IDENTIFICATION OF NOVEL MODIFIERS OF CHROMOSOME INHERITANCE

USING A GENETICALLY SENSITISED DROSOPHILA MODEL

Thesis submitted by

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STATEMENT OF THE CONTRIBUTION OF OTHERS

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ABSTRACT

Loss of chromosome cohesion has been implicated as a significant contributor to chromosome missegregation and aneuploidy. A central and essential figure mediating chromosome cohesion is the cohesin complex. This multi-subunit protein is conserved in all eukaryotes studied and related proteins are also present in bacteria. Reduction of cohesin subunits and cohesin regulators has been conclusively demonstrated to perturb normal chromosome cohesion and segregation. Cohesin not only has a central role in maintaining cohesion between sister-chromatids between S phase and the onset of anaphase in both mitosis and meiosis. The cohesin complex is also essential in the formation and maintenance of chiasmata between homologous chromosomes during meiosis.

The study presented in this thesis aimed to identify regulators of chromosome cohesion and segregation by employing a genetic screening approach using a modified cohesin subunit (*Rad21*^{NC}) that perturbs chromosome segregation by impeding chromosome separation at anaphase. At the commencement of this study it was clear that much of the molecular network underlying chromosome segregation remained to be determined and that metazoan species, such as *Drosophila*, could provide unique insights into these processes. The aim of this study was to identify novel regulators of chromosome cohesion and segregation. To achieve this, a chromosome missegregation model was used that employed a non-cleavable form of the cohesin component *Rad21* to impede chromosome segregation. When ectopically expressed in the replicating cells of the developing *Drosophila* eye this cleavage-resistant variant produced a reduced and disorganised eye phenotype. The underlying cellular phenotype demonstrated increased levels of tetraploidy, aneuploidy, lagging chromosomes and fragments of broken chromosomes. This eye phenotype proved modifiable by second site heterozygous mutations in genes encoding known regulators of cohesin and chromosome segregation, such as *NippedB*, *Separase* and *Cyclin B*. Following this characterisation the chromosome missegregation phenotype was utilised as a screening tool to identify regulators of chromosome segregation capable of modifying chromosome cohesion. The genetic screen performed as part of this study identified 133 candidate loci that were able to modify the GMR>*Rad21*^{NC} chromosome missegregation model and thereby have

been linked to chromosome segregation. The screen was carried out using mutant alleles of loci that fell within the breakpoints of deletions previously identified as GMR>*Rad21*^{NC} modifiers. The number of modifying loci is greater than originally anticipated, however, this may reflect the molecular complexity of chromosome segregation, the inter-connectedness of cellular pathways and the multi-functional nature of proteins.

The presence of RAD21^{NC} cohesin complexes on chromosomes has a detrimental effect on the cell following the onset of anaphase. The direct effects of the expression of *Rad21*^{NC} on chromosome segregation were visualised using neuroblast chromosomes, with a significant number of tetraploid and aneuploid cells observed, as well as lagging and broken chromosomes. Impeding chromosome segregation during *Drosophila* eye development was shown to cause significant levels of cell death and altered cell cycle progression, producing a disorganised (rough) and reduced adult eye. This GMR>*Rad21*^{NC} rough-eye phenotype was employed to perform a genome-wide screen for second site modifier loci capable of either suppressing or enhancing the RAD21^{NC} eye phenotype. The genetic screen identified 133 individual modifier loci as candidate chromosome cohesion and segregation regulators. In-depth analyses of published studies revealed that many of the loci identified had established links to cohesin, chromosome segregation, mitosis and meiosis, indicating that the screen had successfully identified regulators of chromosome cohesion and segregation. However, several of these loci had not previously been implicated in chromosome cohesion and segregation and are very interesting for further investigation as novel regulators of these processes.

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LIST OF ABBREVIATIONS

aa	Amino acid
BL	US *Drosophila* stock centre at Bloomington, Indiana
bp	Base Pair
BSA	Bovine serum albumin
CDK	Cyclin-dependent kinase
cDNA	DNA that has been synthesized from a messenger RNA template
CO_2	Carbon dioxide
Df	Deficiency
ddH2O	Double-distilled water
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
ECL	Enhanced chemi-luminescence
EDTA	Ethylenediaminetetraacetic acid
G_0	Parental generation
G_1	First generation
G_2	Second generation
GMR>	GMR-Gal4 induced transgene expression
kb	Kilobase
kDa	Kilodalton
LB	Luria Bertani media
MI	Meiosis I – first meiotic division
MII	Meiosis II – second meiotic division
MF	Morphogenetic furrow
mRNA	Messenger ribonucleic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ND	Non-disjunction
PBS	Phosphate buffered saline
PBT	Phosphate buffered saline with 0.1% Tween-20
PCR	Polymerase chain reaction
PD	Precocious division (of chromosomes)
RNA	Ribonucleic acid
rpm	Revolutions per minute
SAC	Spindle assembly checkpoint
SAP	Shrimp alkaline phosphatase
SB	Squishing buffer
SDS	Sodium dodecylsulphate
SMC	Structural maintenance of chromosomes
TAE	Tris-acetate-EDTA
Tris-Cl	Tris-chloride
TE	Tris-EDTA
UAS	Upstream activation sequence