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The regulation of male-specific transcripts by sex determining genes in *Drosophila melanogaster*

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The accumulation of male-specific transcripts in various genotypes of *Drosophila melanogaster* was analysed by Northern blot hybridization. The genotypes were either hetero- or homozygous for a mutation in one of the genes for somatic sex determination. The aim was to study the role of these genes in regulating male-specific transcription in soma or germ line. All intersexual phenotypes and pseudomales irrespective of their genotypic sex accumulate the male-specific somatic RNA demonstrating that it is regulated by these genes. In contrast to this, the transcript from the male germ line could only be detected in those mutant genotypes that have the male sex chromosome constitution of X/Y, although its synthesis is not dependent on the presence of a Y chromosome. It is, therefore, not under the control of the sex determining loci but directly regulated by the primary signal for sex determination, namely the ratio of X chromosomes to autosomes.

Key words: Drosophila/male-specific RNAs/sex determining genes/soma/germ line

Introduction

In *Drosophila*, sex determination is regulated by the ratio of X chromosomes (X) to sets of autosomes (A). An X/X;A/A genotype with a ratio of 1.0 results in female development, whereas a ratio of 0.5 (genotype: X/Y;A/A) leads to male development. The Y chromosome is without any effect on sexual differentiation. The basic signal of the X:A ratio is then processed by some regulatory genes responsible for sex determination and dosage compensation (for review, see Baker and Belote, 1983; Nöthiger and Steinmann-Zwicky, 1985). Genetic analyses of those genes suggest that they function together as parts of a regulatory hierarchy.

Four autosomal genes are known which are involved in the determination of the somatic sex. Two of them, namely transformer (*tra*; Sturtevant, 1945) and transformer-2 (*tra-2*; Watanabe, 1975) transform X/X flies into sterile pseudomales. The mutations intersex (*ix*; Morgan *et al.*, 1943) and doublesex (*dsx*; Hildreth, 1965) on the other hand transform flies to intersexes. While *ix* acts only in X/X flies, *dsx* can transform both X/X and X/Y genotypes although sex-specific alleles exist (see Baker and Belote, 1983).

According to the model of Baker and Ridge (1980) the expression of the bifunctional *dsx* locus is controlled by the products of the other three genes in the following manner. In X/Y;A/A genotypes the three genes are in the OFF position, thus leaving the *dsx* locus in its ground status, i.e. dsx^m ON and dsx^f OFF. This situation allows male differentiation to occur whereas female differentation is repressed. In X/X;A/A genotypes, on the other hand, the *tra*, *tra-2*, and *ix* locus are set to ON. This switches dsx^m to OFF and dsx^f to ON, leading to female differentiation and repressing male one.

Temperature shift experiments with temperature-sensitive *tra-2* alleles suggest that $tra\text{-}2^+$ function is required throughout development for normal sexual differentiation (Belote and Baker, 1982). The same is probably true for the other three genes, too (see Baker and Belote, 1983). Analysis of the activity of the genes for the yolk proteins (YPs) provided further evidence that the functional products of these regulatory loci are required in differentiated cells of the adult fly. The female-specific synthesis of the YPs was shown to be dependent on the four sex determination regulatory genes (Postlethwait *et al.*, 1980; Bownes and Nöthiger, 1981; Ota *et al.*, 1981). Furthermore, using the $tra\text{-}2^{ts}$ alleles it could be demonstrated that a functional *tra-2* gene product is necessary to initiate and maintain the transcription of the female-specific *YP* genes (Belote *et al.*, 1985).

The present study is aimed at extending these results by examining, at the molecular level, how the accumulation of male-specific transcripts is controlled by these four regulatory genes. Since it was shown by transplantation experiments that the four regulatory loci do not interfere with sex determination in the germ line (Marsh and Wieschaus, 1978; Schüpbach, 1982) special attention is paid to differences in the response of somatic or germ line expression. This was possible due to the cloning of the genes that are exclusively expressed either in the male accessory glands or in spermatogenic stages (Schäfer, 1986).

The results presented here show that somatic male-specific transcription is dependent on the sex determination regulatory hierarchy. Thus, pseudomales or intersexes, irrespective of their genotype, accumulate transcripts specific for the male accessory gland. In contrast to this, the RNA specific for the male germ line can only be detected in transformed flies with an X:A ratio of 0.5 and is, therefore, not under the direct control of the regulatory genes.

Results

Transcript accumulation in X/0 flies

Description of clones. Out of the isolated set of clones for male-specific transcripts in *D. melanogaster* (Schäfer, 1986) two representatives were used in this study. As an example for somatic transcription in males the clone *mst(2)ag-15* was used. This clone encodes a poly(A)$^+$ RNA of ~350 nucleotides length which is exclusively accumulated in the male accessory glands, the so-called paragonia. The second clone, named *mst(3)gl-9* codes for an ~450-nucleotide RNA which is transcribed only in the male germ line. An additional RNA species of ~1.5 kb length which is not germ line-specific can sometimes be detected by cross-hybridization to this clone. The clone *YP1* which encodes the mRNA for the yolk protein 1 (Barnett *et al.*, 1980) served as a control since the YP synthesis in genotypes with altered somatic sex was well characterized (see Introduction).

X/0 analysis. The importance of the Y chromosome for male

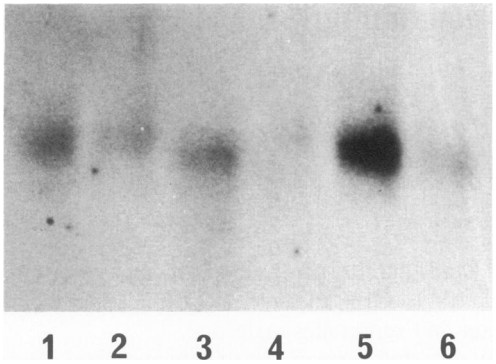

Fig. 1. Hybridization of the nick-translated clone *mst(3)gl-9* to a Northern transfer of RNAs from *Drosophila* males with different numbers of the Y chromosome. *X/Y/Y:* **(1)** 2 μg of poly(A)$^+$ and **(2)** 20 μg of total RNA; *X/Y:* **(3)** 2 μg of poly(A)$^+$ and **(4)** 20 μg of total RNA; *X/0:* **(5)** 10 μg of poly(A)$^+$ and **(6)** 50 μg of total RNA.

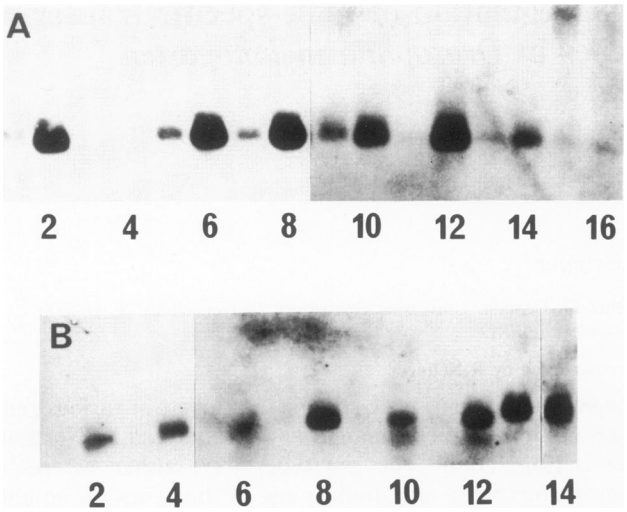

Fig. 2. Hybridization of the nick-translated clone *mst(2)ag-15* to Northern transfers of RNAs from various genotypes. The odd lanes contain total RNA and the even lanes poly(A)$^+$ RNA unless stated otherwise. The sources for the RNAs were the following genotypes (the respective phenotypes are listed in Table I). **Panel A: 1** and **2:** *X/Y;tra-2/+;* **3** and **4:** *X/X;tra-2/+;* **5** and **6:** *X/Y;tra-2/tra-2;* **7** and **8:** *X/X;tra-2/tra-2;* **9** and **10:** *X/Y;tra-2^{OTF}/tra-2^{OTF};* **11** and **12:** *X/X;tra-2^{OTF}/tra-2^{OTF};* **13** and **14:** *X/Y;ix/ix^2;* **15** and **16:** *X/X;ix/ix^2.* **Panel B: 1** and **2:** *X/X;dsx/dsx;* **3** and **4:** *X/Y;dsx/dsx;* **5** and **6:** *X/Y;dsx^m/+;* **7** and **8:** *X/Y;dsx^m/dsx;* **9** and **10:** *X/Y;dsx^{Mas}/+;* **11** and **12:** *X/X;dsx^{Mas}/+;* **13:** *X/Y;dsx^m/dsx^D;* **14:** total RNA from *X/X;dsx^m/dsx^D.*

Table I. Experimental flies and summary of results

Genotype	Sexual phenotype	Transcript accumulation[a]		
		YP1	*ag-15*	*gl-9*
X/X;+/+	female	+	−	−
X/Y;+/+	male	−	+	+
X/X;tra/+	female	+	−	−
X/X;tra/tra	pseudomale	−	+	−
X/Y;tra/+	male	−	+	+
X/Y;tra/tra	male	−	+	+
X/X;tra-2/+	female	+	−	−
X/X;tra-2/tra-2	pseudomale	−	+	−
X/Y;tra-2/+	male	−	+	+
X/Y;tra-2/tra-2	sterile male	−	+	+
X/X;tra-2^{OTF}/+	female	+	−	−
X/X;tra-2^{OTF}/tra-2^{OTF}	incomplete pseudomale	+	+	−
X/Y;tra-2^{OTF}/+	male	−	+	+
X/Y;tra-2^{OTF}/tra-2^{OTF}	male	−	+	+
X/X;ix/+	female	+	−	−
X/X;ix/ix^2	intersexual	+	+	−
X/Y;ix/+	male	−	+	+
X/Y;ix/ix^2	male	−	+	+
X/X;dsx/+	female	+	−	−
X/X;dsx/dsx	intersexual	+	+	−
X/Y;dsx/+	male	−	+	+
X/Y;dsx/dsx	intersexual	+	+	(+)
X/X;dsx^D/+	intersexual	+	+	−
X/X;dsx^D/dsx	pseudomale	−	+	−
X/X;dsx^D/dsx^m	intersexual	+	+	−
X/Y;dsx^D/+	male	−	+	+
X/Y;dsx^D/dsx	male	−	+	+
X/Y;dsx^D/dsx^m	male	−	+	+
X/Y;dsx^m/+	male	−	+	+
X/Y;dsx^m/dsx	intersexual	+	+	(+)
X/X;dsx^{Mas}/+	intersexual	+	+	−
X/Y;dsx^{Mas}/+	male	−	+	+

[a] *YP1*: the female-specific yolk protein 1 mRNA; *ag-15*: the transcript from the male accessory gland which is encoded by *mst(2)ag-15*; *gl-9*: the germ line-specific RNA homologous to *mst(3)gl-9*; the symbols stand for: + detectable, (+) detectable only with an *in vitro* RNA transcript as hybridization probe, − not detectable.

fertility and its regulatory role during spermatogenesis are well documented (for reviews, see Hess and Meyer, 1968; Hennig, 1985). It could, therefore, be argued that the germ line-specific transcript can only be accumulated in genotypes carrying a Y chromosome. This in turn would render experiments with most intersexual genotypes meaningless since the majority of the mutants affect only X/X and not X/Y genotypes (see Introduction). Therefore, it was tested whether both clones can detect their complementary RNAs in males with null, one or two Y chromosomes. Figure 1 clearly shows that the transcript homologous to *mst(3)gl-9* is at least as abundant in X/0 males as it is in X/Y and X/Y/Y flies; in other words, the Y chromosome is not necessary for the transcription of the respective gene. The same was found for the somatic transcript of *mst(2)ag-15* (data not shown). This was expected since the Y chromosome is active only in spermatogenic stages.

Transcript accumulation in mutant genotypes

Table I summarizes the data of the different hybridizations to transfers of RNAs isolated from the various genotypes. The control hybridizations with the clone *YP1* demonstrate that the YP mRNA is accumulated in females, all intersexual phenotypes, even in the incomplete pseudomales of the genotype *X/X;tra-2^{OTF}/tra-2^{OTF}*, but not in pseudomales or in males. This is in accordance with the published experiments (Postlethwait *et al.*, 1980; Bownes and Nöthiger, 1981; Ota *et al.*, 1981; Belote *et al.*, 1985) and demonstrates that the isolated RNAs are not contaminated or mislabelled.

Accumulation of the somatic transcript. The RNA homologous to clone *mst(2)ag-15* can be found in males, pseudomales and intersexual phenotypes, irrespective of their genotype but not in females (see Figure 2 as an example and Table I). These data reveal a strict correlation between the presence of male morphological characteristics and the ability to accumulate the male ac-

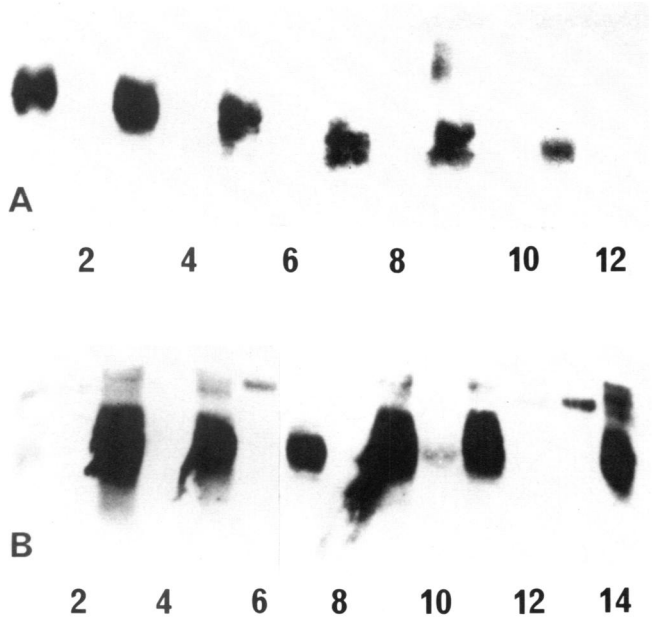

Fig. 3. Hybridization of an *in vitro* RNA transcript from the coding region of the germ line-specific clone *mst(3)gl-9* to Northern transfers of total RNAs. The RNAs were isolated from the following genotypes (for the respective phenotype see Table I). **Panel A: 1:** *X/Y;tra/tra;* **2:** *X/X;tra/tra;* **3:** *X/Y;tra/+;* **4:** *X/X;tra/+;* **5:** *X/Y;tra-2/tra-2;* **6:** *X/X;tra-2/tra-2;* **7:** *X/Y;tra-2/+;* **8:** *X/X;tra-2/+;* **9:** *X/Y;tra-2^{OTF}/tra-2^{OTF};* **10:** *X/X;tra-2^{OTF}/tra-2^{OTF};* **11:** *X/Y;ix/ix²;* **12:** *X/X;ix/ix².* **Panel B: 1:** *X/Y;dsx/dsx;* **2:** *X/X;dsx/dsx;* **3:** *X/Y;dsx/+.* **4:** *X/X;dsx/+;* **5:** *X/Y;dsx^{D}/dsx;* **6:** *X/X;dsx^{D}/dsx;* **7:** *X/Y;dsx^{D}/+;* **8:** *X/X;dsx^{D}/+;* **9:** *X/Y;dsx^{m}/+;* **10:** *X/Y;dsx^{m}/dsx;* **11:** *X/Y;dsx^{m}/dsx^{D};* **12:** *X/X;dsx^{m}/dsx^{D};* **13:** *X/X;dsx^{Mas}/+;* **14:** *X/Y;dsx^{Mas}/+.*

cessory gland RNA. In addition, they show that the accumulation and probably the synthesis of this RNA are not under the direct control of the X:A ratio, but are regulated by those four sex determining genes.

Accumulation of the germ line transcript. The detection of the germ line-specific transcript of *mst(3)gl-9* in the various genotypes was difficult for two reasons. (i) Even in fertile males the corresponding RNA is of low abundance. (ii) The RNA has only a short poly(A) tail which in general does not allow an enrichment by an oligo(dT)-cellulose chromatography step. As a consequence, with nick-translated hybridization probes complementary sequences could be found in X/Y males only (data not shown).

Using *in vitro* synthesized radioactively labelled RNA as a probe the transcript could be detected in two intersexual phenotypes as well, making these flies the only ones where all three RNAs accumulate (see Figure 3 and Table I). Two aspects deserve special emphasis. One is that these are the only intersexual genotypes with an X/Y sex chromosome constitution. The second is that the RNA probe can reproducibly detect the germ line transcript in those two intersexes but never in any pseudomales which are all of the female chromosomal sex (i.e. X/X;A/A). This demonstrates that the sex determining genes do not regulate the accumulation of the spermatogenic RNA. Instead, the X:A ratio is governing the ability to accumulate and probably to synthesize this germ line-specific RNA.

Discussion

The genes for the *Drosophila* transcripts analyzed in this study are typical of many eukaryotic structural loci in that their expression is restricted to special tissues at certain times of development. More important is their additional characteristic, namely that they are regulated in a sex-specific manner. That offers a way to analyze how the regulatory hierarchy governing somatic sexual differentiation manages to guarantee the male-specific expression of these genes.

The experiments reported here show that the accumulation of the male accessory gland transcript is clearly under the control of those regulatory genes. This could be either by directly governing the transcription or indirectly in the sense that only the formation of the paragonia is dependent on those genes. The transcription of *mst(2)ag-15* is then merely a consequence of the fact that paragonia are present. In either case, the sex determination regulatory genes have the expected role for the control of the somatic male-specific gene expression, too. In other words, the male accessory gland RNA is the male-specific counterpart to the well analyzed female-specific yolk proteins (Postlethwait *et al.*, 1980; Bownes and Nöthiger, 1981; Ota *et al.*, 1981) and their RNAs (Belote *et al.*, 1985; this work). It is noteworthy that the partly male and partly female morphological characteristics in the intersexual and incomplete pseudomale phenotypes (Baker and Ridge, 1980) can also be observed at the molecular level. All these flies accumulate the female-specific YP RNA as well as the male-specific accessory gland transcript and this is most likely true at the level of the single fly although it is not yet proven.

For the accumulation of the germ line-specific transcript the situation is different. Only those two intersexual genotypes with an X/Y sex chromosome constitution detectably accumulate this RNA. Although the three different X/X pseudomales have the better developed internal male genitalia they must accumulate considerably less, if any, of the testis-specific RNA. The data, therefore, confirm the transplantation experiments by Marsh and Wieschaus (1977) and by Schüpbach (1982) which demonstrated that the regulatory genes governing somatic sexual differentiation do not act in the germ line. Rather, the X:A ratio is the main regulatory element for the accumulation of the male germ line transcript. This is identical to the situation reported for the normal female germ cell differentiation (Schüpbach, 1985).

As already discussed by Schüpbach (1982) 'the failure of transplanted germ cells of one chromosomal sex to differentiate into fully functional gametes of the other sex does not necessarily mean that the sexual pathway of a germ cell is cell autonomously regulated. The criterion (i.e. the capability to produce fully functional gametes) may be too stringent.' Indeed, Nöthiger *et al.* (cited after Nöthiger and Steinmann-Zwicky, 1985) have rarely observed spermatogenic cells, such as spermatocytes, spermatids and even immotile sperm in the testes of pseudomales. These observations do suggest that the gonadal soma may influence the germ line in such a way that the male pathway is sometimes even used by X/X germ cells although their karyotype prevents normal spermatogenesis.

According to the criterion of the experiments reported here, the accumulation of a germ line-specific RNA, the gonadal soma did not succeed in imposing the male pathway on the X/X germ cells. This does not exclude the possibility that sometimes germ cells in pseudomales do synthesize the testis-specific transcript since this would not be detected by the method employed. Ways to overcome this limit of sensitivity could only be the analysis of spermatogenic cells in testes of pseudomales by either the use of *in situ* hybridization to transcripts or the use of antigens specific for the male germ line. Work in this direction is currently under way.

Materials and methods

Drosophila culture

D. melanogaster were grown at 23°C on a standard cornmeal – molasses – yeast agar medium containing propionic acid and supplemented with live yeast.

Fly stocks

For description of the markers and balancers see Lindsley and Grell (1968).

Wild-type: Oregon R.

tra. (Sturtevant, 1945) #1: B^SY/y w; mwh tra cp ri/TM3, Sb Ser.
#2: B^SY; th st tra cp ri p^p/TM3, Sb Ser.
Pseudomales were produced by crossing heteozygous females of stock #1 with homozygous *tra* males of stock #2.

tra2. (Watanabe, 1975) #1: B^SY; pr cn tra-2 bw/SM5.
#2: B^SY; pr cn tra-2/SM5.
Pseudomales were generated by crossing heterozygous females of stock #1 with heterozygous males of stock #2.

tra-2^{OTF}. (Fujihara *et al.*, 1978): B^SY; tra-2^{OTF}/SM1.
The incomplete pseudomales were produced by crossing heterozygous females to homozygous *tra-2^{OTF}* males.

ix. (Morgan *et al.*, 1943) B^SY; pr cn ix/SM5.

ix^2. (Meyer and Edmondson, 1951): ab^2 ix^2 bw sp^2/In(2L)Cy In(2R)Cy, Cy dp^{lvl} Bl cn^2 L^4 sp^2.
Intersexual flies were generated by crossing heterozygous *ix^2* females with homozygous *ix* males.

dsx. (Hildreth, 1965): y/B^SY; dsx p^p/TM6.

dsx^m. (A.Garen, unpublished): B^SY; st dsx^m ca/TM3, Sb Ser.

dsx^D. (Fung and Gowen, 1957): B^SY/X/T(1;3)OR60, y; dsx^D Sb e/TM6.

dsx^{Mas}. (Mischaikow, 1959): y; T(1;3)OR60/dsc^{Mas}/TM3, Sb Ser.
To produce the different intersexuals or pseudomales heterozygous flies of the appropriate stocks were crossed.

Molecular analysis

RNA isolation, Northern transfers and radiolabelling procedures of the *Drosophila* genomic clones were performed as already described (Schäfer, 1986).

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