THE CONSEQUENCES OF NULLOSOMY FOR A CHROMOSOMAL REGION AFFECTING CYCLIC AMP PHOSPHODIESTERASE ACTIVITY IN DROSOPHILA

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ABSTRACT

A study of Drosophila nullosomic for chromomere 3D4 shows that this region of the genome is necessary for male fertility, normal female fertility and normal oogenesis. Males nullosomic for 3D4 lack normal, motile sperm. Females nullosomic for this region exert a maternal influence on their progeny which results in a diversity of imaginal defects. The observation that chromomere 3D4 is the most probable locus for a chromosomal region which affects cAMP phosphodiesterase activity, and which may contain a structural gene for the enzyme, prompts the hypothesis that the diverse physiological effects caused by nullosomy for 3D4 are the result of an aberrant cAMP metabolism.

A survey of the genome of *Drosophila melanogaster*, employing segmental aneuploidy to locate presumptive structural genes for cyclic nucleotide phosphodiesterases, has uncovered a region of the *X* chromosome which exhibits the aneuploid effects on cAMP phosphodiesterase activity expected for a structural gene. Duplication and deficiency mapping places this region within chromomeres 3D3 and 3D4, between Notch and diminutive, with 3D4 being the most probable location (KIGER and GOLANTY 1977). However, the presence of any lethal or visible locus between Notch and diminutive has been previously excluded (LEFEVRE 1974). In view of the supposedly vital role of cAMP phosphodiesterase in regulation of cellular cAMP levels (AMER and KREIGBAUM 1975) this observation is intriguing.

Males and females nullosomic for the region affecting cAMP phosphodiesterase activity have been constructed. These flies have less than 30% of the total cAMP phosphodiesterase activity of euploid flies (KIGER and GOLANTY, unpublished data). This observation suggests either that duplicate structural gene(s) for this enzyme exist elsewhere in the genome, or that we are dealing with a regulatory locus and that, presumably, the structural gene for the enzyme is located elsewhere. Regardless of which of these cases may be operative, the question arises: Is there any functional defect associated with nullosomy for this region? Is the loss of 70% of the activity of cAMP phosphodiesterase possible without an effect on the phenotype of the fly? A possible answer to these questions is presented in the results described below, in which the phenotypes of males and females nullosomic for chromomere 3D4 are more closely investigated.

TABLE 1

Stocks

Stock	Cytology	Source
Df(1)N^{8}/Basc; Dp(1;3)w^{+67k27}/+	deficiency 3C1–3D6	E. B. LEWIS
[listed in the text as *Dp(1;3)G^{spot}*]	duplication 3A5 or 7–3F1	G. LEFEVRE
Df(1)N^{8}/Basc; Dp(1;2)w^{+51b7}/+	duplication 3C2–3D6	G. LEFEVRE
Df(1)N^{64i16}/Basc; SM1, Cy Dp(1;2)w^{+51b7}/+	deficiency 3C3–3D4	W. J. WELSHONS
C(1)DX, y w f/Df(1)N^{64i16}; SM1, Cy Dp(1;2)w^{+51b7}/+		
rst Df(1)N$^{71h24-5}$/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C4–3D4	M. M. GREEN
C(1)DX, y w f/rst Df(1)N$^{71h24-5}$; Dp(1;2)w^{+51b7}/+		
Df(1)N^{64j15}/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C4–3D3	W. J. WELSHONS
C(1)DX, y w f/Df(1)N^{64j15}; Dp(1;2)w^{+51b7}/+		
y w^{a} Df(1)N^{54l9}/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C6–3C10	W. J. WELSHONS
C(1)DX, y w f/y w^{a} Df(1)N^{54l9}; Dp(1;2)w^{+51b7}/+		
Df(1)dm^{75e19}/Basc (or *FM7*)	deficiency 3C12–3E4	G. LEFEVRE
y^{2} Df(1)w, spl ec sn^{3}/w^{+}Y/C(1)DX, y f/w^{+}Y	duplication 2D2–3D3	D. LINDSLEY
dm/Basc		E. B. LEWIS

METHODS

The stocks employed in this study are detailed in Table 1, and a cytological map of the relevant portion of the *X* chromosome can be found in the previous paper (KIGER and GOLANTY 1977). LINDSLEY and GRELL (1968) describe the chromosomes and mutations employed. The crosses performed are listed in Table 2 and are referred to by number in the text. All of the data presented are based on matings involving single nullosomic males or females to 3 or 4 flies of the opposite sex.

RESULTS

In order to confirm the relationship of the deficiencies employed to the diminutive locus, which is the only genetic marker close to the region affecting cAMP phosphodiesterase activity, cross 1 was performed. The results presented in Table 3 show that the *dm* locus is situated to the left of the right breakpoint of *Df(1)N^{8}* and to the right of the right breakpoints of *Df(1)^{64i16}*, *Df(1)N$^{71h24-5}$*, and *Df(1)N^{64j15}*, *i.e.*, the latter three chromosomes are not deficient for *dm^{+}*. This is

TABLE 2

Crosses performed

Cross 1 *Df(1)/Basc* ♀ × *dm* ♂ or *dm/Basc* ♀ × *Df(1)N; Dp(1;2)w^{+51b7}/+* ♂

Cross 2 *Df(1)N/Basc* ♀ × *y^{2} Df(1)w spl ec sn^{3}/w^{+}Y* ♂

Cross 3 *C(1)DX, y w f* ♀ × *Df(1)N/w^{+}Y* ♂

Cross 4 *Df(1)dm^{75e19}/Basc* ♀ × *Df(1)N; Dp(1;2)w^{+51b7}* (or *Dp(1;3)G^{spot}*)/+ ♂

Cross 5 *Df(1)N/Df(1)dm^{75e19}* ♀ × *Basc; Dp(1;2)w^{+51b7}/+* ♂

Cross 6 *Df(1)N/Df(1)dm^{75e19}; Dp(1;2)w^{+51b7}/+* ♀ × *Basc* ♂

TABLE 3

The relationship of the deficiencies employed to the diminutive locus

Genotype	Phenotype
Df(1)N^{8}/dm	diminutive, Notch
Df(1)N^{64i16}/dm	nondiminutive, Notch
Df(1)N$^{71h24-5}$/dm	nondiminutive, Notch
Df(1)N^{64j15}/dm	nondiminutive, Notch
Df(1)dm^{75e19}/dm	diminutive

consistent with the locus given by LEFEVRE (1976) for *dm* as 3D5 or the interband between 3D4 and 3D5.

To construct males nullosomic for 3D4, or 3D3 and 3D4, it is necessary to complement the lethality associated with deficiency for *N*. This is done in cross 2 by introducing the $w^{+}Y$ (duplication 2D2–3D3 which carries N^{+}. The results of this cross are presented in Table 4. *Df(1)N^{8}/w^{+}Y* males are not recovered, due at least to nullosomy for dm^{+}, which is lethal (G. LEFEVRE, personal communication). The inability of *Dp(1;2)w^{+51b7}* to complement the lethality of the *Df(1)N^{8}* (see stock in Table 1) may be due to the presence of a recessive lethal outside the deficiency, since nullosomy for 3C1 is not lethal (SORSA, GREEN and BEERMANN 1973). The other deficiencies tested all survive as males.

The fertility of individual *Df(1)N/w^{+}Y* males was tested in cross 3. *Df(1)N^{64i16}/w^{+}Y* and *Df(1)N$^{71h24-5}$/w^{+}Y* males are sterile and examination shows that they lack motile sperm. These males are nullosomic for 3D4 and possibly for 3D3 [the endpoints of the $w^{+}Y$ duplication are difficult to define and it may extend only to 3D2 (G. LEFEVRE, personal communication)]. The presence of *Dp(1;2)w^{+51b7}* restores fertility to males hemizygous for these chromosomes, indicating that the locus necessary for fertility lies between the right breakpoint of $w^{+}Y$ and that of *Dp(1;2)w^{+51b7}* (see stocks in Table 1). In contrast, *Df(1)N^{64j15}/w^{+}Y* males are fertile, and consequently the locus necessary for fertility must be to the right of the right breakpoint of *Df(1)^{64j15}* and in 3D4.

Females nullosomic for the region affecting cAMP phosphodiesterase activity are produced in cross 4 and survive because of complementation for N^{+} and dm^{+}

TABLE 4

*Phenotypes of nullosomic and monosomic males**

	w^{+}Y; +	*Y; Dp(1;2)$^{+51b7}$*
Df(1)N^{8}; +	—	—
Df(1)N^{64i6}; +	+^{S}	+^{F}
Df(1)N$^{71h24-5}$; +	+^{S}	+^{F}
Df(1)N^{64j15}; +	+^{F}	+^{F}
Df(1)N^{54l9}; +	+^{F}	+^{F}

* + = viable; — = inviable; F = fertile; S = sterile.

TABLE 5

*Phenotypes of nullosomic and monosomic females**

	$Df(1)dm^{75e19}$; +	$Df(1)dm^{75e19}$; $Dp(1;2)w^{+51b7}$
$Df(1)N^{8}$; +	—	—
$Df(1)N^{64i6}$; +	+SS	+F
$Df(1)N^{71h24-5}$; +	+SS	+F
$Df(1)N^{64j15}$; +	+F	+F
$Df(1)N^{54l9}$; +	+F	+F

* + = viable; — = inviable; F = fertile; SS = sterile.

by $Df(1)dm^{75e19}$ and $Df(1)N$ respectively, except in the case of $Df(1)N^{8}$ which fails to survive. In performing cross 4, it is necessary that the $Df(1)N^{8}$ chromosome be accompanied by $Dp(1;3)G^{spot}$, which complements the recessive lethal present in this X chromosome. The results of this cross are presented in Table 5. Females of genotypes $Df(1)N^{64i16}/Df(1)dm^{75e19}$ and $Df(1)N^{71h24-5}/Df(1)dm^{75e19}$ are nullosomic for 3C12–3D4. These females, though viable, are either markedly reduced in fertility or are sterile (see Table 6). Females of genotype $Df(1)N^{64j15}/Df(1)dm^{75e19}$ are nullosomic for 3C12–3D3, and those of genotype $Df(1)N^{54l9}/Df(1)dm^{75e19}$ are not nullosomic. Females of these latter two genotypes are normal and comparable in fertility, a fact noted earlier by G. LEFEVRE (personal communication). Thus the locus responsible, in the nullo-

TABLE 6

Nullosomic females: fertility and maternal influence on progeny

Cross	Maternal genotype	Fertility	Progeny*	Imaginal structures absent (%)	Unevaginated leg discs (%)
5	$Df(1)N^{64i16}/Df(1)dm^{75e19}$	9/20	186	27 (15)	6 (3)
6	$Df(1)N^{64i16}/Df(1)dm^{75e19}$; *SM1, Cy* $Dp(1;2)w^{+51b7}$/+	10/10	985	0 (0)	1 (0.1)
5	$Df(1)N^{71h24-5}/Df(1)dm^{75e19}$	8/20	76	10 (13)	2 (3)
6	$Df(1)N^{71h24-5}/Df(1)dm^{75e19}$; $Dp(1;2)w^{+51b7}$/+	10/10	1090	0 (0)	1 (0.1)
5	$Df(1)N^{64j15}/Df(1)dm^{75e19}$	16/20	892	1 (0.1)	1 (0.1)
6	$Df(1)N^{64j15}/Df(1)dm^{75e19}$; $Dp(1;2)w^{+51b7}$/+	10/10	663	0 (0)	0 (0)

* Total number of progeny obtained from the indicated number of fertile females in six days.

somic state, for female infertility is situated in 3D4. The infertility is not a consequence of homozygosity for a common recessive gene located elsewhere, since normal fertility is restored to $Df(1)N/Df(1)dm^{75e19}$ genotypes by $Dp(1;2)w^{+51b7}$.

When testing the fertility of the females nullosomic for 3D4, obtained from cross 4, it was observed that a striking number of the progeny of these females were defective in one way or another. The most common defect is the absence of one or both metathoracic legs. Less frequently one or both mesothoracic legs may be missing, and this defect is often accompanied by the absence of one or both metathoracic legs. Very rarely, a prothoracic leg may be absent, but the low frequency of this defect may reflect the need for prothoracic legs in emergence from the pupal case. The absence of a haltere is not uncommon, and missing first abdominal hemitergites, genitalia and wings have been noted, as have cleft thoraxes. These defects are shared more or less equally by all of the progeny genotypes obtained from mothers nullosomic for 3D4. The nature of the defects observed are those expected from the failure of specific imaginal discs or histoblasts to either form or develop normally (POSTLETHWAIT and SCHNEIDERMAN 1973). Flies with missing parts have been dissected and, in most cases, show no traces of any unevaginated imaginal structures. The nature of the defects suggests that females nullosomic for 3D4 produce defective eggs, and that the defects observed in the progeny are the result of a maternal influence on development. The diversity of specific defects indicates a wide spread physiological disorder which may also be responsible for the observed reduction in fertility of the mothers.

Crosses 5 and 6 were performed to test the hypothesis that a maternal influence is the cause of the defective progeny of female nullosomic for 3D4. The progeny genotypes of crosses 5 and 6 are identical. The crosses differ only in whether the mother is nullosomic for 3D4, and $Dp(1;2)w^{+51b7}$ is introduced paternally, or the mother is monosomic for 3D4, and $Dp(1;2)w^{+51b7}$ is introduced maternally. The results of these crosses are presented in Table 6 where the proportion of fruitful matings are recorded along with the total number of adult progeny produced and examined. In all cases, defective progeny were dissected and examined for unevaginated imaginal structures. Only unevaginated leg discs were observed, and these instances are recorded separately from progeny totally lacking structures. The results demonstrate conclusively that maternal nullosomy for 3D4 is responsible for the observed sterility and reduced fertility of such mothers and for the occurrence of defective progeny. The data also show that females nullosomic for 3C12–3D3 have no demonstrable defect.

DISCUSSION

The results presented here demonstrate that chromomere 3D4 is necessary for male fertility, normal female fertility and normal oogenesis. As shown in the preceding paper (KIGER and GOLANTY 1977), aneuploidy for 3D4 affects cAMP phosphodiesterase activity in a manner consistent with the hypothesis that a structural gene for the enzyme is present in this chromomere. In spite of the fact that the average chromomere of Drosophila contains enough DNA to

code for twenty average size polypeptides (FRISTROM and YUND 1973), it is tempting to speculate that the pleiotropic effects of nullosomy for 3D4 are all a consequence of the absence of a gene for a specific cAMP phosphodiesterase. Thus, an aberrant cAMP metabolism may underlie the diverse physiological disorders described above.

The hypothesis that duplicate structural genes with different chromosomal locations may exist is supported by the results obtained thus far. The fact that nullosomic females and males are viable and morphologically normal but exhibit defects associated with the reproductive system suggests that the duplicate genes for cAMP phosphodiesterase have evolved different functions of a tissue-specific nature. Before this hypothesis can be accepted, however, it is necessary to prove that 3D4 contains a structural gene for cAMP phosphodiesterase rather than a regulatory gene whose product is required for the normal production of cAMP phosphodiesterase activity, above the basal level observed in flies nullosomic for 3D4. The genetic mapping of a cAMP phosphodiesterase with an altered amino acid sequence to this chromomere would constitute such proof.

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LITERATURE CITED

AMER, M. S. and W. E. KREIGHBAUM, 1975 Cyclic nucleotide phosphodiesterases: properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. J. Pharm. Sci. **64:** 1–37.

FRISTROM, J. W. and M. A. YUND, 1973 Genetic programming for development in *Drosophila*. CRC Critical Reviews in Biochemistry **1:** 537–570.

KIGER, J. A., JR. and E. GOLANTY, 1977 A cytogenetic analysis of cyclic nucleotide phosphodiesterase activities in Drosophila. Genetics **85:** 609–622.

LEFEVRE, G., JR., 1974 Exceptions to the one band-one gene hypothesis. Genetics **77:** s39. ——, 1976 A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. pp. 31–66. In: *Genetics and Biology of Drosophila*, Vol. 1a. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, London.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. No. **627**: Washington, D. C.

POSTLETHWAIT, J. H. and H. A. SCHNEIDERMAN, 1973 Developmental genetics of Drosophila imaginal discs. Ann. Rev. of Genet. **7:** 381–433.

SORSA, V., M. M. GREEN and W. BEERMANN, 1973 Cytogenetic fine structure and chromosomal localization of the white gene in *Drosophila melanogaster*. Nature, N.B. **245:** 34–37.

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