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In vitro characterization of human REV1 low-fidelity DNA polymerase

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Whenever DNA damage fails to be repaired, it is encountered by translesion synthesis machinery in order to maintain genomic stability. The main players of translesion synthesis are low-fidelity DNA polymerases. Their functioning can lead to mutagenesis and chemotherapy resistance, and alterations in their activity or expression can lead to various cancers.

REV1 is perhaps the most important of translesion polymerases since it possesses not only a catalytic function, but also a structural function. The former consists in REV1 being primarily a deoxycytidyl transferase enabled by its catalytic domain forming special interactions with the incoming cytidine and located approximately in the middle of the protein. The structural function is performed by the C-terminal domain of REV1 allowing it to serve as a platform for the assembly of other low-fidelity polymerases of translesion synthesis. Studies of human REV1 are hampered by the general difficulty of the isolation of the protein, as well as it being prone to the cleavage of the C-terminal domain.

This work introduces a new method for isolation of intact and highly-active hREV1 from *E. coli* and *S. cerevisiae*. The *S. cerevisiae* preparation was chosen for in-depth analysis, as it is the first full-sized hREV1 protein without a tag isolated from a eukaryotic expression system.

The purified protein demonstrated primarily deoxycytidyl transferase activity when bypassing undamaged nucleotides and the most wide-spread lesions AP-site and 8-oxoguanine. It was shown that the G-G intrastrand crosslink caused by a popular chemotherapy agent cisplatin could not be efficiently bypassed by REV1 *in vitro*. Analysis of DNA lesions that allow to elucidate the mechanism of REV1 (1,N6-etenoadenine and 7-deazaadenine/7-deazaguanine) demonstrated that disrupting Watson-Crick base pairing of the template nucleotide did not inhibit REV1. However, disrupting Hoogsteen base pairing leads to a significant decrease of its activity, which presents biochemical evidence for a unique mechanism of hREV1 active site functioning, in line with previously obtained structural data.

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