NIH DNA Repair
Feb, 2005
Dr. Judy Campisi

Genome maintenance systems, cancer and aging

Cancer

Major cause of mortality after about the mid-point of maximum life span

Why does it happen?

Why doesn't it happen more often?

Tumor suppressor mechanisms curtail the development of cancer throughout the life span

Two Classes of Tumor Suppressors

- ***CARETAKERS -- act on the genome***

Prevent cancer by preventing mutations
Damage prevention and repair

- ***GATEKEEPERS -- act on cells***

Prevent potential cancer cells from forming tumors

Apoptosis - causes potential cancer cells to die
Cellular senescence - prevents their growth

Caretaker tumor suppressors are longevity assurance genes (e.g., BLM, WRN, TELOMERES)

Gatekeeper tumor suppressors can be antagonistically pleiotropic

ANTAGONISTIC PLEIOTROPY

(evolutionary hypothesis of aging)

What's good for you when you are young

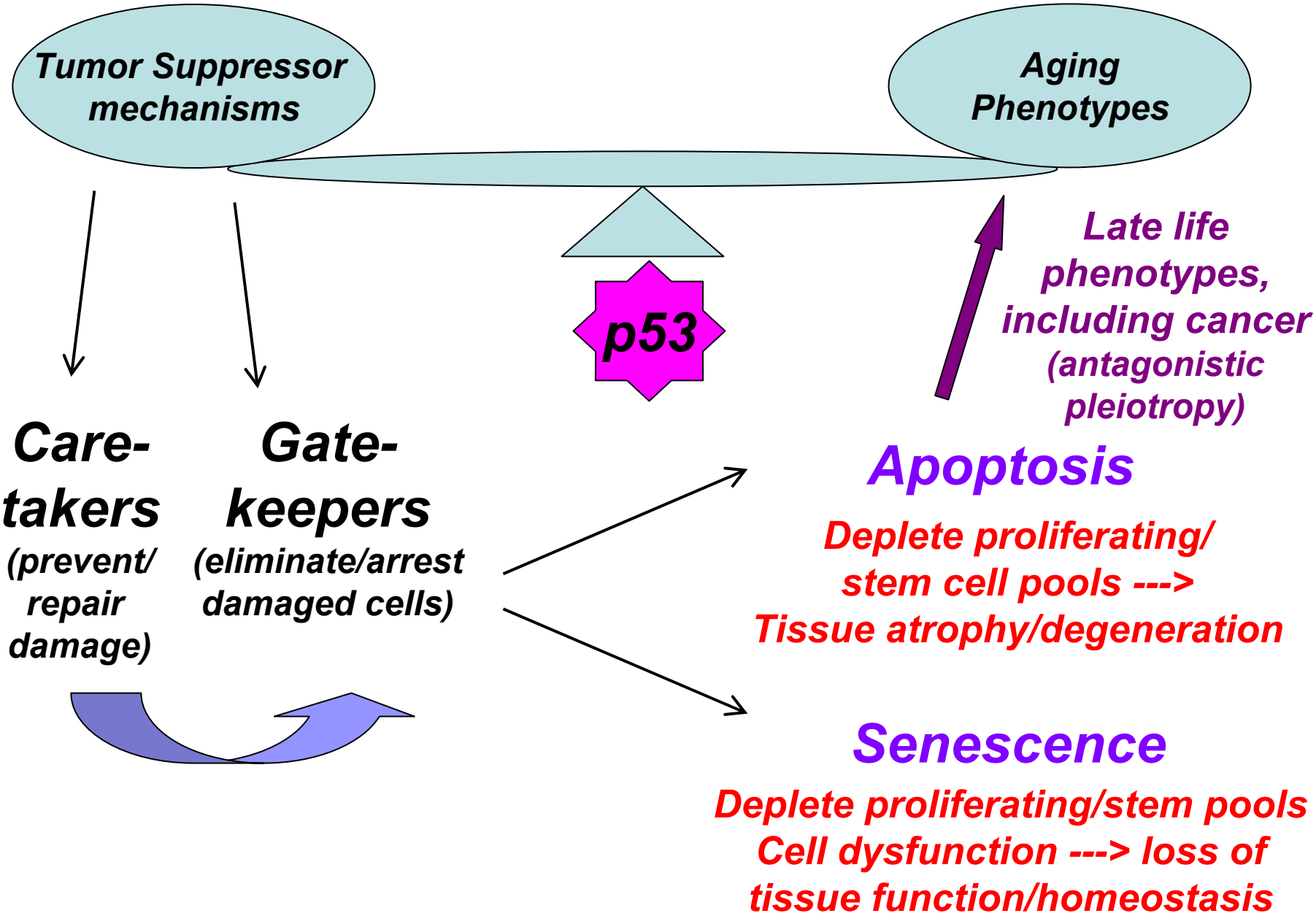
can be bad for you when you are old.

Why might gatekeeper tumor suppressors -- be antagonistically pleiotropic??

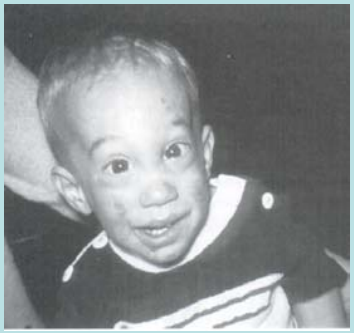
***APOPTOSIS** -- culls defective cells…..*
but deplete tissues of cells

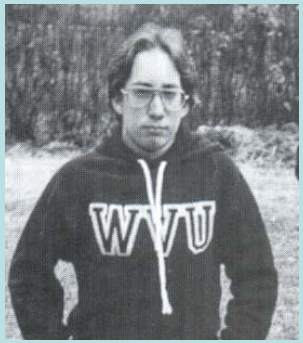
***CELLULAR SENESCENCE** -- arrests proliferation of defective cells …..*
but senescent cells are dysfunctional

Suppressing cancer costs -- aging



Bloom Syndrome





Life Span = 2-3 decades

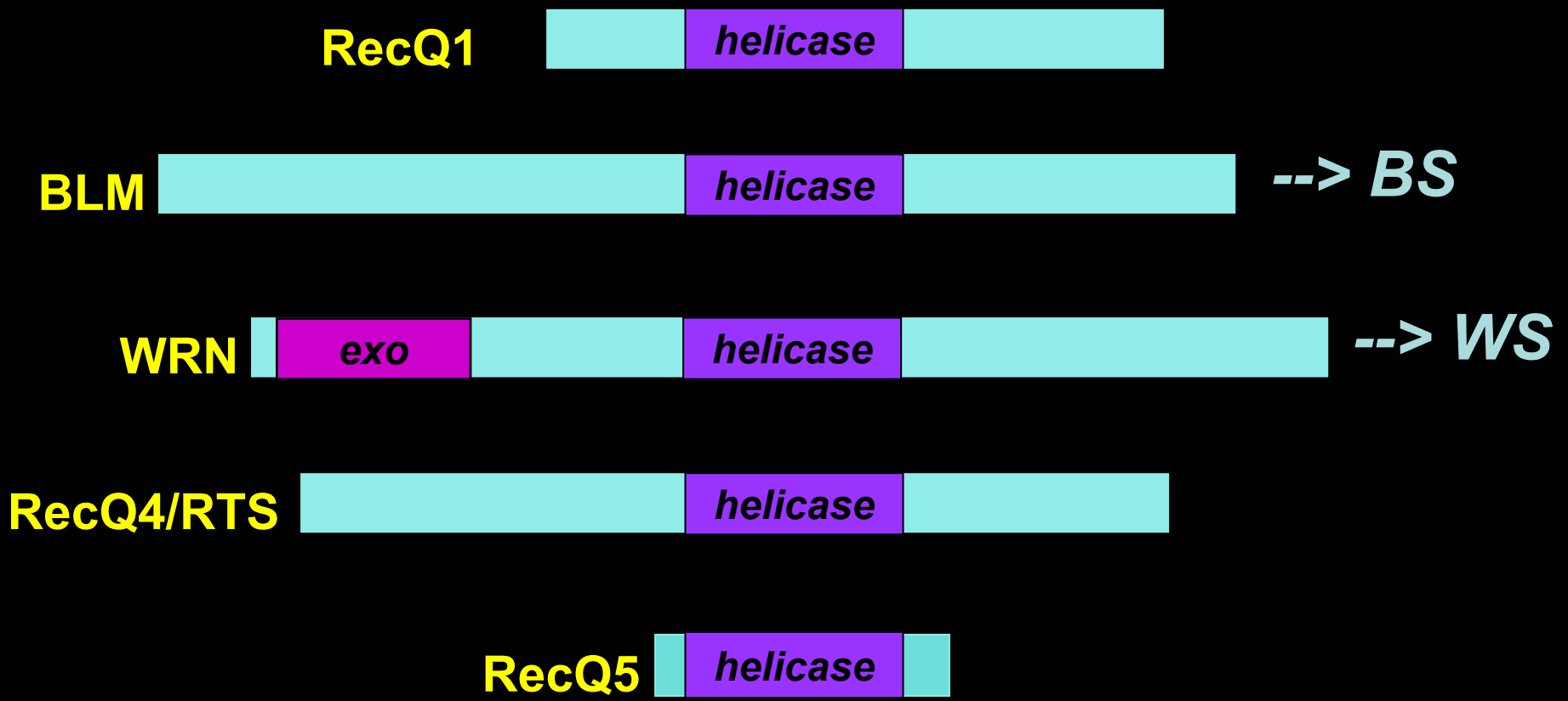
Small from birth
**Cancer prone (age-type)*
Sun-sensitive skin rash
Immune-deficient
Type II diabetes
Male infertility

Werner Syndrome

Life Span = 4-5 decades

Normal until puberty
**Premature aging*
Cancer prone (mesenchyme)
Cardiovascular disease
Type II diabetes
Cataracts

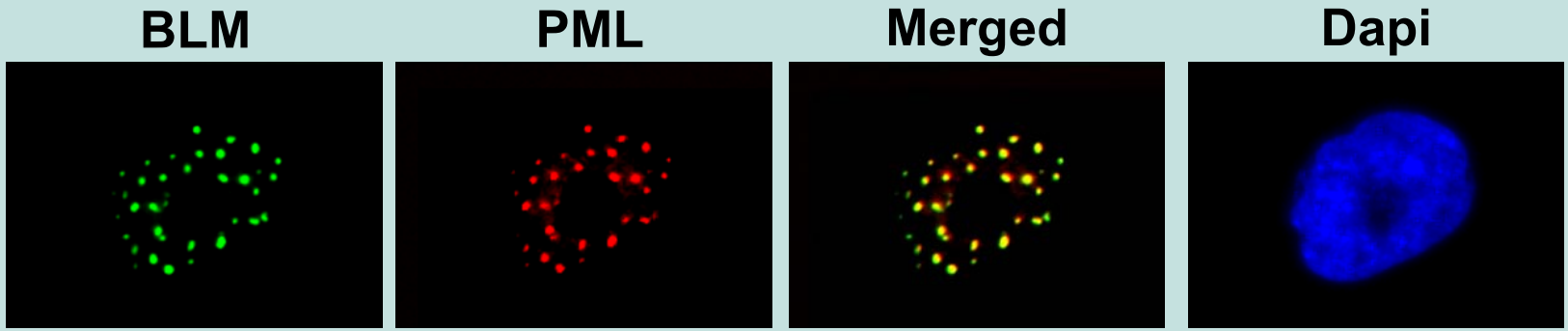
Human RECQ-like helicases



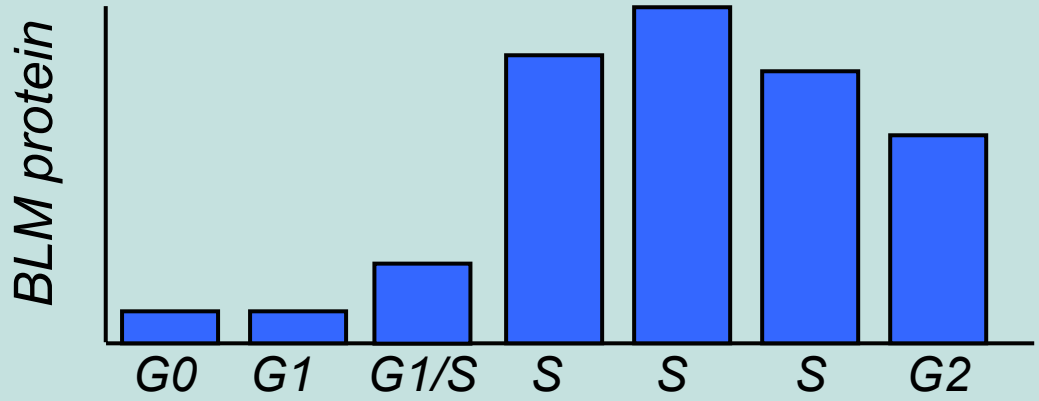
BLM, WRN
-- no obvious tissue-specificity
-- no obvious developmental specificity

What are the unique functions of
WRN and BLM that might account for
the unique phenotypes of WS and BS?

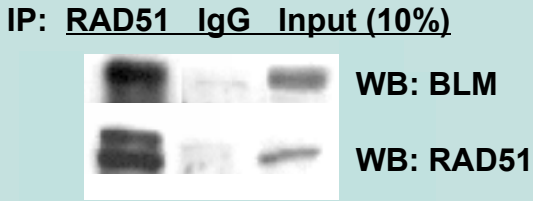
BLM localizes to PML bodies



BLM is expressed in S phase



BLM interacts with RAD51



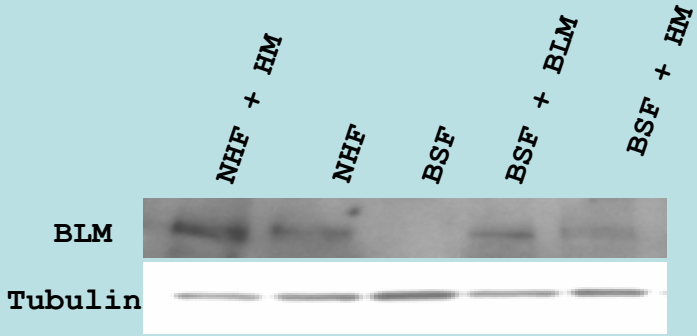
Role in homologous recombination

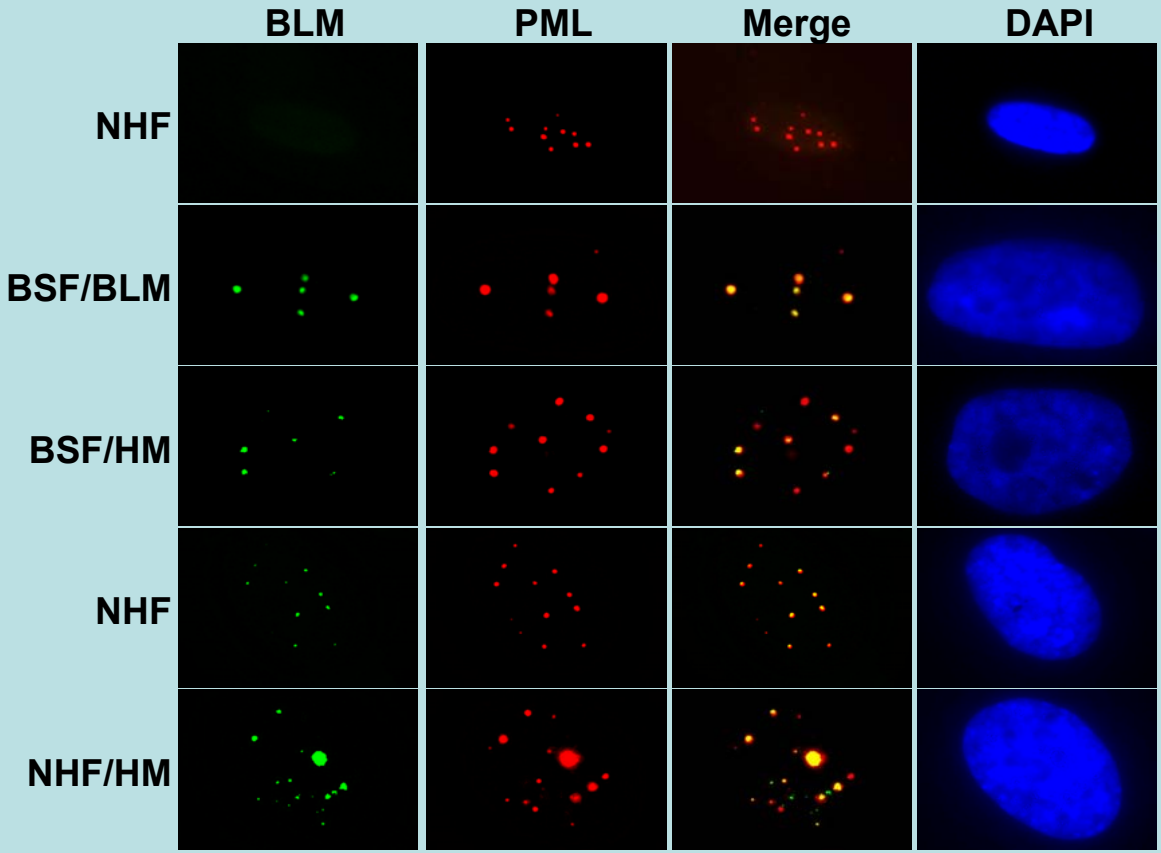
Bischof et al., J Cell Biol 153: 367 (2001)

Isogenic 'normal' human BS fibroblasts +/- BLM

'Normal' = telomerized

'Normal' = p53 checkpoints intact

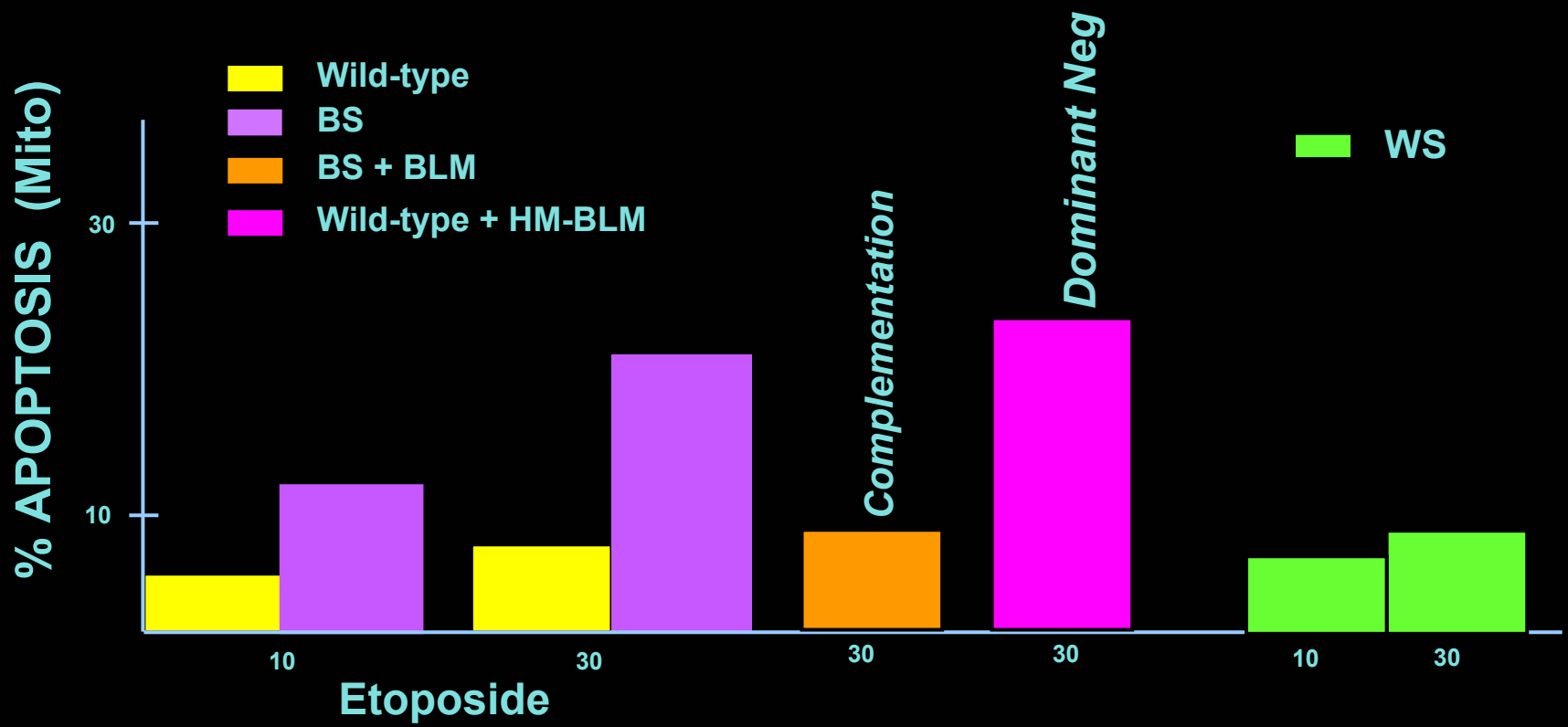




Davalos & Campisi, J Cell Biol 162: 1197 (2003)

BS cells are mildly hypersensitive to DNA damaging agents:

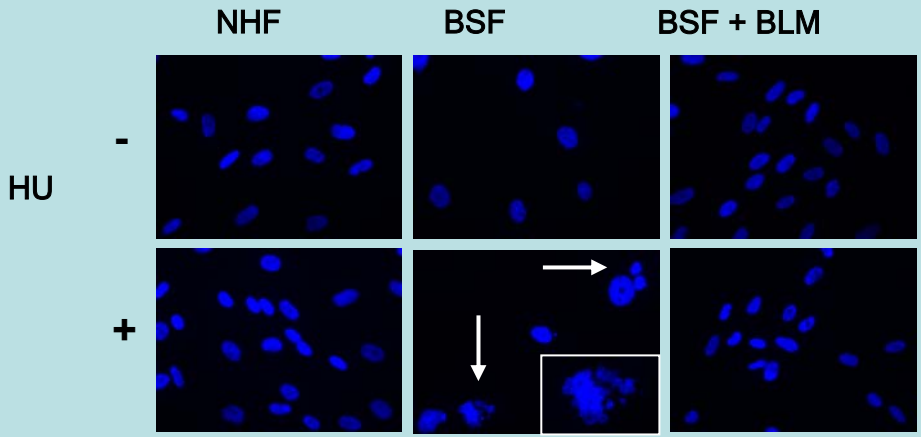
APOPTOSIS

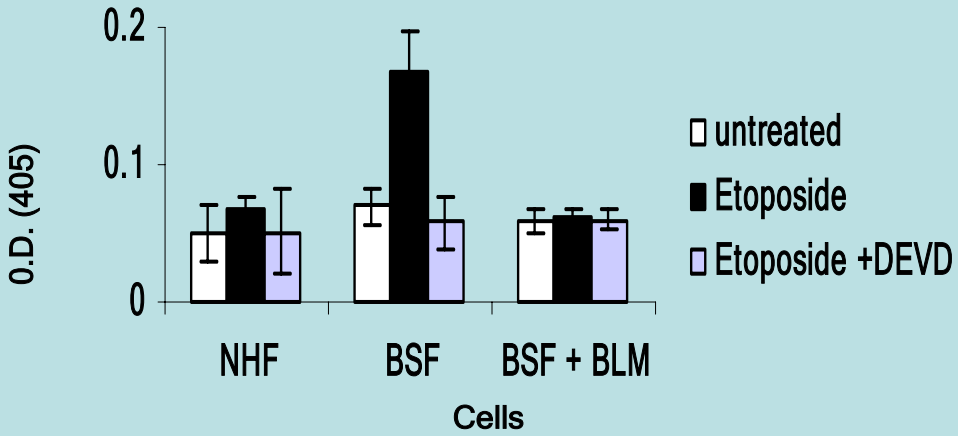


Also..Bleomycin, X-rays

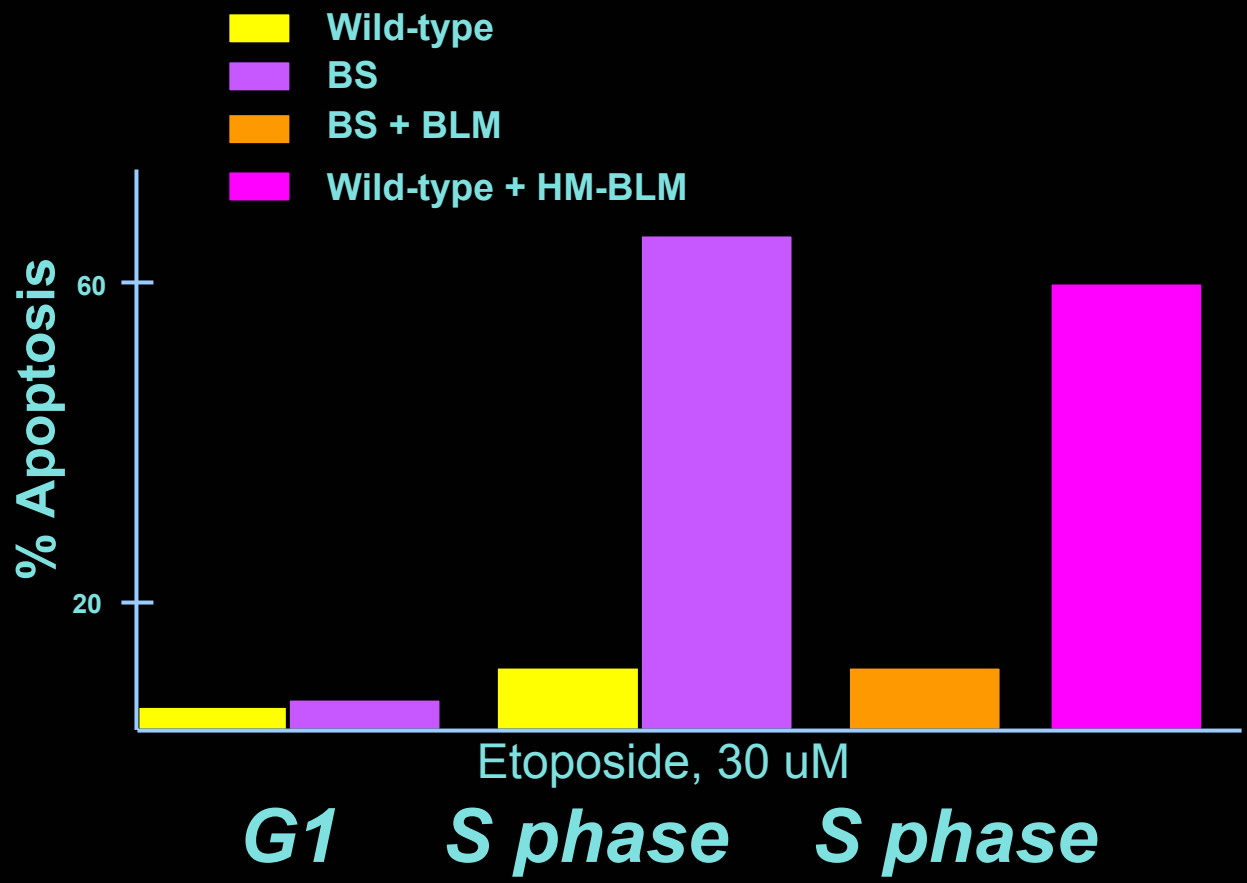
Exponentially growing cells

Damaged BS fibroblasts die by apoptosis



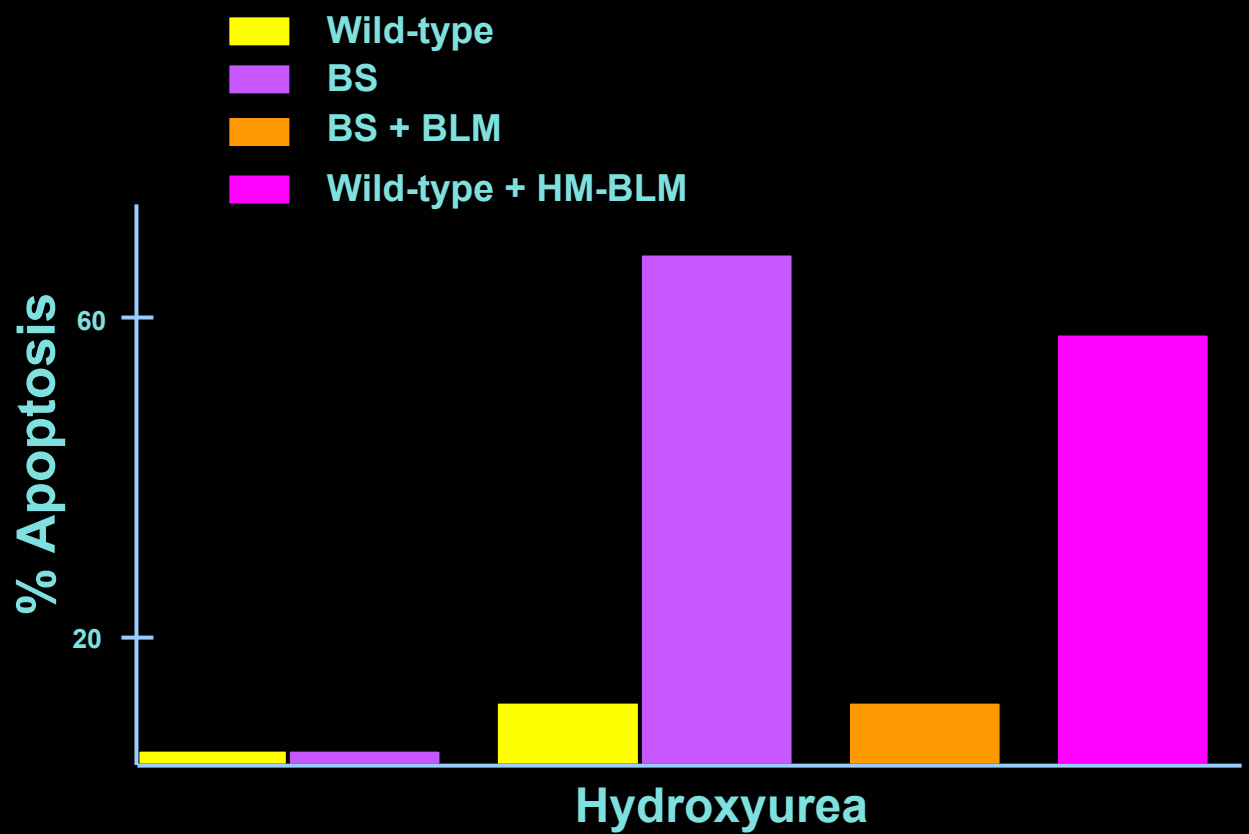


Hypersensitivity of BS cells confined to S phase



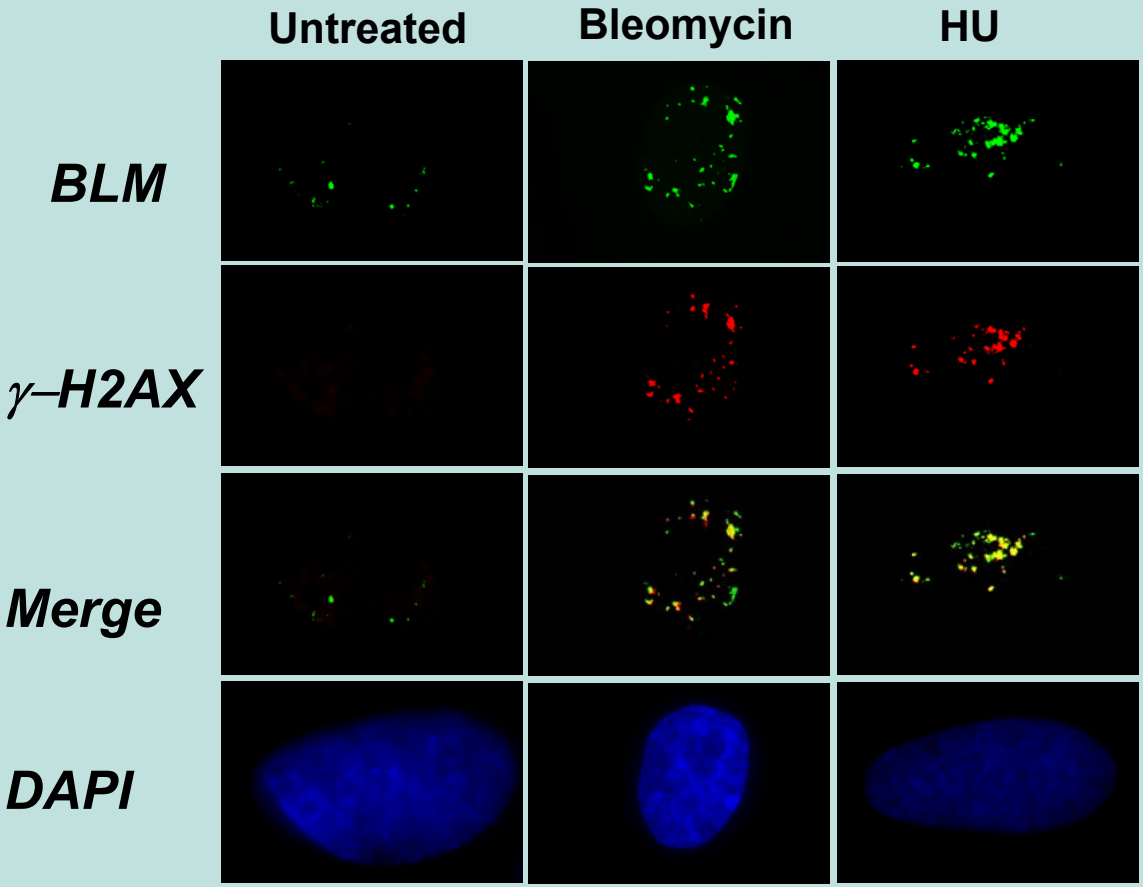
% Labeled Nuclei: <5% >80% >80%

Hypersensitivity of BS cells in S phase due to stalled replication forks



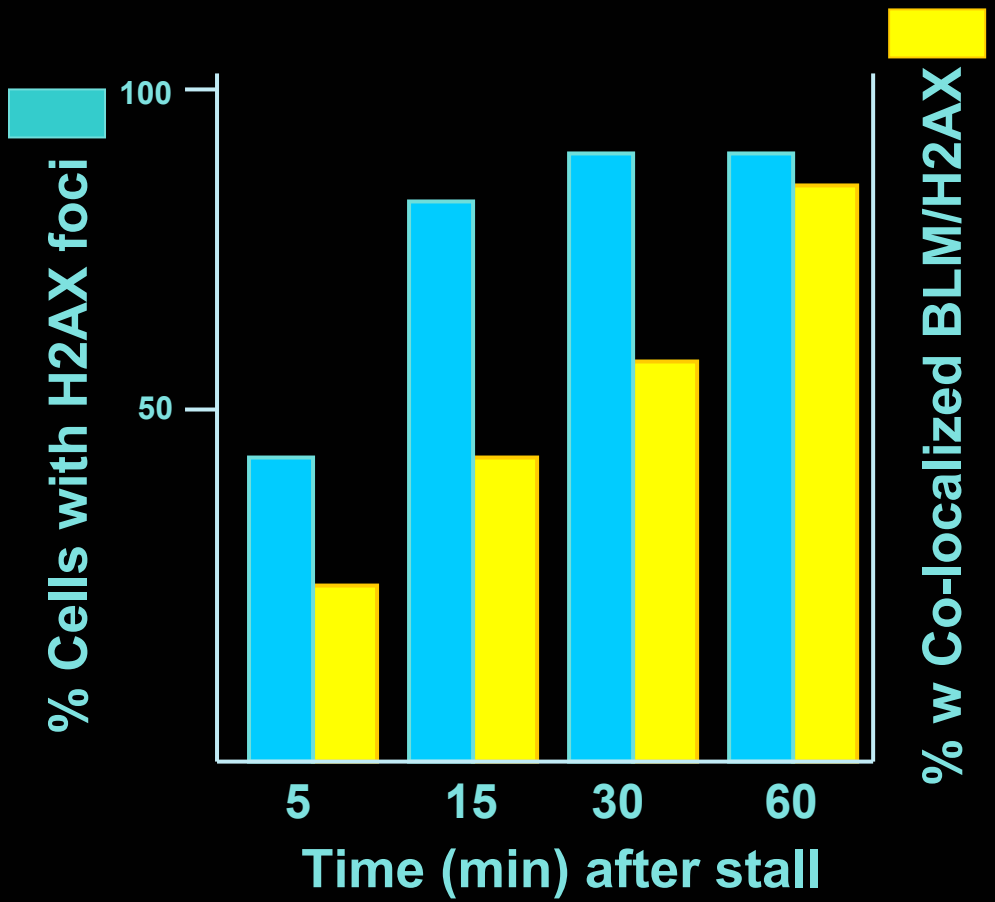
G1 *S phase* *S phase*

Stalled replication forks develop DSBs; BLM localizes to replication forks with DSBs

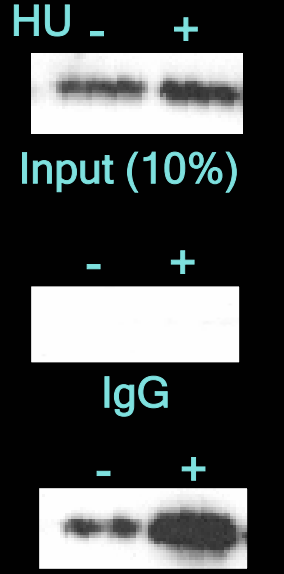


Co-localization: ***60-70%*** ***>95%***

BLM responds rapidly to damaged replication forks



Complex with γ-H2AX



BS cells undergo apoptosis at high frequency when replication forks stall

Endogenous oxidative damage continually stalls replication forks

BS cells are continually faced with conditions that favor cell death

Why are children with BS small?

BS children continually lose cells by apoptosis
(during embryogenesis and throughout life)

APOPTOSIS SUPPRESSES CANCER

Why are children with BS cancer prone (not progeroid)?

≈70% BS cells undergo apoptosis when replication forks stall

What happens to the survivors?

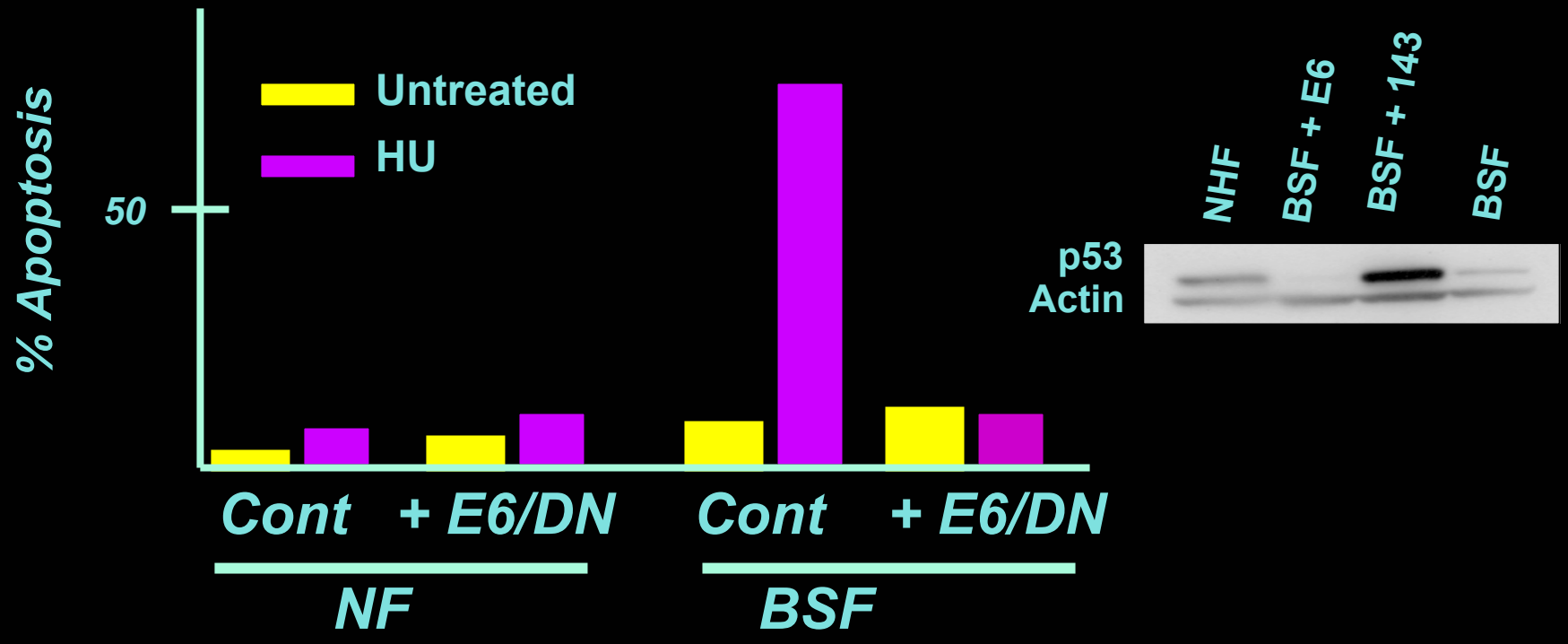
Synchronize survivors in S phase

+ HU:

20-30% survival

(Survival is stochastic)

Apoptotic death of BS cells is p53-dependent



p53 prevents survival of BS cells with damaged replication forks (cells at risk for faulty repair)

BS cells are under pressure to lose p53 function in order to survive damage during replication

Loss of p53 function + susceptibility to mutations ----> very high risk for developing cancer

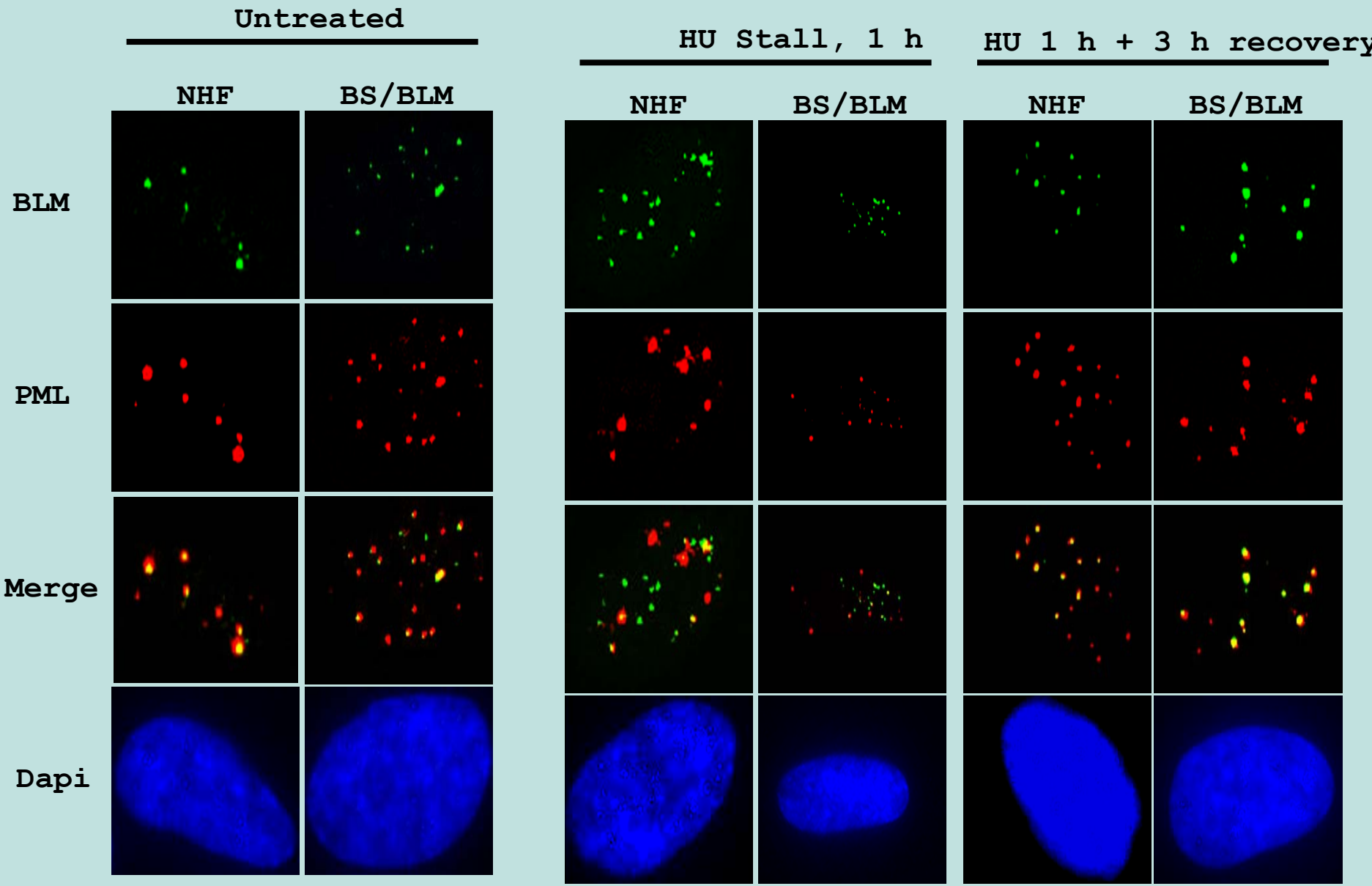
Children with BS die of cancer before high rate of apoptosis can produce progeroid symptoms

What is the function of BLM at damaged replication forks?

What is the role of p53?

Davalos et al., Cell Cycle 3: 1579 (2004)

BLM moves *transiently* from PML bodies to replication forks with DSBs

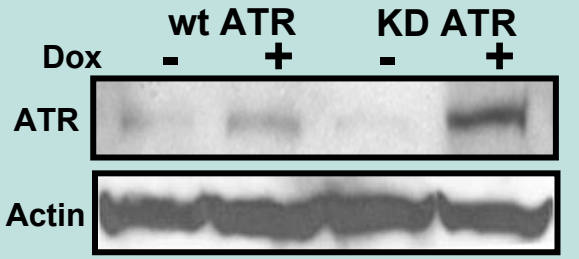


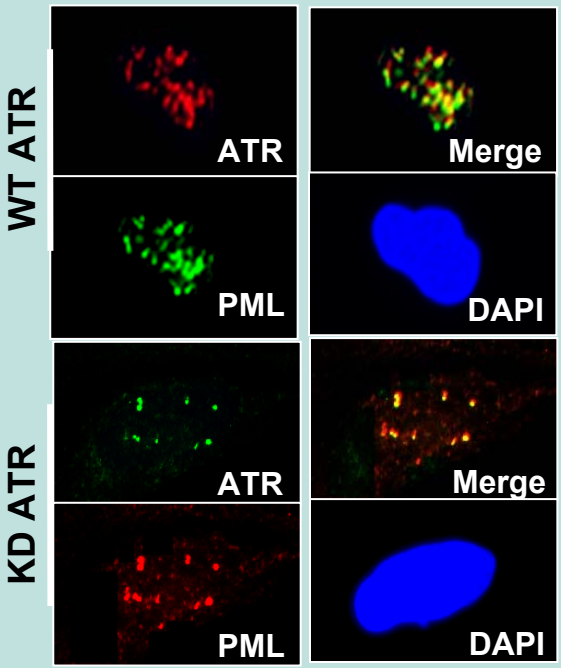
What regulates BLM movement from PML bodies to damaged replication forks?

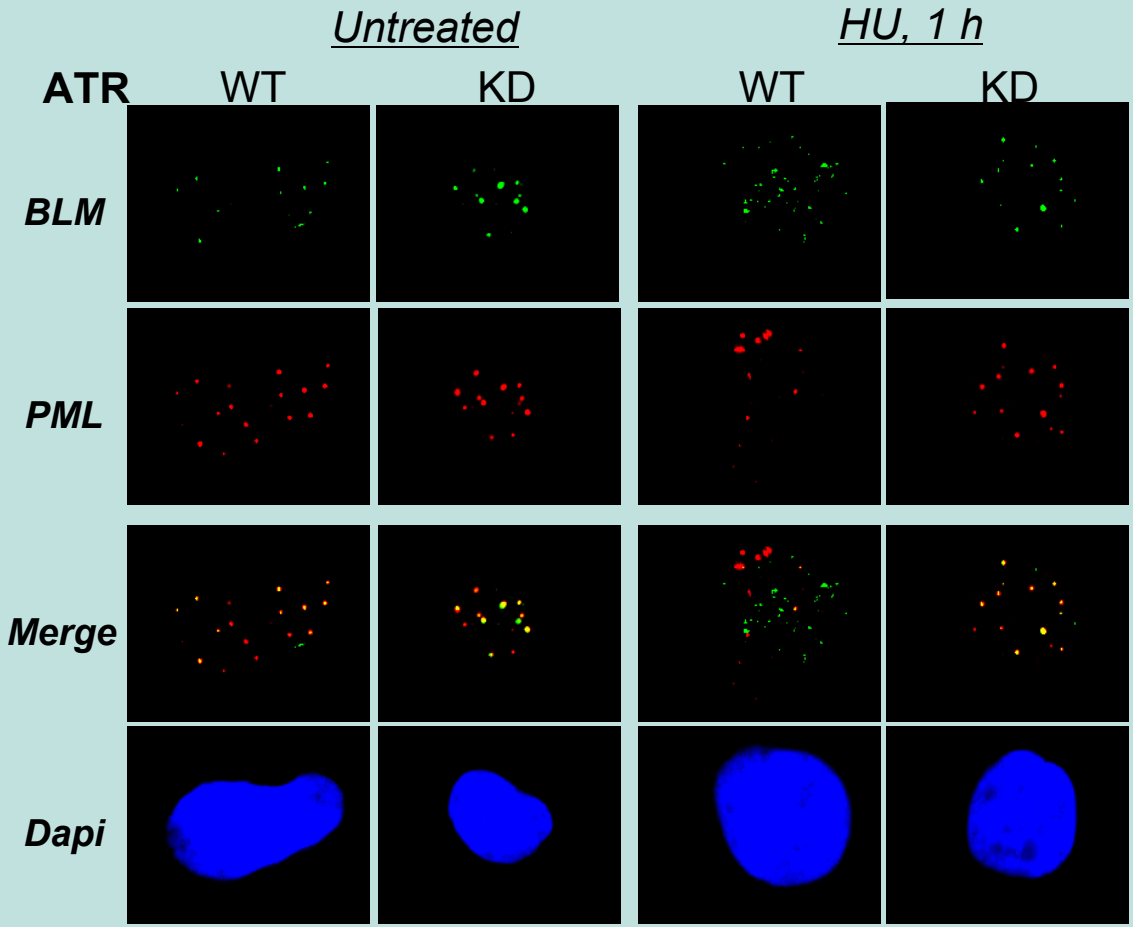
ATM, ATR damage signaling kinases?

ATR required for BLM translocation to damaged replication forks

ATR-deficient human Fb (TF) + dox-inducible flag-KD-ATR (dominant negative)
Primary NHF, WT or ATR-deficient (Seckle syndrome)

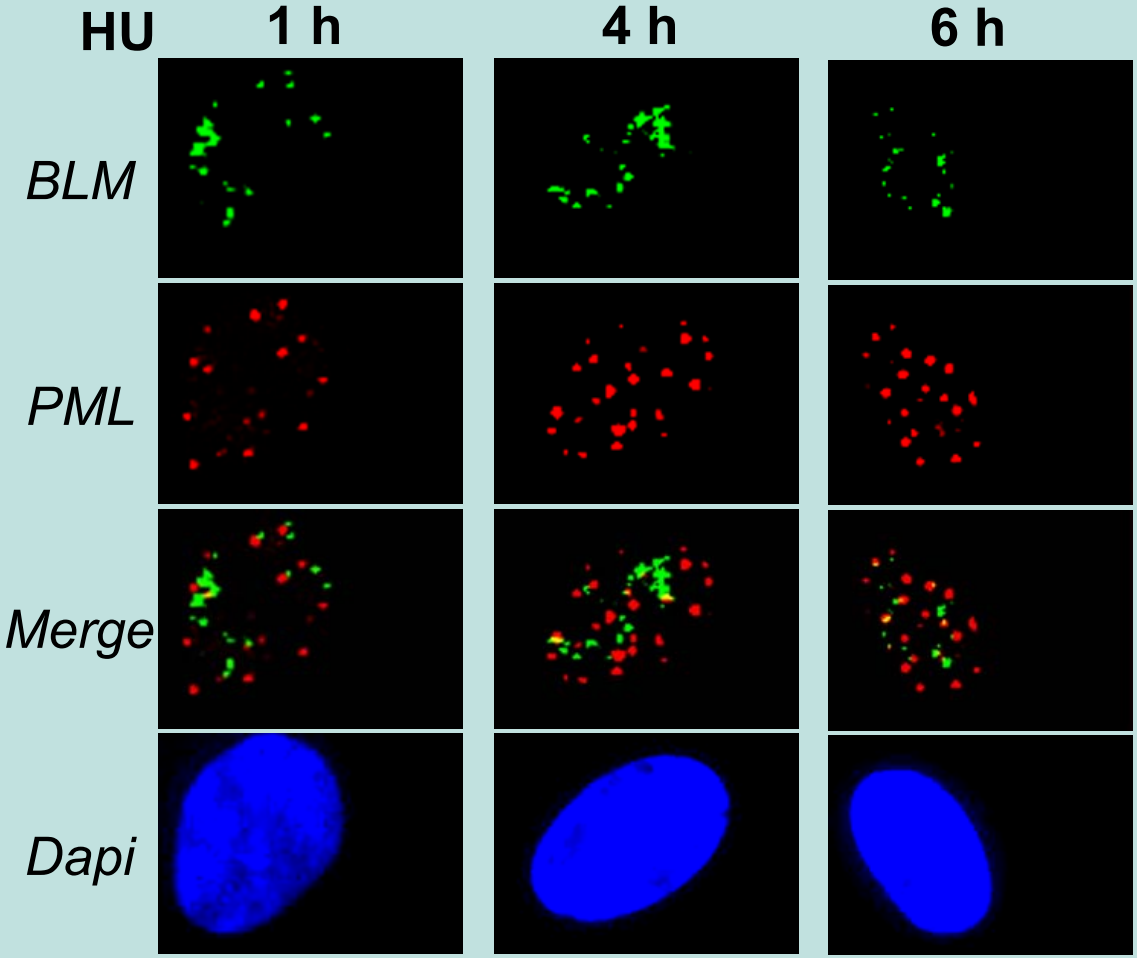






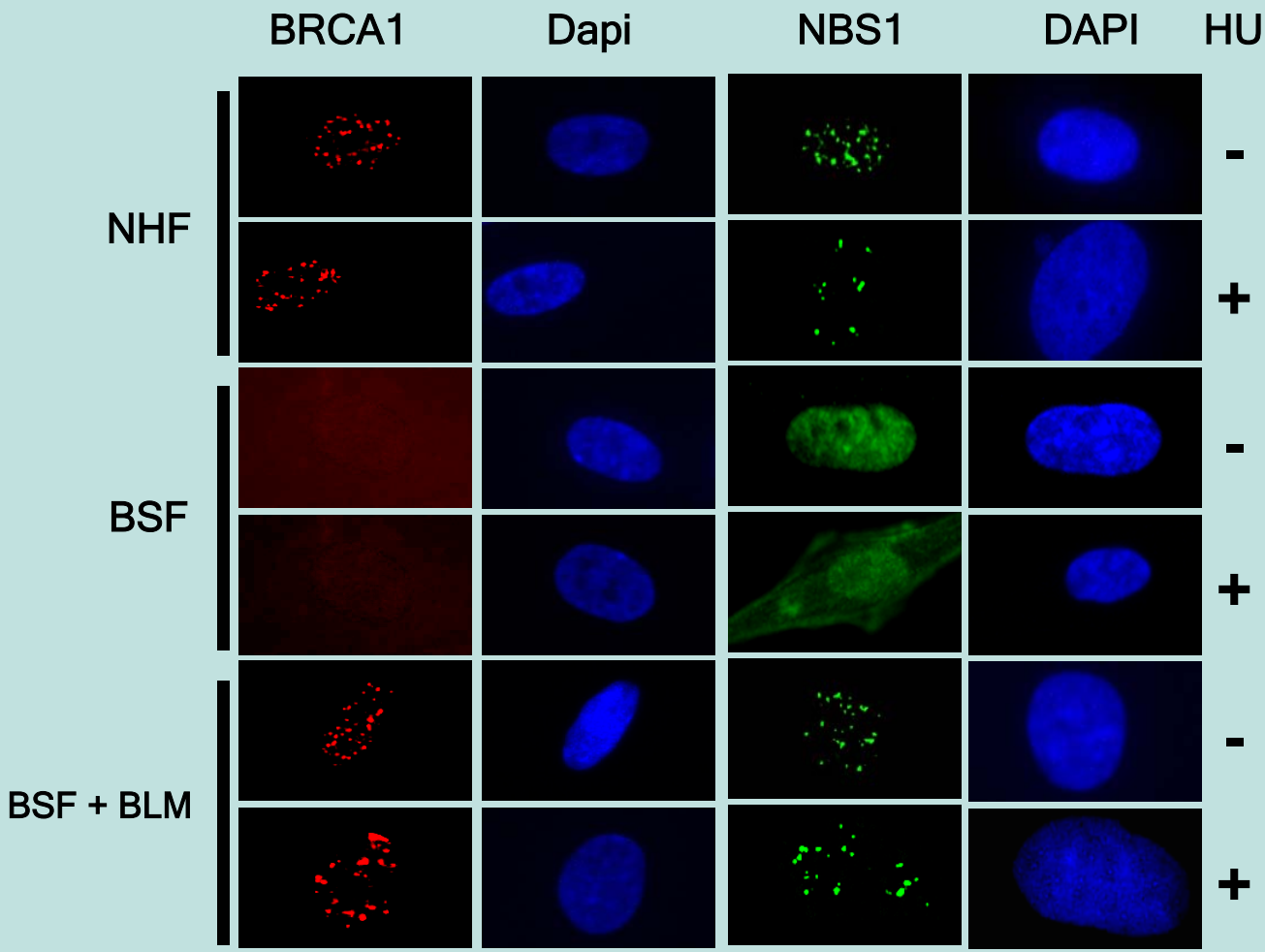
ATM required for BLM return to PML bodies

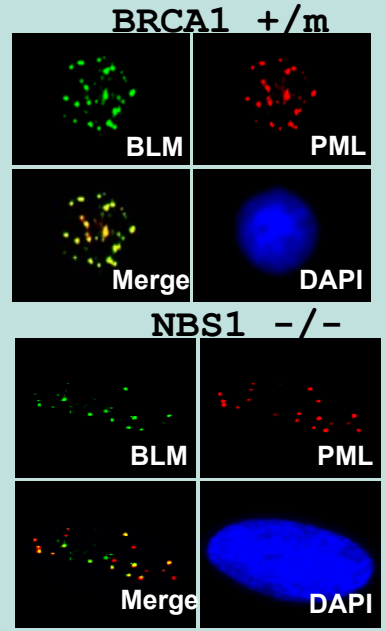
AT human fibroblasts, +/- telomerase

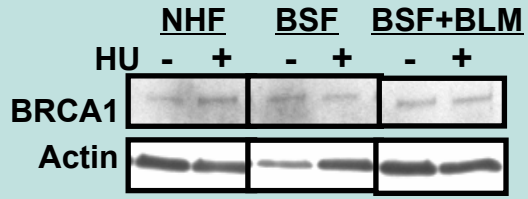


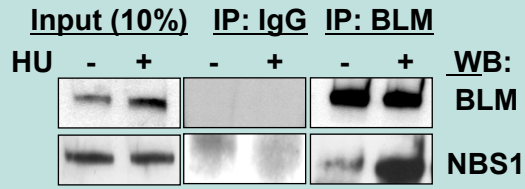
Structural function for BLM at damaged replication forks ?

BLM required for BRCA1, NBS1 recruitment to damaged replication forks

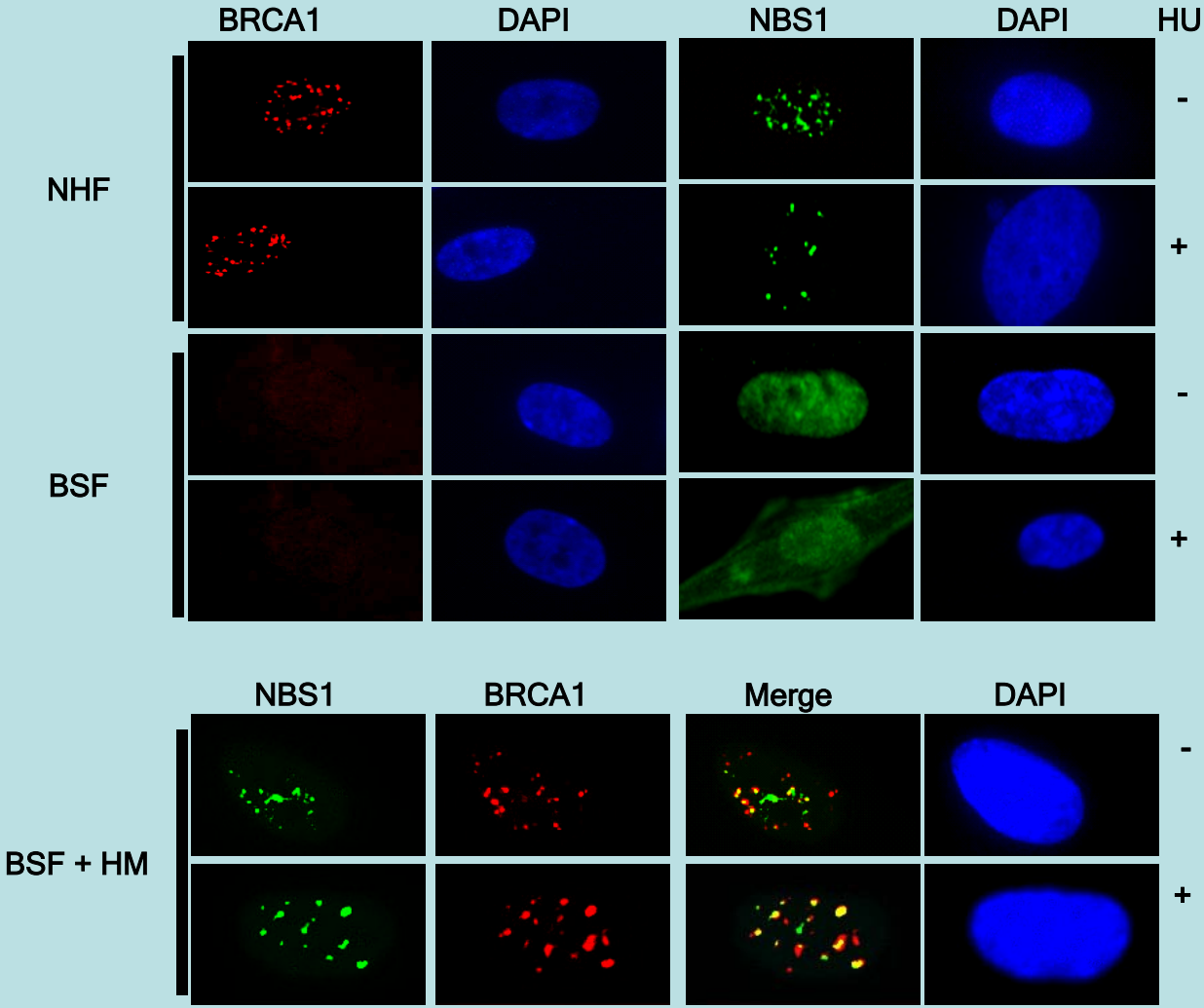




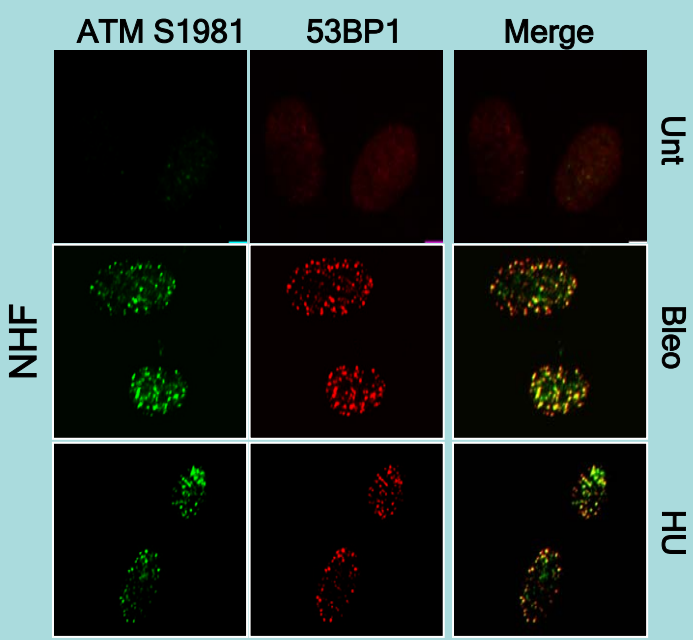


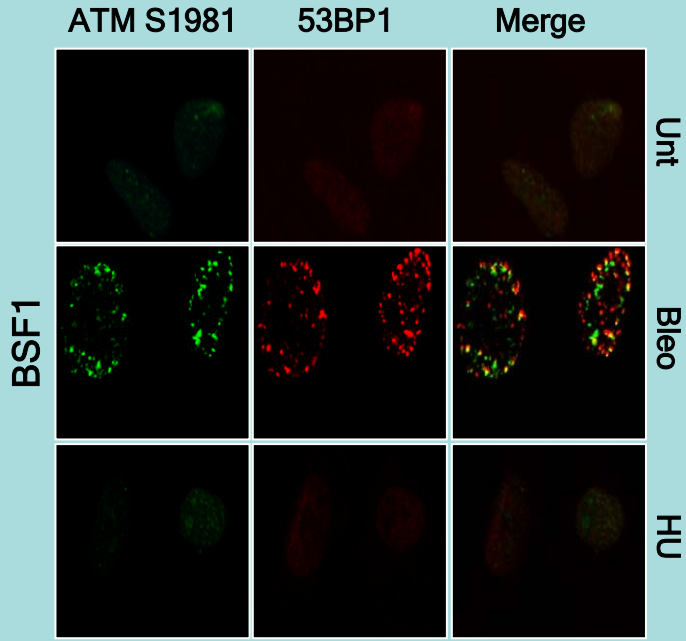


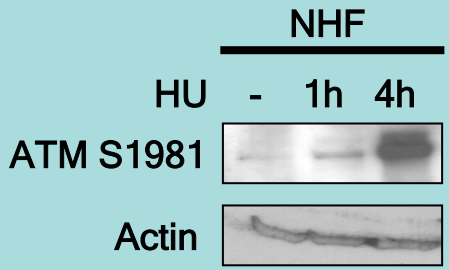
Catalytically inactive BLM restores BRCA1, NBSI localization to damaged replication forks

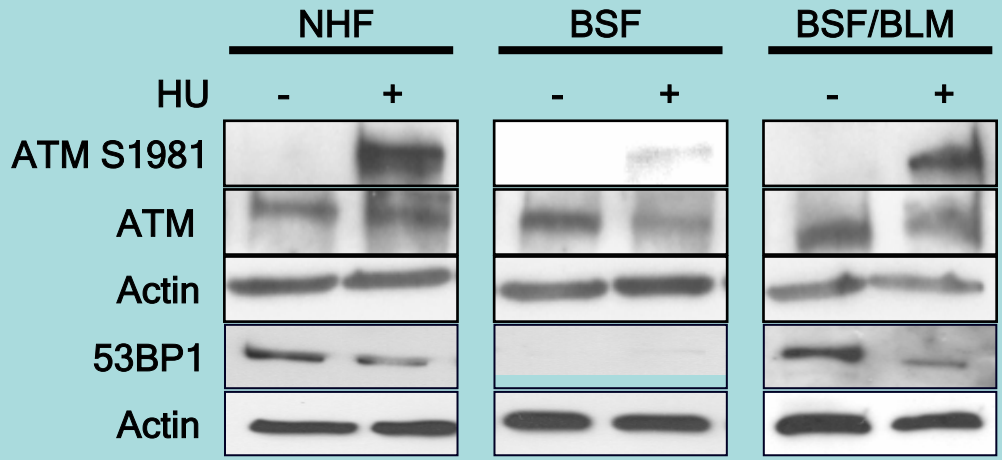


BLM required for 53BP1 recruitment and ATM activation



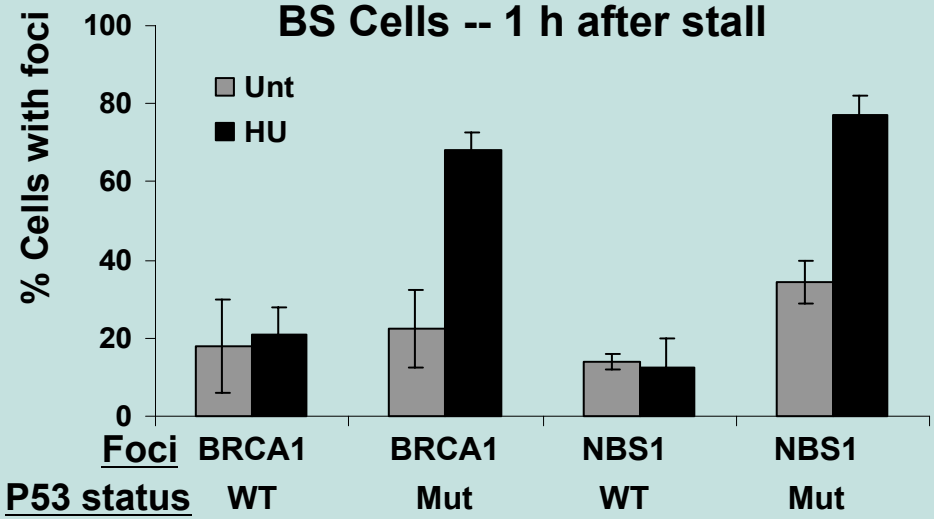


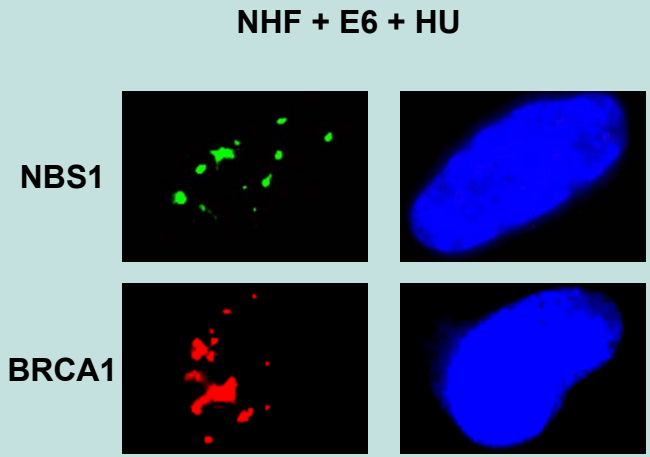


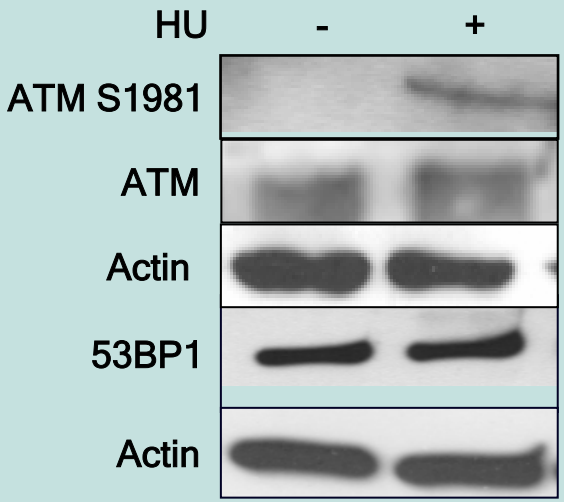


What is the role of p53 in BLM function?

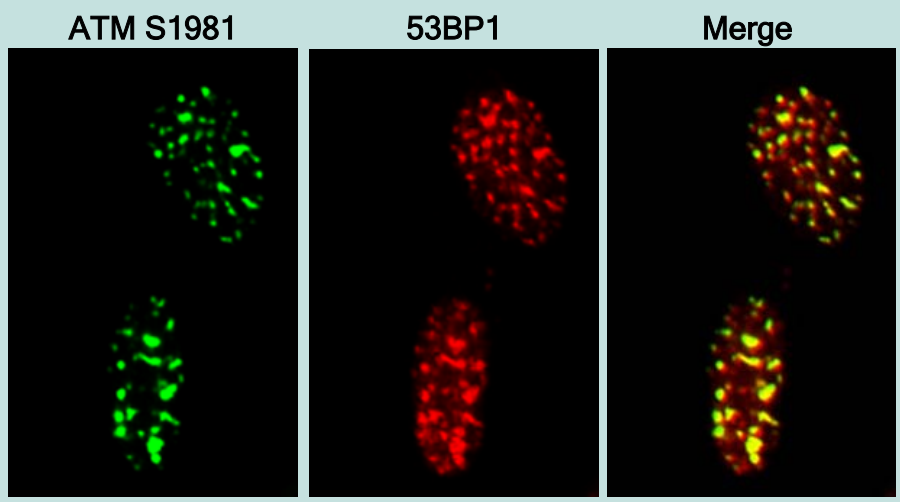
p53 prevents repair complexes from forming in absence of BLM







BSF + DN-p53



p53 orchestrates assembly of repair complexes at replication forks with DSBs (prevent error-prone repair?)

BLM is crucial for orchestrated, p53-dependent repair complex assembly

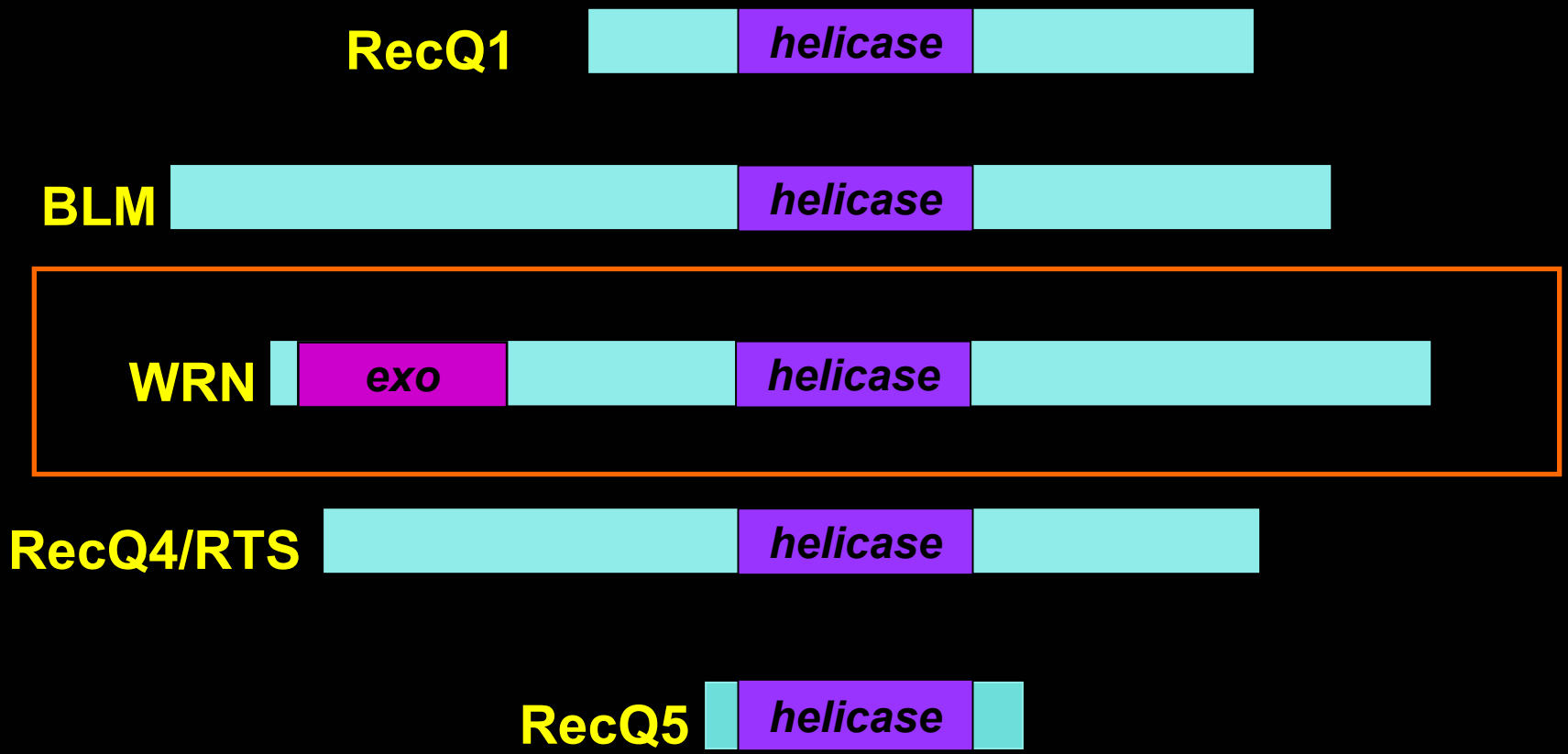
BLM + p53 deficiency ---> genomic instability, (cancer)

Werner Syndrome

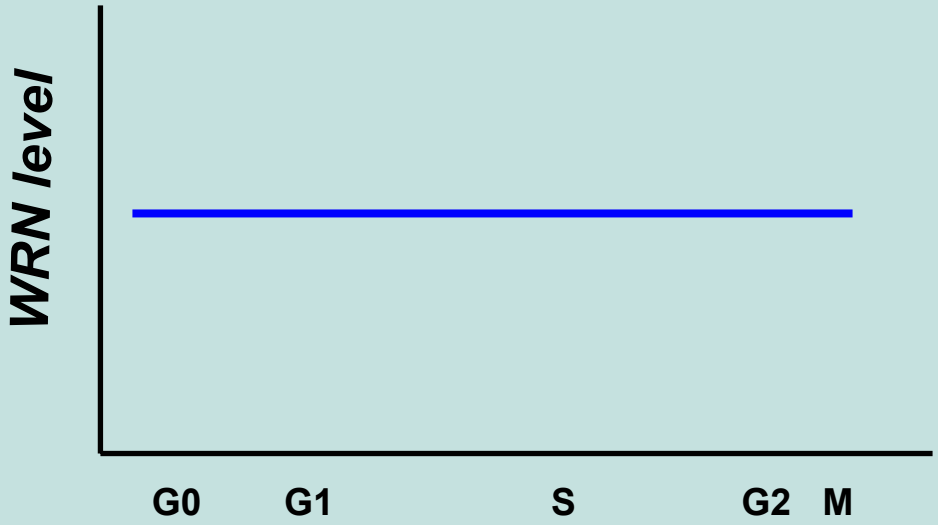
Life Span = 4-5 decades

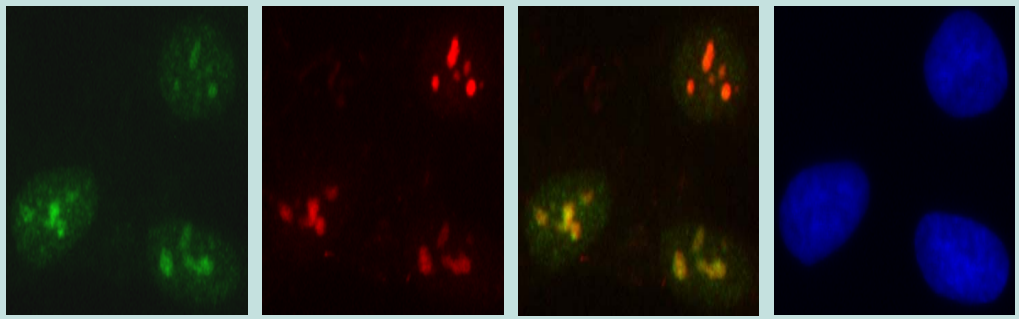
Normal until puberty
**Premature aging*
Cancer prone (mesenchyme)
Cardiovascular disease
Type II diabetes
Cataracts

Human RECQ-like helicases

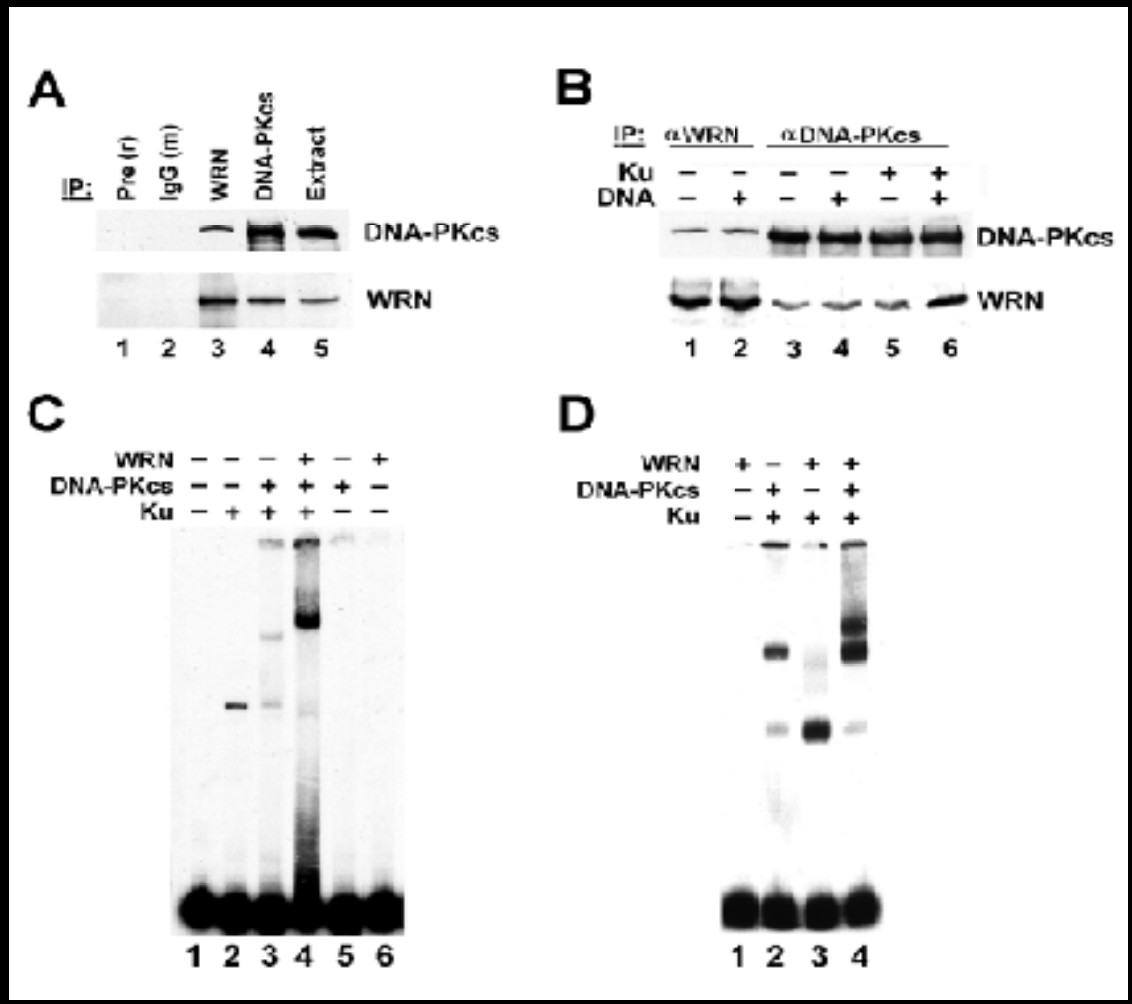


WRN is not cell cycle regulated; Localizes to nucleoli



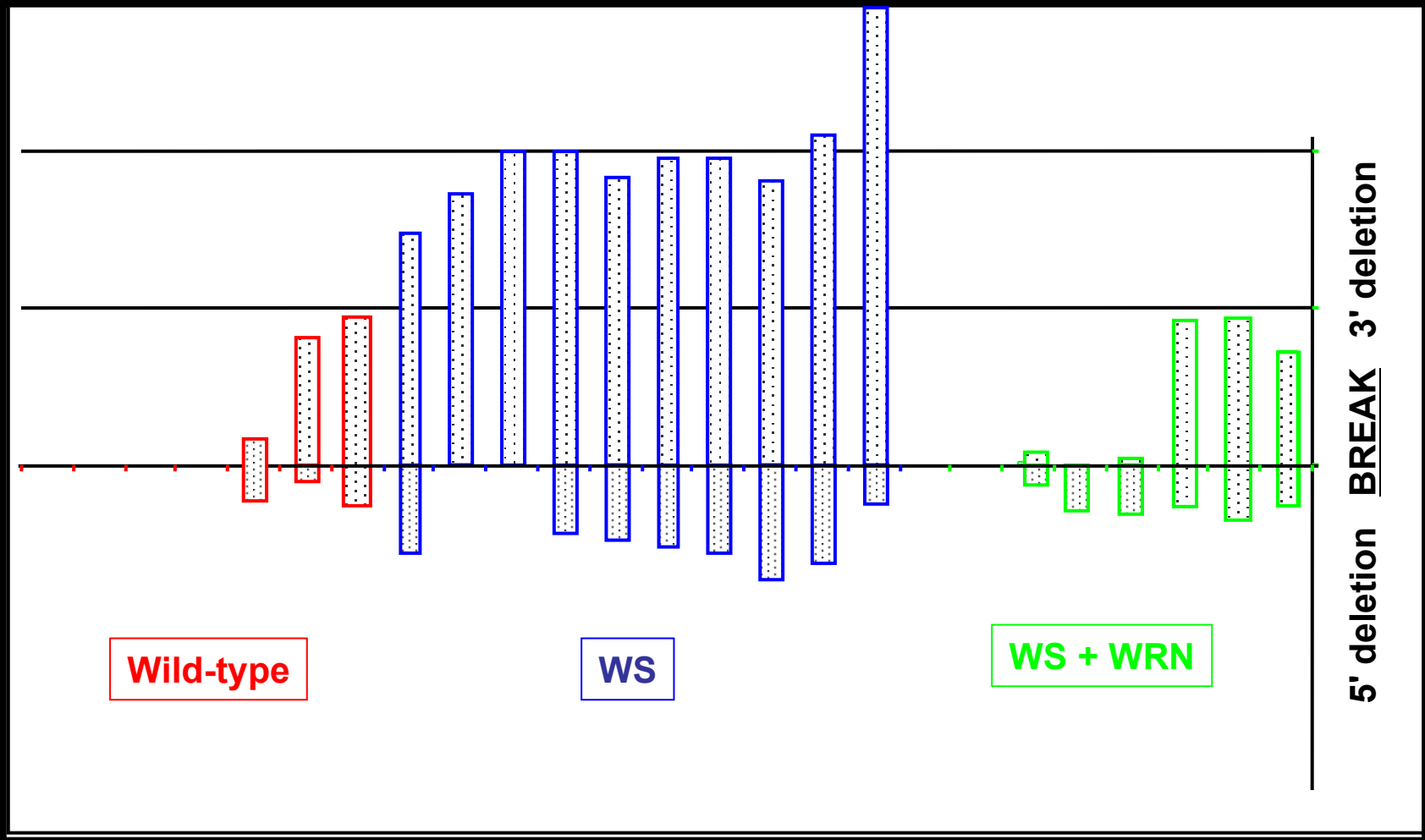


WRN interacts with components of NHEJ



Yannone et al., J Biol Chem 276: 38242 (2001)

WRN deficiency causes extensive 3' deletion at non-homologously joined ends



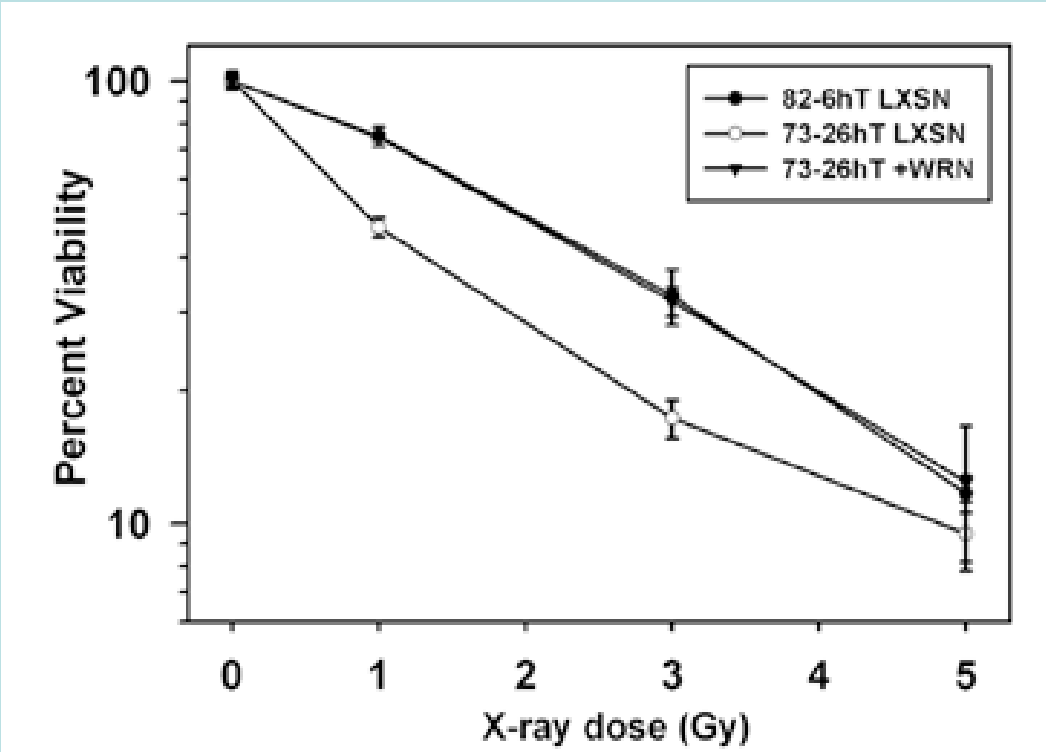
Oshima et al., Cancer Res 62: 574 (2002)

WRN can interact with NHEJ components and participates in NHEJ reactions

NHEJ requires a helicase to search for microhomology and exonuclease to process broken ends

Cells defective in NHEJ are extremely sensitive to IR
WS cells are mildly IR-sensitive

WS cells are mildly X-ray sensitive



Under what circumstances is the WRN-NHEJ interaction relevant?

Dysfunctional telomeres resemble DNA DSBs, substrates for NHEJ

WRN deficiency accelerates senescence

(in contrast to BLM deficiency, which increases apoptosis)

(premature accumulation of senescent cells --> loss of tissue function & homeostasis, promotes late life cancer)

Abrogated by telomerase (epithelial stem cells)

Thanks!

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***Collaborators**: Sandy Chang (MDAnderson), David Chen/Steve Yannone (LBNL), Nathan Ellis (Sloan-Kettering), Junko Oshima (U WA), Robert Shiestl (UCLA)*