Acharyya, M., and R.N. Chatterjee. University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019 India. In situ transcription analysis of chromatin template activity of the X chromosomes of sex-transformed flies of *Drosophila melanogaster*.

In *Drosophila melanogaster* sex is determined by the ratio of number of X chromosomes (X) to the sets of autosomes (A). Several mutants including *tra, tra-2, ix* and *dsx* have phenotypes which also suggest that the wild type functions of the loci play important roles in somatic sexual differentiation in *D. melanogaster*. The *transformer* (*tra*), *transformer-2* (*tra-2*) or *intersex* (*ix*) mutants affect somatic sex differentiation only in females. The null mutations at *double sex* (*dsx*) affect both XX and XY individuals causing them to develop as phenotypically intersex. Smith and Lucchesi (1969) claimed that altered sexual physiology does not have any role in dosage compensation of X-linked gene in *D. melanogaster*. However, it is not clear from the data whether the altered sexual physiology of the sex transformed flies has any role to set and /or maintain the transcription of the X-chromosome by altering the chromatin template activity of the X-chromosome or not. In view of this reason, the present investigation was undertaken to examine the relative template activity of X chromosome in sexually transformed flies of *Drosophila melanogaster* with the use of *E. coli* RNA polymerases.

For the present experiments, *in situ* transcription assay was carried out on the fixed cytological preparation of salivary gland chromosomes of third instar larvae of the sexually transformed flies as described by Chatterjee (1985) using DNA dependent *E. coli* RNA polymerase holoenzyme obtained from Sigma Chemicals. Reaction of *in situ* transcription was carried out on the fixed polytene chromosome preparation by addition of 3 units of purified RNA polymerase holoenzyme on each slide in combination with assay mixture (Chatterjee, 1985). Reactions were terminated by placing the slide in 5% cold TCA w/v containing 0.01 M $Na_4P_2O_7 \bullet 10\ H_2O$. Unincorporated nucleoside triphosphate was removed by repeated washing in 5% cold TCA and the preparations were then processed for autoradiography. Exposure time was 15 days.

Table 1 gives the grain number (together with statistical analysis) over the X chromosome and autosomal arm (2R). Data reveal that the relative template activity as measured by the X/A ratio of grain number in wild type male and *tra/tra* male is very close to that in female. Thus, the ratio of the number of silver grains on the X chromosomal segment (1A-10F) to that on autosomal segment (56F-60F) in XX; *tra/tra* transformed flies is fairly concordant with that of XY; *tra/tra* flies. In fact, the male phenotype produced by *tra* mutation had no recognizable influence on chromosomal organization of the paired X's. Similarly, when the relative template activity of the X chromosome of either XX; *dsx/dsx* or XX, *ix/ix* flies was compared to normal females, no appreciable change in the X chromosomal organization was noted. On the other hand, in XY, *dsx/dsx* and XY; *ix/ix* flies, the relative template activities were comparable to normal males. The statistical analysis of the data further shows a positive correlation between the grain number over the X chromosome to that of the autosome (2R) in all individuals.

On the basis of these observations it appears that it is the X:A ratio and not the sexual phenotype of the adult flies that sets the level of template activity of the X chromosome. Thus it has been observed that when the X:A ratio is

Table 1. Data on ^{3}H-UMP labeling pattern of an X chromosomal segment (1A-10F) and an autosomal segment (56A-60F) of the normal and sex determination mutant strains of *D. melanogaster* under *in situ* transcription condition with *E. coli* RNA polymerase.

Genotype	X:A ratio	Phenotypic sex	Number of nuclei examined	Mean grain no. with ± S.E.		X/2R with S.E.	Correlation coefficient (r)
				X chromosome (1A-10F)	2R (56A-60F)		
XX; +/+	1.00	Female	18	542.81 ± 47.38	305.30 ± 29.53	1.76 ± 0.11	0.96
XY; +/+	0.50	Male	19	515.83 ± 38.10	297.11 ± 28.17	1.73 ± 0.19	0.94
XX; tra/tra	1.00	Male	17	531.24 ± 44.96	291.92 ± 25.51	1.81 ± 0.18	0.93
XY; tra/tra	0.50	Male	19	421.95 ± 36.26	266.93 ± 24.29	1.58 ± 0.12	0.82
XX; ix/ix	1.00	Intersex	19	331.38 ± 38.14	184.43 ± 25.01	1.79 ± 0.09	0.91
XY; ix/ix	0.50	Male	20	301.07 ± 42.15	171.16 ± 29.21	1.75 ± 0.11	0.85
XX; dsx/dsx	1.00	Double-sex	17	437.21 ± 34.91	249.17 ± 27.12	1.75 ± 0.12	0.86
XY; dsx/dsx	0.50	Double-sex	18	401.61 ± 31.19	241.94 ± 19.81	1.65 ± 0.12	0.92

1.0 (as in the case of XX; *tra/tra* males) irrespective of sexual phenotype, the template activity of the X chromosome is set at a female level. Similarly, in the case of XX; *dsx/dsx* individuals, the X chromosome is transcribed at female level. On the other hand, in XY; *dsx/dsx* individuals, the level of template activity is set at a male level. In summary, our data clearly indicate that the sex determining mutants, *tra*, *ix* and *dsx* have no role in regulating the template organization of the X chromosome(s) (see Table 1) for dosage compensation.

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Alatortsev, V.E. Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq.46, Moscow, 123182, Russia. Genetic loci in the *Pgd-K10* region of the *Drosophila* X chromosome.

The *Pgd-K10* region of the X chromosome is one of the most genetically investigated areas of the *Drosophila melanogaster* genome. The fine genetic structure of this region has been determined in several independent studies based on saturation of this region by lethal mutations and by rearrangements and complementation analysis (Gvozdev *et al.*, 1973; Perrimon, *et al.*, 1984; Alatortsev and Tolchkov, 1985). For the descriptions of the individual complementation groups, see Lindsley and Zimm (1992).

There are some additions to the information about earlier described complementation groups. First, our complementation analysis showed that group *N2* (Gvozdev *et al.*, 1977) coincides with group *l(1)C204* (Perrimon *et al.*, 1985), as well as groups *N7* and *l(1)JA127*. Thus, two pairs of groups were correctly jointed (Lindsley and Zimm, 1992). They were designated as *wapl* and *l(1)2Ea*, respectively. Second, the *l(1)90* mutation representing separate complementation group complements the *JC105* deletion and must be situated to the left of *wapl*, between the *Pgd* and *wapl* loci.

Contiguous and overlapped DNA fragments from the *Pgd-K10* region were cloned in several laboratories in the course of chromosomal walks along the Canton (Haenlin *et al.*, 1985), Oregon (Dura *et al.*, 1987), and *gt* w^a (Alatortsev, 1987) X chromosomes, and the physical map for the region was constructed. Molecular approaches allowed to expand our knowledge about genetic structure of the region. Thus, cluster containing four *Cytochrome P450* genes was found in the interval between *wapl* and *pn* loci (Gandhi *et al.*, 1992; Frolov and Alatortsev, 1994). Recently the *Vinculin* (*Vinc*) gene was described between the *2Ea* and *pcx* loci (Alatortsev *et al.*, 1997).

Current arrangement of genetic loci in the *Pgd-K10* interval is shown in Figure 1.

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- Pgd - l90* - wapl* - P450* - pn - 2Ea* - Vinc* - pcx - kz - K10 -

Figure 1. Arrangement of genetic loci in the *Pgd-K10* region of the *Drosophila X* chromosome. The orientation is from centromere-distal (left) to centromere-proximal (right). Added or changed loci are marked by asterisks (see text).