

History of DNA Repair: Chinese Hamster Cells and DNA Repair --- **A Long-Lasting Affair**

Larry H. Thompson

BBR Program

Lawrence Livermore National Laboratory

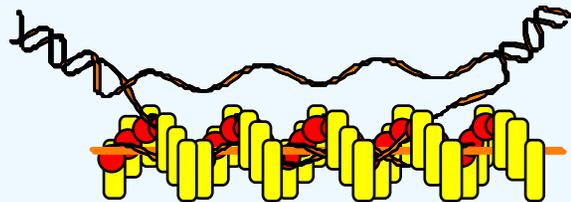
Livermore, California, USA

May 18, 2004

Chinese hamster cells meet DNA repair:
an entirely acceptable affair

Larry H. Thompson

Bioessays 20, 589-597, 1998



Thompson, L.H. and Suit, H.D.
(1967). Proliferation
kinetics of x-irradiated
mouse L cells studied with
time-lapse photography. I.
Experimental methods and
data analysis. *Int. J.
Radiat. Biol.* 13, 391-397.

Thompson, L.H. and Suit, H.D.
(1969). Proliferation
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time-lapse photography. II.
Int. J. Radiat. Biol. 15, 347-
362.

Thompson, L.H. and
Humphrey, R.M. (1969).
Response of mouse L-P59
cells to x-irradiation in the
G2 phase. *Int. J. Radiat.
Biol.* 15, 181-184.

Goal in Toronto in 1969: the isolation of somatic cell mutants



EMS award, 1986

Isolation of Temperature-Sensitive Mutants of L-Cells*

L. H. Thompson,[†] R. Mankovitz,[‡] R. M. Baker,[§] J. E. Till,
L. Siminovitch, and G. F. Whitmore

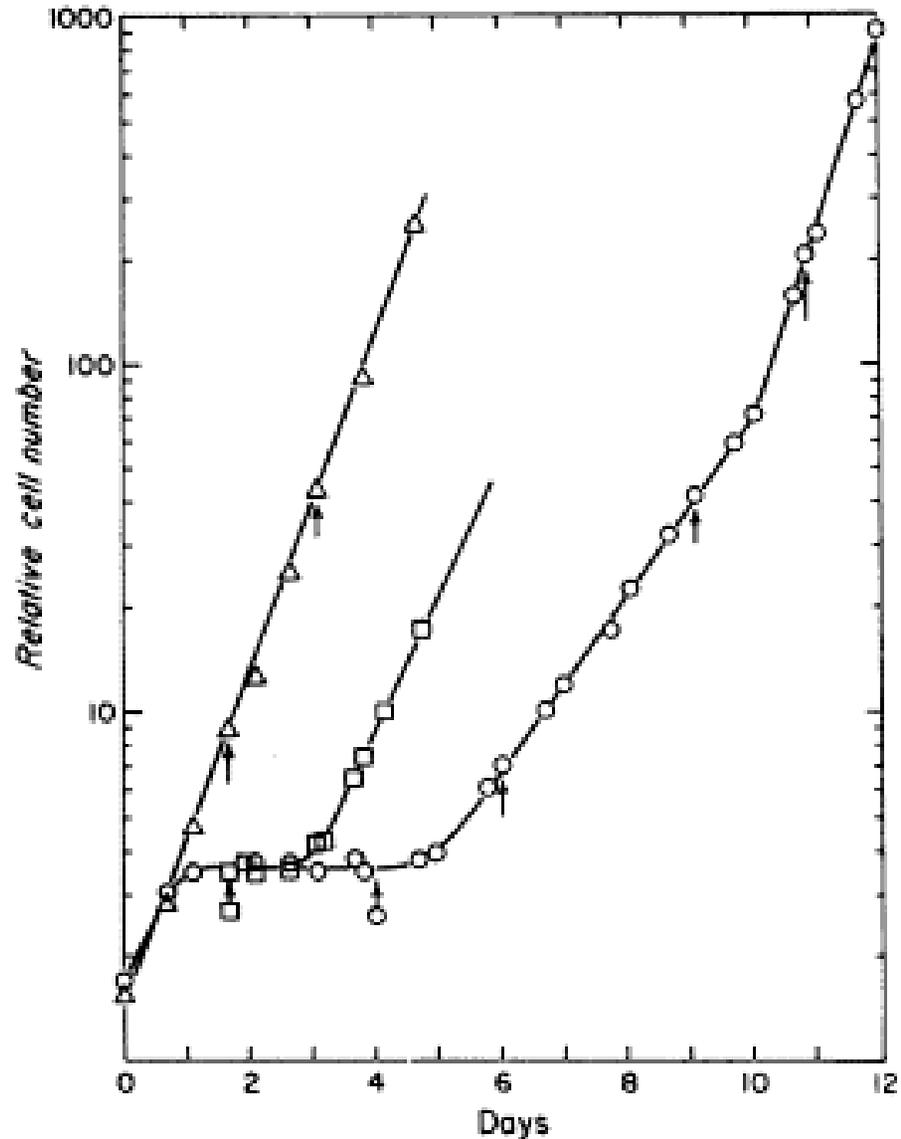
DEPARTMENTS OF MEDICAL BIOPHYSICS AND MEDICAL CELL BIOLOGY, UNIVERSITY OF TORONTO;
AND THE ONTARIO CANCER INSTITUTE, TORONTO, ONTARIO

Communicated by François Jacob, March 16, 1970

Abstract. Procedures are described for the isolation of conditional lethal mutants of mouse L-60T cells. The mutant lines were temperature sensitive by the following criteria: (a) colony-forming ability, (b) growth in suspension culture, and (c) rate of uptake of tritiated-thymidine.

The development and use of conditional lethal mutations has considerably facilitated the analysis of function in viruses and bacteria; as a result, the main features of regulation in these materials have been outlined. However, it is not yet clear how applicable these models of regulation are to eucaryotic cells and, in particular, whether or not they provide a basis for understanding the processes involved in the differentiation of mammalian cells. Although techniques have been developed for genetic studies with somatic cell hybrids,^{1, 2} progress in this area has been severely inhibited by the limited numbers and kinds of mutants available. Drug resistant lines^{3, 4} and a few auxotrophs⁵⁻⁷ have been reported,

Mouse tsA1 has a dramatic temperature-sensitive growth phenotype at 38.5°C.



The gene defective in tsA1 is later identified.

- Colwill, R.W. and Sheinin, R. (1983). ts A1S9 locus in mouse L cells may encode a novobiocin binding protein that is required for DNA topoisomerase II activity. *Proc Natl Acad Sci U S A* 80, 4644-4648.
- Brown, C.J., Powers, V.E., Munroe, D.L., Sheinin, R., and Willard, H.F. (1989). Gene on short arm of human X chromosome complements murine tsA1S9 DNA synthesis mutation. *Somat Cell Mol Genet* 15, 173-178.
- Disteché, C.M., Zacksenhaus, E., Adler, D.A., Bressler, S.L., Keitz, B.T., and Chapman, V.M. (1992). Mapping and expression of the ubiquitin-activating enzyme E1 (Ube1) gene in the mouse. *Mamm Genome* 3, 156-161.

Should we switch to haploid frog cells? --- NO

Proceedings of the National Academy of Sciences
Vol. 65, No. 2, pp. 337-344, February 1970

Stable Haploid Cultured Cell Lines from frog Embryos*

Jerome J. Freed† and Liselotte Mezger-Freed

THE INSTITUTE FOR CANCER RESEARCH, FOX CHASE, PHILADELPHIA, PENNSYLVANIA

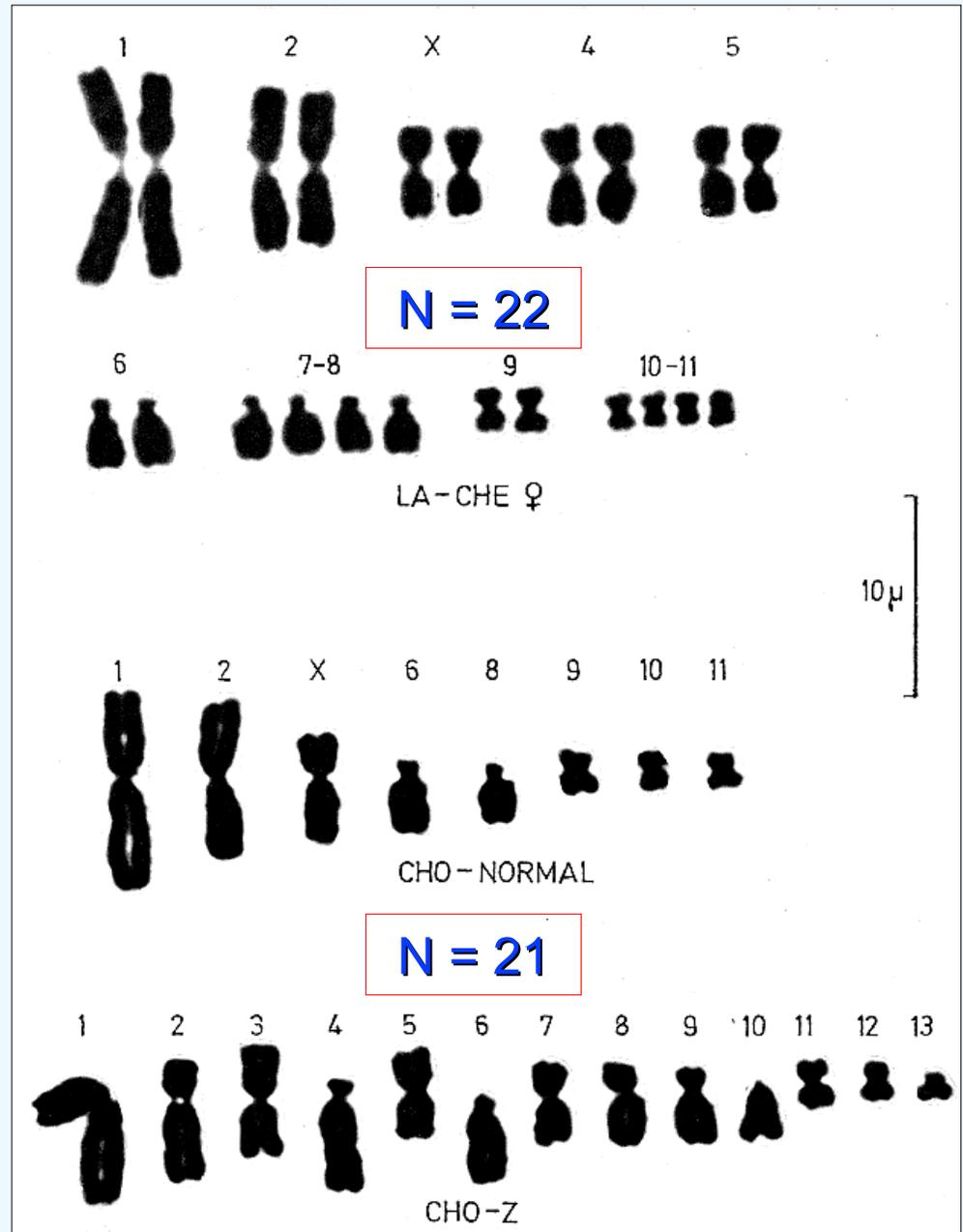
Communicated by Robert Briggs, October 31, 1969

Abstract. Two haploid cell lines have been established from androgenetic embryos of the frog, *Rana pipiens*; one line has been maintained in culture for 150 generations, the other for 200 generations. Karyotypes of the two lines agree well with the standard for the species although some chromosomes show small differences in length. The cells multiply in the same defined basal medium used for culture of other anuran cell lines; this medium consists of the usual amino acids, vitamins, and serum macromolecules plus an exogenous purine source. Both the haploids resemble “permanent” cell lines in their prolonged multiplication in culture. The two lines differ in their mode of growth, one being epithelial-like, the other forming an overlapping meshwork of fibroblast-like cells. Both have the low plating efficiency characteristic of “unaltered” cells. These two lines are exceptional in their ability to compete successfully with the diploid variants which arise by endomitosis or cell fusion and which usually overgrow the haploid population. The more vigorous line, RPH 68.2A, should provide the long-desired haploid material for genetic studies in cell culture.

Chinese hamster ovary (CHO cells)

Tjio, J. H. and Puck, T. T. (1958). Genetics of somatic mammalian cells. II. chromosomal constitution of cells in tissue culture. *J. Exp. Med.* 108, 259-271

Deaven, L.L. and Petersen, D.F. (1973). The chromosomes of CHO, an aneuploid Chinese hamster cell line. *Chromosoma* 41, 129-144.



Why use CHO hamster cells as a model system?

- Subdiploid, stable karyotype (but highly rearranged)
- Growth in both monolayer and suspension culture
- Highly amenable to synchronization by centrifugal elutriation
- Yields unusually quantitative data (e.g. survival curves and cell cycle studies)
- ~ 10,000 publications;
~ 800 on DNA repair
(~ 40,000 for HeLa cells)

HeLa cells spread rampantly.

Gartler, S.M. (1967). Apparent Hela cell contamination of human heteroploid cell lines: Genetic markers as tracers in cell culture. *Nature: Natl Cancer Inst Monogr* 217, 167-195.

Heneen, W.K. (1976). HeLa cells and their possible contamination of other cell lines: karyotype studies. *Hereditas* 82, 217-248.

Solov'ev, V.D., Khesin, I.a.E., Amchenkova, A.M., and Gulevich, N.E. (1977). Problema kontaminatsii perevivaemykh kletochnykh linii kletkami HeLa. *Vopr Virusol* 246-251.

Lavappa, K.S. (1978). Survey of ATCC stocks of human cell lines for HeLa contamination. *In Vitro* 14, 469-475.

Seed stocks of human cell lines deposited at the American Type Culture Collection (ATCC) have been examined for cross-contamination with HeLa cells using Giemsa-banded marker chromosomes. **Sixteen additional cell lines investigated have been found to exhibit marker chromosomes typical of HeLa cells.** Quinacrine fluorescence studies further revealed the absence of Y chromosome in these lines. **These observations indicate that the lines are HeLa derivatives.**

A Mammalian Cell Mutant with a Temperature-Sensitive Leucyl-Transfer RNA Synthetase

(somatic cell genetics/CHO cells/conditional lethality/inhibition of protein synthesis)

L. H. THOMPSON*, J. L. HARKINS, AND C. P. STANNERS

The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Canada, M4X 1K9

Communicated by Theodore T. Puck, July 13, 1973

ABSTRACT A cell mutant of the Chinese hamster ovary line, which is temperature sensitive for protein synthesis, is specifically defective *in vivo* in its ability to charge tRNA with leucine. Cytoplasmic extracts exhibited temperature-sensitive leucyl-tRNA synthetase activity. It is, therefore, highly likely that the mutant has a structural alteration in leucyl-tRNA synthetase. The low leakiness and low reversion rate of this mutant, combined with the specificity of the defect in its protein-synthesizing machinery, make it an appealing tool for investigating regulatory mechanisms in animal cells.

MATERIALS AND METHODS

Cells and Culture Conditions. The wild-type cells were a clone of the Chinese hamster ovary line (CHO) (8), which has a stable karyotype consisting of 21 chromosomes with little cell-to-cell variation (9). Cells from frozen stocks of this line and of the mutant *tsH1* were maintained in exponential growth in suspension culture at 34°. The growth medium was α -minimal essential medium (10) supplemented with antibiotics and 10% fetal-bovine serum (Flow Laboratories). Tem-

Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*n*-nitrosoguanidine; *ts*, temperature sensitive; CHO, Chinese hamster cell urine.

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Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells

(complementation analysis/mutagen sensitivity/tester strains/7-bromomethylbenz(a)anthracene)

LARRY H. THOMPSON*, DAVID B. BUSCH†‡, KERRY BROOKMAN*, CAROLYN L. MOONEY*, AND DONALD A. GLASER†

*Biomedical Sciences Division, L-452, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, California 94550; and †Department of Molecular Biology, University of California, Berkeley, California 94720

Contributed by Donald A. Glaser, February 17, 1981

ABSTRACT Mutant lines of Chinese hamster ovary cells that show hypersensitivity to killing and mutagenesis by UV light were analyzed by genetic complementation analysis to determine whether defects in different gene loci might underlie a common cellular phenotype. To facilitate rapid screening of mutant clones, a procedure was devised that allowed presumptive complementation to be assessed on the basis of the frequency of UV-resistant cells after fusion by polyethylene glycol. Four classes were identified among 44 clones tested. By using drug-resistance markers for selection of hybrid cells in crosses between UV mutant and wild type, a mutant from each of the four classes was shown to behave as phenotypically recessive. Hybrids were also isolated from crosses between each of the pair combinations of the four mutants. All such hybrids were relatively resistant to UV killing, providing confirmation of the complementation classes. When mutants representing the four UV-complementation classes were tested with the polyaromatic hydrocarbon 7-bromomethylbenz(a)anthracene, complementation was again seen for all pair combinations. These results suggest that each class of mutants represents a biochemical defect that plays a common role in the repair of both UV-induced and chemically induced lesions in the DNA.

tation groups on the basis of their responses to either UV or the chemical mutagen 7-bromomethylbenz(a)anthracene (7-Br-MeBA). These results show that different genetic defects can underlie the UV-sensitive phenotype and suggest the possibility of a genetic analysis of repair functions in CHO cells.

MATERIALS AND METHODS

Cells and Culture Conditions. The wild-type CHO lines, SC1 and AA8, and culturing techniques have been described (11); UV-sensitive clones were obtained from AA8 (9, 10). Mutants designated UV-24 and UV-41 correspond, respectively, to lines previously labeled as 60-21 (10) and 361-112-10b (12). Hybrids are designated by the prefix "Hy" and a suffix for the clone number. Doubling times of mutants and hybrids were 13-16 hr.

Exposure to UV. Cells were exposed to germicidal UV on plastic dishes as described (11).

Isolation of Drug-Resistant Lines. For hybridization, drug-resistance markers were introduced into certain lines. Thioguanine resistance was obtained at 12 μ M and ouabain resistance

Role of DNA repair in mutagenesis of Chinese hamster ovary cells by 7-bromomethylbenz[*a*]anthracene

(mutagen sensitivity/mutation induction/sister chromatid exchange/thioguanine resistance/ouabain resistance)

LARRY H. THOMPSON, KERRY W. BROOKMAN, ANTHONY V. CARRANO, AND LARRY E. DILLEHAY

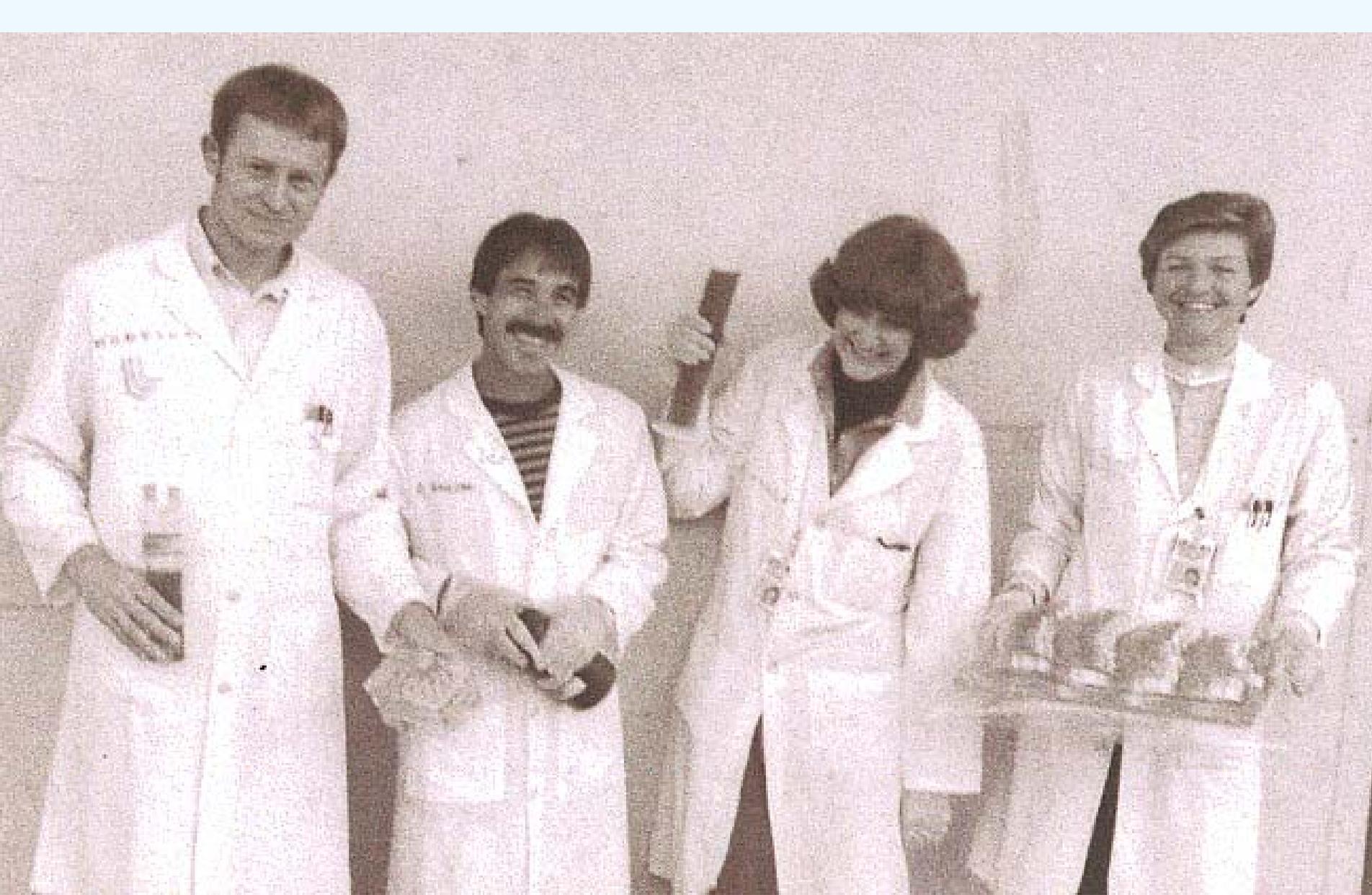
Biomedical Sciences Division, L-452, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, California 94550

Communicated by Richard B. Setlow, September 17, 1981

ABSTRACT The role of DNA repair in mutagenesis was studied in normal, repair-proficient Chinese hamster ovary cells and in two mutant strains that are deficient in excision repair. By using the mutagen 7-bromomethylbenz[*a*]anthracene (7-BrMeBA) and the technique of alkaline elution of DNA, the mutants were found to be defective at or before the incision step of excision repair. Dose-responses were determined for cell killing, mutation induction at three loci, and sister chromatid exchanges over a survival range of 1.0-0.1 after 7-BrMeBA treatment. The mutants were

one study presented exceptional results (15). Moreover, enhanced viral mutagenesis in UV-treated host cells has suggested the presence of mutagenic repair (16, 17). In rat hepatoma cells the increase in x-ray-induced mutations under conditions of uncoupling of oxidative phosphorylation has also been interpreted as reflecting error-prone repair (18).

The recent isolation of repair-deficient mutant strains of Chinese hamster ovary (CHO) cells provides a new system in which the involvement of repair in mutagenesis can be quan-

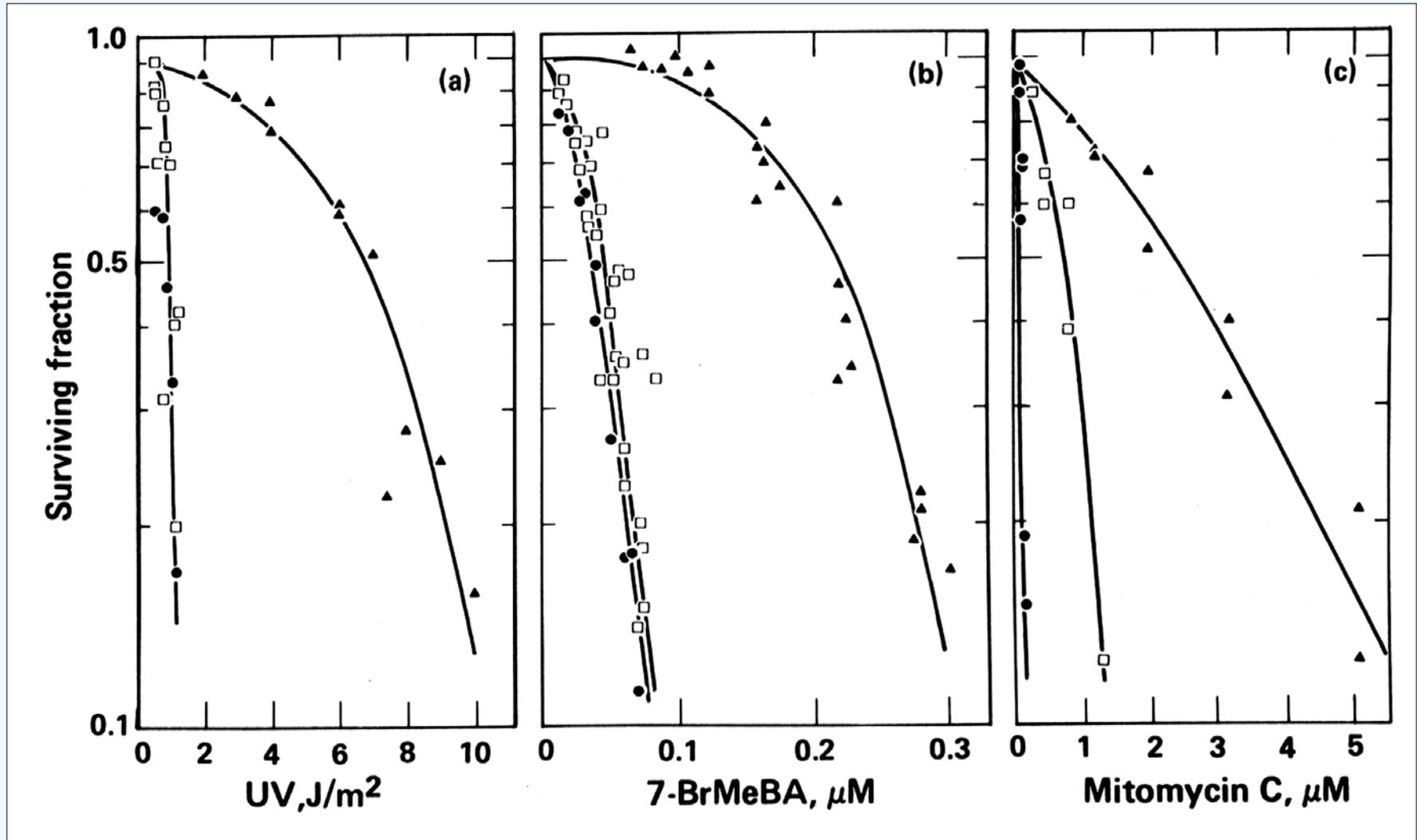


Mutagen sensitivity profiles for *ERCC1* and *ERCC2/XPD* CHO UV-sensitive mutants

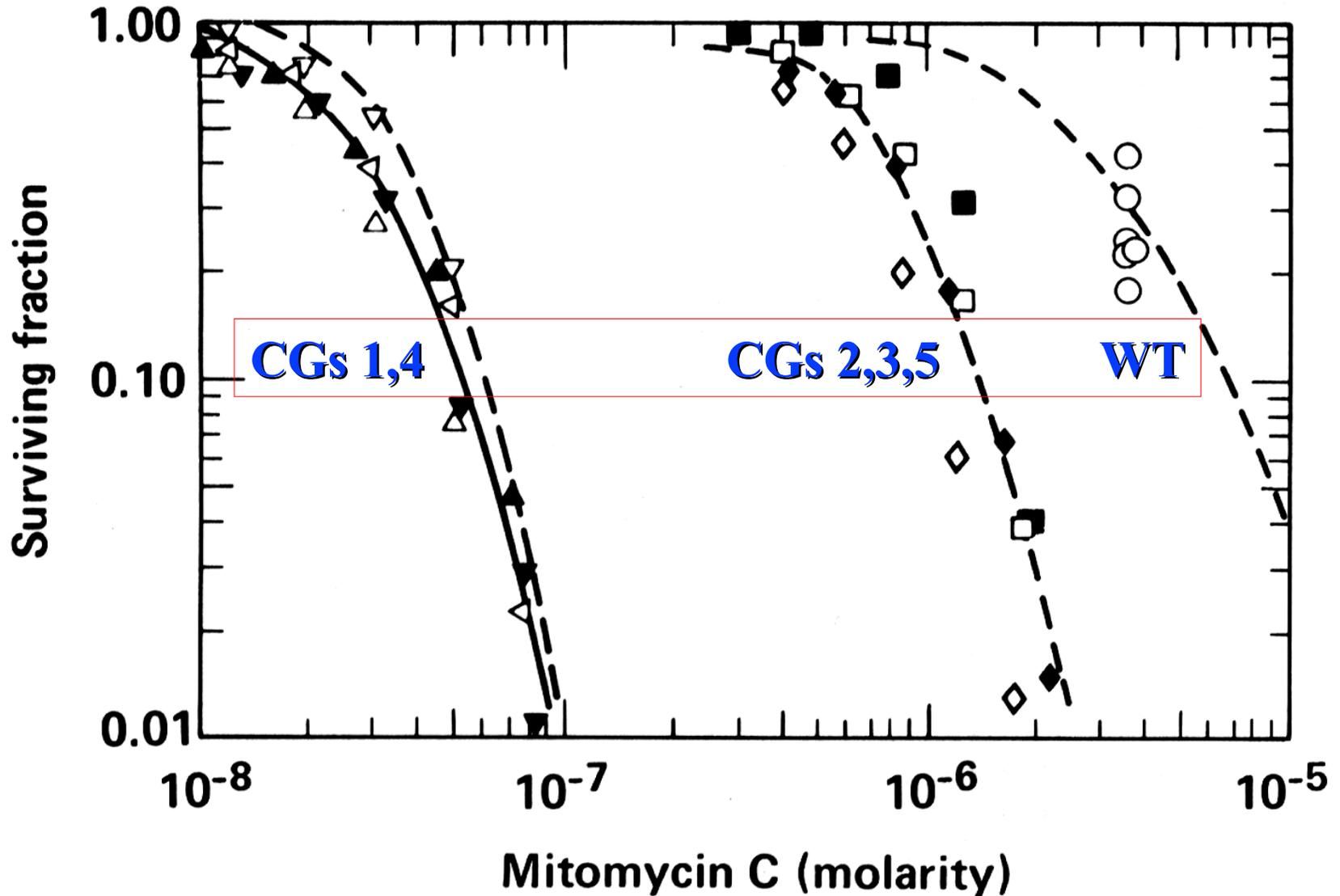
UV photoproducts

Bulky monoadducts

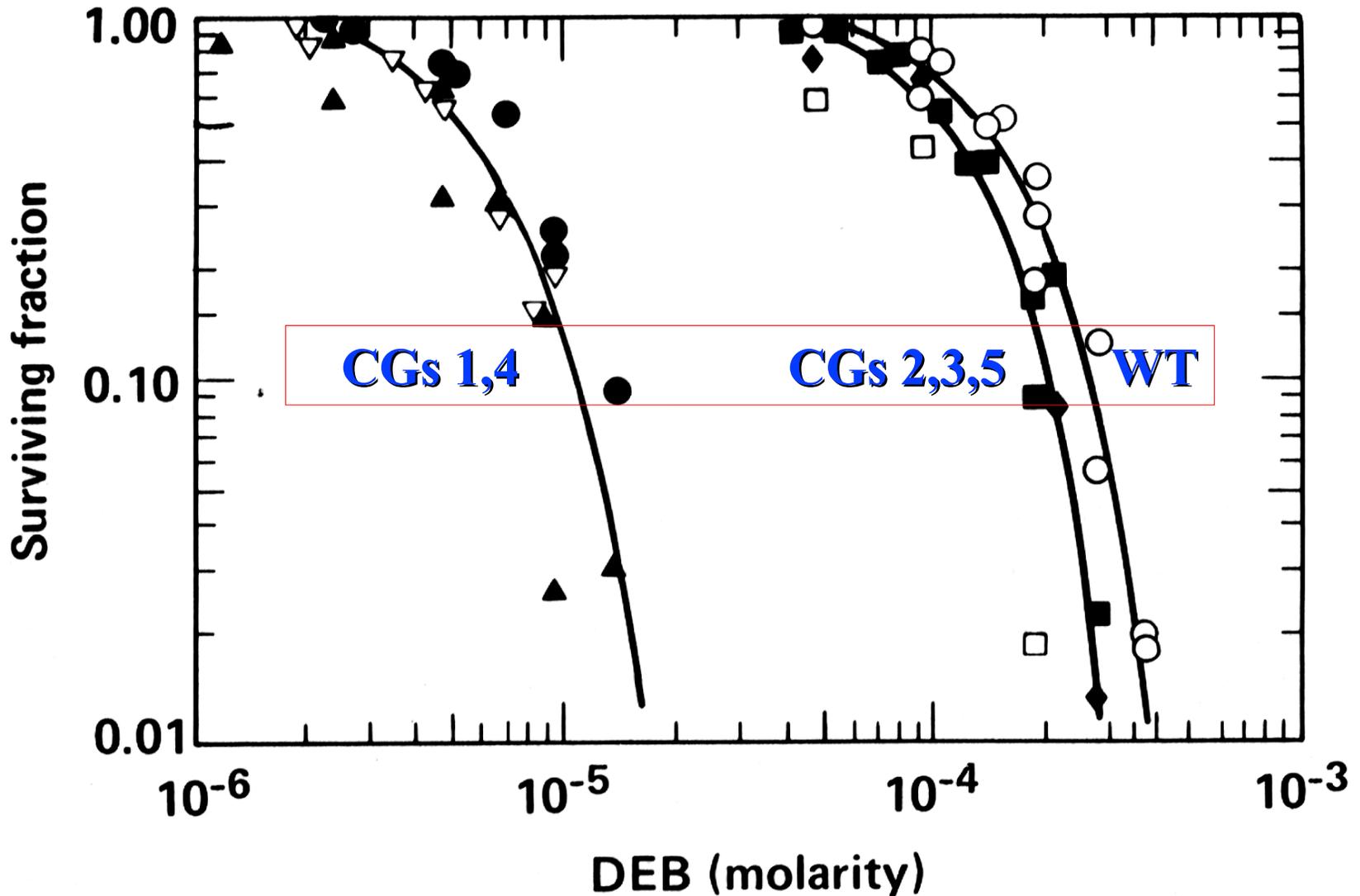
Crosslinks



Differential sensitivity of *ERCC1-ERCC5* mutants to mitomycin C

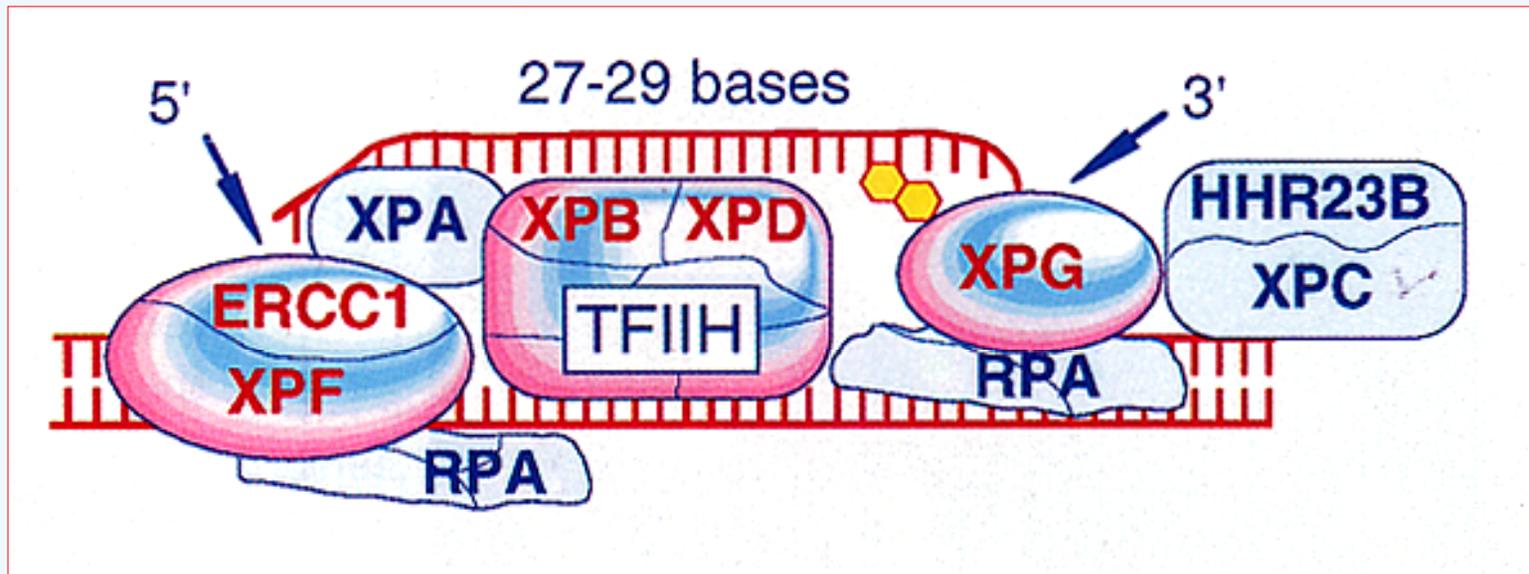


Differential sensitivity of *ERCC1-ERCC5* mutants to diepoxybutane



NER nucleases and helicases:

ERCC1-ERCC5 proteins are key enzymes in the recognition and dual incision at bulky adducts



ERCC1 = no XP group

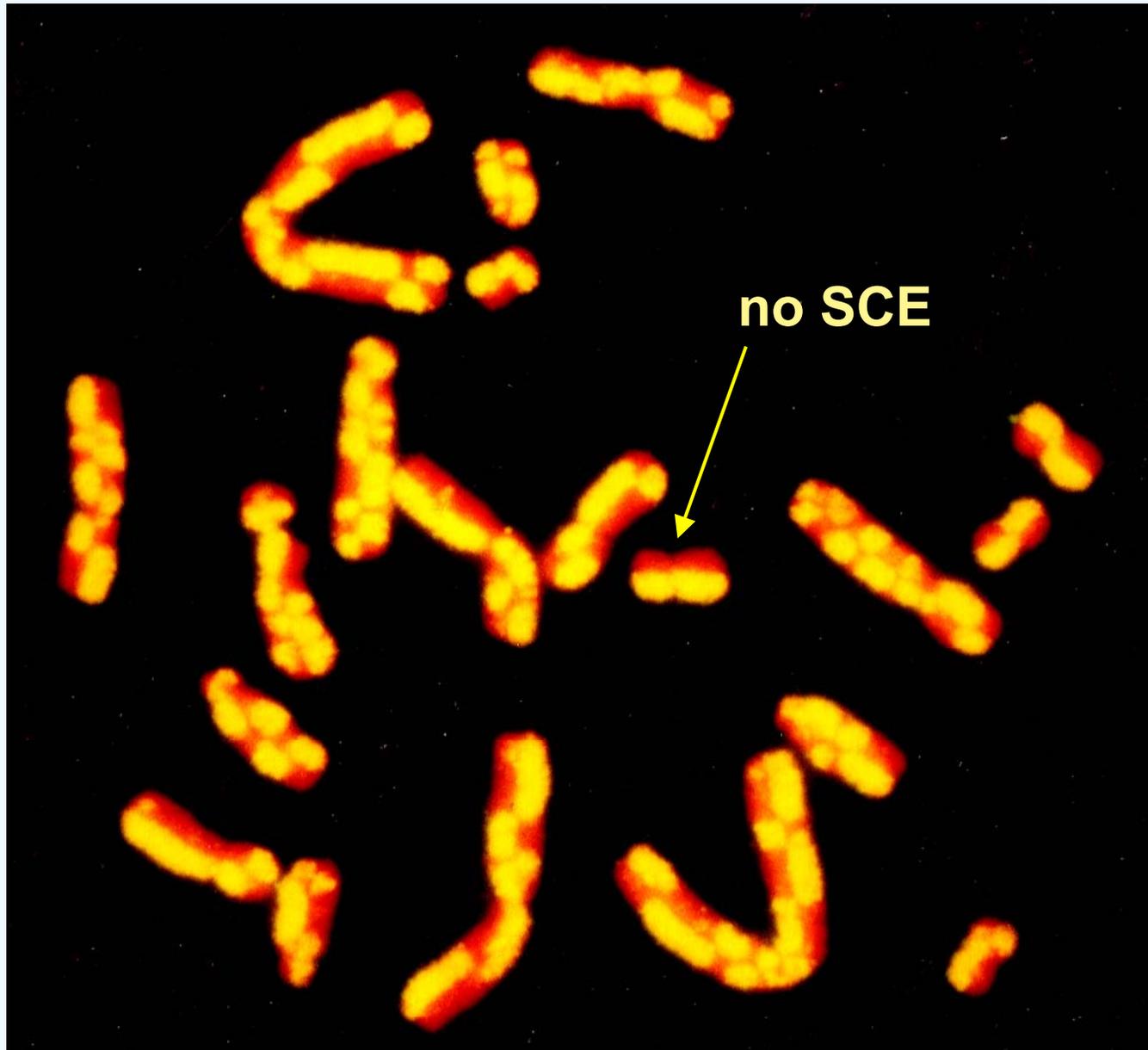
ERCC2 = XPD

ERCC3 = XPB

ERCC4 = XPF

ERCC5 = XPG

Excessive sister-chromatid exchange in CHO *xrcc1* EM9 cells



XRCC mutants are a motley bunch!



Available online at www.sciencedirect.com



DNA Repair 2 (2003) 655–672

**DNA
REPAIR**

www.elsevier.com/locate/dnarepair

Mini review

The mammalian *XRCC* genes: their roles in DNA repair and genetic stability

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^b *Department of Toxicogenetics—MGC, Leiden University Medical Center, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands*

^c *Department of Molecular Cell Genetics, the Ludwig Rydygier University of Medical Sciences, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland*

Received 31 March 2003; received in revised form 3 April 2003; accepted 3 April 2003

= CHO mutants

Repair pathway	Gene symbol	Chromosome position (human)	Mutant cell line ^a
BER	<i>XRCC1</i>	19q13.2	EM7, EM9, EM-C11, EM-C12

Single-strand break repair

HRR	<i>XRCC2</i>	7q36.1	irs1
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Double-strand break repair

<i>XRCC3</i>	14q32.3	irs1SF
<i>RAD51L2/RAD51C</i>	17q23–24	CL-V4B, irs3
<i>XRCC11/BRCA2/FANCD1</i>	13q12–13	V-C8
<i>RAD51D ?</i>		
<i>XRCC9/FANCG</i>	9p13	UV40, NM3

Fanconi anemia replication fork breakage pathway

NHEJ	XRCC4	5q13.1-3	XR-1, M10
	XRCC5/Ku80	2q35	xrs series, XR-VB series, sxi-2,3
	XRCC6/G22P1	22q11-13	Only known from knockout mouse cells
	XRCC7/PRKDC	8q11	V3, scid, irs20, SX9, XR-C1, XR-C2
	LIG4	13q33-34	SX10
Other	XRCC8	n.k.	irs2, V-C4, V-E5, V-G8, CM1, CM3, CM6

Double-strand break repair

Unknown function

The evolution of CHO DNA repair genetics at LLNL: *life gets harder with age*

Mutant isolation & characterization
A little hemizyosity goes a long way
1978-1980



Chromosomal mapping
Which chromosome has the repair gene?
1985-1993



Gene cloning & characterization
Chinese hamster cells love foreign DNA
1988-1998



Targeting vector construction &
gene targeting (knockouts)
No beginner's luck
2000-present

CHO UV5 cells carrying human chromosome 19



An unexpected mutant from the semi-automated hunts...

Busch, D. B., Zdzienicka, M. Z., Natarajan, A. T., Jones, N. J., Overkamp, W. I. J., Collins, A., Mitchell, D. L., Stefanini, M., Botta, E., Riboni, R., Albert, R. B., Liu, N., and Thompson, L. H. A CHO mutant, **UV40, that is sensitive to diverse mutagens and represents a new complementation group of mitomycin C sensitivity**. *Mutat. Res.*, 363: 209-221, 1996.

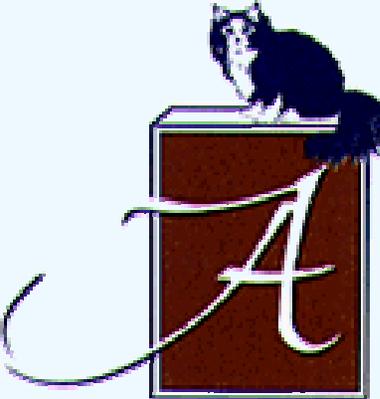
David Busch: A brilliant scientist & physician succumbs to leukemia in 2002



David B. Busch, PhD & MD

Many were affected by David's passing





ADVENT HILL
Cattery



The human *XRCC9* gene corrects chromosomal instability and mutagen sensitivities in CHO UV40 cells

(DNA repair/radiation sensitivity/chromosome aberrations/sister chromatid exchange)

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*Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA, 94551; †Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284; and ‡Department of Environmental and Toxicologic Pathology, Armed Forces Institute of Pathology, Washington, DC 20306

Edited by Charles M. Radding, Yale University School of Medicine, New Haven, CT, and approved June 16, 1997 (received for review December 12, 1996)

ABSTRACT The Chinese hamster ovary (CHO) mutant UV40 cell line is hypersensitive to UV and ionizing radiation, simple alkylating agents, and DNA cross-linking agents. The mutant cells also have a high level of spontaneous chromosomal aberrations and 3-fold elevated sister chromatid exchange. We cloned and sequenced a human cDNA, designated *XRCC9*, that partially corrected the hypersensitivity of UV40 to mitomycin C, cisplatin, ethyl methanesulfonate, UV, and γ -radiation. The spontaneous chromosomal aberrations in *XRCC9* cDNA transformants were almost fully corrected whereas sister chromatid exchanges were unchanged. The *XRCC9* genomic sequence was cloned and mapped to chromosome 9p13. The translated *XRCC9* sequence of 622 amino acids has no similarity with known proteins. The 2.5-kb *XRCC9* mRNA seen in the parental cells was undetectable in UV40 cells. The mRNA levels in testis were up to 10-fold higher compared with other human tissues and up to 100-fold higher compared with other baboon tissues. *XRCC9* is a candidate tumor suppressor gene that might operate in a postreplication repair or a cell cycle checkpoint function.

XRCC6 (*Ku70*), and *XRCC7* (*SCID*) (22–26). The Ku/DNA-PK $_{cs}$ pathway appears to account for rejoining of a substantial fraction of the double-strand breaks resulting from ionizing radiation and from gene rearrangement in germ-line lymphoid cells (27).

The CHO mutant UV40 represents a new complementation group that is unique among hamster cell mutants (28). UV40 exhibits hypersensitivity to multiple classes of mutagens and shows pronounced spontaneous chromosomal instability (~25% abnormal metaphases). Isolated as being hypersensitive to UV (4-fold), UV40 cells seem to possess normal nucleotide excision repair and were reported to be hypersensitive to mitomycin C (MMC; 11-fold), ethyl methanesulfonate (EMS; 10-fold), methyl methanesulfonate (MMS; 5-fold), and ionizing radiation (2-fold) (28). UV40 is also unusual among rodent mutants in combining broad-spectrum sensitivity with a 3-fold elevated level of SCE. We report here the isolation of a human cDNA and genomic sequence, which efficiently corrects the chromosomal instability of UV40 and partially corrects mutagen sensitivity. We speculate that the product of the *XRCC9* gene may participate in a postreplication repair pathway or a cell cycle checkpoint function.

Ionizing radiation produces a complex mixture of strand breaks

The Fanconi anaemia group G gene *FANCG* is identical with *XRCC9*

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*These authors contributed equally to this work.

Fanconi anemia (FA) is an autosomal recessive disease with diverse clinical symptoms including developmental anomalies, bone marrow failure and early occurrence of malignancies¹. In addition to spontaneous chromosome instability, FA cells exhibit cell cycle disturbances and hypersensitivity to cross-linking agents¹. Eight complementation groups (A–H) have been distinguished², each group possibly representing a distinct FA gene³. The genes mutated in patients of complementation groups A (*FANCA*; refs 4,5) and C (*FANCC*; ref. 6) have been identified, and *FANCD* has been mapped to chromosome band 3p22–26 (ref. 7). An additional FA gene has recently been mapped to chromosome 9p (ref. 8). Here we report the identification of the gene mutated in group G, *FANCG*, on the basis of complementation of an FA-G cell line and the presence of pathogenic mutations in four FA-G patients. We identified the gene as human *XRCC9*, a gene which has been shown to complement the MMC-sensitive Chinese hamster mutant UV40, and is suspected to be involved in DNA post-replication repair or cell cycle checkpoint control^{9,10}. The gene is localized to chromosome band 9p13 (ref. 9), corresponding with a known localization of an FA gene.

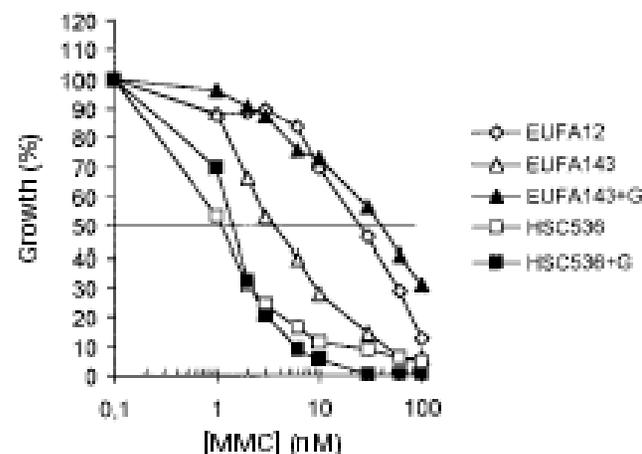
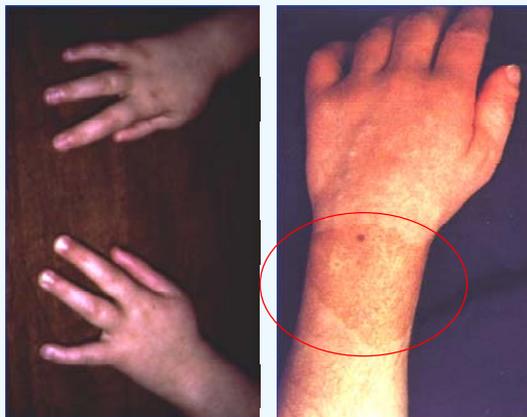


Fig. 1 Complementation of cross-linker hypersensitivity by pFAG9. MMC-induced growth inhibition showing specific hypersensitivity of EUFA143 (FA-G) lymphoblasts is shown. A level equal to that of wild-type cells (EUFA12) was restored after transfection with pFAG9, except in complementation group C lymphoblasts (HSC538).

by 350 bp. The remainder of the pFAG9 insert appeared identical to the sequence reported for *XRCC9*.

Clinical phenotype and genetics of Fanconi anemia



- Progressive **aplastic anemia** due to loss of bone marrow stem cells
- Predisposition to **cancer**, including squamous cell carcinoma
- 15% incidence of acute myeloid leukemia in FA children, i.e ~15,000 x increase
- 70% of patients have diverse **developmental abnormalities** in upper limbs, skeleton, GI tract, skin, kidney, heart, and CNS
- Clinical diagnosis from testing lymphocytes for chromosomal **sensitivity to the crosslinking agents** diepoxybutane or mitomycin C
- Genetically complex with ~12 genes; 8 cloned

How much is a knockout mutant worth ---
in lost technicians, disgruntled postdocs, and DOE \$\$?

No knockout

APE1

Knockouts

XPD

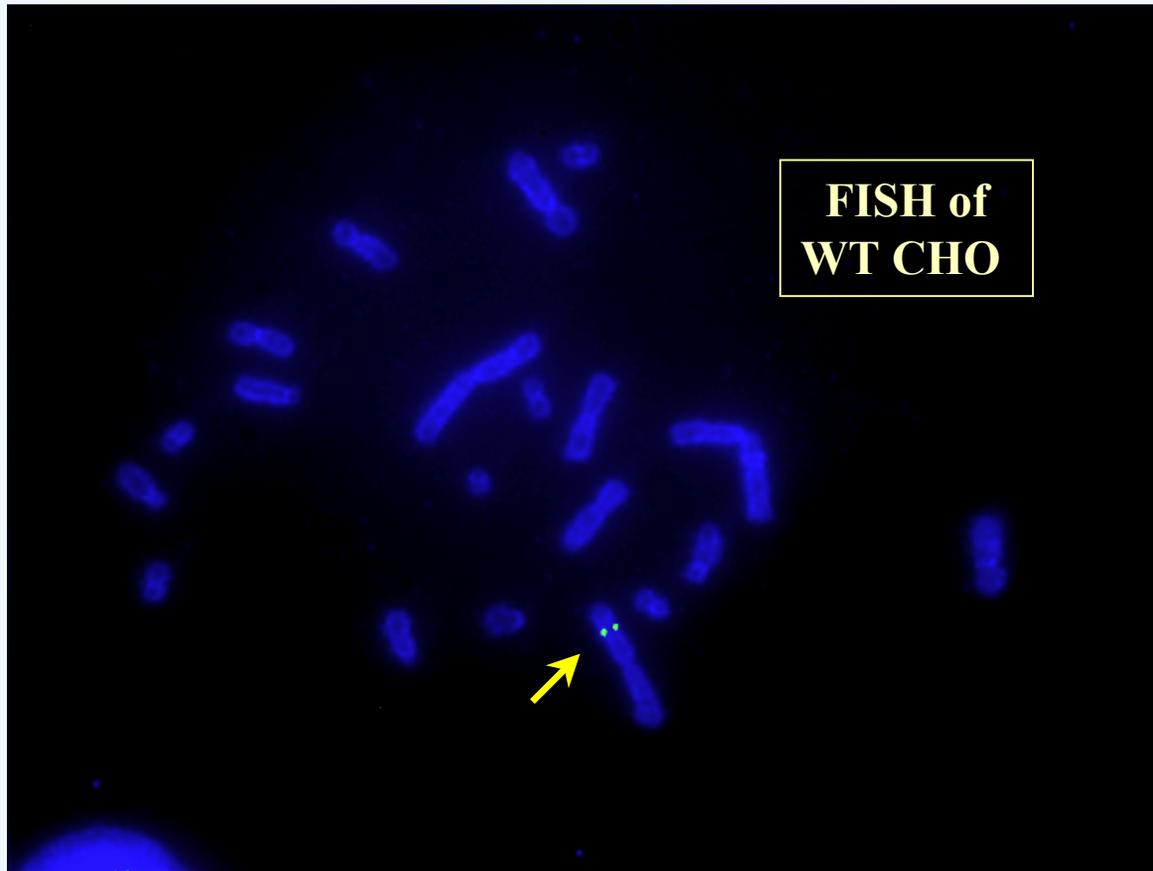
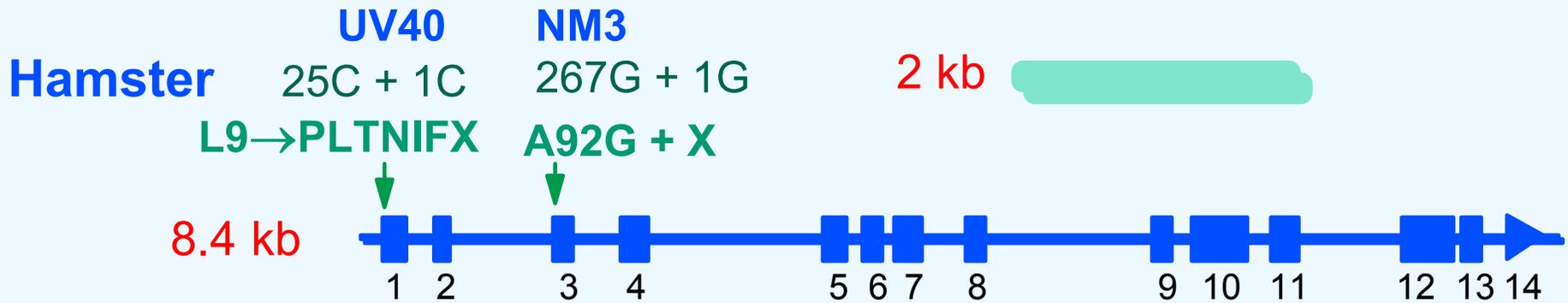
FANCG

RAD51C (1 allele)

RAD51D

APE1, XPD, & FANCG are single-copy genes

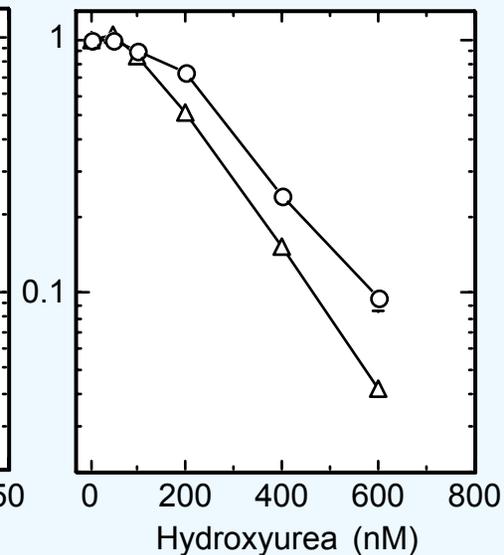
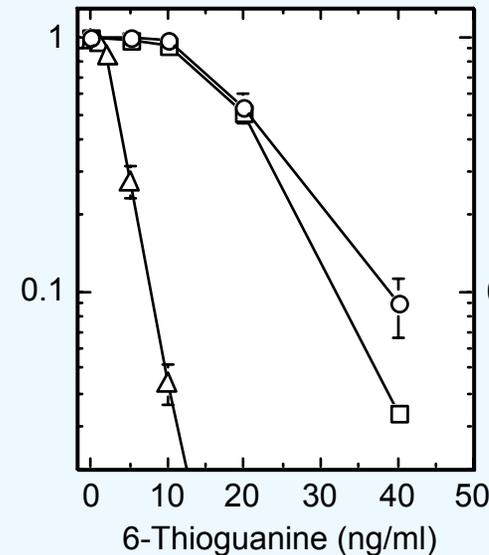
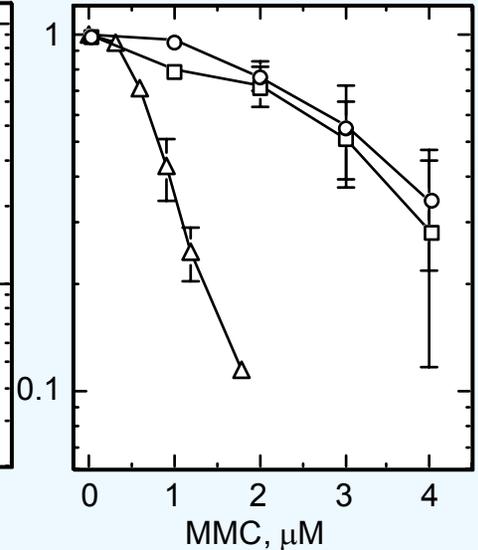
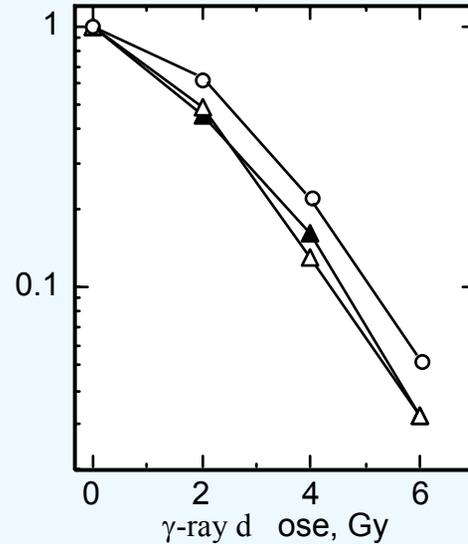
Hamster *FancG* Gene Structure and Mutations



FGKO40 cells are **NOT** specifically sensitive to DNA cross-linking agents

Agent fold sensitivity (D_{37})

Diepoxybutane (crosslinks)	7
Thioguanine	5
Methyl methanesulfonate	4
Methyl nitrosourea	4
Mitomycin C (crosslinks)	3
ENU	3
CNU (crosslinks)	3
Chlorambucil (crosslinks)	2
UV-C	1.5
Camptothecin	1.2
Hydroxyurea	1.2
Ionizing radiation	1.2
Paraquat	1.0
Thymidine	1.0
Hydrogen peroxide	0.8



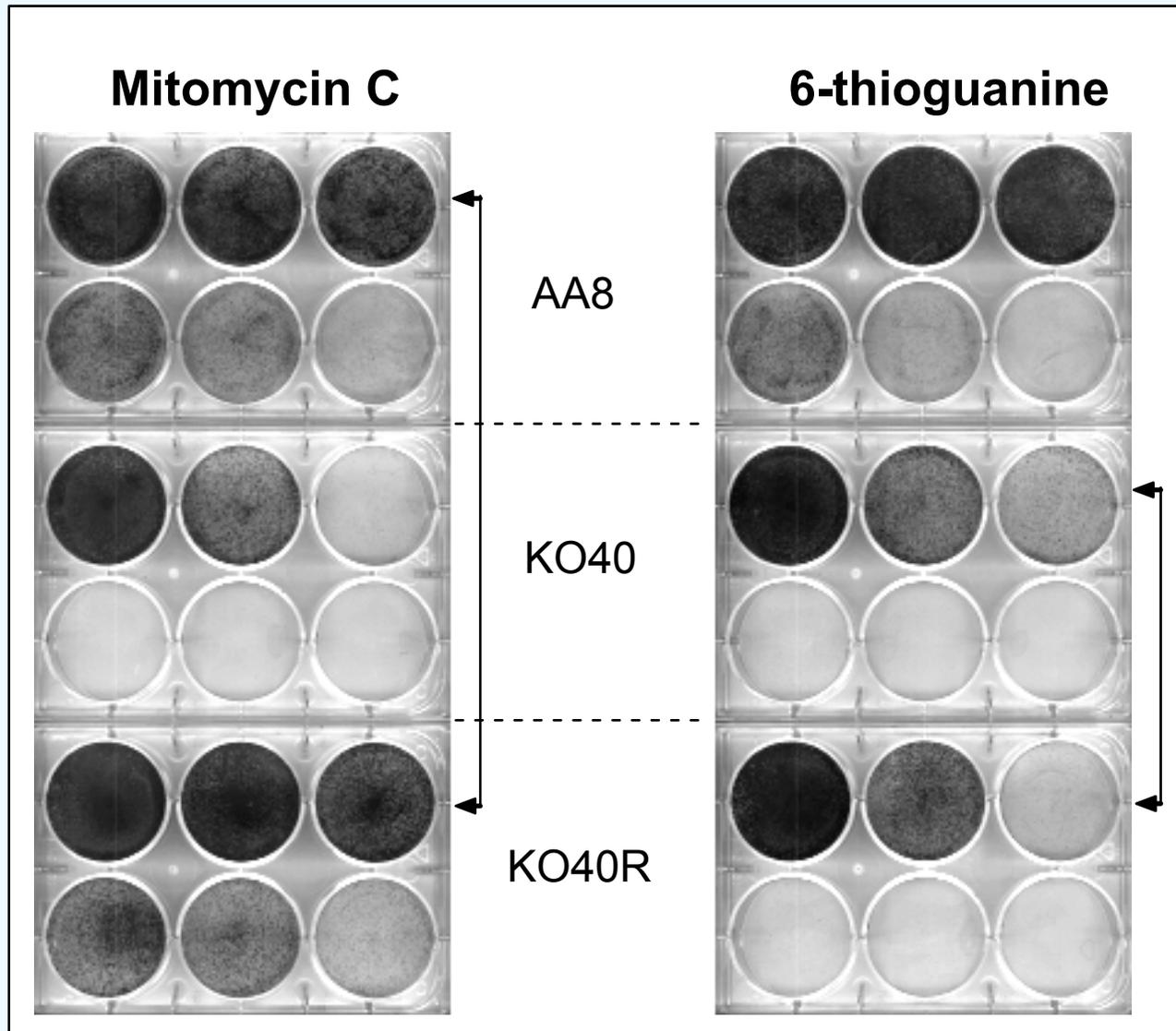
Reduction in *hprt* mutation rate in KO40 cells

**To be updated
after publication
of data**

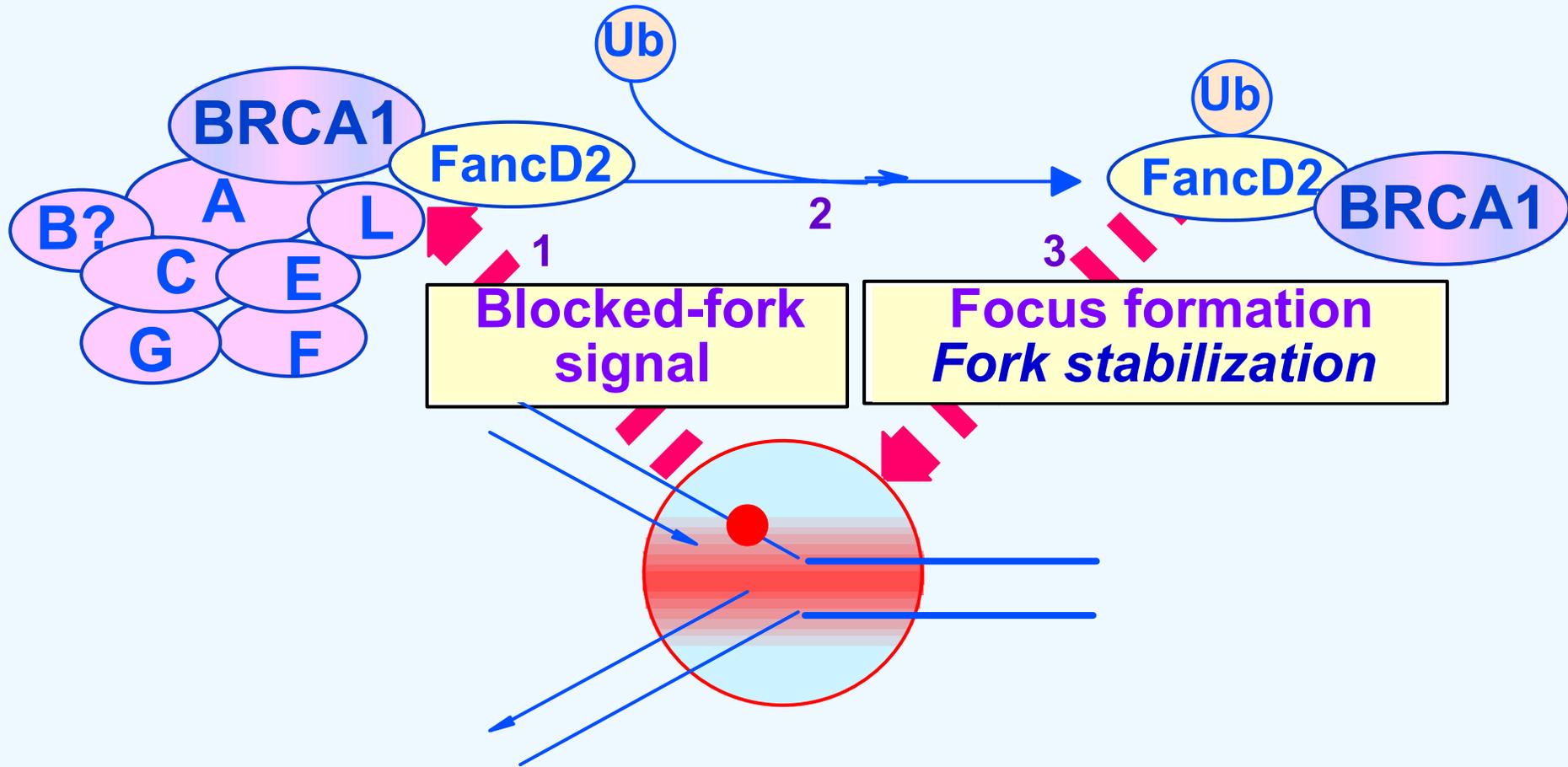
KO40 cells have increased spontaneous rates of gene amplification
at *dhfr* and *CAD* loci (like DNA-PKcs mutant of CHO cells)

**To be updated
after publication
of data**

KO40 revert to MMC resistance at a high rate

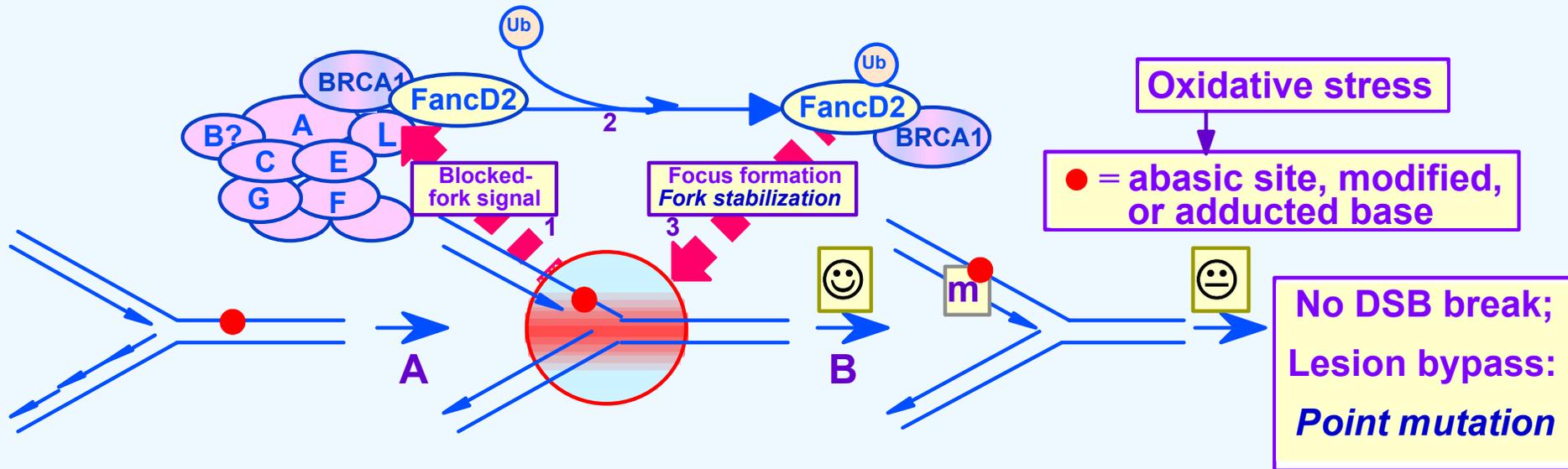


Monoubiquitination of FANCD2 is essential for resistance to crosslinks and other DNA damage



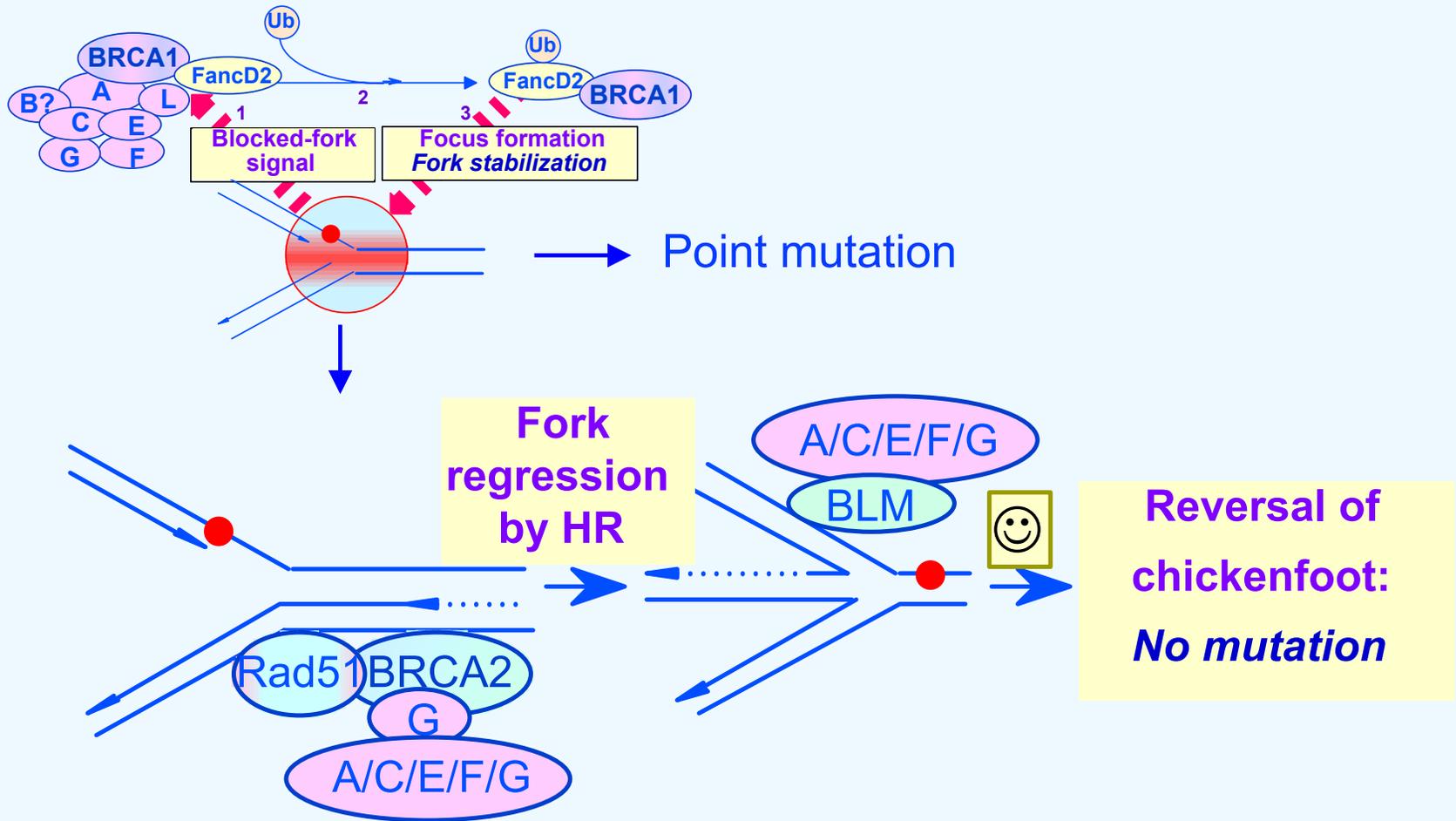
A new molecular model for FA:

Activation of **FANCD2** by replication-fork-blocking lesion, leading to **stabilization** of the blocked fork



- FA proteins allow translesion, mutagenic polymerases to bypass blocking lesions.
- Blocked forks may provide the signal for an S phase checkpoint.

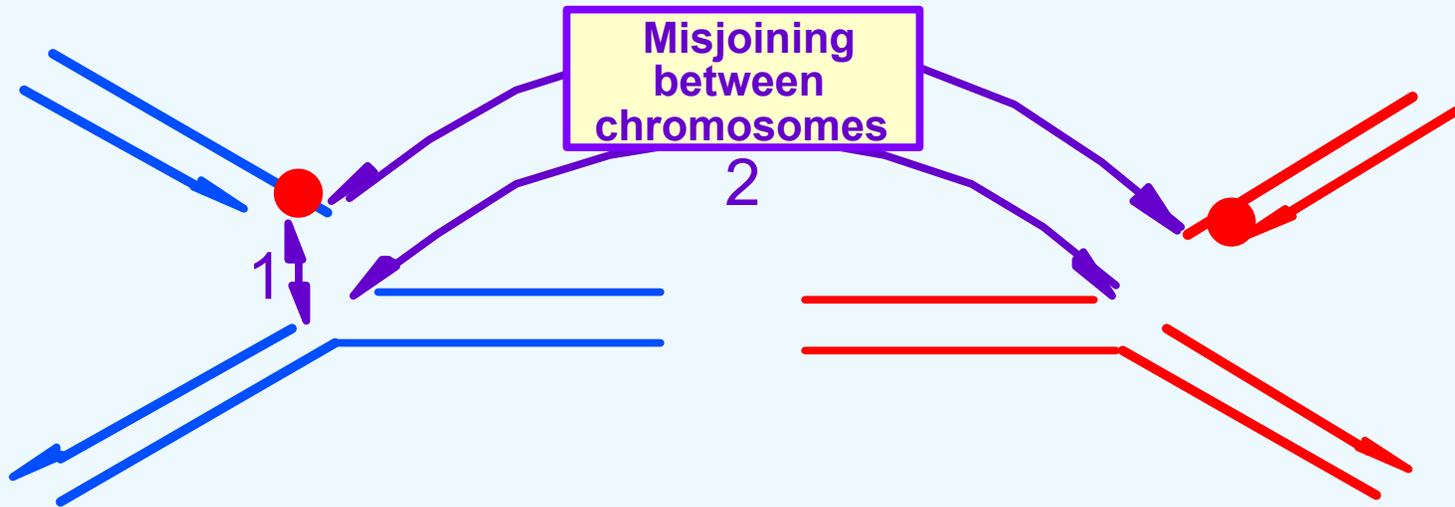
FA proteins allow the nonmutagenic, HR (homologous recombination) pathway to bypass a blocking lesion



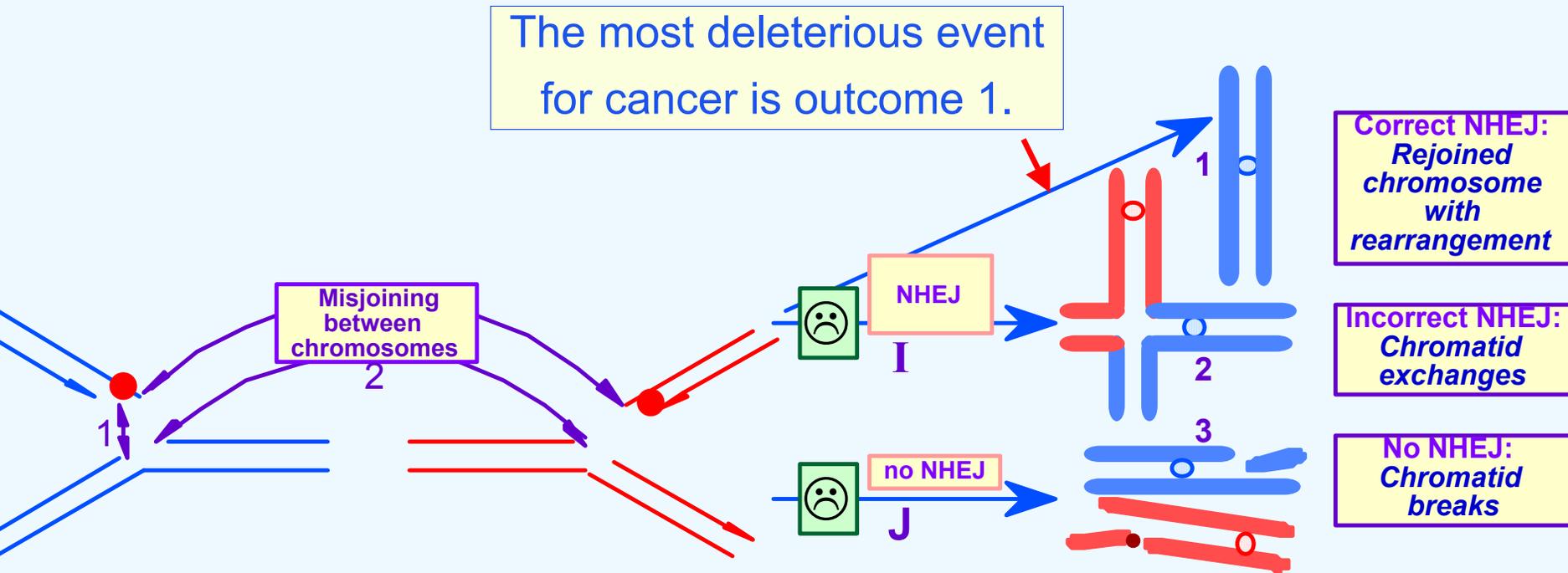
FA proteins may allow lesion bypass by interfacing with the HR machinery.

Without FA proteins, more chromosome rearrangements occur.

DSBs from broken forks interact through error-prone nonhomologous end joining (NHEJ).

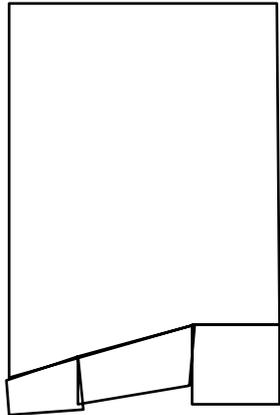


Without FA proteins, at metaphase there are more breaks, as well as **rearrangements** & exchanges arising from misrepair.

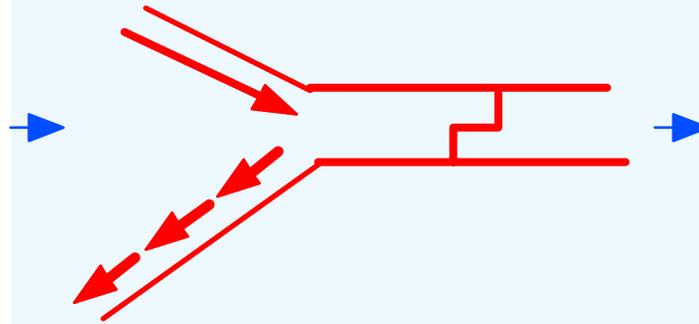


KO40 cells have increased spontaneous rates of gene amplification at *dhfr* and *CAD* loci (like DNA-PKcs mutant of CHO cells)

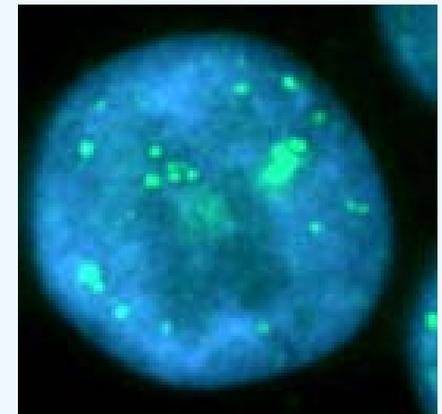
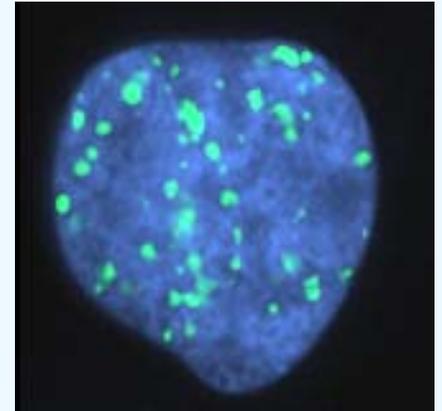
Synchronize cells in early S-phase



Treat for 30 min with MMC
100% survival of WT cells



Sample over time & measure nuclear foci for γ H2AX and Rad51



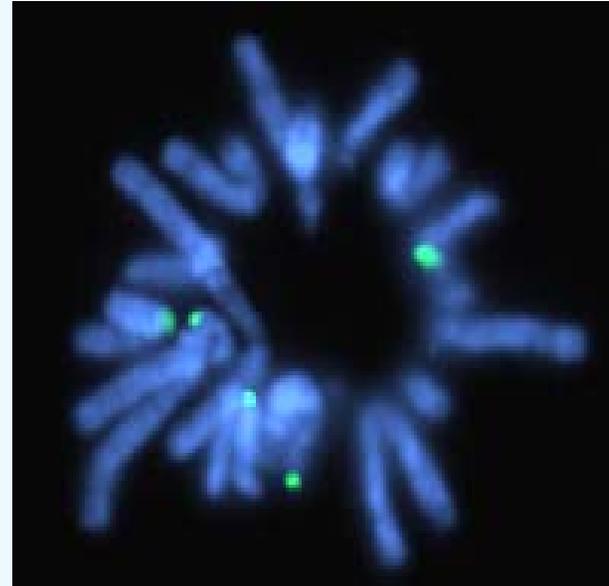
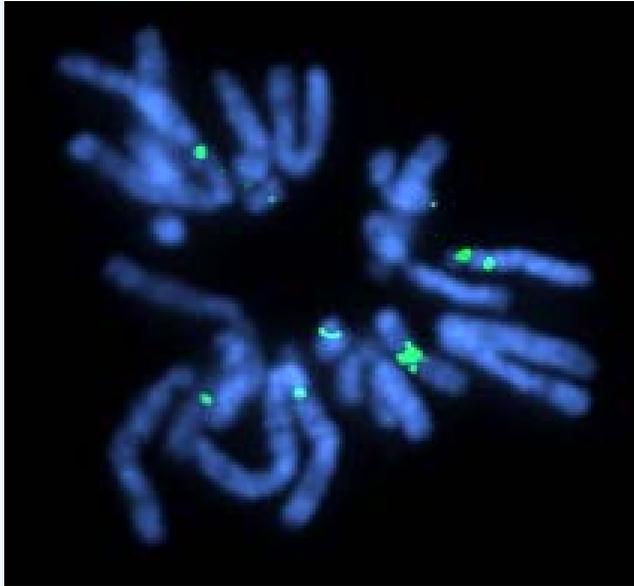
**KO40 exhibit more gH2AX foci than wild-type cells
in response to MMC treatment**

**To be updated
after
publication of
data**

**KO40 exhibit more Rad51 foci than wild-type cells
in response to MMC treatment**

**To be updated
after publication
of data**

Persistent foci at mitosis do not imply chromosomal breaks.



- ➡ **WT CHO cells treated in early S phase with mitomycin C; S.F. = 100%**
- ➡ **Persistent γ H2AX foci 8 hr post-treatment when cells are dividing**
- ➡ **Are double-strand breaks present in these foci?**



Mutation Research 486 (2001) 217–247

DNA Repair

www.elsevier.com/locate/dnarepair

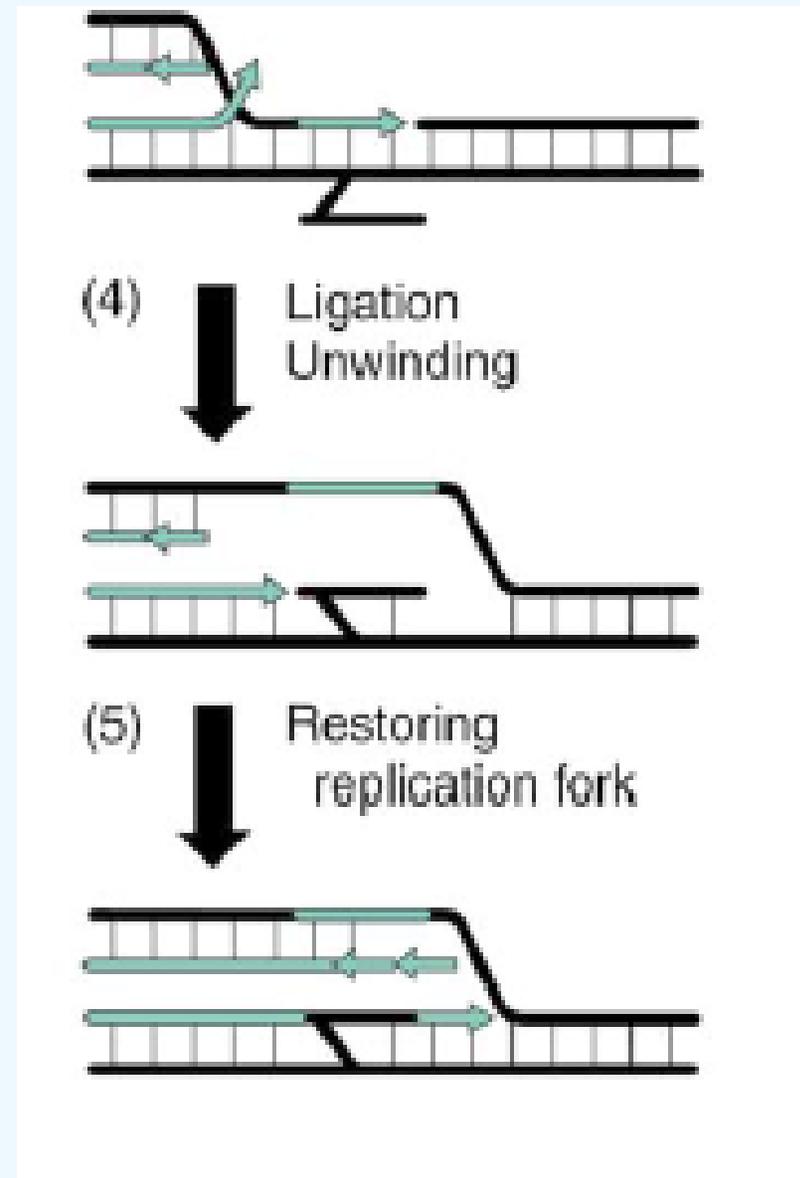
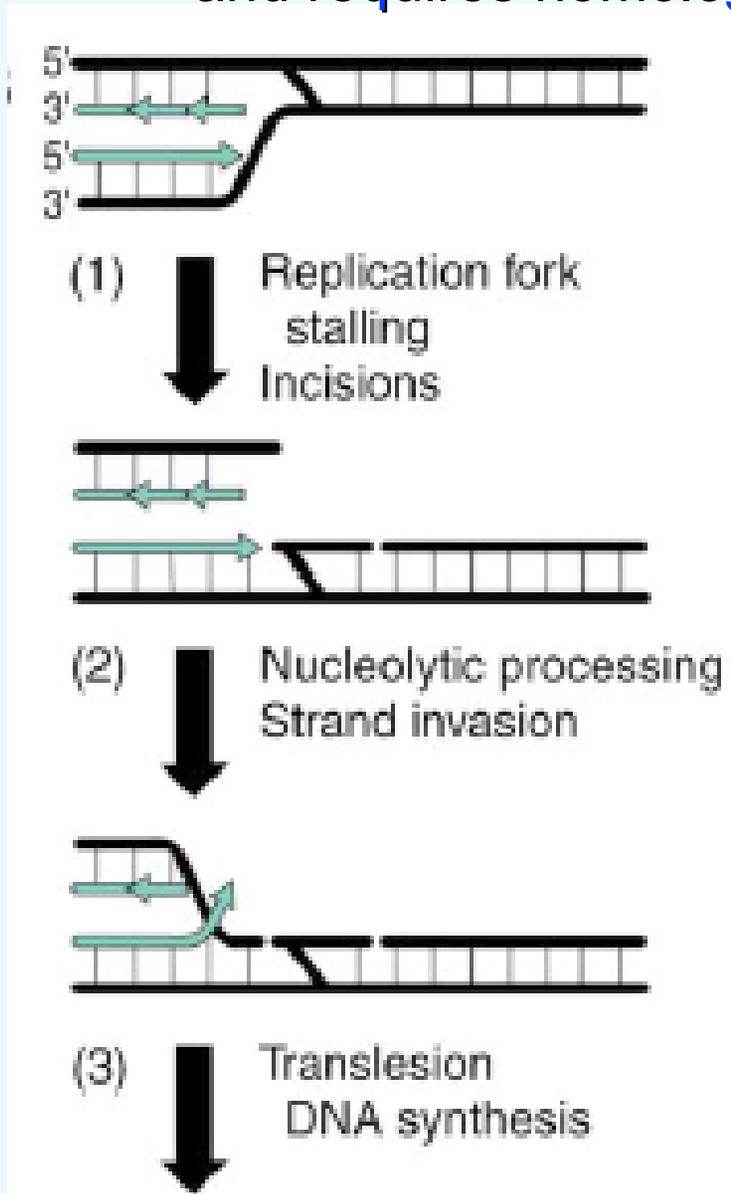
Community address: www.elsevier.com/locate/mutres

Mini review

Repair of DNA interstrand cross-links

Mies L.G. Dronkert^a, Roland Kanaar^{a,b,*}

Passage of DNA replication forks through crosslinks is error prone and requires homologous recombination proteins



Summary of studies that suggest more double-strand breaks occurring during replication in KO40 *fancg* cells



Higher frequencies of γ H2AX foci in S-phase cells



Higher frequencies of Rad51 foci in S-phase cells

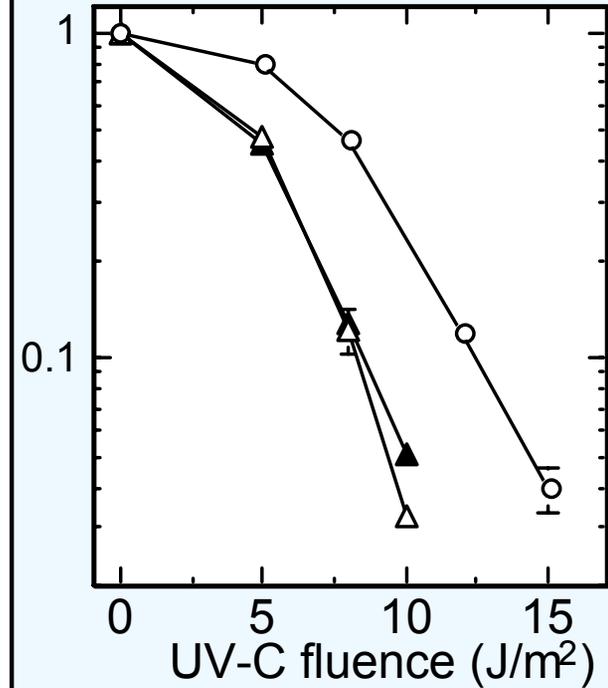
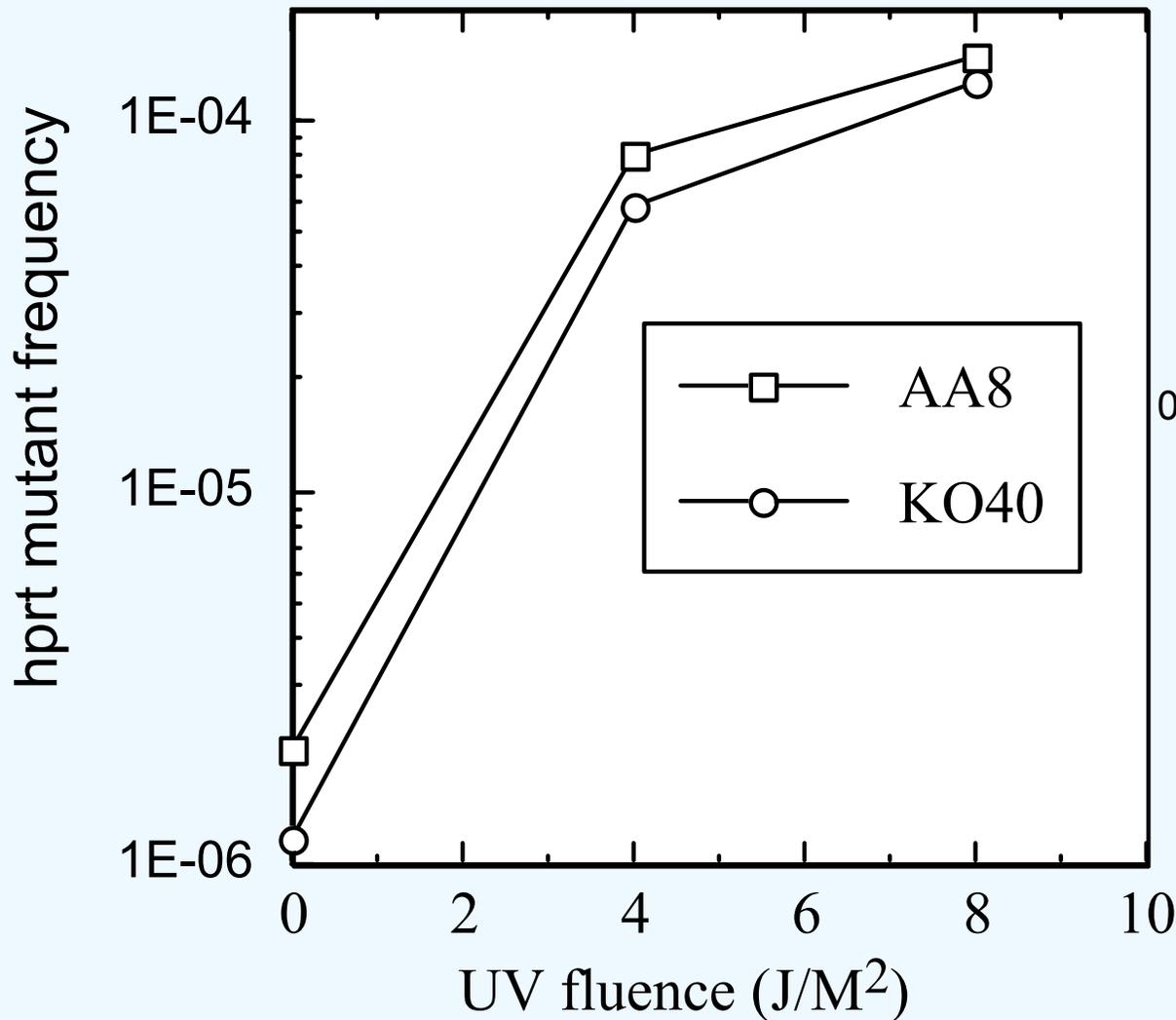


Increased rates of gene amplification, a phenotype shared with mutant cells defective in NHEJ DSB repair



A reduction in *hprt* mutation rate

Modest hypomutability for UV-C induced mutations at the *hprt* locus KO40 *fancg* cells



Who got me started

Bill Dewey, PhD Program at MD Anderson
and NIH training grant

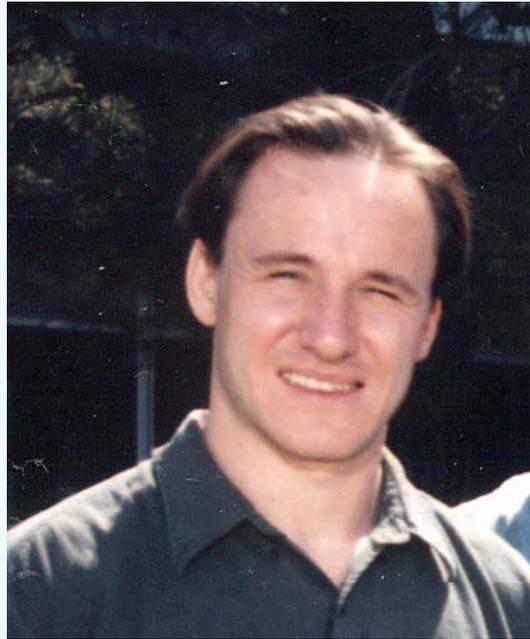
Herman Suit, PhD from UT GSBS, Biophysics

Ron Humphrey, Laboratory and thesis comm.

Gordon Whitmore & Louis Siminovitch, OCI

Mortimer Mendelsohn, position at LLNL

Postdoctoral scientists



Gerald Adair

Larry E. Dillehay

Cynthia A. Hoy

Christine A. Weber

Nigel J. Jones

Keith Caldecott

Robert Tebbs

Christopher Parris

Nan Liu

Sally Kadkhodayan

Ian McConnell

James George

Clodagh Finnegan

Lisa Wrischnik

John Hinz

N. Alice Yamada



Who did much of the work



Robert Tebbs
(senior staff)
Gene targeting,
Nuclear foci



Alice Yamada (postdoc)
Cell synchrony, protein assays



John Hinz
(postdoc)
Cell survival,
fluctuation
experiments

Casey Cat:
“FancG
KO? ...so
what?...”



Who did much of the work



Ed Salazar
Gene knockouts &
characterization

Kathryn Segalle



Vicki Kopf
KO mutant
characterization

Peter Nham



Life is not a dress rehearsal.

**anonymous
psychiatrist**