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The Genetic Analysis of *snf*: A Drosophila Sex Determination Gene Required for Activation of *Sex-lethal* in Both the Germline and the Soma

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ABSTRACT

Our analysis demonstrates that *snf* is a positive regulator of *Sex-lethal* in both the germline and the soma. In the germline, unregulated expression of *Sex-lethal* can bypass the requirement for snf^+ gene function, implying that *snf* is required for *Sex-lethal* activity in the germline. This conclusion is supported by the finding that the *Sex-lethal* transcription pattern is abnormal in a *snf* mutant background. In the soma, activation of *Sex-lethal* appears to be sensitive to *snf* gene dosage only when the probability of *Sex-lethal* activation has been otherwise reduced. We also show that the activity of one of the constitutive *Sex-lethal* alleles (Sxl^{M1}) is sensitive to *snf* gene dosage, demonstrating that, in spite of its constitutive behavior in some assays, Sxl^{M1} is still subject to some regulation. In spite of *snf*'s role in the somatic activation of *Sex-lethal*, no lethal alleles of *snf* were isolated in a screen of ~25,000 chromosomes. The observation that the existing *snf* mutations present a lethal phenotype only in certain genetic backgrounds suggests that *snf* is required, but is not essential, for the activation of *Sex-lethal* in the soma. In contrast, *snf* does appear to be essential for activation of *Sex-lethal* in the germline, as evidenced by its female-sterile phenotype.

SEXUAL development in the fruit fly *Drosophila melanogaster* is one of the best characterized systems to study the complex gene interactions necessary for the initiation and maintenance of developmental decisions. The sex-specific decisions leading to somatic sexual differentiation, the vital process of *X* chromosome dosage compensation and differentiation of the germline are all controlled by the binary switch gene *Sex-lethal* (*Sxl*) (HODGKIN 1990; PAULI and MAHOWALD 1990; SLEE and BOWNES 1990; STEINMANN-ZWICKY, AMREIN and NOTHIGER 1990). When *Sxl* is ON, the female-specific pathway is chosen; conversely when *Sxl* is OFF, the male-specific pathway is chosen. Recessive loss of function mutations are female-specific lethals, whereas dominant gain of function mutations are male-specific lethals.

At a molecular level activation of *Sxl* appears to be quite complex. In the soma, the initial response of *Sxl* to the *X* chromosome to autosome ratio is under transcriptional regulation (ERICKSON and CLINE 1991; KEYES, CLINE and SCHEDL 1992). Once female-specific expression of *Sxl* is achieved, it regulates its own activity as well as the downstream genes by alternative RNA splicing (BELL *et al.* 1988; NAGOSHI *et al.* 1988; INOUE *et al.* 1990; BELL *et al.* 1991; SAMUELS, SCHEDL and CLINE 1991). In contrast to the soma, little is known about how *Sxl* is activated in the germline, although it is possible that it too is activated by a similar two step process (SALZ, CLINE and SCHEDL 1987; SAMUELS, SCHEDL and CLINE 1991).

Genetic studies have suggested that another locus, in addition to *Sxl*, is required for a similar set of sex-specific functions in both the germline and the soma (GOLLIN and KING 1981; OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). *snf*'s role (also named *fs(1)1621*, *sans-fille*, and *liz*), in germline sex determination was determined directly from the genetic analysis of a single allele, snf^{1621}. Females homozygous for snf^{1621} are sterile (GANS, AUDIT and MASSON 1975). In the mutant neither the oocyte nor the nurse cells differentiate. Instead the germarial cells continue to divide, resulting in the formation of ovarian tumors (GOLLIN and KING 1981). The similarity of *snf* and *Sxl* mutant phenotypes in the germline suggests that they disrupt the same process, germline-sex determination (OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988).

In contrast to *snf*'s role in germline sex determination, its role in somatic sex differentiation and *X* chromosome dosage compensation can only be inferred by an unusual genetic interaction between mutations at the *snf* and *Sxl* loci. Females that are doubly heterozygous for a *Sxl* null mutation and snf^{1621} (or a deficiency of *snf*) show a cold-sensitive dominant lethal interaction (CLINE 1988; OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). The double heterozygous females have reduced viability, presumably due to upsets in dosage compensation. In addition, many of these surviving females show signs of sex transformations. In addition to its zygotic requirement for *snf* function, a maternal requirement can also be inferred from these data. All

daughters (*snf/Sxl* and *+/Sxl*) from *snf/+* mothers and *Sxl* fathers have reduced viability, irrespective of their genotype with respect to *snf*.

Although genetic interactions between mutations at different loci are useful in identifying genes required in similar processes, they tell us little about their actual function. Thus to understand the role of *snf* in both the germline and the soma we have extended the genetic analysis of *snf*. Our results indicate that *snf* is a positive regulator of *Sxl*: *snf* function is required to activate *Sxl* both in the early embryo and in the germline. Surprisingly, in an extensive screen for new mutations, we recovered only one new allele of *snf*. This new allele behaves in a similar fashion to the original allele in both the germline and the soma. The failure to isolate lethal alleles of *snf* suggests that zygotic *snf* function is not essential for *Sxl* activation in the early embryo.

MATERIALS AND METHODS

Note on nomenclature: A considerable amount of confusion surrounds the naming of the *snf* locus. *snf* was initially isolated in a screen for female-sterile mutations and named *fs(1)1621* (GANS, AUDIT and MASSON 1975; GOLLIN and KING 1981). Once identified as a sex determination gene it was renamed *sans-fille* (*snf*) (OLIVER, PERRIMON and MAHOWALD 1988) and *liz* (STEINMANN-ZWICKY 1988). Although we will use the three letter designation *snf* (pronounced sniff) in this report, we would like to point out that the name *sans-fille*, which means "daughterless" in French, is inconsistent with the phenotype of the mutation since mutant females are simply sterile and produce neither sons nor daughters.

Fly stocks and culture conditions: Mutations and rearrangements not described in the text or listed in Table 1 are described in Lindsley and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987, 1990).

Unless otherwise indicated, flies were reared at room temperature which ranged from 22° to 25° on a standard cornmeal, yeast molasses, agar medium.

Cytological analysis: The salivary gland chromosomes of individuals heterozygous for a rearrangement and a wild-type chromosome were examined by phase contrast microscopy after staining with lacto-acetic-orcein. Cytological breakpoints were assigned using the photographs in LEFEVRE (1976) and SORSA (1988).

Isolation of γ-ray induced mutations that do not complement *snf*^{1621}: This screen was necessarily complicated by the fact that there was, at the time, no duplication for the region that was viable in males (see below on how we made one). Virgin collection in this screen was kept to a minimum by including *l(1)44ts* , which is lethal at 29° but not at 25° (KOMITOPOULOU *et al.* 1983). Males hemizygous for an isogenized X chromosome marked with *yellow* were exposed to 4000 rad γ-irradiation delivered from a ^{60}Co source and mated *en masse* to *l(1)44ts/FM3* virgin females at 29°. Only virgin female progeny are produced in the next generation because both *l(1)44ts* and FM3 males are lethal. Individual stocks of each mutagenized X chromosome were established by mating a single *y/FM3* virgin to an FM7 male and then in the next generation selecting out the *FM7/FM3* females. Each stock was then tested for complementation with *snf*^{1621}.

Construction of *Dp(1;2)4FRDup*–a duplication that covers the 4C11-12; 4F11-12 interval and is viable in males: To facilitate the genetic analysis of this region of the *X* chromosome, a duplication was needed. However, all preexisting duplications were lethal to males because they also include a copy of a dosage sensitive region, located at position 3F (CLINE 1988; OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). When present in two copies in males, this region causes lethality. Our strategy for isolating a duplication that covers *Df(1)JC70* but does not include a copy of the dosage sensitive region was to induce a deficiency in a preexisting duplication *Dp(1;2)w*$^+$*64b13*. Males that were *Df(1)A113; Dp(1;2)w*$^+$*64b13/+* were exposed to 4000 R γ-irradiation and crossed to females homozygous for *yellow* and *white*. Out of the ≈50,000 chromosomes screened, 22 males that carried the duplication were selected, of which 5 were fertile. The extent of the remaining duplication was determined by complementation with several deficiencies in the area. The reduced duplication (*Dp(1;2)4FRDup*) that was used in this study covers *Df(1)JC70* and *Df(1)ovoG6*, but not *Df(1)RC40*. It also contains the wild-type copies of both *Notch* and *white*, which can be used to determine the absence or presence of the duplication. In the text we have abbreviated *Dp(1;2)4FRDup* as 4FRDup.

Isolation of mutations in the 4C11-12; 4F11-12 interval: The screen was designed to isolate mutations within *Df(1)JC70* that are either lethal in females, female sterile or that are either "sonless" or "daughterless" (Figure 1). Our selection criteria made no assumptions with respect to the mutant phenotype in males. Males isogenic for an *X* chromosome marked with *w* were fed either 0.025 M ethyl methanesulfonate (EMS) or 0.007 M diexpoxybutane (DEB) according to the method of LEWIS and BACHER (1968) and then mated to *C(1)DX ywf;* 4FRDup (*w*$^+$)/+ virgins. The duplication-carrying male progeny of this cross (*w;* 4FRDup/+) were mated individually to several *yw Df(1)JC70/FM7c* females. Progeny from each vial were scored for the induction of a mutation within the deficiency by scoring the phenotype of *w/y w Df(1)JC70* females. In each case the *w/FM7c* females, balanced for the mutagen treated chromosome, were used to establish a stock of each putative mutation.

Only those mutations that retested over either *Df(1)ovoG6* or *Df(1)DEB4D* were analyzed further. *Df(1)DEB4D* was isolated in a pilot screen for DEB-induced lethals within *Df(1)JC70*. The pilot screen was virtually identical to the one described here.

Determination of the order of and the distance between *snf* and *fs(1)1059* by recombination: Because recombination between *fs(1)1059* and *snf* mutations carried in *trans* will produce a wild-type recombinant, the frequency of fertile chromosomes recovered is indicative of the distance between the two loci. The order of the flanking markers (*white* and *cut*) present on the fertile chromosome is indicative of the order of the two genes. To map *fs(1)1059* relative to *snf*, sons recombinant for the flanking markers *white* and *cut* (*w*$^+$ *ct* and *w ct*$^+$ males) were collected from among the progeny of *w snf*^{1621} *ct/fs(1)1059* females. To screen for recombination between *snf* and *fs(1)1059*, each putative recombinant male was mated to *Df(1)JC70/FM7* females and the non balancer female progeny tested for fertility. Of 6397 males, 1106 were recombinant for the flanking markers, *w* and *ct*. These males were then tested for a recombination event between *snf* and *fs(1)1059*. Two *w*$^+$ *ct* recombinant chromosomes were found to be fertile when heterozygous with *Df(1)JC70*; these two chromosomes represent half of all recombination events between *snf* and *fs(1)1059*. From these data we can conclude that the two genes are 0.06 cM apart and that *snf* is distal to *fs(1)1059*.

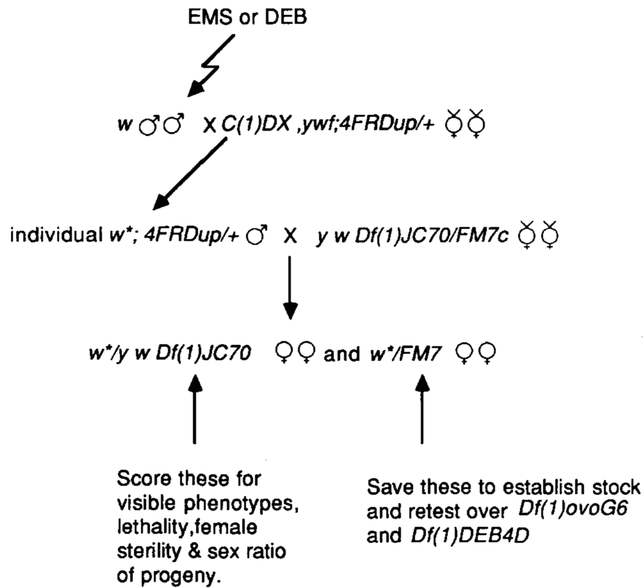

FIGURE 1.—Crossing scheme for mutant isolation in the 4C11-12;4F11-12 interval described in MATERIALS AND METHODS. * indicates a mutagen treated chromosome.

RNA analysis: Poly(A$^+$) RNA was isolated and Northern blots carried out as previously described (SALZ *et al.* 1989).

RESULTS

Isolation and characterization of new *snf* alleles: To determine the effect of a complete loss of *snf* function, we initiated three large scale screens to isolate new *snf* alleles. In all we screened approximately 25,000 chromosomes using three different mutagens.

Initially we screened for γ-ray induced *snf* alleles that did not complement *snf*^{1621} (described in MATERIALS AND METHODS). Because females heterozygous for a deficiency of the locus and *snf*^{1621} are viable, but sterile, new mutants were to be identified based on a sterile phenotype when heterozygous with *snf*^{1621}. In this screen we also expected to isolate new *Sxl* alleles by virtue of the fact that the double heterozygous *Sxl*, *snf*$^+$/*Sxl*$^+$, *snf* females have reduced viability and are sterile. In a screen of ≈10,000 *X* chromosomes we identified three female-specific lethal mutations that map by recombination to the *Sxl* locus (data not shown). No mutations that map within the *snf* region were isolated in this screen.

The next screens were broader in scope and were facilitated by the construction of a male-viable duplication of the cytogenetic interval containing *snf* (see MATERIALS AND METHODS). Within this interval, we screened for mutations, based solely on their phenotype in females (Figure 1): We screened for female-specific lethals, non-sex-specific lethals, female-steriles and females that produce progeny in aberrant sex ratios (a "daughterless" or "sonless" phenotype). About 10,000 EMS-treated *X* chromosomes and

TABLE 1

Cytology of chromosome deficiencies used in this study

Name	Cytology[a]	Reference
Df(1)JC70	4C11-12;5A3-4	CRAYMER AND ROY (1980)
Df(1)svbEH[b]	Not visible, removes *fl(1)301, hnt, svb, ovo*	EBERL AND HILLIKER (1988)
Df(1)ovoG6[b]	4C11-12;4F1,2	OLIVER, PERRIMON AND MAHOWALD (1987)
Df(1)ovoG7[b]	4A4;4F1,2	OLIVER, PERRIMON AND MAHOWALD (1987)
Df(1)RC40	4B;4F1,2	CRAYMER AND ROY (1980)
Df(1)ovo44	4A4-5;4F11-12	STEINMANN-ZWICKY (1988)
Df(1)HC44	3E;4F11-12	CRAYMER AND ROY (1980)
Df(1)DEB4D	4E1,2;4F11-12	This study

[a] All breakpoints are based on our own cytology.
[b] *Df(1)ovoG6* and *Df(1)ovoG7* were originally published as *Df(1)ovoD1rG6* and *Df(1)ovoD1rG7*, respectively. *Df(1)svbEH* was originally published as *l(1)EH587* and identified as a small deletion by OLIVER, PERRIMON and MAHOWALD (1987) that removes *hnt*, *svb* and *ovo*. We have also shown that it removes another locus *fl(1)302* (STEINMANN-ZWICKY 1988) which is located in 4C adjacent to *hnt* (data not shown).

5,000 DEB-treated *X* chromosomes were screened for mutations within the 4C11-12;4F11-12 interval. In all 16 mutations were isolated: 8 female-steriles and 8 non-sex-specific lethals. Each was tested for complementation with the deficiencies listed in Table 1, which divide the 4C11-12; 4F11-12 interval into 5 regions. Mutations that fell within the same region were tested for complementation among themselves as well as with previously identified mutations that map within each region. The results of this analysis are summarized in Figure 2.

Three mutations were localized to the same interval as *snf* (4F1, 2-4F11, 12) by complementation with several deficiencies. Whereas two of these mutations map by recombination to the *Sxl* locus (1–19), the third mutation, *e8H*, maps by recombination close to the *snf* locus (1–11) (data not shown). *e8H* appears to be an allele of *snf* because it does not complement *snf*^{1621}, but does complement the only other known mutation within this interval, *fs(1)1059*. *e8H*/*snf*^{1621} females are sterile and have ovarian tumors. Surprisingly, and in contrast to *snf*^{1621}, females homozygous for *e8H* are fertile and have equal numbers of male and female progeny (data not shown).

***snf*^{e8H} also displays a lethal synergistic interaction with *Sxl*:** Previous studies have shown that *snf*^{1621} or a deficiency of *snf* show a cold sensitive lethal interaction with null alleles of *Sxl* (CLINE 1988; OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). In our experimental conditions this interaction is fairly weak (Table 2, cross A). Thus we might not detect an interaction with *snf*^{e8H} since it is a weaker mutation than *snf*^{1621}. We therefore made the assay more sensitive by the addition of a mutation at another sex determination locus. By reducing the dose of either *daughterless* (*da*^{2}/+) in the mother or

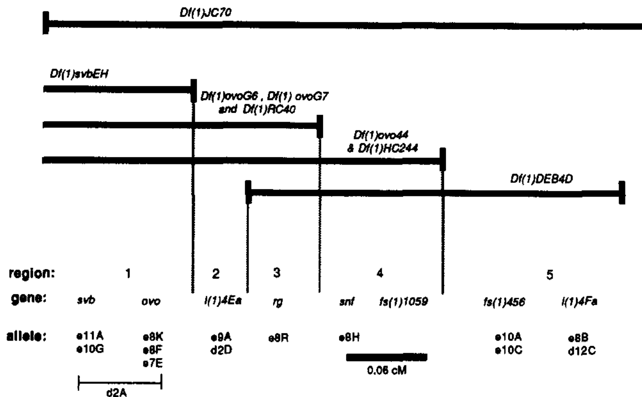

FIGURE 2.—Genetic organization of the 4C11-12;4F11-12 cytogenetic interval. Deletions are indicated by heavy horizontal lines. DEB induced mutations are preceded by a lowercase d; EMS induced mutations are preceded by a lower case e. **Region 1:** Three lethal mutations were identified as alleles of *shavenbaby* (*svb*) by a lack of complementation with each other and with svb^{YP17b} (WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984). One of these alleles, *d2A* is also an allele of *ovo* as determined by complementation with ovo^{D1rS1} (OLIVER, PERRIMON and MAHOWALD 1988). In addition, three EMS-induced female sterile mutations were identified as alleles of *ovo*. Previous studies had also localized a locus named *lozenge-like* (*lzl*) to this region. However, recent molecular studies suggest that the *lzl* phenotype is apparently due to the misexpression of an aberrant *ovo* gene product (GARFINKEL, LOHE and MAHOWALD, 1992; MEVEL-NINIO, TERRACOL and KAFATOS 1991). All of the existing *lzl* mutations are associated with an insertion of a gypsy transposable element in the same orientation within the dominant ovo^{D1} locus. **Region 2:** Two alleles of a new lethal complementation group, *l(1)4Ea*, were identified within this region. **Region 3:** The *rugose* (*rg*) gene is the only known gene within this region. Although the original mutation, rg^1, has a rough eye phenotype with no effect on viability, the *rg* allele we isolated is a semilethal. It is likely that lethality is due to mutant *rg* function since this allele fully complements the neighboring lethal, *l(1)4Ea*. Our data suggest that the *rg* gene is required for an essential function that is not eye-specific. **Region 4:** *snf* and *fs(1)1059* (also reported as *fs(1)K575* and *fs(1)M60*) are located within this region. One additional allele of *snf* was identified by complementation with snf^{1621}. Although no alleles of *fs(1)1059* were isolated in our screens, others have reported the isolation of several EMS-induced alleles (GANS, AUDIT and MASSON 1975; MOHLER 1977; KOMITOPOULOU *et al.* 1983; PERRIMON *et al.* 1986). Recombination analysis has placed *snf* 0.06 cM distal to *fs(1)1059* (see MATERIALS AND METHODS for details). **Region 5:** Two complementation groups have been identified within this region. Two new alleles of *fs(1)456* were identified by complementation with *fs(1)456* (GANS, AUDIT and MASSON 1975) and two alleles of a newly identified lethal complementation group *l(1)4Fa.* were identified. The order of these two genes with respect to each other is not known.

sisterless-a (*sis-a*/+) in the zygote the lethal interaction between *snf* and *Sxl* is enhanced. For example even though daughters from snf^{1621}/+ mothers and Sxl^{f1} fathers show only slightly reduced viability, daughters from snf^{1621}/+ mothers and Sxl^{f1}, *sis-a* fathers rarely survive (compare crosses A and B in Table 2). Similarly, daughters from snf^{1621}/+; da^2/+ mothers and Sxl^{f1} fathers seldom survive (data not shown).

The data in Table 2 (crosses C, D and E) demonstrate that even though snf^{e8H} appears to be a weaker

TABLE 2

Dominant synergism between *Sxl*, *sis-a* and the mutations in 4F1,2-4F11,12

Cross[a]	Parental genotype	Genotype and number of progeny and their viability (%) relative to the number of control males recovered				Control males
A	*1621/FM7* × *Sxl/Y*	*1621/Sxl* 30 (26%)	*Sxl/FM7* 69 (60%)	*1621/Y* 96 (83%)		*FM7/Y* 116
B	*1621/FM7* × *Sxl, sis/Y*	*1621/Sxl, sis* 2 (1%)	*Sxl, sis/FM7* 9 (6%)	*1621/Y* 149 (100%)		*FM7/Y* 150
C	*e8H/FM7* × *Sxl, sis/Y*	*e8H/Sxl, sis* 41 (51%)	*Sxl, sis/FM7* 46 (58%)	*e8H/Y* 61 (76%)		*FM7/Y* 80
D	*e8H/e8H* × *Sxl, sis/Y*	*e8H/Sxl, sis* 0 (<0.5%)				*e8H/Y* 196
E	*e8H/e8H* × *Sxl*	*e8H/Sxl* 0 (<0.3%)				*e8H/Y* 398
F	*1059/FM7* × *Sxl, sis/Y*	*1059/Sxl, sis* 122 (100%)	*Sxl, sis/FM7* 182 (100%)	*1059/Y* 229 (100%)		*FM7/Y* 65

[a] Full genotypes of the crosses: *cm* Sxl^{f1} *ct/Y* males or *cm* Sxl^{f1} ct *sis-a/Y* males were crossed to the following females: *y cho* snf^{1621} *ct v g f/FM7*, *fs(1)1059* v^{24}*/FM7*, *w* snf^{e8H}*/FM7*, or *w* snf^{e8H}*/w* snf^{e8H}.

allele than snf^{1621} it still displays a strong lethal synergistic interaction with the sex determination loci. *Sxl*, *sis-a*/+,+ female progeny from mothers *heterozygous* for snf^{e8H} have reduced viability (cross C). The lethal synergistic interaction can be further enhanced by reducing the amount of snf^+ product produced by the mother. *Sxl*, *sis-a*/+,+ females from mothers *homozgyous* for snf^{e8H} are not viable (cross D). The importance of the maternal requirement for snf^+ function is further demonstrated by the observation that *Sxl*/+ females from mothers homozygous for snf^{e8H} are also not viable (cross E).

The *snf* mutations are the only identified mutations within 4F1,2–4F11,12 that interact with *Sxl*: The failure to recover lethal alleles of *snf* is somewhat surprising in light of previous studies that suggested that *snf* also plays a vital role in the soma. This conclusion was based on the observation that a deficiency chromosome that lacks at least from 4F1,2 to 4F11,12 interacts with mutations at the *Sxl* locus (CLINE 1988; OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). Because snf^{1621} interacts in the same way it was presumed that the *snf* locus is solely responsible for this interaction (OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-

ZWICKY 1988). However, given that another locus, *fs(1)1059*, is also located within the same interval as *snf*, it is a formal possibility that it too could interact with *Sxl*. The data in Table 2 (cross F) demonstrate that *fs(1)1059* fully complements *Sxl, sis-a*. Thus it appears that the *snf* locus is the only identified locus within 4F1,2–4F11,12 that displays a lethal synergistic interaction with *Sxl*.

***snf*'s function in the germline:** The similarity of the ovarian tumor phenotype observed in *snf* mutations compared to that found in germline-specific mutations of *Sxl* (SALZ, CLINE and SCHEDL 1987) suggests that they both disrupt the same process. STEINMANN-ZWICKY (1988) further refined the relationship between *Sxl* and *snf* by demonstrating that the female sterility of snf^{1621} homozygous females can be suppressed by Sxl^{M1}, a constitutive allele of *Sxl*. Although the phenotypes of double mutant combinations can be used to place genes in a regulatory hierarchy, they can also result from allele-specific interactions that are not interpretable in the same manner (for example, see the interaction between Sxl^{M1} and *snf* in the soma described in the next section). The following observations suggest that the germline suppression of the *snf* mutant phenotype is not due to allele-specific interactions. The sterility of snf^{1621} can be suppressed by either of two constitutive alleles Sxl^{M1} or Sxl^{M4}. Both snf^{1621}, Sxl^{M1}/*Df(1)DEB4D* and snf^{1621}, Sxl^{M1}/*Df(1)DEB4D* females are fertile. Moreover, Sxl^{M1} can equally suppress both alleles of *snf*: both *Df(1)DEB4D*, Sxl^{M1}/snf^{e8H} females and *Df(1)DEB4D*, Sxl^{M1}/snf^{1621} females are fertile. Because the suppression is not due to allele-specific interactions between *snf* and Sxl^{M1}, the suppression is likely to be due to the ability of the unregulated expression of *Sxl* to bypass the germline requirement for snf^{+}. From these data snf^{+} appears to be a positive regulator of *Sxl* in the germline.

Consistent with this hypothesis is the observation that the *Sxl* transcription pattern is altered in females homozgyous for snf^{1621} (Figure 3). In wild type females, three major size classes of RNAs are observed when a *Sxl* cDNA is used as a probe: 4.2, 3.3 and 1.9 kb. In contrast, in snf^{1621} females the 1.9-kb RNA is not detectable and there is a marked reduction in the level of the 3.3-kb size class. Similar abnormal transcription patterns are also obtained with females without a germline (SALZ *et al.* 1989). Thus snf^{1621} mutant females appear to be missing the two germline-dependent *Sxl* RNAs.

***snf*'s function in the soma:** Although *snf* function appears to be genetically upstream of *Sxl* in the germline, its role in the soma appeared, from analogous experiments, to be downstream of *Sxl* (STEINMANN-ZWICKY 1988). We suspected that these seemingly contradictory results in the two tissues might be due

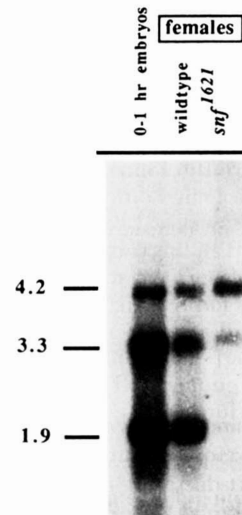

FIGURE 3.—*Sxl* transcription pattern in snf^{1621} mutant females. Northern blot hybridization of poly(A^{+}) RNA (≈5 μg per lane) from 0–1-hr embryos, wild-type females and homozygous snf^{1621} mutant females probed with the female *Sxl* cDNA described in BELL *et al.* (1988).

to allele specific interactions, rather than tissue specific differences. To determine whether this was the case, we compared in parallel experiments the snf^{1621}-Sxl^{M1} and snf^{1621}-Sxl^{M4} interaction.

Males hemizygous for either Sxl^{M1} or Sxl^{M4} do not survive, presumably due to upsets in dosage compensation that result from the inappropriate expression of *Sxl*. Yet the male lethality can be partially suppressed by *snf* mutations at 18° (17% of the expected snf^{1621}, Sxl^{M1} males are recovered), seemingly placing *snf*'s somatic function downstream of *Sxl* (data not shown). If this were the case then we would expect *snf* mutations to block the male lethality of any and all *Sxl* constitutive alleles. However, *snf* does not suppress the male-lethal phenotype of Sxl^{M4} (No snf^{1621}, Sxl^{M4} males were ever recovered out of over 500 progeny scored at either 18° or 25°). The allele specificity of the interaction between *snf* and Sxl^{M1} suggests to us that Sxl^{M1} requires snf^{+} gene function for fully constitutive expression.

Sxl^{M1}'s unique requirement for *snf* function can also be assayed in females. Females heterozygous for deletions which remove two positive regulators of *Sxl*, *sis-a* and *sisterless-b* (*sis-b*) are lethal (CLINE 1988). The data presented in Table 3 demonstrates that this lethality is suppressible by either Sxl^{M1} or Sxl^{M4}. Suppression by Sxl^{M1}, however, is dependent on two wildtype copies of *snf*. *sis-b, sis-a*/snf^{1621}, Sxl^{M1} females have a relative viability of only 16%. In contrast, the ability of Sxl^{M4} to suppress the female lethality is independent of *snf*: *sis-b, sis-a*/snf^{1621}, Sxl^{M4} females are fully viable.

If snf^{+} does function as a regulator of *Sxl*, then under some circumstances *snf* mutations should behave like *Sxl* null alleles. For instance, null alleles of

TABLE 3

snf^{1621} **blocks the constitutive behavior of** *Sxl^{M1}*, **but not of** *Sxl^{M4}* **in females**

snf^X, Sxl^X	Relative viability of *$sis\text{-}b^-$, $sis\text{-}a^-/snf^X$, Sxl^X* females (*n*)	*Relative viability of $sis\text{-}b^-$, $sis\text{-}a^-/+$* females (*n*)	*Reference females $sis\text{-}b^-$, $sis\text{-}a^-/snf^X$, Sxl^X $Dp(sis\text{-}b^+)$, $Dp(sis\text{-}a^+)/+$ (n)*
snf^{1621}, Sxl^+	<0.5% (0)	2.3% (5)	217
snf^+, Sxl^{M1}	114% (127)	<0.9% (0)	111
snf^{1621}, Sxl^{M1}	16% (38)	0.4% (1)	240
snf^+, Sxl^{M4}	130% (114)	1.1% (1)	88
snf^{1621}, Sxl^{M4}	107% (133)	<0.5% (0)	124

Relative viability is defined as the number (*n*) of experimental animals relative to the number of control sisters recovered from the same cross. The crosses were as follows: *y snf^X, Sxl^X/FM7* females × *Df(1)sc19, Df(1)N71/Y; DD(2)Ha, y^+/+* males. The full genotypes of the *snf^X, Sxl^X* chromosomes are: 1) *y cho snf^{1621} ct sn v g f*; 2) *y Sxl^{M1} ct sn v*; 3) *y cho snf^{1621} Sxl^{M1} ct sn v*; 4) *y cm Sxl^{M4}* and 5) *y cho snf^{1621} Sxl^{M4}cm.*

Sxl suppress the male lethality that results from the simultaneous duplication of *sis-a* and *sis-b* (CLINE 1988). Like *Sxl* mutations, *snf^{1621}* can suppress the male lethality of the double duplication (Table 4, crosses A and B). The inability of *snf^{1621}* to suppress the male lethality associated with the double duplication as completely as a null allele of *Sxl* is not totally unexpected because *snf* mutations apparently do not prevent *Sxl* activation, but instead only reduce the probability of *Sxl* activation.

Consistent with previous studies, the data in Table 4 suggest that *snf^+* is also required maternally to activate *Sxl* in the embryo. However, because differences in male viability can be attributed to differences in genetic background (CLINE 1988), we repeated these crosses in such a way that variations in genetic background were kept to a minimum. These data are presented in Table 4 (crosses C and D). In each cross we determined the relative viability of the same isogenic *snf^+* chromosome. *snf^+, $Dp(sis\text{-}b^+)$, $Dp(sis\text{-}a^+)/+$* sons from mothers heterozygous for a deletion that uncovers *snf* are more viable (13% relative viability) than *snf^+, $Dp(sis\text{-}b^+)$, $Dp(sis\text{-}a^+)/+$* sons from *snf^+* females (0.5% relative viability). Thus, suppression of the double duplication male lethality by mutations in *snf* results in part from an apparent maternal effect.

DISCUSSION

***snf* activates *Sxl* in the germline:** Mutations at the *snf* locus result in abnormal ovarian development. During normal oogenesis, the oogonia divide to give rise to a number of cystoblasts. Each cystoblast undergoes four cell divisions to produce a syncytium of 16 interconnected cells. One of these cells develops into an oocyte and the remaining cells 15 cells become the nurse cells (KING 1970). In *snf* mutations the cystocytes appear to undergo uncontrolled cell divisions, resulting in an ovarian tumor phenotype. The similarity of *snf* and *Sxl* mutant phenotypes in the germline suggests that *snf* is a sex determination gene. We present two lines of evidence that demonstrate that *snf* is required to activate *Sxl* in the germline. First, the *snf* mutant phenotype can be suppressed by either of two constitutive alleles of *Sxl*. Thus unregulated expression of *Sxl* can bypass the requirement for *snf^+* gene function. Second, an analysis of the *Sxl* transcription pattern demonstrates that in *snf* mutant females the two germline-dependent *Sxl* transcripts are missing. A detailed comparison of the structures of the different female-specific RNAs has found that the germline and the somatic RNAs are very similar in structure (SAMUELS, SCHEDL and CLINE 1991). Thus it appears that the germline RNAs, like the somatic RNAs, are processed by a female-specific splicing mechanism. Although we expect that the failure to activate *Sxl* in the germline does result in the processing of the germline-dependent *Sxl* RNAs in the male mode, the fact that we did not observe these RNAs in our experiments is not unexpected given the fact that we examined the expression pattern in whole females. The ovaries in *snf* mutant females are rudimentary, suggesting that the germline-specific transcripts are making only a minimal contribution to the overall expression pattern. In contrast, the ovary in wild-type females is well developed, consequently the germline transcripts are likely to make a more substantial contribution to the total expression pattern.

***snf* activates *Sxl* in the soma:** Our data demonstrate that activation of *Sxl* is sensitive to both the maternal and zygotic gene dosage of *snf* in certain genetic backgrounds. Not only do *snf* mutations present a female-lethal phenotype if the probability of *Sxl* activation has been otherwise reduced, they also suppress the male-lethal phenotype associated with an increased probability of *Sxl* activation. Whether *snf* is essential for *Sxl* function can not be determined until we unequivocally determine the null phenotype of *snf*. Although we screened over 25,000 chromosomes for new *snf* alleles, the fact that we isolated only one new *snf* allele (whereas we isolated five additional *Sxl* mutations), makes us uncertain as to whether we have identified the null phenotype. However, our observation does suggest the possibility that the null phenotype is not a lethal. A nonessential requirement for

TABLE 4

Suppression of the male lethality of the *Dp(sis-b⁺), Dp(sis-a⁺)* chromosome

Cross	Genotype of mother with respect to snf^x, Sxl^x	Relative viability of snf^x, Sxl^x; $DpDp/+$ males (*n*)	Relative viability of +; $DpDp/+$ males (*n*)	Relative viability of snf^x, Sxl^x; +/+ males (*n*)	Reference males +/+; +/+ (*n*)
A	$Sxl^{f1}/FM7$	59% (45)	<1.3% (0)	88% (67)	76
B	$snf^{1621}/FM7$	17% (34)	6% (12)	122% (246)	201
C	$Df(snf^-)/+$		13% (13)		102
D	$Sxl^{f1}/+$	44% (96)	0.5% (1)	97% (210)	217

Relative viability is assessed by comparing the number (*n*) of experimental animals to the number of control animals recovered from the same cross. The full genotype of the crosses were as follows:
A) *y w* Sxl^{f1} *ct/FM7* ♀♀ × *Df(1)sc19, Df(1)N71/Y; DD(2)Ha*, $y^+/+$ ♂♂.
B) *y cho* snf^{1621} *ct sn v g f/FM7* ♀♀ × *Df(1)sc19, Df(1)N71/Y; DD(2)Ha*, $y^+/+$ ♂♂.
C) *y w Df(1)JC70*, snf^-*/y w* ♀♀ × *Df(1)sc19, Df(1)N71/Y; DD(2)Ha*, $y^+/+$ ♂♂.
D) *y w* Sxl^{f1} *ct/y w* ♀♀ × *Df(1)sc19, Df(1)N71/Y; DD(2)Ha*, $y^+/+$ ♂♂.

snf^+ function can be explained if there are one or more other genes with functions similar to snf^+ that are required to activate *Sxl* in the embryo. Such redundant functions have been proposed to explain the viable phenotypes of mutations in other genes with predicted vital functions. For instance, no lethal mutations were isolated in the Drosophila fasciclin I gene, a neural cell adhesion molecule (ELKINS *et al.* 1990). A mutant phenotype was only observed when mutations in *fas I* are combined with a mutation in the *abl* tyrosine kinase gene.

Our results also indicate that the activation of a specific gain of function allele of *Sxl*, Sxl^{M1}, is sensitive to gene dosage of *snf*, whereas the activation of another gain of function allele, Sxl^{M4}, is independent of *snf* gene function. We observed that the sensitivity of Sxl^{M1} to *snf* gene dosage was detectable in males under all circumstances, and in females when the *X* to autosome ratio had been reduced (by simultaneously reducing the dosage of *sis-a* and *sis-b*). Previous studies have also shown that Sxl^{M1} is also sensitive to the *X* to autosome ratio in the germline (NOTHIGER *et al.* 1989; STEINMANN-ZWICKY, SCHMID and NOTHIGER 1989). Only when the ratio is 1:1 in the germline is Sxl^{M1} found to be truly constitutive.

These results are consistent with the molecular nature of both constitutive alleles. The mutant phenotypes of both Sxl^{M4} and Sxl^{M1} appear to be due to insertions of foreign DNA within 1 kb of each other. These insertions apparently cause *Sxl* to be processed in the female mode irrespective of the *X* to autosome ratio (MAINE *et al.* 1985). The location of the insertion associated with Sxl^{M4} is within or very close to the male-specific exon, suggesting that it might block the male-specific splicing alternative. This forces the female-specific alternative to be used under all circumstances. The location of the insertion associated with Sxl^{M1}, on the other hand, is 1 kb away from the male-specific exon, suggesting that it functions by a different mechanism. Thus it is probable that it requires at least some of the factors normally necessary to enter into the female mode of activity. Our data suggest that one of these factors is the *snf* gene product.

Where in the process of *Sxl* activation is *snf* likely to function? The experiments described here provide compelling evidence that *snf* is required to activate *Sxl* in both the germline and the soma. Interestingly, the signals required to activate *Sxl* in the two tissue types appear to be different: Whereas an *X* to autosome ratio of 1:1 is sufficient for activation of *Sxl* in the soma, germline activation of *Sxl* requires, in addition to a ratio of 1:1, an inductive signal from the female gonadal soma (NOTHIGER *et al.* 1989; STEINMANN-ZWICKY, SCHMID and NOTHIGER 1989). Since it is likely that *snf* has the same function in both the germline and the soma, we propose that *snf* facilitates the interpretation of the *X* to autosome ratio. Alternatively *snf* may function downstream, or independently, of the signals required to initiate *Sxl* activation, thus maintaining *Sxl* activity. Whether *snf* regulates *Sxl* at the level of sex-specific transcription or sex-specific RNA processing remains to be determined.

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