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THE LACK OF EFFECT OF TUNICAMYCIN ON CILIA REGENERATION IN TETRAHYMENA PYRIFORMIS

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SUMMARY

Tetrahymena pyriformis cultures were starved, deciliated and incubated in a reciliation medium in the presence and absence of tunicamycin. This antibiotic was shown to have no effect on the rate of reciliation or the appearance of the organisms even when present in relatively large amounts, although cell division was blocked. A small inhibition of protein synthesis was noted in the presence of tunicamycin, but mannose incorporation was totally abolished. It was concluded that the reciliation of these organisms takes place by a mechanism that does not require the de novo synthesis of glycoprotein.

INTRODUCTION

Tunicamycin is an acetylglucosamine-containing antibiotic which has been shown to be a potent inhibitor of UDP-GlcNAc:dolichyl-phosphate GlcNAc-1-phosphate transferase (1-3), an initial step in glycoprotein glycosylation. Frisch et al. (4) have shown that cell division and conjugation can be inhibited completely by exposing this organism to as little as 1.6 mg/ml of this antibiotic. The incorporation of labeled glucosamine into alcohol precipitable material was inhibited 40% with little effect on protein synthesis, thus indicating the participation of glycoprotein in the mating process. Richmond (5) also demonstrated an inhibitory effect of tunicamycin on the conjugation process but did not observe a significant drop in glucosamine incorporation.

The sensitivity of this organism to tunicamycin prompted us to investigate the effect of tunicamycin on the reciliation process to determine if glycoprotein synthesis is involved. Studies by Child (6) and Rosenbaum and Carlson (7) showed that Tetrahymena could be deciliated and would rapidly reciliate in a cyclohexamide sensitive process, thus providing evidence that the formation of new cilia required de novo protein synthesis. Guttman and Gorovsky (8) reported that starved cells recover

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motility the same as growing cells and require RNA and protein synthesis, but the basal level of protein synthesis is much lower in starved cells which makes it easier to demonstrate changes in protein synthesis that are induced by deciliation. Since we had shown (9) that this organism synthesizes glycoproteins by a dolichol requiring pathway which is apparently identical to that in higher animals it seemed reasonable to assume that tunicamycin might block reciliation. These cells were grown under the conditions previously described.

METHODS AND MATERIALS

Materials. Tunicamycin was provided by Dr. Alan Elbein, who received it as a gift from Dr. Robert Hamill, Eli Lilly Co. The antibiotic was purified by reversed phase high performance liquid chromatography (10), and the major 256 nm absorbing peak was used for the studies reported below. Dr. Alan Elbein provided both the 2-[^{3}H]-mannose (16 C_i/mmol), a product of Amersham, and the 4,5-[^{3}H]-leucine, obtained from New England Nuclear Co. Tetrahymena pyriformis strain B_4 was generously provided by Dr. Martin Gorovsky.

Deciliation and Reciliation. Cells were harvested from a culture of Tetrahymena containing approximately 700,000 cells/ml by centrifuging at 150 x g for 15 min. The pelleted cells were suspended in 0.07 volume of the starvation medium which was a 10 mM Tris-HCl buffer, pH 7.3, and a sample of the suspension sufficient to yield a final concentration of approximately 100,000 cells/ml was added to a liter of the same buffer and incubated 24 hr at 28°C. At the end of this time, the Tetrahymena were deciliated by the procedure of Gorovsky and Calzone (12), and allowed to reciliate. The percentage of reciliated cells was determined on the basis of cell motility as described by Guttman and Gorovsky (8).

Determination of [^{3}H]-Leucine Incorporation. Deciliated cells prepared as described above were incubated in the reciliation medium with 10 μC_i of [^{3}H]-leucine in a total volume of 1.2 ml, in the presence and absence of tunicamycin. At various intervals, 100 μl aliquots were removed and added to a 1.5 ml centrifuge tube containing 100 μl of a concentrated suspension of incubated cells (to aid in the precipitation of the samples) and 200 μl of cold 10% TCA. The contents of the tubes were well mixed and centrifuged. The acid insoluble pellets were washed 3 times with cold 10% TCA. The washed pellets were incubated overnight at room temperature with 400 μl of tissue solubilizer and counted in a scintillation counter.

Determination of [^{3}H]-Mannose Incorporation. The incorporation of labeled mannose by the deciliated cells was determined in the same way as described above for leucine incorporation, except that the incubations were carried out in the presence of 10 μCi of 2-[^{3}H]-mannose.

RESULTS AND DISCUSSION

The Effect of Tunicamycin on the Reciliation of Deciliated Tetrahymena. In agreement with the results of Frisch et al. (4), we found that cells would not multiply in their normal growth medium in the

presence of as little as 1 μg of tunicamycin. After 24 hr the cells appeared to be alive, but had not multiplied and were somewhat rounded in appearance. In Figure 1, the effect of 2 different levels of the antibiotic on the reciliation of the deciliated organisms is shown. From these data and microscopic observation it appeared that tunicamycin had no effect on the reciliation process. It should be noted that the tunicamycin used in these experiments was very highly purified and was shown to inhibit the incorporation of Glc-NAc-1-phosphate into GlcNAc-pyrophosphoryl-dolichol approximately 50% at a concentration of 15 ng/ml when used in an in vitro system (10). Therefore it might be expected that if the formation of new cilia involved the participation of glycoprotein it would be inhibited by the concentrations of the antibiotic used, which were in excess of those shown to totally abolish conjugation.

The Effect of Tunicamycin on Leucine Incorporation in Deciliated Tetrahymena. Deciliated Tetrahymena were allowed to reciliate under exactly the same conditions which were employed in the experiment shown in Figure 1 in the presence of [^{3}H]-leucine. The uptake of leucine into protein by these organisms in the presence and absence of tunicamycin is shown in Figure 2. It can be seen that the incorporation of leucine into protein was depressed only approximately 15%. Guttman and Gorovsky (8) showed that the advantage of using starved cells for studying the

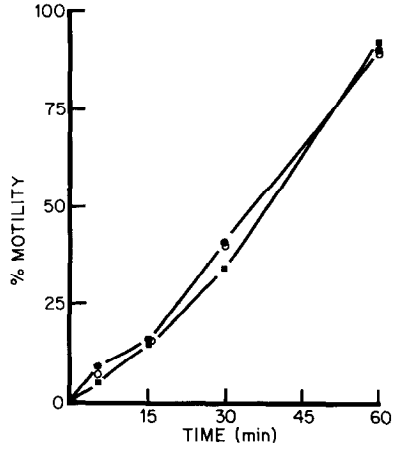

Figure 1. The Recovery of Motility by Deciliated Tetrahymena

Starved cells were deciliated and allowed to reciliate in the presence of zero, •, 1 μg/ml, o, or 5 μg/ml, ▪, Tunicamycin. The incubation conditions and the procedure for determining reciliation are described in the text.

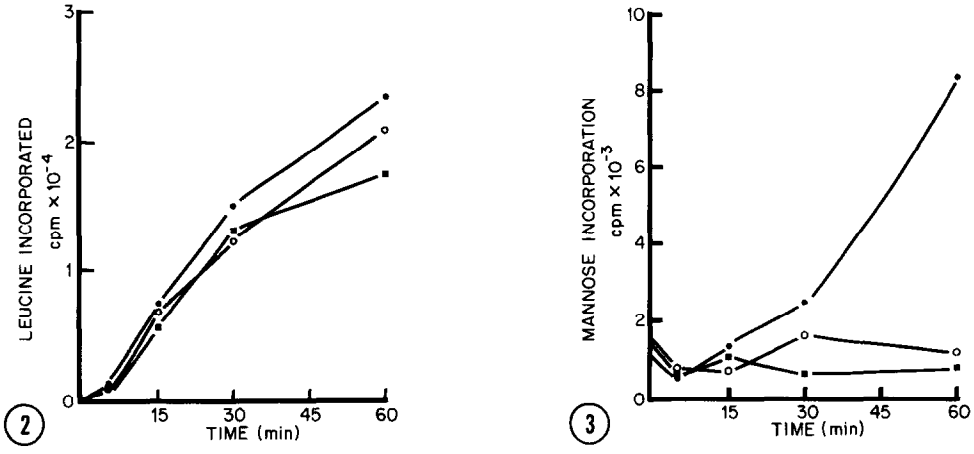

Figure 2. The Effect of Tunicamycin on [^{3}H]-Leucine Incorporation

Deciliated cells were incubated with radioactive leucine under the conditions described in Figure 1 and the incorporation of the amino acid was determined as described in the text. Tunicamycin concentrations: zero, •, 1 μg/ml, o, or 5 μg/ml, ▪.

Figure 3. The Effect of Tunicamycin on [^{3}H]-Mannose Incorporation

Deciliated cells were incubated with labeled mannose under the conditions described in Figure 1 and mannose incorporation was determined as described in the text. Tunicamycin concentrations: zero, •, 1 μg/ml, o, or 5 μg/ml, ▪.

reciliation process was that they incorporated large amounts of radioactivity into proteins induced by deciliation which could be detected easily as compared to growing cells. It is very clear from the results shown in Figure 2 that the primary effect of tunicamycin was not to inhibit the synthesis of proteins required in the regeneration of cilia, which is the case when deciliated cells are incubated with actinomycin or cyclohexamide.

The Effect of Tunicamycin on Mannose Incorporation. The incorporation of 2-[^{3}H]-mannose, a widely applied method of estimating glycoprotein synthesis, was investigated under exactly the same conditions that were employed in the previous experiments. The results of this experiment which are given in Figure 3 show that the incorporation of labeled mannose into acid-precipitable macromolecules is abolished completely when reciliation occurs in the presence of as little as 1 μg/ml of tunicamycin.

Taken together, the results reported in this paper provide strong evidence that the regeneration of new cilia by Tetrahymena pyriformis does

not require the participation of the dolichol-linked glycoprotein synthesizing system. The use of starved cells in these experiments should have amplified any effects of the antibiotic on the system and rendered it more sensitive to inhibition. Even in the presence of a relatively high concentration of purified antibiotic, that was shown to completely inhibit glycoprotein synthesis, the rate and extent of reciliation could not be distinguished from the controls. The relatively small inhibition of protein synthesis also did not seem to affect reciliation. The most obvious conclusion from these experiments is that glycoprotein synthesis is not involved in the formation of the cilia.

ACKNOWLEDGEMENT

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