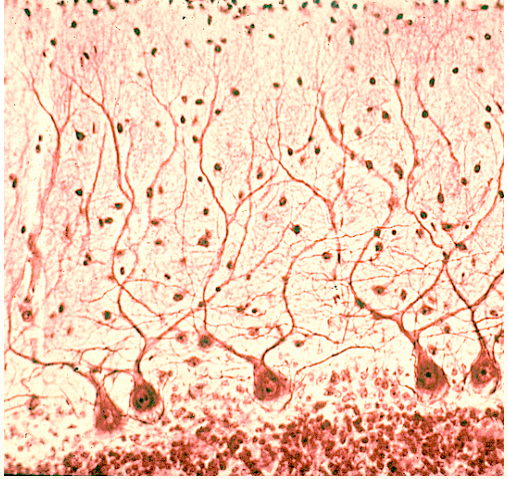
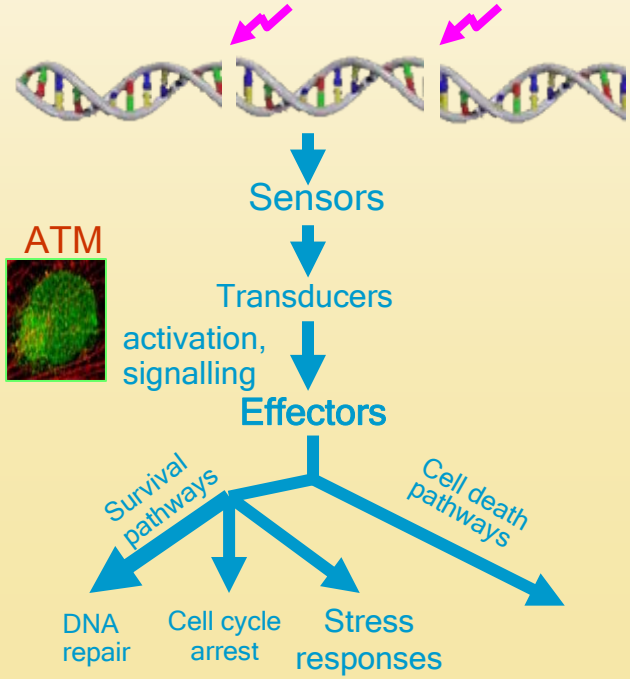


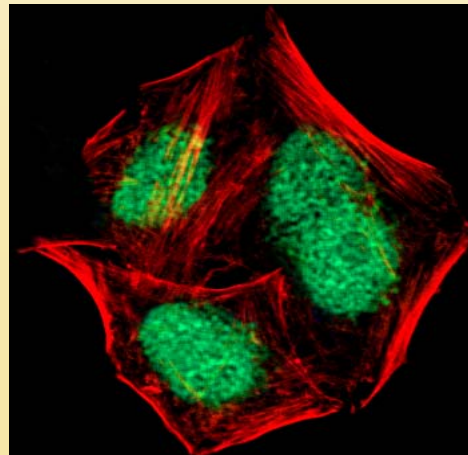
DNA repair Interest Group Cancer Center/Signal Transduction Program Seminar

Burnham Institute for Medical Research

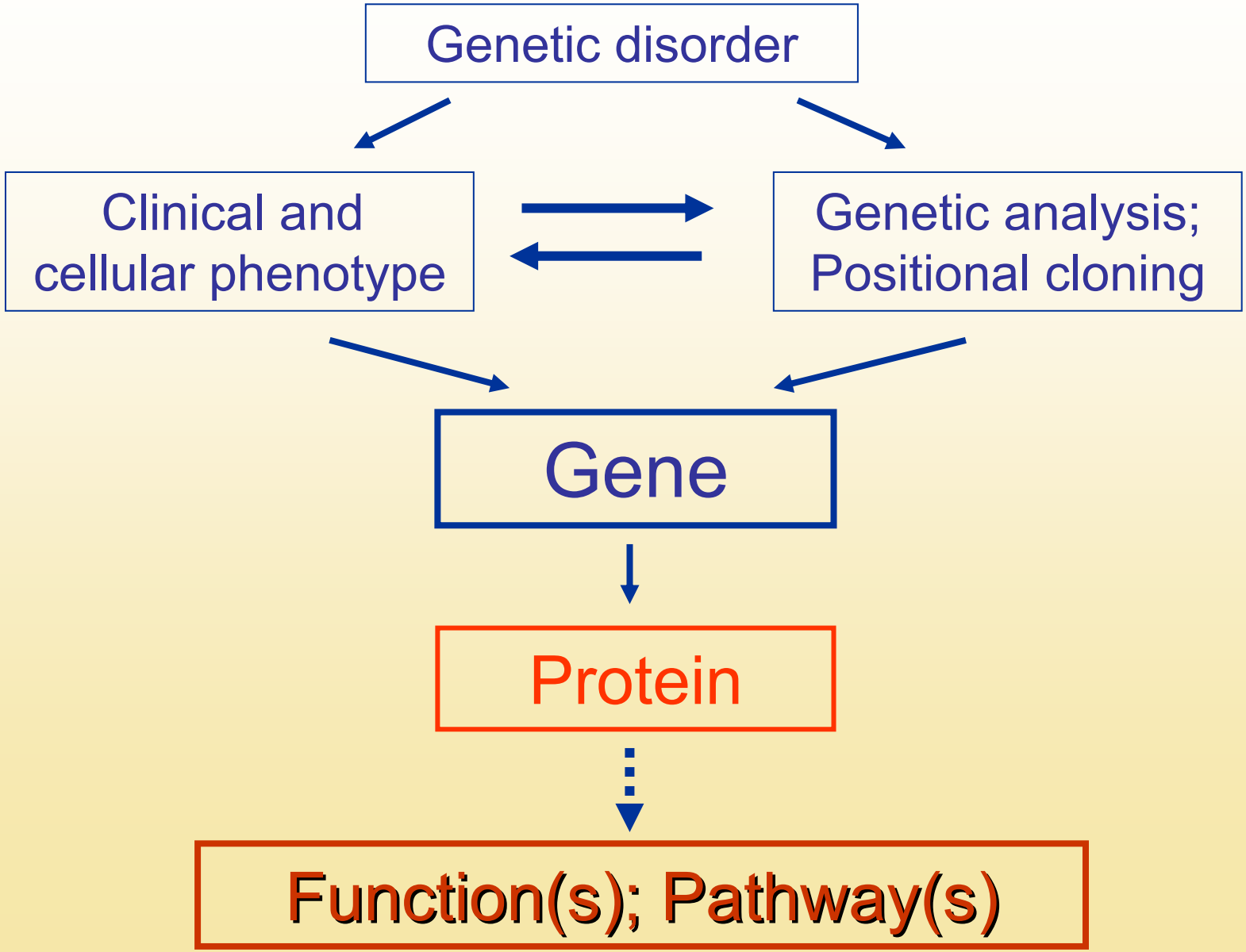
ATM: The Art of Maintaining Genome integrity



The A-T Group
Tel Aviv University

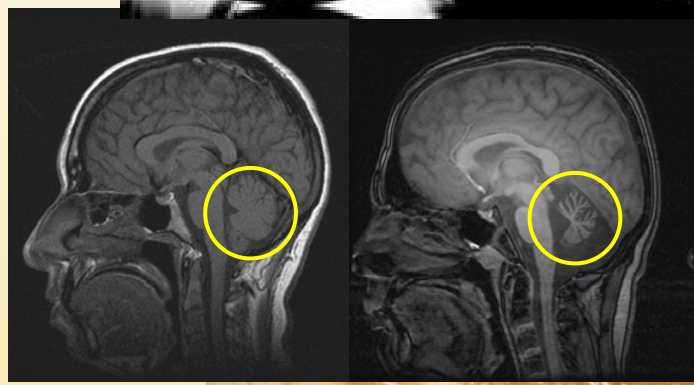


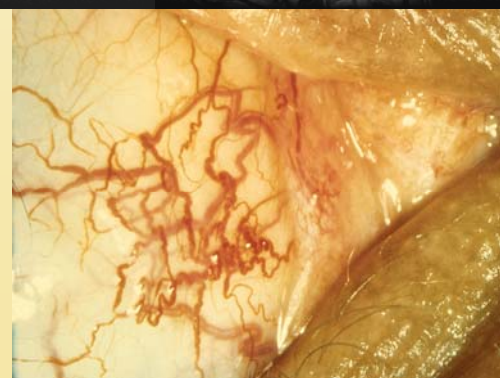
From Genetic Disorder to Function



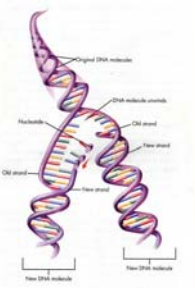
Ataxia-Telangiectasia

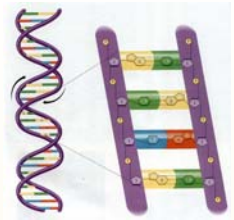
- Autosomal recessive
- Cerebellar ataxia (loss of Purkinje cells), severe neuromotor dysfunction
- Telangiectasia
- Immunodeficiency (B&T cells); recurrent infections
- Thymic and gonadal dysgenesis
- Retarded growth
- Premature aging
- Genomic instability
- Cancer predisposition
- Acute sensitivity to ionizing radiation
- **A profound defect in cellular responses to double strand breaks in the DNA**

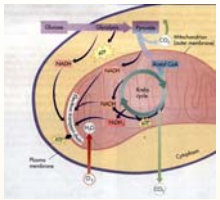


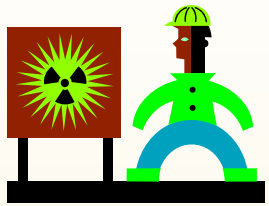


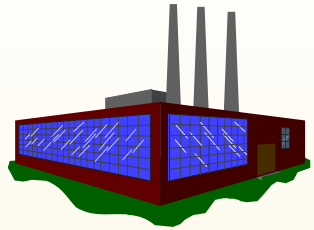
DNA Damage

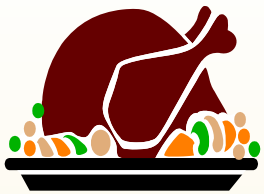


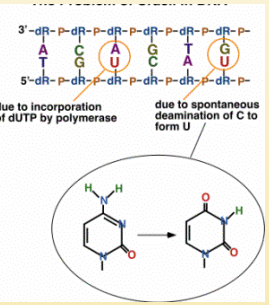


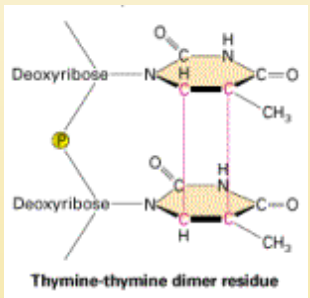


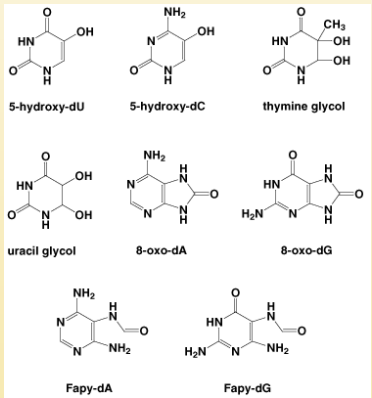


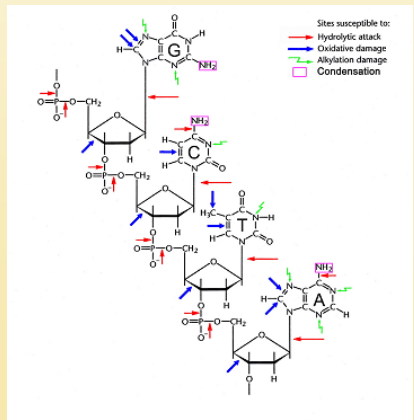


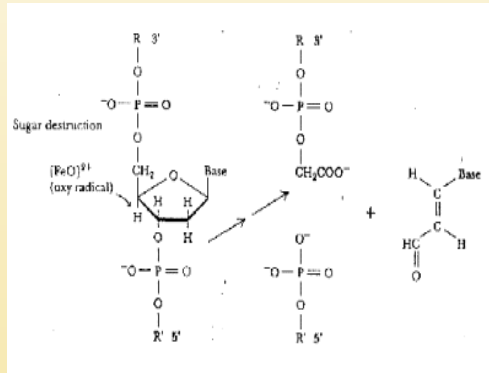




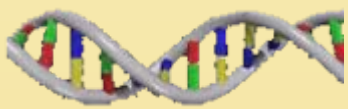


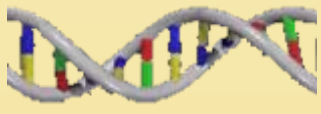




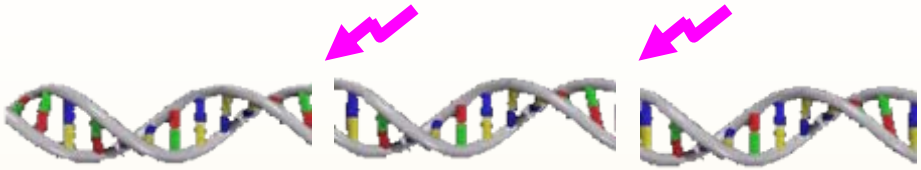


Double strand breaks





Repair DNA Double Strand Breaks



Physiologic Double-Strand DNA Break/Repair

1. RAG1,2 breaks (RAG1,2)
2. Class switch breaks (AID, UNG, APE)

Pathologic Double-Strand DNA Break/Repair

1. Ionizing radiation
2. Oxidative free radicals
3. Replication across a nick

cleavage phase

cleavage phase

joining phase

Nonhomologous DNA End Joining (NHEJ)

Ku70/86
Artemis:DNA-PKcs
XRCC4:DNA ligase IV

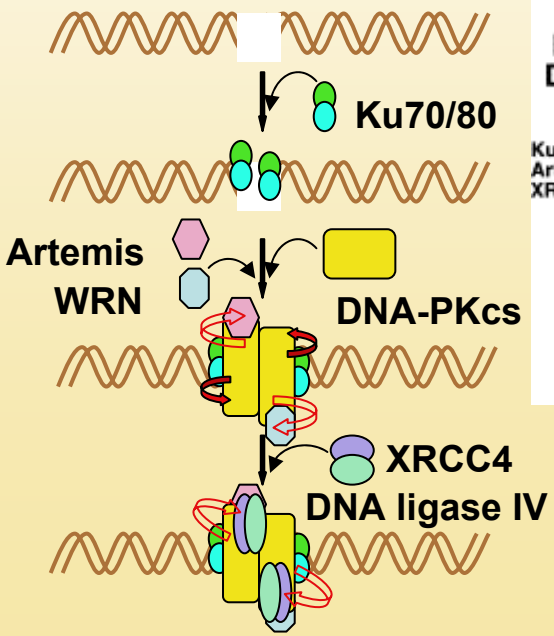
Homologous Recombination (HR)

RAD50, MRE11, XRS2, RAD51, 52, 54, 55, 57

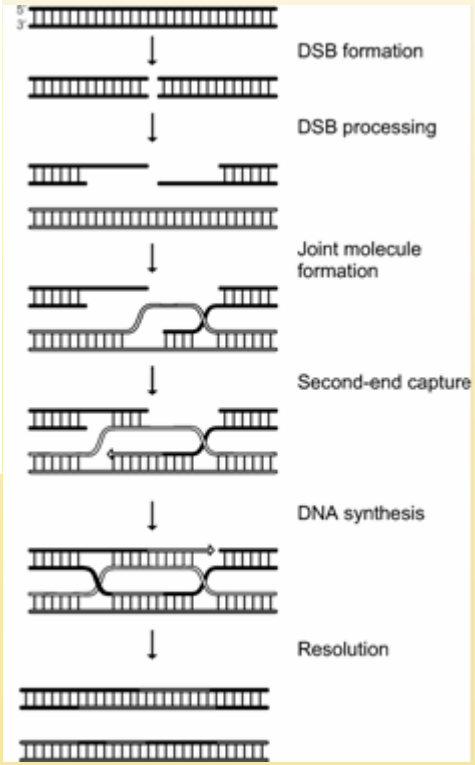
entire cell cycle

late S, G2

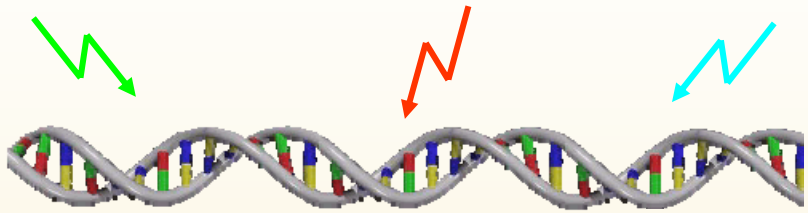
NHEJ



HR

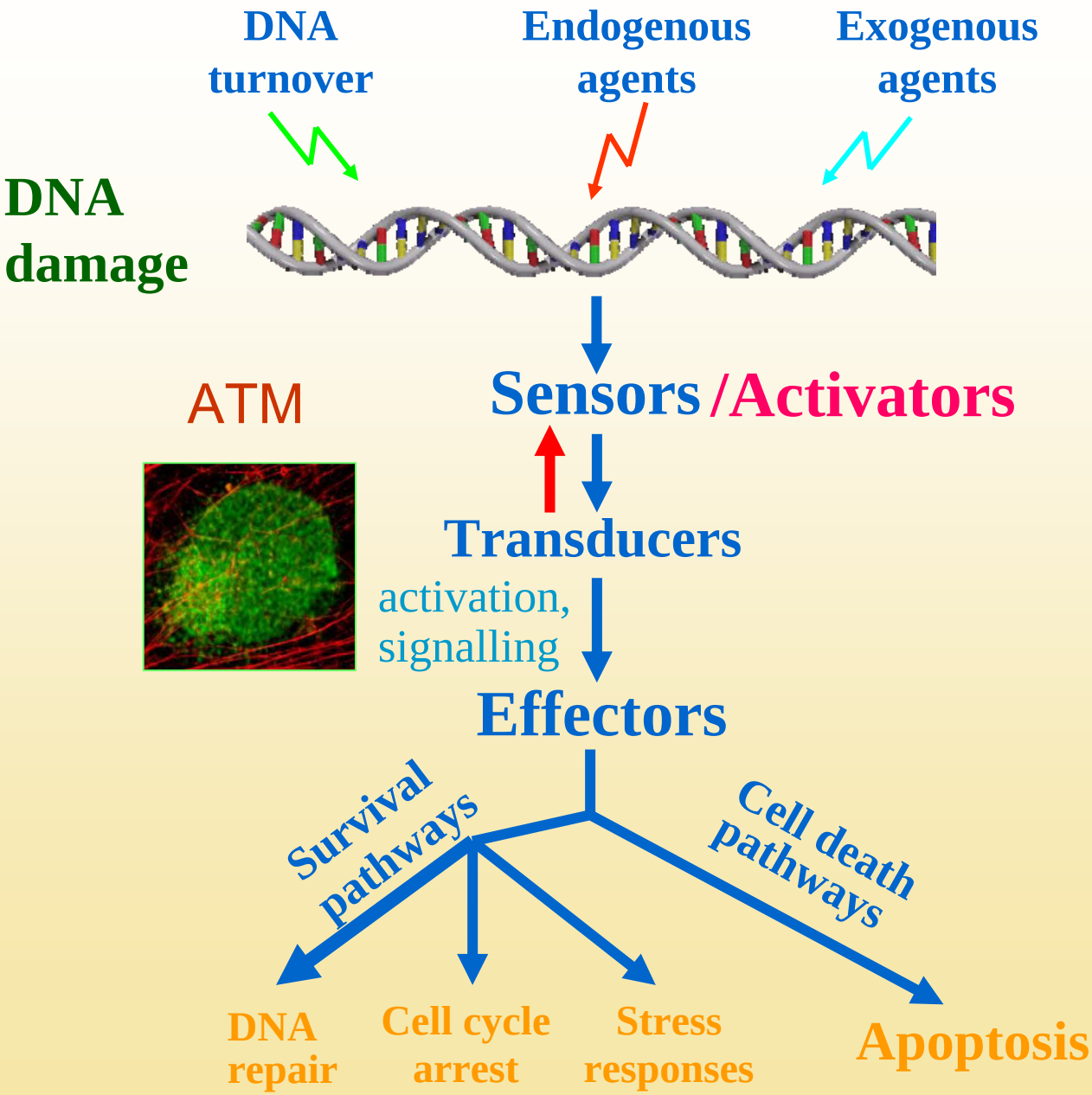


The DNA Damage Response

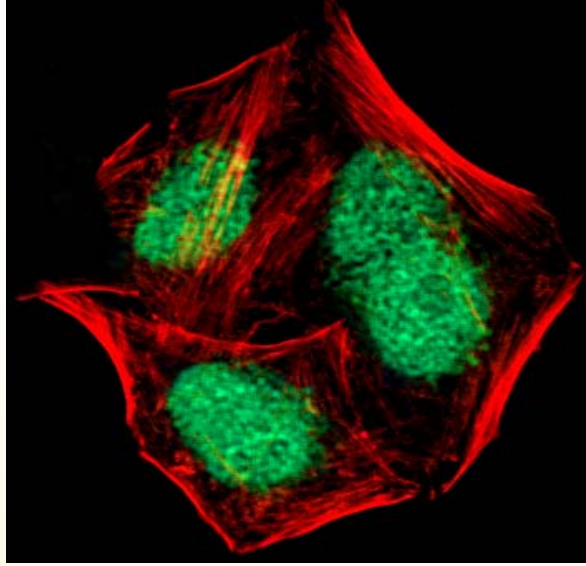


- DNA repair
- Cell cycle checkpoints
- Affects numerous cellular processes
- Modulates gene expression
- Concerted action of numerous, interlocked signaling pathways creating an intricate web
- Activated instantaneously and vigorously, particularly by DSBs

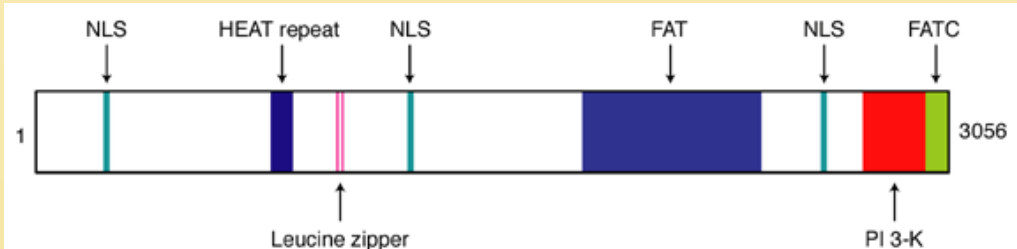
Cellular Responses to DNA Damage



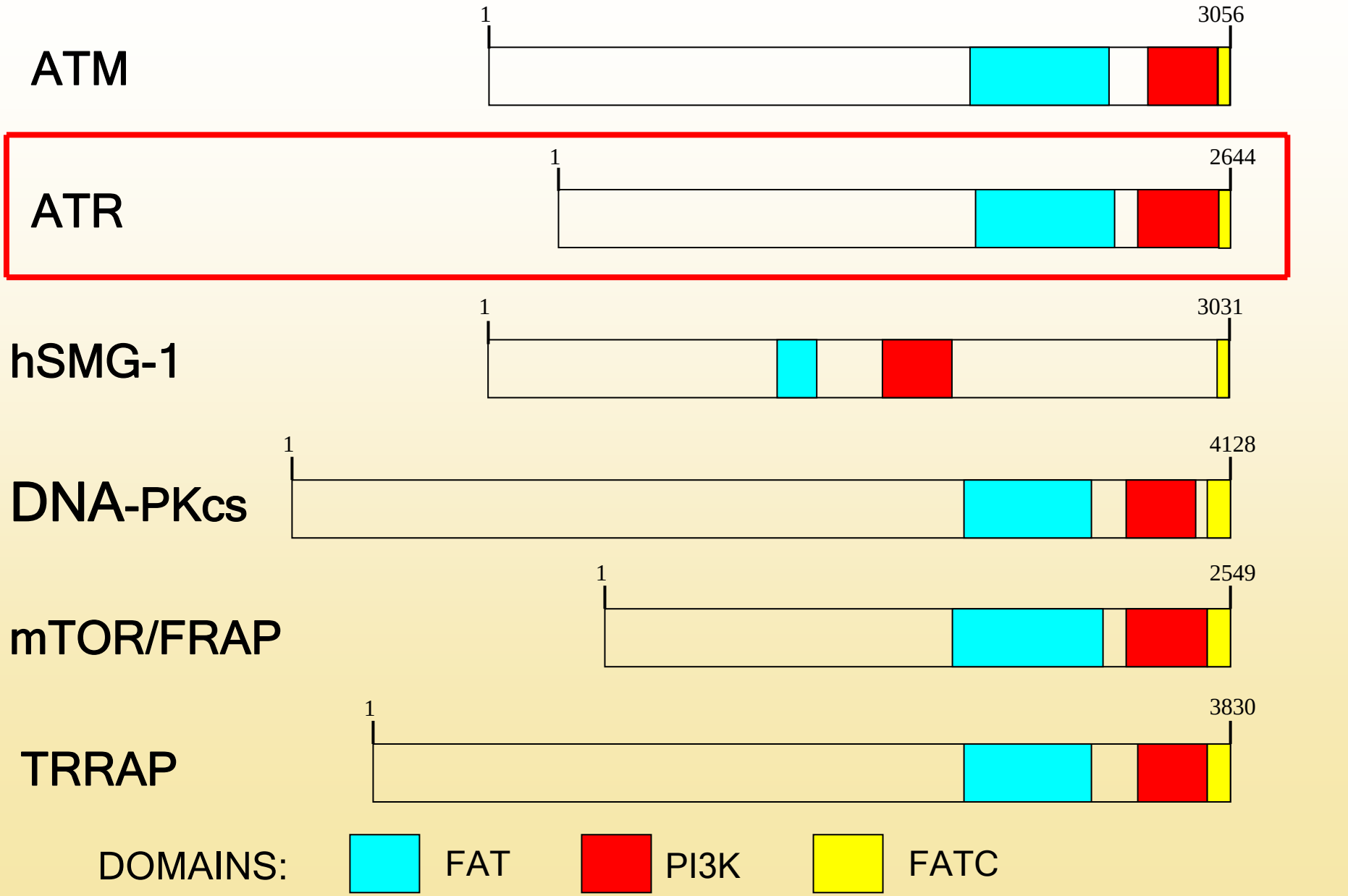
The ATM Protein



- Identified by positional cloning of the A-T gene
- Completely missing or inactivated in A-T patients
- A large (370 kDa), nuclear phosphoprotein
- A carboxy-terminal PI3-kinase-like motif
- Protein kinase activity (serine-threonine)
- ATM targets: preference for SQ or TQ
- **The primary activator of the cellular response to double strand breaks (DSBs)**
- Activated by DSBs



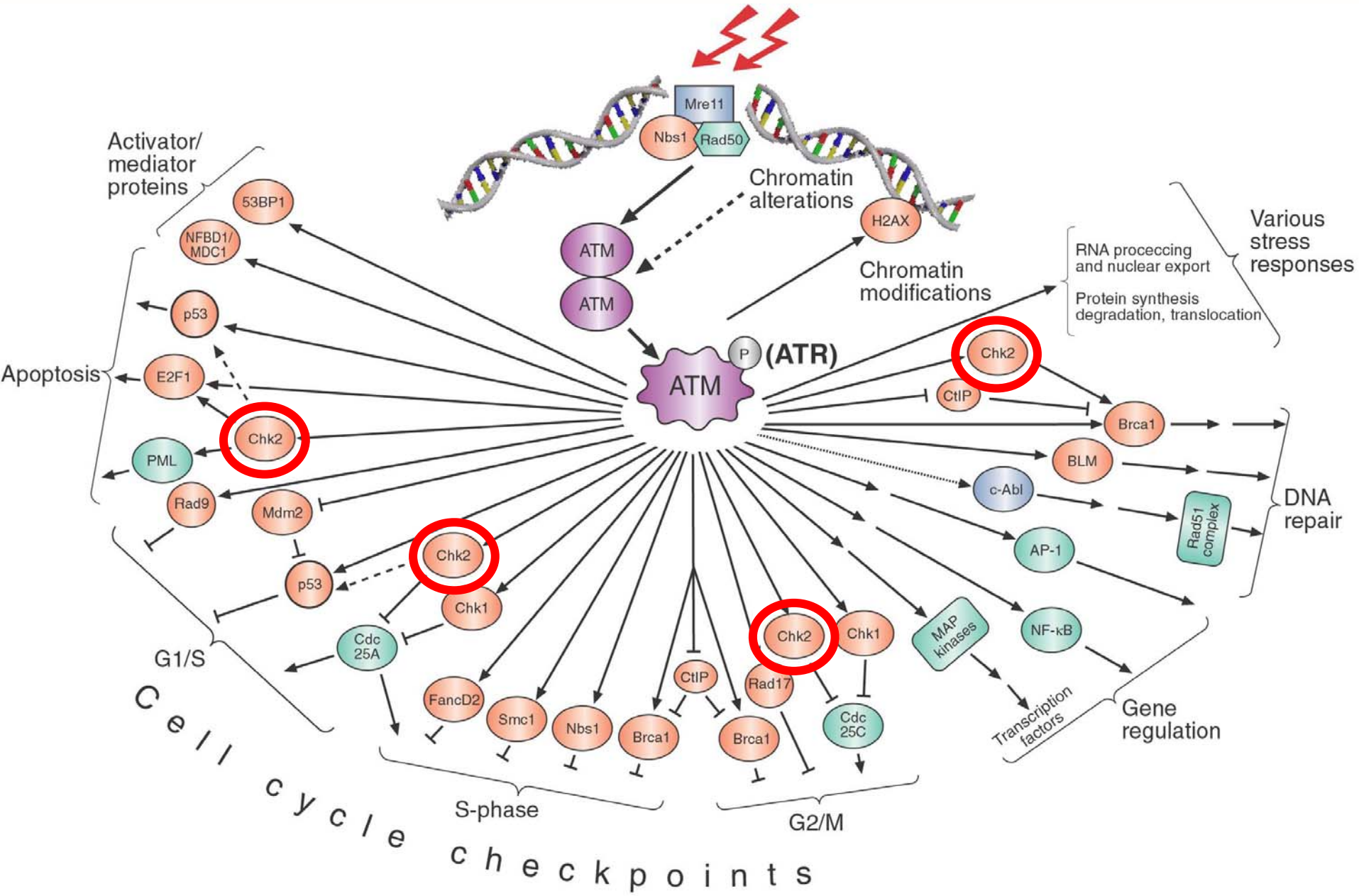
PI3-Kinase-Related Protein Kinases (PIKKs)



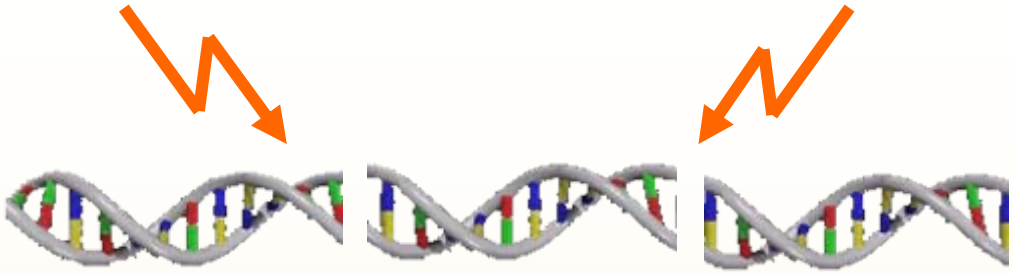
ATM

Control of signaling networks by phosphorylation of key players

ATM-Mediated DNA Damage Responses



DSBs

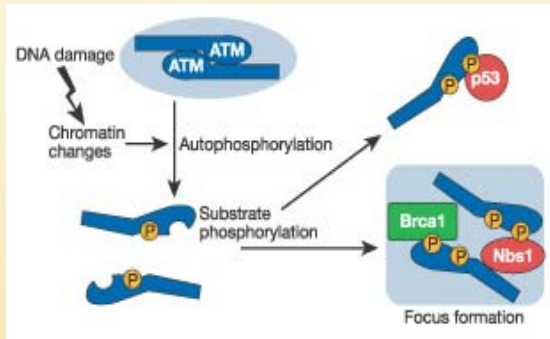


ATM

Recruitment to DSB sites (Andegeko et al., 2001)

Dormant dimers turn into active monomers (Bakkenist & Kastan, 2003)

Activated ATM



Phosphorylation

Effectors

Survival pathways

Cell death pathways

DSB repair

Cell cycle checkpoints

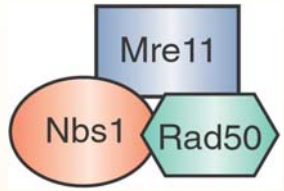
Stress responses

Apoptosis

DSBs

binding to DSBs;
damage processing

MRN complex



Processed
DNA lesions

ATM

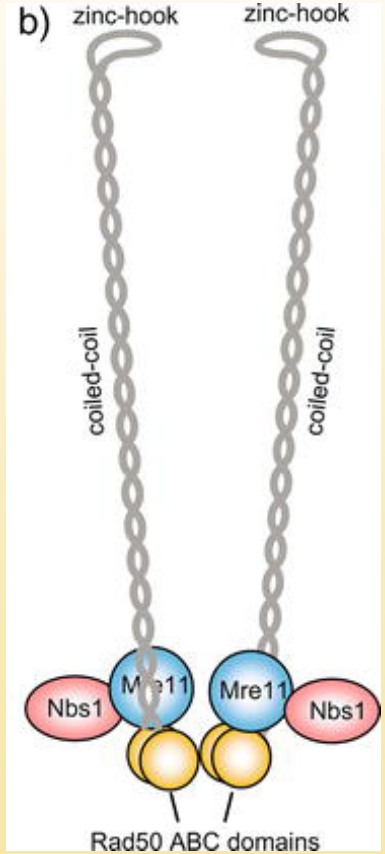
Activation
Association with
processed DSBs

Activated
ATM

phosphorylation

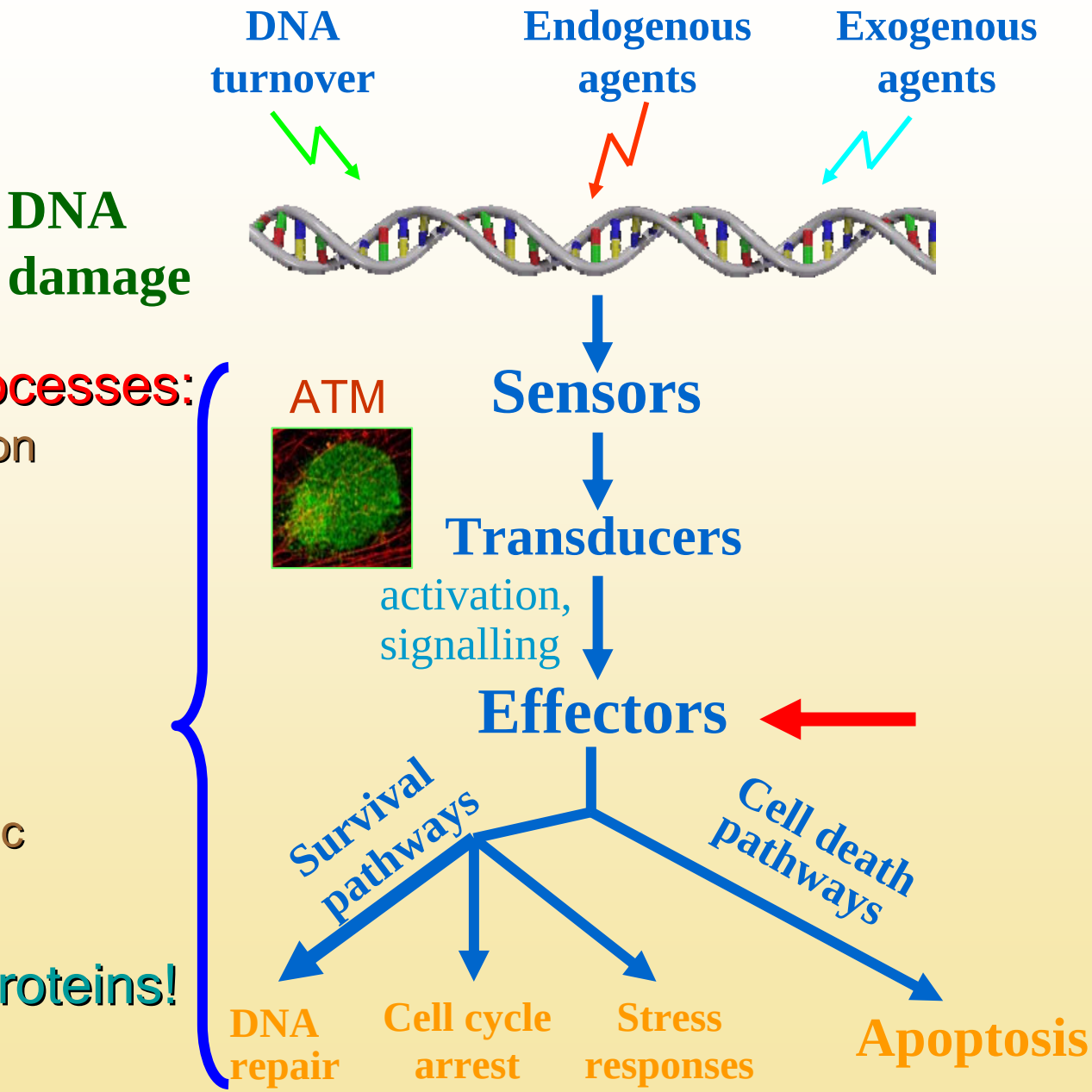
Target proteins

Signaling pathways



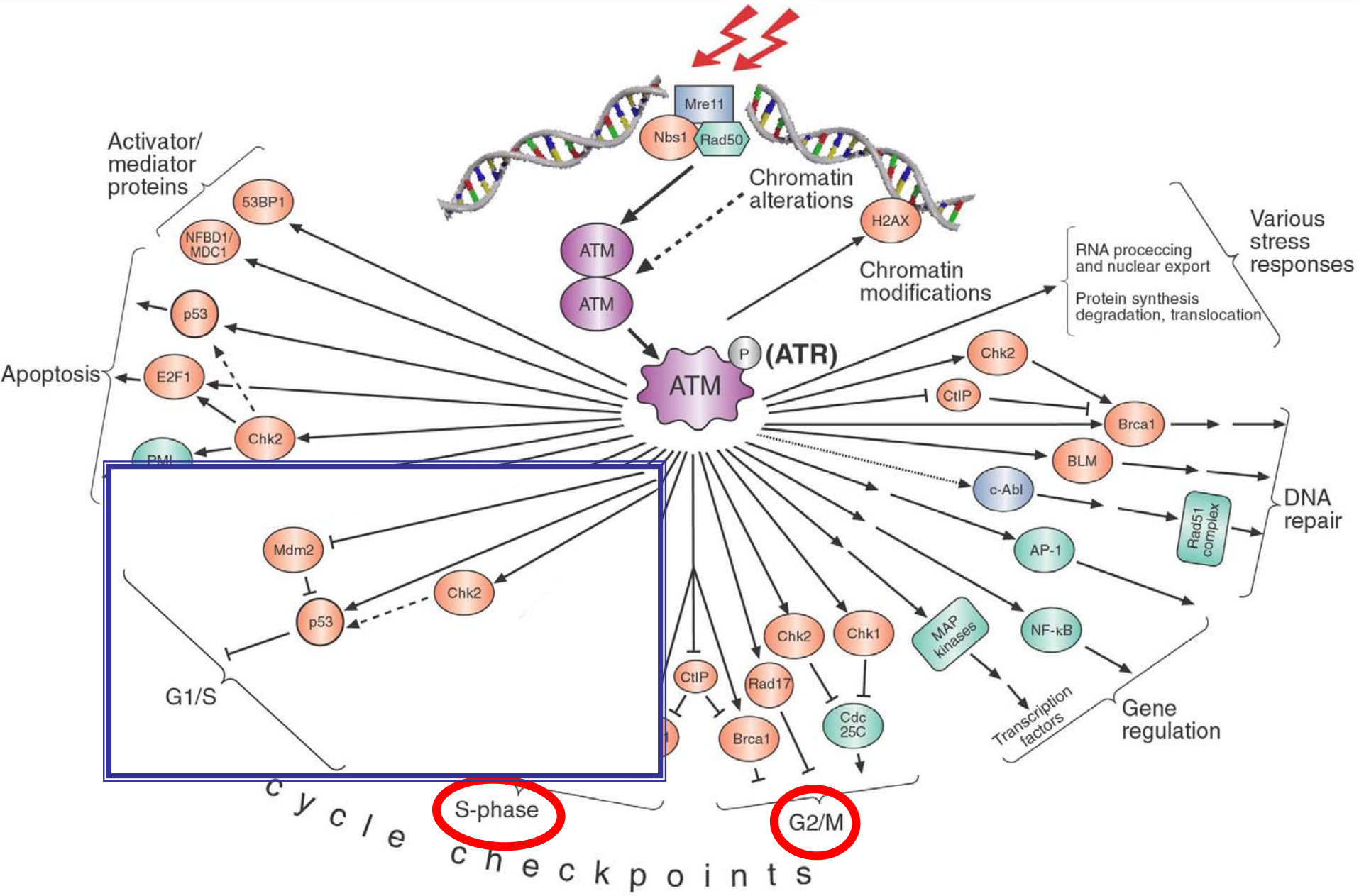
T. Uziel et al., 2003

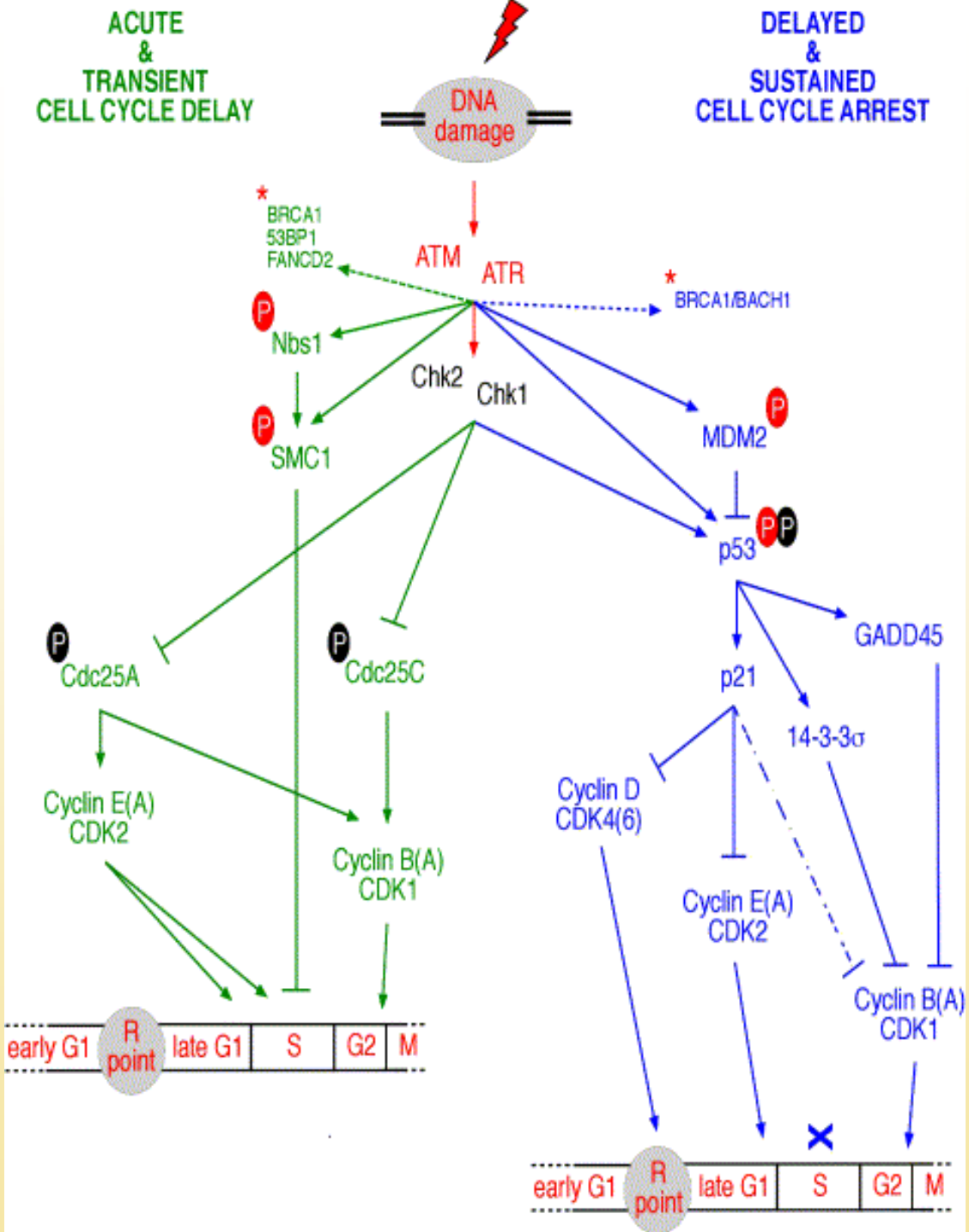
Cellular Responses to DNA Damage



The SHARP Map, May 20, 2006

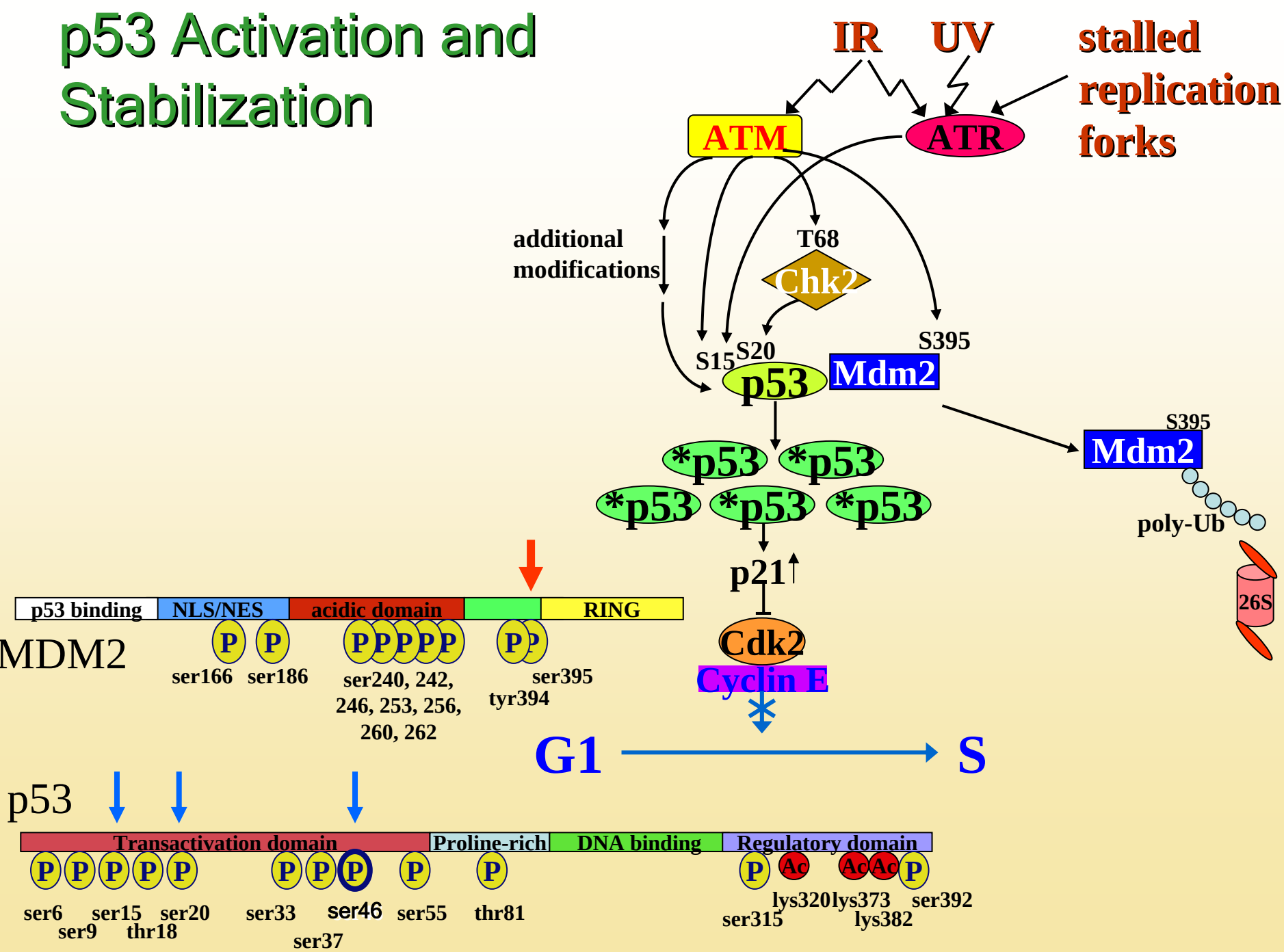
ATM-Mediated DNA Damage Responses



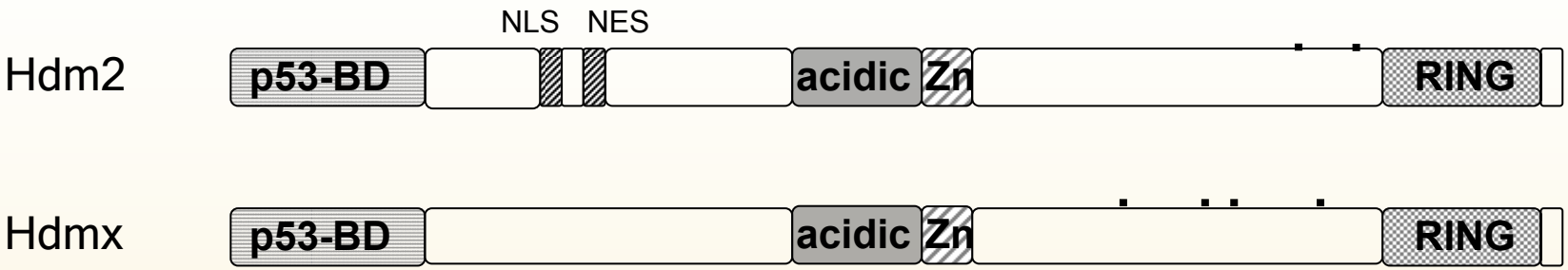


J. Lukas et al., 2004

p53 Activation and Stabilization



Aother Player in the p53 Control Loop: Mdmx



Yaron Pereg

Dganit Shkedy

Rami Khosravi

Sharon Biton

Aart G. Jochemsen (Leiden)

Petra de Graaf

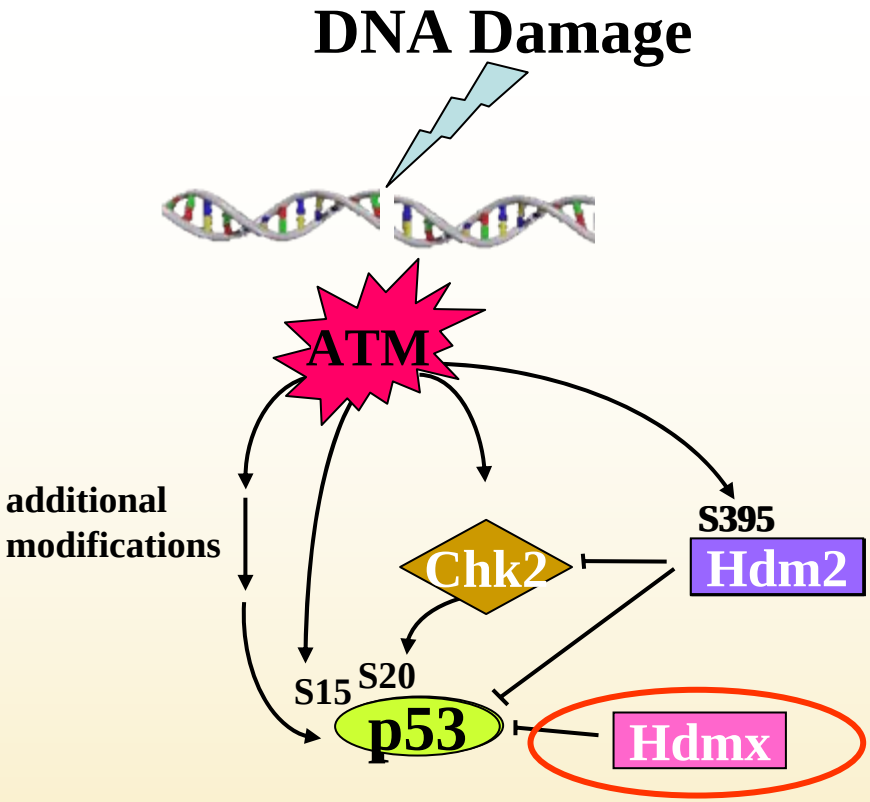
Eric Meulmeester

Wolf Lehmann (Heidelberg)

Marina Edelson-Averbukh

Koji Okamoto (Tokyo)

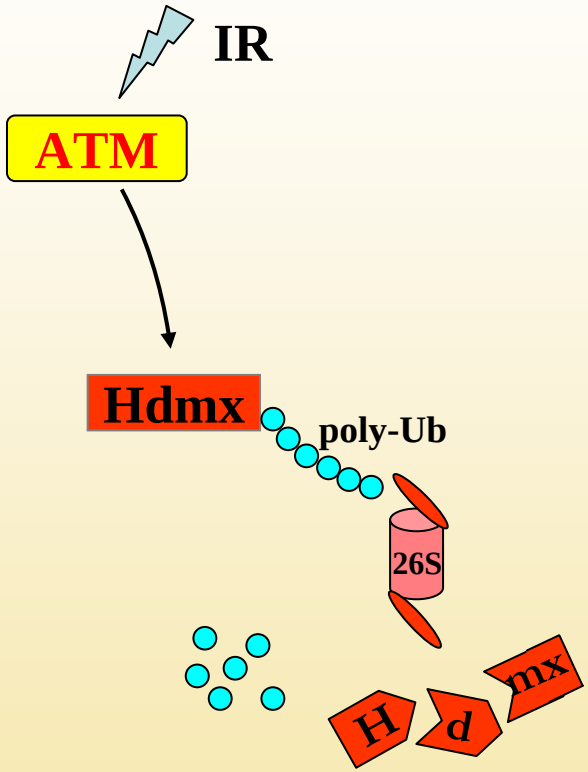
Yoichi Taya (Tokyo)

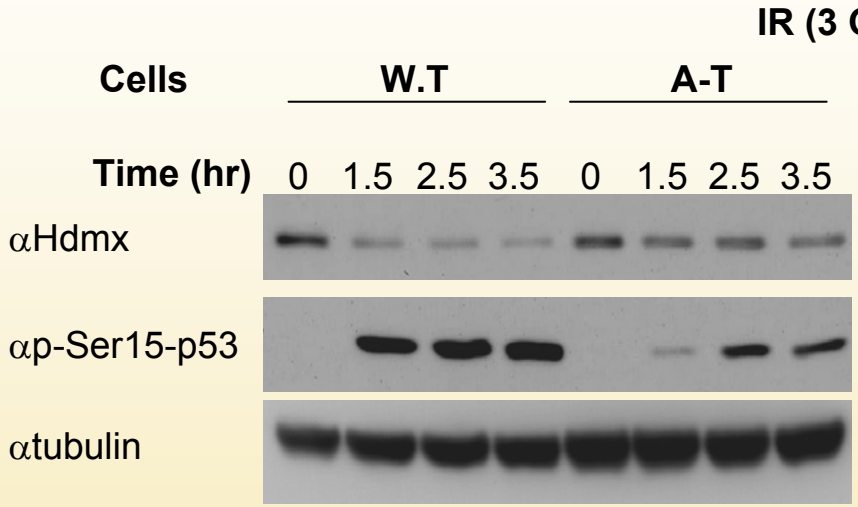


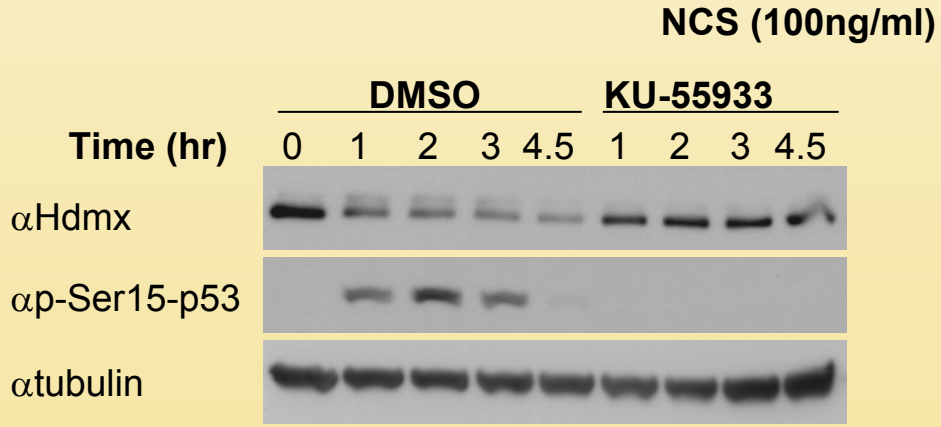
Following DNA damage Hdmx is degraded in an Hdm2-dependent manner (Pan & Chen, 2003; Kawai et al., 2003)

Is Hdmx degradation ATM-dependent?

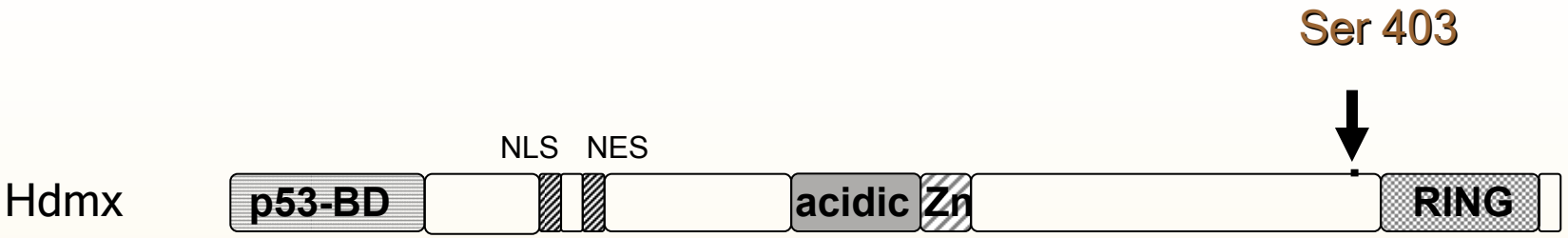
ATM Dependence of Damage-Induced Degradation of Hdmx

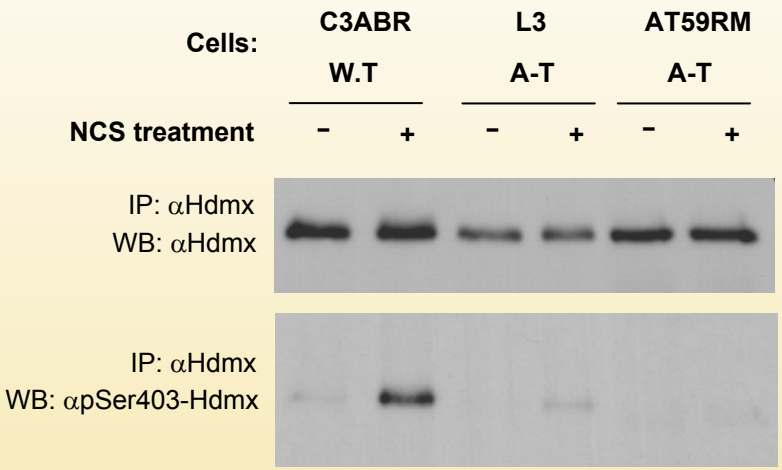


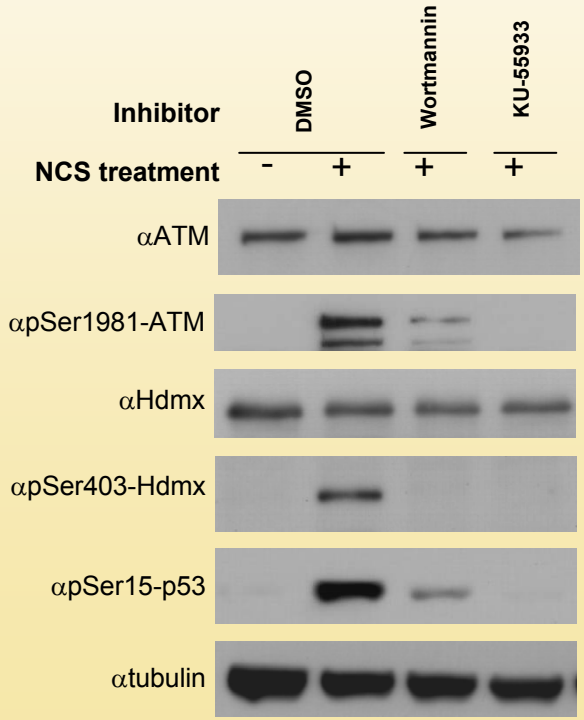




ATM-Mediated Phosphorylation of Ser403

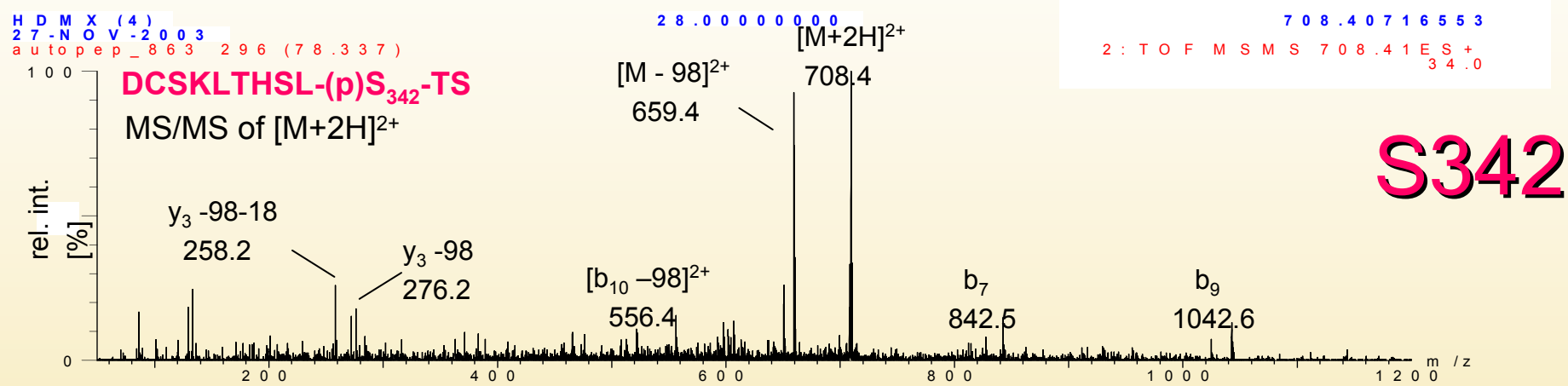


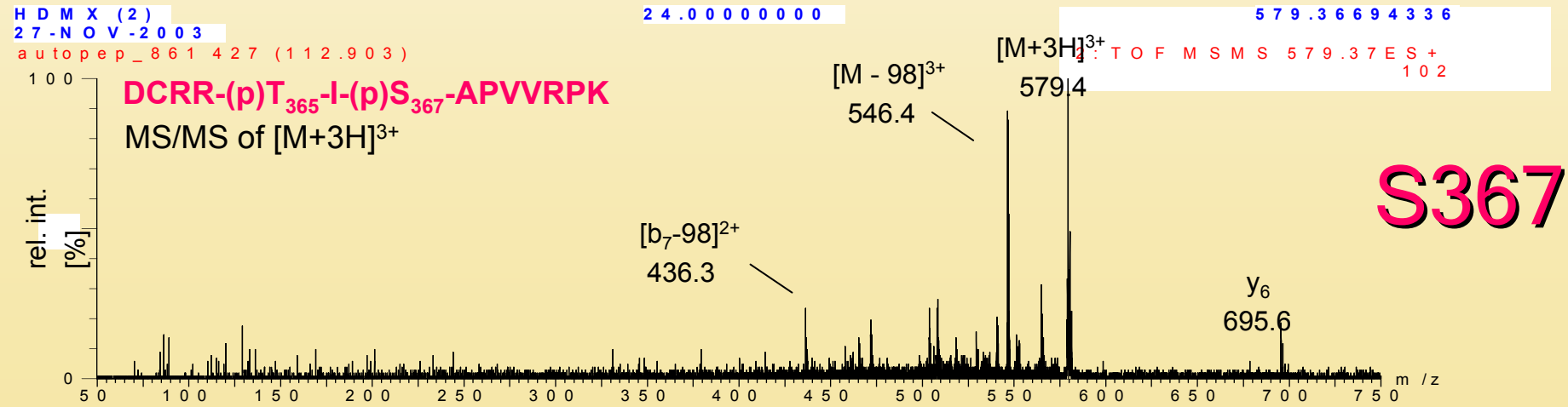




Identification of Protein Phosphorylation using Mass Spectrometry

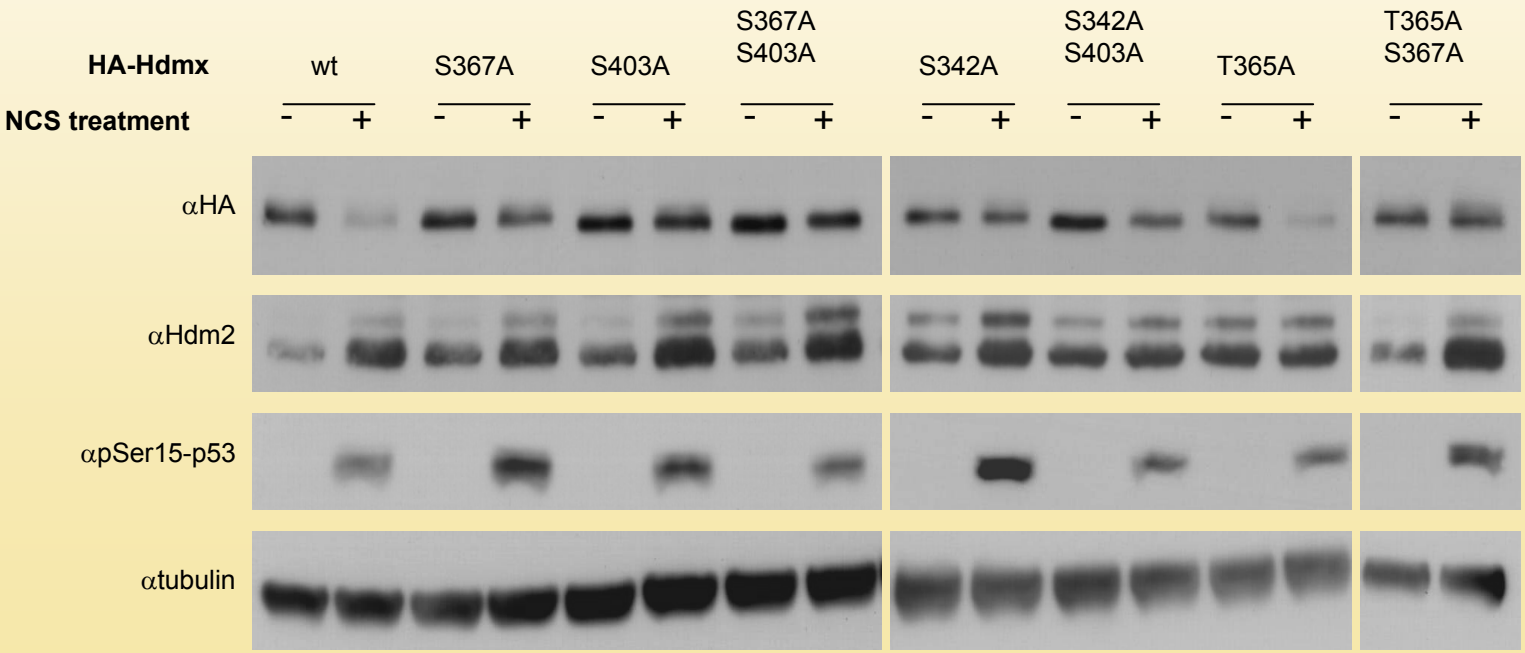
Wolf Lehmann



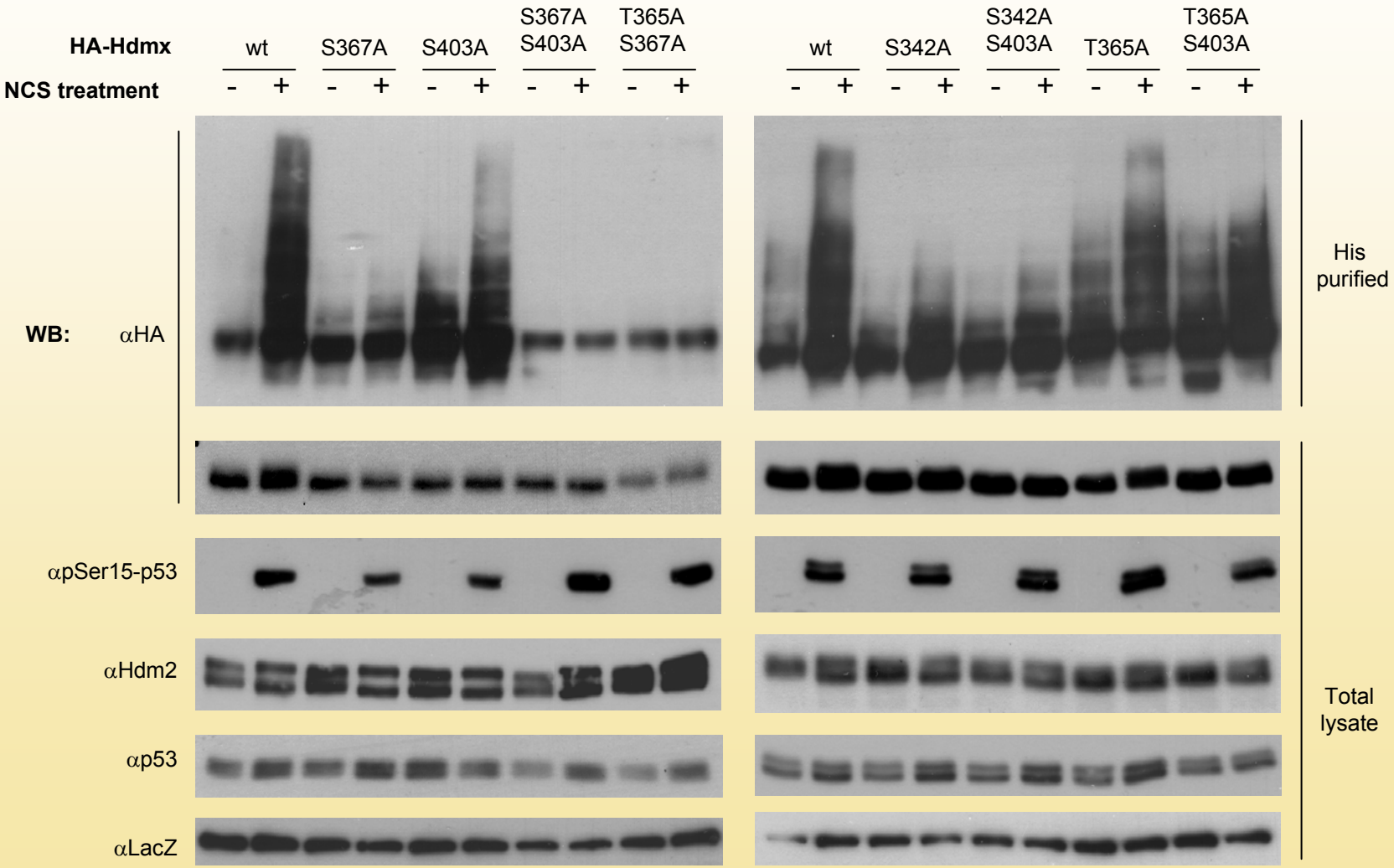


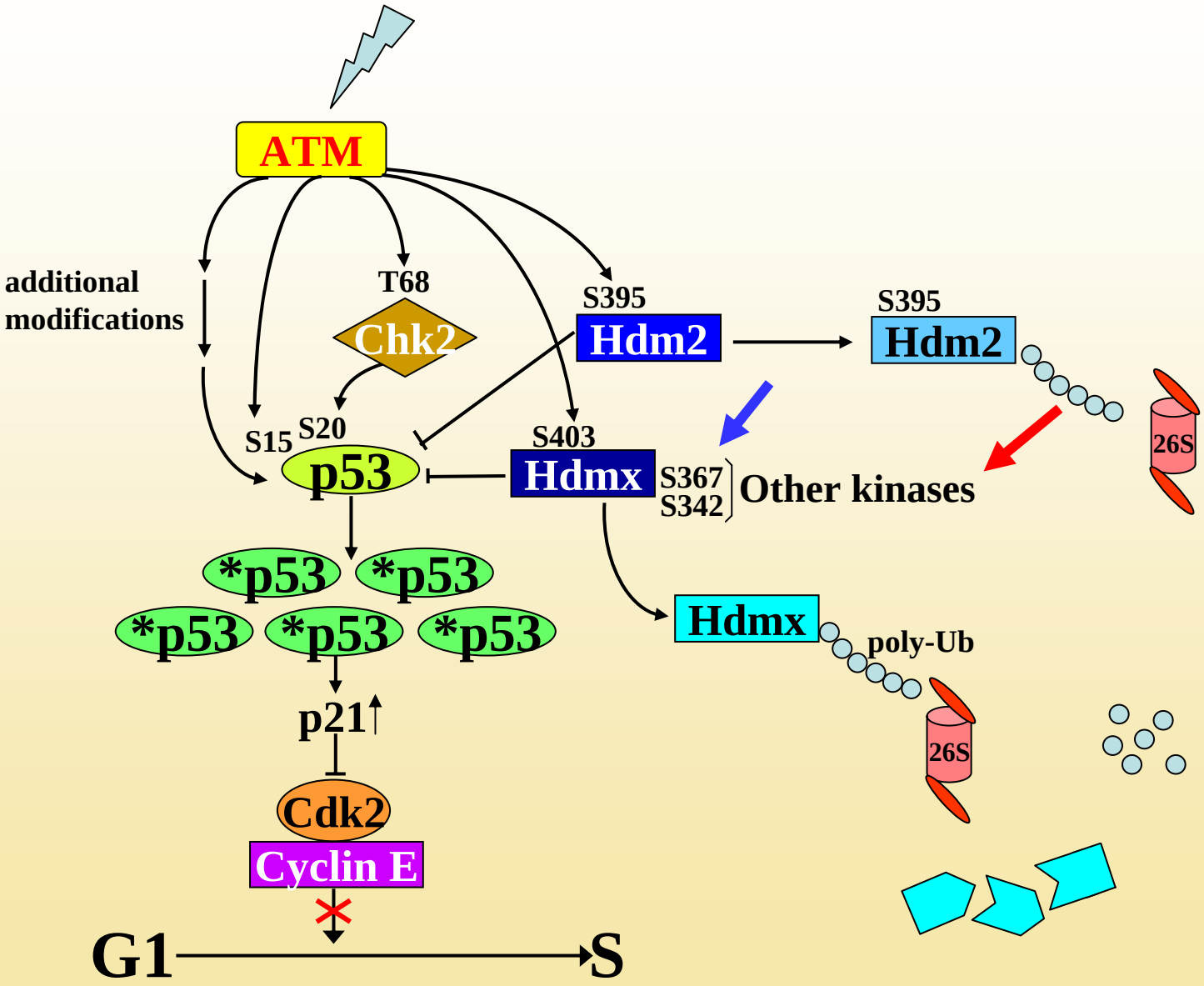
Role of Phosphorylations in Hdmx Degradation

Hdmx

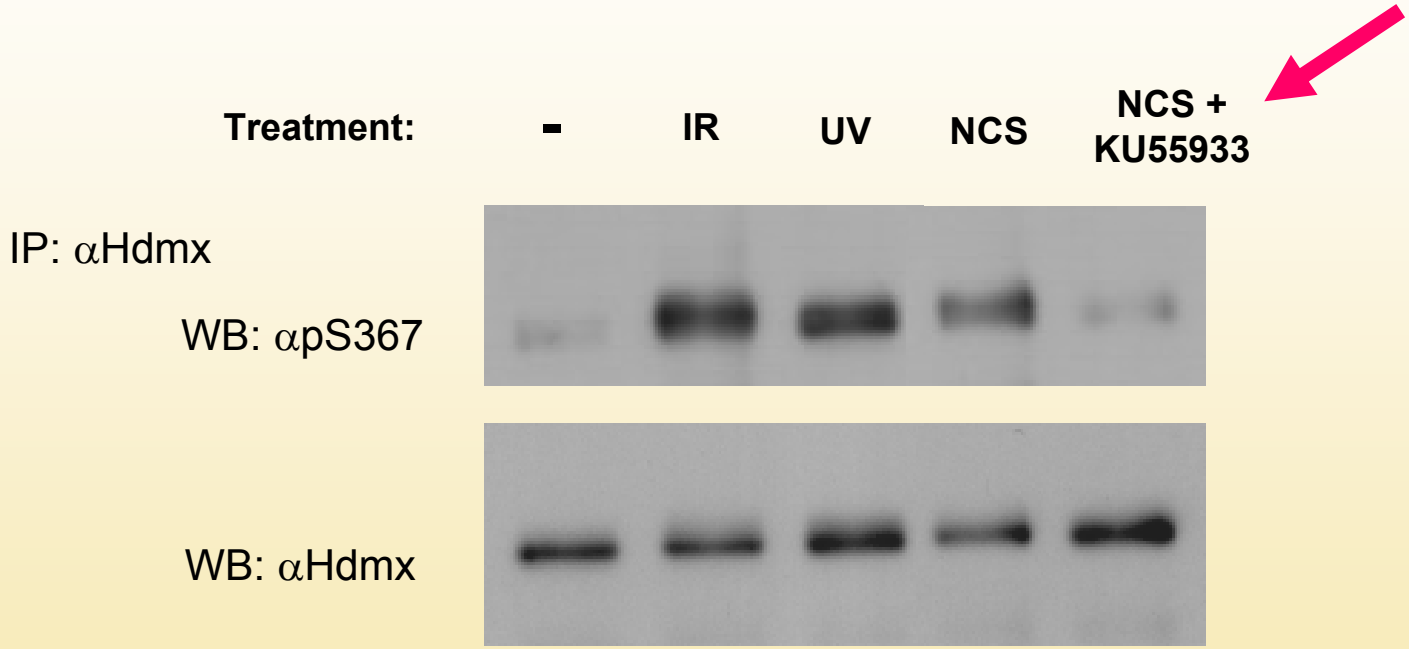


Role of Hdmx Phosphorylations in its Ubiquitination



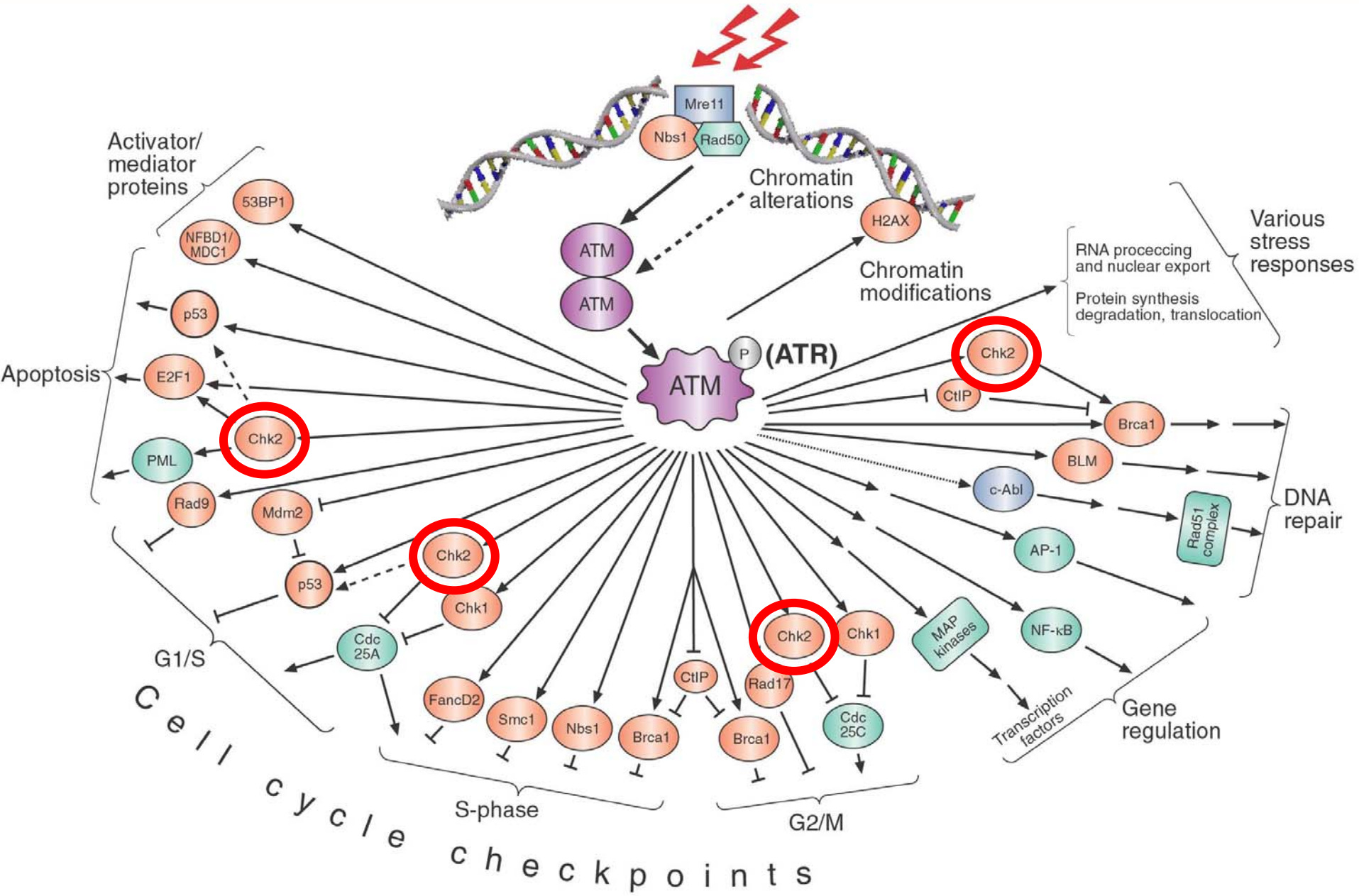

ATM
additional
modifications
T68
Chk2
S15
S20
p53
S395
Hdm2
S403
Hdmx
S367
S342
Other kinases
S395
Hdm2
26S
*p53
*p53
*p53
*p53
*p53
p21↑
Cdk2
Cyclin E
G1
S
Hdmx
poly-Ub
26S

Ser367 Phosphorylation in Response to DNA Damage

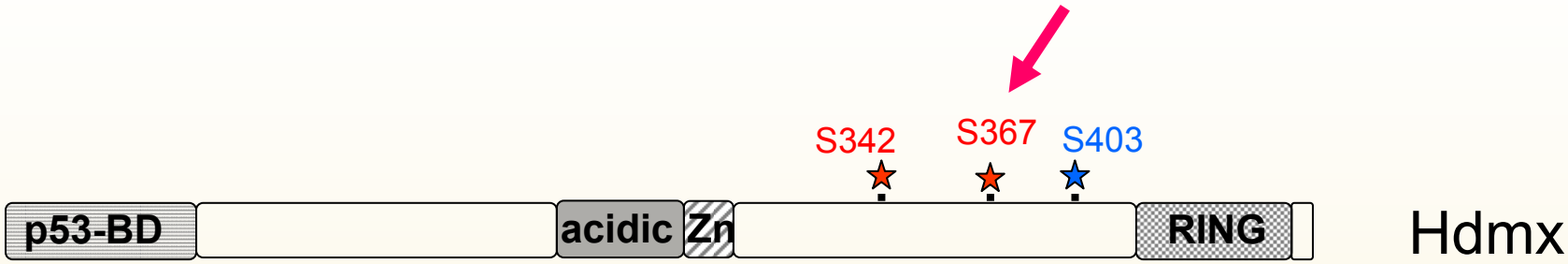


Candidate kinase?

ATM-Mediated DNA Damage Responses

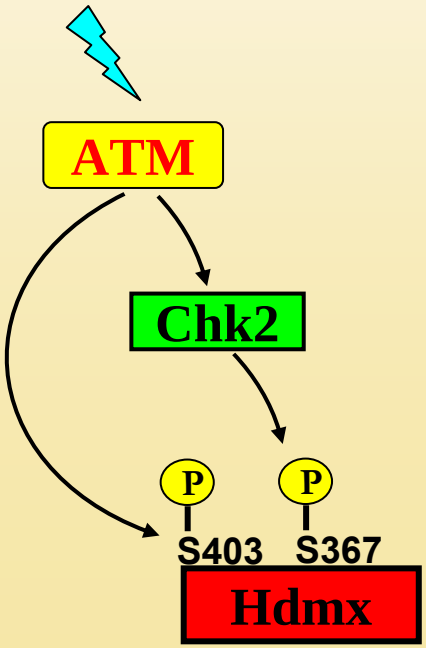


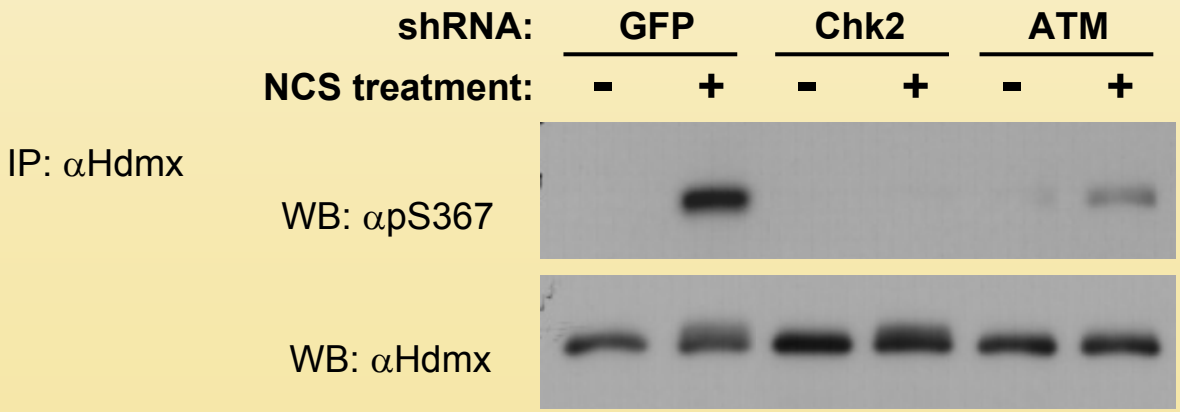
Chk2: Candidate Kinase for Ser367 Phosphorylation



Chk2 consensus phosphorylation site: R X X S

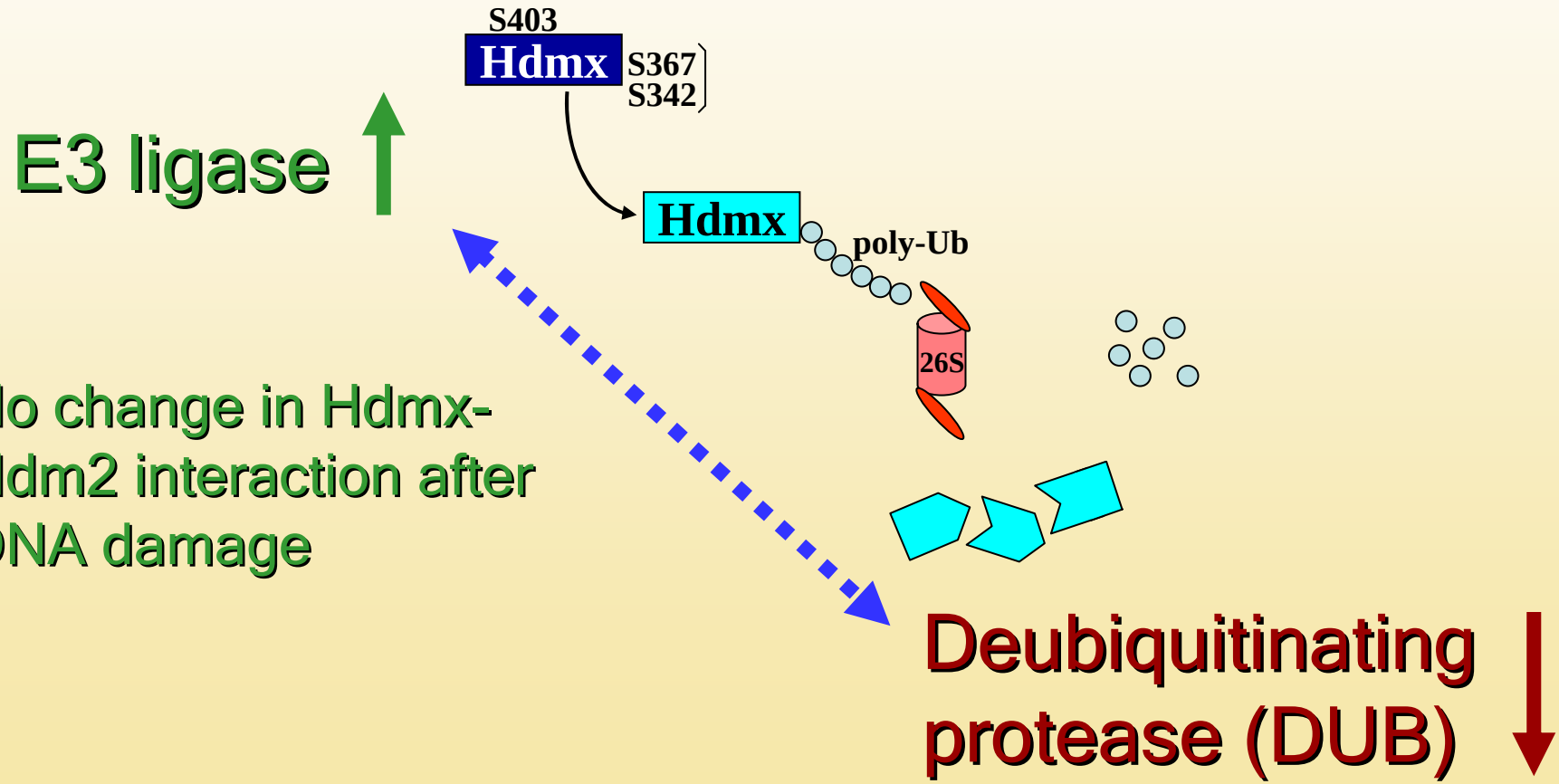
Hdmx: R T I S — S367





Mechanism, Mechanism....

Protein degradation via the proteasome pathway

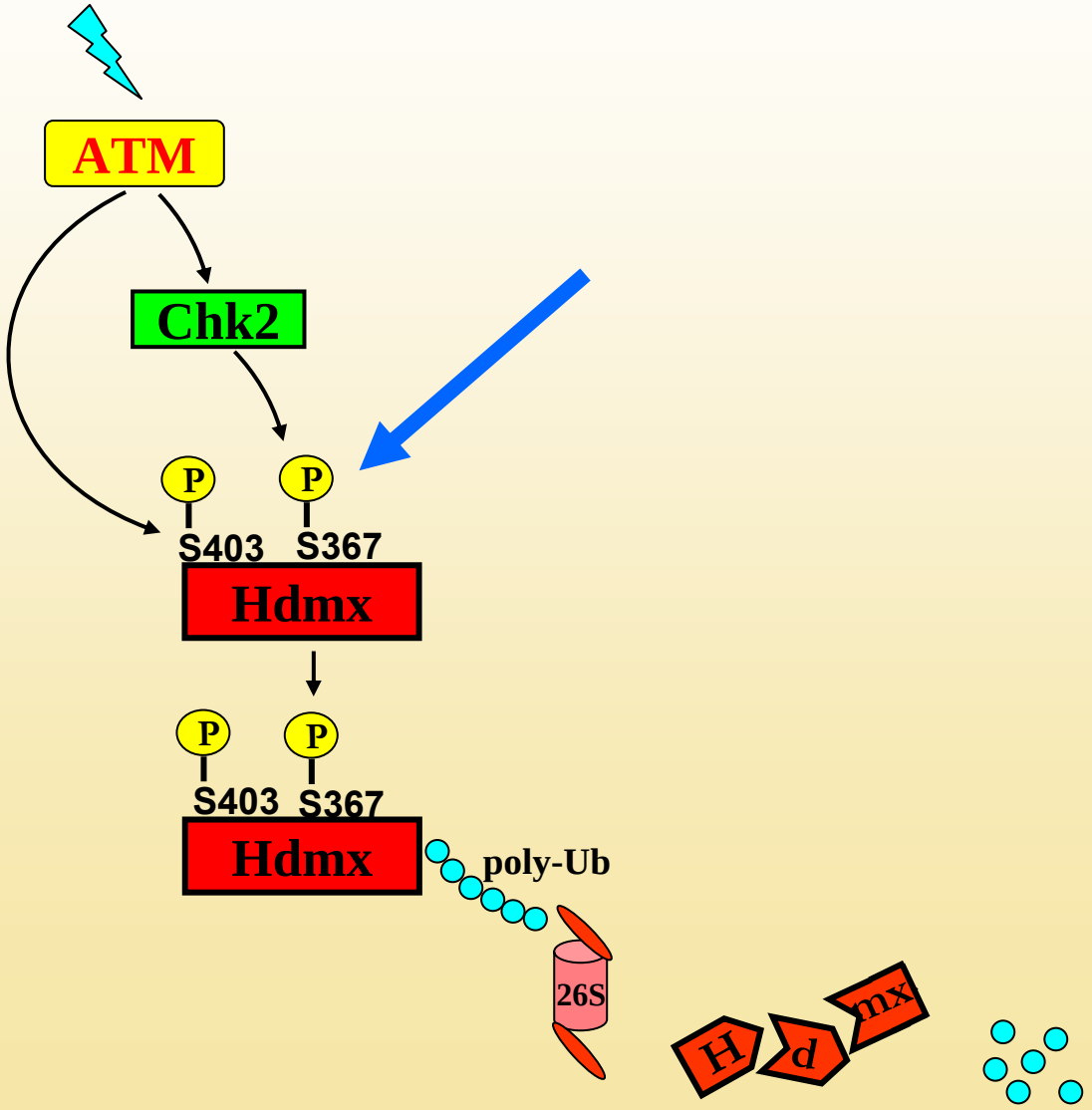


HAUSP is a DUB for Hdmx

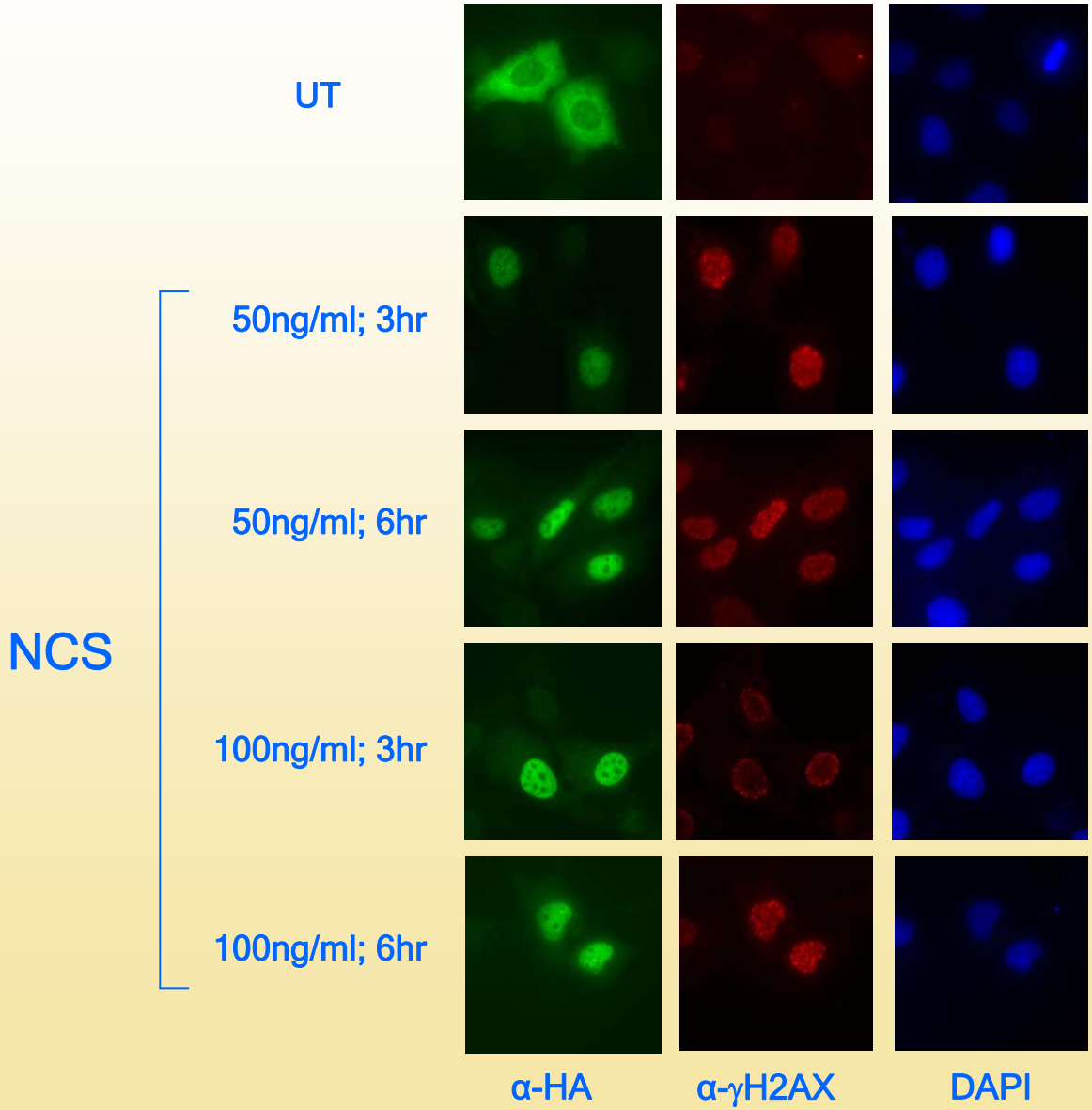
E. Meulmeester et al., 2005

- Herpes virus-associated ubiquitin specific protease (also known as USP7)
- Previously known to deubiquitinate p53 and Hdm2
- HAUSP binds, deubiquitinates and stabilizes Hdmx
- DNA damage does not alter HAUSP's enzymatic activity, stability or subcellular localization
- DNA damage reduces HAUSP's binding to Hdmx and Hdm2 but NOT p53
- The reduction in HAUSP-Hdmx affinity is ATM-dependent and is due to phosphorylation of Hdmx

Significance of S367 Phosphorylation



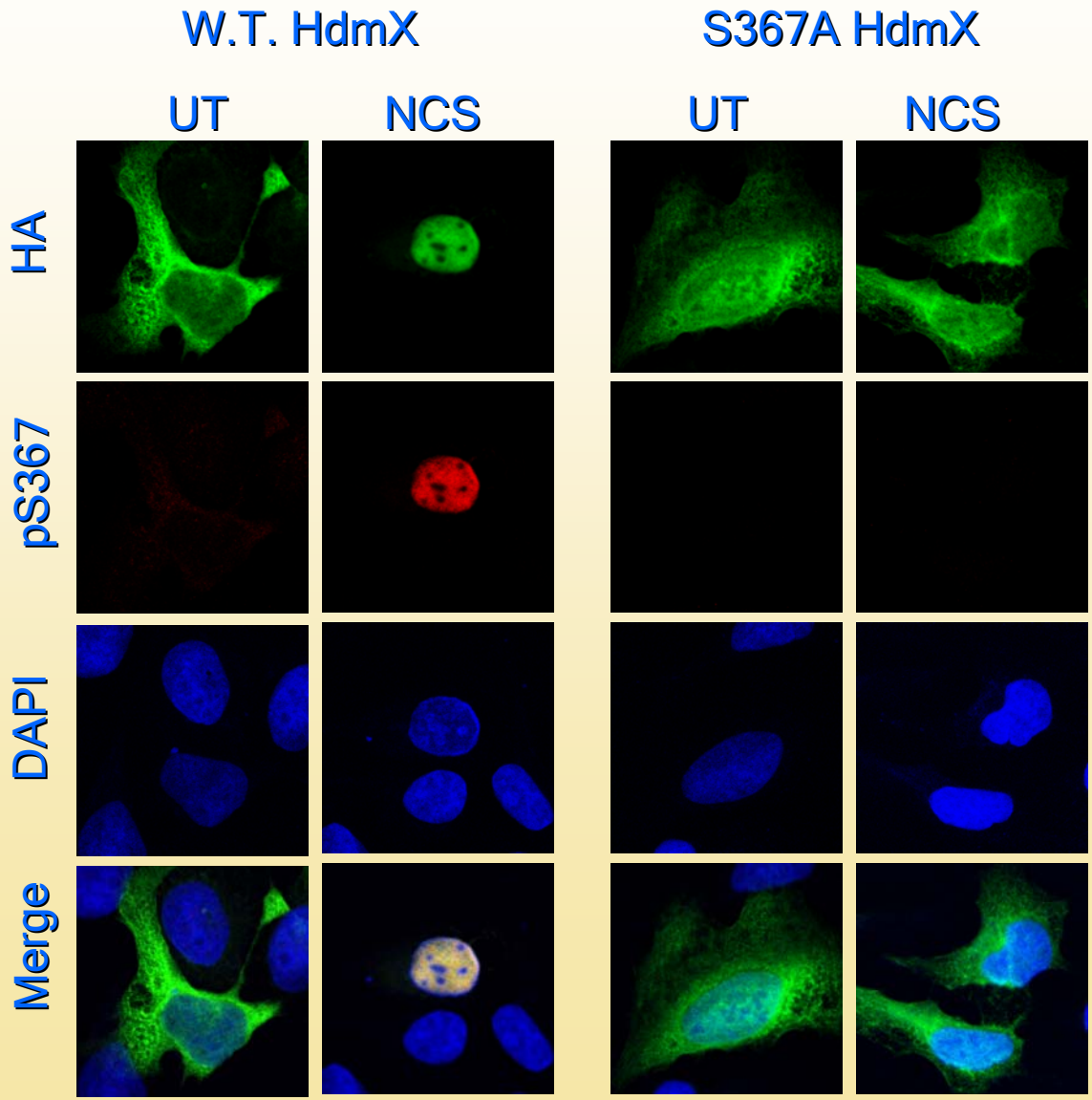
Nuclear Accumulation of Hdmx in Response to DSB



Mediated by Hdmx phosphorylations?

Nuclear import or nuclear retention?

Nuclear Accumulation of Hdmx in Response to DSB is Dependent on S367



Ser403 is NOT required for nuclear accumulation of Hdmx

S367 phosphorylation takes place in the nucleus!

Hdmx undergoes nuclear retention

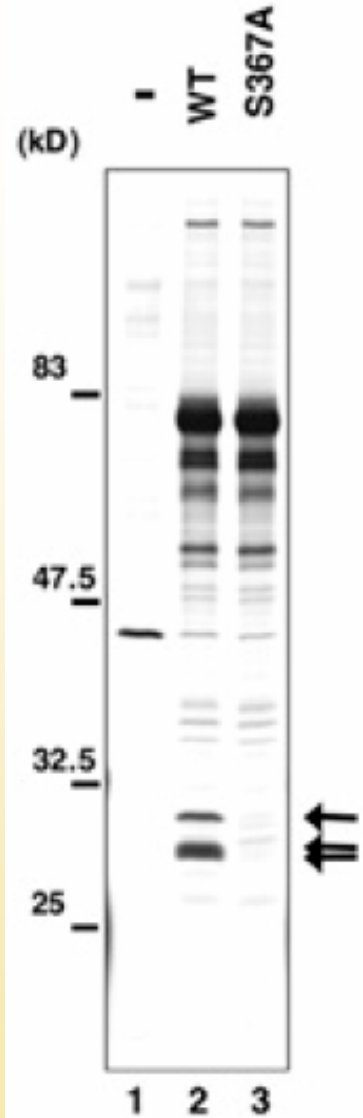
S367 Phosphorylation Creates a 14-3-3 Binding Site

K. Okamoto et al., 2005

14-3-3 binding motif: RXXXS(P)XP

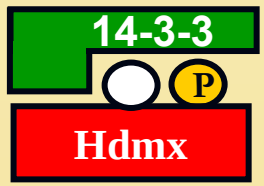
Hdmx: RRTIS(P)AP

T365 pS367

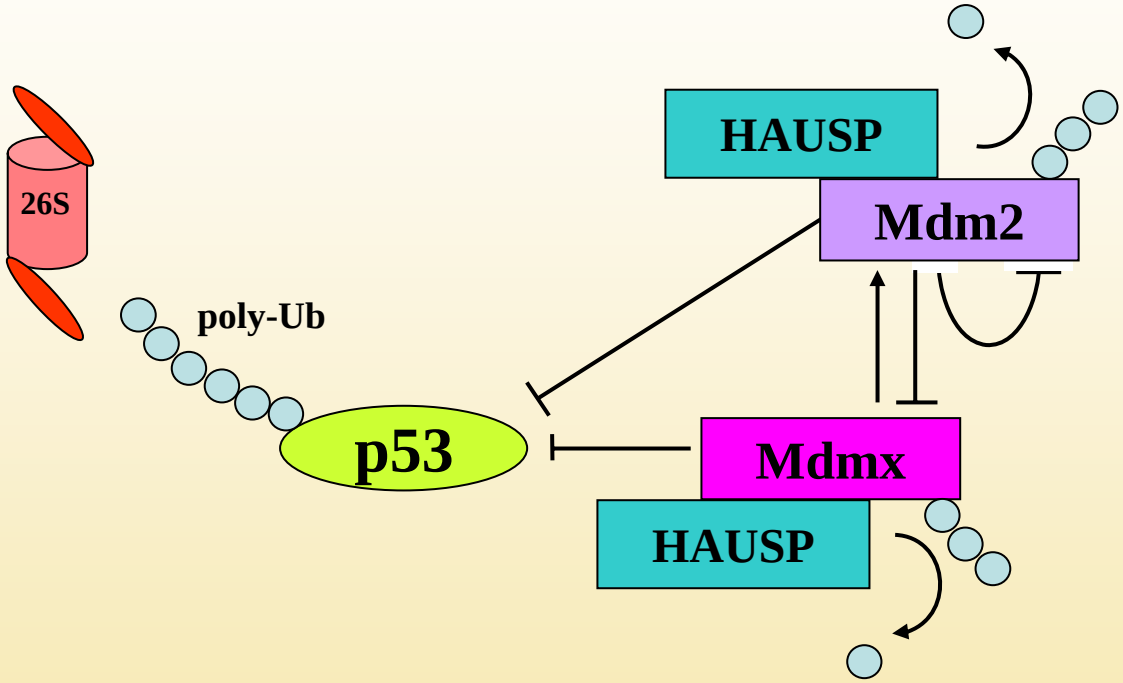


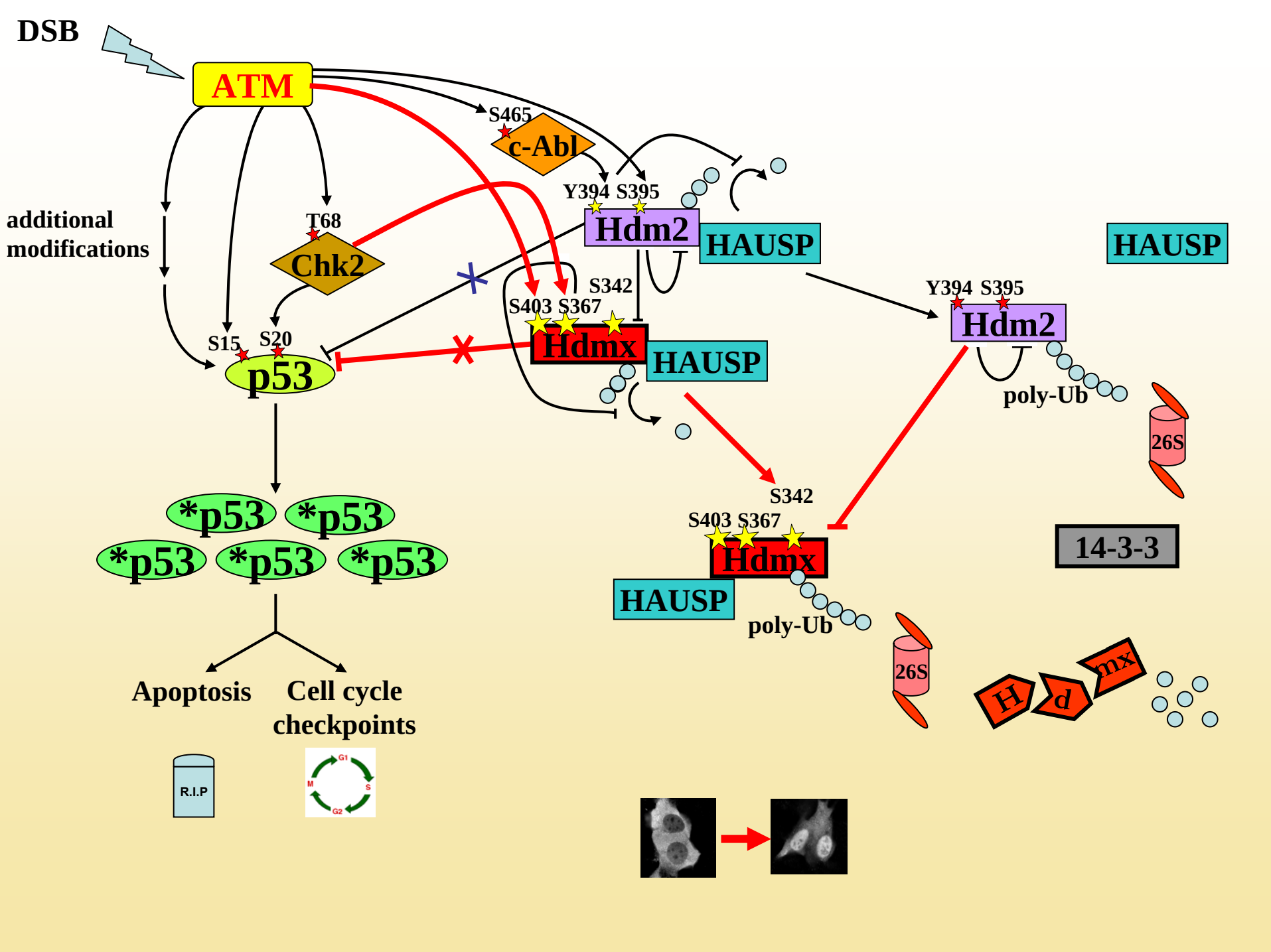
14-3-3 binding is enhanced by DNA damage and is important for Hdmx degradation

14-3-3 binding is essential for nuclear accumulation of Hdmx

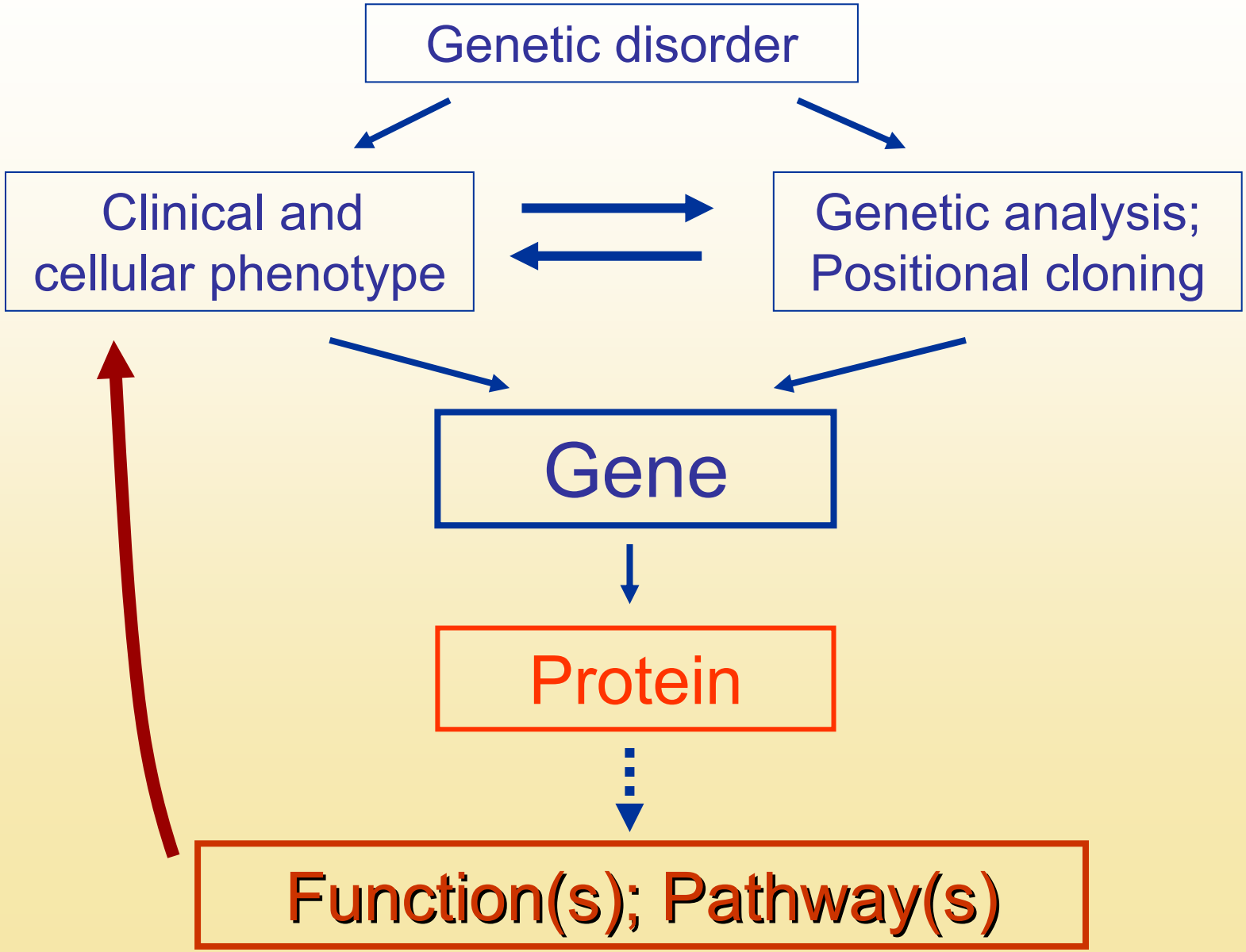


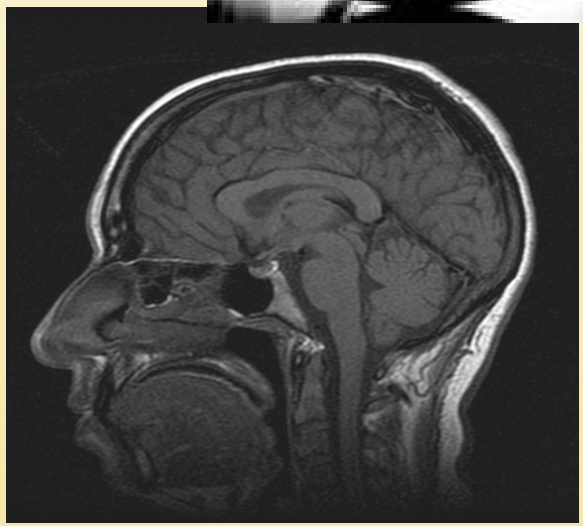
Non-Stressed Cells



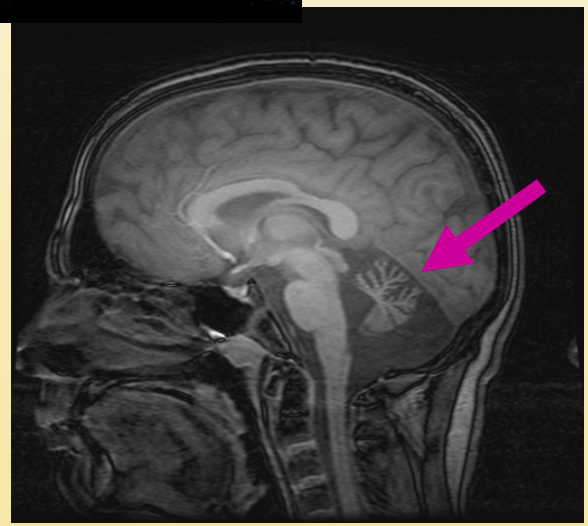

DSB
ATM
S465
c-Abl
Y394 S395
Hdm2
HAUSP
HAUSP
additional
modifications
T68
Chk2
S342
S403 S367
Hdmx
HAUSP
Y394 S395
Hdm2
S15 S20
p53
poly-Ub
26S
*p53
*p53
*p53
*p53
*p53
S342
S403 S367
Hdmx
14-3-3
HAUSP
poly-Ub
26S
Apoptosis
Cell cycle
checkpoints
R.I.P
G1
S
G2
M
H
d
mx

From Genetic Disorder to Function



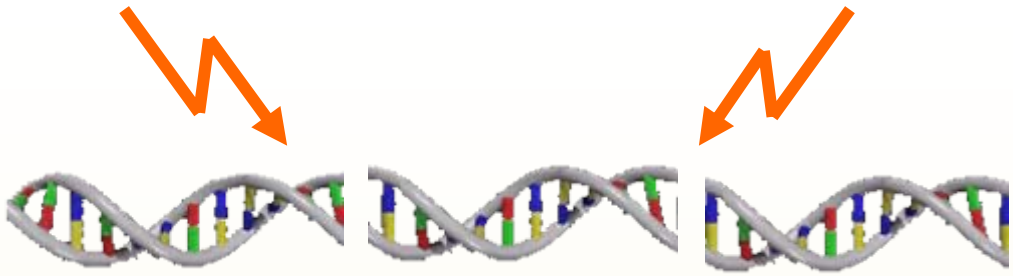


Normal brain



A-T Patient

DSBs



ATM

Observations in genomic instability syndromes…

Activated **ATM**

Phosphorylation

Effectors

Survival pathways

Cell death pathways

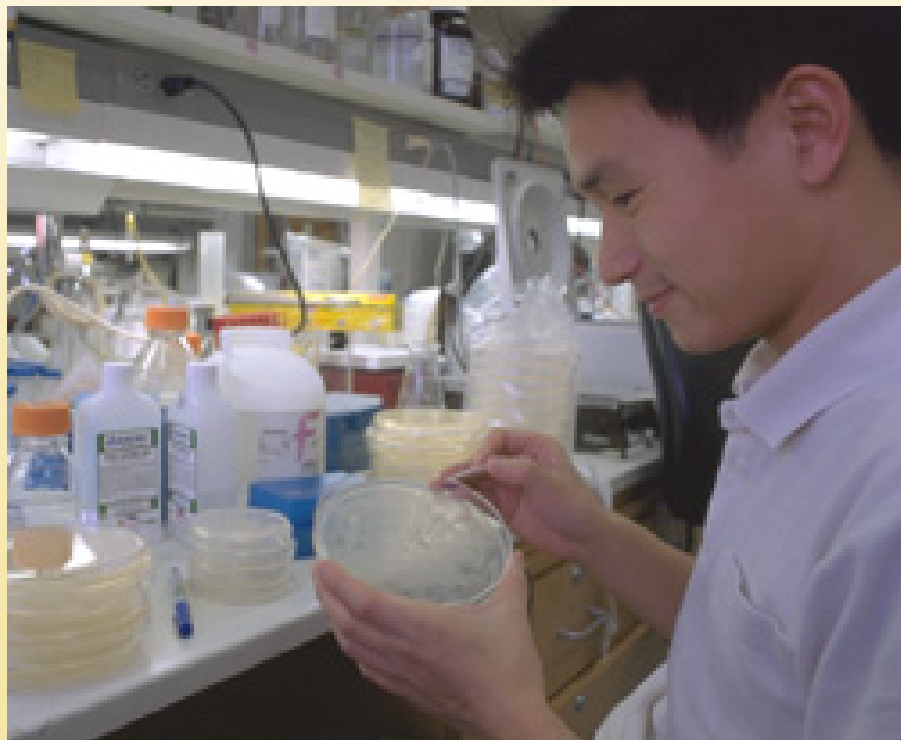
DSB repair

Cell cycle checkpoints

Stress responses

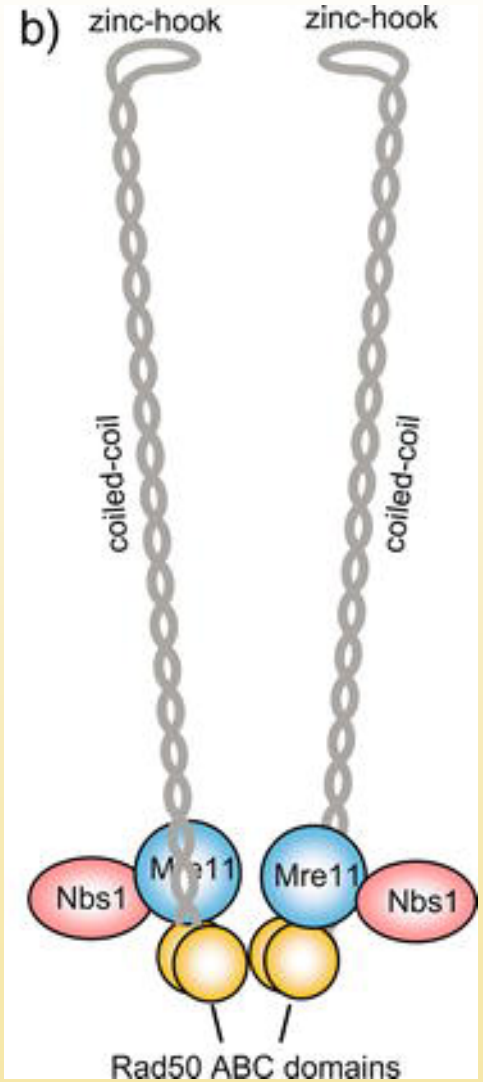
Apoptosis

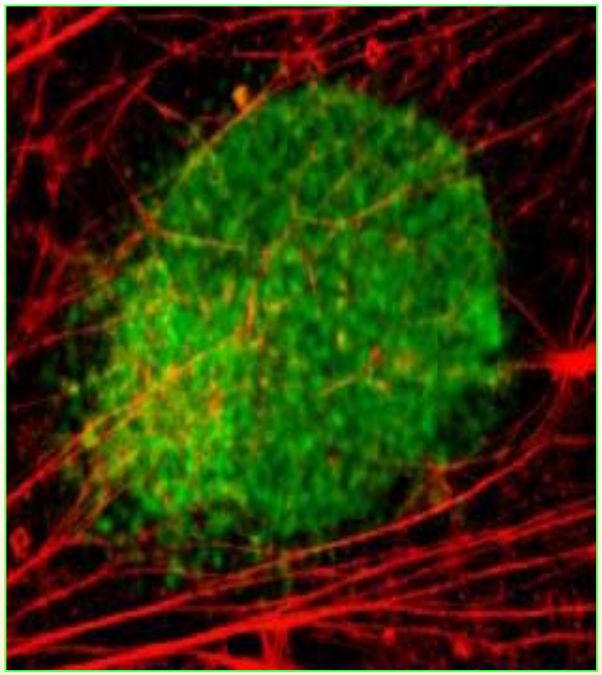
Cross-talk Between the Clinic and the Lab



A-T-Like Disease (A-TLD)

- Has most of the major characteristics of A-T with later onset and slower progression
- Hypomorphic mutations in the *MRE11* gene
- A-TLD is the only A-T-like disorder in which there is no mutation in the *ATM* gene and the ATM protein is intact and active





Tamar Uziel:

Is the response of ATM to DNA damage dependent on functional MRN complex?

Requirement of the Mre11-Rad50-Nbs1 Complex for ATM Activation by DNA Damage

(Uziel et al., 2003)

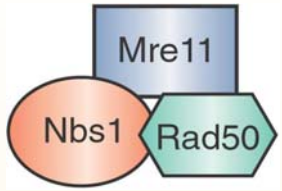
A-TLD cells:

- Defects in all parameters of ATM activation and in downstream pathways
- Phenotypic reconstitution of A-TLD cells by ectopic expression of Mre11 restores normal ATM activation
- Knocking down Mre11 results in defective ATM activation

DSBs

binding to DSBs;
damage processing

MRN complex



Processed
DNA lesions

ATM

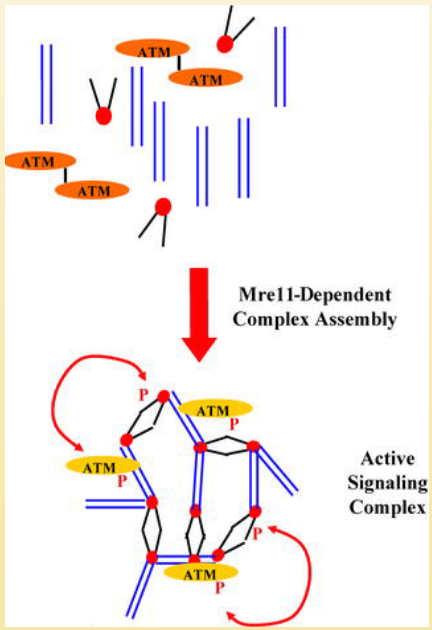
Activation
Association with
processed DSBs

Activated
ATM

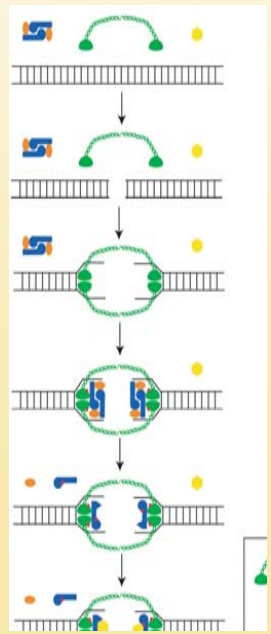
phosphorylation

Target proteins

Signaling pathways

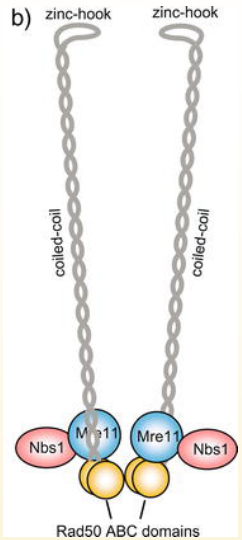


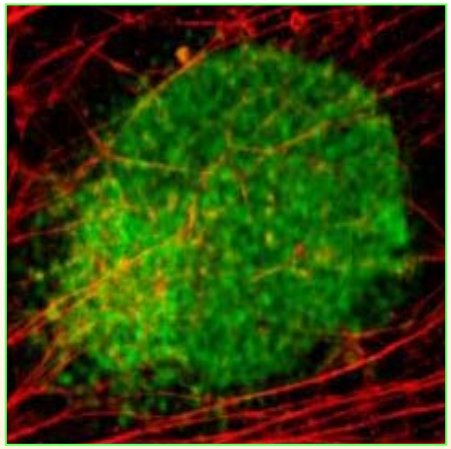
Costanzo et al., 2004

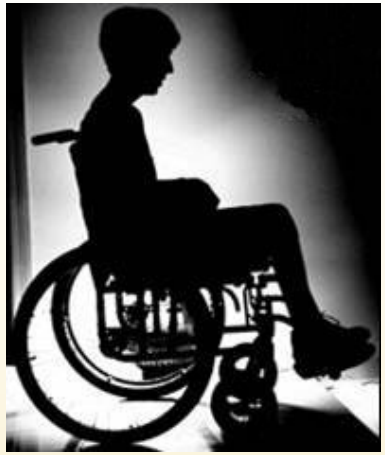


Paull & Lee, 2005

Insights from Understanding A-TLD

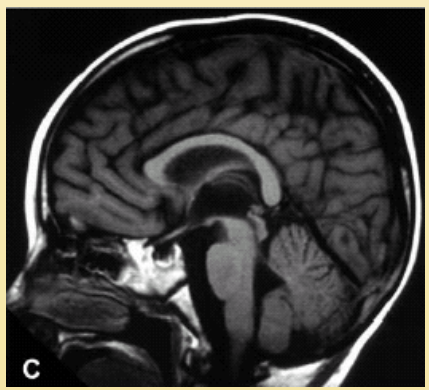




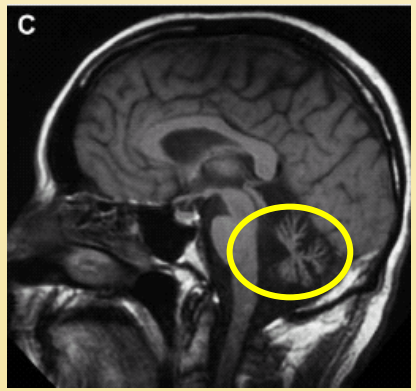


- Inability to activate ATM properly is sufficient to cause an A-T-like phenotype
- **The cerebellar degeneration in A-T patients probably results from their defective response to DNA damage**

Normal



A-T



ATM Localization and Function in Neuronal Cells

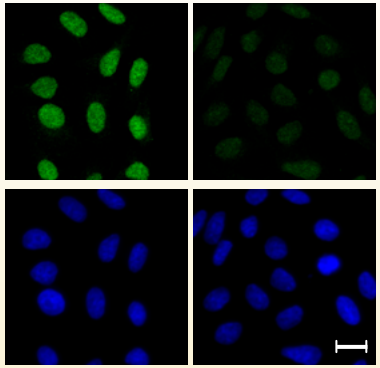
- Human neuron-like cells:
  - In vitro differentiation of neuroblastoma cell lines
  - Neuronal differentiation of human embryonic stem cells (with B. Reubinoff)
  - Cerebellar neural stem cells (with NeuralStem, Inc.)
- Murine cerebellar cells (Ari Barzilai):
  - Tissue sections
  - Slice cultures

ATM is Nuclear Human Neuron-Like Cells

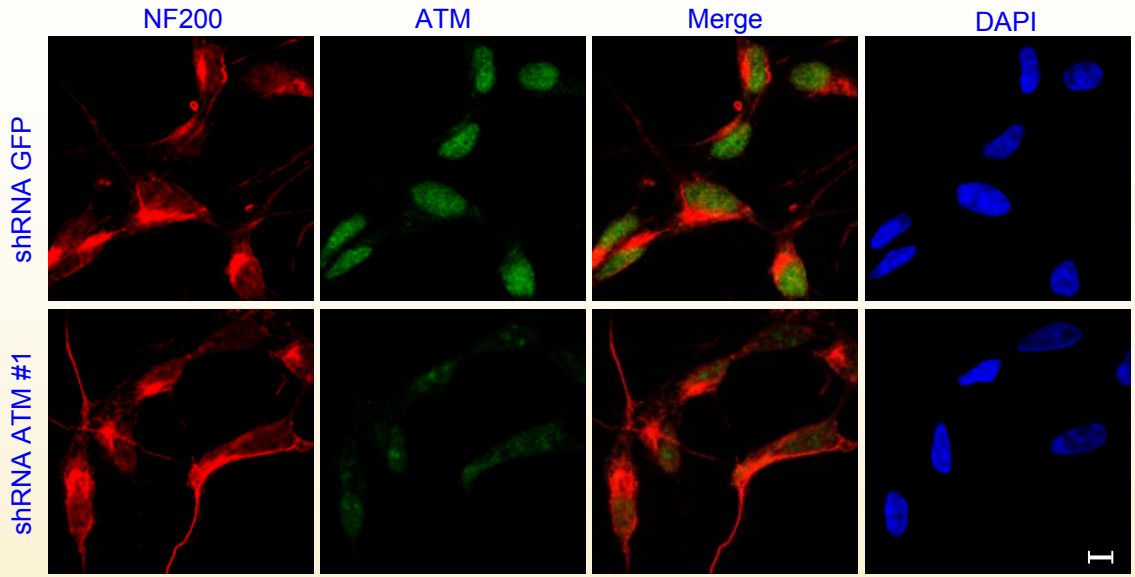
Sharon Biton

HeLa cells

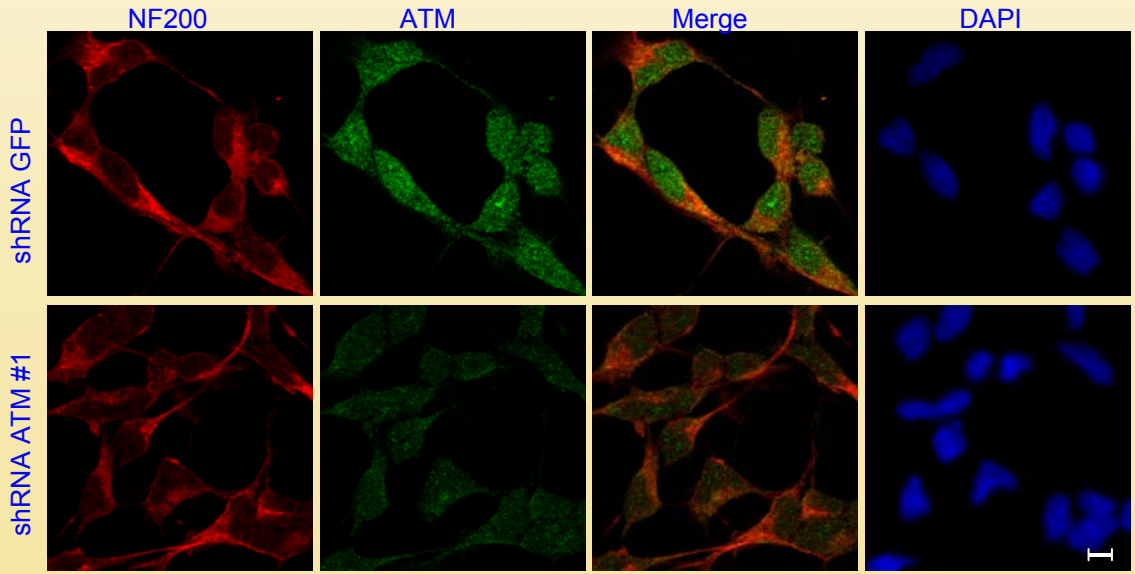
shRNA: LacZ | shRNA: ATM



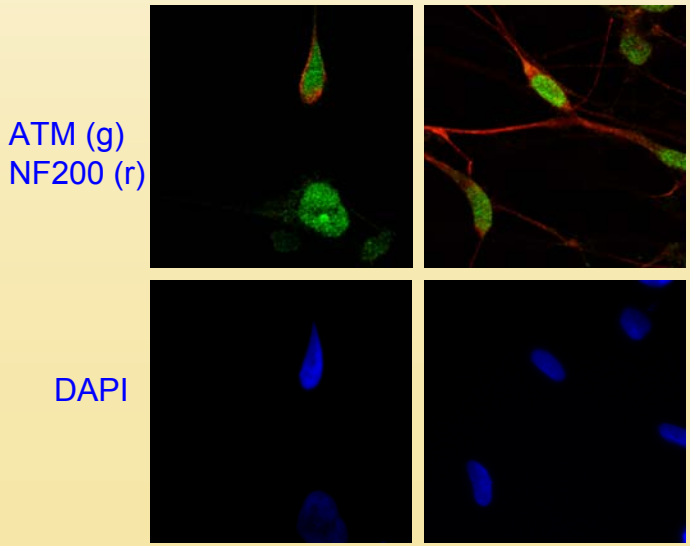
SH-SY5Y NLCs



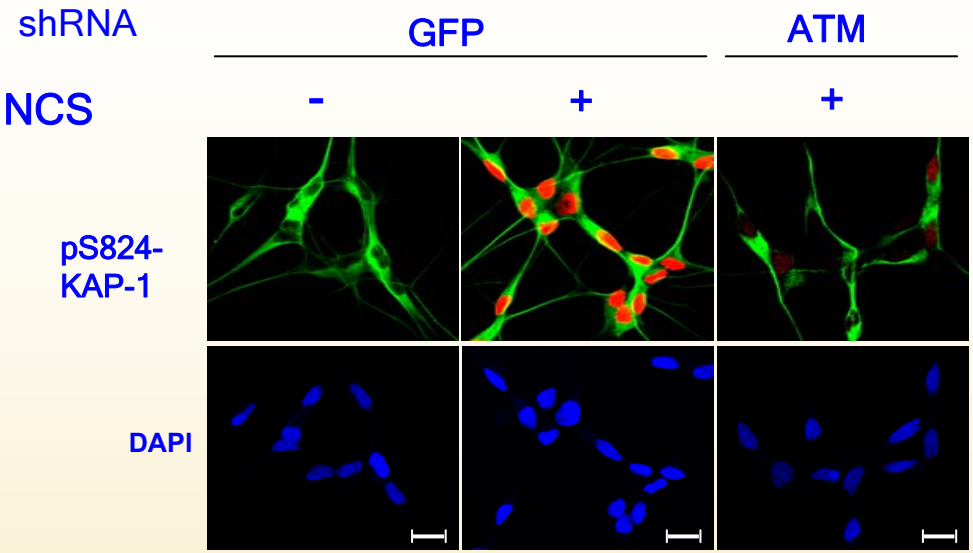
LA-N-5 NLCs

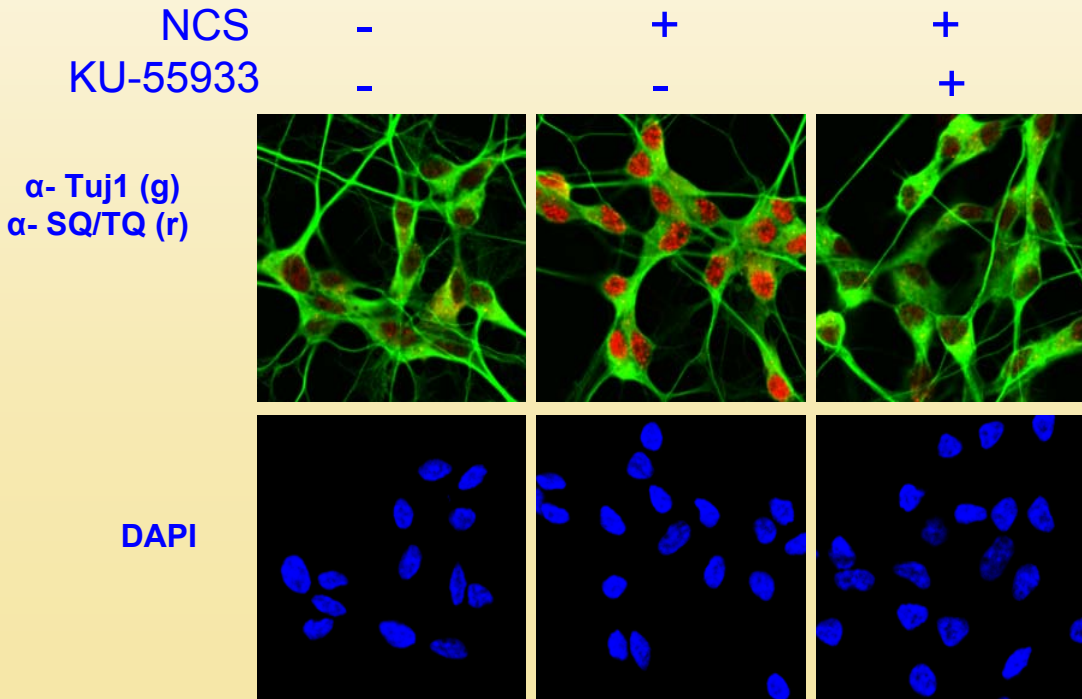


Differentiated hESC



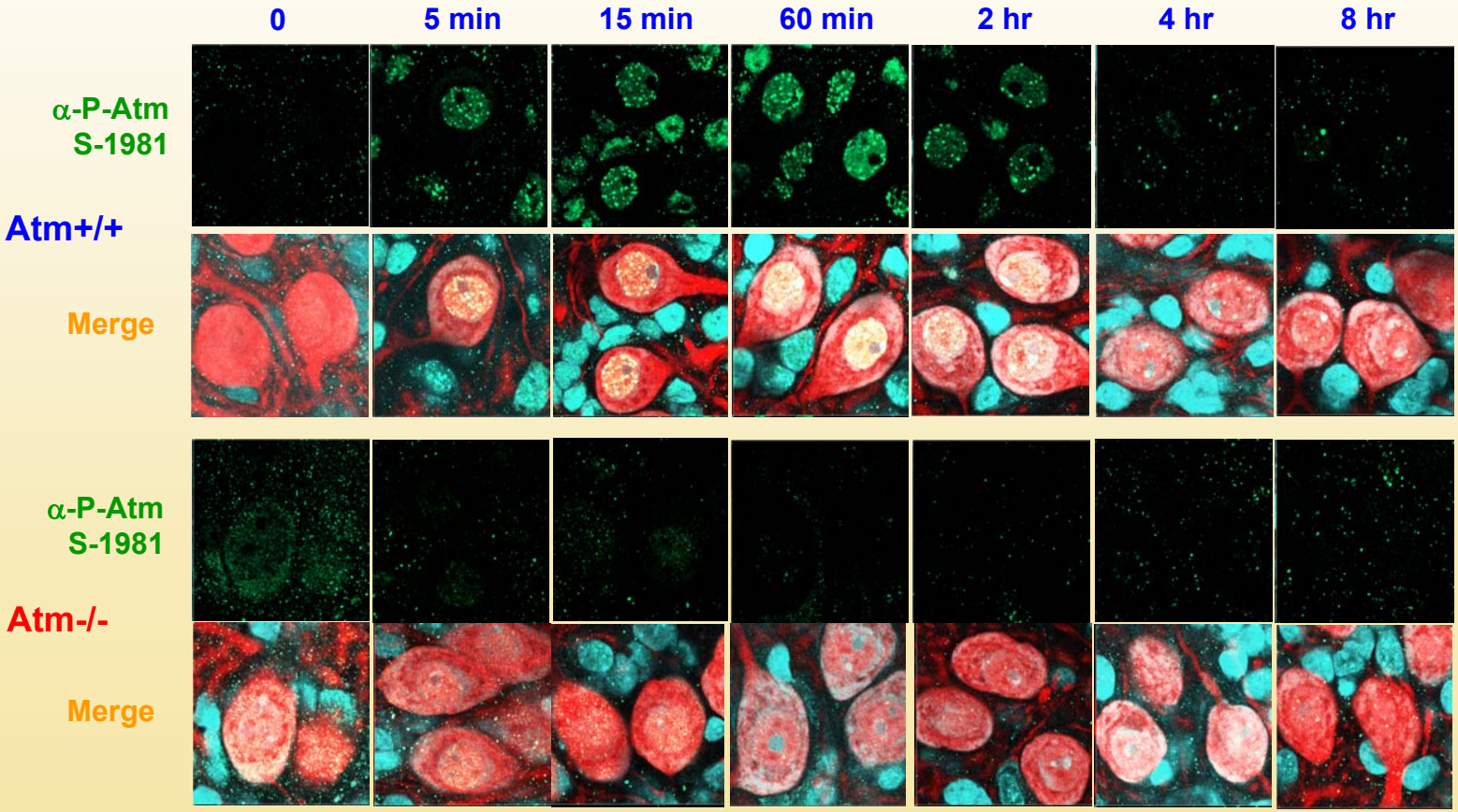
ATM- Dependence DNA Damage Responses



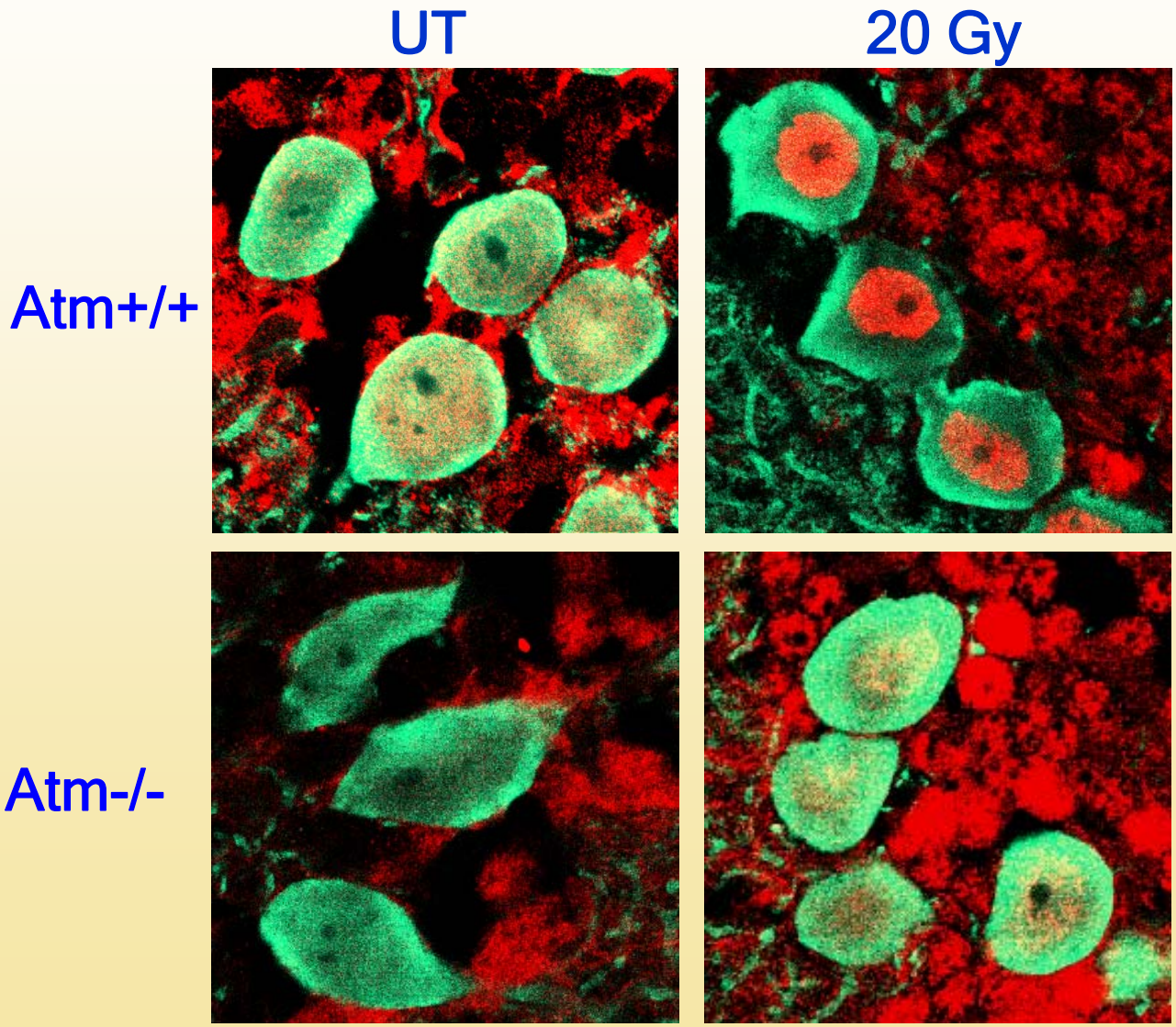


ATM-Dependent DNA Damage Responses in Mouse Cerebellar Neurons

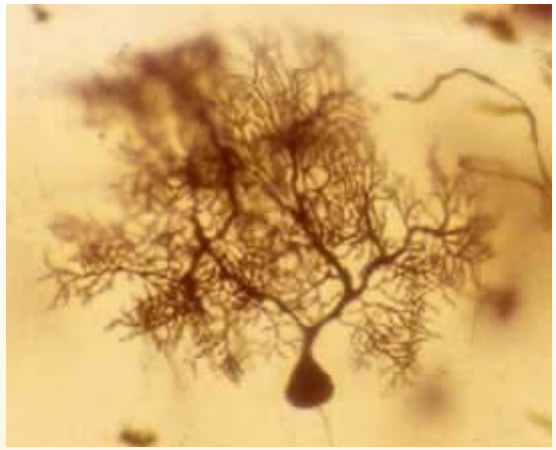
Inbal Dar
Ari Barzilai



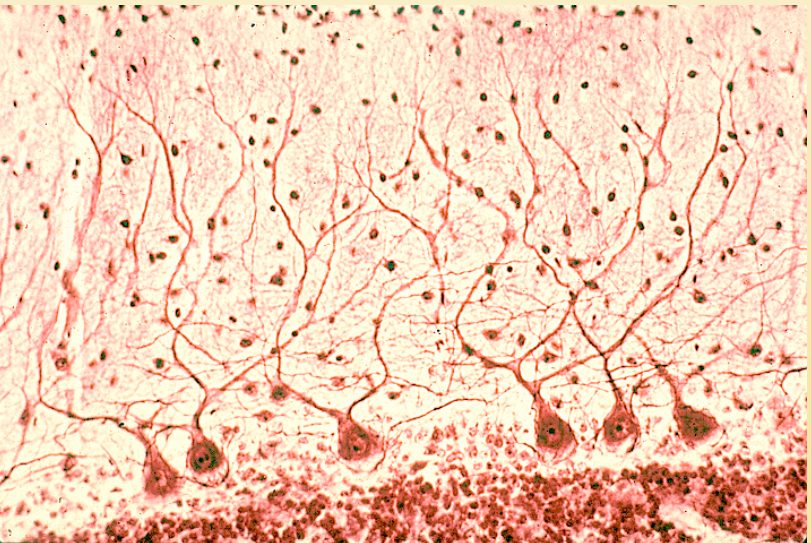
ATM Autophosphorylation in Mouse Cerebellar Neurons



Importance of the DNA Damage Response in Neurons

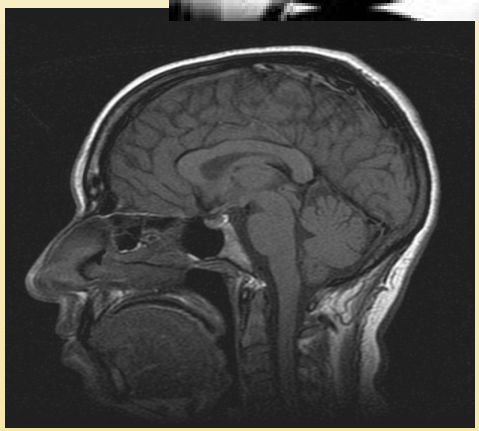


- Long life-span
- Extensive metabolic activity
- Oxidative stress
- High transcriptional activity

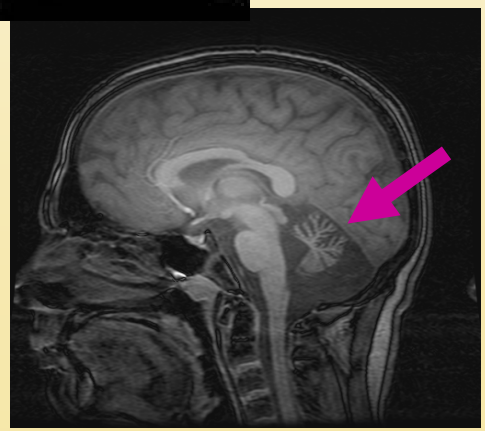


Differences between the DNA damage response in proliferating and post-mitotic cells…

New treatment Modalities?



Normal brain

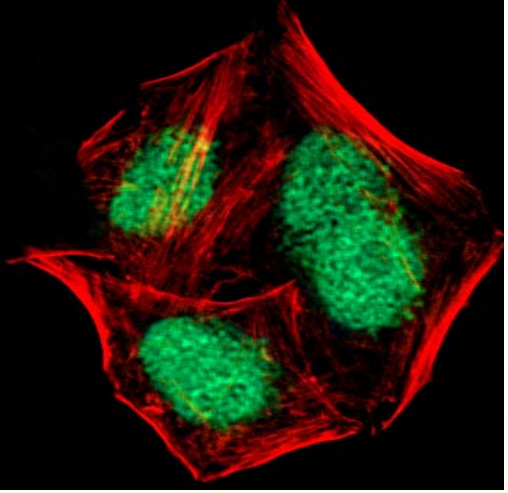


A-T Patient

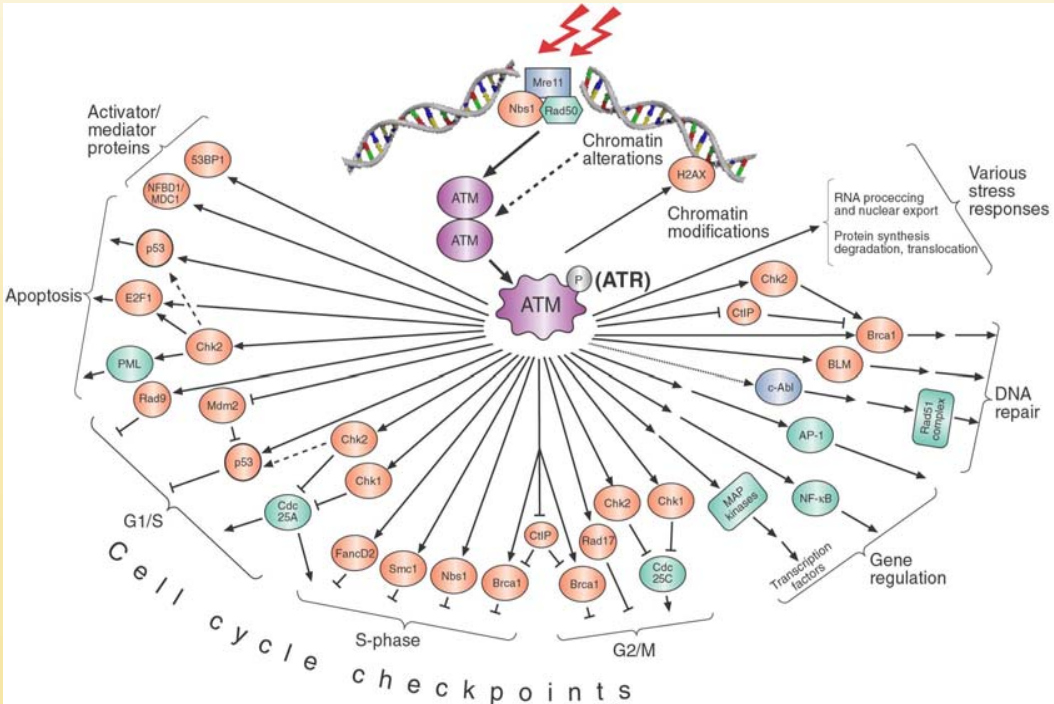
High Throughput Drug Screening

- Readout representing the DNA damage response
- Search for drug that will stimulate ATM-redundant functions
- Stimulation as low as 10% of the normal response may lead to considerably milder phenotype
- Possible mechanism: stimulation of redundant kinases?

Center for Chemical Genomics, National Human Genome Research Institute, NIH



Thank You


Mre11
Nbs1
Rad50
Chromatin alterations
H2AX
ATM
ATM
Chromatin modifications
Activator/ mediator proteins
53BP1
NFBD1/ MDC1
p53
E2F1
Apoptosis
Chk2
PML
Rad9
Mdm2
p53
Chk2
Chk1
Cdc 25A
G1/S
ATM
P
(ATR)
RNA proccecing and nuclear export
Protein synthesis degradation, translocation
Various stress responses
Chk2
CtIP
Brca1
BLM
c-Abl
Rad51 complex
DNA repair
AP-1
MAP kinases
NF-κB
Transcription factors
Gene regulation
Chk2
Chk1
CtIP
Rad17
Brca1
Cdc 25C
FancD2
Smc1
Nbs1
Brca1
S-phase
G2/M
Cell cycle checkpoints