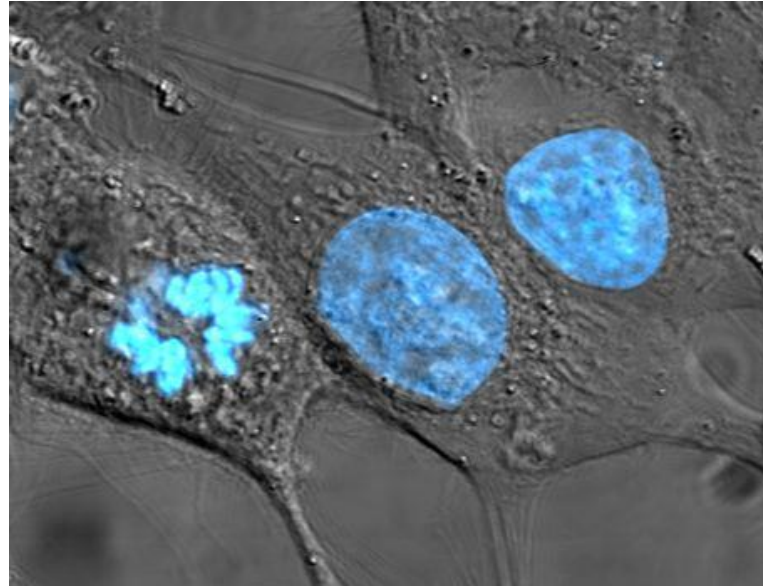


**Understand the mechanisms which prevents carcinogenesis
by studying a family
of caretaker tumor suppressors**

**28th Mid Year Meeting
Indian Academy of Sciences
Faculty Hall, Indian Institute of Science
Bengaluru, 30th June to 1st July, 2017**

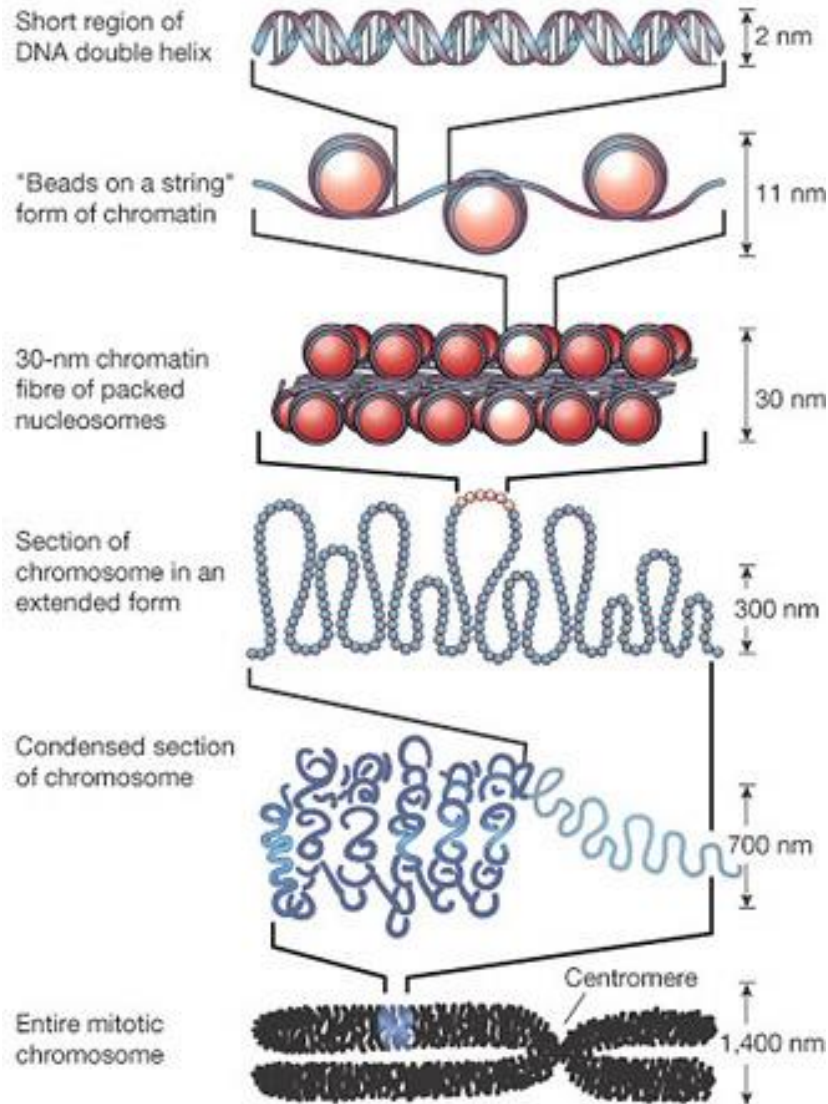
**Sagar Sengupta, Ph.D.
National Institute of Immunology,
New Delhi, India**

Sub-cellular organelles

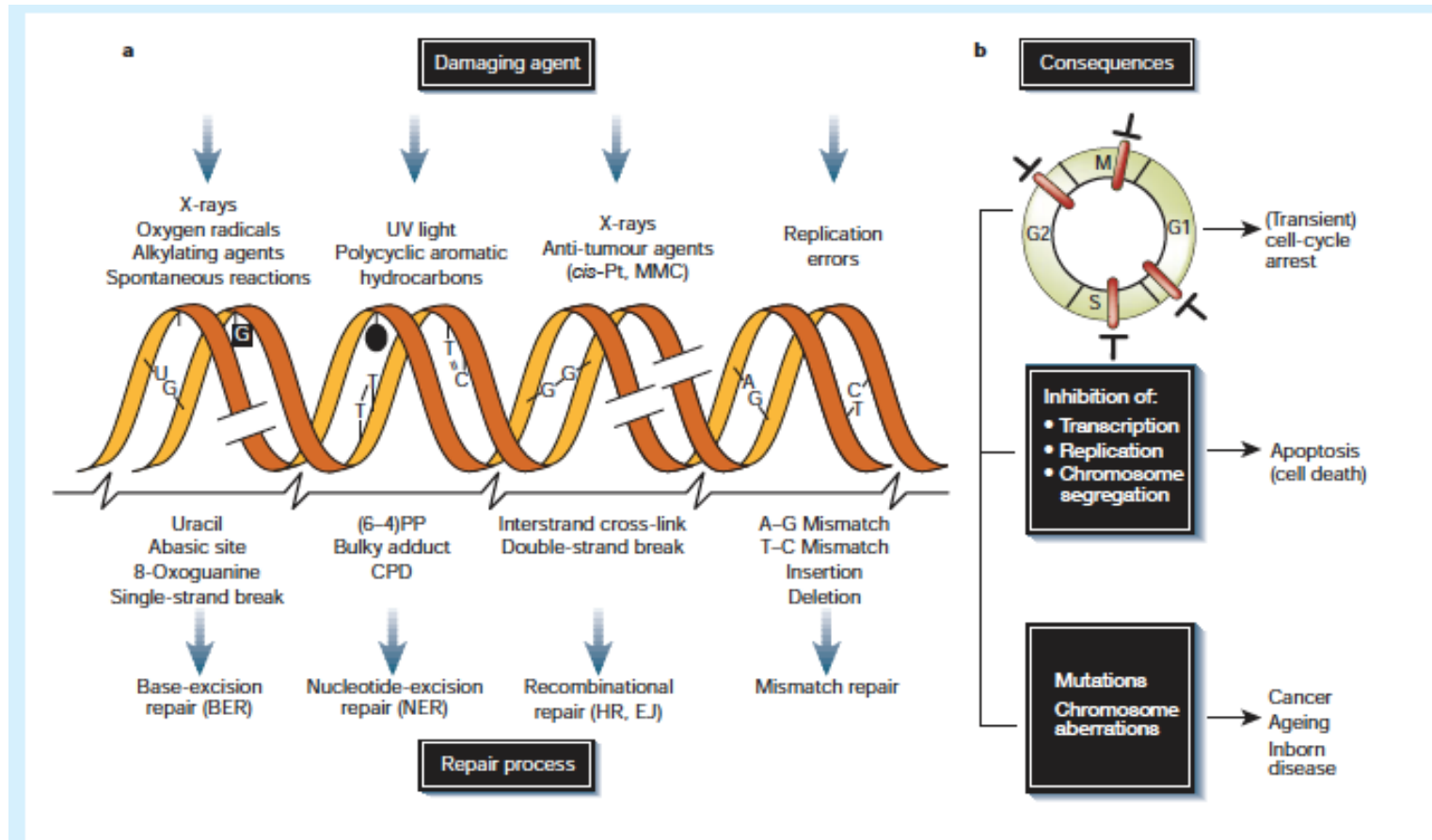


Intracellular Compartment	Percent of Total Cell Volume	Approximate Number per Cell
Cytosol	54	1
Mitochondria	23	1700
Rough and smooth ER cisternae	12	1
Nucleus	8	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

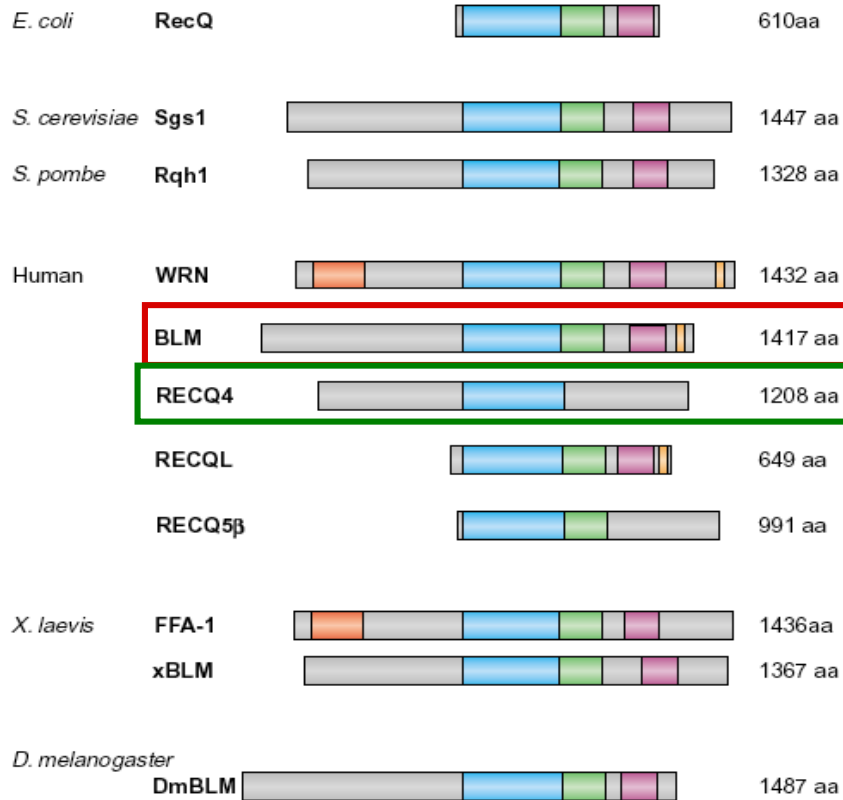
Higher order chromatin organization



DNA damage accumulation in cells lead to chromosomal instability

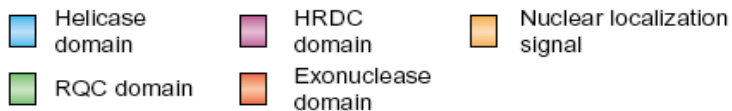


RecQ helicase family



- Mutations in BLM, RECQL4 and WRN cause cancer predisposition syndromes in human
 - Bloom Syndrome
 - Rothmund Thomson Syndrome
 - Werner Syndrome

Key:



TRENDS in Cell Biology

Bloom Syndrome: a cancer prone disorder

Clinical Symptoms:

- Type 2 diabetes
- Proportional dwarfism
- Sun induced erythema
- Male infertility, female subfertility
- Immune deficiency
- Early onset cancer pre-disposition
- BS patients predisposed to all types of cancer



BLM interact with proteins involved in

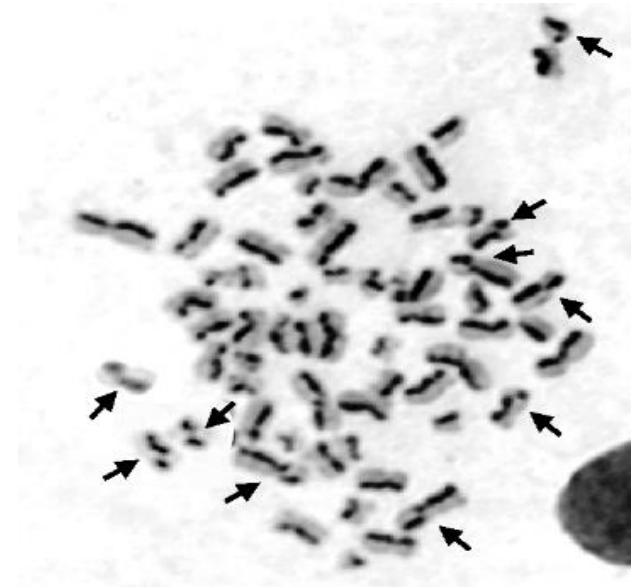
- Genome surveillance
- Homologous recombination
- Tumor suppressors

BLM functions in

- DNA damage sensing
- DNA repair and recombination

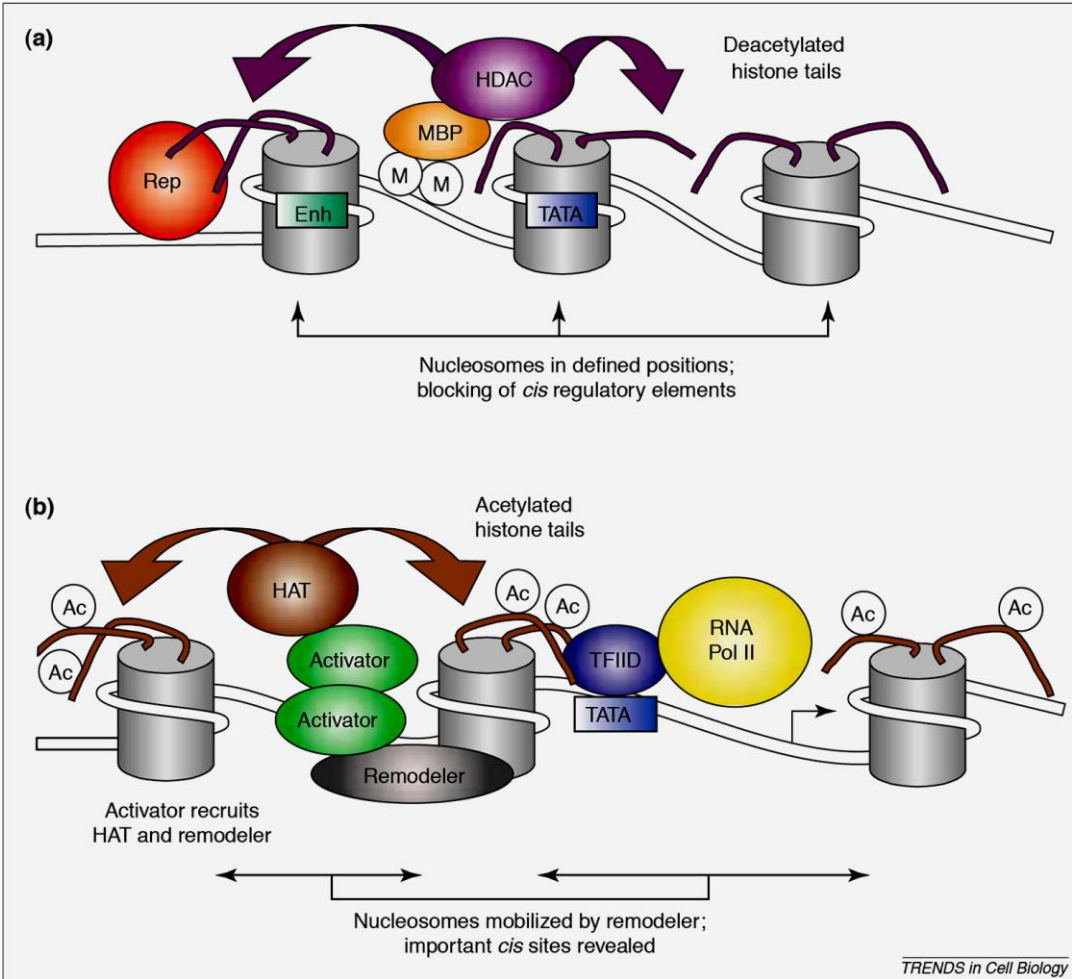
Cellular Phenotypes

- Micronuclei formation
- Quadriradials
- Increase in endogenous levels of DNA damage
- Hyperrecombination (Sister Chromatid Exchange)

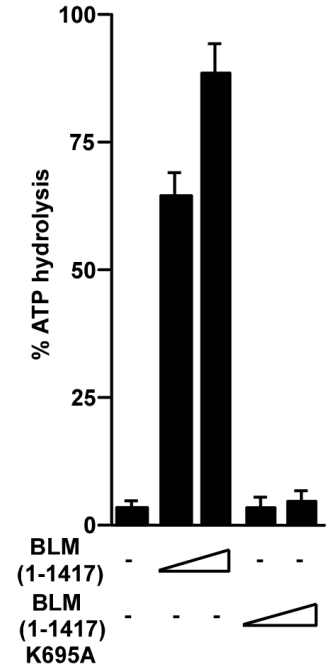
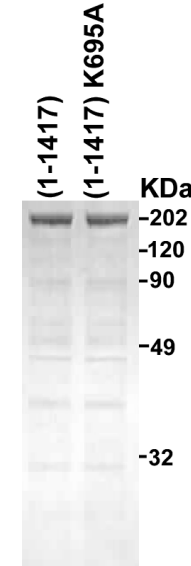
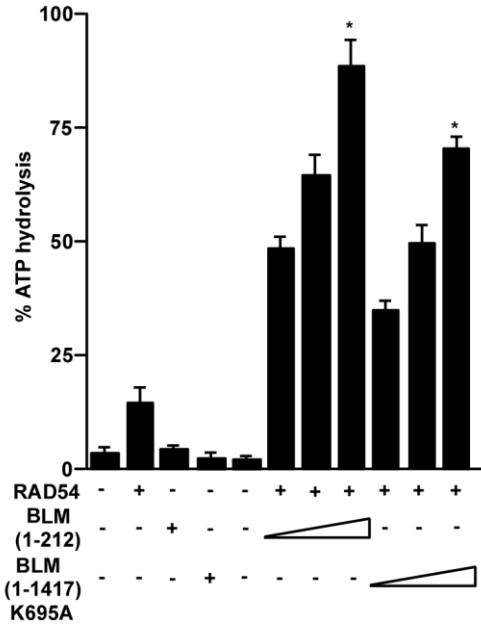
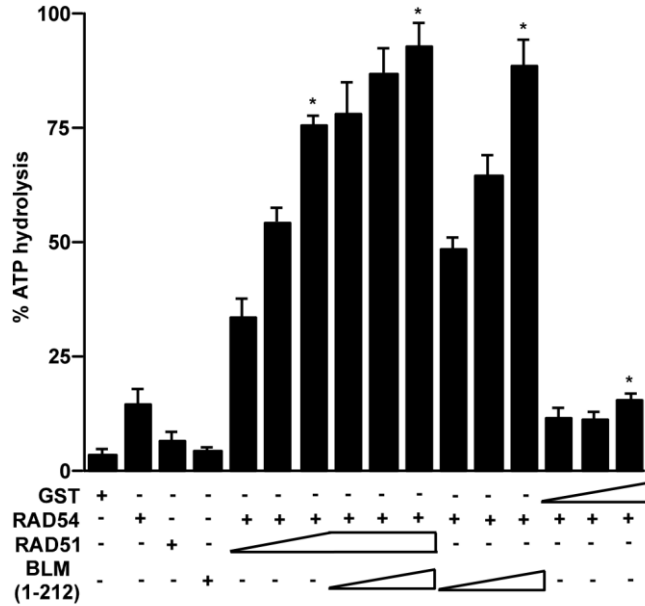


Does BLM have a role in remodeling the chromatin?

Repressed and active chromatin

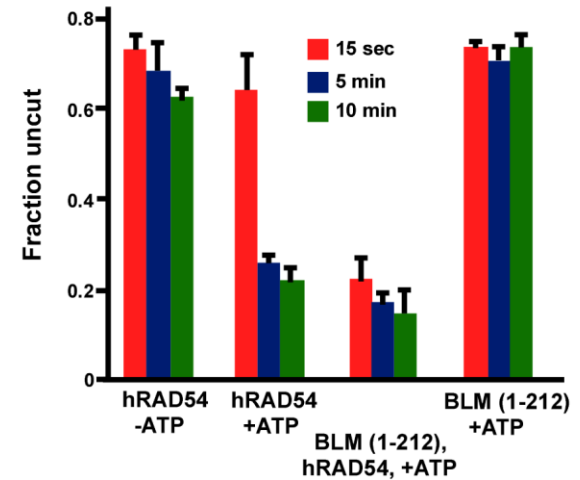
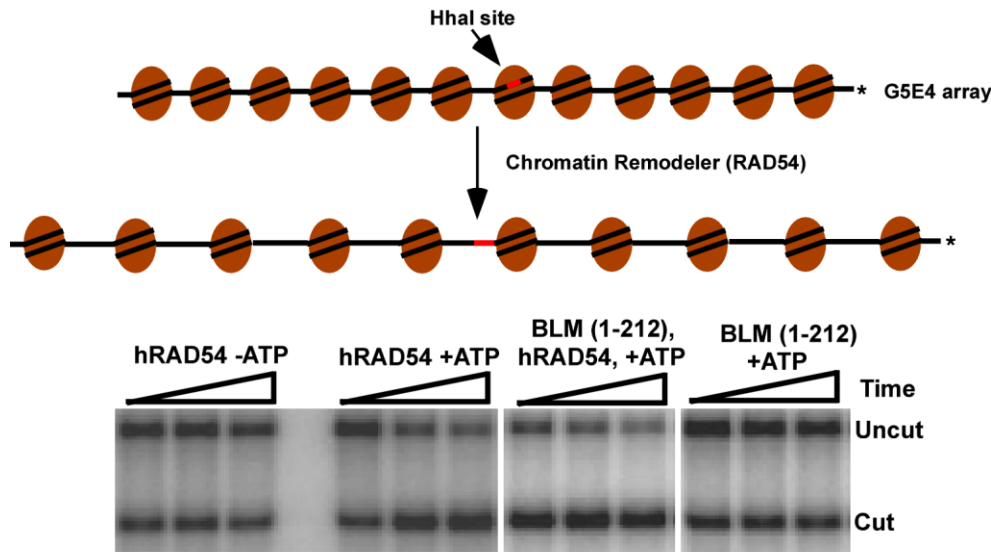


BLM stimulates the ATPase activity of RAD54



Does BLM stimulate the ATP hydrolysis dependent chromatin remodeling activity of RAD54?

BLM stimulates chromatin remodeling activities of RAD54

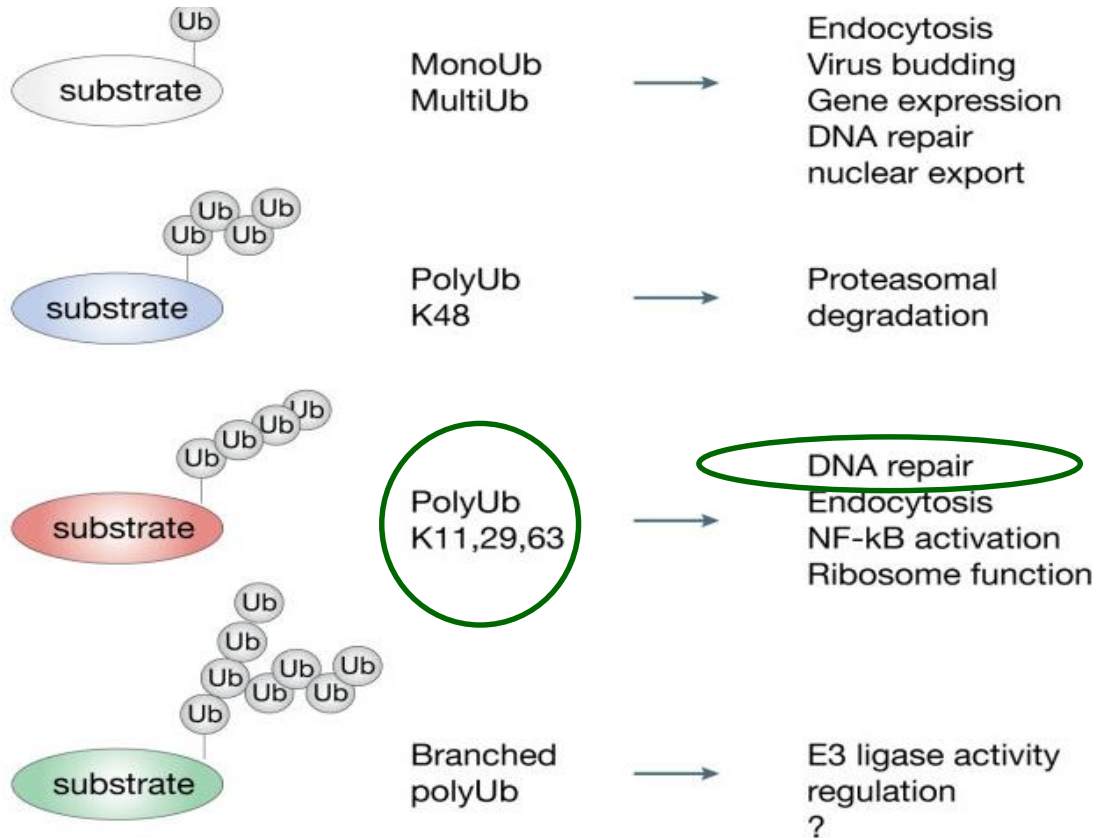


How does BLM recognize DNA damage?

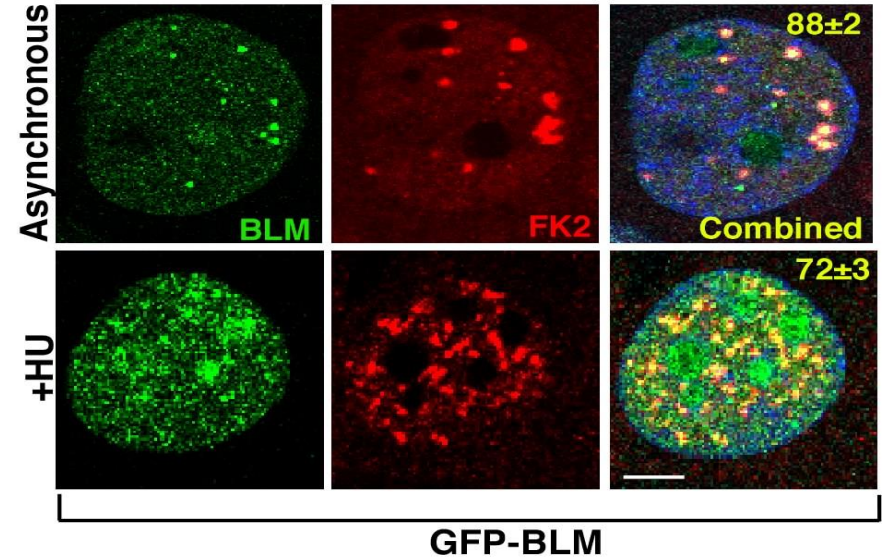
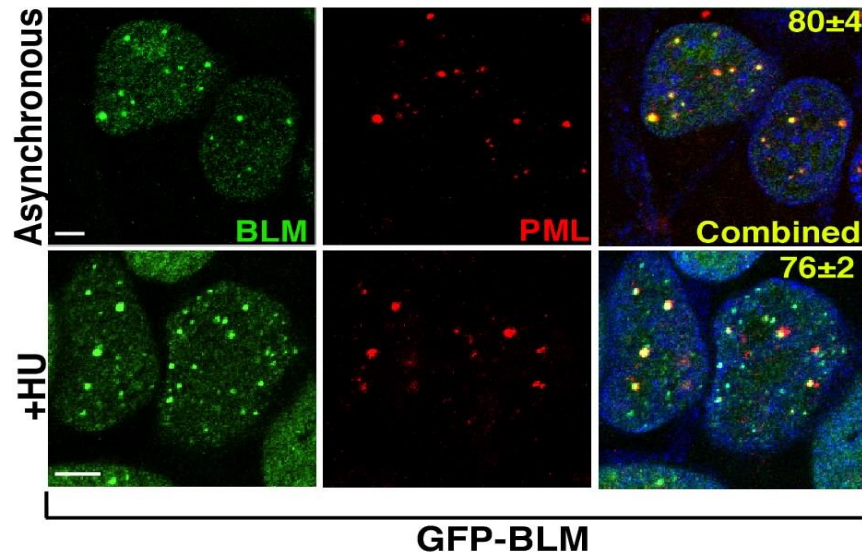
Different types of ubiquitylation



- 76 amino acids
- 8.5 kDa protein
- Ubiquitous in nature

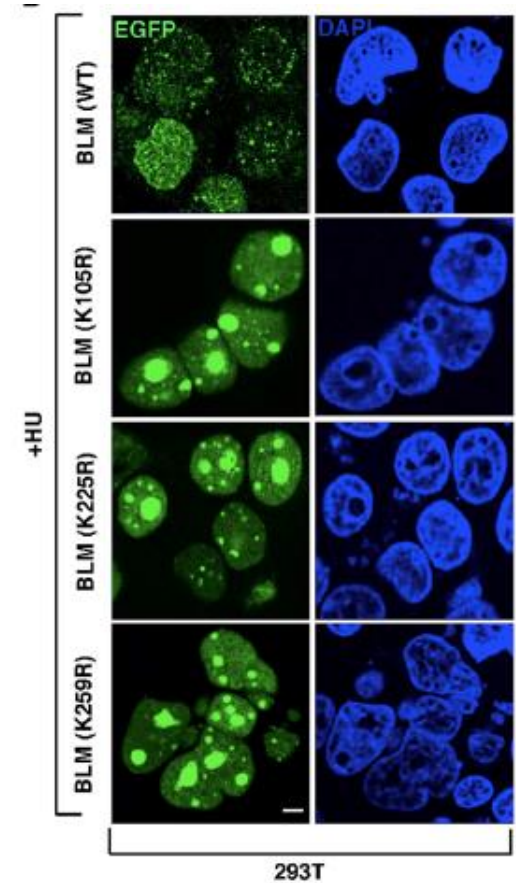
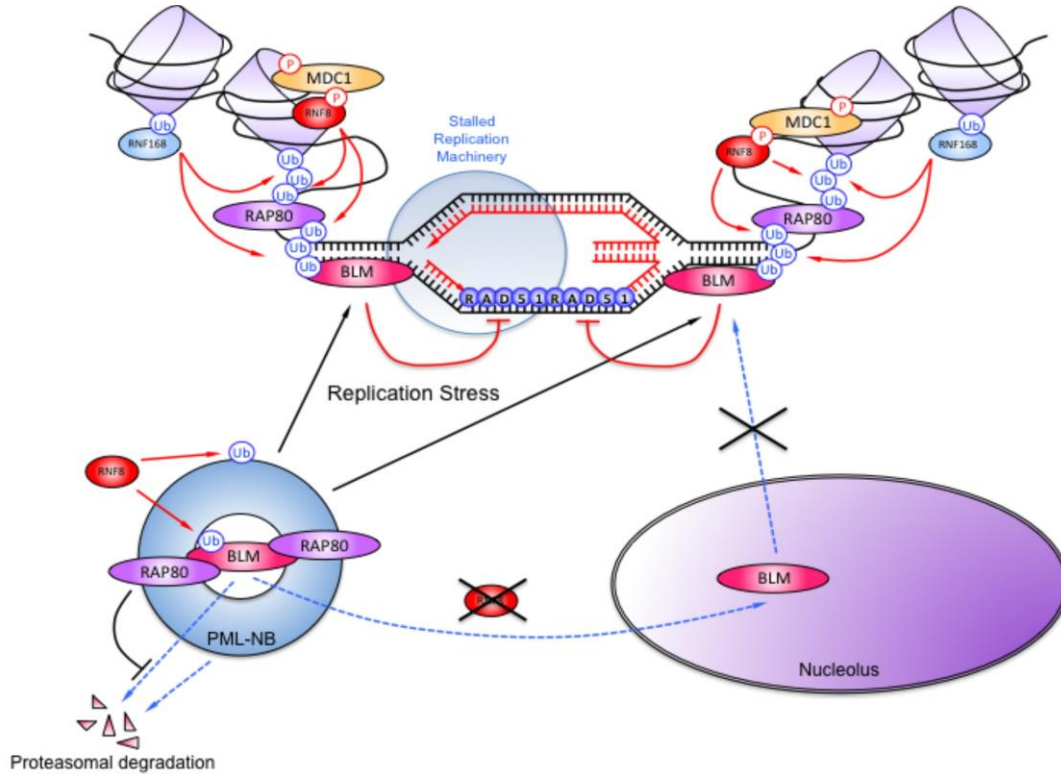


BLM localizes with sites of ubiquitylation after DNA damage



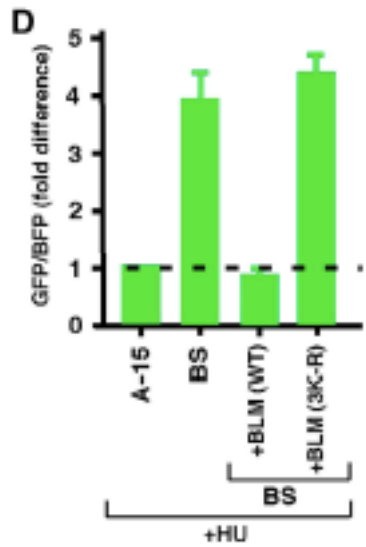
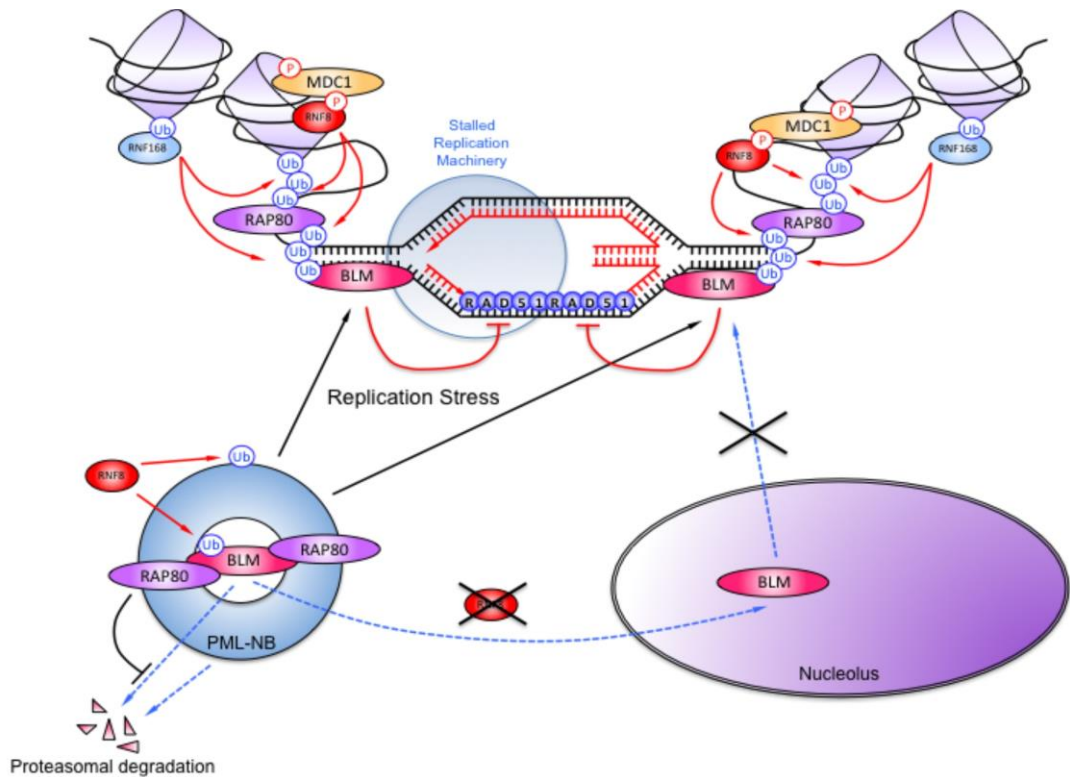
Hypothesis: Is BLM itself ubiquitylated after DNA damage?

Recruitment of BLM to the site of DNA damage depends on specific ubiquitylation events

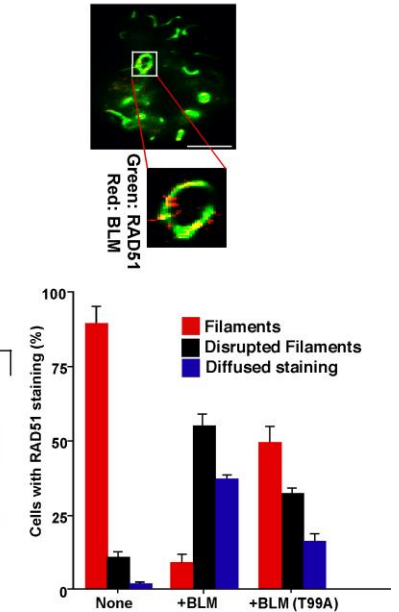
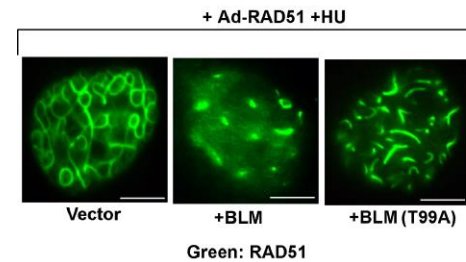
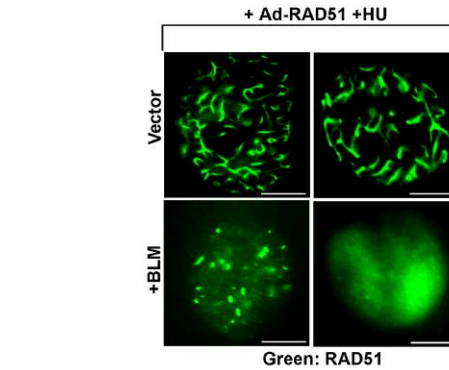
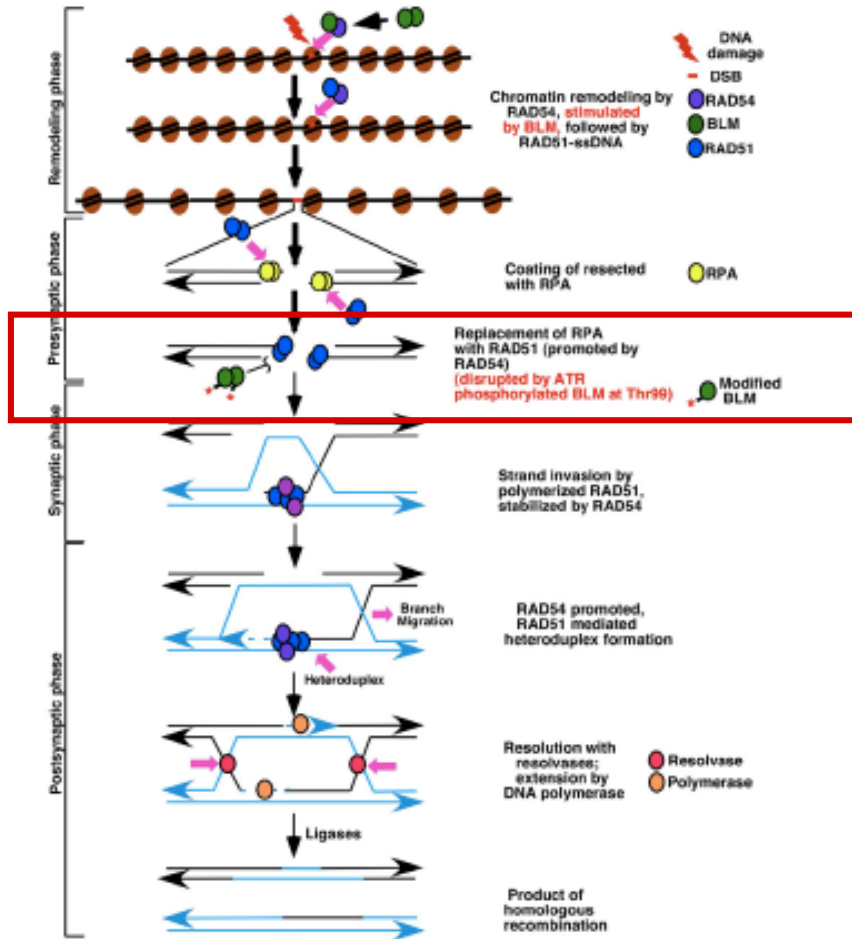


**How does the recruited BLM affect the DNA repair process,
homologous recombination?**

Recruitment of BLM to the site of DNA damage allows optimal level of homologous recombination



BLM inhibits homologous recombination by disrupting RAD51 nucleofilaments



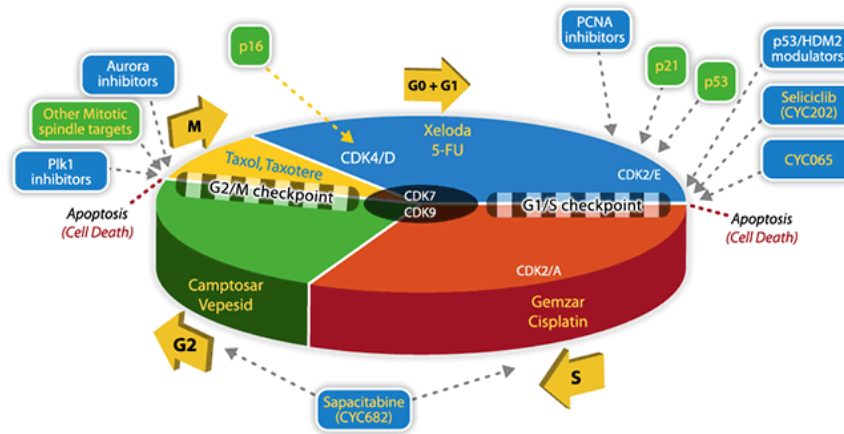
Tripathi et al., (2007) J Cell Biol 178(1): 9-14.

Tripathi et al., (2008) Carcinogenesis, 29(1): 52-61.

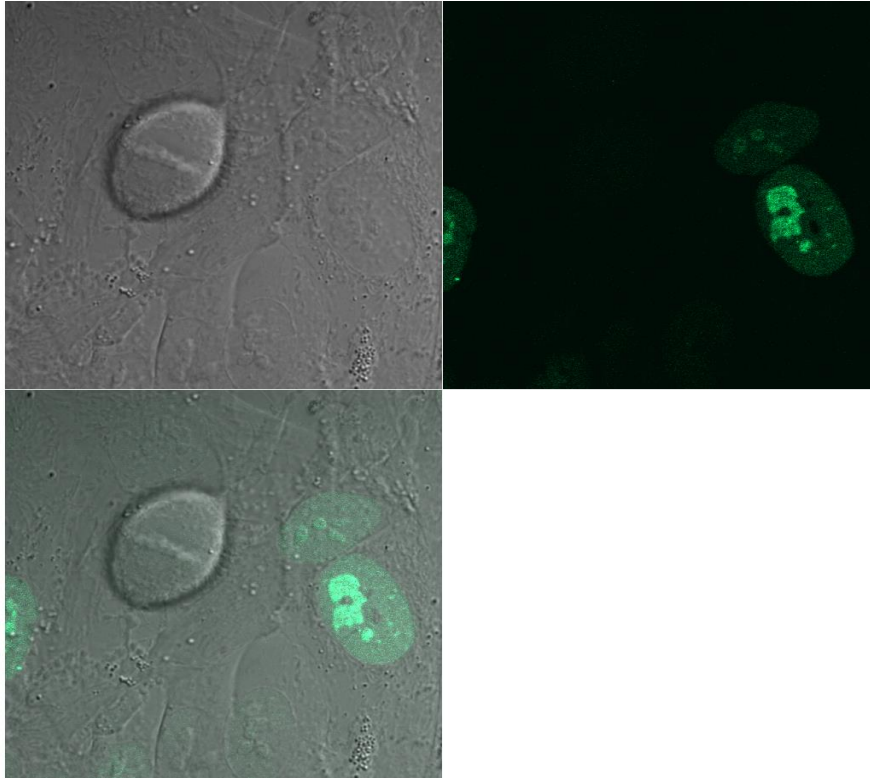
Srivastava et al. (2009) J Cell Sci. 122(Pt 17): 3093-3103.

What is the mechanism of turnover of BLM during the cell cycle?

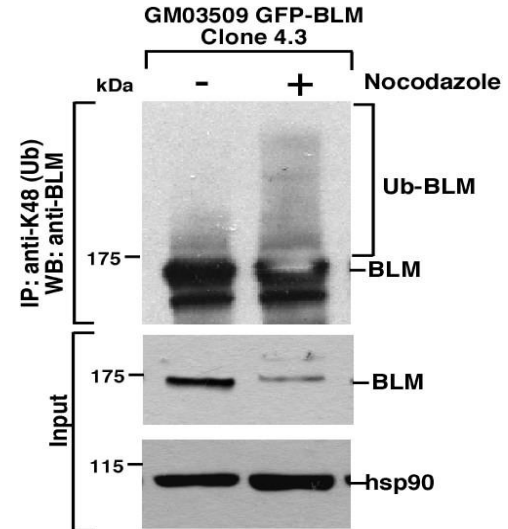
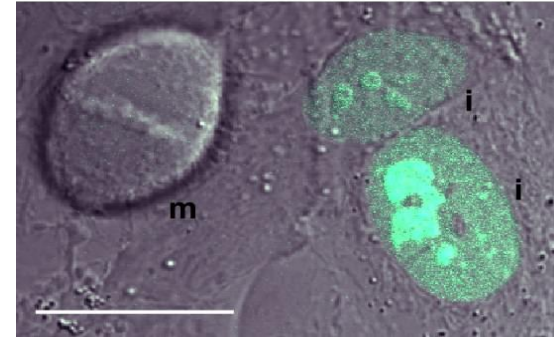
Cell Cycle



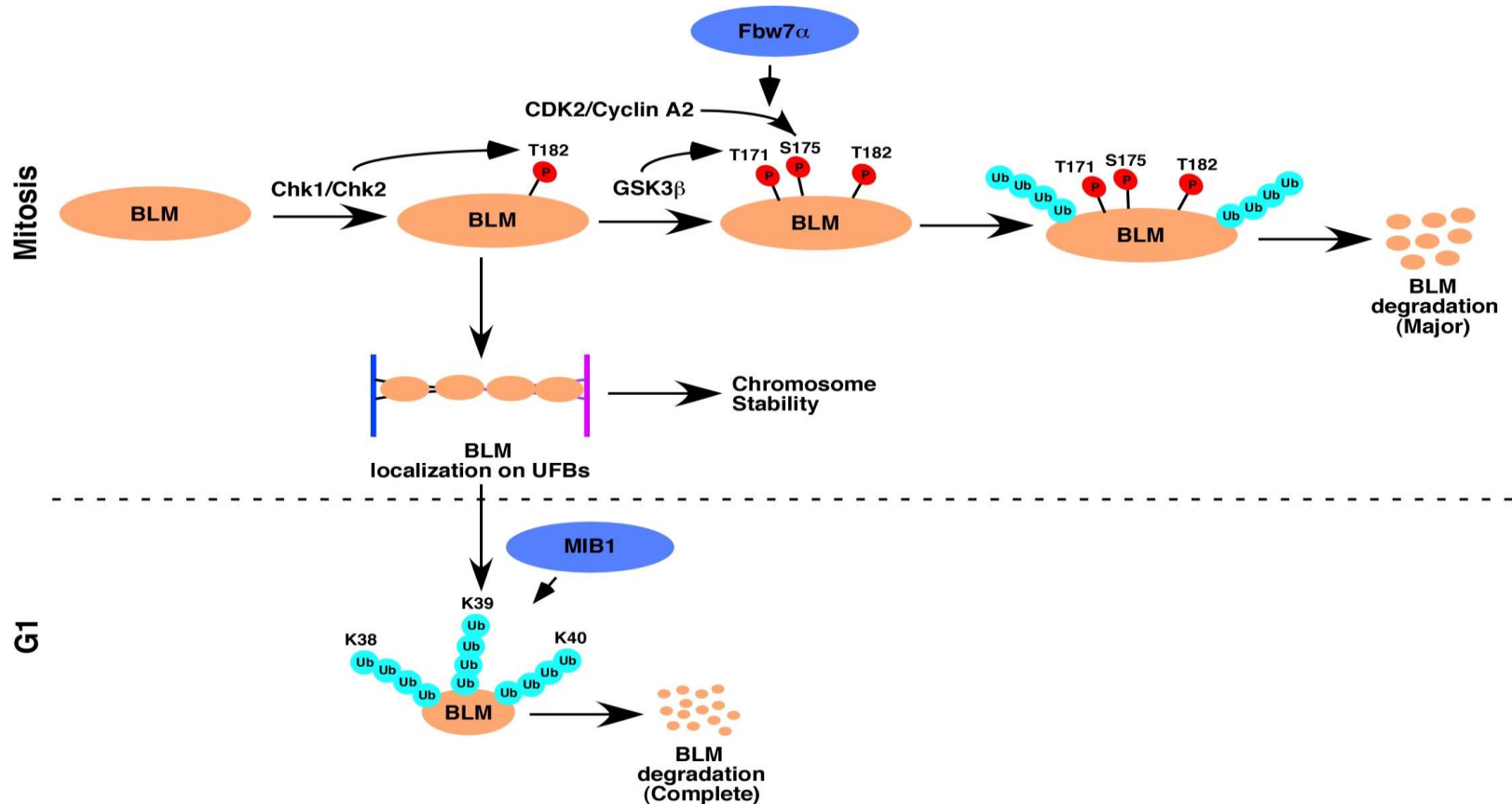
BLM undergoes turnover during mitosis



GM03509 GFP-BLM Clone 4.3
(Asynchronous)

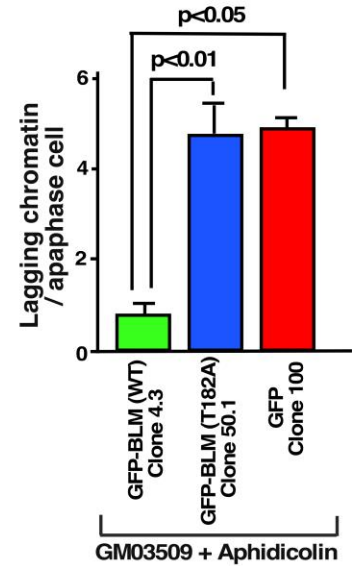
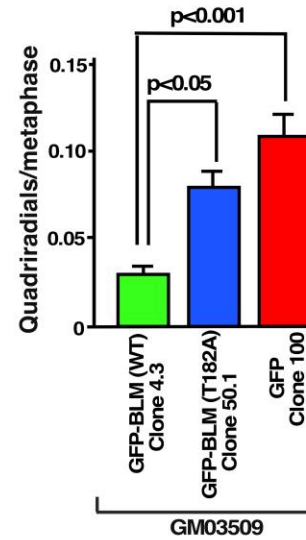
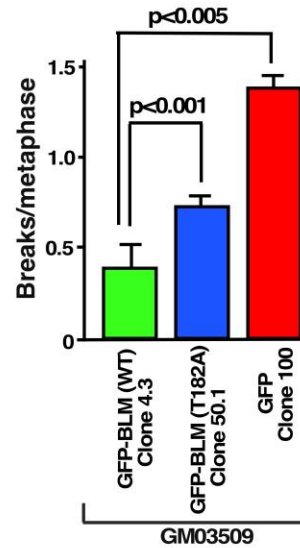
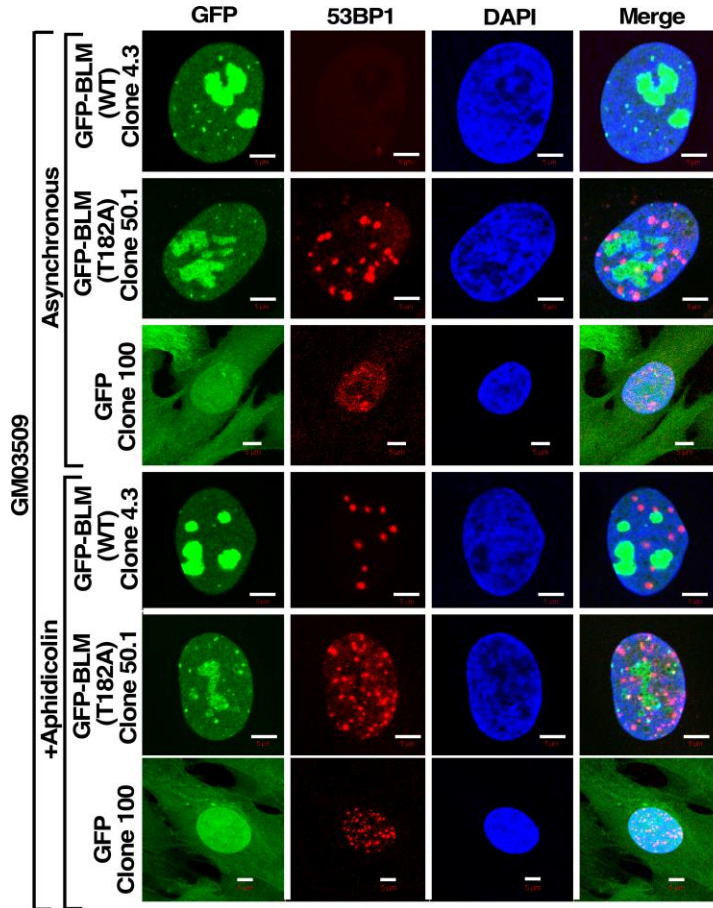


Sequential phosphorylation of BLM controls its ubiquitylation and turnover during mitosis



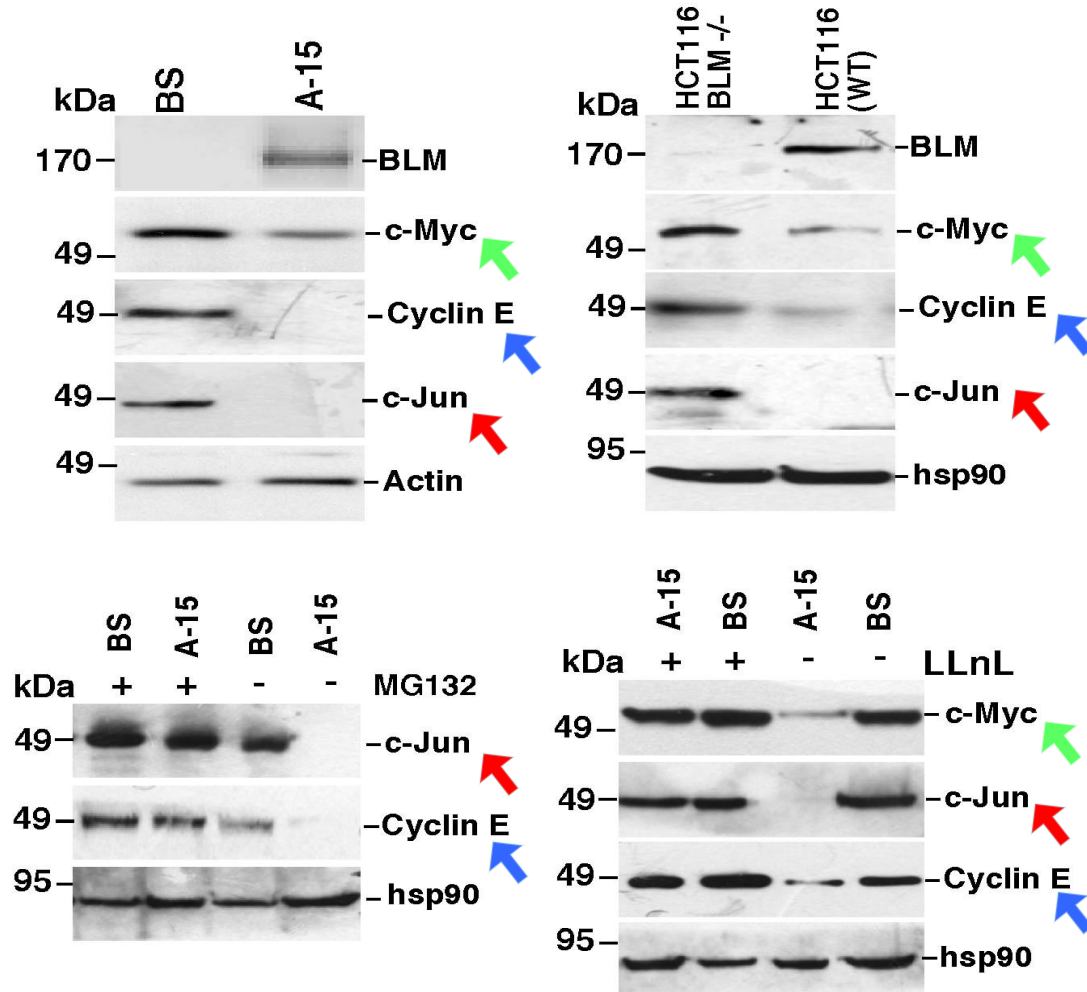
What happens if BLM cannot undergo cell cycle dependent turnover?

Lack of BLM turnover leads to genome instability

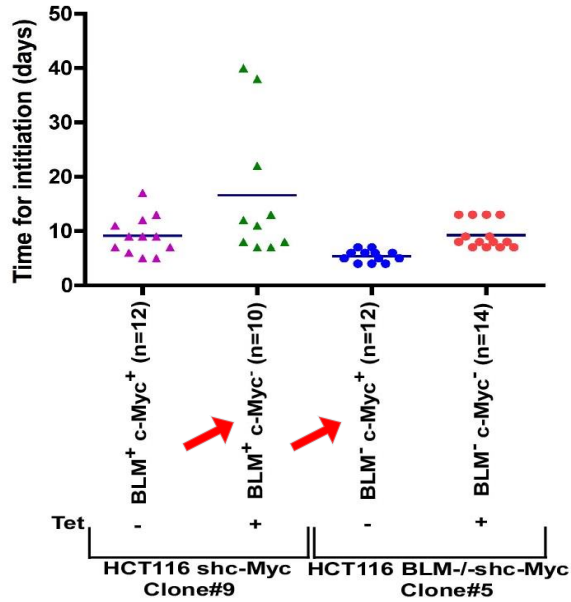


Can BLM carry out its function as a tumour suppressor by controlling the turnovers of oncoproteins?

Loss of BLM enhances the stability of multiple Fbw7 substrates



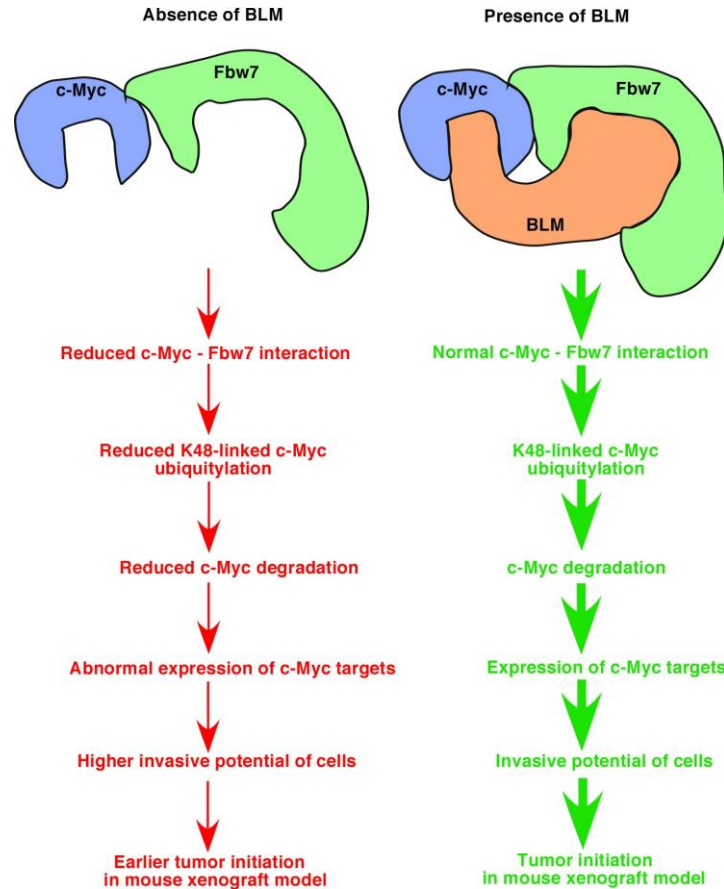
BLM negatively regulates c-Myc mediated tumor initiation



Genotype	Tumor initiation (Mean ± SD)	p value	Consequence
BLM ⁺ c-Myc ⁺ vs BLM ⁺ c-Myc ⁻	9.167±1.036 16.60±3.988	0.022	Initiation delayed
BLM ⁺ c-Myc ⁺ vs BLM ⁻ c-Myc ⁺	9.167±1.036 5.417±0.3128	0.044	Initiation advanced
BLM ⁺ c-Myc ⁻ vs BLM ⁻ c-Myc ⁺	16.60±3.988 5.417±0.3128	0.006	Initiation advanced

Xenograft model in nude mice

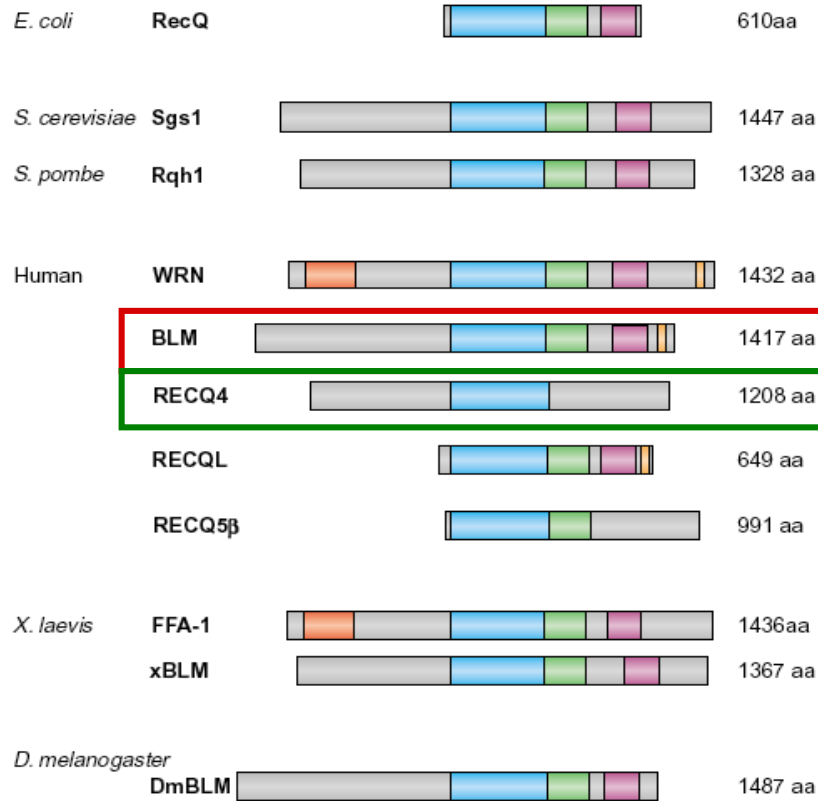
BLM enhances E3 ligase-mediated degradation of c-Myc



“Is BLM helicase a global tumor suppressor?”

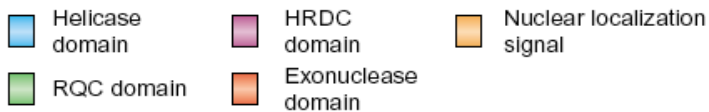
Does mitochondria contribute to genome stability?

RecQ helicase family



- Mutations in BLM, RECQL4 and WRN cause cancer predisposition syndromes in human
 Bloom Syndrome
 Rothmund Thomson Syndrome
 Werner Syndrome

Key:



TRENDS in Cell Biology

Rothmund Thomson Syndrome: a disorder related to mitochondrial dysfunction

Clinical Symptoms:

- Poikilodermal lesions
- Juvenile cataract
- Skeletal abnormalities
- Radial ray defects
- Alopecia
- Predisposition towards certain forms of cancer (Osteosarcoma and Lymphoma)

RECQL4 interact with

- Mitochondrial polymerases
- Tumour suppressor p53
- Proteins involved in DNA repair

RECQL4 functions in

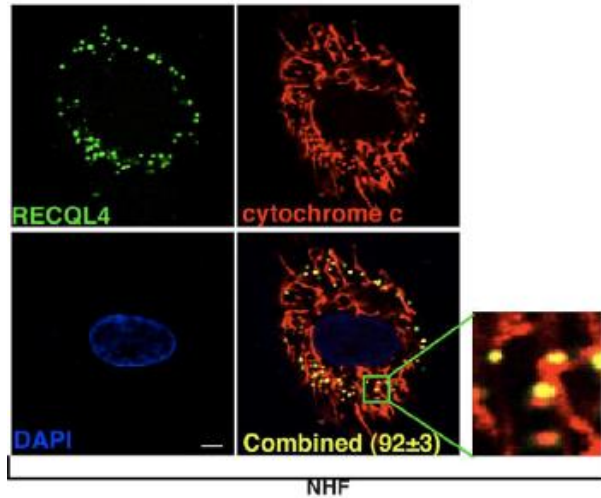
- Mitochondrial and nuclear DNA replication and repair

Cellular Phenotypes

- Abnormalities in chromosome 8



RECQL4 is a mitochondrial helicase

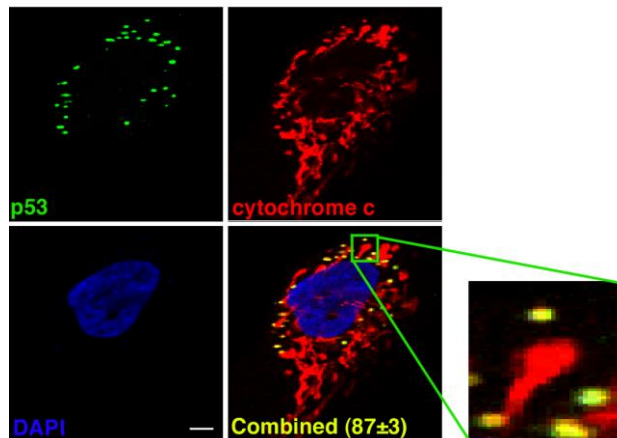


RECQL4 and p53 interaction leads to masking of their Nuclear Localization Signals (NLSs)

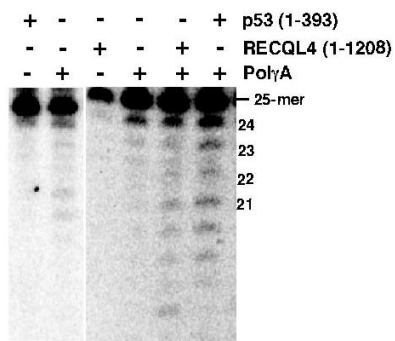
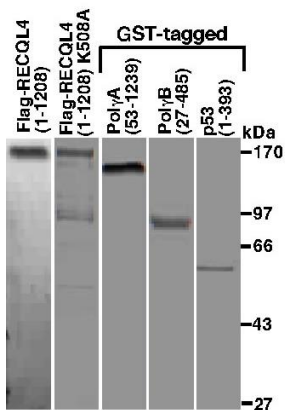
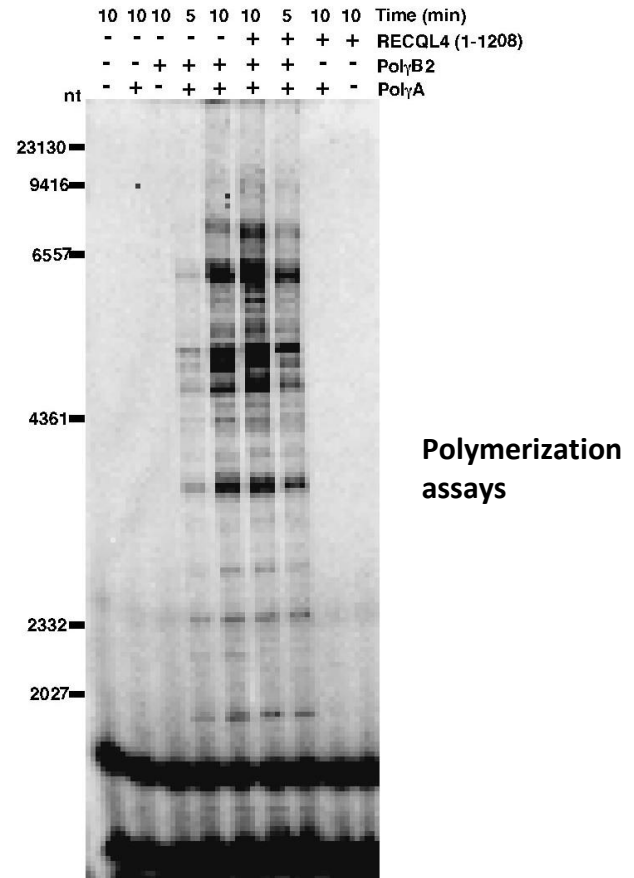
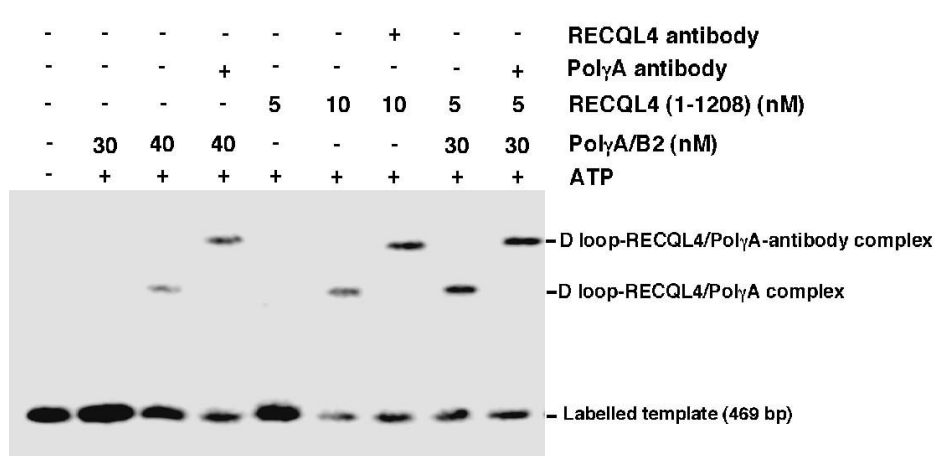
mtDNA is required for both p53 and RECQL4 to localize to the mitochondria.

RECQL4 localizes to the nucleus only during S-phase. In all other phases endogenous RECQL4 is present predominantly in the mitochondria.

RECQL4 has a functional Mitochondrial Localization Signal (MLS)



RECQL4 and p53 enhances the functions of mitochondrial DNA polymerase (PolyA/B2)

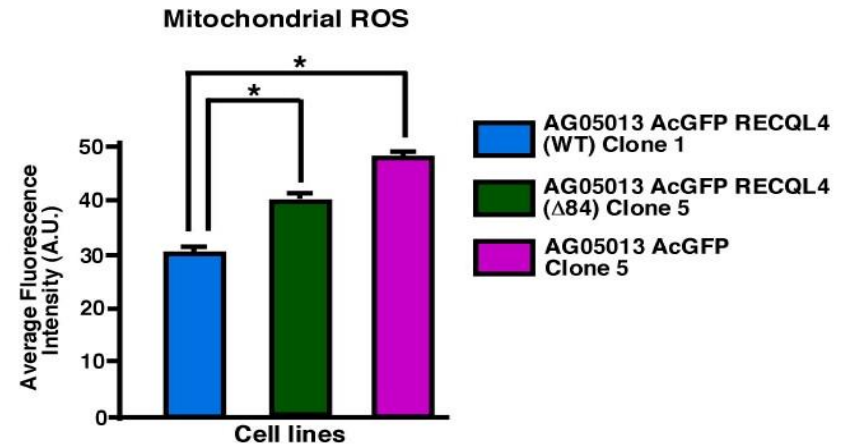
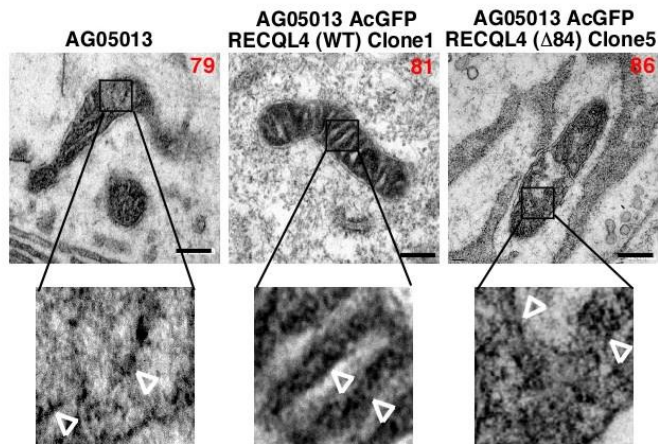
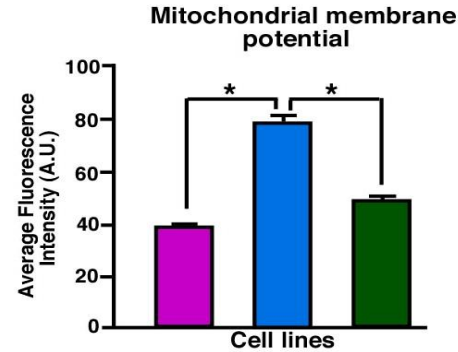
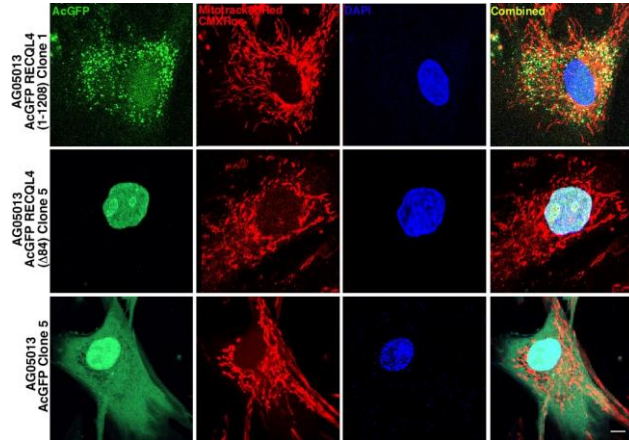


Gel retardation assays

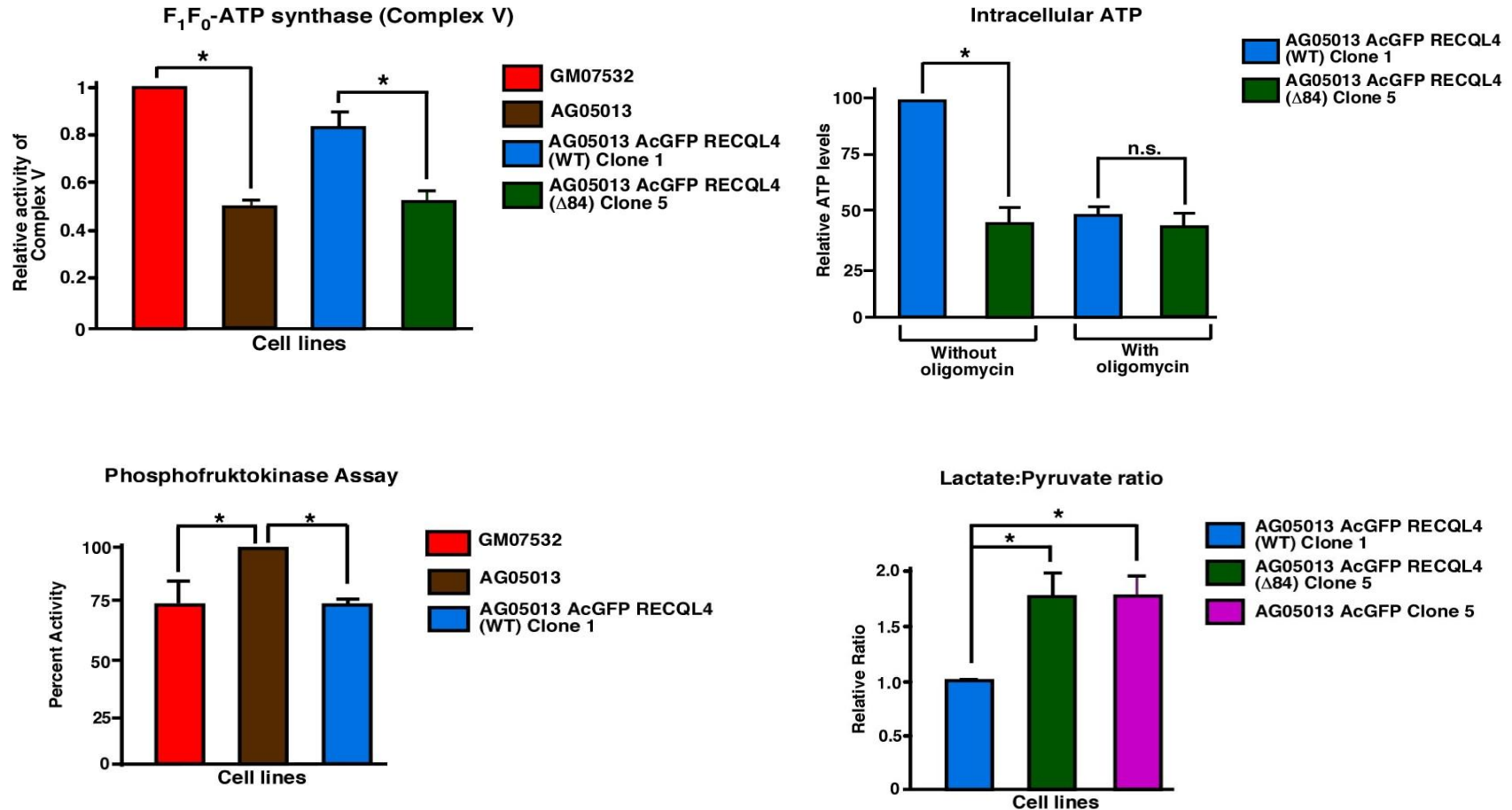
Exonuclease assays

How does mitochondrial functions contribute to carcinogenesis?

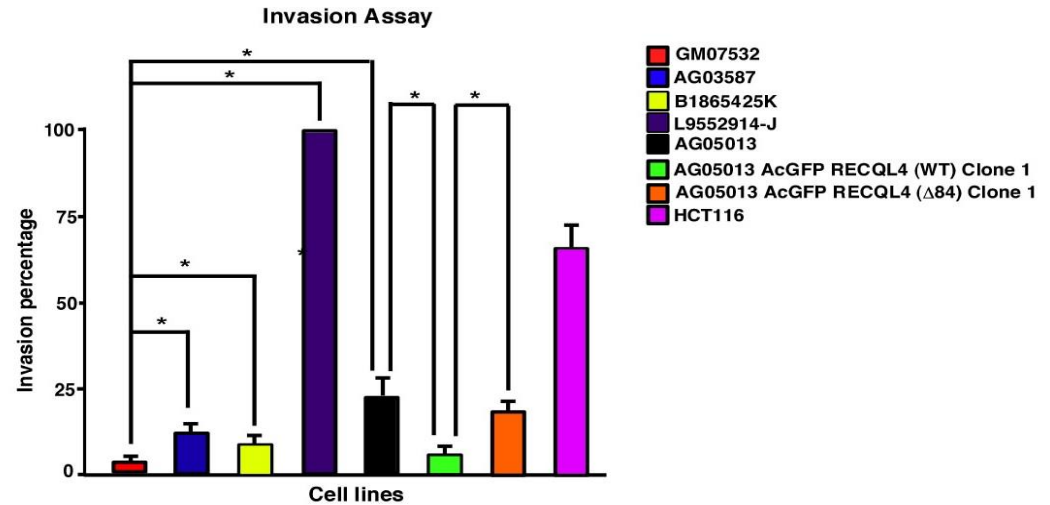
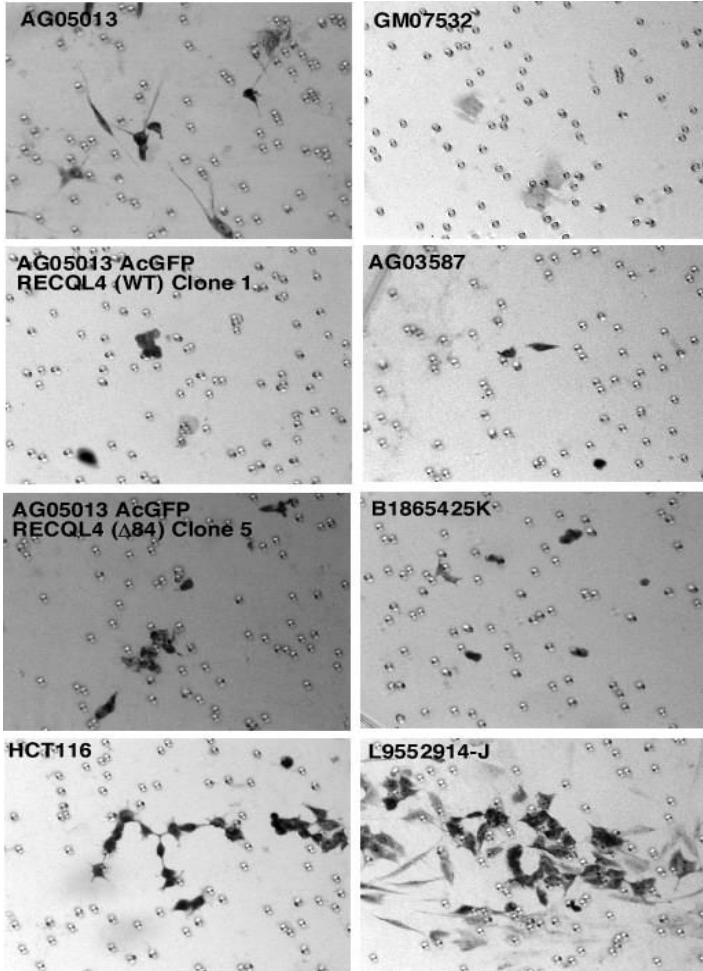
Lack of mitochondrial RECQL4 leads to dysfunctional cristae and increased membrane potential



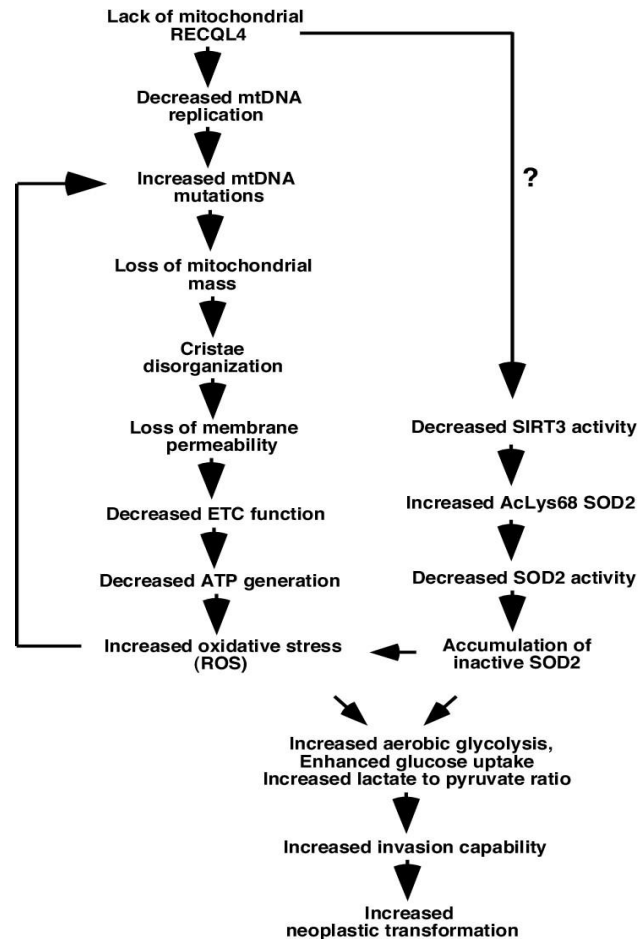
Cells lacking mitochondrial RECQL4 generate ATP by glycolytic shift



Invasive property of cells increases in absence of mitochondrial localization of RECQL4



Nuclear encoded RECQL4 affects neoplastic transformation by affecting mitochondrial functions



Acknowledgement

Past Members:

Sarabpreet Kaur
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Suhas Sampat Kharat
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Raina Priyadarshini
Richa Mudgal
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