Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

Sex-specific regulation of the *male-specific lethal-1* dosage compensation gene in *Drosophila*

Melanie J. Palmer,[1] Ron Richman,[2,3] Liz Richter,[2] and Mitzi I. Kuroda[2–4]

[1]Department of Entomology, Oklahoma State University, Stillwater, Oklahoma 74078 USA; [2]Department of Cell Biology, [3]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030 USA

Dosage compensation in *Drosophila* occurs by a twofold increase in transcription per copy of X-linked genes in males (XY) compared with females (XX). *msl-1* is one of four genes that are essential for dosage compensation in males, and MSL-1 protein is associated specifically with the male X chromosome. To explore the basis for the sex specificity of dosage compensation, we examined MSL-1 expression in males, females, and dosage compensation mutants. MSL-1 protein levels are negatively regulated by *Sxl* in females, resulting in male-specific expression of MSL-1. In addition, *msl-2* is required for translation and/or stability of MSL-1 in males. Furthermore, the wild-type pattern of MSL-1 localization to the X chromosome is dependent on *mle* and *msl-3* function, although a subset of sites are stained with MSL-1 antibodies in these mutants. Collectively, these data provide the first evidence for an order of *msl* gene function and suggest that male-specific expression of MSL-1 plays a key role in the sex specificity of dosage compensation.

[*Key Words*: Dosage compensation; sex determination; *Drosophila*]

Received December 2, 1993; revised version accepted January 27, 1994.

In many animals, failure to dosage compensate sex-linked genes leads to a lethal genetic imbalance. Although the mechanisms of dosage compensation are varied, all result in global regulation of the expression of genes that are physically linked. In *Drosophila melanogaster*, dosage compensation occurs in males, producing a twofold increase in the level of X-linked gene transcripts relative to transcripts produced from each X chromosome in females (for review, see Lucchesi and Manning 1987; Henikoff and Meneely 1993; Kuroda et al. 1993). The enhanced expression of X-linked genes in male nuclei is thought to result from the interaction of male-specific *trans*-acting factors with numerous *cis*-acting sequences on the X chromosome. Four candidates for *trans*-acting dosage compensation regulators have been characterized (for review, see Lucchesi and Manning 1987). Mutations in these genes, *maleless*, and *male-specific lethal-1*, *male-specific lethal-2*, and *male-specific lethal-3* (collectively referred to as the *msl's*) result in the death of homozygous males as third-instar larvae or early pupae. This lethal phenotype correlates with a failure to dosage compensate X-linked genes (Belote and Lucchesi 1980a; Okuno et al. 1984; Breen and Lucchesi 1985).

Two of the *msl's*, *msl-1* (Palmer et al. 1993) and *mle* (Kuroda et al. 1991), have been cloned, and both proteins (MSL-1 and MLE) show a striking association with the X chromosome in male, but not female nuclei, supporting their proposed roles as dosage compensation regulators. MSL-1 contains acidic regions similar in character to segments found in a diverse group of proteins involved in transcription and chromatin modeling (Palmer et al. 1993 and references therein). The acidic regions of these proteins are thought to contact more basic chromosomal proteins such as histones, mediating nucleosome assembly or release (Earnshaw et al. 1987; Philpott and Leno 1992; Chen et al. 1994). MLE contains motifs that place it in a superfamily of ATP-binding proteins with known or putative helicase activity (Gorbalenya et al. 1989). MLE displays a stronger similarity to proteins with known RNA, rather than DNA helicase activity (Lee and Hurwitz 1993), suggesting that MLE may interact with nascent transcripts rather than with DNA regulatory elements.

Several lines of evidence suggest that alteration of chromatin structure may provide, at least in part, the biochemical basis for dosage compensation in *Drosophila*. A histone H4 isoform, uniquely acetylated at lysine 16 (H4Ac16), is preferentially associated with the male X chromosome in *Drosophila* polytene nuclei (Turner et al. 1992). The pattern of distribution of H4Ac16 on the X chromosome is strikingly similar to that of the regulatory proteins MLE and MSL-1, and its presence on the X requires the wild-type function of all of the *msl* genes (Bone et al. 1994). In addition, the higher transcriptional activity of the male X chromosome in

[4]Corresponding author.

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

larval polytene nuclei correlates with its more diffuse cytological appearance (Offerman 1936; Mukherjee and Beerman 1965). These observations suggest that there are distinct X chromatin structures in male and female nuclei (Dobzhansky 1957) and that chromatin structure may play an integral role in dosage compensation.

One of the outstanding questions in *Drosophila* dosage compensation is the basis for male specificity. Differential regulation of sex determination and dosage compensation begins with the assessment of the X to autosome (X:A) ratio early in development (for review, see Cline 1993). When the ratio is 1.0 (XX:2A), the *Sex-lethal* (*Sxl*) gene is activated, resulting in female-specific sexual differentiation and basal levels of X chromosome transcription (Cline 1978, 1984, 1988; Lucchesi and Skripski 1981). When the X:A ratio is 0.5 (XY:2A), the absence of functional *Sxl* products results in male-specific sexual differentiation and hypertranscription of the X chromosome. Although it is clear that *Sxl* must negatively regulate *msl* function in females, the direct target of *Sxl* regulation is not known.

Both MLE and H4Ac16 are present at appreciable levels in female nuclei and thus do not appear to be direct targets of *Sxl* regulation (Kuroda et al. 1991; Turner et al. 1992). However, neither protein is preferentially associated with the X chromosomes in females. To investigate the basis for the male specificity of dosage compensation, we have examined the regulation of *msl-1* in wild-type males and females and in dosage compensation mutants. We found that MSL-1 protein is present in males and absent or greatly reduced in females, correlating with its male-specific function. The *Sxl* gene is important for this sex-specific regulation, as MSL-1 accumulates and associates with the X chromosomes in *Sxl* mutant females. The wild-type function of *msl-2* is required for translation and/or stability of MSL-1 in males, as MSL-1 protein levels are decreased or absent in *msl-2* mutants. Our data suggest that MSL-1 localization to the X chromosome is partially independent of *mle* and *msl-3*. Collectively, these results provide the first evidence for an order of *msl* gene function and suggest that male-specific expression of MSL-1 plays a key role in the sex specificity of dosage compensation.

Results

Sxl *regulates the level of MSL-1 protein in females*

Dosage compensation genes that are regulated by *Sxl* would be expected to show differential expression in females, which require *Sxl* expression for viability, and in males, which lack functional SXL protein. *mle* protein is expressed in both males and females and therefore has been proposed to be dependent on one or more of the other *msl's* for its male-specific X chromosome localization and function (Kuroda et al. 1991; Gorman et al. 1993). *msl-1* transcripts are found in similar abundance in both sexes and show no detectable differences in splicing, suggesting that *msl-1* expression is not regulated at the transcriptional level (Palmer et al. 1993). Therefore, a key question is whether the male specificity of MSL-1 X chromosome association is a result of the regulation of MSL-1 expression or the regulation of its activity. Because anti-MSL-1 antibodies directed against central portions of MSL-1 did not perform well on Western blots (Palmer et al. 1993), we prepared polyclonal antibodies directed against the full-length protein (see Materials and methods). We first examined MSL-1 protein levels in larvae, because a critical time for *msl-1* function is in the late larval stages (Belote and Lucchesi 1980a). These antibodies detect a 170-kD protein in crude extracts of wild-type male larvae that is absent, or present at substantially reduced levels, in extracts from *msl-1* mutant male larvae and wild-type female larvae, respectively (Fig. 1A). MSL-1 appears to be a relatively labile protein (R. Richman, unpubl.); thus, additional minor bands detected in male lanes may be proteolytic breakdown products of MSL-1. In a parallel blot, MLE staining is detectable in both males and females, confirming that differences in MSL-1 detection are not simply the result of

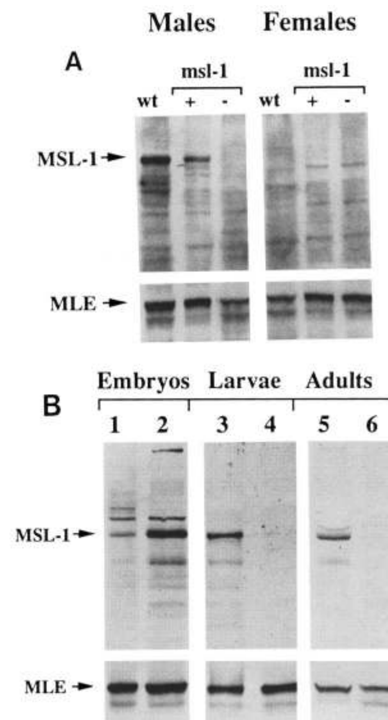

Figure 1. (*A*) Comparison of MSL-1 protein levels in male and female larvae by Western blot. MSL-1 is present in wild-type (wt) and heterozygous *msl-1* males (+) but is absent from homozygous *msl-1* males (−). MSL-1 is not detected in females of any genotype: Wild-type (wt), heterozygotes (+), or *msl-1* homozygotes (−). Detection of MLE from a duplicate blot is shown as a control for protein loaded. (*B*) Detection of MSL-1 in embryos and sexed adults by Western blot: (1) 0- to 1-hr embryos, (2) 0- to 16-hr embryos, (3) male larvae, (4) female larvae, (5) adult males, (6) adult females. Lanes shown are from the same gel. The identity of higher molecular-weight bands detected in the embryonic lanes is not known. Detection of MLE from a duplicate blot is shown below.

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

differences in protein loading. In Figure 1B, we show that a band of the appropriate size to be MSL-1 is also present in unsexed embryos and is prominent in adult males but not in adult females.

We asked whether *Sxl* function was required for the differential expression of MSL-1 in males and females. The early lethality associated with both gain-of-function and null *Sxl* mutations precludes examination of *msl* expression in most *Sxl* backgrounds (Cline 1978). However, some combinations of *Sxl* alleles do result in partial complementation (Cline 1984), allowing some female progeny to survive beyond embryogenesis. In one such combination, ~50% of females heteroallelic for a null *Sxl* allele, Sxl^{f1}, and a partial loss-of-function *Sxl* allele, Sxl^{fhv1}, survive to the third-instar larval stage (Cline 1980; Lucchesi and Skripski 1981) and are mosaic for *Sxl* expression (Gorman et al. 1993). If *msl-1* expression is negatively regulated by *Sxl*, then the absence of SXL in some nuclei should result in detectable levels of MSL-1 protein in these mutant females. As shown in Figure 2, the 170-KD band, which is barely detectable in crude extracts from wild-type females, is clearly present in extracts from Sxl^{f1}/Sxl^{fhv1} females, demonstrating that *Sxl* negatively regulates the level of MSL-1 protein.

The 170-kD protein detected in Western blot analysis is considerably larger than that predicted by the *msl-1* open reading frame of 2855 nucleotides (~105 kD) (Palmer et al. 1993). We have also observed aberrant migration of various *msl-1* fusion proteins expressed in *Escherichia coli* (M. Palmer, unpubl.). Anomalous electrophoretic migration is characteristic of a number of proteins that contain structural features such as clustering of proline residues and acidic and/or basic residues, both of which are present in MSL-1 (Kimelman et al. 1984; Verma 1984; Query et al. 1989). Furthermore, posttranslational processing such as glycosylation and phosphorylation (see Palmer et al. 1993) may also contribute to slower migration of MSL-1 on SDS-polyacrylamide gels.

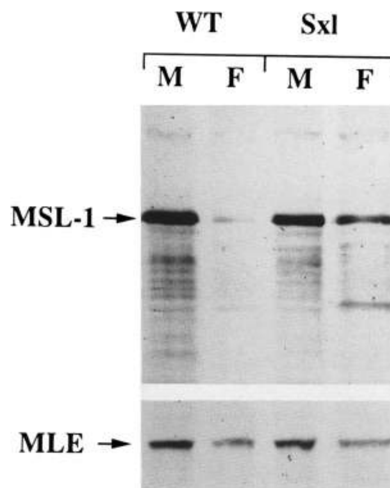

Figure 2. Comparison of MSL-1 protein levels in wild-type and *Sxl* mutant larvae by Western blot. MSL-1 is present in wild-type and Sxl^{fhv1} males (lanes *1*,*3*) and in Sxl^{f1}/Sxl^{fhv1} females (lane *4*). In this experiment, a faint band of the correct mobility to be MSL-1 is also seen in wild-type females (lane *2*). Detection of MLE from a duplicate blot is shown as a control for protein loaded.

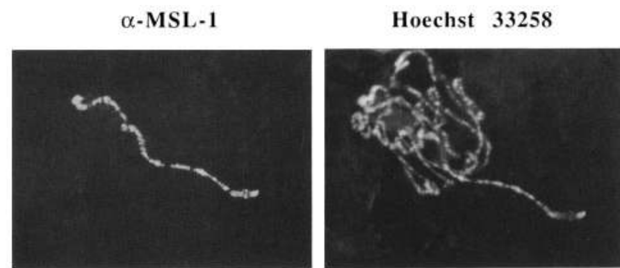

Figure 3. MSL-1 exhibits a male pattern of X chromosome staining in Sxl^- XX nuclei. Immunolocalization of MSL-1 in a polytene chromosome squash from a Sxl^{f1}/Sxl^{fhv1} female. (*Left*) MSL-1 association with the paired X chromosomes; (*right*) The same nucleus stained with Hoechst 33258 to show all chromosome arms.

MSL-1 associates with the female X chromosomes in Sxl$^-$ *nuclei*

Because MSL-1 is normally absent or present at very low levels in wild type XX nuclei, we asked whether the accumulation of MSL-1 seen in XX nuclei that lack SXL leads to the male pattern of X chromosome localization. We found that Sxl^{f1}/Sxl^{fhv1} nuclei that lack SXL staining acquire a male pattern of MSL-1 association with the X chromosome (Fig. 3). Conversely, nuclei that show a wild-type pattern of SXL staining lack MSL-1 immunostaining (data not shown). Therefore, MSL-1, like MLE and H4Ac16 (Gorman et al. 1993; Bone et al. 1994), exhibits a wild-type male localization pattern in Sxl^- XX nuclei. These results are consistent with previous studies demonstrating that some nuclei from Sxl^{f1}/Sxl^{fhv1} females have higher levels of X-linked transcription, correlating with X chromosomes that appear decondensed relative to wild type (Lucchesi and Skripski 1981).

msl-2 *positively regulates MSL-1 levels*

The regulation of *msl-1* by *Sxl* might be direct, at the level of RNA transport or translation, or indirect, possibly acting through one of the other *msl* genes. Because the *msl* phenotypes are all similar, no genetic epistasis has been established for this group of regulators. MLE expression is not affected in the other *msl* mutants, but X chromosome localization of MLE is dependent on each of the *msl's* (Gorman et al. 1993). To determine whether MSL-1 expression is dependent on *msl* function, we performed Western blot analysis of crude cellular extracts isolated from *msl* mutant male larvae (Fig. 4A). MSL-1 protein is absent in homozygous *msl-1* larvae but is present in extracts from male larvae homozygous for *msl-3* and *mle* mutations. In contrast, MSL-1 is unde-

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

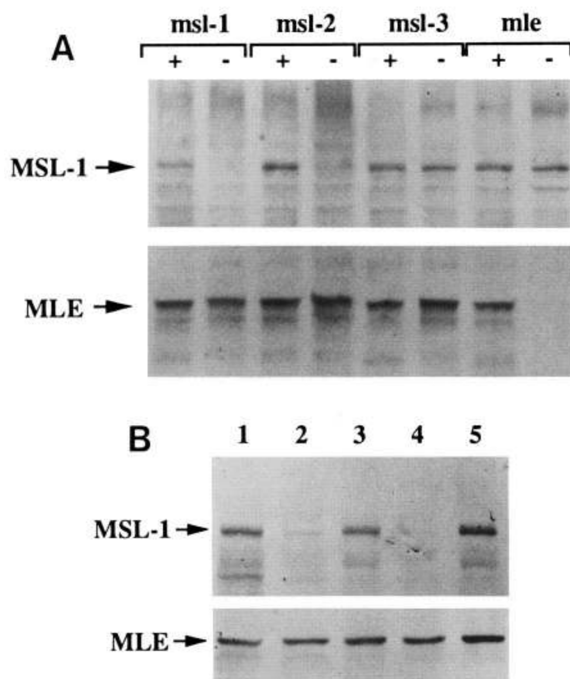

Figure 4. Western blot analysis of wild-type and *msl* mutant larvae. (*A*) Pairs of lanes compare heterozygous and homozygous *msl* mutant male siblings. MSL-1 is not detectable in homozygous *msl-1* and *msl-2* mutants but is present in *msl-3* and *mle* mutants. A duplicate blot reacted with anti-MLE antibodies is shown below as a control for protein loaded. MLE is present in all except the *mle* mutant lane. (*B*) Blot comparing MSL-1 levels in wild-type males (lane *1*), wild-type females (lane *2*), Sxl^{f1}/Sxl^{fhv1}; *msl-2*/+ females (lane *3*), Sxl^{f1}/Sxl^{fhv1}; *msl-2*/*msl-2* females (lane *4*), and Sxl^{fhv1}/Y; *msl-2*/+ males (lane *5*). As in males, MSL-1 accumulation in *Sxl* females is dependent on wild-type *msl-2*.

tectable in extracts from homozygous *msl-2* mutant male larvae, even when the lane is overloaded. Therefore, the level of MSL-1 appears to be dependent on the expression of *msl-2* but not on *msl-3* or *mle*.

Because *msl-2* mutant male larvae are less healthy than *mle* and *msl-3* mutant males (M. Kuroda, unpubl.), we wanted to rule out the possibility that the lack of MSL-1 in *msl-2* mutants is the result of a nonspecific effect on protein stability. In that case, MSL-1 levels would not be decreased in Sxl^{f1}/Sxl^{fhv1} female larvae that lack a functional *msl-2* allele, as the health of these larvae is indistinguishable from their siblings. To test this possibility, we prepared total cellular extracts from Sxl^{f1}/Sxl^{fhv1}; *msl-2*/*msl-2* female larvae and examined *msl-1* protein levels (Fig. 4B). MSL-1 is absent or present at very low levels (comparable with wild-type females) in Sxl^{f1}/Sxl^{fhv1}; *msl-2*/*msl-2* mutant females, whereas their Sxl^{f1}/Sxl^{fhv1}; *msl-2*/+ siblings have substantial levels of MSL-1. Therefore, using two different genetic tests, we found that *msl-2* expression positively regulates MSL-1.

These results suggest that *msl-2* regulates the translation or stability of MSL-1 protein in males. However, another possibility is that *msl-2* encodes a general factor required for transcription of *msl-1* in both sexes. Because of the difficulty in obtaining sufficient poly(A)$^+$ RNA from *msl-2* mutant males, we compared *msl-1* transcripts from *msl-2*/+ and *msl-2*/*msl-2* mutant females by Northern analysis (Fig. 5A). Regulation of *msl-1* by *msl-2* does not appear to occur at the transcriptional level, because all three *msl-1* transcripts are present in *msl-2* mutants. The *msl-1* transcripts are also present in adult females that lack a germ line (Fig. 5B), supporting the hypothesis that *msl-1* is transcribed in somatic tissues of females and negatively regulated at the post-transcriptional level.

mle and msl-3 are required for the wild-type pattern of MSL-1 association with the X chromosome

The similarity of *msl* mutant phenotypes (for review, see Lucchesi and Manning 1987), and the dependence of MLE on the other *msl* functions for X chromosome localization (Gorman et al. 1993), suggests that *msl* wild-type functions may be interdependent. The apparent absence of MSL-1 in *msl-2* mutants suggests, however, that there may be epistasis in *msl* action. The fact that *msl-1* lacks a recognizable DNA-binding motif suggests that X-specific localization is likely to depend on interactions with other chromosomal proteins. Therefore, although

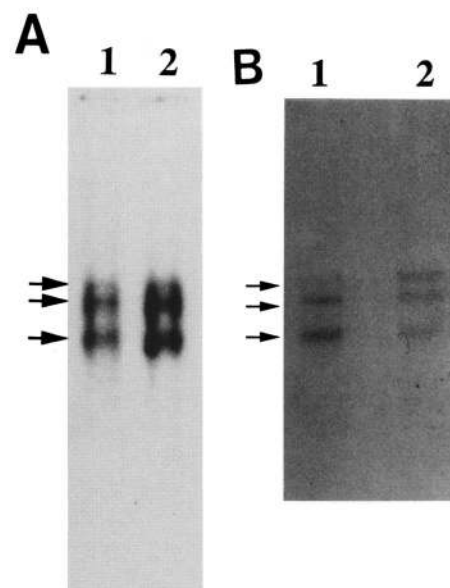

Figure 5. (*A*) All three *msl-1* transcripts are present in *msl-2* mutants. Northern blot comparing *msl-1* transcripts in *msl-2* mutant females (lane *1*) and *msl-2*/+ females (lane *2*). Approximately 10 μg of poly(A)$^+$ RNA was loaded in each lane. The probe used contained the entire *msl-1* open reading frame (Palmer et al. 1993). (*B*) *msl-1* transcription in females is not limited to the germ line. Northern blot comparing the female progeny of wild-type mothers (lane *1*) and tud^1 mothers (lane *2*). All three *msl-1* transcripts are present in daughters of tud^1 mothers, which lack a germ line.

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

mle and *msl-3* are not required for MSL-1 expression or stability in male nuclei, they may still be required for X-specific localization of MSL-1. To determine whether MSL-1 association is dependent on *msl-3* or *mle*, we examined MSL-1 localization in these mutant backgrounds.

Homozygous mutant *msl* males die as late larvae to early pupae and are greatly delayed in their development (Belote and Lucchesi 1980b; Belote 1983). As a consequence, they contain very small salivary glands with polytene chromosomes that are difficult to spread. We examined MSL-1 immunostaining in *mle* and *msl-3* mutant males and occasionally detected faint staining of one chromosome per nucleus (data not shown). However, because of the poor morphology of polytene chromosomes in homozygous *msl* males, we adopted the strategy utilized by Gorman et al. (1993) to examine MSL-1 staining in Sxl^{f1}/Sxl^{fhv1} female larvae that also lack functional *msl* alleles. Because MSL-1 associates with the X chromosomes in Sxl^{f1}/Sxl^{fhv1} nuclei that lack SXL staining (Fig. 3), we performed double-labeling of Sxl^{f1}/Sxl^{fhv1}; *msl* larvae to determine whether removal of *msl-2*, *msl-3*, or *mle* function results in the loss of MSL-1 X chromosome staining in these nuclei.

As expected, MSL-1 was not detected on either *Sxl; msl-1* (data not shown) or *Sxl; msl-2* polytene chromosomes (Fig. 6a), because these larvae lack MSL-1 protein. However, in contrast to the wild-type pattern of several hundred X chromosome sites seen in *Sxl*; msl^+ larvae (Fig. 3), *Sxl; msl-3* and *Sxl; mle* larvae have ~40 sites of MSL-1 immunostaining on the X chromosomes (Fig. 6c,e). As in wild-type MSL-1 localization, the subset of sites seen in the mutant backgrounds coincides with interbands rather than in the regions of highest DNA content. The MSL-1 sites consistently stain brighter in the *msl-3* mutants than in the *mle* mutants (M. Kuroda, unpubl.), although bright staining can be seen in some *mle* nuclei (e.g., Fig. 6e). Owing to difficulty in analysis of the relatively weak anti-MSL-1 staining seen in most *Sxl; mle* mutant nuclei, a definitive comparison of the staining patterns in the two mutant backgrounds has not yet been accomplished. However, a preliminary study of the ~40 sites suggests that they are not simply the brightest staining sites in the wild-type MSL-1 pattern but that they represent a distinct subset that appears very similar in the two mutant backgrounds.

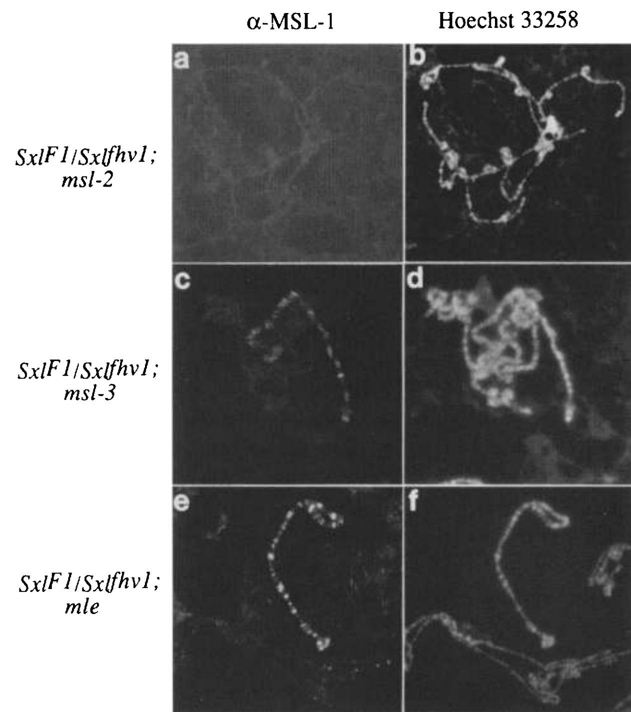

Figure 6. Anti-MSL-1 immunostaining of polytene chromosomes from *Sxl; msl* mutant females. The nuclei shown have followed a male developmental fate, as judged by the failure to stain with anti-SXL antibodies (data not shown). The *left* panels (*a,c,e*) are stained with anti-MSL-1 antibodies. The *right* panels (*b,d,f*) show the same nuclei stained with Hoeshst 33258 to view all chromosome arms. MSL-1 staining is not detected on the X chromosomes in Sxl^{f1}/Sxl^{fhv1}; *msl-2* females (*a*) but is detected on a subset of bands on the X chromosomes in Sxl^{f1}/Sxl^{fhv1}; $msl\text{-}3^P$ (*c*) and in Sxl^{f1}/Sxl^{fhv1}; mle^1 females (*e*).

Discussion

Sxl *regulation of MSL-1 expression*

MSL-1 protein is present in males and is absent (or at very low levels) in females. The *Sxl* gene is important for this sex-specific regulation, as MSL-1 accumulates and associates with the X chromosomes in *Sxl* mutant females. MLE and H4Ac16 also accumulate on the X chromosomes of *Sxl* females (Gorman et al. 1993; Bone et al. 1994), but this regulation appears indirect, as MLE and H4Ac16 are present at appreciable levels in both sexes (Kuroda et al. 1991; Turner et al. 1992). Because localization of both MLE and H4Ac16 is dependent on *msl-1* function (Gorman et al. 1993; Bone et al. 1994), these observations suggest that the male-specific expression of MSL-1 could account, at least in part, for the sex specificity of dosage compensation.

Sxl encodes a protein with an RNA-binding domain that regulates the splicing of its own pre-mRNA and that of at least one downstream sex determination gene, *transformer* (Boggs et al. 1987; Bell et al. 1988; Sosnowski et al. 1989; Inoue 1990). *Sxl* does not regulate splicing of *msl-1* mRNA, suggesting that *Sxl* regulates *msl-1* by either an indirect or novel regulatory mechanism. Interestingly, two of three *msl-1* transcripts contain poly(U) stretches in the 3'-untranslated region that resemble SXL-binding sites (Palmer et al. 1993). Therefore, we are currently investigating the possibility that *Sxl* may directly regulate *msl-1* expression, perhaps by affecting RNA transport or translation. However, because the smallest *msl-1* transcript lacks the 3' poly(U) stretches, and all three *msl-1* transcripts are found in somatic tissues in females (Fig. 5B), it is also possible that *msl-1* is not a direct target of *Sxl* but is instead regulated by the availability of another protein (e.g.,

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

MSL-2, see below) whose expression may be under the control of *Sxl*.

The *msl* genes are probably not the only dosage compensation functions regulated by *Sxl*. Although *Sxl* mutant females are postulated to die because of a failure to negatively regulate dosage compensation, mutations in any of the known *msl's* do not suppress the female lethality of *Sxl* mutants (Skripsky and Lucchesi 1982; Uenoyama et al. 1982; Uenoyama 1984), except in the case of a *Sxl* allele that retains function early in development (Cline 1984). In addition, the *runt* gene is dosage-compensated during embryonic development in *msl-1*, *msl-2*, or *mle* mutants (Gergen 1987). Thus, although MSL-1 may be a key regulator of dosage compensation in the larval stages, another as yet unidentified gene may play this role earlier in development.

msl *regulation of MSL-1 expression and activity*

To investigate the possibility that MSL-1 expression is affected by one of the other *msl's*, we examined the expression of MSL-1 in *msl* mutants. We found MSL-1 to be present in *mle* and *msl-3* mutants but to be drastically reduced or absent in *msl-2* mutants. This suggests that *msl-2* expression positively regulates the level of MSL-1 protein in male nuclei and that the potential absence of *msl-2* function in XX nuclei could prevent stable accumulation of MSL-1. These observations provide the first demonstration of epistasis in *msl* expression. *msl-1* transcripts are present in *msl-2* mutant females (Fig. 5A); thus, like *Sxl* regulation of MSL-1, regulation by *msl-2* appears to be post-transcriptional. MSL-1 has several regions rich in proline, serine, threonine, and glutamic acid (PEST sequences) that may signal rapid turnover (Rogers et al. 1986), several potential glycosylation sites, and >40 potential phosphorylation sites (Palmer et al. 1993). *msl-2* could catalyze a post-translational modification of the MSL-1 polypeptide that protects it from rapid turnover or could stablize MSL-1 by direct interaction, perhaps targeting it to the X chromosome. If *msl-2* regulation of *msl-1* expression is direct, then *msl-2* may provide the regulatory link between *Sxl* and the other *msl's*.

In addition to regulation of MSL-1 at the level of expression or protein stability, we show that the wild-type pattern of MSL-1 localization is at least partially dependent on wild-type *msl-3* and *mle*. However, MSL-1 is present at a reproducible pattern of ~40 sites in each of these mutants. A very important caveat is that the mutant alleles used in this study could retain partial function. If so, then the MSL-1 binding seen may actually be attributable to residual *msl-3* or *mle* function. However, this is inconsistent with the observation that these 40 sites are not simply the brightest sites of staining in the wild-type MSL-1 pattern. Furthermore, we observe similar MSL-1 staining patterns in two independent *mle* mutants, mle^1 (Fig. 6e) and mle^{pml8} (data not shown). The mle^{pml8} allele carries a deletion of three of the seven conserved helicase motifs and can encode only the amino-terminal half of the MLE protein (Kuroda et al. 1991). The lesion in the mle^1 allele has not been identified, but immunostaining reveals no detectable MLE protein in *Sxl*; mle^1 female larvae, and mle^1 males produce no detectable MLE on chromosomes or on Western blots (L. Richter and M. Kuroda, unpubl.).

MLE (Kuroda et al. 1991), MSL-1 (Palmer et al. 1993), and MSL-3 (unpublished work cited in Gorman et al. 1993 and Henikoff and Meneely 1993) are all associated with the male X chromosome. MLE and MSL-1 are colocalized in the same discrete banding pattern on the X chromosome (Bone et al. 1994). These results, along with the dependence of MLE on the other *msl's* for X chromosome localization, have led to the hypothesis that the *msl* proteins interact and function as a complex (Gorman et al. 1993; Kuroda et al. 1993; Palmer et al. 1993; Bone et al. 1994). That MSL-1 is also dependent on *mle* and *msl-3* for its wild-type pattern of X chromosome localization supports this hypothesis. In addition, the residual binding of MSL-1 in these mutants suggests that there is an order to complex assembly. A model for the assembly of factors on the X chromosome that is consistent with our results is presented in Figure 7.

The finding that MSL-1 is present at a subset of sites in *mle* and *msl-3* mutants raises the question as to whether or not an associated increase in X-linked transcription occurs in these mutants. Whereas MSL-1 is present at ~40 sites in the absence of *mle* or *msl-3*, H4Ac16 is not detected on the X chromosome over background levels in any of the *msl* mutants (Bone et al. 1994). Thus, if the post-translational modification of histone H4 is integral to dosage compensation, it appears that a significant increase in transcription occurs only when the full complement of *msl* proteins is present.

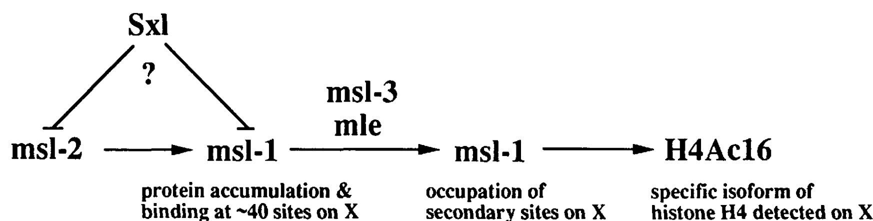

Figure 7. Model for the order of *msl* action based on MSL-1 expression and immunolocalization studies. *msl-2* is required for MSL-1 accumulation and male X chromosome association at ~40 interspersed sites. *Sxl* protein negatively regulates MSL-1 accumulation in females, either directly or through an intermediate regulatory gene such as *msl-2*. The function of *msl-3* and *mle* leads to the association of MSL-1 with many additional sites along the length of the X chromosome. Ultimately, the functions of all the *msl's* are required for the presence of histone H4 acetylated at lysine 16 (H4Ac16) on the male X. The presence of H4Ac16 on the male X correlates with dosage compensation of X-linked genes in *Drosophila* (Bone et al. 1994).

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

A critical component of dosage compensation that remains elusive is the identity of *cis*-acting dosage compensation sequences that direct binding of regulators to the X chromosome. Our results suggest that perhaps the failure, thus far, to identify a discrete dosage compensation *cis*-acting sequence is attributable to the lack of a recognizable consensus sequence at most X-linked regions. The majority of sites might have a relatively low affinity for dosage compensation regulators. The subset of MSL-1-binding sites that are independent of *mle* and/or *msl-3* are relatively evenly spaced along the length of the X chromosome. It is possible that these sites may dictate initial recognition and high-affinity binding of dosage compensation regulators, followed by the stabilization of weaker affinity intervening segments on the X chromosome. Although speculative, this hypothesis might explain the difficulty in obtaining full rather than partial dosage compensation of X-linked clones inserted on autosomes (Su Qian and V. Pirrotta, pers. comm.). Transgenes inserted on autosomes might lack these potentially critical flanking sites that could function to organize or stabilize the regulators at intervening regions. *msl-1* (and *msl-2*) function could be critical to the initial assembly of dosage compensation regulators at these discrete sites.

Materials and methods

Drosophila *stocks*

Flies were raised on standard cornmeal–yeast–agar–molasses medium containing propionic acid as an antifungal agent. The *msl-1*$^{\gamma216}$ and *msl-1*$^{\gamma269}$ alleles are described in Palmer et al. (1993). All stocks not specifically mentioned in the text are described in Lindsley and Zimm (1992).

Crosses to generate *msl* mutants for Western blots were perfomed at 18°C as follows: *mle* mutants: *y; mle*^{pml8} *cn bw/CyO* T(1;2)B80 y^+ females×*y*/Y; *mle*^{pml8} *cn bw/CyO* T(1;2)B80 y^+ males. *msl-1* mutants: *y; msl-1*$^{\gamma269}$ *cn bw/CyO* T(1;2)B80 y^+ females× *y*/Y; *msl-1*$^{\gamma216}$ *cn bw/CyO* T(1;2)B80 y^+ males. *msl-2* mutants: *y; msl-2 cn bw/CyO* T(1;2)B80 y^+ females×*y*/Y; *msl-2 b pr cn wxt bw/CyO* T(1;2)B80 y^+ males. *msl-3* mutants: *msl-3*^{P} *red/TM3* females×*msl-3*^{MAK} *red e/TM3* males. Homozygous *mle, msl-1,* and *msl-2* larvae were identified by the absence of the y^+ marker, present on the *CyO* y^+ balancer chromosome (J. Botas, pers. comm.). Homozygous *msl-3*^{P}/*msl-3*^{MAK} larvae were identified by their red Malpighian tubules, due to the presence of the *red* marker. The *msl-3*^{P} allele is the original *mle-3*^{1} allele (Uchida et al. 1981). *msl-3*^{MAK} is an X-ray-induced allele (M. Keene and T. Hazelrigg, pers. comm.).

Crosses to generate *Sxl; msl* double mutants for immunostaining were performed at 25°C as follows: *Sxl* females: *cm Sxl*^{fhv1} *ct* females×*Sxl*^{f1} *oc ptg v*/Y males. *Sxl; msl-1: cm Sxl*^{fhv1} *ct; msl-1*$^{\gamma216}$ *cn bw* females×*Sxl*^{f1} *oc ptg v*/Y; *msl-1*^{b} *cn bw/SM1* males. *Sxl; msl-2*: *cm Sxl*^{fhv1} *ct; msl-2 b pr cn wxt bw* females×*Sxl*^{f1} *oc ptg v*/Y; *msl-2 cn bw/CyO* males. *Sxl; msl-3*: *cm Sxl*^{fhv1} *ct; msl-3*^{P} *red* females×*Sxl*^{f1} *oc ptg v*/Y; *msl-3*^{P} *red/TM6B* males. *Sxl; mle*: *cm Sxl*^{fhv1} *ct; pr mle*^{1} females×*Sxl*^{f1} *oc ptg v/Y; pr mle*^{1}*/SM1* males. Homozygous *mle, msl-1,* and *msl-2* squashes were identified cytologically. In heterozygous *msl/Balancer* squashes, the second chromosome is largely asynapsed. Homozygous *msl-3*^{P} larvae were identified by the *red* mutation and the absence of the *TM6B* dominant marker *Tb*.

Crosses to generate *Sxl; msl-2* larvae for Western blots are as follows: *cm Sxl*^{fhv1} *ct; msl-2 b pr cn wxt bw* females×*Sxl*^{f1} *oc ptg v*/Y; *msl-2 cn bw/In (2LR) Gla Bc Elp* males. Homozygous *msl-2* mutants were identified by the absence of the *Bc* larval marker. In all cases, larvae were sexed by gonad size as viewed through the cuticle.

Preparation of antisera

Affinity-purified polyclonal antibodies specific for MSL-1 were raised in rabbits. A plasmid, placMSL-1, encoding a putative full-length MSL-1 open reading frame was constructed by ligating *msl-1* genomic sequences to *msl-1* cDNA sequences in the vector pMTL23 (Chambers et al. 1988). A 538-bp *Nco*I–*Eco*RI genomic fragment beginning at the first in-frame ATG codon and a 2446-kb *Eco*RI–*Hind*III cDNA fragment encompassing the remainder of the *msl-1* open reading frame (Palmer et al. 1993) were inserted sequentially into the pMTL23 polylinker. The *msl-1* protein was subsequently expressed in *E. coli* by induction of the *lac* promoter with IPTG as described previously (Kuroda et al. 1991).

The anti-MLE antibodies used were raised against either the amino-terminal 225 amino acids or the full-length MLE protein expressed from an *mle* cDNA cloned into pET8 (Novagen). Induction of MLE expression in *E. coli* was performed according to the manufacturer's instructions. Antibodies were raised and affinity purified essentially as described previously (Kuroda et al. 1991).

Western blots

Third-instar larvae were homogenized using a Dounce homogenizer in 1× Laemmli buffer with the following proteinase inhibitors: 1 μg/ml of pepstatin, 1 μg/ml of leupeptin, 1 mM benzamidine, 10 μM aprotinin, 1 μg/ml of antipain, 1 μg/ml of soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride. Crude extracts were boiled for 5 min, and ~30 μg of protein was electrophoresed through a 4% stacking–6% polyacrylamide–SDS Laemmli gel. Proteins were transferred to nitrocellulose using an EC transfer apparatus, optimizing for transfer of high-molecular-weight proteins (0.5 A for 2.5 hr) as described (Matsudaira 1987). Western blots were performed using an alkaline phosphatase goat anti-rabbit IgG detection system (Promega).

Immunostaining of polytene chromosomes

Polytene chromosomes were prepared as described (Kuroda et al. 1991). Affinity-purified rabbit anti-MSL-1 antibodies were employed at a concentration of 1:20. Mouse anti-SXL antibodies obtained from ascites fluid were used at a dilution of 1:2000. Donkey FITC- and Texas Red-conjugated secondary antibodies (Jackson ImmunoResearch) were used at a 1:200 dilution.

Acknowledgments

We thank Daniel Bopp and Paul Schedl for providing the anti-SXL antibodies, Monica Gorman and Bruce Baker for providing *Sxl; msl* stocks, Irina Solovyeva for assistance with Northern blots, and Tiffany Molony for construction of placMSL-1. We are grateful to S. Elledge, R. Kelley, W. Mattox, G. Murphy, and S. Qian for critical reading of the manuscript. This work was supported by National Institutes of Health grant GM45744 and National Science Foundation grant DMB-9158176 to M.I.K. and under Oklahoma Agricultural Experiment Station (OAES) proj-

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

ect H-2139 to M.J.P. M.I.K. is a Searle scholar and an assistant investigator of the Howard Hughes Medical Institute.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

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.

Belote, J.M. 1983. *Male-specific lethal* mutations of *Drosophila melanogaster*. II. Parameters of gene action during male development. *Genetics* **105:** 881–896.

Belote, J.M. and J.C. Lucchesi. 1980a. Control of X chromosome transcription by the *maleless* gene in *Drosophila*. *Nature* **285:** 573–574.

———. 1980b. *Male-specific lethal* mutations of *Drosophila melanogaster*. *Genetics* **96:** 165–186.

Boggs, R.T., P. Gregor, S. Idriss, J.M. Belote, and M. McKeown. 1987. Regulation of sexual differentiation in *D. melanogaster* via alternative splicing of RNA from the *transformer* gene. *Cell* **50:** 739–747.

Bone, J.R., J. Lavender, R. Richman, M.J. Palmer, B.M. Turner, and M.I. Kuroda. 1994. Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes & Dev.* **8:** 96–104.

Breen, T.R. and J.C. Lucchesi. 1985. Analysis of the dosage compensation of a specific transcript in *Drosophila melanogaster*. *Genetics* **112:** 483–491.

Chambers, S.P., S.E. Prior, D.A. Barstow, and N.P. Minton. 1988. The pMTL nic-cloning vectors. I. Improved pUC polylinker regions to facilitate the use of sonicated DNA for nucleotide sequencing. *Gene* **68:** 139–149.

Chen, H., B. Li, and J.L. Workman. 1994. A histone-binding protein, nucleoplasmin, stimulates transcription factor binding to nucleosomes and factor-induced nucleosome disassembly. *EMBO J.* (in press).

Cline, T.W. 1978. Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with *daughterless*. *Genetics* **90:** 683–698.

———. 1980. Maternal and zygotic sex-specific gene interactions in *Drosophila melanogaster*. *Genetics* **96:** 903–926.

———. 1984. Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. *Genetics* **107:** 231–277.

———. 1988. Evidence that *sisterless-a* and *sisterless-b* are two of several discrete "numerator elements" of the *X/A* sex determination signal in *Drosophila* that switch *Sxl* between two alternative stable expression states. *Genetics* **119:** 829–862.

———. 1993. The *Drosophila* sex determination signal: How do flies count to two? *Trends Genet.* **9:** 385–390.

Dobzhansky, T. 1957. The X-chromosome in the larval salivary glands of hybrids *Drosophila insularis*×*Drosophila tropicalis*. *Chromosoma* **8:** 691–698.

Earnshaw, W.C., K.F. Sullivan, P.S. Machlin, C.A. Cooke, D.A. Kaiser, T.D. Pollard, N.F. Rothsfield, and D.D. Cleveland. 1987. Molecular cloning of a cDNA for CENP-B, the major centromeric autoantigen. *J. Cell Biol.* **104:** 817–829.

Gergen, J.P. 1987. Dosage compensation in *Drosophila*: Evidence that *daughterless* and *Sex-lethal* control X chromosome activity at the blastoderm stage of embryogenesis. *Genetics* **117:** 477–485.

Gorbalenya, A.E., E.V. Koonin, A.P. Donchenko, and V.M. Blinov. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair, and expression of DNA and RNA genomes. *Nucleic Acids Res.* **17:** 4713–4730.

Gorman, M., M.I. Kuroda, and B.S. Baker. 1993. Regulation of the sex-specific binding of the maleless dosage compensation protein to the male X chromosome in *Drosophila*. *Cell* **72:** 39–49.

Henikoff, S. and P.M. Meneely. 1993. Unwinding dosage compensation. *Cell* **72:** 1–2.

Inoue, K., K. Hoshijima, H. Sakamoto, and Y. Shimura. 1990. Binding of the *Drosophila Sex-Lethal* gene product to the alternative splice site of the *transformer* transcript. *Nature* **344:** 461–463.

Kimelman, D., L.A. Lucher, K.H. Brackman, J.S. Symington, M. Ptashne, and M. Green. 1984. Synthesis in *Escherichia coli* of human adenovirus type 12 transforming proteins encoded by early region 1A 13S mRNA and 12S mRNA. *Proc. Natl. Acad. Sci.* **81:** 6300–6304.

Kuroda, M.I., M.J. Kernan, R. Kreber, B. Ganetsky, and B.S. Baker. 1991. The *maleless* protein associates with the X chromosome to regulate dosage compensation in *Drosophila*. *Cell* **66:** 935–947.

Kuroda, M.I., M.J. Palmer, and J.C. Lucchesi. 1993. X chromosome dosage compensation in *Drosophila*. *Sem. Dev. Biol.* **4:** 107–116.

Lee, C.-G. and J. Hurwitz. 1993. Human RNA helicase A is homologous to the maleless protein of *Drosophila*. *J. Biol. Chem.* **268:** 16822–16830.

Lindsley, D.L. and G.G. Zimm. 1992. *The genome of Drosophila melanogaster*. Academic Press, San Diego, CA.

Lucchesi, J.C. and J.E. Manning. 1987. Gene dosage compensation in *Drosophila melanogaster*. *Adv. Genet.* **24:** 371–429.

Lucchesi, J.C. and T. Skripski. 1981. The link between dosage compensation and sex differentiation in *Drosophila melanogaster*. *Chromosoma* **82:** 217–227.

Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene diflouride membranes. *J. Biol. Chem.* **21:** 10035–10038.

Mukherjee, A.S. and W. Beerman. 1965. Synthesis of ribonucleic acid by the X chromosomes of *Drosophila melanogaster* and the problem of dosage compensation. *Nature* **207:** 785–786.

Offerman, C.A. 1936. Branched chromosomes as symmetrical duplications. *J. Genet.* **32:** 103–116.

Okuno, T., T. Satou, and K. Oishi. 1984. Studies on the sex-specific lethals of *Drosophila melanogaster*. VII. Sex-specific lethals that do not affect dosage compensation. *Jpn. J. Genet.* **59:** 237–247.

Palmer, M.J., V.A. Mergner, R. Richman, J.E. Manning, M.I. Kuroda, and J.C. Lucchesi. 1993. The male specific lethal one (*msl-1*) gene of *Drosophila melanogaster* encodes a novel protein that associates with the X chromosome in males. *Genetics* **134:** 545–557.

Philpott, A. and G.H. Leno. 1992. Nucleoplasmin remodels sperm chromatin in *Xenopus* egg extracts. *Cell* **69:** 759–767.

Query, C.C., R.C. Bentley, and J.D. Keene. 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. *Cell* **57:** 89–101.

Rogers, S., R. Wells, and M. Rechsteiner. 1986. Amino acid sequences common to rapidly degraded proteins: The PEST hypothesis. *Science* **234:** 364–368.

Skripsky, T. and J.C. Lucchesi. 1982. Intersexuality resulting

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

from the interaction of sex-specific lethal mutations in *Drosophila melanogaster*. *Dev. Biol.* **94:** 153–162.

Sosnowski, B.A., J.M. Belote, and M. McKeown. 1989. Sex-specific alternative splicing of RNA from the *transformer* gene results from sequence-dependent splice site blockage. *Cell* **58:** 449–459.

Turner, B.M., A.J. Birley, and J. Lavender. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila melanogaster*. *Cell* **69:** 375–384.

Uchida, S., T. Uenoyama, and K. Oishi. 1981. Studies on the sex-specific lethals of *Drosophila melanogaster*. III. A third chromosome male-specific lethal mutant. *Jpn. J. Genet.* **56:** 523–527.

Uenoyama, A., A. Fukunaga, and K. Oishi. 1982. Studies on the sex-specific lethals of *Drosophila melanogaster*. V. Sex transformation caused by interactions between a female-specific lethal, *Sxl*, and the male-specific lethals *mle(3)132*, *msl-2*, and *mle*. *Genetics* **102:** 233–243.

Uenoyama, T. 1984. Studies on the sex-specific lethals of *Drosophila melanogaster*. VIII. Enhancement and suppression of *Sxl*. *Jpn. J. Genet.* **59:** 335–348.

Verma, I. 1984. From c-fos to v-fos. *Nature* **308:** 317–322.

Downloaded from genesdev.cshlp.org on August 22, 2022 - Published by Cold Spring Harbor Laboratory Press

Sex-specific regulation of the male-specific lethal-1 dosage compensation gene in Drosophila.

M J Palmer, R Richman, L Richter, et al.

Genes Dev. 1994, **8:**
Access the most recent version at doi:10.1101/gad.8.6.698

References This article cites 41 articles, 14 of which can be accessed free at:
http://genesdev.cshlp.org/content/8/6/698.full.html#ref-list-1

License

Email Alerting Service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or **click here.**

horizon a PerkinElmer company
Streamline your research with
Horizon Discovery's ASO tool

Copyright © Cold Spring Harbor Laboratory Press