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Review

Organelle plasticity and interactions in cholesterol transport and steroid biosynthesis

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ABSTRACT

Steroid biosynthesis is a multi-step process controlled by pituitary hormones, which, via cAMP-dependent signaling pathways, drive tissue-specific steroid formation. Steroidogenesis begins with the transport of the substrate, cholesterol, from intracellular stores into the inner mitochondrial membrane, where the steroidogenic enzyme CYP11A1 converts cholesterol to pregnenolone. This process is accelerated by hormones and involves a number of proteins and protein–protein interactions. Indeed, cholesterol, stored in lipid droplets and membranes, is transferred through a hormone-induced complex of proteins derived from the cytosol, mitochondria, and other organelles termed the transduceosome to the outer mitochondrial membrane. From there, cholesterol reaches CYP11A1 through outer/inner membrane contact sites. Thus, cholesterol transfer is likely achieved through a hormone-dependent reorganization of organelles and protein distribution and interactions. The findings reviewed herein suggest the presence of a hormone-dependent organelle communication network mediated by protein–protein interactions and inter-organelle trafficking, resulting in the efficient and timely delivery of cholesterol into mitochondria for steroid synthesis.

Contents

Abbreviations: cAMP, cyclic AMP; ACAT, acyl-CoA cholesterol acyltransferase; ACSL4, acyl-CoA synthetase 4; ANT, adenine nucleotide transporter; BN-PAGE, blue native polyacrylamide gel electrophoresis; CPT, carnitine palmitoyltransferase; ER, endoplasmic reticulum; ETC, electron transfer chain; FDX, ferredoxin; FDXR, ferredoxin reductase; IDH2, isocitrate dehydrogenase 2; IMM, inner mitochondrial membrane; LAM, lipid droplet-associated membrane; LD, lipid droplet; MAM, mitochondria-associated membrane; MDH2, malate dehydrogenase 2; MFNs, mitofusins; MPTP, mitochondrial permeability transition pore; OMM, outer mitochondrial membrane; PKA, protein kinase A (cAMP-dependent); ROS, reactive oxygen species; SE, sterol ester; TCA, tricarboxylic acid cycle or citric acid cycle; TG, triglyceride; TSPO, translocator protein (18-kDa); VDAC, voltage dependent anion channel.

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1. Introduction

Steroids are critical mediators of numerous processes in the body, from the regulation of development and reproduction to behavior. Under physiological conditions, steroidogenic cells store a low amount of steroid hormones but in response to circulating pituitary hormones adrenal and gonadal cells rapidly produce large amounts of glucocorticoids, mineralocorticoids, progestins, androgen, and estrogen to respond to various physiological needs. The placenta and brain also have the ability to make steroids that are targeted to satisfy specific needs of these tissues. At present, there is no evidence that pituitary hormones control placental and brain steroidogenesis. Steroid biosynthesis begins at the mitochondrion, where cholesterol, transferred from intracellular stores to the outer and then inner mitochondrial membrane (IMM), is metabolized by the cytochrome P450 side chain cleavage enzyme CYP11A1, located in the matrix side of the IMM.

Although steroidogenic cells constitutively produce a certain amount of steroids, this process is accelerated by the pituitary trophic hormones, adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), and follicle stimulating hormone (FSH), which induce the production of cyclic AMP (cAMP) (Jefcoate, 2002; Simpson and Waterman, 1988). This rise in intracellular cAMP results in the mobilization of free cholesterol (Garren et al., 1971). This cholesterol is transferred to mitochondria to be used for the production of steroids. The transfer of hydrophobic cholesterol has been proposed to occur in three steps: (i) integration at the outer mitochondrial membrane (OMM), where it remains segregated from the structural membrane cholesterol; (ii) movement from the OMM to the IMM; and (iii) loading onto CYP11A1 at the matrix side of the IMM. Although not much was known about the first step, the identification of a hormone-induced multiprotein complex composed of cytosolic and OMM proteins at the OMM controlling the rate of steroid formation named the transduceosome (Liu et al., 2006; Rone et al., 2009a) enhanced our understanding of the mechanisms underlying cholesterol entry into the OMM and will be discussed later in this review. It has been proposed that the transfer of cholesterol across the hydrophilic intra-mitochondrial membrane space occurs at specialized contact sites where there is apposition between the OMM and the IMM (Stevens et al., 1985; Thomson, 2003). This was recently confirmed by the identification of a bioactive, multimeric protein complex spanning the OMM–IMM unit that is responsible for the hormone-induced import, segregation, targeting, and metabolism of cholesterol (Rone et al., 2012) Thus, cholesterol import into the OMM through the transduceosome and passage into the IMM through the contact sites bring cholesterol to CYP11A1, which is present in this multimeric complex at the OMM–IMM contact sites, for enzyme loading and conversion to pregnenolone (Hall, 1985; Jefcoate, 2002; Rone et al., 2012). Pregnenolone, the precursor of all steroids, can then be converted to tissue-specific steroid hormones through steroidogenic enzymes located in mitochondria or the endoplasmic reticulum (ER), though the exact mechanism is currently unknown.

The identified multiprotein enzyme complex formed by transduceosome proteins and the OMM–IMM contact sites containing CYP11A1 is able to transfer the substrate cholesterol to its site of metabolism without equilibration with and diffusion into the surrounding environment (Rone et al., 2012). Such supramolecular complexes have been referred to as protein machines or metabolons (Srere, 1987).

As mitochondria are cholesterol-poor organelles, the mobilization of cholesterol from different cytosolic sources, such as lipid droplets (LDs), the ER, and others, to mitochondria ensures the continuation of steroid production. The identification of the transduceosome, which allows the association of mitochondria with cytosolic proteins and multiple organelles, provides the missing physical link between organelles that represent potential sources of the steroidogenic cholesterol and mitochondria, the site of cholesterol metabolism. The regulation of inter-organelle interactions with the mitochondria should provide further flexibility of cholesterol sources and regulation of downstream steroid formation.

In this review, we discuss the intracellular mechanisms by which steroidogenesis is initiated and tightly regulated, focusing on the transfer of cholesterol via protein–protein and inter-organelle interactions to CYP11A1. We first discuss the initial transfer of cholesterol to the IMM through the transduceosome and the formation of the steroidogenic metabolon. Then, we discuss the role mitochondrial morphology plays in steroidogenesis and the roles that other intracellular organelles, specifically the ER and LDs, play in making cholesterol available for steroidogenesis via their interactions with mitochondria.

2. Transduceosome and steroidogenic metabolon: hormone-dependent mitochondrion-targeted cholesterol import and metabolism

In 2006, we identified an OMM protein complex termed the transduceosome that propagates cAMP signaling and initiates cholesterol transfer to the mitochondria, thus functioning as a sieve at the OMM by separating and restricting cholesterol for steroidogenic activity (Liu et al., 2006). This regulated, controlled flow of cholesterol into mitochondria is accomplished by anchoring of the cytosolic components of the transduceosome, acyl-CoA binding domain-containing 3 (ACBD3), protein kinase A regulatory subunit I alpha (PKA-RIα), and the hormone-induced mitochondrion-targeted steroidogenesis acute regulatory protein (STAR) to the OMM proteins translocator protein (18 kDa, TSPO) and voltage-dependent anion channel (VDAC). While the initial components of the proposed metabolon allow cholesterol transfer to the OMM, the transduceosome does not identify the key mechanism of this metabolon of cholesterol transfer to the IMM and CYP11A1. Therefore, to further understand this process and identify the proposed metabolon regulating the rate-limiting step in steroid production, we utilized blue native-polyacrylamide gel electrophoresis (BN-PAGE), chemical crosslinking, and mass spectrometry. In early 2012, we reported the presence of an 800-kDa protein complex composed of OMM and IMM proteins, including TSPO, VDAC, AAA + ATPase ATAD3, and CYP11A1 (Rone et al., 2012). A schematic representation of the transduceosome and steroidogenic metabolon is shown in Fig. 1A. While each of these proteins is