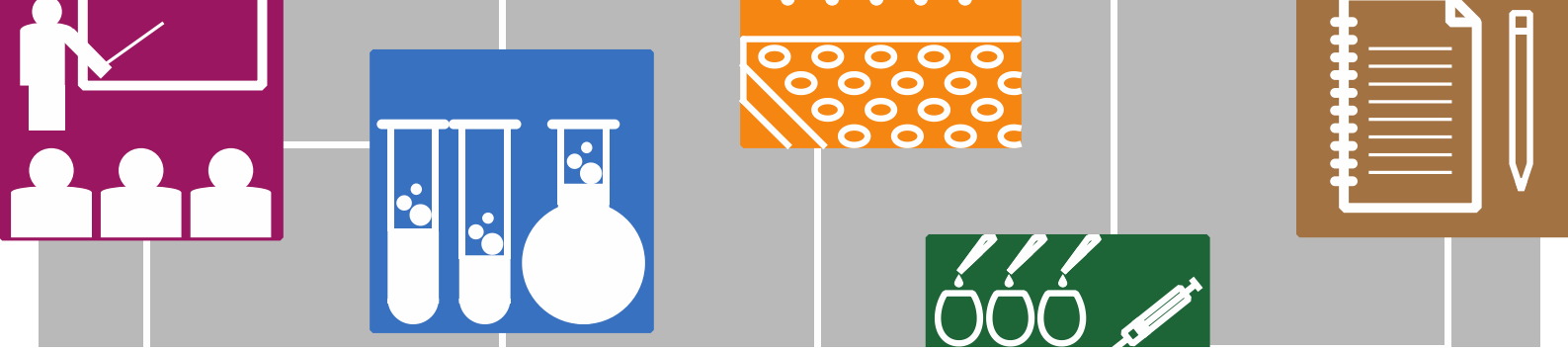


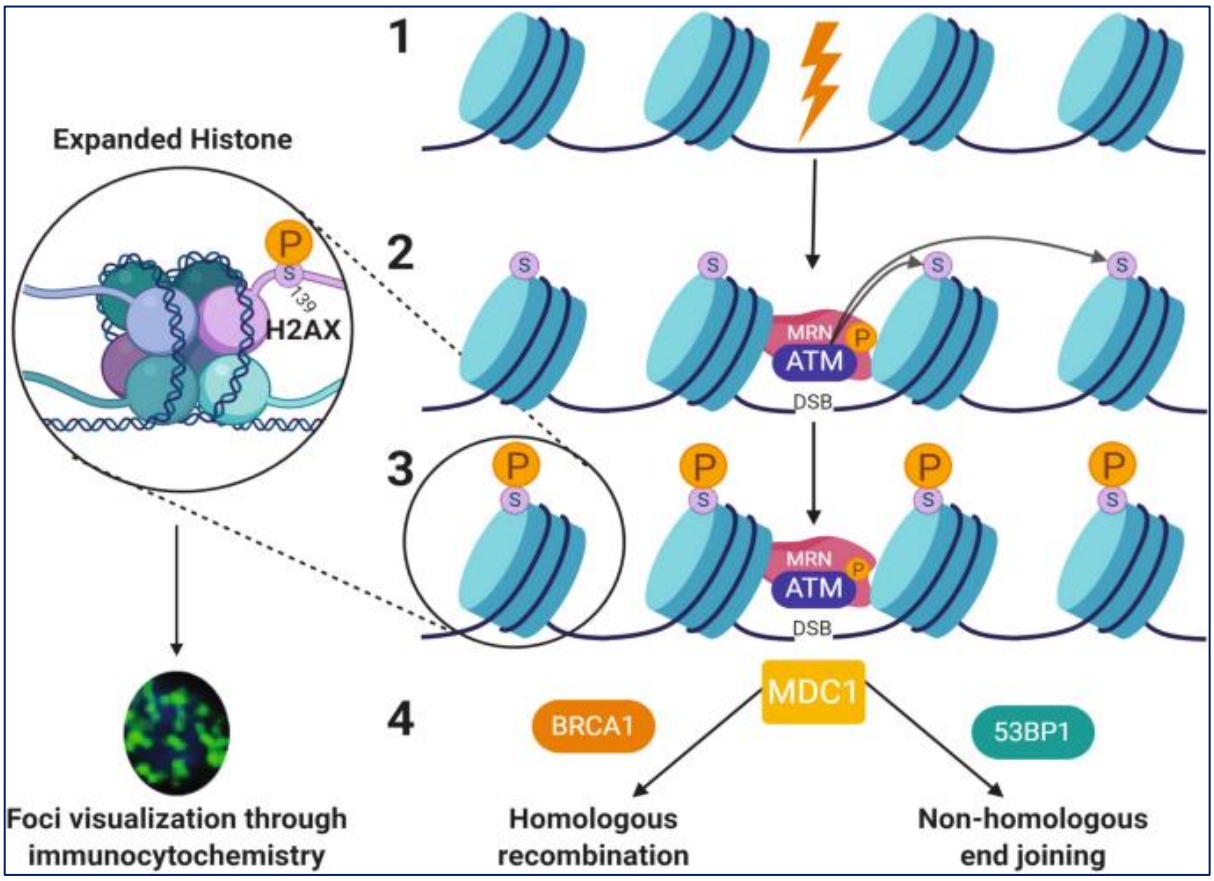
Gamma H2AX assay

Ionizing irradiation induces base damage, single strand breaks and double strand breaks in the DNA. Most DNA double strand breaks (DNA DSB) are efficiently repaired within minutes up to hours after exposure. Residual DNA damage is considered to be the main cause of the cell lethal effect of radiation. Indeed, for a large number of cell lines, a clear correlation between residual DNA damage at 24 hours after exposure and clonogenic cell death has been reported. DNA DSB can be visualized with an antibody against gamma H2AX (yH2Ax) labeled with a fluorescent marker. yH2Ax is the phosporylated form of the histone protein H2AX. Phosphorylation of H2AX occurs rapidly at site of DSBs so staining for yH2Ax allows for quantification of DNA DSB via counting of yH2Ax foci (red) in cells by fluorescence microscopy. To verify the nuclear localization of the foci, the cell nucleus can be visualized by DNA staining with DAPI (blue).

Counting the nuclear yH2Ax foci 30 minutes and 24 hours after irradiation can provide information on how efficiently DNA repair occurs in a specific cell. In addition, comparison of this repair ability in the presence of absence of a drug can indicate whether or not the drug is able to interfere with efficient DNA repair.

An outline of the background is provided in the figure below.

Tip: By counting and comparing the nuclear yH2Ax foci you will find compounds that prevent adequate DNA repair after 24 hours. You can e.g., calculate the ratio of foci at 30 min. vs. 24 hours. A high ratio would indicate good repair, a low ratio suggests an inadequate repair. Note that a skilled observer might already identify the most inhibitory compounds without actual counting.



H2AX is a histone protein component (in purple in the magnification). When a DSB occurs, a serine in H2AX proteins close to the DSB is rapidly phosphorylated giving rise to gammaH2AX. Antibodies directed against the phosphorylated serine of gammaH2AX are used to visualize the protein which indirectly indicates where DSBs are. Counting the number of these stained foci is indicative of the DNA damage in the cell nucleus (here shown in green). (Illustration from Noubissi et al. Sci Rep. 2021).