Increased ER–mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress

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In the section given below, PtdIns(3,4,5)P_3 was on four occasions incorrectly printed instead of the correct Ins(1,4,5)P_3.

We apologise for this mistake.

Increased mitochondrial Ca^{2+} drives the adaptive metabolic boost observed during early phases of ER stress

Increases in mitochondrial respiration and ATP production are often consequences of increases in mitochondrial Ca^{2+} (Green and Wang, 2010). In order to determine whether early phases of ER stress induced by tunicamycin increased mitochondrial Ca^{2+} , we treated cells expressing cytosolic or mitochondrial aequorins with histamine [which evokes Ins(1,4,5)P_3-dependent Ca^{2+} release] and compared their mitochondrial Ca^{2+} uptake. We observed that histamine led to a mitochondrial Ca^{2+} uptake that was significantly higher in tunicamycin-pretreated cells ($P<0.05$; 4 hours) than in untreated cells (Fig. 6A). Cytosolic Ca^{2+} increased similarly in tunicamycin-treated and untreated cells (Fig. 6B). These results indicate that the differences in mitochondrial Ca^{2+} levels are not due to altered Ca^{2+} release mediated by the Ins(1,4,5)P_3 receptor but to an enhanced mitochondrial Ca^{2+} uptake, presumably due to the increased apposition of ER and mitochondrial Ca^{2+} channels. By using a different dye, Fura-2, we monitored the peak cytosolic Ca^{2+} levels after thapsigargin addition, reflecting the kinetics of Ca^{2+} release after sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibition. After 4 hours of tunicamycin treatment, the thapsigargin-induced Ca^{2+} peak was increased, and it was further elevated by inhibition of mitochondrial Ca^{2+} uptake using Ru360 (Fig. 6C). These results suggest that, besides the Ins(1,4,5)P_3-receptor-mediated direct Ca^{2+} transfer from the ER to neighboring mitochondria, an additional phenomenon associated with the early phases of ER stress involves Ca^{2+} leak from the ER, also resulting in mitochondrial Ca^{2+} uptake. Indeed, no mitochondrial Ca^{2+} uptake following the thapsigargin-induced Ca^{2+} leak was observed in *Mfn2*-knockout cells (Fig. 6D), which is reflected by the lack of effect of Ru360. This result further indicates that juxtaposition of mitochondria with the ER is necessary for the mitochondrial Ca^{2+} uptake evoked by Ca^{2+} leak during early phases of ER stress.

Finally, to test whether mitochondrial Ca^{2+} levels control the metabolic mitochondrial boost, we measured oxygen consumption rates resulting from OXPHOS in the presence of the Ins(1,4,5)P_3 receptor inhibitor xestospongin B or the mitochondrial Ca^{2+} uptake inhibitor RuRed. We observed that both xestospongin B and RuRed decreased the rate of oxygen consumption after tunicamycin treatment (Fig. 7A,B), which confirms that increased mitochondrial Ca^{2+} uptake, resulting from ER–mitochondrial coupling, is necessary for the metabolic response observed during early phases of ER stress. Therefore, in order to evaluate whether the early metabolic boost forms part of an adaptive response triggered by ER stress, we inhibited mitochondrial Ca^{2+} uptake and measured cell viability [through propidium iodide (PI) incorporation] and $\Delta\psi_m$. We observed that the inhibition of mitochondrial Ca^{2+} uptake during the early phase of ER stress increased cell death (PI-positive cells) and also decreased $\Delta\psi_m$ at 48 hours (Fig. 7C).

In total, the results presented in this study suggest strongly that Ca^{2+} transfer resulting from enhanced ER–mitochondrial coupling leads to a localized increase in mitochondrial metabolism, thus providing energetic substrates key for a cellular adaptive response in face of ER stress. Further experiments will determine whether this bioenergetic response is necessary for improving the energetic state of the ER, and therefore its folding capacity, or, as it is restricted to perinuclear zones, for the activation of a specific nuclear transcriptional program that participates in the cellular adaptation to stress.

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