

Role of cholesterologenesis and isoprenoid synthesis in DNA replication and cell growth

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CHOLESTEROL SYNTHESIS AND CELL GROWTH

Over the past few years, there has developed overwhelming evidence that cholesterol, and undoubtedly other isoprenoids, play a critical and, with few exceptions, an essential role in the growth of all eucaryotic cells. The earliest evidence of such a relationship was the finding of Sreere, Chaikoff, and Dauben (1) that rapidly growing tissues such as the brains of newborn rats actively synthesize cholesterol from two-carbon fragments, whereas the adult brain, which shows little cellular turnover, carries out *de novo* cholesterologenesis at only very slow rates. This observation anticipated many subsequent studies that demonstrated that there is, in general, an excellent correlation between the rates of cell replication and the rates of cholesterol synthesis.

Thus, rapidly proliferating tissues such as the intestine have high levels of both cholesterol synthesis and 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis (Fig. 1). By contrast, an organ such as the kidney, which undergoes only very slow cell turnover, is virtually devoid of *de novo* cholesterol synthesis and has very little HMG-CoA reductase activity (2-5). The one exception to this generalization is the adult liver, which has a slow cell turnover, but actively synthesizes cholesterol. It is assumed that, as the major source of serum cholesterol, *de novo* cholesterologenesis by the liver is required primarily for this export function, rather than for the cholesterol needs of cell proliferation. Consistent with this correlation between cell proliferation and the activity of the cholesterol biosynthetic pathway, the rapid liver regeneration that follows partial hepatectomy is characterized by a very rapid rate of cholesterol synthesis (6). As noted below, Trentalance et al. (7) have extended this observation by demonstrating that in the early stages of such hepatic regeneration, the activity of HMG-CoA reductase, but not overall chole-

sterogenesis, is accelerated, and only after 16 hr of hepatic regeneration does the rate of cholesterol synthesis first exceed that of the control liver.

The correlation between cell proliferation, cholesterol synthesis, and HMG-CoA reductase has also been demonstrated in a number of tissue culture systems. Chen, Heninger, and Kandutsch (8) first showed that phytohemagglutinin-stimulated mouse lymphocytes undergo a sixfold increase in acetate incorporation into cholesterol 24 hr before the cells entered the S-phase of the cell cycle. Huneeus, Wiley, and Siperstein (9) have shown that, following release of baby hamster kidney cells from a double thymidine block, there is an increase in HMG-CoA reductase activity which precedes the first cell doubling. Habenicht, Glomset, and Ross (10) demonstrated a similar increase in HMG-CoA reductase and cholesterol synthesis in both mouse fibroblasts and in smooth muscle cells following stimulation with platelet-derived growth factor (PDGF). Interestingly, it is likely that rapidly proliferating cells can satisfy their needs for cholesterol by increased uptake of exogenous cholesterol. Goldstein and Brown (11), and more recently Witte et al. (12), demonstrated an increase in low density lipoprotein receptors in cells undergoing proliferation, suggesting that such cells can satisfy at least some of their cholesterol requirements by an increased uptake of cholesterol-rich LDL.

REQUIREMENT FOR CHOLESTEROL IN CELL GROWTH AND PROLIFERATION

The studies cited above demonstrated a correlation between cholesterologenesis, cell growth, and proliferation but did not directly address the question of a requirement for cholesterol in cell replication. Cholesterol is a com-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; PDGF, platelet-derived growth factor; LDL, low density lipoproteins.

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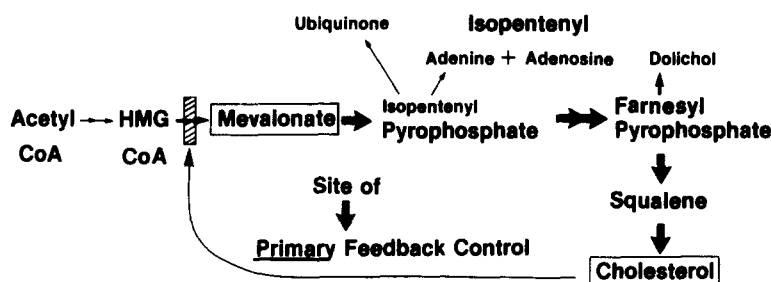


Fig. 1. Pathway of isoprenoid synthesis.

ponent of the lipoprotein membranes of the nucleus, mitochondria, and microsomes, as well as the plasma membrane. It is not surprising, therefore, that a supply of cholesterol would be required for the membrane proliferation that must accompany cell growth. It has, in fact, been known for many years that insects and crustaceans, which have little if any capacity to synthesize cholesterol, must ingest exogenous sterols in order to grow and survive (13).

Cholesterol synthesis is under feedback control (14, 15), which operates by regulating the production of mevalonic acid (16–18) (Fig. 1). When exogenous cholesterol is not available, the synthesis of mevalonate is therefore increased, and the supply of cholesterol, as well as of other isoprenoids, is thereby maintained. Brown and Goldstein (19) have elegantly reviewed this control of isoprenoid biosynthesis under various physiologic circumstances. The most critical test of a true requirement for cholesterol in cell growth, therefore, necessitated a method of specifically inhibiting the production of mevalonate in the absence of a supply of exogenous cholesterol.

The first evidence that such inhibition of cholesterol synthesis would inhibit cell growth was provided by Chen, Kandutsch, and Wymouth (20), who demonstrated that treatment of mouse L cells with either 25-hydroxy- or 20-hydroxycholesterol, compounds that inhibit HMG-CoA reductase, would block the growth and proliferation of these cells. Brown and Goldstein (21) carried out similar experiments and noted that 1–2 days of treatment with such oxygenated sterols greatly reduced cell proliferation. Subsequently, the studies of Goldstein, Helgeson, and Brown (22) and of Kaneko, Hazama-Shimada, and Endo (23) showed that treatment for 6 to 9 days with compactin, a very potent competitive inhibitor of HMG-CoA reductase, also prevented cell proliferation in tissue culture systems.

Brown and Goldstein (21), Kandutsch and Chen (24), and Cornell et al. (25) demonstrated that the addition of either mevalonate or cholesterol would reverse the inhibition of cell proliferation caused by the oxygenated sterols. The compactin inhibition of cell growth and proliferation was shown to be similarly reversed by

adding either cholesterol or mevalonate (22). Subsequently, however, Kaneko et al. (23) made the important observation that, even in the presence of cholesterol, added mevalonate was needed to restore proliferation to compactin-treated cells, an observation that was subsequently confirmed by Brown and Goldstein (19). These last two studies indicated that other isoprenoid products of mevalonate, such as dolichol and ubiquinone, are probably also required for cell growth. This conclusion is consistent with the finding that the activity of HMG-CoA reductase is increased at 8 hr following partial hepatectomy, whereas cholesterol synthesis does not increase above normal until the 16th hr of hepatic regeneration (7). This series of experiments left little doubt that cholesterol and/or mevalonate serve essential functions, primarily to provide the needed structural cholesterol in cell growth and proliferation. The mechanism by which cholesterogenesis might control cell growth and replication was not specifically examined in these studies.

ROLE OF ISOPRENOIDS IN DNA SYNTHESIS

Despite the convincing evidence that cholesterol itself is required for cell proliferation, our laboratory first raised the possibility that the production of mevalonic acid, in addition to serving as a precursor for the structural cholesterol requirements of cell growth, might regulate cell proliferation by playing a direct role in DNA replication (9). Kandutsch's laboratory (8, 24) had previously reported that, following prolonged treatment of either normal or phytohemagglutinin-stimulated L cells with 25-hydroxycholesterol, a decrease in DNA synthesis eventually occurred. This effect, however, was observed only after 28–30 hr of exposure to the oxygenated sterols, and it therefore seemed likely, as the authors concluded, that the observed effects on DNA synthesis were secondary to depleting the cells of cholesterol. That decreased cell transformation results from inhibiting cholesterol synthesis with oxygenated steroids has more recently been confirmed by Astruc et al. (26, 27), as well as by Chen (28) and Cornell et al. (25, 29). In contrast with these results, Kaneko et al. (23) reported

that such inhibition of mevalonate and cholesterol synthesis by compactin has no effect on DNA synthesis.

The aims of our studies were, first, to examine a possible acute role for mevalonate in initiating DNA replication, and secondly, to determine whether such an effect of mevalonate is independent of its function as a cholesterol precursor (9). Initially the relationship between HMG-CoA reductase, and hence mevalonate and DNA synthesis, was examined in synchronized BHK-21 cells during the various stages of the normal cell cycle. The results demonstrated that there is a consistent rise in HMG-CoA reductase at or just prior to each S-phase DNA replication. This observation suggested that HMG-CoA reductase might have an acute function in the replication of DNA. In fact, when HMG-CoA reductase activity was blocked by treatment with compactin, the normal S-phase increase in DNA synthesis was specifically prevented. Significantly, the reinitiation of DNA replication by mevalonate was rapid, occurring within minutes after its addition to compactin-inhibited cells. Furthermore, in many cases, an excess of mevalonate resulted in an elevation of DNA synthesis even above that of control cells. By contrast, cholesterol, although added in amounts sufficient to cause significant intracellular cholesterol accumulation, had no effect upon DNA synthesis in the compactin-treated cells. It is noteworthy that, in the presence of cholesterol, this effect of mevalonate depletion and replacement is specific for S-phase DNA replication, with no influence of mevalonate on DNA synthesis being detected in either G₁ or G₂ phases of the cell cycle. These data provided the first evidence that, independent of its function in cholesterol synthesis, the mevalonate synthesized by HMG-CoA reductase plays an acute role in initiating DNA replication.

A number of other investigators, notably Habenicht et al. (10), Larson et al. (30), and most recently Fairbanks, Witte, and Goodman (31), have subsequently also shown that depletion of mevalonate will inhibit DNA synthesis by a mechanism that is independent of cholesterol synthesis, in that inhibition of DNA synthesis by compactin treatment could be reversed by the addition of mevalonate, but not by cholesterol.

The exact mechanism by which mevalonate can initiate DNA replication remains unknown; however, this laboratory has produced preliminary evidence that isopentenyl adenine and its hydroxylated derivative, zeatin, (but not dolichol or ubiquinone) can largely replace the role of mevalonate in initiating DNA replication (32).

More recent studies have attempted to dissect the function of mevalonate in controlling DNA replication and cell growth by dissociating the cholesterol and cholesterol-independent requirements for mevalonate during the cell cycle (33). Use was made of two inhibitors of cholesterol synthesis, TMD (trimethyl decal), a specific inhibitor of cholesterol synthesis acting beyond meva-

lonate at squalene cyclization, and compactin, acting to inhibit mevalonate synthesis. With this approach, it could be shown that cholesterol itself is required for cell growth during the early G₁ phase of the cell cycle, whereas the noncholesterol requirement for mevalonate, which can largely be replaced by isopentenyl adenine, is not detectable early in G₁ and appears to be localized to the G₁-S interphase of the cell cycle. The inability of isopentenyl adenine to stimulate DNA replication when added early in the G₁ phase of the cell cycle has been confirmed by the studies of Perkins, Ledin, and Stubbs (34), as well as by those of Larson et al. (30). Interestingly, Fairbanks et al. (31) have shown that, in PDGF-stimulated human fibroblasts, compactin would inhibit and mevalonate would restore DNA replication during a "window" from the 10th to 15th hrs after adding PDGF, DNA synthesis being measured at 24 hr.

These findings have therefore indicated that mevalonate serves at least two functions in cell growth and proliferation (Fig. 2). First, as described above, mevalonate can, when conditions require, provide sufficient amounts of structural cholesterol for the maintenance and synthesis of the plasma and intracellular membranes needed for cell growth. The second function of mevalonate, independent of its role as a cholesterol precursor, is to serve an essential role in the initiation of DNA replication. The exact mechanism by which a noncholesterol product of mevalonate, for which isopentenyl adenine or related compounds are candidates, may initiate DNA replication remains to be determined. Nonetheless, as indicated in Fig. 2, this finding has uncovered an unexpected direct link between the pathway of cholesterol synthesis and DNA replication.

LOSS OF FEEDBACK CONTROL OF CHOLESTEROL SYNTHESIS IN MALIGNANT STATES

The finding that the synthesis of mevalonic acid has an essential function in DNA replication raises the possibility that a derangement in mevalonate synthesis might play a role in carcinogenesis. As noted earlier, the liver (14, 15), and in some species (35) all tissues, normally demonstrate a negative feedback mechanism in which exogenous cholesterol, by inhibiting the synthesis of mevalonic acid (16-18), depresses the overall de novo synthesis of endogenous cholesterol. Our laboratory has demonstrated that this feedback response to dietary cholesterol is lost when cells undergo malignant transformation. Loss of cholesterol feedback control in cancer cells was initially demonstrated in the mouse hepatoma BW-7756 (6). Similar observations have been made in a total of eleven minimal deviation rat hepatomas, which consistently show this defect regardless of

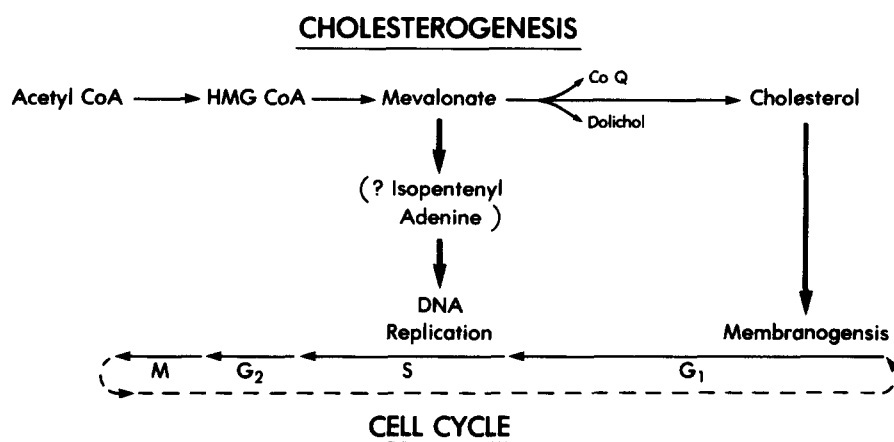


Fig. 2. Role of cholesterol synthesis in the cell cycle and DNA synthesis.

cell proliferation rate (36). The latter tumors have been widely used as models by which to study biochemical changes that are specific for malignancy. As a result, the loss of the cholesterol feedback system in all such minimal deviation tumors examined *in vitro* (37) and *in vivo* (36, 38) indicates that the development of this biochemical defect may be specific for malignant change rather than a nonspecific consequence of the cell dedifferentiation that can follow malignant transformation. This feedback deletion has also been demonstrated in spontaneous human hepatomas (6, 37). Moreover, loss of the cholesterol feedback system is not restricted to hepatomas since this defect has also been demonstrated in two types of leukemia, the L2C leukemia of the guinea pig (39) and a rat leukemia (40). The complete loss or marked impairment of the cholesterol feedback system following cholesterol feeding has, with only a single exception, been confirmed in all cancers thus far studied (41–45). To date, the only tumor that has demonstrated a significant feedback response to dietary cholesterol feeding is the L-1210 leukemic cell (46). Further, this biochemical defect has been demonstrated *in vitro* (47). At least one laboratory has not been able to demonstrate loss of this feedback mechanism under tissue culture conditions (48); however, others have documented that deranged cholesterol feedback control is present in cancer cells grown in tissue culture (49).

The mechanism of the loss of the cholesterol feedback system in malignant cells remains unknown. The defect is not simply a consequence of rapid cell proliferation since, as noted, the regenerating liver possesses a sensitive cholesterol feedback mechanism (6). It has been recently shown by Barnard, Erickson, and Cooper (50) that there is a 70–80% decrease in chylomicron remnant receptors in a hepatoma grown *in vivo*, a finding that might account for a decrease in the response of the tumors to exogenous cholesterol. However, this suggestion is made less likely by the findings that labeled cholesterol readily penetrates tumors (6), that exogenous cholesterol accu-

mulates in hepatomas (51), and finally, that the intracellular membranes of hepatomas contain a higher concentration of cholesterol than does normal liver (52–54), despite which fact cholesterol synthesis in these tumor cells is not depressed. It is of interest that cholesterol synthesis in malignant cells is also not affected by the fasting-induced inhibition of cholesterol synthesis that is characteristic of normal cells, an observation that suggests a more general failure of tumors to respond to the regulatory factors that normally control cholesterol synthesis (55).

Not too surprisingly, the cholesterol feedback defect in malignant cells has been localized to the site of HMG-CoA reductase action (56), a finding that has been independently shown by Goldfarb and Pitot (57) and by Kandutsch and Hancock (43). Brown, Dana, and Siperstein (58) reported that HMG-CoA reductase isolated from tumor cells has the same cold sensitivity, as well as identical kinetic and structural properties as does the HMG-CoA reductase isolated from normal cells. In addition, the enzyme from tumors appears to be antigenically very similar to that from liver. More recently, however, Feingold et al. (59) have demonstrated that the activation state of HMG-CoA reductase in three minimal deviation hepatomas is consistently higher than that observed in normal liver. Further, this effect appears to be characteristic of tumors since the enzyme from fetal liver and rapidly regenerating liver cells shows an activation state similar to that of the enzyme from normal liver. This finding, then, represents the first indication of a fundamental difference in the HMG-CoA reductase of tumors as compared to normal tissue.

LOSS OF FEEDBACK CONTROL IN PREMALIGNANT CELLS

The loss of cholesterol feedback control in malignant cells could, of course, represent a consequence rather

than an early event in the process of carcinogenesis. It was, therefore, important to determine whether this defect in feedback regulation followed or preceded the malignant transformation. A number of studies have now demonstrated that the cholesterol feedback lesion is, in fact, present in the precancerous state. The administration of the potent hepatocarcinogen aflatoxin B-1 to a rat causes loss of the cholesterol feedback system within a few days; yet in the dose of aflatoxin administered, tumors appear only after 6–9 months (39, 60, 61). The effect of aflatoxin is organ-specific in that the feedback regulation of the intestine is retained in the aflatoxin-treated animals, and aflatoxin, under the conditions employed, does not produce intestinal tumors (62). The observations of defective cholesterol feedback control in premalignant cells have been extensively confirmed and extended, primarily by Sabine's laboratory (63–67). There is also some evidence that this premalignant lesion occurs in human beings (68). The consistency with which the loss of the cholesterol feedback system occurs in malignant cells, coupled with the finding that this defect precedes the appearance of cancers under premalignant conditions, supports the hypothesis that deranged cholesterol synthesis may be involved in the early stages of carcinogenesis.

SUMMARY

In summary, there is now ample evidence that cell growth, as well as DNA replication, is closely linked to cholesterol synthesis in a manner that has only recently been appreciated. Mevalonic acid, the product of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, is now known to serve at least two functions in the cell cycle. First, mevalonate acts as a precursor of the cholesterol that is needed for cell membrane synthesis, and second, independent of cholesterol synthesis, mevalonate has been shown to serve a rapid and essential initiator function in DNA replication. These findings, coupled with the fact that loss of the cholesterol feedback control of mevalonic acid represents the most consistent biochemical defect so far identified in the malignant and premalignant states, suggests the possibility that uncontrolled synthesis of mevalonate may play a role in deranged DNA synthesis and malignant transformation. ■

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