TRANSFER RIBONUCLEIC ACID METHYLASE ACTIVITY DURING MORPHOGENESIS IN *ARTHROBACTER CRYSTALLOPOIETES*

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The tRNA methylase activity of *Arthrobacter crystallopoietes* was found to change as the organism progressed through its morphogenic cycle. The activity was highest in the coccoidal cells and decreased to a value of approximately 60% lower for logarithmically growing rod cells. The results suggest that the qualitative changes may be due to nontranscriptional control mediated by specific inhibitors.

INTRODUCTION

Growth of *Arthrobacter crystallopoietes* in complex media is accompanied by distinct morphological changes that occur in an exclusively vegetative life cycle. When coccoidal cells are introduced into a fresh medium, a predivisional lag phase occurs during which the cells elongate and develop into rods. Toward the end of logarithmic growth, the cells become progressively smaller as the large rods fragment into smaller rods which shorten into coccoidal cells. Nutritional control of the morphogenetic cycle has been reported by Ensign and Wolfe (1). In a chemically defined glucose-salts medium, *A. crystallopoietes* does not exhibit the typical changes in morphology but rather divides as spheres. Only after the addition of certain inducer compounds do the coccoids undergo the morphological changes typical of growth in complex media. The relative simplicity, reproducibility, and synchrony as well as the nutritional control of the morphological changes exhibited during the growth cycle of *A. crystallopoietes* have led to its use for studies of regulatory mechanisms of bacterial morphogenesis.

Morphogenesis in some phylogenetically higher organisms and tissues has been shown to be accompanied by both qualitative and quantitative changes in transfer RNA (tRNA) methylase activity (2). In contrast, organisms showing no differentiation during their life cycle have a constant tRNA methylase activity (3). In the present study, we have examined tRNA methylation in *A. crystallopoietes* to determine first, if changes in the methylases exist and second, whether any observed changes can be correlated with phenomena known to vary during *Arthrobacter* morphogenesis.

METHODS

Growth conditions

Arthrobacter crystallopoietes (ATCC 1581) was grown in a medium containing (w/v) yeast extract (0.25%), tryptone (0.5%), and glucose (0.1%) at a pH of 7.0. Cultures were started by inoculating 500 ml medium (in a two-liter flask) with a 24-hr culture to an A_{485} = 0.04. Cultures were incubated at 30 C while shaking at 150 rpm on a New Brunswick model VS rotary shaker.

Preparation and assay of tRNA methylase

At various times after inoculation, cells were harvested by centrifugation at 4 C, washed twice in Tris HCl buffer (0.01 *M*, pH 8.2) containing $MgCl_2$ (3×10^{-4}*M*) and finally suspended (25% w/v) in the same buffer. Cells were disrupted by sonic treatment and the cell debris removed by low speed centrifugation prior to centrifugation at 100,000 × g for 1 hr. Aliquots of the resulting supernatant were taken for protein determination (4) and used as the source of tRNA methylase. The methylase activity of the extracts was measured according to Pillinger and Borek (3) Using [^{14}C-methyl] -*S*-adenosylmethionine with a specific activity of 28.9 mCi/m*M* (New England Nuclear). A assay containing no heterologous tRNA was made at each protein concentration to correct for background.

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RESULTS AND DISCUSSION

The tRNA methylase activity of *A. crystallopoietes* of different ages was measured by the *in vitro* transfer of the ^{14}C-methyl group from *S*-adenosylmethionine to *E. coli* tRNA (Figure 1). Since tRNA is normally fully methylated with respect to homologous enzyme, *E. coli* tRNA provides a uniform standard for comparing differences between preparations of *Arthrobacter* methylases. The methylase activity is measured in a crude cell extract, presumably, as in other organisms, representing the net activity of many tRNA methylases. Although net activities complicate the interpretation, several trends are apparent. The net specific activity of the tRNA methylases, represented by the slopes in Figure 1, decreases from a maximum for coccoidal cells to a value approximately 60% lower for logarithmically growing rods. This decrease in net specific activity (number active molecules/total protein) may represent either a uniform change in all tRNA methylases or a change in specific classes of methylases. To distinguish between these possibilities it is necessary to consider the maximum values of methylase activity at saturation level of enzyme, which is proportional to the number of tRNA sites methylated. If a change in specific activity between enzyme preparations represents a proportional decrease in all classes of methylases, the resulting curves should have different slopes but the same final saturation level. Conversely, if a change in specific activity represents the loss of specific classes of enzyme, the resulting curves for different preparations will have not only different slopes but different final levels at enzyme saturation. From Fig. 1 the maximum number of E. coli tRNA sites are methylated by the *Arthrobacter* extract from cocciodal cells, while extracts form rod cells methylate 80% fewer sites. Like the specific activity, the methylating capacity of the crude extracts appears to decrease during the transition from coccoid to rod morphology, which together suggest that specific classes of methylases are changing independently. Wong *et al.* (5) have reported similar results for *Neurospora* with the conclusion that specific classes of methylases vary as a function of life cycle. Their conclusion was supported by the isolation and quantitation of specific methylated nucleosides obtained from tRNA hydrolysates.

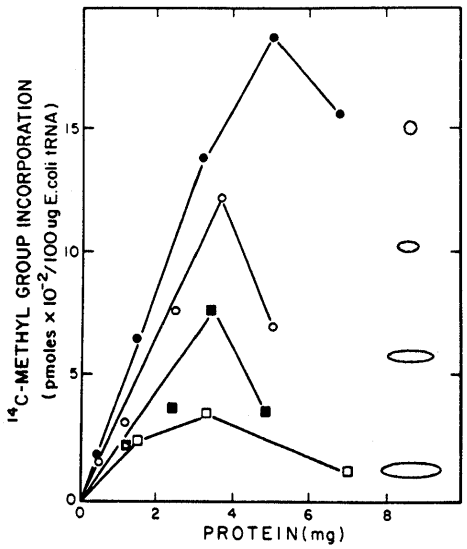

FIGURE 1. *In vitro* assay of tRNA methylase activity. Transfer RNA methylating enzymes from *Arthrobacter crystallopoietes* were prepared and assayed from cells in various stages of the growth cycle (Methods). Protein content of the cell extract was determined by the method of Lowry *et al.* (4). o—o 4 hr; ■—■ 10 hr; □—□ 12 hr; ●—● 24 hr. The morphology characteristic of each cell age assayed is shown diagrammatically to the right.

Although a loss or reduction of some specific methylase (s) in the crude extract from various stages could account for the results thus far discussed, the analysis is complicated by the observation that, after reaching saturation levels, the measured methylating capacity decreases. This reduction was found to be not due to the presence of RNase or a breakdown of *S*–adenosylmethionine. A similar pattern has been shown to represent a methylase inhibitor (6), the activity of which is dependent on the absolute concentration. A specific methylase inhibitor in our system was not detected by the assay method of Sharma and Borek (7), but this by no means eliminates the possibility that one (or more) exists. On the contrary, an inhibitor, varying in amount as a function of cell age and acting on specific methylases or uniformly on all, would explain the observed results.

Previous studies using *A. crystallopoietes* have indicated many parameters that vary in conjunction with the changes in morphology. Changes in membrane permeability to glucose and amino acids have been observed (8) and Krulwich and Ensign (9) have shown that compounds which induce

rod formation alter glucose metabolism. Ferdinandus and Clark (10) have shown that the activities of certain enzymes associated with lipid metabolism change and can be correlated with morphogenesis. At the present time there is no *a priori* relationship between the changes observed for the tRNA methylases and any of the phenomena which have been observed during *Arthrobacter* morphogenesis (11). Our results do show that the pattern of tRNA methylation in the procaryotic organism, *A. crystallopoietes,* changes quantitatively during the growth cycle and suggest that qualitative changes may result from nontranscriptional control mediated by specific inhibitors as found in many examples of eucaryotic cells.

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