

# Sinalização de Resposta ao Dano de DNA

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Abril/2018

Danos DNA



Detector



Responder

# DNA Damage Response (DDR)

*“The DNA damage response is a network of cellular pathways that sense DNA damage and replication stress and sets in motion a choreographed response to protect the cell and ameliorate the threat to the organism.”*

Harper & Elledge, *Mol Cell*, 2007; Jackson & Bartek, *Nature*, 2009

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Quebra de dupla fita



Exposição de fita simples

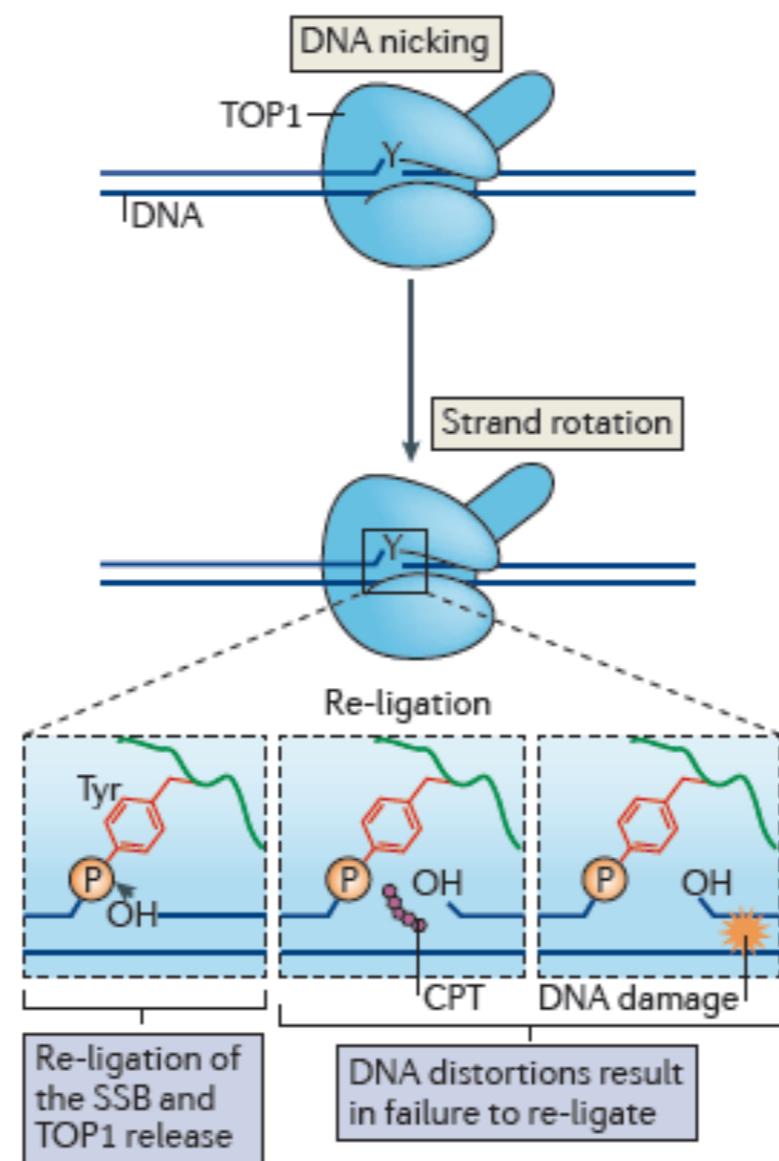


Como surgem essas estruturas?

# Quebra de dupla fita

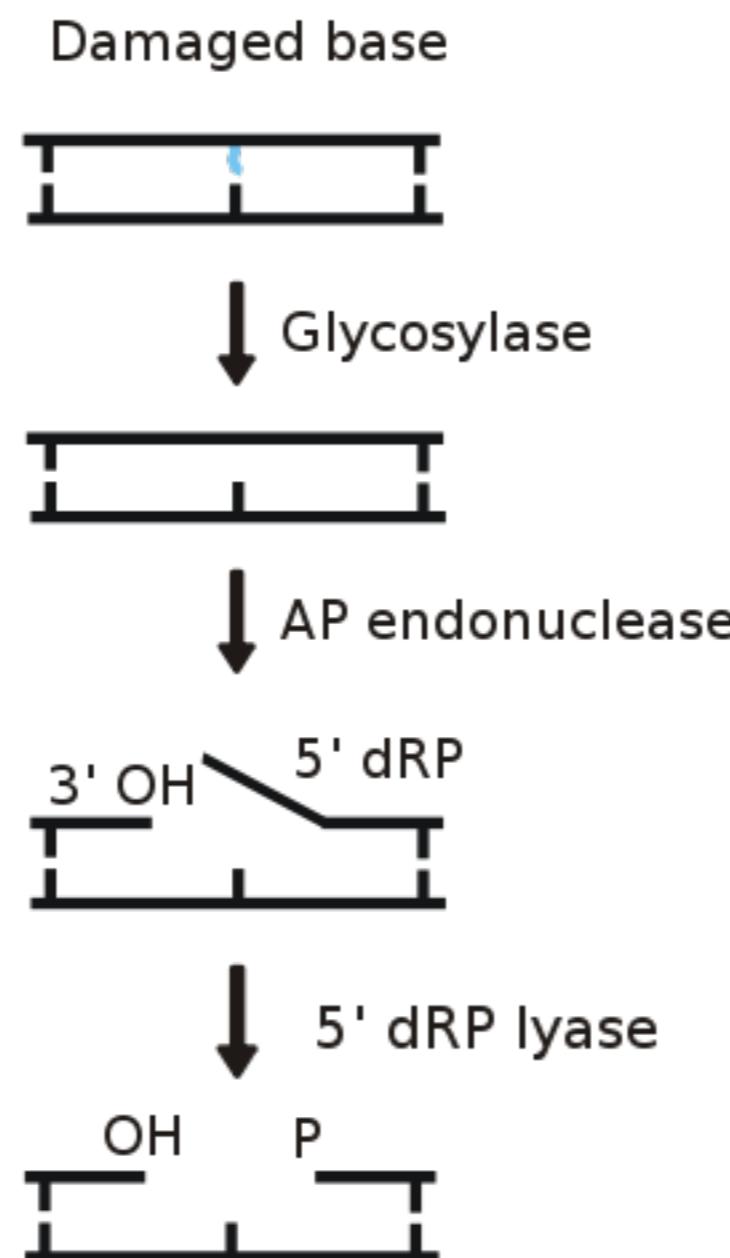
- Alta transferência de energia - Ex. Radiação Ionizante;
- Eventos em duas etapas, iniciando com a quebra de uma fita simples;

# Pela ação de drogas



Stingele & Jentsch, *Nat Rev Mol Cell Bio*, 2015

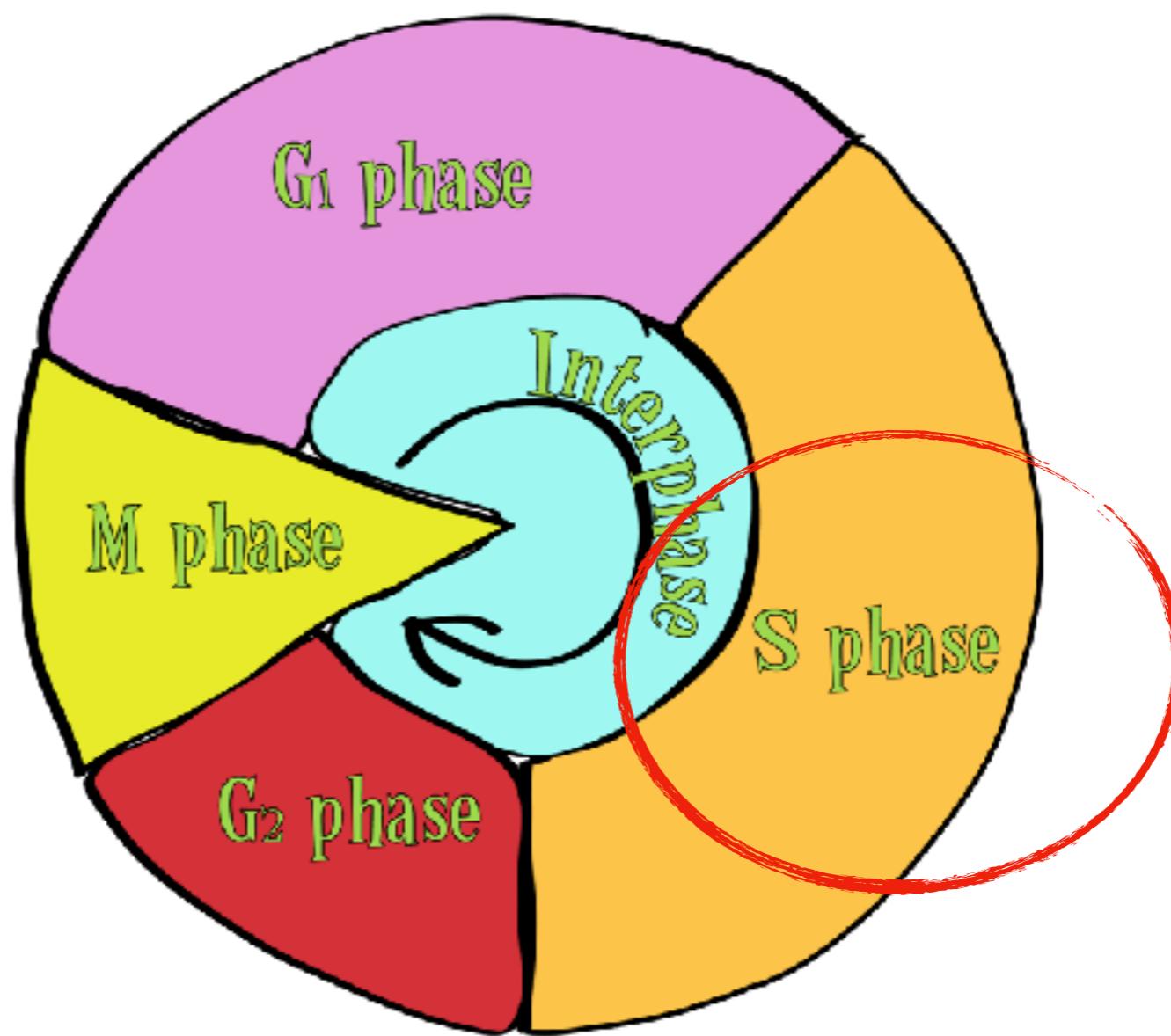
# Evolução de lesões menores



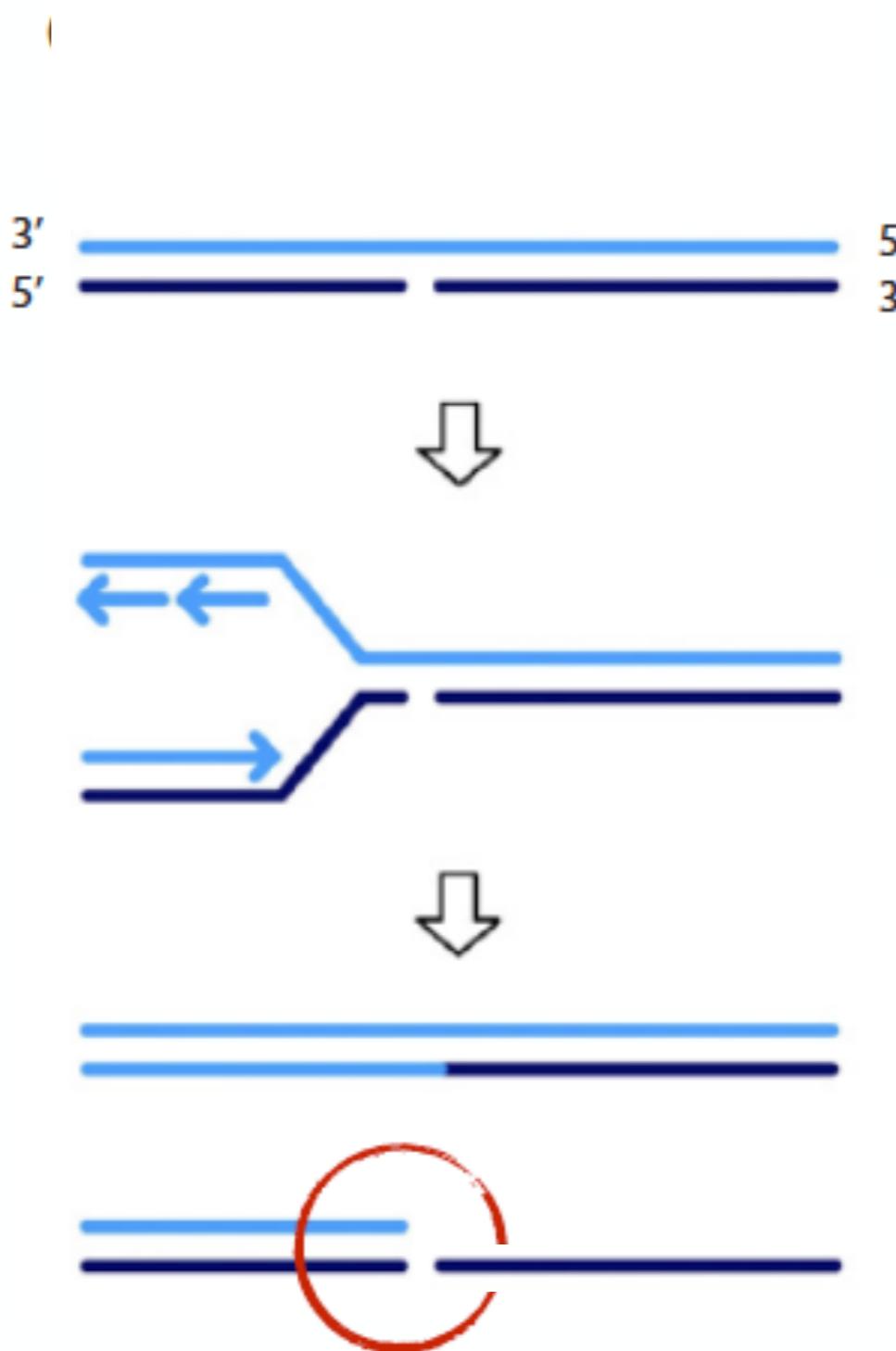
# Quebras de fita simples



# Influência fase ciclo celular



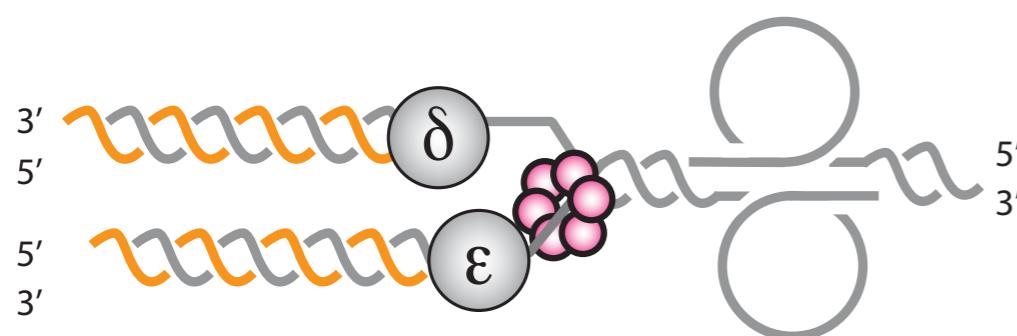
# Quebras de fita simples



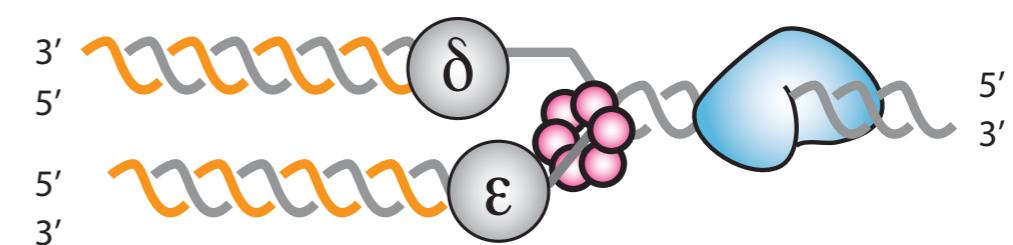
Ainda sobre desafios na fase S

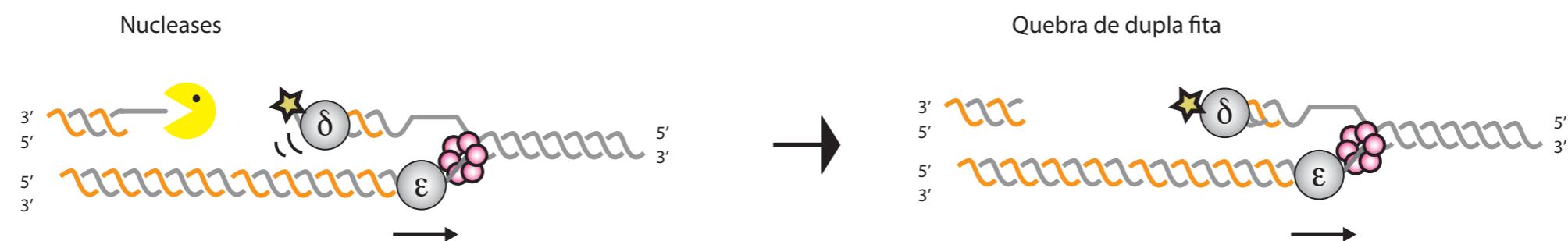
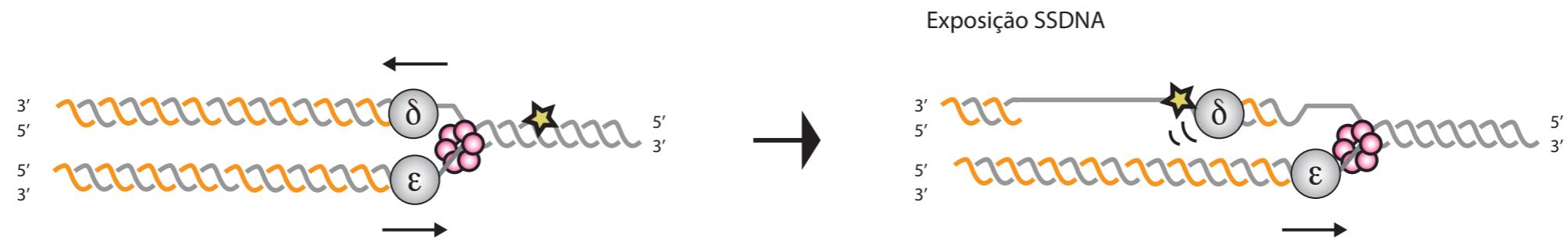
# Barreiras a Forquilha de Replicação

Estruturas secundárias



Complexos transcripcionais

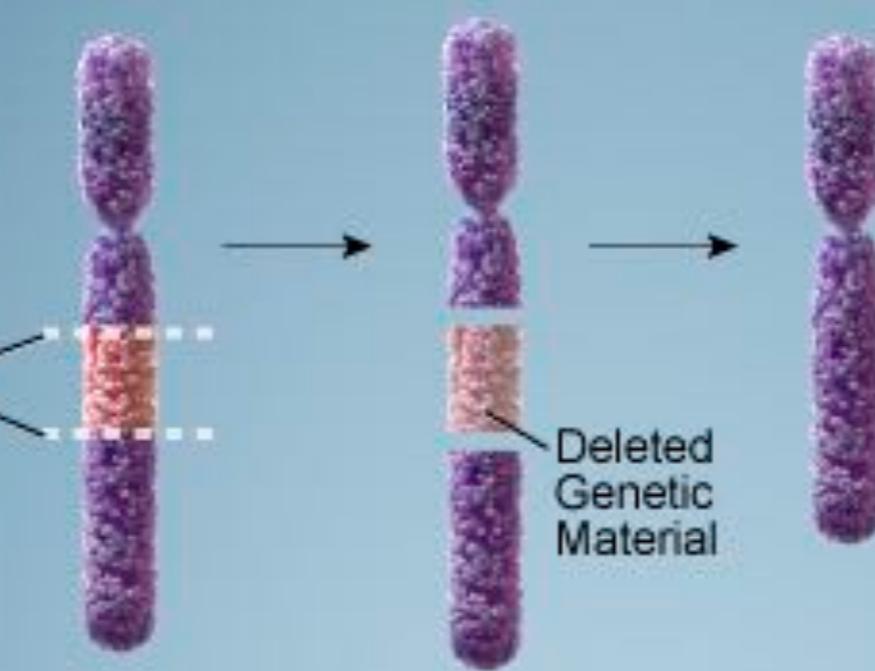




Por que a quebra de dupla fita é  
um problema?

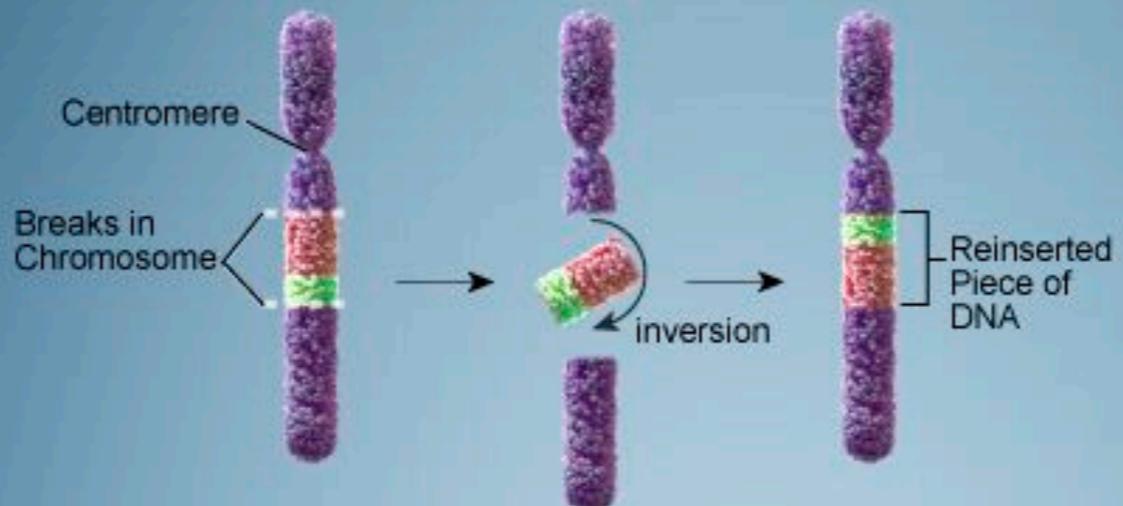
## Deletion

Breaks in Chromosome

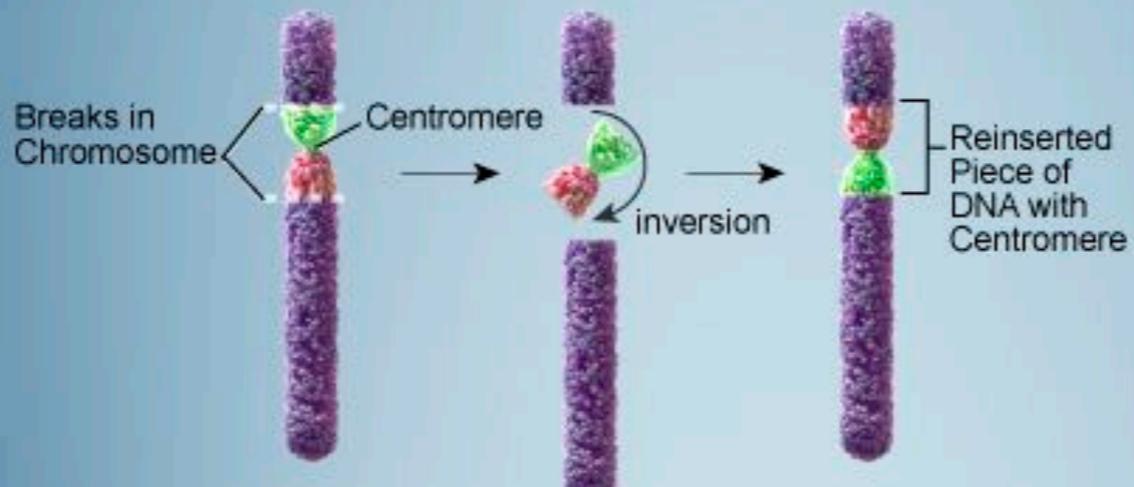


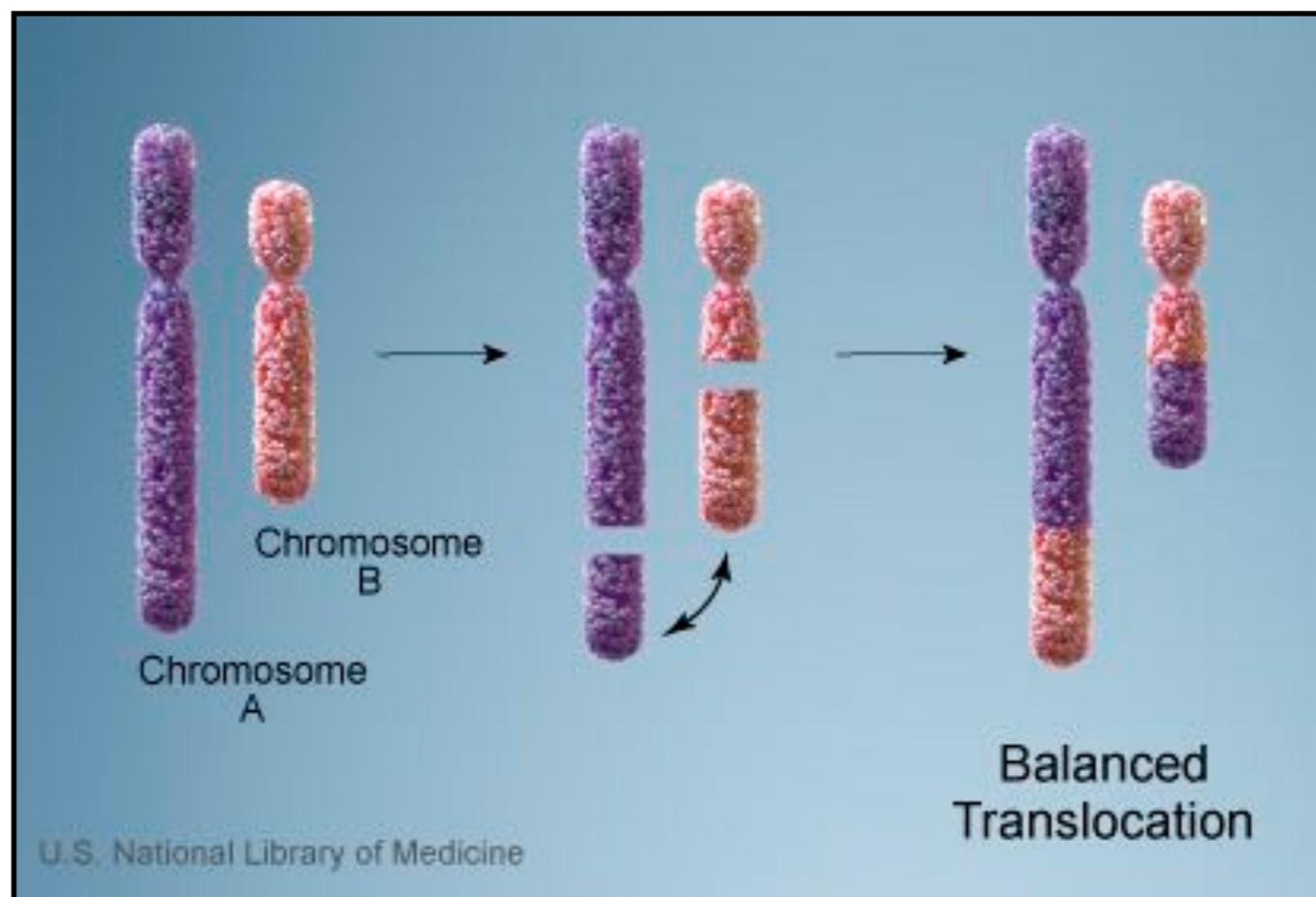
U.S. National Library of Medicine

### Paracentric Inversion



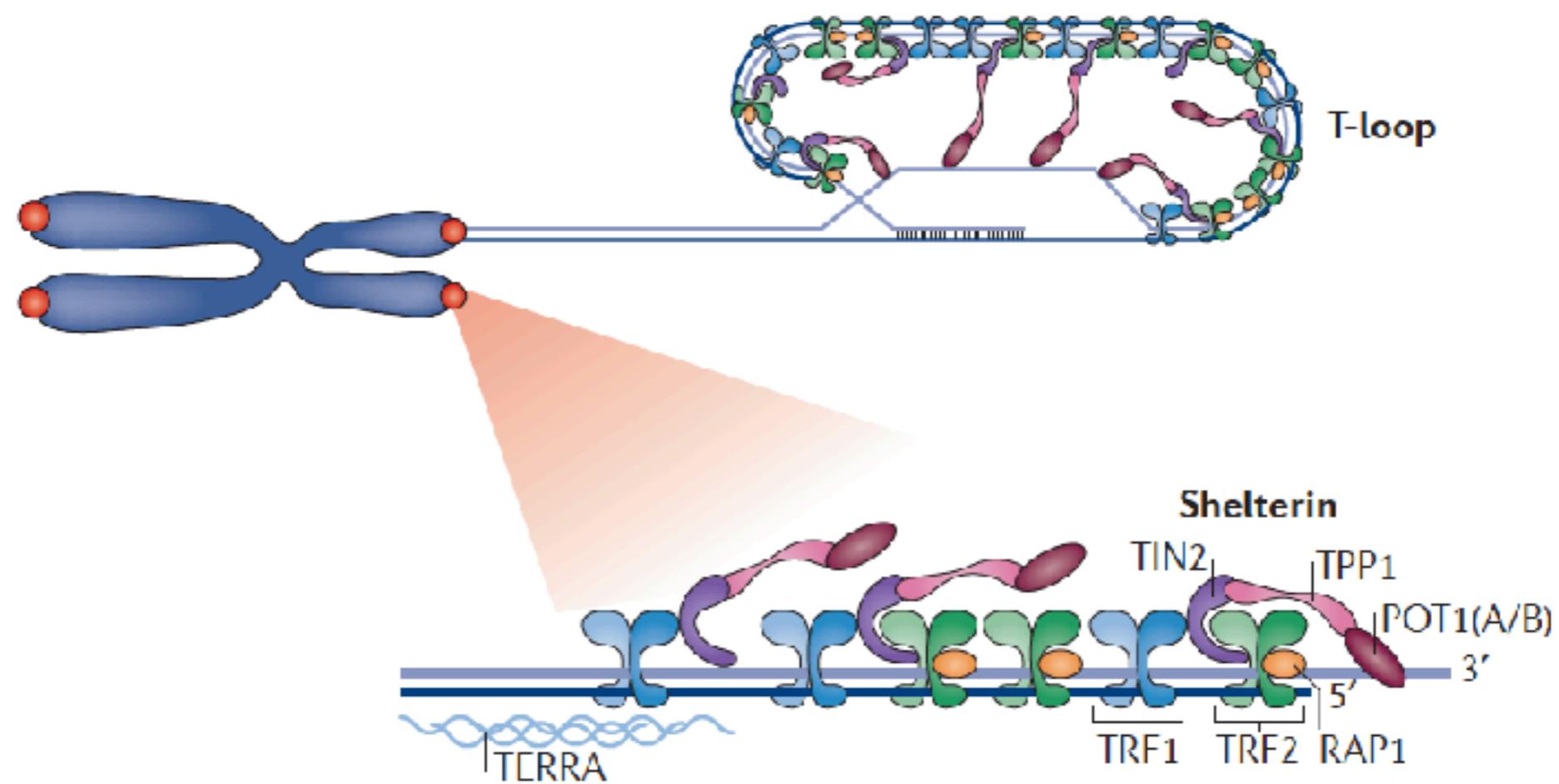
### Pericentric Inversion





Extremidades dupla fita expostas  
precisam ser prevenidas ou  
protegidas

# Telômeros

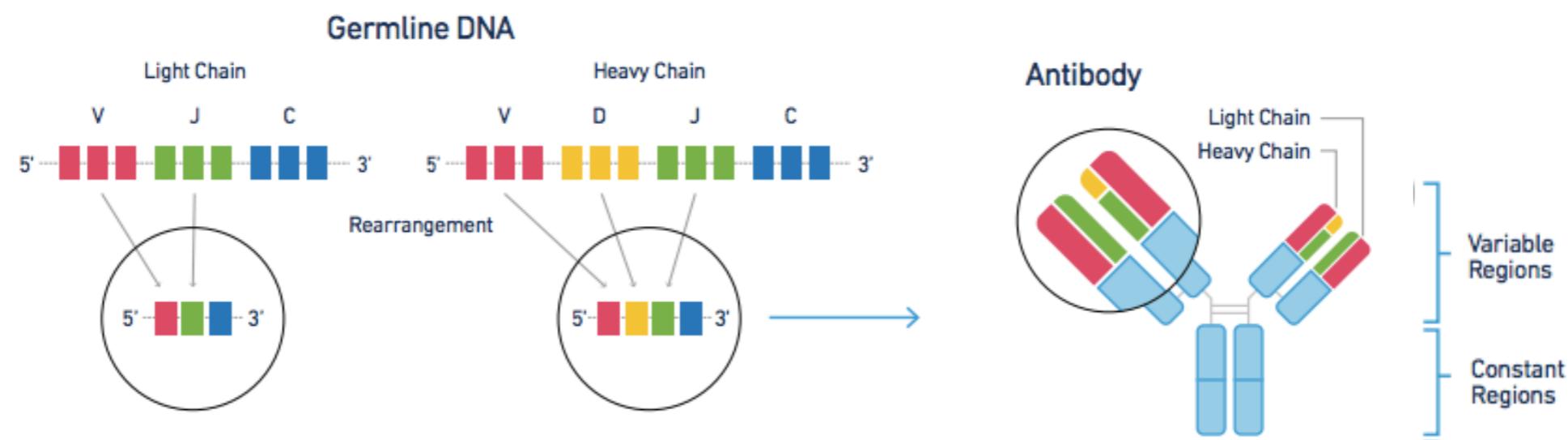


Lazzerini-Denchi & Sfeir, *Nat Rev Mol Cell Bio*, 2016

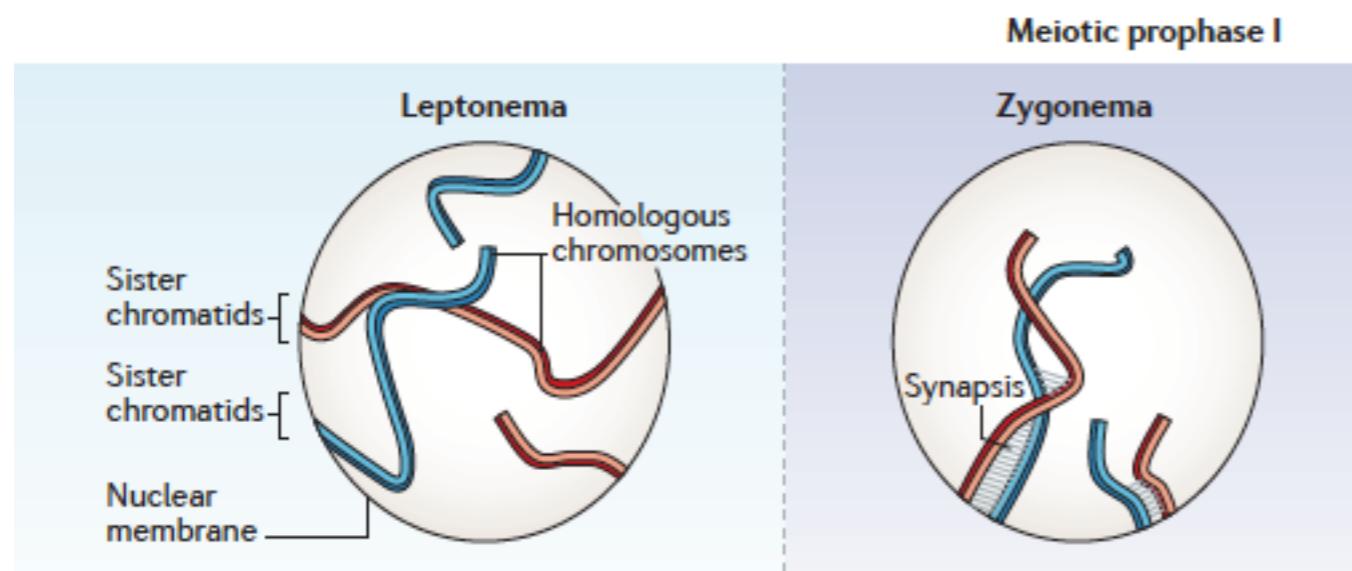
Quebras de dupla fita são  
sempre deletérias?

Quebra de dupla fita programada

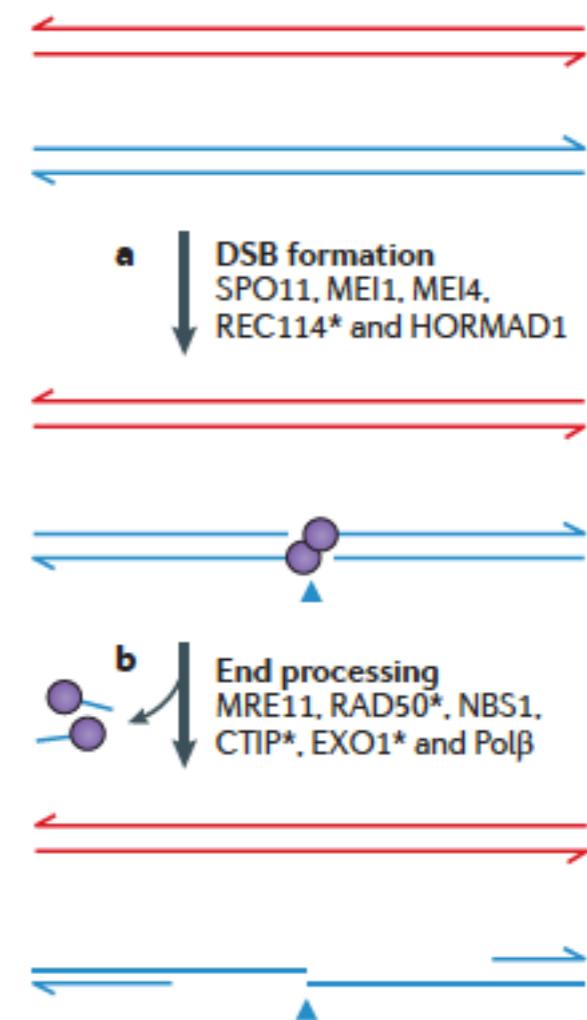
# Recombinação VDJ



# Meiose



Baudat *et al.*, *Nat Rev Gen*, 2013



# Breve histórico sobre sinalização de dano

# The Daily Mirror

CERTIFIED CIRCULATION LARGER THAN ANY OTHER PICTURE PAPER IN THE WORLD

No. 3,611.

Published at One Shilling,  
one New Penny.

FRIDAY, MAY 21, 1915

One Halfpenny.

"DEVILRY, THY NAME IS GERMANY!": SOLDIERS, TRAPPED BY  
A GAS CLOUD, LIE UNCONSCIOUS IN THE TRENCHES.



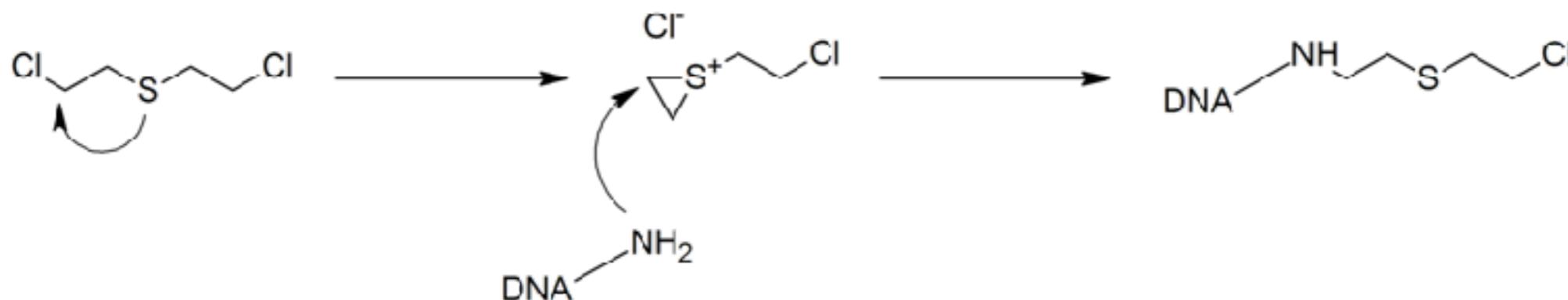
SCIENCE PHOTO LIBRARY

## The Reaction of Mustard Gas with Nucleic Acids *in vitro* and *in vivo*

BY P. BROOKES AND P. D. LAWLEY

*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3*

(Received 1 April 1960)



*Nature* Vol. 254 March 20 1975

# Different drugs arrest cells at a number of distinct stages in G<sub>2</sub>

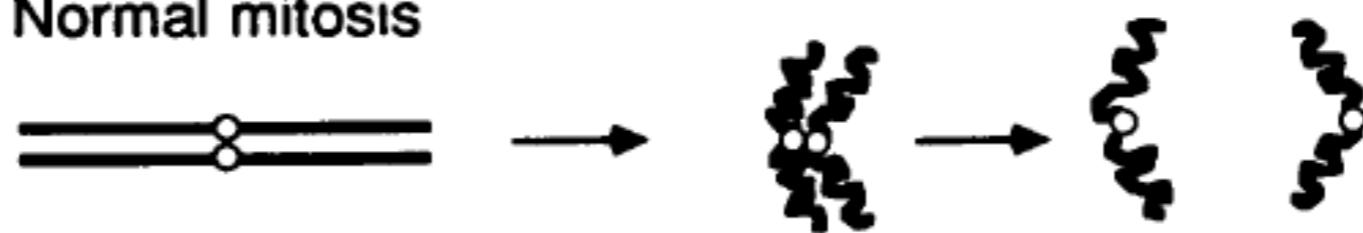
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ROBERT A. TOBEY

*Cellular and Molecular Radiobiology Group,  
Los Alamos Scientific Laboratory,  
University of California,  
Los Alamos, New Mexico 87544*

Received December 5, 1974; revised January 23, 1975.

**Normal mitosis**



*“The broken chromosome is an inadequate substrate for chromosome condensation or for some later step and mitosis is blocked”*

# Problema intrínseco ao DNA

*Proc. Natl Acad. Sci. USA*  
Vol. 79, pp. 2942-2946, May 1982  
Cell Biology

## Mechanism by which caffeine potentiates lethality of nitrogen mustard

(cell cycle/DNA damage/DNA repair/mitotic delay/nuclear fragmentation)

CHING C. LAU AND ARTHUR B. PARDEE\*

Department of Pharmacology, Harvard Medical School, 250 Longwood Avenue, Boston, Massachusetts 02115; and Division of Cell Growth and Regulation, Sidney Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115

*Contributed by Arthur B. Pardee, February 4, 1982*

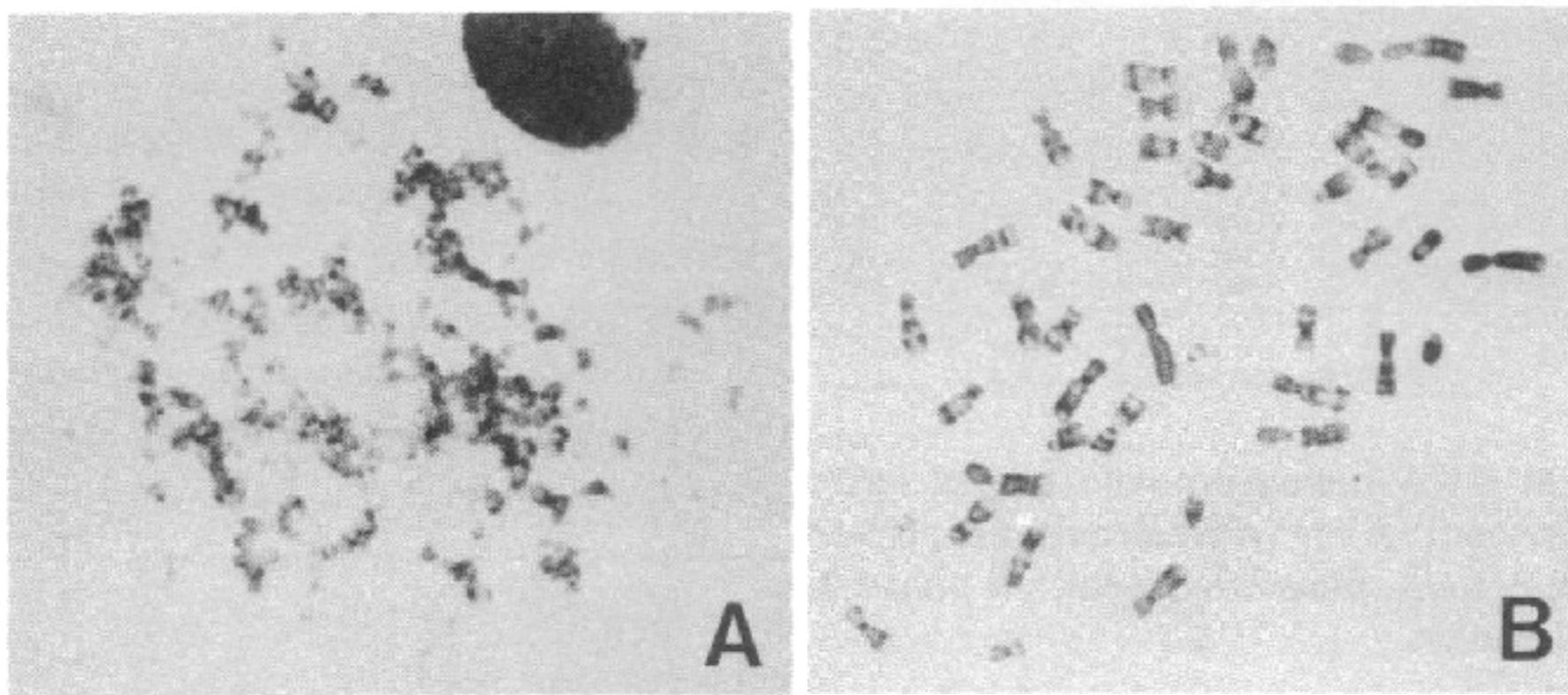
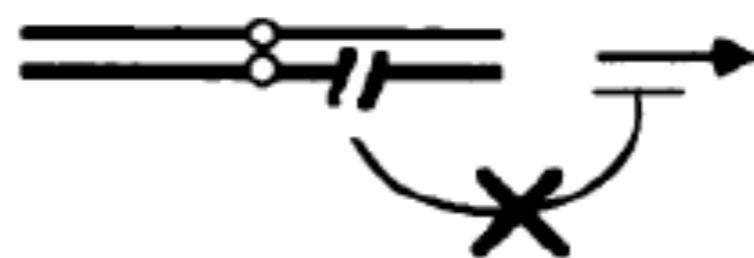


FIG. 5. Typical chromosome spreads of HN2-treated cells with (A) or without (B) caffeine post-treatment. Caffeine was added immediately after HN2 was removed and Colcemid (0.2  $\mu$ g/ml) was added 5 hr later. Mitotic cells were harvested 3 hr later and metaphase chromosomes were spread and stained with Giemsa stain. ( $\times 525$ .)

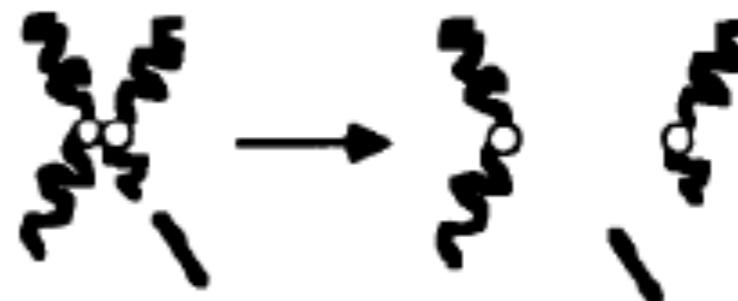
**Table 1. Effect of cycloheximide on enhanced lethality and nuclear fragmentation induced by caffeine**

Addition	% survival		% intact nuclei	
	Without HN2	With HN2	Without HN2	With HN2
Nothing	100	85	100	93
Caffeine	94	23	99	31

Relief of dependence

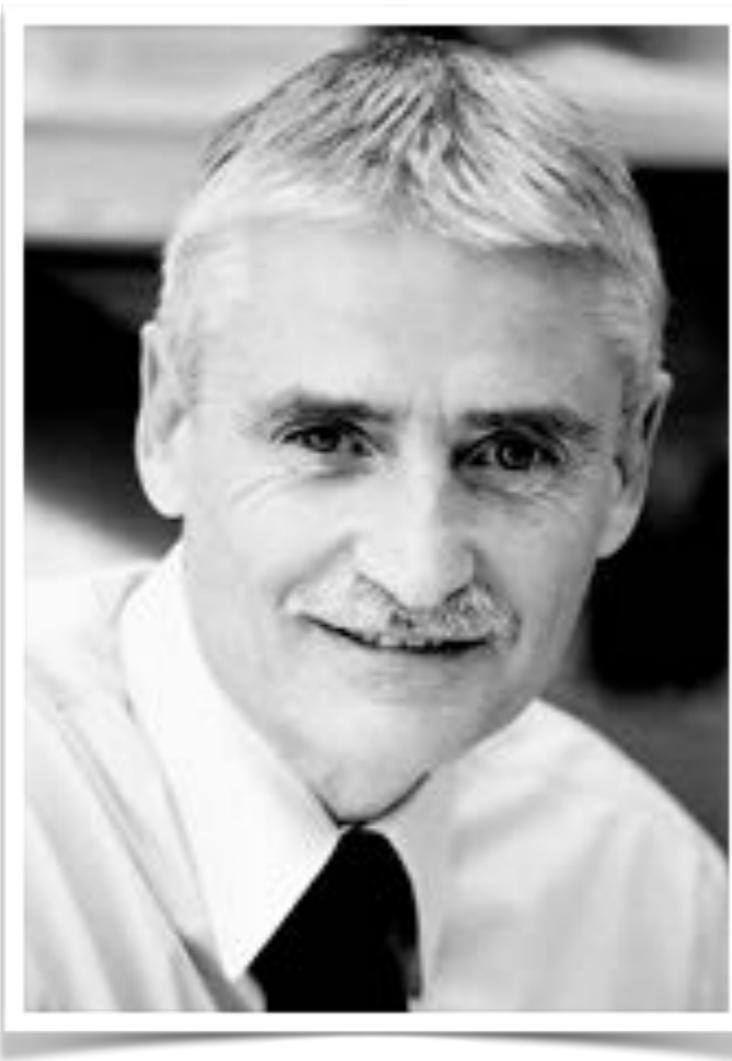


Error

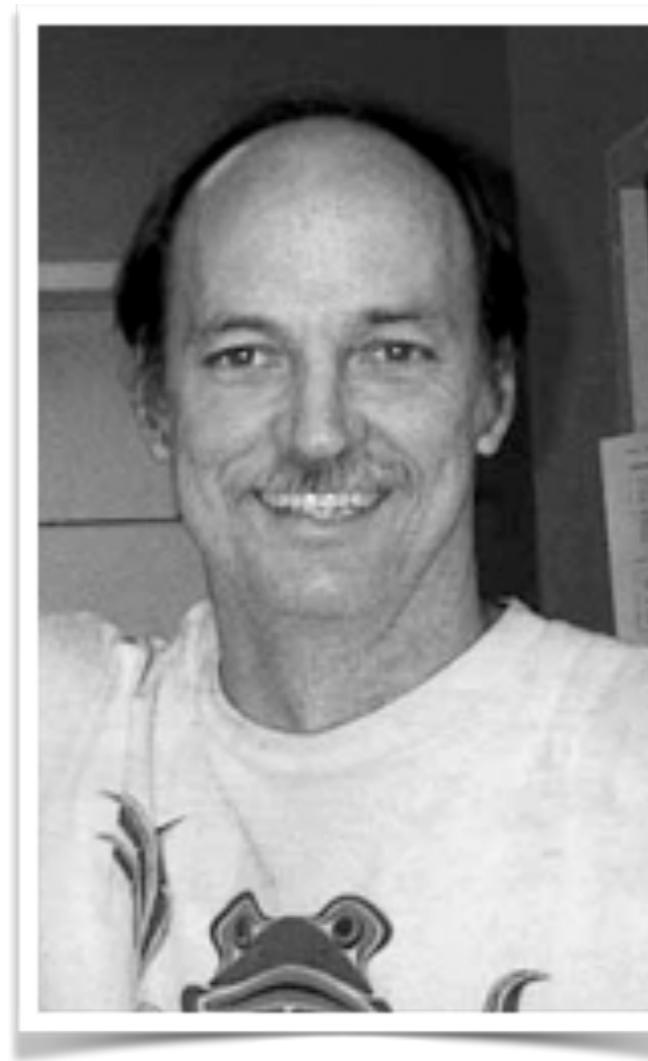


Como provar a existência  
desse mecanismo?

Lee Hartwell

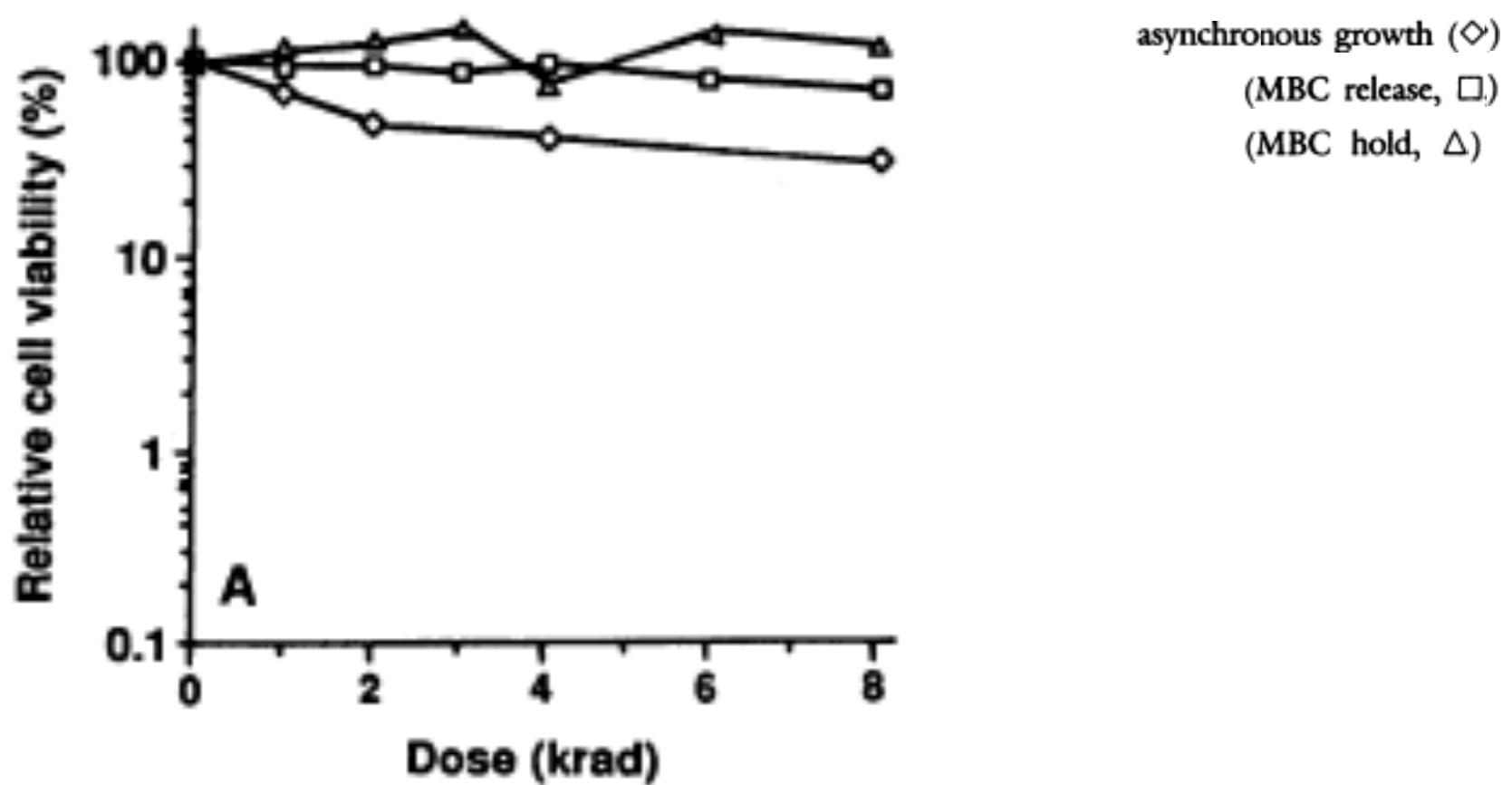


Ted Weinert

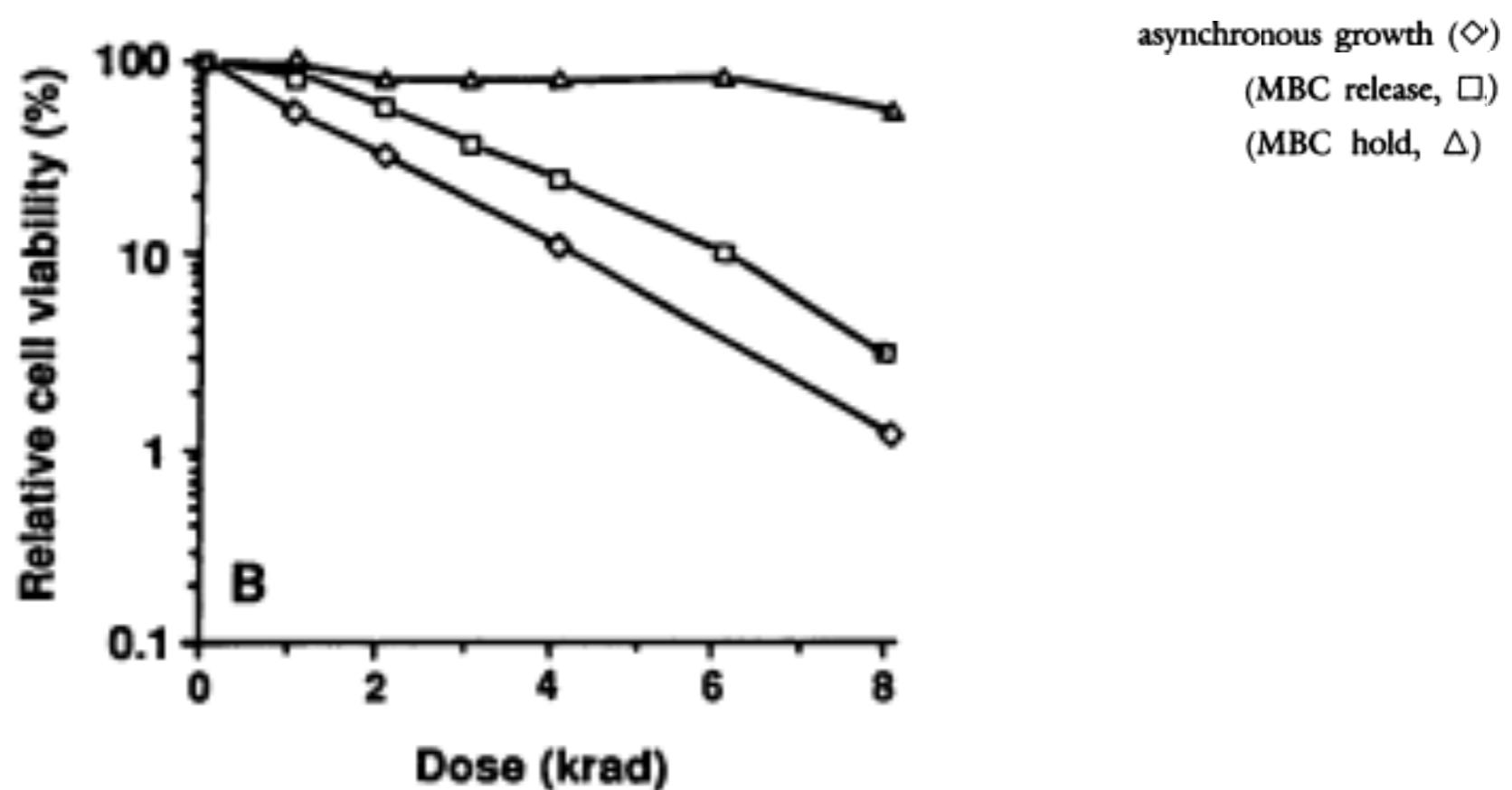


- Selecionaram mutantes que apresentavam sensibilidade a radiação (mutantes *RAD*);
- Avaliaram se essa sensibilidade seria dependente da progressão do ciclo celular;

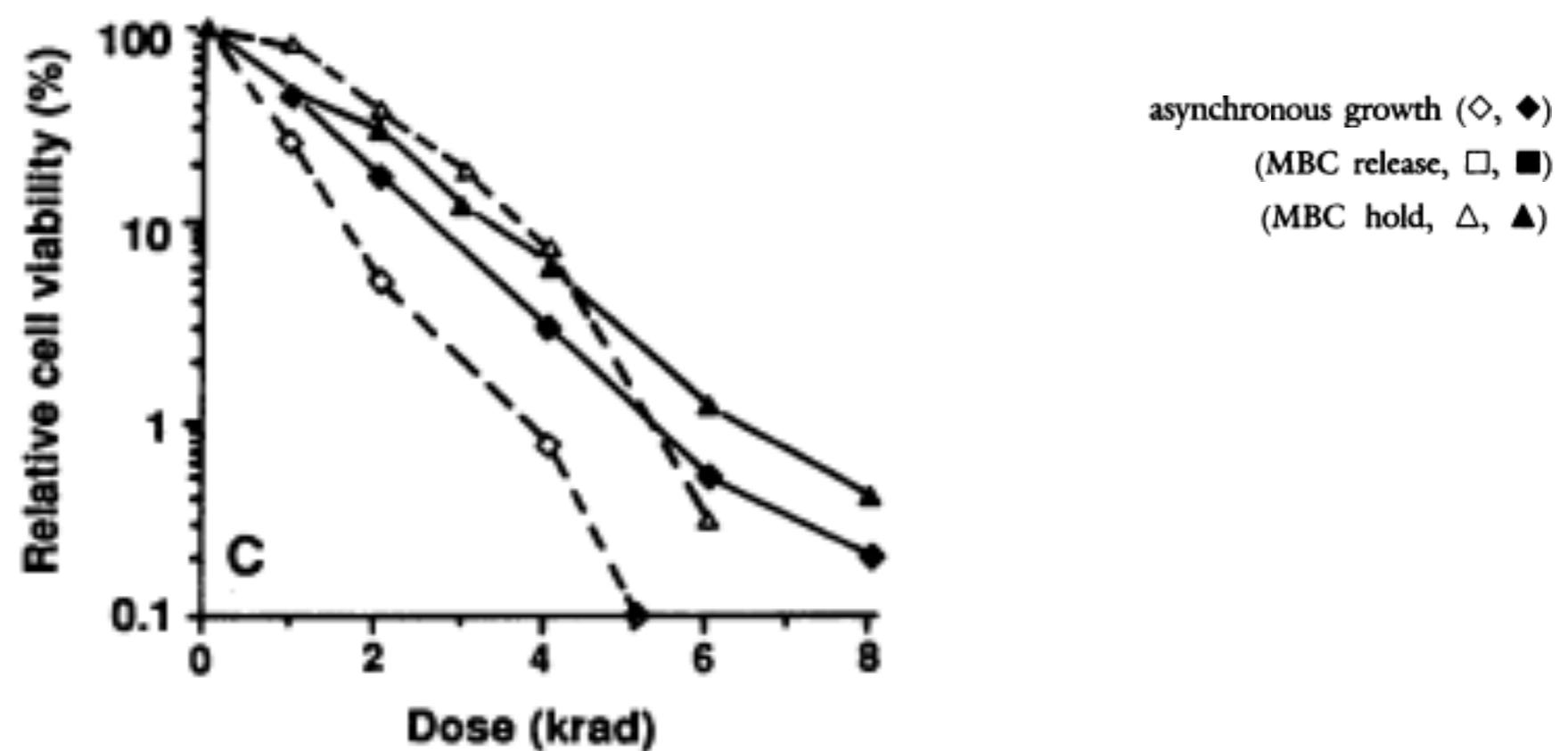
WT



# *rad9Δ*



# *rad52Δ* (pont.) ou *rad18Δ* (cont.)



# The *RAD9* Gene Controls the Cell Cycle Response to DNA Damage in *Saccharomyces cerevisiae*

TED A. WEINERT AND LELAND H. HARTWELL

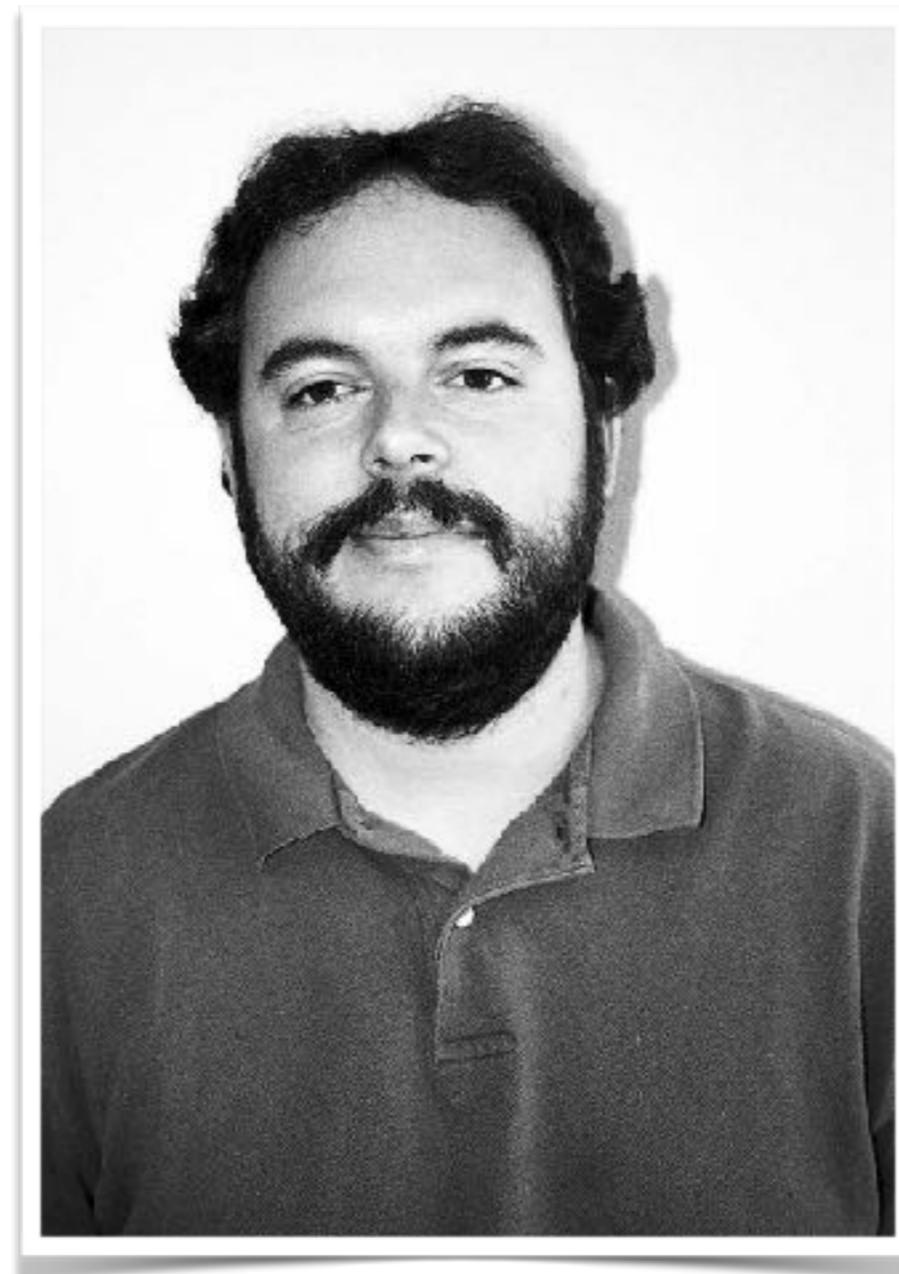
SCIENCE, VOL. 241

# **Checkpoints: Controls That Ensure the Order of Cell Cycle Events**

**LELAND H. HARTWELL AND TED A. WEINERT\***

**SCIENCE, VOL. 246**

Stephen Elledge



# DNA Damage Uninducible (DUN)

Estresse de replicação



DUN1 (quinase)



Metabolismo de nucleotídeos

Cell, Vol. 75, 1119–1127, December 17, 1993, Copyright © 1993 by Cell Press

## **DUN1 Encodes a Protein Kinase That Controls the DNA Damage Response in Yeast**

**Zheng Zhou\*** and **Stephen J. Elledge\*†**

\*Howard Hughes Medical Institute and  
Verna and Marrs McLean Department of Biochemistry

†Institute for Molecular Genetics  
Baylor College of Medicine  
Houston, Texas 77030

Reforça a idéia de TRANSDUÇÃO de informação

# Organização da via de RDD

**Sensores** - Detectam o estímulo;

**Transdutores** - Transferem o sinal;

**Mediadores** - Amplificam o sinal;

**Efetores** - Respondem ao sinal;

“DSB”



Exposição de “SSDNA”



MRN

Sensores

RPA

ATM

Transdutores

ATR

MDC1/53BP1/BRCA1/PTIP

Mediadores

9-1-1/TopBP1/CLASPIN

Chk2

Efetores

Chk1

**Table 1.** Factors involved in DNA strand break repair and damage signaling in budding yeast and mammals

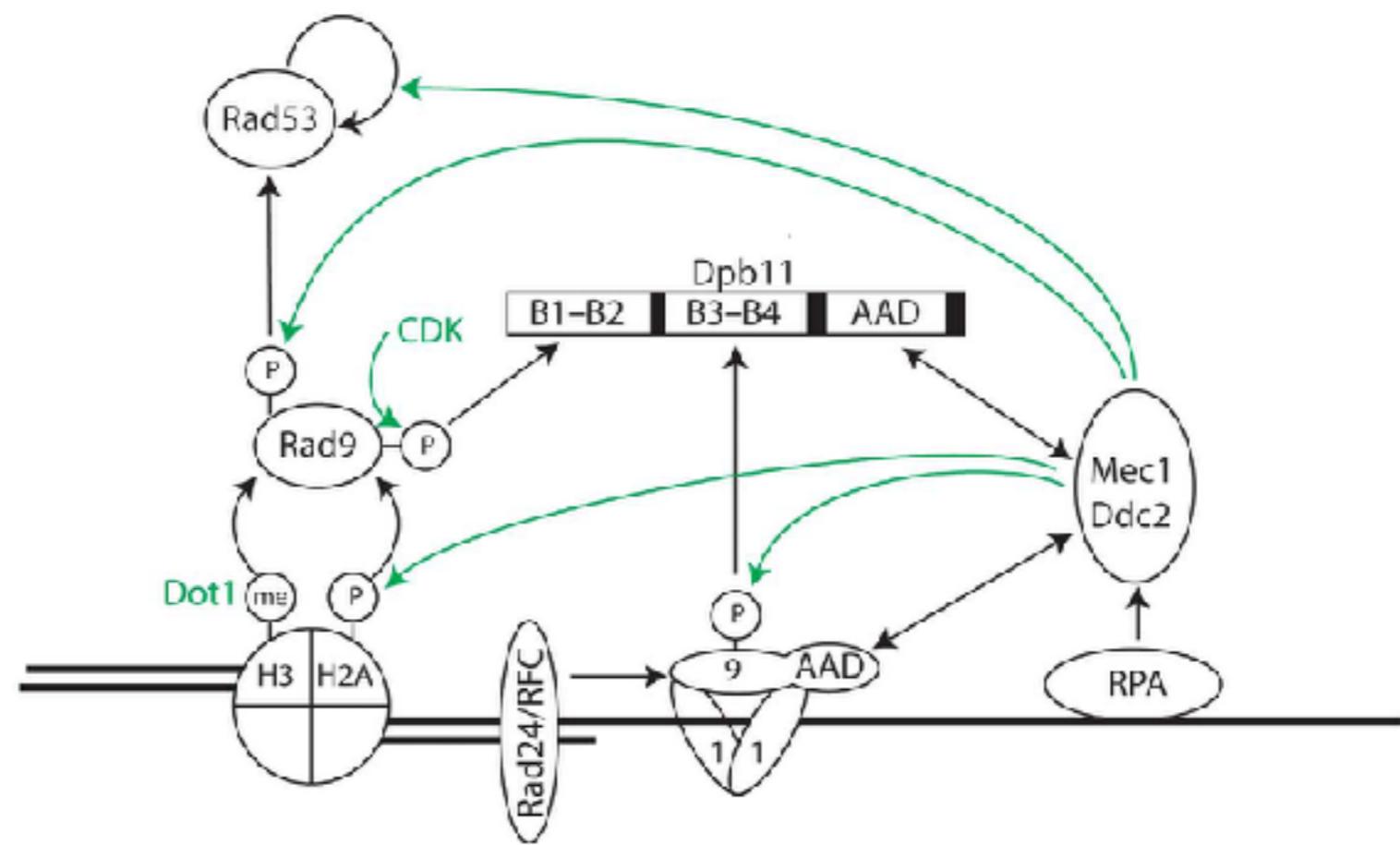
	Mammals	Yeast ( <i>S. cerevisiae</i> )
<b>DNA damage signaling</b>		
Sensors	MRN RPA (+RFC-like, PCNA-like checkpoint clamp)	MRX Rfa (+RFC-like, PCNA-like checkpoint clamp)
Transducers	ATM ATR-ATRIP	Tel1 Mec1-Ddc2
Mediators		
ATM signaling	53BP1, MDC1, BRCA1, MCPH1 PTIP	Rad9
ATR signaling	TopBP1 Claspin	Dpb11 Mrc1
Effectors	CHK1 CHK2	Chk1 Rad53

Pollo & Jackson, *Genes Dev*, 2011

**Table 1.** Factors involved in DNA strand break repair and damage signaling in budding yeast and mammals

	Mammals	Yeast ( <i>S. cerevisiae</i> )
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Pollo & Jackson, *Genes Dev*, 2011



Pfander & Diffley, *EMBO J*, 2011

“DSB”



MRN

ATM

MDC1/53BP1/BRCA1/PTIP

Chk2

Exposição de “SSDNA”



Sensores

Transdutores

Mediadores

Efetores

RPA

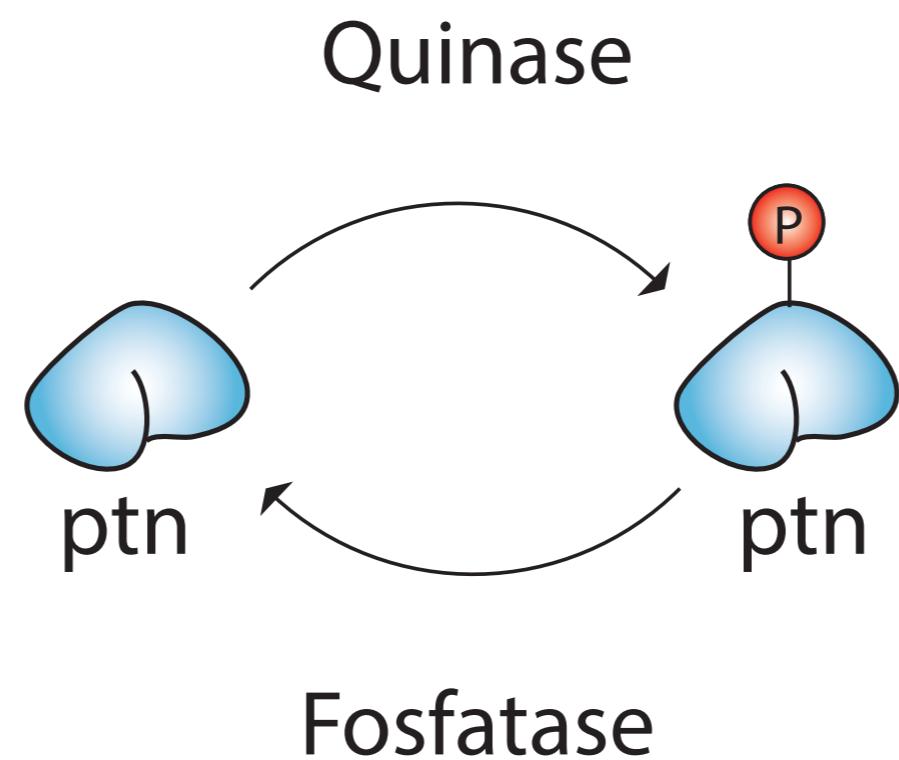
ATR

9-1-1/TopBP1/CLASPIN

Chk1

Por que a fosforilação é um evento importante nessa via de sinalização?

- Diversificam a natureza química das ptns;
- São modificações reversíveis;



# Repertório funcional fosforilações

- Regulação alostérica;
- Regulação degradação;
- Regulação interação entre ptgs;

“DSB”



Exposição de “SSDNA”



MRN

Sensores

RPA

ATM

Transdutores

ATR

MDC1/53BP1/BRCA1/PTIP

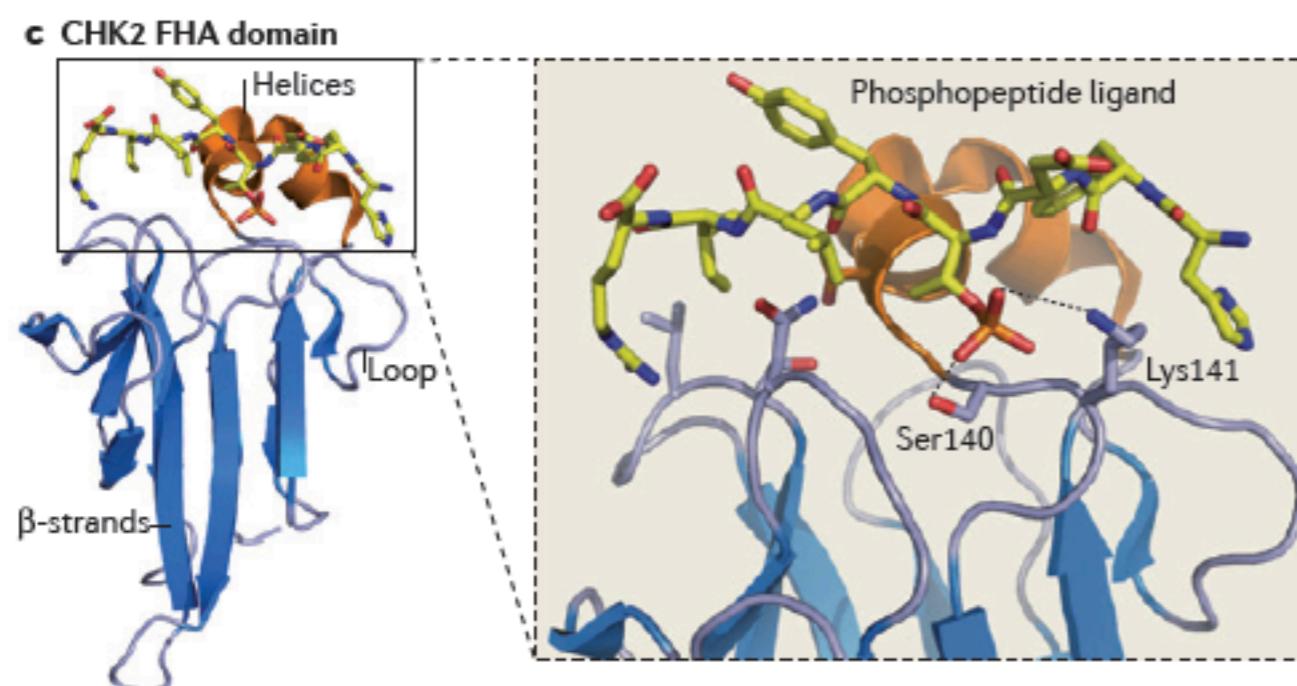
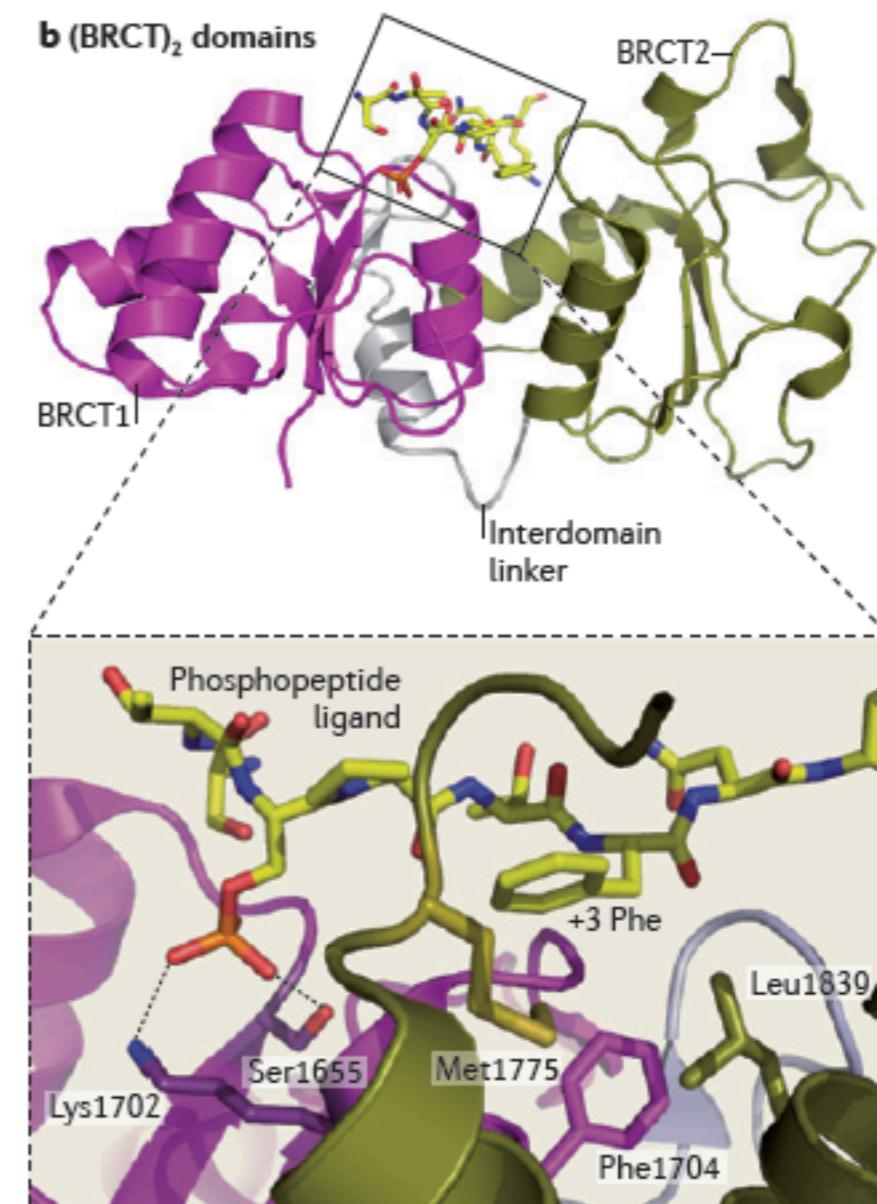
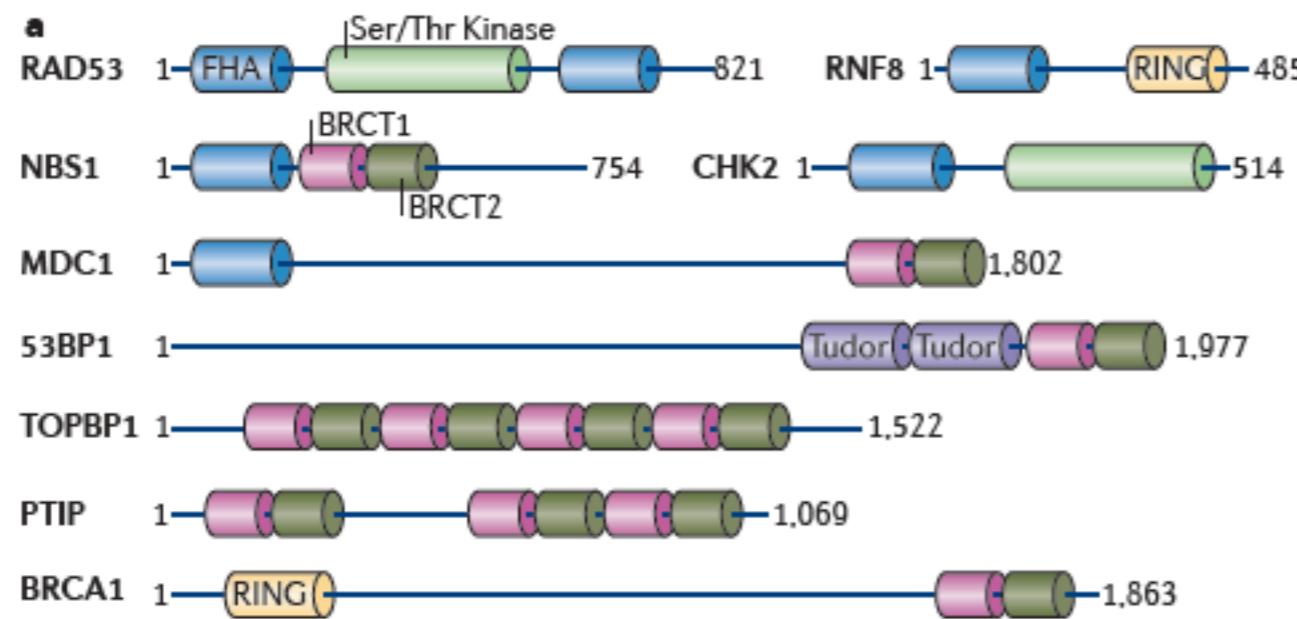
Mediadores

9-1-1/TopBP1/CLASPIN

Chk2

Efetores

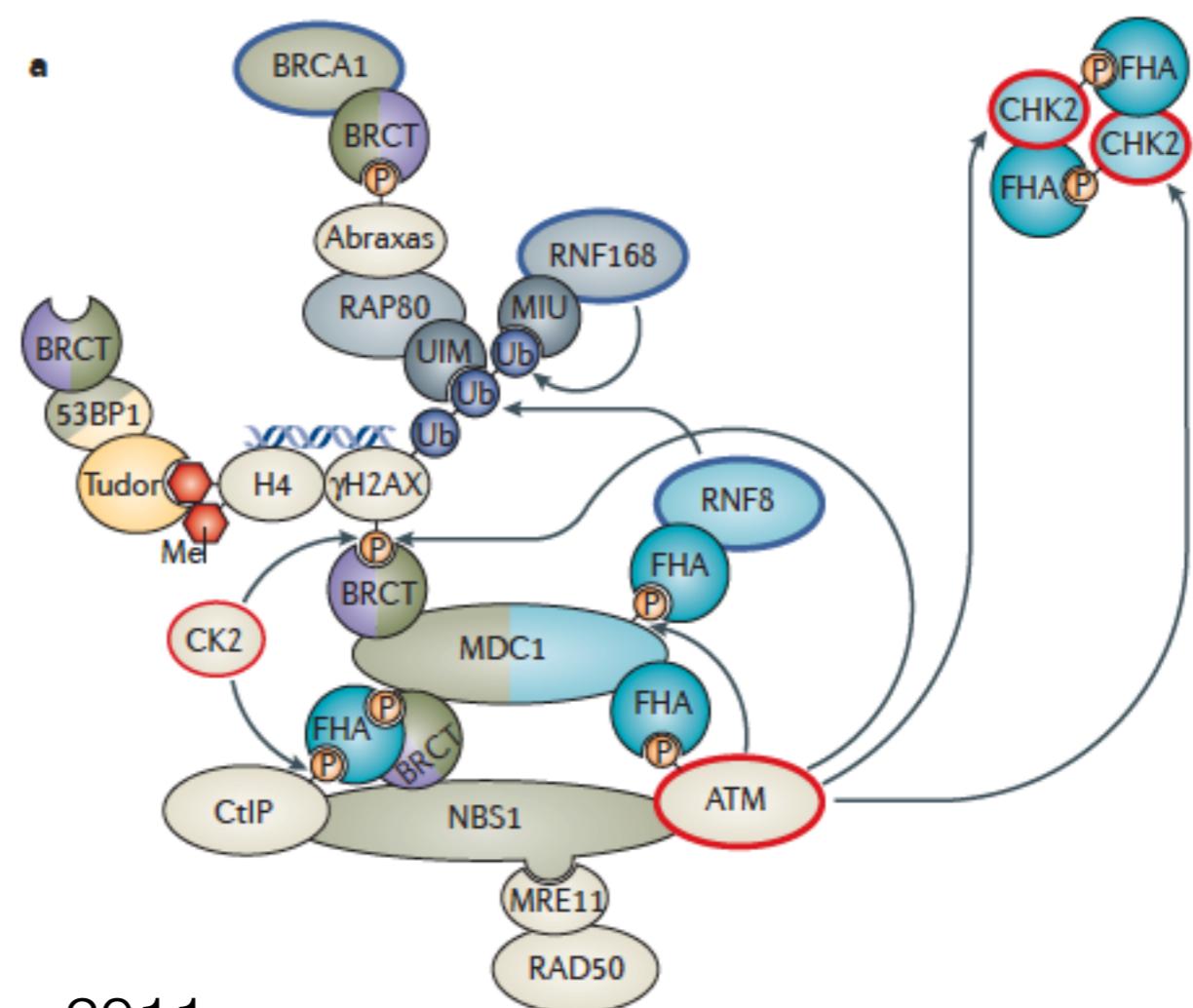
Chk1



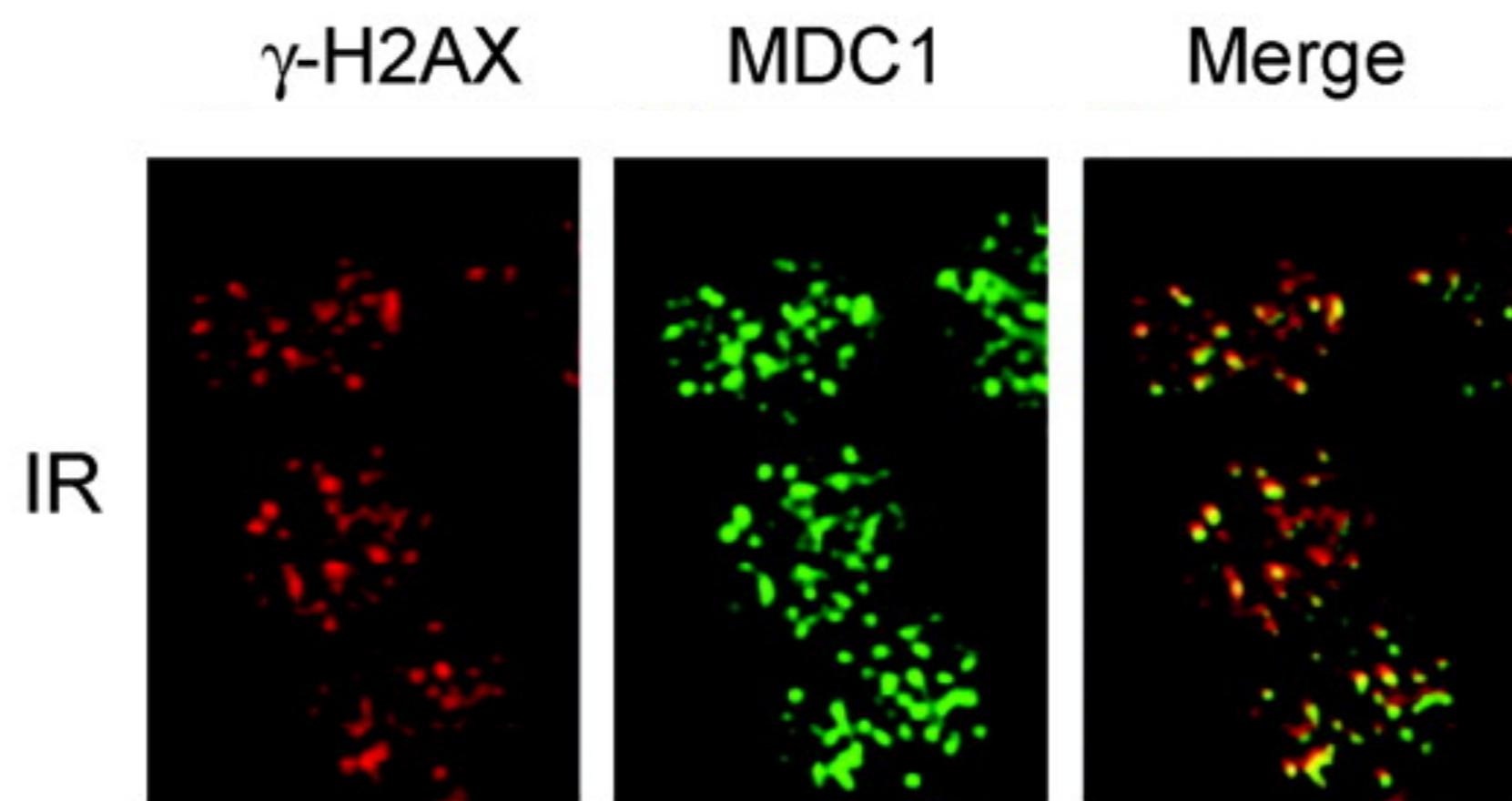
# DNA DR

*“The DNA damage response is a network of cellular pathways that sense DNA damage and replication stress and **sets in motion a choreographed response to protect the cell and ameliorate the threat to the organism.**”*

Harper & Elledge, *Mol Cell*, 2007; Jackson & Bartek, *Nature*, 2009



Resposta dependente de localização



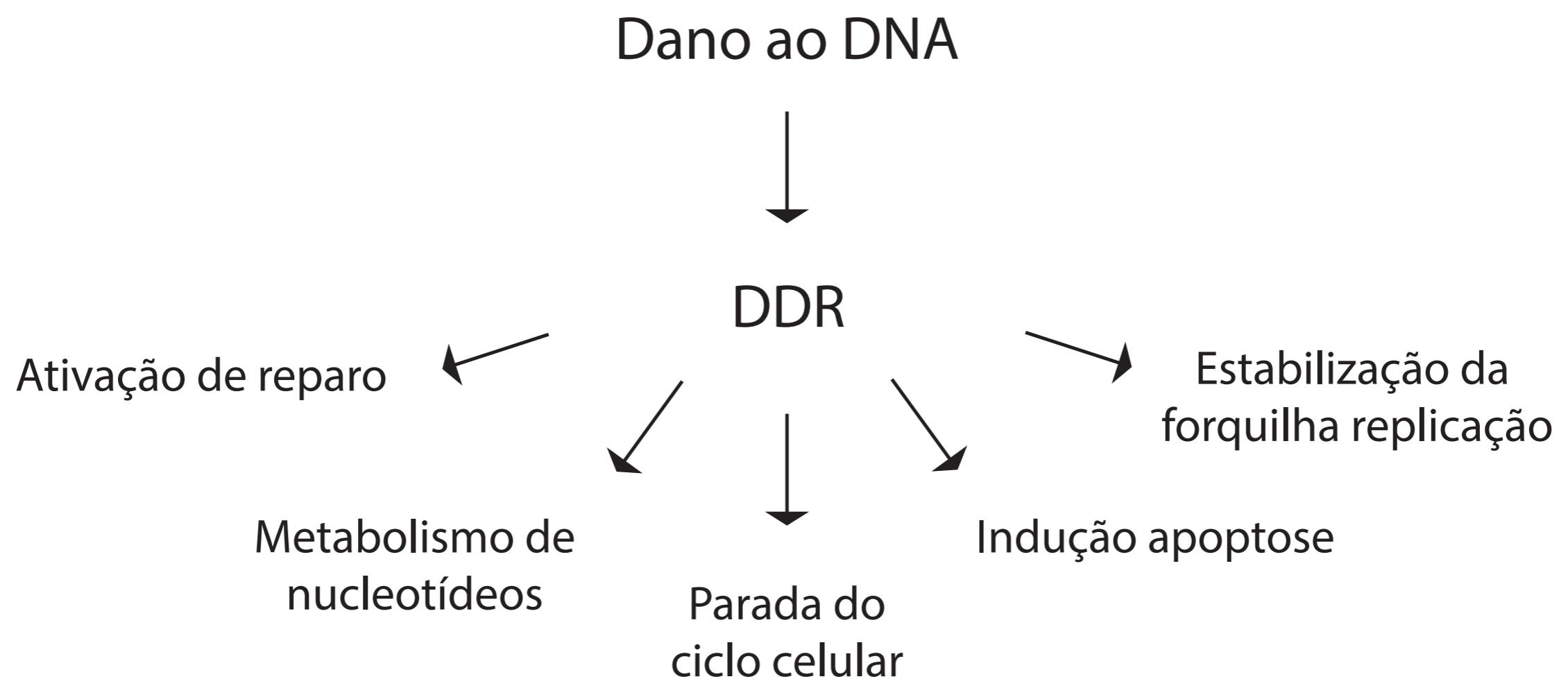
Pandita, *Cancer Research*, 2007

# DNA DR

*“The DNA damage response is a network of cellular pathways that sense DNA damage and replication stress and sets in motion a choreographed response to protect the cell and ameliorate the threat to the organism.”*

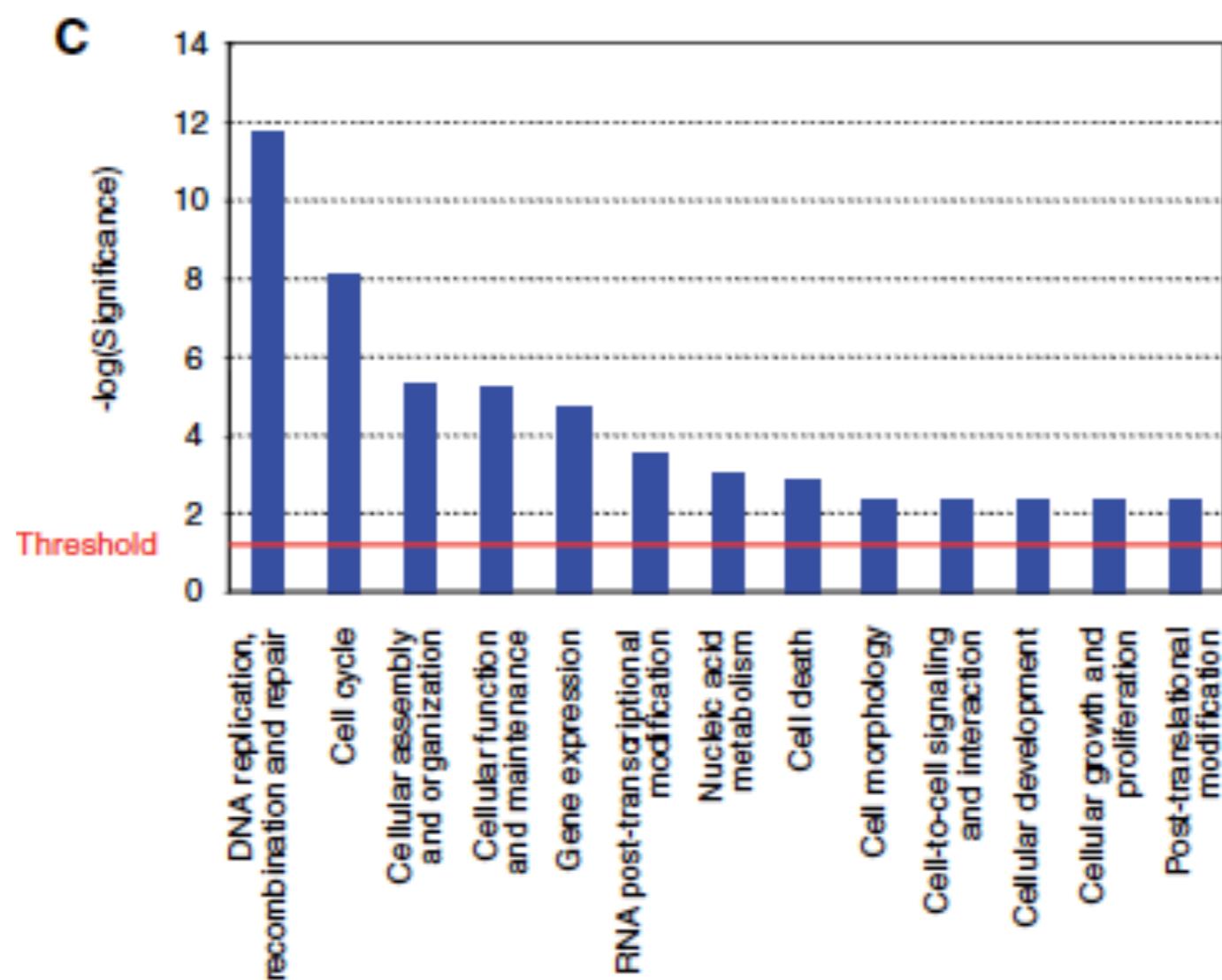
Harper & Elledge, *Mol Cell*, 2007; Jackson & Bartek, *Nature*, 2009

Como ?



# ATM and ATR Substrate Analysis Reveals Extensive Protein Networks Responsive to DNA Damage

Shuhei Matsuoka, *et al.*  
*Science* 316, 1160 (2007);



NATURE 2015

# The core spliceosome as target and effector of non-canonical ATM signalling

Maria Tresini<sup>1</sup>, Daniël O. Warmerdam<sup>2</sup>, Petros Kolovos<sup>3</sup>, Loes Snijder<sup>1</sup>, Mischa G. Vrouwe<sup>4</sup>, Jeroen A. A. Demmers<sup>5</sup>, Wilfred F. J. van IJcken<sup>6</sup>, Frank G. Grosveld<sup>3</sup>, René H. Medema<sup>2</sup>, Jan H. J. Hoeijmakers<sup>1</sup>, Leon H. F. Mullenders<sup>4</sup>, Wim Vermeulen<sup>1</sup> & Jurgen A. Marteijn<sup>1</sup>

Molecular Cell 51, 423–439, August 22, 2013 ©2013 Elsevier Inc. 423

## NEK8 Links the ATR-Regulated Replication Stress Response and S Phase CDK Activity to Renal Ciliopathies

Hyo Jei Claudia Choi,<sup>1</sup> Jia-Ren Lin,<sup>1</sup> Jean-Baptiste Vannier,<sup>2</sup> Gisela G. Slaats,<sup>3</sup> Andrew C. Kile,<sup>1</sup> Renee D. Paulsen,<sup>1</sup> Danielle K. Manning,<sup>4</sup> David R. Beier,<sup>4</sup> Rachel H. Giles,<sup>3</sup> Simon J. Boulton,<sup>2</sup> and Karlene A. Cimprich<sup>1,\*</sup>

<sup>1</sup>Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA 94025, USA

<sup>2</sup>Clare Hall Laboratories, London Research Institute, Blanche Lane, South Mimms EN6 3LD, UK

<sup>3</sup>Department of Nephrology and Hypertension, University Medical Center Utrecht, Heidelberglaan 100, 3584CX Utrecht, the Netherlands

<sup>4</sup>Brigham and Women's Hospital, Division of Genetics, Boston, MA 02115, USA

**“** What emerged more slowly, however, was an appreciation that DDR mechanisms ... are essential for cancer avoidance **”**

<b>Kinase</b>	<b>Syndrome</b>	<b>Cancer Susceptibility</b>
ATR	Seckel Syndrome	Oropharyngeal Cancer Syndrome
* ATM	Ataxia Telangiectasia	Lymphomas & Leukemias
Chk1	-	Endometrial & Stomach
Chk2	Li-Fraumeni Syndrome	Breast & Colon

Kastan, *Mol Cancer Res*, 2008 Bartek & Lukas, *Cancer Cell*, 2003

# Ataxia Telangiectasia

- Neurodegeneração, imunodeficiência, câncer;
- Células isoladas pacientes A-T;
- Mutantes hipomórficos “naturais”;

<b>Kinase</b>	<b>Syndrome</b>	<b>Cancer Susceptibility</b>
ATR	Seckel Syndrome	Oropharyngeal Cancer Syndrome
ATM	Ataxia Telangiectasia	Lymphomas & Leukemias
Chk1	-	Endometrial & Stomach
Chk2	Li-Fraumeni Syndrome	Breast & Colon

Kastan (2008) *Mol Cancer Res*, Bartek & Lukas (2003) *Cancer Cell*

# *Seckel Syndrome*

- Disturbios desenvolvimento, câncer;
- Mutações hipomórficas em ATR;

The EMBO Journal Vol.17 No.1 pp.159–169, 1998

## **Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints**

**William A.Cliby<sup>1,2</sup>, Christopher J.Roberts<sup>1,3</sup>,  
Karlene A.Cimprich<sup>4,5</sup>, Cheri M.Stringer<sup>1</sup>,  
John R.Lamb<sup>1</sup>, Stuart L.Schreiber<sup>3</sup> and  
Stephen H.Friend<sup>1,6</sup>**

GENES & DEVELOPMENT 14:397–402

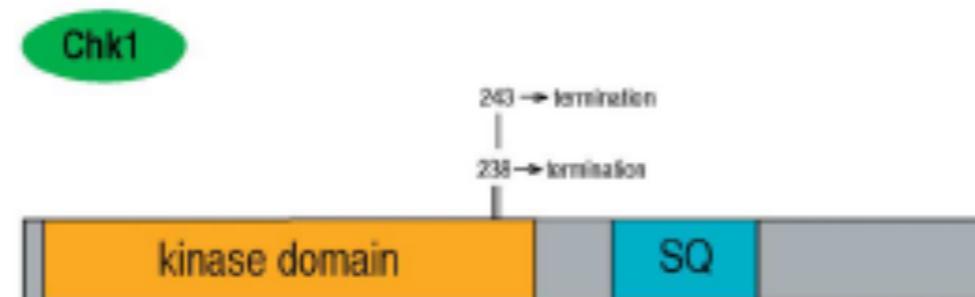
# ***ATR disruption leads to chromosomal fragmentation and early embryonic lethality***

**Eric J. Brown and David Baltimore<sup>1</sup>**

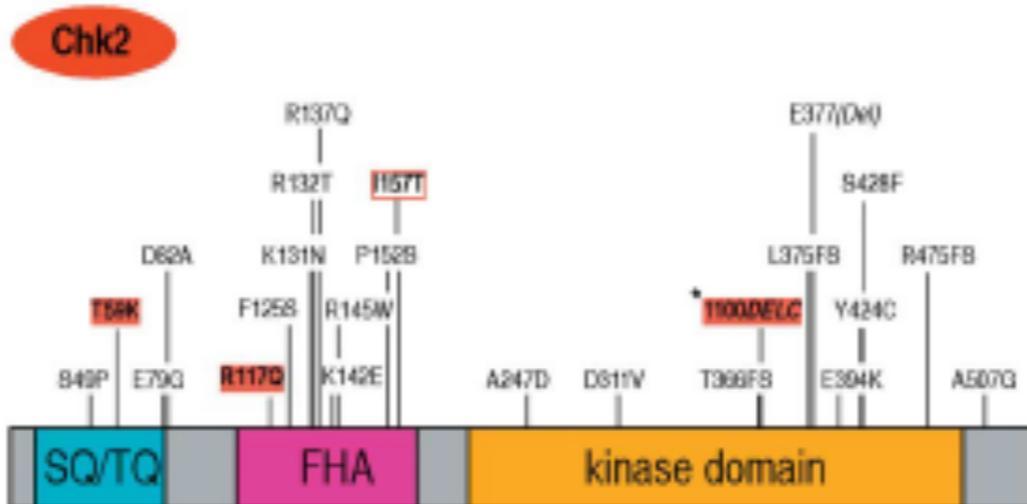
California Institute of Technology, Division of Biology,  
Pasadena, California 91125 USA

Received November 16, 1999; revised version accepted  
January 10, 2000.

<b>Kinase</b>	<b>Syndrome</b>	<b>Cancer Susceptibility</b>
ATR	Seckel Syndrome	Oropharyngeal Cancer Syndrome
ATM	Ataxia Telangiectasia	Lymphomas & Leukemias
* Chk1	-	Endometrial & Stomach
* Chk2	Li-Fraumeni Syndrome	Breast & Colon

**A**

*Aberrant in:* endometrial, gastric, and colorectal carcinomas.

**B**

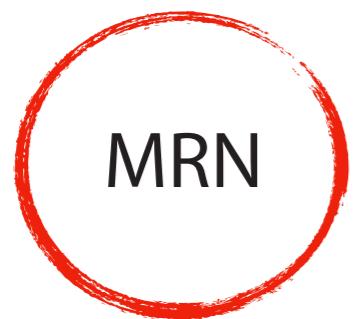
*Identified mutations in:* carcinoma of the breast, colon, lung, bladder, ovary, and vulva; sarcomas; lymphomas (AML, NHL); Li-Fraumeni syndrome.

Síndromes associadas a outros  
componentes da via de RDD

# *Nijmegen Break Syndrome (NBS)*

- Microcefalia, imunodeficiência, câncer;
- Mutação gene *NBS1*;
- Ptn parte do complexo MRN;
- NBS tb é conhecida como variante A-T;

“DSB”



ATM

MDC1/53BP1/BRCA1/PTIP

Chk2

Sensores

Transdutores

Mediadores

Efetores

Exposição de “SSDNA”



RPA

ATR

9-1-1/TopBP1/CLASPIN

Chk1

# *Werner or Bloom Syndromes*

- Instabilidade genômica; câncer
- Mutação gene *NBS1*;
- Disfunções *WRN* e *BLM*;
- Helicase família RecQ;

“DSB”



MRN

ATM

MDC1/53BP1/BRCA1/PTIP

Chk2

Exposição de “SSDNA”



Sensores

Transdutores

Mediadores

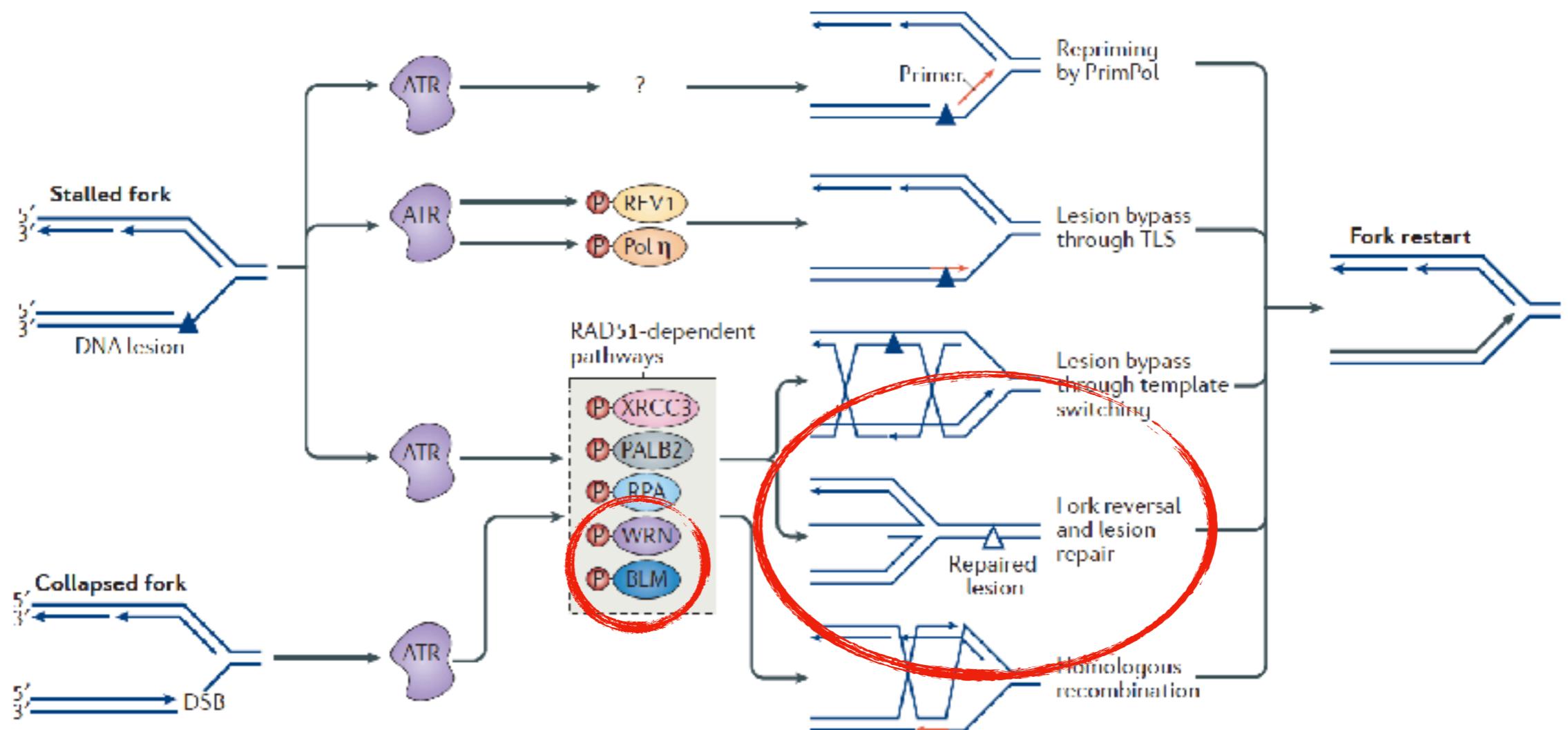
Efetores

RPA

ATR

9-1-1/TopBP1/CLASPIN

Chk1



Via de RDD como alvo terapêutico  
seletivo contra o câncer

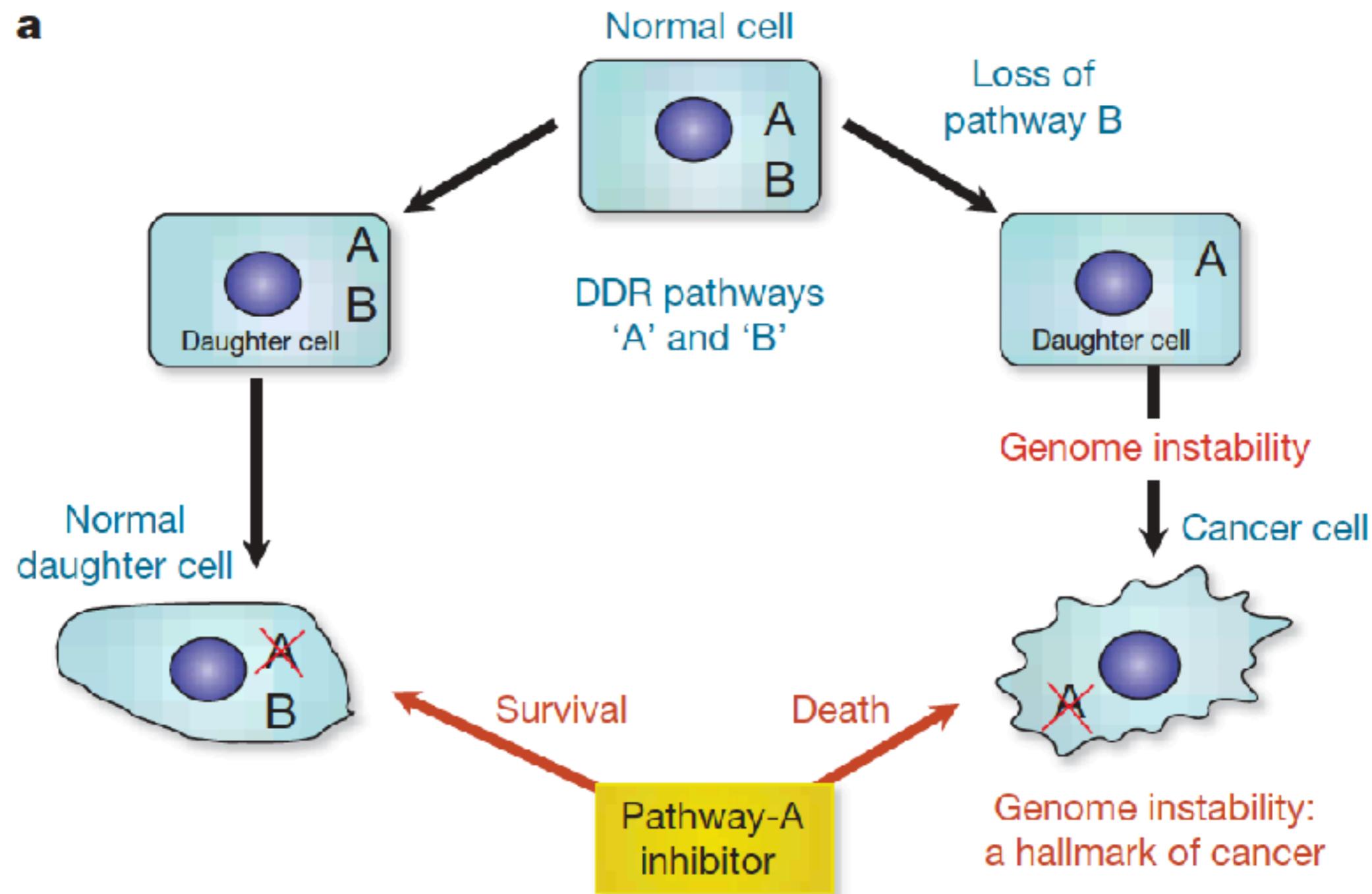
# Princípio da Radio ou Quimioterapia

**Table 3 | Examples of DNA-damaging drugs used to treat cancer**

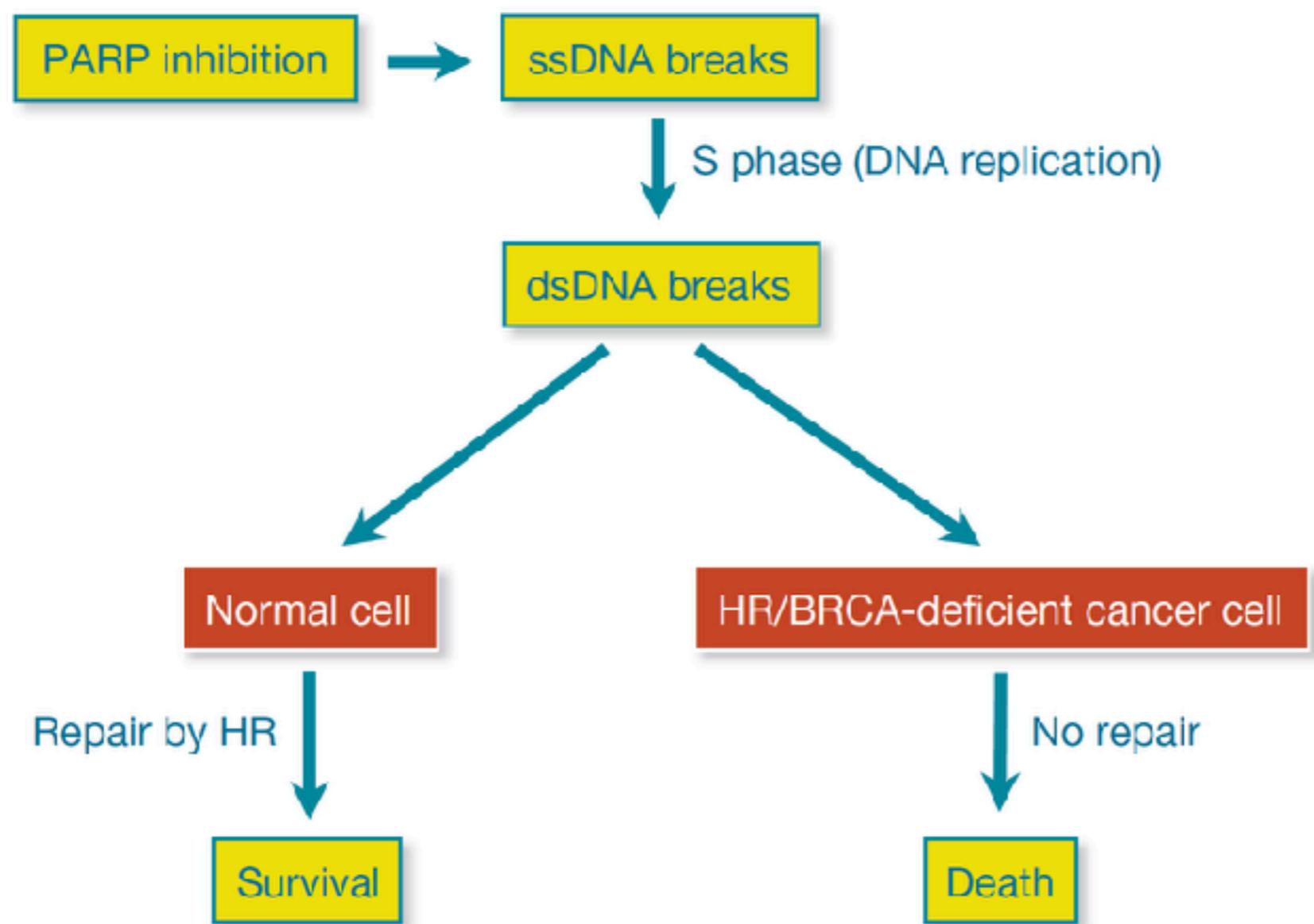
Cancer treatment	Types of DNA lesions induced
Radiotherapy and radiomimetics	
Ionizing radiation	SSBs, DSB, base damage
Bleomycin	
Monofunctional alkylators	
Alkylsulphonates	Base damage, replication lesions, bulky DNA adducts
Nitrosourea compounds	
Temozolamide	
Bifunctional alkylators	
Nitrogen mustard	DSBs, DNA crosslinks, replication lesions, bulky DNA adducts
Mitomycin C	
Cisplatin	
Antimetabolites	
5-Fluorouracil	Cytotoxic metabolite, inhibits base pairing leading to base damage and replication lesions
Thiopurines	
Folate analogues	
Topoisomerase inhibitors	
Camptothecins (Topo I)	DSBs, SSBs, replication lesions; anthracyclines also generate free radicals
Etoposide (Topo II)	
Anthracyclines (doxorubicin, epirubicin, daunorubicin) (Topo II)	
Replication inhibitors	
Aphidicolin	DSBs, replication lesions
Hydroxyurea	

See text for details (modified from ref. 87).

Jackson & Bartek, *Nature*, 2009

**a**

**b**



Jackson & Bartek, *Nature*, 2009



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