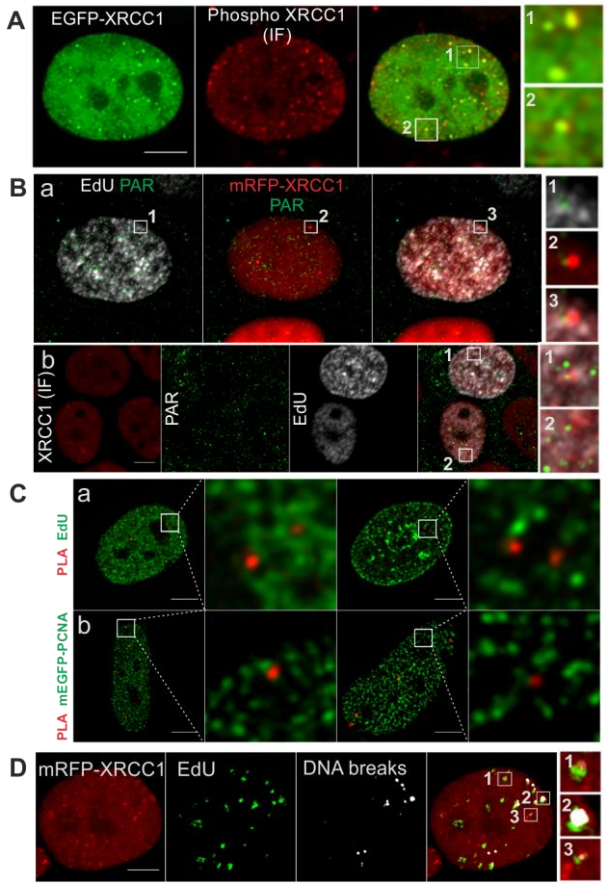
**Suppl. Data Set 4.**
**Localization of poly(ADP-ribose) molecules, XRCC1-PCNA repair complexes, and phosphorylated XRCC1 molecules, in relation to location of XRCC1-containing nuclear bodies.**




***Supplementary Figure 4.*** *Localization of post-translationally modified repair proteins and XRCC1-PCNA complexes in relation to XRCC1-containing nuclear bodies.*
***A****. Immunofluorescent staining of XRCC1 molecules phosphorylated by CK2 kinase (red) shows that such molecules accumulate within the large XRCC1 foci, detected by imaging the fusion protein EGFP-XRCC1 (green). These structures contain both unmodified and modified forms of the protein, as shown in the enlarged microscopy images.*
***B****. Poly(ADP-ribose) chains (IF, green) always accumulate outside of XRCC1 foci (mRFP-XRCC1 (a) or XRCC1 detected by IF (b)) (red), at the same time overlapping replication sites (a, b) (magnified images in (a and b)). Therefore, XRCC1-containing nuclear bodies are presumably always located in the regions outside or adjacent to single-strand DNA breaks.*
***C****. XRCC1-PCNA complexes in nuclei of HeLa cells, detected by proximity ligation assay. These images demonstrate that in wild-type, non-transfected and in transfected replicating cells the sites where the two proteins interact in a complex are located in the vicinity of the regions of replication (a,b).*
***D.*** *DNA breaks (improved TUNEL assay, white) are localized outside or within the periphery of XRCC1 foci (mRFP-XRCC1, red), but within replication sites (EdU, green).*
*Scale bars: 5 µm, ROIs (A,C): 2.5 x 2.5 µm, (B): 3.2 x 3.2 µm, (D): 2.1 x 2.1 µm.*

In order to establish the location of activated, post-translationally modified repair protein molecules in relation to XRCC1-containing nuclear bodies, we examined nuclei of cells expressing mRFP-XRCC1 in which phosphorylated molecules of XRCC1 and poly(ADP-ribose) (PAR) were detected using immunofluorescence (Suppl. Fig. 4A,B). Apparently, the conspicuous repair foci are the areas of accumulation of both unmodified XRCC1 molecules and the ones containing the sites phosphorylated by CK2 kinase (Suppl. Fig. 4A). Phosphorylation of this type is required for stabilization of the protein, the stimulation of the interactions and complex formation with other repair factors, and high efficiency of repair of SSBs (1). Moreover, it has been shown that it reduces affinity of XRCC1 to DNA (2). Thus the presence of phosphorylated and unmodified molecules inside the nuclear bodies we describe here (Suppl. Fig. 4A) may suggest that there is an exchange of molecules between the nuclear bodies and the sites of DNA damage. Suppl. Fig. 4A demonstrates that XRCC1 molecules phosphorylated by CK2 are accumulated inside nuclear bodies. This observation supports the notion that the nuclear bodies formed in response to DNA damage serve as the molecular reservoirs responsible for storage, delivery and exchange of the repair factors, in unmodified and modified forms, prepared for functional complex formation at the sites of DNA lesions. Interestingly, however, poly(ADP-ribose) polymers were never found within XRCC1 foci. Moreover, their accumulation in cell nuclei was often detected in localizations adjacent to these large nuclear structures (Fig. 3B, Suppl. Fig. 4B). The images in Suppl. Fig. 4B also show that the PAR chains colocalize with the replication sites while being adjacent to XRCC1-containing nuclear bodies. Among many roles played by poly(ADP-ribosyl)ation there is formation of PAR chains in response to DNA damage. They are engaged in regulation of protein-protein or protein-nucleic acids interactions and play a role of scaffolds that mediate the recruitment and binding of the repair factors and regulate protein degradation. XRCC1 has been shown to bind PAR polymers at the sites of DNA damage. This mediates its interactions with PARP1 or its binding to the DNA strands (3–8). The images presented in Fig. 3B and Suppl. Fig. 4B prove that the accumulation of PAR chains, most likely marking the location of a DNA lesion, always occurs outside of the regions of accumulation of XRCC1. Thus we infer that binding of XRCC1 molecules to PAR chains, known to be attached to the repair protein factors and nucleic acids directly at the location of the DNA lesion, takes place outside of the XRCC1 repair foci, frequently in the regions adjacent to these structures. Therefore, we conclude that the conspicuous XRCC1-containing nuclear bodies do not surround the single-stranded breaks located within the regions of replication (Fig. 3B, Suppl. Fig. 4B).

Suppl. Fig. 4 also demonstrates formation of XRCC1-PCNA complexes in response to damage. If XRCC1 molecules are recruited to a DNA lesion in a replication site to perform a repair task, they would be expected to recruit other repair proteins and interact with them. The question of direct interactions between repair factors, like XRCC1 and PCNA, and formation of multiprotein complexes cannot be solved by direct imaging and colocalization analysis. Due to limited spatial resolution, proximity between XRCC1 foci and replication sites seen in microscopy images does not demonstrate direct interaction between XRCC1 molecules and components of the replication or DNA repair machinery. In order to search for protein-protein interactions we employed proximity ligation assay (PLA). XRCC1 was previously shown to directly interact with PCNA (9), therefore, we attempted to detect protein complexes containing XRCC1 and PCNA. PLA demonstrated that XRCC1 and PCNA indeed formed a limited number of detectable complexes,

or components of large multiprotein complexes, positioned in close proximity of replication sites (Suppl. Fig. 4C). The presence of XRCC1-PCNA complexes detected using PLA suggests that a small proportion of XRCC1 molecules recruited to SSBs in replicating regions indeed form complexes with PCNA.

The images presented in Suppl. Fig. 4D (as well as in Fig. 3A) constitute a direct proof for the localization of DNA breaks labeled using an improved TUNEL assay for direct detection of free DNA ends. DNA lesions (both single- and double-strand breaks) are not surrounded by the repair factors and they are localized outside or within the periphery of XRCC1 foci (Fig. 3A, Suppl. Fig. 4D). However, the simultaneous detection of regions of replication shows that DNA breaks are localized within the areas of active replication (Suppl. Fig. 4D).

**References**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