Replication stress and DNA damage response in solid tumours

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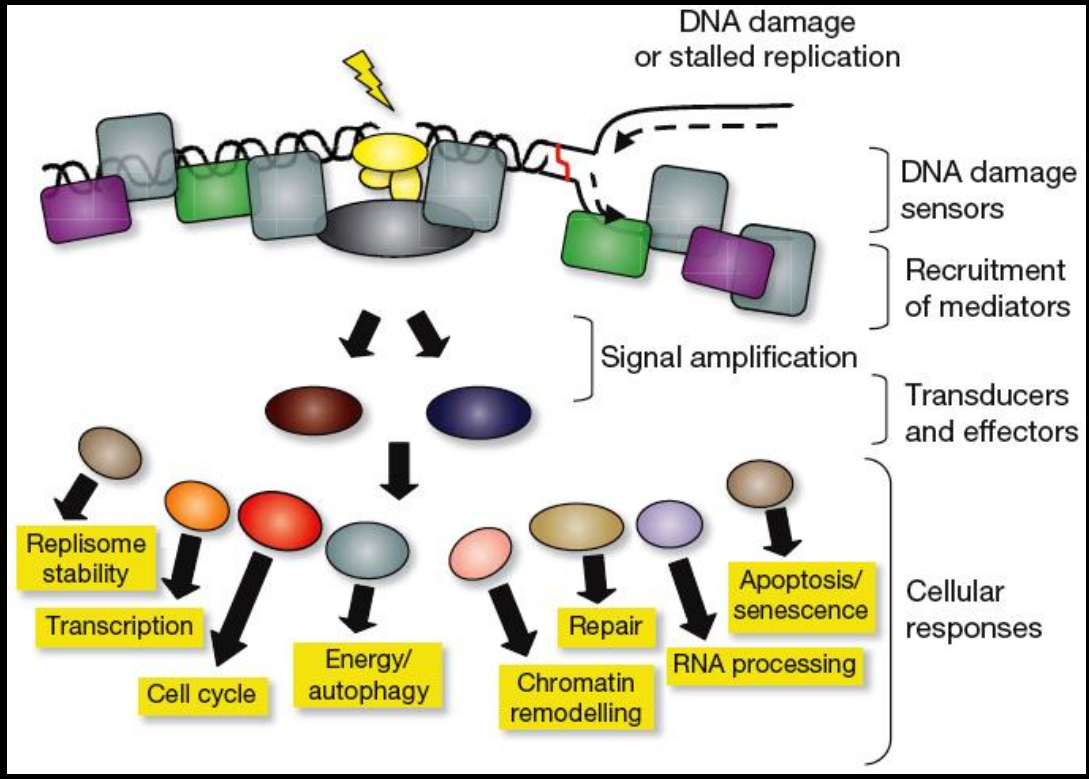
Danish Cancer Society Research Centre, Copenhagen



Jackson and Bartek, Nature 2009

Topics:

A) Replication stress: an emerging Hallmark of cancer - in tumor pathogenesis and chromosomal instability

A) Cellular response to DNA double strand breaks and its relevance to cancer

B) Synthetic lethality vs. viability (fitness) principles in DDR-targeted cancer treatment

DNA damage response (DDR) and cancer

A) DNA damage causes cancer (through mutations)

B) The major cancer treatment modalities radio/chemotherapy operate largely through DNA damage

C) DNA damage causes harmful side-effects of therapy in normal tissues (hair loss, bone marrow, gastrointestinal)

D) DDR defects are common in cancer; promote tumorigenesis and influence responses to treatment

E) DDR provides a biological barrier against cancer progression early in human tumour development!

Kastan & Bartek, Nature (2004)

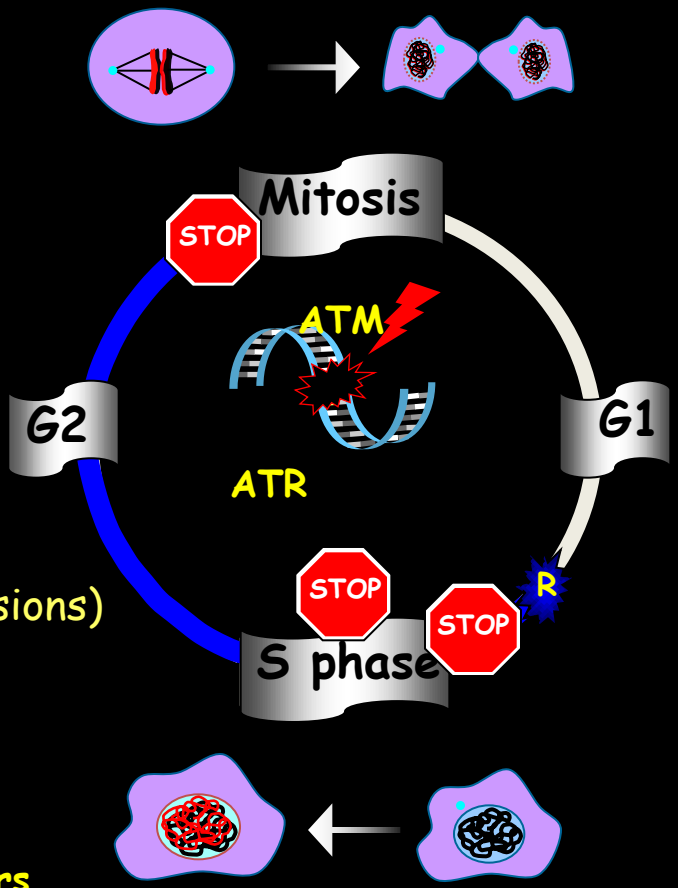
How to avoid genomic instability?

At any given time $5x10^9$ cells in an adult human body actively cycle, and genome integrity is constantly being undermined by endogenous (replication arrors, reactive oxygen species) and exogenous (UV, ionizing radiation, chemical polutants, cigarette smoke...) insults....

50.000 single strand breaks
10 double strand breaks
10 interstrand crosslinks
10.000 de-purinations
600 de-aminations
2.000 oxidative lesions
5.000 alkylation lesions

(Endogenous/spontaneous lesions)

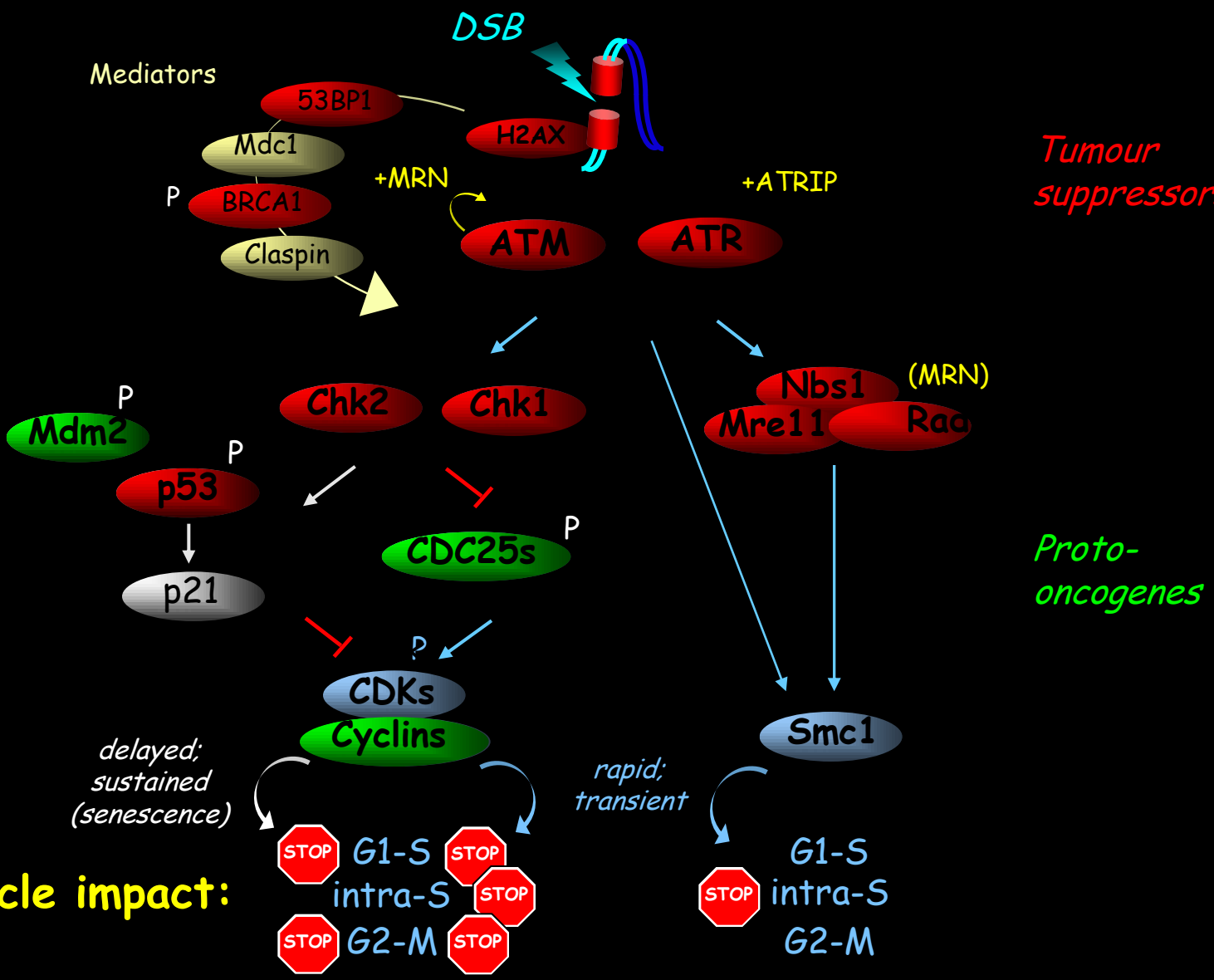
Activated oncogenes
Inactivated tumour suppressors



Cell division
DNA repair
STOP Cell Cycle CHECKPOINTS
Cell death
Senescence

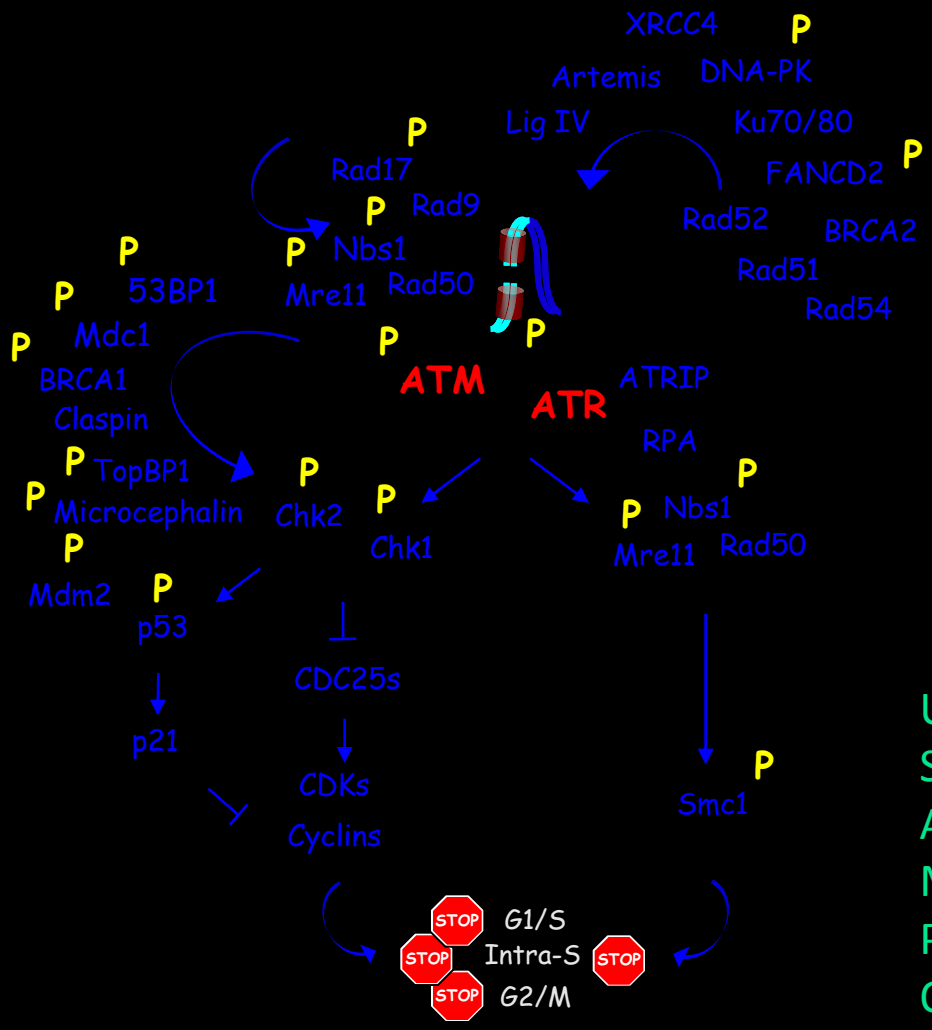
DNA damage signaling underlies most, if not all, types of cellular senescence!!

Tumour suppressors in DNA damage checkpoint signalling



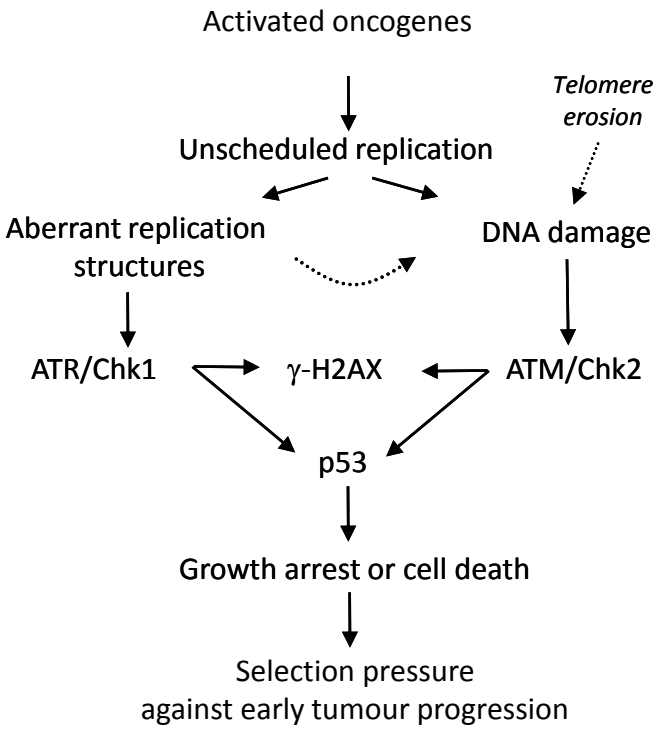
Kastan M and Bartek J, Nature 432: 316-323, 2004

DNA damage response is propelled by *protein phosphorylations*



Ubiquitylation***
Sumoylation
Acetylation*
Methylation**
Parsylation
Other modifications?

The DDR machinery as an intrinsic anti-cancer barrier:

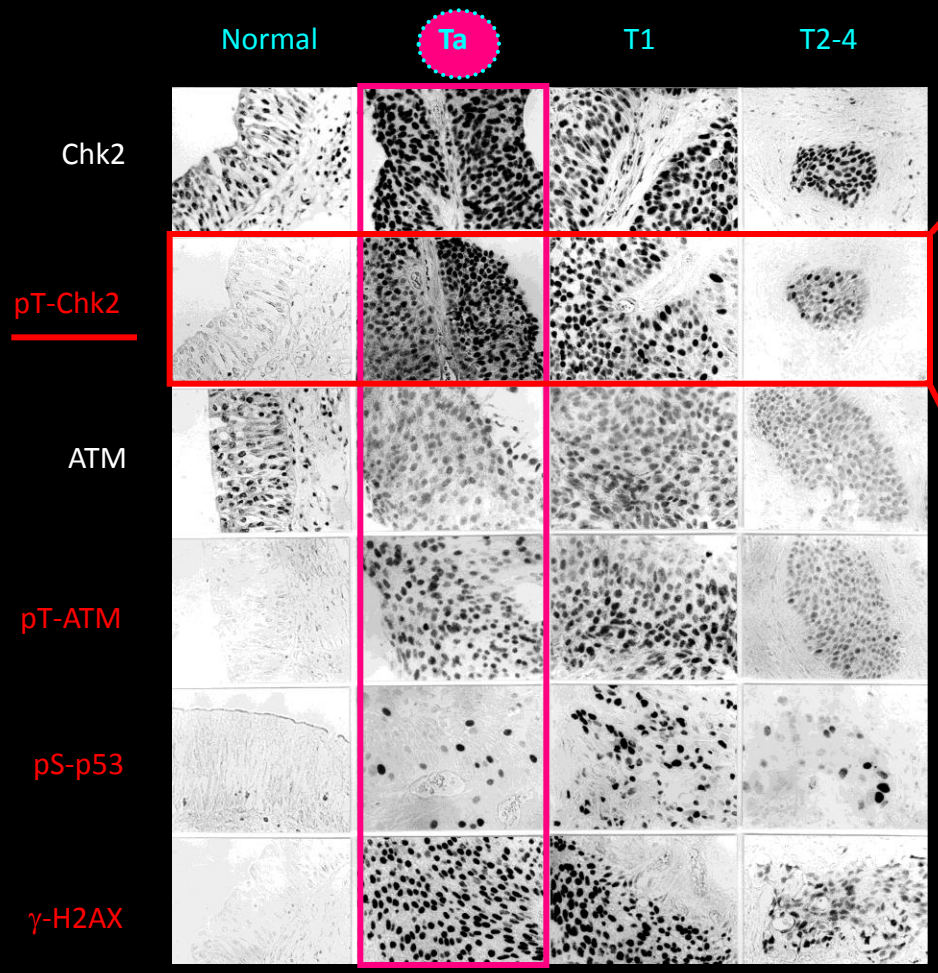


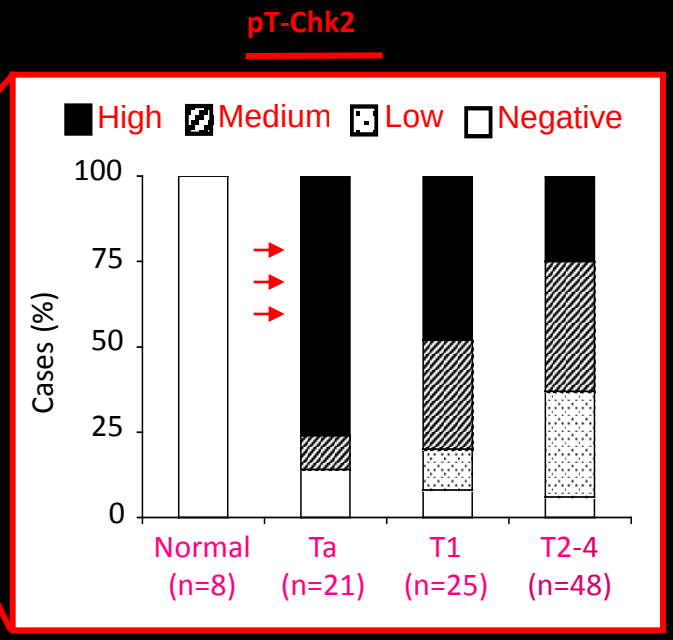
Tumors are under (replication) STRESS!! : High endogenous DNA damage!! (=distinct from normal tissues: hence could be targeted in cancer treatment)

Bartkova et al. Nature 2005
Gorgoulis et al. Nature 2005
Bartkova et al. Nature 2006
DiMicco et al. Nature 2006
Halazonetis, Gorgoulis, Bartek: Science 2008

The ATR/ATM-activated network: inducible barrier to constrain tumour development. Tumour-associated defects in the DDR (ATM, Chk2,p53 etc.) may rescue growth at the expense of genomic instability and tumour progression.

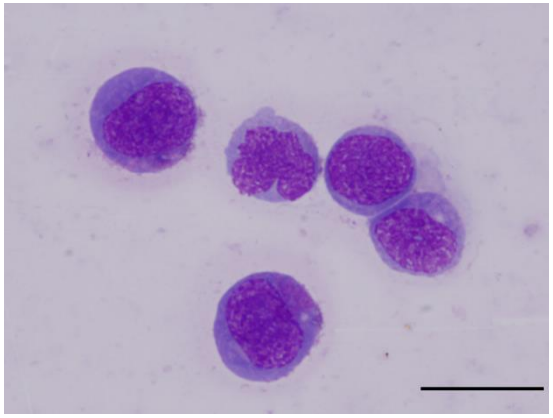
Constitutive activation of DNA damage signaling in *early* *bladder tumours*



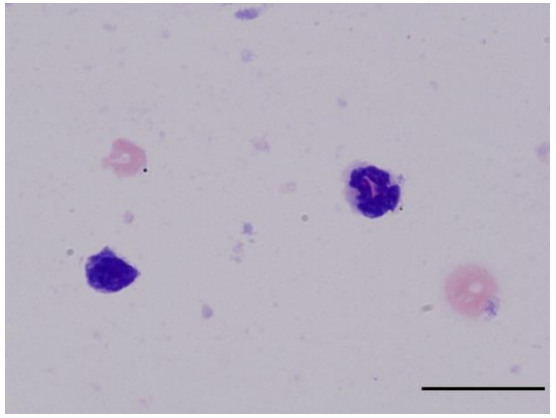


DDR activation is maximal in the early pre-invasive lesions and precedes occurrence of defects in the ATM-Chk2-p53 pathway.

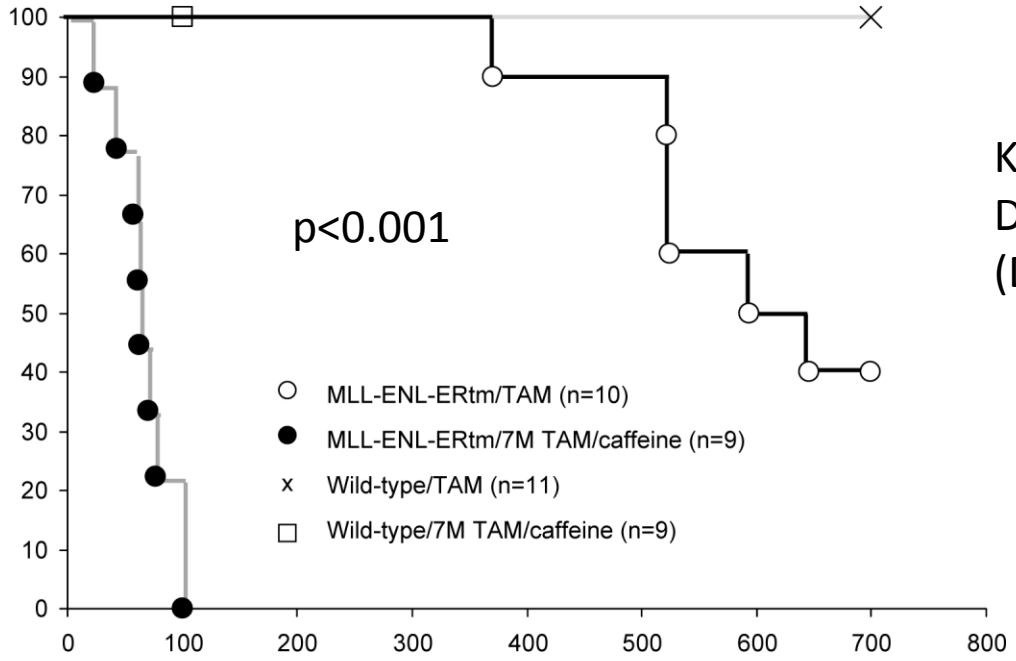
Caffeine treatment in vivo (inhibition of DDR kinase signaling) leads to accelerated MLL-ENL-induced leukemogenesis and kinetics of Leukemia Stem Cells



+ caffeine



- caf.



Kinetics of leukemia Development (Disease-free survival)

Days of TAM

Takacova et al., *Cancer Cell*, 2012

Replication stress (RS): an emerging 'Hallmark of cancer'

Causes: Colisions of DNA replication, transcription and co-transcriptional processes (RNA maturation/splicing, mRNA transport), DNA-RNA hybrids: R-loops, fork reversal/collapse, DNA cross-links, nucleotide depletion...

Consequences:
☹ Enhanced genomic instability – fuels tumor progression !
☹ Enhanced tumor heterogeneity and fitness/survival, resistance to therapy!
(Burrell et al. Nature 501: 338-45, 2013)
☺ Vulnerability to inhibitors of fork protection (e.g. ATR/CHk1 inhibitors)
(Capetillo and Brown labs; Bartek et al. Nat.Struct.Mol. Biol. 19: 5-7, 2012)

Cell's defense (‚buffering RS'): Through fork protection/restart mechanisms, and replication checkpoint: major determinants = ATR-Chk1 signalling and RPA protein abundance (threshold!!) – these protect replication forks from stress and potential collapse and ‚chromosomal catastrophy'
(Toledo et al. Cell 155: 1088-1103, 2013)

And a novel ATR-Chk1 checkpoint at the nuclear membrane
(Kumar et al. Cell 158: 633-46, 2014)

10 most significant focal deletions in human cancer (>3000 tumor specimens examined) & fragile sites!

- (ref: Beroukhim et al., Nature 2010)

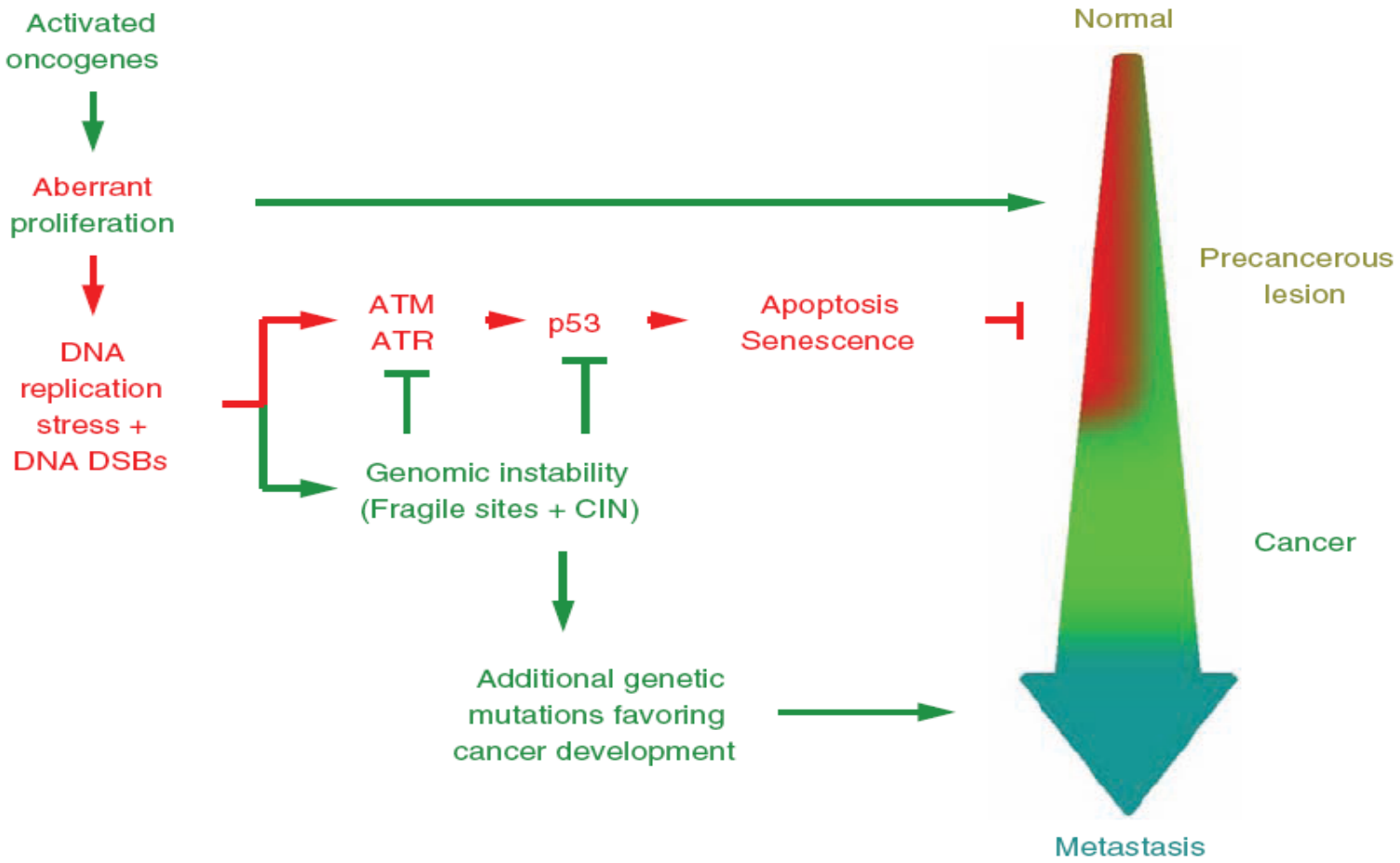
#	Gene Targeted	Frequency (%)	Type
1	CDKN2A/B	17.1	TSG
2	FHIT	13.5	FRA3B**
3	WWOX	10.9	FRA16D**
4	PTPRD	6.9	Large Gene (Fra?)*
5	MACROD2	7.0	FRA-chr20**
6	PARK2	7.4	FRA6E**
7	RB1	7.6	TSG
8	LRP1B	7.1	FRA2F**
9	FAT1	7.9	Near-Telomere*
10	PDE4D	7.8	FRA5H**

- TSG, tumour suppressor gene; FRA-chr20, recently identified fragile site in chromosome 20.

REVIEW 7 MARCH 2008 VOL 319 SCIENCE

An Oncogene-Induced DNA Damage Model for Cancer Development

Thanos D. Halazonetis,[1]* Vassilis G. Gorgoulis,[2] Jiri Bartek[3]



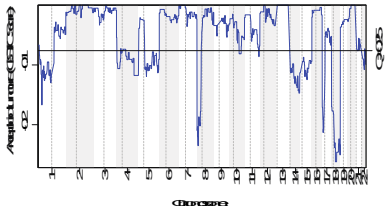
Model

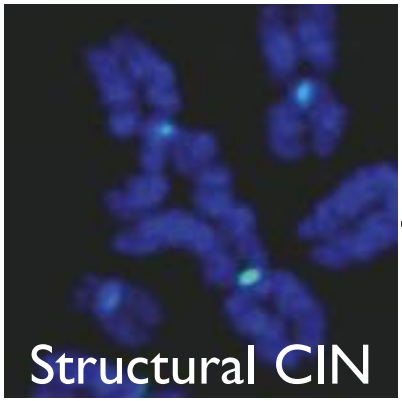
(Burrell at al. Nature 494: 492-6, 2013)

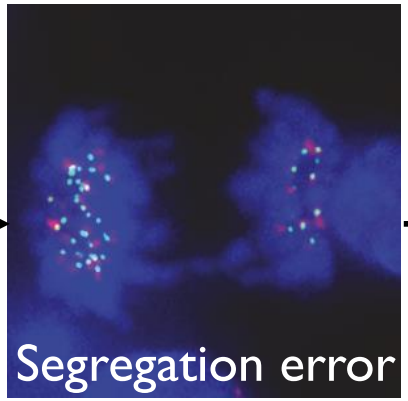
Exogenous Nucleosides

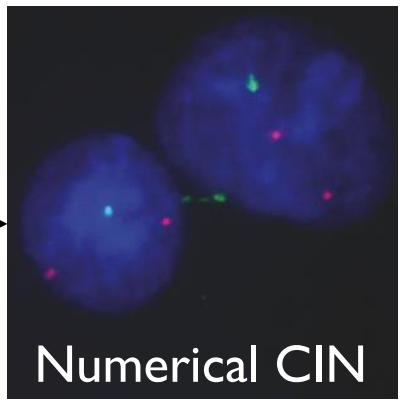
Replication stress

18q loss?
PIGN,MEX3C, ZNF516



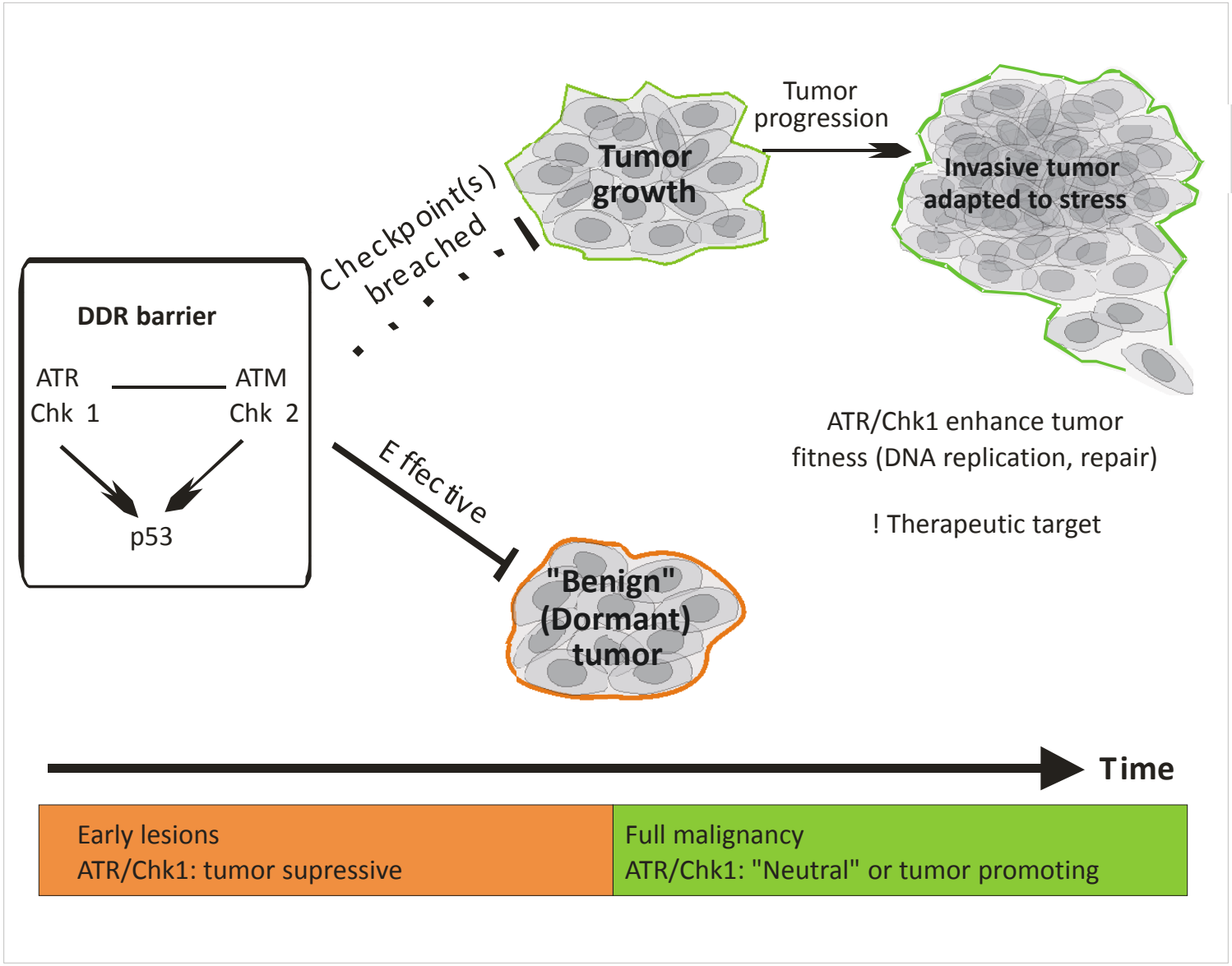






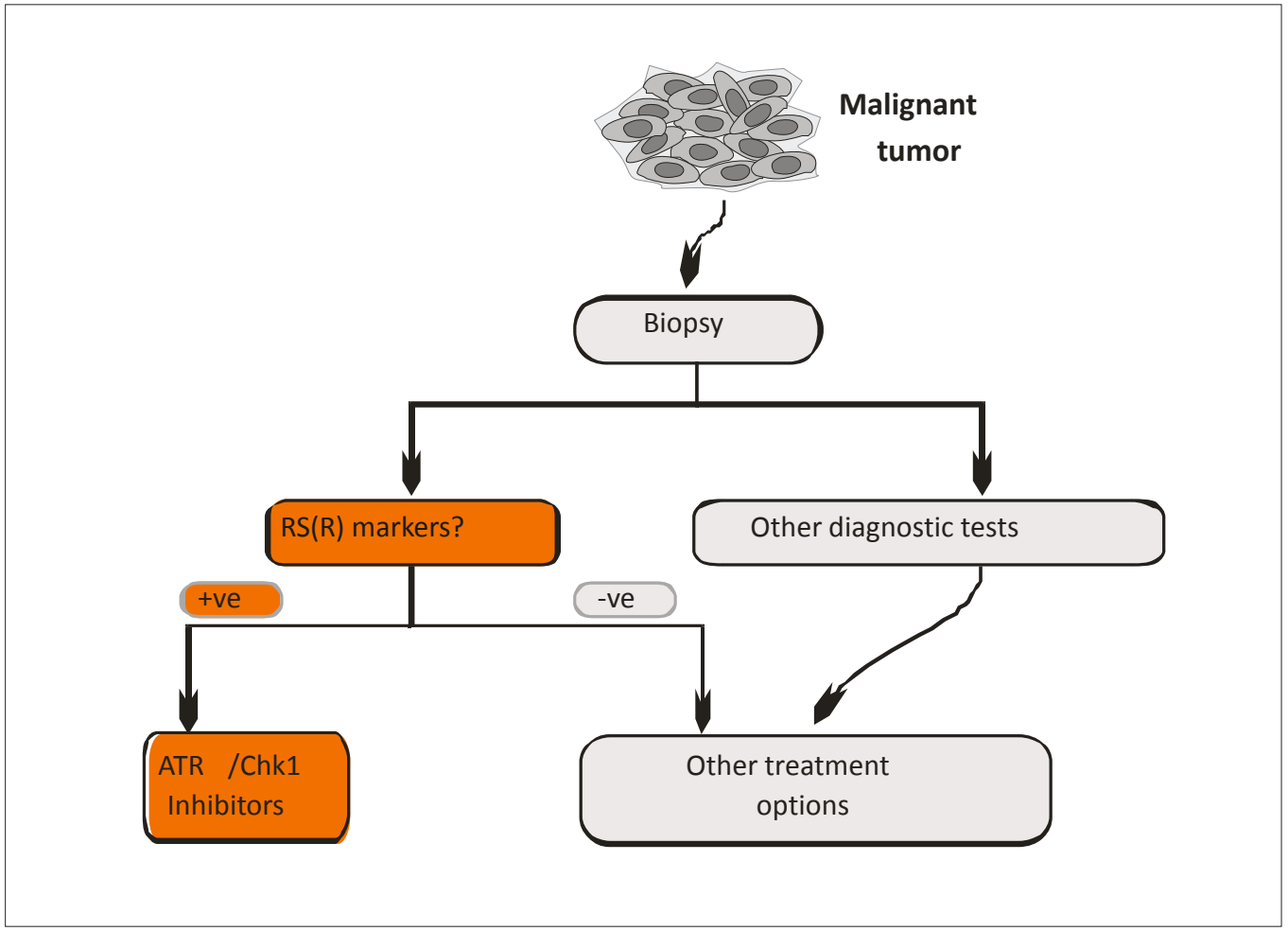
Shift from focus on mitotic defects to Replication stress as a major cause of CIN ?

Distinct roles of the ATR/Chk1 pathway during multistep tumorigenesis



Bartek et al. Nat.Struct.Mol. Biol. 19: 5-7, 2012

Potential exploitation of replication stress as a target for cancer therapy

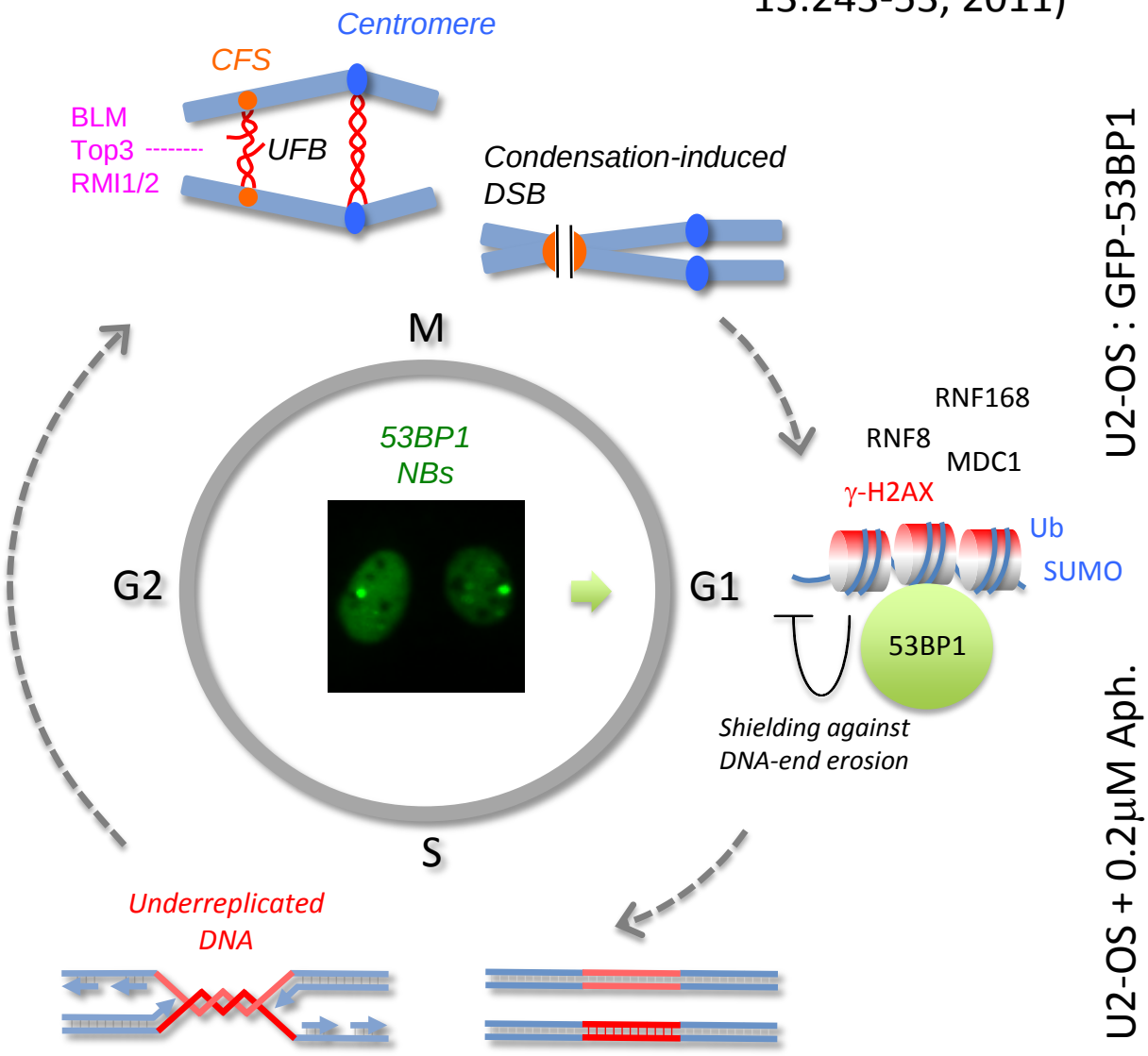


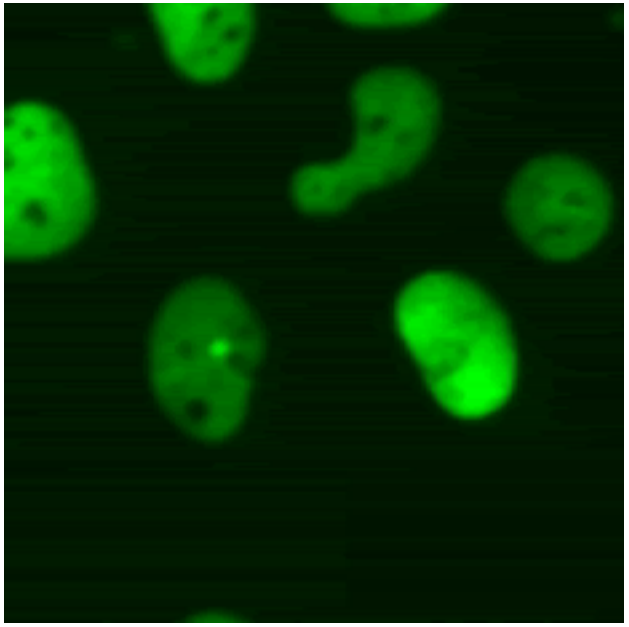
Many tumors feature enhanced replication stress (RS): Biomarkers of RS and/or activated RS response (RSR) could help select patients who might benefit from treatment with e.g. inhibitors of ATR or Chk1 kinase (Thresholds and benefits vs. potential side-effects??)

Bartek et al. Nat.Struct.Mol. Biol. 19: 5-7, 2012

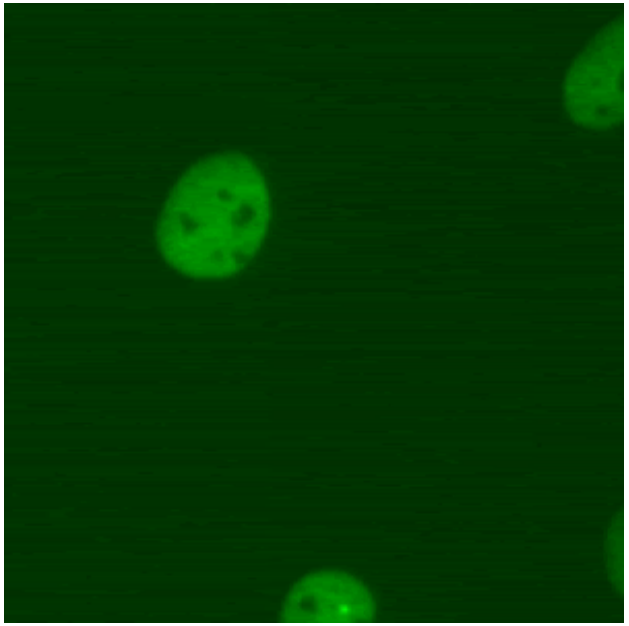
53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress

(Lukas et al. Nature Cell Biol. 13:243-53, 2011)





U2-OS : GFP-53BP1

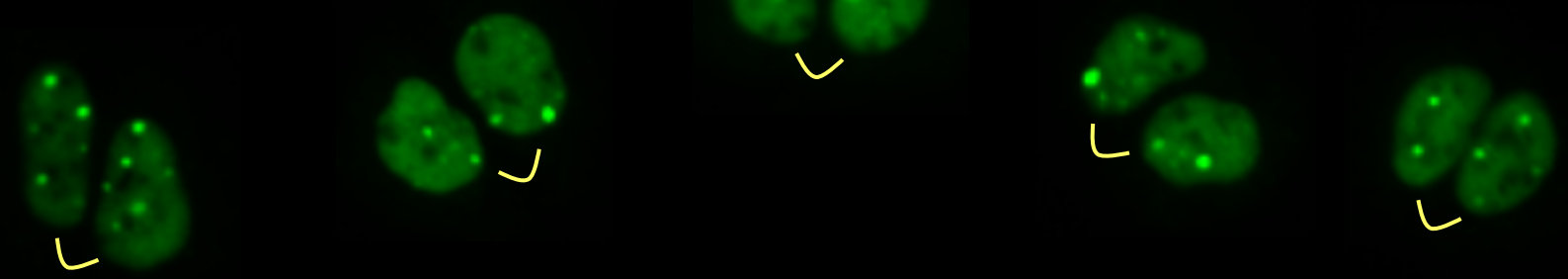


U2-OS + 0.2μM Aph.

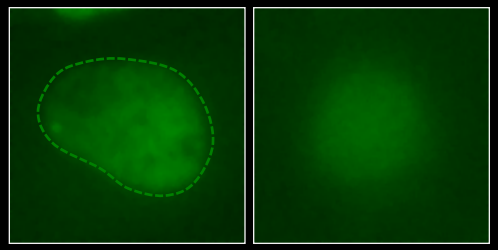
53BP1 nuclear bodies in newly-born daughter cells are 'symmetrical'

after normal cell division

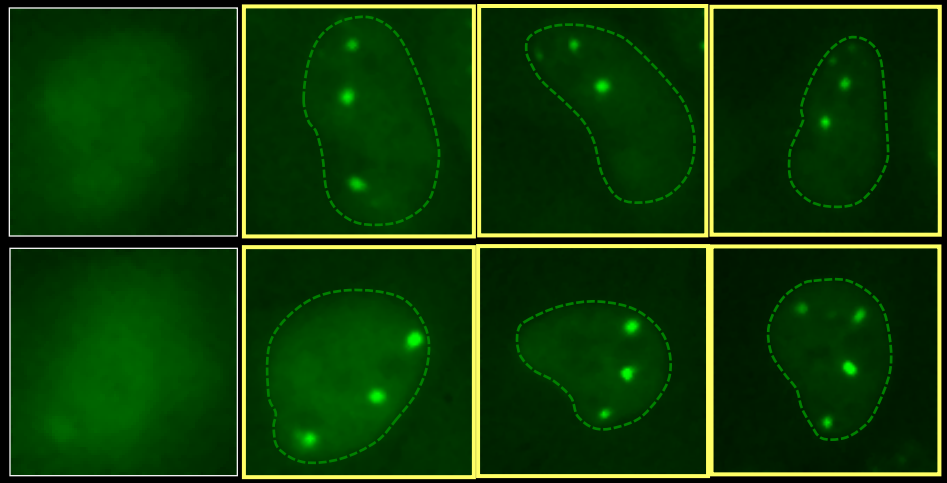
G1 daughter cells (α53BP1)



GFP-53BP1



G2 phase M phase



Telophase/ G1

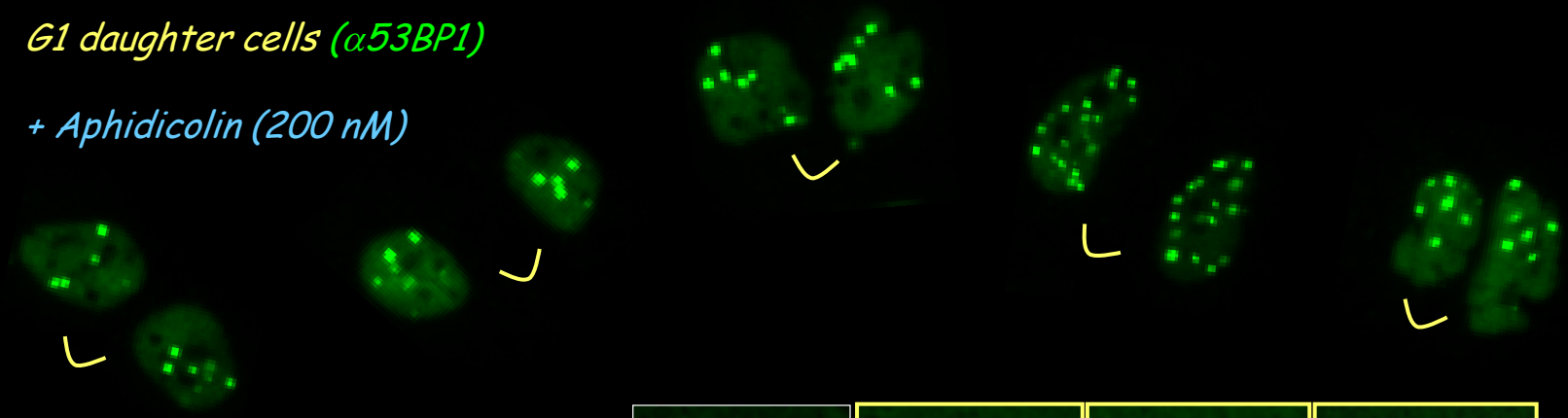
G1 phase

53BP1 nuclear bodies in newly-born daughter cells increase in number & remain 'symmetrical'

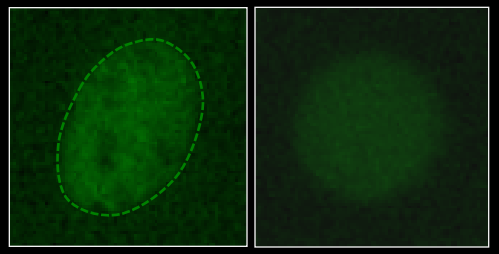
after aphidicolin-induced replication stress

G1 daughter cells (α53BP1)

+ Aphidicolin (200 nM)

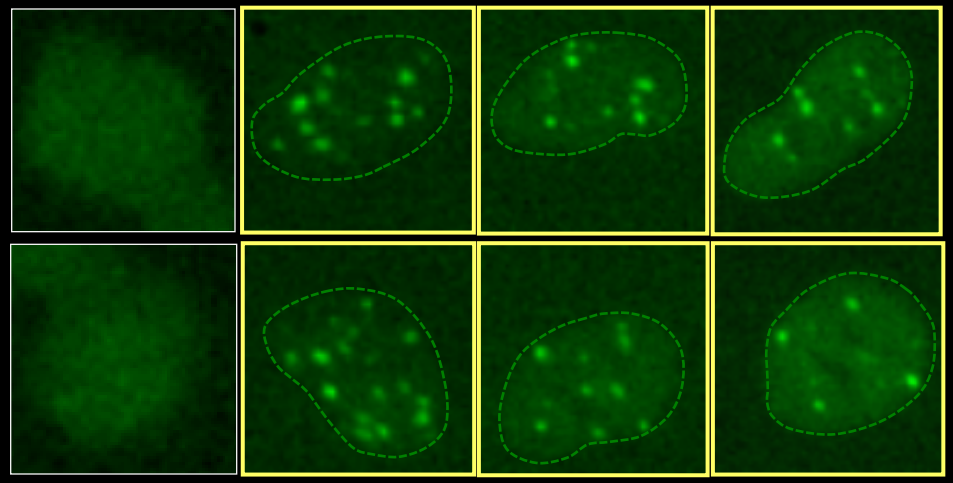


GFP-53BP1



G2 phase M phase

+ Aphidicolin (200 nM)



Telophase/ G1

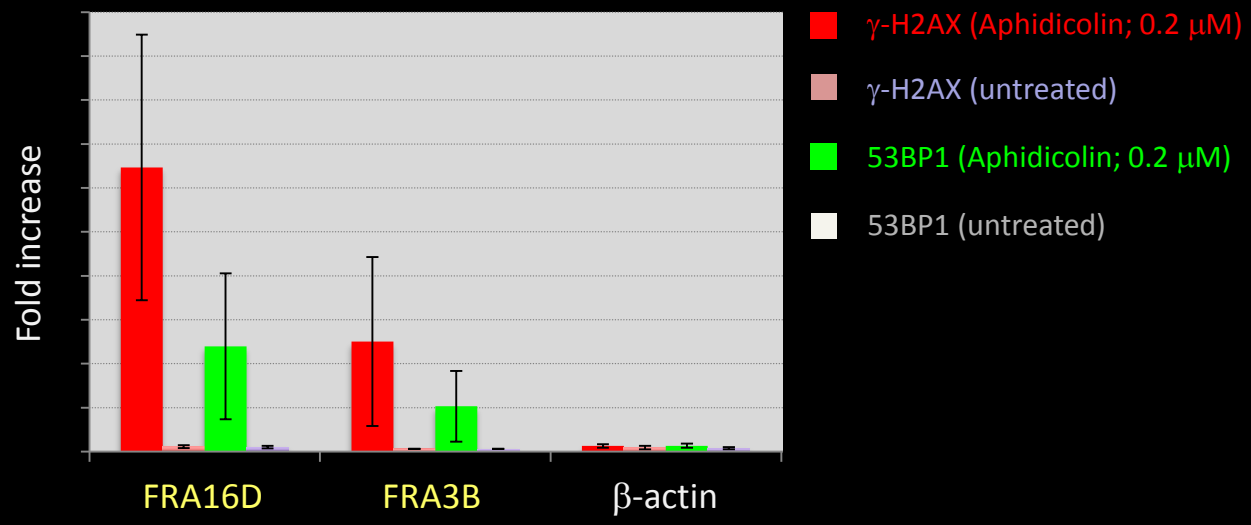
G1 phase

Chromosomal sites prone to encounter replication problems

that might be transmitted to mitosis:

! Relevance for human diseases – oncogenesis…!

**Common fragile sites*

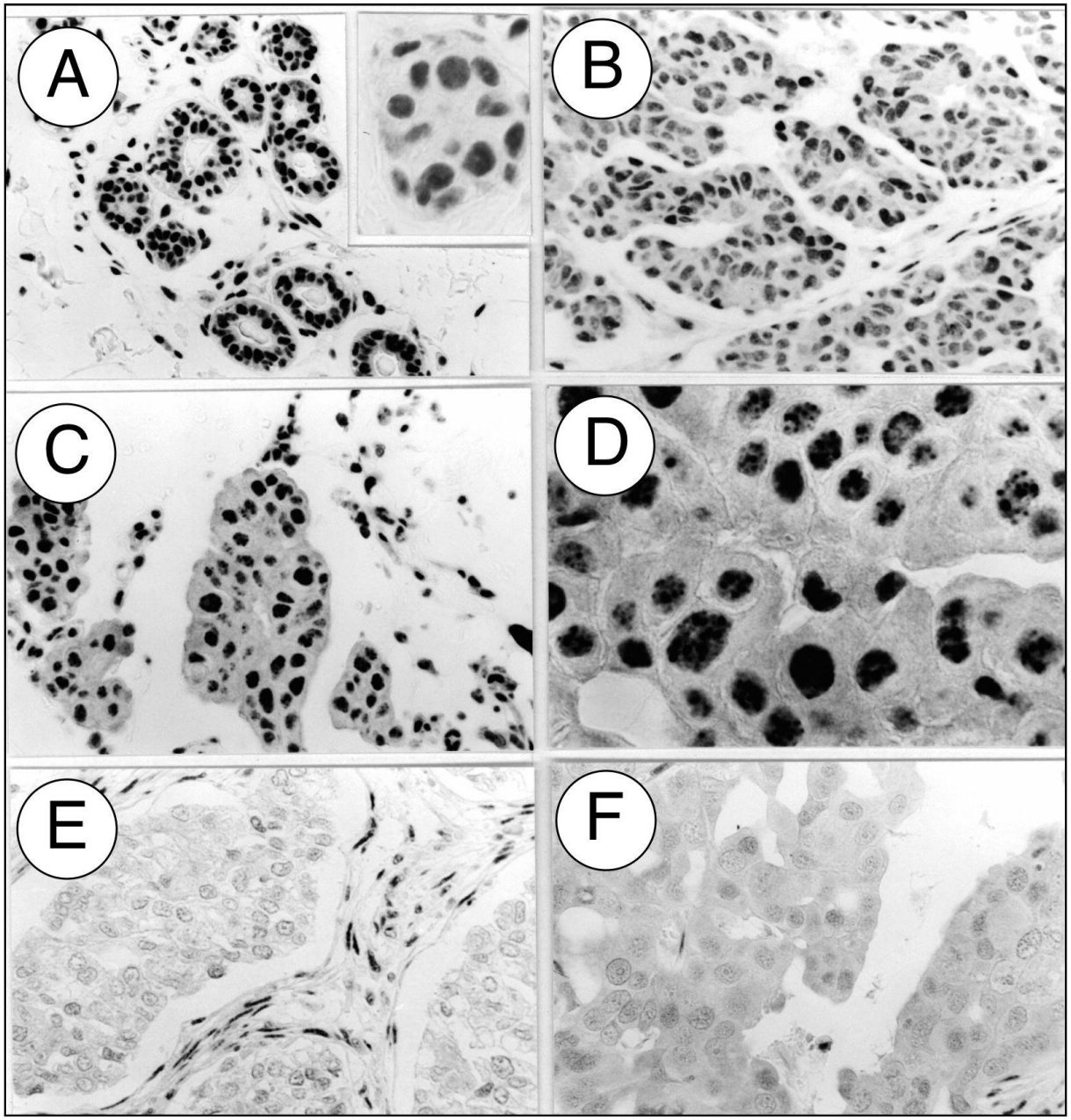


53BP1 as a candidate Biomarker

IHC staining for 53BP1:
normal human breast (A)
and
breast carcinomas (B-F)

Loss of 53BP1 in subsets of human tumors

Significance??

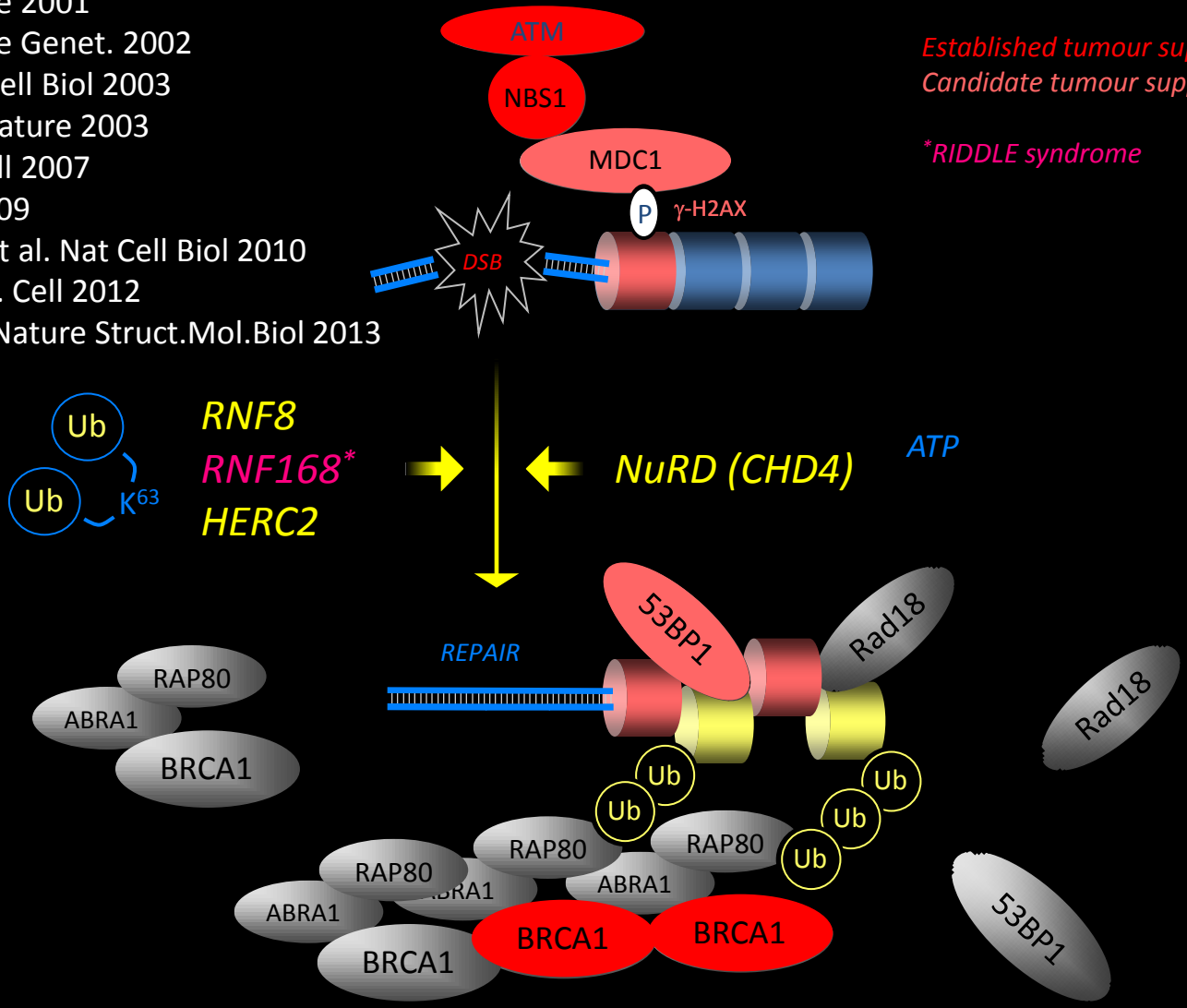


Pathways involved' in 'licensing' of damaged chromatin to reduce adverse effects of DNA breakage

Falck et al. Nature 2001
Falck et al. Nature Genet. 2002
Lukas et al, Nat Cell Biol 2003
Goldberg et al. Nature 2003
Mailand et al. Cell 2007
Doil et al. Cell 2009
Bekker-Jensent et al. Nat Cell Biol 2010
Gudjonsson et al. Cell 2012
Watanabe et al. Nature Struct.Mol.Biol 2013

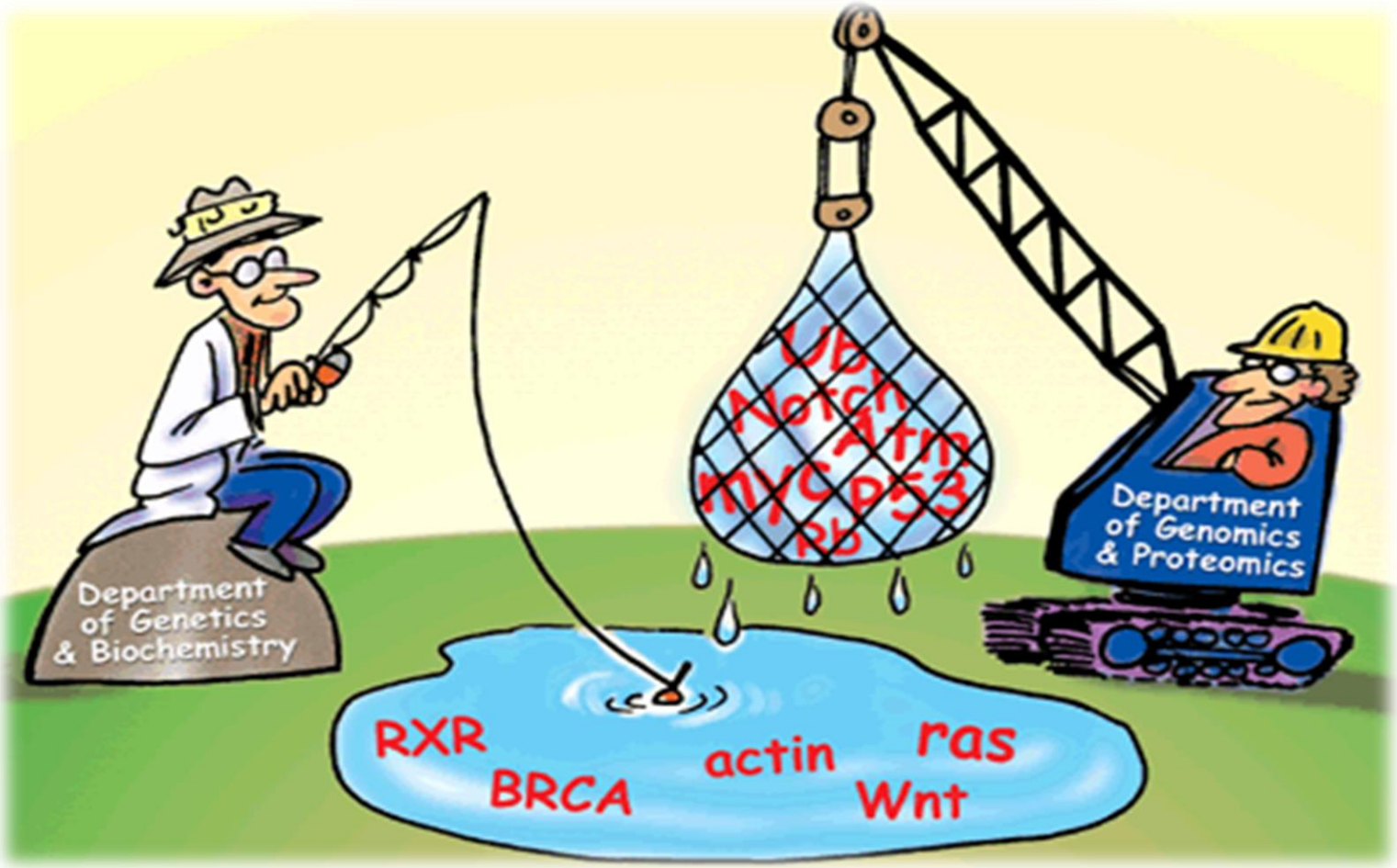
Established tumour suppressors
Candidate tumour suppressors

**RIDDLE syndrome*



Biomarkers for Clinical Oncology: Therapeutic Targets and Surrogate Endpoints

Example of Synthetic viability in treatment resistance



Traditional vs. High-throughput Approach

The synthetic lethality principle: discovery of treatment sensitivities (e.g. sensitivity to PARP inhibitors in BRCA1/2-deficient tumors

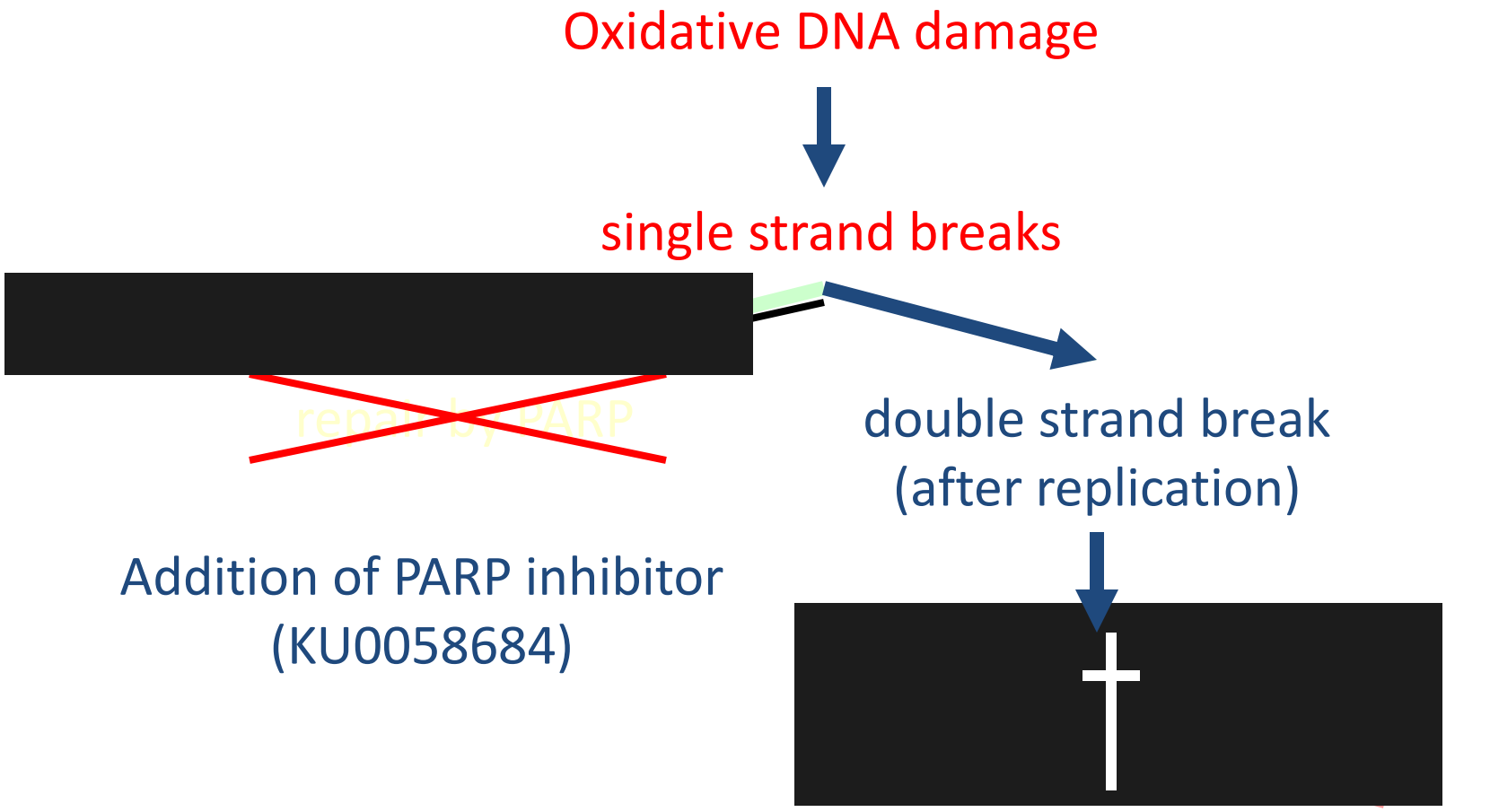
The synthetic viability principle: acquired tumor fitness/resistance to treatment (e.g. resistance to PARP inhibitors in BRCA1/2 deficient tumors with concomitant loss of **53BP1, JMJD1C, or Rev7** : hence re-gained ability to repair DNA breaks by HR)

Examples of targets for cancer therapy
within the DNA damage response machinery:

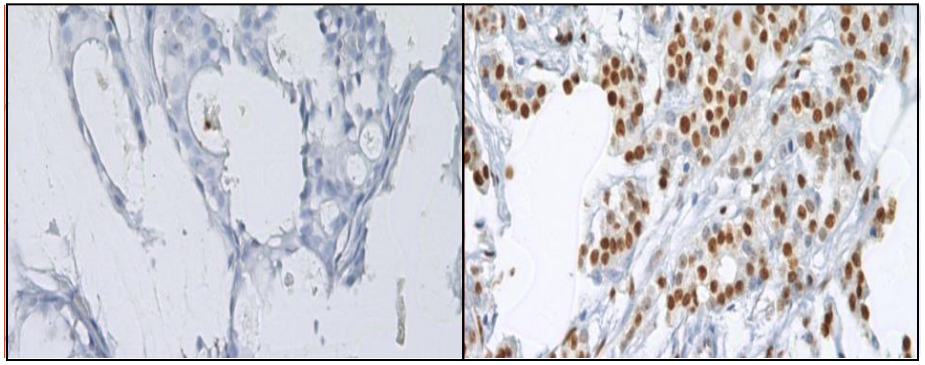
Checkpoint kinases/replication stress targeting (Chk1, ATM, ATR, DNA-PK, Chk2)
DNA repair enzymes (PARP1, MGMT...)
Inhibitors of protein-protein interactions (e.g. p53-mdm2)

Telomerase inhibitors
Proteasome inhibitors (Velcade)

Rationale of therapy by PARP inhibitor



Low 53BP1 expression is associated with triple-negative and BRCA1/2-mutated breast cancer



Resistance to PARP inhibitors (*synthetic viability*)

BRCA1	53BP1	Response
Wt	Wt	Resistant
Mut.	Wt	Sensitive
Mut	Mut	Resistant

Features	Normal 53BP1 (%)	Aberrant 53BP1(%)	p^*
Not Triple-negative	861 (87.0)	14 (50.0)	
Triple-negative	129 (13.0)	14 (50.0)	**0.000004**
Non-BRCA1/2	1079 (94.0)	29 (76.3)	
BRCA1/2	69 (6.0)	9 (23.7)	**0.00001**

and:

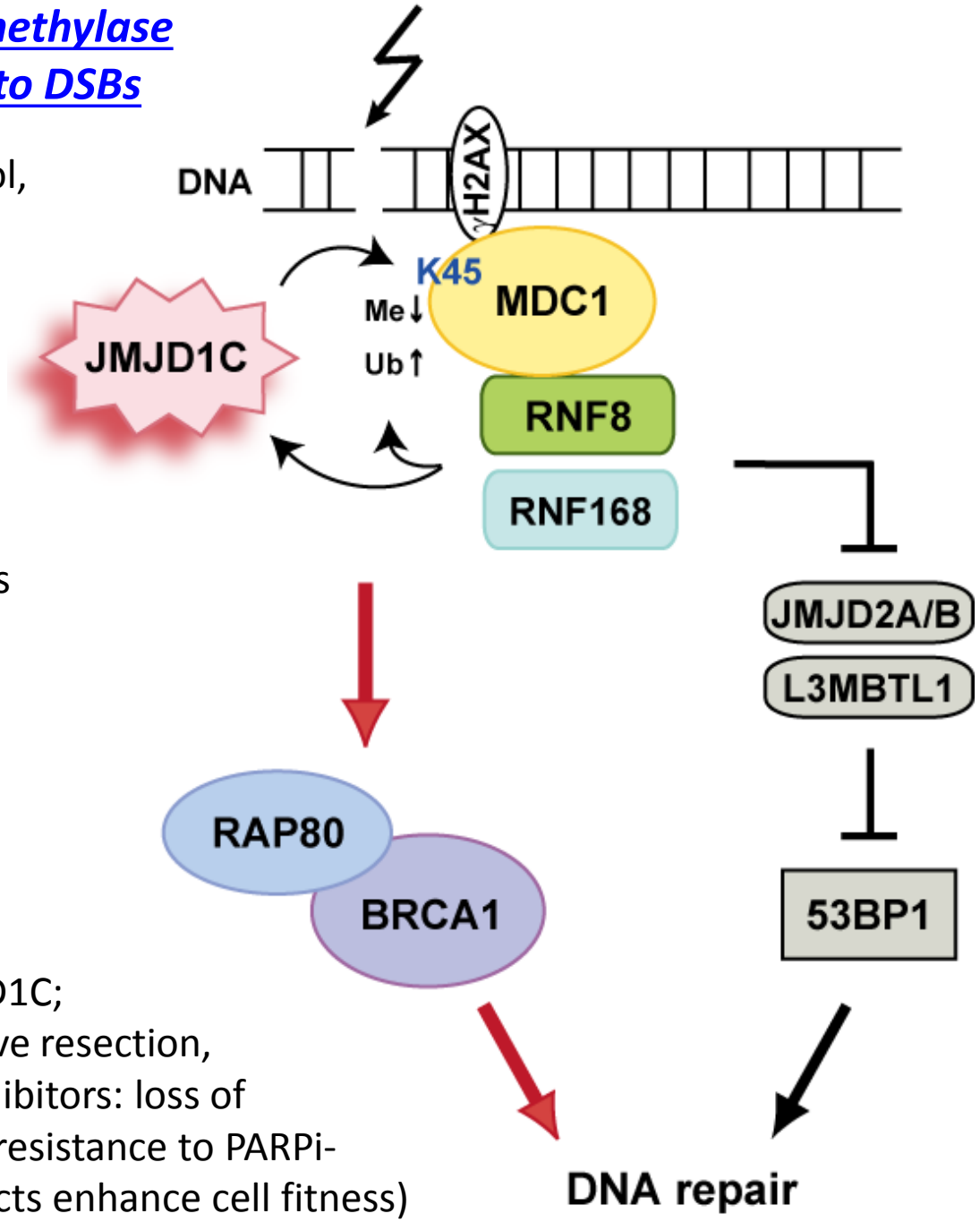
Enhanced fitness – survival of cancer cells with BRCA1 defects upon 53BP1 loss (*synthetic viability*) due to partly rescued DNA repair

Bouwman et al, Nat Struct Mol Biol 2010

MODEL

JMJD1C demethylase in response to DSBs

(Watanabe et al. Nature Struct.Mol.Biol, Nov. 2013)



JMJD1C demethylase:

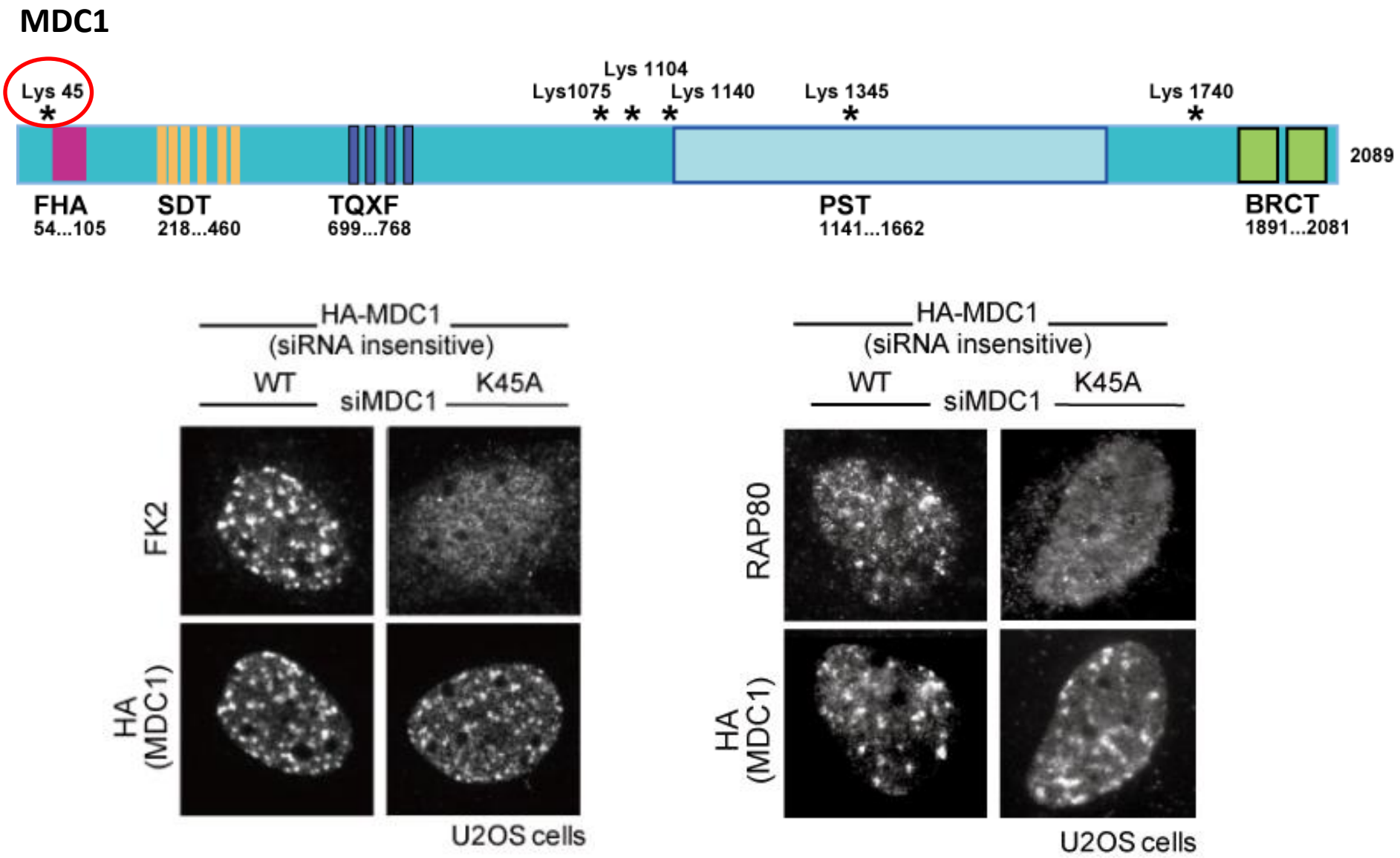
* = selective for the BRCA1 branch of the DSB chromatin response

*RNF8 binds to, and physically protects (stabilizes) JMJD1C after DNA damage

*JMJD1C de-methylates MDC1-K45, helps recruit RNF8 to MDC1-P, promotes MDC1/'FK2 ubiquitylations' and recruitment of Rap80/BRCA1

*Subset of breast cancers: loss of JMJD1C; JMJD1C-Rap80/BRCA1 oppose excessive resection, & impact responses to IR and PARP inhibitors: loss of JMJD1C in BRCA1-deficient ca. causes resistance to PARPi- example of 'Synthetic viability' (2 defects enhance cell fitness)

Methylation of Lysine 45 on MDC1 regulated by JMJD1C: MDC1 is required for ubiquitin conjugates followed by recruitment of RAP80 at DNA damage sites



DNA damage response in personalized cancer therapy: emerging principles and 'hopes'

A) Cancer cells are 'addicted to' stress support mechanisms – exploitable for therapy!

B) Examples of successful treatment strategies targeting DDR are emerging (e.g. PARPi), yet individualized approach is essential!

C) New predictive biomarkers and validated targets are required to select patients and predict response to treatment!

A) Cancer stem cells should be better understood in terms of their radio/chemo-resistance and targeted: (GBM stem cells are more sensitive to PARP inhibitors: Venere et al. CDD 2014).

B) There are more potential new targets in DDR pathways and synthetic lethalities with other mechanisms to be discovered!!!

Our external collaborators

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& coll.

University of Aarhus

Torben Ørntoft
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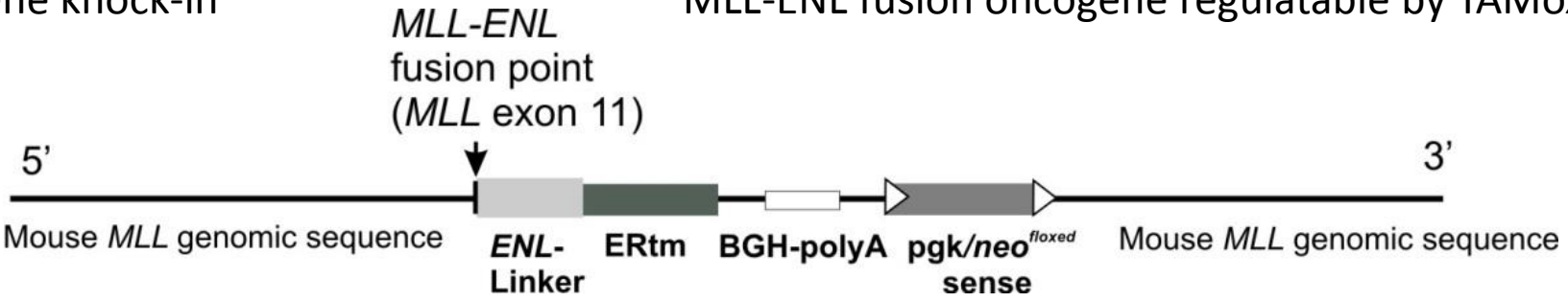
Replication stress and tumorigenesis - some of the open questions:

Also in mice? Also hematological tumors? Is DDR a barrier? Senescence/Death? Tissue specificity?

*Mouse model for MLL-ENL induced leukemia (V. Divoky & S. Tacacova, Univ. Olomouc, CR)

gene knock-in MLL-ENL fusion oncogene regulatable by TAMoxifen



Mice: MLL-ENL-ERtm +/- TAM vs.: Wild-type +/- TAM controls

Phenotypes/parameters:

Disease course, cell phenotypes, BM vs. Spleen, gene+cytokine expression profiles, DDR signaling

*MLL-ENL-ERtm/+TAM mice develop myeloproliferative disease (expansion of mature neutrophils

- early hyper-proliferative phase (100%-penetrant) (cell colonies +self-renewaL: TAM-dependent

*By 8-9 months of TAM: BM-prolif. blocked – transition to senescence/cell death (BM depletion)

Spleen-slower proliferation, partial senescence,

*50% MLL-ENL-ERtm/+TAM mice develop myeloid leukemia/terminal (latency 592+/-112 days !!)

(here c-Kit+ve leukemia stem cell subpopulation expands)

Takacova et al., *Cancer Cell*, in press