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Changes in Protein Isoprenylation during the Growth of Suspension-Cultured Tobacco Cells[1]

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Isoprenylation facilitates the association of proteins with intracellular membranes and/or other proteins. In mammalian and yeast cells, isoprenylated proteins are involved in signal transduction, cell division, organization of the cytoskeleton, and vesicular transport. Recently, protein isoprenylation has been demonstrated in higher plants, but little is currently known about the functions of isoprenylated plant proteins. We report that inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (lovastatin) or prenyl:protein transferases (perillyl alcohol) severely impair the growth of cultured tobacco (*Nicotiana tabacum*) cells but only when added within the first 2 d following transfer to fresh medium, before any increase in culture volume is detectable. This "window" of sensitivity to inhibitors of protein isoprenylation correlates temporally with an increase in [^{14}C]mevalonate incorporation into tobacco cell proteins in vitro. We have also observed a marked increase in farnesyl:protein transferase activity at this early time in the growth of tobacco cultures. In contrast, type I geranylgeranyl:protein transferase activity does not change significantly during culture growth. Although these events coincide with the replication of DNA, 1 μM lovastatin-treated cells are capable of DNA synthesis, suggesting that lovastatin-induced cell growth arrest is not due to inhibition of DNA replication. Together, these data support the hypothesis that protein isoprenylation is necessary for the early stages of growth of tobacco cultures.

The mevalonate pathway leads to the synthesis of various plant isoprenoids, including sterols, pigments, electron carriers, and growth substances (Brown and Goldstein, 1980; Bach, 1987; Goldstein and Brown, 1990). Mevalonate-derived compounds have also been shown to be incorporated into a variety of plant cell proteins (Randall et al., 1993; Swiezewska et al., 1993). In mammalian and yeast cells, isoprenylated proteins contain an isoprenoid (a 15-carbon farnesyl or a 20-carbon geranylgeranyl moiety) in thioether linkage to a Cys residue at or near the carboxy terminus of the protein (Maltese and Erdman, 1989; Farnsworth et al., 1990; Maltese, 1990; Reiss et al., 1990; Rilling et al., 1990). Many isoprenylated proteins are further modified by proteolysis and carboxymethylation of the isoprenylated Cys residue (Clarke et al., 1988; Gutierrez et al., 1989; Maltese et al., 1990). The isoprenoid moiety (often in concert with other hydrophobic adducts) is believed to facilitate interactions between the isoprenylated protein and intracellular membranes (Hancock et al., 1989; Glomset et al., 1990; Jackson et al., 1990) and/or other proteins (Kuroda et al., 1993; Beranger et al., 1994).

Several proteins having an isoprenoid adduct have been identified in mammalian and yeast cells. These include the low-molecular-weight Ras-like GTP-binding proteins, the γ subunits of heterotrimeric G proteins, and nuclear lamins (Wolda and Glomset, 1988; Casey et al., 1989; Farnsworth et al., 1989; Hancock et al., 1989; Vorburger et al., 1989; Didsbury et al., 1990; Fukada et al., 1990; Kawata et al., 1990; Maltese and Robishaw, 1990; Maltese et al., 1990; Mumby et al., 1990; Yamane et al., 1990). These proteins are involved in signal transduction, regulation of cell division, intracellular vesicle transport, and organization of the cytoskeleton. For example, isoprenylation is required for the membrane association and function of Ras (Jackson et al., 1990), a signal-transducing protein that regulates cell division in response to growth factors. Consistent with a role for protein isoprenylation in signal transduction, isoprenylation of heterotrimeric G protein γ subunits is necessary for cellular responses to various hormones and extracellular signals (Fukada et al., 1990). At least one isoprenylated protein (Rac) has been shown to regulate actin polymerization (Didsbury et al., 1990; Ridley et al., 1992), and other isoprenylated GTP-binding proteins (i.e. Rabs) have been shown to participate in intracellular vesicle transport (Goud et al., 1988; Balch, 1990; Khosravi-Far et al., 1991; Kinsella and Maltese, 1992; Rothman and Orci, 1992; Seabra et al., 1992). Nuclear lamins, which form a proteinaceous matrix on the inner surface of the nuclear envelope, are also isoprenylated (Wolda and Glomset, 1988; Farnsworth et al., 1989; Vorburger et al., 1989; Lutz et al., 1992). These nuclear intermediate filament proteins mediate interactions between chromatin and the nuclear envelope and regulate envelope disassembly and reassembly during mitosis.

Cultured tobacco (*Nicotiana tabacum*) cells have been shown to incorporate derivatives of mevalonate into proteins with masses similar to those of mammalian small Ras-like GTP-binding proteins and nuclear lamins (Randall et al., 1993). Consistent with the hypothesis that protein isoprenylation is necessary in plants for signal transduction, cell growth regulation, cytoskeletal organization, and membrane biogenesis, evidence has accumulated in recent years for the existence of plant small Ras-like GTP-binding

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proteins, heterotrimeric G proteins, and nuclear lamins (Fairley-Grenot and Assmann, 1991; Frederick et al., 1992; Li and Roux, 1992; McNulty and Saunders, 1992; Terryn et al., 1993; Tong et al., 1993; Bowler et al., 1994; Verma et al., 1994). In addition, a number of novel plant cDNAs have been identified that encode substrates for the tobacco farnesyl:protein transferase (Biermann et al., 1994). However, the only plant protein that has been shown to be isoprenylated in vivo is an *Atriplex nummularia* protein (ANJ1) homologous to the bacterial molecular chaperone DnaJ (Zhu et al., 1993).

To date, three prenyl:protein transferases have been identified in mammalian and yeast cells, one farnesyl:protein transferase and two geranylgeranyl:protein transferases (Reiss et al., 1990; Kohl et al., 1991; Moores et al., 1991; Seabra et al., 1992). Tobacco cell extracts have also been shown to contain farnesyl:protein transferase and geranylgeranyl:protein transferase type I activities (Randall et al., 1993; Biermann et al., 1994). These enzymes prenylate proteins containing a "CaaX" sequence at the carboxy terminus, where C is Cys, a is usually an aliphatic amino acid, and X is one of several possible amino acids. The carboxy-terminal amino acid of the CaaX motif determines whether the protein is a substrate for the farnesyl:protein transferase or the geranylgeranyl:protein transferase type I (CaaX sequences terminating in Ser, Met, Ala, Cys, or Gln are substrates for the farnesyl:protein transferase, whereas CaaX sequences terminating in Leu are substrates for the geranylgeranyl:protein transferase type I) (Reiss et al., 1990; Moores et al., 1991; Randall et al., 1993). In contrast, the geranylgeranyl:protein transferase type II from mammalian and yeast systems prenylates XXCC, CCXX, or CXC carboxy termini (Moores et al., 1991; Seabra et al., 1992). A similar activity in plants is likely but has not been characterized.

Since many isoprenylated proteins have been implicated in regulation of cell growth and division, we wished to characterize the relationship between protein isoprenylation and the growth of suspension-cultured tobacco cells. Here we show a temporal correlation between sensitivity of tobacco cell growth to inhibitors of protein isoprenylation, a complex pattern of detectable mevalonate labeling of tobacco proteins, and a peak of farnesyl:protein transferase activity, suggesting a role for isoprenylated proteins in the control of growth of tobacco cultures.

MATERIALS AND METHODS

Tobacco Tissue Culture

All cultures were suspensions of the tobacco (*Nicotiana tabacum* cv Bright Yellow-2) cell line BY-2 and were grown in Murashige-Skoog liquid medium (Murashige and Skoog, 1962) containing 0.9 μM (0.2 mg/L) 2,4-D at 26 ± 1°C in continuous, fluorescent light. Cultures were started by adding 3 mL of stationary phase cells to 30 mL of fresh medium or, in some experiments, by adding 1.5 mL of stationary phase cells to 15 mL of fresh medium. Growth of tobacco cultures was monitored by measuring cell volumes after 10 min of settling and by microscopic examination of the cells (to monitor cell size and cell viability).

Protein Labeling with [^{14}C]Mevalonic Acid

Tobacco cultures were incubated for 16 h in Murashige-Skoog liquid medium containing 0.9 μM 2,4-D and 10 μM lovastatin, after which 35 μL of settled cells were labeled in the same medium (70 μL total volume) for 4 h with 7 μCi/mL (*R,S*)-[2-^{14}C]mevalonolactone (50 mCi/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO). Cells were then homogenized in 1 volume of 2× SDS-PAGE sample buffer (Laemmli, 1970). Prior to electrophoresis on 14% SDS-polyacrylamide gels (Laemmli, 1970), samples were heated at 90°C in sample buffer for 4 min and particulates were removed by microcentrifugation at 10,000*g* for 2 min. Following electrophoresis, gels were soaked in Amplify fluorographic reagent (Amersham) for 30 min at room temperature, dried, and used to expose Kodak XAR-5 film for 2 to 8 weeks at −80°C.

Prenyl:Protein Transferase Assays

Prenyl:protein transferase reaction mixtures containing 5.0 μM Ras-CAIM, Ras-CAIL, or Ras-SVLS, 50 to 100 μg of tobacco protein, 20 mM $MgCl_2$, 5 mM DTT, 50 mM Hepes (pH 7.5), and 20 μCi/mL of either [^{3}H]farnesyl PPi or [^{3}H]geranylgeranyl PPi (15 Ci/mmol, American Radiolabeled Chemicals) in a total volume of 125 μL were incubated at 30°C for 40 min (Randall et al., 1993). The rate of incorporation was linearly related to the amount of tobacco protein in the reaction mixture (Randall et al., 1993). Two 50-μL portions of the reaction were terminated with 1 M HCl in ethanol. The precipitates were then collected and washed with ethanol on Whatman GF/A glass fiber filters and counted by liquid scintillation.

Tobacco cell extracts were prepared by homogenization in a 4°C mortar with 1 mL of homogenization buffer (500 mM mannitol, 6 mM EGTA, 50 mM Hepes, pH 7.4, 5 mM DTT, 0.1 mg/mL aprotinin, 0.01 mg/mL leupeptin, 1 mM PMSF) per gram of tissue. Extracts were then centrifuged at 10,000*g* for 10 min at 4°C. Protein concentrations were measured by the method of Bradford (1976). Following protein determination, BSA was added to a final concentration of 1 mg/mL to deter proteolysis of the plant proteins.

The Ras proteins used for prenyl:protein transferase assays were encoded by plasmids containing a 3′ truncated yeast *ras1* gene under the control of the lac promoter (Moores et al., 1991). The plasmids (obtained with permission from Merck Research Laboratories, West Point, PA) were modified by site-directed mutagenesis such that different constructs encoded 26-kD Ras proteins with different carboxy-terminal tetrapeptide sequences (Ras-CAIM, Ras-CAIL, or Ras-SVLS). Ras-CAIM was used as a farnesyl:protein transferase substrate, Ras-CAIL was used as a geranylgeranyl:protein transferase type I substrate, and Ras-SVLS was used as a negative control (it lacks a carboxy terminal Cys residue). These proteins were expressed and purified as described previously (Randall et al., 1993; Biermann et al., 1994).

[^{3}H]Thymidine Labeling

Four identical tobacco BY-2 cultures were started at 6-h intervals from a single stationary phase culture. DNA synthesis was then measured at various times by incubating 100 μL of settled cells in the presence of 5 μCi [*methyl*-^{3}H]thymidine (85 Ci/mmol, Amersham) for 30 min at 24°C in conditioned culture medium (200 μL total volume). The cells were frozen at −80°C after removal of the culture medium and stored until all time points were taken. To measure incorporation of [^{3}H]thymidine into DNA, the samples were thawed in the presence of 100 μL of 25% TCA and homogenized with a pestle designed to fit a 1.5-mL microfuge tube. After a 10-min incubation on ice, the precipitates were sedimented by microcentrifugation at 10,000*g* for 5 min and the supernatants were discarded. The pellets were then resuspended in 100 μL of 12.5% TCA at 4°C and resedimented. The pellets were again resuspended in 100 μL of 12.5% TCA at 4°C and a 10-μL portion of each suspension was collected on GF/A glass fiber filters, washed with 5 mL of acetone, dried, and counted by liquid scintillation. Another 10-μL portion of each suspension was used for protein determination (Bradford, 1976).

RESULTS

Effect of Isoprenoid Pathway Inhibitors on the Growth of Tobacco Cultures

Lovastatin is a specific inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (Alberts et al., 1980; Brown and Goldstein, 1980; Bach, 1987; Goldstein and Brown, 1990; Maltese, 1990), which catalyzes the first committed step in the biosynthesis of all isoprenoids (Brown and Goldstein, 1980; Goldstein and Brown, 1990). To determine the sensitivity of tobacco cells at different stages of growth to lovastatin, tobacco suspension cultures were treated with 1 μM lovastatin at various times following transfer to fresh medium. Culture growth was strongly inhibited when 1 μM lovastatin was added 1 or 2 d after transfer but not when it was added 3 or 5 d after transfer (Figs. 1A and 2). Thus, a window of sensitivity to lovastatin exists in this cell line during the lag phase of growth. Furthermore, this inhibition can be relieved by exogenous mevalonic acid (Crowell and Salaz, 1992; Randall et al., 1993), confirming that growth inhibition is caused by a defect in isoprenoid synthesis. These results indicate that products of the mevalonate pathway are required prior to observable increases in settled cell volume.

Since the mevalonate pathway leads to a myriad of products, many of which might be required for cell growth, we tested the hypothesis that inhibition of protein isoprenylation is responsible for lovastatin-induced cell growth arrest. Accordingly, we tested the ability of perillyl alcohol, a monoterpene inhibitor of protein isoprenylation (Crowell et al., 1994; Gelb et al., 1995), to block tobacco cell culture growth. Perillyl alcohol at 1 mM caused growth inhibition (Figs. 1B and 2) and, like lovastatin, was effective only when added prior to the 3rd d after transfer to fresh medium. On the other hand, 1 mM limonene (Figs. 1C and 2) had no effect on cell growth (this related monoterpene

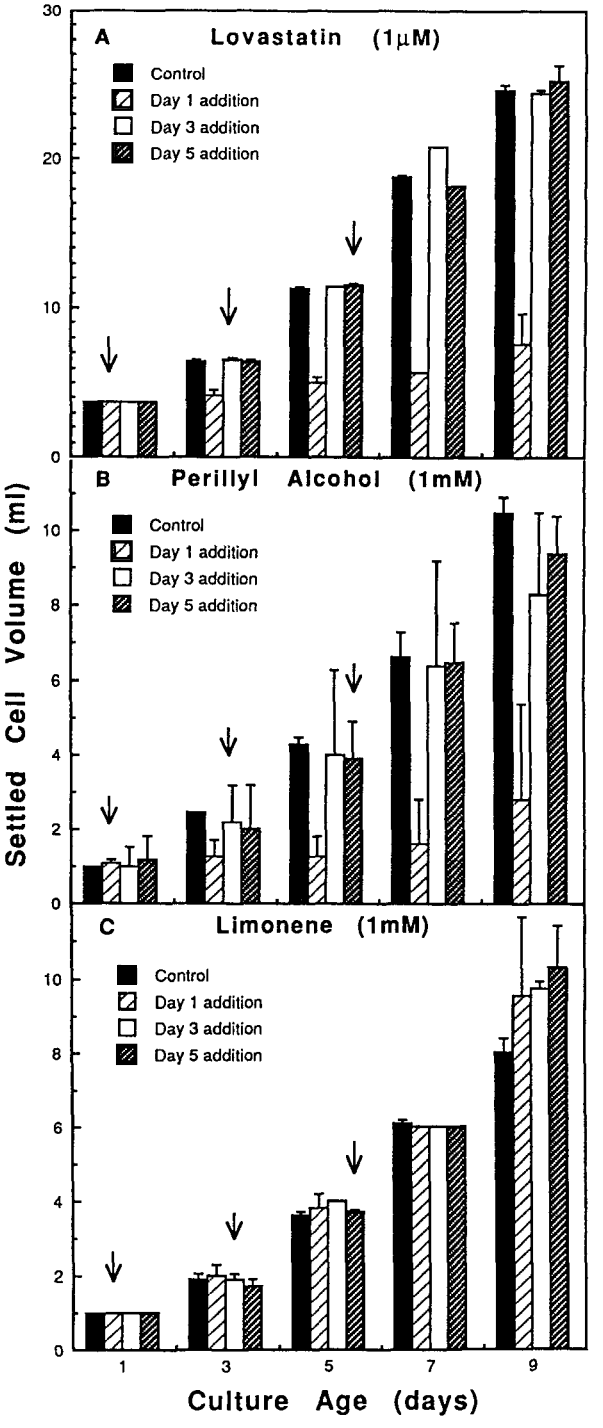

Figure 1. Lovastatin and perillyl alcohol inhibit growth of tobacco BY-2 cells when added within the first 2 d of culture. Compounds were added at various times after transfer of stationary phase cells to fresh medium and culture volumes were monitored as a function of time. A, Lovastatin (1 μM) was added to 30-mL cultures; B, perillyl alcohol (1 mM) was added to 15-mL cultures; C, limonene (1 mM) was added to 15-mL cultures. Times of addition of these compounds are indicated by arrows. Error bars represent SD.

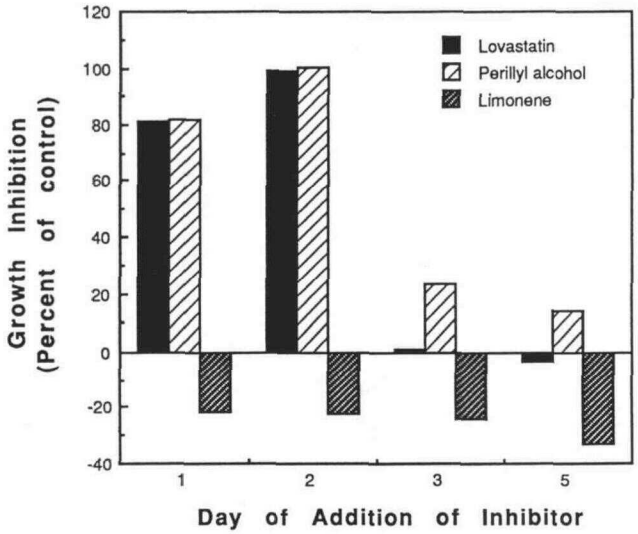

Figure 2. Percent inhibition of growth of cultured tobacco cells by lovastatin and perillyl alcohol as a function of time of addition. Growth inhibition was calculated according to the formula:

$$\text{Percent growth inhibition} = \left[1 - \frac{(Vt_f - Vt_o)}{(Vu_f - Vu_o)}\right] \times 100$$

where Vt_f is the final volume of the treated culture, Vt_o is the initial volume of the treated culture, Vu_f is the final volume of the untreated culture, and Vu_o is the initial volume of the untreated culture. These data are from one representative experiment not shown in Figure 1.

has been shown to be ineffective at inhibiting protein isoprenylation at 1 mM, indicating that growth inhibition by perillyl alcohol is probably not due to nonspecific effects of monoterpenes [Crowell et al., 1994; Gelb et al., 1995]). Consistent with these growth effects being caused by inhibition of protein isoprenylation, 1 mM perillyl alcohol inhibited the incorporation of [^{14}C]mevalonic acid derivatives into tobacco cell proteins, whereas 1 mM limonene did not (Fig. 3). These results suggest that protein isoprenylation may be required for initiation of growth of stationary phase tobacco cells.

Incorporation of [^{14}C]Mevalonic Acid Derivatives into Protein during the Growth of Tobacco Cultures

The results described above suggest that protein isoprenylation may be essential to initiate growth of tobacco cells in culture. To test for quantitative and qualitative changes in protein isoprenylation during the growth of tobacco cultures, cells were analyzed for the incorporation of [^{14}C]mevalonic acid derivatives into protein at various times after transfer to fresh medium. A dramatic difference in protein labeling was observed between cells analyzed within the first 2 d after transfer and those analyzed at later times (Fig. 4). Cells labeled 1 to 2 d after transfer incorporated more mevalonate-derived material into protein than did cells labeled 3 to 8 d after transfer. More importantly, the pattern of labeled proteins was more complex in cells analyzed 1 to 2 d after transfer. These data suggest that an increase in the abundance and/or isoprenylation of various proteins occurs within the first 2 d after transfer of stationary phase tobacco cells to fresh medium. These changes correlate temporally with the window of sensitivity of tobacco cultures to lovastatin and perillyl alcohol.

Prenyl:Protein Transferase Activities during the Growth of Tobacco Cultures

To determine whether the increased mevalonate labeling of tobacco proteins might be accompanied by increased prenyl:protein transferase activities, protein extracts prepared from tobacco cultures at various times after transfer were analyzed for farnesyl:protein transferase and geranylgeranyl:protein transferase type I activities. Farnesyl:protein transferase assays contained Ras-CAIM and [^{3}H]farnesyl PPi, whereas geranylgeranyl:protein transferase type I assays contained Ras-CAIL and [^{3}H]geranylgeranyl PPi. Farnesyl:protein transferase activity was highest in extracts prepared from cells harvested 1 to 2 d after transfer (Fig. 5), whereas geranylgeranyl:protein transferase type I activity was much lower and showed no significant changes during culture growth. The increase in farnesyl:protein transferase activity occurred before any appreciable increase in settled cell volume. The increase in farnesyl:protein transferase activity thus correlated temporally with the increased mevalonate labeling of tobacco proteins (Fig. 4) and with the sensitivity of tobacco cells to lovastatin and perillyl alcohol (Figs. 1 and 2).

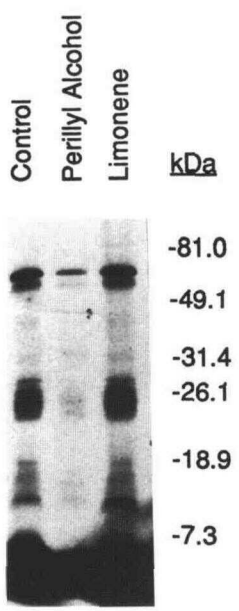

Figure 3. Inhibition of protein isoprenylation by perillyl alcohol in tobacco cultures. Tobacco cultures were incubated in the presence of 10 μM lovastatin for 16 h, after which 35 μL of settled BY-2 cells were labeled with (*R,S*)-[2-^{14}C]mevalonolactone in the presence or absence of 1 mM perillyl alcohol or limonene as described in "Materials and Methods." Twenty micrograms of total cellular protein were loaded in each lane. Molecular mass markers are indicated on the right.

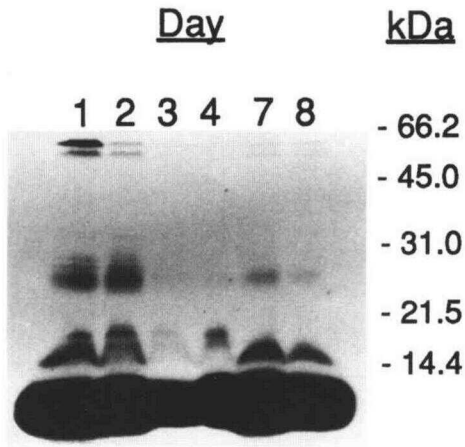

Figure 4. Time course of [^{14}C]mevalonate labeling of proteins during the growth of tobacco cultures. At the indicated times after transfer of stationary phase cells to fresh medium, tobacco cultures were incubated in the presence of 10 μM lovastatin for 16 h, after which 35 μL of settled BY-2 cells were labeled with (*R,S*)-[2-^{14}C]mevalonolactone as described in "Materials and Methods." Twenty micrograms of total cellular protein were loaded in each lane. Molecular mass markers are indicated on the right.

[^{3}H]Thymidine Incorporation during the Growth of Tobacco Cultures

The results described above suggest that protein isoprenylation may be required for the initiation of growth of tobacco cultures. Critical events in the growth of these cells must occur within 3 d after transfer of stationary phase cultures to fresh medium, prior to measurable increases in settled cell volume. Evidence in the literature suggests that tobacco cultures undergo multiple cell divisions prior to increases in cell or culture volume (Iraki et al., 1989; Suzuki et al., 1992). Since DNA replication must precede cell division, we tested the hypothesis that the window of sensitivity of tobacco cells to lovastatin and perillyl alcohol, the increased mevalonate labeling of tobacco proteins in culture, and the peak of farnesyl:protein transferase activity are temporally related to DNA replication. As shown in Figure 6, tobacco cells initiated DNA replication 1 d after transfer of stationary phase cells to fresh medium, well before any detectable increase in settled cell volume. This result is supported by a recent report that tobacco BY-2 cells begin DNA replication within 24 h after transfer of stationary phase cells to fresh medium and divide immediately thereafter (Suzuki et al., 1992). Furthermore, as shown in Figure 7, tobacco cell growth is blocked by aphidicolin, an inhibitor of eukaryotic DNA synthesis, but only when added within the first 2 d after transfer. This result strongly suggests that the efficient incorporation of thymidine into DNA early in the culture of tobacco cells is due to active DNA synthesis. Thus, the changes in protein isoprenylation described above correlate temporally with DNA replication in tobacco BY-2 cultures. It is interesting that the results shown in Figure 6 suggest synchronous DNA replication in tobacco cells following transfer to fresh medium.

The temporal correlation between sensitivity of tobacco cell growth to lovastatin and perillyl alcohol, increased mevalonate labeling of tobacco proteins, increased farnesyl:protein transferase activity, and DNA replication initially suggested that prenylated proteins might be required for DNA replication in cultured tobacco cells. This hypothesis has received experimental support in other systems (Huneeus et al., 1980; Siperstein, 1984; Langan and Volpe, 1987; Sepp-Lorenzino et al., 1991; Sinensky et al., 1994). However, as shown in Figure 6, addition of 1 μM lovastatin to cultured BY-2 cells on the day of transfer did not dramatically affect the incorporation of [^{3}H]thymidine into

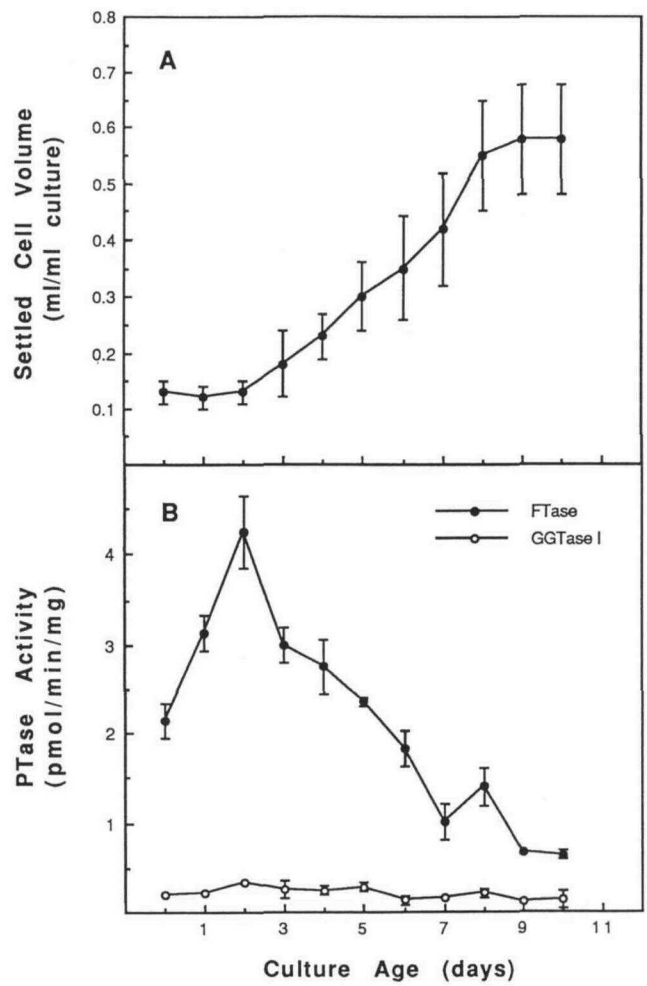

Figure 5. Time course of prenyl:protein transferase (PTase) activities during the growth of tobacco cultures. A, Increase in BY-2 culture volume as a function of time. B, At the indicated times after transfer of stationary phase cells to fresh medium, extracts were prepared from BY-2 cultures as described in "Materials and Methods." These extracts were then analyzed for farnesyl:protein transferase activity (FTase) or geranylgeranyl:protein transferase type I activity (GGTase I). Prenyl:protein transferase activities are given in picomoles of product formed per minute per milligram of tobacco protein. The d 0 extract was prepared 1 h after transfer to fresh medium. That farnesyl:protein transferase activity is higher at d 0 than at d 10 indicates rapid induction of farnesyl:protein transferase activity following transfer. Error bars represent SD.

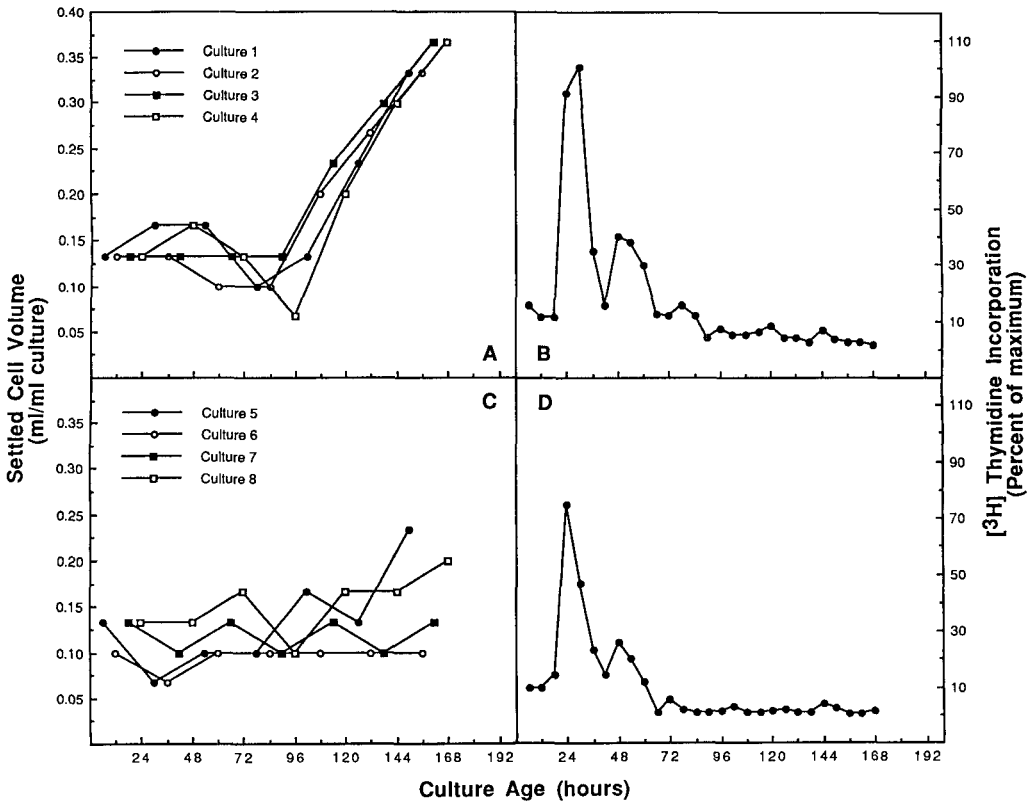

Figure 6. Effect of lovastatin on time course of incorporation of [^{3}H]thymidine into DNA. A, Time-dependent growth of four identical, untreated tobacco cultures. B, At the indicated times, 100 μL of cells shown in A were analyzed for incorporation of [*methyl*-^{3}H]thymidine into TCA-precipitable material. All values are normalized to total protein and expressed as percent of maximum. C, Time-dependent growth of four identical, lovastatin-treated tobacco cultures. D, At the indicated times, 100 μL of cells shown in C were analyzed for incorporation of [*methyl*-^{3}H]thymidine into TCA-precipitable material. All values are normalized to total protein and expressed as percent of maximum for the untreated cultures.

DNA (only a 20% decrease was observed), even though growth was strongly inhibited. These data argue that inhibition of growth of cultured tobacco cells in the presence of 1 μM lovastatin is probably not due to an inhibition of DNA synthesis.

DISCUSSION

The data shown herein reveal a temporal correlation between sensitivity of tobacco cell growth to inhibitors of protein isoprenylation, increased mevalonate labeling of tobacco cell proteins, high farnesyl:protein transferase activity, and DNA replication. These events all occur within the first 3 d after transfer of stationary phase tobacco cells to fresh medium. This correlation suggests that one or more isoprenylated proteins may play a crucial role in the initiation of growth of tobacco cultures. Consistent with this hypothesis, several [^{14}C]mevalonate-labeled proteins are detectable predominantly in cells analyzed 1 to 2 d after transfer.

The addition of lovastatin or perillyl alcohol after the third day of culture had no effect on the growth of the culture, arguing against nonspecific toxicity of these inhibitors. Interestingly, cells treated on d 0 with 1 μM lovastatin efficiently incorporated [^{3}H]thymidine into DNA, suggesting that inhibition of cell growth was not due to inhibition of DNA replication. However, inhibition of DNA replication cannot be ruled out altogether as the cause of lovastatin-induced cell growth arrest, because catastrophic effects on growth could result from even a slight inhibition of DNA replication. Furthermore, higher concentrations of lovastatin may severely inhibit DNA replication. Future experiments will focus on defining the point of cell growth arrest in lovastatin-treated cultures.

In mammalian cells, lovastatin decreases [^{3}H]thymidine incorporation into newly synthesized DNA by $\geq$90% (Huneeus et al., 1980; Siperstein, 1984; Langan and Volpe, 1987; Sepp-Lorenzino et al., 1991; Sinensky et al., 1994). Indeed, it has been suggested that the isoprenylation and subsequent processing of prelamin A is necessary for DNA synthesis in F9 teratocarcinoma cells, since expression of a processed, mature lamin A renders these cells insensitive to inhibition of DNA synthesis by lovastatin (Sinensky et al., 1994). Lamin-like proteins have been described in plants, but their role in DNA replication and/or cell division is unclear (Frederick et al., 1992; Li and Roux, 1992; McNulty and Saunders, 1992; Tong et al., 1993).

To further define the role of isoprenylated proteins in the initiation of growth of tobacco cultures, it will be necessary to identify proteins that accumulate and/or become isopre-

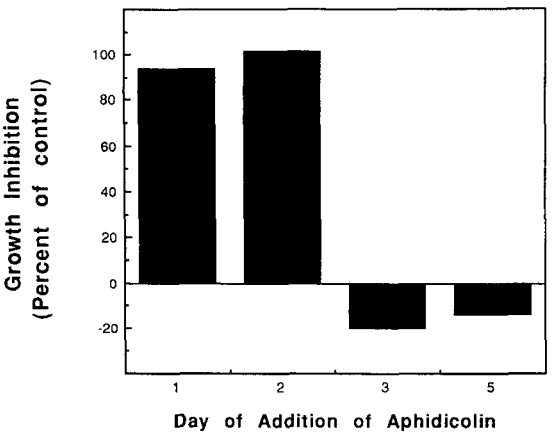

Figure 7. Percent inhibition of growth of cultured tobacco cells by aphidicolin as a function of time of addition. Growth inhibition was calculated according to the formula:

$$\text{Percent growth inhibition} = \left[1 - \frac{(Vt_f - Vt_o)}{(Vu_f - Vu_o)}\right] \times 100$$

where Vt_f is the final volume of the treated culture, Vt_o is the initial volume of the treated culture, Vu_f is the final volume of the untreated culture, and Vu_o is the initial volume of the untreated culture. These data are from one representative experiment.

nylated during the first 3 d of culture growth (the possibility that other products of mevalonate are required for initiation of growth of tobacco cells must be examined as well, since multiple aspects of isoprenoid biosynthesis are affected by lovastatin and, perhaps, by perillyl alcohol). We have recently described an expression screening procedure for identifying cDNAs that encode isoprenylated proteins (Biermann et al., 1994). This procedure has been utilized to identify several novel proteins that can be isoprenylated in vitro, and the temporal expression of the corresponding genes in tobacco cultures is now under investigation. These studies will further our understanding of the functions of isoprenylated plant proteins and their role in the growth and division of plant cells.

NOTE ADDED IN PROOF

The results shown in Figure 5 have been supported by recent observations of farnesyl:protein transferase gene expression in cultured tobacco cells (D. Zhou, D. Qian, C.L. Cramer, Z. Yang, personal communication).

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