THE MUTATIONAL BASIS FOR THE “ALLELIC” MODIFIER MUTANTS, ENHANCER AND SUPPRESSOR OF HAIRLESS, OF DROSOPHILA MELANOGASTER

DAVID NASH

Department of Genetics, University of Alberta, Edmonton 7, Alberta, Canada

Received June 2, 1969

Hairless, \((H,\) chromosome 3, 69.5) of Drosophila melanogaster, is modified by two mutations, an enhancer \((E(H))\) and a suppressor \((Su(H))\), which are remarkable in having opposite effects upon \(H\), yet both mapping at approximately the same location (50.5) on chromosome 2. A functional interpretation of these two mutants, based upon phenotypic observations, was presented by Nash (1965). \(Su(H)\) was considered a deficiency mutation, whereas \(E(H)\) appeared to have gained function. Nash and Woloshyn (1969) showed that \(E(H)\) reverts to wild type with a frequency higher than is normal for back mutation. They proposed that this effect might be due to unequal crossing over and therefore concluded that \(E(H)\) is probably a duplication even though cytogenetic evidence was lacking. An extension of these two hypotheses is that cytologically identifiable duplications and deficiencies of the chromosomal region carrying the modifiers might act, respectively, as enhancers and suppressors of Hairless.

The following paper reports on studies relative to this suggestion. The positive results obtained incidentally indicated the chromosomal location of the mutants and, as a result, it has been possible to conduct a careful search for a duplication at that location in the \(E(H)\) mutant chromosome.

MATERIALS AND METHODS

Stocks: The following stocks were used: 1. \(H/LVM:\) derived from a selection line \((L)\) of Nash (1965). \(H\) and \(LVM\) (an unmarked third chromosome inversion) constitute a stable balanced-lethal system. 2. \(E(H)/E(H);H/LVM:\) obtained by repeated backcrossing of \(E(H)/+;H\) flies to \(H/LVM\) and then made homozygous for \(E(H)\). This stock is subject to constant selection against reversion of \(E(H)\) to \(E(H)^+\) (Nash and Woloshyn 1968); when non-\(E(H)\) segregants appear they are phenotypically similar to flies from the \(H/LVM\) stock. It is inferred, therefore, that there is a uniform and similar genetic background in both stocks, at least with respect to minor modifiers of Hairless. 3. Three stocks bearing duplications of the \(Adh\) (Alcohol dehydrogenase) locus (chromosome 2, 50.1 Grell, Jacobson and Murphy 1965) and one with a deficiency for the same region, all produced by E. H. Grell of the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee; these stocks are balanced by \(Cy\) inversions of unknown origins on the second chromosome. \(Dp(2;2)Adh2, T(2;3)DpAdh1\) and \(Df(2L)64j\) act as lethal balancers. \(Dp(2;2)Adh3\), which is homozygous viable, is maintained in balance by a recessive lethal located to the left of the duplication and dissociable from it by recombination (Grell, personal communication). 4. an attached-\(XY\) strain \((XX/X\tilde{Y}vB)\) from the Division of

Biology, California Institute of Technology, Pasadena. All stocks and experimental cultures were maintained at 20°C on the yeast-sucrose medium of Nash and Bell (1968).

The test for interaction of Hairless and the chromosomal aberrations: To test the effects of the four chromosomal aberrations upon the expression of Hairless, each was crossed to the H/LVM stock. The general nature of these crosses is shown in the caption to Table 2. Four types of progeny with differentiable phenotypes (CY/+;H/+), Aberration/+;H/+, Cy/+;LVM/+ and Aberration/+;LVM/+ were produced. The first two classes were compared for the degree of interaction between H and the aberration. The expression of Hairless was measured by the number of bristles (macrochaetae) missing from the dorsal surface of the head.

Cytological observations: Grell's cytological and genetic studies (1968 and unpublished) indicate that the locus of Su(H) is in or about region 35 of Bridges' (1942) cytological map of Drosophila melanogaster salivary gland chromosomes. This region is characterized by uncertain cytological appearance in salivary nuclei and is subject to ectopic pairing. It is known that an increase in the clarity of the region can be achieved if an extra Y chromosome is present in the genome (Lefevre, personal communication). Consequently, females from the duplication, deficiency and E(H)/E(H); H/LVM stocks were crossed to an attached-XY male and salivary glands from females of genetic constitution X/X, prepared by the method of Plaut and Nash (1964), were observed with phase microscopy.

The absence of the Cy inversion, indicated by unambiguously uninverted 2L chromosome arms, was established cytologically before a preparation was inspected for the chromosomal aberrations other than E(H).

RESULTS

Table 1 describes the aberrations. Figure 1 illustrates most of the critical cytological observations made in compiling Table 1. Dp(2;2)Adh2 and Dp(2;3)Adh3 are apparently both tandem duplications. T(2;3)DpAdh1 is extremely complex and has not previously been described fully. Df(2)64j was first described by Grell (1968) with respect to Bridges' (1935) cytological map, which differs sufficiently from the later map (1942) to account for the slight alteration reported here.

TABLE 1
The cytogenetic characteristics of four chromosomal aberrations with suspected Hairless modifier activity

<table>
<thead>
<tr>
<th>Designation of rearrangement*</th>
<th>Cytology† (new map order)</th>
<th>Modifier activity</th>
<th>Region responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(2L)64j</td>
<td>21-34D1</td>
<td>35B10-40</td>
<td>suppresses</td>
</tr>
<tr>
<td>Dp(2;2)Adh2</td>
<td>21-35C1</td>
<td>32D3-40</td>
<td>enhances</td>
</tr>
<tr>
<td>Dp(2;2)Adh3‡</td>
<td>21-35A</td>
<td>34B-40</td>
<td>slight suppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>probably not attributable to duplication</td>
</tr>
<tr>
<td>T(2;3)DpAdh1‡</td>
<td>21-33E</td>
<td>89A-81</td>
<td>enhances</td>
</tr>
<tr>
<td></td>
<td>100-89A</td>
<td>33E-35D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30A-31D</td>
<td>35A-40</td>
<td></td>
</tr>
</tbody>
</table>

* After Grell (1968).
† Bridges' (1942) map
‡ The resolution of DpAdh1 and 3 is sufficiently difficult to limit identification of the breaks only to subdivisions. DpAdh3 may extend some way into 35B, but not further than 35B5.
Table 2 shows data on the phenotypic expression of Hairless in females from the crosses between H/LVM and these four stocks. Dp(2;2)Adh2 and T(2;3)DpAdh1 enhance Hairless. Dp(2;2)Adh3, the smallest duplication, possibly suppresses Hairless. There are also significant differences attributable to the different Cy balancers.

The deletion of Df(2)64j, shown by Grell (1968) to cover Su(H), judged by the lethality of Su(H)/Df(2)64j heterozygotes (Su(H)/Su(H) is also lethal), acts phenotypically as Su(H). Interruption of wing vein L5 was used to identify some Df(2)64j/+;H/+ individuals when all head bristles were present. Although Su(H) is known occasionally to produce such interruptions in the absence of H, this is not the case in the present cross: A progeny test of 50 non-Cy flies, with complete sets of head bristles, and with or without wing abnormality showed that interruptions correlated exactly with the presence of the H gene in suppressed condition.

The modifiers are mimicked by the aberrations far more closely than the numerical analysis above shows, for the changes in frequency of effect at different bristle sites and changes in the numbers of “sockets” or bristle vestiges (see Nash 1965) are also similar.

Figures 2 and 3 show examples of Bridges’ segment 35 in E(H) heterozygotes. The three somatically paired segments in Figure 2 show no clear-cut evidence of

TABLE 2

The interaction of Hairless and four second chromosomal aberrations with suspected modifier activity

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Number of dorsal head bristles lost</th>
<th>Flies scored</th>
<th>Mean* loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 7 8 9 10 11 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Df(2L)64j</td>
<td>29 10 1 . . . . . . . . . . . . . .</td>
<td>40</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td>Cy</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>52</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td>Dp(2;2)Adh2</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>113</td>
<td>9.5±1.5</td>
</tr>
<tr>
<td>Cy</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>110</td>
<td>4.7±1.5</td>
</tr>
<tr>
<td>T(2;3)DpAdh1</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>114</td>
<td>8.2±1.3</td>
</tr>
<tr>
<td>Cy</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>117</td>
<td>4.7±1.4</td>
</tr>
<tr>
<td>Dp(2;2)Adh3</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>80</td>
<td>3.3±1.2</td>
</tr>
<tr>
<td>Cy</td>
<td>. . . . . . . . . . . . . . . . . .</td>
<td>80</td>
<td>5.1±1.4</td>
</tr>
</tbody>
</table>

The cross Aberration H* / H* + ; + H H* H* yields four types of progeny:

(1) Aberration H* / + ; + H H* H* yields four types of progeny:

(2) Aberration H* / + ; + H H* H* yields four types of progeny:

(3) Aberration H* / + ; + H H* H* yields four types of progeny:

(4) Aberration H* / + ; + H H* H* yields four types of progeny:

Females of types 1 and 2 are compared in the above table. Males show effects consistent with the above results, but the means differ according to the direction of the cross (H female parent or H male parent), as would be expected. Approximately equal numbers of females from each of the two possible crosses were used.

Six of the seven pairs of head bristles (macrochaetae) are affected by H.

* Standard deviations shown are calculated from the raw data. The differences between controls (Cy) and aberrations were tested statistically after angular transformation, assuming the number of bristles missing is a fraction of 12. All differences are significant at the .001 level. A test for heterogeneity amongst the controls also shows dissimilarity at the .001 level.
(a) Complete chromosome arms 2L and 3R. Synapsing of homologs is complete. From the translocation break points, 33F and 89A (T), the chromosomes can be traced to their tips and to the centric regions (C). The portions of 2L proximal and distal to the translocation are synapsed due to the presence of a duplication of region 30A–31D, which is inserted between duplicate copies of region 35A–35D. This latter duplication is most likely to carry the \textit{Adh} and \textit{Su(H)} genes.
any duplication. Figure 3, however, shows the region in the unpaired state. Without the supporting evidence that \textit{E(H)} reverts and that two duplications in this region act as enhancers, this micrograph would be inconclusive. However, considering that evidence and some of the points raised later, it seems reasonable to claim that Figure 3 actually shows a new duplication, \textit{Dp(2;2)\textit{E(H)}}:\textit{35B6–8; 35B8–10}.

**DISCUSSION AND CONCLUSIONS**

The finding of \textit{Hairless} modifier activity in chromosomal aberrations which likely include the \textit{E(H)} and \textit{Su(H)} chromosomal site(s) allows identification of the most probable region within which these mutants are located as shown in Figure 4.

The region with extra material shown in Figure 3 is exactly that in which it is deduced that \textit{Su(H)} and \textit{E(H)} are likely to be located. Thus it is concluded that these two modifiers of \textit{Hairless} are, respectively, an amorph (or at least a hypomorph) for a locus which interacts with \textit{Hairless} and a small duplication which includes the same locus. It should be noticed that the locus of \textit{Adh}, which is included in all three duplications and the deficiency, must be to the left of \textit{Su(H)}, which is in accordance with \textit{Grell et al.'s} (1965) mapping of \textit{Adh}.

\textit{DpAdh3}, the smallest inversion, far from enhancing \textit{H}, apparently suppresses it. Were this to prove an effect of the duplication \textit{per se}, rather than of the total chromosome bearing it, it would be necessary to speculate upon its significance. However, \textit{DpAdh3} is not a complete \textit{Su(H)} mimic, being homozygous viable. Such small, statistically highly significant effects are commonly found to accompany single-chromosome substitutions. For example, the different \textit{Cy} controls are similarly statistically distinguishable. In the circumstances, conjecture about the \textit{DpAdh3} effect seems premature. The necessity for an exercise of judgement in this case, implies an opposite judgement with respect to the other aberrations, of course.

The establishment of \textit{E(H)} as a duplication leads to reinterpretation of recombination in \textit{E(H)}/\textit{Su(H)} flies. The data presented by \textit{Nash} (1965) gave an estimate of 0.28\% recombination between \textit{E(H)} and \textit{Su(H)}. The figure seemed too large to allow a definitive suggestion that the mutants interfered with the same cistron. However, if \textit{E(H)} is a duplication, two relatively common means to loci. (b) All three copies of region 35A–D are synapsed, but the transposed segment, 30A–31D, is unsynapsed with its homologs in the normal position in 2L. The translocation break points (T), distal portions of 3R and a small part of the wild type 2L (+) are also unsynapsed. (c) Similar to (b), except that most of 2L distal to the translocation is unsynapsed. Instead, region 30A–31D in the wild type 2L is synapsed with its transposed homolog. As a result a small unsynapsed region, 31E–33E (U), is present associated with the duplication complex. (d) \textit{Dp(2;2)Adh2} heterozygote. (e) and (f) \textit{Dp(2;2)Adh3} heterozygotes. Precise analysis of this duplication is difficult. The probable limits of the duplication are shown, as well as the likely site of \textit{Su(H)}, 35B6–9, which should fall outside these limits if the lack of interaction of the duplication with \textit{H} is correctly interpreted. (g) \textit{Df(2)64j} heterozygote. The segment 31D is marked in (b)–(g) to identify the narrow region which is a useful pointer to the correct identification of the vicinity of the aberrations. Magnifications: (a) 355 $\times$; (b)–(g), 915 $\times$. 
Figure 2.—Three examples of the $E(H)$ chromosome in heterozygous condition. In each case homologous pairing is complete. (a) and (b) show reasonably clear cytology, but no satisfactory evidence of a duplication. (c) illustrates the common condition of the region, which is subject to ectopic pairing even, as here, in the presence of an extra $Y$ chromosome. Without the extra $Y$ cytological studies on the region are almost impossible. The proposed location of $E(H)$ (35B6-9) and the distinctive region 31D are indicated. Magnification 915 ×.

Figure 3.—A rare example of an $E(H)/+$ nucleus showing asynapsis in the heterozygous chromosome region. The right-hand homolog shows extra material in the region 35B which is contained within the region where $E(H)$ seems to be located. Magnification: (a), 685 ×; (b), 1605 ×.
the production of "recombinants" i.e., unmodified Hairless phenotypes, are available. Firstly, a recombination could result in the incorporation of the Su(H) lesion into one or other of the duplicate parts of E(H) and, secondly, one of the duplicate parts could be lost by recombination without incorporating Su(H) into the remaining copy. The "recombination" frequency in each case is related to the length of the duplication and not to the length of the portion of the genome specifying the modifier activity. There is thus no objection to considering E(H) and Su(H) as producing their effects on Hairless flies as a result of changes in a smaller genetic unit than that involved in recombination.

To allow for recombination, it must be argued that at least a part of the region present in duplicate in E(H) is present once in Su(H). Considering the small size of Dp(2;2)E(H), it is unlikely that Su(H) would be cytologically identifiable as a deficiency, even if one were present. However, there is every reason for supposing that Su(H) could just as well be a point mutation.

The instability of E(H) (Nash and Woloshyn 1968) is now accounted for as an unequal crossing over phenomenon. However, the reciprocal product, a triplication, has not been identified with certainty. Three flies with few chaetae on their entire bodies have been observed in E(H)/E(H) cultures, but were infertile; their occurrence there and in no other stock during extensive breeding of Hairless flies suggests that the reciprocal recombinant in unequal crossing over does occur.

On the basis of Gowen's (1933) observation of triploids containing one and two doses of Hairless and its homozygous lethality, it was supposed that the H mutant completely lacks normal activity (Nash 1965). Although several alleles of H have been described with different levels of expressivity, placing H^t (the allele used in the above experiments), H^s (a "strong" allele) and H^e in different genetic backgrounds shows that these three alleles have morphologically similar effects. The original differences ascribed to them must have been artifacts of

Figure 4.—The deduced cytological location of Hairless modifier activity.
genetic background (NASH 1969). Attempts to produce a cytologically observable deletion of the $H$ locus have so far been unsuccessful and none is known to the author, so that the original conclusion has not been checked by the most obvious test.

The explanation of the physiological basis of the interaction between the Hairless mutant and its modifiers—the ultimate aim of this study—is still in doubt. The approach to normality of $Su(H)/Su(H)^+;H/H^+$ flies might suggest that a simple balance between the dosage of the two loci is all that is necessary for normal development. However, although it is hardly worthwhile indulging in lengthy speculation, it can be said unequivocally that this simple model is insufficient. If it were correct, we would expect to find that $E(H)/E(H);H^+/H^+$ flies, with a ratio of dosages similar to that found in $Su(H)^+/Su(H)^+;H/H^+$ would exhibit a Hairless phenotype, which is not the case; such flies are phenotypically wild type. Thus we must consider the absolute dosage of the $H^+$ as being more critical than the number of copies of $Su(H)^+$ found in the genome. Presumably some complex interaction between the two genes or their products is involved. As a further step, transplantation studies are presently being undertaken to try to define whether the interactions and actions of the two gene loci can be considered cell-autonomous or whether they involve cell or tissue interactions during development of the fly.

**SUMMARY**

Elucidation of the nature of two known mutant modifiers of Hairless ($E(H)$ and $Su(H)$) was sought. A deficiency and two duplications of the locus of $Su(H)$ were found to act, respectively, as a suppressor and as enhancers of Hairless. It is concluded that $Su(H)$ is a defective gene and $E(H)$, an enhancer "allelic" to $Su(H)$, a duplication. Cytological studies on $E(H)$ confirm the presence of a small duplication in chromosome region 35B6-10, which, on genetic grounds, is the likely location of $E(H)$.

The author is grateful to E. H. Grell for supplying four aberrations used in this study and for his helpful comments. The work was supported by the National Research Council of Canada (operating grant A3269).

**LITERATURE CITED**


