

XPC DNA REPAIR PROTEIN REGULATION IN THE CONTEXT OF  
THE G1/S CELL CYCLE CHECKPOINT

Tabitha M. Hardy

Submitted to the faculty of the University Graduate School  
in partial fulfillment of the requirements  
for the degree  
Doctor of Philosophy  
in the Department of Microbiology and Immunology,  
Indiana University

August 2010

Accepted by the Faculty of Indiana University, in partial  
fulfillment of the requirements for the degree of Doctor of Philosophy.

---

Martin L. Smith, Ph.D. -Chair

---

Wilbert A. Derbigny, Ph.D.

Doctoral Committee

---

Brittney-Shea Herbert, Ph.D.

June 16, 2010

---

Ann Roman, Ph.D.

For Mom: Thank God for you, the wind beneath my wings.

In memory of Perry L. Hardy (Dad):

“You raise me up, so I can stand on mountains,

You raise me up, to walk on stormy seas

I am strong when I am on your shoulders,

You raise me up, to more than I can be.”

“You Raise Me Up” –by Josh Groban

## ACKNOWLEDGEMENTS

I would like to thank my research mentor and committee chair, Dr. Martin L. Smith, for his guidance, direction, and patience throughout this process. Thank you so much for taking me in and for training me at a time when I wasn't sure if I could finish. I would also like to thank you for your encouragement and for saying things like "you're going to get out, if it kills us all!" I am also extremely grateful to Dr. MA Suresh Kumar for his constant support and for helping me in various capacities throughout this process. Additionally, I would also like to thank my advisory committee, Drs. Derbigny, Herbert, and Roman for standing by me, lending their support, advice, suggestions, and often a listening ear.

I would like to thank the Department of Microbiology and Immunology, especially Dr. Hal Broxmeyer for his support, direction, and work with the Bridges to the Doctorate and Harper's Scholars programs. I would also like to thank the incredible Micro office and support staff: Cindy, Janis, Audrey, LaSonya, Cathy, Linda, Tricia, Tasha, Laverne and Ms. Cookie for their love and encouragement and for all of the smiles and laughs along the way.

I would also like to thank the IU School of Medicine Graduate Division, the IUSM Office of Multicultural Affairs, the IUPUI Graduate Office, and the IUPUI Office of Diversity, Equity, and Inclusion for helping me in various capacities.

Finally, I want to acknowledge my friends and family. Thank you all so much for your incredible love and support throughout this process, for always listening through the ups and the downs, for thoughts, prayers, and tissues, for talking me down from the ledge many days...I am truly grateful. To my Pasadena Heights family thank you for truly

becoming family, for providing my spiritual home away from home, and for your love and support. To my ISC family thank you for providing my outlet and for allowing me to be free and just sing. To my UPnGO family thank you for allowing me to serve you and know that your labor is not in vain. To my biological family, where would I be without you! Your continued love, care, and support even when you didn't quite know what I was doing or how long it would take, have made all the difference. You mean the world to me! Lastly, I would like to give honor to God with whom all things are possible.

## **ABSTRACT**

Tabitha M. Hardy

### **XPC DNA REPAIR PROTEIN REGULATION IN THE CONTEXT OF THE G1/S CELL CYCLE CHECKPOINT**

DNA is subject to various types of damage that can impair cellular function or cause cell death. DNA damage blocks normal cellular processes such as replication and transcription and can have catastrophic consequences for the cell and for the organism. It has long been thought that the G1/S cell cycle checkpoint allows time for DNA repair by delaying S-phase entry. The p53 tumor suppressor pathway regulates the G1/S checkpoint by regulating the cyclin-dependent kinase inhibitor p21Waf1/Cip1, but p53 also regulates the nucleotide excision DNA repair protein XPC. Here, using p53-null cell lines we show that additional mechanisms stabilize XPC protein and promote nucleotide excision repair (NER) in concert with the G1/S checkpoint. At least one mechanism to stabilize and destabilize XPC involves ubiquitin-mediated degradation of XPC, as the ubiquitin ligase inhibitor MG-132 blocked XPC degradation. The retinoblastoma protein RB, in its unphosphorylated form actually stabilized XPC and promoted NER as measured by host-cell reactivation experiments. The data suggest that XPC protein and XPC-mediated NER is tightly linked to the G1/S checkpoint even in cells lacking functional p53.

Martin L. Smith, Ph.D. - Chair

## TABLE OF CONTENTS

<b>LIST OF TABLES</b> .....	ix
<b>LIST OF FIGURES</b> .....	x
<b>LIST OF ABBREVIATIONS</b> .....	xi
<b>INTRODUCTION</b> .....	1
DNA damage and types of repair.....	1
Nucleotide Excision Repair .....	1
XPC.....	5
Regulation of XPC.....	8
Regulation of XPC by ubiquitination .....	9
DNA repair and the cell cycle.....	10
Cell cycle checkpoints and DNA Repair .....	11
Regulation of the cell cycle and XPC by p53 .....	13
RB and DNA repair .....	15
<b>MATERIALS AND METHODS</b> .....	16
Cell lines .....	16
Cell transfection plasmids.....	16
Immunoblotting.....	18
Western blot antibodies.....	19
Recombinant proteins .....	20
Immunoprecipitation.....	20
Host Cell Reactivation .....	21

CAT ELISA .....	23
Pulse Chase Analysis .....	23
Statistical Analysis.....	24
<b>RESULTS .....</b>	<b>25</b>
XPC protein expression and DNA repair activity in the presence of RB.....	25
RB activation of XPC does not require ongoing protein synthesis .....	29
XPC and RB immunoprecipitation .....	30
RB deletion mutants.....	33
XPC and G1/S cyclins .....	33
XPC ubiquitination .....	42
XPC and MDM2.....	44
<b>DISCUSSION .....</b>	<b>47</b>
<b>FUTURE DIRECTIONS.....</b>	<b>54</b>
The XPC complex.....	54
NER co-factors.....	54
Chemotherapeutic implications .....	55
<b>REFERENCES.....</b>	<b>57</b>
<b>CURRICULUM VITAE</b>	

## LIST OF TABLES

Table 1. Plasmids used in H1299 and Saos cell transfection experiments .....	17
Table 2. Western blot antibodies .....	19
Table 3. Recombinant proteins .....	20

## LIST OF FIGURES

Figure 1.	Basic mechanism for nucleotide excision DNA repair .....	4
Figure 2.	XPC is rate-limiting and required for NER DNA repair .....	6
Figure 3.	Diagram of the G1/S checkpoint .....	14
Figure 4.	Diagram of host cell reactivation assay .....	22
Figure 5.	RB enhances XPC-mediated HCR of a cisplatin-damaged reporter gene .....	27
Figure 6.	RB stabilizes XPC .....	31
Figure 7.	Putative interaction between RB and XPC.....	35
Figure 8.	Structure of RB and Rb deletion mutants.....	37
Figure 9.	RB mutants fail to stabilize XPC .....	38
Figure 10.	Cyclin E counteracts RB stabilization of XPC.....	39
Figure 11.	Ubiquitin ligase inhibitor MG-132 rescues XPC .....	41
Figure 12.	Cyclin E may act as a signal for the ubiquitination of XPC.....	43
Figure 13.	MDM2 may be involved in the negative regulation of XPC.....	45
Figure 14.	Schematic of a possible mechanism of XPC regulation.....	50

## LIST OF ABBREVIATIONS

DNA	deoxyribonucleic acid
BER	base excision repair
MMR	mismatch repair
NER	nucleotide excision repair
CPD	cyclobutane pyrimidine dimmers
UV	ultraviolet radiation
TC-NER	transcription coupled nucleotide excision repair
G-NER	global genomic nucleotide excision repair
CS	Cockayne's Syndrome
RNA	ribonucleic acid
XP	xeroderma pigmentosum
NSCLC	nonsmall cell lung carcinoma
WT	wild type
DDB	DNA damage binding protein
Cul4a	cullin 4a
RB	retinoblastoma
CDK	cyclin-dependent kinase
MDM	murine double minute
SDS	sodium dodecyl sulfate
NCI	national cancer institute
HCR	host cell reactivation

CAT	chloroamphenicol acetyltransferase
ELISA	enzyme-linked immunosorbent assay
CMV	cytomegalovirus
CHX	cycloheximide
DN	dominant negative
shRNA	short-hairpin ribonucleic acid
ANOVA	analysis of variance