Molecular basis for the DNA damage-induced interaction between cytochrome c and the histone chaperone SET/TAF-Iβ

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During the DNA damage response, nucleosome eviction by histone chaperones provides access of repair machinery to DNA injuries. The histone chaperone and oncoprotein SET/*template-activating factor*-Iβ (SET) engages DNA repair response. Upon DNA insults, mitochondrial cytochrome *c* (C*c*) reaches the cell nucleus, where it binds SET so as to inhibit its histone chaperone activity. SET functions as a homodimer in which each monomer consists on an N-end α-helix dimerization domain (residues 1-80), a globular α/β domain (a.k.a. *earmuff*; 81-225) and a low-complexity acidic region (LCAR; 226-277). Our previous data demonstrate that SET dimerization and *earmuff* domains are sufficient to bind both histones and C*c*. To further characterize the SET-C*c* complex, we deployed a methodological approach combining Electron Paramagnetic Resonance (EPR) and Nuclear Magnetic Resonance (NMR) with Small-Angle X-ray Scattering (SAXS). For such purpose, we have designed five single cysteine mutants across a SET construct lacking its disordered region, named SET-ΔC (residues 1 to 225). Cysteine residues were bound to either nitroxide spin or ^{19}F probes. Continuous-wave EPR spectra of the spin probe and chemical-shift perturbations of ^{19}F resonances were assessed to determine those regions from SET-ΔC interacting with C*c*. Paramagnetic Relaxation Enhancement NMR (PRE-NMR) measurements induced by the SET spin probe onto the C*c* surface deciphered those residues of the hemeprotein involved in the complex formation. SAXS experiments enabled to obtain a low-resolution model of the SET-C*c* complex. Altogether, our findings indicate that C*c* recognizes the globular domains of SET, where histones bind to, so providing a molecular basis for histone chaperone inhibition activity.