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Essential role for mevalonate synthesis in DNA replication

(thymidine/DNA synthesis/compactin/hydroxymethylglutaryl-CoA reductase/cholesterol)

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ABSTRACT **The relationship between 3-hydroxy-3-methylglutaryl (HMG) CoA reductase activity [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] and DNA synthesis was studied in synchronized cultures of BHK-21 cells. During a 24-hr period of cell replication, two phases of accelerated thymidine incorporation into DNA corresponding to two S phases of the cell cycle occurred. A marked increase in activity of HMG CoA reductase was consistently observed at or just prior to each of these peaks of DNA synthesis. Moreover, when HMG CoA reductase activity was suppressed by the competitive inhibitor compactin, the normal S-phase burst of DNA synthesis was specifically and totally prevented. Finally, the compactin-induced inhibition of DNA synthesis could be completely reversed within minutes by the addition of mevalonate, the product of the HMG CoA reductase reaction. By contrast, addition of cholesterol-rich lipoproteins had no effect upon DNA synthesis in compactin-treated cells. These data demonstrate that HMG CoA reductase activity, and therefore the production of mevalonate, plays an essential role in the synthesis of DNA specifically during the S phase of the cell cycle. Moreover, the results indicate that this function of mevalonate in regulating DNA replication is independent of its conversion to cholesterol.**

A large body of evidence has accumulated indicating that *de novo* cholesterogenesis plays an important role in cell growth. Except in a tissue such as liver, which secretes cholesterol into the bloodstream, there is a well-documented correlation between the activity of hydroxymethylglutaryl (HMG) CoA reductase [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] and cell replication. Rapidly proliferating tissues, such as intestine and developing brain, demonstrate high rates of cholesterol synthesis and increased activities of HMG CoA reductase, the rate-limiting enzyme and the source of mevalonate, on the pathway of cholesterogenesis (1–4). By contrast, adult brain and kidney, which have very slow cell turnover, have low rates of *de novo* cholesterogenesis and little HMG CoA reductase activity (2, 3, 5). Moreover, adult tissues that are stimulated to divide, such as regenerating liver (6) or phytohemagglutinin-treated lymphocytes (7), develop both increased HMG CoA reductase activity and accelerated overall cholesterol synthesis. It is also a consistent characteristic of both malignant (6, 8–12) and premalignant (10) cells that they lose the feedback regulation of HMG CoA reductase and of cholesterogenesis which characterizes normal tissues.

Furthermore, there are ample data indicating that the role in cell growth played by the cholesterol synthetic pathway may be an essential one. Several studies have demonstrated that if HMG CoA reductase activity is blocked for 1–3 days in mammalian cells in tissue culture, cell growth (13) and DNA synthesis (14, 15) eventually decrease and the cells die unless supplied with either cholesterol or a cholesterol precursor, such as mevalonate. In addition, invertebrates, which cannot synthesize cholesterol, require a source of exogenous cholesterol to survive (16). Cholesterol influences both the rigidity and fluidity of the membrane lipid bilayer (17, 18); thus, the above findings have been reasonably interpreted as indicating that cholesterol itself is required during the cell cycle if cells are to grow and divide. In spite of these studies relating cholesterogenesis to cell growth and division, the manner in which cholesterol synthesis and the regulation of the cell cycle may be articulated is not understood.

As part of a detailed examination of how cholesterol synthesis in general and HMG CoA reductase activity in particular may influence cell replication, a study was undertaken to determine whether these reactions of cholesterogenesis may specifically influence the synthesis of DNA in the various phases of the cell cycle. Synchronized BHK-21 cells were used to examine the relationship between HMG CoA reductase (i.e., mevalonate production) and DNA synthesis during each step of the cell cycle. We observed that a marked rise in HMG CoA reductase activity consistently occurs at or just preceding the increased incorporation of [^{3}H]thymidine into DNA that characterizes the S phase of the cell cycle. More significantly, evidence is presented which strongly suggests that mevalonate production—independent of its function in cholesterol synthesis—plays an essential and specific role in regulating S-phase DNA replication.

MATERIALS AND METHODS

Reagents. [*methyl*-^{3}H]dThd (20 Ci/mmol, 1 Ci = 3.7 × 10^{10} becquerels), [2-^{14}C]acetic acid (sodium salt) (51–59 Ci/mmol), [1,2-^{3}H]cholesterol (50 Ci/mmol), [3-^{14}C]HMG CoA (40–60 Ci/mol), and Aquasol were obtained from New England Nuclear. [5-^{14}C]Mevalonate (15 Ci/mol) was obtained from Research Products (Elk Grove Village, IL). D,L-[5-^{3}H]Mevalonic acid (dibenzyl ethylene diamine salt) was purchased from New England Nuclear.

Thymidine, glucose-6-phosphate (monosodium salt), D,L-mevalonic acid lactone, and NADP$^+$ were obtained from Sigma. The ion-exchange resin AG-1-X8, 200–400 mesh formate form, was purchased from Bio-Rad. Inorganic reagents were analytical-reagent grade, supplied by Baker. Glucose-6-phosphate dehydrogenase was obtained from Boehringer Mannheim; HMG CoA was from P-L Biochemicals.

Minimum essential medium with Earle's salts was obtained from the University of California, San Francisco Cell Culture Facility. Minimum essential media were supplemented with a 1.0% (vol/vol) minimum essential medium vitamin solution,

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Abbreviations: HMG CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; FCS, fetal calf serum.

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1% (vol/vol) minimum essential medium amino acid solution 50× concentrated, 1% (vol/vol) fungizone, 0.5% (vol/vol) neomycin solution (100,000 μg/ml), and penicillin (10,000 units/ml). All of these culture media supplements were obtained from GIBCO. Either fetal calf serum (FCS) (340 μg of cholesterol per ml) or delipidized FCS (22 μg of cholesterol per ml), prepared as described below, was incorporated into each of the culture media at a final concentration of 12% (vol/vol). Two-chamber tissue culture slides were obtained from Lab-Tek. The emulsion for autoradiography was Kodak type NTB-3.

Compactin. The inhibitor (ML-236B) was the gift of R. B. Fears (Beecham Pharmaceuticals) and of Akiro Endo (Tokyo Nōkō University). Compactin was brought into solution as described (19).

Cell Culture. Cultures of BHK-21 cells (a nonmalignant, transformed fibroblast cell line) were obtained from the Cell Culture Facility of the University of California, San Francisco. Stock cultures of these cells were grown as monolayers in 150-cm^2 flasks maintained in a 5% CO_2 atmosphere at 37°C in a humidified incubator. The stock cells were maintained by subculture every 4 days at a split ratio of 1:4.

All experiments were carried out in 60 × 15 mm petri dishes at a cell concentration of approximately 25×10^3 cells in 3 ml of medium. They were allowed to grow in this medium for 24 hr before being synchronized.

Synchronization was obtained by double-excess dThd block of exponentially growing cells (20). dThd (2.5 mM) was added to supplemented minimum essential medium containing 12% FCS. After 12 hr, the cultured cells were washed three times with 3 ml of phosphate-buffered saline without calcium and magnesium and incubated again for 8 hr at 37°C in 3 ml of normal medium. When specified, delipidized serum was substituted for whole serum during this incubation period. After 8 hr, a second 12-hr incubation in 2.5 mM dThd was carried out in minimum essential medium containing either whole FCS or delipidized serum, as specified. The dThd block was released by washing the cells three times with warmed phosphate-buffered saline, after which they were studied under the incubation conditions described in the individual experiments. At all time points, cells were examined by light microscopy and monitored for viability by determining trypan blue exclusion. Only exponentially growing cells were used for study.

DNA Synthesis. Cells were incubated with [*methyl*-^{3}H]dThd (1 μCi/ml) for 15 min. They were then washed three times with 3 ml of medium containing 0.6 mM dThd and three times with 3 ml of phosphate-buffered saline. Cells were then scraped from the petri dish with a rubber policeman, and 300 μl of 2 mM sodium dodecyl sulfate was added to disrupt the cell membranes. Five milliliters of cold 5% trichloroacetic acid was then added to precipitate the DNA; the precipitate was filtered on a Millipore filter-AA (0.8 μm), washed with 15 ml of 5% cold trichloroacetic acid, and dried, and the tritium content of the DNA was determined in Aquasol.

HMG CoA Reductase. The rate of conversion of [3-^{14}C]HMG CoA to [^{14}C]mevalonate was measured in detergent-solubilized extracts of the BHK-21 cells as described by Brown and Goldstein (21) and modified by Avigan *et al.* (22) and D. C. Cohen, M. H. Wiley, and M. D. Siperstein (unpublished data). Duplicate determinations of HMG CoA reductase in 10 experiments consistently agreed within 8%. In the experiment presented in Fig. 1, one determination of HMG CoA reductase was made on each cell culture dish.

Incorporation of [^{14}C]Acetate and [^{14}C]Mevalonate into Cholesterol. Monolayers of BHK-21 cells were incubated for 15 min with either [^{14}C]acetate (2 μCi/ml) or [^{14}C]mevalonate (2 μCi/ml) at 37°C in 3 ml of supplemented minimum essential medium containing 12% delipidized serum. After incubation, the medium from each petri dish was transferred into a 50-ml screwtop glass tube; the cells then were dissolved in 1 ml of 0.1 M NaOH and added to the medium from the corresponding petri dish. To each 4 ml of this mixture, 7 ml of ethanol/KOH/H_2O, 5:1:2 (vol/vol) and 10^5 cpm of [1,2-^{3}H]cholesterol, as an internal standard, were added. Total β-hydroxy sterols were isolated as the digitonide as described (23).

Delipidized Serum. To prepare lipoprotein-poor serum, we adjusted the density of FCS to 1.215 g/cm^3 with solid KBr and centrifuged the mixture in a Beckman 30 rotor at 105,000 × *g* for 48 hr at 4–10°C. After centrifugation, the bottom fraction was dialyzed for 36 hr and then passed successively through 0.45-μm and 0.22-μm Millipore filters. The resulting preparation contained 22 μg of cholesterol per ml. The final concentration in the incubation media was therefore 2.6 μg/ml.

Preparation of Cholesterol-Rich Lipoproteins. The cholesterol-rich lipoprotein fraction was prepared by centrifuging FCS at density 1.21 for 48 hr at 105,000 × *g*. The upper 10 ml of solution was collected, dialyzed against 1 liter of 0.15 M NaCl/0.3 mM acetate for 48 hr at 5°C, and passed through Millipore filters as described above. The resulting solution contained 890 μg of cholesterol per ml. The cholesterol concentration of the media prepared with this cholesterol-rich lipropotein was 106 μg/ml.

Protein Determination. Protein concentrations were measured by the method of Lowry *et al.* (24), with bovine serum albumin as the standard.

Cholesterol. Cholesterol was determined by gas/liquid chromatography by means of an OV-17 column and a Hewlett-Packard (model 5830A) gas/liquid chromatograph.

Cell Number. At the time intervals noted, cells were scraped from the petri dishes with a rubber policeman and diluted in 1 ml of phosphate-buffered saline. An aliquot of 20 μl was added to 20 μl of 0.1% trypan blue and the cells were counted in a hemocytometer.

Autoradiography. Synchronized monolayers were grown on two-chamber slides. When the dThd block was released, cells were washed as described before and fixed in Carnoy's fixative, dehydrated, and dried overnight. Autoradiography was carried out for 12 days.

RESULTS

Thymidine Incorporation into DNA During BHK-21 Cell Cycle. Fig. 1 illustrates the incorporation of [^{3}H]dThd into DNA during the various phases of the BHK-21 cell cycle. DNA synthesis in this cell line reached a maximum 4 hr after the dThd block was released, then decreased to a minimum between 10 and 12 hr, and increased again at a time corresponding to a second S-phase peak 14 hr after removal of the dThd block. For reasons that are not clear, in this experiment no detectable incorporation of [^{3}H]dThd into DNA occurred at the 12-hr timepoint. This was the only sample in numerous experiments (see Figs. 2 and 3) in which DNA synthesis was negligible. Autoradiography confirmed these results by showing a 16-fold increase in labeled cells (i.e., from 5% to 80%) over the 3-hr period after block release, followed by a decrease to a minimum labeled cell number of 10% between 8 and 12 hr after block release.

As indicated in Fig. 1, the total number of cells doubled between 10 and 12 hr after the dThd block was removed; this time corresponds to a cell cycle of 12.5 hr, a figure which agrees with that previously reported for BHK-21 cells grown at 37°C (25). At the time that the dThd block was released, the BHK cells typically appeared extended and flat, their normal appearance

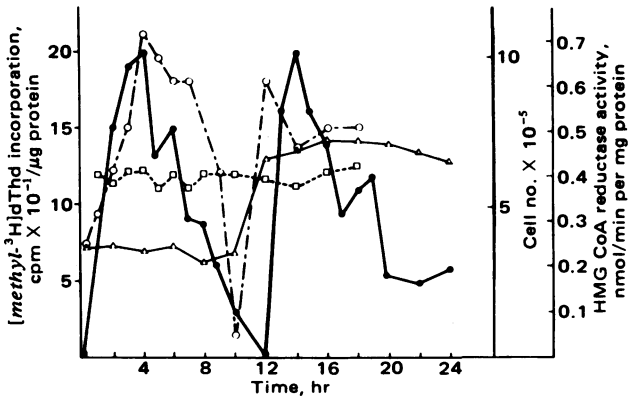

FIG. 1. Cells were grown in supplemented minimum essential medium with 12% (vol/vol) FCS and were synchronized by the double-dThd block technique. After the block was released, the cells were divided into three groups and samples from each group were harvested every hour for 24 hr for the following analyses: ●, [^{3}H]dThd incorporation; ○, HMG CoA reductase activity in synchronized cells; □, HMG CoA reductase in unsynchronized cells; △, determination of cell number per petri dish.

when entering the S phase. At approximately 10 hr, while they were undergoing mitosis, the cells began to round up and became loosely attached to the dish surface. By 24 hr the BHK cells had reached confluency and metabolic events during the cell cycle could not be studied further.

HMG CoA Reductase Activity. The activity of HMG CoA reductase was assayed over an 18-hr period, corresponding to almost two cell cycles. As indicated by the typical experiment shown in Fig. 1, HMG CoA reductase activity underwent marked fluctuations during the various phases of the cell cycle, at times during the cycle increasing to twice that of the unsynchronized cells examined concomitantly. Reductase activity began to increase 2 hr after release of the dThd block, reached a maximum at 4 hr, and then declined to a minimum at 10 hr. Thereafter the HMG CoA reductase activity again increased during the second cell cycle, peaking during this cycle at a time corresponding to 12 hr after the release of the dThd block.

It is striking that the HMG CoA reductase activity consistently increased and reached a maximum just prior to or at the time that S-phase dThd incorporation into DNA increased to its maximum. This effect was seen more clearly in the second S-phase peak of the two cell cycles studied, where the maximum HMG CoA reductase activity always preceded the S-phase burst of [^{3}H]dThd incorporation into DNA. Similar results (not shown) were obtained in four additional experiments.

To confirm that these marked changes in HMG CoA reductase activity were related to the phases of the cell cycle, these experiments were repeated with BHK cells synchronized by an alternate procedure. A uniform population of synchronized mitotic cells can easily be obtained because cells can be detached from the dish surface at this phase in the cycle by gentle rotation on a Yankee shaker (A. H. Thomas, Philadelphia, PA) at 40–60 rpm. When such mitotic cells were collected and centrifuged as decribed (26), [^{3}H]dThd incorporation and HMG CoA reductase activity were both very low (i.e., 22 cpm per μg of protein and less than 18 pmol/min per mg, respectively). If these cells were reincubated and allowed to grow for 5 hr to reach a period corresponding to S phase ([^{3}H]dThd incorporation, 86 cpm per μg of protein), HMG CoA activity increased more than 10-fold (i.e., to 192 pmol/min per mg of protein). Thus, the changes in HMG CoA reductase activity previously observed between G_1 and S periods could be confirmed in cells prepared by this second means of synchronization.

Incorporation of [^{14}C]Acetate and [^{14}C]Mevalonate into Sterols. Table 1 illustrates the incorporation of [^{14}C]acetate and [^{14}C]mevalonate into sterols at various time intervals after release of synchronized BHK-21 cells from the dThd block. The results demonstrate that there is a lower level of sterol synthesis from both substrates at 9–10 hr than at 12 hr after initiation of cell growth. This course of sterol synthesis approximately parallels that of HMG CoA reductase. However, these data indicate that over the 15-min course of the study a maximum of only 9 ng of mevalonate would have been converted into sterols. The cells incubated in whole serum were exposed to a total of 124 μg of cholesterol in 3 ml of medium. Sterol synthesis from added mevalonate could therefore have increased the overall cholesterol concentration of the medium by no more than 0.007%.

Effect of Inhibition of HMG CoA Reductase on DNA Synthesis. In view of the close temporal relationship between the activity of HMG CoA reductase and S-phase DNA synthesis in the synchronized BHK cells, studies next were carried out to determine whether blocking HMG CoA reductase activity would influence DNA synthesis. Compactin (ML-236B) is a very potent competitive inhibitor of HMG CoA reductase activity, and the addition of this compound will almost completely block the synthesis of cholesterol *in vitro* (19, 27, 28). In preliminary studies it was shown that at a compactin concentration of 2.5 μM, sterol synthesis in the BHK-21 cell line was inhibited over a 1-hr period by at least 95%. This inhibition of cholesterol synthesis was sustained as long as compactin was present in the medium.

As shown in Fig. 2, when compactin (2.5 μM) was added to synchronized BHK-21 cells 1 hr prior to assay for DNA synthesis, the S-phase burst of DNA synthesis was completely prevented. This effect, moreover, was observed for each of the two cell cycles studied and in each of a total of three experiments.

Initial evidence for the specificity of the compactin inhibition of DNA synthesis was provided by the fact that throughout the 13 hr of the experiment, the compactin-treated BHK-21 cells remained morphologically normal, adhered to the surface of the tissue culture dishes, and consistently showed exclusion of trypan blue in at least 95% of the cells. Moreover, the 1-hr compactin treatment caused no change in total cell numbers at any time during the cell cycle.

Mevalonate Reversal of Blocked DNA Synthesis. That the effect of compactin on DNA synthesis was due specifically to an inhibition of mevalonate production was demonstrated by the fact (Fig. 2) that the addition of 0.4 mM mevalonate *during the 15 min* of the [^{3}H]dThd incorporation completely reversed the compactin inhibition of S-phase DNA synthesis. Again, this effect was apparent in both of the two cycles examined during the 13-hr experiment.

Table 1. Cholesterol synthesis by BHK-21 cells from acetate and mevalonate

Time after block release, hr	[^{14}C]Acetate incorporation*	[^{14}C]Mevalonate incorporation*
8	2.4	0.6
10	3.7	1.04
12	10.0	4.42

Cells were grown in supplemented media and synchronized. For the last 20 hr of the dThd block, the cells were kept in delipidized serum and were collected 8, 10, and 12 hr after block release. Cells were incubated for 15 min in either [^{14}C]acetate or [^{14}C]mevalonate before being harvested.

* pmol/min per dish.

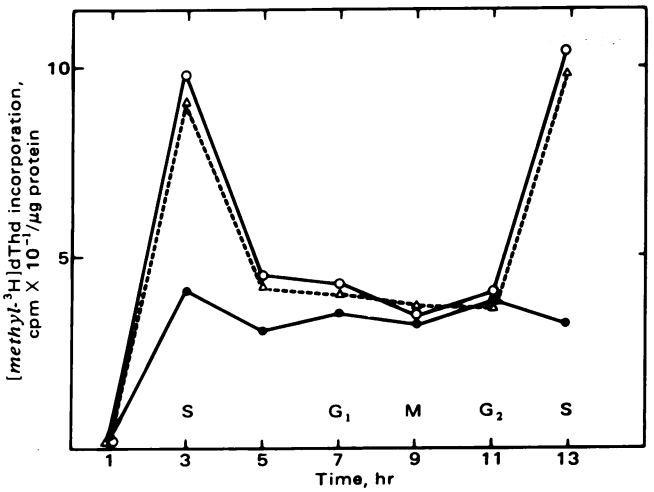

FIG. 2. Cells were grown in supplemented minimum essential media with 12% (vol/vol) FCS for 24 hr and synchronized. After the block was released, the cells were divided into three groups and samples from each group were harvested every 2 hr. [³H]dThd incorporation was determined on cells treated as follows: O, control cells, [³H]dThd incorporation determined during the last 15 min before harvest; ●, 2.5 μM compactin added 1 hr and [³H]dThd added 15 min before harvest; △, 2.5 μM compactin added 1 hr and 0.4 mM mevalonate and [³H]dThd added during 15 min before harvest.

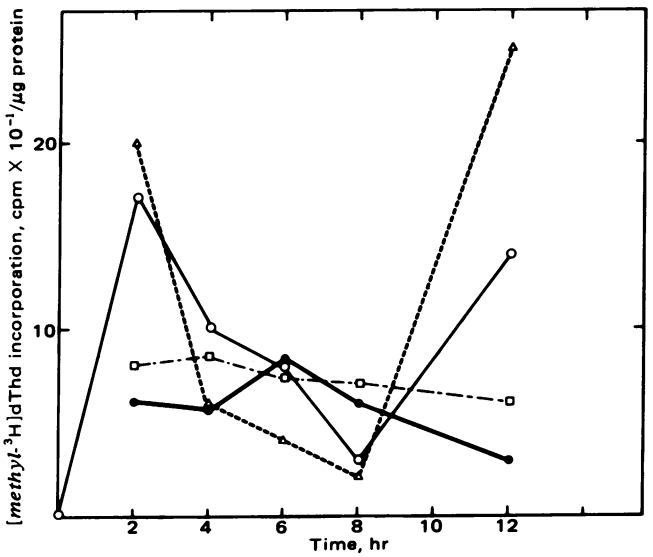

FIG. 3. Cells were grown in supplemented minimum essential media with 12% (vol/vol) FCS for 24 hr and then maintained in medium containing delipidized serum. After the dThd block was released, the monolayers were divided into four groups and samples from each group were harvested every 2 hr. [³H]dThd incorporation was determined in cells treated as follows: O, control cells, [³H]dThd added during last 15 min before harvest; ●, 2.5 μM compactin added for last 2 hr and [³H]dThd during the last 15 min before harvest; △, 2.5 μM compactin added for 2 hr and [³H]dThd and 0.4 mM mevalonate for 15 min before harvest; □, 2.5 μM compactin added 2 hr and [³H]dThd and cholesterol-rich lipoprotein (to yield a final cholesterol concentration of 106 μg/ml) added 15 min before harvest.

Effect of Cholesterol and Mevalonate on Compactin-Induced Inhibition of DNA Synthesis in Cells Grown in Delipidized Serum. Because the experiment described in Fig. 2 was carried out in the presence of the relatively high concentrations of cholesterol normally present in whole serum, it seemed unlikely that the compactin-induced inhibition of DNA synthesis could be the result of decreasing the cholesterol content of the cell. Direct measurement of the cholesterol content of the BHK-21 cells in fact confirmed that a 2-hr exposure to compactin had no detectable effect upon their cholesterol content (i.e., control, 17 $\mu g/10^6$ cells; compactin, 19 $\mu g/10^6$ cells). This possibility was more directly examined by repeating the study with BHK-21 cells grown in medium containing delipidized serum. As shown in Fig. 3, inhibition of HMG CoA reductase activity by the addition of compactin once more resulted in complete elimination of S-phase DNA synthesis in both of the cell cycles examined during this experiment. Mevalonic acid, when added for the last 15 min of each experimental period, again caused a complete reversal of the compactin-induced inhibition of DNA synthesis during the first cell cycle studied; even more dramatically, during the second S phase the addition of mevalonic acid not only reversed the compactin inhibition, but resulted in an acceleration of DNA synthesis to a level of approximately twice that of the untreated control cells.

In marked contrast to the striking effect of mevalonic acid on DNA synthesis, the addition of cholesterol-rich lipoproteins, despite causing the cholesterol content of the cells to increase by 64%, was completely incapable of reversing the compactin inhibition of S-phase DNA synthesis.

This experiment indicates that the HMG CoA reductase requirement for DNA synthesis is the result of a dependence of S-phase DNA production upon the availability of mevalonic acid itself rather than on any increment of cholesterol that might have resulted from increased mevalonate synthesis.

Specificity of Mevalonate-Induced DNA Synthesis for S-Phase DNA. It is apparent from the data in Figs. 2 and 3 that the effect of compactin is remarkably specific for the S-phase burst of DNA synthesis in that the compactin block of mevalonate production did not significantly influence DNA synthesis during G_1, M, or early G_2 phases of the cell cycle. Moreover, as indicated by the experiments shown in Figs. 2 and 3, addition of mevalonate affected DNA synthesis only during the S phase of the cycle.

DISCUSSION

The major finding of the present study is that mevalonate, or a product of mevalonate, is essential for the initiation of DNA replication, specifically during the S phase of the cell cycle. The first indication in this study that mevalonate production might play a role in DNA synthesis was the finding that in synchronized cultures of BHK-21 cells, HMG CoA reductase activity and, hence, mevalonate synthesis regularly increase at or just prior to the S-phase acceleration of DNA synthesis. This finding raised the possibility that HMG CoA reductase might play some role in initiating or controlling DNA synthesis in the normal cell cycle. This hypothesis was strengthened by the observation that when HMG CoA reductase was blocked by the competitive inhibitor, compactin, the S-phase burst of DNA synthesis was completely inhibited, an observation which indicated that the presence of mevalonate might well be required for DNA synthesis. That the effect of compactin in blocking DNA synthesis was in fact mediated through an inhibition of mevalonate production was then directly demonstrated by the finding that the compactin-induced inhibition of S-phase DNA synthesis was completely reversed within minutes after the addition of mevalonate. In fact, the addition of relatively large amounts of mevalonate to the compactin-blocked cells stimulated DNA synthesis even to levels well above those of the control cell population.

Several lines of evidence indicate that the requirement for mevalonate production in S-phase DNA synthesis is independent of its conversion to cholesterol. First, cells incubated in

media containing whole serum were exposed to cholesterol concentrations of 4.1 mg/dl, a level that has been shown to be adequate to supply the cellular cholesterol requirements in tissue culture (21) and that permitted growth to confluence in the present experiments. Despite the presence of such ample amounts of cholesterol, blockage of mevalonate synthesis for only 1 hr completely prevented DNA replication. No detectable depletion of cellular cholesterol occurred during this brief exposure to compactin. On the other hand, the restoration of S-phase DNA synthesis was observed within minutes after addition of mevalonate, during which time only picomole amounts of mevalonate were incorporated into a cellular cholesterol pool of more than 30 nmol. Finally, when cells were grown in media containing delipidized plasma, mevalonate promptly reversed the compactin-induced block of DNA synthesis, whereas the addition of relatively large concentrations of cholesterol in the form of cholesterol-rich lipoproteins, while increasing the cholesterol concentration in the cell by 64%, was totally ineffective in reversing the inhibition of DNA synthesis. Thus, it would appear that either mevalonate itself or an isoprenoid product of mevalonate stimulates S-phase DNA synthesis by a mechanism that is independent of its role in sterol synthesis.

The effect of mevalonate on DNA synthesis appears to be specific for the DNA replication that takes place in the S phase of the cell cycle. The data in Figs. 2 and 3 clearly demonstrate that treatment with compactin blocks DNA synthesis only at this phase of the cell cycle and has no effect upon the residual DNA synthesis of G_2, M, and G_1 phases. In addition, treatment with mevalonate did not significantly enhance DNA synthesis during any but the S phase of the cycle. This surprising specificity of the mevalonate effect for this phase of DNA synthesis suggests that mevalonate, or a derivative, may be required for the activation of DNA polymerase, in all likelihood the α-DNA polymerase, which functions specifically during the S phase of the cell cycle to effect the replication of the DNA chain (29, 30). In this regard, any possible effect of mevalonate on the earlier steps of DNA synthesis [e.g., on thymidine kinase, which shows maximum activity at the G_2 phase of the cell cycle (31)] would have been reflected in the DNA synthesis at G_2, M, and G_1 phases, which, as noted, were in fact not influenced by the availability of mevalonate.

HMG CoA reductase, then, probably has at least two independent roles in regulating cell division. First, as is well established, the mevalonate produced by this enzyme can, if necessary, provide adequate amounts of cholesterol for the long-term maintenance of plasma membrane integrity. Second, as demonstrated in the present study, mevalonate, either itself or perhaps through some intermediate product, appears to be required for the very rapid activation of DNA polymerase-catalyzed DNA replication. In fact, as shown here, not only does the absence of mevalonate prevent normal, S-phase DNA synthesis, but an excess of mevalonate can, within a few minutes, stimulate DNA replication to supernormal levels.

Although the exact chemical structure of the mevalonate-derived stimulator of DNA synthesis remains to be established, the unexpected finding that the activity of HMG CoA reductase and, hence, mevalonate production play essential regulatory roles in S-phase DNA synthesis indicates an important functional link between these two apparently unrelated biochemical processes. Moreover, these findings, coupled with our earlier observation that the feedback control of this enzyme is consistently and uniquely lost during the uncontrolled cell growth of malignant states (6, 8–11), suggest that HMG CoA reductase may have far more important physiologic and pathologic functions in normal and deranged cell replication than has heretofore been suspected.

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