Amine-specificity of the inactivating ornithine decarboxylase modification in *Physarum polycephalum*

John L. A. MITCHELL, Gail K. MITCHELL and David D. CARTER
Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, U.S.A.

(Received 7 April 1982/Accepted 27 May 1982)

The enzyme catalysing the polyamine-stimulated modification of *Physarum* ornithine decarboxylase *in vivo* was partially purified and its activity on purified ornithine decarboxylase was examined with respect to its specificity for various amines. Spermidine, spermine and several polyamine analogues strongly promoted this reaction *in vitro* (apparent K_m in the 0.1–0.5 mM range), whereas putrescine (apparent K_m 5.33 mM) and several related diamines were not nearly as effective. In agreement with this, sensitivity studies performed *in vivo* also suggested that cellular spermidine, and not putrescine, is critical in modulating ornithine decarboxylase activity by this post-translational control. Unlike putrescine, or other diamines, 1,3-diaminopropane demonstrated a functional similarity to the polyamines in stimulating this reaction. This study has demonstrated a method whereby non-physiological amines capable of depressing ornithine decarboxylase activity by this natural feedback mechanism can be readily identified for further evaluation of their potential use in the experimental and medical control of polyamine biosynthesis.

Putrescine, spermidine and spermine are intimately involved in many aspects of eukaryotic cell growth, and the regulation of polyamine biosynthesis appears to be an important control element in cell proliferation and differentiation (for references see Heby, 1981). Consequently artificial manipulation of polyamine biosynthesis by specific inhibitors has been found to be effective, not only in the experimental modification of cell proliferation, but also in the medical limitation of the growth of certain tumours (Marton, 1981; Jänne *et al.*, 1978, 1981) and blood parasites (McCann *et al.*, 1981). Maintenance of normal cellular concentrations of polyamines appears to be associated with an extreme sensitivity of the biosynthetic enzymes ornithine decarboxylase (EC 4.1.1.17) and *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) to fluctuations in their products, the polyamines. The mechanism of this important product feedback is not well understood. Simple allosteric inhibition of the biosynthetic enzymes is not found, and rather putrescine and the polyamines are thought to influence directly the synthesis and degradation of these enzymes by several diverse means. Understanding this complex of regulatory mechanisms is of obvious importance to scientific and medical advances in the control of polyamine biosynthesis.

Some evidence has been presented that the poly-

amines may inhibit the synthesis of the ornithine decarboxylase enzyme (Clark & Fuller, 1975; McCann *et al.*, 1979; Kallio *et al.*, 1977). The diamines may alternatively (or additionally) repress ornithine decarboxylase activity by stimulating the synthesis and availability of an anti-enzyme protein that specifically binds and inactivates this enzyme (Heller *et al.*, 1976; McCann *et al.*, 1977; Pegg *et al.*, 1978; Kallio, 1978; McCann *et al.*, 1980; Heller & Canellakis, 1981). Others have speculated that ornithine decarboxylase may be inactivated post-translationally by covalent putrescine addition (Russell, 1981) or by phosphorylation with a polyamine-dependent protein kinase (Atmar & Kuehn, 1981).

In the simple eukaryote *Physarum polycephalum* external polyamines have been shown to induce an unidentified post-translational modification in the ornithine decarboxylase protein that renders it inactive *in vivo*, even though this form of the enzyme can be assayed *in vitro* in the presence of relatively high concentrations of the coenzyme pyridoxal 5'-phosphate (Mitchell *et al.*, 1978, 1981a). The protein catalysing this conversion of active (A-form) enzyme into inactive (B-form) enzyme has been isolated and the reaction shown to require spermidine (Mitchell *et al.*, 1981b). Sufficient conversion-factor protein has now been partially purified to

permit extensive evaluation of the polyamine-specificity of this reaction. The present study therefore is the first time a known mechanism of ornithine decarboxylase modification *in vivo* has been investigated for diamine or polyamine requirements, and analogue substitution, *in vitro*. This information, in comparison with corresponding diamine, polyamine and analogue studies *in vivo* is valuable in our attempts to understand the apparently complex polyamine feedback control of this enzyme, and the possible use of polyamine analogues to modify cellular polyamine biosynthesis experimentally.

Experimental

Chemicals

Pyridoxal 5'-phosphate, L-ornithine, Hepps [4-(2-hydroxyethyl)-1-piperazinepropanesulphonic acid], dithiothreitol, EDTA, cycloheximide and the chloride salts of putrescine, spermidine, spermine, diaminoethane, cadaverine, diaminoethane, diaminoheptane, 1,4-diaminobutan-2-one and 1,3-diamino-2-hydroxypropane were purchased from Sigma Chemical Co. Methylglyoxal bis(guanyldiguanidine) {1,1'-[(methylethanediyldene)dinitrilo]diguanidine}, 1,3-diaminopropane and 3,3'-iminodipropylamine were purchased from Aldrich Chemical Co. 1,4,7-Triaminoheptane and 1,3,6-triaminohexane were gifts from Merrell International Research Laboratories, Strasbourg, France. L-[1-¹⁴C]Ornithine (50 Ci/mol) was purchased from Amersham/Searle Corp.

Culture techniques

Cultures of *Physarum polycephalum* were maintained and sampled as described by Mitchell & Rusch (1973). Only exponentially growing shake-flask cultures of asynchronous microplasmodia were used in these experiments.

Assay of ornithine decarboxylase activity

The assay was performed as described by Mitchell & Kottas (1979). A-form enzyme activity was determined with the assay medium 10 mM-Hepps/NaOH buffer (pH 8.0)/0.5 mM-dithiothreitol/0.5 mM-EDTA/0.5 μ M-pyridoxal 5'-phosphate/0.1 mM-L-[1-¹⁴C]ornithine (0.02 μ Ci). Total assayable enzyme was determined with assay medium containing Hepps buffer, pH 8.4, and 100 μ M-pyridoxal 5'-phosphate along with the EDTA and dithiothreitol. The difference between the activities observed in these assays was attributed to the B-form enzyme. One unit of activity was designated to be 1 μ mol of CO₂ released/min at 25°C.

Purification of ornithine decarboxylase

Acetone-dried pellets of water-washed *Physarum* microplasmodia (approx. 25 g of protein) were suspended in 5 litres of ice-cold 50 mM-potassium phos-

phate buffer, pH 7.4, containing 0.5 mM-EDTA, 15 mM-2-mercaptoethanol and 20 μ M-pyridoxal 5'-phosphate. The homogenate suspension was filtered into a suspension of 1600 ml of DEAE-cellulose equilibrated in the same buffer. This slurry was then poured into a column (31 cm $\times$ 10.5 cm) that contained a 500 ml bed of the resin, washed with 2 litres of buffer and batch-eluted with buffer containing 0.2 M-KCl. The enzymically active fractions (about 250 ml, eluted at approx. 1.75 litres) were concentrated to 50 ml by filtration on an Amicon hollow-fibre (H1X-50) membrane. This was applied to a Sephacryl S-300 column (60 cm $\times$ 5.0 cm) pre-equilibrated in buffer without KCl, and eluted in 20 ml fractions at 50 ml/h. The most active four or five fractions were applied to a column (8.0 cm $\times$ 2.3 cm) of DEAE-Sephacel, washed with 2 column volumes of buffer and eluted with a linear 0–0.2 M-KCl gradient in 600 ml of buffer. The most active fractions were pooled and concentrated to 8 ml on an Amicon PM-10 ultrafiltration membrane. The concentrated sample was then applied to a column of Ultrogel AcA-34 (2.5 cm $\times$ 90 cm) and eluted at 18 ml/h. The peak of eluted activity, which was concentrated to 2 ml on an Amicon PM-10 ultrafiltration membrane, represented an 800-fold purification of approx. 13 units of 80–100% A-form ornithine decarboxylase that was quite stable at –20°C in the presence of 30% (v/v) glycerol.

Assay of A-form–B-form converting protein

A known amount (0.001–0.005 unit) of partially purified form-A ornithine decarboxylase was contained in a 0.2 ml reaction mix consisting of 20 mM-Hepps buffer (pH 8.0)/0.5 mM-EDTA/1.0 mM-spermidine/2.5 mM-dithiothreitol/20% (w/v) poly(ethylene glycol) and crude homogenate or isolated conversion factor. Immediately after mixing, 0.02 ml samples were removed and duplicate assays performed to determine A-form and total (A-form + B-form) ornithine decarboxylase activity. These assays were repeated after 1 h at 25°C and the loss in the A-form enzyme, in comparison with the stable total activity, was used to indicate enzyme modification.

Preparation of A-form–B-form converting protein

Microplasmodia from exponential culture were centrifuged and washed in distilled water at 23°C for 2 min. This material was again pelleted and homogenized by sonication in 20 mM-Hepps buffer, pH 8.0, containing 0.5 mM-EDTA and 3 mM-dithiothreitol. The precipitate produced between 40 and 70% saturation with (NH₄)₂SO₄ at 4°C was resuspended in 30 ml of this buffer containing 50 mM-NaCl and was desalted and depigmented on a column of Sephadex G-25 (200 ml bed volume). This was then diluted to 500 ml with the same

buffer (also containing 50 mM-NaCl) and applied to a 50 ml DEAE-Sephacel column. After a washing with 100 ml of buffer, the conversion-factor activity was eluted with a 0.05–0.25 M-NaCl linear gradient (800 ml), where 20 ml fractions were collected at about 50 ml/h. Samples (0.1 ml) of each fraction were dialysed against the buffer without salt for 2 h in a Microdialyzer (Bethesda Research Laboratories) and 0.09 ml portions of the desalted fractions assayed for enzyme-modification activity. The active fractions were pooled and the protein precipitated by 70% saturation with $(\text{NH}_4)_2\text{SO}_4$. The pellet was resuspended in 10 ml of buffer containing 0.3 M-NaCl and applied to an Ultrogel AcA-44 column (2.5 cm $\times$ 84 cm). Buffer containing 0.3 M-NaCl was run through this column at 15 ml/h, with 7 ml fractions being collected. The peak of enzyme activity was determined on desalted fractions as indicated above, and the peak fractions were combined, concentrated to 2 ml on an Amicon PM-10 ultrafiltration membrane and stored at -20°C in buffer containing 30% glycerol.

Results

The isolation procedure that we reported previously produced a conversion factor that was unstable in the A-form–B-form conversion assay, resulting in a cessation of catalytic activity after

45–60 min (Mitchell *et al.*, 1981b). Our current methods for partial purification of this converting protein, and the indicated improved assay conditions that include poly(ethylene glycol), result in a reaction that proceeds at constant velocity for at least 90 min. This assay procedure is capable of complete conversion of low concentrations of substrate (e.g. 0.008 unit of A-form ornithine decarboxylase/ml) to product, B-form enzyme. In the presence of high substrate concentrations, however, reaction velocity declines quickly as the product accumulates, suggesting product inhibition through its competition for the enzyme. In the kinetic studies reported in the present paper, the substrate concentration of 0.017 unit/ml was utilized throughout to avoid both substrate depletion and product feedback, thereby allowing reactions linear with time for at least 90 min, even at saturating polyamine concentrations.

As previously reported (Mitchell *et al.*, 1981b), isolated conversion-factor protein will not catalyse this modification of ornithine decarboxylase without the presence of a polyamine, a requirement not satisfied by inorganic cations. In the experiments summarized in Table 1 we have examined the specificity of the stimulation of this reaction through comparison of reaction saturation by various diamines and polyamines. Although the V_{max} values were approximately the same for all the compounds for which it could be determined, there were striking differences

Table 1. Comparison of the ability of various amines to stimulate the enzyme-catalysed modification of ornithine decarboxylase *in vitro*

The A-form–B-form conversion assay was performed as described in the text with 0.017 unit of partially purified ornithine decarboxylase, sufficient partially purified converting factor to modify 10–40% of the substrate in 60 min, and various amine concentrations. The amine saturation curves were graphed as double-reciprocal plots with the K_m values determined by linear-regression analysis of the six to eight data points per compound. Several of the diamines were only marginally effective in stimulating any conversion, even at 4 mM. K_m determinations could not be performed on these because the very high diamine concentrations required for activity led to protein precipitation and direct inhibition of ornithine decarboxylase activity. In many cases multiple separate K_m determinations were made, and the value presented is the mean, followed by the standard deviation and sample size.

Compound	Apparent K_m (mM)
Polyamines	
Spermidine $\text{NH}_2\text{--}[\text{CH}_2]_4\text{--NH--}[\text{CH}_2]_3\text{--NH}_2$	0.35 (± 0.147) ($n = 6$)
Spermine $\text{NH}_2\text{--}[\text{CH}_2]_3\text{--NH--}[\text{CH}_2]_4\text{--NH--}[\text{CH}_2]_3\text{--NH}_2$	0.11 (± 0.023) ($n = 5$)
3,3'-Iminodipropylamine $\text{NH}_2\text{--}[\text{CH}_2]_3\text{--NH--}[\text{CH}_2]_3\text{--NH}_2$	0.47 (± 0.04) ($n = 4$)
1,3,6-Triaminohexane $\text{NH}_2\text{--}[\text{CH}_2]_2\text{--CH(NH}_2\text{)--}[\text{CH}_2]_3\text{--NH}_2$	0.52
1,4,7-Triaminoheptane $\text{NH}_2\text{--}[\text{CH}_2]_3\text{--CH(NH}_2\text{)--}[\text{CH}_2]_3\text{--NH}_2$	0.39
Diamines	
1,2-Diaminoethane	>6
1,3-Diaminopropane	0.41 (± 0.028) ($n = 4$)
1,4-Diaminobutane (putrescine)	5.33 (± 0.40) ($n = 4$)
1,5-Diaminopentane (cadaverine)	2.35
1,6-Diaminohexane	>6
1,7-Diaminoheptane	>6

in the apparent K_m values for these amines. As shown in Table 1, spermine was most effective in stimulating this reaction. Approximately 3 times as much spermidine or spermidine analogues were required to produce an equivalent reaction.

The variable stimulation by the diamines is noteworthy. Putrescine, which many regard as most critical in the feedback modulation of ornithine decarboxylase activity, was found to be 10-fold less effective than the polyamines at stimulating this modification of ornithine decarboxylase *in vitro*. Cadaverine was effective at somewhat lower concentrations than putrescine, but longer-chain diamines were almost ineffective. In contrast, 1,3-diaminopropane was almost as potent as spermidine in stimulating this reaction. 1,3-Diamino-2-hydroxypropane and 1,4-diaminobutan-2-one were also tested in this series, but their effectiveness was not determined because of their strong inhibition of the ornithine decarboxylase from *Physarum*. Methylglyoxal bis(guanyldiazide), a compound known to inhibit *S*-adenosylmethionine decarboxylase (Williams-Ashman & Schenone, 1972) and to compete with polyamines for cellular uptake (Clark & Fuller, 1975), was also tested and found to be unable to stimulate the enzyme-catalysed modification of ornithine decarboxylase at concentrations up to 4 mM. Similarly cycloheximide, which induces rapid conversion of A-form into B-form enzyme *in vivo* (Mitchell *et al.*, 1976), was found to be inactive in the direct stimulation of this reaction *in vitro*.

On the basis of the above results one might expect that all of the polyamines tested and none of the diamines except diaminopropane would be effective in stimulating this inactivating modification of ornithine decarboxylase *in vivo*. To test this, cultures of *Physarum* were exposed to 0.5 mM concentrations of the various diamines and polyamines and the units of A-form enzyme converted into B-form enzyme in 30 min were evaluated. The experiment shown in Table 2, which typifies the results of six such experiments, demonstrates many deviations from the expected amine responses *in vivo*. Spermidine was consistently found to be more effective than spermine in this modification *in vivo*, even though *in vitro* the reaction was sensitive to 3-fold lower concentrations of spermine. Of the spermidine analogues 3,3'-iminodipropylamine was also found to be consistently more effective than spermine, yet triaminoheptane and triaminoheptane were essentially inactive.

Most of the diamines tested, including diaminopropane, failed to elicit a response *in vivo*. Putrescine, however, was found to be very active. When cultures were exposed to 1–2 mM-putrescine the response after 30 min was approximately equivalent to that of spermidine. At low concentrations (e.g. 0.2 mM) the response to putrescine was considerably delayed with respect to equivalent concentrations of spermidine, and the maximal response achieved after 60–90 min was only 40–45% of that for spermidine (Table 3). A 12 h pretreatment of a

Table 2. Comparison of the ability of various amines to stimulate the conversion of A-form into B-form ornithine decarboxylase *in vivo*

The indicated amines were added at 0.5 mM to replicate 50 ml cultures of exponential-phase *Physarum* microplasmodia in 500 ml shaking flasks. Within 1 min of addition of the compound a 5 ml sample was withdrawn, pelleted and frozen as described in the text. Sampling was repeated after an additional 30 min of culturing. The A-form and total ornithine decarboxylase activities were assayed for all samples as described in the text, and the absolute change in A-form enzyme activity, as well as the change relative to the spermidine flask, were tabulated. The slight increase in A-form enzyme activity during this time interval noted in several flasks was designated as negative conversion of A-form into B-form enzyme.

Compound (at 0.5 mM)	Conversion of A-form into B-form enzyme in 30 min period (units/mg of protein)	% of conversion induced by spermidine
Control (no addition)	–30	0
Polyamines		
Spermidine	1680	100
Spermine	732	43.6
3,3'-Iminodipropylamine	1092	65.0
1,3,6-Triaminoheptane	318	18.9
1,4,7-Triaminoheptane	–84	0
Diamines		
1,3-Diaminopropane	–60	0
1,4-Diaminobutane (putrescine)	1122	66.8
1,4-Diaminopentane (cadaverine)	–96	0
1,6-Diaminoheptane	138	8.2
1,7-Diaminoheptane	–6	0

Table 3. Comparison of spermidine- and putrescine-induced A-form-B-form modification of ornithine decarboxylase *in vivo*

This experiment was similar to that in Table 2 except amine concentrations and lengths of exposure were varied. The amount (units) of A-form ornithine decarboxylase modified in each experiment depends on the specific activity of the cultures on that day and the percentage of this activity in the A-form at the start of the experiment. The units of A-form enzyme converted in this experiment, as in that of Table 2, may therefore not be exactly comparable with that of the similar experiments done at different times.

Treatment		Conversion of A-form into B-form enzyme (units/mg of protein)	% of conversion induced by equivalent spermidine treatment
Compound	Time (min)		
Control	30	50	—
0.2 mM-Spermidine	20	1400	—
	40	1700	—
	60	2250	—
0.2 mM-Putrescine	20	-200	0
	40	500	29.4
	60	900	40.0
1.0 mM-Spermidine	30	2550	—
1.0 mM-Putrescine	30	2650	104.0

culture with 9.0 mM-methylglyoxal bis(guanylhydrazone) completely prevented any response to up to 3.0 mM-putrescine (results not shown).

Discussion

During exponential growth *Physarum* contains approx. 1–2 mM-putrescine, 0.5–1 mM-spermidine and no measurable spermine (Mitchell & Rusch, 1973; Mitchell *et al.*, 1978). These concentrations are much lower than the K_m for putrescine activation of the modification of ornithine decarboxylase *in vitro* yet slightly above that for spermidine. It is therefore strongly suggested that spermidine concentrations may be of greater importance than putrescine in this mechanism of feedback control of ornithine decarboxylase. This conclusion is supported by the inability of putrescine to induce this conversion *in vivo*, in the presence of methylglyoxal bis(guanylhydrazone), and by the delayed response *in vivo* to low concentrations of putrescine in comparison with spermidine. As noted previously, however, the 1–2 mM cellular putrescine concentrations may still feedback directly on ornithine decarboxylase activity in this organism through competition for the active site (K_i 0.45 mM) (Mitchell *et al.*, 1978).

The involvement of spermidine or spermine, as distinct from putrescine, in the regulation of ornithine decarboxylase activity has been reported in several other eukaryote systems. Paulus & Davis (1981) found *Neurospora* ornithine decarboxylase to be most responsive to cellular spermidine concentrations. In yeast cells Tyagi *et al.* (1981) found ornithine decarboxylase to be post-translationally activated by the polyamines, and Tabor (1981) reported that spermine was specifically responsible for repression of ornithine decarboxylase. Spermi-

dine and spermine are also reported to negatively regulate HeLa-cell ornithine decarboxylase at lower concentrations than putrescine or non-physiological diamines (Branca & Herbst, 1980). Consistent with control of ornithine decarboxylase activity by spermidine or spermine in mammalian tissue is the observation that methylglyoxal bis(guanylhydrazone), an inhibitor of spermidine and spermine biosynthesis, actually stimulates ornithine decarboxylase activity in the presence of elevated putrescine concentrations (Heby *et al.*, 1973; Fillingame & Morris, 1973).

In addition to the polyamine-sensitive component of the modulation of ornithine decarboxylase activity, the mammalian enzyme is sensitive to putrescine and non-physiological diamines, at the level of either enzyme synthesis or the production of an ornithine decarboxylase-inhibitory anti-enzyme (Kallio *et al.*, 1977; Pegg *et al.*, 1978; Bethell & Pegg, 1979; McCann *et al.*, 1980; Heller & Canelakis, 1981). It is clear from the present study that these diamine-dependent effects are not of major consideration in the A-form-B-form ornithine decarboxylase conversion reaction of *Physarum*.

Although putrescine is not a good stimulator of this modification of ornithine decarboxylase, we may note the otherwise absence of specificity as demonstrated by the large number of polyamines that were approximately equally effective in stimulating this reaction. This observation is consistent with the interpretation that the polyamine is not a substrate for the reaction, but rather that it serves a catalytic role in promoting the appropriate conformational interaction between A-form ornithine decarboxylase and the conversion factor. In support of this we have already shown that the polyamines are more effective than putrescine at inducing conformational change in ornithine decarboxylase

(Mitchell *et al.*, 1978), and the conversion factor itself has a high affinity for spermidine and spermine (J. L. A. Mitchell, unpublished work). It has also been noted that ^{14}C -labelled spermidine used to stimulate this reaction does not become covalently associated with the product, B-form ornithine decarboxylase (J. L. A. Mitchell, unpublished work). The precise role of the polyamine in this reaction has yet to be determined; however, this role is not merely to serve as a cation, because this reaction is not promoted by inorganic cations or most diamines, even at concentrations 10 times that of the polyamines.

The large difference between the apparent K_m values for putrescine and 1,3-diaminopropane, a non-physiological analogue, is noteworthy. The ornithine decarboxylase modification reaction may be thought to recognize specifically the $\text{NH}_2\text{--}[\text{CH}_2]_3\text{--NH}_2$ group, as this is a common feature of the polyamines found to be effective in stimulating this reaction. Inconsistent with this theory, however, is the observation that triaminoheptane, which does not contain this group, mimicked spermidine in stimulating this enzyme modification. Although it is not obvious why diaminopropane, like the polyamines, stimulated this reaction, it is clear that functionally this diamine may be more closely related to spermidine and spermine than to putrescine. Indeed, diaminopropane has not been found to be a particularly good putrescine analogue in stimulating *S*-adenosylmethionine decarboxylase activity *in vivo* (Mamont & Danzin, 1981) or inhibiting HTC-cell ornithine decarboxylase (McCann *et al.*, 1980) even though it was readily taken into the cell. Furthermore, unlike putrescine, it is not a good substrate in the propylamine transferase reaction (Raina & Jänne, 1975). The many studies that have used this diamine as a putrescine analogue, in attempts to distinguish between putrescine and polyamine effects, should be tempered by this possible functional relation between diaminopropane and the polyamines. Such polyamine-like activity may be at least partially responsible for the effectiveness of this analogue in limiting mammalian polyamine biosynthesis.

The use of non-physiological di- and poly-amines to alter polyamine biosynthesis experimentally is confounded by another problem demonstrated in the present study. Although diaminopropane and the polyamines were all effective stimulators of the modification of ornithine decarboxylase *in vitro*, only spermidine, and to a smaller extent spermine, were very effective *in vivo*. This difference was most probably due to poor recognition of the other compounds by the membrane putrescine- and spermidine-transport mechanisms in *Physarum*.

The control of polyamine biosynthesis is a complex regulatory mechanism that appears to contain

many product-sensitive reactions. By isolating one of the critical ornithine decarboxylase-modifying reactions and analysing its polyamine/putrescine-specificity *in vitro* and *in vivo*, we have at least begun to understand this complex feedback regulatory system.

This work was supported by Grant no. 81-16 from the American Cancer Society, Illinois Division, and Biomedical Research Support Grant RR-7176 from the National Institutes of Health.

References

- Atmar, V. J. & Kuehn, G. D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5518–5522
- Bethell, D. R. & Pegg, A. E. (1979) *Biochem. J.* **180**, 87–94
- Branca, A. A. & Herbst, E. J. (1980) *Biochem. J.* **186**, 925–931
- Clark, J. L. & Fuller, J. L. (1975) *Biochemistry* **14**, 4403–4409
- Fillingame, R. H. & Morris, D. R. (1973) *Biochemistry* **12**, 4479–4487
- Heby, O. (1981) *Differentiation* **19**, 1–20
- Heby, O., Sauter, S. & Russell, D. H. (1973) *Biochem. J.* **136**, 1121–1124
- Heller, J. S. & Canellakis, E. S. (1981) *J. Cell. Physiol.* **107**, 209–217
- Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1858–1862
- Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
- Jänne, J., Alhonen-Hongisto, L., Seppänen, P. & Siimes, M. (1981) *Med. Biol.* **59**, 448–457
- Kallio, A. (1978) *Acta Chem. Scand. Ser. B* **32**, 759–764
- Kallio, A., Löfman, M., Pösö, H. & Jänne, J. (1977) *FEBS Lett.* **79**, 195–199
- Mamont, P. S. & Danzin, C. (1981) *Adv. Polyamine Res.* **3**, 123–135
- Marton, L. J. (1981) *Med. Biol.* **59**, 458–461
- McCann, P. P., Tardif, C. & Mamont, P. S. (1977) *Biochem. Biophys. Res. Commun.* **75**, 948–954
- McCann, P. P., Tardif, C., Hornsperger, J.-M. & Böhlen, P. (1979) *J. Cell. Physiol.* **99**, 183–190
- McCann, P. P., Tardif, C., Pegg, A. E. & Diekema, K. (1980) *Life Sci.* **26**, 2003–2010
- McCann, P. P., Bacchi, C. J., Clarkson, A. B., Jr., Seed, J. R., Nathan, H. C., Amole, B. O., Hunter, S. H. & Sjoerdsma, A. (1981) *Med. Biol.* **59**, 434–440
- Mitchell, J. L. A. & Kottas, G. E. (1979) *FEBS Lett.* **102**, 265–268
- Mitchell, J. L. A. & Rusch, H. P. (1973) *Biochim. Biophys. Acta* **297**, 503–516
- Mitchell, J. L. A., Campbell, H. A. & Carter, D. D. (1976) *FEBS Lett.* **62**, 33–37
- Mitchell, J. L. A., Carter, D. D. & Rybski, J. A. (1978) *Eur. J. Biochem.* **92**, 325–331
- Mitchell, J. L. A., Yingling, R. A. & Mitchell, G. K. (1981a) *FEBS Lett.* **131**, 305–309

- Mitchell, J. L. A., Augustine, T. A. & Wilson, J. M. (1981b) *Biochim. Biophys. Acta* **657**, 257–267
- Paulus, T. J. & Davis, R. H. (1981) *J. Bacteriol.* **145**, 14–20
- Pegg, A., Conover, C. & Wrona, A. (1978) *Biochem. J.* **170**, 651–660
- Raina, A. & Jänne, J. (1975) *Med. Biol.* **53**, 121–147
- Russell, D. H. (1981) *Biochem. Biophys. Res. Commun.* **99**, 1167–1172
- Tabor, C. W. (1981) *Med. Biol.* **59**, 272–278
- Tyagi, A. K., Tabor, C. W. & Tabor, H. (1981) *J. Biol. Chem.* **256**, 12156–12163
- Williams-Ashman, H. G. & Schenone, A. (1972) *Biochem. Biophys. Res. Commun.* **46**, 288–295