simple manner here, but it uses what we call exchange forces to generate the saturation. The quarks must come in three varieties, red, blue, and yellow. The force has the effect of exchanging colors. The mathematical theory of this is rather simple. In fact the equations for such a theory were written down over 20 years ago by Yang and Mills, who saw no application of them but published them because they looked so beautiful and symmetrical. With this theory there are eight kinds of gluons (depending on which pairs of colors they exchange). The saturated states are those which have no net color, that is, which are completely neutral as to color. A single quark or two quarks could not be neutral, but a group of three is indifferent to color if one is red, one blue, and one yellow.

They must be in just the condition we need to explain how three u quarks can appear to be in the same state in spite of the exclusion principle.

We have been led by two different arguments to this need for colored quarks.

If experiments continue to confirm the need for quarks in protons, this is the way the theory will apparently develop: quarks of three colors, so nine in all, and eight kinds of gluons. This part sounds elaborate but is mathematically simple. And a long range force—which sounds simple but appears mathematically a bit unnatural. Suggestions to explain this long range force, such as Kauffmann's, all seem a little awkward and without an inner beauty we usually expect from truth. But sometimes the truth is discovered first and the beauty or "necessity" of that truth seen only later. At least it seems now we have a very good guess to work on.

Beside our eight gluons and nine quarks there would still be the electron, muon, photon, graviton, and two neutrinos, so we would still leave a new proliferation of particles to be analyzed by the next generation. Will they find them all composed of yet simpler elements at yet another level?

Female Steroid Hormones and Target Cell Nuclei

The effects of steroid hormones on target cell nuclei are of major importance in the induction of new cell functions.

Bert W. O'Malley and Anthony R. Means

Studies designed to elucidate the sequence of events responsible for steroid hormone effects in endocrine target cells have led many investigators to consider the nucleus as the primary site of hormone action. Numerous experiments have supported the suggestion that steroid hormones regulate cell function by influencing the synthesis of proteins in the target tissue (*1–5*). In most instances, the stimulation of such protein synthesis by steroid hormone is preceded by quantitative and often qualitative changes in the synthesis cellular RNA. Stimulation of nuclear, rapidly labeled heterogeneous RNA followed by increased production of ribosomal RNA and often transfer RNA are frequently observed effects on RNA metabolism (*6–7*). It is likely, but has not been proved, that the messenger RNA (mRNA) of animal cells is a component of the giant heterogeneous nuclear RNA. Additional support for a primary effect of steroids on nuclear gene transcription is provided by the ability of actinomycin D and other inhibitors of RNA synthesis to block most steroid hormone–mediated cell responses. General theories in which stimulation of mRNA is regarded as the primary event should not be overestimated, however, since some evidence suggests other possibilities (*8*). On the other hand, recent experiments have conclusively demonstrated that steroid hormones are capable of inducting a net increase in specific mRNA molecules in target cells (*9–12*).

If steroids do in fact regulate nuclear gene transcription, certain considerations should be compatible with such a theory. There must be a mechanism for limiting the steroid-induced response to target tissues. There should be a defined sequence of events which results in the transport of a steroid molecule to its presumed nuclear site of action following penetration of the target cell membrane. The existence of mediators or "second messengers" must be delineated. The steroid hormone itself or an intracellular mediator should be capable of interacting at certain predetermined sites in the nucleus prior to alterations in DNA transcription. Changes in nuclear RNA synthesis should finally result in a net increase in the amounts of specific mRNA's, which should, in turn, be limited to steroid hormone target tissue and inducible by only the steroid in question. Increases in the intracellular concentrations of these mRNA molecules should precede fluctuations in the rate of synthesis of the corresponding specific proteins. Over the past decade many experimental data relating to these theoretical considerations have accumulated. In this article we summarize the evidence favoring our prejudice that the target cell nucleus is a major determinant in steroid hormone induction of new cell functions. Our discussions are limited primarily to the activity of estrogen in the rat uterus and chick oviduct and the activity of progesterone in the chick oviduct because most experimental data on mechanisms of female sex steroid action emanate from these model systems. However, the generality of these observations as applied to mechanisms of action of all steroid hormones has been recently reviewed (*13–15*).

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Steroid Hormone Receptors

The concept of steroid hormone "receptors" initially resulted from studies by Jensen and Jacobson (*16*) in which physiologic amounts of radioactive estradiol were injected into immature rats. It was noted that target tissue alone was capable of retaining 17β-[^{3}H]estradiol against a marked concentration gradient with blood. These observations were confirmed by both biochemical and autoradiographic methods (*17*), and were eventually extended to numerous vertebrate species (*14*). Upon homogenization of uterine target tissue, a soluble protein capable of binding [^{3}H]estradiol was shown to exist in the cytoplasm (obtained in the supernatant fraction after centrifugation at 105,000*g*) by Toft and Gorski (*18*). This binding protein was considered to be a "receptor" because of its limitation to estrogen target tissue, its high binding affinity for estrogens (dissociation constant, $K_d \sim 10^{-10}$), and its specific attraction for only biologically active estrogens, either naturally occurring or synthetic. The estrogen binding molecule is heat labile, nondialyzable, and precipitable with ammonium sulfate. When homogenates of uterus were incubated with various enzymes, the molecular binding was destroyed by proteolytic enzymes but not by ribonuclease or deoxyribonuclease. This result suggested that at least the active molecular binding site might be protein in nature.

Upon ultracentrifugation in a sucrose gradient, this cytoplasmic hormone-protein complex sedimented as a discrete band at about 8*S* (*18, 19*). Addition of 0.3*M* potassium chloride to these gradients resulted in the reversible transformation of this 8*S* complex to a more slowly sedimenting 4*S* form (*20*). Thus the cytoplasmic receptor appeared to contain a 4*S* estradiol binding unit which under conditions of low ionic strength underwent association with other binding entities to form an 8*S* complex. Recent experiments have indicated that in the presence of ionic conditions in the presumed physiological range, an intermediate 6*S* form may predominate (*21*). In any event, it appears that the sedimentation behavior can vary in relation to concentration and ionic conditions and, although probably artifactual, it is a useful and reliable method for identifying steroid binding proteins (*22*). At present there is no way to determine the exact size or configuration of a receptor in its natural state in the unbroken cell.

The generality of the estrogen receptor has been demonstrated by a variety of studies in which vagina, mammary gland, pituitary, and hypothalamus have been used, such tissues being obtained from the rat, mouse, human being, and other species (*14*).

The chick oviduct, a specific target tissue for progesterone, exemplifies a similar type of interaction with progestational steroids. When a chick treated with estrogen was subsequently injected with [^{3}H]progesterone, the major fraction of labeled steroid was detected in the cytoplasm and nucleus of the oviduct (*23*). Within this tissue, the cytoplasmic radioactivity appeared to be present in a macromolecular complex. The cytosol progesterone binding macromolecule had a sedimentation coefficient of 3.8*S* during sucrose gradient centrifugation in the presence of 0.3*M* KCl and aggregated to 5*S* and 8*S* when the ion concentration was low (no KCl) (*24*). The oviduct binding component showed a striking affinity for progesterone ($K_d \sim 8 \times 10^{-10}$ at 4°C) and appeared to comprise only 0.02 percent of the cytosol protein. The cytosol progesterone binding macromolecule was unequivocally distinguished from plasma transcortin by agarose gel chromatography, discontinuous polyacrylamide electrophoresis, isoelectric gradient chromatography, and protamine sulfate precipitation.

Indirect physical-chemical calculations suggested that the molecule was a protein which existed in the shape of a prolate ellipsoid with a monomeric molecular weight of approximately 90,000. The calculated axial ratio showed that the protein was a macromolecule with a length 14 to 18 times greater than its width (*24*). This explained an apparent discrepancy of molecular weight upon analysis by different techniques.

The binding protein in the cytosol was specific for oviduct and showed very little affinity for estrogens (estradiol, estrone), mineralocorticoids (aldosterone), glucocorticoids (cortisol), or progesterone precursors and inactive metabolites. The tissue concentration of progesterone binding protein was increased tenfold by prior estrogen treatment (*25*). This estrogen-mediated increase in progesterone binding protein correlated quite closely with the estrogen-induced quantitative enhancement of the oviduct-progesterone response (avidin synthesis). For these reasons, it was thought that the combined evidence was compatible with the concept that this progesterone binding molecule was in fact a physiologic receptor for the hormone and that the formation of this steroid hormone-receptor complex is an obligatory initial step in steroid hormone action.

Progesterone binding components have been partially characterized in the uterine cytosol fraction of the guinea pig (*26*), rabbit (*27–31*), rat (*27, 29–33*), mouse (*33*), and human being (*28*). Studies performed in several species have provided evidence for an estrogen-responsive, progesterone binding system in the uterus (*34, 35*) and vagina (*35, 36*). In the guinea pig, fluctuations in the progesterone binding capacity of the uterine cytosol fraction have been measured during the estrous cycle (*37*), but the factors responsible for the variation in the concentration of uterine progesterone binding sites during the cycle have not been established.

In a recent study, Leavitt *et al.* (*38*) found an estrogen-dependent 7*S* progesterone binding component in the hamster uterus. The occurrence of cyclic variations in the cellular concentrations of progesterone binding sites was indicated by increases in the concentration of progesterone receptor during diestrus (day 3) to a maximum at proestrus on day 4 coincident with an increase in the concentration of estradiol in the serum (*38*). The number of progesterone binding sites decreased slowly after ovariectomy at proestrus and the level was rapidly restored by exogenous administration of estrogen, confirming that the increase in the number of uterine progesterone binding sites during the cycle depends on the presence of estrogen.

Transfer of the Hormone-Receptor Complex to the Nucleus

After exposure of uterine tissue to ^{3}H-labeled 17β-estradiol, two intracellular sites of hormone binding were noted: one in the cytoplasm and another in the nucleus (*39*). Subsequently, nuclear binding appeared to predominate. The pioneering studies of Jensen and his associates (*40*) and of Gorski *et al.* (*1*) have led to the concept that an estrogen-induced conformational change occurs in the uterine cytoplasmic receptor protein and that

this is followed by the translocation of the hormone-receptor complex to the nucleus. This translocation process was an attractive hypothesis since it placed the hormone-receptor complex in a compartmental location adjacent to the site of hormone-induced changes in gene expression.

That a temperature-dependent (37°C) intracellular transfer of protein-bound estradiol from the cytosol to the nucleus actually occurs was initially demonstrated in the rat uterus (*40*). Incubation of 17β-[^{3}H]estradiol with uterine tissue in vitro led to an accumulation of a salt-extractable (0.3*M* KCl) form of the estrogen-receptor complex from a preparation of nuclei. This nuclear hormone-receptor complex sedimented at 5*S* during sucrose gradient centrifugation and was undetectable in nuclei of target tissue not previously exposed to estrogen. While nuclear 5*S* receptor appeared during exposure to hormone, an apparent concomitant depletion in the total quantity of cytoplasmic 8*S* receptor occurred. Cell-free exposure of preparations of nuclei to [^{3}H]estradiol and cytoplasmic receptor led to accumulation of extractable 5*S* hormone-receptor complex from the nuclei, but no extractable 5*S* complex was noted when [^{3}H]estradiol alone was incubated with uterine nuclei. It was these observations that led to the "two-step" hypothesis of Jensen *et al.* (*40*) according to which the 5*S*-estradiol receptor complex extracted from nucleus is thought to represent an altered form of the cytoplasmic receptor. Recent evidence suggests that upon interacting with estrogen, the binding protein undergoes a physical change permitting it to relocate to a nuclear position (*41*). A new equilibrium is then achieved in which up to 90 percent of the estrogen bound to receptor is in the nuclear position. Because of this equilibrium, any estimation of the correlation between tissue response and estrogen binding must allow for both nuclear and cytoplasmic estrogen-receptor complexes being equally correlated with responses after equilibrium is reached.

Most of the conclusions we have discussed are based on experiments with uterine tissue in vitro or on experiments conducted under cell-free conditions. An important recent methodologic advance has permitted a better understanding of estrogen-receptor translocation to the nuclear compartment in living tissue and has thus enabled investigators to correlate the translocation process with the biologic responses of uterine tissue to various estrogenic compounds (*42*). The quantitative assay for determination of the number of nuclear estrogen-receptor sites relies on the exchange of [^{3}H]estradiol with unlabeled estradiol bound to target cell nuclei in living tissue under various physiological states. Clark *et al.* (*43*) have been able to demonstrate that the translocation of estrogen receptor to the nucleus occurs under the influence of endogenous estrogen during the estrous cycle. The number of estrogen-receptor sites that are formed is a dose-dependent phenomenon that shows a striking positive correlation with early uterine physiological responses to estrogen administration (*44*).

Because progesterone was thought to act in the nucleus to influence gene transcription, it became pertinent to establish whether this hormone was also bound to a macromolecular receptor in the nucleus of the target cell. After stimulation with the hormone, purification of oviduct nuclei and extraction with salt revealed that such a nuclear receptor was present and that it was almost identical to the receptor found in the cytoplasm of the same cells (*23*). In experiments similar to those performed with estrogen in rat uterus, it was demonstrated that no appreciable quantities of nuclear receptor were found in purified oviduct nuclei from unstimulated animals. However, upon exposure to progesterone, an increase in extractable nuclear receptor protein occurred simultaneously with a diminution in the amount of cytoplasmic receptor (*23*). It appeared again that this steroid hormone was also capable of initiating a translocation process which resulted in an accumulation of receptor-bound intracellular [^{3}H]progesterone in the target cell nucleus.

A subsequent series of experiments conducted under cell-free conditions proved to be of considerable interest. As with estrogen and uterus, direct incubation of [^{3}H]progesterone with purified oviduct nuclei led to little steroid being bound, and progesterone binding was shown to be dependent on the simultaneous presence of its receptor in the reaction medium. However, it was further shown that the progesterone-receptor complex could only bind well to nuclei of oviduct target tissue and that nuclei of nontarget tissue such as lung, spleen, heart, and intestine demonstrated little capacity to "accept" and retain the hormone-receptor complex (*23*).

This observation led to the "nuclear acceptor hypothesis," according to which target cell nuclei should contain acceptor sites with a specific affinity for the receptor molecules. Moreover, similar experiments performed with rat prostate after administration of androgens confirmed that this hypothesis was generally valid for sex steroids and their target cell nuclei (*45*).

It seemed that for the progesterone receptor to bind to the oviduct nuclei, it first had to be complexed with a progestational steroid and then allowed to undergo a time- and temperature-dependent transformation, presumably to a structural form capable of interacting with the nuclear acceptor sites. The intranuclear location of these acceptor sites was confirmed when, upon fractionation of nuclei exposed to ^{3}H-labeled hormone receptor, the radioactive complex could be found attached to the chromatin (*23, 46, 47*).

Hormone-Receptor Binding to DNA

Because it has been reported that deoxyribonuclease releases bound estradiol from uterine nuclei, DNA is implicated in the nuclear binding of the hormone-receptor complex (*48*). The binding of uterine estrogen receptors to DNA has also been demonstrated to occur in vitro (*49, 50*). The binding is of sufficient strength to withstand centrifugation of the DNA through sucrose gradients to which up to 0.1*M* KCl has been added. These observations have been confirmed by means of DNA-cellulose chromatography (*51, 52*). Both cytosol and nuclear receptor forms of the complex were shown to bind to DNA, and partial purification of the receptor by ammonium sulfate precipitation appeared to enhance binding to DNA (*51*). This reaction has some of the properties of nuclear binding of estradiol in vivo and in vitro in that the binding to DNA is disrupted by 0.3*M* KCl and there exists only a limited number of high-affinity binding sites on DNA for the receptor. In fact DNA-cellulose chromatography has been used to effect receptor purification (*51, 52*). In a recent report it was calculated that if a 1 : 1 interaction occurs between estradiol and receptor, the

available DNA binding sites are saturated at a level of two receptors for every 10^7 nucleotides (DNA), or 500 receptor molecules per quantity of DNA found in a single nucleus (*50*). Although DNA has many of the characteristics one would expect of the nuclear acceptor, a lack of binding specificity is evident. In fact, estrogen receptor from rat uteri can interact with DNA from calf thymus, salmon sperm, *Escherichia coli*, and *Bacillus subtilis*. It appears then that the acceptor site is more complex and that other components of chromatin may act to modify receptor binding to DNA.

Purified chick DNA has the capacity to bind and retain the [^{3}H]progesterone-receptor complex (*53*). At high concentrations of DNA, essentially all of a limited quantity of hormone receptor could be bound to DNA. This binding affinity did not appear to be uniquely sequence-specific as DNA from a heterologous eukaryotic species (calf) showed a similar capacity to bind the chick progesterone-receptor complex (*12*).

After elution of either crude or purified progesterone receptor from a diethylaminoethyl cellulose (DEAE) column, the molecule can be resolved into two apparent subfractions or subunits termed A and B (*54*). Both subunits bind specifically with biologically active progestins for which they have a high affinity and low capacity. It is of considerable interest that only subunit A became bound to purified DNA (*53*). The B unit had no such capacity.

Although it is possible that this DNA interaction was simply a nonspecific absorption phenomenon, it was subsequently noted that binding to DNA was a high-affinity reaction ($K_d \simeq 3 \times 10^{-10} M$) and that there were a limited number of binding sites (one receptor bound for every 10^6 nucleotide pairs of DNA). Thus, the receptor (A subunit) has a high affinity for the binding sites of oviduct DNA and the DNA has a limited number of sites.

In studies of the specificity of binding in relation to the nucleotide sequences of DNA, there appeared to be a limited degree of species specificity. Although receptor DNA interactions are of great potential interest because of the known effects of steroid hormones on gene expression, no good evidence exists to support the notion that a steroid hormone-receptor complex can bind to specific polydeoxynucleotide sequences.

Hormone-Receptor Interaction with Chromatin Nonhistone Proteins

When [^{3}H]estradiol is incubated directly with preparations of target tissue chromatin in vitro, very little of it becomes bound to the chromatin. However, incubation of the preformed [^{3}H]estradiol-receptor complex from uterus with uterine chromatin results in significant retention of the complex on chromatin (*55*). Removal of histone (basic) proteins prior to incubation exposes even more receptor binding sites (*50*). Similar results for androgen-receptor interactions with male target (for example, prostate) and nontarget tissues have been reported by researchers at three laboratories (*55, 56*).

Studies of the recombination of steroids, or steroid-receptor complexes, and chromatin in cell-free systems reveal that very little [^{3}H]progesterone becomes bound to target cell (oviduct) chromatin in the absence of receptor protein (*47, 55, 57*). Substantial amounts of [^{3}H]progesterone were recovered bound to oviduct chromatin when the [^{3}H]progesterone was first combined with the oviduct cytosol receptor. Unmetabolized [^{3}H]progesterone-receptor complex could be reextracted intact by treatment of chromatin with 0.3*M* KCl after incubation in vitro. Binding was specific for progesterone receptor alone because the binding of steroid could not be enhanced by extracellular binding proteins such as transcortin nor even by other cytosol preparations from nontarget tissues. Furthermore, these results demonstrated that progesterone receptor complexes bind to oviduct chromatin to a much greater degree than to nontarget chromatin such as that from spleen, heart, lung, or hen erythrocytes (*46*).

In attempts to determine the fraction of chromatin responsible for the binding of the hormone-receptor complex to chromosomes, histone proteins were selectively dissociated from chromatin and the chromatin was subsequently reconstituted by sequential dialysis. In this manner, "hybrid" chromatins were also prepared in which histones from other tissues or species were substituted during reconstitution. Binding of receptor to this reconstituted chromatin was similar to binding to the intact native chromatin of oviduct. Moreover, the capacity to bind the steroid-receptor complex was completely retained by hybrid chromatins containing histones from a nontarget tissue of a different species, for example, calf thymus. Finally, because oviduct chromatin from which all histones had been removed still displayed a more extensive binding than spleen chromatin containing no histones, it was concluded that histones themselves were not primarily responsible for the specificity of receptor binding (*46, 47*).

Preliminary experiments in which the dissociated chromatin was treated with ribonuclease prior to reconstitution suggested that chromosomal RNA was also not of major importance for the extensive binding. However, if during reconstitution the nonhistone (acidic) proteins of chromatin were removed, the chromatin lost most of its capacity to bind the progesterone-receptor complex (*46*).

In more detailed investigations of the importance of nonhistone proteins in regulating receptor binding, nonhistones and histones were dissociated from the chromatin of oviduct and erythrocytes. These proteins were then separated from the DNA, but it was possible to reconstitute most of the nonhistones back to the DNA by gradient dialysis. In certain instances the nonhistones and histones of the chromatin of one tissue were reconstituted with the DNA of another tissue to form hybrid chromatins. An immunochemical method employing specific anitserums against chromatin nonhistone proteins of various tissues was used to monitor the nonhistone fraction during reconstitution experiments to substantiate formation of hybrid chromatins (*58*).

Oviduct cytosol, containing the [^{3}H]-progesterone-receptor complex, was then incubated separately with the intact, reconstituted, or hybrid chromatins. Reconstituted oviduct chromatin binds progesterone-receptor complex in a manner quantitatively similar to that of intact native oviduct chromatin. However, when the nonhistone protein of erythrocyte is inserted onto the oviduct DNA during reconstitution, this hybrid loses its enhanced ability to bind receptor and resembles native erythrocyte chromatin. Conversely, insertion of nonhistone protein from oviduct into erythrocyte chromatin bestows binding capacity to this hybrid chromatin resembling that of native oviduct (*47, 58*). These experiments demonstrate that the enhanced acceptor ca-

pacity of target tissue chromatin for the hormone receptor of that tissue can be transferred to a nontarget DNA through transfer of the nonhistone (acidic) protein fraction. Additional experiments have localized this "acceptor capacity" to a certain subfraction of the total nonhistone proteins of the target cell chromatin (*58*).

As with the binding to DNA already discussed, only one of the putative progesterone receptor units, subunit B, has this capacity to interact with the nonhistone proteins of oviduct tissue (*53*). Thus, only if one considers the combined characteristics of both the A subunit (affinity for DNA) and the B subunit (affinity for nonhistone protein) of the progesterone receptor, can the full potential of crude intact receptor be accounted for. It appears then that both DNA and a nonhistone protein fraction of target cell chromatin play a positive role in forming the acceptor sites for uterine receptor in uterine chromatin and progesterone receptor in oviduct chromatin.

In summary, it can be speculated that after entry of the steroid-receptor complex into the nuclear compartment, the initial molecular interaction of the steroid-receptor complex with chromatin may occur in two parts, a high-affinity reaction occurring between the receptor subunit A and chromatin DNA and another high-affinity reaction occurring between a specifier subunit B of the intact native receptor and the nonhistone acceptor proteins (*12*). If this hormone-receptor complex is actually the inducer unit for steroid hormone modulation of nuclear RNA transcription, then this initial binding to the genome may prove to be of major importance to steroid hormone action.

Steroid Hormone Effects on the Cell Cycle

A necessary prerequisite of the study of hormone effects on mitosis in a target tissue is a knowledge of the amount and chronology of mitotic activity and cell cycle parameters in the absence of hormonal influence. In the immature oviduct, mitoses are infrequent (*59*). The mean mitotic index (MI) of the surface epithelium was 0.43 in the 7-day-old chicks (*60*). This reflects the slow natural growth of the oviduct that continues until sexual maturation. A single injection of estrogen markedly stimulated mitosis in the oviduct. There was a rapid rise in MI between 9 and 12 hours. The MI reached a peak of 2.3 at 18 hours after treatment and the amount of mitotic activity began to fall at 24 hours and continued to drop until 42 hours. The frequency of cells in mitosis 48 hours after injection with estrogen was more than twice that observed in the unstimulated oviduct.

When chicks are given a second injection of estrogen 24 hours after the first, a second rise in MI is observed. The patterns of changes in MI following single and double treatments could be explained in terms of a hormone-induced stimulation of division in a single population of cells. The cells appeared to progress through the cycle in a parasynchronous fashion. Estrogen treatment resulted in a similar stimulation of the rate of DNA synthesis in oviduct, leading to a dramatic increase in total DNA consistent with the hormonally induced cell proliferation (*60*).

Within a variety of tissues it has been shown that after an appropriate stimulus, nonproliferating cells can be stimulated to divide. In general, proliferative stimuli act to stimulate cells in the G1 (prereplicative) phase of the cell cycle to enter S phase (synthesis of DNA), G2 phase and then mitosis, or G2 cells to undergo mitosis. To ascertain the exact stage at which cells are responsive to hormone in the immature oviduct, chicks were given simultaneous injections of hormone and either fluorodeoxyuridine (FdU) or hydroxyurea (HU). If the estrogen acted to stimulate G1 cells to enter S phase, the inhibitors of DNA synthesis should have blocked the hormone-induced stimulation of mitosis. If these hormones stimulated a G2 population to undergo mitosis, the inhibitors should not block the early hormone-induced rise in MI. Both FdU and HU inhibited the stimulation of mitosis that normally occurred after treatment with estrogen; thus the stimuli causing proliferation acted prior to the completion of DNA synthesis, that is, in G1 or S. In the unstimulated oviduct, G2 = 1.75 hours and S = 7.3 hours, but a rise in MI was not observed until 12 hours after administration of the hormone. It appears, therefore, that estrogen stimulates G1 cells to enter S phase. These results are similar to those showing that estrogen stimulates cell proliferation in the uterine and vaginal epithelia of the mouse (*61*).

Progesterone also stimulated a small fraction of cells to undergo mitosis. A small, but significant rise in MI was observed 12 and 18 hours after treatment. The frequency of cells in mitosis dropped below the control level at 24 hours and remained low until 48 hours, with or without a second injection of progesterone (*60*). These data indicate that although progesterone can act as a stimulus for proliferation of a small population of cells in the surface epithelium of the immature oviduct, this steroid appears to exert its predominant inhibiting action by blocking the normal progress of proliferating cells through the cell cycle. The basis and relationship between these two actions of progesterone are not clear.

Effects of Steroids on Chromatin Composition and Conformation

In chick oviduct, differentiation of epithelial cells and synthesis of specific proteins such as ovalbumin and lysozyme occur in response to estrogen administration (*2*). Previous data have suggested that there is transcriptional control of both the differentiation process and of cell-specific protein synthesis. Based upon the hypothesis that changes in gene transcription during differentiation reflect, in part, changes in the tissue-specific pattern of gene restriction, changes occurring in the chemical composition and physical properties of oviduct chromatin were investigated during estrogen-mediated tissue differentiation.

Quantitative analysis of the chromatin from various stages of oviduct development demonstrated that while the amounts of histone varied randomly, the amounts of nonhistones increased during the first few days of differentiation and decreased gradually until completion of development (15 days of estrogen treatment) (*62*). The amounts of chromatin associated with RNA followed a similar pattern. Moreover, the capacity of the intact chromatins to serve as templates for RNA synthesis in vitro with bacterial polymerase also increased during the first few days of differentiation and then decreased during the final stages of development.

To clarify the relationship and the role of the nonhistones in the tissue-specific restriction of DNA, the antigenic properties of nonhistone protein-DNA complexes isolated from different organs were determined and then compared with those of the corresponding native (intact) chromatins. Antibodies were produced in rabbits against a preparation of oviduct nonhistone protein complexed to DNA (nucleoacidic protein) which was prepared from oviduct chromatin of chicks that had been injected with an estrogen for 15 days (*63*). The results of these studies were as follows: (i) The nucleoacidic proteins isolated from chromatin complexed to DNA were good immunogens which initiated the formation of complement-fixing antibody. (ii) The antibodies reacted strongly with the preparations from the homologous organ (chick oviduct), whereas the affinity for preparations of nucleoacidic proteins from heterologous sources (liver, heart, spleen) was very low, this indicating that the structure or composition of the antigenic sites on the nonhistone proteins in chromatin is organ specific. (iii) During the development of chick oviduct, the antigenic sites for acidic proteins underwent marked alterations which probably involved changes in the species of nonhistone proteins in addition to possible structural alterations of already existing proteins.

Structural analyses of oviduct chromosomal protein-DNA complexes were made by means of circular dichroism (CD) performed under standardized conditions. During estrogen-stimulated development of the oviduct, the chromatin DNA displayed a gradual increase in the magnitude of ellipticity measured at 275 nanometers. Other studies suggest that this increase in ellipticity may represent an "opening" of the DNA, that is, removal of proteins from areas of the DNA (*64*). Thus the CD analysis of oviduct chromatin supports the concept that an alteration in the composition or steric conformation, or both, of target cell chromatin occurs during steroid hormone-induced differentiation. These studies, together with the data showing that major quantitative and qualitative changes occur in the nucleoacidic proteins of chromatin, provide additional evidence that differentiation represents progressive alterations in chromatin biochemistry which may result in changes in cell structure and function (*62*).

Steroid Hormone Effects on RNA Synthesis

Since it is now clear that the steroid hormone-receptor complex enters the nucleus and binds to target cell chromatin, it seems logical to consider that the synthesis of RNA plays a major role in the primary mechanism of action. It has not yet been possible to demonstrate directly that the chromatin binding results in an increased rate of nuclear transcription. However, much evidence exists to support the hypothesis that steroid hormones do act upon the nucleus of target cells.

The first demonstration that estrogen stimulates the incorporation of precursor into total cell RNA came from the laboratory of Mueller and his associates (*65*). Initially, the responses to estrogen were measured in terms of hours. These studies and those from the laboratories of Gorski and Hamilton led to an argument about whether the stimulation of RNA synthesis was a cause or consequence of estrogen-mediated translation (*66, 67*). Examining the reaction more and more promptly after hormone administration, investigators finally demonstrated that within 2 minutes of a single injection of estrogen, a 40 percent increase occurred in the synthesis of rapidly labeled nuclear RNA (*68*). The estrogen stimulation of this activity was biphasic: there was an initial peak at 20 to 30 minutes, then the activity decreased abruptly and remained low for 2 hours after which time it increased once again and remained high for at least 24 hours (*69, 70*). Moreover, the first phase of this response was prevented by actinomycin D but was not blocked by cycloheximide (*71*). These data strongly suggest that the initial response of the nuclear apparatus is not dependent upon the continued synthesis of protein.

The nature of the rapidly labeled nuclear RNA formed during the first few minutes of estrogen action is still uncertain. Initial studies of the base composition of the RNA suggested that it was ribosomal-like (*69*). However, more recent studies on the sedimentation characteristics of this RNA and kinetics of stimulation of nucleoplasmic RNA polymerase II yield evidence of a more DNA-like nature (*72–74*). Moreover, stimulation of synthesis of this rapidly labeled nuclear RNA seems to be mandatory for the appearance in the cytoplasm of a group of specific estrogen-induced proteins (*75*). Within a few hours after injection, estrogen has resulted in stimulation of both ribosomal precursor RNA, 28*S*, 18*S*, and 5*S* ribosomal RNA and 4*S* transfer RNA (*4, 13, 72, 76*). Thus the synthesis of all classes of uterine RNA are eventually enhanced by estrogen.

Another approach to the investigation of changes in RNA synthesis is to utilize chromatin template activity. By this procedure one can estimate the percentage of the total genome which is available for transcription. Chromatin is used as the sole source of DNA but an excess of exogenous RNA polymerase is provided (usually from *E. coli*). Teng and Hamilton (*77*) described a 25 percent stimulation of template capacity of rat uterine chromatin within 30 minutes after the injection of estrogen to adult ovariectomized animals. Similar observations were made by Church and McCarthy (*78*) using chromatin isolated from endometrium of castrated rabbits. In these experiments, however, effects were observed as early as 10 minutes after estrogen administration. Enhancement of chromatin template activity in the rat uterus has also been observed by Barker and Warren (*79*). More recently, Glasser *et al.* (*74*) examined template activity in the rat uterus. Care was taken to monitor the chemical composition of chromatin during the isolation procedure, and in these experiments, the effects of estrogen were first demonstrable at 1 hour and the effects of estrogen on chromatin activity was paralleled by the stimulation of RNA polymerase I.

Chromatin template activity has also been shown to be stimulated by estrogen in chick oviduct (*7, 57*). By the technique of nearest-neighbor base analysis it has been shown that the qualitative changes in template activity occur in concert with quantitative differences in the nature of RNA products (*7*). Comparison of the base frequencies of RNA synthesized by chromatin from control and estrogen-treated chicks revealed considerable differences. In general, the RNA tended to become more AU-rich (adenine and uracil) during hormone treatment. Here again the data suggest that estrogen must promote the synthesis of new species of RNA during differentiation of the oviduct.

All classes of RNA are also eventually increased in the oviduct in re-

sponse to estrogen. Changes occur in the pattern of ribosomal precursor RNA as judged by polyacrylamide gel electrophoresis (*80*). Ribosomal 28*S* and 18*S* RNA species are increased (*81*) and there are marked effects on the synthesis of 4*S* and 5*S* RNA's (*7*). These increased rates of synthesis of ribosome-associated RNA species are accompanied for the first 7 days after estrogen treatment by a continuous accumulation of cytoplasmic ribosomes (*81, 82*).

Limited use has been made of the technique of DNA-RNA hybridization for studying effects of the female sex steroids on the uterine transcriptional apparatus. Church and McCarthy (*78*) employed this method to examine the effect of estrogen on the appearance of new populations of nuclear RNA sequences and obtained evidence that estrogen very rapidly induces synthesis of different populations of RNA molecules. The technique is of limited use for hormone sensitive tissues, however, because under the conditions normally used, only changes in repetitive sequences of DNA can be analyzed. Thus the portion of the genome containing unique sequences responsible for synthesis of most mRNA molecules is not assayed under the conditions used by Church and McCarthy (*78*). Recent methodological advances have allowed the genome to be effectively subdivided into unique and repeating sequences of DNA (*83*). Although no one has attempted to apply these new techniques to the investigation of the effects of estrogen on the mammalian uterus, in this laboratory we have utilized hydroxylapatite chromatography to subdivide the chick oviduct genome into unique and repetitive DNA sequences (*84*). We found no detectable differences in the renaturation profiles of oviduct DNA at various times of estrogen stimulation. The data suggest that estrogen is not acting through major gene duplication or deletion and offer evidence that the new proteins required for oviduct growth may arise from differential gene transcription.

It has also been found that DNA with unique sequences is transcribed in the oviduct with 25 to 30 percent of the resulting RNA being processed into the cytoplasm. Moreover, estrogen apparently causes an increase in the extent of transcription of unique gene sequences. Thus, although the amount of RNA transcribed from unique sequence DNA that is processed into the cytoplasm does not appear to vary as a result of estrogen treatment, qualitative differences seem to exist in the cytoplasmic mRNA populations at different stages of estrogen-induced differentiation.

Steroid Hormone Effects on RNA Polymerase Activity

Gorski was the first to report an effect of estrogen on RNA polymerase activity assayed in a crude nuclear pellet obtained from uteri of immature rats (*85*). Within 1 hour after a single injection of estradiol there was an increase in the activity of magnesium-dependent RNA polymerase. These observations were subsequently confirmed with a highly purified preparation of nuclei which had been stripped of the outer membrane by treatment with detergent (*70, 86*). A second polymerase activity was also shown to be stimulated by estrogen but required 12 hours of hormone treatment (*70, 86*). This enzyme activity required a high concentration of ions and showed a preference of Mn^{2+}. It was subsequently demonstrated that the Mg^{2+}-dependent enzyme (polymerase I) was restricted to the nucleolus and synthesized ribosomal RNA. On the other hand, the high salt enzyme activity (polymerase II) was located in the nucleoplasm and synthesized a product with a DNA-like base composition (*87*). Thus it appeared that estrogen stimulated synthesis of ribosomal RNA before any apparent effect on DNA-like RNA formation.

In further investigations of the effect of estrogen on uterine polymerase I activity, Barry and Gorski (*88*) obtained results suggesting that estrogen evokes an increase in the rate of chain elongation within 1 hour but does not affect the number of growing chains. Such experiments imply that estrogen stimulates the activity but not the number of polymerase molecules, and that its effect at this stage of the reaction is only minimally due to transcription of additional template.

Estrogen has also been shown to increase the activity of RNA polymerase in the chick oviduct during hormone-mediated differentiation (*2, 89*). In this tissue, there seems to occur a concomitant increase in polymerase I and II that is first demonstrable at about 6 hours. By 48 hours there is an eight- to tenfold increase in the polymerase I activity. It seems certain that the effect of estrogen is to enhance the activity of existing polymerase molecules as well as to increase the number of initiation sites on the DNA. This interpretation stems from the fact that during differentiation many additional cell-specific proteins appear that are under transcriptional control. Therefore, although estrogen stimulates RNA polymerase activity in both uterus and oviduct, it may do so by two different mechanisms.

For several years the rapid but transient stimulation of uterine RNA synthesis by estrogen could not be explained because neither chromatin template nor RNA polymerase seemed to be increased at the same time. However, by utilizing stringent kinetic conditions of assay, Glasser *et al.* (*74*) have shown that a rapid but transient increase in polymerase II activity can be demonstrated 15 minutes after estrogen administration. Moreover, the kinetics of this increase occur in concert with the previously documented stimulation of rapidly labeled nuclear RNA synthesis. The initial enhancement of polymerase II activity occurs prior to any detectable increase in the activities of chromatin template or polymerase I. In fact, stimulation of polymerase I first occurs at 1 hour and reaches maximum activity at 4 hours. These data therefore confirm the results of several investigators regarding the time course of activation of the nucleolar RNA polymerase I by estrogen (*85, 86*).

The studies of Glasser *et al.* (*74*) also offer evidence that may help to solve another problem of estrogen action in the uterus—whether some or all of the effects on transcription require continued protein synthesis. The activation of polymerase II is biphasic. When actinomycin D is injected 30 minutes before the estrogen, both of the increases in activity are abolished. However, the injection of cycloheximide before the injection of actinomycin D does not prevent the initial rapid increase in polymerase II. In fact, there is an increase over the effect seen with estrogen alone. Cycloheximide affects the synthesis of rapidly labeled RNA in the same way (*71*). On the other hand, the secondary increase in polymerase II which begins 2 to 3 hours after estrogen injection is completely abolished by cycloheximide. These data could explain why in the earlier studies from Gorski's laboratory (*66*), and in a more recent report from Nicolette and Babler (*90*), it was maintained that stopping

uterine protein synthesis prevented an estrogen-induced enhancement of RNA synthesis. Investigations were usually made 2 to 6 hours after hormone administration, considerably after the rapid but transient first phase of the RNA stimulation curve. Recent results from DeAngelo and Gorski (*75*) also revealed that a very early and specific event in the induction of a specific protein (induced protein) by estrogen is blocked by actinomycin D but not by puromycin or cycloheximide. Thus it seems that an early increase in the activity of RNA polymerase II could be necessary for the subsequent biochemical events in the action of estrogen on the uterus (*74*).

Raynaud-Jammet and Baulieu (*91*) were the first workers to demonstrate an effect of estrogen on uterine RNA polymerase activity in vitro. The estrogen had to be incubated with uterine cytosol in order for the response to occur, presumably because the reaction is time- and temperature-dependent. Similar observations have been made by Arnaud *et al.* (*92*) and by Mohla *et al.* (*93*). Arnaud *et al.* (*92*) suggested that only the 5*S* form of the estrogen-receptor complex would stimulate uterine RNA synthesis in vitro and that this form acted specifically on nucleolar RNA polymerase I. Moreover, these workers (*94*) suggested that phosphorylation of the 5*S* receptor resulted in enhanced ability of the complex to stimulate RNA synthesis. Mohla *et al.* (*93*) also reported a 40 to 60 percent stimulation of uterine polymerase activity in vitro and showed that prior incubation of the estrogen with the cytosol is necessary for the "transformation" of the cytosol receptor to the 5*S* form. Mohla *et al.* used these results to support their hypothesis that receptor transformation is an important step in estrogen action. Furthermore, they suggested that one of the biochemical functions of estradiol may be to induce conversion of the receptor protein to an active form that can enter the nucleus, bind to acceptor molecules, and induce RNA synthesis.

Recent studies in our laboratory have revealed that estrogen may indeed result in a change in chromatin template which allows an increased number of binding sites for RNA polymerase. These studies have been performed under conditions which will distinguish spurious binding on nicked regions of the DNA from true promoter regions. The role of the hormone receptor in this process, however, has yet to be elucidated.

For studies of the effect of progesterone on RNA polymerase activity, investigators have used primarily the chick oviduct as a model system (*2, 89*). Administration of progesterone to immature chicks not previously treated with estrogen produces little effect for the first 5 hours. Between 5 and 10 hours there is a considerable increase in the activities of both polymerase I and polymerase II. The maximum effect occurs at about 24 hours. Thus the increase in polymerase activity anticipates the induction of avidin synthesis.

The effect of progesterone on polymerase activity in oviducts from chicks previously treated with estrogen for 14 days is considerably different. A transient but significant decrease in activity occurs 2 hours after a single injection of progesterone (*2*); this is followed by an increase which peaks at 24 hours, and again this increased polymerase activity precedes the induction of avidin synthesis. This same type of response curve is seen for both rapidly labeled nuclear RNA and the polymerase which is presumably responsible for its synthesis (*2*). The reason for the initial decrease in activity cannot be the withdrawal of estrogen. It is probable that progesterone acts in two ways: (i) to induce mRNA specifically to synthesize avidin and (ii) to antagonize estrogen. In any event the decrease of estrogen-mediated events by progesterone is widespread among the biochemical events examined to date.

Effects of Steroids on Production and Translation of Messenger RNA

One of the most elusive problems in studies of the action of steroid hormones on the nucleus is that of obtaining direct evidence that the generation of mRNA is a rate-limiting step. Measurements of transcriptional changes have indicated that estrogen does, in fact, play a role at this level of cell regulation. Most of the major changes induced in estrogen-sensitive target tissues by administration of the hormone are blocked by prior treatment of the tissues with actinomycin D (*2, 13, 75*). Moreover, as already discussed, estrogen stimulates the synthesis of rapidly labeled nuclear RNA, and RNA polymerase activity is altered as is the template capacity of nuclear chromatin. Analysis of the RNA products by hybridization, and nearest-neighbor base analysis, also reveal marked changes in response to estrogen (*2, 77, 84, 95*). None of these studies, however, constitute direct proof of alterations in the transcription of specific structural genes.

The only definitive way to prove the existence of the ovalbumin mRNA was to demonstrate that it would support the unambiguous translation of ovalbumin in a cell-free protein synthesis system. It would then be necessary to show the absolute dependence on estrogen administration. We chose to use a modified rabbit reticulocyte lysate as the protein synthesis system. Since ovalbumin has a molecular weight of 45,000, an 8*S* to 17*S* RNA fraction was isolated from hen oviduct polysomes and was subsequently shown to contain ovalbumin mRNA activity (*10, 11*). Proof that the reaction product was authentic ovalbumin has been gained by several procedures: (i) interaction with a specific antiserum to purified ovalbumin; (ii) solubilization of the immunoprecipitate and analysis on sodium dodecyl sulfate gels; (iii) ion-exchange chromatography on carboxymethyl cellulose followed by reprecipitation with antiovalbumin; and (iv) the construction of peptide maps (*10, 96*).

The ovalbumin mRNA activity was specific for RNA isolated from oviduct of estrogen-stimulated chicks and was primarily found in the 8*S* to 17*S* fraction of polysomal RNA. The amount of synthesis was increased by addition of protein synthesis initiation factors. Moreover, inhibitors of chain initiation, such as edeine or aurin tricarboxylic acid, or of general protein synthesis, such as puromycin or cycloheximide, completely block ovalbumin synthesis directed by the oviduct mRNA fraction. Ribonuclease destroys the messenger activity whereas deoxyribonuclease does not. Steroid hormone-receptor complexes had no effect on the translation of ovalbumin mRNA.

This system for translating the ovalbumin mRNA with fidelity allowed us to look at the hormonal regulation of this specific messenger. Oviduct from laying hens, in which ovalbumin is being synthesized at its maximal rate, contains the greatest amount of ovalbumin mRNA activity. On the other hand, there is no detectable mRNA in ovalbumin from the unstimulated immature oviduct of the 7-day-old chick. Stimulation of these animals with estrogen for 4, 10, or 16 days leads to

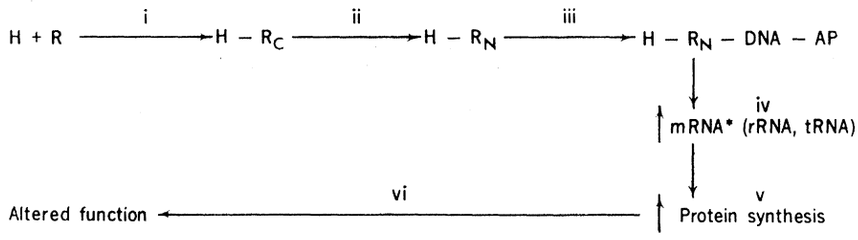

Fig. 1. Biochemical steps in steroid hormone action: (i) hormone (H) entering the target cell binds to a specific cytoplasmic receptor (R) and (ii) forms a hormone-receptor complex (H-R_C) which is transported to the nucleus (H-R_N) where (iii) it binds to specific acceptor sites on the genome [chromatin DNA and nonhistones (or acidic proteins, AP)]. This is followed by (iv) activation of the transcriptional apparatus that results in the appearance of new RNA species; (v) transport of the hormone-induced RNA to the cytoplasm; and (vi) the steroid-mediated functional response that is characteristic of the target tissue. Among the components of such a system which may bring about functional changes in the target cell are enzymes, structural or regulatory proteins, and nuclear events that are subject to amplification.

increasing activity of the extractable messenger. However, when chicks treated with estrogen for 16 days are subsequently withdrawn from hormone for 16 days, the ovalbumin mRNA activity again becomes very low. Finally the administration of estrogen to these animals for 1, 2, or 4 days after the 16-day withdrawal period leads once more to a progressive increase in ovalbumin messenger. These data reveal that indeed the amounts of extractable ovalbumin mRNA from oviduct are directly dependent upon estrogen stimulation. Moreover, the changes in ovalbumin mRNA in response to estrogen paralleled or slightly anticipated the changes in oviductal accumulation of ovalbumin (*97*).

To better assess the changes in mRNA after estrogen treatment, kinetic analyses of mRNA activity were made and the rate of ovalbumin synthesis was calculated (*9*). At various times after injection of estrogen to chicks subjected to a 16-day withdrawal period, the rate of ovalbumin synthesis was assayed in vitro with minces of oviduct. The rate of synthesis of this specific protein was time-dependent and peaked at 18 hours after the injection of steroid. The approximate half-life of the messenger was calculated from the descending limb of the induction curve to be 8 to 10 hours. Similar studies were then performed except that ovalbumin mRNA was extracted and the activity was quantified in the translation system. There was a remarkable parallelism between the rate of ovalbumin synthesis and the amount of available mRNA. Again, mRNA could be detected prior to ovalbumin synthesis, and the half-life of the mRNA was 8 to 10 hours.

Thus it appears that estrogen acts in the nucleus to promote the synthesis of mRNA's which code for the cell-specific proteins. Since ovalbumin represents nearly 60 percent of the protein synthesized in oviduct gland cells under the influence of estrogen, it was questioned whether the ovalbumin mRNA was transcribed from single copy DNA or whether this might represent an instance of gene amplification. The ovalbumin mRNA was purified by a variety of procedures until its activity, tested in the protein synthesis system, had been increased some 100-fold compared to a preparation of total oviduct RNA. The partially purified mRNA was incubated with RNA-directed DNA polymerase from avian myeloblastosis virus in order to produce a copy of radioactively labeled DNA which would be the complement of ovalbumin mRNA. The ^{3}H-labeled DNA produced was sized on alkaline sucrose gradients and shown to contain fragments up to 2000 nucleotides in length. When the ^{3}H-labeled DNA was reacted with excess ovalbumin mRNA, 90 percent of the labeled DNA formed a stable hybrid with the mRNA indicating that the ^{3}H-labeled DNA was indeed a complementary copy. A fraction of the ^{3}H-labeled DNA was then used in a DNA-excess hybridization experiment (*98*). Whole DNA was sheared to 400 nucleotide lengths and incubated with the ^{3}H-labeled DNA at an excess of $10^7 : 1$. Complementary ^{3}H-labeled DNA hybridized to chick DNA with a $C_0t_{1/2}$ (measures of reassociation; concentration (mole) $\times$ seconds per liter) of 480. Under similar conditions of second-order kinetics, single copy or unique-sequence DNA hybridizes with a $C_0t_{1/2}$ of 420 (*84*). Thus the complementary ^{3}H-labeled DNA has hybrization properties which suggest that the ovalbumin gene is only represented one time in the oviduct genome (*99*). These data suggest, then, that estrogen may act at the level of transcription to stimulate production of numerous copies of a single gene. This type of hormonal regulation would lead to a high intracellular concentration of ovalbumin mRNA and subsequently of ovalbumin itself.

In fact, recent studies have been completed in our laboratory in which this ^{3}H-labeled complementary DNA copy of the ovalbumin mRNA has been used to calculate the exact number of mRNA copies in each oviduct cell prior to and during hormonal stimulation. After estrogen treatment, up to 15,000 molecules of ovalbumin mRNA may accumulate in a single oviduct cell. Withdrawal of the hormone results in a decreased concentration of ovalbumin mRNA to $\sim$ 10 molecules per cell. This molecular probe (^{3}H-labeled complementary DNA) will remain as a powerful tool for monitoring transcription of specific genes.

Available data strongly suggested that estrogen acts in the nucleus to promote the synthesis of mRNA's which are necessary for the subsequent actions of this steroid on growth and differentiation. An important question that remained unanswered, however, was the general applicability of this mechanism to the actions of other hormones. In the chick oviduct, progesterone has been shown to control specifically the synthesis of the egg-white protein avidin (*2*, *100*). Unlike estrogen, no marked changes occur in total cell RNA synthesis, and polysome profiles are seemingly unaltered (*2*, *101*). Avidin represents no more than 0.1 percent of the total egg-white protein. Consequently, it followed that the mRNA for this protein might also be present in small amounts. Extraction of total RNA from estrogen-stimulated hen oviducts proved to be less than satisfactory as a means of quantitation. When such RNA preparations were tested in the heterologous protein synthesis system, it was not always possible to demonstrate avidin synthesis by a specific immunoprecipitation procedure. In order to assure reproducible results, it was necessary to effect a partial purification of the messenger fraction. We were able to take advantage of the fact that many mRNA's including the one for avidin contain, at the 3′ terminal end, an extensive sequence of poly-

adenylate residues. The presence of a poly(A) sequence was shown by Brawerman *et al.* (*102*) to allow the mRNA to be selectively adsorbed to nitrocellulose filters. Application of this procedure to oviduct RNA results in a one-step 50-fold purification of avidin (and ovalbumin) mRNA (*103*). This simple procedure allowed us to measure routinely and consistently the avidin mRNA activity which appears in oviduct in response to progesterone (*104*).

Avidin mRNA is only present in the 8*S* to 17*S* fraction of oviduct polysomal RNA. Moreover, it has been shown by sucrose gradient analysis to have an average sedimentation of 9*S*. This would be expected if the message were to code for a protein of approximately 15,000 daltons. This is, in fact, the molecular weight of a single subunit of avidin. Avidin mRNA activity is also abolished by ribonuclease and no avidin is synthesized when inhibitors of peptide chain initiation or elongation are present in the cell-free system. Again, direct addition of progesterone, estrogen, or adenosine 3′,5′-monophosphate has no effect on the translation process.

Avidin mRNA activity is highest in oviducts of mature laying hens where progesterone stimulation is maximal. No activity can be demonstrated in the unstimulated immature chicks or in oviducts from animals which have received multiple injections of estrogen. However, after a single injection of progesterone, avidin mRNA activity can be detected within 6 hours (*9*). Maximum concentrations are achieved by 18 hours, which is considerably before the maximum amount of avidin appears in the tissue. These data suggest that both estrogen and progesterone act on the nucleus of target cells to promote the synthesis of mRNA's. This response seems to be a rate-limiting step in the subsequent production of specific proteins.

Summary

The data discussed herein demonstrate the great variation in target-tissue response that can occur after administration of steroid hormones. The female sex steroids can exert regulatory effects on the synthesis, activity, and possibly even the degradation of tissue enzymes and structural proteins. Each response, nevertheless, appears to be dependent on the synthesis of nuclear RNA. In many instances, the steroid actually promotes a qualitative change in the base composition and sequence of the RNA synthesized by the target cell, implying a specific effect on gene transcription. Most important is our direct quantitative evidence that sex steroids cause a net increase in the intracellular amounts of specific mRNA molecules in target tissues.

It thus appears that we are discovering a pattern of steroid hormone action which includes (Fig. 1): (i) uptake of the hormone by the target cell and binding to a specific cytoplasmic receptor protein; (ii) transport of the steroid-receptor complex to the nucleus; (iii) binding of this "active" complex to specific "acceptor" sites on the genome (chromatin DNA and acidic protein); (iv) activation of the transcriptional apparatus resulting in the appearance of new RNA species which includes specific mRNA's; (v) transport of the hormone-induced RNA to the cytoplasm resulting in synthesis of new proteins on cytoplasmic ribosomes; and (vi) the occurrence of the specific steroid-mediated "functional response" characteristic of that particular target tissue.

To elucidate fully the mechanism of steroid hormone action we must study the biochemistry of the process by which information held by the steroid hormone-receptor complex is transferred to the nuclear transcription apparatus. If our assumptions are correct, we should ultimately be able to discover how this hormone-receptor complex exerts a specific regulatory effect on nuclear RNA metabolism. Such regulation might be achieved (i) by direct effects on chromatin template leading to increased gene transcription and thus RNA synthesis; (ii) by activation of the polymerase complex itself; (iii) by inhibition of RNA breakdown; or (iv) by intranuclear processing of large precursor molecules so that smaller biologically active sequences are produced, and (v) by transport of RNA from the nucleus to the cytoplasmic sites of cellular protein synthesis.

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Innovation in Industry and the Diffusion of Technology

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The impact of technological change on economic growth (*1*), industrial productivity, and international competition and trade has been widely recognized. There is a rapidly developing interest in such issues as the environment for advances in science (*2*) and technology (*3*) and the contribution and relationship of basic science to technology (*4*). Recent debate has focused on the questions of whether and how to provide incentives to firms to innovate and to spend greater amounts on research and development, and whether and how to reduce the barriers to innovation faced by firms (*5*). A wealth of hypotheses and case studies of the process through which technology is created, developed, and used by firms is available and should provide a useful perspective in dealing with these questions. The sources of more than 2000 case studies, the industries or innovations studied, and the sizes of the samples are summarized in Table 1. A simple concept of the factors that limit and determine a firm's effectiveness in innovation and of the phases and relationships in the innovative process is presented below; this concept allows one to compare the findings from these diverse sources.

In this article, I present what we know—or think we know—about the process of innovation by firms. How do characteristics of the environment affect firms' innovation? What factors and information affect the creation and acceptance of ideas for new products? What factors are related to effective development efforts? What do we know about the acceptance of innovations in the market and about the creation of new firms based on technology? Finally, past work in this field

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