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Characterisation of novel Histone H3 and RAD9 mutants defective in the DNA damage response in *Saccharomyces cerevisiae*

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A thesis submitted to the National University of Ireland, Galway for the degree of Doctor of Philosophy

Supervisor: Professor Noel F. Lowndes
Co-supervisor: Doctor Muriel Grenon

February 2013
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# Abbreviations

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<th>Full Form</th>
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<tbody>
<tr>
<td>ATM</td>
<td>Ataxia telangiectasia mutated</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast and ovarian cancer type-1 susceptibility gene</td>
</tr>
<tr>
<td>BRCT</td>
<td>BRCA1 carboxyl terminal</td>
</tr>
<tr>
<td>BER</td>
<td>Base excision repair</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum album</td>
</tr>
<tr>
<td>CAD</td>
<td>Chk1 activation domain</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin-dependent kinase</td>
</tr>
<tr>
<td>ChIP</td>
<td>Chromatin Immunoprecipitation</td>
</tr>
<tr>
<td>CPT</td>
<td>Camptotheclin</td>
</tr>
<tr>
<td>DNA-PKcs</td>
<td>DNA-dependent protein kinase catalytic subunit</td>
</tr>
<tr>
<td>DDR</td>
<td>DNA damage response</td>
</tr>
<tr>
<td>DSB</td>
<td>Double-strand break</td>
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<tr>
<td>FACS</td>
<td>Fluorescence Activated Cell Sorting</td>
</tr>
<tr>
<td>G1</td>
<td>Gap phase 1</td>
</tr>
<tr>
<td>G2</td>
<td>Gap phase 2</td>
</tr>
<tr>
<td>HAT</td>
<td>Histone Acetyltransferase</td>
</tr>
<tr>
<td>γ-H2A</td>
<td>Phosphorylation of histone H2A on serine 129</td>
</tr>
<tr>
<td>HHT2</td>
<td>Histone H3 Copy-II</td>
</tr>
<tr>
<td>HHF2</td>
<td>Histone H4 Copy-II</td>
</tr>
<tr>
<td>HR</td>
<td>Homologous recombination</td>
</tr>
<tr>
<td>HU</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>IR</td>
<td>Ionizing radiation</td>
</tr>
<tr>
<td>M</td>
<td>Mitosis</td>
</tr>
<tr>
<td>MDC1</td>
<td>Mediator of DNA damage checkpoint 1</td>
</tr>
<tr>
<td>MMS</td>
<td>Methylmethane sulphonate</td>
</tr>
<tr>
<td>MMR</td>
<td>Mismatch Repair</td>
</tr>
<tr>
<td>MRX</td>
<td>Mre11-Rad50-Xrs2</td>
</tr>
<tr>
<td>NER</td>
<td>Nucleotide excision repair</td>
</tr>
<tr>
<td>NHEJ</td>
<td>Non-homologous end joining</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PIKK</td>
<td>Phosphoinositide 3-kinase related kinase</td>
</tr>
<tr>
<td>PTM</td>
<td>Post-translational modification</td>
</tr>
<tr>
<td>RCAF</td>
<td>Replication-Coupling assembly factor</td>
</tr>
<tr>
<td>RFC</td>
<td>Replication factor C</td>
</tr>
<tr>
<td>RPA</td>
<td>Replication protein A</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Single stranded DNA</td>
</tr>
<tr>
<td>SCD</td>
<td>SQ/TQ cluster domain</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>TopI</td>
<td>Topoisomerase I</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris-buffered saline</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>YFP</td>
<td>Yellow Fluorescent Protein</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-Type</td>
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DECLARATION

I, Mary Walsh declare that the work presented in the thesis has been acquired and analysed by myself with the exception of:

Chapter 2:
- Figure 2.2A and 2.2B. Histone mutant screen and drop test analysis was performed by Thomas Costelloe.

Chapter 3:
- Figure 3.5 was performed by Thomas Costelloe.

Chapter 4:
- Figure 4.1 was performed by Noel Lowndes
- Figure 4.2 was performed by Jean Soulier
- Figure 4.3 was performed by Aisling O’Shaughnessy
- Figure 4.4 was performed in part by Aisling O’Shaughnessy
- Figure 4.5A was performed by Aisling O’Shaughnessy and Figure 4.5B by Achille Pellicioli
The DNA damage response (DDR) is a signal transduction cascade, which regulates cell cycle progression, gene transcription and DNA repair. Histones also play a vital role in the activation of the DDR and have been shown to be necessary for checkpoint protein interactions with chromatin, including Rad9, the first checkpoint protein discovered. Here, we study the role of these two types of proteins in the DDR.

In a screen to identify DNA damage sensitive histone mutants, specific residues of histone H3 were found to be involved in resistance to Camptotecin (CPT). We investigated further the role of two of these residues. We found that serine 28, which is highly conserved and phosphorylated in higher cells, is neither phosphorylated nor necessary to the DDR. However, when glutamate residue 50 was mutated to glycine, we observed defective growth phenotype, a slower S phase progression and sensitivity to a range of S phase damaging agents. H3E50 is located in the H3 αN helix, a region known to be important for cell viability and nucleosome stability. We observed that h3E50G and h3E50A cells have a strong checkpoint recovery defect along with the prolonged presence of Rad52 foci in agreement with chromatin structure being key to DSB repair in S phase.

We also studied mutations in Rad9 that induce a strong defect in checkpoint recovery from the G2/M checkpoint. These mutations affect two of the 20 possible CDK phosphorylation sites on Rad9 and are important for cell cycle phosphorylation. The Rad9 protein contains 20 putative Cdk phosphorylation sites. We have identified two of these sites important for recovery of cells from the G2/M checkpoint. Our results suggest that rad9S494A+S618A might be defective for specific Rad9 function including non homologous end joining, the other pathway of choice for DNA double-strand break repair.

Taken together, this work provides new leads to study the connection between chromatin structure and the cellular response to DNA damage.