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Mitochondria-Associated Endoplasmic Reticulum Membranes in Insulin Signaling

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Hepatic insulin resistance is a key feature of type 2 diabetes, and the consequent dysregulation of glucose and lipid output from the liver are important contributors to the observed hyperglycemia and hyperlipidemia. While excessive lipid accumulation (1) and defective signaling by protein kinase C (2) have been implicated in the past, the full panoply of molecular mechanisms involved is still not defined. Deeper insights would therefore be welcome in the quest to identify new therapeutic approaches to the disease (3).

Mitochondrial metabolism and its dysfunction are well-known to contribute to metabolic dyshomeostasis in type 2 diabetes, with impaired glucose oxidation resulting from a failure to fully activate the intramitochondrial pyruvate dehydrogenase (PDH) complex (4). Originally described as sites for the exchange of phospholipids between organelles (5), mitochondria-associated membranes (MAMs) represent close contact sites through which endoplasmic reticulum (ER) communicates with mitochondria supporting the transfer not only of lipids but also the exchange of calcium (Ca^{2+}) ions and other species. MAMs play important roles in several signal transduction pathways and the relevance of the ER–mitochondria interface to human diseases, including Alzheimer disease, cancer, and lysosomal storage disease (6), is slowly emerging. In this issue, Tubbs et al. (7) demonstrate the potential importance of MAM integrity in insulin action and resistance in hepatocytes.

The association between ER and mitochondrial homeostasis and insulin signaling has recently been a topic of intense investigation and debate (8–10). Importantly, a reduction in ER-mitochondrial cross talk, achieved by liver-specific ablation of the regulator of mitochondrial fusion, mitofusin (Mfn2), causes mitochondrial dysfunction, insulin resistance, and impaired glucose tolerance (11). Moreover, the target for insulin signaling mammalian target of rapamycin complex 2 (mTORC2) is localized to MAMs and regulates their integrity (8). Tubbs et al. reinforce the concept that MAMs play a critical role in insulin signaling with an innovative new approach.

The techniques routinely used to study the MAMs are based on analysis of ER-mitochondria contact sites via electron microscopy, intracellular localization of MAM markers (such as FACL-4 or SigmaR1) in combination with microscopy techniques, or isolation of MAMs through subcellular fractionations followed by Western blot analysis (12).

Tubbs et al. optimize an in situ proximity ligation assay using a protein of the outer mitochondrial membrane (voltage-dependent anion channel, VDAC1), the inositol 1,4,5-trisphosphate receptor (IP3R) for the ER membranes or the molecular chaperone glucose-regulated protein 75 (Grp75) as probes (Fig. 1).

The new method extends the capabilities of traditional immunoassays to include direct detection of protein interactions and modifications with high specificity and sensitivity. With this, and complementary techniques, the group coordinated by Jennifer Rieusset (7) clearly demonstrates a strong relationship between MAM integrity and efficient insulin action in hepatic cells (Fig. 1). Indeed, they demonstrate in vitro and in vivo that insulin action is directly linked to the MAM formation and maintenance and show that this is disrupted in murine models of type 2 diabetes.

Tubbs et al. also show that protein kinase B (PKB, also known as Akt) is a critical kinase involved at the MAM level, possibly interacting with IP3R1 and probably mediating its phosphorylation, in turn regulating ER Ca^{2+} release through IP3R1. However, it is possible that other members of the IP3R family beyond type 1 may also

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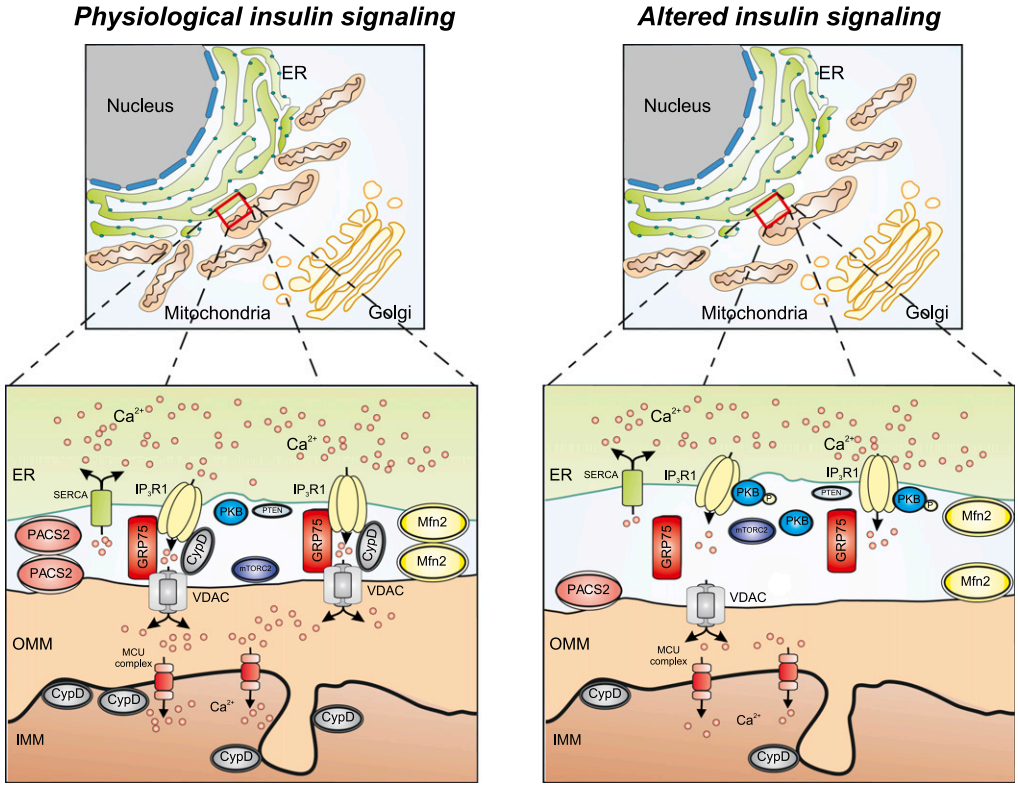

Figure 1—MAM integrity is required for the correct insulin signaling and action in hepatocyte. CypD, cyclophilin D; IMM, inner mitochondrial membrane; MCU, mitochondrial Ca^{2+} uniporter; OMM, outer mitochondrial membrane; P, phosphorylated; PACS2, phosphofurin acidic cluster sorting protein 2; PTEN, phosphatase and tensin homolog; SERCA, sarcoendoplasmic reticulum Ca^{2+} transport ATPase.

be involved. Indeed, IP3R type 3 is the most abundant IP3R isoform present at the MAM, and it seems to be the only isoform whose induced PKB phosphorylation modulates effectively the release of Ca^{2+} from the ER (8,13).

Importantly, the authors show that enhancement of MAM formation restores hepatic insulin signaling. However, whether the original alterations cause, or result from, insulin resistance is still unknown. It will be interesting, therefore, to follow up this work to understand which precise signal transduction mechanisms (Ca^{2+} signals, reactive oxygen species, lipids, or all of these?) are involved. Studies using liver cells from healthy or type 2 diabetic human donors will also be necessary to confirm the relevance of this phenomenon to the disease in humans.

An intriguing aspect of the present study is that the role that cellular Ca^{2+} homeostasis plays in insulin signaling has previously been unclear. Whereas several G-protein–coupled receptor-linked hormones, such as vasopressin (14), clearly lead to increases in both cytosolic and mitochondrial Ca^{2+} in liver cells (15) by gating IP3R, there is little evidence for changes in the concentration of these ions in response to insulin (16). One possible mechanism reconciling these earlier studies and the present study is that a low, tonic level of Ca^{2+} outflow from the ER into mitochondria is required to ensure that insulin-derived signals are able to activate PDH (17). It is also conceivable that insulin causes a highly localized redistribution of Ca^{2+} between the ER and mitochondria close to MAMs.

Finally, it will be important to learn whether MAM integrity is required in other cell types as well as in hepatocytes. Glucose-induced Ca^{2+} changes play well-defined roles in the control of insulin secretion from pancreatic β-cells (18), whose failure ultimately drives the progression toward frank type 2 diabetes (19). Whereas mitochondrial integrity has been shown to be critical for normal glucose sensing in β-cells (20), the role of ER-mitochondria contacts (i.e., MAMs) has not been examined. Given that β-cells are probably also targets for insulin action (21), alterations in MAM interaction in these cells may be of particular importance. Likewise, the role of MAMs in muscle tissue, where normal mitochondrial functions are vital to avoid insulin resistance (2), is also an important question.

Understanding the interplay and the signaling specificity between ER and mitochondria thus appears to be an important new goal in diabetes research. We hope the article by Tubbs et al. (7) and future work may provide the impetus for the development of new molecularly targeted drugs, bringing the prospects of a new day for efficient diabetes treatment closer to reality.

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