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DIHYDROTESTOSTERONE AND THE INITIATION OF PROTEIN SYNTHESIS BY PROSTATE RIBOSOMES

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SUMMARY

The binding of the initiator [^{35}S]methionyl-tRNA$_f$ to prostate ribosomal particles is dependent on GTP and on a prostate cytosol fraction. The cytosol activity decreases rapidly after the rat is castrated. An increase in the cytosol activity can be observed almost immediately (within 10 min) after an intravenous injection of dihydrotestosterone into the castrated rat.

In many target cells, steroid hormones can bind firmly to specific proteins called steroid receptors. It is not clear at this time how these receptor molecules, when combined with steroids, trigger the hormone action. Since the effect of steroid hormones on nuclear RNA synthesis in responsive cells can take place rapidly, it has generally been assumed that they may act by regulating gene transcription in the cell nuclei [1].

It has also been suggested that some steroid hormones may act in certain stages of post-transcriptional processes that lead to gene translation (*i.e.*, protein synthesis). For example, Tomkins *et al.*[2] have proposed that the steroid–receptor complex may inhibit a hypothetical "translational repressor" activity, either by a direct interaction with the repressor or by inhibiting its synthesis. Similarly, Ohno[3] has considered the possibility that the receptor protein itself is a "translational block" which binds mRNA and prevents it from translation, but that this suppressive activity is released by steroid binding to the receptors.

Earlier, we suggested a model in which a steroid–receptor complex not only can participate in the regulation of gene transcription, but also can bind to a specific RNA product and regulate its processing and/or utilization [4]. More recently, we provided experimental results showing that certain ribonucleoprotein particles in the rat uterus and ventral prostate

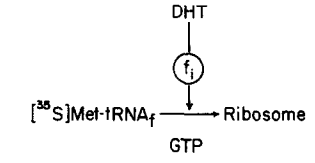

Fig. 1. Assay scheme for the effect of dihydrotestosterone (DHT) on the cytosol factors involved in the formation of an initiation complex.

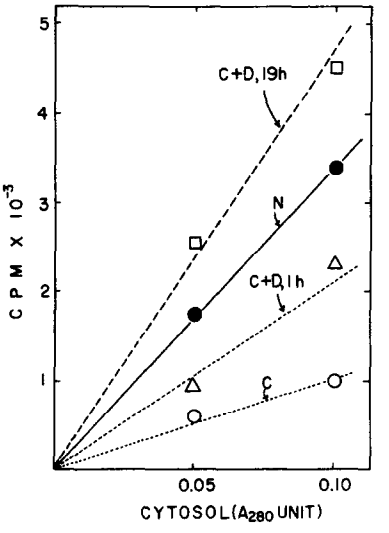

Fig. 2. Effect of castration and dihydrotestosterone administration on the prostate cytosol factors involved in the binding of [^{35}S]methionyl-tRNA$_f$ by prostate ribosomal particles [9]. [^{35}S]Methionyl-tRNA$_f$ was prepared by charging of rat liver tRNA with [^{35}S]methionine (29 Ci/mmol) by means of an *Escherichia coli* synthetase. The aminoacylated product is known to be essentially the initiator methionyl-tRNA and is suitable for measurements of the formation of an initiation complex in protein synthesis. The ribosomal particles were obtained from the ventral prostate of castrated rats. The cytosol preparations were prepared from normal rats (●), rats castrated 19 h previously (○), and castrated rats injected intraperitoneally with DHT (2·5 mg in 0·5 ml sesame oil per rat) one hour (△) or 19 h (□) earlier. The reaction mixture, in a final volume of 0·3 ml, contained ribosomes (2·3 A_{260} unit), GTP (1·3 mM), dithiothreitol (3·0 mM, $MgCl_2$ (5 mM), KCl (90 mM), Tris–HCl buffer (20 mM, pH 7·5, [^{35}S]methionyl-tRNA$_f$ (48,400 c.p.m., 0·08 A_{260} unit), and a cytosol preparation in the quantities shown. The mixture was incubated at 30° for 10 min, and the radioactivity retained by a Millipore membrane after filtration was measured. The binding activity (1400 c.p.m.) in the absence of an additional cytosol fraction was deducted from the data.

may bind, respectively to the estrogen–receptor and dihydrotestosterone(DHT)–receptor complexes [5, 6].

To understand further the possible effect of androgens on post-transcriptional processes, we have studied the early effect of DHT on the factors required for the initiation of protein synthesis on ribosomes of the rat ventral prostate. In one such investigation, we characterized the initiation process by measuring the ability of prostate ribosomal particles to bind [^{35}S]methionyl-tRNA$_f$, which could be considered as the initiator aminoacyl-tRNA in eukaryotic cells (Fig. 1). The binding activity was found to be dependent on the presence of GTP, ribosomal particles, and a cytosol preparation, and to have many properties similar to those reported for the liver and for reticulocyte systems [7, 8]. If the amounts of ribosomal particles, GTP, and [^{35}S]methionyl-tRNA were kept constant, the binding activity was linearly proportional to increasing amounts of prostate cytosol preparation added to the experimental tube, until a plateau level was reached.

Using this assay method, we have compared the cytosol preparations of normal, androgen-deficient, and androgen-supplemented rats for their ability to support the formation of initiation complex. As shown in Fig. 2, we found that the prostate cytosol preparation from rats castrated 19 h earlier was considerably less active than that from normal rats. The loss of cytosol activity after castration could be prevented by administration of DHT to the rats immediately after castration. A very significant stimulation of the activity can also be noted 1 h after the injection of the androgen into the castrated rats. Many additional experiments indicated that the reduction in cytosol activity occurs within one hour after castration. The androgen effect seems to occur immediately, for we have repeatedly observed a 20–40% increase in the cytosol activity within 10 min after intravenous injection of DHT into castrated rats [9].

We are now studying whether the androgen-dependent cytosol activity is due to the protein and/or RNA factors. Attempts are being made to demonstrate the androgen effect in *in vitro* systems. Our findings suggest that the effect of a steroid–receptor complex on the post-transcriptional process may be more direct than that considered previously.

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