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The Structure of the Human Mitochondrial β-ketoacyl-ACP synthase

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Life depends on fatty acids. The practical consequences of this dependency are illustrated by the capacity of the fatty acid synthase inhibitors cerulenin and C75 to induce apoptosis in certain cancer cell lines, leaving healthy cells unaffected. The traditional view on *de novo* fatty acid synthesis in mammals confines it to the cytosolic multifunctional fatty acid synthase. Evidence of *de novo* fatty acid synthesis in isolated mitochondria and the cloning of nuclear genes encoding mitochondrially targeted proteins homologous to the prokaryotic fatty acid synthases revolutionizes this view and adds a novel unexplored dimension to eukaryotic fatty acid biosynthesis. The mitochondrial fatty acid synthesis system is proposed to provide the medium chain length fatty acids for synthesis of the 8 carbon (C8) lipoic acid, a co-factor to pyruvate dehydrogenase essential for respiration. We have expressed, purified and characterized the product of the putative human mitochondrial ß-ketoacyl ACP synthase (*mt*KAS) suspected to catalyze all the carbon-carbon bond forming reactions in mitochondrial fatty acid synthesis. The X-ray crystal structure of the mtKAS to 2.05 Å resolution and of its complex with a C6-acyl substrate complex to 1.65 Å have been determined. The low sensitivities of the human *mt*KAS to cerulenin and C75 were unexpected in that the *Arabidopsis mt*KAS is very sensitive to cerulenin. A bulky amino acid restricting access to the far end of the fatty acid binding pocket provides a possible explanation for the low affinity, and thereby an explanation for the successful use of these inhibitors in inducing apoptosis in cancer cell lines.

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Aminoglycoside binding to hiv-1 dis rna kissing-loop complex: from crystals to cells

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All retroviral genomes consist in two homologous single stranded RNAs linked near their 5' ends. HIV-1 Dimerization Initiation Site (DIS) is a strongly conserved stem-loop in the 5' region of the genomic RNA. It was shown *in vivo* that alteration of the DIS strongly reduces viral infectivity. We have previously solved crystal structures of the DIS kissing-loop complex [1], [2]. Analysis of these crystal structures revealed an unexpected resemblance between the DIS kissing-loop and the 16 S ribosomal aminoacyl-tRNA site (A-site), which is the target of aminoglycoside antibiotics. We have shown that some aminoglycosides specifically bind to the DIS kissing-loop complex with an affinity and geometry similar to that observed in the A-site [3].

In agreement with these previous results, we have now solved crystal structures of the DIS kissing-loop complex bound to four aminoglycosides: neamine, ribostamycin, neomycin and lividomycin solved at 1.8 to 2.2 A resolution. Electron density maps are of excellent quality, revealing most of the RNA and drug hydration. These structures show that two aminoglycosides are bound per kissing-loop complex and strongly stabilize the loop-loop interaction. In spite of the difference in topology with the ribosomal A-site, the DIS loop-loop complex is specifically recognized by the antibiotic. Rings 1,2 and 3 of aminoglycosides are required for specific recognition, whereas rings 4 and 5, interacting through non-specific contacts, are required to improve the affinity. Importantly, the binding was also observed by chemical probing in infected cells and in viral particles, showing that the DIS remains accessible to these drugs *in vivo* and is therefore a valuable new viral target. These structures provide an excellent starting point for designing potential new drugs targeted against the viral RNA.

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