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Dosage compensation and sex determination in *Drosophila:* mechanism of measurement of the X/A ratio+

RAGHAVENDRA GADAGKAR*, VIDYANAND NANJUNDIAH***, N. V. JOSHI* and H. SHARAT CHANDRA* **

* Centre for Theoretical Studies, ** Microbiology and Cell Biology Laboratory and ICMR Centre for Genetics and Cell Biology, Indian Institute of Science, Bangalore 560 012, India and *** Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay 400 005

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Abstract. We propose a molecular mechanism for the intra-cellular measurement of the ratio of the number of X chromosomes to the number of sets of autosomes, a process central to both sex determination and dosage compensation in *Drosophila melanogaster.* In addition to the two loci, *da* and *Sxl,* which have been shown by Cline (*Genetics,* **90**, 683, 1978)and others to be involved in these processes, we postulate two other loci, one autosomal (ω) and the other, X-linked (π). The product of the autosomal locus *da* stimulates ω and initiates synthesis of a limited quantity of repressor. *Sxl* and π ,both of which are X-linked, compete for this repressor as well as for RNA polymerase. It is assumed that *Sxl* has lower affinity than π for repressor as well as polymerase and that the binding of polymerase to one of these sites modulates the binding affinity of the other site for the enzyme. It can be shown that as a result of these postulated interactions transcription from the *Sxl* site is proportional to the X/A ratio such that the levels of Sxl^+ product are low in males, high in females and intermediate in the intersexes. If, as proposed by Cline, the Sxl^- product is an inhibitor of X chromosome activity, this would result in dosage compensation. The model leads to the conclusion that high levels of Sxl^+ product promote a female phenotype and low levels, a male phenotype. One interesting consequence of the assumptions on which the model is based is that the level of Sxl^+ product in the cell, when examined as a function of increasing repressor concentration, first goes up and then decreases, yielding a bell-shaped curve. This feature of the model provides an explanation for some of the remarkable interactions among mutants at the *Sxl, da* and *mle* loci and leads to several predictions. The proposed mechanism may also have relevance to certain other problems, such as size regulation during development, which seem to involve measurement of ratios at the cellular level.

Keywords X-Chromosome transcription; sex-lethal mutations; maternal effect; RNA polymerase; size regulation.

Introduction

Bridges (1925) has shown that in *Drosophila melanogaster* the sexual phenotype is determined by the ratio of the number of X chromosomes (X) to the number of sets of autosomes (A). This X/A ratio, or Bridges' ratio, also regulates the rate at

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which most X-linked genes are transcribed (Lucchesi, 1973; Maroni and Plaut, 1973; Stewart and Merriam, 1978; Chandra, 1979). As a result, in flies with an integral number of chromosomes, the level of activity of enzymes coded by X-linked genes is proportional to the number of copies of the structural gene divided by the Bridges' ratio (Chandra, 1979). The end result of this regulatory process, known as *dosage compensation,* is that the phenotype resulting from two doses of a given X-linked gene in the female (AAXX) is equal to that resulting from one dose in the male (AAXY). This is the consequence of the single X chromosome in the male being transcribed at rougly twice the rate as each of the two chromosomes in the female. *D. melanogaster* is able to sustain wide variation in X/A ratios, and it has therefore been possible to show that dosage compensation operates over a variety of chromosome constitutions. Since both the sexual phenotype and dosage compensation appear to be cell-autonomous properties (Bridges, 1930; Lakhotia and Mukherjee, 1969), an intriguing feature of these two phenomena is the mechanism by which the X/A ratio is assessed within cells. Mutations which interfere with the capacity to measure the X/A ratio and, as a consequence, affect dosage compensation or sex determination, might provide insight into the molecular mechanisms involved in these processes.

Cline (1978, 1980) has made an elegant study of the following mutations which appear to fulfil such a purpose. (i) Daughterless (*da*) is a temperature-sensitive autosomal recessive (2-41.5) (Bell, 1954; Cline, 1976). Homozygous females leave behind only male offspring because the daughters die during embryonic development. Daughters can be rescued from *da/da* mothers following early injection of wild type (da^+) egg cytoplasm (Bownes *et al*., 1977), suggesting that the daughterless phenotype is caused by the absence of some diffusible product coded for by the da^+ locus, (ii) Sex-lethal, male-specific (Sxl^{M1}), is an X-linked mutation (1-19.2) (Cline, 1978), lethal to males and, curiously, also a dominant suppressor of *da.* (iii) Sex-lethal, female-specific, (Sxl^{F1}), is also X-lined (Muller and Zimmering, 1960; Zimmering and Muller, 1961), 0.007 recombination units away from Sxl^{M1} (Cline, 1978). It was isolated as a dominant mutation but later studies have shown that it normally behaves as a recessive and that its occasional dominant character is dependent on some undefined elements of the genetic background and on certain environmental conditions (Cline, 1978).

Cline has shown that the effects of these mutations can be explained on the following bases. (a) A maternal factor is produced by da^+, the wild type allele of the *da* locus. In a fertilized egg whose X/A ratio corresponds to that of a female, this factor activates transcription at the *Sxl* locus. (b) The Sxl^{M1} locus is the control region of the *Sxl* gene and the Sxl^{F1} locus is the structural part, (c) The Sxl^+ product is essential for females and lethal for males. (d) The Sxl^{M1} mutation makes the synthesis of Sxl^+ product constitutive, that is, independent of stimulation by the da^+ factor. Based on these and other results, Cline has made the conjecture that the Sxl^+ product might itself be involved in dosage compensation and sex determination (Cline, 1978, 1979a). The mechanism by which X/A ratio is measured in the embryo is, however, an undefined aspect of Cline's interpretation.

In this paper we (i) present a model to show how measurement of the X/A ratio can be effected; (ii) show that the level of *Sxl*$^+$ product is proportional to the X/A ratio; and, (iii) postulate that there is a quantitative relationship between the sexual phenotype and the *Sxl*$^+$ product such that increasing cellular concentration of this product leads to increasing 'femaleness' while decreasing concentrations result in 'maleness'. The model also provides an explanation for the interactions among some of the related mutants affecting sex determination and dosage compensation.

The reasoning which led us to this model has been briefly outlined in a recent publication (Gadagkar *et al.*, 1981).

The model

Qualitative aspects

The model (figure 1) consists of five components: (i) the *da*$^+$ factor, produced in the mother and stored in the egg; (ii) a postulated autosomal site ω capable of

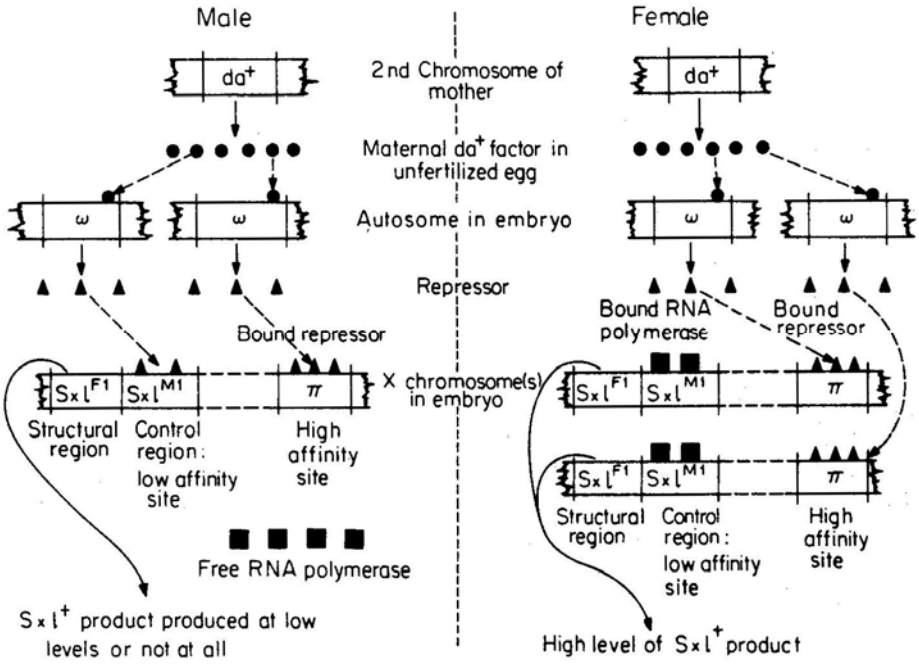

Figure 1. A model for the measurement of the ratio of the number of X chromosomes to the number of sets of autosomes.

The *da*$^+$ factor (●)is produced in excess by the maternal gene *da*$^+$ and stored in the egg. Following fertilization, this factor binds to a specific autosomal site ω in the embryo resulting in the production of a small quantity of repressor (▲). In the male embroyo (left half of the figure), there is only one X chromosome and therefore only one set of low affinity *Sxl* and high affinity π sites. The repressor is able to bind significantly to both these sites. As a result, on the average, little or no RNA polymerase (■) binds to the *Sxl* site and little or no *Sxl*$^+$ product is produced. In the female embryo (right half of the figure), there are two X chromosomes but the same quantity of repressor as in the male. This quantity of repressor is just sufficient to significantly block the π sites. RNA polymerase binds to *Sxl* and initiates synthesis of the *Sxl*$^+$ product. Females are viable at high levels of *Sxl*$^+$ product and males at low levels.

binding da^+ factor and releasing repressor; (iii) the *Sxl* locus, which in our model is the *low affinity site,* capable of binding both the repressor and RNA polymerase; (iv) a postulated *high affinity site* π, also on the X chromosome, capable of binding both repressor and RNA polymerase with a much higher affinity than the *Sxl* locus; and (v) *RNA polymerase* which binds to both the high and low affinity sites and whose binding to the low affinity site results in transcription at the *Sxl* locus. RNA polymerase binds to *Sxl and* π with an affinity which is less than that of the repressor for these two sites. Binding of RNA polymerase to either *Sxl* or π reduces its binding to the other site.

In the mother the da^+ locus produces an *excess* of da^+ factor which is stored in the egg. Following fertilization, the da^+ factor binds to ω; this results in the synthesis of a *small* quantity of repressor. Both male and female embryos have two copies fo ω and would therefore have the same quantity of repressor. In contrast, the number of *Sxl* and π sites is two each in the female and one each in the male. Repressor and RNA polymerase compete for binding to the low affinity *Sxl* and high affinity π sites. However, the affinity of the repressor for either of these sites is higher than that of the polymerase. Therefore the π site is preferentially bound by the repressor. Since the female has two π sites, these get bound to a significant extent by the repressor. However, repressor concentrations are limiting and therefore allow for polymerase binding to the low affinity *Sxl* sites; this leads to synthesis of significant amounts of the Sxl^+ product. In the male, on the other hand, there is only one copy each of the high and low affinity sites. The repressor thus practically saturates both these sites. Consequently, the low affinity *Sxl site* hardly binds RNA polymerase and little or no Sxl^+ product is produced. In a qualitative sense Sxl^+ product will therefore be made in the female but not in the male.

Our model also provides a ready explanation for the mutants discussed by Cline (1978). Eggs of *da/da* flies lack da^+ factor and therefore also lack repressor. In the absence of repressor, RNA polymerase binds preferentially to the π sites. As a result, affinity of the *Sxl* site for polymerase is reduced and thus a negligible amount of Sxl^+ product is produced. The Sxl^+ product being essential for females, this leads to the daughterless phenotype. The male lethal phenotype of Sxl^{M1} suggests that Sxl^+ product is produced in these males despite the low X/A ratio. Sxl^{Ml} also acts as a suppressor of the daughterless phenotype. Both these effects of this remarkable mutation have a simple explanation in terms of our model: the mutation increases the affinity of the *Sxl* site for RNA polymerase, more RNA polymerase binds to it than in the wild type, and this leads to male lethality and survival of the daughters of *da/da* mothers.

A situation in which two sites compete for repressor and RNA polymerase resulting in the regulation of transcripion from these sites, is in fact known to exist in the bacteriophage lambda (Ptashne *et al.,* 1976, 1980; Walz *et al.,* 1976) where operator-promoter complexes for the genes *cro* and *cI* are close to each other and

are regulated by the same repressor. The *cro* operator-promoter complex, analogous to the π site in our model, has a higher affinity for repressor and RNA polymerase than the *cI* operator-promoter complex, analogous to the *Sxl* site. As a result, transcription from the *cI* promoter first increases and then decreases as a function of repressor concentration. In our model transcription from the *Sxl* site behaves in an identical fashion, also as a function of repressor concentration, and this leads to the model's many interesting features (see below).

Quantitative aspects

Relationship of level of Sxl^+ product to viability: Since Sxl^+ product is assumed to regulate the rate of transcription of the X chromosomes, the level of Sxl^+ product per X chromosome is used here as the standard of comparison among different genotypes. We assume that the viability of a genotype varies with the level of Sxl^+ product per X chromosome and that a male is maximally viable at levels of Sxl^+ product lower than that at which females are maximally viable. Males are assumed to be inviable at levels of Sxl^+ product above those in the intersex and females at levels below. Clearly, a number of factors other than the level of Sxl^+ product must be contributing to the reduced viabilities of metamales (AAAXY), metafemales (AAXXX) and intersexes (AAAXX). However, we assume that the only effect in terms of the contribution of Sxl^+ product is that increasing levels of Sxl^+ product reduce male viability and decreasing levels reduce female viability. This assumption is consistent with the observation that Sxl^{F1} males (presumably with no Sxl^+ product) are fully viable as are Sxl^{M1}/Sxl^{M1} females (Cline, 1978). Thus we define levels of Sxl^+ product above those occurring in the intersex as the region of female viability. Conversely levels of Sxl^+ product below those in the intersex are defined as the region of male viability. It should be noted that this represents a modification of Cline's (1978) 'all-or-none' assumption that Sxl^+ product is essential for females and lethal for males.

Computation of levels of Sxl^+ product: The calculations made in this paper refer to the binding equilibria between repressor and polymerase on the one hand and the low (*Sxl*) and high (π) affinity sites on the other. Binding is assumed to be Michaelian (non-cooperative) except that polymerase binding to either *Sxl* or π depresses its binding affinity to the other. Details are given in the legend to figure 2. The values of the parameters used as well as the range within which each can vary without affecting our conclusions are given in table 1. The result of these calculations is an expression for the equilibrium binding of RNA polymerase to the low affinity *Sxl* site. We assume that the level of this binding is directly reflected in the level of Sxl^+ product within the cell.

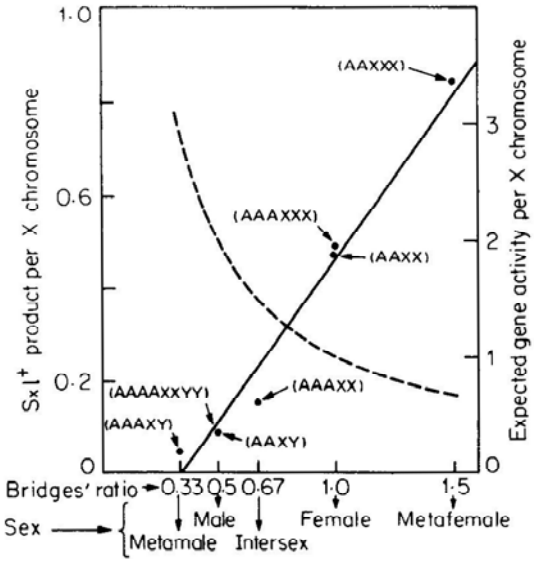

Figure 2. Levels of *Sxl*$^+$ product per X chromosome (left ordinate and solid line) and expected values of gene activity per X chromosome locus (right ordinate and broken line) (Chandra, 1979); both are expressed as a function of Bridges' ratio. Levels of *Sxl*$^+$ product were computed by solving for the following binding equilibria: $K_{LR} \times [L] \times [R] = [LR]$; $K_{LP} \times [L] \times [P] = [LP]$; $K_{HR} \times [H] \times [R] = [HR]$; $K_{HP} \times [H] \times [P] = [HP]$; where, [L], [H], [R] and [P] denote the free concentrations of low affinity sites (*Sxl*), high affinity sites (π), repressor, and RNA polymerase, respectively, and [LR], [LP], [HR] and [HP] are the concentrations of the bound complexes. The affinities of the reactions are denoted by K_{LR}, K_{LP}, K_{HR} and K_{HP} respectively.

The variable affinity in the binding of RNA polymerase to the two sites is simulated using the following equations:

$$K_{LP} = \frac{K^{o}_{LP}}{1 + \left(A \frac{[HP]}{[H_o]}\right)^{n}} \; ; \; K_{HP} = \frac{K^{o}_{HP}}{1 + \left(A \frac{[LP]}{[L_o]}\right)^{n}}$$

where, K^{o}_{LP} is the affinity of RNA polymerase to the *Sxl site* when no polymerase is bound to the π site, K^{o}_{HP} is the affinity of RNA polymerase to the π site when no polymerase is bound to the *Sxl* site; $[L_o]$, and $[H_o]$ are the total concentrations of *Sxl* and π, and A and n are constants. Thus, as binding of polymerase to the π site increases, K_{LP} decreases; when a fraction 1/A of the π sites is bound by polymerase, K_{LP} becomes half of K^{o}_{LP} and, finally, when all the π sites are occupied by polymerase, the affinity falls by a factor of $1 + A^n$. Binding of polymerase to *Sxl* reduces its affinity for π in a like manner.

Conservation conditions yield the following set of equations: $[LP] + [LR] + [L] = [L_o]$; $[HP] + [HR] + [H] = [Ho]$; $[LR] + [HR] + [R] = [R_o]$; $[LP] + [HP] + [P] = [P_o]$;where, $[R_o]$and $[P_o]$ are the total concentrations of repressor and polymerase respectively.

For various sets of constants, $[L_o]$, $[H_o]$, $[R_o]$, $[P_o]$, K_{HR}, K_{LR}, K^{o}_{HP}, K^{o}_{LP}, A and n, the above equations were solved iteratively. $[L_o]$ and $[H_o]$ were taken as unity and scaled with the number of X chromosomes while $[R_o]$ and $[P_o]$ were scaled with the number of sets of autosomes. After these computations were completed, the gene coding for RNA polymerase II was shown to be on the X chromosome (Greenleaf *et al.* 1980). $[P_o]$ was therefore also scaled with the number of X chromosomes and the results do not alter any of our conclusions. The values of the various parameters used are given in table 1.

Table 1. Values of parameters used and their range of tolerance.

	Value used in the calculation	Range tolerated*
Repressor (R_0)	1.5	1.3 −1.7
RNA Polymerase (P_0)	5	2 −10
Affinity of polymerase for the low affinity site ($K°_{LP}$)	1	0.5 −2.0
Affinity of polymerase for the low affinity site in the Sxl^{M1} mutation	10	5 −40
Affinity of polymerase for the high affinity site ($K°_{HP}$)	100	10 −500
Affinity of repressor for the low affinity site (K_{LR})	100	50. −150
Affinity of repressor for the high affinity site (K_{HR})	10^5	2.5×10^4 −∞
A†	3.00	2.75 −3.50
n†	4.00	3.75 −4.50

* When the value of any parameter is outside this range, either the level of Sxl^+ product does not increase as a function of Bridges' ratio or the mutants do not behave as described in the text.

† These are constants in the equations used to simulate the variable affinity in the binding of RNA polymerase to the high and low affinity sites. See legend to figure 2 for details.

Numerical results

General Remarks

The level of Sxl^+ product per X chromosome increases in proportion to Bridges' ratio (figure 2). Triploids and tetraploids are extremely close to their diploid counterparts in their levels of Sxl^+ product per X chromosome, suggesting that it is indeed the X/A *ratio* rather than the level of X or A separately that is being measured. By applying the criteria for viability given earlier, one can see that (i) *da* is lethal in the female but not in the male; (ii) Sxl^{M1} is lethal in the male but not in the female; (iii) either one or two doses of Sxl^{M1} will rescue the daughters of *da/da* mothers and (iv) Sxl^{F1} is recessive because the level of Sxl^+ product per X chromosome in an Sxl^{F1}/Sxl^+ individual, though half of the normal level, is still within the region of female viability (table 2).

Mutations that can arise in the system

By varying the values of the parameters used beyond the assumed limits of viability of the wild type we are able to predict the kinds of mutations that can arise in this system (table 1). Many interesting consequences follow from the observation that the level of Sxl^+ product, when assessed as a function of increasing repressor concentration, first goes up and then comes down (figures 3A and B). Thus, whereas the region of female viability is confined to one continuous interval of repressor concentrations, males survive only at very low or high repressor levels.

Table 2. *Sxl*$^+$ product per X chromosome in wild type and mutant flies.

	Genotype	*Sxl*$^+$ product per X chromosome*
1.	*Wild type male*	*0.08*
2.	Sons of *da/da* mothers	0.10
3.	Sxl^{M1} male	0.40
4.	2, above, with Sxl^{M1}	0.52
5.	*Wild type female*	*0.47*
6.	Daughters of *da/da* mothers	0.09
7.	Sxl^{M1}/Sxl^{M1} female	0.68
8.	6, above, with Sxl^{M1}/Sxl^{M1}	0.47
9.	6, above, with Sxl^{M1}/Sxl^{+}	0.28
10.	Sxl^{F1}/Sxl^{+} female	0.24
11.	*Intersex* (AAAXX)	*0.15*
12.	Intersex offspring (AAAXX) of *da/da* mothers	0.13
13.	11, above, with Sxl^{M1}/Sxl^{M1}	0.48
14.	12, above, with Sxl^{M1}/Sxl^{M1}	0.60
15.	*Metamale* (AAAXY)	*0.05*
16.	15, above, with Sxl^{M1}	0.34
17.	*Metafemale* (AAXXX)	*0.84*
18.	Metafemale offspring (AAXXX) of *da/da* mothers	0.08

* These values were calculated as described in legend to figure 2. The parameters used are those listed in table 1.

Thus we have the apparently paradoxical situation in which a partial reduction in repressor levels leads to male-specific lethality, whereas totally eliminating the repressor restores viability to the male but results in female lethality. These predictions are mirrored respectively in the male-specific autosomal lethal, *mle* (Belote and Lucchesi, 1980a, b; Fukunaga *et al.,* 1975; Tanaka *et al.,* 1976) and in the *da* mutation (Cline, 1978). The other autosomal, male-specific lethals *msl-1, msl-1*^{b}*, msl-2* (Belote and Lucchesi, 1980a, b) are expected to be similar to *mle* in this respect.

The basis for the curious interaction between the *da* and Sxl^{M1} mutations is also brought out by Figs. 3A and B. Sxl^{M1} increases the level of *Sxl*$^+$ product above that of the wild type over the entire range of repressor concentrations. This has the effect of making the male nonviable over the entire range and of restoring viability to those females in which the repressor concentration is *simultaneously* lowered to near zero levels. Sxl^{M1}, therefore, is a male-specific lethal mutation which also has the property of rescuing the daughters of *da/da* mothers.

The observation that the level of *Sxl*$^+$ product first increases and then decreases with repressor concentration is central to explaining yet another curious result. This is the recent finding of Skripsky and Lucchesi (1980) that females of the genotype *mle/mle;* Sxl^{F1}/Sxl^{+} develop, with a low penetrance, male secondary sexual characteristics (sex-combs). Referring to figure 3, the bell-shape of the curve implies that if the effect of the *mle* mutation is to partially reduce repressor concentration, it would lead to unacceptably high levels of *Sxl*$^+$ product in the male. In the female, on the other hand the levels are slightly reduced but still within the region of viability. In combination with one dose of Sxl^{F1}, which by itself reduces the level of *Sxl*$^+$ product by one half, *mle/mle* would further lower the

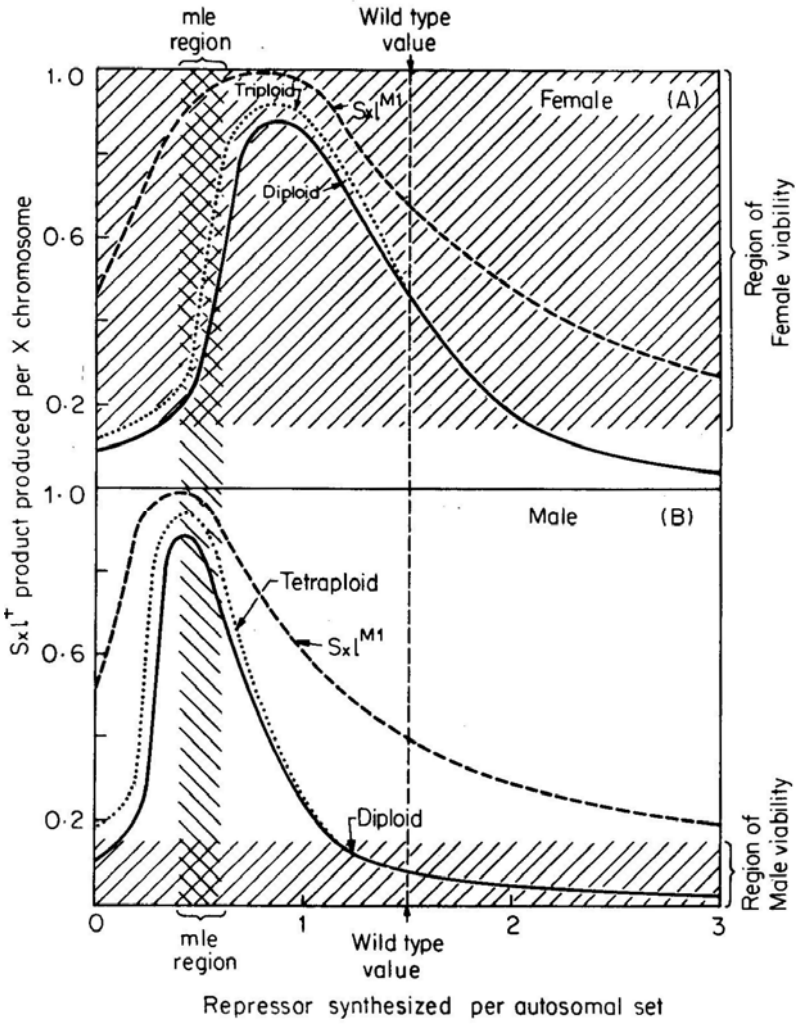

Figure 3. Levels of *Sxl*$^+$ product per X chromsome as a function of repressor synthesized per autosomal set in the female (A) and male (B).

Solid lines refer to the wild type, dotted lines to tetraploid male and triploid female and broken lines to the *Sxl*^{M1} male and female. The levels of *Sxl*$^+$ product corresponding to the regions of male and female viability and also the levels of repressor corresponding to the *mle* mutation are shown as hatched areas. Note that the range in levels of *Sxl*$^+$ product tolerated by a male is much narrower than that tolerated by a female. Metafemales have an extreme female phenotype and a rate of transcription per X chromosome lower than that in the normal female (Lucchesi *et al.* 1974; Stewart and Meriam, 1975). In terms of our model metafemales should have a level of *Sxl*$^+$ product higher than that of a normal female. Thus the level in normal females is expected to be somewhat below 1.0, the theoretical maximum value for *Sxl*$^+$ product per X chromosome. *Sxl*^{F1} is a recessive mutation (Cline, 1978). Since *SxlF1/Sxl*$^+$ females (presumably with half the wild type levels of *Sxl*$^+$ product) are fully viable, the level of *Sxl*$^+$ product in an intersex should be less than 0.5. The male, as a result, can only have a relatively narrow region of viability, ranging from zero to some value below 0.5 of *Sxl*$^+$ product per X chromosome. The *da* mutation corresponds to a zero level of repressor. The value of $[R_o]$=1.5, considered as the wild type value, is indicated by the broken vertical line running through both panels.

level of Sxl^+ product and bring it to the neighbourhood of the male value. Consequently, such flies, if they survive, ought to show male-like characters.

The consequences of varying the affinities of the *Sxl* and π sites for repressor and RNA polymerase have been examined by us. A summary of these results and the properties of the various mutations, known as well as predicted, and the interactions among them are given in summary form in table 3 and figure 4.

Table 3. Properties of mutations, known and predicted.*

Mutation	Reference	Phenotype	Expected change at the molecular level	Can be rescued by	Will rescue the effects of
Sxl^{F1}	Cline (1978)	Female lethal	Inactive *Sxl* product	None	Sxl^{M1}, *mle*
da	Cline (1978)	Daughterless	No repressor, leading to low levels of Sxl^+ product in females	Sxl^{M1}	K_{LR} *low*
mle	Belote and Lucchesi (1980a, b); Fukunaga *et al.* (1975); Tanaka *et al.* (1976)	Male lethal	Lower than normal levels of repressor, leading to high levels of Sxl^+ product in males	Sxl^{F1}, K_{HP} *high* or K_{HR} *low*	None
Sxl^{M1} or K_{LP} *high*	Cline (1978); also see figure 4A	Male lethal	Higher than normal levels of Sxl^+ product in males	Sxl^{F1}	K_{HP} *high*, K_{HR} *low*, *da*
K_{LR} *low*	Predicted, see Fig. 4B	Male lethal	Higher than normal levels of Sxl^+ product in males	*da*	K_{HP} *high*, K_{HR} *low*
K_{HP} *high*	Predicted, see figure 4C	Female lethal	Lower than normal levels of Sxl^+ product in females	Sxl^{M1}, K_{LR} *low*	*mle*
K_{HR} *low*	Predicted, see figure 4D	Female lethal	Lower than normal levels of Sxl^+ product in females	Sxl^{M1}, K_{LR} *low*	*mle*
ω-	Predicted	Female lethal	No repressor, leading to lower than normal levels of Sxl^+ product in females	Sxl^{M1}	K_{LR} *low*

* K_{LP} and K_{LR} are, as defined in legend to Figure 2, the affinities of the *Sxl* site to RNA polymerase and repressor respectively; similarly, K_{HP} and K_{HR} are the affinities of the π site to the polymerase and repressor respectively. The mutation K_{LP} *high* is one which results in an increase in K_{LP} ; K_{LR} *low* is a mutation which results in a decrease in K_{LR} ; K_{HP} *high* results in an increase in K_{HP} whereas K_{HR} *low* leads to a decrease in K_{HR} .

Changes in the affinity of the repressor or polymerase to these sites can be brought about by muations in the sites themselves or by mutations affecting the properties of the repressor or polymerase. The former would behave as X-linked mutations and cannot be rescued by injection of cytoplasm from wild type eggs into defective eggs while the latter would behave as autosomal mutations and can be rescued by injection of cytoplasm from wild type eggs. The mutation Sxl^{Ml}, which results in an increase in K_{LP}, is expected to be of the former kind because it is X-linked.

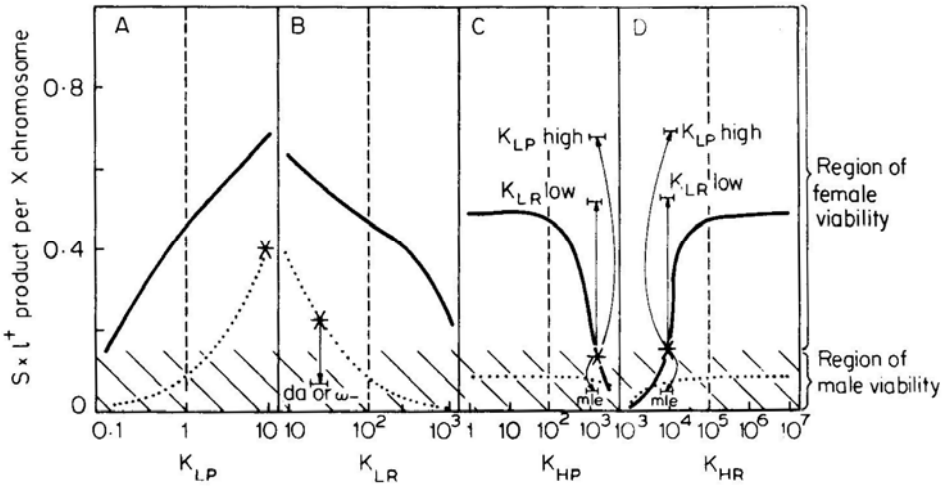

Figure 4. Levels of *Sxl*$^+$ product per X chromosome in the female (solid line) and male (dotted line) as a function of variations in the values of K_{LP} (Panel A), K_{LR} (Panel B), K_{HP} (Panel C) and K_{HR} (Panel D).

The panels A, B, C and D illustrate respectively the consequences of the four mutations *K_{LP} high, K_{LR} low, K_{HP} high* and *K_{HR} low*. The values considered as wild type for each of these affinities are indicated by broken vertical lines running through the middle of each panel. The values considered as mutant for each of these affinities are indicated by an asterisk in each panel. The region of male viability is indicated by the hatched areas whereas the region of female viability is unhatched. The two male-specific lethal mutations *K_{LP} high* and *K_{LR} low* can rescue females carrying either of the two female-specific lethal mutations *K_{HP} high* and *K_{HR} low* by restoring high levels of *Sxl*$^+$ product in them. This is indicated by means of long vertical arrows in panels C and D. On the other hand neither of the two female-specific lethals can rescue the male-specific lethals *K_{LP} high* and *K_{LR} low* because they do not bring about any significant reduction in the high levels of *Sxl*$^+$ product occurring in such genotypes. As shown by the arrow in Panel B, the mutations *da* and ω rescue males carrying the male-specific lethal mutation *K_{LR} low* by bringing down the amount of *Sxl*$^+$ product to a level at which males are viable. The *mle* mutation fails to rescue females carrying either of the two female-specific lethal mutations (*K_{HP} high and K_{HR} low)* because it further brings down the already low level of *Sxl*$^+$ product. This is shown by the short arrows in panels C and D. In all panels, the arrows indicate the levels of *Sxl*$^+$ product reached as a result of combining the mutation denoted against the arrow with the mutation illustrated in the panel. The values of affinities used here to represent the different mutations are arbitrary. See also table 3.

Discussion

The model discussed here provides a molecular mechanism for understanding how the X/A ratio can be measured in the cells of a developing embryo. The measurement is effected by means of a series of interactions initiated by the *da*$^+$ factor which result in a characteristic levels of *Sxl*$^+$ product in the cell. This *Sxl*$^+$ product is assumed to function as an inhibitor in regulating the rate of transcription of X-linked genes (Cline, 1978).

We wish to leave open the question whether the *Sxl*$^+$ product regulates transcription of the X chromosome *en bloc* or whether there are several *Sxl*$^+$-like products regulating transcription in different sets of X-linked genes (see Chandra, 1979 for a review). We have also not discussed the consequences of duplications and deletions of the *Sxl* locus (Cline, 1978) because we do not know whether the

duplications and deletions include both the *Sxl* and π sites or not. Since the model requires that the two sites, *Sxl* and π, function in a coordinated fashion, it is not possible to predict the consequences of separating them. Nor have we discussed the results of Stewart and Merriam (1975) which seem to suggest that in flies with 2½ X chromosomes the relationship between dosage compensation and the Bridges' ratio breaks down irrespective of which chromosome arm is retained as the extra segment. These data cannot be simply interpreted in terms of our model because we do not know if the postulated relationship between Sxl^+ product and the Bridges' ratio (figure 2) also breaks down in these flies. It is possible that while this relationship is retained in such flies, breakdown occurs at the level of regulation of the rate of transcription by the Sxl^+ product. Resolution of this problem will depend to a significant extent on our understanding whether the X chromosome is regulated in a piecemeal or *en bloc* manner.

We now wish to make a few remarks regarding the implications of our model for the broader problems of dosage compensation and sex determination. We postulate that increasing levels of Sxl^+ product promote a female phenotype and, correspondingly, decreasing levels, a male phenotype. Independently of its effect on sexual phenotype, increasing levels of Sxl^+ product per X chromosome would lead to decreasing levels of X-linked gene products. Thus we consider the Sxl^+ product as having two primary roles, one in determining the sexual phenotype and the other, in dosage compensation. There are several other genes affecting sex determination (Baker and Ridge, 1980). The picture we have is that the Sxl^+ gene product initiates the pathway determining the sexual phenotype and that the other genes act subsequently.

Three predictions can be made about the role of the Sxl^+ product in dosage compensation. (i) Flies carrying the mutation Sxl^{F1} should have little or no Sxl^+ product. The rate of transcription of the X chromosome in such flies should therefore be higher than that in individuals carrying the wild type allele. This is consistent with the recent observations of Lucchesi and Skripsky (1981). (ii) Flies carrying the mutation Sxl^{M1} should have higher levels of Sxl^+ product than wild type individuals (table 2). The rate of transcription of the X chromosome in such flies should therefore be lower than in their wild type counterparts. Lucchesi and Skripsky (1981) have studied males of this genotype but their data did not permit them to distinguish between a lower rate of transcription and under-replication of the X chromosome. (iii) We expect the mutation *mle* to interfere with dosage compensation in the male by lowering the rate of transcription of the X chromosome; in the female, on the other hand, this mutation should have little or no effect (figures 3A and B). This, is consistent with recent experimental data (Belote and Lucchesi, 1980a).

Three classes of data have a bearing on the relationship between Sxl^+ product and sex determination. One has to do with the sexual phenotype of flies carrying one or more of the mutations which form components of our model. For example, if Sxl^+ product is also involved in sex determination, we would predict that the sexual phenotype of (i) Sxl^{M1}/Sxl^{M1} and Sxl^{M1}/Sxl^+ females would shift in the direction of metafemales; (ii) Sxl^{F1}/Sxl^+ females would shift in the direction of

intersexes; and (iii) daughters of *da/da* mothers rescued by a single copy of Sxl^{M1} would more closely resemble intersexes than those rescued by two copies of Sxl^{M1}. A second class has to do with the effects of mutations which modulate the level of Sxl^{+} product in flies which already have an abnormal sexual phenotype. For example, an individual of the constitution AAAXX, which would normally develop as an intersex, might be expected to develop as a male under the influence of *da* and as a female under the influence Sxl^{M1} (table 2). This is in fact the observation of Cline (1981). Reasoning along the same lines, we predict that *da* would decrease the 'femaleness' of a metafemale while Sxl^{M1} would decrease the 'maleness' of a metamale along a male-female continuum (table 2). The third class of results pertains to the sexual phenotype in islands of mutant cells of one sex in a genetic background consisting of wild type cells of the other sex. In gynandromorphs, or in mosaics with viable Sxl^{M1} male tissue in a background of Sxl^{M1}/Sxl^{+} female tissue, our model suggests that the male tissue should exhibit phenotypic features of a female. Similarly, when viable, Sxl^{F1}/Sxl^{F1} female tissue within a Sxl^{F1}/Sxl^{+} background would show male characteristics. These are indeed the observations reported by Cline (1979a, b).

Finally, the following predictions can be made about the interaction of Sxl^{F1} with Sxl^{M1} and *mle*. Males carrying the mutations Sxl^{M1} or *mle* are inviable because, according to the model, there would be an overproduction of the Sxl^{+} product. Sxl^{F1} is assumed to be a mutation in the structural part of the *Sxl* locus leading to the production of inactive *Sxl* product. Since Sxl^{F1} males–which presumably have no Sxl^{+} product at all–are viable, it follows that Sxl^{F1} should rescue male embryos carrying Sxl^{M1} or *mle*. This Prediction appears to be confirmed by the behaviour of two new alleles at the Sxl^{F1} region (Cline, 1981). Both these mutant alleles rescue Sxl^{M1} males from lethality. Data are not yet available for the interaction of *mle* with *SxlM1*.

We wish to point out that while some of the predictions made here are a direct consequence of the molecular mechanism we have proposed for the measurement of the X/A ratio, others follow from our quantitative approach to Cline's qualitative model for the behaviour of the *Sxl* and *da* mutations (Cline, 1978).

To account for certain experimental observations on levels of alcohol dehydrogenase activity in maize, Schwartz (1971) has proposed a 'gene competition' model which has certain features similar to our model for the measurement of the X/A ratio. According to Schwartz, the level of gene activity is related to the availability of a factor for which a group of genes competes. This factor, he assumes, is present in limiting concentrations. Schwartz's experimental results, which are consistent with this interpretation, suggest that such models are plausible in eukaryotic systems. Schwartz has in fact suggested that such a gene competition model may explain certain features of dosage compensation in *D. melanogaster* (Schwartz, 1973).

A feature of our model for dosage compensation is that it permits the measurement of *ratios* of the concentrations of two molecular species. This is brought out by the observation that the levels of Sxl^{+} product in the triploid female and the tetraploid male remain very close to those in their diploid counterparts throughout the range of repressor concentrations (figures 3A and B). In many

developing systems, the fate of a cell depends on its relative position with in the cell mass (Wolpert, 1971). A means for a cell to determine its relative position is to measure, for instance, the ratio of two substances ('morphogens') whose concentration gradients across the cell mass are in opposite directions. The consequences implied in looking at the problem of regulative development in such a manner are being examined by us.

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