

Mechanisms of DNA Repair by Photolyase and Excision Nuclease

**Nobel Lecture in Chemistry
Stockholms Universitet**

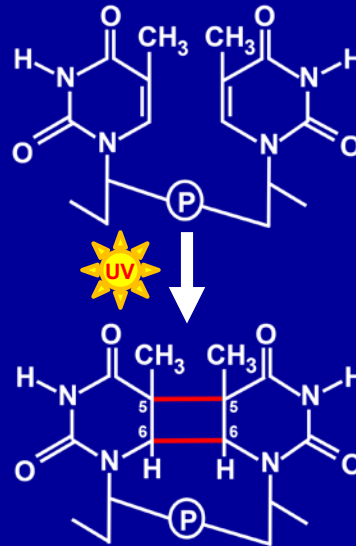
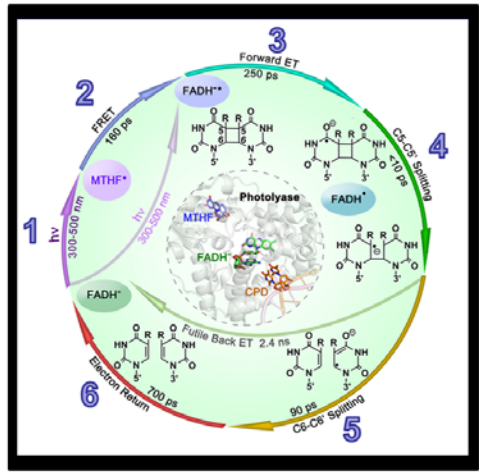
December 8, 2015

Aziz Sancar

**Department of Biochemistry and Biophysics
University of North Carolina School of Medicine
Chapel Hill, North Carolina**

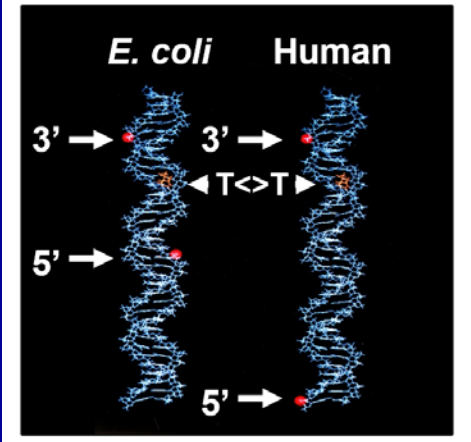
Outline

Photolyase

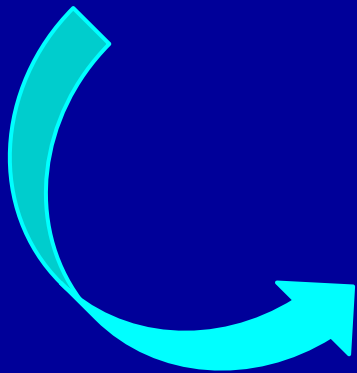
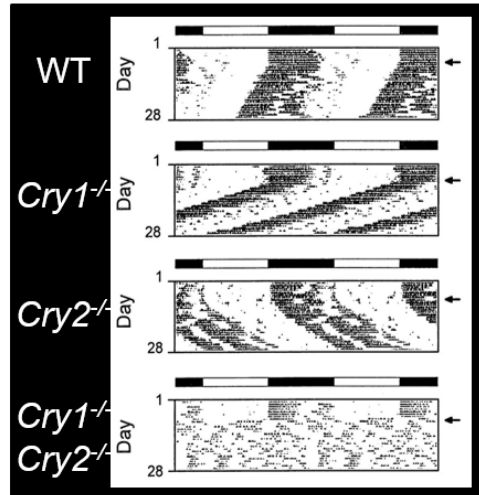


Thymine Dimer (T<=>T)

Nucleotide Excision Repair

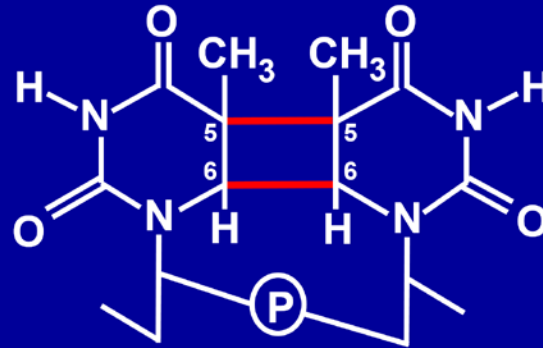
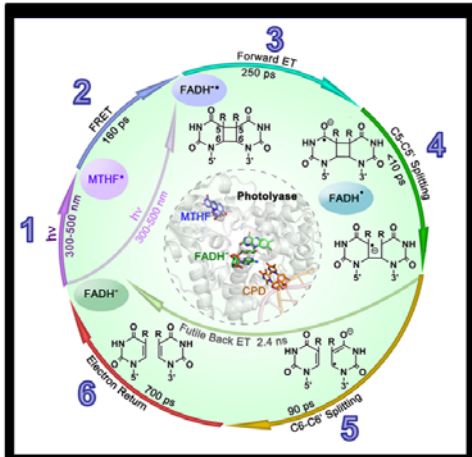


Cryptochrome



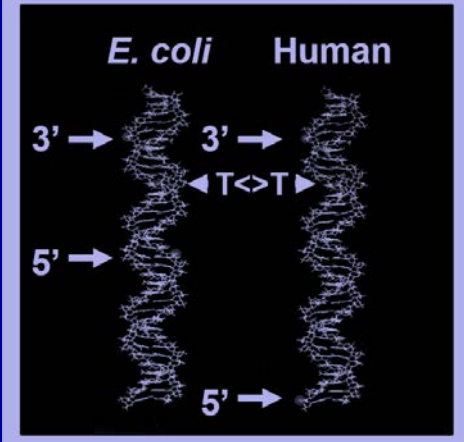
Outline

Photolyase

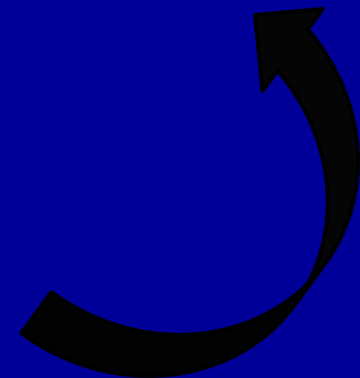
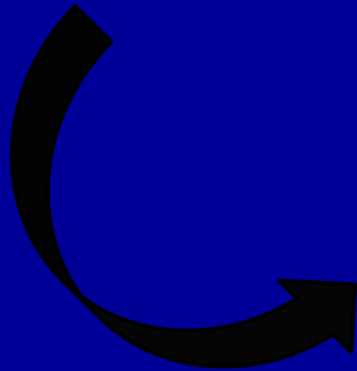
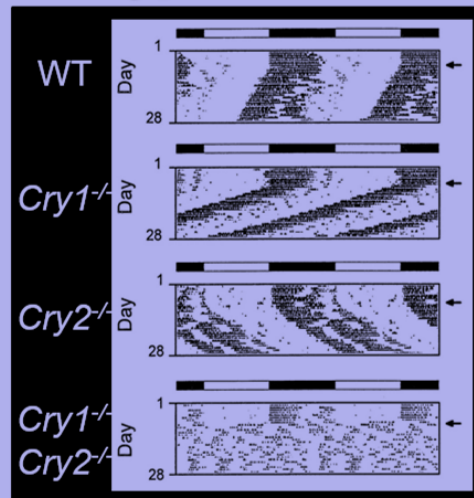


Thymine Dimer (T<T>)

Nucleotide Excision Repair



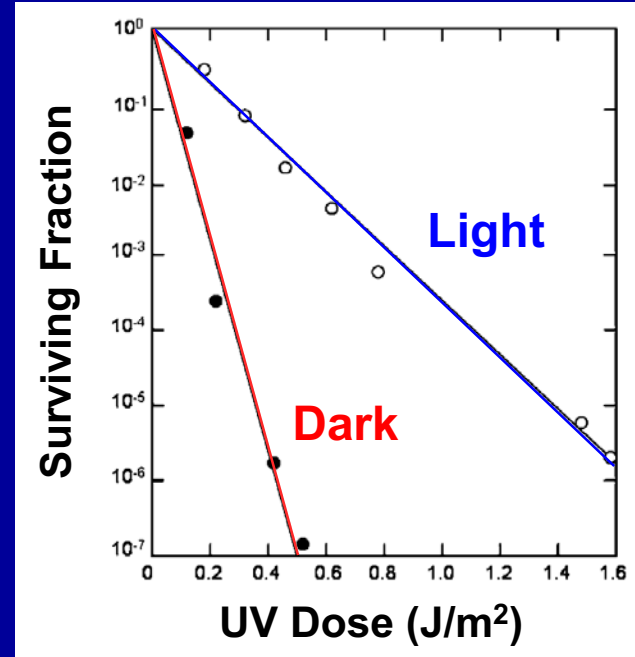
Cryptochrome



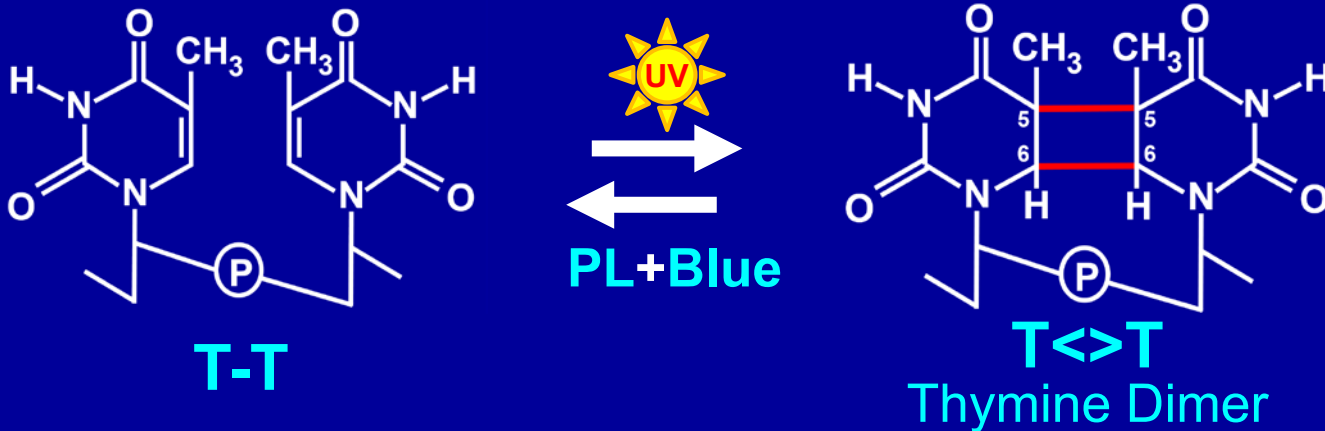
Photoreactivation (DNA Repair)



Rupert and Sancar, UT Dallas, 2009



Sancar A and Rupert CS (1978) *Gene* 4:295-308



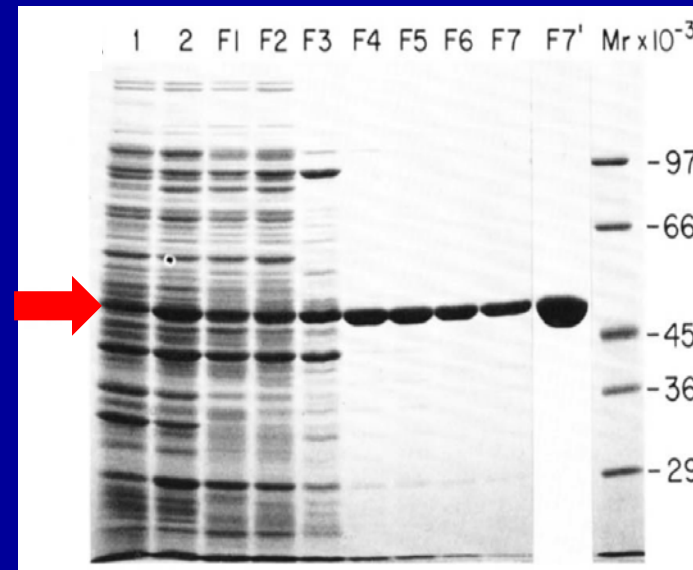
Cloning and Purification of Photolyase

Electron micrograph of the plasmid containing *Phr*



Sancar A (1977) PhD Dissertation, UT Dallas

Purified photolyase protein has bright blue color



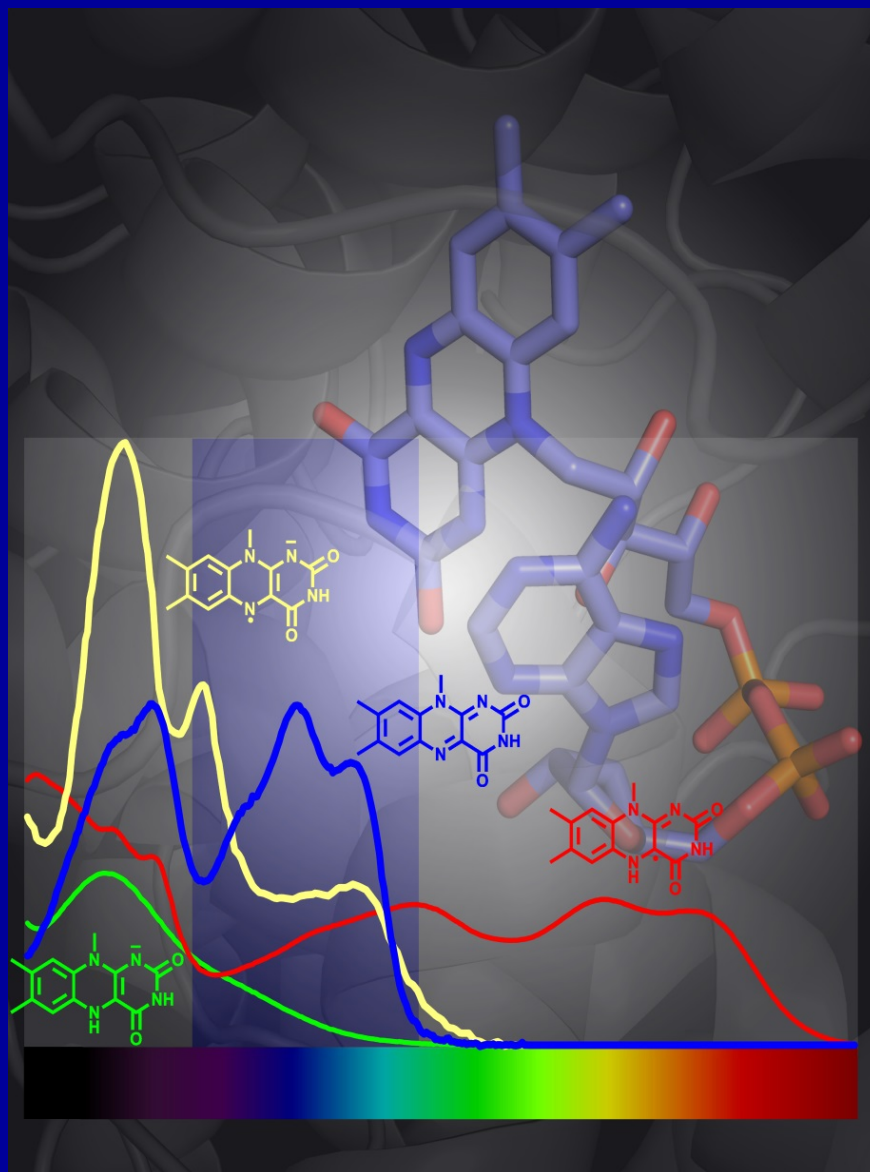
Sancar A, et al (1984) *JBC* 259:6028-32

Sancar A and Sancar GB (1984) *JMB* 172:223-7

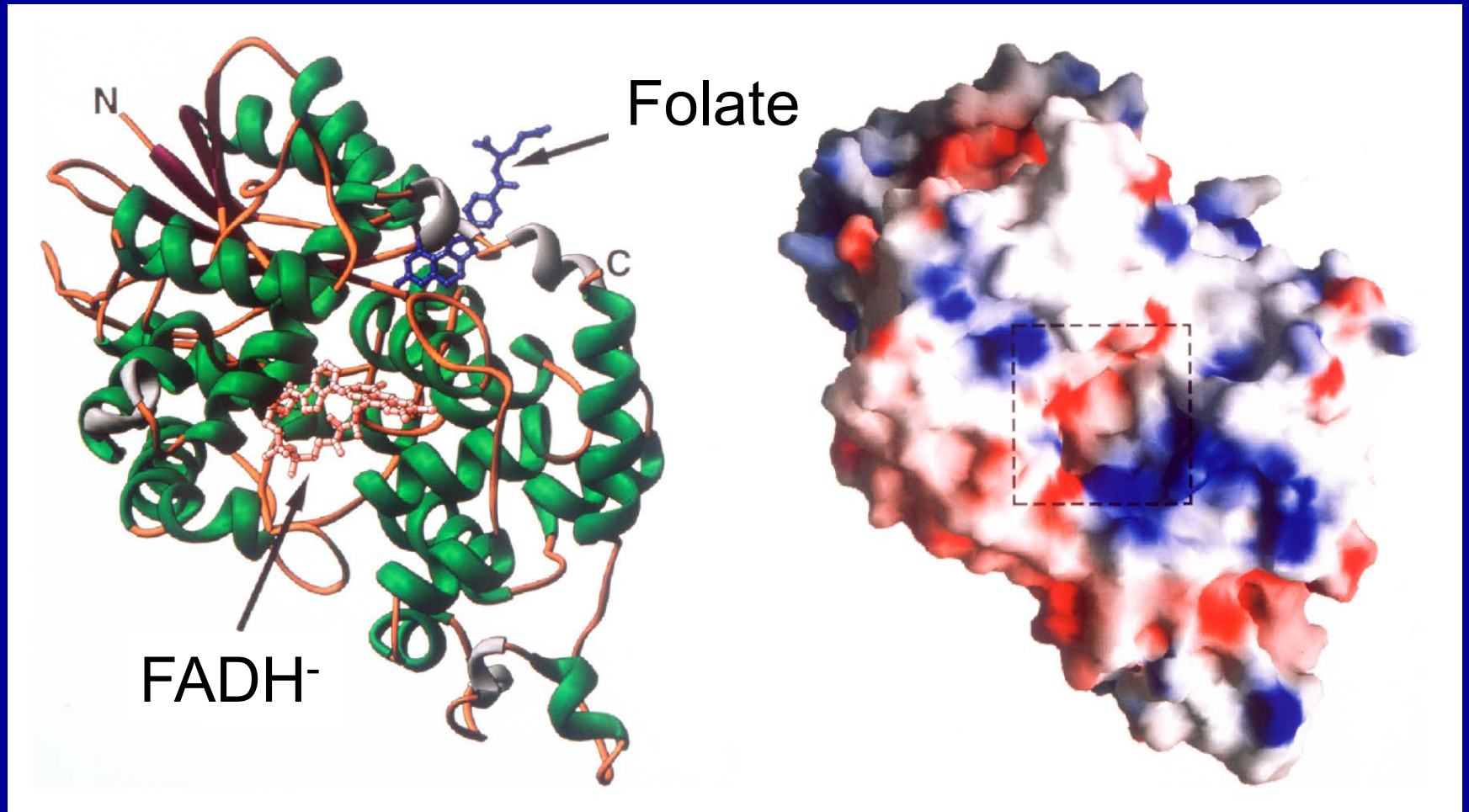
Photolyase Contains Two Cofactors

FAD
(catalyst)

Folate
(solar panel)

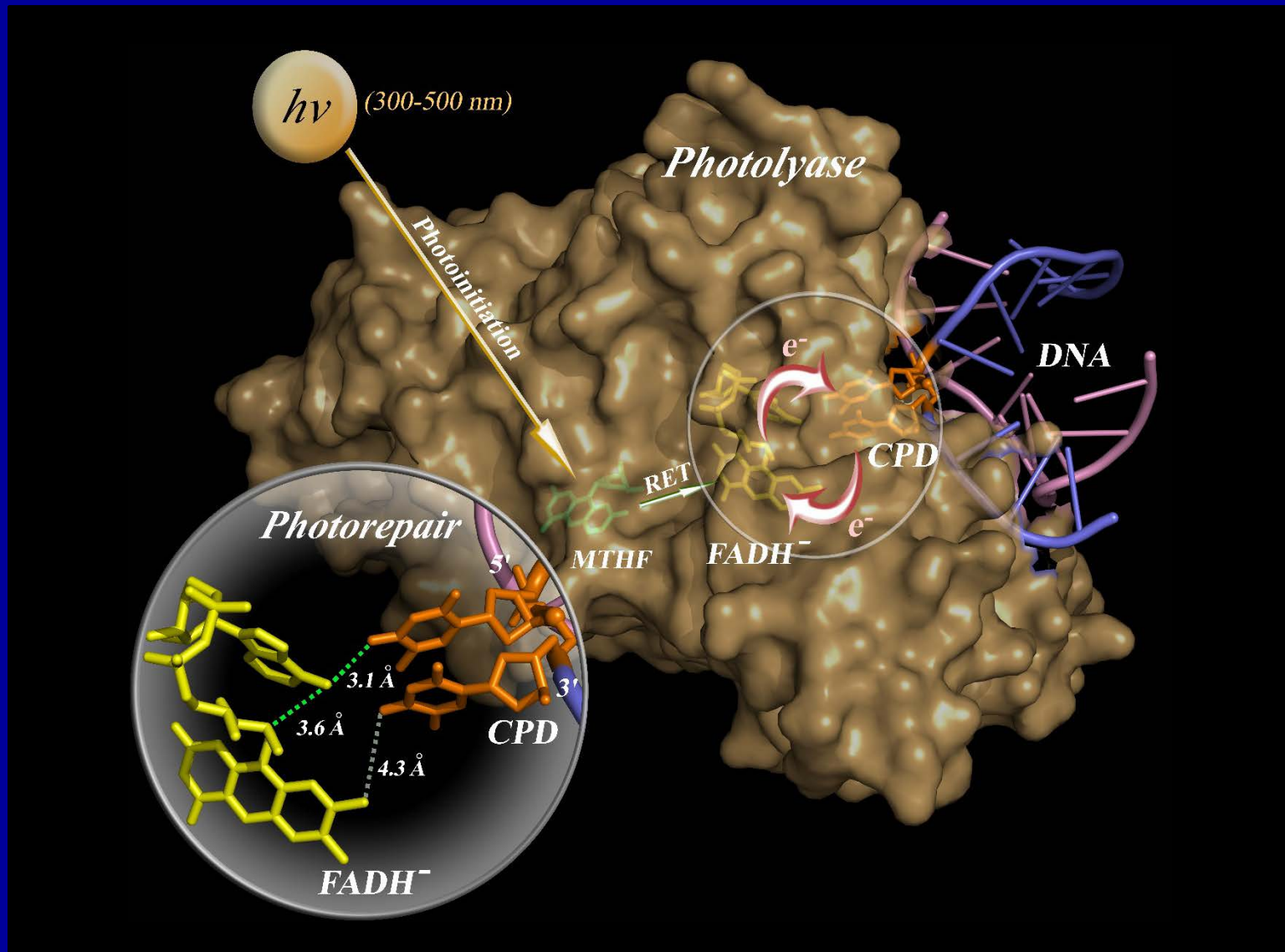


Structure of Photolyase



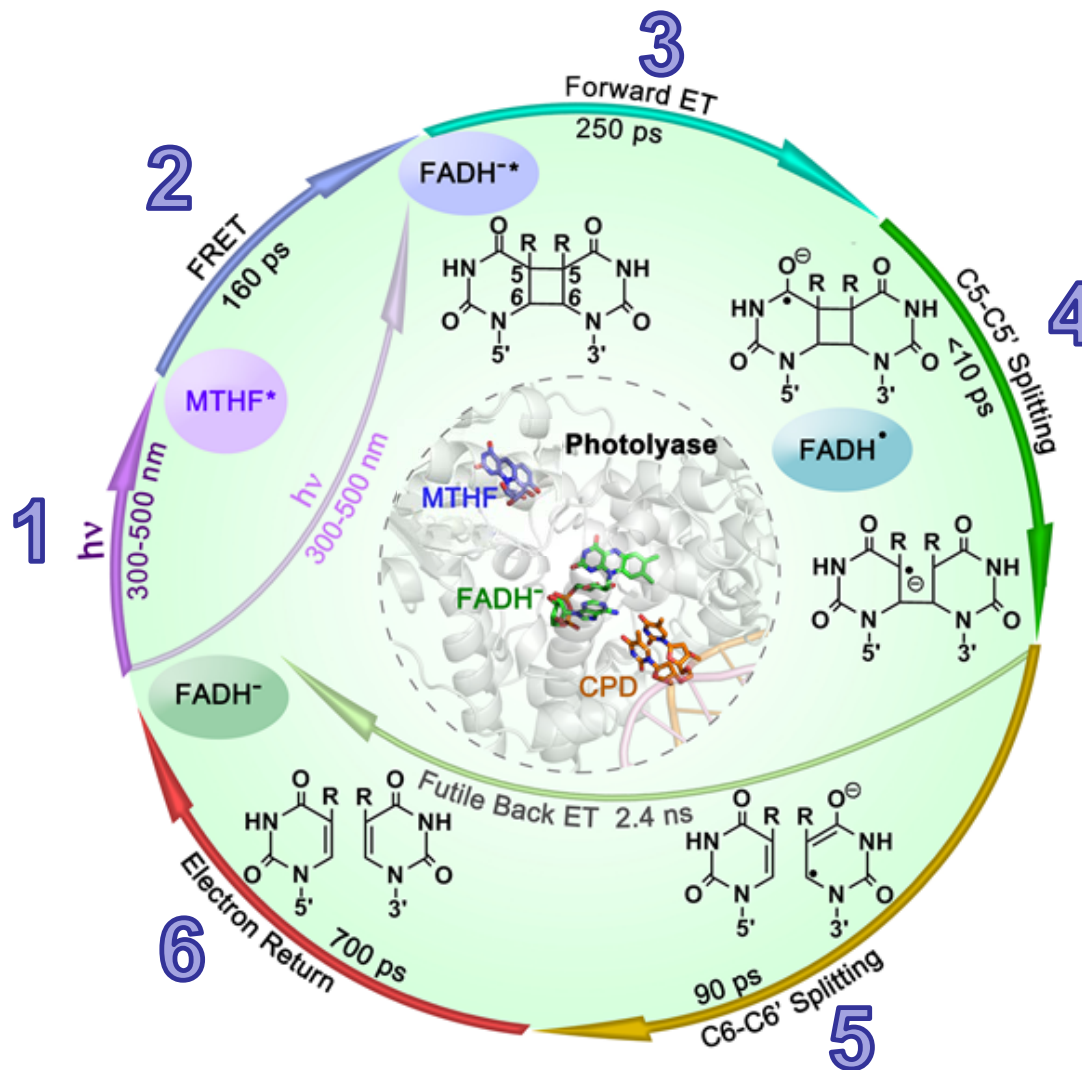
Park HW, et al (1995) *Science* 268:1866-1872

Reaction Mechanism of Photolyase



Liu Z, et al (2011) *PNAS* 108:14831-36
Tan C, et al (2014) *J Phys Chem A* 118:10522-30

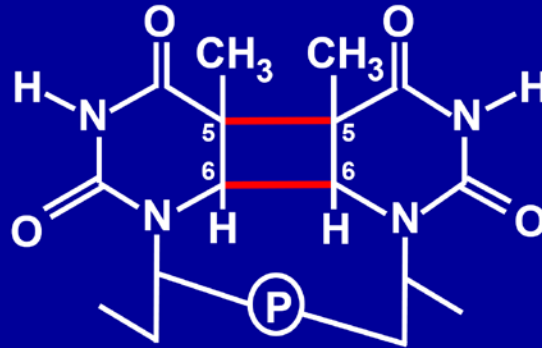
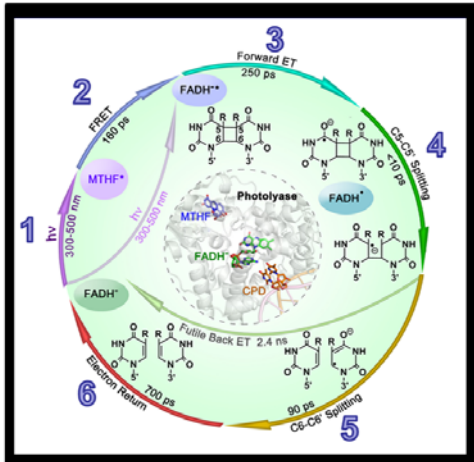
Ultrafast Kinetics of Photolyase



Liu Z, et al (2011) *PNAS* 108:14831-36
Tan C, et al (2014) *J Phys Chem A* 118:10522-30

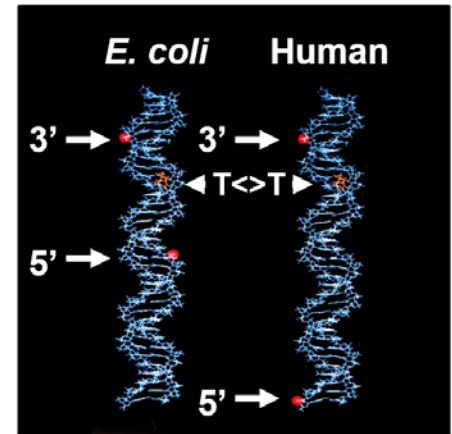
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Photolyase

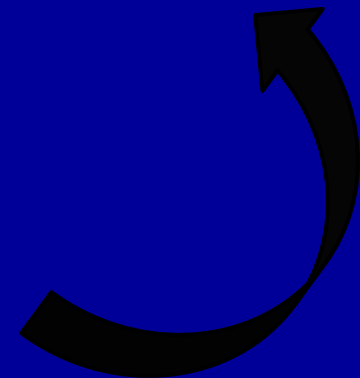
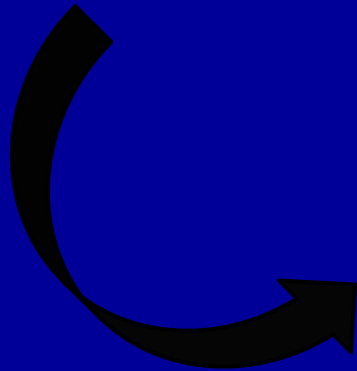
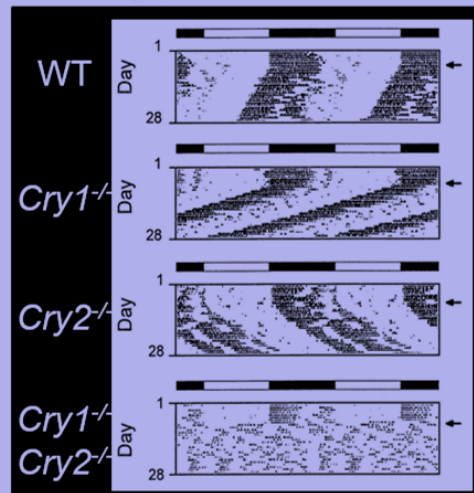


Thymine Dimer (T \leftrightarrow T)

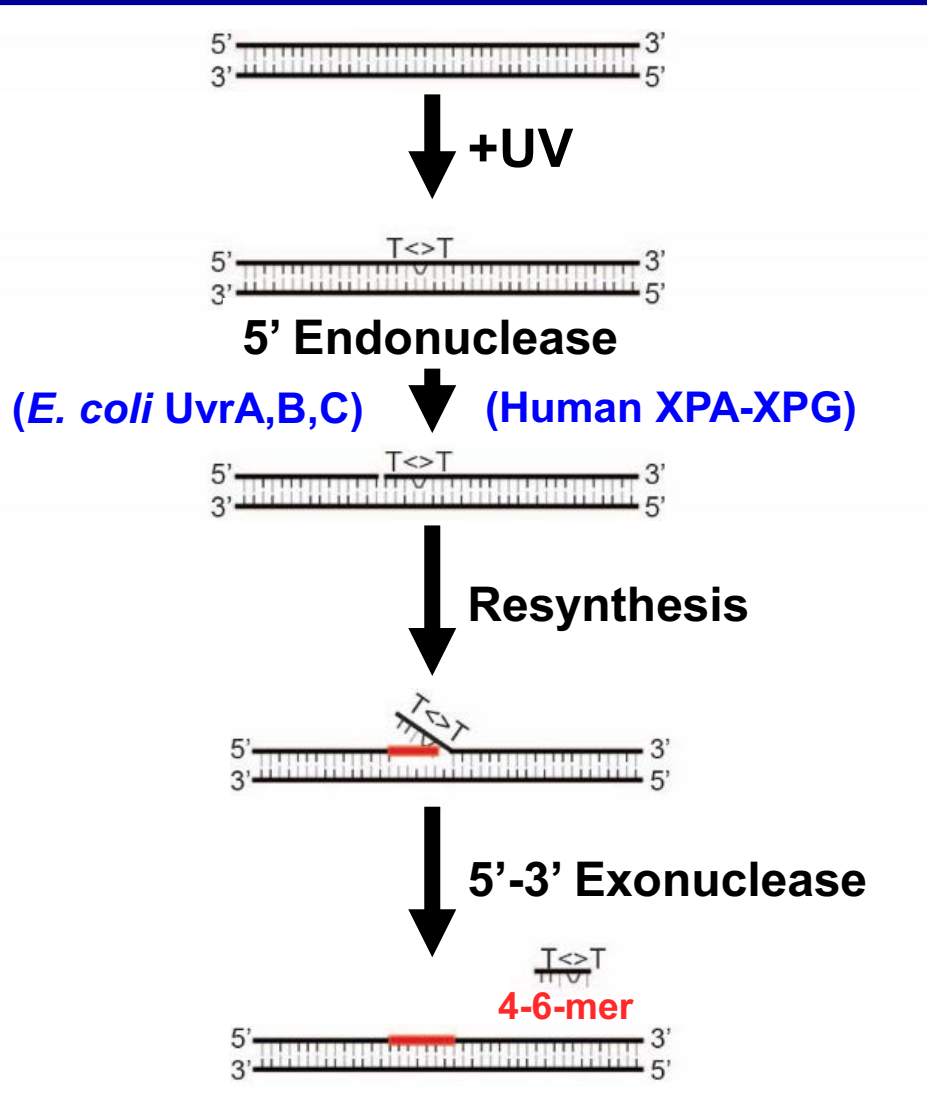
Nucleotide Excision Repair



Cryptochrome

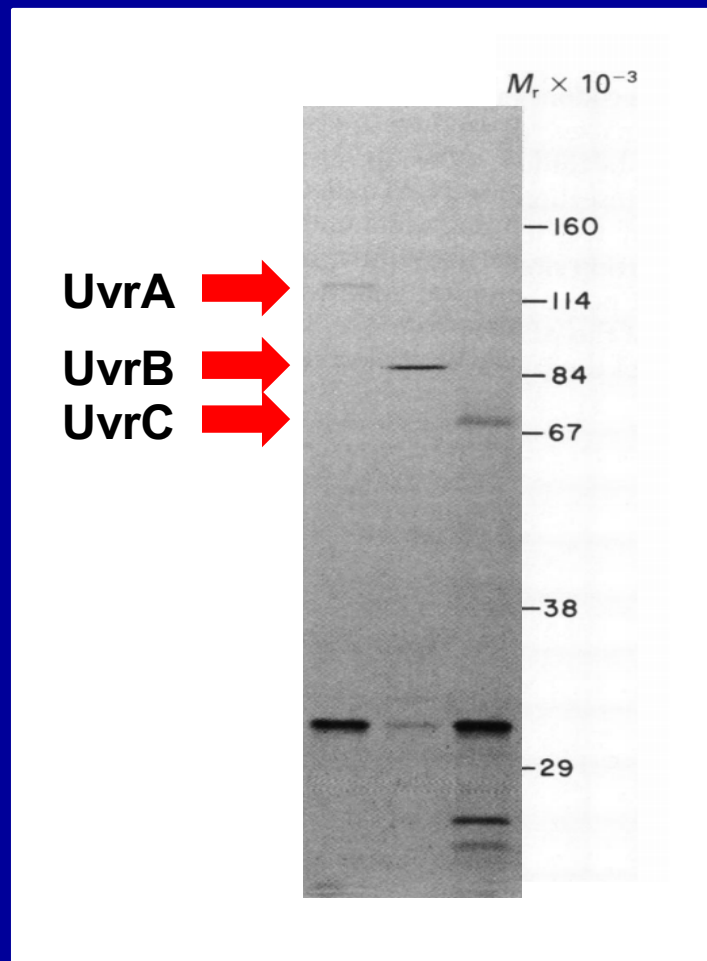


Model for UV Repair Circa 1982



- 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.

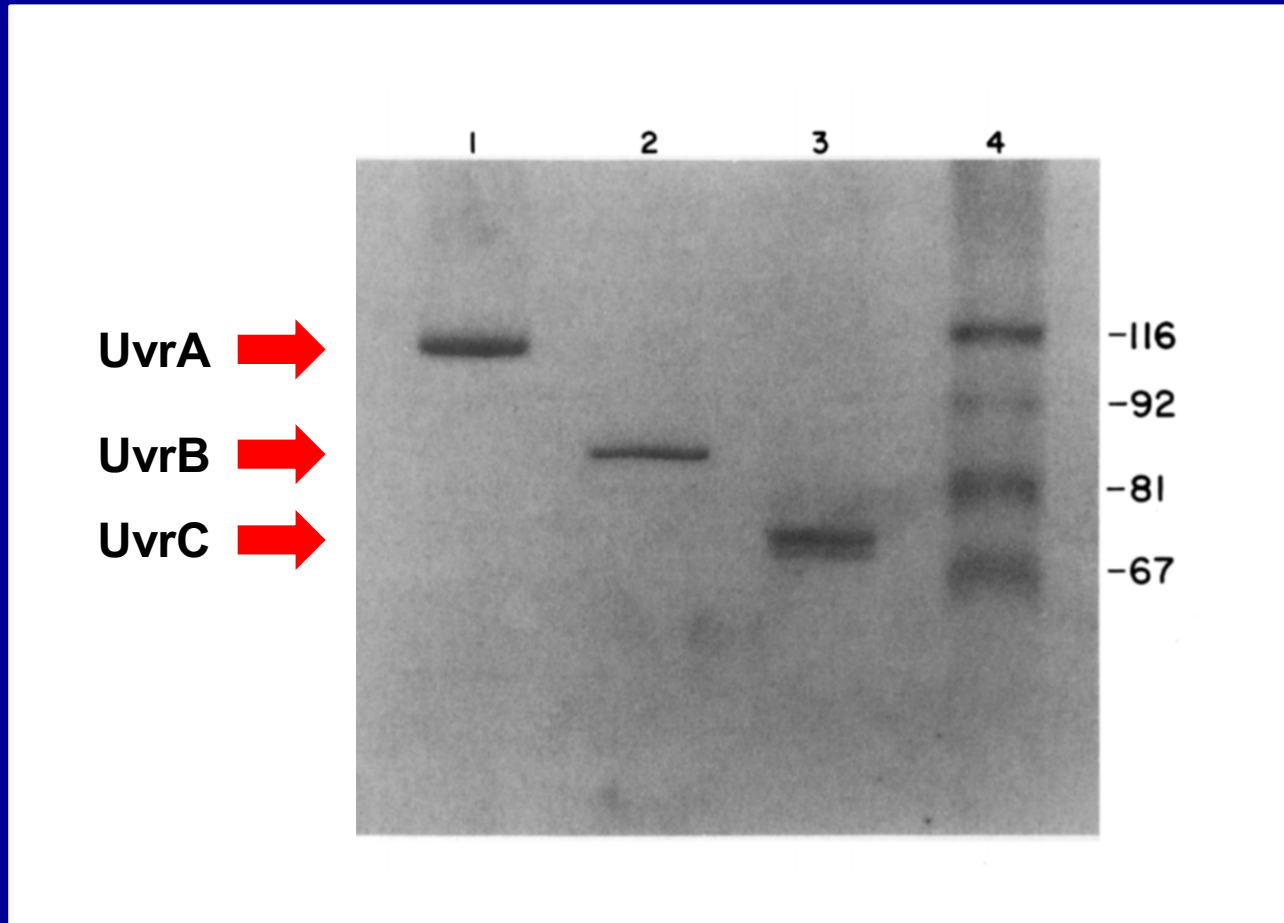
Identification of the *E. coli* Excision Repair Proteins by the Maxicell Method



Sancar A *et al* (1979) *J Bacteriol* 137:692-93
Sancar A *et al* (1981) *PNAS* 78:5450-54

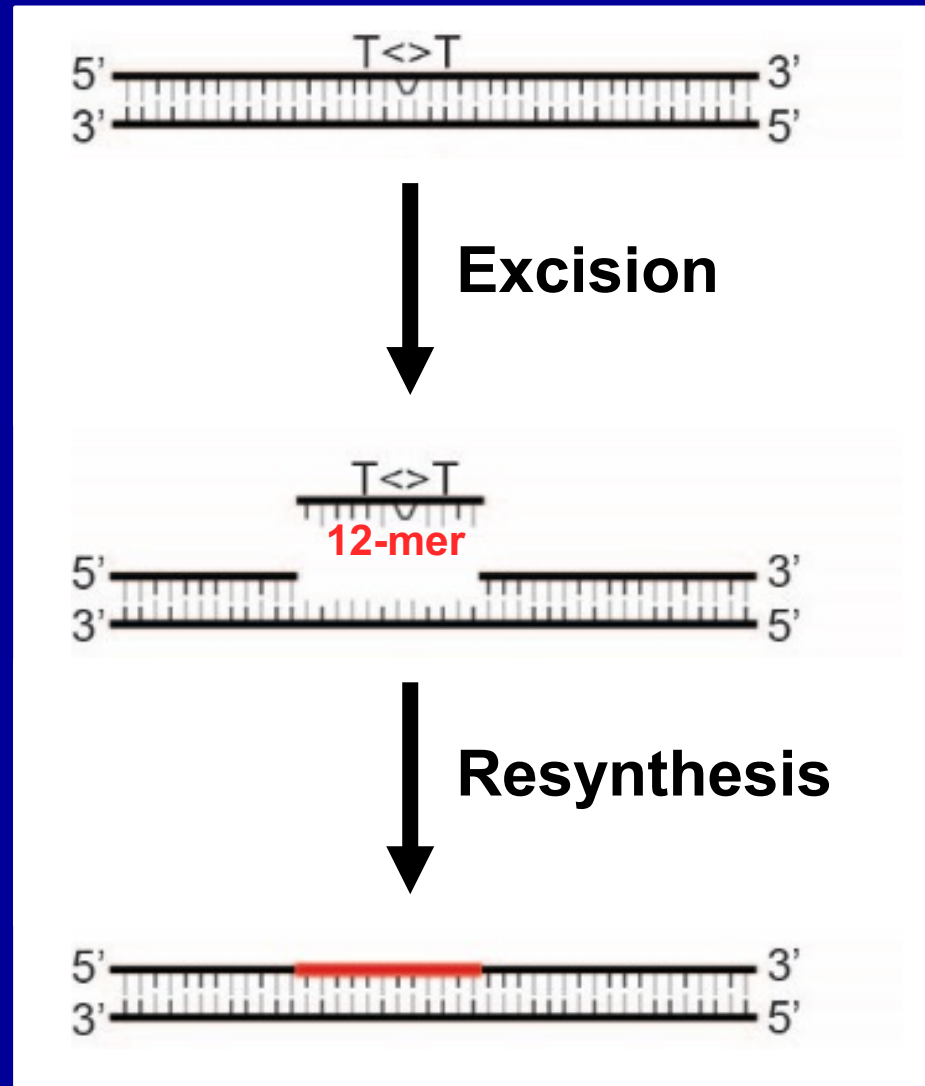
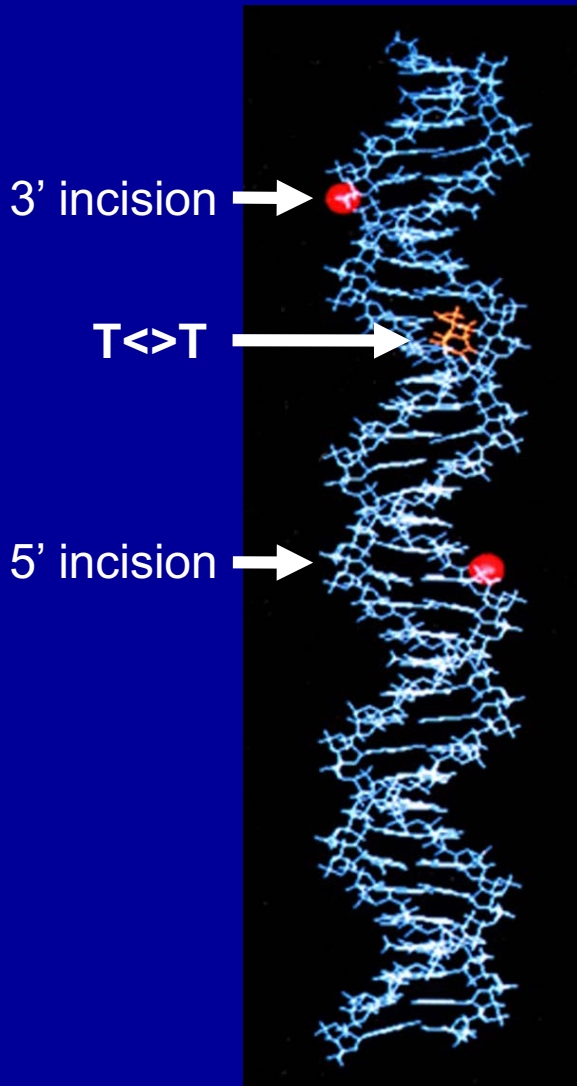
Sancar A *et al* (1981) *JMB* 148:63-76
Sancar A *et al* (1981) *JMB* 148:45-62

Purification of *E. coli* Excision Repair Proteins



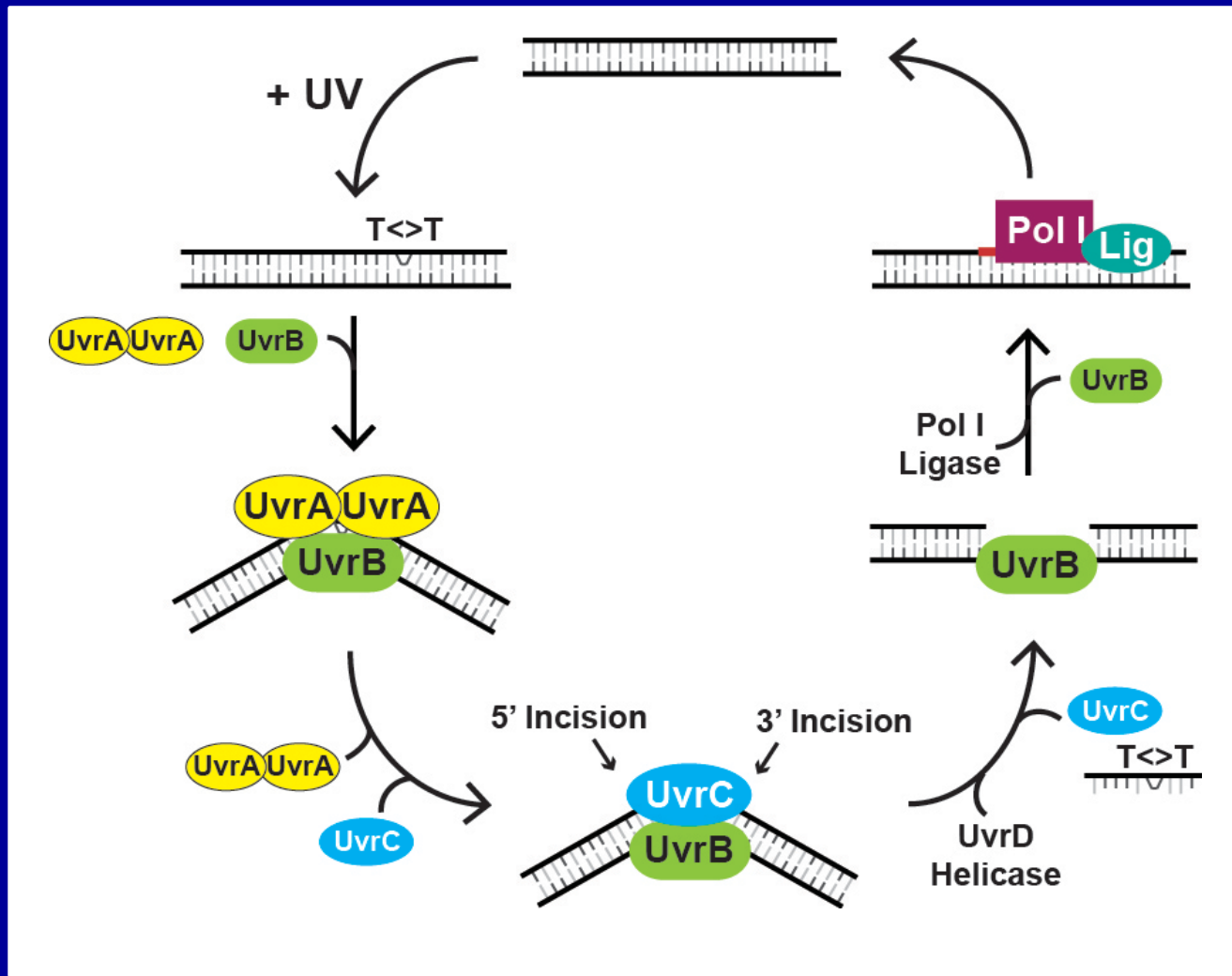
Sancar A and Rupp WD (1983) *Cell* 33:249-60

Dual Incisions in *E. coli* Excision Repair



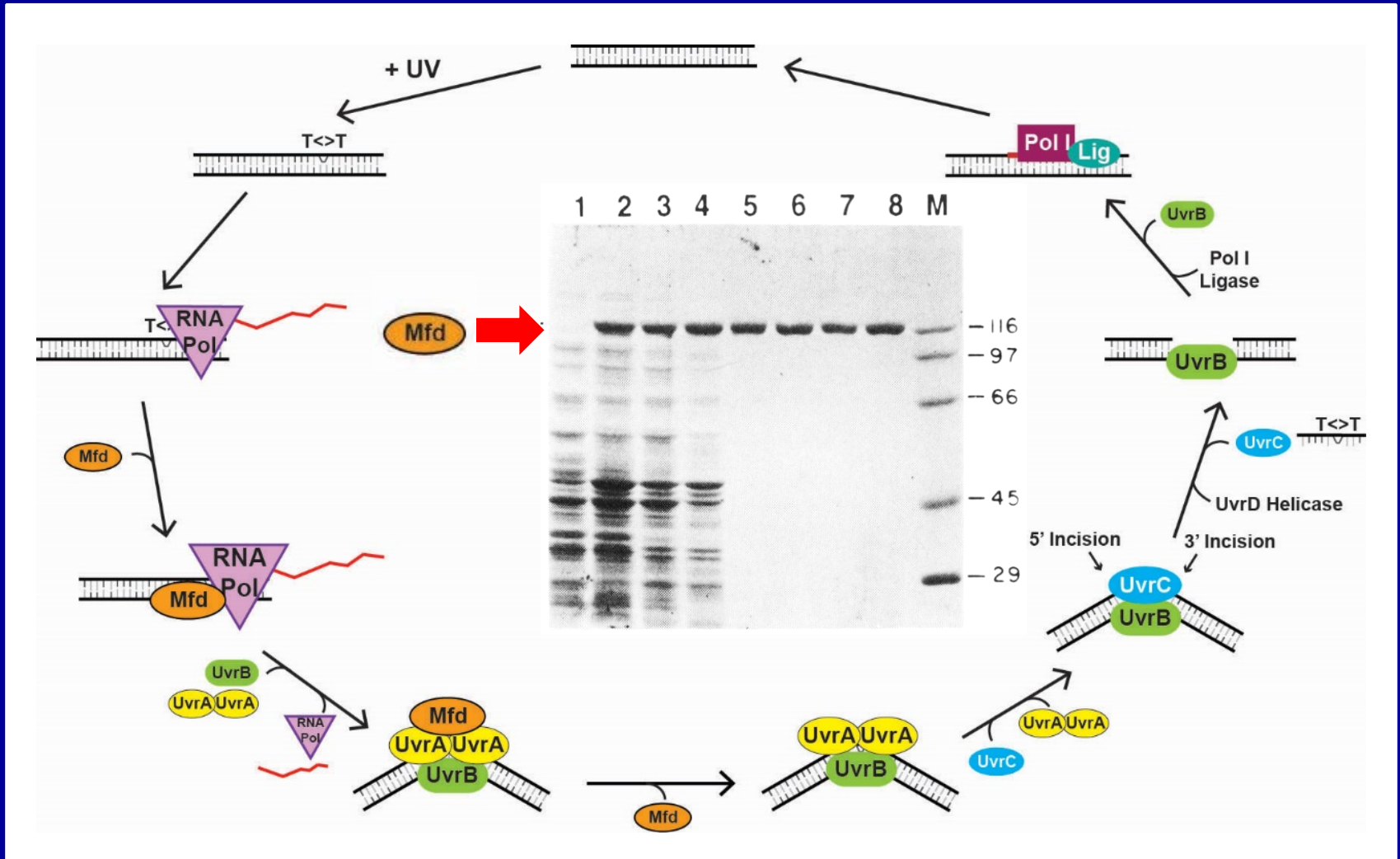
Sancar A and Rupp WD (1983) *Cell* 33:249-60
Sancar A (1994) *Science* 266:1954-56

Mechanism of Excision Repair in *E. coli*



Lin JJ & Sancar A (1992) *Mol Microbiol* 6:2219-24

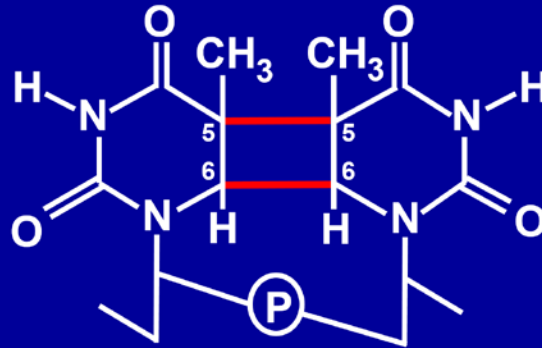
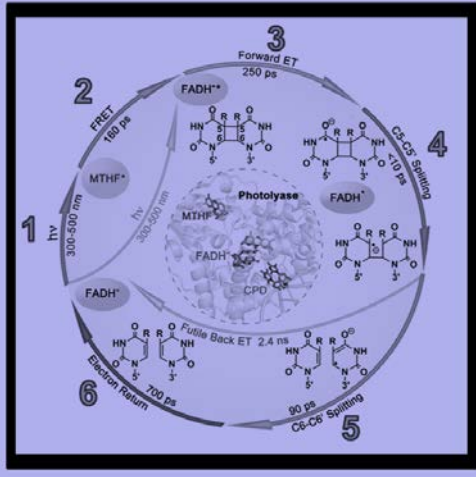
Mechanism of Transcription Coupled Repair



Selby CP & Sancar A (1993) *Science* 260:53-58

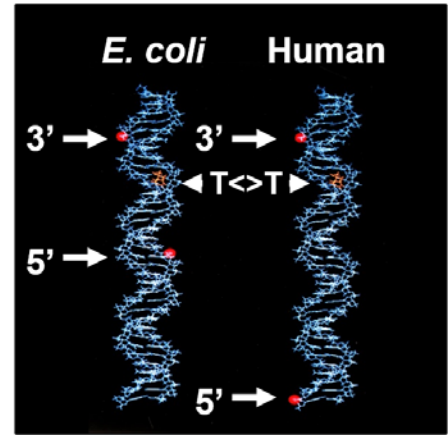
Outline

Photolyase

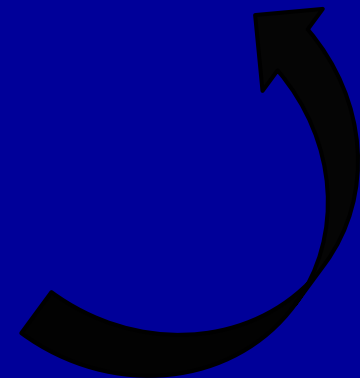
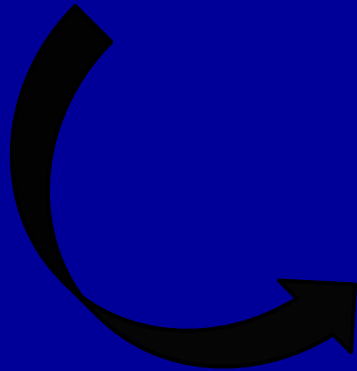
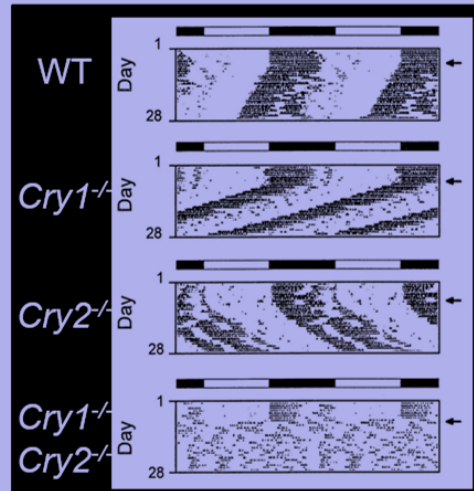


Thymine Dimer (T \leftrightarrow T)

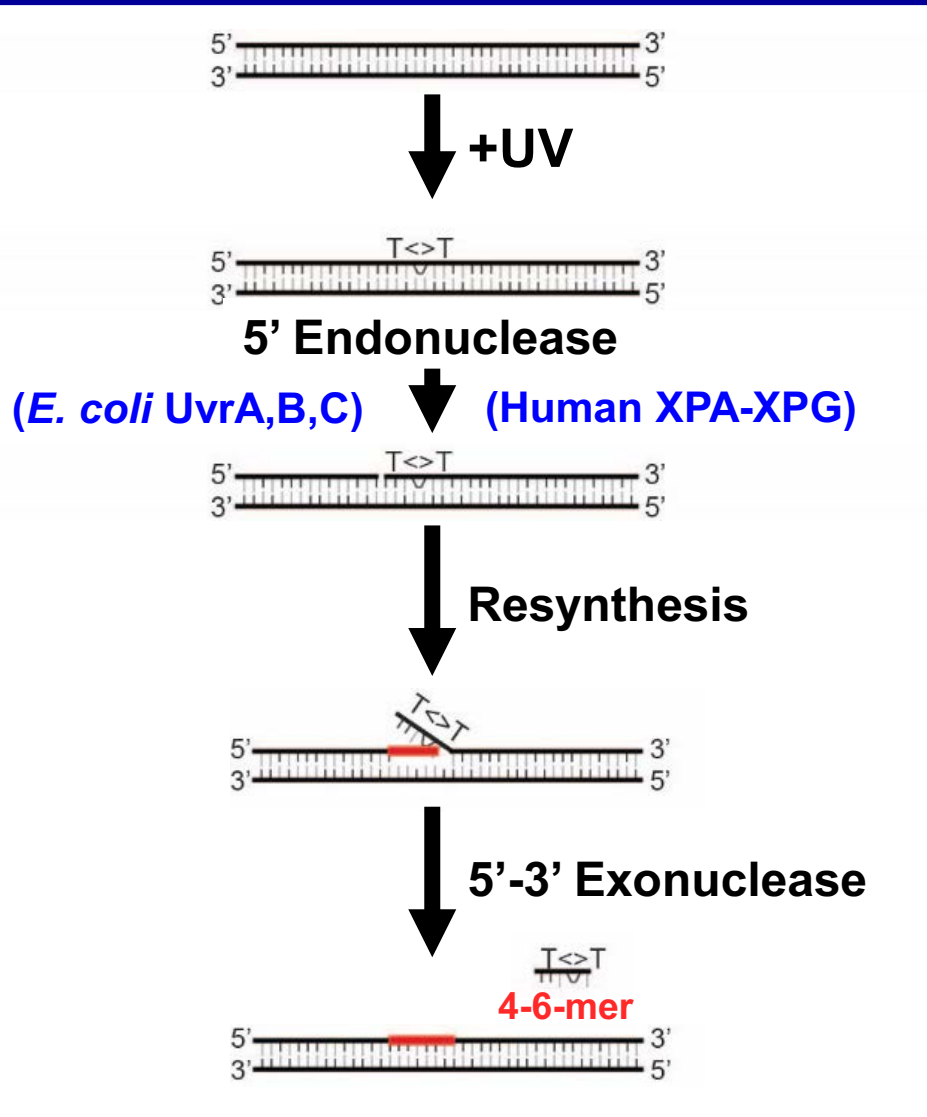
Nucleotide Excision Repair



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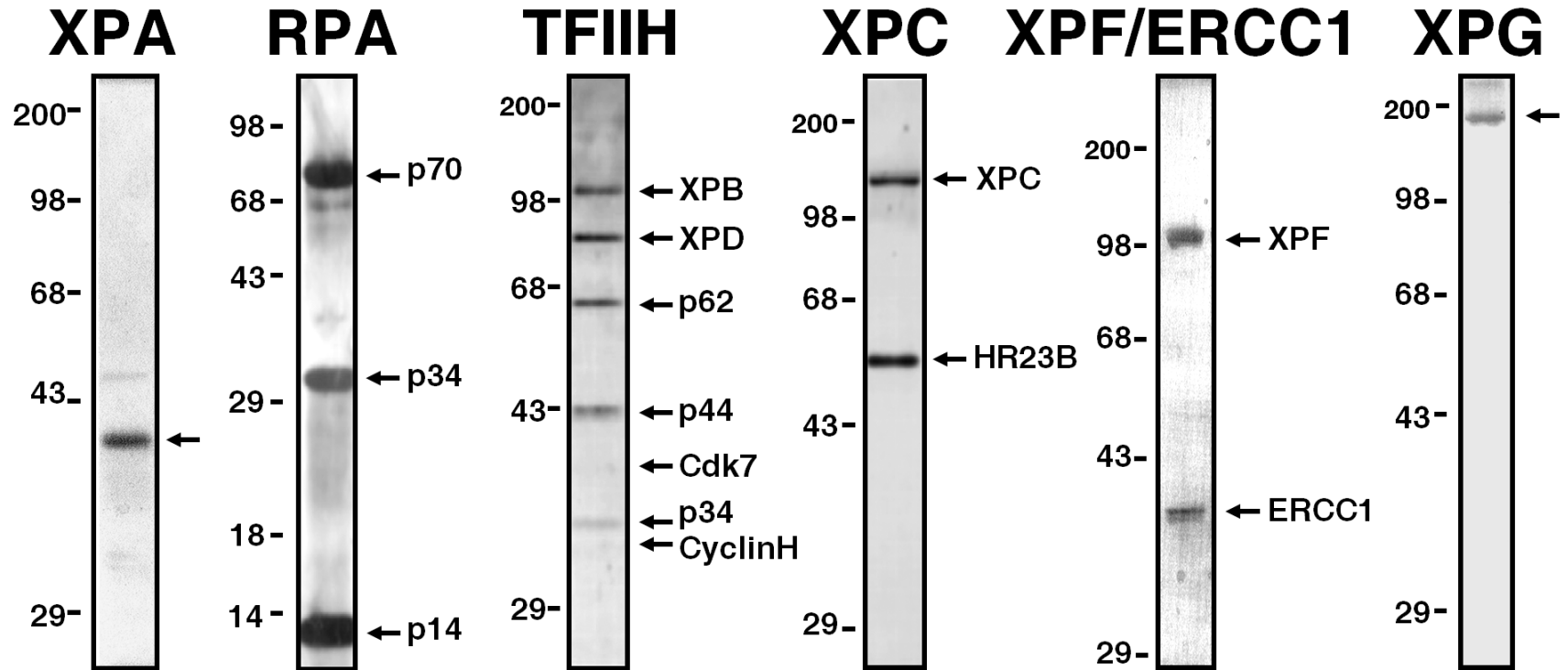
Xeroderma Pigmentosum

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



Halpern J, *et al* (2008) *Cases J* 1:254

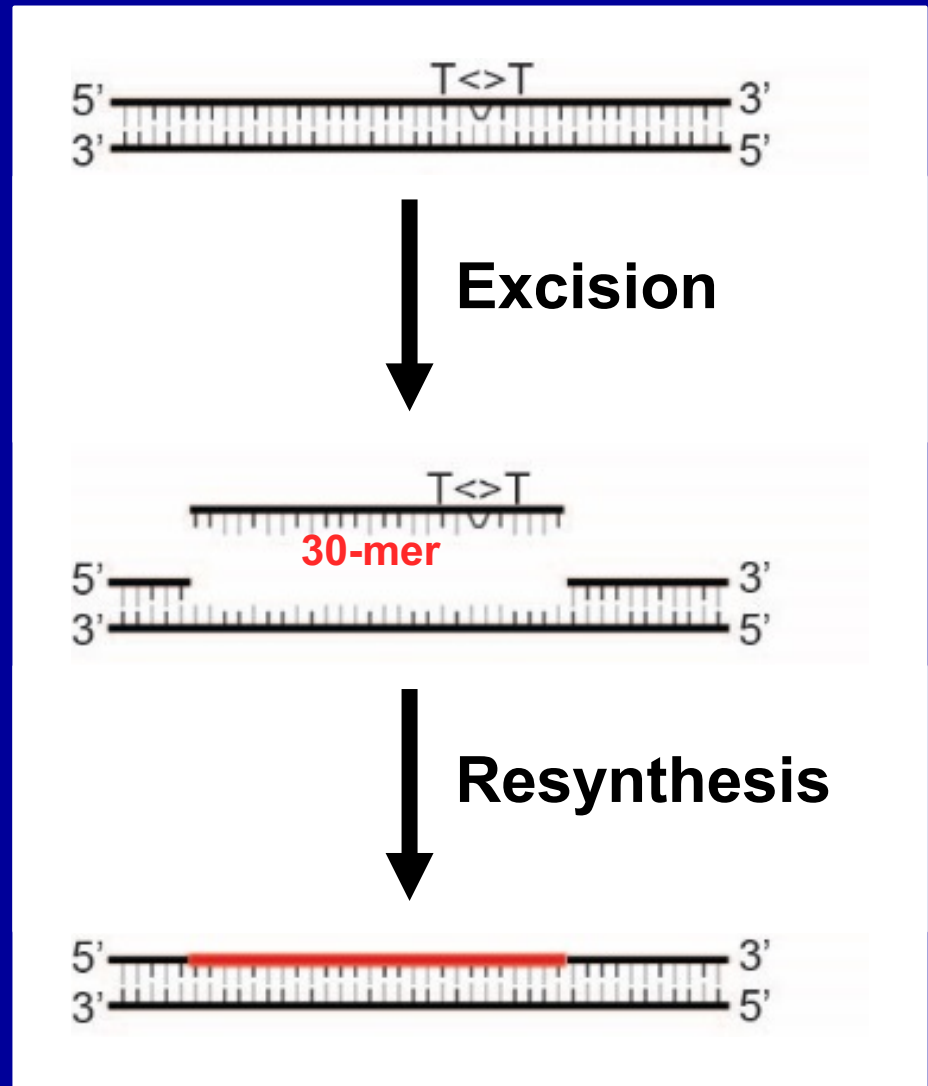
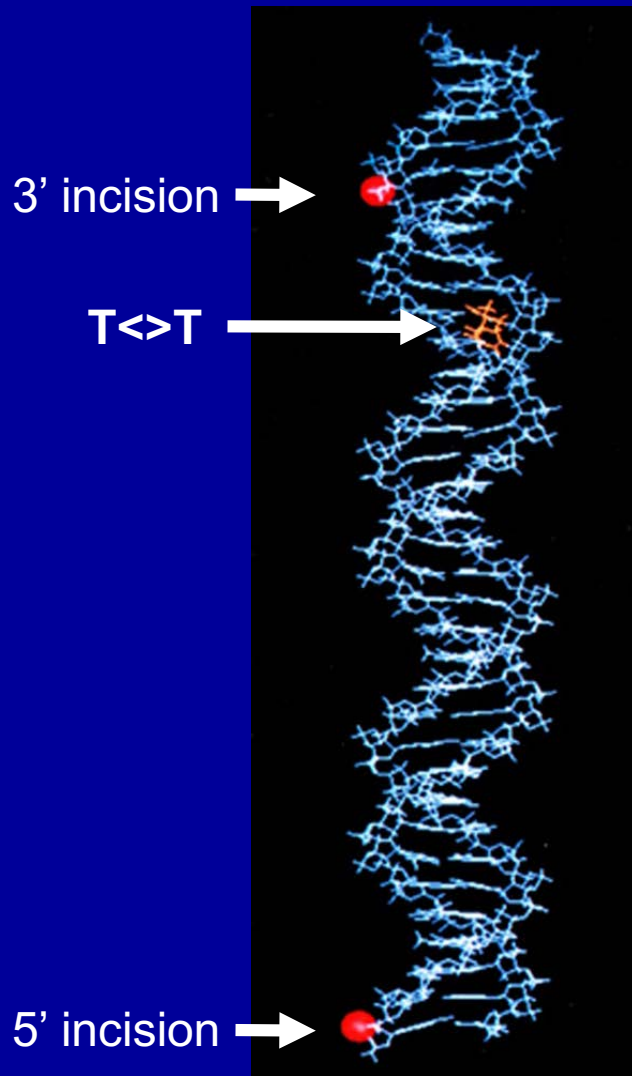
Human Excision Repair Factors



Mu D, et al (1995) *J Biol Chem* 270:2415-18

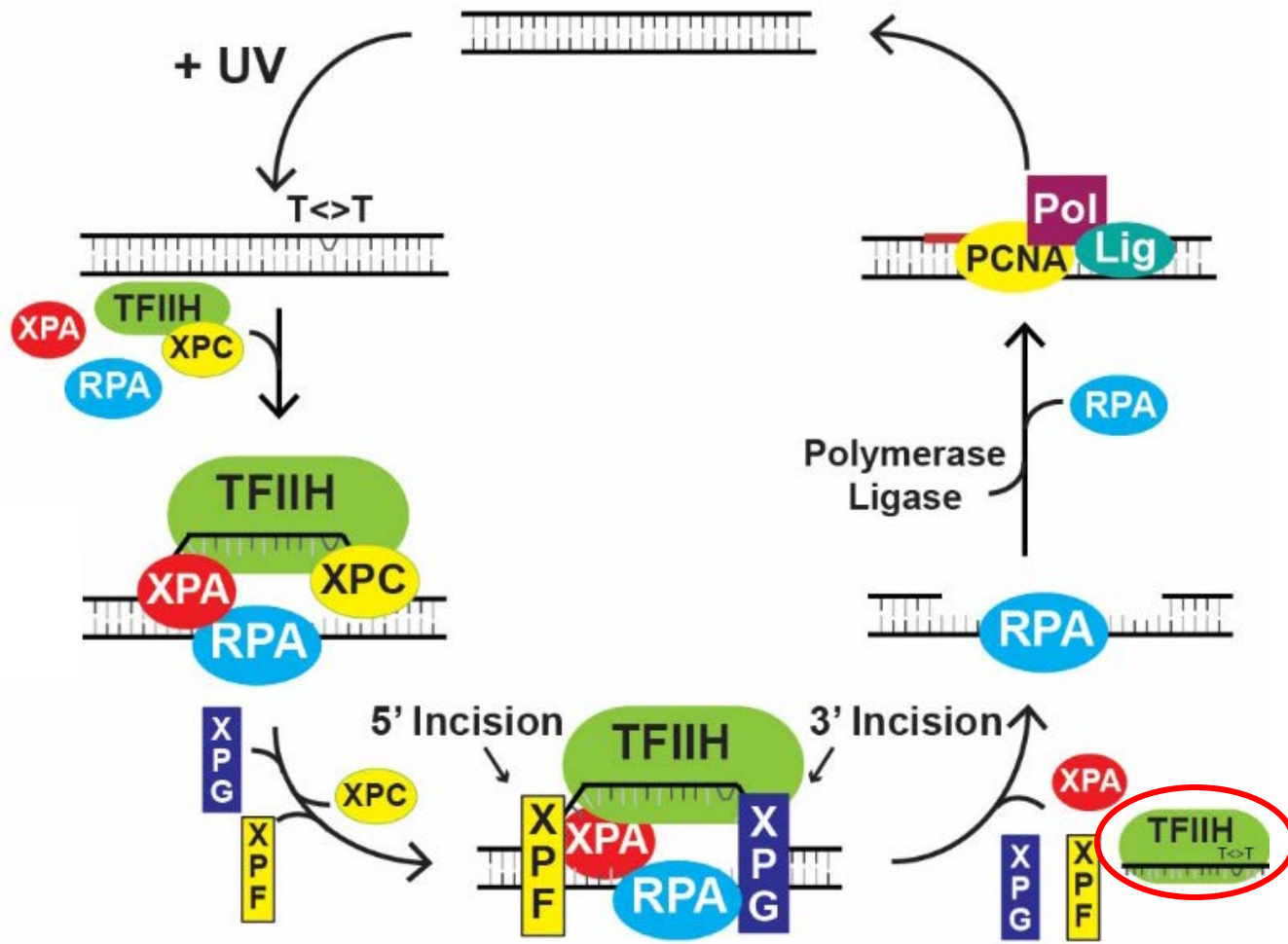
Mu D, Hsu DS, Sancar A (1996) *J Biol Chem* 271:8285-94

Dual Incisions in human Excision Repair

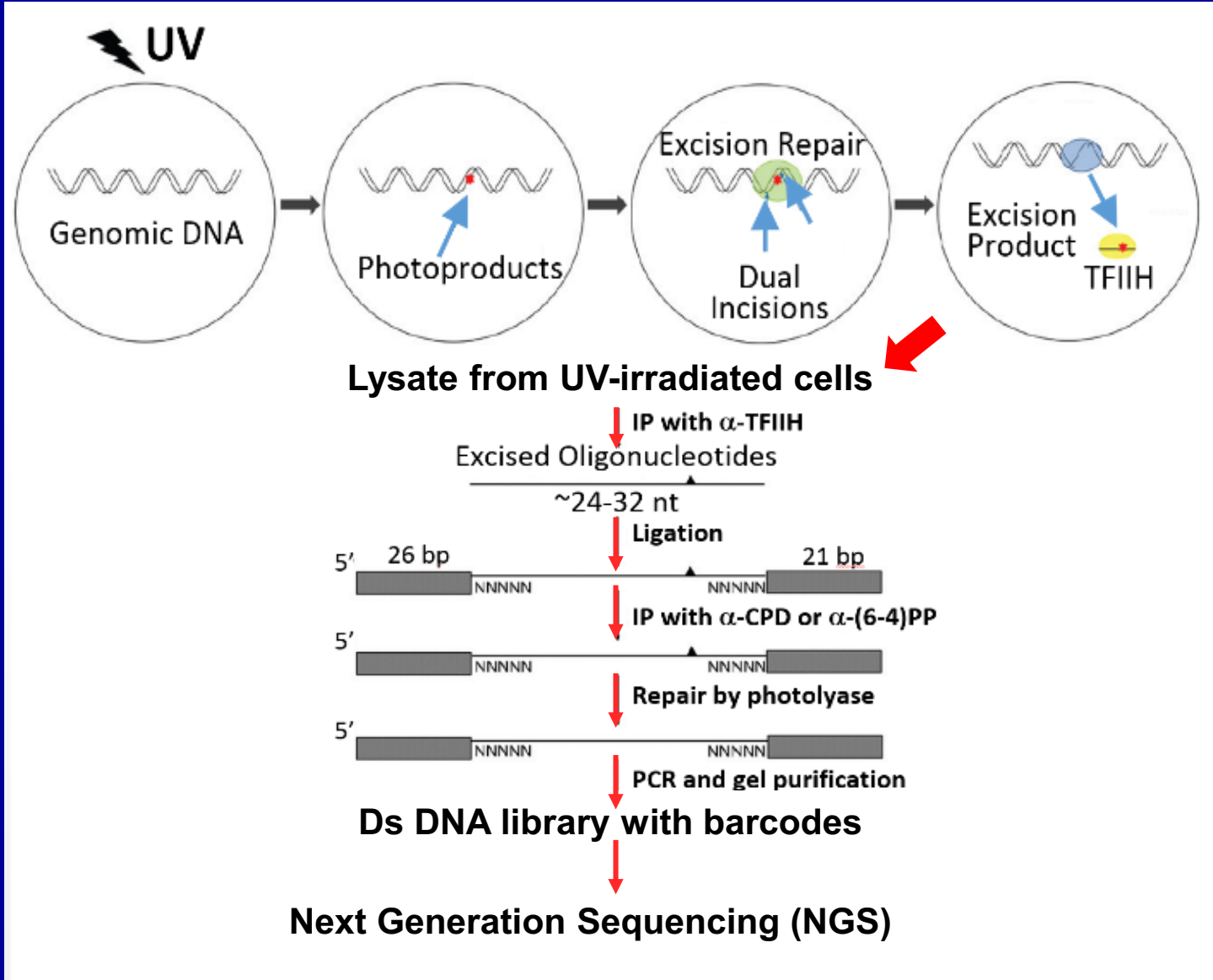


Huang JC, *et al* (1992) *PNAS* 89:3664-68
Sancar A (1994) *Science* 266:1954-56

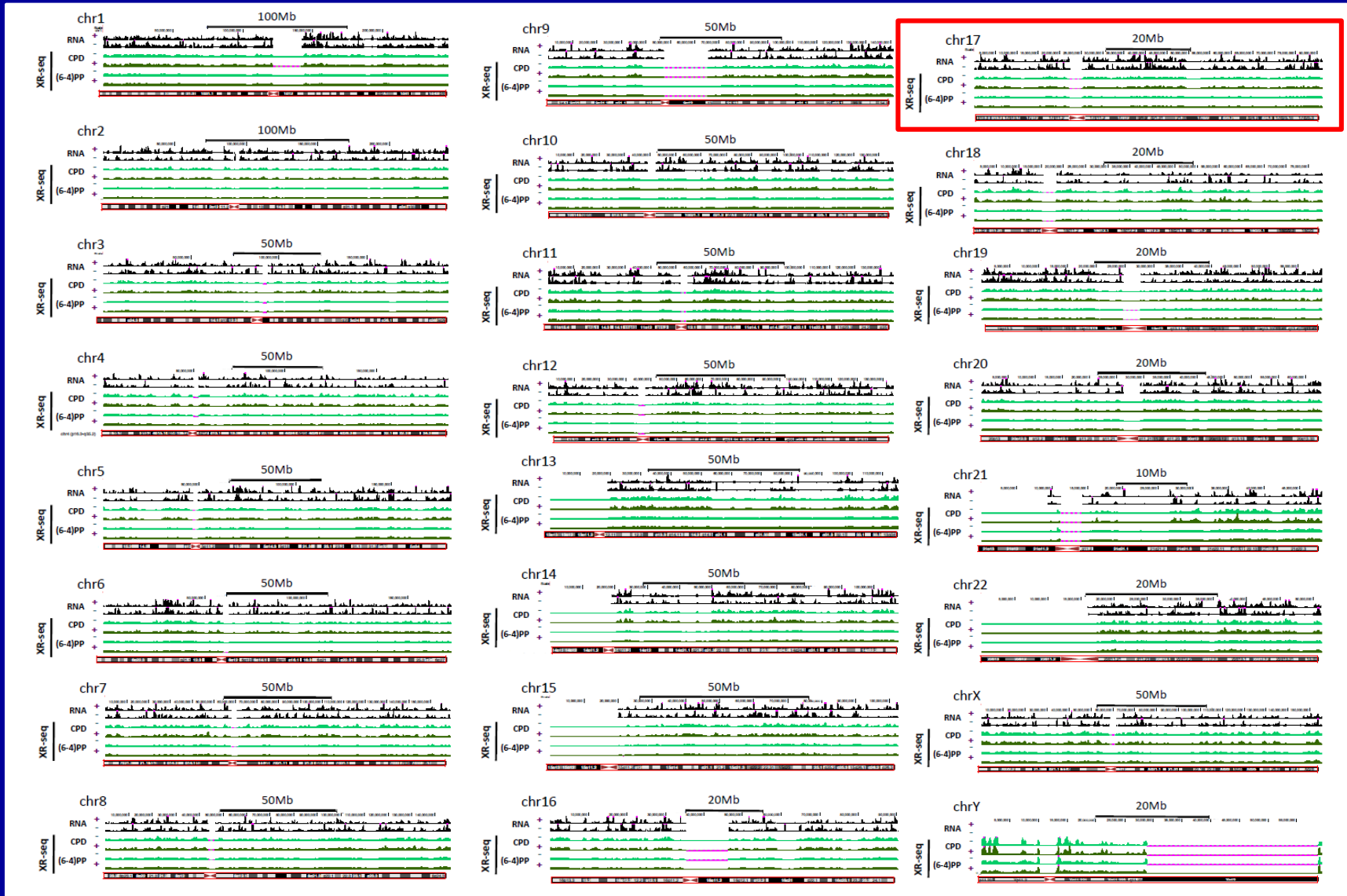
Mechanism of Excision Repair in Humans



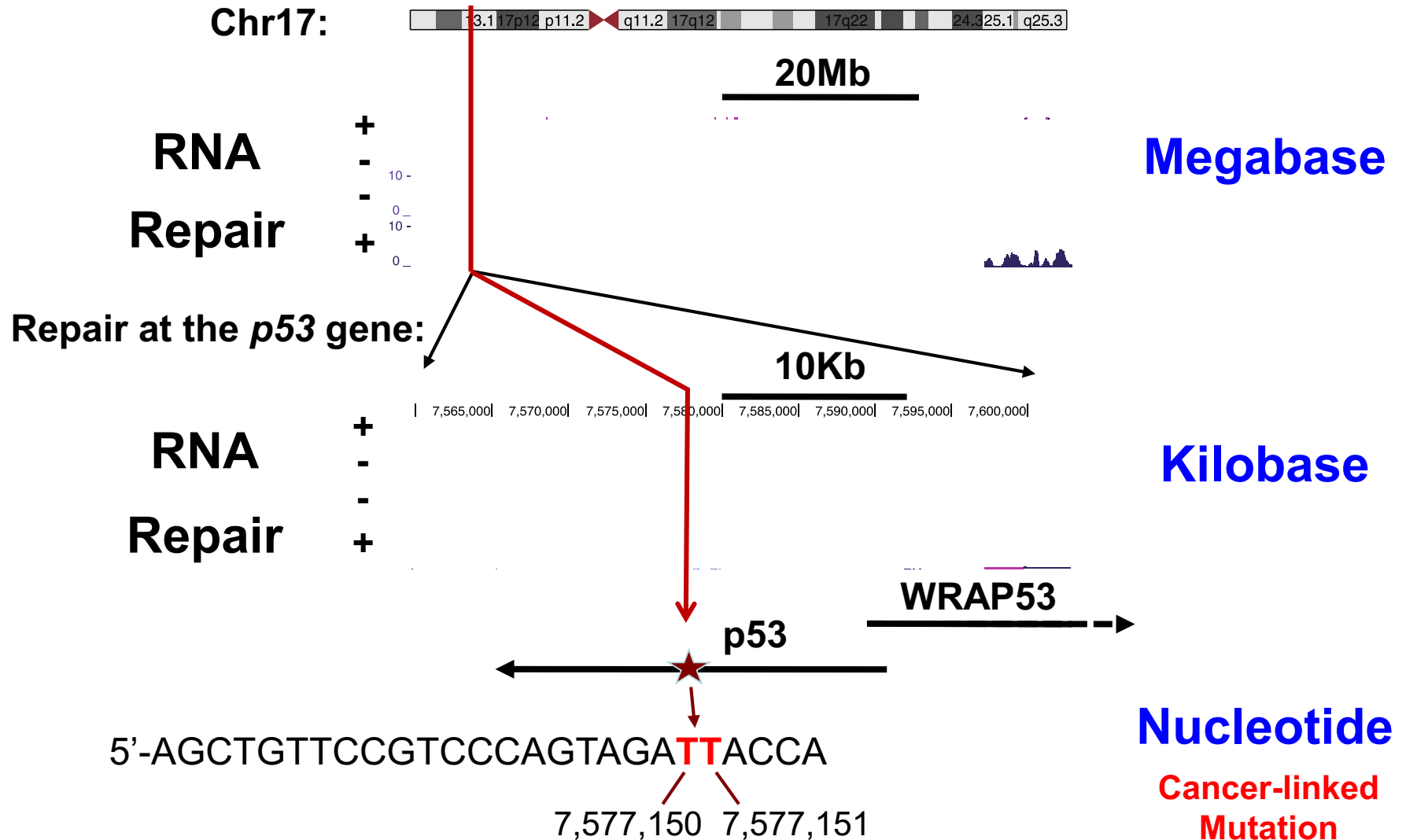
Mapping the Excised Oligomer in Humans



Excision Repair Map of the Human Genome

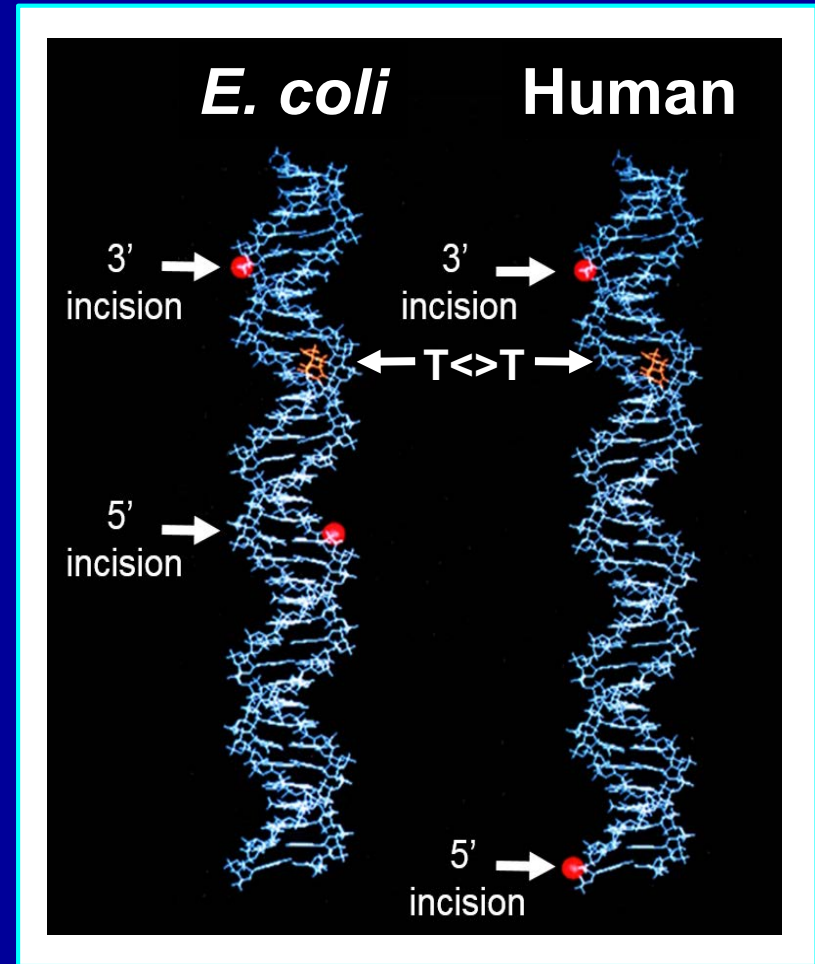


Excision Repair of *p53* at Single Nucleotide Resolution



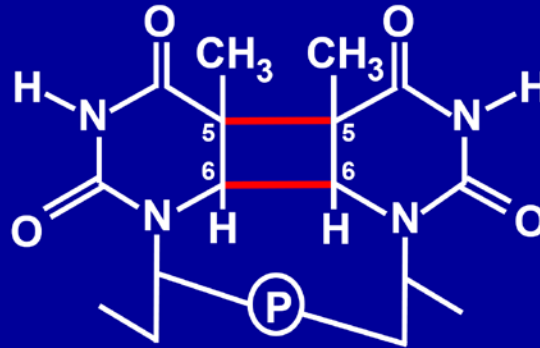
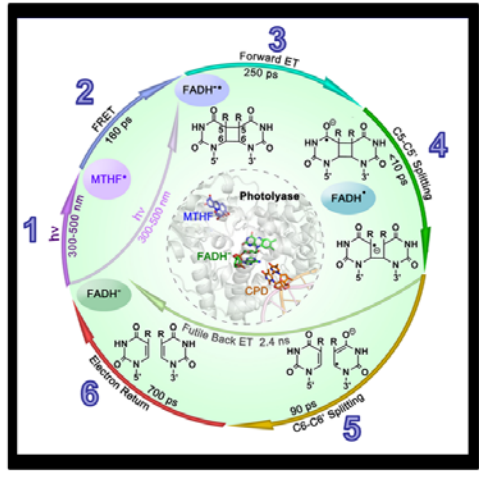
Excision Repair

- 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.



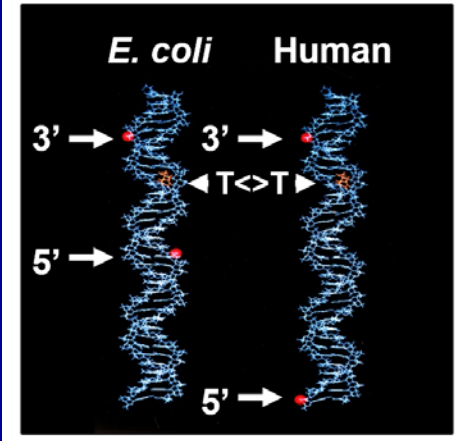
Outline

Photolyase

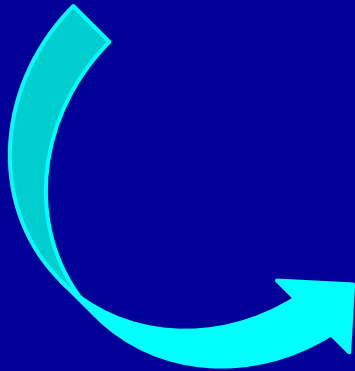
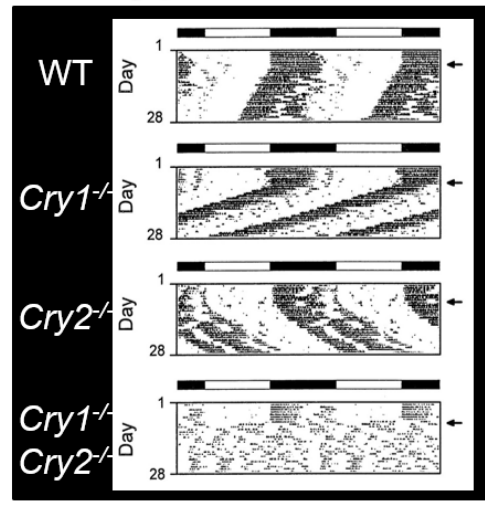


Thymine Dimer (T \leftrightarrow T)

Nucleotide Excision Repair



Cryptochrome



Cryptochrome

- Humans do not have photolyase

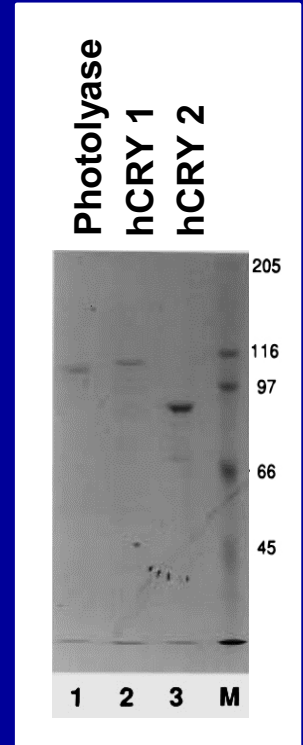
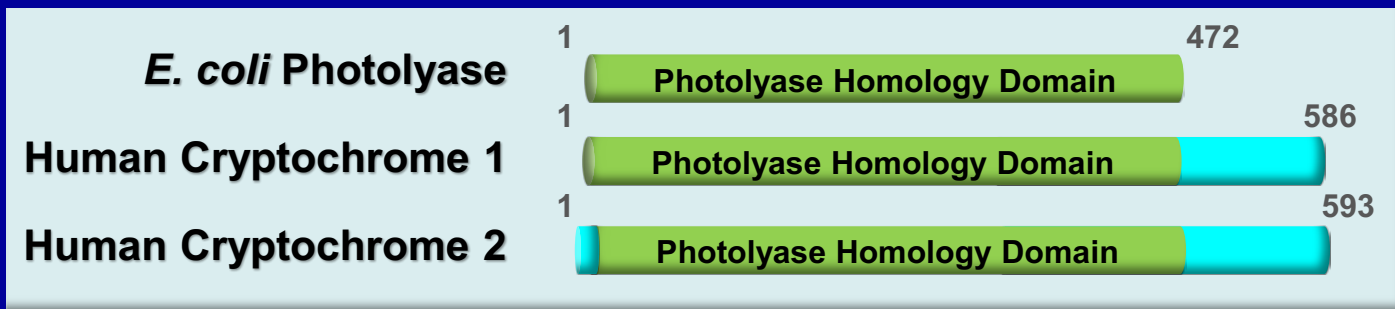
Li YF, *et al* (1993) *PNAS* 90:4389-93

- Humans have a photolyase homolog

Adams MD, *et al* (1995) *Nature* 377:3-174

- Humans have 2 photolyase paralogs

Hsu DS, *et al* (1996) *Biochemistry* 35:13871-77



Photolyase



Cryptochrome

Brautigam CA, *et al* (2004) *PNAS* 101:12142-47

Jetlag, Cryptochrome, and the Circadian Clock

Determined that human CRYs are not repair proteins

Discovered genetic evidence that human CRYs are clock proteins

Spring
1996

May - June
1996

June - August
1996

May - November
1998

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

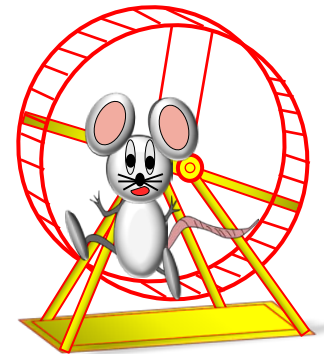
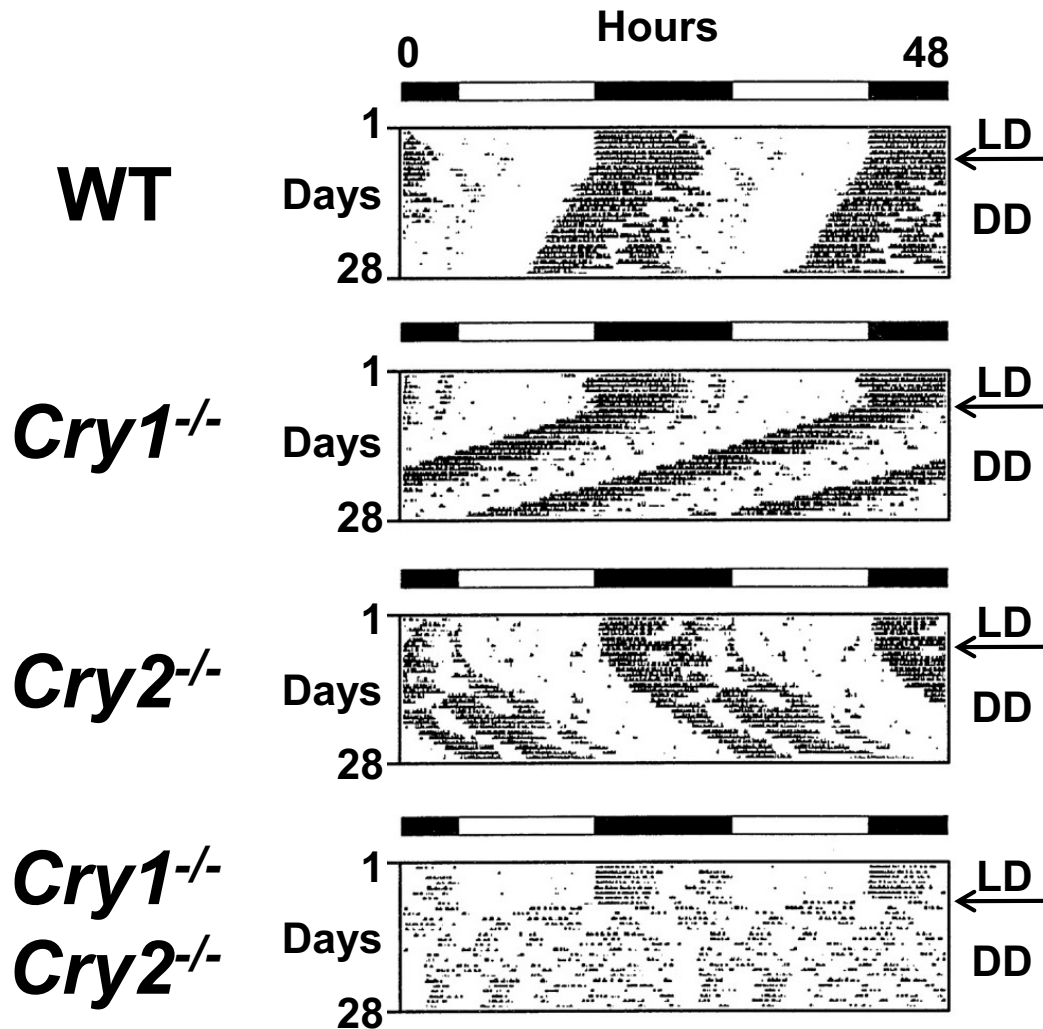
Wrote the human CRY paper claiming CRYs are circadian proteins



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

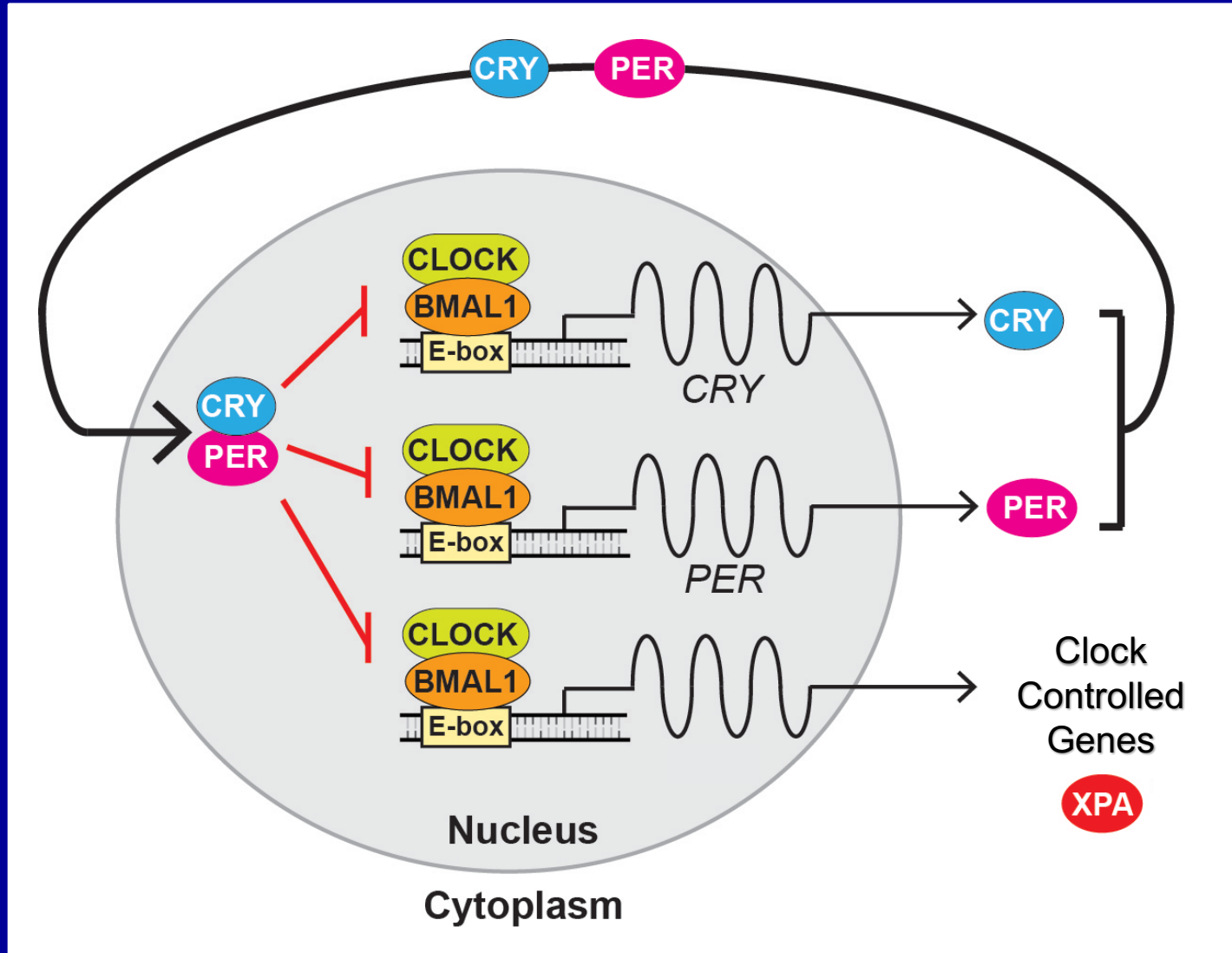


Thresher RJ, *et al* (1998) *Science* 282:1490-94
Vitaterna MH, *et al* (1999) *PNAS* 96:12114-19

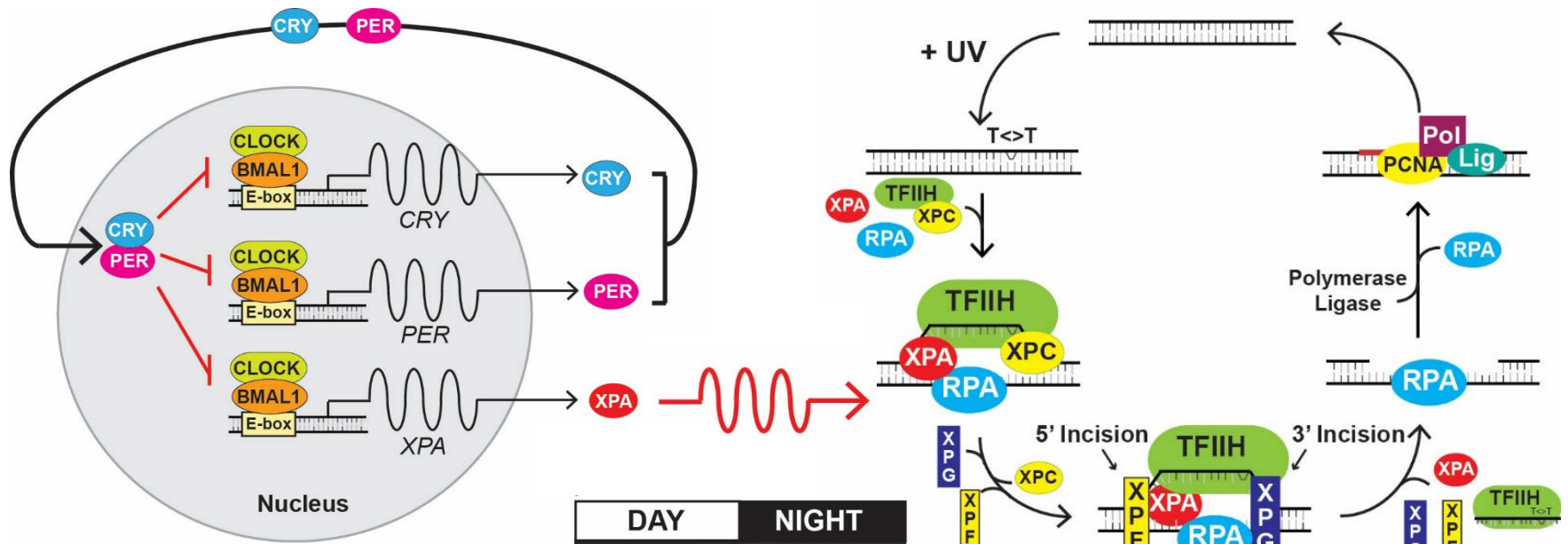
Mammalian Clock Genes/Proteins (1996-2000)

- 1) CRYPTOCHROME (Flavoprotein)
- 2) PERIOD (PAS domain)
- 3) CLOCK (bHLH-PAS)
- 4) BMAL1 (bHLH-PAS)

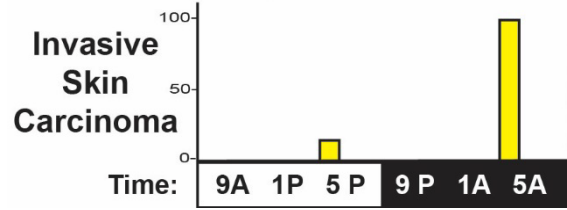
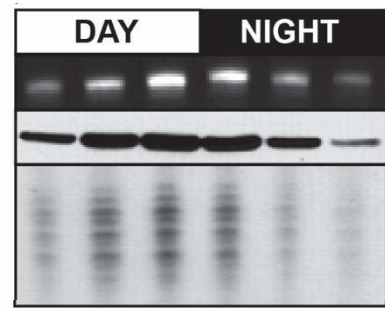
Circadian Control Mechanism



Circadian Control of Excision Repair



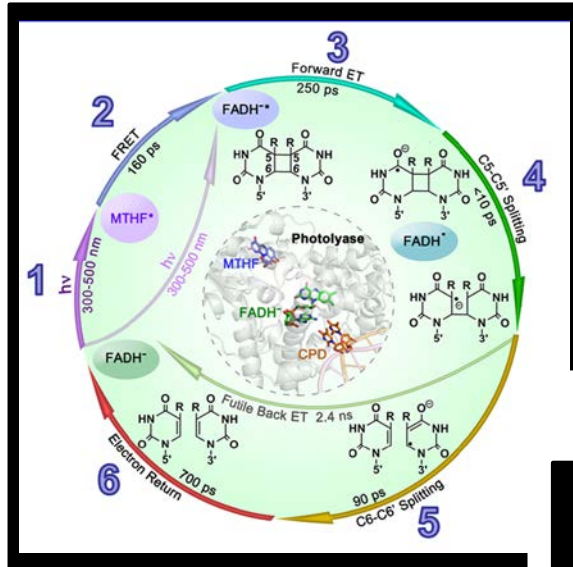
Nucleus
Cytoplasm



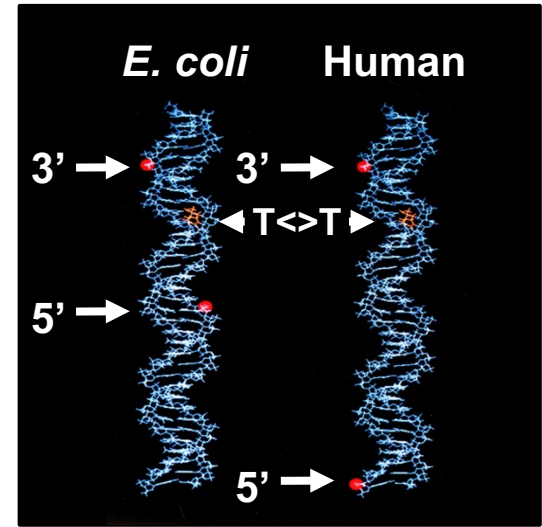
Kang T, et al (2010) PNAS 107:4890-95
Gaddameedhi S, et al (2011) PNAS 108:18790-95

Summary

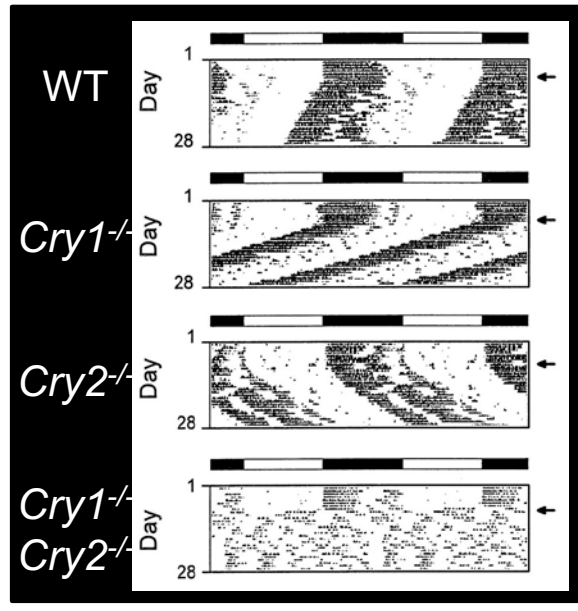
Photolyase



Nucleotide Excision Repair



Cryptochrome



Acknowledgments

Sancar Lab Members

Adar, Sheera
Ahn, Kyujeong
Akan, Zafer
Annayev, Yunus
Araujo, Francisco
Arat, Nezahat
Arnette, Robin
Asimgil, Hande
Bereketoglu, Sidar
Berrocal, Gloria
Bessho, Tadayoshi
Bondo, Eddie
Bouyer, James
Branum, Mark
Cakit, Ceylan
Cantürk, Fazile
Capp, Christopher
Carlton, Wendi
Chiou, Yi-Ying
Choi, Jun-Hyuk
Croteau, Deborah
Dawut, Lale
Denaro, Tracy

DeRocco, Vanessa
Ensch-Simon, Ingrid
Erkmen, Gulnihal Kulaksiz
Gaddameedhi, Shobhan
Gauger, Michele
Han, Chih-Chiang (Eric)
Hara, Ryujiro
Hassan, Bachar
Heenan, Erin
Hsu, Shiao-Wen (David)
Hu, Jinchuan
Huang, Juch-Chin (JC)
Husain, Intisar
Hutsell, Stephanie
Jiang, Gouchun
Kang, Tae-Hong
Karaman, Muhammet
Kavakli, Ibrahim (Halil)
Kawara, Hiroaki
Kazantsev, Aleksey
Kemp, Michael
Kim, Sang-Tae
Lee, Jin-Hyup

Levy, Michael
Li, Wentao
Li, Ywan-Feng
Lin, Jing-Jer
Lindsey-Boltz, Laura
Malhotra, Khushbeer
Matsunaga, Tsukasa
McDowell-Buchanan, Carla
Meganck, Rita
Miyamoto, Yasuhide
Mo, Jinyao
Morrison, Lydia
Mu, David
Myles, Gary
Nichols, Anne
Ögrünç, Müge
Orren, David
Özer, Zahide
Özgür, Sezgin
Ozkan-Dagliyan, Irem
Öztürk, Nuri
Park, Chi-Hyun
Partch, Carrie
Payne, Gillian
Payne, Nicola

Petit, Claude
Phillips, A. Meleah
Rastogi, Promila
Reardon, Joyce
Sar, Funda
Selby, Christopher
Sercin, Ozdemirhan
Shields, Katie
Sibghat-Ullah
Smith, Frances
Song, Sang-Hun
Svoboda, Daniel
Thomas, David
Thompson, Carol
Thresher, Randy
Ünsal-Kaçmaz, Keziban
Vagas, Elif
Van Houten, Ben
Wakasugi, Mitsuo
Worthington, Erin (Nikki)
Yang, Yanyan
Ye, Rui
Yilmaz, Seçil
Zhao, Xiaodong (Jerry)
Zhao, Shaying

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Aksoy, Muzaffer
Rupp, W. Dean
Howard-Flanders, Paul

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Collaborators

Bambara, Robert
Chaney, Stephen
Cordeiro-Stone, Marila
Deisenhofer, Johann
Griffith, Jack
Hearst, John
Heelis, Paul
Hurwitz, Jerard
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