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ON THE FACTORS AFFECTING THE INITIATION OF PROTEIN SYNTHESIS IN THE RAT VENTRAL PROSTATE: ANDROGENS, POLYAMINES AND CONJUGATED PROTEINS*

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SUMMARY

In the ventral prostate of certain strains of rats, androgens may have a very rapid effect on the initiation of protein synthesis by an antiandrogen sensitive process that may not be dependent on the synthesis of new RNA or protein. The primary effect appears to be at the step of met-tRNA$_f$ binding to the initiation factor EIF-1. Several stimulatory and inhibitory factors that can affect initiation factor activity are present in the prostate cytosol fraction. The low molecular weight inhibitory fraction contains polyamines which, at concentrations below 0.01 mM, can significantly inhibit this activity. The macromolecular effector fraction contains conjugated proteins (prostin H) that can destabilize met-tRNA$_f$ binding to initiation factor(s) and can also stimulate the incorporation of met-tRNA$_f$ into the newly synthesized protein. Upon enzymatic or acid proteolysis, prostin H releases the inhibitory components which behave like polyamines. It is possible that certain specific protein-bound polyamines play important roles in the hormonal regulation of protein synthesis in mammalian cells.

The androgen–receptor complex of the rat ventral prostate can interact with nuclear chromatin, certain divalent cations, and nucleoside triphosphates [1]. This finding together with the observations that the nuclear RNA polymerase activity of the rat prostate can be enhanced very rapidly by androgens *in vivo* [2, 3] and by certain preparations of androgen-receptor complex in cell-free systems [4], is in agreement with the view that the nuclear androgen–receptor complex may participate directly in the regulation of RNA synthesis. However, the possibility, that androgens and their receptors may be involved in certain post transcriptional processes [5, 6] cannot be eliminated at this time.

Our studies have shown that the prostate androgen–receptor complex can bind to polyribonucleotides and certain nuclear ribonucleoprotein particles [5–8] or to the ribosomal subunit particles (but not to the 80 S monosome or polysomes) [1]. We also have reported that, in certain strains of rats, androgens can within 10–20 min enhance the ability of the prostate cytosol proteins to promote [^{35}S]met-tRNA$_f$ (initiator tRNA) binding to prostate ribosomes [9]. To confirm that this binding represents the initiation of protein synthesis, we have further characterized the individual steps involved in the initiation process and found that they are very similar to those proposed for other eukaryotic systems (Fig. 1). In the initiation process, met-tRNA$_f$ first interacts with GTP and a protein initiation factor EIF-1 to form an initiator complex [met-tRNA$_f$-GTP-EIF-1] that can be trapped by nitrocellulose membranes. This complex then binds to the 40 S (but not to the 60 S) ribosomal subunit to form a 40 S initiation complex that can, in the presence of other factors, bind mRNA and a 60 S subunit to form an 80 S ribosomal complex and then initiate peptide bond formation.

As shown in Fig. 2, the effect of castration and injection of 5α-dihydrotestosterone can be seen by analysis of the activity of the cytosol protein in promoting binding of the radioactive initiator tRNA to the 40 S ribosomal particle. More recent studies have

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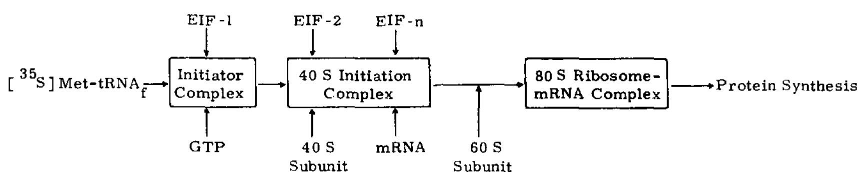

Fig. 1. Steps involved in the initiation of protein synthesis in rat ventral prostate.

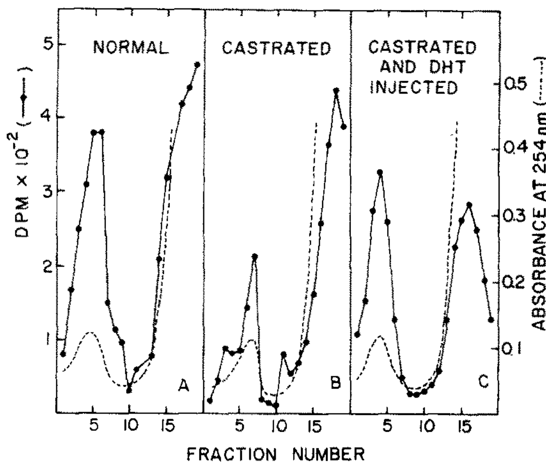

Fig. 2. Effect of androgen *in vivo* on the prostate cytosol initiation factor EIF-1 activity. Adult Carworth CFE male rats (300 g body weight) were used. Two groups of rats were castrated and, 16 h later, one group was injected i.v. with 20 μg of 5α-dihydrotestosterone (in 0.1 ml saline containing 10% ethanol) and another with the vehicle solution alone, 10 min before they were killed. The cytosol preparations were obtained from the normal control (A), castrated (B), and castrated and androgen-treated (C) rats by the method described previously [9]. The preparations were assayed for their ability to promote [^{35}S]met-tRNA$_f$ binding to the 40 S prostate ribosomal particles from the normal rats. The assay medium (0.3 ml) contained 300 μg cytosol proteins, 0.6 A_{260} unit of 40 S ribosomal particles, 404,000 c.p.m. [^{35}S]met-tRNA$_f$ (0.9 ρ mol) in 5.9 μg liver tRNA, 2 mM GTP, 5 mM mgCl$_2$, 25 mM KCl, 3 mM dithiothreitol, 10 μg poly (A.U.G.) and 20 mM Tris–HCl, pH 7.5. The mixture was incubated at 30°C for 30 min and fixed with 5 μl of 25% glutaraldehyde (adjusted by IN NaOH to pH 6). Two tenth ml of the fixed sample was layered on the top of a linear 5–20% sucrose gradient medium containing 5 mM MgCl$_2$, 25 mM KCl, and 20 mM Tris–HCl, pH 7.5 and centrifuged at 54,000 rev./min for 110 min in a Beckman swinging bucket rotor No. 56. Fractions (0.2 ml each) were analyzed for absorbancy at 254 nm and for the radioactivity that could be retained on Millipore HA filters [9]. The fractions were numbered from the bottom of the centrifuged samples. The 40 S ribosomal particles sedimented near fraction number 5.

shown that the androgen effect can be detected by measurement of the formation of the initiator complex in the absence of prostate ribosomal particles. Thus, the androgen effect appears to be on the cytosol initiation factor EIF-1 that can bind the initiator tRNA.

In our laboratory, Dr. E. Castañeda has shown that this rapid effect was not affected significantly by high doses of actinomycin D (200 μg/100 g body weight) and cyclohexamide (10 mg/100 g body weight), which can inhibit the incorporation of [^{3}H]-uridine into RNA by 75% and that of [^{3}H]-leucine into proteins by 95%, respectively in experimental animals. This observation, as well as the rapidity of the response, suggest that the androgen effect may not necessarily depend on the new synthesis of RNA or proteins. Since cyproterone acetate (3 mg/100 g body weight), a potent antiandrogen that can inhibit receptor binding of androgens in the prostate, can prevent this rapid response, the androgen–receptor complex may be involved in this hormonal effect; other possibilities, however, cannot be excluded.

If the androgen effect is independent of the synthesis of new RNA or protein, the hormone-induced alteration in the initiation factor (EIF-1) activity may be dependent on the modification or interaction of the existing factors that affect the activity in the prostate cells. During the purification of the prostate initiation factor, we found that certain macromolecular fractions are inhibitory whereas others are stimulatory. The nature and the biological significance of these effectors are not clear. By Sephadex gel filtration of the prostate preparations, it is possible to separate

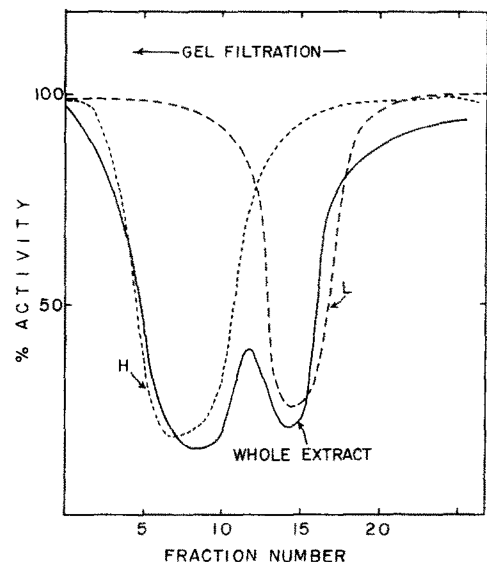

Fig. 3. Inhibition of [^{35}S]met-tRNA$_f$ binding to initiation factor EIF-1 by prostate extracts. Rat ventral prostate was homogenized in 4 vol. of ice-cold water. The homogenate was heated at 90° for 20 min and centrifuged at 15,000 rev./min for 15 min in a Sorvall centrifuge. The supernatant (whole extract) was dialyzed in ice-cold water overnight and the dialysate was concentrated to the same vol as that of the original homogenate. Four ml each of the undialyzed whole extract, the dialyzed (protein H fraction) samples, and the dialysate (prostin L fraction) were individually filtered through Sephadex G-75 gel columns (1.5 cm. × 19 cm.), eluted with ice-cold water, and collected in 2 ml fractions. The binding assay was performed in 0.3 ml of a mixture containing 0.05 ml of the prostate fraction, EIF-1 (10 μg protein) extracted from liver ribosomes [17], 101,000 c.p.m. [^{35}S]-met-tRNA$_f$ (0.3 pmole), 1 μg liver tRNA, 1 mM GTP, 25 mM KCl, 3 mM dithiothreitol, and 20 mM Tris–HCl, pH 7.5. The incubation was carried out at 20°C for 5 min and stopped by dilution of the mixture with the buffered medium containing 25 mM KCl, 3 mM dithiothreitol, 20 mM Tris–HCl, pH 7.5. The diluted mixture was filtered through a millipore HA filter for measurement of the [^{35}S]-initiator complex formed. The percent activity remaining was calculated by comparison with the result of the tube containing no prostate sample.

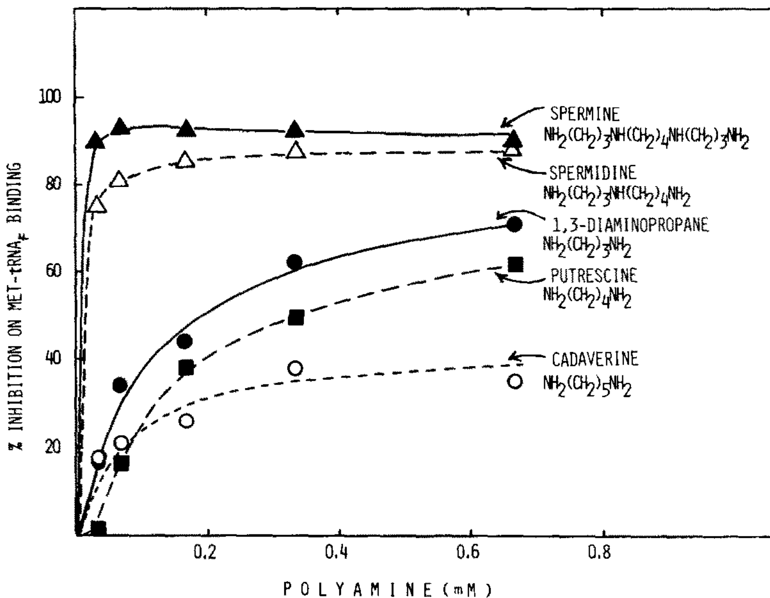

Fig. 4. Inhibitory effect of polyamines on [^{35}S]-met-tRNA$_f$ binding by initiation factor EIF-1. The assay was performed as described in Fig. 3, in the presence of various polyamines at the concentrations shown on the abscissa.

these factors into two groups: a high- and a low-molecular-weight fraction. For convenience, and since the ventral prostate contains the greatest abundance of these factors among many tissues which we have tested, we have called the fractions prostin H and L respectively. The activity of both is also found in dorsal prostate and seminal vesicles but the testis and liver have very low activity. The coagulating gland, spleen, muscle, and blood contain very little or no activity. Both the H and L fractions inhibit initiator tRNA binding by the partially purified prostate or liver initiation factor EIF-1 (Fig. 3), but only the prostin H fraction can significantly enhance the incorporation of [^{35}S]-methionine from initiator tRNA into the acid precipitable protein fraction in the presence of ribosomes. These prostin factors have been purified further by DEAE-cellulose column chromatography.

Both factors are stable when maintained at 90° for 20 min and can be separated from inorganic ions by Sephadex gel filtration. Their action does not appear to be that of degrading the initiator tRNA or GTP. Upon extensive treatment with trypsin, pronase, acid, or alkali, prostin H materials released low molecular weight substances that behaved very much like the inhibitors in the prostin L fraction in regard to activity and fractionation. Under conditions at which DNA and RNA would be hydrolyzed (by nuclease or brief acid and alkali treatment), such a conversion was not observed and the activity of prostin H and L was not affected. The active molecules in the prostin H fraction were not extractable by ether, hexane, or methylene chloride. Thus prostin H appears to be a protein conjugate of a low molecular weight inhibitor.

The above studies also suggest that the inhibitory component (or components) is not a simple oligonucleotide, lipid, or oligopeptide. In fact, it is very likely that polyamines in the prostin L preparation are responsible for the prostin L activity, since the rat prostate is rich in various polyamines [10] that have been found to affect, among others, tRNA structure [11] and protein synthesis [12], including met-tRNA$_f$ binding [13]. As shown in Fig. 4, we found that some polyamines at a concentration below 0.01 mM can inhibit the initiator tRNA binding of the partially purified initiation factor EIF-1 from rat liver or prostate under the conditions of our assay. The inhibitory action of prostins and polyamines may be similar to the effect of Mg^{2+} which, at millimolar concentrations, can dissociate the initiation complex [13, 14]. In our assay system polyamines were active at very low concentrations and demonstrate some structural specificities.

If the inhibitory units are polyamines, the prostin H activity may be due to polyamine-bound proteins. By gradient centrifugation in a 5–20% sucrose gradient medium containing 25 mM KCl and 20 mM Tris–HCl buffer pH 7.5, the prostin H activity was found to associate with three major fractions having sedimentation coefficients of 2 S, 3 S, and 5 S whereas the prostin L activity remained near the top of the tube. These forms were also separable by use of a long Sephadex gel column. Whereas the heterogeneity of the prostin H components may be due to nonspecific association of polyamines to various proteins (including covalent binding by transamidases [15, 16]), it is also plausible to consider the possibility that different prostins are involved in the specific regulation

of the synthesis of different proteins at the initiator tRNA binding step. It should be noted also that the Mg^{2+} and polyamine effect on met-tRNA binding is not observed with a highly purified eukaryotic initiation factor EIF-1, possibly due to the loss of an unknown factor [13, 14]. We have also found that certain prostate cytosol proteins may limit the inhibitory action of prostins and polyamines. It is conceivable that these factors together with prostins and polyamines, may play specific roles in the hormonal regulation of the translational process in mammalian cells.

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DISCUSSION

Clark. Did I understand you to say that the Wistar animals did not respond?

Liao. That's right. So far, only a few groups responded and many others did not.

Clark. Do you think that it's just a preparative technicality?

Liao. I think so, but it is also possible that there are physiological differences between the responsive and unresponsive animals.

Clark. So, now are you saying it might be stress-related or just endogenous?

Liao. Well, we saw more clear response with the adrenalectomized and castrated rats than with the castrated animals. This may be related to the endogenous androgen labels in the experimental animals.

King. Did you say that the receptor complex only binds to the ribosome subunit? If so, what happens when you get a complete ribosome? Does the complex come off?

Liao. The receptor complex does not bind to the 80 *S* monosome or polysomes *at all.* We have thought about the possibility that the receptor complex may play a role in joining the two subunit particles. It is a very interesting hypothesis but has not been proved experimentally.

Spelsberg. In forming your 80 *S* particle where does the messenger come from? Is it endogenous to the particle?

Liao. If we would start from the 40 *S* subunit particles, we would use A_pU_pG containing oligoribonucleotide.

Jensen. Your finding that this phenomenon is inhibited by cyproterone acetate is most interesting. As you say, this suggests but does not prove that the androgen–receptor complex is actually causing the effect. One wonders whether this might represent a non-nuclear action of the hormone–receptor complex. Do other inhibitors of complex formation, such as flutamide, also inhibit the response? Also are you able to demonstrate an effect *in vitro* and to determine whether the cytoplasmic or the nuclear form of the complex is active?

Liao. Dr. Evan Castañeda was working on this particular aspect when she was in our laboratory. She was able to show that flutamide could suppress the androgen effect *in vivo.* We have not been able to show the effect in tissue incubation or cell-free systems.

Crabbé. Dr. Liao, may I ask you what led you to try adrenalectomy to get "better results"? Along the same lines did you ever examine the situation in hypophysectomized animals?

Liao. We did adrenalectomy simply trying to lower the endogenous steroids as much as possible. With the rats responsive to castration, Dr. Castañeda was able to see that hypophysectomy (for 1 day) alone can result in some reduction of the initiation factor activity in the prostate cytosol. Animals hypophysectomized and castrated responded to the same extent as those castrated but not hypophysectomized.

Crabbé. So the animals without gonads and without adrenals behave the way hypophysectomized animals do. Are there significant differences between hypophysectomized animals and animals that have undergone both adrenalectomy and gonadectomy?

Liao. The extent of reduction in the initiation factor activity of the prostate cytosol preparation after hypophysectomy was not as great as that seen after castration. However, we used rats within one day after they were operated on and we do not know what the long term effect of the surgery would be.