Biomarkers of exposure to environmental and endogenous leukaemogens

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The Open University
Ring Road North
Sports Pavilion
North Parking
Childrens Centre
Wimpey 3
Wimpey 2
Wimpey 1
North Lane
R.A.F. Hut
North Spur
Offices 6
Pentz
Meacham
Briggs
Gardiner 2
Gardiner 1
Science Preparation Laboratories
M Block
Shop
Bank
Mathematics
North
Venables Building
West
East
South
South West
South East
Earth Sciences
K Block
F Block
E Block
D Block
C Blk
B Block
A Block
Wilson Building
H Block
Chambers Building
S & T Workshop
East Temporary Building
Inner East Parking
Outer East Parking
East Parking
East Lane
Ring Road East
Ring Road West
Outer West Parking
Inner West Parking
Horlock
Crowther
West
North
South
Building
Jennie Lee Library
Walton Hall
Christodoulou Rooms
Refectory
Perry Building
East Perry Building
BBC Production Centre
Berrill Building
Reception
Parking
Visitors
V10 Brickhill Street
Walton Drive
to Reception
Bus stops
St Michael's Church
Church Parking
South Entrance
South Entrance Parking
Groveway H9

Introduction

- Most cancers are preventable
- DNA damage appears to be involved in carcinogenesis
- How can we put these two facts together to make progress in the battle against cancer?

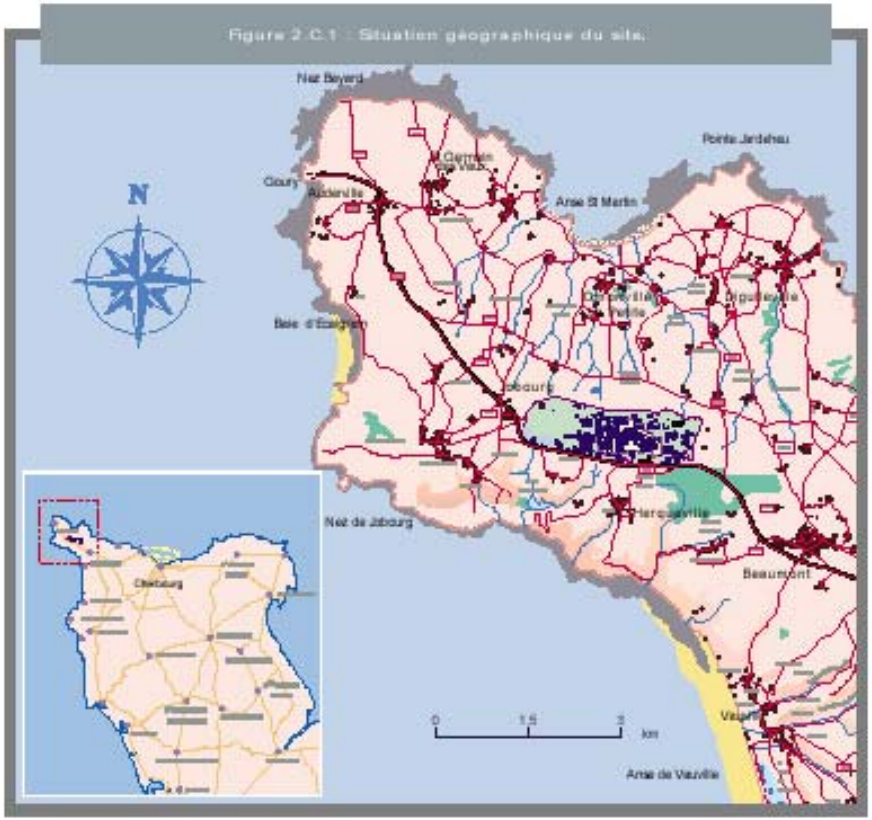


Photo 2.C.1 : Paysage maritime

Photo 2.C.2 : Paysage rural et industriel

“Nobody wants dirt on their DNA”

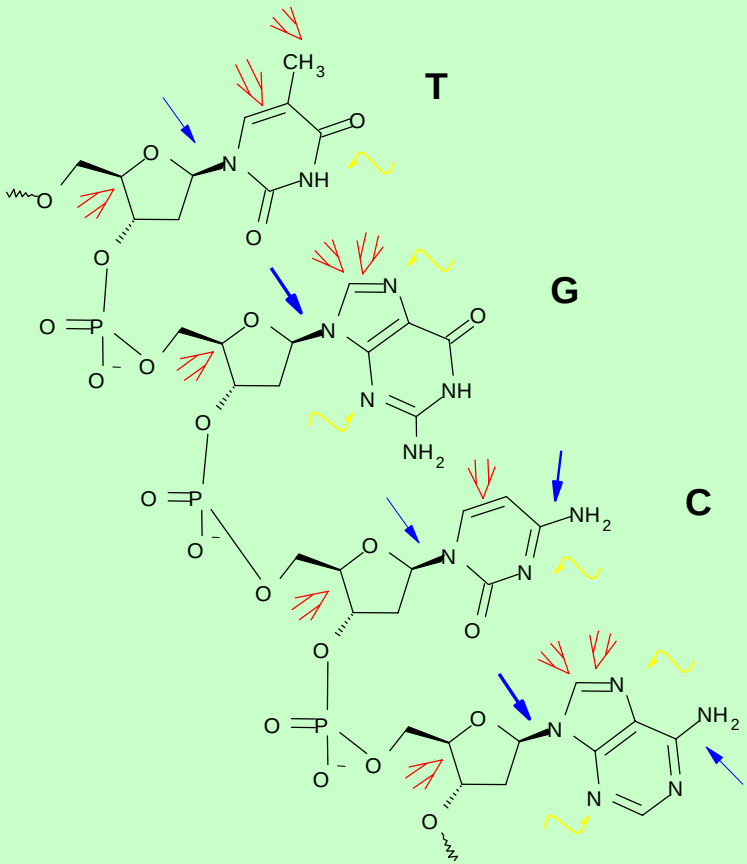
Anon.

Stability of DNA

- “Although DNA is the carrier of genetic information, it has limited stability”
- “All biological macromolecules spontaneously decompose”

Lindahl, *Nature*, 1993, *362*, 709

Chemical instability of DNA

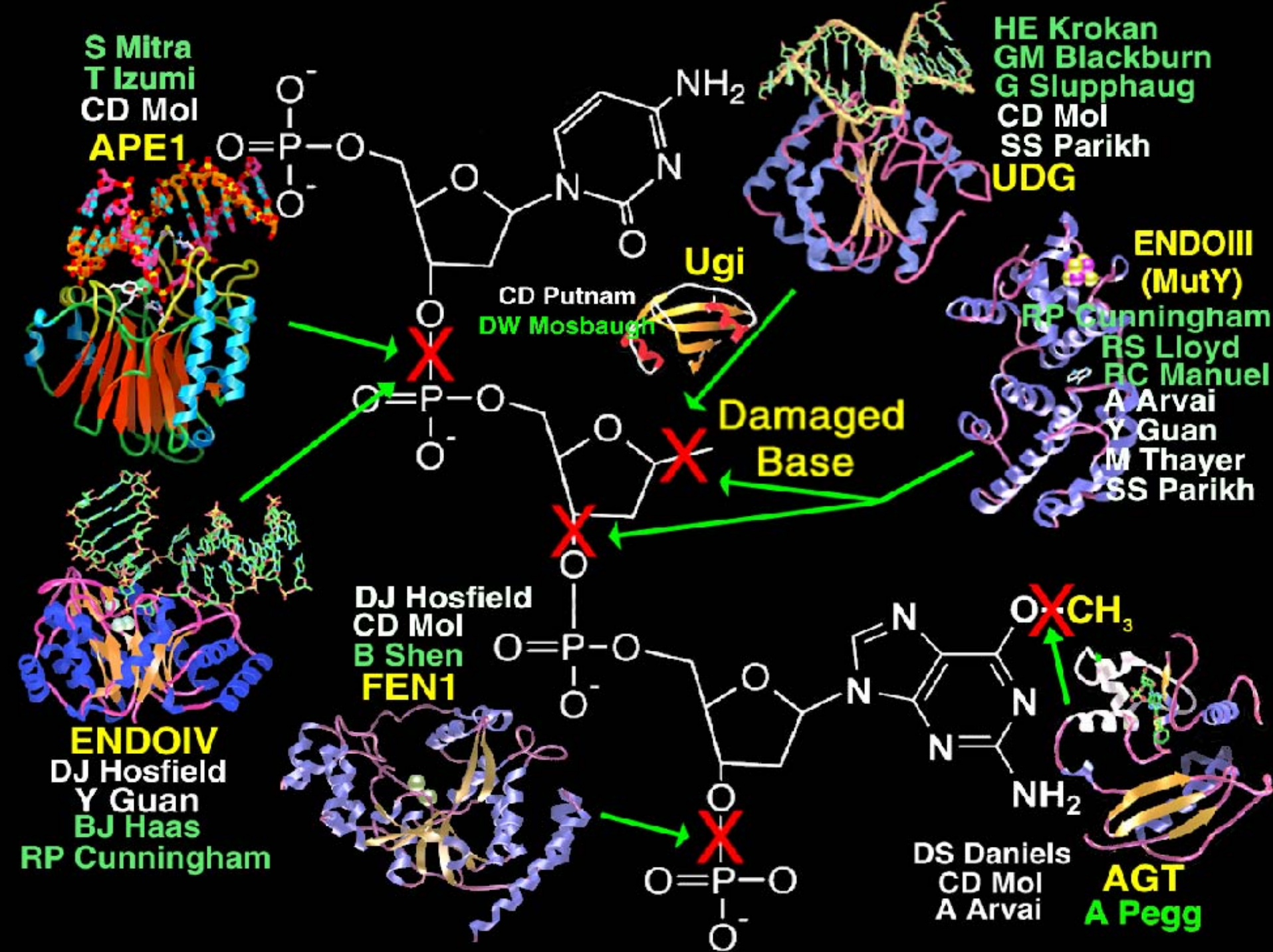


Hydrolytic attack

Oxidative damage

Non-enzymatic methylation by S-adenosyl methionine

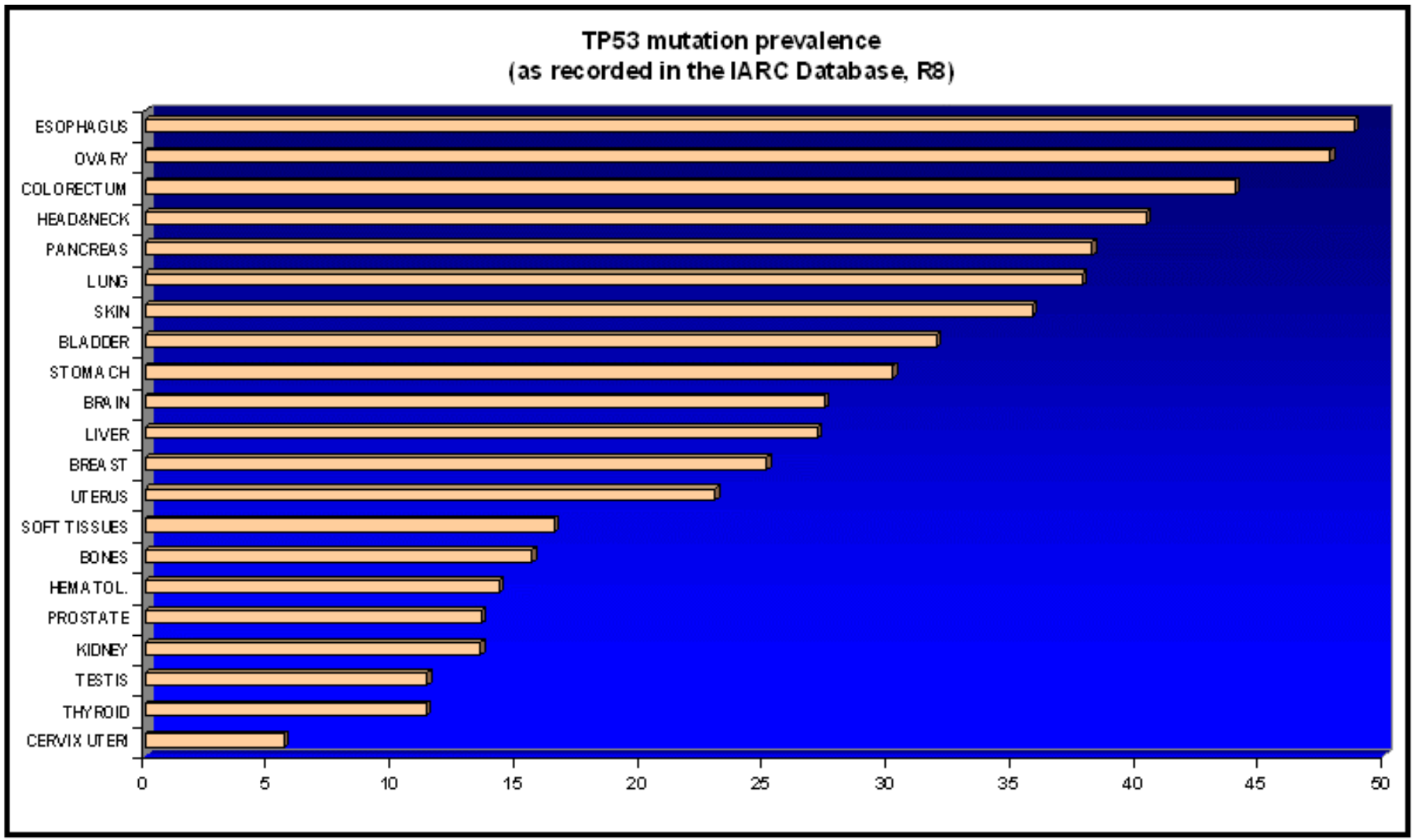
from Lindahl, 1993


S Mitra
T Izumi
CD Mol
APE1
HE Krokan
GM Blackburn
G Slupphaug
CD Mol
SS Parikh
UDG
Ugi
CD Putnam
DW Mosbaugh
ENDOIII
(MutY)
RP Cunningham
RS Lloyd
RC Manuel
A Arvai
Y Guan
M Thayer
SS Parikh
Damaged
Base
DJ Hosfield
CD Mol
B Shen
FEN1
ENDOIV
DJ Hosfield
Y Guan
BJ Haas
RP Cunningham
DS Daniels
CD Mol
A Arvai
AGT
A Pegg

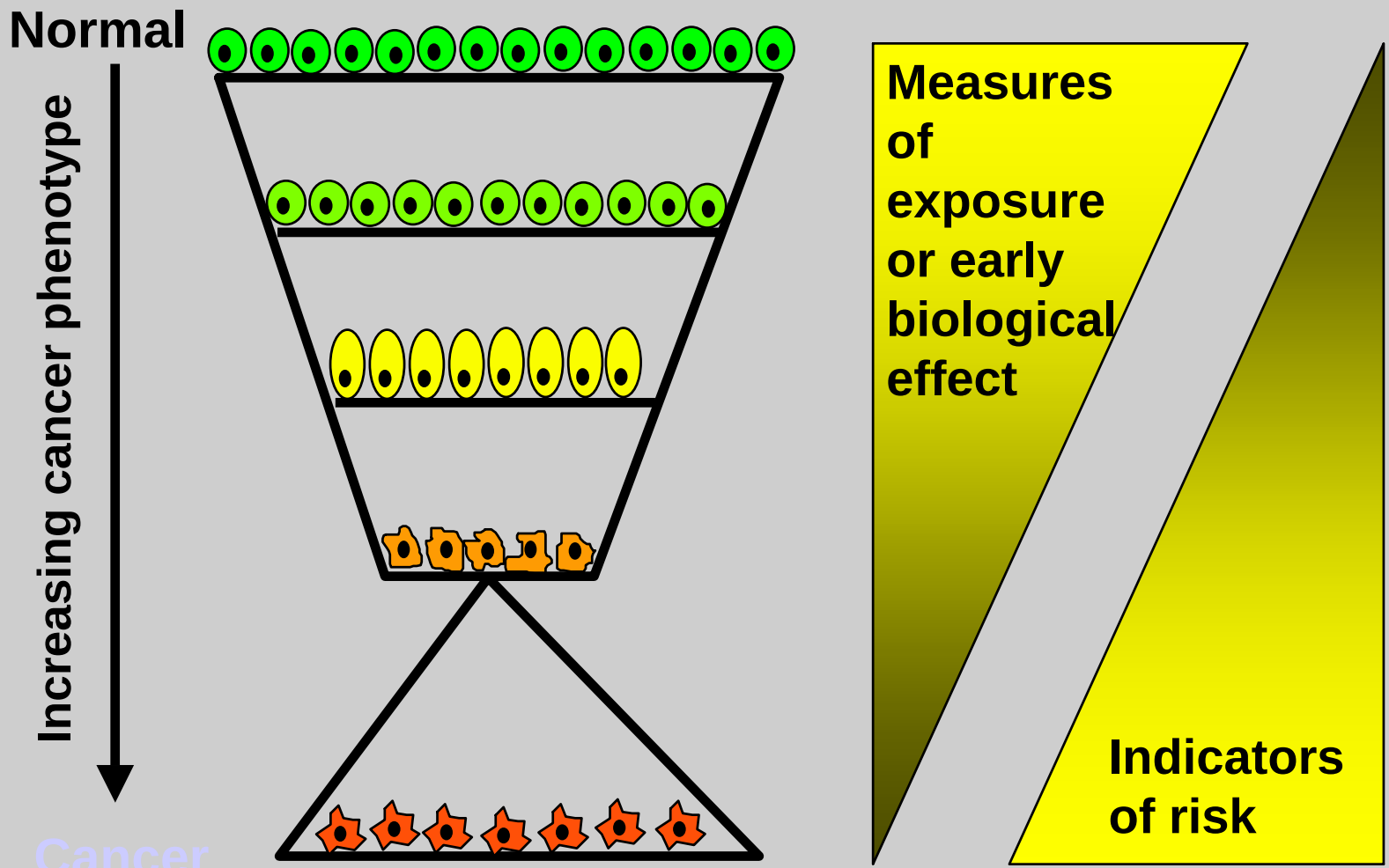
How relevant is DNA damage to cancer?

- The human genome is subject to thousands of damage events per day per cell
- There are many reactive intermediates arising from cellular physiology (e.g., ROS, RNS)
- Potent evidence for the importance of DNA damage in carcinogenesis comes from the observation that the p53 gene is mutated in up to 50% of human cancers

Prevalence of p53 mutations



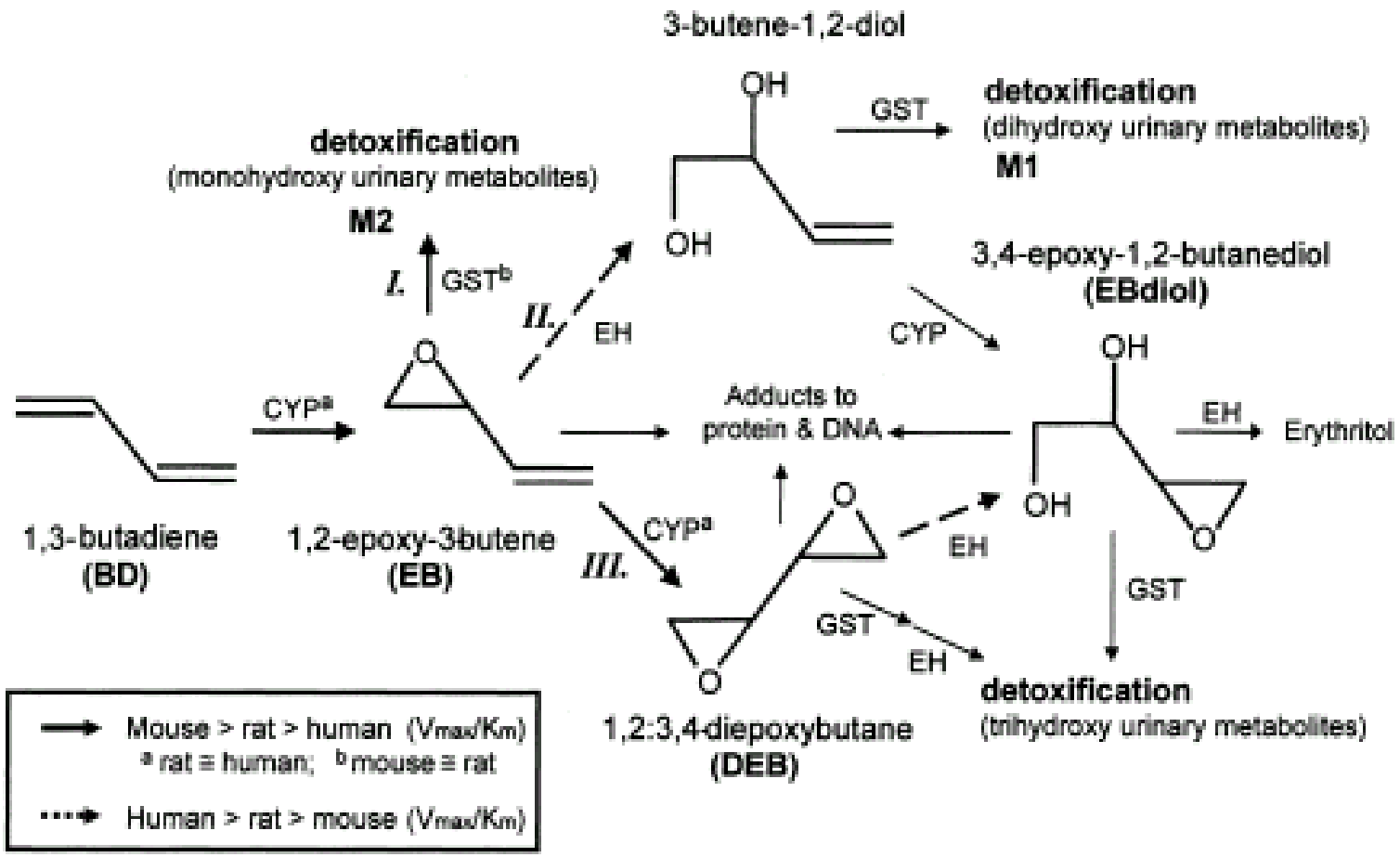
Biomarkers of exposure and risk



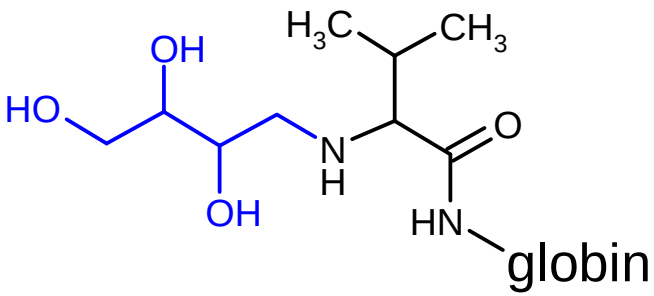
1,3-Butadiene

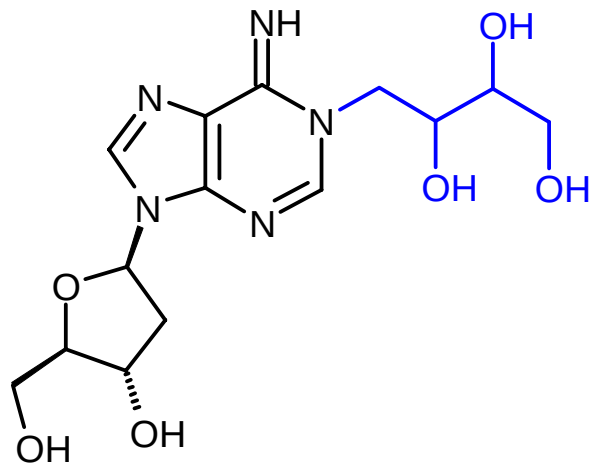
- Evidence of the carcinogenic hazard of 1,3-butadiene comes from two main sources: firstly, studies of human populations exposed in the workplace and, secondly, from investigations carried out in laboratory animals
- In 1999 an IARC working group concluded that 1,3-butadiene was probably a human carcinogen (group 2A)
- In the absence of compelling human epidemiology that fact that 1,3-butadiene displayed a similar metabolic pattern in experimental animals as humans and that both DNA and protein adducts were detected in exposed humans provided key pieces of evidence to support the evaluation

1,3-Butadiene



DNA and protein adducts





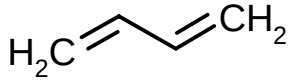
1,3-butadiene forms characteristic adducts with both globin and DNA

1,3-Butadiene

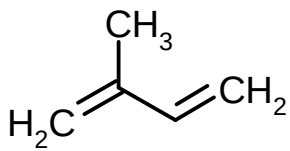
- In the UK an environmental air standard for 1,3-butadiene has been set at 1 ppb using an NOAEL of 1,000 ppb
- DNA adducts to 1,3-butadiene can be detected at >1,000 ppb but the relationship between adducts and biological effect remains unclear
- However, detection of 1,3-butadiene adducts at levels of <10 ppb is difficult

Isoprene - a possible endogenous exposure

- Isoprene is a ubiquitous natural precursor to many important biological molecules and is structurally very similar to 1,3-butadiene and undergoes similar metabolism
- Humans exhale isoprene



1,3-butadiene

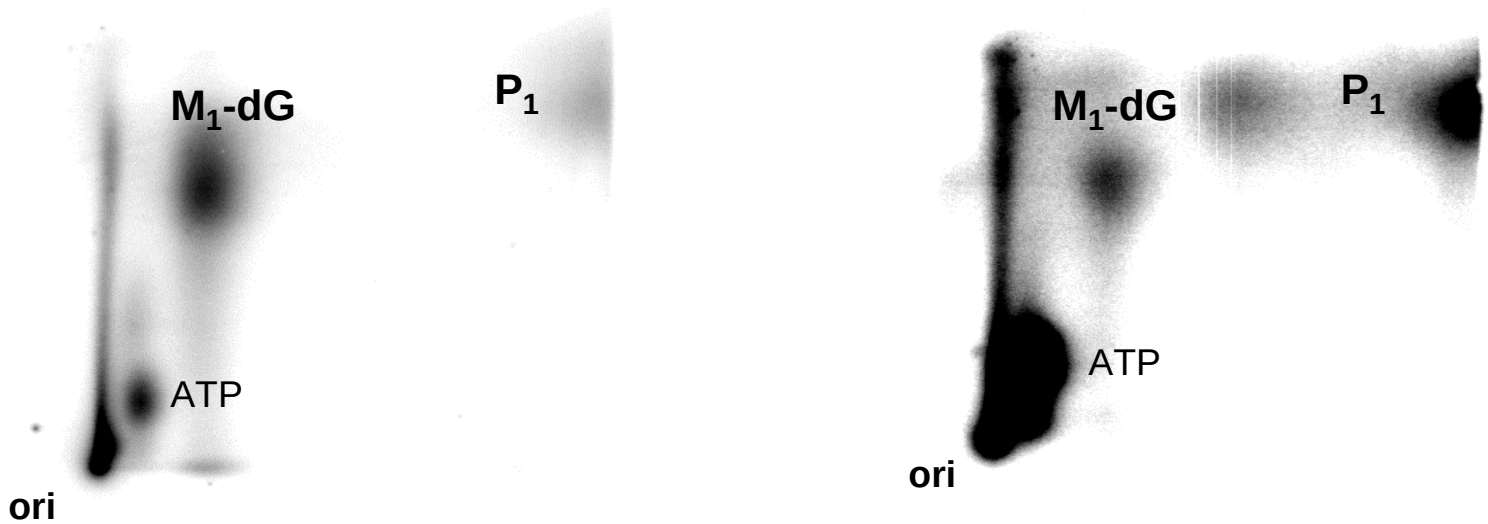


Isoprene

Increasing sensitivity

- The ^{32}P-postlabelling assay developed by Randerath and Randerath and colleagues revolutionised the detection of DNA damage
- The identity of the adduct(s) did not need to be known *a priori*
- One modified base in a billion (10^9) could be detected
- A number of environmental genotoxicants have been detected (PAH, tobacco smoke)

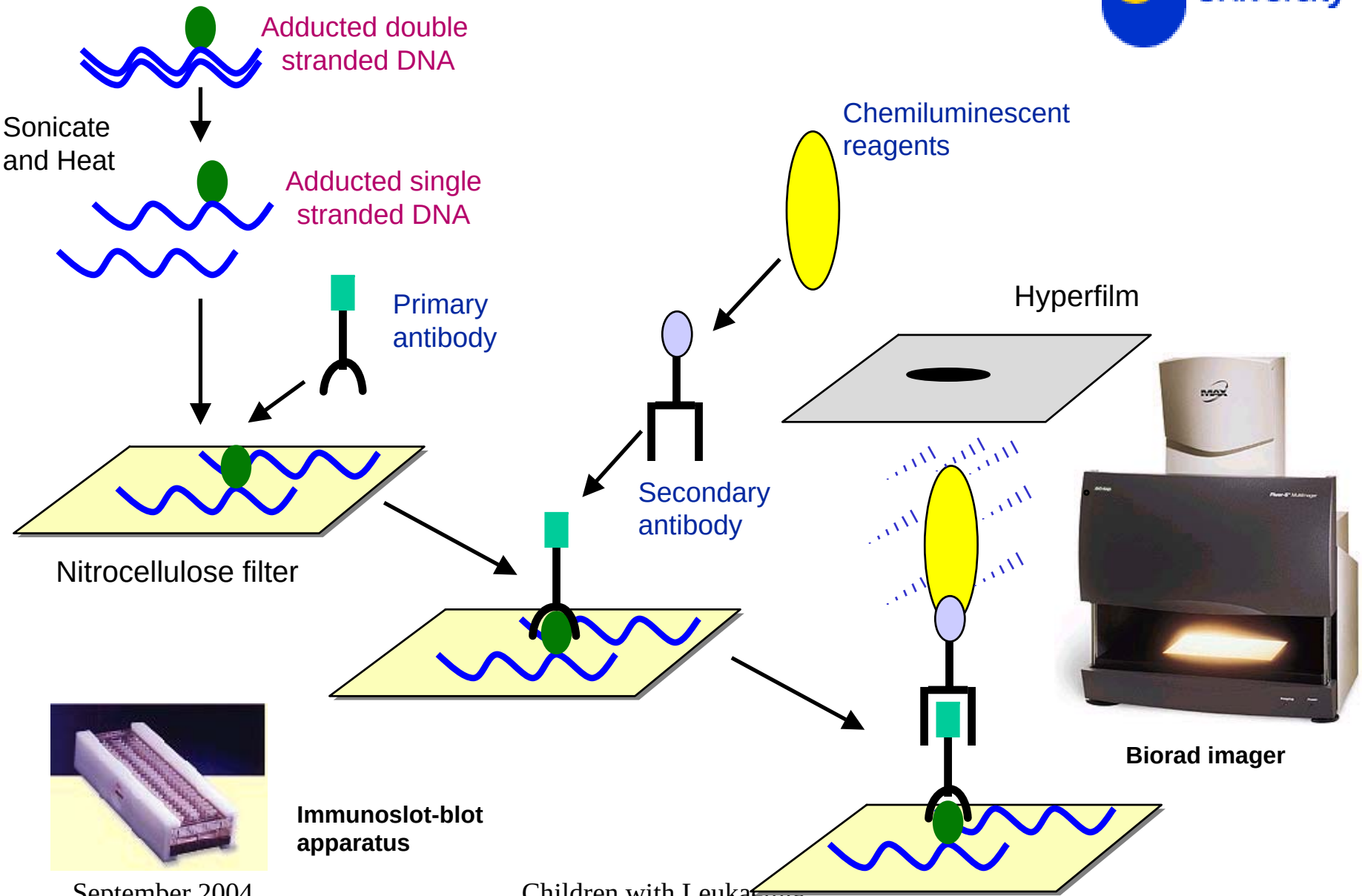
^{32}P-postlabelling



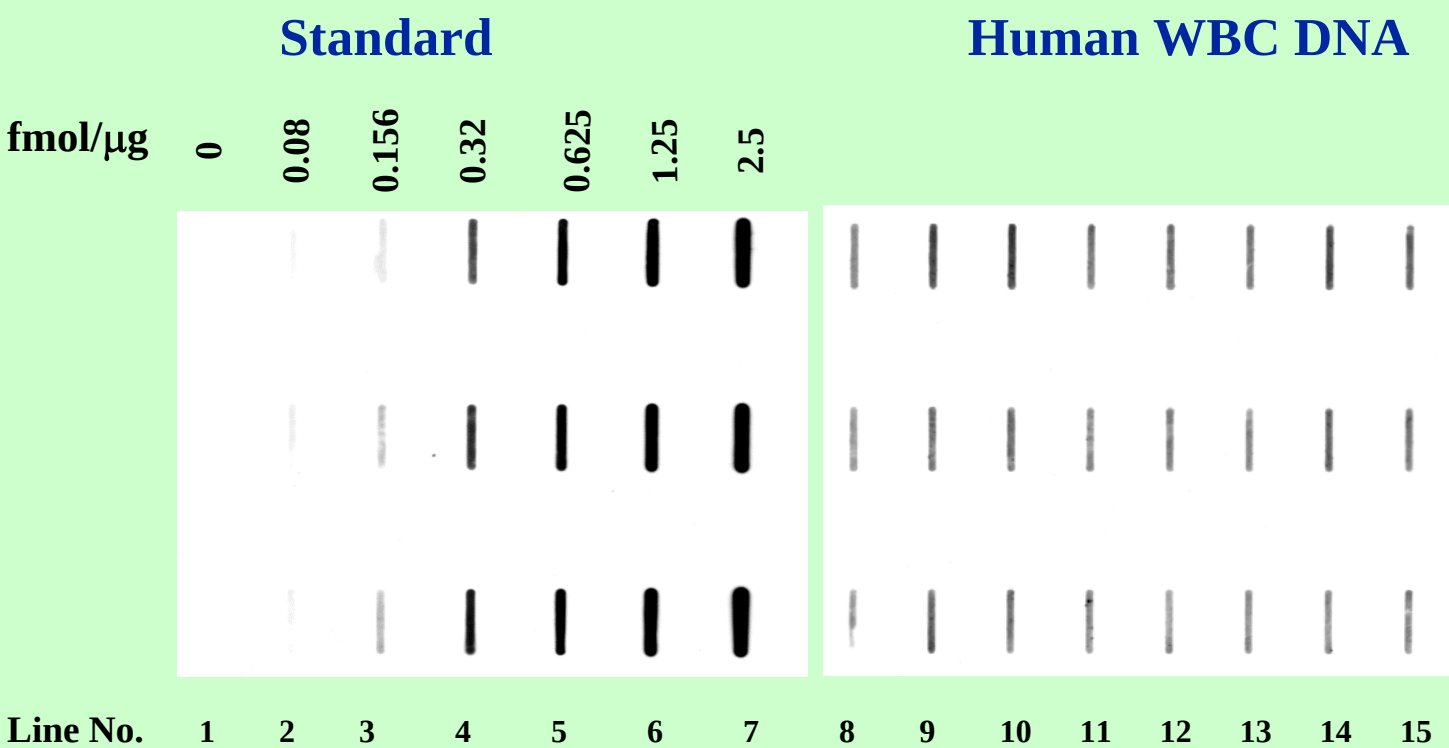
Adducts are separated as 3',5'-diphosphates with ^{32}P introduced into the 5' position

Leuratti et al., 1998

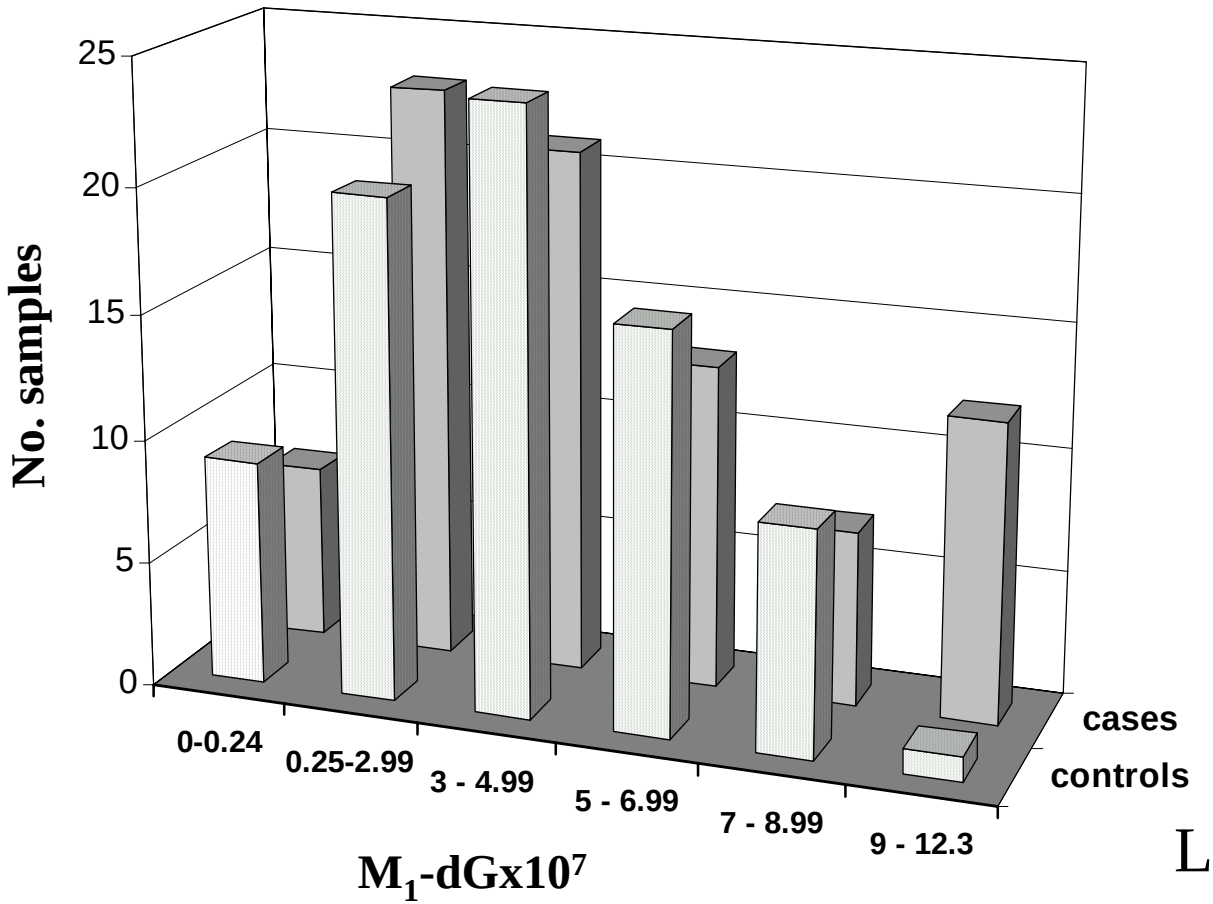
Schematic representation of the immunoslot-blot procedure



M_1dG immunoslot blot



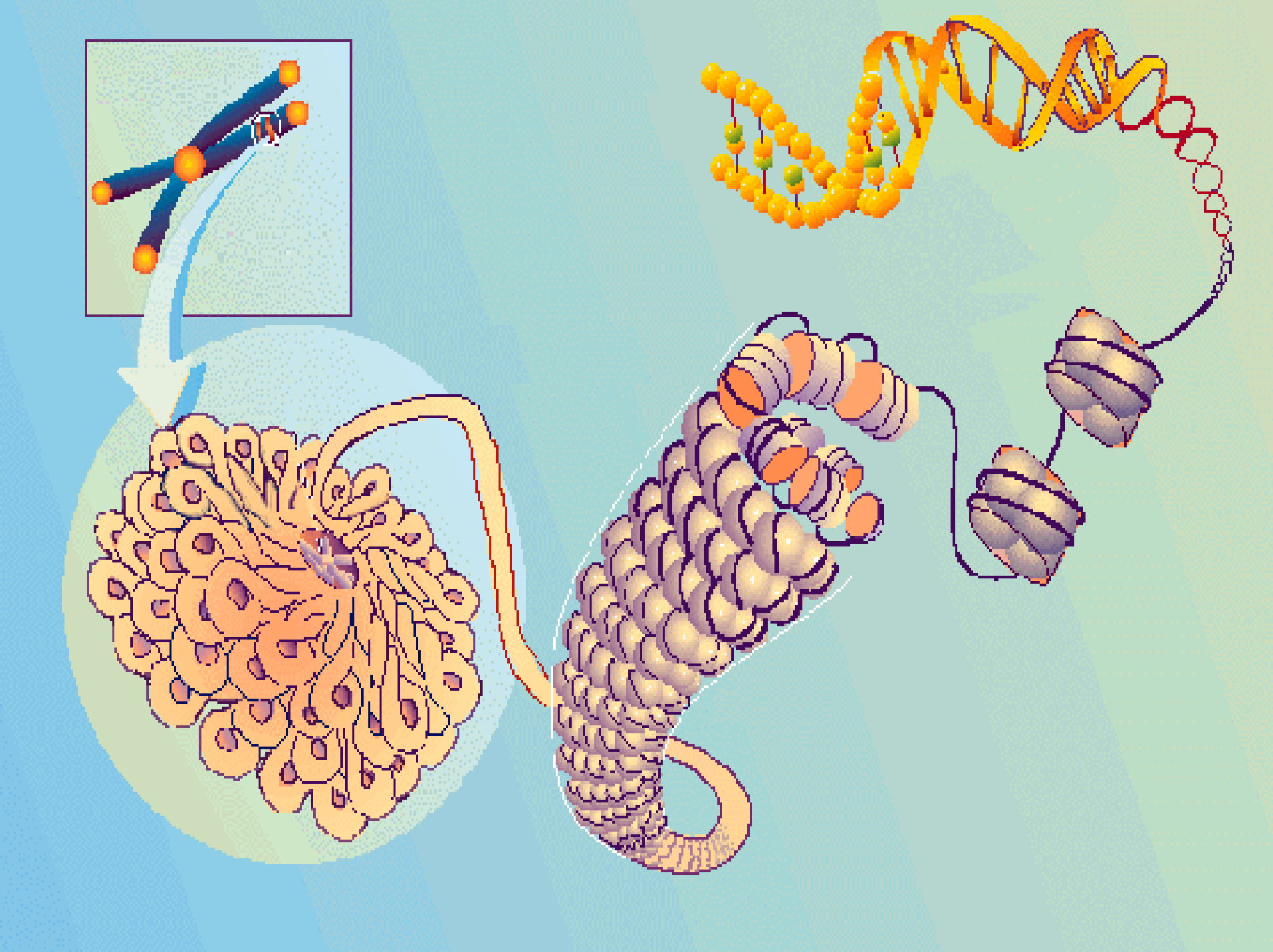
M_1dG in colon DNA



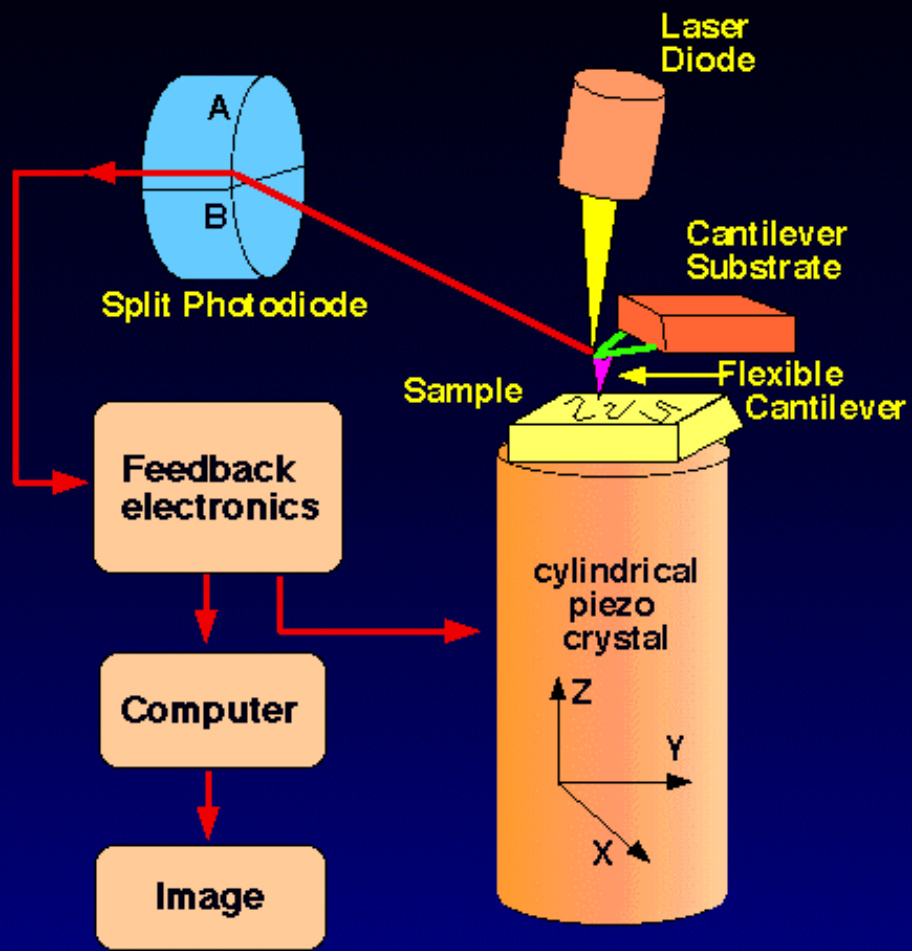
Leuratti *et al.*, *CEBP, 2001*

A new tool for DNA adduct localisation

- Most methods for DNA adduct measurement effectively measure average levels of damage across the genome but p53 results suggest that certain types of adduct are likely to be much more important for cancer risk
- Atomic force microscopy (AFM) has the potential to allow physical localisation of specific types of DNA damage within particular sequences



Atomic Force Microscope Sensing System



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Molecular Imaging Group

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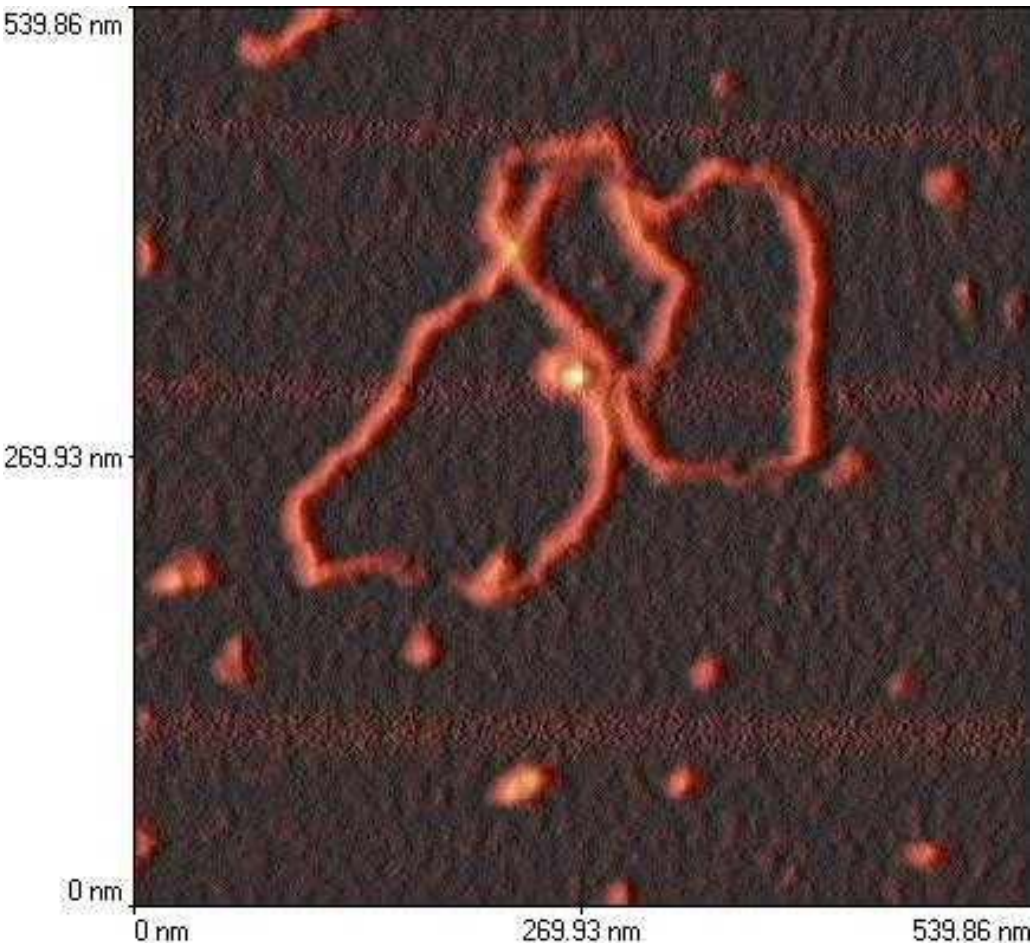
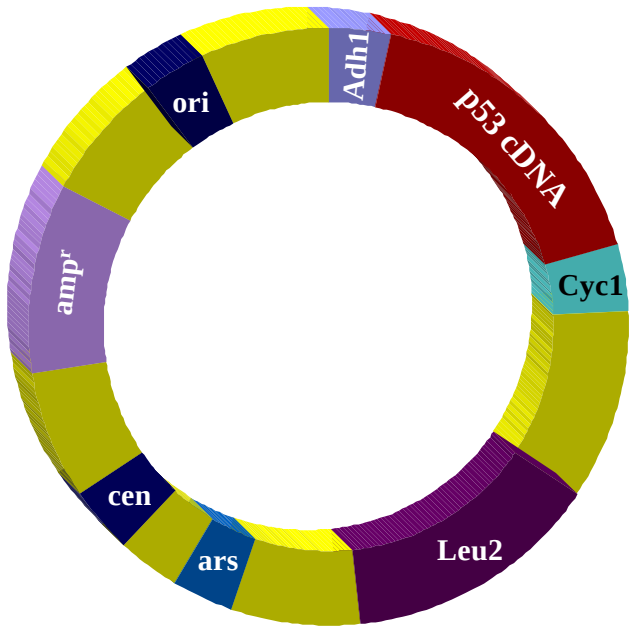
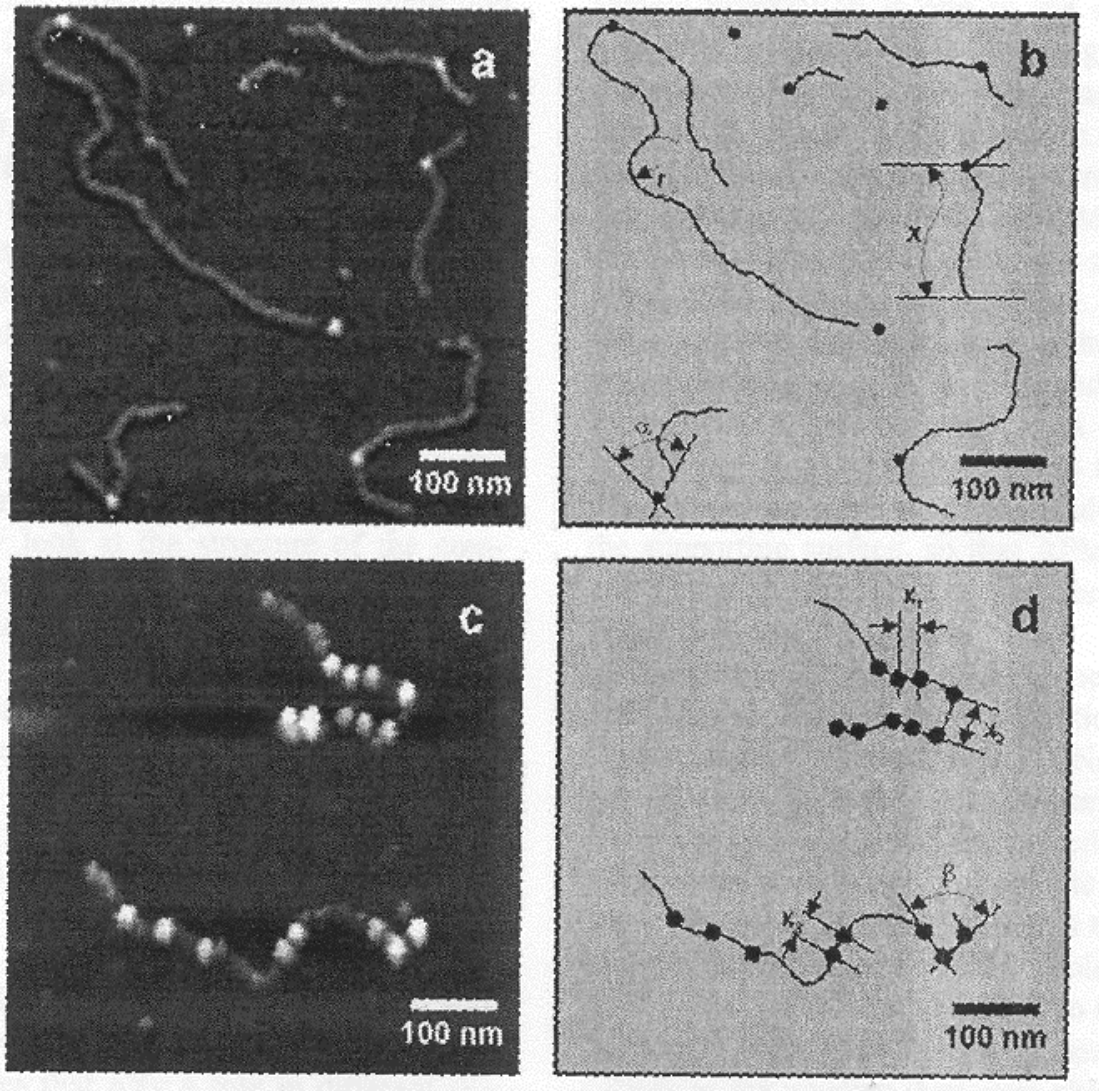
AFM in practice

Visualisation of single DNA molecules





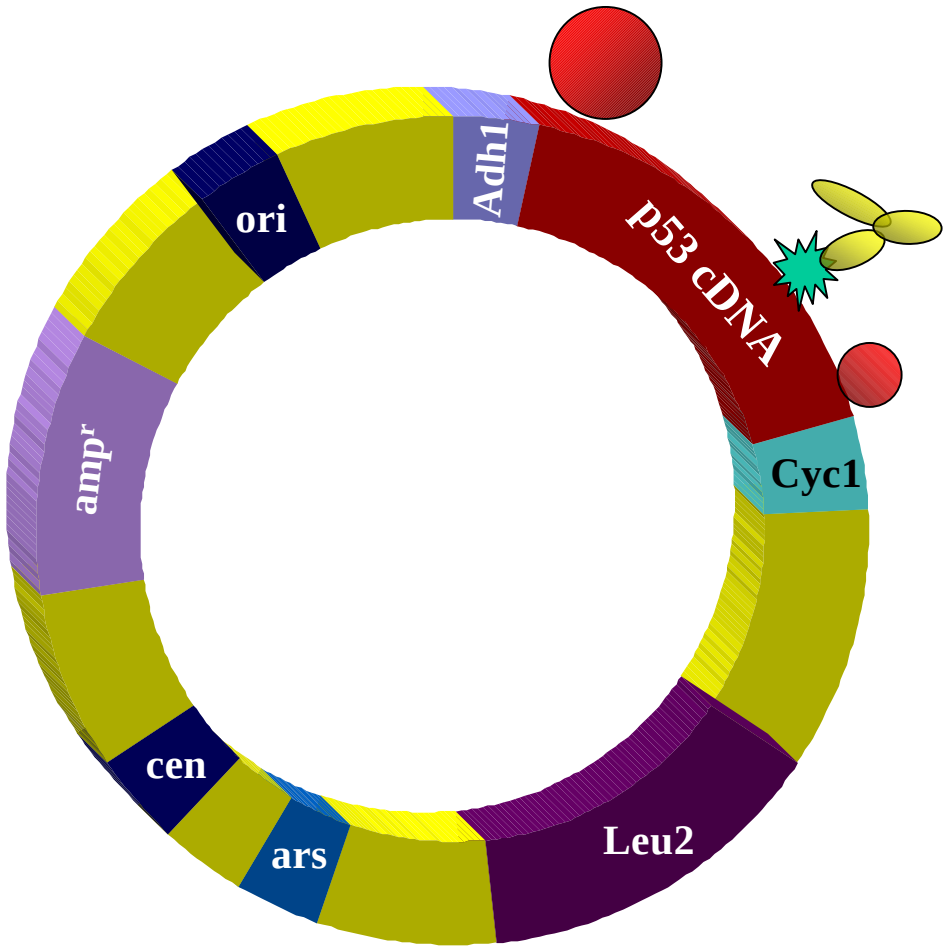
Physical mapping of DNA damage



DNA-protein complexes

Van Noort S.J.T. *et al.*, Nucl. Acids.Res., 1999

Mapping DNA damage



Summary (1)

- There is good evidence that measurement of DNA damage can be a useful indicator not only of exposure but also of risk of disease
- Numerous methods are available for detection of DNA damage at levels relevant to “real” human exposure
- Overall the major risks for adult human cancers come from subtle interactions of exogenous and endogenous factors for which DNA adducts can be informative quantitative markers

Summary (2)

- However, exposures to known leukaemogenic chemicals (such as benzene and butadiene) are low
- Collecting blood and tissue samples from children to extract DNA or proteins for adduct analysis is difficult for good reasons
- Given the rarity of the outcome it seems unlikely that biomarkers of DNA damage are going to be particularly useful in studies of leukaemia in children

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The Open University

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FOOD STANDARDS AGENCY