Summary

Cell death-inducing DFF[DNA fragmentation factor]-like effector-a (CIDEa), may initiate apoptosis by disrupting a complex consisting of 40-kDa caspase-3-activated nuclease DFF40 and its 45-kDa inhibitor DFF45. We measured the levels of mRNA CIDEa in rattus norvegicus tissues and detected high levels of RNA in white adipose tissue. We have confirmed the presence of CIDEa in mitochondria and importance of CIDE-C domain for this localization. We have also performed immunodetection of subcellular fractions of HeLa cells adapted for a tetracycline-inducible CIDEa expression. We have observed redistribution, enhanced upon treatment with camptothecin or valinomycin, of CIDEa to nucleus. CIDEa content increased in the nuclear fraction but decreased in cytosolic fraction in cells treated to initiate apoptosis. We hypothesize that CIDEa is sequestered in mitochondria while transfer of this potentially dangerous protein from mitochondria into nucleus intensifies or even initiates apoptosis.

Mitochondria in numerous cell types, especially in cultured cells, form a reticular network undergoing constant fusion and fission. The three dimensional (3D) morphology of these networks however has not been studied in detail to our knowledge. We have investigated insulinoma INS-1E and hepatocellular carcinoma HEP-G2 cells transfected with mitochondria-addressed GFP. Using 4Pi microscopy, 3D morphology changes responding to decreased oxidative phosphorylation and/or energetic status could be observed in these cells at an unprecedented 100 nm level of detail. In INS-1E cells cultivated at 11 mM glucose, the mitoreticulum appears predominantly as one interconnected mitochondrion with a nearly constant 262±26 nm tubule diameter. When cultured at 5 mM glucose, INS-1E cells show 311±36 nm tubules coexisting with numerous flat cisternae. Similar interconnected 284±38 nm and 417±110 nm tubules were found in HEP-G2 cells cultivated at 5 mM and hyperglycemic 25 mM glucose, respectively. With rotenone inhibiting respiration to ~10%, disintegration into several reticula and numerous ~300 nm spheres or short tubules was observed. Deenergization by uncoupling additionally led to formation of rings and bulky cisternae of 1.4±0.4 µm diameter. Rotenone and uncoupler acted synergically in INS-1E cells and increased fusion (ongoing with fission) forming bowl-like shapes. Thus we have revealed previously undescribed details for shapes upon mitochondrial disintegration and clearly demonstrated that high resolution 3D microscopy is required for visualization of the mitochondrial network.