stantiated by their differing ability to activate RNA polymerases and replenish the cytosolic estradiol and progesterone receptors. Both estrogens, estradiol and moxestrol, increase the number of polymerase molecules involved in transcription and also increase chain elongation, whereas the antiestrogen, RU 16117 would appear to have an inhibitory effect.

11. Microfluorimetric method to detect specific oestradiol receptors in different cell types or cell suspensions from target tissues, D. BECCATI, G. LANZA, I. NENCI and A. PIFFANELLI, Istituto di Anatomia Patologica, Universita di Ferrara, 44100 Ferrara, Italy

Fluorescent antibody localization of oestradiol-17β by immuno-fluorescence sandwich technique has been developed and applied to the demonstration and quantitation of specific oestradiol receptors in different cell types on cell suspensions from target tissues. Using this method the quantity of high affinity receptors occupied or unoccupied by endogenous oestradiol can be measured. Methodological details are given.

So far it has been possible to investigate, *in intact cells,* the temperature-dependent two-step mechanism suggested for the interaction of oestradiol with target cells, the intranuclear attachment and, lastly, the fate of oestradiol–receptor complexes.

Moreover, applying this technique to the study of cell populations from breast and endometrial tumours it is possible to differentiate the heterogenous cell populations containing cytoplasmic receptor within a single tumour.

The quantitation of these hormone-independent mixed cell clones might be useful to predict the response of each tumour to endocrine therapy.

Defects in the transformation and subsequent translocation of cytoplasmic receptor or in its final binding to chromatin (which may well occur in the presence of abundant cytoplasmic receptors) have also been revealed in these preparations of intact cells.

D. Biosynthesis of steroid-hormone receptors, A. HUGHES, P. SZENDRO, CECILIA TERAN, JANET KIELHORN, W. SIERRALTA, G. STONE, M. LITTLE and P. W. JUNGBLUT, Max-Planck-Institut für Zellbiologie, D 294 Wilhelmshaven, Postfach 1009, Germany

The responses of tissues to steroid hormones are mediated by interaction with specific cytoplasmic receptors and subsequent transfer of the steroid–receptor complexes into the nucleus. In consequence, a depletion/replenishment cycle of cytoplasmic receptors is a common phenomenon in the course of action of every steroid hormone. This cycle has been studied for the soluble cytoplasmic estradiol receptor in rat uteri, and for both the soluble and structure-bound receptor in pig uteri [1].

Following the injection of estradiol into ovariectomized, estrogen-primed rats (4 × 1 μg E-2 in oil, every second day, last injection 3 days before experiment), there is a rapid fall in estrogen receptor levels (depletion), compared to control values, followed at 5–6 h after injection by a replenishment phase. Such a response follows a subcutaneous, intraperitoneal or, in the present case, an intrauterine injection of estradiol. This last technique involves injection into the uterine lumen *via* the cervices. The bi-cornuate structure of the rat uterus allows for a treated and a control horn in the same animal. Actinomycin D-injected 2 h earlier or together with estradiol, had no effect on the control (zero time) levels or on the initial depletion step, but definitely inhibited, although not completely, the replenishment phase. Puromycin, injected 2 h before estradiol, induced a small drop in the zero time receptor levels, but had no effect on the replenishment phase unless injected 5 h after estradiol, resulting in a temporary arrest of the replenishment phase. Both sets of experiments show that replenishment does represent resynthesis. The puromycin-induced fall in control levels further indicates that receptor synthesis persists in animals which are deprived of their main hormonal sources. This latter point was confirmed by a 3 months study of uterine estradiol receptor levels in ovariectomized or ovariectomized/hypophysectomized rats kept under controlled conditions [1]. Not only was estradiol receptor present throughout this period, there were also considerable, but irregular fluctuations in receptor levels. Similar results were obtained with ovariectomized/adrenalectomized rats.

The response of the pig uterine soluble cytoplasmic E-2 receptor to estradiol is similar to that of the rat. Following the extraction and characterisation of specific estradiol receptors from pig uterine microsomes, the existence of a biosynthetic receptor sequence became apparent [1]. Uterine microsomes, extracted with low ionic strength buffer containing estradiol, and analyzed by density gradient centrifugation and agargel electrophoresis, contain a "basic" 3·5S receptor and an "acidic" 4·5S receptor. The interrelationship of these receptor forms with the soluble cytoplasmic receptor was established following a series of experiments using the intrauterine injection technique via the cervical route. Ovariectomized, pre-treated pigs, with one horn disconnected from the corpus uteri (control horn), were injected with estradiol and then killed at various times after injection, when the levels of the microsomal and cytosol receptor were determined. The soluble receptor, as expected, showed an initial rapid depletion. Replenishment started about 5 h after injection. In contrast, the concentration of basic 3·5S receptor extracted from the microsomal fraction rose steeply between 60 and 90 min after the intrauterine application of estradiol. The control horn levels of all receptors are, by comparison, unchanged. The data from these and similar experiments strongly suggest that the basic 3·5S receptor is an early product of receptor biosynthesis.

This small receptor can be dimerised to a basic 4·5S molecule on warming. Both this dimer and the extracted acidic 4·5S molecule are reversibly dissociated by protonation into 3·5S basic and 3·5S acidic subunits respectively, the latter subunit probably being the authentic *in vivo* component, which dimerises during the extraction procedure [2]. The formation of stable dimers requires the presence of estradiol. Comparison of these data for the microsomal receptor with those available for the soluble cytoplasmic receptor forms shows certain similarities. Uterine cytosol extracts, extracted at pH 7·5 with low ionic strength buffer, and run in gradients containing buffered 0·4 M KCl, display only acidic receptors sedimenting at 4S and 5S. The radioactivity sedimenting at 5S can be shifted to the 4S position by protonation, the reverse shift is accomplished by proton withdrawal and accelerated by warming. Similar to the estradiol requirement for the formation of stable microsomal 4·5S dimers, stable cytosol 5S-estrogen complexes are derived from estradiol charged 4S molecules but not from estrone complexes. On the basis of such evidence, the major receptor biosynthetic pathway *in vivo* is presumed to be: basic microsomal 3·5S → acidic microsomal 3·5S → cytosol 4S → cytosol 5S. The commonly observed 8–10S cytosol receptor is thought to be a storage form, which can be activated when necessary. Although the bio-

synthetic sequence just proposed is derived from data on the estrogen receptor, the discovery of microsomal receptors for DHT and progesterone strongly indicates that it is generally applicable to all steroid hormone receptors [2].

Concerning the regulation of receptor biosynthesis and its integration into a scheme of steroid hormone action, some observations can be made. On the basis of results from both the rat and pig experiments, we believe that the receptor is used once only, and that no recycling is involved. Only 10 min after estradiol injection can any heat-exchangeable estradiol-receptor complexes be detected in the soluble phase. These can be accounted for by the small amounts of estradiol still left in the injected solution, which on homogenization occupies free receptor sites *in vitro*. Accordingly, 30 min after injection, when the residual intraluminal fluid is free of estradiol, no exchange is observed. At this same time point, more than 80% of the total uterine estradiol content is found in the particulate ("nuclear") fraction, indicating that *in vivo* newly formed estradiol-receptor complexes are immediately transferred to the nucleus.

Converging lines of evidence – deriving from the receptor biosynthetic receptor sequence and from the *in vitro* interconversion studies [1, 3, 4] – suggest that the (soluble) 5S hormone–receptor complex is the active component *in vivo*. Whether this complex arises by dimerisation of the 4S-hormone–receptor monomer or by addition of a non-hormone binding entity to the 4S unit is still unsettled [1, 4, 5]. The latter possibility is difficult to envisage in view of the fact that the *in vitro* conversions between small and large forms of both the soluble and the structure-bound receptors apparently involve the same mechanisms.

Despite extensive investigations, the reactions underlying the enhancement of transcription by the active steroid receptor complex have yet to be elucidated. Any possible mechanism of action must cope with the simultaneous occurrence of individual receptors for various steroid hormones within a single cell [6].

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12. Estradiol receptor translocation from cytoplasm to nucleus in the embryonic chick mullerian duct cell, C. S. TENG and C. T. TENG, Department of Cell Biology, Baylor College of Medicine, Houston, Texas, U.S.A.

Sex steroids are involved in the normal development of embryonic Mullerian duct (Md). To what extent does steroid hormone effect Md development is not well known. In order to understand the steroid-tissue interaction at the molecular level, an estradiol (E_2) receptor has been isolated from the cytoplasm of 10–15 day embryonic female chick left Md. The embryonic cytoplasmic E_2 receptor has characteristics similar to those of other sex organs previously described. This communication reports that after *in vivo* chorioallantoic injection of E_2 (10–40 μg E_2/egg) to the 15 day chick embryos for 2 h, 45–95% of the initial concentration of E_2 receptor in the cytoplasm of the chick Md cell were translocated into the nucleus. The process of translocation is dependent on the amount of E_2 administered *in vivo*. At 6 h after *in vivo* E_2 administration about 30% replenishment of the initial content of the cytoplasmic receptor was observed in the cytoplasm. The *in vivo* non-radioactive E_2 exposed Md nuclei could exhibit saturable exchange with [^{3}H]-E_2 *in vitro*. The optimal condition for exchange is at 37°–41°C for 1–2 h. The [^{3}H]-E_2 receptor complex extracted from the exchanged nuclei has the sedimentation coefficient of 5–6S, and its isoelectric point is 6·8. The nuclear E_2 binding sites of the developing Md cell were calculated to be 1·66, 2·22, 2·63 and 2·50 pmol/mg DNA (or approximately 2500, 3300, 4000 and 3800 sites/nucleus), the corresponding dissociation constants are 3·0, 3·1, 3·1, and 3·0 nM, for the developmental stages of the 10th, 12th, 15th, and 18th-day embryo respectively. In summary we conclude that: (a) E_2 receptor translocation from cytoplasm to nucleus does take place in the embryonic sex organ. (b) The translocation is dependent on the concentration of the administered E_2 (c) The number of E_2 binding sites in the nuclei increase linearly from day 10 to day 12 of incubation, then level off from day 12 to day 18 of incubation. (Supported by NIH Grant HD-08218-03).

13. Post-transcriptional nuclear control of protein synthesis by progesterone, P. TUOHIMAA and E. SÖDERLING, Department of Biomedical Sciences, University of Tampere, SF-33520, Finland

Avidin is secretory protein of avian oviduct. It is induced by progestagenic compounds. In this study the nuclear and cytoplasmic mRNA activities coding for avidin has been studied up to 24 h after the administration of progesterone. One-day-old immature Leghorn chicks were injected daily with 0·5 mg of diethylstilbestrol in order to stimulate the oviducts to grow. On day 10 they were injected with 5 mg progesterone. Oviducts of 30 animals were pooled for each RNA extraction. Nuclear pre-mRNA was isolated with a sucrose gradient centrifugation, salt and phenol extraction (Georgiev, G. P. & Samarina, O. P., in *Advances in Cell Biology* 2:47, 1971). Cytoplasmic mRNA was isolated with an antibody precipitation of the avidin polysomes. Thereafter the polysomal mRNA was extracted with phenol in the presence of a low salt concentration and high pH (pH 9·0) (Brawerman *et al., Biochemistry* 11 637, 1972). The messenger activity of pre-mRNA and polysomal mRNA was tested in a cell-free system derived from rabbit reticulocytes. The injection of progesterone clearly increases the messenger activity for avidin in the polysomal mRNA fraction. On the other hand, we can find some avidin mRNA activity also in the immature, untreated chick oviducts. Also nuclear pre-mRNA for avidin can be translated in a heterologous cell-free system. However, the messenger activity of nuclear RNA shows changes opposite to that of the polysomal RNA. The avidin coding activity of pre-mRNA decreases to almost zero at 2 h after the administration of progesterone. Concomitantly with the changes of avidin coding activities of nuclear and cytoplasmic RNA there is an enhancement of ribonuclease T_2 activity in the nucleus and cytoplasm. The present results suggest that the transfer and cleavage of the nuclear pre-mRNA may be a consequence of ribonuclease activation and might be a locus of non-transcriptional control of protein synthesis.