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The Site of Function of the Y Chromosome in *Drosophila melanogaster* Males

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Summary. The Y chromosome is essential for fertility in *D. melanogaster* males. An analysis of 126 *pal*-induced Y chromosome mosaics indicated that its function is only required in the germ line of fertile males. This analysis also showed that approximately $^1/_4$ of all *pal*-induced Y chromosome mosaics had an XO/XYY constitution and hence that they resulted from somatic nondisjunction. Preliminary evidence suggests that *pal*-induced somatic nondisjunction can occur at the second or subsequent cleavage divisions.

Introduction

Very early in the history of *Drosophila* genetics it became clear that the Y chromosome is necessary for fertility in *D. melanogaster* males because flies lacking a Y (XO males), although phenotypically and behaviorally indistinguishable from XY males, were invariably sterile (Sturtevant, 1915; Bridges, 1916). The testes of XO males appear normal, but various defects are apparent in the late stages of spermiogenesis and sperm motility is never observed (reviewed by Hess and Meyer, 1968; Williamson, 1976). More recent evidence has led Lifschytz and Hareven (1977) to suggest that genes on the Y are indispensable for developmental processes that take place already in the primary spermatocyte and for normal meiosis.

A question that often arises in studying various aspects of sperm formation is the anatomical site at which Y-linked male fertility factors (Brosseau, 1960) function. Stern and Hadorn (1938) investigated this problem by carrying out reciprocal transplantations of larval testes between fertile (XY) and sterile (lacking a part of the Y chromosome essential for fertility) males. They were able to show that even when fusion of normal and sterile testes occurred, the progeny that were recovered from either host or donor were always derived from the XY testes. These experiments, as well as the experiments of Seidel (1963), suggest that the Y-linked fertility factors are active only in the testes (although one cannot rule out the possibility that these factors might function outside the testes prior to their removal from the donor). However, the testes are comprised of tissues originating from two very different cell lineages: germ and mesodermal cells (e.g. Gehring et al., 1976). Several lines of evidence suggest that Y-linked fertility factors function in the germ line:

(1) Y-linked male sterile mutations were not recovered as mosaics from XY sons of EMS-treated males but were recovered from sons that carried an additional untreated Y (Williamson, 1970a).

(2) Williamson (1970b) and Ayles et al. (1973) reported that the onset of sterility when males carrying Y-linked temperature sensitive sterility mutations were transferred from permissive to restrictive temperature, and the onset of fertility when such males were transferred from restrictive to permissive temperature ranged from 4 to 12 days. This period roughly corresponds to the time it takes for a primary spermatocyte to differentiate into a mature spermatozoon.

(3) Structures resembling amphibian lampbrush chromosomes are present in the nuclei of primary spermatocytes during their growth phase. The available cytogenetic and biochemical evidence (Hess and Meyer, 1968) suggests that these lampbrush loops are a cytological manifestation of the synthetically active fertility factors of the Y.

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Taken together, these experiments suggest that at least some Y-linked fertility factors must function in the germ line of a fertile male. However, these results do not indicate as to whether one or more of these factors might also act in the mesodermal components of the testis, or in other somatic regions early in development. In this report we present evidence that all fertility factors of the Y chromosome function only in the germ line. In addition, we report some previously undescribed properties of the paternal loss (*pal*) mutation (Baker, 1975).

Material and Methods

In principle, it is possible to determine the site of function of the Y chromosome via pole cell transplantations (reviewed by van Deusen, 1976) or, as will be shown in this paper, from mosaic analysis. We crossed y/y^+ Y; *pal*/*pal* males to *y*/*y* females and screened their sons for *pal*-induced mosaicism of the y^+ Y chromosome (for descriptions of genetic variants, see Lindsley and Grell, 1968). In these mosaics the presence or absence of the Y in most cuticular regions can be determined by bristle color (dark vs. yellow) and, in the germ line, by crossing them to *y*/*y* females and testing their sons' fertility and body color. All mosaics were crossed to a group of 4–6 *y*/*y* females. In addition, many mosaics were also crossed to one or two additional sets of 3–5 C(1)RM, *y*/*O* and/or *y v f mal*^{bz} females. No attempt was made to maximize the number of offspring produced by a given fertile mosaic male or to count all the offspring that were produced. A male was considered to be sterile if, after matings with at least four young *y*/*y* females for a period of more than five days, it did not produce any offspring.

As a control, 65 dark and 30 yellow males were simultaneously isolated from the same culture vials or bottles from which some mosaic males were isolated and crossed singly to 4–6 *y*/*y* females. All 65 dark (y/y^+ Y; pal^+/pal) males were fertile and all but one of the yellow males (y/O; pal^+/pal) were sterile. (Further analysis showed that this exceptional yellow fertile male, as well as the 2 mosaic males that gave rise only to yellow and fertile sons and daughters, carried a Y chromosome and that all 3 Y's in question lost the y^+ allele). These preliminary observations provided the rationale for our experiment. Since all males carrying a Y are fertile and all males lacking a Y are sterile, it is expected that all Y-chromosome mosaic males carrying a Y in all tissues in which the Y-linked fertility factors must function will be fertile and all other mosaics will be sterile. It is then possible to correlate fertility vs. sterility with the presence or absence of the Y chromosome in various body parts and thus to determine the fertility site for function of the Y chromosome.

Results and Discussion

The Y Chromosome Functions Only in the Germ Line. We obtained 139 Y chromosome mosaic males. The cuticle of 9 was at least in part haplo-4, 2 died less than 5 days after isolation without producing any offspring, and progeny tests revealed that 2 mosaics were the result of loss of the y^+ allele during the first few cleavage divisions (see above). The analysis below is only based upon the remaining 126 mosaics. On an average, each cuticular landmark was yellow in 39.1% of the mosaics and dark in the remaining 60.9%. The distances between various cuticular landmarks were calculated (Kankel and Hall, 1976) and a fate map constructed. This map resembled maps reported by others (see, for example, Garcia-Bellido and Merriam, 1969; Hotta and Benzer, 1972; Baker, 1975).

In our sample, 66 mosaics were fertile and 60 were sterile. If we assume that the anatomical site of function of the Y-linked fertility genes originates from a single narrowly-localized region on the blastoderm surface and then proceed to fate map this region the result is clearcut: the fertility focus maps to the general area of the blastoderm that, according to embryological (Huettner, 1923), experimental (Illmensee, 1976), and genetic (Gehring et al., 1976) analyses, gives rise to the germ cells. The coincidence between map positions of the Y fertility focus and the germ cells becomes especially obvious when these data are compared with data described elsewhere (Nisani, 1977a). The map position of the germ line depicted there (Nissani, 1977a; Fig. 1) was calculated for technical reasons from the partial sample (179 out of 215) of germinal completes. But some of the males in the present study must have been germinal mosaics so, to make the comparison presented in Table 1 more meaningful, the data in Nissani's (1977a) report were used to recalculate the distances between the germ cells and various cuticular landmarks for the entire collection of 215 mosaics. The similarity in distances, especially posterior ones, and the almost identical interrelationships that exist among the landmarks (rows 1 and 2, Table 1), leave no doubt that

Table 1. Comparative fate map positions of Y chromosome fertility focus and of germ cells

Cuticular landmark:	Anal plate	Clasper	Sixth hemi-tergite	Fifth hemi-sternite	Third hemi-tergite	Third hemi-sternite	Humeral bristles	Antenna
Distance in sturts from germ cells[a]	19.9	20.8	30.3	32.8	35.9	36.4	45.9	49.4
Distance in sturts from Y fertility focus	18.1	19.6	31.2	31.7	40.1	38.5	55.2	57.9

[a] Recalculated from 215 $\overline{XY}$, *y B*/$/y^+$Y fertile mosaic males, including 36 germinal mosaics (from Fig. 1, Nissani, 1977a)

the map position of the Y chromosome fertility focus is coincident with the map position of the germ cells, and indicate therefore that all Y-linked fertility factors must function only in the germ line. We shall now consider this last conclusion in more detail.

Firstly, it is now well established that in a large sample of mosaics any two anatomical regions, irrespective of how close to each other their primordia are on the blastoderm surface, will occasionally differ in genotype (e.g., Garcia-Bellido and Merriam, 1969). This is also true for the germinal and mesodermal components of the gonads which have unlike genetic constitutions in some 30% of all mosaics (Gehring et al., 1976; Nissani and Fellinger, 1978). Yet, in our sample of 126 mosaics, the mosaic boundary never passed between the germ cells and the fertility focus: not even one male gave rise to some yellow and sterile sons. This can be explained only by the assumption that activity of the Y chromosome in the germ line is a necessary condition for male fertility (Williamson, 1970a).

Secondly, these data show that activity of the Y chromosome in the germ line is sufficient for male fertility:

(1) In mapping the Y fertility focus we made the assumption that only one focus was involved. If two or more separate foci were involved, it would have been very difficult to explain the coincidence in map positions of the germ cells and the Y fertility focus.

(2) On an average, each cuticular landmark was yellow in 39.1% of the mosaics. Perhaps more relevant here is the frequency of yellow cuticle for three posterior landmarks (genitalia, sixth tergite, fifth sternite) which, as in previous experiments involving *pal*-induced mosaicism, was somewhat higher: 48.7%. If there is a single fertility focus, the similar proportion of sterility among mosaic males ($60/126 = 47.6\%$) can be readily accounted for (Nissani, 1977b). But if 2 or more foci are involved, such as the germ line and the mesodermal components of the testis, the proportion of sterile males in our sample of 126 mosaics should have been much higher than the average proportion of yellow cuticle among posterior landmarks.

pal-Induced Y Chromosome Mosaics and Somatic Nondisjunction. The cross of a fertile $y//y^+$ Y mosaic male to y/y females should produce dark sons and yellow daughters. This was found to be the case for 47 out of the 66 fertile mosaics in our sample; the only exceptions here were an occasional nondisjunctional yellow son (frequency $32:10{,}879 < 0.003$) and a dark daughter (frequency $8:10{,}300 < 0.001$). But, unexpectedly, 17 mosaics gave rise to large numbers of dark daughters. (There were also two cases with 0.05 and 0.01 ratios of dark to yellow daughters. These two will be arbitrarily grouped together with the above 47 mosaics). That the occurrence of these dark daughters is due to their 17 fathers' mosaicism for the Y chromosome is clear from the following considerations: 1. Among 65 dark control males which were identical in all respects to their mosaic brothers, none produced dark daughters. The only exceptions were an occasional nondisjunctional yellow male (frequency $20:5735 < 0.004$) and a dark female (frequency $11:5945 < 0.002$). 2. Such females were rarely observed in all cultures that gave rise to the homozygous *pal* fathers of our mosaics. 3. When crossed to *yvfmal*^{bz} and/or C(1)RM/O females these mosaics gave again rise to dark daughters and dark sons, respectively, indicating that these observations are not due to some peculiarities of the *yellow* stock used in this study.

An extensive investigation of these 17 exceptional mosaic males and their descendants, which will not be presented here, disclosed that their germ cells carried an extra Y chromosome: they had a y/y^+ Y/y^+Y germinal constitution instead of the expected y/y^+Y constitution (Grell, 1969). The most likely explanation for the occurrence of these males is that they resulted from early somatic nondisjunction of the Y. This is in agreement with Baker's (1975) inference that approximately 5.5% of *pal*-induced X chromosome mosaics were XXX/XO mosaics and explains previous observations that some *pal*-induced C(1)RM, $y//y^+$Y chromosome mosaic females when crossed to males with unmarked Y's gave rise consistently to dark daughters (e.g. Nissani and Fellinger, 1978). An alternative explanation is that an extra Y chromosome is present only in the germ line due to some irregularities in the development of the germ line in some Y chromosome mosaic males. According to the somatic nondisjunctional alternative some or all dark somatic tissues of these mosaics carry 2 Y's, according to the second alternative, only the germ line does. Fortunately, males with 2 y^+Y's can be accurately distinguished from males with one y^+Y in at least one somatic region: the second posterior cells of the wings of an individual with 2 y^+Y's have extra bristles (Brosseau, 1960; Lindsley and Grell, 1968). To ascertain the reliability of this difference, 76 males were subjected to progeny tests and the numbers of bristles on their one pair of bilateral second posterior cells were counted. Among 57 males shown by progeny tests to carry a single y^+Y, the numbers of bristles on their 114 second cells ranged from 0 to 2, and the mean number of bristles per wing was 0.29. Among 19 males shown by progeny tests to carry 2 y^+Y's, the range was 2–10 and the mean 5.1. The need to count the number of bristles on second posterior cells became apparent only after most of the mosaics were discarded and thus this test was applied only to a sample of 15 (in our collection of 126 mo-

saics) which were generated for this purpose. Out of 10 fertile males in this group, 8 produced only y/y^+Y sons and y/y daughters, and 2 produced, in addition, $y/y^+Y/y^+Y$ sons and $y/y/y^+Y$ daughters. In the first group of 8 mosaics, 9 second posterior cells were dark and 7 yellow and not one of these 16 cells had more than one bristle. In contrast, one second posterior wing cell of each male in the second group was dark and they had 7 and 5 bristles; the other wing cell of each was yellow and had no bristles. We may therefore conclude that about $^1/_4$ of *pal*-induced mosaics in this study resulted from somatic non-disjunction.

pal-Induced Somatic Nondisjunction can Occur After the First Cleavage Division. In studies involving chromosomal-loss mosaics, it is often of interest to know whether this loss can occur only at the first cleavage division or also later in development (cf. Hotta and Benzer, 1972; Baker, 1975; Baker and Hall, 1976; Kankel and Hall, 1976; Férrus and Garcia-Bellido, 1977). A similar question can be asked with regard to *pal*-induced somatic nondisjunction. If somatic nondisjunction occurs only at the first cleavage division, then all dark landmarks should carry 2 Y's; if it occurs later then dark landmarks with either one Y or 2 Y's should co-exist in the same fly. That is, when somatic nondisjunction occurs after the first division it gives rise to O//Y//YY triple mosaics. In a sample of 15 mosaics one sterile male had two dark wings; one second posterior cell had 8 bristles and the other had no bristles. More data are required to confirm this observation and to estimate its frequency, but it does suggest that this male was a triple mosaic and, hence, that *pal* can induce somatic nondisjunction after the first cleavage division.

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Recent experiments (J.L. Marsh and E. Wieschaus: Nature, 1978, **212**, 249–251) show that some XO males can be rendered completely fertile by transplantation of XY germ cells. This confirms our conclusion that normal sperm formation does not require the presence of a Y chromosome in any somatic tissue.