# Evidence for a Recombination-Independent Pathway for the Repair of DNA Interstrand Cross-Links Based on a Site-Specific Study with Nitrogen Mustard†

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ABSTRACT: DNA−DNA interstrand cross-links are thought to be important for the cytotoxicity of many chemotherapeutic agents. To study this more definitively, adduct site-specific methods are used to construct a plasmid with a single nitrogen mustard interstrand cross-link (inter-HN2-pTZSV28). Replication efficiency (RE = [colonies from (inter-HN2-pTZSV28)/(control with no cross-link)]) is ~0.3 following transformation into *Escherichia coli*, implying that the cross-link is repaired. The commonly accepted pathway for cross-link repair, which involves both nucleotide excision repair (NER) and recombination, is ruled out since RE is ~0.3 in a Δ*recA* strain. Non-RecA-directed recombination such as copy-choice is also unlikely. However, NER is involved since RE was ~0.02 in strains deficient in NER. Base excision repair is not important since RE is ~0.3 in strains deficient in 3-methyladenine DNA glycosylases I and II, FAPY DNA glycosylase, both known apurinic/apyrimidinic endonucleases, or DNA deoxyribophosphodiesterase. Another hypothetical repair pathway hinging on a 5′ → 3′ exonuclease activity is unlikely since RE is ~0.3 in cells deficient in either the 5′ → 3′ exonuclease activities of DNA polymerase I, exonuclease VII, or RecJ. Thus, aside from NER, it is unclear what else participates in this recombination-independent repair pathway, although a pathway involing NER followed by replicative bypass of the lesion is the current working hypothesis. Psoralen interstrand cross-links appear *not* to be repairable by this second pathway, which may have implications for the relative cytotoxicity of interstrand cross-links from different agents.

A variety of anticancer, chemotherapeutic agents are bifunctionally reactive and, therefore, can cross-link biological macromolecules, notably DNA. The ability of these DNA cross-links to interfere with DNA replication and, ultimately, to cause cytotoxicity appears to be essential to this anticancer activity (Colvin, 1982; Ludlum, 1986; Kohn & Gibson, 1986; Hemminki & Ludlum, 1984; Pratt et al., 1994). DNA−DNA interstrand and intrastrand as well as DNA−protein cross-links each form, raising the questions of which cross-link is important for cytotoxicity and chemotherapeutic efficacy, and why? Where it has been studied, the formation of DNA−protein cross-links does not appear to correlate with cytotoxicity (Erickson et al., 1980). Interstrand cross-links have been implicated in the case of nitrosoureas (Erickson et al., 1980; Zlotogorski & Erickson, 1984; Dolan et al., 1986, Samson et al., 1986; Brennard et al., 1986) and are likely to be relevant for the psoralens [Liu et al., 1984; Piette et al. (1988) and references therein]. In contrast, intrastrand cross-links have been implicated for *cis*-diamminedichloroplatinum(II) (*cis*-DDP)[1] and its derivatives (Roberts & Thompson, 1979; Zamble & Lippard, 1995). Cytotoxicity from a variety of mustards correlates with the formation of interstrand cross-links in many (O'Conner & Kohn, 1990; Aida & Bodell, 1987), but not all (O'Conner et al., 1991), cases. Older studies on sulfur mustard seemed to imply a dominant role for intrastrand cross-links in a simple model system (Lawley et al., 1969).

The discussion in the previous paragraph raises two interrelated issues. (1) It is not obvious why different kinds of cross-links should be responsible for cytotoxity in the case of different agents (e.g., BCNU vs *cis*-DDP). (2) When a particular cross-linking agent is reacted with DNA, invariably all of these cross-links, as well as other adducts, are formed more or less randomly around the genome, which makes it difficult to determine what adduct is responsible for what biological end point and why. Both of these issues can be addressed if the biological consequences of individual DNA adducts can be studied, e.g., by using adduct site-specific techniques, which permit the construction of vectors that contain adducts of defined chemical structure at known genome locations (Singer & Essigmann, 1991; Loechler, 1996). A variety of approaches have been used to study monoadducts and DNA−DNA intrastrand cross-links, which both have modifications in a single strand of DNA. In addition, we developed a general strategy to do adduct site-specific work with interstrand cross-links (Ojwang et al., 1989; Grueneberg et al., 1991).

We chose to begin our studies with nitrogen mustard, because it and its derivatives (e.g., cyclophosphamide and melphalan) are used as widely as any anticancer drugs, although less is known about their precise mechanism of

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[1] Abbreviations: HN2, nitrogen mustard; 1-M1-HN2-X1, a partially duplex oligonucleotide covalently linked by a single nitrogen mustard interstrand cross-link (for details see Materials and Methods); inter-HN2-pTZSV28, a plasmid containing a single nitrogen mustard interstrand cross-link; C-pTZSV28, a plasmid constructed identically to inter-HN2-pTZSV28 but lacking the cross-link; *cis*-DDP, *cis*-diamminedichloroplatinum(II); BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; Kf, Klenow fragment of DNA polymerase I; ss, single stranded; ds, double stranded; SD, standard deviation; RE, replication efficiency (see text and footnote *b* in Table 1); RRE, relative replication efficiency (see text and footnote *c* in Table 1); PAGE, polyacrylamide gel electrophoresis; PAG, polyacrylamide gel; AP, apurinic/apyrimidinic.

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