NATO Advanced Study Institute
DNA Damage and Repair

Oxygen Radical Effects, Cellular Protection and Biological Consequences

October 14-24, 1997
Antalya, Turkey
Tekirova Corinthia Hotel & Resort

Organizing Committee Sponsorship Introduction Objectives Structure of the ASI Lecturers Participants Institute Venue Program Application Abstracts Application Form

Last Update: October 6, 1997

ORGANIZING COMMITTEE:

Dr. Miral Dizdaroglu, *Director of the Advanced Study Institute*, Senior Scientist, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Dr. Barry Halliwell, Professor of Biochemistry, Department of Pharmacology, University of London, King's College, London SW3 6LX, UK

Dr. Ali E. Karakaya, Professor of Toxicology, Faculty of Pharmacy, Gazi University, 06330 Hipodrom, Ankara, Turkey

Dr. Hans E. Krokan, Professor of Molecular Biology, UNIGEN Center for Molecular Biology, Norwegian University of Science and Technology, N-7005 Trondheim, Norway

Dr. Jacques Laval, Research Director, Institut Gustave Roussy, 94805 Villejuif Cedex, France

Sponsorship:

This NATO Advanced Study Institute (ASI) is sponsored by the NATO Scientific and Environmental Affairs Division. Additional support is provided by the Biotechnology Division, National Institute of Standards and Technology, USA, the Danish Centre for Molecular Gerontology, Denmark, and the Turkish Society of Toxicology, Turkey.

INTRODUCTION

An Advanced Study Institute (ASI) is a high-level tutorial course of ten working days duration where a carefully defined subject is treated in depth by lecturers of international standing. ASIs contribute to dissemination of scientific knowledge and the formation of international scientific contacts. Presentations are made by the lecturers to about 60-80 ASI students who are mostly at the post-doctoral level. However, this does not exclude students who are about to obtain their doctoral degrees, and may also include other appropriately qualified senior scientists. Attendance at ASIs is open to all suitably qualified applicants irrespective of nationality. ASI students normally come from NATO countries; those from non-NATO countries may only receive financial support from the NATO grant if they are from NATO Cooperation Partner countries. ASI students are chosen by the organizing committee for their appropriate qualifications following their responses to advertisement of the ASI. Students are required to stay for the entire duration of the ASI to ensure full interaction.

NATO countries: Belgium, Canada, Denmark, France, Germany, Greece, Iceland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Turkey, United Kingdom, Unites States of America.

NATO Cooperation Partner countries: Albania, Armenia, Azerbaijan, Belarus, Bulgaria, Czech Republic, Estonia, Georgia, Hungary, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, the former Republic of Macedonia, Moldova, Poland, Romania, Russia, Slovak Republic, Slovania, Tajikistan, Turkmenistan, Ukraine, Uzbekistan.

OBJECTIVES

Damage to DNA by both exogenous and endogenous sources is increasingly regarded as highly important in the initiation and progression of cancer and in the occurrence of other pathological events. DNA damage caused by reactive oxygen-derived species, also called oxidative DNA damage, is the most frequent type encountered by aerobic cells. Mechanistic studies of carcinogenesis indicate an important role of this type of damage to DNA. There is also strong evidence to support the role of oxidative DNA damage in the aging process.

DNA damage is opposed in vivo by repair systems. If not repaired, DNA damage may lead to detrimental biological consequences. Therefore, the repair of DNA damage is regarded as one of the essential events in all life forms. In recent years, the field of DNA repair flourished due to new findings on DNA repair mechanisms and the molecular basis of cancer. In 1994, DNA repair enzymes have been named Science magazine's Molecule of the Year.

There is an increasing awareness of the relevance of DNA damage and repair to human health. A detailed knowledge of mechanisms of DNA damage and repair, and how individual repair enzymes function may lead to manipulation of DNA repair in cells and ultimately to an increase of the resistance of human cells to DNA-damaging agents. Our knowledge in this field has increased vastly in recent years. The time is ripe to convene a NATO-ASI meeting of scientists of international standing from the fields of biochemistry, molecular biology, enzymology, biomedical science and radiation biology to analyze these questions in detail and to teach the student participants the basics and new developments of the field of DNA damage and repair. The lecturers will present and discuss the state-of-the-art knowledge and recent developments in this research field, and its pertinence to human health. In this meeting, we expect the interactions between the lecturers and participants to be synergistic and challenging, and to contribute greatly to dissemination of scientific knowledge and the formation of international scientific collaborations.

STRUCTURE of the ASI

Lectures of 45-50 min will be presented by 26 lecturers (see the following page for the list of lecturers), followed by a discussion of 15-30 min. In addition, there will be a section of contributed papers consisting of oral and poster presentations. Abstracts submitted by the applicants will be chosen by the organizing committee as an oral or a poster presentation in this section of the meeting. Furthermore, there will be invited papers to be presented in this section as well. A book summarizing the lectures will be published by Plenum Publishing Corporation. The final program of the meeting will be announced soon. Please check this web site for further updates.

LECTURERS

Their Affiliations and Topics

Dr. Steven A. Akman, Wake Forest Comprehensive Cancer Center, Winston-Salem, North Carolina 27157, USA. *"Mapping reactive oxygen-induced DNA damage at nucleotide resolution."*

Dr. Okezie I. Aruoma, University of London King's College, London SW3 6LX, UK. *"Novel application of oxidative DNA damage to study antioxidant actions of plant extracts."*

Dr. Serge Boiteux, UMR217 CNRS/CEA, Dpt. Radiobiologie et Radiopathologie, 92265-Fontenay aux Roses , FRANCE. *"Repair of 8-oxoguanine in eukaryotes: the OGG1 enzymes."*

Dr. Vilhelm A. Bohr, Chief of the Laboratory of Molecular Genetics, National Institute on Aging, NIH, Baltimore, MD 21224, USA. *"Repair and transcription in premature aging syndromes."*

Dr. Jean Cadet, CEA/Department de Recherche Fondamentale sur la Matiere Condensee, SCIB/LAN, F-38054 Grenoble Cedex 9, FRANCE *"Oxidative base damage to DNA; recent mechanistic aspects."*

Dr. Bruce Demple, Professor of Toxicology, Harvard School of Public Health, Boston, MA 02115, USA. *"Roles of AP*

endonucleases in repair and genetic stability."

Dr. Walter A. Deutsch, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA. *"Drosophila ribosomal protein S3 contains N-glycosylase, abasic site, and deoxyribophosphodiesterase DNA repair activities."*

Dr. Miral Dizdaroglu, Senior Scientist, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. *"Mechanisms of oxidative DNA damage; lesions and their measurement."*

Dr. Paul W. Doetsch, Professor of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322, USA. *"Bypass of DNA damage by RNA polymerases: implications for DNA repair and transcriptional mutagenesis."*

Dr. Errol C. Friedberg, Professor, University of Texas Southwestern Medical Center Dallas, TX 75235, USA. *"Nucleotide excision repair in eukaryotes: yeast as a model system."*

Dr. Matthew B. Grisham, Professor of Physiology and Biophysics, Louisiana State University School of Medicine, Shreveport, LA 71130. *"Modulation of nitrosation and oxidation reactions by superoxide and nitric oxide."*

Dr. Arthur P. Grollman, Professor of Pharmacology and Medicine, State University at Stony Brook School of Medicine, Stony Brook, NY 11794, USA. *"Oxidative DNA damage; mechanisms of mutagenesis and repair."*

Dr. Lawrence Grossman, Professor of Biochemistry, Johns Hopkins University, Baltimore, MD 21205. *"DNA repair as a biomarker in aging and for skin and lung cancer epidemiology studies."*

Dr. Barry Halliwell, Professor of Biochemistry, University of London King's College, London SW3 6LX, UK. *"Oxidative DNA damage and human health."*

Dr. Philip C. Hanawalt, Professor of Biology, Stanford University, Stanford, CA 94305, USA. *"Role of transcription-coupled DNA repair in human health."*

Dr. Ali E. Karakaya, Professor of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, Turkey. *"Genotoxicity tests; applications for occupational exposure."*

Dr. Kazimierz S. Kasprzak, Research Chemist, National Cancer Institute, NIH, Frederick. MD 21702, USA. *"Studies on oxidative genotoxicity of human metal carcinogens: recent developments."*

Dr. Hans E. Krokan, Professor of Molecular Biology, Norwegian University of Science and Technology, N-7005 Trondheim, Norway. *"Human uracil DNA glycosylase: gene structure, regulation and structural basis for enzyme catalysis."*

Dr. Yoke W. Kow, Department of Radiation Oncology, Emory University School of Medicine, Atlanta, GA 30335, USA. *"Mechanism of action of Escherichia coli formamidopyrimidine N-glycosylase."*

Dr. Jacques Laval, Research Director, Institut Gustave Roussy, 94805 Villejuif Cedex, France. *"Repair of DNA damaged by free radicals."*

Dr. Tomas Lindahl, Deputy Director of Research, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire, EN6 3LD, UK. *"Mechanisms of repair of endogenous DNA damage."*

Dr. Stuart Linn, Professor of Biochemistry, University of California, Berkeley, CA 94720. *"The chemical bases for hydrogen peroxide toxicity and DNA damage."*

Dr. Steffen Loft, Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, DENMARK. *"Measurement of oxidative damage to DNA nucleobases in vivo: interpretation of nuclear levels and urinary excretion of repair products."*

Dr. Joe Lunec, Centre for Mechanisms of Human Toxicity, University of Leicester, Leicester, LE1 9HN, UK. *"Effects of vitamin E supplementation on in vivo oxidative DNA damage in normal individuals."*

Dr. Sankar Mitra, The University of Texas Med. Branch at Galveston, Sealy Center for Molecular Science, Galveston, TX 77555-1079, USA. *"Regulation of the major human AP-endonuclease, a multi-functional protein by oxidative stress."*

Dr. Etsuo Niki, Professor of Chemistry, University of Tokyo, 4-6-1 Komaba, Megura, Tokyo, Japan. *"Action of antioxidants against oxidative stress."*

Dr. Susumu Nishimura, Senior Executive Director, Banyu Tsukuba Research Institute, Tsukuba, 300-33, Japan. *"8-Hydroxyguanine in DNA: its formation by oxygen radicals, repair and implication in mutation/carcinogenesis."*

Dr. Hiroshi Ohshima, Chief, Unit of Endogenous Cancer Risk Factors, International Agency for Research on Cancer, 69372 Lyon Cedex 08, France. *"DNA damage induced by reactive nitrogen-species."*

Dr. Nancy Oleinick, Professor, Case Western Reserve University, Cleveland, OH 44106- 4942, USA. *"Modification of radiation-induced DNA damage by chromatin organization."*

Dr. Ryszard Olinski, Department of Clinical Biochemistry, University School of Medical Sciences, 85-092 Bydgoszcz, POLAND. *"Estimation of free radical-induced DNA base damages in cancerous and HIV-infected patients and in healthy subjects."*

Dr. Mehmet Öztürk, Professor of Molecular Biology, Bilkent University, 06533 Bilkent, Ankara, Turkey. *"p53 tumor suppressor gene: its role in DNA damage response and cancer."*

Dr. Dennis J. Reeder, Leader, DNA Technologies Group, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. *"Free radicals, DNA damage and p53 expression: a review of interrelationships with apoptosis and prospects for diagnosis."*

Dr. Joyce T. Reardon, Professor of Biochemistry, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA. *"Molecular mechanisms of nucletide excision repair in mammalian cells."*

Dr. Clemens von Sonntag, Max-Planck-Institut für Strahlenchemie, Stiftstr. 34-36, D-45413 Mülheim a.d. Ruhr, GERMANY. *"Mechanistic studies of radiation-induced DNA damage"*

Dr. Shinya Toyokuni, Department of Pathology, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, JAPAN. *"Detection of 8-hydroxy-2´-deoxyguanosine by a monoclonal antibody N45.1 and its application."*

Dr. Susan S. Wallace, Professor of Microbiology, University of Vermont College of Medicine, Burlington, VE 05405, USA. *"Processing and consequences of oxidative DNA lesions."*

Dr. John F. Ward, Professor of Radiobiology, University of California San Diego, La Jolla, CA 92093, USA. *"Ionizing radiation damage to DNA: a challenge to repair systems."*

PARTICIPANTS

FROM NATO COUNTRIES

Bertrand de Wergifosse, Cellular Biology Laboratory, Université Catholique de Louvain-UCL, Place Croix du Sud, 4 - Bâtiment Carnoy, B-1348 Louvain-la-Neuve, BELGIUM

Kevin Lenton, Centre de Recherche en Gerontologie et Gereatrie, Universite de Sherbrooke, 1036 Belvedere Sud, Sherbrooke, QC, J1H 4C4, CANADA

M. Vakur Bor, Department of Clinical Biochemistry, Aarhus University Hospital, Norrebrogade 44, DK-8000 Aarhus C, DENMARK

Mette Christiansen, Danish Center for Gerantology, University of Aarhus, Langelandsgade 140, bygn. 510, 8000 Aarhus C, DENMARK

Tanja Thybo Frederiksen, Danish Center for Gerantology, University of Aarhus, C.F. Mollers Alle, Bldg. 130, 8000 Aarhus C, DENMARK

Allan Weiman, Department of Clinical Pharmacology, Q7642, Rigshospitalet, 20, Tagensvej, DK-2200 Copenhagen N, DENMARK

Anja Wellejus, Department of Pharmacology, University of Copenhagen, 18.5 Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, DENMARK

Valerie Abalea, Laboratoire de Biologie Cellulaire, INSERM U456, 2, Avenue du Pr. L. Bernard, 35043 Rennes Cedex, FRANCE

Martine Defais, CNRS, Institut de Pharmacologie et Biologie Structurale, 205 route de Narbonne, 31077 Toulouse Cedex, FRANCE

Christa Baumstark-Khan, DLR, Institute of Aerospace Medicine, Radiobiolgy Unit, Linder Hoehe, D-51147 Köln, GERMANY

Nair Sreejayan, University of Munich, Klinikum Grosshadern, Department of Medicine D-81377 München, GERMANY

Alexander Georgakilas, Laboratory for Rad. Mol. Genetics, National Centre for Scientific Res., "Demokritos" Aghia Paraskevi, 153 10 Athens, GREECE

Kostas Haveles, Laboratory for Rad. Mol. Genetics, National Centre for Scientific Res."Demokritos" Aghia Paraskevi, 153 10 Athens, GREECE

Lampros Papandreou, University of Ionnina, Medical School , Department of Obstetrics and Gynaecology, 2nd Obstetrics Clinic, GR-451 10 Ionnina, GREECE

Chryssostomos Chatgilialoglu, Consiglio Nazionale Delle Richerche, Via P. Gobetti, 101, 40129 Bologna, ITALY

Antonia M. Pedrini, Instituto di genetica Biochimica, ed Evoluzionistica del CNR, Via Abbiategrasso, 207, 27100 Pavia, ITALY

Niels Aarsaether, Department of Molecular Biology, HIB-University of Bergen, 5020 Bergen, NORWAY

Sangeeta Bharati, UNIGEN Center for Molecular Biology, Norwegian University for Science and Technology, N-7005 Trondheim, Norway

Svein Bjelland, Stavanger College, School of Science and Technology, Ullandhaug, PO Box 2557, N-4004 Stavangar, NORWAY

Hilde Nilsen, UNIGEN Center for Molecular Biology, Norwegian University for Science and Technology, N-7005 Trondheim, NORWAY

Kristin Solum, UNIGEN Center for Molecular Biology, Norwegian University for Science and Technology, N-7005 Trondheim, NORWAY

Teresa Roldan-Arjona, Faculdad de Ciencias, Departamento de Genetica, Universidad de Cordoba, 14071-Cordoba, SPAIN

Rafael Rodriguez, Faculdad de Ciencias, Departamento de Genetica, Universidad de Cordoba, 14071-Cordoba, SPAIN

Bulbin Akbasak, Department of Biology and Genetics, Faculty of Medicine, Inonu University, Malatya, TURKEY

Nursen Basaran, Department of Toxicology, Faculty of Pharmacy, Hacettepe University Ankara, TURKEY

Mustafa Birincioglu, Department of Pharmacology, Faculty of Medicine, Inonu University, Malatya, Turkey

Sema Burgaz, Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, TURKEY

Belma Giray, Department of Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, TURKEY

Bensu Karahalil, Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, TURKEY

Asuman Karakaya, Department of Toxicology, Faculty of Pharmacy, University of Ankara, Ankara, TURKEY

Okyay Kilinc, Department of Molecular Biology and Genetics, Bogazici University, Istanbul, TURKEY

Hilal Ozdag, Department of Molecular Biology and Genetics, Bilkent University, Ankara, TURKEY

Ibrahim Pirim, Department of Genetics, Faculty of Medicine, Ataturk University, Erzurum, TURKEY

Semra Sardas, Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, TURKEY

Sema Senturker, Biochemistry Department, Faculty of Medicine, Osman Gazi University Eskisehir, TURKEY

Sinan Suzen, Gulhane Military Health Academy, Ankara, TURKEY

Aysen Tezcaner, Department of Biological Sciences, Middle East Technical University, Ankara, TURKEY

Berrin Tunca, Department of Molecular Biology, Faculty of Medicine, Uludag University, Bursa, TURKEY

Kezban Unsal, Department of Molecular Biology and Genetics, Bilkent University, Ankara, TURKEY

Ismet Yilmaz, Department of Biochemistry, Faculty of Medicine, Inonu University, Malatya, TURKEY

Suzanne Cholerton, Department of Pharmacological Sciences, University of Newcastle Upon Tyne, Newcastle NE2 4HH, UK

Andrew Jenner, Pharmacology Department, King's College London University, London, SW3 6LX, UK

Tracy Melvin, MRC Radiation and Genomic Stability Unit, Chilton, Oxon, OX11 ORD, UK

Elizabeth Bailey, Laboratory of Toxicology, Harvard School of Public Health, Boston, MA 02115, USA

George J. Broder, Fox Cancer Center, Radiology 1553 Cherry Lane Rydal, PA 19046, USA

Richard M. Burger, Laboratory of Chromosome Biology, The Public Health Research Institute, New York, NY 10016, USA

Jonathan A. Eisen, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA

Kevin Geiss, Department of Zoology, Miami University, Oxford, OH 45056, USA

Sandra Gunselman, Colorado State University, Department of Environmental Health Fort Collins, CO 80523, USA

Leonora Lipinski, Laboratory of Molecular Genetics, National Institute on Aging, NIH Baltimore, MD 21224, USA

Monica McTigue, Health Sciences Center, Department of Pharmacology, University of New York at Stony Brook, Stony Brook, NY 11794-8651, USA

Henry Rodriguez, Department of Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA

Ivan Roussyn, Curriculum in Toxicology, University of North Carolina at Chapel Hill Chapel Hill, NC 27599-7400, USA

David M. Sonntag, University of Cincinnati, Department of Environmental Health, Cincinnati, OH 45220, USA

Robertus Stierum, Laboratory of Molecular Genetics, National Institute on Aging, NIH Baltimore, MD 21224, USA

Steve Waters, Department of Cancer Biology, Wake Forest Comprehensive Cancer Center, Winston-Salem, NC 27157, USA

Dmitry Zharkov, Department of Pharmacology, State University of New York at Stony Brook, Stony Brook, NY 11794-8651, USA

FROM NON-NATO COUNTRIES

Armen K. Nersesyan, Cancer Research Centre, Yerevan State University, Yerevan, ARMENIA

Anton Tontchev, Laboratory of Electron Microscopy, Department of Anatomy and Histology, Medical University of Varna, BG-9002 VARNA, BULGARIA

Maarit Lankinen, Department of Clinical Biochemistry Laboratory of Molecular Hematology, Tempere University Hospital PO Box 200 33521 Tempere, FINNLAND

Vitalija Simkeviciene, Institute of Biochemistry, Department of Bioorganic Compounds Technology, Mokslininku 12, 2600 Vilnius, LITHUANIA

Saule Uleckiene, Laboratory of Environmental Carcinogens, Lithuanian Oncology Center, Polocko 4, 2007 Vilnius, LITHUANIA

Jan Barciszewski, Institute of Biorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, POLAND

Pawel Jaruga, Department of Clinical Biochemistry, Medical Academy, 85-094, Bydgoszcz, POLAND

Bogdan Constantinescu, Institute of Atomic Physics, PO BOX MG-6, Bucharest, ROMANIA

Rita Balamirzoeva, Department of Ethnic Populations Genetics, Institute of History, Arch. and Ethnogr., Daghestanian Center of Russian Academy of Sciences, 367030, Makhachkala, Daghestan, RUSSIA

Machbuba Pulatova, Institute of Chemical Physics Russian Academy of Sciences Kosigyns Street, 4 Moscow, 117977, Russia

Alla A. Oudalova, Russian Institute of Agricultural Radiology & Agroecology, 249020 Obninsk, Kaluga Region, RUSSIA

Olga M. Rozanova, Laboratory of Cytogenetics, Institute of Theor. and Exper. Biophysics Pushchino, Moscow Region, 142292, RUSSIA

Vladimir Sharygin, Institute of Chemical Physics Russian Academy of Sciences Kosigyns Street, 4 Moscow, 117977, Russia

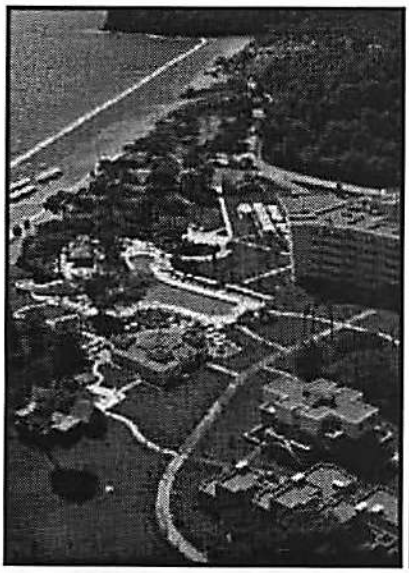
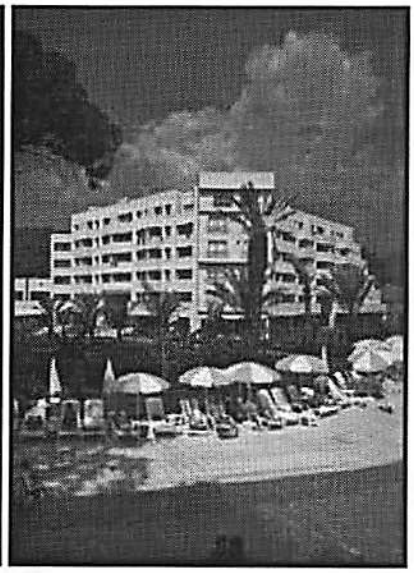
Elizabeth Bilsland, Gothenburg University, Lundsbergs Laboratory Department of Molecular Biology Medicinaregatan 9C S-413 90 Gothenburg, Sweden

Cyril Privezentzev, National Academy of Science of Ukraine, The A.O. Kovalevsky Institute of Biology of the Southern Sea, Nakhimova Ave. 2, 335011 Sevastopol, UKRAINE

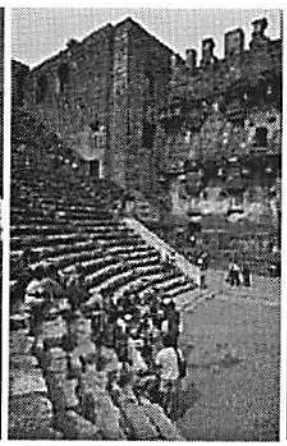
INSTITUTE VENUE

The ASI will be held at the five-star **Tekirova Corinthia Hotel & Resort** (Tel. +90-242-821-4750; Fax +90-242-821-4635), which is located 70 km (approx. 1 hour by car) from the International Airport of Antalya. Transport to and from the hotel will be provided. Turkish Airlines has four daily flights from Istanbul to Antalya. Many European Airlines have direct flights to Istanbul. The cost of accommodation at the hotel is $65/person/day in a double bed- room and $90/day in a single-room with full board (including all five-star hotel facilities). ASI students supported by NATO grant will share a double bed-room with another ASI student. **Smoking will be prohibited in the meeting rooms.**

Tekirova Corinthia Hotel & Resort

Please check the following web site for information on Antalya and the related areas: http://www.turkey.org/antalya.htm


SCIENTIFIC PROGRAM

1st Day, Oct. 14, Tuesday

Morning

9.00-9.30	Welcome and introductory remarks (committee members) Chairperson: Nancy L. Oleinick
9.30-10.30	Barry Halliwell, *"Oxidative DNA damage and human health."*
10.30-11.00	Coffee Break
11.00-12.00	Philip C. Hanawalt, *"Role of transcription-coupled DNA repair in human health."*
Afternoon	Chairperson: Barry Halliwell
15.30-16.30	Miral Dizdaroglu, *"Mechanisms of oxidative DNA damage; lesions and their measurement."*
16.30-17.30	Jean Cadet, *"Oxidative base damage to DNA: recent mechanistic aspects."*
17.30-18.00	Coffee Break
18.00-19.00	Jacques Laval, *"Repair of DNA damaged by free radicals."*

Evening

20.00-22.00	Welcoming Cocktail

2nd Day, Oct. 15, Wednesday

Morning	Chairperson: Jacques Laval
9.00-10.00	Susumu Nishimura, *"8-Hydroxyguanine in DNA: its formation by oxygen radicals, repair and implication in mutagenesis/carcinogenesis."*
10.00-11.00	Serge Boiteux, *"Repair of 8-oxoguanine in eukaryotes: the OGG1 enzymes."*
11.00-11.30	Coffee Break
11.30-12.30	Stuart Linn, *"Sequence preferences for cleavage of duplex DNA by Fe^{+2} and hydrogen peroxide."*
Afternoon	Chairperson: Philip C. Hanawalt
16.00-17.00	Steven A. Akman, *"Mapping reactive oxygen-induced DNA damage at nucleotide resolution."*
17.00-17.30	Coffee Break
17.30-18.30	Joyce Reardon, *"Molecular mechanisms of nucleotide excision repair in mammalian cells."*

3rd Day, Oct. 16, Thursday

Morning	Chairperson: Steve A. Akman
9.00-10.00	Tomas Lindahl, *"Mechanisms of repair of endogenous DNA damage."*
10.00-11.00	Arthur P. Grollman, *"Oxidative DNA damage; mechanisms of mutagenesis and repair."*
11.00-11.30	Coffee Break
11.30-12.30	Hans E. Krokan, *"Human uracil DNA glycosylase: gene structure, regulation and structural basis for enzyme catalysis."*

Afternoon

16.00-18.00	Poster session

4th Day, Oct. 17, Friday

Morning	Chairperson: Errol C. Friedberg
9.00-10.00	Susan S. Wallace, *"Processing and consequences of oxidative DNA base lesions."*
10.00-11.00	Bruce Demple,*"Roles of AP endonucleases in repair and genetic stability."*
11.00-11.30	Coffee Break
11.30-12.30	Walter A. Deutsch, *"Drosophila ribosomal protein S3 contains N- glycosylase, abasic site, and deoxyribophosphodiesterase DNA repair activities."*
Afternoon	Chairperson: John F. Ward
16.00-17.00	Clemens von Sonntag, *"Mechanistic studies of radiation-induced DNA damage"*
17.00-17.30	Coffee Break
17.30-18.30	Shinya Toyokuni, *"Detection of 8-hydroxy-2´-deoxyguanosine by a monoclonal antibody N45.1 and its application."*

5th Day, Oct. 18, Saturday

Morning	Chairperson: Clemens von Sonntag
9.00-10.00	John F. Ward, *"Ionizing radiation damage to DNA: a challenge to repair systems."*
10.00-11.00	Nancy Oleinick, *"Modification of radiation-induced DNA damage by chromatin organization."*
11.00-11.30	Coffee Break
11.30-12.30	Sankar Mitra, *"Regulation of the major human AP-endonuclease, a multi-functional protein by oxidative stress."*
Afternoon	Chairperson: Wilhelm Bohr
16.00-17.00	Mehmet Öztürk *"p53 tumor suppressor gene: its role in DNA damage response and cancer."*
17.00-17.30	Coffee Break
17.30-18.30	Steffen Loft, *"Measurement of oxidative damage to DNA nucleobases in vivo: interpretation of nuclear levels and urinary excretion of repair products."*
Evening	
20.00	Gala Dinner

6th Day, Oct. 19, Sunday

Break

7th Day, Oct. 20, Monday

Morning	Chairperson: Stuart M. Linn
9.00-10.00	Vilhelm A. Bohr, *"DNA repair and transcription deficiencies in premature aging syndromes."*
10.00-11.00	Paul W. Doetsch, *"Bypass of DNA damage by RNA polymerases: implications for DNA repair and transcriptional mutagenesis."*
11.00-11.30	Coffee Break
11.30-12.30	Joe Lunec, *"Effects of vitamin E supplementation on in vivo oxidative DNA damage in normal individuals."*
Afternoon	Chairperson: Susumu Nishimura
16.00-17.00	Dennis J. Reeder, *"Free radicals, DNA damage and p53 expression: a review of interrelationships with apoptosis and prospects for diagnostics."*
17.00-17.30	Coffee Break
17.30-18.30	Lawrence Grossman, *"DNA repair as a casual factor in chronic diseases in the population."*
Evening	Chairperson: Hans Krokan
20.00-22.00	Oral poster presentations

Henry Rodriguez, USA, *"Genomic Gene Enrichment for LMPCR Analysis."*

Teresa Roldán-Arjona, Spain,	*"Substrate Specificity of Eukaryotic Nth Homologues."*
Andrew Jenner, UK,,	*"Hypochlorous Acid-Induced Base Damage in Isolated Calf Thymus DNA."*
Leonora Lipinski, USA,	*"Processing of Oxidative DNA Damage in Familial Alzheimer Disease."*
Tanja Thybo Frederiksen, Denmark,	*"Gene Specific Formation and Repair of 8-Hydroxyguanine in Mammalian Cells in vivo."*
Maarit Lankinen, Finnland,	*"Radiation-Induced DNA Strand Breaks in Human Hematopoietic Cells Measured Using the Comet Assay."*
Chryssostomos Chatgilialoglu, Italy,	*"Free Radical Chemistry Associated with C-1 Position of Nucleosides."*
Mustafa Birincioglu, Turkey,	*"Is Free Radical Scavenging Action of Ace Inhibitors Related to Their Protective Effect on Reperfusion Arrhythmias in Rats?"*
Ibrahim Pirim, Turkey,	*"The Optimization of the PCR-Restriction Isotyping for APO-E Genotype."*
Uleckiene Saule, Lithuania,	*"Studies on Genotoxicity of Occupational Exposures."*
K. S. Haveles, Greece,	*"Effects of Tris and Phenol in ?-Irradiated DNA Samples."*

8th Day, Oct. 21, Tuesday

Morning	Chairperson: Arthur P. Grollman
9.00-10.00	Errol C. Friedberg, *"Nucleotide excision repair in the yeast Saccharomyces cerevisiae."*
10.00-11.00	Lawrence Grossman, *"Studies on the role of RNA polymerase in DNA repair."*
11.00-11.30	Coffee Break
11.30-12.30	Hiroshi Ohshima, *"DNA damage induced by reactive nitrogen-species."*
Afternoon	
16.00-18.00	Poster session

9th Day, Oct. 22, Wednesday

Morning	Chairperson: Dennis J. Reeder
9.00-10.00	Kazimierz S. Kasprzak, *"Studies on oxidative genotoxicity of human metal carcinogens: recent developments."*
10.00-11.00	Yoke W. Kow, *"Mechanism of action of Escherichia coli formamidopyrimidine N-glycosylase."*
11.00-11.30	Coffee Break
11.30-12.30	Matthew B. Grisham, *"Interactions between superoxide and nitric oxide: implications in DNA damage and repair."*
Afternoon	Chairperson: Matthew B. Grisham
15.30-16.30	Okezie I. Aruoma, *"Novel application of oxidative DNA damage to study antioxidant actions of plant extracts."*
16.30-17.00	Coffee Break
17.00-18.00	Ali E. Karakaya, *"Genotoxicity tests; applications for occupational exposure."*
Evening	Chairperson: Joe Lunec
20.00-22.00	Oral poster presentations

Graciela Spivak, USA,	*"Repair of Oxidative Damage in Transcribed and Non-Transcribed DNA Strands in Human Cells ."*
M. K. Pulatova, Russia,	*"Drugs and DNA Synthesis System: The Biochemical Mechanisms of DNA Damage, Repair and Protection."*
Dmitry O. Zharkov, USA,	*"Cloning and Characterization of Mouse 8- Oxoguanine DNA Glycosylase/AP Lyase (MOGG1)."*
Nair Sreejayan, Germany,	*"Effect of Bile Acids on Lipid Peroxidation: The Role of Iron."*
Martine Defais, France,	*"Role of the Hamster RAD51 Protein in DNA Damage Response and Homologous Recombination."*
J. Barciszewski, Poland,	*"Mechanism of Kinetin Formation IN DNA."*
Sandra J. Gunselman, USA,	*"Use of Infrared Spectral Models in Cancer Research and Their Potential Clinical Applications."*
Svein Bjelland, Norway,	*"5-Formyldeoxyuridine-Induced Mutagenesis in Bacteria and Mammalian Cells."*
Kevin J. Lenton. Canada,	*"DNA Base Damage in Human Lymphocytes."*

Stephen B. Waters, USA,	*"Factors Affecting 5-Methylcytosine to Thymine Transitions in the P53 Gene of Colorectal Cancers."*
Hilde Nilsen, Norway,	*"Gene and Promoter Structure of the Murine Uracil-DNA Glycosylase."*
Hilal Özdag, Turkey,	*"Germline Mutation Analysis of DNA Repair Related BRCA1 and BRCA2 Tumor Supressor Genes."*
Semra Sardas, Turkey,	*"Evaluation of DNA Damage in Lymphocytes of Cancer Patients Under Gamma-Radiation Therapy by Single Gel Electrophoresis"*

10th Day, Oct. 23, Thursday

Morning	Chairperson: Paul W. Doetsch
9.00-10.00	Ryszard Olinski, *"Estimation of free radical-induced DNA base damages in cancerous and HIV-infected patients and in healthy subjects."*
10.00-10.30	Coffee break
10.30-11.30	Etsuo Niki, *"Action of antioxidants against oxidative stress."*
Afternoon	
16.00-18.00	Poster session
Evening	
20.00-22.00	Closing cocktail

11th Day, Oct. 24, Friday

Departure

APPLICATION

Attendance at the ASI is open to all suitably qualified applicants irrespective of nationality. Financial support will be provided for scientists from NATO countries, and for those from NATO Cooperation Partner countries (see Introduction for the list of the countries). The applicants should be scientists at the post-doctoral level. However, this does not exclude students who are about to obtain their doctoral degrees, and may also include other appropriately qualified senior scientists. ASI students will be chosen by the organizing committee from the applicants on the basis of their scientific qualifications. The NATO grant will be used to cover accommodation and, in exceptional cases, also the travel expenses of the accepted applicants. Accepted ASI students will be required to present a poster on their work during the meeting. All abstracts will be presented as posters. In addition, up to ten abstracts will be chosen as oral presentations of 5-10 min duration. ASI students are required to bring 2-3 slides or transparencies on their posters to give an oral presentation and answer questions. Those who are chosen to give oral presentations will be announced during the meeting.

The number of ASI students who will be supported by the NATO grant is limited to 70 at this ASI. Applicants who are not accepted to receive financial support, or other scientists who are not eligible for support by the NATO grant may still attend the meeting provided they are accepted as ASI students and they pay for their own hotel expenses. **All participants in this ASI must stay at the designated Institute Venue, which is the Tekirova Corinthia Hotel & Resort (see Institute Venue).** Participants who desire to stay at other hotels, but to attend the meeting at the designated Institute Venue will not be accepted to this ASI. There is no registration fee for members of academic institutions. A registration fee of $200 will be charged to applicants from the industry. A deposit of $150 on chargeable living expenses will be requested from ASI students who are accepted to receive financial support. This deposit will be non-refundable in the event of late cancellation by the applicant. ASI Students must stay for the entire duration of the meeting to ensure full interaction.

The application deadline is July 1, 1997. For application, submit the application form, a resume of no more than three pages, and a brief explanation why you would like to participate in this ASI to the address below. A letter of recommendation from your supervisor is requested. The use of e-mail is encouraged. Applicants will be notified of the decision of the organizing committee within 4-6 weeks following the deadline. Please watch this web site for further updates.

Dr. Miral Dizdaroglu
National Institute of Standards and Technology
Bldg. 222/A353
Gaithersburg, MD 20899, USA

Tel. +1-301-975-2581
Fax. +1-301-330-3447
E-mail: miral@nist.gov

Important Note: *NATO does not take out any health or accident insurance for lecturers and students in the ASI meeting; such insurance is an individual responsibility. NATO and the organizers do not assume any responsibility, either in this context or for any other liability.*

ABSTRACTS

Abstracts should be prepared according to the following instructions:

1. The abstract should be written in English.
2. The abstract should be typed on a page with 2.5 cm space on both sides and on the top.
3. The abstract should be informative and consist of the following.
    a. TITLE; short and clear in the first line or two (all capital letters).
    b. Complete names of the authors, with the presenting author listed first, and the affiliations of the authors.
    c. The body of the abstract should be typed in single space, lower case; with CG Times (if possible) and 12 point font size (laser printer or electric typewriter preferable). Abbreviations may be used after first defining them.
    d. Single space should be left between title, names of authors and body of abstract.
4. The abstract should not exceed 250 words.

APPLICATION FORM

- On-Line application
- Download postscript application form
- Print text application form

Application deadline: July 1, 1997

Applications via Mail - Please send to:

Dr. Miral Dizdaroglu
National Institute of Standards and Technology
Bldg. 222/A353
Gaithersburg, MD 20899, USA

along with a resume of no more than three pages, a brief explanation why you would like to participate in this ASI and a letter of recommendation from your supervisor why you should attend this meeting.

NATO Advanced Study Institute

DNA Damage and Repair

Oxygen Radical Effects, Cellular Protection and Biological Consequences

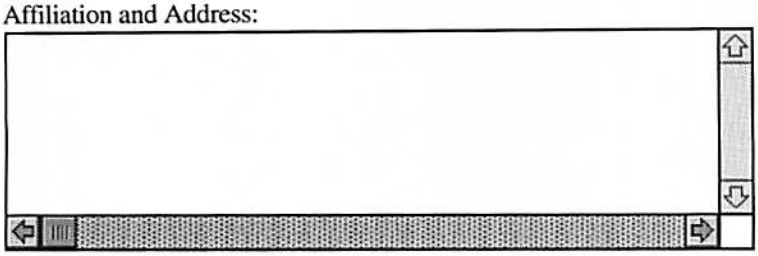
October 14-24, 1997, Tekirova/Antalya, Turkey

Last Name:

First Name:

Citizenship:

Affiliation and Address:



Telephone: Fax:

E-mail:

Post-Doctoral Fellow? Ph.D. Student? Senior Scientist?

Year of completion of Ph.D.:

Support for travel is requested: Yes No

Support for accommodation is requested: Yes No

In case of yes, please explain reasons for the request and give the size of the requested support (in US dollars) and E-mail to miral@nist.gov:

Will you present a poster on your work? Yes No

Also please e-mail to miral@nist.gov:

- a resume of no more than three pages
- a brief explanation why you would like to participate in this ASI
- a letter of recommendation from your supervisor why you should attend this meeting.

This page is maintained by Dr. Hillary S. Gilson: hillary.gilson@nist.gov.

NATIONAL SCIENCE FOUNDATION
4201 WILSON BOULEVARD
ARLINGTON, VIRGINIA 22230

Directorate for Education
and Human Resources

April 8, 1997

ASI#960617

Mr. Jonathan A.Eisen
Stanford University
Herrin Hall #356
Stanford, California 94305-5020

Dear Mr. Eisen:

It is a pleasure to inform you that you have been nominated by the director of a NATO Advanced Study Institute (ASI) to be a candidate for a National Science Foundation (NSF) travel award to attend the above-referenced ASI this year.

If you are selected, NSF will provide support in the amount of $1000 for transportation and miscellaneous expenses incurred in attending the ASI.

To be eligible to receive an award, you must:

* be either a predoctoral student or a person who has not held a doctoral degree more than three years,

* be a United States citizen or permanent resident alien, and

* be planning to attend an ASI **as your principal reason for travel to Europe** at the time.

In order to be eligible for NSF travel funds, the international travel support awardee must use a **U.S. flag carrier** when such service is available. This requirement is not negotiable.

Please return to us two completed copies of the enclosed IVDF Form 102 (do not answer
item 15, "Amount Requested for Airline" and please sign both copies) together with one
copy of the Letter of Invitation in the mailing envelope provided. You may

NATIONAL SCIENCE FOUNDATION
4201 WILSON BOULEVARD
ARLINGTON, VIRGINIA 22230

Directorate for Education
and Human Resources

May 16, 1997

ASI#960617

Mr. Jonathan Eisen
7G Barnes House
Stanford, CA 94305

Dear Mr. Eisen:

It is a pleasure to inform you that the National Science Foundation will provide support in the amount of $1,000 for transportation and miscellaneous expenses for you to attend the above-referenced Advanced Study Institute this year.

This award is subject to the conditions in F.L. 27, Attachment to International Travel Grant. Because the use of U.S. flag carriers by international travel support awardees is required by law* when such service is available, please pay particular attention to the requirement for use of U.S. flag carriers as stated in F.L. 27.

If you are unable to attend the Advanced Study Institute, you must inform the National Science Foundation and return all funds to NSF.

Within the next ten days the amount of this award will be direct deposited to the bank or financial institution identified by you for this purpose.

Within 60 days after completion of your travel, you must submit NSF Form 250, International Travel Report Form to the Foundation. Contrary to the directions on the Form 250 and F.L. 27, only one copy of the completed form is to be submitted to the ASI Travel Awards Program at NSF.

Sincerely,

Richard P. Metcalf
Associate Program Director
Graduate Fellowships

Attachments
F.L. 27
NSF Form 250

*International Air Transportation Fair Competitive Practices Act of 1974 known as the "Fly America Act."

Please return to us two completed copies of the enclosed NSF Form 192 (do not answer item 15, "Amount Requested for Airfare" and please sign both copies) together with one copy of the letter of invitation from the Institute Director, a brief curriculum vitae, and a completed NSF Form 1310. In addition an NSF Form 1379 also must be submitted. The Debt Collection Improvement Act of 1996 requires federal agencies to transfer funds electronically; therefore, sections I, III, & IV of NSF Form 1379 should be completed and returned with the travel award application forms. (Further instructions for Form 1379 are found on the back of the form.) The set of forms should take approximately twenty minutes to complete. Upon receipt of your forms, NSF will contact you **if it is able to provide travel funds.**

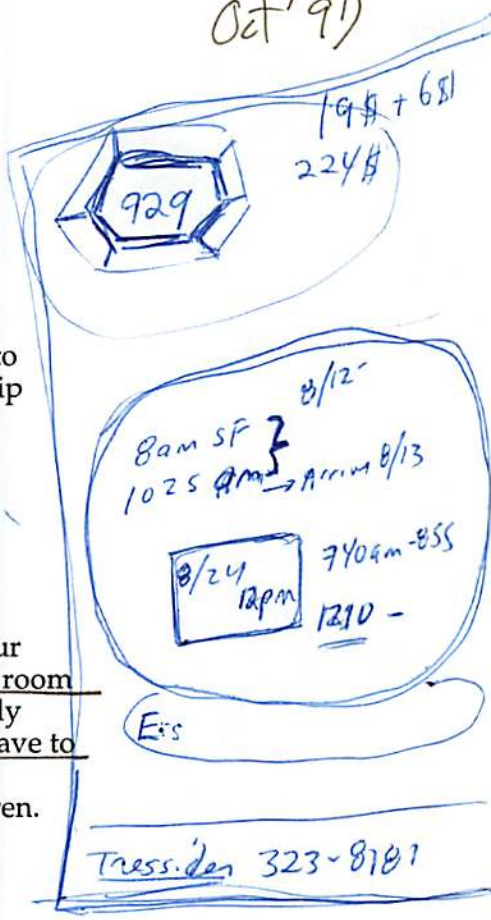
Please submit your application as soon as possible. If you have any questions, please feel free to contact us by Internet at *nato-asi@nsf.gov* or by phone at 703-306-1630.

Sincerely,

Richard P. Metcalf
Associate Program Director
Graduate Fellowship Program

Enclosures

Turkey Oct 97



From: miral@enh.nist.gov
Date: Fri, 27 Dec 1996 12:13:18 -0500

Subject: NATO Meeting

Dear colleague;

Some facts about the NATO meeting are slowly emerging. I would like to inform you about them as early as possible so that you can plan your trip early.

1. The arrival date is October 13, 1997. The meeting will start on Oct. 14. The departure date is Oct. 24.

2. The grant will pay for your room and all your meals including your drinks (also including five-star Hotel facilities such as tennis, sauna, Turkish bath, swimming) for eleven days. If you would like to bring your spouse or a friend with you, you could do so. We will arrange a double room for you. The additional cost for the second person will be approximately $35-40/day, again including everything as mentioned above (you will have to take care of this one). If you would like to bring your children, please let me know. I will have to get information about the price for the children.

3. I know that ten days are quite a long time. But, I think the meeting itself and the meeting site are so attractive that you will be able to stay for the entire duration of the meeting (I hope very much so). To make your stay more interesting and worthwhile, we will have the following events in addition to the talks: welcoming party; conference dinner with typical Turkish entertainment; a boat trip of 4-5 h duration on the sea visiting some ancient sites; a bus trip to ancient cities Perge, Aspendos and Side; a visit to the city of Antalya, its wonderful harbor and famous archeological museum, and maybe more. Of course, you can add things like sunshine, warm weather and warm Mediterranean waters to all these attractions. Please visit the following web site for some information about the region: http://www.turkey.org/antalya.htm. I will send you more information including some brochures soon.

Application
-1 photo
-cover letter

Doris

4. I would like you to plan your airplane trip as early as possible, and give me some possible dates and what airline you would like to fly. As your hotel costs, your flight will be paid for by the grant. Here is a suggestion: Turkish Airlines flies daily from New York JFK Airport to Istanbul (non-stop flight leaving at 6 pm in a comfortable four-engine Airbus plane). It arrives around 10.50 am in Istanbul with a connecting flight at 2 pm to Antalya arriving at 3.15 pm. You will be picked up at the Antalya Airport and driven to the hotel (about one hour drive). For those who will be coming from Europe, you can take a Turkish Airlines flight from any major European city and connect to another flight to Antalya. Turkish Airlines also flies directly from Tokyo to Istanbul. At present, Turkish Airlines flies from Istanbul to Antalya four times a day. Of course, you may choose to fly with a different airline to Istanbul. But if we can book you all for the same airline, we may get a discount, or even some free tickets. I am aware that not all of you live in New York and you have to get to New York first. This part will also be included in your ticket provided by us.

Furthermore, I am open to suggestions for alternative flying routes.

5. Please tell your post docs, associates and senior scientists in your department/institute about the meeting. Everyone can apply to attend the meeting. Qualified applicants will be chosen for a support from the NATO grant. In the next few days, I will have a web site with more information about the meeting and how to apply. I will let you know its address as soon as it is finished. Soon, I will also have a tentative program and send it to you for your approval.

I am looking forward to hearing from you soon. I wish you all a happy, prosperous and successful New Year.

Please acknowledge the receipt of this e.mail. Thanks very much. – Did 12/27/96

Miral Dizdaroglu
NIST, Bldg. 222/A353
Gaithersburg, MD 20899, USA

Tel. 301-975-2581
Fax. 301-330-3447

UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

January 30, 1997

Dear Colleague,

Enclosed please find a brochure about the hotel where the NATO Meeting will be held and some information about the ancient sites in the vicinity of Antalya. Please do not hesitate to contact me if you have any questions about hotel or anything else about the meeting.

With my best regards,

M. Dizdaroglu

Miral Dizdaroglu
NIST, Bldg. 222/A353
Gaithersburg, MD 20899

Tel. 301-975-2581
Fax. 301-330-3447
E.mail: miral@nist.gov

Jonathan —
Please send your CV + reprints to Dizdaroglu with note to the effect that you are the person I recommended from the lab to join the meeting. See you Wednesday.
Phil

NIST

UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

December 5, 1996

Dr. Philip C. Hanawalt
Department of Biological Sciences
Stanford University
Stanford, CA 94305

Dear Dr. Hanawalt;

I am pleased to inform you that NATO has approved my grant application to organize the proposed meeting on "DNA Damage and Repair: Implications for Human Health and Aging" to be held in Antalya, Turkey in 1997. The meeting will be held during the period of 13 to 23th of October, 1997. Please mark these dates on your calendar. I will let you know further details in near future. Thanks very much again for your agreeing to participate in this meeting as a lecturer.

Would you please confirm your participation in this meeting?

With my best wishes,

M. Dizdaroglu

Miral Dizdaroglu
NIST, Bldg. 222/A353
Gaithersburg, MD 20899, USA

Tel. 301-975-2581
Fax. 301-330-3447
e.mail: miral@nist.gov

email confirmation Dec 5 96

NIST

UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

May 20, 1997

Mr. Jonathan A. Eisen
Herrin Hall #356
Department of Biological Sciences
Stanford University
Stanford, CA 94305-5020

PK 188 Tekirova
Kemer, Antalya
Ph. 242 821 47 50 PBX
Fax 242 821 47 59

Dear Mr. Eisen,

I am pleased to inform you that you have been selected as one of the 70 student participants in the NATO Advanced Study Institute (ASI) *"DNA Damage and Repair; Oxygen Radical Effects, Cellular Protection and Biological Consequences"* to be held in Antalya, Turkey, October 14-24, 1997. Your hotel expenses including your meals will be paid by the NATO grant for the duration of the ASI (eleven nights). The arrival and departure days are October 13 and October 24, respectively. You must stay for the entire duration of the meeting to ensure full interaction. At present, we cannot make any commitment to cover your travel expenses because of the tight budget. ***Please make your travel arrangements as early as possible to ensure your timely arrival at the meeting.***

All participants in this ASI must stay at the designated Institute Venue, which is the Tekirova Corinthia Hotel & Resort. Participants who desire to stay at other hotels, but to attend the meeting at the designated Institute Venue are not acceptable. All ASI students supported by the NATO grant will share a double bed-room with another ASI student. You are requested to submit a deposit of $150 on chargeable living expenses. This deposit will be non-refundable in the event of late cancellation by you. It will be returned to you during the meeting. Please submit your deposit ($150) by bank transfer to the following account:

Account No. : 4299-2137353 NATO ASI 960617
Bank Code: SWIFT Code: ISBKTRIS
Holder of Account: M. Miral Dizdaroglu
Name and Full Address of Bank Branch:
Turkiye Is Bankasi, Baskent Branch,
Ataturk Bulvari 191/C,
06684 Kavaklidere-ANKARA/TURKEY

NIST

You are invited to submit an abstract and present a poster on your work during the meeting. The subject should be related to the general theme of the ASI, and present original research performed by you and your coworkers. All abstracts will be presented as posters. In addition, up to ten abstracts will be chosen as oral presentations of 5-10 min duration. Those who are chosen to give oral presentations will be announced during the meeting. Thus you are asked to bring 2-3 slides or transparencies on your poster to give an oral presentation and answer questions in case your poster is chosen as an oral presentation. Please see the attached sheet for instructions how to prepare your abstract.

I would like to ask you to inform me about your acceptance at your earliest convenience by fax or e.mail. If we do not hear from you by **July 31, 1997**, we will assume that you are no longer interested in participating in this ASI and we will offer your position to an alternate.

You can get all the information and the latest updates on the meeting by checking the WEB site on: **http://indigo15.carb.nist.gov/natoasi**

Please do not hesitate to contact me if you have any questions concerning the ASI and your participation. We would like to congratulate you for your selection to attend the ASI. We look forward to seeing you at the Tekirova Corinthia Hotel & Resort, Antalya, Turkey.

Sincerely yours,

Dr. Miral Dizdaroglu
Director of the ASI
National Institute of Standards and Technology
Bldg. 222/A353
Gaithersburg, MD 20899, USA

Tel.: +1-301-975-2581
Fax.: +1-301-330-3447;
E.mail: miral@nist.gov

Important note: *NATO does not take out any health or accident insurance for participants in the ASI meeting; such insurance is an individual responsibility. NATO and the organizers do not assume any responsibility, either in this context or for any other liability.*

Turkey

11:54:13 -0800 (PST)
From: miral@enh.nist.gov
Date: Thu, 02 Jan 1997 14:52:58 -0500
Subject: NATO Meeting

Daer Colleague;

I prepared a tentative program for the meeting. I would like very much to hear your comments and suggestions for improvement. Please let me know all that at your earliest convenience.
Thanks very much.

Miral Dizdaroglu
NIST, Bldg. 222/A353
Gaithersburg, MD 20899, USA

Tel. 301-975-2581; Fax. 301-330-3447

http://indigo15.cstl.nist.gov/natoasi

NATO Meeting PROGRAM (Tentative)

1st Day, Oct. 14
A.M.

Welcome and introductory remarks (committee members)

Barry Halliwell , "Oxidative DNA damage and human health."
Philip C. Hanawalt, "Role of transcription-coupled DNA repair in human health."

P.M.

Miral Dizdaroglu, "Mechanisms of oxidative DNA damage; lesions and their measurement."
Jacques Laval, "Repair of DNA damaged by free radicals."

EVENING: WELCOMING COCKTAIL

2nd Day, Oct. 15
A.M.

Stuart Linn, "The chemical bases for hydrogen peroxide toxicity and DNA damage."
Susumu Nishimura, "8-Hydroxyguanine in DNA: its formation by oxygen radicals, repair and implication in mutagenesis/carcinogenesis."

P.M.

Tomas Lindahl, "Mechanisms of repair of endogenous DNA damage."
Hans E. Krokan, "Human uracil DNA glycosylase: gene structure, regulation and structural basis for enzyme catalysis."

3rd Day, Oct. 16
A.M.

Arthur P. Grollman, "Oxidative DNA damage; mechanisms of mutagenesis and repair."
Aziz Sancar, "Molecular mechanisms of excision repair in humans."

FREE AFTERNOON

4th Day, Oct. 17
A.M.

Susan S. Wallace, "Processing and consequences of oxidative DNA lesions." Bruce Demple, "Roles of AP endonucleases in repair and genetic stability."

P.M.

Contributed papers, oral session

5th Day, Oct. 18
A.M.

John F. Ward, "Ionizing radiation damage to DNA: a challenge to repair systems."
Nancy Oleinick, "Modification of radiation-induced DNA damage by chromatin organization."

P.M.

Mehmet =D6zt=FCrk, "p53 tumor suppressor gene: its role in DNA damage response and cancer."
Hiroshi Ohshima, "DNA damage induced by reactive nitrogen-species."

EVENING: GALA DINNER

6th Day, Oct. 19, Break

7th Day, Oct. 20
A.M.

Vilhelm A. Bohr, "Repair and transcription in premature aging syndromes."
Paul W. Doetsch, "Bypass of DNA damage by RNA polymerases: implications for DNA repair and transcriptional mutagenesis."

P.M.

Contributed papers, poster session

8th Day, Oct. 21
A.M.

Paul Modrich, "Mechanisms of DNA mismatch correction."
Errol C. Friedberg, "Nucleotide excision repair in eukaryotes: yeast as a model system."

P.M.

Dennis J. Reeder, "Free radicals, DNA damage and p53 expression: a review of interrelationships with apoptosis and prospects for diagnostics."
Lawrence Grossman, "DNA repair as a biomarker in aging and for skin and lung cancer epidemiology studies."

9th Day, Oct. 22
A.M.

Kazimierz S. Kasprzak, "Studies on oxidative genotoxicity of human metal carcinogens: recent developments."
Matthew B. Grisham, "Modulation of nitrosation and oxidation reactions by superoxide and nitric oxide."

FREE AFTERNOON

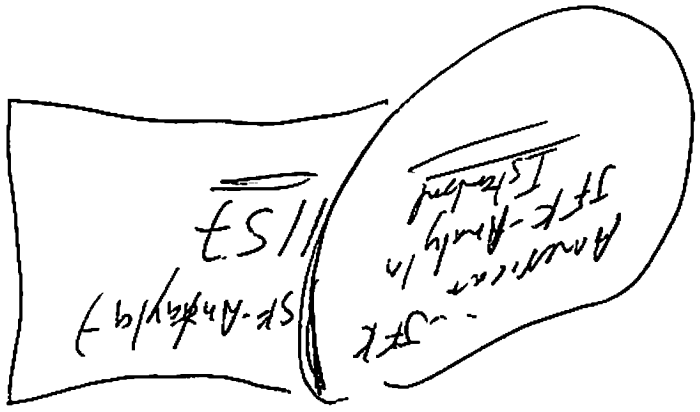
10th Day, Oct. 23
A.M.

Ali E. Karakaya, "Genotoxicity tests; applications for occupational exposure."
Etsuo Niki, "Action of antioxidants against oxidative stress."

P.M.

Contributed Papers

11th Day, Oct. 24, Departure



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10·14·97

In the beginning

75 people Nancy Olnick

Barry Halliwell - O_2 DNA Damage & Human Health

O_2 would probably not be approved as a drug by the FDA

O_2 → 95% Mitochondrial ATP synthesis

O_2 → 5% Superoxide (O_2^-)

Hydrogen peroxide (H_2O_2) } Reactive Oxygen Species

↓ Fe, Cu

Hydroxyl radical (-OH)

Production of Reactive O Species

Intentional

Accidental

Nitrous Oxide Also Important

Antioxidant Defenses

Scavengers (SOD, Glutathione)

Binders (Fe-Ferritin, Cu-CuO_2, Vit C + E?)

Fe, Cu

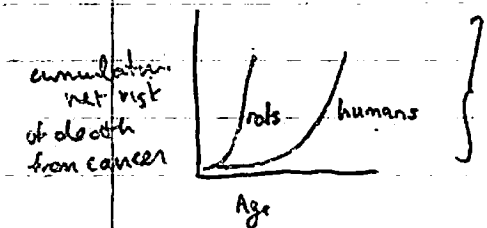
V.t E, C

Many plant compounds

Equilibrium

O_2 production —| protection → O_2 damage

Ames 1988



} can this be explained by O_2 damage

Assessing O_2 damage in vivo

1. Steady state (e.g. measure 8-O-G in DNA)
2. Total damage

O_2^- → $ONOO^-$ → nitrosation/oxidation, oxidation methionine, 8-nitrosoguanine

NO^-

C-8-OH-adduct radical → Fapy-G, 8-hydroxyguanine

Human disease - can either be caused ↓ protection or ↑ production

O_2 damage

1. Lipid peroxidation → → → end products can modify DNA
2. DNA damage
3. Protein damage → → → alter repair or polymerase damage
4. Ca^{++} ↑ → leads to ↑ in many enzymes

All bases get damaged

$OH^- \longrightarrow$ DNA $\rightarrow$ A, C, T, G

$H_2O_2 \longrightarrow$ DNA No direct damage

With information of what lesions each agent causes you can then use pattern of DNA damage to infer likely causative agent

For example

If H_2O_2 causes strand breaks by $\uparrow Ca$ and activating nucleases then H_2O_2 should not cause base damage

If H_2O_2 causes strand breaks by causing $OH^- \uparrow$ then should get base damage

Another example - Cig. smoke

- Cigarette smoke contains many O_2^-
- Most DNA-alterations induced by cig. smoke = deamination product

Parkinson's disease

- Substantia nigra die off leads to $\downarrow$ dopamine
-

DNA Damage → (P53) → Cell cycle
→ Repair
→ ER

P53

© mutations in tumors

Phil Hanawalt - TCR

① GGR - efficiency affected by seq. context + chromatin
TCR - only of tx-strand; regs. pol II, arrested pol II may recruit repair enzymes

② Ecol: vs Eukaryotes

pol removed pol removed?

③ TCR or Not

CPD's
6-4's
x-links
BPDE BPDE
T-glycol

④ Stall polymerase? Polymerase leave DNA

CPD - stalls + pol II stalls
AF - no stall + no stalls
AAF - stall + pol II falls off

⑤ XP + CS ; cancer prone vs. dulp problems
-Some overlap
GGR TCR

⑥ proteins

	# of patients	# w/ CS
CSA,B	140	140
XPB	3	3
XPD	52	2
XPG	12	6

DNA Damage → (P53) → Cell cycle
→ Rep
→ ER

(P53)

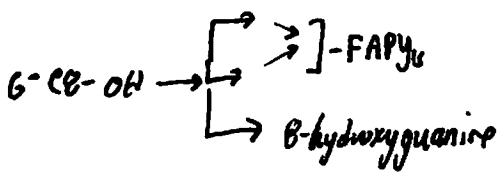
@mutations in tumors

Miral Dizdaroglu

SU

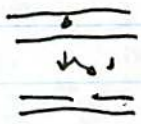
Hydroxyl radical ...

- products produced depend on presence/absence of O_2
  (i.e. - get different products if O_2 present vs. absent)



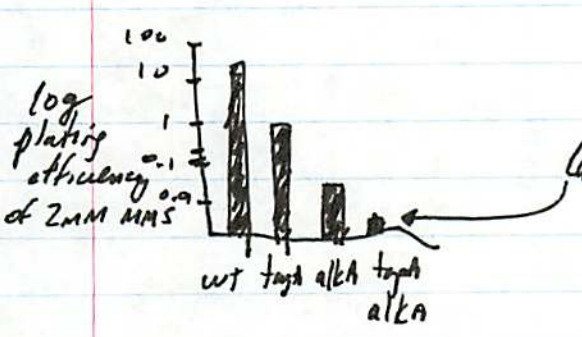
labelled
Ratio of these two groups
can tell something

J. Laval - Repair of O_2 Lesions



Hypoxanthine
~~Ethylating~~ Ethenecobase
C-8-oxoguanine

Alkylation
-can lead to ring opening



Can complement this mutant w/ human 3-Me-A -- this is how cloned.

Something in these cells can repair Hypoxanthine

		3Me-A glycosylase	Hyp 20/T
Human	ANPG	1	0.15
Rat	AOPG	1	0.15
Yeast	MAG	1	0.004
E coli	AlkA	1	0.0012

Diff specificity

Ratio of Activity of enzyme of hypox.: 3Me-A.

Hypoxanthine activity doesn't depend on what base is there.

Ethene- lesions

- AlkA + ANPG can recognize & excise ethano- Adenine
- mammalian enzymes remove it well
- yeast + E.coli have v. low activity

[redacted]

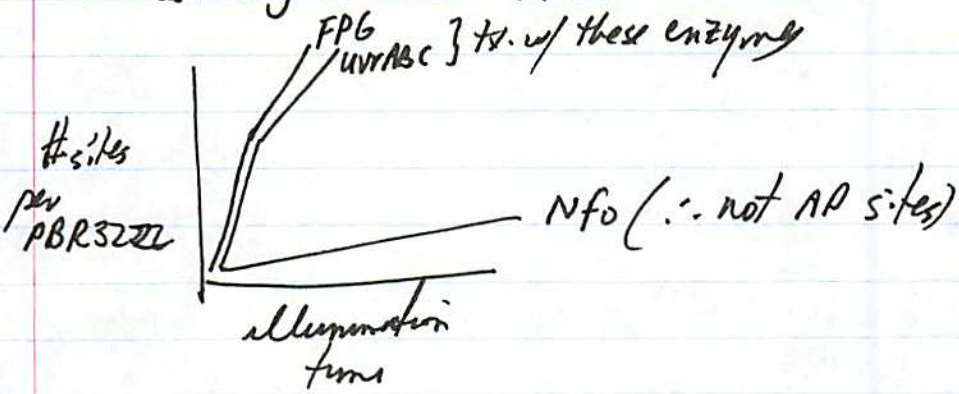
C-8-Oxo-Guanine

- Fpg protein
  - 1 zinc/molecule
  - footprint, 3nt 5', 2 nt 3'

3 Activities
- B-glycosylase
- AP lyase
- dRPase

AP site → Nfo
dRP → Fpg

Can recognize 8-oxo-G; FPG



Fpg Zinc finger

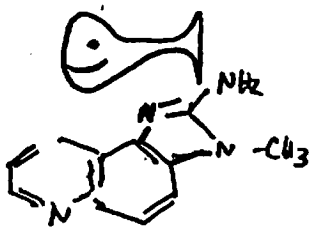
Mutate any of the Cys → lose almost all activity.

Ecol. fpg-mutY double mutant
used to examine mutation protection properties of diff fpg mutants

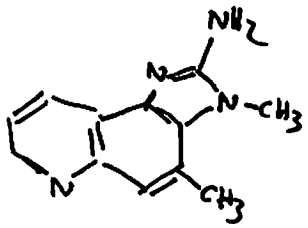
All protein can bind to nicks generated by Fpg

S. Ishimura

Isolated mutagens from Sardines



IQ



Discovered two peaks w/ HPLC of DNA tx. w/ these

Isolated enzyme from E. coli that cleaved this = MutM = same as Fpg

MutM
MutY
MutT

Isolated activity from mouse

Ogg 1 = human mutM?

- 8 exons
- multiple splice forms

} Similar to Ogg1 from yeast

All isoforms suppress Ecoli mutY + mutM mutations

Brochemical Activity like yeast Ogg1

- β-elimination at 5'

The Ogg1 Enzymes : Serge Boiteaux

Cloning yeast gene

- tried cloned activity but never got completely pure
- used genetics to complement Ecoli fpg-mutY mutants
  - these mutants have very high mutation rate in Miller assay
- isolated two ID plasmids encoding yeast gene that complemented this
- extracts from Ecoli w/ this plasmid showed 8-oh G repair activity

OGG1 ← PIF1 →

- NO detectible seq. similarity to Fpg

YEAST

- does NOT removed Fapy-G very well, but it does remove it. Cannot remove FAPY-A.

- some similarity to Nth
  - including Ntg1, Ntg2 } from yeast

- Ogg1 mutants ... spontaneous mutator w/ higher GC → TA transversion

XP

Joyce Reardon

- Intro
- XP - only talking about Global Genome Repair
- Human excision

AAF	++++
Cisplatin	+++
6-4	+++
8-oG	++
TT	++
Cholesterol	+++
Mismatch	+/-
O-6-MeG	+/-
N6-MeA	+/-
TG	+
Abasic	++

} Repair activity

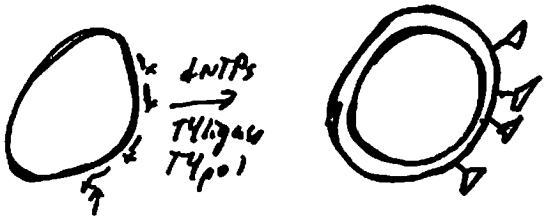
Psor. monoadduct +
cisplatin 1-2 G6 +/-

Basal Excision

XPA + RPA	Damage Recognition, Recruitment
TFIIH	Preincision complex recruitment
XPB	3'-5' Helicase
XPD	5'-3' "
p62	
p52	
p44	
p34	
CAK	
XPC	Binds DNA, part of pre-incision complex
XPG	3' incision
XPF-ERCC1	5' incision

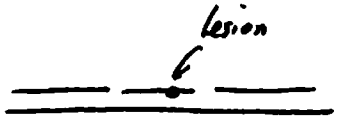
DNA Substrates + Repair Assay

Circular



Placement of label depends on whether you want to look at 5', 3' incisions

Linear



Damage Recognition

don't know exact details

- regs XPA + RPA
- XPA + RPA interact

XPA interacts w/ 2 of 3 subunits

- both binds DNA but v. weak (eg Engels + Legerski show 5-10x ↑ in binding when together)

Recruitment of Repair Factors

Protein interaction

XPA + TFIIH

XPC + TFIIH

XPC + RAD23b

} stable complex

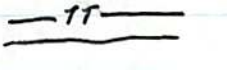
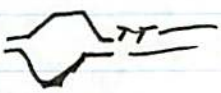
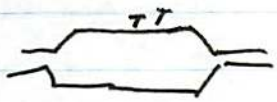
DNA unwinding

- Helical deformation at damaged site enlarged

Unwinding

XPB - helicase
XPD " helicase
XPC - stabilizes - unwound region + protects DNA from digestion by endonuclease

Mimicing Bubble

		Required? XPC	TFIIH
	no bubble	yes	yes
	3' bubble	no	
	5' bubble	no	
	3'+5' bubble	no	partially

binding and cleaving bubbles

Nuclease Recruitment

- 3' incision probably occurs 1st + then 5' soon after

- XPG interacts w/ TFIIH + RPA
- XPF-ERCC1 interacts w/ RPA + XPA

- XPG has non-catalytic role

- if XPF-ERCC1 is omitted XPG can make 3'
- if XPG is omitted --no 5' incision made by XPF-ERCC1

- also 5' excision products accumulate more quickly

XPG

mutant DB12 ... binds DNA but no nuclease
... no incision 5' to 3'
... but w/ XPF-ERCC1 present 5' incision can occur

∴ XPG seems to have non-catalytic role

Post-incision

- Dissociation
- Damage containing oligo is released even w/o repair synthesis
- Some repair prots go into solution
  " " " stay w/ oligo
  " " " stay on DNA

Repair synthesis

RPA
pol δ, ε
some ligase but don't know which

Repair patch assay

patch size { - use 3 dNTPs + dNTPαS
- T7 cleavage
- sequencing gel } can identify patch sequence } patch covers almost exactly 3'+5' incision sites. ∴ not much extension either way

But this is in vitro

Other lesion

- Thymine glycol } usually repaired by BER
- 8-oxo-6

but look at NER

- why?... XP ~~severity~~ uv sensitivity correlates w/ neurologic symptoms

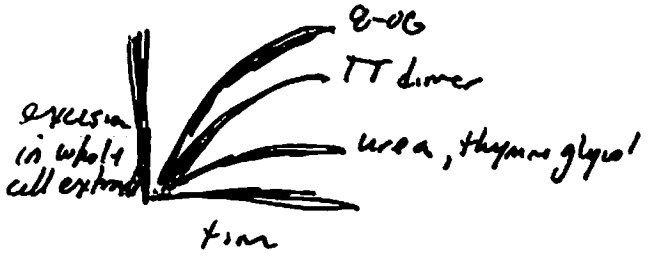
Robbins suggested XP neurological symptoms due to unrepaired O2 damage

- also Cn + Sancar showed UvrABC can repair TG

So

	Excision in HeLa extract	Excision in AAB extract	Excision in Mutants in AAB	Excision in Mixed mutant extracts
8-oxo-6	+ not as efficient	+ v. efficient	-	+
Thymine-glycol	+ not as efficient	+ efficient	-	+
Urea				
TT dimer	+	+	-	+

Are these ~~do~~ repaired at physiologically relevant rate? - She asks.

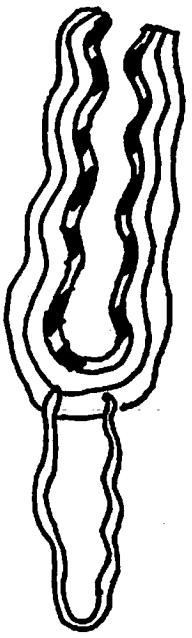


showed that repair of these is dependent on NER proteins in reconstituted system

Cooper et al

XPG ~~requires~~ dependent BER (also regs CS-proteins)

Suggests XP-proteins maintain genomic integrity in fully differentiated neurons.



?'s

- suggests ERCC1-XPF can cut duplex DNA when in complex w/ TFIIH ... etc ∴ ERCC1-XPF may not be ss-DNA specific

- suggests XPD + XPB may be reqd for oligo release

- suggests XPA, RPA can somehow sense distortion in helix ... and may slow down in translocating along DNA ...

- XPG dominant negative effect
- also seen w/ XPA

Rad4-Rad1 double-mutant
slightly higher mutation rate } reported by Serge

L. Grossman

- deletion of 3' end of UvrA protein leads to lack of recognition

Tom Lindahl - Repair of Endogenous DNA damage

Endogenous damage

- base loss
- 3-Me-A (formed by S-Aden-Met among many things)
- oxygen damage
  - strand breaks
  - base lesions

} Don't know rate of damage in vivo. Don't know concentration of O_2 in-vivo. Don't know localization of DNA damage.

Small alkylating agents alkylate DNA in-vivo about the same as in-vitro. ∴ Chromatin doesn't matter

Repair enzymes (sort of provide evidence that damage in-vivo is important)

How measure rate of formation of lesions?

- equilibrium levels... are from a balance between damage + repair. Like cytosine deamination (it is hard to detect U in DNA because it is repaired very rapidly)

formation → U ← repair

- suggests same is true for 8-OH-G

But could inhibit repair

Measuring 8-OH-G

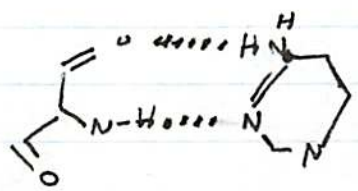
- estimating levels in DNA depends on way DNA treated
- suggests best way is to ~~[illegible]~~ inhibit repair
- suggests Mt-DNA should have more 8-OH-G because less chromatin + more damage

~~Most genes~~

hOgg1

3p25

- human 8-oxy-guanine glycosylase
- they cloned longer form than many other groups
- specific for 8-O-G opposite C
- v. little AP lyase activity



Suggests that the mechanism of repair is NOT base flipping.

hOGG1 near VHL

3p25
- XPC nearby
- VHL nearby

} Other groups ~~dont~~ have looked for + not found Mutations in hOgg1 associated with cancers.

Mouse MOGG1 KO
- should allow better estimation of 8-O-G formation
- mice not available yet

Other lesions induced by ionizing radiation

Referred to work by M. ral D. that showed most ancient DNA cannot be amplified by PCR because it contains lots of purine/pyrimidine damage.

Human Nth
- easy to express
- purified

} Making mouse KO

Reconstitution of BER in-vitro

glycosylase (HAP)
XRCC1
Ligase III
polB

XRCC1 is a scaffolding protein in BER
- has binding sites for Ligase III and polB + PARP

in-vitro
~90% efficiency of U repair in ~15 minutes

Ligase III: two different forms w/ different C-termini
- BRCT domain
- this region is the part that interacts w/ XRCC1

XRCC1
- inhibits strand displacement by polB

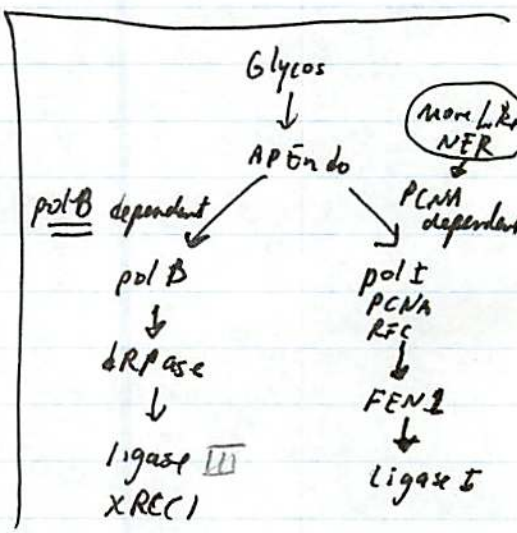
Long patch BER

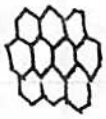
FEN1 = DNAse IV
- structure specific nuclease is required for longer patch repair
- polymerase
- 5' nuclease } this region similar to FEN1

DNA polymerase

- XPG cannot substitute for FEN1

PCNA
- stimulates long patch BER



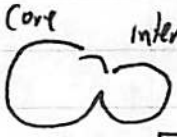


Ligases

Ligase I - for okazaki

III - BER has BRCT domain

IV - long patch BER has BRCT domain



Yeast

- found lig4 ... (Yeast genome sequence is wrong)
- not required
- ~~protein~~
- lig4 mutants not obviously defective in repair
- lig4 mutant defective in recombination

Lig4 in humans co-purifies w/ XRCC4

- XRCC4 hamster mutants have same phenotype as KU mutants (DNA-PK too)

- protein = 100KD in humans
- no introns in animals
- KU also has intronless genes

- Lig4 KO in mouse = embryonic lethal

Structure-function - Grollman

Sequence effects appear to be important

Does 8-OG miscode
Is it mutagenic
How does it affect DNA structure
How does ~~does~~ is it recognized

Does 8-OG miscode

C:A ratio put opposite 8-OG

pol α	1:200	replication enzymes put A in
δ	1:5	
III-Ecoli	1:8	
B	4:1	repair enzymes put C in
I-Ecoli	7:1	
δ-yeast	5:1	

Diff between repair & replication

Extension past 8-O-G

8-O-G : C extended poorly
8-O-G : A extended well ∴ Mutations quite likely

Is 8-OG mutagenic

Duplex vector - mutation analysis complicate by NER
∴ Put 8-O-G in sigle-strand vector
- site-specific

G:T mutations increase

How does 8-O-G affect structure

Recognition Fpg + MutY

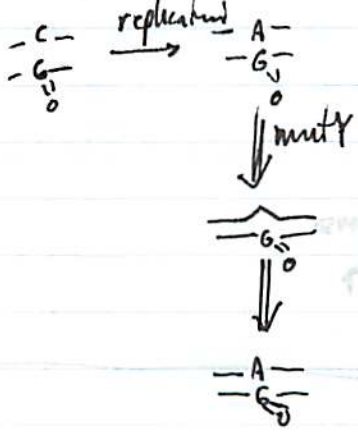
Fpg

- used NMR to determine structure of this Zn-finger
- suggests recognition is v. important because repair enzymes are in low abundance

MutY

- very sensitive to any modification to adenine (doesn't work)
- binding doesn't change much but catalytic activity does

8-o-6 pathway



MutY

- doesn't have 1st conserved in Nths
- has been x-tallized
- does have conserved D138

Ung1 Mt form
- much more widely expressed than nuclear forms
- in all tissues... highest in muscle (skeletal + heart)

Ung2 Nuclear form
- high in testes, colon, intestine, placenta, thymus
- most expressed in proliferating tissue

		Ung1	Ung2
promoter elements	TATA-box	−	−
	MIC	+	+
	Sp1	+	+
	E2F	+	−

Mouse + Human Mito-localization seqs may be amphiphilic helices

RPA binding
- Ung2 + RPA interact w/ RPA
- Sequences required for interaction w/ RPA are similar

RPA binding 2
- Ung2 has a 2nd RPA binding region
  - N-term
  - shows similarity to N-term of 6 TBP

p
Ung2 ... stains unevenly in nuclease
... pos. then correlates w/ RPA staining
... spots show up more in S phase
... RPA 10X more abundant
... Ung2 spot staining decr. quicker than RPA spots

→ Replication Factories

Inhibition of UDG w/ antibodies

- does not inhibit DNA replication but does not inhibit UDG

Why Ung in mtDNA?
- maybe to repair Oz damage

Susan Wallace : Processing and consequences of oxidative base damage

Properties of Nei, Nth

- recognize all pyrimidine ring opening products

Nth + MutY

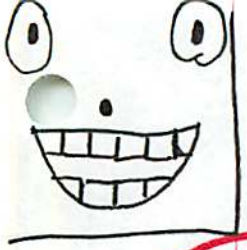
- no overlapping substrate specificity BUT sequences v. simila.

Nei + Fpg

- little overlapping specificity BUT sequences v. similar
- produce same products at AP site
- both have dRPase activity
- Nei will cleave 8-oxo-A
- similar K_d's
- different footprints
- double-mutants -- hypersensitive to H_2O_2 + ionizing radiation

Can closely spaced lesions give rise to DSB's

- don't cleave abasic sites as well nearby but they will
- what about opposite strands



o_i_g B_r_n_ ↑

How well do they cleave opposite strand break?

Base damage OR Base loss
- cleavage is greatly reduced if immediately opposite strand break
- if 3 nts opposite - cleavage 0%
- if 6 nts " - cleavage 100%

Processing of Uracil Glycol

But only if opposite A-
- U-Glycol efficiently elongated once incorporated
- T-Glycol not efficiently " " " "

What is incorporation opposite U-Glycol

	what inserted	mutagenic
abasic site	A>G>T	+
urea	A>G	+
	A>G>T	+
		poor

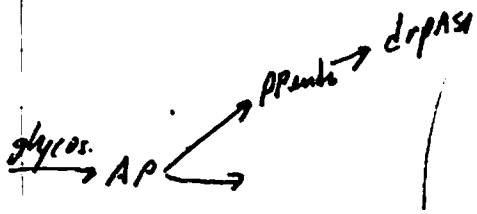
NOIR
NOB
BORING
BORN
BONG
BOG
BIG
BRIG
BRING
GRM
GOD
ROB
RING
RIG

5-HU
U-Glycol
dihydrouracil
} all derived from C but pair as T so ∴ should be mutagenic.

HU protein binds gaps

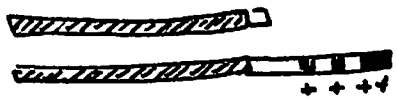
BRUCE DEMPLE - AP Endonucleases

Class I - β-lyase enzymes, abasic residue at 3' end,
Class II - act hydrolytically + cleave just to 5' side
- require dRPase



Class II

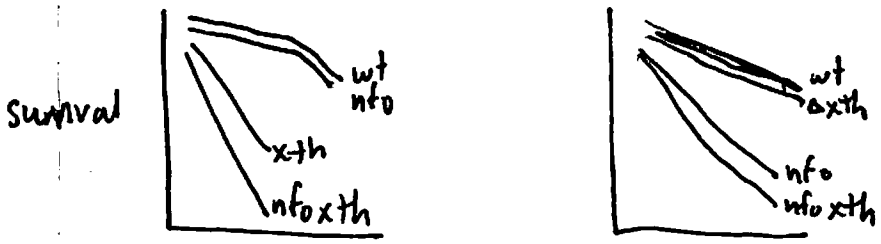
- includes Xth -
- Nfo family - (some relationship to TPases + DNase I)



(∴ A rule prob. doesn't work)

APN1 mutants

- incr. spont mutation rate (AT→CG ↑ most)
- sensitive to damage
- incr. tpon mobilization

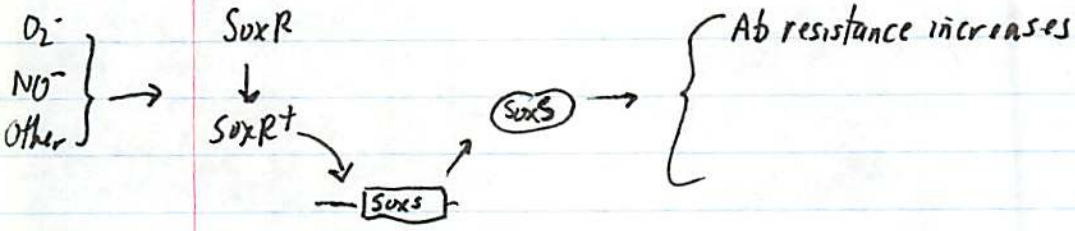


Uv exposure

Bleomycin

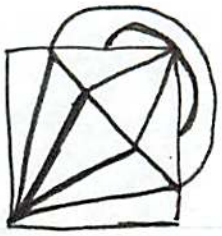
Nfo

Nfo induced
- normally at v. low levels in cell
- induction due to O_2 stress
  - SoxR undergoes Δ in response to redox Δ
  - SoxS then induced
  - this induces many things including Nfo

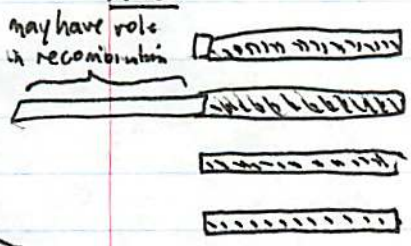


- Nfo mutants hypersensitized to macrophage killing
  (macrophage kill w/ NO)
  (i.e inhibit NO ~~killing~~ synthesis cells don't die as much)

- Nfo deficient bacteria accumulate damage ~~that~~
  (i.e tx w/ bleomycin) that can only be repaired w/ Nfo



APE



APE - AP Endo; exo very low

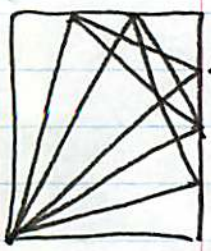
Rrp1 - AP Endo; exo activity; some role in recombination

ExoA -

ExoIII - this is the least similar to ExoIII

- Suggest human enzyme has evolved to be Abasic site specialist

- APE can complement yeast APN1



APE1

- can cleave almost ANY abasic site -- don't need ring
- can cleave abasic site w/ mismatch 3'
- cannot cleave abasic site w/ mismatch 5'

} ∴ Probably need "Normal" DNA 5' to site

Are proteins coordinated

APE1 interacts w/ polβ and Ku70

- in two hybrid APE1 + polβ interact
- APE1 does not bind well after excision
- polβ's ability to excise abasic residue incr w/ APE presence
- APE interacts w/ Ku70

Walter Deetsch - Drosophila S3 is N-glycosylase, abasic site + dRPase activity

[redacted]

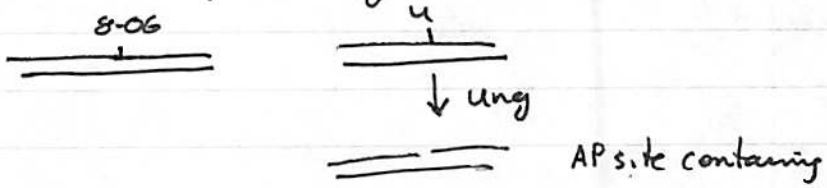
Why examine S3?

- Cloned PO riboprotein in Drosophila w/ APEndo antibody
  - some APEndo activity

- S3 in humans is deficient in XP-D?

- S3 in Drosophila has AP lyase activity
  - P. Doetsch showed that S3 identified some UV photoproduct

① overexpressed S3 in mutM strain as GST-tagged protein
② used to measure repair in synthetic oligos



Activity is very labile

③ S3-GST has activity
  - [redacted] - N-glycosylase activity
  - some β-lyase activity

④ γ-irradiated substrate
  - only liberates 2-modified bases (8-0-6 + fpg)
  - similar Kcat/Km for both

- why?
- yeast S3?
-

In vivo?

-Is it in nucleus?

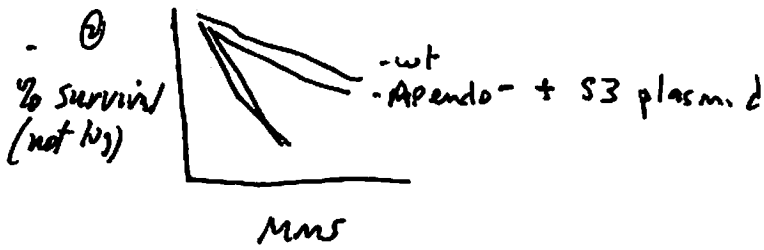
-has nuclear localization signal

-associated w/ nuclear matrix < westerns are too clean.

-

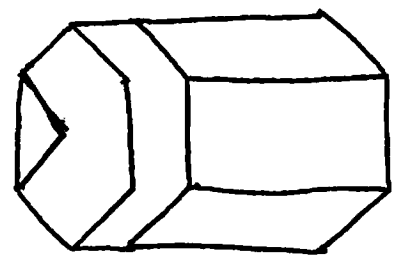
-In vivo f(x)

Complements mutator phenotype of mutM



- S3 has dRPase activity

- Protection of FA-C cells against MMC using dS3 gene



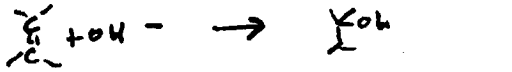
John Ward

Effects of Radiation on Water

$$H_2O \rightarrow H_2, H_2O_2, OH^-, H^+, e_{aq}$$

OH RXN's

Abstraction 

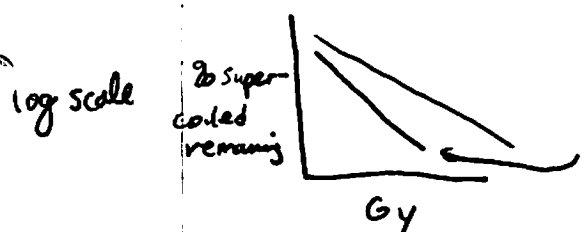
Addition 

Oxidation

DNA $\xrightarrow{Gy}$ Assay supercoiling w/o denaturing

↘ ↗

tx. w/ Fpg or other enzymes

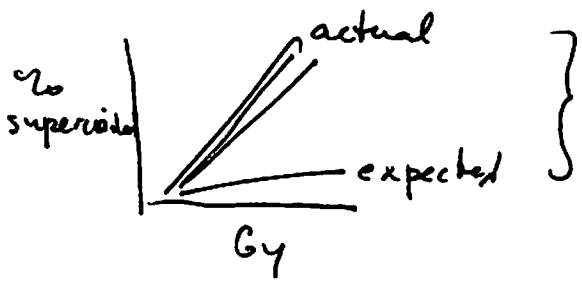


Ssb +	Ø	1
Ssb +	Fpg	2.3
Ssb +	Nth	1.5

} Ratio of strand breaks induced by fpg

↖ Claims that these #'s closely parallel those from GCMS (~4:1 ratio expected)

In mammalian cells

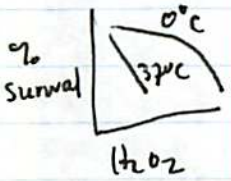


} Why is the actual yield so different from expected?

Is the distribution within the DNA different.

Suggests Peroxyl radicals are formed

- peroxyl radicals do not give rise to strand breaks
- one SSB could cause large change in DNA conformation (why?)
- may be explanation for repair induction at low radiation doses



Is endogenous O_2 damage comparable to γ-ray damage?

- no ... diff. mutation induction
- diff. % of diff types of damage
- diff. Amt. of damage required to kill 63% of cells

Why?

(B) it is DSB's that are important ... and diff types of damage give diff. freqs of DSB's

(C) is it OH^- radicals that cause these strand breaks?

→ (D) scavengers protect from effects

(E) but α-particles kill more / gray than γ yet produce less OH^-/gray

(suggests these #'s are wrong)

Multiply Damaged Sites (MDS)

- get many MDS's w/ γ-rays
  - SSB opposite base damage
  - base damage near base damage

Repair of DSBs

① may lose a base
② misrejoining (Lobrich et al 1996)

Nancy Olenick - chromatin modification of damage

Chromatin organization + damage

X-rays:
- active sequences contain higher level of damage than inactive sequences

Accessibility of DNA/chromatin to OH

- DSB yield ↑ w/ ~~remo~~ disruption of chromatin

DNA protein x-links

- measure amt of protein bound to DNA
- DNA-prot x-links still form readily even when most of the histones are gone
- suggests the proteins that are x-linked are nuclear matrix prots.

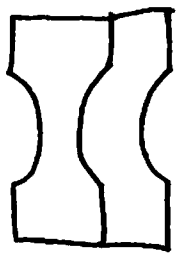
Nuclear Matrix

- some proteins always found associated w/ matrix
- but mostly enormous variability
- where is the damage?

isolate nuclei --> cut w/ Rest Enzymes

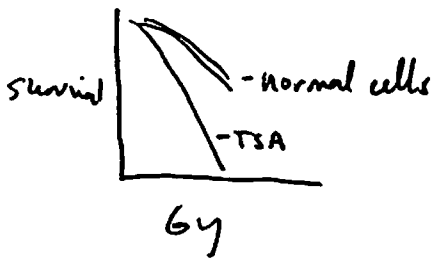
∴ DNA protein X-links form in matrix.

Histone Acetylation



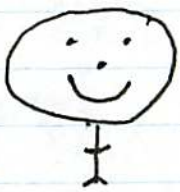
- manipulating histone acetylation
- Norman chromatin
- TH induces chromatin opening

NaButyrate - - - inhibits histone deacetylation
Trichostatin A - - - more specific



Many genes turned on w/ TSA + NaButyrate

Human APE : Sankar Mitra



GÜMÜS

- Mammalian APE has low level of phosphoesterase activity compared to Ecoli Nth

Which part of BER is rate limiting?

w/ O_2 damage

- apparently APE is limiting... in whole cell extract if you add BER enzymes (as extra) only addition of APE1 leads to increase in repair

P. - but APE1 not limiting for Ung repair

Proposes two BER pathways

APE1 - **Regulation**

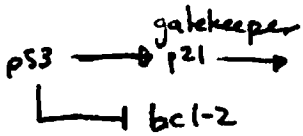
- also a redox activator of tx. factors
- also a neg. regulator of nCaRE seq. containing promoters
- Ku70/80 required for neg. regulation
- APE1 has nCaREa + nCaREb sequences + appears to autoregulate itself
- activated by a variety of reactive oxygen species

- claims adaptive response occurs involving activation of BER to remove damage that would trigger apoptosis

Outlook: P53

DNA Repair is not a problem for cells that are not dividing

Caretakers - protect genome
Gatekeepers - arrange cell to allow repair

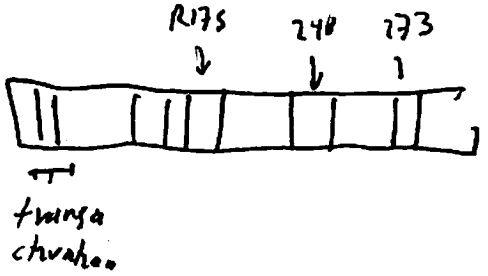


1979 - p53 cloned

Liver G→T v. high
Lung G→T v. high
Colon G→A

Aflotoxin vs. BPDE

- both attack G's
- mostly the same G's



p53 binding sequency

- very degenerate
  ∴ can regulate many genes

Overexpress P53 → cell cycle arrest

P53 ... induces apoptosis

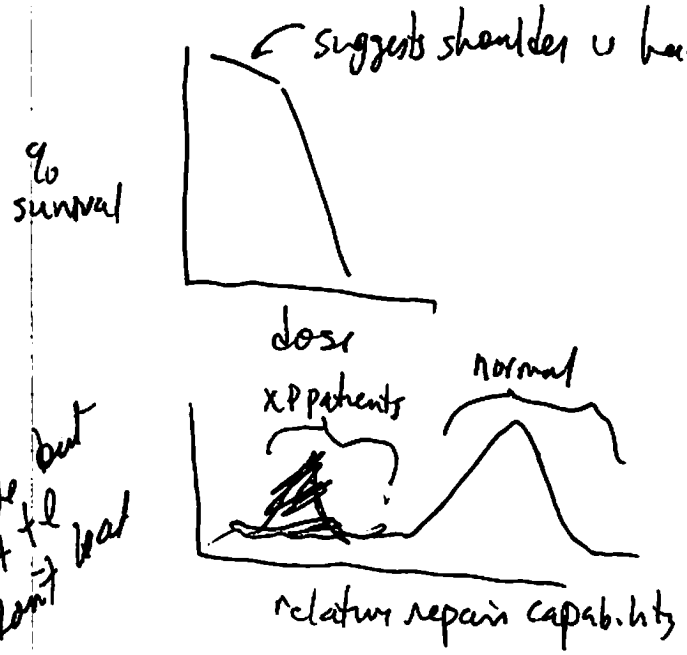
Other P53 roles

- cell differentiation
- repair
- devlp
- replication

DNA damage response

- p53 protein accumulates after ionizing radiation
- p21 transcripts increase
- p21 protein increases
- MDM2 protein increases
- in some cell types -- induction varies
- cell cycle arrest (G1, G2)
  - same cells w/ mutant p53 don't arrest
  - induces apoptosis

I would assume



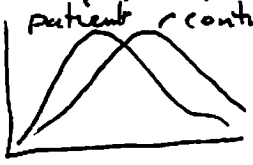
How rule out that tx + tl defects don't lead ↓ HCR.

Use % cat activity

Could it be due to tx or tl or uptake defect

Clinical test

- used stored lymphocytes for assay



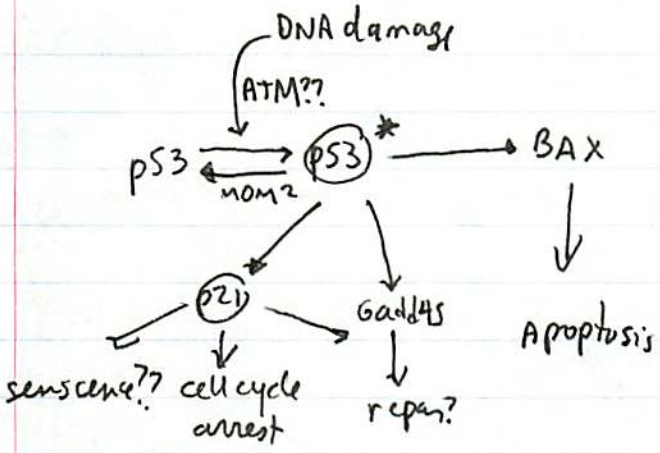
P53 mutations

- 35↗ w/ low repair ... seq. p53 genes
  - in Basal Cell Carcinoma -- pt mutations
  - Squamous Cell " ... allelic loss

Basal Cell Carcinoma

- mainly in whites
- one area in Taiwan w/ high levels
- high levels of arsenic

MODEL



P73

Alternatin splicing
- Homologous to p53
- Highest %sim in DNA bindy domain
- Hotspots are conserved

p73β has additional 200 aa

	p53	p73
DNA damage induction	+	−
Tx activation	+	+
cell cycle arrest	+	+
apoptosis	+	+
monoallelic expression	−−	+
LOH	+	?
mutation	+	?

p73 vs p53

Why so many P53 somatic mutations and no somatic mutations in repair genes

Will Bohr : Aging

Repair differences between different genes / regions
- P53 repaired very well
- mt DNA -- no NER
- telomeres -- more repair than expect for non-txd gene

Mitochondria
- some repair
- repair enzymes present
- no repair of TT dimers
- good repair of alkylating agents
- 4 nitro-quinoline is repaired well too
- age associated increase in O_2 damage
  (but many don't see a very dramatic increase)

Gene specific repair of 8-O-6

8-O-6 repaired in mt DNA

- use Fpg
- very good repair in mt

But very few G's so % repair may not be best measure

- 8-O-6 specific endonuclease in mt
  - activity resembles Ogg1
  - 25-30 kDa
  - ds DNA specific
  - prefers 8-O-G : C
  - V. low abundance
  - activity appears to incr. w/ aging (as has PARP)

Repair in inactive genes

d globin	low	} why differences?
telomere	lower	
X-linked 754	lowest	

- diff. in how ready for tx.

Repair - in-vivo labelling

- appears to start in matrix and then extend to loops

Recruitment to matrix?

- PCNA may play a role ...
  - forms insoluble complex associated w/ nuclear matrix (w/ TFIIH, PZI .. XPA)
  - seems to be defective in XPA

PCNA complex of matrix

Aging Diseases

- Werner's
- CS ... humans don't have large increase in cancer ... mouse does have increase
- XP
- AT
- Hutchinson-Gulford syndrome

Is CS a tx or repair defect?

- lower tx. in intact cells of CS than normal
- basal pol II tx. is reduced in CS-cells (sensitive to α-amanatin)
- can be complemented by addition of CS-B
- chromatin appears to be looser

RAD54 -- interacts w/ RAD51
MOT1
SNF2
SWI

when deplete proteins from extract tx in CS cells decreases more (~20) than tx in normal cells

- In vitro w/ plasmid w/ G-less cassette
  - no tx in CSA or CSB extracts
  - but repair is OK (i.e. extract is good)
  - normal polI / polIII tx. in extracts

CSB -- interacts w/ TFIIH, XPG, XPA

suggests CS-B might be involved in altering chromatin structure

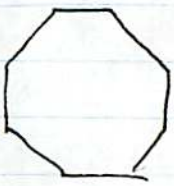
CSB sensitive to DNA quality

- txn in CS extracts is hypersensitive to DNA quality
  - if purify DNA more carefully tx is OK
  - "bad" DNA has some sort of lesion

Aziz thinks CS-B is an elongation factor

Site specific mutations in CS-B

Werner's

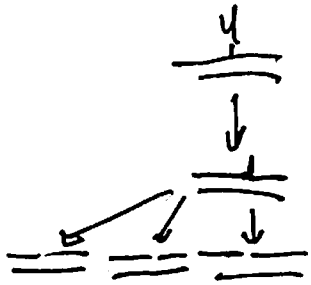


- may be sensitive to 4NQO (when in arrest)
- mutator phenotype
- many apparent repair deficiencies (including TCR)
- defect in txn in vitro + in vivo
- protein has 3'-5' + 5'-3' helicase activity
- seems to interact w/ topoisomerase (like SGS1)

Paul Doetsch : RNA pol & DNA damage

Ecoli + other RNA polymerases

- what lesions block which polymerases



	KlenTaq	SP6	T7	Ecoli RNApol	Ecoli DNApol
+P: works but pauses	U	+	+		
+Gs works w/ gap	Abasic sites	+P	+P		
	Strand breaks				
as induced by	-Nth	-	-		
	-Nfo	-	-		
	-Hot Alkali	-	-		
	Abasic site insertion	A	A	A	
	Abasic site on nts	+	+		
	Dihydrouracil			+insert A	+insert A
	~~O6~~-Me6			+U	+T
	~~8~~-oxo-G			+AC opposite G	+AC opposite G (larger gaps quit larger gaps in RNA)
	Gaps	+	+G		
as gaps get larger, ability to jump gap decreases	1 base		+G	+G	
	3 "		+G		
	6-9 "		+G		
	≥ 10		+G		
	correct insertion near gap	+	+	+	

Bypass efficiency is lower for Ecoli RNApol.

Lesion Bypass / Tx miscoding

maybe explains why txn is mutagenic

1. No TCR
2. Net decr. repair due to RNA pol. occupancy of template
3. tx mutagenesis
4. "fixation" of mutation when replication occurs

In vitro

- inclusion of U in template
- addition of UNG while txn is occurring ...
  - Ung repair inhibited

Tx mutagenesis

tx mutagenesis

- suggests miscoding of transcripts means that DNA damage on template strand could have "extra" effect

- tested w/ GFP model system

Retromutagenesis

→ mutant transcripts → cell could be released from non-growth state

↓

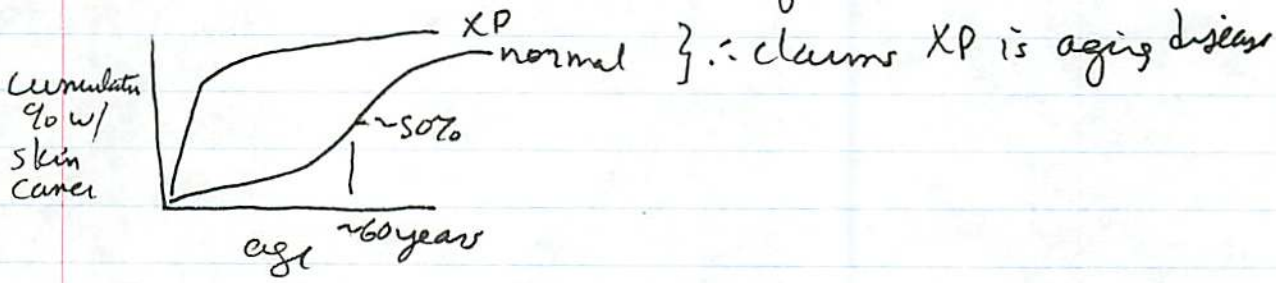
fixation of mutation because allowed to grow

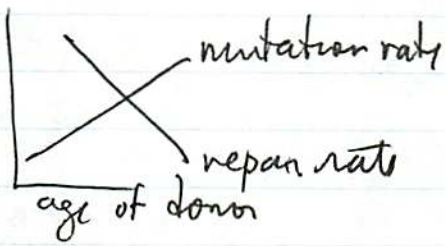
Larry Grossman - Human

Skin cancer increases towards the poles in recent times.

XP

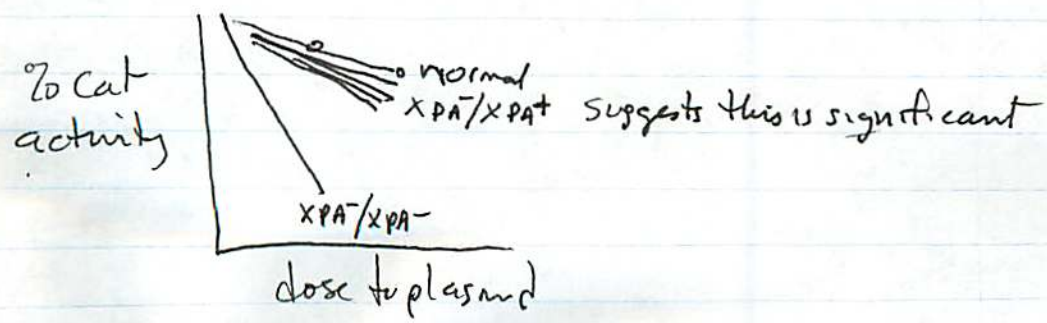
- discovered 1803 Moritz Kaposi
- 1/250,000 recessive frequency
- 2000x incr. in skin cancer.
- 10-20x incr. risk on internal factor.





Host cell reactivation

- using pCMVcat



Errol Friedberg

NER in yeast "profound genetic complexity in all eukaryotes"

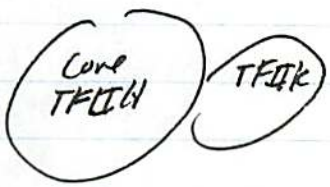
But there is a Rad16 like gene in humans.

Rad17 } no known human homologs
Rad16 }

Suggests humans may not have homologs because they have XPC

Reqd for NER

Rad 1, 2, 10, 4, (4, 7, 16, 23 : other
Rfa 1, 2, 3 = RPA1
(Rad 3 Ssl1, Ssl2, TFB1, TFB2, TFB3, TFB4) = ~~TFIIH~~ core TFIIH



TFIIK = CCL1, kIN28

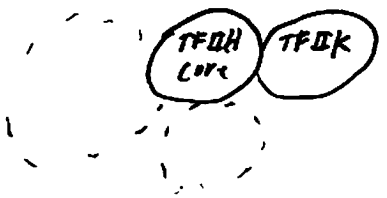
whole complex = holo TFIIH

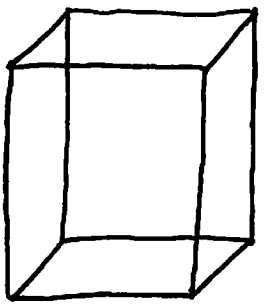
TCR = Rad26, Rad28
Rad26 reqd.
Rad28 not reqd.

} can also study TCR by examining recovery of RNA synthesis after UV.

Rad16 or Rad7 recover RNA synthesis faster than wt. Calls this pathway txn independent DNA repair.

Big Txn Complexes





Big repair complexes

(A) ① whole cell yeast extracts
② fractionation
③ westerns
} many non-TFIIH components associate w/ TFIIH

whole fraction can complement in-vitro repair defects

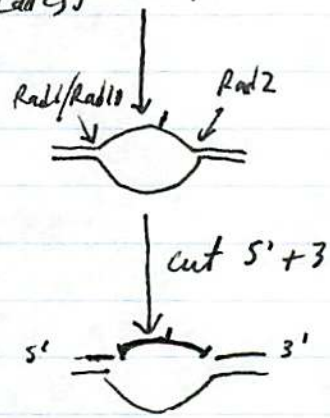
(B) ① Flag Rad14 (his tag)
② Ni-Nta column
③ cofractionization of many NER/TFIIH ~~complexes~~ proteins

Also show cofractionation after DNAse tx. ∴ Not just a DNA binding activity.

Why make such a complex?
- constant survey of genome

specific proteins

Rad3
Rad25 } open up bubble for txn and/or repair

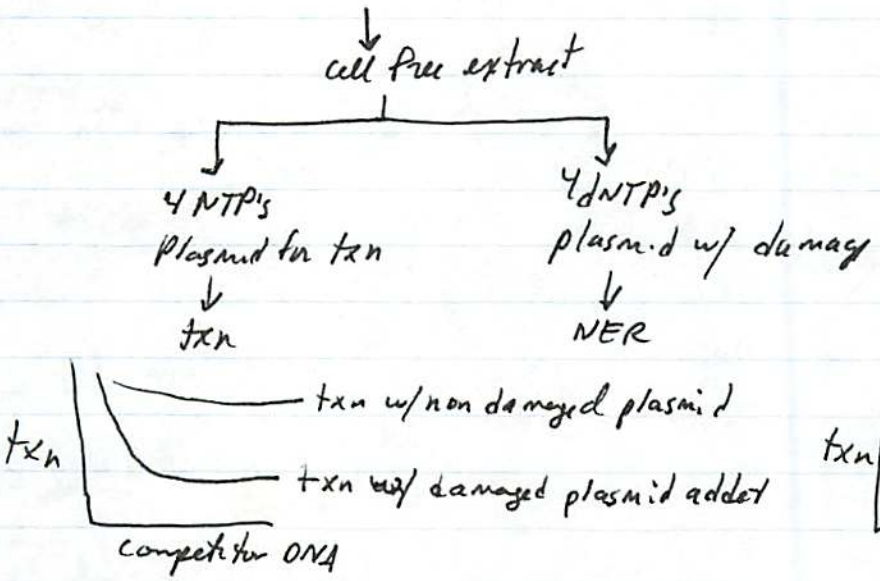


what about other proteins?

{ Rad7 interacts with Rad16 + Rad4
Rad4 " " Rad23 } non. tx. strand repair

Transcription + repair roles of TFIIH?

- is there any competition



Competition

Inhibition of pol II txn requires active NER
- Rad14 mutants don't show competition
- Rad1, 10, 7, 16, 4, 2 all show no competition
- dNTP's required
- if allow time for repair 1st ... inhibition is alleviated
- if add whole TFIIH inhibition is alleviated (somewhat)
- addition of core TFIIH, or TFIIB, TFIID no alleviation

Suggests DNA damage pushes an equilibrium between txn + repair complexes towards repair

Rad26 does not show reduction in pol II txn by competition. ∴ Suggests Rad26 is involved in shifting between txn + repair complexes

Larry Grossman : RNA pol.

NER in E. coli:

UvrA dimerizes → UvrA UvrA binds DNA ← UvrB+ATP → UvrA UvrA UvrB = helicase (cryptic UvrB helicase) ← uvrC → UvrA·UvrA + excision

UvrA + UvrA ⇌ UvrA·UvrA (ADP+Pi ↔ ATP)

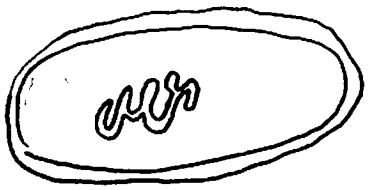
In vitro vs In vivo

- UV irradiate E. coli:
- study protein ↑ over time w/ UV
  - uvrA+B maximize ~2 hrs after UV
  - uvrC doesn't do much

- 60% of damage repaired before max. induction
- synthesis after this point is CAM dependent

- fractionation

	before uv	after UV
cytoplasm	uvrB, uvrC	uvrB
membrane		uvrA, uvrB
DNA	uvrA	uvrA



6-4 Ab
- moves to inner membrane also
-

Repair complex
- recruitment of pol B to complex depends on UvrA, uvrC, recA
- 17 prots
  including NER, tr, gyrase proteins

- DNA membrane fraction does not contain all factors required for NER
  - reqs ribo NTP's
  - sensitive to rif

Supercoiling assay for UvrAB helicase vs ~~TFIIH~~

~~Start to finish~~	E coli	TFIIH
Energy source	ATP, dATP	ATP, dATP
Sensitive to ATPγS	+	+
Req Mg	+	+
Direction	5'-3'	Bidirectional

- RNA polymerase addition enhances UvrAB supercoiling
- suggests RNA pol Δ's DNA structure
- nicking occurs on strand opposite where uvrAB bind