

Regulation of a Sesquiterpene Cyclase in Cellulase-Treated Tobacco Cell Suspension Cultures¹

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ABSTRACT

The regulation of an elicitor-inducible sesquiterpene cyclase in tobacco (*Nicotiana tabacum*) cell suspension cultures was investigated. Sesquiterpene cyclase activity was absent from control cell cultures but induced to a maximum within 15 hours of cellulase addition to the cell cultures. The induction of the cyclase activity was correlated with an absolute amount of the cyclase protein as measured in immunoblots. Both the *in vivo* synthesis rate, measured as the incorporation of [³⁵S]methionine by cell cultures into immunoprecipitable cyclase protein, and the cyclase mRNA translational activity, measured as the incorporation of [³⁵S]methionine into immunoprecipitable cyclase protein synthesized by *in vitro* translation of isolated RNA, were maximal at that time when the increase in cyclase enzyme activity was maximal. Using thiouridine to selectively label and isolate *de novo* synthesized mRNA, the *in vitro* translation products encoded by the newly synthesized RNA from elicitor-treated, but not control, cell cultures contained immunoprecipitable cyclase protein. These results suggest that the induction of the sesquiterpene cyclase in elicitor-treated cell cultures is primarily regulated by transcriptional control of the cyclase gene.

When fungal elicitors are added to tobacco cell suspension cultures, the cultures cease sterol accumulation and instead synthesize and secrete sesquiterpenoids (23, 25). Previous work correlated the decline in sterol biosynthesis with a suppression of squalene synthetase enzyme activity, and the induction of sesquiterpenoid biosynthesis with an induction of a sesquiterpene cyclase enzyme activity (23, 25). Because these two enzymes are positioned at a putative branch point in the isoprenoid biosynthetic pathway, the induction of one enzyme and the suppression of the other were interpreted as an important mechanism controlling carbon flow and hence, end product formation (25). This simplistic model assumed that FPP³ was equally available to both enzymes and that sesquiterpenoid and sterol biosynthesis were not localized to different subcellular compartments. The mechanisms respon-

sible for the regulation of these two enzyme activities are currently unknown.

Studies of phytoalexin biosynthesis in plants other than tobacco have provided interesting and important insights into the mechanisms utilized by plants in regulating these biochemical pathways. Of the particular pathways studied, regulation of phenylpropanoid phytoalexin biosynthesis in parsley and bean and diterpene phytoalexins in castor bean are those best understood. For example, the induction of PAL, a key enzyme in the phenylpropanoid pathway, has been correlated with changes in the *de novo* synthesis of the enzyme protein (2, 11), the translational activity of the mRNA coding for the PAL polypeptide (2, 12), the absolute amount of the PAL mRNA, and the transcription rate of the PAL gene (14, 17). Similar data have been reported for a number of other enzymes of the phenylpropanoid pathway in both parsley and bean (reviewed in Ref. 7). The regulation of casbene accumulation in castor bean appears similarly complex. Casbene synthetase is a key branch point enzyme of diterpene biosynthesis catalyzing the single step conversion of geranylgeranyl diphosphate to casbene, a potent fungal toxin. Casbene synthetase is absent from control plant tissue but induced to high levels upon challenge with *Rhizopus stolonifer* (9). Changes in the translational activity of the casbene synthetase mRNA have also been described (18). Recently, Lois and West (16) documented transcriptional control of the casbene synthetase gene in nuclear run-off type experiments.

To determine whether similar mechanisms are responsible for controlling sesquiterpene metabolism in tobacco cells, we have focused our initial efforts on a sesquiterpene cyclase. The sesquiterpene cyclase is a soluble, apparently cytoplasmic enzyme (U Vögeli, J Chappell, unpublished observations) that catalyzes the conversion of FPP to 5-*epi*-aristolochene (29), a bicyclic sesquiterpene intermediate predicted for the ultimate production of capsidiol. Capsidiol is one of several abundant sesquiterpenoids found in tobacco tissues challenged with pathogens or fungal elicitors (1, 5, 10, 22, 28). In previous work, characterization of the purified cyclase and generation of poly- and monoclonal antibodies were reported (26). Using the cyclase antibodies, the current work presents evidence that the induction of the sesquiterpene cyclase enzyme activity is mediated by transcriptional control of the cyclase gene.

MATERIALS AND METHODS

Isotopes

[³⁵S]Methionine (1066 Ci/mmol) was purchased from ICN. [5,6-³H]Uridine (39.3 Ci/mmol) was obtained from New

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³ Abbreviations: FPP, farnesyl diphosphate; PAL, phenylalanine ammonia-lyase.

England Nuclear. *Trans,trans*-[1-³H]farnesyl diphosphate (87 mCi/mmol) was synthesized according to the method of Schechter and Bloch (21) and Popjak *et al.* (20).

Cell Cultures and Cellulase Treatment

Cell cultures of *Nicotiana tabacum* cv KY14 were maintained in Murashige-Skoog medium as described previously (5). Cell cultures in their rapid phase of growth, corresponding to 3 d after subculturing, were used for the induction studies. The cell cultures were incubated with 0.2 to 0.3 μ g of cellulase (*Trichoderma viride*, type RS, Onozuka) per mL of cell culture for the indicated lengths of time before collecting the cells by light suction filtration onto Whatman filter paper. In preliminary experiments, the amount of cellulase used was optimized for maximal stimulation of capsidiol production and induction of cyclase enzyme activity.

Cyclase Enzyme Assay

Cells (0.5 g) were homogenized in a mortar and pestle with 1 mL of 80 mM potassium phosphate buffer (pH 7), 20% (w/v) glycerol, 10 mM sodium metabisulfite, 10 mM sodium ascorbate, 15 mM MgCl₂, and 5 mM β -mercaptoethanol. The homogenate was centrifuged in an Eppendorf centrifuge for 10 min at 12,000g. For the cyclase assay, 5 to 10 μ L of the supernatant, corresponding to 3 to 10 μ g protein, was incubated in a total volume of 75 μ L containing 5 mM β -mercaptoethanol, 150 mM Tris/HCl (pH 7), 30 mM MgCl₂, and 5% (w/v) glycerol. The reaction was initiated by the addition of 3 nmol of [³H]FPP. After incubation for 5 to 10 min at 35°C, the reaction was chilled in an ice bath, and the reaction products were partitioned into 200 μ L of *n*-hexane. After a brief centrifugation, an aliquot of the *n*-hexane (100–150 μ L) was reacted with 50 mg of silica powder to remove any contaminating FPP or farnesol generated by phosphatase activity. The radioactivity remaining in the *n*-hexane was then determined by scintillation counting and taken as the measure of the 5-epi-aristolochene produced. The reaction products were previously characterized by TLC and GC-MS and consisted of 40 to 60% farnesol and 20 to 40% 5-epi-aristolochene (25, 29). Cyclase activity is expressed as nmol of aristolochene produced/h·mg protein. Protein concentration was determined by the Bradford (4) method.

In Vivo Synthesis Rates of Proteins

[³⁵S]Methionine (100 μ Ci) was added to 10 mL of control or cellulase-treated cell cultures. After a 2 h incubation, during which 70 to 80% of the methionine was taken up, cells were collected by filtration and frozen. Extracts were prepared as described above for the cyclase enzyme assay. Protein content and cyclase enzyme activity were determined, and the amount of radioactivity incorporated was determined by TCA precipitation. About 4 to 6% of the radioactivity taken up by either control or cellulase-treated cell cultures was incorporated into TCA-precipitable material. Equal amounts of incorporated radioactivity (~100,000 dpm) were analyzed directly by SDS-PAGE/fluorography (3, 13) and used to determine the amount of radioactivity incorporated into immunoprecipitable cyclase protein.

RNA Isolation and In Vitro Translation

Total RNA was isolated according to the method of Mohnen *et al.* (19) modified by Vögeli-Lange *et al.* (27). Twelve micrograms of total RNA were used in a 15 μ L *in vitro* translation assay (rabbit reticulocyte lysate; Amersham) containing 10 μ Ci of [³⁵S]methionine. The incorporation of radioactivity into TCA-precipitable material was determined in 1- μ L aliquots. Samples containing equal amounts of TCA-precipitable radioactivity (~100,000 dpm) were used for immunoprecipitations and analysis of the *in vitro* translation products by SDS-PAGE/fluorography (3, 13).

In Vivo Labeling of RNA with Thiouridine and [³H]Uridine

Control cell cultures and cultures treated with cellulase for 2 h were used for these *in vivo* labeling studies. 4-Thiouridine at 1 mM and [³H]uridine at 0.03 μ M were added to the cultures for an additional 2 h incubation. Total RNA was isolated from the cell cultures as described above. The newly synthesized, thiouridine/[³H]uridine containing RNA was isolated according to the method of Woodford *et al.* (30). Briefly, 1 mg of total RNA was adjusted to 50 mM sodium acetate (pH 5.5), 0.1% SDS, 0.15 M NaCl, and 4 mM EDTA (buffer A). After denaturing of the RNA at 65°C for 10 min, the RNA was chilled on ice and incubated with 1.5 mL of packed Affigel 501 (Bio-Rad) for 2 h at room temperature. The slurry was poured into a syringe, washed with 5 volumes of buffer A followed by 5 volumes of buffer A containing 0.5 M NaCl to remove nonspecifically bound RNA. The thiouridine-labeled RNA was eluted with buffer A containing 10 mM β -mercaptoethanol. Fractions with the highest radioactivity were pooled and concentrated by ethanol precipitation. The efficiency of the selection for newly synthesized RNA was determined by comparing the specific radioactivity of the total RNA with the specific radioactivity of the eluted RNA. The proportion of cyclase mRNA translational activity in the nonthiouridine and thiouridine RNA populations was determined by *in vitro* translation/immunoprecipitation analysis.

Immunological Techniques

Immunoblot analysis of proteins separated by SDS-PAGE was described previously (15, 24, 26). Specific immunoprecipitation of the radioactive cyclase protein produced *in vivo* or *in vitro* was similar to that of Mohnen *et al.* (19). In a typical experiment, the same amount of TCA-precipitable radioactivity from each sample was subjected to immunoprecipitation analysis. The amount of antisera required for maximal precipitation efficiency was determined previously. The immunoprecipitations were analyzed by SDS-PAGE/fluorography (19). To quantify the radioactivity in an immunoprecipitate, an aliquot was counted directly in a scintillation counter. To correct for background, the value obtained with a preimmune serum was subtracted. Determining the amount of radioactivity associated with the cyclase protein resolved by SDS-gel electrophoresis of the immunoprecipitates gave identical results to directly counting aliquots of the immunoprecipitates.

Each experiment was repeated several times, and results of a representative experiment are shown. The relative trends were highly reproducible from experiment to experiment. For

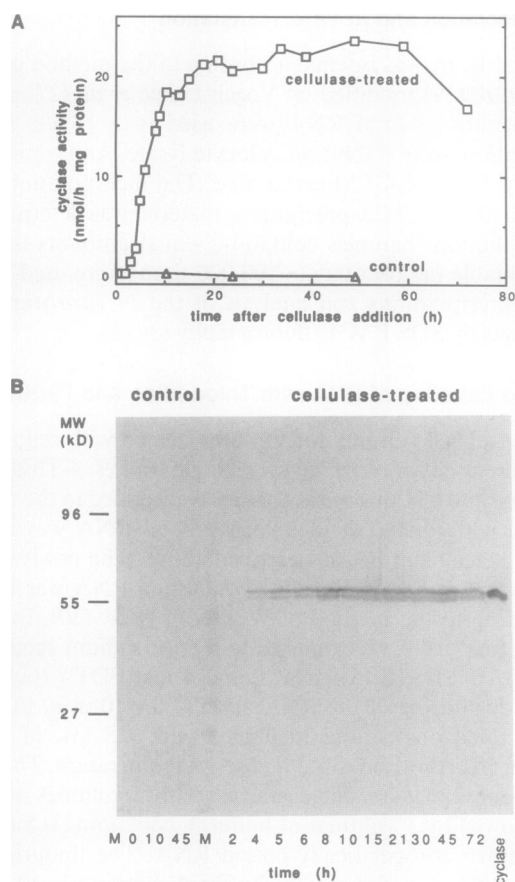


Figure 1. Induction of sesquiterpene cyclase enzyme activity and immunodetection of the cyclase protein in cellulase-treated cell cultures. Sesquiterpene cyclase enzyme activity (A) was measured in extracts prepared from control (Δ) and cellulase-treated (\square) cell cultures collected at the indicated times of treatment. Aliquots of 200 μ g protein from each of the extracts were also separated by SDS-PAGE and probed for the cyclase protein by immunoblotting (B). The lane marked "cyclase" contains 1 μ g of purified cyclase protein.

example, the maximal cyclase mRNA translational activity was always found 5 to 6 h after cellulase addition, even though the absolute amount of the translational activity varied 20 to 30% between independent experiments.

RESULTS AND DISCUSSION

Induction of the Cyclase Enzyme Activity and Polypeptide

Time courses for the induction of the cyclase enzyme activity and the absolute level of the cyclase protein in cellulase-treated cell cultures are shown in Figure 1. As reported previously (26), no cyclase enzyme activity was detectable in control cell cultures. However, the enzyme activity increased sharply in the cellulase-treated cell cultures after a 2-h lag, reached a maximum 12 to 14 h later, and remained at that level for the next 40 h. The induction of the cyclase enzyme activity was paralleled by an accumulation of the cyclase polypeptide(s) (Fig. 1B). By comparison with an immunoblot containing known amounts of purified cyclase protein, the

cyclase polypeptide level was estimated to be approximately 0.15 to 0.3% of the total soluble protein when the enzyme activity was maximal.

De Novo Synthesis of the Cyclase Protein

At various times after initiation of the cellulase treatment, control and cellulase-treated cultures were incubated for an additional 2-h interval with [35 S]methionine, and extracts of the *in vivo* synthesized proteins prepared. To determine if the *in vivo* synthesized proteins were prepared. To determine if the *in vivo* labeled proteins were challenged with cyclase antibodies, and the immunoprecipitates were evaluated by SDS-PAGE/fluorography (Fig. 2B). Incorporation of radioactivity into aliquots of the immunoprecipitates was also quantified, and those values were compared with the changes in cyclase

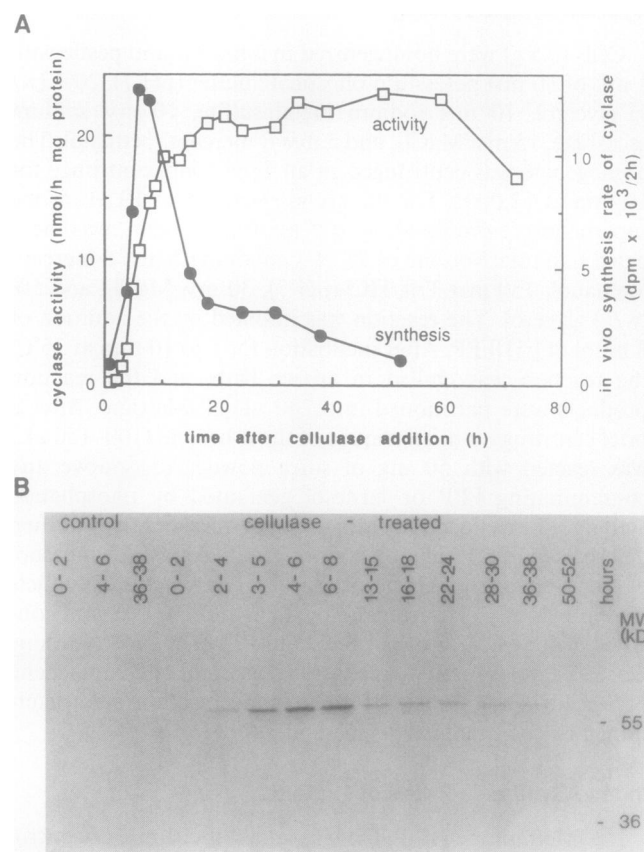


Figure 2. Changes in the *in vivo* synthesis rate of the sesquiterpene cyclase protein in relationship to the cyclase enzyme activity in cellulase-treated cell cultures. Control and cellulase-treated cell cultures were pulse-labeled with [35 S]methionine for 2-h intervals at the times indicated. Extracts were prepared from the cells, and aliquots containing equal amounts of TCA precipitable radioactivity were challenged with cyclase polyclonal antibody. The amount of radioactive, immunoprecipitable cyclase protein was either quantified by counting an aliquot of the immunoprecipitates (\bullet) in a scintillation counter (A) or visualized by SDS-PAGE/fluorography (B). The same cell cultures were used for this experiment and that depicted in Figure 1. Hence, the sesquiterpene cyclase enzyme activity (\square) was as measured in Figure 1A.

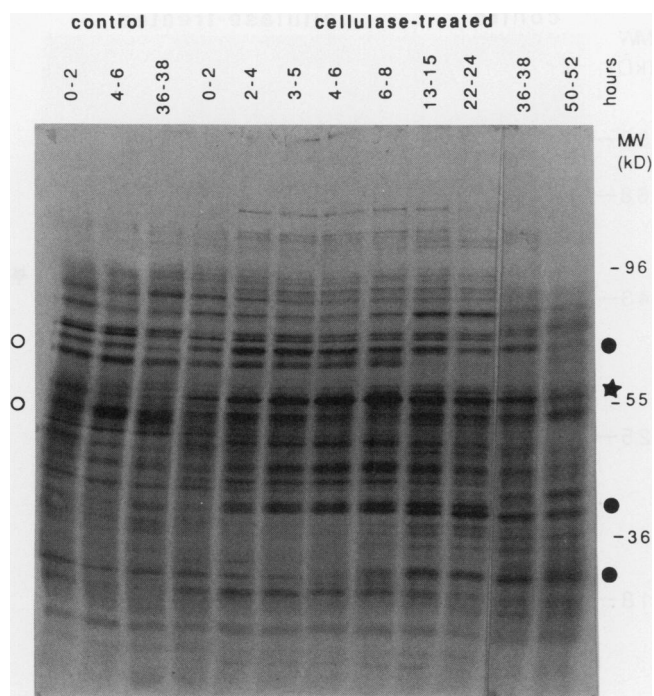


Figure 3. Comparison of the total proteins synthesized *in vivo* by control and cellulase-treated cell suspension cultures. Aliquots of equal TCA-precipitable radioactivity from the cellular extracts described in Figure 2 were separated by SDS-PAGE and the radioactive proteins visualized by fluorography. ●, Proteins whose apparent synthesis rates are enhanced by cellulase treatment; ○, those suppressed; ★, region of the gel corresponding to the mol wt of the sesquiterpene cyclase protein.

enzyme activity (Fig. 2A). Maximum synthesis of the cyclase polypeptide occurred 6 h after cellulase addition to the cultures and coincided with that time when the induction of enzyme activity was maximal. Approximately 2% of the TCA-precipitable radioactivity was incorporated into the cyclase protein at this time. The major polypeptide of 63.5 kD observed in the SDS-PAGE/fluorogram co-migrated with the predominant band observed in the immunoblot of Figure 1. No radiolabeled cyclase polypeptides were immunoprecipitable from control cell cultures incubated with [³⁵S]methionine at any time throughout the experiment.

To gain a broader understanding of the response of the cell cultures to the cellulase treatment, changes in the pattern of the total *de novo* synthesized proteins were also investigated by SDS-PAGE/fluorography (Fig. 3). The proteins synthesized in the cellulase-treated cultures were similar to those in the control cell cultures over the duration of the experiment (greater than 38 h). Nonetheless, induction and suppression in the apparent synthesis rates of a few polypeptides were noted. For example, the synthesis rates of polypeptides approximately 33, 42, 44, and 72 kD were induced, whereas the synthesis rates of a 52 and 68 kD polypeptide were suppressed. Furthermore, these changes did not occur coordinately; some were observed within 4 h of cellulase treatment, whereas others did not occur until after 12 h of cellulase treatment. And, although the changes noted here are consistent with

changes in the *de novo* synthesis rates of particular proteins, it should be noted that such changes could also result from alterations in the rates of catabolism of the particular proteins, or a combination of *de novo* synthesis and catabolism.

Induction of the Cyclase mRNA Translational Activity

At various times after initiation of the cellulase treatment, total RNA was isolated from control and cellulase-treated cells and translated *in vitro* in the presence of [³⁵S]methionine. To measure the cyclase mRNA translational activity, the incorporation of radioactivity into *in vitro* translation products immunoprecipitable with the cyclase antibodies was quantified (Fig. 4A). The specificity of the immunoprecipitation reactions was further evaluated by SDS-PAGE/fluorography (Fig. 4B). No *in vitro* translation products directed by RNA isolated from control cell cultures were precipitable with the cyclase antibodies. However, a transient induction of the

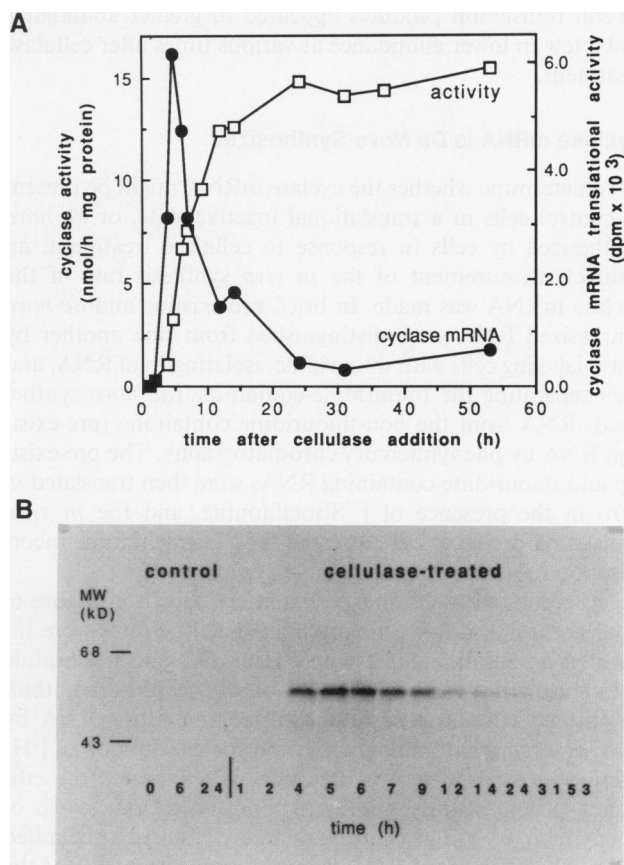


Figure 4. Induction of the sesquiterpene cyclase mRNA translational activity in relation to the cyclase enzyme activity in cellulase-treated cell cultures. RNA isolated from cells collected at the indicated time of treatment were translated *in vitro* in the presence of [³⁵S]methionine, and aliquots containing equal amounts of TCA-precipitable radioactivity were challenged with cyclase polyclonal antibody. The amount of radioactive, immunoprecipitable cyclase protein was quantified by counting an aliquot of the immunoprecipitates (●) in a scintillation counter (A), or visualized by SDS-PAGE/fluorography (B). Sesquiterpene cyclase enzyme activity (□) was measured in separate extracts prepared from the same cell cultures (A).

cyclase mRNA translational activity was observed with RNA from cellulase-treated cell cultures. The time-course change in the cyclase mRNA translational activity was very similar to that observed for the *in vivo* labeling of cyclase protein (compare Figs. 2A and 4A). At that time when cyclase mRNA translational activity was maximal, 5 to 6 h after cellulase addition to the cell cultures, incorporation of radioactivity into the cyclase protein accounted for 2 to 3% of the total incorporation into *in vitro* translation products.

To determine if the changes in the *in vivo* synthesized proteins by the cellulase-treated cell cultures observed in Figure 3 were a reflection of a changing mRNA population, total RNA isolated from control and cellulase-treated cell cultures was translated *in vitro* in the presence of [³⁵S]methionine, and the pattern of *in vitro* synthesized proteins compared by SDS-PAGE/fluorography (Fig. 5). Similar to the findings of the *in vivo* synthesis experiment, the overall patterns of the *in vitro* synthesized proteins were very similar between control and cellulase-treated cell cultures. However, several translation products appeared in greater abundance and a few in lower abundance at various times after cellulase treatment.

Cyclase mRNA is De Novo Synthesized

To determine whether the cyclase mRNA might be present in control cells in a translational inactive state, or *de novo* synthesized by cells in response to cellulase treatment, an indirect measurement of the *in vivo* synthesis rate of the cyclase mRNA was made. In brief, pre-existing and *de novo* synthesized RNA were distinguished from one another by pulse-labeling cells with thiouridine, isolating total RNA, and then separating the thiouridine-containing (*de novo* synthesized) RNA from the non-thiouridine containing (pre-existing) RNA by phenylmercury chromatography. The pre-existing and thiouridine-containing RNAs were then translated *in vitro* in the presence of [³⁵S]methionine, and the *in vitro* translation products were assessed for [³⁵S]methionine incorporation into immunoprecipitable cyclase protein.

The results of such an experiment are shown in Figure 6. Both control and 2-h cellulase-treated cell cultures were incubated an additional 2 h with [³H]uridine and thiouridine before isolating total RNA. The newly synthesized, thio-substituted RNA was separated from pre-existing RNA by phenylmercury chromatography, and the distribution of [³H]uridine incorporation was determined to estimate the efficiency of the affinity chromatography step. One cycle of phenylmercury affinity chromatography efficiently separated the newly synthesized RNA from the pre-existing RNA; the specific radioactivity of the RNA retained by the column (newly synthesized), representing about 1 to 2.5% of the total RNA, was at least 70-fold higher than in the RNA that did not bind to the phenylmercury matrix (pre-existing; Fig. 6C). More than 75% of the radioactivity recovered was associated with the newly synthesized RNA fraction, and a comparison of the specific radioactivity of the newly synthesized RNA to the initial RNA loaded onto the phenylmercury affinity column indicated a 14-fold enrichment for the RNA synthesized during the 2-h labeling period.

To determine how the cyclase mRNA segregated in the

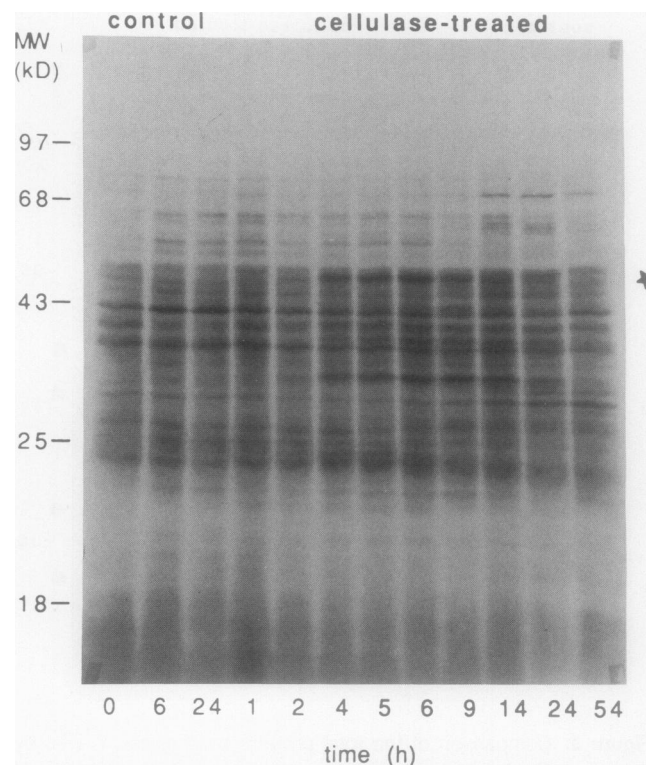


Figure 5. Comparison of the total *in vitro* translation products produced by RNAs isolated from control and cellulase-treated cell suspension cultures. Aliquots of equal TCA-precipitable radioactivity from the *in vitro* translations described in Figure 4 were separated by SDS-PAGE, and the radioactive proteins visualized by fluorography. *, Region of the gel corresponding to the mol wt of the sesquiterpene cyclase protein.

various RNA fractions, the *in vitro* translational activity of each fraction for the cyclase polypeptide was measured. In contrast to the findings of Cramer *et al.* (6), unlabeled and thiouridine-labeled RNA from control and cellulase-treated cell cultures translated equally well. Also, thiouridine labeling did not alter the induction of the cyclase translational activity (compare lanes 8 [thiouridine-labeled] and 9 [unlabeled] in Fig. 6), nor did it induce the cyclase translational activity (odd lanes).

Thiouridine-labeled RNA from cellulase-treated cell cultures, but not labeled RNA from control cell cultures, contained translationally active cyclase mRNA (Fig. 6, compare lanes 5 and 6). For the newly synthesized RNA from the cellulase-treated cells, immunoprecipitable cyclase accounted for 2.4 to 2.8% of the [³⁵S]methionine incorporated in an *in vitro* translation. For comparison, the RNA from cellulase-treated cell cultures that did not bind to the affinity column (pre-existing RNA) contained 3 times less cyclase translational activity (Fig. 6, lane 4). This was not unexpected. During the first 2 h after addition of cellulase to the cell cultures, cyclase mRNA would be expected to accumulate, albeit at a much lower rate than observed during the thiouridine labeling period, 2 to 4 h after addition of cellulase. Thus, the RNA isolated from the cellulase-treated cells would be expected to contain a pool of pre-existing cyclase mRNA. In fact, RNA

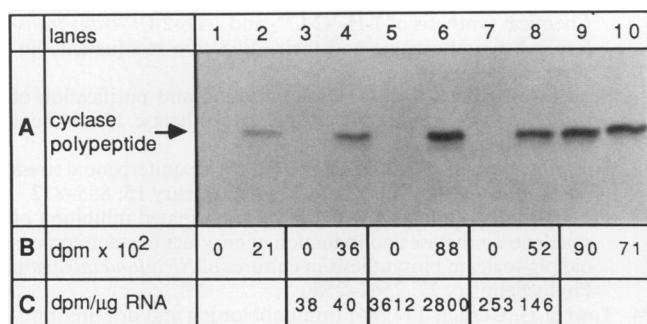


Figure 6. *De novo* synthesis of the cyclase mRNA in cellulase-treated cell cultures. [³H]Uridine and thiouridine were added to control (lanes 3, 5, 7) and cellulase-treated (lanes 4, 6, 8) cell cultures 2 h after initiation of the cellulase treatment. After a further 2-h incubation (4 h total), the cells were harvested, and total RNA was isolated (lanes 7, 8). Aliquots of these RNA samples were fractionated by phenylmercury affinity chromatography into pre-existing (RNA not containing thiouridine; lanes 3, 4) and newly synthesized (RNA containing thiouridine; lanes 5, 6) RNA populations. Lanes 1 and 2 represent RNA isolated from control and cellulase-treated cell cultures collected after only the initial 2 h incubation (no [³H]uridine or thiouridine labeling). Lane 9 represents RNA isolated from a cell culture treated for 4 h with cellulase only (no [³H]uridine or thiouridine labeling). An aliquot of this RNA was subjected to phenylmercury affinity chromatography, and lane 10 represents the fraction of the RNA that did not bind to the column material. The specific radioactivity of the different RNA fractions was measured (C), expressed as the radioactivity incorporated/μg RNA, and represents the efficiency of the labeling/chromatography steps (compare lane 7 with lanes 3 and 5; lane 8 with lanes 4 and 6). Immunoprecipitable [³⁵S]methionine-labeled sesquiterpene cyclase protein synthesized *in vitro* by translation of the different RNA fractions was visually assessed by SDS-PAGE/fluorography (A) and quantified by counting an aliquot of the immunoprecipitates in a scintillation counter (B). Equal amounts of TCA-precipitable radioactivity from the *in vitro* translations were challenged with the cyclase polyclonal antibody.

isolated from 2-h cellulase-treated cell cultures contained a similar amount of translationally active cyclase mRNA (Fig. 6, lane 2) as that found associated with the pre-existing RNA fraction from the thiouridine-labeled, induced cells (Fig. 6, lane 4). No translationally active cyclase mRNA bound non-specifically to the affinity column, nor did the chromatography step alter the cyclase translational activity (in Fig. 6, compare lanes 9 and 10).

CONCLUSIONS

Tobacco cell suspension cultures respond to elicitor treatment by the induction of a limited number of proteins. One of the induced proteins has been identified as a sesquiterpene cyclase, an enzyme that catalyzes a reaction committing carbon to the production of extracellular, antimicrobial sesquiterpenoids (25, 29). As previously discussed, an additional physiological consequence of this induction is the possible diversion of carbon from the general isoprenoid biosynthetic pathway, and hence, the depletion of isoprenoids necessary for actively growing cells, such as sterols (23, 25). The current results have demonstrated that: (a) the induced sesquiterpene

cyclase enzyme activity is correlated with the absolute amount of the cyclase protein; (b) the induced sesquiterpene cyclase is *de novo* synthesized; (c) the changes in the *de novo* synthesis rate of the cyclase protein are correlated with changes in the cyclase mRNA translational activity; and (d) by the thiouridine-labeling technique, that the cyclase mRNA is *de novo* synthesized. Because the validity of the thiouridine-labeling technique as a measure of the transcription rate of a gene has been verified (6, 30), we conclude that the induction of the sesquiterpene cyclase enzyme activity in elicitor-treated tobacco cell suspension cultures is primarily regulated by transcriptional control of the cyclase gene.

Comparison of the present findings to the regulation of phytoalexin biosynthesis in other systems reveals similarities in the mechanism of control. Transcriptional control for inducibility of many of the enzymes of the phenylpropanoid phytoalexin pathway in parsley (17) and french bean (14), as well as casbene synthetase (16), a commitment enzyme for diterpene phytoalexin biosynthesis in castor bean, has been established by nuclear run-off experiments. Furthermore, the DNA sequences 5' to the chalcone synthase gene from bean have proven sufficient to confer elicitor inducibility onto a heterologous gene (8). Whether similar DNA sequences control the transcriptional rate of genes coding for enzymes of the isoprenoid phytoalexin biosynthetic pathways remains to be determined.

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