Proc. Nat. Acad. Sci. USA
Vol. 71, No. 1, pp. 30–34, January 1974

Mechanism of Inhibition of Eukaryotic Protein Synthesis by Trichothecene Fungal Toxins

(H-HeLa cells/yeast/polyribosomes/peptidyl transferase activity/initiation)

ERIC CUNDLIFFE, MICHAEL CANNON, AND JULIAN DAVIES

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisc. 53706

Communicated by R. H. Burris, September 6, 1973

ABSTRACT **The 12,13-epoxytrichothecenes, a group of sesquiterpenoid fungal antibiotics, inhibit protein synthesis in eukaryotic cells but do not share a common mode of action. Trichodermin stabilizes polyribosomes, prevents their disaggregation by puromycin, and also prevents the release of nascent peptides from ribosomes by puromycin. Nivalenol, T-2 toxin, and verrucarin A cause rapid and almost quantitative breakdown of polyribosomes in H-HeLa cells, a process which is inhibited by anisomycin, cycloheximide, or trichodermin. Similar effects of trichodermin, nivalenol, and verrucarin A are also observed in yeast spheroplasts. We conclude that nivalenol, T-2 toxin, and verrucarin A are potent and highly selective inhibitors of polypeptide chain initiation in eukaryotes, whereas trichodermin inhibits chain elongation and (or) termination. We have compared the structural formulae of various trichothecenes and suggest that the presence of substituents on carbon-15 of the common trichothecene ring may be important in determining the precise modes of action of this group of compounds.**

The 12,13-epoxytrichothecenes comprise a group of closely-related sesquiterpenoid antibiotics (toxins) produced by various fungal genera, namely *Fusarium*, *Trichoderma*, *Myrothecium*, and *Cephalosporium*. These compounds, which have wide-ranging biological activity against eukaryotic cells, are toxic to fungi, protozoa, insects, yeasts, plants, and animals and to various mammalian cell lines in tissue culture (for a comprehensive review, see ref. 1). The verrucarins (see Fig. 1*a*) are among the most potent cytotoxic substances known. The primary anatomical lesions in animals treated with trichothecenes include damage to the proliferating cells of the intestinal and gastric mucosa, bone marrow, and spleen. Frequently there is a characteristic lag of several hours before the onset of visible symptoms of toxicity. Cells in tissue culture develop abnormal chromosomes in the presence of trichothecenes; these antibiotics also disrupt the mitotic process.

Trichothecene-producing fungi are responsible for the spoilage of cereal crops and various fruits and they are probably of great importance in mycotoxicosis, since there is circumstantial evidence implicating trichothecenes in the poisoning of animals [e.g., moldy corn toxicosis of cattle, poultry, and pigs; fescue foot disease of cattle; alimentary toxic aleukia of humans; and stachybotryotoxicosis of humans and cattle (2)].

In mammalian cells in culture, trichothecenes first inhibit protein synthesis and then DNA synthesis, whereas synthesis of RNA is not severely affected (3–5). Similar effects were observed in protozoan cultures (6). The general conclusion from the work reported here, namely that the trichothecenes are inhibitors of eukaryotic protein synthesis, was established directly by Ueno and colleagues (7) who showed that nivalenol (Fig. 1*d*) inhibits protein synthesis *in vitro* in a rabbit-reticulocyte extract programmed with poly(U). Subsequently, trichodermin was shown to inhibit *in vitro* polypeptide chain termination with mammalian ribosomes (refs. 8–10, and W. Tate and C. T. Caskey, personal communication). By contrast, fusarenone X (Fig. 1*e*) caused disaggregation of polyribosomes in mouse fibroblasts (12), an effect which was inhibited by cycloheximide. Here, we describe the effects of a number of trichothecenes, singly an in combination with

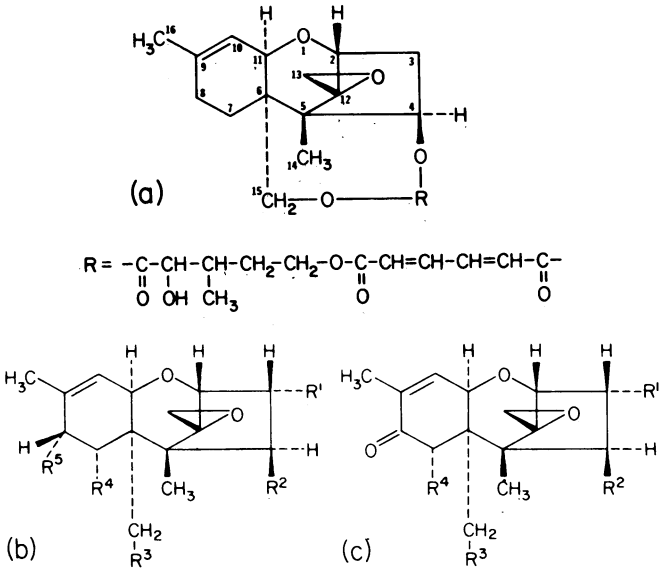

FIG. 1. The structure of various 12,13-epoxytrichothecenes. (*a*) Verrucarin A. The verrucarins and roridins differ in the composition of R. (*b*) Trichodermin and T-2 toxin. For trichodermin R^1 = H, R^2 = O·CO·CH_3, R^3 = R^4 = R^5 = H. For T-2 toxin R^1 = OH, R^2 = R^3 = O·CO·CH_3, R^4 = H, R^5 = O·CO·CH_2·HC(CH_3)(CH_3). (*c*) Nivalenol and fusarenone X. For nivalenol R^1 = R^2 = R^3 = R^4 = OH. For fusarenone X, R^1 = R^3 = R^4 = OH, R^2 = O·CO·CH_3. For trichothecolone R^1 = R^3 = R^4 = H, R^2 = OH. For trichothecin R^1 = R^3 = R^4 = H, R^2 = O·CO·HC = CH·CH_3.

Abbreviations: TMNa buffer, 10 mm Tris, 15 mM $MgCl_2$, and 140 mM NaCl adjusted with HCl to pH 7.6 at 20°; Me_2SO, dimethylsulfoxide.

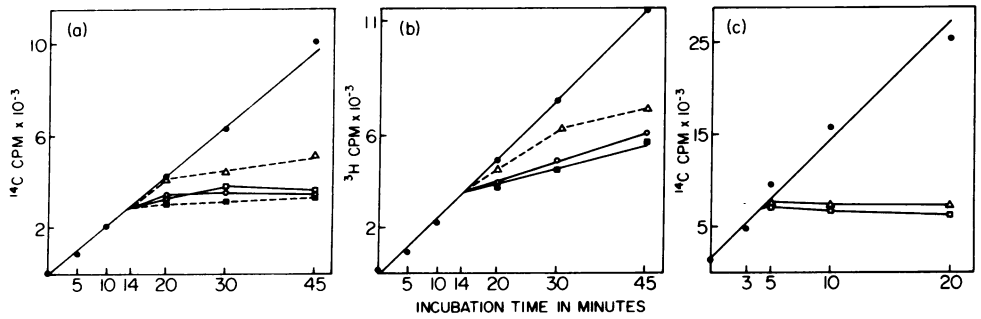

FIG. 2. Effects of trichothecenes on the synthesis of protein and RNA. Yeast spheroplasts (40-ml sample) prepared as described in *Materials and Methods* were mixed with [^{3}H]uridine (50 μg). Samples (0.5-ml) were removed into ice-cold 10% trichloroacetic acid (0.5 ml) containing 0.25 mg/ml of uridine and 0.5 mg/ml of leucine at the times indicated. After 14 min incubation, spheroplast samples were mixed with various drugs (50 μg/ml) and further aliquots removed as illustrated. H-HeLa cells in 35 ml of medium AL (13) were mixed with [^{14}C]leucine (3 μCi) and aliquots (0.5 ml) removed into trichloroacetic acid after 0, 3, and 5 min of incubation. Samples of cells were then mixed with various drugs (25 μg/ml) and further aliquots removed as illustrated. All aliquots were processed and counted as described in *Materials and Methods*. (*a*) Uptake of leucine and (*b*) uptake of uridine by yeast spheroplasts; (*c*) uptake of leucine by H-HeLa cells. ●, control; △, T-2 toxin added; □, trichodermin added; ○, verrucarin A added; ■, cycloheximide added. For both (*b*) and (*c*), results with verrucarin A and trichodermin gave identical plots. (The numbers on the ordinates have been multiplied by 10^{-3})

other antibiotics, on protein synthesis in intact H-HeLa cells and in yeast spheroplasts to characterize more precisely the mechanism of their inhibitory activity.

MATERIALS AND METHODS

Growth of H-HeLa Cells. H-HeLa cells were grown in Medium B containing 10% serum protein (12) at 37° with orbital shaking in glassware treated with Siliclad (Clay-Adams, Parsippany, N.J.). For experiments involving the incorporation of radioactive leucine into nascent proteins, cells were harvested at 10,000 × *g* for 5 min in a Sorvall RC-2B centrifuge and resuspended in a similar medium AL (13) lacking amino acids and serum. In such cases, experiments were concluded within 45 min of the change of medium. In all experiments, the H-HeLa cells used were growing exponentially at densities of 3.5 to 4 × 10^{5} cells per ml.

Sampling of H-HeLa Cells and Preparation of Lysates. Samples (5 ml or 10 ml, as specified in legends to tables and figures) were pipetted into an equal volume of frozen, crushed TMNa buffer (10 mM Tris, 15 mM $MgCl_2$, and 140 mM NaCl adjusted with HCl to pH 7.6 at 20°) which was allowed to thaw slowly at 0°. The cells were collected by centrifugation at 10,000 × *g* for 5 min at 4° in a Sorvall centrifuge with a SS-34 rotor; pellets were resuspended in 0.3 ml TMNa buffer and Nonidet P40 (Shell Chemical Co., New York) detergent (0.5% v/v final concentration) was added. Lysis occurred within 5 min at 0°. Pancreatic ribonuclease (10 μg/ml, final concentration) was added to those lysates derived from cell cultures incubated with [^{3}H]leucine (see legend to Fig. 6 and ref. 14).

Sucrose Density-Gradient Analysis. H-HeLa cell lysates (0.3 ml) were layered onto 5 ml of 15–30% (w/v) sucrose gradients made up in TMNa buffer. Centrifugation was at 4° at 35,000 rpm for 40 min (polyribosome experiments) or 45,000 rpm for 75 min (RNase-treated lysates) in the Spinco ultracentrifuge with an SW50.1 rotor. Absorbance by components in the gradients was measured at 254 nm in an Isco model DUA-2 gradient analyzer pumping at 1 ml/min and with a chart speed of 20 mm/min. For gradients containing radioactively labeled nascent proteins, 50 fractions each of 0.1 ml were collected on Whatman 3 MM paper discs after passage through the flow cell. These were washed twice in 5% (w/v) trichloroacetic acid, twice in ethanol:ether (50:50 v/v) and once in ether. Each wash was for 5 min. After the discs were dried, the radioactivity they retained was estimated by liquid scintillation using a toluene based fluor. To analyze polyribosomes from spheroplasts of *Saccharomyces cerevisiae* before or after exposure to a specific drug, the method of Cannon *et al.* (15) was employed. Syntheses of both RNA and protein in spheroplasts were monitored in a double-labeling experiment using [^{3}H]uridine and [^{14}C]-leucine (15). Protein synthesis in H-HeLa cells in the absence or presence of drug was studied by measuring uptake of [^{14}C]-leucine. Each sample was transferred directly into ice-cold 10% trichloroacetic acid and processed and counted as described previously (15).

All toxins were dissolved in dimethylsulfoxide (Me_2SO) so that final concentrations of Me_2SO in experimental reaction mixtures were always less than 1% (v/v). In control experiments, Me_2SO did not contribute to any of the effects reported here.

Anisomycin and cycloheximide were dissolved (5 mg/ml) in water. Puromycin dihydrochloride was dissolved (10 mg/ml) in TMNa buffer. [*5,6*-^{3}H]Uridine (42.4 Ci/mmol) was obtained from the New England Nuclear Corp., Boston, Mass. L-[*U*-^{14}C]Leucine (342 mCi/mmol) was obtained from the Radiochemical Center, Amersham, as was L-[*4,5*-^{3}H] leucine (58 Ci/mmol).

RESULTS

Each of the antibiotics used in this study is a potent inhibitor of protein synthesis both in H-HeLa cells and in yeast spheroplasts (Fig. 2). Also, in agreement with others (3–6, 11) we find that the trichothecenes do not primarily inhibit RNA synthesis.

The effects of various trichothecenes upon polyribosome profiles in intact H-HeLa cells and in yeast spheroplasts are illustrated in Fig. 3*a–f*. In other experiments (data not given) polyribosome profiles indistinguishable from controls (Fig. 3*a*) were obtained from H-HeLa cells following treatment with anisomycin (200 μg/ml) or cycloheximide (200 μg/ml). For treatment of yeast spheroplasts with cycloheximide, see Fig. 4*c*. The trichothecenes caused rapid and virtually com-

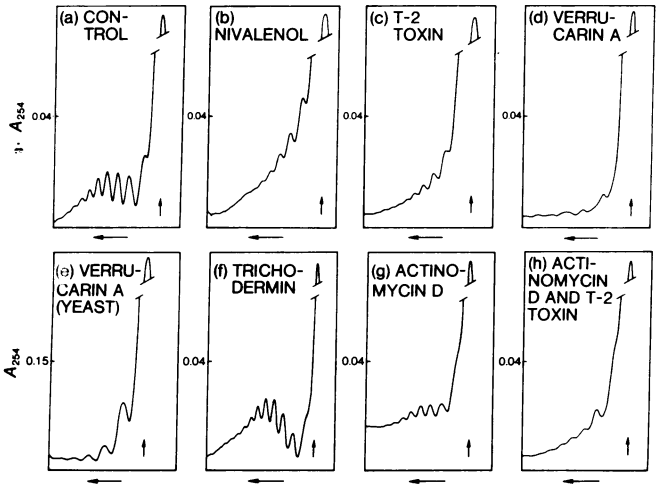

FIG. 3. Effects of trichothecenes on polyribosomes *in vivo*. H-HeLa cells (10 ml) in medium B supplemented with serum protein were sampled and lysed as described in *Materials and Methods*. Polyribosome profiles derived from H-HeLa cells incubated in the absence of antibiotics [control(*a*)] and from H-HeLa cells (except where indicated as yeast spheroplasts) incubated with antibiotics: (*b*) nivalenol (15 μg/ml) for 1 min, (*c*) T-2 toxin (25 μg/ml) for 15 sec, (*d*) verrucarin A (25 μg/ml) for 1 min, (*e*) verrucarin A (25 μg/ml) for 1 min (yeast spheroplasts), (*f*) trichodermin (25 μg/ml) for 1 min, (*g*) actinomycin D (10 μg/ml) for 7 min, and (*h*) actinomycin D (10 μg/ml) plus T-2 toxin (25 μg/ml) together for 1 min. [Experimental conditions for using yeast spheroplasts (*e*) are described in *Materials and Methods*.] In all cases, the position of the 80S ribosome peak is marked with a *vertical arrow* and the left side of each tracing represents the bottom of the gradient. The *horizontal arrow* under abscissa represents the direction of sedimentation of polyribosomes.

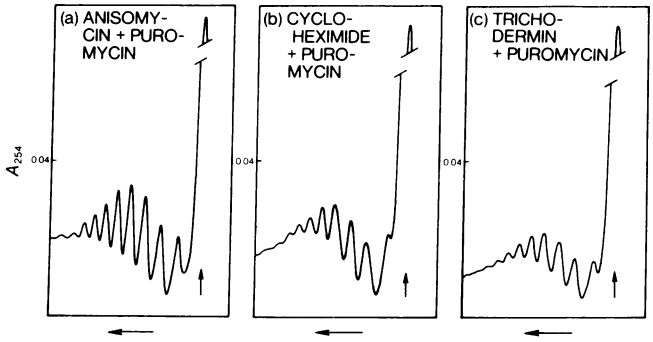

FIG. 5. Effects of antibiotics on blocking the puromycin-induced degradation of polyribosomes. The experiment was carried out as described for Fig. 3. Prior to sampling, H-HeLa cells were incubated with antibiotics: (*a*) anisomycin (250 μg/ml) for 1 min then puromycin (50 μg/ml) for 1 min, (*b*) cycloheximide (250 μg/ml for 1 min then puromycin (50 μg/ml) for 1 min, and (*c*) trichodermin (25 μg/ml) for 1 min then puromycin (50 μg/ml) for 1 min. Treatment with puromycin alone (not shown) caused disappearance of all polyribosomal material.

plete breakdown of polyribosomes. The exception is trichodermin which did not alter the polyribosome profile. When breakdown occurred, it was essentially complete within 60 sec and was much more rapid than that observed in the presence of actinomycin D (Fig. 3*g*). Also, when H-HeLa cells were exposed to T-2 toxin and to actinomycin D simultaneously, polyribosomes were degraded at the rapid rate characteristic of the effects of T-2 toxin alone (Fig. 3*h*). In yeast spheroplasts (data not shown), T-2 toxin induced only slow breakdown of polyribosomes (half-life of 10–15 min). This relatively slow effect may result from impermeability of the yeast cell to T-2 toxin; cell-free systems from yeast are extremely sensitive to the toxin (D. Schindler, personal communication). Over 50% of the polyribosomes disappeared after treatment with nivalenol, T-2 toxin, or verrucarin A (Fig. 3*a–e*). This point is of considerable significance (see below) as is the fact (data not given) that when trichothecene concentrations were increased there was no qualitative or quantitative change in polyribosome patterns (e.g., from 1 to 100 μg/ml in the case of T-2 toxin added to H-HeLa cells, and from 25 to 100 μg/ml for verrucarin A acting on yeast spheroplasts).

To discriminate between the various possible modes of action of trichothecenes, we studied their effects, in combination with other antibiotics of known action, on polyribosome profiles. As shown in Fig. 4*a* and *b*, pretreatment of H-HeLa cells with anisomycin prevented breakdown of polyribosomes when verrucarin A or T-2 toxin was added subsequently. Similarly (Fig. 4*c* and *d*), pretreatment of yeast spheroplasts with cycloheximide prevented the breakdown of polyribo-

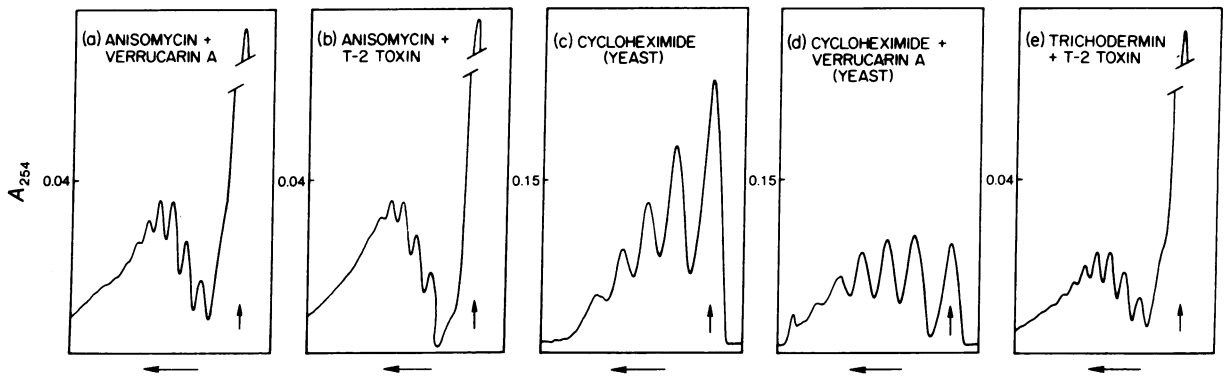

FIG. 4. Effects of other antibiotics on trichothecene-induced breakdown of polyribosomes *in vivo*. The experiment was carried out as described in the legend to Fig. 3. Prior to sampling, H-HeLa cells or yeast spheroplasts were incubated with antibiotics: (*a*) anisomycin (250 μg/ml) for 3 min then verrucarin A (25 μg/ml) for 1 min (H-HeLa cells), (*b*) anisomycin (250 μg/ml) for 3 min then T-2 toxin (25 μg/ml) for 1 min (H-HeLa cells) (*c*) cycloheximide (25 μg/ml) for 3 min (yeast spheroplasts) (*d*) cycloheximide (25 μg/ml) for 2 min then verrucarin A (25 μg/ml) for 1 min (yeast spheroplasts), and (*e*) trichodermin (25 μg/ml for 3 min then T-2 toxin (25 μg/ml) for 1 min (H-HeLa cells).

TABLE 1. *Effects of anisomycin, cycloheximide, and trichodermin on the release of nascent peptides from ribosomes of H-HeLa cells by puromycin in vivo*

Treatment	% Nascent peptides on ribosomes
Anisomycin	100
Anisomycin then puromycin	97
Cycloheximide	100
Cycloheximide then puromycin	89
Trichodermin	100
Trichodermin then puromycin	97
Controls: No drug added	100
Puromycin alone	13

H-HeLa cells in medium AL(13) were incubated at 37° with [^{3}H]leucine to label nascent peptides (as in Fig. 6) before protein synthesis was stopped by the addition of anisomycin (250 μg/ml, final concentration), cycloheximide (250 μg/ml), or trichodermin (25 μg/ml). Two minutes after adding the antibiotic, aliquots (5 ml) were removed and puromycin (50 μg/ml) was added to the remaining cells. After 1-min incubation, further samples were taken. All samples were lysed, treated with ribonuclease, and analyzed as described for Fig. 6 and in *Materials and Methods*. The progress of puromycin-induced peptide release was followed by comparing the ratio of total ^{3}H in nascent peptides on ribosomes:total absorbance of ribosomal material in each sucrose gradient. Values of this ratio obtained from control lysates in the presence of a given drug (not treated with puromycin) were normalized to 100%. The amount of ribosomal material present in a given gradient was estimated from the area under the A_{254} nm peak.

somes in the presence of verrucarin A. Both anisomycin and cycloheximide are known inhibitors of peptide chain elongation (for a review, see ref. 16). Similar results were obtained when nivalenol was added after pretreatment with anisomycin (data not shown). In other experiments (Fig. 4*e*), trichodermin resembled anisomycin in preventing disaggregation of polyribosomes in the presence of other trichothecenes.

Finally, we examined the effects of puromycin in H-HeLa cells treated with anisomycin, cycloheximide, or trichodermin. In this system, as in many others, puromycin causes extensive degradation of polyribosomes as a consequence of stripping nascent polypeptides from functional ribosomes (see ref. 16). Anisomycin, cycloheximide, and trichodermin each prevented both breakdown of polyribosomes (Fig. 5*a–c*) and release of nascent peptides from ribosomes (Table 1) in the presence of puromycin. In the experiments reported in Table 1, the ribonuclease treatment degrades polyribosomes to 80S monosomes without displacing the nascent peptides and, by localizing the 80S monosomes in sucrose density gradients, facilitates the estimation of the amount of nascent peptide material present per ribosome (see ref. 14). A typical sucrose gradient profile of an extract of H-HeLa cells treated in this way is shown in Fig. 6, and the data reported in Table 1 are derived from a number of such experiments.

DISCUSSION

With the exception of trichodermin, all the trichothecenes studied here resembled fusarenone X (11) in causing the disaggregation of eukaryotic polyribosomes *in vivo* (Fig. 3*a–f*). The extreme rapidity of this process (essentially complete within 60 sec) compared with the much slower rate of dis-

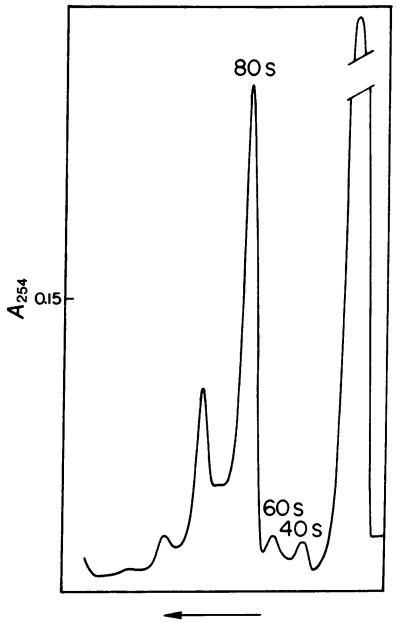

FIG. 6. Sucrose gradient analysis of a H-HeLa cell lysate treated with ribonuclease. H-HeLa cells in medium AL(13) were incubated with [^{3}H]leucine (10 μCi/ml, final concentration) for 3 min before samples (5-ml) were removed and lysed as described in *Materials and Methods*. Before centrifugation on sucrose density gradients, lysates were treated with pancreatic ribonuclease (10 μg/ml) for 5 min at 0°.

aggregation observed in the presence of actinomycin D (Fig. 3*g*) convinces us that these effects cannot be attributed to inhibition of mRNA synthesis by trichothecenes. This conclusion is in agreement with our precursor-incorporation data (Fig. 2) and with the previous work of others (3–5, 11). Since disaggregation of polyribosomes induced by trichothecenes was inhibited by antibiotics such as cycloheximide or anisomycin, which inhibit the elongation of nascent polypeptides (16), we conclude that this process involved "run-off" of ribosomes from mRNA, i.e., the process involved protein synthesis.

This interpretation apparently conflicts with results presented in a recent paper by Carrasco *et al.* (17). Here, the authors claim that the trichothecenes (verrucarin A, trichodermin, and fusarenone X) are potent inhibitors of peptide bond formation, as assayed with human-tonsil ribosomes. Although it is possible in principle to cause partial (up to 50% maximum) degradation of polyribosomes with any inhibitor of polypeptide chain elongation (for a discussion of this point, see refs. 16 and 18) we do not believe that such considerations apply here for three reasons. First, we observed more than 50% breakdown of polyribosomes in the presence of trichothecenes (Fig. 3). Second, the amount of polyribosome run-off did not decrease as the trichothecene concentration was increased. Third, the rate of breakdown of polyribosomes in the presence of trichothecenes was very much greater than that induced by actinomycin D; in general low concentrations of inhibitors of polypeptide chain elongation cause breakdown to occur at a rate closely resembling that with actinomycin D (E. Cundliffe, unpublished data). Accordingly, we conclude that those trichothecenes which cause breakdown of polyribosomes do so by inhibiting polypeptide chain initiation in a highly specific fashion.

The results of Carrasco *et al.* (17) were obtained using cell-free systems with isolated ribosome monomers. In the past, similar systems have given ambiguous results as to the exact modes of action of antibiotics in bacteria, e.g., lincomycin and the streptogramins (16). Our results contrast with those for pactamycin, which preferentially inhibits polypeptide chain initiation only at carefully selected low concentrations but which inhibits chain elongation at higher concentrations (19).

Trichodermin apparently prevents polypeptide chain elongation and, since this compound inhibits puromycin-induced release of peptidyl tRNA (the puromycin reaction) *in vivo*, it almost certainly inhibits either the peptidyl transferase reaction or the translocation process (for a discussion, see refs. 14 and 16). Recent evidence favors inhibition of the peptidyl transferase reaction (17). This difference in mode of action between trichodermin and other trichothecenes studied here is apparent from the data in Fig. 4*f* which showed that polyribosome breakdown induced by T-2 toxin was inhibited by trichodermin.

After examining the structural formulae of the various toxins studied (Fig. 1*a–c*), we suggest that the nature of substituents on carbon-15 may be particularly significant in determining their precise modes of action. In trichodermin, carbon-15 is part of an unsubstituted methyl group, whereas the other trichothecenes used here have oxygen-containing substituents on carbon-15. We suggest that the 12,13-epoxytrichothecenes probably bind to ribosomes, perhaps covalently via opening of the epoxide ring, and that the presence or absence of substitutents on C-15 (a prominent position of the molecule as judged from space-filling models) determines their precise modes of action. This line of reasoning leads us to predict that compounds such as trichothecin and trichothecolone (see Fig. 1*c*) will resemble trichodermin in mode of action whereas diacetoxyscirpenol (as trichodermin but R^1 = OH, R^3 = $O \cdot CO \cdot CH_3$, see Fig. 1*b*) and HT-2 toxin (as T-2 toxin but R^2 = OH, see Fig. 1*b*) will be inhibitors of eukaryotic polypeptide-chain initiation.

Recently, trichodermin has been shown to effect peptidyl transferase activity and thus inhibit polypeptide chain termination (refs. 8–10, and W. Tate and C. T. Caskey, personal communication). Although our results could support this suggestion (see also ref. 17), it should be noted that peptidyl transferase activity is an integral part of the termination process and that specific inhibition of the termination event might be a rare phenomenon. However, this could occur if a drug were to interact directly with one or other of the peptide release factors rather than with the ribosome itself. In any event, the 12,13-epoxytrichothecenes are closely related chemically and if they all bind to the same ribosomal site(s) it will be interesting to discover how relatively minor modifications of the trichothecene molecule can perturb ribosomal function in different ways.

Note added in proof. The importance of substitution on carbon-15 of the epoxytrichothecenes has been confirmed in subsequent studies in which we have shown that diacetoxyscirpenol and HT-2 toxin resemble T-2 toxin in inhibiting polypeptide chain initiation, and trichothecin and trichodermol resemble trichodermin in mode of inhibition of eukaryotic protein synthesis. (Cannon, M., unpublished observations, Schindler, D., *Nature*, in press). We wish to thank Dr. Calvin McLaughlin for providing us with a preprint of work describing evidence that trichodermin blocks the activity of peptidyl transferase required for normal termination of polypeptide chains in eukaryotic protein synthesis. (Wei, C.-M, Hansen, B. S., Vaughan, M. H., Jr., and McLaughlin, C. S., *Proc. Nat. Acad. Sci*, in press).

We are extremely grateful to Dr. Frank Strong for discussions and for providing samples of many of the trichothecenes. Generous samples of trichodermin and verrucarin A were provided by Drs. W. O. Godtfredsen and Ch. Tamm. Drs. R. R. Rueckert, T. Matthews, and K. C. Medappa very kindly instructed us in the techniques necessary for experimentation with mammalian cells in culture. This work was supported by grants from the National Institutes of Health (AI 10076), The Medical Research Council of Great Britain, and Grant no. IN-35 from the American Cancer Society.

1. Bamburg, J. R. & Strong, F. M. (1971) in *Microbial Toxins*, eds. Kadis, S., Ciegler, A. & Ajl, S. J. (Academic Press Inc., New York), Vol. 7, pp. 207–292.
2. Eppley, R. M. & Bailey, W. J. (1973) *Science* **181**, 758–760.
3. Tatsuno, T. (1968) *Cancer Res.* **28**, 2393–2396.
4. Ueno, Y. & Fukushima, K. (1968) *Experimentia* **24**, 1032–1033.
5. Ohtsubo, K., Yamada, M. & Saito, M. (1968) *Jap. J. Med. Sci. Biol.* **21**, 185–194.
6. Nakano, N. (1968) *Jap. J. Med. Sci. Biol.* **21**, 351–357.
7. Ueno, Y., Hosoya, M., Morita, Y., Ueno, I. & Tatsuno, T. (1968) *J. Biochem. (Tokyo)* **64**, 479–485.
8. Wei, C.-M. & McLaughlin, C. S. (1973) *Fed. Proc.* **32**, abstr. 1538.
9. Hansen, B. S. & Vaughan, M. H., Jr. (1973) *Fed. Proc.* **32**, abstr. 1539.
10. Stafford, M. E. & McLaughlin, C. S. (1973) *J. Cell. Physiol.*, in press.
11. Ohtsubo, K., Kaden, P. & Mittermayer, C. (1972) *Biochim. Biophys. Acta* **287**, 520–525.
12. Medappa, K. C., McLean, C. & Rueckert, R. R. (1971) *Virology* **44**, 259–270.
13. Butterworth, B. E., Hall, L., Stoltfus, C. M. & Rueckert, R. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3083–3087.
14. Cundliffe, E. & McQuillen, K. (1967) *J. Mol. Biol.* **30**, 137–146.
15. Cannon, M., Davies, J. E. & Jimenez, A. (1973) *FEBS Lett.* **32**, 277–280.
16. Cundliffe, E. (1972) "Antibiotic inhibitors of ribosome functions," in *Molecular Basis of Antibiotic Action*, Authors Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H. & Waring, M. J. (Wiley Interscience, New York), pp. 278–379.
17. Carrasco, L., Barbacid, M. & Vazquez, D. (1973) *Biochim. Acta* **312**, 368–376.
18. Cundliffe, E. (1967) *Mol. Pharmacol.* **3**, 401–411.
19. Stewart-Blair, M. L., Yanowitz, I. S. & Goldberg, I. H. (1971) *Biochemistry* **10**, 4198–4206.