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Author

Tokunaga, Chiyoko

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THE PROBLEM OF AUTONOMY OR NONAUTONOMY OF GENE EFFECTS IN MOSAICS.

BY

CHIYOKO TOKUNAGA

DEPARTMENT OF ZOOLOGY AND THE LAWRENCE RADIATION LABORATORY

UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA 94720

Running title: GENE EFFECTS IN MOSAICS

Dedicated to Dr.Curt Stern on the occasion of his 70th Birthday.

Abstract

The dominant autosomal gene Msc (Multiple sex combs) of Drosophila causes the appearance of sex combs on the second and third legs and a reduction of size of sex combs on first legs. The effect of Msc was studied in mosaic flies in which not-Msc spots were present on a Msc background. The mosaics were due to somatic crossing over induced by X-rays. The analysis of the mosaics suggested autonomy of the action of Msc, provided that the somatic crossing over occurs less frequently proximally to the Msc locus than distally. This unusual ratio of somatic crossing over was confirmed by a separate experiment with the gene Kinked, whose locus is very close to Msc.

In *Drosophila melanogaster* normal males carry a special organ on their forelegs, the sex comb. No sex comb is formed on the second and third legs. In males homozygous for the recessive second chromosome mutant *esc* (*extra sex combs*) a sex comb may be formed on all three pairs of legs though with reduced penetrance in the second and third legs. Males which are heterozygous for *esc* may, as a result of somatic crossing over, possess cells which are homozygous for the gene. Such males are genetic mosaics, consisting of *esc*/+ and *esc*/*esc* cells. The question of autonomy or nonautonomy of the effects of the *esc*/*esc* genotypes arises when the *esc*/*esc* cells happen to be in a region of the second and third legs in which not-mosaic *esc*/*esc* males form sex combs. If *esc*/*esc* acts autonomously then even small spots of *esc*/*esc* tissue will be able to differentiate sex comb teeth. If the homozygous genotypes act nonautonomously, then no sex comb structures will differentiate in small spots of *esc*/*esc* embedded in the bulk of *esc*/+ cells. It has been shown, that the developmental processes which lead to extra sex combs in case of *esc*/*esc* belong to the class of autonomous gene action (1). During the earlier work with mutants affecting the formation of sex combs several dominant genes (such as *Msc*, *Multiple sex combs*) were described which place sex combs on the second and third legs. It seemed interesting to determine whether these dominant mutants act autonomously as does the recessive *esc*. Although similar in effect in not-mosaic males there is an important difference in the determination of the sex combs in mosaics. In males heterozygous for the recessive gene *esc*, the critical *esc*/*esc* spots are surrounded by the overwhelming mass of phenotypically normal *esc*/+ tissue, while in males heterozygous for a dominant gene the critical +/+ spots are surrounded by the overwhelming mass of dominant mutant tissue. Stated differently, the *esc*/*esc* cells are derived from pre-crossing over (in phenotypically normal) *esc*/+ cells while in case of dominant mutant the not-dominant +/+ cells are derived from pre-crossing over cells heterozygous for the dominant mutant.

The penetrance of most mutants carrying sex combs on the second and third legs is so low that only a fraction of the mutant legs have a comb. If a comb is formed it consists usually of only very few teeth. The mutant Msc is exceptional in that the penetrance for sex combs on the second legs is very high. This makes Msc suitable for an analysis of autonomy or nonautonomy of gene action in the formation of sex combs on the second legs. Moreover Msc also affects the sex combs on the first legs. Paradoxically this latter effect consists of a reduction of number of teeth. While normal males have about 10 teeth per first leg combs Msc males have about 6 teeth only. To cite a specific finding, Oregon R males had 10.75 ± 0.18 (S.E.) teeth per comb while Msc/+ males had 5.6 ± 0.12 per comb((Tokunaga(2) where standard deviations are given)). The study of autonomy or nonautonomy of the effects of Msc may therefore include the determination of the size of first leg sex combs.

Material and Methods

Multiple sex combs (Msc, 3-48.0) is located in the right arm of chromosome 3, close to the kinetochore, and associated with a small inversion (2). In homozygous state it is a zygotic lethal. It is not known whether it is also cell lethal.

Mosaics for Msc were obtained by means of X-ray induced somatic crossing over in male larvae of the genotype Msc/+. As a marker for spots resulting from somatic crossing over which is independent of Msc, the X-linked recessive gene yellow (y, 1-0.0) was used. Specifically the males carried y on the first chromosome and y^+ present in a translocation of the tip of the X chromosome to the distal part of the right arm of one of the third chromosomes which carries Msc. The body color of these males is not-y except when somatic crossing over removes the y^+ translocation from a cell which after replication results in a spot with y coloration.

Two different third chromosome with a y^+ translocation were used. The first synthesized by Dr.E.Novitski, contains a translocation of the extreme tip of the X chromosome to very close to the distal end of 3R. Confirming a preliminary analysis made by Dr.Novitski I found that the X-chromosomal tip is translocated to somewhere between A and B of section 99 of the salivary chromosome map. The short distal tip of 3R is absent. Msc was introduced into this T(1;3) chromosome which was used in experimental series 1. The second third chromosome used to obtain mosaics is known as In(3LR)C269 (3). The left arm of this pericentric inversion chromosome is composed of a short proximal part of 3L and a short distal part of 3R. The right arm is composed of a long proximal part of 3R followed by a long distal part of 3L, which is viable in homozygous state. Msc and sc^{J4} were introduced into this chromosome by crossing over resulting in a very long chromosome arm which carries Msc proximally and y^+ of sc^{J4} (3) at its tip. The C269 chromosome was used in experimental series 2. The dosage of X-rays administered to larvae of different ages - 24-48, 48-64 and 48-72 hours after oviposition - was 1500r.

The legs of the irradiated adult males were scrutinized under the dissecting microscope (X100 and X160) for the presence of y spots in the sex comb area of each leg. The mosaic legs were then mounted in André medium for detailed inspection under the compound microscope.

The sex comb area of the second leg was, in analogy to that of the first leg (4), somewhat arbitrarily defined. It is that distal region of the basitarsus which includes as its proximal boundary two distal bristles of the longitudinal rows No.6 to 8 and including the one or two distal bractless bristles 6.5. The width of the area was given by the distance of longitudinal row 6 to 8 ((for terminology, see(1)). [illegible]

There were some mosaic legs in which the pigmentation difference between yellow and wild type was not clearly developed due perhaps to somatic induction of abnormal pigmentation by X rays. Only unmistakable spots resulting from somatic crossing over are included in the data presented in this report.

Results

A. The sex combs of mosaic second legs.

If the sex comb area of a mosaic second leg consists of cells with the genotype Msc^+/Msc^+ (= +/+) and the rest of the leg of cells of the genotype Msc/+ and if the +/+ cells act autonomously in development, then no sex comb will form in the +/+ area. On the other hand, if the +/+ cells act nonautonomously, then the +/+ tissue would form a sex comb, its frequency depending on the penetrance of the nonautonomous developmental mechanism. (This latter penetrance may or may not be similar to that of the sex comb of Msc/+, not-mosaic legs).

The following analysis is based on data from yellow spots within the sex comb area of the second leg of males which had been 24-48 hours old when X-rayed. In the experimental series 1, a total of 26 second legs from 25 males with yellow spots were found; in experimental series 2, 27 second legs from 26 males (Table 1). In each series one of the males was mosaic on each of the two second legs. In all but one of the [illegible], one or more sex comb teeth had been formed. The exception was a second leg in series 1. There, 23 out of 24 second legs had a sex comb giving a frequency of occurence of 95.8%; in series 2, 100% of 25 mosaic second legs had formed a sex comb (Table 2; in each series, one male with a mosaic spot in both second legs was excluded from this table).

In order to compare these frequencies of occurence with those found in not-mosaic legs of Msc/+ males, the frequencies of sex combs in two control groups were determined: (a) in the not-mosaic second leg of those individuals which had one mosaic leg and (b) in the not-mosaic brothers of mosaic males. In these controls the frequencies of second leg sex combs were about 90% (87.5% and 91% respectively) in series 1 and 100% in series 2 (Table 2).

These data permit two alternative interpretations. The first assumes autonomy of the effect of the Msc gene. According to this interpretation, the not-yellow teeth in mosaic second legs have the same genotype in respect with Msc, Msc/+, as has the main tissue of the males, and the yellow teeth also have the same Msc/+ genotype. The difference between the not-yellow and yellow teeth is a difference in genotype in respect to y. In the y^+ tissue the y^+ translocation has been retained while in the y tissue it has been removed by somatic crossing over. The region of the crossover would have to be proximally to the tip of the chromosome 3 but distally to the locus of Msc. Accordingly the ratio of somatic crossing over proximally: distally of the Msc locus would be 5:21 (0.24) in series 1 and 6:21 (0.29) in series 2 (Table 1). (Specifically, the 5:21 ratio in series 1 has to be slightly adjusted to account for the fact that the penetrance of Msc in this series is somewhat lower than in series 2.)

The alternative to the assumption of a low frequency of somatic crossing over proximally to and a high frequency distally to Msc consists of the assumption of nonautonomy of the Msc phenotype in mosaics. According to this interpretation the majority of yellow comb teeth have the genotype Msc$^+$/Msc$^+$ resulting from crossing over proximal to Msc and thus removing from the chromosome not only the y^+ translocation but also Msc. The fact that the yellow, supposedly Mac$^+$/Msc$^+$ cells differentiate sex comb teeth would be due to the influence of the surrounding Msc/+ cells. Mosaics without yellow teeth would then be regarded as consisting of Msc$^+$/Msc$^+$ cells in which the surrounding Msc/+ cells had not induced the formation of sex combs. It might be expected in this case that the frequency of a yellow sex combs in mosaic legs might be lower than the frequency of not-yellow sex combs in the not-mosaic controls since nonautonomy in the former spots might often lead to lesser degree of differentiation than in the latter in which both the influence of the not-mosaic tissues and of the Msc/+ genotype of the comb forming cells themselves would lead to comb formation. The data show, however, that the frequency of mosaic legs with yellow teeth is not significantly less than in the controls (Table 2). In experiment 1, mosaics with yellow teeth constitute (9+11)/24=0.83 of all mosaics and 21 of 24=0.88 of the control (a). In experiment 2, yellow teeth in mosaics are somewhat less frequent than the combs in control (a), 80%(20/25) vs. 100%, but the small sample size of the mosaics does not permit a definite conclusion.

Two further comparisons were made on the basis of the data listed in experiment 1. The first was between the sex comb size, defined as number of yellow, if any, plus not-yellow teeth of the mosaic leg and the not-mosaic leg of the same male as control. Under the assumption of autonomy of Msc$^+$ the not-mosaic combs should show no difference from the mosaic combs since the presence of yellow teeth presumably was caused by the same Msc/+ genotype of the yellow teeth as that of the not-yellow teeth. The second comparison was restricted to the size of the combs with yellow teeth as compared to the size of the combs of the controls. In experiment 1, the first comparison was based on 24 males and the second on 20 males, In experiment 2, the two comparisons were based on 25 and 20 males, respectively. The results of the two-way analysis of variance revealed that there are no significant differences between the combs of the mosaic legs and the combs of the second legs on the other side of the same male in either of the two experimental series. (($P > 0.75$ in the first, $P > 0.5$ in the second comparison in experiment 1; $P > 0.75$ in the first, $P > 0.25$ in the second in experiment 2.)

These results are compatible with the assumption that the majority of yellow cells are Msc/+ and act autonomously, but the same results can be interpreted as being due to the genotype Msc^+/Msc^+ of the yellow cells which are subject to nonautonomous expression of tooth formation.

B. Mosaic sex combs on the first leg.

As mentioned earlier, Msc reduces the sex comb size of the first leg. If a Msc^+/Msc^+ spot in the sex comb area develops autonomously in an otherwise Msc/+ leg, the number of teeth would be expected to be greater than the number of teeth expected on a not-mosaic Msc/+ sex comb. Yellow spots which covered a part of the sex comb of the first leg were found in 40 legs from 39 males in experiment 1, and 23 legs from 22 males in experiment 2. These mosaics were induced in 24-48 hours old larvae. The size of the mosaic combs represented by teeth number was compared with the size of the not-mosaic combs of the other first leg of the same Msc/+ male. For the paired comparison, 38 males in experiment 1, and 21 males in experiment 2 were available. The average number of teeth per comb was similar in the mosaics and not-mosaic legs. There were 8.08 ± 0.20 (S.E.) and 8.08 ± 0.13 teeth respectively in experiment 1 (n=38), and 7.62 ± 0.21 and 7.76 ± 0.15 in experiment 2 (n=21).

A two-way analysis of variance of each comparison revealed that the sizes of the mosaic and not-mosaic combs are not significantly different (P=1 in experiment 1, P=0.5-0.75 in experiment 2). The same analysis of sex comb mosaics induced in 48-72 hours old larvae showed similar results. In experiment 1, 48-64 hours old larvae, and in experiment 2, 48-72 hours old larvae were irradiated and the induced mosaic sex combs were analyzed. For the paired comparisons of the sex comb size, 44 males in experiment 1 and 28 males in experiment 2 were available, in which a comb mosaic on the first leg is compared with the not-mosaic comb on the homologous first leg of the same male. In experiment 1 (n=44), the average numbers of teeth per comb were 8.34±0.16 and 8.11±0.19 in mosaic and not-mosaic combs respectively. In experiment 2 (n=28) the numbers were 7.68±0.33 and 7.89±0.24. A two-way analysis of variance of each comparison indicated that the sizes of the mosaic and not-mosaic combs are not significantly different (P=0.1-0.25 in exp.1, P=0.25-0.50 in exp.2).

The above analysis again indicates that if the majority of the y spots represent Msc^+/Msc^+, the Msc allele acts nonautonomously. If however the majority of the y spots are Msc/+ then nonautonomy of Msc^+ can not be proven by the above data.

Appendix

C. Size of yellow spots in two series of experiments.

When the sizes of y spots in the sex comb area in the two experimental series are compared, one finds that the spots in experiment 2 are larger on the average than in experiment 1. The comparisons were based on the number of y teeth in mosaic combs of first legs. In the 24-48 hours series, the average number of y teeth per comb in experiment 1 (1.73±0.17, n=40) was smaller than in experiment 2 (3.30±0.34, n=23). An analysis of variance indicated that the difference was significant ($P<0.001$). In the mosaic combs induced at 48-64 hours in experiment 1 and at 48-72 hours in experiment 2, the average number of y teeth per mosaic combs in experiment 1 (1.41 ± 0.14, n=46) was smaller than in experiment 2 (2.10 ± 0.22, n=30), the difference being significant ($P<0.01$). The mean size of the mosaic combs as well as of the not-mosaic combs, did not show any significant differences between the two age groups that were irradiated in different developmental periods.

The larger size of induced spots suggests their earlier origin. The larger size of the spots found in experiment 2 as compaired to the size in experiment 1 may have been due to a difference in developmental speed of the different genetic constitutions of the two experimental series. If this is the case, the larva of experiment 1 developed faster than those of experiment 2.

Discussion

The main data presented and analysed in this report are compatible with either of two alternative interpretations: the effect in mosaicism is either autonomous or nonautonomous. If autonomous, then the fraction of somatic crossing over proximal to the kinetochore of chromosome 3R to that distally must be much smaller than expected from the general impression that somatic crossing over is mostly occuring in or close to the region of pericentric heterochromatin. If the effect of Msc is nonautonomous then no specific expectation regarding the location of crossovers are required since it should make little difference whether a crossover proximally to Msc resulted in y; Msc^{+}/Msc^{+} cells or a crossover distally to Msc resulted in y; Msc/+ cells.

A decision between the alternatives of autonomy or nonautonomy rests on knowledge of the ratio of somatic crossovers proximally to those distally of the Msc locus. Such knowledge must come from the use of a mutant whose locus is close to that of Msc and whose expression is known to be autonomous. The dominant gene Kinked (Ki, 3-47.6) for kinked bristles fulfills these conditions. Using Novitski's chromosome 3 whose right arm is distally marked by y^+ and determining the frequencies of somatic crossing over proximally and distally to Ki in flies who were heterozygous for a Ki-carrying third chromosome that was marked by y^+, a ratio of crossovers proximally and distally to Ki of 7:20 (=0.35) was obtained (48-72 hours old larvae irradiated with 1500r). The ratio signifies that somatic crossing over proximally to the locus of Ki is only a third as frequent as crossing over distally. This agrees in principle with recent experiments with Ki and Sb on 3R by Garcia-Bellido (6). The same should be the case for crossing over on either side of Msc whose locus is so close to that of Ki and from which the frequency of crossing over proximally may be reduced still further since it is associated with a small inversion (2). While it remains true that crossing over in or near the heterochromatin is disproportionally more frequent than expected from the relative shortness of the heterochromatin region as expressed in salivary gland chromosome it must be admitted that not enough attention has been given to the fact that somatic crossing over in euchromatin, i.e. more distal regions does occur. It frequency seems to vary in different chromosomes.

insert 35 in 3rd line of p.11

Given a low frequency of somatic crossing over proximally to Msc, the majority of yellow spots should have the genotype Msc/+. Since the cells of those spots still contain the mutant allele Msc, it is obvious that they could form sex comb teeth, autonomously.

As shown above in the study of the second leg mosaics, the assumption of autonomy of Msc implies that the ratios of yellow areas without Msc to those with Mss correspond to the ratios of proximal to distal crossing over. In experiment 1 the ratio was 5:21(0.24), and in experiment 2, 4:21(0.29), similar to the ratio of 7:20(0.35) for Ki. This ratio was determined on mosaics of the sternoplurae which develop from the same disc as that of the second leg (4). It may be concluded that Mss acts autonomously. It thus belongs to the considerable number of genes who act autonomously and whose phenotypes in mosaics are determined by competence to respond to an invariant prepattern, or, to use Wolpert's terminology (7), to respond to an invariant pattern of positional information.

Autonomy and nonautonomy of genic expression are not absolute phenomena. When the genotype of a cell is changed by somatic crossing over it may soon after express its new constitution. This would be autonomous action. It is however also possible that the genotype of the cell before it is changed excerts an effect on the cell, which persists in the future independently of the later changed genotype. In the experiments reported here, autonomy of Mss has been established following induction of somatic crossing over in leg discs of at most 72 hours old larvae. Had X-irradiation been applied to older larvae a critical period might have been discovered after which a changed Mss genotype could not have been able to express itself, e.g. in differentiation of sex comb teeth on second legs. Garcia-Bellido and Merriam (8) have coined the term "perdurance" to signify the persistence of gene-initiated processes in cells which are no longer able to respond to a new genotype. Perdurance is thus a type of nonautonomous action.

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References

1. Tokunaga, C. and C. Stern (1965) "The developmental autonomy of extra sex combs in *Drosophila melanogaster*." Develop. Biol., 11:50-81.
2. Tokunaga, C. (1966) "Msc: Multiple sex combs." Drosoph. Inform. Serv., 41:57.
3. Lindsley, D. L. and E. H. Grell (1968) "Genetic variations of *Drosophila melanogaster*." Carnegie Inst. Wash. Publ. No.627.
4. Tokunaga, C. (1962) "Cell lineage and differentiation on the male foreleg of *Drosophila melanogaster*." Develop. Biol., 4:489-516.
5. Stern, C., et. al. (1963) "The cell lineage of the sternopleura in *Drosophila melanogaster*." Develop. Biol., 7:365-378.
6. Garcia-Bellido, A. (1972) "Some parameters of mitotic recombination in *Drosophila melanogaster*." Molec. Gen. Genetics, 115:54-72.
7. Wolpert, L. (1969) "Positional information and the spatial pattern of cellular differentiation." J. Theoret. Biol., 25:1-47.
8. Garcia-Bellido, A. and J. R. Merriam (1971) "Genetic analysis of cell heredity in imaginal discs of *Drosophila melanogaster*." Proc. Nat. Acad. Sci. USA, 68:2222-2226.

Table 2.

Penetrance of sex comb (in parenthesis) on the second leg of Msc/+ males, in mosaics, in not-mosaics on the opposite second leg of the same male (control a) and in randomly chosen 100 not-mosaic brothers (control b).

Experim. series	Number of legs	Number of mosaics with sex comb				Number of not-mosaic combs	
		y teeth only	+ teeth only	y and + teeth	Total	control a	control b
1	24	9	3	11	23 (95.8%)	21 (87.5%)	
control b	200						182 (91%)
2	25	0	5	20	25 (100%)	25 (100%)	
control b	200						200 (100%)

Table 1. Mosaic teeth pattern of the sex combs of second legs and the not-mosaic combs on the opposite second leg of the same Msc/+ male.

Experiment 1				Experiment 2			
Number of males	Mosaic: Number & order of + & y teeth in a comb (prox.---distal)	Mosaic: Total teeth per comb	Not-mosaic: Total teeth per comb	Number of males	Mosaic: Number & order of + & y teeth in a comb (prox.---distal)	Mosaic: Total teeth per comb	Not-Mosaic: Total teeth per comb
1	2+ 3y 1+	6	0	1	3y 3+	6	5
1	1+ 2y 1+	4	4	1	2y 1+ 1y	4	3
1	3+ 1y	4	2	1	2+ 2y 1+	5	4
1	2+ 1y	3	1	1	1+ 2y 1+	4	3
1	2+ 1y	3	2	1	2+ 1y 1+	4	3
1	1+ 1y	2	3	1	1+ 1y 1+	3	1
1	1+ 1y	2	1	1	2y 2+	4	4
2	1y 1+	2	3	2	2y 1+	3	3
2	1y 1+	2	2	1	1+ 2y	3	5
1	2y	2	2	1	6+ 1y	7	6
1	2y	2	1	2	4+ 1y	5	3
1	1y	1	4	1	3+ 1y	4	6
1	1y	1	3	1	3+ 1y	4	4
3	1y	1	2	2	3+ 1y	4	3
1	1y	1	1	2	1+ 1y	2	4
1	1y	1	0	1	1y 1+	2	2
1	3+	3	0	1	5+	5	5
2	1+	1	1	1	4+	4	6
1	0	0	4	1	3+	3	5
1	1y	1		1	2+	2	3
	1+	1		1	1+	1	1
				1	2+ 1y	3	
					3+	3	
Total 25	26y & 24+			26	31y & 68+		
Average teeth per comb		1.92±0.25	1.92±0.25			3.67±0.26	3.68±0.28