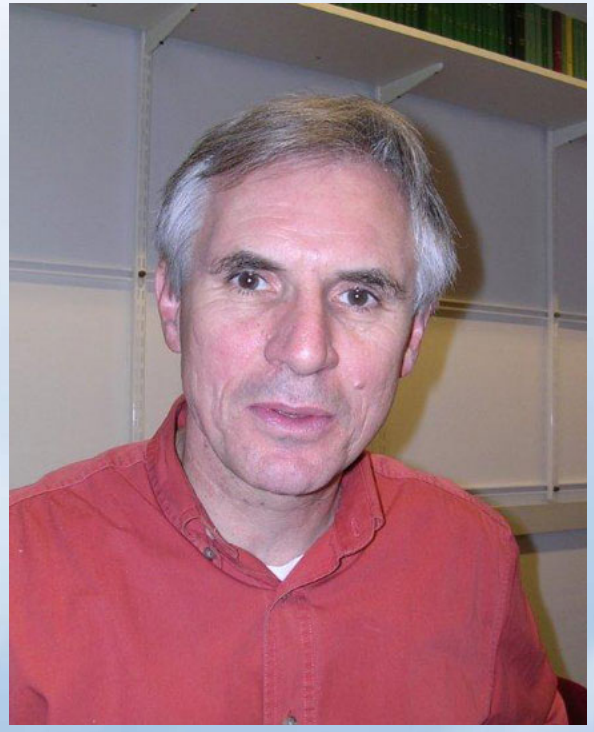
Leon Mullenders, Ph.D.
Professor of Toxicogenetics,
Department of Human Genetics,
Leiden University Medical Centre

Cellular Defenses against Radiation Injury

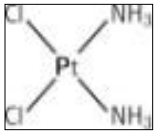


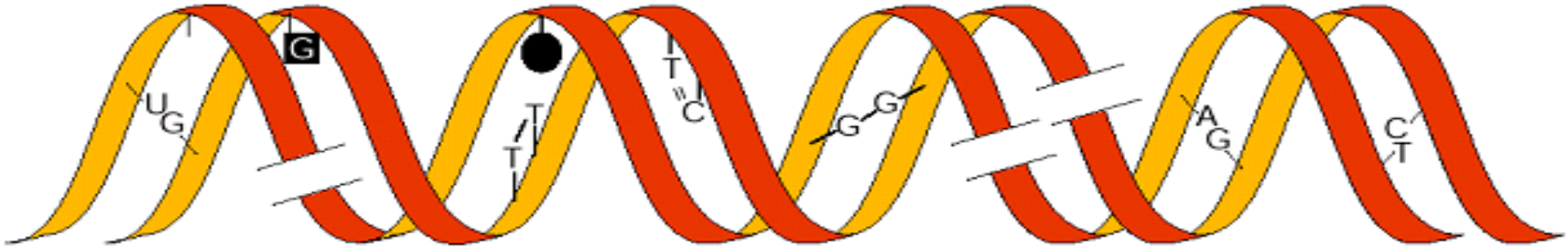
Radiation Epidemiology & Dosimetry Course

DNA DAMAGE AND HUMAN DIEASE

EXOGENOUS FACTORS

ENDOGENOUS FACTORS



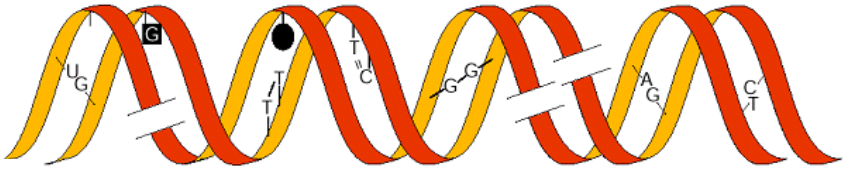


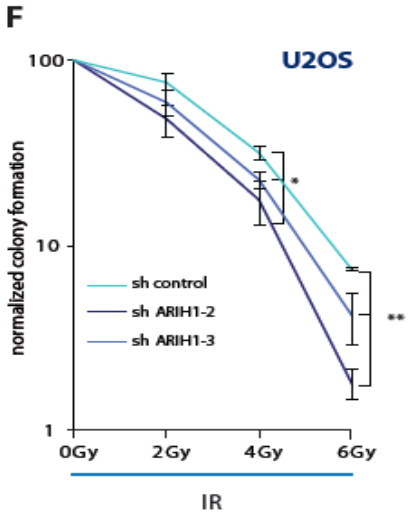
Base damage | **Bulky adducts** | **Crosslinks** | **DNA breaks** | **Mispaired bases**

Toxicity | **Genomic Instability** | **Homeostasis**

Cancer | **Neurodegeneration** | **Developmental problems** | **Ageing**

DNA DAMAGE RESPONSE 1





The DNA Damage Response

A network of pathways that protects against DNA damage

transcription responses

cell cycle arrest

DNA repair

apoptosis

chromatin remodeling

Damage bypass

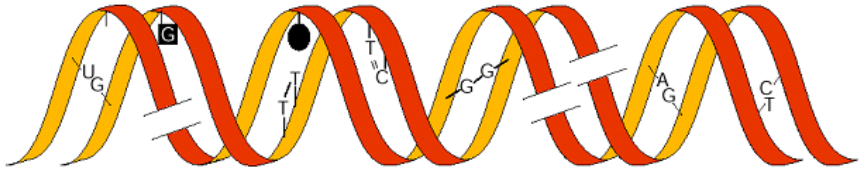
RNA splicing*

translation responses **

mitochondrial responses

* Sollier et al, Mol Cell 2014 ** von Stechow et al, MCB 2015

DNA DAMAGE RESPONSE 2



SENSORS

RAD50
MRE11
NBS1…

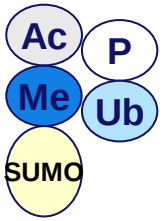
TRANSDUCERS

ATM, ATR
53BP1, MDC1
BRCA1….

EFFECTORS

Chk1,Chk2
p53……

DNA Damage signalling



Kinases
Phosphatases
Ubiquitin ligases
Dubs etc.

transcription responses

cell cycle arrest

DNA repair

apoptosis

chromatin remodeling

Damage bypass

RNA splicing*

translation responses **

mitochondrial responses

* Sollier et al, Mol Cell 2014 ** von Stechow et al, MCB 2015

SUMMARY 1 (DDR)

- Maintenance of genomic integrity relies on functional DDR
- DDR encompasses initial DNA damage sensing up to translation control
- DDR is tightly regulated by posttranslational modifications
- The number of DDR genes is rapidly increasing and thereby the number of potential risk factors that contribute to DNA damage mediated disease.
- Factors related to human disease(cancer)

  Cell cycle checkpoints/ apoptosis

  DNA repair factors and chromatin remodelers

  Factors involved in DNA damage signaling

DOUBLE STRANDED DNA BREAKS

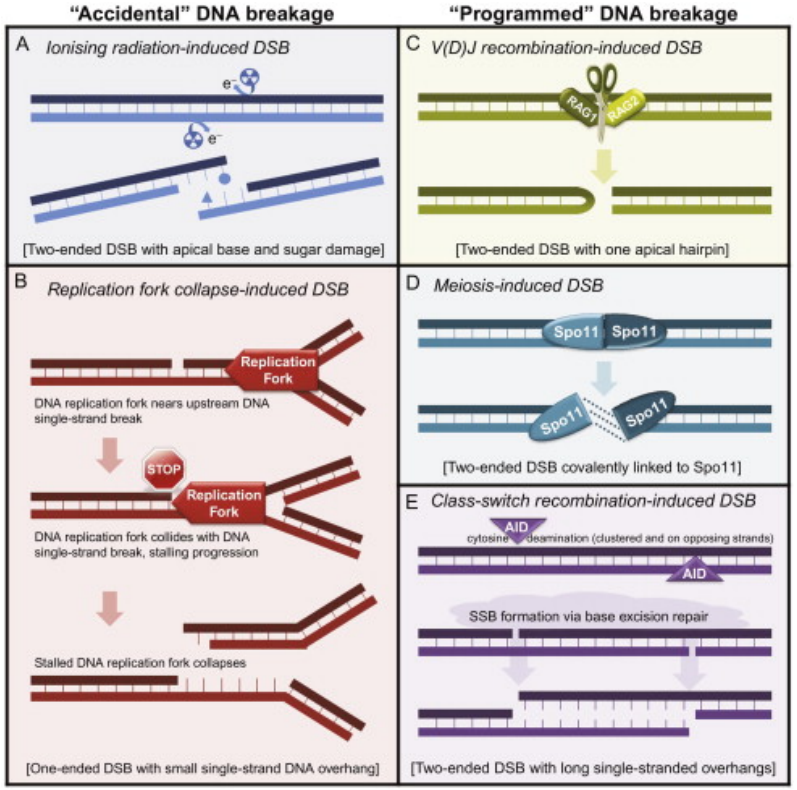
Programmed DSBs

- **V(D) J recombination**
- **Class-switch recombination**
- **Meiosis**

Accidental DSBs

- **DNA replication and transcription**

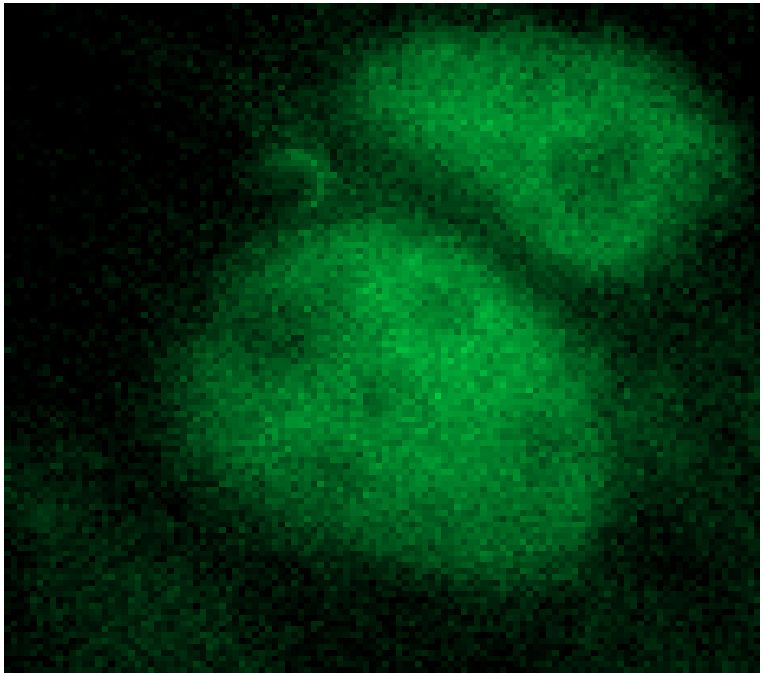
- **Ionizing Radiations**
  - DNA base damage
  - DNA strand breaks
  - Clustered DNA damage



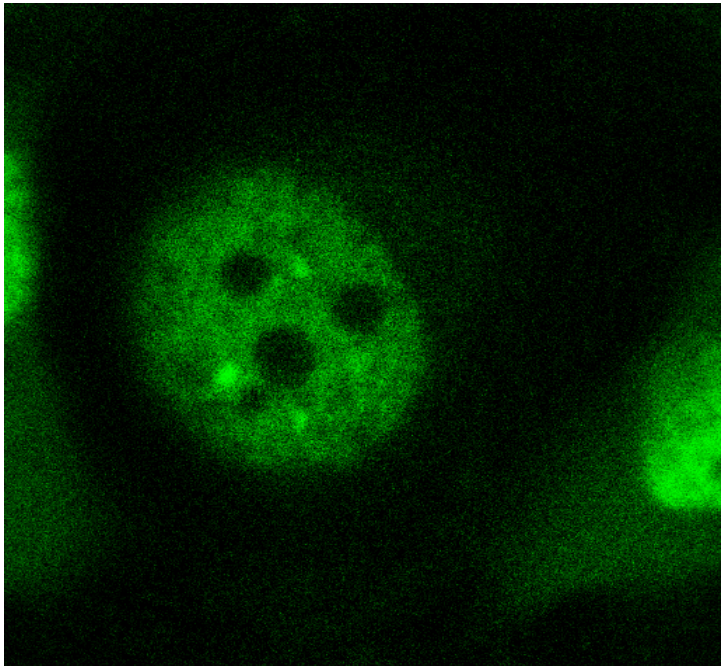
Goodarzi et al, Advances in Genetics, 2013

DYNAMICS OF DNA REPAIR

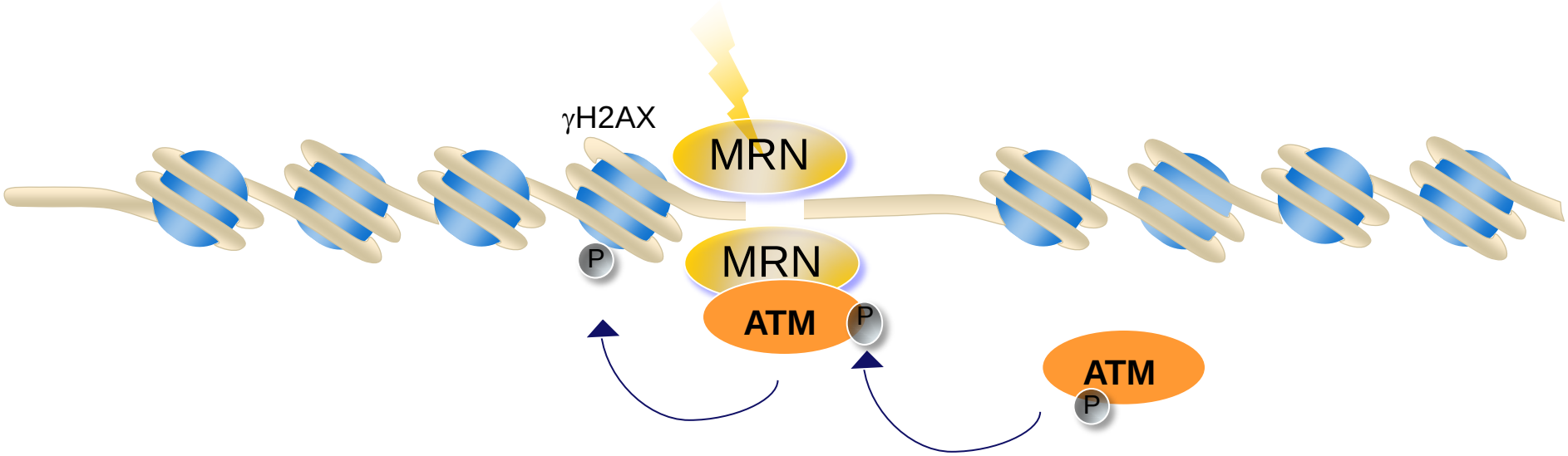
Recruitment of XPG to local UV damage



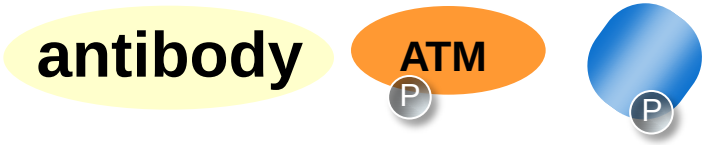
Recruitment of 53BP1 to laser induced damage



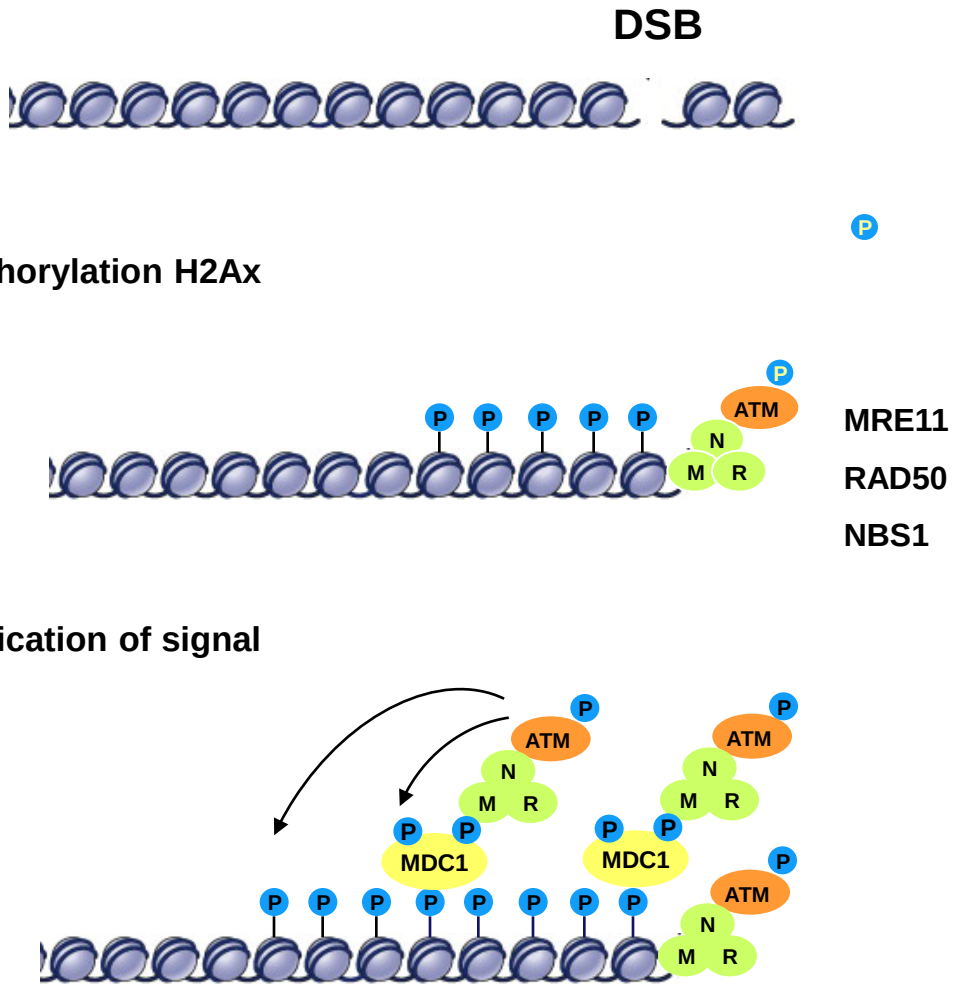
Double-strand break (DSB)

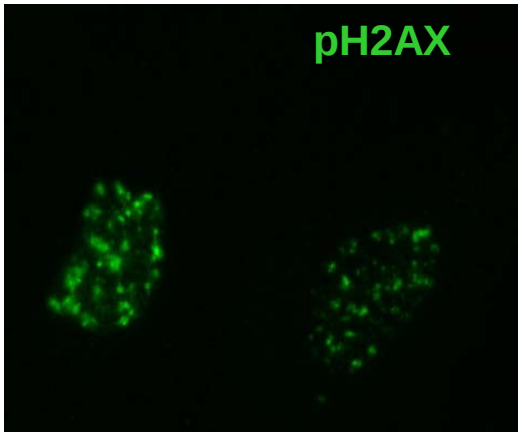


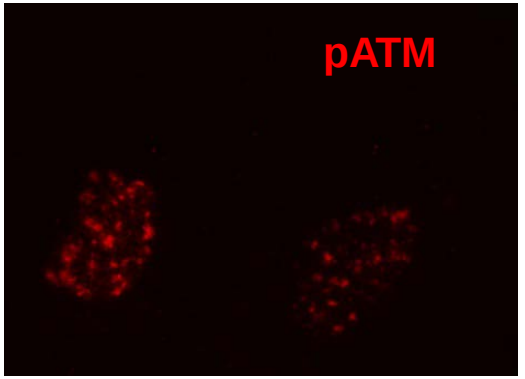
Cell cycle regulation and DSB repair



CHROMATIN AND THE DSB RESPONSE 2





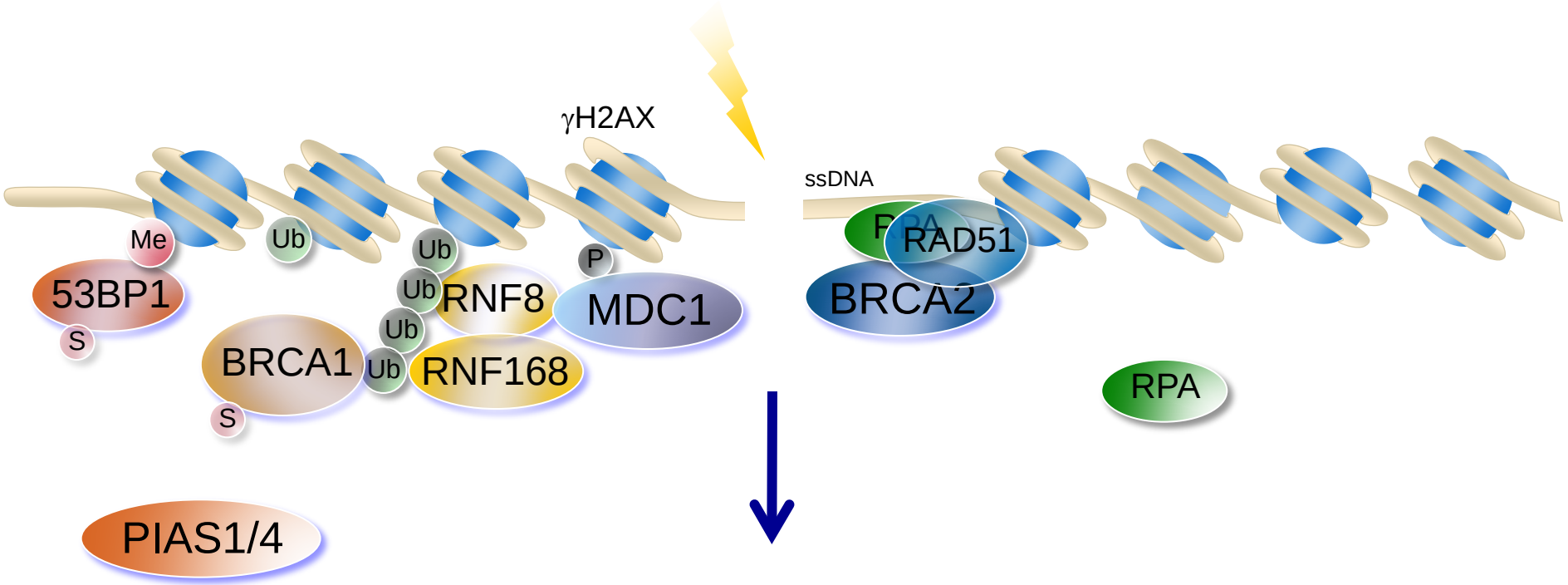


Focus

Local concentration of protein

CHROMATIN AND THE DSB RESPONSE 3

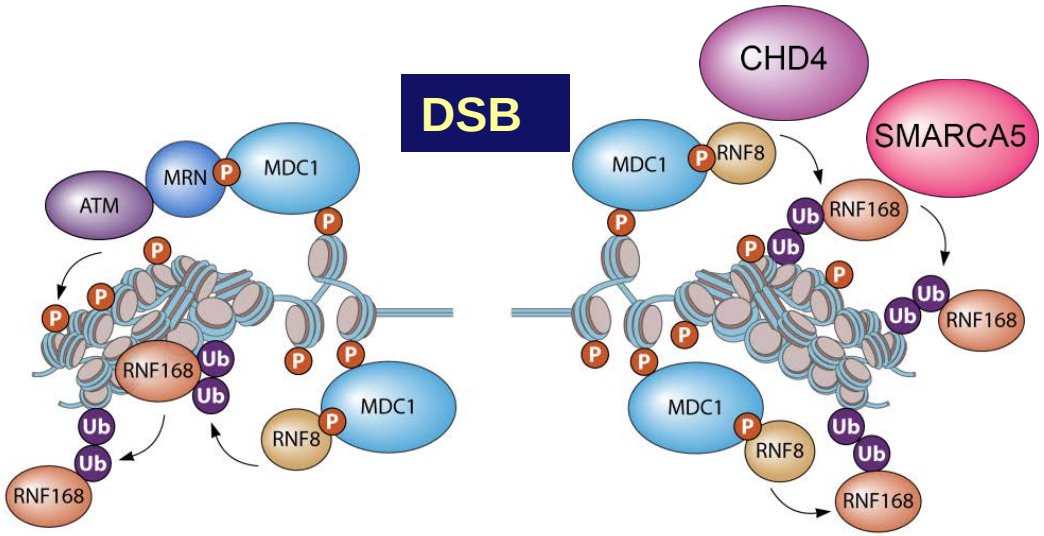
Double-strand break (DSB)



Cell cycle regulation and DSB repair

Van Attikum and Gasser, Trends Cell Biol. (2009)

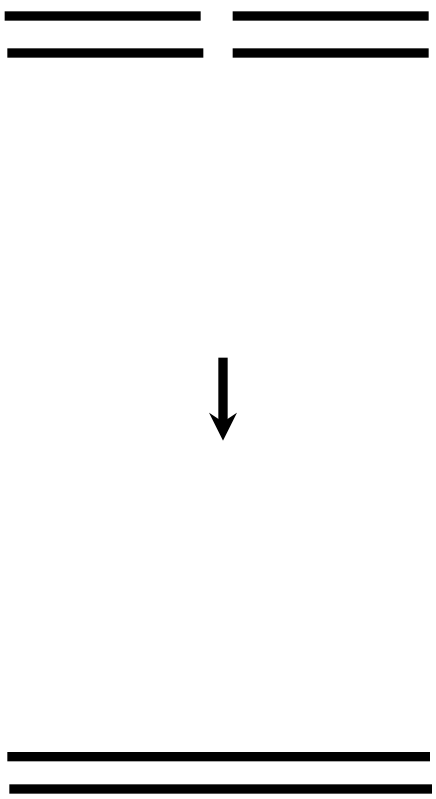
CHROMATIN AND THE DSB RESPONSE 4



Smeenk *et al.*, J. Cell Biol. (2010)
Smeenk *et al.*, J. Cell Sci. (2012)
Luijsterburg *et al.*, EMBO J. (2012)
Klement *et al.*, J. Cell Biol. (2014)

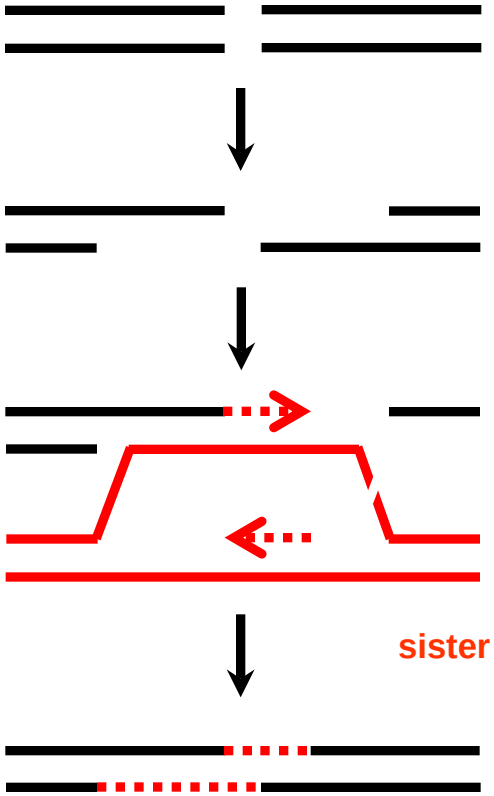
DSB-REPAIR PATHWAYS

non-homologous endjoining



- **G1, S, G2**

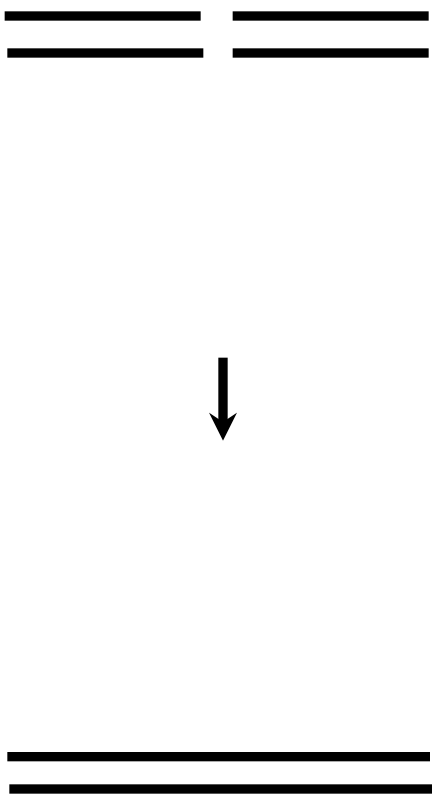
homologous recombination



- **Late S, G2**

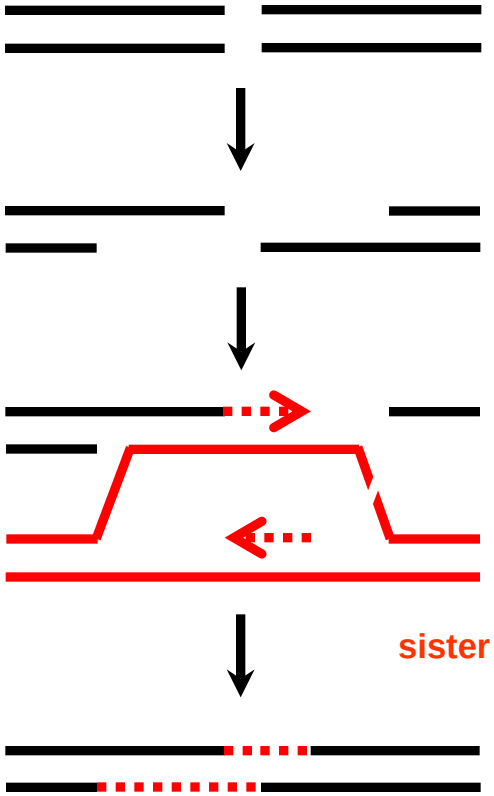
DSB-REPAIR PATHWAYS

non-homologous endjoining



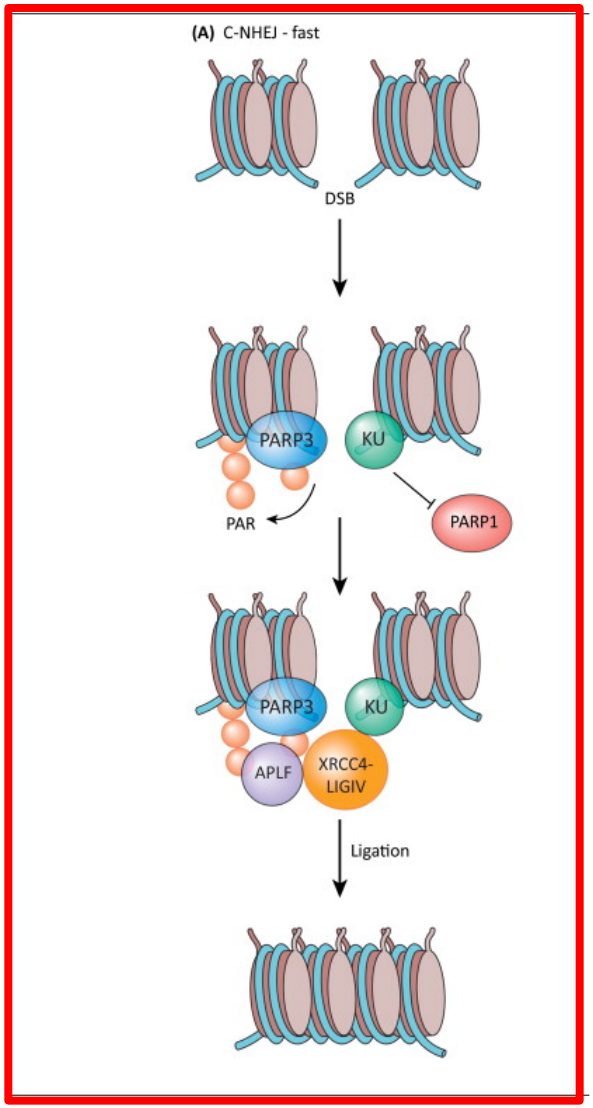
- **G1, S, G2**

homologous recombination



- **Late S, G2**

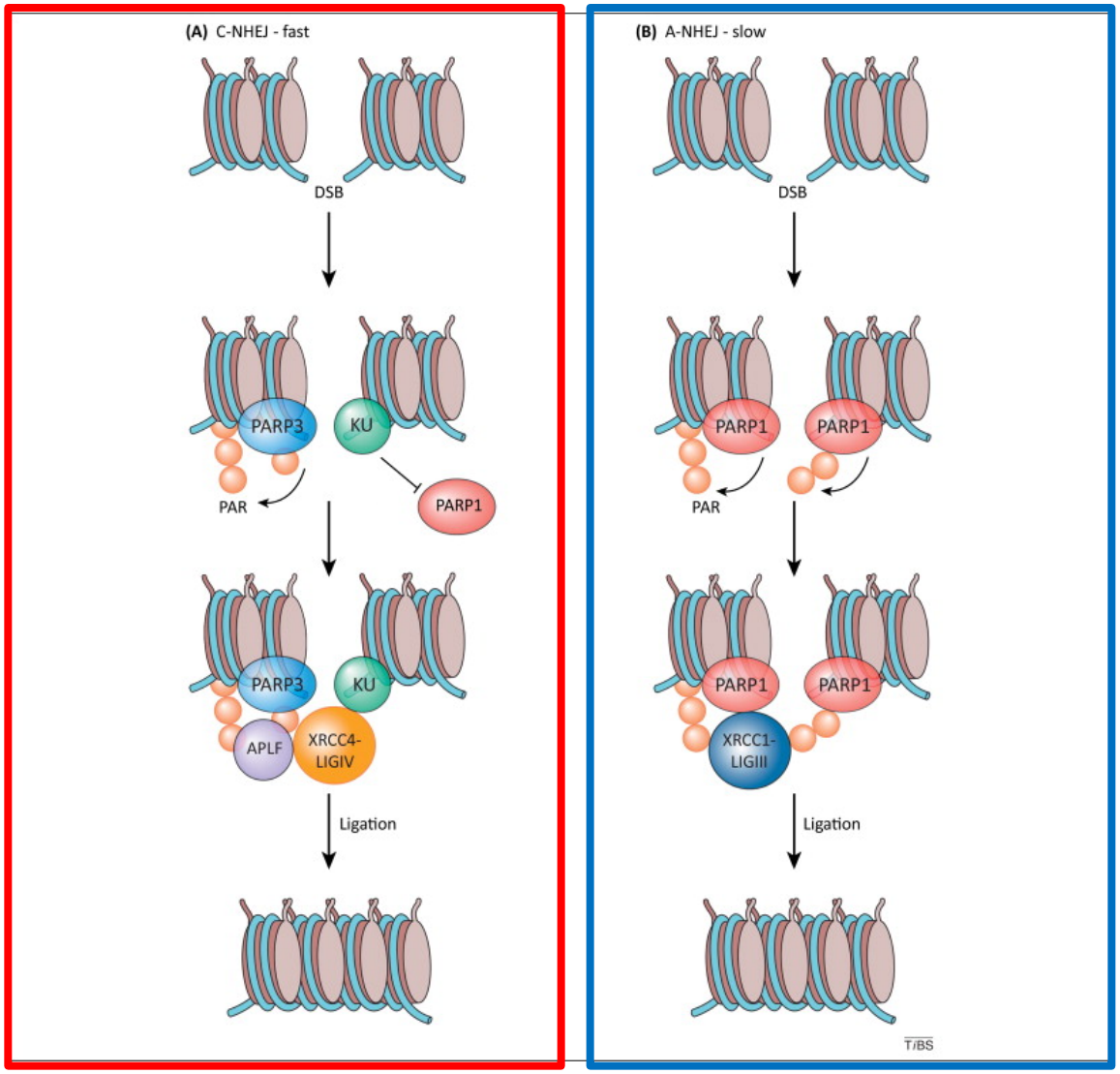
NON-HOMOLOGOUS ENDJOINING



Canonical NEHJ
fast

Pines et al Trends in Biochemical Sciences, 2013

NON-HOMOLOGOUS ENDJOINING



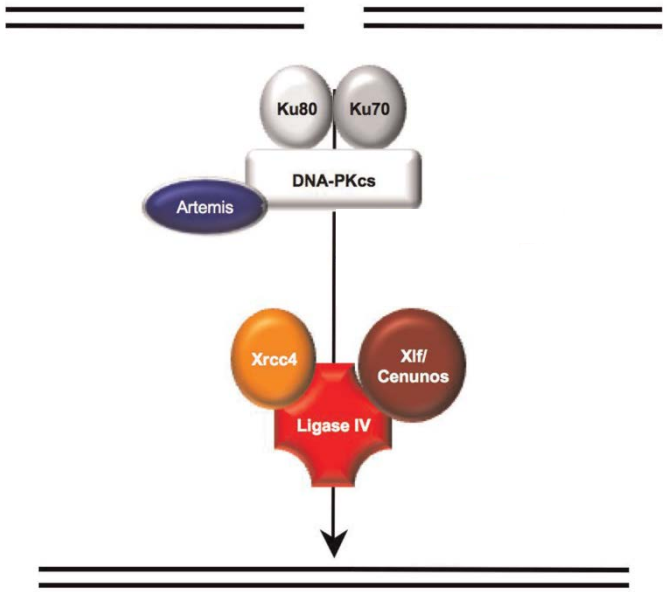
Canonical NEHJ
fast

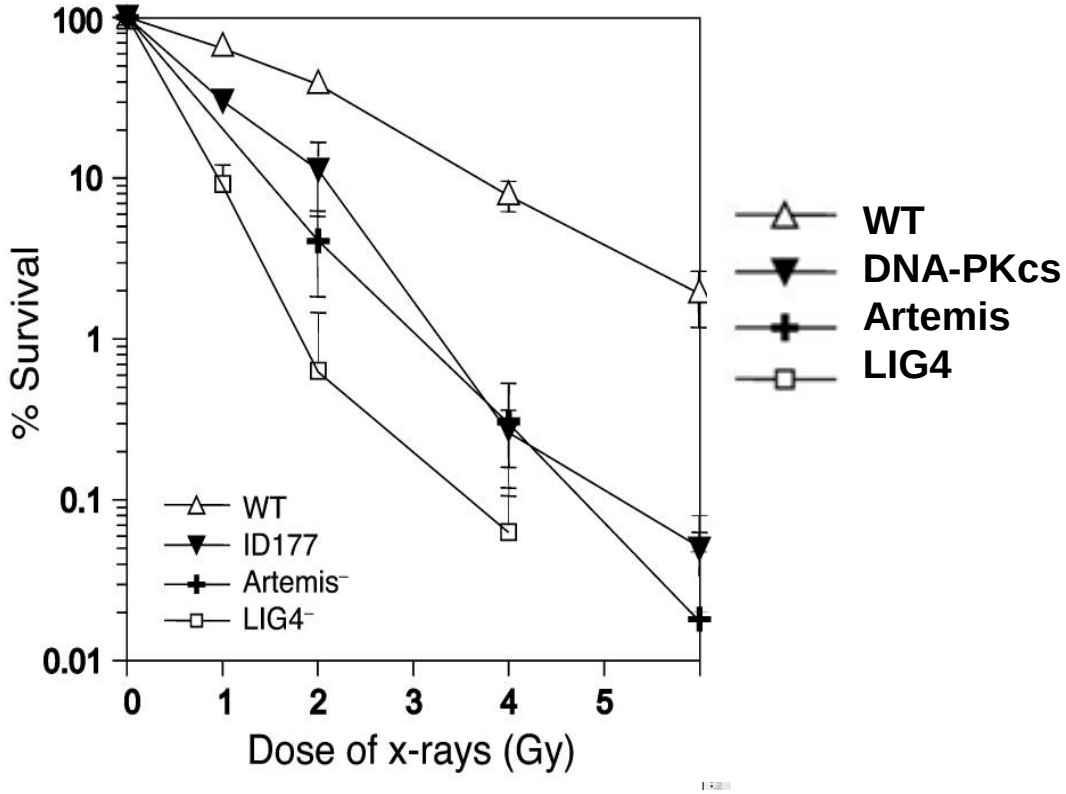
Alternative NEHJ
slow

Pines et al Trends in Biochemical Sciences, 2013

SCID patients: XLF, Ligase IV, XRCC4, Artemis and DNA-PKcs

Ataxia telangiectasia patients





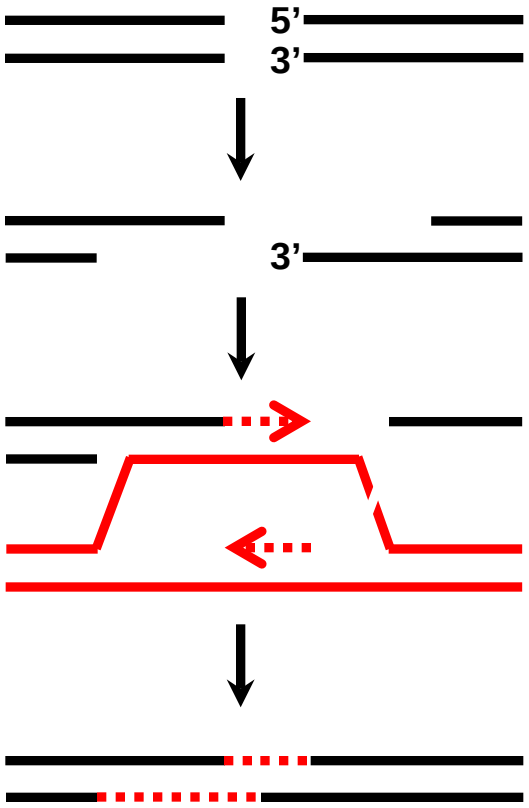
van der Burg et al., 2009

MODEL FOR RECOMBINATION (SIMPLE)

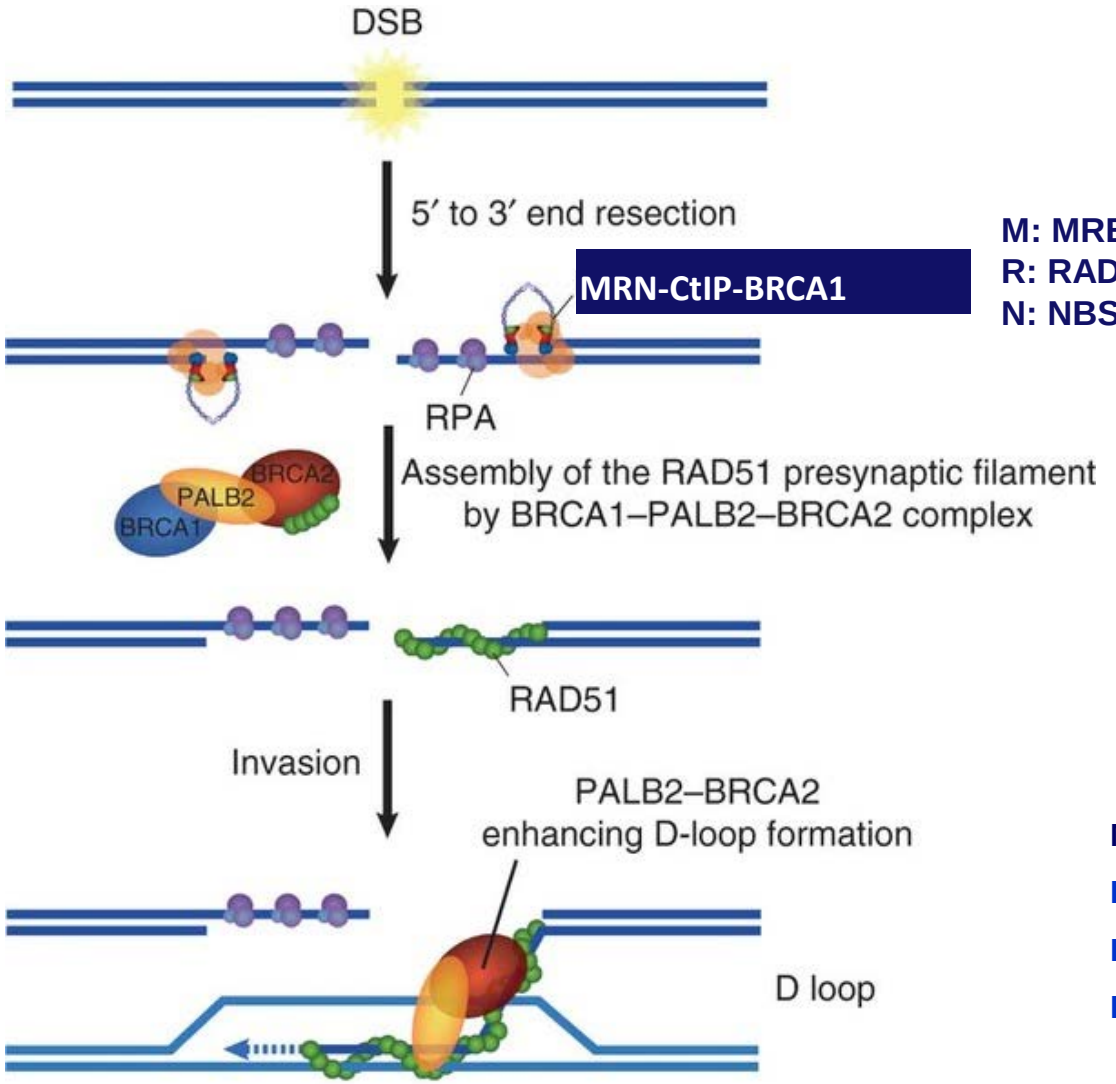
Presynaptic: **resection**

Synaptic: **strand invasion and DNA synthesis**

Postsynaptic: **resolution**



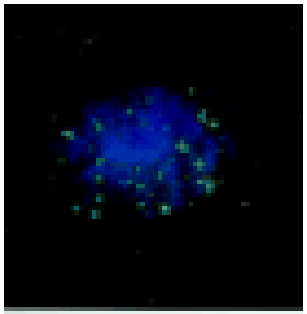
BRCA2: ESSENTIAL FOR RAD51 FILAMENT FORMATION



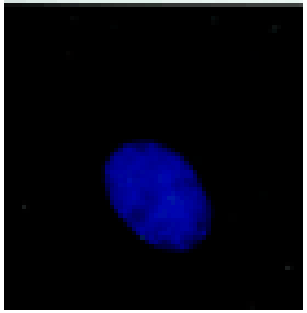
M: MRE11
R: RAD50
N: NBS1

RAD51 foci

Wild-type



BRCA2 mutant



Mediators (RAD52 group):

RAD51AP, RAD52

RAD51B, C, D, XRCC2, XRCC3

RAD54, RAD54B,

Southelp et al, 2003

SYNDROMES RELATED TO HR DEFECTS

SYNDROMES	GENE	FUNCTION in HR
Nijmegen breakage syndrome (NBS)	NBS1, RAD50	resection
Ataxia telangiectasia like disease (ATLD)	MRE11	resection
Fanconi anemia (group D1)	BRCA2 RAD52 group*	RAD51 loading
Hereditary breast cancer	BRCA1, 2, RAD52 group**	various steps in recombination
Bloom syndrome (RecQ-like)	BLM	resection, resolution
Seckel syndrome	CtIP	resection

* RAD51C, PALB2, BRIP1

** RAD51C, D, XRCC2, PALB2, RAD50, NBS1, BRIP1

SUMMARY (DSB repair)

- In human cells repair of DSB is mediated by error prone NHEJ and error free HR depending on the stage of cell cycle.

- Repair of DSB requires

  core factors

  kinases, ubiquitin ligases, Dubs, SUMO ligases to induce

  posttranslational modfications of chromatin(histones) and proteins

  chromatin remodelers

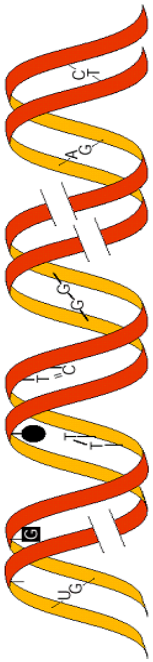
- Mutations in Core factors, Kinases, Ubiquitin ligases and chromatin remodelers are linked to radiosensitive human disorders and disease

  Kinase ATM (Ataxia telangiectasia)

  Ubiquitin ligase RNF8 (Riddle syndrome)

  Chromatin remodeler CHD4 (cancer)

CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE



DNA Damage Signaling

Damage bypass

Chromatin Remodeling

apoptosis

DNA repair

Cell cycle Arrest

Transcription responses

No/misrepair replication

Genetic alterations

?

Epigenetic alterations

Genomic Instability

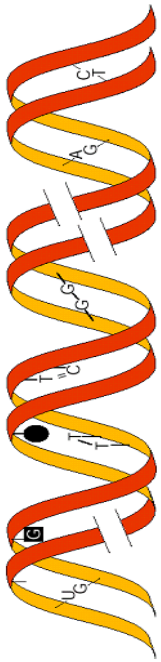
Disease cancer

Acute (early) response
DNA damage response (DDR)

Transmission of DNA damage

Disease

CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE



cell to cell signaling

Delayed genomic instability

Bystander effects

Adaptive response

Reactive oxygen driven stress response ?

Disease cancer

NON TARGETED EFFECTS

Disease

Low dose ionizing radiation

below 200 mGy (UNSCEAR) or 100 mGy (others, EU)

dose rate 0.1mGy min $^{-1}$(UNSCEAR)

Starting point

Radiation acts primarily by inducing DNA damage in somatic and germ cells

- DNA base damage
- DNA strand breaks
- Clustered DNA damage

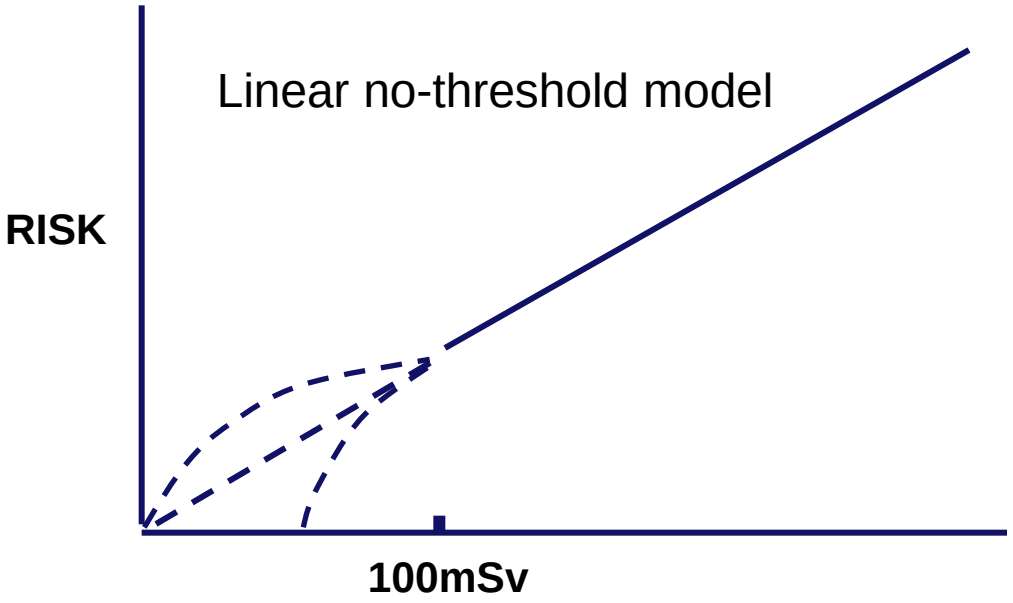
spontaneous DNA damage in cultured cells: 1 x 10^{-7} lesions/base

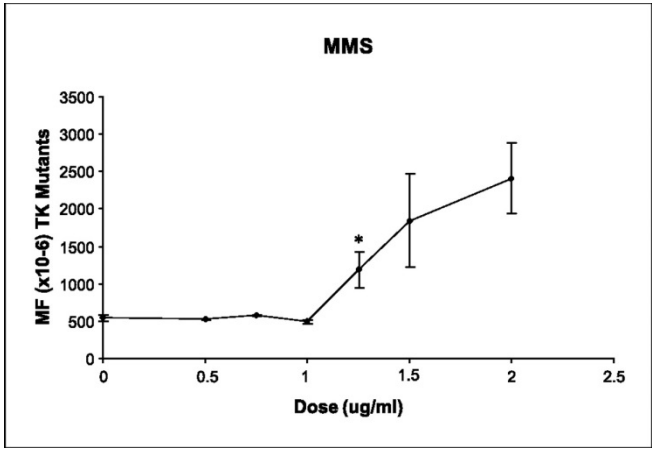
100 mGy: - 10-20% of spontaneous oxidative damage

- increased DSB: 4 DSB

Cramers et al, Rad Res 2011

LOW DOSE IR RISK MODELS





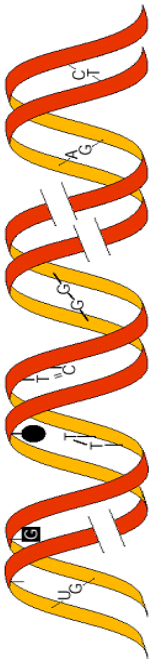
Shareen H. Doak et al. Cancer Res 2007

- Dose response relationships
- Mechanisms

- Do the processes that drive carcinogenesis due to high IR dose also contribute to low dose radiation carcinogenesis ?

- Are biological responses at low and protracted doses similar to those observed at high acute doses?

CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE



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Cell cycle Arrest

Transcription responses

Acute (early) response
DNA damage response (DDR)

No/misrepair replication

Genetic alterations

Genomic Instability

Disease cancer

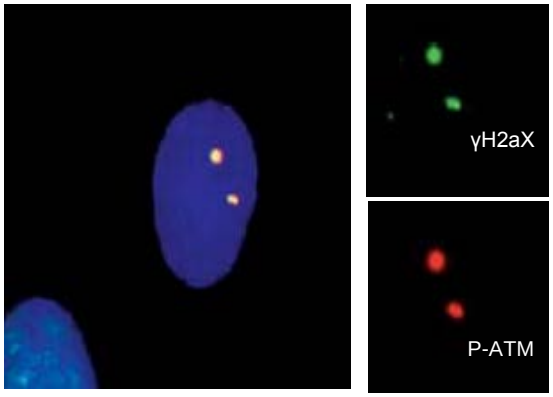
?

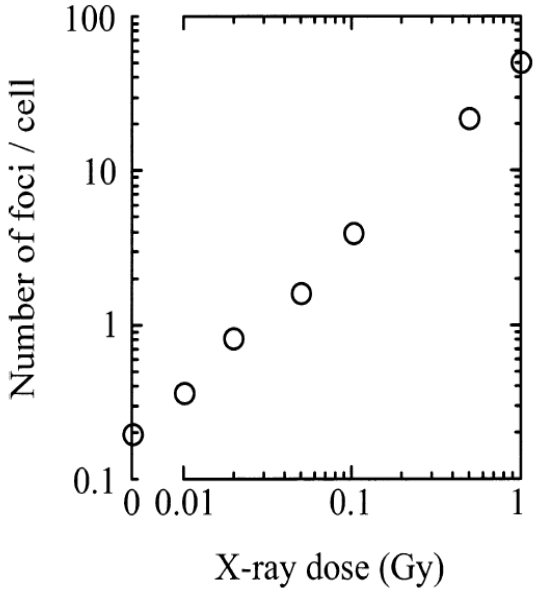
Epigenetic alterations

Transmission of DNA damage

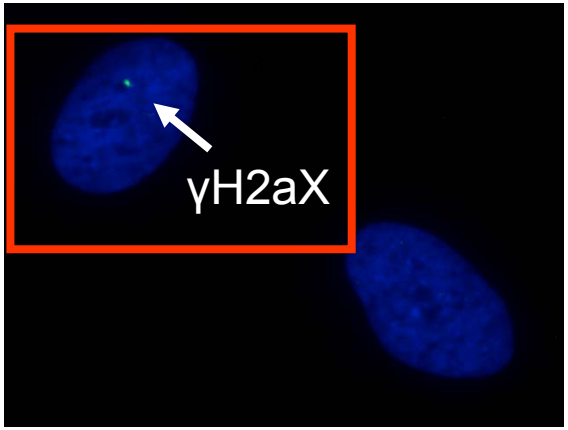
Disease

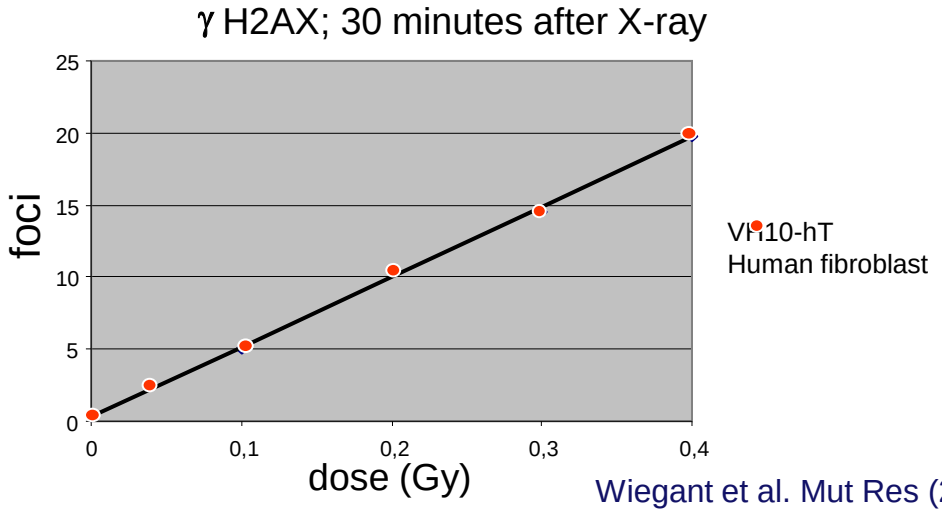
DNA DAMAGE SIGNALLING
PHOSPHORYLATION OF ATM and H2AX (FOCI)





Suzuki et al. Radiation Res (2006)





Wiegant et al. Mut Res (2006)

PHOSPHO PROTEOMICS

SILAC (unbiased)

Light

Heavy

Radiation
Chemicals

control

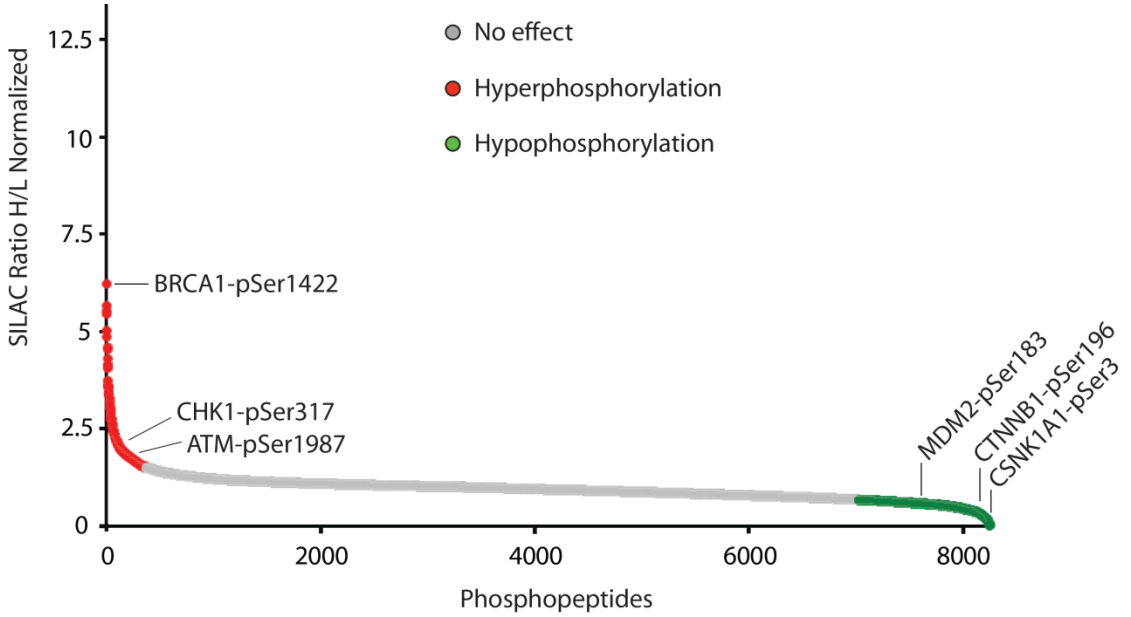
4hr

Phosphopeptide enrichment

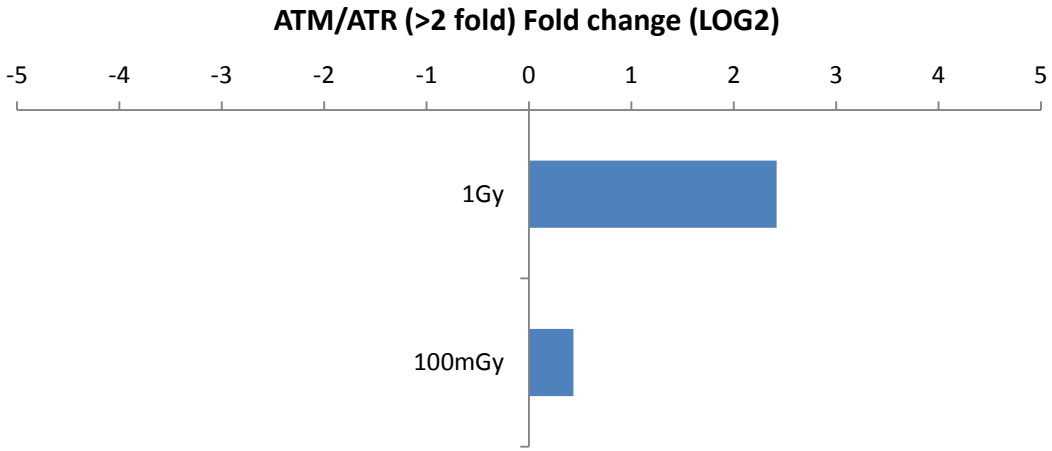
Nano LC-MS/MS

Phosphopeptide identification and quantification

F



PHOSPHOPROTEOME PROFILE
4HRS AFTER 1Gy AND 100MGy X-RAYS



Gene name	Positions within proteins	Modified sequence	100 mGy	1 Gy
Atm	1891	_SA**T**PANSDSESENFLR_	1,1662	1,1604
Atm	1895	_SATPAN**S**DSESENFLR_	1,0004	1,0564
Atm	1987	_SPTFEEG**S**QGTTISSLSEK_	1,3599	2,7644
Atm	3006	_QSL**S**DTDQSFNK_	1,9786	2,9375

S(p)1981

Kozlov et al, JBC, 2011

INDUCED NETWORKS

Kinase	cisPlatin[1]	Neocarzinostatin[2]	20mGy[3]	500mGy[3,4]
DNA repair	**Yes**	**Yes**	Yes (weak)	**Yes**
Cell cycle / mitose	**Yes**	**Yes**		
Chromatin	**Yes**	**Yes**		**Yes**
RNA synthesis		**Yes**		**Yes**
Splicesome		**Yes**	**Yes**	**Yes**
MAPK, PKA	**Yes**	**Yes**	**Yes**	**Yes**
Cytoskeleton/ microtubuli	**Yes**		**Yes**	

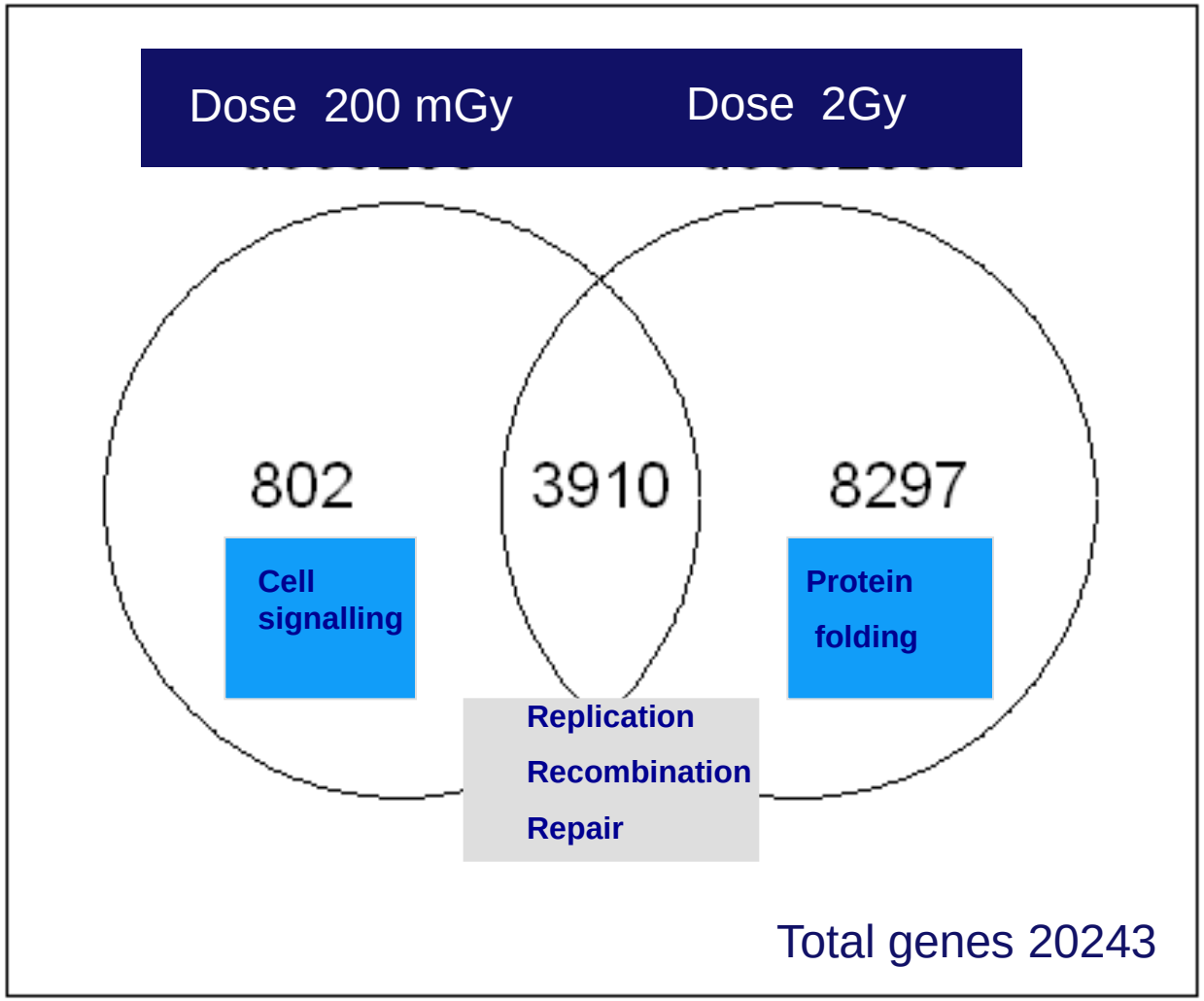
1. Pines et al, MCB, 2011

2. Bensimon et al, Science sign, 2010 Chromatin

3. Yang et al, PlosOne, 2010

4. Bennetzen et al, Mol Cell Prot, 2010 Chromatin

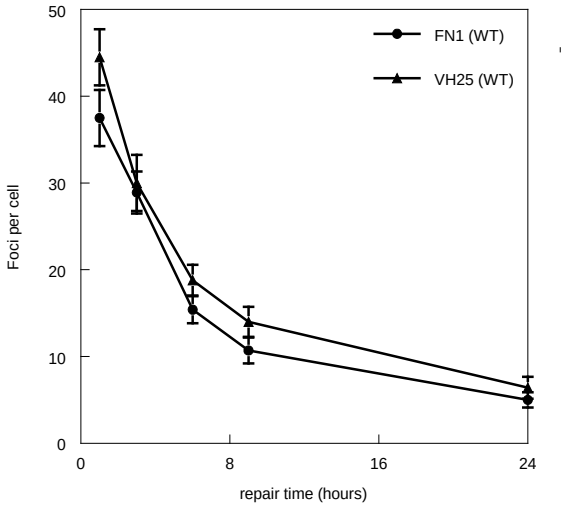
HIGH DOSE AND LOW DOSE TRANSCRIPTOMICS
LYMPHOCYTES OF 106 INDIVIDUALS

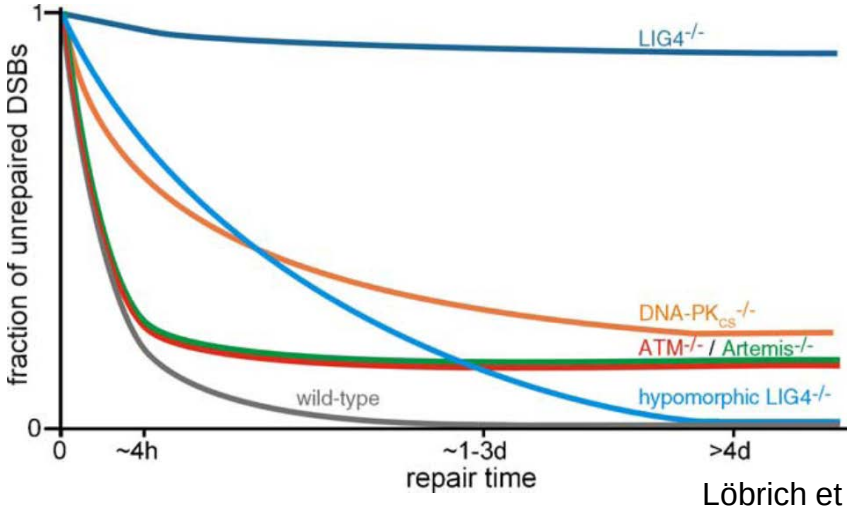


Meglicz, Mišovic, Vrieling and Giphart-Gassler, 2013

REPAIR OF DSB IN HUMAN CELLS

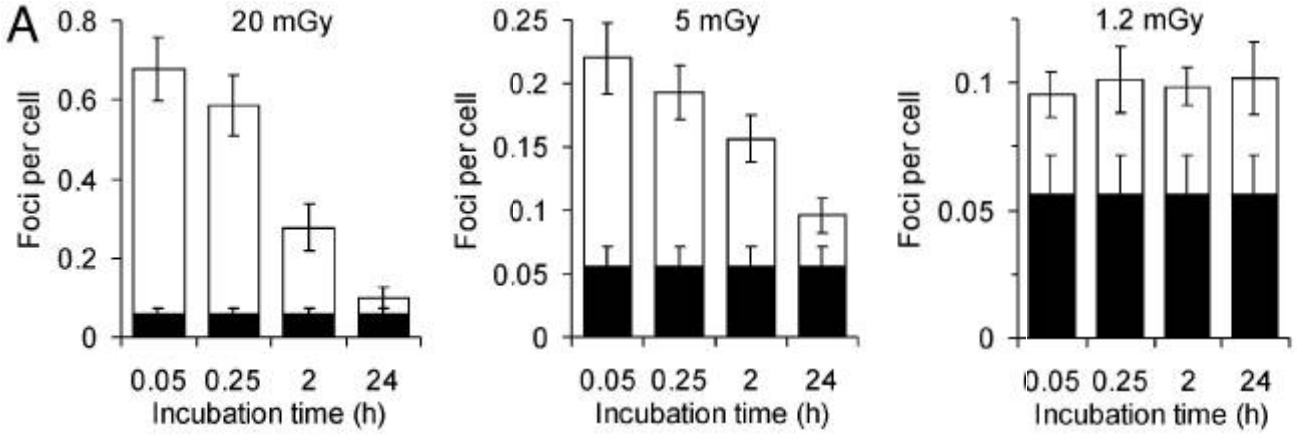
γH2AX foci after 2 Gy X-ray





Hetero chromatin

Löbrich et al , 2005



Rothkamm et al . Proc Natl Acad Sci. 2003

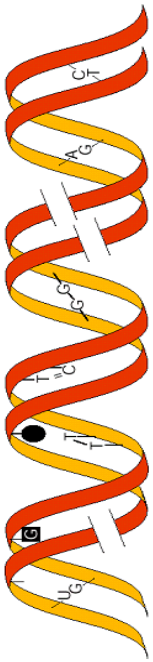
SUMMARY 3 (LOW DOSE RESPONSE)

- No global threshold for DNA damage signaling (20-50mGy) ATM, H2AX, p53, CHk1/2 (Short et al, Rad Research 2005).
- Lack of information on the impact of gene defects on genome instability at low dose
- Threshold in G2 checkpoint activation (100mGy) (Krempler et al, Cell Cycle, 2007
- Apoptosis occurs at very low dose (2mGy) (Portess et al, Cancer Research 2007)
- Repair rates are the same at high and low dose (Suzuki et al, Rad Research 2006).
- A single DSB can activate the repair complex formation (Rodrigue et al, 2006). However, DSB repair is abrogated at 1.2 mGy (Rothkamp et al, PNAS 2003)

SUMMARY 3 (LOW DOSE RESPONSE)

- At present no generally agreed patterns of gene expression changes associated with exposures to different doses or dose rates.
- Profound inter-individual variation in gene expression patterns.
- Cellular studies on radiation and proteomics are limited and inconclusive with respect to dose or dose rates.
- There are indications that low dose and low-dose rate responses differ from high dose and high dose rate responses for endpoints such as gene expression and proteomics.

CELLULAR AND TISSUE RESPONSES TO DNA DAMAGE



DNA Damage Signaling

Damage bypass

Chromatin Remodeling

apoptosis

DNA repair

Cell cycle Arrest

Transcription responses

No/misrepair replication

Genetic alterations

Genomic Instability

Disease cancer

?

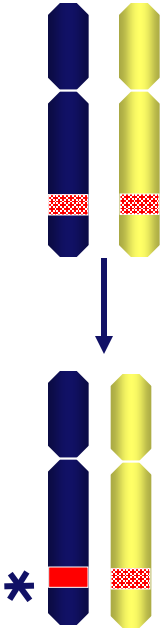
Epigenetic alterations

Acute (early) response
DNA damage response (DDR)

Transmission of DNA damage

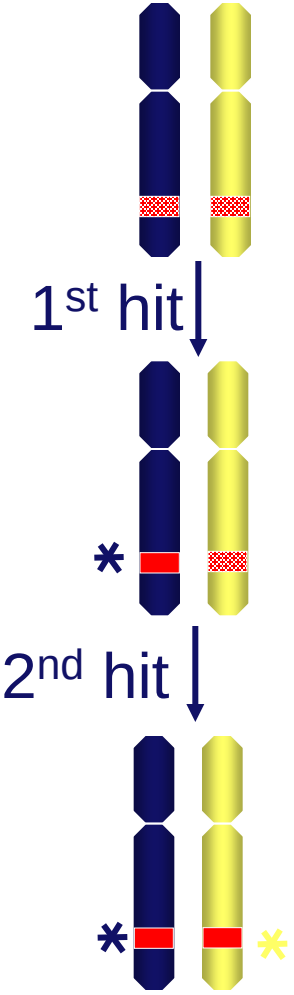
Disease

proto-oncogenes



dominant

tumorsuppressor genes



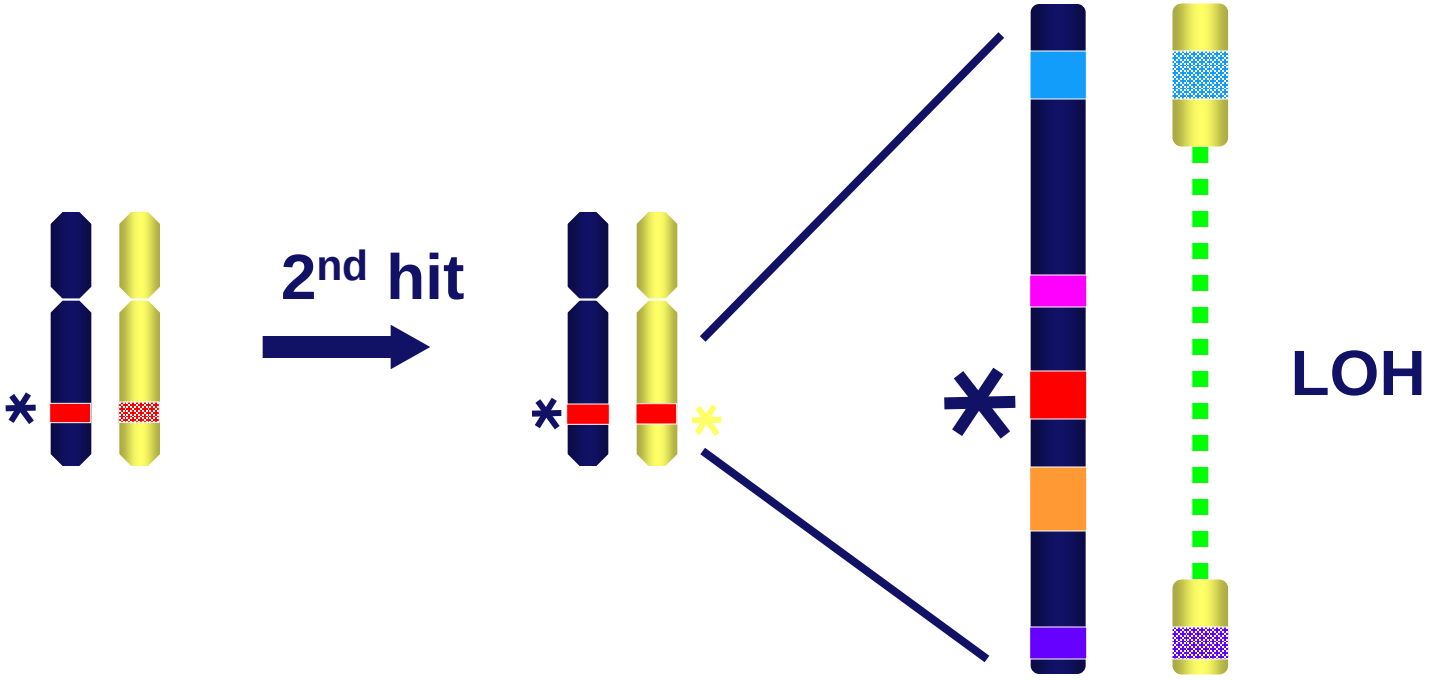
recessive

hereditary forms of cancer

Deregulated function

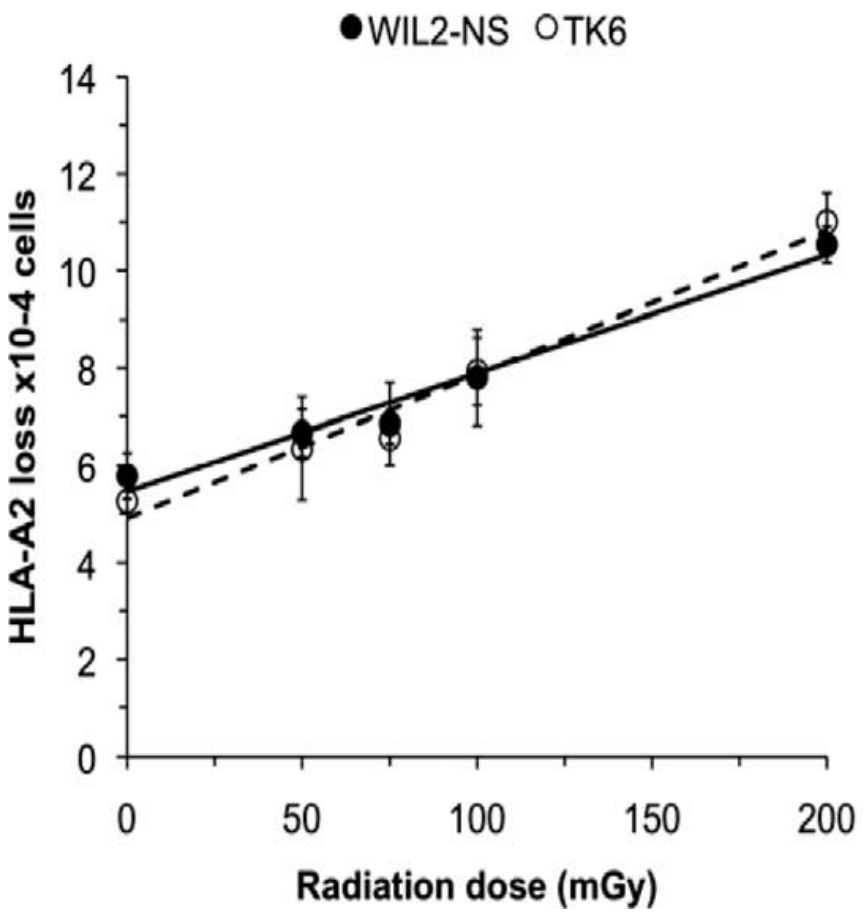
loss of function

LOSS OF HETEROZYGOSITY (LOH)

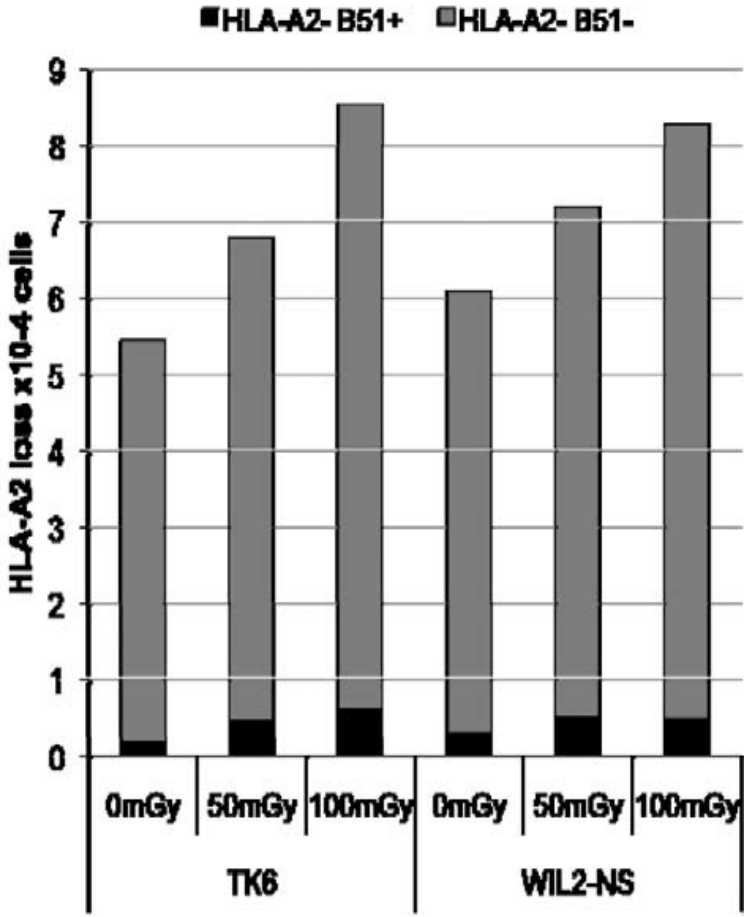


LOH IN HUMAN LYMPHOBLASTOID CELLS

A.

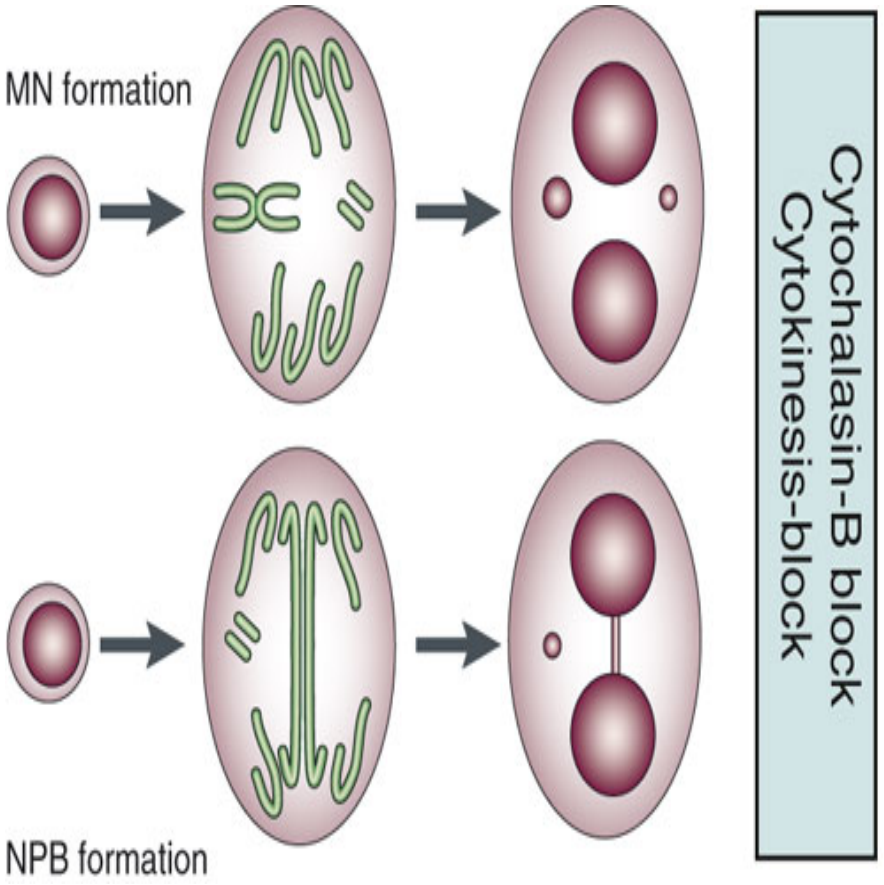


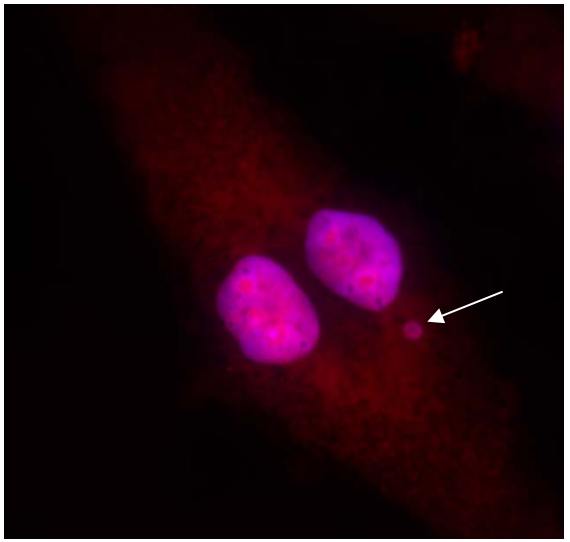
B.

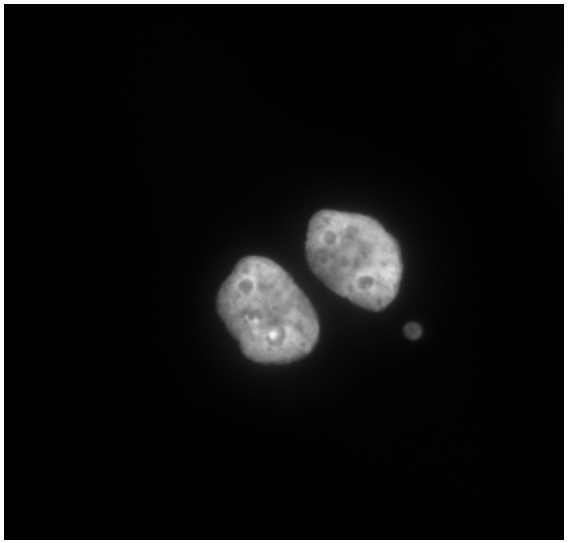


Boei et al, Rad Res 2011

MICRONUCLEI (MN) INDUCTION (THE CBMN-ASSAY)

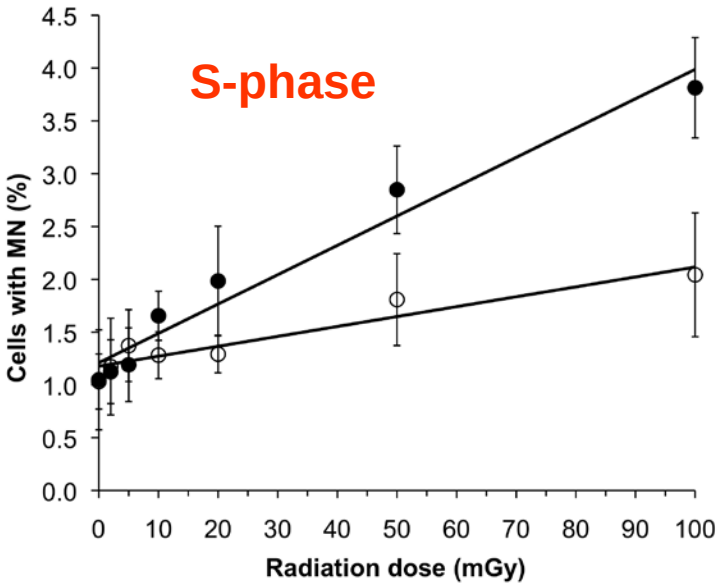


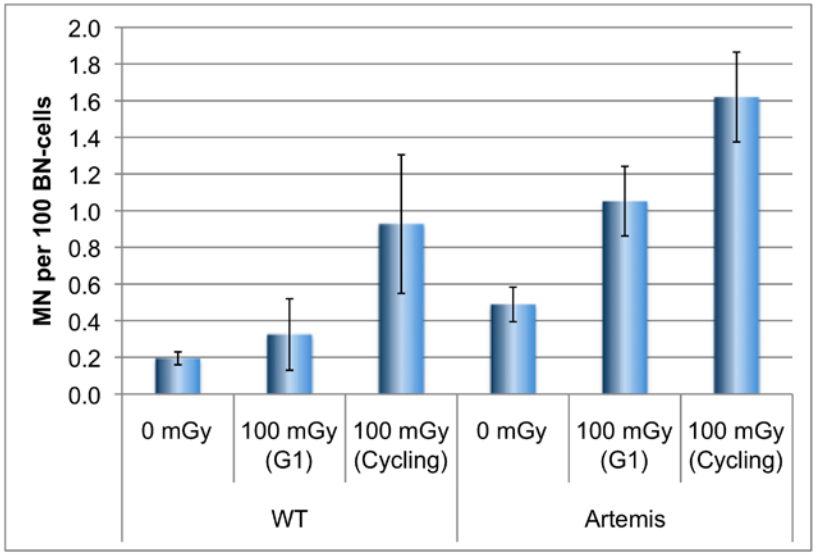


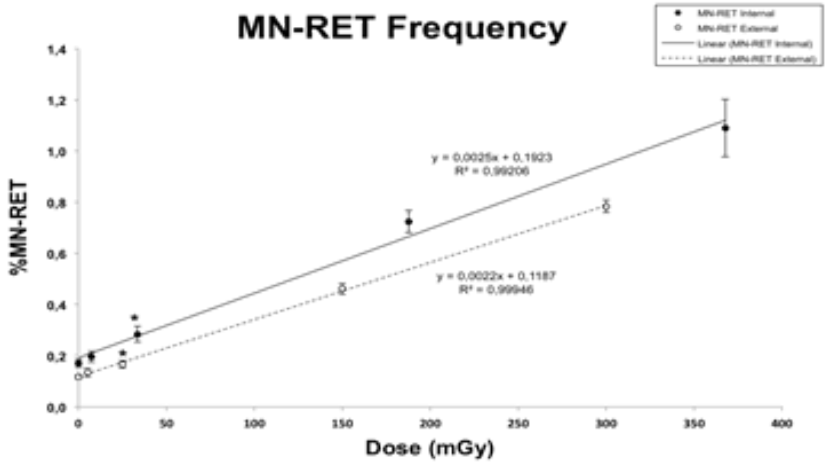


Fenech, Nature Methods, 2007

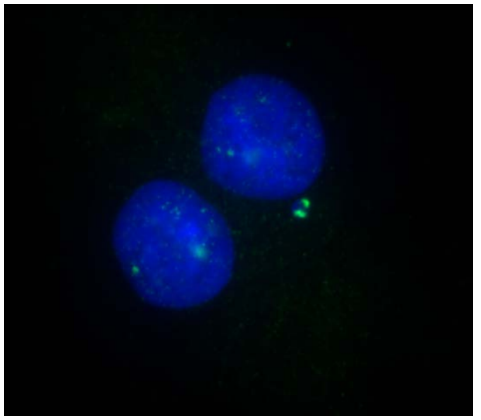
MICRONUCLEI (MN) INDUCTION USING HUMAN FIBROBLASTS





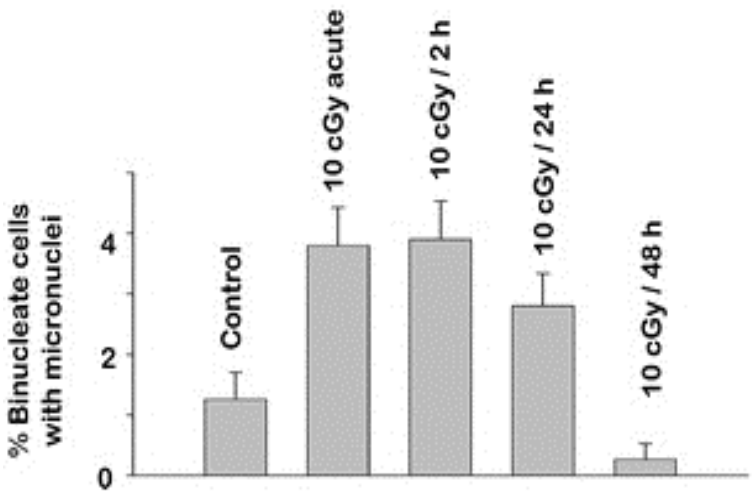


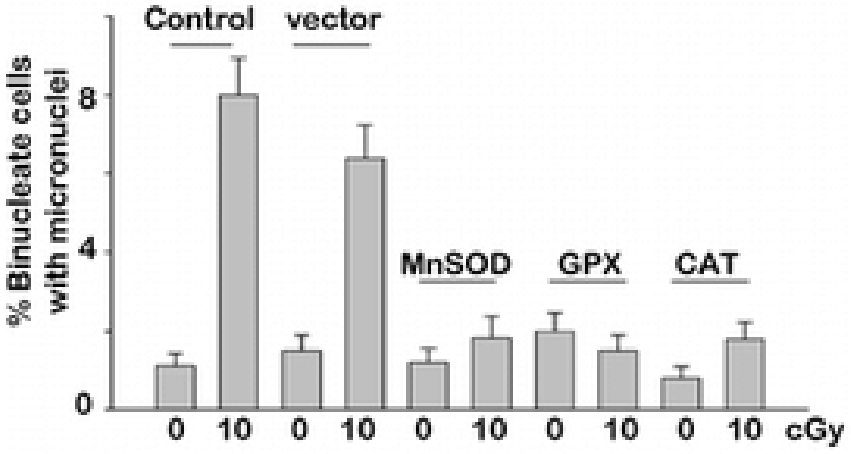
Manning et al, Mut Res 2014



Boei et al, Rad Res 2011.

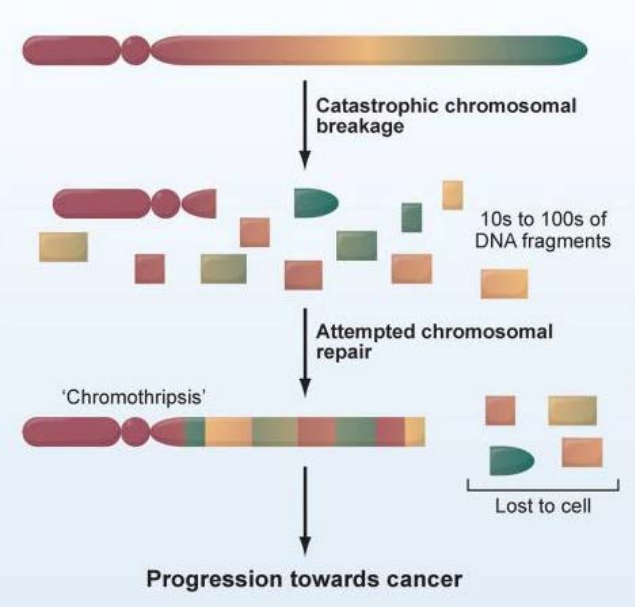
MICRONUCLEI (MN) INDUCTION AND DOSE RATE





De Toledo et al, Rad Res 2006

CHROMOTHRIPSIS AND CANCER



Stephens et al, Cell, 2011

Micronuclei

Crasta et al, Nature 2012

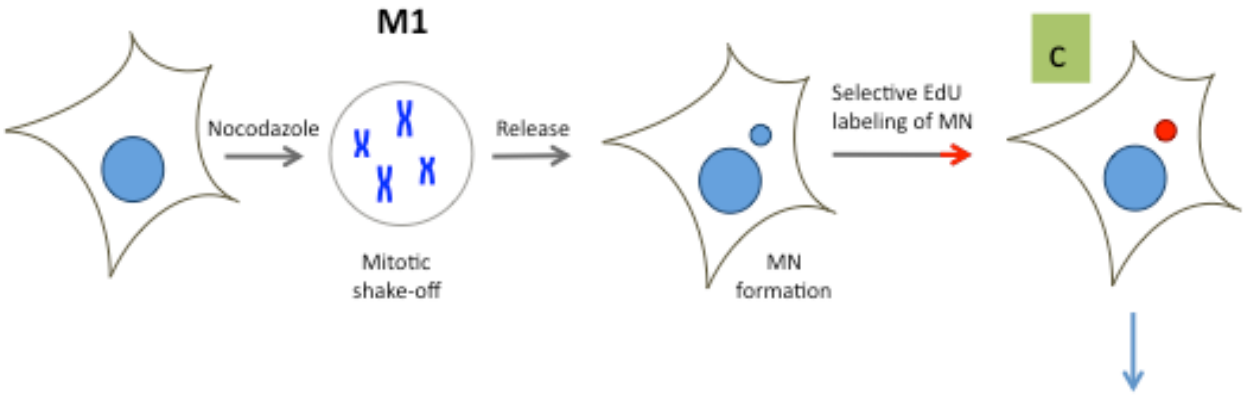
Frequency of chromothripsis In tumours (2012)	3% of diverse cancer cell types 25% of bone cancer 18% of neuroblastoma

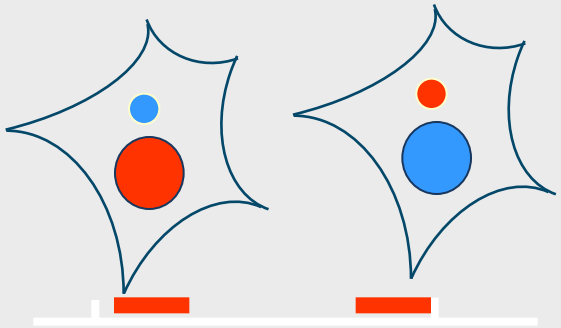
Forment et al, Chromothripsis and cancer: causes and consequences of chromosome scattering, Nature Reviews 2012

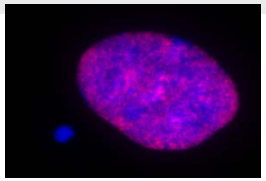
Stephens et al, Massive chromosome rearrangements acquired a single catastrophic event during cancer development, Cell 2011

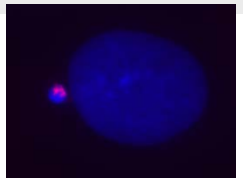
CHROMOTHRIPSIS AND MN

Figure 2





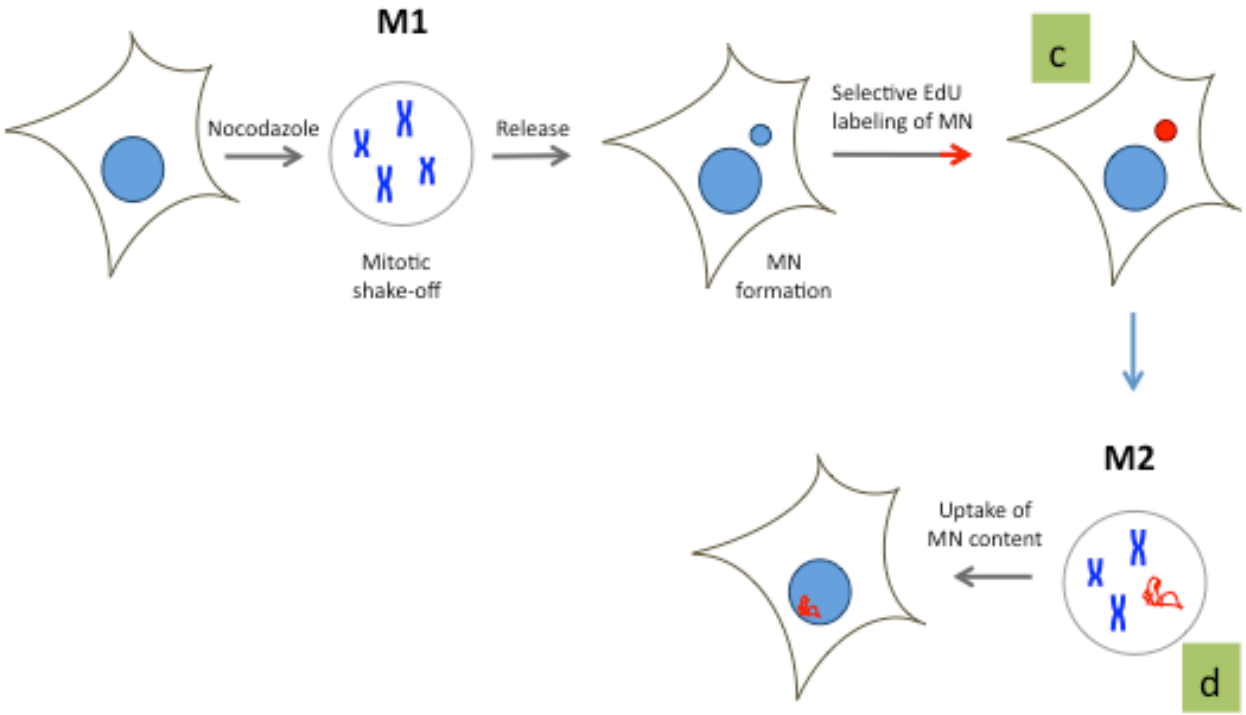


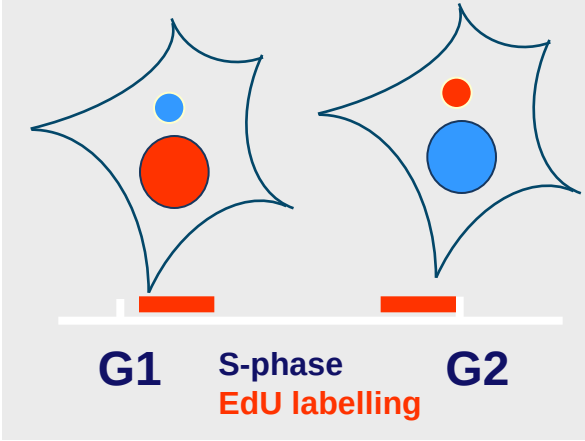


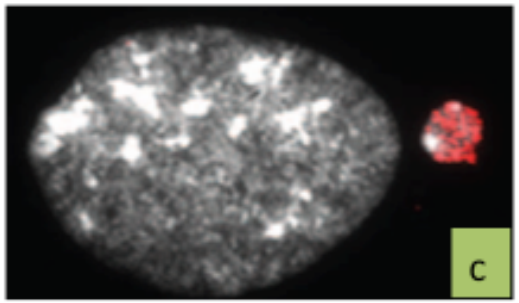
Crasta et al, Nature 2012

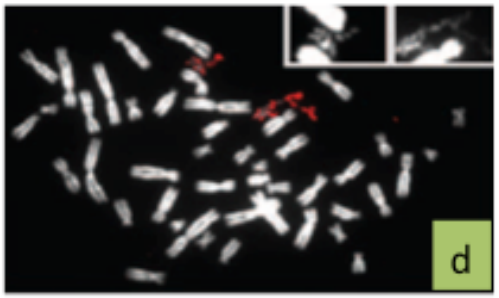
CHROMOTHRIPSIS AND MN

Figure 2



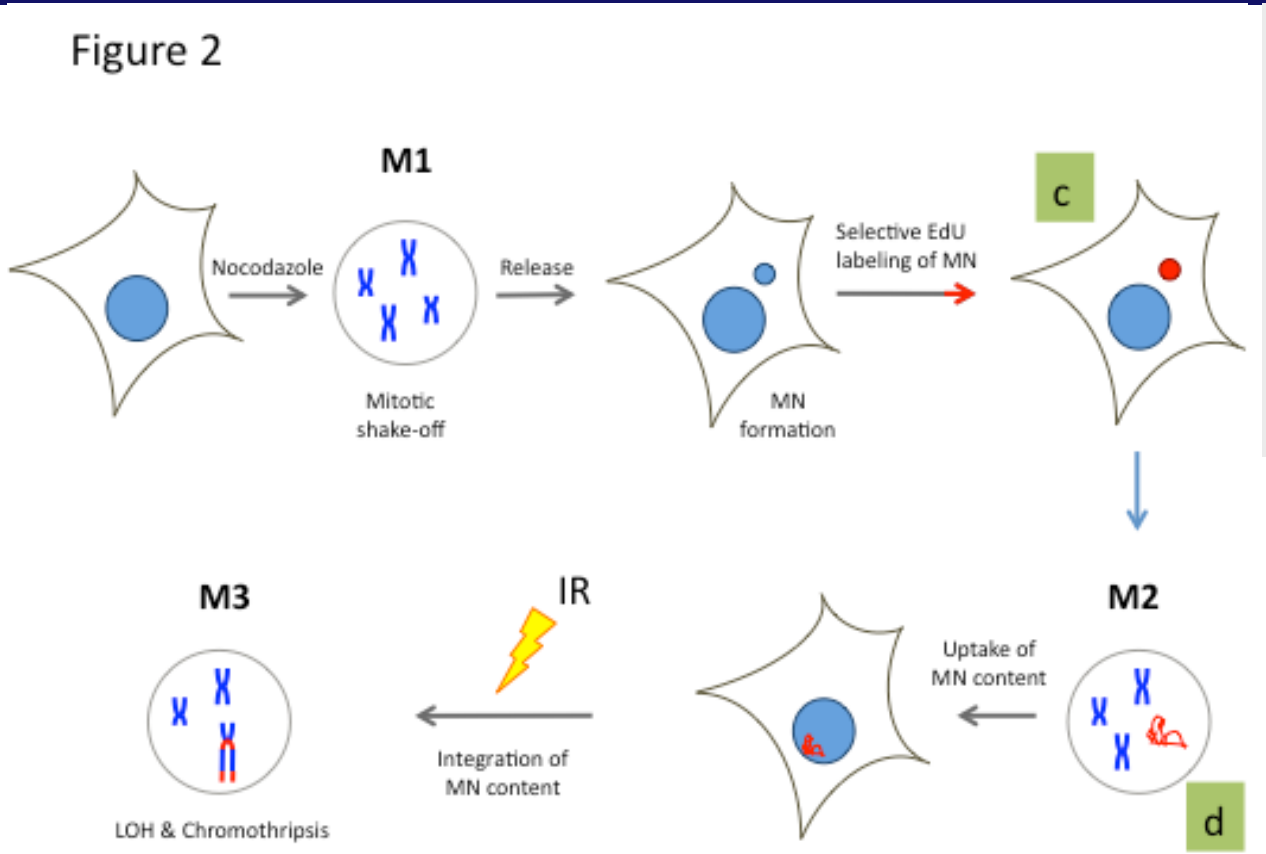


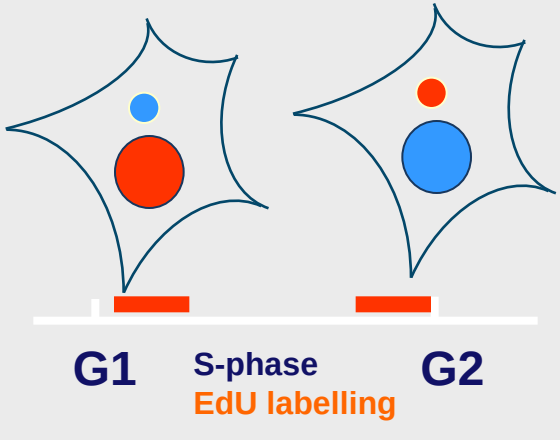


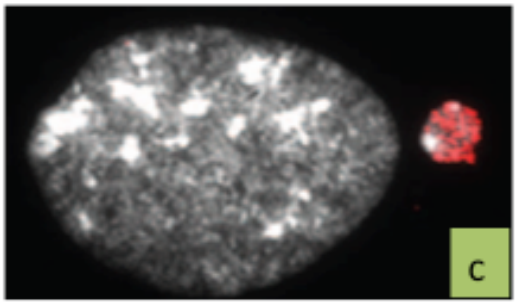


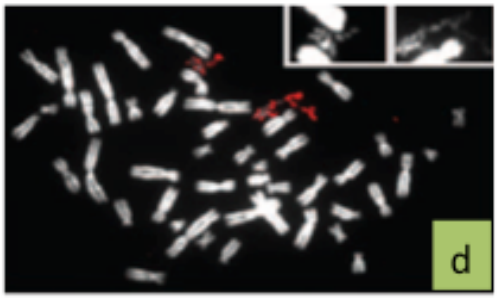
Crasta et al, Nature 2012

CHROMOTHRIPSIS AND MN



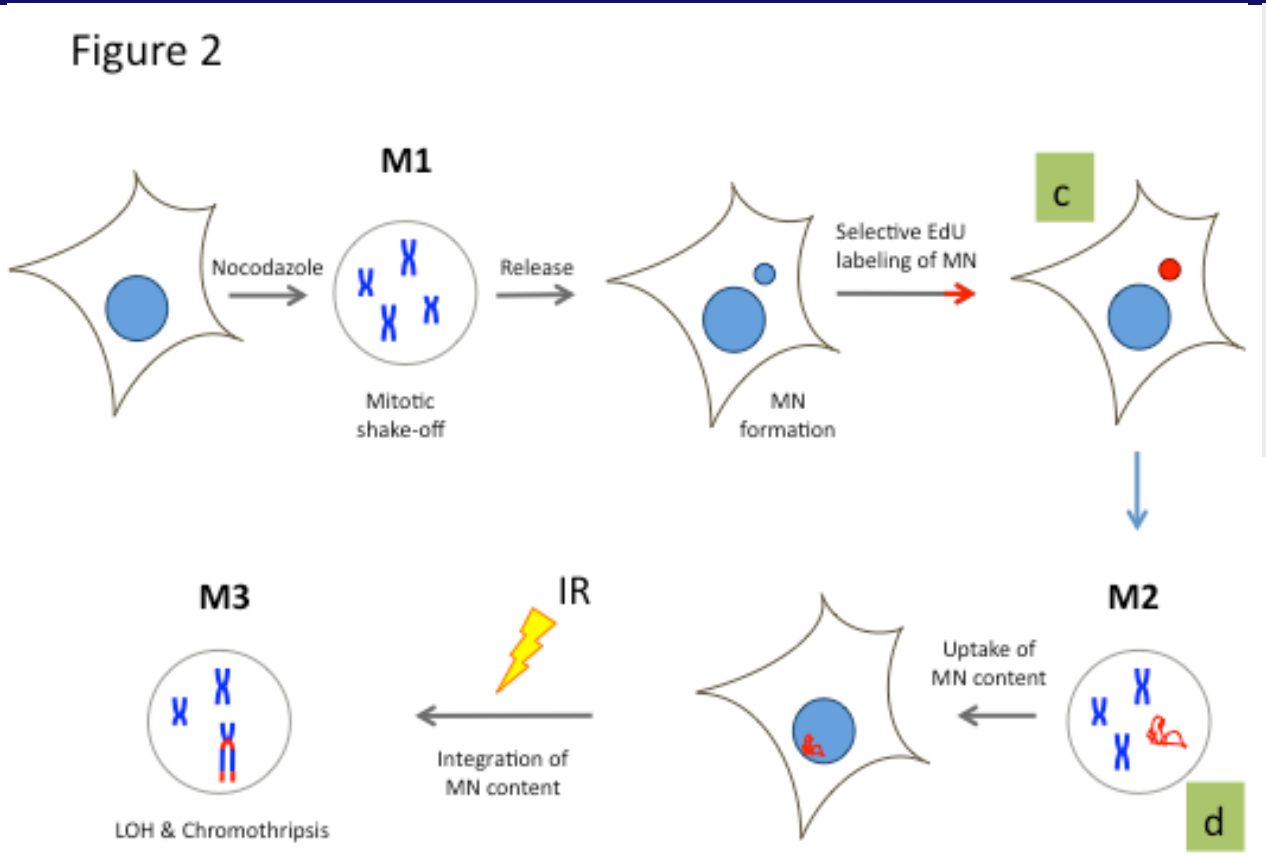


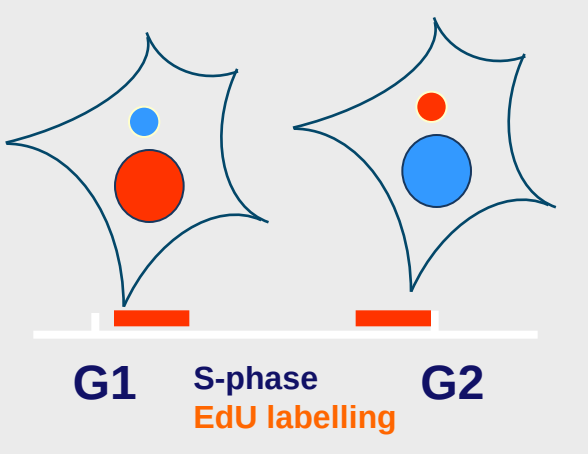


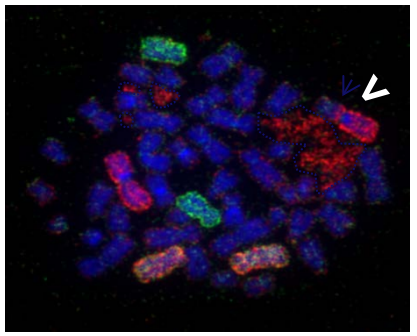


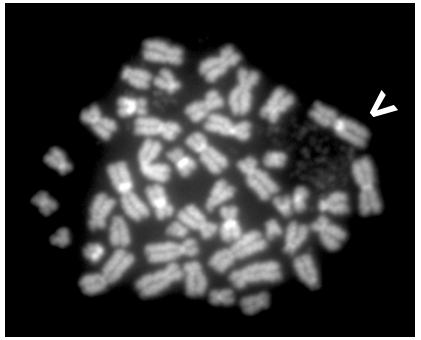
Crasta et al, Nature 2012

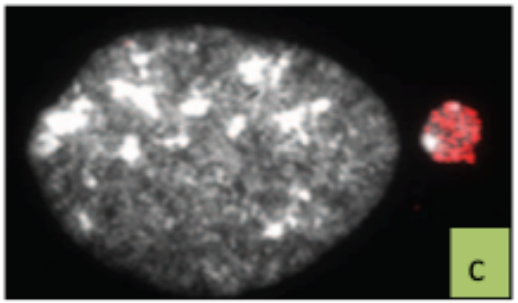
CHROMOTHRIPSIS AND MN

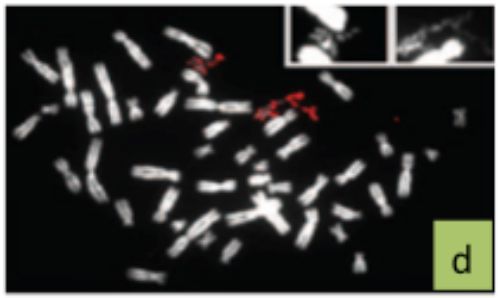








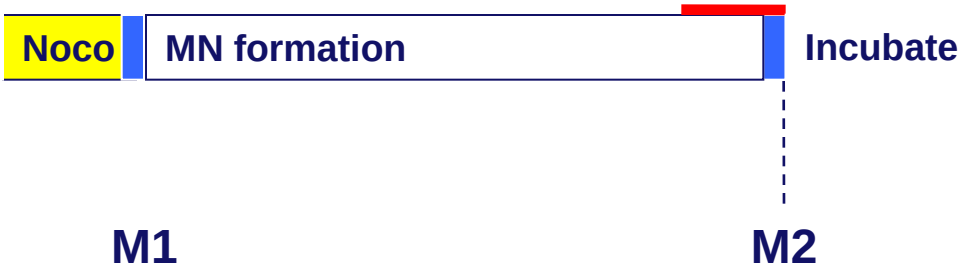




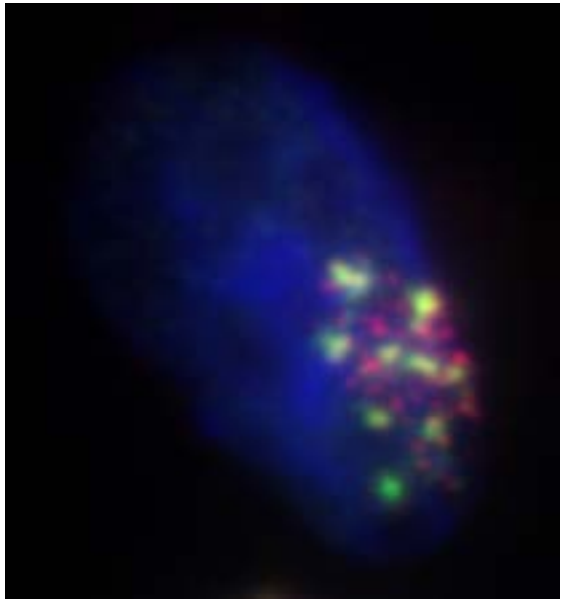
Crasta et al, Nature 2012

Mayak river population , unpublished results

UPTAKE OF FRAGMENTED CHROMOSOMES AFTER SECOND MITOSIS



= Shake-off = Presence of EdU



Boei et al, unpublished

SUMMARY 4

- No low dose threshold for cancer related genetic damage in spite of very effective DNA damage response
- Chromosomal damage is enhanced in cells from human DDR syndromes
- Cellular responses to high and low dose are partly similar and partly specific (transcriptomics, proteomics)
- Mechanism of low dose cancer induction might involve genetic damage mediated chromothrypsis

Questions and Answers

U.S. Department of Health and Human Services
National Institutes of Health | National Cancer Institute
www.dceg.cancer.gov/RadEpiCourse
1-800-4-CANCER
Produced May 2015