Mechanisms of DNA Repair by Photolyase and Excision Nuclease

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Outline

Photolyase

Thymine Dimer (T<>T)

Nucleotide Excision Repair

E. coli   Human

3'    3'    T<>T

5'    5'    

Cryptochrome

WT

Cry1/ 

Cry2/

Cry1/ 

Cry2/
Photoreactivation (DNA Repair)


Rupert and Sancar, UT Dallas, 2009

![Diagram of thymine dimer repair](image)

**Thymine Dimer**

**PL + Blue**

**T-T**

**T<>T**

**UV**
Cloning and Purification of Photolyase

Electron micrograph of the plasmid containing *Phr*

Sancar A (1977) PhD Dissertation, UT Dallas

Purified photolyase protein has bright blue color

Photolyase Contains Two Cofactors

FAD (catalyst)  
Folate (solar panel)

Structure of Photolyase

Reaction Mechanism of Photolyase

Ultrafast Kinetics of Photolyase

Outline

Thymine Dimer (T<>T)

Photolyase

Nucleotide Excision Repair

Cryptochrome

E. coli  Human

3’ 3’

T<>T

5’ 5’
Model for UV Repair Circa 1982

- Thymine dimers are removed from the genome in both *E. coli* and humans.

- Excised thymine dimers were reported to exist in oligonucleotides 4-6 nt in length.

- Excision is genetically controlled by *Uvr* genes in *E. coli* and *XP* genes in humans.

- Following excision, the repair gap is filled in and ligated.

- Excised dimers remain within the cell.
Identification of the *E. coli* Excision Repair Proteins by the Maxicell Method

Purification of *E. coli* Excision Repair Proteins

Dual Incisions in \textit{E. coli} Excision Repair

3' incision

\text{T<>T}

5' incision

Excision

12-mer

Resynthesis

Sancar A and Rupp WD (1983) \textit{Cell} 33:249-60
Sancar A (1994) \textit{Science} 266:1954-56
Mechanism of Excision Repair in *E. coli*

Mechanism of Transcription Coupled Repair

Outline

Thymine Dimer (T<>T)
Model for UV Repair Circa 1982

- Thymine dimers are removed from the genome in both *E. coli* and humans.

- Excised thymine dimers were reported to exist in oligonucleotides 4-6 nt in length.

- Excision is genetically controlled by *Uvr* genes in *E. coli* and *XP* genes in humans.

- Following excision, the repair gap is filled in and ligated.

- Excised dimers remain within the cell.

- +UV

- 5' Endonuclease
  - *(E. coli UvrA,B,C)*
  - *(Human XPA-XPG)*

- Resynthesis

- 5'-3' Exonuclease
  - *4-6-mer*
Xeroderma Pigmentosum

Patients lacking excision repair XP proteins (XPA-XPG) have 5,000 higher incidence of skin cancer

Human Excision Repair Factors

Dual Incisions in human Excision Repair

3’ incision

T<>T

5’ incision

Excision

30-mer

Resynthesis

Mechanism of Excision Repair in Humans

Mapping the Excised Oligomer in Humans

Lysate from UV-irradiated cells
- IP with $\alpha$-TFI IH
- Excised Oligonucleotides
- $\sim$24-32 nt
- Ligation

5' 26 bp __________ 21 bp
NNNNNN

5' NNNNN

5' NNNNN

IP with $\alpha$-CPD or $\alpha$-(6-4)PP

Repair by photolyase

PCR and gel purification

Ds DNA library with barcodes

Next Generation Sequencing (NGS)

Excision Repair Map of the Human Genome

Excision Repair of \textit{p53} at Single Nucleotide Resolution

Chr17:

- RNA Repair
  - +
  - -

Repair at the \textit{p53} gene:

- RNA Repair
  - +
  - -

- \textit{p53}
  - 5'-AGCTGTTCCGTCGCCAGTAGA 
  - TTACCA
  - 7,577,150 7,577,151

Cancer-linked Mutation

Excision Repair

- Nucleotide excision repair is initiated by dual incisions in both *E. coli* and humans.

- Excision is genetically controlled by the evolutionarily unrelated *Uvr* genes in *E. coli* and *XP* genes in humans.

- Dual incisions remove an oligomer of ~12 nucleotides in *E. coli* and ~30 nucleotides in humans.

- Following excision, the repair gap is filled in and ligated.

- By capturing the excised oligomers, we have generated an excision repair map of the whole human genome.
Outline

Thymine Dimer (T<>T)

Photolyase

Nucleotide Excision Repair

Cryptochrome

E. coli  Human

3' 3'  T<>T

5' 5'
• Humans do not have photolyase
• Humans have a photolyase homolog
• Humans have 2 photolyase paralogs

E. coli Photolyase

Human Cryptochrome 1

Human Cryptochrome 2

Photolyase Homology Domain

Photolyase

Cryptochrome

Jetlag, Cryptochrome, and the Circadian Clock

- **Spring 1996**: Traveled to Turkey to visit family and on my return flight read the AA Inflight Magazine article by William Schwartz, “Internal Timekeeping” about jetlag and the circadian clock.

- **May - June 1996**: Determined that human CRYs are not repair proteins.

- **June - August 1996**: Discovered genetic evidence that human CRYs are clock proteins.

- **May - November 1998**: Wrote the human CRY paper claiming CRYs are circadian proteins.
Clock and Circadian Clock

- Clock is a Time Keeping Object/System
  - Mechanic
  - Electronic
  - Molecular (Circadian Clock)

- Circadian Clock is an innate timekeeping molecular mechanism that maintains daily rhythmicity in biochemical, physiological and behavioral functions independent of external input.
Cryptochrome is Essential for the Circadian Clock

Mammalian Clock Genes/Proteins (1996-2000)

1) CRYPTOCHROME (Flavoprotein)

2) PERIOD (PAS domain)

3) CLOCK (bHLH-PAS)

4) BMAL1 (bHLH-PAS)
Circadian Control Mechanism

Circadian Control of Excision Repair

Summary

**Photolyase**

**Cryptochrome**

**Nucleotide Excision Repair**

E. coli  Human

3' → 3' → T<>T

5' → 5'
# Acknowledgments

## Sancar Lab Members

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Acknowledgments

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![NIH Logo](image)

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