My life over and under the microscope, as

a cancer scientist and patient

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Who am I?

My life over the microscope . . .

Scientific training and experience

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B.A. Cell Biology, University of Connecticut 1975

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Field crew, US. Forest Service, summers of 1972, 1973

Campbell et al., “Man's Activities and Subsequent Gypsy Moth Egg-mass Density Along the Forest Edge”, *Environmental Entomology* (1976)

Gypsy moths laying egg masses

Campbell et al., “Man's Activities and Subsequent Gypsy Moth Egg-mass Density Along the Forest Edge”, *Environmental Entomology* (1976)

Almost complete defoliation of a hillside by gypsy moth caterpillars

Scientific training and experience (cont.):

Research Assistant, Institute of Medical Research of Bennington, Bennington, Vermont (1977 – 1980)

Rhode and Paradiso, *"Parvovirus Genome: Nucleotide Sequence of H-1 and Mapping of Its Genes by Hybrid-Arrested Translation" J. Virology* (1983)

Scientific training and experience (cont.)

Research Assistant, Dept. Medicine, UC San Diego School of Medicine (1981 - 1983)

Goulian et al., Parvovirus DNA Replication. *In* Mechanisms of DNA Replication and Recombination, (ed. N. Cozarelli), pp. 367-379, Alan Liss, Inc., New York (1983).

My family has an extensive family cancer history:

My mother died of early onset (age 52) breast cancer

All five of her siblings died of cancer (ovarian, breast
bladder and lung)

My father was diagnosed with prostate cancer and died of treatment complications

A younger brother (still alive) was diagnosed with prostate cancer

A younger sister died of breast cancer at age 64

Another younger sister was diagnosed with ovarian and breast cancer

This extensive history of cancer led me to pursue a career as a senior cancer scientist in order to better understand the malignant forces killing my family, which also threatened to kill me

Scientific training and experience (cont.)

Ph.D. Cell Biology, University of Vermont 1987

Mentor: Nick Heintz, Ph.D.

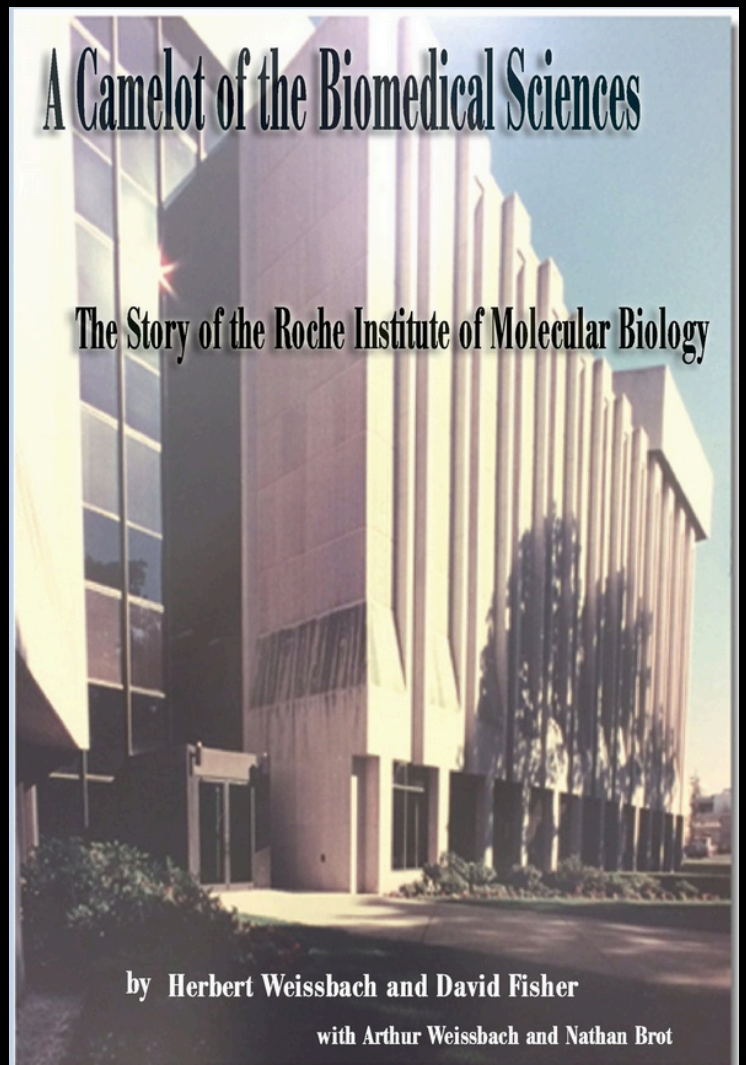
Dissertation title: “Identification of an Origin of DNA Replication in a Mammalian Chromosome”

Burhans et al., *Proc. Natl. Acad. Sci.* (1986); 83 (20) pp. 7790-7794

Burhans et al., *Biochemistry* (1986); 25 (2) pp. 441-449

Scientific training and experience (cont.)

Postdoctoral training: Roche Institute of Molecular Biology 1987 – 1992



Scientific training and experience (cont.)

Postdoctoral training:

Roche Institute of Molecular Biology 1987 – 1992

Sydney Udenfriend (Founding Director) The Scientist (1995): "Camelot In Nutley, N.J.: Roche Institute Of Molecular Biology Remembered"

"In 1967, establishment of the Roche Institute of Molecular Biology (RIMB) by Hoffmann-La Roche received wide news coverage. In a relatively short time, the Institute established itself as a world-class research and training center and maintained this reputation for almost 30 years . . . the Science Citation Index has always rated RIMB among the top four or five independent institutes in the United States (*Science Watch*, **4**:2, 1993)."

Scientific training and experience (cont.)

Postdoctoral training:

Roche Institute of Molecular Biology 1987 – 1992

Mentor – Melvin DePamphilis, Ph.D.

Burhans et al., *"Identification of an origin of bidirectional DNA replication in mammalian chromosomes."* *Cell* (1990) 62 (5) pp. 955-65.

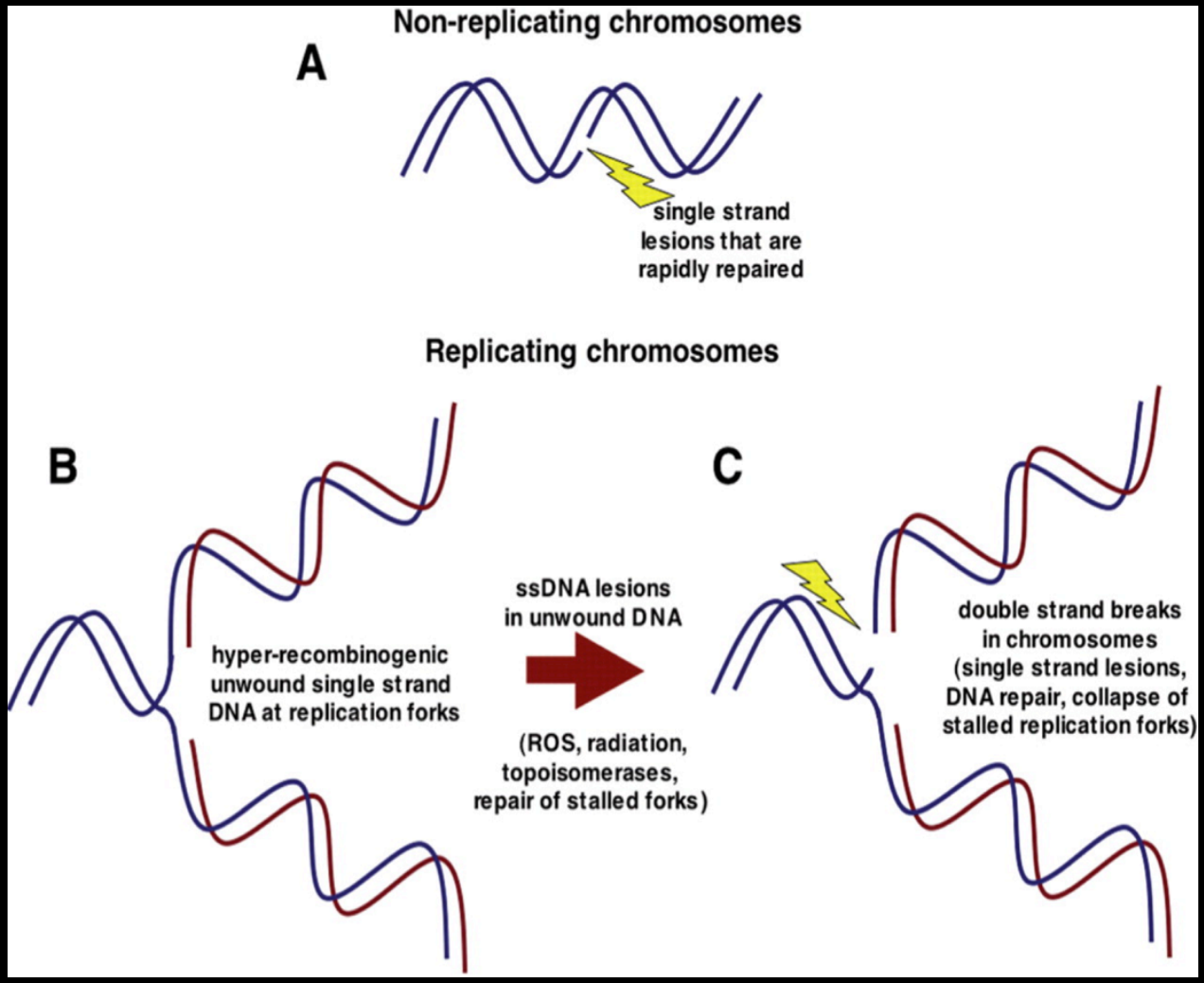
Burhans et al. *"Emetine allows identification of mammalian origins of DNA replication by imbalanced DNA synthesis, not through conservative nucleosome segregation". EMBO J.* (1990) 9 (9) pp. 2911ff

Burhans, W. and Huberman, J. *"DNA replication origins in animal cells: a question of context?"* *Science* 13, 639-640 (1994).

Most of my 26 year career as a senior scientist has been spent investigating the relationships between DNA replication stress and genetic instability in cancer

What is DNA replication stress and how does it promote genetic instability in cancer?

The unique structure of replicating DNA underlies genetic instability that drives cancer and other age-related disorders

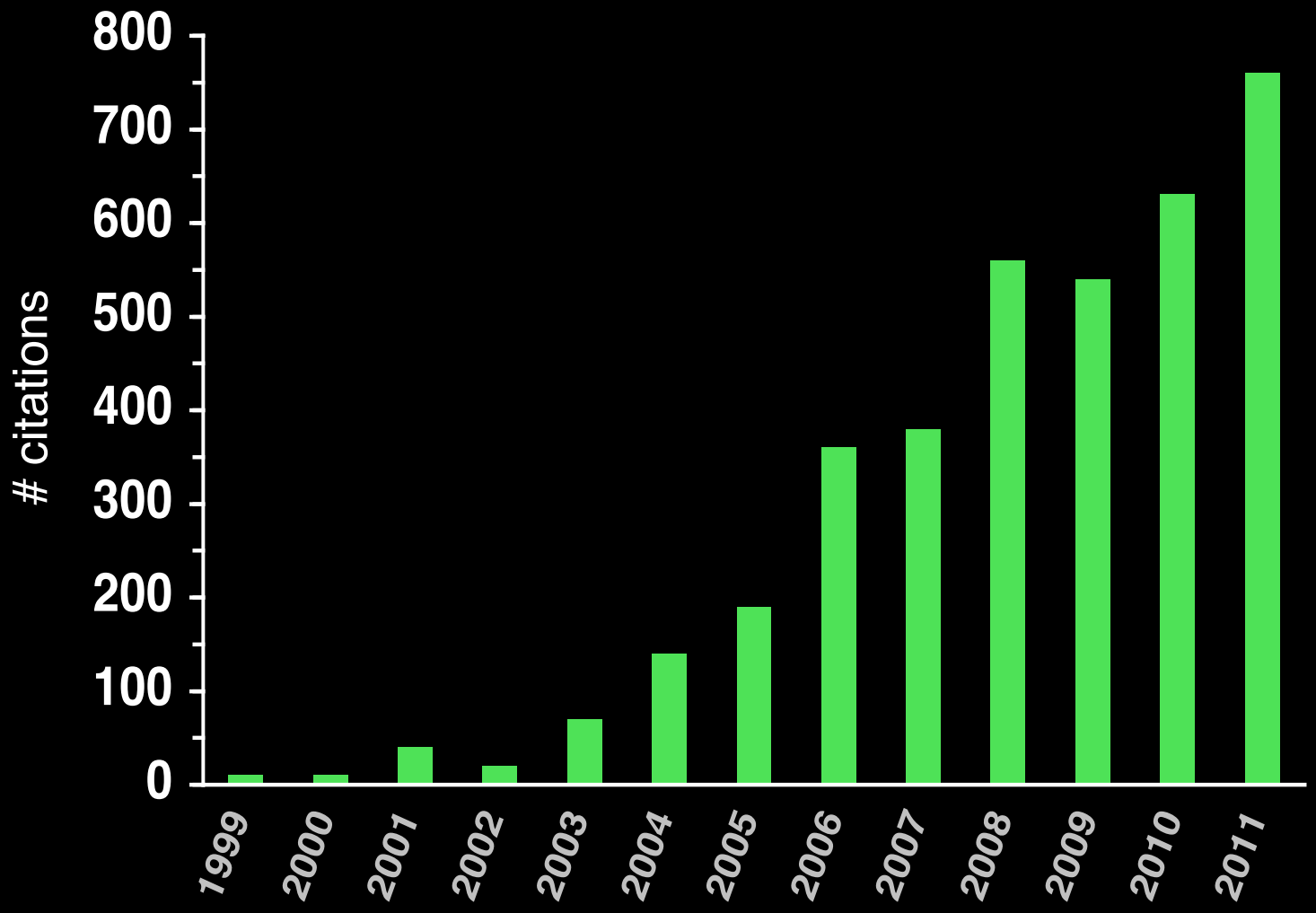


"DNA replication stress, genetic instability and aging"

Burhans and Weinberger, Nuc. Acids Res. (2007)

Citations per year identified by Google Scholar

that link “DNA replication stress” AND “cancer”

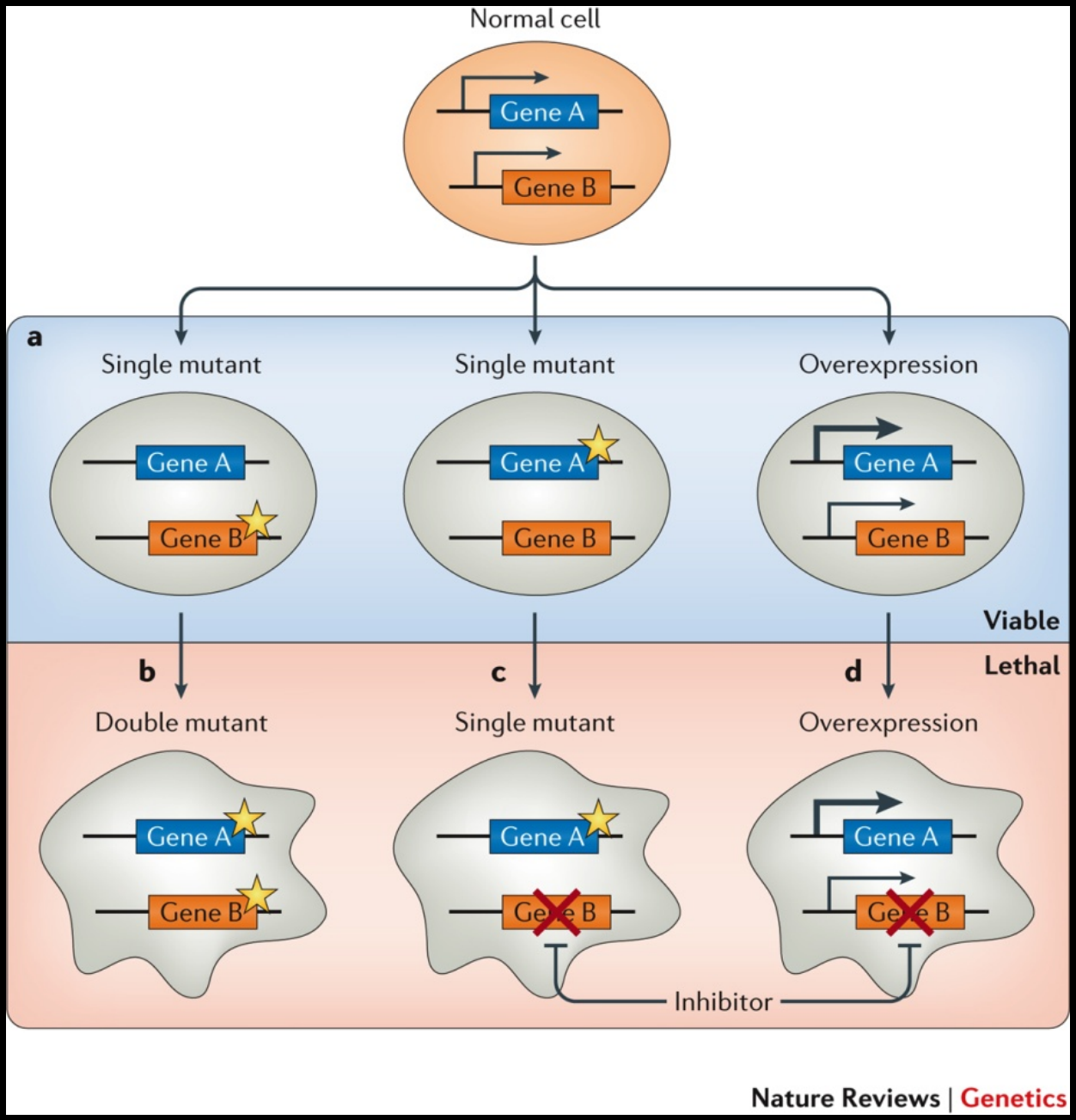


Synthetic lethality (or synthetic sickness)

Phenomenon first described in Drosophila by Calvin Bridges in 1922

First referred to as synthetic lethality by Theodore Dobzhansky in 1946

Synthetic lethality and cancer



= drug inhibition

From:

DNA Replication and

Human Disease

Cold Spring Harbor Laboratory Press (2006)

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DNA Replication and Cancer

William C. Burhans
Roswell Park Cancer Institute
Buffalo, New York 14222

Antony M. Carr
Genome Damage and Stability Centre
University of Sussex
Brighton, United Kingdom BN1 9RQ

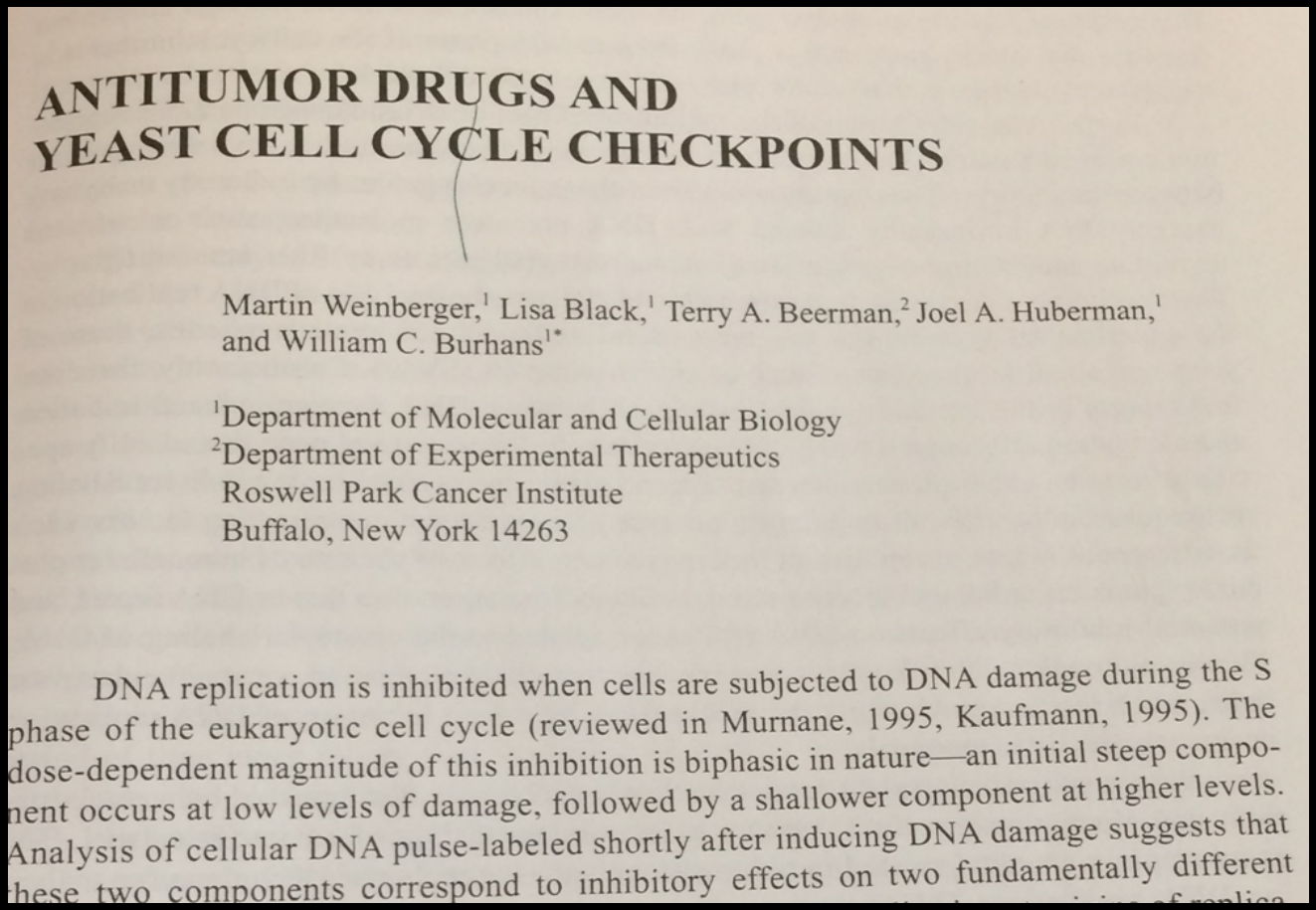
Geoffrey M. Wahl
Gene Expression Laboratory
The Salk Institute
La Jolla, California 92037

CANCER IS A COLLECTION OF DISEASES with different etiologies associated with a variety of molecular defects. Nevertheless, the cancer phenotype is invariably associated with loss of growth control leading to inappropriate or excessive proliferation of cells, and eventually, malignancy. The requirement for faithful duplication of genetic information in proliferating cells places DNA replication at the heart of the problem of cancer. For example, many of the molecular defects that lead to aberrant cell proliferation occur in pathways that converge on regulatory events controlling entry into S phase. This includes G_1 checkpoint pathways that block entry into S phase in response to DNA damage and other stresses (Chapter 18), as well as pRb and E2F-dependent pathways that up-regulate proteins required for licensing origins of DNA replication during reentry of cells into the cell cycle and at early stages of tumor progression (Chapters 3 and 16). In fact, the induction of licensing proteins in proliferating cells underlies their efficacy as diagnostic and prognostic markers for cancer (Chapter 25). Once cells enter S phase, the unique susceptibility to DNA damage of unwound DNA at replication forks can lead to genome instability, which is another salient feature of most, if not

FUTURE PERSPECTIVES

The connections between DNA replication and cancer summarized here and in other chapters of this volume undoubtedly will drive the development of more effective diagnostic, prognostic, and therapeutic strategies for treating cancer. This includes "synthetic lethality" therapeutic approaches employing drugs that act synergistically with replication-related and other defects in cancer cells to specifically target these cells for elimination (Kaelin 2005). Two recent examples of this approach are the use of G_2 checkpoint-abrogating drugs to selectively eliminate cancer cells that accumulate DNA damage during S phase due to G_1 checkpoint defects (for review, see Kawabe 2004), and the increased sensitivity of BRCA1- and BRCA2-defective cancer cells to inhibitors of poly (ADP-ribose) polymerase (McCabe et al. 2005). Another effective approach may

In 1995 my lab screened a panel of ~ 100 hypomorphic *S. cerevisiae* mutants for synthetic lethal interactions between these mutations and a DNA-damaging drug called adozelesin



ANTITUMOR DRUGS AND YEAST CELL CYCLE CHECKPOINTS

Martin Weinberger,[1] Lisa Black,[1] Terry A. Beerman,[2] Joel A. Huberman,[1] and William C. Burhans[1*]

[1]Department of Molecular and Cellular Biology
[2]Department of Experimental Therapeutics
Roswell Park Cancer Institute
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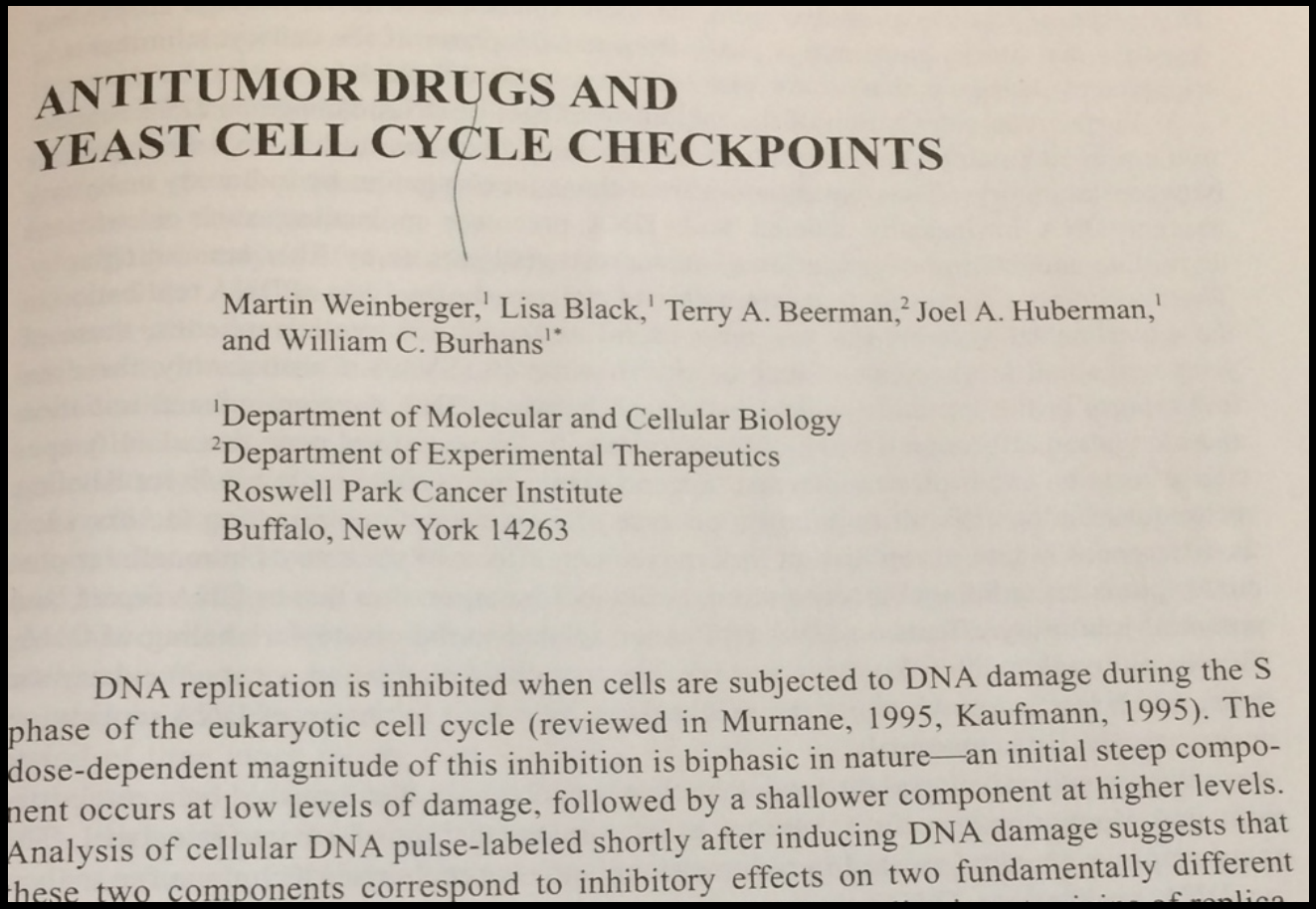
DNA replication is inhibited when cells are subjected to DNA damage during the S phase of the eukaryotic cell cycle (reviewed in Murnane, 1995, Kaufmann, 1995). The dose-dependent magnitude of this inhibition is biphasic in nature—an initial steep component occurs at low levels of damage, followed by a shallower component at higher levels. Analysis of cellular DNA pulse-labeled shortly after inducing DNA damage suggests that these two components correspond to inhibitory effects on two fundamentally different

Mutations in genes encoding proteins required for initiation of

DNA replication were hypersensitive to this drug



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Pezcoller Foundation Symposia

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GENOMIC INSTABILITY AND IMMORTALITY IN CANCER

Edited by

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114:141–151.
Wang, Y., Huq, M. S., Cheng, X., and Iliakis, G. (1996). Evidence for activities inhibiting in trans in
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82:685–687.

DISCUSSION

Hartwell: How do you interpret the increased death of the *orc2* mutan
following treatment with adozelesin?

Burhans: What I think is happening is that at the semipermissive temp
initiation function is partially compromised by the mutation.These cells are sick co
to the congenic wild type cells, even in the absence of drug treatment. Presuma
decrease in viability that occurs when we treat them with adozelesin sort of kic
over the edge by triggering an inhibitory effect that further decreases the fun
orc2p. Our results don't prove this, because the possibility remains that, when we
orc2 mutant strain with drug, we are simply making sick cells even sicker in a non
way. To address this question, we are using other types of assays to look for dif
effects in the *orc2* mutant strain compared to wild type cells.

Wahl: You went from inhibition of SV40 DNA replication to yeast. But you
say anything about drugs and cellular replication origins.

My life under the microscope . . .

I was diagnosed with Stage 3B prostate cancer in 2013

Treatment overview:

Androgen deprivation therapy (ongoing)

Radiation therapy – 45 fractions (2013)

Provenge (immunotherapy) (2015)

Olaparib (PARP inhibitor) (2015)

Keytruda immunotherapy (2016)

ATR inhibitor (2018)

Chemotherapy (docetaxel, cabazitaxil, carboplatin (since 2018)

My life under the microscope . . .

in 2015 genetic testing revealed that my germline

harbored a pathogenic mutation in the

breast cancer gene BRCA2

My life under the microscope . . .

At the time, very few urological oncologists were aware of the fact that the breast cancer gene BRCA2 was known to play a role in a small proportion of cases of prostate cancer

BRCA2 plays an important role in homologous recombination mediated repair of double-strand breaks in DNA . . .

. . . which means it operates in a genetic pathway my lab

has investigated for dozens of years

Defects in BRCA2 and the related breast cancer gene BRCA1

make cells susceptible to a class of drugs

that inhibit another protein that plays a role in homologous

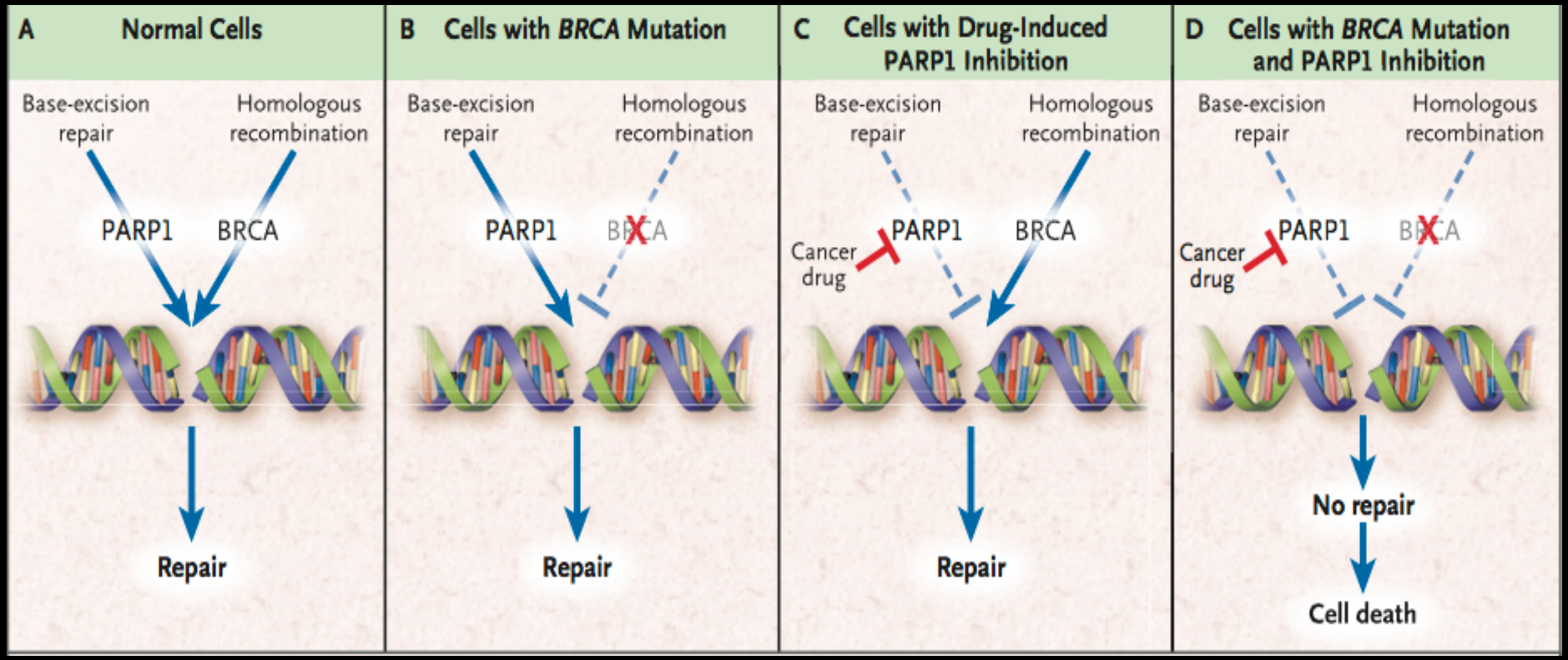
recombination repair of ds DNA breaks called

“poly-ADP ribose polymerase”, or “PARP”, via a mechanism

that involves synthetic lethal interactions

Synthetic lethal interactions between mutations in BRCA proteins

and drugs that inhibit poly (ADP) ribose polymerase (PARP1)



NEJM (2009)

Over and under the microscope . . .

My life over and under the microscope . . .

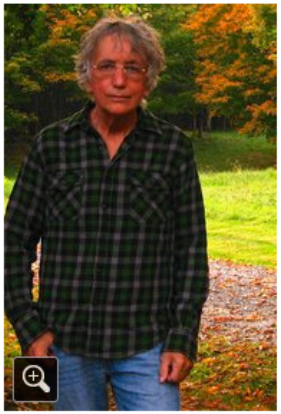
As a scientist with expertise in DNA repair pathways,

I have played an unusually active role in planning

my treatment

From the New York Times

Magazine, May 15, 2016



William C. Burhans
BUZZ BURHANS

A Researcher Becomes the Patient

My laboratory at a major cancer center has spent many of the last 24 years studying DNA replication and DNA damage-response pathways that require BRCA proteins, which suppress tumors, as well as PARP (polyADP ribose polymerase) proteins. Ten years ago, I wrote a book chapter in which I predicted (based on preclinical studies) that PARP-inhibiting drugs would one day provide effective treatment of BRCA-positive breast and ovarian cancer. A few short months ago, a drug called olaparib became the first PARP inhibitor approved for treatment of ovarian or breast cancer. Three years ago, I was diagnosed with an aggressive prostate adenocarcinoma caused by a rare BRCA2 mutation, which was most likely inherited from my mother, who died of breast cancer when she was in her 40s. Radiation treatment and treatment with a number of anti-cancer drugs at the cancer center where I work has failed to stop the growth or metastasis of this tumor for more than a few months. My oncologists know very little about BRCA-positive prostate cancer, which is also rare, and had not heard of olaparib. This has occasionally resulted in an unusual and somewhat disorienting experience. Unlike most patients who sit in exam rooms and furiously scribble information relayed by their oncologists, my oncologists are sometimes scribbling information I relay to them, such as how to spell “olaparib”! After arranging mostly on my own for treatment with olaparib as an experimental drug almost one year ago, tumor progression has been halted, and recent bone scans indicate that my bone metastases are melting away. The success of my treatment reflects, in part, the extraordinary promise of personalized medicine that targets specific cancer-causing mutations, although the role I’ve played in my treatment perhaps takes personalized medicine to the extreme. ***William C. Burhans****, 64, Buffalo* ◆

Eventually olaparib stopped working – sequencing of tumor cell DNA from a biopsy performed at Dana-Farber revealed that the BRCA2 mutation had reverted, meaning that the BRCA2 protein it encodes has resumed wild type function.

I next participated in a clinical trial at Dana-Farber that tested an inhibitor of the Ataxia telengectasia - related (ATR) protein, which functions in a constitutively active DNA damage checkpoint pathway that my laboratory first reported in 2003 (Miao et al., *J. Biol. Chem* (2003).

Current treatment is with conventional chemotherapies that are doing a good job of knocking down disease.

Acknowledgements

I would like to thank the following clinicians for their wonderfully competent and compassionate care:

Marcus Sikorsky, P.A. (RPCI)

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Atish Choudhury, M.D. (Dana-Farber)

Monica He, B.S. (Dana-Farber)

Samuel Singer, M.D. (Sloan Kettering)

Johann de Bono, M.D. (Institute of Cancer Research, ICR London)