

Bleomycin-induced γ H2AX foci map preferentially to replicating domains in CHO9 interphase nuclei

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© 2014, Springer Science+Business Media Dordrecht. Exposure to DNA damaging agents triggers phosphorylation of histone variant H2AX (generating γ H2AX) in large chromatin regions flanking DNA lesions, allowing their immunodetection as nuclear foci. Even though a predominance of γ H2AX foci in euchromatin has been postulated, foci positioning when DNA insult occurs in replicating eu- or heterochromatin regions has not been extensively explored. Labeling of interphase nuclei with 5-ethynyl-2'-deoxyuridine (EdU) pulses has revealed that DNA replication is temporarily and spatially regulated: euchromatin replicates in early S (ES) and heterochromatin along mid and late S (MS/LS) phases. In order to map DNA damage with respect to replicating domains, the distribution of γ H2AX foci induced by the radiomimetic agent bleomycin was analyzed in CHO9 interphase nuclei by delineating euchromatic (H3K4me3+) and replicating (EdU+) regions. Quantification of overlapping pixels and 3D inter-object overla