RECESSIVE GENETIC SCREEN FOR MISMATCH REPAIR COMPONENTS IN *BLM*-DEFICIENT ES CELLS

A dissertation submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

by *Ge Guo*

The Wellcome Trust Sanger Institute
University of Cambridge

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DECLARATION

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specially indicated in the text. None of the material presented herein has been submitted previously for the purpose of obtaining another degree.

Ge Guo

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ABSTRACT

RECESSIVE GENETIC SCREEN FOR MISMATCH REPAIR COMPONENTS IN *BLM*-DEFICIENT ES CELLS

Phenotype-driven recessive genetic screens in diploid organisms require a strategy to render the mutation homozygous. Although homozygous mutant mice can be generated by breeding, a reliable method to make homozygous mutations in cultured cells has not been available, limiting recessive screens in culture. Cultured embryonic stem (ES) cells provide access to all of the genes required to elaborate the fundamental components and physiological systems of a mammalian cell, as well as genes involved in differentiation. It has been established that in *Blm*-deficient cells, homozygous daughter cells can be readily segregated from cells carrying heterozygous mutations, presumably through mitotic recombination between non-sister chromatids. In this study, I have exploited the high rate of mitotic recombination in *Blm*-deficient ES cells to generate a genome wide library of homozygous mutant cells from heterozygous mutations induced with a revertible gene trap retrovirus. This library is composed of nearly 10,000 individual gene trap clones. To further investigate the use of this library, a recessive genetic screen has been carried out to identify cells with defects in DNA mismatch repair (MMR) that exhibit resistance to 6-thioguanine. Multiple homozygous mutants in mismatch repair homologue 6 (*Msh6*) were recovered, providing confirmation of the effectiveness of this recessive genetic screen. *Dnmt1* was recovered as a novel MMR gene from this screen. It was verified that Dnmt1-deficient ES cells exhibit micro-satellite instability. *Dnmt1* mutant mice are predisposed to certain type of cancers. The finding that *Dnmt1* is a novel MMR gene provides new insights into the mechanistic role of *Dnmt1* in cancer. Importantly, the combination of insertional mutagenesis in *Blm*-deficient ES cells opens a new approach for phenotype based recessive genetic screens in ES cells.

LIST OF FIGURES

Figure 1-1. Approaches to generate homozygous mutations in ES cells --- 7-9
Figure 1-2. DNA mismatch repair in *E.coli* ---------- 21
Figure 1-3. DNA mismatch recognition in Eukaryotes ---------- 23
Figure 1-4. DNA mismatch repair in MNNG and 6TG cytotoxity ---------- 31
Figure 1-5. Schematic representation of basic gene trap strategies --------- 45-46
Figure 1-6. Recombinant retroviral vectors and viral production ---------- 52
Figure 1-7. Principle of self inactivating (SIN) retroviral vectors ---------- 54
Figure 2-1. Splinkerette PCR ---------- 73
Figure 3-1. Cytotoxicity of 6TG in mouse ES cell lines ---------- 84
Figure 3-2. Genomic instability in *Blm*-deficient ES cells ---------- 88
Figure 3-3. Gene-targeted *Blm* alleles ---------- 89
Figure 3-4. Generation of *Hprt*$^{+/+}$ *Blm* deficient ES cells ---------- 92-93
Figure 3-5. EMS mutagenesis and 6TG screens ---------- 97
Figure 4-1. Revertible gene trap virus ---------- 104
Figure 4-2. Schematic the construction of 3'LTR for revertible retroviral vectors ---------- 107-109
Figure 4-3. Retroviral gene trap vectors ---------- 110
Figure 4-4. Construction of a revertible retroviral vector driven by CMV Promoter /enhancer ---------- 111
Figure 4-5. Viral titer by pBaORNeo, pBaERNeo and four individual pCBaORNeo vectors ---------- 114
Figure 4-6. Schematic of retroviral gene trap vectors and viral titres -------- 115
Figure 4-7. Southern-blot analysis of the integrated provirus from RGTV-1 117
Figure 5-1. Screening strategy for 6TG resistant (6TG^{R}) gene trap mutants ---------- 124
Figure 5-2. Approaches to identify gene trap mutations ---------- 125-126
Figure 5-3. Identification of *Msh6* gene trap mutations ---------- 128

Figure 5-4. Molecular analysis of Msh6 mutations ---------- 130
Figure 5-5. Quantitative Southern-blot analysis (QTSouthern) of STA gene trap mutants ---------- 134
Figure 5-6. Southern-blot analysis of gene trap clone STA1.2 135
Figure 5-7. QTSouthern analysis of gene trap mutants obtained in the STB screen ---------- 138
Figure 5-8. Southern-blot analysis of *Dnmt1* gene trap mutations ---------- 141
Figure 5-9. Molecular analysis of gene trap *Dnmt1* mutants ---------- 142
Figure 5-10. Southern-blot analysis of *Tgif* gene trap mutants ---------- 145
Figure 5-11. Cre reversal assay of *Tgif* gene trap mutation ---------- 146
Figure 5-12. RT-PCR analysis of *Tgif* expression ---------- 147
Figure 5-13. Characterization of a translocated *Parp-2/Rbpsuh* locus ------ 150-151
Figure 5-14. Characterization of the gene trap mutation in STC4-F11------- 154-155
Figure 5-15. Southern analysis of *Msh6* locus in gene trap clones ---------- 161
Figure 5-16. Southern analysis of *Mlh1* locus in gene trap clones ---------- 162
Figure 5-17. Southern analysis of *MSh2* locus in genetrap clones ---------- 163
Figure 5-18. Southern analysis of *Dnmt1* locus in gene-trap clones --------- 164
Figure 5-19. 5'RACE amplification of the splicing junction ---------- 167
Figure 5-20. Proviral/host junctions identified by SpPCR ---------- 168
Figure 6-1. Construction of the slippage vector, P-Slip ---------- 176
Figure 6-2. Gene-targeting of P-Slip cassette into *ROSA26* locus ---------- 178
Figure 6-3. Generation *Dnmt1* knockout cells by gene-targeting ---------- 181
Figure 6-4. 5' portion of *Dnmt1* coding sequence ---------- 183
Figure 6-5. Expression analysis of Dnmt1 ---------- 184
Figure 6-6. Determination of DNA methylation ---------- 186

LIST OF TABLES

Table 1-1. Homologues of bacterial MMR genes in S.cerevisiae and Mammals ---------- 22
Table 3-1. Survival rates of cells in 6-TG ---------- 95
Table 3-2. EMS mutated NGG cells and 6TG screens ---------- 98
Table 5-1. Gene trap mutation in STA clones ---------- 132
Table 5-2a.Gene mutated in Bi-allelic gene trap STB clones ---------- 139
Table 5-2b.STB gene trap clones ---------- 140
Table 5-3. STC gene trap clones ---------- 158-160
Table 6-1. Mutation rate of P-Slip in gene trap mutants ---------- 180
Table 6-2. Mutation rate of P-Slip in Dnmt1- deficient cells ---------- 187
Table 6-3. Sequence analysis of P-Slip mutations ---------- 190
Table 6-4. Homologous recombination with Rb targeting vectors -------- 192

TABLE OF CONTENT

DECLARATION II
ACKNOWLEDGEMENT III
ABSTRACT IV
LIST OF FIGURES V
LIST OF TABLES VII
TABLE OF CONTENT VIII
1.1 The mouse as a genetic model 1
1.2 Mouse ES cells as a genetic tool 2
1.3 Approaches to generate homozygous mutations in ES cells 3
1.3.1 Sequential gene-targeting 3
1.3.2 High concentration G418 selection 3
1.3.3 Induced mitotic recombination 5
1.3.4 Elevated mitotic recombination in *BLM*-deficient cells 6
1.3.4.1 Bloom's syndrome 10
1.3.4.2 *BLM*, Bloom's syndrome gene 10
1.3.4.3 Enzyme activity, an untypical DNA helicase 11
1.3.4.4 BLM in DNA replication 12
1.3.4.5 A model for sister-chromatid exchange caused by *BLM*-deficiency 13
1.3.4.6 Proteins interacting with BLM 14
1.3.4.7 Mouse models of Bloom's syndrome 16
1.3.4.8 Elevated LOH rate in *Blm*-deficient mouse ES cells 17
1.4 Recessive genetic screens in mammalian cells 17
1.5 DNA mismatch repair 18
1.5.1 DNA mismatch repair in bacteria 19
1.5.2 DNA mismatch repair in eukaryotes 20
1.5.3 MMR in homologous recombination 25
1.5.4 DNA mismatch repair in meiosis 27
1.5.5 MMR in DNA damage surveillance 28
1.5.5.1 MMR deficiency causes DNA methylation damage tolerance 28
1.5.5.2 Two models for the function of MMR in DNA damage surveillance 29

1.5.5.3 MMR deficiency causes tolerance to 6-thioguanine (6TG) 32
1.5.5.4 Molecular basis of MMR in DNA damage surveillance 32
1.5.5.5 MED1/MBD4, a methyl-CpG binding protein involved in DNA damage surveillance 33
1.5.6 A genetic screen for genes that protect the *C. elegans* genome against mutations 35
1.5.7 MMR deficiency in Cancer 36
1.6 Mutagenesis in mice and embryonic stem cells 37
1.6.1 Forward genetics, phenotype-based screens 37
1.6.2 Reverse genetics, transgenic animals and gene-targeting 40
1.6.3 Insertional mutagenesis, the gene trap approach 42
1.6.3.1 Gene trap methods 42
1.6.3.2 Gene trap mutagenesis in genetic screens 47
1.6.3.2.1 Expression screens 47
1.6.3.2.2 Gene trap in phenotype-driven screens 49
1.6.3.3 Methods for introducing gene trap vectors into cells 50
1.6.3.3.1 Electroporation 50
1.6.3.3.2 Retroviral based gene transfer 50
1.6.3.4 Gene trap “hot spots” 56
1.7 Thesis project 57
2.1 Vectors 59
2.1.1 The Slippage construct, P-Slip 59
2.1.2 Gene-targeting vectors 60
2.1.3 Retroviral vectors 61
2.2 Cell culture 63
2.2.1 ES cell culture 63
2.2.2 Chemicals used for selection in ES cells 63
2.2.3 Transfection of DNA into ES cells by electroporation 64
2.2.4 Rb-targeting using Isogenic and non-isogenic gene targeting vectors 64
2.2.5 Gene-targeting of *ROSA26/Slip-TV1* construct 65
2.2.6 Cre-mediated recombination 65

2.2.7 Clonal survival assay 65
2.2.8 EMS mutagenesis in ES cells 66
2.2.9 Retroviral approaches 66
2.2.9.1 Producing retrovirus by transient transfection 66
2.2.9.2 Viral Infection 67
2.2.9.3 Determination of the transfection efficiency 67
2.2.9.4 Titration of the retrovirus 67
2.2.10 Gene trap mutagenesis and 6TG resistance screen 68
2.2.10.1 Gene trap mutants by RGTV-1 retrovirus on NGG5-3 cells. 68
2.2.10.2 Screen for 6TG resistant gene trap mutants 68
2.2.11 Fluctuation analysis of the MSI rate of P-Slip. 69
2.3 DNA methods 69
2.3.1 Probes 69
2.3.1.1 General probes 69
2.3.1.2 PCR amplified genomic DNA probe 70
2.3.1.3 cDNA probe 71
2.3.2 Southern blotting and hybridization 71
2.3.3 Isolation of proviral/host junction by Splinkerette PCR 74
2.3.4 Cre-*loxP* mediated reversal assay of integrated retrovirus 76
2.3.5 Quantitative Southern analysis (QTSouthern) 76
2.3.6 Sequencing analysis of the recovered P-Slip cassette 77
2.3.7 Determination of the CpG methylation pattern 77
2.4 RNA methods 78
2.4.1 RT-PCR 78
2.4.2 RT-PCR primers 78
2.4.3 Northern blotting and hybridation 79
2.4.4 Isolation of the 5’ end of gene trap transcripts by 5’RACE 80
3.1 Introduction 83
3.1.1 Screen for 6TG resistance mutants 83
3.1.2 MMR system and 6TG tolerance in ES cells 83
3.1.3 6TG resistance caused by deficiency in *Hprt* gene 85

3.1.4 EMS mutagenesis in ES cells 86
3.1.5 *Blm* deficient ES cells in 6TG resistance screen 87
3.2 Results 90
3.2.1 Construction of the *Blm* deficient ES cells carrying two copies of an *Hprt* minigene 90
3.2.2 NGG cells are 6TG sensitive 94
3.2.3 Screening for 6TG resistant mutants by EMS mutagenesis 96
3.2.3.1 EMS treatment of *Blm* deficent NGG cells 96
3.2.3.2 Screen for 6TG resistant mutants at low cell density 96
3.2.3.3 Screen for 6TG resistance cells at high cell density 99
3.3 Discussion 99
3.3.1 Construction of NGG (*Blm*$^{tm3/tm4}$, *Gdf-9* $^{tm1/tm3}$) cells 99
3.3.2 Screen for 6TG resistant mutants 100
4.1 Introduction 102
4.1.1 Gene trap mutagenesis, *SAβgeo* gene trap cassette 102
4.1.2 A revertible retroviral gene trap vector design 102
4.2 Results 105
4.2.1 Construction of revertible retroviral backbones 105
4.2.2 Efficiency of retroviral vectors for transferring autonomous genes 106
4.2.3 Revertible retroviral gene trap vectors 112
4.2.4 The function of *loxP* sites in integrated provirus 113
4.2.5 The structure of integrated proviruses 116
4.3 Discussion 116
4.3.1 Construction of recombinant retroviral vectors 116
4.3.2 Factors that affect retroviral vector efficiency 118
4.3.3 Revertible gene trap vector, a useful tool for genetic screen in *Blm* deficient ES cells 119
5.1 Introduction 121
5.1.1 Screen strategy 121
5.1.2 Approaches to identify gene trap mutations 121
5.2 Results 127

5.2.1 Gene trap mutant library (GT library) on *Blm*-deficient ES cells..... 127
5.2.2 STA screen..... 127
5.2.2.1 *Msh6*, a most frequently identified STA clones..... 127
5.2.2.2 Expression of *Msh6* is reduced in gene trap mutants..... 129
5.2.2.3 Cre-mediated reversal of gene trap mutations..... 131
5.2.2.3 The copy number of gene trap insertions..... 133
5.2.3 STB screen..... 136
5.2.3.1 *Dnmt1* gene trap mutant..... 136
5.2.3.2 *Tgif* gene trap mutant..... 143
5.2.3.3 Identification of a complex locus, Parp-2/Rbpsuh..... 148
5.2.4 STC screen..... 152
5.2.5 Single allelic or non-revertible gene trap mutants..... 156
5.3 Discussion..... 165
5.3.1 Summary..... 165
5.3.2 High throughput analysis of gene trap mutations..... 165
5.3.3 LOH efficiency on different genomic locus..... 169
5.3.4 Genomic coverage of gene trap mutagenesis..... 170
6.1 Introduction..... 171
6.1.1 Increased spontaneous mutation in MMR deficient cells..... 171
6.1.2 Microsatellite instability as a hallmark of MMR deficiency..... 171
6.1.3 Determination of mutation rate by Luria-Delbruck fluctuation analysis 172
6.1.4 DNA mismatch repair deficiency leads to increased homologous recombination between diverged sequences..... 173
6.2 Results..... 174
6.2.1 Construction of the P-Slip slippage cassette..... 174
6.2.2 Gene-targeting P-Slip in *ROSA26* locus..... 177
6.2.3 Determination of MSI rate of the targeted P-Slip cassette in gene trap mutants..... 179
6.2.4 Generation and characterization of Dnmt1-deficient ES cells by gene-targeting..... 179
6.2.4.1 Expression analysis of Dnmt1..... 182

6.2.4.2 Functional analysis of Dnmt1 activity 185
6.2.5 Dnmt1 deficiency in the Dnmt1 knockout cells causes increased MSI 188
6.2.6 The puromycin resistant ES cells carry a single copy of the targeted P-Slip cassette. 188
6.2.7 Mutated CA repeat in Puromycin resistant ES cells..... 189
6.2.8 Dnmt1 does not block homeologous recombination 191
6.3 Discussion 193
6.3.1 Summary 193
6.3.2 MSI in *Dnmt-1* deficient cells is not a result of changes in MMR gene expression 194
6.3.3 DNA replication, repair and methylation are coordinated processes .. 195
6.3.4 Evidence of links between DNA methylation and DNA mismatch repair 196
6.3.5 Blm affects DNA mismatch repair 199
Part I: A system for recessive screens 201
7.1 *Blm*-deficient cells..... 201
7.2 Gene trap mutagenesis 202
7.3 Broad applications of the Blm-deficiency in recessive screens..... 202
7.4 Transponson-mediated mutagenesis..... 203
7.5 Combination of deletional mutations with *Blm*-deficiency 205
7.6 RNA interference (RNAi), a new era for mutagenesis 206
Part II: DNA methylation and mismatch repair surveillance..... 209
7.7 *Dnmt1*, a MMR surveillance gene..... 209
References..... 212