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.
NCI, NIH, Bethesda, MD

Vilhelm A. Bohr, M.D., Ph.D.
NIA, NIH, Baltimore, MD

• 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
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| **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 |

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<td><strong>Patrick Sung</strong>&lt;br&gt;Univ of Texas</td>
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<td><strong>Steven A. Leadon, UNC</strong></td>
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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
Reflections on how I was led into DNA repair
June 20, 2000

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

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)
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 photochemical 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...
1974

XP4BE
First XP variant patient

XP5BE – XP6BE
First XP-D patients
XP11BE
First XP-B
patient
(XP/CS)
WORKSHOP ON

"REPAIR MECHANISMS IN MAMMALIAN CELLS"

Paul H.M. Lohman, Ph.D.

Dirk Bootsma, 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 Ely 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

Secretary 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 Noordwijk beach. 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. Buses 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 accommodation 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. Leunen 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

16.30 - 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
15.00 - 18.30 h   free
19.00 - 19.30 h   excursion to "De Keukenhof"
19.30 - 20.30 h   apéritif
"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. Rösch
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

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. Klümek, 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. Campagnoli, 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
PARTICIPANTS

ARLETT, C.F., MRC Cell mutation unit, University of Sussex, Falmer, Brighton, England

BACCHETTI, S., Laboratory for Molecular Genetics, Leiden State University, Leiden, The Netherlands

BEN-ISHAI, R., Department of biology, Technion - Israel Institute of Technology, Haifa, Israel

BERENDS, W., Laboratory for biochemistry and biophysics, Technical University, Delft, The Netherlands

BLEICHRODT, J.F., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

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

BRENT, T.P., St. Jude Children's Research Hospital, Memphis, Tennessee 38101, USA

BRIDGES, B.A., MRC Cell mutation unit, University of Sussex, Falmer, Brighton, England

CAMPAIGNARI, F., Laboratory of genetical biochemistry, Biology Division, EURATOM joint research center, 21020 Ispra, Varese, Italy

CLEAVER, J.E., Laboratory of radiobiology, University of California, San Francisco, Calif. 94112, USA

CLERICI, L., Laboratory of genetical biochemistry, Biology Division, EURATOM joint research center, 21020 Ispra, Varese, Italy

COHEN, E.M., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

CORNELIS, J.J., Laboratoire de biophysique et radiobiologie, Université libre de Bruxelles, 67 Rue des Chevaux, 1640 Rhode-St-Genèse, Belgium

DEAN, Tunstall laboratory, Broad Oak Road, Sittingbourne, Kent, England

DEVORET, R., Laboratoire d'enzymologie, CNRS, 91400 Orsay, Yvelines, France

ELKIND, M.M., Experimental radiopathology unit, Medical Research Council, Hammersmith Hospital, London W12, England

EMMELOT, P., Nederlands Kanker Instituut, Sarphatistraat 108, Amsterdam, The Netherlands

ERRERA, M., Laboratoire de biophysique et radiobiologie, Université libre de Bruxelles, 67 Rue des Chevaux, 1640 Rhode-St-Genèse, Belgium

EVANS, H.J., MRC Clinical and population cytogenetics unit, Western general hospital, Crewe Road, Edinburgh, Scotland

FALASCHI, A., Laboratorio di genetica biochimica ed evoluzionistica, C.N.R., Via Sant'Epifanio 14, 27100 Pavia, Italy

FOX, B.W., Paterson laboratories, Christie Hospital and Holt Radium Institute, Wilmslow road, Withington, Manchester, England

FOX, M., Paterson laboratories, Christie Hospital and Holt Radium Institute, Wilmslow road, Withington, Manchester, England
GROSSLAND, L., Graduate department of biochemistry, Brandeis University, Waltham, Mass., USA

HOWARD-FLANDERS, P., Department of radiology, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510, USA

JUNG, E.G., Department of dermatology, University of Heidelberg, Voss-Straße 2, Heidelberg, W.-Germany

KIHLMAN, B.A., Department of genetics and plant breeding, Royal Agricultural College of Sweden, Uppsala 7, Sweden

KLEIJER, W.J., Department of cell biology and genetics, Medical Faculty, Rotterdam, The Netherlands

KLIMEK, M., Institute of biophysics, Czechoslovak Academy of Sciences, Krakovská 135, 61265 Brno, Czechoslovakia

LEHANN, A.R., MRC Cell mutation unit, University of Sussex, Falmer, Brighton, England

LETT, J.T., Department of radiology and radiation biology, Colorado State University, Fort Collins, Colorado 80521, USA

LINDAHL, T., Department of chemistry, Karolinska Institute, 10401 Stockholm, Sweden

LOHMANN, P.H.M., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

LUTZNER, M.A., Department of health, education and welfare, Public Health Service, National Institute of Health, Bethesda, Maryland 20014, USA

MATHELET, M.M., Laboratory of genetical biochemistry, Biology Division, EURATOM joint research center, 21020 Ispra, Varese, Italy

MATTERN, J.E., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

MEYN, R.E., The University of Texas at Houston, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, Texas 77025, USA

MIKHelson, V.M., Laboratory of radiation cytology, Institute of cytology of the Academy of Sciences of the USSR, Leningrad, USSR

NUZZO, F., Laboratorio di genetica biochimica ed evoluzioneistica, C.N.R., Via Sant'Epifanio 14, Z1 100 Pavia, Italy

NIKAIoD, O., Paterson Laboratories, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester, England

OOSTERBAAN, R.A., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

ORMEROD, M.G., Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Clifton Avenue, Belmead, Sutton, Surrey, England

PAINTER, R.B., Laboratory of radiobiology, University of California, San Francisco, Calif. 94112, USA

PARKER, D.R., Department of radiation genetics and chemical mutagenesis, Leiden State University, Leiden, The Netherlands

PARRY, E., Department of genetics, University college of Swansea, Singleton Park, Swansea, Gwent, W. Glamorgan, Wales

PARRY, J.M., Department of genetics, University college of Swansea, Singleton Park, Swansea, Gwent, W. Glamorgan, Wales

PATERSON, M.C., Laboratory for Molecular Genetics, Leiden State University, Leiden, The Netherlands

PEDRINI, M.A., Laboratorio di genetica biochimica ed evoluzionistica, C.N.R., Via Sant'Epifanio 14, Z1 100 Pavia, Italy

RAUTH, A.M., Department of medical biophysics, University of Toronto, Ontario cancer institute, 500 Sherbourne Street, Toronto 5, Ontario, Canada

REGAN, J.D., Biology division, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tennessee 37830, USA

ROBBINS, J.H., National Institutes of Health, Bethesda, Maryland 20014, USA

ROBERTS, J.J., Chester Beatty research institute, Institute of cancer research, Pollards Wood research station, Chalfont St-Giles, Bucks, England

RÖSCH, A., Laboratory for Molecular Genetics, Leiden State University, Leiden, The Netherlands

RUPP, W.D., Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510, USA

SARMA, Temple University, Health Sciences Center, School of Medicine, Fels Research Institute, Philadelphia, Pennsylvania 19140, USA

SIMONS, J.W.I.M., Department of radiation genetics and chemical mutagenesis, Leiden State University, Leiden, The Netherlands

SLOE, H., Laboratory of radiobiology, University of California, San Francisco, Calif. 94112, USA

SMETS, L., Nederlands Kanker Instituut, Sarphatistraat 108, Amsterdam, The Netherlands

SOBLSKY, F.H., Department of radiation genetics and chemical mutagenesis, Leiden State University, Leiden, The Netherlands

STICH, H.F., The University of British Columbia, Cancer research center, Vancouver 8, Canada

STRAUSS, B.S., Department of microbiology, The University of Chicago, 5724 South Ellis Avenue, Chicago, Illinois 60637, USA

VAN DEN BERG, H.J., Department of experimental pathology and cancer research, University of Leiden, 171 Woodhouse Lane, Leeds, England

VELDHUIJSEN, G., Medical Biological Laboratory TNO, P.O. Box 45, Rijswijk 2100, The Netherlands

WEERD-KASTELEIN, E.A.de, Department of cell biology and genetics, Medical Faculty, Rotterdam, The Netherlands

ZEELAND, A.A., Department of radiation genetics and chemical mutagenesis, Leiden State University, Leiden, The Netherlands
Genetic Heterogeneity in Xeroderma Pigmentosum: Complementation Groups and Their Relationship to DNA Repair Rates

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

KENNETH H. KRAMER*, HAYDEN G. COON, ROBERT A. PETINGA, SUSANNA F. BARRETT, ANN E. RAHEJ, AND JAY H. ROBBINS

Dermatology Branch and Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20204

Communicated by Auron B. Pearson, October 1, 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 radiation/unscheduled nucleotide DNA synthesis. The nuclei in binucleate 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 un fused mononucleate 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 mononucleate 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 [3H]thymidine into DNA segments synthesized to replace regions containing UV-induced pyrimidine dimers (1, 2, 4). This [3H]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 (7-9) 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 binucleate heterokaryons, both of the nuclei in the fused cells perform more unscheduled DNA synthesis than the nuclei of the unfused mononucleate 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 that 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 XPK1MSF Abbreviations: XP; xeroderma pigmentosum; UV; ultraviolet.

* Present address: Department of Dermatology, University of Miami School of Medicine, Box 520675, Miami, Fls. 33162

(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°C in a 5% CO2-95% air incubator with more than 90% humidity. They were used after two to seven additional passages.

Cell Fusion. One- to 3-day-old cover slip cultures in 60-mm petri dishes were used for irradiation and fusion, the latter performed by modification of the method of Yamashita et al. (15). Cultures, cooled on ice, were washed with 0.25% Hank's balanced salt solution and covered with 0.1 ml cold, serum-free medium containing about 100 hemoglobinizing units [standardized with 0.65%/v/v chick thymocyte deoxycytidine 5'-triphosphate (dCTP)-inactivated Sendai virus (14). Two minutes later, about 0.1 ml of cold, serum-free medium containing 5 X 10^6 cells (to be fused with those already on the cover slip) was dropped on each cover slip. After 30 min in the cold, the dishes were placed in the 37°C incubator. Three hours later, the cover slips were covered with serum-containing medium (5 ml per dish), and the incubation was continued.

UV Irradiation. About 1 hr after addition of inactivating virus, the cultures were washed twice with phosphate-buffered saline (PBS) at room temperature and covered with phosphate-buffered saline (1 ml per dish). Immediately after irradiation with 300 ergs/cm^2 of 254 nm UV light, the phosphate-buffered saline was removed and 2 ml of Hank's base medium (16, (Office Laboratory or NIH Media Unit containing human serum, 20% fresh, heparinized, filtered human plasma, and 20 units/ml of [3H]thymidine (specific activity, 17-23 Ci/mmol; Amersham-Searle) was added. The dishes were placed in the incubator for 3 hr. The cultures were then washed and fixed. Unirradiated cover slip cultures were processed in parallel with the irradiated cultures.

Autoradiography and Their Analysis. Autoradiographs, prepared with NTB-3 emulsion (Eastman Kodak), were exposed at 4°C for 7 days (4 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 cover slip cultures not treated with virus, grains over lightly labeled (1, 2, 5, 6) nuclei from 100 consecutively observed mononucleate cells were counted. Virus-treated cover slips were scanned under low power for areas containing mononucleate or multilobate cells that had the most light labeling. In such areas, grains were counted over the 100 nuclei of 50 consecu-
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

1 Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014 (U.S.A.) and 2 Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, Rotterdam (The Netherlands)

(Received July 24th, 1975)
(Accepted July 28th, 1975)

Summary

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

Introduction

Patients with xeroderma pigmentosum (XP) develop malignancies and photodamage 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; 3HThdR, tritiated thymidine; NIH, National Institutes of Health.

Collaboration in determination of 5 XP complementation groups

1975
One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells

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

MIROSŁAWA PROTIĆ-SABLJAČ* and KENNETH H. KRAMER†
Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Richard B. Sclaf, 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, 5630 base pairs; pSV2cat/SVgpt, 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 cot 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 pSV2cat/SVgpt, UV inactivation of CAT expression was greater in the xeroderma pigmentosum group A and D lines (ID₅₀ = 56 J m⁻²) than in the other human cell lines tested (normal, ataxia telangiectasia, Lesch-Nyhan, retinoblastoma) (ID₅₀ = 680 J m⁻²). ID₅₀ is the dose that reduces the percentage of CAT activity by 50% along the exponential portion of the dose-response curve). The ID₅₀ of the CAT inactivation curve was 67 J m⁻² for pSV2cat and for pRSVcat in the xeroderma pigmentosum group A cells. The similarity of the ID₅₀ data in the xeroderma pigmentosum group A cells for three plasmids of different size and promoters implies that 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 pigmentosus 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 pigmentosus 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 non-replicating 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 acetyl-transferase (CAT)), inserted between viral promoters (simian virus 40 (SV40) or Rous sarcoma virus (RSV) long terminal repeat (LTR) and SV40 polyadenylation sequences that permit gene expression in mammalian cells. In addition, the

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To whom reprint requests should be addressed.

1985
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

<table>
<thead>
<tr>
<th></th>
<th>XP</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transitions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC → AT</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>GC → AC</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>AT → GC</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>Transversions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC → TA</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>GC → CG</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>AT → TA</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>AT → CG</td>
<td>66</td>
<td>61</td>
</tr>
</tbody>
</table>

Abbreviations: SV40, simian virus 40; bp, base pairs.
*Permanent address: Department of Medical Microbiology, University of Lund, Malmo General Hospital, Malmo, Sweden.
**To whom reprint requests should be addressed.
Xeroderma Pigmentosum

Cutaneous, Ocular, and Neurologic Abnormalities in 830 Published Cases

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

1987

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

Myung Moo Lee, M.D.

Ken Kraemer, M.D.

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 7202, 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 pigmentosus 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-
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 83 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.)

METHODS

Patient Selection

We evaluated patients with the typical clinical features of xeroderma pigmentosum 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 (i.e., known teratogens) 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 detection of skin cancers. From the Laboratory of Molecular Carcinogenesis (K.H.K.), the Dermatology Branch (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.

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).

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>AGE/SEX</th>
<th>BEFORE TREATMENT* (2 Yr)</th>
<th>DURING TREATMENT* (2 Yr)</th>
<th>AFTER TREATMENT* (12–14 Mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/F</td>
<td>43 (21.5)</td>
<td>3 (1.5)</td>
<td>18 (18.0)</td>
</tr>
<tr>
<td>2</td>
<td>12/F</td>
<td>39 (18.5)</td>
<td>3 (2.0)</td>
<td>29 (38.7)§</td>
</tr>
<tr>
<td>3</td>
<td>17/M</td>
<td>23 (11.5)</td>
<td>6 (3.0)</td>
<td>20 (20.0)</td>
</tr>
<tr>
<td>4</td>
<td>39/M</td>
<td>10 (5.0)</td>
<td>3 (1.5)</td>
<td>4 (3.4)</td>
</tr>
<tr>
<td>5</td>
<td>10/M</td>
<td>8 (4.0)</td>
<td>9 (4.5)</td>
<td>10 (10.0)</td>
</tr>
</tbody>
</table>

*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.

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318:1633-1637 (June 23), 1988
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 Lamberti, 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 cancers in patients with XP, although acting by different mechanisms for the two types of skin cancer.

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

1994 – fold increase in skin cancer in XP
Photoproduction 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)

DOUGLAS E. BRASH, SARASWATHY SEETHARAM, KENNETH H. KRAMER, MICHAEL M. SELDMAN, AND ANDERS BEEDERG

*Department of Human Carcinogenesis and Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892; and Genentech

Pharmaceutical Co., South San Francisco, CA 94080

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ABSTRACT The role of UV radiation-induced photoproducts in initiating base substitution mutations to human cells was examined by measuring photoproduction frequency distributions and mutations in a sup+ DNA gene on a shuttle vector (p2189). The vector was transfected into DNA repair-deficient cells (xeroderma pigmentosum, complementation group A) and into normal cells. Frequencies of cytotoxic photoproducts and pyrimidine-photopyrimidines (6-4) photoproducts varied by as much as 88-fold at different dipyrimidine sites in the gene. All transition mutations occurred at dipyrimidine sites, predominantly at cytosines, with a 17-fold difference in mutation frequencies for different sites. Removal of more than 92% of the cytotoxic photoproducts by in vitro photoreactivation before transformation reduced the mutation frequency while preserving the mutation distribution, indicating that the cytosine-containing cyclobutane dimer was the major mutagenic lesion at these sites and that 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-photopyrimidines (6-4) photoproducts correlated with the mutation frequency, even in the absence of excision repair. Mutations at hot spots occurred at sites with low or high frequency of photoproduction formation and mutation cold spots occurred at sites with many photoproducts. These result suggest that although photoproducts are required for UV mutagens, the prominence of most mutation hot spots and cold spots is primarily determined by DNA repair features rather than 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 GC to AT transitions (2-8). It has long been anticipated that identification of the mutagenic UV photoproduction 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 distributions might reveal why different carcinogens activate different photoproducts even in the same tissue type (12).

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 photoproductions implicated in mutagenesis, the cyclobutane dimer and the pyrimidine-photopyrimidine (6-4) photoproduction (ref. 3 and 4 and references therein), in a shuttle vector sup+ DNA gene (6). The vector was transfected with UV in vitro and the photoproduction 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 p2189 was restriction digested and end labeled following UV treatment, so the photoproduction frequencies measured were identical to those transformed into the cells. The DNA was end labeled by filling in the unique EcoRI site (top strand) or the base pair 229 Xho I site (bottom strand). To detect the presence of cyclobutane dimers and pyrimidine-photopyrimidines (6-4) photoproducts at individual base pairs, end-labeled DNA was incubated with T4 dimer-specific endonuclease (a gift from A. G. Green, Stanford University) or 1 M piperidine, respectively (12). Samples were electrophoresed and quantitated as described (12) or quanitated by scanning the autoradiograms with an LKB Ultrascan laser densitometer using known radioactivity standards. The variation of measurements of replicate samples was <2%. The plasmid p2189.1 (SV40) was treated with 344-nm UV radiation from a germicidal lamp as described (12, 14). Escherichia coli DNA photolyase (a gift from A. Naour, University of North Carolina) was used as described (14) to photolyze pyrimidine dimers but not (6-4) photoproducts (12). The extent of photoreactivation was measured by the T4 endonuclease V assay on supercoiled DNA (11) and on defected sequence DNA (12). Similar viruses of SV40 (pSV40)-transformed xeroderma pigmentosum (XP21BE) and normal (GM0637) cell lines were obtained from the Institute for Medical Research (Manchester, UK) and grown as described (13, 14). XP21BE fibroblasts have been shown to have 2% of normal DNA excision repair (15). Mutation spectra were determined as described (6, 13).

RESULTS

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

Abbreviations: SV40, simian virus 40.
3To whom reprint requests should be addressed.
4Present address: Department of Medical Microbiology, Malmo General Hospital, University of Lund, Malmo, Sweden.

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

S Faghri,1 D Tanuma,1 K H Koerner,1 J J DiGiovanni2

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 (55%), abnormal characteristics at birth (35%), scoliosis (30%), infections (36%), photosensitivity (46%), maternal pregnancy complications (39%) and definitive DNA repair (34%) that was delayed, or have defects in the 19th year of life (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 multifocal developmental defects, recurrent infections and a high mortality at a young age. Abnormal characteristics at birth and pregnancy complications, unreported 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, sulfur deficient hair.1 When the hair from TTD patients is observed under polarizing microscopy, it displays a distinctive alternating light and dark banding pattern, called “tiger tail banding.”2-4 TTD results from mutations in one of several different DNA repair genes (XPD, XPD, or XPD) and TTDN1, a gene of unknown function.5 Although XPB and XPD mutations are also seen in xeroderma pigmentosum, a disease with a 900-fold increase in skin cancer,6-8 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 1975, two cases were described under the term “trichothiodystrophy,” which encompasses a wide spectrum of non- cutaneous findings, to describe the unifying feature.9 The name reflects the brittle, sulfur deficient hair seen in all TTD patients (from Greek, trichos meaning hair; thio; sall; def; dys; faulty; troph; nourishment). Several synonyms have been used to describe the clinical features of these patients. FIBD,10 BIDs,11 and RDS12 describe six features of TTD: Photosensitivity, trichothiodystrophy, brittle hair, Intelectual impairment, Decreased stature. In order to avoid 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 analyzed the frequency of the clinical findings described in an effort to characterize the spectrum of the disease better.

We modified this review after a similar study on xeroderma pigmentosum.

METHODS
We developed a standardised spreadsheet listing more than 200 clinical and laboratory characteristics. The search was limited 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 as a subsequently reported paper, the data were consolidated. For each case, we searched PubMed, Medline, Web of Science, and the reference sections of published articles. Search terms included trichothiodystrophy, TTD, Trichothiodystrophy, Pallister syndrome, FIBD, BIDs, RDS, and XPD.

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 concerning clinical features of TTD and a confirmed DNA repair abnormality, but the clinical overlap 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 following clinical or laboratory abnormalities (1) presence of brittle hair and/or hair shaft abnormalities; (2) tiger tail banding with polarized 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, 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

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.

http://videocast.nih.gov/PastEvents.asp?c=5
Transcription coupled repair of oxidative DNA damage
Feb 16, 1999
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

1. $^3$H-TdR Label
2. Damage
3. Repair in Presence of Brd Urd
4. Extract DNA
5. Restrict
6. Neutral CsCl Gradient
7. Separate or Slot Blot
8. 0 hr. + Cleavage
9. 24 hr. + Cleavage
10. Proteinase K, Dialysis
11. Alkaline Gel Transfer Hybridization
12. Southern Blot
13. Use Poisson Distribution To Calculate Adduct Frequency
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

Vilhelm A. Bohr, Charles Allen Smith, Diane S. Okumoto, and Phillip C. Hanawalt
Department of Biological Sciences
Stanford University
Stanford, California 94305

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/m²), 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, average genome repair.
Strand Specificity of the repair of pyrimidine dimers in the CHO DHFR gene after UV irradiation

<table>
<thead>
<tr>
<th>UV dose</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>40</th>
<th>40</th>
<th>40</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hrs Repair</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>T4 endo</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Transcribed Strand

Non-transcribed Strand
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