A recessive genetic screen to discover components in miRNA pathways using *piggyBac*-mediated insertional mutagenesis in *Blm*-deficient mouse embryonic stem cells

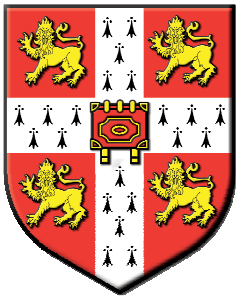
Meng Li

A dissertation submitted in partial fulfilment of the requirements for the award of the degree of

Doctor of Philosophy

of the

University of Cambridge



The Wellcome Trust Sanger Institute

Sidney Sussex College, Cambridge

Declarations

This thesis is submitted on partial fulfilment of the requirements for the degree of Doctor of Philosophy of the University of Cambridge. It describes the work carried out at the Wellcome Trust Sanger Institute from October 2006 to August 2010. Unless otherwise indicated, the research is my own and not the product of collaboration.

This thesis does not exceed the word limit of 60,000 as set by the Degree Committee for the Faculty of Biology.

Meng Li

25th August 2010

Acknowledgements

My foremost gratitude goes to Prof. Allan Bradley for being an inspirational mentor with his broad vision to science. For the past four years, he has provided me with guidance, support and patience, helping me through the difficulties and frustrations. I am aware of the fact that without his open-minded approach to science and his encouragement, I would not be able to have the freedom to pursue my own ideas and build up confidence in conducting independent research.

Many thanks to the Bradley group, current members and alumni, for being great people to work with, and always being there for help and advice. Special acknowledgement goes to Kosuke Yusa, for making his resources available to me and from whom I have learnt a great deal and had so many instructive discussions. Also thanks to Qi Liang, for introducing me to many basic techniques and providing me with much of the starting materials to start my projects. Thanks to Stephen Pettitt, Yue Huang and Wei Wang (Mr.), who are also working on *piggyBac* and *Blm*-deficient ES cell systems, for all the instructive discussions and sharing of resources. I am very grateful to Frances Law, Alastair Beasley and James Cooper for tissue culture support; Haydn Prosser and Hiroko Yusa for providing me with useful plasmids; Roland Rad and George Vassiliou for useful discussions and having laughs with. I would like also to thank many great people from other labs in the Sanger Institute; Daniel Turner and Sabine Eckert for their expertise in conducting Illumina sequencing of my samples, Zeming Ning for helping me with bioinformatics analysis and Tomas Fitzgerald for array CGH analysis.

Thanks to my thesis committee members Julie Ahringer, Bill Skarnes, and David Adams for their comments and advice throughout my PhD. Thanks too to Seth Grant, Jyoti Choudhary and Jürg Bähler for hosting me in their labs during my first PhD year rotations and to Mark Colin and Marcelo Coba for guiding me through the rotations.

Robert, you are always here for me and this work would not be as it is without your constant support. I look forward to our life together as husband and wife. Thank you for proof-reading of this thesis too.

Mum and Dad, thank you so much for all your love and support emotionally and financially in whatever I chose to do and always being there for me even you are thousands of miles away in China when I am faced with difficult decisions and feeling sad.

Thanks to my future in-laws, Andy and Julie Phipps for being so welcoming, generous and treating me as a true family member for the past six years.

Abstract

Genome-wide recessive genetic screens complement reverse genetic approaches to ascribe gene function in biological pathways of interest without prior knowledge. The ability to conduct such screens in cultured embryonic stem cells requires a tractable mutagenic system combined with a *Blm*-deficient background to efficiently generate and convert heterozygous mutations to homozygosity in parallel on a genome-wide scale.

The first part of this thesis describes the establishment of a novel mutagenic strategy based on remobilisation of a single copy *piggyBac* transposon targeted within the genome. This strategy has the significant advantage over conventional co-transfection methods for transposon delivery in their ease of mutant library construction while maintaining a single copy of the mutagen per cell for the subsequent establishment of the genotype-phenotype causality.

The second part of this thesis concerns the development of reporter systems to conduct genetic screens using the established mutant pools, for the identification of novel factors in the miRNA biogenesis pathways and dissecting possible differential regulators in the two branches of the miRNA downstream effector pathways, i.e. miRNA mediated mRNA decay and translational repression. To our knowledge, this is the first attempt to use non-hypothesis driven genetic approach to identify novel components in this pathway in mammals. Preliminary screening with one of the reporter system has revealed a homozygous mutant in a known effector in the miRNA-mediated repression, *Ago2*.

A final part of this thesis presents a separate part of the research during the PhD to advance the *piggyBac* transposon technology in large genomic DNA delivery. This work has demonstrated a giant cargo capacity of to up to 100 kb for *piggyBac* transposons. The integrations of giant *piggyBac* transposons are intact, they can be expressed stably and can be remobilised from the genome. Giant *piggyBac* transposons open new doors to many applications in basic mammalian genetics and gene therapy, which are not possible with existing methods.

Contents

Chapter 2 – Materials and methods

Abbreviations

°C	degrees centigrade
4-OHT	4-hydroxyltamoxifen
6-TG	6-thioguanine
Ago	Argonaute
BAC	bacterial artificial chromosome
Blm	Bloom syndrome gene
bp	base pair
bghpA	bovine growth hormone poly-adenylation signal
BrdU	5-bromo-2-deoxyuridine
Bsd	blasticidin resistant gene
CAG	the hybrid cytomegalovirus enhancer/chicken β actin promoter
CMV	cytomegalovirus promoter
CGH	comparative genomic hybridisation
DMSO	dimethylsulfoxide
ESCC	ES cell-specific cell cycle-regulating
FIAU	1-(2-deoxy-2-fluoro-1- D-arabinofuranosyl)-5-iodouracil
Huc	human ubiquitin C promoter
IRES	Internal ribosome entry site
LB	Luria-Bertani broth
LOH	loss of heterozygosity
miRNA	microRNA
MMR	mismatch repair
MOPS	3-morpholinopropane-1-sulfonic acid, $C_7H_{15}NO_4S$
Neo	neomycin resistant gene
nt	nucleotides
o/n	over night
PB	*piggyBac* transposon
PB5 or PB3	*piggyBac* 5’ (or 3’) inverted terminal repeat

PBase	*piggyBac* transposase
PB ITRs	*piggyBac* transposon inverted terminal repeats
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PGK	phosphoglycerate kinase
Pri-miRNA	Primary microRNA transcript
Pre-miRNA	Precusor microRNA transcript
Puro	puromycin resistant gene
PuroΔtk	fusion gene of puromycin *N*-acetyletransferase gene (puro) and truncated herpes simplex viral type 1 thymidine kinase gene (Δtk)
rbgpA	rabbit β-globin poly-adenylation signal
RISC	RNA-induced silencing complex
RNAi	RNA interference
r.p.m	revolutions per minute
RT-PCR	reverse transcripton- PCR
SCE	sister chromatid exchange
SDS	Sodium lauryl sulfate, $C_{12}H_{25}SO_4Na$
siRNA	small interfering RNA
TBS	Tris buffered saline

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of tables