Genetic and developmental analysis of the sex-determining gene ‘double sex’ (dsx) of Drosophila melanogaster

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Abstract: Sex determination in Drosophila depends on the ratio of X chromosomes to sets of autosomes (X:A). This chromosomal signal is used to regulate a few control genes whose state of activity selects either the male or the female sexual pathway. We have studied the structure and function of dsx (double sex) which appears to be the last regulatory gene on whose function the sexual pathway eventually depends. We have mutagenized the locus, varied the doses of dominant dsx-mutations and wildtype alleles, and combined different dsx-alleles with recessive mutations in other sex-determining genes, such as ix, tra-2 and tra. The locus dsx harbours two genetic functions, dsxm to implement the male program, dsxf to implement the female program. We found that dsxm and dsxf can mutate independently although most mutations abolish both functions. We conclude that dsxm and dsxf each have their specific domain, but also share a large region of DNA that is essential for both functions. We present evidence that the dominant mutations correspond to a constitutive expression of the male-determining function dsxm, with the simultaneous abolishment of the female-determining function dsxf. This effect can be counteracted by two doses of expressed dsxf so that a female phenotype results. The products of one dose of expressed dsxm and one dose of expressed dsxf in the same cell appear to neutralize each other which leads to a null phenotype. The mutant combinations suggest that the product of dsxf requires the products of ix+, tra-2+ and tra+ to become functional.

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Genetic and developmental analysis of the sex-determining gene ‘double sex’ (dsx) of Drosophila melanogaster

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Summary

Sex determination in Drosophila depends on the ratio of X chromosomes to sets of autosomes (X: A). This chromosomal signal is used to regulate a few control genes whose state of activity selects either the male or the female sexual pathway. We have studied the structure and function of dsx (double sex) which appears to be the last regulatory gene on whose function the sexual pathway eventually depends. We have mutagenized the locus, varied the doses of dominant dsx-mutations and wildtype alleles, and combined different dsx-alleles with recessive mutations in other sex-determining genes, such as ix, tra-2 and tra.

The locus dsx harbours two genetic functions, dsxm to implement the male program, dsxf to implement the female program. We found that dsxm and dsxf can mutate independently although most mutations abolish both functions. We conclude that dsxm and dsxf each have their specific domain, but also share a large region of DNA that is essential for both functions. We present evidence that the dominant mutations correspond to a constitutive expression of the male-determining function dsxm, with the simultaneous abolishment of the female-determining function dsxf. This effect can be counteracted by two doses of expressed dsxf so that a female phenotype results. The products of one dose of expressed dsxm and one dose of expressed dsxf in the same cell appear to neutralize each other which leads to a null phenotype. The mutant combinations suggest that the product of dsxf requires the products of ix+, tra-2+ and tra+ to become functional.

Introduction

In Drosophila, the ratio of X chromosomes to sets of autosomes (X: A) is the only discriminator between male (XYAA) and female (XXAA) development (Bridges, 1921). All processes related to sex, i.e. dosage compensation and sex determination in the soma and in the germ line, depend on the X: A ratio. This quantitative signal, however, does not directly control the sex differentiation genes, but uses a small number of regulatory genes whose state of activity then selects either the male or the female pathway. Seven genes are known so far that govern sexual differentiation of somatic cells, namely da, sis-a, Sxl, ix, tra-2, tra and dsx. Of these, dsx (double sex), described by Hildreth (1965), occupies a special position: Mutant combinations suggest that it lies at the end of a regulatory pathway and that the functional state of dsx actually determines the sexual phenotype of somatic cells (Baker & Ridge, 1980). Mutations at dsx can be recessive or dominant, and they can affect both chromosomal sexes, or only XY, or only XX. The mutations cause an intersexual phenotype at the cellular level as if the male and the female programs were simultaneously expressed in the same cell. The dsx-locus is also special since it seems to harbour two functions, one required for male development and one for female development (for review see Baker & Belote, 1983; Nöthiger & Steinmann-Zwicky, 1985; for sis-a see Cline, 1986).

In an attempt to learn more about structure, function and regulation of dsx, we undertook a genetic and developmental analysis of this locus and studied the phenotypic effects of various mutant alleles and genetic combinations.

2. Materials and Methods

Unless noted otherwise, all crosses were done at 25 °C. The flies were reared on standard food (corn meal, sugar, yeast, agar). For genetic symbols see Lindsley & Grell (1968). The mutation dsx° was described by...
Duncan & Kaufman (1975), dsx<sup>mas</sup> by Nöthiger et al. (1980); dsx<sup>2</sup> is another dominant allele of dsx, found in W. Gehring's laboratory in Basel.

2.1. Screening of mutants at the dsx-locus

Males with the genotype X/Y. B<sup>+</sup>; mwh<sup>7</sup> jv p<sup>+</sup> were fed with EMS (Lewis & Bacher, 1968), and massmated to Basc; Sh/TM3, Ser females. Progeny was raised at room temperature. Male parents were discarded after five days to prevent clustering of new mutations. Single F<sub>1</sub> males of the genotype Basc/Y. B<sup>+</sup>; mwh<sup>7</sup> jv p<sup>+</sup>/TM3, Ser and single females of the genotype +/Basc: mwh<sup>7</sup> jv p<sup>+</sup>/TM3, Ser were crossed with two th st r e p Df(dsx) bx sr e<sup>/</sup>TM3, Sh Ser flies. (The Df(dsx) was recovered as a 'revertant' of dsx<sup>o</sup> and identified as a deficiency described as Df(3R), dsx<sup>b+85</sup> in Duncan & Kaufman, 1975.)

The single crosses were set up at 29 °C in an attempt to isolate also temperature-sensitive mutants. In the F<sub>2</sub>, new dsx-mutants as well as lethal factors were uncovered by the chromosome carrying the deficiency Df(dsx), and could be kept over the TM3 chromosome as a balanced stock. Flies expressing a new dsx mutation were mounted in Faure's solution and studied under a compound microscope.

2.2. Complementation analysis

Crosses were performed between new dsx-alleles inter se and also with previously existing mutants. The resulting flies were checked for fertility and afterwards mounted for microscopical inspection of their sexually dimorphic structures. Recessive lethals which are located within Df(dsx) were tested for complementation; they were also combined with a viable dsx-allele to see whether any of the lethals displayed a dsx phenotype.

2.3. Induction of reversions of two dominant dsx mutations, dsx<sup>o</sup> and dsx<sup>+</sup>

(a) dsx<sup>o</sup> Sb e/TM6 males were treated with EMS (Lewis & Bacher, 1968) and crossed with y w females. The progeny was screened for normal and fertile Sb females.

(b) Males with the genotype X/Y. B<sup>+</sup>; dsx<sup>+</sup>/TM3, Sh Ser e were irradiated with 4000 r (Philips MG160, 2 mm Al filter, 25 cm distance, 150 kV, 14 mA, 6 min) and crossed with e females. Sb<sup>+</sup> Ser e<sup>+</sup> daughters showing a normal female phenotype indicated a dsx<sup>+</sup> revertant. Revertants were testcrossed and analysed over different dsx-alleles and over Df(dsx).

2.4. Dosage effects

The effect of dsx<sup>o</sup> in the presence of two doses of dsx<sup>+</sup> was studied in (1) triploid females (3X;3A), (2) triploid intersexes (2X;3A) and (3) normal diploids (2X;2A). For this purpose, animals with the genotypes (1) XX/X;dsx<sup>o</sup>/+ /+ , (2) XX;dsx<sup>o</sup>/+ /+, and (3) XX/Y. dsx<sup>+</sup> ; dsx<sup>o</sup> /+ were constructed and their sexually dimorphic structures were compared with those of X/X; dsx<sup>o</sup> /+. The Y. dsx<sup>+</sup> is a translocation of the region 84D10, 11; 85A1-3 to the Y chromosome; besides dsx<sup>+</sup>, it also carries p<sup>+</sup> and Rg(pbx), and is described as T(Y;3) P92 in Duncan & Kaufman (1975).

Table 1. Scheme for quantifying the sexual phenotype of flies in abdominal segments A7–A9

| Abdominal segments with values assigned to the structures of wild-type males and females |
|----------------------------------|------------------|------------------|
| A7 (7 tergite/sternite)          | A8 (Female genitalia) | A9 (Male genitalia) |
| Structure | p<sup>n</sup> | w<sup>e</sup> | Structure | p | w | Structure | p | w |
| T7<sup>+</sup> | 0-7 | 3 | VP | 0-56 | 3 | CL | 0-12 |
| S7 | 0-3 | | T8 | 0-34 | 3 | SP | 0-04 |
| SPT | 0-1 | | | | | LP | 0-14 |
| | | | GA | 0-38 | | |
| | | | HY | 0-14 | | |
| | | | PE | 0-18 | | |

* A value (p) was ascribed to each structure so that the sum of these values added up to 1-0 for the imaginal derivatives of one abdominal segment in a normal wild-type female or male. In the intersexes, the structures were most of the times smaller and incomplete and sometimes absent, yielding a sum considerably below 1-0 (see Fig. 1).

* Abbreviations: T7, S7, tergite 7, sternite 7; VP, vaginal plate; T8, tergite 8; SPT, spermatheca; CL, clasper; SP, sperm pump; LP, lateral plate; GA, genital arch; HY, hypandrium; PE, penis with parameres.

* For the calculation of the 'sex index' (see Fig. 2), the values of the three segments were given a weight (w) roughly proportional to the size of the imaginal structures in normal flies. Thus, for each genotype the lengths of the shaded columns in Fig. 1 were multiplied by 3 for A7 and A8, and by 4 for A9; the three values obtained were then added and divided by 10 (3 + 3 + 4). In this way, a value between 0-0 (completely male) and 1-0 (completely female) was obtained (for explanation see Fig. 1). This value places an intersex on a line between a male and a female (see Fig. 2).

For illustration of the structures see Fig. 3.
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2.5. Ovary transplantation

After we had found that $\frac{XX}{Y}$. $d sx^+$; $d sx^0/+ \text{ were sterile females with well developed ovaries}$, we tested whether such ovaries could give rise to functional eggs if transplanted into normal females (series A and B), and whether $\frac{XX}{Y}$. $d sx^+$; $d sx^0/+ \text{ would become fertile if provided with normal ovaries}$ (series C and D). Ovaries were transplanted between larvae of the late 3rd instar as described by Ursprung (1967).

2.5.1. Series A and B

Host larvae were obtained by crossing Basc; TM3, Ser/Sb females to Fsl(K1237)/Y males. Fsl(K1237) is a fully penetrant, dominant mutation that sterilizes females by blocking oogenesis before stage 4 (see King, 1970 for description of stages); the mutation acts only in the germ line (Busson et al. 1983). Since K1237 host ovaries remain small, donor ovaries can develop without competition (Monod & Poulson, 1936). (The Fsl(K1237) mutation was kindly provided by M. Gans, Gif-sur-Yvette.)

Donors were produced by crossing C(1)RM, y v/ Y.dsx$^+$; red/red females to y/Y.dsx$^+$; $d sx^0$ Sb e/red males. Half of the female larvae had the genotype C(1)RM, y v/ Y.dsx$^+$; $d sx^0$ Sb e/red; the other half carried three doses of $d sx^+$ and is also listed in Table 2. After transplantation, surviving female hosts were test-crossed to either y/Y, $d sx^{maj}$/TM3, Sb Ser males (series A), or to X/Y. $d sx^+$; $d sx^{maj}$/TM2, Ubx males (series B). The genotype of the donor ovary, abbreviated as $d sx^0$ and $d sx^+$ in the right-most column in Table 2, was inferred from the genetic markers seen in the offspring. The C(1)RM, y v chromosome is abbreviated as $XX$ in Table 2.

2.5.2. Series C and D

Donor ovaries from y w$^w$/mal larvae were injected into C(1)RM, y v/ Y.dsx$^+$; $d sx^0$ Sb e/red hosts (series C) or into Basc/T(1; Y; 3) H1; $d sx^0$ Sb e/+ hosts (series D). The T(1; Y; 3) H1 is an X chromosome that carries a duplication for $d sx^+$ and that eliminates aldehyde oxidase (AO) activity and that can be used as a histochemical marker (Janning, 1976). Thus, donor and host ovaries could be distinguished and we could determine whether a donor ovary had attached to the host's gonads.

2.6. Interaction of $d sx$-alleles with mutations at other sex-determining loci

(a) To study the epistatic relation between $d sx$ alleles and transformer (tra), the following double-mutants were constructed, both in X/X and X/Y animals: (1) tra $d sx^0$/tra $d sx^+$, (2) tra $d sx^0$/tra $d sx^1$, (3) tra $d sx^{maj}$/tra $d sx^+$ and (4) tra $d sx^0$/tra +. The recombinant chromosome tra $d sx^+$ was obtained by irradiating X/Y; $d sx^0$ Sb e/+/tra + st tra cp ri p$^+$ males with 2500 r and screening their male offspring for an
induced recombinational event between $st$ and $ri$ or $p$. Individual recombinant males were tested for the presence of $tra$ and $dsx$ in $cis$ on the $Sb$ chromosome. (The symbol $dsx^{a\beta}$ designates alleles that have lost both functions, $m$ and $f$.)

(b) The interaction of $dsx^{a\beta}$ with $ix$, $tra-2$ and $tra$ was studied in the following six genotypes:

1. $X/X$; $ix$/SM5; $dsx$0 $Sb$ e/+
2. $X/X$; $ix$/$ix$; $dsx^{a\beta}$ $Sb$ e/+,
3. $X/X$; $tra-2$ +/+; $dsx$0 $Sb$ e/+,
4. $X/X$; $tra$/+ + $dsx^{a\beta}$ $Sb$ e,
5. $X/X$; $tra-2$ +/+; $tra$ $dsx$0 $Sb$ e/+,
6. $X/X$; +/SM5; $tra$ $dsx^{a\beta}$ $Sb$ e/+

Two genotypes, $X/X$; +/SM5; $dsx^{a\beta}$ $Sb$ e/+ and $X/X$; $dsx^{a\beta}$ $Sb$ e/TM6, served as references.

The sexually dimorphic structures of these flies were microscopically analysed. For this purpose, the flies were macerated in hot 10% NaOH, washed in H2O, and mounted under coverslips in Faure’s solution. For abdominal segment 7 and the genitalia, the sexual, genitalia (segment 8), and one for the male genitalia (segment 9). The values are quantitative and do not indicate whether the structures were morphologically normal or abnormal.

The values for the structures of these three segments were used to calculate a ‘sex index’ which characterizes the intersex by assigning a place to each of the genotypes on a line between normal male and normal female (see Table 1, Fig. 2).

3. Results

3.1. Mutagenesis

From a total of 7218 single crosses, 4854 had progeny. Among these, eight new $dsx$ alleles and 40 recessive lethals were recovered within the deficiency of $dsx^{a\beta}$- (referred to as $Df(dsx)$ in this paper). The $dsx$ alleles fall into three categories:

(i) $dsx^{a\beta}$, recessive, affecting both chromosomal sexes, 6 alleles. These mutations transform both chromosomal sexes, although with different expressivity, into intersexes of the type described by Hildreth (1965) and Baker & Ridge (1980) for the original $dsx$-allele. Our new alleles were tested in trans with Hildreth’s (1965) original $dsx$ allele and with $Df(dsx)$. The allele $dsx^{a1}$ gives the same strong intersexual phenotype as the original $dsx$ allele which presumably corresponds to a loss of function (Baker & Ridge, 1980). Four mutants ($dsx^{a2}$, $dsx^{a1}$, $dsx^{a3}$, $dsx^{a4}$) have a much stronger effect on XX than on XY animals. The latter look almost normal, but display slightly rounded analia, an incomplete penis apparatus, and are sterile. In XX-animals, all four mutants give a clear intersexual phenotype in combination with Hildreth’s (1965) $dsx$-allele. All chromosomes carrying a new $dsx$ mutation were homozygous lethal in both sexes; since they are viable over $Df(3R)$ $dsx^{a\beta}$, they must have suffered lethal hits outside the deficiency. A sixth mutant was lost before a stock could be established.

No complementation was found among the five alleles, except for $X/Y$; $dsx^{a1}$/$dsx^{a1}$ that were fertile males. In combination with the strong $dsx^{a1}$-allele, the four weaker alleles showed a weak phenotype in $XY$ and a strong phenotype in $XX$-flies. We conclude that these alleles are leaky and can still make some $dsx^{-}$-product that is at least partially active in chromosomal males.

(ii) $dsx^{i}$, recessive, affecting only XX animals, one allele. Animals with genotype $X/Y$; $dsx^{i}$/dsx1 have a strong $dsx$-phenotype whereas $X/Y$, $dsx^{i}$/dsx1 are normal, fertile males. Transheterozygotes of the male-specific mutation $dsx^{i}$ and our $dsx^{i}$ yield fertile males and females indicating full complementation of the two mutations. The allele $dsx^{i}$ was discovered and kindly provided by A. Garen and briefly described as $dsx^{i}$ by Baker & Ridge (1980).

(iii) $dsx^{om}$, dominant, affecting only XX animals, one allele. Animals with genotype $X/Y$; $dsx^{om}$/+ are always sterile and show a very weak $dsx$ phenotype (reduced number of vaginal teeth, cleft in dorsal anal plate). $X/Y$; $dsx^{om}$/dsx show a strong $dsx$-phenotype with more male than female characteristics. $XY$-animals remain unaffected.

Lethal mutations within the $dsx$-deficiency. Forty lethals were detected within the interval of $Df(dsx)$. None of them gave an intersexual phenotype in
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combination with a dsx-allele; conversely, none of the eight new dsx alleles was lethal over the deficiency. The complementation pattern is complex and yielded 29 different complementation groups (Leist, 1983). The lethals were not analysed further.

3.2. Dominant mutations at the dsx-locus

(a) Description. So far, three different spontaneous dominant mutations at the dsx-locus were recovered: dsx<sup>o</sup> (Fung & Gowen, 1957; Duncan & Kaufman, 1975), dsx<sup>Mas</sup> (Mischakow, 1959; Nothiger et al. 1980), dsx<sup>r</sup> (kindly provided W. Gehring, Basel). They produce very similar phenotypes, and the following description thus applies to all three of them.

The terminalia of X/X; dsx<sup>o</sup>/+ flies carry abnormal male and female elements, with the female genital set anterior to the male set (Fig. 3a). The male genital structures are well developed, but the penis apparatus is reduced, the hypandrium mostly absent, and many bristles are abnormal. The female vaginal plate is always present, but reduced in size and with abnormal bristles. Between the vaginal plates, a mass of yellow, chitinized tissue is often enclosed, identified as a secondary rudimentary penis apparatus. The anal plates are arranged in a left–right position as in males, but the shape corresponds to a sexually intermediate form. The bristle pattern is a mosaic of male, female and intermediate bristles. The basitarsus of the foreleg are present, but they remained rudimentary in most flies studied. X/X; dsx<sup>o</sup>/Df(ds) flies are completely normal, i.e. look like females, but were sterile, a fact already noticed by Gowen & Fung (1957). The sixth tergite was darkly pigmented; the basal row of bristles on the foreleg showed a slight dsx-sex comb; the outer genitalia and analia were as in normal females, except that the vaginal plates and the seventh tergite carried a reduced number of bristles; the internal genitalia were also female, but not always complete. The parovaria, the uterus and the receptaculum seminis were absent in about 1/5 of the flies studied. Ovaries were present, but they remained rudimentary in most cases; some 40% of the animals also contained traces of testicular tissue.

(b) Description of 'reversions'. Among 2500 X/X; dsx<sup>o</sup>/+/+ flies, testing X/X; dsx<sup>o</sup>/Df(ds) flies, X/X; dsx<sup>o</sup>/dsx<sup>o</sup> flies as well as X/X; dsx<sup>o</sup>/Df(ds) flies are sterile pseudomales with perfect external and internal male genitalia and analia (Duncan & Kaufman, 1975; Nothiger et al. 1980; Baker & Ridge, 1980). The inner genitalia are male; the gonads are rudimentary testes and resemble those found in X/X; tra/tra pseudomales (Seidel, 1963).

Flies with the genotype X/X; dsx<sup>o</sup>/dsx<sup>r</sup> flies are sterile pseudomales with perfect external and internal male genitalia and analia (Duncan & Kaufman, 1975; Nothiger et al. 1980; Baker & Ridge, 1980) and female pattern. The differences between the two genotypes were insignificant. The 2X; 3A dsx<sup>o</sup> that arose in the same cross, the 2X; 3A dsx<sup>r</sup> flies looked very similar. Both had typically male sex combs that contained a reduced number of bristles. The segmentation pattern was variable, with seventh and eighth tergites sometimes being present, sometimes absent. The pigmentation of the fifth and sixth tergites was a mosaic for the male and female pattern. The differences between the two genotypes were insignificant. The 2X; 3A dsx<sup>o</sup> contained fewer external male genitalia than the 2X; 3A dsx<sup>r</sup> controls, which, in view of the masculinizing action of dsx<sup>r</sup>, is paradoxical. The effect cannot be ascribed to dsx<sup>r</sup>, but must be the result of differences in the genetic background which is known to influence the sexual phenotype of triploid intersexes.

All three 'revertants' were tested in trans with dsx<sup>x</sup>, dsx<sup>y</sup>, Df(dsx), and dsx<sup>o</sup>. These combinations revealed that the 'revertants' were recessive dsx-alleles of the type dsx<sup>o</sup>, as were the 'revertants' of dsx<sup>p</sup> and dsx<sup>Mas</sup> described by Duncan & Kaufman (1975) and by Belote et al. (1985). Over dsx<sup>Mas</sup> or Df(dsx) they showed an intersexual phenotype in both chromosomal sexes; over dsx<sup>p</sup> Sb<sup>+</sup> they transformed XX animals into pseudomales; with dsx<sup>x</sup> or dsx<sup>y</sup>, they gave an intersexual or normal phenotype, depending on the chromosomal sex.

3.3. Effects of variable doses of dsx<sup>o</sup> and dsx<sup>r</sup>

(a) Sexual phenotype of triploids and diploids

(1) Triploid flies with the genotype XX/ X; dsx<sup>o</sup>+/+ + looked like females, but were sterile, a fact already noticed by Gowen & Fung (1957). The sixth tergite was darkly pigmented; the basal row of bristles on the foreleg showed a slight dsx-sex comb; the outer genitalia and analia were as in normal females, except that the vaginal plates and the seventh tergite carried a reduced number of bristles; the internal genitalia were also female, but not always complete. The parovaria, the uterus and the receptaculum seminis were absent in about 1/5 of the flies studied. Ovaries were present, but they remained rudimentary in most cases; some 40% of the animals also contained traces of testicular tissue.

(2) Triploid intersexes with XX/Y; dsx<sup>o</sup>+/+ + displayed the general features of triploid intersexes, i.e. a mosaic pattern of male and female elements (Stern, 1966; Laüg, 1969), rather than the intersexual phenotype of dsx mutants. When compared to their siblings 2X; 3A dsx<sup>x</sup> that arose in the same cross, the 2X; 3A dsx<sup>p</sup> flies looked very similar. Both had typically male sex combs that contained a reduced number of bristles. The segmentation pattern was variable, with seventh and eighth tergites sometimes being present, sometimes absent. The pigmentation of the fifth and sixth tergites was a mosaic for the male and female pattern. The differences between the two genotypes were insignificant. The 2X; 3A dsx<sup>o</sup> contained fewer external male genitalia than the 2X; 3A dsx<sup>r</sup> controls, which, in view of the masculinizing action of dsx<sup>r</sup>, is paradoxical. The effect cannot be ascribed to dsx<sup>r</sup>, but must be the result of differences in the genetic background which is known to influence the sexual phenotype of triploid intersexes.

(3) Diploid flies with the genotype XX/ Y; dsx<sup>x</sup>; dsx<sup>o</sup>+/+ + looked like normal females, but were sterile (Fig. 3b). In contrast to the triploid flies with one dose of dsx<sup>o</sup> (see above), their ovaries were well developed containing all oogenic stages up to mature eggs, and all the other internal structures appeared also normal. They mated, and few of them even laid eggs, that, however, were never fertilized. Microscopical inspection of the receptaculum seminis showed that sperm were present at 0, 0.5, 2 or 24 h after copulation, but...
they were invariably immotile. In wild type control females, on the other hand, motile sperm were found at all times tested up to 24 h after copulation. It thus seems that the sperm became immobilized immediately after entry into the gonoducts of \( XX/Y.\ dsx^+;\ dsx^0\ Sb\ e/ red \) females.

(b) Ovary transplantation

The results, summarized in Table 2, show that \( XX/Y.\ dsx^+;\ dsx^0\ Sb\ e/ red \) ovaries, when attached to the gonoducts of a normal female, can in fact produce eggs that can be fertilized and give rise to adult flies. The experiment indicates that the gonadal soma of \( XX/Y.\ dsx^+;\ dsx^0\ Sb\ e/ red \) females must therefore reside outside of the germ line and the gonadal soma. The fact that normal sperm is instantly inactivated in the seminal receptacle of these females suggests a focus in the gonoducts which are the derivatives of the genital disc. The situation is reminiscent of meta-

(c) Flies with two dominant dsx-alleles, produced by ovary transplantation

The transplantation series A and B differ in the genotype of the tester males (Table 2) which in series B carry a \( Y \) chromosome with a duplication of \( dsx^+ \).

In series A, part of the progeny will have the genetic constitution \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \). Twelve such flies were produced by the three females with attached \( dsx^0 \) ovaries. They were phenotypically male and sterile with rudimentary testes, resembling \( X/X;\ tru/tru \) pseudomales in every respect (Fig. 3a). This observation confirms earlier results obtained with transplanted pole cells of \( X/X;\ dsx^0/\text{ma}^* \) embryos (Nöthiger et al. 1980).

In series B, the 12 females with attached \( dsx^0 \) ovaries produced an interesting genotype with two dominant \( dsx^-\)alleles and one dose of \( dsx^+ \) (\( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \)). We obtained 46 animals of this genotype all of which were typical pseudomales (Fig. 3c). A microscopic inspection disclosed that the 6th sternite was evenly covered with bristles, which is a female characteristic; the 6th sternite of normal males is devoid of bristles. The analia of \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) are slightly rounded compared with those of \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \). The genitalia are male, but morphologically not quite normal (compare Fig. 3c with 3a). These characteristics suggest that the additional dose of \( dsx^+ \) has a very weak feminizing effect.

Table 2. Transplantation of ovaries

| Series | Host* | Donor* | Tester male* | No. of surviving hosts | No. of fertile females | No. of attached donor ovaries |
|--------|-------|--------|--------------|------------------------|------------------------|-------------------------------|
| A      | Fs(1) K1237/Basc; TM3, Ser/+ | \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) or \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) | X/Y; dsx^0/\text{ma}^* /TM3 | 53 | 10 | 3 dsx^0 |
| B      | Fs(1) K1237/Basc; TM3, Ser/+ | \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) or \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) | X/Y; dsx^0/\text{ma}^* /TM2 | 111 | 27 | 12 dsx^0 |
| C      | \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \) | y w f^*mal/+/+ | 8 | 0 | 1 |
| D      | X.\ dsx^+/X; dsx^0/\text{ma}^* | y w f^*mal/+/+ | 23 | 0 | 2 |

* For complete genotypes of hosts and donors see Materials and Methods. The genotype of the donor ovary in series A and B is abbreviated in the right-hand column as \( dsx^0 \) corresponding to \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \), and as \( dsx^+ \) corresponding to \( XX/Y.\ dsx^+;\ dsx^0/\text{ma}^* \).
Fig. 3. Photographs of terminalia and forelegs of XX flies with variable doses of wild-type (dsx+) and dominant mutant (dsx\textsuperscript{D}, dsx\textsuperscript{Mes}) alleles of dsx. (a) X/X; dsx\textsuperscript{D}/dsx\textsuperscript{Mes} is a normal pseudomale. (b) X\textsuperscript{X}/Y. dsx\textsuperscript{+}; dsx\textsuperscript{D}/+ is practically female. Arrow points to basal row of bristles on the basitarsus of the foreleg; in males, this row is rotated by 90° and the bristles are thick with rounded tips (see SC in (a). (c) X\textsuperscript{X}/Y. dsx\textsuperscript{+}; dsx\textsuperscript{D}/dsx\textsuperscript{Mes} is essentially pseudomale, but the male genitalia are slightly less well developed than in (a), the analia are more round, and sternite 6 (S6) carries bristles. (d) X/X; dsx\textsuperscript{D}/+ is typically intersexual: note intermediate shape and position of sex comb bristles (arrow SC), presence of abnormal and incomplete male and female genital structures, position, shape and bristle pattern of anal plates. Female structures (underlined): AN\textsubscript{S}, anal plates; SPT, spermatheca; T7, tergite 7; T8, tergite 8; VP, vaginal plates. Male structures: AN\textsubscript{D}, anal plates; CL, claspers; GA, genital arch; HY, hypandrium; LP, lateral plates; PE, penis; SC, sex comb.
The function of the locus can be deduced from the mutant phenotypes, as summarized in Table 3. Recessive mutations that behave like null alleles show that $dsx^m$ is required for male development, and $dsx^f$ for female development. In the absence of either product, flies develop as intersexes with a phenotype that suggests that both sets of sex differentiation genes are simultaneously expressed in a cell. Shape and position of the bristles of the sex comb, e.g. are intermediate between the male and female type, similar to that shown in Fig. 3d. This suggests that the sex differentiation genes are constitutively active, and that the wild-type function of $dsx^m$ is to repress the female set, that of $dsx^f$ to repress the male set so that, in a normal fly, only one set of sex differentiation genes is expressed. The same conclusion was reached by Baker and his collaborators (review see Baker & Belote, 1983; Belote et al. 1985).

The dominant $dsx$ mutations are particularly informative. So far, three spontaneous dominant alleles ($dsx^D$, $dsx^{max}$, $dsx^U$) and one weaker allele ($dsx^u$, induced by EMS) have been found. In combination with a wildtype $dsx^+$ allele, the dominant mutations transform XX-zygotes into intersexes of the same type as do the recessive alleles; over $Df(dsx)$, $dsx^{max}$ or $dsx^U$, the strong spontaneous alleles transform XX-zygotes into pseudomasculines, but have no effect on XY-animals. They thus behave as constitutive mutations for $dsx^m$ while at the same time lacking $dsx^f$ function. The complementary dominant mutation, i.e. one that has a dominant feminizing effect on XY zygotes, has not been found. This fact has implications for our interpretation of the structure and regulation of the $dsx$-locus (see later).

Since $X/X$; $dsx^D/Df(dsx)$ is a pseudomale, the intersexual phenotype of $X/X$; $dsx^D/+$ must be ascribed to the wild-type allele. In $X/X$ animals, this wildtype allele is apparently expressed in the female mode, yielding the female-specific product of $dsx^f$. It is intriguing that $X/X$; $dsx^D/+$, in which $dsx^m$ and $dsx^f$ are simultaneously expressed, produces the same intersexual phenotype as $X/X$; $dsx^D/dsx^D$ in which no functional products are formed. If we introduce M and F to designate the functional products of $dsx^m$ and $dsx^f$, respectively, we can say that M and F neutralize each other which leads to abolishment of both functions so that neither the male nor the female differentiation genes are repressed.

We remember that $dsx^D$ in trans with all $dsx^{max}$
Table 3. The dsx-locus.

| Sex chromosomes | Types of mutationsa | Sex chromosomes | Pattern of activity at dsx-locus | Consequences on sex-specific differentiation genes | sex |
|-----------------|---------------------|-----------------|----------------------------------|--------------------------------------------------|-----|
|                 | dsx (dsx<sup>mt</sup>) | dsx<sup>m</sup> | dsx<sup>+</sup> | dsx<sup>0</sup> |                                |     |
| XX              | F                   | F               | F                     | Male set repressed                            | Female |
| XY              | M                   | M               | M                     | Female set repressed                           | Male  |

<sup>a</sup> Genetic analyses reveal two functions (complementation units) in dsx; the male-directing dsx<sup>m</sup> and the female-directing dsx<sup>+</sup> function (see text).

<sup>b</sup> In normal wild-type flies, dsx is regulated in such a way that either dsx<sup>m</sup> or dsx<sup>+</sup> is expressed. The respective products, M or F, are used the repress either the female set or the male set of sex differentiation genes. Absence of the required product results in intersexuality (Ø) due to expression of male and female differentiation genes. The allele dsx<sup>0</sup> expresses M constitutively.

0, female; Ø, male; F, intersexual.

alleles and with dsx<sup>+</sup> leads to male development; of the mutant alleles, only the male-specific dsx<sup>+</sup> results in intersexual development of X/X; dsx<sup>0</sup>/dsx<sup>m</sup>. This indicates that an intact functional product of dsx is required to neutralize dsx<sup>+</sup>. This neutralization could occur at the protein level. We arrive at this conclusion because the functional product of dsx appears to be a dimer or multimer, as suggested by the observation that dsx<sup>688</sup> and dsx<sup>501</sup>, 2 alleles of the dsx<sup>+</sup> type, complement in XY animals to give normal fertile males. It is therefore conceivable that the normal products of dsx<sup>m</sup> and dsx<sup>+</sup> which are both made in X/X; dsx<sup>0</sup>/+ can also aggregate, but such a heteromer would be non-functional.

Considering that XX/Y. dsx<sup>+</sup>; dsx<sup>0</sup>/+ produces a phenotype that is essentially female, and XX/Y. dsx<sup>+</sup>; dsx<sup>0</sup>/dsx<sup>m</sup> a phenotype that is essentially male, it appears as if F was titrated against M. If M and F were to form random aggregates, we would expect to find a minority of pure M-homomers in the XY animals and a few pure F-homomers in the latter. These homomers could lead to some intersexuality. We have in fact observed that XX/Y. dsx<sup>+</sup>; dsx<sup>0</sup>/+ are sterile 'females', and XX/Y. dsx<sup>+</sup>; dsx<sup>0</sup>/dsx<sup>m</sup> 'males' display some weak female characteristics (see Results).

We are aware that our titration model is rather naive, but we were struck by its simplicity and accuracy in accounting for the observed phenotypes. It can also accommodate the phenotype of triploid intersexes carrying a dsx<sup>0</sup>-allele (X/X; 3A). These flies display a mosaic of male and female structures, as do regular triploid intersexes, and show no signs of the presence of a dominant masculinizing mutation, such as more and larger male structures of a dsx-phenotype. The mosaic character of regular triploid intersexes is thought to result from the ambiguous value of the X:A signal that is read by some cells as male, by others as female (Baker & Belote, 1983; Steinmann-Zwicky & Nöthiger, 1985a, b). In X/X; 3A with one dose of dsx<sup>0</sup>, the constitutive expression of dsx<sup>m</sup> in those cells that embark on the male pathway is, of course, without consequences; in the cells that respond by implementing the female program, the one dsx<sup>0</sup> is counteracted by the two dsx<sup>+</sup> alleles now expressing F, a situation that will lead to a female phenotype.

4.2. Regulation of dsx

How is dsx regulated so that in a normal male the locus expresses the male-determining M-function of dsx<sup>+</sup>, and in a normal female the F-function of dsx<sup>0</sup>? – The epistatic relations displayed by double mutants have been used to infer a genetic hierarchy with the gene Sxl at the top and dsx at the end of the cascade; the role of tra-2, tra and ix is to mediate between Sxl and dsx (for reviews see Nöthiger & Steinmann-Zwicky, 1985; Belote et al. 1985).

We want to discuss two questions: (1) do all these genes act in a cascade by forming a single chain? and 2) is dsx regulated at the transcriptional or post-transcriptional level?

If the genes were arranged in a single chain, e.g. Sxl tra-2 tra ix and if the purpose of the cascade was to achieve expression of either dsx<sup>m</sup> or dsx<sup>+</sup>, then recessive (lack of function) mutations in either Sxl, tra-2, tra or ix should result in XX animals becoming transformed into pseudomales, whereas dominant (constitutive) mutations in any one of these four genes should transform XY animals into pseudofemales.

The predictions are fulfilled for recessive mutations in Sxl, tra-2, and tra, but not for the two alleles at the ix-locus; and dominant mutations with the expected phenotype are only known for Sxl, but not for tra, tra-2 or ix. Therefore, we think that tra-2, tra and ix do
not form a cascade, but are separately regulated and function in parallel; their concerted action achieves that $dsx$ makes a functional F-product. The genetic data which show two functions, $dsx_m$ and $dsx_s$, are compatible with the existence of two cistrons. Formally, we can imagine that expression of $dsx_m$ is the basic state, and that M represses F and other female-specific genes. When $dsx_m$ is repressed (by $tra^{+}$ and other upstream genes), $dsx_s$ can become active leading to F. Such a model, however, in which $dsx_s$ is OFF as a consequence of $dsx_m$ being on, and is ON whenever $dsx_m$ is OFF, is unlikely on several arguments:

- the constitutive mutations $dsx^0$, $dsx^{mas}$ and $dsx^*$ only act in cis and do not prevent the correct expression of $dsx_s$ by a wild-type allele in trans, as revealed by the intersexual phenotype of $X/X'; dsx^0/+;$
- mutations that abolish the $m$-function (3 alleles, Garen & Lepesant, pers. comm.) do not lead to constitutive expression of $dsx_s$ although the $f$-function is intact. In fact, no dominant constitutive mutations exist for $dsx_s$ whereas four are known for $dsx_m$;
- most mutations induced by EMS—which thus are mainly point mutations—abolish $m$ and $f$ simultaneously; the $m$ and $f$ functions appear small relative to the common function.

We want to propose that the $dsx$-locus is not regulated at the transcriptional level, but produces the same primary transcript(s) in XX and XY animals. This transcript has male-determining function. The female-determining transcripts could be made by splicing whereby $tra-2^*$ and $tra^+$ cooperate to remove or abolish the M-function. The ensuing product is non-functional, and acquires its functionality as F only after the product of $ix^*$ has further modified the transcript(s) or has combined with the $dsx$ protein.

The following arguments lend support to this hypothesis:

- when none of the genes $Sxl$, $tra-2$, $tra$, $ix$ are active, $dsx$ expresses the male function. Thus, the $M$-function represents the basic state of $dsx$-expression;
- when $tra$ or $tra-2$ is inactivated by mutations, XX animals develop as pseudomales. Thus, both genes, $tra-2^*$ and $tra^+$ cooperate in XX animals to abolish the $M$-function, say by removing the $m$-domain. But the resulting product is non-functional; it is only ‘demasculinized’ and does not yet function as F, as revealed by mutations in $ix$. When this gene is mutated, but $tra-2^*$ and $tra^+$ are active, XX animals turn into intersexes. Thus, $ix^*$ is required in XX animals to convey F-function to the $dsx$-product after $tra-2^*$ and $tra^+$ have modified the basic M-product. (We know that $tra-2^*$ and $tra^+$ are active in $X/X$; $ix/ix$ because such flies are intersexes whereas $X/X$; $ix/ix$; $tra/tra$ or $X/X$; $ix-2/ix-2$ are pseudomales.) This view predicts that the genotype $X/X'; ix/ix$; $dsx^0/dsx^{*}$, in which lack of $ix^*$ leaves the product of $dsx^0$ non-functional, should produce a pseudomale comparable to $X/X$; $dsx^0/dsx^{*}$. This is practically the case although some residual activity of F is noticeable, probably due to $ix$ being a leaky allele (see Figs. 1, 2);
- genotype $X/X$; $dsx^0/D(dsx)$ is a pseudomale showing that the mutant product of $dsx^0$ is resistant to the action of $tra-2^*$, $tra^+$ and $ix^*$, three genes that, we conclude, must be active in $X/X$; $dsx^0/+$. Since a functional F-product is formed by the wild-type allele. Reducing the dose of wild-type alleles at $tra-2$, $tra$, or $ix$ reduces the amount of F-product in $dsx^0/+$. This effect is dose-sensitive, these genes behave like structural genes performing an enzymatic reaction.

We think that the presented evidence, although indirect, is more supportive of a post-transcriptional than of a transcriptional mode of regulation. The preliminary molecular data are compatible with both views (Belote et al. 1985).

The locus of $dsx$ has recently been cloned (Belote et al. 1985). It is rather large comprising some 30 kb. We can extrapolate from the recombinational and molecular data of the $rosy$ locus which has a genetic length of 0.005 cM and a molecular size of 4.1 kb (Coté et al. 1986), our recombination distance of 0.03 cM between $dsx^0$ and $dsx^{mas}$ corresponds to some 24 kb, an estimate that is in good agreement with the molecular data. The $tra$-gene, on the other hand, is at least 10 times smaller, with the relevant information being contained within some 2 kb (Butler et al. 1986; McKeown et al. 1987). Northern analysis of the $dsx$ RNA show that sex-specific transcripts do in fact exist as anticipated on the basis of the genetic data. But the pattern of transcripts is more complex than expected, and sex-specific differences were so far only found in pupae and adults, but not in larvae (Belote et al. 1985). This latter result, however, must be due to insufficient sensitivity of the assays, since cloning analyses had shown that proper expression of $dsx$ is required during larval development (Baker & Ridge, 1980). Direct observations also indicate that $dsx$ is differentially expressed in male and female larvae. We conclude this because $X/Y$; $dsx^0/D(dsx)$ larvae are phenotypically male with testes anlagen and male genital discs (unpubl. obs.). These pseudomales are indistinguishable from $X/Y$; $dsx^0/*$ normal male larvae, but differ from $X/X$; $dsx^+/dsx^-$ female larvae.

The cloning of the sex-determining genes is currently under way (Belote et al. 1985; Maine et al. 1985a, b; Butler et al. 1986; McKeown et al. 1987). The molecular probes will allow us to test the hypotheses put forward by developmental geneticists, and they will eventually reveal the regulatory network of the genes governing sex determination.
Sex determination in Drosophila

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References

Baker, B. S. & Ridge, K. (1980). Sex and the single cell: on the action of major loci affecting sex determination in Drosophila melanogaster. Genetics 94, 383-423.

Baker, B. S. & Belote, J. M. (1983). Sex determination and dosage compensation in Drosophila melanogaster. Annual Review of Genetics 17, 345-393.

Belote, J. M., McKeown, M. B., Andrew, D. J., Scott, T. N., Wolfner, M. F. & Baker, B. S. (1985). Control of sexual differentiation in Drosophila melanogaster. Cold Spring Harbor Symposia on Quantitative Biology 50, 603-614.

Bridges, C. B. (1921). Tripletoid intersexes in Drosophila melanogaster. Science 54, 252-254.

Butler, B., Pirrotta, V., Irminger-Finger, I. & Nothiger, R. (1986). The sex-determining gene tra of Drosophila: molecular cloning and transformation studies. EMBO Journal 5, 3607-3613.

Busson, D., Gans, M., Komitopoulou, K. & Masson, M. (1983). Genetic analysis of three dominant female sterile mutations located on the X-chromosome of Drosophila melanogaster. Genetics 105, 309-325.

Cline, T. W. (1986). A female-specific lethal lesion in an X-linked positive regulator of the Drosophila sex determination gene, Sex-lethal. Genetics 113, 641-663.

Coté, B., Bender, W., Curtis, D. & Chovnick, A. (1986). Molecular mapping of the rosy locus in Drosophila melanogaster. Genetics 112, 769-783.

Duncan, F. W. & Kaufman, T. C. (1975). Cytogenetic analysis of chromosome 3 in Drosophila melanogaster. Mapping of the proximal portion of the right arm. Genetics 80, 733-752.

Epper, F. (1981). Morphological analysis and fate map of the intersexual genital disc of the mutant double-sex dominant in Drosophila melanogaster. Developmental Biology 88, 104-114.

Fung, S. T. C. & Gowen, J. W. (1957). The developmental effect of a sex-limited gene in Drosophila melanogaster. Journal of Experimental Zoology 134, 515-532.

Gowen, J. W. & Fung, S. T. C. (1957). Determination of sex through genes in a major sex locus in Drosophila melanogaster. Heredity 11, 397-402.

Grüter, A. (1983). Genetische Feinstrukturanalyse am doublesex-Locus von Drosophila melanogaster. Diploma Thesis, University of Zurich, Switzerland.

Hildreth, P. E. (1965). Doublesex, a recessive gene that transforms both males and females of Drosophila melanogaster into intersexes. Genetics 51, 659-678.

Hilfiker, A. (1983). Gendosisanalyse X-chromosomaler und autosomaler Geschlechtsbestimmungsfaktoren unter Berücksichtigung der Temperatur bei Drosophila melanogaster. Diploma Thesis, University of Zurich, Switzerland.

Janning, W. (1976). Entwicklungsgenetische Untersuchungen an Gynandren von Drosophila melanogaster. IV. Vergleich der morphogenetischen Anlagepläne larvaler und imaginaler Strukturen. Wilhelm Roux's Archives of Developmental Biology 179, 349-372.

Janning, W., Labhart, C. & Nöthiger, R. (1983). Cell lineage restrictions in the genital disc of Drosophila revealed by Minute gynandromorphs. Wilhelm Roux's Archives of Developmental Biology 192, 337-346.

King, R. C. (1970). Ovarian development in Drosophila melanogaster. New York: Academic Press.

Laugé, G. (1969). Influence de la temperature d'élevage sur l'expression des caracteres sexuels externes et internes des intersexues triploides de Drosophila melanogaster. Comptes Rendues de l’Académie des Sciences Paris 255, 1798-1800.

Leist, Ch. (1983). Komplementationsanalyse von 40 rezessiven Letalfaktoren in der double-sex Region (84F 2-3; 84F 16) von Drosophila melanogaster. Diploma Thesis, University of Zurich, Switzerland.

Lewis, E. B. & Bacher, F. (1968). Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Drosophila Information Service 43, 193.

Lindsay, D. L. & Grill, E. H. (1968). Genetic variations of Drosophila melanogaster. Carnegie Institution of Washington Publication No. 627.

Maine, E. M., Salz, H. K., Schell, P. & Cline, T. W. (1985a). Sex-lethal, a link between sex determination and sexual differentiation in Drosophila melanogaster. Cold Spring Harbor Symposium on Quantitative Biology 50, 595-604.

Maine, E. M., Salz, H. K., Cline, T. W. & Schell, P. (1985b). The Sex-lethal gene of Drosophila: DNA alterations associated with sex-specific lethal mutations. Cell 43, 521-529.

McKeown, M., Belote, J. M. & Baker, B. S. (1987). A molecular analysis of transformer, a gene in Drosophila melanogaster that controls female sexual development. Cell 48, 489-499.

Mischaikow, E. (1959). Mas: Masculinizer. Drosophila Information Service 33, 98.

Monod, J. & Poulson, D. F. (1936). Specific reactions of the ovary to interspecific transplantation among members of the melanogaster group of Drosophila. Genetics 22, 257-263.

Nöthiger, R., Roost, M. & Schüpbach, T. (1980). ‘Masculinizer’ is an allele of ‘doublesex’. Drosophila Information Service 55, 118.

Nöthiger, R. & Steinmann-Zwicky, M. (1985). Sex-determination in Drosophila. Trends in Genetics 1, 209-215.

Roost, M. (1978). Das Zusammenwirken geschlechtsbestimmender Gene bei Drosophila melanogaster unter besonderer Berücksichtigung der Mutation doublesex Dominant. Diploma Thesis, University of Zurich, Switzerland.

Schüpbach, T., Wieschaus, E. & Nöthiger, R. (1978). A study of the female germ line in mosaics of Drosophila. Wilhelm Roux's Archives of Developmental Biology 184, 41-56.

Seidel, S. (1963). Experimentelle Untersuchungen über die Grundlagen der Sterilität von transformer (tra) Männchen bei Drosophila melanogaster. Zeitschrift für Vererbungslehre 94, 215-241.

Steinmann-Zwicky, M. & Nöthiger, R. (1985a). The hierarchical relation between X-chromosomes and autosomal sex-determining genes. EMBO Journal 4, 163-166.

Steinmann-Zwicky, M. & Nöthiger, R. (1985b). A small region on the X chromosome of Drosophila regulates a key gene that controls sex determination and dosage compensation. Cell 42, 877-887.

Stern, C. (1966). Pigmentation mosaicism in intersexes of Drosophila. Revue Suisse de Zoologie 73, 339-355.

Ursprung, H. (1967). In vivo culture of Drosophila imaginal discs. In ‘Methods of Developmental Biology’ (ed. F. H. Wilt and N. K. Wessells), pp. 485-492. New York: T. Y. Crowell.