The importance of the promoter in *Drosophila* dosage compensation

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Abstract

Dosage compensation is the equalisation of gene expression from unequal doses of genes. *Drosophila* males up-regulate transcription from their single X chromosome to equal that from the two female X chromosomes. Five *male-specific lethal* (*msl*) genes are required in males, and encode the main agents of the up-regulation. At least these proteins, together with either or both of two non-coding RNAs, form the MSL chromatin-modifying complex. Female-specific translational repression of a key component, *msl2*, limits the complex to males. The MSL complex binds to the X chromosome at hundreds of distinct loci, acetylates nucleosomes, and de-condenses the chromatin. Together with possibly many co-factors, the transcriptional up-regulation caused by MSL complex appears to counteract repressive factors to achieve an average effect of transcriptional doubling.

Here, I have studied the initiation of MSL regulation on the X chromosome with a variety of approaches. In order to study early events, dosage compensation was induced in females with ectopic expression of *msl2* from the tetracycline system. However, low background expression without activation prohibited further studies. To identify novel factors that affect dosage compensation, a reporter gene system based on variable eye size was evaluated. The system provided a dose-dependent phenotype, but could not report additional up-regulation by the MSL complex, and was thus unsuitable for the proposed mutational screen.

The quantifiable *lacZ* gene was measured in a strict comparison of expression from an eye-specfic (GMR) or a constitutive (*armadillo*) promoter. At defined locations on the X chromsome, *armadillo-lacZ* acquired local compensation, but *GMR-lacZ* did not. Further modifications upstream of *GMR-lacZ* increased the response, and confirmed the importance of the promoter in attraction of dosage compensation. To corroborate this with the established importance of genic sequences in MSL attraction, a combinatorial model of attraction is proposed. The relative importance of early or constitutive expression was also tested, by providing *GMR-lacZ* with extra expression through the tetracycline system. A burst of embryonic expression, and constitutive expression, were both insufficient to increase dosage compensation of the transgene. Finally, the compensation of GMR-mediated transgenes was confounded by 'transvection' effects of chromosome pairing. This effect may have wider implications on the study of compensation at individual genes.

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Now it is done. I've learnt so much.

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Aside from standard molecular biology abbreviations, and *le Système international d'unités* (SI) conventions, the following abbreviations are used:

~	approximately	MRE	MSL recognition element
6PGD	6-phosphogluconate dehydrogenase	msl	male-specific lethal
abbrv	abbreviations	NURF	nucleosome remodeling factor
a.k.a.	also known as	p	plasmid
arm	armadillo	PCNA	proliferating cell nuclear antigen
BAD	biotinylation activation domain	RFP	red fluorescent protein
β-gal	β-galactosidase	roX	RNA on the X
ChIP	Chromatin Immuno-precipitation	RT	reverse transcriptase
DRE	DNA replication-related element	rtTA	reverse tTA
FUM	fumarase	SCF	DNA supercoiling factor
G	generation	SV40	Simian vacuolating virus 40
G6PD	glucose-6-phosphate dehydrogenase	Sxl	Sex-Lethal
GFP	green fluorescent protein	tet	tetracycline
GMR	Glass multimer reporter	tetO	tetracycline operator (TRE)
H3K36	histone 3, lysine 36	TetR	tetracycline repressor
H3K36me3	tri-methylated H3K36	TRE	tetracycline response element
H3S10	histone 3, serine 10	TRF2	TBP-related factor
H4K16	histone 4, lysine 16	tsp	transcription start point
HA	hemaglutanin	tTA	tetracycline-controlled transcriptional activator
HAT	histone acetyl transferase	tTS	tetracycline-controlled transcriptional silencer
hid	head involution defective	UAS	upstream activation sequence
HIS	6 histidine residues	UTR	un-translated region
hsp	heat-shock protein	X-gal	5-bromo-4chloro-3indolyl-β-D-galactopyranoside
IRES	internal ribosome entry site	y	yellow
LB	Luria-Bertani	YFP	yellow fluorescent protein
mle	maleless	w	white
mof	males-absent on the first	WT	wild-type