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A different level of *X*-chromosomal transcription in an In(1)BM^2 (reinverted) strain and in its hyperploid derivatives resolves an *X*-coded regulatory activity for dosage compensation in *Drosophila*

A. S. MUKHERJEE AND MITA GHOSH

Genetics Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

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Summary

The transcriptional competence of the *X*-chromosome of a mutant strain of *Drosophila melanogaster*, [in(1)BM^2 (reinverted)], and of hyperploid derivatives with different additional segments of the *X*-chromosome has been examined. The single *X* in the mutant male shows twice as much puffiness and RNA synthesis as does that in the normal male, revealing a level of *X*-coded activity in addition to the normal male and female levels. Feulgen cytophotometry reveals no duplication of DNA content in the mutant *X*. When duplication for the segments 1A-3E, 9A-20F, 11A-20F and 16A-20F of the *X*-chromosome are combined in the male with the mutant chromosome, the super-hyperactivity of the mutant *X* is completely abolished. In combination with the B^s. *Y* duplication, which contains 16A7-B2, the two-fold activity is also completely suppressed.

The mutant chromosome can appear in three discrete manifestations, namely, highly flabby, intermittently flabby and normal, suggesting a leaky nature of the mutant. The effect is also temperature-sensitive. Our results suggest that there may be a modulator gene complex (M$^+$) in the 16A7-B2 region as well as regulators elsewhere on the *X*, which in combination influence the hyperactivity of the male *X* in *Drosophila*. We suggest that the In(1)BM^2 (reinverted) chromosome carries a hypomorphic mutation of M$^+$(M^m). The results presented here and earlier data on various *X*-chromosomal and autosomal hyperploids are discussed in the light of a model for dosage compensation in *Drosophila*.

Introduction

The hyperactivity of the *X*-chromosome in male *Drosophila* has been soundly established (Mukherjee & Beermann, 1965; Korge, 1970; Maroni & Plaut, 1973*a*, b). That it is a feature of most *Drosophila* species (Mukherjee & Chatterjee, 1976; Lakhotia & Mukherjee, 1972; Lakhotia, 1971) and applies to the individual organism as a whole and not just to polytene chromosomes has also been well documented (Belote & Lucchesi, 1980 *a*, *b*). It is now generally accepted that this hyperactivity of *X*-chromosomal genes in males is the transcriptional manifestation of the dosage compensation of *X*-linked genes (Stewart & Merriam, 1980; Baker & Belote, 1983).

Transcriptional as well as translational assays of *X*-coded products of *Drosophila melanogaster* have revealed that there are usually only two levels of *X*-coded activity, those of the normal male and female (1*X*2A and 2*X*2A vs. 3*X*3A). Yet, at least five sub-levels of transcription can be identified if the analysis is extended to flies with abnormal genotypes, e.g. 3*X*2A, 2*X*3A and 1*X*3A. Among flies with normal and abnormal genetic constitution, metafemales (3*X*2A) have the least and metamales (1*X*3A) have the highest activity per *X*-linked gene copy. While analyses of transcription and *X*-coded enzyme activity in various euploids and hyperploids have lent support to a model dependent only on the activity of autosomally coded positive regulatory factors (Lucchesi, 1973; Maroni & Lucchesi, 1980), analyses of sex lethal genes (*see* review by Baker & Belote, 1983) have clearly shown that *X*-coded activity is directed by an interplay of *X*-linked and autosomal regulatory loci (see Discussion). In a preliminary communication, we reported a mutant strain, In(1)BM^2 (reinverted) of *D. melanogaster* (Majumder *et al.* 1978; Ghosh & Mukherjee, 1983), in which a sixth level of transcription has been recognized, being the highest level so far identified. In this mutant, the *X*-chromosome is frequently 1·5 to 2 times as puffy as the *X* in a normal male, but shows a mosaic expression ranging from extremely puffy throughout (flabby) to intermittent flabby (intermediate) to nearly normal throughout (normal). The mutant phenotype is temperature-sensitive (Ghosh, Banerjee & Mukherjee, 1985). In this communication, we present the results of our assays of *X*-chromosome transcription in this mutant, and also in hyperploid derivatives in

which the mutant *X* has been combined with proximal and distal duplications. Analyses of these results provide us with important clues to the existence of *X*-chromosomal regulatory loci and their mode of function.

2. Materials and Methods

(i) Drosophila *stocks*

The wild-type strain Oregon R^+, the mutant strain, In(1)BM^2 (reinverted, to be designated as *rv* mutant), the translocation stocks, T(*X*; *Y*)B10, T(*X*; *Y*)B29, T(*X*: *Y*)B36, T(*X*; *Y*)J8, T(*X*; *Y*)J2 and T(*X*; *Y*)B44, and $yf/+/B^s Y.y^+$; $y^2\ w^i\ ct^6\ f/B^s Y.y^+$ were used for the present investigation. The protocol of genetic crosses used to generate different duplications of *X*-chromosomal segments is described by Stewart & Merriam (1975). The details of the break points and the markers (B^s or y^+) are given in Table 1 (see Stewart & Merriam, 1975; Maroni & Lucchesi, 1980). The $B^s . Y$ (Bar of Stone) is an altered *Y* chromosome in which the segment 16A7–B2 of the *X* is attached to the long arm of the $Y(Y^L)$. The phenotype of a male containing the normal *X* and $B^s . Y$ is manifested as strip Bar eye. The details of the stocks are given in Lindsley & Grell (1968). The male-specific lethal mutant, mle^{ts}, has been used for comparison only and therefore used only in Table 4 in a generalized way as mle^{ts} (for all alleles of mle^+ and msl^+).

Adults and all development stages were reared at 18 ± 1 °C, as this temperature appears to be permissive for expression of the *rv* mutant.

The *X*-chromosome in the mutant strain, In(1)BM^2(*rv*), is derived from an inversion stock in which the segment 16A1-20F of the *X*-chromosome (break points are at 15F-16A1 and at 20D-F) was inverted (Majumder *et al.* 1978). In the *rv* mutant the segment has been reinverted to its original sequence and no change in band pattern is detectable. However, frequently ectopic pairing between 15F-16A1 and the β-heterochromatin at 20E-F has been observed. From this we believe that some β-heterochromatin material might be transposed to 15F-16A1 consequent to reinversion.

In(1)BM^2(*rv*) strains hyperploids for different segments of the proximal and distal parts of the *X*-chromosome were generated by appropriate crosses between the *rv* mutant and particular translocation- or duplication-bearing males. The following hyperploid male genotypes could be generated: *rv*/16A-20F, *rv*/9A-20F, *rv*/11A-20F, *rv*/1A-3E and $rv/B^s . Y$. The *rv*/11A-20F and *rv*/16A-20F genotypes include an additional dose of the 16A7-B2 region (carried by $B^s . Y$). The *rv*/1A-3E hyperploid does not carry an additional 16A7-B2 region. Since the translocation strain T(*X*; *Y*)B10 carried a free *Y*-chromosome, the hyperploid *rv*/16A-20F might have an additional *Y*-chromosome. However, the presence of the *Y* slightly decreases the overall expressivity of the flabby character by the *rv* mutant, and hence does not interfere with our results (Ghosh, unpublished data, D. Bose, unpublished data). All hyperploids manifested themselves as phenotypic males. The presence of the duplications in these hyperploids was confirmed cytologically. *rv*/1A-5C and *rv*/1A-8C genotypes could not be obtained.

(ii) *Cytological preparation*

Salivary glands from pre-sexed mature third instar larvae were excised in *Drosophila* Ringer solution at pH 7·0, fixed in acetomethanol, stained in a mixture of aceto-carmine and aceto-orcein and squashed in a drop of lacto-orcein. To verify the age of larvae, a few active puffs, characteristic of the specific stage, were used as landmarks (Ashburner, 1972).

(iii) *Autoradiographic monitoring of RNA synthesis*

Mature late third instar male and female larvae (approximately 90 h after hatching) of Oregon R^+, of the *rv* mutant and of the different hyperploid males were sacrificed. The excised glands were incubated in [^{3}H]uridine (conc. 200 μCi/ml, sp. activity 12700 mCi/mM; obtained from BARC, Bombay) for 10 mins. Labelled salivary glands were fixed and squashed preparations of chromosomes were made and processed for autoradiography with Kodak AR10 stripping film as described by Lakhotia & Mukherjee (1969). The exposure time was 18 days. The number of

Table 1. *Genetic and cytological information on the translocations used*

Translocations used	Break points		Markers selected for duplications	Number of 16A expected in the duplications		
	X	*Y*		*X*	*Y*	Total
T(*X*; *Y*) B29	3E	L	y^+	1	—	1
T(*X*; *Y*) B36	5C	S	B^s (did not survive)	1	1	2
T(*X*; *Y*) J2	9A	S	y^+	2	—	2
T(*X*; *Y*) B44	11A	L	B^s	2	1	3
T(*X*; *Y*) B10	15EF	L	B^s	2	1	3

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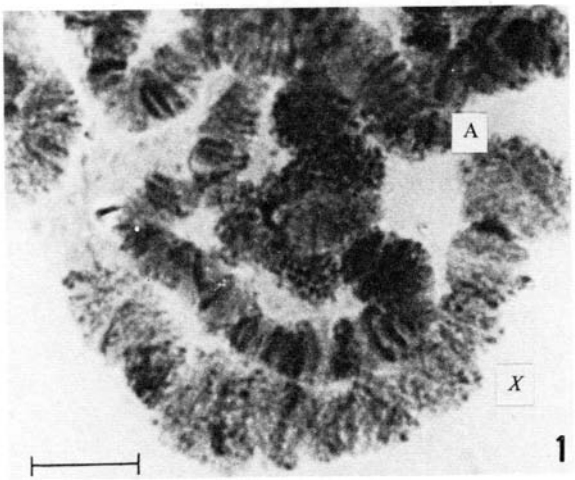

Fig. 1. Salivary gland chromosomes of In(1)BM² (*rv*) male showing the highly inflated stumpy *X*-chromosome (flabby). Bar in all Figures represent 10 μm.
X = *X*-chromosome, A = autosome.

silver grains over the *X*-chromosomal segments (1A-20A) and that over the autosomal segments (56A-60F: Chromosome 2R) were scored from different nuclei and the ratios (*X*/2R) of silver grain number were used for the evaluation and assessment of the relative transcriptional activity of the regions of the *X*. The labelling over the *X*-chromosomal segments outside the duplication as well as that over the 56A-60F region of 2R in each nucleus served as internal controls.

(iv) *Cytophotometry*

For relative quantitation of DNA, chromosomal squash preparations after fixation were stained by the Feulgen–Rossenbeck technique (Stowell, 1945). The preparations were scanned only when there was no overlying cytoplasm and no twisting of the chromosomes concerned. The transmittance values of a specific segment of the *X* (1A-2EF) and of the autosomal segment (58F-60F) were obtained with an MPV II (Leitz) cytophotometry system in conjunction with a 547 nm interference band filter attached to an Orthoplan (Leitz) microscope. The transmittance values were transformed into integrated absorbance (AE), using the two area-one wavelength method as described by Garcia & Iorio (1966). This transformation procedure eliminates the distributional error as far as practicable.

Photomicrographs have been taken using a Zeiss Photomicroscope III, usually with a 100× oil immersion objective.

3. Results and Discussion

As reported earlier (Majumder *et al.* 1978), the *X* chromosome in the larval salivary gland of an In(1)BM² (*rv*) male (Figs. 1, 2*a*) is 1·5–2·0 times as

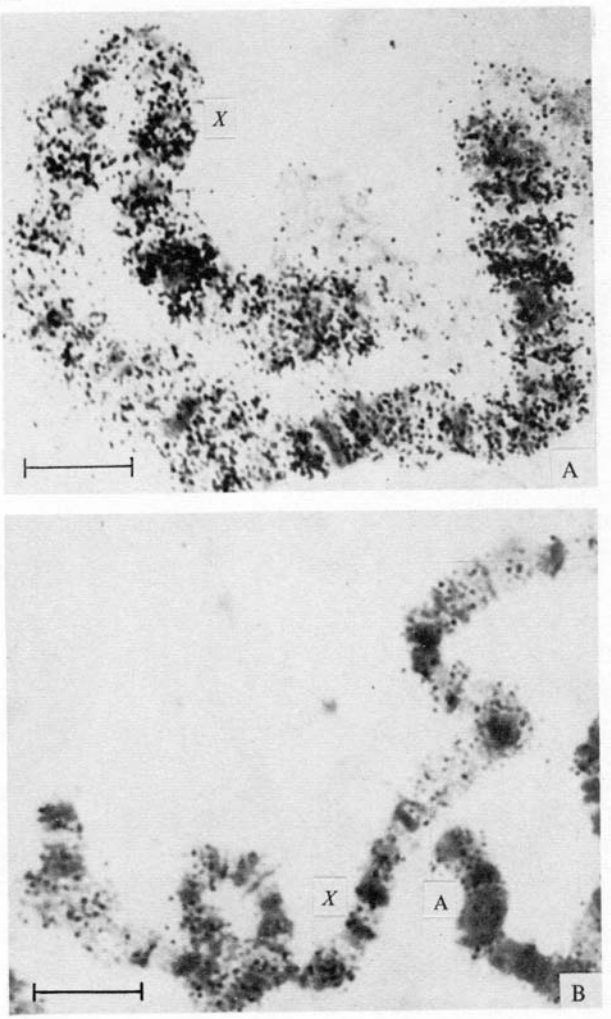

Fig. 2. Autoradiograms showing [³H]uridine labelling on the flabby *X*-chromosome of (A) In(1)BM² (*rv*) male and (B) Normal (Oregon R⁺) male.

puffy as that in Oregon R⁺ male (Fig. 2*b*). The *X*-chromosome in the homozygous female of the same mutant strain is not different from that in the Oregon R⁺ female. [³H]uridine autoradiograms reveal that the *X*-chromosome in the mutant male has a visibly higher labelling density (Fig. 2). The superactive *X*-chromosome in the nuclei of In(1)BM² (*rv*) males manifests itself in three configurations, viz. (*a*) extremely puffy (termed flabby, Fig. 3*a*), (*b*) intermittently puffy and non-puffy (intermediate, Fig. 3*b*) and (*c*) normal male-like (termed normal, Fig. 3*c* as compared to wild type, Fig. 3*d*) in the proportion of approximately 2:1:1, respectively. The three types of structure, i.e. flabby, intermediate and normal, of the *rv* mutant male *X*-chromosomes are observed in the same salivary gland, exhibiting mosaicism.

Earlier analyses of transcription as well as G6PD and 6PGD enzyme levels in males hyperploid for different segments of the *X*-chromosome have yielded interesting information (Stewart & Merriam, 1975; Maroni & Lucchesi, 1980). The common finding in these studies was that the segments involved in the

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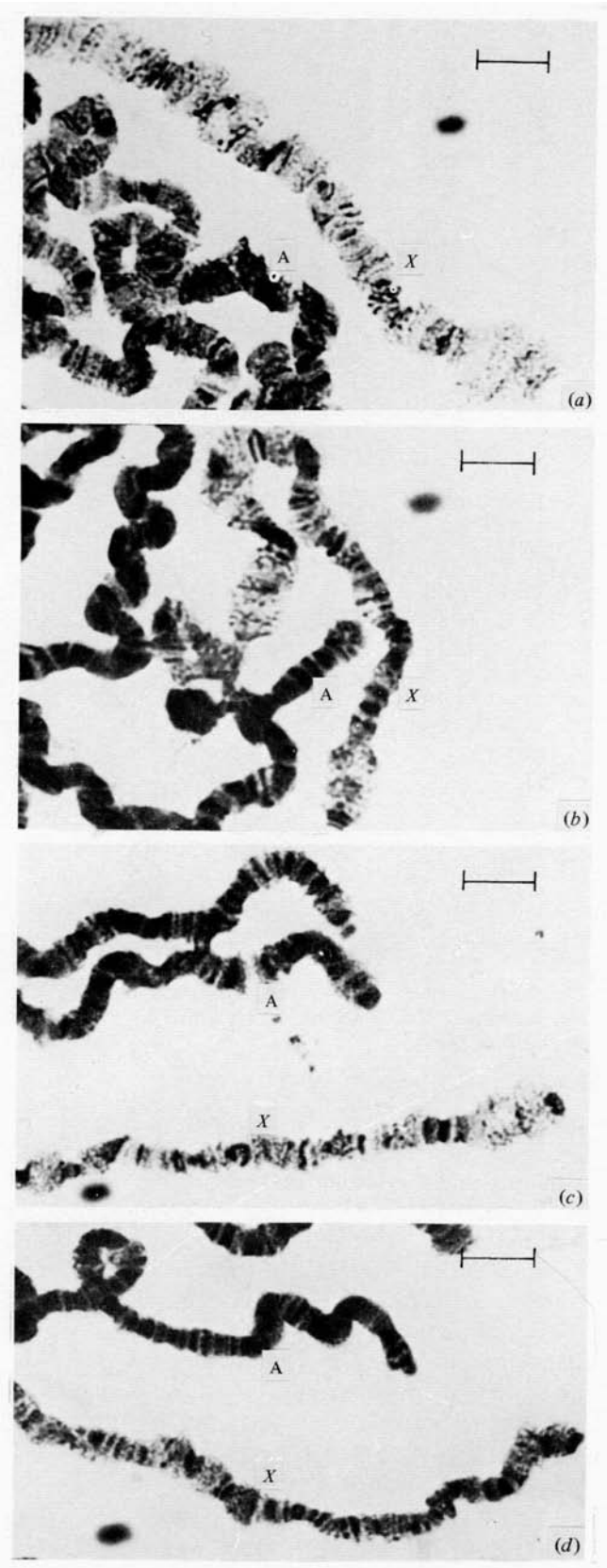

Fig. 3. Salivary gland chromosomes showing the three configurations of the *X*-chromosome of In(1)BM² (*rv*) male. (*a*) Flabby *X*-chromosome – an extreme expression, with only a few condensed bands; (*b*) Intermediate *X*-chromosome (partly flabby, partly normal) and (*c*) Normal *X*-chromosome showing normal width as in (*d*) the normal (wild type) male.

hyperploidy were individually hyperactive over their entire length (ranging from 10 to 80% of the *X*-chromosome). However, two findings in these reports were also extremely significant, viz. (*a*) hyperploidy larger than 1·25 *X*-chromosomal length could not be generated when the duplication was from the distal end, and (*b*) hyperploids for proximal segments, almost as large as the entire *X* could be generated without difficulty. Maroni & Lucchesi (1980) suggested that the failure to generate hyperploids with distal duplications of the *X* might be due to their lack of an additional copy of haplo-insufficient (in the hyperploid male) segment 15F-17A. Similarly, our studies of the *rv* chromosome reveal that (*a*) hyperploid males with an *rv* chromosome and all different proximal segments could be successfully generated, at least up to the 3rd instar larval stage, (*b*) hyperploid males could not be generated with a distal segment larger than 1A-3E. Why hyperploid with 1A-3E (in the absence of B^s) could be generated but not with larger segments remains conjectural. The relative [³H]uridine labelling intensity indices measured by the ratio of the number of silver grains on the *X*-segment (1A-20A) to that on the 2R segment (56A-60F) are 5·09, 4·82 and 4·38 respectively, in the three classes as compared to 3·63, 3·89 and 3·76 in normal males, normal females and mutant females, respectively (Table 2).

The labelling density frequency profile for the *X*/A grain ratio presented in Fig. 4 reveals that the range of labelling density is usually between 3·0 and 6·0 in normal males and females, while it is shifted toward the higher density especially for flabby and intermediate classes of the mutant male. The modal values for normal males, normal females, *rv* mutant females and flabby, intermediate and normal types of *rv* mutant males are 5·0, 5·0, 4·5, 5·5, 5·5 and 5·5 respectively.

It may be mentioned that the expressivity of the flabby *X* in this mutant strain varies, depending upon the rearing temperature. Both at lower (approximately 16–18 °C) as well as higher (28 °C) than normal rearing temperatures (i.e. 23 °C), the frequency of flabby *X*-chromosomes is considerably increased, e.g. below 23 °C (Ghosh *et al.* 1985) or decreased, e.g. above 23 °C (Bose *et al.*, unpublished).

In order to find out whether the extreme puffiness of the *X*-chromosome of *rv* mutant male larvae is due to increased DNA content, integrated absorbance values (with a 547 nm band filter for Feulgen preparations) were compared. Our results reveal that respectively the single *X* chromosomes of the normal male and the mutant male are not significantly different in their DNA content (AE_X/AE_A ratios are 0·52 and 0·60).

Our attempts to generate hyperploids with Dp (1A-5C) and Dp (1A-8C) have not been successful, possibly because they did not bear the 15F-17A segment as a duplication. Among the hyperploids with proximal segments, those with Dp (11A-20F) and Dp (16A-20F) had an additional copy of 16A-B as B^s was

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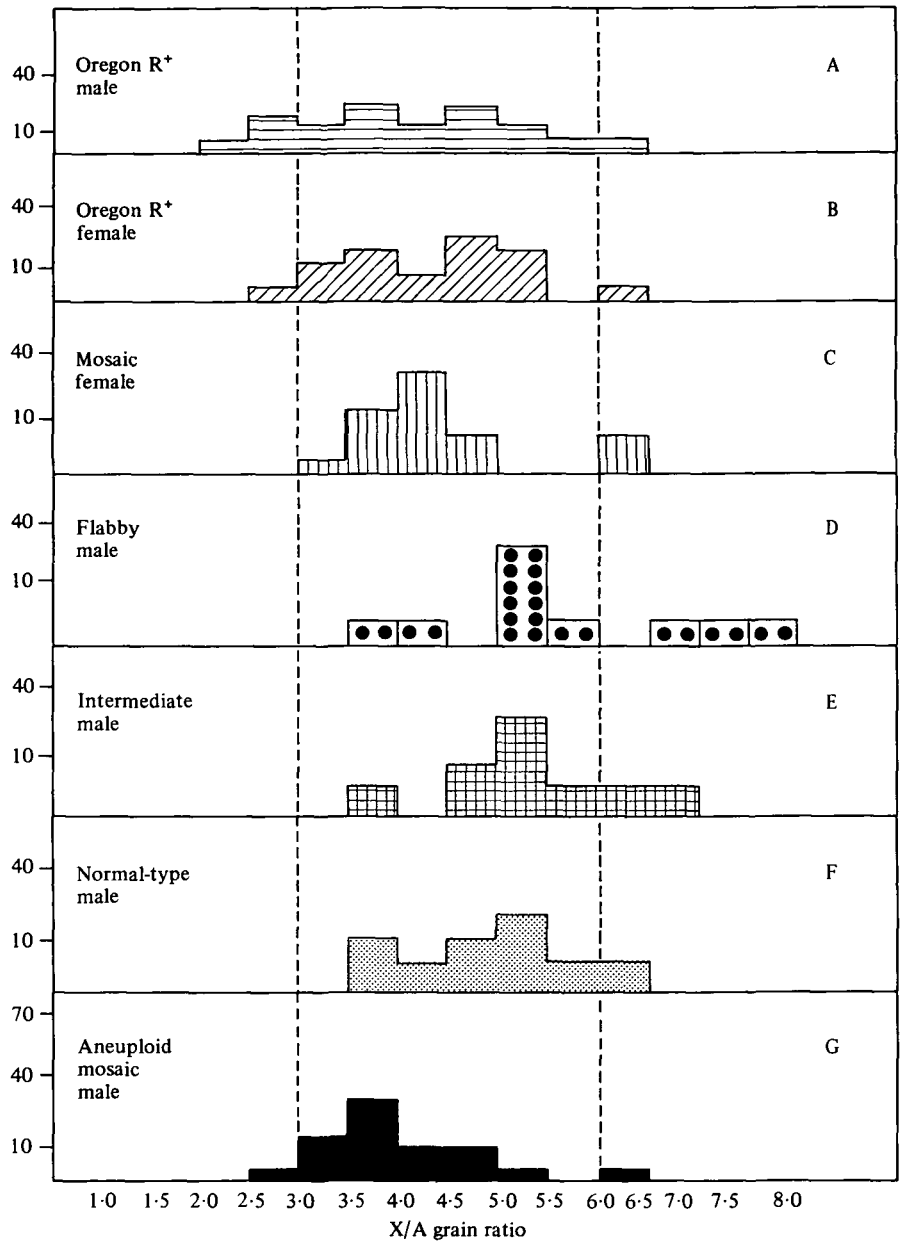

Fig. 4. [^{3}H]uridine labelling density profile showing the frequencies of the grain ratios (X/A) in Oregon R$^+$ male (A), Oregon R$^+$ female (B), In(1)BM^2 (*rv*) female (C), In(1)BM^2 (*rv*) male – flaby (D), Intermediate (E), normal (F) and *rv*/aneuploid male (G). The dotted lines indicate the range of variation relative to the Oregon R$^+$ male and female and the direction of shift in the mutants. The profiles for the flabby (D), intermediate (E) and normal (F) classes show clear shift of the *mode* towards the higher value.

Table 2. [^3H]*uridine labelling density on the* X*-chromosome of different genotypes and sexes as measured by* X/*A grain ratio*

Strain and sex	Number of chromosomes scored	Grain ratio ± S.E. 1A-20A 56A-60F	1A-15F 56A-60F	16A-20A 56A-60F
Oregon R$^+$ (male)	20	3·63 ± 0·24	3·00 ± 0·20	0·65 ± 0·06
Oregon R$^+$ (female)	20	3.89 ± 0.21	3.10 ± 0.18	0.79 ± 0.05
In(1)BM^2 (*rv*) female	20	3·76 ± 0·12	3·03 ± 0·12	0·72 ± 0·04
In(1)BM^2 (*rv*) flabby (male)	20	5·09 ± 0·39**	3·82 ± 0·32**	1·27 ± 0·08**
In(1)BM^2 (*rv*) intermediate (male)	20	4·82 ± 0·39**	3·54 ± 0·15**	1·27 ± 0·11**
In(1)BM^2 (*rv*) normal (male)	20	4·38 ± 0·27*	3·24 ± 0·19**	1·13 ± 0·11**
In(1)BM^2 (*rv*)/Dp (16A-20F) (male)	20	3.56 ± 0.18	2.74 ± 0.13*	0.82 ± 0.06**

* $P < 0·05$; ** $P < 0·01$, when compared with Oregon R$^+$ male.

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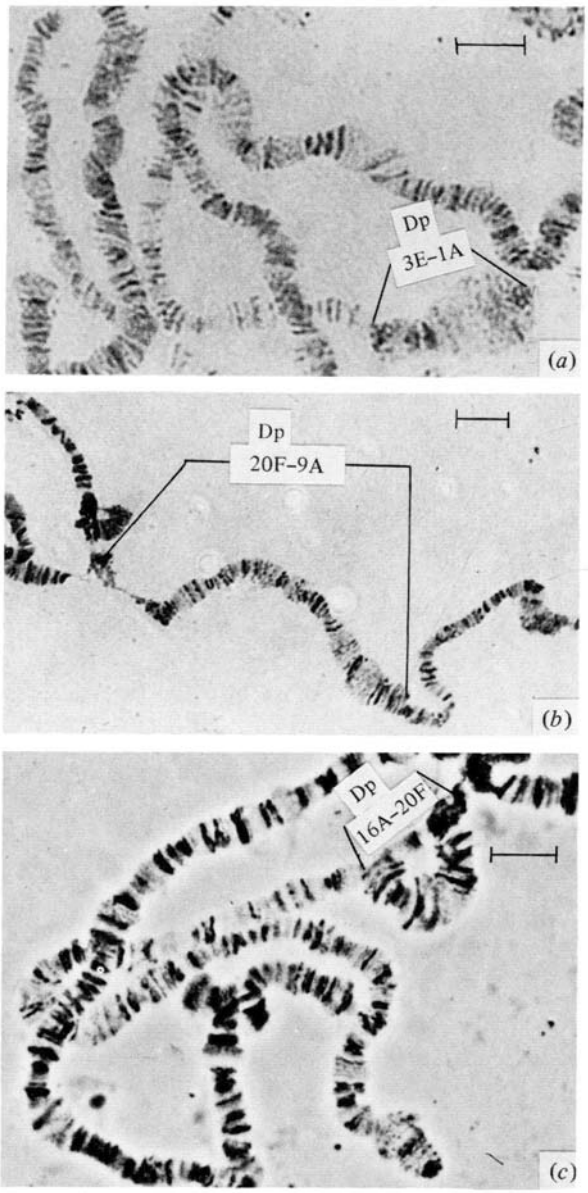

Fig. 5. Photomicrographs of segmental aneuploid *X*-chromosomes in In(1)BM² (*rv*) male, duplicated for the segments – (*a*) 1A-3E, (*b*) 9A-20F and (*c*) 16A-20F. The regions 1A-3E, 9A-20F and 16A-20F are bracketed in the respective figures. Note the increase in width of the aneuploid segment (dosage effect).

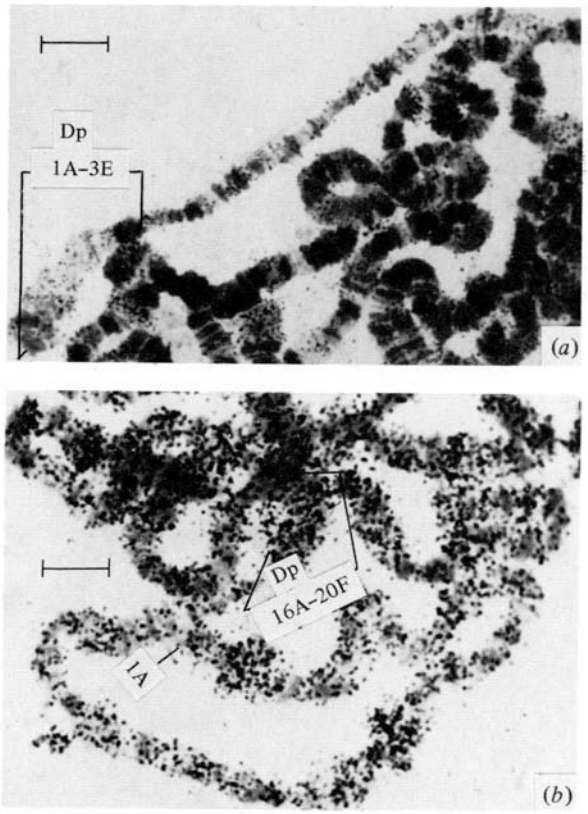

Fig. 6. Autoradiograms showing [³H]uridine labelling on the *X*-chromosomes of *rv*/aneuploid male with duplications (bracketed), (*a*) duplication in the distal segment 1A-3E and (*b*) duplication in the proximal segment 16A-20F. Note while the duplicated regions show dosage effect, the haplo segments reveal a near normal male level grain density (compared to the diplo-autosomal level of grain density).

included in the complements (see Table 1) and all yielded healthy larvae, and often developed to adults. It is possible that hyperploids for distal segments could be generated if an additional piece of 16A-B (or of the haplo-insufficient region 15F-17A) were provided in the genome. We are working on this possibility. In all of the *rv* hyperploids examined, each of the diplo-*X* segments involved in the hyperploidy showed a morphology characteristic of that shown by the equivalent segment in a normal male (a dosage effect for hyperactivity) (Prasad, Duttagupta & Mukherjee, 1981; Prasad-Sinha & Mukherjee, 1985). The remaining part of the *X* containing only one homologue also showed uniformly normal male-like puffiness, being neither flabby nor at the lower level characteristic of a female (Fig. 5*a–c*). Similarly, in a DP(1A-3E)/In(1)BM² (*rv*) hyperploid, the haplo-*X* segment shows the normal male level of activity. The reduction in this case is suggestive of the existence of regulatory signals as proposed by Steinmann (1984) (since Steinmann's model assumes regulatory loci interspersed on the *X* whereas the modulator hypothesis proposes the existence of one master-type modulator gene complex, see later).

Autoradiograms of [³H]uridine incorporation over the polytene chromosomes of hyperploid mutant male nuclei revealed that the superhyperactivity (flabby condition) of the In(1)BM² (*rv*) chromosome was invariably abolished (fig. 6, Table 3). The grain count ratio (*X*/A) in the mutant male (1A-15F/56A-60F) was highly comparable to that for the Oregon R⁺ male. For 1A-20A/56A-60F, the grain number on the paired segments have not been halved as this may give distributional error. As expected, the paired hyperploid segments had twice as much activity as the haplo segment (i.e. it showed a dosage effect for hyperactivity), rather than showing a female level of transcription or a flabby type transcription level. This was true for all hyperploids with proximal segments of the

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Table 3. *Mean [³H]uridine grain incorporating (mean X/A grain ratio ± S.E.) on different segments of X-chromosome and autosome (56A-60F regions on 2R chromosome have been taken as autosomal segments)*

Strain and sex	*X* load	1A-20A[a] 56A-60F ± S.E.	1A-15F 56A-60F ± S.E.	1A-3E 56A-60F ± S.E.	11A-20A 56A-60F ± S.E.	9A-20A 56A-60F ± S.E.	16A-20A 56A-60F ± S.E.
Oregon R⁺ (male)	1	3·63 ± 0·24	3·00 ± 0·20	0·59 ± 0·04	1·65 ± 0·05	1·80 ± 0·02	0·65 ± 0·06
Oregon R⁺ (female)	2	3·89 ± 0·21	3·10 ± 0·18	0·67–0·05	1·79 ± 0·04	1·91 ± 0·05	0·79 ± 0·05
In(1)BM² (*rv*) (female)	2	3·76 ± 0·12	3·03 ± 0·15	0·66 ± 0·04	1·66 ± 0·03	1·76 ± 0·03	0·72 ± 0·04
In(1)BM² (*rv*) (male) (average of three classes)	1	4·77 ± 0·17	3·72 ± 0·14	0·80 ± 0·04	2·26 ± 0·04	2·40 ± 0·05	1·05 ± 0·07
In(1)BM² (*rv*)/Dp (1A-3E) (male)	1·15	3·72 ± 0·14	3·04 ± 0·14	0·69 ± 0·03	—	—	0·68 ± 0·03
In(1)BM² (*rv*)/Dp (9A-20F) (male)	1·60	3·86 ± 0·10	3·04 ± 0·12	—	—	2·00 ± 0·02	0·82 ± 0·02
In(1)BM² (*rv*)/Dp (11A-20F) [male(+ $B^s Y$)]	1·50	3·80 ± 0·11	3·00 ± 0·11	—	—	1·80 ± 0·04	0·80 ± 0·04
In(1)BM² (*rv*)/Dp (16A-20F) [male(+ $B^s Y$)]	1·25	3·56 ± 0·18	2·74 ± 0·13	—	—	—	0·82 ± 0·06

[a] Entire *X* complement minus the *β*-heterochromatin (20B-20F).

X-chromosome, as large as 9A-20F and as small as 16A-20F as well as with the distal segment 1A-3E (Fig. 6). Moreover, the In(1)BM² (*rv*) *X*-chromosome shows normal male activity when duplicated for B^s alone (B^s contains 16A7-B2, Lindsley & Grell, 1968) (Fig. 7). These results clearly show that while the mutant is leaky in the hemizygous condition in the hyperploid male, with at least one normal copy of 16A-B, each segment is transcribed at a level characteristic of the same segment in a normal male. In contrast, Maroni & Lucchesi (1980) found that while each of the diplo-*X* segments in a hyperploid – Oregon R⁺ male showed a nearly normal male level of activity, the haplo segments in those males showed lower than the haplo-*X* activity of the normal male. They attributed this lower activity for haplo segments in the hyperploid male as resulting from a pre-disposed constancy in the overall level of *X*-chromosome transcription. While Prasad *et al.* (1981) could not verify this, Prasad-Sinha & Mukherjee (1985) substantiated this finding in cellular mosaics for the aneuploid segment 9A-11A. The finding is not substantiated, however, in aneuploids containing such duplications which also include 16A-20F (Prasad-Sinha, unpublished). The results of the present investigation reveal that the haplo segment external to the hyperploid segment has a lower activity than the same segment of the mutant *X* has in the absence of a duplication; however, this reduced activity is close to that in the wild type (hyperactive) male *X* and not less. Therefore, this reduction in activity does not compensate the near double activity in the hyperploid region as might be predicted by the constancy hypothesis. The labelling density frequency profile presented in Fig. 4 (bottom) reveals that in the hyperploid males, there is only one profile (and little or no mosaicism) and the modal value of *X*/A is shifted toward the left to nearly 3·7.

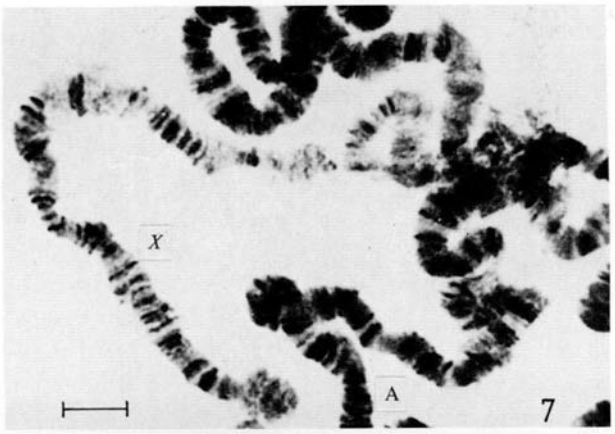

Fig. 7. Salivary gland chromosomes of In(1)BM² (*rv*) male with B^s. *Y* segment showing no flabby expression of the *X*-chromosome. The B^s. *Y* is not cytologically recognizable except the ectopic pairing (arrow).

To account for these results we propose that this tendency to normal activity of the *X* chromosome in In(1)BM² (*rv*) hyperploid males, in the presence of B^s or 16A-B from another source, is due to an inhibitory factor released from certain genes located on the *X*-chromosome (possibly interspersed on several regions) which are triggered by the action of a 'modulator' located at the region 16A-B and which might be responsible for the organizational status (i.e. template form) of the entire *X*-chromosome. This proposition has been discussed at length by Mukherjee (1982).

Several models have been proposed to explain dosage compensation in *Drosophila*. That proposed by Muller (1950) hypothesized a negative control system operating to reduce expression in the female. The competition model (Maroni & Plaut, 1973*a*, *b*; Schwartz,

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1973), on the other hand envisaged regulation by a positive activation mechanism to increase expression in the male. These models have been discussed at length by Stewart & Merriam (1980) as well as by Lucchesi (1977). Stewart & Merriam (1980) tested the two models by well designed experiments and concluded that neither could explain the regulation of male *X* hyperactivity or dosage compensation in *Drosophila*. They further suggested that modulation of gene activity by dosage compensation occurs in stepwise fashion and that in each step the difference of an entire *X* chromosome is recognized.

In a recent review, Baker & Belote (1983) have pointed out the need for an expanded model to explain the effects of the *Sxl*$^+$ (Sex lethal gene, 1:6E1-7B7), *da*$^+$ (daughterless, 2:31CD-F) and *msl*$^+$ (male lethal genes, autosomal, 2:36F7-37B8) loci with respect to sex determination and dosage compensation in *Drosophila*. As pointed out by them, many aspects of the observed data cannot be explained solely in terms of the *X*:A ratio (the primary level), rather one should consider the pathway by which information about the ratio is communicated to the autosomal sex specific lethals that determine terminal differentiation at the secondary level. In consideration of the results on enzyme activity in *X*-chromosomal segmental aneuploids and supersexes obtained by Lucchesi, Rawls & Maroni (1974) and Stewart & Merriam (1975), Baker & Belote (1983) commented that the control of dosage compensation may involve more than just the activity or inactivity of the *msl's* (male-specific lethals) and that there may be other levels of control that can finely tune the activity of the *X*-chromosome to give the range of observed levels of chromosomal transcription. Baker & Belote (1983) made two propositions to explain the initial determination of sex and dosage compensation: (1) That sex determination and dosage compensation have only two states, either male or female, and that a single irreversible early event determines both sex and *X*-chromosomal transcription level. (2) That the primary level of determination of sex and dosage compensation involves a combination or interaction of both the *X*:A ratio and maternally synthesized products present in the egg. As discussed by Baker & Belote (1983), their model also cannot provide a unified explanation for the activities obtained in metamales, metafemales and triploid intersexes on the one hand and segemental hyperploid males on the other. For this reason, and from the results obtained from earlier work including that presented here, we propose a model (*a*) that assumes an interaction between at least two determining regulatory products without the prior requirement for discrimination between the sexes and (*b*) that lends itself to a molecular explanation.

The model is represented in a schematic form in Fig. 8. The basic features of the model are as follows. (1) A modulator gene complex (M^+) is located within the segment 16A-B of the *X*-chromosome. (2) The product(s) of the *Sxl*$^+$ gene complex at 6E1-7B7 (*Sxl*; 1·0:19·2; Belote & Lucchesi, 1980*b*), after being itself modulated (by some early determining gene product such as that of *da*$^+$), monitors the *X*/A ratio and switches on M^+. (3) Each M^+ after its activation produces by itself and/or activates a number of regulatory loci (R) to produce, a quantum of inhibitor molecules the amount of which is determined by the extent of activation of M^+. (4) The inhibitor molecules interact stoichiometrically with enhancer molecules coded by activator loci on the autosomes (e.g. *mle*, *msl*, etc.); the quality and quantity of the unreacted inhibitor and enhancer molecules (i.e. free inhibitors and enhancers) determine the level of transcription. (5) There is an optimum range of the quanta of unreacted molecules that can be tolerated

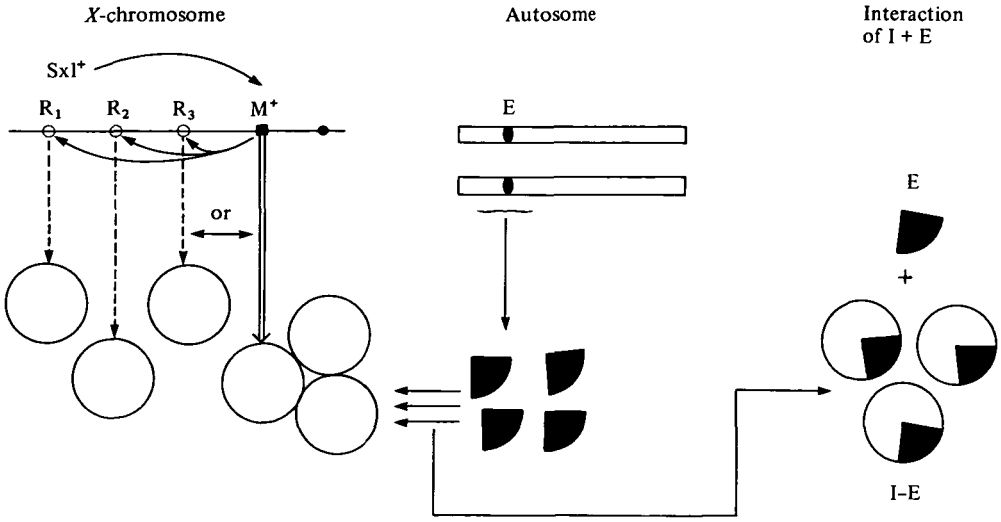

Fig. 8. Diagrammatic representation of the proposed model of events leading to hyperactivity. The sequential events are: (1) *Sxl*$^+$ after monitoring *X*/A ratio switches on M^+, (2) M^+ itself and/or through activation of certain regulatory loci (R_1, R_2, R_3) produces a defined quantum of inhibitor substance per genome, (3) The inhibitors interact stoichiometrically with the Enhancer signals from the autosomal activator loci, (4) The unreacted molecules of inhibitors or enhancers decide the hyperactivity or no hyperactivity.

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Table 4. *Assessment of Enhancer/Inhibitor ratio as visualized in the modulator hypothesis and the consequent gene activities in different genomic complements as expected on the basis of the equation and as observed*

	X/A complement	Sex phenotype	M/*Sxl* ratio	*Ratio of E/I* units	Total no. of E_u or I_u[a]	Expected activity per gene	Observed activity per gene
(1)	*XY* 2A	Normal male	1:1	4:3 (1·33)	$E_u = 1$	2	2
(2)	*XX* 2A	Normal female	2:2	2:3 (0·66)	$I_u = 2$	1	1
(3)	*XY* 3A	Metamale	1:1	2:1 (2·00)	$E_u = 3$	3	3
(4)	*XXY* 3A	Intersex	2:2	1:1 (1·00)	$I_u/E_u = 0$	< 1·5	< 1·5
(5)	*XXX* 3A	Triploid female	3:3	2:3 (0.66)	$I_u = 3$	1	1
(6)	*XXX* 2A	Metafemale	3:3	4:9 (0.45)	$I_u = 5$	0.7	0.33[b] (?)
(7)	*XY* 2A [+Dp16A (*B*^{s}. *Y*)]	Hyperploid (16A)	3:1	2:3 (0.66)	$I_u < 2$	1	1.0
(8)	*X* [In(1)BM^2 (*rv*)] *Y* 2A	Male (mutant)	1:1	2:1 (2·00)	$E_u = 2$	3	3.00±0.50
(9)	(*XX*) [In(1)BM^2 (*rv*)] 2A	Homozygous mutant female	2:2	1:1 (1·00)	$I_u/E_u = 0$ (or some I_u)	< 1·5	1·0
(10)	In(1)BM^2 (*rv*)/Dp 16A-20F (or 9A-20F) *Y* 2A (*B*^{s}*Y*)	Hyperploid mutant male	3:1	4:3 (1·33)[c]	$E_u < 1$	< 2·0	< 2·0
(11)	*XY* 2A (*mle/mle*)	*mle* male	1:1	2:3 (0·66)	$I_u = 1$	1·0	1·0

Assumptions: 1. Each M$^+$ produces 3 units of inhibitors. 2. Each E$^+$ produces 2 units of activators. 3. Each M^m produces 2 units of inhibitors. 4. Each *mle*-mutant enhancer produces 1 unit of activator. 5. An *E/I* ratio of 1·33 produces 2·0 equivalents of product in the male. Other expected amounts have been calculated on that ratio equivalent [that is each ratio was multiplied by (2·0+1·33 = 1·5) 1·5 to obtain the expected activity per cell].

[a] E_u, Unreacted enhancer; I_u, unreacted inhibitor.

[b] Only this observed value does not correspond accurately with the expected value.

and that decides the normal viability of the male or female (see Baker & Belote, 1983).

Our model implies that there must be a quantitative relation between the number of M$^+$ loci and the number of *Sxl*$^+$ loci, and between the number of M$^+$ loci and the number of R loci in order to realise a viable male or female level of transcription. For example, if there are two copies of M$^+$ but only one of *Sxl*$^+$ the activation of M$^+$ will be subnormal [e.g. in 1*X* (+Dp 16A-20F) 2A]. Consequently, even if the number of R loci is increased, unless the dose of *Sxl*$^+$ is also increased, the activity (i.e. number of free enhancer molecules) remains more or less like that of the normal male. The balance of the three regulatory systems, i.e. *Sxl*$^+$:M$^+$:R$^+$, therefore, decides the level of transcription (and perhaps viability). As proposed in the model, the modulator (M$^+$), by itself or by activating the R loci, produces a specified quantum of the inhibitor signal (*I*) per haploid complement in a normal male or female. This quantum is taken as 3 units for the sake of discussion. The autosomal activators produce, again arbitrarily, 2 units of enhancers (*E*) per haploid set. Thus 4 units of enhancers could be produced per genome. In the normal male (1*X*2A), if 3 units of *I* would bind stoichiometrically with 3 units of *E*, one unreacted *E* (designated as E_u) would be left. This would result in hyperactivity of the male *X*, i.e. a male level transcription. In the normal female (2*X*2A) there would be 6 units of *I* and 4 units of *E*. The result that would follow would be a female level transcription due to an inhibitory effect on transcription by the unreacted *I*'s (i.e. I_u) (Table 4).

On the basis of these hypothetical data the ratio of E:I in males is 4:3 (= 1·33) and in females it is 2:3 (= 0.66). These two ratios lead to activities per gene of 2 and 1, respectively (normalised total activity of the gene in a female). This relation then generates a constant (k) equivalent to 1·5 for the equation, $q = E/I \times [k]$, where, q is the activity per gene. The constant may be determined by the M$^+$/*Sxl*$^+$ ratio which is 1:1 in all normal euploids, but may be different in certain aneuploids. The relation is translated into the expected and observed activity in Table 4. It may be noted that while the *E/I* ratio decides the potential level of transcription, the absolute level of unreacted inhibitor or repressor actually determines the genetic activity through appropriate molecular kinetics. It is evident that the observed values for all euploids and some aneuploids fit with the expected values of activity per gene. The implications of the equation to segmental aneuploids with otherwise normal male and female genomic complements are under investigation.

Now, if a mutation occurs in the modulator gene complex, the inhibitor signal may either not be produced at all (a null mutant), be produced in less amount (a hypomorph) or may be defective (an antimorph or neomorph). We suggest that in In(1)BM^2 (*rv*) the modulator gene complex (M$^+$) is mutated to a hypomorph for the reason that it appears to be leaky in males and in two doses (females) gives normal female activity. As shown in Table 4, if we assume that the hypomorph M^m produces only two units of the inhibitor instead of three, then in an In(1)BM^2 (*rv*) male, two units of *I* would react with two of the four units of *E* leaving 2 units (twice as much as in the wild-type male) of unreacted enhancers (E_u). This results in four units of gene activity per *X*-chromoso-

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mal gene. Similarly in an In(1)BM^2 (*rv*) homozygous female with 2 doses of *Sxl*$^+$ and two of M^m, 4 units of inhibitors (2 for each M^m) would be produced. These four units would react with all four units of enhancers, leaving either no unreacted enhancer or inhibitor or a little amount of inhibitor (assuming different levels of leakiness). This would result in the same gene activity as in the normal female (Table 4).

If we now examine the data In(1)BM (*rv*)/Dp 16A-B hyperploids (which contain the mutated M and 16A-B duplication as well) in the light of this model we obtain the following results. M^m/M$^+$ [M$^+$ is present in Dp (16A-B)] should produce 5 units of inhibitors and the two doses of autosomal activators should produce 4 units of enhancers. The reaction would leave 1 unit of the inhibitor unreacted that should then lead to a level of activity more like that of the normal female. The observed level is, however, close to that of the normal (wild type) male. This discrepancy could be explained if the signal from one dose of *Sxl*$^+$ can not activate more than one modulator, in which case only three *I* signals will be produced instead of 5, leaving one unreacted enhancer as in the normal male. The model fits reasonably well with the data obtained from metamales (*XY*3A), triploid intersexes (*XXY*3A) and triploid females (*XXX*3A) as well as the enhancer defective mutants (*mle*^{ts}, *msl*, etc.) (Table 4). However, the results on G6PD and 6PGD levels in metafemales (3*X*2A) obtained by Lucchesi, Rawls & Maroni (1974) and Stewart & Merriam (1975), and the results of Chatterjee (1985) on *in situ* transcription, do not accurately fit with this model. For some reason, not well explained, Faizullin & Gvozdev (1973) reported a different result with metafemales, which is more compatible with our model.

During the studies of the past decade at least eleven regulatory genes that function at some stage during the establishment of sex and/or dosage compensation have been recognized in the *Drosophila* genome (Baker & Belote, 1983). Some of them act early in development and function in both sex determination and dosage compensation (*Sxl*, *da* and *her*), others may function either in establishing the sex alone (*tra*, *tra*-2, *dsx*, *ix*) or dosage compensation alone (*mls*, *msl*-1, *msl*-2, *msl*-3). Leaving aside the intricacies of the mechanism of action of these genes, it may be mentioned that both sex determination and dosage compensation may require a common primary regulatory event followed by a series of independent secondary regulatory events. Further studies of different combinations of the different regulatory mutations will be necessary in order to elucidate the sequence of events determining hyperactivity. We have been able to resolve the action of the Modulator in more detail in normal 1*X*2A and 2*X*2A backgrounds and have located it by genetic analysis. These results will be published elsewhere. Our preliminary results on the assay of activity of proteins (105000 *g* supernatant) fractionated by gel filtration obtained from the *rv* mutant male and female have lent support to a molecular explanation of the model (Mukherjee & Ghosh, in preparation).

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