THE GENETIC IDENTIFICATION OF A HETEROCHROMATIC SEGMENT ON THE *X* CHROMOSOME OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

An autosomal euchromatic maternal-effect mutant, *abo* (= abnormal oocyte), interacts with, or regulates the activity of, the heterochromatin of the sex chromosomes of *Drosophila melanogaster*. It is shown that this interaction or regulation with the *X* chromosome involves a specific heterochromatic locus or small region that maps to the distal penultimate one-eighth of the basal *X*-chromosome heterochromatic segment.

ALTHOUGH the basal heterochromatin of the *X* chromosome of *Drosophila melanogaster* (symbol: *Xh*) is large both cytologically (COOPER 1959) and in DNA content (RUDKIN 1965), of the hundreds of loci identified on the *X* chromosome only *bb* (bobbed), *NO* (the nucleolus organizer) and conceivably *su(f)* (suppressor of forked) are located in the heterochromatic region (LINDSLEY and GRELL 1968). Recently, a new genetic property of *Xh* has been demonstrated (SANDLER 1970). It was shown that females homozygous for an autosomal recessive maternal-effect mutant, *abo* (abnormal oocyte), produce eggs whose probability of supporting zygotic development increases with increasing numbers of either the *Y* chromosome or doses of *Xh* present in either the mutant mother or in her abo^+ progeny. In this report we show that there is a specific locus or small region of *Xh* (symbol: Xh^{abo}) responsible for this rescue that maps to the distal six-eighths to seven-eighths of the basal *X* heterochromatin.

The basic experiment diagnostic of *abo* is as follows: sister *abo/abo* and *abo/+* females carrying normal *X* chromosomes are crossed with abo^+/abo^+ males carrying an attached-*XY* chromosome and no other sex chromosome. The regular progeny, that is, those that receive a single maternal *X* chromosome, can be either *X/XY* females or *X/0* males according to the sex chromosome constitution of the fertilizing sperm: *X/XY* female offspring of homozygous *abo* mothers survive 70 percent as often as in the control, while the *X/0* male offspring of mutant mothers survive only 6 percent as well as in the control. The relative survival of these two classes is expressed as "♂-recovery" which is defined as the ratio of the sex-ratios in experimental and control sets (see footnote ¶, Table 1). A mapping of the region of *Xh* responsible for rescuing eggs from

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TABLE 1

The results of crossing females homozygous for the sex-linked recessive y *and either heterozygous (C) or homozygous (E) for* abo *by males carrying* $Y^{S}X\cdot Y^{L}$, In(1)EN, y B *and the indicated* X*-chromosome free duplication marked by* y^{+}

Duplication	Size*	*bb*†	*su(f)*‡	Frequency exceptions		Type of progeny§ Regular $y\ B\ ♀♀$	Regular $+♂♂$	Exceptional $B♀♀$	Exceptional $y♂♂$	♂-recovery¶
NONE	—	—	—	—	C	7066	9760‖	—	—	0.12
				—	E	4763	810‖	—	—	
Dp(1;f)1187	<0.3	—	—	0.50	C	2253	3225	2485	2944	0.13
				0.48	E	1052	201	936	228	
Dp(1;f)1205	0.7	—	—	0.49	C	1815	2485	1862	2224	0.11
				0.48	E	1367	198	1323	133	
Dp(1;f)1144	1.1	—	—	0.49	C	630	1752	441	1871	0.14
				0.45	E	674	258	581	190	
Dp(1;f)1346	2.0	+	—	0.00	C	933	1533	0	0	0.15
				0.00	E	1570	394	0	0	
Dp(1;f)856	3.0	+	—	0.01	C	2453	2968	0	37	0.17
				<0.01	E	2965	592	4	5	
Dp(1;f)1173	3.2–3.6	+	—	0.01	C	1762	2396	3	36	0.29
				<0.01	E	1946	766	0	5	
Dp(1;f)3	3.7–4.0	+	+	0.01	C	3463	5423	1	49	0.32
				<0.01	E	2081	1031	0	10	

* As fraction of mitotic chromosome 4; KRIVSHENKO and COOPER in LINDSLEY and GRELL (1968).
† From LINDSLEY and GRELL (1968) and confirmed by us in crosses to *C(1)DX*.
‡ From LINDSLEY and GRELL (1968) and/or confirmed or determined by us.
§ The relatively rare exceptions resulting from nondisjunction in the parental females have been excluded from these data.
‖ These males are *y* owing to the absence of a $y+$ homolog in the parental males.
¶ "♂-recovery" is computed among regular progeny only as (♀♀ in control) *X* (♂♂ in experimental) ÷ (♀♀ in experimental) *X* (♂♂ in control).

the *abo*-induced lethality is accomplished by performing this cross using abo^{+} males that carry, in addition to the attached-*XY* chromosome, any one of a series of *X*-chromosome duplications.[1] These duplications all carry the centromere of the *X* chromosome, a portion of *Xh* starting at the centromere and proceeding distally for some length, and the tip of the *X* chromosome including the normal allele of *y*. In any such cross, the regular female progeny (as opposed to instances in which the attached-*XY* chromosome and the duplication nondisjoined in the parental male) will carry a maternal *X* chromosome and the paternal attached-*XY*; the regular male progeny will carry a maternal *X* chromosome and whatever free duplication is involved in the cross. Any change in ♂-recovery, therefore, should reflect a change in the survival of the *X*/duplication-bearing males.

The results of this experiment are shown in Table 1. The duplications are listed in order of increasing size. The presence or absence of the *bb* locus (which is about in the middle of *Xh*) and the *su(f)* locus (which is near the heterochromatic-euchromatic border) is also shown. It may be noted in passing that those

[1] The duplications used here are those constructed and described by KRIVSHENKO and COOPER, except for *Dp(1;f)3* which was constructed by WELTMAN and described by LINDSLEY and SANDLER. References and full descriptions of all of these are found in LINDSLEY and GRELL (1968).

duplications that lack the *bb* locus segregate, in male meiosis, at random with respect to the attached-*XY* chromosome, while the presence of the *bb* locus results in the regular separation of the two sex chromosomes.

A clear separation of *bb* and Xh^{abo}, with Xh^{abo} occupying the more distal position, is made by *Dp(1;f)1144* and *Dp(1;f)1346*. The similarity of the results with *Dp(1;f)1173* and *Dp(1;f)3* implies that Xh^{abo} is separable from, and proximal to, *su(f)*. The cytological lengths of the duplications suggest that Xh^{abo} is in the distal fourth of *Xh*.

This position can be made somewhat more precise by a consideration of some previously published data (SANDLER 1970). It was shown that the relative survival from *abo/abo* mothers, compared with control, was 25%–30% for *XY* males and 50%–53% for *XX* females. This implies either that the *Y* chromosome rescues less than the *X* chromosome, or that, owing to differences in development between the two sexes, male zygotes are inherently less rescuable. That the latter obtains was indicted by the facts that (1) $XX^{-}Y$ females (X^{-} symbolizes an *X* chromosome deficient for most of *Xh*, $In(1)sc^{4L}sc^{8R}$) have a relative survival of 53% comparable to the 50%–53% for *XX* females, and (2) XX^{-} females have a relative survival of 14%, which is higher than the 6% relative survival exhibited by *X0* males. These observations strongly suggest (1) a difference in rescuability of males and females, (2) the identity of rescue by the *X* and *Y* chromosomes, and (3) that the heterochromatin contained in $In(1)sc^{4L}sc^{8R}$ does not effect rescue. This chromosome does contain the distal-most one-eighth of *Xh* implying that Xh^{abo} is proximal to this and therefore in the penultimate eighth of *Xh*.

It seems very likely that Xh^{abo} is the necessary function in *Xh* that can be inferred from the lethality of $In(1)sc^{4L}sc^{8R}/Dp(1;f)1346$ males (LINDSLEY and GRELL 1968) that are deficient for the region between the right breakpoint of $In(1)sc^{4}$ and *bb*.

In these experiments, we have encountered one unexplained peculiarity. The experiments just referred to imply that *Xh*, at least in an intact *X* chromosome, and a *Y* chromosome rescue to the same extent. Since *Dp(1;f)3* carries all of *Xh*, it too should rescue as efficiently as the *Y*. However δ-recovery using $y^{+}Y$ (a *Y* chromosome with the y^{+} tip of the *X* analogous to the free duplications) was 0.86 compared to 0.32 for *Dp(1;f)3* (Table 2). Moreover, in the previous experiments of SANDLER (1970), the $y^{+}Y$ was not different from a normal *Y*, but in the present experiments a normal *Y* resulted in a δ-recovery of only 0.61 (Table 2).

The *X* chromosomes used in the experiments reported here differ from those used by SANDLER in that our *X* chromosomes carry *y* to permit identification of duplication-bearing progeny. A comparison of results from the two *abo* stocks is also shown in Table 2. It can be seen that both the $y^{+}Y$ and *Dp(1;f)3* exhibit very different δ-recovery values in the two stocks but that in both cases the *Y* is a more efficient rescue agent than is the duplication. However, the male recovery value for $y^{+}Y$ in the y^{+};*abo* stock of 0.42 is similar to the 0.48 reported by SANDLER with this same stock, for a normal *Y*, suggesting that the y^{+};*abo* stock has not changed since the earlier report.

TABLE 2

The results of crossing females heterozygous (C) or homozygous (E) for abo *from balanced* abo/In(2LR)Cy *stocks with either normal* (y+) *or* y*-bearing* (y) X *chromosomes by* $Y^{S}X\text{-}Y^{L}$, In(1)EN, y B *males carrying either* y+Y, *a normal* Y *chromosome, or* Dp(1;f)3

Parental ♀	♂		Progeny* ♀♀	♂♂	♂-recovery†
y;abo XY/y+Y		C	1939	1983	0.86
		E	1695	1488	
y;abo XY/Y		C	2117	2154	0.61
		E	1334	824	
‡*y;abo XY/Dp(1;f)3*		C	3464	5472	0.32
		E	2081	1041	
y+;abo XY/y+Y		C	1479	1647	0.42
		E	1243	577	
§*y+;abo XY/Y*		C	5616	5487	0.48
		E	4311	2017	
y+;abo XY/Dp(1;f)3		C	2205	2758	0.17
		E	2214	478	

* Exceptional and regular progeny are summed in the cases in which they are distinguishable.
† Computed as in Table 1.
‡ Data from Table 1.
§ Data from SANDLER (1970); his Table 3.

From these observations it seems reasonably clear that different wild-type *X* chromosomes and different wild-type *Y* chromosomes can carry *abo*-responsive regions of differing efficacy. While it is not clear what the physical basis of these differences is, the observations are reminiscent of those showing that the heterochromatin of normal *X* chromosomes from different stocks can contain very different numbers of rRNA cistrons (RITOSSA, ATWOOD and SPIEGELMAN 1966, SPEAR and GALL 1973).

In summary, it has been previously argued from the interactions between *Xh* and *abo*, that the product of the autosomal locus interacts with (most likely regulates) the activity of some function or functions of the heterochromatin of the sex chromosomes. The present observations show that this interaction is not with heterochromatin in general, but rather with a specific, mappable, locus or small region on the *X* chromosome, and, by inference, with an homologous segment on the *Y* chromosome.

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