# ACTIVITY OF TRANSFER RNA METHYLTRANSFERASES DURING INSECT METAMORPHOSIS

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ABSTRACT

Activity of Transfer RNA Methyltransferases

During Insect Metamorphosis

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Transfer RNA (tRNA) methyltransferases were partially purified from Manduca sexta prepupa larvae and 2-day-old pupae. The purification procedure involved pH 5 precipitation, gel filtration to remove endogenous inhibitors and activators, and ammonium sulfate precipitation. To ensure maximal activation of all preparations, 0.36 M ammonium acetate was used in the reaction mixture. Escherichia coli B tRNA was the methyl accepting substrate, and $^{14}CH_3$-S-adenosylmethionine was the methyl donor. The prepupa larvae had a specific activity of 140 units/mg protein. The 2-day-old pupae had a specific activity of 430 units/mg protein.

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I thank Dr. Roller for providing the insects.

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# INTRODUCTION

Transfer ribonucleic acids (tRNA) are macromolecules of prime importance to the functioning of the cell. These molecules are charged with amino acids and transport them to the ribosome-messenger RNA (mRNA) complex. At this complex the tRNA "reads" a message encoded in the mRNA and orders the amino acids into a sequence which becomes a polypeptide. Proteins could not be produced in the cell without the proper functioning of the tRNA.

Transfer RNA methyltransferases are a group of enzymes which transfer methyl groups from S-adenosylmethionine to preformed tRNA (Borek and Srinivasan, 1966). The enzymes bring about qualitative changes in the tRNA. These enzymes are found in the cytoplasm of cells where they undertake the methylating reactions (Starr and Sells, 1969).

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# REVIEW OF LITERATURE

Transfer RNAs are composed of the four basic nucleotides with a few minor modifications. The four basic nucleotides are adenylic acid, guanylic acid, cytidylic acid, and uridylic acid. A typical tRNA has approximately 75 nucleotides and a great variety of combinations and permutations. The chain of nucleotides is folded into a compact bundle with great stability. There is extensive hydrogen bonding between many of the bases which stabilizes the structure. For simplicity tRNAs are usually diagramed in a cloverleaf structure as in Figure 1.

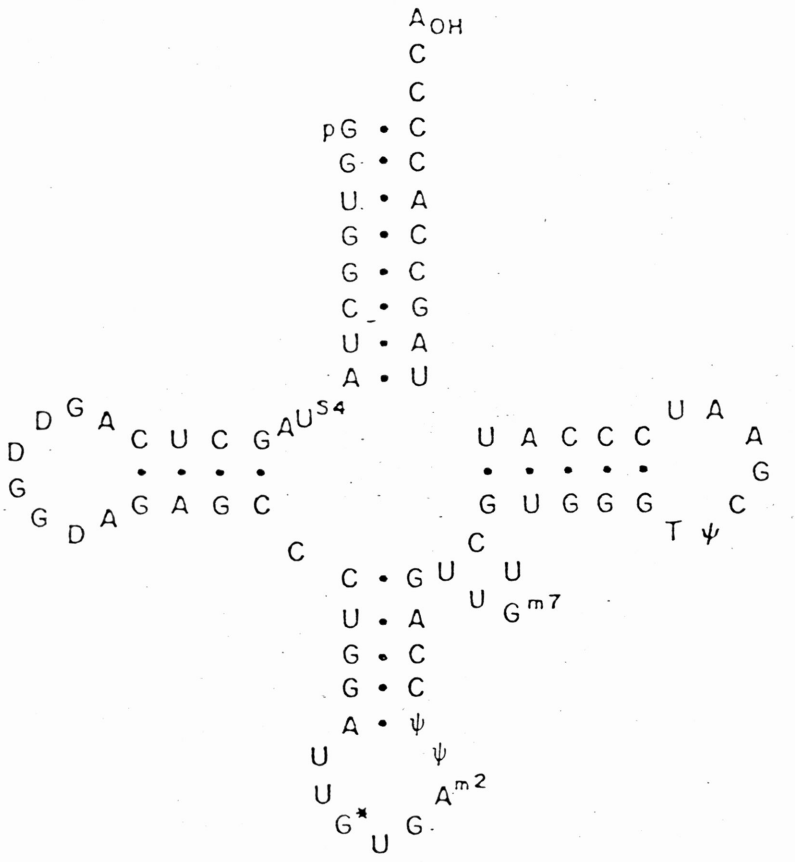


Figure 1. Schematic representation of $tRNA^{His}$ in Salmonella typhimurium.

Amino acids bind to the hydroxyl end of the molecule. Each tRNA binds only one of the types of amino acids. There are, however, many more kinds of tRNAs than there are types of amino acids. Three of the nucleotides in the anticodon loop are termed the anticodon. The anticodon hydrogen-bonds to the complimentary three nucleotides of the messenger RNA. These three nucleotides of the mRNA are termed the codon. The other loops of the tRNA bind to the ribosome for stability. The codon-anticodon recognition is what determines which tRNA will be next to deliver its amino acid to the growing polypeptide.

The tRNA methyltransferases are located almost exclusively in the cytoplasm (Starr and Sells, 1969). There are an unknown number of methyltransferases and their populations vary from species to species and even from organ to organ (Srinivasan and Borek, 1963; Turkington and Riddle, 1970). The tRNA methyltransferases are tRNA specific, base specific, and position specific in their methylations (Kerr and Borek, 1972). Baguley and Staehelin (1970) purified rat liver 1-adenine methyltransferase and used it to methylate yeast tRNA which was deficient in 1-methyladenine. The oligonucleotides produced by enzymatic digestion of the rat liver tRNA and the yeast tRNA were identical with respect to 1-methyladenine. The enzyme from rat liver methylated _in vitro_ the sites the yeast enzyme omitted _in vivo_. The recognition system of the enzymes must therefore be something other than a short sequence of nucleotides. Perhaps the recognition site is a part of the tertiary structure of the tRNA molecule.

A few natural inhibitors of the tRNA methyltransferases have been discovered. Kerr (1972) discovered a system consisting of glycine-N-methyl-

transferase and glycine. Glycine-N-methyltransferase competes with tRNA methyltransferases for S-adenosylmethionine. Glycine-N-methyltransferase also produces S-adenosylhomocysteine which is a more potent inhibitor of the tRNA methyltransferases than of glycine-N-methyltransferase. Nicotinamide in a concentration of 5mM was found by Halpern et al. (1971) to inhibit tRNA methyltransferases. There also is a nicotinamide methyltransferase which acts like the glycine methyltransferase above.

Hormones also exert some control over the tRNA methyltransferases. Sharma et al. (1971) showed that uteri of ovarectomized rat, rabbit, and pig had up to a 50% reduction in tRNA methyltransferase capacity. Physiological doses of estradiol restored the methyltransferase capacity in the uteri. Turkington (1969) demonstrated that insulin and prolactin elevated the tRNA methyltransferase capacity of mammary gland tissue.

Transfer RNA methyltransferases can also be activated. Monovalent cations ($K^+$, $Na^+$, $NH_4^+$) in concentrations from 0.15M to 0.4M provoke a 3- to 20-fold increase in methylation rate. Rodeh et al. (1967) found that 0.36M ammonium ion increased the total tRNA methyltransferase capacity of rat liver extracts. However, there are also systems which do not respond to ammonium ion (Kerr, 1970; McFarlane, 1969). Biogenic polyamines also stimulate the incorporation of methyl groups (Leboy, 1970). Cadaverine, putrescine, spermine, and spermidine at physiological concentrations increase both specific activity and capacity of tRNA methyltransferases.

The tRNA methyltransferases of tumor tissues differ both quantitatively and qualitatively from those of normal tissues. Tsutsui et al. (1966) demonstrated that in many tumorous tissues the tRNA methyl transferases are hyperactive compared to their normal counterparts.

Sharma (1973) demonstrated that tRNA methyltransferases are qualitatively different. Thus the enzymes isolated from tumorous tissue show a higher specific activity and new methylating activity (Mittelman et al., 1967).

Differentiating tissue also shows increased tRNA methyltransferase activity. Hancock et al. (1967) demonstrated increased activity in embryonic mouse and rabbit liver. Borek (1971) showed similar results with a wide variety of tissues.

The tRNA methyltransferases of regenerating tissue, however, do not show increased activity. Rodeh et al. (1967) compared the enzymes from normal and regenerating rat liver. They found the specific activity and extent of methylation identical to that of normal tissue.

The purpose of methylating tRNA has not been clarified to date. The methyl groups may be involved in any of the known functions of tRNA. Shugart et al. (1968) demonstrated that methyl-free tRNA accepted amino acids at a much lower rate than normal tRNA. Thus methylation may be a requirement for amino acid charging. Methylation may also be a requirement at the translational level. Thiebe and Zachau (1968) showed that if the modified base Y next to the anticodon of yeast $tRNA^{Phe}$ is excised the translational ability disappears. Fittler and Hall (1966) demonstrated that removal of the isopentenyl group next to the anticodon of tRNA also eliminated the ribosomal binding ability.

## OBJECTIVE OF RESEARCH

The objective of this research project was to examine whether any changes occur in the activity and pattern of methylation of tRNA by methyltransferases partially purified from the prepupa and 2-day-old pupa of Manduca sexta (tobacco hornworm). The activity determinations were completed. However, the pattern determinations were not. This report will discuss the activities of the tRNA methyltransferases.

# DETERMINATION OF SPECIFIC ACTIVITY OF ENZYMES

## MATERIALS AND METHODS

### Insects

Prepupa larvae and 2-day-old pupae of the tobacco hornworm were obtained from Dr. Roller of the Texas A&M University Biology Department.

### Chemicals

S-Adenosylmethionine-$^{14}CH_3$ with a specific activity of 51.8 Curies per mole was obtained from ICN Isotope and Nuclear Division. Escherichia coli B was obtained from Schwarz/Mann Biochemicals. The Cm-Sephadex C-50 gel was obtained from Sigma Chemical Company. All other chemicals used were of analytical grade.

### Enzyme Preparation

All of the following steps were carried out at 4C. Larvae and pupae were homogenized in a Waring blender at top speed for 30 seconds in 6 volumes of Buffer H. Buffer H, or homogenizing buffer, is composed of the following: 0.01M Tris-HCl (pH 7.4), 0.01M NaCl, 0.0015M $MgCl_2$, and 0.001M β-Mercaptoethanol. The homogenate was centrifuged in a SS-34 rotor in a RC2-B Sorvall centrifuge at a force of 20,000g for 20 minutes. The supernatant was brought to pH 5 with 1.0M glacial acetic acid. The protein which precipitated was collected by centrifugation at a force of 10,000g for 15 minutes. The precipitate was resuspended in 0.05M phosphate buffer (pH 6.0) containing 0.001M β-Mercaptoethanol. The resulting suspension was centrifuged at a force of 30,000g for 10 minutes. The supernatant

was passed through a Cm-Sephadex C-50 gel column equilibrated with the pH 6 phosphate buffer. The column passed protein but absorbed cations. The eluent was brought to 60% saturation by slowly adding crystalline ammonium sulfate. The precipitate was collected by centrifugation in the above centrifuge at a force of 30,000g for 10 minutes. The precipitate was resuspended in 0.01M Tris-HCl (pH 8.2) for the assay.

Assay of tRNA Methyltransferases

The tRNA methyltransferase activity of enzyme preparations was determined by using $^{14}CH_3$-S-adenosylmethionine as methyl donor and Escherichia coli B tRNA as methyl accepting substrate. The assay method was based on the assay system of Tsutsui et al. (1966). The reaction mixture contained 0.04M Tris-HCl (pH 8.2), 0.004M β-Mercaptoethanol, 0.36M ammonium acetate, 30 µg tRNA, 0.2 µCurie $^{14}CH_3$-S-adenosylmethionine (specific activity 51.8 Curies per mole), varying amounts of enzyme preparation, and distilled water to make up a final volume of 0.25 ml. The assays were conducted in duplicate with duplicate blanks. The tubes were incubated at 37C for 1 hour with mild shaking in a New Brunswick Scientific reciprocal water bath shaker model R76. The reaction was stopped by placing the tubes in ice for 5 minutes, adding 0.25 ml of 10% trichloroacetic acid to precipitate the tRNA, and adding 120 µg Escherichia coli B tRNA carrier per tube. The tRNA was allowed to precipitate for 30 minutes at 4C. The precipitate was collected on Whatman glass fiber GF/C filter paper which had been dipped in 0.1M EDTA to reduce nonspecific adsorption. The tubes were rinsed 4 times with 5% trichloroacetic acid. The precipitate on the filter paper was washed 3 times each ether-absolute ethanol 1:1 (volume:volume) and diethyl ether. A heat lamp was used to dry the filters. Scintillation

vials with 10 ml of counting fluid were used to determine counts. The counting fluid contained 4.0 g 2,5-Diphenyloxazole (PPO) and 0.10 g 1,4-bis-(2-(5-Phenyloxazlyl) )-benzene (POPOP) per liter of scintanalyzed toluene. A Beckman liquid scintillation counter model LS-250 was used to determine counts. The counting efficiency was 94.45%.

Protein Determination

Protein concentration was determined by the Lowry method (Lowry et al. 1950) using crystalline bovine serum albumin as the standard. Spectrophotometric measurements were made on a Hitachi spetrophotometer.

RESULTS

In this study we have chosen to measure the activity of the enzymes as the amount of methyl group incorporated at saturation. Changes in the incorporation of methyl group could result from changes in concentration of activators or inhibitors, changes in enzyme concentrations, or changes in enzyme specificity. We eliminated the effect of activators and inhibitors to the best of our ability by using an ion exchange column to trap cations and adding ammonium acetate (0.36M) to maximally activate all reaction mixtures. Our results indicate qualitative changes in the methyltransferases because we are measuring the extent of tRNA methylation.

A large number of individuals were used in each enzyme preparation of both prepupa larvae and 2-day-old pupae. Thus, variations found in individuals have been eliminated. Three activity determinations were carried out and averaged for each developmental stage. The results are

presented in Table I.

TABLE I

SPECIFIC ACTIVITY OF tRNA METHYLTRANSFERASES

PURIFIED FROM DIFFERENT DEVELOPMENTAL STAGES

| Developmental Stage | Specific Activity[a] |
|---|---|
| Prepupa larva | 140 |
| 2-day-old pupa | 430 |

[a]Specific Activity = picomoles $^{14}C$ incorporated/ mg protein/30 µg tRNA/hour.

## CONCLUSION

The 2-day-old pupa shows a total tRNA methyltransferase activity three times that of the prepupa larva.

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