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Map expansion around ts-mutations in genes controlling cAMP metabolism in **D.melanogaster.**

Two ts-mutations in genes controlling cAMP metabolism were obtained following treatment with EMS and screening for ts-lethality on media supplemented with beta-adrenoblockator propranolol and phosphodiesterase (PDE) inhibitor theophylline (Savvateeva & Kamyshev 1981). Mutation ts155 leads to propranolol-dependent ts-lethality during postembryonic development at 29°C and to the increase in both cAMP content and adenylate cyclase (AC) activity. Mutation ts398 leads to inhibitor-independent ts-lethality and to increased PDE activity. Since in ts398 mutants (1) AC activity is higher than normal and is readily activated at 29°C; (2) activity of PDE-I assayed in heat-pretreated homogenates is higher than normal, and (3) boiled extracts of ts398 are potent activators of the wild type and its own PDE-I, the mutation is presumed to affect calmodulin, which is known to be stable at boiling and capable of activating both AC and PDE-I (Cheung 1980). Data on Ca^{2+} and EGTA effects suggest that ts398 presumably increases Ca^{2+}-binding activity of calmodulin (Savvateeva et al. in press).

To map both mutations in X-chromosome mutant males were mated to yctvf females, F_1 progeny was reared at 25°C and F_b at 29°C (in case of ts155 on media supplemented with propranolol), ts-lethality being the character to be mapped. Crossovers were scored in the survived male progeny. The obtained results (Table1) revealed quite unexpected facts: a region containing a ts-mutation exceeded the standard map distance approximately on 4.7 map units, while the distance between y-f in both cases fitted well the expected 56.7.

The suggestion that the observed map expansion resulted from the experimental procedure when F_b had to be reared at 29°C could be considered only while analyzing each case separately but not while comparing them. However, the necessity of mapping the mutations left the revealed peculiarity as a mere curiosity.

The next step in mapping was delimiting the interval around ts-mutations by the most proximate markers. Subtraction of the minimal distance for ts155--7.7 map units--from the map position of ct (1-20.0) localized the mutation in the vicinity of cv (1-13.7). When cv ct was used as a marker stock for mapping ts155 (Table 2), the distance between cv and ct appeared to be 27.5 map units instead of the expected 6.3. Considering this overwhelming result as a distortion due to a small sample size, we repeated mapping using y cv ct f stock (Table 2). The result appeared to be the same: while the distance between cv and ct was again 27.2, the distance between y and f did not differ significantly from the standard due to some reduction in recombination in the regions y-cv and ct-f. According to the data presented in Table 1, the map position of ts398 could be determined as 5.9 map units to the right from v, i.e., 1-38.9. Delimitation of the region by the markers dy (1-36.2) and wy (1-41.9) revealed the map expansion, which was evident in comparison with both the standard map and the results obtained while crossing Canton-S males to dy wy females, the increment being the same as in Table 1. At this stage it became evident that the map expansion around the ts-mutations deserved a special attention.

As to map position of ts398 the subtraction of the minimal distance

Table 1. Genetic mapping of ts155 and ts398.
(Exchanges around ts-mutations are underlined.)

y 1 2 ct 3 v 4 f
x x x x
ts155

No. of exchange, crossover phenotype	No. of flies	%
1 ct v f	130	11.32
2 y	49	4.26
3 y ct	112	9.75
4 y ct v	185	16.11
5 normal	14	1.21*
1-3 ct	3	0.26
1-4 ct v	26	2.26
2-3 y v f	-	
2-4 y f	6	0.52
3-4 y ct f	23	2.00
1-2-3 v f	6	0.52
1-2-4 f	13	1.13
2-3-4 y v	-	
1-3-4 ct f	1	0.08
1-2-3-4 v	1	0.08
P-type y ct v f	579	
Total No.	1148	

y_16.9_ts_7.7_ct_12.7_v_22.2_f
24.6 34.9
37.3
59.5

y 1 ct 2 v 3 4 f
x x x x
ts398

No. of exchange crossover phenotype	No. of flies	%
1 ct v f		11.77
2 v f	53	7.69
3 f	34	4.93
4 y ct v	103	14.95
1-2 y v f	2	0.29
1-3 y f	4	0.58
1-4 ct v	34	4.93
2-3 y ct f	-	
2-4 v	15	2.18
3-4 normal	3	0.43
1-2-3 ct f	-	
1-2-4 y v	-	
2-3-4 y ct	-	
1-3-4 y	-	
1-2-3-4 ct	-	
P-type y ct v f	364	
Total No.	689	

y_17.0_ct_10.2_v_5.9_ts_22.5_f
27.2 28.4
33.1
55.6

* coincidence 2.6

Table 2. Genetic mapping of ts155 and ts398, using proximate flanking markers.

cv 1 2 ct
x x
ts155

No. of exchange phenotype	No. of flies	%
1 ct	26	4.8
2 cv	88	16.1
1-2normal	18	3.3*
P-type cv ct	414	
Total No.	546	

cv 8.1 ts 19.4 ct
27.5
* coincidence 4.3

dy 1 2 wy
x x
ts398

1 wy	72	6.7
2 dy	23	2.1
1-2 normal	8	0.8*
P-type dy wy	965	
Total No.	1068	

dy 7.5 ts 2.9 wy
10.4
* coincidence 5.2

Canton-S/dy wy		
wy	76	2.1
dy	106	2.7
P-type normal	1869	
dy wy	1643	dy 4.9 wy
Total No.	3964	

y 1 cv 2 3 ct 4 f
x x x x
ts155

No. of exchange phenotype	No. of flies	%
1 cv ct f	66	6.4
2 ct f	13	1.3
3 y cv	59	5.7
4 y cv ct	299	28.9
1-2 y ct f	1	0.1
1-3 cv	1	0.1
1-4 cv ct	14	1.3
2-3 normal	96	9.3*
2-4 ct	2	0.2
3-4 y cv f	8	0.8
1-2-3 y	-	
1-2-4 y ct	-	
2-3-4 f	2	0.2
1-3-4 cv f	-	
1-2-3-4 yf	-	
P-type y cv ct f	472	
Total No.	1033	

y 7.9 cv 11.1 ts 16.1 ct 31.4 f
19.0
27.2
58.6
* coincidence 125.5

Table 3. Percent of recombination in different temperature regimes experienced in divelopment of F_1.

y 1 w 2 ct 3 4
x x x x
ts398 wy

No. of exchange crossover phenotype	Temperature regimes: 25° constant	29° embryo I instar	29° pupae	29° constant
1. w ct	0.2	0.3	0.5	0.4
2. ct	14.4	13.9	15.0	16.1
3. normal	21.3	21.4	20.2	21.8
4. y w ct wy	3.9	2.4	2.4	3.1
1-2. y ct				0.05
1-3. y	0.05			0.1
1-4. w ct wy				
2-3. y w	0.9	1.2	1.2	1.8
2-4. ct wy	0.2	0.2	0.4	0.6
3-4. wy	0.4		0.3	0.4
1-2-3. w	0.05			
1-2-4. y ct wy				
2-3-4. y w wy	0.2			0.05
1-3-4. y wy				
1-2-3-4 w wy				
No. of flies	2431	2264	1712	2561
Distances				
ct-ts	22.9	22.6	21.7	24.2
ts-wy	4.7	2.6	3.1	4.1
y-ts	38.9	38.2	38.8	43.3
ct-wy	27.6	25.2	24.8	28.3
y-wy	43.6	40.8	41.9	47.4

2.9 from the position of wy gave 39.0 which did not differ from the above 38.9, i.e., the mutation could be localized independently from the marker stock used and from the map expansion observed. Using Df(1)KA10 (11A1; 11A7) and Df(1)v^{65b} (9F12/13; 11A8-9), it became possible to place ts398 to the right of 11A8-9.

In the case of ts155 the map expansion which increased with the shortening of the distance between the flanking markers did not allow the localization of the mutation on the genetic map. The usage of Df(1)N73 (5C2; 5D5-6) and Df(1)C149 (5A8-9; 5C5-6) placed ts155 between 5C5-6 - 5D5-6.

What are the reasons for map expansion around EMS-induced ts-mutations, which are traditionally presumed to be point mutations, i.e., in any case affecting the structure of a gene rather than that of a chromosome? If we address Tables 1 and 2 once more, we can note that (1) double exchanges in the region containing ts-mutation occur easier than those that involve one site near ts-mutation and the other in the different region (for example 3-4 vs. 2-3 for ts398, 1-2 vs. 2-3 for ts155); (2) among the triple crossovers are more frequent the ones that involve two exchanges around ts-mutations; (3) on short, approximately equal distances (6.3 map units between cv and ct; 5.7 between dy and wy) double exchanges occur easily, and the occurrence of single exchanges is also greater; the coincidence being significantly greater than 1; (4) the magnitude of map expansion around ts155 is significantly less in y-ct region than in cv-ct one, that hardly could be observed provided the mutation resulted from cytologically-visible rearrangement.

It could be suggested that the elevated temperature known to increase recombination would have been the major factor responsible for the observed map expansion. However, since only the F_b progeny was reared at 29°C, the temperature might have affected the viability of crossovers but not the frequency of recombination. As seen from Table 1, the preferential survival of crossovers could not explain the region-specific map expansion around each mutation, since the same

marker stocks experienced the same temperature regimes. It is the position of ts-mutation that determines the crossover frequency: y ct is abundant in the case of ts155, since it results from a single exchange, but is absent in the case of ts398, where it would have emerged only after a triple exchange.

The data on temperature effects on the recombination itself was obtained using the "pupal system" of Grell (1973) which allows the registration of the consequences of heat-shocks experienced by the synchronous population of F_1 oocytes. According to the method y w ct females were mated to ts398 wy males and allowed to lay eggs for 4 hr and the egg samples were either set for development at constant temperatures 25°C and 29°C or given 24 hr heat-shocks at 29°C. The 1st shock covered the end of embryonic stage--the beginning of the 1st larval instar, the 2nd given between 120-144 hr after egg-laying, coincided with the premeiotic synthesis of DNA (Grell 1973). The development of F_b took place at 29°C. The obtained results (Table 3) have shown that in all variants except the permanent development at 29°C ts398 maps in the same position, while the maximal temperature-sensitivity shows the region ct-wy, which contains the ts-mutation, due to increase or decrease in double exchanges. When in the same experimental design ts398 wy is substituted for dy wy, neither dy-wy region, nor ct-wy region deviate from the standard map distances and no map expansion is observable (data to be published in detail elsewhere). Thus the phenomenon of map expansion seems to deserve more attention than a mere by-product of mapping experiments, since in the similar experiments in Kiger's group (Salz et al. 1982), they found the reduced recombination around the EMS-induced muation dunce^{M14}, which lead to the decreased activity of PDE-II. In the case of ts398, provided the mutation is in the structural gene coding for calmodulin, the enlarged recombination might have been the result of the high degree of internal homology revealed in the amino acid sequence of calmodulin (Cheung 1980). A gene coding for such a protein could facilitate conjugational conformations for an unequal crossing over. The structure of the gene affected by ts155 may be suspected to have high internal homology as well. The recombinational properties of dunce^{M14} and ts398, which affect learning ability, and of ts155, affecting locomotor activity, make the system of gene control of cAMP metabolism rather promising for further studies on genetic control of the second messengers functions important for cell regulation and neural plasticity.

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Serotonin affects locomotor activity in **Drosophila** via cAMP system.

Serotonin injected into 3-day old virgin Drosophila females was shown to produce the pronounced dosage-dependent increase in locomotor activity, the effect being maximal at the 3rd hour after the injection (Kamyshev etal. 1983). The study of the fate of H^3-serotonin in the Drosophila organism leads to the conclusion that the increase in locomotor activity results from the stimulatory action of serotonin itself, while the rather long latency is likely to be related to N-acetylserotonin effects. The latter metabolite was shown to be the only product of H^3-serotonin conversion, its production being mostly intensive immediately after H^3-serotonin injection and its excretion being rapid enough to make the substance undetectable by the end of the second hour when about 50% of injected serotonin was still present in Drosophila tissues.

It is well known that in many cases serotonin produces its effects via cyclic AMP system: the serotonin-sensitive adenylate cyclase is found in nervous tissue of various insects and its pharmacological properties are similar to those of serotonin receptors in mammals and molluscs (Evans 1980). This work was designed to test the possibility that the effects of serotonin on locomotor activity are mediated via cAMP.

cAMP content was measured in virgin 3-day old females (10 flies per sample) of wild-type strain Canton-S using standard cAMP determination kit (Amersham, England). Serotonin creatinine sulfate (Reanal, Hungary, 20 ng of serotonin-base in 0.2 µl of saline) was injected using the previously described technique (Kamyshev et al. 1983).

The dynamics of the increase in cAMP content following serotonin injection (Fig. 1) resembles the dynamics of the development of its effects on locomotor activity (Kamyshev et al. 1983), i.e., the pronounced effect becomes evident only after a rather long latency of about 2 hr. Thus, it seems likely that the changes in locomotor activity level result from the changes in cAMP content and this is in accordance with the data on positive correlation between cAMP content and locomotor activity in Drosophila ts-mutants with impaired cAMP metabolism (Savvateeva & Kamyshev 1981). The more complicated question is why both effects of serotonin have such a long latency. The intensive production of N-acetylserotonin following the injection of H^3-serotonin might have been responsible for the delay in the manifestation