DEVELOPMENT OF A REPORTER GENE ASSAY TO IDENTIFY CONTROL ELEMENTS REQUIRED FOR DOSAGE COMPENSATION IN *DROSOPHILA MELANOGASTER*

A thesis presented in partial fulfilment of the requirements
for the Degree of Master of Science in Genetics
at Massey University, Palmerston North,
New Zealand

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1998

ABSTRACT

Dosage compensation (equalisation of X-linked gene products) occurs in *Drosophila melanogaster* by a two-fold transcriptional increase of X-linked gene expression in the male. This involves the binding of four proteins, MSL-1, MSL-2, MSL-3 and MLE (collectively known as the MSLs), to hundreds of sites along the length of the male X. The MSLs are thought to recruit MOF, a histone acetyl transferase, which facilitates the increase in transcriptional activity of X-linked genes. The DNA sequences required to target the MSL complex to the X chromosome (known as dosage compensation regulatory elements, or DCREs) remain elusive, despite numerous attempts over the last ten years to identify them. DCREs are thought to be present at multiple sites along the length of the X chromosome, as antibodies to the MSLs bind to hundreds of sites along the X, and autosomal genes transduced to the X usually become dosage compensated.

The first objective of this study was to develop a reporter gene assay to screen for DCREs that would minimise problems previously encountered. A construct consisting of the constitutive *armadillo* promoter fused to the *lacZ* reporter gene (called *arm-lacZ*) was flanked by insulator elements which block the repressive effects of the autosomal chromatin environment. Fragments of X-linked DNA were inserted upstream of the *armadillo* promoter with the premise that males carrying one copy of an autosomal insertion of this construct would express twice the level of β-galactosidase as females. Transgenic flies carrying autosomal insertions of X-linked fragments plus *arm-lacZ* were generated and one dose males and females were assayed for β-galactosidase activity using a spectrophotometric assay. In all cases, males and females expressed the same level of *lacZ*. This suggests that no DCREs that could confer dosage compensation onto *arm-lacZ* were present in the X-linked fragments. *arm-lacZ* is capable of being dosage compensated as males and females carrying one copy of an X-linked insertion of *arm-lacZ* produce a 2:1 male to female ratio. This implies that DCREs of the 'strength' required to dosage compensate *arm-lacZ* are rarer than previously thought.

A second method of dosage compensation that is independent of the MSLs is thought to occur in *Drosophila*. The X-linked gene *runt* is dosage compensated in the absence

of the MSLs. It is possible that *runt* is sex specifically regulated by the female specific *Sex lethal* protein (*Sxl*). *Sxl* down-regulates *msl-2* in females by binding to $(U)_8$ or $A(U)_7$ sequences in the *msl-2* 5' and 3' untranslated regions (UTRs) of the mRNA. *runt* mRNA contains three *Sxl* binding sites in its 3'UTR, as do 20 other X-linked genes. The second objective of this project was to determine if *Sxl* could down regulate a gene in females, purely by the addition of three *Sxl* binding sites to the 3'UTR. *Sxl* binding sites were inserted into the 3'UTR of *arm-lacZ* in the form of a 40 bp synthetic linker containing three of the sites, and also as a 170 bp fragment from the *runt* 3'UTR. β-galactosidase assays of flies carrying the *Sxl* binding sites from *runt* showed that males expressed an average of 1.31 to 1.46 times the level of *lacZ* than females. This shows that *Sxl* can down-regulate a gene if there are *Sxl* binding sites in its 3'UTR, however, to achieve two-fold regulation, additional factors may be required, or topologically, the sites may not have been in the right position in the 3'UTR for optimal activity of *Sxl*. Flies carrying the synthetic linker expressed the same level of β-galactosidase in both sexes which suggests that either additional elements within the 3'UTR are required, or that the spacing between the sites is critical for the action of *Sxl*.

ACKNOWLEDGEMENTS

A big thank you to my supervisor, Dr. Max Scott for your brilliant guidance, friendliness and for always being available to give help and advice. I really appreciate it.

Also many thanks to my family for all your love and support and for showing an interest in my work. I would like especially to thank Dad for patiently fixing my endless computer problems which would have otherwise driven me insane!

To the Blands (and Lesley), thanks for your encouragement and support and for yummy Sunday dinners. Special thanks to Chris for the barbecuing lessons (I hope to get my BBQ - Bachelor of Barbecuing - soon!) and to Marian for your enthusiasm and for being a mother figure, it means a lot to me.

To the friends I have made at Massey - Kirsty, BJ, Kathryn, James, Amanda and many others, thanks for your friendship, we have had heaps of fun times and I will miss the friendly Massey atmosphere.

To everyone in the Fly Spot, past and present - Max, Kathryn, Sheralee, Lewis, Xuelei, Jörg and Rebecca, a great team of friendly people who helped make my time in the lab really enjoyable. I wont miss the blowflies, though!!

Thank you to Massey and the (former) Department of Microbiology and Genetics for providing me with funding which has made my Master's a lot easier.

Finally, to my fiancé Ross, thanks for your love, encouragement, friendship and emails that I looked forward to each day. See you in Auckland!

ABBREVIATIONS

ß	beta
Δ	delta
λ	lambda
bp	base pairs
°C	degrees Celsius
dNTP	dinucleotide triphosphate
DNase	deoxyribonuclease
DNA	deoxyribonucleic acid
F	female
g	gram
kb	kilobase pairs
mRNA	messenger ribonucleic acid
µ	micro
m	milli
M	male
M	molar
min	minute
nm	nanometer
PCR	polymerase chain reaction
RNase	ribonuclease
RNA	ribonucleic acid
rpm	revolutions per minute
UV	ultra violet
U	units
UTR	untranslated region
V	volts
v/v	volume per volume
w/v	weight per volume

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