**virilizer regulates Sex-lethal in the germline of Drosophila melanogaster**

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**SUMMARY**

In *Drosophila*, the gene *Sex-lethal (Sxl)* is required for female development. It controls sexual differentiation in the soma, dosage compensation and oogenesis. The continuous production of SXL proteins in XX animals is maintained by autoregulation and depends on *virilizer (vir)*. This gene is required in somatic cells for the female-specific splicing of *Sxl* primary transcripts and for an unknown vital process in both sexes. In the soma, clones of XX cells lacking *Sxl* or *vir* are sexually transformed and form male structures; in the XX cells mutant for *Sxl* extensively proliferate, but are unable to differentiate.

We now studied the role of *vir* in the germline by generating germline chimeras. We found that XX germ cells mutant for *vir*, in contrast to cells mutant for *Sxl*, perform oogenesis. We show that the early production of SXL in undifferentiated germ cells is independent of *vir* while, later in oogenesis, expression of *Sxl* becomes dependent on *vir*. We conclude that the early SXL proteins are sufficient for the production of eggs whereas the later SXL proteins are dispensable for this process.

However, *vir* must be active in the female germline to allow normal embryonic development because maternal products of *vir* are required for the early post-transcriptional regulation of *Sxl* in XX embryos and for a vital process in embryos of both sexes.

Key words: *Drosophila*, Sex determination, Oogenesis, Autoregulation, Maternal effect, *Sxl*, *virilizer (vir)*

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**INTRODUCTION**

*Sx-lethal (Sxl)* is the key gene for sex determination in *Drosophila melanogaster*. It controls three different pathways: somatic sex determination, dosage compensation, which equalizes the amount of X-chromosomal transcripts in males and females, and oogenesis. The primary signal for sex determination in the soma is the ratio of X chromosomes to sets of autosomes (X:A) (Bridges, 1921, 1925; reviewed by Cline, 1993). If this ratio is 1.0 (XX:AA), *Sxl* is transcriptionally activated. As a consequence, the sex-determining genes *transformer (tra)* and *doublesex (dsx)* are female-specifically expressed, which leads to female somatic differentiation.

If the X:A ratio is 0.5 (X:AA), *Sxl* and *tra* remain inactive, and the bifunctional locus *dsx* is expressed in the male mode, which results in male somatic differentiation (for reviews see Belote, 1989, 1992; Steinmann-Zwicky et al., 1990; Cronmiller and Salz, 1994; Cline and Meyer, 1996). In the absence of SXL protein, the dosage compensation gene *male-specific lethal-2 (msl-2)* expresses a functional product and promotes, together with the products of the other male-specific lethal genes (*msl-1, msl-3, mle*) and *males-absent on the first (mof)*, the hypertranscription of the single X chromosome in males (Belote and Lucchesi, 1980; Kelley et al., 1995; Zhou et al., 1995; Hilfiker et al., 1997; for reviews see Kuroda et al., 1993; Baker et al., 1994; Gorman and Baker, 1994). Mutations in *Sxl* or upstream of it or in the *msl* genes lead to sex-specific lethality because of disturbance of dosage compensation. Mutations downstream of *Sxl* in the somatic sex determination pathway cause sexual transformation.

The differential expression of *Sxl* is achieved in two steps. The first level of control is transcriptional. Products of numerator and denominator genes, which form the X:A signal regulate an establishment promoter, *P<sub>e</sub>*. In XX embryos, this promoter is activated at blastoderm stage and produces an early SXL protein. Later, *Sxl* is regulated by alternative splicing. Around early gastrulation, transcription from *P<sub>e</sub>* is switched off and a maintenance promoter, *P<sub>m</sub>* becomes active in both sexes. The early SXL protein, present only in females, promotes the female-specific splicing of its own pre-mRNA, thus starting an autoregulatory loop. In males, which have no preexisting SXL protein, the *Sxl* mRNA includes an exon (#3) with several in-frame stop codons that terminate translation prematurely (Bell et al., 1988; Bopp et al., 1991; Keyes et al., 1992; Wang and Bell, 1994). Three additional genes are known to be involved in the female-specific splicing of *Sxl* pre-mRNA: *sans-fille (snf)* (Salz, 1992; Albrecht and Salz, 1993), *female-lethal-2-d (fl(2)d)* (Granadino et al., 1990, 1992) and *virilizer (vir)* (Hilfiker and Nöthiger, 1991; Hilfiker et al., 1995). Females mutant for *snf* are sterile. Certain alleles of *fl(2)d* and *vir* cause female-specific lethality and a sexual transformation of somatic cell clones into male tissue. Both effects result from a male-specific expression of *Sxl* in XX animals. In addition, all three genes have a vital function in both sexes.

Regulation and function of *Sxl* in the germline are less well understood. Females mutant for *Sxl<sup>1504</sup>*
specifically disrupts the germline function, and females with transplanted pole cells mutant for the null allele Sxl\(^{0}\), develop ovaries with tumorous cysts due to an excessive proliferation of the germ cells and to a partial transformation into spermatocytes (Schüpbach, 1985; Steinmann-Zwicky et al., 1989; Bopp et al., 1993). The genes snf, fl(2)J, ovo and ovarian tumor (ota) are required for proper expression of Sxl in XX germ cells (Salz, 1992; Granadino et al., 1992; Oliver et al., 1993; Pauli et al., 1993). Mutations in any of these genes cause tumorous ovaries. This shows that Sxl is necessary for the differentiation of female germ cells. The initial expression of Sxl is probably regulated by a cell-autonomous signal, the germline X:A ratio, which is measured by elements different from those used to determine the X:A ratio in the soma and by an inductive signal that is provided by somatic cells (Nöthiger et al., 1989; Steinmann-Zwicky et al., 1989; Steinmann-Zwicky, 1993; Granadino et al., 1993; Horabin et al., 1995; reviewed by Steinmann-Zwicky, 1992). It was recently shown that, in parallel to the soma, Sxl in the germline is also maintained by autoregulation (Hager and Cline, 1997). In our paper, we analyzed this second step of regulation and asked whether Sxl in germ cells depends on vir, which is involved in the autoregulation of Sxl in somatic cells.

MATERIALS AND METHODS

General techniques

Unless noted otherwise, all crosses were done at 25°C. Flies were reared on standard food (corn meal, sugar, yeast, agar, Nipagin). For genetic symbols, see Lindsley and Zimm (1992).

vir alleles

vir\(^{1ts}\) is a temperature-sensitive allele that transforms XX animals into intersexes at 29°C; vir\(^{2}\) causes female-specific lethality. Both alleles have no effect on XY animals. vir\(^{6}\) is lethal for females and semilethal for males. vir\(^{3}\) and vir\(^{4}\) are lethal for both sexes (Hilfiker and Nöthiger, 1991; Hilfiker et al., 1995). Df(2R)vir130 is a deficiency that uncovers Frd, twi and vir.

Clones in the germline

Cell clones homozygous for vir were generated by the FLP-DFS technique (Chou and Perrimon, 1992; Chou et al., 1993; Xu and Rubin, 1993). Females of the genotype w; P[\(\text{osk}\)\(^{+}\); FRT]42D, P[\(\text{osk}\)\(^{+}\); \(\text{y}^{+}\); \(\text{y}\)\(^{+}\); \(\text{Dfd}(\text{F1})::\text{lacZ}\)]/CyO were crossed to y w FL122.16/y; P[\(\text{osk}\)\(^{+}\); FRT]42D, P[\(\text{osk}\)\(^{+}\); \(\text{y}^{+}\); \(\text{Dfd}(\text{F1})::\text{lacZ}\)]/CyO males. To induce mitotic recombination, their offspring was heatshocked for 30 minutes at 37°C between 24 and 32 hours after oviposition. w/y w FLP; FRT vir bw/FRT ovo\(^{D}\) females were then tested for fertility. The same technique was used to generate clones mutant for Sxl in females with the genotypes y w cm Sxl\(^{0}\) et P[\(\text{mini}\) w]; FRT]101A\(^{+}\) ovo\(^{D}\) \(\times\) P[\(\text{mini}\) w]; FRT]101A; FLP\(^{D}\) and y w cm Sxl\(^{0}\) \(\times\) P[\(\text{mini}\) w]; FRT]101A ovo\(^{D}\) \(\times\) P[\(\text{mini}\) w]; FRT]101A; FLP\(^{D}\). They were heatshocked for 1 hour at 37°C between 24 and 32 hours after oviposition.

Staining procedures

Embryos of different stages derived from homozygous vir germ cells were stained with anti-SXL antibody as described by Bopp et al. (1993). The paternal X chromosome was marked with Df(1)F1::lacZ (Zeng et al., 1994) to distinguish XX and XY embryos. Adult ovaries with clones of stem cells homozygous for vir and Sxl were stained with anti-SXL antibody and DAPI (Bopp et al., 1993).

Examination of moribund embryos

Females with germ cells mutant for vir\(^{3}\) and vir\(^{4}\) were crossed to males carrying the following enhancer trap constructs: Sxl\(_{11}\):lacZ (Keyes et al., 1992), hb::lacZ, en::lacZ, ftz::lacZ, hh::lacZ. Their embryos were stained with X-Gal and DAPI according to standard protocols.

Transplantation of larval gonads

Gonads of third instar larvae of XY; cn vir\(^{2}\) bw/Df(2R)vir130 and XY; cn vir\(^{2}\) bw/Df(2R)vir130 males were transplanted (Ursprung, 1967) into adult females and cultured in their abdomen for 6 days. Afterwards, the gonads were analysed by phase-contrast microscopy.

Transplantation of pole cells

Females homozygous for osk\(^{301}\) were crossed to Oregon R wild-type males. Due to a maternal effect of osk, the resulting embryos lack endogenous pole cells and served as hosts for transplanted pole cells (Lehmann and Nüsslein-Volhard, 1986). The donor embryos were obtained from a cross of y sn\(^{1}\); vir\(^{3}\) bw/T(2;3)Xa females with B\(^{5}\)Y; Df(2R)vir130/SM5 males. For technical details, see Van Deusen (1976) and Steinmann-Zwicky et al. (1989).

RESULTS

XX germ cells homozygous for vir are able to complete oogenesis

In XX animals, somatic cell clones that become homozygous mutant for vir switch to the male pathway (Hilfiker et al., 1995). We now tried to determine the sexual fate of XX; vir germ cells. The FLP-DFS technique was used to generate clones homozygous for vir\(^{2}\), vir\(^{3}\), vir\(^{4}\) and vir\(^{6}\) in heterozygous vir\(^{0}\) females (see Materials and Methods). These females are sterile due to ovo\(^{D}\) unless they lose this dominant female-sterile mutation by mitotic recombination and the resulting stem cells homozygous for vir are able to form eggs.

Germ cells mutant for any of the four tested vir alleles were able to complete oogenesis (summarized in Table 1). Females with germ cells mutant for the female-specific lethal allele vir\(^{2}\) gave rise to male and female offspring in a 1:1 ratio when mated to vir\(^{+}\) males, and to purely male offspring when mated to homozygous vir\(^{2}\) males. Females with germ cells mutant for the stronger vir\(^{6}\) allele produced no daughters, even when the father contributed a vir\(^{+}\) allele. But even of the male offspring, only a few per cent survived to adulthood. In contrast to these two alleles, females with germ cells mutant for the strong alleles vir\(^{3}\) and vir\(^{4}\) formed eggs, but no larvae hatched (except very few escapers with vir\(^{2}\)). In addition, the fecundity was also affected: the number of females laying eggs as well as the number of eggs produced per female were significantly reduced for vir\(^{6}\) (18 eggs per female in 3 days, n=48) and even more so for vir\(^{3}\) (9 eggs per female in 3 days, n=48), compared to 57 eggs for vir\(^{2}\) (n=52).

All these phenotypes were rescued if the clones were made in females that carried a transgene containing a genomic 10 kb fragment with the vir\(^{+}\) gene (M. Niessen, unpublished). This shows that the defects are due to vir and not to any other unknown mutations that become homozygous after mitotic recombination. Such mutations would not be rescued by the vir\(^{+}\) transgene.

Thus, XX germ cells mutant for vir, in contrast to somatic cells, are not sexually transformed. The embryonic defects that occur despite a paternal vir\(^{+}\) gene point to a maternal effect and the reduced number of eggs to a vital function in female germ cells.
Maternal vir product is required for the autoregulation of Sxl

Since earlier results had shown that vir is required for female-specific expression of Sxl (Hilfiker et al., 1995), we looked at the expression of Sxl in embryos derived from females with germ cells mutant for vir. To monitor the early transcriptional expression of Sxl, we introduced a SxlPe::lacZ construct. In addition, the embryos were stained with anti-SXL antibody. To distinguish the two sexes, XX embryos were marked with Dfd(F1)::lacZ transmitted by the paternal X chromosome. In homozygous vir2/f embryonic germ cells, the establishment promoter of Sxl was activated at blastoderm and SXL protein was uniformly distributed. At the beginning of gastrulation, the expression of SXL became mosaic and finally disappeared (Fig. 1B-D).

All vir alleles are truly recessive. vir2/f, vir4ts females are viable when they derive from heterozygous vir2/f female germ cells (Hilfiker et al., 1995), but did not survive when they derived from homozygous vir2/f germ cells (Table 1). Females with germ cells mutant for the stronger allele vir4 never produced any daughters, even when the father contributed a vir+ allele. Similar to vir2/f, vir2/f embryos, vir6/vir+ embryos correctly initiated transcription of Sxl, but failed to maintain expression (Fig. 1E,F). Thus, maternal vir+ product is necessary for the autoregulation of Sxl during early development of XX embryos.

Maternal vir product has a vital function for embryogenesis

Germ cells mutant for the strong alleles vir3 and vir4 were able to form eggs, but no larvae hatched except for very few escapers with vir3. Even with vir6, the viability of XY embryos was strongly reduced and XX animals were completely inviable. The genotype of the father had no influence on survival. The eggs were fertilized as shown by the presence of dividing nuclei in developing embryos. The developmental potential of heterozygous vir embryos is variable: some were arrested already at blastoderm stage, others died as differentiated embryos or rarely as larvae. To determine the lethal period and to identify possible specific defects, several enhancer trap lines were introduced via the father (see Materials and Methods). In vir3/+ embryos, the establishment promoter of Sxl was activated and the segmentation genes hunchback (hb), fushi tarazu (ftz), en (engrailed) and hedgehog (hh) were correctly expressed. This suggests that the known maternal components necessary for formation of the anterior-posterior axis are normally distributed. vir4 embryos are more strongly affected. Already at blastoderm stage, reporter genes like hh and Sxl were not expressed. vir4/+ embryos showed a general disturbance in morphology with an irregular distribution of nuclei.

SxlM partially rescues the germline defects

We tested whether constitutive alleles of Sxl can rescue any of the three germline defects of vir, namely the reduced egg production of the females themselves, the maternal effect on the autoregulation of Sxl in XX embryos, and the maternal effect on the viability of XX and XY embryos.

We used two constitutive mutations of Sxl, SxlM1 and SxlM4 (Cline, 1978; Maine et al., 1985). SxlM1 is not fully constitutive and still depends to some extent on the regulation by vir, snf and fl(2) d. SxlM1 male flies, but survive if they are also mutant for vir2 (Hilfiker et al., 1995), snf621 (Steinmann-Zwicky, 1988; Salz, 1992) or fl(2) d (Granadino et al., 1992). On the other hand, vir2/vir4, snf621 and fl(2) d females are rescued by SxlM1, SxlM4 is fully constitutive and male mutant for SxlM4 cannot be rescued by mutations in vir, snf or fl(2) d.

Experimental females with SxlM4; vir/vir germ cells were tested for the three germline defects. Presence of SxlM4 neither increased production of eggs in females with germ cells mutant

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<td>vir4/vir4</td>
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**Table 1. Sex and viability of offspring derived from females with vir/vir germ cells**

**Fig. 1.** Expression of Sxl in Oregon R wild-type male and female embryos (A) and in embryos derived from females with homozygous vir germ cells (B-F). (B) Uniform activation of SxlPe::lacZ in a vir2/vir2 embryo at blastoderm stage. Gradual loss of SXL protein in developing vir2/vir2 embryos (C,D) and in vir3/+ embryos (E,F). The embryos were stained with X-Gal (blue) and anti-SXL antibody (brown). The expression of Dfd::lacZ (arrows) identifies these embryos as XX.
were themselves homozygous for cells. If XX embryos, derived from homozygous independent of female germ cells and a vital maternal effect that are both despite the presence of SXL protein, develop as strongly reported previously that XX animals with these genotypes, were sexually transformed (Table 2, lines I, II). It has been maternal Sxl females. Thus, the autoregulation of Sxl, depending on Sxl has no germ cells. This rescue must be zygotic since derived from these eggs. Thus, Sxl M4; vir 2f has a vital function in vir 6 /vir 2f for vir since vir 2f/vir 2f and vir 2f/vir 6 embryos derived from heterozygous mothers are rescued by Sxl M4 (Hilfiker et al., 1995). Thus, the zygotic function of Sxl M4 comes too late or is too weak to rescue XX animals that obtain neither maternal nor zygotic vir + product.

**SXL protein is reduced in ovaries mutant for vir**

*Sxl* is necessary for the differentiation of female germ cells. A specific allele, *Sxl* M4, disrupts only the germline activity of *Sxl*. *Sxl* M4 females are viable, but sterile due to an excessive proliferation of undifferentiated germ cells (Bopp et al., 1993). Since the ovarian phenotypes of *Sxl* M4 and *f4* are different, and since *Sxl* M4 rescues the defects in female embryos derived from vir 6 germ cells only zygotically, *Sxl* function is expected to be independent of *vir* in germ cells. To test this assumption, ovaries with vir clones were stained with anti-SXL antibody to monitor the expression of *Sxl*. In wild-type females, SXL protein is present in nurse cells and follicle cells, but not in the oocyte (Fig. 2A,B).

SXL protein was present in clones of all vir alleles at the tip of the germarium, but the amount in nurse cells decreased already at stage two. In later stages, SXL was nearly or totally absent. Despite the reduction of SXL protein, no tumorous cysts were observed as they occur in *Sxl* M4 ovaries (Fig. 2C-E). Thus, it is possible that SXL is only needed for the first steps of oogenesis in the germarium.

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**Table 2. Effects of Sxl M on vir/vir germ cells and their offspring**

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<tr>
<td>I</td>
<td>Sxl M4; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
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<tr>
<td></td>
<td></td>
<td>85♂</td>
<td>55♂ (2)</td>
<td>15♂ (2)</td>
<td>15♂ (2)</td>
<td>15♂ (2)</td>
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<td>II</td>
<td>Sxl M4/+; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
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<td></td>
<td></td>
<td>55♂ (2)</td>
<td>19♀ (3)</td>
<td>19♀ (3)</td>
<td>19♀ (3)</td>
<td>19♀ (3)</td>
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<tr>
<td>III</td>
<td>Sxl M1; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
<td>+/Y; vir 2f/vir 2f</td>
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<tr>
<td></td>
<td></td>
<td>114♂</td>
<td>74♂ (1)</td>
<td>440♂</td>
<td>39♀</td>
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</tr>
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</table>

Full maternal genotype: aw Sxl M/w FLP; FRT vir bw/FRT ovo D; by w FLP/+; FRT vir bw/FRT ovo D.

n.d. = not determined.

1) Also viable without Sxl M.
2) Rescued by Sxl M, but sexually transformed by vir.
3) Rescued by Sxl M, female phenotype due to vir +.
4) Escapers.
5) snf used to introduce Sxl M from father.

for vir 2f and vir 4 did it rescue the lethality of embryos derived from these eggs. Thus, *vir* has a vital function in female germ cells and a vital maternal effect that are both independent of Sxl (Table 2, line III).

*Sxl* M4 was able to rescue the daughters of females with vir 6 germ cells. This rescue must be zygotic since Sxl has no maternal effect on zygotic activity of Sxl (Cline, 1978; Bopp et al., 1993). *Sxl* M4; vir 2f/vir + animals developed as normal females. Thus, the autoregulation of Sxl, depending on maternal *vir*, can be bypassed by *Sxl* M4 in the embryo. *Sxl* M4; vir 2f/vir 6 animals from *Sxl* M4; vir 2f germ cells and *Sxl* M4; vir 6/vir 2f from *Sxl* M4; vir 6 germ cells were also rescued, but were sexually transformed (Table 2, lines I, II). It has been reported previously that XX animals with these genotypes, despite the presence of SXL protein, develop as strongly masculinized intersexes since *vir* is also necessary for the female-specific splicing of *tra* (Hilfiker et al., 1995).

*Sxl* M1 can be introduced either by the female or by the male if he is mutant for snf 1621 or *vir* M1; *Sxl* M1 fathers were able to rescue *Sxl* M1; vir 6/vir + daughters from mothers with vir 6 germ cells. If XX embryos, derived from homozygous vir germ cells, were themselves homozygous for *vir* 2f or transheterozygous for *vir* 2f/vir 6 , the allele *Sxl* M1, in contrast to *Sxl* M4, was not able to rescue them, irrespective of maternal or paternal inheritance (Table 2, lines I, II, IV, V). This failure to rescue is not due to the genetic constitution of the zygote, but to the maternal lack of *vir* since *vir* 2f/vir 2f and *vir* 2f/vir 6 embryos derived from heterozygous mothers are rescued by *Sxl* M4 (Hilfiker et al., 1995). Thus, the zygotic function of *Sxl* M4 comes too late or is too weak to rescue XX animals that obtain neither maternal nor zygotic *vir* + product.
**Sxl** is required for the early steps of oogenesis

To test if **Sxl** and **vir** mutations really disrupt different processes in oogenesis, we generated germline clones in *Sxl<sup>f1</sup>/ovo<sup>D</sup> and *Sxl<sup>f4</sup>/ovo<sup>D</sup> females at the same stage as the **vir** clones. In contrast to **vir**, these females never laid any eggs. *Sxl<sup>f4</sup> clones showed the typical phenotype with tumorous cysts, which we never observed in **vir** clones. These results suggest that **Sxl** is necessary for the early phase of differentiation of germ cells and that **vir** is involved in the regulation of **Sxl** only later when oogenesis is already initiated and can be completed without **Sxl**.

**Male germ cells are not affected by **vir**

The gene **vir** has a female-specific function in the soma and the germline. In addition, it plays a role in an important vital process in somatic cells of both sexes. As a last point, we examined a possible role in the male germline. By transplanting whole larval gonads of the lethal genotypes *XY; vir<sup>3</sup>/Df(2R)vir<sup>130</sup>* and *XY; vir<sup>3</sup>/Df(2R)vir<sup>130</sup>* into agametic **XY** host flies, we could show that sperm was formed after culturing the gonads in the abdomen of adult females. We also observed a typical phenotype of tumorous cysts in both cell types.

**DISCUSSION**

Besides its requirement for the female-specific splicing of **Sxl** and **tra** and a yet unknown vital process in the soma of both sexes, we found that **vir** has several functions in the female germline: maternal product of **vir** is required for the regulation of **Sxl** in daughters and for the viability of both sexes. In addition, **vir** is necessary in the female germline for a normal rate of egg production and for the maintenance of late **Sxl** activity. The early production of **SXL**, however, is independent of **vir** and is sufficient to initiate and execute oogenesis. Finally, **vir** is dispensable for spermatogenesis.

**Table 3. XY germ cells mutant for a lethal **vir** allele perform spermatogenesis when transplanted into a male host**

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<tr>
<th>Genotype of integrated germ cells*</th>
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<tr>
<td><em>T(2;3)Xa/SM5</em></td>
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<tr>
<td><em>Df(2R)vir130/T(2;3)Xa</em></td>
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<tr>
<td><em>vir&lt;sup&gt;3&lt;/sup&gt;/SM5</em></td>
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<tr>
<td><em>vir&lt;sup&gt;3&lt;/sup&gt;/Df(2R)vir130</em></td>
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*Adult flies were tested for fertility with *cn vir<sup>fs</sup> bw/cn vir<sup>fs</sup> bw* females, and the genotype of the integrated germ cells was determined from the phenotype of the progeny. Of 113 adult male hosts, only 7 were fertile.

**Functions of **vir** in oogenesis**

In contrast to somatic cells, XX germ cells do not need **vir** for their female identity. Oogenesis can proceed normally in **vir** mutant germ cells. However, germ cells mutant for strong alleles which affect the vital somatic function form much smaller clones. Either some of these mutant germ cells die, or they divide and differentiate more slowly. Since this defect cannot be rescued by a constitutive allele of **Sxl**, the affected function must be independent of **Sxl**. This conclusion is further supported by **vir<sup>2f</sup>**, which reduces expression of **Sxl** in germ cells but allows egg production at a normal rate. The vital functions of **vir**, both in somatic and female germ cells, are unknown. It is possible, however, that **vir** is involved in the same process in both cell types.

**Maternal functions of **vir**

**Daughterless** (**da**), **hermaphrodite** (**her**), and **snf** are known to have a maternal effect on the viability of daughters. **XX** embryos from **da** or **her** mutant mothers fail to initiate **Sxl** (Cline, 1980, 1993; Albrecht and Salz, 1993; Pultz and Baker, 1995). Our work has now identified **vir** as a further component that is maternally required for the regulation of **Sxl** in **XX** embryos. Unlike **da** and **her**, but similar to **snf**, it acts at the level of post-transcriptional control. The establishment promoter of **Sxl** is activated both in **vir<sup>2f</sup>+** and **vir<sup>2f</sup>/vir<sup>2f</sup>** embryos derived from **vir** mutant mothers, but **Sxl** expression is not maintained. The lethality of **vir<sup>2f</sup>+** embryos indicates that maternal product of **vir** is responsible for the autoregulation of **Sxl** during early development. In addition, maternal **vir** function is also required for viability of offspring of both sexes as shown by strong alleles prompting the vital function. For such alleles, neither the autoregulation of **Sxl** nor the vital process can be rescued by a paternal **vir**+ allele. The reason may be that the zygotic gene is either not yet active or not yet sufficiently active.

**Regulation and function of **Sxl** and **vir** in the female germline**

Most genes of the somatic sex determination cascade are dispensable within germ cells (Marsh and Wieschaus, 1978; Schüpbach, 1982; Granadino et al., 1993; Steinmann-Zwicky, 1993, 1994a; Horabin et al., 1995). **Sxl**, although necessary for oogenesis, does not have a master regulatory function for sex determination in the germline, as indicated by the following observations. (1) Expression of **Sxl** in germ cells is first detected in 16- to 20-hour-old embryos (Horabin et al., 1995). A male-specifically expressed gene, however, is already observed in germ cells of 10-hour-old embryos, suggesting that some aspect of sexual development must have been determined prior to expression of **Sxl** (Staab et al., 1996). (2) **Sxl<sup>f4</sup>/Sxl<sup>f1</sup>** and
**Fig. 3.** Model for the regulation and function of Sxl and vir in the female germ line. Sxl is activated by a somatic signal and the germline X:A ratio. Early SXL is necessary for the female development of germ cells, as indicated by mutations in Sxl, snf, fl(2) d, ovo or otu which block oogenesis. Later in oogenesis, expression of Sxl becomes dependent on vir, and perhaps again on snf, fl(2) d, ovo and otu (interrupted arrow). This later SXL product, however, is no longer necessary for the differentiation of eggs, but its function is unknown (interrupted arrow). In addition to the regulation of Sxl, vir has an unknown vital function in germ cell development and a maternal effect on embryogenesis.

Sxl<sup>f5</sup>/Sxl<sup>f1</sup> larvae have female gonads by the criteria of size and morphology; only as adults do the germ cells form abnormal multicellular cysts. Sxl does not seem to control the sex-specific differentiation of germ cells in larvae, but is required later for oogenesis (Steinmann-Zwicky, 1994b). (3) XY germ cells containing Sxl<sup>M1</sup> or Sxl<sup>M4</sup> are not feminized, but instead form fertile sperm (Steinmann-Zwicky, 1993).

The expression of Sxl in the germline depends on inductive signals from the gonadal soma and on an autonomous signal given by the germline X:A ratio, which is measured by elements different from those used to determine the somatic X:A ratio (Granadino et al., 1993; Steinmann-Zwicky, 1993). Thus, initiation of Sxl is different in soma and germline. We now showed that, in a second phase, expression of Sxl in differentiating cysts becomes dependent on vir. This parallels the regulation of Sxl in somatic cells. In contrast to the soma, however, the consequences of misexpression of Sxl are different: germ cells are not sexually transformed.

The different germline phenotypes of Sxl and vir mutations may have trivial reasons. A perdurance effect of VIR protein made in heterozygous stem cells prior to mitotic recombination could maintain the production of SXL protein in vir clones. However, the tested females continued to lay eggs even two weeks after the induction of clones and after several rounds of cell divisions. By this time, VIR protein should have been diluted or eliminated and should no longer be able to regulate Sxl. A second possibility would be that the first 24 hours of development in heterozygous condition before the induction of clones determine the female fate of a germ cell irreversibly. In all previous experiments with ovaries mutant for Sxl<sup>M1</sup> and with transplanted pole cells mutant for Sxl<sup>M4</sup>, the germ cells were homozygous mutant from the beginning (Schüpbach, 1985; Bopp et al., 1993). But even when we induced Sxl clones under the same conditions as the vir clones, the females remained sterile as in earlier experiments showing that Sxl is necessary after the induction of the clones. Therefore, the phenotypic differences of Sxl and vir mutants are caused by different requirements for the two genes.

Based on these results, we suggest the following model (Fig. 3). The initiation mechanism of Sxl in the germline is unknown. Early SXL protein, however, is necessary in gonial cells at the tip of the germarium in the adult ovary for female development. Later in oogenesis, the germ cells may become independent of the primary signals by the soma and the X:A ratio and maintain SXL production by autoregulation (Hager and Cline, 1997). This later post-transcriptional regulation depends on vir. Late SXL protein, however, is no longer necessary for the female differentiation of germ cells, once these cells have embarked on the oogenic pathway. It is not yet clear what other functions SXL has in later stages of oogenesis. SXL protein is redistributed during oogenesis (Bopp et al., 1993). In stem cells and early cystoblasts in the gerarium, SXL is predominantly cytoplasmic. During the mitotic divisions, the level of cytoplasmic SXL drops drastically. In the cluster of 16 cells, the protein becomes concentrated in the nuclei of the cystocytes. This transition of SXL protein may reflect the early and late functions of Sxl. We conclude that SXL is required for a short period during the transition from stem cells to cystoblasts. Lack of SXL during this phase results in tumorous cysts.

snf and fl(2) d are two other genes involved in the autoregulation of Sxl in the soma. Salz (1992) and Granadino et al. (1992) showed that these two genes are also required for oogenesis. Mutant females produce no eggs, but instead form multicellular cysts. We can only speculate why mutations in snf, fl(2) d and vir have different consequences in the germline. They may still all affect the autoregulation of Sxl, as they do in somatic cells, but at different times. It is possible that the production of early SXL protein depends on a post-transcriptional regulation that requires snf and fl(2) d, whereas vir may only act later in oogenesis.

The regulation of Sxl in the germline is certainly more complex than in the soma, and the germline function of this gene is also less clear. A comparative analysis of the regulation and function of Sxl in soma and germline is therefore worthwhile and may contribute to our understanding of how a gene operates in different cell types.

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