History of DNA Repair: Four decades of studies of DNA Repair at NIH and The first twenty-four years of the DNA Repair Interest Group

Kenneth H. Kraemer, M.D.
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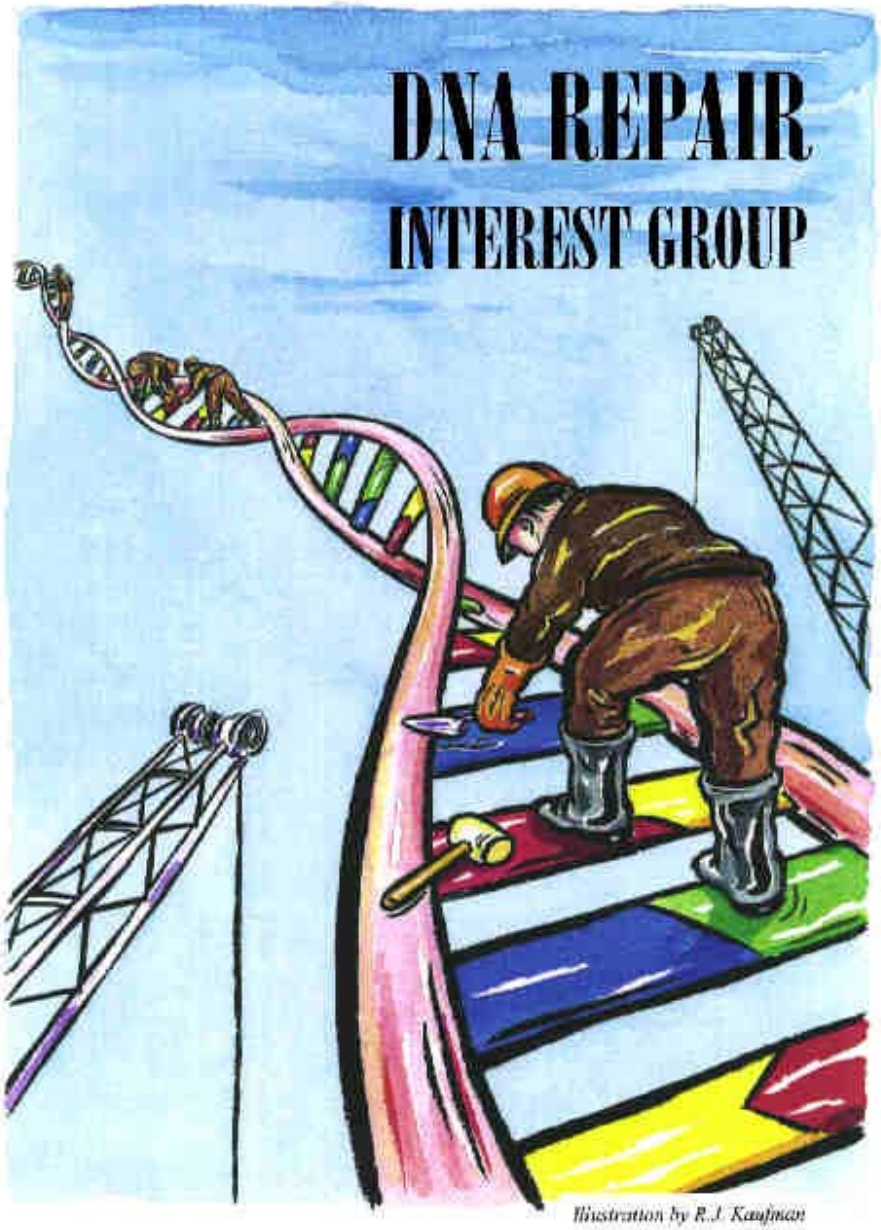
NIA, NIH, Baltimore, MD

Update of: Bohr, V.A. and Kraemer, K.H.: The DNA Repair Interest Group: a global village DNA Repair 4: 405-406 (2005) (editorial)



- Group began 1985 (Building 37 – Bohr/Kraemer)
- Videoconferences began 1995
- Monthly videoconferences
- 14 linked sites across US
- 130+ lectures archived at http://videocast.nih.gov
- e-mail list: >1400 subscribers worldwide
- website

To join send a request to kraemerk@nih.gov

http://videocast.nih.gov/PastEvents.asp?c=5

14 Linked Sites in DNA Repair Interest Group Videoconferences - 2009

- **NIH – Bethesda, MD; NIA, Baltimore, MD; NCI, Frederick, MD; NIEHS, Research Triangle Park, NC**
- **National Labs – Lawrence Livermore, Livermore, CA; Brookhaven, Upton, NY**
- **Universities- State University of New York, Stony Brook, NY; Univ of Kentucky, Lexington, KY; Univ of Michigan, Ann Arbor, MI; Univ of North Carolina, Chapel Hill, NC; Wake Forest Univ, Winston-Salem, NC; Oregon Health & Science Univ, Portland, OR; Univ of Pittsburgh, Pittsburgh, PA; Georgetown Univ, Washington, DC; M.D. Anderson, Smithville, TX**

http://videocast.nih.gov/PastEvents.asp?c=5

DNA Repair Interest Group 2009 Seminar Series		
Tom Misteli	March 17	Spatial Genome Organization in the Formation of Translocations and DNA Repair
Dan Yarosh, AGI Dermatics	January 27	Intercellular Communication of DNA Damage its Repair
DNA Repair Interest Group 2008 Seminar Series		
David Ferguson, U of Michigan	November 18	Roles of Mammalian Mre11 in Genomic Stability and Development
Kyungjae Myung, NHGRI	October 28	Evolutionary Conserved Pathways Suppress Genomic Instability
Stephanie McElhinny, NIEHS	June 17	Division of Labor at the Eukaryotic Replication Fork
Claudia Wiese, LBNL	June 17	Promotion of Homologous Recombination and Genomic Stability by RAD51AP1
Sankar Mitra U of Texas Galveston	May 20	Condensed History of DNA Repair with Emphasis on Small Lesion Repair
Patricia Opresko, U Pitt	April 15	Roles for RecQ Helicases in Telomere Preservation
David Johnson, U of Texas	March 18	E2F1, a Chromatin Accessibility Factor for DNA Repair
Nancy Thomas, UNC	February 19	Molecular Epidemiology of BRAF and NRAS Mutations in Melanomas
DNA Repair Interest Group 2007 Seminar Series		
Guo-Min Li, Univ of Kentucky	November 20	Mechanism of DNA Mismatch Repair
Wolf Heyer, UC Davis	October 16	Roles of the Snf2-Like Motor Protein Rad54
Sam Wilson, NIEHS	June 19	History of DNA Repair
R. Stephen Lloyd Portland, Oregon	May 15	Crossing Oxidative DNA Damage Threshold - Loss of NEIL1 DNA Glycosylase
Masaaki Moriya, SUNY	April 17	DNA Repair and Mutagenesis of ROS-Generated Lesions
Zac Pursell, NIEHS, Paul Wilson, Livermore Karin Scarpinato, WFU	March 20	DNA Repair - Young Investigator Showcase
Laura J. Niedernhofer Univ of Pittsburgh	February 20	Accelerated Aging and Cancer in ERCC1-XPF-Deficient Mouse Models
Dan Camerini-Ortero, NIDDK	January 16	Meiotic DNA Double-Strand Break Repair in Mice and Humans

DNA Repair Interest Group 2006 Seminar Series		
Stephen Meyn, Toronto	December 19	Human Telomeric Proteins TRF1, TRF2 and TIN2 Participate in Novel Cellular Response to DNA Damage
William Kaufmann, UNC	November 21	Human Intra-S Checkpoint Response to UV-induced DNA Damage
Betsy Sutherland, Brookhaven	October 17	Endogenous and Radiation-Induced DNA Damage Clusters and Their Repair
Yie Liu, NIA	September 19	Repair and Maintenance of Eroded Telomeres in Mice
Stuart Linn Univ of California at Berkeley	June 20	History of DNA Repair - Life in the Serendipitous Lane: Excitement and Gratification in Studying DNA Repair
Yossi Shiloh, Tel Aviv University	May 23	ATM: A Sentry at the Gate of Genome Stability
DNA Repair Interest Group	April 18	Young Investigator Showcase - Short Talks at Different Sites
Ian Hickson, Oxford University	March 21	Genomic Instability and Cancer: Insights from Bloom Syndrome
Ben van Houten, NIEHS	February 21	In Search of Damage: Structure-Function Studies of Bacterial Nucleotide Excision Repair Proteins
Ken Kraemer, Vilhelm Bohr, Ben van Houten	January 17	Tribute to Larry Grossman Ph.D. 1924 - 2006
Vesna Rapic-Otrin, U of Pittsburgh	January 17	The UV-DDB-Based Ubiquit E3 Ligases and NER
DNA Repair Interest Group 2005 Seminar Series		
Orlando Scharrer, SUNY	December 20	Mechanisms of Damage Recognition and Dual Incision in Human Nucleotide Excision Repair
Susan Lees Miller, U of Calgary	November 15	The Role of Protein Phosphorylation in Nonhomologous End Joining
Joanne Sweasy, Yale	October 18	Is There a Link Between DNA Polymerase Beta and Cancer?
Jonathan Eisen, TIGR	September 20	More Questions Than Answers: Insights into DNA Repair Processes from Genome Sequencing Projects
Kurt W. Kohn, NCI	June 14	Personal History of DNA Damage and Repair Research 1960-2005
Steve West, Oxford	May 17	Introduction to New Insights into Mechanisms of BRCA2 Regulated Double Strand Break Repair (Entire Lecture Will be Posted Later)
Kyungjae Myung, NHGRI	April 19	Suppression and Formation of Gross Chromosomal Rearrangements: Cross Talk in DNA Metabolism
Miguel Garcia-Diaz, Kyungjae Myung & John Hinz	April 19	DNA Repair - Short Talks by Young Investigators
John Hinz, LLNL	April 19	Distinct Genomic Instabilities Associated with Deficiencies in Homologous Recombination or FANCD2 Monoubiquitination
Wei Yang, NIDDK	March 15	DNA Repair in 3D
Judy Campisi, UC Berkeley	February 15	Genome Maintenance Systems, Cancer and Aging
Michael Seidman, NIA	January 18	Repair & Mutagenesis of Triplex Targeted Interstrand Crosslinks

DNA Repair Interest Group 2004 Seminar Series		
Thomas Wilson, U of Michigan	December 21	Nonhomologous End-Joining: Lessons from Yeast
Vilhelm Bohr, NIA	December 21	DNA Repair Interest Group Tribute to Erling Christen Seeberg, Ph.D., 1946-2004
Susan Olson Oregon Health Sci Univ	November 16	Exploring Cellular and Molecular Aspects of Fanconi Anemia from a Cytogenetic Perspective
Roel Schaaper, NIEHS	October 19	Novel Mutators and Correction of Replication Errors in E Coli
Kenneth H. Kraemer, NCI	September 21	Clinical, Molecular & Epidemiologic Studies of Xeroderma Pigmentosum & Related Disorders of DNA Repair
Sikandar Khan, NCI	June 15	Two Essential Splice Lariat Branchpoint Sequences in one Intron in a Xeroderma Pigmentosum DNA Repair Gene
Diane Cabelof	June 15	The Ability of a Cell to Repair DNA Damage Influences Aging and Cancer
Francesca Storici, NIEHS	June 15	Mechanisms of Recombination and Double Strand Break Repair in Yeast with Targeted Oligonucleotides and the Delitto Perfetto Approach
Larry Thompson, LLNL	May 18	History of DNA Repair: CHO Cells and DNA Repair - a Long-Lasting Affair
David Wilson, NIA	April 20	Regulation of Central Steps in Human Base Excision Repair
Alan Lehmann, Sussex Univ	March 16	Translesion Synthesis and Polymerase Switching in Human Cells
Tom Kunkel, NIEHS	March 16	DNA Repair Interest Group Tribute to Dale W. Mosbaugh, Ph.D., 1953-2004
Dmitry Gordenin, NIEHS,	February 17	Inhibition of Mismatch Repair by Cadmium
Steve Kowalczykowski, UC Davis	January 20	Biochemistry of Recombinational DNA Repair: Common Themes
DNA Repair Interest Group 2003 Seminar Series		
Andre Nussenzweig, NCI	December 16	The Role of DNA Breaks in Genomic Instability
Lei Li , M.D. Anderson	November 18	DNA Damage Cell Cycle Checkpoint: Beyond Buying Time for Repair
Alain Sarasin, Institut Gustave Roussy	October 14	Xeroderma Pigmentosum: Role of Pol ETA in UV-Induced Mutagenesis
Satya Prakash, U of Texas	September 16	Studies of Human DNA Repair Diseases in Yeast
Satya Prakash	September 16	Translesion Synthesis DNA Polymerases of Yeast and Humans
Karen Vasquez, MD Anderson	June 17	Processing Site-Specific DNA Lesions by DNA Repair and Recombination
Tom Rosenquist, SUNY	June 17	NEIL Proteins and Base Excision Repair in Mice
John Bradsher, NCI	June 17	Roles of the Cockayne Syndrome Proteins in Nucleotide Excision Repair and Transcription
Errol Friedberg, U of Texas	May 20	Honest Jim Revealed - The Writings of James D. Watson
Qingyi Wei, M.D. Anderson	April 15	DNA Repair Function, Polymorphisms and Cancer Risk in the General Population
Sankar Mitra, U of Texas	March 11	Co-ordination of Oxidative Damage Repair in the Mammalian Genome
Stephen J. Elledge, Baylor	March 05	Sensing and Responding to DNA Damage
John Taylor, NIEHS	January 21	DNA Repair Gene Polymorphisms and Cancer Risk

DNA Repair Interest Group 2002 Seminar Series		
John Tainer, UC Berkeley	December 17	Conformational Controls and DNA Repair Coordination
Rob Sobol, U of Pittsburgh	November 12	DNA Base Damage and Repair Intermediates: Out of the Pan and into the Fire
Al Fornace, NCI	October 15	Convergence of the p53 and MAP Kinase Stress Signaling Pathways after UV Radiation
Dale Ramsden, UNC	September 17	DNA Double Strand Break Repair
David Chen, LBNL	June 18	Role of DNA-PK in Cellular Responses to DNA Damage
Mark J. Schofield, NIH	May 21	DNA Mismatch Repair
Sunitha Yanamadala, U of Michigan	May 21	Role of Mismatch Repair Proteins in Signaling P53 and Apoptosis
Federica Marini - U of Pittsburgh	May 21	A Human DNA Helicase Homologous to the DNA Crosslink Sensitivity Protein MUS308
DNA Repair Interest Group	April 16	Tribute to Dr David Busch, Ph.D., M.D. July 25, 1953 to April 11
Philip Hanawalt, Stanford	April 16	Half a Century of DNA Repair: An Historical Perspective
Alan Tomkinson, U of Texas	March 19	Mechanisms of DNA End Joining
Yves Pommier, NCI	February 19	Nucleotide Excision Repair-Dependent Cytotoxicity of a Novel Anticancer Agent, Ecteinascidin 743
Thomas Kunkel, NIEHS	January 15	Recent Studies of DNA Mismatch Repair
DNA Repair Interest Group 2001 Seminar Series		
Richard Wood, U of Pittsburgh	December 18	Tolerating Damaged DNA
J. C. States, U of Louisville	November 13	Cisplatin Regulation of XPA Expression in Ovarian Cancer Cells
Douglas E. Brash, Yale	October 24	UV, p53 and Skin Cancer Lecture Presented to the Royal College of Pathologists, London, England
Daniel Yarosh, Applied Genetics	October 16	Reduction of Skin Cancer in Xeroderma Pigmentosum Patients Treated Topically with DNA Repair Enxymes
James Cleaver, UCSF	June 19	History of DNA Repair: Mending Human Genes
Bill Copeland, NIEHS	May 15	Mitochondrial DNA Replication Fidelity and Mitochondrial Diseases
Peter Beernink, LLNL	March 20	Crystal Structure of Human Apurinic/Apyridinimic Endonuclease I
Yong Hwan Jin, NIEHS	March 20	The 3'-5' Exonuclease of DNA Polymerase d is Redundant with Rad27/Fen1 for Processing of Okazaki Fragments
Robert M. Brosh, NIA	March 20	Molecular Interactions of the Werner Syndrome Protein
Vilhelm Bohr, NIA	February 20	DNA Repair Defects in Premature Aging Disorders
Mats Ljungman U of Michigan	January 16	Stopped in its tracks - RNA pol II as a sensor for DNA damage

DNA Repair Interest Group 2000 Seminar Series		
Patrick Sung Univ of Texas	December 19	Functional Interactions Among RAD52 Group Proteins in Recombination and Repair
Zhigang Wang, Univ of KY	November 21	Translesion Synthesis by the UmuC Family of DNA Polymerases
Yoshihiro Matsumoto, Fox Chase	October 17	Functions of PCNA in Base Excision Repair
Kenneth Kraemer, NCI	September 19	Clinical and Molecular Features of Xeroderma Pigmentosum and Related Disorders of DNA Repair
Richard Setlow, Brookhaven	June 20	Refections On How I Was Led Into and Onto DNA Repair.
Peter Glazer, Yale Univ	April 18	Targeted genome modification via DNA triple helix formation
Robert Levine	March 21	Mutagenesis Induced by Endogenous DNA Adducts in Human Cells
Robert Sobol, NIEHS	March 21	Mutagenesis and dRP Lyase Activity in DNA ß-Polymerase Dependent Base Excision Repair in Mouse Cells
Steffen Emmert, NCI	March 21	The xeroderma pigmentosum group C gene leads to selective repair of cyclobutane pyrimidine dimers rather than 6-4 photoproducts.
Steve Matson, UNC	February 15	Two E. Coli Mismatch Repair Enzymes, DNA Helicase II and MUTL, Interact to Catalyze Efficient Unwinding of Duplex DNA
John Essigman, MIT	January 18	Cellular responses to the DNA damaging agent Cisplatin
Miral Dizdaroglu	January 14	Mechanisms of Oxidative DNA Damage and the Processing by BER
DNA Repair Interest Group 1999 Seminar Series		
Gilbert Chu Stanford	December 14	Recognizing DNA damage.
Rodney Nairn, Moderator	November 16	Meeting Report: DNA Repair and Mutagenesis
Larry Thompson, LLNL	October 19	Homologous recombinational repair and its role in cancer.
Roger Woodgate	September 14	Translesion DNA synthesis: From mutations in bacteria to the xeroderma pigmentosum variant phenotype in humans
Jim George & Nan Liu – LLNL	June 15	NER Activity in the XPD mutant, V-H1 Role of XRCC2 in homologous recombinational repair
Lawrence Grossman - JHU	May 25	Four decades of DNA Repair: From populations of molecules to populations of people
Karin Drotschmann- NIEHS	March 23	Mutator phenotype of yeast strains heterozygous for mutations in the Msh2 gene
Bruce McKay - Univ of Michigan	March 23	The role of p53 in DNA repair and recovery of RNA synthesis following UV-irradiation
P.J. Brooks- NIAAA	March 23	Repair of a cyclopurine lesion in DNA by nucleotide excision repair: Implications for neurodegeneration in xeroderma pigmentosum.
Steven A. Leadon, UNC	February 16	Transcription - Coupled Repair of Oxidative DNA Damage
Carl Anderson, Brookhaven	January 19	DNA Damage Activates P53 via Phosphorylation-Acetylation

DNA Repair Interest Group
1998 Seminar Series

Al Fornace	December 15	The role of the tumor suppressor p53 and its effector genes on DNA repair
Shyam Sharan & Mikhail Kashlev, ABL-BRP	October 20	1) Functional Analysis of Murine Brca2: Role in DNA Repair - 2) Mechanism of Transcription Processivity: Comparative studies of Prokaryotic and Eukaryotic RNA Polymerases
Steve Matson, UNC	September 22	Protein-protein interactions that modulate the activity of DNA helicase II
Phil Hanawalt, Stanford	May 19	Parallels between P53 dependent excision-repair in mammalian cells and the SOS response in bacteria
Miria Stefanini	April 21	Nucleotide Excision Repair Defective Syndromes: Genotype-Phenotype Relationships.
Thomas Kunkel, NIEHS	March 17	Studies of Eukaryotic DNA Mismatch Repair
Betsy Sutherland, Bookhaven	February 17	The Case of the UV-sensitive rice: an international detective story.
Dan Bogenhagen, SUNY	January 20	Repair of Abasic Sites in DNA Using Mitochondrial Enzymes

Selected DNA Repair Videos

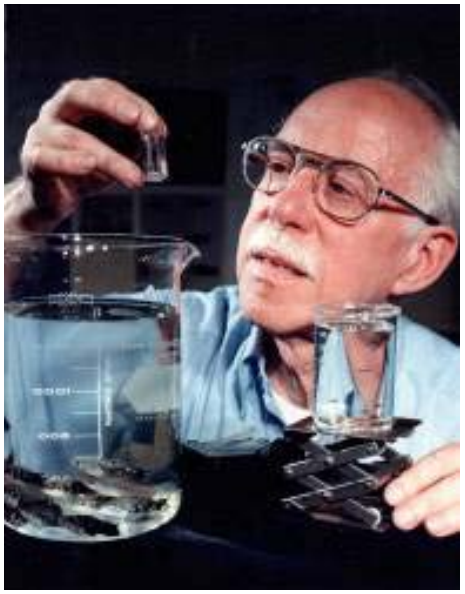
- 1960's Setlow demonstrates importance of CPD in DNA
- 1960–70's Fighter pilot Grossman studies DNA repair enzymes in bacteria
- 1968 Cleaver reports repair defect in xeroderma pigmentosum
- 1980's Hanawalt describes transcription coupled repair
- Leadon – retracted work

http://videocast.nih.gov/PastEvents.asp?c=5

Richard B. Setlow, Ph.D.

Brookhaven National Lab,
Upton, NY

Reflections on how I was led into DNA repair
June 20, 2000



Richard Setlow, Ph.D. 1989

http://videocast.nih.gov/PastEvents.asp?c=5

Lawrence Grossman, Ph.D.

Johns Hopkins School of Public Health, Baltimore, MD

Four decades of DNA repair: From populations of molecules to populations of people

May 25,1999

Arthur Grollman, Ph.D.

Larry Grossman, Ph.D.

2004

http://videocast.nih.gov/PastEvents.asp?c=5

James E. Cleaver, Ph.D.

University of California San Francisco, San Francisco, CA

Mending human genes
June 19, 2001

Ken Kraemer, M.D.

James Cleaver, Ph.D.

2008

http://videocast.nih.gov/PastEvents.asp?c=5

Kenneth H. Kraemer, M.D.

CCR, NCI, Bethesda, MD

1971
Harlem Hospital, NY

1973
Dermatology Branch, NCI

Partnership approach to studies of rare diseases with defective DNA repair: Patient – Researcher – NIH (Scientific Community) Robbins / Kraemer

- Identify new patients with unusual features
- Bring to NIH for extensive clinical evaluation and counseling
- Make recommendations to family and local doctors for management and treatment
- Establish cell lines for laboratory study
- Attempt to correlate clinical abnormalities with cellular defects
- Publish studies of patients and their cells
- Make well characterized cell lines available to general scientific community – deposit in cell banks (ATTC, Human Genetic Mutant Cell Repository)

Reprinted from ANNALS OF INTERNAL MEDICINE Vol. 80, No. 2, Feb. 1974
Printed in U.S.A.

Xeroderma Pigmentosum

An Inherited Disease with Sun Sensitivity, Multiple Cutaneous Neoplasms, and Abnormal DNA Repair

Moderator: JAY H. ROBBINS, M.D. *Discussants:* KENNETH H. KRAEMER, M.D., MARVIN A. LUTZNER, M.D., BARRY W. FESTOFF, M.D., and HAYDEN G. COON, Ph.D,
Bethesda, Maryland

Xeroderma pigmentosum is a hereditary disease clinically manifested primarily on sun-exposed skin, which develops abnormal pigmentation and malignant tumors. Mental retardation, areflexia, and other neurological abnormalities are seen in some patients. Only one biochemical defect has been found: cells from various tissues repair ultraviolet-induced deoxyribonucleic acid (DNA) damage slowly, compared with normal cells. Cell fusion studies show that genetic complementation can occur between fibroblasts from certain pairs of patients, thus overcoming the DNA-repair defect in each member of the pair and demonstrating the heterogeneity of the genetic lesion. The patients at NIH who have slow DNA repair comprise four distinct complementation groups, indicating that at least four mutations can cause defective DNA repair.

DR. JAY H. ROBBINS*: Xeroderma pigmentosum is a rare autosomal recessive disease in which patients develop solar damage, pigmentation abnormalities, and malignancies in the areas of skin exposed to sunlight (1-4). These cutaneous lesions sometimes occur in association with abnormalities of other organ systems, including the nervous system (3-6).

In 1968 Cleaver (7) reported that skin fibroblasts from these patients are unable to repair normally a certain type of ultraviolet (UV)-induced damage in their deoxyribonucleic acid (DNA). Soon thereafter, Epstein and co-workers (8) reported that xeroderma pigmentosum epidermal cells show this DNA repair defect in vivo. These exciting findings suggested that these patients' DNA-repair defects might cause their clinical abnormalities, particularly their malignancies, through somatic mutation resulting from their unrepaired UV-damaged DNA (7, 8).

During the 5 years since Cleaver's discovery, this DNA-repair defect has been studied in fibroblast strains from approximately 60 patients with xeroderma pigmentosum. In the Dermatology Branch of the National Cancer Institute we have performed photobiological studies of the DNA-repair process in various types of cells from 15 of these patients, representing 12 kindreds (9-15). We have also evaluated their clinical histories and the manifestations of their disease. We report here the results of these clinical and laboratory studies and describe new forms of xeroderma pigmentosum discovered in the NIH series. The results of recent studies, by cell fusion methods (15), of the genetic heterogeneity of the DNA-repair defect are also presented.

It is first necessary, however, to describe some of

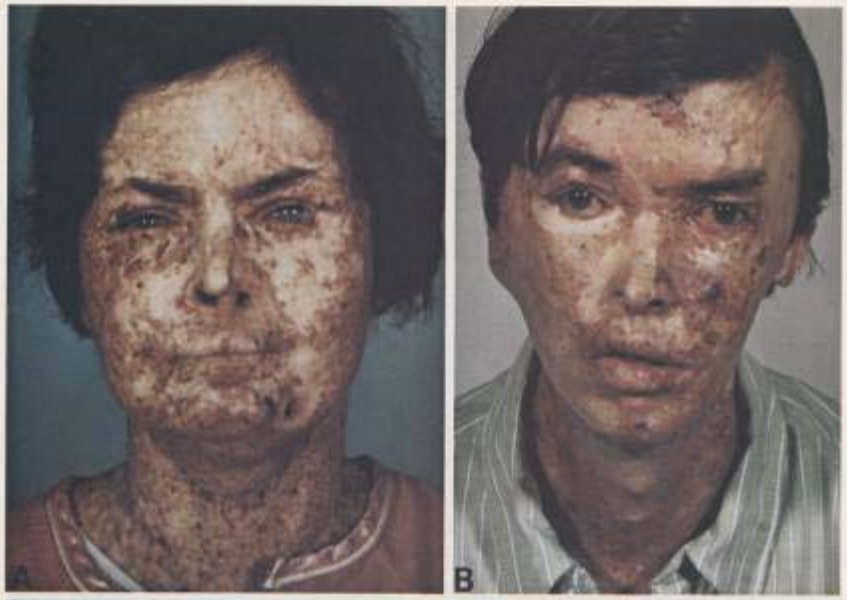
* Senior Investigator, Dermatology Branch, National Cancer Institute.

► An edited transcription of a Combined Clinical Staff Conference at the Clinical Center, Bethesda, Maryland, by the National Cancer Institute, National Institutes of Health, U.S. Department of Health, Education, and Welfare.

Jay H. Robbins, M.D.

1974

XP4BE
First XP variant patient



XP5BE – XP6BE
First XP-D patients

Figure 4. A. The face and neck of Patient 1, whose cells have only 15% to 25% of the normal DNA repair rate, show many of the cutaneous manifestations of xeroderma pigmentosum. Areas of hypopigmentation and numerous freckles with different intensities of pigmentation are apparent. The opacified left cornea was later replaced with a corneal transplant. The deformities of the nose and left palpebral fissure primarily result from surgical removal of tumors. B. Patient 4, whose cells have no detectable defect in UV-induced ^{3}HTdR incorporation has had very severe clinical manifestations of xeroderma pigmentosum. The white, relatively disease-free areas of skin (under right eye, right nasal and cheek areas, left temple) are skin grafts obtained several years earlier from sun-protected, relatively uninvolved skin. Several tumors are present. Note the injected conjunctiva and the destruction of the left lower eyelid. C. Patient 5 (right) and Patient 6 (left) with their mother. These patients have 25% to 55% of the normal DNA repair rate. The parents of patients with xeroderma pigmentosum have no clinical manifestations of the disease. The nose of Patient 5 has undergone several surgical procedures. Affected members of a family have similar clinical manifestations. D. Back and buttocks of Patient 6, showing the absence of freckles in areas protected from the sun.

1974

XP11BE
First XP-B patient (XP/CS)

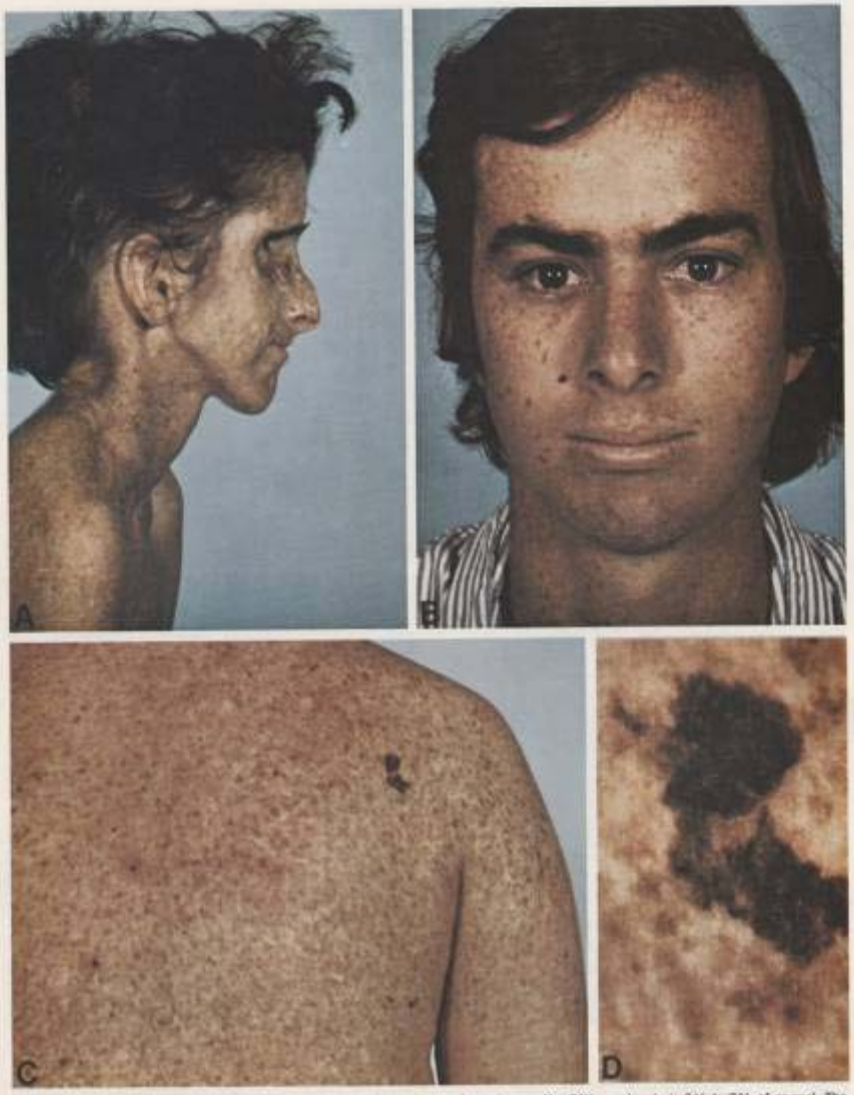
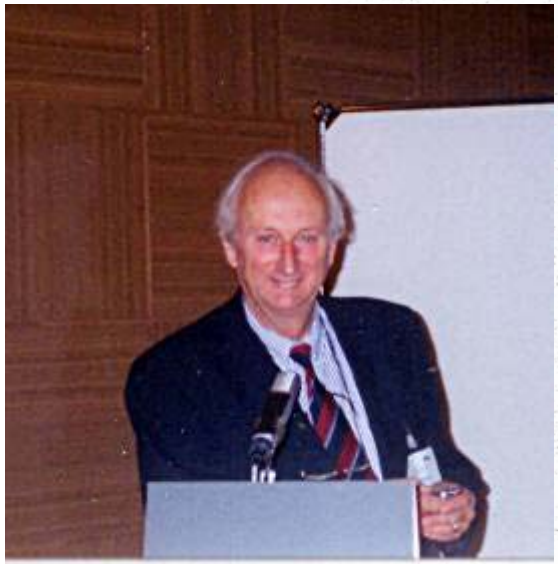


Figure 5. A. Patient 11, who has Cockayne's syndrome and xeroderma pigmentosum. Her DNA repair rate is 3% to 7% of normal. The freckles and small areas of hypopigmentation are similar to those of the other patients. Her beak-like nose, sunken eyes, and loss of subcutaneous tissue are similar to these manifestations in other patients with Cockayne's syndrome. B. Patient 2, who has the same level of DNA repair as Patient 1 (Figure 4), has had very few tumors. His face and eyes have escaped most of the more serious sequelae of xeroderma pigmentosum. C. Numerous freckles on the back of Patient 1. Note the diversity of their size, shape, and intensity of pigmentation. D. The large, irregularly shaped, deeply pigmented lesion on Patient 1's right shoulder, shown here in higher magnification, was histologically proved to be a lentigo. Note the small areas of hypopigmentation in the surrounding skin.

1974

WORKSHOP ON

"REPAIR MECHANISMS IN MAMMALIAN CELLS"



Dirk Bootsma, Ph.D.

Paul H.M. Lohman, Ph.D.

Noordwijkerhout, The Netherlands,
May 6 - 10, 1973

1973

ORGANIZERS:

P.H.M.Lohman, Ph.D.
Medical Biological Laboratory TNO
Rijswijk 2100, The Netherlands

D.Bootsma, Ph.D.
Department of cell biology and genetics
Medical Faculty
Rotterdam, The Netherlands.

Secretary: miss Elly Looy
Technical assistance: miss Milou Sluyter

The organizers gratefully acknowledge the European Molecular Biology Organisation, EURATOM, Unilever Research, Shell Research and the Dutch Ministry of Public and Environmental Health for the financial support which made this workshop possible.

GENERAL INFORMATION

Secretarial office for the workshop: Dr.P.H.M.Lohman, Medical Biological Laboratory TNO, P.O.Box 45, Rijswijk 2100, The Netherlands.
Tel.: (0)15 - 120330

The address of the conference center:

"De Leeuwenhorst"
Langelaan 3
Noordwijkerhout, The Netherlands
Tel.: (0) 2523 - 2741

Location of the center: "De Leeuwenhorst" in Noordwijkerhout, near Leiden, is situated about 5 miles from Noordwijkbeach. It is surrounded by bulbflower-fields which at that time are expected to be in full colour. "De Leeuwenhorst" is a former seminary and consequently has a large number of single bedrooms, equipped with one bed, a wardrobe, a table and a chair and a private shower. A small number of double bedrooms is available. Next to the congress center a lovely park is situated where you can walk. Free cards for this park are available at the information desk of the congress center.

Arrival: The congress center is not easily reached by train; participants wishing to be met at the train station in Leiden or Schiphol airport are requested to inform the secretary of their expected time of arrival.

Social events: Thursday, May 8, a visit to the famous bulbflower exposition "De Keukenhof" is offered to you by the organizing committee. Busses will leave the congress center at 15.00 h and will bring you back at 18.30 h from "De Keukenhof". The visit is followed by the "Workshop Dinner" at 19.30 h in "De Leeuwenhorst".
Since the number of accompanying wives is relatively small, no ladies programme has been organized.

If you want hotel accomodation before or after the meeting, please contact the secretary. For the invited participants rooms have been reserved in the congress center from Sunday afternoon May 6 till Thursday morning May 10.

OUTLINE OF THE WORKSHOP

The workshop will be a discussion meeting of invited participants. These participants will be experts in the field of repair mechanisms in mammalian cells in the widest sense. This implies that not only repair mechanisms in irradiated cells will be discussed. Also studies of mammalian cells treated with chemical agents will be an important part of the discussion. The scope of the workshop makes it possible to discuss the role of repair enzymes in radiosensitivity, carcinogenesis and/or mutagenesis in mammalian cells.
The workshop is divided into five sessions:

session 1a: "Evidence for removal of DNA lesions"

session 1b: "Evidence for "bypassing" of DNA lesions"

session 2: "Mutations affecting DNA repair in mammalian cells"

session 3: "Enzymes involved in DNA repair in mammalian cells"

session 4: "Consequences of repair, misrepair or defective repair"

and an opening session on models of DNA repair in mammalian cells.

Each of the sessions will be introduced by one speaker during 30 min. The invited participants will be scheduled as "discussants" in the field of each session. The chairman of each session will organize the session in such a way that after the introductory paper a discussion will take place containing prepared contributions of the discussants which are of limited time (15 min). After the contributions of the discussants, other participants will have the opportunity for informal discussion.

The workshop organizers, P.H.M.Lohman and D.Bootsma, are intending to summarize the discussions in a paper that will be published in Mutation Research. Before submitting the paper to the journal the summary will be sent to all discussants for their comments.

PROGRAMME

SUNDAY, May 6

16.00 - 19.30 h	arrival and registration participants
19.30 - 20.30 h	dinner
21.00 h	opening session

MONDAY, May 7

08.00 - 09.00 h	breakfast
09.00 - 13.00 h	session 1a
13.00 - 14.00 h	lunch
14.00 - 18.00 h	session 1b
19.00 - 20.00 h	dinner
	evening free, bar is open

TUESDAY, May 8

08.00 - 09.00 h	breakfast
09.00 - 13.00 h	session 2
13.00 - 14.00 h	lunch
14.00 - 15.00 h	free
15.00 - 18.30 h	excursion to "De Keukenhof"
19.00 - 19.30 h	apéritif
19.30 - 20.30 h	"Workshop Dinner"
	evening free, bar is open

WEDNESDAY, May 9

08.00 - 09.00 h	breakfast
09.00 - 13.00 h	session 3
13.00 - 14.00 h	lunch
14.00 - 18.00 h	session 4
19.00 - 20.00 h	dinner
	evening free, bar is open

THURSDAY, May 10

08.00 - 09.00 h	breakfast
09.00 h	departure

(during the evenings, rooms are available for informal discussions)

Opening session on models of DNA repair in mammalian cells

Chairman : A. Rörsch

P. Howard Flanders: "Rates of exchange in the currency of repair"
B.A. Bridges : "Repair and misrepair"

Session 1a: "Evidence for removal of DNA lesions"

Chairman : R.B. Painter

Introductory speaker : B.S. Strauss

Discussants : R. Ben-Ishai, M.M. Elkind, J.T. Lett, M.G. Ormerod, J.J. Roberts

Session 1b: "Evidence for "bypassing" of DNA lesions"

Chairman : P. Howard Flanders

Introductory speaker : W.D. Rupp

Discussants : A.R. Lehmann, R.E. Meyn, A.M. Rauth, M. Errera

Session 2: "Mutations affecting DNA repair in mammalian cells"

Chairman : H.J. Evans

Introductory speaker : J.E. Cleaver

Discussants : W.J. Kleijer, J.H. Robbins, E.G. Jung, M.C. Paterson, J.D. Regan, M. Klímek, B.W. Fox, M. Fox

Session 3: "Enzymes involved in DNA repair in mammalian cells"

Chairman : R. Devoret

Introductory speaker : L. Grossman

Discussants : T. Lindahl, A. Falaschi, F. Campagnari, T.P. Brent, S. Bacchetti, G. Veldhuisen, H. Slor

Session 4: "Consequences of repair, misrepair or defective repair"

Chairman : F.H. Sobels

Introductory speaker : H.F. Stich

Discussants : J.M. Parry, C.F. Arlett, B.A. Kihlman, M.M. Elkind, V.M. Mikhelson, J.J. Roberts, M. Sarma

1973

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Genetic Heterogeneity in Xeroderma Pigmentosum: Complementation Groups and Their Relationship to DNA Repair Rates

(ultraviolet radiation/mutations/unscheduled DNA synthesis/somatic cell genetics)

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Communicated by Aaron B. Lerner, October 4, 1974

ABSTRACT **Fibroblast strains from 12 patients with xeroderma pigmentosum had lower than normal rates of DNA repair, as determined by autoradiographic studies of ultraviolet-induced unscheduled nuclear DNA synthesis. The nuclei in binuclear cells, obtained by fusing fibroblasts from certain pairs of these strains, had a greater rate of DNA repair than the nuclei of either strain's unfused mononuclear cells. These results indicate that complementary corrections of the strains' repair defects had occurred in the fused cells. Four complementation groups were found, indicating that at least four mutations caused decreased DNA repair among these 12 strains. The unfused mononuclear cells of each group had a characteristic rate of repair that differed from the rates of the other groups.**

In xeroderma pigmentosum (XP), a rare disease of autosomal, recessive inheritance, sun-exposed skin develops severe solar damage, pigmentation changes, and neoplasms (1). XP cells repair DNA damaged by ultraviolet (UV) light more slowly than normal cells (1–3). Defective repair in cells from most XP patients can be detected by measuring the rate at which the cells incorporate [^{3}H]thymidine into DNA segments synthesized to replace regions containing UV-induced pyrimidine dimers (1, 2, 4). This [^{3}H]thymidine incorporation reflects DNA repair replication and is manifested autoradiographically as unscheduled DNA synthesis (1, 2, 4–6).

XP is one of the few human diseases (7, 8) in which somatic cell genetic studies have successfully shown different mutations to be responsible for an observed phenotype. When fibroblast strains from certain pairs of XP patients are fused in culture to form binuclear heterokaryons, both of the nuclei in the fused cells perform more unscheduled DNA synthesis than the nuclei of the unfused mononuclear cells of either strain (1, 9). De Weerd-Kastelein *et al.* (9) first demonstrated this genetic heterogeneity in XP and have found three complementation groups (10). We have found four complementation groups among the 12 XP strains we have studied, and we have shown that all the strains within a complementation group have a similar rate of unscheduled DNA synthesis that is characteristic for each group.

MATERIALS AND METHODS

Fibroblast Strains. Twelve XP and three normal control donor strains were from the American Type Culture Collection, Rockville, Md. Ten of the XP strains were from patients of the NIH series (1). These XP strains and strains XPKMSF (11) and XP1LO (12) are identified by the nomenclature for XP strains (13). Fibroblasts were grown without antibiotics in modified Ham's F12 medium (14) supplemented with 5% fetal calf serum at 37° in a 5% CO_2–95% air incubator with more than 95% humidity. They were used after two to seven additional passages.

Cell Fusion. One- to 3-day-old coverslip cultures in 60-mm petri dishes were used for irradiation and fusion, the latt performed by a modification of the method of Yamanaka ar Okada (15). Cultures, cooled on ice, were washed with col Hanks' balanced salts solution and covered with 0.1 ml cold, serum-free medium containing about 100 hemagglutina ing units [standardized with 0.03% (v/v) chick erythrocytes of β-propiolactone-inactivated Sendai virus (14). Ten minute later, about 0.1 ml of cold, serum-free medium containin 5×10^4 cells (to be fused with those already on the cover slips) was dropped on each coverslip. After 20 min in the cold the dishes were placed in the 37° incubator. Three hours late the coverslips were covered with serum-containing mediun (3 ml per dish), and the incubation was continued.

UV Irradiation. About 16 hr after addition of inactivatec virus, the cultures were washed twice with phosphate-buffered saline (pH 7.4) at room temperature and covered with phosphate-buffered saline (1 ml per dish). Immediately after irradiation with 300 erg/mm² of 254 nm UV light (16), the phosphate-buffered saline was removed and 2 ml of Hanks' base medium 199 (Difco Laboratories or NIH Media Unit) containing bicarbonate, 20% fresh, heparinized, filtered human plasma, and 20 μCi of [*methyl*-^{3}H]thymidine (specific activity, 17–25 Ci/mmol; Amersham-Searle) was added. The dishes were placed in the incubator for 3 hr. The cultures were then washed and fixed. Unirradiated coverslip cultures were processed in parallel with the irradiated cultures.

Autoradiograms and Their Analysis. Autoradiograms, prepared with NTB-3 emulsion (Eastman Kodak), were exposed at 4° for 7 days (8 days in the case of Exp. 1 of Table 1), then developed and stained. Grain production and counting efficiencies were constant for the range of grain counts in these experiments.

On coverslip cultures not treated with virus, grains over "lightly labeled" (1, 2, 5, 6) nuclei from 100 consecutively observed mononuclear cells were counted. Virus-treated coverslips were scanned under low power for areas containing multinucleate cells that had the most light labeling. In such an area, grains were counted over the 100 nuclei of 50 consecu-

Abbreviations: XP, xeroderma pigmentosum; UV, ultraviolet.

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TABLE 2. *Assignment of the 12 XP fibroblast strains to their unscheduled DNA synthesis complementation groups**

	XP1LO	XPKMSF	XP12BE	XP11BE	XP1BE	XP2BE	XP3BE	XP8BE	XP10BE	XP5BE	XP6BE	XP7BE
XP1LO	0											
XPKMSF	0	n										
XP12BE	0	0	0									
XP11BE	+	+	+	0								
XP1BE	+	+	+	+	0							
XP2BE	+	+	+	+	0	0						
XP3BE	+	n	+	+	0	0	0					
XP8BE	n	n	+	+	0	0	0	n				
XP10BE	+	n	+	+	0	0	0	0	0			
XP5BE	+	+	n	+	+	+	+	n	+	0		
XP6BE	n	n	+	+	+	n	n	+	+	0	n	
XP7BE	+	+	+	+	+	+	+	n	+	0	0	0

* +, Complementing; 0, noncomplementing; n, not studied. Fibroblast strains listed in the vertical columns were fused to those listed in the horizontal columns. Complementation was determined autoradiographically by visual inspection (e.g., see Fig. 2) or by grain count analysis (as in Figs. 3–5).

Relationship of complementation groups to residual DNA repair levels

1975

Mutation Research, 33 (1975) 327—340

FIVE COMPLEMENTATION GROUPS IN XERODERMA PIGMENTOSUM

K.H. KRAEMER[1], E.A. DE WEERD-KASTELEIN[2], J.H. ROBBINS[1], W. KEIJZER[2], S.F. BARRETT[1], R.A. PETINGA[1] AND D. BOOTSMA[2]

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Summary

A collaborative study was undertaken to determine the relationship betwe the three DNA repair complementation groups in xeroderma pigmentosu found at Erasmus University, Rotterdam, and the four groups found at t National Institutes of Health, Bethesda. The results of this study reveal th there are five currently known complementation groups in xeroderma p mentosum.

Introduction

Patients with xeroderma pigmentosum (XP) develop malignancies and p.. mentation abnormalities on areas of skin exposed to sunlight [10]. Skin fibroblasts from most patients with this autosomal recessive disease are unable to perform excision repair of UV-induced pyrimidine dimers in their DNA as rapidly as normal fibroblasts [2,3,10]. This defective repair can be manifest as a decreased rate of UV-induced unscheduled DNA synthesis (UDS) [2,3,10]. In 1972 investigators at Erasmus University, Rotterdam, found two complementation groups in XP by showing that nuclei in heterokaryons formed by fusing fibroblasts from certain pairs of such repair-defective XP patients performed UV-induced UDS at a normal rate [11]. Subsequently a third complementation group was found among the Rotterdam XP strains [12].

The demonstration of genetic heterogeneity for DNA repair among the Rotterdam XP strains prompted investigators at the National Institutes of Health

Abbreviations: XP, xeroderma pigmentosum; UDS, unscheduled DNA synthesis; ^{3}HTdR, tritiated thymidine; NIH, National Institutes of Health.

TABLE III

COMPARISON OF UV-INDUCED UDS IN THE REPRESENTATIVE XP STRAINS MEASURED IN BOTH INSTITUTES

Complementation group	Strain	UV-induced UDS (% of normal rate)	
		Rotterdam[a]	NIH[b]
A	Control donors	100	100
	XP25RO	<5	<2
	XP12BE		<2
	XP1LO	<5	<2
B	XP11BE	4	4.8
C	XP4RO	10—15	12.9
	XP1BE		19.8
	XP2BE	24—27	13—18
D	XP5BE	10	27.1
E	XP2RO	40—60	60

[a] Expressed as a percentage of the levels found in normal cells after a UV dose of 10 J/m^2.
[b] Expressed as a percentage of the levels found in normal cells after a UV dose of 30 J/m^2.

Collaboration in determination of 5 XP complementation groups

1975

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Genetics

One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells

(DNA repair/ultraviolet radiation/host cell reactivation/skin cancer)

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Communicated by Richard B. Setlow, June 24, 1985

ABSTRACT **We have developed a host cell reactivation assay of DNA repair utilizing UV-treated plasmid vectors. The assay primarily reflects cellular repair of transcriptional activity of damaged DNA measured indirectly as enzyme activity of the transfected genes. We studied three plasmids (pSV2cat, 5020 base pairs; pSV2catSVgpt, 7268 base pairs; and pRSVcat, 5027 base pairs) with different sizes and promoters carrying the bacterial *cat* gene (CAT, chloramphenicol acetyltransferase) in a construction that permits *cat* expression in human cells. All human simian virus 40-transformed cells studied expressed high levels of the transfected *cat* gene. UV treatment of the plasmids prior to transfection resulted in differential decrease in CAT activity in different cell lines. With pSV2catSVgpt, UV inactivation of CAT expression was greater in the xeroderma pigmentosum group A and D lines (D_0 = 56 J·m^{-2}) than in the other human cell lines tested (normal, ataxia-telangiectasia, Lesch–Nyhan, retinoblastoma) (D_0 = 680 J·m^{-2}) (D_0 is the dose that reduces the percentage of CAT activity by 63% along the exponential portion of the dose–response curve). The D_0 of the CAT inactivation curve was 50 J·m^{-2} for pSV2cat and for pRSVcat in the xeroderma pigmentosum group A cells. The similarity of the D_0 data in the xeroderma pigmentosum group A cells for three plasmids of different size and promoters implies they all have similar UV-inactivation target size. UV-induced pyrimidine dimer formation in the plasmids was quantified by assay of the number of UV-induced T4 endonuclease V-sensitive sites. In the most sensitive xeroderma pigmentosum cells, with all three plasmids, one UV-induced pyrimidine dimer inactivates a target of about 2 kilobases, close to the size of the putative CAT mRNA.**

Cells from patients with the autosomal recessive, cancer-prone disease xeroderma pigmentosum are hypersensitive to killing by UV radiation and have defective repair of UV-damaged DNA (1). This DNA repair defect has been measured by demonstrating reduced proliferation in xeroderma pigmentosum cells of UV-treated infecting viral particles (2), or viral DNA (3), a process known as "host cell reactivation."

We have developed a host cell reactivation assay utilizing UV-treated recombinant DNA expression vectors. By using nonreplicating plasmids we are able to examine the influence of precisely quantified DNA damage on plasmid transcription and to study excision repair independently of postreplication repair. We chose three plasmids of known sequence with differing size and with different promoters. They all carry the same bacterial gene, *cat* [coding for chloramphenicol acetyltransferase (CAT)], inserted between viral promoters [simian virus 40 (SV40) or Rous sarcoma virus (RSV) long terminal repeat (LTR)] and SV40 polyadenylylation sequences that permit gene expression in mammalian cells. In addition, the

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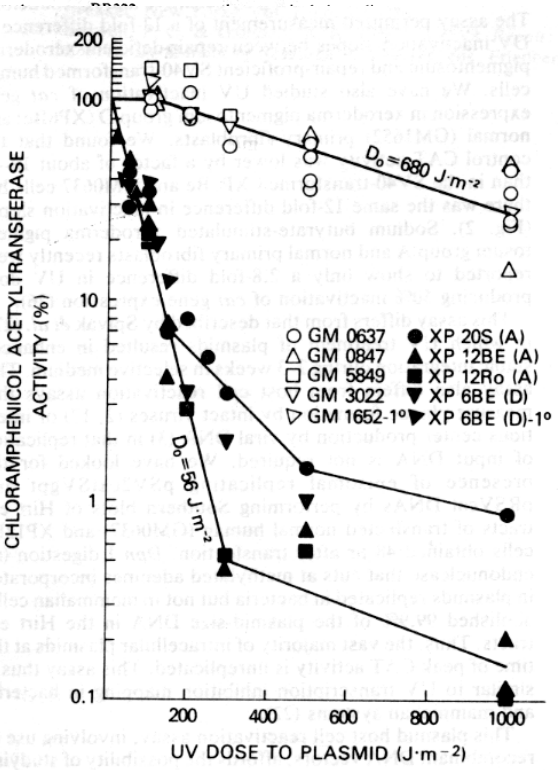

FIG. 2. Transient expression of *cat* gene in SV40-transformed xeroderma pigmentosum, ataxia-telangiectasia, retinoblastoma, Lesch–Nyhan, and normal human cells and primary human skin fibroblasts transfected with UV-treated pSV2catSVgpt DNA. The data for primary line XP6Be were obtained with pRSVcat.

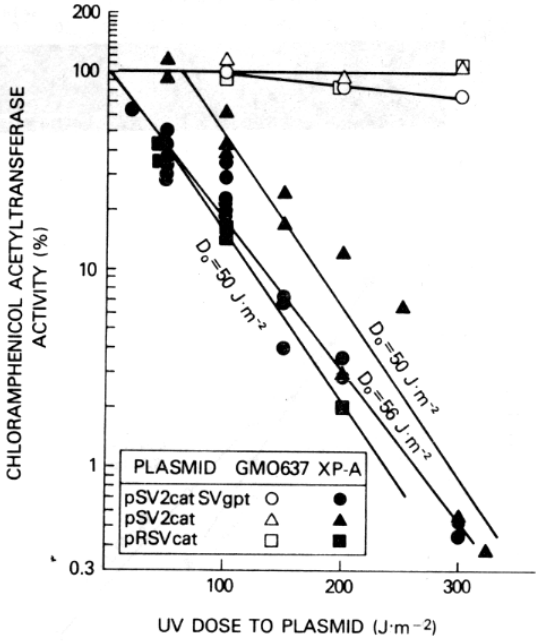

FIG. 3. Transient expression of *cat* gene in SV40-transformed xeroderma pigmentosum group A and normal human cells transfected with UV-treated plasmids. Normal (GM0637) (open symbols) and xeroderma pigmentosum group A (XP12Be, XP20s, and XP12Ro) (closed symbols) SV40-transformed cells were transfected with UV-treated plasmids containing the *cat* gene: pSV2catSVgpt (circles), pSV2cat (triangles), pRSVcat (squares). Zero UV-dose CAT activities for the normal cells were 0.45, 0.62, and 1.2 nmol·mg^{-1}·min^{-1} and for the xeroderma pigmentosum cells were 0.02–0.27, 0.14–0.45, and 1.8 nmol·mg^{-1}·min^{-1} after transfection with 10 μg of the plasmids pSV2catSVgpt, pSV2cat, and pRSVcat per plate, respectively.

Use of shuttle vector plasmid to measure DNA repair in XP cells

1985

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8273–8277, November 1986
Genetics

Restricted ultraviolet mutational spectrum in a shuttle vector propagated in xeroderma pigmentosum cells

(DNA repair/suppressor tRNA/ultraviolet carcinogenesis)

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Communicated by Aaron B. Lerner, July 7, 1986

ABSTRACT **A shuttle vector plasmid, pZ189, carrying a bacterial suppressor tRNA marker gene, was treated with ultraviolet radiation and propagated in cultured skin cells from a patient with the skin-cancer-prone, DNA repair-deficient disease xeroderma pigmentosum and in repair-proficient cells. After replication in the human cells, progeny plasmids were purified. Plasmid survival and mutations inactivating the marker gene were scored by transforming an indicator strain of *Escherichia coli* carrying a suppressible amber mutation in the β-galactosidase gene. Plasmid survival in the xeroderma pigmentosum cells was less than that of pZ189 harvested from repair-proficient human cells. The point-mutation frequency in the 150-base-pair tRNA marker gene increased up to 100-fold with ultraviolet dose. Sequence analysis of 150 mutant plasmids revealed that mutations were infrequent at potential thymine-thymine dimer sites. Ninety-three percent of the mutant plasmids from the xeroderma pigmentosum cells showed G·C→A·T transitions, compared to 73% in the normal cells ($P < 0.002$). There were significantly fewer transversions ($P < 0.002$) (especially G·C→T·A) and multiple base substitutions ($P < 0.00001$) than when pZ189 was passaged in repair-proficient cells. The subset of mutational changes that are common to ultraviolet-treated plasmids propagated in both repair-proficient and xeroderma pigmentosum skin cells may be associated with the development of ultraviolet-induced skin cancer in humans.**

Xeroderma pigmentosum is an autosomal recessive disorder marked by sun sensitivity and a >1000-fold-increased frequency of neoplasms in skin exposed to sunlight (1). This disorder is one of the best known examples of the relationship between exposure to a mutagenic agent and tumorigenesis. Cultured cells from individuals with xeroderma pigmentosum are hypersensitive to ultraviolet radiation and have a reduced capacity for DNA repair (2, 3). In particular, cells from the xeroderma pigmentosum complementation group A have a profound defect in DNA repair and are almost totally unable to excise ultraviolet photoproducts (2–4). Mutation data from xeroderma pigmentosum cells would be of considerable interest, particularly in light of our understanding of the role of mutagenesis in the activation of certain oncogenes (5).

To acquire this information, we used the shuttle vector plasmid pZ189 (6), which replicates in human and bacterial cells and which carries a small bacterial marker gene, the activity of which can be determined in a standard microbiological assay (Fig. 1). pZ189 contains the origin of replication and large tumor (T)-antigen gene from SV40, information for replication and maintenance in bacteria from pBR327, and the marker gene *supF*, which encodes a suppressor tRNA that suppresses an amber mutation in the β-galactosidase gene (*lacZ*) in an indicator strain of *Escherichia coli*. Bacterial colonies containing plasmids with mutant or wild-type suppressor tRNA genes can be identified by color (wild-type are blue, mutants are light blue or white). Plasmids with mutant tRNA genes can be isolated and the sequence of the 150-bp marker gene determined in a single operation (7). This (8) and other (9–11) vectors have been used to study ultraviolet mutagenesis in repair-proficient mammalian cells.

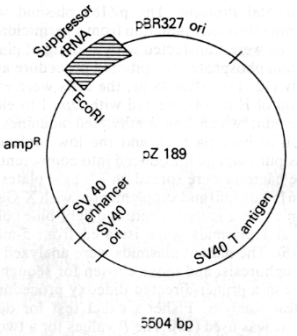

FIG. 1. Schematic diagram of plasmid pZ189, the shuttle vector used for mutagenesis studies. The plasmid contains simian virus 40 (SV40) sequences permitting growth in mammalian cells and pBR327 sequences enabling replication in bacteria. The approximately 150-base-pair (bp) suppressor tRNA marker sequence is flanked by sequences essential for survival of the plasmid in bacteria [pBR327 origin of replication and the ampicillin-resistance gene (amp^R)] thereby reducing the frequency of detection of large deletions in this region.

MATERIALS AND METHODS

Cells and Plasmid. The DNA repair-deficient, SV40-transformed, xeroderma pigmentosum cell line XP12BE(SV40) was derived from the skin of a xeroderma pigmentosum patient of complementation group A (4), who by age 20 has had 98 documented cutaneous basal-cell carcinomas. Fibroblasts from this patient have been shown to have <2% of normal DNA excision repair (4). The DNA repair-proficient SV40-transformed human fibroblast cell line GM0637(SV40) was derived from the skin of a healthy 18-year-old woman. Cell lines were obtained from the Institute for Medical Research (Camden, NJ) and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

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Abbreviations: SV40, simian virus 40; bp, base pair(s).

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Michael Seidman, Ph.D.

2004

Use of replicating shuttle vector plasmid to measure DNA repair and mutagenesis in XP cells

Table 3. Types of single or tandem base substitutions in ultraviolet-treated pZ189 replicated in xeroderma pigmentosum (XP) or normal human cells

	No. of changes	
	XP	Normal
Transitions	67 (94%)	61 (75%)
G·C → A·T	66 (93%)	59 (73%)
A·T → G·C	1 (1%)	2 (2%)
Transversions	4 (6%)	20 (25%)
G·C → T·A	0	8 (10%)
G·C → C·G	1 (1%)	5 (6%)
A·T → T·A	3 (4%)	6 (8%)
A·T → C·G	0	1 (1%)

1986

Reprinted from the Archives of Dermatology
February 1987, Volume 123

Review Article

Xeroderma Pigmentosum

Cutaneous, Ocular, and Neurologic Abnormalities in 830 Published Cases

Kenneth H. Kraemer, MD; Myung M. Lee; Joseph Scotto, MS

• **Quantitative frequencies of clinical abnormalities in xeroderma pigmentosum were estimated by abstracting published descriptions of 830 patients in 297 articles obtained from a survey of the medical literature from 1874 to 1982. The median patient age was 12 years with nearly equal numbers of male and female patients. Cutaneous symptoms (sun sensitivity or freckling) had a median age of onset of between 1 and 2 years. Forty-five percent of the patients described had basal cell carcinoma or squamous cell carcinoma of the skin. The median age of first nonmelanoma skin cancer among patients with xeroderma pigmentosum was 8 years, more than 50 years less than that among patients with skin cancer in the United States. Melanomas were reported in 5% of patients. Ninety-seven percent of the reported basal and squamous cell carcinomas and 65% of the melanomas in patients with xeroderma pigmentosum occurred on the face, head, or neck. Seventy percent probability of survival was attained at age 40 years, a 28-year reduction in comparison with the US general population. Ocular abnormalities were reported in 40% of the patients described and were restricted to tissues exposed to ultraviolet radiation (lid, conjunctiva, and cornea) and included ectropion, corneal opacity leading to blindness, and neoplasms. Neurologic abnormalities were found in 18% of the cases reported, consisting of progressive mental deterioration, hyporeflexia or areflexia, and progressive deafness in some patients in association with dwarfism and immature sexual development. There was scant information concerning the efficacy of any therapeutic regimen.**

(*Arch Dermatol* 1987;123:241-250)

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From the Laboratory of Molecular Carcinogenesis (Dr Kraemer and Mr Lee) and the Biostatistics Branch (Mr Scotto), National Cancer Institute, Bethesda, Md. Mr Lee is now with Northwestern University School of Medicine, Chicago.

Reprint requests to the National Cancer Institute, Bldg 37, Room 3E24, Bethesda, MD 20892 (Dr Kraemer).

Xeroderma pigmentosum is a rare genetic disease with clinical and cellular hypersensitivity to ultraviolet radiation and defective DNA repair. Patients with xeroderma pigmentosum experience sun-induced cutaneous and ocular abnormalities, including neoplasia. Some patients have, in addition, progressive neurologic degeneration. Xeroderma pigmentosum, thus, serves as a model disorder linking defective DNA repair with clinical abnormalities and neoplasia. Quantitative information concerning the frequency of various clinical features of xeroderma pigmentosum would be useful in guiding patient management and in increasing understanding of the manifestations of defective DNA repair. To obtain such information rapidly, and to complement the establishment of a long-term prospective Xeroderma Pigmentosum Registry (Xeroderma Pigmentosum Registry, c/o Department of Pathology, Room C520, Medical Science Bldg, New Jersey Medical School, 100 Bergen St, Newark, NJ 07103), we performed a systematic study of 830 cases in 297 published articles obtained by surveying the medical literature from 1874 to 1982, a span of 108 years.

MATERIALS AND METHODS

A standard form for abstracting published case reports of xeroderma pigmentosum was prepared to probe for 207 items of clinical or laboratory information. A separate form was prepared for each patient mentioned in a report. Since we utilized the information only to the extent stated in the case report, there was, consequently, extensive information on some patients and minimal information on others. No attempt was made to gather unpublished information on the reported cases. The information on the abstracting form was converted into a computer-compatible code and entered into a mainframe computer (IBM 370). The data were analyzed using a statistical analysis and data retrieval system (SAS, SAS Institutes Inc, Cary, NC).

Utilizing resources of the library of the National Insti-

Myung Moo Lee, M.D.

Ken Kraemer, M.D.

1987

XP patients have 50 yr reduction in age of onset of skin cancer

1987

PREVENTION OF SKIN CANCER IN XERODERMA PIGMENTOSUM WITH THE USE OF ORAL ISOTRETINOIN

KENNETH H. KRAEMER, M.D., JOHN J. DIGIOVANNA, M.D., ALAN N. MOSHELL, M.D., ROBERT E. TARONE, PH.D., AND GARY L. PECK, M.D.

Abstract To confirm reports that skin cancer can be prevented with retinoids, we conducted a three-year controlled prospective study of oral isotretinoin (also called 13-*cis* retinoic acid) in five patients with xeroderma pigmentosum who had a history of multiple cutaneous basal-cell or squamous-cell carcinomas. Patients were treated with isotretinoin at a dosage of 2 mg per kilogram of body weight per day for two years and then followed for an additional year, without the drug. Before, during, and after treatment, biopsies of all suspicious lesions were performed, and skin cancers were surgically removed.

The patients had a total of 121 tumors (mean, 24; range, 8 to 43) in the two-year interval before treatment. During two years of treatment with isotretinoin, there were 25 tumors (mean, 5; range, 3 to 9), with an average reduction in skin cancers of 63 percent ($P = 0.019$). After the drug was discontinued, the tumor frequency increased a mean of 8.5-fold (range, 2- to 19-fold) over the frequency during treatment ($P = 0.007$).

Although all patients experienced mucocutaneous toxic effects, and triglyceride, liver-function, or skeletal abnormalities developed in some, high-dose oral isotretinoin was effective in the chemoprophylaxis of skin cancers in patients with xeroderma pigmentosum. (N Engl J Med 1988; 318:1633-7.)

ABOUT 500,000 people in the United States are treated each year for basal-cell or squamous-cell carcinoma of the skin.[1] In patients with xeroderma pigmentosum — an extremely rare, autosomal recessive disorder with an incidence of about one per million and characterized by sun sensitivity and a deficiency in the repair of ultraviolet-damaged DNA[2,3] — these skin cancers develop at a frequency more than 1000 times that in the general population.[3,4] Thus, patients with xeroderma pigmentosum are ideal candidates for studies of cancer prevention in humans.

The prevention of cancer with pharmacologic agents is a newly developing area of investigation.[5] Several studies in humans have used retinoids to prevent a variety of premalignant disorders.[6-10] By contrast, except in studies of recurrent bladder tumors,[7] there are few data concerning the prevention of overtly malignant lesions in humans. Although there have been some reports of prevention of skin cancers with the use of retinoids,[6-8,11-20] no long-term prospective study comparing the rates of tumor formation before, during, and after treatment has been published.

We have conducted a three-year, controlled, prospective study to determine whether high-dose oral isotretinoin (2 mg per kilogram of body weight per day) is effective in preventing the development of new skin cancers in patients with xeroderma pigmentosum. Control was achieved by comparing the frequency of tumors in each patient during treatment with that before and after treatment.

From the Laboratory of Molecular Carcinogenesis (K.H.K.), the Dermatology Branch (J.J.D. and G.L.P.), and the Biostatistics Branch (R.E.T.) of the National Cancer Institute, and the Skin Diseases Branch (A.N.M.) of the National Institute of Arthritis, Musculoskeletal, and Skin Diseases, Bethesda, Md. Address reprint requests to Dr. Kraemer at the National Cancer Institute, Bldg. 37, Rm. 3E24, Bethesda, MD 20892.

METHODS

Patient Selection

We evaluated patients with the typical clinical features of xeroderma pigmentosum[2,3] and a high frequency of skin cancers (more than two skin cancers per year for the previous two years) who were referred to the National Institutes of Health (NIH) from throughout the United States. For each patient, records were obtained from all referring physicians to determine the number of histologically diagnosed skin cancers that had occurred in the two years before treatment. Lesions that had been treated without histologic examination — with cryosurgery, for example — were not included in the determination of pretreatment tumor incidence. The selection criteria also required that women able to bear children be willing to use measures to avoid pregnancy during treatment with isotretinoin (a known teratogen) and excluded patients with deeply invasive or metastatic tumors. The purpose and plan of the study, as approved by the Clinical Research Subpanel of the National Cancer Institute, and the anticipated side effects were explained to each patient or parent, and written informed consent was obtained.

Base-Line Studies

Before treatment began, base-line color photographs of the entire skin surface, including close-ups (with a ruled marker) of the face, extremities, and abnormal lesions, were taken to facilitate the detec-

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318:1633-1637 (June 23), 1988

John J. DiGiovanna, M.D.

2008

Ken Kraemer, M.D.

Cancer chemoprevention in XP patients

Table 1. Number of Skin Cancers in Patients with Xeroderma Pigmentosum before, during, and after Therapy with Oral Isotretinoin (2 mg per Kilogram per Day).

PATIENT	AGE/SEX	BEFORE TREATMENT* (2 YR)	DURING TREATMENT* (2 YR)	AFTER TREATMENT† (12–14 MO)
		number (number per year)		
1	19/F	43 (21.5)	3 (1.5)	18 (18.0)
2	12/F	37 (18.5)	4 (2.0)	29 (38.7)‡
3	17/M	23 (11.5)	6 (3.0)	20 (20.0)
4	39/M	10 (5.0)	3 (1.5)	4 (3.4)
5	10/M	8 (4.0)	9 (4.5)	10 (10.0)

*Cancers studied by biopsy during the first two months of treatment were all documented by photography to have been present before treatment and thus were included in that category.

†Cancers studied by biopsy after treatment were not present in photographs taken during treatment.

‡Treatment was resumed after nine months because of the large number of tumors that appeared after the drug was discontinued.

1988

Reprinted from the Archives of Dermatology
August, 1994 Volume 130

STUDY

The Role of Sunlight and DNA Repair in Melanoma and Nonmelanoma Skin Cancer

The Xeroderma Pigmentosum Paradigm

Kenneth H. Kraemer, MD; Myung-Moo Lee, MD; Alan D. Andrews, MD; W. Clark Lambert, MD, PhD

Background and Design: The frequency of melanoma and nonmelanoma skin cancer is increasing rapidly in the United States. However, the linkage of these cancers to sun exposure has been questioned because of differences in anatomic site distribution. To obtain insights into the development of these skin cancers, we examined reports of 132 patients with xeroderma pigmentosum (XP), an inherited cancer-prone, DNA repair–deficient disorder with marked clinical and laboratory UV hypersensitivity.

Results: Malignant skin neoplasms were present in 70% of the patients with XP at a median age of 8 years, which is 50 years earlier than in the US white population. Fifty-seven percent of the patients had basal cell or squamous cell carcinoma, and 22% had melanoma. The frequency of melanomas, like the frequency of nonmelanoma skin cancers (basal cell and squamous cell carcinomas), anterior eye cancers, and tongue cancers, but unlike that of internal neoplasms, was increased 1000-fold or more in patients with XP who were younger than 20 years. As in the general population, the anatomic distribution of melanomas was different from that of nonmelanomas in the patients with XP.

Conclusions: These data suggest that (1) DNA repair plays a major role in the prevention of cutaneous cancers in the general population and (2) sunlight exposure is responsible for the induction of melanoma as well as nonmelanoma skin cancer in patients with XP, although acting by different mechanisms for the two types of skin cancer.

(Arch Dermatol. 1994;130:1018-1021)

From the Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Md (Drs Kraemer and Lee); the Department of Dermatology, Columbia University College of Physicians and Surgeons, New York, NY (Dr Andrews); and the Departments of Laboratory Medicine and Pathology and Dermatology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ (Dr Lambert). Dr Lee is now with the Division of Dermatology, Department of Family Practice, US Air Force Medical Corps, Yokota (Japan) Air Base Hospital.

THE FREQUENCY of both melanoma and nonmelanoma skin cancer is increasing rapidly in the United States.[1-3] The degree of association of these cancers with sun exposure has been questioned because of the different anatomic site distribution of melanomas vs that of nonmelanoma skin cancers.[1,3] Patients with xeroderma pigmentosum (XP), which is a rare, hereditary disorder with marked clinical and cellular UV hypersensitivity and defective DNA repair in all cells, develop numerous skin cancers.[4-7] In a search for insight into factors that are important in the development of melanoma and nonmelanoma skin cancers in humans, we analyzed information obtained from reports to the Xeroderma Pigmentosum Registry, Newark, NJ.

RESULTS

A total of 132 patients with XP were registered: 72 males and 60 females. The group was young, with a median age of 16 years; 123 of the patients were younger than 40 years. Malignant skin neoplasms were reported in 93 (70%) of the patients. The median age for development of the first skin cancer was 8 years (among the 64 patients with this information). This is approximately 50 years younger than the median age of development of skin cancer in the US general population.[1] Many patients with XP had a history of multiple skin cancers: 73 patients had more than one cancer (20 of these had both basal cell carcinoma and melanoma), and 32 patients had more than 20 skin cancers each (based on the 82 reports in which the number of skin cancers was specified).

See Methods on next page

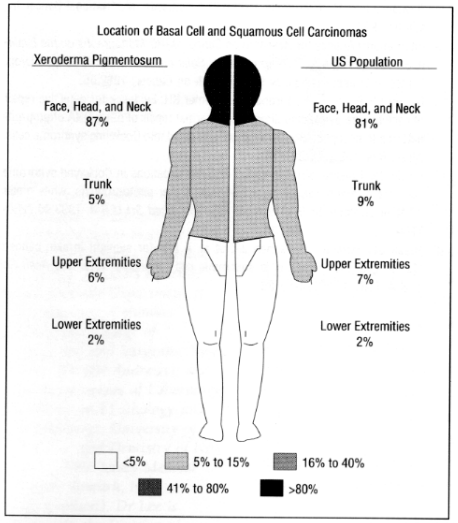

Figure 1. *Location of nonmelanoma skin cancers in patients with xeroderma pigmentosum compared with the US white population. The anatomic location of 401 basal cell or squamous cell carcinomas reported in the patients with xeroderma pigmentosum is compared with that of 26 817 cancers in the US white population (from Scotto et al[1]).*

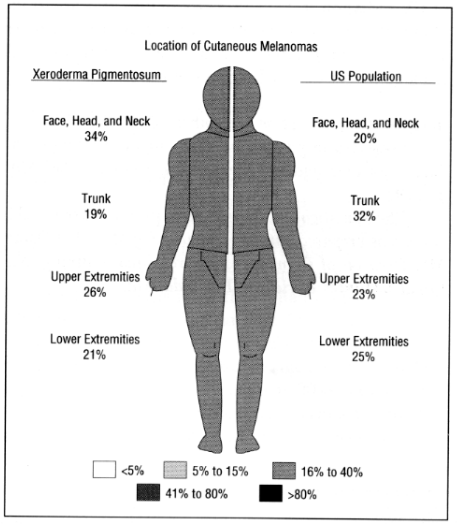

Figure 2. *Location of melanomas in patients with xeroderma pigmentosum compared with the US population. The anatomic location of 58 cutaneous melanomas in the patients with xeroderma pigmentosum is compared with that of 5844 melanomas in the US population (from Young et al[8]).*

1000 – fold increase in skin cancer in XP

1994

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3782–3786, June 1987
Genetics

Photoproduct frequency is not the major determinant of UV base substitution hot spots or cold spots in human cells

(UV carcinogenesis/DNA structure/xeroderma pigmentosum/DNA repair/shuttle vector)

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Communicated by Evelyn M. Witkin, February 13, 1987

ABSTRACT **The role of UV radiation-induced photoproducts in initiating base substitution mutations in human cells was examined by measuring photoproduct frequency distributions and mutations in a *supF* tRNA gene on a shuttle vector plasmid transfected into DNA repair-deficient cells (xeroderma pigmentosum, complementation group A) and into normal cells. Frequencies of cyclobutane dimers and pyrimidine-pyrimidone (6-4) photoproducts varied by as much as 80-fold at different dipyrimidine sites within the gene. All transition mutations occurred at dipyrimidine sites, predominantly at cytosine, with a 17-fold variation in mutation frequency between different sites. Removal of >99% of the cyclobutane dimers by *in vitro* photoreactivation before transfection reduced the mutation frequency while preserving the mutation distribution, indicating that (*i*) cytosine-containing cyclobutane dimers were the major mutagenic lesions at these sites and (*ii*) cytosine-containing non-cyclobutane dimer photoproducts were also mutagenic lesions. However, at individual dipyrimidine sites neither the frequency of cyclobutane dimers nor the frequency of pyrimidine-pyrimidone (6-4) photoproducts correlated with the mutation frequency, even in the absence of excision repair. Mutation hot spots occurred at sites with low or high frequency of photoproduct formation and mutation cold spots occurred at sites with many photoproducts. These results suggest that although photoproducts are required for UV mutagenesis, the prominence of most mutation hot spots and cold spots is primarily determined by DNA structural features rather than by the frequency of DNA photoproducts.**

The majority of base substitution mutations, whether chemically or physically induced or spontaneous, appear to be located at DNA lesions (1, 2). In the case of UV radiation, the lesions appear to be dipyrimidine photoproducts that initiate G·C to A·T transitions (2–8). It has long been anticipated that identification of the mutagenic UV photoproduct in human cells would provide a missing link in the etiology of sunlight-related human skin cancers, in oncogene activation by point mutations (9), and in the pathogenesis of xeroderma pigmentosum, a human disease with defective photoproduct excision and markedly elevated skin cancer incidence (10). For example, the lesion distribution might reveal why different carcinogens activate different protooncogenes even in the same tissue type (11).

Recently it has become possible to measure mutation spectra in mammalian cells following treatment of DNA with UV or other DNA-damaging agents by using shuttle vector plasmids (5–8). We have now measured the frequencies of two UV photoproducts implicated in mutagenesis, the cyclobutane dimer and the pyrimidine-pyrimidone (6-4) photoproduct (refs. 3 and 4 and references therein), in a shuttle vector *supF* tRNA gene (6). The vector was treated with UV *in vitro* and the photoproduct frequency was measured. The vector was transfected into repair-deficient and normal human cells, and the number of mutations at different sites was determined (6). By *in vitro* photoreactivation of cyclobutane dimers prior to transfection, we determined that cyclobutane dimers and non-cyclobutane dimer photoproducts contributed to base substitution mutations. The frequency of these photoproducts at mutated sites did not correlate with the mutation frequency at the same sites, even in the absence of excision repair.

MATERIALS AND METHODS

Site-Specific Determination of Cyclobutane Dimers and (6-4) Photoproducts. Form I plasmid pZ189 was restriction digested and end labeled following UV treatment, so the photoproduct frequencies measured were identical to those transfected into the cells. The DNA was 3′ end labeled by filling in the unique *Eco*RI site (top strand) or the base pair 239 *Xho* II site (bottom strand). To detect the presence of cyclobutane dimers and pyrimidine-pyrimidone (6-4) photoproducts at individual base pairs, end-labeled DNA was incubated with T4 dimer-specific endonuclease (a gift from A. Ganesan, Stanford University) or 1 M piperidine, respectively (12). Samples were electrophoresed and quantitated as described (12) or quantitated by scanning the autoradiograms with an LKB Ultroscan laser densitometer using known radioactivity standards. The variation of measurements of replicate samples was <20%.

Plasmid Treatment and Mutagenesis. pZ189 (6) was treated with 254-nm UV radiation from a germicidal lamp as described (13, 14). *Escherichia coli* DNA photolyase (12) (a gift from A. Sancar, University of North Carolina) was used as described (14) to monomerize pyrimidine dimers but not (6-4) photoproducts (12). The extent of photoreactivation was measured by the T4 endonuclease V assay on supercoiled DNA (13) and on defined sequence DNA (12). Simian virus 40 (SV40)-transformed xeroderma pigmentosum (XP12Be) and normal (GM0637) cell lines were obtained from the Institute for Medical Research (Camden, NJ), and grown as described (13, 14). XP12Be fibroblasts have been shown to have <2% of normal DNA excision repair (15). Mutation spectra were determined as described (6, 13).

RESULTS

Photoproduct Frequency and Mutation Frequency. The frequency of UV photoproducts was measured at 58 of the 72

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Abbreviation: SV40, simian virus 40.

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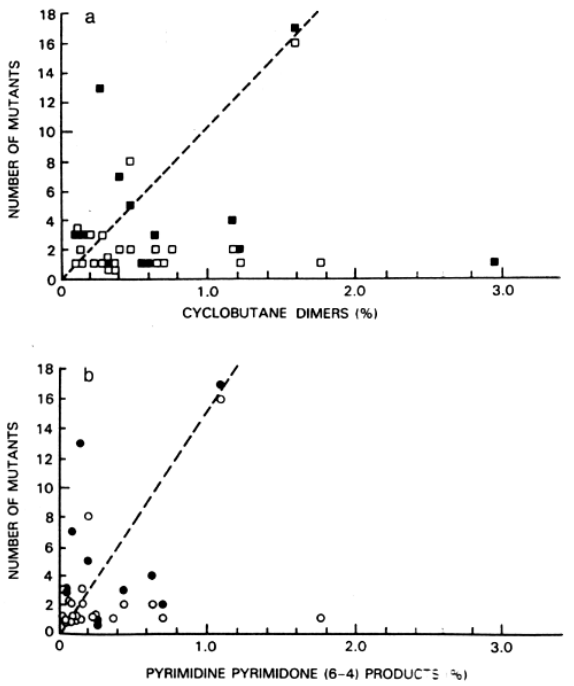

FIG. 3. Relationship of cyclobutane dimer and pyrimidine-pyrimidone (6-4) photoproduct frequency to the number of transition mutants found in pZ189 propagated in xeroderma pigmentosum [XP12BE (SV40)] or repair-proficient [GM0637 (SV40)] human cells. (*a*) Cyclobutane dimer frequency versus number of transition mutants. (*b*) Pyrimidine-pyrimidone (6-4) photoproduct frequency versus number of transition mutants. ● and ■, xeroderma pigmentosum cells; ○ and □, repair-proficient cells. Mutations were assigned to the 3′ base of a photoproduct where possible. Attribution of mutations to the 5′ base did not alter the results. The dashed line in each graph indicates a theoretical linear relationship between photoproduct frequency and mutation frequency assuming that the mutations at base pair 156 are proportional to the photoproduct frequency.

1994

EDITOR'S CHOICE

Trichothiodystrophy: a systematic review of 112 published cases characterises a wide spectrum of clinical manifestations

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ABSTRACT

Trichothiodystrophy (TTD) is a rare, autosomal recessive disease, characterised by brittle, sulfur deficient hair and multisystem abnormalities. A systematic literature review identified 112 patients ranging from 12 weeks to 47 years of age (median 6 years). In addition to hair abnormalities, common features reported were developmental delay/intellectual impairment (86%), short stature (73%), ichthyosis (65%), abnormal characteristics at birth (55%), ocular abnormalities (51%), infections (46%), photosensitivity (42%), maternal pregnancy complications (28%) and defective DNA repair (37%). There was high mortality, with 19 deaths under the age of 10 years (13 infection related), which is 20-fold higher compared to the US population. The spectrum of clinical features varied from mild disease with only hair involvement to severe disease with profound developmental defects, recurrent infections and a high mortality at a young age. Abnormal characteristics at birth and pregnancy complications, unrecognised but common features of TTD, suggest a role for DNA repair genes in normal fetal development.

Trichothiodystrophy (TTD) is a rare, autosomal recessive disease, in which patients have brittle, sulphur deficient hair.[1 2] When the hair from TTD patients is observed under polarising microscopy, it displays a diagnostic alternating light and dark banding pattern, called "tiger tail banding"[3 4] (fig 1). TTD results from mutations in one of several different DNA repair genes (*XPB*, *XPD* or *TTDA*)[5 6] and *TTDN1*, a gene of unknown function.[7] Although XPB and XPD mutations are also seen in xeroderma pigmentosum, a disease with a 1000-fold increase in skin cancer,[8 9–11] TTD patients have not been reported to have an increase in cancer.

TTD patients display a wide variety of clinical features, including cutaneous, neurological, and growth abnormalities. As a result, a variety of names have been used to describe the disease. In 1979, Price coined the term "trichothiodystrophy," which encompasses a wide spectrum of neurocutaneous findings, to describe the unifying feature.[12] The name reflects the brittle, sulfur deficient hair seen in all TTD patients (from Greek, tricho- meaning hair; -thio-, sulfur; -dys-, faulty; -trophy, nourishment). Several acronyms have been used to describe the clinical features of these patients. PIBIDS,[13] IBIDS[14 15] and BIDS[16] describe six features of TTD: Photosensitivity, Ichthyosis, Brittle hair, Intellectual impairment, Decreased fertility, and Short stature. In order to assess the prevalence of the reported clinical features of TTD, we performed an extensive literature review to find all published case reports of patients with TTD. We analysed the frequency of the clinical findings described in an effort to characterise the spectrum of the disease better.

We modelled this review after a similar study on xeroderma pigmentosum.[9]

METHODS

We developed a standard Excel spreadsheet listing more than 200 clinical and laboratory characteristics. The search was restricted to published information in reports, and no effort was made to obtain unpublished data on the reported patients. This approach results in underreporting of characteristics not noted at the time of publication. However, when reported patients were identifiable as being the same individual in a subsequently reported paper, the data were consolidated. We searched PubMed/Medline, Web of Science, and the references cited in retrieved articles. Search terms were trichothiodystrophy, TTD, Tay syndrome, Pollitt syndrome, PIBIDS, IBIDS, and BIDS.

The most definitive clinical criteria include microscopic examination of hair shafts for tiger tail banding and structural abnormalities and the analysis of hair shaft sulfur content. However, diagnostic criteria for TTD have evolved over the decades since these reports have been published. As a result, some reports included patients with convincing clinical features of TTD and a confirmed DNA repair abnormality, but the clinical workup did not include hair analysis. In order to standardise selection of patients, we chose criteria, which determined whether or not a case report was included. Inclusion criteria were based on having at least two of the four following clinical or laboratory abnormalities: (1) presence of brittle hair and/or hair shaft abnormalities; (2) tiger tail banding with polarised microscopy; (3) decreased sulfur or cystine content of hair; and (4) DNA repair abnormality. While any one of these features is highly suggestive of TTD,[9 11 17] we required a minimum of two features to confirm the diagnosis. We chose criteria which we reasoned would allow us to capture reports of most patients with TTD and which were important in forming the basis for the various subtypes which have led to our current understanding of the disease. These criteria were developed in order to provide a uniform approach to inclusion of case reports with varied amounts of information and published over more than

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Review

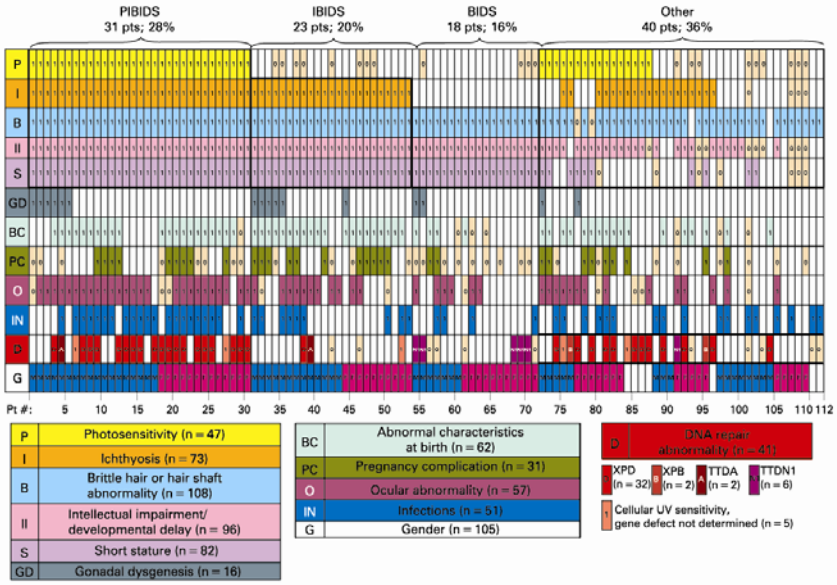
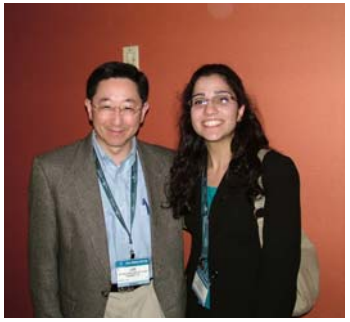


Figure 4 A clinical array of features reported in the literature on 112 trichothiodystrophy patients. Each column of rectangles represents clinical features of one reported patient. Presence or absence of each feature is indicated in each rectangle of a column. Abnormal clinical features reported are indicated by "1" in a coloured rectangle. Normal reported features are indicated by "0" in a tan rectangle. Unreported features are blank. The rows represent P (yellow)—photosensitivity (n = 47 cases); I (orange)—ichthyosis (n = 73 cases); B (powder blue)—brittle hair or hair shaft abnormality (n = 108); II (pink) —intellectual impairment (n = 96 cases); GD (grey) —gonadal dysgenesis (n = 16 cases); BC (light green) —abnormal birth characteristics (n = 62 cases); PC (dark green) —pregnancy complications (n = 31 pregnancies); O (maroon)—ocular abnormality (n = 57 cases); IN (royal blue) —infections (n = 51 patients); D (red)—DNA repair abnormality (n = 41); D in red rectangle—*XPD* (n = 32 cases); B in striped red rectangle—*XPB* (n = 2 cases); A in striped red rectangle—*TTDA* (n = 2); N in striped pink rectangle—*TTDN1* (n = 6 cases); I in red rectangle—cellular ultraviolet (UV) hypersensitivity, gene not determined (n = 5); G—gender (n = 105 patients); blue rectangles—males (n = 54 cases); pink rectangles—females (n = 51 cases). Patients whose clinical features fulfil the criteria for PIBIDS (28%), IBIDS (20%), BIDS (16%) and those that do not (OTHER) (36%), are grouped by bold outline (decreased fertility is ignored in this grouping due to inability to assess in children.)

Pregnancy complications and increased death from infection in TTD

Myung Moo Lee M.D.



Salma Faghri

2008

Philip C. Hanawalt, Ph.D.

Stanford University, Palo Alto, CA

Half a century of DNA repair: An historical perspective

April 16, 2002

J. M. Egly, Ph.D.

Phil Hanawalt, Ph.D.

2004

http://videocast.nih.gov/PastEvents.asp?c=5

Steven Leadon, Ph.D.

University of North Carolina, Chapel Hill, NC

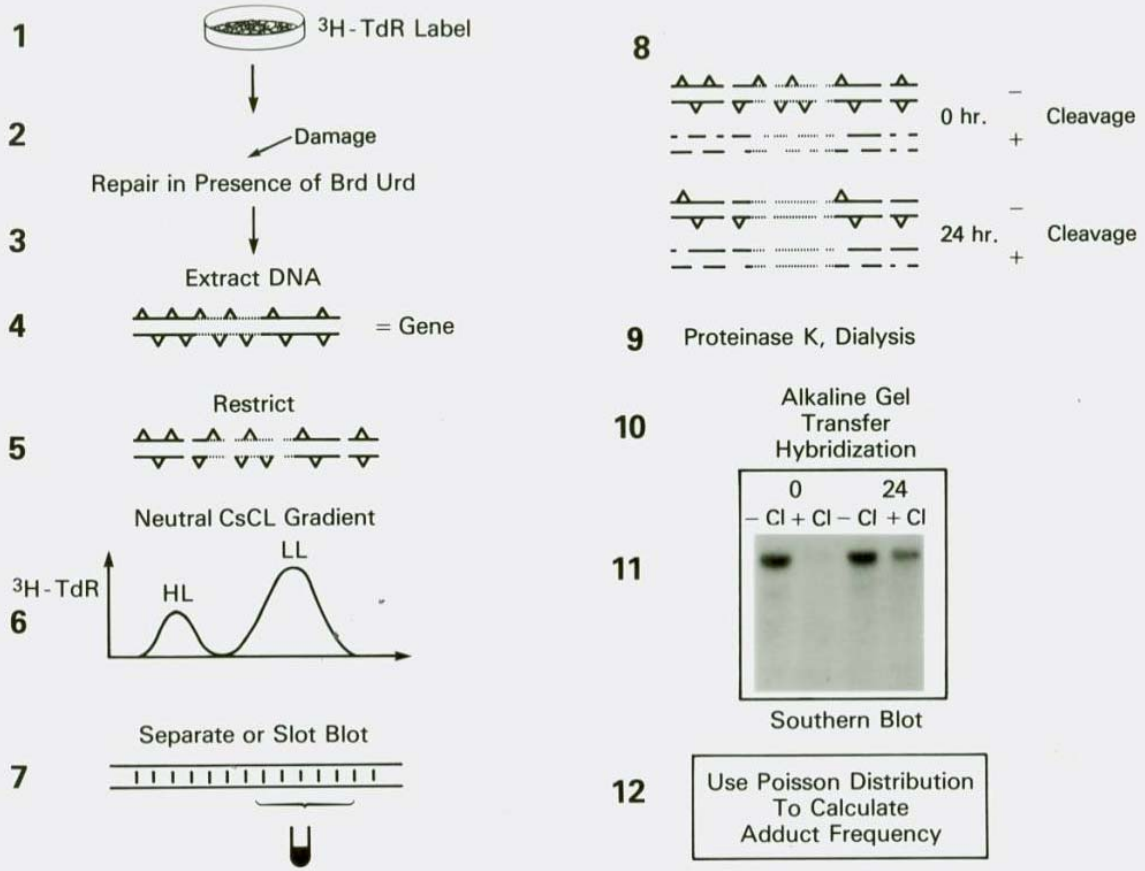
Transcription coupled repair of oxidative DNA damage
Feb 16, 1999

http://videocast.nih.gov/PastEvents.asp?c=5

Gene Repair and TCR
Hanawalt lab in the early eighties

- Southern quantitation
  - Vilhelm Bohr
  - Allan Smith
  - Isabel Mellon
  - Diane Okumoto
- Other assays
  - Tony Leadon
    - Lesion specific ab
  - Jean Michel Vos
    - Crosslink repair

Methodology to study Gene Specific damage and Repair



Cell, Vol. 40, 359–369, February 1985, Copyright © 1985 by MIT 0092–8674/85/020359–11 $02.00/0

DNA Repair in an Active Gene: Removal of Pyrimidine Dimers from the DHFR Gene of CHO Cells Is Much More Efficient than in the Genome Overall

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Summary

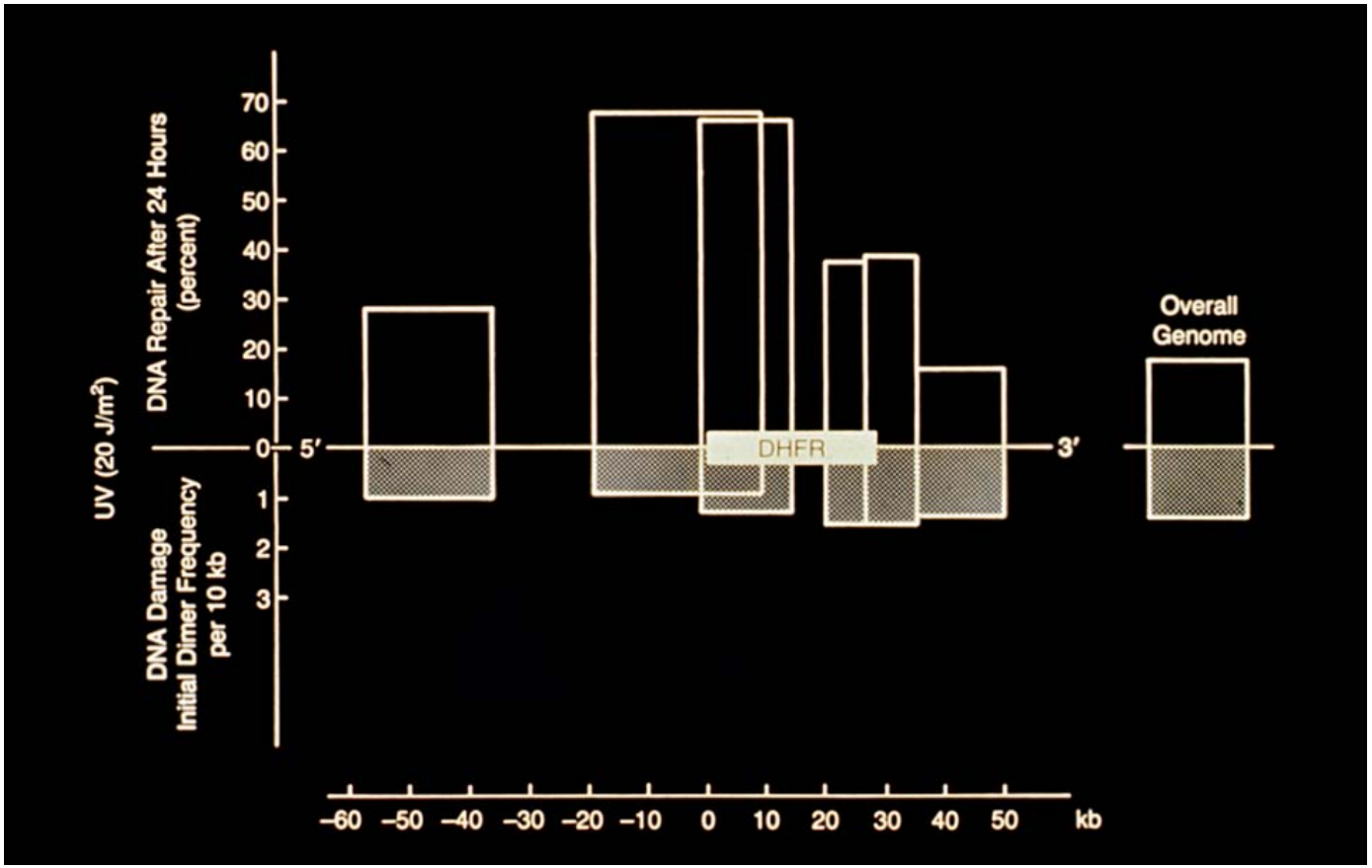
DNA repair was measured in the dihydrofolate reductase gene in Chinese hamster ovary cells, amplified for the gene, by quantitating pyrimidine dimers with a specific UV-endonuclease. More than two thirds of the dimers had been removed from a 14.1 kb restriction fragment of the gene by 26 hr after irradiation (20 J/m^2), while little removal was detected in fragments upstream of the gene and only 15% were removed from the genome overall. This suggests that damage processing can vary according to function or activity of affected sequences, which has general implications for correlations of DNA repair with survival and mutagenesis. Perhaps preferential repair of vital sequences facilitates UV-resistance of these cells despite low overall repair levels.

highly radioactive sequence-specific probes to detect specific restriction fragments in the genome with an enzymatic assay to measure frequencies of pyrimidine dimers alone. We also used a cell line in which this gene had been amplified to about 100 copies (Johnston et al., 1983). The increased copy number results in overproduction of the gene product and consequent cellular resistance to methotrexate (MTX) (Alt et al., 1978). Maintenance of the cells in MTX ensures continued activity of the DHFR genes.

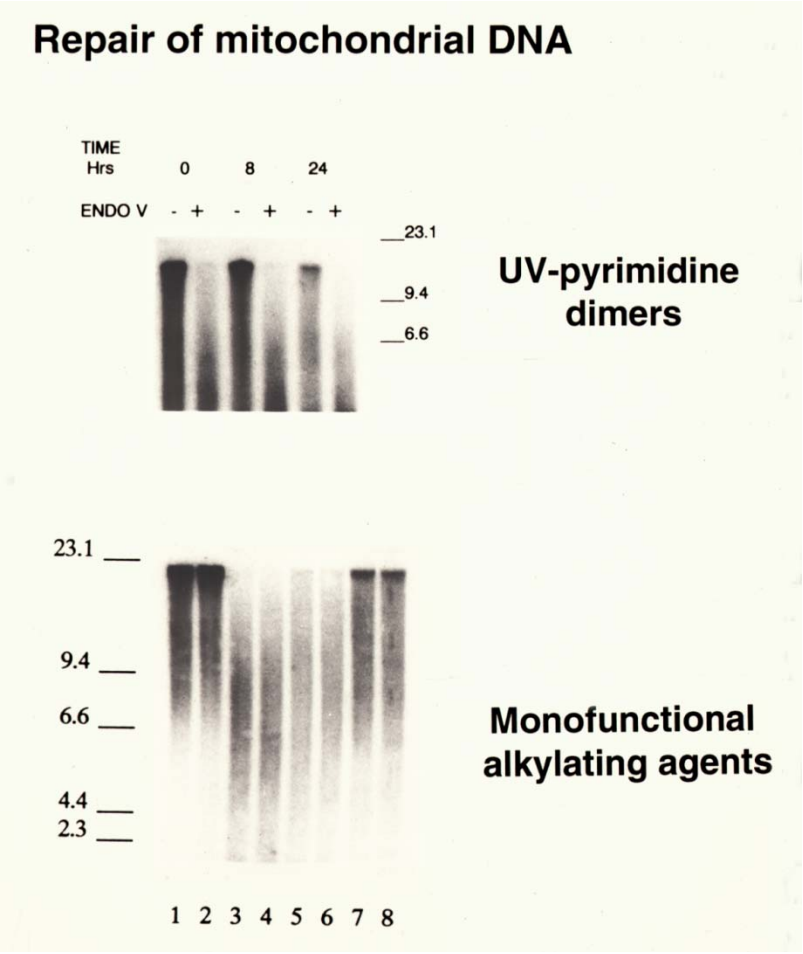
After reaction with a specific UV-endonuclease, restricted DNA was electrophoresed in alkaline gels to separate the full-length strands from the products of incision at sites of pyrimidine dimers. The quantity of hybridization to full-length fragments was used to calculate the fraction of molecules containing no endonuclease sensitive sites (ESS). The overall frequency of ESS per restriction fragment was then derived using the Poisson expression.

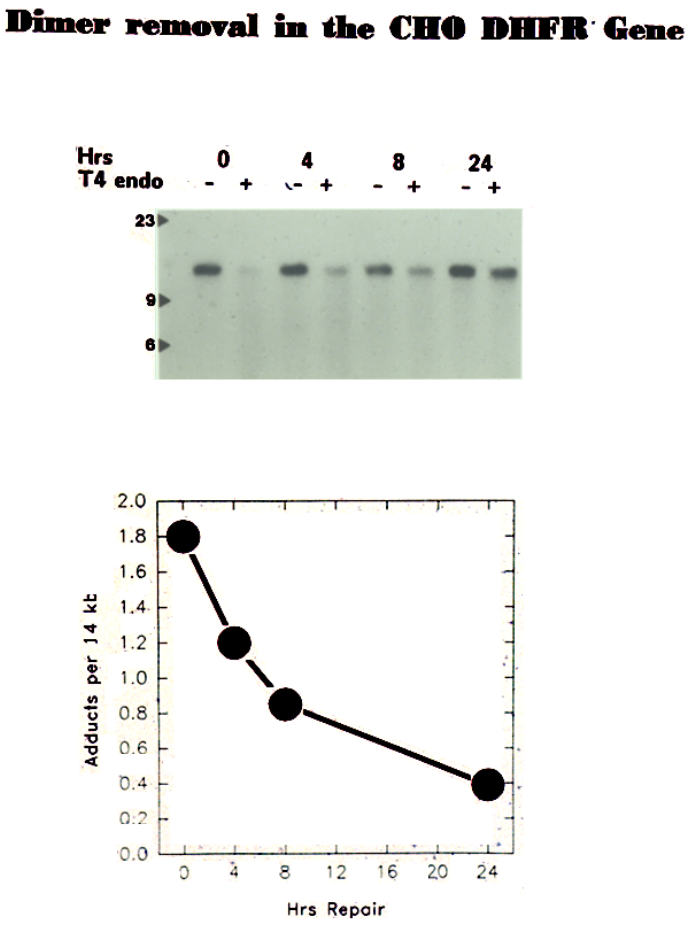
Although the initial frequency of pyrimidine dimers formed in the DHFR gene was found to be similar to that in the genome as a whole, their removal from the DHFR gene was markedly greater. In addition, we observed little or no removal from two genomic fragments located on the amplified unit at least 15 kb upstream of the gene. This is the first demonstration of preferential excision repair in a

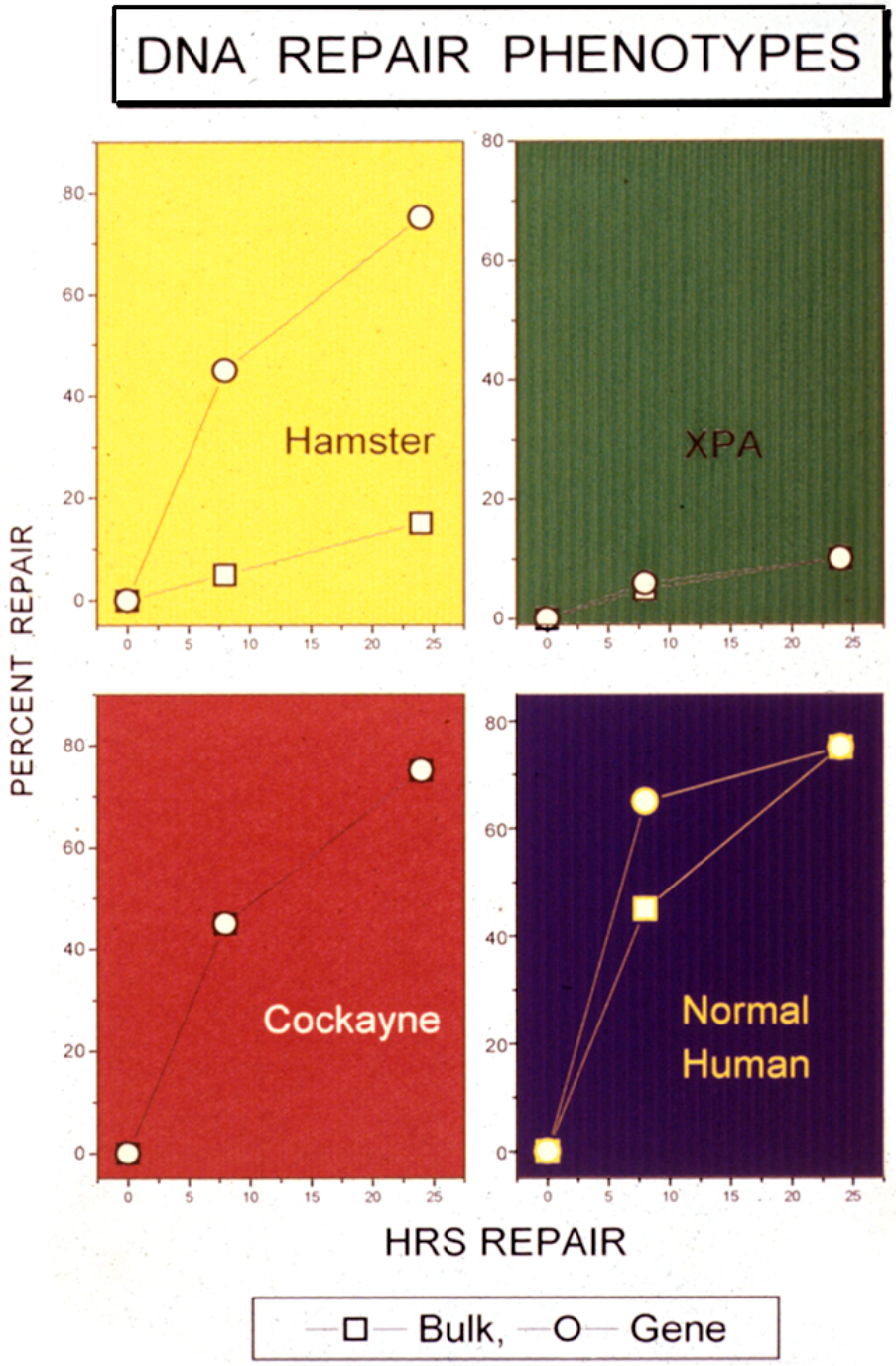
DHFR Gene Repair Domain



Gene Repair of UV and alkylation damage in nuclear and mitochondrial DNA





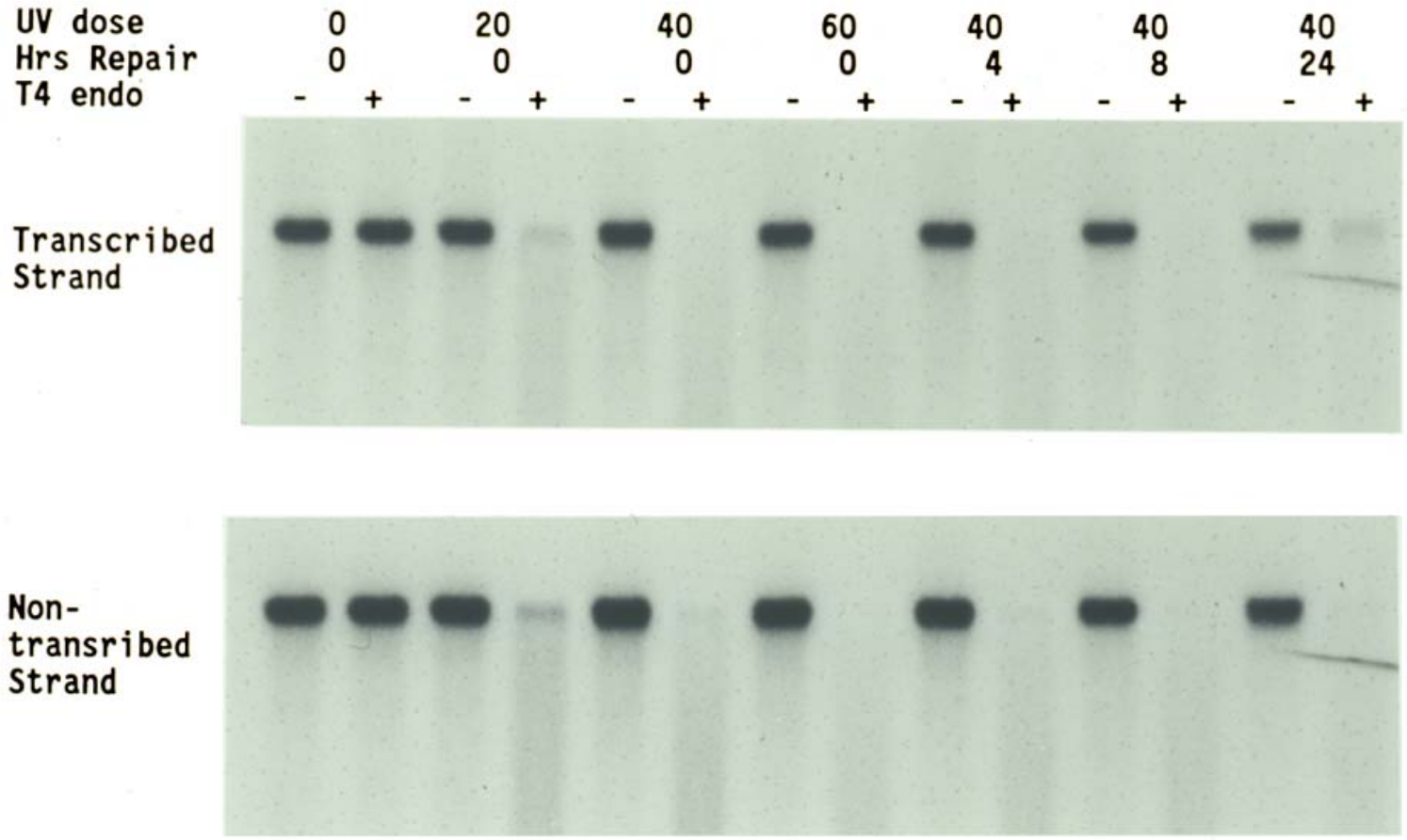


Rodent cell paradox

Rodent cells repair only active genes and their UV survival is similar to that in human cells.

UV survival correlates better with active gene repair than overall, avareage genome repair

Strand Specificity of the repair of pyrimidine dimers in the CHO DHFR gene after UV irradiation



Gene Repair and TCR (TC-NER)

- Gene Repair
  - Does not always correlate with transcriptional activity
  - Correlation to mutation hotspots
  - Chromatin structure ?
  - Gene “importance”
- TCR (TC-NER)
  - Phenomenon
  - Associated with strand bias of repair
  - Mechanistically not yet understood

GENE REPAIR WITH ANTIBODIES TO BRDU OR LESIONS (TG)

Gene repair with antibodies to repair patches (BrdU) or lesions (TG)

↓

Damaged DNA is sheared

↓

Treated with antibody

↓

Precipitated with Farr assay – separates into the repaired and damaged DNA

↓

Slot blot and probe the damaged DNA for presence of specific genes