Mechanism and kinetics of recruitment and dissociation of RPA protein involved in the repair of DNA protein crosslinks (DPCs)

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DNA-protein crosslinks (DPCs) are among the most toxic types of DNA damage, interfering with fundamental cellular processes, such as DNA replication and transcription [1]. Several widely used chemotherapeutics, including cisplatin, camptothecin (CPT) derivatives, and 5-aza-dC, induce DPCs [2]. In eukaryotic cells, replication protein A (RPA) acts as a hub protein during DNA replication, recombination, and repair. Furthermore, RPA is explored as a prognostic marker and therapeutic target in cancer [3]. In light of its major involvement in repair responses, we sought to characterize the dynamics of RPA recruitment and dissociation at DPC sites in response to CPT. CPT treatment induced an accumulation of RPA foci, indicating the formation of ssDNA at DPCs, a likely intermediary step in subsequent repair processes. Foci appeared immediately after CPT treatment, and their average intensity indicated a rapid increase in RPA for up to 120 minutes, where after the increase continued at a slower rate. CPT disrupted cell cycle progression, with damaged cells failing to divide for up 48 hours, in addition to a two-fold nuclear enlargement, which indicated that the CPT-induced cycle blockade did not halt cell growth. In contrast to actively replicating cells, G1 and G2 cells did not exhibit rapid RPA foci accumulation nor cell cycle arrest. It was only after these cells entered the subsequent S phase that RPA foci were formed. Furthermore, no comparable impediment in cycle progression was observed. This indicates that DPCs formed in G1 and G2 cells were efficiently resolved, allowing for successful S phase entry and subsequent progression through the cell cycle, which contrasts the persistent damage and cycle blockade observed in cells treated while in S phase. Blocking MRN complex activity via MRE11 inhibitor treatment revealed that the initial RPA accumulation was in large part due to the unfolding of DNA rather than its resection. Taken together, our findings reveal a differential involvement of RPA during DPC resolution, which seems to depend on cell cycle stage.

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