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# Characterisation of mutants influencing epigenetic gene silencing in the mouse

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## **Declaration**

I carried out the work described in this thesis between March 2004 and February 2006 in the School of Molecular and Microbial Biosciences, The University of Sydney, and between February 2006 and October 2007 in the Department of Population Studies and Human Genetics, Queensland Institute of Medical Research. I performed these experiments myself, except where otherwise stated. I have not presented this material for the purpose of obtaining any other degree.

Tim Bruxner

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## **Publications arising from this thesis**

### *Journal publications*

Blewitt, M. E., Vickaryous, N. K., Hemley, S. J., Ashe, A., Bruxner, T. J., Preis, J. I., Arkell, R., and Whitelaw, E. (2005). An N-ethyl-N-nitrosourea screen for genes involved in variegation in the mouse. *Proc Natl Acad Sci U S A* *102*, 7629-7634.

### *Book chapters*

Bruxner, T. J., and Whitelaw, E. (In press) Transgenerational Epigenetic Inheritance, In *Epigenetics*, J. Töst, ed. (Perth, UK: Prepress Projects Ltd).

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## Abstract

The field of epigenetics emerged primarily from studies in *Drosophila*, and is now being studied intensively by mammalian biologists. In order to increase our knowledge of epigenetic gene control in the mouse, I have studied modifiers of epigenetic gene silencing.

My main method of investigation involved the characterisation of mutants from a sensitised ENU mutagenesis screen performed previously in our laboratory. The screen was carried out in an FVB/NJ strain carrying a variegating GFP transgene expressed in erythrocytes. To date we have recovered 12 dominant (D) and seven recessive (R) mutant mouse lines from this screen that display altered transgene expression. We have named these *Mommes* (Modifiers of murine metastable epialleles). I investigated the phenotype and attempted to identify the underlying causative mutation of two of these *Momme* mutants.

*MommeD6* is a semi-dominant, homozygous lethal mutation that acts as a suppressor of variegation with respect to the GFP transgene. This mutation has a large effect on the level of expression of the transgene in expressing cells, but little effect on the percentage of cells expressing the transgene. *MommeD6* is linked to a 2.5 Mbp interval on chromosome 14. *MommeD9* is a semi-dominant, homozygous lethal mutation that acts as an enhancer of variegation with respect to the GFP transgene. Mutants have a tendency to become obese as they age, show abnormal haematology profiles, and females develop infertility. *MommeD9* is linked to a 17.4 Mbp region on chromosome 7.

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I produced and studied a strain carrying the same GFP transgene but in a new strain background, C57BL/6J. This strain provided an opportunity to look for strain-specific modifiers of expression of the GFP transgene. Several regions were mapped to chromosomal locations. Further work will be needed to identify the genes involved. This mouse will be useful in future mutagenesis screens of this type.

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## List of abbreviations

129	129P4/RrRk
<i>a</i>	<i>agouti</i> (null allele)
<i>A</i>	<i>Agouti</i> (wildtype allele)
<i>A<sup>vy</sup></i>	<i>agouti viable yellow</i>
<i>Axin<sup>Fu</sup></i>	<i>axin fused</i>
bp	base pair
BSA	bovine serum albumin
C57	C57BL/6J
CpG	cytosine-phosphate-guanine sequence
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DEPC	diethyl pyrocarbonate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
Dnmt	DNA methyltransferase
dpc	days post coitum
dsRNA	double stranded RNA
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetic acid
ENU	N-ethyl-N-nitrosourea
F <sub>1</sub>	first filial generation
FVB	FVB/NJ
g	constant of gravitational acceleration
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GFP	green fluorescent protein
HAT	histone acetyltransferase
HDAC	histone deacetylase
HMTase	histone methyltransferase
HP1	heterochromatin protein 1
IAP	intracisternal A particle
ICR	imprinting control region

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LTR	long terminal repeat
Mel18	synonym for Pcgf2 (polycomb group ring finger 2)
miRNA	microRNA
<i>Momme</i>	<i>Modifier of murine metastable epiallele</i>
MOPS	3-[N-morphino]propanesulfonic acid
MQW	Milli-Q water
mRNA	messenger RNA
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEV	position effect variegation
PGC	primordial germ cell
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
rRNA	ribosomal RNA
SDS	sodium dodecyl sulfate (lauryl sulfate sodium salt)
SINE	short interspersed nuclear element
siRNA	short interfering RNA
snoRNA	small nucleolar RNA
SNP	single nucleotide polymorphism
SSC	sodium chloride, tri-sodium citrate buffer
TAE	tris, acetic acid, EDTA buffer
TBE	tris, boric acid, EDTA buffer
TE	tris, EDTA buffer
TRD	transmission ratio distortion
Tris	tris-hydroxymethyl-methylamine
tRNA	transfer RNA
UV	ultraviolet
v	volume
w	weight
<i>Xist</i>	inactive X specific transcripts
Xp	paternal X chromosome

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