A Highly Conserved Signal Controls Degradation of 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) Reductase in Eukaryotes*

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Sterol synthesis by the mevalonate pathway is modulated, in part, through feedback-regulated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR). In both mammals and yeast, a non-sterol isoprenoid signal positively regulates the rate of HMGR degradation. To define more precisely the molecule that serves as the source of this signal, we have conducted both pharmacological and genetic manipulations of the mevalonate pathway in yeast. We now demonstrate that farnesyl diphosphate (FPP) is the source of the positive signal for Hmg2p degradation in yeast. This FPP-derived signal does not act by altering the endoplasmic reticulum degradation machinery in general. Rather, the FPP-derived signal specifically modulates Hmg2p stability. In mammalian cells, an FPP-derived molecule also serves as a positive signal for HMGR degradation. Thus, both yeast and mammalian cells employ the same strategy for regulation of HMGR degradation, perhaps by conserved molecular processes.

The mevalonate pathway is responsible for the biosynthesis of numerous essential molecules including prenyl groups, coenzyme Q, dolichol, and sterols such as cholesterol (1). 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) is a key enzyme of the pathway and is rate-determining for cholesterol synthesis in mammals (2, 3). The mevalonate pathway is modulated in large part by feedback control of the amount of HMGR protein (1), and a significant portion of HMGR feedback control occurs through regulation of HMGR degradation (4–6). HMGR is an integral endoplasmic reticulum (ER) membrane protein, and its degradation occurs without exit from the ER (6–8). The non-catalytic, N-terminal transmembrane anchor of HMGR is both pharmacologically and genetically required for normal regulation of Hmg2p, called HRD genes (12). Our earlier work has shown that the yeast HMGR isozyme Hmg2p is degraded in a regulated manner with many similarities to the analogous process in mammals. Through the use of genetic selections and screens, we have been able to identify genes required for the degradation of Hmg2p, called HRD genes (11), and genes required for normal regulation of Hmg2p degradation.

Concurrently with these screens, we have been studying the nature of the mevalonate-derived signals that control Hmg2p stability. Hmg2p degradation is regulated by an unknown signal from the mevalonate pathway (Fig. 1a). Inhibiting early pathway enzymes, such as HMGR itself or HMG-CoA synthase, decreases the rate of Hmg2p degradation (6). These early pathway blocks decrease the availability of a downstream signal for degradation. Conversely, inhibiting the enzyme squalene synthase, which is downstream of HMGR, stimulates degradation and ubiquitination of Hmg2p (12). Furthermore, the degradation-enhancing effect of squalene synthase inhibition is abolished by simultaneous inhibition of HMGR degradation.

These pharmacological studies imply that the signal for Hmg2p degradation is a pathway molecule between mevalonate and squalene (Fig. 1a). The most reasonable candidate for this signal is farnesyl diphosphate (FPP) or an off-pathway FPP derivative. The idea that FPP, or a derivative, is a positive signal for Hmg2p degradation is particularly interesting since there is accumulating evidence from in vitro and in vivo studies that farnesol, an FPP-derivative, is a signal for regulation of mammalian HMGR stability (13–17).

We have now tested the hypothesis that FPP provides a molecular signal for control of Hmg2p stability using unique genetic opportunities available in yeast. In conjunction with pharmacological and biochemical approaches, we have constructed yeast strains that allowed either overexpression or down-regulation of specific mevalonate pathway genes (Fig. 1b). Our results indicated that FPP was indeed a source of a signal for Hmg2p degradation in yeast, demonstrating that there is striking conservation for this mode of HMGR regulation among eukaryotes.

EXPERIMENTAL PROCEDURES

Materials and Reagents—All enzymes were obtained from New England Biolabs (Beverly, MA). Chemical reagents were obtained from Sigma. Lovastatin and zaragozic acid were generously donated by Merck. Terbinafine was commercially obtained as a 1% Lamisil® solution from Novartis (East Hanover, NJ). ECL™ chemiluminescence immunodetection reagents were from Amersham Pharmacia Biotech. The anti-Myc 9E10 antibody was used as a cell culture supernatant.

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‡ The abbreviations used are: HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; FPP, farnesyl diphosphate; ER, endoplasmic reticulum; ZA, zaragozic acid; Lov, lovastatin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; kh, kilobase pair; HSV, herpes simplex virus; FACS, fluorescence-activated cell sorter; wt, wild type; HA, hemagglutinin; GFP, green fluorescent protein.

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FIG. 1. Manipulation of the mevalonate pathway. A, representation of the mevalonate (Met) pathway indicating key enzymes and their inhibitors. B, construction of a MET3 promoter-regulated allele by “promoter switching.” A mevalonate pathway gene, ERGX, was placed under control of the MET3 promoter through targeted integration of a promoter-switch plasmid at the ERGX genomic locus. The plasmid consisted of a non-functional, 5′ portion of the ERGX gene placed behind the MET3 promoter (P_{MET3}). Integration resulted in a functional copy of the ERGX gene placed under control of the MET3 promoter and a non-functional, deleted copy behind the native promoter.

Photomicrographs of representative yeast transformants. A, the Met− phenotype (left) with expression of ERG5 from the P_{MET3} promoter (right). B, the wild-type phenotype (left) with expression of ERG5 from the endogenous MET3 promoter (right).
Squalene Synthase Levels Determined the Degree of Hmg2p Degradation—The above results indicated that ZA altered Hmg2p degradation by decreasing squalene synthase activity. In that case, genetic down-regulation of the squalene synthase gene (ERG9) should also increase Hmg2p degradation. Because mevalonate pathway enzymes are essential for yeast viability, a null allele of ERG9 in yeast results in cell death (32, 33). Therefore, we made a conditional allele of squalene synthase by placing the wild-type ERG9 gene under control of the MET3 promoter (22), which is repressed by the presence of high extracellular concentrations (>0.5 mM) of mevalonolactone (23). We constructed a “promoter-switch” plasmid that contained a truncated version of erg9 placed behind the MET3 promoter. Targeted integration of this plasmid into the ERG9 locus resulted in the creation of a single, functional copy of ERG9 under control of the regulated MET3 promoter (P\text{MET3}) (Fig. 1b). This plasmid was used to transform a methionine prototroph yeast strain to allow growth in any concentration of methionine. The strain also co-expressed 1Myc-Hmg2p and Hmg2p-GFP allowing a complete characterization of Hmg2p degradation. When grown in the absence of methionine, normal regulated Hmg2p degradation was observed in the yeast strain that expressed squalene synthase from the MET3 promoter (P\text{MET3-ERG9}).

Genetic down-regulation of squalene synthase enhanced Hmg2p degradation in a manner identical to inhibition with ZA. After 15 h growth in 2 mM methionine, Hmg2p degradation in the P\text{MET3-ERG9} strain was increased. This was indicated by a lower steady-state level of Hmg2p-GFP and 1Myc-Hmg2p in the P\text{MET3-ERG9} strain compared with the wild-type strain (Fig. 3, a and b, P\text{MET3-ERG9} versus wt). The effect of down-regulation was similar to that in the wild-type strain after 15 h incubation in the presence of ZA (P\text{MET3-ERG9} with wt, +ZA). Hmg2p-GFP in the P\text{MET3-ERG9} was stabilized by the addition of lovastatin to a similar degree as the stabilization of Hmg2p-GFP by lovastatin addition in the wild-type strain (P\text{MET3-ERG9} compared with wt, +ZA +Lov), indicating that enhanced Hmg2p degradation caused by squalene synthase down-regulation or ZA addition was still regulated by the mevalonate pathway.

It was possible that enhanced Hmg2p degradation was the result of cell death inadvertently caused by squalene synthase down-regulation rather than the build-up of a positive signal for degradation. Cells containing the P\text{MET3-ERG9} allele ceased to grow after 15 h incubation in 2 mM methionine (6 doublings) (see below, Fig. 8b). However, when the P\text{MET3-ERG9} cells from this 15-h time point were transferred to media without methionine, they retained the same plating efficiency and growth curve as identically treated wild-type ERG9 cells (see below, Fig. 8a).

4 R. Gardner and R. Hampton, unpublished observations.
Squalene synthase overexpression decreased Hmg2p degradation. Effect of squalene synthase overexpression on 1Myc-Hmg2p degradation. Otherwise identical strains expressing squalene synthase from the wild-type promoter (wt) or from a single, integrated allele with the GAPDH promoter (P_{GAPDH}-ERG9) were subjected to a cycloheximide chase assay. Lysates for each indicated time point after addition of cycloheximide were made and immunoblotted to determine Hmg2p levels.

Fig. 8b), indicating that these cells were still viable.

The enhanced Hmg2p degradation by ZA addition or squalene synthase down-regulation required the HRD gene-encoded proteins. Enhanced degradation by squalene synthase or ZA addition was completely eliminated by the presence of the hrd1Δ allele (Fig. 3b, P_{MET3}-ERG9, hrd1Δ, and wt +ZA, hrd1Δ), which normally stabilizes Hmg2p (11). This indicated that the degradation-enhancing effect of squalene synthase down-regulation or ZA addition required a functional HRD pathway and was not due to aberrant degradation by an alternate pathway. Furthermore, identical steady-state levels of 1Myc-Hmg2p in the wild-type, hrd1Δ strain and the P_{MET3}-ERG9, hrd1Δ strain indicated that the lower steady-state levels of Hmg2p in the P_{MET3}-ERG9 strain were due only to enhanced degradation and not reduced translation efficiency.

Additionally, we observed that overexpression of squalene synthase stabilized Hmg2p. In the strains previously described in Fig. 2, which overexpressed squalene synthase by the presence of the P_{GAPDH}-ERG9 allele, both versions of Hmg2p were significantly stabilized. This was observed as both an increase in the steady-state level of 1Myc-Hmg2p (Fig. 4, P_{GAPDH}-ERG9 versus wt) and Hmg2p-GFP,4 and as a decrease in 1Myc-Hmg2p degradation when squalene synthase was overexpressed (Fig. 4, P_{GAPDH}-ERG9 versus wt). Thus, squalene synthase overexpression had the opposite effect on Hmg2p degradation as squalene synthase down-regulation as squalene synthase down-regulation.

Down-regulation of Farnesyl-diphosphate Synthase Stabilized Hmg2p—The above studies implicated the substrate of squalene synthase, FPP, as a central molecule in the regulation of Hmg2p stability. Manipulation of squalene synthase predicted to increase FPP levels hastened Hmg2p degradation, whereas manipulation of squalene synthase predicted to decrease FPP levels slowed Hmg2p degradation. We wanted to test further the hypothesis that FPP was the source of the positive signal for Hmg2p degradation, by eliminating FPP production. This could be accomplished by inhibition of farnesyl-diphosphate synthase (FPP synthase), which generates FPP as a product. Unfortunately, no drugs are currently available that inhibit yeast FPP synthase in vivo. Therefore, we again used a genetic approach to lower FPP synthase production. As with the other enzymes of the mevalonate pathway, yeast cells that contain a null allele of FPP synthase are not viable (34), so we generated a conditional allele of the FPP synthase coding region (ERG20) that resulted in the wild-type ERG20 gene placed under control of the MET3 promoter, similar to ERG9 described above. When grown in the absence of methionine, normal regulated Hmg2p degradation was observed in the yeast strain that expressed FPP synthase from the MET3 promoter (P_{MET3}-ERG20).4

In contrast to enhanced Hmg2p degradation caused by squalene synthase down-regulation, FPP synthase down-regulation resulted in stabilization of Hmg2p. When the P_{MET3}-ERG20 strain was grown 15 h in 2 mM methionine, Hmg2p was exceedingly stable, as indicated by both a higher steady-state level and decreased degradation of 1Myc-Hmg2p (Fig. 5, P_{MET3}-ERG20 versus wt, Lovastatin (50 μg/ml final concentration) was added to the indicated sample (4ZA) at the same time as addition of cycloheximide. Lovastatin (50 μg/ml final concentration) was added to the indicated wild-type strain (wt, +Low) at the same time as addition of methionine. b, effect of FPP synthase down-regulation on Hmg2p-GFP steady-state levels. Cells expressing FPP synthase from either the wild-type promoter (wt) or the MET3 promoter (P_{MET3}-ERG20) were grown for 15 h at 30 °C in the presence of 2 mM methionine. ZA (10 μg/ml final concentration) or lovastatin (4ZA; 25 μg/ml final concentration) was added to the appropriate cultures, and all cultures were grown an additional 4 h at 30 °C. Hmg2p-GFP fluorescence was analyzed by flow cytometry.

Thus, Hmg2p stabilization was caused by FPP synthase down-regulation, further strengthening the model that FPP was the source of a positive signal for Hmg2p degradation.

FPP Synthase Down-regulation Also Blocked Hmg2p Ubiquitination—The covalent attachment of ubiquitin is a critical and regulated step in Hmg2p degradation (12). Because FPP synthase down-regulation stabilized Hmg2p, we also deter-
Regulated ubiquitination of 1Myc-Hmg2p in cells expressing FPP synthase from the wild-type promoter (wt) or the MET3 promoter (P\text{MET3}-ERG20). Cells were grown 15 h at 30 °C in the presence of 2 mM methionine. Ubiquitination assays were performed in the presence of no drug (−), 25 μg/ml lovastatin (Lov), or 10 μg/ml ZA (ZA). For the wild-type strain, lovastatin was added 30 min prior to cell lysis, and ZA was added 10 min prior to cell lysis. For the P\text{MET3}-ERG20 strain, ZA was added either 10 min (ZA10) or 60 min (ZA60) prior to cell lysis. Upper panels are the result of anti-HA (α-HA) immunoblotting for covalently linked HA-tagged ubiquitin. Lower panels are the result of parallel immunoblotting of an aliquot (1/8 total volume) of the same immunoprecipitates with the 9E10 anti-Myc antibody (α-myc) to assess total immunoprecipitated Hmg2p.

Fig. 6. FPP synthase down-regulation decreased Hmg2p ubiquitination. Regulated ubiquitination of 1Myc-Hmg2p in cells expressing FPP synthase from the wild-type promoter (wt) or the MET3 promoter (P\text{MET3}-ERG20). Cells were grown 15 h at 30 °C in the presence of 2 mM methionine. Ubiquitination assays were performed in the presence of no drug (−), 25 μg/ml lovastatin (Lov), or 10 μg/ml ZA (ZA). For the wild-type strain, lovastatin was added 30 min prior to cell lysis, and ZA was added 10 min prior to cell lysis. For the P\text{MET3}-ERG20 strain, ZA was added either 10 min (ZA10) or 60 min (ZA60) prior to cell lysis. Upper panels are the result of anti-HA (α-HA) immunoblotting for covalently linked HA-tagged ubiquitin. Lower panels are the result of parallel immunoblotting of an aliquot (1/8 total volume) of the same immunoprecipitates with the 9E10 anti-Myc antibody (α-myc) to assess total immunoprecipitated Hmg2p.

Down-regulation of FPP synthase caused a drastic decrease in the level of Hmg2p ubiquitination (Fig. 6, P\text{MET3}-ERG20 versus wt, no drug lanes). This effect was similar to the addition of lovastatin to the wild-type strain during the ubiquitination assay (wt, Lov lane). Furthermore, the addition of ZA for 10 min during the ubiquitination assay, which normally increases Hmg2p ubiquitination, had no effect on Hmg2p ubiquitination in the FPP synthase down-regulated strain (wt ZA lane versus P\text{MET3}-ERG20,ZA10 lane). This effect of FPP synthase down-regulation on ZA action was identical to addition of drugs, such as lovastatin or ZA, which normally altered the steady-state levels of Hmg2p-GFP (top right panel). Similarly, down-regulation of squalene synthase or FPP synthase had no effect on the steady-state levels of the unregulated 6Myc-Hmg2p and 6Myc-Hmg2p-GFP (bottom left panel). Thus, pharmacological or genetic manipulations that altered the cellular levels of FPP correspondingly altered Hmg2p ubiquitination in a manner consistent with its role as a positive degradation signal.

Genetic Manipulations Did Not Alter General ER Degradation—The above genetic experiments were consistent with FPP being the source of a positive signal for Hmg2p degradation. However, the results could also be explained by effects on the machinery for ER degradation, rather than on the signal con-trolling Hmg2p degradation. To test this, we examined if these genetic manipulations altered the degradation of 6My-Hmg2p, a previously described unregulated mutant of Hmg2p (11). Degradation of 6My-Hmg2p requires the same degradation pathway as normal Hmg2p (11), but 6My-Hmg2p degradation is not regulated by signals from the mevalonate pathway. Thus, if down-regulation of mevalonate pathway enzymes altered only the regulatory signal for Hmg2p degradation, then 6My-Hmg2p degradation should be unaffected.

The effect of squalene synthase or FPP synthase down-regulation on 6My-Hmg2p degradation was determined in a strain that was isogenic to the wild-type strain described above, except that unregulated 6My-Hmg2p and 6My-Hmg2p-GFP were co-expressed rather than 1Myc-Hmg2p and Hmg2p-GFP. As shown in the top left panel of Fig. 7, 6My-Hmg2p-GFP steady-state levels were unaffected by addition of drugs, such as lovastatin or ZA, which normally altered the steady-state levels of Hmg2p-GFP (top right panel). Similarly, down-regulation of squalene synthase or FPP synthase had no effect on the steady-state levels of the unregulated 6My-Hmg2p-GFP (bottom left panel), whereas these perturbations appropriately altered the steady-state levels of the regulated Hmg2p-GFP (bottom right panel). The co-expressed 6My-Hmg2p was similarly unresponsive to the same pharmacological or genetic manipulations that affected 1Myc-Hmg2p or the optical Hmg2p-GFP reporter. These results indicated that squalene synthase and FPP synthase down-regulation affected the regulation of Hmg2p stability and not the process of ER degradation.

Squalene Epoxidase Down-regulation Did Not Alter Hmg2p Degradation—The above genetic experiments provided strong evidence that FPP levels within the cell controlled the rate of Hmg2p degradation. However, it was not clear if this feature was unique to FPP or if other downstream products could also affect Hmg2p degradation. Squalene epoxidase is the next enzyme after squalene synthase in the pathway and is responsible for the formation of squalene epoxide from squalene (Fig. 1a). We wondered if altering squalene epoxidase levels would...
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Fig. 8. Squalene epoxidase down-regulation or inhibition had no effect on Hmg2p-GFP steady-state levels. a, effect of squalene epoxidase down-regulation on Hmg2p steady-state levels. Cells expressing squalene epoxidase from either the wild-type promoter (wt) or the MET3 promoter (P_{MET3-ERG1}) or squalene synthase from the MET3 promoter (P_{MET3-ERG9}) were grown for 15 h at 30 °C in the presence of 2 mM methionine. Hmg2p-GFP fluorescence was analyzed by flow cytometry. b, left panel, cells containing either the conditional alleles of squalene synthase (P_{MET3-ERG9}), FPP synthase (P_{MET3-ERG20}), or squalene epoxidase (P_{MET3-ERG1}) were grown to mid-log phase (A_{600} approximately 0.5) in media that did not contain methionine. New cultures containing 2 mM methionine were inoculated from these previously grown cultures to an initial A_{600} of 0.01 and then incubated for 32 h at 30 °C. The new A_{600} for these cultures was measured at the indicated time points and plotted versus time. Growth of each strain was compared with the wild-type strain (wt). Right panel, new cultures, which contained no methionine, were inoculated from the previous 15-h methionine-grown cultures to an initial A_{600} of 0.01 and then incubated for 32 h at 30 °C. The new A_{600} for these cultures was measured at the indicated time points and plotted versus time. c, effect of terbinafine (Tb), a squalene epoxidase inhibitor, on Hmg2p-GFP steady-state levels. Cells were treated with either no drug, 40 μM terbinafine (Tb), or 10 μM/ml ZA (ZA) and grown for 4 h at 30 °C. Hmg2p-GFP steady-state fluorescence was analyzed by flow cytometry.

DISCUSSION

HMGR degradation is regulated by signals generated downstream in the mevalonate pathway. The identity of these signals and the mechanism by which they control HMGR degradation is currently not known. To understand better the nature of this regulatory mechanism, we have examined the regulated degradation of the yeast HMGR isozyme Hmg2p through genetic and pharmacological manipulation of the mevalonate pathway.

The results detailed in this work strongly implicated the mevalonate pathway product FPP as the source of the positive signal for Hmg2p degradation. ZA addition or squalene synthase down-regulation, both predicted to increase FPP levels in the cell, increased Hmg2p degradation. Conversely, addition of lovastatin, FPP synthase down-regulation, or squalene synthase overexpression, all predicted to decrease FPP levels in the cell, stabilized Hmg2p. Identical Hmg2p steady-state levels in degradation-deficient strains containing either normal or down-regulated squalene synthase levels indicated that FPP levels affected only Hmg2p degradation.

Utility of the MET3 Promoter—In order to examine the molecular signals for Hmg2p degradation, we required a way to manipulate the mevalonate pathway at any enzymatic step. Drugs that inhibit some of the pathway enzymes are available, such as lovastatin and ZA. However, there are no currently available drugs that inhibit enzymes between HMGR and squalene synthase. Furthermore, because the products of the mevalonate pathway are essential for yeast viability, null alleles of mevalonate pathway genes result in cell death. Therefore, we required a way to alter the level or activity of mevalonate pathway enzymes in a controllable, yet viable, manner.

To accomplish this, we manipulated the levels of key mevalonate pathway enzymes by placing them under the control of a regulated promoter. We chose the MET3 promoter (22), which can be repressed by incubation of cells in high concentrations of methionine (>0.5 mM) (23). In all cases, the conditional alleles were similar to null alleles in that cells carrying the conditional alleles, when continuously incubated in methionine, were unable to grow after a few doublings. However, unlike cells with null alleles, cells carrying the conditional alleles could be induced to grow by transferring them to media containing no methionine. This type of genetic manipulation provided a facile way to alter the expression of target genes and could be used, in theory, to create regulated alleles of any yeast gene.

FPP as the Source of the Signal for Hmg2p Degradation—Manipulation of FPP levels by both pharmacological and genetic means resulted in the expected changes to Hmg2p degradation consistent with FPP, or a derivative, as a positive signal for Hmg2p degradation. The idea that intracellular levels of FPP, or a derivative, serve to modulate Hmg2p degradation in yeast paralleled similar observations in mammalian cells. It has been proposed that degradation of mammalian HMGR is regulated by the intracellular levels of farnesol, a derivative of FPP (13–17). It may also be the case that farnesol regulates Hmg2p degradation.

One way to distinguish between FPP and farnesol as the
regulatory signal would be by elimination of the pyrophosphatase activity required to convert FPP to farnesol (17). Recently, two pyrophosphatases, LPP1 and DPP1, that appear to convert FPP to farnesol in vitro have been described (39). Null alleles of the genes encoding these enzymes have been made in yeast, and the cells are viable. We examined Hmg2p degradation in these strains and found that the absence of Lpp1p and Dpp1p had no effect on Hmg2p regulated degradation. This could mean that either FPP, not farnesol, is the signal for HMGR degradation in yeast or that these enzymes are not solely responsible for the conversion of FPP to farnesol in vivo. In either case, it is apparent that FPP serves as the source of a signal for HMGR degradation in both mammals and yeast.

In mammalian cells, there is an additional sterol signal that acts to modulate HMGR degradation. The addition of sterols to mammalian cells results in increased degradation of HMGR (4, 5). However, this downstream signal from sterols does not accelerate HMGR degradation in the absence of the upstream FPP-derived signal (4, 17), indicating that sterols provide an additional positive signal for HMGR degradation that works only in conjunction with the FPP-derived signal. In this study, our results demonstrated that no other pathway product downstream of FPP was required for Hmg2p degradation in yeast. That is inhibition of squalene epoxidase activity or down-regulation of squalene epoxidase had no stabilizing effect on Hmg2p degradation. However, yeast may contain abundant stores of sterols, and so the existence of other signals cannot be ruled out by these negative results. We are further exploring, by both genetic and pharmacological means, whether an additional signal downstream of squalene can act in conjunction with FPP to hasten Hmg2p degradation.

Mechanisms by Which FPP Regulates Hmg2p Degradation—The identification of FPP, or a derivative, as the positive signal for HMGR degradation in yeast leads to several models for regulation of Hmg2p degradation. The simplest model for the regulatory mechanism is that the FPP-derived signal acts as an allosteric regulator and physically binds to the transmembrane domain of Hmg2p, altering its susceptibility to degradation. Alternatively, the FPP-derived molecule could modify or interact with a separate effector protein that alters Hmg2p stability. The effector could act to promote or prevent Hmg2p degradation, as either of these possibilities have precedent in cellular degradation (40, 41). It is also possible that the FPP-derived signal directly affects the structure of the ER membrane. By this model, the Hmg2p transmembrane domain would respond to the altered membrane by becoming increasingly susceptible to degradation. In this regard, it is interesting that the distance from the ER surface of key residues in the Hmg2p transmembrane domain is critical in regulation of Hmg2p stability.3

We are currently exploring the mechanism by which FPP acts to regulate Hmg2p degradation. It is clear that both yeast and mammalian cells measure FPP production as a means to control HMGR degradation. This conservation in signaling strategy, together with the similar location and machinery required for HMGR degradation, indicates that the mechanism for regulation of HMGR degradation may also be conserved among eukaryotes.

A final and important note should be made regarding a recent study on a fascinating side effect of the HMGR inhibitor lovastatin. It has been demonstrated that the closed β-lactone ring form of this particular statin can directly inhibit the proteasome (29). At first thought, it might be reasonable to conclude that this action is responsible for the stabilizing effects of lovastatin on Hmg2p, which is degraded by the proteasome (11). However, several observations show quite clearly that all actions of lovastatin we have observed were due to altered signal production and not proteasome inhibition. Degradation of 6Myc-Hmg2p, which is proteasome-dependent, is unaffected by doses of lovastatin that slow Hmg2p degradation (Ref. 11 and Fig. 7). Furthermore, the pathway inhibitor L659,699, which inhibits HMGR-Coa synthase but contains no lactone moiety, is equally effective at stabilizing Hmg2p as lovastatin (12). Third, genetic manipulations of HMGR-Coa synthase (6), or FPP synthase (this work), which slow production of the degradation signals but do not involve drugs, have identical actions to lovastatin on Hmg2p degradation but not 6Myc-Hmg2p degradation. Finally, lovastatin decreases ubiquitination of Hmg2p (Ref. 12 and this study), an effect opposite of that observed when the proteasome is compromised or inhibited (11). Although proteasome inhibition by the closed ring form of lovastatin is interesting and perhaps important clinically, it plays no obvious or important role in the actions of this drug on regulating Hmg2p stability in yeast and is most likely not involved in mammalian HMGR degradation, for similar reasons.

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