MSL-1 PLAYS A CENTRAL ROLE IN ASSEMBLY OF THE MSL COMPLEX WHICH MEDIATES DOSAGE COMPENSATION IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Dosage compensation in *Drosophila melanogaster* is achieved by a twofold increase of transcription of X-linked genes in males. This involves the binding of four proteins, MSL-1, MSL-2, MSL-3 and MLE (collectively known as the MSLs) which are believed to act as a multi-protein complex, to hundreds of sites along the length of the X chromosome. MOF, a putative histone acetyl transferase, is thought to be also associated with MSLs and plays a role in hypertrascription of X-linked genes. Overexpression of either a C-terminal or N-terminal domain of MSL-1 leads to male-specific lethality which is probably due to association with other MSLs to form a non-functional complex.

One aim of this study was to identify whether any known MSLs and/or unknown protein binds with the C-terminal domain of MSL-1. A second aim was to further define the domain of MSL-1 which interacts to MSL-2. Initial attempts to identify the protein which interacts the C-terminal domain of MSL-1 by either genetics analysis or co-immunoprecipitation were inconclusive. Thus, an alternative approach of affinity chromatography of epitope-tagged MSL-1/MSL-complex was followed. Transgenic flies which express either a FLAG-tagged N-terminal region of MSL-1 or FLAG tagged C-terminal domain following heat shock were generated. These lines were crossed with other transgenic lines to co-express the MSL-1 domain with Either MSL-2, MSL-3, MLE or MOF. FLAG affinity chromatography of protein extracts prepared from these flies showed that MSL-2 co-purifies with the N-terminal domain of MSL-1 (aa 85 - 263), whereas MOF and MSL-3 co-purify with the C-terminal domain of MSL-1 (aa 705 - 1039). MLE does not appear to associate with either region of MSL-1. Further, the C-terminal domain of MSL-1 also bound specifically to a glutathione S-transferase-MOF fusion protein. Co-expression of MSL-2 rescued males from the lethal effect which was caused by overexpression of the N-terminal domain of MSL-1. However, co-expression of either or both MOF and MSL-3 with the C-terminal domain of MSL-1 did not improve male viability. This suggests that additional factors may bind to the FC/MOF/MSL-3 complex. Finally, MLE also bound to GST-MOF fusion protein, suggesting a direct interaction between MLE and MOF. These findings suggest that MSL-1 plays a central in assembly of the MSL multi-protein complex that is required to achieve dosage compensation.

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ABBREVIATION

α	alpha
aa	amino acid
N-terminal	amino terminal
β	beta
Δ	delta
C-terminal	carboxy terminal
°C	degrees Celsius
bp	base pairs
dNTP	dinucleotide triphosphate
DNase	deoxyribonuclease
DNA	deoxyribonucleic acid
EMS	ethyl methane sulfanoic acid
g	gram
GST	glutathione S-transferase
hsp	heat shock promoter
kb	kilobase pairs
kD	kilodalton
μ	micro
L (l)	liter
m	milli
M	molar
nm	nanometer
%	percent
PCR	polymerase chain reaction
RNase	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
U	units
UTR	untranslated region
V	volts
v/v	volume per volume
w/v	weight per volume

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1. INTRODUCTION

1.1 SEX DIFFERENTIATION AND DOSAGE COMPENSATION

Sex differentiation is often determined by differences in the number of copies of a single chromosome (Reviewed by Baker *et al.*, 1994). In such cases, a process of dosage compensation may evolve to allow the different number of copies of genes in the two sexes to produce the same amount of functional product. In *Drosophila*, the primary determinant of sex is the ratio of the number of X-chromosomes (X) to sets of autosomes (A). When *Drosophila* has one X-chromosome to two sets of autosomes (1X:2A = 0.5), it evolves into a male (Reviewed by Belote, 1992). An X:A ratio of 1 leads flies to develop into females. This process of somatic sex determination is largely cell autonomous, and there appears to be no hormonal component involved. Although all male *Drosophila* possess a Y-chromosome, it does not play any role in sex determination.

This leads to an interesting fact: there are many important X-linked genes on the X-chromosome. With only one X-chromosome in males and two in females, cells will produce one dose of X-linked gene product and two doses respectively. This raises a question of possible different phenotypes of these X-linked genes in male and female, as the result of different dosage. However, X-linked genes do not show signs of producing unequal amounts of gene product between male and female. In fact, these gene products are manufactured equally in both male and female (Kuroda *et al.*, 1993). This is achieved by a mechanism called dosage compensation. Different organisms have evolved what appear to be different mechanisms to equalise X-linked gene expression in the sexes. In mammals, one of the two X chromosomes in females is transcriptionally inactivated (Reviewed by Willard and Salz, 1997). In *Caenorhabditis elegans*, equalisation of X-linked gene products is achieved by decreasing the activity of genes on both X

chromosomes in hermaphrodites (XX), to reach the level of gene products produced in males (X) (Gorman and Baker, 1994). In *Drosophila melanogaster*, which has a different mode of dosage compensation, the rate of transcription of the single male X chromosome is hyperactivated twofold relative to each female X chromosome (Lucchesi and Manning, 1987).

1.2 THE DISCOVERY OF DOSAGE COMPENSATION IN *DROSOPHILA*

In 1931, Herman Huller published an observation that eye-pigment conferred by the hypomorphic X-linked allele w^a was identified in hemizygous XY males and in homozygous XX females. However, females do not produce two doses of pigment, rather they are producing an equal amount of pigment as male *Drosophila*. Similar results have been obtained for other X-linked genes, suggesting that there must be a mechanism which is able to compensate the dosage difference of X-linked genes between the two sexes.

1.3 CYTOLOGICAL OBSERVATIONS

In *Drosophila* salivary gland polytene nuclei, the male X-chromosome is distinct from autosomes and the female X-chromosome in both appearance and chromatin structure. It is puffier and paler in comparison with the female X-chromosome (Dobzhansky, 1957). This difference is not due to an increased DNA content in the male X-chromosome. Aronson *et al.* (1954) used UV microspectrophotometry to show that the paired female X-chromosome contains twice the amount of DNA material as a single male X-chromosome. Therefore, the difference between the X-chromosomes of the two sexes is either a distinct chromatin configuration or an accumulation of gene products.

1.4 TRANSCRIPTION AUTORADIOGRAPHY EXPERIMENTS

In 1965, Mukherjee and Beerman prepared polytene chromosome squashes from *Drosophila melanogaster* third instar larval salivary glands which were used as the subjects for short pulse labelling with [^{3}H]uridine (Muller, 1950). By using autoradiography, one is able to determine the level of incorporation of labelled uridine along the chromosome arms (Henikoff and Meneely, 1993). The grain counts on the male X-chromosome are greater than both the left arm of autosome 3 and the regions of paired female X-chromosomes. However, unpaired female X-chromosome still seem to have a silver grain count, and it is significantly higher than half of the value for paired regions of the same chromosome. All the observations lead to the fact that a single male X-chromosome is transcriptionally more active than a single female chromosome, and the dosage compensation is at the level of RNA synthesis. The characteristic bloated appearance could be the consequence of accumulation of nascent RNA.

1.5 SEX-LETHAL (*Sxl*)

The *Sex-lethal* gene plays a pivotal role in the processes of somatic sex determination as well as in the process of dosage compensation (Reviewed by Parkhurst and Meneely, 1994). The structure of *Sex-lethal* comprises 10 exons and two promoter regions. The use of different promoters, different exons, and different polyadenylation sites leads to the production of at least 10 different RNA species (Palmer *et al.*, 1994), with varying patterns of expression. SXL has a special feature of auto-regulation in that the protein turns on its own *Sxl* gene and produces more of its gene product (Bernstein *et al.*, 1995;

MacDougall *et al.*, 1995). When the ratio X:A is 1, as females are produced, the *Sxl* locus is making SXL protein which represses the pathway of dosage compensation and results in basal transcription of both X chromosomes (Lucchesi *et al.*, 1982). Whereas if the ratio is 0.5, male development and hypertranscrption occurs as a consequence of lacking SXL protein (Reviewed by Kelly and Kuroda, 1995). Null mutations in *Sxl* are lethal in females - presumably both X chromosomes are hypertranscribed. Conversely, constitutive expression of *Sxl* causes male lethality (Cline, 1978).

1.6 MALE-SPECIFIC LETHAL (*msl*)

There are four genes identified which are responsible for dosage compensation (Reviewed by Lucchesi, 1996). The identification of these four genes is significant for the understanding of the mechanism of dosage compensation. Genetic screens (using the chemical mutagen ethyl methane sulfanoic (EMS)) designed to isolate mutations affecting essential biochemical or physiological processes unique to males or females have uncovered several sex-specific lethal mutations that affect dosage compensation, *maleless* (*mle*) (Fukunaga *et al.*, 1975), *male-specific lethal-1* (*msl-1*) (Belote and Lucchesi, 1980), *male-specific lethal-2* (*msl-2*) (Belote and Lucchesi, 1980), and *male-specific lethal-3* (msl-*3*) (Lucchesi *et al.*, 1982). These are collectively called *msls*. All *mle*, *msl-1* and *msl-2* gene loci are located at chromosome 2, and *msl-3* is located at chromosome 3. The products of the *msl* genes are necessary to maintain an equivalent level of most X-linked gene transcripts in males relative to females. A loss of function of any *msl* through mutation has lethal effects during late larval development in males but has no detectable effect in females.

Genetic evidence suggests that the lethality produced by these *male-specific lethal* genes is directly related to the presence of only one X chromosome in males. Fukunana *et al.*

(1975) and Belote & Lucchesi (1980b) have shown that the male specific lethality is not related to the sexual differentiation. The absence of interaction between sex transforming genes (*dsx* and *tra-2*) and *male-specific lethal* genes (*msl-1* and *msl-2*), and between *dsx* and *tra-3* and *mle*, is proved by introducing sex-transforming genes into the genotype of individuals that are homozygous for *msl* genes. Also, the presence of the Y chromosome has no effect. Individuals of two sexes with zero, one or two Y chromosomes showed no effect on the sex-specific lethality and are viable. Another indication for male-specific lethality is that the difference in the number of X chromosomes has no effect on individuals as the lethality could be due to a difference in dosage of a specific X-linked gene or the number of X chromosomes as a whole. Experiments showed that the duplication of small fragments of X chromosome one at a time does not reduce the lethality caused by male-specific gene mutations in males.

It has been found that male homozygous mutations for any of the *male-specific lethal* genes exhibit a level of X-linked gene activity that is 50-65% of that seen in the wild type, whereas no reduction is observed in the amount of autosomal enzymes tested. Breen and Lucchesi (1986) showed that at the restrictive temperature, the loss of function of *mle* significantly reduced the steady state RNA levels from the X-linked gene, *Sgs-4*, as compared to the transcript levels of the autosomal gene, *Sgs-3*.

1.7 SEX-SPECIFIC LOCALISATION OF MSLs

Since the cloning of *mle* (Kuroda *et al.*, 1991), *msl-1* (Palmer *et al.*, 1993), *msl-2* (Zhou *et al.*, 1995) and *msl-3* (Gorman *et al.*, 1995), the antibodies to their encoded products reveal that all four MSL proteins bind hundreds of specific sites along the male X chromosome (Bashaw and Baker, 1995; Gorman *et al.*, 1995; Kelly *et al.*, 1995; Kuroda *et al.*, 1991; Zhou *et al.*, 1995). None of these four MSLs is associated with X chromosomes in

females, although MLE is associated with 30-40 autosomal sites (Kuroda *et al.*, 1991). MSLs also associate with autosomes in males - *mle* is associated with 30-40 autosomal sites (Kuroda *et al.*, 1991), *msl-1* and *msl-3* are associated with 10-20 autosomal sites (Kelly *et al.*, 1995; Gorman *et al.*, 1995), and *msl-2* is associated with 20-30 autosomal sites and it is co-localised with MSL-1 at these sites (Kelly *et al.*, 1995; Zhou *et al.*, 1995). All of these four *msls* are not processed sex-specifically at the RNA level, and each of the *msl* proteins is produced in both sexes except *msl-2* (Kelly *et al.*, 1995; Zhou *et al.*, 1995). Male-specific lethal binding sites on the male X chromosome are consistent with studies showing that genes transposed from an autosome onto the X chromosome frequently become hypertranscribed, while X-linked genes translocated to an autosome remain dosage compensated. As these post-translocated genes produce various amounts of dosage compensation, it is possible that X-linked *cis*-acting sequences are able to confer hypertranscription on different genes depending on their chromatin context where the genes are placed (Kuroda *et al.*, 1993). The *cis*-acting sites on the male X chromosome could be bound by *trans*-acting MSL proteins and result in dosage compensation (Bone and Kuroda, 1996).

Protein analysis of the association of MSLs with the male X chromosome has been investigated in various mutant backgrounds (Lucchesi and Manning, 1987). Each of the MSL proteins must be functional in order to observe the wild-type chromatin-pattern of the remaining three, suggesting that MSLs act in a heteromeric protein complex (Lucchesi, 1996). Western blotting showed that the MLE is expressed in male homozygous for a mutant at each of the other *msl* loci, which means the other *msls* do not regulate *mle* at the level of protein expression (Gorman *et al.*, 1993). The MSL-1 protein is present in both homozygous mutations for *mle* and *msl-3* in larvae, but not in *msl-2* homozygous mutation or female larvae. Experiments also showed the MSL-1 and MSL-2 proteins can be co-immunoprecipitated by either anti-MSL-1 or anti-MSL-2 from protein extracts of

male larvae (Kelly *et al.*, 1995). Since the MSL-1 and MSL-2 proteins are at low levels or absent in females, neither was immunoprecipitated from female extracts as expected.

Ectopic expression of MSL-2 protein in females decreases viability and delays development since MSL-2 protein is not normally present in females (Kelly *et al.*, 1995). This experiment is conducted by constructing transgenic lines expressing the *msl-2* open reading frame under control of the heat shock 83 promoter (hsp83). The hsp83 allows constitutive activity of *msl-2* in both soma and germline, and can be heat shocked for further induction. Interestingly, MSL-1 protein levels are also significantly increased in H83M2 transgenic females, suggesting that the presence of MSL-2 may be required for translation or stability of MSL-1 protein. This result supports the fact that *msl-1* transcripts are present in homozygous mutation for *msl-2* at larval stages but no functional MSL-1 protein forms at late developmental stages.

In flies which are mosaic for *Sxl* expression, MSL proteins are only associated with the X chromosome in cells which are not expressing SXL. Since there are several types of transcripts which are produced by each of the *mle*, *msl-1* and *msl-3*, and all of these appear to be equivalent in both males and females, the regulation of these transcripts by SXL protein is not direct. Therefore the only MSL protein, MSL-2, which is solely produced in males, can be the key linkage between SXL, MSL complex and dosage compensation (Bashaw and Baker, 1995) (details see Section 1.9)

In polytene chromosome squashes the male X-chromosome is more open and diffuse than that of the females (Dobzhansky, 1957). It has been proposed that this altered chromatin configuration is important in allowing hypertranscription to occur. One of the components for hypertranscription is the recognition of the acetylated isoform of histone H4 (H4Ac16) (Turner *et al.*, 1995). It is a histone H4 acetylated at lysine 16 in the N-terminal region. Using the technique of immunolabelling, a specific acetylated isoform is

not only detected predominantly on the male X chromosome, coincidently, it also has the same pattern of association with the X chromosome as that of the MSLs. H4Ac16 is not detected on the X chromosome in homozygous mutation for *msls* males, correlating with the lack of dosage compensation in these mutants. Conversely, in *Sxl* mutants, H4Ac16 is detected on the X chromosomes in females which have inappropriate hypertranscription as a consequence. All of the above suggest that synthesis or localisation of H4Ac16 is controlled by the dosage compensation regulatory hierarchy (Lee *et al.*, 1993), and may potentially be involved in dosage compensation through interaction with the products of the *msl* genes.

1.8 NON-CODING RNAS ARE INVOLVED IN DOSAGE COMPENSATION

The mutants *roX1* and *roX2* were isolated in an enhancer detector screen for mushroom body expression of the reporter gene *lacZ* (Han *et al.*, 1996). The expression of both *roX1* and *roX2* are restricted to the neuron cells of adult male flies (Amrein and Axel, 1997). The two genes are X-linked and lack significant open reading frames, suggesting they may encode non-coding RNAs (Amrein and Axel, 1997; Meller *et al.*, 1997). Mutations in any one of the *msl* genes prevent the expression of *roX1* and *roX2*, and ectopic expression in females of the normally male-specific *msl-2* gene induces expression of *roX1* and *roX2* (Amrein and Axel, 1997; Meller *et al.*, 1997). Expression of *roX1* is dependent on *Sxl* but is independent of the Y chromosome and *tra* which is a downstream effector of *Sxl* (Meller *et al.*, 1997). *In situ* hybridisation of *roX1* probes to the male third instar larval salivary gland reveals a subcellular localisation of *roX1* RNA identical to that of MSL-2 which binds to the X chromosome (Meller *et al.*, 1997). *RoX1* recognises, or paints, the X chromosome of males in a similar mechanism as the mammalian *Xist* non-coding RNA which coats the inactive X chromosome. *In situ* hybridisation of a chromosome containing

roX1 region was transposed to the Y or the 2nd chromosome and showed binding of *roX1* to the transposed X-linked chromatin as well as to the X chromosome (Meller *et al.*, 1997). This result suggests that *roX1* can act either in *trans* or in *cis* in contrast to *Xist* which can only spread in *cis* along chromatin from the X-inactivation centre.

MLE consists of RNA binding domains (Kuroda *et al.*, 1991). It would be tempting to speculate that *roX1* is required for the binding of MLE to the male X. However, the localisation of MLE to the male X appeared undisrupted by mutations in *roX1* (Meller *et al.*, 1997). Meller *et al.* (1997) suggested that there is a family of non-homologous RNAs including *roX1* and *roX2*, which are functionally redundant. They proposed that the binding of the MSL complex to the X activates the male-specific RNA which facilitates a change in chromatin structure leading to hypertranscription of the male X chromosome.

1.9 A MODEL FOR THE REGULATION OF DOSAGE COMPENSATION

Studies showed that the four histones comprise the nucleosome core and participate in the transcriptional regulation of numerous genes acting as suppressors of transcription (Turner, 1991). The core histones undergo several post-translational modifications, including acetylation at the N-termini, leading to the suggestion that the structure and function of chromatin could be altered through an enzymatic pathway. The finding that histone acetylation pre-exists transcription showed the modification is not a consequence of transcription but is most likely a prerequisite (Turner *et al.*, 1992). This suggests that histone acetylation can stabilise the binding of transcriptional factors to mucleosomal DNA, and may play a role in initiating or in maintaining the accessibility of transcriptional regulatory elements in chromatin. Acetylation of lysine at the N-terminus neutralises the positive charge of histones. Experiments illustrate that removal or acetylation of the histone H4 N-terminal tails facilitate the interaction of the

transcriptional factors USF and GAL4-AH where the highest DNA binding affinity is obtained (Turner *et al.*, 1992). As is described above, the H4Ac16 may play an important role in loosening the chromatin structure and increasing the accessibility of transcriptional factors in association with the male X chromosome in *Drosophila* (Bone *et al.*, 1994).

MLE has short sequences that identify it as a member of one of two superfamilies of nucleic acid helicases (Richter *et al.*, 1996). It is highly homologous to human RNA helicase A and bovine nuclear DNA helicase II, for which a DNA and RNA unwinding activity has been illustrated. This helicase-like MLE protein is possibly targeting a stable RNA which is the mediator for the association of MLE with the MSL complex, since RNase treatment excludes the possibilities of interaction with nascent transcripts, protein-protein interaction with the MSL complex, or direct DNA binding (Gorman *et al.*, 1993; Kuroda *et al.*, 1991; Richter *et al.*, 1996).

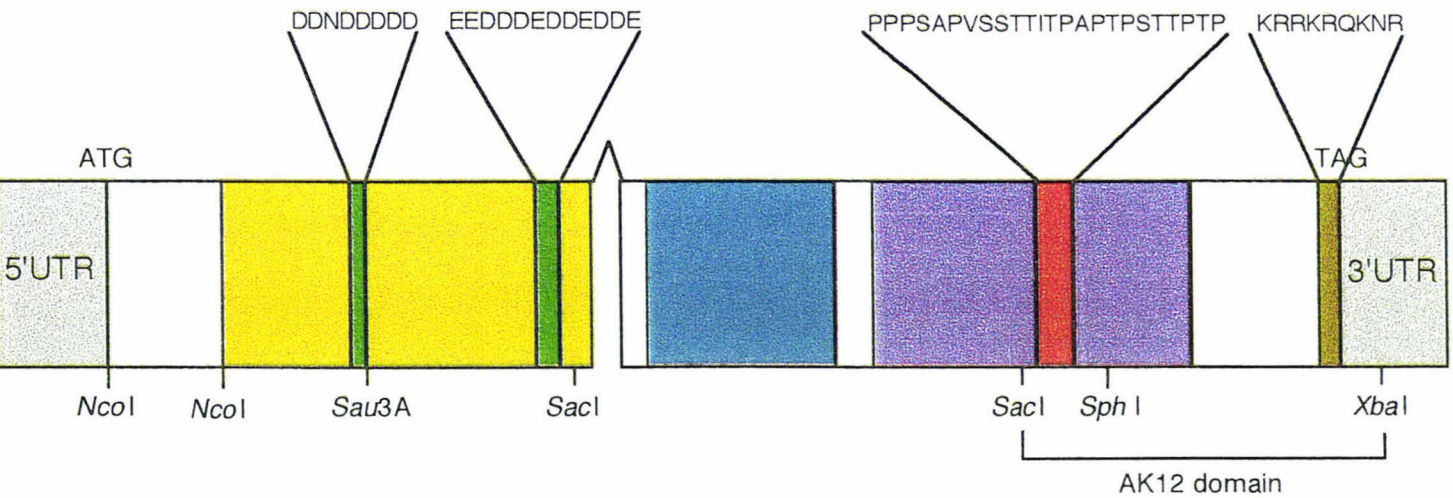
Male-specific lethal-1 encodes a 1039 aa protein with a highly acidic N terminus that includes two short stretches composed almost entirely of aspartate or aspartate and glutamate residues as well as numerous interspersed glutamate doublets (Figure 1). These characteristics are common to a large group of proteins such as nucleolin and nucleoplasmin which are thought to be involved in transcription regulation and chromatin modelling. Direct contacts may possibly be made between acidic regions of these proteins, including MSL-1, and basic chromosomal proteins such as histones. This may lead to alterations of nucleosome positioning or conformation. Also in the central region of the MSL-1, there are many serine (S), threonine (T) and proline (P), residues, which form S/T/P motifs that could be sites of phosphorylation by protein kinases, and therefore regulate the activity of the protein and perhaps the MSL complex.

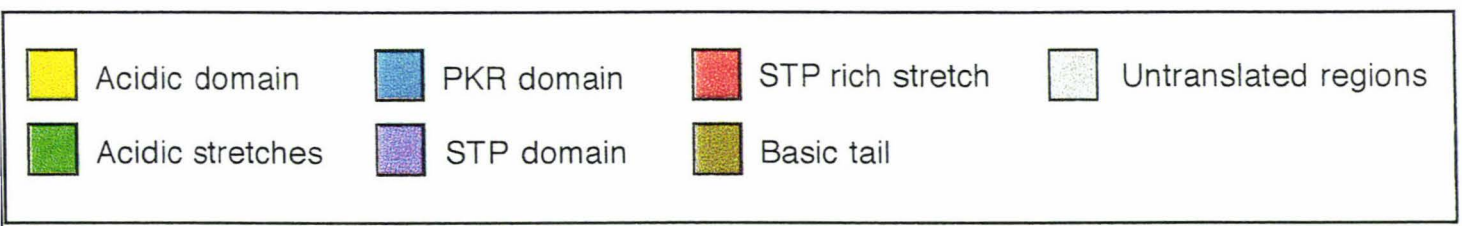
MSL-3 contains two chromo domains (Lucchesi, 1996). This feature is interesting as the secondary structure of a single domain appears to be unstable, and may require interaction

Figure 1. Diagrammatic Representation of The Open Reading Frame of *msl-1*

Protein coding sequences are drawn as boxes and the putative domains are coloured. The amino acid sequence of the asp/glu-rich stretches within the acidic domain is given using the standard one letter code as is the sequence of the basic tail and a highly ser/thr/pro-rich sequence within the STP domain.

Structure of *male specific lethal* (*msl-1*) Gene





with another chromo domain or with some globular domain in another part of the protein to yield a stable configuration. Stabilisation may also be achieved by the interaction between chromo domains of different proteins. Chromo domains share a characteristic with proteins that are responsible for repression, as several proteins are found to participate in transcriptional repression (Koonin *et al.*, 1995). However, chromo domains are also found in proteins known to function as transcriptional activators, suggesting that the function of the chromo domain is to deliver regulatory proteins to their site of action, whether they exert a positive or a negative effect on gene activity. Therefore the presence of MSL-3 indicates the potential for this type of regulation may be involved in dosage compensation.

As described above, MSL-2 is present only in males and is co-localised with the other three MSLs (Kelly *et al.*, 1995; Bashaw and Baker, 1996). With a perfect inverse correlation between the presence of SXL and the presence of MSL-2, it makes MSL-2 a likely governor of the MSL complex for transcriptional enhancement (Bashaw and Baker, 1995). Studies showed that the SXL protein, which is a RNA binding protein whose known function is to regulate pre-mRNA splicing, may target the *msl-2* gene that produces non-functional MSL-2 in females (Bashaw and Baker, 1997). Experiments suggest that there is a difference in the splicing of *msl-2* pre-mRNA between the two sexes. A 133 nucleotide intron in the 5' untranslated region (UTR) of *msl-2* is retained in females but removed in males (Bashaw and Baker, 1995; Zhou *et al.*, 1995). There are potential SXL-binding sites in the 3' UTR as well as the two potential SXL-binding sites present in the male-specific intron in the 5' UTR. That sequence in the 3' UTR plays a role in the regulation of MSL-2 expression (Bashaw and Baker 1997). It is demonstrated by removing the 3' UTR that leads to detectable binding of MSL-2 to the X chromosome in females. In addition to detecting MSL-2 associated with 50-60 sites on the X chromosomes of these transgenic females, all three of the other MSLs are also associated at these same sites. This suggests that the expression of MSL-2 due to deletion of the 3'

UTR results in the assembly of a male-type MSL complex, indicating SXL could act through these sites at some level other than splicing to control MSL-2 expression since male *msl-2* transcripts have possessed these regions. Therefore the presence of potential SXL-binding sites and the alternative splicing event make the 5' UTR a strong candidate for a region that may contain other sequences crucial to the regulation of MSL-2.

The encoded protein MSL-2 consists of 769 amino acid residues and it contains a RING finger (C_3HC_4 zinc finger) at the N-terminus of the protein, with a coiled coil at the central domain followed by positively and negatively charged segments that flank a metallothionein-like domain which has eight conserved and two non-conserved cysteines (Bashaw and Baker, 1995; Zhou *et al.*, 1995). The RING finger is related to the classical zinc finger and is found in a large group of proteins with potential for DNA or protein-protein interactions. Interactions between the zinc fingers of transcription factors, SP1 and TFIIIA, and thionein have been demonstrated *in vitro* (Zhou *et al.*, 1995). These observations suggest a possible novel mechanism, based on the intramolecular exchange of metal ions, that may modulate the activity of MSL-2. Since MSL-2 is the only MSL protein containing sequence elements that can be implicated in DNA binding, it is possible that MSL-2 interacts directly with male X-chromosomal DNA and at the same time targets the putative MSL complex to male X. When a twofold transcriptional rate is reached, MSL-2 may undergo a conformational change that allows the lowering of RING finger affinity for the zinc atoms, which may be captured by the metallothionein-like portion of the protein, restricting the transcriptional rate so that it does not reach beyond twofold. When the male X-chromosome is hypotranscribed, the condition is reversed and a twofold transcription is re-established.

Dosage compensation of the *runt* gene during embryogenesis is dependent on *Sxl* but independent of the *msl* genes (Gergen, 1987), suggesting that there is another possible kind of regulation for dosage compensation. This hypothesis is supported by the

experiment of female SXL mutants which can not be rescued by mutation of the MSLs (Kelly and Kuroda, 1995). With 20% of the genetic material located on the X-chromosome, more screening is needed in order to find other dosage-compensation-related genes.

1.10 DISCOVERY OF THE *males-absent on the first (mof)* GENE

Hilfiker *et al.* (1997) have recently discovered a possible fifth male-specific lethal gene which may be involved in the regulatory process of dosage compensation. *Males-absent on the first* (*mof*) has been identified by using the phenotype of male-specific lethality to screen the X chromosome of *Drosophila melanogaster* for EMS-induced mutations. Mutant *mof* males can develop to the third larval instar of the pre-pupal stage but fail to metamorphose and to hatch, whereas the viability of mutant female is unaffected. Staining of polytene chromosomes with anti-MSL antibodies suggests that the association of MSL-1 and MSL-2 with the male X-chromosome of mutant *mof* larvae is slightly reduced. However, the association of MLE is substantially reduced, and the H4 isoform, H4Ac16, appears to be absent (Gu *et al.*, 1998). Other evidence showed that the ectopic expression of MSL-2 in females can be rescued by expressing the MOF protein from one wild-type copy of *mof*, indicating *mof* has a functional role in dosage compensation. MOF contains the signature motif for the acetyl coenzyme A binding site found in numerous and diverse acetyl transferases (Hilfiker *et al.*, 1997). Therefore MOF could be one of the histone acetyl transferases which may be responsible for the particular histone acetylation (i.e. H4Ac16) associated with the male X chromosome, and consequently provides a functional link between nucleosomal modification and the transcriptional enhancement.

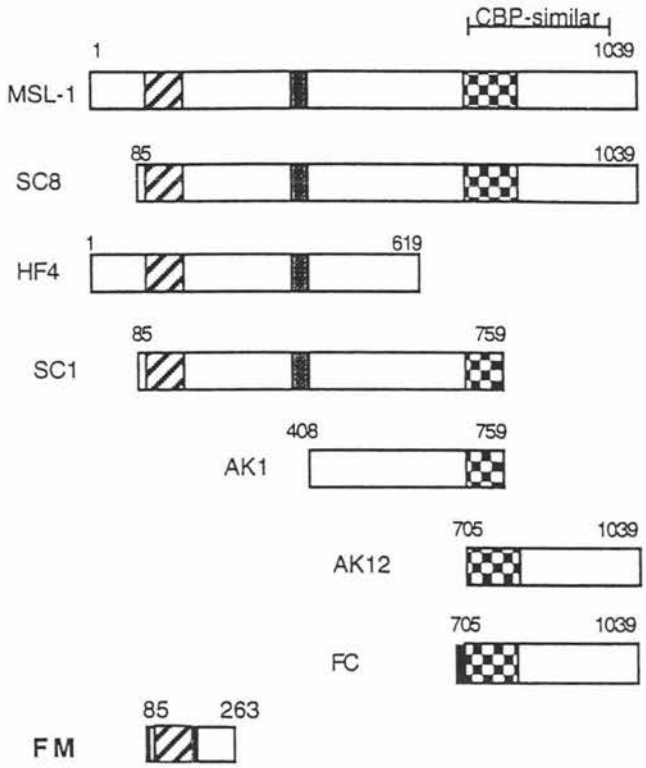
1.11 OVEREXPRESSION OF DOMAINS OF MSL-1 in *D. melanogaster*

MSL-1 (Figure 1) consists of several domains (Lucchesi, 1998). The first third of the protein (amino acid 1 to 409) contains the acidic region which is discussed in Section 1.8. The next region is a P/K/R domain which is from amino acid 410 to 634aa, which contains a predominance of proline (P), lysine (K) and arginine (R). The final region is the STP domain which is located from amino acid 702 to 877 aa. It contains several stretches rich in serine, threonine and proline. There is a basic tail which possesses seven basic amino acids out of the final nine amino acids (Figure 1).

Plasmid pAK12 contains the 1.17 kb region of *msl-1* 3' end bounded by *Sac*I and *Xba*I restriction sites inserted into the expression vector pCaSpeR-hs (Table 1). In this construct, expression of the C terminal domain of MSL-1 is under the control of heat shock promoter 70 (*hsp70*) (A. Knox, unpublished results). The restriction fragment codes for the basic tail and approximately half of the STP domain. This construct was microinjected (Section 2.18) into recipient *yw* stocks (Table 3) and homozygous flies were obtained (A. Knox, unpublished results). In these transgenics (AK12 flies), overexpression of the C-terminal domain (amino acid 705 - 1039, Figure 2) was achieved when raised at 30°C and heat shocked daily for one hour at 37°C (Section 2.21). Under these conditions, males showed a significant decrease in viability compared to either the injection stocks or transgenic with full-length MSL-1 (Dr M. Scott, unpublished results). It is proposed that the lethality is caused by the competition with functional MSL-1 for binding to a protein required for dosage compensation. Overexpression of MLE, MSL-2 or MSL-3 one at the time with the C-terminal domain shows no significant improvement in the viability of heterozygous AK12 males (M. Scott, unpublished results). This suggests that either the C-terminal domain is bound to more than one MSL protein or the domain binds to an unknown factor which is required to achieve twofold dosage

Figure 2. Dominant-Negative Versions of MSL-1.

The numbers at the beginning and end of each construct indicate the region of MSL-1 which is expressed in transgenic flies. The FLAG tag (DYKDDDDK - shaded black) is at the amino terminus of the protein encoded by the FC and FM constructs. Cross-hatched region is a predicted amphipathic α-helix (aa 96-172). The shaded region is a highly acidic stretch (aa 708-801). The region between aa 712 to 988 shows similarity to amino acids 863 to 1117 of mouse CBP.


CBP-similar
1
1039
MSL-1
85
1039
SC8
1
619
HF4
85
759
SC1
408
759
AK1
705
1039
AK12
705
1039
FC
85
263
FM

compensation. The recent finding of the MOF protein suggests it could be a possible target for the C-terminal region of MSL-1.

Similar experiments were carried out for transformants carrying the FMS construct (Figure 2) and results showed there is a significant reduction in male viability when heat shocked daily (Section 2.21) (Dr. M. Scott, unpublished results). The FMS protein lacks the first 84 amino acids of MSL-1, and contains a FLAG octapeptide which is tagged at the amino terminus. A FMS line was crossed with lines carrying null mutation for either *msl-1, msl-2, msl-3* or *mle*. These crosses would produce progeny with reduced concentration of 50 % of normal *msl-1, msl-2, msl-3* and *mle*, and overexpressed FMS when heat shocked daily (Section 2.21). Male viability was significantly reduced if the males of FMS were heterozygous for *msl-2*, however, there was no significant difference in the relative viability of heterozygous *msl-1, msl-3* and *mle* males compared to their respective wild-type siblings (Dr. M. Scott, unpublished results). These results indicate that in FMS males the concentration of MSL-2 available for dosage compensation is limiting. Further, the viability of males which overexpressed both FMS and MSL-2 was significantly improved compared to males which expressed only the FMS (Dr. M. Scott, unpublished results), suggesting that FMS interacts with MSL-2. Similarly experiments were carried out with transformants lines carrying the ANT which express aa 85-759 of MSL-1 with a FLAG tag at N terminus (Figure 2). Overexpression of the ANT protein caused a decrease in male viability which however was significantly improved if MSL-2 was co-expressed. This indicates that the domain which interacts with MSL-2 should be in the first two-thirds of MSL-1.

1.12 RESEARCH AIMS AND SPECIFIC OBJECTIVES

This study has two aims. The main aim is to determine whether MOF, or other factor(s), associate with the C-terminal domain of MSL-1. The second aim is to define the MSL-2 binding domain of MSL-1.

1.12.1 Factor(s) Associated With The C-Terminal Domain of MSL-1

The first aim of this project is to determine what factor, either one of the MSLs, MOF or an unidentified factor, associates with the C-terminal domain of MSL-1. Both biochemical and genetic approaches were used to identify the interacting factor(s).

The specific objectives are as follows:

1. Screen for EMS induced mutation which enhance or suppress male lethality caused by overexpression of the C-terminal domain.
2. 2. To generate transgenic flies which express a (FLAG) tagged C-terminal domain of MSL-1.
3. To clone the *mof* gene and make transgenic *Drosophila* lines which express MOF.
4. To make and transform *E. coli* with a plasmid designed to express a glutathione S-transferase MOF fusion protein.
5. To determine which protein(s) co-purify with the FLAG-tagged C-terminal domain of MSL-1 over a FLAG affinity column.
6. To determine which protein (s) co-purify with the GST-MOF fusion protein over a glutathione affinity column.
7. To determine if males can be rescued from the dominant-negative effects of overexpression of the C-terminal domain of MSL-1 by co-expression of any of the MSLs (or combination).

1.12.2 Define MSL-2 Binding Domain of MSL-1

The second aim is to further define the domain of MSL-1 which interacts with MSL-2.

The specific objectives are as follows:

1. To generate transgenic flies which express amino-terminal region of MSL-1 (aa 85 - 263) with a FLAG - tag FM (Figure 2).
2. To determine if overexpression of the FM protein causes male-specific lethality. If so, determine if FM can be rescued by co-expression of MSL-2.
3. To determine if MSL-2 co-purifies with the FMS, ANT, and FM proteins over a FLAG affinity column.