Glucocorticoid and developmental regulation of uteroglobin synthesis in rabbit lung

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The synthesis of uteroglobin in rabbit lung was studied after the administration of glucocorticoids to intact adult animals as well as during the late stages of rabbit development. The synthesis of uteroglobin was compared with levels of translatable uteroglobin mRNA in the lung. Uteroglobin synthesis was determined both by incorporation of [³⁵S]methionine into the protein by lung explants incubated *in vitro* and by radioimmunoassay measurements of uteroglobin concentration in lung. Lung poly(A)-containing mRNA, isolated by oligo(dT)–cellulose chromatography, was translated in cell-free systems and the activity of uteroglobin mRNA was determined after immunoprecipitation. Dexamethasone administration increased about 2-fold the synthesis of lung uteroglobin compared with the controls. The effect of cortisol was more moderate. Both glucocorticoids did not affect the degradation rate of lung uteroglobin, but produced increases in the translatable levels of uteroglobin mRNA parallel to those observed for uteroglobin synthesis. During the late stages of rabbit development, both the synthesis of lung uteroglobin and the translatable levels of its mRNA increase in parallel about 12-fold in a biphasic fashion. A first increase occurred between 2 days before and 2 days after birth. Starting at 5 days of age, there was a second increase in both parameters, which at 12 days of age reached values close to those observed in adult rabbits. Our results suggest that the rate of lung uteroglobin synthesis could be mainly determined by the translatable levels of its mRNA.

Uteroglobin (Beier, 1968) or blastokinin (Krishnan & Daniel, 1967) is a small secretory protein composed of two identical subunits of 70 amino acids each (Ponstingl *et al.*, 1978; Popp *et al.*, 1978). It was initially discovered in the rabbit uterus, where its synthesis is regulated by ovarian steroid hormones by a mechanism involving variations in the levels of its mRNA (Bullock *et al.*, 1976; Atger & Milgrom, 1977; Beato *et al.*, 1980; Müller & Beato, 1980). Therefore this system is receiving considerable attention as a model for the study of the mechanisms of action of ovarian steroid hormones.

A uteroglobin-like protein was also found in other tissues of the rabbit, particularly in the lung (Noske & Feigelson, 1976). On the basis of immunological and chemical studies (Beato & Beier, 1978; Torkkeli *et al.*, 1978), it appears that uterine and lung

uteroglobins are very similar, if not identical. Moreover, both proteins are synthesized as a larger precursor (pre-uteroglobin) containing a *N*-terminal additional peptide (Beato & Nieto, 1976; Bullock, 1977; Malsky *et al.*, 1979; Savouret *et al.*, 1980). However, the uteroglobin concentration in the lung does not seem to be regulated by ovarian hormones, but it appears to be controlled by glucocorticoids (Feigelson *et al.*, 1977; Torkkeli *et al.*, 1978). A subsequent report indicates that this control could be at a post-transcriptional level (Savouret *et al.*, 1980). On the other hand, it has been reported that the uteroglobin concentration in lungs of newborn rabbits is considerably lower than in adult lung, which may be related to the effects of glucocorticoids on the maturation of the lung (Torkkeli *et al.*, 1978).

In the above studies, the synthesis of lung uteroglobin has been estimated from the concentration of the protein in the tissue. However, a study of the synthesis *in vitro* of lung uteroglobin (i.e. by

Abbreviations used: poly(A)⁺ mRNA, poly(A)-containing mRNA; SDS, sodium dodecyl sulphate.

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incorporation of radioactive precursors) has not been performed. Therefore in the present work we have studied the synthesis of lung uteroglobin not only by radioimmunoassay determinations but also by measuring the relative synthesis of the protein in lung explants. Simultaneously, we have determined the activity of the lung uteroglobin mRNA by translation in cell-free systems. We have examined the changes occurring in these parameters after the administration of glucocorticoids as well as during development. Our results suggest that the variations in lung uteroglobin synthesis occur concomitantly with parallel changes in the translatable levels of its mRNA.

Materials and methods

Materials

Dexamethasone sulphate (Dexa-Scheron) was from Schering A.G. Cortisol 21-phosphate was supplied by Sigma Chemical Co. L-[^{35}S]Methionine (800–1000 Ci/mmol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Oligo-(dT)-cellulose (type T_2) was purchased from Collaborative Research (Waltham, MA, U.S.A.). *Staphylococcus aureus* suspension (Pansorbin) was from Calbiochem-Behring (La Jolla, CA, U.S.A.). Glassware and solutions used in the preparation of RNA were either autoclaved or treated with 0.1% diethyl pyrocarbonate. Phenol was distilled before use.

Animals

New Zealand female rabbits weighing about 2.5 kg were injected intramuscularly during 4 days with two daily doses of dexamethasone (5 mg in water) or cortisol phosphate (10 mg in water). At 12 h after the last injection, the animals were killed and bled, and the lungs were aseptically removed. Small portions of lung were used for the preparation of explants and the remaining tissue was immediately frozen at -76°C and used for RNA preparation and radioimmunoassay of uteroglobin as described below. Foetuses were obtained by uterotomy from time-dated pregnant rabbits.

Incubation of explants

Lung explants were incubated essentially as described for endometrial explants (Nieto & Beato, 1980). Small pieces of lung (1–2 mm) were pre-incubated in culture medium for 1 h at 37°C , followed by incubation in fresh medium containing $20\mu\text{Ci}$ of [^{35}S]methionine/ml. At the end of the incubation the tissue was homogenized in 20 mM-sodium phosphate (pH 7.5)/0.15 M-NaCl, containing 0.5% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.7 mM-phenylmethanesulphonyl fluoride, 0.02% NaN_3 and 1 mM-methionine (PBS-D

medium), and the homogenate was centrifuged at 100 000 g for 1 h. Portions of the supernatant were taken for determination of tissue proteins and radioactivity incorporated into proteins (Nieto & Beato, 1980) as well as for immunoprecipitation as described below.

Preparation of poly(A) $^+$ mRNA

Extraction of lung nucleic acids was performed essentially by the method of Brawerman (1974). Frozen tissue was homogenized with 5 vol. (v/w) of 0.1 M-Tris/HCl (pH 9)/0.5% (w/v) SDS/2 mM-EDTA and 5 vol. of phenol saturated with the above buffer. The phenolic phase was separated by centrifugation (9000 g for 10 min) and extracted with 2.5 vol. of buffer. The combined aqueous phases were extracted three times with phenol, and the nucleic acids were precipitated overnight at -20°C with 2 vol. of ethanol and 0.1 vol. of 2 M-LiCl. The nucleic acids, dissolved in 0.5 M-NaCl/0.5% (w/v) SDS/10 mM-Tris/HCl (pH 7.5), were heated at 60°C for 5 min and the poly(A) $^+$ RNA was purified by chromatography on oligo(dT)-cellulose (Arneemann *et al.*, 1977).

Cell-free protein synthesis

Wheat-germ extracts were prepared as described by Marcu & Dudock (1974), and the lung poly(A) $^+$ mRNA was translated under conditions previously described (Beato & Nieto, 1976), with $100\mu\text{Ci}$ of [^{35}S]methionine/ml as labelled amino acid. Preparation of reticulocyte lysates and translation of lung mRNA were done as described by Pelham & Jackson (1976); [^{35}S]methionine ($80\mu\text{Ci}/\text{ml}$) was used as labelled amino acid. Usually cell-free protein synthesis was done in $25\mu\text{l}$ assay mixtures. Portions of these were used for immunoprecipitation and measurement of radioactivity incorporated into proteins.

Immunoprecipitation

The products synthesized by explants or in the cell-free systems were immunoprecipitated with a monospecific anti-uteroglobin γ -globulin (Arneemann *et al.*, 1977). Samples of the homogenates of explants (30–40 μl) were diluted with 0.2 ml of PBS-D medium containing 0.1% bovine serum albumin and incubated either for 5 h at 25°C or overnight at 4°C with 80 μg of the antibody. Then 8 μl of *Staphylococcus aureus* suspension was added and the tubes were centrifuged at 3000 g for 5 min. The supernatants were aspirated and the pellets washed three times with the same buffer. The washed pellets were resuspended in 0.1 ml of 8 M-urea/1% SDS, heated for 10 min at 100°C and used either for electrophoretic analysis or for determination of radioactivity after mixing with a scintillation mixture (Beato & Nieto, 1976). Cell-free

products were immunoprecipitated in a similar way. Portions of the cell-free assays (5 μ l), diluted with 0.1 ml of PBS-D medium containing 0.1% bovine serum albumin, were pre-treated with 2 μ l of *Staphylococcus aureus* and the mixtures were centrifuged at 10000 *g* for 10 min. The supernatants were immunoprecipitated as described above. Immunoprecipitation was performed in duplicate, and the values of blank assays (containing either no antibody or an excess of unlabelled pure uteroglobin) were subtracted.

Radioimmunoassay of uteroglobin

Pre-weighed lung tissue was homogenized in PBS-D medium and the homogenate centrifuged at 20000 *g* for 20 min. The supernatant was used to measure both tissue protein and uteroglobin as described previously (Nieto & Beato, 1980). Preliminary experiments demonstrated the parallelism between the assay curves obtained with lung homogenates and the standard of pure uterine uteroglobin.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Immunoprecipitates were analysed by electrophoresis in gels containing 12.5% (w/v) acrylamide, 1.25% (w/v) *NN'*-methylenebisacrylamide, 1% (w/v) SDS and 8 M-urea (Swank & Munkres, 1971). After electrophoresis, the gels were cut into 2.2 mm slices. Each slice was incubated at 70°C for 8–12 h with 0.5 ml of 2% SDS, after which 5 ml of scintillation mixture was added for measurement of radioactivity.

Results

Synthesis of uteroglobin by lung explants

Lung explants incubated *in vitro* incorporate [35 S]methionine into proteins in an essentially linear fashion for at least 6 h (Fig. 1a). At this time, about 6% of the newly synthesized proteins are secreted into the medium (Fig. 1b). The labelled proteins from the tissue and the medium were immunoprecipitated with anti-uteroglobin γ -globulin to observe the time-course of uteroglobin synthesis. The amount of newly synthesized uteroglobin also increases linearly in the tissue during the incubation, and after a lag period of about 1 h the protein is secreted into the medium. This lag is similar to that observed for the secretion of uteroglobin by endometrial explants (Nieto & Beato, 1980) and it could represent the time needed for the intracellular transport of newly synthesized uteroglobin. At 6 h of incubation, about 15% of the newly synthesized uteroglobin has been secreted into the medium. Referred to the total proteins present in the system (medium + tissue), uteroglobin represents about 1.5% of the proteins

Fig. 1. Kinetics of incorporation of [35 S]methionine into total and immunoprecipitable proteins by lung explants incubated *in vitro*.

Lung explants (20–30 mg) were preincubated for 1 h in 1 ml of Dulbecco's medium containing 0.1 mM-methionine. Then, they were incubated for the indicated times in fresh medium containing 20 μ Ci of [35 S]methionine/ml. The tissue was homogenized and centrifuged at 100000 *g* for 1 h. Samples from the supernatant and from the incubation medium were taken for determinations of tissue proteins, radioactivity incorporated into total proteins (O) and immunoprecipitable uteroglobin ($\blacktriangle$). Values are referred to 1 mg of tissue protein.

synthesized by lung explants. Kinetics similar to those described above were obtained by using lung explants from rabbits treated with dexamethasone as described in the Materials and methods section (results not shown), although the percentage of uteroglobin synthesis increased 2-fold (see Table 1).

The evidence that the immunoprecipitable radioactivity was newly synthesized uteroglobin was substantiated by analysis of the immunoprecipitates by SDS/polyacrylamide-gel electrophoresis. Uteroglobin is composed of two identical subunits joined

Table 1. *Effect of glucocorticoids on the synthesis of lung uteroglobin*

Rabbits were treated with dexamethasone or cortisol as described in the Materials and methods section. Lungs were excised and used both for measurement of uteroglobin by radioimmunoassay and for preparation of explants. Explants were incubated for 2 h in presence of [35 S]methionine. The tissue + medium were homogenized and centrifuged at 100 000g for 1 h. Incorporation of radioactivity into total protein and uteroglobin were measured in the supernatant as trichloroacetic acid-precipitable radioactivity and immunoprecipitation respectively. Tissue protein was also measured. Values are means $\pm$ S.E.M. calculated for the numbers of animals in parentheses.

Treatment	Incorporation of [35 S]methionine (c.p.m./ μ g of tissue protein)		Uteroglobin (%)	Uteroglobin $\dagger$ (μ g/mg of tissue protein)
	Total protein	Uteroglobin		
Control (7)	168 $\pm$ 17	2.32 $\pm$ 0.24	1.5 $\pm$ 0.22	1.60 $\pm$ 0.1
Dexamethasone (7)	143 $\pm$ 12	4.05 $\pm$ 0.57*	3.1 $\pm$ 0.35*	2.90 $\pm$ 0.2*
Cortisol (4)	141 $\pm$ 20	3.30 $\pm$ 0.65	2.4 $\pm$ 0.30	2.10 $\pm$ 0.3

* Significantly different from control by Student's *t* test, $P < 0.01$.

$\dagger$ Determined by radioimmunoassay.

Fig. 2. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the immunoprecipitated protein synthesized by lung explants*

The immunoprecipitated products synthesized by lung explants as described in Fig. 1 were analysed in 12.5%-acrylamide gels containing 8 M-urea either in the presence (○) or in the absence (●) of 2-mercaptoethanol. The gels were fractionated into 2.2 mm slices for radioactivity measurements. The arrows indicate the position of the dimer (UGL₂) and the subunit (UGL₁) of the uteroglobin marker run in parallel.

by disulphide bonds, and therefore its electrophoretic mobility changes after reduction of these bonds (Beato & Rungger, 1975; Nieto *et al.*, 1977). Fig. 2 shows that the labelled immunoprecipitated product moves on electrophoresis at the position of authentic uteroglobin both in the presence and in the absence of 2-mercaptoethanol.

Effect of glucocorticoid treatment on lung uteroglobin synthesis

The effect of glucocorticoids on the synthesis of lung uteroglobin was investigated by measuring the radioactivity incorporated into uteroglobin by explants from control rabbits and from animals treated either with dexamethasone or with cortisol. The results summarized in Table 1 show that neither dexamethasone nor cortisol significantly affects the synthesis of total proteins compared with the controls, but increases the synthesis of uteroglobin. Dexamethasone increases about 2-fold the synthesis of uteroglobin, expressed either per mg of tissue protein or as percentage of the total protein synthesis, whereas the effect of cortisol appears to be more moderate. The content of uteroglobin in the lung of the animals used in each experiment was also determined by radioimmunoassay. The results, also shown in Table 1, indicate that there is a good correlation between uteroglobin concentration in the lung and the synthesis of the protein observed in lung explants. The increase in uteroglobin synthesis produced by glucocorticoids does not seem to be a consequence of decreased degradation of the protein, since glucocorticoid treatment did not produce significant changes in the stability of uteroglobin as measured by pulse-chase experiments in lung explants (results not shown).

Translation of lung mRNA in cell-free systems

In order to measure the levels of translatable uteroglobin mRNA in the lung, we established the conditions for accurate translation of this mRNA. Lung poly(A)⁺ mRNA isolated by oligo(dT)-chromatography was translated in two cell-free systems: reticulocyte lysate and wheat-germ extract. Fig. 3 shows the incorporation of [35 S]methionine into proteins obtained in the reticulocyte lysate supple-

Fig. 3. Synthesis of total and immunoprecipitable proteins by reticulocyte lysates supplemented with lung mRNA

Protein synthesis was carried out for 30 min at 37°C in 25 µl assay mixtures supplemented with the indicated amounts of lung mRNA isolated by oligo(dT)-cellulose chromatography. Samples of the assay mixtures were taken for determination of radioactivity incorporated into total (●) and immunoprecipitable (△) proteins. Values have been corrected for endogenous incorporation in the absence of added mRNA. The inset shows the percentage of immunoprecipitable products synthesized at the different concentrations of mRNA.

mented with different amounts of lung mRNA. Total protein synthesis is linear up to mRNA concentrations of 10 µg/ml, reaching a plateau at 40 µg/ml. The synthesis of immunoprecipitable products follows more linear kinetics, and hence the percentage of immunoprecipitable products is constant at low mRNA concentrations and increases with increasing RNA concentration (Fig. 3, inset). This percentage can reach more than 10% at mRNA concentrations of 80 µg/ml. Curves similar to that described above were obtained by translating lung mRNA in the wheat-germ extract. In this system the percentage of immunoprecipitable products can reach 20% (results not shown). In the reticulocyte lysate, optimal protein synthesis was obtained at 100 mM-K⁺ and 0.25 mM-Mg²⁺, whereas in the wheat-germ system optimal concentrations of K⁺ and Mg²⁺ were 80 mM and 3 mM respectively.

Fig. 4 shows the electrophoretic analysis of the immunoprecipitable products synthesized in both the reticulocyte and the wheat-germ systems. Pre-uteroglobin is the polypeptide synthesized in both systems, in agreement with previous reports (Bullock, 1977; Savouret *et al.*, 1980). The radioactivity

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the immunoprecipitable products synthesized in the wheat-germ system (a) and in the reticulocyte lysate (b)

The total products synthesized in the wheat-germ system (see the Materials and methods section) or in the reticulocyte lysate (see Fig. 3) were immunoprecipitated either in the absence (●) or in the presence (○) of an excess of unlabelled uteroglobin. The immunoprecipitates were analysed in polyacrylamide gels in the presence of 2-mercaptoethanol as described in Fig. 2. The arrows indicate the position of the uteroglobin markers run in parallel gels.

of the pre-uteroglobin peak could be displaced if the immunoprecipitation was carried out in the presence of an excess of unlabelled uteroglobin, as expected from the common antigenic determinants shared by uteroglobin and its precursor (Beato & Nieto, 1976; Savouret *et al.*, 1980).

Effect of glucocorticoids on the translatable levels of lung uteroglobin mRNA

To test whether the glucocorticoid-mediated increase in lung uteroglobin synthesis was due to a correlative increase in the activity of uteroglobin mRNA, lung mRNA from control and glucocorticoid-treated rabbits was isolated and its ability to direct protein synthesis was measured in cell-free systems. The uteroglobin-mRNA activity was also

Table 2. *Effect of glucocorticoids on the translatable levels of lung uteroglobin mRNA*

Poly(A)⁺ mRNA was isolated from the lungs of the animals used in the experiments described in Table 1. The mRNA was translated in reticulocyte-lysate and wheat-germ systems. Incorporation of [³⁵S]methionine into total protein and pre-uteroglobin were measured by trichloroacetic acid precipitation and immunoprecipitation respectively. The mRNA was translated at three different concentrations and the translatable activity was calculated from the linear slope of the assay curve. Values are means $\pm$ S.E.M. calculated for the numbers of animals in parentheses.

Translation system	Treatment	mRNA yield ($\mu\text{g/g}$ of tissue)	10 ⁻³ $\times$ Incorporation of [³⁵ S]methionine (c.p.m./ μg of mRNA)		Pre-uteroglobin synthesis (% of total protein)
			Total protein	Pre-uteroglobin	
Reticulocyte	Control (7)	83 $\pm$ 7	131 $\pm$ 10	1.8 $\pm$ 0.12	1.4 $\pm$ 0.13
	Dexamethasone (7)	84 $\pm$ 6	129 $\pm$ 10	3.5 $\pm$ 0.36*	2.7 $\pm$ 0.22*
	Cortisol (4)	86 $\pm$ 5	111 $\pm$ 14	2.1 $\pm$ 0.29	2.1 $\pm$ 0.51
Wheat germ	Control		45 $\pm$ 3	0.48 $\pm$ 0.02	1.0 $\pm$ 0.06
	Dexamethasone		49 $\pm$ 3	0.90 $\pm$ 0.08*	1.8 $\pm$ 0.10*
	Cortisol		41 $\pm$ 3	0.55 $\pm$ 0.04	1.4 $\pm$ 0.18

* Significantly different from control by Student's *t* test, $P < 0.01$.

Fig. 5. *Uteroglobin synthesis and uteroglobin-mRNA activity in the lung during rabbit development*

The percentage of uteroglobin synthesis (□) was measured in lung explants incubated *in vitro* for 2 h in the presence of [³⁵S]methionine. The concentration of uteroglobin in the lung (●) was measured by radioimmunoassay. The uteroglobin-mRNA activity (▲) was determined from the percentage of immunoprecipitable products synthesized in the reticulocyte lysate under conditions of linear response (see Fig. 3). Values are means $\pm$ S.E.M. (vertical bars) for three to six animals from different litters.

measured after immunoprecipitation of the total products. In several experiments, and as additional control, the immunoprecipitates were further subjected to SDS/polyacrylamide-gel electrophoresis,

and the peak of synthesized pre-uteroglobin was then measured. The results (Table 2) allow establishment of several features. A similar amount of poly(A)⁺ mRNA was obtained from the lungs of control and treated animals, and these mRNA samples have similar activity in directing protein synthesis in cell-free systems. Compared with the controls, treatment with dexamethasone increases about 2-fold the activity of uteroglobin mRNA, expressed either per mass of mRNA (and hence per mass of lung tissue) or as a percentage of total protein synthesis. Treatment with cortisol also increases the activity of uteroglobin mRNA, although to a lesser extent, in agreement with the results obtained by measuring both the relative synthesis of uteroglobin and its concentration in the lung (see Table 1).

Synthesis of lung uteroglobin during rabbit development

The synthesis of lung uteroglobin during development of rabbits was also studied by simultaneous measurements of the relative synthesis in explants, the uteroglobin content in the lung and the translatable levels of uteroglobin mRNA (Fig. 5). In 28-day fetuses (i.e. 2 days before birth) the activity of uteroglobin mRNA accounts for about 0.12% of total mRNA activity and the uteroglobin content is about 0.12 $\mu\text{g}/\text{mg}$ of soluble tissue protein. Between that age and 2 days after birth, these values increase 4–5-fold and then appear to reach a plateau, which is maintained up to day 5. Afterwards, there are new and parallel increases (2–3-fold) in both parameters, which at day 12 reach values close to those found in adult animals. The values of relative synthesis of the protein also follow, as a whole, a

pattern similar to that observed for uteroglobin-mRNA activities and uteroglobin concentration in the tissue. The total increase in lung uteroglobin synthesis (measured either as relative synthesis in explants or as content in the tissue) between 28-day foetuses and adult rabbits is about 12-fold.

Discussion

The results reported here indicate that rabbit lung explants could be used to measure the synthesis of uteroglobin in lung. So far this synthesis has been estimated from the concentration of uteroglobin in lung, but this estimation could not necessarily reflect the actual synthesis, as differences in the accumulation of the protein could occur.

With [^{35}S]methionine as labelled amino acid, radioactive uteroglobin accounts for about 1.5% of the proteins synthesized by lung explants from normal rabbits. This percentage is probably an overestimation, since uteroglobin is rich in methionine as compared with other proteins. With [^{35}S]methionine as labelled precursor, overestimations of the relative synthesis of uteroglobin has been observed in endometrial explants (Nieto & Beato, 1980) or on translation of uterine uteroglobin mRNA in frog oocytes (Beato & Rungger, 1975). Uteroglobin represents about 9% of the proteins synthesized by endometrial explants from pseudo-pregnant rabbits (Nieto & Beato, 1980), in which about 50% of the cell population are producing uteroglobin (Müller & Beato, 1980). Thus, if the rates of uteroglobin synthesis were similar in endometrial and in lung cells, about 10% of the total lung cells could be producing uteroglobin.

The present results also show that lung explants secrete newly synthesized uteroglobin into the incubation medium, and therefore this system *in vitro* could be useful for the study of uteroglobin secretion in lung. The secretion rate of the protein seems to be lower in the lung than in the endometrium, since lung explants secrete in a 6 h incubation only about 15% of the newly synthesized uteroglobin as compared with 70% secreted by endometrial explants in the same period (Nieto & Beato, 1980).

The percentages of uteroglobin synthesis in lung and in endometrium appear to correlate well with the concentrations of uteroglobin mRNA in both tissues measured by hybridization to complementary DNA. Thus uteroglobin mRNA represents about 1.5% of the total mRNA of the endometrium from pseudo-pregnant rabbits (in which the relative synthesis of uteroglobin accounts for about 9%) (Beato *et al.*, 1980; Heins & Beato, 1981) and about 0.3% of the endometrial mRNA in animals treated with oestradiol (in which uteroglobin synthesis represents about 1.8%). In lung, uteroglobin mRNA accounts for

about 0.1% (Savouret *et al.*, 1980) to 0.4% (Heins & Beato, 1981) of the total mRNA (with a uteroglobin synthesis of about 1.5%). The above percentages also indicate that uteroglobin mRNA is translated *in vivo* about 6-fold more efficiently than the average cellular mRNA both in the endometrium and in the lung. This preferential translation is also observed in cell-free systems, particularly when excess of mRNA over ribosomes is attained and hence competition of mRNA for ribosomes occurs. In these conditions, the percentage of pre-uteroglobin synthesized increases progressively (Fig. 3), even reaching more than 10%. In conditions of ribosome excess over mRNA (likely to occur *in vivo*), the percentage of pre-uteroglobin synthesis appears to be essentially independent of the mRNA concentration and close to that obtained in lung explants.

Our results obtained by measuring the relative synthesis of uteroglobin in lung explants confirm the induction of lung uteroglobin synthesis by glucocorticoids previously reported on the basis of measurement of uteroglobin concentrations in the lung (Feigelson *et al.*, 1977; Torkkeli *et al.*, 1978). The increases in uteroglobin synthesis produced by glucocorticoids are rather small (2-fold at most), but may be due to the fact that the animals were not adrenalectomized, and hence endogenous glucocorticoids are present. Glucocorticoid-mediated stimulations of the same order have been typically reported by other authors (Torkkeli *et al.*, 1978; Savouret *et al.*, 1980). Also in agreement with these reports, we found that cortisol is less efficient than dexamethasone in inducing uteroglobin synthesis. Our results also indicate that glucocorticoids produce increases in the translatable activity of lung uteroglobin mRNA parallel to those observed for the synthesis of the protein. This finding contrasts with the results of Savouret *et al.* (1980), which indicate that there is no change in the lung uteroglobin mRNA (measured either by translation or by hybridization experiments) after the dexamethasone-mediated increase in uteroglobin concentration in lung. Besides the age of the animals and the schedule of hormonal treatment, a possible explanation for these discrepant results could be that these latter experiments had been performed at a late time in the glucocorticoid induction at which the levels of uteroglobin mRNA could have fallen to nearly normal values while the content of stored uteroglobin in the lung could remain still increased. Alternatively, it is possible that the observed increase in the translatable activity of lung uteroglobin mRNA could reflect an effect of glucocorticoids on the translatability of pre-existing mRNA, as discussed below.

We also show that there are important increases in lung uteroglobin synthesis during development. The factors responsible for these increases are not

yet known, but glucocorticoid controls might be involved. For instance, it is well known that glucocorticoids have a stimulatory effect on lung maturation at late stages of foetal development (Tierney, 1974), a time at which the synthesis of lung uteroglobin was shown to increase rapidly. Also at this time, there are increases in the serum glucocorticoid concentrations in foetuses of several mammalian species (Bassett & Thorburn, 1969; Mitchell *et al.*, 1981). The developmental changes in the synthesis of uteroglobin are well correlated with parallel variations in the activity of its mRNA, which, together with the results obtained by glucocorticoid administration to adult rabbits, seem to indicate that lung uteroglobin synthesis is mainly determined by the translatable levels of its mRNA.

Certainly, our experiments cannot distinguish whether the variations in translatable activity of uteroglobin mRNA actually represent differences in the amount of this mRNA or whether they reflect differences in the translatability of the mRNA. Thus it is possible that uteroglobin mRNA could be modified to render it more efficiently translatable both *in vivo* and in cell-free systems. It is also possible that mechanisms such as preferential utilization of the uteroglobin mRNA might play a role in the regulation of uteroglobin synthesis in the lung.

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References

- Arnemann, J., Heins, B. & Beato, M. (1977) *Nucleic Acids Res.* **4**, 4023–4036
- Atger, M. & Milgrom, E. (1977) *J. Biol. Chem.* **252**, 5412–5418
- Bassett, J. M. & Thorburn, G. D. (1969) *J. Endocrinol.* **44**, 285–286
- Beato, M. & Beier, H. M. (1978) *J. Reprod. Fertil.* **53**, 305–314
- Beato, M. & Nieto, A. (1976) *Eur. J. Biochem.* **64**, 15–25
- Beato, M. & Rungger, D. (1975) *FEBS Lett.* **59**, 305–309
- Beato, M., Arnemann, J., Heins, B., Müller, H. & Nieto, A. (1980) in *Steroid Induced Uterine Proteins* (Beato, M., ed.), pp. 351–368, Elsevier/North-Holland, Amsterdam
- Beier, H. M. (1968) *Biochim. Biophys. Acta* **160**, 289–291
- Brawerman, G. (1974) *Methods Enzymol.* **30**, 605–612
- Bullock, D. W. (1977) *Biol. Reprod.* **17**, 104–107
- Bullock, D. W., Woo, S. L. C. & O'Malley, B. W. (1976) *Biol. Reprod.* **15**, 435–443
- Feigelson, M., Noske, J. G., Goswami, A. K. & Kay, E. (1977) *Ann. N.Y. Acad. Sci.* **286**, 273–286
- Heins, B. & Beato, M. (1981) *Mol. Cell. Endocrinol.* **21**, 139–150
- Krishnan, R. S. & Daniel, J. C., Jr. (1967) *Science* **158**, 490–492
- Malsky, M., Bullock, D. W., Willard, J. J. & Ward, D. N. (1979) *J. Biol. Chem.* **254**, 1580–1585
- Marcu, K. & Dudock, B. (1974) *Nucleic Acids Res.* **1**, 1385–1397
- Mitchell, B. F., Seron-Ferré, M., Hess, D. L. & Jaffe, R. B. (1981) *Endocrinology* **108**, 916–924
- Müller, H. & Beato, M. (1980) *Eur. J. Biochem.* **112**, 235–241
- Nieto, A. & Beato, M. (1980) *Mol. Cell. Endocrinol.* **17**, 25–39
- Nieto, A., Ponstingl, H. & Beato, M. (1977) *Arch. Biochem. Biophys.* **180**, 82–92
- Noske, I. & Feigelson, M. (1976) *Biol. Reprod.* **15**, 704–713
- Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256
- Popp, R. A., Foresman, K. R., Wise, L. D. & Daniel, J. C., Jr. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5516–5519
- Ponstingl, H., Nieto, A. & Beato, M. (1978) *Biochemistry* **17**, 3908–3912
- Savouret, J. F., Loosfelt, H., Atger, M. & Milgrom, E. (1980) *J. Biol. Chem.* **255**, 4131–4136
- Swank, R. T. & Munkres, K. D. (1971) *Anal. Biochem.* **39**, 462–477
- Tierney, D. F. (1974) *Annu. Rev. Physiol.* **36**, 209–231
- Torkkeli, T. K., Krusius, T. & Jänne, O. (1978) *Biochim. Biophys. Acta* **544**, 578–592