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Review

Underlying mechanisms for sterol-induced ubiquitination and ER-associated degradation of HMG CoA reductase

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ABSTRACT

Accelerated ubiquitination and subsequent endoplasmic reticulum (ER)-associated degradation (ERAD) constitute one of several mechanisms for feedback control of HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol and nonsterol isoprenoids. This ERAD is initiated by the accumulation of certain sterols in ER membranes, which trigger binding of reductase to ER membrane proteins called Insigs. Insig-associated ubiquitin ligases facilitate ubiquitination of reductase, marking the enzyme for extraction across the ER membrane through a reaction that is augmented by nonsterol isoprenoids. Once extracted, ubiquitinated reductase becomes dislocated into the cytosol for degradation by 26S proteasomes. In this review, we will highlight several advances in the understanding of reductase ERAD, which includes the discovery for a role of the vitamin K_2 synthetic enzyme UBIAD1 in the reaction and demonstration that sterol-accelerated ERAD significantly contributes to feedback regulation of reductase and cholesterol metabolism in livers of whole animals.

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1. Introduction

The endoplasmic reticulum (ER)-localized enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase catalyzes synthesis of mevalonate, a rate-limiting reaction in the branched pathway that produces sterols such as cholesterol and nonsterol isoprenoids including farnesyl pyrophosphate (Fpp), geranylgeranyl pyrophosphate (GGpp), dolichol, heme, ubiquinone, and the vitamin K_2 subtype menaquinone-4 (MK-4) (Fig. 1) [1,2]. Cholesterol, the bulk end-product of the mevalonate pathway, plays a well-known role in the maintenance of membrane integrity. In addition, cholesterol is a precursor in synthesis of steroid hormones as well as vitamin D and is an important component of lipoproteins that ferry the sterol and other lipids throughout the body. Nonsterol end-products of mevalonate metabolism play crucial roles in a variety of cellular processes. These range from electron transport (heme and ubiquinone), asparagine-linked glycoprotein synthesis (dolichol), and coagulation (vitamin K_2) to signal transduction, cell growth, and migration (Fpp and GGpp) [2]. A constant supply of nonsterol isoprenoids is required for optimal cell function; however, cells must avoid the overaccumulation of cholesterol, which can form solid crystals that are highly toxic. This toxicity extends to the whole animal; excess levels of circulating cholesterol initiates atherosclerosis, a major risk factor for coronary artery disease

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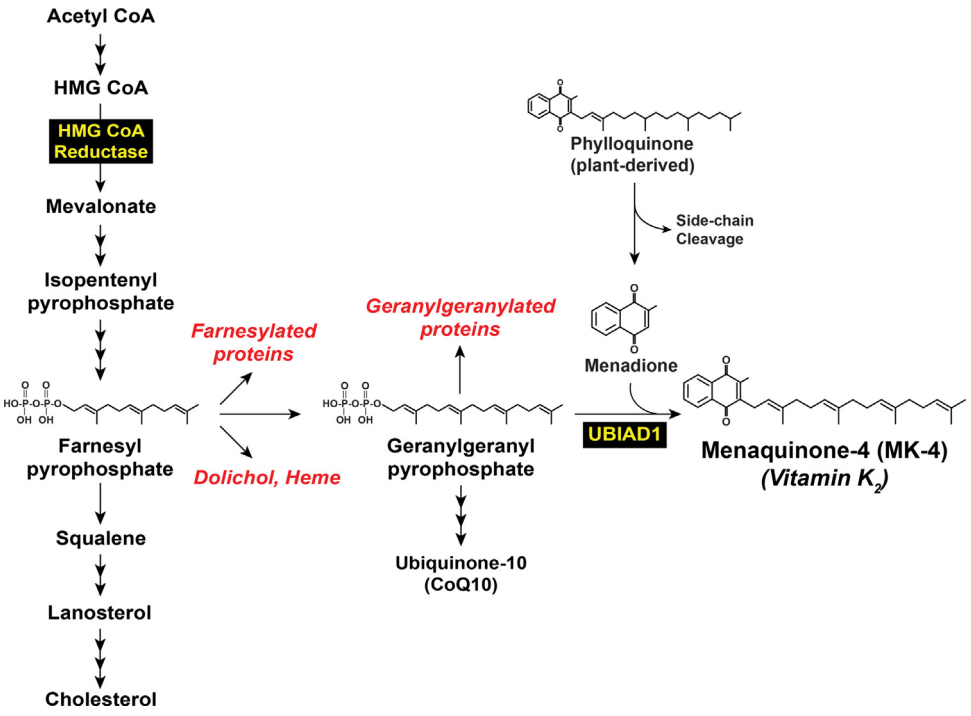

Fig. 1. Biosynthesis of cholesterol and nonsterol isoprenoids in mammalian cells.

[3]. The delicate balance between synthesis of sterol and nonsterol isoprenoids is achieved through a multivalent feedback regulatory system that exerts stringent control on levels and activity of reductase [4].

Results of studies employing compactin, a competitive inhibitor of reductase isolated from fungi by Endo and co-workers [5,6], first revealed the multivalent feedback regulation of reductase. When cells were cultured in medium supplemented with fetal calf serum (FCS), reductase levels were suppressed and cholesterol was synthesized at low rates owing to receptor-mediated uptake of low density lipoproteins (LDLs) [7]. Cholesterol liberated from internalized LDL suppressed reductase through the multivalent feedback regulatory system. Depriving cells of cholesterol by incubating them in the absence of lipoproteins and the presence compactin disrupted the reductase regulatory system, eliciting a marked increase (up to 200-fold) in the amount of reductase protein [8]. The addition to cells of LDL or oxysterols, which bypass the LDL-receptor for cellular uptake, partially suppressed reductase. However, complete reversal of the compactin-induced increase in reductase required treatment of cells with small amounts of mevalonate together in addition to exogenous sterols. Together, these findings provided a basis for the concept that sterol and nonsterol isoprenoids mediate multiple feedback mechanisms that govern the levels of reductase.

Sterol and nonsterol isoprenoids differentially inhibit reductase through multiple mechanisms including: 1) inhibiting transcription of the reductase gene; 2) blocking translation of the reductase mRNA; and 3) accelerating degradation of the reductase protein (Fig. 2) [1]. Sterols mediate transcriptional regulation of reductase by blocking proteolytic activation of sterol regulatory element-binding proteins (SREBPs). This family of membrane-bound transcription factors enhance transcription of genes encoding reductase and other cholesterol biosynthetic enzymes as well as the LDL-receptor [9]. Translational regulation is mediated by an unknown nonsterol isoprenoid through a reaction that may be mediated by the complex 5′-untranslated region of the reductase mRNA [1]. Sterol and nonsterol isoprenoids combine to accelerate ER-associated degradation (ERAD) of reductase through a mechanism mediated by the ubiquitin/proteasome system [10–12]. This ERAD reduces the half-life of reductase protein from 12 h in compactin-treated cells to less than 1 h in sterol-replete cells.

2. Insigs, major players in transcriptional and post-transcriptional mechanisms for feedback regulation of HMG CoA reductase

Mammalian HMG CoA reductase is comprised of 887 or 888 amino acids; the protein can be roughly divided into two distinct domains (Fig. 3**A**). The N-terminal domain of reductase anchors the protein to ER membranes and consists of eight transmembrane domains separated by short hydrophilic loops [13]. The hydrophilic C-terminal domain of reductase projects into the cytosol where it exerts all of the catalytic activity of the enzyme [14]. Two early observations indicate that the membrane domain plays a key role in ERAD of reductase. First, expression of the truncated, soluble C-terminal domain of reductase rescues the cholesterol auxotrophy of reductase-deficient Chinese hamster ovary (CHO) cells [15]. However, this protein was found to be very stable and sterols failed to accelerate its degradation. The second observation was provided by studies of a fusion protein between the membrane domain of reductase and soluble β-galactosidase [16]. Degradation of the reductase-β-galactosidase fusion protein was accelerated by sterols in a manner similar to that of wild type, full-length reductase. Considered together, these key findings suggest a scenario in which the membrane domain of reductase directly or indirectly senses membrane-embedded sterols, triggering reactions that cause the entire enzyme to become susceptible to proteolytic degradation. This degradation can be blocked by inhibitors of the 26S proteasome, which leads to the accumulation of ubiquitinated forms of reductase [11].

Insight into the mechanism for sterol-accelerated ERAD of reductase emerged from the transcriptional axis of the multivalent reductase regulatory system. Proteolytic activation of SREBPs requires Scap, which contains an N-terminal membrane domain

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