P006 Mitochondrial localization regulates Calcium entry, but not ER Calcium release

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Whether different subsets of mitochondria play distinct roles in shaping intracellular Ca^{2+} signals is presently unresolved. Here, we over-express of the dynactin subunit dynamitin in order to inhibition the fission factor dynamin-related protein (Drp-1), and relocalise mitochondria from the plasma membrane towards the nuclear periphery in HeLa cells. The impact of these changes on free calcium concentration in the cytosol ($[Ca^{2+}]_c$), mitochondria ($[Ca^{2+}]_m$) and ER ($[Ca^{2+}]_{ER}$) was then monitored with specifically-targeted aequorins and mitochondrially-targeted fluorescent probes (mitopericam). Whilst dynamitin over-expression increased the number of close contacts between the ER and mitochondria by 2.5-fold, assessed using organelle-targeted GFP variants and spinning disc confocal microscopy, histamine-induced changes in organellar $[Ca^{2+}]$ were unaffected over a range of histamine concentrations. By contrast, Ca^{2+} influx elicited significantly smaller increases in $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ in dynamitin-expressing than in control cells. Moreover, using mitochondrially-targeted dsRed and confocal imaging, we demonstrate that mitochondria are highly motile in Hela cells. These data suggest that (a) the strategic localisation of a subset of mitochondria beneath the plasma membrane is required for normal Ca^{2+} influx, but that (b) sites of transfer of Ca^{2+} ions between mitochondria and the ER are highly mobile, questioning the existence of a stable protein complex responsible for Ca^{2+} exchange.