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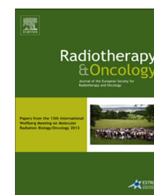

Review

# Ionizing-radiation induced DNA double-strand breaks: A direct and indirect lighting up

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ABSTRACT

The occurrence of DNA double-strand breaks (DSBs) induced by ionizing radiation has been extensively studied by biochemical or cell imaging techniques. Cell imaging development relies on technical advances as well as our knowledge of the cell DNA damage response (DDR) process. The DDR involves a complex network of proteins that initiate and coordinate DNA damage signaling and repair activities. As some DDR proteins assemble at DSBs in an established spatio-temporal pattern, visible nuclear foci are produced. In addition, post-translational modifications are important for the signaling and the recruitment of specific partners at damaged chromatin foci. We briefly review here the most widely used methods to study DSBs. We also discuss the development of indirect methods, using reporter expression or intra-nuclear antibodies, to follow the production of DSBs in real time and in living cells.

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Ionizing radiation (IR) produce a wide variety of DNA lesions among them double-strand breaks (DSBs), considered to be the major actor responsible for cell death. If unrepaired or improperly repaired, DSBs contribute to chromosomal aberrations, which may lead to human disorders including cancer [1]. Consequently different approaches have been undertaken to identify the mechanisms involved in the production, signaling and repair of DSBs.

The production of DSBs can be quantified by biochemical techniques such as the pulsed field gel electrophoresis (PFGE). In addition, DSBs production can be followed by cell imaging either globally, with the neutral comet assay, or damage specific, through immunostaining of marker proteins or recruitment of fluorescent proteins to the breaks. To analyze the recruitment of signaling and/or repair proteins, a clear understanding of the DNA damage response (DDR) is needed, supported by the development of cell imaging after IR or, more recently, after microlaser irradiation. We will briefly introduce the panel of biochemical and cell imaging techniques. Their insights into the DSBs repair kinetics, largely obtained by microlaser irradiation and fluorescent protein recruitment, will be presented. Since such techniques based on the overexpression of protein might generate artifacts, indirect approaches have also been developed. We discuss here the potential benefits of using intracellular antibodies, in particular directed against post-translational modifications of DDR proteins, as well as the use of different reporter systems.

## Human DNA damage response after ionizing radiation treatment

In mammalian cells, the production of DSBs initiates a global cellular response, including checkpoint signaling and repair (Fig. 1) [2]. The MRN (MRE11/RAD50/NBS1) complex binds to DSBs (Fig. 1B and C) and facilitates the activation of ATM (Ataxia Telangiectasia Mutated), a key PI3K related kinase in the DDR[3]. At the break site, ATM autophosphorylates, allowing its activation and the subsequent phosphorylation of numerous substrates in the surrounding chromatin. Among ATM substrates, H2AX – an H2A histone variant called γH2AX when phosphorylated – is considered as one of the earliest markers of the DSB signaling [4]. H2AX phosphorylation reaction is amplified by the recruitment of MDC1 (Fig. 1C and D), a central player of the DDR through its interaction with γH2AX [5]. The accumulation of DDR proteins at damaged sites leads to the formation of foci, visible after staining under

*Abbreviations:* bp, base-pair; DDR, DNA damage response; DNA-PKcs, catalytic subunit of the DNA-dependent protein kinase; DSB, DNA Double-Strand Break; HR, homologous recombination; IR, ionizing radiation; IRIF, ionizing radiation induced foci; LET, Linear Energy Transfer; MRN, MRE11–RAD50–NBS1 complex; NCO, noncrossover; NHEJ, non-homologous end-joining; PARP, poly ADP ribose polymerase; PI3K, phosphoinositide 3-kinase; PFGE, pulse field gel electrophoresis; ssDNA, single-stranded DNA; SSB, DNA single-strand break.

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microscopic examination. The signaling amplification by MDC1 participates to the recruitment of multiple DDR members (Fig. 1D–F), such as RAP80, 53BP1, KAP-1 and BRCA1 [6]. The recruitment of 53BP1 and BRCA1 proteins in ionizing-radiation-induced foci (IRIF), triggered by γH2AX and the MDC1 binding, is also dependent on the participation of the RNF8/RNF168 E3-ubiquitin ligases [7,8]. The overall signaling pathway leads to the downstream phosphorylation of CHK2, p53 and CDC25, triggering

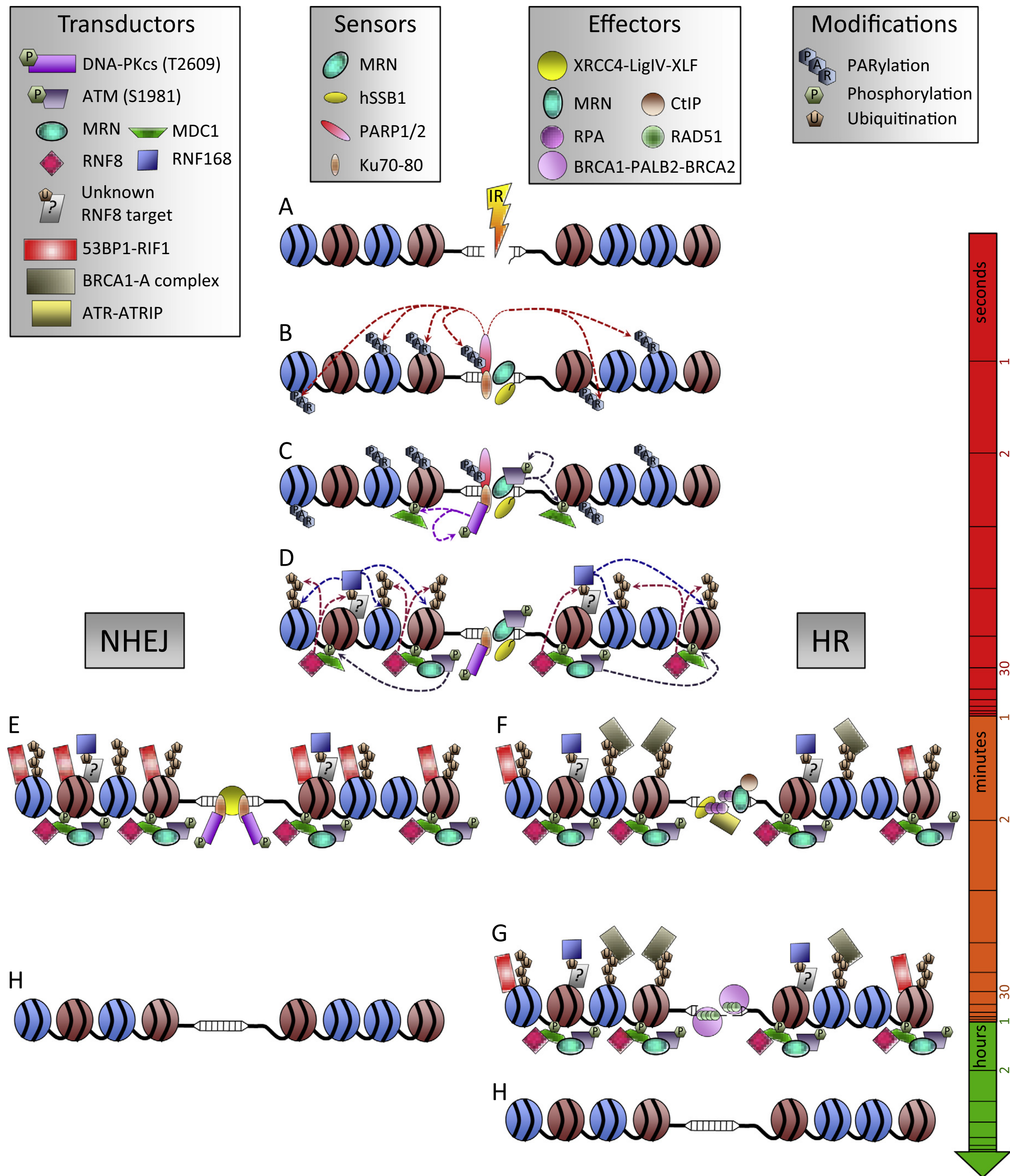


**Fig. 1.** Kinetics of DDR protein recruitment and modification at IR-induced DSBs. (A) Exposure to ionizing radiations (IR) induces DSBs. (B) Sensor proteins immediately recognize DSB formation. These include PARP1, which induces its own PARylation together with the PARylation of the surrounding chromatin and many DDR actors. MRN and Ku70-80 interact with DSB ends, while hSSB1 binds to the ssDNA regions. (C) H2AX phosphorylation happens in the first seconds after IR. ATM and DNA-PKcs are recruited to DSB through their interaction with MRN and Ku80, respectively. ATM and DNA-PKcs autophosphorylation drive their activation and phosphorylate H2AX (γH2AX), close to the DSB site (H2AX-containing nucleosomes are shown in red) and MDC1 rapidly binds γH2AX. (D) Modification of the chromatin flanking the DSB within the first minute after IR. MDC1 recruits more MRN–ATM complexes, which phosphorylate more distal H2AX. RNF8, recruited through its interaction with MDC1, promotes the ubiquitylation of a yet unidentified non-nucleosomal target. Once ubiquitylated, this protein is recognized by RNF168 that initiates H2A and H2AX ubiquitylation. RNF8/RNF168 then promotes K63 ubiquitin chain formation. (E and F) Factors determining the DSB repair pathway choice take place during the first minutes after IR. (E) NHEJ pathway. 53BP1 and RIF1 are recruited *via* 53BP1 binding to H4-K20me2, therefore preventing BRCA1 accumulation and inhibiting resection. The XRCC4-LigIV-XLF complex promotes DSB ligation. (F) HR pathway. BRCA1 complex binds to K63 ubiquitin chains at DSB, leading to 53BP1 exclusion. CtIP cooperates with MRN to initiate resection, removing Ku70-80 from DSB ends. ssDNA overhangs, bound by hSSB1 and RPA, recruit ATR-ATRIP. (G) The BRCA1–PALB2–BRCA2 complex recruits RAD51 to DSB around 30 min after IR. (H) Loss of DSB markers after repair completion. The right arrow outlines the timing of events after DSB appearance, with a simplified logarithmic scale.

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