

### BOX 3 - Histone H2AX -

Histones not only have a role in packing the genomic DNA but also influence DNA transcription and repair.

Each nucleosome consists of a linker histone H1 and an octamer of core histones: an H3-H4 tetramer flanked by two H2A-H2B dimers. Each of the separate units is subject to covalent modifications (178) and the degree, order and combinations of these modifications determine the ability to wrap the DNA and influence transcription and repair processes.

Histone H4 (32) and H2AX (387) (one of the subfamilies of H2A) are known to undergo modifications upon DSB formation. The H2AX proteins form 2-25% of the H2A histone pool in mammalian cells. They are phosphorylated by phosphatidylinositol-3OH-kinases on the Serine 136 and 139 positions in mice upon DSB formation (374). Phosphorylation is performed by ATR, ATM and DNA PKcs (48, 124). Phosphorylated H2AX is called  $\gamma$ -H2AX and forms foci within 1-5 minutes, reaching a maximum level within 10 minutes, which can persist for hours (374). Phosphorylation of H2AX spans some megabases (2-30Mbp) of chromatin around the DSB (373, 374) and takes place within a few seconds. Studies on  $\gamma$ -H2AX foci reveal that each single focus may correspond to one single DSB (373). A single focus is estimated to contain approximately 2000  $\gamma$ -H2AX molecules (374).

Foci not only form as a response to DSBs induced by exogenous treatments and agents, but also to DSBs formed by cell regulated processes, i.e. V(D)J recombination (62), class switch recombination (342) or replication (collapsed replication forks) (124).  $\gamma$ -H2AX foci co-localize with many DSB repair associated proteins involved in NHEJ, such as MRE11, RAD50 and NBS1 and in homologous recombination, such as BRCA1 and RAD51. Absence of H2AX does not abrogate the initial recruitment of these proteins to DSBs (57) but it is required for formation of BRCA1 foci (57, 337), but not RAD51 foci (58). In support of H2AX involvement in DSB repair is the fact that cells from *H2AXKO* mice are sensitive to IR and possibly slightly to cross linking agents, show reduced homologous recombination frequencies (58) and display increased chromosomal instability (14, 58). The mice themselves show growth retardation, display impaired class switch recombination (but not V(D)J recombination) and the males are sterile due to meiotic defects. Mouse *H2AXKO* mutations in a p53 deficient background reveal a similar phenotype, but in addition show enhanced tumor susceptibility (15, 56). This observation supports the idea that H2AX is a protein with an important role in signaling and localizing DNA damage to other proteins.