ON THE MECHANISM OF ADAPTATION TO PROTEIN SYNTHESIS INHIBITORS BY TETRAHYMENA

Facilitation, Cross Adaptation, and Resensitization

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ABSTRACT

Tetrahymena is able to adapt to the presence of sublethal concentrations of many drugs which inhibit a wide variety of cellular functions. In spite of the generality of this phenomenon in Tetrahymena, the mechanism of adaptation at the cellular and molecular levels is unknown. This study deals mainly with adaptation to the protein synthesis inhibitors, cycloheximide and emetine. The physiological response of Tetrahymena to sublethal concentrations of these drugs is an immediate cessation of cell division for a period of time dependent on the drug concentration, followed by an abrupt resumption of exponential growth at a constant rate. By measuring the length of the growth lags under a variety of experimental conditions, we have confirmed several observations made by Frankel and coworkers, and provide evidence for two new phenomena associated with adaptation to cycloheximide: (a) adaptation to cycloheximide also results in adaptation of cells to emetine, another protein synthesis inhibitor not closely related structurally to cycloheximide. We have termed this phenomenon cross adaptation, (b) exposure to concentrations of cycloheximide too low to cause any growth lags or inhibition of protein synthesis significantly shortens the time required by cells to adapt to higher concentrations of cycloheximide. We have termed this phenomenon facilitation. Facilitation shows some degree of specificity in that facilitation with cycloheximide has no effect on adaptation to emetine. From this, we infer the existence of two distinct systems involved in adaptation to cycloheximide, one of which shows a higher degree of specificity towards cycloheximide than the other. We also show that transfer of adapted or facilitated cells to drug-free medium results in a gradual but complete resensitization. The kinetics of resensitization suggest that the cellular machinery responsible for adaptation and facilitation does not leave the cell, but is simply diluted out during cell division.

INTRODUCTION

Tetrahymena has the remarkable ability to adapt to a wide range of drugs at initially inhibitory concentrations (1). This phenomenon was originally described by Frankel in the amicronucleate strain GL-C (2) and subsequently termed “recovery.” We have elected to use the term “adaptation” introduced by Rasmussen and Zeuthen (3) since this response occurs in the continued pres-
ence of a drug, rather than after its removal. The most studied example in *Tetrahymena* is adaptation to the protein synthesis inhibitor, cycloheximide (1, 4, 5). Adaptation to cycloheximide has recently been demonstrated also in cultured plant cells (Rosa, 6).

The objectives of this study were to confirm and extend to syngen I previous observations on cycloheximide adaptation and to examine more closely the mechanism of induction and the specificity of the response. Specifically, since adaptation to cycloheximide is reported not to cause adaptation to the unrelated drug colchicine (1), we investigated the possibility of interaction between adaptation to cycloheximide and adaptation to emetine, another protein synthesis inhibitor. While emetine has no close structural similarity to cycloheximide (see, however, reference 7), both drugs appear to have a similar mode of action in that they inhibit translocation by eukaryotic cytoplasmic ribosomes (8–10). It is not yet known whether both drugs act by binding at the same site, although the isolation of cycloheximide-resistant mutants of *Saccharomyces cerevisiae* that yield cell-free extracts resistant to cycloheximide but not to emetine (11) suggests that these drugs may act at different sites. This study was undertaken in view of (a) the generality of the phenomenon of adaptation in *Tetrahymena*, (b) the intrinsic interest of the mechanism of the response, and (c) the practical importance of understanding the cellular response to a widely used protein synthesis inhibitor, such as cycloheximide.

We describe below our finding that *Tetrahymena* also adapts to the presence of emetine and that cycloheximide and emetine can induce cross adaptation to each other. We have also detected a very sensitive and specific response to cycloheximide, which we have termed "facilitation." Finally, we report evidence that adaptation and facilitation are completely reversible phenomena; the adapted (or facilitated) state decays with a half-life of one doubling time during growth after removal of the drug.

**MATERIALS AND METHODS**

**Strains**

All experiments reported in this paper were performed with wild-type strain D1968-5 of *Tetrahymena pyriformis*, syngen I, previously described (12).

**Media**

The cells were grown and exposed to the antibiotics in PYY/P+S medium (12), which contains proteose peptone, yeast extract, salts, penicillin, and streptomycin. The penicillin and streptomycin were present to prevent bacterial contamination and have no effect on growth rate or the response to the drugs studied in this paper. In preliminary experiments, some variability in the duration of growth lags caused by emetine (but not by cycloheximide) was observed with different batches of proteose peptone. For this reason, all of the experiments involving emetine were performed in medium containing proteose peptone from a single batch (Difco control number 551192, Difco Laboratories, Detroit, Mich.).

**Antibiotics**

Cycloheximide and emetine HCl were obtained from Sigma Chemical Co., St. Louis, Mo. Streptimidone (A grade) was obtained from Calbiochem, La Jolla, Calif. Glutarimide was obtained from Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N.Y. Stock solutions were prepared with sterile, distilled H2O and used immediately. The structures of these compounds are shown in Fig. 1.

**Growth Conditions**

Cultures were grown in 500- or 125-ml culture flasks in an Eberbach shaking water bath (Eberbach Corp., Ann Arbor, Mich.) at 30°C at 150 oscillations per minute.

**Cell Counts**

Culture samples, diluted with filtered 0.89% (wt/vol) NaCl, were counted with a Celsoscope (Particle Data, Inc., Elmhurst, Ill.), as previously described (12).

**Centrifugation**

Cells were pelleted by centrifuging 10-ml aliquots in 15-ml conical test tubes in a clinical centrifuge at 700 g for 30 s at room temperature. Exponentially growing...
cells treated in this manner resume exponential growth after resuspension at the original rate without any apparent lag in growth.

**Calculation of growth lags**

A regression line was calculated for the points in the rising portion of a curve such as that shown in Fig. 2, and the intersection of this line with the horizontal portion of the curve was taken as the end of the lag period. The length of the lag period was taken to be the difference between this time and the time of addition of the drug. The error bars in the figures and the deviations in the table represent the 95% confidence intervals obtained from the regression analyses.

**RESULTS**

**Effect of Cycloheximide on Strain D1968-5**

Exposure of exponentially growing cells to concentrations of cycloheximide from 0.01 to 1.0 μg/ml results in an immediate inhibition of cell division. After a lag period, roughly proportional to the log of the cycloheximide concentration, the cells abruptly resume exponential growth at a rate lower than that exhibited before exposure to the drug. The effect of 0.25 μg/ml of cycloheximide on wild-type cells is shown in Fig. 2, and the concentration dependence of this growth lag is shown in Fig. 3. The concentration dependence of the growth lag is similar to the concentration dependence of the division delay induced by cycloheximide in heat-synchronized cells of strain GL-C (4). After the resumption of exponential growth, the cells are said to have “adapted” to the presence of cycloheximide. These adapted cells grow at a lower rate in cycloheximide (Fig. 2) but attain normal culture densities when grown to stationary phase (data not shown). If adapted cells are washed by centrifugation and resuspended in medium containing the same concentration of cycloheximide, no lag in growth is observed.

**Facilitation**

In order to investigate the inducibility of adaptation to cycloheximide, the response to cycloheximide after preincubation with low concentrations of cycloheximide was examined. It was found that preincubation of cells in concentrations of cycloheximide lower than those that cause a detectable lag in growth or decrease in growth rate (<0.01 μg/ml) significantly reduces the growth lag exhibited on subsequent exposure to a higher cycloheximide concentration. This phenomenon, which we have termed “facilitation,” is illustrated in Fig. 4. The decrease in the growth lag is roughly proportional to the log of the preincubation concentration, and the decrease due to a given concentration is greater after longer preincubation periods, as illustrated in Fig. 5. Preincubation for periods longer than 12 h, however, does not further
FIGURE 4 Effect of preincubation on the growth lag in 0.25 μg/ml cycloheximide. Exponentially growing cells were diluted into drug-free medium or medium containing either 2.5 or 0.25 ng/ml cycloheximide and incubated for 12 h. Samples from each culture were then transferred to medium with (○) or without (●) 0.25 μg/ml cycloheximide and incubated for 5 h. Key to the symbols: curves 1 and 2, cells preincubated with 2.5 ng/ml cycloheximide; curves 3 and 4, cells preincubated with 0.25 ng/ml cycloheximide; curves 5 and 6, cells preincubated in drug-free medium.

decrease the growth lag caused by subsequent exposure to 0.25 μg/ml cycloheximide (data not shown).

Adaptation to Emetine in Strain D1968-5

Before investigating the relationship between adaptation to cycloheximide and adaptation to emetine, the response of *Tetrahymena* to emetine alone was examined. The effect of emetine on the growth of exponential cultures of strain D1968-5 was found to be similar to the effect of cycloheximide, except that adaptation to emetine occurs within the range of 1-20 μg/ml. Approximately 30-fold higher concentrations of emetine (corresponding to a 15-fold greater molarity) are required to produce a growth lag similar to that produced by a given concentration of cycloheximide. For example, the growth lag of wild-type cells exposed to 7.5 μg/ml emetine is identical to the growth lag due to 0.25 μg/ml cycloheximide.

Emetine-adapted cells, when washed and resuspended in drug-free medium, immediately resume growth at a rate similar to that of cells grown continuously in drug-free medium. This observation, and the fact that *Tetrahymena* can adapt to and resume exponential growth in the continued presence of emetine, suggest that in *Tetrahymena* the effect of emetine is reversible. This reversibility of inhibition of growth by emetine in *Tetrahymena* is in contrast to its reported irreversible inhibition of protein synthesis in human HeLa cells (8).

Cross Adaptation between Cycloheximide and Emetine

The experiments described below were conducted to study the response to one drug (emetine or cycloheximide) of cells adapted to the other drug. In the first experiment, the response of cycloheximide-adapted cells to emetine was studied. Unadapted, cycloheximide-adapted, and emetine-adapted cells were washed by centrifugation and resuspended in both drug-free medium and medium containing 7.5 μg/ml emetine. The results of this experiment are shown in Fig. 6 A. Cycloheximide-adapted cells (curve no. 3) exhibited no growth lag when resuspended in emetine. Control (unadapted) cells showed the full 2.5-h lag (curve no. 5). Control cells adapted to emetine
showed no lag when resuspended in the same concentration of emetine (curve no. 4). Thus, it is apparent that cells adapted to cycloheximide respond to a subsequent emetine treatment in a manner identical to that of cells previously adapted to emetine. We have termed this phenomenon "cross adaptation," meaning more precisely, cycloheximide-induced cross adaptation to emetine.

Two additional points in Fig. 6 A are worth noting. The absence of a growth lag in unadapted cells resuspended in drug-free medium (curve no. 1) supports the statement made in Materials and Methods that the centrifugation procedure employed does not detectably affect the growth of the cells. Second, cycloheximide-adapted cells resuspended in drug-free medium (curve no. 2) resume growth at the same rate as unadapted cells (curve no. 1), rather than at the lower rate characteristic of adapted cells in cycloheximide; thus the inhibitory effect of cycloheximide on the growth rate of adapted cells is immediately reversible.

The reciprocal effect of emetine-induced cross adaptation to cycloheximide is shown in Fig. 6 B. This experiment was identical to the experiment of Fig. 6 A, except that the washed cells were resuspended in medium with and without 0.25 μg/ml cycloheximide rather than 7.5 μg/ml emetine. A comparison of curves 4 (emetine-adapted cells resuspended in cycloheximide) and 5 (unadapted, control cells resuspended in cycloheximide) shows that adaptation to emetine shortens the growth lag exhibited upon subsequent exposure to cycloheximide, although the effect is not as complete as that caused by adaptation to cycloheximide itself (curve no. 3). Thus, this experiment demonstrates the occurrence of an emetine-induced cross adaptation to cycloheximide, although this effect appears to be somewhat less efficient than the reciprocal phenomenon demonstrated in the previous experiment. Curve 2 provides evidence that the inhibitory effect of emetine on the growth rate of adapted cells (like the effect of cycloheximide) is immediately reversible when the drug is removed.

**Lack of Facilitation with Emetine**

Since adaptation to one drug can be induced by treatment with the other drug, it was of interest to determine whether a similar situation holds true.
for facilitation, i.e., whether facilitation also occurs with emetine and whether cross facilitation occurs. This was done by investigating the effect of preincubation with low concentrations of cycloheximide or emetine on the response to a high concentration of emetine and the effect of preincubation with a low concentration of emetine on the response to a high concentration of cycloheximide.

The following results were obtained: (a) preincubation of cells with a low concentration of emetine (0.075 μg/ml) does not decrease the growth lag exhibited upon subsequent exposure to a 100-fold higher concentration of emetine (7.5 μg/ml); (b) preincubation with 2.5 ng/ml cycloheximide does not decrease the lag caused by subsequent exposure to a high concentration of cycloheximide (0.25 μg/ml). This result is significant in that this preincubation treatment decreases the lag caused by subsequent exposure to a 100-fold higher concentration of cycloheximide (0.25 μg/ml) by 60% (Fig. 4, curves 2 and 6), (c) preincubation with a low concentration of emetine (0.075 μg/ml) does not decrease the lag caused by subsequent exposure to a high concentration of cycloheximide (0.25 μg/ml). Thus, there is no evidence for any cross facilitation between cycloheximide and emetine.

**Resensitization of Adapted Cells**

The next series of experiments was conducted to study the kinetics of loss of the adapted state during growth after removal of the drug and to determine whether the cells return completely to the unadapted state under these conditions. The results show that when cycloheximide- or emetine-adapted cells are grown for several generations in drug-free medium, they gradually become resensitized. In Fig. 7, the duration of the growth lag produced in cycloheximide- or emetine-adapted cells when reexposed to either cycloheximide or emetine (expressed as a percentage of the duration of the growth lag induced in unadapted cells by these concentrations of cycloheximide or emetine) is plotted as a function of the number of generations of growth of the adapted cells in drug-free medium. If it is assumed that the length of the growth lag reflects the degree of resensitization, the loss of the adapted state of cycloheximide- and emetine-adapted cells appears to be exponential, with a half-life approximately equal to one doubling time in drug-free medium.

![Figure 7 Resensitization after (a) adaptation and (b) facilitation.](image-url)
The results described in the preceding paragraph are in apparent disagreement with a previous report to the effect that resensitization to cycloheximide occurs at a lower rate and is only 80% complete after seven generations of growth in drug-free medium (5). Although this disagreement could be attributed to differences between heat-synchronized, amicronucleate cells of strain GL-C and exponentially growing cells of strain D1968-5 of syngen 1, another explanation, possibly more plausible in view of the results reported in this study, is that the cells used in the previous study were not washed sufficiently and therefore remained in a facilitating concentration of cycloheximide during growth in what was thought to be drug-free medium. Under those circumstances, the lag produced in these incompletely washed cells by reexposure to a high concentration of cycloheximide would have been less than that produced in unadapted (or unfacilitated) cells, which would have led to the inference that resensitization was incomplete.

This prediction was justified by the results of the experiment given in Table I. In that experiment, cycloheximide-adapted cells were washed completely free of cycloheximide and resuspended in either drug-free medium or in medium containing facilitating concentrations of cycloheximide (to simulate incomplete washing), grown for seven generations, and then reexposed to the original concentration of cycloheximide (0.25 μg/ml). The lag exhibited by the completely washed cells was identical to the control lag induced in previously unadapted cells, showing that resensitization was complete after seven generations of growth in drug-free medium. Cells that were grown in 0.1 ng/ml for the seven generations, however, exhibited a lag that was 20% shorter than the control lag (which would give the impression that resensitization was only 80% complete), mimicking the results previously reported (5).

**Resensitization of Facilitated Cells**

An experiment similar to those of the previous section was performed to determine whether facilitated cells become resensitized to cycloheximide when grown in drug-free medium. The results of that experiment, also shown in Fig. 7, indicate that facilitated cells, when removed from preincubation concentrations of cycloheximide, gradually become resensitized over the course of several generations. The rate of resensitization of facilitated cells in drug-free medium is similar to the rate of resensitization of adapted cells in drug-free medium: the facilitated state, like the adapted state, decays with a half-life of approximately one doubling time.

### Table I

**Effect of Facilitating Concentrations of Cycloheximide on the Degree of Resensitization**

| Incubation conditions | Growth lag in 0.25 μg/ml cycloheximide | Control lag |
|-----------------------|---------------------------------------|-------------|
| **Before centrifugation** | **After centrifugation** | **min** | **%** |
| 0.25 μg/ml cycloheximide, 150 min | 0.25 ng/ml cycloheximide, 15 h | 98 ± 5 | 68.5 ± 3.3 |
| 0.25 μg/ml cycloheximide, 150 min | 0.1 ng/ml cycloheximide, 15 h | 118 ± 9 | 82.5 ± 6.2 |
| 0.25 μg/ml cycloheximide, 150 min | drug-free medium, 15 h | 142 ± 6 | 99.3 ± 4.1 |
| drug-free medium, 150 min | drug-free medium, 15 h | 143 ± 2 | — |

Exponentially growing cells were diluted twofold into 0.25 μg/ml cycloheximide, or fourfold into drug-free medium, and incubated for 150 min. Cells for each culture were then centrifuged and resuspended in drug-free medium twice. Samples of washed cells from the cycloheximide-treated culture were diluted 32-fold into medium containing 0.25 ng/ml cycloheximide, 0.1 ng/ml cycloheximide, or drug-free medium and incubated for 15 h (seven generations). A sample of washed cells from the untreated control culture was diluted 32-fold into drug-free medium and also incubated for 15 h. (This procedure resulted in an estimated 320,000-fold dilution of the cycloheximide in the original culture, so that the only significant amounts of cycloheximide present during the 15-h incubation period were those added to the 0.25 and 0.1 ng/ml cultures.) After 15 h, samples from each culture were added to drug-free medium and to medium containing 0.25 μg/ml cycloheximide. These cultures were then incubated for 5 h to obtain growth curves from which the lags shown in the Table were calculated.
Response of Strain D1968-5 to Streptimidone and Glutarimide

We have also investigated the response of *Tetrahymena* to streptimidone, a protein synthesis inhibitor (13) structurally related to cycloheximide (Fig. 1). It was found that streptimidone has effects similar to those of cycloheximide. Thus, it induces adaptation and facilitation to its own presence and cross adaptation to emetine. Furthermore, streptimidone and cycloheximide show cross adaptation and cross facilitation to each other. The molar concentration of streptimidone required to produce a given effect is about four times greater than the concentration of cycloheximide required to produce the same effect (C. T. Roberts, Jr., unpublished observations). This difference in effective concentration between streptimidone and cycloheximide is probably due to differing affinities of the two drugs for the same receptor, rather than to different sites of action, since a cycloheximide-resistant mutant derived from strain D1968-5 (14) is also more resistant to streptimidone, although not to emetine.

As can be seen in Fig. 1, cycloheximide contains a glutarimide moiety that is considered necessary for its biological activity (13). Glutarimide itself, however, does not inhibit cell growth (or, presumably, protein synthesis) at concentrations up to 1 mg/ml (C. T. Roberts, Jr., unpublished observations). To determine whether glutarimide could still induce facilitation or adaptation in the absence of any effect on growth, the effect of glutarimide on responses to cycloheximide and emetine was investigated. It was found that concentrations as high as 1 mg/ml do not decrease the growth lags exhibited on subsequent exposure to high concentrations of either cycloheximide or emetine.

DISCUSSION

Adaptation to Cycloheximide, Streptimidone, and Emetine, and Cross Adaptation between These Drugs

This study adds streptimidone and emetine to the long list of drugs to which *Tetrahymena* can adapt. The effect of sublethal concentrations of these drugs on exponentially growing cells, i.e., the immediate but temporary cessation of cell division followed by a resumption of exponential growth at a slightly slower rate, is very similar to the effect of cycloheximide. That this similarity is more than a superficial coincidence is indicated by our demonstration of cross adaptation between these drugs, i.e., the partial or complete adaptation of cells to one drug produced by adaptation to another drug.

We view adaptation to the protein synthesis inhibitors studied here as a process involving the interaction of the drug with a specific receptor. This interaction results in the appearance of an "effector," which is responsible for adaptation, i.e., the lowered biological activity of a given external drug concentration. Cross adaptation, in this context, could result from the appearance of the same effector in response to different drugs, or of different effectors with similar functions. Cross adaptation, then, could be a trivial consequence of two drugs being structurally so similar that they interact with the same receptor (though with possibly different affinities) to produce the appearance of the same effector of adaptation. Cross adaptation between cycloheximide and streptimidone is probably an example of this trivial case, since these drugs are structurally very similar. Cross adaptation between cycloheximide and emetine, however, is more difficult to explain on this basis, since these drugs are not closely related structurally. (A vague structural similarity has been proposed [7], and although chemical groups thought to be necessary for the cycloheximide molecule's biological activity [15] are either absent or substituted in emetine, these need not be the chemical groups important for the induction of adaptation.) Nevertheless, as discussed below, our results demonstrate qualitative differences between the responses to cycloheximide and emetine, which suggest that cross adaptation between cycloheximide and emetine may not have the trivial basis suggested above for cycloheximide-streptimidone cross adaptation.

Concerning the nature of the effector of adaptation, there are several possibilities for its mode of action: (a) the replacement of the "target" macromolecule by another with the same function in protein synthesis, but with lowered affinity for the drug; (b) an increase in the number of target macromolecules so as to "titrate out" the drug. These two possibilities appear unlikely since they would require a stoichiometric replacement or increase in the target protein, at a time when most protein synthesis is inhibited. In addition, a gradual recovery of the growth rate might have been expected, instead of the abrupt resumption of growth at a constant rate actually observed; (c)
circumvention of the requirement for the functioning of the target macromolecule. Since *Tetrahymena*, as well as other eukaryotic cells, possesses two apparently distinct and complete protein synthesis systems, such an adaptation mechanism could involve the cycloheximide-resistant synthesis on mitochondrial ribosomes (15) of proteins normally synthesized on cytoplasmic ribosomes; (d) a decrease in the internal concentration of the drug (or a possibly more active derivative of it), either through a detoxifying enzyme, altered permeability, failure to activate, or active exclusion of the drug from the cell (the latter possibly by the contractible vacuole) (1). Detoxification would eventually result in a decrease in the concentration of the drug in the medium. Since this does not occur with cycloheximide (1), this mechanism seems unlikely. The uptake of 14C-labeled cycloheximide during adaptation is currently under investigation to evaluate the possibilities of altered permeability and exclusion. Although some of the above possibilities are not likely to account singly for adaptation, their action in combination with others is not ruled out.

**Two Components of the Adaptation Response to Cycloheximide in *Tetrahymena***

In this study, we have discovered an effect of cycloheximide at very low concentrations, in the neighborhood of 0.25 ng/ml ($8.0 \times 10^{-19}$ M). Although cycloheximide, at these concentrations, has no effect on cell growth or protein synthesis in *Tetrahymena* (4), an effect was detected in the form of a decreased growth lag when cells preincubated with the lower concentration were challenged with 1000-fold higher concentrations. We have termed this phenomenon facilitation. The question can be raised as to whether facilitation and adaptation to cycloheximide represent the result of a single response system, with the empirical differences detected merely reflecting different strengths of the same response, or whether there are qualitative differences between the two responses. The use of emetine has provided evidence that appears to support the second possibility. We have shown that treatment with a high concentration of cycloheximide (0.25 µg/ml) results in adaptation to that concentration of cycloheximide and to 7.5 µg/ml emetine. In contrast, facilitation with a low concentration of cycloheximide (2.5 ng/ml), sufficient to reduce by 60% the growth lag exhibited when cells are subsequently challenged with 0.25 µg/ml cycloheximide, has no effect on the length of the growth lag caused by subsequently challenging these cells with 7.5 µg/ml emetine. On the basis of these results, we postulate the existence of two systems involved in the cellular response to cycloheximide. System I, responsible for facilitation, is induced by low concentrations of cycloheximide but not by correspondingly low concentrations of emetine; this response affects the kinetics of adaptation to cycloheximide but not to emetine. Thus system I, in its establishment, maintenance, and mode of action, discriminates between cycloheximide and emetine. System II, responsible for the classical adaptation response to cycloheximide originally described by Frankel (1), for adaptation to emetine and for cross adaptation (described above), is induced and maintained in response to higher concentrations of cycloheximide or emetine; this response exhibits little or no discrimination between cycloheximide and emetine. Further clarification of the relationship between these two systems requires knowledge of the molecular basis of their responses.

**Nature of the Adaptation Systems**

The kinetics of resensitization of facilitated and adapted cells support some further inferences concerning the nature of systems I and II, respectively. The observation that both the facilitated and adapted states decay with a half-life of one doubling time upon removal of cycloheximide suggests that the effector components required for facilitation and adaptation are diluted out as a result of cell division. Thus, while these components themselves appear to be stable, they appear to require the presence of cycloheximide for their induction and maintenance. These findings also suggest that they cannot diffuse or be transported out of the cell easily, and that they may therefore be (or be bound to) macromolecules. Additional growth delays are obtained upon challenging cells adapted to cycloheximide with a higher concentration of the drug (1; C. T. Roberts, Jr., unpublished observations; and T. Wang, personal communication). This suggests that the concentration of the effector is to some extent determined by the concentration of cycloheximide.

Concerning the production of the effector molecules, our experiments do not allow a distinction
Multiplicity of Cellular Receptors for Cycloheximide

The several responses by Tetrahymena to cycloheximide (facilitation, adaptation, and inhibition of protein synthesis) raise the issue of whether these effects are mediated by one or by several cellular receptors (i.e., components that can bind cycloheximide). The observations that facilitation by cycloheximide confers no protection against emetine while adaptation does and that the cycloheximide concentrations that induce facilitation and adaptation vary by as much as four orders of magnitude suggest that these responses involve different receptors. Since cycloheximide concentrations that induce facilitation do not detectably inhibit protein synthesis (4) or growth, it is probable that at least one receptor involved in facilitation is different from that involved in the inhibition of protein synthesis. The latter receptor is presumed to be a ribosomal component, since cycloheximide is thought to act at the peptidyl ribosomal site (9). Our results, then, suggest the existence of at least two (and perhaps more) functionally distinct cellular receptors for cycloheximide. The fact that very low concentrations of cycloheximide are effective in inducing facilitation supports the inference that at least one receptor has a very high affinity for cycloheximide.

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