Molecular Genetic Aspects of Sex Determination in *Drosophila*

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And the rib, which the LORD GOD *had taken from man, made he a woman.* Genesis 2:22, King James Version.

Summary

Analysis of the mechanisms underlying sex determination and sex differentiation in Drosophila has provided evidence for a complex but comprehensible regulatory hierarchy governing these developmental decisions. It is suggested here that the pattern of sexual differentiation and dosage compensation characteristic of the male is a default regulatory state. Recent results have provided, in addition, some surprising and intriguing conclusions: (1) that several of the critical controlling genes produce more transcripts than was predicted from the genetic analyses; (2) that setting of the alternative sex-specific states of the doublesex (dsx) locus involves differential transcript processing; and (3) that some aspects of sexual differentation require the prolonged action of certain elements of the regulatory hierarchy. These findings are discussed in connection with the current model of sex determination in Drosophila.

Introduction

Sexual differences have probably aroused human interest since the proverbial serpent did its bit. Among biologists, sexual differences have been the subject of studies from almost every possible perspective – physiological, evolutionary, developmental, genetical, behavioral, neurological, etc. As molecular geneticists, our interest is in the process of sexual differentiation. What are the regulatory mechanisms that differentially control gene expression during development to produce a male or female? Although the primary chromosomal sex-determining mechanisms have been described in a great variety of species,[1,2] it is only in the past decade that we have begun to understand in three organisms – the yeast *Saccharomyces cerevisiae*,[3] the nematode *Caenorhabditis elegans*,[4] and the fruit fly *Drosophila melanogaster*[5,6] – the regulatory cascades that translate this initial step in sexual development into a sexually mature adult. Here we focus on recent work in *Drosophila melanogaster* that has given a molecular as well as genetical understanding of some of the genes controlling sexual differentiation.

The System

The cell lineages producing the soma and germline of the Drosophila adult separate from each other, and from the larval progenitor cells, during embryonic development. In the embryo and larvae, the progenitor cells of the adult soma are undifferentiated dividing cells, morphologically indistinguishable from one another, until they stop dividing and differentiate in the pupae. Nevertheless, it is during the embryonic and larval periods that many of the regulatory events occur that determine the patterns of sexual differentiation of the adult. It is with these regulatory events that we are concerned.

Sex in Drosophila is determined by the ratio of X chromosomes to sets of autosomes (X:A ratio) and is independent of the Y chromosome. The X:A ratio is also the primary determinant of dosage compensation, a process that equalizes the amount of product produced by the genes on the male's single sex chromosome and the female's two sex chromosomes.[7] A number of the genetic components involved in the assessment of the X:A ratio and the initial transmittal of that decision have been recently identified and subjected to extensive genetic analysis. Here we will focus on the consequences of this initial decision (work on these initial events themselves has been previously reviewed;[8] for more recent studies on this topic see Refs. 8 and 9).

The Regulatory Hierarchy Controlling Sex

The assessment by a cell of its X:A ratio during embryogenesis (in flies each cell determines its own sex; there are no known hormonal components to sex determination) is transmitted by means of a set of regulatory genes to: (1) the genes on the X chromosome whose activities are dosage-compensated; and (2) the genes throughout the genome whose products are responsible for generating the somatic sexual dimorphisms of the adult; and (3) the germline.

The cascade of regulatory genes that controls somatic sex determination and X transcription in females is relatively elaborate; in contrast the situation in males is simple. A major hypothesis we elaborate here is that a male develops when there is not an active signal generated by the assessment of X:A ratio, and these regulatory genes are consequently expressed in default states. By 'default state' we do not mean that all of these regulatory genes are inactive in a male – they are not – but rather that their expression (or lack of expression) is determined solely by their interactions with the general cellular transcriptional and translational machinery and not by any sex-specific control. Conversely, a female develops when an active signal from the X:A ratio causes the expression of additional regulatory genes.

Only one sex-determination regulatory gene is known that functions in males (Fig. 1). This gene, *doublesex* (*dsx*), is unusual in that it has active but opposite genetic functions in the two sexes.[10] In males the active function of *dsx* is to repress the genes that are involved in female sexual differentiation. Male differentiation, not being repressed, occurs. No functions are known that are necessary only in males for *dsx* to be expressed in this manner. This suggests the hypothesis that the *dsx* male function is the normal form of expression of this gene and will simply occur unless there is information to the contrary.

In females the *Sxl* locus is activated in response to the X:A ratio (Fig. 1). The Sxl^+ function is necessary in females for somatic sexual differentiation, germline function, and the female transcriptional level of X-linked genes.[6] To regulate sexual differentiation in all somatic cells the Sxl^+ gene is believed to activate two other regulatory genes, *transformer* (*tra*) and *transformer-2* (*tra-2*). The tra^+ and $tra\text{-}2^+$ products allow the expression of the *dsx* female function which acts to repress male sexual differentiation.[10] Female differentiation, not being repressed, occurs. The repression of male differentiation is carried out by the *dsx* gene in conjunction with another female-specific regulatory gene, *intersex* (*ix*).[10]

Dosage compensation in flies is achieved by transcribing the male's X chromosome at a rate equal to that of the female's two X chromosomes.[7] This hypertranscription of the male's X chromosome requires the products of four male-specific lethal (msl) loci (Fig. 1).[11] No genes are known that function between the assessment of the X:A ratio and the activation of the msl loci in males. Thus we suggest that the default states of the msl loci are such that they are normally expressed. In females, the control of X-linked gene transcription is also achieved via the Sxl^+ locus, which functions to prevent the expression of the msl loci.[12]

Molecular Analysis of Regulatory Genes

The molecular analyses of *Sxl*,[13,14] *tra*[15,16] and *dsx*[16] (and our unpublished results) are consistent with the genetic models of this hierarchy and suggest molecular mechanisms for how these genes interact. All three genes have been cloned and mapped by use of chromosomal rearrangements. Northern blot analyses reveal complex patterns of transcriptional regulation for all three genes.

Each gene has sex-specific transcripts whose sexual and temporal patterns of expression are consistent with the genetic analyses. In the cases of *Sxl* and *tra* there are female-specific transcripts in agreement with the finding that these genes have female-specific functions. These female-specific transcripts appear by the times when these genes are

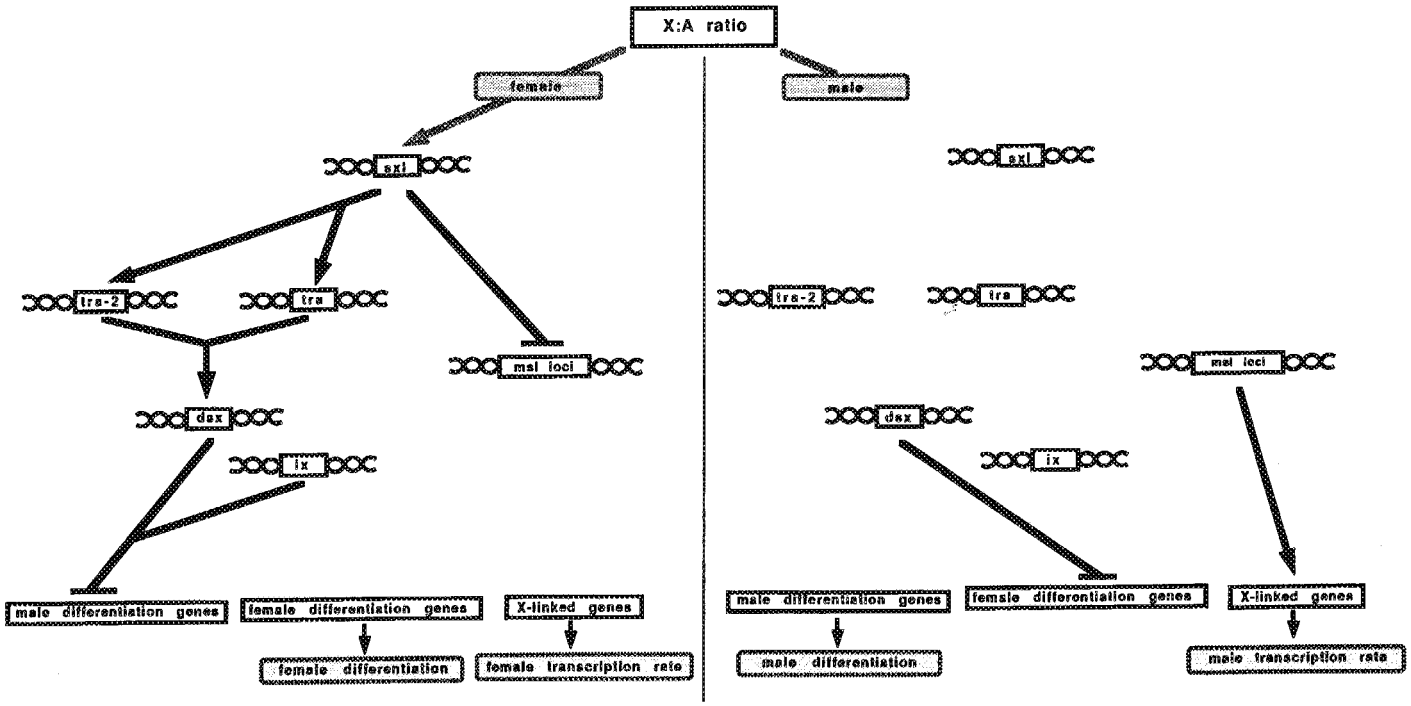

Fig. 1. *Outline of the regulatory hierarchy controlling sexual differentiation and dosage compensation in Drosophila. Arrows between genes indicate positive control. A bar at the end of a line indicates negative control.*

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known to function from developmental studies: in the embryo and in the late larval period for *Sxl* and *tra*, respectively. The *dsx* locus produces both male- and female-specific transcripts beginning in the late larval period, consistent with the finding that *dsx* has different roles in controlling sexual differentiation in the two sexes and is expressed by the pupal period. Given the concordance of the sexual and temporal patterns of these transcripts with the patterns of activity of these genes inferred from genetic studies, it is likely that these transcripts represent the functional products of these genes. It should be noted, however, that for both *Sxl* and *dsx* there are more sex-specific transcripts than are needed to account for the genetic results.

All three of these genes have additional transcripts, whose patterns of appearance are unexpected. The *Sxl* locus has sex-non-specific and male-specific transcripts. The *tra* locus also has a sex-non-specific transcript and the *dsx* locus has two sex-non-specific transcripts. The patterns of appearance of these unexpected transcripts are related to those of the other transcripts from these genes, implying that these transcripts are not from overlapping but from unrelated genetic functions. For both *tra* and *Sxl*, the sex-non-specific and male transcripts occur at the same times as the female-specific transcripts. Disappearance of the sex-non-specific *dsx* transcripts is at least roughly, and perhaps exactly, coincident with the appearance of the sex-specific *dsx* transcripts.

There are several pieces of data which substantially delimit the possible functions of these transcripts. For both *Sxl* and *tra* there are tiny deletions that remove all or parts of these genes, including the regions encoding the sex-non-specific and male transcripts. These deletions are homozygous viable in males where they have no detectable phenotypic effect. Thus these sex-non-specific transcripts (and male transcripts in the case of *Sxl*) may be produced solely as a necessary consequence of some aspect of determination or the transcriptional machinery in the cell and be without biological functions. Alternatively, they may encode functions too subtle to detect, or may function to negatively regulate the genes removed by the respective deletions (i.e. they may represent negative auto-regulatory functions).[13,15]

The sex-specific transcription patterns of these genes suggest that a major aspect of their regulation is at the level of transcription and transcript processing. However, the temporal pattern of these transcripts is not sufficient to explain these genes' observed hierarchical relationship. In particular the transcript pattern of *dsx* changes at the end of the larval period in a similar fashion in both sexes: the sex-non-specific transcripts cease to be produced and sex-specific transcripts appear. In females, the female-specific transcripts from the *Sxl* and *tra* genes are present substantially before this time, and in males no regulatory function is known that functions between the assessment of the X:A ratio and *dsx*. This suggests to us that an additional regulatory signal is expressed at the end of the larval period that is responsible for this change in the *dsx* transcription pattern. It is intriguing that at about this time the transcriptional activities of a large array of genes are altered in preparation for pupariation by changes in the titers of the steroid hormone ecdysone and juvenile hormone.

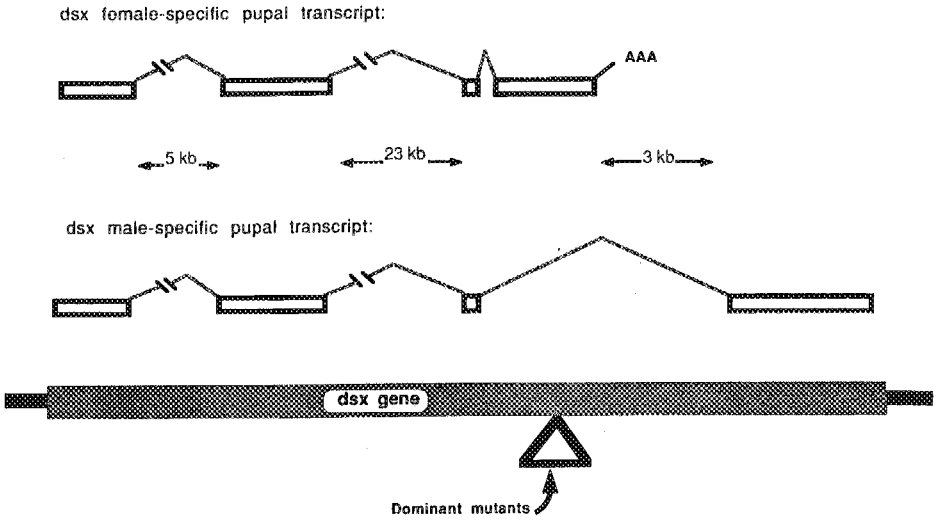

Fig. 2. *Relationship between the sex-specific pupal transcripts at the* dsx *locus. Open boxes represent exons. The 5′ end of the gene is to the left. The extent of the* dsx *gene (shaded bar) and the location of the dominant mutations in the gene is represented at the bottom of the figure. The 3′ end of the male-specific transcript has not been located.*

The signal initiating the production of the sex-specific *dsx* transcripts at the end of the larval period is probably the same in the two sexes, since the male- and female-specific *dsx* transcripts are quite similar in structure (Fig. 2). Mapping and sequencing of cDNAs has shown that the male- and female-specific *dsx* transcripts have in common most, if not all, of their 5′ exon (it has not been established if they have the same 5′ end) and identical second and third exons. They differ by the presence of 3′ sex-specific exons. This suggests that a regulatory event common to the two sexes allows the production of a primary *dsx* transcript which is then differentially processed in a sex-specific manner. In females this processing is achieved by the products of the *tra-2* and *tra* genes. We do not know at what level the *tra* gene's products are controlling *dsx* expression; the data are consistent with regulation of splicing, poly-A addition or transcript termination.

That the *tra* and *tra-2* gene products are the sole difference between males and females in the regulation of *dsx* expression during the larval and pupal period is suggested by the following considerations. An active function of the *dsx* locus is required in both males and females for normal sexual differentiation: null *dsx* mutants transform both sexes into intersexes. However, the absence of either the *tra* or *tra-2* function in a chromosomally female individual does not transform that individual into an intersex, but rather into a male. This implies that the *dsx* male function is expressed in a female in the absence of the products of the tra loci.[10] Thus females have everything necessary to process the primary dsx transcript into the male product, but fail to do so only because the wild-type products of the tra loci lead to the alternative (female) pattern of RNA processing.

This view is supported by the analysis of dominant *dsx* mutants. There are four dominant *dsx* mutants (Ref. 10 and unpublished) and all have similar properties: they are null for the *dsx* female function and express the *dsx* male function regardless of chromosomal sex. Thus if a *dsx* dominant mutant is the only *dsx* allele in a chromosomally female individual, the individual is transformed into a male. Molecularly, three of these dominant mutants are associated with insertions

of middle repeat sequences into *dsx* and the fourth mutant is a tiny deletion. All four are located within the 3′ female-specific *dsx* exon. We hypothesize that the mutations in some way prevent the processing of the primary *dsx* transcript into the female-specific mRNAs, and it is instead processed to yield the male mRNA in the inappropriate sex.

The Downstream Genes

The ultimate function of this regulatory hierarchy is to control the genes whose products are differentially expressed in the somatic cells of the two sexes. *A priori* these regulatory genes could act at one point during development to set the pathway of sexual differentiation irreversibly. Alternatively they could act throughout development to allow the expression of the appropriate developmental program.

These possibilities have been examined through the use of temperature-sensitive *tra-2* alleles (*tra-2*^{ts}).[17] Chromosomally female individuals homozygous for *tra-2*^{ts} develop as females at the permissive temperature of 16 °C but as males at the restrictive temperature of 29 °C. By shifting such individuals from one temperature to the other at various times it was found that this regulatory hierarchy is required throughout much of development for female sexual differentiation. Surprisingly, even within single cell lineages sex is not determined at one time, but rather different aspects of sex are determined at different times. One interpretation of this result is that different sexual differentiation functions need to be controlled at different times within a cell.

At least some of the individual genes under the control of this hierarchy require the continuous presence of the functional products of these regulatory genes for the initiation and maintenance of their expression. The best-studied sex-specific terminal differentiation functions are the three yolk protein (YP) genes. They are expressed only in females and are under the control of the sex-determination regulatory genes described above.[5] Temperature-shift experiments with homozygous *tra-2*^{ts} females showed that yolk protein synthesis is temperature-sensitive in the adult and is regulated at the level of transcription (or transcript stability).[18] Moreover, functional *tra-2* product is needed for both the initiation and maintenance of their transcription.

The YP genes are probably representative of many terminal sexual differentiation genes in that they need information about tissue, developmental stage, and sex for their expression. For the YP genes some of this additional trans-acting regulatory information is provided hormonally by 20-hydroxyecdysone and/or juvenile hormone.[19] This control appears to be independent of sex, since the titers of 20-hydroxyecdysone are the same in males and females during the pupal and adult periods.[20] It is likely that eclosion triggers the hormonal events necessary for YP gene expression. The sex-specific adult pattern of YP gene expression can be accounted for by the *dsx*$^{+}$ gene acting in the fat body of the male to prevent their expression regardless of hormone concentration, whereas in females the YP genes are not repressed and can thus respond to hormonal signals.

The sex-determination regulatory hierarchy also actively controls sexual courtship behavior in the adult.[21] Homozygous *tra-2*^{ts} females reared to adulthood at the permissive temperature do not display male courtship behavior. However, if they are then shifted to the restrictive temperature for several days, some male courtship behavior is often exhibited. This suggests that the differentiated state of the adult CNS is not immutably fixed at some earlier stage of development, but rather that a particular state of differentiation is maintained by the active control of gene expression in the adult CNS.

Not all of the genes under the control of this hierarchy are regulated like the YP genes.[22] The expression of a gene encoding an adult male-specific transcript is not affected by temperature shifts of chromosomally female *tra-2*^{ts} adults. There are several possible interpretations of this finding.[22] One we find attractive is based on the observation that this male-specific transcript is found in a tissue, the paragonia (a somatic tissue associated with the testis), that is present only in males. It may be that the paragonia is irreversibly determined to be male at some time prior to adulthood, and this then precludes the *tra-2* gene's expression in this tissue.

An Overview

The genetic and molecular analyses of the regulatory hierarchy governing sex and dosage compensation in Drosophila provide a substantial outline of how this central developmental process is regulated. We suggest that the pattern of sexual differentiation and dosage compensation characteristic of a male is a default regulatory state. In the absence of information to the contrary (i.e. that the organism, or cell, is female) the *msl* loci will be expressed and elicit hypertranscription of the X chromosomal genes and the *dsx* locus will function to bring about male differentiation. If, on the other hand, the X:A ratio is perceived as female, a signal is generated that results in the switching of these regulatory genes from their default states to those characteristic of a female. This is achieved by activating the *Sxl* locus, which in turn has at least two functions. With respect to X transcription, *Sxl*$^{+}$ expression results in turning off the *msl* loci. With respect to sex determination, *Sxl*$^{+}$ expression results in the activation of the *tra-2* and *tra* loci, whose products impose an alternative pattern of RNA processing on the pupal transcript from the *dsx* locus. This model has several caveats. Additional genes may be involved in the regulation of sex determination, and their discovery could modify this view; searches for new sex-determination regulatory genes are under way.[16] Secondly, the molecular characterizations of the *Sxl*, *tra* and *dsx* loci reveal more transcripts than expected from genetic analysis, as well as transcripts whose patterns of expression are not predicted. Depending on the nature of the functions (if any) of these transcripts it may be necessary to modify the model.

The control of sexual differentiation by this hierarchy appears to be negative: in females it functions to insure that male-specific sexual differentiation functions are not expressed, while in males it functions to prevent the expression of female sexual differentiation functions. In the absence of the functioning of this regulatory hierarchy in either sex (e.g. a null *dsx* mutant) both male and female sexual differentiation functions are expressed, resulting in intersexual development. From an evolutionary perspective this is consistent with a view of sexuality having arisen in an ancestral organism in which both male and female sexual differentiation occurred (i.e. a hermophrodite) by the acquisition of the ability to turn off either male or female differentiation functions.

One of the relatively unexpected features of sex determination in Drosophila is the span of developmental time across which the sex-determination regulatory hierarchy functions to regulate gene expression – from shortly after fertilization into adulthood. Sexual differentiation is also a surprisingly dynamic process. Although the

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sexual phenotype of some parts of a fly, such as the sexually dimorphic parts of the cuticle, obviously become immutably fixed during development, this is not the case for all aspects of sexual differentiation. For both courtship behaviour and the YP genes, their regulation depends on the continuous functioning of this regulatory hierarchy in the adult.

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REFERENCES

1 WHITE, M. J. D. (1973). *Animal Cytology and Evolution*. 3rd edn. Cambridge University Press, Cambridge, U.K.

2 BULL, J. J. (1983). *Evolution of Sex Determining Mechanisms*. Benjamin Cummings, Menlo Park.

3 NASMYTH, K. A. (1982). Molecular genetics of yeast mating type. *Annu. Rev. Genet.* **16**, 439–500.

4 HODGKIN, J., DONIACH, T. & SHEN, M. (1985). The sex determination pathway in the nematode *Caenorhabditis elegans*: variations on a theme. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 585–593.

5 BAKER, B. S. & BELOTE, J. M. (1983). Sex determination and dosage compensation in *Drosophila melanogaster*. *Annu. Rev. Genet.* **17**, 345–393.

6 CLINE, T. W. (1985). Primary events in the determination of sex in *Drosophila melanogaster*. In *Origin and Evolution of Sex* (ed. H. O. Halvorson and A. Monroy), pp. 301–327. Alan R. Liss, New York.

7 LUCCHESI, J. C. & MANNING, G. (1987). Genetics of dosage compensation in *Drosophila melanogaster*. *Adv. in Genetics* (in press).

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

9 STEINMANN-ZWICKY, M. & NOTHIGER, R. (1985). A small region on the X chromosome of Drosophila regulates a key gene that controls sex determination and dosage compensation. *Cell* **42**, 877–887.

10 BAKER, B. S. & RIDGE, K. A. (1980). Sex and the single cell. 1. On the action of major loci affecting sex determination in *Drosophila melanogaster*. *Genetics* **39**, 383–423.

11 BELOTE, J. M. & LUCCHESI, J. C. (1980). Control of X chromosome transcription by the maleless gene in Drosophila. *Nature* **285**, 573–575.

12 LUCCHESI, J. C. & SKRIPSKY, T. (1981). The link between dosage compensation and sex determination in *Drosophila melanogaster*. *Chromosoma* **82**, 217–227.

13 MAINE, E. M., SALZ, H. K., SCHEDL, P. & CLINE, T. W. (1985). The Sex-lethal gene of Drosophila: DNA alterations associated with sex-specific lethal mutations. *Cell* **43**, 521–529.

14 MAINE, E. M., SALZ, H. K., SCHEDL, P. & CLINE, T. W. (1985). Sex-lethal, a link between sex determination and sexual differentiation in *Drosophila melanogaster*. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 595–604.

15 MCKEOWN, M., BELOTE, J. & BAKER, B. S. (1987). A molecular analysis of *transformer*, a gene in *Drosophila melanogaster* that controls female sexual differentiation. *Cell* (in press).

16 BELOTE, J. M., MCKEOWN, M. B., ANDREW, D. J., SCOTT, N. T., WOLFNER, M. F. & BAKER, B. S. (1985). Control of sexual differentiation in *Drosophila melanogaster*. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 605–614.

17 BELOTE, J. M. & BAKER, B. S. (1982). Sex determination in *Drosophila melanogaster:* analysis of *transformer*-2, a sex determining locus. *Proc. Natl Acad. Sci. USA* **79**, 1568–1572.

18 BELOTE, J. M., HANDLER, A. M., WOLFNER, M. F., LIVAK, K. L. & BAKER, B. S. (1985). Sex-specific regulation of yolk protein gene expression in Drosophila. *Cell* **40**, 339–348.

19 POSTLETHWAIT, J. H. & HANDLER, A. (1979). The roles of juvenile hormone and 20-hydroxyecdysone during vitellogenesis in isolated abdomens of *Drosophila melanogaster*. *J. Insect Physiol.* **25**, 455–460.

20 HANDLER, A. M. (1982). Ecdysteroid titers during pupal and adult development in *Drosophila melanogaster*. *Devl. Biol.* **93**, 73–82.

21 BELOTE, J. M. & BAKER, B. S. (1987). The genetic control of the development and expression of a sex-specific behavioral pattern in Drosophila. (submitted).

22 DIBENEDETTO, A. J., LAKICH, D. M., KRUGER, W. D., BELOTE, J. M., BAKER, B. S. & WOLFNER, M. W. (1986). Sequences expressed sex-specifically in *Drosophila melanogaster* adults. *Devl. Biol.* (in press).

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