- The first exam will take place on Wednesday October 29, from 1:30pm-3:30pm. The exam will take place in room **CE1103** (i.e. our usual room). Please arrive a bit earlier (by 1:25pm) to find your place and get installed.
- Auxiliary material (books, computer, cell phone, handouts, printouts etc.) is not allowed.
- Please bring your Camipro card.

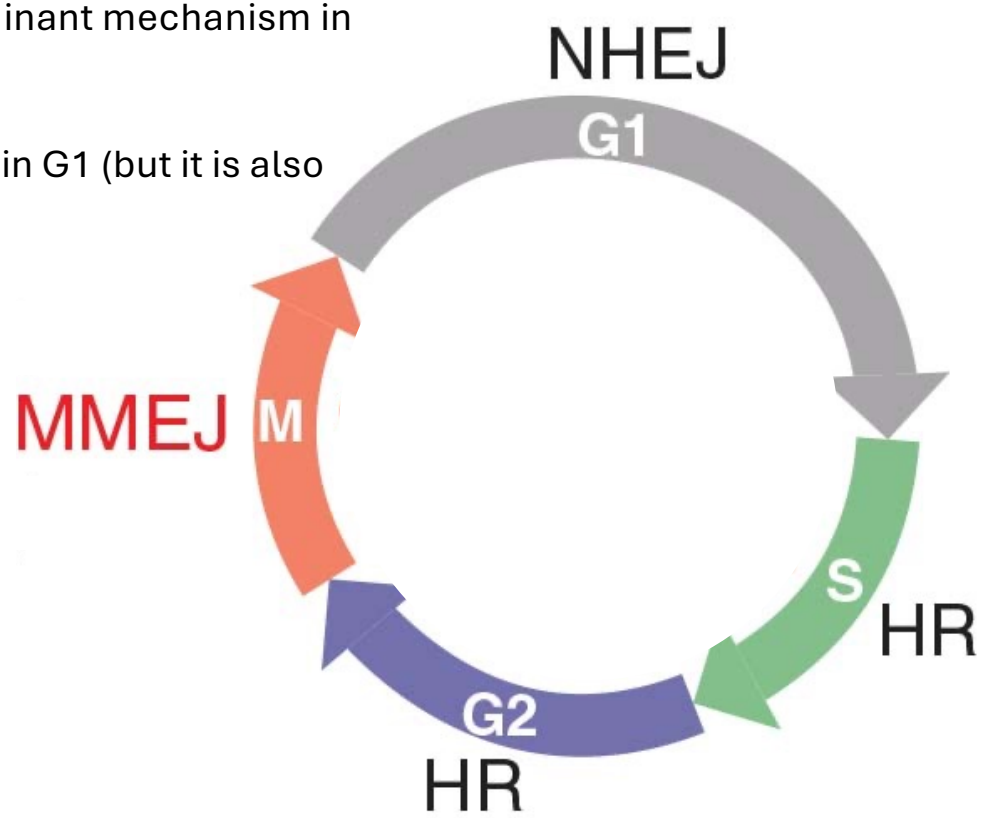
I am a bit confused between the **DNA repair mechanisms**: the ones that act throughout all the **cell cycle**, and the ones that are checkpoint-specific. For example, when a DSB occurs, what is the first factor recruited, and how does the cell cycle step matter in this?
If I understood correctly, **ATR is activated upon ssDNA breaks or replication stress**, while **ATM is activated after dsDNA breaks**. But we know that when DSBs occur, they get resected by nucleases, so would ATM still be recruited? Or would the ssDNA rather be coated by RPA, recruiting ATR-ATRIP?
So overall I think I am having trouble connecting everything we learned about DNA breaks… An overall summary would be immensely helpful.

Repair of DNA Double-Strand Breaks During the Cell Cycle

HR: active in S and G2 (predominant mechanism in S/G2)

NHEJ: active and predominant in G1 (but it is also active in S and G2)

MMEJ: active in M



...MMEJ activity in mitosis repairs persistent DSBs that originate in S phase.
Of note, NHEJ and HR are not active in mitosis.

Brambati et al., Science **381**, 653–660 (2023)

Regulation of End Resection During the Cell Cycle Determines Pathway Choice

G1: 53BP1 is recruited to DNA ds breaks in an ATM-dependent manner. **53BP1** (in cooperation with RIF1, shieldin,and others) **blocks end resection** by nucleases.
→**DNA ds breaks are repaired by NHEJ**

S/G2: End resection does occur to initiate **HDR**. Phosphorylation of 53BP1 prevent stable recruitment of 53BP1 at DSBs. **CDK1** phosphorylates CtIP, NBS1 and other factors stimulating end resection (phosphorylated CtIP stimulates the nuclease activity of MRN to initiate end resection; long range resection is done by EXO1 or DNA2 nucleases). End resection by nucleases is also promoted by **BRCA1**.

PARP Inhibitor Resistance

Strong evidence

Not discussed in this course:

Resistance mechanisms	Cause of resistance	Clinical evidence
(i) Increased drug efflux	- Upregulation of ABC transporters	- No evidence
(ii) Decreased PARP trapping	- Loss or decreased trapping of PARP1 - Loss of PARG	- Trapping-diminishing PARP1 mutation in PARPi-resistant tumour - No evidence
(iii) Restoration of HR	- Reactivation of *BRCA1/2* - Loss of 53BP1 - Loss of Shieldin factors - Loss of CTC/Polα - Loss of DYNLL1/ATMIN	- Mutations in patients and PDXs - Low expression and mutations in PDXs - Low expression and mutations in PDXs - No evidence - No evidence
(iv) Stabilization of stalled forks	- Loss of PTIP - Loss of EZH2	- No evidence - No evidence

PDX: patient derived xenografts

Trends in Cell Biology

Figure 2. Modes of Resistance to PARP Inhibitors (PARPi). An overview of the four distinct categories of PARPi resistance mechanisms. In each category (left column), all molecular mechanisms that have been identified in preclinical studies are mentioned (middle column). In addition, whether direct clinical evidence for PARPi resistance has been observed in primary tumor material or PDX-models until this date is indicated (right column). Abbreviations: HR, homologous recombination; PARP, poly(ADP-ribose) polymerase; PDX, patient-derived xenograft.

- PARG: Poly (ADP-ribose) glycohydrolase; removes poly ADP-ribose moieties
- **Loss of 53BP1, shieldin, CTC1 →increased 3' overhangs at DSBs**

→ reactivates resection and HR in BRCA1 cells (in BRCA1 deficiency resection is inhibited). **BRCA2 deficient cells are not acquiring PARP inhibitor resistance by loss of 53BP1!**

From: Trends Cell Biol 29, 820 (2019)

- Loss of 53BP1 in BRCA1-deficient cells restores, to some degree, homologous recombination in a manner that depends on the activation of end resection.
- …loss-of-function mutations in *53BP1* or shieldin lead to PARPi resistance in BRCA1 deficiency (but not BRCA2 deficiency!).

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Activation of ATR

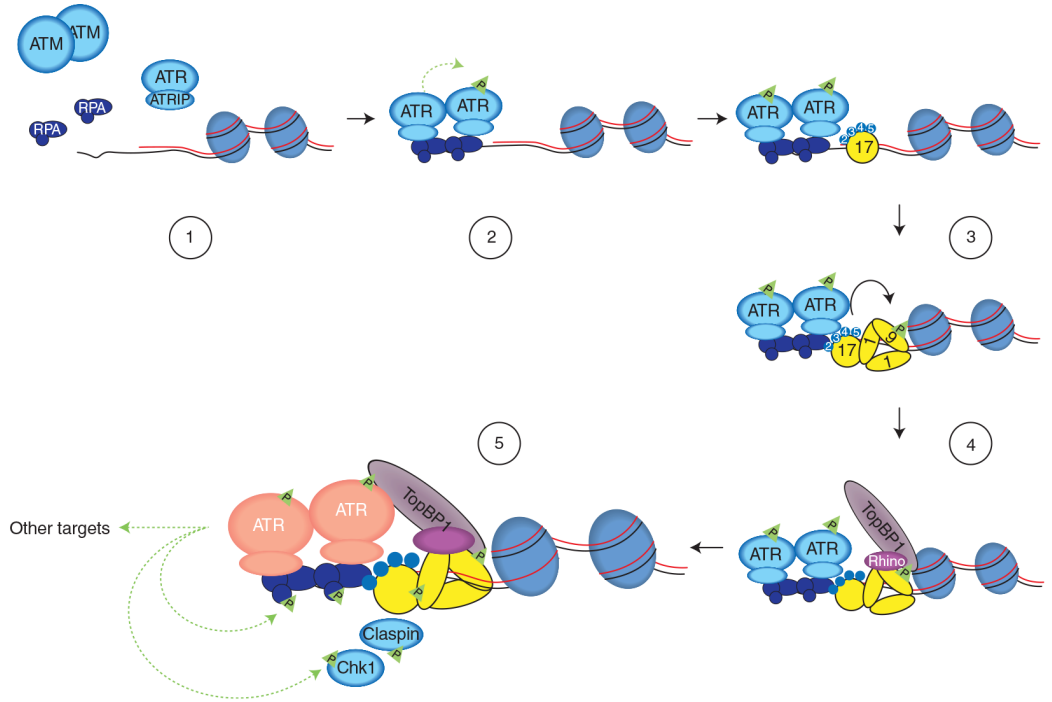


Figure 4. A fail-safe, multistep mechanism for ATR activation. Increased amounts of ssDNA are generated by resection of DNA ends or by uncoordinated DNA unwinding and synthesis at replication forks. Extensively resected DNA ends are no longer recognized by ATM efficiently. Once coated by RPA, ssDNA recruits the ATR-ATRIP complex (1), and promotes ATR *trans*-autophosphorylation (2). RPA-ssDNA also promotes the recruitment of the Rad17-Rfc2-5 clamp loader to junctions between ssDNA and dsDNA, and the loading of Rad9-Rad1-Hus1 (9-1-1) checkpoint clamps onto dsDNA (3). TopBP1 interacts with phosphorylated Rad9 and with Rhino, which associates with 9-1-1 (4). The TopBP1 recruited to dsDNA by 9-1-1 and Rhino engages the ATR-ATRIP complex on RPA-ssDNA through the ATR autophosphorylation site T1989. This process enables TopBP1 to stimulate ATR-ATRIP to its full capacity (pink) on ssDNA (5). TopBP1 may also function as a scaffold to facilitate ATR substrate recognition. This multistep process for ATR activation ensures that ATR is only activated when both ssDNA and ssDNA/dsDNA junctions are present at sites of DNA damage and are recognized by DNA damage sensors, providing a fail-safe but versatile mechanism to signal DNA damage. The dashed green lines represent phosphorylation events, and the solid black line represents the loading of 9-1-1 by the Rad17-RFC2-5 complex.

From: Cold Spring Harbor Perspect Biol 2013;5:a01276

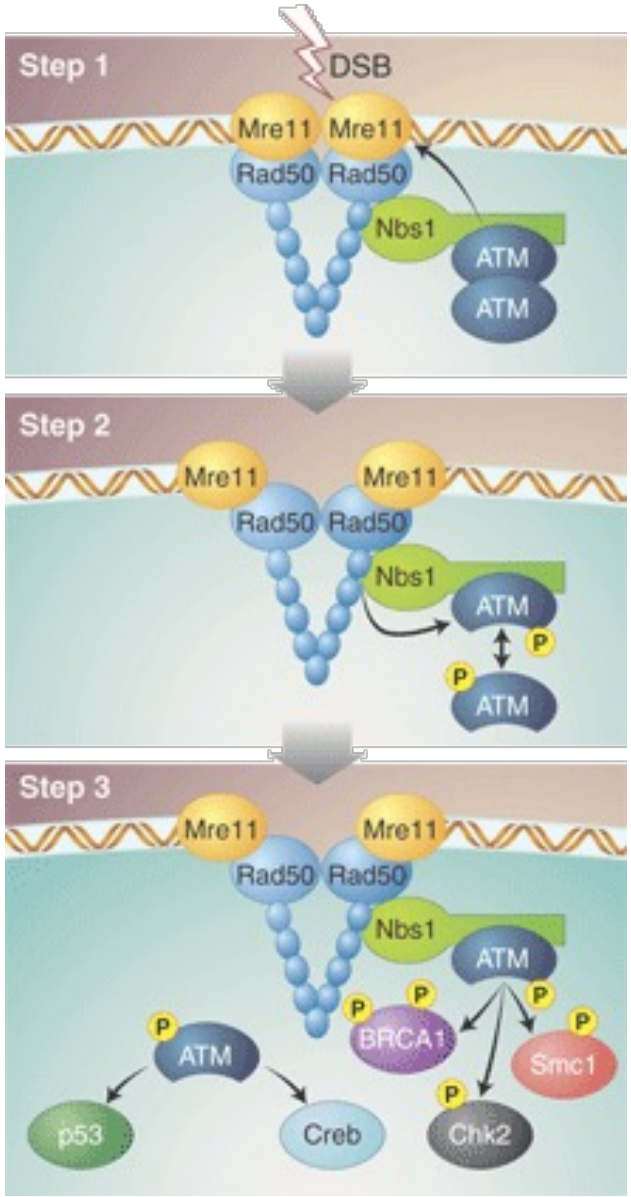
Roles of ATR

ATR phosphorylates hundreds of proteins (involved in DNA replication, repair, checkpoint control etc)

- Checkpoint activation and cell cycle arrest, senescence or apoptosis (through phosphorylation of CHK1 and phosphorylation and **stabilization of p53**).
- ATR phosphorylates H2AX on Ser139 during replication stress, forming γH2AX (Though H2AX phosphorylation is mainly attributed to ATM).
- Stimulates gene expression (via p53 and others) of crucial resection factors such as BRCA1, CTIP, and BLM.
- Stimulation of DNA repair reaction: e.g. ATR phosphorylates PALB2 which facilitates the loading of RAD51 onto the single stranded DNA.
- …

Activation of ATM

The MRN complex and ATM activation. (Step 1) The induction of **DSBs** in DNA leads to prompt **recruitment** of **MRN** complexes. These complexes form a bridge between free DNA ends via the coiled-coil arms of Rad50 dimers. Inactive ATM dimers are recruited to the DSBs through interaction with the carboxyl terminus of Nbs1. (Step 2) Activating signals are delivered to ATM dimers, possibly through a conformational change in Nbs1. **ATM** undergoes **phosphorylation at Ser^{1981}** accompanied by its conversion from a **dimer to** a **monomer**. The MR complex may also trigger a conformational change in ATM that stimulates substrate recruitment. (Step 3) Activated ATM monomers either remain in the vicinity of the DSB, where they phosphorylate colocalized substrates, or diffuse away from the DSB sites to phosphorylate nuclear substrates, such as p53.



Roles of ATM

ATM phosphorylates hundreds of proteins (involved in DNA replication, repair, checkpoint control, etc)

- Checkpoint activation and cell cycle arrest, senescence or apoptosis (through phosphorylation of CHK2, and phosphorylation and **stabilization of p53**).

- Chromatin remodeling: ATM phosphorylates H2AX on Ser139 near DNA double-strand breaks, forming γH2AX

→ attraction of DNA repair complexes

- Phosphorylation of other chromatin factors (e.g. KAP1) which relax chromatin structure

- Promotes expression (via p53 and others) and activity of DNA repair factors

- …

In G1, MRN and ATM bind first, followed by recruitment of 53BP1, which prevents end resection.
In G2, 53BP1 can also bind subsequently to MRN and ATM, but not in a stable manner. Thus, end resection is favored followed by HDR.