The *Drosophila* sex determination gene *snf* encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein

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Alternative splicing controls the expression of many genes, including the *Drosophila* sex determination gene *Sex-lethal*. Previous studies have suggested that *snf* plays a role in regulating *Sex-lethal* splicing. Here, we demonstrate that *snf* is an integral component of the machinery required for splice site recognition. We have cloned *snf* and found that it has sequence homology to the mammalian U1A and U2B'' snRNP proteins. Moreover, we establish that *snf* encodes a *Drosophila* protein shown previously to have functional similarity to U1A. Finally, with the isolation and analysis of a null mutation, we demonstrate that *snf* is an essential gene. These studies provide the first demonstration, in a multicellular organism, that mutations in a U1 snRNP protein alter splicing *in vivo*.

[Key Words: *snf* gene; *Drosophila*; sex determination; snRNP protein; pre-mRNA splicing]

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Control of RNA splicing has proven to be a common means of regulating differential gene expression (for review, see McKeown 1990; Green 1991). Although much is known about the biochemistry of the splicing reaction *in vitro*, the mechanisms regulating alternative splice site choices remain largely speculative. To further our understanding of the mechanisms of splicing regulation, we have chosen to use the *Drosophila* sex determination system because of its accessibility to genetic techniques (for review, see Belote 1992; Burtis and Wolfner 1992; McKeown and Madigan 1992; Steinmann-Zwicky 1992; Cline 1993; Cronmiller and Salz 1994). By focusing on the identification of mutations that disrupt specific alternative splicing events, we hope to identify products that play a crucial role in splicing regulation irrespective of whether they are structural or regulatory components of the splicing machinery. In particular, our approach has been to identify and characterize mutations that disrupt the sex-specific splicing pattern of *Sex-lethal* (*Sxl*).

Sxl is a binary switch gene that regulates the three major aspects of sexual cell fate: somatic sexual differentiation, germ-line development, and X-chromosome dosage compensation (for review, see Cline 1993; Cronmiller and Salz 1994). If *Sxl* is "on", the female developmental pathway is selected; alternatively, if *Sxl* is "off", male development ensues. Because of its role in the vital process of dosage compensation, loss-of-function mutations result in female-specific lethality and gain-of-function mutations result in male-specific lethality. Although the on/off regulation of *Sxl* is controlled prima-

rily at the level of RNA splicing, female-specific expression is initially regulated at the level of transcription (Keyes et al. 1992). Once female-specific expression is initiated, a switch from the female-specific promoter to the nonsex-specific promoter results in the production of nonsex-specific pre-mRNAs (Salz et al. 1989; Keyes et al. 1992). These pre-mRNAs are differentially spliced such that only the female-specific mRNA contains a large open reading frame (ORF) (Bell et al. 1988; Samuels et al. 1991). One of the first splicing factors to be identified as necessary for the on/off regulation of *Sxl* was *Sxl* itself (Cline 1984; Bell et al. 1991; Keyes et al. 1992). *Sxl* is believed to control its own splicing by the SXL protein binding its own pre-mRNA to block recognition of the male-specific exon (Sakamoto et al. 1992; Horabin and Schedl 1993a,b). In addition to *Sxl* itself, genetic studies have identified two other genes that regulate *Sxl* splicing: *fl(2)d* (Granadino et al. 1990, 1991, 1992) and *snf* (Oliver et al. 1988; Steinmann-Zwicky 1988; Salz 1992; Albrecht and Salz 1993; Bopp et al. 1993; Oliver et al. 1993). Although genetic studies have suggested that *fl(2)d* is required throughout development to maintain the female-specific splicing pattern of *Sxl*, no molecular data are available to elucidate further its role in splicing regulation.

In this paper we investigate the molecular mechanism by which *snf* regulates the sex-specific splicing of *Sxl*. *snf* was first identified as a positive regulator of *Sxl* in the germ line based on the analysis of a single allele, *snf*¹⁶²¹ (Oliver et al. 1988, 1993; Steinmann-Zwicky 1988; Salz

Here, we demonstrate that *snf* is a U1 snRNP protein and is therefore an integral component of the machinery required for splice site recognition. We have cloned the *snf* gene and found that it encodes a nuclear protein with homology to the mammalian U1A and U2B' small nuclear ribonucleoprotein particle (snRNP) proteins. Moreover, we have established that *snf* encodes a *Drosophila* protein shown previously to have functional similarity to the U1 snRNP-specific U1A protein. Finally, with the isolation and analysis of a null mutation, we show that *snf* function is not limited to sex determination: A complete loss of *snf* function results in embryonic lethality. Taken together, these results strongly suggest that *snf* plays a direct role in alternative splice site selection. Although biochemical studies will be required to establish the exact role of *snf* in splicing, the data presented here underscore the importance of snRNP proteins in regulating alternative splicing events in vivo.

Results

Identification of the snf genomic region

As the first step toward cloning the *snf* gene, we screened for P-element insertions that failed to complement

*snf*¹⁶²¹. Because P elements are thought to transpose preferentially into genomic regions close to their original insertion sites (Tower et al. 1993), we mobilized a P[*lacW*] element, located in 4F1,2, within the coding sequence of the closely linked *deadhead* (*dhd*) gene (Salz et al. 1994). Although females homozygous for this P-element insertion, designated *dhd*^{P8}, are female-sterile, *dhd*^{P8} fully complements *snf*¹⁶²¹ and is therefore not allelic to *snf*.

Of ~700 male-viable derivative lines established after mobilization of the P-element insertion in *dhd*^{P8}, one new P-element insertion, *snf*^{PR110}, failed to complement both *snf*¹⁶²¹ and *dhd*^{P8}. Excision of the P element within *snf*^{PR110} did not revert either the *snf* or the *dhd* mutant phenotypes, suggesting that *snf*^{PR110} had both a P-element insertion and an additional chromosomal rearrangement. Cloning of the genomic DNA flanking the P-element insertion site in *snf*^{PR110} revealed that *snf*^{PR110} contained both a P-element insertion and a deletion of ~3-kb of genomic DNA on the 5' side of *dhd* (Fig. 1). Another P element-derived deletion, *snf*^{IA2}, was characterized at the molecular level and found to be identical to *snf*^{PR110}; however, the P element had excised from *snf*^{IA2} (Fig. 1). These data located the sequences required for *snf* function to within ~3 kb of *dhd*.

The snf gene encodes a 1.0-kb non-sex-specific transcript

Genomic DNA fragments from the region 5' of *dhd* were used as probes on Northern blots to identify a single 1-kb non-sex-specific RNA adjacent to *dhd* [Salz et al. 1994 and data not shown]. The possibility that *snf* encodes a non-sex-specific transcript is consistent with our understanding of *snf* function, as genetic studies had shown that *snf* mutations can affect *Sxl* expression in both

Figure 1. Molecular map of the region surrounding *snf*. The extent of the deletions in the region and the position of inserted P elements are indicated at the top of the diagram by heavy lines and inverted triangles, respectively. *snf*^{PR110} was identified in a screen to mobilized the P element within *dhd*^{P8} (see Materials and methods). *snf*^{fA2} and *snf*^{fJ210} are derivatives of *snf*^{PR110} (see Materials and methods). (□) Exons; (thin lines) intervening sequences. Restriction enzyme sites are indicated by (B) *Bam*HI, (E) *Eco*RI, (K) *Kpn*II, (Xb) *Xba*I, and (X) *Xho*I. Arrows labeled P1–P7 show the location and orientation of primers used for PCR amplification. The location of the 4.8-kb *Xho*I fragment that rescues *dhd* mutations is indicated (lower right). In the expanded view of the *snf* locus (lower left), the small open boxes represent untranslated regions.

males and females (Steinmann-Zwicky 1988; Salz 1992). To determine whether this RNA corresponds to *snf*, several corresponding cDNAs were isolated from a head cDNA library (Itoh et al. 1986); one of these cDNAs, λ KE-14, was characterized further. When used as a probe on Northern blots, λ KE-14 detected a 1-kb non-sex-specific RNA that is missing in homozygous *snf*^{PR110} and *snf*^{IA2} animals but not in *dhd*^{P8} animals (Fig. 2). Instead, a 0.9-kb RNA was detected in both *snf*^{IA2} and *snf*^{PR110} animals but not in *dhd*^{P8} animals. Southern blot and RNase protection analysis demonstrate that the deletions associated with *snf*^{PR110} and *snf*^{IA2} remove the second exon of this transcriptional unit and break within the intron (Figs. 1 and 3). Thus, it is likely that the 0.9-kb transcript produced by these deletion alleles is a truncation of the wild-type message. This finding, in conjunction with the finding that *snf*¹⁶²¹ is a missense mutation within the coding region of this 1-kb RNA (see below), identifies this mRNA as a transcript of the *snf* gene.

snf encodes a RRM protein with homology to two similar snRNP proteins: U1A and U2B''

The insert within the λ KE-14 cDNA, as well as the corresponding genomic region, was sequenced and found to have an ORF of 216 amino acids that would produce a protein with an apparent molecular mass of 28 kD (Fig. 3). The predicted protein contains two RNA recognition motifs (RRMs), one at the amino terminus and the other at the carboxyl terminus (for review, see Kenan et al. 1991). This protein shows extensive overall similarity to two closely related human snRNP proteins, U1A and

U2B'' (Habets et al. 1987; Sillekens et al. 1987). U1A and U2B'' are two highly similar RNA-binding proteins that bind different target RNAs. U1A binds U1 snRNA, and U2B'' binds U2 snRNA. Between SNF and these two human proteins, the amino acid identity is 72% and 70%, respectively (Fig. 4). For just the amino terminal RRM domain, which is responsible for the snRNA-binding specificity of U1A and U2B'', the identity is even stronger: 82.5% with U1A and 79% with U2B'' (Scherly et al. 1990a,b; Bentley and Keene 1991; Jessen et al. 1991; Kenan et al. 1991; Tsai et al. 1991). Finally, the amino-terminal RRM domain of the SNF protein also shows extensive homology to U1A in *Xenopus* (69% identity), to U2B'' in potato (54% identity), but not to U1A in yeast (14.5% identity) (Kenan et al. 1991; Scherly et al. 1991; Simpson et al. 1991; Liao et al. 1993). Interestingly, even though the function of the carboxy-terminal RRM is not known, it too is highly conserved between *Drosophila*, human, *Xenopus*, and potato (Fig. 4B).

We also found that the SNF protein is identical to a previously cloned *Drosophila* gene, *D25*, reported to have functional similarity to U1A (Harper et al. 1992). Although Harper et al. (1992) did not publish a cytological location for *D25*, *D25* must be *snf* because the sequences of the cDNAs are virtually identical. We found only five differences between the sequence of the *snf* cDNA and that published by Harper et al. (1992); none of these differences results in any change in the coding potential (Fig. 3).

*snf*¹⁶²¹ contains a single point mutation within the amino-terminal RRM

To establish further that we have cloned the *snf* gene, we characterized the molecular lesion associated with *snf*¹⁶²¹ by amplifying and sequencing the genomic DNA from this mutant allele and its parent chromosome. The *snf*¹⁶²¹ mutation resulted from a G to A transition (at position 209, Fig. 3), substituting a histidine for an arginine residue in the amino terminal RRM (at position 49, Fig. 4B). As illustrated in Figure 4B, this arginine is conserved in both U1A and U2B'' proteins. Moreover, in vitro structure/function studies have shown that this residue is crucial for human U1A to bind its target RNA (Nagai et al. 1990; Jessen et al. 1991).

SNF is a nuclear protein recognized by an antibody specific for mammalian U2B''

Previously, a monoclonal antibody (mAb 4G3) directed against the human U2B'' was shown to detect a *Drosophila* nuclear antigen that is similar in size to the predicted 28-kD SNF protein (Habets et al. 1989; Amero et al. 1992; Bopp et al. 1993). Is this antigen SNF? The epitope for mAb 4G3 has been mapped to the carboxy-terminal RRM of U2B'' (Habets et al. 1989). Thus, if mAb 4G3 was specific for SNF, then the 28-kD protein would not be detected in extracts from either *snf*^{IA2} or *snf*^{PR110} flies because both of these alleles carry deletions that remove the second exon of the gene, including

Figure 2. Identification of the *snf* transcriptional unit. Northern transfer of 5- μ g samples of poly(A)⁺ RNA prepared from sexed adults from wild-type, *snf*^{IA2}, *snf*^{PR110}, and *dhd*^{P8} homozygous flies probed with the *snf* cDNA. To control for the amount of RNA loaded onto each lane, the blot was subsequently reprobbed with 5C actin DNA (Fryberg et al. 1983). The positions of the RNA size standards are indicated at left.

The sex determination gene *snf* is a snRNP protein

tgccatcgct	gttgaccagc	aaattgtgtg	gcttcaaatc	gcggtgcagt	atccaattga	gatgcaaata	ctccagaccc	-835														
tttagcgctca	tgatggcata	ggccttgatg	ttgcgctgcg	ttaatataat	cttggttatcc	ttgatgatca	cctccagatc	-755														
cgtgtccatg	aagtcgaaaa	ccagcgagac	attcgaaagt	tgaccgaaaa	cgctccaccaa	gccaatgata	ttctcgtgct	-675														
gcagctcctg	caatatcttg	atctcacgca	gcgctgtcct	gttgataccg	tcgctgcat	cctcgcgcga	tcctctcttg	-595														
atcttcttta	cgccacaat	ttggttggtc	accgtgtcgc	gcgccttgta	aactgtggca	aactgcgggtg	cgaagtggga	-515														
atgcgcacat	tgttatggat	cgttgggttg	atatccgttg	cgcgggactc	acctgacct	cgccgaggaa	ggatagcttg	-435														
gcatagcgtt	ccgttttgtc	attcgcattg	ggcagcatcg	tttaggagcc	tcataaatag	cacgaaaatg	actgaaatgc	-355														
tgccaggccg	aatcaaaaaca	attcaagttc	gcaaacgcta	tcgatggccg	ataaccgatg	gacgccggta	gggctatcg	-275														
cgcgccccaa	aaaaaatact	agatttcaga	gtctattaag	aataatgcaa	aaacagcgta	ttgctccttt	attgctaate	-195														
aattttatag	tttaacaagt	tatgcaaata	atacacaact	gtcaatttaa	attagctgca	tgtgattgtc	atttaatttt	-115														
agctactttt	agctatgtgc	agtttaacaa	cactgccaaa	caccggtgcg	ataacatata	gatagaccgc	cccaaacgat	-35														
1 ↓-snf ^{J210} breakpoint																						
gccttctgct	atcgatgaca	gactcgtttt	ttttCTCAAC	AATACAGAGC	AAATTGGTTG	ATTTTGTGTCG	AATTAAGTGA	46														
ATAAACGCA	CGACAGG	ATG	GAG	ATG	CTA	CCC	AAC	CAA	ACG	ATT	TAC	ATC	AAC	AAT	CTG	AAC	GAG	AAG	114			
			M	E	M	L	P	N	Q	T	I	Y	I	N	N	L	N	E	K			
ATC	AAG	AAG	GAG	GAG	CTA	AAG	AAG	TCG	CTC	TAT	GCG	ATT	TTC	TCG	CAA	TTC	GGC	CAA	ATT	CTG	GAC	180
I	K	K	E	E	L	K	K	S	L	Y	A	I	F	S	Q	F	G	Q	I	L	D	
A-snf ¹⁶²¹																						
ATT	GTG	GCC	CTA	AAA	ACG	CTC	AAA	ATG	GCG	GCG	CAG	GCA	TTT	GTG	ATC	TTC	AAG	GAG	ATC	GCG	AGC	246
I	V	A	L	K	T	L	K	M	R	G	Q	A	F	V	I	F	K	E	I	G	S	
GCT	TCG	AAT	GCC	CTG	CGC	ACC	ATG	CAG	GGC	TTC	CCG	TTC	TAC	GAC	AAG	CCC	ATG	CAG	ATC	GCC	TAC	312
A	S	N	A	L	R	T	M	Q	G	F	P	F	Y	D	K	P	M	Q	I	A	Y	
TCC	AAA	TCC	GAT	TCG	GAT	ATT	GTG	GCC	AAG	ATA	AAG	GGT	ACC	TTC	AAG	GAG	CGC	CCC	AAG	AAG	GTC	378
S	K	S	D	S	D	I	V	A	K	I	K	G	T	F	K	E	R	P	K	K	V	
AAG	CCA	CCA	AAA	CCA	GCG	CCG	GGT	ACC	GAT	GAG	AAG	AA	gtgagtatcc	caattgctaa	ttgggcccgt							446
K	P	P	K	P	A	P	G	T	D	E	K	K										
♦♦♦♦ -snf ^{PR110} & snf ^{JA2}																						
ttagattcaa	tcatttgttg	ccaacggcca	tggtgctacc	atttgttget	ccctgttgcc	tcgcaaaaac	ccaatgtttt	526														
cattcgcaag	cctgaaatcc	tttttcttac	taaaacatt	tcgcatttaa	ccaatgttag	tttttggcca	gaattatgga	606														
cttgaataac	aagaggtgtc	tcaagtcttc	ataatatctt	tagttatcaa	ctcctttttg	ctctttaatc	gatgcgattt	686														
gagtttcagt	tgcaaaatgt	ttcatttttg	ttcatttttg	cgcgacaaaa	atccgtagta	atcctagttt	tctgggatgt	766														
gcgaatgact	aggcaaaaaa	aaaatgcaac	ttcatgtgac	actaagacaa	ctcctttttt	ttttcgttcg	cacag G GAC	845														
							D															
G-KE14																						
AAG	AAG	AAG	AAG	CCG	AGC	AGC	GCC	GAG	AAC	TCG	AAC	CCG	AAC	GCA	CAG	ACC	GAA	CAG	CCG	CCG	AAC	911
K	K	K	K	P	S	S	A	E	N	S	N	P	N	A	Q	T	E	Q	P	P	N	
CAG	ATC	CTC	TTC	CTC	ACC	AAT	CTG	CCC	GAG	GAG	ACC	AAC	GAG	ATG	ATG	CTG	TCC	ATG	CTG	TTC	AAT	977
Q	I	L	F	L	T	N	L	P	E	E	T	N	E	M	M	L	S	M	L	F	N	
C-KE14																						
CAG	TTC	CCC	GGC	TTC	AAG	GAG	GTG	CGT	CTT	GTG	CCG	AAT	CGT	CAC	GAC	ATC	GCC	TTT	GTG	GAG	TTC	1043
Q	F	P	G	F	K	E	V	R	L	V	P	N	R	H	D	I	A	F	V	E	F	
ACC	ACC	GAG	TTG	CAG	AGC	AAT	GCC	GCC	AAG	GAG	GCG	CTG	CAG	GGC	TTC	AAG	ATT	ACG	CCG	ACG	CAC	1109
T	T	E	L	Q	S	N	A	A	K	E	A	L	Q	G	F	K	I	T	P	T	H	
T-KE14																						
GCC	ATG	AAG	ATA	ACG	TTC	GCC	AAG	AAG	TGA	ACGCAACT	CCAGTCGCCG	GGCGTTCCAG	CAACTCCAAT	1177								
A	M	K	I	T	F	A	K	K	*													
↓																						
ATAATTTAA	ACTATAGAAT	AAATCGATAT	AAACAAACA	actacgagta	ttccatatgc	ccatatccct	cccatatccc	1257														
gatttcgccc	aaagagtctc	taaggctaa	aagtagagaa	tagaatagtt	tattgttatt	gtgtagatct	a	1328														

Figure 3. Nucleotide and deduced amino acid sequence of the *snf* genomic locus. The genomic nucleotide sequence corresponding to the *snf* cDNA is indicated by uppercase letters; the flanking and intervening nucleotide sequences are in lowercase. The putative translation product in the single-letter amino acid code with the RRM domains in bold is presented below the nucleotide sequence. The polyadenylation signal is underlined, and the polyadenylation site is marked by a double-shafted arrow. The exact location of the *snf*^{J210} breakpoint is indicated by an arrow, and the location of the *snf*¹⁶²¹ missense mutation is shown. In addition, the approximate location of the *snf*^{PR110} and *snf*^{JA2} mutations are indicated (♦). There are no nucleotide differences between the D25 cDNA (Harper et al. 1992; GeneBank accession number M89775) and the corresponding regions of this genomic sequence. On the other hand, the *snf* cDNA that we isolated, λKE-14, has 5 nucleotide differences from this sequence that do not result in an alteration of coding potential. Three of these differences are illustrated; however, a dinucleotide insertion (GA; after nucleotide 37) present in the *snf* cDNA of λKE-14 is not shown. The genomic sequence reported here has been assigned GenBank accession number L29521.

the carboxy-terminal RRM (see Fig. 1). As illustrated in Figure 5A, mAb 4G3 recognizes a 28-kD protein in wild-type animals but not in either *snf*^{JA2} or *snf*^{PR110} animals.

Similarly, no cross-reacting antigen was detected in whole-mount immunohistochemical staining of tissues from *snf*^{JA2} homozygous animals (Fig. 6C,E). The failure

Figure 5. The SNF protein is recognized by an antibody directed against the mammalian U2B'' throughout development. (A) Western transfer of protein extracts from homozygous adult wild-type, *snf^{IA2}*, *snf^{PR110}*, *snf^{e8H}*, and *snf¹⁶²¹* females probed with mAb 4G3. (B) Developmental profile of the SNF protein. Proteins were extracted from wild-type adults and staged embryos and larvae. The ages of the embryos are in hours after egg deposition (25°C). The Western blot contains equal amounts of protein in all lanes except for the 0- to 30-min embryo collection, which contained about half the amount of protein (data not shown). The locations of protein size standards (Bio-Rad) (in kD) are indicated at left.

to detect a protein in the 3' deletion mutations establishes that mAb 4G3 is specific for SNF protein.

To determine the temporal distribution of the SNF protein, we probed Western blots containing protein extracts from different stages of development with mAb 4G3 and found the protein present in all stages examined (Fig. 5B). To examine more precisely the subcellular localization of SNF, mAb 4G3 was used to stain whole-mount embryos and larval tissues. We found that mAb 4G3 staining is localized to the nucleus in all tissues throughout most of development (Fig. 6B,D; data not shown). For example, we found staining localized to the polytene nuclei of salivary glands from third-instar larvae (Fig. 6D). These results are consistent with those obtained previously by Amero et al. (1992) who found that mAb 4G3 detected an antigen that is associated with many sites throughout the polytene chromosomes. As might be expected, we found that the staining of chromosomes is not limited to the salivary gland. During the early embryonic mitotic divisions, we also observed staining to condensed chromosomes (data not shown). Together with the developmental Western analysis, these data clearly demonstrate that SNF is a nuclear protein that is expressed throughout development.

Because genetic data have demonstrated a requirement for maternal *snf* function, we examined the expression of SNF in early embryos and during oogenesis. When West-

ern blots containing protein extracts from newly laid eggs (0–30 min of development) were probed with mAb 4G3, we found that the 28-kD SNF protein was present (Fig. 5B). In contrast to most of development, whole-mount antibody staining of these early embryos revealed that SNF was non-nuclear; mAb 4G3 staining was homogeneously distributed throughout these syncytial embryos (Fig. 6A). Several mitotic divisions later, SNF was restricted to the nuclei (Fig. 6B; data not shown). To determine whether SNF is likely to be among the proteins made by the nurse cells and deposited into the oocyte, we examined the distribution of mAb 4G3 antigens in the ovary. All nuclei in both the germ-line and the somatic components of the ovary were found to be stained by mAb 4G3. Furthermore, we found high levels of staining in the cytoplasm of the nurse cells, suggesting that the SNF protein is deposited into the developing

Figure 6. The SNF protein is nuclear throughout most of development. Whole-mount immunostaining of wild-type and homozygous *snf^{IA2}* embryos and salivary glands with mAb 4G3. *snf^{IA2}* embryos and larvae were collected from a *snf^{IA2}/snf^{IA2}*, *P(w⁺, dhd⁺)/P(w⁺, dhd⁺)* stock. (A) The protein is distributed uniformly in a preblastoderm wild-type embryo. (B) The protein is nuclear in all cell types in a wild-type cellular blastoderm-stage embryo. (C) No protein is detected in homozygous *snf^{IA2}* embryos preblastoderm (top) or cellular blastoderm embryos. (D) The protein is nuclear in a salivary gland from a wild-type third-instar larva. (E) No protein is detected in salivary glands from a homozygous *snf^{IA2}* third-instar larva.

oocyte when the nurse cells dump their contents (data not shown).

The null phenotype of snf is a non-sex-specific lethal

Although previous genetic studies have shown that mutations in *snf* disrupt the establishment of the *Sxl* female-specific splicing pattern in both the germ line and the soma (Albrecht and Salz 1993; Bopp et al. 1993; Oliver et al. 1993), the most extreme allele isolated until now, *snf*¹⁶²¹, is associated only with female sterility, suggesting that *snf* may only be essential in the female germ line (Gans et al. 1975; Salz 1992). However, based on genetic criteria, this allele appears to retain some function (Gollin and King 1981). Similarly, the 3' deletion mutant allele, *snf*^{IA2}, is not a candidate for a null allele because it retains significant amounts of *snf* gene activity (see below).

To determine unequivocally the null phenotype, we screened directly for deletions of the entire *snf*-coding sequence in a manner that was independent of phenotype. The P element in *snf*^{PR110} was remobilized and 347 balanced stocks were established on the basis of eye color reversion. Each stock was then screened for deletions of the first *snf* exon by a combination of PCR and Southern analysis. Of 335 homozygous viable stocks, none contained deletions of the remaining *snf* exon. In contrast, 12 lethal derivatives all appeared to contain larger deletions. One of these deletion derivatives, *snf*^{J210}, appeared to contain a breakpoint within the first exon. Sequencing revealed that *snf*^{J210} does delete the entire coding sequence of *snf*, starting at nucleotide 21 of the 5'-untranslated region and extending into, but not beyond, the *dhd*-coding sequence (Figs. 1,3).

Animals homozygous for *snf*^{J210} were found to be embryonic lethals (data not shown). Two lines of evidence demonstrated that the lethal phenotype is attributable to lack of *snf* function. First, the lethality mapped by both recombination and deficiency mapping to the same interval as the *snf* locus. Within this region there are only two other known lethals, *l(1)4Fa* and *l(1)4Fb*, both of which complement *snf*^{J210} (Salz 1992; H. Salz, unpubl.). Second, the lethality is not attributable to the simultaneous deletion of both *dhd* and *snf* because the lethality is not rescued by a transgene carrying a copy of *dhd*⁺.

Genetic characterization of the new snf alleles

Although *snf*^{J210} is a non-sex-specific lethal, its function in germ-line sex determination is still evident when one examines the ovaries of viable combinations of *snf* mutations. For instance, *snf*^{J210}/*snf*^{IA2} (in the presence of a transgene carrying a copy of *dhd*⁺; see Fig. 1) and *snf*^{J210}/*snf*¹⁶²¹ females produced ovarian tumors.

Surprisingly, and in contrast to either *snf*^{J210}/*snf*^{IA2} females or *snf*¹⁶²¹ homozygous females, females homozygous for *snf*^{IA2} are fertile (in the presence of a transgene carrying a copy of *dhd*⁺; see Fig. 1). The observation that the *snf*^{IA2} deletion mutation is homozygous viable suggests that the carboxyl terminus of the protein is not essential for function. In vitro structure/function

analysis of the mammalian U1A protein have shown previously that a fragment containing only the amino-terminal RRM binds U1 snRNA with the same affinity as the native protein (Jessen et al. 1991; Tsai et al. 1991).

The role of *snf* in somatic sex determination can be assessed by the strength of a female-lethal synergistic interaction with *Sxl* (Oliver et al. 1988; Steinmann-Zwicky 1988; Salz 1992). When assessing the function of *snf*^{J210}, we found that the lethal synergistic interaction it displayed with *Sxl* was surprisingly weak when compared with the *Sxl*-*snf*¹⁶²¹ lethal synergistic interaction (Table 1; cf. crosses A and D). Similarly, the lethal synergistic interaction *snf*^{IA2} displays with *Sxl*, is clearly weaker than the *Sxl*-*snf*¹⁶²¹ lethal synergistic interaction (Table 1; cf. crosses B and C to A). The *Sxl*-*snf*^{IA2} lethal synergism was only detectable when mothers were homozygous for *snf*^{IA2} (cf. crosses B and C).

Because *snf*¹⁶²¹ produces a stronger phenotype than loss-of-function mutations, it can be classified as a gain-of-function mutation. Gain-of-function mutations, like *snf*¹⁶²¹, whose mutant phenotypes are suppressed by an extra copy of the wild-type gene, are thought to disrupt the processes in which they normally participate (Salz 1992; Albrecht and Salz 1993; H. Salz, unpubl.). The similarity in phenotype between *snf*¹⁶²¹ and the viable loss-of-function mutations further suggests that complexes containing the SNF¹⁶²¹ protein interfere with the establishment of the *Sxl* autoregulatory loop by functional inactivation of U1 snRNPs. Taken together, these results demonstrate that *snf*¹⁶²¹ is an antimorphic or dominant-negative mutation (Muller 1932; Herskowitz 1987).

Discussion

In *Drosophila*, the choice between male and female development requires that *Sxl* activity be tightly controlled throughout development. In females, *Sxl* maintains its activity state by an autoregulatory feedback loop in which SXL protein directs the female-specific splicing pattern of its own pre-mRNA (Cline 1984; Bell et al. 1991; Keyes et al. 1992; Sakamoto et al. 1992; Horabin and Schedl 1993a,b). Although previous studies had shown that *snf* also participates in the establishment of the *Sxl* autoregulatory loop, it remained to be determined whether *snf* was directly involved in female-specific splicing (Albrecht and Salz 1993; Bopp et al. 1993; Oliver et al. 1993). Our finding that the SNF protein is localized to the nucleus and has extensive homology to biochemically characterized RNA-binding proteins strongly suggests that SNF directly influences splicing regulation. Furthermore, by characterizing an unambiguous *snf* null mutation, we show that the function of *snf* is not limited to sex determination: A complete loss of *snf* function results in embryonic lethality.

snf encodes a U1 snRNP protein

We show here that *snf* encodes a nuclear protein with strong homology to two highly similar snRNP proteins,

Table 1. Female-lethal synergism between *snf* and *Sxl*

Cross ¹	Maternal genotype	Percent relative viability ^a genotype, and the number of progeny (<i>n</i>) recovered from each cross ^b			
		<i>snf/Sxl, sis-a</i> females	<i>Sxl, sis-a/FM7</i> females	nonbalancer males (<i>n</i>)	control males (<i>n</i>)
A	<i>snf</i> ¹⁶²¹ /FM7	1.3 (3)	19 (44)	<i>snf</i> ¹⁶²¹ /Y 106 (244)	FM7/Y (230)
B	<i>snf</i> ^{IA2} /FM7	261 (397)	230 (349)	<i>snf</i> ^{IA2} /Y 259 (394)	FM7/Y (152)
C	<i>snf</i> ^{IA2} <i>snf</i> ^{IA2}	55 (327)	—	—	<i>snf</i> ^{IA2} /Y (595)
D	<i>snf</i> ¹²¹⁰ /FM7	122 (276)	131 (297)	<i>snf</i> ¹²¹⁰ (0)	FM7/Y (227)
E	<i>dhd</i> ^{P8} /FM7	248 (201)	232 (188)	<i>dhd</i> ^{P8} /Y 184 (149)	FM7/Y (81)
<i>snf</i> ⁺ control					

The lethal synergistic interaction between *snf* and *Sxl* is enhanced by the addition of a mutation at another sex determination locus, *sisterless-a* (*sis-a*) as described in Salz (1992).

^aRelative viability is defined as the number (*n*) of experimental animals relative to the number of control males recovered from each cross. To facilitate comparisons of relative viability between the different crosses, balancer males are the most appropriate control males. However, because these males are not as viable as nonbalancer males our estimates of viability are overestimates.

^b*cm Sxl*¹¹ *ct sis-a*/Y males were crossed to the following females: (A) *w snf*¹⁶²¹ *ct*/FM7c; (B) *w snf*^{IA2}/FM7c; (C) *w snf*^{IA2}/*w snf*^{IA2}; *P*(*w*⁺, *dhd*⁺)/*P*(*w*⁺, *dhd*⁺); (D) *w snf*¹²¹⁰/FM7c; (E) *w dhd*^{P8}/FM7c.

U1A and U2B''. These two proteins, although very similar, bind different target RNAs (for review, see Kenan et al. 1991). U1A binds the U1 small nuclear RNA (snRNA) and is incorporated into the U1 snRNP complex, whereas U2B'' (in conjunction with U2A') binds the U2 snRNA and is incorporated into the U2 snRNP complex. In many respects, SNF resembles U2B'' more closely than U1A. Not only does SNF have a similar overall structure and molecular weight to U2B'' but it also contains a 5-amino-acid motif within the amino-terminal RRM domain (marked 5-MER in Fig. 4B) that is identical to that found in U2B''. In the human protein, this pentamer region has been shown to be necessary for the U1A protein to discriminate between U1 and U2 RNA (Scherly et al. 1990a,b; Bentley and Keene 1991). Functional studies, on the other hand, demonstrate that SNF is a U1 snRNP protein. An epitope-tagged SNF protein immunoprecipitates from both *Drosophila* and human cell extracts, U1 snRNA but not U2 snRNA (Harper et al. 1992). Moreover, R. Mancebo and S. Mount have recently determined that the SNF protein is present in purified *Drosophila* U1 snRNP complexes (pers. comm.). Clearly, *snf* encodes a U1 snRNP protein. Whether SNF is the only U1A-like protein in *Drosophila* remains to be determined.

Role of *snf* in establishing the female-specific splicing pattern of *Sxl*

Although the fact that a *snf* null allele is a non-sex-specific lethal establishes that *snf* is required for developmental processes unrelated to sex determination, its role in regulating *Sxl* can provide us with valuable insights

into its biochemical function. As illustrated in Figure 7, the female-specific SXL protein promotes exon skipping of its own pre-mRNA by binding directly to intron sequences surrounding the male-specific exon, thereby preventing the general splicing machinery from recognizing the male-specific 5' splice site (Sakamoto et al. 1992; Horabin and Schedl 1993a,b). Because it is also required to establish the female-specific splice site choice, *snf* is likely to function in conjunction with *Sxl* to bias the general splicing machinery against the recognition of the male 5' splice site. These studies suggest that as a component of a U1 snRNP, the SNF protein modulates 5' splice site recognition. Because U1 snRNPs function early in the splicing pathway, many studies have already implied that differential binding of the U1 snRNP to the 5' splice site plays a role in the regulation of splice site choice (Rosbash and Séraphin 1991; Hoffman and Grabowski 1992; Siebel et al. 1992; Eperon et al. 1993; Nandabalan et al. 1993). Our studies, however, provide the first demonstration in a multicellular organism that mutations in a U1 snRNP protein alter splicing in vivo.

As a generic component of the U1 snRNP, SNF might be required for the recognition of all 5' splice sites. Our genetic data are certainly consistent with such a conclusion: The *snf* null phenotype is a non-sex-specific lethal. Given this scenario, how do we explain the observation that the viable loss-of-function and gain-of-function mutations appear to affect the regulation of *Sxl* preferentially? In the mammalian system, relative levels of splicing factors have been shown to interact in a concentration-dependent manner to influence splice site selection (Ge and Manley 1990; Krainer et al. 1990; Mayeda and

Figure 7. Model of the autoregulatory splicing regulation of the *Sxl* pre-mRNA. Genetic and molecular studies from several laboratories have suggested a detailed model for the sex-specific splicing regulation of the *Sxl* pre-mRNA (see text for references). The regulation of *Sxl* expression can be divided into a two-step process. Initially, female-specific expression is regulated at the level of transcription (not shown). After cellular blastoderm, there is a switch in promoters from the early female-specific promoter to a non-sex-specific promoter. The resulting pre-mRNAs are differentially spliced in males and females. In females, the presence of the SXL protein promotes skipping of the male-specific exon (exon 3), resulting in the production of protein. SXL promotes its own pre-mRNA splicing by binding to poly(U) tracks in the introns surrounding the male-specific exon, thus preventing the general splicing machinery from recognizing the male-specific 5' splice site and forcing exon 2 to be joined to exon 4. In males, on the other hand, there is no SXL protein present; therefore, the 5' male-specific splice site is recognized and exon 3 is retained. Boxes represent exons; broken lines represent the splicing events.

Krainer 1992; Eperon et al. 1993; Mayeda et al. 1993). Thus, one possibility is that the simultaneous reduction of both *snf* and *Sxl* activity affects 5' splice site choice, in this case favoring selection of the male-specific 5' splice site. For instance, although *snf^{IA2}* is a weak loss-of-function mutation, it nonetheless displays a recessive lethal synergistic interaction with *Sxl*. Similarly, the dominant-negative mutation *snf¹⁶²¹* displays a dominant lethal synergistic interaction with *Sxl*. Although there are a number of ways the SNF¹⁶²¹ protein could interfere with the process in which it normally participates, the nature of the *snf¹⁶²¹* missense mutation suggests that the SNF¹⁶²¹ protein has an altered binding affinity for its target RNA. *snf¹⁶²¹* is a missense mutation that substitutes a histidine for a conserved arginine in its amino-terminal RRM domain. In vitro studies have shown that mutation of this conserved arginine can either lower or completely abolish the binding affinity of human U1A for its target RNA (Nagai et al. 1990; Jessen et al. 1991).

A similar argument based on stoichiometry can also explain the sensitivity of the germ line to reductions of *snf* gene function. *snf* mutations disrupt *Sxl* regulation in the germ line without the simultaneous reduction of *Sxl* function—a requirement in the soma to see a mutant effect. For example, both *snf¹⁶²¹* homozygous females and *snf^{IA2}/snf¹²¹⁰* females are sterile irrespective of the genetic background. In the germ line, unlike the soma,

the SXL protein is predominately cytoplasmic (Bopp et al. 1991, 1993). Thus, in the germ line, it would appear that only a limited amount of SXL protein is transported to the nucleus where it functions and, therefore, it is always rate limiting (Bopp et al. 1993). Consequently, even small reductions in the level of SNF protein would cause an imbalance in the germ line. In the soma, on the other hand, all of the SXL protein is nuclear localized, suggesting that it is not rate-limiting (Bopp et al. 1991).

Even as a component of a U1 snRNP, SNF might not be required for all splicing events. The fact that *snf* has greater sequence homology to U2B'' than to U1A suggests that the function of *snf* may not precisely parallel that of the mammalian U1A protein. Perhaps *snf* is similar to the yeast U1A protein, which is not essential for the recognition of most 5' splice sites (Liao et al. 1993). In yeast, the absence of U1A has no effect on the assembly or function of U1 snRNPs, either in vitro or in vivo. By analogy, *snf* function would not be required for the function of most U1 snRNPs, but only for regulated splice sites, such as the *Sxl* male 5' splice site. If correct, this model predicts that a null mutation would not eliminate splicing but would instead only disrupt specific regulated splicing events. This possibility can now be tested.

Materials and methods

Note on nomenclature

A considerable amount of confusion surrounds the naming of the *snf* locus. *snf* was initially identified in a screen for female-sterile mutations and named *fs(1)1621* (Gans et al. 1975). Once identified as a sex determination gene it was renamed *sans-fille* (*snf*) (Oliver et al. 1988) and *liz* (Steinmann-Zwicky 1988). Although we have constantly used the acronym *snf*, (pronounced sniff), others still identify the locus as *fs(1)1621* and *liz* in the literature. In light of the fact that *snf* is an snRNP protein whose null phenotype is a non-sex-specific lethal, none of the names assigned to the *snf* locus are consistent with its phenotype. We therefore propose to retain the acronym *snf* and rename the locus splicing necessary factor.

Fly stocks and culture conditions

snf¹⁶²¹ is described in Lindsley and Zimm (1992) as *fs(1)A1621*. *snf^{esH}*, *Df(1)DEB4D*, *Dp(1;2)4FRDup*, are described in Salz (1992), and *dhd^{P8}* and the *dhd⁺* transgene *P(w⁺, dhd⁺)* are described in Salz et al. (1994). The remaining mutations and balancer chromosomes are either described in the text or in Lindsley and Zimm (1992). All crosses were carried out at room temperature, which ranged from 22°C–25°C, on a standard cornmeal, yeast, molasses, agar medium.

Isolation of P-element-induced *snf* mutations

To isolate P-element-induced *snf* mutations, a P[*lacW*] enhancer trap element (Bier et al. 1989) within the *dhd* gene was mobilized and screened for mutations that did not complement *snf¹⁶²¹*. To mobilize the P-element insertion, *w dhd^{P8}/FM7c* virgins were mated to males containing a source of transposase, $\Delta 2-3$ (Robertson et al. 1988), of genotype *w; Sb $\Delta 2-3$ /TM6*. The F₁ males (*w dhd^{P8}; Sb $\Delta 2-3$ /+*) were mated to *C(1)DX y w f*

virgins. It is in the germ line of these F_1 males that the P element can transpose. Single male progeny of this cross that carried a new insertion event (recognized by darker eye color than *dhd*^{P8} males, which have light yellow eyes) were crossed to *Df(1)DEB4D/FM7c* virgins. Fertility tests of the females with the deficiency from each vial were scored for an eggless female-sterile phenotype. One event, PR110, had this phenotype and was found not to complement *snf*¹⁶²¹.

Derivatives of *snf*^{PR110} were isolated as follows: *snf*^{IA2} was isolated in a similar screen as described above except that it was selected for the loss of the P-element insertion as judged by the loss of the w^+ eye color marker. *snf*¹²¹⁰ was isolated in a screen that allowed the recovery of lethals. The P element in *snf*^{PR110} was mobilized by crossing *w snf*^{PR110}/*FM7c* virgins to *w; Dr/TMS, Δ2-3* males. The F_1 males of genotype, *w snf*^{PR110};+/TMS, Δ2-3 were mated to *y w Df(1)ovoG6/FM7c* virgins. *Df(1)ovoG6* is an X chromosome deficiency which does not uncover the *snf* locus (Oliver et al. 1987). Females of the genotype *w snf*^{PR110}/*y w Df(1)ovoG6* in which the P element had remobilized (recognized because the females have white eyes) were individually mated to *FM7c* males and stocks established in the next generation. Males from 335 derivative lines were viable and were therefore screened by PCR for the loss of the first *snf* exon (P2–P3 amplification product; Fig. 1) without the concomitant loss of the second *dhd* exon (P6–P7 amplification product; Fig. 1). None of these lines were deleted for the *snf* gene. Twelve lines contained homozygous lethal derivatives and therefore could not be analyzed by PCR. Consequently, the breakpoints were characterized by genomic Southern blots. One line, *snf*¹²¹⁰ was identified as a small deletion that only extended through the first exon of the *snf* gene.

Molecular analysis

Unless otherwise noted, standard molecular techniques followed the procedures described in Ausubel et al. (1987). Five cDNAs corresponding to *snf* were obtained by screening a *Drosophila* head cDNA library in λgt11 (Itoh et al. 1986) using either a ~2.5-kb *Bam*HI–*Kpn*I or a ~2.8-kb *Kpn*I–*Eco*RI genomic fragment as probes (Fig. 1). One cDNA clone, λKE-14, was characterized further and found to contain a 788-bp insert that hybridized exclusively to the altered transcript in *snf*^{PR110} (Fig. 2). This cDNA was then subcloned into pBluescript (Stratagene) for sequence analysis. Unidirectional deletions of pBS/KE-14 were made using exonuclease III and mung bean nuclease (New England Biolabs) as described (Henikoff 1984). These inserts were sequenced by the dideoxy chain termination method (Sanger et al. 1977) using Sequenase v2.0 (U.S. Biochemical). The genomic subclones, 2.5-kb *Bam*HI–*Kpn*I and 2.8-kb *Kpn*I–*Eco*RI were also sequenced in a similar manner. Data base searches and alignments were generated by the BLAST programs at NCBI using the BLAST network service (Altschul et al. 1990).

RNA from adult flies was isolated as described in Chomczynski and Sacchi (1987). Northern transfers and RNase T2 protection assays were carried out as described previously (Flickinger et al. 1992).

Sequencing of the mutant alleles was carried out by direct cycle sequencing of gel-purified PCR-amplified genomic DNA fragments with the CircumVent kit according to the manufacturer's directions (New England Biolabs). In short, 1 μg of genomic DNA from wild-type or mutant *snf* alleles was amplified in a MJ Research PCT-100 thermocycler using 0.25 μM of each primer, 0.2 mM each dNTP (Promega), 2.5 units of *Taq* DNA polymerase (Boehringer Mannheim) in 100 μl of a standard buffer containing 3 mM MgCl (Ausubel et al. 1987). The geno-

mic DNA from *snf*¹⁶²¹ homozygotes and its parent chromosome were PCR amplified with the P2–P4 primer pair, whereas DNA from *snf*¹²¹⁰ heterozygotes was amplified using the P2–P7 and P1–P5 primer pairs, respectively (Fig. 1). Forty cycles of the following parameters were used: 30 sec at 94°C, 45 sec at 49°C, 90 sec at 72°C, followed by a final 5-min extension step at 72°C. The following primers were used: P1, 5'-CGTCCACCAAGC-CAATGATA-3'; P2, 5'-CTCAACAATACAGAGCAAAT-3'; P3, 5'-GAATCGGATTGGAGTAG-3'; P4, 5'-AGATCTACA-CAATAACAATAAA-3'; P5, 5'-GATTGCTGCTTACGCCTT-3'; P6, 5'-GTTCGTTTATGTATTGCA-3'; and P7, 5'-AAT-TGCTTCTTAGTGCCT-3'.

Protein analysis

Immunoblot analysis. Adult flies, embryos, and larvae were collected and frozen on dry ice and then homogenized in ~150 μl of 2× SDS loading buffer. Samples were boiled for ~5 min and then centrifuged for 1 min at 4000g to pellet the debris. Samples (4–30 μl) were loaded on a 12.5% SDS-polyacrylamide gel and electrophoresed. Proteins were electrophoretically transferred to a PVDF membrane (Tropix) as described (Towbin et al. 1979). The membrane was blocked and then incubated with a 1:10 dilution of culture supernatant containing mAb 4G3 (Habets et al. 1989). The primary antibody was visualized using chemiluminescence with the Western-Light kit according to the manufacturer's directions (Tropix). In the immunoblots shown in Figure 5, the only antigen detected by mAb 4G3 is a 28-kD protein. However, we and others have observed that a larger cross-reacting protein is sometimes detected by this antibody in both *Drosophila* and HeLa cell extracts (Amero et al. 1992; Tazi et al. 1993; T. Flickinger, unpubl.). Because the larger protein is similar in size to U1A and is detected in purified U1 snRNPs, it was identified as U1A (Amero et al. 1992; Tazi et al. 1993). Interestingly, we do not detect this 37-kD protein in extracts from either *snf*^{IA2} or *snf*^{PR110} animals under conditions where we do detect it in extracts from wild-type animals. Although the origin of this cross-reacting protein has yet to be determined, its absence in *snf* mutant extracts suggests the possibility that it is encoded by the *snf* gene. However, because we have no evidence of any alternative splicing within the *snf* gene, it is more likely that the expression of this 37-kD antigen represents a protein complex containing the SNF protein.

Immunolocalization in whole mounts The fixation and antibody staining to embryos and salivary glands were performed as described in Salz et al. (1994). The mAb 4G3 was diluted 1:10, stained with a biotinylated goat anti-mouse secondary antibody (Chemicon), diluted 1:5000 and visualized with the Vectastain ABC elite HRP signal detection system according to the manufacturer's instructions.

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References

- Albrecht, E.B. and H.K. Salz. 1993. The *Drosophila* sex determination gene *snf* is utilized to establish the female-specific splicing pattern of *Sex-lethal*. *Genetics* **134**: 801–807.
- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410.
- Amero, S.A., G. Raychaudhuri, C.L. Cass, W.J. van Venrooij, W.J. Habets, A.R. Krainer, and A.L. Beyer. 1992. Independent deposition of heterogeneous nuclear ribonucleoproteins and small nuclear ribonucleoprotein particles at sites of transcription. *Proc. Natl. Acad. Sci.* **89**: 8409–8413.
- Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Siedman, J.A. Smith, and J. Struhl, eds. 1987. *Current protocols in molecular biology*. Greene Publishing/Wiley-Interscience, New York.
- Bell, L.R., E.M. Maine, P. Schedl, and T.W. Cline. 1988. *Sex-lethal*, a *Drosophila* sex determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. *Cell* **55**: 1037–1046.
- Bell, L.R., J.I. Horabin, P. Schedl, and T.W. Cline. 1991. Positive autoregulation of *Sex-lethal* by alternative splicing maintains the female determined state in *Drosophila*. *Cell* **65**: 229–239.
- Belote, J.M. 1992. Sex determination in *Drosophila melanogaster*: From the X:A ratio to *doublesex*. *Sem. Dev. Biol.* **3**: 319–330.
- Bentley, R.C. and J.D. Keene. 1991. Recognition of U1 and U2 small nuclear RNAs can be altered by a 5-amino acid segment in the U2 small nuclear ribonucleoprotein partical (snRNP) B'' protein and through interactions with U2 snRNP-A' protein. *Mol. Cell. Biol.* **11**: 1829–1839.
- Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall, S. Barbel, L. Ackerman, R. Carretto, T. Uemura, E. Grell, L. Jan, and Y. Jan. 1989. Searching for pattern and mutation in the *Drosophila* genome with a P-lacZ vector. *Genes & Dev.* **3**: 1273–1287.
- Bopp, D., L.R. Bell, T.W. Cline, and P. Schedl. 1991. Developmental distribution of female-specific *Sex-lethal* proteins in *Drosophila melanogaster*. *Genes & Dev.* **5**: 403–415.
- Bopp, D., J.I. Horabin, R.A. Lersch, T.W. Cline, and P. Schedl. 1993. Expression of the *Sex-lethal* gene is controlled at multiple levels during *Drosophila* oogenesis. *Development* **118**: 797–812.
- Burtis, K.C. and M.F. Wolfner. 1992. The view from the bottom: Sex-specific traits and their control in *Drosophila*. *Sem. Dev. Biol.* **3**: 331–340.
- Chomezynski, P. and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159.
- Cline, T.W. 1984. Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. *Genetics* **107**: 231–277.
- Cronmiller, C. and H.K. Salz. 1994. The feminine mystique: The initiation of sex determination in *Drosophila*. In *Molecular biology of sex differentiation* (ed. S.S. Wachtel), pp. 171–203. Academic Press, San Diego, CA.
- Eperon, I.C., D.C. Ireland, R.A. Smith, A. Mayeda, and A.R. Krainer. 1993. Pathways for selection of 5' splice sites by U1 snRNPs and SF2/ASF. *EMBO J.* **12**: 3607–3617.
- Flickinger, T.W., N.J. Maihle, and H.-J. Kung. 1992. An alternatively processed mRNA from the avian *c-erbB* gene encodes a soluble, truncated form of the receptor that can block ligand-dependent transformation. *Mol. Cell. Biol.* **12**: 883–893.
- Fryberg, E.A., J.W. Mahaffey, B.J. Bond, and N. Davidson. 1983. Transcripts of the six *Drosophila* actin genes accumulate in a stage- and tissue specific manner. *Cell* **33**: 115–123.
- Gans, M., C. Audit, and M. Masson. 1975. Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster*. *Genetics* **81**: 683–704.
- Ge, H. and J.L. Manley. 1990. A protein factor, ASF, controls cell-specific alternative splicing of SV40 early pre-mRNA in vitro. *Cell* **62**: 25–34.
- Gollin, S.M. and R.C. King. 1981. Studies on *fs(1)1621*, a mutation producing ovarian tumors in *Drosophila melanogaster*. *Dev. Genet.* **2**: 203–218.
- Granadino, B., S. Campuzano, and L. Sánchez. 1990. The *Drosophila melanogaster fl(2)d* gene is needed for the female-specific splicing of *Sex-lethal* RNA. *EMBO J.* **9**: 2597–2602.
- Granadino, B., A.B. San Juan, and L. Sánchez. 1991. The gene *fl(2)d* is required for various Sxl-controlled processes in *Drosophila* females. *Wilhelm Roux's Arch. Dev. Biol.* **200**: 172–176.
- Granadino, B., A.B. San Juan, P. Santamaria, and L. Sánchez. 1992. Evidence of a dual function in *fl(2)d*, a gene needed for *Sex-lethal* expression in *Drosophila melanogaster*. *Genetics* **130**: 597–612.
- Green, M.R. 1991. Biochemical mechanisms of constitutive and regulated pre-mRNA splicing. *Annu. Rev. Cell Biol.* **7**: 559–599.
- Habets, W.J., P.T.G. Sillekens, M.H. Hoet, J.A. Schalken, A.J.M. Roebroek, J.A.M. Leunissen, W.J.M. van de Ven, and W.J. van Venrooij. 1987. Analysis of a cDNA clone expressing a human autoimmune antigen: Full-length sequence of the U2 small nuclear RNA-associated B'' antigen. *Proc. Natl. Acad. Sci.* **84**: 2421–2425.
- Habets, W.J., M.H. Hoet, B.A.W. de Jong, A. van der Kemp, and W.J. van Venrooij. 1989. Mapping of B cell epitopes on small nuclear ribonucleoproteins that react with human autoantibodies as well as with experimentally-induced mouse monoclonal antibodies. *J. Immunol.* **143**: 2560–2566.
- Harper, D.S., L.D. Fresco, and J.D. Keene. 1992. RNA binding specificity of a *Drosophila* snRNP protein that shares sequence homology with mammalian U1-A and U2-B'' proteins. *Nucleic Acids Res.* **20**: 3645–3650.
- Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**: 351–359.
- Herskowitz, I. 1987. Functional inactivation of genes by dominant negative mutations. *Nature* **329**: 219–222.
- Hoffman, B.E. and P.J. Grabowski. 1992. U1snRNP targets an essential splicing factor, U2AF65, to the 3' splice site by a network of interactions spanning the exon. *Genes & Dev.* **6**: 2554–2568.
- Horabin, J.I. and P. Schedl. 1993a. Regulated splicing of the *Drosophila Sex-lethal* male exon involves a blockage mechanism. *Mol. Cell. Biol.* **13**: 1408–1414.
- . 1993b. *Sex-lethal* autoregulation requires multiple cis-acting elements upstream and downstream of the male exon and appears to depend largely on controlling the use of the male exon 5' splice site. *Mol. Cell. Biol.* **13**: 7734–7746.
- Itoh, N., J.R. Slemmon, D.H. Hawke, R. Williamson, E. Morita, K. Itakura, and P.M. Salvaterra. 1986. Cloning of *Drosophila* choline acetyltransferase cDNA. *Proc. Natl. Acad. Sci.* **83**: 4081–4085.

- Jessen, T.-H., C. Oubridge, C.H. Teo, C. Pritchard, and K. Nagai. 1991. Identification of molecular contacts between the U1A small nuclear ribonucleoprotein and U1 RNA. *EMBO J.* **10**: 3447–3456.
- Kenan, D.J., C.C. Query, and J.D. Keene. 1991. RNA recognition: Towards identifying determinants of specificity. *Trends Biochem. Sci.* **16**: 214–220.
- Keyes, L.N., T.W. Cline, and P. Schedl. 1992. The primary sex-determination signal of *Drosophila* acts at the level of transcription. *Cell* **68**: 933–943.
- Krainer, A.R., G. Conway, and D. Kozak. 1990. The essential pre-mRNA splicing factor SF2 influences 5' splice site selection by activating proximal sites. *Cell* **62**: 35–42.
- Liao, X.C., J. Tang, and M. Rosbash. 1993. An enhancer screen identifies a gene that encodes the yeast U1 snRNP A protein: Implications for snRNP protein function in pre-mRNA splicing. *Genes & Dev.* **7**: 419–428.
- Lindsley, D. and G.G. Zimm. 1992. The genome of *Drosophila melanogaster*. Academic Press, San Diego, CA.
- Mayeda, A. and A.R. Krainer. 1992. Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell* **68**: 365–375.
- Mayeda, A., D.M. Helfman, and A.R. Krainer. 1993. Modulation of exon skipping and inclusion by heterogeneous nuclear ribonucleoprotein A1 and pre-mRNA splicing factor SF2/ASF. *Mol. Cell. Biol.* **13**: 2993–3001.
- McKeown, M. 1990. Regulation of alternative splicing. In *Genetic engineering* (ed. J.K. Setlow), pp. 139–181. Plenum Press, New York.
- McKeown, M. and S.J. Madigan. 1992. Sex determination and differentiation in invertebrates: *Drosophila* and *Caenorhabditis elegans*. *Curr. Opin. Cell Biol.* **4**: 948–954.
- Muller, H.J. 1932. Further studies on the nature and causes of gene mutations. *Proc. 6th Int. Congr. Genet.* **1**: 213–252.
- Nagai, K., C. Oubridge, T.H. Jessen, J. Li, and P.R. Evans. 1990. Crystal structure of the RNA-binding domain of the U1 small nuclear ribonucleoprotein A. *Nature* **348**: 515–520.
- Nandabalan, K., L. Price, and G.S. Roeder. 1993. Mutations in U1 snRNA bypass the requirement for a cell type-specific RNA splicing factor. *Cell* **73**: 407–415.
- Oliver, B., N. Perrimon, and A.P. Mahowald. 1987. The *ovo* locus is required for sex-specific germ line maintenance in *Drosophila*. *Genes & Dev.* **1**: 913–923.
- Oliver, B., N. Perrimon, and A.P. Mahowald. 1988. Genetic evidence that the *sans fille* locus is involved in *Drosophila* sex determination. *Genetics* **120**: 159–171.
- Oliver, B., Y.-J. Kim, and B.S. Baker. 1993. *Sex-lethal*, master and slave: A hierarchy of germline sex determination in *Drosophila*. *Development* **119**: 897–908.
- Robertson, H.M., C.R. Preston, R.W. Phillis, D.M. Johnson-Schultz, W.K. Benz, and W.R. Engels. 1988. A stable genomic source of P element transposase in *Drosophila melanogaster*. *Genetics* **118**: 461–470.
- Rosbash, M. and B. Séraphin. 1991. Who's on first? The U1 snRNP-5' splice site interaction and splicing. *Trends Biochem. Sci.* **16**: 187–190.
- Sakamoto, H., K. Inoue, I. Higuchi, Y. Ono, and Y. Shimura. 1992. Control of *Drosophila Sex-lethal* pre-mRNA splicing by its own female-specific product. *Nucleic Acids Res.* **20**: 5533–5540.
- Salz, H.K. 1992. The genetic analysis of *snf*: A *Drosophila* sex determination gene required for activation of *Sex-lethal* in both the germline and the soma. *Genetics* **130**: 547–554.
- Salz, H.K., E.M. Maine, L.N. Keyes, M.E. Samuels, T.W. Cline, and P. Schedl. 1989. The *Drosophila* female-specific sex determination gene, *Sex-lethal*, has stage-, tissue-, and sex-specific RNAs suggesting multiple modes of regulation. *Genes & Dev.* **3**: 708–719.
- Salz, H.K., T.W. Flickinger, E. Mittendorf, A. Pellicena-Palle, J.P. Petschek, and E.B. Albrecht. 1994. The *Drosophila* maternal effect locus *deadhead* encodes a thioredoxin homolog required for female meiosis and early embryonic development. *Genetics* **136**: 1075–1086.
- Samuels, M.E., P. Schedl, and T.W. Cline. 1991. The complex set of late transcripts from the *Drosophila* sex determination gene *Sex-lethal* encodes multiple related polypeptides. *Mol. Cell. Biol.* **11**: 3584–3602.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Scherly, D., W. Boelens, N.A. Dathan, W.J. van Rooij, and I.W. Mattaj. 1990a. Major determinants of the specificity of interaction between small nuclear ribonucleoproteins U1A and U2B'' and their cognate RNAs. *Nature* **345**: 502–506.
- Scherly, D., N.A. Dathan, W. Boelens, W.J. van Venrooij, and I.W. Mattaj. 1990b. The U2B'' RNP motif as a site of protein-protein interaction. *EMBO J.* **9**: 3675–3681.
- Scherly, D., C. Kambach, W. Boelens, W.J. van Venrooij, and I.W. Mattaj. 1991. Conserved amino acid residues within and outside of the amino-terminal ribonucleoprotein motif of U1A small nuclear ribonucleoprotein involved in U1 RNA binding. *J. Mol. Biol.* **219**: 577–584.
- Siebel, C.W., L.D. Fresco, and D.C. Rio. 1992. The mechanism of somatic inhibition of *Drosophila* P-element pre-mRNA splicing: multiprotein complexes at an exon pseudo-5' splice site control U1 snRNP binding. *Genes & Dev.* **6**: 1386–1401.
- Sillekens, P.T.G., W.J. Habets, R.P. Beijer, and W.J. Venrooij. 1987. cDNA cloning of the human U1 snRNA-associated A protein: Extensive homology between U1 and U2 snRNP specific proteins. *EMBO J.* **6**: 3841–3848.
- Simpson, G.G., P. Vaux, G. Clark, R. Waugh, J.D. Beggs, and J.W.S. Brown. 1991. Evolutionary conservation of the spliceosomal protein, U2B''. *Nucleic Acids Res.* **19**: 5213–5217.
- Steinmann-Zwicky, M. 1988. Sex determination in *Drosophila*: The X-chromosomal gene *liz* is required for *Sxl* activity. *EMBO J.* **7**: 3889–3898.
- . 1992. Sex determination of *Drosophila* germ cells. *Sem. Dev. Biol.* **3**: 341–347.
- Tazi, J., U. Kornstadt, F. Rossi, P. Jeanteur, G. Cathala, C. Brunel, and R. Luhrmann. 1993. Thiophosphorylation of U1-70K protein inhibits pre-mRNA splicing. *Nature* **363**: 283–286.
- Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci.* **76**: 4350–4354.
- Tower, J., G.H. Karpen, N. Craig, and A.C. Spradling. 1993. Preferential transposition of *Drosophila* P elements to nearby chromosomal sites. *Genetics* **133**: 347–359.
- Tsai, D.E., D.S. Harper, and J.D. Keene. 1991. U1snRNP-A protein selects a ten nucleotide consensus sequence from a degenerate RNA pool presented in various structural contexts. *Nucleic Acids Res.* **19**: 4931–4936.

The *Drosophila* sex determination gene *snf* encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein.

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