DNA REPAIR AND THE FANCONI ANEMIA PATHWAY:
INSIGHTS INTO FEMALE MEIOSIS AND MITOSIS

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BSc (Hons I)

PH.D THESIS
Declaration

This thesis contains no material which has been accepted for the award of any other Degree or Diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made to the text. I give consent to the final version of my thesis being made available worldwide when deposited into the University’s Digital Repository, subject to the provision of the Copyright Act 1968.

I hereby certify that the work embodies in this thesis contains a published paper of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication (Appendix I)

Wai Shan Yuen

26th October 2012
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I would like to sincerely thank my supervisors, Keith and Julie, for giving me this opportunity. I am grateful and honoured for their guidance and wisdom that they have shown me throughout this project.

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I am eternally grateful to my mum and my brother, who have shown me so much love, patience, and understanding throughout these years. For without them, I am nothing.

To Kaini, my best bud, it’s always better when we’re together. I miss the fun and crazy times with you. Thanks for being there for me, always. To Korey and all my friends, here and back home, thanks for your love and your neverending support. To Timbre, Zee and Bear for all of your love and company.

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Abstract

There are numerous intrinsic and extrinsic factors that cause DNA damage. Without proper DNA repair, such damage would cause genomic instability, premature aging and cancer. The Fanconi Anemia (FA) pathway is important for the repair and resolution of interstrand crosslinks. The key events of this pathway are the ubiquitination of a FA protein, FANCD2, and its localisation onto sites of DNA damage as nuclear foci. Of interest, Gametogenetin (GGN) has been found previously to interact directly with the FA protein responsible for this ubiquitination. In this thesis, using siRNA knockdown, I examined first the role of GGN1 in HeLa cell growth and survival. The phenotypic similarities of GGN1 depleted cells and FA-deficient fibroblasts led me to investigate the role of GGN1 in the FA pathway. It was found that GGN1 was important for the localisation but not the ubiquitination of FANCD2. In addition, an automated method for FANCD2 foci quantification and analysis was developed.

In comparison to mitotic cells, oocytes spend the majority of their life arrested in prophase I and this would make them vulnerable to DNA damage. This could in turn lead to female infertility and embryo abnormalities. However, there is little known about the DNA repair capacity of oocytes to respond to such damages. It was discovered, in this thesis, that oocytes have a high tolerance for interstrand crosslinks (ICLs) as compared to double stranded breaks. In combination with the expression of FA transcripts and proteins, this suggested that oocytes might have an active ICL repair. It was determined in this thesis that although oocytes had the ability to detect DNA damage, the FA pathway was found to be inactive during meiosis and only initiated upon embryo formation. The data presented in this thesis also suggest that FANCD2 might have a separate role in meiosis.
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<tr>
<td>8-oxoG</td>
<td>8-oxo-7,8-dihydroguanine</td>
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<tr>
<td>9-1-1 complex</td>
<td>Rad9-Rad1-Hus1 Complex</td>
</tr>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ATM</td>
<td>Ataxia Telangiectasia Mutated</td>
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<tr>
<td>ATR</td>
<td>ATM and Rad3-related</td>
</tr>
<tr>
<td>ATRIP</td>
<td>ATR Interacting Protein</td>
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<tr>
<td>BLM</td>
<td>Bloom Syndrome Protein</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>CCD</td>
<td>Cytochalasin D</td>
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<tr>
<td>Cdc</td>
<td>Cell Division Cycle</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>Chk</td>
<td>Checkpoint Kinase</td>
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<tr>
<td>COC</td>
<td>Cumulus Oocyte Complex</td>
</tr>
<tr>
<td>CRISP2</td>
<td>Cysteine-Rich Secretory Protein 2</td>
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<tr>
<td>D2-L</td>
<td>Monoubiquitinated FANCD2</td>
</tr>
<tr>
<td>D2-S</td>
<td>Non-ubiquitinated FANCD2</td>
</tr>
<tr>
<td>ddh2o</td>
<td>Double Distilled Water</td>
</tr>
<tr>
<td>DDR</td>
<td>DNA Damage Response</td>
</tr>
<tr>
<td>dHJ</td>
<td>Double Holliday Junction</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle Media</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulphoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleicacid</td>
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<tr>
<td>DNA-PK</td>
<td>DNA-dependent Protein Kinase</td>
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<tr>
<td>DSB</td>
<td>Double Stranded Break</td>
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<tr>
<td>E</td>
<td>Embryonic Day</td>
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<tr>
<td>FA</td>
<td>Fanconi Anemia</td>
</tr>
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<td>Fetal Calf Serum</td>
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<tr>
<td>G</td>
<td>Guanine</td>
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<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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GFP          Green Fluorescent Protein
GGN          Gametogenetin
GGNBP        Gametogenetin Binding Protein
GV           Germinal Vesicle
GVBD         Germinal Vesicle Breakdown
H2AX         Histone 2A Variant Member X
HA           Human Influenza Haemagglutinin
HCG          Human Chorionic Gonadotrophin
HEK293       Human Embryonic Kidney Cells
HeLa         Human Cervical Adenocarcinoma Cells
Hep-2        Human Laryngeal Epithelial Cells
HR           Homologous Recombination
ICL          Interstrand Crosslinks
ID complex   FANCD2-FANCI Complex
IR           Ionising Radiation
IU           International Units
IVM          In Vitro Maturation
kDa          Kilodalton
Mad2         Mitotic Arrest Deficient 2
MDC1         Mediator of DNA Damage Checkpoint Protein 1
MII          Metaphase II
MMC          Mitomycin C
MRN Complex  MRE11-Rad50-Nbs1 Complex
mRNA         Messenger RNA
NCS          Neocarzinostatin
NER          Nucleotide Excision repair
NES          Nuclear Export Sequence
NLS          Nuclear Localisation Sequence
NOXA         Phorbol-12-myristate-13-acetate-induced Protein 1
OAZ3         Ornithine Decarboxylase Antizyme 3
PB1/2        Polar Body Extrusion
PBS          Phosphate Buffered Saline
PCNA         Proliferating Cell Nuclear Antigen
PGC          Primordial Germ Cells
PMSG         Pregnant Mare Serum Gonadotrophin
PN           Pronucleus
<table>
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<td>POG</td>
<td>Proliferation of Germ Cells</td>
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<td>DNA Polymerase v</td>
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<tr>
<td>Pro-MI</td>
<td>Prometaphase I</td>
</tr>
<tr>
<td>PUMA</td>
<td>p53 Upregulated Modulator of Apoptosis</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
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<tr>
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<td>Quantitative Polymerase Chain Reaction</td>
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<td>Recombination Activating Gene</td>
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<td>Reactive Oxygen Species</td>
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<td>Reverse Transcription PCR</td>
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<td>Spindle Assembly Complex</td>
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