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

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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

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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

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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