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HORMONE-INDUCED TRANSFORMATION OF RECEPTOR PROTEINS*

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During the course of steroid-induced translocation to the nuclei of their respective target cells, extranuclear receptor proteins for estrogenic and androgenic hormones undergo temperature-dependent alteration that can be recognized both by a change in sedimentation properties and the acquisition of an ability to bind to isolated nuclei or chromatin. Warming the uterine cytosol receptor with estradiol increases the sedimentation coefficient of the complex from 3·8 S to 5·3 S, whereas similar incubation of the prostatic cytosol receptor with dihydrotestosterone decreases the sedimentation coefficient from 3·8 S to 3·0 S; in each case the transformed complex resembles that extracted from target cell nuclei after hormone administration *in vivo*. Treatment of isolated uterine nuclei with transformed estradiol-receptor complex not only increases their RNA polymerase activity but also changes the nearest neighbor frequency spectrum of the RNA produced, in the same manner as does estradiol administered *in vivo*. Transformed estrogen-receptor complex from calf uterine nuclei has been isolated in a form that is homogeneous both on gel electrophoresis and on analytical ultracentrifugation; the aminoacid composition of this apparently pure receptor protein, which we have named estrophilin, has been determined, and rabbits have been immunized for the preparation of specific antibodies.

DISCUSSION

McKerns:

I was very interested in your experiment on incubation of isolated nuclei with your transformed receptor estrogen complex. Your analysis of the type of RNA showed it to be predominantly ribosomal, indicating presumably that the complex went to the nucleolus rather than to the cytoplasm of the nucleus. I was not clear about your buffer systems. Was this one that would favour ribosomal RNA or have you tried both the high and low ionic strength ones?

Jensen:

You mean the assay of the polymerase activity? This is carried out with magnesium under low salt conditions that would favour so-called polymerase I. It isn't a simple situation, however. If it were just polymerase I being stimulated, it should be insensitive to α-amanitin. It turns out, both in our hands and those of others who have studied this, that about 30% of the stimulation is sensitive to α-amanitin and 70% is not. One of the questions we are trying to answer is whether one or both of these polymerases are stimulated preferentially and whether there is a sequential type of response, similar to the finding by Glasser and associates that, *in vivo*, polymerase II is stimulated first and then polymerase I. Maybe we are not looking early enough. We are using 30 min of incubation with the nuclei. We should really see what happens after 5 min.

Kolpakov:

I should like to ask about a special protein factor. Is there really a factor that promotes transport of the steroid complex into nuclei or not? Some years ago in the literature I read that there is a special protein factor which promoted transport of the steroid complex into the nuclei. What is your opinion about that mechanism?

Jensen:

I don't know about this report, and we have no evidence that there is a factor necessary to promote the translocation of the complex.

Crabbé:

I'd like to ask whether according to you there is such a thing as a shuttle for receptors moving, after being loaded with steroid, from cytoplasm to the nucleus and then being available again once they have moved back to the cytoplasm after being unloaded.

Jensen:

We know little or nothing about how the receptor gets out of the nucleus or even how the hormone gets out. There are two schools of thought. Some feel that there may be metabolic conversion of the estradiol in the nucleus to something that doesn't bind as tightly, perhaps estrone. We don't find any estrone there because it goes out right away. My own feeling is that the transformed estrogen receptor complex comes into the nucleus and binds to some macromolecule in the nucleus called an "acceptor". This association causes a change in the conformation of the receptor protein, decreasing its affinity for the steroid and resulting in hormone release. Whether the steroid leaves the receptor protein in the nucleus and comes out to encounter a new receptor molecule and repeat the process or whether the receptor leaves the estradiol in the nucleus and then moves out to be recycled are interesting speculations. Our present state of knowledge does not permit any firm conclusions.

Vorob'ev:

Have you evidence for the action of your purified receptor

*The full text will be published soon in this Journal.

on isolated chromatin? Have you tried to add purified protein to isolated chromatin and then to study its transcriptional activity?

Jensen:

So far we don't have a good answer with the highly purified material. The partially purified, transformed receptor is still quite active when incubated either with whole nuclei or with isolated uterine chromatin which is then used as template with the polymerase enzyme extracted from the nuclei.

Ritzén:

It is known from other systems, especially cell hybridization experiments where in effect one nucleus can be placed into the cytoplasm of another cell, that when a dormant nucleus is activated there's a tremendous influx of all kinds of cytoplasmic proteins into the nucleus. Do you have any information about other proteins besides the cytoplasmic receptors, that move from the cytoplasm to the nucleus, and is there any quantitation of the total amount of cytoplasmic soluble proteins that enter the nucleus when the cell is activated?

Jensen:

This is a good question. The only protein one can follow going into the nucleus is the receptor protein by using the radioactive steroid as the marker. I don't know if I quite understand your question on quantitative correlation.

Ritzén:

Just to quantitate the total amount of protein that is transferred from the cytoplasm into the nucleus to see whether the receptor might be half a percent or 1 ‰, etc., of the proteins that enter the nucleus from the cytoplasm. Is there a specific transport mechanism for the receptor or is it just one of the proteins that are sucked into the nucleus by the nuclear sponge as it increases?

Jensen:

I'm sure that this kind of study could be done but it hasn't been yet. You have to have some way of finding the protein in the nucleus, if only tiny amounts. Perhaps fluorescent antibodies would be a possible way.

De Hertogh:

I would like to come back to the previous question regarding the flux of steroids in and out of the cell and of the nucleus. In a physiological situation, we may consider that the uterus is submitted to a continuous perfusion of estradiol, at a rate which is dependent on the plasma concentration. The latter, as far as we know in the rat, is changing rather slowly (in terms of several hours), so that a state of equilibrium may exist between plasma and tissue levels.

Such an equilibrium can be experimentally obtained by long term infusion of labeled hormone to the animal (De Hertogh *et al.*, *Endocrinology* **88**, (1971) 165–174). In such a steady state situation, there is a continuous influx of labeled hormone from the blood to the cell, and from the cytosol to the nucleus.

This can be shown by chase-experiments, in which [^{3}H]-estradiol is replaced by ^{14}C-estradiol in the infusion fluid, after the steady state has been obtained with the former label. In this case, the ^{14}C-hormone replaces the 3H-hormone progressively, without modification of the total hormone concentration in the tissue. This process is an active one and involves all subcellular fractions, implying a recycling of the estrogen from nucleus to the cytosol. (De Hertogh *et al.*, *J. steroid Biochem.* **4**, (1973) 289–320).

Whether this turnover of the steroid moiety is accompanied by a similar turnover of the receptor protein is another story.

In mouse fibroblasts, Ischii *et al.* (*Biochemistry*, **11** (1972) 3896–3904) have suggested that there was a turnover of triamcinolone acetonide binding protein from cytosol to nucleus and back from nucleus to cytosol. This replenishment of cytosol binding protein took place in the absence of protein synthesis. What do you think about the uterus?

Jensen:

I agree with you that, as far as the steroid goes, the turnover is much more rapid than we had originally assumed from the overall uptake and retention curves *in vivo*. When one compares the depletion of the cytosol receptor after a physiologic dose of the hormone with the amount of estradiol present in the nucleus at the point of maximum depletion, one finds that 5 times as much receptor has disappeared as there is estradiol present in the nucleus. This is what suggests to us that the estradiol may be re-cycling. I don't know about the receptor recycling in the uterus.

Kellie:

If we consider that the cytosol receptor is a ferry carrying estradiol across to the nucleus, this could explain how estradiol stimulates the nucleus. If you now suggest the converse, namely that the purpose of the estradiol is to conduct the cytosol receptor into nucleus you are then faced with the difficult problem of how the cytosol receptor is replaced. Could you comment on that?

Jensen:

I think the cytosol receptor is re-synthesized rather rapidly, and the depletion of the cytosol receptor by its translocation to the nucleus is a stimulus to re-synthesis. Our original experiments, and subsequent more extensive studies by Sarff and Gorski demonstrate that the administration of cycloheximide will prevent the restoration of the cytosol receptor level that ordinarily follows estradiol-induced depletion. In Dr. de Hertogh's case, where he has a continuous infusion of estradiol, I think the receptor is being continuously resynthesized.

Kellie:

Some years ago we measured the cytosol receptor concentration in the rat uterus and we were able to show that throughout the estrus cycle the concentration varied quite substantially.

Jensen:

Whether one likes to think of the receptor bringing the hormone in or the hormone bringing the receptor in, at this stage of knowledge one is entitled to his choice. If the receptor serves only to transport the hormone into the nucleus, one is then left with the question of how the estradiol exerts its action now that it is there. In that case, it must act with another receptor. There have been some who say the "real receptor" should be present in small amounts, only 10 molecules or so per nucleus, rather than the several thousands that come in from the cytoplasm. We prefer to think that the hormone brings the protein in, changing it to an active form. Our working hypothesis is that the transformed receptor protein is a modulating factor in the whole RNA polymerase system, perhaps analogous but probably not identical with the modulating factors that have been elucidated for RNA polymerase systems in procaryotic cells, that enables the transcription system to operate in an optimal manner.