Mutations affecting the indirect flight muscles of *Drosophila melanogaster*

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* *To our deep sorrow Ilan Ivan Deak died on 29 July, 1979. This paper presents the results of the work done by the members of his group.*

**SUMMARY**

The development of the indirect flight muscles of *Drosophila melanogaster* was studied by analysing mutations that cause flightlessness. Twenty-five mutations on the X-chromosome and two on the third chromosome were examined. The X-chromosomal mutations form ten complementation units. The ten loci were assigned preliminary map positions by meiotic recombination and deficiencies and duplications. The two autosomal mutations represent two genes. Gynandromorph analyses suggest that many of these mutations have their primary effect in the presumptive thoracic muscle region of the embryo. The mutations cause a variety of characteristic defects, such as absence of the bulk of the thoracic muscle mass, or absence of only one of the two fibrillar muscle groups. Electronmicroscopic studies of sixteen mutants representing all twelve loci reveal abnormal myofibrillar organization in some of these mutants, e.g. aberrant or missing Z-bands, or absence of the thin filaments. Mutant protein patterns, obtained by SDS-polyacrylamide gel electrophoresis, show the following differences from wild type: ten mutants are characterized by absence or reduction of the 54 K protein, and most mutants exhibit a reduction and modification of the 80 and 90 K proteins. The absence or reduction of the 54 K protein was strongly correlated with aberrant Z-bands.

**INTRODUCTION**

Myogenesis is a complex morphogenetic process involving the differentiation of myogenic cells into highly specialized muscle tissue. Many aspects of this process have been elucidated using vertebrate (Dienstman & Holtzer, 1975), and invertebrate *in vivo* or *in vitro* systems (Finlayson, 1975). However, wide gaps exist in our understanding of myofibrillogenesis, particularly in the ways how differential gene expression creates the diversity of musculature.

Similarly complex systems, such as the nervous system (Hall, 1978) and visual system (Campos-Ortega, 1980) have been analysed using appropriate behavioural mutants of *Drosophila.* Application of the same method to studies...
on muscle development requires mutations that interrupt the developmental process at various levels. The indirect flight muscles with their distinct characteristics and defined time of differentiation during metamorphosis are most suitable for investigation. The behavioural phenotype 'flightlessness' is easily recognized in mutagenesis screens and was therefore used to select new muscle mutations (Hotta & Benzer, 1972; Deak, 1977; Homyk & Sheppard, 1977; Koana & Hotta, 1978; Homyk et al. 1980).

An extensive analysis of such mutations may provide insights into the network of genes that are involved in myogenesis. The technique of fate mapping (Hotta & Benzer, 1970) allows distinction between mutations whose primary target tissue are the muscles, and mutations that affect other tissues required for normal muscle development (Guth, 1968; Nüesch, 1968). Further information can be collected by comparing the morphological and behavioural abnormalities, as well as the protein patterns exhibited by different alleles of the same locus and by different loci, and by correlating biochemical and ultrastructural aberrations (Mogami, Nonomura & Hotta, 1981).

**MATERIALS AND METHODS**

**Flies**

The Oregon-R (Ore) strain of *Drosophila melanogaster* was taken as wild-type control. Mutant stocks were kept homozygous or balanced over either *In(l)FMb, y*w* l*z* B* (Merriam, 1969) or *In(l)FM6, y*w* sc*d* dm B*. For a description of mutations and chromosomes used for mapping and balancing see Lindsley & Grell (1968). The X-chromosomal deletions employed (Table 1) were kindly supplied by Dr E. Wieschaus. The mutants *fltO* and *rdgB* were isolated and characterized by Koana & Hotta (1978) and Hotta & Benzer (1970) respectively, *l(l)mysts* by Wright (1960), and *ewg* by Hanratty (unpublished). The source of the mutations *flw1*, *flw2*, *hdp2*, *int1*, *up1*, *up2*, *vtw1*, and *vtw2* has been described previously (Deak, 1977). Revertants of *Bx (hdpSBLA, hdpRBE1*, Lifschytz & Green, 1979) were generously supplied by Dr E. Lifschytz. As these mutants do not correspond to the previously characterized mutant *hdp2* (see Results), they have been renamed as *heldwing* alleles (*hldSBLA, hldRBE1*).

**Mutagenesis and mutant isolation**

A number of X-chromosomal mutations affecting adult musculature were induced by ethyl methanesulphonate (EMS) as described by Lewis & Bacher (1968). Wild-type males were fed for 24 h with 0.025 M EMS dissolved in a 1% sucrose solution, and then were mass mated to *C(l)RM, y f* females. All of the F1 male progeny were subsequently tested for flightlessness by gently releasing them onto a flat surface. Putative flightless mutants were re-examined and only retained if the mutant had no apparent cuticular defect which might interfere with flight ability, i.e. curled wings, incised wings, etc. Other criteria to be
Flightless mutants of Drosophila melanogaster fulfilled before further analysis was carried out were: either gaps in the fluorescence pattern of the musculature under polarized light, or indentations of the thorax characteristic of the previously isolated muscle mutant indented-thorax (int). Such males were mated to C(1)RM, y f females and the following mutant lines were established (in parentheses are the previously used laboratory designations for these mutants, reported in Deak et al. 1980). Flutter, Fluv (88), Flus (280); flap-wing, flw3 (671), flw4 (725); gumper, gmp1 (696), gmp2 (628); grounded, gnd1 (694), gnd2 (623), gnd3 (200); heldup, hdp3 (405), hdp4 (299), hdp5 (13); indented-thorax, int3 (421), int4 (719); lethal(l)93pupal-temperature sensitive, l(l)93pts (93); standby, sdby (794); upheld, up3 (1121).

Gynandromorph analysis

Mosaic flies were produced using the unstable ring-X-chromosome R(l)wnc. Virgin females heterozygous for this chromosome were mated to males carrying the muscle mutation (x) and the cuticular marker y. Loss of the ring-X-chromosome in some of the cells of the R(l)wnc/y (x) F1 female zygotes, resulted in mosaic flies, in which male cuticle was marked with y while female cuticle was phenotypically wild type. The distribution of male and female cuticular landmarks was recorded in the mosaic progeny and their behaviour, i.e. wing position and ability to fly, were scored. Mosaic control flies without a muscle mutation behaved like non-mosaic wild type.

Values for the frequencies with which selected structures were of different genotype were obtained, and represented on a fate map. As flight ability can only be scored as a single parameter but may result from the combined action of two or more bilaterally symmetrical foci, the data were analysed according to the ‘domineering’ or ‘submissive’ models for interacting foci (Hotta & Benzer, 1972).

Morphological investigation

Frozen or plastic embedded sections were prepared and stained as previously described (Deak, 1977), or stained with 0.5% toluidine blue, and examined under a light microscope. For electron microscopy isolated thoraces were opened ventrally in insect Ringer solution, fixed for one hour at 4 °C in 0.05 M cacodylate buffer (pH 7.2) containing 2.5% glutaraldehyde and 5% sucrose, washed in buffer for 15 min, post fixed in buffered 1% OsO4 for 1–3 h, dehydrated in graded acetone, and embedded in epon. After staining in uranyl acetate and lead, sections were photographed in a Siemens 102 electron microscope.

Biochemical analysis

Freshly emerged flies (0–2 h) and 5- to 8-day-old flies were collected, and crude extracts were prepared by homogenizing the flies in 15 mM Tris-HCl, pH 7.2, 5 mM ethyleneglycol-bis- (β-aminoethylether)-N,N'-tetraacetic acid
(EGTA), 0.3 M sucrose. The homogenate was centrifuged at 800 g, and the myofibrillar pellet washed three times with the homogenization buffer. Then the chitin particles and cell debris were separated from the myofibrillar suspension by a short centrifugation pulse. The suspended myofibrils were sedimented in an Eppendorf centrifuge and dissociated in 15 mM Tris-HCl, pH 7.2, 1% sodium dodecylsulphate (SDS), 1 mM dithiothreitol (DTT), 1% β-mercaptoethanol, 5% glycerol by boiling them for 3 min. The dissociated myofibrils were again separated from the debris by centrifugation in order to avoid subsequent degradation by debris-associated proteases.

Electrophoretic analysis was carried out on one-dimensional SDS-polyacrylamide gels (Laemmli, 1970). In order to screen for major changes in the protein pattern, equal amounts of protein were loaded on 7 and 10% gels, and after electrophoresis, the proteins were stained with 0.25% Coomassie brilliant blue, followed by destaining in 50% methanol, 7% acetic acid.

RESULTS AND DISCUSSION

Isolation of mutants

Some $6 \times 10^4$ males with mutagenized X-chromosomes were screened for flightlessness. Of 1200 males retained, approximately 100 individuals were shown to possess sex-linked mutations satisfying the criteria outlined in Materials and Methods. This report deals with 25 of these X-chromosomal mutants. In addition the mutants $rsd$ (raised) and $sr$ (stripe) on the third chromosome were also analysed.

Stocks were established and the mutations were mapped using a $y$ $cho$ $cv$ $vf$, $y^+$ or $y$ $cho$ $cv$ $sn^3$ $v$ $g^2$ $f$ chromosome. Complementation tests were performed in all pairwise combinations. Based on the mapping and complementation studies the mutations fell into ten cistrons. Due to incomplete penetrance, most of the mutants could only be mapped to approximate positions (Fig. 1). In other
laboratories sex-linked flightless mutations have been isolated at similar map positions (Koana & Hotta, 1978; Homýk & Sheppard, 1977; Homýk, Szidonya & Suzuki, 1980). Complementation tests between the mutants of the different laboratories, however, have not yet been made.

**Phenotype of the mutants**

The phenotype of the mutants *flw*, *hdp*, *int*, *up*, and *vtw* has been reported previously (Deak, 1977). Newly isolated alleles of these mutants behaved in a similar manner, i.e., the mutant flies were flightless, held their wings in an abnormal position, and sometimes exhibited variable jump ability. A number of these mutations, while recessive with regard to their wing position, showed semi- or complete dominance with regard to flight ability. This was particularly true of *hdp*3, *hdp*A, *hdp*5, *int*, *rsd*, *up*1, and *up*2, all of which were still flightless when heterozygous for a wild-type allele. Additionally, these mutants displayed a strong degree of intercistronic non-complementation. Up to 30% of *up*/+ *rsd*/+ females had abnormal wing position, even though these two mutations lie on the first and third chromosome, respectively. This intercistronic non-complementation is an intriguing phenomenon which could suggest that the two genes or rather their products may interact in some way. For *int* and *up*, which exhibit a readily distinguishable phenotype, *up* is epistatic in that 70–80% of the *int*/*up* heterozygous females had upheld wings whereas only 20–30% held their wings ventrolaterally or had thoracic indentations.

The mutant *Flu*1 (1–37·0) is phenotypically similar to the mutant *Hk*1 (1–30·0; Kaplan & Trout, 1969). *Flu*1 heterozygotes, when etherized, vigorously shook their legs and fluttered their wings in a scissoring motion. Their flight ability was reduced and they displayed an impaired jump response. Homozygous flies were completely flightless and showed greater agitation upon etherization. *Flu*2 animals, on the other hand, were fully recessive with respect to their flightless phenotype and displayed no hyperkinetic activity. As no extensive attempt has been made to separate the two phenotypes by recombination, it is possible that *Flu*1 may contain two closely linked mutations, one affecting nervous activity and one affecting flight ability.

The mutant *l(I)93p*ts lies between *cv* (1–13·7) and *sn* (1–21·0) and is a temperature-sensitive pupal lethal. Preliminary temperature-shift experiments indicated that the mutant has its temperature-sensitive period in late third instar and early pupal stages. Shifts from 22 to 29 °C at these times resulted in less than 5% survival relative to *C(I)DX, yf* or *FM6* controls. Shifts at later pupal stages, just prior to eclosion, resulted in an increased penetrance of muscle defects, as well as in incomplete rotation of the external male genitalia, and subsequent sterility. *l(I)93p*ts did not complement with *l(I)mys*ts (1–21·7), an embryonic lethal lacking mesodermal derivatives (Wright, 1960; Newman & Wright, 1981). *l(I)93p*ts therefore might well be an allele of *l(I)mys*, although lack of complementation has been found for the clearly non-allelic mutations *up* and *rsd*.
Table 1. List of X-chromosomal deficiencies (Df) and duplications (Dp) used for the cytological localization of flightless mutations

<table>
<thead>
<tr>
<th>Deficiency or duplication</th>
<th>Breakpoints</th>
<th>Mutants uncovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP(l;f)s*</td>
<td>1A</td>
<td>ewg*</td>
</tr>
<tr>
<td>Df(l)HF366</td>
<td>3E8; 5A7</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)RC40</td>
<td>4B1; 4F1</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)C149</td>
<td>5A8-9; 5C5-6</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)N73</td>
<td>5C2; 5D5-6</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)HA32</td>
<td>6E4-5; 7A6</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)ctJ{4,6}</td>
<td>7A2; 7C1</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)ct{268-48}</td>
<td>7A5-6; 7B8-C1</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)sn{128}</td>
<td>7D1; 7D5-6</td>
<td>Fl(1)93p+</td>
</tr>
<tr>
<td>Df(l)RA2</td>
<td>7D10; 8A4-5</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)KA14</td>
<td>7F1-2; 8C6</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)CS2</td>
<td>8E; 9D</td>
<td>gmp*, Flw*</td>
</tr>
<tr>
<td>Df(l)ctJ{135}</td>
<td>9B1; 10A1</td>
<td>Flw*</td>
</tr>
<tr>
<td>Df(l)ras-v{17}Go</td>
<td>9E3-4; 10A4-5</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)RA37</td>
<td>10A7; 10B17</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)KA7†, §§</td>
<td>10A9; 10F10</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)N105§</td>
<td>10F7-8; 11C4-D1</td>
<td>Flu</td>
</tr>
<tr>
<td>Df(l)JA26</td>
<td>11A1; 11D-E</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)N12</td>
<td>11D1-2; 11F1-2</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)C246</td>
<td>11; 12A1-2</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)g†</td>
<td>12A; 12E</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)HA92</td>
<td>12A6-7; 12D3</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)KA9</td>
<td>12E1; 13A5</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)SBLA</td>
<td>17A; 17B?</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)N19</td>
<td>17A; 18A2</td>
<td>hld{SBLA, RBE}1*</td>
</tr>
<tr>
<td>Df(l)JA27</td>
<td>18A; 18D</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)mal‡</td>
<td>18F; heterochr.</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)mal‡</td>
<td>18F; 19E</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)mal‡</td>
<td>19A; heterochr.</td>
<td>—</td>
</tr>
<tr>
<td>Df(l)DCB1-35b</td>
<td>19F; 20F</td>
<td>sdby,</td>
</tr>
</tbody>
</table>

* Mutant wing position uncovered in heterozygotes over Df.
† Lethal over Df.
‡ Df/ + exhibits abnormal wing position.
§ Df/ + has a hyperkinetic phenotype.
|| Flight ability reduced in heterozygote over Df.

iftO*, located proximal of f is a mutant with normal wing position (Koana & Hotta, 1978). It did not complement with sdby situated between sn(1-21-0) and v(1-33-0), which has ventrolaterally held wings. Transheterozygotes held their wings normally, but were completely flightless. No further tests were undertaken to determine their possible allelism.
Flightless mutants of Drosophila melanogaster

![Diagram showing cytogenetic analysis of the int-up region. The limits of seven deficiencies extending from 10F8 to 13A5 are shown; interrupted lines indicate uncertain breakpoints. Each deficiency was examined in trans with the recessive mutations fw, wy, s, rdgB, g, ty, int, and up. The cytological location of each of the marker mutations, as well as the probable location of int and up, is shown. Mutants uncovered by the deficiency are given in bold print under the line representing the deficiency. None of the deficiencies uncovered int or up.]

**Deficiency analysis**

Twelve of the flightless mutations were uncovered on the basis of an abnormal wing phenotype, flightlessness, or lethality when in trans with the appropriate deletion (see Table 1). None of the deletions exposed gnd, hdp, or vtw alleles. As expected, ewg, which maps distal to y (Deak, 1977), was covered by Dp(l; f)se8 and therefore lies in 1A.

l(l)93p18, which did not complement l(l)myst18 was lethal at all temperatures when in trans with Df(l)shC128, a deficiency that includes l(l)myst18.

All flw alleles proved to be lethal over Df(l)yL15, but were viable over Df(l)C52 with a flw phenotype. Since hemizygous flw males, however, are viable, we are dealing with a complex non-allelic interaction between flw and Df(l)yL15. As this holds true for all flw alleles isolated independently, the flw and the lethal phenotype must both be due to the flw mutation itself.

Flu1 heterozygotes show a reduced flight ability and a hyperkinetic phenotype. Since the two deficiencies Df(l)KA7 and Df(l)N105 themselves exhibit a dominant hyperkinetic phenotype, we could not localize Flu1. However, only Flu1/ Df(l)N105 flies, but not Flu1 over other deficiencies, were further reduced in their flight ability. Thus Flu1 may well lie within this region.

Two additional mutations isolated elsewhere, hldSBLA (Lifschytz & Green, 1979) and fltO2 (Koana & Hotta, 1978), were also included in this study. fltO2, which maps to the right of f, lies within Df(l)DCB1–35b. sdb, shown to be non-complementing with fltO2, was also non-complementing with this deficiency.
Table 2. *Summary of focus mapping for the flightless mutants gmp¹, gnd², Flu¹, sdby, and ewg*

(Sturt distances between cuticular landmarks and the ipsilateral behavioural focus are presented for each of the five mutations. The distances between left and right foci were calculated according to Hotta & Benzer (1972), and represent averages ± s.d. derived from the five cuticular landmarks; half of this value gives the distance between the foci and the ventral midline of the embryo. Abbreviations for the cuticular landmarks: Ar, arista; L1, first leg; Hum, humeral bristle; Psc, posterior scutellar bristle; Terg2, second abdominal tergite.)

<table>
<thead>
<tr>
<th>Mutant</th>
<th>No. of gynanders</th>
<th>No. of flightless gynanders</th>
<th>Distance in sturts to cuticular landmarks</th>
<th>Distance left-right foci</th>
<th>Region of blastoderm affected</th>
<th>Probable focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>gmp¹*</td>
<td>98</td>
<td>56</td>
<td>36-5</td>
<td>14-0±1-9</td>
<td>Ventral thoracic</td>
<td>Muscle</td>
</tr>
<tr>
<td>gnd²†</td>
<td>61</td>
<td>31</td>
<td>36-9</td>
<td>19-2±2-0</td>
<td>Ventral thoracic</td>
<td>Muscle?</td>
</tr>
<tr>
<td>Flu¹</td>
<td>58</td>
<td>34</td>
<td>21-0</td>
<td>6-2±3-4</td>
<td>Anterior ventral</td>
<td>Nerve?</td>
</tr>
<tr>
<td>sdby</td>
<td>87</td>
<td>50</td>
<td>25-0</td>
<td>27-2±7-6</td>
<td>Ventral thoracic</td>
<td>Nerve?</td>
</tr>
<tr>
<td>ewg</td>
<td>161</td>
<td>84</td>
<td>36-0</td>
<td>3-6±2-3</td>
<td>Ventral thoracic</td>
<td>Muscle</td>
</tr>
</tbody>
</table>

For fate maps the reader is referred to Janning (1978, fig. 14) and Hall (1978, fig. 4).
* The weaker allele gmp² also maps to this region.
† Results based on 30 gynanders suggest that gnd¹ also maps in the ventral thoracic region.
**Flightless mutants of Drosophila melanogaster**

Both mutations in trans with *Df(l)DCBl-35b* rendered the flies completely flightless, but gave normal wing positions. As expected, *hld*SBLA, placed in region 17A by Lifschytz & Green (1979), was also uncovered by *Df(l)N19*. None of the *hdp* alleles tested was uncovered by this deficiency, and therefore *hdp* must lie to the left of *hld*SBLA.

**The int-up region**

Two of the mutations studied, *int* and *up*, map to the same region, approximately equidistant from the markers *wy* (1-41-9) and *g* (1-44-4), slightly to the right of *s* (1-43-0) at about 1-43-5 ± 0-3. Though resulting in separate and distinct phenotypes (Deak, 1977), these mutations and their alleles did not complement each other, and trans heterozygotes displayed a predominantly *up* phenotype.

*int*, *up* and six flanking mutations have been mapped with respect to seven deficiencies covering the region 10F8 to 13A5. All of the flanking mutations are uncovered by at least one of the deficiencies. The extent of each deficiency and the cytological location of each of these mutations is shown in Fig. 2.
Table 3. Summary of the light- and electron-microscopic analysis of 16 mutants compared to Oregon wild-type flies
(Three to six flies of each mutant, aged 3–5 days were scored as described in Materials and Methods).

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Amount of muscle</th>
<th>Light-microscope appearance of fibres</th>
<th>Myofibrillar organization</th>
<th>Z-band structure</th>
<th>No. of Z-bands/100 μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DLMs</td>
<td>DVMs</td>
<td>TDTs</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Oregon ewg</td>
<td>Normal</td>
<td>Normal</td>
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<td>Normal</td>
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<tr>
<td>Fln¹</td>
<td>Normal</td>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>flw</td>
<td>Scant</td>
<td>Scant</td>
<td>Normal</td>
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<tr>
<td>gmp¹</td>
<td>Normal</td>
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<tr>
<td>gnd¹</td>
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<tr>
<td>hdp²*</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Disorganized</td>
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<tr>
<td>hdp²</td>
<td>Normal</td>
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<td>Normal</td>
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<td>Normal</td>
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<tr>
<td>int*</td>
<td>Reduced</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>int¹**</td>
<td>Reduced</td>
<td>Reduced</td>
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<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>l(l)93p¹</td>
<td>Scant</td>
<td>Normal</td>
<td>Reduced</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>rsd</td>
<td>Reduced</td>
<td>Reduced</td>
<td>Normal</td>
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<tr>
<td>sdby</td>
<td>Normal</td>
<td>Normal</td>
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<td>Normal</td>
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<tr>
<td>sr</td>
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<td>vtw²</td>
<td>Scant</td>
<td>Reduced</td>
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</tr>
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</table>

Abbreviations: DLMs, dorsal longitudinal muscles; DVMs, dorsoventral muscles; TDT, tergal depressor of the trochanter of the second leg.

* 'Degeneration' mutant.
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Fig. 4. Light micrographs of serial cross sections through the thorax of wild-type and mutant flies, aged 3 to 5 days. Dorsal is up. (a) Toluidine-blue stained 15 μm frozen section of a wild-type thorax. (b) Toluidine-blue-stained 1 μm epon-embedded cross section of ewg. dlm's are absent, while dvm's are present and appear normal. in, intestine. (c) Longitudinal 15 μm frozen section of sdbv stained for α-GpDH. All of the indirect flight muscles are present, but the fibres appear thinner and less compact (arrows) than in wild type. (d) α-GpDH-stained longitudinal frozen section of int. The bulk of the muscle mass is present but highly disorganized, and partly accumulated underneath the cuticular indentation (*). Abbreviations are the same as in Fig. 3.
Neither \textit{int} nor \textit{up} was uncovered by any of these deficiencies; however, as the breakpoints in the 12A1–2; 12A6–7 region are unclear, it is possible that both \textit{int} and \textit{up} lie within this region. This would be in accordance with the mapping data, as both mutations lie to the right of \textit{s} and to the left of \textit{g}. Although \textit{rdgB} has previously been reported to map to 1–42–7 (Harris & Stark, 1977), it is included within \textit{Df(l)HA92} and \textit{Df(l)g1}, which places it to the right of \textit{s} (1–43–0). Both \textit{int} and \textit{up} map to the left of \textit{rdgB}, which is further evidence that these mutations lie somewhere within 12A. Alternatively, \textit{int} and \textit{up} may not behave as hypo- or amorphs and thus would not be detected by deficiency analysis.

\textbf{Foci of the mutants}

Gynandromorph fate mapping suggests that the three genes \textit{int}, \textit{up}, and \textit{hdp} have their primary site of action in the presumptive thoracic musculature, while two others, \textit{flw} and \textit{vtw} may function in the fat body and tracheae, respectively (Deak, 1977). In addition, we have constructed preliminary fate maps of \textit{ewg}, \textit{Flu1}, \textit{gmp1}, \textit{gnd2}, and \textit{sdby} in order to discriminate between cuticular and nerve/muscle sites of action for these genes. Fifteen pairs of surface landmarks, wing position and flight ability, were scored for being wild type or mutant in mosaic flies (Table 2). For the five mutations tested, the majority of gynandromorphs were flightless. Therefore, the data were treated according to the domineering model (Hall, 1978), which gave positive values for all stunt distances. The small numbers of gynandromorphs scored and the large distances between the behavioural and cuticular landmarks (16 sturts and more) do not allow precise mapping. Therefore, we have refrained from presenting fate maps. But all five mutations listed in Table 2 appear to have their foci in the ventral region of the embryo.

Of the five mutants examined \textit{ewg} and \textit{gmp1} mapped to the ventral thoracic region of the blastoderm. Embryological studies by Poulson (1950) have shown that this region contains the primordial mesoderm from which musculature arises. Other mutations that affect the indirect flight musculature, such as \textit{up}, \textit{hdp}, \textit{int}, \textit{fli F1}, \textit{flt H}, \textit{flt D0} (Hotta & Benzer, 1972; Deak, 1977; Koana & Hotta, 1978; and Homyk 1978), also map within this region. We therefore conclude that \textit{ewg} and \textit{gmp1} have their primary defects in the thoracic musculature. Of the remaining three mutants, \textit{gnd2} mapped somewhere more dorsally from the focus of \textit{gmp1}; \textit{Flu1} and \textit{sdby} mapped to the anterior region of the blastoderm. Since it is difficult to distinguish between muscle and nerve primordia in the anterior region of the embryo, we were unable to determine which of these two tissues is affected.
Effects of the mutations on the morphology of the thoracic musculature

For further insight into the nature of the lesions that cause flightlessness, we examined the mutants for gross morphological and ultrastructural abnormalities. We grouped the mutants on the basis of different types of abnormalities in their thoracic muscles. For comparison the organization of the thoracic muscles of a wild-type fly is shown in outline in Fig. 3 (for description see Zalokar, 1947; Shafiq, 1963; Crossley, 1978), and the ultrastructure is pictured in Fig. 5(a). The results are summarized in Table 3.

Group 1: large parts of dorsal longitudinal and dorsoventral muscles absent

The mutations $flw$, $vtw$, and their alleles showed the most drastic aberrations in that most of the dorsal longitudinal muscles (DLMs) as well as most of the dorsoventral muscles (DVMs) were lacking (Deak, 1977). No histological evidence was found for degeneration, and it thus seems that some of these muscles are not formed. With regard to myofibrillar organization, the small amount of muscle present was highly disturbed (Fig. 5c); thin and thick filaments were hardly recognizable. Gynandromorph analysis suggested that tissues other than musculature are primarily affected by $flw$ and $vtw$ (Deak, 1977). Therefore, we speculate that these two genes play auxiliary roles in the formation of muscle tissue.

Group 2: DLMs absent

The mutations $ewg$, $l(1)93P^{ts}$, and $sr$ specifically affected the DLMs which were often missing in such flies. The ultrastructure of the remaining muscles of these three mutants, however, approached that of the wild type. Although the DLMs and DVMs, except for their location and orientation, appear identical at the level of morphological, histological, and biochemical analysis (Deak, 1976), results from extirpations and transplantations of imaginal discs (Zalokar, 1947; Deak et al. 1980) suggested that they arise from different cell populations. The action of $ewg$, $l(1)93P^{ts}$, and $sr$ on the DLMs, but not the DVMs, supports this conclusion.

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Fig. 6. Ultrastructural details of dlm’s from adult males of four mutants. (a) $up$. Electron-dense structures consisting of repeated electron-dense bands (arrow), which are connected by thin filaments. (b) $hdp^2$, 5-day-old. Disorganized myofibrils (mf) and one almost normal fibril with Z- and H-bands present. (c) $int$, freshly eclosed and (d) $int$, 5-day-old. The young flies contain abnormal Z-bands and a fairly normal myofibril (mf) structure, whereas the older flies completely lack Z-bands. mi, mitochondria. (e) and (f) $rsd$, 5-day-old. Myofibrils are disorganized and only contain thin filaments. (e) longitudinal section, (f) cross-section. The dense middle zone of the thick filaments forms a structure similar to the H-band in normal flies (arrow).
Group 3: Z-bands and myofibrils affected

(a) Slight disturbance. In animals, mutant for Flu, gmp, gnd, and sdby, the normal complement of muscles was present and only slightly affected at the light microscopic level. The fibres particularly of the DLMs were often thin and loosely packed (see sdby, Fig. 4c). Also at the electron microscopic level these
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mutants were almost indistinguishable from the wild type. sdby and gnd, however, showed irregular cross striation, the fibrils did not lie parallel to each other and occasionally contained electron-dense structures as in up\(^2\) and up\(^3\) (see group 3b).

(b) Strong disturbance. In int, up, and their alleles and in rsd the indirect flight muscles were also present, but in contrast to group 3a, they were badly disorganized (Fig. 4d), mostly lacking Z-bands (Fig. 6a, c, d). Although hdp and its alleles cause large parts of the thoracic musculature to be absent, they were included in this group, since they also showed a significant reduction or absence of Z-bands. When Z-bands were present they were always crooked and disorganized, as in up\(^2\), up\(^3\), hdp, and in freshly eclosed int flies (Fig. 5f). All of the X-chromosomal mutations of this subgroup mapped in the muscle primordium itself.

The mutations in this subgroup display some special features. The alleles hdp, int, and int\(^4\) had a much less extreme phenotype at eclosion than a few days later (Figs. 6c, d). In 5 to 15-day-old adult flies the cross striation became increasingly irregular: Z-bands fused or disappeared and the myofibrils were crooked. This process of ‘degeneration’ proceeded from the centre of the fibrils towards their attachment sites. It was already described for late pupae of hdp\(^8\) by Hotta & Benzer (1972).

up\(^2\) and its more extreme allele up\(^3\) commonly contained electron-dense structures (Fig. 6a), occasionally also seen in gnd and sdby. Similar structures were described as ‘striated bundles’ by Koana and Hotta (1978) in the mutant flt 0 and were also found after heat lesion in Calliphora muscle (Auber & Auber-Thomay, 1978); they could represent Z-band material.

The mutant rsd was particularly interesting for its lack of thin filaments (Figs. 6e, f). The thick filaments formed irregular myofibrillar bundles with occasional cross striation reminiscent of the H-zone in normal muscle (Fig. 6e). Rarely thin filaments were found associated with electron-dense structures appearing to be Z-band material. This phenotype was also observed in rsd fibrillar muscles cultured in vitro from embryonic cells (Fenner, 1981). It has been shown (Lang, Wyss & Eppenberger, 1981) that such flies fail to synthesize the actin III isoform in their fibrillar muscles. However, rsd is probably not the structural gene for actin III since cloned DNA-segments of actin genes did not hybridize anywhere near the presumed position of rsd on the salivary gland chromosome (Tobin et al. 1980; Fyrberg, Kindle, Davidson & Sodia, 1980). Therefore, the rsd locus may be somehow involved in the regulation of actin III. hdp, int, up, rsd, and their alleles seem to be involved in the establishment or maintenance of the detailed architecture of the myofibril, and of the Z-bands in particular.
Electrophoresis of flight-muscle proteins

Twenty-two flightless mutants with morphologically abnormal flight muscles were analysed by electrophoresis in an attempt to correlate defects in myofibrillar ultrastructure with specific changes in protein constitution. Myofibrillar extractions were prepared as described on pp. 63–64. Contamination from muscles other than indirect flight muscles was slight, since protein patterns of myofibrils prepared from homogenized whole flies, thoraces, or isolated indirect flight muscles were identical.

Compared to wild type, the electrophoretic patterns of mutant flies, showed a number of specific differences (Fig. 7). The protein band with an estimated molecular weight of 54 K was absent in hdp\(^3\), \(^4\), \(^5\), int\(^3\), rsd, up\(^3\), and reduced in hdp\(^2\), int, flw\(^b\), \(^4\). Samples of young hdp\(^2\) or int flies had a stronger 54 K band than older flies; the degree of reduction paralleled the degree of muscle ‘degeneration’ (p. 77). It is striking that in all those mutants in which this band was affected, the Z-bands were absent or their structure was highly disturbed. This correlation suggests that the 54 K protein is a major component of the Z-band.

The two protein bands with approximate molecular weights of 80 and 90 K were reduced in the mutants with morphologically abnormal flight muscles. This was often paralleled by the appearance of new bands of slightly lower molecular weights (Fig. 7). Interestingly, the proteins migrating in this region were affected by all the mutations, whereby the banding pattern varied among mutants (Fig. 7). Thus, it appears that mutations causing abnormal muscle morphology also lead to abnormal banding patterns of a particular class of proteins.

Proteins known to migrate in the same region on SDS-polyacrylamide gels (80–100 K) are the larval serum proteins (Roberts & Brock, 1981). In myofibrillar extracts of wild-type flies, the larval serum proteins disappear at eclosion, but in some of the mutants they persist into adult life (Dubuis, 1981). This suggests a connection between abnormal muscle formation and persistence of these proteins.

CONCLUDING REMARKS

The aim of our work was to identify and study some of the genes that are involved in the complex process of myogenesis. We chose the indirect flight muscles of the adult fruit fly to investigate muscle development. Obviously, our screen selecting for flightlessness will only detect mutations that perturb flight ability but still allow development to adulthood. Mutations of genes that affect basic steps in myogenesis, such as the segregation of the mesoderm from other larval or adult precursor cells, or that exert functions necessary for all muscle types, will cause lethality.

Our study has detected a number of X-chromosomal genes some of which have very specific effects on a particular type of muscle, such as the DLMs, or on particular structures of the myofibre. Surprisingly, however, no mutant was found
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in which a major protein was absent or noticeably changed. We see two possible explanations for this: (i) such mutants may be lethal because the protein they specify is required in all types of muscles; or (ii) there are multigene families coding for the major proteins (Fyrberg et al. 1980; Tobin et al. 1980) whereby more than one member of the family is active in the indirect flight muscles. At present, we cannot distinguish between these and other possibilities.

What is the function of the genes that we have identified? We can speculate that their products may be involved in the assembly of proteins of the indirect flight muscles. Or they may specify minor proteins, such as may be present in specific attachment sites; these would remain undetected in our gels.

We consider our investigations as a first step towards identifying and characterizing the genes that are involved in myogenesis. Further analyses will have to include lethal mutations in genes not yet identified, and potentially lethal alleles of the genes studied currently, so that indispensable functions will also be found.

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