THE X-LINKED *LSP1α* GENE OF *DROSOPHILA MELANOGASTER* IS NOT ACETYLATED BY MOF, BUT IS SEX-SPECIFICALLY REGULATED BY INDIVIDUAL COMPONENTS OF THE MSL COMPLEX

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ABSTRACT

Male *Drosophila melanogaster* double the transcription of most of the genes on their single X chromosome, to equal that from the two female X chromosomes, in a process termed dosage compensation. This process is mediated by the MSL complex, consisting of both protein and non-coding RNA components. This complex is only active in males due to the presence of MSL2, which is not translated in females.

The X-linked *Lsp1α* gene of *Drosophila melanogaster* appears to escape dosage compensation, and exhibits two-fold higher levels of expression in females compared to males. The apparent lack of dosage compensation of *Lsp1α* could be due to the promoter being more active in females than in males, or to a lack of regulation by the MSL complex. In this study, the mechanism by which this happens has been addressed. *Lsp1α* is expressed exclusively in the fat body tissue of third instar larvae, and forms part of a multi-protein complex that acts as a nutrient reservoir during pupariation. In this study it has been shown that transgenes, in which the reporter gene, *lacZ,* is under the control of the *Lsp1α* promoter, exhibit variable levels of increased activity in female compared to male third instar larvae. At high levels of transgene expression, activity of the transgene is equal in female and male larvae. When the expression of the transgene is low, the activity of the transgene is much higher in female compared to male larvae. This increased sensitivity of the *Lsp1α* promoter to position effects in females appears to be mediated by one or more components of the MSL complex. Females ectopically expressing MSL2 exhibit decreased levels of transgene activity. Furthermore, overexpression of MSL1 causes an increase in the activity of transgenes subject to strong position effects.

Despite these findings, the sex-specific regulation of the *Lsp1α* promoter does not account for the non-dosage compensated appearance of *Lsp1α.* Instead, unlike control dosage compensated X-linked genes, *Lsp1α* is not enriched for a histone modification, acetylation of lysine 16 of histone H4 that is essential for dosage compensation by the MSL complex.

The developmental stage at which the four genes flanking *Lsp1α* are expressed has been determined using northern RNA hybridization. Expression of the gene immediately 3' of *Lsp1α* could not be detected at any developmental stage using northern RNA hybridization or in adults by RT-PCR. However, the two genes flanking *Lsp1α* are expressed in equal levels in male and female *Drosophila* as determined by quantitative RNase protection analysis. Furthermore, the regions between *Lsp1α* and these flanking dosage compensated genes do not prevent dosage compensation of an X-linked *arm-lacZ* reporter gene.

Bioinformatic analysis shows that *Lsp1α* is present in three species closely related to *D. melanogaster* but is absent in more distantly related species. It is probable that because of its recent evolutionary origin, the *Lsp1α* gene lacks the DNA sequences that are required to attract the MSL complex. More generally, a model is proposed in which dosage compensation involves binding of the MSL complex to DNA sequences in actively transcribed regions with possible limited spreading to closely associated active genes.

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The creation of transgenic *Drosophila* lines in this study has been approved under the protocol number GMO 00/MU/51 by the Massey University Genetic Technology Committee.

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ABBREVIATIONS

ATP	adenosine 5'-triphosphate
BDGP	Berkeley *Drosophila* Genome Project
bp	base pair
BSA	bovine serum albumin
°C	degrees Celsius
ca.	approximately
cDNA	copy deoxyribonucleic acid
CES	chromatin entry site
ChIP	chromatin immunoprecipitation
cpm	counts per minute
CsCl	cesium chloride
CTD	C-terminal domain
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2-deoxycytidine 5'-triphosphate
DF	dilution factor
dGTP	2'-deoxyguanosine 5'-triphosphate
DHS	DNaseI hypersensitive site
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiothreitol
dTTP	2'-deoxythymidine 5'-triphosphate
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
g	gram
GFP	green fluorescent protein
h	hour
H3K20	lysine 20 of histone H3
H3S10P	phosphorylated serine 10 of histone H3
H4K16ac	acetylated lysine 16 of histone H4
HA	hemagglutinin

HAT	histone acetyl transferase
HCl	hydrochloric acid
HDAC	histone deactylase
HMT	histone methyl transferase
IGEPAL	octylphenylpolyethylene glycol
kb	kilobase-pairs
kDa	kilodaltons
KOAc	potassium acetate
L	litre
LB	Luria-Bertani (media or broth)
M	molar, moles per litre
mg	milligram
μL	microlitre
mL	millilitre
Milli-Q water	water purified by a Milli-Q ion exchange column
μM	micromolar, micromoles per litre
mM	millimolar, millimoles per litre
min	minute
M_r	relative molecular mass (g mol^{-1})
mRNA	messenger ribonucleic acid
MSL	male specific lethal
NaOAc	sodium acetate
nmol	nanomole
nt	nucleotide
ORF	open reading frame
PCR	polymerase chain reaction
pH	-Log $[H^+]$
poly(A)$^+$	polyadenylated
RNA	ribonucleic acid
RNAPII	RNA polymerase II
RNase	ribonuclease
RNAi	RNA interference
RT-PCR	reverse transcriptase-polymerase chain reaction
rpm	revolutions per minute

s	second
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphism
ss	single stranded
TE	10 mM Tris, 1 mM EDTA (pH 8.0)
TEMED	N, N, N', N'-tetramethyl-ethylendiamin
Tris	tris(hydroxymethy)aminomethane
Tween-20	polyoxyethylenesorbitan monolaurate
UTR	untranslated region
UV	ultra violet light
V	volt (m^2 kg s^{-3} A^{-1})
WT	wild-type
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight