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# tRNA methylases in the bovine lens

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*The tRNA methylating enzymes were examined in different sections of steer and calf lens. The methylase activity was highest in the equatorial region of steer and calf lens. It decreased in the polar region and no activity could be detected in the nuclear region of either steer or calf lens.*

**Key words:** RNA, methyltransferases, enzyme activity, crystalline lens, lens epithelium cell, lens cortex, lens fiber cells, lens nucleus, enzyme assays, carbon isotopes, radiometry, cattle.

The vertebrate lens is a continuously differentiating organ. It consists of 3 cellular elements with different biological activities: (1) an outer monolayer of epithelial cells, (2) a cortical region of cells which are recently differentiated from the cuboidal epithelial cell to an elongated fiber cell, and (3) an inner core of completely differentiated, enucleate fibers. It has been shown that these 3 types of cells have distinct, characteristic patterns of protein and nucleic acid synthesis.<sup>1-4</sup>

Transfer RNA (tRNA) is one of the cardinal components of the protein-synthesizing machinery. The tRNA methylases, which methylate the tRNA at the macro-

molecular level, confer an individuality on the number and distribution of methylated bases in the tRNA in a species- and organ-specific manner.<sup>5</sup> A requirement for methylation of the tRNA in order for it to interact expeditiously with its amino acid-activating enzyme has been shown by Shugart and associates.<sup>6</sup> The methylating enzymes undergo a variety of changes in biological systems which are being subjected to shifts in regulatory mechanisms, such as developing embryonic tissue,<sup>7</sup> metamorphosing insects,<sup>8</sup> and a number of tumor tissues.<sup>9</sup> The mammalian lens also presents a system in which cells are undergoing changes in their properties, and provides an opportunity to investigate possible relationships between the methylation of tRNA and growth, aging, and differentiation of the lens.

This report describes the purification and assay of the tRNA-methylating enzymes from different areas of the bovine lens.

## Material and methods

Steer and calf lenses were obtained from eyes of freshly slaughtered animals. The eyes were

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brought to the laboratory packed in ice. The lenses were separated from the rest of the globe and stripped of their lens capsules, which included the epithelial cells. The stripped lenses were divided into 3 fractions:<sup>10</sup> (1) the equatorial region, which encompasses cells undergoing the differentiation from epithelial to fiber cells, (2) the polar region, which includes the older parts of recently formed cortex fibers, and (3) the most central part of the nucleus, which contains the oldest part of the lens.

**Preparation of the enzyme extract.** As preliminary studies on the soluble and insoluble portions of complete, unfractionated homogenates of lenses could not detect any measurable level of tRNA methylase activity, the following procedure was used to concentrate the methylating enzymes to a detectable level.<sup>11</sup>

The 3 fractions of lens tissue were subjected to identical isolation procedures. The tissue was homogenized in four volumes of 0.25M sucrose, 0.01M MgCl<sub>2</sub>, 0.005M 2-mercaptoethanol; the homogenate was centrifuged at 105,000 × g for one hour and the supernatant decanted. The supernatant was brought to 0.1 per cent protamine sulfate concentration and allowed to stand for 30 minutes. The solution was then centrifuged at 20,000 × g for 30 minutes and the precipitate was extracted with a volume of 0.3M sodium succinate equal to the volume of the wet lens tissue from which the precipitate was obtained, pH 6.0.<sup>11</sup> The solution was recentrifuged for 15 minutes at 10,000 × g and the supernatant was used as the enzyme extract in all assays. All operations were carried out at 4° C. Hurwitz and associates<sup>11</sup> found with this procedure a quantitative recovery of the methylating enzyme present in solution in their system.

**Assay for tRNA methylase activity.** The conditions used were similar to those of Baliga and associates.<sup>8</sup> The incubation mixture contained Tris-HCl buffer, pH 8.2, 50 μmoles, 5 μmoles of MgCl<sub>2</sub>, 5 μmoles of 2-mercaptoethanol, 10 μmoles of methyl-<sup>14</sup>C S-adenosylmethionine (Tracerlab, SA = 1.5 × 10<sup>7</sup> c.p.m. per micromole), 400 μg of *Escherichia coli* B tRNA (General Biochemicals Inc. "stripped tRNA"), and varying amounts of enzyme extracts in a total volume of 1 ml.

The assay mixture was incubated at 37° C. for the desired time and the reaction was stopped by the addition of an equal volume of 10 per cent trichloroacetic acid. The precipitate was washed thoroughly with 5 per cent trichloroacetic acid, taken up in 0.2N NH<sub>4</sub>OH, and was transferred to a planchet for counting in a gas-flow low background counter. Controls without tRNA were treated identically to determine the background level. To determine the maximum extent to which an enzyme preparation was able to methylate tRNA, increasing amounts of enzyme were suc-

cessively added to the substrate until a further increase in enzyme concentration did not increase the amount of incorporated label. The total number of micromoles of <sup>14</sup>C-methyl incorporated into 1 mg. of RNA was a measure of the extent of methylation.

## Results

The tRNA methylases can be characterized by their rates of reaction and by the extent to which the methylation of a heterologous substrate can proceed with increasing concentration of the enzyme. The specific activities calculated per milligram of protein of the enzyme preparation as well as per gram of wet weight of the three regions of the lens and the saturation levels of tRNA methylase activities are listed in Table I. The methylases have the highest capacity of methylation and are found to have the highest specific activity in the actively differentiating equatorial region. In the polar area the enzymes drop in activity with the greatest decrease found in the adult. In the most central part of the nucleus, the enzyme activity drops to essentially zero in both calf and steer. This is not due to an inhibition of the methylating enzymes as mixing of nuclear and equatorial enzymes extracts causes no reduction in enzyme activity. These changes in methylating activity appear also when the activity is calculated per unit wet weight of the lens region, although the differences are somewhat smaller.

Fig. 1 shows the time course of the methylation reaction for steer and calf equatorial and polar regions. It can be seen that in the equatorial region the steer and calf enzymes are essentially the same in both rate of methylation and extent of methylation. In the polar fractions of both calf and steer lens the enzymes have a lowered capacity to methylate at saturation levels, with the polar steer methylases the lowest. In the case of the calf, the enzymes from the polar region will methylate only to 50 per cent of the level of the equatorial methylases and in the polar region of the steer lens the level

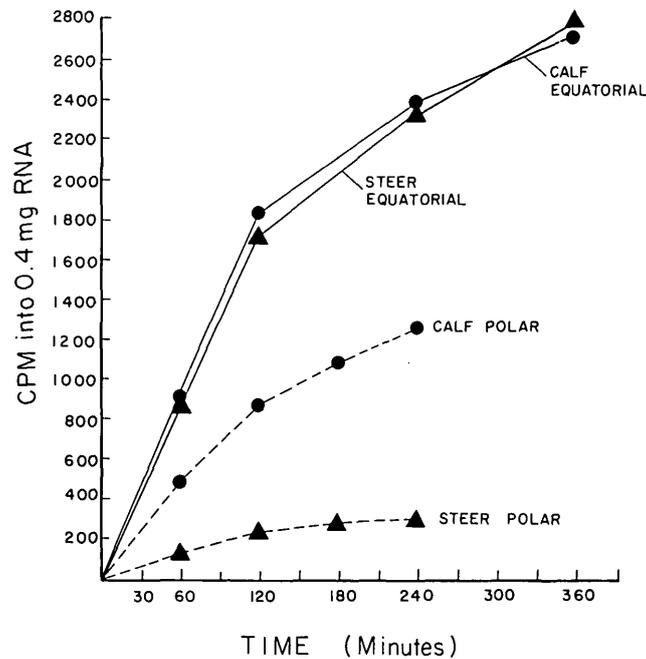


Fig. 1. Time course of  $^{14}\text{C}$ -methyl incorporation by enzyme extracts from the equatorial and polar regions of steer and calf lenses. The assay tubes all contained  $400\ \mu\text{g}$  of *E. coli* B tRNA as substrate.

**Table I.** Distribution of tRNA methylase activity in different regions of the bovine lens. The data represent an average of 6 experiments. Specific activity = micromoles of  $^{14}\text{C}$ -methyl incorporated into 1 mg. of RNA per milligram of protein of the enzyme extract in 30 minutes. Extent of methylation = total number of micromoles of  $^{14}\text{C}$ -methyl incorporated into 1 mg. of RNA

	Wet weight of starting material (Gm.)	Protein in enzyme extract (mg./ml.)	Specific activity	Activity per gram of wet weight of lens	Extent of methylation
<i>Steer</i>					
Equatorial	20	3.95 ( $\pm 0.31$ )	36 ( $\pm 4.0$ )	142 ( $\pm 15$ )	420 ( $\pm 36$ )
Polar	15	8.6 ( $\pm 1.1$ )	5.0 ( $\pm 0.6$ )	43 ( $\pm 5$ )	15 ( $\pm 2$ )
Nuclear	2	9.3 ( $\pm 1.0$ )	1	9.3	1
<i>Calf</i>					
Equatorial	20	3.3 ( $\pm 0.16$ )	38 ( $\pm 2.0$ )	127.6 ( $\pm 6.7$ )	405 ( $\pm 39$ )
Polar	10	4.9 ( $\pm 0.5$ )	15 ( $\pm 1.7$ )	73.5 ( $\pm 8$ )	205 ( $\pm 18$ )
Nuclear	1	13.0 ( $\pm 1.5$ )	1	13	1

is down to 15 per cent. The shape of the curves in Fig. 1 excludes the possibility that the differences in the extent of methylation in different regions of the lenses might be due to differences in the rate of

inactivation of the enzyme in different regions.

In order to examine the products of the methylases large scale enzyme incubations were carried out; the tRNA was isolated

**Table II.** <sup>14</sup>C-methyl nucleosides isolated from 2 mg. samples of RNA methylated by enzyme extracts from steer and calf lens

Nucleoside	c.p.m.	
	Steer	Calf
N <sup>2</sup> -Methyl guanosine	9,000	5,900
N <sup>2</sup> , N <sup>2</sup> -Dimethyl guanosine	9,400	5,700

and the identity of the methylated bases was determined according to the method of Hall.<sup>12</sup> Table II gives the results from samples methylated by steer and calf equatorial enzymes. The recovery of radioactive material applied to the column was 85 to 90 per cent. The predominance of methylated guanines is characteristic of enzymes from mammalian tissues.<sup>13</sup>

### Discussion

It is apparent that the enzymes which alter the structure of transfer RNA, the tRNA methylases, are highly active in the equatorial region of the lens which contains cells actively participating in differentiation. The enzyme activity is reduced by a factor of 2 in the polar region of the calf lens and by a factor of 7 in the steer polar cells. The enzyme activity is virtually absent in the central part of the nucleus, in which no further structural alteration can be discerned. Thus, the methylases are localized in those parts of the lens which are undergoing differential gene activation and repression leading to extreme alterations in the structure and metabolism of the cells. Many of these changes involve protein synthesis in the cell, including both the specific proteins synthesized<sup>1-3</sup> and the components of the protein-synthesizing system.<sup>4, 14</sup> Our findings suggest alterations in the structure of yet another component of the protein synthesizing apparatus in these cells—the transfer RNA's. Specific tRNA's have been shown to have regulatory functions in other systems: chain initiation,<sup>15</sup> amber (nonsense) suppression,<sup>16-18</sup> and missense

suppression.<sup>19</sup> The alterations in structure and conformation due to methylation of the tRNA's may be one of the factors involved in the control of terminal lens cell differentiation.

As the determinations of the methylation activities were carried out on supernatants obtained from homogenates by removing the albuminoid, it seemed necessary to consider the possibility that variations in the amount of this insoluble protein may, by absorption, remove methylating enzymes from the homogenates. To test this possibility, we determined the total insoluble protein of the 3 regions of the lens of steer and calf. In the steer the values were 1.48, 2.30, and 6.77 per cent protein per gram of wet weight in the equatorial, the polar, and the nuclear part of the lens, respectively. In the calf the corresponding values were 1.03, 1.12, and 1.18 per cent. It will be immediately noted that the amount of albuminoid per gram of wet weight of the polar region of the calf is lower than that of the equatorial region of the steer. Nevertheless, the methylating activity of the polar region of the calf is significantly lower than that of the equatorial region of the steer. On the other hand, the concentration of the albuminoid in the nucleus of the calf lens is significantly lower than in the polar region of the steer but, nevertheless, the methylating activity in the calf nucleus is much lower than in the polar region of the steer. The quantity of albuminoid, therefore, does not show any correlation to the methylating activity in various parts of the lens. It is, of course, possible, though barely probable, that there are qualitative differences in the albuminoid of different lens regions at different ages which are related to its ability to absorb the methylating enzyme, but this would only mean that such qualitative changes related to differentiation or aging are responsible for the inactivation of the methylating enzymes.

### REFERENCES

1. Papaconstantinou, J.: Biochemistry of bovine lens proteins. II. The  $\gamma$ -crystallins of adult

- bovine, calf and embryonic lenses, *Biochim. Biophys. Acta* **107**: 81, 1965.
2. Takata, C., Albright, J. F., and Yamada, T.: Lens fiber differentiation and gamma crystallins: Immunofluorescent study of Wolffian regeneration, *Science* **147**: 1299, 1965.
  3. Steward, J. A., and Papaconstantinou, J.: Lactate dehydrogenase isozymes and their relationship to lens cell differentiation, *Biochim. Biophys. Acta* **121**: 69, 1966.
  4. Reeder, R., and Bell, E.: Short- and long-lived messenger RNA in embryonic chick lens, *Science* **150**: 71, 1965.
  5. Srinivasan, P. R., and Borek, E.: Enzymatic alteration of nucleic acid structure, *Science* **145**: 548, 1964.
  6. Shugart, L., Novelli, G. D., and Stulberg, M. P.: Isolation and properties of undermethylated phenylalanine transfer ribonucleic acids from a relaxed mutant of *Escherichia coli*, *Biochim. Biophys. Acta* **157**: 83, 1968.
  7. Simon, L., Glasky, A. J., and Rejal, T. H.: Enzymes in the central nervous system. I. RNA methylase, *Biochim. Biophys. Acta* **142**: 99, 1967.
  8. Baliga, B. S., Srinivasan, P. R., and Borek, E.: Changes in the tRNA methylating enzymes during insect metamorphosis, *Nature* **208**: 555, 1965.
  9. Tsutsui, E., Srinivasan, P. R., and Borek, E.: tRNA methylases in tumors of animal and human origin, *Proc. Nat. Acad. Sci. U. S.* **56**: 1003, 1966.
  10. Dische, Z., Devi, A., and Zelmenis, G.: Influence of age and cataract formation on the ribonucleic acid of the lens. I. Changes in various fractions of ribonucleic acid of rat lenses during aging, *Amer. J. Ophthal.* **51**: 993, 1961.
  11. Hurwitz, J., Gold, M., and Anders, M.: The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. III. Purification of soluble ribonucleic acid methylating enzymes, *J. Biol. Chem.* **239**: 3462, 1964.
  12. Hall, R. H.: A general procedure for the isolation of "minor" nucleosides from ribonucleic acid hydrolysates, *Biochemistry* **4**: 661, 1965.
  13. Smith, J. B., and Dunn, D. B.: The occurrence of methylated guanines in ribonucleic acids from several sources, *Biochem. J.* **72**: 294, 1959.
  14. Papaconstantinou, J.: Molecular aspects of lens cell differentiation, *Science* **156**: 338, 1967.
  15. Marcker, K.: The formation of N-formylmethionyl-sRNA, *J. Mol. Biol.* **14**: 63, 1965.
  16. Capecchi, M. R., and Gussin, G. N.: Suppression *in vitro*: Identification of a serine sRNA as a "nonsense" suppressor, *Science* **149**: 417, 1965.
  17. Engelhardt, D. L., Webster, R. E., Wilhelm, R. C., and Zinder, N. D.: *In vitro* studies on the mechanism of suppression of a nonsense mutation, *Proc. Nat. Acad. Sci. U. S.* **54**: 1791, 1965.
  18. Smth, J. D., Abelson, J. N., Clark, B. F., Goodman, H. M., and Brenner, S.: Studies on *amber* suppressor tRNA, *Cold Spring Harbor Symp. Quant. Biol.* **31**: 479, 1966.
  19. Carbon, J., Berg, P., and Yanofsky, C.: Missense suppression due to a genetically altered tRNA, *Cold Spring Harbor Symp. Quant. Biol.* **31**: 489, 1966.