The synthesis of ribosomal RNA and ribosomal protein and their incorporation into ribosomes in the uterus of the oestrogen-stimulated immature rat

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The effect of oestrogen on the synthesis of ribosomal proteins in the uterus of the immature rat has been investigated. Stimulated synthesis peaks, at 6–7-times control levels, 12 h after a single administration of the hormone. The stimulated synthesis and incorporation of newly made proteins into ribosomal particles exhibit very similar kinetics. The incorporation of newly made rRNA into ribosomes mirrors that of ribosomal protein but lags several hours behind the peak of oestrogen-stimulated rRNA synthesis.

Cell-free protein synthesis *Immunoassay*

1. INTRODUCTION

A major feature of oestrogen-induced hypertrophy in the uterus of the immature rat is the synthesis of the components of ribosomes and the formation of new ribosome particles (review [1]). The synthesis of mature 28 S and 18 S rRNA peaks, at up to 12-times control levels, 4 h after the administration of the hormone [2] but there follows a considerable delay before the peak, at 12 h, in the incorporation of the newly made rRNA and ribosomal proteins into cytoplasmic polysomes [3,4]. Here, we have investigated the oestrogen-stimulated synthesis of ribosomal proteins and found that this also lags well behind the synthesis of rRNA. However, there appears to be little or no lag between the synthesis of ribosomal protein and its incorporation into ribosomes.

Abbreviations: oestradiol-17β, 1,3,5(10)-estratriene-3, 17β-diol; ELISA, enzyme-linked immunosorbant assay

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2. MATERIALS AND METHODS

The source and maintenance of immature female rats, the administration of oestradiol-17β and the preparation of acid-insoluble fractions have been described [2].

Uterine polysomes [3] and ribosomal proteins [4] were also prepared as previously described except that the final protein precipitation was with 5 vols acetone at −20°C for 16 h [5]. They were dissolved and used to immunize rabbits as in [6].

Cell-free protein synthesis was performed by the method of [7] using rabbit reticulocyte lysate (Amersham International). Syntheses were performed in a total volume of 25 μl for 90 min, at 30°C in a gently shaking water bath after which the reaction was stopped by the addition of 1 μl of 10 mM puromycin dihydrochloride and continuation of the incubation for a further 10 min. This also served to release nascent polypeptide from the ribosomes.

Ribosomal proteins labelled by cell-free protein synthesis were removed from the reticulocyte lysate by double immunoprecipitation. The proteins were bound by an excess of anti-ribosomal protein antibody. This antibody was then precipi-

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tated by addition of an anti-rabbit antibody at optimal proportions [8]. Before any of this could be done, however, ribosomal subunits, which would interfere with the reaction, had to be removed from the protein synthesising mixture. The mix was diluted to 5 ml with detergent-supplemented PBS (170 mM NaCl, 1.8 mM KH_2PO_4, 10 mM Na_2PO_4, pH 7.4, containing 0.05% BSA, 0.02% Triton X-100 and 0.02% sodium deoxycholate) and the ribosomal subunits were removed from suspension by centrifugation at 230 000 × *g* (50 000 rpm) in an SW 50.1 rotor of a Beckman L2 65B ultracentrifuge, for 4 h at 4°C. Aliquots of the supernatants were diluted to 1 ml with the above buffer and to each was added 10 μl normal mouse serum, followed by 10 μl sheep anti-mouse gamma-globulin antibody. The samples were incubated for 4 h at 4°C and the resulting precipitate was removed by centrifugation for 15 min in an Eppendorf centrifuge. Material that bound antibodies non-specifically was removed by this procedure. To the supernatant fraction was added 20 μl of a purified gamma-globulin containing rabbit anti-ribosomal protein antibody. This was then incubated at 37°C for 1 h, with mixing. Subsequently, 10 μl of a purified gamma-globulin containing donkey anti-rabbit gamma-globulin antibody, was added to effect precipitation. This was achieved by incubation overnight (16 h) at 4°C, after which the precipitate was collected by centrifugation for 15 min in an Eppendorf centrifuge. The pellet was washed 3 times with detergent-supplemented PBS, digested with 300 μl Protosol (New England Nuclear) overnight at 60°C, and counted in a Beckman LS 8100 scintillation counter, using toluene–PPO (5 g/l) as scintillant.

The purified gamma-globulin fraction was prepared by ammonium sulphate precipitation. Just prior to precipitation saturated $(NH_4)_2SO_4$ was adjusted to pH 6.5 by the addition of 2 N NaOH. A 2 ml sample of serum was stirred at room temperature while $(NH_4)_2SO_4$ solution was slowly added. When the serum sample had been made 33% with respect to $(NH_4)_2SO_4$ saturation, the suspension was stirred at room temperature for a further 30 min. The gamma-globulins were collected by centrifugation at 1000 × *g* in a Beckman benchtop centrifuge and redissolved in 2 ml of 0.9% (w/v) saline. The precipitation was then repeated. The final precipitate was redissolved in and dialysed against PBS buffer overnight at 4°C and the solution centrifuged at 20 000 × *g* (12 000 rpm) in a Sorval RC-5 (HB4 rotor) to remove any insoluble precipitate.

Competition enzyme-linked immunosorbant assay (ELISA) was performed in microtitre plates (Falcon) employing horseradish peroxidase conjugated to goat anti-rabbit gamma-globulin in an enzyme immunoassay essentially as in [9]. The volume was reduced to 100 μl per well and absorbance at 492 nm was assayed directly using a Titertek multiscan spectrophotometer. Immunoblotting was performed as in [10].

3. RESULTS

Highly purified rat liver ribosomal protein was used to raise antibodies in rabbits as described in the methods section. Fig.1 shows the binding properties of various macromolecules incubated with anti-ribosomal protein serum. Ribosomal proteins bound strongly to the antibody and allowed the detection of as little as 10 ng protein. Intact ribosomes also interacted with the antisera but were approximately one-fifth as active as free ribosomal proteins, presumably because some antigenic sites

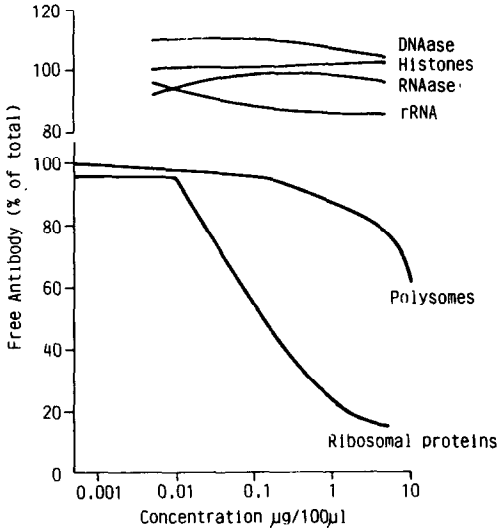

Fig.1. The specificity of rabbit anti-ribosomal protein. The indicated macromolecules were used to compete with purified ribosomal protein for the antibody in competition ELISA assay as described in section 2. Those macromolecules that did not show extensive binding are, for reasons of clarity, plotted on a separate axis.

were shielded in the ribonucleoprotein particle. A selection of other basic proteins were ineffective competitors for the antibody as were the proteins of a post-ribosomal supernatant. Ribosomal RNA exhibited a small reaction with the antisera. This reaction was not unexpected as it was difficult to exclude all traces of rRNA from the immunogen or to exclude all traces of ribosomal protein from the rRNA probe. Either source of contamination could have resulted in the observed reaction. The specificity of the immune sera was also analyzed by immuno-blotting and showed that the antibodies were polyconal and reacted with all the major ribosomal proteins. They did not react with blots of the proteins of a post ribosomal supernatant (not shown).

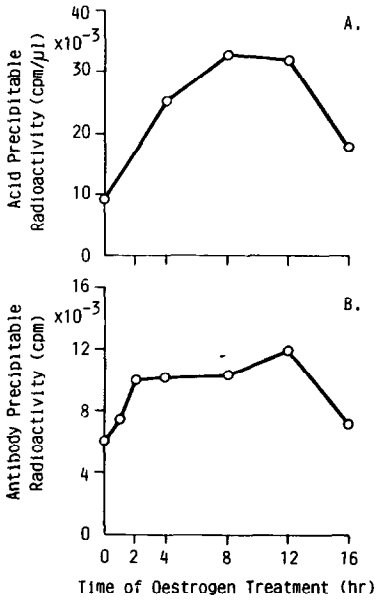

Fig.2. The effects of oestrogen on the synthesis by uterine polysomes of (A) total protein and (B) ribosomal protein. Groups of 6 immature female rats were injected with oestradiol-17β at various times before death. Polysomes were prepared from the uteri of these animals as described in section 2 and 0.2 $A_{260\,nm}$ units were incubated in a final volume of 25 μl of messenger-dependent lysate (Amersham International) for 90 min at 30°C. (A) Duplicate 1 μl aliquots of incubation mixture were taken for the measurement of acid-precipitable radioactivity. (B) 10^6 cpm of acid-precipitable material from the incubation mix was precipitated with rabbit anti-ribosomal protein antibody as described in section 2.

Anti-ribosomal protein gamma globulin, purified from the antisera, was used to follow the effects of oestrogen on the synthesis of ribosomal proteins. Uterine polysomes were incubated in a cell-free reticulocyte translation system as described in section 2. Puromycin was then used to release nascent polypeptide and the ribosomal material was removed from the translation mix by centrifugation. This was necessary to ensure that the proteins of the ribosomes did not compete with the translation product in binding the antibody. Aliquots of the supernatant, containing 10^6 cpm of acid-insoluble translation product, were challenged with pre-immune serum and the non-specifically bound polypeptide removed by centrifugation. The supernatant was then rechallenged with purified gamma globulin containing anti-ribosomal protein antibodies. The pre-immune serum precipitated negligible quantities of radioactivity while the post-immune serum precipitated 0.5 and 1.2% of the acid-insoluble counts in untreated and 12 h oestrogen-treated rats, respectively. Fig.2

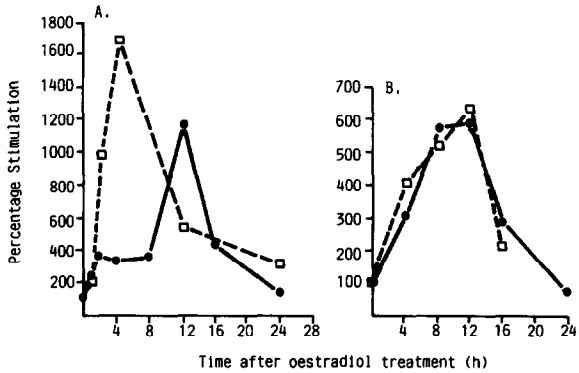

Fig.3. (A) The effect of oestradiol-17β on the synthesis of rRNA and its incorporation into uterine ribosomes. The curve of the incorporation of precursor into newly made rRNA (□---□) is derived from the data of [2] and [11] by measuring the area under peaks of radioactive 28 S and 18 S RNA separated on 2.7% polyacrylamide gels. The curve of the incorporation of newly made rRNA into polysomal ribosomes (●——●) is from the data of [3]. (B) The effect of oestradiol-17β on the synthesis of ribosomal proteins and their incorporation into uterine ribosomes. The curve of the incorporation of precursor into newly made ribosomal protein (□---□) is derived from the data of fig.2. The curve of the incorporation of newly made ribosomal proteins into polysomal ribosomes (●——●) is from [3].

illustrates the effect of oestrogen on the synthesis of ribosomal protein and compares it with the effect of the hormone on total protein synthesis measured as the incorporation of [^{35}S]methionine into acid-insoluble product. Because the data of fig.3B were derived from a constant amount of newly made protein (10^6 acid-insoluble cpm), the results indicate the extent to which oestrogen stimulated the synthesis of ribosomal proteins over and above its effects on total protein synthesis. The overall effect of the hormone on ribosomal protein synthesis approximated to the sum of the two curves of fig.2A,B. Such a curve is illustrated in fig.3 and is compared with our previously published data for the synthesis of rRNA and for the incorporation of newly made rRNA and ribosomal proteins into intact ribosomes.

4. DISCUSSION

For a number of years, this laboratory has studied oestrogen-stimulated ribosome synthesis as a key event in the hormone-induced hypertrophy of the uterus. We have noted that the rapid stimulation of rRNA synthesis was followed by increased incorporation of rRNA into new ribosomal particles but that these two events were separated by a substantial lag of ~8 h (fig.3). We examined the effects of oestrogen on the synthesis of ribosomal proteins in an effort to determine whether the timing of their synthesis could explain the delay in the incorporation of newly made rRNA into ribosomes. The results appeared to indicate that such an explanation was possible in that it was not until well after the peak of rRNA synthesis that ribosomal protein synthesis was stimulated to a similar extent. However, studies of the pool sizes and turnover of both the proteins and the RNA would be necessary to clarify this point.

To assemble active ribosomes, the cell must synthesize approx. 80 different proteins and 4 different rRNA species. In all cases investigated, the accumulation of ribosomal components has been shown to be tightly coordinated. In no case was excess rRNA or ribosomal proteins found in cellular pools. Thus, inhibition of ribosomal protein synthesis with puromycin resulted in reduced rRNA accumulation [12] and inhibition of rRNA synthesis with actinomycin D resulted in reduced ribosomal protein accumulation [13,14]. Similar findings were reported with yeast mutants defective in the synthesis of rRNA or ribosomal proteins [15,16] and in the changing rates of ribosome synthesis during cellular differentiation [17]. The coordination did not, however, appear to be at the level of synthesis. Indeed, in most cases, inhibited synthesis of one component did not effect the synthesis of the other [12,13,16]. The reduced accumulation of the latter was due to increased degradation. Clearly, the turnover of the component molecules of the ribosome, as well as the pool sizes of the ribosomal proteins are further parameters that could influence the kinetics of oestrogen-induced uterine ribosome synthesis. Nothing is currently known, for instance, of the percentage of newly made uterine rRNA that enters mature cytoplasmic ribosomes or of any effect oestrogen might exert on the process. It is known that the hormone profoundly stimulates the activity of cytoplasmic ribonuclease and results in a corresponding decrease in the activity of an endogenous ribonuclease inhibitor ([18,19], Brockdorff and Knowler, unpublished) but the role of these proteins in ribosome accumulation by the hormone-stimulated tissue is conjectural.

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