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Dolichol delays G_1-arrest for one cell cycle in human fibroblasts subjected to depletion of serum or mevalonate

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Summary

It is well-established that some product(s) or metabolite(s) of mevalonate is (are) critical for growth of mammalian cells. In the search for this (these) compound(s) it seems meaningful to distinguish between compounds needed for *cell cycle progression* in proliferating cells and compounds needed for *growth activation* of arrested cells. By using time-lapse video recording we have studied the possible regulatory role of cholesterol, dolichol and mevalonate in the cell cycle of human diploid fibroblasts (HDF). HDF, which are serum-dependent, were rapidly growth-arrested in the first part of G_1 upon removal of serum factors. They also responded to mevinolin (an HMG CoA reductase inhibitor) by a similar G_1-block, indicating that a mevalonate-derived product is involved in the G_1-located cell cycle control of HDF. Interestingly, dolichol counteracted the G_1-block caused by both types of treatment. Hence, the early G_1-cells could traverse the remainder of the cell cycle and divide despite depletion of serum or mevalonate. We also demonstrated that addition of dolichol resulted in a significant decrease in the rate of protein degradation. This protein stabilizing effect may constitute the mechanism by which dolichol delays the G_1-arrest of HDF.

Key words: dolichol, cell cycle, fibroblasts, cell growth, mevalonate.

Introduction

It is well known that mevalonate is required for growth of mammalian cells (Brown and Goldstein, 1980; Siperstein, 1984; Goldstein and Brown, 1990) as well as constituting the key metabolite in the biosynthesis of cholesterol and a variety of sterol and non-sterol isoprenoid (e.g. dolichol, isopentenyladenine and ubiquinone) derivatives (Brown and Goldstein, 1980; Goldstein and Brown, 1990). The formation of mevalonate, from 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA), is catalyzed by HMG CoA reductase, which is the principal regulatory step in the pathway (Brown and Goldstein, 1980). The regulation of HMG CoA reductase has been the subject of intense research mainly because cholesterol is of importance in the pathogenesis of arteriosclerosis (Endo and Hasumi, 1989). In contrast, data from several laboratories suggest that the mevalonate-derived product (still not identified) that is critical for cell growth, is an isoprene of non-sterol type (Quesney-Huneeus et al., 1980; Brown and Goldstein, 1980; Habenicht et al., 1980; Yachnin, 1982; Larsson and Zetterberg, 1986; Doyle and Kandutsch, 1988).

In the search for mevalonate-derived compounds involved in growth control much attention has been aimed at prenylated proteins (Schmidt et al., 1984; Glomset et al., 1990). Farnesylation of the proto-oncogene product $p21^{ras}$ has especially attracted interest (Casey et al., 1989; Schafer et al., 1989; DeClue et al., 1991; Jakóbisiak et al., 1991). Even if prenylation of proteins is necessary for cell growth, there is still no evidence that this processing plays a major role in growth regulation.

It is unclear whether isoprenoid-induced growth regulation is mediated by one single compound or by several compounds, and it is also unknown whether various growth conditions require different mevalonate-derived compounds. Hence, it would be interesting to elucidate whether there are any differences in the isoprenoid requirements between *growth activated cells* and *cycling cells*. In the present study we have investigated this matter by using a genetically stable cell system, i.e. human diploid fibroblasts (HDF), in which the possible growth-regulatory role of cholesterol, dolichol and mevalonate was studied. The data obtained demonstrate that dolichol has the capability of counteracting the G_1-specific arrest following depletion of mevalonate or serum, but only in the first cell cycle.

Materials and methods

Materials

Mevinolin was obtained from Merck, Sharp and Dohme, and was converted to its sodium salt before experimental use. All other

chemicals were from Sigma. The type of dolichol used was dolichol-20 (C100). Radiochemicals were from Amersham.

Cell culture

Low-passage (passage 2-6) human diploid fibroblasts (HDF), obtained from Flow Inc., were grown in monolayers in tissue-culture flasks maintained in a 95% air/5% CO_2 atmosphere at 37°C in a humidified incubator. Cells were cultured in a 1:1 mixture of Modified Eagle's Medium (MEM) and Ham's F-12 medium, supplemented with 10% (v/v) Foetal Calf Serum (FCS).

For experimental purposes, cells were cultured in tissue-culture flasks or dishes. Cells were seeded at a density of 2,000-5,000 cells per cm^2. The experiments were started 48-72 hours later, at which time a cell density of 7,000-20,000 cells/cm^2 had been reached.

Time-lapse video recording

Cell number measurements, as well as determinations of cell cycle position and intermitotic time for individual cells, were performed by the use of time-lapse video recording (TLV). This was done by placing a 25 cm^2 flask containing exponentially growing HDF in an upright microscope with an attached video camera system for time-lapse analysis. The temperature of the medium was kept at 37°C by a stage heater. A more detailed description of the technique is presented elsewhere (Larsson and Zetterberg, 1986).

[^{3}H]thymidine labelling and autoradiography

In the indicated experiments a small glass coverslip was placed on the bottom of a 25 cm^2 flask. After seeding of the cells the flask was subjected to TLV as described above. Cells growing on the glass coverslip were thereby filmed. When an appropriate cell density was reached the proliferating cells were shifted to new fresh serum-supplemented medium. 24 h later the cells were pulse-labelled (30 min) with [^{3}H]thymidine (1 μCi/ml, 5 Ci/mmol), after which the coverslip was removed and the cells fixed in 95% (v/v) ethanol. The investigated area was then re-identified and photographed. Thereafter autoradiography was carried out, as previously described (Larsson and Zetterberg, 1986). From the video recordings the cell cycle position (measured as time elapsed after last mitosis) for individual cells on the photograph was determined. These age-determined cells were then identified on the autoradiograph. In this manner the percentage of [^{3}H]thymidine-labelled cells (representing cells in S-phase) or unlabelled cells (representing cells in the pre-replicative phase, G_1) at various cell cycle positions could be scored. Thereby, the cells were subdivided into 2-h age-classes (0-1.9 h, 2-3.9 h and so on). The mean value of each age-class was based on measurements of 5-10 cells.

Flow cytometry

Exponentially growing HDF cultivated in 75-cm^2 bottles were washed in EDTA solution (0.2 mM) briefly at room temperature. EDTA was removed and the cells were exposed to a 0.25% trypsin solution at 37°C until they detached from the plastic surface (normally 2-5 min). The cells were suspended in medium containing 10% FCS. After 5 min of centrifugation at 5,000 revs/min, the supernatant was removed. The cells were washed once in 0.2 M tris-buffered saline and fixed by adding 1 ml 95% ethanol, drop by drop, under vigorous stirring. The suspension was kept in a refrigerator (4°C) until further analysis was completed.

The cells were stained in a buffered solution containing 0.14 M NaCl, 0.11 M Tris-HCl (pH 7.0), and 50 mg ethidium bromide per 1,000 ml solution; 50 μl RNase (100,000 i.u./ml in 0.9% NaCl solution) was added to every 5 ml stain solution. The cellular DNA content was then determined by a flow cytophotometer.

Determination of N-linked glycosylation

Total incorporation of [^{3}H]glucosamine into N-linked glycoproteins was determined according to the protocol of Carson and Lennarz (1981). Thereby, the cell cultures in 2 ml dishes were labelled with D-6-[^{3}H]glucosamine (5 μCi/ml, 25.2 Ci/mmol) over a 3-h period. Thereafter the cells were rinsed with phosphate-buffered saline and treated with ice-cold trichloroacetic acid (TCA). The radioactivity in TCA-insoluble material was determined and normalized to protein content.

Determination of protein synthesis and degradation

Overall protein synthesis of cultured cells was determined after pulse-labelling (30 min) with [^{3}H]leucine (10 μCi/ml) by measuring the radioactivity in the TCA-insoluble materal. Corrections were made for variation in protein content.

Studies of overall degradation of short-lived proteins were performed by exposing exponentially growing cells cultured in the presence of serum to a 30-min pulse-labelling with [^{3}H]leucine (20 μCi/ml), after which the cells (after rinsing the cells 4 times in PBS containing 2 mM unlabelled leucine) were shifted to the indicated experimental medium containing 2 mM unlabelled leucine (in order to minimize re-incorporation of released [^{3}H]leucine) for various periods (2-24 h). Therafter the media and cell lysates were harvested and treated with ice-cold 10% TCA. Samples of the TCA-insoluble cell material and the TCA-soluble media and cell material were then taken for determination of radioactivity. The radioactivity in the TCA-soluble material, which measures the release of [^{3}H]leucine during the chase periods, was corrected for variation in radioactivity in the TCA-precipitate and for variation in protein content.

Determination of protein content

Protein content of cell lysates was determined by a dye-binding assay (Bradford, 1976), with a reagent purchased from Bio-Rad. Bovine serum albumin was used as a standard.

Lipid supplementations

Exogenous lipids, whose effects on cell cycle progression were assayed, were re-dissolved prior to experimental use in 99.5% ethanol at appropriate concentrations. In every set of experiments equal volumes of ethanol were added to the controls.

Results

From DNA distribution curves obtained by flow cytometry the fractions of cells in different cell cycle phases of exponentially growing HDF were calculated in accordance with the method of Baisch et al. (1975). By knowing the mean generation time (16.5 h), which was determined by time-lapse video recording (TLV), and considering the exponential growth pattern (Von Foerster, 1959), the mean lengths of the cell cycle phases were calculated: G_1, 7.2 h; S, 6.8 h; G_2+M, 2.5 h. These values are in good agreement with data reported by others (Stein and Atkins, 1986; Moorhead and Defendi, 1963; Defendi and Manson, 1963; Macieira-Coelho et al., 1966).

Fig. 1A,B shows the effects of dolichol and mevalonate on HDF growth-arrested by mevinolin or serum depletion, as measured by determining the cell number by TLV. It is shown that dolichol failed to stimulate proliferation of any of these arrested cells. As expected mevalonate overcame the mevinolin-induced growth inhibition, but had no effects on serum-depleted cells. Other concentrations of dolichol

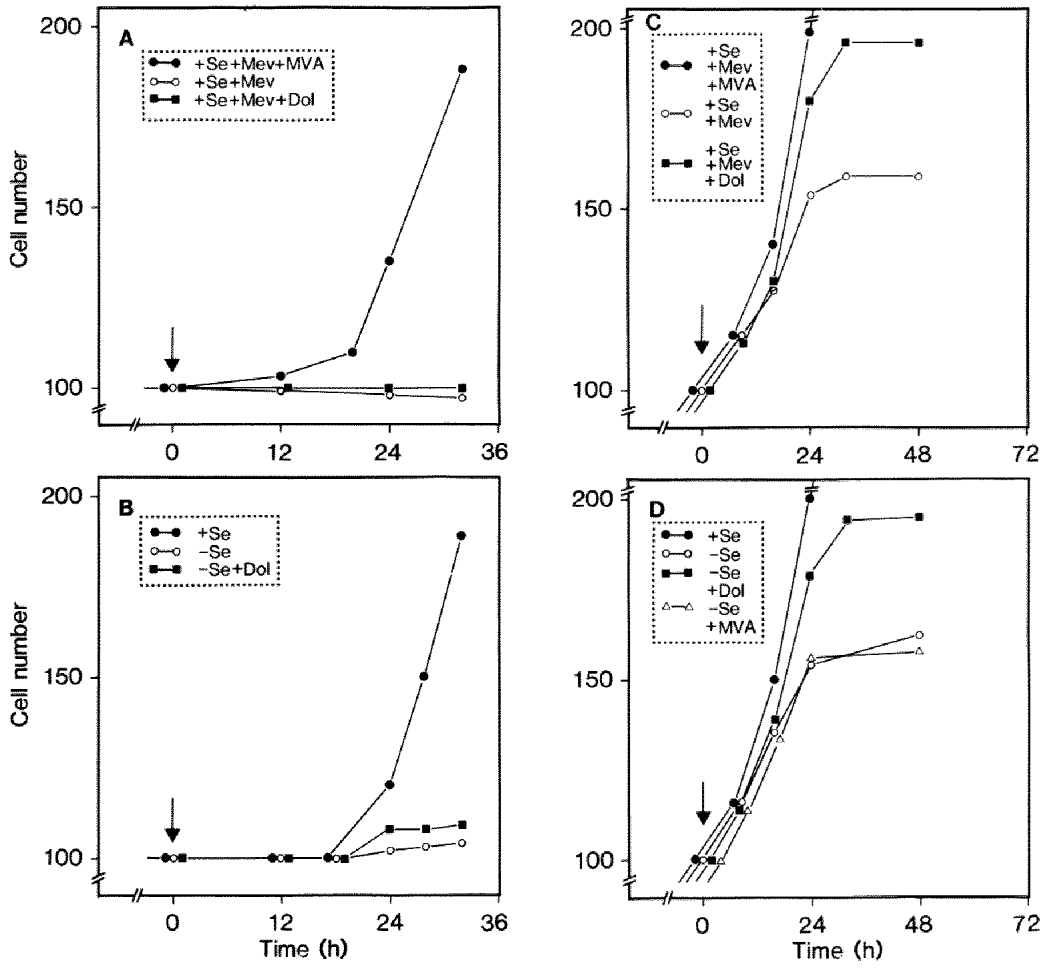

Fig. 1. (A,B) HDF were growth-arrested by treatment with 25 µM mevinolin (A) or serum depletion (B) for 72 h, after which they were shifted to new fresh medium containing the indicated supplements. The cell number in the investigated surface areas was determined by TLV. The cell number at the onset (indicated by an arrow) of stimulation is normalized to 100. (C,D) Exponentially growing HDF, cultured in medium supplemented with 10% FCS, were treated with indicated media and compounds for 48 h. The cell number in the investigated surface areas was determined by TLV. The cell number at the onset (indicated by an arrow) of treatment is normalized to 100.

and mevalonate were also tested but this did not change the result (data not shown). Cholesterol also failed to stimulate proliferation in arrested cells (data not shown).

In Fig. 1C,D it is shown that treatment with mevinolin or serum-free medium efficiently blocked the proliferation of cycling cells, i.e. cells which were exponentially growing before treatment. In fact, the kinetics of these two different types of growth inhibitions were almost identical. As can be seen, the cells were unable to double in cell number. Instead, the cells increased by approximately 60% in number, whereupon they became stationary. This kind of cell cycle kinetics might be explained by a stringent cell cycle-specific control since only cells having passed a 'restriction point' are able to complete their cell cycle (as will be discussed below). Mevalonate prevented the mevinolin-induced arrest, but failed to counteract that caused by serum depletion. Interestingly, dolichol caused a substantial counteraction of both types of blocks and the cells nearly doubled in number. This might imply that dolichol prevents the cell cycle-specific arrest in the first cell cycle, while it fails to retain cells for a second cycle. In order to study this matter in detail we have conducted a set of cell cycle experiments.

Since it is well known that cell cycle regulation occurs mainly in the G_1-phase (Pardee et al., 1981; Zetterberg and Larsson, 1985), it was interesting to characterize the G_1-phase of proliferating HDF in detail. Fig. 2 shows the kinetics of the transit between G_1- and S-phase of exponentially growing HDF as measured by determining the fraction of [^{3}H]thymidine-labelling at various cell cycle positions (see materials and methods). Cell cycle position is defined as time (h) elapsed after completion of mitosis. As can be seen, there are no labelled cells younger than 4 h, after which there is an increasing number of labelled cells until a cell cycle position of 10-11 h is reached. These data imply that the length of the G_1-phase of HDF varies between 4 and

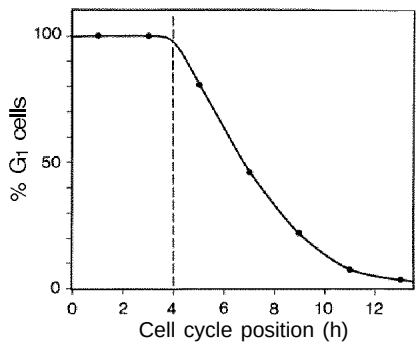

Fig. 2. Percentage of G_1-cells as a function of cell cycle position in exponentially growing HDF (see Materials and methods).

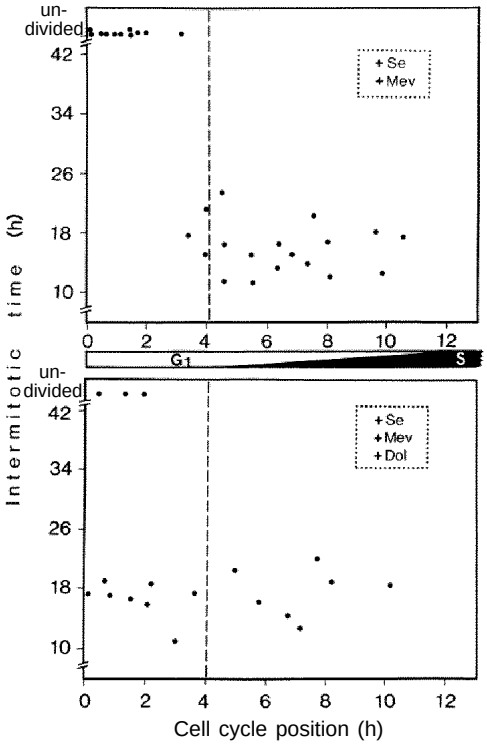

Fig. 3. Relationship between cell cycle position (i.e. time elapsed after mitosis), at the onset of treatment with mevinolin, and intermitotic time for individual cells. Exponentially growing HDF were exposed to new fresh serum-supplemented media containing 25 μM mevinolin (upper) or mevinolin and dolichol (1 μg/ml) (lower). Cell cycle position and intermitotic time was determined by TLV. A schematic cell cycle model showing the transit between G_1 and S is shown between the upper and lower panels.

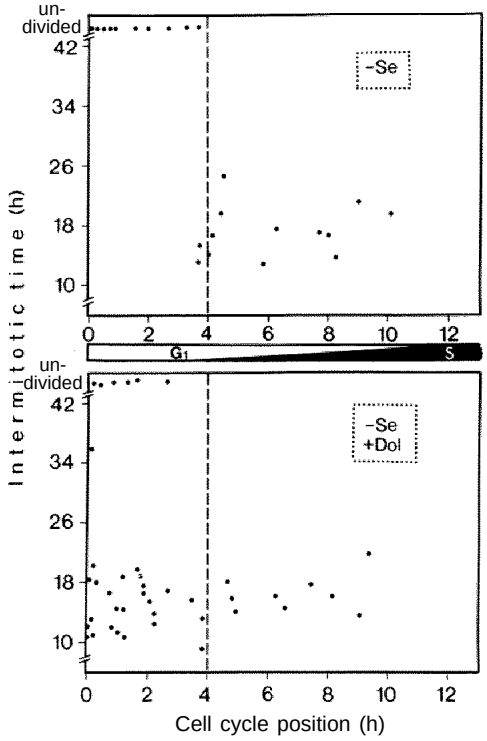

Fig. 4. Relationship between cell cycle position, at onset of serum depletion, and intermitotic time. Exponentially growing HDF were exposed to serum-free medium (upper) or serum-free medium and dolichol (1 μg/ml) (lower).

11 h, which is consistent with the case of murine fibroblasts (Zetterberg and Larsson, 1985).

In Fig. 3A the effects of treatment with mevinolin on cycling HDF are shown. As can be seen, mevinolin prevented cells younger than 3.5-4 h (i.e. cells at cell cycle positions shorter than 3.5-4 h at the time of onset of treatment) from traversing the remainder of the cell cycle and subsequently undergoing cell division. In contrast, cells at later stages of the cell cycle were not affected. These data taken together with the data in Fig. 2 imply that only cells in the first invariable part of G_1 are responsive to growth inhibition. As shown in Fig. 3B, addition of dolichol along with mevinolin caused a substantial counteraction of the mevinolin-induced cell cycle block. A cell cycle-specific block similar to that caused by mevinolin was obtained by treating cycling HDF with serum-free medium (Fig. 4). Dolichol exhibited an effective counteractive effect also in this case.

The data in Figs 3 and 4 fully explain the growth curves shown in Fig. 1C,D. From the rapid inhibitory effects of mevinolin and serum depletion on the cell cycle progression of young G_1 cells (Figs 3 and 4) it follows that only cells in late G_1, S and G_2 can undergo cell division. Since these responsive G_1 cells account for a substantial fraction (more than 30% if considering the logarithmic age-density curve for proliferating cells) of the whole cell population, the increase in cell number would have been limited to 60-70% after growth inhibition (compare with Fig. 1C,D). In contrast, if dolichol was added along with the inhibitory agents most of the postmitotic G_1-cells completed their cell cycle and underwent cell division. Therefore, these dolichol-treated cells could double in cell number (Fig. 1C,D). However, they could not progress through a second cell cycle after completion of the first cycle (Fig. 1C,D).

Fig. 5 summarizes the effects of cholesterol, dolichol and mevalonate on G_1-progression of HDF treated with mevinolin or serum-free medium. Cholesterol had no effect at all on either mevalonate-depleted or serum-depleted cells. As expected, mevalonate prevented cell cycle arrest due to mevinolin but had no effect on cells inhibited by serum-free treatment.

In order to investigate whether there are any changes in overall N-linked glycosylation in HDF exposed to mevinolin or serum depletion, we measured the incorporation of [^{3}H]glucosamine into acid-insoluble proteins. As can be seen in Table 1 mevinolin caused a 38.6% decrease in the [^{3}H]glucosamine incorporation after a 24-h treatment, whereas there was only a 16.7% decrease after a 3-h treatment. Interestingly, serum depletion caused a 25.4% decrease in the incorporation as early as after 3 h, while there was no further decrease during the next 21 h. However, in none of these cases did dolichol exhibit any counteractive effect.

We also investigated whether addition of dolichol had any effect on overall protein synthesis and overall protein

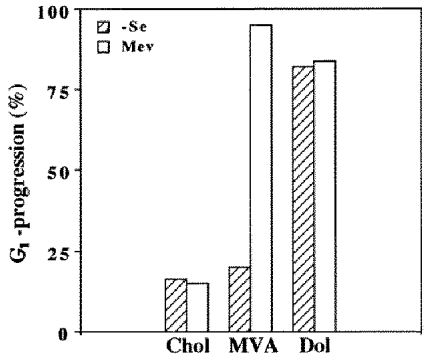

Fig. 5. Percentage of cells undergoing G_1-progression. In separate experiments the effects of cholesterol (10 μg/ml), mevalonate (100 μg/ml) and dolichol (1 μg/ml) on the cell cycle progression of cells treated with mevinolin (25 μM) or serum-free medium were measured (compare with Figs 3 and 4). Thereby the percentages of postmitotic G_1 cells (i.e. cell younger than 4 hours at onset of treatment) capable of undergoing cell division are determined.

Table 1. *N-linked glycosylation in serum- and mevalonate-depleted cells*

Treatment	Incorporation of [^{3}H]GlcN (% of control) 0-3 h	21-24 h
+ Serum	100	100
− Serum	74.6	77.1
− Serum + Dol (1 μg/ml)	62.8	62.1
+ Serum + Mev (25 μM)	83.3	61.4
+ Serum + Mev (25 μM) + Dol (1 μg/ml)	86.8	54.3

Exponentially growing HDF were shifted to new fresh media with indicated supplements for 3 h or 24 h. [^{3}H]glucosamine (5 μCi/ml) was added for 3 h (0-3 and 21-24 h, respectively). Thereafter the cells were harvested for measurement of N-linked glycosylation. The obtained disints/min values were corrected for variation in protein content, and are indicated as percentages of the controls (+ Serum).

degradation. As shown in Table 2, a 4-h treatment with serum-free medium led to a 35% decrease in protein synthesis, measured as incorporation of [^{3}H]leucine into TCA-insoluble cell lysate. In contrast, treatment with mevinolin only exerted marginal inhibitory effects on this processing. Addition of dolichol did not affect protein synthesis to any significant extent (Table 2).

Next we studied the rate of protein degradation in HDF. We were interested in short-lived proteins, since such proteins have been reported to be involved in growth control (Rossow et al., 1979; Pardee et al., 1981). This was done by measuring the release of amino acid following pulse-chase labelling with [^{3}H]leucine. Fig. 6A shows that the rate of overall protein degradation of HDF growing in the presence of serum and in the absence of mevinolin was considerably higher during the first 8 h than after 24 h. Serum depletion increased the rate of protein degradation up to 25% (at 8 h), whereas treatment with mevinolin only resulted in a small increase (7% at most). Interestingly, addition of dolichol was followed by a decrease in protein degradation (Fig. 6B). These effects were most prominent after 4 and 8 h. In fact, these stabilizing effects compensated for the increase in protein degradation that was caused

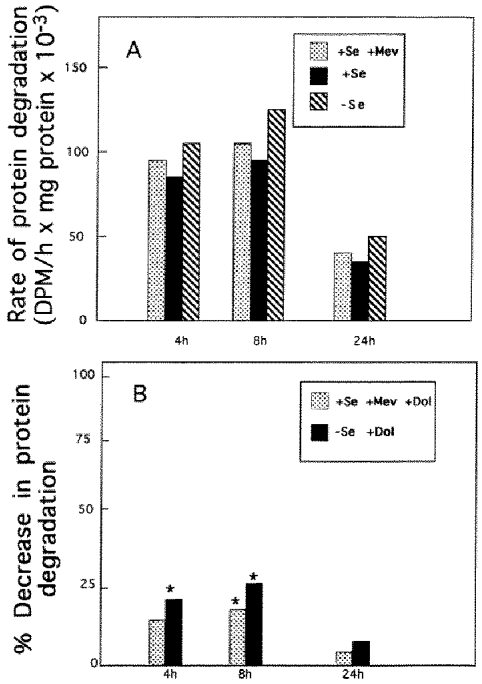

Fig. 6. (A) Rate of protein degradation of HDF following treatment with mevinolin (25 μM) or serum-free medium, as compared to untreated control cells. Exponentially growing HDF were pulse-labelled with [^{3}H]leucine, whereupon they were shifted to the experimental media for various periods. For details see Materials and methods. The rate of protein degradation, measured as release of labelled amino acid/h, after 4, 8 and 24 h was determined. The indicated values represent mean values of duplicate determinations. The standard deviations were less than 10% of the means. (B) Decrease in protein degradation as a result of addition of dolichol to the experimental media. The procedures were exactly identical with those described in Fig. 6A with the exception that separate experimental media (as indicated in the inset legend) also contained dolichol (the final concentration was 1 μg/ml). The effects of dolichol on the rates of protein degradation, as related to cells treated with mevinolin and serum-free medium without addition of dolichol, were determined. The indicated values represent mean values of duplicate determinations. $P<0.05$ is indicated by *.

Table 2. *Rate of protein synthesis*

Treatment	Incorporation of [^{3}H]leucine (% of controls) 4 h	24 h
+ Serum	100	100
− Serum	65	53
− Serum + Dol (1 μg/ml)	59	51
+ Serum + Mev (25 μM)	92	89
+ Serum + Mev (25 μM) + dol (1 μg/ml)	89	91
+ Serum + CHX (0.25 μg/ml)	55	40
+ Serum + CHX (0.25 μg/ml) + dol (1 μg/ml)	52	41
+ Serum + CHX (0.75 μg/ml)	21	15
+ Serum + CHX (0.75 μg/ml) + dol (1 μg/ml)	18	17

Exponentially growing HDF were shifted to new fresh serum-supplemented medium containing indicated compounds for 4 or 24 h whereupon the cells were pulse-labelled (30 min) with [^{3}H]leucine (10 μCi/ml). The obtained disints/min values were corrected for variation in protein content, and are expressed as percentages of the controls (+ Serum). CHX, cycloheximide.

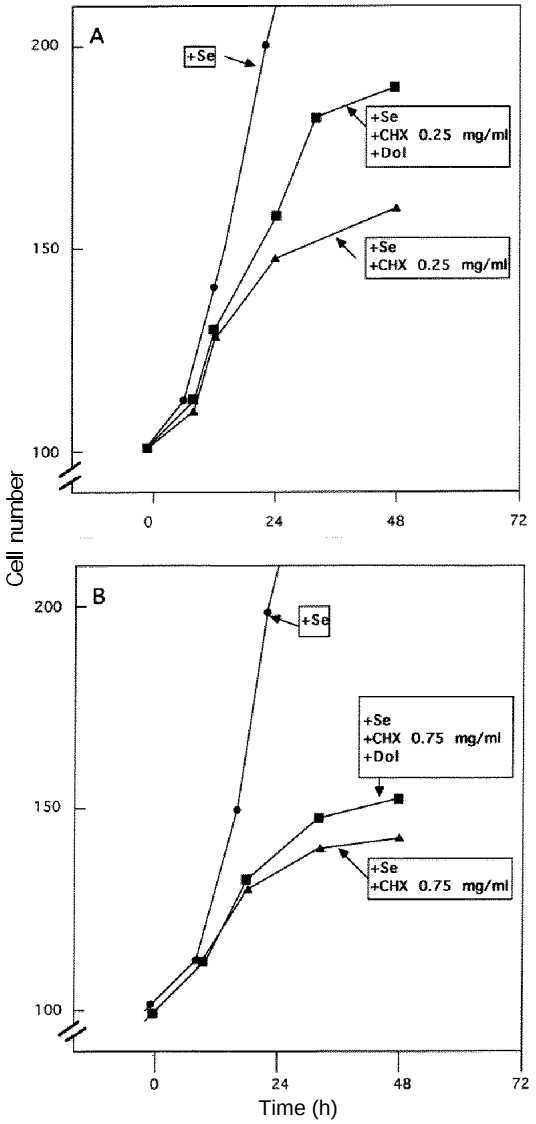

Fig. 7. (A,B) Exponentially growing HDF, cultured in medium supplemented with 10% FCS, were treated as indicated for 48 h. The cell number in the investigated surface areas was determined by TLV. The cell number at the onset (indicated by an arrow) of treatment is normalized to 100. CHX, cycloheximide.

by the treatment with mevinolin or serum-free medium (compare with Fig. 6A). These stabilizing effects following addition of dolichol may explain why dolichol delays the cell cycle exit for one cell cycle. In Fig. 7A it is shown that a 45% decrease in de novo protein synthesis, following addition of cycloheximide (0.25 μg/ml) (Table 2), prevented exponentially growing HDF from doubling in cell number. In a similar way as after treatment with mevinolin or serum-free medium (compare with Fig. 1), the cells increased by only 50-60% whereupon they became stationary. However, upon addition of dolichol along with cycloheximide (0.25 μg/ml) the cells almost doubled in cell number (Fig. 7A). In contrast, when the cells were exposed to an 80-85% inhibition in overall protein synthesis, induced by 0.75 μg cycloheximide/ml (Table 2), dolichol failed to overcome the growth inhibition (Fig. 7B). As shown in Table 2 dolichol did not counteract the inhibitory effects of cycloheximide on de novo protein synthesis.

Discussion

The three major findings of this study are: (1) mevalonate depletion blocks growth of cycling HDF in a cell cycle-specific manner which from a kinetic point of view is identical to the block caused by serum depletion; (2) dolichol can counteract the cell cycle-specific block caused by mevinolin and serum depletion. However, dolichol is only effective for one cell cycle, and fails to stimulate arrested cells; (3) addition of dolichol to the cells results in a stabilization of short-lived proteins. This stabilization may explain why the cell cycle arrest is delayed for one cell cycle.

The kinetic resemblance between the aforementioned cell cycle blocks suggests that common mechanisms may be involved in growth control of HDF. Since mevinolin is a potent inhibitor of HMG CoA reductase, which catalyzes the conversion of HMG CoA to mevalonate, it seems reasonable to assume that some metabolite or product of mevalonate is involved in the mechanisms controlling cell cycle traverse. So far most studies of isoprenoid growth regulation have been done using growth-activated cells. In quiescent 3T3-cells, smooth muscle cells and human fibroblasts addition of PDGF increased the activity of HMG CoA reductase multifold (Habenicht et al., 1980; Fairbanks et al., 1984). If this increase in enzymatic activity was inhibited by compactin or mevinolin the cells failed to initiate DNA synthesis. However, addition of mevalonate, but not cholesterol, restored entry into S-phase (Habenicht et al., 1980; Fairbanks et al., 1984). These studies strongly suggest that some mevalonate-derived metabolite or product is involved in the processes triggering initiation of DNA synthesis in growth-activated cells. Similar results have been obtained in other types of growth arrested cells activated by growth factors or serum (Quesney-Huneeus et al., 1980; Perkins et al., 1982; Yachnin, 1982; Langan and Volpe, 1987).

In contrast, comparatively few studies have focused on the role of mevalonate in growth regulation of *cycling cells*. In any event, as shown in this study and in other studies (Sinensky and Logel, 1985; Larsson and Zetterberg, 1986; Doyle and Kandutsch, 1988; Keyomarsi et al., 1991), it is evident that mevalonate depletion causes a G_1-arrest in cycling mammalian cells. In the study of Keyomarsi et al. (1991) various types of cells, including human fibroblasts (WI38), were blocked in the G_1-phase following treatment with lovastatin (mevinolin). Whereas we focused particular interest on the kinetics of how cells exit from G_1, they primarily studied how the cells resume the cell cycle after the exit (Keyomarsi et al., 1991). However, their kinetic data are in agreement with our results, and support our finding that the cells are arrested in the early part of G_1 following treatment with mevinolin. Also in concurrence with our results they found that lipids other than mevalonate fail to stimulate the cells blocked by mevinolin (Keyomarsi et al., 1991).

The second major finding of this study is the ability of

dolichol to prevent cycling HDF from G_1-arrest during one cell cycle. Following addition of dolichol approximately 80% of the cells at cell ages of 0-4 h were able to progress through the cell cycle despite depletion of serum or mevalonate.

The most well-known biological role of dolichol is its participation in N-linked glycosylation where in its phosphorylated form it acts as a carrier of oligosaccharides in the assembly of glycoproteins (Struck and Lennarz, 1980). Since protein glycosylation is of critical importance for several cellular processes, e.g. function of growth factor receptors (Carpenter and Cohen, 1977), it is conceivable that this process may have a regulatory influence on cell growth. In Swiss and Balb 3T3 cells a connection between N-linked glycosylation and proliferation has been demonstrated (Larsson, 1985; Harrington and Pledger, 1987; Kabakoff et al., 1990). Furthermore, in a study by Adair and Cafmeyer (1987) of mouse L-1210 cells synchronized by centrifugal elutriation it was shown that the levels of dolichyl phosphate were increased linearly through the cell cycle, reaching a value in late S-phase twice that of early G_1. On the basis of those interesting data, we investigated whether dolichol exerts its effect on G_1-progression through increasing the level of glycoprotein synthesis. However, as shown in this study, addition of dolichol to HDF had no significant effect on the uptake of glucosamine. Therefore, apart from the possibility of a minor cell-cycle-specific dolichol-induced increase in N-linked glycosylation, which might be difficult to detect in an asynchronous population, the present data suggest that the added dolichol does not stimulate glycoprotein synthesis. This is also compatible with findings that exogenous dolichol is only to a very limited extent phosphorylated (Rip et al., 1985), which is a prerequisite for participation in N-linked glycosylation (Struck and Lennarz, 1980), after uptake in cultured cells. Therefore, it seems likely that the added dolichol interferes with G_1-progression of HDF by other mechanisms than by increasing N-linked glycosylation. Such mechanisms might concern changes in cell membranes. It has in fact been shown that dolichol decreases membrane stability, increases phospolipid fatty acid fluidity as well as it increases membrane permeability (Valtersson et al., 1985; deRopp and Troy, 1985). In this respect long-chain dolichol was most effective (Valtersson et al., 1985; deRopp and Troy, 1985). A more recent study by Knudsen and Troy (1989) suggested that dolichol derivatives induce a non-bilayer organization of phospholipid molecules in phosphatidylethanolamine/phosphatidylcholine vesicles. The physiological role of such changes is still unknown. One possibility is that the dolichol-induced changes in membrane lipids may interfere with the function of membrane proteins. Such interactions between membrane lipids and proteins would in turn affect a variety of cellular functions. In terms of cell growth, changes in activity of membrane-associated proteases would be of regulatory importance. An interrelationship between protein turnover and cell growth in several cell types has been reported (Shilo et al., 1979; Lockwood et al., 1982; Cholchini et al., 1989), and it is well documented that the G_1-progression of normal cells is dependent on the biosynthesis of labile proteins (Rossow et al., 1979; Pardee et al., 1981). In fact the third major finding of the present study was that addition of dolichol to cells depleted of serum or mevalonate caused a significant decrease in the overall degradation of short-lived proteins. This effect might be a result of an interaction between membrane lipids and membrane-bound proteases. The stabilization of labile proteins could explain the delay in the G_1-arrest of HDF which is brought about by dolichol. This notion is supported by the finding that the addition of dolichol partially counteracted the cell-cycle-inhibitory effect of cycloheximide. Hence the drop in labile cell-cycle-regulatory proteins following treatment with cycloheximide could be compensated for by an increased protein stabilization. The idea that dolichol delays G_1-arrest through its protein stabilizing effect would also explain why dolichol fails to stimulate quiscent cells to growth. In order to stimulate quiescent cells a decreased protein degradation is not sufficient, since the expression of growth regulatory genes is also required (Kelly et al., 1983).

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