

## **Identification of Mammalian Proteins that Cross-Link DNA in the Presence of Nitrogen Mustards**

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The antitumor activity of nitrogen mustard drugs is commonly attributed to their ability to induce DNA-DNA cross-links through consecutive alkylation of two nucleophilic sites within the DNA duplex. However, recent studies suggest that the formation of DNA-protein cross-links may also contribute to the antitumor effects of these widely prescribed agents. Through the use of affinity capture and tandem mass spectrometry, numerous proteins were identified that cross-link DNA following exposure of Chinese hamster ovary (CHO) and human cervical carcinoma (HeLa) nuclear protein extracts to mechlorethamine in the presence of double-stranded oligodeoxynucleotides. The identified proteins encompass a broad range of cellular functions, including chromatin regulation, DNA replication and repair, cell cycle control, transcription regulation, and architectural/structural applications. The extent of mechlorethamine-induced DNA-protein cross-linking in several of the identified proteins was examined by Western blot analysis, and our results indicate a dose-dependent response. As the formation of DNA-protein cross-links would likely interfere with crucial cellular processes such as DNA replication and transcription, our findings suggest that DNA-protein cross-linking may contribute to the cytotoxicity and antitumor activity of nitrogen mustards in mammalian cells.