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The Role of Replication Stress in Ochratoxin A Genotoxicity

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The mycotoxin and food contaminant Ochratoxin A is one of the most potent renal carcinogens known to date, but its mechanism of carcinogenicity still needs to be resolved to improve science-based assessment of human health risks associated with dietary exposure to OTA. While *in vitro* and *in vivo* studies suggest that disruption of mitosis coupled with compensatory stimulation of cell proliferation may promote genomic instability and tumorigenesis in rat kidneys *in vivo*, the causal chain of events preceding perturbation of mitosis by OTA remains to be established. As mitotic aberrations may result from replicative stress, the present work aimed to investigate if OTA interferes with DNA replication using the DNA fiber assay. This technique relies on the sequential pulse labeling of cells with thymidine analogs such as 5-iodo-2′-deoxyuridine (IdU) and 5-chloro-2′-deoxyuridine (CldU) and subsequent visualization of thymidine analogues incorporated into replicating DNA using indirect immunofluorescence. Analysis of replication forks dynamics revealed a significant reduction in replication fork velocity in human renal epithelial cells (HK-2) exposed to OTA at concentrations $\geq$10 μM for 1 hour. Analysis of individual track lengths indicated global slowing of fork progression in response to OTA rather than shortening of individual tracks as seen in cells exposed to the alkylating agent cisplatin. In support of the mild but significant effects of OTA on replication fork velocity, analysis by Western blot and/or immunofluorescence demonstrated a significant, concentration-related increase in γH2AX and pChk1 (S317) in cells treated with OTA. Importantly, costaining with CldU revealed γH2AX foci exclusively in cells with newly replicated DNA, thus supporting a mechanistic link between DNA replication and induction of γH2AX foci by OTA. Moreover, visualization of γH2AX in the extended chromatin fiber assay revealed a concentration-dependent increase in γH2AX along replicative chromatin fibers. Taken together, these data provide first experimental evidence for perturbation of the S-phase replisome machinery by OTA and suggest replication stress as an early key event in OTA genotoxicity.