Biochem. J. (1978) **172**, 227–231
Printed in Great Britain

Thyrotropin-Stimulated Recruitment of Free Monoribosomes on to Membrane-Bound Thyroglobulin-Synthesizing Polyribosomes

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(*Received* 14 *July* 1977)

Treatment of ox and dog thyroid slices *in vitro* with either thyrotropin or dibutyryl cyclic AMP elicited a variety of changes in polyribosome distribution. The most marked and consistent responses were decreases in both free and membrane-bound monoribosomes with a concomitant increase in the specific peak of thyroglobulin-synthesizing polyribosomes. On some occasions there was a shift towards heavier aggregates in the free polyribosomes. The increase in the amount of thyroglobulin-synthesizing polyribosomes was not accompanied by a shift in its location on the gradients. These changes were apparent within 30min of thyrotropin addition and within 60min of the addition of dibutyryl cyclic AMP. It is suggested that the major initial effect on translation of both thyrotropin and dibutyryl cyclic AMP is to stimulate the recruitment of pre-existing free monoribosomes on to pre-existing unloaded or under-loaded thyroglobulin mRNA molecules.

The thyroid gland synthesizes large amounts of a specific protein, thyroglobulin, which represents the precursor and storage form of thyroid hormones. Many aspects of thyroid metabolism, including protein synthesis, are under pituitary control via the hormone thyrotropin, which exerts its effects by altering intracellular concentration of 3′:5′-cyclic AMP (Dumont, 1971). Indeed, total protein synthesis in the thyroid gland has been shown to be stimulated by thyrotropin, both *in vivo* (Pavlovic-Hournac *et al.*, 1971) and *in vitro* (Lecocq & Dumont, 1972; Sherwin & Tong, 1976; Wagar *et al.*, 1973). This effect is rapid (less than 30min) and accompanied by a shift in polyribosome distribution towards heavier aggregates, suggesting that thyrotropin may act, at least in part, at the translational level (Lecocq & Dumont, 1972, 1973; Sherwin & Tong, 1976). A specific effect of thyrotropin on the synthesis of thyroglobulin has been suggested (Lissitzky *et al.*, 1969; Pavlovic-Hournac *et al.*, 1971; Salabe *et al.*, 1973), but its demonstration has been made difficult by the extreme abundance of thyroglobulin and its degradation products and by the concomitant thyrotropin-induced stimulation of thyroglobulin degradation (Pavlovic-Hournac & Delbauffe, 1976).

Thyroglobulin is synthesized on a population of large membrane-bound polyribosomes (De Nayer *et al.*, 1973; Vassart & Dumont, 1973). These thyroglobulin-synthesizing polyribosomes are readily visible as a discrete peak on sucrose gradients since they are so large, comprise most of the polyribosomes and can now be isolated in an undegraded state (Davies *et al.*, 1977). Accordingly, any change in the quantity of thyroglobulin-synthesizing polyribosomes will be evident as a change in the area under the specific peak, whereas any marked change in the loading of ribosomes will be apparent as a lateral shift of the peak (Vassart *et al.*, 1970, 1971). Thus the potential for thyroglobulin synthesis can be estimated directly from polyribosome profiles without interference from the degradation of thyroglobulin that may occur simultaneously. It was the aim of the present study to investigate whether thyroglobulin or cyclic nucleotides had any specific effect on the quantity or size of thyroglobulin-synthesizing polyribosomes.

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Materials and Methods

Slices of thyroid gland from ox or dog were incubated as described earlier (Lecocq & Dumont, 1972). Immediately after incubation the tissue was frozen in liquid N_2 and polyribosomes isolated as described previously (Davies *et al.*, 1977). The frozen tissue was pulverized in a mortar and homogenized in at least 5vol. of buffer A (0.2M-Tris/HCl, pH8.5; 50mM-KCl; 25mM-$MgCl_2$; 0.2M-sucrose) containing 3mg of yeast RNA (Koch–Light, Colnbrook, Bucks, U.K.)/ml in a motor-driven Teflon/

glass homogenizer. The homogenate was centrifuged at 800*g* for 10min in a Sorvall SS 34 rotor and the 'nuclear' pellet was resuspended in 1.0ml of buffer A (without RNA) and re-centrifuged for 5min at 27000*g* to compact the pellet and remove contaminating cytoplasm. The pellet was retained and the supernatant added to the original 800*g* supernatant. These combined supernatants were centrifuged for 10min at 27000*g* to pellet the bound polyribosomes. This pellet was resuspended in 1.0ml of buffer A (without RNA), re-pelleted for 5min at 27000*g* and retained. The supernatant was combined with the original 27000*g* supernatant, layered over a 3ml pad of 2M-sucrose in buffer A and centrifuged at 240000*g* for 3h ($r_{av.}$ 5.7cm) in a Spinco 65 rotor to pellet free polyribosomes. Occasionally, the centrifugation at 800*g* was omitted so that the nuclei and bound polyribosomes were pelleted together.

The free polyribosomes were resuspended in buffer B (50mM-Tris/HCl, pH7.5; 20mM-KCl; 10mM-$MgCl_2$). Samples (0.2ml) were layered on linear 15–50% (w/v) sucrose gradients in buffer B (4.8ml) and centrifuged at 255000*g* ($r_{av.}$ 8.78cm) for 30min in a Spinco SW 56 rotor. The washed bound polyribosome pellet was resuspended in buffer A containing 100 units of heparin/ml, 1% Brij 58 and 1% sodium deoxycholate, incubated for 10min, and clarified by centrifugation at 27000*g* for 5min. The released polyribosomes were layered on identical gradients and also centrifuged for 30min. The 800*g* pellet ('nuclear' polyribosomes) or the combined 800*g* plus 27000*g* pellets were resuspended in buffer A containing 2% (v/v) Triton X-100 and processed similarly to the bound polyribosomes. All operations were conducted at 0–2°C. After gradient centrifugation, the tube contents were analysed as described by Lecocq & Dumont (1972).

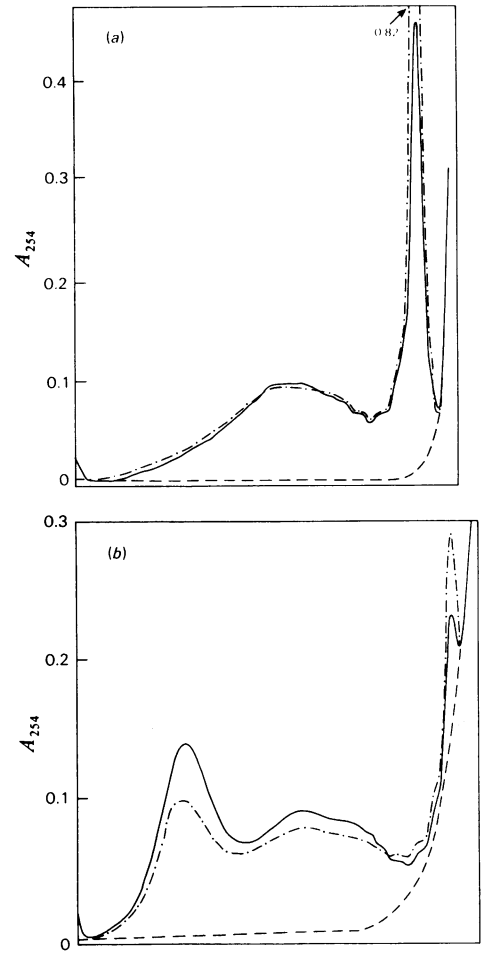

Fig. 1. *Effect of thyrotropin on the distribution of free and membrane-bound polyribosomes in ox thyroid*
Slices were incubated with or without 10munits of thyrotropin/ml for 1h before polyribosome extraction (see the Materials and Methods section). Profiles represent the extract from 0.25g for the free polyribosomes (*a*) and 0.2g for the bound polyribosomes (*b*) with (——) and without (– · –) thyrotropin. The broken line represents a gradient overlayered with resuspension buffer alone. The direction of sedimentation is from right to left.

Results

The profiles depicted in Fig. 1 show ribosomal distributions from ox thyroid slices incubated for 1h in the presence or absence of 10munits of thyrotropin/ml (Lecocq & Dumont, 1972). The free polyribosomes showed little change, whereas the free monoribosomes decreased to about one-half of the initial amount after thyrotropin treatment (Fig. 1*a*). The bound polyribosomes (Fig. 1*b*) also exhibited a loss in monoribosomes, but showed an increase in larger polyribosomes, especially the peak of very large thyroglobulin-synthesizing polyribosomes. The profiles from slices treated with dibutyryl cyclic AMP are not shown, since they were almost superimposable over the profiles from thyrotropin-treated tissue. Planimetry of different regions of these profiles, after allowing for incomplete pelleting of free polyribosomes through the sucrose pads, showed virtually no change in total ribosomal material during incubation for 1h. This suggests that the hormone stimulates the recruitment of pre-existing free monoribosomes into membrane-bound polyribosomes. Very similar responses were found in about two-thirds of the 15 experiments carried out with ox thyroid.

The profiles in Fig. 2 show ribosome distributions of ox thyroid incubated for 30 or 60min with thyro-

tropin or dibutyryl cyclic AMP. The main difference between this experiment and that shown in Fig. 1 is that the thyroid was highly hypertrophic and weighed almost 40g, compared with an average of about 20g for ox thyroid. This kind of tissue, which may have originated from animals illegally treated with anti-thyroid drugs, routinely had less free monoribosomes and more free polyribosomes (Fig. 2*a*) than did the less distended tissue (Fig. 1*a*). However, even in this tissue, both thyrotropin and dibutyryl cyclic AMP caused a decrease in the number of free monoribosomes (Fig. 2*a*) similar to that found earlier (Fig. 1*a*), but in this case the hormone caused a shift towards heavier free-polyribosome aggregates. With the bound polyribosomes, after treatment for 30min, thyrotropin caused a marked decrease in the number of monoribosomes and small polyribosomes and an increase in the peak of thyroglobulin-synthesizing polyribosomes, where dibutyryl cyclic AMP was much less effective (Fig. 2*b*). However, after treatment for 60min (Fig. 2*c*), thyrotropin had little additional effect, whereas dibutyryl cyclic AMP had become almost as effective as thyrotropin in stimulating formation of thyroglobulin-synthesizing polyribosomes. Results similar to these were found in the remaining one-third of the experiments, usually with enlarged thyroids. Although there were minor differences between responses of free polyribosomes in different experiments, certain features were consistent in all the experiments with ox thyroid slices. Both thyrotropin and dibutyryl cyclic AMP always evoked a decrease in monoribosome content, with the greatest effect (in terms of total amount of material) on the free monoribosomes. They also always stimulated an increase in the peak of large thyroglobulin-synthesizing polyribosomes, and the effect of thyrotropin was apparent sooner than that of dibutyryl cyclic AMP. They also had little effect on the total amount of ribosomal material recovered after incubation. Of major importance, however, is the fact that the increase in height of the peak of thyroglobulin polyribosomes was never accompanied by a discernible lateral shift in its location.

The results of experiments with slices of thyroid from dog were qualitatively similar to those from ox, in so far as both thyrotropin and dibutyryl cyclic AMP elicited a marked decrease in free monoribosomes with a concomitant increase in the height

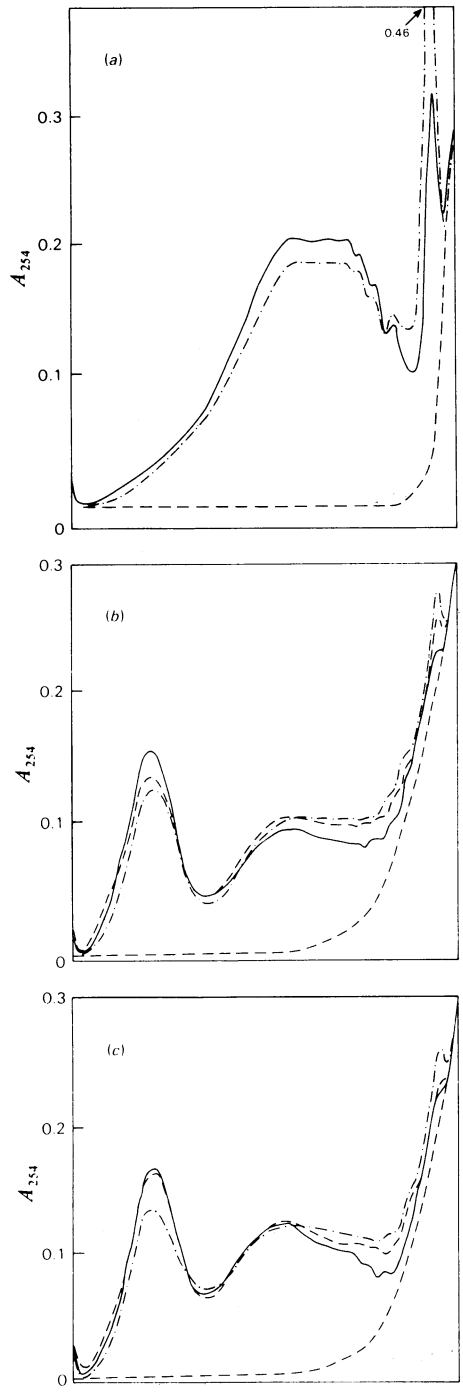

Fig. 2. *Effect of thyrotropin and dibutyryl cyclic AMP on the distribution of free and bound polyribosomes from a 'distended' ox thyroid*

The protocol was identical with that in Fig. 1 except that an enlarged thyroid was used, incubation was for 30min (*b*) or 60min (*a* and *c*) and the total bound polyribosome fraction (centrifugation at 800*g* omitted) was analysed (*b* and *c*). -·-, No addition; ——, 10munits of thyrotropin/ml added; ----, 0.4mM-dibutyryl cyclic AMP added.

of the peak of thyroglobulin-synthesizing polyribosomes, without causing its lateral displacement on the gradient. However, even though experiments involving mixing of the homogenates from dog with those of ox showed no difference in the degradation of the polyribosomes in the extract from the different sources, dog thyroid routinely yielded profiles exhibiting a much greater proportion of monoribosomes and a much less obvious peak of thyroglobulin-synthesizing polyribosomes.

Discussion

The results in the present paper with ox thyroid support those reported earlier for dog thyroid (Lecocq & Dumont, 1973), where thyrotropin and dibutyryl cyclic AMP caused a shift in polyribosome distribution towards heavier aggregates. The interpretation, at that time, was either that thyrotropin had caused a general increase in the initiation of mRNA translation (ribosome recruitment) or that it had promoted the specific translation of larger mRNA molecules at the expense of smaller ones. By using sodium deoxycholate-treated postmitochondrial supernatant as the source of ribosomes, this earlier study dealt with a mixture of free and bound ribosomes that are preferentially enriched in free polyribosomes (Blobel & Potter, 1967) and did not demonstrate the presence of intact thyroglobulin-synthesizing polyribosomes.

The hormone-stimulated increase in membrane-bound thyroglobulin-synthesizing polyribosomes found here with both ox and dog has interesting characteristics. First, it takes place mainly at the expense of free monoribosomes (Figs. 1*a* and 2*a*). Secondly, as judged by the depth of sedimentation of these polyribosomes, it is not accompanied by an increase in their mean size (Figs. 1*b*, 2*b* and 2*c*). Thirdly, it is either accompanied by only a slight increase (Fig. 2*a*) or no change in free polyribosomes (Fig. 1*a*). These characteristics eliminate the possibility that thyrotropin causes a general stimulation of polypeptide-chain initiation, or even a specific stimulation of the recruitment of monoribosomes on pre-existing, normally loaded mRNA coding for thyroglobulin (Vassart *et al.*, 1971). Indeed, in this latter case, the mean size of these polyribosomes would increase. Fourthly, since large thyroglobulin-synthesizing polyribosomes were never evident in the free-polyribosome fraction, the hormone could not have stimulated the binding to membranes of pre-existing unbound thyroglobulin-synthesizing polyribosomes. These responses were similar in all the tissues examined (normal and distended ox thyroid and dog thyroid), even though the basal polyribosome content (and presumably the physiological status of the tissue) was considerably different.

In the absence of any significant loss of mRNA coding for thyroglobulin during incubation without hormones (Seed & Goldberg, 1965; Vassart & Dumont, 1973), the present results strongly imply that thyrotropin increases the number of mRNA molecules coding for thyroglobulin available for translation. This could be achieved either by the accumulation of newly synthesized mRNA coding for thyroglobulin or by translation of a population of previously unloaded or under-loaded mRNA. The rapidity of polyribosome aggregation in response to thyrotropin (Fig. 2*b*) makes a purely transcriptional effect highly unlikely. Indeed, even in the chicken oviduct, maximally stimulated by oestrogens, mRNA coding for ovalbumin accumulates much more slowly (McKnight *et al.*, 1975). Moreover, evidence has been provided that the first effect of thyrotropin is primarily at the level of translation (Lecocq & Dumont, 1973; Sherwin & Tong, 1976). Thus the observed increase in thyroglobulin-synthesizing polyribosomes after thyrotropin treatment must correspond to the recruitment of monoribosomes on to 'spare' mRNA molecules coding for thyroglobulin. Such untranslated mRNA has been observed in reticulocytes (Marbaix *et al.*, 1976) and in unfertilized eggs (Gurdon, 1974). Hypothetical mechanisms for activation of mRNA coding for thyroglobulin could involve specific phosphorylation reactions on messenger ribonucleoprotein particles coding for thyroglobulin or capping of its 5′ end (Rottman *et al.*, 1974).

The hormone-induced recruitment of free monoribosomes into membrane-bound polyribosomes found in the present paper with thyroid is strikingly similar to the response evoked by the plant hormone indol-3-ylacetic acid (Davies, 1976). Although this great similarity in responses cannot implicate a common primary mode of action for these diverse hormones, they do suggest that hormones from widely different tissues can have a similar effect on the translation mechanism.

The work was performed within the framework of the Association Contract Euratom–University of Brussels–University of Pisa, and thanks to a grant (Action Concertee) of the Ministère de la Politique Scientifique. E. D. was supported by grants from the University of Nebraska Research Council and Euratom. G. V. is Chargé de Recherche, Fonds National de la Recherche Scientifique.

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