Journal of Steroid Biochemistry, 1974, Vol. 5, pp. 1009–1011. Pergamon Press. Printed in Great Britain.

GENERAL DISCUSSION

McKerns:

Unlike steroid hormones, the trophic hormones don't seem to need any binding proteins. We have found some direct effects of trophic hormones on isolated nuclei.

Figure 1 represents RNA synthesis in purified nuclei (McKerns K. W.: *Biochim. biophys. Acta* **349** (1974) 465). There is about 0·3 μg of DNA in each incubation. The two bars on the left represent the response with a high-ionic strength buffer with Mn^{2+} that is 0·4 M with respect to ammonium sulfate. In that buffer system the nuclei synthesize principally messenger RNA. The control contains [U-^{14}C] glucose-6-phosphate and $NADP^+$. Incubation was for 10 min. HCG at picomolar concentrations had quite a marked effect. Nuclei incubated in low-ionic strength buffer and synthesizing principally ribosomal RNA, was also markedly stimulated with picomolar concentrations of HCG (Fig. 2). Nuclei were incubated in the low-ionic strength buffer medium with glucose-6-phosphate and NADP. Here the label is [6-^{14}C] orotate. The toxin α-amanitin is a specific inhibtor of polymerase II associated with mRNA synthesis. α-Amanitin had little effect on the synthesis of rRNA stimulated by HCG. In other experiments it was shown that α-amanitin greatly reduced mRNA synthesis stimulated by HCG in nuclei incubated in the high-ionic strength buffer. In summary, with various experiments, it was shown that the isolated nuclei contain the complete pentose phosphate pathway for the metabolism of glucose-6-phosphate to PP-Rib-P, the complete *de novo* pathways for biosynthesis of purine and pyrimidine nucleotides, as well as the usual templates and polymerase enzymes. LH and HCG stimulate glucose-6-phosphate dehydrogenase in nuclei to provide the rate-limiting substrate PP-Rib-P required for purine and pyrimidine nucleotide biosynthesis. The α and β subunits of HCG have no direct effect, but they were found to inhibit on a mole to mole basis the response to the intact HCG. These experiments were done with corpus luteum tissue from the pig, but we have used also human, cow and rat.

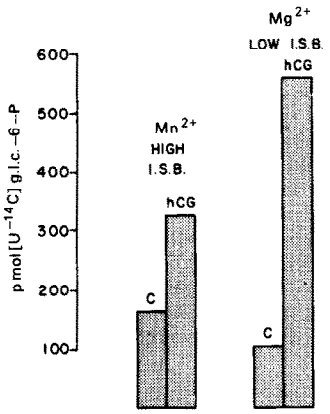

Fig. 1 (McKerns)

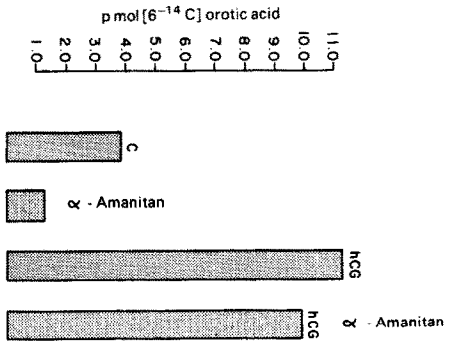

Fig. 2 (McKerns)

Lindner:

I would like to ask Dr. McKerns whether he has evidence that human chorionic gonadotrophin actually penetrates the nucleus of intact corpus luteum cells.

McKerns:

These nuclei have intact membranes and they do respond to gonadotropin. ^{125}I-labelled LH does enter the nucleus. Brig Saxena (Cornell Medical Center) has evidence of penetration of the α and β subunits.

Kuss:

Dr. Schrader has shown some kinetic data on steroid receptor interaction. Is there some explanation for the rather low association rate constant.

Schrader:

In our hands the dissociation constant for binding of hormone to receptor measured by equilibrium techniques is not identical to the constant derived as the ratio of the rate constants. One would tend to trust the equilibrium measurement since it is independent of the pathway of the reaction and to suggest that binding of hormone to receptor is not as simple as we think. One possibility would be the fact that the receptor may be in a conformational state in a cell to which normally hormone does not readily bind. There may be some sort of induced fit or some small population of receptor which will bind hormone; when hormone is present the equilibrium shifts in favour of the binding form and that is the rate limiting step. That is one explanation for it. Another very good possibility is that I have a large artifact in my assocation rate constant, although these studies have been done many times and yield the identical results.

Rousseau:

I would like to ask Dr. Schrader whether his A component of progesterone receptor displays preferential affinity for certain kinds of DNA or whether DNA binding is the same with all DNAs he tried.

Schrader:

We did one study to determine whether there was any difference in binding between chick DNA and other DNAs. In our hands, we found that the receptor bound poorly to a procaryotic DNA, DNA from b-subtilis. That was done a year and a half ago. I haven't gone back and repeated it, but I would say the overwhelming evidence to date is the fact that the binding of the type that we are looking at ($K_d = 10^{-10}$ M) probably will not show DNA specificity. However, we must keep in mind that fact that if our ideas about how genes get "turned on" are in any way correct, something has to be recognizing the nucleotide sequence at particular gene sites.

Jensen:

In the case of the transformed estrogen receptor, we did some experiments a couple of years ago with Geoffrey Zubay, using a number of different DNAs that he had prepared. Once you transformed or activated the estrogen–receptor complex, it bound to all the DNAs, but the one it bound best to was phage DNA. So I would agree with Dr. Schrader that with plain DNA there doesn't seem to be much specificity.

Munck:

I should like to ask Dr. Schrader if it's possible that the A and B proteins might come from different cells within the oviduct?

Schrader:

That's a good question. We studied that by looking at the ratio of A protein to B protein during differentiation of the oviduct. The undifferentiated oviduct consists of essentially a homogeneous population of undifferentiated epithelial cells. It differentiates into a highly structured organ containing many cell types. The ratio of the two proteins remains constant during the experiments.

Jensen:

I would like to ask Dr. Schrader if you take your A complex, your B complex and a mixture of the two and sediment these in sucrose gradients, what do you see?

Schrader:

No re-combination to form an 8S material whatsoever so far in our hands.

Jensen:

But do the A and B complexes run the same?

Schrader:

Yes, A and B sediment identically. I haven't tried the two materials purified on sucrose gradients. I have run them on agarose. I can show you the agarose profile if you'd like to see it.

Jensen:

No, I just wondered if you put them together whether they run the same.

Schrader:

are separated on SDS-polyacrylamide gel.

Mousseron-Canet:

Did you obtain some data concerning the isoelectric point of your progesterone receptors?

Schrader:

We reported an isoelectric point of 4·5 for that molecule. The isoelectric point was a heterogeneous pattern between pH4 and pH5. The progesterone receptor complex, unlike the androgen receptor protein is not stable at its isolelectric point and the steroid comes off the receptor at below pH6·0. By the time you get to the isoelectric point you haven't got very much of the receptor left. Until we have an independent assay for the receptor protein itself I think that the isoelectric point I found when I did the experiment could change.

Jensen:

Your substances are more acidic than some of the other receptor proteins.

Schrader:

I'd like to ask Dr. Vorob'ev who reported some very interesting studies on RNA. Could you explain to us the conditions of the hybridization reactions that you used. This is most critical to the interpretation of hybridization whether or not you have faithfully reconstructed DNA–RNA complexes.

Vorob'ev:

We used a DNA-driven reaction to characterize the RNA population. We carried out hybridization in the presence of excess DNA in 0·3 M NaCl, 0·03 M Na citrate at 68°C. We used Tetrahymena pyriformis RNA as a control in all hybridization experiments. For competition hybridization we selected appropriate time and DNA/RNA ratio to reveal hybridization specificity. Hybrids were treated with RNAase.

I think that one of the main questions is what happens after hormone–receptor complexes are bound to chromatin. I would like to mention our experiments made with Dr. I. Konstantinova on chromatin preparations which we isolated from livers after injection of cortisone. We compared chromatin from normal animals and chromatin from animals which were treated by cortisone. We isolated chromatin and transcribed RNA from this preparation and then isolated the product of transcription, RNA, and used this RNA in molecular hybridization experiments.

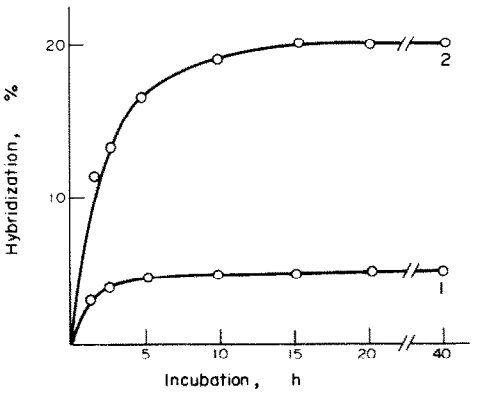

Fig. 1 (Vorob'ev)

Hybridization experiments revealed a higher relative content of RNA molecules synthesized from repetitive nucleotide sequences of DNA during *in vitro* transcription. On Fig. 1 we can see the percentage of hybridization on the

ordinate against hours of hybridization of RNA transcribed from chromatin. The first curve refers to normal chromatin and the second curve to chromatin activated by cortisone. One can see an increase in hybridization capacity meaning that cortisone activated specifically repetitive sequences of DNA in chromatin. If you compare in competitive experiment (Fig. 2) the properties of RNA which has been transcribed *in vitro* with RNA synthesized *in vivo* after cortisone administration, you can see that they are similar in their hybridization properties. The liver chromatin preparations used as templates in this cell-free system retain their ability to support synthesis of RNA molecules specific for the hormone-activated state of liver cells. Further experiments were undertaken to elucidate the question of what protein fractions must be present to maintain the hormone-activated state of chromatin revealed in these experiments. We extracted certain protein fractions from chromatin with NaCl solutions of different molarities. Such protein-depleted chromatin samples were used in our experiments as templates for *in vitro* RNA synthesis. It is known, that with the extraction of chromatin with 0·6–0·7 M NaCl, lysine-rich histone, F1, and a considerable amount of non-histone proteins are removed and among them most of the hormone–receptor complexes. In the first series of experiments we used chromatin which was depleted in proteins extracted in 0·7 M NaCl.

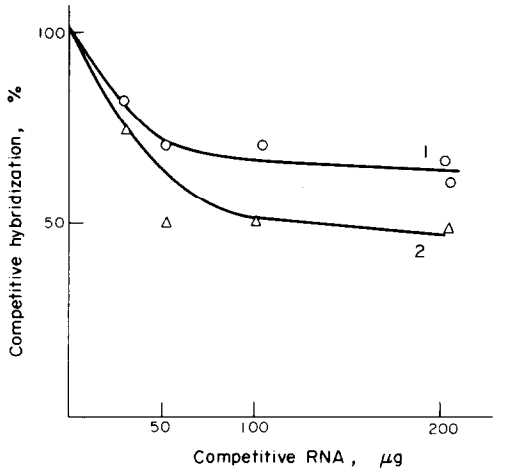

Fig. 2 (Vorob'ev)

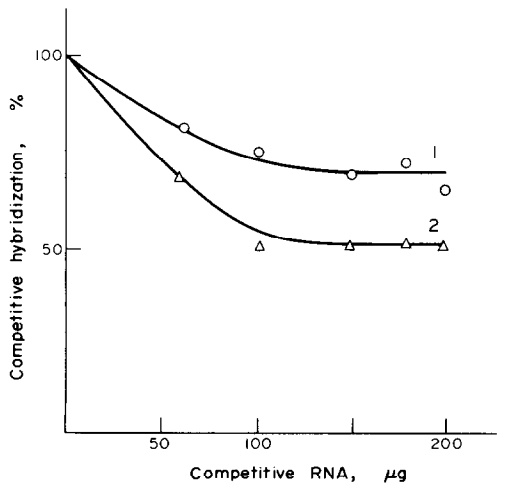

Fig. 3 (Vorob'ev)

Figure 3 presents the competitive hybridization experiments with RNA synthesized on such protein-depleted chromatin template. We used labelled RNA transcribed *in vitro* and for competition we used unlabelled RNA which was extracted from liver cells. Curve 2 shows competition with RNA liver cells from the animals which received cortisone and curve 1—with RNA from normal animals. You can see a difference which means that there is variation in RNA population. This difference is the same as that observed when we compare RNA transcribed from normal chromatin. So we can say with certainty that the protein which was extracted in this experiment is not indispensable for the maintenance of hormonal specificity of chromatin as a template.

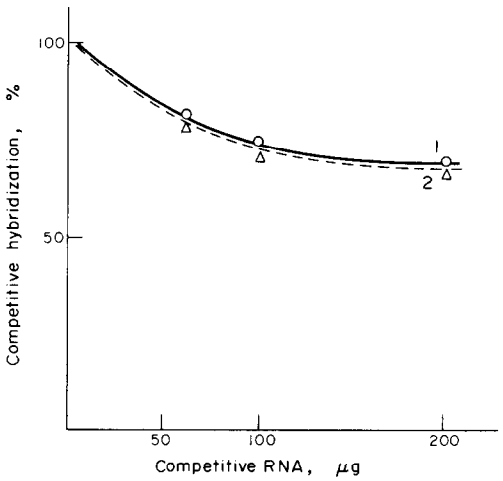

Fig. 4 (Vorob'ev)

Figure 4: These changes disappeared completely after the treatment of chromatin with 1 M sodium chloride which means that the presence of protein fractions removed from chromatin with sodium chloride in the range of 0·7–1 M is required to maintain the hormone-specific pattern of transcription. It is known that histone fractions F2α and

removed from chromatin in this range of molarity. Now we are trying to isolate these non-histone proteins to elucidate the role of these proteins in template specificity of hormone-activated chromatin.