Maternal administration of dexamethasone stimulates choline-phosphate cytidylyltransferase in fetal type II cells

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Administration of dexamethasone to pregnant rats at 19 days gestation increased phosphatidylcholine synthesis (45%) from radioactive choline in type II cells. This enhanced synthesis of phosphatidylcholine was accompanied by an increased conversion of choline phosphate into CDP-choline. Similar results were obtained by incubating organotypic cultures of 19-day-fetal rat lung with cortisol. The increased conversion of choline phosphate into CDP-choline correlated with an enhanced choline-phosphate cytidylyltransferase activity (31% after dexamethasone treatment; 47% after cortisol exposure) in the cell homogenates. A similar increase (26% after dexamethasone treatment; 39% after cortisol exposure) was found in the microsomal-associated enzyme. No differences in cytosolic enzyme activity were observed. The specific activity of the microsomal enzyme was 3–4 times that of the cytosolic enzyme. Most of the enzyme activity was located in the microsomal fraction (58–65%). The treatments had no effect on the total amount of enzyme recovered from the cell homogenates. These results, taken collectively, are interpreted to indicate that the active form of cytidylyltransferase in type II cells is the membrane-bound enzyme and that cytidylyltransferase activation in type II cells from fetal rat lung after maternal glucocorticoid administration occurs by binding of inactive cytosolic enzyme to endoplasmic reticulum.

INTRODUCTION

Pulmonary surfactant present in the alveolar lining layer prevents alveolar collapse and transudation of fluid at low lung volumes (Goerke, 1974). The ability of pulmonary surfactant to lower the surface tension at end expiration is of critical importance at birth (Avery & Mead, 1959). The production of surfactant is stimulated near term (Perelman *et al.*, 1981). During this period the content of lung phosphatidylcholine increases dramatically (Rooney *et al.*, 1976), and this increase is correlated with enhanced rates of phosphatidylcholine synthesis (Maniscalco *et al.*, 1978). Numerous studies have suggested that lung maturation, especially the onset of surfactant synthesis, is regulated by endogenous glucocorticoids (Ballard, 1982). Glucocorticoids stimulate pulmonary phosphatidylcholine synthesis, and it appears that this effect is mediated by an activation of choline-phosphate cytidylyltransferase (EC 2.7.7.15) (Rooney *et al.*, 1979; Possmayer *et al.*, 1979; Brehier & Rooney, 1981). We have demonstrated that the glucocorticoid action on surfactant-associated phosphatidylcholine synthesis occurs ultimately at the level of the alveolar type II cells and involves a fibroblast-derived polypeptide, fibroblast–pneumonocyte factor, which stimulates cytidylyltransferase (Post *et al.*, 1986). This enzyme may regulate the CDP-choline pathway for phosphatidylcholine formation in whole lung and in alveolar type II cells (Tokmakjian & Possmayer, 1981; Post *et al.*, 1982, 1984a,b). The mechanism of activation of cytidylyltransferase in the lung remains unclear. A number of mechanisms have been proposed, including covalent modification (Radika & Possmayer, 1985) and changes in lipid environment (Weinhold *et al.*, 1981,

1984). The latter studies suggested that cytidylyltransferase was regulated by a redistribution of enzyme between cytosol and endoplasmic reticulum. The activity of the cytosolic form of the enzyme was low compared with that of the microsomal form (Weinhold *et al.*, 1981), and enzyme activation occurred by association of the inactive cytosolic enzyme with endoplasmic membranes (Weinhold *et al.*, 1981, 1984). A redistribution-activation mechanism for controlling cytidylyltransferase activity has been observed in various cells (Sleight & Kent, 1983; Vance & Pelech, 1984; Wright *et al.*, 1985). Consequently, I examined whether the stimulation of cytidylyltransferase activity in fetal lung after maternal administration of glucocorticoids occurs in the type II cells and is regulated by a translocation mechanism.

EXPERIMENTAL

Materials

Pregnant Sprague–Dawley rats of known gestation age were obtained from Charles River Laboratories, Wilmington, MA, U.S.A. All radioactive chemicals were from New England Nuclear, Boston, MA, U.S.A. Unlabelled biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Dexamethasone and cortisol were purchased from Steraloids, Wilton, NH, U.S.A.

Cell cultures

Organotypic cultures were prepared from fetal rat lungs as previously described (Post *et al.*, 1984c). Briefly, the lungs were dissociated into single cells and the monodispersed cells were inoculated on to Gelfoam

Abbreviation used: MEM, minimal essential medium.

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sponges to form organotypic cultures. The cultures were incubated in MEM containing 10% (v/v) fetal-calf serum and placed in an atmosphere of 5% CO₂ in air at 37 °C. After 20 h of incubation, the cultures were used for incorporation or enzyme studies.

Glucocorticoid treatment

On day 19 of gestation, pregnant rats were injected intraperitoneally with dexamethasone (0.2 mg/kg) in dimethyl sulphoxide. Controls were similarly injected with vehicle alone. Approx. 24 h after the injections, the fetuses were delivered by hysterotomy, killed, and their lungs isolated. The freshly excised lungs were used for the preparation of organotypic cultures as described above. After 20 h of culture, the type II cells were isolated (Post *et al.*, 1984c) and the cells were resuspended in 0.145 M-NaCl/50 mM-Tris/HCl (pH 7.4)/50 mM-NaF/2.5 mM-EDTA (hereafter termed 'Tris/saline'). The cells were ruptured with 50 strokes of a Dounce homogenizer and then subjected to subcellular fractionation.

For the study of the effect of cortisol on the cytidyltransferase activity of type II cells in organotypic cultures directly, cultures prepared from 19-day-fetal rat lungs were incubated in serum-free MEM with and without 100 nM-cortisol. After 20 h of incubation, the type II cells were isolated, resuspended in Tris/saline and subsequently homogenized as above.

Preparation of subcellular fractions

The cell homogenate was centrifuged at 300 g for 10 min, and the resulting supernatant was centrifuged at 12000 g for 10 min. The (post-mitochondrial) supernatant from the latter procedure was centrifuged at 100000 g for 60 min. The (microsomal) particulate material was resuspended in Tris/saline, and the cytosolic and microsomal fractions were assayed for cytidyltransferase activity.

Choline-phosphate cytidyltransferase assay

Assay mixtures contained 20 mM-Tris/succinate (pH 7.8), 6 mM-MgCl₂, 8 mM-CTP and 4 mM-[Me-¹⁴C]-choline phosphate (sp. radioactivity 0.15 µCi/µmol) in a reaction volume of 200 µl. Each incubation was started by the addition of enzyme (up to 100 µg of protein) and incubated for 30 min at 37 °C. The reaction was terminated by boiling for 2 min and the protein was removed by centrifugation (1000 g for 10 min). A 50 µl sample was taken from the supernatant, mixed with 2 nmol of unlabelled CDP-choline and 4 nmol of unlabelled choline phosphate as carriers, and subjected to t.l.c. on precoated silica-gel G plates (J. T. Baker Chemical Co.). The plates were developed in ethanol/water/conc. NH₃ (sp.gr. 0.88) (50:25:1, by vol.) for the isolation of the reaction product, CDP-choline. The CDP-choline spots were detected with iodine vapour, scraped into scintillation vials and assayed for radioactivity.

Incorporation studies

Organotypic cultures prepared from lungs of fetal rats treated with either dexamethasone or vehicle were incubated for 5 h in MEM containing 1 µCi of [Me-³H]choline/ml. At the end of this period the type II cells were isolated from the sponges. The incubations were terminated by extracting the cells with chloroform/methanol (Bligh & Dyer, 1959). Phosphatidylcholine was

isolated from the lipid extract by t.l.c. on silica-gel G plates with chloroform/methanol/water (65:35:4, by vol.) as eluent. The remaining aqueous phase was assayed for choline-containing intermediates.

Pulse-chase studies

Organotypic cultures prepared from lungs of 19-day-fetal rats were incubated in MEM supplemented with 1 µCi of [Me-³H]choline/ml. After 20 h of incubation the radioactive medium was removed and the cultures were washed with unlabelled MEM. The cultures were incubated in MEM in the presence or absence of 100 nM-cortisol, and the type II cells were isolated from the cultures 24 h after the addition of cortisol. The cell suspension was extracted with chloroform/methanol (1:2, v/v) and phosphatidylcholine was isolated. The aqueous layer remaining after the lipid extraction was analysed for choline-containing compounds.

Measurement of choline-containing metabolites

A sample (250 µl) of the aqueous layer (2.5 ml) remaining after the lipid extraction was subjected to h.p.l.c. on an ion-exchange column (Aminex A-27) in an isocratic mode (Mazzola & Kent, 1984). The choline phosphate and CDP-choline fractions were recovered from the column in scintillation vials and assayed for radioactivity.

Other methods

The protein concentrations were measured by the Bradford assay (Bradford, 1976; Spector, 1978), with bovine serum albumin as standard. Radioactivity was measured in an Aquasol-based (New England Nuclear) scintillation system.

RESULTS

The effect of maternal dexamethasone administration on choline incorporation into phosphatidylcholine and choline-containing metabolites by type II cells is shown in Fig. 1. Dexamethasone treatment increased phosphatidylcholine synthesis by approx. 45%. This increase correlated with a significant decrease in the ratio of radioactive choline phosphate to CDP-choline, indicating that choline-phosphate cytidyltransferase activity was stimulated by the treatment.

The activity of choline-phosphate cytidyltransferase in subcellular fractions of fetal lung type II cells from control and dexamethasone-treated rats is shown in Table 1. The specific activity in the microsomal fraction was 4 times that in the cytosol. As shown in Fig. 2, approx. 65% of the total activity was recovered in the microsomal fraction. It should be noted that about 75.0 ± 1.6% (mean ± S.E.M.) of total cytidyltransferase activity was recovered, whereas complete recovery of protein was achieved. Dexamethasone increased the activity of cytidyltransferase in the post-mitochondrial supernatant of the fetal type II cells by approx. 31% (Table 1). A similar increase was found in the microsomal fraction, whereas no differences were observed between the cytosolic fractions of type II cells from control and treated fetal rat lungs. Dexamethasone had no significant effect on the subcellular distribution of cytidyltransferase (Fig. 2). However, the results showed a trend in the direction of a shift of activity from cytosol to microsomal fraction. In addition, the recovery of

Fig. 1. Effect of dexamethasone administration to pregnant rats on choline incorporation into choline phosphate, CDP-choline and phosphatidylcholine of fetal type II cells

Pregnant rats were treated as described in Table 1. Organotypic cultures were prepared from the fetal lungs. After overnight incubation, the cultures were incubated for 5 h in the presence of radioactive choline and the radioactivity incorporated into choline phosphate, CDP-choline and phosphatidylcholine was determined. The values are means $\pm$ S.E.M. of four experiments carried out in quadruplicate. Significance was determined by Student's *t* test. Key: CONT, control; DEX, dexamethasone.

Fig. 2. Effect of maternal dexamethasone administration on subcellular distribution of choline-phosphate cytidyltransferase in fetal type II cells

The pregnant rats were treated as described for Table 1. Fetal type II cells were isolated, homogenized and fractionated. Enzyme activity was assayed in the different subcellular fractions. The values presented are means $\pm$ S.E.M. of three experiments carried out in duplicate. Key: CONT, control; DEX, dexamethasone.

cytidyltransferase was not affected by the treatment (control, $75.0 \pm 1.6\%$; dexamethasone, $75.8 \pm 5.3\%$; mean $\pm$ S.E.M.).

In previous studies, we have found that glucocorticoids act indirectly on the type II cells (Post *et al.*, 1984b; 1986; Floros *et al.*, 1985; Smith, 1979). The glucocorticoid effect on phosphatidylcholine synthesis by type II cells is mediated via a polypeptide, fibroblast-pneumonocyte factor, which is produced and secreted by fetal lung fibroblasts in response to glucocorticoids. In order to test the effect of glucocorticoids on cytidyltransferase activity in type II cells directly, we incubated organotypic cultures (containing fibroblasts and type II cells) of fetal rat lung with cortisol and subsequently isolated the type II cells. A pulse-chase experiment demonstrated that the cortisol-stimulated phosphatidyl-

choline formation from radioactive choline in type II cells was accompanied by a decrease in the ratio of choline phosphate to CDP-choline (Fig. 3). This observation indicates again that cortisol stimulates the cytidyltransferase activity in fetal type II cells. The stimulation of the activity by cortisol is most likely mediated by fibroblast-pneumonocyte factor (Post *et al.*, 1986).

Table 2 shows that exposure of organotypic cultures to cortisol resulted in a significant increase in specific activity in the post-mitochondrial supernatant (47%) and in the microsomal fraction of the type II cells (39%). The cortisol treatment did not affect the distribution of the enzymic activity (Fig. 4). Most of the activity was located in the microsomal fraction (approx. 58%).

Table 1. Effect of maternal treatment with dexamethasone on choline-phosphate cytidyltransferase activity in fetal type II cells

Pregnant rats were injected with either dexamethasone or vehicle alone at 19 days of gestation, and the fetuses were delivered at 20 days. Organotypic cultures were prepared from the fetal lungs. After overnight incubation, the type II cells were isolated from the organotypic cultures, and enzyme activity was assayed. Values are means $\pm$ S.E.M. of three experiments each carried out in duplicate. Significance was determined by Student's *t* test: n.s., not significant.

Subcellular fraction	Activity (nmol/min per mg of protein)		Treated/control	P
	Control	Dexamethasone		
Homogenate	1.07 ± 0.07	1.39 ± 0.06	1.31 ± 0.04	< 0.001
Cytosol	0.40 ± 0.04	0.39 ± 0.06	1.00 ± 0.03	n.s.
Microsomal	1.75 ± 0.26	2.19 ± 0.31	1.26 ± 0.02	< 0.001

Fig. 3. Effect of cortisol on choline metabolism in fetal type II cells

Organotypic cultures of 19-day-fetal rat lung were incubated for 20 h with radioactive choline and then incubated for 24 h in MEM in the presence (CORT) or absence (CONT) of cortisol. The radioactivity associated with choline phosphate, CDP-choline and phosphatidylcholine was determined. The values are representative for two experiments carried out in quadruplicate and are expressed as means $\pm$ S.E.M.

Table 2. Effect of cortisol on choline-phosphate cytidyltransferase activity of fetal rat lung type II cells in organotypic cultures

Organotypic cultures were prepared with lung cells from fetal rats at 19 days of gestation and incubated with and without cortisol. After 20 h of incubation, the type II cells were isolated from the organotypic cultures and enzyme activity was assayed. Values are means $\pm$ S.E.M. of four experiments each carried out in duplicate. Significance was determined by Student's *t* test: n.s., not significant.

Subcellular fraction	Activity (nmol/min per mg of protein)		Treated/control	P
	Control	Cortisol		
Homogenate	0.91 $\pm$ 0.06	1.34 $\pm$ 0.17	1.47 $\pm$ 0.19	< 0.05
Cytosol	0.51 $\pm$ 0.07	0.67 $\pm$ 0.08	1.31 $\pm$ 0.16	n.s.
Microsomal	1.42 $\pm$ 0.08	1.98 $\pm$ 0.09	1.39 $\pm$ 0.06	< 0.001

Again, a trend in the direction of a translocation of activity from cytosol to microsomal fraction was observed. Furthermore, cortisol had no significant effect on the recovery of choline-phosphate cytidyltransferase (control, 88.7 ± 4.5 ; cortisol, 75.8 ± 2.7 ; mean $\pm$ S.E.M.).

DISCUSSION

In this paper I provide evidence that increased surfactant-associated phosphatidylcholine synthesis, which occurs after maternal dexamethasone administration (Rooney *et al.*, 1976, 1979; Gross *et al.*, 1980, 1983; Post *et al.*, 1986), involves an activation of choline-phosphate cytidyltransferase in the fetal type II cells. This enhancement in cytidyltransferase activity was observed in total cell homogenates (post-mitochondrial supernatant) as well as in the microsomal fraction, but was not found in the cytosol. The latter contradicts a preliminary report by Viscardi *et al.* (1985), who

reported that dexamethasone and tri-iodothyronine increased cytidyltransferase activity in the cytosolic fraction of fetal rat lung type II cells. Activation of cytosolic cytidyltransferase has also been described for fetal rabbit lungs after oestrogen treatment (Chu & Rooney, 1985). The increase was mediated by endogenous phospholipids (Chu & Rooney, 1985). However, studies with type II cells from fetal rabbit lung have demonstrated that exposure to fatty acids results preferentially in an enhancement of microsomal enzymic activity (Aeberhard *et al.*, 1986).

My observation that the microsomal specific activity of cytidyltransferase was approx. 4 times the cytosolic specific activity is in agreement with the results obtained with the fetal rabbit type II cells (Aeberhard *et al.*, 1986). These results support the idea that cytidyltransferase in the type II cells is activated by a translocation of inactive cytosolic enzyme from cytosol to microsomal fraction. Although the results point to the direction of a

Fig. 4. Effect of cortisol exposure of organotypic cultures on subcellular distribution of choline-phosphate cytidylyltransferase activity in fetal type II cells

Organotypic cultures were exposed to cortisol and type II cells were isolated. The cells were homogenized, fractionated and assayed for enzyme activity. The values are means $\pm$ S.E.M. of four experiments carried out in duplicate. Key: CONT, control; CORT, cortisol.

translocation of cytidylyltransferase in type II cells after glucocorticoid treatment, the subcellular redistribution of the enzyme was not statistically significant. Most of the activity was already located in the microsomal fraction before the treatment. These results do suggest, however, that the glucocorticoid effect is too modest to have a statistically significant impact on the distribution of the enzyme. Subcellular redistribution in association with increased cytidylyltransferase activity has been reported in choline-depleted type II cells from adult rat lung (Tesan *et al.*, 1985). A translocation of enzyme in the absence of altered enzyme activity was found in adult type II cells incubated in the presence of pulmonary surfactant (Tesan *et al.*, 1985). In addition, numerous studies with non-pulmonary cells have demonstrated that cytidylyltransferase is regulated by enzyme-membrane interactions rather than by changes in the number of enzyme molecules (Sleight & Kent, 1983; Pelech *et al.*, 1983, 1984a; Vance & Pelech, 1984; Wright *et al.*, 1985). Several factors have been implicated in the control of cytidylyltransferase binding to membranes. For example, studies with lung (Weinhold *et al.*, 1984) and liver (Pelech *et al.*, 1983) have demonstrated that fatty acids facilitate the binding of the pulmonary enzyme to microsomal membranes. In addition, studies with type II cells from fetal rabbit lung have shown that an increase in the intracellular concentration of fatty acids resulted in increased phosphatidylcholine synthesis (Aeberhard *et al.*, 1986). This increase in phosphatidylcholine synthesis was accompanied by an enhanced activity of choline-phosphate cytidylyltransferase in the microsomal fraction. Hence, the involvement of fatty acids in the promotion of cytidylyltransferase binding to endoplasmic-reticulum membranes in fetal type II cells

must be considered. On the other hand, although it has been reported that phorbol esters stimulate translocation of cytidylyltransferase in HeLa cells (Pelech *et al.*, 1984b), no significant release of fatty acids was demonstrated (Cook & Vance, 1985).

Another mechanism by which cytidylyltransferase association to endoplasmic-reticulum membranes may occur involves a protein-dephosphorylation reaction. Previous studies with liver have suggested that cytidylyltransferase may be interconverted between two forms: a phosphorylated form, which does not bind to membranes, and a dephosphorylated form, which associates with the endoplasmic reticulum (Vance & Pelech, 1984). A similar regulation mechanism has been suggested for the pulmonary enzyme (Radika & Possmayer, 1985).

In summary, the effect on surfactant phosphatidylcholine production after maternal glucocorticoid administration is mediated by an activation of cytidylyltransferase in type II cells. The augmented cytidylyltransferase activity can be explained most easily through a translocation of the enzyme from cytosol to the microsomal fraction. Further experiments are needed for confirmation and for the identification of factors involved in the translocation process.

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REFERENCES

- Aeberhard, E. E., Barrett, C. T., Kaplan, S. A. & Scott, M. L. (1986) *Biochim. Biophys. Acta* **875**, 6-11
- Avery, M. E. & Mead, J. (1959) *Am. J. Dis. Child.* **97**, 517-523
- Ballard, P. L. (1982) in *Lung Development: Biological and Clinical Perspectives* (Farrell, P. M., ed.), vol. 2, pp. 205-253, Academic Press, New York
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911-917
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248-254
- Brehier, A. & Rooney, S. A. (1981) *Exp. Lung Res.* **2**, 273-287
- Chu, A. J. & Rooney, S. A. (1985) *Biochim. Biophys. Acta* **252**, 346-356
- Cook, H. W. & Vance, D. E. (1985) *Can. J. Biochem. Cell Biol.* **63**, 145-151
- Floros, J., Post, M. & Smith, B. T. (1985) *J. Biol. Chem.* **260**, 2265-2267
- Goerke, J. (1974) *Biochim. Biophys. Acta* **344**, 241-261
- Gross, I., Wilson, C. M., Ingleson, L. D., Brehier, A. & Rooney, S. A. (1980) *J. Appl. Physiol.* **48**, 872-877
- Gross, I., Ballard, P. L., Ballard, R. A., Jones, C. T. & Wilson, C. M. (1983) *Endocrinology* (Baltimore) **112**, 829-837
- Maniscalco, W. M., Wilson, C. M., Gross, I., Gobran, L., Rooney, S. A. & Warshaw, J. B. (1978) *Biochim. Biophys. Acta* **530**, 333-346
- Mazzola, G. & Kent, C. (1984) *Anal. Biochem.* **141**, 137-142
- Pelech, S. L., Pritchard, P. H., Brindley, D. N. & Vance, D. E. (1983) *J. Biol. Chem.* **258**, 6782-6788
- Pelech, S. L., Cook, H. W., Paddon, H. B. & Vance, D. E. (1984a) *Biochim. Biophys. Acta* **795**, 433-440
- Pelech, S. L., Paddon, H. B. & Vance, D. E. (1984b) *Biochim. Biophys. Acta* **795**, 447-451
- Perelman, R. E., Engle, M. E. & Farrell, P. M. (1981) *Lung* **159**, 53-80
- Possmayer, F., Casola, P. G., Chan, F., Hill, S., Metcalfe, I. L., Stewart-DeHaan, P. J., Wong, T., Las Heras, J., Gammal, E. & Harding, P. G. R. (1979) *Biochim. Biophys. Acta* **574**, 197-211

- Post, M., Batenburg, J. J., Schuurmans, E. A. J. M. & Van Golde, L. M. G. (1982) *Biochim. Biophys. Acta* **712**, 390–394
- Post, M., Batenburg, J. J., Smith, B. T. & Van Golde, L. M. G. (1984a) *Biochim. Biophys. Acta* **795**, 522–557
- Post, M., Batenburg, J. J., Van Golde, L. M. G. & Smith, B. T. (1984b) *Biochim. Biophys. Acta* **795**, 558–563
- Post, M., Torday, J. S. & Smith, B. T. (1984c) *Exp. Lung Res.* **7**, 53–65
- Post, M., Barsoumian, A. & Smith, B. T. (1986) *J. Biol. Chem.* **261**, 2179–2184
- Radika, K. & Possmayer, F. (1985) *Biochem. J.* **232**, 833–840
- Rooney, S. A., Wai-Lee, T. S., Gobran, L. & Motoyama, E. (1976) *Biochim. Biophys. Acta* **431**, 447–458
- Rooney, S. A., Gobran, L. I., Marino, P. M., Maniscalco, W. M. & Gross, I. (1979) *Biochim. Biophys. Acta* **572**, 64–76
- Sleight, R. E. & Kent, C. (1983) *J. Biol. Chem.* **258**, 831–835
- Smith, B. T. (1979) *Science* **204**, 1094–1095
- Spector, T. (1978) *Anal. Biochem.* **86**, 142–146
- Tesan, M., Ancheschi, M. M. & Bleasdale, J. E. (1985) *Biochem. J.* **232**, 705–713
- Tokmakjian, S. & Possmayer, F. (1981) *Biochim. Biophys. Acta* **666**, 176–180
- Vance, D. E. & Pelech, S. L. (1984) *Trends Biochem. Sci.* **9**, 75–77
- Viscardi, R. M., Simon, R. H. & Weinhold, P. A. (1985) *Pediatr. Res.* **19**, 165A
- Weinhold, P. A., Feldman, D. A., Quade, M. M., Miller, J. C. & Brooks, R. L. (1981) *Biochim. Biophys. Acta* **665**, 134–144
- Weinhold, P. A., Rounsifer, M. E., Williams, S. E., Brubaker, P. G. & Feldman, D. A. (1984) *J. Biol. Chem.* **257**, 10315–10321
- Wright, P. S., Morand, J. N. & Kent, C. (1985) *J. Biol. Chem.* **260**, 7919–7926

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