Tagetitoxin Inhibits RNA Synthesis Directed by RNA Polymerases from Chloroplasts and Escherichia coli*

Dennis E. Mathews and Richard D. Durbin

From the Department of Plant Pathology, University of Wisconsin-Madison and the Agriculture Research Service, United States Department of Agriculture, Madison, Wisconsin 53706

Tagetitoxin, a chlorosis-inducing phytotoxin produced by Pseudomonas syringae pv. tagetis, inhibits RNA synthesis directed by chloroplast RNA polymerase. In isolated chloroplasts, tagetitoxin quickly and specifically reduced the incorporation of [3H]uridine into RNA. When it was added to transcriptionally active chloroplast protein extracts, the toxin directly inhibited incorporation of [32P]UTP into RNA. In addition, tagetitoxin inhibited in vitro RNA synthesis directed by the RNA polymerase from Escherichia coli. In vitro transcription reactions directed by chloroplast RNA polymerase or E. coli RNA polymerase are inhibited at tagetitoxin concentrations less than 1 μM. Nuclear RNA polymerase II purified from wheat germ was only affected at tagetitoxin concentrations greater than 10 μM during in vitro transcription. Tagetitoxin concentrations as high as 1 mM did not affect in vitro transcription reactions directed by RNA polymerase from bacteriophage T7 or SP6.

Several plant pathogenic bacteria of the genus Pseudomonas are known to produce toxins capable of causing chlorotic symptoms in host plants (Durbin, 1981; Daly and Deverall, 1983). The toxin produced by Pseudomonas syringae pv. tagetis, trivially named tagetitoxin, appears to be unique in that the resulting chlorosis is confined to the plant apex (Trimboli et al., 1978; Mitchell and Durbin, 1981). This pattern of chlorosis arises because tagetitoxin treatment prevents new chlorophyll accumulation but does not appear to reduce existing chlorophyll levels (Lukens, 1983). Despite the lack of chlorophyll, the growth rate and morphology of chlorotic leaves remain unaffected in toxin-treated plants as long as heterotrophy can be maintained.

The toxin can be purified from culture filtrates of P. syringae pv. tagetis (Mitchell and Durbin, 1981) and a tentative structure has been assigned (M, 416) (Mitchell et al., 1989).

The effects of tagetitoxin treatment appear to be largely confined to chloroplasts, although it was previously shown that the toxin is not a direct inhibitor of photosynthesis or chlorophyll biosynthesis (Lukens, 1983). In leaves of tagetitoxin-treated seedlings, proplastids, which would normally differentiate into chloroplasts, fail to develop beyond a very rudimentary stage (Jutte and Durbin, 1979; Lukens and Durbin, 1985). These rudimentary chloroplasts appear to have intact envelopes, but the internal membrane systems are greatly reduced and severely disorganized. Plastid 70 S ribosomes fail to accumulate in both light- and dark-grown, toxin-treated seedlings. Accordingly, plastid-encoded polypeptides normally translated on these ribosomes cannot be detected. In addition, the levels of chloroplast ribosomal as well as messenger RNAs are severely reduced in leaves of toxin-treated seedlings (Lukens et al., 1987).

Despite evidence for a chloroplast-specific mechanism, no direct effect of tagetitoxin on chloroplast metabolism has been demonstrated. Here we present evidence, based on experiments with isolated chloroplasts and chloroplast extracts, that tagetitoxin is a selective inhibitor of chloroplast RNA polymerase. We propose that this is the primary mechanism that is ultimately responsible for all of the effects, including chlorosis, observed in tagetitoxin-treated plants. We show that in vitro RNA synthesis directed by the RNA polymerase from Escherichia coli is also inhibited by tagetitoxin. However, in vitro transcription directed by nuclear RNA polymerase II from wheat germ is much less sensitive to the toxin and the RNA polymerases from two bacteriophage, SP6 and T7, are unaffected by the toxin even at high concentrations. The effect of tagetitoxin on nuclear RNA polymerases from several other organisms is described in the following paper.

MATERIALS AND METHODS

Preparation of Tagetitoxin—Tagetitoxin was purified from culture filtrates of P. syringae pv. tagetis using the modified procedure reported by Lukens and Durbin (1985). The toxin was purified to a clear, glassy residue and was estimated to be 70–100% pure, based on its specific activity in a zinnia apical chlorosis bioassay (Mitchell and Durbin, 1981).

Growth of Plants—Peas (Progress No. 9, Old's Seed Co., Madison, WI) were grown in Jiffy Mix with an 18-h light/6-h dark photoperiod at a constant temperature of 18 °C. Seedlings were harvested 8 days after sowing. Prior to harvesting, seedlings were kept in darkness 14–16 h to deplete chloroplasts of starch and then, immediately before harvesting, illuminated for 30–45 min.

In Organello Incorporation Reactions—The conditions for isolation of intact chloroplasts and in organello incorporation reactions were based on the procedure described by Nivison and coworkers for optimal in organello protein synthesis (Nivison et al., 1996). Incorporation reactions contained 350 mM Sorbitol, 33 mM HEPES-KOH (pH 8.3), 1 mM Dithiothreitol, 12.5 mM MgCl2, 9.5 mM ATP, and 200 μM each of Isoeucine and Threonine. When uridine incorporation into RNA was monitored, [5,6-3H]uridine (40 Ci/mM) was added to give a final concentration of 20 μCi/ml and when thymidine incorporation into DNA was monitored, [6-3H]thymidine (23 Ci/mM) was added to give a final concentration of 20 μCi/ml. Incorporation of Methionine into protein was monitored by adding [35S]methionine (1120 Ci/mM) to give a final concentration of 40 μCi/ml. Reactions were started by adding chloroplasts to the reaction mixture and bringing the final chlorophyll concentration to approximately 0.1 mg/ml/
In vitro transcription reactions contained 40 ml of the reaction mixture and were started by adding MgCl₂ and placing vials in a 37 °C water bath. After 15 min, reactions were stopped by adding EDTA to a final concentration of 20 mM. Aliquots were spotted on DE81 filters which were then processed as described above.

In Vitro Transcription with Nuclear RNA Polymerase II from Wheat Germ Nuclei—Nuclear RNA polymerase II purified from wheat germ was generously provided by D. Hager (University of Wisconsin-Madison) and was tested for sensitivity to tagetitoxin in nonpromoter-directed in vitro transcription reactions. In vitro transcription reactions contained 25 mM Tris-HCl (pH 7.9), 8 mM MgCl₂, 25 mM ammonium sulfate, 0.5 mM ATP, GTP, and CTP, 15 μM [α-³²P]UTP, 250 μg/ml heat-denatured calf thymus DNA, and various amounts of tagetitoxin. Transcription assays were carried out for 20 min at 30 °C and terminated by addition of EDTA to a final concentration of 20 mM. Aliquots were spotted on DE81 filters which were then processed as described above.

RESULTS

In Organello Synthesis of Protein, RNA, and DNA—When tagetitoxin was added to isolated, intact chloroplasts, there was no significant effect on the ability of these chloroplasts to incorporate [³⁵S]methionine into protein during the remainder of the 90-min incubation period (Fig. 1A). In contrast, the same treatment quickly reduced incorporation of [³H]uridine into RNA (Fig. 1B) and caused a small (but reproducible) decrease in the incorporation of [³H]thymidine into DNA (Fig. 1C).

The effect of other additions or deletions on [³H]uridine incorporation confirms that tagetitoxin was indeed inhibiting RNA synthesis within intact chloroplasts (Table I). Incorporation was abolished by actinomycin D, indicating that it was the result of DNA-dependent RNA synthesis (Krakow and Kumar, 1980). The addition of ribonuclease had little effect, indicating that incorporation occurred within intact organelles. The intactness of the organelles was confirmed by examination with a phase contrast microscope (Kuhn and von Wettstein, 1961). Additionally, tagetitoxin was as effective at inhibiting incorporation when light was used to generate the ATP (via photophosphorylation) needed to drive [³H]uridine incorporation as it was when ATP was supplied exogenously (data not shown). This is significant because only intact chloroplasts have the capacity to utilize light energy, in the absence of other energy sources, to generate the ATP needed to convert [³H]uridine into [³H]UTP (Hartley and Ellis, 1973). The octapeptide ɑ-amanitin (Wolfgang, 1982), which inhibits nuclear RNA polymerase II and, to a lesser extent, nuclear RNA polymerase III, did not significantly affect [³H]uridine incorporation, indicating little nuclear contamination. Microscopic examinations confirmed that chloroplast preparations were essentially free of contaminating nuclei. The insensitivity of the final chloroplast preparation to the bacterial RNA polymerase inhibitor rifampicin indicated that bacterial contamination was minimal. In the absence of exogenously supplied ATP, addition of acetate did not enhance the incorporation reactions. Since acetate is a nonoxidizable substrate that can be utilized by bacteria but not by chloroplasts or mitochondria, this also would suggest that any bacterial contribution to [³H]uridine incorporation was minimal. In addition, when bacterial contamination was assayed by direct plating of the final chloroplast preparation onto nutrient media, it was estimated that these preparations contained less than 1 bacterium per 100,000 chloroplasts. Therefore, we conclude that the [³H]uridine incorporation inhibited by tagetitoxin in these reactions can be attributed to chloroplasts and not to contaminating bacteria, nuclei, or mitochondria.

Chloroplasts in Vitro Transcription—Transcriptionally active extracts were prepared from lysed pea chloroplasts according to the method described by Grussem and coworkers.
The effect of various treatments on the incorporation of \[^3H\]uridine into RNA with isolated chloroplast preparations

Intact chloroplasts were isolated from 8-day-old pea seedlings and incubated with \[^3H\]uridine and 9.5 mM ATP under standard conditions described under "Materials and Methods." The effect of various additions or deletions to the incubation mixture on overall \[^3H\]uridine incorporation is presented as a percent of the incorporation in a control reaction under standard conditions. Values represent the mean of at least three independent experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[^3H]Uridine incorporation % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>+ actinomycin D (10 (\mu)g/ml)</td>
<td>2</td>
</tr>
<tr>
<td>+ ribonuclease (10 (\mu)g/ml)</td>
<td>92</td>
</tr>
<tr>
<td>+ rifampicin (10 (\mu)g/ml)</td>
<td>91</td>
</tr>
<tr>
<td>- ATP</td>
<td>0.7</td>
</tr>
<tr>
<td>- ATP, + 5 mM acetate</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of tagetitoxin on the \textit{in organello} incorporation of \[^35S\]methionine, \[^3H\]uridine, and \[^3H\]thymidine. A, the incorporation of \[^35S\]methionine into protein by intact chloroplasts was monitored by removing aliquots of the reaction mixture at intervals and measuring the level of trichloroacetic acid-insoluble radioactivity. The incubation mixture was divided 9 min after chloroplasts were added to start the incorporation reaction and combined with either water (○) or tagetitoxin (○) to a final concentration of 100 \(\mu\)M. B, the incorporation of \[^3H\]uridine into RNA by intact chloroplasts was monitored by removing aliquots of the reaction mixture at intervals and measuring the level of trichloroacetic acid-insoluble radioactivity. The incubation mixture was divided 9 min after chloroplasts were added to start the incorporation reaction and combined with either water (○) or tagetitoxin (○) to a final concentration of 100 \(\mu\)M. C, the incorporation of \[^3H\]thymidine into DNA by intact chloroplasts was monitored by removing aliquots of the reaction mixture at intervals and measuring the level of trichloroacetic acid-insoluble radioactivity. The incubation mixture was divided 9 min after chloroplasts were added to start the incorporation reaction and combined with either water (○) or tagetitoxin (○) to a final concentration of 100 \(\mu\)M.

Fig. 2. Effect of tagetitoxin on \textit{in vitro} transcription with chloroplast extracts. Autoradiogram of \[^32P\]UMP-labeled RNA which was transcribed in \textit{vitro} with chloroplast extracts and separated on 10% polyacrylamide, 80% urea gels. Chloroplast extracts were combined with \[^32P\]UTP without added plasmid DNA (lane 1) or with a plasmid containing the \textit{trnM2} gene (lanes 2-7). This plasmid is derived from pUC8 and contains the spinach chloroplast \textit{trnM2} gene which is transcribed and processed to a mature 75-nucleotide transcript. The product of a controlled reaction without added tagetitoxin is in lane 2. Tagetitoxin was included in transcription reactions at a final concentration of 0.1 \(\mu\)M (lane 3), 0.5 \(\mu\)M (lane 4), 1.0 \(\mu\)M (lane 5), 5.0 \(\mu\)M (lane 6), and 10.0 \(\mu\)M (lane 7).

(Gruissem \textit{et al.}, 1986b). Endogenous chloroplast DNA was removed during preparation of the extracts making subsequent \textit{in vitro} transcription dependent on the addition of a DNA template. No significant incorporation of \[^32P\]UTP into RNA occurred in these extracts without addition of a DNA template (Fig. 2, lane 1). Significant incorporation resulted from addition of a DNA template with a chloroplast gene (Fig. 2, lane 2). Addition of tagetitoxin to \textit{in vitro} transcription reactions using the chloroplast extract reduced incorporation of \[^32P\]UTP into RNA (Fig. 2, lanes 3-7). The template used in these reactions was a plasmid containing the spinach chloroplast \textit{trnM2} gene (Gruissem and Zurawski, 1985a). In spinach chloroplast extracts the \textit{trnM2} gene is transcribed and processed into a mature 75 nucleotide \textit{tRNA}^Met. The quantitative effect of tagetitoxin on \textit{in vitro} transcription was determined by excising bands that migrated at approximately 75 nucleotides (the size expected for the mature spinach chloroplast \textit{tRNA}^Met) and measuring the radioactivity by liquid scintillation counting. When the dose-response curve for tagetitoxin inhibition of chloroplast \textit{in vitro} transcription is compared to the dose-response curve for inhibition of chloroplast \textit{in organello} transcription, it is apparent that the \textit{in vitro} reaction is more sensitive (Fig. 3).

Tagetitoxin inhibited \[^32P\]UTP incorporation to a similar extent when other templates were used in the chloroplast transcription reactions. Among these templates was a plasmid containing the transcription unit \textit{trnM2-35A2} in which transcription of the \textit{trnM2} coding region is initiated from the

Table I

\begin{tabular}{|c|c|}
\hline
Treatment & \[^3H\]Uridine incorporation % control \\
\hline
Control & 100 \\
+ actinomycin D (10 \(\mu\)g/ml) & 2 \\
+ ribonuclease (10 \(\mu\)g/ml) & 92 \\
+ rifampicin (10 \(\mu\)g/ml) & 91 \\
- ATP & 0.7 \\
- ATP, + 5 mM acetate & 0.7 \\
\hline
\end{tabular}

Downloaded from http://www.jbc.org/ by guest on August 15, 2017
Chloroplast extracts were used to direct incorporation of [\(^{32}\)P]UTP plasmid containing the trnM2 gene. Incorporation of [\(^{32}\)P]UTP was into RNA in vitro with varying concentrations of tagetitoxin included. The amount of [\(^{32}\)P]UTP was quantitated by excising the area of the gel where the 75-nucleotide tRNA\(^{\text{ts}}\) transcript migrated and measuring radioactivity directly. Incorporation of [\(^{32}\)P]UTP in vitro.

RNA synthesis tions with chloroplast extract in the absence of tagetitoxin. The resulting [\(^{32}\)P]UMP-labeled plasmid containing the trnM2 gene as the DNA template was carried out in the absence of tagetitoxin. The resulting [\(^{32}\)P]UMP-labeled RNA was purified and reincubated in chloroplast extracts under the same conditions used during in vitro transcription except that [\(^{32}\)P] UTP was not present. Incorporation of [\(^{32}\)P]UTP was increased by exciting the area of the gel where the 75-nucleotide tRNA\(^{\text{ts}}\) transcript migrated and measuring radioactivity directly. Incorporation of [\(^{32}\)P]UTP in vitro.

Effect of Tagetitoxin on RNA Stability—When [\(^{35}\)S]UMP-labeled RNA was recovered from the initial in vitro transcription reaction. Lanes 2–4 represent RNA reisolated from a second incubation in chloroplast extracts with no tagetitoxin (lane 2), 1 mM tagetitoxin (lane 3), or 10 \(\mu\)M tagetitoxin (lane 4).

spinach chloroplast psbA promoter (Gruissem and Zurawski, 1985a) and another plasmid containing the spinach chloroplast trnS1 transcription unit which encodes tRNA\(^{\text{ts}}\). Expression of the spinach chloroplast trnS1 gene differs from the trnM2 gene in that it does not require a 5'-upstream promoter element (Gruissem et al., 1986a).

Effect of Tagetitoxin on RNA Stability—When [\(^{35}\)S]UMP-labeled RNA was recovered from in vitro transcription reactions with chloroplast extract in the absence of tagetitoxin and then reincubated in chloroplast extracts in the presence of tagetitoxin, there was no apparent effect on RNA stability per se (Fig. 4). Thus, tagetitoxin appears to reduce [\(^{35}\)S]UTP incorporation by inhibiting RNA synthesis, rather than by enhancing RNA degradation in the chloroplast extracts.

DISCUSSION

We have presented evidence that tagetitoxin is a direct inhibitor of chloroplast RNA synthesis. When tagetitoxin was added to isolated, intact chloroplasts, the rate of [\(^{3}H\)]uridine incorporation into RNA was rapidly reduced. At a final tagetitoxin concentration of 1 mM, incorporation of [\(^{3}H\)]uridine was almost completely abolished. Other additions and deletions confirmed that the [\(^{3}H\)]uridine incorporation was indeed the result of DNA-dependent RNA synthesis within intact chloroplasts.

While tagetitoxin reduced the rate of [\(^{3}H\)]uridine incorporation into RNA in isolated chloroplasts, it had no effect on the incorporation of [\(^{35}\)S]methionine into protein. This demonstrates that the earliest measurable effects of tagetitoxin are selective, which is in agreement with previous results indicating that the toxin does not inhibit photosynthetic electron transport when added to isolated chloroplasts (Lukens, 1983). The prevention of chloroplast RNA synthesis in vivo would eventually have an impact on chloroplast protein synthesis, but in isolated chloroplasts, protein synthesis is thought to represent the elongation of nascent polypeptides from preexisting transcripts. It has been reported that RNA synthesis in isolated chloroplasts can be limited by inhibitors, such as cordycepin and actinomycin D, without affecting the incorporation of labeled amino acids into proteins (Blair and
Tagetitoxin treatment also appeared to decrease the incorporation of \[^{32}P\]UTP into RNA in isolated chloroplasts. However, the effect on chloroplast DNA synthesis was about 100-fold less than the effect on RNA synthesis. Since DNA replication in prokaryotes and eukaryotes requires synthesis of RNA to serve as 3' primers for the initiation of DNA strands (Kornberg, 1980), it is possible that the effect of the toxin on chloroplast DNA synthesis is an indirect result of the inhibition of chloroplast RNA synthesis. It is noteworthy that tagetitoxin does not inhibit DNA polymerase I from *E. coli* during "nick translation" reactions (data not shown).

Chloroplast in *vitro* RNA synthesis was even more sensitive to tagetitoxin than in *organello* RNA synthesis. The incorporation of \[^{32}P\]UTP into RNA using transcriptionally active chloroplast extracts was virtually abolished when the tagetitoxin concentration was 10 \(\mu\)M. The increased sensitivity of chloroplast extracts suggests that the chloroplast envelope may present a partial barrier to tagetitoxin. Since the toxin limited incorporation of \[^{32}P\]UTP into RNA but did not affect RNA stability per se in the chloroplast extracts, the reduced incorporation of \[^{32}P\]UTP into RNA can be attributed to a decreased rate of RNA synthesis and not enhanced degradation.

The effect of tagetitoxin on RNA synthesis directed by several other RNA polymerase enzymes was tested in *vitro*. Tagetitoxin inhibited *in vitro* RNA synthesis directed by *E. coli* RNA polymerase almost as effectively as it inhibited *in vitro* RNA synthesis directed by chloroplast RNA polymerase. However, *in vitro* transcription directed by RNA polymerase II from wheat germ nuclei was not significantly inhibited until the concentration of toxin was greater than 100 \(\mu\)M. The RNA polymerase from the bacteriophage SP6 or T7 was unaffected by tagetitoxin at concentrations as high as 1 \(\text{mM}\).

Sensitivity of *in vitro* RNA synthesis to tagetitoxin, thus, seems to depend on the type of RNA polymerase. The RNA polymerase from *E. coli* is well characterized and consists of a catalytically active core polymerase which has a subunit composition of \(\alpha_2\beta\beta'\) (Chamberlin, 1982; Losick and Chamberlin, 1976; von Hippel et al., 1984). An additional subunit, \(\sigma\), is present in the holoenzyme and is necessary for promoter recognition. Chloroplast RNA polymerase has not been definitively characterized, although there is considerable evidence to suggest that it resembles the RNA polymerase of *E. coli*. Open reading frames have been identified in the chloroplast genome which have considerable DNA sequence homology with genes for all three subunits of the *E. coli* core RNA polymerase (Sijben-Müller et al., 1990; Ohyama et al., 1990; Shinozaki et al., 1986; Hudson et al., 1988). There is evidence that these putative genes for chloroplast RNA polymerase subunits are transcribed and that they contribute functionally to the synthesis of chloroplast RNA (Sijben-Müller et al., 1986; Hudson et al., 1988; Little and Hallick, 1988; Ruz and Kössel, 1988; Purton and Gray, 1989). In addition, many chloroplast promoter elements resemble typical prokaryotic promoters which suggests that the two types of RNA polymerase are similar (Gruissem and Zurawski, 1985; Hanley-Bowdoin and Chua, 1987). Although an open reading frame with homology to the *E. coli* \(\sigma\) subunit has not been found in the chloroplast genome, there is evidence for a chloroplast RNA polymerase subunit with \(\sigma\)-like activity (Bulow and Link, 1988; Lerbs et al., 1988).

Nuclear RNA polymerase II has a much more complex subunit composition than that of *E. coli* RNA polymerase, yet DNA sequencing of major subunit genes has revealed certain regions of conservation (Allison et al., 1986; Falkenburg et al., 1987). This may account for the partial sensitivity of wheat germ RNA polymerase II found here, as well as the observation (Steinberg et al., 1990) that RNA polymerase III from other organisms is quite sensitive to tagetitoxin. The RNA polymerases from bacteriophages SP6 and T7 which appeared completely insensitive to tagetitoxin, are very different from *E. coli* RNA polymerase and in each case consist of a single polypeptide capable of initiating and transcribing RNA from DNA templates containing an SP6 or T7 specific promoter (Bautz, 1976; Butler and Chamberlin, 1982).

*E. coli* is not the only prokaryote with an RNA polymerase sensitive to tagetitoxin. The toxin inhibited *in vitro* transcription directed by RNA polymerase from the cyanobacterium *Anabaena* 7120 (generously provided by R. Haselkorn, University of Chicago) and RNA polymerase from tagetitoxin-producing strains of *P. syringae* pv. *tagetis* was also sensitive to the toxin in *vitro* (data not shown). How a toxin-producing strain of *P. syringae* pv. *tagetis* might protect itself from self-toxicity is unknown. In fact, we have not observed that tagetitoxin causes growth inhibition of any bacteria, including *E. coli*. It is possible that toxin uptake is blocked or that the toxin is metabolized to a non-toxic form.

The results of a more detailed investigation of the mechanism of tagetitoxin inhibition of *E. coli* RNA polymerase will be presented elsewhere. These studies have shown that inhibition is not a function of DNA concentration, indicating that tagetitoxin does not act by binding to the DNA template. Also, in these studies we have shown that tagetitoxin can inhibit chloroplast and *E. coli* RNA polymerase during the elongation phase of RNA synthesis.

We have shown that tagetitoxin is capable of inhibiting RNA synthesis directed by chloroplast RNA polymerase in *organello* and *in vitro*. We propose that tagetitoxin has a similar effect on chloroplast RNA synthesis in *vivo*. Such a mechanism would be sufficient to account for the effects observed in tagetitoxin-treated plants.

In higher plants, the development of chloroplasts from undifferentiated proplasts takes place rapidly and is completed during the early phase of cell growth. During this period when chloroplasts are increasing in both volume and number, there is a dramatic increase in the accumulation of chloroplast transcription and translation products (for reviews see Mullet, 1988; Gruissem, 1989). Although many chloroplast proteins are nuclear encoded and translated on cytoplasmic 80 S ribosomes, the chloroplast genome does contain the genes for rRNAs and tRNAs along with mRNAs for many ribosomal and photosynthetic proteins. Inhibition of chloroplast RNA synthesis would obviously have the most profound impact on developing chloroplasts in which the demand for de novo RNA synthesis is great. This could explain why chlorosis is only observed in those tissues in which chloroplast maturation is incomplete at the time of toxin treatment. However, even though tagetitoxin does not appear to reduce existing chlorophyll levels, it cannot be assumed that mature chloroplasts are unaffected by the toxin. The sensitivity of *in organello* transcription to tagetitoxin did not appear to vary with the age of pea seedlings from which chloroplasts were isolated (data not shown). Thus, *in planta* tagetitoxin may inhibit RNA synthesis in mature chloroplasts without resulting in a net decrease in the existing chlorophyll level.

Since we have not attempted to determine the *in planta* distribution of tagetitoxin, we cannot rule out the possibility that the pattern of symptom expression may partially reflect an uneven distribution of the toxin. In previous studies, however, there was no indication that tagetitoxin could reduce chlorophyll levels in mature green tissues despite attempts to
introduce the toxin into these areas by subepidermal injection, vacuum infiltration, or placing cut stems, with mature leaves attached, directly into toxin solution (Lukens, 1983).

Tagetitoxin is the first compound to be identified which could be used to selectively inhibit plastid transcription in higher plants. Despite the apparent similarity with E. coli RNA polymerase, plastid RNA polymerase from higher plants is not sensitive to the prokaryotic transcription inhibitor rifampicin (Ellis, 1982). We can only speculate, therefore, as to precisely what effect the specific inhibition of plastid transcription might have in vivo. Plastid translation, on the other hand, can be inhibited by any of several 70 S ribosome translation inhibitors such as streptomycin, lincomycin, β-threo-chlamenihicil, or spectinomycin (Kirk and Tilney-Bassett, 1975). It is noteworthy that symptoms resulting from treatment with these inhibitors resemble the symptoms observed in tagetitoxin-treated plants, i.e. plastid development and chlorophyll accumulation is prevented in tissues where chloroplasts are undergoing maturation, while existing chlorophyll levels do not appear to be reduced in tissues containing mature chloroplasts. Additionally, as with tagetitoxin, treatment of plants with 70 S ribosome translation inhibitors does not immediately affect growth rate or morphology.

The evidence presented in this study is consistent with a primary mechanism for tagetitoxin in which the toxin selectively inhibits plastid RNA polymerase. Preliminary results from this study and additional studies in progress indicate that the plastid nuclear RNA polymerases (I, II, and III) are much less sensitive to tagetitoxin than plastid RNA polymerase. Such a mechanism would make tagetitoxin unique among pythoxytins and among known RNA synthesis inhibitors. It is the first reported inhibitor that could allow for the selective inhibition of plastid RNA synthesis in planta. Tagetitoxin may, thus, provide a unique tool with which to study plastid RNA synthesis and processing, as well as nuclear/plastid interactions.

Acknowledgment—We thank Dr. Wilhelm Gruissem for providing plasmid templates and for helpful suggestions regarding plastid in vitro transcription reactions.

REFERENCES

Gruissem, W., and Zurawski, G. (1985a) EMBO J. 4, 1637–1644
Gruissem, W., and Zurawski, G. (1985b) EMBO J. 4, 3375–3383
Little, M. C., and Hallick, R. B. (1968) J. Biol. Chem. 263, 14302–14307

498 Tagetitoxin Inhibition of RNA Polymerases
Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and Escherichia coli.
D E Mathews and R D Durbin


Access the most updated version of this article at [http://www.jbc.org/content/265/1/493](http://www.jbc.org/content/265/1/493)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/265/1/493.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/265/1/493.full.html#ref-list-1](http://www.jbc.org/content/265/1/493.full.html#ref-list-1)