A CYTOGENETIC ANALYSIS OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITIES IN DROSOPHILA

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Manuscript received October 20, 1976

ABSTRACT

The genome of *Drosophila melanogaster* has been surveyed for chromosomal regions which exert a dosage effect on the activities of cAMP phosphodiesterase or cGMP phosphodiesterase. Two regions increase cAMP phosphodiesterase activity when present as duplications. A region of the *X* chromosome increases cAMP phosphodiesterase activity when duplicated and decreases that activity when deficient. This region has been delimited to chromomeres 3D3 and 3D4, with 3D4 being the most probable locus, and may contain a structural gene for cAMP phosphodiesterase. A region on the third chromosome, 90E–91B, increases cAMP phosphodiesterase activity when duplicated but has no affect on the activity when deficient. Two regions increase cGMP phosphodiesterase activity when present as duplications. A region of the *X* chromosome, 5D–9C, increases cGMP phosphodiesterase activity when duplicated, but smaller duplications covering this region fail to show such an increase, indicating that a single locus is not responsible for the increase observed for the larger duplication. A region of the third chromosome, 88C–91B, also increases cGMP phosphodiesterase activity when duplicated. Smaller duplications covering this region show smaller increases than that observed for the larger duplication, suggesting that at least three loci between 88C and 91B contribute to the observed increase by that region. Deficiencies covering region 88C–91B do not affect cGMP phosphodiesterase activity. No locus for a presumptive structural gene for cGMP phosphodiesterase has been found. Limitations of the use of segmental aneuploidy in locating structural genes for enzymes are discussed.

CYCLIC AMP and cyclic GMP perform important regulatory roles in cellular physiology. Their cellular concentrations are correlated with the physiological state of the cell and are controlled by the synthetic activities of adenylate and guanylate cyclases, utilizing ATP and GTP, and by the degradative activities of phosphodiesterases, converting them to 5′-AMP and 5′-GMP respectively. The nature and regulation of these two enzyme systems are currently subjects of intensive biochemical study. Abnormalities of cyclic nucleotide metabolism increasingly are being associated with diseased states in man and other organisms (ROBINSON, BUTCHER and SUTHERLAND 1971; AMER and KRIEGHBAUM 1975).

Current knowledge of the genetics of cyclic nucleotide metabolism in higher organisms is limited primarily to studies of cAMP metabolism which have been performed with mammalian cell line mutants (BOURNE *et al.* 1975). An im-

portant result of these studies is that cAMP does not appear to be necessary for cell viability and may be a "luxury" molecule for individual cells. If cyclic nucleotides are "luxury" molecules, unnecessary for cell viability, then a full appreciation of their roles can be gained only if they are considered in the context of cellular associations and of the whole organism. This realization has prompted us to begin an investigation of the genetics of cyclic nucleotide metabolism in *Drosophila melanogaster* with the purpose of contributing to an understanding of its complexity and of developing the genetic means to manipulate cyclic nucleotide levels *in vivo* by control of the appropriate enzyme activities. The identification of structural genes for enzymes controlling cyclic nucleotide levels would provide a starting point for such studies.

An approach that we have chosen to identify genes controlling cyclic nucleotide levels is to utilize segmental aneuploidy to locate presumptive structural genes for cAMP and cGMP phosphodiesterases. This approach is based on the assumption that a fly bearing a duplication for a chromosomal segment containing the structural gene for an enzyme will show a proportionate increase in the activity of that enzyme compared to a euploid fly; conversely, deficiency for such a segment will result in a proportionate decrease in the activity of that enzyme. This assumption has been verified for a number of Drosophila enzymes (see HALL and KANKEL 1976, for a recent summary of such work). Segmental aneuploidy has been successfully employed to delimit previously unmapped presumptive structural genes for dopa decarboxylase (HODGETTS 1975) and acetylcholinesterase (HALL and KANKEL 1976). The general validity of this underlying assumption is subject to some question, however. The activities of glucose-6-phosphate dehydrogenase, α-glycerophosphate dehydrogenase and isocitrate dehydrogenase, whose structural genes are mapped, have been shown to be affected by duplications of chromosomal segments other than those containing the structural genes. It is conjectured that these segments contain regulatory genes for the enzyme activities studied (RAWLS and LUCCHESI 1974).

We report here a survey of the entire genome of *Drosophila melanogaster* employing duplication aneuploids, which reveals two regions that increase cAMP phosphodiesterase activity: a region of the *X* chromosome, which shows the deficiency response expected for a structural gene; and a region of the third chromosome, which does not show the expected deficiency response. The *X* chromosome region affecting cAMP phosphodiesterase activity has been delimited to one or possibly two chromomeres. Two regions have also been found which increase cGMP phosphodiesterase activity in duplication aneuploids, but neither of these regions demonstrate the properties expected for a region containing the structural gene for that enzyme.

MATERIALS AND METHODS

Production of aneuploid Drosophila: Most segmentally aneuploid flies were produced by crossing the *Y*; autosome translocation stocks described by LINDSLEY *et al.* (1972) and the *Y*;*X* translocation stocks described by STEWART and MERRIAM (1973, 1975) or others provided by J. MERRIAM. Some flies aneuploid for the *X* chromosome were produced using stocks carrying

insertional translocations and/or deficiencies involving the *X* chromosome or, in one instance, a tandem duplication of a portion of the *X* chromosome. The details of most such crosses and the identification of aneuploid progeny have been described by LINDSLEY *et al.* (1972) and STEWART and MERRIAM (1973, 1975), and their application for studies similar to those described here have been discussed by O'BRIEN and GETHMANN (1973), HODGETTS (1975) and HALL and KANKEL (1976). The stocks that we have employed are detailed in Table 1, and the cytology of some of the *X* chromosome duplications and deficiencies used are shown in Figure 1. LINDSLEY and GRELL (1968) describe the other chromosomes and genetic markers employed.

In comparisons of the enzyme activities of particular autosomally aneuploid flies with euploid flies, sibs of equivalent ages (1–3 days) and age distributions (1 day) from the same culture bottle were used in order to minimize sources of possible variation in activities. In comparsons of *X* chromosome aneuploids and euploids it was not always possible to compare sibs; in these cases mated females of different genotypes were permitted to lay eggs in the same culture bottle in order that aneuploids and euploids would experience identical environmental conditions during development. Flies of the same sex were always compared even though males and females do not differ significantly in activities on a weight basis (Table 2).

Enzyme assays: Flies (2–5) were weighed on a Cann Electrobalance and then homogenized at 0°C in a glass mortar with motor-driven teflon pestle in 1.0 ml of buffer containing 80 mM Tris-HCl, pH 8.0; 20 mM $MgCl_2$; 20 mM 2-mercaptoethanol; and 0.2% bovine serum albumin. The homogenate was then diluted to a concentration of 1 fly/ml with the same buffer before use. Assays were carried out in duplicate by mixing 0.05 ml of homogenate with 0.05 ml of [^{3}H]cAMP at concentrations of 20, 8, 4, 2×10^{-6}M or of [^{3}H]cGMP at concentrations of 80, 40, 20, 8×10^{-6}M, giving a final volume of 0.10 ml containing all components at half the concentrations listed above. This range of concentrations allows the detection of phosphodiesterases with K_m values in the micromolar range, which are those generally believed to be responsible for regulating intracellular levels of cyclic nucleotides (AMER and KREIGHBAUM 1975). All assays were incubated at 30°C for 10 minutes and then quenched by heating to 90°C for 2 minutes in a hot block. Upon cooling, 0.02 ml of carrier containing 2.5 mg/ml cAMP; 2.5

TABLE 1

Stocks

Stock	Cytology	Source
	X chromosome	
Df(w-ec)^{64d}, cm ct sn^3/Basc; Dp(1;3)w^{+67k27}/+	deficiency 3C2–3F1	G. LEFEVRE
[listed in the text as *Df(1)N^{8t}* and *Dp(1;3)G^{spot}*]	duplication 3A5 or 7–3F1	G. LEFEVRE
Df(w-ec)^{64d}, cm ct sn^3/Basc;Dp(1;2)w^{+51b7}/+	duplication 3C2–3D6	G. LEFEVRE
Df(1)N^8/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C1–3D6	E. B. LEWIS
Df(1)N^{64i16}/Basc; SM1, Cy Dp(1;2)w^{+51b7}/+	deficiency 3C3–3D4	W. J. WELSHONS
rst Df(1)N$^{71h24-5}$/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C4–3D4	M. M. GREEN
Df(1)N^{64j15}/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C4–3D3	W. J. WELSHONS
y w^a Df(1)N^{54l9}/Basc; Dp(1;2)w^{+51b7}/+	deficiency 3C6–3C10	G. LEFEVRE
Df(1)w^{-67k30}/Basc; Dp(1;2)w^{+51b})/+	deficiency 3C2–3C6	G. LEFEVRE
Df(1)dm^{75e19}/Basc	deficiency 3C12–3E4	G. LEFEVRE
y^2 Dp(1;1)^{69a20} sn^3/y^{2}In(1)dl-49, w m^2 g^4	duplication 3A–8C	M. M. GREEN
y^2 Df(1)w, spl ec sn^3/w$^+$Y/C(1)DX, y f/w$^+$Y	duplication 2D2–3D3	D. LINDSLEY
T(X;Y)B36/FM7	5C;Y^S	J. MERRIAM
T(X;Y)B171/FM7	5D;Y^L	J. MERRIAM
T(X;Y)B138/FM7	7E;Y^L	J. MERRIAM
T(X;Y)J8/FM7	8C;Y^L	J. MERRIAM
T(X;Y)B26/C(1)DX, y w f	9C;Y^S	J. MERRIAM
T(X;Y)B10/FM7	15EF;Y^L	J. MERRIAM

TABLE 1—Continued

Stock	Cytology	Source
Second chromosome		
$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y/$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y; In(2LR)SM1, al^{2} Cy cn^{2} sp^{2}/Sco		D. LINDSLEY
C(1)RM, y/$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y/T(Y;2)/In(2L+2R)Cy, Cy cn^{2}		
(or *In(2LR)SM1, al^{2} Cy cn^{2} sp^{2}*)		D. LINDSLEY
where *T(Y;2)* is:	with breakpoints:	
T(Y;2)H116	24F;Y^{L}	
H52	27E;Y^{S}	
L52	30F;Y^{L}	
R15	35B-C;Y^{S}	
B110	38C;Y^{L}	
L138	39C;Y^{S}	
B177	41;Y^{L}	
J59	43A;Y^{S}	
B26	43E-F;Y^{L}	
H136	44C;Y^{L}	
L23	45F;Y^{S}	
H144	47F;Y^{L}	
L110	50C;Y^{L}	
R14	52E;Y^{L}	
H149	54F;Y^{S}	
L107	57B;Y^{L}	
P59	59B;Y^{S}	
Third chromosome		
$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y/$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y; In(3LR)TM6, ss^{-} bx^{34e} Ubx^{67b} e/Sb		D. LINDSLEY
C(1)M3, y^{2} bb/$Y^{S}X{\cdot}Y^{L}$, In(1)EN, y/T(Y;3)/In(3LR)TM6, ss^{-} bx^{34e} Ubx^{67b} e		D. LINDSLEY
where *T(Y;3)* is:	with breakpoints:	
T(Y;3)B141	64E;Y^{S}	
G122	67C;Y^{L}	
H156	70C;Y^{S}	
D228	74A;Y^{L}	
J162	79D;Y^{S}	
L132	83C-D;Y^{S}	
L136	83E-F;Y^{S}	
R36	86B;Y^{L}	
G48	88C;Y^{S}	
L142	89C;Y^{L}	
B116	90E;Y^{L}	
A89	91B;Y^{L}	
B93	93F-94A;Y^{S}	
G73	96A;Y^{L}	
R128	97F;Y^{S}	
Fourth chromosome		
spa^{pol}/spa^{pol}		M. M. GREEN
C(1)RM, y/$Y^{S}X{\cdot}Y^{L}$, In(1)EN, v f B; C(4)RM, ci ey^{R}/0		R. J. MACINTYRE

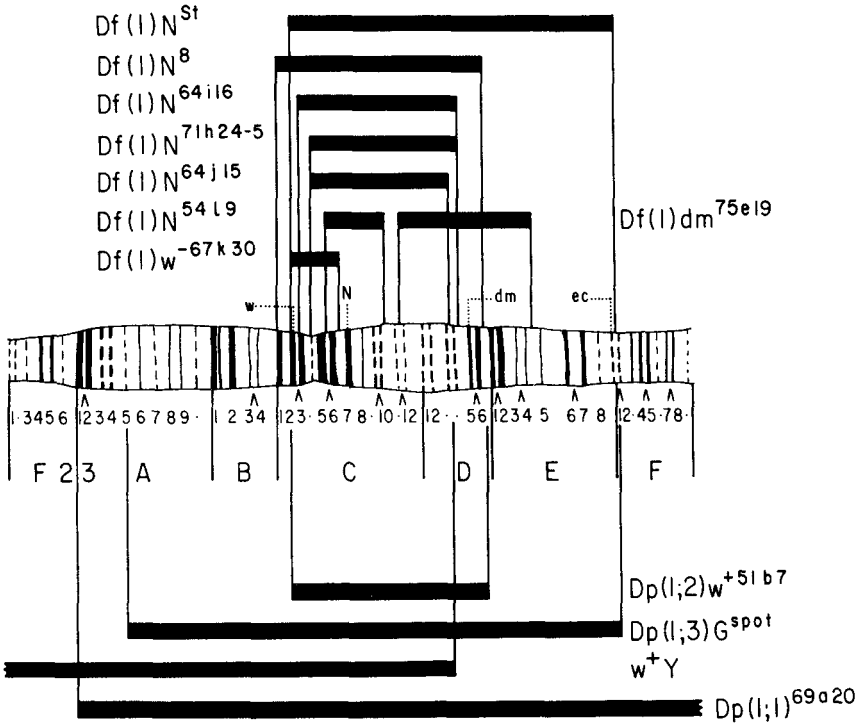

FIGURE 1.—A drawing of a segment of the *X* chromosome of *Drosophila melanogaster* showing the cytological extent of some of the deficiencies and duplications discussed in the text. Redrawn from Bridges' revised map (LINDSLEY and GRELL 1968) by Hana Van Campen.

mg/ml 5′-AMP; and saturated with adenosine or 0.02 ml of carrier containing cGMP, 5′-GMP and guanosine in the same concentrations were added respectively to the assays for cAMP phosphodiesterase and cGMP phosphodiesterase. Chromatography of 0.025 ml of each assay mixture was performed on Whatman 3MM paper overnight in a descending system using 0.5 M NH_4Ac, pH 7.0 and 100% ethanol (2:5). The 5′-nucleotide monophosphates and nucleosides were identified under a UV lamp, cut from the paper, and eluted from the paper with 3.0 ml of 0.01 M HCl at 90°C for 15 minutes. One ml aliquots of the acid solutions were counted in 10 ml of scintillation fluid (0.2g POPOP, 3g PPO, 250 ml Triton X-114 and 750 ml xylenes).

[^{3}H]cAMP and [^{3}H]cGMP were obtained from New England Nuclear Corp. in ethanol solution. The ethanol was removed by lyophylization prior to dilution to the required specific activity since ethanol inhibits the phosphodiesterases under study. Specific activities of 100–130

TABLE 2

Cyclic nucleotide phosphodiesterase activities in females and males

	Females*	Males*
V_{max} cAMP Hydrolysis	17.9 ± 1.8	20.7 ± 2.6
V_{max} cGMP Hydrolysis	24.6 ± 3.6	26.1 ± 6.6

* Cumulated data from several euploid genotypes.

mCi/mmole were generally used in assays. Unlabeled nucleotides and nucleosides were obtained from Sigma Chemical Co.

Enzyme activities are measured in units of picomoles 5′-nucleotide/minute/mg of fly. The conversion of cyclic nucleotide to nucleoside must be monitored and the quantity added to the amount of 5′-nucleotide produced during incubation because in the crude homogenate employed for the assay some dephosphorylation of the 5′-nucleotides occurs. For example, in some assays as much as 30% of the 5′-AMP produced may be converted to adenosine. The means of duplicate assays were plotted by the method described by EADIE (1952) and HOFSTEE (1952) to obtain the V_{max} and K_m for each activity in each aneuploid and euploid studied. Comparisons between aneuploids and euploids are presented as a relative activity ($V_{max}^{aneuploid}/V_{max}^{euploid}$). We naively expect the relative activity to be 1.5 if the structural gene for the enzyme in question is present as a duplication in the aneuploid or 0.5 if the aneuploid is deficient for the structural gene. Statistical treatment of data was carried out according to HOEL (1954). All indicated confidence limits are the 95% confidence limits calculated from the t distribution.

RESULTS

Survey of the entire genome using duplication aneuploids: Segmental aneuploidy was used to survey the genome simultaneously for regions which might contain the structural genes of cAMP phosphodiesterase and cGMP phosphodiesterase. The ratio of $V_{max}^{duplication}/V_{max}^{euploid}$ for each activity was independently determined twice. This provided a mean value and a range for the two determinations for each enzyme activity. For a sample of size 2, the range is a good approximation of the standard deviation which might be expected if a larger sample size had been chosen (HOEL 1954). If the mean of the two determinations for a particular segment was larger than 1.2, or if the range suggested that a higher mean might be found upon further study, additional determinations of the relative activity were made until we were satisfied with the final mean. This rather subjective approach is biased toward providing more reliable data for those segmental aneuploids showing higher relative activities, and thus the data for all segments of the genome do not have equal significance. The results of this survey are shown in Figure 2. The cGMP phosphodiesterase data for the segment 88C–91B on the third chromosome stand out as the only region with a relative activity near 1.5 The cAMP phosphodiesterase data do not point to a unique segment as a candidate for the locus of the presumptive structural gene. The two regions showing the highest relative activity for cAMP phosphodiesterase are the distal half of the X chromosome (tip–9C) and the region on the third chromosome (88C–91B) which is high for cGMP phosphodiesterase activity.

Detailed study of the X *chromosome using duplication aneuploids*: In the above survey, the X chromosome was examined using the stock *T(X;Y)B26/C(1)DX, y w f*, which segregates females duplicated for either the distal or proximal half of the X chromosome, as well as euploid sisters. Because the data of STEWART and MERRIAM (1975) for 6-phosphogluconate dehydrogenase (6PGD), whose structural gene is located in the distal half of the chromosome, do not show the expected relative activity of 1.5 for flies duplicated for the distal half of the chromosome, we felt that the large size of the duplication employed might influence the relative activity observed. We therefore surveyed the X chromosome employing smaller duplications with the result shown in Figure 3.

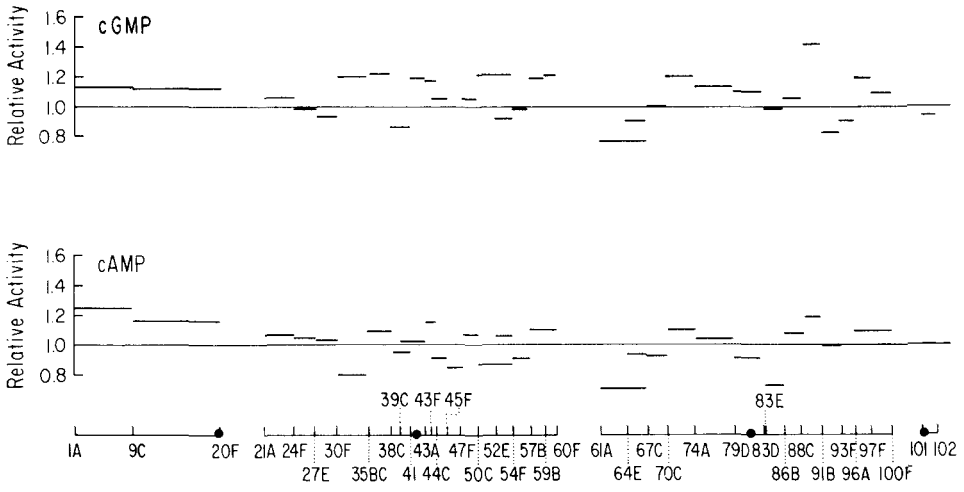

FIGURE 2.—A map of the relative activities of cAMP and cGMP hydrolysis by segmental aneuploids. The standard salivary gland chromosome band designations for breakpoints are given for (from left to right) the *X* chromosome, 2nd chromosome, 3rd chromosome and 4th chromosome. In the case of the 4th chromosome, the relative activity is that for triplo-4 to haplo-4 females. Dots represent the relative positions of the centromeres.

The first step taken was to assay segmental aneuploids duplicated for quarters of the *X* chromosome: tip–5D; 5D–9C; 9C–15EF; 15EF–centromere. Females carrying a duplication for tip–5D show a relative activity of 1.5 for cAMP phosphodiesterase, confirming our hypothesis that the size of the duplication might influence the observed relative activity. Unexpectedly, females carrying the duplication 5D–9C show a relative activity of 1.5 for cGMP phosphodiesterase.

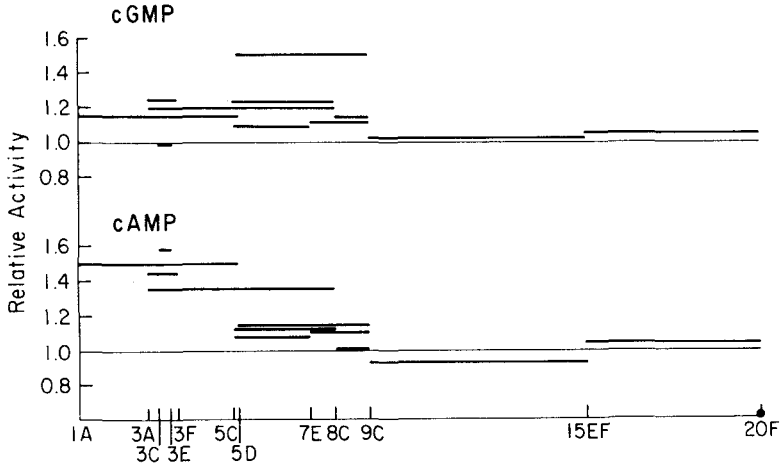

FIGURE 3.—An *X*-chromosome map of the relative activities of cAMP and cGMP hydrolysis by segmentally aneuploid females.

TABLE 3

Relative activities of cAMP phosphodiesterase in selected duplication aneuploids

Duplication	$V_{max}^{duplication}/V_{max}^{euploid}$ Females	Males
Dp(1;1)^{69a20}	1.36 ± 0.29	
Dp(1;3)G^{spot}	1.44 ± .040	1.77 ± 0.15
Dp(1;2)w^{+51b7}	1.58 ± 0.26	
w^{+}Y		0.89 ± 0.20

Analyses of smaller duplication aneuploids permit a more precise localization of the region responsible for the increase in cAMP phosphodiesterase activity (Figure 3 and Table 3). The smallest duplication to contain this region is *Dp(1;2)w^{+51b7}* (3C2–3D6). It is also contained in *Dp(1;3)G^{spot}* and *Dp(1;1)^{69a20}*. The *w^{+}Y* (duplication 2D2–3D3) overlaps most of *Dp(1;2)w^{+51b7}* and *Dp(1;3)-G^{spot}* and yet does not contain the responsible region (Table 3). This region then must lie to the right of the *w^{+}Y* duplication and to the left of the right end of *Dp(1;2)w^{+51b7}* and, thus, be included in 3D4–3D6. This assignment is subject to the following qualification. The precise endpoints of the *w^{+}Y* duplication are cytologically difficult to define, and it is possible that it only extends to 3D2 (G. LEFEVRE, personal communication). The region is certainly included in 3D3–3D6, or within four chromomeres.

Efforts to further localize a region between 5D and 9C responsible for increasing cGMP phosphodiesterase activity when present as a duplication were not successful. This region was examined using smaller duplications: 5C–7E; 7E–9C; 5C–8C; 8C–9C. No smaller duplication showed the increase in cGMP phosphodiesterase activity exhibited by the duplication for 5D–9C, nor did the sum of the relative activities for either two small duplications equal that of the larger (Figure 3). Thus it seems unlikely that there is a single locus between 5D and 9C with the dosage property expected for the structure gene for cGMP phosphodiesterase.

TABLE 4

Relative activities of cyclic nucleotide phosphodiesterases in selected deficiency aneuploids

Deficiency	$V_{max}^{duplication}/V_{max}^{euploid}$ cAMP Hydrolysis	cGMP Hydrolysis
Df(1)N^{8t}	0.685 ± 0.066	1.50 ± 0.57
Df(1)N^{8}	0.756 ± 0.170	1.22 ± 0.45
Df(1)N$^{71h24-5}$	0.638 ± 0.077	0.95 ± 0.08
Df(1)N^{64i16}	0.725 ± 0.093	1.10 ± 0.15
Df(1)N^{64j15}	0.815 ± 0.063	1.02 ± 0.13
Df(1)N^{54l9}	0.996 ± 0.089	1.12 ± 0.16
Df(1)w^{-67k30}	1.12 ± 0.054	0.95 ± 0.25
Df(1)dm^{75e19}	0.662 ± 0.028	1.20 ± 0.23

Mapping of the w–ec *region using deficiency aneuploids:* Duplication mapping places the *X* chromosome region affecting cAMP phosphodiesterase activity between *w* and *ec* and near *dm* (Figure 1). Deficiencies for all or parts of this region are available. The cAMP phosphodiesterase activities of flies heterozygous for some of these deficiencies and the *Basc* balancer were compared with sibs homozygous for *Basc*. The results presented in Table 4 show that a locus, deficiency for which significantly decreases cAMP phosphodiesterase activity, lies to the right of the left breakpoint of $Df(1)dm^{75e19}$ and to the left of the right breakpoints of $Df(1)N^{71h24-5}$ and $Df(1)N^{64i16}$ and is included in 3C12–3D4.

Combining the results of both the duplication and deficiency mappings, it is possible to say that a locus with the dosage properties expected for the structural gene for cAMP phosphodiesterase is included in chromomeres 3D3 and 3D4. The right breakpoint of $Df(1)N^{64j15}$ (between 3D3 and 3D4) occurs at a point which is critical to the assignment of a precise locus. $Df(1)^{64j15}/Basc$ heterozygotes show a depression in cAMP phosphodiesterase activity intermediate between those heterozygous deficiencies which do and do not contain this locus (Table 4). Cytological examination places band 3D4 between the right breakpoints of $Df(1)N^{64j15}$ and those of $Df(1)N^{71h24-5}$ and $Df(1)N^{64i16}$ (G. LEFEVRE, personal communication). Genetic evidence indicating that the right breakpoints of these three deficiencies are not identical will be presented in the following paper (KIGER 1977). It is possible that the presumptive structural gene for cAMP phosphodiesterase is included in band 3D4 and the decrease in activity observed for $Df(1)N^{64j15}$ heterozygotes is due to an effect of the breakpoint on the expression of the gene in the adjacent 3D4 band. The possibility that both bands 3D3 and 3D4 contain structural genes for the enzyme cannot be ruled out, however. Indeed, it should be noted that none of the deficiencies examined in Table 4 show a relative activity as low as 0.5. This may indicate that either (i) another structural gene for this enzyme is present elsewhere in the genome, (ii) partial compensation by the structural gene in the *Basc* chromosome may take place, or (iii) this locus may represent a regulatory locus rather than the structural gene for cAMP phosphodiesterase.

If the region included in 3D3–3D4, which affects cAMP phosphodiesterase activity, is indeed the structural gene for the enzyme, then the effects of duplications and deficiencies for the region on enzyme activity should be additive rather than dominant or recessive. That this is the case is demonstrated in Figure 4. Homozygous *Basc* flies exhibit the same V_{max} as aneuploid flies of genotype $Df(1)N^{8t}/Basc$; $Dp(1;2)w^{+51b7}/+$. The data also indicate that the cAMP phosphodiesterase activities in both euploid and aneuploid flies have nearly identical K_m values (range 1.8 to 3.1×10^{-6}M) as would be expected if the changes in V_{max} are due to changes in the amount of enzyme activity present. These results are consistent with the hypothesis that the structural gene for this enzyme is within the region being studied, but do not prove that it is the structural gene which is present rather than a regulatory gene for the enzyme.

A finer survey of the third chromosome region affecting both phosphodiesterase activities: The initial survey of the genome using duplications showed that

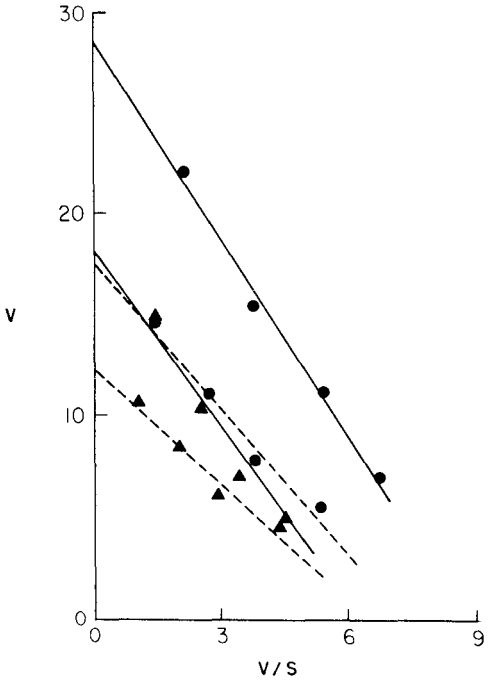

FIGURE 4.—Eadie-Hofstee plot of cAMP hydrolysis by homogenates of euploid and aneuploid flies. The intercept on the ordinate gives the V_{max}, and the K_m is given by the negative slope of the line (v = velocity; s = substrate concentration). *Basc/Basc* ●---●; *Basc/Basc;Dp(1;2)w*$^{+51b7}$/+ ●——●; *Df(1)N*^{81}*/Basc*; *Dp(1;2)w*$^{+51b7}$/+ ▲——▲; *Df(1)N*^{81}*/Basc* ▲---▲.

the third chromosome region 88C–91B causes an increase in the relative activity of cGMP phosphodiesterase to near 1.5 and of cAMP phosphodiesterase to 1.2, the second highest value observed for a segmental duplication affecting cAMP phosphodiesterase activity in the survey. This region was broken into smaller regions (88C–89C; 89C–90E; 90E–91B), aneuploids for which could be recovered in both the duplicated and deficient states. The presence of *Ubx*$^{+}$ at 89E, a haploinsufficient locus (LINDSLEY *et al.* 1972), provides independent evidence that the translocation stocks employed to survey this region yield a deficiency for this locus (expressing the *Ubx* phenotype) and, in combination with the balancer bearing *Ubx*^{67b}, evidence that they yield duplications for this locus (suppressing *Ubx*^{67b}). The results of the survey of the 88C–91B region are presented in Figure 5. In no case do deficiency aneuploids for one of the smaller regions exhibit a decrease in enzyme activity which would be expected if the structural gene for one of the enzymes were present. The increase in cAMP phosphodiesterase activity noted for the region of 88C–91B must be the result of duplication for the region 90E–91B. The increase in cGMP phosphodiesterase activity associated with the region 88C–91B appears to be the result of the sum of the effects observed for each of the smaller duplications.

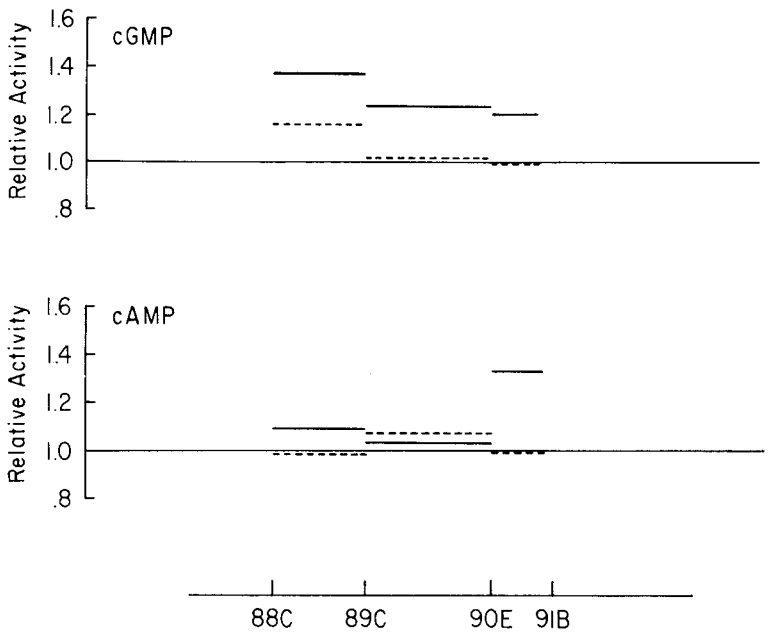

FIGURE 5.—A map of the relative activities of cAMP and cGMP hydrolysis by segmentally aneuploid flies covering the region 88C–91B. Solid lines are the relative activities for duplication aneuploids, and dashed lines are the relative activities for deficiency aneuploids.

DISCUSSION

Segmental aneuploidy is a potentially useful tool for mapping genes that code for known proteins in the absence of known genetic variants of such proteins. Its successful application, however, depends on a number of other factors. First, there should exist a unique locus for the structural gene in question. If an enzyme activity is the result of the contribution of a number of noncontiguous structural genes, then segmental aneuploidy may or may not reveal one or more of these loci. Its successful application would depend on the relative activities of, and the number of, loci involved. Gene duplication, possibly followed by translocation and the acquisition of new functions by the duplicates, is well documented in the history of organisms (OHNO 1970). The recent finding in Drosophila of mutant phenotypes which are suppressed by nonhomologous chromosome duplications suggests that duplicated genes may exist which have not diverged significantly in function (LEFEVRE and WRIGHT 1976).

Second, an enzyme activity should not be subject to any feed-back regulation. If the level of an enzyme activity is critical to the cell, then closely linked, *cis*-dominant, regulatory loci may exist which can modulate the synthesis of the enzyme to buffer against the dosage effects of aneuploidy. Since there is only one small region of the Drosophila genome which shows aneuploid lethality (LINDSLEY *et al.* 1972) such modulation is probably common.

Third, distantly linked regulatory loci may exist which act in a dosage-dependent way to affect an enzyme activity (positively or negatively). Putative examples of such loci have been presented by RAWLS and LUCCHESI (1974). Such loci may act at the level of transcription or translation or code for proteins which act as activators or inhibitors of an enzyme activity through protein-protein interaction.

Finally, it is possible to envision less specific ways in which aneuploidy might affect enzyme activities through general metabolic derangements which would have pleiotropic effects on many enzymes.

The factors contributing to the activities of cyclic nucleotide phosphodiesterases are complex. In the numerous systems in which cyclic nucleotide phosphodiesterases have been characterized biochemically, they have been found to occur in multiple physical and kinetic forms (AMER and KREIGBAUM 1975). Cyclic AMP phosphodiesterase activity can be induced by hormones (UZUNOV, SHEIN and WEISS 1973) and by cAMP or its dibutyryl derivative (D'ARMIENTO, JOHNSON and PASTAN 1972; MAGANIELLO and VAUGHAN 1972; BOURNE, TOMKINS and DION 1973) through mechanisms which require new protein synthesis. Its activity and physical forms can also be rapidly altered by mechanisms which do not appear to require new protein synthesis (PLEDGER, THOMPSON and STRADA 1976; VAN INWEGEN *et al.* 1976). In some systems a protein activator of cAMP phosphodiesterase is known which appears to be under a separate genetic control (LYNCH, TALLANT and CHEUNG 1975).

Although pitfalls may exist in the use of the technique of segmental aneuploidy, and the cyclic nucleotide phosphodiesterase system is biochemically complex, the results presented here identify a genetic locus on the *X* chromosome which has major effects on cAMP phosphodiesterase activity. The dosage effects suggest that this locus may be a structural gene for the enzyme but do not eliminate the possibility of a regulatory gene as the responsible agent. Regardless of the nature of this locus, its identification provides a tool by which we can genetically control the level of cAMP phophodiesterase activity *in vivo*. The fortunate occurrence of deficiencies for this locus and its presence in insertional translocations makes it possible to obtain sibs with different gene dosages and enzyme activities by segregation within a single stock (*e.g.*, Figure 4). A genetic study of the effects of this locus on the adult fly is presented in the following paper (KIGER 1977).

The results also suggest that there is a locus on the third chromosome between 90E and 91B which may exert a regulatory effect on cAMP phosphodiesterase. Duplication of this region markedly increases cAMP phosphodiesterase activity, but a deficiency of the region has no effect on the activity. The inducible nature of cAMP phosphodiesterase suggests that duplication of this region might result in an increase in cAMP concentration which would in turn lead to an increase in cAMP phosphodiesterase activity. The simplest postulate would be that the structural gene for adenylate cyclase may reside here, but the complexity of the system does not compel acceptance of this possibility over others.

The failure of segmental aneuploidy to provide a presumptive structural gene locus for cGMP phosphodiesterase indicates that we have uncovered one or more of the pitfalls outlined above. The results demonstrate that cGMP phosphodiesterase activity (as well as cAMP phosphodiesterase activity) can be markedly affected by duplication of certain regions of the genome. The totality of the data does indicate that cAMP phosphodiesterase and cGMP phosphodiesterase activities are under different genetic controls (Figures 2, 3 and 5; Table 4), a finding which substantiates biochemical inferences. It has been proposed that cAMP and cGMP regulate antagonistic physiological responses in cells (the Yin Yang Hypothesis), and evidence indicating that cellular physiology may be regulated by the ratio of cAMP to cGMP has been presented (GOLDBERG *et al.* 1975). It is interesting to note that duplication for two autosomal regions (30F–35B–C and 50C–54F) appears to have quite opposite effects on cAMP phosphodiesterase and cGMP phosphodiesterase activities (Figure 2). These two regions may bear further study in the light of this hypothesis.

In conclusion, our use of segmental aneuploidy to investigate cyclic nucleotide phosphodiesterase activities has provided a foothold for further investigation of the genetic control of cyclic nucleotide levels. Biochemical investigation of the cAMP phosphodiesterase activity in flies nullosomic for chromomeres 3D3 and 3D4 in comparison to that of euploid flies should increase our understanding of the genetic basis for the multiple enzymatic forms which are present (KIGER and GOLANTY, unpublished data). Physiological studies of nullosomic flies should permit an assignment of biological function to this region.

We wish to thank all of those who generously provided the stocks used in this study. We are particularly indebted to G. LEFEVRE, JR. and to R. J. MACINTYRE for their advice and counsel. This work was supported in part by Public Health Service grant GM 21137.

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