

Cellular Defenses Against Radiation-Induced Carcinogenesis:

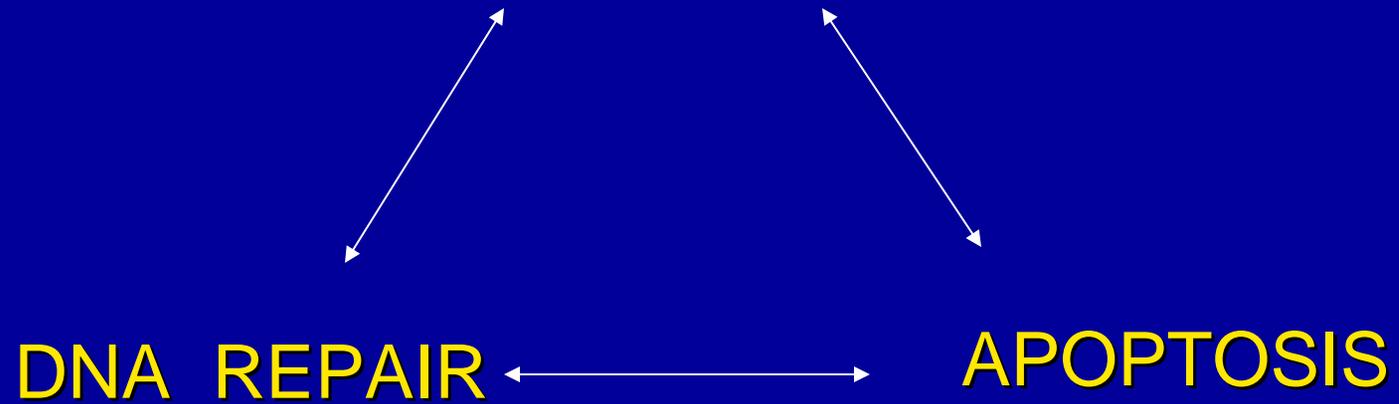
- Cell Cycle Arrest
- DNA Repair
- Apoptosis

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CELL CYCLE ARREST



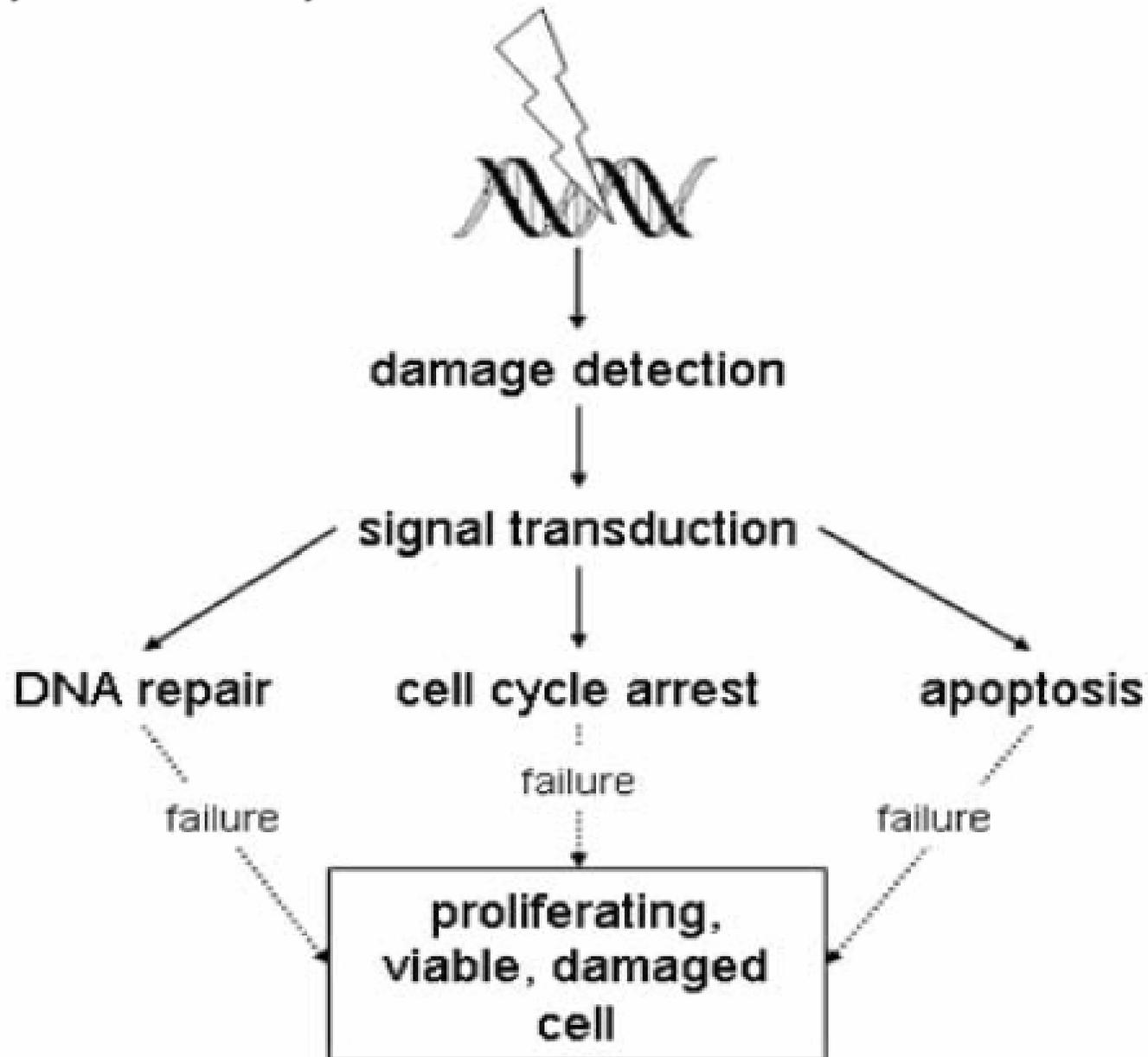
GOALS:

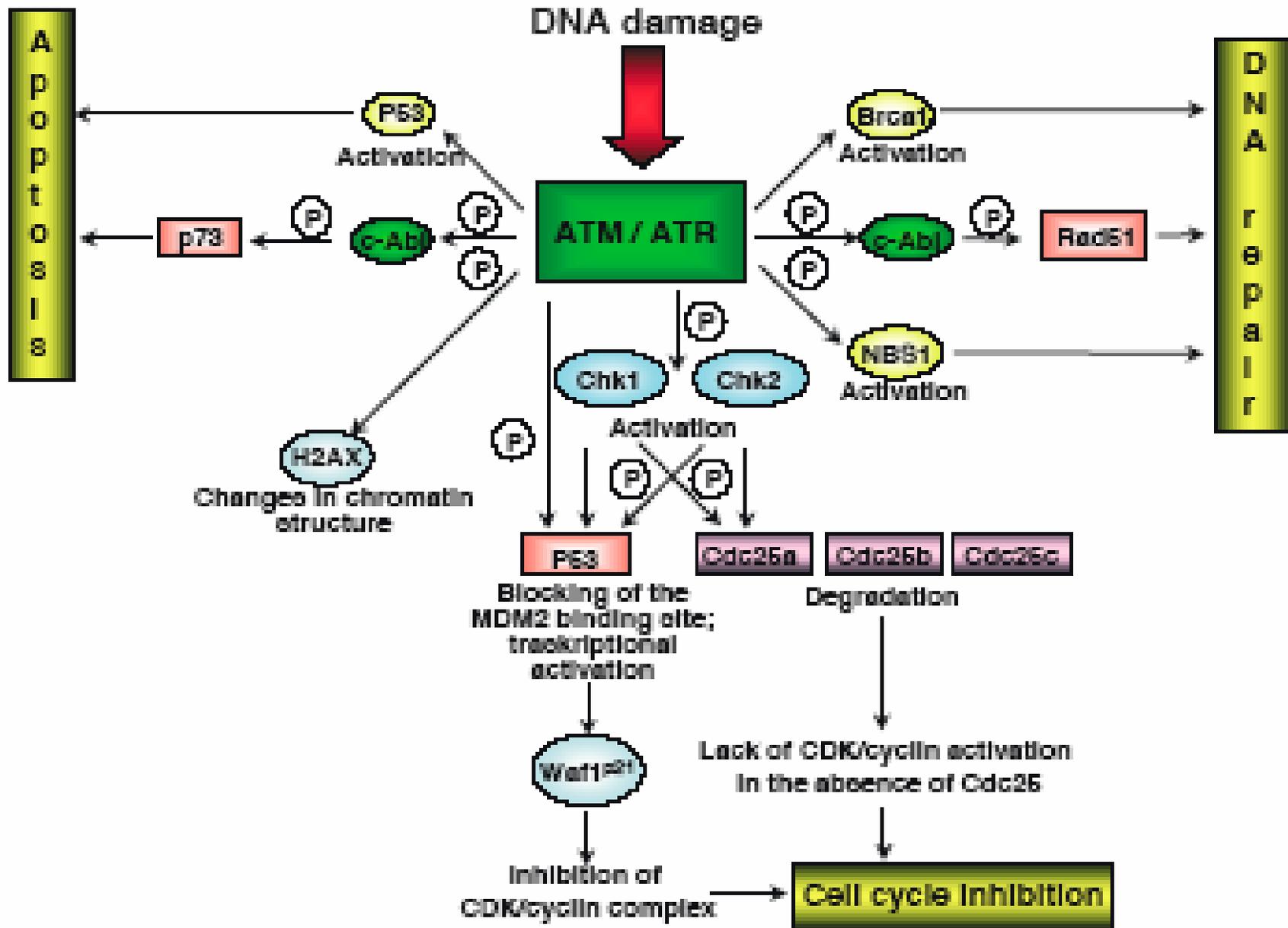
- Provide an overall description of how these three processes work to inhibit transformation.
- Describe how they are mechanistically connected.
- Show how they interact with radiation damage.
- Discuss molecular epidemiology implications for gene-environment interaction studies.
- Review epidemiological biases and confounding issues.

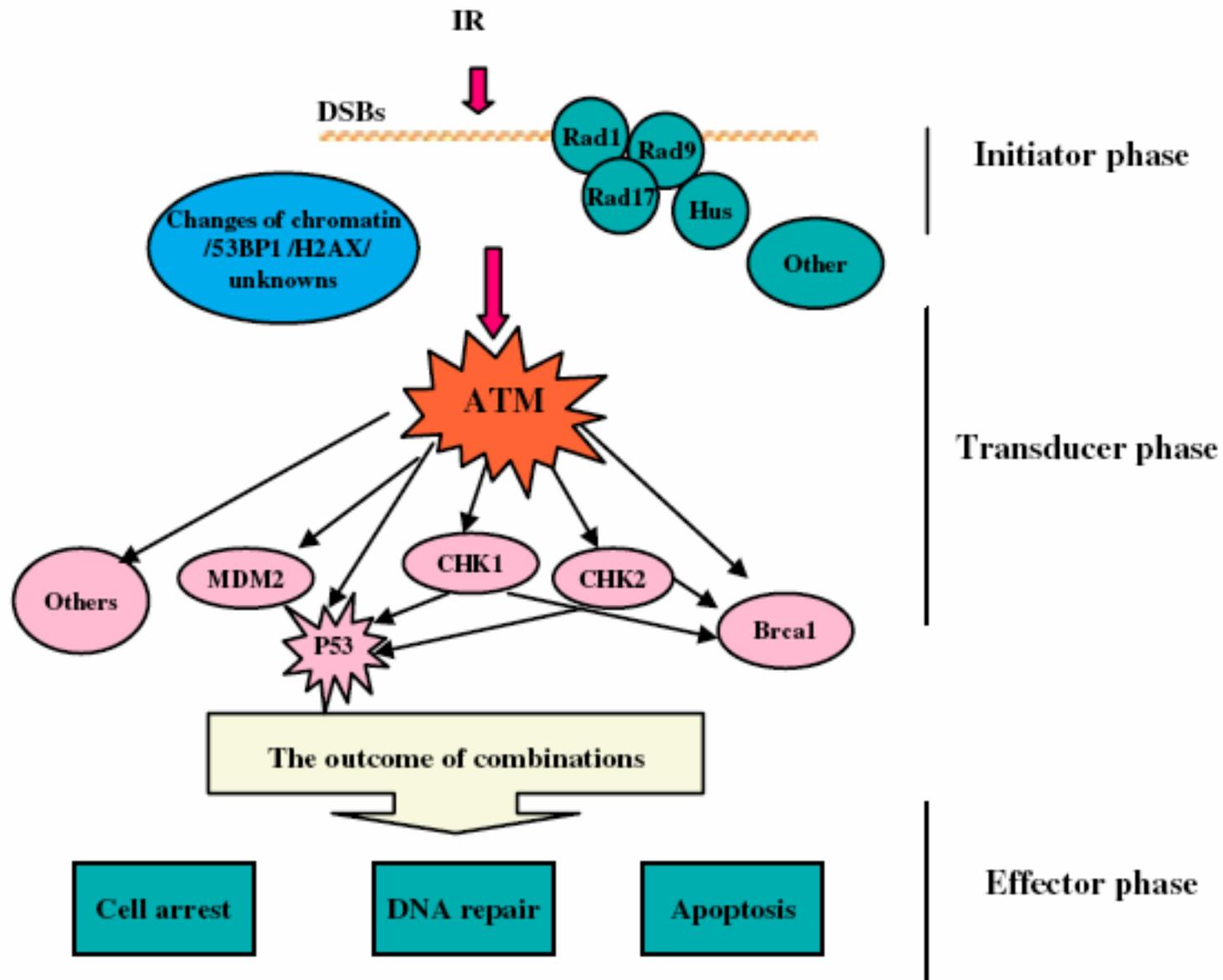
DNA damage is thought to be the primary mechanism by which radiation transforms cells.

Yet, only a small number of cells are actually transformed.

How are most cells protecting themselves from DNA damage-mediated transformation?





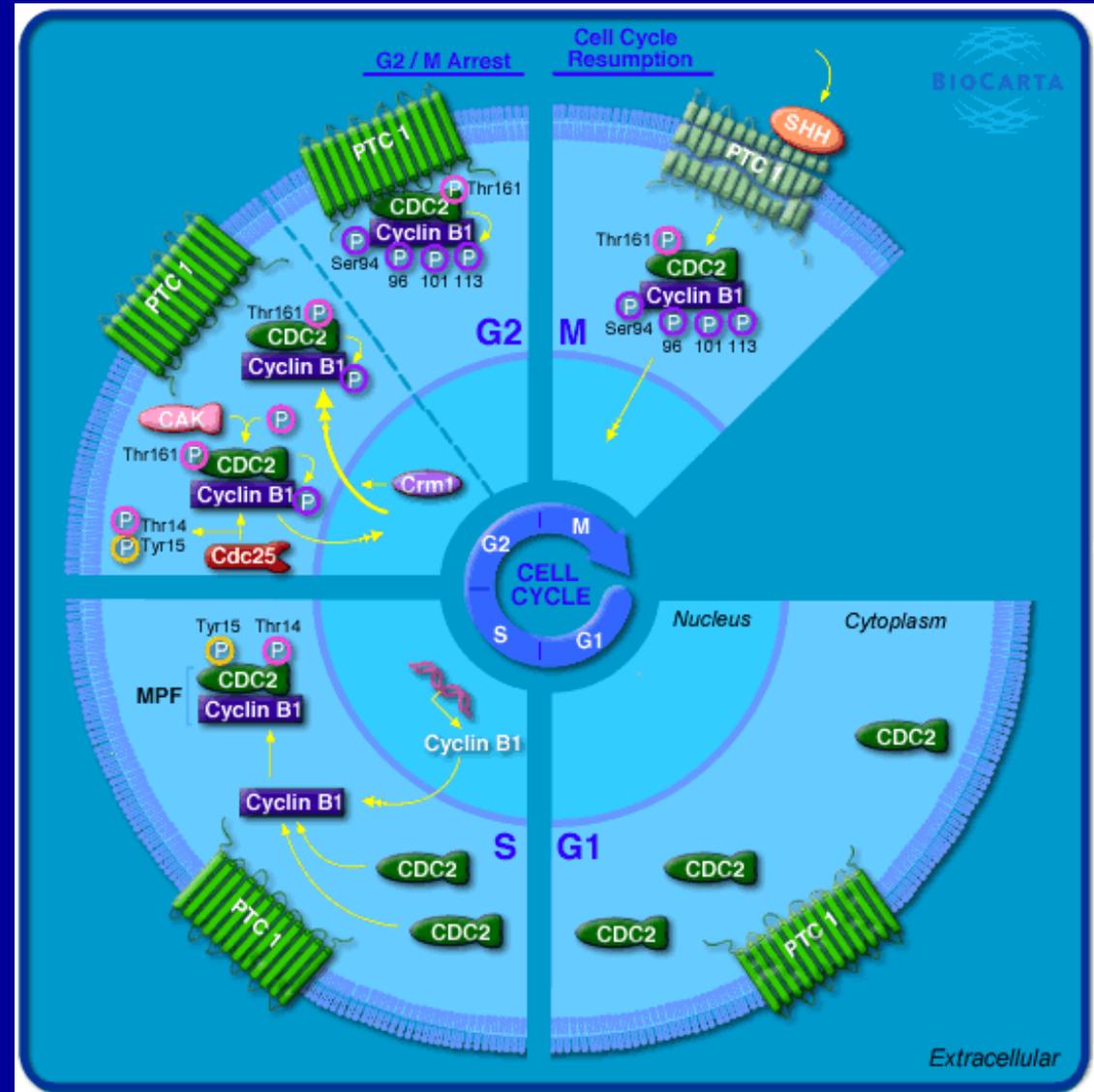


P53 and radiation responses

P Fei and WS El-Deiry

Oncogene (2003) 22, 5774-5783

CELL CYCLE ARREST



Cancer cells “evolve” into a highly unstable phenotype:

Loss of contact inhibition



Loss of anchorage dependence



Tumorogenesis



Metastasis

The ability to undergo successive genetic change suggests that a loss of genetic stability is an early event in carcinogenesis.

Cell cycle control via cell cycle checkpoints, is thought to be a major mechanism by which cells maintain genetic stability.

WHY CHECKPOINTS?

Fidelity of cell division is dependent upon faithful copying and segregation of genetic material, both spatially and temporally. That is, the ordered sequence of specific events is essential to proper execution of the task.

For this reason, cells have developed checkpoints that insure that the previous replication step is complete before the next step begins.

HOW DO CHECKPOINTS WORK?

Checkpoints are governed by phosphorylation activity of a group of proteins called CDK (cyclin dependent kinases).

The CDKs are active only in complexes that contain at least one other protein, called a “cyclin”.

Changes in the cyclin and kinase components of the complexes are the “switches” that control and regulate progression through the cell cycle.

In this model, a cohort of proteins required for progression of a particular phase are activated (or inactivated) by phosphorylation of the cyclin/CDK complexes.

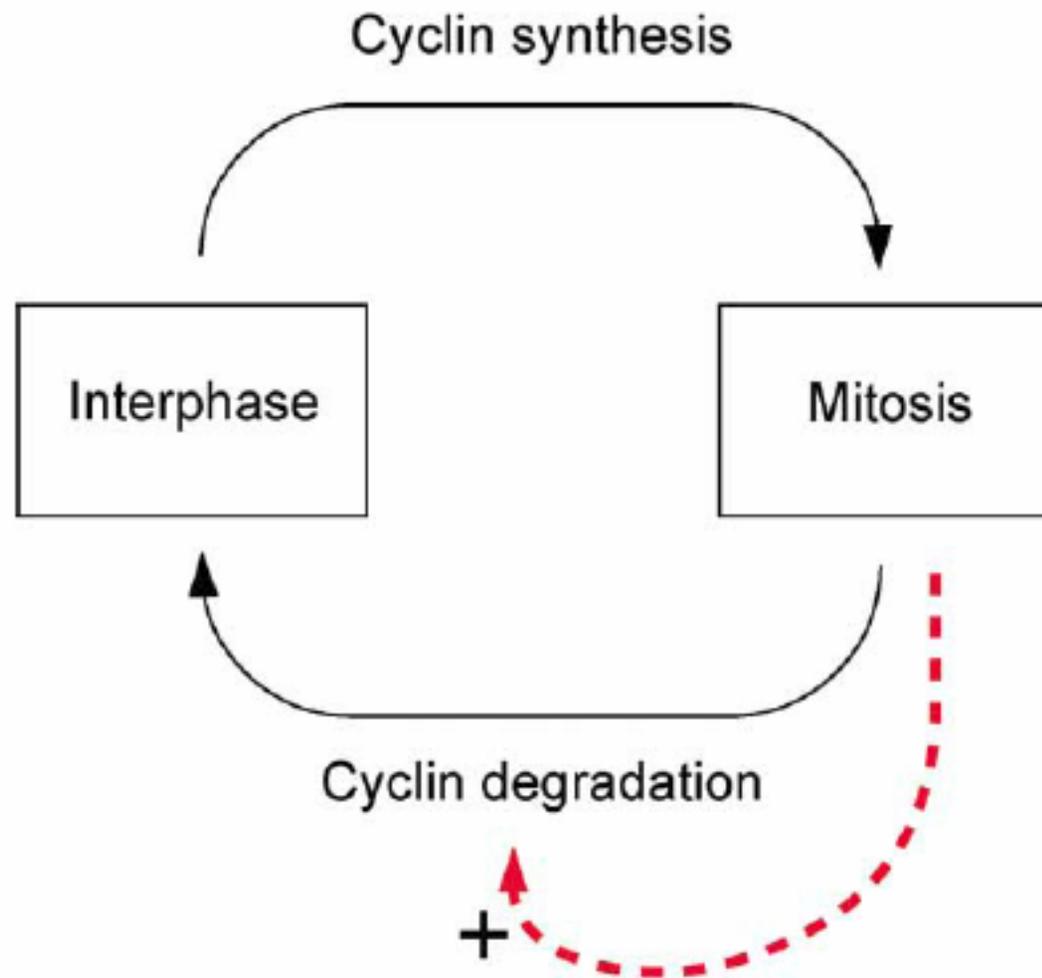
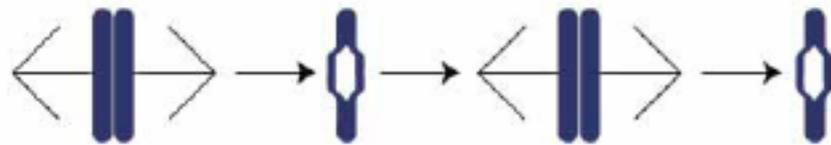


Figure 1. The Original Cyclin-Based Model for the Cell Cycle
The simplest possible model for the cell cycle based on the discovery of cyclin. See text for details.

A

Early Embryonic Cycle



Standard Cycle

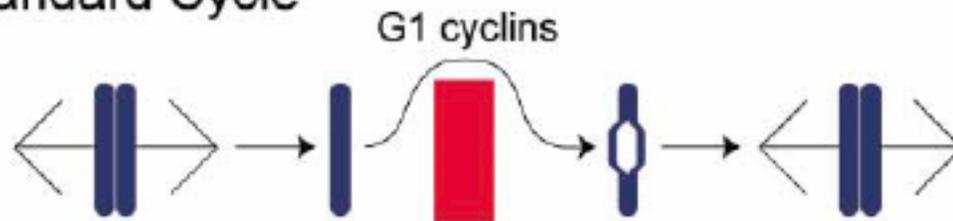


Figure 3. G1 Cyclins Overcome Inhibitors of Cell Cycle Progression
(A) In early embryonic cell cycles, DNA replication begins as soon as cells leave mitosis. In most cell cycles, however, the combination of anaphase promoting factor activity and Cdk inhibitors ensures that cells spend appreciable time in G1 and require the synthesis of G1 cyclins that overcome these inhibitory factors.

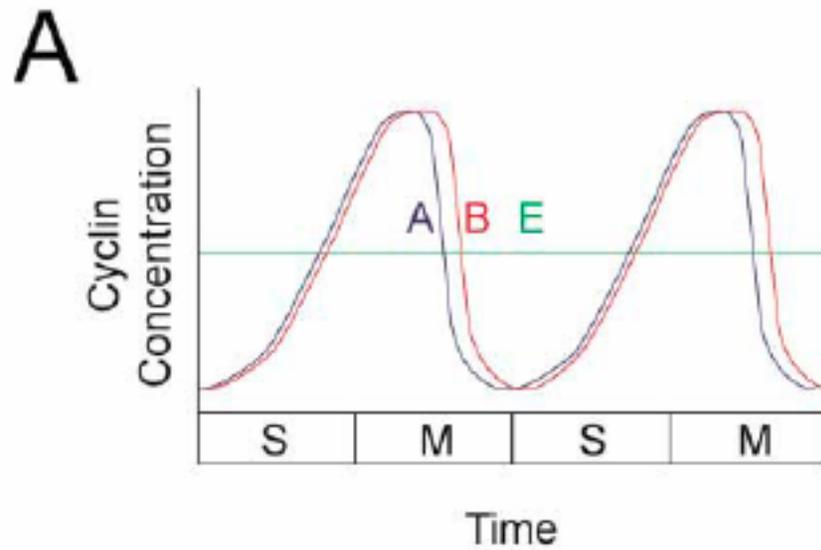
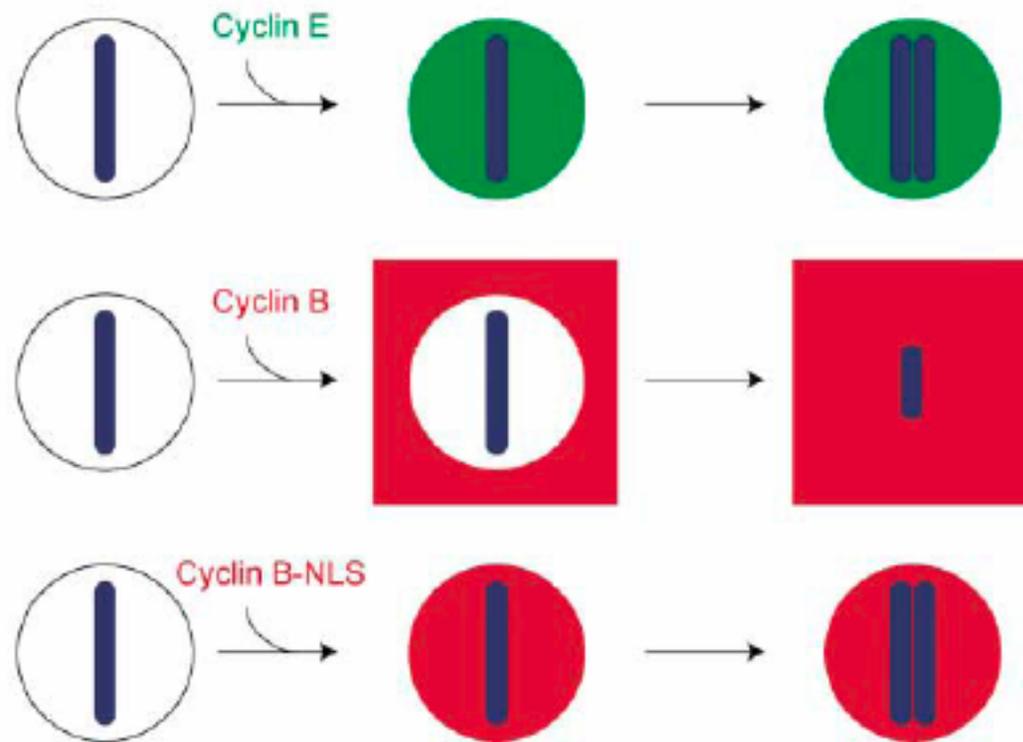


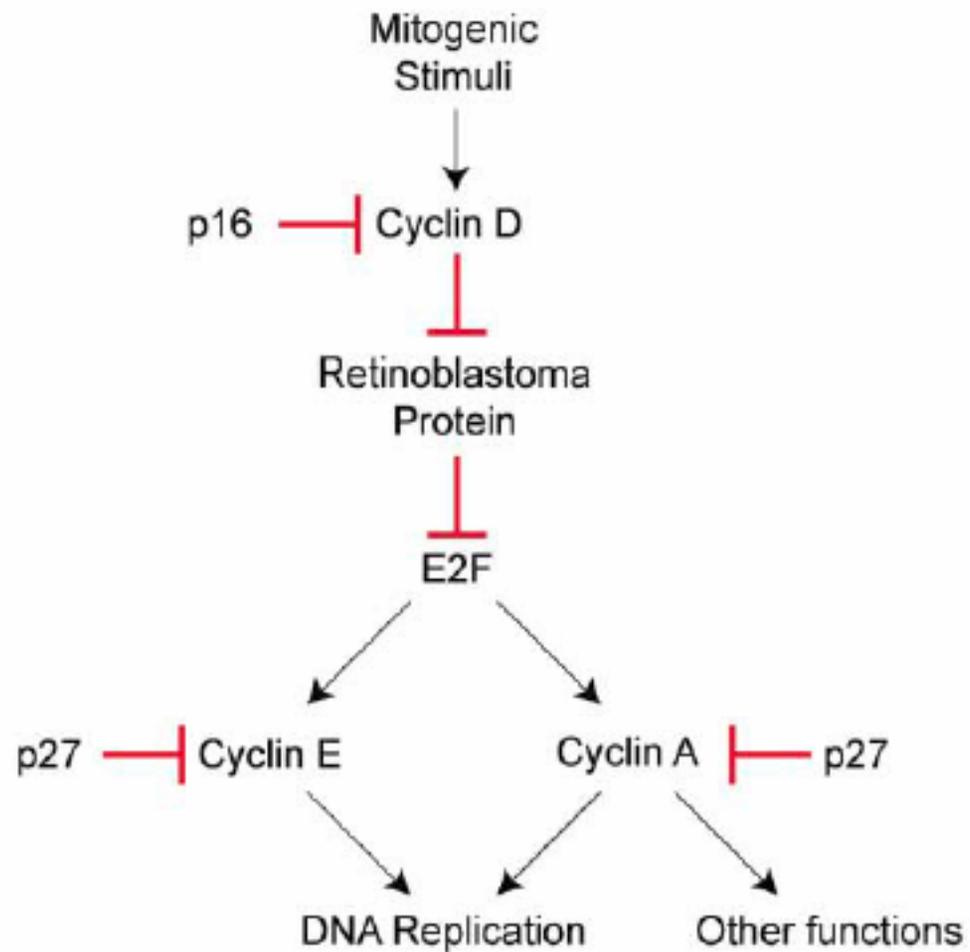
Figure 2. The Activities of Cyclins Are Determined by Their Location
(A) A cartoon of the abundance of cyclins A, B, and E during the early embryonic cell cycles of frogs.

B



(B) The consequences of adding different cyclins to a cell cycle extract depleted of cyclins A, B, and E. The nucleus is shown as a circle containing a single chromosome. Cyclin E enters the nucleus and induces DNA replication, but wild-type cyclin B fails to do so. Adding a nuclear localization sequence to cyclin B allows it to enter the nucleus and induce DNA replication.

B



(B) The relationship between G1 (cyclin D) and S phase cyclins (A and E), growth factors, and Cdk inhibitors in animal cells. See text for details.

In yeast, only a single CDK is used by a sequence of different cyclins that are briefly transcribed and then quickly degraded at specific points in the cell cycle. The cyclin is, therefore, the important regulatory component determining the specificity of the CDK.

In mammalian cells, multiple CDKs appear to be involved:

CDK4 functions early (in response to growth factors)

CDK2 is required to start DNA replication

CDC2 is essential for mitosis

Cyclin

D

E and/or A

A and B

Cyclin/CDK complexes seem to be regulated by a variety of feedback mechanism, both positive and negative, that include:

- Transcription of cyclin
- Degradation of cyclin
- Phosphorylation of CDKs

Negative feedback occurs during development, differentiation, and senescence. It probably acts to stop cell cycle progression when the integrity of the genome has been compromised for some reason.

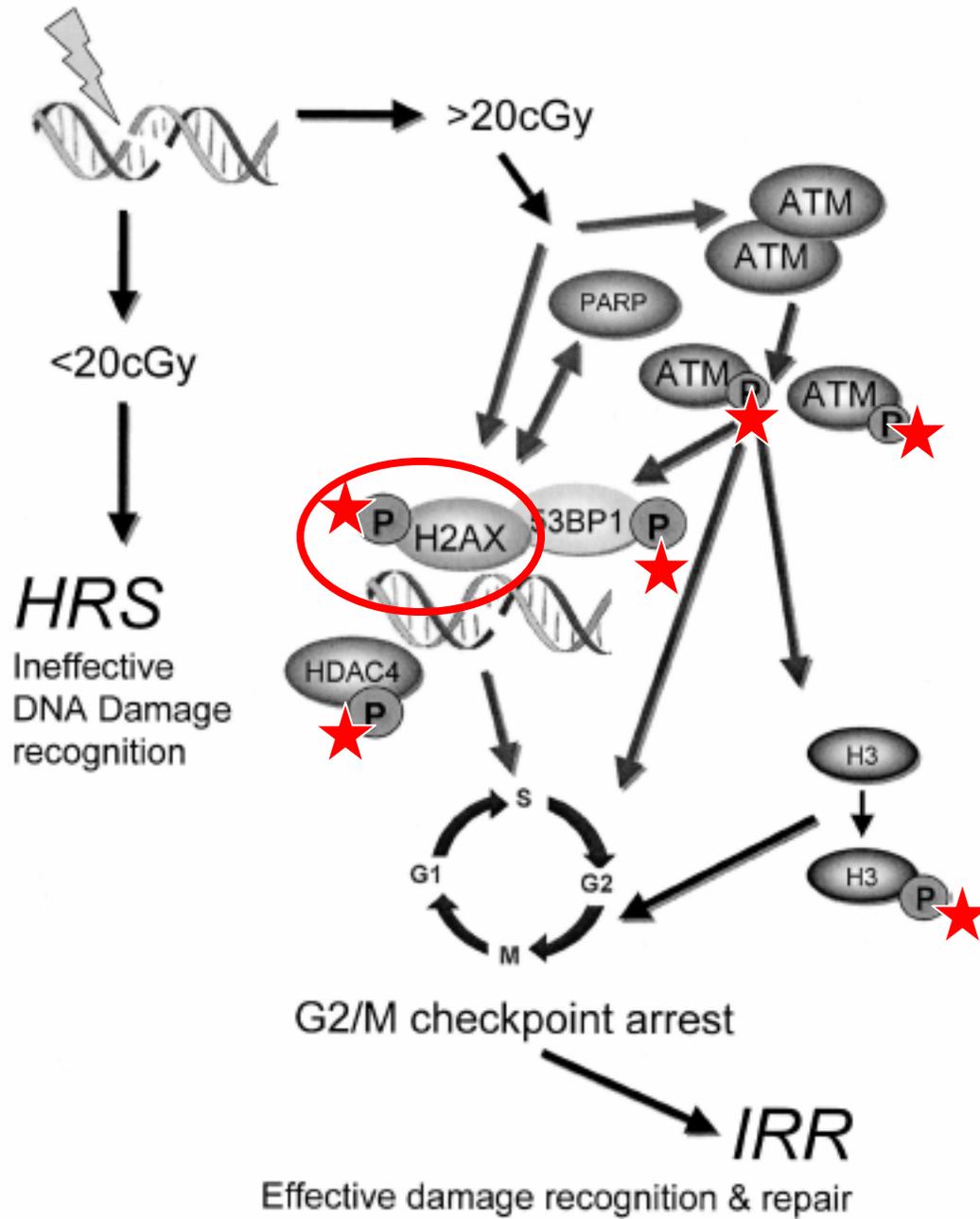
WHAT EFFECT DOES DNA DAMAGE HAVE?

A major challenge to genetic integrity is physical damage to DNA, and it appears that cells have developed strong negative feedbacks in response to DNA damage.

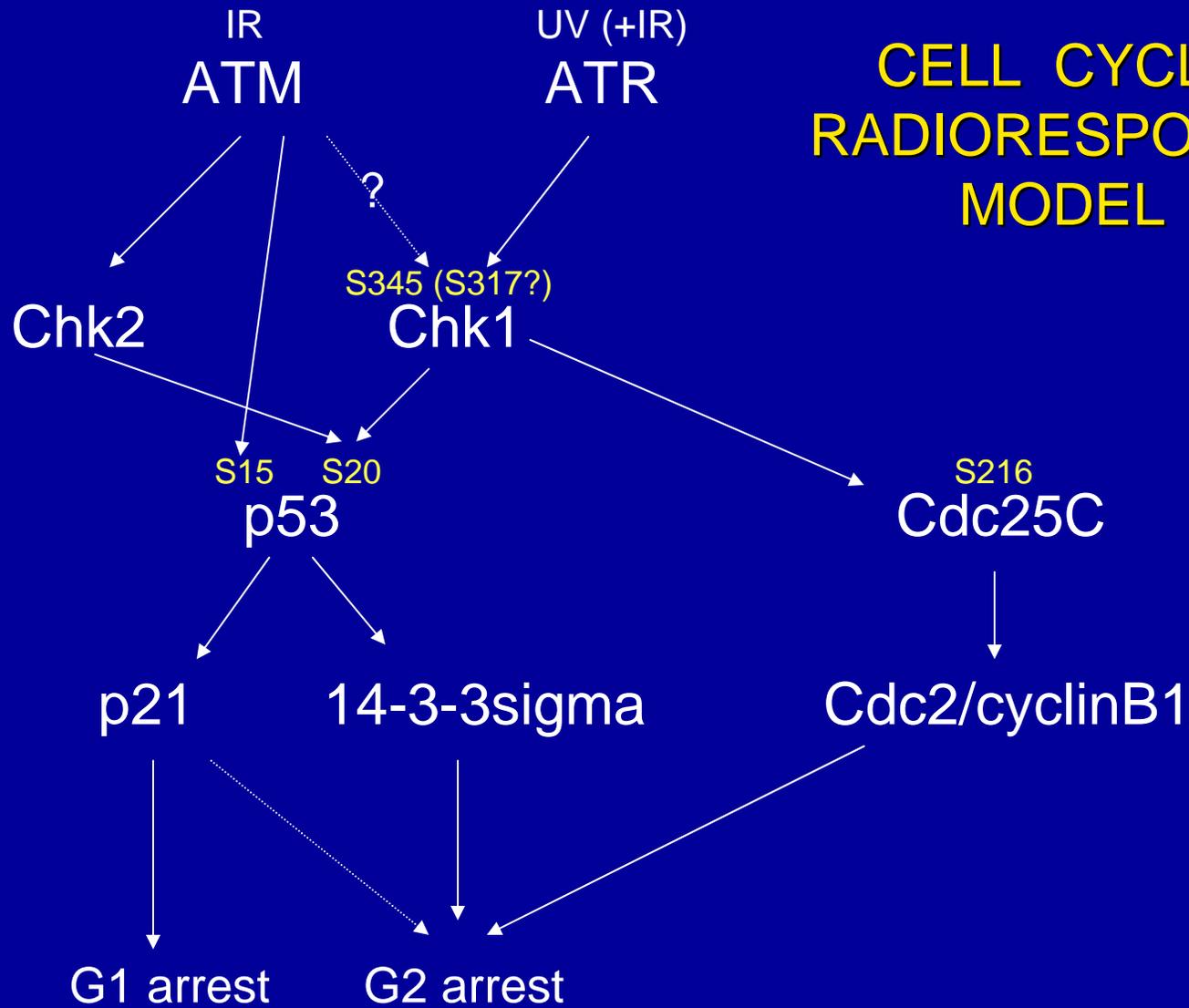
Suppression of cell cycle works in concert with DNA repair to:

1. Allow time for DNA repair
2. To stimulate DNA repair activity

Feedback mechanisms are mediated via intermediate proteins that detect or respond to either the damaging agent or the damage itself and act on the cyclin/CDK complexes to suppress their ability to promote progression to the next stage of the cell cycle. There are probably many checkpoints throughout the cell and only the major ones are known.



CELL CYCLE RADIORESPONSE MODEL



At least two checkpoints are responsive to DNA damage:

- G1-S transition
- G2-M transition

In mammalian cells the G1-S checkpoint is best understood.

G1-S checkpoint:

An early response to DNA damage is induction of p53 by a post-translational mechanism.

P53 then transcriptionally activates a set of p53 dependent genes:

- Gadd45 is a growth arrest DNA damage dependent gene
- p21 inhibits the kinase activity of multiple cyclin/CDK complexes.

The major consequence of p53 induction is either arrest in G1 or apoptosis.

DO DEFECTIVE CHECKPOINTS CAUSE CANCER?

Evidence suggests that the loss of the G1-S checkpoint can result in cancer:

1. p53 is commonly mutated in a wide variety of cancers.
2. p53 mutant cells are typically highly aneuploid and have gene amplifications.
3. Some cancer viruses express proteins that bind to p53.
4. Cells from A-T patients (cancer prone) have abnormal induction of p53.

Evidence for the role of the G2-M checkpoint in cancer is weaker:

1. Cells from A-T patients undergo reduced G2-M arrest in response to DNA damage.
2. Cancer cell lines often have reduced G2-M arrest.
3. Some cancer cells have altered expression of cyclins A, B, and CDC2.

Radiation-Induced G₂ delay in lymphoblasts may be a good biomarker for lung cancer

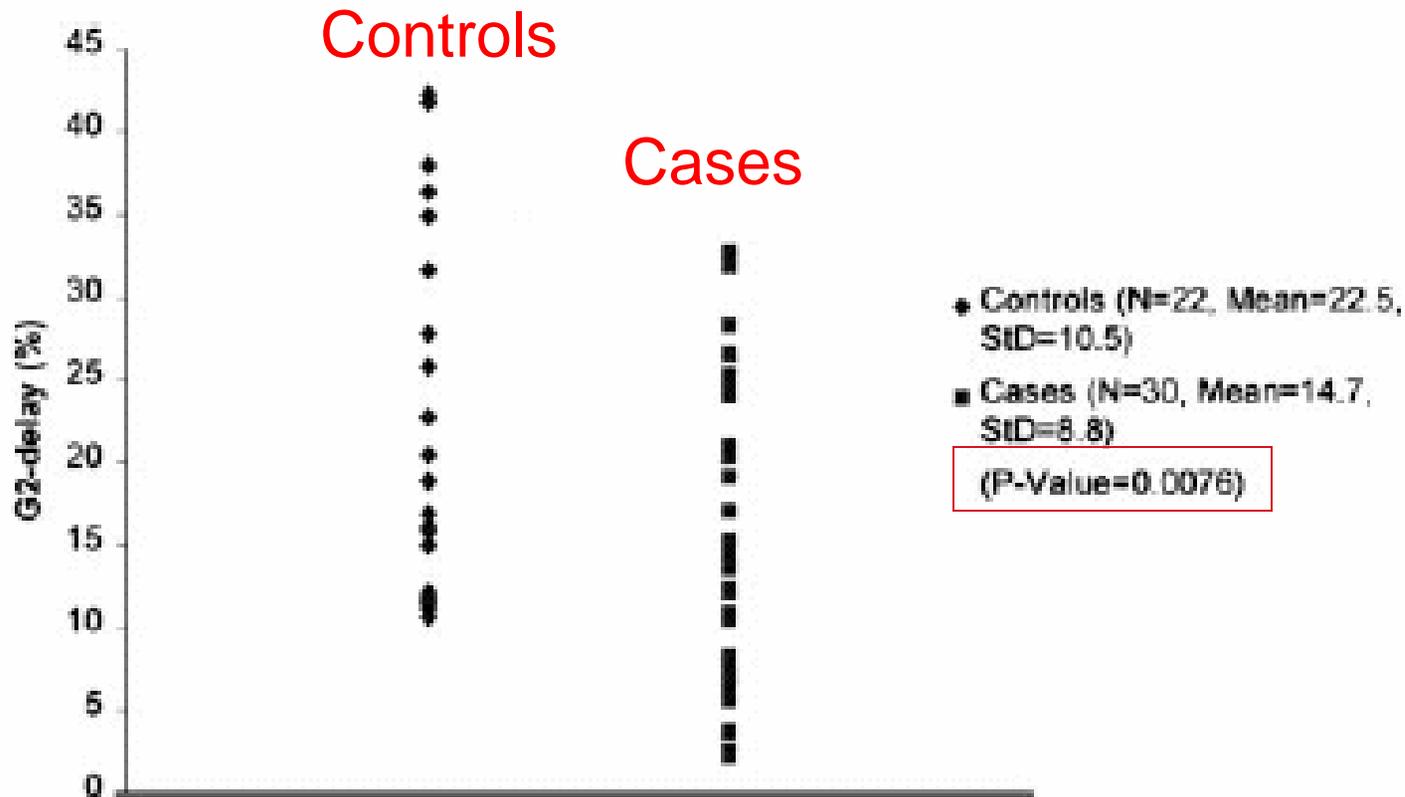


Fig. 3. Distribution of G₂ delay in lymphoblastoid cells of lung cancer cases and controls. Cell lines from 22 normal healthy donors and 30 lung cancer patients were exposed to 2.5 Gy of γ -radiation for 10 h. The values shown are the mean values from three separate experiments.

APOPTOSIS

R. Mirakian et al. / Journal of Immunological Methods 265 (2002) 161–175

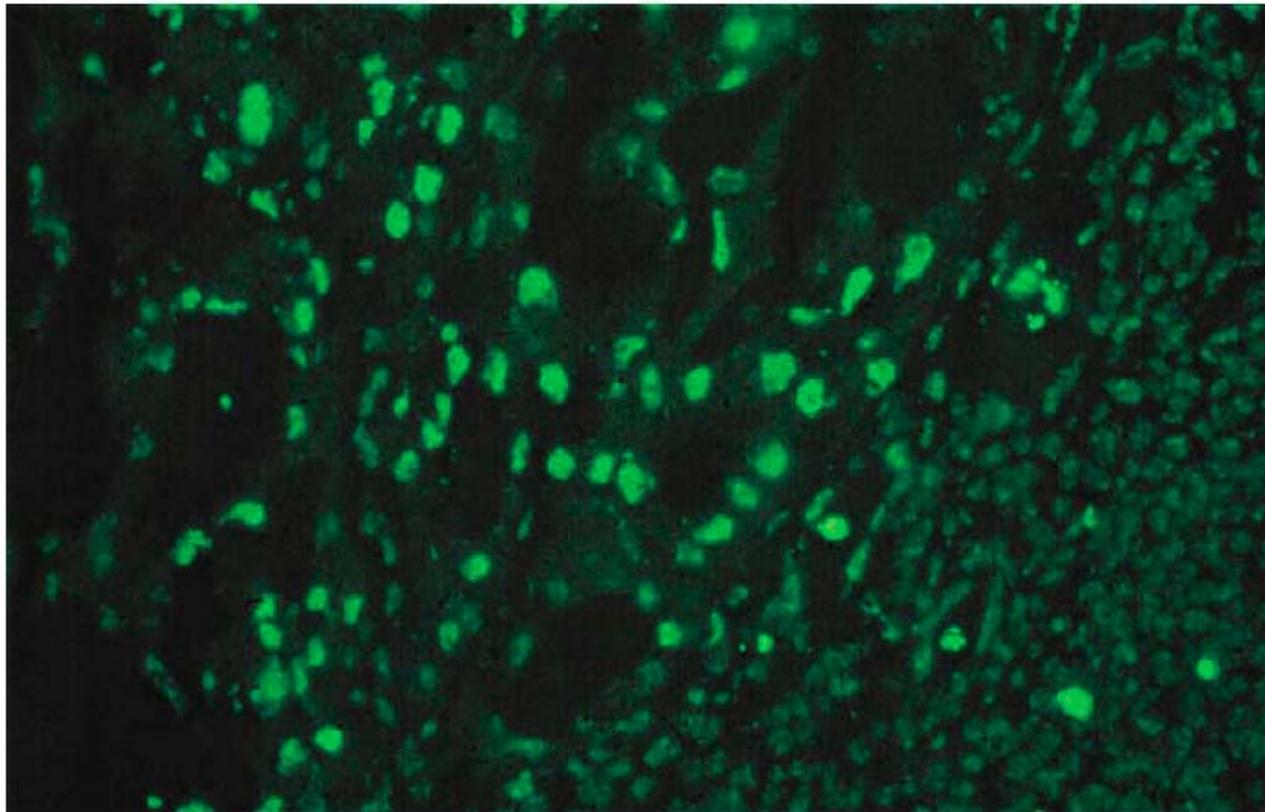
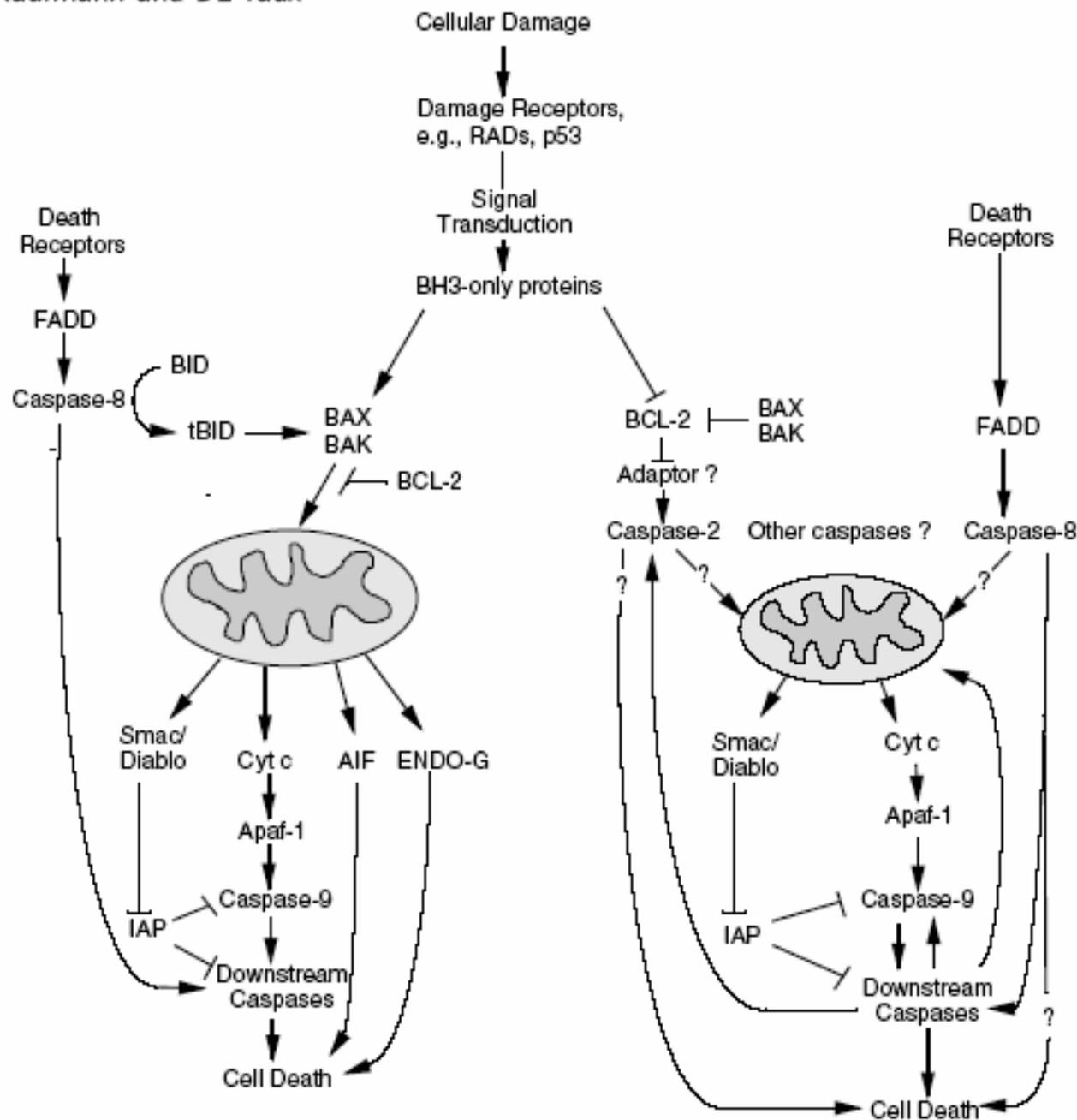


Fig. 1. In situ fluorescence staining of apoptotic cells using the TUNEL technique. Positive staining in nuclei of disrupted follicles in a Hashimoto's thyroiditis gland (magnification $\times 250$).



APOPTOSIS

Science 285:898, 1999

Fas Ligand: A Sensor for DNA Damage Critical in Skin Cancer Etiology

Laurie L. Hill, Allal Ouhtit, Susan M. Loughlin, Margaret L. Kripke, Honnavara N. Ananthaswamy, Laurie B. Owen-Schaub*

DNA-damaged cells can either repair the DNA or be eliminated through a homeostatic control mechanism termed "cellular proofreading." Elimination of DNA-damaged cells after ultraviolet radiation (UVR) through sunburn cell (apoptotic keratinocyte) formation is thought to be pivotal for the removal of precancerous skin cells. Sunburn cell formation was found to be dependent on Fas ligand (FasL), a pro-apoptotic protein induced by DNA damage. Chronic exposure to UVR caused 14 of 20 (70 percent) FasL-deficient mice and 1 of 20 (5 percent) wild-type mice to accumulate p53 mutations in the epidermis. Thus, FasL-mediated apoptosis is important for skin homeostasis, suggesting that the dysregulation of Fas-FasL interactions may be central to the development of skin cancer.

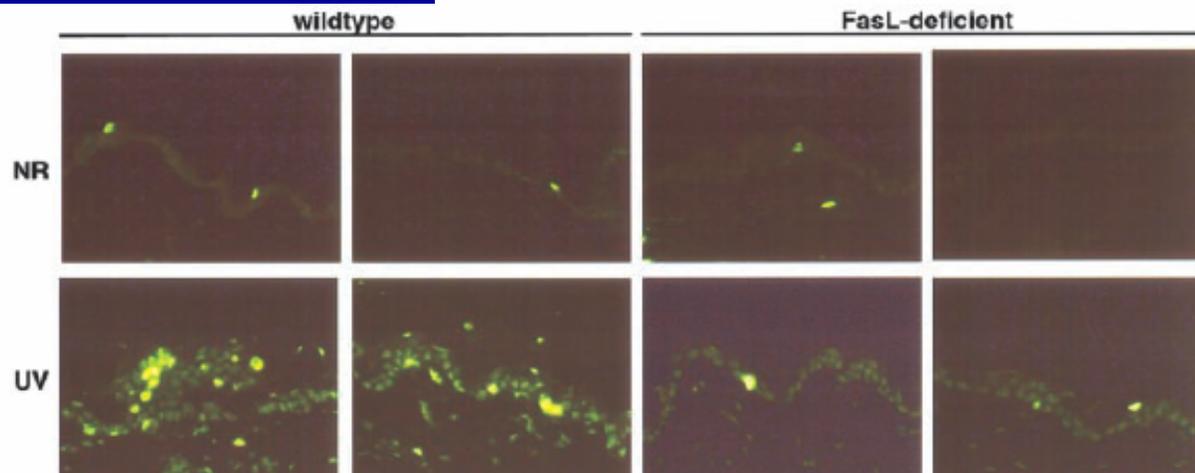


Fig. 2. Sunburn cell induction in wild-type and FasL-deficient (*gld/gld*) mice after UVR. Mice were acutely exposed to UV-B light (5 kJ/m^2), and skin sections were harvested for TUNEL analysis at 0 (NR) and 24 (UV) hours (15). A minimum of four mice (nonirradiated and irradiated) were examined; sections from two individual mice are shown. NR, nonirradiated. Magnification, $\times 10$.

DNA REPAIR



Somatic Mutation and Cancer

Environmental carcinogens

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graph TD; A[Environmental carcinogens] --> B[DNA Damage]; B --> C[DNA Repair]; C --> D[Mutatagenesis]; D --> E[Carcinogenesis];
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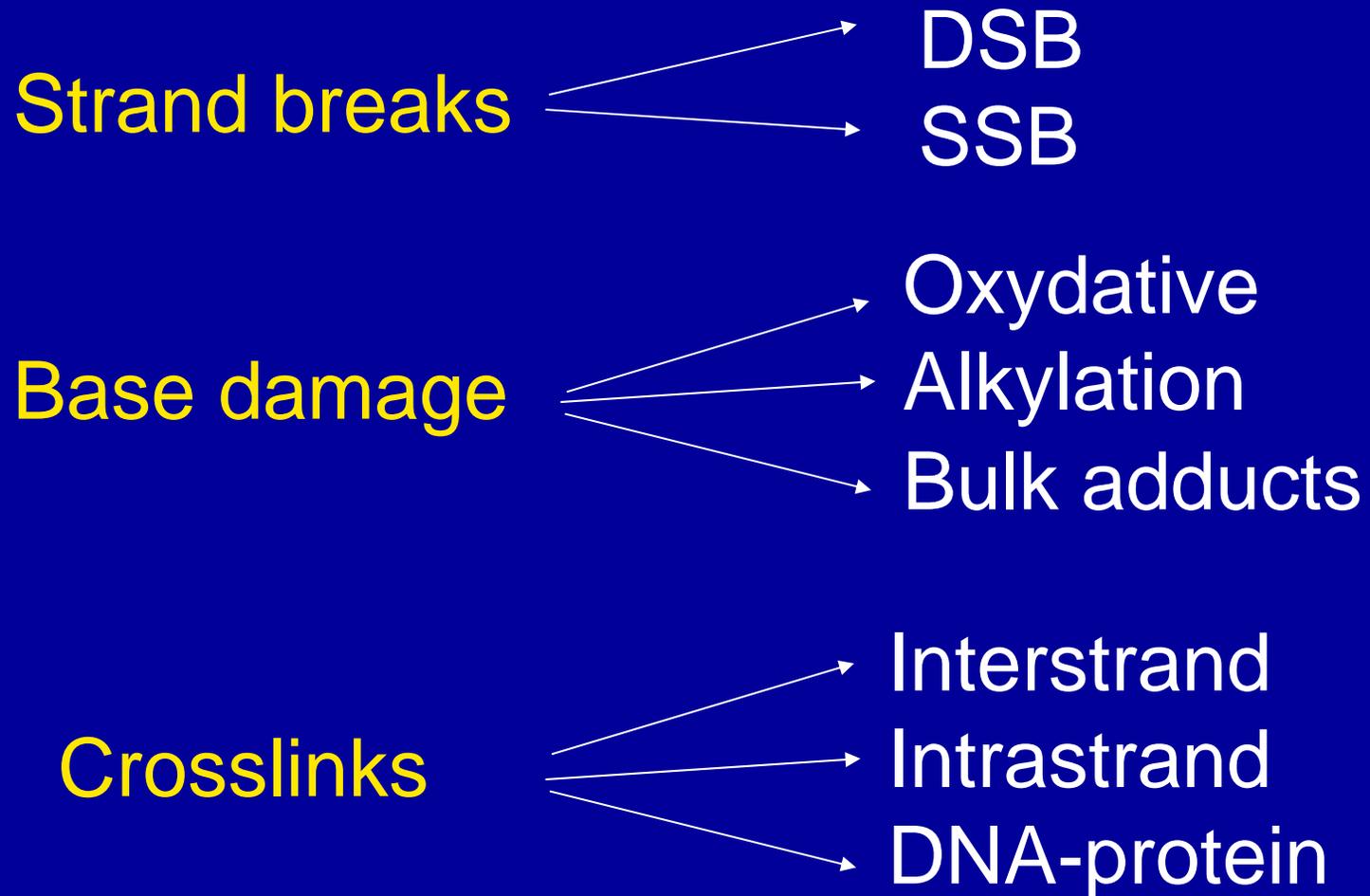
DNA Damage

DNA Repair

Mutatagenesis

Carcinogenesis

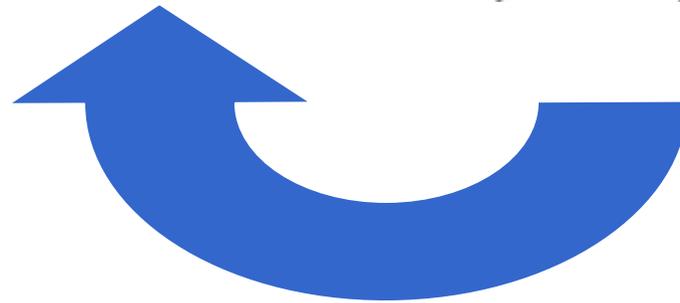
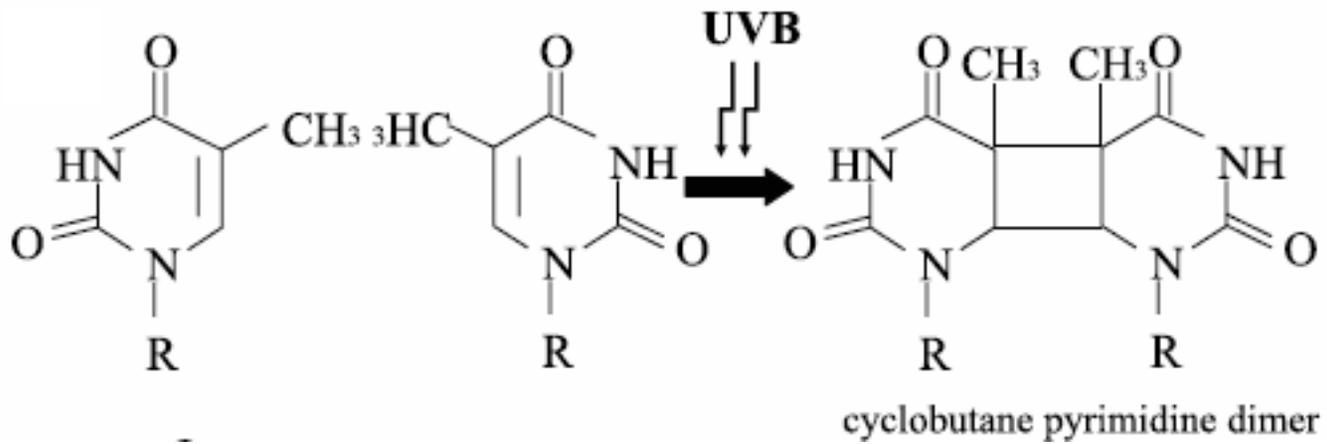
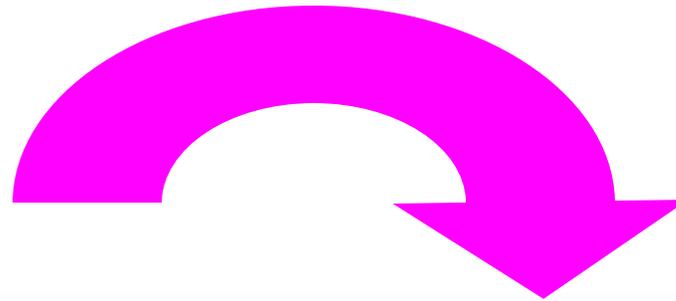
Major classes of DNA damage:



Major DNA repair pathways:

- Non-homologous end joining (NHEJ)
- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- Homologous Recombination Repair
- Illegitimate Recombination Repair
- Mismatch Repair (MMR)

254 nm



>320 nm

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5574-5578, December 1977
Cell Biology

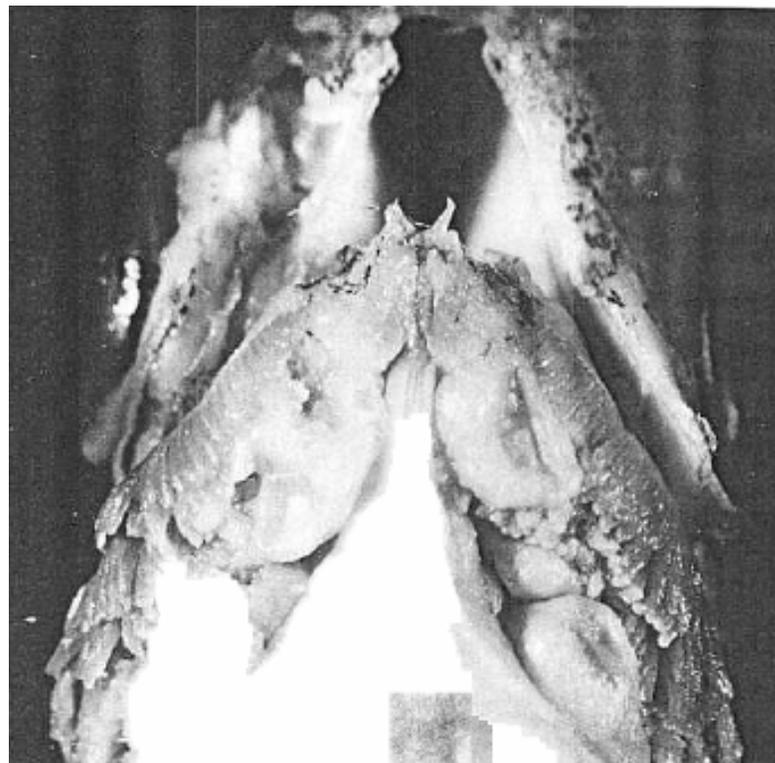
Evidence that pyrimidine dimers in DNA can give rise to tumors

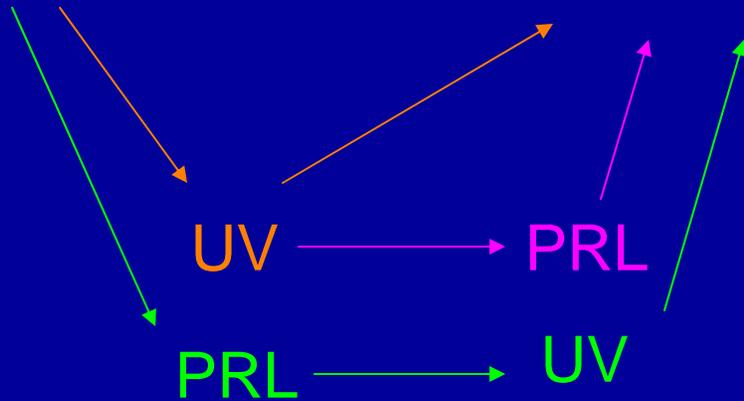
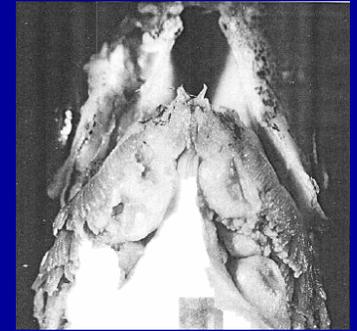
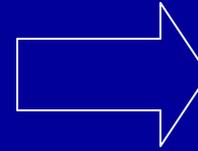
(UV irradiation/photoreactivation/fish/thyroid)

R. W. HART*, R. B. SETLOW, AND A. D. WOODHEAD

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Contributed by R. B. Setlow, September 12, 1977





Fish with thyroid tumors

	Number	Percent
UV (24 J/m ²)	40/40	100%
UV + PRL	0/22	0%
PRL + UV	38/40	95%
untreated	0/22	0%

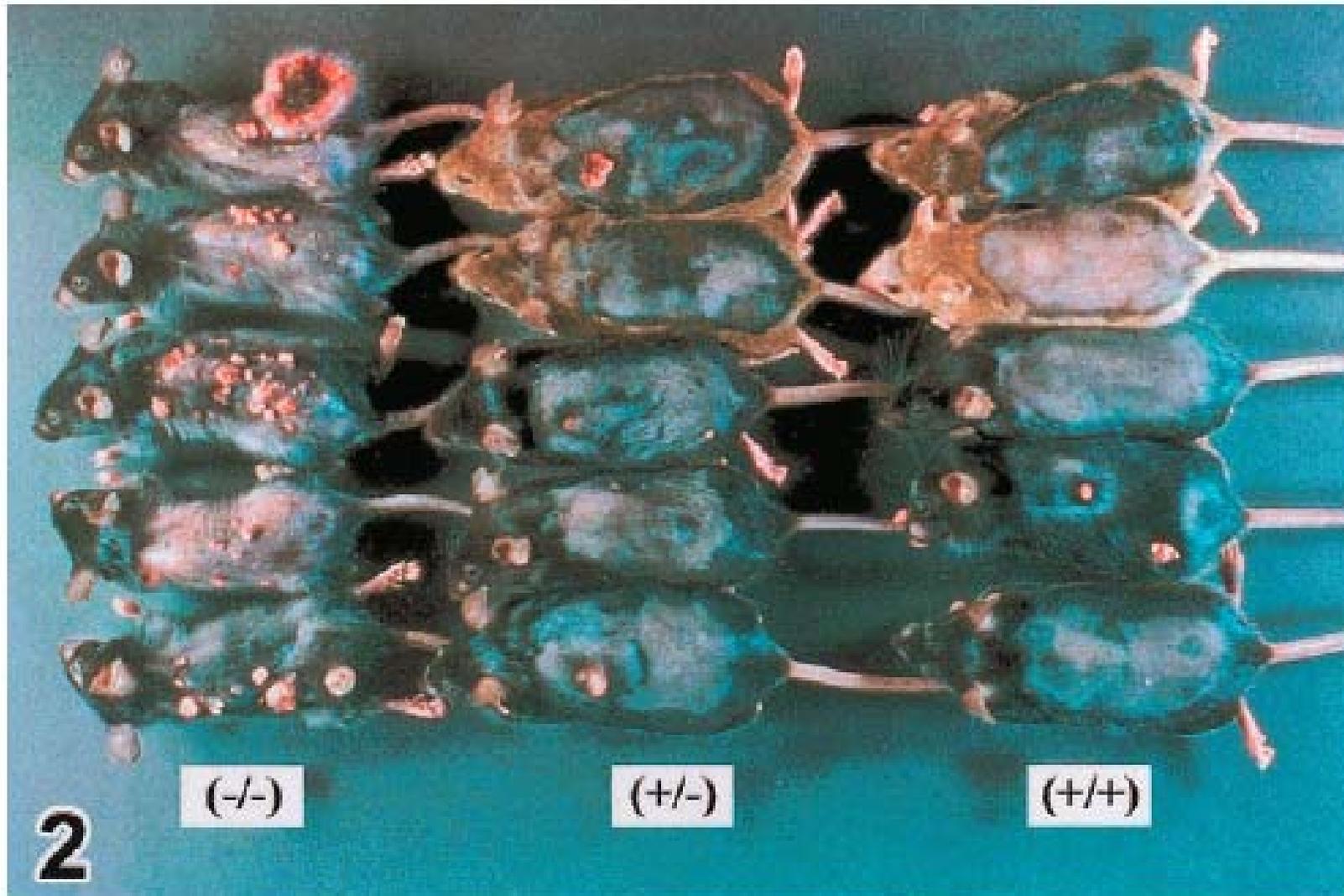


Fig. 2. DMBA-induced skin tumors in *XPA*-deficient (left), heterozygous (middle) and wild-type (right) mice. Tumors are more frequent in *XPA*-deficient mice.

Table 3

Mouse models with inactivated DNA repair genes

Repair system	Type of DNA damage	Inherited human disease	Cancer risk	Transgenic mouse model
Direct repair	Alkyl adducts	?	?	<i>Mgmt</i>
BER	Single-base	?	?	<i>Aag, Ogg, Udg, etc.</i>
NER	Bulky adducts	XP	+	<i>Xpa, Xpb, Xpc, Xpg</i>
		CS	–	<i>Csa, Csb</i>
		XP-CS	+	<i>Xpb, Xpd, Xpg</i>
		TTD	–	<i>Xpd-Ttd</i>
MMR	Base pair mismatch	HNPCC	+	<i>Msh2, Msh3, Msh5, Msh6, Mlh1, Pms1, Pms2</i>
Homologous recombination	Strand breaks, cross-links	?	?	<i>Rad52, Rad54, Rad54B</i>
End joining	Strand breaks, cross-links	?	?	<i>Ku70, Ku80, DNA-PK_{CS}</i>

BER: base excision repair; NER: nucleotide excision repair; MMR: mismatch repair. - = not present (existing patients do not have a cancer phenotype); ? = no patients existing or known.

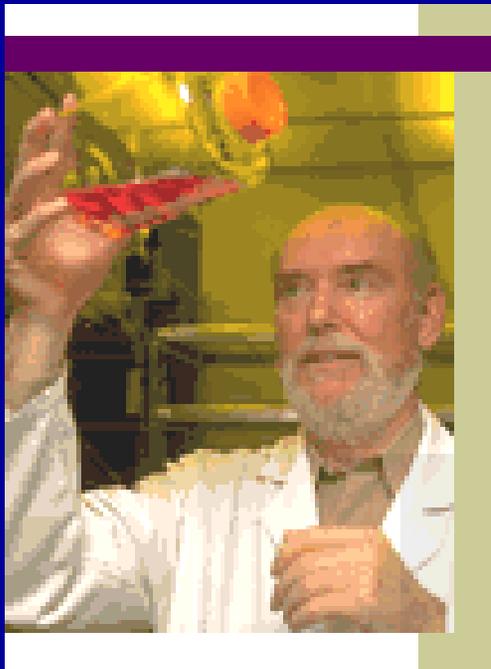
Defective Repair Replication of DNA in Xeroderma Pigmentosum

by

J. E. CLEAVER

Laboratory of Radiobiology,
University of California Medical Center,
San Francisco, California

Normal skin fibroblasts can repair ultraviolet radiation damage to DNA by inserting new bases into DNA in the form of small patches. Cells from patients with the hereditary disease xeroderma pigmentosum carry a mutation such that repair replication of DNA is either absent or much reduced in comparison to normal fibroblasts. Patients with xeroderma pigmentosum develop fatal skin cancers when exposed to sunlight, and so the failure of DNA repair in the skin must be related to carcinogenesis.



“... the failure of DNA repair in the skin *must* be related to carcinogenesis.”

-- James E. Cleaver

Nucleotide Excision Repair (NER)

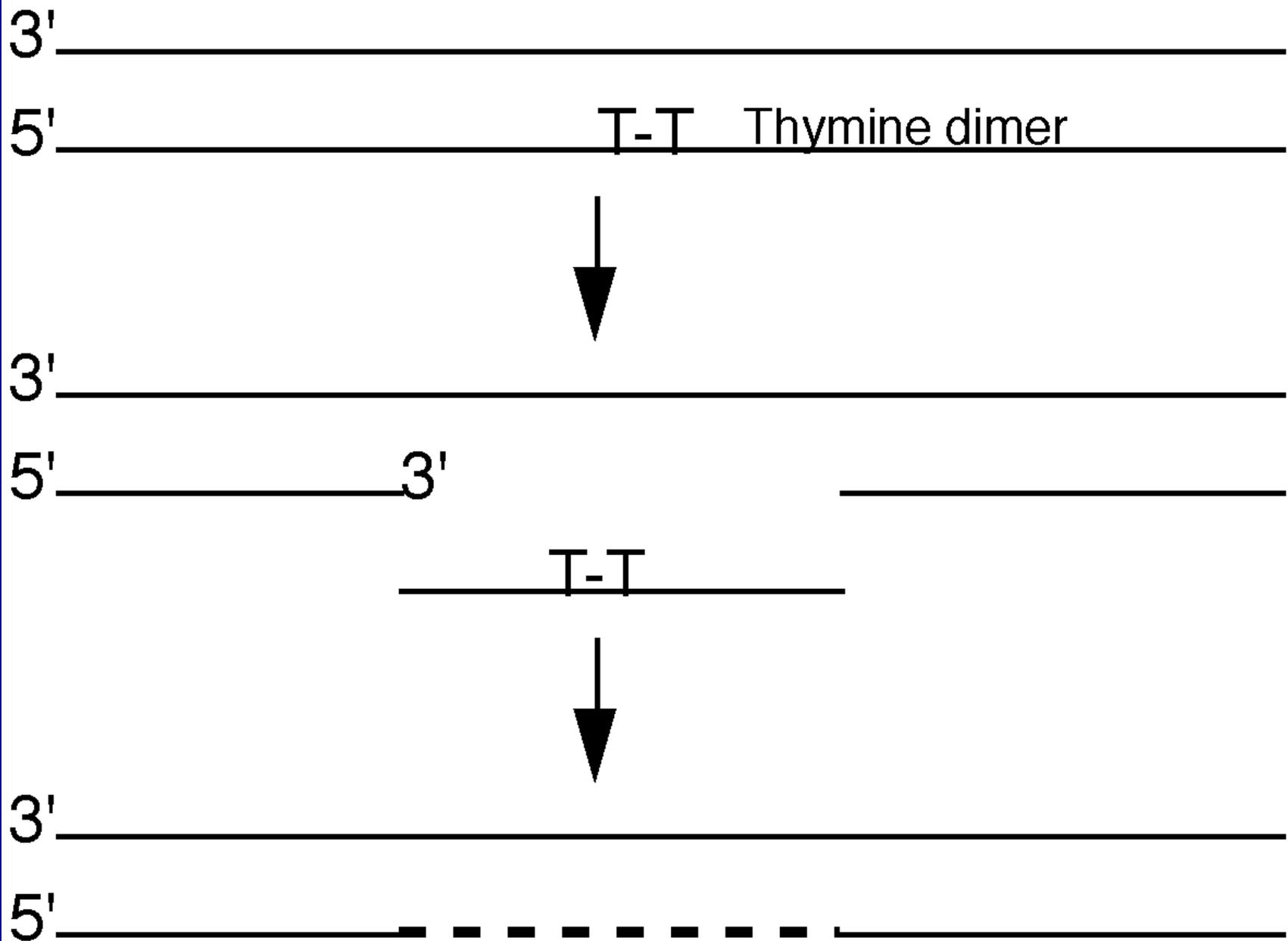
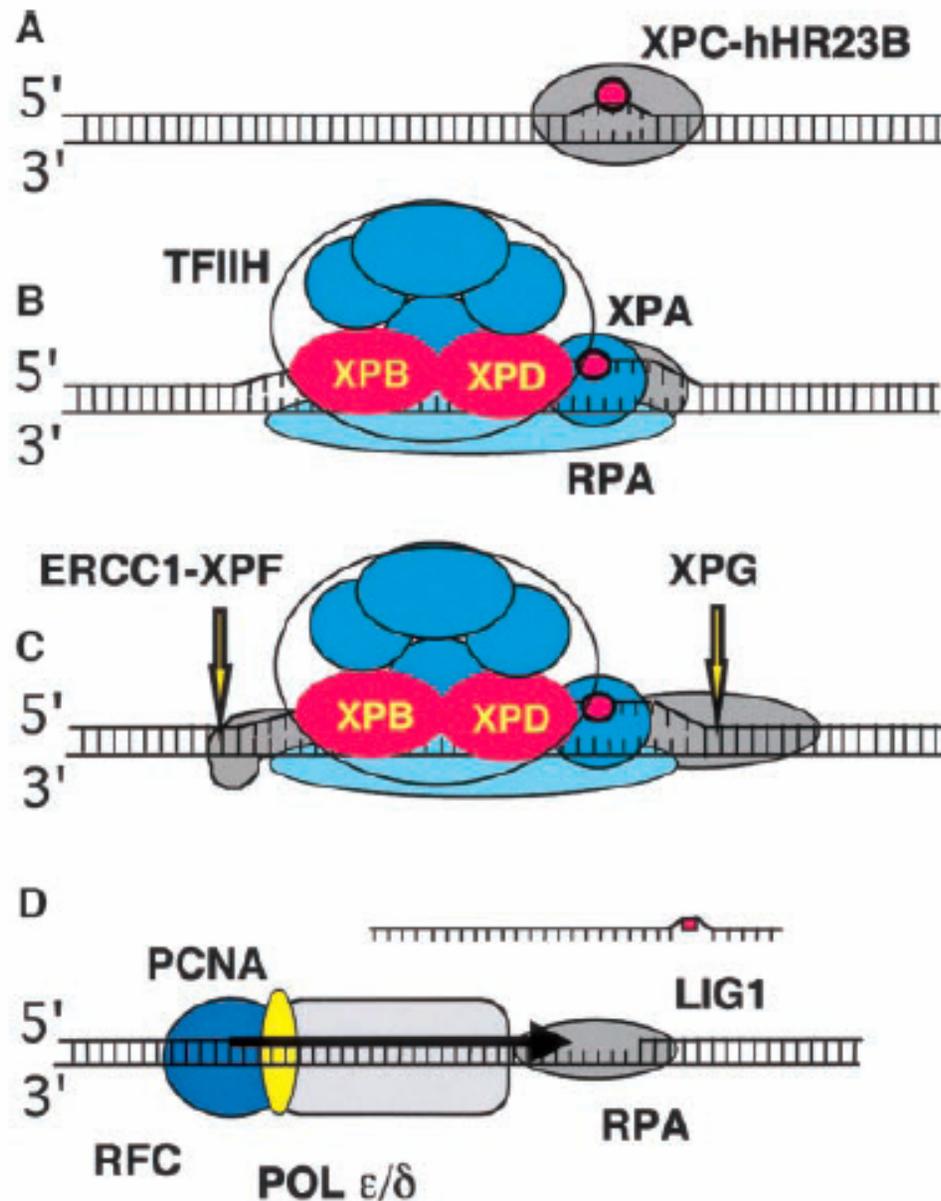
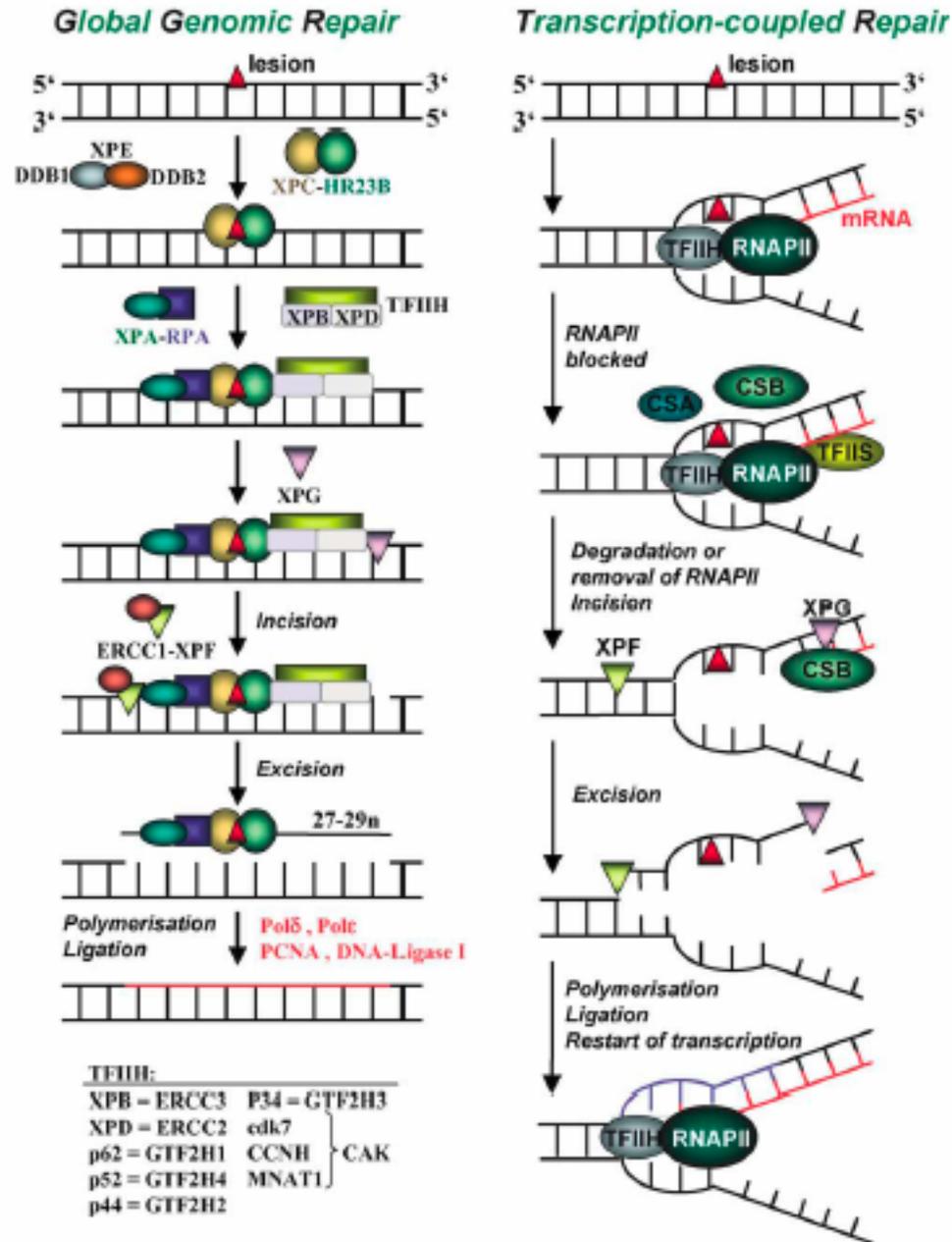


Fig. 3. Nucleotide excision repair in nontranscribed regions (the bulk of DNA). Initially, a distortion is recognized, probably by the XPC-hHR23B protein (A). An open bubble structure is then formed around a lesion in a reaction that uses the ATP-dependent helicase activities of XPB and XPD (two of the subunits of TFIIH) and also involves XPA and RPA (B). Formation of this open complex creates specific sites for cutting on the 3' side by the XPG nuclease and then on the 5' side by the ERCC1-XPF nuclease (C). After a 24- to 32-residue oligonucleotide is released, the gap is filled in by PCNA-dependent POL ϵ or δ and sealed by a DNA ligase, presumably LIG1 (D).



Nucleotide Excision Repair



NER PATHWAY GENES

GENE	ALIASES	DESCRIPTION
CCNH		cyclin H
CDK7		cyclin-dependent kinase 7
CETN2	CALT CEN2	caltractin isoform 1 (Centrin 2)
CKN1	CSA	Cockayne syndrome 1 (classical)
DDB1		damage-specific DNA binding protein 1
DDB2		damage-specific DNA binding protein 2
ERCC1	UV20	excision repair cross-complementing group 1
ERCC2	XPD	excision repair cross-complementing group 2
ERCC3	XPB BTF2 GTF2H RAD25 TFIIH	excision repair cross-complementing group 3
ERCC4	XPF RAD1	excision repair cross-complementing group 4
ERCC5	XPG UVDR XPGC ERCC2	excision repair cross-complementing group 5
ERCC6	CSB CKN2 COFS RAD26	excision repair cross-complementing group 6
GTF2H1		general transcription factor IIH, polypeptide 1
GTF2H2		general transcription factor IIH, polypeptide 2
GTF2H3		general transcription factor IIH, polypeptide 3
GTF2H4		general transcription factor IIH, polypeptide 4
LIG1		ligase I, DNA, ATP-dependent
MNAT1		menage a trois 1 (CAK assembly factor)
RAD23A	HHR23A	RAD23 homolog A
RAD23B	HHR23B P58 HR23B	RAD23 homolog B
RPA1		replication protein A1
RPA2		replication protein A2
RPA3		replication protein A3
XAB2	HCNP	HCNP protein; XPA-binding protein 2
XPA	XP1 XPAC	XP complementation group A

Individuals at risk for skin cancer are at risk for other cancers

Association of Nonmelanoma Skin Cancer with Second Malignancy

The Women's Health Initiative Observational Study

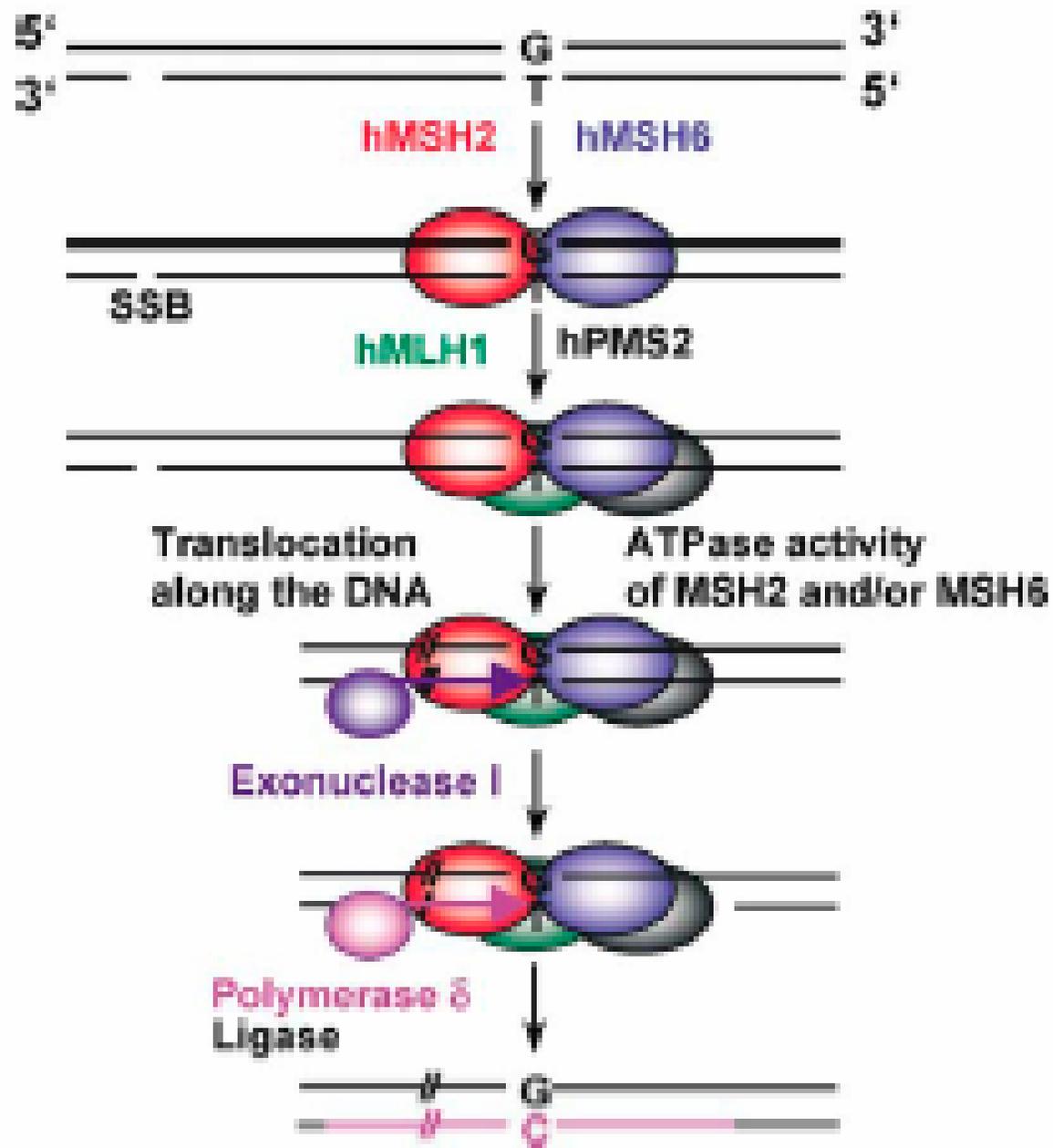
Prevalence and Odds of History of Other Malignancies by Nonmelanoma Skin Cancer History Status at Enrollment

Other history of malignancy	Reported ever having NMSC				OR	95% Wald confidence limits	P value
	No (n = 85,170)		Yes (n = 7665)				
	No.	% ^a	No.	% ^a			
Any other cancer (excluding NMSC)	9927	11.66	1878	24.86	2.30	2.18–2.44	< 0.0001
Breast	4444	5.22	831	10.91	2.09	1.93–2.26	< 0.0001
Ovary	540	0.63	98	1.29	2.01	1.61–2.50	< 0.0001
Endometrium	1302	1.53	264	3.47	2.00	1.74–2.29	< 0.0001
Colon, rectum, bowel, or intestine	727	0.85	124	1.63	1.68	1.38–2.04	< 0.0001
Thyroid	401	0.47	94	1.24	2.60	2.07–3.28	< 0.0001
Cervix	1030	1.21	165	2.17	1.92	1.62–2.28	< 0.0001
Melanoma	885	1.04	299	3.93	3.29	2.87–3.76	< 0.0001
Liver	25	0.03	10	0.13	5.96	2.71–13.11	< 0.0001
Lung	162	0.19	56	0.74	3.43	2.51–4.69	< 0.0001
Brain	43	0.05	9	0.12	2.12	1.02–4.39	0.0429
Bone	51	0.06	13	0.17	2.90	1.55–5.44	0.0009
Stomach	47	0.06	12	0.16	3.17	1.63–6.18	0.0007
Blood (leukemia)	64	0.08	24	0.32	3.58	2.21–5.80	< 0.0001
Bladder	168	0.20	23	0.30	1.26	0.81–1.95	0.3114
Lymphoma	163	0.19	42	0.55	2.73	1.92–3.86	< 0.0001
Hodgkin disease	37	0.04	17	0.22	5.69	3.12–10.39	< 0.0001
Other	979	1.17	209	2.89	2.26	1.94–2.64	< 0.0001

NMSC: nonmelanoma skin cancer; OR: odds ratio.

^a Percentages were based on women with a nonmissing response for the cancer in question who reported no history of nonmelanoma skin cancer (NMSC) and reported a history of NMSC, respectively.

Mismatch Repair



Mismatch repair associated tumors in mouse models

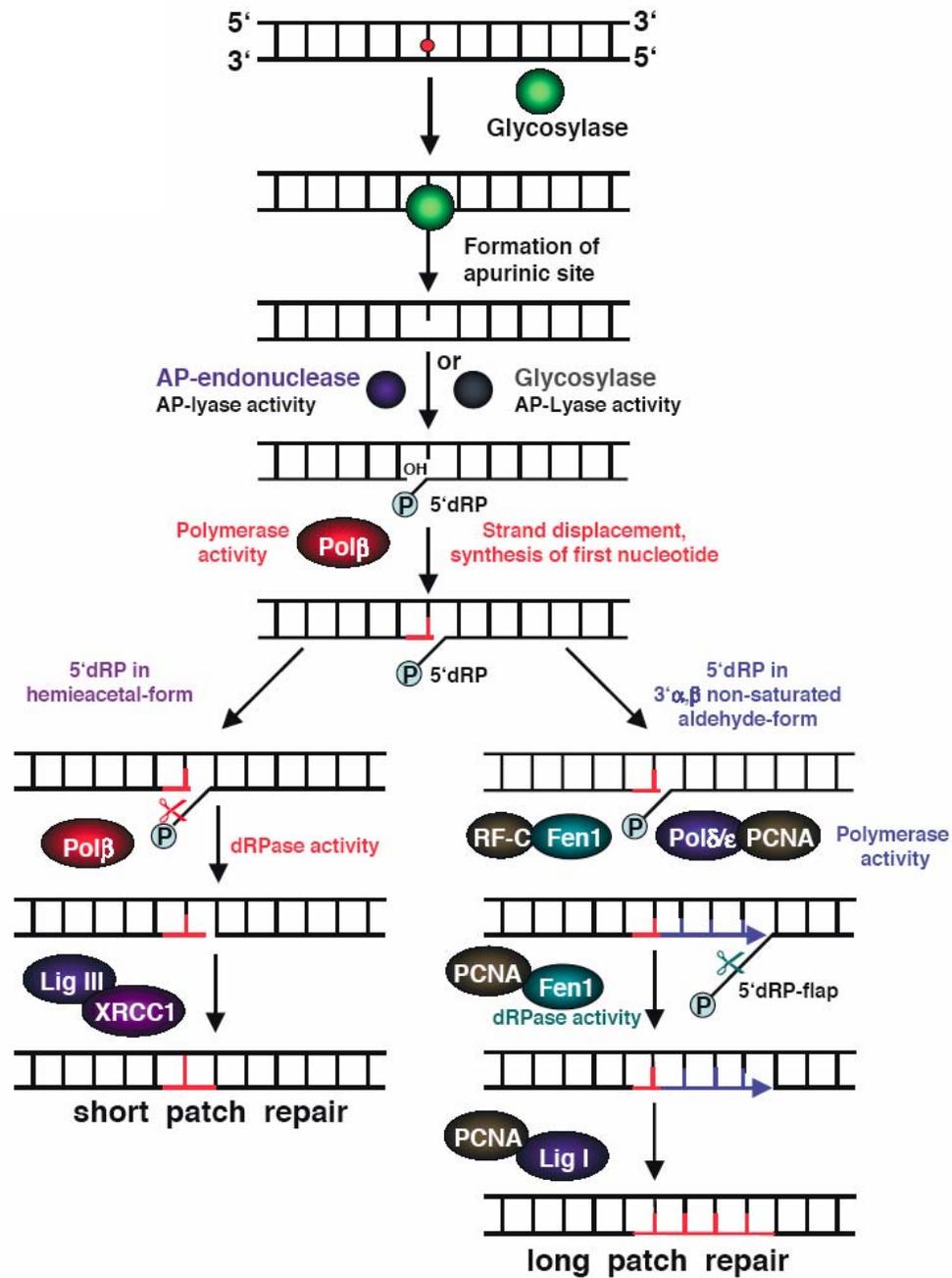
Table 3
Phenotypes of MMR gene knockout mice^a

Mouse	Median survival	Tumor spectrum	Other abnormalities	References
<i>Msh2</i> ^{-/-}	5–6 months	Lymphoma (T-cell) Gastrointestinal and skin cancers in animals that do not succumb to lymphoma	Hyperrecombination	[14,43,44]
<i>Mlh1</i> ^{-/-}	6 months	Intestinal adenocarcinomas Lymphoma	Males and females are infertile (reduced levels of chiasmata)	[45,46]
<i>Msh6</i> ^{-/-}	10 months	Lymphoma (B- and T-cell) Gastrointestinal tumors Uterine tumors	–	[47,48]
<i>Msh3</i> ^{-/-}	Normal life span	No tumors until late age (gastrointestinal tumors)	–	[34,48]
<i>Msh6</i> ^{-/-} ; <i>Msh3</i> ^{-/-}	6 months	Gastrointestinal tumors Non-Hodgkin lymphomas	–	[34,48]
<i>Pms2</i> ^{-/-}	6–9 months	Lymphomas and sarcomas	Males are infertile (abnormal chromosome synapsis in meiosis)	[46,49]
<i>Pms1</i> ^{-/-}	Normal life span	No increased tumor development	–	[46,49]

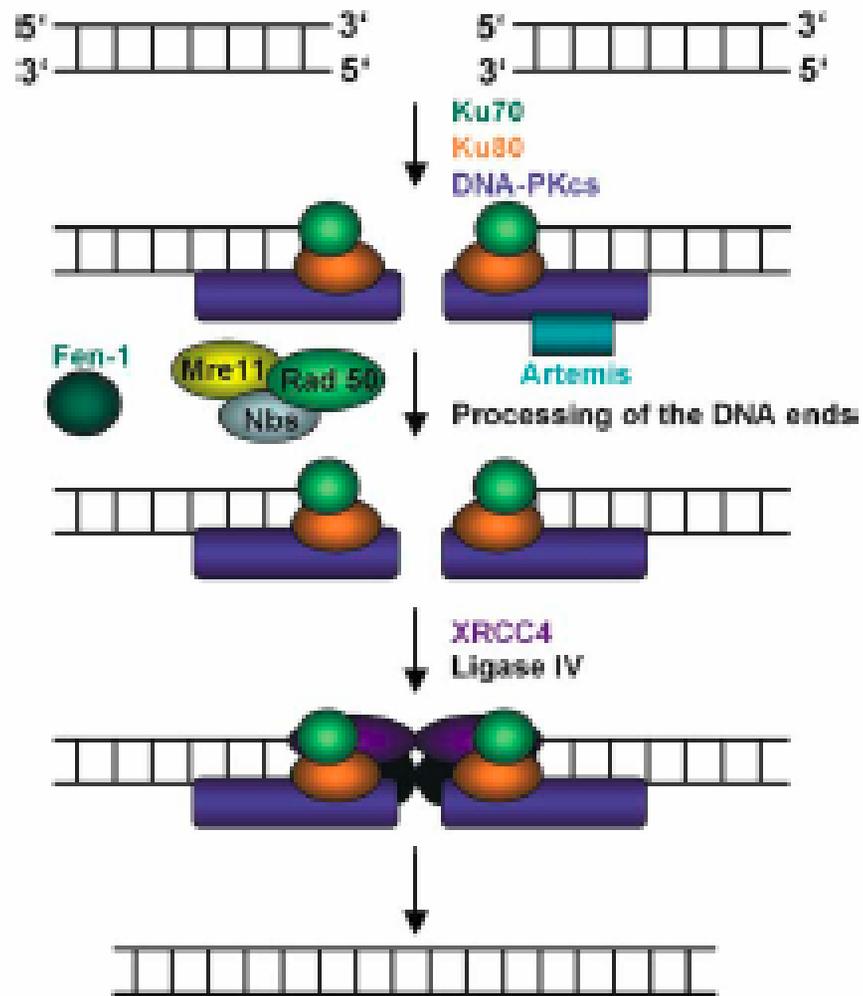
^a Mice heterozygous for the mutations do not show increased tumor formation.

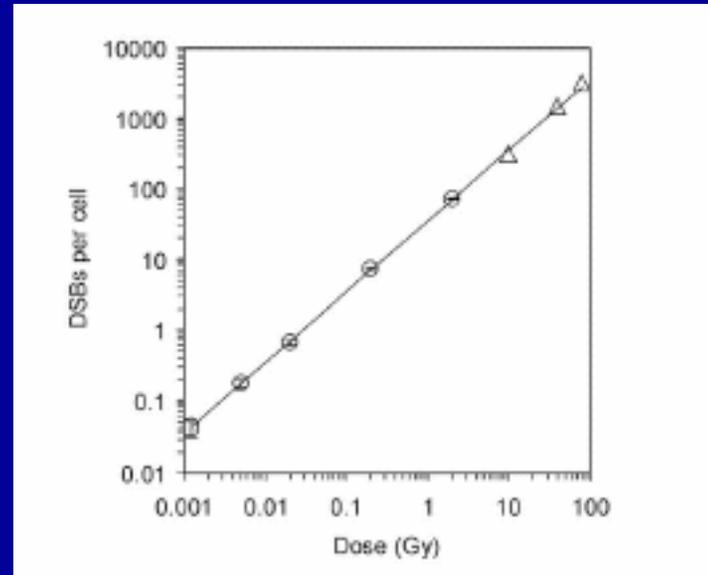
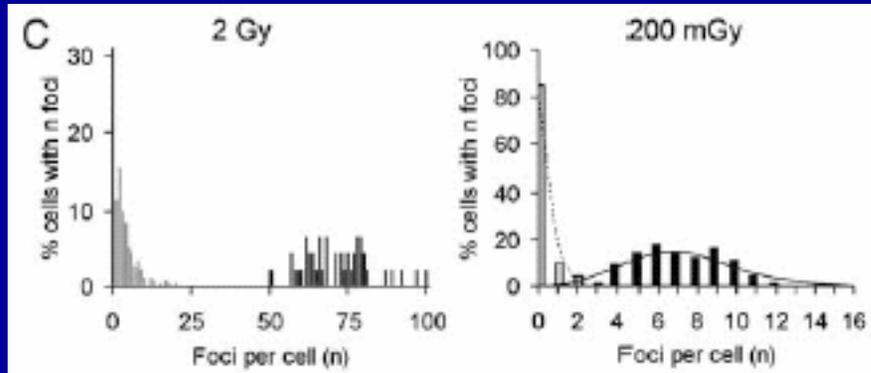
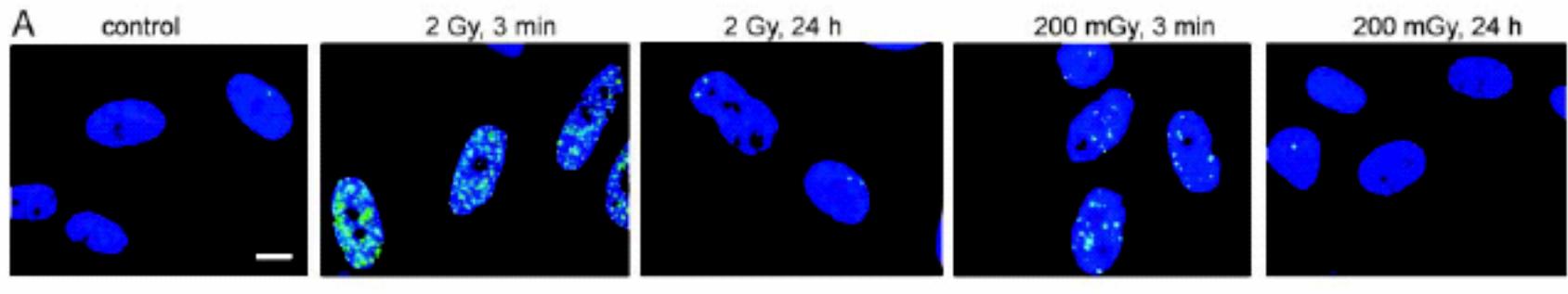
Base Excision Repair

M. Christmann et al. / Toxicology 193 (2003) 3–34



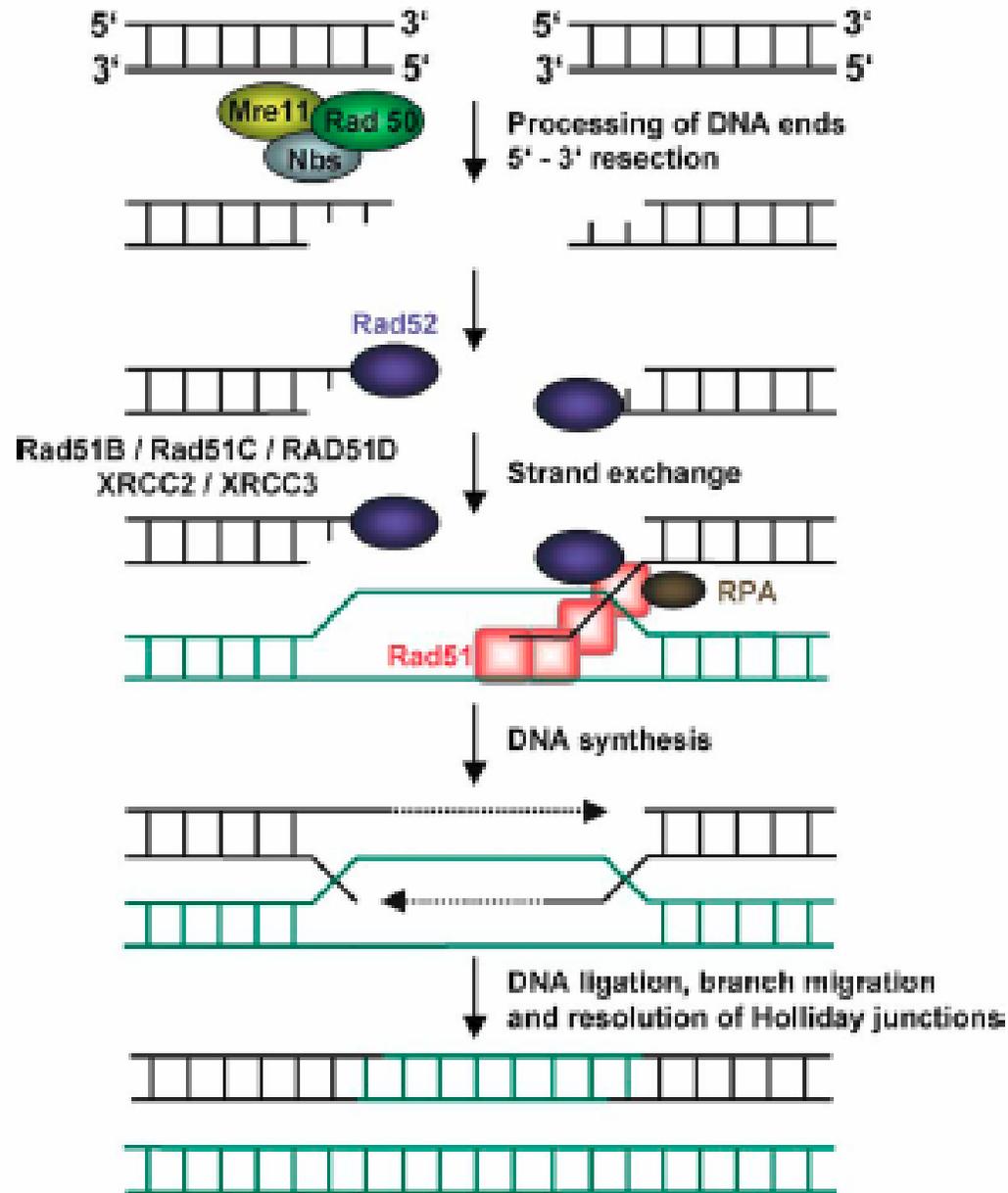
Non-Homologous End Joining (NHEJ)



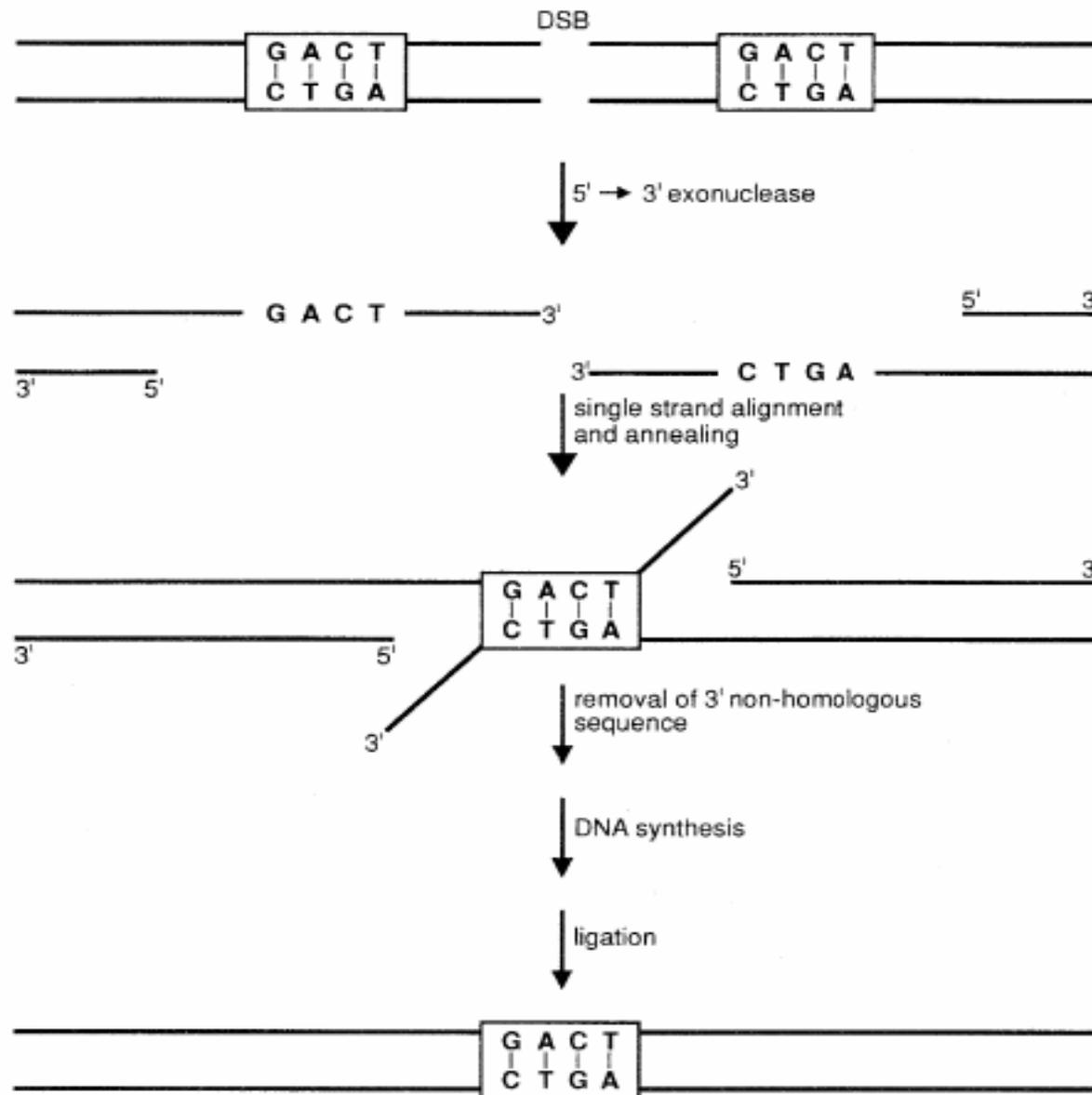


Rothkamm and Lobrick, PNAS, 2003

Homologous Recombination



Illegitimate Recombination



DNA REPAIR PATHWAYS

ERROR-FREE PATHWAYS

base excision repair
nucleotide excision repair
mismatch repair

ERROR-PRONE PATHWAYS

NHEJ
illegitimate recombination

glycosylases
AP-endonucleases
dRp-ases

DNA polymerases
ligases

DNA-PKcs
Ku

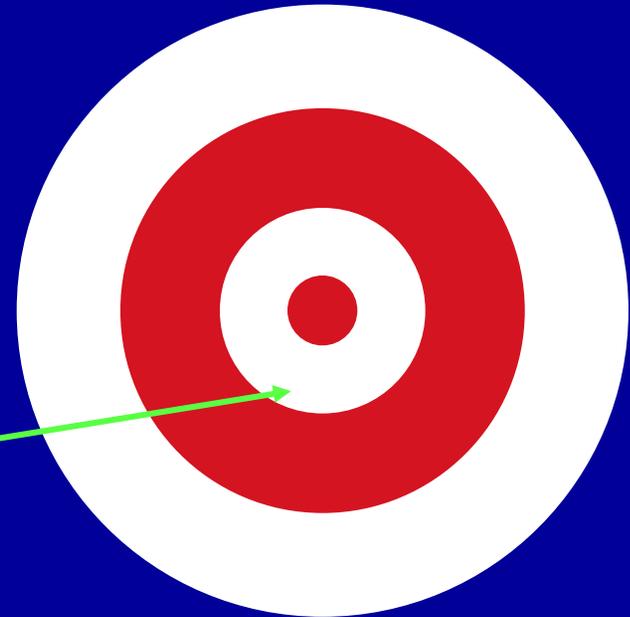
TARGET THEORY



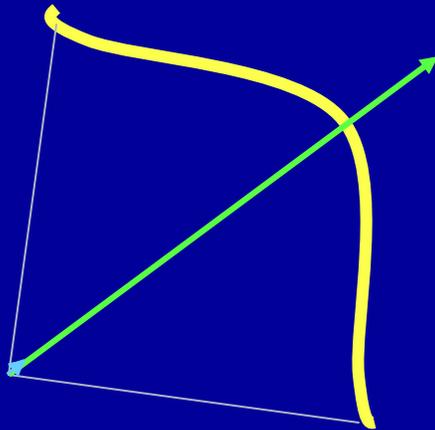
NORMAL CELL



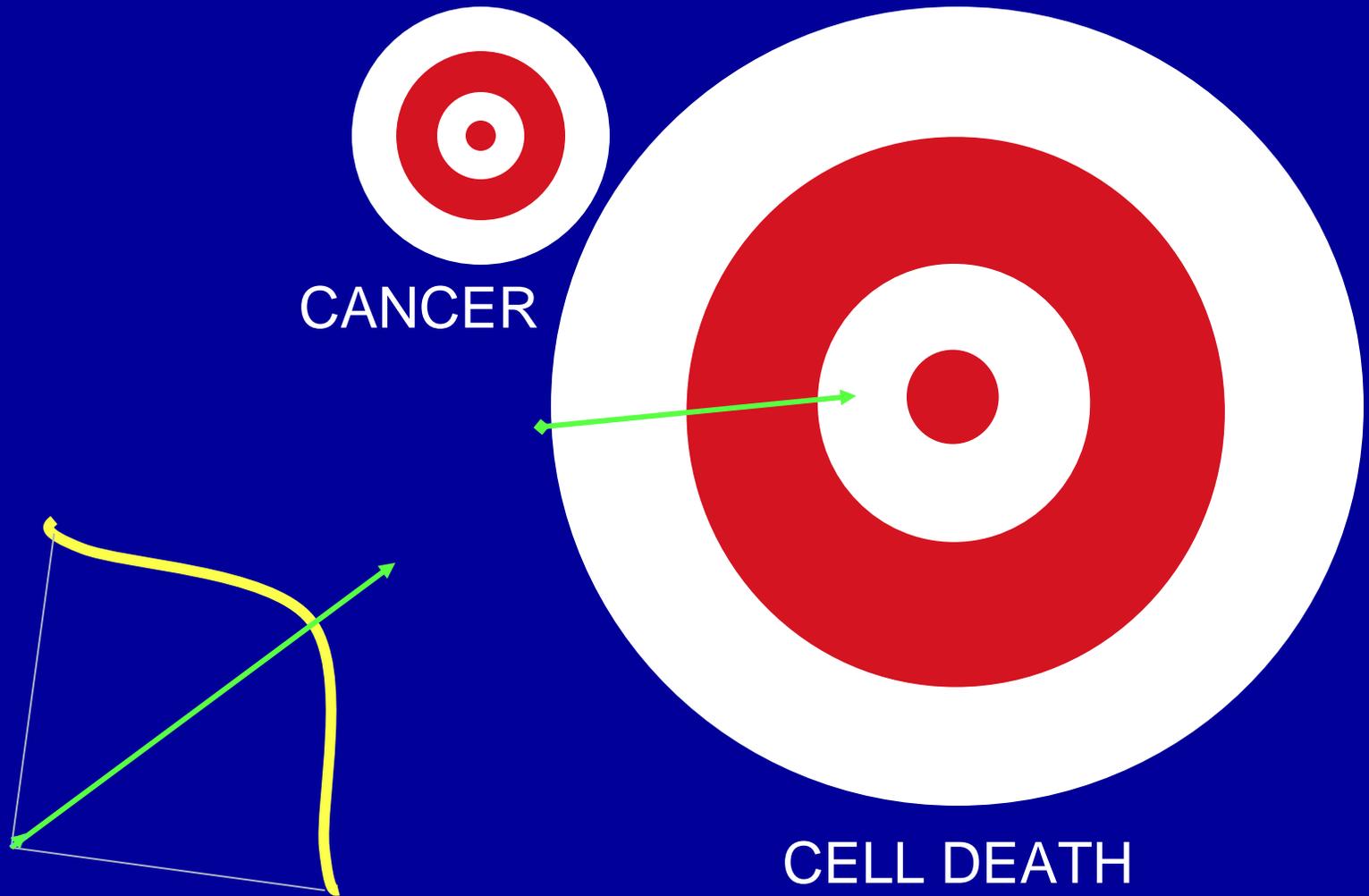
CANCER



CELL DEATH

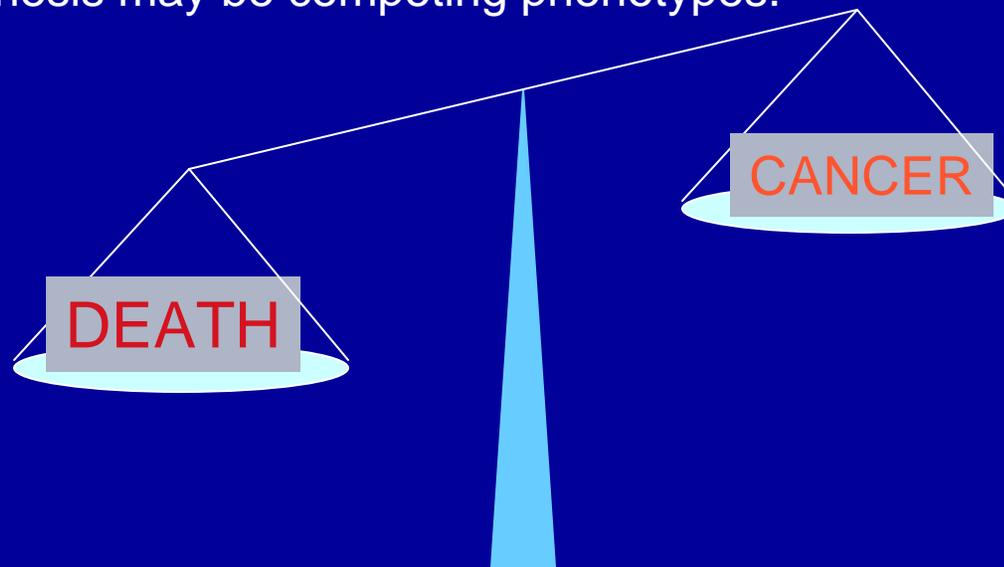


A-T CELL



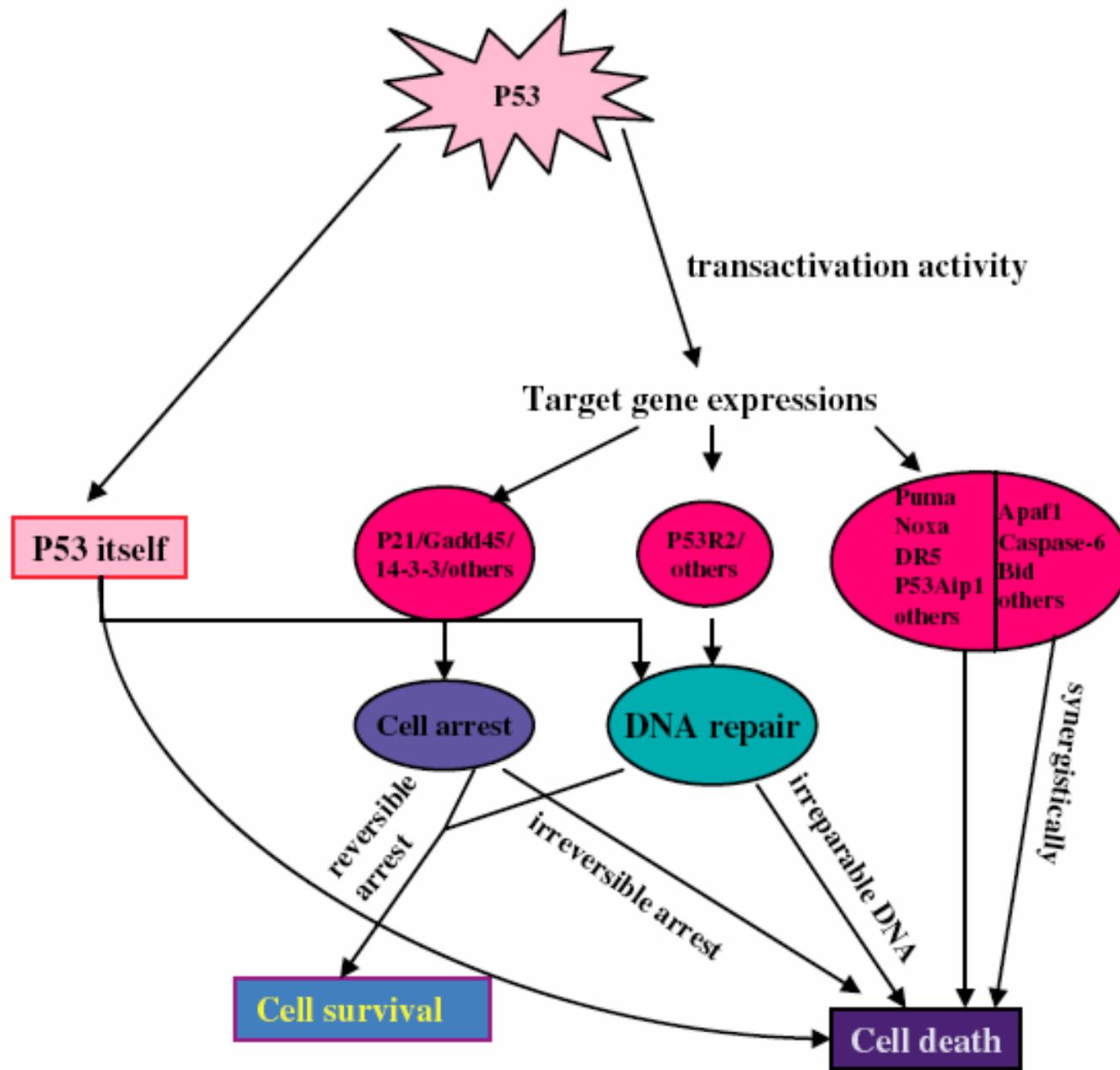
Are radiation sensitivity genes and radiation carcinogenesis genes the same?

- Several radiation sensitivity genes are known (e.g. ATM), but generally these genes confer sensitivity specifically to radiation-induced *killing*.
- Cellular radiosensitivity genes are potential radiation carcinogenesis genes, but association with increased cancer risk has not been established.
- The problem may be that sensitivity to radiation lethality and radiation carcinogenesis may be competing phenotypes.



OTHER TARGET QUESTIONS:

- If DNA repair deficiency predisposes to radiation induced cancer, then what are the mutated target genes that cause cellular transformation?
- What is the mechanism of transformation?



P53 and radiation responses

P Fei and WS El-Deiry

Oncogene (2003) 22, 5774–5783

Li-Fraumeni Syndrome

- Caused by a germline mutation in p53 gene (TP53)
- Characterized by the occurrence of early onset:
 - sarcomas
 - breast cancer
 - brain tumours
 - leukemia
 - adrenocortical tumors

Skin cancers have unique p53 mutations:

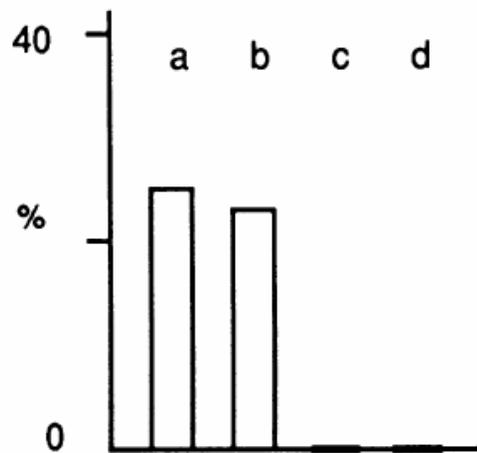
Table 1. Mutations in the p53 gene in invasive squamous cell carcinoma of the skin

Tumor	Age, yr	Sex	Site	Codon	Sequence	Base change	Amino acid change
NI 6	86	♀	Preauricular	7	tCt	C → G	Asp → His
NI 9	77	♀	Chest	56	tcttCa	C → A	Glu → stop
SI 2	82	♂	Preauricular	104/105	gcct	ΔC	Gly → Ala . . . stop
SI 20	82	♂	Temple	104/105	gcct	ΔC	Gly → Ala . . . stop
SI 16	69	♀	Scalp	151	cCccc	C → A	Pro → His
SI 15	69	♀	Hand	152	cccCc	C → T	Pro → Ser
NI 4	76	♂	Front scalp	179	acCa	C → A	His → Asn
NI 3	68	♂	Cheek	245	gcCg	C → A	Gly → Cys
NI 9	77	♀	Chest	245	gCCg	CC → TT	Gly → Asn
SI 13	80	♀	Nose	247–248	aCC*g	CC → TT	Asn-Arg → Asn-Trp
SCC 13	56	♀	Side of face	258	ttCc	C → T	Glu → Lys
NI 11	76	♂	Cheek	278	tCct	C → T	Pro → Ser
SI 1	85	♂	Face	285–286	tCCt	CC → TT	Glu-Glu → Glu-Lys
NI 5	89	♂	Forehead	286	tCct	C → T	Glu → Lys
NI 8	75	♂	Postauricular	317	cccCa	C → T	Gln → stop

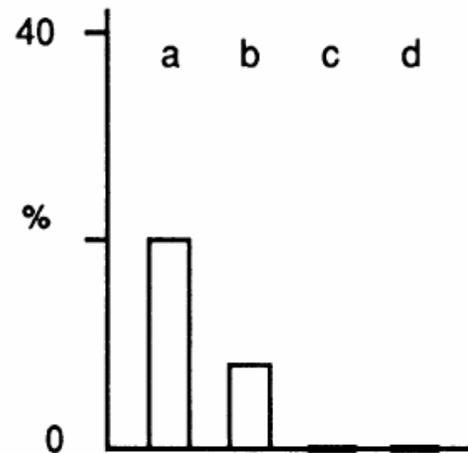
The sequence is written 5' → 3' for the strand containing the pyrimidine. A wild-type allele was observed in all cases except SI 1, SI 15, and SCC 13. Sample NI 9 contained two point mutations. For SI 2, 13, 15, 16, and 20 and NI 4 and 11, an inherited mutation at the site could be excluded based on the presence of a normal sequence in a section of normal tissue or in a second tumor. For SI 13 and 16 and NI 3, 6, and 9, the mutant band was present at less than a 1:1 ratio to the wild-type band; these samples were also those that contained <70% neoplastic cells. SI, Sweden; NI, New York; uppercase letter of sequence, base mutated; ΔC, deletion of a C; C*, cytosine known to be methylated at this site.

Mutation spectrum matched UV mutagenesis and differs from mutations in internal tumors:

p53: squamous cell carcinoma of the skin



UV



p53: internal malignancies

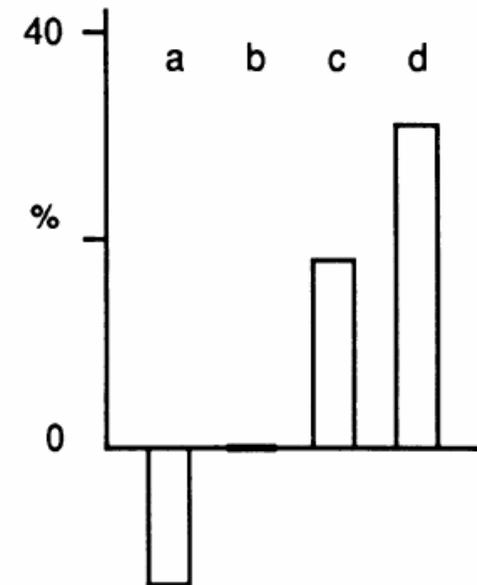
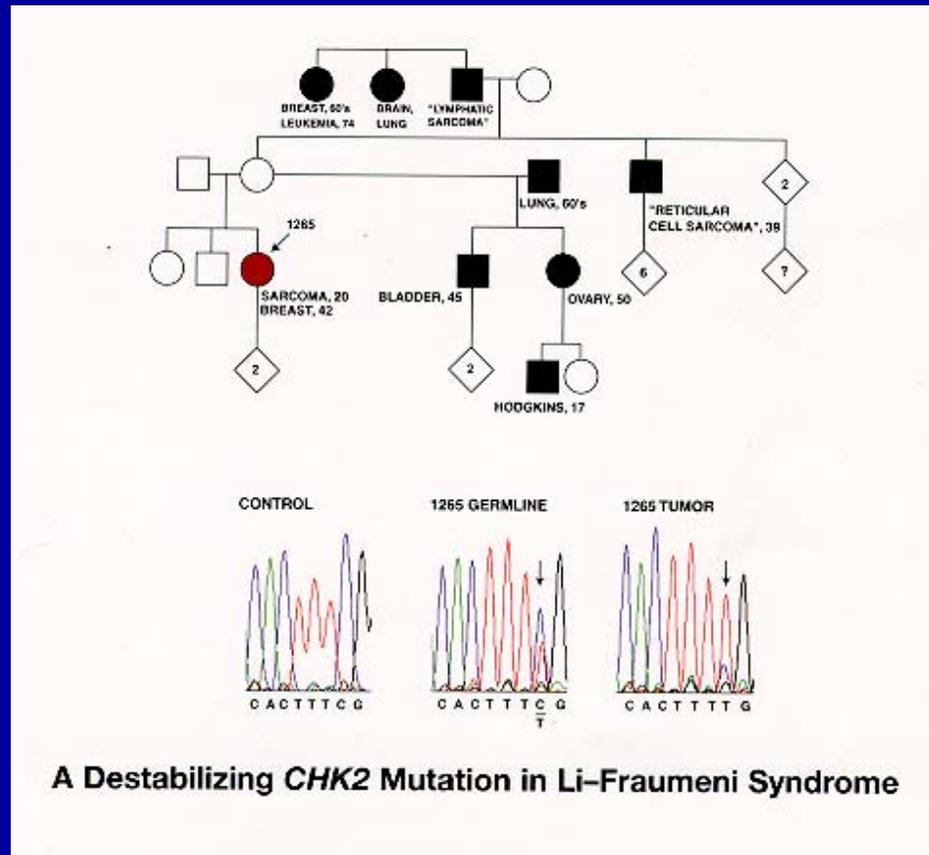


FIG. 2. Diagnostic types of base substitutions in p53 in 13 squamous cell carcinomas of the skin compared with p53 mutations reported in 97 internal malignancies (refs. 25, 27–31, and references therein) and compared with 66 UV mutations studied in endogenous genes in mammalian cells (10, 12). The comparisons are limited to endogenous genes because CG frequencies are underrepresented in mammalian DNA. The vertical axis indicates percentage of total skin squamous cell carcinoma p53 mutations, UV mutations, or internal malignancy p53 mutations that are of mutation type a, b, c, or d. Histograms: a, mutation located at a dipyrimidine site (in excess of the 75% expected randomly); b, CC → TT double-base substitution; c, C → T substitution not at a dipyrimidine site; d, C → T substitution at a CG dinucleotide.

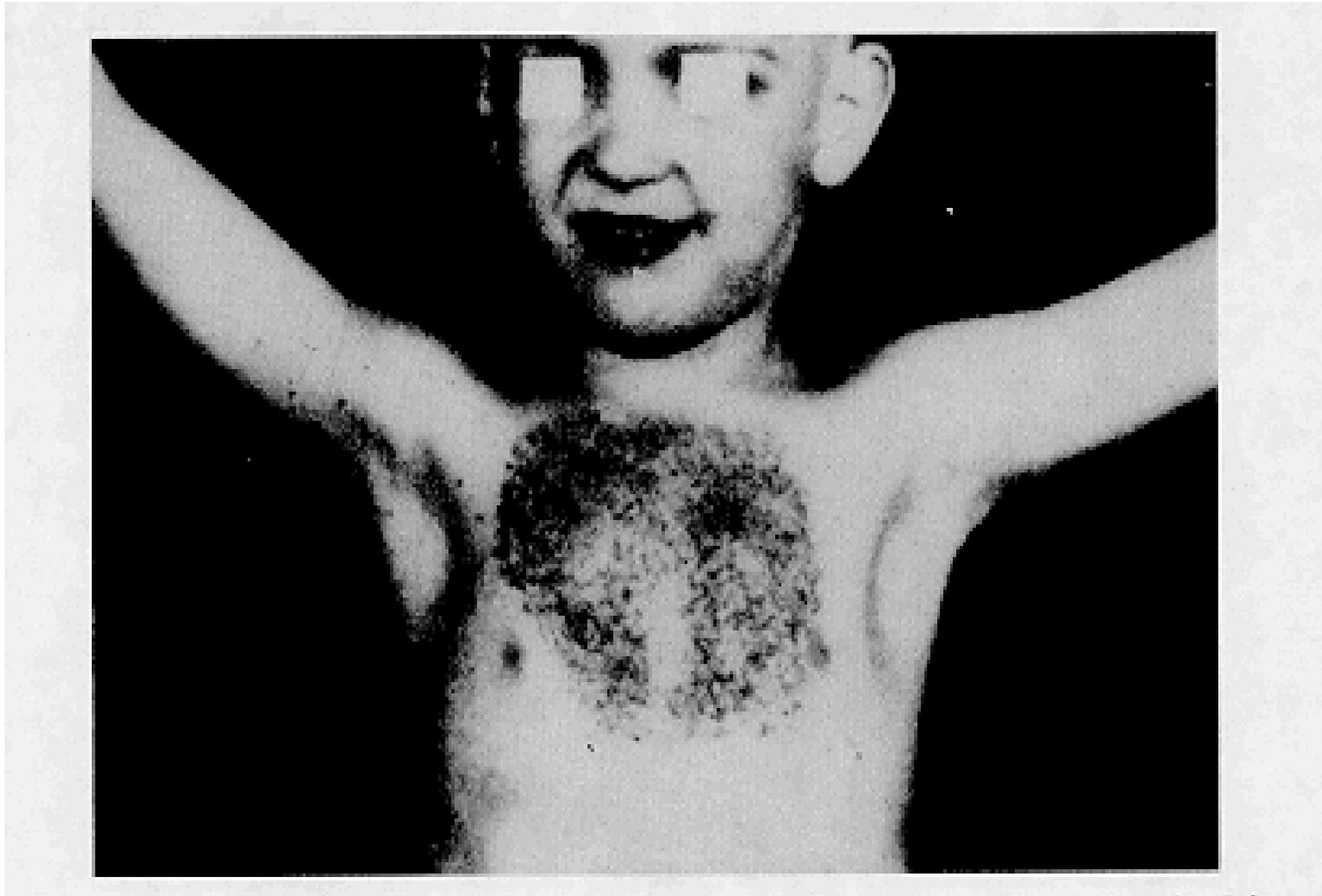
Mutations in *Chk2* produce the Li-Fraumeni phenotype



Family pedigree with proband (1265, *arrow*) diagnosed with both breast cancer and sarcoma. A heterozygous germline mutation in *CHK2* is accompanied by loss of the wild type *CHK2* allele in breast cancer of the proband. The mutant R145W allele encodes an unstable protein. (S.B. Lee et al. *Cancer Res.* 61: 8062, 2001)

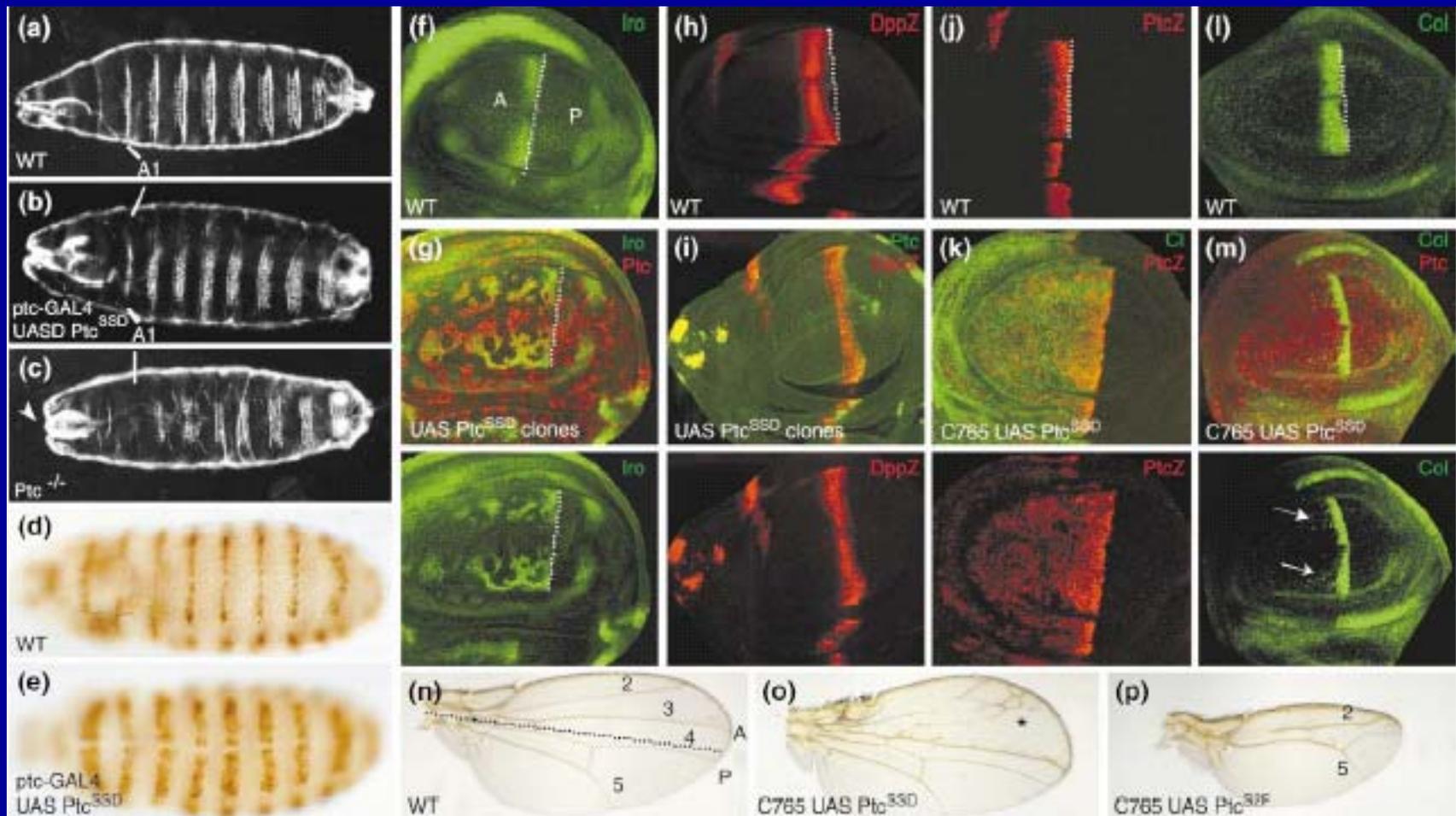
Gorlin Syndrome

- Nevoid basal cell carcinoma syndrome (NBCCS)
- Autosomal dominant disease with high penetrance
- Accounts for ~0.5% of all BCC cases (probably much higher percentage of early onset BCC)
- 20% of the patients also develop medulloblastoma and other cancers.
- Patients treated with radiotherapy develop large numbers of basal cell carcinomas in the radiation field.
- Gene responsible is the human homolog of the “Patched” gene (PTCH) in *Drosophila*, and may be a tumor suppressor in mammalian cells.
- Patched is a transmembrane signal transduction protein upstream of sonic hedgehog, Smoothed, and the proto-oncogene Gli1.
- PTCH heterozygote mice have enhanced sensitivity to radiation-induced teratogenesis.

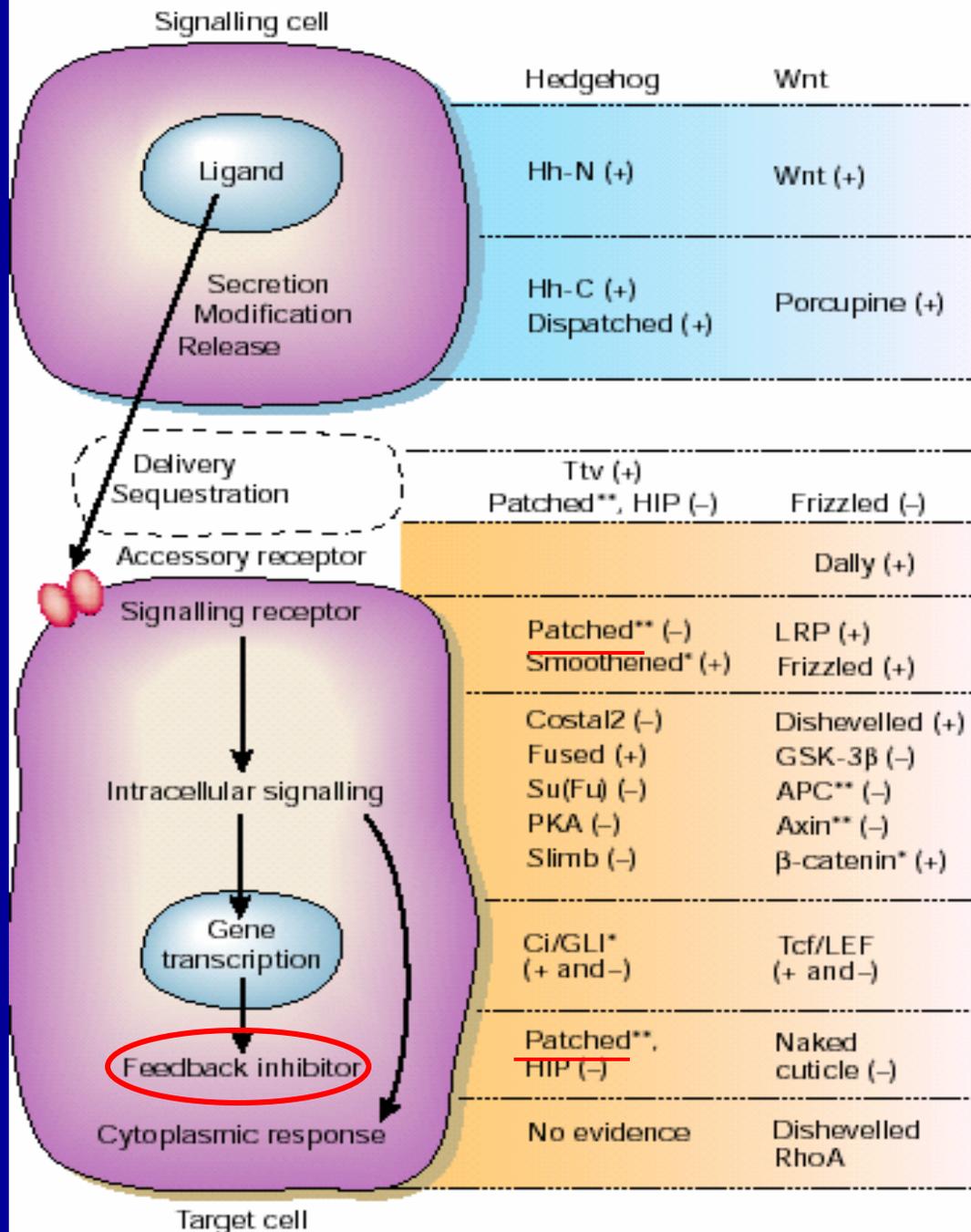


- Very early onset

- Many primary tumors



Verónica Martín, Graciela Carrillo, Carlos Torroja and Isabel Guerrero. The sterol-sensing domain of Patched protein seems to control Smoothed activity through Patched vesicular trafficking, *Curr. Biol.* 11: 601-607 (2001).



Hh and Wnt Pathways

Taipale J and Beachy PA
Nature 411:349-54 (2001)

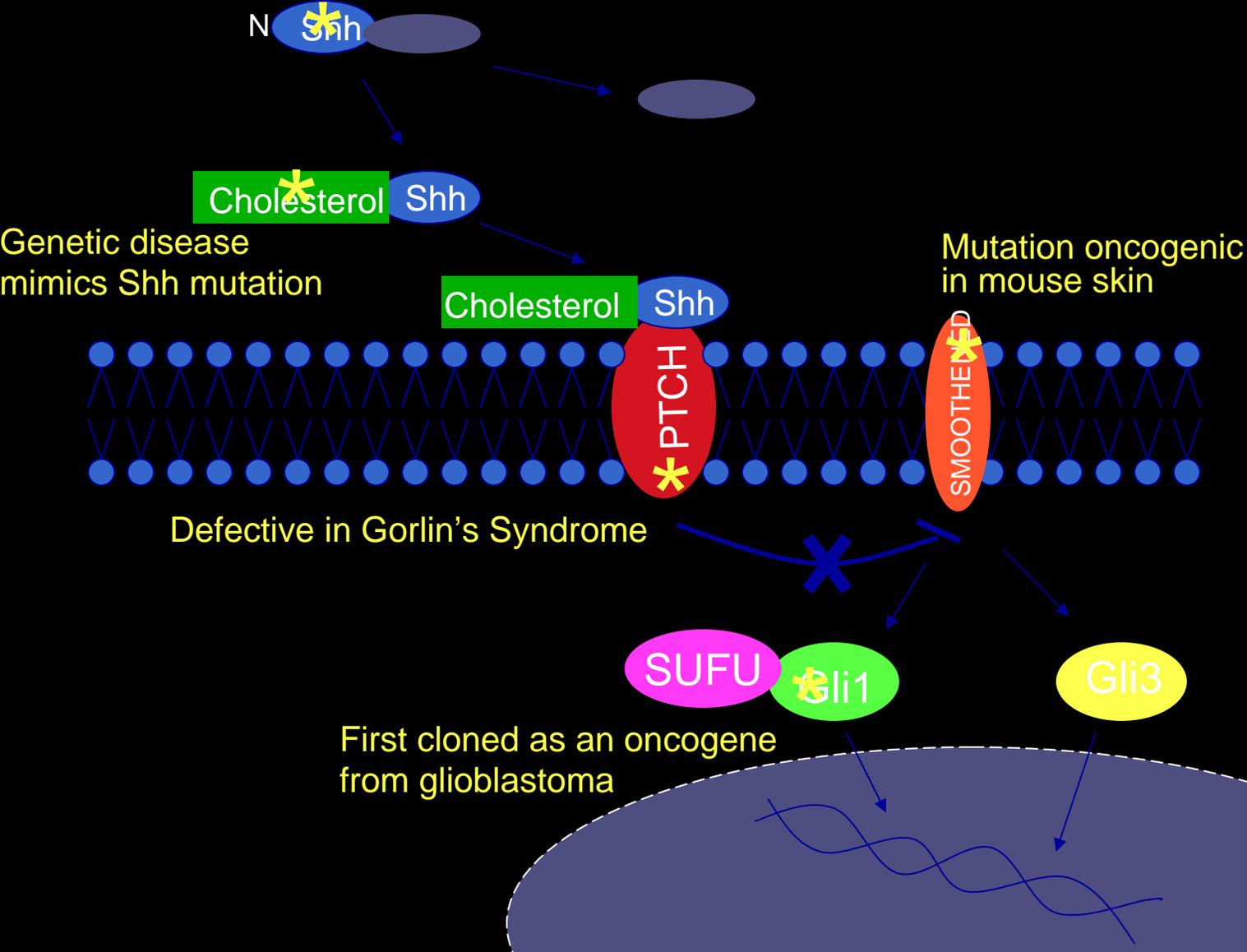
Table 1 Wnt and Hedgehog pathways in cancer

Pathway	Tumour type	Occurrence of mutations in sporadic cases	Familial syndrome, tumour incidence
Hedgehog	Basal cell carcinoma	~50%	BCNS, ~100%
	Medulloblastoma	~25%	BCNS, 1–3%
	Fibrosarcoma	ND	BCNS, low
	Rhabdomyosarcoma	ND	BCNS, very low
Wnt	Colorectal cancer	85%	FAP, very high in untreated cases
	Desmoid tumour	74%	FAP, 10%
	Hepatoblastoma	67%	FAP, <1%

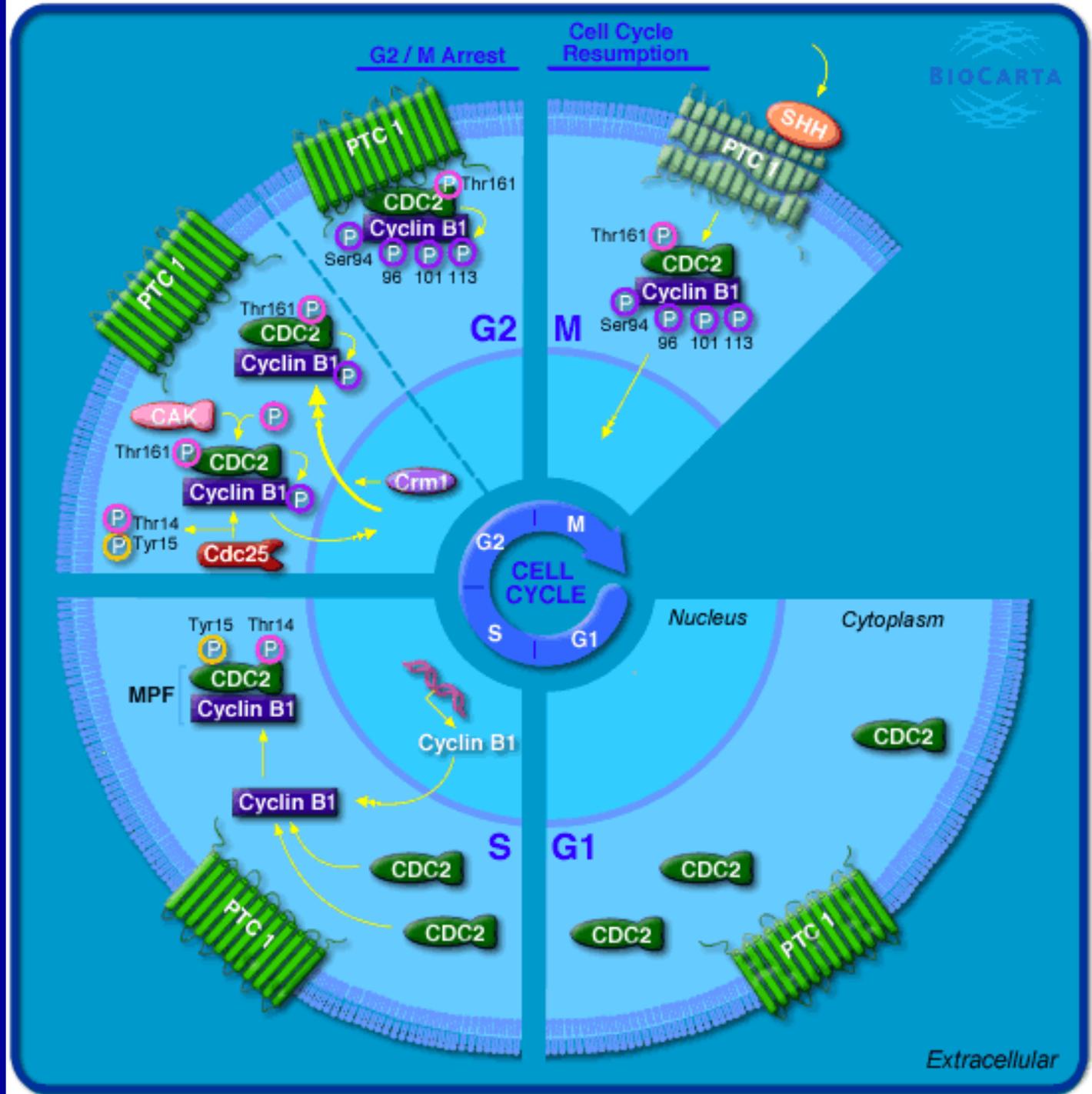
The list presented is not comprehensive and underestimates the prevalence of mutations, as neither all components of a given pathway nor transcriptional targets indicative of pathway activation have generally been examined. Included are cases where clear genetic evidence links increased cancer risk in humans or mice to a germline loss of function of a single copy of a tumour suppressor (*PTCH* in Hh, *APC* in Wnt). ND, no data; BCNS, basal cell nevus syndrome; FAP, familial adenomatous polyposis. (Source: OMIM (<http://www.ncbi.nlm.nih.gov/omim/>) and refs 2, 32, 33, 35, 47, 68–70.)

HEDGEHOG PATHWAY

Overexpression oncogenic in mouse skin



PTCH may play a role in cell cycle regulation



EMBO J. 20:2214, 2001

Radiation Carcinogenesis:

Searching for Gene/Environment Interactions

Gene-Environmental Interactions in Cancer

Which environmental carcinogens?

Which cancers?

Which genes?

Radiation as an Environmental Carcinogen:

- Known to be a human carcinogen for almost 100 years.
 - Strong epidemiological evidence shows clear dose response.
 - High dose risks known with reasonable precision/accuracy.
 - Low dose risks are highly uncertain and model dependent.
 - All tissues believed to be at risk.
 - Some risk incurred at all dose levels (i.e. no threshold).
 - Dosimetry is very good. (What is a “pack-year” anyway?!)
 - All individuals in a population are exposed to some degree.
 - Range of exposures within a population can be quite broad.
- Direct interaction with the target of carcinogenesis, and confines the problem to downstream of DNA damage.
 - Major cellular protective molecular mechanisms known in some degree of detail (e.g. DNA repair and cell cycle arrest).
 - Radiation is a relatively weak carcinogen (room for genetic enhancement).

Question of Sensitive Subpopulations

Do all people have similar risk of radiation-induced cancer, or are there genetically disposed subpopulations of individuals with very high risk?

(i.e. Are there radiation carcinogenesis genes?)

From a public health standpoint, why should we be concerned about radiation carcinogenesis genes?

- Carriers of radiation carcinogenesis genes may represent a subpopulation with significantly elevated risk for radiation-induced cancer, that needs special protection.
- It may be possible to identify high-risk individuals by genotyping.
- Identification of genes associated with increase risk of radiation-induced cancer may help identify fundamental mechanisms of carcinogenesis, and thus identify targets for cancer prevention and cure.

If there are high risk radiation carcinogenesis subpopulations, how many people would fall into these subpopulations?

- The best estimates for cellular radiosensitivity subpopulations are a little less than 10%, but radiation carcinogenesis subpopulations are more difficult to estimate.
- If the subpopulations represent a large percentage of the total population, then their risk levels would have already been incorporated into the population risk estimates.
- If the subpopulations are relatively small, then the subpopulations might have risks substantially larger than the mean risk for the population.

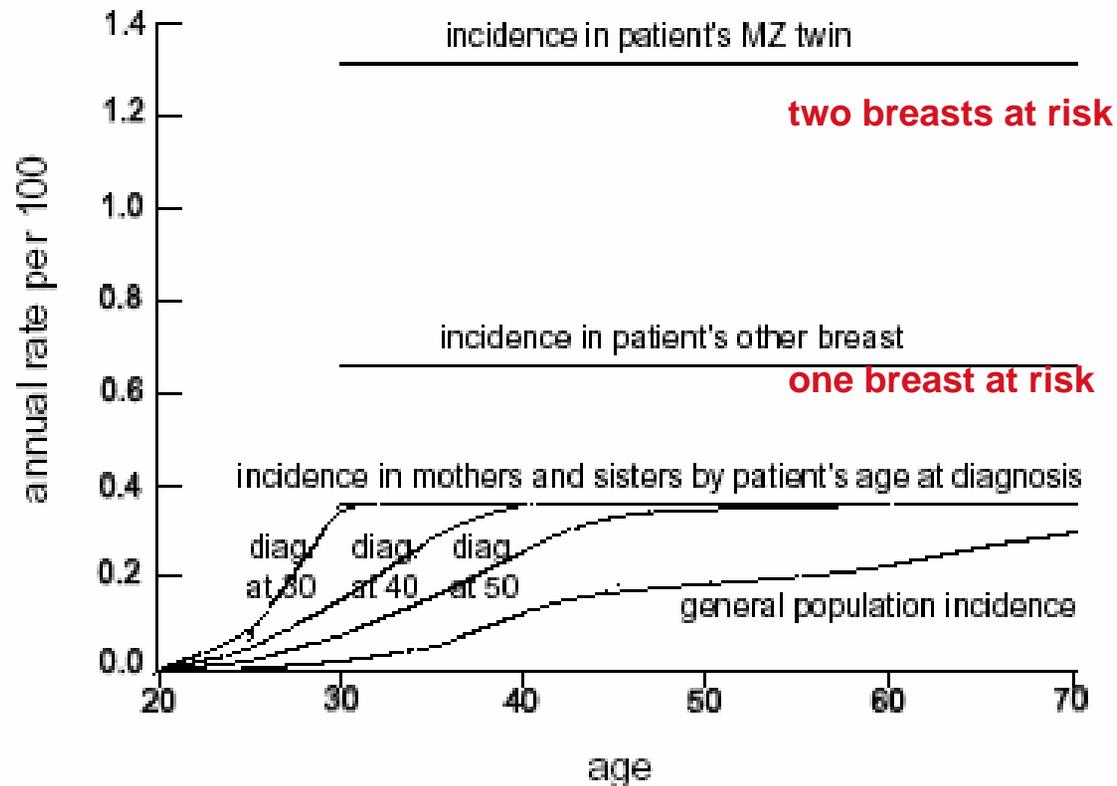


Fig. 1 The suggested pattern of breast cancer incidence in patients' relatives, and general population (Connecticut 1968-72) rates¹⁵

Peto and Mack, *Nature Genetics* 26:411, 2000

“[These data suggest that] a high proportion, and perhaps the majority, of breast cancers arise in a susceptible minority of women.”

What would be the magnitude of their excess risk?

- Difficult to determine, but individual risk could be substantial. (Bomb data for breast cancer suggests as high as 10-20 fold.)
- Could be expressed in different ways (e.g. early onset of common tumors, multiple occurrences of different tumors, increased incidence in specific tissues).

GENETIC SUSCEPTIBILITY TO CANCER

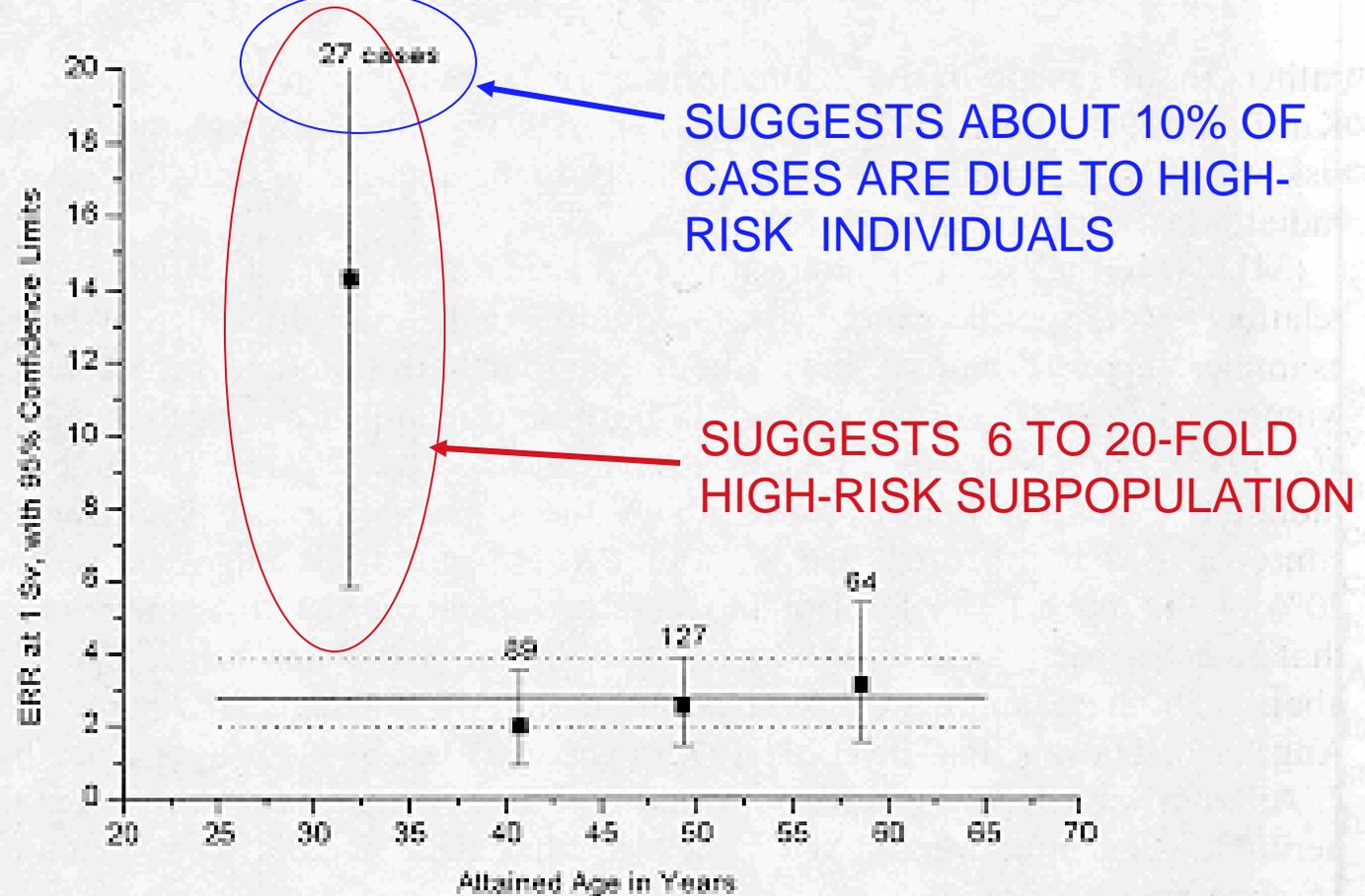


Fig. 5.7. Dose-specific risk of radiation-related breast cancer among female A-bomb survivors exposed before age 20 years of age: overall (horizontal lines) and by age at diagnosis (points with error bars).

Are there any clues to identifying candidate radiation carcinogenesis genes?

- Look at genes associated with a genetic predisposition to cancer.
- Look at genes within radiation signal transduction pathways.
- Look at genes in error-free, rather than error-prone DNA repair pathways (e.g. mismatch repair and base-excision repair).
- Look at genes in cell cycle arrest pathways.

Genetic conditions associated with both high cancer susceptibility and radiation response pathways:

- **Ataxia telangiectasia** is the classic human disease of cellular sensitivity to ionizing radiation (Cells mutated in **ATM** 3-fold more sensitive to cell killing compared to normal). Patients have increased T-cell lymphomas, radiation association is weak.
- **BRCA1** and **BRCA2** are breast cancer susceptibility genes which may play a role in radiation resistance. (Knock out embryos are radiation sensitive, and BRCA1 is phosphorylated by ATM.)
- **Rb** is a cell cycle regulative protein that is defective in **familial retinoblastoma**. Patients have extremely high incidence of retinoblastoma. (Patients appear susceptible to radiation-induced brain cancers, by means of LOH).

(continued)

Genetic conditions associated with both high cancer susceptibility and radiation response pathways:

- **p53** is a radiation response gene involved in radiation-induced cell cycle arrest and apoptosis. (Association of p53 with cellular radioresistance is variable and tissue dependent. **Li-Fraumeni** patients do not appear to have abnormal radiosensitivity. Knock out mice have nearly normal radiosensitivity.)
- Defective **PATCHED** gene results in high incidence of medulloblastoma and spontaneous basal cell carcinomas. There is also an extremely high incidence of radiation-induced basal cell carcinomas. (**Gorlin syndrome** is the only human genetic disease with bona fide increased sensitivity to radiation-induced cancer, and this occurs in the *absence* of cellular radiosensitivity.)

These genetic diseases of radiation sensitivity contribute little to either population or individual risk for radiation carcinogenesis.

- Genetic diseases are very rare in the general population and contribute little to **population risk**.
- Genetic diseases have very high penetrance for cancer phenotype. The patient's baseline risk is so high that radiation can do little to increase **individual risk**.

Low Incidence/High Penetrance

(important to affected individual)

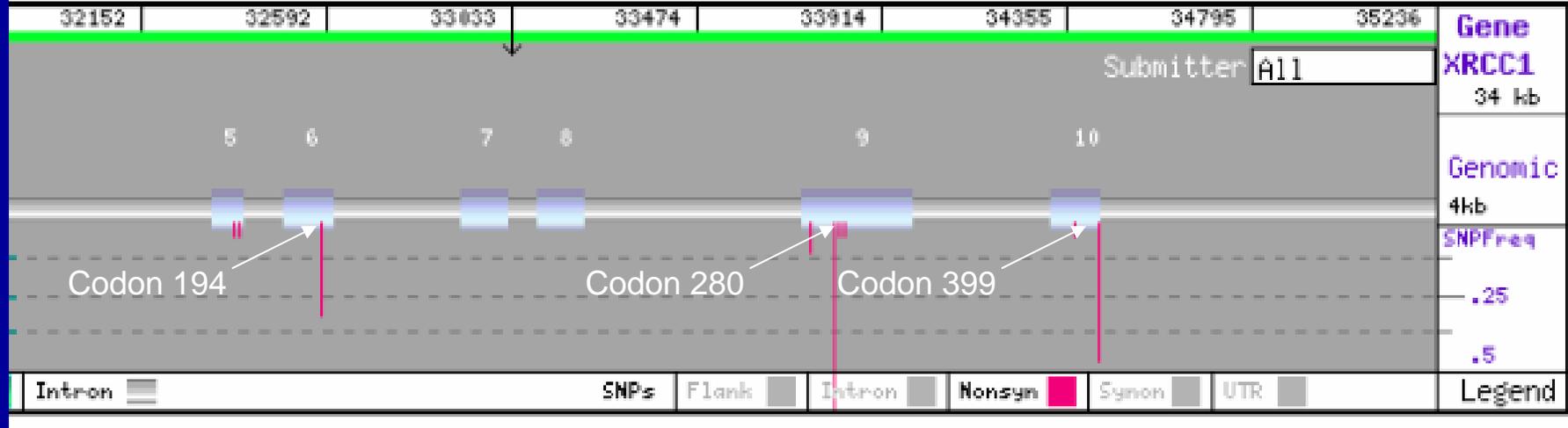
VS.

High Incidence/Low Penetrance

(important to public health)

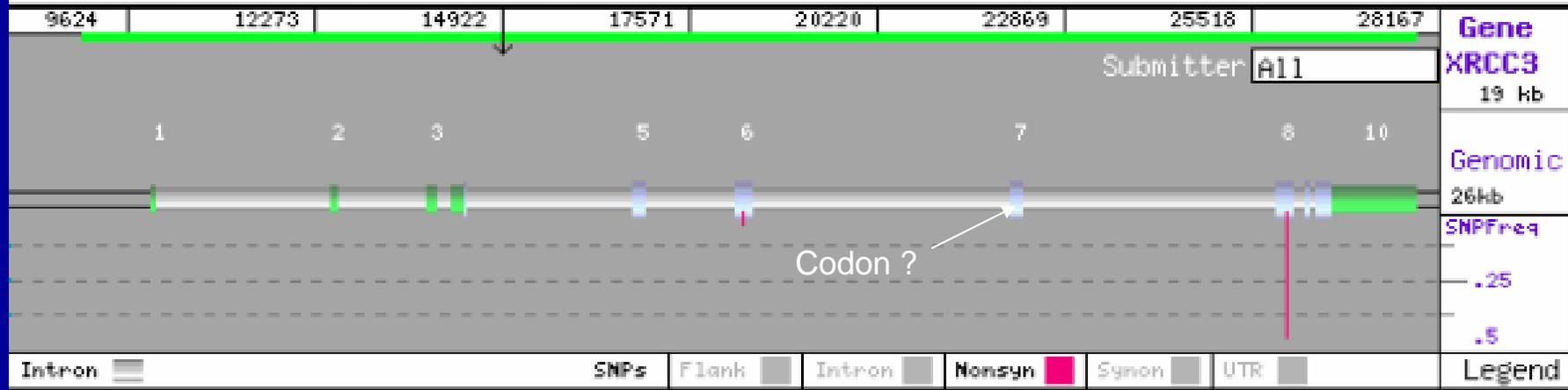
XRCC1

X-ray repair complementing defective repair in Chinese hamster cells 1



XRCC3

X-ray repair complementing defective repair in Chinese hamster cells 3



EPIDEMIOLOGIC ISSUES



Designs for detecting GxE interaction

Interaction is deviation from the expected combined effects of genes (G) and environmental (E) risk factors

We don't all react to environment in the same way...

Radiation is probably the best environmental carcinogen for discovering gene-environmental interactions.

- Dosimetry is better than for any other carcinogen. (Well controlled variable.)
- All tissues are at risk, and their relative sensitivities in normal individuals has been well quantified, at least at higher doses. (Multiple target organs for study.)
- Radioprotective molecular pathways are known with fairly good detail. (Allows for selection of candidate genes, and “binning” of data.)
- Numerous candidate cellular radioresponse biomarkers exist. (Potential to identify good markers for both dose and risk.)
- Large numbers of medically exposed populations for epidemiological study populations. (Good dosimetry and high power.)

Testing for Gene-Environment Interaction

	Envir. -	Envir. +
Genotype -	I_{00}	I_{01}
Genotype +	I_{10}	I_{11}

For simplest binary exposure (E) & genotype (G):
If I_{11} is not a simple function of I_{01} & I_{10} , there is statistical interaction

Testing for GxE Interaction

Express I_{11} as a function of the other rates & divide by baseline incidence I_{00} to get relative risks (which can be approximated by OR for rare diseases)

Additive Model

$$I_{11} = I_{10} + I_{01} - I_{00}$$

$$OR_{11} = OR_{10} + OR_{01} - 1$$

Multiplicative Model

$$I_{11} = I_{10} * I_{01}$$

$$OR_{11} = OR_{10} * OR_{01}$$

Testing for GxE Interaction (cont'd)

If there is statistical interaction, these observed rates will be different than predicted.

Null Hypotheses:

Additive:

$$H_0: OR_{int} = OR_{11} / (OR_{10} + OR_{01} - 1) = 1$$

Multiplicative:

$$H_0: OR_{int} = OR_{11} / (OR_{10} * OR_{01}) = 1$$

GxE in cohort & case-control designs

Cohort study				Case-control study		
Exposure	Genotype	Dis. risk	Rel risk	Case	Cont	Odds ratio
No	No	I	1	A_{00}	B_{00}	1
No	Yes	IR_g	R_g	A_{01}	B_{01}	$OR_g = A_{01}B_{00}/A_{00}B_{01}$
Yes	No	IR_e	R_e	A_{10}	B_{10}	$OR_e = A_{10}B_{00}/A_{00}B_{10}$
Yes	Yes	IR_{ge}	R_{ge}	A_{11}	B_{11}	$OR_{ge} = A_{11}B_{00}/A_{00}B_{11}$

- R_e : Risk among exposed non-carriers (divided by I)
- R_g : Risk among unexposed carriers (divided by I)
- R_{ge} : Risk due to interaction (divided by I)
- Yang & Khoury (1997) Epidemiol Rev 19:33-43

Study designs for GxE

Study design	Advantages	Disadvantages
Case only	Cheaper; may be more efficient	Cannot estimate main effects; Assumes G & E are independent
Case-control (unrelated)	Broad inferences for population based samples	Confounding due to pop. stratification is a danger
Case-control (related)	Minimizes potential for confounding	Overmatching for G & E; Not all cases can be used
Case-parent trios	Avoids confounding; can test for GxE & GxG	Can't test for E alone

See Andrieu & Goldstein (1998) Epi Rev 20:137-147
Goldstein & Andrieu (1999) Monograph JNCI 26:49-54.

Statistical tools for GxE tests

- Case only designs
 - Odds ratios
 - Log linear models
- Case-control designs
 - Chi-square tests on allele frequencies
 - Logistic regression predicting case status
- Case-parent trios
 - Transmission disequilibrium test (chi-square)
 - Conditional logistic regression predicting transmission or occurrence of genotype
 - Log-linear models
 - Chi-square goodness of fit statistics for families

2x2 table for case-only

R_g =risk among G+,E-

R_e =risk among G-,E+

R_{ge} =risk among G+,E+

Cases	G+	G-
E+	a	b
E-	c	d

$$OR_{CA} = R_{ge} / (R_e * R_g) * OR_{co}$$

Where OR_{co} is odds ratio (among controls) relating genotype & exposure

Example of case-only

- If genotype & exposure are independent, $OR_{co} = 1$, so OR_{CA} is a valid measure of GxE interaction
- CP cases from Hwang et al (1995)
- Case-only $OR=5.1$ (1.5-18.5)
 - $OR=5.5$ (2.1-14.8) from case-control
- Genotype of baby, exposure of mother likely independent
- Yang & Khoury (1997)

Cases	G+	G-
E+	a=36	b=7
E-	c=13	d=13

GxE in case-control design

G	E	Case	Control	Estimator
+	+	a	b	$OR_{GE}=ah/bg$
+	-	c	d	$OR_G=ch/dg$
-	+	e	f	$OR_E=eh/fg$
-	-	g	h	--

Multiplicative Interaction: $OR_{GE}/(OR_G * OR_E)$

Hwang et al. (1995) Cleft palate, TGFA & maternal smoking

Smoking	TGFA	Cases	Controls	OR*	95%CI
Non-smoker	No C2	36	167	Ref	
	C2	7	34	0.76	0.36-1.56
Smoker	No C2	13	69	0.88	0.35-2.19
	C2	13	11	7.02	1.78-27.6

*Adjusted for maternal age & parity

Hwang et al. (1995) Am J Epidemiol 141:629-636

Maternal CYP1A1 genotype & smoking with low birth weight in baby

	Mat CYP1A1	# <2500	# \geq 2500	Odds Ratio
All mothers	AA	91	334	1.01 (ns)
	Aa & aa	68	248	
Only smoking mothers	AA	18	57	2.58 (p=0.02)
	Aa & aa	22	27	

Dwyer et al (2004; in press)

Slide courtesy of Dr. Terry Beaty

Another example Pesticide exposure, GSTP1 genotype & risk to Parkinson's

	GSTP1 codon 105	Parkinson's		Odds Ratio
		Cases	Controls	
All subjects	Ile/Ile	33	39	1.31 (ns)
	Val/-	62	56	
Only exposed (pesticides)	Ile/Ile	7	14	5.33 (p=0.004)
	Val/-	32	12	

Dwyer et al (2004; in press)

Slide courtesy of Dr. Terry Beaty

Sample size & Power calculations using Quanto

- Gauderman (2002) STAT MED 21:35-50
- <http://hydra.usc.edu/gxe>
- Windows based program to calculate sample size or power to detect GxE interaction for
 - Case-Control
 - Case-Sib control
 - Case-parent trio
 - Case only

Download Quanto

- <http://hydra.usc.edu/gxe>
- Click on Quanto
- Specify
 - Design
 - Hypothesis
 - Genetic model
 - Exposure prevalence
 - Disease risk model
 - Notice marginal effects change
 - Power vs sample size
- Calculate

Quanto output

Outcome:	Disease
Design:	Unmatched case-control (1:1)
Hypothesis:	Gene-environment interaction
Desired power:	0.800000
Significance level:	0.0500, 2-sided
Gene	
Mode of inheritance:	Dominant
Allele frequency:	0.1000
Binary environmental factor	
Prevalence	0.1000
Disease model	
*P0: 0.000764	kp: 0.001000
RG: 2.0000	*RbarG: 1.9999
RE: 2.0000	*RbarE: 1.9998
RGE: 1.0000	(*indicates calculated value)

Quanto output (cont'd)

Parameter	Null	Full	Reduced
Interaction	bGE=0	bGE,bG,bE	bG,bE
Gene	bG=0	bG	----
Environment	bE=0	bE	----

N							
RGE	Interaction	Gene	Environment	P0	RbarG	RbarE	
1.0000	Cannot calculate	177	281	0.000764	1.9999	1.9998	
2.0000	1480	111	131	0.000723	2.3617	2.6356	
3.0000	558	80	82	0.000685	2.7219	3.2688	
4.0000	341	62	59	0.000652	3.0807	3.8999	
5.0000	249	51	46	0.000622	3.4384	4.5292	

N is the number of cases required for the desired power
 The required number of controls is 1xN

Minimum Sample Size

Hwang et al (1994)
AJE 140:1029-103

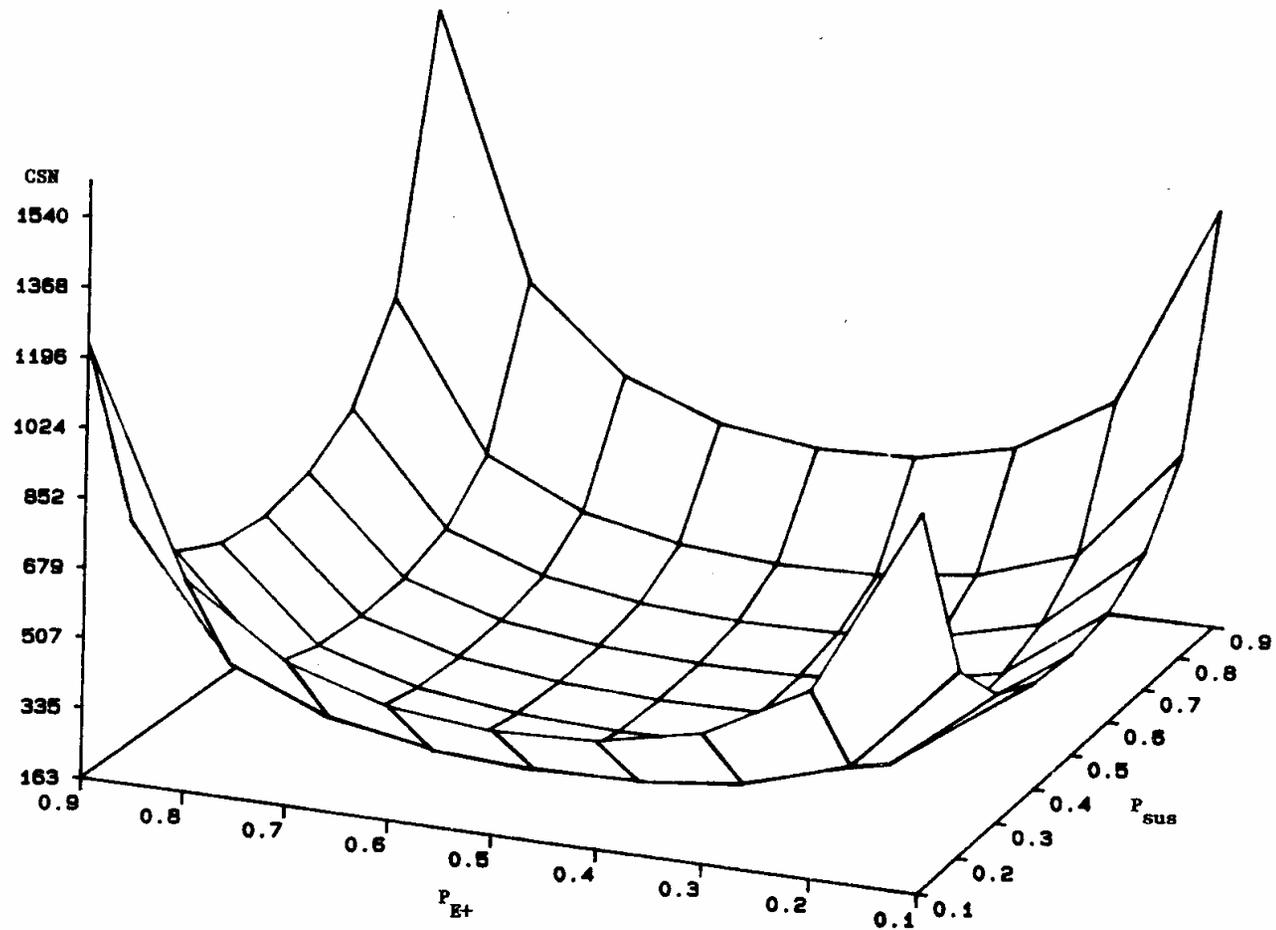


FIGURE 1. Number of cases required for 80% power at a 5% Type I error in a case-control study designed to detect gene-environment interaction with two controls per case over a series of frequencies of exposure and proportions of susceptibility. The proposed odds ratio of interaction (OR_{intr}) equals 4. The odds ratio of disease given exposure among non-susceptibles equals unity. The odds ratio of disease given the susceptible genotype among nonexposed individuals equals unity.

...depends on strength of OR_{Int}

1034 Hwang et al.

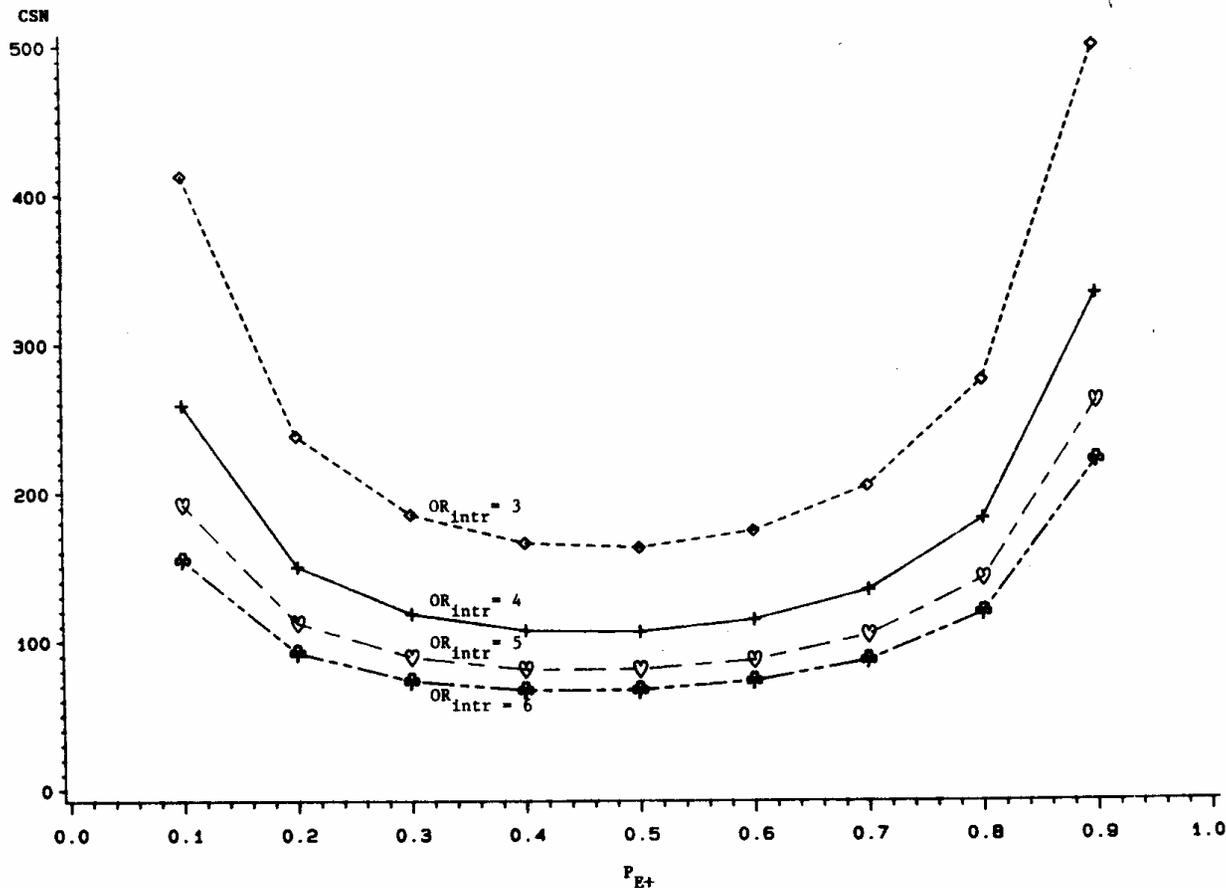
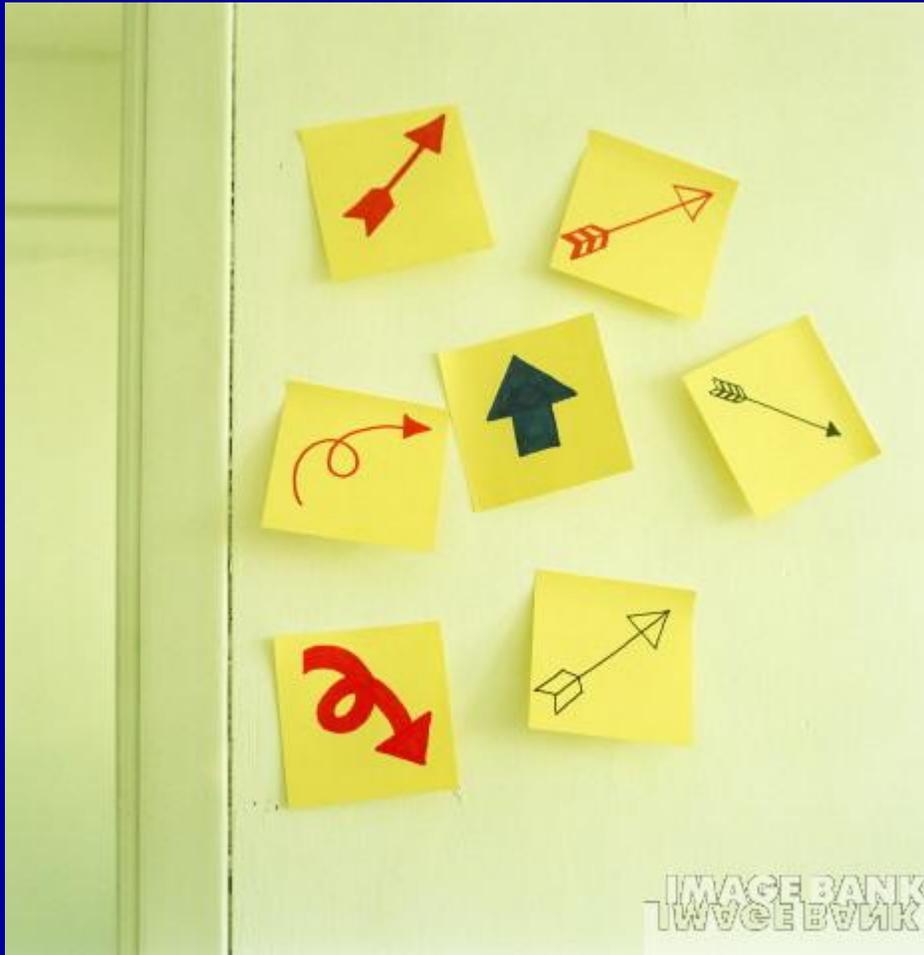


FIGURE 2. Minimum number of cases required for 80% power at a 5% Type I error in a case-control study designed to detect gene-environment interaction. Four values for odds ratios of interaction (3, 4, 5, 6) are considered. The proportion of susceptibles in the general population is 50%. The proposed odds ratio of disease given exposure among non-susceptibles equals unity. The odds ratio of disease given the susceptible genotype among nonexposed individuals equals unity. The case-control study recruits two controls per case.

Slide courtesy of Dr. Terry Beaty



CONFOUNDERS:

AGE

SKIN COLOR

DOSE

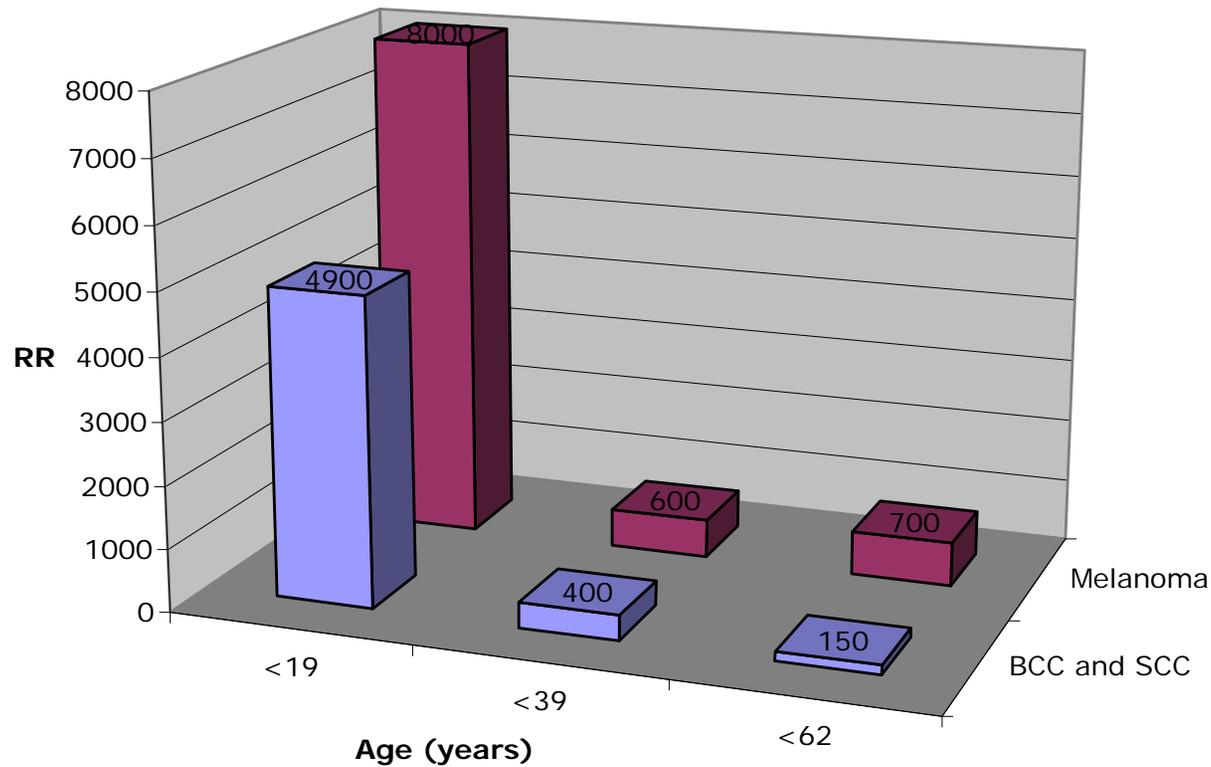
AGE



The Bare Facts of Aging
Photograph by Sarah Leen

National Geographic Magazine, November 2002

RR for XP patients by age



If age dependence of RR in XP holds true for “normal” variants, then a strong negative multiplicative interaction may be involved.

adapted from Kraemer et al. *Arch Dermatol* 130:1018, 1994

WERNER SYNDROME

- A disease of accelerated aging.
- Gene (WRN) encodes a helicase (RecQ) involved in DNA repair and DNA replication.
- Normal aging may involve decrease in DNA repair.
- Scleroderma-like skin changes.
- Increased incidence of malignancy: GI tract, lung, kidney, ovary, breast.



Age and DNA Repair Capacity (DRC) in BCC

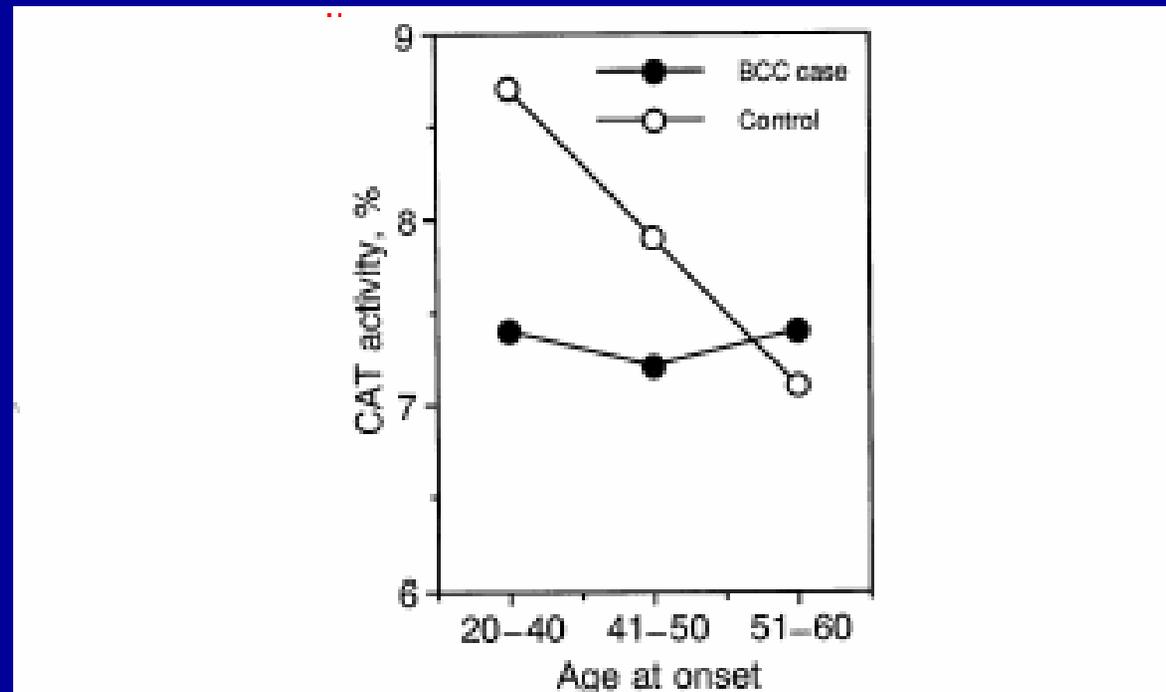


FIG. 1. Relationship between age at first BCC and DRC. The age-related decline in DRC among controls in comparison with that of age-matched cases is displayed. The linear-regression modeling and statistical tests of these data are presented in Table 5.

Age-Dependent Reactive Oxygen Species (ROS)

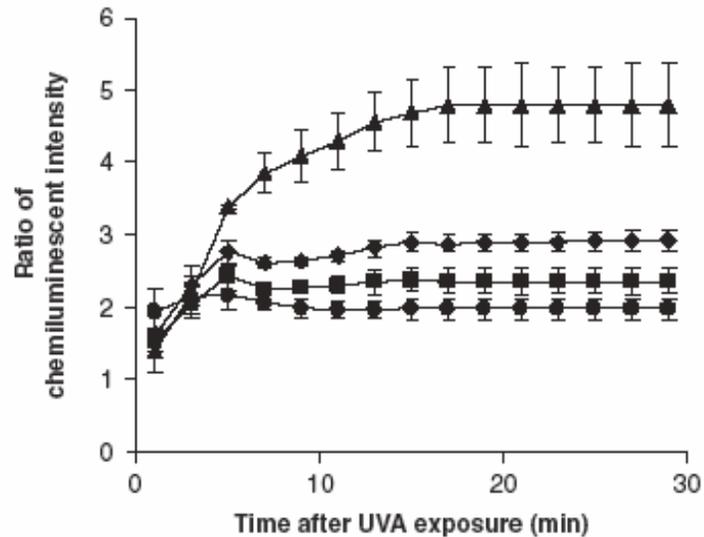
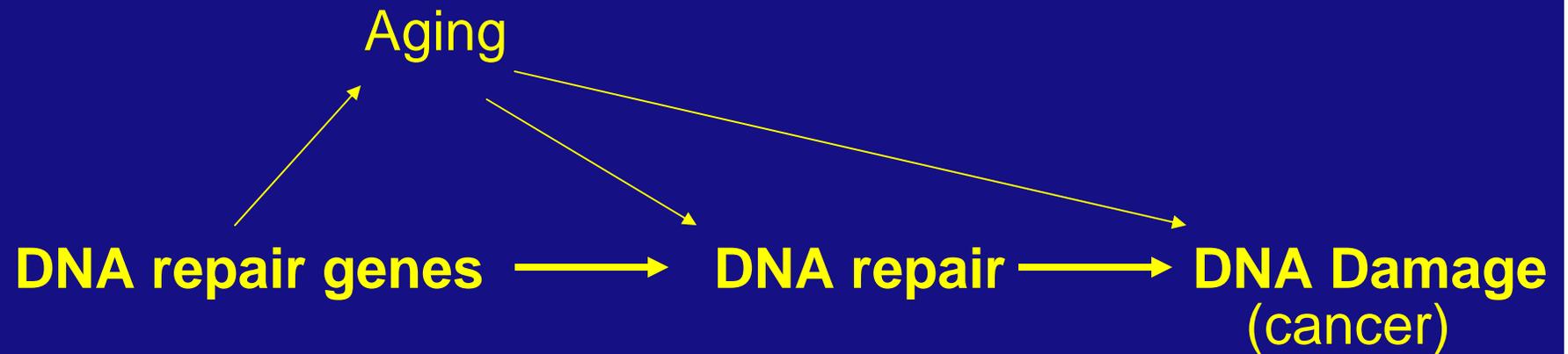
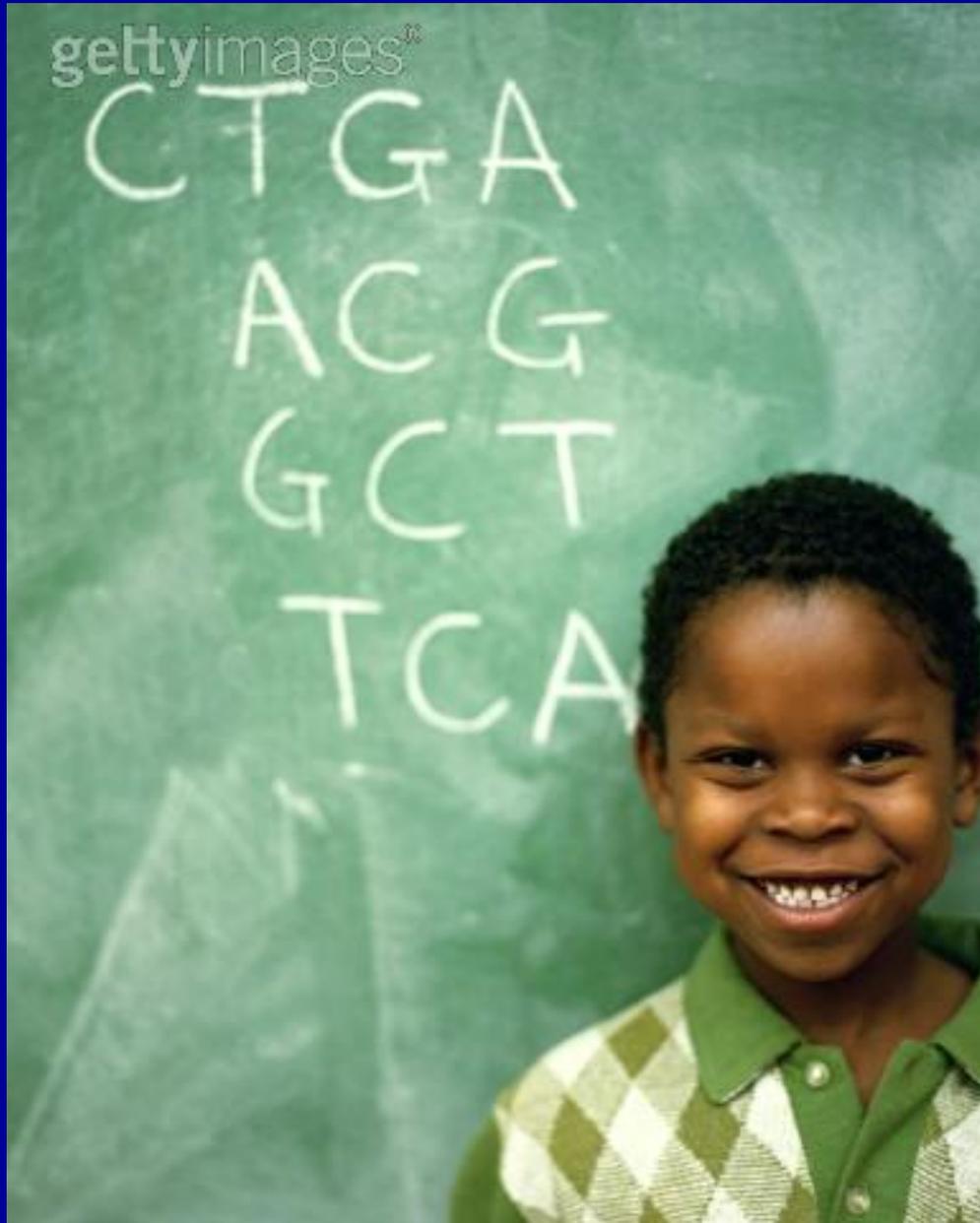


Figure 3. Age-dependent enhancement of the ratio of chemiluminescent intensities due to the ROS generation in UVA-exposed skin to those in the untreated skin of hairless rats aged 8 (●), 24 (■), 48 (◆), and 80 (▲) weeks. Data are expressed as the means \pm standard deviations of six rats in each experiment.



The multiple pathways through which aging can interact in the gene to cancer pathway, makes aging an important confounder that needs to be carefully adjusted for.

Epidemiological limitation is that we can only adjust for chronological age and not biological age.



SKIN COLOR GENETICS

Skin color is a powerful risk modifier:

Whites have ~100-fold higher BCC incidence than blacks.

Whites have ~10-fold higher SCC incidence than blacks.

SKIN COLOR GEOGRAPHY



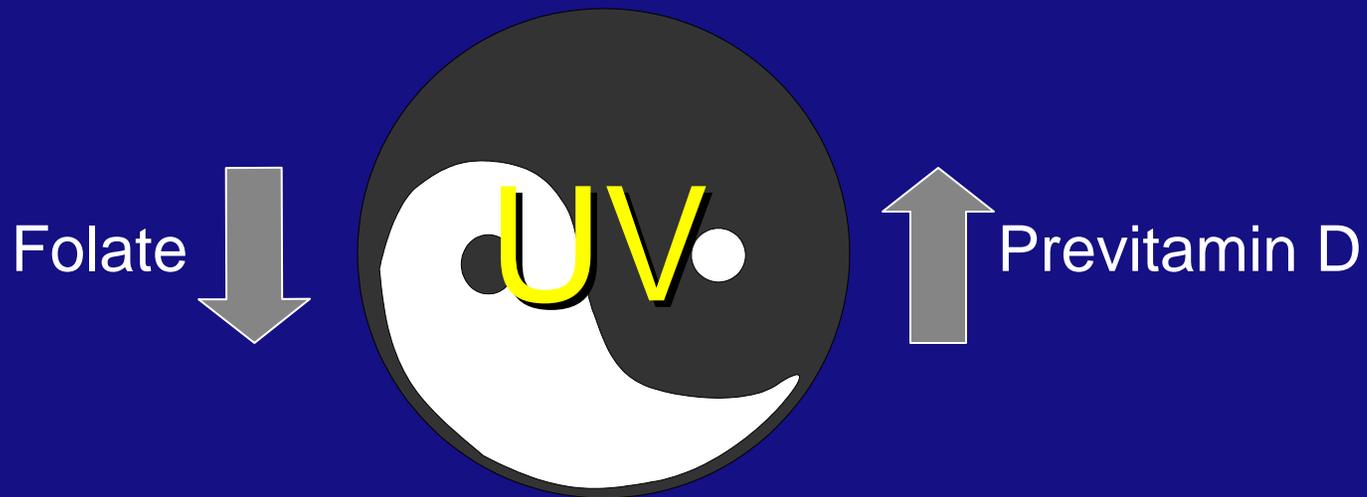
Photograph by Sarah Leen;
map created by George
Chaplin

Australian Aborigine
Glenys Martin holds a
map of human skin
colors based on global
ultraviolet radiation
intensity and
precipitation levels.

PROBLEM:

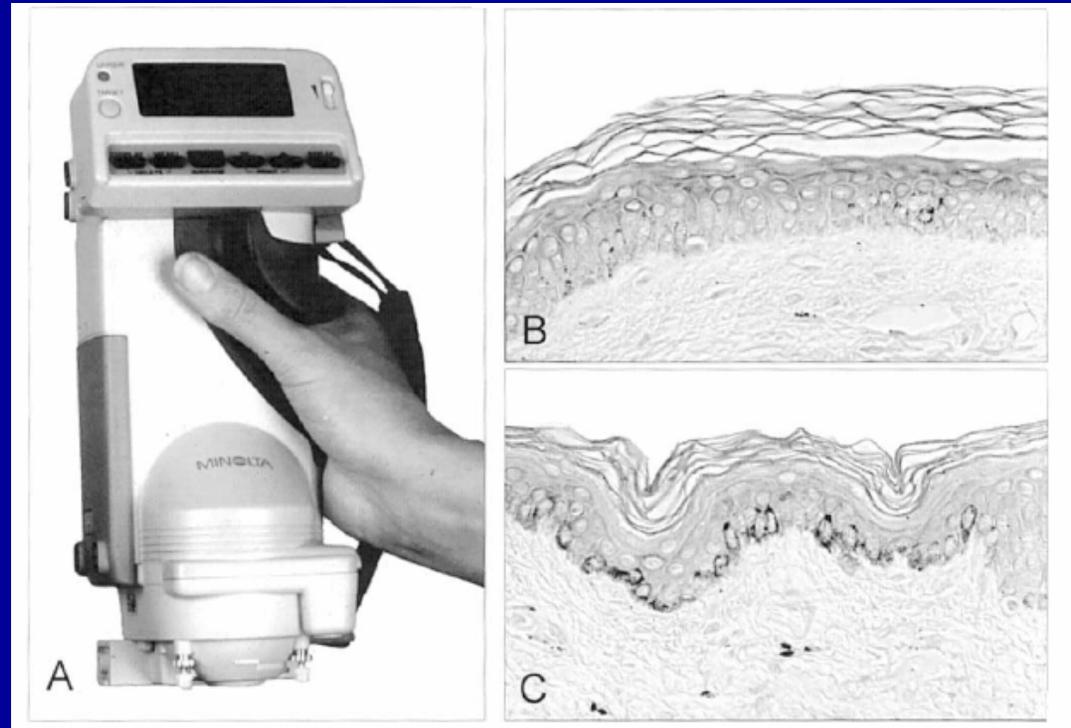
Sun burn and skin cancer are not thought to affect reproductive success. So what is the evolutionary pressure selecting for skin color correlation with UV exposure?

Competing Nutrient Hypothesis of Skin Color



Jablonski and Chaplin, *J Human Evolution* 39:57, 2000

Melanin density is inversely related to skin cancer risk.



Arm melanin (%)	No. of controls	CMM*		BCC*		SCC*				
		No. of subjects	OR*	95% CI*	No. of subjects	OR	95% CI	No. of subjects	OR	95% CI
Adjusted for age										
≥3.00	44	6	1.0		8	1.0		8	1.0	
2.00–2.99	75	22	2.2	0.8, 5.8	19	1.4	0.6, 3.5	28	2.1	0.9, 5.2
1.00–1.99	75	41	4.1	1.6, 10.4	40	2.9	1.3, 6.8	36	2.6	1.1, 6.1
<1.00	38	31	6.2	2.3, 16.6	41	6.3	2.6, 15.1	25	4.2	1.7, 10.8
Linear trend				$p < 0.01$			$p < 0.01$			$p < 0.01$

Melanocortin-1 receptor gene (MC1R) may control for skin type



American Journal of Epidemiology
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Vol. 159, No. 9
Printed in U.S.A.
DOI: 10.1093/aje/kwh120

Does the Addition of Information on Genotype Improve Prediction of the Risk of Melanoma and Nonmelanoma Skin Cancer beyond That Obtained from Skin Phenotype?

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American J. Epidemiology 159, 2004

A young girl with brown hair, seen from the back, is wearing a pink swimsuit with white polka dots. Her back is covered in a white sunscreen. She is standing on a beach with the ocean waves in the background. The word "DOSE" is written in pink capital letters in the upper right quadrant of the image.

DOSE

IMAGE
TWICE

Altered Epidemiologic Paradigm:

Radiation Dose is NOT the Exposure

Genotype = Exposure

Dose = **Effect Modifier** or **Confounder**

GENES

CONFOUNDING VARIABLES

Aging genes
(I.e. non-DNA repair)

Age

DNA repair genes

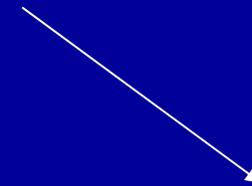
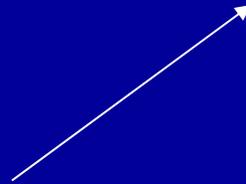
DNA repair

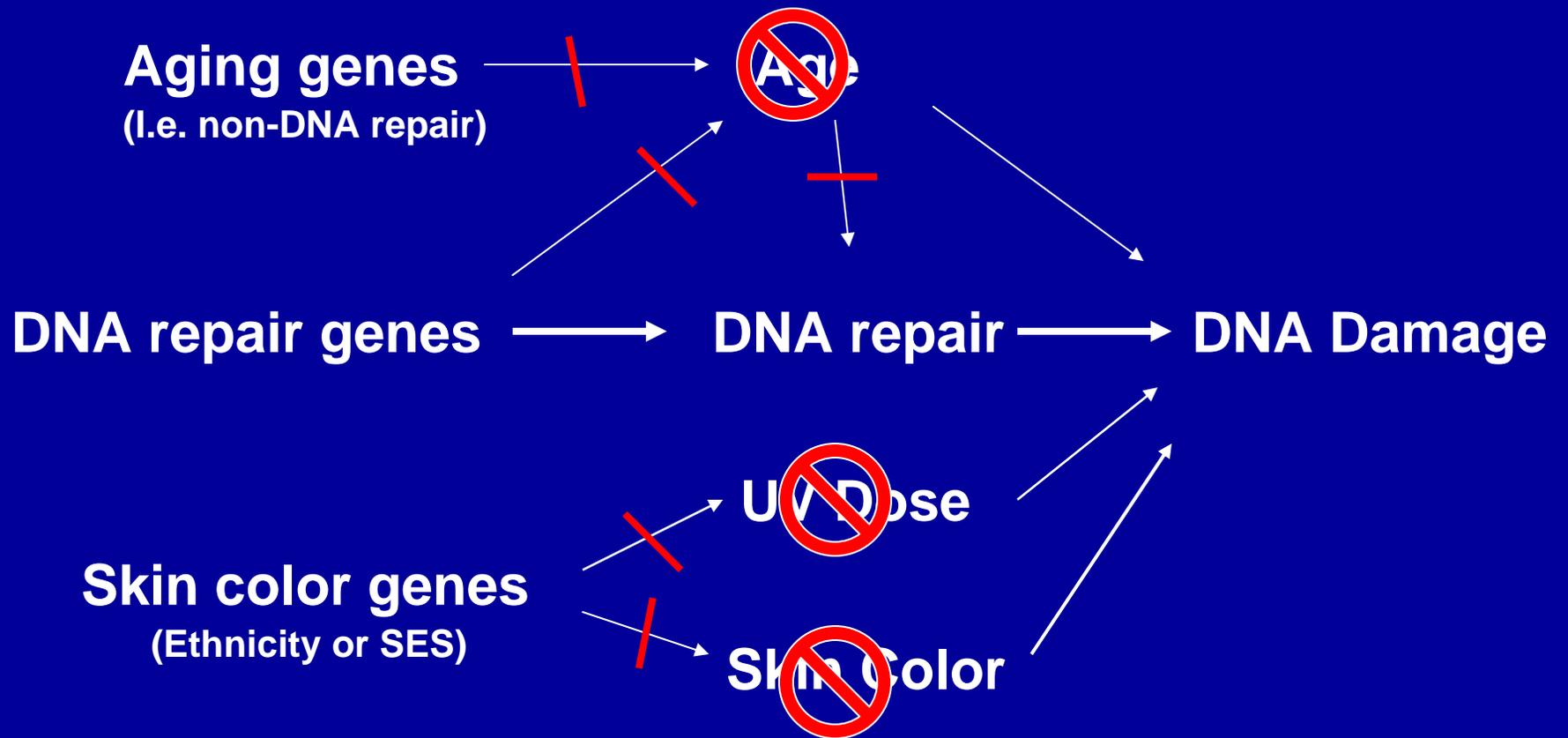
DNA Damage

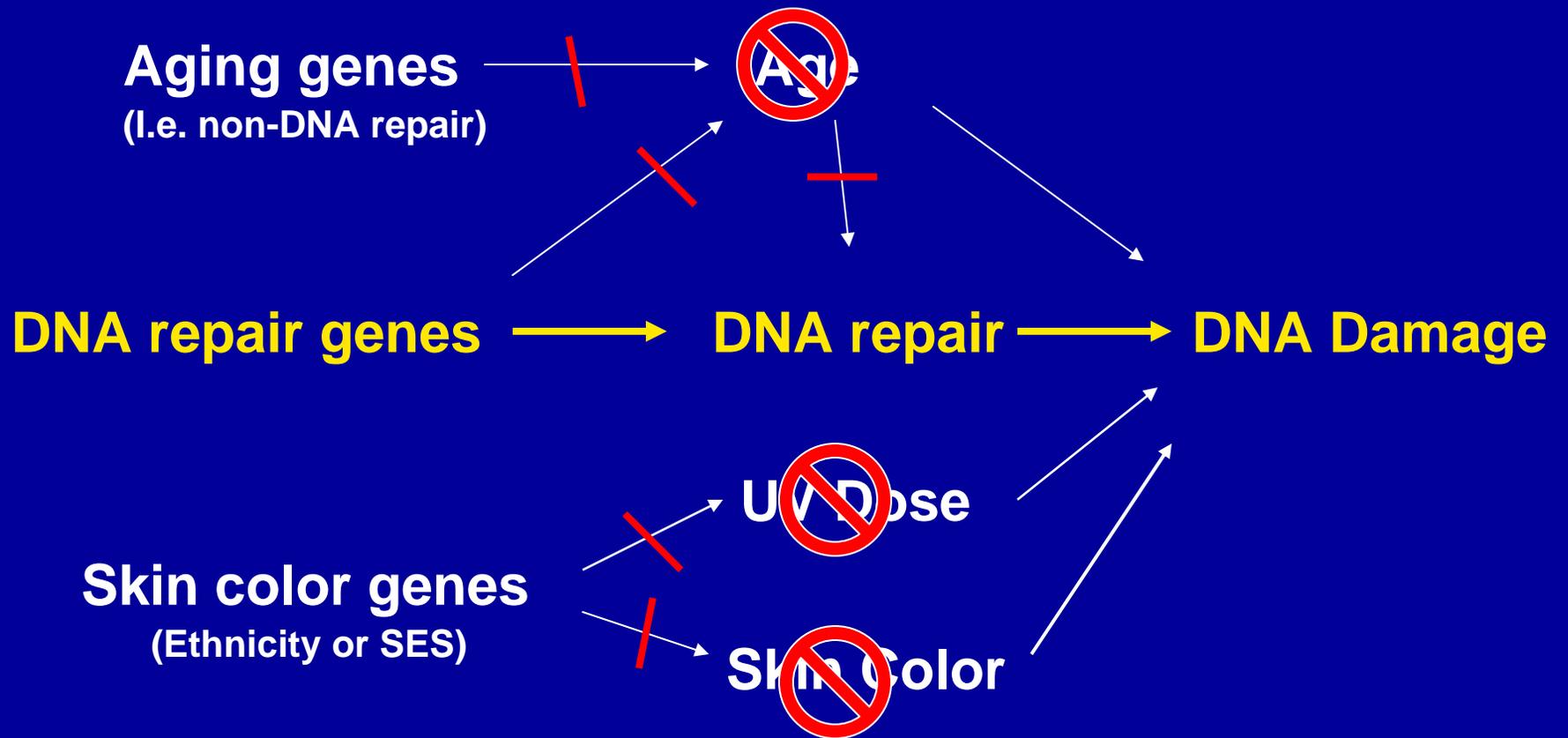
Skin color genes
(Ethnicity or SES)

UV Dose

Skin Color







TAKE-HOME MESSAGE:

- Because of good dosimetry, large numbers of medically exposed individuals, multiple tissues at risk, and reasonably strong mechanistic models, ionizing radiation is probably the **best carcinogen** for studying gene/environment interactions in humans.
- Genetic conditions which alter cellular radioresponses **without increasing cellular radiosensitivity** might provide the most promising area for discovering radiation carcinogenesis genes.
- Carefully selected **DNA repair, cell cycle, and signal transduction** pathways may offer unique opportunities for discovery.
- The **PTCH gene pathway** may offer a good opportunity for discovering high frequency/low penetrance radiation carcinogenesis genes.
- Basic mechanistic research has the potential to suggest **candidate genes** and polymorphisms, and provide intermediate phenotype **biomarkers of susceptibility**.
- Epidemiology remains the **gold standard** for proving gene/environment interactions, and basic sciences needs to pose hypotheses in a way that can be addressed by epidemiology.
- **Genetic epidemiology strengthens environmental epidemiology.**