COMPARTMENTATION OF THE S-ADENOSYLMETHIONINE POOL IN DEVELOPING CHICK EMBRYO CEREBRAL HEMISPHERES, AS DEMONSTRATED BY A FINGERPRINT STUDY OF 18 S RIBOSOMAL RNA

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1. Introduction

In a previous work [1] we studied the variation of the rate of methylation of 18 S and 29 S ribosomal RNA's (rRNA) of chick embryo cerebral hemispheres during the period when morphologically undifferentiated cells were transformed into neuroblasts and spongioblasts (6 to 14 days of embryonic life). After incubation with [^{3}H] methyl methionine, the specific activities of 29 S and 18 S rRNA measured after a 4 hr pulse were similar at 6, 10 and 14 days, but that of S-adenosylmethionine (SAMe), the methyl-donor decreased. Therefore the relative specific activities (RNA specific activity to SAMe specific activity) of 29 S and 18 S rRNA used for the estimation of the rates of methylation increased (about 70% between 6 and 14 days). In contrast, with [^{3}H] uridine as a precursor, the relative specific activity of the rRNA's did not change.

Taking into consideration that there is no independent turnover of methyl groups in mature 29 S and 18 S rRNA, two possible interpretations of these results, discussed in details in [1], were proposed.

i) The number of methyl groups per molecule of rRNA increases, owing to the expression of new rRNA-methylases during cell differentiation.

ii) The specific activity of the SAMe pool used by rRNA-methylases is constant and not decreasing like that of the total pool that we measured. The cellular SAMe pool would thus be compartmentalized.

To choose between these possibilities, we have analyzed ^{14}C-methyl labelled 18 S rRNA by fingerprinting techniques. It will be shown that neither the number nor the nature of methylated oligonucleotides from 18 S rRNA change with age. Therefore the results suggest that the SAMe pool is compartmentalized in developing chick embryo cerebral hemispheres.

2. Methods

Cerebral hemispheres fragments were incubated for 8 hr in the presence of [^{14}C] methyl methionine (53 mCi/mmole, 5 μCi/ml incubation medium) [2]. Cytoplasmic RNA was extracted by cold phenol at pH 7.6 [3]. Low molecular weight RNA's and contaminating DNA were removed after LiCl precipitation of rRNA's [3]. 29 S and 18 S rRNA's were separated on a 5–20% linear sucrose gradient, centrifuged for 14 hr at 63,500 g. The 18 S peak fractions were collected and reprecipitated with ethanol. RNA was digested with T_1 ribonuclease and the products were fractionated by two dimension electrophoresis according to Sanger et al. [4], Brownlee et al. [5], as applied by Fellner [6] to methyl-labelled *E. coli* rRNA.

3. Results and discussion

The autoradiographs of fingerprints of ^{14}C-methyl 18 S rRNA from cerebral hemispheres of 6, 10 and 14 day-old chick embryos are shown in fig. 1 and the

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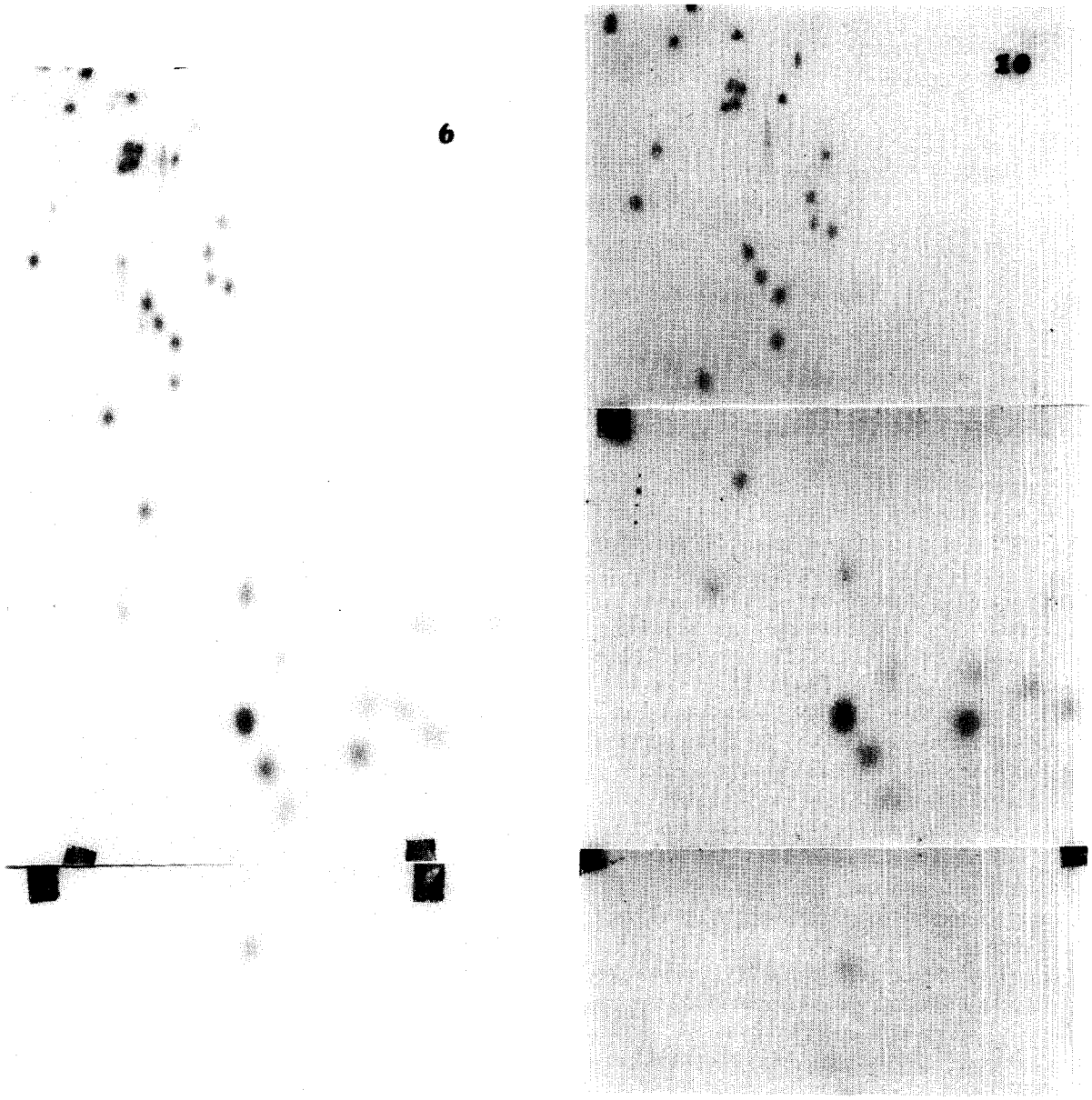

Fig. 1. Fingerprints of T_1 ribonuclease digest of the 18 S rRNA of chick embryo cerebral hemispheres. Labelling of the cerebral cells and RNA extraction were carried out as described under Methods. 18 S rRNA was digested with T_1 ribonuclease and bacterial alkaline phosphatase. Fractionation of hydrolysis products was achieved by electrophoresis: first dimension (from right to left) on a strip of cellulose acetate in the presence of 5% acetic acid/7 M urea (pH 3.5). Second dimension (from top to bottom) on DEAE-paper in 7% formic acid.

key to the autoradiographs in fig. 2. 41 well-resolved major spots were observed. 4 further minor spots (shown by broken circles) were present in low and variable amounts. The fingerprints at 6, 10 and 14 days were virtually identical. However, two small but definite differences were observed in the RNA

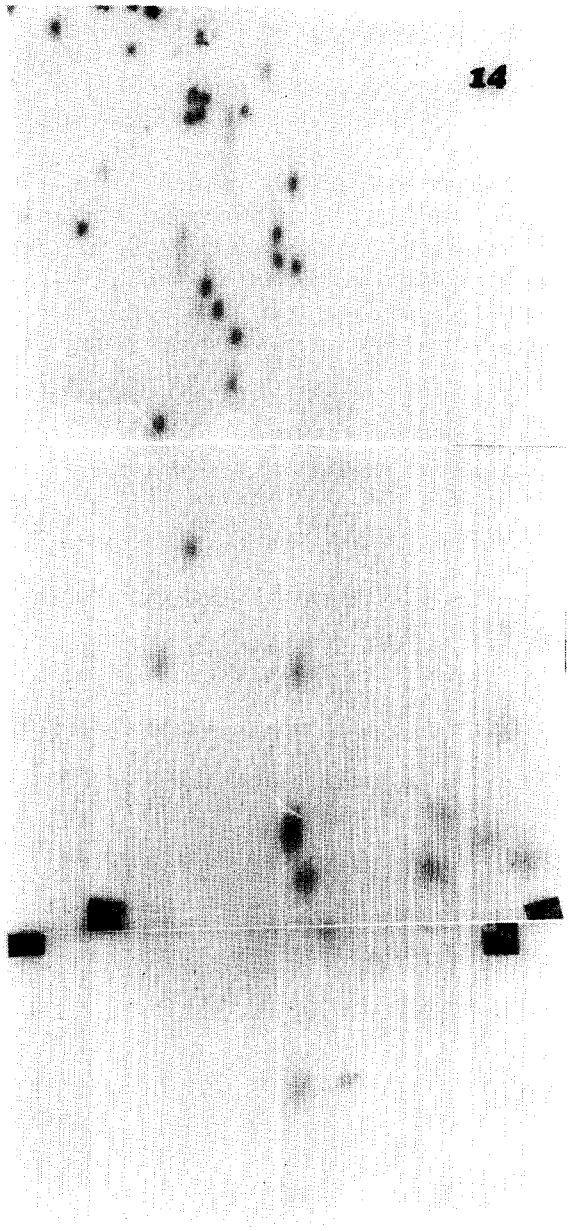

Fig. 1. (Cont.)

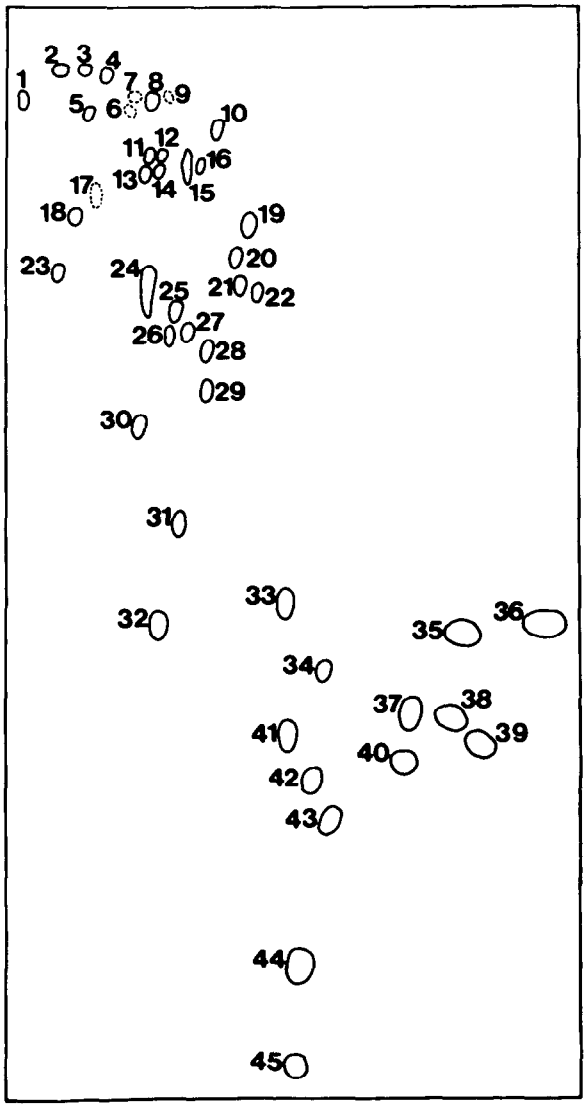

Fig. 2. Key to fingerprints. The autoradiograph of 18 S rRNA from 6 day-old embryos was used as the basis of the plan shown above.

from 10 day-embryos. Spots 24 and 26 appear to be absent. In the other samples these 2 spots "tail" markedly, showing that they may be progressively degraded during electrophoresis, presumably by the acid conditions. Their absence in the 10-day RNA might be due to their complete decomposition in this case. Spot 36 was found only at 6 days on the border of the autoradiograph and had migrated out at 10 and 14 days.

The results indicate that the number and the nature of methylated oligonucleotides from 18 S rRNA do not change with age, thus that no new rRNA

methylases are expressed during the period of cerebral cell differentiation that we are studying. It can be concluded that the real relative specific activity of rRNA as well as the specific activity of the SAMe pool used by rRNA methylases do not change with age and this implies a compartmentation of the cellular SAMe pool.

Our previous work [1] did not permit us to decide whether the rates of methylation and of synthesis of rRNA were coupled or not during differentiation. The results reported now show that the specific activity of methyl labelled rRNA evolves similarly to that of uridine-labelled RNA [3]. Thus, rates of methylation and of synthesis are effectively coupled at the period of cell transformation.

SAMe is involved in many cytoplasmic metabolic pathways. In contrast, the methylation of rRNA is a nucleolar process [7]. It might be tentatively assumed that the SAMe pool utilized by rRNA-methylases is nuclear (or nucleolar) and it is not unlikely that cytoplasmic and nuclear pools vary in a different way at a period of cell type transformation.

The fingerprint pattern of chick 18 S rRNA is more complex than that of *E. coli* rRNA [6] and more spots were resolved in chick than in HeLa cells 18 S rRNA [8]. A more detailed study of the nature of the methylated components of chick rRNA is under investigation.

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References

[1] C. Judes and M. Jacob, Brain Res., manuscript submitted.
[2] C. Judes, M. Sensenbrenner, M. Jacob and P. Mandel, Brain Res., in press.
[3] C. Judes and M. Jacob, Brain Res., in press.
[4] F. Sanger, G.G. Brownlee and B.G. Barrell, J. Mol. Biol. 13 (1965) 373.
[5] G.G. Brownlee and F. Sanger, J. Mol. Biol. 23 (1967) 337.
[6] P. Fellner, European J. Biochem. 11 (1969) 12.
[7] E.F. Zimmerman and B.W. Holler, J. Mol. Biol. 23 (1967) 149.
[8] M. Salim, R. Williamson and B.E.H. Maden, FEBS Letters 12 (1970) 109.