Ornithine decarboxylase production *in vitro* by using mouse cDNA

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Microgram quantities of ornithine decarboxylase (ODC, EC 4.1.1.17)-specific mRNA were synthesized by transcription techniques *in vitro*, by using a plasmid containing mouse cDNA coding for this enzyme. The homogeneous RNA preparation was then used for cell-free synthesis of ODC protein, in rabbit reticulocyte lysates. Analysis of products translated *in vitro* by polyacrylamide-gel electrophoresis revealed predominantly one protein produced, with M_r approx. 54000, which was immunoprecipitable by anti-ODC serum. Two-dimensional gel-electrophoretic analysis showed that the protein ODC synthesized *in vitro* had a pI of approx. 5.4, similar to the native enzyme isolated from mouse tissues. In addition, quantification of activity and protein amount showed that the enzyme synthesized *in vitro* had a specific activity of approx. 63000 units (nmol/min)/mg, consistent with the purified mouse kidney enzyme's specific activity of approx. 47000 units/mg. An average of nearly 200 pg of ODC protein was produced *in vitro* from various RNA preparations. These data demonstrate that ODC-specific mRNA and active ODC protein can be produced by '*in vitro*' technology, which should prove useful in studying functional and structural characteristics of these molecules.

INTRODUCTION

The mechanism of regulation of ornithine decarboxylase (ODC), the initial enzyme in the biosynthesis of polyamines, has been widely studied. These investigations were initiated by observations of the highly inducible enzyme activity by growth stimuli, and identification of the biological half-life as one of the shortest of any mammalian protein (for references, see Tabor & Tabor, 1984; Pegg, 1986). In mammalian cells, the control of enzyme activity is regulated by alterations of intracellular ODC protein amount, brought about by transcriptional (Kontula *et al.*, 1984; Gilmour *et al.*, 1985), translational (Kahana & Nathans, 1985a; Kanamoto *et al.*, 1986) and post-translational regulation (Glass & Gerner, 1986; Holtta & Pohjanpelto, 1986; Dircks *et al.*, 1986).

Initial studies on ODC activity regulation were hampered by the small amount of enzyme protein present in most tissues and cell cultures (Kameji *et al.*, 1982; Seely *et al.*, 1982). The availability of high-titre antisera (Kameji *et al.*, 1982; Seely & Pegg, 1983; Isomaa *et al.*, 1983) and production of mutant cell lines (McConlogue & Coffino, 1983; Choi & Scheffler, 1983; Kahana & Nathans, 1984) which greatly overproduce ODC protein have facilitated the investigation of this enzyme, and have been used to isolate cDNA sequences specific for ODC protein (Kontula *et al.*, 1984; Kahana & Nathans, 1984; McConlogue *et al.*, 1984). Though other work has detailed the effects of polyamines in regulating ODC in a feedback-type mechanism at the translational level (Kahana & Nathans, 1985b; Persson *et al.*, 1986) and by influencing the stability of ODC protein (Persson *et al.*, 1984; Glass & Gerner, 1986, 1987;

Holtta & Pohjanpelto, 1986), the detailed mechanism by which these effects occur is unknown.

In an attempt to re-create a system *in vitro* for ODC degradation, our initial goal was to acquire sufficient quantity of ODC protein for study. We have taken advantage of recent techniques of transcription *in vitro* (Melton *et al.*, 1984) and translation *in vitro* of cloned cDNA to produce ODC-specific mRNA and protein from a previously characterized ODC cDNA (Kahana & Nathans, 1984, 1985a). In the present paper we describe this methodology, which enables the production of sufficient quantities of easily obtainable ODC mRNA and protein for detailed analysis of potential polyamine interactions with these molecules.

MATERIALS AND METHODS

Materials

L-[³⁵S]Methionine (1120 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). A bacterial absorbant, *Staphylococcus* A (IGSORB), was purchased from the Enzyme Center (Malden, MD, U.S.A.). Ampholytes (pH 4–6.5, 3.5–9.5) were obtained from LKB (Gaithersburg, MD, U.S.A.). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). SP6 RNA polymerase, plasmid pGEM3, RNasin and RQ1 DNAase were purchased from Promega Biotec (Madison, WI, U.S.A.).

Transcription and translation *in vitro*

A plasmid derived from pmODC-1 was given by Dr. Daniel Nathans (Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine,

Abbreviation used: ODC, ornithine decarboxylase (EC 4.1.1.17)

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Baltimore, MD, U.S.A.). A 1692 bp fragment of ODC cDNA was derived from this mouse ODC cDNA clone initially described by Kahana & Nathans (1985a), by digestion of pmODC-1 with endonucleases *Sal*I and *Pvu*II. The fragment contains 69 bp of the 5' leader sequence, the complete coding sequence and 240 bp of the 3' non-transcribed sequence. The *Sal*I and *Pvu*II recognition sites were converted into an *Eco*RI site and a *Bam*HI site, respectively, and inserted into the multiple cloning site of plasmid pSP65 (ProMega Biotec) 5' to the promoter for the bacteriophage SP6 RNA polymerase (Fig. 1). The plasmid DNA as received was transferred into *Escherichia coli* DH1, and plasmid DNA was prepared essentially as described by Maniatis *et al.* (1982).

ODC mRNA was prepared *in vitro* as described by Melton *et al.* (1984), with as a template the pmODC-1-derived DNA digested with restriction endonucleases *Bam*HI and *Pvu*II. Then 15 units of SP6 RNA polymerase was added to a reaction mixture (final volume 100 μ l) consisting of 40 mM-Tris/HCl, pH 7.5, 6 mM-MgCl₂, 2 mM-spermidine, 10 mM-NaCl, 1 mM-dithiothreitol, 125 units of RNasin, 0.5 mM each of ATP, CTP, UTP and GTP, and 3 μ g of DNA template. After 2 h at 37 °C, 2 units of RQ1 DNAase was added to digest the template DNA and the incubation was continued for 15 min. The sample was extracted once with phenol/chloroform/3-methylbutan-1-ol (25:24:1, by vol.) which had been equilibrated with 10 mM-NaCl/10 mM-Tris/HCl (pH 7.5)/1 mM-EDTA, then extracted once with chloroform/3-methylbutan-1-ol (24:1, v/v), precipitated in 70% (v/v) ethanol at -20 °C for over 2 h, collected by centrifuging at 15000 g for 15 min, and passed over a 'spun-column' of Sephadex G-50 (Maniatis *et al.*, 1982), which had been equilibrated with 10 mM-Tris/HCl (pH 8.0)/0.1 M-NaCl/0.1 mM-EDTA. The RNA was recovered from the excluded volume and used directly in the translation reaction.

To determine the size of reaction products, samples of the ODC mRNA made *in vitro* were electrophoresed through a 1.0% agarose gel in the presence of formaldehyde (Maniatis *et al.*, 1982), with calf liver 28 S (4800 bp) and 18 S (1850 bp) and *E. coli* 23 S (2904 bp) and 16 S (1541 bp) RNA as markers. The gel was stained with ethidium bromide as described by Maniatis *et al.* (1982) and photographed.

Cell-free synthesis of ODC in rabbit reticulocytes was performed as recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.), by using 5 μ Ci of [³⁵S]methionine per reaction. ODC mRNA was added at the indicated final concentration in a volume of 10 μ l, and reactions were incubated at 30 °C for 1 h. The reactions were terminated by addition of 80 μ l of 4 $\times$ SDS solubilization buffer (2% SDS, 5% β -mercaptoethanol and 3% sucrose in 300 mM-Tris/HCl, pH 7.0). For solubilization before protein separation by two-dimensional electrophoresis, 100 μ l of isoelectric-focusing lysis buffer (8 M-urea, 4% β -mercaptoethanol, 5% Nonidet P-40, 2% ampholytes, pH 3.5-9.5) was added after completion of the translation reaction.

One-dimensional SDS/polyacrylamide-gel electrophoresis was performed by a slight modification of the method of Laemmli (1970). Bisacrylamide was replaced with *NN'*-diallyltartardiamide (Bio-Rad), so that gel slices could be dissolved by the addition of 2% periodic

acid. The method of O'Farrell (1975) was used for the separation of proteins by two-dimensional electrophoresis. Ampholytes of pI ranges 3.5-9.5 and 4-6.5 were used in a 1:1 ratio.

Immunoprecipitation

After translation of ODC mRNA *in vitro*, the samples were diluted with 170 μ l of buffer containing 0.05 M-sodium/potassium phosphate, pH 7.2, 0.1 mM-EDTA, 1.0 mM-dithiothreitol, 20 μ M-pyridoxal phosphate and 100 μ M-phenylmethanesulphonyl fluoride (ODC Assay Buffer), followed by addition of 5 μ l of a 1:500 dilution of primary antiserum (characterized by Seely & Pegg, 1983). After 1 h at 20 °C, 5 μ l of 10% (w/v) suspension of formalin-fixed *Staphylococcus A* was added, and 20 min later the complexes were collected by centrifugation at 7500 g for 2 min. Proteins were eluted from the *Staphylococcus A* at 100 °C for 5 min in 50 μ l of 4 $\times$ SDS solubilization buffer.

ODC activity

After translation *in vitro* of ODC mRNA, the samples were diluted with 170 μ l of ODC assay buffer, 6 μ Ci of L-[1-¹⁴C]ornithine (52.4 mCi/mmol) was added, and reactions were incubated at 37 °C for 30 min. ODC activity was determined by the liberation of ¹⁴CO₂ during the reaction period. One unit of enzyme activity is defined as 1 nmol of ornithine decarboxylated/min at 37 °C.

RESULTS

Transcription *in vitro* of the linearized plasmid derived from pmODC-1 by using SP6 RNA polymerase yields a single RNA species. The sequence of the DNA template is described in Fig. 1. The transcription reaction *in vitro* was performed and the RNA electrophoresed on an agarose gel as described in the Materials and methods section. The photograph in Fig. 2 shows that a highly homogeneous RNA preparation is obtained (lanes b and c), whose size is consistent with the expected 1713 bp. From three separate transcription reactions, using 3 μ g of plasmid DNA, 11, 13 and 17 μ g of RNA were produced in a 2 h reaction at 37 °C. Some preparations contained minor amounts of ODC RNA in the range 700-1690 bp (results not shown). However, these RNA preparations yielded the same protein products as that shown in Fig. 3. The results show that large quantities of RNA are produced in an efficient system which is capable of reutilizing the initial DNA template.

The addition of ODC mRNA produced by transcription *in vitro* to a rabbit reticulocyte-lysate cell-free translation system yields one predominant peptide, of *M_r* 54000 (Fig. 3a). Detectable synthesis of the protein *in vitro* is not evident until a concentration of 250 ng of RNA per reaction mixture is reached (Fig. 3a, lane f), and this concentration represents the optimum for synthesis *in vitro*. Addition of higher concentrations of RNA does not lead to a greater production of this peptide (results not shown). In the absence of exogenously added RNA (Fig. 3a, lane a), a radiolabelled peptide of *M_r* 43000 is produced, presumably resulting from non-synthetic labelling of endogenous protein (Jackson & Hunt, 1983). To determine whether the protein synthesized *in vitro* from ODC mRNA corresponds to actual ODC peptides, immunoreactivity was

Fig. 1. Sequence of the *Bam*HI/*Pvu*II-digested DNA template used for synthesis of ODC mRNA *in vitro*

The ODC cDNA sequence is composed of the 1692 bp fragment from *Sal*I and *Pvu*II digestion of pmODC-1 (Kahana & Nathans, 1985a). Translation is initiated 69 bp from the 5' end of that fragment and is terminated at bp 1452. Transcription from the SP6 promoter adds 15 bp contributed by the vector, and three C residues from the *Eco*RI linker to the 5' end, and three G residues by the *Bam*HI linker to the 3' end, yielding a transcript of 1713 bases.

Fig. 2. Agarose-gel electrophoresis of DNA produced by transcription *in vitro* of pmODC-1 derived DNA

The linearized plasmid derived from pmODC-1 was transcribed *in vitro* as described in the Materials and methods section, and the RNA separated by 1.0%-agarose-gel electrophoresis and photographed. Lane (a) represents RNA markers, and lane (b) 10 μ g and lane (c) 5 μ g of pmODC-1-derived plasmid transcribed *in vitro*.

determined by precipitation with anti-ODC antiserum. These results are shown in Fig. 3(b). Lane (a) shows the protein profile from a standard translation *in vitro* using 250 ng of ODC mRNA. Lane (b) represents those proteins immunoprecipitated by the specific antiserum, and lane (c) non-specific protein absorbed by *Staphylococcus A*. The major protein at M_r approx. 54000 is specifically precipitated, along with two other minor species, at approx. M_r 38000 and 30000. Note that the radiolabelled protein shown in lane (a) is from a single

lysate incubated with 250 ng of ODC mRNA, whereas the protein seen in lane (b) (immunoprecipitated with anti-ODC antiserum) was obtained from half of a similar lysate (see Fig. 3 legend for more detail). Thus the intensity difference between lanes (a) and (b) in Fig. 3(b) reflect differences in the starting protein content after the translation reaction. The protein pattern observed here is similar to that shown for synthesis *in vitro* of ODC mRNA isolated from mouse kidney (Kontula *et al.*, 1984).

To characterize further ODC protein synthesized in this system, we analysed the distribution of proteins translated *in vitro* by two-dimensional electrophoresis. Mouse ODC has a heterogeneous pI of approx. 5.4 (Seely *et al.*, 1985; McConlogue & Coffino, 1983), and the protein made *in vitro* has a very similar isoelectric mobility (Fig. 4a). The non-specific protein labelled in the absence of RNA is shown in Fig. 4(b). The two forms of ODC seen in mouse kidney are not apparent under our conditions, but there is evidence of charge heterogeneity. The nature of this heterogeneity is unknown, and represents a minor amount of the total ODC protein made in this cell-free system.

To quantify the amount of ODC protein made in each translation reaction, ODC activity and radioactivity incorporation into the M_r -54000 species were determined from various RNA preparations. These data are shown in Table 1. ODC activity was determined as described in the Materials and methods section from two separate RNA preparations. These data are shown as Expts. 1 and 2 in Table 1. The activities measured represent radioactivities of approx. 30000 c.p.m. of $^{14}\text{CO}_2$ liberated per reaction, which were completely inhibited by incubating replicate samples with α -difluoromethylornithine, the enzyme-activated irreversible inhibitor of ODC, before enzyme assay (results not shown). For determination of protein content, samples were fractionated by SDS/polyacrylamide-gel electrophoresis, and bands were detected by autoradiography. The M_r -54000 band was excised and solubilized in 2% periodic acid, and radioactivity was determined by scintillation counting. These data are shown as Expts. 3 and 4 in Table 1. ODC sequence data predicts 15 methionine residues per molecule of protein (Kahana & Nathans, 1985a), and, assuming that all the methionine incorporated is radiolabelled, the specific radioactivity can be used to derive an estimate of the amount synthesized. Four independent reactions from two different translations *in vitro* yielded radioactivity in

Fig. 3. Cell-free synthesis of ODC in rabbit reticulocyte lysates from ODC mRNA produced by transcription *in vitro*

(a) Various concentrations of ODC mRNA were added to a translation mixture *in vitro*, and incubated for 1 h at 30 °C. Proteins were fractionated by SDS/polyacrylamide-gel electrophoresis and detected by autoradiography. Lane (a) no addition, (b) 1 ng, (c) 10 ng, (d) 50 ng, (e) 150 ng, (f) 250 ng of ODC mRNA. (b) Immunoprecipitation of proteins translated *in vitro* by anti-ODC antiserum. Duplicate reticulocyte lysates containing 250 ng of ODC mRNA were analysed for ODC protein after incubation at 30 °C. Total protein from one lysate was loaded directly on a single-dimensional SDS/polyacrylamide gel (lane a). The other lysate was divided into two parts, diluted with buffer, as described in the Materials and methods section, and then incubated with (lane b) or without (lane c) anti-ODC antiserum, to distinguish immunoreactive proteins to anti-ODC antiserum (lane b) from proteins which might be non-specifically precipitated by *Staphylococcus* A.

protein corresponding to 171–211 pg of ODC per reaction (approx. 85000 c.p.m. of [³⁵S]methionine), by the assumptions just stated. These values should represent the minimum amount produced, on the assumption that all the methionine incorporated is radiolabelled. This leads to a calculated specific activity of this protein of approx. 63000 units/mg, similar to that of the purified mouse kidney enzyme (47000 units/mg) (Seely *et al.*, 1982). The calculation of specific activity in this study could be an overestimate of the real value, owing to possible underestimation of the quantity of protein produced *in vitro*. It is not known whether the lower- M_r species have catalytic activity, and, since these are not included in the calculations, this would contribute to potential variance.

DISCUSSION

We have been able to produce ODC protein from cloned cDNA sequences by using techniques of transcription and translation *in vitro*. That the major protein translated *in vitro* maintained the characteristics of ODC isolated from cells was based on the following criteria: (a) M_r of approx. 54000 and immunoprecipitation by anti-ODC specific antiserum (Fig. 3), (b) two-dimensional electrophoresis, which reveals a pI of approx. 5.4 (Fig. 4), consistent with that for the purified enzyme (Seely & Pegg, 1983); and (c) an enzyme specific activity very similar to that of purified mouse kidney ODC (Table 1). The procedure described here yields ODC protein in sufficient quantity and specific radioactivity to allow further investigations of ODC structure, and indicate that the protein can be used to study specific

aspects of ODC regulation. The two polypeptides, of M_r approx. 38000 and 30000, which contain ODC sequences, as is evident from the immunoprecipitation results (Fig. 3b), may result from initiation at internal start sites on the mRNA. AUG codons in phase with the first AUG codon are located between 387 and 417 bp and between 660 and 684 bp downstream from the first AUG codon. Initiation of translation at these codons would yield proteins of similar M_r values to those observed in Fig. 3(b).

In the presence of intracellular polyamines, ODC has a half-life as short as 15 min (Tabor & Tabor, 1984; Pegg, 1986; Glass & Gerner, 1987), one of the shortest turnover rates known for mammalian proteins. The mechanism for ODC degradation is unknown, but appears not to involve ubiquitin conjugation or lysosomes, which are thought to be two major pathways for intracellular protein catabolism (Glass & Gerner, 1987). ODC protein produced by the '*in vitro*' methodologies described here could be used to characterize further the specific mechanism of ODC degradation. Rechsteiner and co-workers have elegantly shown that micro-injection of radiolabelled proteins can be used to determine specific degradation pathways *in vivo* (Rote & Rechsteiner, 1983; Hough & Rechsteiner, 1984). Sufficient radiolabel is recovered in essentially a single protein to allow detection of ODC protein turnover after micro-injection (Table 1), and to study potential degradative pathways *in vivo*. This system allows the use of an endogenous cellular protein, and comparisons could be made in a manner similar to that described for other exogenous protein substrates. In addition, ODC produced *in vitro* could also serve as a substrate for the

Fig. 4. Two-dimensional electrophoresis of proteins translated *in vitro* after addition of ODC mRNA to rabbit reticulocyte lysates

ODC mRNA was added at a final concentration of 250 ng to the rabbit reticulocyte-lysate translation mixture. After 1 h at 30 °C, the reaction was terminated, and the proteins were separated by two-dimensional electrophoresis and detected by autoradiography. (a) +ODC mRNA; (b), no addition of exogenous RNA. Arrows indicate the position of the peptide specific for the reaction containing ODC mRNA.

Table 1. Determination of the amount of ODC protein and activity produced by translation *in vitro*

ODC was translated *in vitro* after addition of 250 ng of ODC mRNA, and ODC activity and protein content were determined as described in the Materials and methods section. The numbers in parentheses refer to the number of separate reactions per experiment.

Expt. no.	ODC activity (units/ reaction)	ODC protein (pg/reaction)	Specific activity (units/mg)
1	0.012 ± 0.004 (3)		
2	0.012 ± 0.0008 (4)		
3		211 ± 82 (2)	
4		171 ± 7 (2)	
Average	0.012	191	62827

isolation of a specific proteinase(s) and cofactors which could be responsible for the rapid degradation rate observed *in vivo*.

It has been proposed that a specific amino acid sequence may dictate the intracellular stability of proteins. These sequences [termed PEST regions; proline (P)-, glutamic acid (E)-, serine (S)- and threonine (T)-rich] have been found in 12 proteins with intracellular

half-lives of less than 2 h (Rogers *et al.*, 1986). ODC as analysed by this schema has optimum PEST-site regions predicting rapid turnover. As suggested by Rogers *et al.* (1986), a direct test of this hypothesis would be to induce alterations in these sequences and measure the intracellular stability of the protein. ODC produced by '*in vitro*' methodologies would be suitable for these types of experimental manipulation, specifically by methods of site-directed mutagenesis (Smith, 1985) to modify individual amino acids.

The methods described here also show that transcription *in vitro* can be used to generate large quantities of ODC-specific mRNA. Other results indicate that ODC synthesis *in vivo* is regulated at the translational level by polyamines (Kahana & Nathans, 1985b; Persson *et al.*, 1986). Spermidine and spermine appear to exert a direct and selective inhibitory effect on the translation of ODC mRNA in the reticulocyte-lysate system (Kameji & Pegg, 1987). Reticulocyte lysates contain high concentrations of polyamines (up to 600 μ M-spermidine and 60 μ M-spermine). Thus the failure here to detect measurable ODC synthesis in unfiltered lysates with mRNA contents less than 250 ng (Fig. 3a) could be due to the inhibitory effect of endogenous polyamines, which is only overcome at high mRNA concentrations. The mechanism for polyamine translational inhibition is unknown, but interaction with the unusually long 5' untranslated region of ODC mRNA has been postulated to have an effect on translatability of the message (Pegg, 1986). It is evident that RNA secondary structure can affect translation *in vivo* (Kozak, 1986; Pelletier & Sonenberg, 1985), and polyamines could be acting in a similar manner to alter specifically ODC mRNA. Manipulation of the 5' upstream regions of the ODC cDNA, and production of mRNA *in vitro* by using the plasmid construction described here, would allow a direct determination of sequences involved in polyamine inhibition of ODC translation.

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REFERENCES

- Choi, J. H. & Scheffler, I. E. (1983) *J. Biol. Chem.* **258**, 12601–12603
- Dircks, L., Grens, A., Slezynger, T. C. & Scheffler, I. E. (1986) *J. Cell. Physiol.* **126**, 371–378
- Gilmour, S. K., Avdalovic, N., Madard, T. & O'Brien, G. T. (1985) *J. Biol. Chem.* **260**, 16439–16444
- Glass, J. R. & Gerner, E. W. (1986) *Biochem. J.* **236**, 351–357
- Glass, J. R. & Gerner, E. W. (1987) *J. Cell. Physiol.* **130**, 133–141
- Holttä, E. & Pohjanpelto, P. (1986) *J. Biol. Chem.* **261**, 9502–9508
- Hough, R. & Rechsteiner, M. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 90–94
- Isomaa, V. V., Pajunen, A. E., Bardin, C. W. & Janne, O. A. (1983) *J. Biol. Chem.* **258**, 6735–6740
- Jackson, R. J. & Hunt, T. (1983) *Methods Enzymol.* **96**, 50–74
- Kahana, C. & Nathans, D. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3645–3649

- Kahana, C. & Nathans, D. (1985a) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1673–1677
- Kahana, C. & Nathans, D. (1985b) *J. Biol. Chem.* **260**, 15390–15393
- Kameji, T. & Pegg, A. E. (1987) *J. Biol. Chem.* **262**, 2427–2430
- Kameji, T., Murakami, Y., Fujita, K. & Hayashi, S. (1982) *Biochim. Biophys. Acta* **717**, 111–117
- Kanamoto, R., Utsunomiya, K., Kameji, T. & Hayashi, S. (1986) *Eur. J. Biochem.* **154**, 539–544
- Kontula, K. K., Torkkeli, T. K., Bardin, C. W. & Janne, O. A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 731–735
- Kozak, M. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2850–2854
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor
- McConlogue, L. & Coffino, P. (1983) *J. Biol. Chem.* **258**, 12083–12086
- McConlogue, L., Gupta, M., Wu, L. & Coffino, P. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 540–544
- Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Pegg, A. E. (1986) *Biochem. J.* **234**, 249–262
- Pelletier, J. & Sonenburg, N. (1985) *Cell* **40**, 515–526
- Persson, L., Seely, J. E. & Pegg, A. E. (1984) *Biochemistry* **23**, 3777–3783
- Persson, L., Holm, I. & Heby, O. (1986) *FEBS Lett.* **205**, 175–178
- Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234**, 364–368
- Rote, K. R. & Rechsteiner, M. (1983) *J. Cell. Physiol.* **116**, 103–110
- Seely, J. E. & Pegg, A. E. (1983) *J. Biol. Chem.* **258**, 2496–2500
- Seely, J. E., Pösö, H. & Pegg, A. E. (1982) *Biochemistry* **21**, 3394–3399
- Seely, J. E., Persson, L., Sertich, G. J. & Pegg, A. E. (1985) *Biochem. J.* **226**, 577–586
- Smith, M. (1985) *Annu. Rev. Genet.* **19**, 423–462
- Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–790

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