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Isolation of well purified mitochondria and mitochondria associated membranes (MAM) from animal tissues and cell cultures.

Recent studies have shown, that close apposition of the endoplasmic reticulum (ER) to the mitochondrial surface enables the uptake of  $\text{Ca}^{2+}$  by mitochondria via a low-affinity  $\text{Ca}^{2+}$  uniporter in a very efficient way, because it can be exposed to a microdomain of  $\text{Ca}^{2+}$  concentration ( $50\mu\text{M}$ ) higher than in the bulk cytosol during cell stimulation and needed for uniporter activation ( $10\mu\text{M}$ ).

Due to the low cellular content of MAM fraction, based on the experience of our laboratories, we propose a well-tried isolation protocol, which enables isolation of high-purity MAM and mitochondria fractions from animal tissues and cell cultures in a sufficient amount that can be used for proteomic and other molecular biology studies.

Interactions between the mitochondria and the ER can be important for the generation of the proper calcium signals but also in cell death and in the biosynthesis and trafficking of phospholipids between the two organelles. The identification of the molecular components of such contacts can be potentially useful for a wide range of studies in different physiological and pathological cellular processes.

Mariusz R Wieckowski, Carlotta Giorgi, Magdalena Lebieczinska, Jerzy Duszynski and Paolo Pinton (2009) **Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells**. Nature Protoc. 2009; 4 (11): 1582-90.