The Female-Determining Gene F of the Housefly, Musca domestica, Acts Maternally to Regulate Its Own Zygotic Activity

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ABSTRACT

In Musca domestica, the common housefly, female development requires the continuous activity of the sex-determining gene F from early embryogenesis until metamorphosis. To activate F in embryogenesis, two conditions must be met: There must be no male-determining M factor in the zygotic genome, and the egg must be preconditioned by F activity in the maternal germ line. This maternal activity can be suppressed by introducing an M factor into the maternal germ line, which causes all offspring, including those that do not carry M, to develop as males. By transplantation of pole cells (germline progenitor cells) we have constructed such females with a genetically male germ line and, simultaneously, males with a genetically female germ line carrying a constitutive allele of F [F\text{dominant} (F^{D})]. Crosses between these animals yielded offspring that, despite the presence of M in the maternal germ line, were of female sex, solely due to zygotic F\text{\textsuperscript{D}} brought in via the sperm. This shows that zygotic F function alone is sufficient to promote female development and that in the wild-type situation, maternal F product serves no other function but to activate the zygotic F gene.

In most wild strains of the housefly (Musca domestica L.), sex determination is controlled by Y-chromosomal or autosomal M factors that are equivalent in their effect, but are not necessarily identical (Perje 1948; Milani 1967; Dübendorfer et al. 1992; Schmidt et al. 1997a). When M is present, the key gene for female development, F, remains inactive, which results in male development. Female development depends on the continuous activity of the F gene from early embryogenesis until metamorphosis (Hilfliker-Kleiner et al. 1993), and this F gene product is present when M is absent from the genome. Thus, F is the pivotal gene that determines femaleness in a cell when functional and maleness when nonfunctional.

The switch function of F is demonstrated by two mutations with opposite effects. The dominant allele F\text{dominant} (F^{D}) determines femaleness also in the presence of M factors (Rubini et al. 1972) and is therefore considered a constitutive allele (Nöthiger and Steinhann-Zwicky 1985; Inoue and Hiyoshi 1986). F\text{masculinizer} (F\text{man}) on the other hand, is a loss-of-function mutation which, when homozygous, causes male development even when the animals have no M (Schmidt et al. 1997b). These findings support the view that activity of F is the major requirement for the determination of female sex.

Experiments by Hilfliker-Kleiner et al. (1994) have shown that absence of zygotic M is not sufficient to guarantee female development: Genetically male (M/+) pole cells (the germline progenitor cells), when transplanted into females, nonautonomously differentiate into functional eggs. All of these, however, give rise to males, even if the zygotic genome has no M. Such animals are, therefore, called NoM males. The masculinizing maternal effect of M is abolished by the simultaneous presence of F\text{D} in the maternal germ line. In this case, genetically female offspring (with no M and no F\text{D}, but with two F\text{\textsuperscript{+}} alleles) are females. This rescue must be the result of constitutive F function in the germ line and shows that female development of an embryo not only depends on the absence of zygotic M, but also on the previous activity of F in the maternal germ line.

In normal development, the consequence of maternal F activity may be the accumulation of F product in the eggs, necessary to activate the zygotic F directly or indirectly. Under this assumption, any egg suffering a male-determining maternal effect because of the presence of M in the maternal germ line should be redirected to the female pathway if the father contributed a constitutive F\text{D} to the zygote. Our results show that this is the case. The experiment was possible since sex determination in the germ line of the housefly is nonautonomous in both sexes and thus allows F\text{D} pole cells to develop into functional sperm (Hilfliker-Kleiner et al. 1994).

MATERIALS AND METHODS

M. domestica stocks were kept in transparent plastic containers and fed with dry powdered milk and sugar water. Eggs were collected in black film boxes and larvae reared on standard wheat bran medium according to the protocol of Hilfliker-Kleiner et al. (1994). Pole cell transplantations were...
carried out according to the method developed for Drosophila by Van Deusen (1977), with some modifications making allowance for the larger and more flaccid eggs of Musca (Hilflcker-Kleiner et al. 1994).

Since small populations of larvae are difficult to raise on standard medium, larvae obtained from transplanted embryos or from single-pair crosses were raised on pig dung, which proved optimal even for just a few individuals (Schmidt and Bächli 1996). Before use, the dung was frozen and thawed to kill the occasional egg or embryo possibly deposited before collection from the stable.

The sex-determining genes and autosomal markers used in this study are described by Schmidt et al. (1997a), and lists of housefly mutations are presented in the reviews by Milano (1967, 1975). The males used in our experiments had no Y chromosome, but carried the M factor on the third chromosome (MIII). The constitutive F allele on chromosome IV, F0, was marked in cis with the very closely linked dominant mutation Bald abdomen (Ba, map distance to F0 <0.03 cM; Schmidt et al. 1997b). All embryos used as donors for the transplantation of pole cells were homozygous for the marker brown body (bwb) on chromosome III.

RESULTS

The main goal of our experiments was to eliminate F function in the female germ line, but ensure F activity in the zygote. This was achieved by two simultaneously performed series of pole cell transplantations, yielding females that produced eggs from an M/+ germ line with masculinizing maternal effect and hence without F activity (Figure 1, series I) and males that had F0 in the germ line, producing sperm that could contribute F0 to the zygote (Figure 1, series II).

Table 1 lists the crosses we designed to produce donor and host embryos, such that 75% of the embryos were of the genotypes required for the transplantations. This was crucial for the experiment because, despite this optimization of genotypes, embryonic lethality, unpredictable pole cell integration, and random crossing of the resulting adults brought the chances for a single pair cross with two germline-chimeric partners of the anticipated genotypes down to about 1 per 200 transplanted embryos. A major advantage for the experimental set up was the possibility to produce unisexual clutches of eggs in Musca.

Embryos of exclusively female sex, such as the recipients of series I, were obtained by crossing standard females (XX;+/-+) to males with female genotype (NOM males) obtained from a stock with the maternal-effect mutation Ag (Vanossi Este and Rovati 1982). All female carriers of this dominant mutation are arrhenogenic, i.e., produce mostly NOM males (and, with variable but generally low frequency, some intersexes). Zygotic Ag, irrespective of whether contributed by the egg or by the sperm, has no effect on the somatic development of either sex. Hence, Ag stocks represent populations in which sex is exclusively determined by a maternal

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**Figure 1.**—Experimental set up for the transplantation of pole cells to generate eggs that originate from genetically male pole cells (series I) and sperm that carry F0, i.e., originate from genetically female pole cells (series II). Such transplantations are possible because germine differentiation in Musca is not cell-autonomous, but controlled by the surrounding soma. Endogenous germ lines are not shown. Large ellipses symbolize the recipient animals, and the smaller inserted ellipses the implanted pole cells. White stands for female genotype, and black for male genotype. In series II, only one of the three possible donor genotypes is shown. For the genetic crosses necessary to produce the four types of donor and recipient embryos, see Table 1, series I and II. Genetic symbols: bwb, brown body; M, male-determiner; F0, F Dominant, Ba, Bald abdomen.
effect and the males have no male-determining factor to pass on to their offspring. Embryos of exclusively male sex, such as the donors of series I and the recipients of series II, were obtained by crossing males homozygous for M III (out of an M III/M III; F D/+ stock) to standard-type females. There was no way to generate a population of embryos that all had the F D allele, but crossing M/+; F D/+ females to M/+; +/+ males gave a 50% yield of F D donor embryos (Table 1, series II).

When animals resulting from the two transplantation series were crossed, we could recognize among their offspring those that derived from a maternal germ line without F activity (genotype M/+), but with F D from the paternal germ line, by their bwb Ba phenotype (Figure 1). Whether they also carried M factors was tested by outcrossing (see below).

We transplanted 1044 embryos of the genotypes specified in Table 1, and obtained 249 adults, 107 females and 142 males, which we combined as single pairs. The 35 supernumerary males were crossed to Ag/+ females that exert a male-determining maternal effect comparable to that of a maternal M. Seven out of the 107 single-pair crosses yielded offspring of the bwb Ba phenotype (Table 2, lines 1-3), disclosing that their mother had integrated M/+ pole cells and that their father contributed the allele F D. These animals were exclusively females. All offspring of the bwb Ba phenotype were males, showing, as an internal control, that the masculinizing maternal effect of M in the female germline was complete. Among the 35 Ag/+ mothers (Table 2, footnote 1) there was also one interesting case whose Ba + offspring, i.e., without F D, were all males because of the maternal effect of Ag. The only daughters produced by this female were those that inherited a paternal F D, as shown by their Ba phenotype.

Offspring that were females because of paternally contributed F D were crossed to NoM males of the Ag stock to determine the number of M III factors in their genomes: If they had none, their Ba + offspring (F +) were exclusively female. If they had just one M, they produced Ba + offspring of both sexes, and if they had two, their Ba + progeny were exclusively male. This was done with 26 of the 74 females derived from exclusively donor gametes and 5 of the 87 daughters of the Ag/+ mother, which revealed all combinations of F D and M (8 M/M; F D Ba/+; 18 M/+; F D Ba/+; and 5 +/+; F D Ba/+). This result proves that all animals that originate from an M/+ or Ag/+ female germ line and receive F D via the fertilizing sperm develop as females, irrespective of whether they are devoid of M or carry one or even two M factors. Thus, the presence of F D in the zygotic genome is sufficient to direct a male-predicted embryo to the female developmental pathway.

### DISCUSSION

An M factor, when introduced into the female germ line, predetermines all developing oocytes for male development, even if the resulting zygotes do not themselves contain M (Figure 2A). The experiments described here were designed to analyze this male-determining maternal effect for the purpose of understanding the control of maternal and zygotic sex determination in the wild type.

Our transplantation experiments show that a paternally provided F D allele becomes active in the zygote and determines normal female development also when the egg, because of a maternal effect, was predetermined to develop as a male (Figure 2C) and even in the presence of zygotic M. From this result it follows that zygotic F activity is necessary and sufficient for female somatic sex determination and that the maternal contribution (disruptable by M in the female germline) is.

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**TABLE 1**

Crosses to produce donor and recipient embryos for the transplantation of pole cells

<table>
<thead>
<tr>
<th>Series</th>
<th>Female × male</th>
<th>Genotype and sex of offspring</th>
<th>Offspring used as</th>
<th>Reference to Figure 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+/+ × Ag/+ or +/+ × F D</td>
<td>M III/+ and M III/bwb male</td>
<td>Female recipient</td>
<td>Left side, white</td>
</tr>
<tr>
<td></td>
<td>bwb/bwb × bwb M III/bwb M III</td>
<td></td>
<td>Male donor</td>
<td>Left side, black</td>
</tr>
<tr>
<td>II</td>
<td>+/+ × M III/M III bwb M III/bwb; F D Ba/+ × bwb M III/bwb</td>
<td>M III/+ male; bwb M III/bwb M III; F D Ba/+ female; bwb M III/bwb; F D Ba/+ female; bwb M III/bwb; F D Ba/+ female</td>
<td>Male recipient</td>
<td>Right side, black</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female donors</td>
<td></td>
<td>Right side, white</td>
</tr>
</tbody>
</table>

1. NoM males; for an explanation of this stock see text.

2. Only these embryos, representing 50% of the donors of series II, carry the F D Ba chromosome and thus have pole cells of the desired genotype. As embryos, they are not distinguishable from their brothers.
TABLE 2  
Offspring obtained from the combination of females and males with transplanted pole cells

<table>
<thead>
<tr>
<th>Genotype of transplanted gametes of only one parent</th>
<th>Genotype of transplanted gametes of both parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-D female, F+ male</td>
<td>F-D female, F+ male</td>
</tr>
<tr>
<td>F-D female, F- male</td>
<td>F-D female, F- male</td>
</tr>
</tbody>
</table>

- **Offspring from donor-derived germ line of only one parent:**
  - F-D female, F+ male: 2 cases, 1 female, 1 male.
  - F-D female, F- male: 4 cases, 2 females, 2 males.

- **Offspring from donor-derived germ line of both parents:**
  - F-D female, F+ male: 6 cases, 3 females, 3 males.
  - F-D female, F- male: 10 cases, 5 females, 5 males.

Note: The number of cases obtained for each type of germ line combination.

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The results indicate that the male-determining maternal effect of M is, in the female germ line, caused by the lack of maternal F product, rather than by perdurance of maternal M. This is shown by two main facts: First, when present in the female germ line concomitantly with M, it rescues the maternal effect, such that those embryos that carry either M or F (but two F alleles) are again females (Hilfer-Kleiner et al., 1994; see also Figure 2B). Second, if the female germ line does not contain M, but is made deficient for the F function by transplantation of homozygous F- male cells into normal females, the same male-determining maternal effect is seen as with maternal M (Schmidt et al., 1997b). These observations, together with the results described here, are convincing evidence that the male-determining maternal effect of M in the female germ line is the result of lacking maternal F function and that this function is required only for the activation of the zygotic F.

This interpretation can also explain a puzzling phenomenon we encountered when analyzing M factors with incomplete expressivity. One such factor is represented by a truncated, ring-shaped Y chromosome, R(YS). X/R(YS) animals can be intersexual, but most of them develop as morphologically normal, fertile males. However, about 40% of these males and intersexes accumulate yolk proteins in their hemolymph, a typically female trait (Hediger et al., 1998). When introduced into the female germ line, R(YS) exerts a maternal effect causing the development of NoM males that, surprisingly, do not even show a trace of yolk proteins in their hemolymph (Figure 3). Thus, the masculinizing materi-
Regulation of Sex-Determining Genes

Figure 2—Experimental situations explaining the sex-determining mechanism of the wild type: (A) male-determining maternal effect of M; (B) rescue of this maternal effect by maternal, constitutively active F⁰ (data for A and B from Hiflaker-Kleiner et al. 1994); (C) rescue of maternal effect by paternal contribution of F⁰ (in zygotes with or without M, this article); (D) inferred mechanism in the wild-type female; and (E) mechanism in the wild-type male.

Figure 3.—Western blots of hemolymph samples with yolk proteins visualized with anti-Musca-vitellin-antibody. The arrowhead marks the major yolk proteins of M.domestica. (A) wild-type controls, X/Y male (C♂) and X/X female (C♀); (B) animals with zygotic R(YS), males (R♂) and intersexes (♂♀) from standard X/X mothers; (C) animals derived from eggs with masculinizing maternal effect, originating from a maternal X/R(YS) germ line, males carrying the maternally inherited R(YS) chromosome (R♂) and males without R(YS) or any other M factor (Nom♂). None of these males (0 out of 13 and 0 out of 14, respectively) had any detectable yolk proteins in the hemolymph.

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The maternal effect of R(YS) is even stronger than the effect of its presence in the zygote, again suggesting that the maternal effect does not come about by the perdurance of maternal M product. Rather, R(YS) in the maternal germ line rigorously shuts down maternal F activity, which masculinizes all offspring, whereas in the zygote, it could only produce an ambiguous signal.

A sex-determining maternal effect has also been demonstrated in the blowfly Chrysomya rufa. In this species, the dominant allele F' must be present in the maternal germ line if the eggs are to develop as females. In the absence of maternal F', all eggs develop as males, even when the zygote itself receives F' from a father with transplanted pole cells (Ullrich 1984). Thus, F' is a genetic element with exclusively maternal activity and no sex-determining effect in the zygote. The same has been shown in Drosophila melanogaster for the gene da (daughterless), which, besides somatic functions in both sexes, is required maternally, but not zygotically, for female sex determination (Cronmiller and Cline 1986). This is in conspicuous contrast to the situation in Musca, where M and F both function maternally and zygotically, rendering homology of F in Musca to F' in Chrysomya and to da in Drosophila very unlikely.

Positive autoregulation of the key gene for female development is not only a feature of F in Musca, but also of Sex-lethal (Sxl) in D. melanogaster (Bell et al. 1991). However, this parallel does not identify F as the homologue of Sxl in Drosophila. A highly conserved homologue of Sxl does exist in Musca, but it is equally expressed in females and males and thus is not a candidate for F (Meise et al. 1998).

In conclusion, our results demonstrate that the zygotic function of the F gene, indispensible for female development, is regulated by two antagonistic factors: It is activated by its own product from the maternal germ line, but is blocked by M, the male-determining genetic control element, which, in standard housefly strains, is carried by the Y chromosome. Comparisons with other insect systems give us no clues as to the nature of the genes M and F, but we are currently trying to identify these genes molecularly to find out how they and their products control sexual development in Musca.
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LITERATURE CITED


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