

REGULATION OF WILD-TYPE AND MUTANT P53 THROUGH
DNA-MEDIATED CHARGE TRANSPORT

Thesis by
Wendy Mercer Geil

In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology

Pasadena, California

2012

(Defended September 29, 2011)

© 2012

Wendy Mercer Geil

All Rights Reserved

Acknowledgements

I would like to thank my adviser, Professor Jackie Barton, for giving me this project and for the support and encouragement she has given over the years. Also, I thank my committee, Doug Rees, Shu-ou Shan, and Jack Beauchamp for pointing me in the right direction, giving me advice, and brainstorming on various aspects of my project.

Many thanks go out to Maureen Renta for keeping the lab together and encouraging me when things were rough.

I thank Kate Augustyn, Nicolai K. Andersen, and Rebecca Chen (SURF) for p53 camaraderie. Katie Schaefer deserves appreciation for a fresh look at the problem and many hours of brainstorming and experiments. Many thanks go to Eddie Merino, for teaching me how to approach experiments in an organized and meaningful way.

I especially thank Amie Boal and Joey Genereux for teaching me how to write for a scientific audience. I have appreciated the insights that Paul Lee, Marisa Buzzeo, Russ Ernst, John Phillips, Eric Olmon, and Brian Zeglis have given me over the past 6 years. Many thanks go out to Susan Shadle for being the best undergraduate mentor ever. I thank Paul Nelson and the Caltech Toastmasters for help with public speaking.

To my friends Katy Muzikar, Christine Romano, Anna Maltsev, Jenn Stockdill, Pam Sontz, Cindy and Joey Genereux, Melanie Yen, Katie Brenner, Stephanie Johnson, Natalie Muren, and Andy and Lesley Monroe: thank you so much friendship and support throughout grad school. This would not have happened without you all.

Most of all, I thank my parents, Tom and Janice Mercer, sister LeeAnn Mercer, and husband Ethan Geil for endless support, boundless encouragement, and many, many phone calls. I love you, more than words could ever express.

ABSTRACT

The global transcription factor p53 controls many cellular processes, including the cellular response to oxidative stress. It had been determined that that dissociation of wild type p53 from its promoter site can occur upon DNA-mediated oxidation. In this work, we use site-directed mutagenesis to construct charge-deficient mutants of p53; the chemistry of DNA-mediated oxidation of p53 was examined using these mutants.

The control point for p53 oxidation through DNA-mediated charge transport (DNA CT) is cysteine 275. Using differential thiol labeling and detection of modified peptides with mass spectrometry, we demonstrated that cysteines 124 and 141 in superstable p53 form a terminal disulfide bond upon DNA-mediated oxidation. This leads to a conformational change that inhibits DNA from binding by p53. The disulfide formed between cysteines 124 and 141 is a result of a series of disulfide bond exchange across the protein from the DNA base stack.

We also investigated the dependence of p53 oxidation on DNA sequences. ESMA analysis of biologically derived p53 recognition sequences with varying quantities of guanine doublets and triplets showed efficient p53 oxidation to depend on the presence of low energy GG or GGG sites. Moreover, consistent results were found with biologically derived promoter sequences. Sequence S100A2, with guanine triplets on the same strand, showed the most oxidation of p53, followed by ODC1 and caspase-1. We confirmed these sequence-specific effects by measuring the change in expression level of the genes after induction of DNA CT *in vivo*. S100A2 mRNA levels decreased after photooxidant and light treatment, reflecting the oxidation and dissociation of p53 from the S100A2

site. However, caspase-1 and ODC1 mRNA levels remained the same, indicating less DNA-mediated p53 oxidation.

The results from this study illustrate how protein oxidation at a distance through DNA CT contributes to cellular signaling. This oxidative signaling can control how p53 regulates gene expression under oxidative stress, and this signaling may be disrupted in cancerous cells.

TABLE OF CONTENTS

List of Figures and Tables	viii
Chapter 1: DNA-Mediated Charge Transport in a Biological Context	1
Chapter 2: Oxidation of p53 through DNA-Mediated Charge Transport: Dependence on p53 Sequence	17
Chapter 3: Biological Effects of DNA Sequence on DNA-Mediated p53 Regulation	54
Chapter 4: Conclusions	84
Appendix 1: Protein Overexpression and Purification Protocol for p53	88
Appendix 2: Sequencing Results for p53 Mutants	91

LIST OF FIGURES AND TABLES

Chapter 1

Figure 1.1 DNA structure	5
Figure 1.2. Representation of oxidative DNA CT	6
Figure 1.3. Pathways regulated by p53	8
Figure 1.4. Representation of the p53 crystal structure	12

Chapter 2

Figure 2.1. Model of DNA CT oxidation leading to p53 disulfide bond formation and/or protein-DNA cross-linking	23
Figure 2.2. DNA sequences used in this study	24
Table 2.1. K_d 's for mutants bound to the Gadd45 recognition element	30
Figure 2.3. a) example of data from an EMSA with C141S p53 b) data compiled from gel shift experiments with the mutants	31
Figure 2.4. Example of a SDS-PAGE gel assay	33
Figure 2.5. Results of the SDS-PAGE analysis on the cross-linking effects of DNA CT on various p53 mutants	34
Figure 2.6. Flow chart of MS protocol	37
Figure 2.7. Cysteine MS spectra for an irradiated sample of DNA and p53SS....	38
Table 2.2. Oxidation state of cysteine residues in p53	39
Figure 2.8. Structural environment of cysteine residues that could be involved in disulfide bond formation	43
Table 2.3. Distance between cysteine residues	44

Chapter 3

Figure 3.1. DNA sequences used in this experiment	62
Figure 3.2. EMSA example	67
Figure 3.3. Gel shift assay results for naturally derived sequences	68
Figure 3.4. Synthetic vs. natural sequence results for the oxidation of p53	69
Figure 3.5. Procedure for induction of DNA CT inside of cells for RT-qPCR analysis	70
Figure 3.6. RT-qPCR results from conditions leading to p53 oxidation <i>in vivo</i>	71
Figure 3.7. RT-qPCR results from other conditions leading to p53 oxidation <i>in vivo</i>	72