DNA Oxidation by Charge Transport in Mitochondria†

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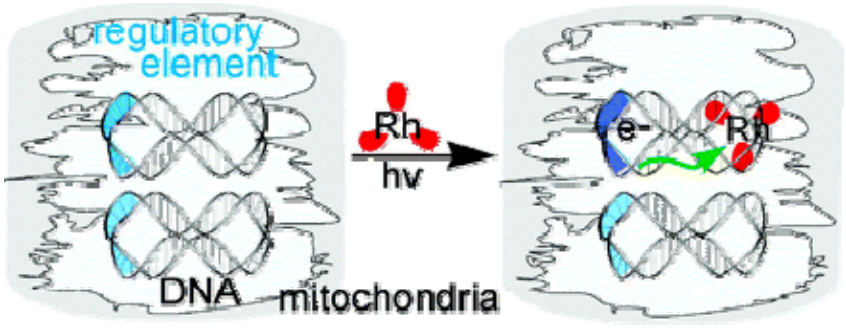

Abstract:

Sites of oxidative DNA damage in functioning mitochondria have been identified using a rhodium photooxidant as a probe. Here we show that a primer extension reaction can be used to monitor oxidative DNA damage directly in functioning mitochondria after photoreaction with a rhodium intercalator that penetrates the intact mitochondrial membrane. The complex $[Rh(phi)_2bpy]Cl_3$ (phi = 9,10-phenanthrenequinonediimine) binds to DNA within the mitochondria and, upon irradiation, initiates DNA oxidation reactions. Significantly, piperidine treatment of the mitochondria leads to protein-dependent primer extension stops spaced every ~20 base pairs. Hence, within the mitochondria, the DNA is well covered and packaged by proteins. Photolysis of the mitochondria containing $[Rh(phi)_2bpy]^{3+}$ leads to oxidative DNA damage at positions 260 and 298; both are mutational hot spots associated with cancers. The latter position is the 5'-nucleotide of conserved sequence block II and is critical to replication of the mitochondrial DNA. The oxidative damage is found to be DNA-mediated, utilizing a charge transport mechanism, as the Rh binding sites are spatially separated from the oxidation-prone regions. This long-range DNA-mediated oxidation occurs despite protein association. Indeed, the oxidation of the mitochondrial DNA leads not only to specific oxidative lesions, but also to a corresponding change in the protein-induced stops in the primer extension. Mitochondrial DNA damage promotes specific changes in protein-DNA contacts and is thus sensed by the mitochondrial protein machinery.