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Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl-CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion

(cholesterol/end-product feedback regulation/protein degradation/energy metabolism)

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ABSTRACT **An AMP-activated protein kinase has been reported to phosphorylate rodent 3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA reductase; (*S*)-mevalonate:-NAD$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.88] at Ser-871, thereby lowering its catalytic activity [Clarke, P. R. & Hardie, D. G. (1990) *EMBO J.* 9, 2439–2446]. To explore the physiologic role of this reaction, we prepared a cDNA encoding a mutant form of hamster HMG-CoA reductase with alanine substituted for serine at residue 871. When overexpressed in transfected cells, the wild-type enzyme, but not the Ser-871 to Ala mutant, was labeled with [^{32}P]phosphate, confirming Ser-871 as the site of phosphorylation. The wild-type enzyme, but not the mutant enzyme, showed reduced activity when the cells were harvested with the phosphatase inhibitor KF, confirming phosphorylation as a mechanism for inactivation within the cell. Despite the lack of phosphorylation, the posttranscriptional feedback regulation of the mutant enzyme was normal, as indicated by reduced activity when cells were incubated with mevalonate, 25-hydroxycholesterol, or low density lipoprotein. Moreover, the mutant enzyme showed a normal acceleration of degradation when the transfected cells were incubated with sterols. Cells expressing the wild-type enzyme showed a decreased incorporation of [^{14}C]pyruvate into sterols when ATP was depleted by incubation with 2-deoxy-D-glucose. No such reduction was seen in cells expressing the Ser-871 to Ala mutant enzyme. We conclude that the AMP-activated protein kinase does not play a role in end-product feedback regulation of HMG-CoA reductase, but rather it comes into play when cellular ATP levels are depleted, thereby lowering the rate of cholesterol synthesis and preserving the energy stores of the cell.**

3-Hydroxy-3-methylglutaryl-CoA reductase [HMG-CoA reductase; (*S*)-mevalonate:NAD$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.88] is a 97-kDa membrane-bound enzyme of the endoplasmic reticulum that produces mevalonate, which is required for synthesis of cholesterol and isoprenoids in animal cells (1). When cells are depleted of mevalonate or when mevalonate demands are increased, the amount of HMG-CoA reductase protein increases by several hundredfold over basal levels. This increase results from a combination of enhanced transcription of the gene, enhanced translation of the mRNA, and reduced degradation of the enzyme (1). These changes are all reversed when sterols and/or mevalonate are provided to the cells.

In addition to this classic end-product feedback regulation, which affects the level of enzyme protein, the activity of HMG-CoA reductase can be altered reversibly by a cycle of phosphorylation and dephosphorylation (2). Phosphorylation occurs on a serine (residue 871 in the hamster sequence) that is near the C terminus of the protein (3). The reaction is catalyzed by a unique kinase that uses ATP as a phosphate donor and requires AMP as an activator (4, 5). Phosphorylation decreases the catalytic activity of the enzyme by ≈80%. Although some groups have speculated that phosphorylation accelerates the degradation of HMG-CoA reductase protein (6, 7), other groups have found no evidence for such an effect (8).

The AMP-activated kinase also phosphorylates and inactivates acetyl-CoA carboxylase, thereby potentially inhibiting the synthesis of fatty acids as well as cholesterol (4, 5). Hardie (4, 5) has suggested that the AMP-activated kinase functions to conserve energy by inhibiting anabolic pathways when cellular ATP levels are depleted and AMP levels increase. This conclusion is supported by the finding of increased phosphorylation of HMG-CoA reductase in hepatocytes that have been incubated with fructose, which raises AMP levels (9). This hypothesis is also consistent with the presence of a similar AMP-activated kinase in organisms as primitive as insects and plants, which suggests a fundamental cell-autonomous role such as energy conservation (4, 5).

Other investigators (2, 10–12) have provided evidence to support the idea that phosphorylation of HMG-CoA reductase in liver may mediate responses to insulin or may counteract the pattern of diurnal rhythm of HMG-CoA reductase in this organ. To date, however, there are no convincing data to correlate a change in the phosphorylation state of HMG-CoA reductase with an overall change in the rate of cholesterol biosynthesis in liver or any other tissue (13).

To explore the regulatory role of phosphorylation, in the current experiments we have prepared a cDNA encoding a mutant form of hamster HMG-CoA reductase with Ala substituted for Ser-871 and transfected it into several cell types. As expected, this enzyme is no longer phosphorylated significantly in cultured cells. Nevertheless, it shows normal posttranscriptional feedback suppression by sterols and mevalonate. Whereas normal cells show a decreased incorporation of pyruvate into cholesterol after ATP depletion, this regulation is abolished in transfected cells expressing the Ser-871 to Ala mutant form of HMG-CoA reductase. These data support the notion that a major function of the AMP-activated kinase is to suppress cholesterol biosynthesis (and presumably fatty acid biosynthesis) in response to a decrease in the energy stores of the cell.

EXPERIMENTAL PROCEDURES

Materials and Methods. Standard techniques of molecular biology were used for plasmid constructions and cell transfections (14). Newborn calf lipoprotein-deficient serum ($d <$ 1.215 g/ml) and human low density lipoprotein (LDL) ($d =$

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Abbreviations: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; LDL, low density lipoprotein.

1.019–1.063 g/ml) were prepared as described (15). We obtained Tran^{35}S-label (1100 Ci/mmol; 1 Ci = 37 GBq) from ICN; sodium [2-^{14}C]pyruvate (10.4 mCi/mmol) and [^{32}P]phosphate (9000 Ci/mmol) from DuPont/New England Nuclear; (*RS*)-[5-^{3}H]mevalonolactone (60 Ci/mmol) from American Radiolabeled Chemicals (St. Louis); 2-deoxy-D-glucose from Aldrich; and protein A-Sepharose CL-4B from Sigma. A polyclonal antibody directed against the C-terminal 60-kDa domain of human HMG-CoA reductase (16) was prepared by repeated injections of New Zealand White rabbits with 50 μg of purified bacterially expressed protein in Freund's adjuvant (kindly provided by Kenneth L. Luskey and Johann Deisenhofer of this institution). All other reagents were obtained from previously described sources (17, 18).

Plasmids. pCMV7Red, a plasmid encoding wild-type Chinese hamster HMG-CoA reductase, was constructed by subcloning a *Bam*HI/*Hpa* I fragment of pRed227 (19) into a *Bam*HI/*Sma* I site in pCMV7 (20). A single amino acid substitution (Ser-871 to Ala) was created in pCMV7Red by oligonucleotide-directed mutagenesis (21) of a 1.6-kb *Sma* I/*Sac* I fragment from pCMV7Red subcloned into bacteriophage M13mp19. A 1.3-kb *Pfl*MI/*Eco*RV fragment encoding the C-terminal 200 amino acids and 3′ untranslated region of HMG-CoA reductase was then excised from the mutated M13 clone and subcloned into pCMV7Red to produce pCMV7Red (Ser-871 to Ala).

Cell Culture. Cells were grown in monolayers at 37°C in an atmosphere of 5–7% CO_2/93–95% air. Stock cultures of UT-2 cells (22) were grown in medium A (a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's minimum essential medium containing 100 units of penicillin and 100 μg of streptomycin per ml) supplemented with 10% (vol/vol) fetal calf serum and 0.2 mM sodium mevalonate. Transfected UT-2 cell lines (see below) were grown in medium A supplemented with 10% lipoprotein-deficient serum. Simian COS-M6a cells were cultured in medium B (Dulbecco's modified Eagle's minimum essential medium containing 100 units of penicillin and 100 μg of streptomycin per ml) supplemented with 5% fetal calf serum.

Transfections. Wild-type and mutant plasmids encoding HMG-CoA reductase were stably transfected into UT-2 cells, a reductase-negative mevalonate auxotroph (22). On day 0, 5×10^5 UT-2 cells were seeded into each 100-mm Petri dish. On day 1, each monolayer was cotransfected with 9.5 μg of wild-type or mutant plasmid DNA and 0.5 μg of pSV2Neo (23) using a modified calcium phosphate precipitation method (14). On day 2, the cells were refed with medium A supplemented with 10% fetal calf serum, 0.2 mM sodium mevalonate, and G418 (700 μg/ml). On day 8, the cells were incubated with selective medium composed of medium A supplemented with 10% lipoprotein-deficient serum and G418 (700 μg/ml) but containing no mevalonate. Visible clones were picked, subcloned, and maintained in the same medium. The stably transfected UT-2 cell lines expressing the wild-type and mutant HMG-CoA reductase are designated TR-1891-3 and TR-1899 cells, respectively.

Plasmids were transiently transfected with COS cells by the DEAE-dextran method (14). On day 0, 3×10^5 cells were seeded into each 60-mm Petri dish. On day 1, each monolayer was transfected with 3 μg of plasmid DNA and used for experiments on day 3.

HMG-CoA Reductase Activity and Cholesterol Synthesis. HMG-CoA reductase activity was measured as described (15) in detergent-solubilized cell extracts (20–40 μg of protein) in the presence of either 50 mM KCl or 50 mM KF (13) for 20 min (COS cells) or 60 min (transfected UT-2 cells) at 37°C and is expressed as pmol of [^{14}C]HMG-CoA converted to [^{14}C]mevalonate per min per mg of detergent-solubilized protein.

Cholesterol synthesis was assayed by measuring the rate of conversion of [5-^{3}H]mevalonate and [2-^{14}C]pyruvate into squalene, lanosterol, and cholesterol (22, 24). Cell monolayers were washed with phosphate-buffered saline (PBS) and then incubated with buffer A (50 mM Hepes/100 mM NaCl/1 mM $CaCl_2$/10 mM KCl/10% lipoprotein-deficient serum, pH 7.4) containing 1 mM D-glucose, various concentrations of 2-deoxy-D-glucose, 0.5 mM sodium [^{3}H]mevalonate (0.15 mCi/ml), and 1.5 mM sodium [^{14}C]pyruvate (1.9 μCi/ml). After 1 h of incubation at 37°C, the cells were washed and dissolved in 1 ml of 0.1 M NaOH. Each sample was saponified and extracted with petroleum ether, and radioactive squalene, lanosterol, and cholesterol were isolated and quantified by thin-layer chromatography as described (22, 24). To measure fatty acid synthesis, the aqueous phases from the petroleum ether extractions were acidified with concentrated HCl and extracted with petroleum ether and subjected to thin-layer chromatography (22, 24).

Immunoprecipitation of ^{35}S- and ^{32}P-Labeled HMG-CoA Reductase. Cell monolayers were preincubated for 1 h with either methionine/cysteine-free medium B or phosphate-free medium B, after which either 100–150 μCi of Tran^{35}S-label or 300 μCi of [^{32}P]orthophosphate per ml was added. After incubation for 1 h, the cells were washed with PBS, scraped into 1 ml of PBS, and centrifuged for 30 sec at 4°C. The cell pellet was solubilized with SDS-containing buffer (18) and spun in a microcentrifuge for 5 min. The supernatant was incubated with preimmune IgG (10 μg/ml) for 30 min at 4°C, followed by 30 μl of 50% (vol/vol) protein A-Sepharose CL-4B. The resulting supernatant (after centrifugation) was incubated with 16 μg of rabbit anti-HMG-CoA reductase IgG per ml for 1.5 h at 4°C followed by addition of protein A-Sepharose CL-4B. The pellet was washed five times with SDS-containing buffer (18) and subjected to SDS/7% PAGE (18). In cells incubated with [^{32}P]orthophosphate, all buffers during the washing, harvesting, solubilization, and immunoprecipitation contained phosphatase inhibitors (50 mM sodium fluoride/10 mM β-glycerophosphate/0.1 mM sodium vanadate). Gels were calibrated with prestained molecular mass standards (Bio-Rad).

Cellular ATP Concentrations. Cells were washed twice with buffer B (50 mM Tris·HCl/154 mM NaCl, pH 7.4), after which 1 ml of boiling distilled water was added to each dish. The cells were heated in a microwave oven for 10 sec and scraped from the dish at 4°C. The cell extracts were then centrifuged, and the supernatants were assayed for ATP by a luciferin-luciferase assay with the ATP bioluminescence CLS kit (Boehringer Mannheim) and for content of cellular protein with the BCA protein assay (Pierce).

RESULTS

We used standard techniques of *in vitro* mutagenesis to prepare a plasmid encoding a Ser-871 to Ala mutant form of hamster HMG-CoA reductase. The cDNAs encoding either the wild-type or the Ser-871 to Ala mutant were placed under the control of the CMV promoter and were introduced into monkey COS cells for transient expression (Fig. 1). The plasmids encoding the wild-type and mutant enzymes produced equal amounts of protein as revealed by [^{35}S]methionine labeling and immunoprecipitation (lanes 1 and 2). When the cells were incubated with [^{32}P]phosphate, the wild-type enzyme was labeled, but the Ser-871 to Ala mutant was not (lanes 4 and 5), confirming Ser-871 as the major site for phosphorylation (4).

When the COS cells expressing the wild-type enzyme were harvested in the presence of KCl, a high level of enzyme activity was observed (Table 1, Exp. 1). The activity was much lower when the cells were harvested in the presence of KF, which inhibits phosphatases (13, 25). These findings are

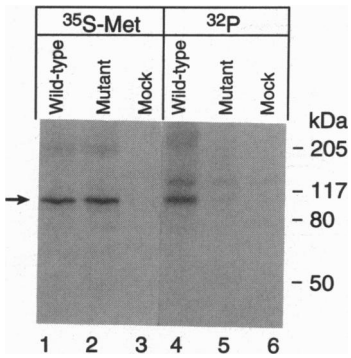

FIG. 1. Immunoprecipitation of radiolabeled HMG-CoA reductase in COS cells transfected with wild-type and mutant cDNAs. Cells were transiently transfected with 3 μg of one of the following plasmids: lanes 1 and 4, pCMV7Red; lanes 2 and 5, pCMV7Red (Ser-871 to Ala); lanes 3 and 6, pCMV7 vector. On day 3, the cells were radiolabeled with either Tran^{35}S-label for 1 h (lanes 1–3) or [^{32}P]orthophosphate for 2 h (lanes 4–6). After incubation, the cells were harvested in the presence of phosphatase inhibitors, and the detergent-solubilized cell extracts were immunoprecipitated with anti-HMG-CoA reductase IgG followed by SDS/PAGE. The gel was exposed to Kodak XAR film for 24 h at −70°C. Arrow denotes position of HMG-CoA reductase.

interpreted to indicate that a major proportion of HMG-CoA reductase is phosphorylated and therefore poorly active in cells (13, 25). It becomes activated by phosphatases during cell harvest, and this is prevented by KF. In contrast, the Ser-871 to Ala mutant showed similar activities when harvested in the presence of KCl or KF, again indicating that this enzyme was not phosphorylated significantly in the cells. Similar results were obtained in another experiment, in which a higher level of expression was obtained (Table 1, Exp. 2).

To study the effects of phosphorylation on regulation of HMG-CoA reductase activity, we transfected the cDNAs encoding the wild-type and the Ser-871 to Ala mutation into UT-2 cells, a line of Chinese hamster ovary cells (CHO cells) that lacks HMG-CoA reductase activity (22). Stably transfected cells were isolated, and regulation of HMG-CoA reductase was measured. When cells expressing the wild-type enzyme were grown in lipoprotein-deficient serum lacking sterols, they had relatively high levels of HMG-CoA reductase activity (Fig. 2*A*). When harvested in the presence of KF, the activity was reduced by ≈80%, indicating that most of the enzyme was in a phosphorylated state. When the cells were incubated with mevalonate, 25-hydroxycholesterol, or LDL as a cholesterol donor, the activity of the enzyme was reduced by 30–60%. This reduction occurred solely by posttranscriptional mechanisms since gene expression was driven by the nonregulated CMV promoter. The

Table 1. HMG-CoA reductase activity in COS cells transiently transfected with wild-type and mutant cDNAs

Transfected cDNA	HMG-CoA reductase activity, pmol per min per mg of protein		
	KCl	KF	KF/KCl
Exp. 1			
Wild type	922	326	0.35
Ser-871 to Ala	1203	1150	0.96
Exp. 2			
Wild type	2458	361	0.15
Ser-871 to Ala	3880	4188	1.08

Cells were transiently transfected with 3 μg of either pCMV7Red (wild type) or pCMV7Red (Ser-871 to Ala). On day 3, the cells were harvested for measurement of HMG-CoA reductase activity in the presence of 50 mM KCl or 50 mM KF as indicated. Each value is the average of duplicate determinations.

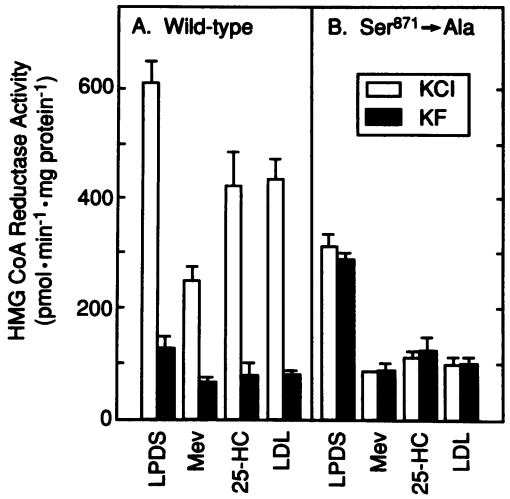

FIG. 2. Regulation of HMG-CoA reductase activity in stably transfected UT-2 cell lines expressing either wild-type (*A*) or mutant (*B*) HMG-CoA reductase. Cells were set up on day 0 at 5 × 10^5 cells per 100-mm dish in 10 ml of medium A containing 10% lipoprotein-deficient serum (LPDS). On day 2, the cells were refed with the same medium A containing 20 μl of ethanol and one of the following additions: none, 30 mM sodium mevalonate (Mev), 2 μg of 25-hydroxycholesterol (25-HC) per ml, or 100 μg protein of human LDL per ml. After 16 h of incubation, the cells were washed with PBS containing either 50 mM KCl or 50 mM KF and harvested with the same buffer. The activity of HMG-CoA reductase was assayed in the presence of 50 mM KCl or 50 mM KF as described. Each bar represents mean ± SE of four experiments.

marked difference in enzyme activity in the presence of KCl and KF persisted in the suppressed cells, indicating that the enzyme existed in the cell largely in a phosphorylated state. Strikingly different results were obtained with the Ser-871 to Ala mutant (Fig. 2*B*). These cells also exhibited relatively high HMG-CoA reductase activities in lipoprotein-deficient serum and were suppressed by mevalonate, 25-hydroxycholesterol, or LDL. However, in contrast to the wild-type enzyme, the Ser-871 to Ala enzyme showed no difference in activity when the cells were harvested in the presence of KCl or KF.

Despite its resistance to phosphorylation, the Ser-871 to Ala enzyme, like wild-type HMG-CoA reductase, showed accelerated degradation in the presence of sterols (Fig. 3). In both cases, incubation of the transfected UT-2 cells with sterols reduced the half-life of the ^{35}S-labeled enzyme from 6–8 h to 2–3 h as determined by immunoprecipitation and autoradiography. Virtually identical results were obtained in an independent experiment.

When UT-2 cells expressing the wild-type or Ser-871 to Ala enzyme were incubated in the presence of increasing concentrations of 2-deoxy-D-glucose, the level of ATP declined equally in both cell types as a result of inhibition of glycolysis (Fig. 4*A*). In cells expressing the wild-type HMG-CoA reductase, the incorporation of [^{14}C]pyruvate into sterols was reduced in proportion to the decline in ATP levels (Fig. 4*B*). In contrast, the cells expressing the Ser-871 to Ala mutant did not show any significant decrease in [^{14}C]pyruvate incorporation into sterols despite equal ATP depletions. The rate of incorporation of [^{14}C]pyruvate into fatty acids declined markedly and equally in both cell types (Fig. 4*C*). The incorporation of [^{3}H]mevalonate into sterols was not affected by this level of ATP depletion (Fig. 4*D*).

DISCUSSION

The current experiments indicate that Ser-871 is the major site for phosphorylation of hamster HMG-CoA reductase in

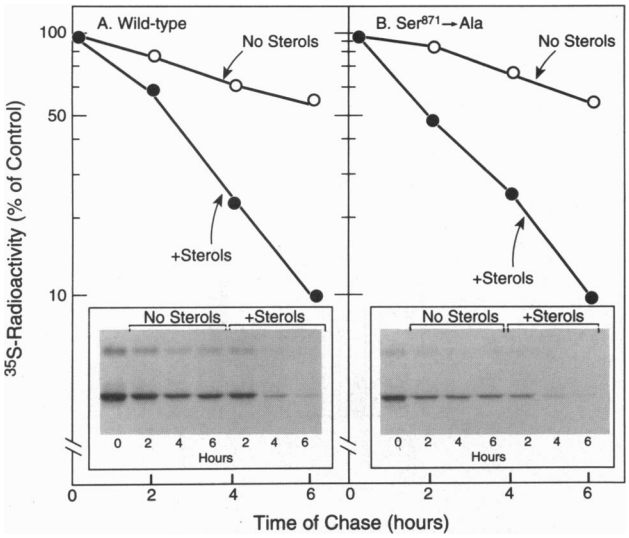

FIG. 3. Turnover of ^{35}S-labeled wild-type and mutant HMG-CoA reductase in stably transfected UT-2 cells. Cells were set on day 0 at 2×10^5 cells per 60-mm dish in 3 ml of medium A containing 10% lipoprotein-deficient serum. On day 2, cells were refed with the same medium containing 100 μM sodium mevalonate and 100 μM compactin. After incubation for 16 h, the cells were pulsed with Tran^{35}S-label for 1 h. The cells were then washed with PBS and incubated with the same medium supplemented with 0.3 mM unlabeled methionine and cysteine in the absence (○) or presence (●) of sterols (1 μg of 25-hydroxycholesterol per ml, 10 μg of cholesterol per ml, and 10 mM sodium mevalonate). After chasing for the indicated time, detergent-solubilized cell extracts were immunoprecipitated with anti-HMG-CoA reductase antibody, followed by SDS/PAGE and autoradiography (*Insets*) as described in the legend to Fig. 1. Radiolabeled HMG-CoA reductase bands (upper band, 200-kDa dimer; lower band, 97-kDa monomer) were quantified by radiometric Ambis analysis and plotted.

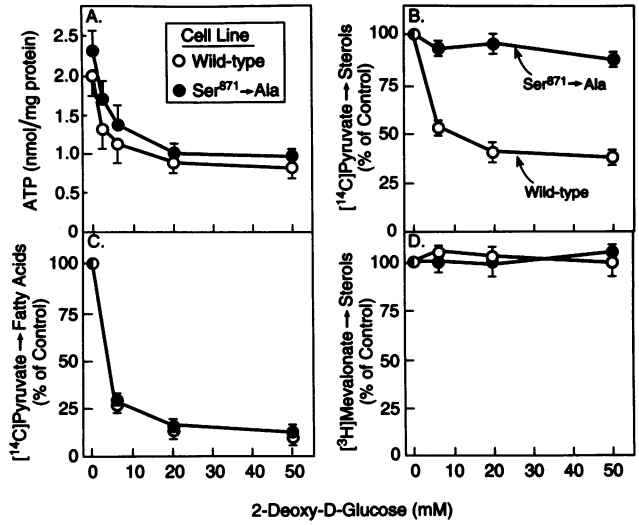

FIG. 4. Effect of 2-deoxy-D-glucose on cellular ATP content (*A*) and incorporation of [^{14}C]pyruvate and [^3H]mevalonate into lipids (*B–D*) in stably transfected UT-2 cells expressing either wild-type (○) or mutant (●) HMG-CoA reductase. Cells were set up on day 0 as described in the legend to Fig. 3. On day 2, each monolayer received 3 ml of medium A containing 10% lipoprotein-deficient serum. (*A*) On day 3, cells were washed with PBS and then incubated with buffer A containing 1 mM D-glucose, 1.5 mM sodium pyruvate, 0.5 mM sodium mevalonate, and the indicated concentration of 2-deoxy-D-glucose. After 1 h, the cells were washed and harvested for measurement of ATP content. Each value represents mean ± SE of three experiments. (*B–D*) On day 3, cells were washed with PBS and incubated with buffer A containing 1.5 mM [^{14}C]pyruvate (1.9 μCi/ml), 0.5 mM [^3H]mevalonate (0.15 mCi/ml), 1 mM D-glucose, and the indicated concentration of 2-deoxy-D-glucose. After 1 h, the cells were washed and dissolved in 1 ml of 0.1 M NaOH. The rates of conversion of [^3H]mevalonate and [^{14}C]pyruvate into squalene, lanosterol, and cholesterol and the conversion of [^{14}C]pyruvate into fatty acids were measured as described. In all experiments, the incorporation into squalene was negligible (<1% of lanosterol plus cholesterol). Each value represents mean ± SE of six experiments. Mean values for "100% of control" were 1.6×10^4 dpm/mg for wild type and 2.2×10^4 for Ser-871 to Ala mutant (*B*), 0.9×10^4 dpm/mg for wild type and 1.6×10^4 for mutant (*C*), 1.8×10^5 dpm/mg for wild type and 1.4×10^5 for mutant (*D*).

monkey COS cells and hamster UT-2 cells, as it is in rat liver cells (3). Substitution of Ser-871 with Ala markedly decreased the incorporation of [^{32}P]phosphate into HMG-CoA reductase in the transfected cells. It also abolished the reduction in enzyme activity that occurs when cells are harvested in the presence of KF, confirming that this reduction is attributable to phosphorylation at Ser-871.

Despite this lack of phosphorylation, the posttranscriptional feedback regulation of the mutant enzyme appeared to be intact. The activity of the Ser-871 to Ala enzyme was reduced in parallel with that of the wild-type enzyme when cells were incubated with mevalonate, 25-hydroxycholesterol, or LDL. Moreover, the mutant enzyme showed a normal acceleration of its rate of degradation when sterols were added to the cells. These data strongly suggest that the AMP-activated kinase does not play a role in the feedback regulation of HMG-CoA reductase by end-products of mevalonate metabolism.

The only regulatory abnormality that we found with the Ser-871 to Ala mutant was the failure of cells to suppress cholesterol biosynthesis when ATP was depleted by incubation with 2-deoxy-D-glucose. These findings confirm the suggestion originally made by Hardie (4, 5) that the major function of the AMP-dependent kinase is to reduce the rate of cholesterol biosynthesis when ATP levels are depleted and AMP levels rise. It is likely that this kinase is also responsible for the decline in fatty acid biosynthesis, as measured by the incorporation of [^{14}C]pyruvate into fatty acids, that occurred when the hamster UT-2 cells were exposed to 2-deoxy-D-glucose.

In the current experiments, as in previous ones with cultured cells (2) and rat liver (13), we found that the majority of wild-type HMG-CoA reductase was phosphorylated and hence relatively inactive under all conditions studied. In studies of rat liver, Zammit and Caldwell (8) suggested that some of this apparent phosphorylation occurs during removal of the liver from the animal, when the liver becomes transiently hypoxic and AMP levels increase. Rapid freezing of the liver produced an enzyme that was less phosphorylated and hence more active. We do not believe that this is the case with cultured cells. In experiments not shown, we harvested the transfected UT-2 cells by quick-freezing or boiling *in situ*. The HMG-CoA reductase enzyme is stable to such treatments (26). Despite these instantaneous harvesting techniques, we found that the enzyme was severalfold more active when harvested in the presence of KCl than in the presence of KF, confirming that the enzyme was highly phosphorylated in the intact cell. The reason for the maintenance of the bulk of HMG-CoA reductase in the phosphorylated state, even when cells are deprived of mevalonate, is not clear. It is possible that cultured cells have relatively high levels of AMP even when grown under optimal conditions of tissue culture. The AMP-activated kinase is extremely sensitive to AMP levels not only because of its AMP activation, but also because the kinase that activates the AMP-activated kinase is itself activated by AMP (4, 5).

The observation that 2-deoxy-D-glucose inhibited [^{14}C]pyruvate incorporation into sterols in cells expressing

wild-type HMG-CoA reductase, but not the Ser-871 to Ala mutant, indicates that the AMP-activated kinase does not inactivate any other enzymes of the cholesterol biosynthetic pathway. If such inactivation had occurred, then the rate of incorporation would have decreased in the cells expressing the mutant enzyme as well as the wild-type enzyme. This finding is in contrast to the sterol-dependent feedback system, which represses the activity of several enzymes of cholesterol biosynthesis, including HMG-CoA synthase, prenyl transferases, and squalene synthetase (1).

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