Arch. Biol. Med. Exp. *13*, 287-293, 1980

Differential Effect of Spermine on Nuclear and Cytoplasmic Transfer RNA Methyl Transferases from *Xenopus laevis* Oocytes

Efecto diferencial de espermina sobre las tRNA metil transferasas nuclear y citoplásmica de oocitos de *Xenopus laevis*

ALDO SOLARI*, JORGE E. ALLENDE**

Departamento de Bioquímica, Facultad de Medicina Norte, Universidad de Chile, Casilla 6671, Santiago 7, Chile

(Recibido el 22 de junio de 1980)

SOLARI, A., ALLENDE, J.E. Differential Effect of Spermine on Nuclear and Cytoplasmic Transfer RNA Methyl Transferases from *Xenopus laevis* Oocytes. (Efecto diferencial de espermina sobre las tRNA metil transferasas nuclear y citoplásmica de oocitos de *Xenopus laevis*). *Arch. Biol. Med. Exp. 13*:287-293, 1980.

X. laevis ovarian tissue or isolated oocytes contain two major tRNA methyl transferase activities capable of methylating total *E. coli* tRNA using S-adenosyl methionine as a methyl donor. These enzymes can be resolved by chromatography on DEAE-cellulose or by gel filtration on Sephadex G-200 into fractions I and II. The tRNA methyl transferase I which is present mainly in the oocyte nuclei, has a molecular weight of 190,000 and an apparent K_m for S-adenosyl methionine of 1.5 μM. The activity of peak II which exists predominantly in the oocyte cytoplasm, has a molecular weight of 125,000 and an apparent K_m for S-adenosyl methionine of 17 μM.

The most striking difference between these two enzymes, however, resides in their response to spermine or magnesium ions. The nuclear enzyme is activated more than 8 fold by spermine and 4 fold by Mg^{2+} while the cytoplsmic activity is slightly inhibited by the polyamine and unaffected by Mg^{2+}. The effect of spermine on the nuclear tRNA methyl transferase is highly dependent on the salt concentration since the stimulatory effect of the polyamine decreases at KCl concentrations above 100 mM becoming inhibitory above 200 mM. Spermine increases 4 fold the V_{max} of the reaction catalyzed by the nuclear enzyme but does not affect its apparent K_m for tRNA which is approximately 2.9 μM. The apparent K_m for tRNA of the cytoplasmic enzyme is 3.3 μM.

One of the major reactions of the post-transcriptional processing of transfer RNA is the methylation of several of its nucleotides catalyzed by RNA methyl transferases (1).

Since *Xenopus laevis* oocytes are presently being used to study the expression of eukaryotic tRNA genes and to dissect out the various processing steps that affect tRNA precursors (2-5), it seemed of interest to undertake a study of the tRNA methyl transferases present in these cells.

*Present address: Department of Biochemistry, University of Connecticut, Health Center, Farmington, Conn. 06032, USA.

**To whom all inquiries about this manuscript should be addressed.

In this communication, we present some of the characteristics of the major tRNA methyl transferase activities found in extracts of *X. laevis* oocytes using S-adenosyl methionine as a methyl donor and bulk *E. coli* tRNA as an acceptor. Interesting differences have been found between the tRNA methyl transferases present in oocyte nuclei and cytoplasm, the most striking of which is the differential effect that the polyamine spermine has on these two activities. The nuclear enzyme is greatly stimulated by this polycation while the cytoplasmic activity is slightly inhibited by it.

MATERIALS AND METHODS

Adenosyl-L-Methionine, S(Methyl-^{3}H) was purchased from Amersham-Searle Corp. *E. coli* transfer RNA was supplied by Calbiochem. 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (Hepes) and non-radioactive S-adenosyl-l-methionine were obtained from Sigma Chemical Co.

Xenopus laevis toads were obtained from the South African Snake Farm, Cape Province, South Africa, and were kept under constant temperature and light conditions. Oocytes were removed from the ovary by manual dissection with watchmaker's forceps and were selected for size. In this work, all oocytes used were full grown, having approximately 1.2 mm in diameter.

Isolation of oocyte nuclei. The procedure employed was similar to that described by Birkenmeier *et al.* (6). Isolated full-grown oocytes were preincubated for 30-60 minutes at room temperature in a buffer containing 10 mM Hepes pH 7.4, 70 mM KCl, 7 mM $MgCl_2$, 1 mM dithiothreitol and 0.1 mM EDTA. The oocytes were punctured in the animal pole with a fine needle and subsequently the expulsion of the nucleus was aided by applying a slight pressure about the oocyte equator with a pair of forceps. Once the nucleus had come out of the cell, it was taken up in a Pasteur pipet and washed free of cytoplasm and yolk by plunging the organelle up and down in the pipet with the same buffer. Careful manipulation of the enucleated oocytes allowed the wound to be sealed without losing more than 10% of the original cytoplasmic material.

Assay for tRNA methyl transferase. The assay incubation mixture contained in a total volume of 0.1 ml: 20 mM Tris-HCl pH 8.4, 100 mM KCl, 5 mM EDTA, 2 mM dithiothreitol, 0.1 mg of bulk *E. coli* tRNA, 40 μM adenosyl-L-methionine-S(methyl-^{3}H) with a specific activity of 50 μCi/μmole and tRNA methyl transferase preparations. The reaction was incubated for 15 minutes at 37° and subsequently it was stopped by addition of 2-3 ml of cold 5% trichloroacetic acid. The precipitated material was retained on glass fiber filters which were extensively washed with 15-20 ml of cold 5% trichloroacetic acid and finally the filters were dried and counted in a liquid scintillation system.

Preparation of crude ovarian extracts as sources of tRNA methyl transferases. Ovarian tissue (30 g) from *X. laevis* mature females was cut into small pieces and homogenized in 40 ml of a solution containing 50 mM Tris-HCl pH 7.5, 200 mM KCl, 3 mM $MgCl_2$, 2 mM mercaptoethanol and 0.5 mM EDTA. The homogenate was centrifuged at 20,000 xg for 15 minutes and the resulting supernatant fluid was centrifuged at 105,000 xg for one hour. The high-speed supernatant fraction was dialyzed during 16 hours against a buffer containing 20 mM Tris HCl, pH 7.5, 3 mM $MgCl_2$, 2 mM β-mercaptoethanol and 0.5 mM EDTA. This crude preparation was used as source of ovarian tRNA methyl transferase for further purification through DEAE-cellulose column chromatography or gel filtration as detailed in the Results.

RESULTS

The high speed supernatant fraction of a homogenate obtained from *X. laevis* ovary has considerable tRNA methyl transferase activity. The results shown in Table 1 demonstrate the capacity of such an extract to catalyze the transfer of methyl groups using adenosyl methionine S(methyl-^{3}H) as methyl donor and RNAs from different sources as acceptors. From these data it is clear that the *E. coli* tRNA is by far the best methyl acceptor and that the homologous tRNA is almost completely inactive presumably because it has been completely modified by these enzymes *in vivo*.

TABLE 1

The capacity of different RNAs to accept methyl groups using *X. laevis* ovarian extracts as source of tRNA methyl transferase

RNA acceptor	*pmoles of (^{3}H) methyl groups incorporated*
E. coli B tRNA	28.6
Wheat germ tRNA	6.6
Yeast tRNA	6.3
X. laevis liver tRNA	2.4
X. laevis oocyte tRNA	0.7
Poly Uridylic acid	0.4

The incorporation of (^{3}H) methyl groups into 0.15 mg of each RNA was tested using 40 μM (^{3}H) methyl S-adenosyl methionine as donor and 0.21 mg of protein of the 105,000 xg supernatant fraction from a *X. laevis* ovarian homogenate as source of t tRNA methyl transferase. The incubation mixture contained in addition 20 mM Tris-HCl pH 8.4, 5 mM EDTA and 100 mM KCl in a final volume of 0.1 ml. After 15 min at 37° the reaction was stopped with cold 5% trichloroacetic acid and the precipitable radioactivity was determined.

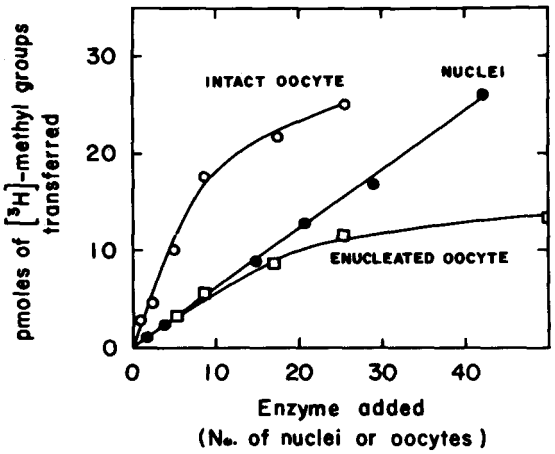

Fig. 1. Distribution of tRNA methyl transferase activity in oocyte nuclei and cytoplasm. X. laevis oocytes, enucleated oocytes and oocyte nuclei were isolated or prepared as described in Methods and extracts were prepared by homogenizing 200 cells or organelles in 300 μl of a buffer containing 10 mM Hepes pH 7.4 70 mM KCl, 7 mM $MgCl_2$, 1 mM dithiothreitol and 0.1 mM EDTA and centrifuging at 20.000 xg. The volumen of the supernatant fraction was measured carefully to determine the amount corresponding to one cell or one nucleus and the tRNA methyl transferase activity was assayed as described in Methods except that 0.4 mM spermine was added to the assay mixture. The symbols shown denote the activity found in whole oocytes (○—○) enucleated oocytes (□—□) and isolated oocyte nuclei (●—●).

The radioactive methyl groups that are incorporated into tRNA by this assay become acid soluble upon treatment with RNase or with acid at 90° for 15 minutes but are not hydrolyzed by the mild alkaline treatment that deacylates aminoacyl-tRNA.

Activity for tRNA methyl transferase can also be found in the extract of nuclei of *X. laevis* oocytes and in the soluble fraction of enucleated cells. Figure 1 shows the relative activity of these enzymes found in the nuclear and cytoplasmic compartments of these cells as compared to the amount found in the total homogenate of the same number of cells. The results obtained indicate that the amount of activity found in the cell nucleus is equivalent to that present in the cytosol and that essentially all the activity found in the total homogenate can be accounted for by the enzyme present in both compartments. This finding demonstrates that there are no great losses of enzyme activity during the isolation and washing of the oocyte nuclei. It must be specified that the tRNA methyl transferase activity of all fractions was assayed in the presence of 0.4 mM spermine which, as will be shown latger, greatly stimulates the nuclear enzyme. Also noteworthy is the fact that the activity of the total homogenate and of the cytoplasm of enucleated cells increases linearly only at low levels of enzyme becoming inhibited by addition of larger amounts of extract. This phenomenon is not observed with the nuclear extract.

DEAE-cellulose chromatography fractionates the tRNA methyl transferase found in the supernatant liquid of an ovarian homogenate into two major peaks that are not well resolved (Fig. 2A). The first peak of activity (I) elutes with approximately 0.1 M KCl and is greatly stimulated by the addition of 0.4 mM spermine. The second fraction (II) elutes at approximately 0.15 M KCl and is slightly inhibited by the presence of this polyamine.

Similar chromatographic fractionation of the activity found in nuclear extracts and in the cytoplasm of enucleated oocytes is shown in Figs. 2B and 2C respectively. Although smaller columns were used because of the reduced amount of material fractionated, it is

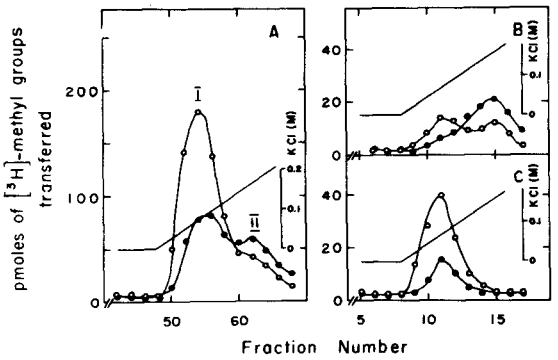

Fig. 2. Fractionation of ovarian tRNA methyl transferases on DEAE-cellulose column chromatography. In part A, DEAE cellulose column (5 × 2 cm) equilibrated with a buffer containing 50 mM Tris-HCl pH 7.5, 3 mM $MgCl_2$, 2 mM β-mercaptoethanol and 0.5 mM EDTA and the 105,000 xg supernatant fraction of and extract of 30 g of *X. laevis* ovary prepared as detailed in Metholds was applied. After extensive washing, the column was eluted with a linear KCl gradient from 0 to 0.4 salt. Fractions of 2 ml were collected and the tRNA methyl transferase was measured in aliquots in the presence (o—o) or abasence (•—•) of 0.4 mM spermine, as described in Methods. In parts B and C a 20,000 xg supernatant solution obtained from extracts of 420 enucleated oocytes (B) or 420 isolated oocyte nuclei (C) were applied to DEAE cellulose columns (2 × 1 cm) and were eluted and assayed in the presence (o—o) or absence (•—•) of 0.4 mM spermine exactly as in part A. The solid line and the internal ordinate indicate th KCl concentrationin the eluted fractions.

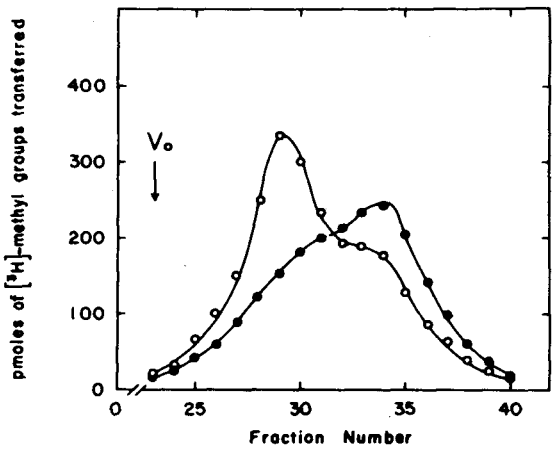

Fig. 3. Separation by gel filtration of tRNA methyl transferase activities of X. laevis ovarian extracts. A Sephadex G-200 column (65 × 1.5 cm) was equilibrated with a buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM KCl, 3 mM $MgCl_2$, 2 mM β-mercaptoethanol and 0.5 mM EDTA, 2.1 mg of protein of partially purified ovarian tRNA methyl transferase was applied. The enzyme preparation was obtained by chromatography of the supernatant fraction of a *X. laevis* ovarian extract on a DEAE-cellulose column eluted between 60 and 200 mM KCl under the conditions described in Fig. 2A. Prior to application to the Sephadex column, the pooled enzyme fractions were concentrated by ultrafiltration. The Sephadex column was eluted with the equilibration buffer at a rate of 9 ml per hour, collecting 2.5 ml fractions. Aliquots were assayed for tRNA methyl transferase activity as described in Methods in the presence (o—o) or absence (•—•) of 0.4 mM spermine.

clear that the nuclear enzyme coincides with the ovarian peak I since it elutes at 0.1 M KCl as a single peak that is highly activated by spermine. The activity of the cytosol obtained from enucleated cells, on the other hand, corresponds to peak II observed in 2A because it elutes mainly at 0.15 M KCl and is inhibited by spermine. This fraction is apparently contaminated with some type I enzyme which becomes especially noticeable in the presence of spermine.

Figure 3 shows the elution pattern obtained by gel filtration on Sephadex G-200 of a mixture of both tRNA methyl transferases from *X. laevis* oocytes partially purified by DEAE-cellulose chromatography. It is evident that this procedure also results in the appearance of two major tRNA methyl transferase activities. The heavier peak is considerably stimulated by spermine as observed with fraction I on DEAE-cellulose while the second peak is inhibited by this compound as seen with peak II in Fig. 2A. Peaks I and II separated by DEAE cellulose chromatography were submitted to gel filtration on the same Sephadex G-200 that had been calibrated with proteins of known molecular weight. The results obtained allowed us to estimate the molecular weight of peak I at 190,000 and that of peak II at 125,000 (not shown).

Figure 4 shows the effect of different concentrations of spermine and Mg^{2+} on the tRNA methyl transferases obtained from isolated nuclei and the cytoplasm of enucleated cells. The nuclear enzyme is activated about 8 fold by concentrations between 0.4 and 1 mM spermine. Higher concentrations of the polyamine reduced the stimulation. The addition of Mg^{2+} at similar concentrations also stimulates the activity of the nuclear enzyme but the metal is less

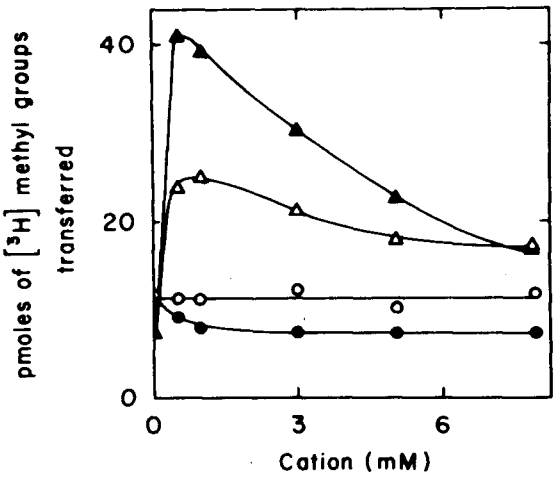

Fig. 4. The effect of Mg^{2+} *and spermine on the nuclear and cytoplasmic tRNA methyl transferase activity of* X. laevis *oocytes.* The tRNA methyl transferase activity was measured in the 20,000 xg supernatant fraction of a homogenate of 15 enucleated oocytes (○ ε ●) or 32 isolated nuclei (△) in the presence of different concentrations of Mg^{2}+ (empty symbols) or spermine (filled symbols). The assay system was essentially as detailed in Methods except that the KCl concentration was 40 mM and that EDTA was omitted from the assays containing Mg^{2+}.

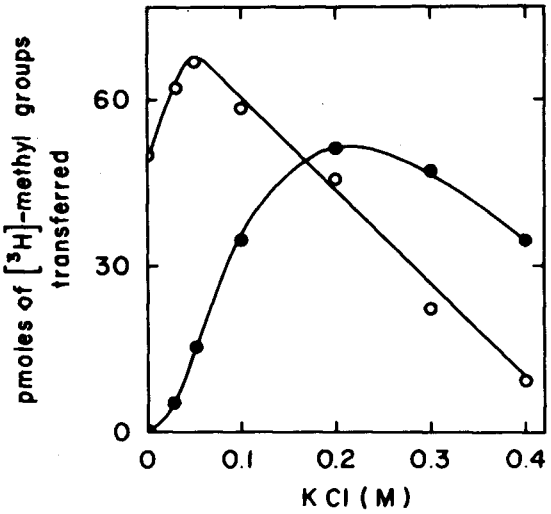

Fig. 5. The effect of KCl on the activity of oocyte tRNA methyl transferase I in the presence and absence of spermine. The activity of tRNA methyl transferase I obtained from DEAE-cellulose column as shown in Figure 2A was assayed at different KCl concentrations in the presence (○—○) or absence (●—●) of 0.4 mM spermine. The assay conditions, with the obvious exception of the KCl concentrations, were the same as described in Methods.

effective than spermine. The activity of the cytoplasmic preparation, on the other hand, is not affected by Mg^{2+} and is inhibited slightly by the addition of spermine. These results corroborate the findings obtained by DEAE-cellulose chromatography regarding the cellular compartments that contain the two enzymes, establishing that peak I is mainly located in the nucleus while peak II is essentially of cytoplasmic origin.

The effect of spermine is highly dependent on the salt concentration of the assay system. Fig. 5 shows that in the absence of spermine peak I of the tRNA methyl transferase shows an absolute dependence on the addition of KCl reaching optimal activity at 200 mM. The presence of 0.4 mM spermine eliminates the requirements for KCl but the addition of this salt inhibits the reaction at concentrations above 0.2 M in the presence of polyamine.

A similar experiment carried out with peak II activity showed that this enzyme is also dependent on KCl, but in this case spermine was inhibitory at KCl concentrations above 50 mM (data not shown).

At KCl concentrations of 20 mM the effect of spermine on the apparent K_m and V_m for tRNA of the peak I or nuclear enzyme was tested. In Fig. 6 it can be seen that the polyamine increases the V_{max} by 4 fold but does not affect the apparent K_m of this enzyme for tRNA which is 2.9 μM. The apparent K_m for tRNA of the cytoplasmic or peak II enzyme is very similar, 3.3 μM. However, these two enzymes differ in their apparent K_{ms} for S-adenosyil methionine which for peak I is of 2.5 μM while for peak II is of 17 μM (data not shown).

DISCUSION

The results presented above demonstrate that *X. laevis* oocytes contain two different tRNA methyl transferases as the major activities than can methylate *E. coli* tRNA. The two activities occupy different cell compartments, one of them occuring predominantly in the nucleus while the other is present preferentially in the cytoplasm. These enzymes differ also in their elution properties

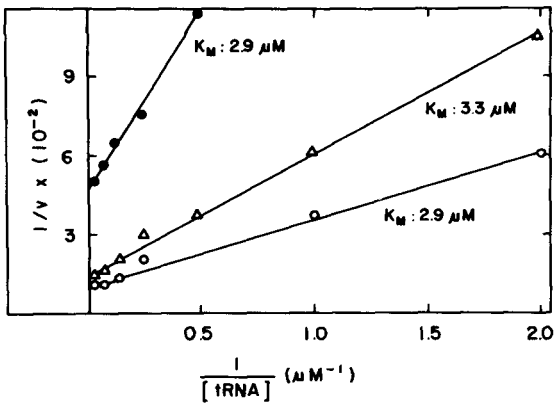

Fig. 6. Double reciprocal plot of the effect of tRNA concentration on the velocity of the reaction catalyzed by tRNA methyl transferase. The effect of different tRNA concentrations on the velocity of the reaction catalyzed by tRNA methyl transferase I was assayed in the presence (○—○) or absence (●—●) of 0.4 mM spermine and in the presence of 40 mM KCl under otherwise standard assay procedures. Similar experiments were carried out with tRNA methyl transferase II (△—△) which was only assayed in the absence of spermine under the exact conditions detailed in Methods. Enzymatic fractions I and II were prepared by DEAE-cellulose column chromatography as in 2A and contained per assay 21 μg of protein respectively.

on DEAE-cellulose chromatography, in their molecular weights, in their apparent Km for S-adenoslyl methionine, and most strikingly in their response to magnesium ions and the polycation spermine.

Spermine has been shown to affect the activity of various reactions involving tRNA (7). The effect of this polycation is apparently a result of its interaction with the polynucleotide structure of tRNA. Such interaction has been shown to occur through X-Ray diffraction studies (8, 9). In this respect it is interesting to note that spermine does not affect the apparent Km of the nuclear tRNA methyl transferase for tRNA but it does increase significantly the V_{max} of this enzyme. Similar results have been observed with a tRNA nucleotidyl transferase (10) and this finding has been interpreted to mean that the polycation accelerates the rate-limiting step of the enzymatic reaction probably by an alteration of tRNA structures that does not involve the region that determines its affinity for the enzyme. Presumably the inhibition of the cytoplasmic enzyme by spermine is also due to the effect of the polycation on the tRNA, in this case spermine would make the tRNAs poorer substrates due to the fact that this enzyme would be acting on a different part of the tRNA molecule. Considerable evidence has been presented regarding the probable physiological role of polyamines in both bacteria and eukaryotes. Of special relevance to our work are the findings of polyamines bound to RNA in oocytes of locusts (11) and in cultured *Xenopus* liver cells (12) as well as the results of Cormick (13) which show that fibroblast nuclei contain a three-fold higher concentration of spermine than the cytoplasm of the same cells. The differential effect of the polyamine on the nuclear and cytoplasmic tRNA methyl transferases reported here may reflect their adaptation to the different spermine concentrations of the two cellular compartments.

The separation of the tRNA methyl transferase activities in the nucleus and cytoplasm of the cell is also interesting in the light of the recent results of Melton *et al.* (5), who have shown that the majority of the base modifications of yeast tRNA precursors occur in the *X. laevis* nucleus but that some of the tRNA precursors are also present in the cytoplasm of these cells. The sequential modification of the bases that accompanies tRNA processing may be partly controlled by compartmentation of the modifying enzymes in the nucleus or cytoplasm of the cell. Obviously, future work in this field should study the specificity of methylation of the activities found in the two cellular compartments. Such studies together with the increasing knowledge we are gaining about tRNA structure should help us to establish the bases of the differential effect of spermine described in this report and the possible physiological role of the separate compartmentation of tRNA methyl transferases.

ACKNOWLEDGEMENTS

This work was part of the doctoral thesis of A. Solari presented to the University of Chile and was supported by grants from the Ford Foundation, UNDP/Unesco project RLA 78/024, the Organization of American States and by the University of Chile.

REFERENCES

1. Kerr, S.J. and Borek, E. (1973) *Enzymes 9:*167-195.
2. Kressman, A., Clarkson, S.G., Pirotta, V. and Birnstiel, M.L. (1978), *Proc. Natl. Acad. Sci. U S A 75:*1176-1180.
3. Schmidt, O., Mao, J. Silverman, S., Hovemann, B., and Soll, D. (1978), *Proc. Natl. Acad. Sci.* USA. *75:*4819-4823.
4. De Robertis, E.M. and Olson, M.V. (1979) *Nature 278:*137-143.
5. Melton, D.A., De Robertis, E.M. and Cortese R., (1980) *Nature 284:*143-148.
6. Birkenmeier, E.H., Brown, D.D. and Jordan, E. (1978) *Cell 15:*1077-1086.
7. Tabor, C.W. and Tabor, H. (1976) *Ann. Rev. Biochem. 45:*285-306.
8. Ladner, J.E., Finch, J.T. Klug, A., and Clark, B.F.C. (1972) *J. Mol. Biol. 72:*99-101.
9. Quigley, G.J., Tester, M.M. and Rich, A. (1978), *Proc. Natl. Acad. Sci.* USA. *75:*64-68.
10. Evans, J.A. and Deutscher, M. (1976) *J. Biol. Chem. 251:*6646-6652.
11. Erdman, G. and Fischer, H.A. (1973) *Histochemie 36:*335-345.
12. Gfeller, R., and Russell, D.H. (1971) *Z. Zellforsch Mikrosk Anat. 120:*321-331.
13. Cormick, F.M. (1978) in *Advances in Polyamine Research* (Campbell, R.A. ed) Academic Press, New York, pp. 173-180.