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Cytological location and lethal interactions of three Minute loci in chromosome 3 of *Drosophila melanogaster*

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SUMMARY

Using seven newly induced duplications, three Minute loci have been located cytologically: $M(3)h^{S\,37}$ in 65F10–11; 66A (a new Minute locus), $M(3)i$ in 67C, and $M(3)h^{y}$ in 68F; 69F. $M(3)h^{S\,37}$ and $M(3)h^{y}$ were previously thought to be allelic because they do not complement for lethality. The finding of Minute mutations with additive or synergistic rather than epistatic interactions makes us suspect that some other Minute mutations have been erroneously called allelic. The involvement of Minute loci in more than one biochemical pathway is discussed in view of the existence of synergistic interactions and of Minute loci without known mutant alleles.

1. INTRODUCTION

In their work on segmental aneuploidy Lindsley *et al.* (1972) made an intensive screen for haplo-insufficient loci in the genome of *Drosophila melanogaster*. Among them, Minute loci constitute by far the largest fraction. One dose of any Minute locus presents a series of phenotypic traits, the more constant being short bristles and delayed emergence of the imagos. Mutations in all these loci are homozygous lethal (see Lindsley & Grell, 1968, for the description of Minutes and other genetic symbols used in the text). Lindsley *et al.* (1972) assigned all known Minute loci to those salivary regions that, showing the haplo-insufficient phenotype of short bristles, were reasonably close to previously published genetic locations of Minute mutations. Besides these, they found seven haplo-insufficient autosomal regions where no Minute loci had been previously reported.

While preparing their book, Lindsley & Grell (1968) followed two criteria to define known Minute mutations as alleles of the same locus. Mutations with similar meiotic locations were considered allelic if data on complementation were not available. Mutations non-complementing for lethality were assigned to the same locus even in the absence of meiotic data. To define two Minute mutations as lesions in the same gene because they do not complement for lethality assumes that in all cases combinations of two Minutes have epistatic effects, as shown by Schultz

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(1929). Additive interactions between different Minute mutations may, however, lead to the erroneous conclusion that they are allelic. One such case will be reported here.

Lately, Minute mutations have become a useful tool to approach developmental problems owing to the property of non-Minute cells to overgrow the surrounding Minute cells in Minute/non-Minute mosaic individuals (Morata & Ripoll, 1975). Two Minutes frequently used for this purpose, $M(3)i^{55}$ and $M(3)h^{S37}$, are located within a region where genetic and cytological data are in conflict. Lindsley *et al.* (1972) placed two known Minute loci in this region: $M(3)i$ between 65DE and 66B, and $M(3)h$ between 67C and 69F. The assignment was probably based on the recombination data locating $M(3)i$ distal to $M(3)h$. However, both loci map genetically proximal to *hairy*, which is cytologically located in 66D2-E1. As will be shown below, both $M(3)i$ and $M(3)h$ lie between 67C and 69F, while the Minute phenotype associated with hypoploidy for region 65DE; 66B corresponds to a Minute locus not previously reported. A mutation at this locus ($M(3)h^{S37}$) had been found but erroneously considered an allele of $M(3)h$ (Lindsley & Grell, 1968).

2. MATERIALS AND METHODS

All alleles of $M(3)i$ described in Lindsley & Grell (1968) have been lost. Some new alleles have been found, their allelism inferred from their meiotic location: $M(3)i^{55}$ (Morata & Ripoll, 1975), and $M(3)i^{G\text{-}1}$, $M(3)i^{G\text{-}2}$ and $M(3)i^{G\text{-}3}$ (Persson, 1976). We chose $M(3)i^{G\text{-}3}$ as representative of this latter group. Of the extant alleles of $M(3)h$ we had access to $M(3)h^{y}$ and $M(3)h^{S37}$. According to Lindsley & Grell (1968), their allelism to $M(3)h$ was inferred from the lethal phenotype of $M(3)h/M(3)h^{y}$ and $M(3)h^{y}/M(3)h^{S37}$ individuals. We made all possible combinations with the four mutations we had, and they indeed behave as belonging to two different complementation groups with respect to lethality.

For a cytological localization of these two Minute loci, a series of duplications, $Dp(3;3)$, covering $M(3)i^{55}$ were induced following a method similar to that of Grell (1969) and Broderick & Roberts (1982). Females homozygous for *mwh* and *jv* (two cuticular marker mutations) were exposed to 4000 rad of X-rays and crossed to *mwh* $M(3)i^{55}/TM1$ males. All *mwh jv*/*mwh* $M(3)i^{55}$ progeny should show a Minute phenotype unless a duplication including the Minute locus or a suppressor of $M(3)i^{55}$ had been induced. The dominant short bristle phenotype was scored in phenotypically *mwh* male progeny, and those individuals not showing it were tested for the presence of a duplication. Out of 5859 male progeny scored, five tandem duplications and two insertional translocations were isolated. Their cytology and phenotype in combination with different Minute mutations is presented in Table 1.

3. RESULTS AND DISCUSSION

From the data shown in Table 1 we can locate cytologically $M(3)i$ in region 67C. All duplications including $M(3)i^{55}$ also include $M(3)i^{G\text{-}3}$ and they most probably are alleles. $M(3)h^{y}$ lies between 68F and 70AB. This interval includes the region where Akam *et al.* (1978) have placed the locus of $M(3)h$, between 69B4 and 69F.

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If the Minute phenotype of *Df(3L)VW3* (69E2-F1; 70C1) (Ashburner *et al.*, D.I.S. 56, 181) is due to haploinsufficiency for $M(3)h^+$, then this locus should be placed between 69E2 and 69F. $M(3)h^{S37}$ is located by our data in 65F. *Df(3)Hn* (66A; 66B) is not reported to produce short bristles (Lindsley & Grell, 1968), and therefore $M(3)h^{S37}$ probably lies between 65F10–11 and 66A. Since $M(3)h^{S37}$ belongs to a locus different from that of $M(3)h^y$ we propose to resurrect its original designation *M(3)hS37*.

Table 1. *Cytology of duplications covering* M(3)i^{55} *and their phenotype over different Minute mutations*

Duplication	Breakpoints	Insertion	$M(3)i^{G\text{-}3}$	Mh^{S37}	$M(3)h^y$
Dp(3; 3)MS1	64DF; 67D8–13	Tandem	WT	WT	M
Dp(3; 3)MS2	65F10–11; 67E5–7	Tandem	—	M	—
Dp(3; 3)MS3	65F10–11; 70A	67E	—	WT	WT
Dp(3; 3)MS4	66C3–5; 68F	65D3–5	WT	M	M
Dp(3; 3)MS5	66E1–2; 70AB	Tandem	—	M	WT
Dp(3; 3)MS6	67A5–9; 67C5–10	Tandem	WT	—	—
Dp(3; 3)MS7	67C1–4; 67D4–11	Tandem	WT	—	—

WT, Wild-type bristles; M, short Minute bristles; —, not tested.

M(3)S37 and $M(3)h^y$ can be separated cytologically and yet they do not complement for lethality. This failure to complement can be explained in either of two ways. First, some combinations of two Minutes could be additive instead of, as is generally assumed, epistatic. Secondly, one of the chromosomes tested here could be doubly mutant. We know that neither chromosome is simultaneously mutant for both Minutes since duplications covering each of them singly have been found (Table 1). However, one of the chromosomes tested could carry a small deficiency including the loci of one Minute and a lethal, while the other chromosome could be doubly mutant: one mutation at the other Minute locus plus the lethal mutation uncovered by the deficiency in the homologous chromosome. Reciprocal recombinants between each of the Minutes and *hairy* (*h*, 3–26·5), which lies between them, were recovered to determine whether either the $M(3)h^y$- or the *M(3)S37*-bearing chromosome carries a lethal mutation in the region of the other Minute locus. Accordingly, *M(3)S37 h*, $M(3)S37^+$ h^+, *h* $M(3)h^y$ and $h^+M(3)h^+$ recombinant chromosomes were recovered and balanced over *TM1* or *TM2* for further testing. Progeny counts from complementation analyses of several of these recombinant chromosomes *inter se* and with the original parental chromosomes are presented in Table 2. The results clearly show that the lethality of the parental chromosomes is only due to the Minute mutations. Therefore, the lethal phenotype of the double heterozygote results from an interaction of *M(3)S37* and $M(3)h^y$ and is not due to any other factor present in either chromosome.

In his analysis of Minute interactions Schultz (1929) studied 16 mutations belonging to 14 loci, which represents about one third of the Minute loci known today. In all cases the double heterozygotes were viable and showed the phenotype of the stronger allele used in the combination. This was true even in individuals heterozygous for three Minutes. Therefore, lethal interactions of the type described

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here between *M(3)S37* and *M(3)h*^{y} seem to be rare. We do not know how frequent this phenomenon is, but it is possible that some of the Minute mutations whose loci have been determined only by complementation analysis could have been misplaced as alleles of known loci because of additive or synergistic interactions. Even though Minute mutations are easy to induce and recognize, Lindsley *et al.* (1972) were still able to find eight new autosomal Minute loci, which is about one

Table 2. *Progeny recovered from crosses of parental Minute-bearing chromosomes to different recombinant chromosomes*

Cross	Bal/Bal	Rec/Bal	PM/Bal	Rec/PM
♀ *M(3)h*^{y}*/TM2* ♂ *h*$^{+}$ *M(3)h*$^{+}$*/TM1*	129	184	182	240
♀*M(3)h*^{y}*/TM2* ♂ *M(3)S37*$^{+}$ *h*$^{+}$*/TM1*	204	180	227	287
♀ *M(3)h*^{y}*/TM2* ♂ *h M(3)h*^{y}*/TM2*	0	163		0
♀ *M(3)h*^{y}*/TM2* ♂ *M(3)S37 h/TM2*	0	744		0
♀ *M(3)S37/TM1* ♂ *h M(3)h*^{y}*/TM1*	0	211		0
♀*M(3)S37/TM1* ♂ *M(3)S37 h/TM1*	0	124		0
♀ *M(3)S37/TM1* ♂ *h*$^{+}$ *M(3)h*$^{+}$*/TM1*	0	254	250	340
♀ *M(3)S37/TM1* ♂ *M(3)S37*$^{+}$ *h*$^{+}$*/TM1*	0	300	203	279
♀ *h*$^{+}$ *M(3)h*$^{+}$*/TM1* ♂ *h*$^{+}$ *M(3)h*$^{+}$*/TM1*	0	153	—	86[a]
♀ *M(3)S37*$^{+}$ *h*$^{+}$*/TM1* ♂ *M(3)S37*$^{+}$ *h*$^{+}$*/TM1*	0	258	—	101[a]

Bal, Balancer chromosome; Rec, recombinant chromosome; PM, parental Minute chromosome; a, homozygous recombinant chromosome.

fourth of the autosomal Minutes described until now. Some new autosomal Minute loci might still be found if, as was the case with *M(3)i* and *M(3)h*, the Minute phenotype of some deficiencies is due to more than one Minute locus. It is possible that mutations in these new loci have already been found but erroneously located, as happened with *M(3)S37*.

Minutes have been thought to be involved in some step of protein synthesis such as translation (see Sinclair, Suzuki & Grigliatti, 1981). This belief is reinforced since deletions of rDNA (Ritossa, Atwood & Spiegelman, 1966*b*) and of 5S rDNA (Procunier & Tartof, 1975) also show a Minute phenotype. The epistatic nature of Minute interactions suggested that Minute loci could be involved in a common pathway. Atwood suggested that they could be coding for different tRNA's (Ritossa *et al.* 1966*a*), which does not seem to be the case (see Sinclair *et al.* 1981, for discussion). More recently, B. Baker has interpreted them as being the sites

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coding for ribosomal proteins (Huang & Baker, 1976) and some molecular data support this hypothesis (Vaslet *et al.* 1980). Since, as has been described here, synergistic interactions are also found, one could speculate that Minute loci can be divided into two groups, each one involved on a different pathway but both related to protein synthesis. Four of the five new third-chromosome Minute regions reported by Lindsley *et al.* (1972) are clustered in the region distal to 65F. Apart from *M(3)S37* no Minute mutations are known in this region. Although so far quite unproven, it is tempting to think that these loci could be related to each other more closely than they are to the rest of the Minute loci known to date in mutant form.

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