Effect of inhibitors of *S*-adenosylmethionine decarboxylase on the contents of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in L1210 cells

Rentala MADHUBALA,* John A. SECRIST, III† and Anthony E. PEGG*‡

*Departments of Physiology and Pharmacology and Cancer Research Center, Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA 17033, U.S.A., and †Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, AL 35255, U.S.A.

Treatment of L1210 cells with either of two inhibitors of *S*-adenosylmethionine decarboxylase (AdoMetDC), namely 5'-deoxy-5'-[*N*-methyl-*N*-[2-(amino-oxy)ethyl]aminoadenosine or 5'-deoxy-5'-[*N*-methyl-*N*-(3-hydrazinopropyl)]aminoadenosine, produced a large increase in the amount of ornithine decarboxylase (ODC) protein. The increased enzyme content was due to a decreased rate of degradation of the protein and to an increased rate of synthesis, but there was no change in its mRNA content. The inhibitors led to a substantial decline in the amounts of intracellular spermidine and spermine, but to a big increase in the amount of putrescine. These results indicate that the content of ODC is negatively regulated by spermidine and spermine at the levels of protein translation and turnover, but that putrescine is much less effective in bringing about this repression. Addition of either spermidine or spermine to the cells treated with the AdoMetDC inhibitors led to a decrease in ODC activity, indicating that either polyamine can bring about this effect, but spermidine produced effects at concentrations similar to those found in the control cells and appears to be the physiologically important regulator. The content of AdoMetDC protein (measured by radioimmunoassay) was also increased by these inhibitors, and a small increase in its mRNA content was observed, but this was insufficient to account for the increase in protein. A substantial stabilization of AdoMetDC occurred in these cells, contributing to the increased enzyme content, but an increase in the rate of translation cannot be ruled out.

INTRODUCTION

Ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) are the key enzymes controlling the biosynthesis of polyamines. These enzymes are subject to regulation by a wide variety of substances (for reviews see [1–5]). It is clear that the polyamines themselves play an important part in this regulation, and it is well documented that the administration of putrescine, spermidine or spermine, which increases cellular polyamine pools, leads to rapid loss of ODC activity [2–13]. Conversely, the decrease in cellular polyamine content by ornithine starvation or the use of competitive ODC inhibitors has been shown to be accompanied by an increase in ODC activity [2,6,12–14]. Although there is general agreement on these findings, it was not entirely clear which of the polyamines is most effective at bringing about the regulation of ODC activity. In particular, it was not apparent whether putrescine, the immediate product of the ODC reaction, itself plays an important role in regulating the activity of this enzyme, or whether its actions are mediated through its conversion into spermidine and spermine. This question could obviously be addressed by application of inhibitors of AdoMetDC, but, until very recently, the only available

inhibitor of this enzyme was MGBG, which is not sufficiently specific for this purpose [15–17]. Recently, several very potent irreversible inhibitors of AdoMetDC have become available [18–20].

We have used two of these inhibitors, MHZPA and MAOEA, to modify polyamine content in L1210 cells. It was found that ODC was increased in response to the decline in spermidine and spermine brought about by these inhibitors, even though putrescine was increased. A spermine synthase inhibitor, AdoS⁺(CH₃)₂ [21,22], was also used to deplete spermine selectively in these cells. These results demonstrated that both spermidine and spermine are active in regulating ODC content. The mechanisms by which ODC was increased by these inhibitors were investigated by measuring the content of its mRNA and the rate of turnover of the protein. The effects of MHZPA and MAOEA on AdoMetDC protein were also studied by using a radioimmunoassay to quantify the enzyme protein, even though most of it was enzymically inactive, owing to reaction with these irreversible inhibitors. Treatment with the inhibitors led to a large increase in the amount of the AdoMetDC protein, but only a small increase in the content of its mRNA. Our results indicate that the synthesis of ODC and AdoMetDC is considerably enhanced in cells which

Abbreviations used: ODC, ornithine decarboxylase; AdoMetDC, *S*-adenosylmethionine decarboxylase; MAOEA, 5'-deoxy-5'-[*N*-methyl-*N*-[2-(amino-oxy)ethyl]aminoadenosine; MHZPA, 5'-deoxy-5'-[*N*-methyl-*N*-(3-hydrazinopropyl)]aminoadenosine; AdoS⁺(CH₃)₂, *S*-methyl-5'-methylthioadenosine; MGBG, methylglyoxal bis(guanyldiazide); MBAG, 1,1'-[(methylethanediyldene)dinitrilo]-bis(3-aminoguanidine).

‡ To whom correspondence should be addressed.

Fig. 1. Stability of ODC and AdoMetDC in L1210 cells treated with MAOEA

Cells were grown for 36 h in the absence (□) or presence (■) of 100 μM -MAOEA, and then 200 μM -cycloheximide was added and samples were taken at various times later as shown. Panel (a) shows results for the activity of ODC, which was expressed as a percentage of that present at the time of addition of cycloheximide. The absolute values were 5.9 ± 0.4 units/mg for control cells and 45.2 ± 2.1 units/mg for the MAOEA-treated cells. Panel (b) shows results for the content of AdoMetDC protein expressed in the same way. The absolute values were 16 ± 3 ng/mg for control cells and 144 ± 29 ng/mg for the MAOEA-treated cells.

ODC activity produced by MAOEA and MHZPA is consistent with the observed intracellular amine content, since the total (putrescine + spermidine + spermine) was substantially increased by the presence of these inhibitors (Table 1).

Analysis by radioimmunoassay showed that the increase in ODC activity in response to MAOEA and MHZPA was due to an elevated amount of ODC protein (Table 1). However, this rise in ODC protein was not mediated by an increased content of mRNA for the enzyme, since analysis by Northern blots and by dot blots showed that there was no significant change in the mRNA content (Table 1). The rise was associated with an increase in the half-life of the enzyme, which was changed from 21 ± 4 min to 72 ± 12 min (Fig. 1), but this is not sufficient to account for the increase in ODC protein. These results suggest that the depletion of spermidine and/or spermine also leads to an increased rate of synthesis of ODC protein, which occurs at the level of translation of the mRNA.

The addition of the spermine synthase inhibitor $\text{AdoS}^+(\text{CH}_3)_2$ led to a decline in the content of spermine in the L1210 cells, which was accompanied by a 2–3-fold increase in the ODC activity and protein (Table 1). Since this increase was much less than the change brought about by MAOEA or MHZPA, which deplete spermine to a lesser extent but also have an effect on spermidine, it appears that spermidine depletion was a major factor in bringing about the decrease in ODC.

The effects of administration of MAOEA and MHZPA on the activity and content of AdoMetDC are shown in Table 2. Although there was a substantial (> 90%) decrease in the activity of this enzyme, the amount of enzyme protein, as measured by radioimmunoassay, rose. This is consistent with the fact that these compounds are irreversible inhibitors of AdoMetDC and the inactivated protein accumulates in the cells. The mRNA for AdoMetDC was analysed by Northern-blot analysis by using a cDNA probe to rat prostate mRNA. The major species of mRNA detected by this procedure had a size of about 3.5 kb, and there was a faint band of size 2.1 kb (results not shown). These sizes are in agreement with results found for bovine, rat and human AdoMetDC mRNA ([32,33]; A. E. Pegg, R. Madhubala, B. Stanley, A. Pajunen, A. Crozat & O. Jänne, unpublished work), although the smaller mRNA

Table 2. AdoMetDC activity, protein and mRNA content in L1210 cells treated with MAOEA and MHZPA

Results shown are means of three determinations which agreed within $\pm 15\%$. The content of mRNA was determined both by counting radioactivity of dot blots (c.p.m./ μg) and by densitometric scanning of Northern blots (units/ μg) as described in the Materials and methods section. In both cases, the results were expressed per μg of RNA isolated.

Treatment and time (h)	AdoMetDC activity (units/mg)	AdoMetDC protein (ng/mg)	AdoMetDC mRNA	
			(c.p.m./ μg)	(units/ μg)
24 h	2.45	20.2	263	1.0
24 h + 100 μM -MAOEA	0.11	94.4	435	2.5
24 h + 50 μM -MHZPA	0.28	91.8	390	2.0
48 h	1.81	14.7	272	1.0
48 h + 100 μM -MAOEA	< 0.1	136	473	2.1
48 h + 50 μM -MHZPA	< 0.1	141	383	2.3
48 h + 0.4 mM- $\text{AdoS}^+(\text{CH}_3)_2$	15.7	130	N.D.	2.7

Table 3. Effect of spermidine and spermine on ODC activity and polyamine contents in cells treated with MAOEA and MHZPA

The cells were harvested for measurement 44 h after the addition shown. All cultures contained 1 mM-aminoguanidine to prevent oxidation of the added polyamines. Results are means $\pm$ s.d. for four or five measurements. For the polyamines, the cell number was not determined directly in this experiment, but these values can be converted into nmol/10⁶ cells by dividing by 8.7.

Treatment	ODC activity (units/mg)	Polyamines (nmol/mg of protein)		
		Putrescine	Spermidine	Spermine
Control	6.6 $\pm$ 0.9	4.0 $\pm$ 0.5	26.9 $\pm$ 1.9	8.5 $\pm$ 1.2
50 μ M-MHZPA	55.3 $\pm$ 3.9	54.3 $\pm$ 8.1	11.4 $\pm$ 1.9	3.9 $\pm$ 0.4
50 μ M-MHZPA + 0.4 μ M-spermine	56.2 $\pm$ 4.8	27.2 $\pm$ 1.9	6.2 $\pm$ 0.3	8.5 $\pm$ 0.7
50 μ M-MHZPA + 5.0 μ M-spermine	0.2 $\pm$ 0.03	< 0.5	4.5 $\pm$ 0.7	29.0 $\pm$ 1.5
50 μ M-MHZPA + 0.5 μ M-spermidine	52.0 $\pm$ 4.9	29.9 $\pm$ 1.0	16.1 $\pm$ 1.9	3.2 $\pm$ 0.2
50 μ M-MHZPA + 1.0 μ M-spermidine	41.9 $\pm$ 4.4	11.9 $\pm$ 4.0	20.6 $\pm$ 3.6	2.1 $\pm$ 0.4
50 μ M-MHZPA + 4.0 μ M-spermidine	2.2 $\pm$ 0.4	0.9 $\pm$ 0.1	31.1 $\pm$ 2.8	2.9 $\pm$ 0.3
100 μ M-MAOEA	55.9 $\pm$ 7.8	54.2 $\pm$ 6.4	10.2 $\pm$ 2.5	3.8 $\pm$ 0.9
100 μ M-MAOEA + 0.4 μ M-spermine	47.0 $\pm$ 13.4	41.5 $\pm$ 1.6	9.1 $\pm$ 0.6	7.0 $\pm$ 0.5
100 μ M-MAOEA + 5.0 μ M-spermine	0.6 $\pm$ 0.3	< 0.5	2.8 $\pm$ 0.1	23.6 $\pm$ 2.8
100 μ M-MAOEA + 0.5 μ M-spermidine	44.8 $\pm$ 2.2	25.9 $\pm$ 4.1	9.3 $\pm$ 0.7	2.9 $\pm$ 0.3
100 μ M-MAOEA + 1.0 μ M-spermidine	47.4 $\pm$ 1.2	21.0 $\pm$ 1.2	17.6 $\pm$ 1.2	2.0 $\pm$ 0.4
100 μ M-MAOEA + 4.0 μ M-spermidine	1.6 $\pm$ 0.4	1.1 $\pm$ 0.5	31.0 $\pm$ 0.8	2.8 $\pm$ 0.3
0.5 μ M-spermidine	9.8 $\pm$ 0.6	3.2 $\pm$ 0.5	20.8 $\pm$ 3.1	5.7 $\pm$ 0.8
1.0 μ M-spermidine	10.0 $\pm$ 0.3	3.3 $\pm$ 0.3	20.0 $\pm$ 1.5	5.6 $\pm$ 0.4
4.0 μ M-spermidine	0.8 $\pm$ 0.3	< 0.5	20.5 $\pm$ 4.4	8.4 $\pm$ 1.9
0.4 μ M-spermine	8.0 $\pm$ 0.4	1.5 $\pm$ 0.2	18.4 $\pm$ 1.4	9.6 $\pm$ 1.1
5.0 μ M-spermine	0.2 $\pm$ 0.05	< 0.5	5.0 $\pm$ 0.7	22.0 $\pm$ 0.6

is less prominent in the L1210 cells. Analysis of the content of AdoMetDC mRNA by Northern blots and by dot blots indicated that there was a small but significant increase in the content of mRNA for this enzyme in response to these inhibitors (Table 2). The increase in mRNA content was, however, only around 2-fold, and this was not sufficient to account for the increased accumulation of the protein. The turnover of the protein was greatly slowed in the presence of the inhibitors (Fig. 1), and this may be a major factor in accounting for the elevated content.

In order to determine the relative importance of spermidine and spermine in the regulation of ODC activity, cells were grown in the presence of MHZPA or MAOEA and various concentrations of these polyamines. The ODC activity and polyamine content of the cells were measured 44 h later (Table 3). Either spermidine or spermine alone was able to decrease the ODC activity to control values or below in the cells treated with the AdoMetDC inhibitors. However, addition of 0.4 μ M-spermine, which restored cellular spermine to control values, was not sufficient to decrease ODC activity. Only when 5 μ M-spermine, which increased cellular spermine substantially above control, was used did ODC decrease completely. Similarly, only when exogenous spermidine was present at concentrations higher than 1 μ M did ODC decline greatly, although there was replenishment of much of the intracellular spermidine concentrations at lower spermidine concentrations (Table 3). The results in Table 3 indicate that either spermine or spermidine can negatively regulate ODC, but suggest that spermidine may be the more physiologically important regulator, since only slight changes in the normal cellular spermidine content are

associated with major alterations in ODC activity (in Table 3, compare the results with 1 μ M- and 4 μ M-spermidine with the control values).

DISCUSSION

The availability of specific inhibitors of AdoMetDC [20,21] and of antibodies [24,28] and cDNA probes for ODC [25,34] and AdoMetDC [29,33] permits more detailed investigation of the regulation of these proteins by the intracellular polyamine concentration. Our results show that putrescine is unlikely to play any significant role in the regulation of ODC activity. Increases in putrescine content of more than an order of magnitude were produced by MAOEA and MHZPA without bringing about any decrease in ODC. It seems clear that the increase in ODC protein is due to the decrease in the cellular contents of spermidine and spermine. The fact that putrescine increases by a greater amount than spermidine and spermine decrease (Tables 1 and 3), leading to a net increase in total diamine plus polyamine content in the cell, shows that putrescine is much less effective in decreasing ODC activity than are the polyamines spermine and spermidine. These results are in agreement with early studies which showed that MGBG treatment led to an increase in ODC activity in rat liver [35–37], but in these experiments the direct effects of MGBG itself cannot be ruled out, since this compound is a polyamine analogue and has numerous other effects in addition to its inhibition of AdoMetDC [2,4,16]. It should be emphasized that, although putrescine does not appear to regulate ODC, it does play an important role in the control of mammalian polyamine concentrations. In addition to acting as a substrate for

spermidine synthase, it both activates AdoMetDC and increases the synthesis of AdoMetDC protein from its precursor [4,33,38].

Our results indicate that either spermine or spermidine can regulate ODC content, but that spermidine may play the more critical role in this regulation, since only a small decrease in spermidine from its normal value is accompanied by a large increase in ODC (Table 3). This suggestion is also in agreement with the finding that $\text{AdoS}^+(\text{CH}_3)_2$, which inhibits spermine synthase and decreases spermine selectively, had a smaller effect on ODC than did MAOEA and MHZPA (Table 1). It is also consistent with previous findings that ODC increased in cells treated with cyclohexylamine [39] or with S-adenosyl-1,8-diamino-3-thio-octane [40]. These compounds inhibit spermidine synthase, and lead to a decrease in spermidine but to a compensatory increase in putrescine and spermine [22,39,40].

The increase in ODC activity brought about by the inhibition of the synthesis of spermidine and spermine clearly does not involve changes in the content of mRNA (Table 1). This conclusion is in agreement with recent studies which have shown that decreased ODC by application of exogenous polyamines [8–10,12–14,26] or analogues [25,41] does not involve a decrease in ODC mRNA. It is apparent that the regulation of ODC synthesis by polyamines is brought about at the translational level. Although exact details of the biochemical mechanism of this translational regulation are not known, the addition of spermidine, spermine or their bis(ethyl) derivatives to reticulocyte lysates selectively inhibits the translation of ODC mRNA [42]. This inhibition may involve the interaction of the polyamines with part of the mRNA, either directly or via a change in the binding of a regulatory protein. However, most of the long 5' leader sequence of ODC mRNA is not needed for this interaction, since a similar inhibition of translation of ODC mRNA was obtained with an mRNA derived from a plasmid containing an ODC cDNA insert which lacks all but 69 bases of this leader sequence (A. E. Pegg, R. Madhubala, T. Kameji & R. J. Bergeron, unpublished work).

In addition to the pronounced effect of polyamines on the translation of ODC mRNA, it is also clear that the degradation rate of ODC protein is influenced by polyamines. Administration of exogenous polyamines decreases the half-life of ODC [9–14], and the decrease in polyamines by use of AdoMetDC inhibitors clearly stabilizes the protein (Fig. 1). At present, little is known about the degradation of ODC protein, which is one of the most rapidly turning-over mammalian proteins, although ODC does contain a strong 'PEST' sequence, and such sequences have been postulated to be important in determination of the rate of protein turnover [43]. An inhibitory protein termed 'antizyme', which appears to be induced by application of putrescine, has been implicated in the decrease of ODC activity [6], and it has been suggested that the combination with this protein might increase the degradation of the ODC protein [24,44]. However, the experiments reported in the present paper do not support the hypothesis that antizyme is involved in the regulation of ODC degradation rates. We obtained no evidence for a change in the amount of antizyme-ODC complex on application of the AdoMetDC inhibitors, since there was a good correlation between the amount of ODC protein and the activity

(Table 1). The apparent absence of antizyme in these cells in which putrescine content is increased by more than an order of magnitude casts doubt on the physiological importance of the induction of antizyme by exogenous putrescine. The possibility that spermidine and spermine interact directly with ODC at the PEST region, or induce post-translational modifications at this region, leading to changes in the degradation rate, would explain the experimental results, and requires further investigation.

It should also be pointed out that there is convincing evidence, based on studies with the lower eukaryote *Neurospora crassa*, that polyamines are compartmentalized in the cell and that only a fraction of total is available for regulation of ODC [45,46]. It is quite possible that this is also the case in mammalian cells, and that spermidine or spermine, which are newly synthesized or have just been taken up, are more important in regulating ODC and AdoMetDC than are pre-existing polyamines. The very rapid increases in the activities of ODC and AdoMetDC seen in lymphoblasts treated with 5'-methylthioadenosine, which inhibits spermine synthase, may be indicative of such a response [47,48].

Our results are also in agreement with the hypothesis that the amount of AdoMetDC is regulated by the content of spermidine and spermine. There was a substantial increase in AdoMetDC protein in the cells treated with MAOEA and MHZPA, and there was a small increase in the AdoMetDC mRNA content. This is consistent with previous studies in which it was found that AdoMetDC activity and mRNA were increased in cells depleted of spermidine by treatment with difluoromethylornithine [49]. There is also evidence that translation of AdoMetDC mRNA is regulated by polyamines [27,42]. However, the stabilization of the degradation of AdoMetDC protein in cells treated with MAOEA and MHZPA was so large that a half-life for the protein could not be calculated accurately over the short period in which cycloheximide can be used without complicating cell toxicity. This stabilization may be due to the binding of the inhibitors, since both MGBG and MBAG, an irreversible inactivator, lead to a very large increase in the amount of the AdoMetDC protein [2,49,50]. Such stabilization may play a large role in the overall increase in AdoMetDC protein, and the extent to which it contributes cannot be calculated easily. Therefore, although the present system is extremely useful for the study of the regulation of ODC, it is not so valuable for the study of AdoMetDC.

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REFERENCES

1. Russell, D. H. (1980) *Pharmacology* **20**, 117–129
2. Pegg, A. E. & McCann, P. P. (1982) *Am. J. Physiol.* **243**, C212–C221
3. Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790
4. Pegg, A. E. (1986) *Biochem. J.* **234**, 249–262
5. Pegg, A. E. (1988) *Cancer Res.* **48**, 759–774
6. Canellakis, E. S., Viceps-Madore, D., Kyriakidis, D. A. & Heller, J. S. (1979) *Curr. Top. Cell. Regul.* **15**, 155–202

7. McCann, P. P. (1980) in *Polyamines in BioMedical Research* (Gaugas, J. M., ed.), pp. 109–123, Wiley, London
8. Kahana, C. & Nathans, D. (1985) *J. Biol. Chem.* **260**, 15390–15393
9. Hölttä, E. & Pohjanpelto, P. (1986) *J. Biol. Chem.* **261**, 9502–9508
10. Persson, L., Holm, I. & Heby, O. (1986) *FEBS Lett.* **205**, 175–178
11. Sertich, G. J., Glass, J. R., Fuller, D. J. M. & Gerner, E. W. (1986) *J. Cell. Physiol.* **127**, 114–120
12. Dirks, L., Grens, A., Slezinger, T. C. & Scheffler, I. E. (1985) *J. Cell. Physiol.* **126**, 371–378
13. McConlogue, L., Dana, S. L. & Coffino, P. (1986) *Mol. Cell. Biol.* **6**, 2865–2871
14. Persson, L., Oredsson, S. M., Anehus, S. & Heby, O. (1985) *Biochem. Biophys. Res. Commun.* **131**, 239–245
15. Jänne, J., Alhonen-Hongisto, L., Nikula, P. & Elo, H. (1985) *Adv. Enzyme Regul.* **24**, 125–139
16. Williams-Ashman, H. G. & Seidenfeld, J. (1986) *Biochem. Pharmacol.* **35**, 1217–1225
17. Porter, C. W. & Sufrin, J. R. (1986) *Anticancer Res.* **6**, 525–542
18. Secrist, J. A., III (1987) *Nucleosides Nucleotides* **6**, 73–83
19. Artamonova, E. Y., Zavalova, L. L., Khomutov, R. M. & Khomutov, A. R. (1986) *Bioorg. Khim.* **12**, 206–212
20. Pegg, A. E., Jones, D. B. & Secrist, J. A., III (1988) *Biochemistry* **27**, 1408–1415
21. Pegg, A. E. & Coward, J. K. (1985) *Biochem. Biophys. Res. Commun.* **133**, 82–89
22. Coward, J. K. & Pegg, A. E. (1987) *Adv. Enzyme Regul.* **26**, 107–113
23. Seely, J. E., Pösö, H. & Pegg, A. E. (1982) *Biochemistry* **21**, 3394–3399
24. Seely, J. E. & Pegg, A. E. (1983) *Biochem. J.* **216**, 701–707
25. Porter, C. W., Berger, F. G., Pegg, A. E., Ganis, B. & Bergeron, R. J. (1987) *Biochem. J.* **242**, 433–440
26. Sertich, G. J. & Pegg, A. E. (1987) *Biochem. Biophys. Res. Commun.* **143**, 424–430
27. Pegg, A. E., Wechter, R. & Pajunen, A. (1987) *Biochem. J.* **244**, 49–54
28. Shirahata, A., Christman, K. & Pegg, A. E. (1985) *Biochemistry* **24**, 4417–4423
29. Pegg, A. E., Kameji, T. & Pajunen, A. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 2046
30. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
31. Seiler, N. (1983) *Methods Enzymol.* **94**, 25–29
32. Mach, M., White, M. W., Neubauer, M., Degen, J. L. & Morris, D. R. (1986) *J. Biol. Chem.* **261**, 11697–11703
33. Pegg, A. E., Kameji, T., Shirahata, A., Stanley, B., Madhubala, R. & Pajunen, A. (1988) *Adv. Enzyme Regul.*, in the press
34. Berger, F. G., Szymanski, P., Read, E. & Watson, G. (1984) *J. Biol. Chem.* **259**, 7941–7946
35. Hölttä, E., Hannonen, P., Pispä, J. & Jänne, J. (1973) *Biochem. J.* **136**, 669–676
36. Alhonen-Hongisto, L., Pösö, H. & Jänne, J. (1980) *Biochem. J.* **188**, 491–501
37. Karvonen, E. & Pösö, H. (1984) *Biochim. Biophys. Acta* **791**, 239–243
38. Kameji, T. & Pegg, A. E. (1987) *Biochem. J.* **243**, 285–288
39. Mitchell, J. L. A., Mahan, D. W., McCann, P. P. & Qasba, P. (1985) *Biochim. Biophys. Acta* **840**, 309–316
40. Pegg, A. E., Tang, K.-C. & Coward, J. K. (1982) *Biochemistry* **21**, 5082–5089
41. Madhubala, R., Secrist, J. A., III & Pegg, A. E. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 2254
42. Kameji, T. & Pegg, A. E. (1987) *J. Biol. Chem.* **262**, 2427–2430
43. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234**, 364–368
44. Murakami, Y. & Hayashi, S. (1985) *Biochem. J.* **226**, 893–896
45. Paulus, T. J., Cramer, C. L. & Davis, R. H. (1983) *J. Biol. Chem.* **258**, 8608–8612
46. Davis, R. H., Krasner, G. N., DiGagni, J. J. & Ristow, J. L. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4105–4109
47. Kubota, M., Kajander, E. O. & Carson, D. A. (1985) *Cancer Res.* **45**, 3567–3572
48. Yamanaka, H., Kubota, M. & Carson, D. A. (1987) *Cancer Res.* **47**, 1771–1774
49. Shirahata, A. & Pegg, A. E. (1986) *J. Biol. Chem.* **261**, 13833–13837
50. Pegg, A. E. (1978) *J. Biol. Chem.* **253**, 539–542

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