Connections Between the TCR Proteins XPG and CSB, Repair of Oxidative DNA Base Damage, and the Radio-Adaptive Response

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A key question that is central to accurately assessing human health risks from environmentally relevant low level (low dose, low dose rate) exposures to ionizing radiation is whether cellular responses measured at the higher doses for which there are strong epidemiological health data and the high doses commonly used in laboratory experiments extrapolate in a linear fashion to low doses. Recent data suggest that they may not. For example, low-dose hypersensitivity for cell killing is well-documented, and we have preliminary data for a low-dose hypersensitivity for chromosomal damage. The radio-adaptive response -- a protective effect for cellular responses to subsequent higher dose ionizing radiation exposures induced by exposure to low doses – is another example of a non-linear response and may reflect a related process. However, the underlying mechanisms of both are poorly understood. In order to improve understanding of carcinogenic risk from low dose exposures, it is necessary to critically examine the relationship between low dose hypersensitivity and the adaptive response and its mechanistic basis. *A compelling model to explain both responses, as suggested by our own work and others, is that critical DNA repair processes require induction in a dose-dependent fashion and therefore are not immediately available following low dose exposures.* This model predicts that induction of the relevant repair process(es) by adaptive exposure should eliminate low-dose hypersensitivity. *The model has significant implications for environmentally relevant continuous low dose, low dose rate exposures.*

Our working hypothesis is that ability to repair oxidative DNA base damage is critical in cell and tissue responses to low doses of ionizing radiation, as well as being a key determinant of the effects of endogenous DNA damage on the organism (in terms of both cancer predisposition and premature aging). If repair of these lesions is not completed before they are encountered by a replication fork or by transcription, DSBs can result indirectly in the first case, or deleterious blocked transcription in the second. Indeed, reduced ability to repair oxidized pyrimidines has been shown to sensitize human cells to ionizing radiation (1), and more rapid removal of these lesions after a challenge dose is induced by prior low dose exposure (2). Preliminary studies have shown that both the damage-inducibility of thymine glycol removal and its efficiency require functions of XPG and CSB (3; M. Weinfeld, pers. comm.), two proteins implicated both in transcription-coupled repair (TCR) and in global base excision repair (BER) of oxidative DNA damage (see below). Strikingly, a recent transcription profiling study identified changes in expression of two DNA repair proteins – XPG and ATM – as correlating with the radio-adaptive response (4). This observation takes on additional significance in view of our finding that XPG is phosphorylated constitutively and hyperphosphorylated after IR, with ATM implicated as a responsible kinase by metabolic labeling studies. We are therefore investigating the possibility that the adaptive response is mediated by induction of base excision repair processes involving XPG and CSB.

DNA repair processes in mammals are regulated by complex interactions including cross-talk between repair pathways and coordination with replication and transcription. The multifunctional protein XPG is essential for the incision step of nucleotide excision repair (NER) and evidently also functions cooperatively with CSB in a non-enzymatic role to initiate TCR (5), a process defective in the severe developmental disorder Cockayne syndrome (CS; 6). In addition, XPG also has an important scaffold role in BER of oxidative DNA damage: Our evidence suggests

that XPG coordinates and stimulates BER by functional interactions with multiple oxidative damage-specific glycosylases and AP endonuclease. It stimulates all three known human glycosylases for removal of oxidized pyrimidines, as well as both the endonuclease and 3'-dRPase activities of APE1. Notably, XP-G/CS fibroblasts are sensitive to both ionizing radiation and H_2O_2, although they have no detectable defect in repair of ionizing radiation-induced DSBs, and repair of a dihydrouracil lesion is significantly decreased in nuclear extracts of XP-G/CS cells. Furthermore, XPG interacts directly with and stimulates CSB, which has been implicated in global BER of oxidative base damage (7) in addition to its required function in TCR of UV damage. Loss of CSB function produces sensitivity to ionizing radiation and oxidizing agents both in human cells and in mice carrying a mutation mimicking that in a human CS-B patient (8), consistent with the idea that CSB, like XPG, is involved in repair of oxidative DNA damage as well as promoting repair of transcription-blocking lesions processed by NER. Whether the roles of XPG and CSB in BER of oxidative DNA damage are functionally connected to their TCR roles remains to be determined, as does the question of how the functions of these proteins are regulated by exposure to low doses of ionizing radiation. Their requirement in the adaptive response as well as in inducible BER, and the relationship between these processes, is under investigation.

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