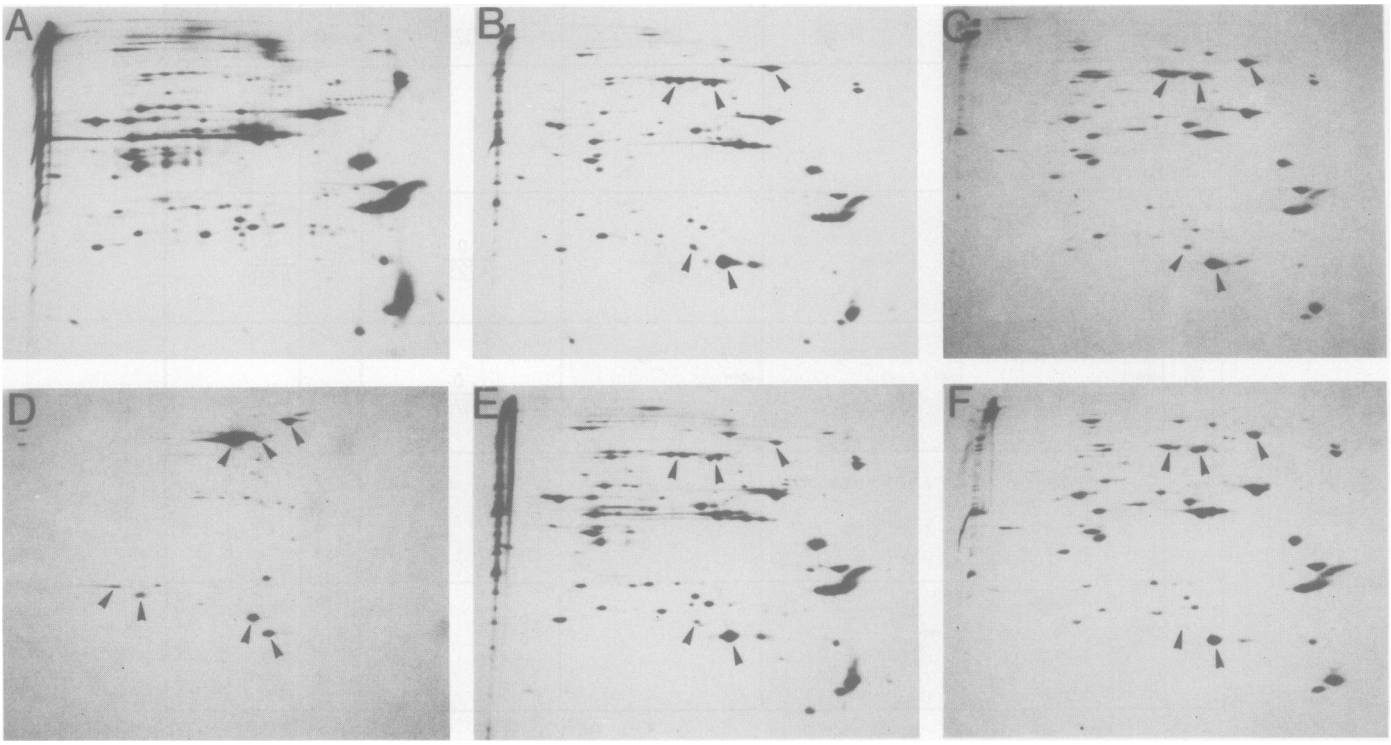

Fig. 6. Mutations synthesizing hsps at low temperature. Autoradiograms of two-dimensional gels of IFM proteins synthesized in normal individual **(A)**, KM75/KM75 **(B)**, HH5/HH5 **(C)**, KM75/+ **(E)** and HH5/+ **(F)** during a 2 h label shortly after eclosion. In **D,** proteins synthesized in normal IFM during heat shock are shown. Major heat shock proteins are indicated by arrowheads.

tide mapping analyses also confirmed the identity of the mutant proteins as hsps (data not shown).

Although all major hsps are accumulated abnormally in the mutant IFM, their rate of synthesis relative to each other is different from that during heat shock. In both alleles (KM75 and HH5), hsp27 and hsp26 are much less actively synthesized than other hsps, the most active ones being hsp84, hsp70 and hsp22 (compare Figure 6 B,C and D). IFM cells of these mutants, however, responded to heat shock in an essentially normal way; synthesis of IFM proteins was reduced, and all hsps, including hsp27 and hsp26, were actively synthesized (data not shown). This indicates that a quantitative difference in hsp synthesis in these mutants is due to the property of the mutation and not to a defect in the *hsp* genes themselves.

In contrast to the standard heat shock response where hsps are induced in most cell types, the constitutive synthesis of hsps in these two mutants was restricted to IFM. Hsps were detected neither in thorax devoid of IFM (Figure 5), nor in individuals at other developmental stages (larvae or embryos).

The effect of KM75 and HH5 mutations on hsp synthesis is dominant; mutant heterozygotes also synthesized hsps without heat shock, although their level of synthesis (especially hsp23) was somewhat lower compared with that in homozygotes (Figures 6,7).

Since structural genes of all major hsps are located on the third chromosome, we asked whether the effect of these third chromosomal actin mutations is restricted to the *hsp* genes which are on the same chromosome. This was tested using a third chromosome balancer *TM1*, which was found to carry an electrophoretic variant of hsp22. When heterozygous flies were constructed having one *TM1* chromosome and another with either KM75 or HH5 mutation, both normal and variant forms of hsp22 were

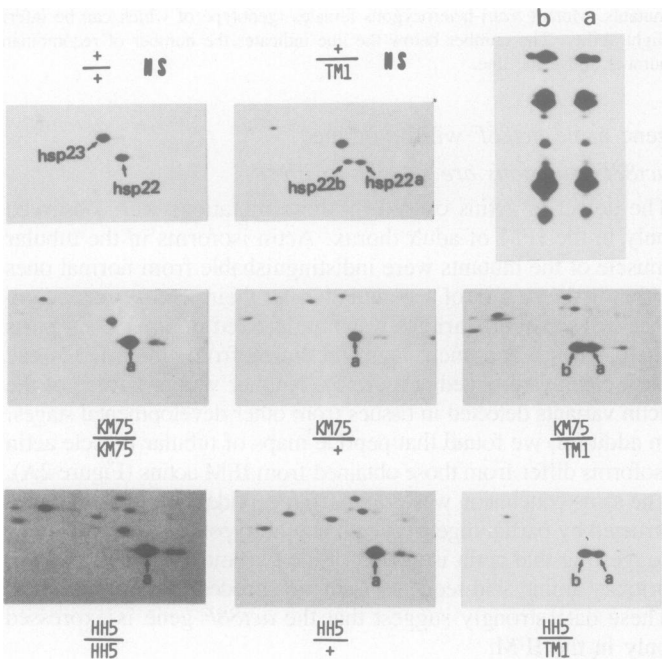

Fig. 7. Activation of hsp22 variants by KM75 and HH5 mutations. Only regions of hsp22 are shown. **A, B:** hsp synthesized during heat shock in animals having two normal third chromosomes **(A)**, or one normal chromosome and one *TM1* chromosome **(B)**. Heterozygotes +/*TM1* synthesized a novel protein designated hsp22b, which was shown to be an electrophoretic variant of hsp22 (hsp22a). **C:** peptide maps of hsp22a **(lane a)** and hsp22b **(lane b)**. **D–I:** hsp synthesized in mutant homo- and heterozygotes' IFM at low temperature. **D:** KM75/KM75, **E:** KM75/+, **F:** KM75/*TM1*, **G:** HH5/HH5, **H:** HH5/+, **I:** HH5/*TM1*. a and b indicate hsp22a and hsp22b, respectively.

Table I. Recombination mapping with respect to dominant flightlessness, actin anomaly and hsp synthesis

KM75	flight$^+$ p42$^-$ HSP$^-$	flight$^-$ p42$^+$ HSP$^+$	others
cu^+sr^-	5	5	0
cu^-sr^+	2	6	0
HH5	**flight$^+$ HSP$^-$**	**flight$^-$ HSP$^+$**	**others**
cu^+sr^-	8	4	0
cu^-sr^+	6	5	0

synthesized (Figure 7). This demonstrates that both KM75 and HH5 mutations activate the hsp22 gene located in *trans*.

Hsp synthesis and actin mutations are inseparable by recombination

We examined the possibility that the two hsp mutants bear, in addition to the actin gene mutation, another mutation which is responsible for the hsp induction at low temperature. Recombination mapping was carried out to see whether the actin anomaly can be separated from abnormal hsp synthesis. Since chromosomal mapping with respect to dominant flightlessness located the two mutations between *cu* (3:50.0) and *sr* (3:62.0), recombinants in which crossing-over had occurred between the two marker genes, were collected and individually tested for the three distinct phenotypes, flightlessness, actin anomaly and hsp synthesis.

Among 18 recombinants from the KM75 strain, seven which were flightless also synthesized both p42 and hsps, whereas 11 had no abnormality in IFM proteins, and were able to fly (Table I). The defect in actin and the abnormal hsp synthesis were never separated from each other in any of the recombinants. The same results were obtained with HH5, although only dominant flightlessness and hsp synthesis were scored (Table I). These results indicate that the genes responsible for dominant flightlessness and synthesis of hsps must be extremely close, if not identical, to the actin structural gene.

Discussion

Identification of Drosophila actin gene mutations

We have described four mutations which affect actin isoforms in the IFM. Our data show that these are either structural gene mutations, or *cis*-acting regulatory mutations of the *act88F* gene. The phenotypes of the actin gene mutations give us some insight into the nature of *act88F* gene expression. Firstly, the effect of actin gene mutations appears exclusively in the adult IFM; none of the actin isoforms (including actin III) in tubular muscles is affected by the mutations. Moreover, we failed to detect any of the actin variants in tissues other than the IFM. We conclude therefore that the *act88F* gene is expressed only in the IFM. This view is consistent with analyses of the temporal and spatial distribution of actin mRNA, which indicate that the *act88F* mRNA is present only in the adult thorax, and is abundant at mid-to-late pupal stages, when the adult musculature differentiates (Fyrberg *et al.*, 1983; Sanchez *et al.*, 1983).

Secondly, the synthesis of actin III in the IFM is restored in a gene dosage-dependent manner in heterozygotes. This indicates that the normal allele of the *act88F* gene encodes actin III. The major *in vitro* translation product of the *act88F* gene, however, has been found to co-focus with actin II (Fyrberg *et al.*, 1983). We do not consider this as an inconsistency. Actin II synthesis is also reduced in three of the four mutations, suggesting the possibility that this isoform is also, at least partly, the *act88F* gene product. The fact that actin III and actin II in IFM have identical peptide maps supports the view that these two isoforms differ only by post-translational modification. Simultaneous increase of actin I and decrease of actin III in the HH5 mutant can be explained if alteration(s) in the protein sequence resulted in incomplete or abnormal modification of the mutant actin. This possibility is currently being investigated.

Since actin is the major component of the thin filaments in muscle, we consider that the dominant flightless character of the mutants is the consequence of the mutations in the *act88F* gene expressed in the IFM.

It may be worth noting that in the IFM of all four actin gene mutations, another thin filament-associated protein, tropomyosin, is also absent or reduced in stained gels (Figure 1). Its synthesis, however, appears to be normal, since incorporation of [^{35}S]-methionine into tropomyosin is fairly high. Genes encoding tropomyosin are also located in chromosomal band 88F, close to the location of the *act88F* gene (Bautch *et al.*, 1982; Karlik *et al.*, 1984b). We consider that it is unlikely that the mutational event(s) which produced the actin gene mutations simultaneously affected the tropomyosin gene. The drastic reduction of tropomyosin is more likely a mere consequence of the absence of normal thin filaments in the mutants' IFM.

Indeed, no myofibrils were visible in any of the four mutations when their thorax was macerated and observed under a phase contrast microscope. This is also true even in KM75 which contains the stable actin variant, p42.

Induction of hsp synthesis by actin gene mutations

Our most important finding is that two of the actin gene mutations cause synthesis of hsps in the absence of inducing stimuli. Cellular responses to heat shock and other stresses have been analyzed and molecular details of the *hsp* genes have been extensively investigated (for a review, see Schlesinger *et al.*, 1982). However, the molecular mechanisms of hsp induction still remain to be clarified. Attempts to analyze this system genetically have been successful in isolating mutations affecting heat shock response in *Escherichia coli* (Neidhardt and Van Bogelen, 1981; Yamamori and Yura, 1982), in yeast (Iida and Yahara, 1984) and in *Dictyostelium* (Loomis and Wheeler, 1982). A systematic search using an elegant method has also been started in *Drosophila* (Bonner *et al.*, 1984). Primary products of the mutant genes have not been identified however, except for the case of *E. coli* (Landick *et al.*, 1984; Grossman *et al.*, 1984).

Our data strongly suggest that mutations in the actin gene, rather than additional independent mutation(s), are the cause of the abnormal hsp synthesis. This is based on three lines of evidence. Firstly, constitutive hsp synthesis is observed in two independently isolated actin gene mutants. Secondly, in both mutants, recombination mapping failed to separate three phenotypes, abnormal hsp synthesis, actin variant synthesis and dominant flightlessness. Thirdly, both the actin anomaly and the hsp synthesis is restricted to the IFM. If a mutation had occurred in genes which control hsp induction upon heat shock, we would expect *hsp* genes to be turned on in all cell types.

The mechanism by which constitutive hsp synthesis is brought about by the mutation in the actin gene remains to be solved. Disruption of normal myofibrillar structure in the mutants is unlikely to be the inducing stimulus for the heat shock response, since a large number of flightless mutants having similar myo-

fibrillar phenotype do not induce hsps at low temperature. Neither is it likely to be due to the absence of normal actin for the following reasons: firstly, the KM88 allele lacks actin III in the IFM, but still has no abnormality in hsp synthesis. Secondly, both hsp mutations show constitutive hsp synthesis even when the synthesis of normal gene product is restored halfway towards normal level in heterozygotes. The constitutive hsp synthesis must, therefore, result from the presence of altered actin proteins in these mutations. The KM129 allele, which does produce an actin variant protein, shows an increase in the level of hsp83 synthesis, although activation of other major hsps is not observed (data not shown). The extent of hsp activation is most likely influenced by differences in the structure and/or the stability of the mutant actin molecules.

Our result that actin gene mutations can cause constitutive hsp synthesis has a parallel in an actin gene mutation found in a human fibroblast cell line (Leavitt and Kakunaga, 1980; Vandekerckhov *et al.*, 1980; Leavitt *et al.*, 1982). In this case, appearance of a mutated β-actin gene variant is accompanied by neoplastic transformation of the cell, disorganization of cytoskeletal architecture and induction of a number of novel polypeptides. Evidence that a hsp is associated with the transforming protein of Rous sarcoma virus (Oppermann *et al.*, 1981), and that synthesis of mammalian hsp is induced by the product of an adenovirus transforming gene (Nevins, 1982) suggest that novel polypeptides observed in transformed fibroblasts might be related to hsps.

We have recently cloned *act88F* genes from the four mutations we have identified. The feasibility of introducing the *act88F* gene by P-factor, mediated transformation (Mahaffey *et al.*, 1985) has allowed us to prove directly the causality of the actin mutations and the *hsp* gene activation (Hiromi, Okamoto, Gehring and Hotta, in preparation). By nucleotide sequencing of the normal and the mutant *act88F* genes, we have identified base sequence alterations in the coding regions resulting in the produciton of the actin variants (Okamoto, Hiromi, Ishikawa, Yamada, Isoda, Maekawa and Hotta, in preparation). Comparison of the sequences in all the mutants will allow us to see whether actin mutations with an hsp anomaly have a special feature in common, either in position or in nature of the mutations within the actin gene.

Materials and methods

Flies

The isolation of the KM75 mutation has been described (Mogami and Hotta, 1981). KM88, KM129 and HH5 originate from a collection of dominant flightless mutants in our laboratory (Mogami and Hotta, 1981, H. Hiromi, personal communication). *Canton-S* wild type strain, from which mutants were isolated, was used as normal standard. Flies were reared at 24 ± 1°C on standard cornmeal-yeast-agar medium.

Chromosomal mapping

Chromosomal mapping with respect to the dominant flightless phenotype was carried out with reference to *ru* (3:0.0), *h* (3:26.5), *th* (3:43.2), *st* (3:44.0), *cu* (3:50.0), *sr* (3:62.0), e^s (3:70.7) and *ca* (3:100.7) [see Lindsley and Grell (1968) for a description of these marker genes]. Females heterozygous for the mutant chromosome and the multiply marked third chromosome (m-chromosome) were mated to males homozygous with respect to the m-chromosomes, and the flight ability of their progeny was scored. Since the two marker genes *cu* and *sr* cause recessive flightlessness, only half of the total recombinants (having neither of the two marker genes) could be tested.

KM75 and HH5 mutations were further mapped with respect to hsp synthesis. Since the dominant flightless phenotype was mapped between *cu* and *sr*, male recombinants having crossing-over points between these marker genes were individually mated to virgin females of the genotype m-chromosome/*TM3* (*TM3* has dominant marker genes *Ser* and *Sb*, in addition to recessive markers *ru* and *e*). Progeny of the appropriate genotype were tested for flight ability, and their IFM proteins were subjected to two-dimensional gel electrophoresis in order to test for accumulation of hsps and actin variants.

Radioisotopic labeling of proteins

Within 1 h after eclosion, flies were injected with 0.1 μl of [^{35}S]methionine (4 – 8 mCi/ml) in Ringer solution. After 2 h of labeling *in vivo*, the reaction was stopped by freezing the flies in a dry ice/acetone mixture. Cold acetone was changed several times, and finally allowed to evaporate under vacuum. Pure preparations of muscles and other tissues are readily available from samples dehydrated in this way (Mogami *et al.*, 1982).

For heat shock treatment, flies were kept at 37°C for 30 min, and then injected with [^{35}S]methionine at room temperature (~24°C), and labeled for 1.5 h at 37°C. The time during which flies were exposed to room temperature never exceeded 5 min.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was carried out essentially as described by O'Farrell (1975). Stained gels were dried between a piece of cellophane membrane (Biorad) and a lucite plate. The dried gel was taped to an intensifying screen (Dupont Cronex Lightning Plus) with the gel side facing the screen, and exposed to Kodak XAR film at −80°C.

Peptide mapping

Peptide maps were made by partial proteolytic digestion with *S. aureus* V8 protease, according to the procedure of Cleveland *et al.* (1977). For two-dimensional peptide maps, gel slices containing the labeled protein fraction were incubated in 100 μl of *S. aureus* protease (0.05 mg/ml in 25 mM Tris-glycine pH 8.3, 1 mM EDTA, 0.1% SDS) for 30 min at 24°C, rinsed with water, and homogenized in 100 μl of lysis buffer of O'Farrell (1975). The digested peptides were separated by non-equilibrium pH gradient electrophoresis (O'Farrell *et al.*, 1977) and visualized by fluorography (Chamberlain, 1979).

RNA purification

Poly(A)$^+$ RNA was purified by the unpublished method of Mikayama. Within 3 h after eclosion adults were frozen in liquid nitrogen, and pulverized in a freezer mill (Spex) with drops of homogenization buffer [0.1 M Tris-Cl pH 8.0, 1% SDS, 5 mM Vanadyl-Ribonucleoside Complex (BRL)]. The powder was suspended in homogenization buffer, extracted three times with phenol, and once with phenol/chloroform. After removal of glycogen by centrifugation in the presence of 4% sarcosine (150 000 *g*, 60 min), RNA was separated from DNA by salting out in 2 M NaCl. Poly(A)$^+$ RNA was prepared by chromatography through oligo(dT)-cellulose (Collaborative Research).

Plasmid purification, hybrid-selection and in vitro translation

The recombinant plasmid, Hd-19, which contains the *Drosophila* actin gene from the 5C locus as a 1.8-kb *Hind*III insert, was a gift of Dr. Sally Tobin. Plasmid DNA was isolated by the alkali lysis method (Birnboim and Doly, 1979) and purified by CsCl-ethidium bromide equilibrium centrifugation.

Hybridization selection experiments were carried out according to the method of Ricciardi *et al.* (1979). 3 μg of poly(A)$^+$ RNA from *CS* and KM75 adults were selected with Hd-19 plasmid DNA fixed on a nitrocellulose filter. Hybridization and washing conditions were identical to those described by Fyrberg *et al.* (1980). *In vitro* translation of mRNA was carried out using a rabbit reticulocyte lysate (Amersham) containing [^{35}S]methionine.

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