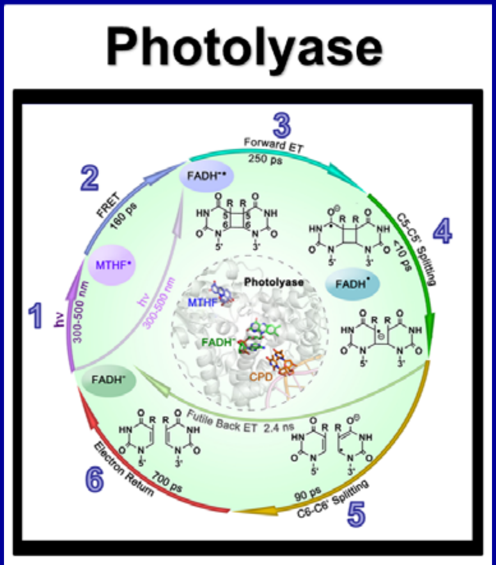
Mechanisms of DNA Repair by Photolyase and Excision Nuclease

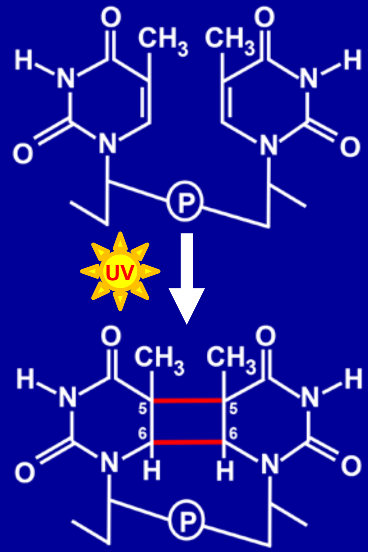
Nobel Lecture in Chemistry
Stockholms Universitet

December 8, 2015

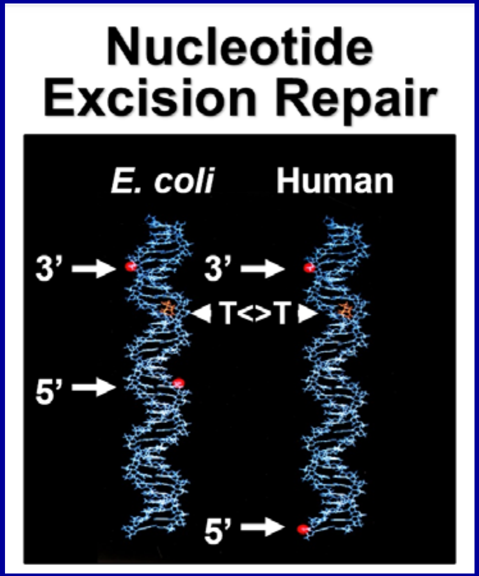
Aziz Sancar
Department of Biochemistry and Biophysics
University of North Carolina School of Medicine
Chapel Hill, North Carolina

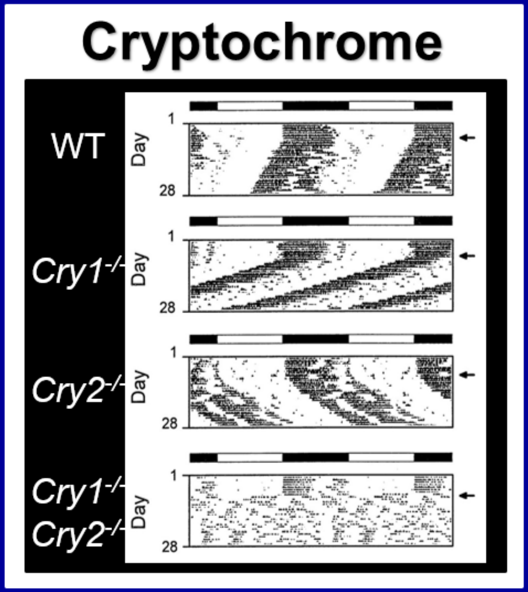
Outline





Thymine Dimer (T<>T)





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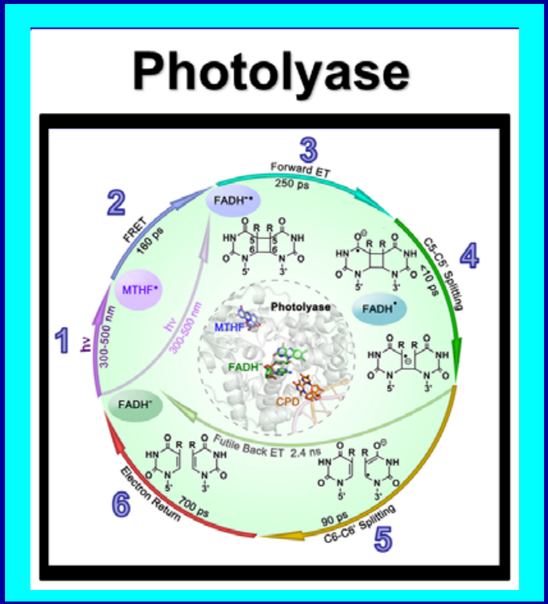

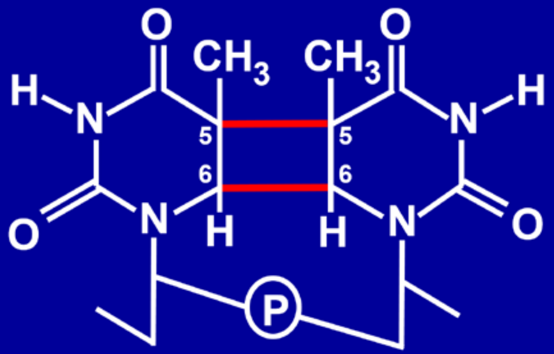

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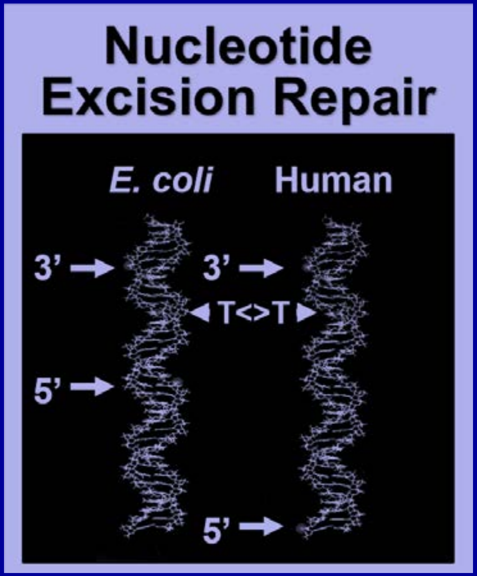
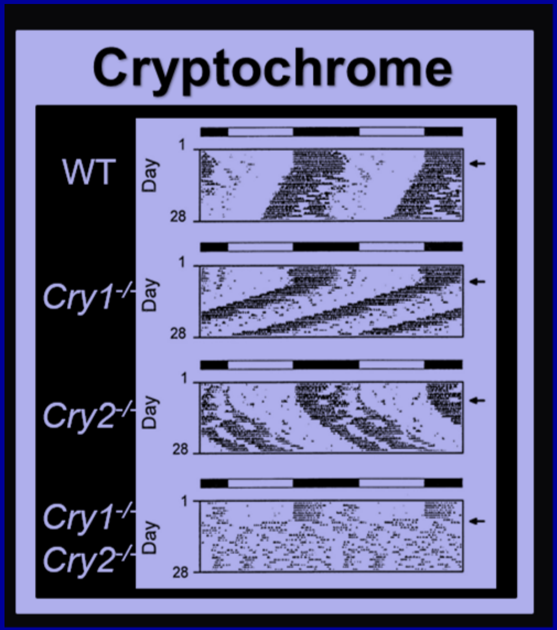
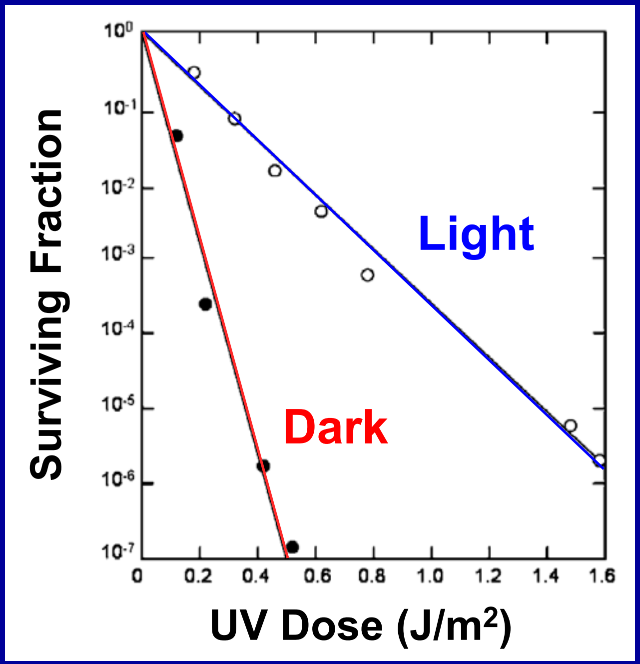


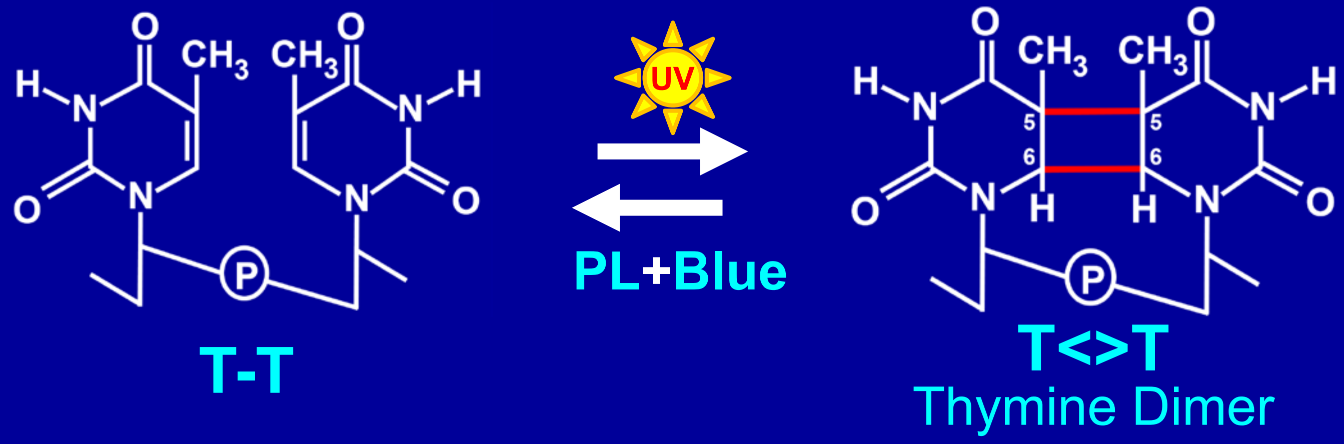


Photoreactivation (DNA Repair)

Rupert and Sancar, UT Dallas, 2009



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

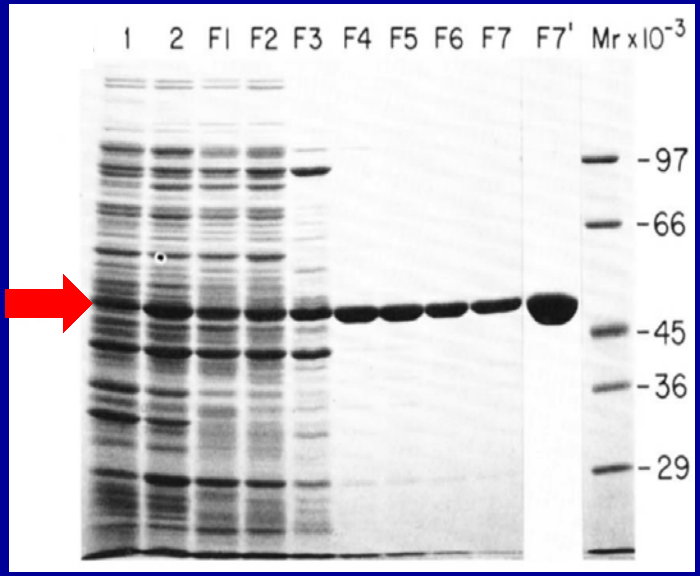


Cloning and Purification of Photolyase

Electron micrograph of the plasmid containing *Phr*

Sancar A (**1977**) PhD Dissertation, UT Dallas

Purified photolyase protein has bright blue color

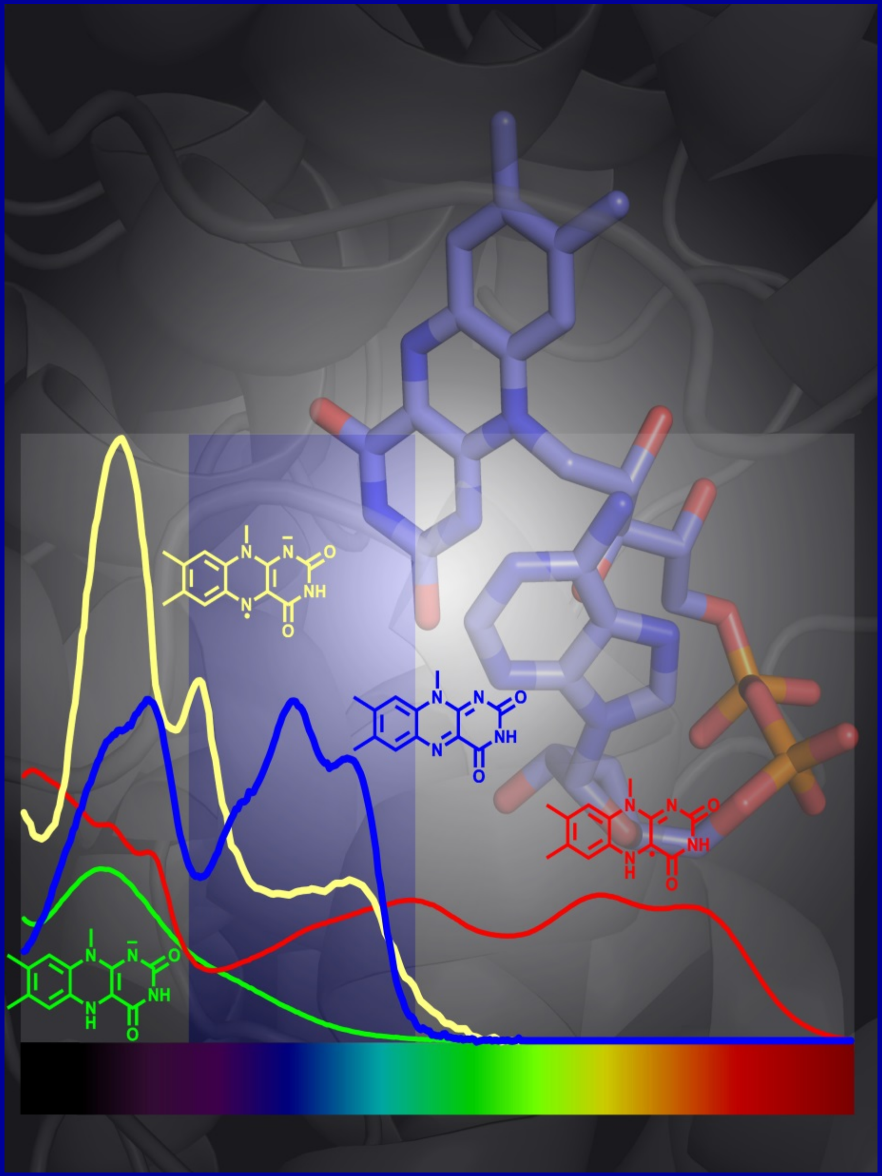


Sancar A, *et al* (**1984**) *JBC* **259**:6028-32

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

Photolyase Contains Two Cofactors

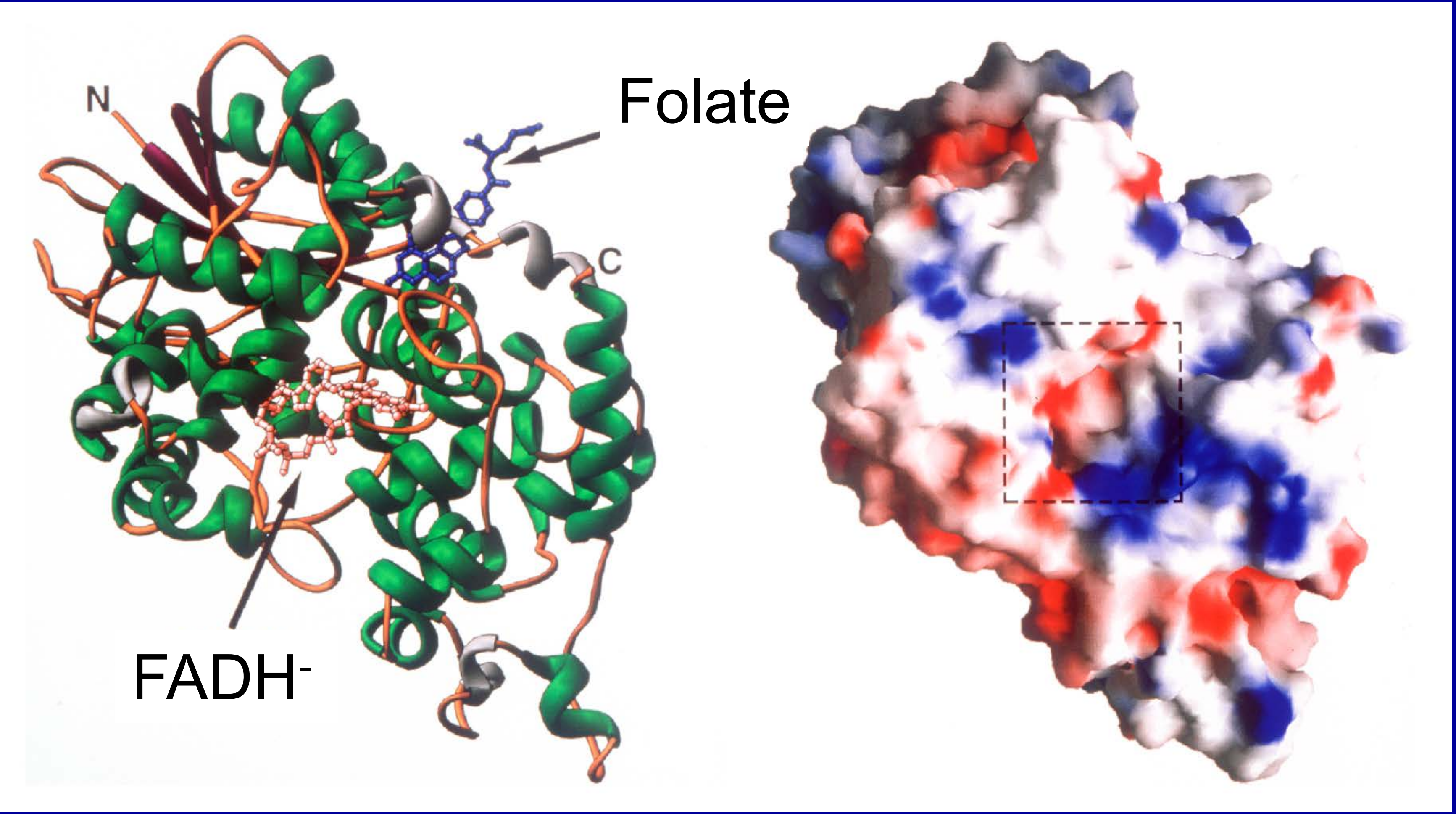
FAD
(catalyst)



Folate
(solar panel)

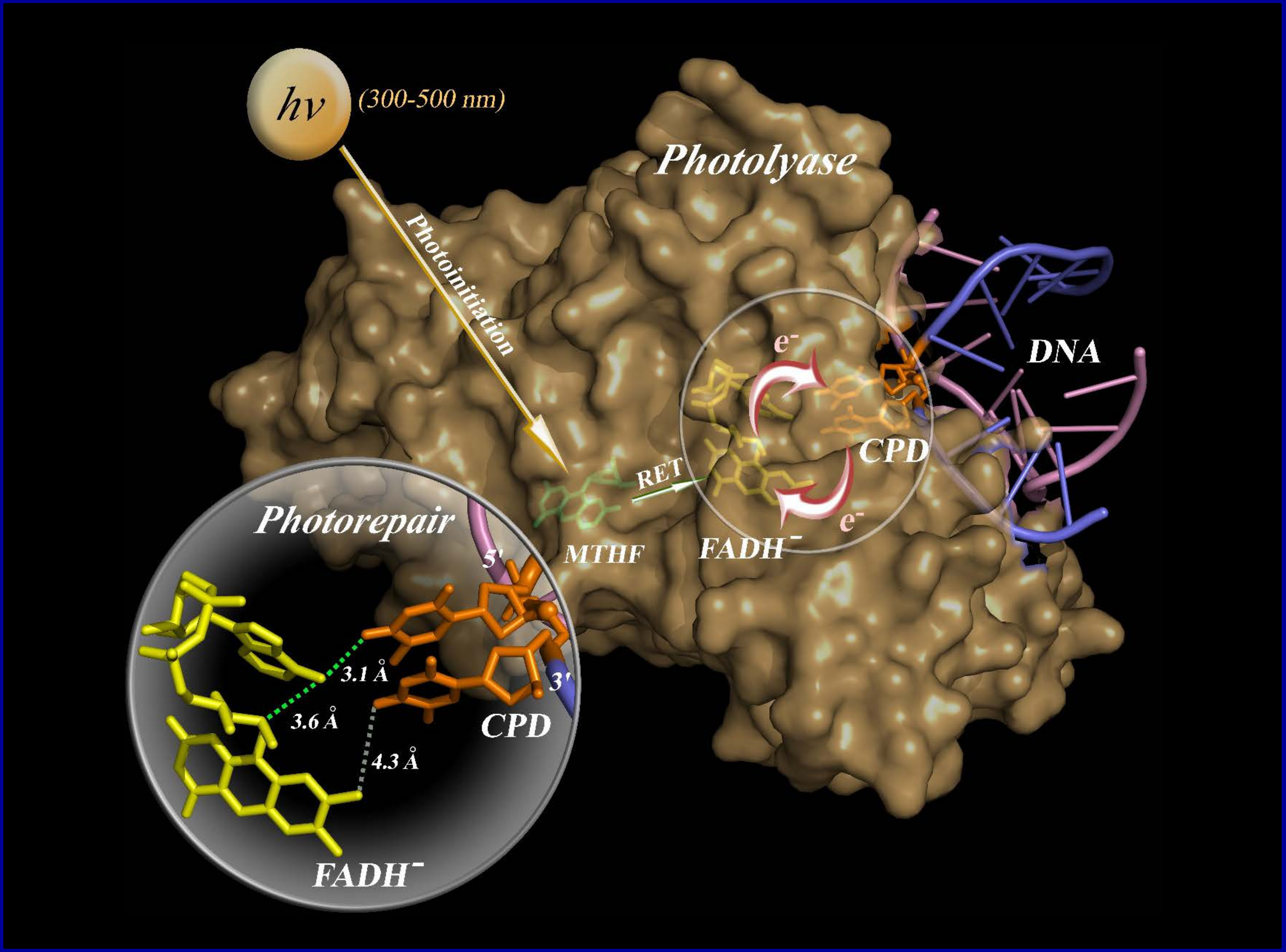
Tan C, *et al* (**2014**) *J Phys Chem A* **118**:10522-30

Structure of Photolyase



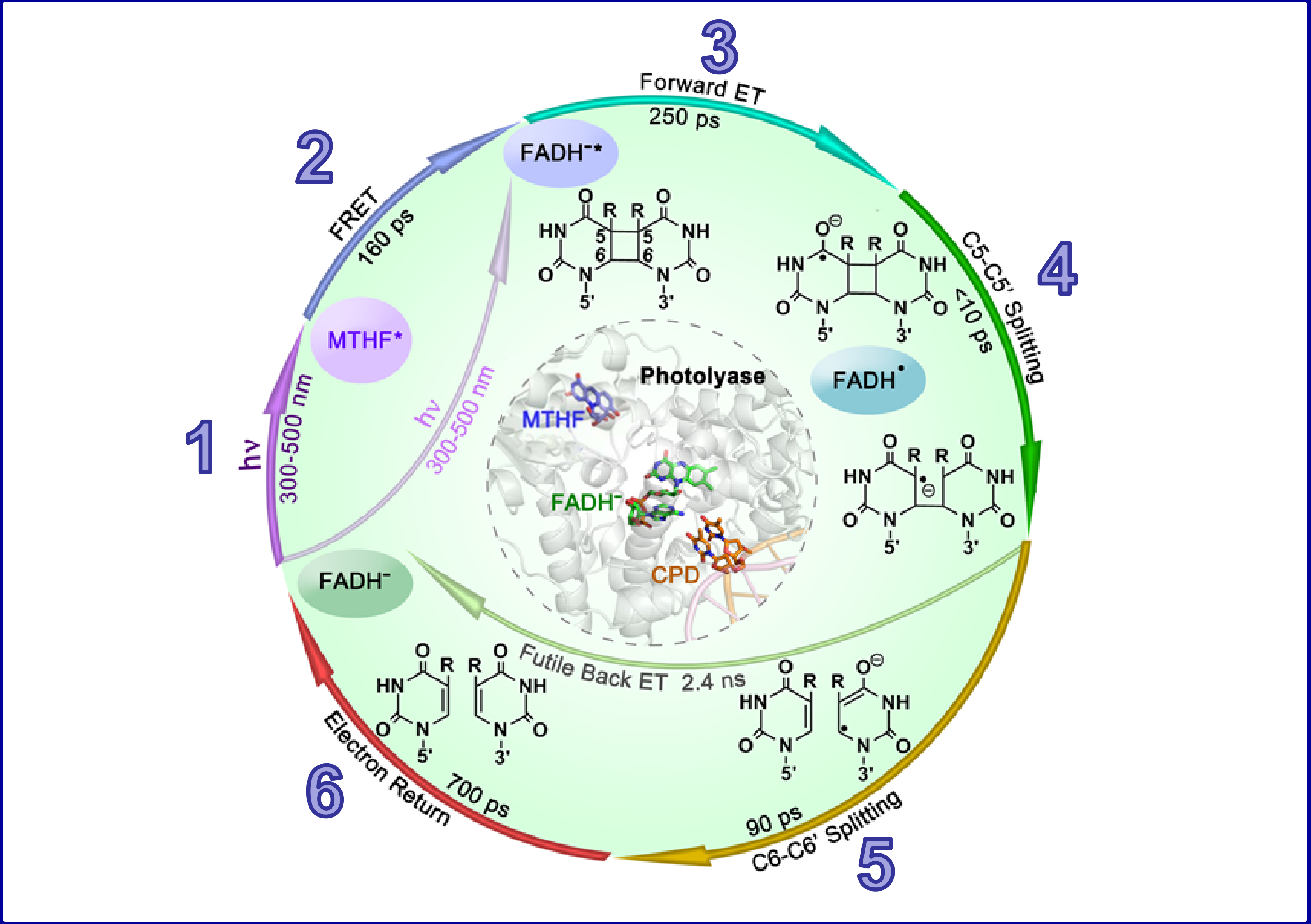
Park HW, *et al* (**1995**) *Science* **268**:1866-1872

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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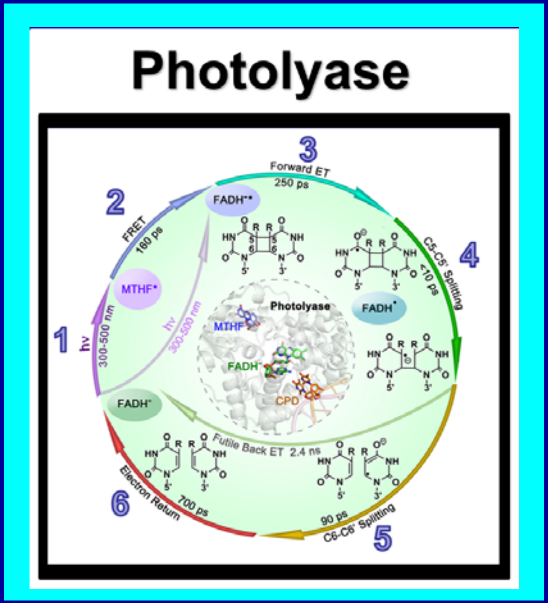
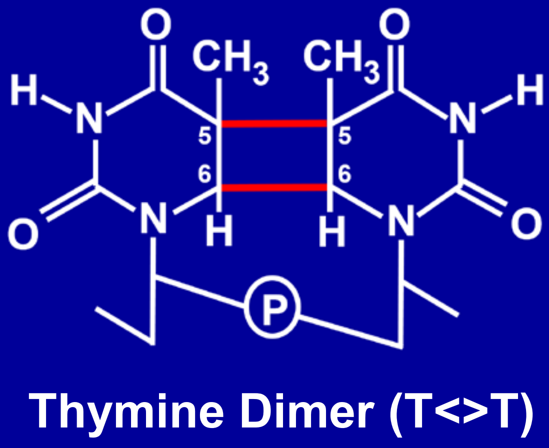
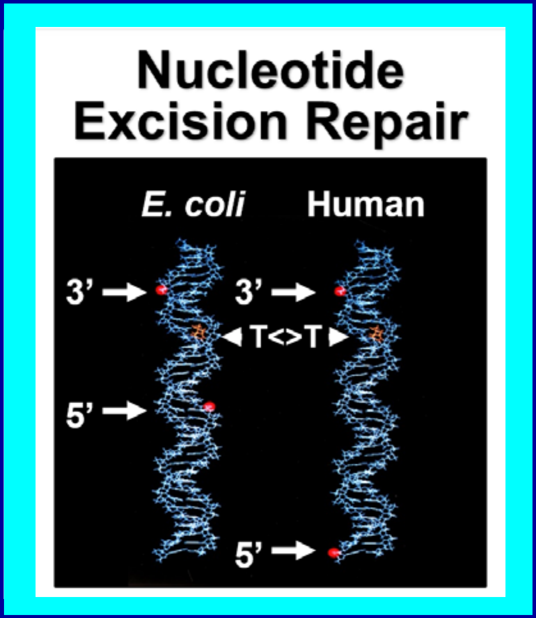
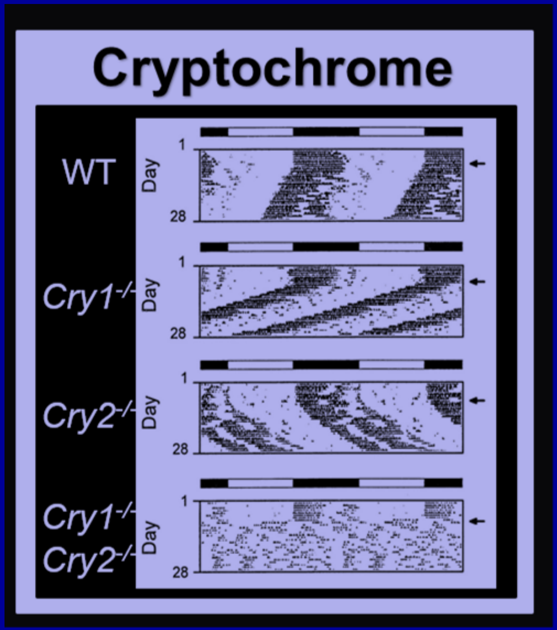
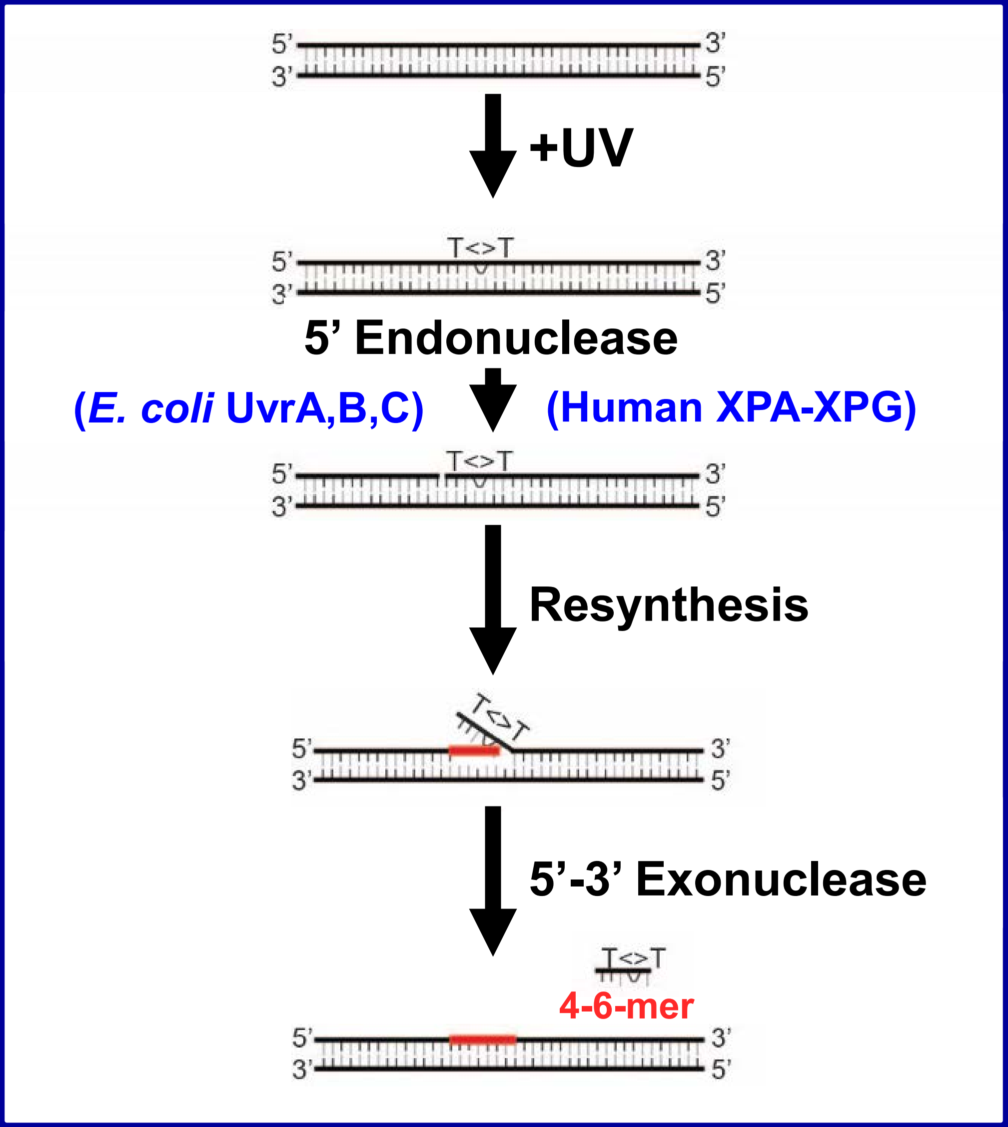

Photolyase


Thymine Dimer (T<>T)


Nucleotide Excision Repair
E. coli
Human
3'
5'
T<>T

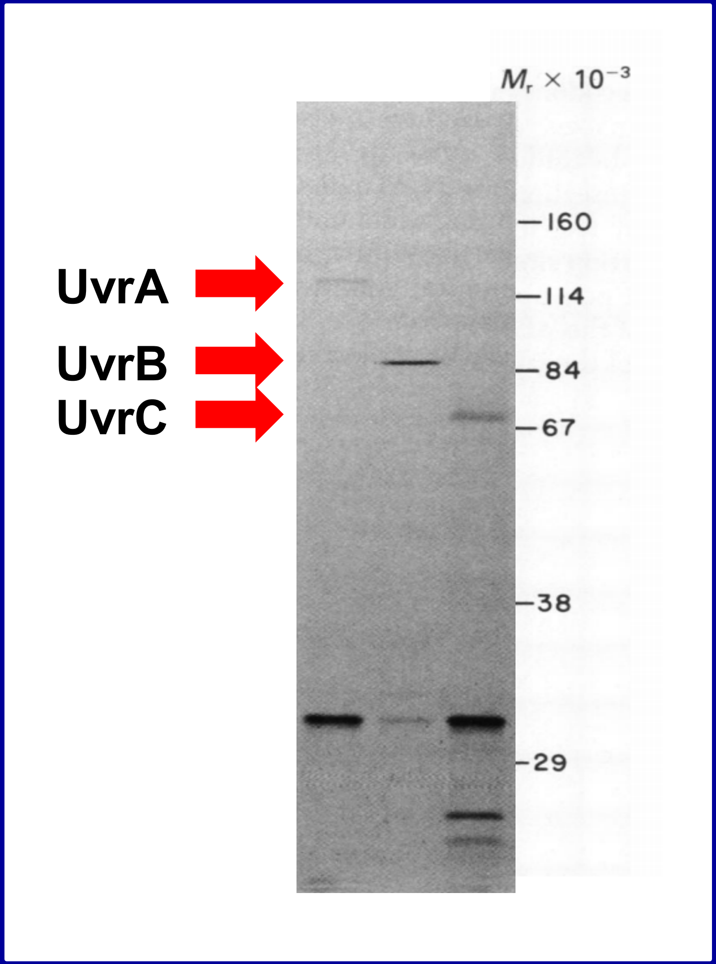

Cryptochrome
WT
Cry1-/-
Cry2-/-
Cry1-/- Cry2-/-
Day
1
28

Model for UV Repair Circa 1982



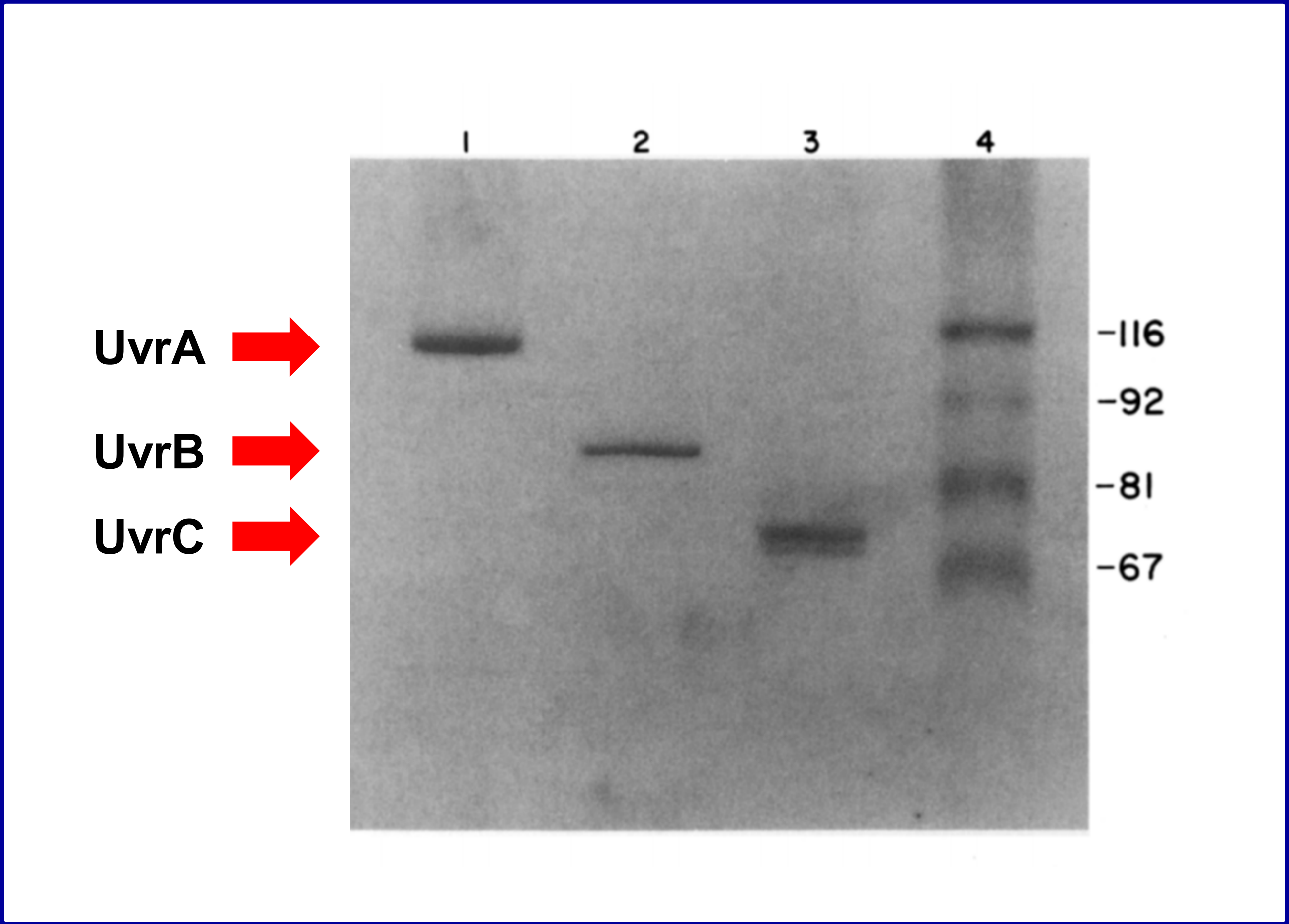
- Thymine dimers are removed from the genome in both *E. coli* and humans.
- Excised thymine dimers were reported to exist in oligonucleotides 4-6 nt in length.
- Excision is genetically controlled by *Uvr* genes in *E. coli* and *XP* genes in humans.
- Following excision, the repair gap is filled in and ligated.
- Excised dimers remain within the cell.

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



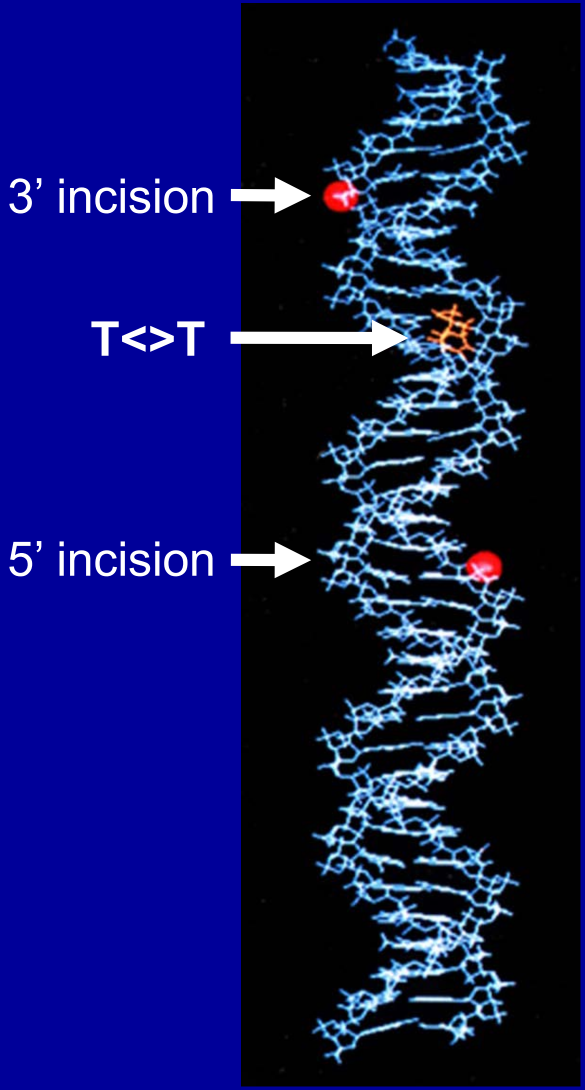
Sancar A *et al* (**1979**) *J Bacteriol* **137**:692-93
Sancar A *et al* (**1981**) *PNAS* **78**:5450-54
Sancar A *et al* (**1981**) *JMB* **148**:63-76
Sancar A *et al* (**1981**) *JMB* **148**:45-62

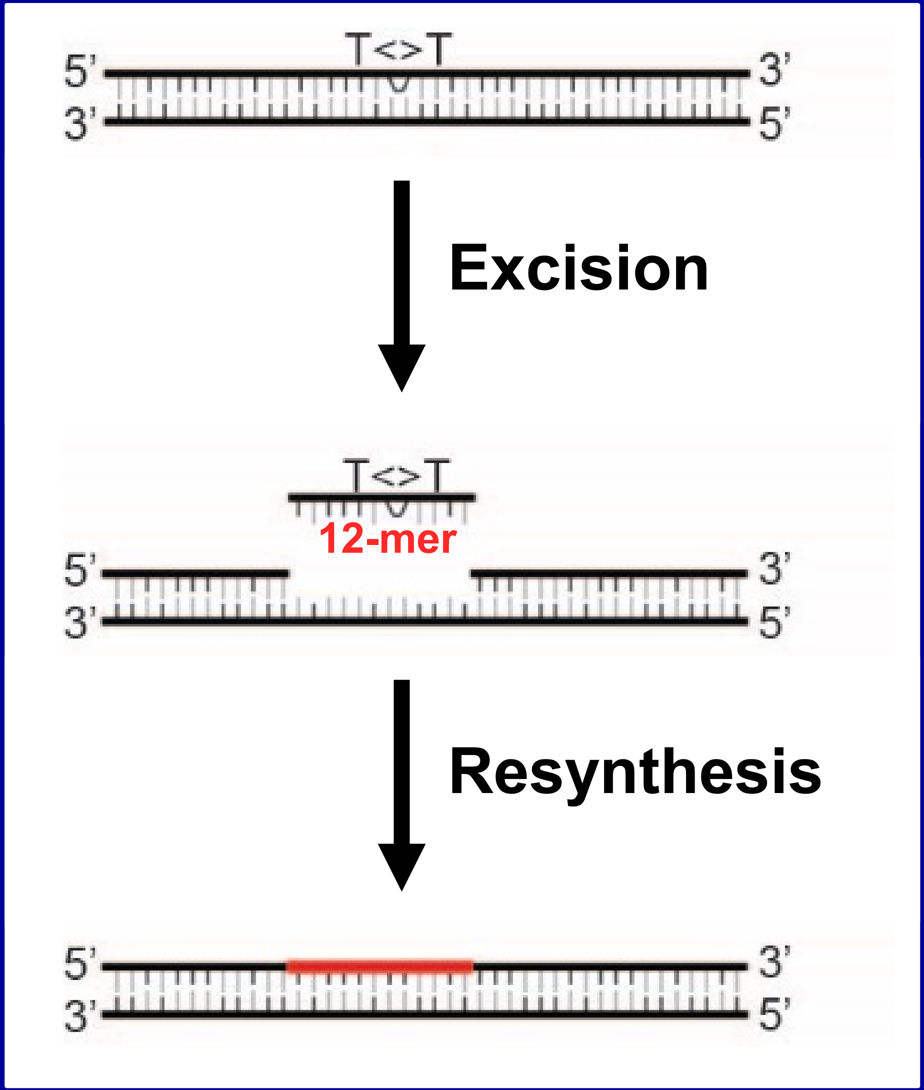
Purification of *E. coli* Excision Repair Proteins



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

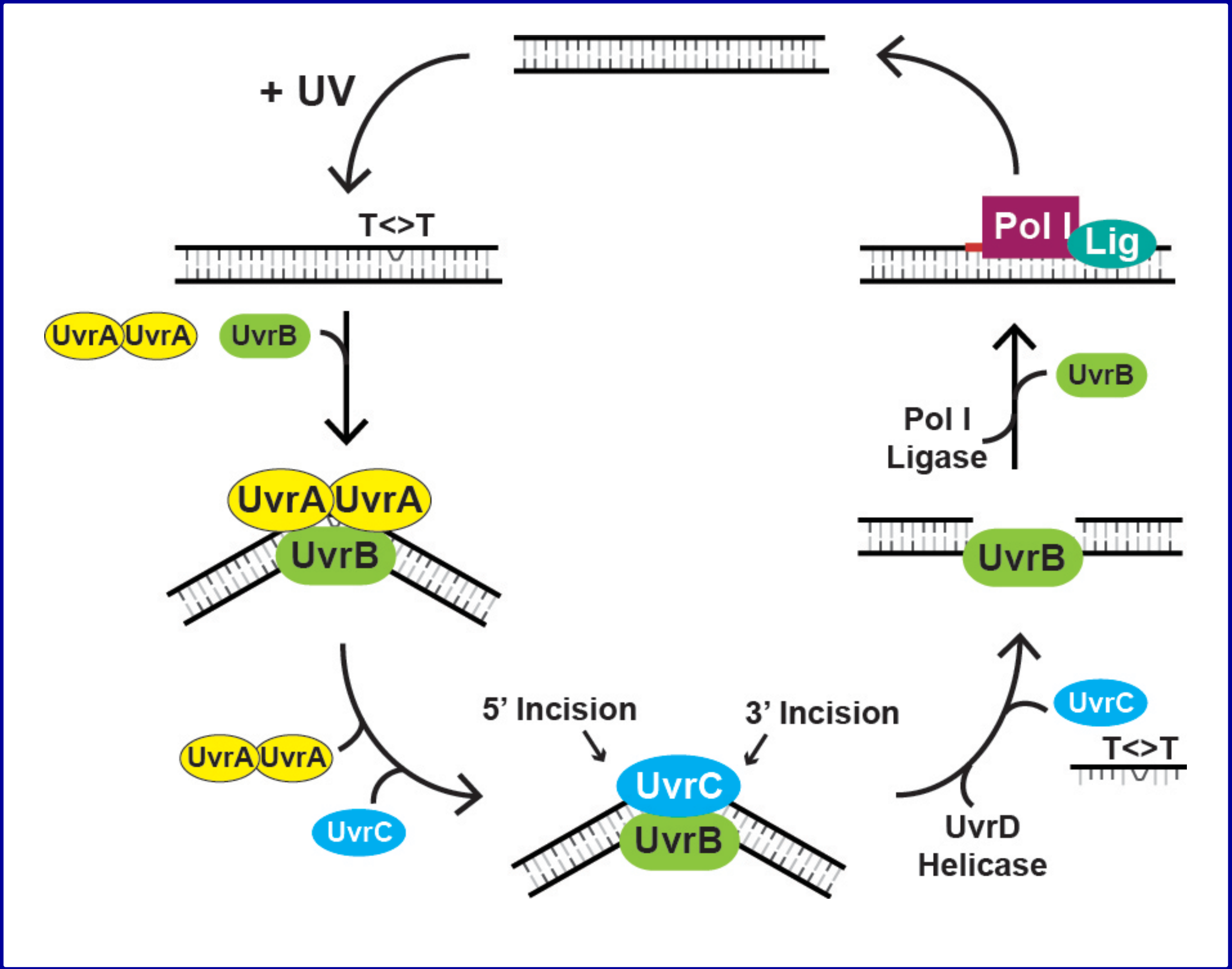
Dual Incisions in *E. coli* Excision Repair





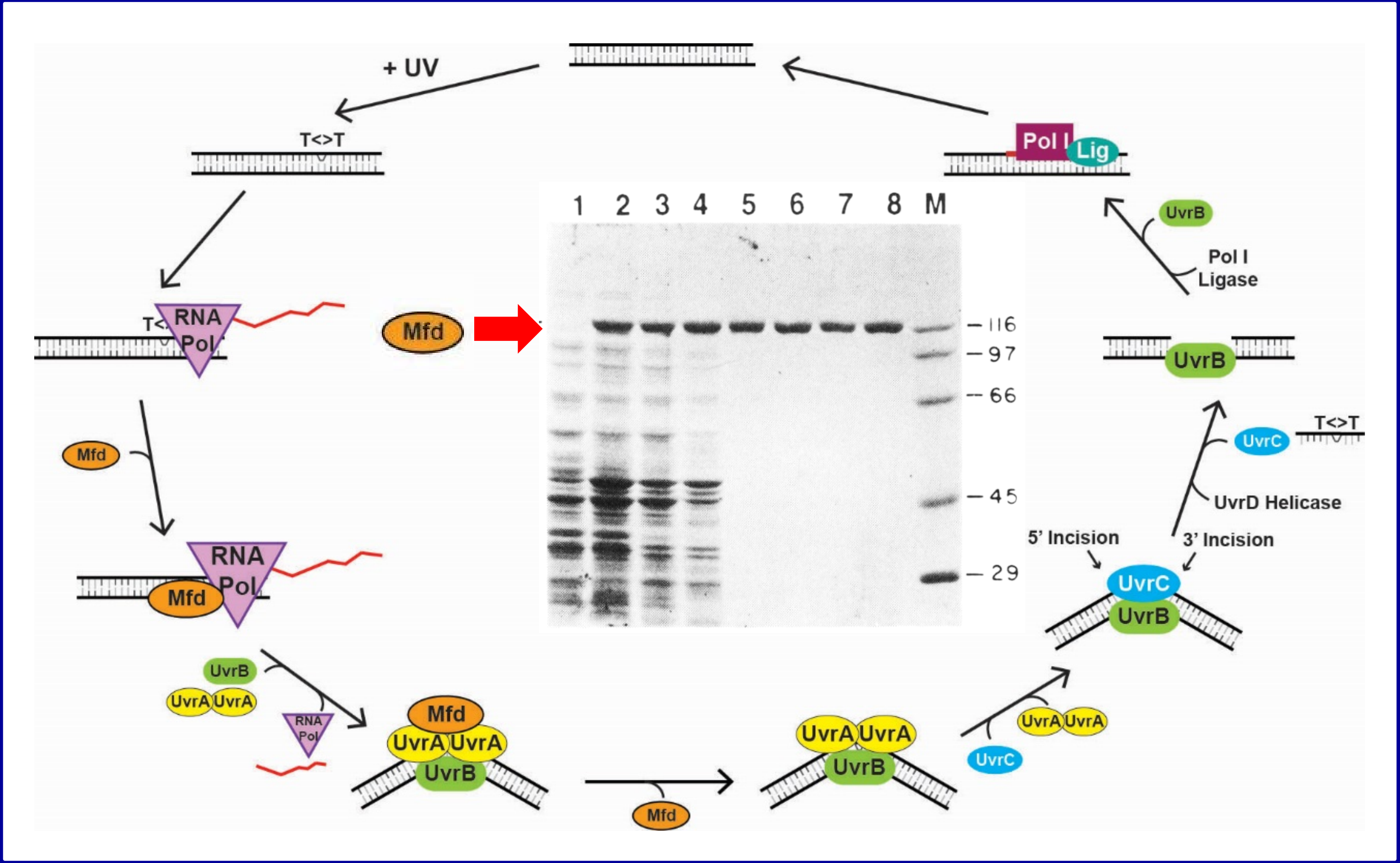
Sancar A and Rupp WD (**1983**) *Cell* **33**:249-60
Sancar A (**1994**) *Science* **266**:1954-56

Mechanism of Excision Repair in *E. coli*



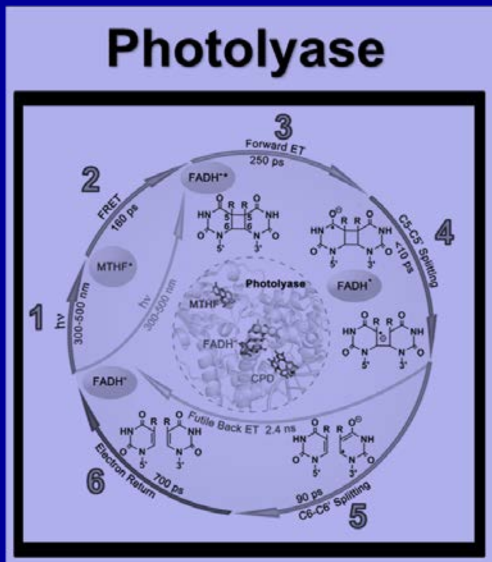
Lin JJ & Sancar A (**1992**) *Mol Microbiol* **6**:2219-24

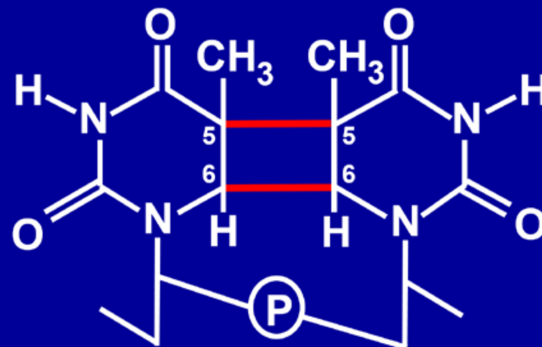
Mechanism of Transcription Coupled Repair



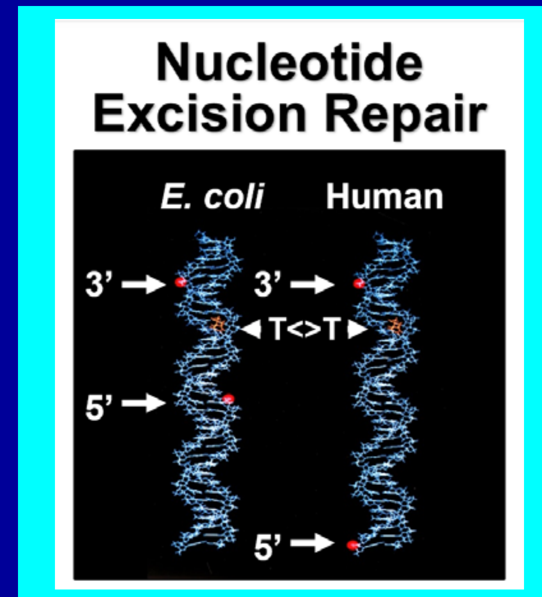
Selby CP & Sancar A (**1993**) *Science* **260**:53-58

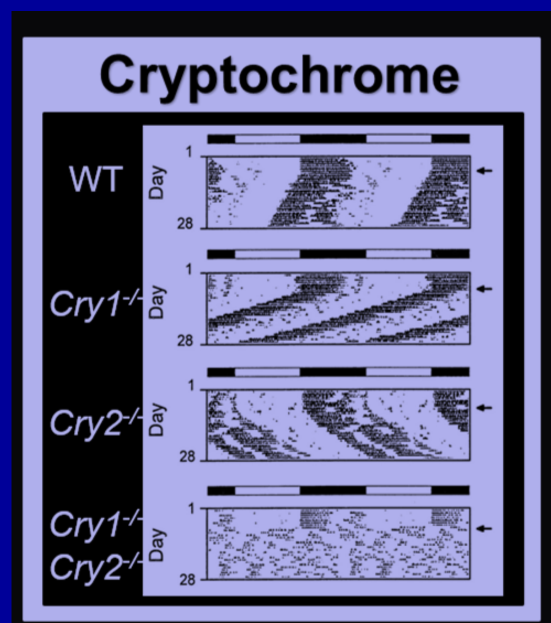
Outline



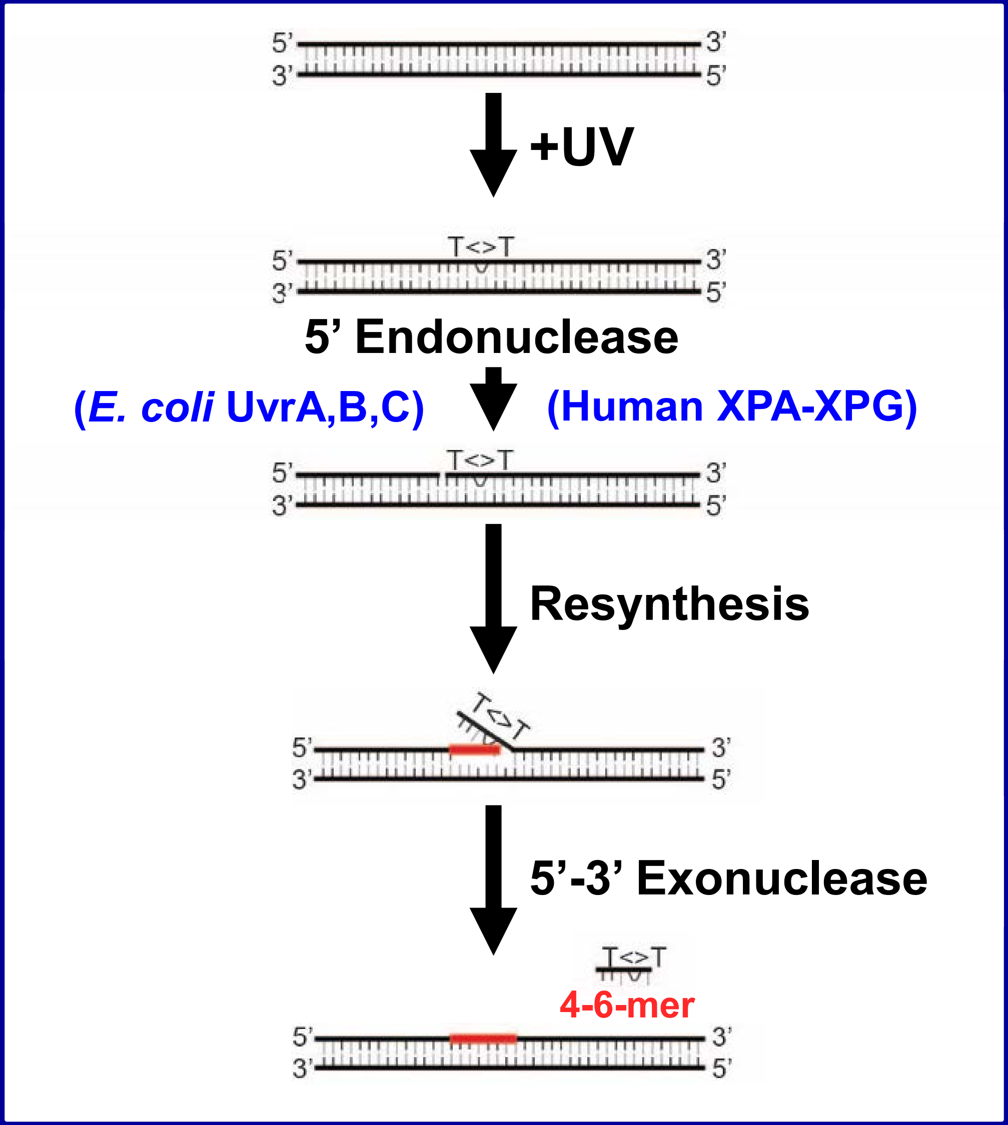


Thymine Dimer (T<>T)





Model for UV Repair Circa 1982



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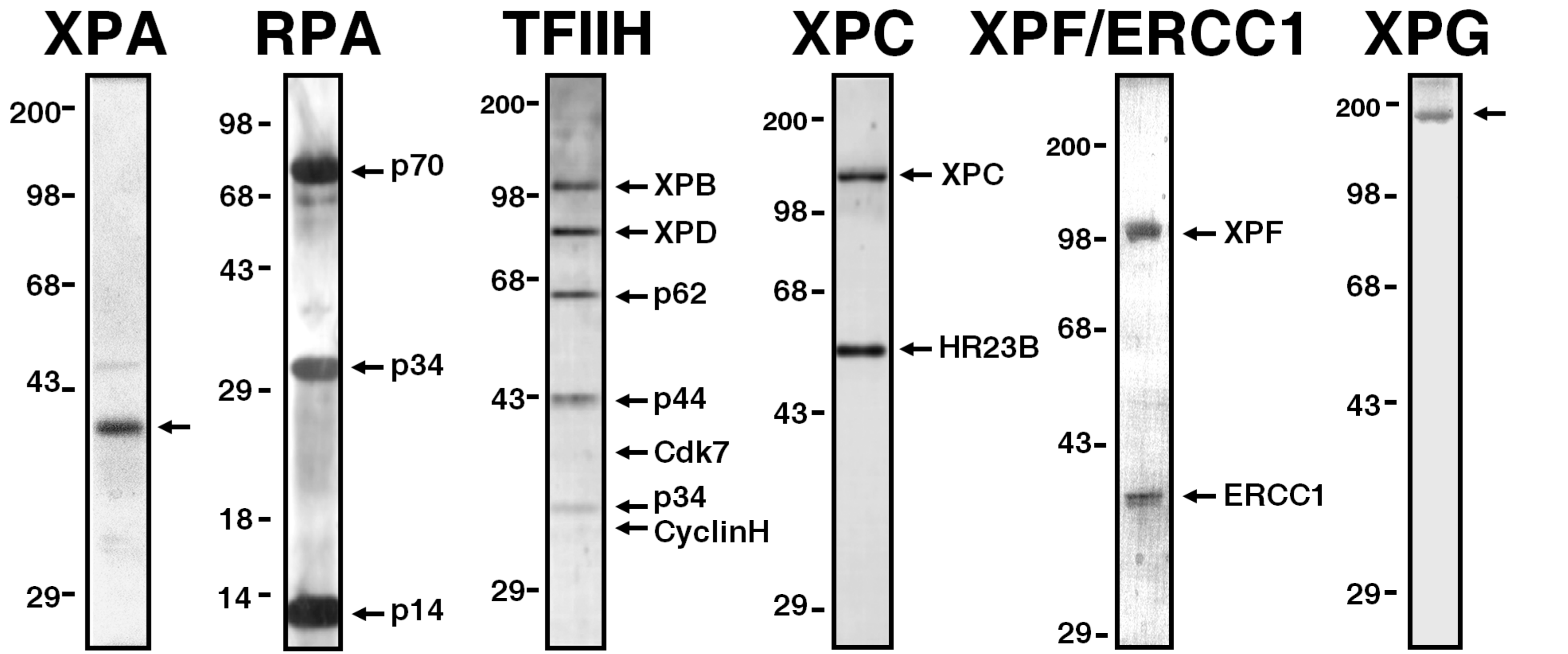
- Excised dimers remain within the cell.

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

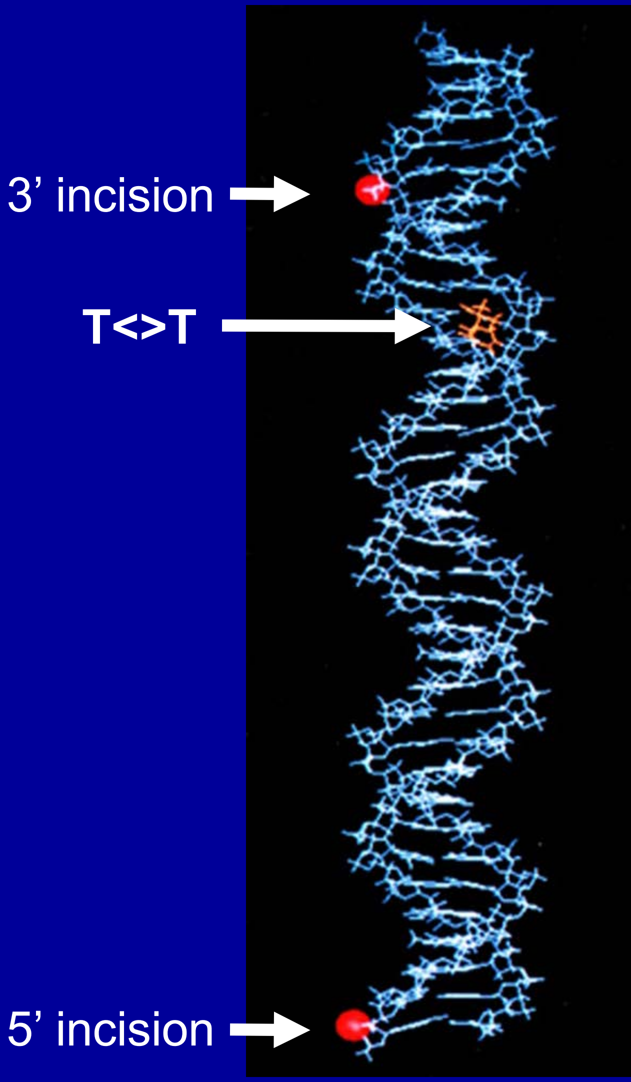
Human Excision Repair Factors

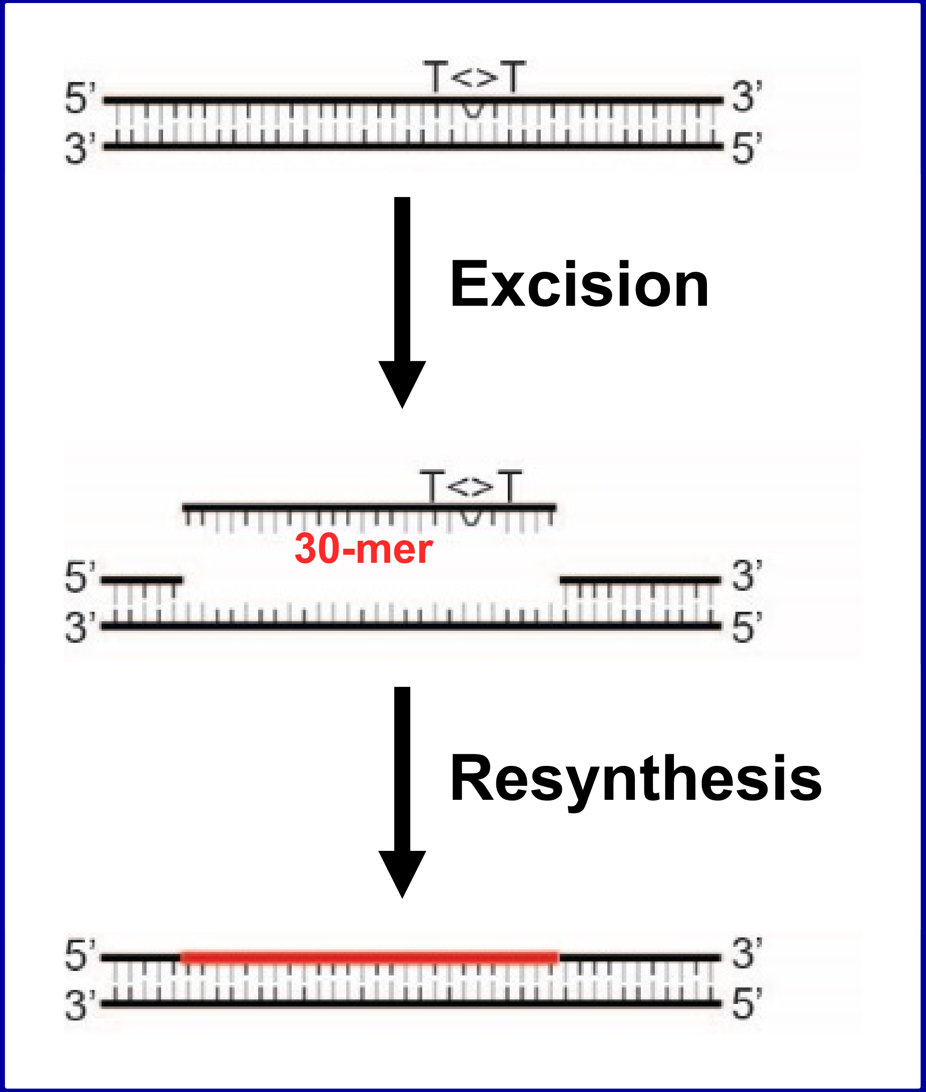


Mu D, *et al* (**1995**) *J Biol Chem* **270**:2415-18

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

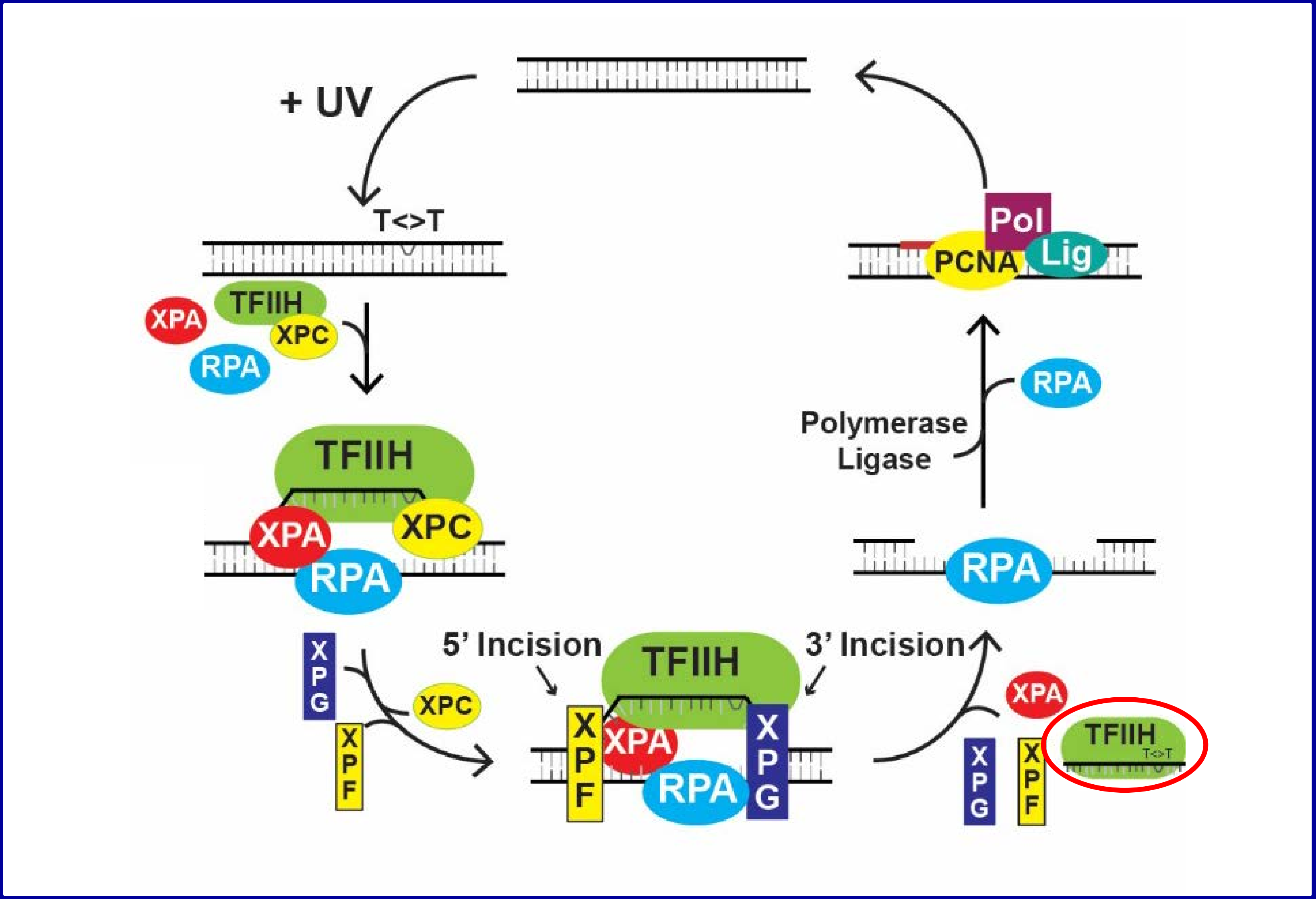
Dual Incisions in human Excision Repair





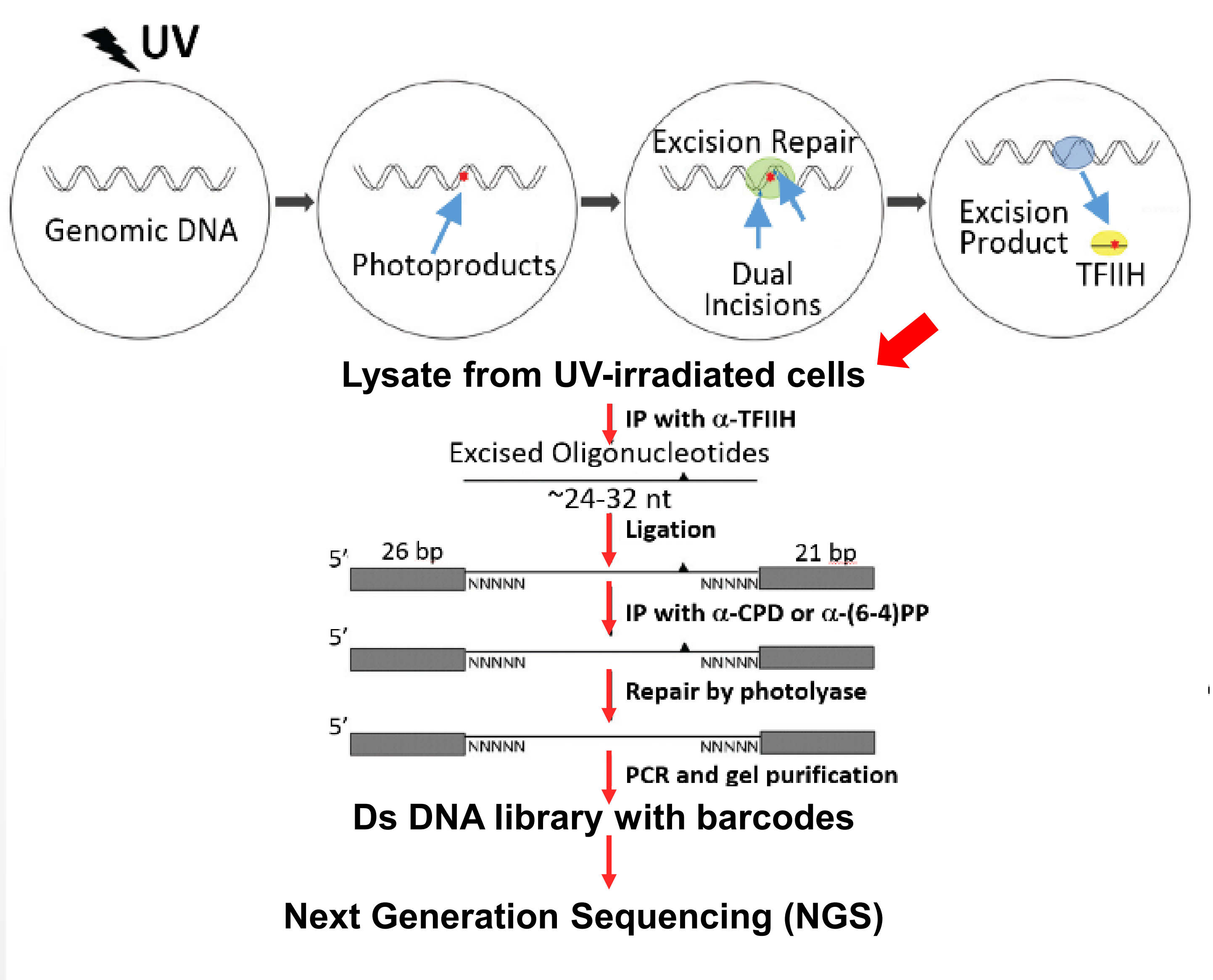
Huang JC, *et al* (**1992**) *PNAS* **89**:3664-68
Sancar A (**1994**) *Science* **266**:1954-56

Mechanism of Excision Repair in Humans



Reardon JT & Sancar A (**2004**) *Cell Cycle* **3**:141-4

Mapping the Excised Oligomer in Humans

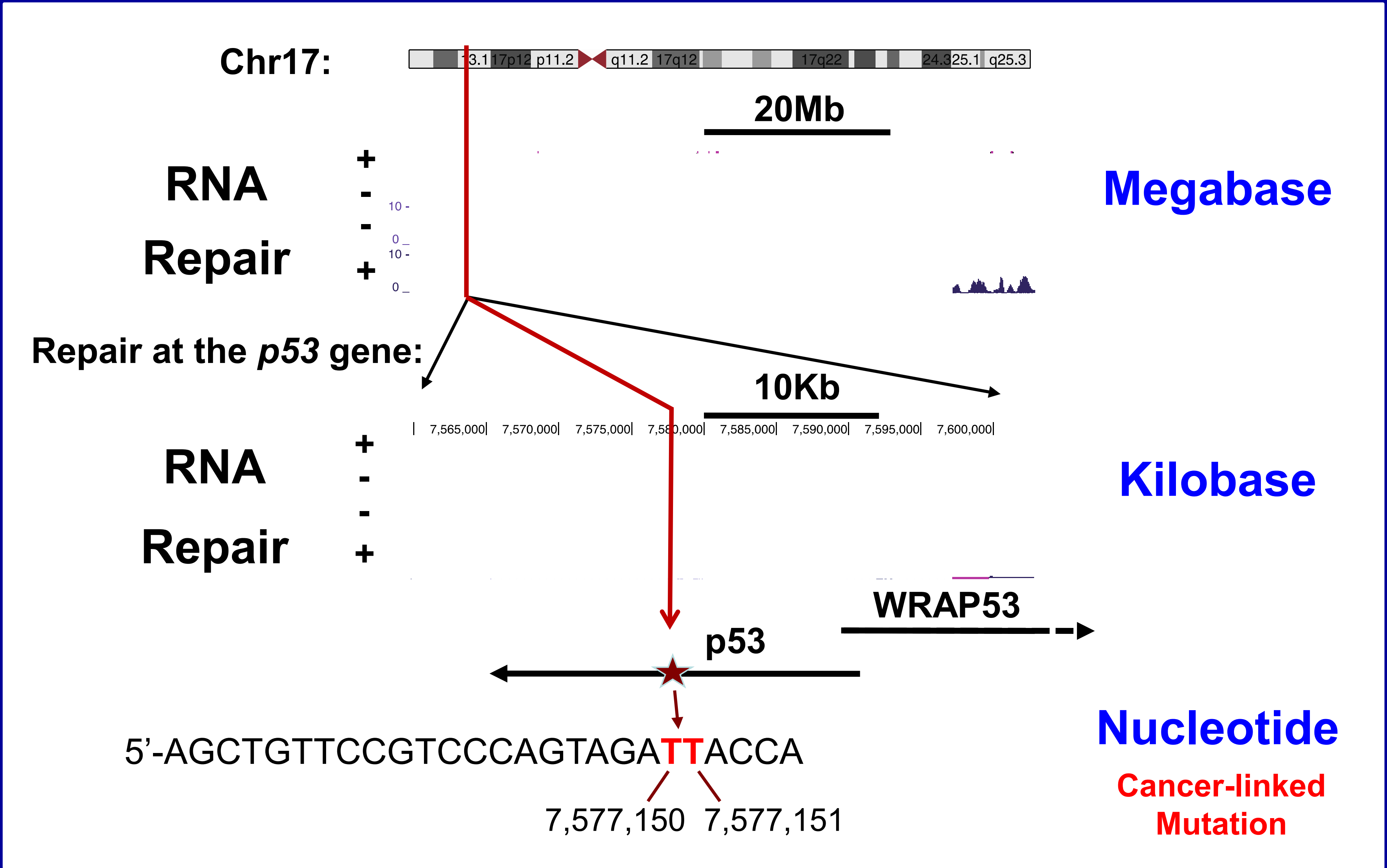


Hu J, Adar S, *et al* (**2015**) *Genes Dev* **29**:948-60

Excision Repair Map of the Human Genome

Hu J, Adar S, *et al* (**2015**) *Genes Dev* **29**:948-60

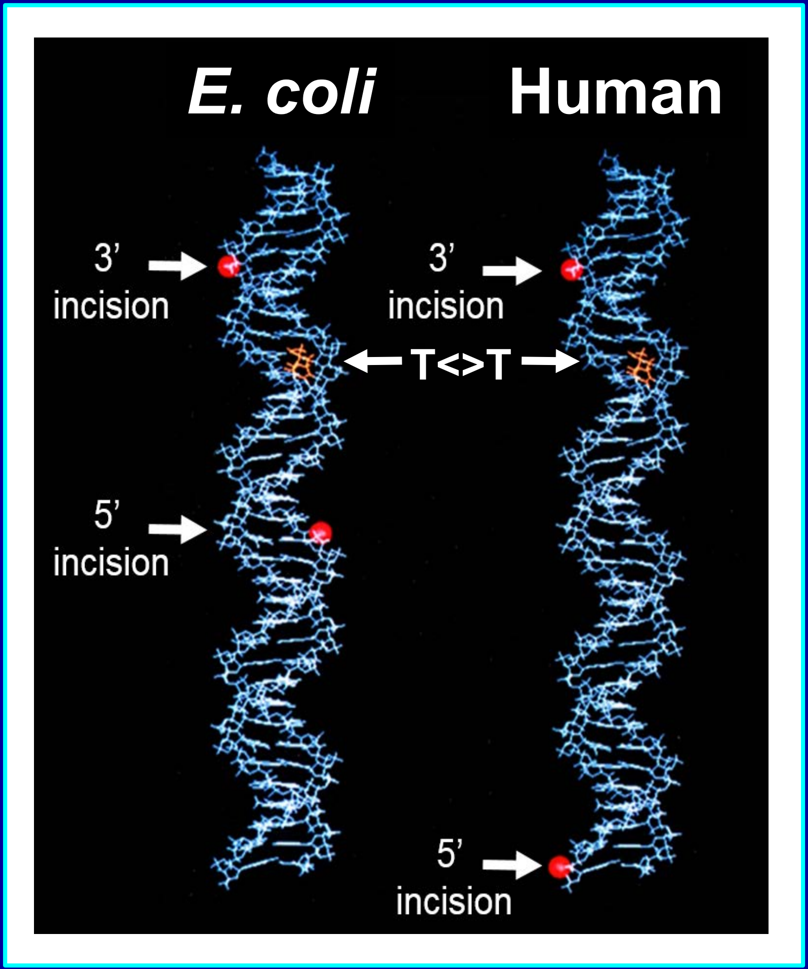
Excision Repair of *p53* at Single Nucleotide Resolution



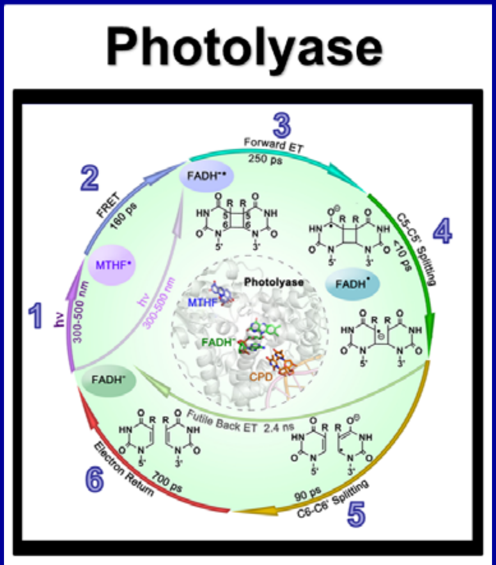
Hu J, Adar S, *et al* (**2015**) *Genes Dev* **29**:948-60

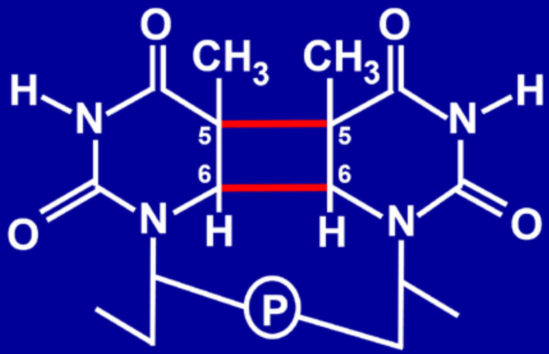
Excision Repair

- 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





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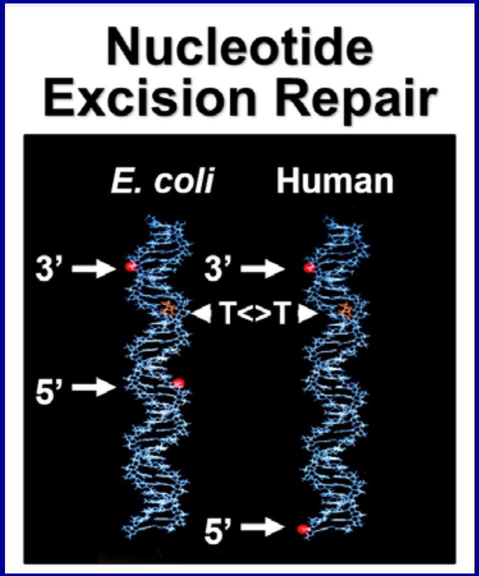
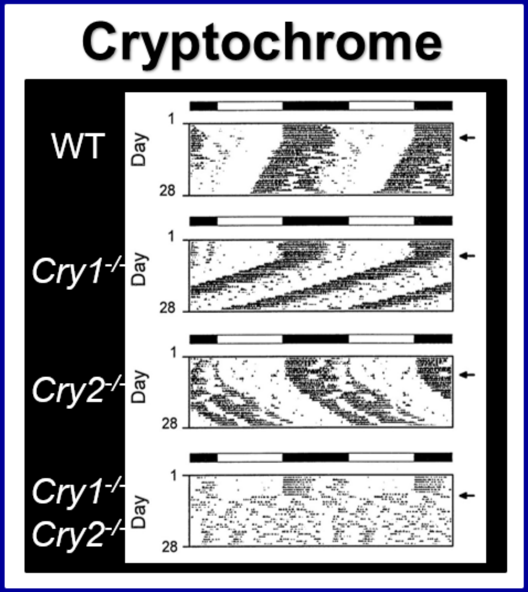




Cryptochrome

- Humans do not have photolyase

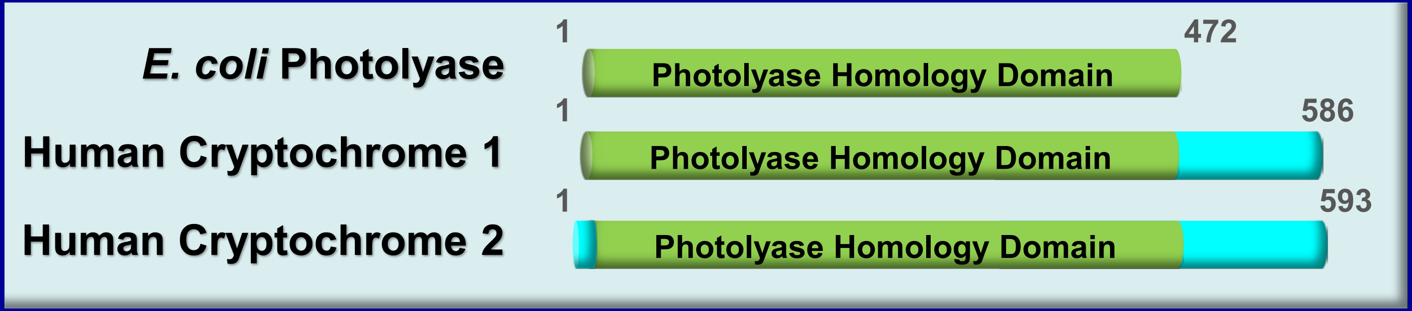
Li YF, *et al* (**1993**) *PNAS* **90**:4389-93

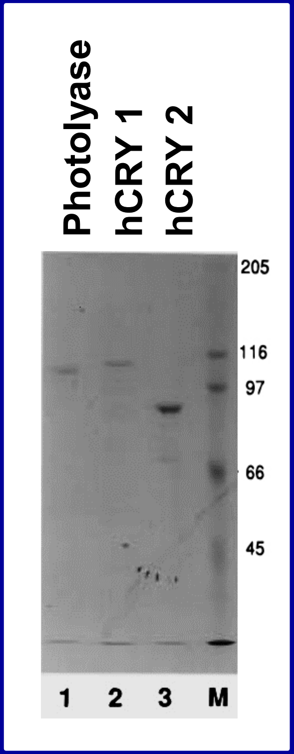
- Humans have a photolyase homolog

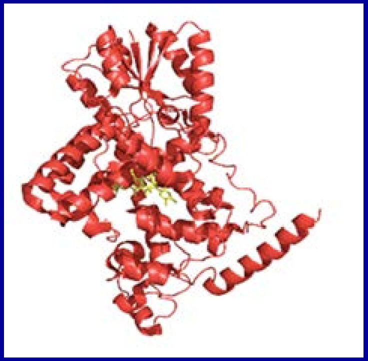
Adams MD, *et al* (**1995**) Nature **377**:3-174

- 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

Spring 1996
Determined that human CRYs are not repair proteins

May - June 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

June - August 1996
Wrote the human CRY paper claiming CRYs are circadian proteins

May - November 1998
Discovered genetic evidence that human CRYs are clock proteins

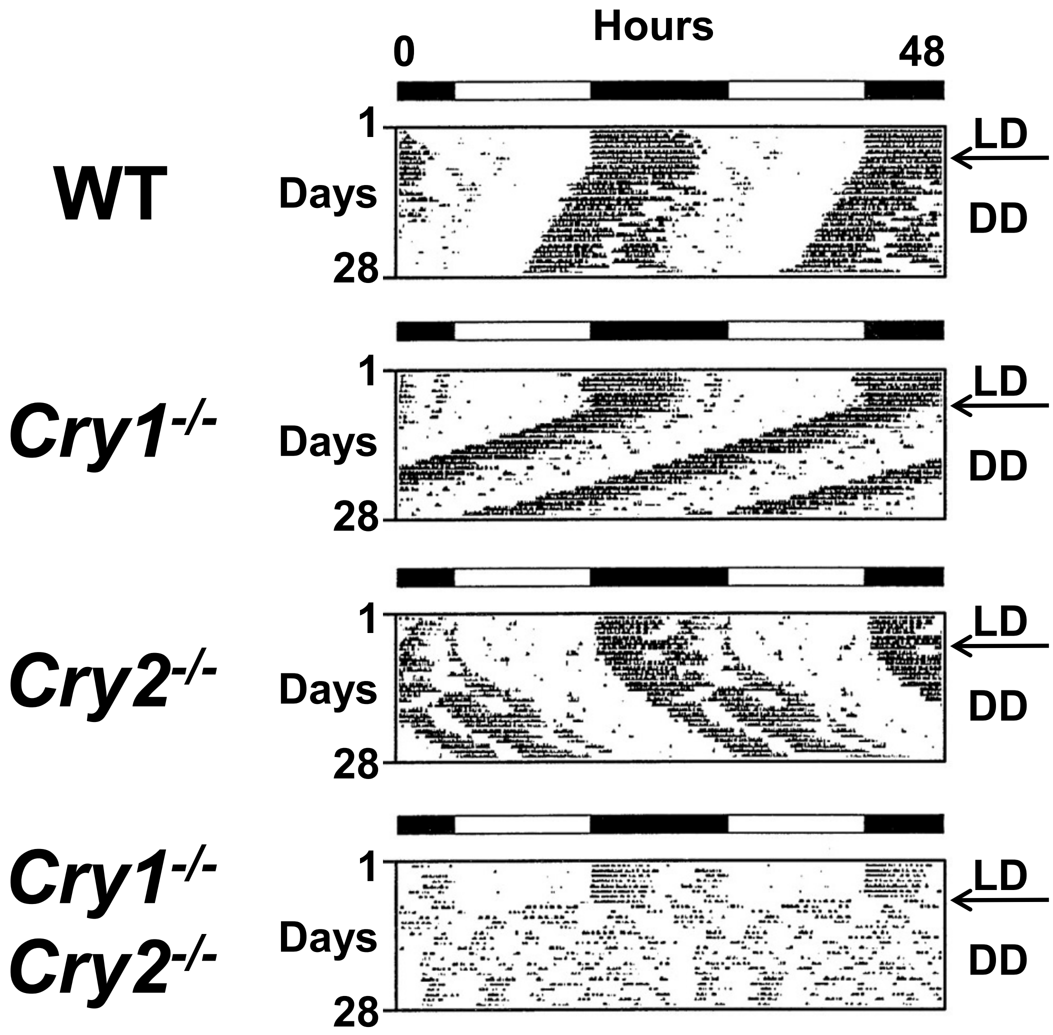
www.NASA.gov

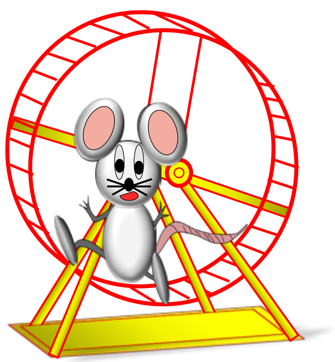
Clock and Circadian Clock

- 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



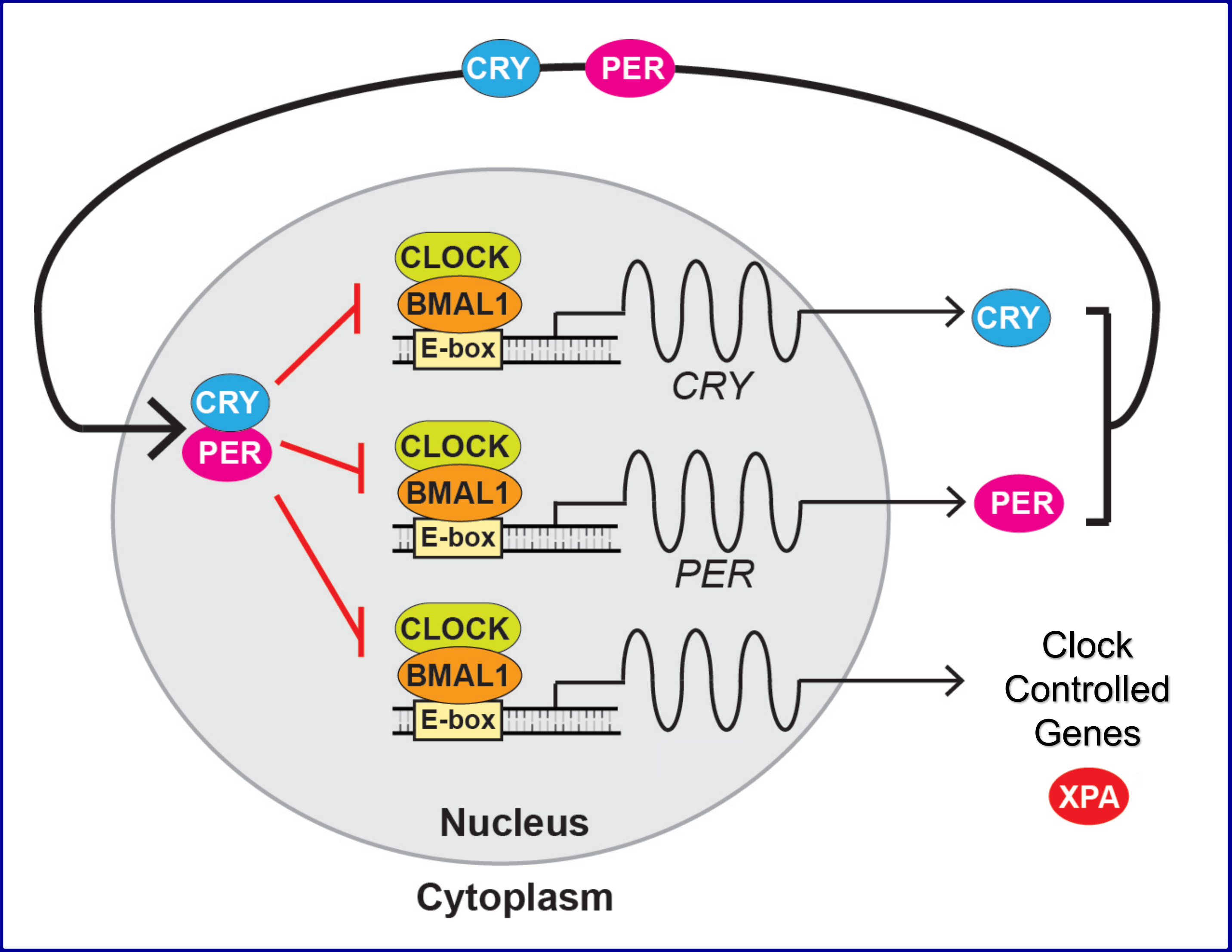


Thresher RJ, *et al* (**1998**) *Science* **282**:1490-94
Vitaterna MH, *et al* (**1999**) *PNAS* **96**:12114-19

Mammalian Clock Genes/Proteins (1996-2000)

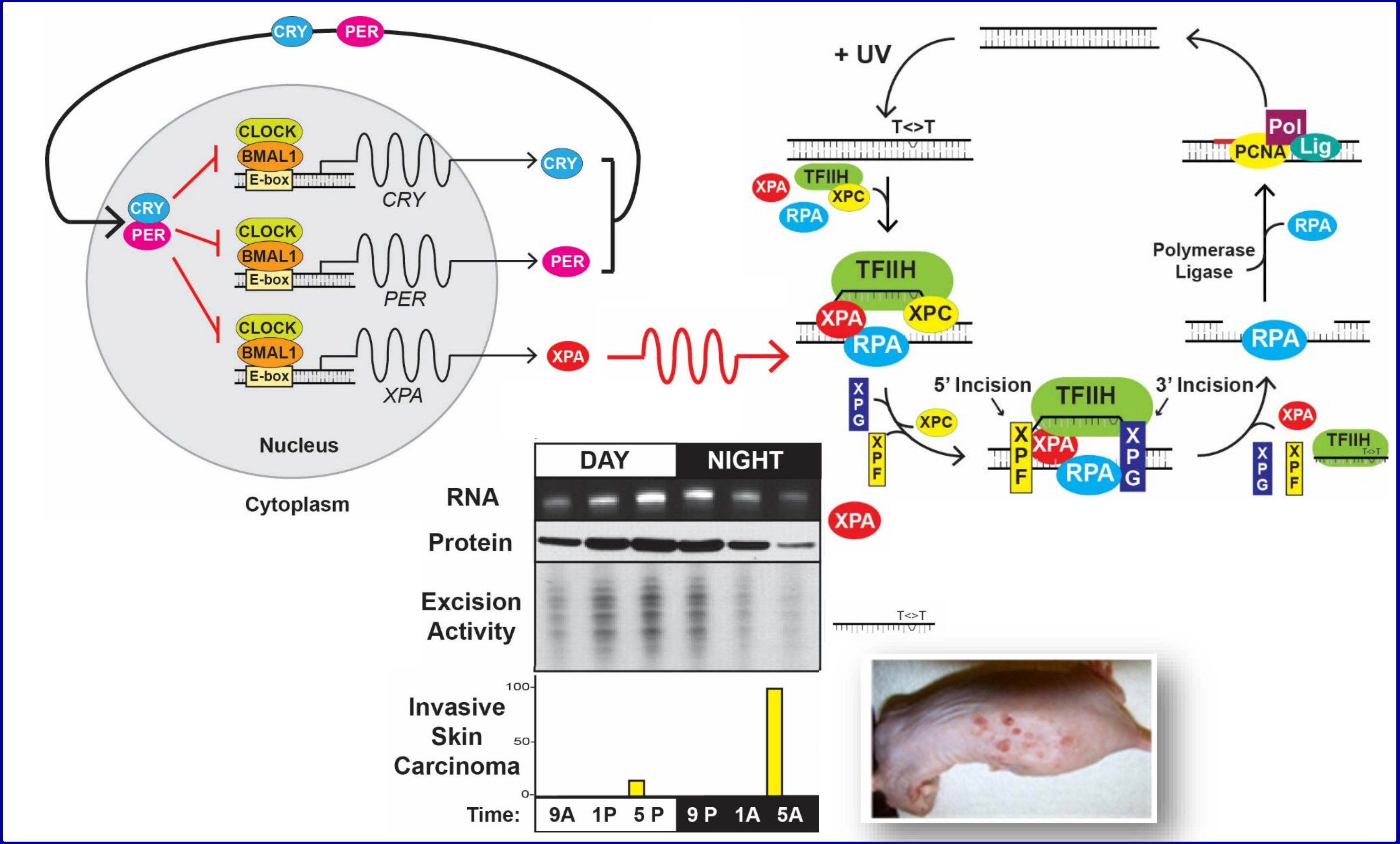
1) CRYPTOCHROME (Flavoprotein)
2) PERIOD (PAS domain)
3) CLOCK (bHLH-PAS)
4) BMAL1 (bHLH-PAS)

Circadian Control Mechanism



Sancar A, *et al* (**2010**) *FEBS Lett* **584**:2618-25

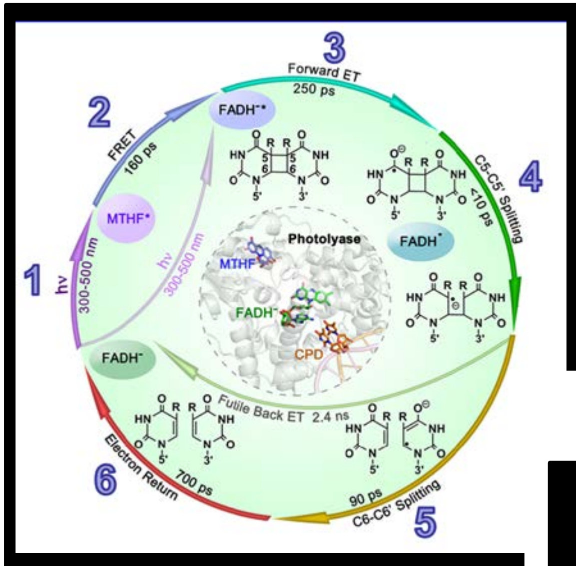
Circadian Control of Excision Repair



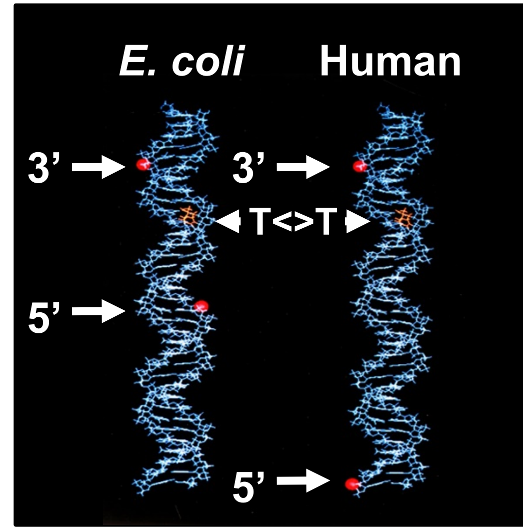
Kang T, *et al* (**2010**) *PNAS* **107**:4890-95
Gaddameedhi S, *et al* (**2011**) *PNAS* **108**:18790-95

Summary

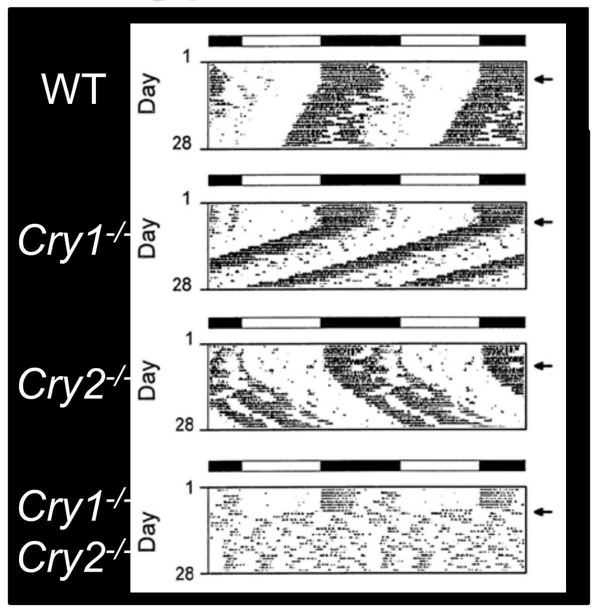
Photolyase



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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Mentors

RUPERT, CLAUD S.
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
Jorns, Marilyn
Kaufmann, William
Kunkel, Thomas
Lieb, Jason
Linn, Stuart
Lippard, Stephen
Modrich, Paul
Rajagopalan, K.V.
Reinberg, Danny
Sancar, Gwendolyn
Smithies, Oliver
Takahashi, Joseph
Taylor, John-Stephen
Thompson, Larry
Van Gelder, Russel
Wold, Marc
Zhong, Dongping

Contributors

Photolyase

Eker, Andries
Sancar, Gwendolyn
Todo, Takeshi
Yasui, Akira

Circadian Clock

Provencio, Ignacio
Reppert, Steven
Rosbash, Michael
Sassone-Corsi, Paolo
Schibler, Ueli
Takahashi, Joseph
van der Horst, Gijsbertus
Young, Michael

Excision Repair

Cleaver, James
Egly, Jean-Marc
Friedberg, Errol
Goosen, Nora
Grossman, Larry
Hanaoka, Fumio
Hanawalt, Philip
Hoeijmakers, Jan
Kisker, Caroline
Prakash, Louise
Prakash, Satya
Tanaka, Kiyoji
Thompson, Larry
Van Houten, Ben
Witkin, Evelyn
Wood, Richard